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PREFACE

First I would like to take this opportunity again to express my sincere thanks to all your support for this "International Conference on Sustainable Biowaste Management 2021 (SBM2021)" , and ‘YOU’ made this conference a great success. I am so privileged and very pleased to have your participation in this conference to deliberate on advancement in biowaste management and its role in biocircular economy.

SBM 2021 will definitely provide you a great opportunity and platform to exchange your views, visions, and experiences on biotechnology, bio-fertilizers and bio-products, and climate change abatements related to biowaste management. The outcome will have immense benefit to Hong Kong, Asia-Pacific partners, and Western countries through knowledge exchange, fostering of collaborations and development of appropriate waste management technologies.

This year we have an overwhelming 438 participants coming from 39 countries all over the world to attend the conference. In addition to 7 keynote presentations, there will be three parallel platform sessions and 3 parallel poster sessions daily for 3 consecutive days with a total of 89 platform presentations and 45 poster presentations. There will be 3 poster presentation sessions each day for 3 days and poster presentations for each day covering 10 specialized themes.

Outstanding papers will be published in special issues of Bioresource Technology and Detritus after review. Best Poster Awards and Young Researcher Awards are also waiting to acknowledge and embrace your knowledge and potential. To encourage the participation from the developing countries, we have set aside some funding to invite prominent scientists to participate the conference.

Due to pandemic Covid-19, we have deferred the conference from 2020 to 2021 and finally we have decided to go have a virtual SBM2021 conference in April 12 to 15, 2021. I look forward to meeting you all at the virtual SBM 2021 and wish you enjoy the conference.

Heartily thanks to all your support for the conference.

Conference Chair
Prof. Jonathan W.C. Wong, PhD, MH, JP
Sino-Forest Applied Research Centre for Pearl River Delta Environment, Institute of Bioresource and Agriculture and Department of Biology
Hong Kong Baptist University
Hong Kong SAR, P.R. China

Table of Contents

International Conference on Sustainable Biowaste Management 2021

Keynote Speeches

SUSTAINABLE BIOWASTE MANAGEMENT IN DEVELOPING COUNTRIES

Agamuthu. P [1,*], Mehran, S.B. [2]

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ABSTRACT. Biowaste comprises of waste originating from agricultural activities, activities involving livestock, food processing plants and from organic portion of municipal solid waste (MSW). This paper summarizes and reviews the generation and management of biowaste from several sources in developing countries. In MSW, approximately more than 50% of MSW is composed of organic waste in developing countries and MSW is mainly disposed of in some forms of landfills. The treatment of other biowaste in developing countries includes usage as animal feed, power generation and conversion into bioproducts such as compost etc. However, majority of biowaste is still managed unsustainably in developing countries. This paper also highlights the reasons for improper management of biowaste. For instance, effective legislations and policies on biowaste management are either absent, or they are not stringently enforced in developing countries. Several technologies for sustainable treatment of biowaste and recommendations for sustainable management are presented.

Keywords: *Biowaste, Food waste, Biomass, 3R, Sustainable waste management.*

Introduction

Biowaste or Biomass refers to the organic portion of waste and it can come from municipal solid waste (MSW) in form of food and garden waste, from agriculture and livestock waste and, at times, from food processing plants. According to Kaza et al. [1], agriculture waste was generated at 3.35 kg/capita/day, whereas MSW produced 44% (884,400,000 tonnes) of organic waste globally in 2016. While the management of biowaste is relatively sustainable in developed countries, the sustainable management of biowaste is lagging in developing countries. This paper sums up the handling of biowaste in developing countries and highlights the causes of unsustainable management of biowaste. Moreover, different technologies are also reviewed for the management of biowaste with focus on resource recovery including energy recovery. Lastly, it also provides recommendations to improvement management of biowaste in developing countries.

Biowaste Management

The source of biowaste from MSW in selected developing countries is shown in Figure 1 below. Organic portion of MSW can range from 42.5% to 77% in developing countries. However, majority of MSW generated in developing countries is unsegregated and is disposed of in landfills or in open dumps (see Figure 2). In comparison to MSW, agricultural waste generation is 4.5 times more than MSW generation throughout the world and is more prevalent in countries with extensive agricultural industries [1].

The qualitative reports of agriculture waste can be seen in [2], since most of these developing countries have big agricultural industries. However, quantitative reports of agriculture and livestock waste from some developing countries and their management are compiled in Table 1 below. In some developing countries, agricultural and livestock waste is openly burnt. Nevertheless, initiatives have been taken to sustainably treat and manage biowaste from agriculture and livestock industry.

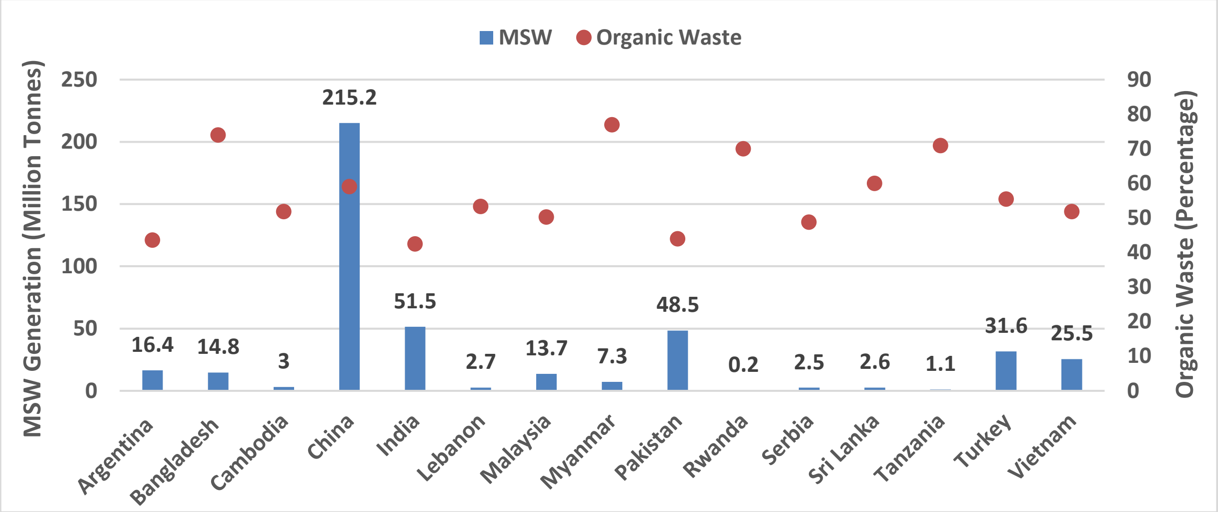

Fig.1. Annual generation of MSW and the percentage of Organic fraction in MSW in selected developing countries (Adapted from [3]).

Table 1. Amount of agriculture waste in selected developing countries (Adapted from [2]).

Countries	Quantity (Million Tonnes)	Description of Agricultural Waste	Management
Bangladesh	43	Agricultural residues, firewood, leaves, branches, twigs, cow dung, rice straw and husks	Biomass is mainly composted. Firewood, leaves, branches, twigs, cow dung, rice straw and husks are used as cooking fuels in villages
China	1,040	Crop straw	40% of agricultural waste is composted (60% is disposed of without treatment)
India	500	Wheat straw, paddy straw, cane trash and other farm wastes	70% of biomass is used as fodder, as fuel for domestic and industrial sectors and for other economic purposes. Rest of waste is used for power generation.
Malaysia	153	Oil palm waste, rice husk and straw, coconut trunk fibres, sugar cane waste, livestock	Electricity generation, animal feed, composting, producing fibreboard, pellets
Pakistan	16.7	Agricultural waste from cotton, rice, maize, wheat and sugarcane crops.	Power generation
Sri Lanka	6.86	Paddy straw, poultry droppings, cow dung	Dung and poultry droppings are used for agriculture lands
Thailand	31.4	Rice and sugarcane waste, wood waste	Utilized as a fuel for power generation. Processes include gasification, combustion, pyrolysis, and biogas.
Vietnam	123	Straw & livestock waste (manure, uneaten feed, and cattle and poultry carcasses)	Used as fertilizer, animal feed, or mushroom cultivation. Approximately 40-50% of livestock waste is treated. Anaerobic digestion is mainly employed (about 32%)

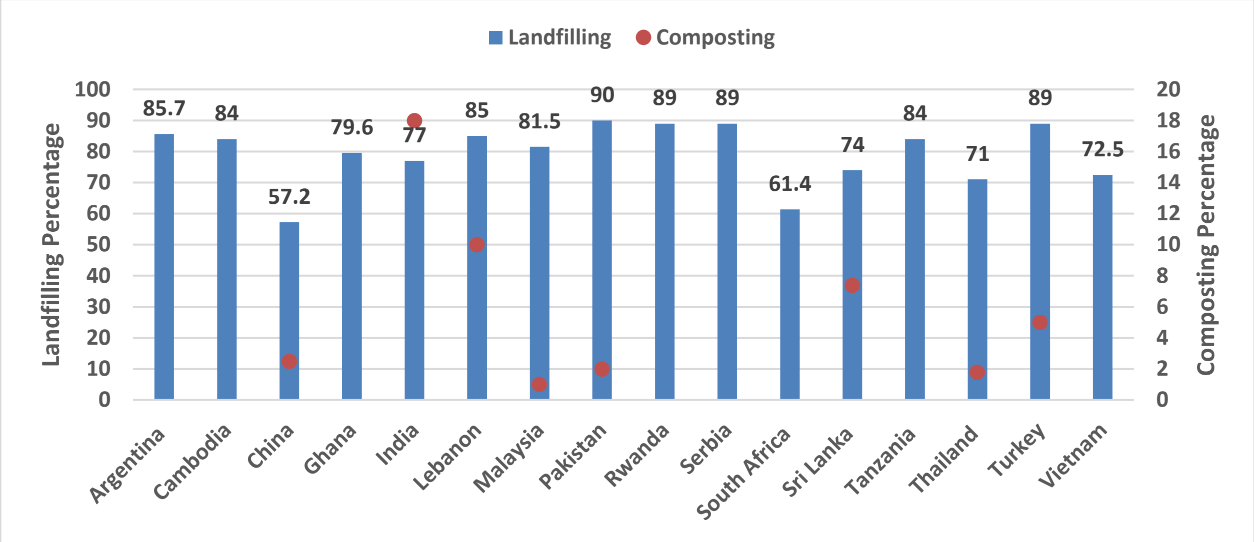

Fig. 2. Rate of landfilling (in both specified and unspecified landfills) and composting of MSW in selected developing countries (Adapted from [2]).

Factors Influencing the Management of Biowaste

There are several factors that can hamper the sustainable management of biowaste. Some of these are listed as follows;

1. Policy Barrier and/or implementation of legislations
2. Market instability / Supply and demand / Economic viability
3. High investment cost
4. Lack of political will, lack of knowledge or skilled personal
5. Unwillingness of industries to change or lack of awareness of relevant stakeholders
6. Unsuitable technological advancements for the host country (due to high capital cost, insufficient feedstock, lack of skilled personal etc.)
7. Immature technology for large-scale implementation of biowaste management

Table 2. Technologies used for the management of biowaste.

Technology	Description
Composting	Organic waste stabilised in aerobic conditions to give biofertilizer. Common in China, Pakistan, India and other nations (see Fig. 2).
Vermicomposting	Organic waste decomposed by earthworms to produce compost. Have been used in Ghana for treating pineapple waste, palm oil mill effluent in Malaysia, leaf litter in India, cow dung with food waste in Uganda and others [6].
Anaerobic Digestion	Stabilises organic waste in anaerobic conditions to produce biogas and biofertilizer. Used in India, China, Vietnam extensively. Smaller plants are used in Cambodia, Laos, Indonesia [7].
Pyrolysis	Converts biomass into granular charcoal, non-condensable gases and pyrolysis oils in anaerobic and high temperature environments. Not widely employed for biomass in the world.
Gasification	Converts biomass into combustible gas mixture in oxidative, high temperature environment. Syngas generated could be used to produce methanol. Gasification plants are operational in India, Uganda [8].

Technologies for Management of Biowaste

Some of the technologies used for the management of biowaste are compiled in Table 2. Note that there are more technologies than mentioned in Table 2 that are being studied and proposed for biomass treatment such as hydrothermal carbonisation [4], torrefaction, fermentation, transesterification, combustion, [5], and others.

Sustainable Development Goals and Biowaste

A total of six sustainable development goals (SDGs) can be associated with sustainable management of biowaste [9]. They are listed below:

1. SDG 2 – Zero Hunger: “End hunger, achieve food security and improved nutrition and promote sustainable agriculture”.
2. SDG 7 – Affordable and Clean Energy: “Ensure access to affordable, reliable, sustainable, and modern energy for all”.
3. SDG 9 – Industry, Innovation and Infrastructure: “Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation”.
4. SDG 12 – Responsible Consumption and Production: “Ensure sustainable consumption and production patterns”.
5. SDG 13 – Climate Action: “Take urgent action to combat climate change and its impacts”.
6. SDG 15 – Life on Land: “Protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss”.

Recommendations

In order to utilize the potential resources offered by biowaste, several initiatives could be taken in developing countries which are recommended below:

1. Create robust database for biowaste generation and determine the potential for bioproducts including energy for respective developing countries
2. Review and revise existing policies such as Energy Policy, Waste or Agriculture Waste Policy, Economic Policy etc.
3. Formulate dedicated policies and regulations on resource circulation of biowaste
4. Provide subsidies, tax exceptions and other economic incentives for establishing recycling and/or waste-to-energy plants for biowaste
5. Create awareness among relevant stakeholders and create opportunities for active participation and cooperation between stakeholders
6. Perform cost analysis, risk assessment of biowaste management to make educated decisions and bring relevant stakeholders on-board
7. Promote research and development of existing and novel technologies for various biomass sources and to make them economically, environmentally and technologically feasible
8. Implementation of SDGs at national level to promote sustainable management of biowaste

Conclusions

While developing countries generate biowaste in plethora amount, the overall management of biowaste is unsustainable. However, there are several technologies that are deployed for energy and/or resource extraction from biowaste in developing countries. These technologies include composting, vermicomposting, anaerobic digestion, gasification and others. Several factors such as absence of dedicated policies and legislations for resource circulation of biowaste, unstable market for bio-products, high investment costs and others hinder the proliferation of abovementioned technologies in developing countries for sustainably handling biowaste. Through achieving the targets of SDGs, biowaste could be managed sustainably in developing countries.

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APPLIED MACHINE LEARNING TO PREDICT CO_2 ADSORPTION ON BIOMASS WASTE-DERIVED POROUS CARBONS

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Biomass waste-derived porous carbons (BWDPCs) are a class of complex materials that have found prominence in sustainable waste management and practical carbon capture. However, their diverse textural properties, the presence of various functional groups, and the varied temperatures and pressures at which they are subjected to during CO_2 adsorption render challenges to understanding their underlying mechanism for CO_2 adsorption. In this study, we employed machine learning to systematically map CO_2 adsorption as a function of the textural and compositional properties of BWDPCs and performed adsorption experiments based on a dataset compiled from literatures. Various tree-based models were devised, where the gradient boosting decision tree (GBDT) had the best predictive performance with R^2 of 0.98 and 0.84 for the training and test data, respectively. The dataset was further classified into regular porous carbons and heteroatom-doped porous carbons, where again the GBDT model had prior and latter R^2 of 0.99 and 0.98 for the training datasets, and 0.86 and 0.79 for test datasets, respectively. Feature importance determination revealed the significance of adsorption parameters > textural properties > compositional properties in the order of precedence for BWDPC-based CO_2 adsorption. ML can accelerate the development of BWDPC-based CO_2 capture application, mitigating climate change and achieving sustainable waste management, simultaneously

Keywords: *Carbon materials, Upcycling, Data analytics, Gradient boosting Decision tree, Low carbon technology, Sustainable waste management.*

MACRO- MESO- AND MICROPLASTICS IN WASTE AND OTHERS

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Plastic is indispensable in contemporary daily life and approximately 50% of plastics are processed for single-use disposable products, which causes huge amount of plastic waste. Plastic waste abandoned in environment can break down into plastic debris, including macro-, meso- and microplastics. Microplastics can enter the food web and be ingested by the human, which may potentially threat ecology, agricultural production and the security of human health. Increasing the recycling and treatment of plastic waste is the main manner to reduce plastic pollution in many countries. However, plastic waste collected in managed waste treatment system may still have the risk to leak plastic debris to environment. Landfill is estimated to store 21-42% of global plastic waste, but has been proved to release microplastics by landfill leachate. Besides, incineration with energy recover accounts for a large proportion of plastic waste treatment. However, unburned material including plastic debris still exist in bottom ash. Organic fertilizer processed from organic waste also contains plastic debris, which is a vehicle for the entry of microplastic into the soil. In rural area, plastic mulching film are widely used for cultivating crops, which may eventually fragment into microplastics with poor recovering strategy. Microplastics will be transported into atmosphere and in aerosols. The state-of-the-art researches about this information relating to microplastics involved in waste management will be presented.

Keywords: *Plastic waste, Landfill, Incineration, Waste Management.*

RESOURCE RECOVERY FROM BIOWASTES: BIOECONOMY PERSPECTIVE FOR ENERGY AND ENVIRONMENTAL SUSTAINABILITY

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Waste-to-welth (w-2-w) and waste-to-energy (w-2-e) concept has gained much momentum in recent years, as on one hand, it offers unique opportunity to handle and dispose solid wastes (municipal waste as well as agro-industrial wastes), and simultaneously provides alternative sources of renewable energy. Solid waste treatment and management is a major issue worldwide. Several countries lack proper basic waste management infrastructure and awareness. Thus, waste-to-energy could be an attractive solution for resource recovery, which eventually offers potential benefits when works on principles of biorefinery. A biorefinery is a facility that integrates biomass conversion processes and equipment to produce bio-products, including biofuels and chemicals. It is analogous to todays' petroleum refinery. By producing several products, a biorefinery takes the advantages of various components present in the biomass and their intermediates, therefore maximizing the value derived from the biomass feedstock. They also help in complete or near-complete utilization of the feedstock and reduction in solid, liquid or gaseous wastes.

Various wastes such as food wastes, agri-crops wastes, municipal solid wastes, etc offer potenhtial opportunites if used as feedtsock for developing bio-based processes for the production of value-added chemicals and fuels. Potential application of such wastes for the production of liqiud and gaseous biofuels and other products on principle of biorefineries has gained more attention for possibilities of bioethanol, biobutanol, biodiesel and other high-value chemicals production, coupled with industrial waste treatment. Two major pathways for these include thermo-chemical conversion and biochemical conversion. However, process integration is key for the techno-economic success.

Keywords: *Resource recovery, Biowastes, Bioeconomy, Energy and Environmental Sustainability.*

BIOCONVERSION OF ORGANIC WASTES TO USEFUL PRODUCTS

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The rapid urbanisation and industrialisation have increased the demand for food, feed, chemicals, materials and energy that have in turn augmented the use of fossil-based resources. In addition, the increment in waste generation has detrimental impacts on environment and natural resources. Owning to the characteristics like high abundance, renewability, ease of accessibility around the globe, valorisation of organic wastes serves as an attractive and potential solution for petro-based resources and waste management issues. Lignocellulosic, agro-industrial and food wastes due to their organic- and nutrient-rich composition have been utilised as a feedstock for production of value-added products via microbial fermentation processes. The process consists of the pre-treatment of the waste biomass, production of value-added product in reactors and downstream processing for the recovery of the product. Various useful products such as bioplastics, biofuels, biopesticides, enzymes, organic acids, biomethane, biohydrogen and bio-fertilisers have been derived from wastes.

The integration of new technologies and holistic approaches for organic waste utilisation will also stimulate the transition towards circular economy that will develop resource-efficient and sustainable policies for long-term benefits. Therefore, feasibility and sustainability of production of various value-added products from wastes and by-products streams will be discussed. The key insights on the pre-treatment strategies and various aspects involved in the sustainable production of chemicals, fuels and materials in a biorefinery approach will be presented.

Keywords: *Wastes, biopolymers, Biofuels, Bioconversion, Value-added products, Circular bioeconomy, Biorefinery.*

SEPARATE COLLECTION OF BIOWASTE IN GERMANY - STATUS QUO AND OPTIMIZATION POTENTIALS

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Since January 1, 2015, biowaste has to be collected separately in Germany according to the Waste Management Act (KrWG, 2012). The EU Waste Framework Directive 2018/851/EU will make the separate collection of biowaste mandatory from 2024.

From the perspective of resource and climate protection, the highest level of sustainability of solid waste management can be achieved by recycling of biowaste with the most comprehensive possible use of its value-adding properties, i.e. by using the energy potential and the nutrient riched compost (organic soil improver and fertilizer), the so-called cascade utilization.

The prerequisite for the highest possible efficiency of the separate collection and recycling system for biowaste is a high collection rate and a low contaminant content. In Germany, separate collection of biowaste has been practiced for almost 40 years. Despite this, the functionality of the system is still in need of improvement and therefore has a high potential for future development.

The current average collection rate for biowaste in Germany is only around 50% and thus significantly lower than that achieved with the separate collection of paper and cardboard as well as glass, with rates over 80%. Collection rates of around 80% can also be achievable with separate collection of biowaste as indicated by the results of the current Germany-wide waste analysis (UBA, 2020).

Detailed analyses of the composition of biowaste in the remaining municipal solid waste itself provide valuable information. Around 88% of the biowaste in the residual waste was kitchen waste, while garden waste makes up the remaining fraction. The following conclusions can be drawn:

- The collection of garden waste via the organic waste garbage can in combination with various bring systems works very well.
- The amount of kitchen waste collected is extremely unsatisfactory.
- The alarmingly high amount of packaged food (18.4%) calls for new strategies to deal with this waste stream.

The content of impurities and foreign matters in the collected organic waste are around 3% but can also reach values of up to 10%. Against the background of the problem of "microplastics", it is imperative that efforts be made to reduce the contaminant load of biowaste.

The presented paper contains the results of a weak point analysis for the determination of defects. Based on these results, solutions are presented to increase the efficiency of the separate collection of biowaste. Many of the optimization approaches are based on findings from best practice separate collection systems.

If it is impossible to increase the performance of separate collection of biowaste, the authors believe that the separate collection of biowaste is at risk.
Keywords: *Biowaste collection in Germany, Status quo, and Optimization potentials.*

PROCESS INTEGRATION FOR A COST-EFFICIENT PRODUCTION OF BIOBASED PRODUCTS

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Due to the increasing need to reduce the environmental footprint and the push towards converting the fossil-based economy into a greener and sustainable economy, biobased technologies have become of increased interest in industrial sectors. Today, lignocellulosic biomass is seen as the main future alternative resource to replace fossil on the production of fuels and chemicals with reduced greenhouse gas emissions. The interest in such raw materials is further enhanced by the fact that they are not only of value for the fuels and chemicals industries, but they can also be relevant for other industrial sectors including food, feed, pharmaceutical and materials, where they can directly provide valuable compounds such as protein, amino acids, oligosaccharides, and phenolics, or can be used to obtain sugars for fermentation purposes. Techno-economic and life cycle assessment studies have shown promising data on the use of biomass-derived sugars in fermentation processes. However, some important points still have to be improved in order to create technologies with enough robustness for implementation in a large scale. Process intensification / integration principles have been considered to improve the techno-economic potential of biobased production. This has been done through strategies such as the use of high solid loading for enzymatic hydrolysis of biomass, simultaneous saccharification and fermentation processes, fermentation with downstream process integration, and fermentation using co-cultivation of microorganisms. Such approaches are seen today as promising strategies able to accelerate the development of new and robust biobased processes.

Keywords: *Lignocellulosic biomass, Biobased products, Process integration, Green technologies, Sustainability.*

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Advance Anaerobic Digestion

INCREASING THE EFFICIENCY OF MECHANICAL-BIOLOGICAL RESIDUAL WASTE TREATMENT THROUGH THE FERMENTATION OF THE LIQUIDS AFTER PRESSING THE ORGANIC FRACTIONS

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ABSTRACT. In 2015, the SUTCO Company has developed and established an anaerobic digestion (AD) technology to treat the organic fractions of municipal solid waste (MSW) in Germany. With this method, the degradable fractions of MSW are pressed and separated by employing a screw press-mesh into solid cake, which is composted in aerobic windrows, and press water, which is feed into anaerobic reactors. For this purpose, a pilot AD has been integrated into an existing mechanical-biological treatment (MBT) facility, received daily about 120 tons of MSW, which is operated by EVA- Abfallentsorgung. The driven motivation and objective of the project is to assess and develop a method to be adopted into MBTs with different operational models and layouts. In the present (pilot-)project, the MSW is separated into organic and inorganic fractions. The organic fractions smaller than 60 mm (corresponds to 65% of the MSW) are mixed with digestate, circulated from the AD, and process water from composting area, and pressed to generate press off leachate “liquid” and press cake “solid”. The generated press water with high content of suspension organic substances is fed into a wet AD. The project objective, along with ensuring the digestion stability in terms of organic degradation (present dissolved organic carbon (DOC) elimination is 83%) and biogas production, is to evaluate the correlation and influence of the each of MSW composition, screw press, digestate and process water on the press water quality and treatment efficiency such as total volume of biogas, generation rate (m3/ Mg waste) and its composition. The team has conducted several trails on site to determine the biogas production in response to different organic loading rate (OLR) and hydraulic retention time (HRT). The result has approved that the increment of OLR in the form of kg DOC in the reactors leading to increasing biogas production, while the specific biogas generation per kg DOC decreased. Likewise, more biogas is produced with increasing HRT. Nevertheless, the total amount of DOC in the press water has increased with increasing the quantity of water mixed with the organic waste. The properties of each of press water, digestate from each reactors, process water, input MSW and press cake were analyzed in the lab. The results showed a high concentration of organic content (above 10,100 mg/l DOC), low ash content (<8% wt./wt.) and suitable pH value (7.3) in press water. The average biogas production was calculated to up to 483 l(N)/ kg oTS, corresponds to 38 m3/ Mg waste to the screw press (<60 mm). The methane and carbon dioxide content lie between 60-65% and 30-40%, respectively. The results indicated that the press water from the organic fraction of MSW is a suitable substrate for AD which gives a high biogas yield and stable operation. However, further investigations should still be implemented to evaluate and reduce the volume of sand settled in anaerobic reactors, reduction of hydrogen sulfide (H2S) by adding of iron hydroxide dose, and feasibility of co-substrate of materials originates from various sources.

Keywords: *Residual waste treatment, Fermentation, Liquid pressed organic fractions.*

Introduction and background description

Waste is the end-step of the material supply-chain cycle or the excess streams result from daily production and consumption activities at residential, commercial, institutional and industrial areas. The urbanization and continuous growth of the global population, which is projected to reach 7.2 billion by 2025 (UNEP, 2005a), have generated additional burdens to the environment via the increasing amount of waste. Among others, the municipal solid waste (MSW) has always been significantly increasing. For instance, in 2016, 2.01 billion tons of MSW was created and it is estimated to achieve 2.59 and 3.40 billion tons per year by 2020 and 2050, respectively (Kaza et al. 2018). The handling of MSW is challenging due to the heterogeneity in quality and quantity. Therefore, MSW is disposed of pricelessly in many developing countries. For example, about 53% and 60% of MSW in middle east and north Africa (MENA) and Latin America was deposited in dumpsite, respectively.

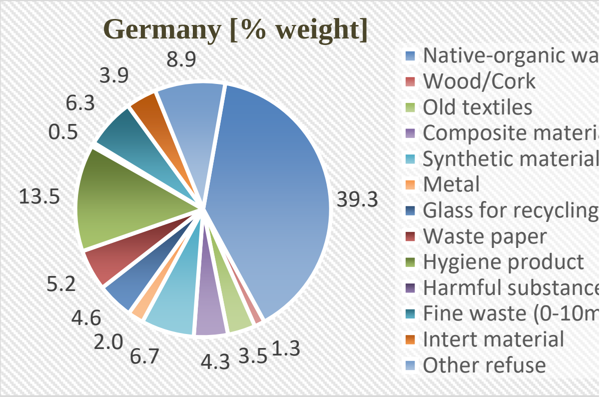


Fig. 1. Composition of residual waste in Germany (source: Dornbusch et al. 2020)

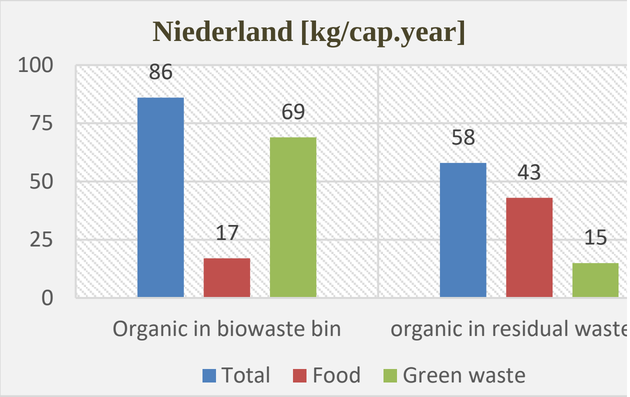


Fig. 2. Collection of organic waste in the Nederland (source: Langveld, G. et al.)

The organic waste, food and green waste, shares the predominant component in the MSW composition. According to the Kaza et al. report in 2018, the biodegradable waste constitutes globally about 44% of MSW. Some developed countries have introduced separate collection of biowaste. Hence, major amount of organic is collected via the biowaste bin at source, for example, 86 kg/cap.y in the Nederland (Figure 2). However, there is still a huge quantity of organic waste that find its destination in the residual waste bins. Figure 1 elaborates the total amount of organic found in the residual waste were 58 kg/cap.y and 50 kg/cap.y in the Nederland and Germany, respectively (Langveld, G. et al. and Dornbusch et al. 2020). The latter corresponds to 39,3% of the residual waste in Germany. The continually increasing amount of waste has demanded multi-dimensional approaches which combines efficient treatment and sustainability. For this, mechanical-biological treatment (MBT) was proven in coping with the growing amount of waste and offers a promising outputs which comply with the respective environmental, legal and regulatory standards.

Integrated anaerobic digestion and mechanical biological residual-waste treatment

In Germany, in the recent scenarios and directives such as Erneuerbare-Energien-Gesetz (EEG 2014) anaerobic digestion (AD) was contained as a sustainable approach that has a significant contribution as part of MBT. The MBT term describes a facility to which different types of waste is delivered and undergo a series of mechanical and biological processes such as crushing and sieving. For instance, the organic fractions are stabilized aerobically via composting. While the recyclables are sorted out for ultimate recycling or recovery. Globally, MBTs can be operated in the absence or presence of AD.

The present paper aims to present and assess a mechanical-biological residual-waste treatment (MBRT) technology which was recently developed to treat residual waste, called MSW henceforward (Figure 3). The facility receives nearly 150 tons MSW per day from residential areas. The MSW contains up to 40 percent of biodegradable waste, however, the households are also served with biowaste bin at source. The collected waste is shredded and passes through a cylindrical trommel with 60 mm circular-mesh diameter. The oversize fractions > 60 mm remain on the sieve are recyclables and handled to prepare high quality refuse derived fuel (RDF) (Figure 3). On the other hand, the fine fractions < 60 mm are predominantly organic. The fresh waste < 60 mm (FW), digestate circulated from AD (GR) and process water collected at rotting area (PZ) are fed into a hopper directly on top of a screw press.

The screw press breaks down input materials into press off leachate “liquid” and press cake “solid”. The press cake (PC) is treated aerobically in order to comply with the Landfill Ordinance (DeponieVerordnung) by stabilizing organic fractions before final disposal. Whereas, the press water (PW) is rich in suspension organic matters and fed into an AD. The AD consists of seven reactors; namely two primary (PrR), two biofilm (BfR), two secondary (SeR) and stirred tank reactors (post treatment reactor PtR). The biogas generated from each reactor is captured and converted by employing a combined heat-power unit (CHP) into energy in the form of electricity and heat. The heat is consumed for AD reactors, drying RDF and buildings. In addition, sand is sucked out from the first three reactors whenever needed and treated before final landfilling.

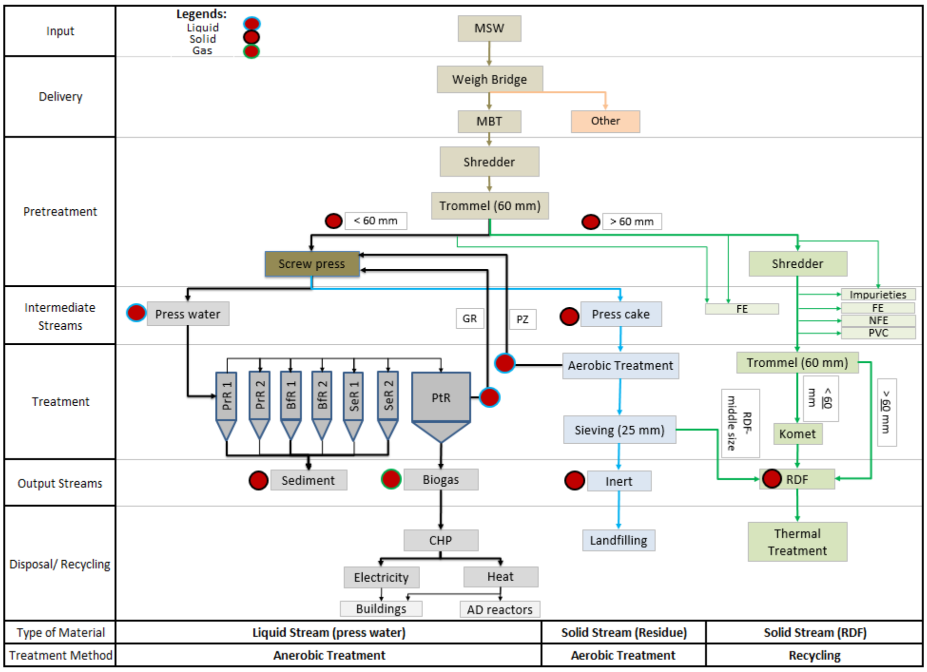


Fig. 3. MBRT plant in Erbenschwang, Germany.

Objectives and research program

The present concept, a multi-purposes project, intents to combine waste treatment and energy generation. The driven goal behind the technology is specifically to assess the suitability of AD with a screw press in order to come up with a model that can be integrated into new and conventional MBTs. Given the practical experiences in the last two years, the project has shown a promising result through treating of waste and renewable energy generation.

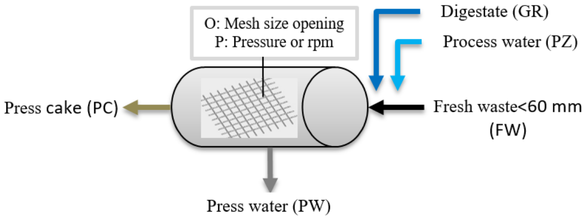


Fig. 4. Input streams to the screw press.

With the commencement of the plant, an operation schedule was developed to monitor the performance which is followed by the operator. Moreover, an academic team from University of Rostock provides scientific support to evaluate and optimize the entire process. To observe the potential impact of each parameter, all the factors were investigated in a set of trials onsite such as waste characteristic, GR/ PZ ratio, mesh-size opening in the outer face of the screw press, pressure of the press and the waste/ fed water ratio (Figure 4). For instance, trial 1 divided into sub-trials when all the feedings were fixed constant and the water/waste (GR+PZ/FW) fed to the press was increasing from 850 to 940 and 1,010 l/Mg (see Table 2, 1-a to 1-c). The GR/PZ ratio was, on the other hand, changing from 4:1 to only GR 4:0. Besides, the screw press was operated under different conditions with various mesh-size openings, e.g. 8, 10 mm, and different pressure, e.g. 4, 4.5 bar while all other remaining inputs were kept unchanged. Also, the mass- and water balances for the entire plant operation were developed. However, they depend greatly on each other. For example, the PC is considered solid stream with up to 50% moisture content.

Lab analysis was executed as a supplementary for onsite trials when regular samples or samples from occasional trials were taken. Table 1 illustrates some parameters analyzed in order to view the physiochemical properties. The ongoing lab analysis during the pilot phase is a fundamental database servers in adjusting the operation and used in the optimization process.

The recent study conducted by Dornbusch et al. 2020 stressed that the fine fractions (0-10 mm) constitutes up to 6,3% of the residual waste in Germany. Given the daily waste fed to the screw press of about 95 Mg/day during trial 1 and the content of fine fractions, sand is transferred from the waste to the AD. It is hence essential to determine the quantity and quality of the sand in order to come up with a proposal to minimize or recycle. However, the sand amount is **extremely low** in the present technology because; 1) the screw press outer face mesh is 5 mm and 2) part of the fine fractions remain in the press cake. Therefore, wet sieving analysis and sand composition experiments were carried out.

Table 1. Lab analysis program for liquid and solid streams.

	TM	oTS	Ash	pH	EC	FOS/TAC	TC	TOC	DOC	TNb	NH4-N	GB21
Liquid	X	X	X	X	X	X	X	X	X	X	X	X
Solid	X	X	X	-	-	X	X	X	X	X	-	X
Sand	X	X	X	X	-	X	X	X	-	X	-	-

Result and Discussion

It was emphasized that the quality of PW, as the main AD input, is important. The properties of PW is significantly influenced by the input streams to the press; namely FW, GR and PZ. Table 2 & 3 summarize the lab results of each streams that fed or generated during the sub-trials of trial 1. However, the PZ quality is unlikely to change significantly and was excluded in trial 1-d.

Table 2. Characteristic of PW samples from sub-trials of trail 1 (output of the press, input to the AD)

Trials	TM [%TS]	oTM [%TS]	pH	EC [µS/cm]	TC [mg/l]	TOC [mg/l]	DOC [mg/l]	TNb [mg/l]	NH4-N [mg/l]
1-a	17.3	8.3	6.9	30,400	13,170	11,860	9,960	2,660	2,322
1-b	15.7	8.1	7.6	34,200	17,220	15,010	11,260	3,300	2,124
1-c	14.8	7.2	7.3	25,200	8,200	6,960	9,270	1,320	2,376
1-d	20.1	10.3	7.1	25,175	17,905	16,120	13,130	4,530	3,118

The following discussions can be concluded from table 2 and 3:

- **TS:** decreased from sub-trial 1-a to 1-c which is comparable to FW & PZ. However, oTM in GR increased from 1-b to 1-c, but the increasing amount of water in 1-c lead to a better dilution and ultimate low oTM.
- **TNb:** the increasing pattern from sub-trial 1-a to 1-b is in agreement with GR because FW remained constant. Decreasing from 1-b to 1-c result from the increment in FW, while GR was not changing. However, it is reverse proportional to PZ, but PZ amount is very small and has low effect on the PW properties.

Table 3. Characteristic of GR and FW from sub-trails of trail 1 (input to the press).

Trials	GR **FW**	TM [%TS]	oTM [%TS]	pH	EC [µS/cm]	TC [mg/l]	TOC [mg/l]	DOC [mg/l]	TNb [mg/l]	NH$_4$-N [mg/l]
1-a	GR	7.6	2.9	8.1	37,700	5,970	3,860	3,170	2,600	2,499
	FW	57.4	29.9			4,640	4,470	4,460	312	
1-b	GR	7.6	2.9	8.3	40,500	9,880	6,430	3,550	3,330	3,024
	FW	56.4	28.4			4,400	4,220	4,150	259	
1-c	GR	12.8	4.9	8.2	40,100	12,230	6,770	4,440	3,380	2,909
	FW	54.7	27.9			3,890	3,730	4,370	223	
1-d	GR	10.3	3.9	8.1	35,550	7,300	4,400	2,725	3,150	3,526
	FW	57.0	31.4			4,390	4,230	4,105	460	

- **DOC:** like in GR and PZ, DOC has increased from sub-trial 1-a to 1-b, however FW decreased slightly. It contributed to that DOC in GR is in dissolved form and pressed simply into PW. The decreasing from 1-b to 1-c while all others were increasing, caused by the huge amount of water lead to dilution. In addition, although GR and FW are not high, but 1-d has a high concentration because of less dilution effect in the absence of PZ.

- **TOC:** increased from sub-trial 1-a to 1-b due to the increasing in GR and PZ, however, slight reduction in FW was observed. Despite a slight increase in GR, TOC in PW from 1-b to 1-c decreased due to the low concentration in FW. The concentration is equal in 1-b and 1-d because of the same TOC in FW in both sub-trials.
- In summary: the properties of all sub-trails are more or less close, except for 1-c caused by the increasing amount of water/waste ratio of 1.01 m^3/Mg fed to the screw press compared to others with less water, e.g. 0.94 m^3/Mg and 0.85 m^3/Mg.

The biogas generation rate is influenced by the input properties and parameters that are converted into biogas. The decisive elements, among others, are TC, TOC and DOC. The PW in sub-trial 1-b has shown the highest specific biogas rate which is contributed to the high TC, TOC and DOC concentrations compared to other sub-trials. Besides, sub-trial 1-b presented higher TNb and lower NH_4-N concentration of 3,300 mg/ and 2,124 mg/l, respectively.

Considering the gas yield of input streams, despite the high gas yield of PZ of 773 l/goTS, it has no huge impact due to its very low volume (one-fifth of the total water). While, FW and GR play significant roles in the biogas production. The high biogas yield of PW in sub-trail 1-b (566.8 l/kgoTS) is interpreted by the high gas yield of GR 185.4 l/kgoTS and appropriate GR+PZ/FW ratio 0.94 m3/Mg (Figure 5). However, the high biogas yield of GR in trial 1-a has not resulted in high biogas yield in PW because the GR+PZ/FW ratio was not high (0.85 m3/Mg) and most of the organics left the screw press with the PC. On the other hand, the low specific biogas yield is in sub-trial 1-c 466 l/goTS is explained by the high GR+PZ/FW ratio 1.01 m^3/Mg which cause ultimate dilution.

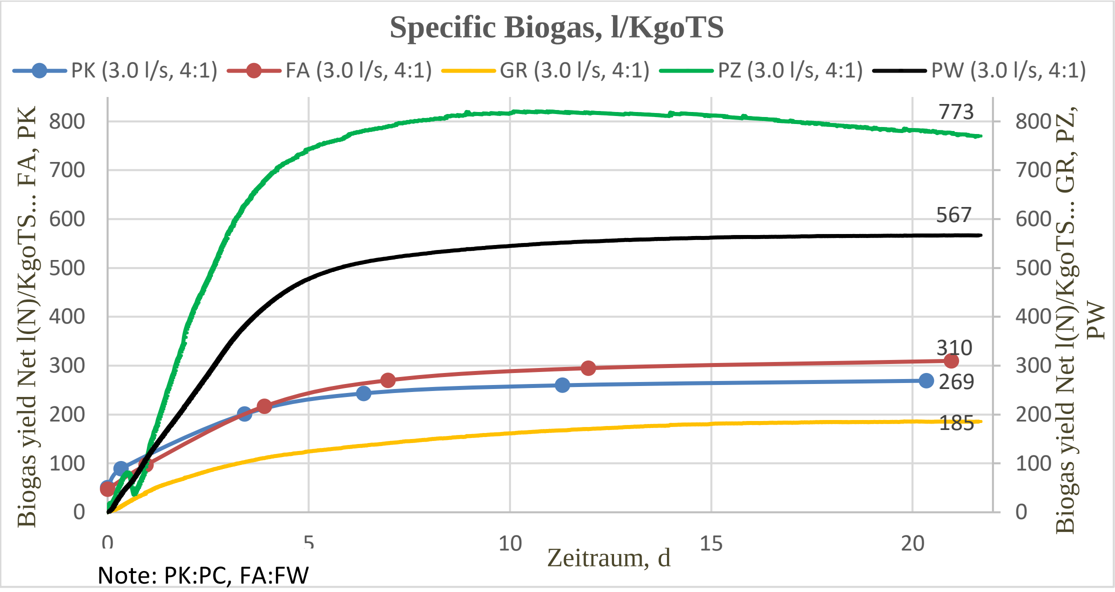
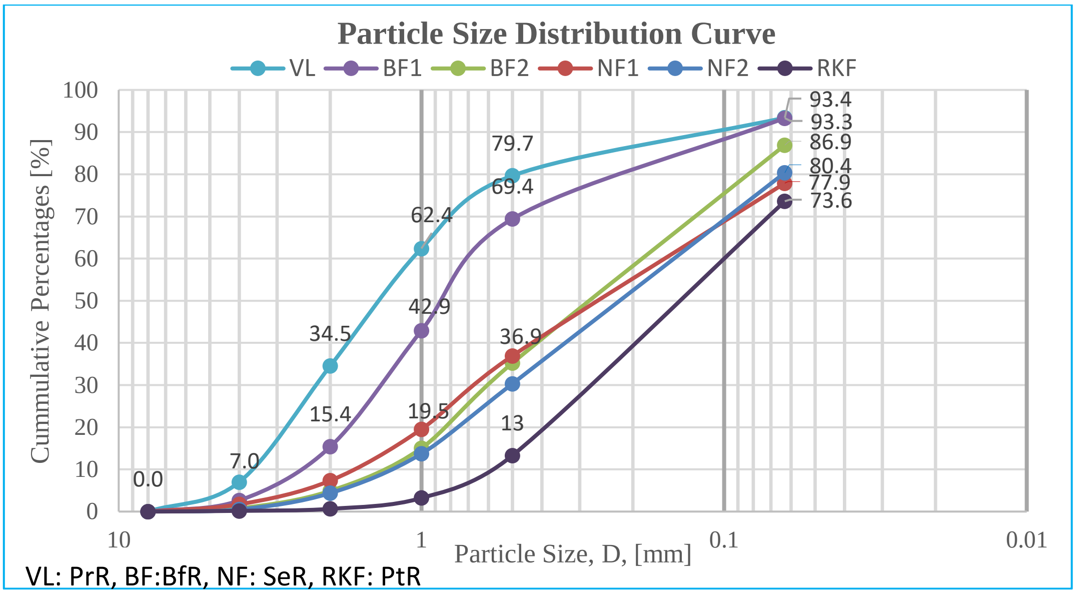


Fig. 5. Specific biogas potential yield of streams in the trial 1-b.

Table 4 presents the volume of biogas generated per ton of PW and FW fed to the screw press. Likewise to the biogas yield, the total gas volume per ton PW and FW are higher in sub-trial 1-b than others. The exclusion of PZ as input stream led to an exceptional increment in the biogas volume per ton FW. This increment encourages the plant operator to still run the plant in the lack of PZ. However, PZ is always available from the aerobic chambers.

The samples used in the sieve analysis were taken from sediment layer in AD reactors to determine the grain size distribution in each reactor and oTS for each size range. The result in Figure 6 shows that the sediment particles are getting smaller towards the PtR. For instance, about 35% of the sediment in PrR is bigger than 2 mm, while it decreased to max 15% and 1% in BRs and in PtR, respectively. In contrast, the percentage of fine particles 0.063 mm has recorded highest in PtR by 60% and minimum in PrR by only 14%.



VL: PrR, BF:BfR, NF: SeR, RKF: PtR

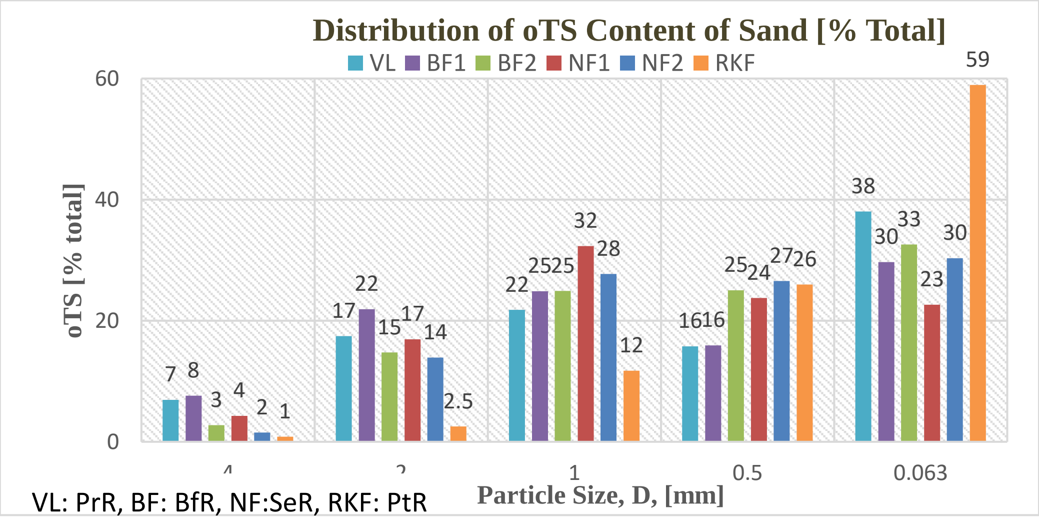
Fig. 6. Particle size distribution curve for sand samples from AD.

Table 4. Potential biogas generation of PW.

Trials	Gas l/kg oTM	oTM [%]	Menge [Mg/h]	oTM [kg/h]	Gas [m3/h]	Gas m^3/Mg PW	Gas m^3/ Mg waste
1-a	418.1	8.27%	10.95	905.62	378.67	34.6	28.5
1-b	566.7	8.05%	16.37	1,318.14	746.97	45.6	45.4
1-c	466.0	7.18%	16.77	1,204.14	561.08	33.5	40.2
1-d	540.4	10.34%	18.62	1,924.05	1,039.76	55.9	68.7

It can be understood from figure 6 that about 93% of the total sand in PrR remain on sieve 0.063 mm. The trend is decreasing towards the PtR as low as 73%. The results is in agreement with literature and contributed to the fact that the PrR is the first container where course fractions settle down and contain heterogeneous particles such as wood, glass, metal and stone. Besides, the small fractions can suspend in PW up to the PtR where a sufficient period of time is guaranteed to settle down.

Figure 7 summarizes the oTS content of each grain size for each sample. The concentration trend is similar to the grain size distribution which is increasing from PrR towards the PtR. In each reactor, the fine fractions such as 0.063 mm and 0.5 mm show high oTS and low content in bigger fractions were observed. This can be concluded with what was witnessed during the analysis that fine fractions are mostly organic material and coarser fractions contain inorganic matters such as glass, metal, stone.



VL: PrR, BF: BfR, NF:SeR, RKF: PtR

Fig. 7. TS content of each grain size per sand sample.

Conclusion

The increasing amount of MSW has urged an appropriate technology to handle waste and convert it into more sustainable products. The present technology can receive waste with different collection systems, mixed waste and residual waste bins, and converted into biogas, RDF and stabilized inert for final disposal. The project aims to optimize the generation of gas, production of high-quality RDF and ensuring the disposal by complying with the Landfill Ordinance. The practical experience since 2019 and the lab results have shown promising trends towards a sustainable circular economy. The plant with screw press guarantees an adequate input quality for AD which ensure biologically stable, safe and energy efficient operation. The biogas yield is in range and methane content is high. The quantity of fine inorganic fractions ends in the AD reactors is very low. In addition, the project studies the evaluation of financial aspects and development of water-, mass- and energy balance as part of the optimization process.

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INTEGRATION APPROACH OF ANAEROBIC CO-DIGESTION AND MICROAERATION AS AN ALTERNATIVE SOLUTION FOR MUNICIPAL ORGANIC WASTE MANAGEMENT

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The organic fraction of municipal solid waste (especially food waste) and sewage sludge have become a huge environmental challenge for the rapidly developing urban area. Among the different technologies for managing organic wastes, anaerobic digestion provides an avenue for valorizing organic waste with concurrent production of biofuel (i.e., biomethane) and organic fertilizer (i.e., digestate). However, issues such as process instability due to volatile fatty acid accumulation (in case of mono-digestion of food waste) and the presence of toxic metals and pathogens (in case of mono-digestion of sewage sludge) adversely affect the biofuel production and land application of fertilizer. We hypothesize that co-digestion of food waste with sewage sludge could eliminate the abovementioned issues by balancing carbon to nitrogen ratio, providing nutrients and sufficient alkalinity, and diluting toxicity. Moreover, strategically supplied controlled amount of oxygen (i.e., microaeration) could further enhance the anaerobic co-digestion of food waste and sewage sludge by improving both hydrolysis of substrates and methanogenesis of intermediate products.

In the first step, biomethane potential (BMP) tests were conducted to exploit the possible synergistic effects (quantified as biomethane yield) of co-substrates at different mixing ratios: i.e., food waste: sewage sludge of 0:1, 4:1, 4:2, 4:3, and 1:0, volatile solids (VS) basis. BMP tests were conducted using a series of 250-mL-serum bottles with the substrate to inoculum ratio of 1.0 and substrate concentration of 1.5 % (VS basis) at the mesophilic condition. The highest methane yield (508.9 NmL/g VS_{added}) of co-digestion at the mixing ratio of 4:1 based on ANOVA test ($p < 0.05$) and Tukey's honestly significant difference test ($\alpha = 0.05$) were further applied to the 6-liters continuous stirring tank reactor. Oxygen gas was fed into the system to raise ORP 25 mV from the reference stage to maintain the microaeration condition. The co-digestion results were evaluated for the steady-state and dynamic performances based on various loading rates compared with mono-digestion. An integration of anaerobic co-digestion with micro-aeration could significantly improve the economic performance of anaerobic digestion technology in managing these waste streams, thereby leading to widespread adoption of this technology in the Asia-pacific regions concurrently addressing the abovementioned environmental, public health, and social issues.

Keywords: *Co-digestion, Microaeration, Food waste, Sewage sludge, Oxidation-reduction potential (ORP).*

THE BEGINNING OF FOOD WASTE AND SEWAGE SLUDGE CO-DIGESTION IN HONG KONG

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Anaerobic digestion of sewage sludge has been adopted for many years by the Drainage Services Department (DSD) of the Government of the Hong Kong Special Administrative Region (HKSARG) as the key sewage sludge stabilisation process, from which biogas is generated as a by-product. Alongside the HKSARG's initiatives in investing in environmental infrastructure and waste-to-energy facilities for smart city development and better utilisation of land in Hong Kong, DSD has also been working on new initiatives to explore feasibility of adopting new and emerging sewage treatment technologies including those for sewage sludge treatment and use of renewable energy. In a recent research study conducted by DSD, an innovative initiative to adopt food waste and sewage sludge co-digestion in Hong Kong was outlined with a view to alleviating Hong Kong's food waste problem. This initiative had subsequently found its way to the 2016 Policy Address of the HKSARG, whereby the Government committed to explore the use of existing sewage treatment works for anaerobic co-digestion of food waste and sewage sludge as an additional part of the network of the organic waste treatment facilities to help raise the food waste treatment capability, based on the concepts of circular economy and "smart city". Planning to set up a trial pilot plant for sewage sludge and food waste co-digestion at the Tai Po Sewage Treatment Works (TPSTW) started in 2016 to confirm the technical feasibility and installation requirements of applying co-digestion technology in Hong Kong.

Before the pilot plant trial, DSD commissioned the University of Hong Kong (HKU) to conduct a series of laboratory tests and analysis since 2014 to identify the optimal operating conditions and metagenomics in the co-digestion process. HKU prepared a bench scale set-up in the laboratory with 1-L semi-continuous medium-size reactors (with 800 mL working volume) and 10-L large-size reactors (with 8 L working volume) which were set to operate at mesophilic conditions (~35˚C). In the medium-sized reactors, the key operating parameters to be investigated were ratios of food waste (FW) to sewage sludge (SS), sludge retention time (SRT) and food waste composition, while the effects of mixing intensity were evaluated in the large-size reactors.

Results of the tests using the medium-size reactors revealed that 1) without any alkali solution addition, total solids of FW:SS ratio of 40:60 was the optimal ratio with the highest methane yield and a good volatile solid reduction (VSR) of 57%; 2) the SRTs of 5, 10, 15 days were insufficient for achieving complete methanogenic digestion; and 3) the food waste composition had a significant impact on the co-digestion performance. As to the large-size reactors, the test results indicated that the mixing intensity is also a factor to be considered in the co-digestion process for achieving optimum biogas yield. The results obtained are valuable for taking forward the pilot plant trial at TPSTW.

Since installation of the co-digestion pilot plant at the TPSTW with its operation began in September 2019, the co-digestion process has been closely monitored and found to be working properly. The technical know-how in using such an innovative sludge treatment process in Hong Kong is being identified.

Keywords: *Food waste, Sewage sludge, Co-digestion.*

RAPID ESTIMATION OF HIGHER HEATING VALUE (HHV) AND BIOCHEMICAL METHANE POTENTIAL (BMP) BY USING ORGANIC WASTES CHARACTERISTICS: APPLICATION IN TROPICAL ENVIRONMENT

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ABSTRACT. The aim of our work was to estimate the Higher Heating Value (HHV) and the Biochemical Methane Potential (BMP) of Organic Wastes (OW) collected in Réunion Island by using their chemical characteristics. On the right hand, measurements of dry matter (DM) and organic matter (OM) contents were carried out by using standardized protocols and on the other hand HHV and BMP were determined by using respectively a bomb calorimeter and an automatic methane potential test system. Firstly, empirical models using DM and OM as predictors from literature were tested to predict HHV and BMP. Secondly, new empirical models for HHV and BMP as a function of their measured components contents (DM and OM) were developed through Linear Regressions. The determination coefficient R^2 and the Standard Error Prediction (SE_P) were used to compare the different equations. The new correlations give accurate results that are closer to measured HHV and BMP.

Keywords: *Waste-to-Energy, Prediction, Regression, Dry Matter, Organic Matter*

Introduction

The progressive decline of fossil fuels reserves (particularly liquid fuels and coal), and their associated harmful effects such as pollution, threatens human health and environment (greenhouse gases associated with global warming) has increased pressure to develop alternative energy program. However, in the present context petroleum serves as a preferred fuel. The increased demand for energy, energy security, and growing environmental concerns has driven the research interest for researchers to think seriously for other alternative sources of energy [1]. In this regard, biomass has come up as a major source of alternative energy having widespread availability and comparatively lower environmental impact than fossil fuels. As considered as biomass, organic waste (OW) can be a new supply for energy production. OW particularly have the potential to become a significant primary energy source [2]. The modern uses of OW take the advantages of modern biomass conversion technologies (combustion, pyrolysis, gasification, fermentation, and anaerobic digestion) for production of heat and electricity, liquid and gaseous transportation fuel, biogas for cooking etc. [3]

In order to assess the energy potential of OW, we challenged to show how helpful is developing a predictive model to determine the HHV and the BMP of Reunion Island materials? There are few references on energetic value of these OW although assessing HV and BMP based on materials from tropical environment. We hypothesise that a generic model built and validate with some HHV and BMP could be used to other OW. Our objectives will be focused on the assessment of energy potential of OW vis-a-vis their potential utilization in waste-to-energy conversion plants using combustion and methanation technology by developing model using DM and OM as predictors.

Material and Methods

Studied samples

OW samples were collected from different regions in Reunion Island. For HHV modelization, 153 samples were studied: 113 OW from livestock, 31 OW from urban residues and 6 OW from agro-industrial residues.

For BMP assessment, 14 samples from livestock OW were considered: 6 poultry effluents, 5 porcine effluents and 3 bovine effluents.

Samples analysis

On the right hand, measurements of dry matter (DM) and organic matter (OM) contents were carried out by using standardized protocols and on the other hand HHV and BMP were determined by using respectively a bomb calorimeter (C7000, IKA, Heitersheim, Germany) and an automatic methane potential test system (AMPTS II, Bioprocess Control, Lund, Sweden).

Equations from literature

Two published equations (PE) were taking into account to predict the HHV of the OW (Table 1). PE were using OM contents from Ebeling *et al.* [4] and DM contents from Thipkhunthod *et al.* [5].

Two equations from literature (EL) were tested to estimate the BMP of the OW (Table 1). Theses EL were built with OM contents and DM contents from a mixture of energetic crops, pig manure slurry, agro-industrial waste, and organic fraction of municipal solid waste as reported by Schievano *et al.* [6].

Table 1: Equations from published studies

Model	Parameters	Equations	Sources
HHV	OM	PE-1 : $0.2916\ OM – 1.893$	[4]
HHV	DM	PE-2 : $0.2679\ DM -0.4573$	[5]
BMP	OM	EL-1 : $BMP = 20.497OM – 1241.534$	[6]
BMP	DM	EL-2 : $BMP = 37.408DM + 57.989$	[6]

New developed models

Simple and multiple linear regressions were adopted to formulate the new equations to predict HHV (H-NE) or BMP (B-NE) of OW samples from OM and DM contents by using R Software [7].

Models accuracy and comparison

The accuracy of the PE and LE was evaluated on the basis of the bias, which is the average difference between the estimated and measured value of HHV or BMP, and the precision was estimated on the basis of the standard error of prediction corrected for bias (SE_P).

For new equations, H-NE and B-NE , the accuracy of the model fit was evaluated through examination of various statistical parameters, including the regression coefficient (R^2), Akaike criteria (AIC) [8], bias and standard error (SE). The best model was considered to be the equation with the lowest AIC. All procedures, including equation construction and statistics, were performed using R [7].

PE and LE were compared respectively to H-NE and B-NE by considering the SE_P: the best model will be the one with the lowest SE_P.

Results and Discussion

HHV prediction

The chemical characteristics of OW used to model HHV were presented in Figure 1. As expected, the constituents, DM and OM, ranges were large and demonstrated a high variability of OW. The results were in accordance with those reported by Yin [9].

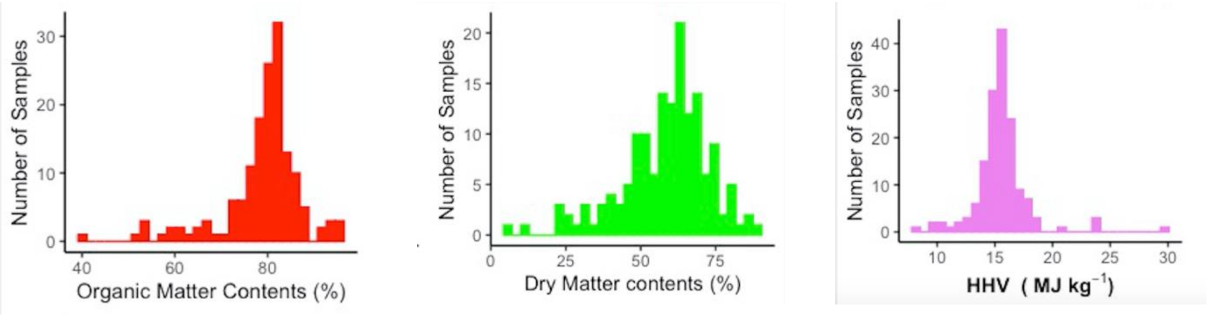

Figure 1: OM contents, DM contents, and HHV of OW used to model the HHV

The Table 2 reported the SE_P of the equations used to assess the HHV either from PE and H-NE. The PE were validated on the basis of our experimental data in order to test the reliability of the estimated HHV. According to the Table 2, the SE_P for LE-1 and LE-2 were respectively 0.850 MJ.kg^{-1} and 3.150 MJ.kg^{-1}. These values were high when compared to the new equations obtained with our OW. The H-NE-2 was the most accurate with a SEP of 0.271 MJ.kg^{-1}.

Table 2: Equations to predict the HHV

Equation type	Parameters	Equations	SE_P
Literature	OM	PE-1: HHV = 0.2196 OM – 1.893	0.850 MJ.kg^{-1}
Literature	DM	PE-2: HHV = 0.2679 DM – 0.4573	3.150 MJ.kg^{-1}
Our study	DM	H-NE-1: HHV = 0.006 DM + 15.200	1.348 MJ.kg^{-1}
Our study	DM^2	**H-NE-2: HHV = 0.027 DM^2 + 8.794**	**0.271 MJ.kg^{-1}**
Our study	DM^{-1}	H-NE-3: HHV = -211.11 DM^{-1} + 29.424	0.453 MJ.kg^{-1}
Our study	OM	H-NE-4: HHV = 0.216 OM – 1.510	0.842 MJ.kg^{-1}
Our study	OM^2	H-NE-5: HHV = 0.002 OM^2 + 6.002	0.827 MJ.kg^{-1}
Our study	OM^{-1}	H-NE-6: HHV = -931.491 DM^{-1} + 7.53	0.868 MJ.kg^{-1}

BMP model

Figure 2 presented the characteristics of OW considered during the BMP modelization. The results showed a high variability between the studied OW.

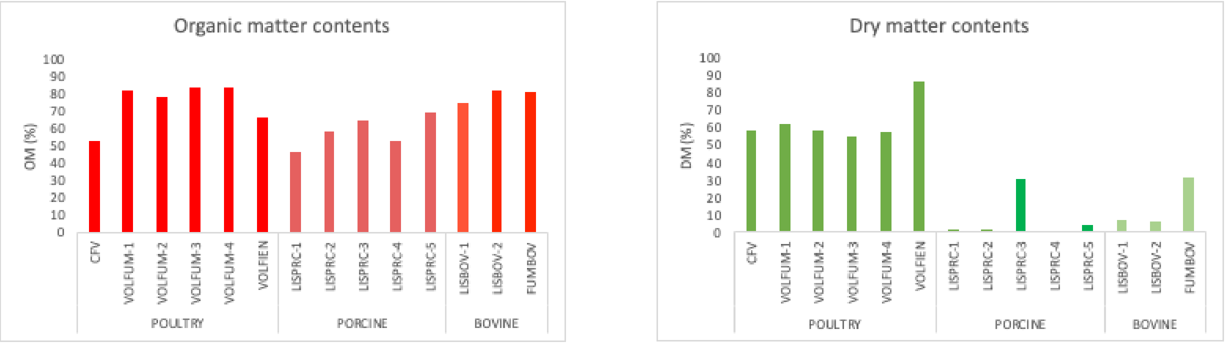

Figure 2: OM and DM contents of OW used to model the BMP

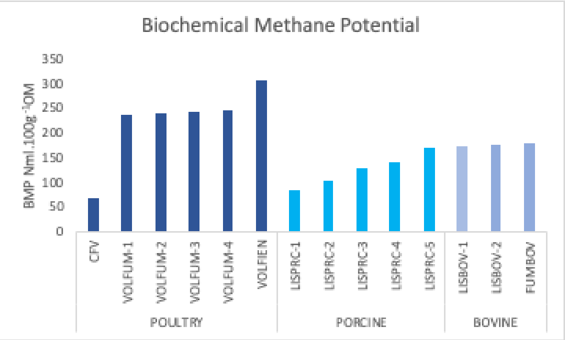

Figure 3 : BMP of OW used to model the BMP

The following Table 3 reported the new equations to predict the BMP of OW. The SE_P values obtained with literature equations were higher than those calculated with the new equations. The lowest SE_P was 25.4 $NmLCH_4.g^{-1}OM$ and was similar with those reported by Schievano *et al.* [6] which was 23.5 $NmLCH_4.g^{-1}OM$.

Table 3 : Equations to predict the BMP

Equation type	Parameters	Equations	SE_P
Literature	OM	EL-1: BMP = 20.497 OM – 1241.534	188.34 $NmLCH_4.g^{-1}OM$
Literature	DM	EL-2: BMP = 37.408 DM + 57.989	929.89 $NmLCH_4.g^{-1}OM$
Our study	DM	B-NE-1: BMP = 1.54 DM + 127.19	37.94 $NmLCH_4.g^{-1}OM$
Our study	DM	B-NE-2: BMP = 3.74 DM	64.00 $NmLCH_4.g^{-1}OM$
Our study	OM	B-NE-3: BMP = 3.83 OM – 88.59	33.78 $NmLCH_4.g^{-1}OM$
Our study	OM	B-NE-4: BMP = 2.59 OM	38.68 $NmLCH_4.g^{-1}OM$
Our study	**DM, OM**	**B-NE-5: BMP = 1.08 DM -2.93 OM – 61.38**	**25.36 $NmLCH_4.g^{-1}OM$**
Our study	DM, OM	B-NE-6: BMP = 1.14 DM + 2.04 OM	26.68 $NmLCH_4.g^{-1}OM$

Conclusion

To conclude, our study demonstrated that DM and OM can be used as predictors either for HHV or BMP. The new correlations are easy to apply and can accelerate the assessment of HHV and BMP of OW.

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COMPARISON OF GOETHITE AND ACTIVATED CARBON ON METHANOGENESIS FROM VOLATILE FATTY ACIDS

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Anaerobic digestion (AD) has become one of the most preferred technologies for recycling energy from biowaste. Nevertheless, AD process is vulnerable to the high organic loading rate and the high concentration of volatile fatty acids (VFAs) tend to accumulate. There are considerable researches aiming to strengthen the anaerobic degradation rate of organics (e.g. acetate, ethanol) by adding conductive iron oxides or carbon materials, such as activated carbon (AC) and biochar etc., in which the strengthened direct interspecies electron transfer (DIET) was suggested to be the main reason. To our best knowledge, there is no previous study comparing the role and function of carbon-based conductive particles and iron oxides in methanogenic process. In present study, the effects of three conductive materials, including AC, iron modified activated carbon (Fe/AC) and goethite (FEOOH), on methanogenesis from VFAs were evaluated. The supplementation of three tested conductive materials could all promote the methane generation significantly as compared to the control reactor without conductive materials addition, especially when high organic acid load was applied. With the initial VFAs concentration of 4 g/L, the maximum methane yield of 266 mL/g-COD was found in the FEOOH reactor, which was 1.6 times higher than that of the control. The differentiation of methane yields could be attributable to the difference of microbial community. AC promoted the growth of the members of *Methanosaeta*, Aminicenantes, and *Smithella*, whereas goethite facilitated the iron-respiring bacteria and syntrophic VFA-oxidizing bacteria *Syntrophomonas* and other unclassified members of *Syntrophomonadaceae*, which perform DIET with *Methanosarcina*. Furthermore, the relationship between microbial community and electron accepting capacity (EAC) and electron donor capacity (EDC) of supplemented conductive materials were evaluated by using representational difference analysis. Results indicated that the higher EDC of goethite compared to AC might be a triggering factor on the selective enrichment of *Methanosarcina* and other electroactive bacteria that are involved in DIET, leading to the improved methanogenic performance under acid stress.

Keywords: *Activated carbon, Conductive particles, DIET, Electron donor capacity, Goethite, Methanogenesis.*

USE OF BIOGENIC RESIDUES FOR THE PRODUCTION OF BIOMETHANE

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ABSTRACT. Biomethane is a climate-friendly, renewable fuel. The DBFZ is planning a pilot plant for the production of biomethane from different biogenic residues. The substrates will be cattle manure, cereal straw, sewage sludge, green cuttings and organic waste. Other products from the plant are solid and liquid nutrient fertilisers such as phosphorus fertiliser as well as HTC char, which can be obtained from fermentation residues, for example. The overall project with its two scenarios, the German raw material potentials for the wastes, residues and by-products which are to be converted in the plant as well as the mass balances of the processes are presented.

Keywords: *Biomethane, Pilot Plant, Biogenic Residues, Resource Potentials, Advanced Fuel.*

Introduction

It is well-known that in 2018 the final energy consumption in the transport sector in Germany was higher than in the year 1990. Despite all the efficiency improvements and further development of engines, no energy reduction took place. The target of reducing the final energy consumption in transport by nearly 40% by 2050 appear unrealistic according to the reference scenarios presented by [1-5]. The targets set by climate protection scenarios for the contribution of the transport sector are even more ambitious. GHG emissions are to be reduced by 95% by 2050 compared to the reference year 1990. According to the reference scenarios [1,3-5] this target will not be achieved. In summary, the transport sector is under enormous pressure to act. Biomethane may be a climate-friendly solution as advanced fuel for the transport sector, if the substrates are compliant with the RED II, annex IX b.

Results and Discussion

Biomass potentials

There are serious differences in the biomasses listed in RED II with regard to mass and fuel potentials. In addition, the relevant material flows need to be narrowed down in terms of mobilisability and optimisability. Therefore, the DBFZ operates a biomass monitoring system since 2019, which can be accessed under http://webapp.dbfz.de. In the Resources Database [6-8], the status of 77 domestic biogenic residues, by-products and wastes can be retrieved for five sectors (agriculture, forestry, municipal waste and sewage sludge, industrial residues, other residues). Ten key items of information are available for the description of the material flow (e.g. theoretical, used, mobilisable potential etc.). Ten different modes of transport and eleven fuel options can be selected, e. g. road, rail, air, waterway respective biomethane, LNG, CNG, bioethanol, biodiesel, alcohol-to-jet, etc. All data are given with minimum, mean and maximum value.

For digestible biomasses in Germany (number: 60) the potentials for mobilisation and optimisation can be indicated with 22.9 million tonnes dry matter resp. 37.4 million tonnes dry matter (see figure 1). The optimisation potential can be divided into material use (23.7 million tonnes dry matter) and energetic use (13.7 million tonnes dry matter).

The ranking of biomasses in terms of their mobilisability potential results in the largest quantities for cereal straw, cattle manure and green waste. The quantities of these three biomasses account for 80% of the considered 60 biomasses [9].

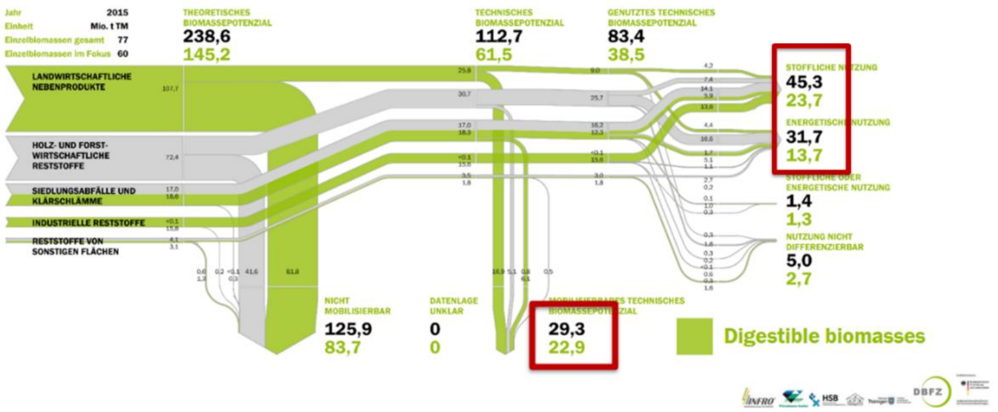

Fig.1. Digestible biomasses (reference year 2015) with potentials for optimisation and mobilisation [8].

Based on the demand in 2015, the impact of biomethane from biogenic residues for the various transport sectors is given in table 1 and [3]. The energy content of the total possible biomethane amounts to 97 to 279 PJ. This means that a total amount of 4% to 11% can be substituted in the transport sector. Of course, the availability of raw materials up to 2030 is subject to certain fluctuations, e.g. for climatic reasons. However, these fluctuations do not change the strategic relevance of biomethane for the transport sector.

Table 1. Potential impact in the transport sector [8].

Vehicles	Potential impact [%]
CNG cars	6 - 19
CNG trucks	13 - 41
LNG trucks	13 - 39
CNG buses	more than 100
LNG inland vessels	more than 100
LNG vessels (bunkering)	87 – more than 100

Description of the pilot plant and the overall project

The plant concept (see figure 2) basically combines anaerobic fermentation with innovative preparation and post-treatment processes such as hydrothermal carbonization (HTC) processes and synthesis-based production of biomethane. Further process steps include the treatment of process water and the separation of valuable by-products as well as the implementation of a demand side management system. The project will be complemented by a potential analysis of the raw materials and preliminary tests in the laboratory to optimize the mixture and process parameters for the pre-treatment and fermentation of the raw materials, as well as technical, economic and ecological assessments. Furthermore, a dissemination concept will be developed and a feasibility study will be conducted to address site issues, market conditions and stakeholder interests for the construction of a commercial plant.

The novelty of the plant is that various established technologies are combined in one plant concept, and for the DBFZ the scale is a novelty. The anaerobic fermentation of biogenic residues is a challenge – especially the fermentation of lignocellulosic substrates such as straw and green cuttings. The biomethane yield will be enhanced by methanation of the biogenic carbon dioxide from the biogas process and hydrogen. On the pilot scale, hydrogen is provided in gas cylinders, on a commercial scale hydrogen should be electrolytically generated with electricity from renewable sources. With this SynBioPTX approach it is possible to contribute to the reduction of GHG emissions and to the provision of renewable biomethane as advanced fuel in the transport sector.

Scenario approach

Two scenarios will be considered: A rural scenario with cattle manure and cereal straw (in total 9.4 t a^{-1}) as substrates and an urban scenario with sewage sludge, green cuttings and organic waste (in total 14.6 t a^{-1}) as substrates. The amounts of hydrogen will be 0.13 t a^{-1} resp. 0.12 t a^{-1} for the scenarios. As products 0.59 t a^{-1} resp. 0.53 t a^{-1} biomethane as well as 4.9 t a^{-1} solid and liquid fertiliser (rural scenario) resp. 2.5 t a^{-1}

fertiliser and 2.9 t a^{-1} HTC char (urban scenario) are expected. The pilot plant will be constructed and operated in 2021 - 2024.

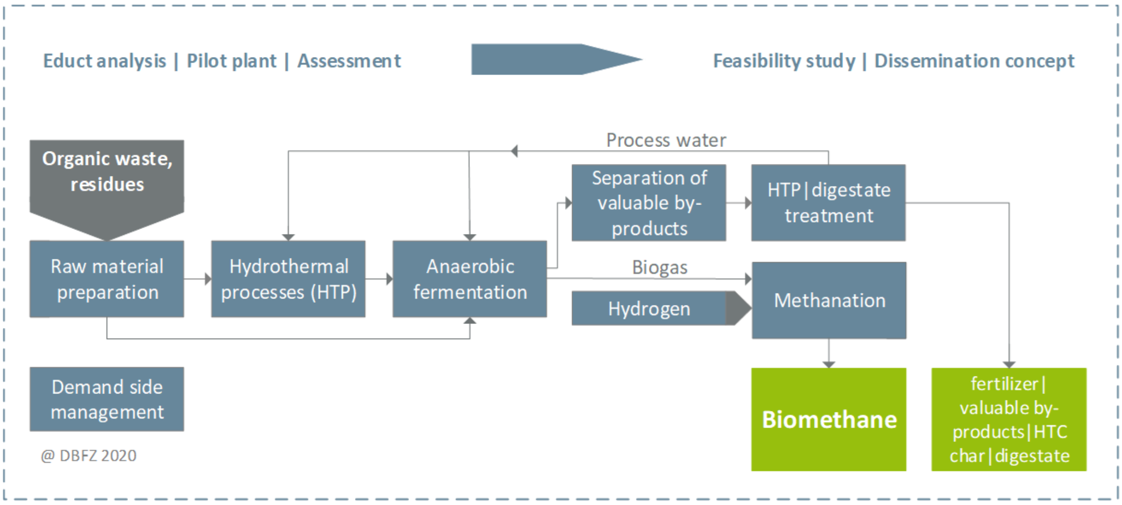

Fig.2. Process steps of the pilot plant, which is to be constructed and operated in the project Pilot-SBG.

Information about the commercial scale

Studies are currently underway to find the optimal scale for a commercial plant. Furthermore it is examined to which extent existing conventional biogas plants in Germany can also be expanded for the concept.

For a commercial plant an hourly production of 1,300 standard cubic metres of biogas is currently assumed as optimal. For this, the input for an urban plant can be considered to be 6.34 t h^{-1} cereal straw and 20.4 t h^{-1} cattle manure (the ratio of straw to manure can still be optimized) and the input for a rural plant can be assumed to be 8.98 t h^{-1} organic waste, 26.9 t h^{-1} sewage sludge and 12.0 t h^{-1} green cuttings. With this 1.71 t h^{-1} resp. 1.75 t h^{-1} biomethane will be produced. The quantities of fertiliser and HTC char generated are accordingly 14.5 t h^{-1} fertiliser for the rural scenario as well as 8.16 t h^{-1}fertiliser and 9.62 t h^{-1} HTC char for the urban scenario. The process water should be recycled.

The feasibility study for a commercial plant is supported by a comprehensive location and stakeholder analysis as well as the consideration of market, infrastructure and regulatory framework.

Acknowledgement

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EFFICIENT CATALYTIC PRODUCTION OF LEVULINIC ACID FROM STARCH-RICH FOOD WASTE USING A BIPHASIC SYSTEM

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Waste biomass such as food wastes is attracting considerable attention as feedstock for biorefinery applications. Catalytic production of sugars and platform chemicals could be an effective technic for recycling a considerable amount of food waste generated across the world. This study investigates the catalytic production of levulinic acid (LA) from bread waste, a common stream of starch-rich food waste generated worldwide. Gamma-valerolactone (GVL) was used as a green co-solvent in binary (GVL/H_2O) monophasic and biphasic systems to investigate LA production; besides H_2O only system served as a baseline. Though hydrolysis of bread waste was efficient in GVL/H_2O (1:1) monophasic solvent (~65 Cmol% in 1 min at 130°C), the maximum LA yield obtained was only ~11-16 Cmol% (150°C, 15-20 min). LA yield and selectivity could be improved by a biphasic solvent system that may facilitate LA production by extracting the target product (LA) in the organic phase. As GVL is completely miscible in H_2O, sodium chloride (NaCl) (30 wt%$_{(aq)}$) was used as a phase modifier to prepare the biphasic reaction medium. Results revealed that LA yield increased up to ~2.5 times in biphasic system (~28 Cmol%, 150°C, GVL/H_2O (1:1)) compared to the monophasic system (~11 Cmol%) following same reaction conditions. The partition coefficient for LA (LA-organic/LA aqueous) achieved was 4.2 in the GVL/H_2O (1:1) (NaCl) (30 wt%$_{(aq)}$) biphasic medium indicating LA was effectively extracted in the organic phase. The biphasic solvent system facilitates selective LA production and could be optimised tuning reaction conditions.

Keywords: *Biorefinery, Catalytic valorisation, Platform chemical, Food waste, Biphasic.*

DECENTRALIZED TREATMENT OF BIODEGRADABLE MUNICIPAL SOLID WASTES

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India is the fastest developing country in the Asian continent. But development pushes many environmental issues such as water pollution, solid waste problem, air and noise pollution, as well as soil contamination. Management of such problems is very essential function in a country to achieve sustainable development. Solid waste management in India is considered as one of the least prioritized functions during the last decades. Although many governmental programs and schemes came into existence during these period such as Nirmal Bharat Abhiyaan and Swacch Bharat Mission in 2014 but were least success. Amongst different types of solid wastes, India is facing difficulties in managing the organic wastes. The most common ways to manage such wastes in India is either by open dumping and uncontrolled burning. These methods are causing severe environmental pollution and health problems. The current study proposes decentralized treatment of such organic wastes by using the two technologies, i.e., composting and anaerobic digestion. The end product from these technologies are compost and methane gas which are beneficial in many ways in the different sectors such as agriculture and energy generation that helps to maintains the sutainability. The composters and digestors of the different capacities were installed at IITG campus and various parameters studied to identify its efficiency. The results indicated the final compost with nearly 3% nitrogen with sufficient phosphorus and potassium levels beneficial for soil health. The study on anaerobic digestion indicated its capability to produce 60-65% methane gas. The study also recommends that decentralized treatement of such wastes at residential complexes or at commercial places will increase its value and the productivity.

Keywords: *Solid waste, Sustainability, Swacch Bharat mission, composting, anaerobic digestion.*

WHAT ELSE DO WE NEED KNOW ABOUT PRETREATMENT?

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Pretreatments, including mechanical, thermal, chemical and biological treatments, are commonly used before anaerobic digestion (AD). These approaches achieve different levels of success by accelerating the hydrolysis efficiency (the rate-limiting step in AD) and enhancing subsequent biogas productivity. While in the enjoyment of the benefits from the pretreatment, new problems that induced during the pretreatment cannot be overlooked. It should be noted that most of pretreatment not only releases easily biodegradable substances but also releases or generates more recalcitrant, such as humic substances and complex aromatic compounds. More dissolved organic matters, e.g. refractory or inhibitory by-products, are surprisingly present in the centrate from the anaerobic digestor with pretreatment. These residual recalcitrant compounds could be problematic during the mainstream treatment or the side-stream centrate treatment, creating membrane fouling and anammox process inhibition. Thus, attention should be given to reduce and/or remove these recalcitrant compounds during the pretreatment or posttreatment. First, relative mild pretreatment methods (such as biological enzymatic pretreatment and combined physicochemical pretreatment) are recommended to reduce the formation of these refractory substances. Secondly, optimization of anaerobic digestion operating conditions e.g. retention time, pH, temperature, could minimize the refractory compounds. In addition, onside addition of conductive materials can facilitate recalcitrant compounds removal and enhance biogas production, which can be an alternative to the conventional pretreatment. At last, advanced oxidation process (AOP) as post-treatment would help to further degrade or oxidize the refractory substances remained in AD effluent. This talk will highlight the problems and challenges that are introduced by various pretreatment methods and propose potential solutions for mitigation.

Keywords: *Anaerobic digestion, Pretreatment, Drawbacks, Advanced oxidation process.*

BIOGAS MONITORING PROGRAMME III: ENERGY EFFICIENCY ASSESSMENT OF 61 BIOGAS PLANTS IN GERMANY – OUTCOMES AND METHODOLOGICAL CHALLENGES

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Anaerobic digestion (AD) poses a sustainable source of electrical energy and heat. An important measure to reach the Paris climate goals is not only the reduction of primary energy consumption, but also improving the efficiencies of existing energy producers and consumers. The development of AD plants in Germany was fostered by the "Renewable Energy Sources Act", resulting in more than 8,700 installed plants in 2016 with a cumulated electrical capacity of about 4.5 GW (Daniel-Gromke et al., 2018). Amendments to the act implemented in 2014 led to stagnation in plant construction and steered operators and planners towards improving efficiencies. One of the aims of the developments conducted towards efficiency assessment methods is to identify sources of losses and emissions. The latter shall be identified if coming from poor CHP efficiencies, flare losses or overly fed digestion systems. Furthermore, the presentation gives an overview of the current state of the German biogas plant inventory in terms of plant sizes and layouts, as well as concepts of energy utilisation and feeding regimes.

Reliable data acquisition is vital for precise mass and energy balances of any energy production facility. Experience has shown that measurements on agricultural biogas plants are ever so often more practice-oriented, e.g. measuring substrate inputs in excavator shovels as a unit. Other important numbers are difficult to determine - like the energy conversion efficiency of CHP engines, which usually degrade over time depending on the working principle and the manufacturer. Those inaccuracies leave blank or fuzzy spots in available balancing methods.

The economic assessment reveals a broad range in terms of profitability of current biogas plants. Still being dependent on feed-in tariffs, premiums turn out to be an effective way of influencing the role of biogas plants in the energy system, e.g. by providing net stabilisation through flexible power provision or by stabilising manure.

The project consortium was able to substantiate tabled data from e.g. energy providers by acquiring real world data from numerous technically diverse biogas plants. The most important results thereof will be presented.

Also, the available methods for balancing biogas plants raise hope for shedding light on the currently dark spots in data recording in agricultural surroundings. Furthermore, approaching the biogas plant balance-wise allows for distinguishing between deficiencies in either the biological substrate degradation or the subsequent biogas conversion step.

Keywords: *Biogas monitoring programme, Energy efficiency assessment, Outcome, Challenges.*

FEASIBILITY OF PRESSURE MEDIATED TWO-PHASE ANAEROBIC DIGESTION TO IMPROVING METHANE AND ITS UNDERLYING MECHANISMS ON HYDROLYSIS

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ABSTRACT. A partial diversion of gas from the LBR to the methanogenic phase at a headspace pressure of 3.3 psi has been shown to enhance methane production. Besides the self-generation gas, four different gas strategies were adopted to maintain the headspace pressure of 3.3 psi using (i) N_2 gas (physical pressure control), (ii) CO_2 gas, (iii) biogas of CO_2:CH_4 = 4:6, and (iv) partial gas diversion of self-generating biogas from LBR. Lower food waste conversion efficiencies including hydrolysis-acidogenic degree and methane recovery were observed in systems with CO_2 gas strategies. The highest acetate production (60 gCOD/kg VS_{added}) was obtained in LBR with self-generation gas pressure, whereas only around 48 gCOD/kg VS_{added} acetate was produced under the other three conditions. About 219 L/kg VS_{added} of methane was produced from integrated system with self-generation gas strategy, which was 10% higher than that with N_2 strategy and at least 30% higher than those with CO_2 partial pressure. Therefore, the present approach provides an effective way for enriching acetate generation, and give a better understanding about the improvement of methane-generating platform in a two-phase AD system.

Keywords: *Food waste, two-phase anaerobic digestion, pressure, hydrogen, methane*

Introduction

Two-phase AD process, particularly, solid-liquid separated system with leach bed reactor (LBR) coupled with up-flow anaerobic sludge blanket (UASB) is attractive for the treatment of food waste (Jung et al., 2018). This separation of hydrolysis-acidogenesis and methanogenesis in the two-phase AD can allow recovery of both hydrogen and methane. However, it is not considered as both technically reliable and commercially attractive to recover hydrogen in acidogenic fermentation process (Li et al., 2009). Recently, biogas upgrading by supplementation of hydrogen to methanogenic reactors has attracted greater attention. Therefore, acidogenic gas enriched with hydrogen is recognized as a source which can be converted to methane via hydrogenotrophic methanogenesis. To reduce hydrogen loss and simultaneously enhance methane production in two-phase AD system, an innovative method of transferring and reutilizing acidogenic off-gas from acidogenic LBR to improve methane recovery in UASB was established by Yan et al. (2016). For this novel integrated system, gas diversion not only affect the methane production related to hydrogen reutilization, but also influence the metabolic processes in LBR due to headspace pressure effect as well as availability of these gases for the LBR metabolic processes. As headspace pressure generated by acidogenic off-gas including hydrogen and CO_2 could participate in metabolic process, not only the simple physical pressure effect but also biochemical pressure effect on solid waste degradation, particularly hydrolysis-acidogenesis, has to be investigated in this integrated system.

Nevertheless, it is impossible to prevent hydrogen consumption in solid-state degradation mainly owing to the gas transfer limitation (Abbassi-Guendouz et al., 2012). This is relevant for homoacetogenesis that convert hydrogen and CO_2 to acetate; therefore, the local accumulation of hydrogen and CO_2 gas and high pressure that would overcome the gas-transfer limitation would strengthen this process. Acetate as well as hydrogen can be directly converted to methane, so, it is expected to improve methane generation by enriching acetate via homoacetogenesis in this integrated AD system. Previous studies enhanced or alleviated homoacetogenesis by injecting or reducing artificial hydrogen and CO_2 gas, but studies on utilizing natural biogas from self-anaerobic process to regulate homoacetogenesis in acidogenic phase is rare.

To better understand pressurized solid-liquid AD process, it is first necessary to compare the food waste conversion efficiency in two-phase AD process with/without headspace pressure and reveal how (a) headspace pressure affects the solid waste degradation in terms of physical and biochemical aspects, and (b) assess the feasibility of different biogas source from conventional AD process on the potential enhancement of acetate.

Material and Methods

Substrate and inoculum

An artificial food waste involving boiled pork, boiled rice, cabbage and bread was applied in this study. The inoculum for two-phase AD was sourced from the anaerobic digestion sludge collected from the Shek Wu Hui wastewater treatment plant, Hong Kong.

Batch experimental operation and analysis

Batch studies were carried out in two-phase AD, in duplicate for different conditions. Food waste, 2 kg, was mixed with 20 % (I/S, wet basis) AD sludge, and 10 % wood chips (10% w/w, wet basis) was used as a bulking agent to facilitate the liquid leaching in LBR. Deionized water was added to adjust the total solids (TS) content to 15% in the LBR. During the experiment, leachate was collected every day from the bottom of LBR and its pH and volume was measured immediately. Around 50 mL leachate was sampled for further chemical analysis. 50% of the leachate removed from LBR was replaced by deionized water and returned back to the reactor from the top after adjusting pH to 6.0 ± 0.2 using 5 M NaOH or 5 M HCl solutions. The residual 50% of leachate was fed into the corresponding UASB after adjusting pH to 7.5 ± 0.2.

The first experiment was conducted to compare the food waste conversion efficiency in two-phase AD process with/without headspace pressure. To justify the physical effect of headspace pressure, the degradation performance of the ambient system with entire gas diversion (without headspace pressure effect) was compared with that of the pressurized integrated system (maintained at 3.3 psi by pumping nitrogen). Meanwhile, controlling headspace pressure (3.3 psi) in LBRs by different gases, such as self-generation gas and nitrogen gas, to evaluate the biochemical effects of headspace pressure on waste degradation was carried out.

The second experiment was set to estimate the feasibility of using different gas sources for acetate enrichment and investigate their effect on overall food waste degradation in this integrated pressurized AD system. Three different gas strategies were adopted to maintain the headspace pressure of 3.3 psi using (i) CO_2 gas (100% CO_2), (ii) biogas of $CO_2:CH_4 = 4:6$, and (iii) partial gas diversion of self-generating gas from LBR. The headspace pressure was controlled by online pressure sensor, and the acidogenic gas beyond the set pressure was transferred into methanogenic reactors.

TS and VS were analysed using APHA 2540. The soluble chemical oxygen demand (sCOD) of daily leachate samples and UASB effluent was analysed using APHA 5520. For the analysis of volatile fatty acids (VFAs) were analysed using GC (HP 6890) with a flame ionization detector (FID). Gas composition were analysed using GC (HP7890) equipped with thermal conductivity detector (TCD) (Yan, Selvam, & Wong, 2016).

Results and Discussion

Food waste conversion efficiency in two-phase AD process with/without headspace pressure

The overall food waste degradation performance in the integrated two-phase AD system is presented in Table 1. In LBRs, the VS removal efficiency of particulate organic waste was increased by the influence of headspace pressure, in particular, when headspace pressure of acidogenic reactor was controlled via self-generation off-gas. Correspondingly, the effect of headspace pressure contributed to higher hydrolysis performance. There was 12% and 18% higher hydrolysis yield in LBRs with headspace pressure by self-generation gas and nitrogen gas, respectively, comparing to that of LBR without headspace pressure. It was in agreement with the result that physical pressure can increase the enzymatic degradation of starch resulting in enhanced degree of hydrolysis pf particulate waste (Hayashi & Hayashida, 1989). Whereas, compared with the effect of physical pressure by pumping nitrogen gas, lower hydrolysis yield was observed in LBR with partial gas diversion. This might be due to the role of hydrogen partial pressure generated by self-generated off-gas, indicate that hydrogen partial pressure could reduce the hydrolysis of particulate waste. Additionally, more acidogenic products were generated in pressurized LBRs compared to that in regular LBR without headspace pressure owing to higher hydrolysis yield in pressurized LBRs. However, the acidogenic degree, the ability of sCOD transformation to metabolic products, was no difference between different LBRs with/without headspace pressure. Arslan et al. (2012) also reported that hydrogen partial pressure has no effect on metabolic acids production from carbohydrates, proteins and lipids rich waste. Therefore, it was concluded that headspace pressure could effectively improve solid waste hydrolysis and acidogenesis, and hydrogen partial pressure showed certain inhibitory impact on hydrolysis and not on acidogenesis.

Both leachate and biogas produced from acidogenic LBRs were fed into UASBs to generate methane in this two-phase AD process. Methane production was primarily related to the degradation of the fermentation products from the leachates and was affected by the leachate quality. Overall, methane production increased with the increasing hydrolysis-acidogenesis yield in leachates. The highest methane yield was obtained in methanogenic phase connected to the pressurized LBRs with self-generation gas pressure, which was 25% higher than that of without pressure control. No hydrogen was detected in biogas from UASBs which were connected to LBRs with entire (0 psi) and partial self-generation gas diversion (3.3 psi), indicating complete utilization of hydrogen in these UASBs and it contributed to 8% of methane recovery. Besides hydrogen, acetate can be directly utilized by methanogens. There was 34% increase in acetate production in pressurized LBR with self-generation gas, which may relate to the other 17% of enhanced methane production. But the increase of methane yield under the nitrogen pressure was comparatively lesser than the self-generation gas diversion (3.3 psi) treatment despite higher sCOD production. Compared to methane production with self-generation gas, lower COD removal efficiency was observed with nitrogen gas pressure treatment. Similar low COD removal with nitrogen was reported earlier (Fuchs et al., 2003). Additionally, large amount of hydrogen was detected in methanogenic gas, indicating incomplete hydrogen conversion due to higher gas flow rate (>10 ml/min), which might be another reason for lower methane production.

Table 1. Food waste degradation performance in two-phase AD process (1st set of experiment)

	VS removal efficiency (%)	Hydrolysis yield (g/kg VS_{added})	Acidogenesis yield (g/ kg VS_{added})	Methane production (L/ kg VS_{added})
Entire gas diversion (0 psi)	63.0 ± 1.7	620.9 ± 7.5	460.4 ± 5.0	175.2 ± 5.0
Pumping nitrogen gas (3.3 psi)	65.1 ± 2.1	733.4 ± 12.4	528.1 ± 7.1	198.0 ± 5.8
Partial gas diversion (3.3 psi)	77.2 ± 2.6	690.1 ± 9.4	534.4 ± 7.4	218.7 ± 7.1

Impact of various gas sources used to maintain headspace pressure

To maintain the headspace pressure in LBRs, self-generated gas (from LBR itself), and biogas containing 40% CO_2 can be applied to drive the reactions to produce acetate. A second set of experiment was carried out to assess the feasibility of self-generation gas pressure from acidogenic or methanogenic phase on the potential of homoacetogenic process in pressurized LBRs. The headspace filled with CO_2 gas was used as the control to identify the effect of CO_2 headspace pressure effect alone on overall particulate waste degradation. Different pressure strategies resulted in the differential leachability of acidogenic LBRs. Varying degrees of clogging were observed from day 6 to day 10 with CO_2 control strategy and biogas strategy reflecting leaching process failure in acidogenic reactor. During this clogging period, remarkable decrease of hydrolysis was observed, particularly in the CO_2 control. Interestingly, overall, the highest VS removal and hydrolysis performance were obtained in control. Proteinaceous material is one of the main components of food waste, and its degradation can be estimated by soluble nitrogen profiles such as total Kjeldahl nitrogen (TKN) and ammonium concentrations. Similar production trend and yield of soluble nitrogen were observed with biogas strategy and self-generation gas strategy while with CO_2 gas strategy, the generation of soluble nitrogen was much higher compared with the other two conditions. Meanwhile, the concentration valerate, an important intermediate during anaerobic degradation of protein (Flotats et al., 2003), was almost two times higher under CO_2 headspace pressure. These results indicated that higher CO2 partial pressure is favourable for the degradation of proteinaceous materials that could justify the higher VS removal efficiency and hydrolysis yield observed in control, compared to other strategies. Although the hydrolytic yield as evidenced by the sCOD concentration was comparatively higher with CO2 strategy, than other two treatments, the acidogenesis, i.e., the transformation efficiency of hydrolysis products to into acetate and other shorts chain acids VFA of CO_2 treatment was lesser than other two treatments that also reflected in less methane yield.

In general, hydrogen partial pressure plays a crucial role in hydrogen production and consumption during acidogenic fermentation. Although the highest hydrogen yield was obtained in LBR with self-generation gas strategy, the estimated amount of hydrogen consumption in LBR with self-generation gas strategy was the highest. Thus, higher hydrogen pressure enables to promote hydrogen consuming process such as propionate production, valerate production and homoacetogenesis (Arslan et al., 2012). Propionate and valerate production can also be related to protein and lipid degradation, and it is difficult to identify whether their generation are closely contributed to hydrogen partial pressure. But it is obvious that the highest acetate production (60 gCOD/kg VS_{added}) was obtained in LBR with self-generation gas pressure, whereas <48 gCOD/kg VS_{added} acetate was produced in other conditions. The higher acetate concentation (30% higher than other treatments) with self-generation gas strategy could be linked to the enhancement of homoacetogenesis. Therefore, hydrogen partial pressure in headspace can be a driving force for homoacetogenesis rather than CO_2 partial pressure. Subsequently, 219 L/kg VS_{added} of methane was produced with self-generation gas strategy, which was at least 30% higher than those with CO_2 strategies.

Conclusions

The headspace pressure could effectively accelerate the anaerobic degradation of particulate waste. The hydrogen partial pressure is not regarded as a limiting factor for solid waste hydrolysis and acidogenesis. In addition, higher CO_2 partial pressure could facilitate protein degradation but interfere hydrogen consumption process. In general, the acidogenic off-gas containing hydrogen and CO_2, was identified as suitable gas to maintain headspace pressure driving the production of more acetate and hydrogen in acidogenic LBR and consequently to improve methane conversion efficiency in UASB.

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MANURE MANAGEMENT FOR METHANE MITIGATION – *IN-VITRO* DETERMINATION OF METHANE EMISSION FROM MANURE FOR IMPROVED INVENTORY MODELLING

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Manure management is associated with the emission of greenhouse gases, with particular regard to methane (CH_4). Even though mitigation options (e.g., biogas treatment) exists, documentation and verification of manure treatment technologies for greenhouse gas (GHG) mitigation is a main barrier towards implementation.

The currently ongoing project *Manure management for methane mitigation - Improved inventory modelling to support policy actions (M4Models)* set out to validate a recently proposed methodology for experimental determination of CH_4 emissions from manure. By integration with country-specific farm models, it could provide estimates of GHG mitigation at farm level, as well as improve estimates of CH_4 emissions and GHG mitigation potentials for national inventories.

The proposed methodology will be briefly presented together with a current status update and presentation will draw attention to the introduction of a market-ready analytical tool using the existing Gas Endeavour® technology platform of BPC Instruments AB (Sweden) to estimate daily CH_4 emissions from manure during storage and the effect of technologies for CH_4 mitigation. This apparatus with automated and high-precision gas monitoring simulates conditions in bulk slurry and allows continuous separation and quantification of CH_4 and CO_2 evolution rates with full kinetic information during the *in-vitro* test in real-time.

Keywords: *Manure Management, Methane Mitigation, Methane Emission, Improved inventory modelling.*

DECENTRALIZED WASTE MANAGEMENT AND GREEN ECONOMY: A CASE STUDY OF PRE-PROCESSED WASTE INPUT TO, 25KG/DAY KITCHEN WASTE BIOGAS PLANT ESTABLISHED AT PRAMATI HILL VIEW ACADEMY SCHOOL, MYSURU, INDIA

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The 25 kg/day kitchen waste biogas plant at Pramati Hillview Academy, Mysuru, India, implemented by NIE-CREST (Centre for Renewable Energy and Sustainable Technologies), The National Institute of Engineering (NIE), Mysore has solved the issue of organic waste disposal of the school to an extent of 100% and created a value chain for waste to wealth. The biogas plant, at present, is partially fulfilling thermal energy demand and has achieved zero organic waste and reduced the carbon footprints. Analysis of data indicates that the plant in a year saves 438 kg of LPG used for cooking, reduces 1.32 tons of CO_2 emission and converts 7.9 tons of organic waste to energy and manure, thus achieving decentralized waste management and green economy.

Keywords: *Waste management, Green economy, Kitchen waste, Biogas plant.*

EFFECT OF WHEAT STRAW PELLET BIOCHAR ADDITION ON FOOD WASTE/SLUDGE ANAEROBIC CO-DIGESTION

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ABSTRACT. Co-digestion overcomes the drawbacks of mono-digestion by balancing the C/N ratio and diluting toxic compounds. However, stable methane production at particularly at high organic loading in co-digestion remains a challenge mainly due to incomplete VFA conversions to methane and consequent VFA inhibition in the system. This study explored the impact of biochar addition in co-digestion to alleviate VFA inhibition and increase methane production was studied with a concentration on dosage and elucidation via improved direct electron transport. Results showed that 10g/L of WSP550°C leads to an increase in methane production of 313±1.4 mL/gVS$_{add}$ and the VS removal efficiency of 50 % compared to biochar free (control). This also corresponded to an increase in methanogenic favorable substrates including acetic acid (>40%) and butyric acid (~20%) concentrations over the control, especially during 12-15 days of co-digestion. The optimized biochar dosage in FW/sludge co-digestion for feature a unique scenario of optimal VFA observation by biochar while maintaining appropriate VFA bioavailability in methane production which, could be a useful strategy to further increase the OLR in anaerobic co-digestion.

Keywords: *Anaerobic co-digestion; Biochar; Volatile fatty acid; Food waste; Methane*

Introduction

Hong Kong generates ~11500 tonnes of municipal solid waste (MSW) per day which includes ~3300 tonnes of food waste (~30%) per day. As the economic development is increased, the population rise and living standards in cities grew, the amount of food waste increased [1]. Acid inhibition is one of the main challenges for the stable operation of the food waste (FW) aerobic digestion system, as it limits the increase in organic loading rate (OLR), affects the capacity of the reactor, and increases the construction cost of the treatment method. Our recent studies on FW/sludge co-digestion have indicated that a high accumulation of intermediate metabolites i.e., VFA including acetic acid, butyric acid and propionic acid and long-chain acids are found in co-digestion systems [2,3]. Biochar in anaerobic digesters reduces time lag until methane production begins, and combined methane production rises [4,5]. In recent years, AD amended with biochar has attracted more attention, due to its potential benefits in the AD process such as stabilizing the digestion process, alleviating inhibitory effect, enhancing buffering capacity, and improving the methane yield. The addition of AD process affects the change in the quality and quantity of producing biogas, for the digestion of organic waste, the addition of biochar 5 to 18 g/L in dry mass, increased methane production by 20-40% [6]. Moreover, biochar in AD will adsorb inhibitors, including ammonia and VFAs by increasing the alkalinity of buffer potential and stabilization of the AD system [7], and decreased the lag phase of microbial development, and ultimately improving the methane yield. As per our knowledge, while we have widely noted the promotion of the AD methods by supplementing various biochar pyrolysis temperatures, there are few analyses of the biochar effects resulting from optimal conditions on the stability of the co-digestion phase at different dosage or concentrations. These dosages used in high solids conditions are expected to be low on a mass basis. Therefore, using an optimal biochar dosage concentration for VFA degradation in co-digestion is crucial for its cost-effective application. The cost will increase with a higher quality of biochar dosages. This detrimental effect may be exacerbated at lower re-used biochar dosage. However, this hypothesis is yet to be investigated. An important parameter for biochar assisted co-digestion is biochar dosage since it has an important influence on the adsorption efficiency of biochar. However, to the best of authors' knowledge, the impact of biochar addition and the consequence of biochar dosage on food waste and waste activated sewage sludge have not been reported. Our objective here is to optimize the biochar dosage in food waste/sludge co-digestion which features a unique scenario of optimal VFA capture by biochar while maintaining appropriate VFA bioavailability for methane assimilation within the reactor.

Material and Methods

Biochar characterization

The wheat straw residues biochar was collected from Biochar Research Centre (UKBRC), University of Edinburgh in UK. WSP550 ℃ was manufactured in a scalable 20 min process optimized for research use, comprising of a rotating kiln heated indirectly and electrically. The biochar was processed at high temperature (550 ℃) with pH 9.96, EC- 1.7(uS/cm) oxygen- 6.92(wt%), CEF-7.15 (cmol/kg), O/C- 0.080 (mol/mol), H/C- 0.37 (mol/mol), Surface adsorption capacity (SA) -26.4 (m^2/g) the elemental analyses as C- 68.26 %, N-1.39 %, P- 0.14 %, K- 1.56 %, ash- 21.25 wt %. This biochar was finally grounded and sieved to attain a fraction of 1-2 mm.

Substrate and inoculum

Food waste was prepared in laboratory according to the composition which was based on Hong Kong diest [8]. Anaerobically digestated sludge was used as the inoculum in the co-digestion studies. The sludges were obtained from Yuen long wastewater treatment plant, Hong Kong. Both sludge and food waste substrates were individually characterized for various physicochemical parameters prior to use in the digestion, as per the protocols in our previous publication [2].

Biochar amended food waste/sludge co-digestion systems.

To evaluate the effect of different dosages of biochar on AD, additional ratios of 2, 5, 10 and 20 g/L biochar were established. The working volume of 400 mL and a provision of continuous mixing in 35 and online gas monitoring via (AMPT-II). The ratio of substrate to inoculum (I/S) on total solids basis 6.04 (11.52 (w/w) on VS basis. The different experimental groups were named R1-2, R2-5, R3-10, R4-20 g/L, and control (without biochar addition) according to the biochar dosage. Specifically, different substrate concentrations of 1.075 g/gVS, 2.15 g/gVS, 4.3 g/gVS and 10.75 g/gVS were used respectively, based on VS.

Analytical methods

The samples were collected from each reactor on the initial day and last day of co-digestion for measurement of TS and volatile solids (VS), and every second day for samples measuring VFAs (acetic acid, butyric acid, propionic acid, iso-butyric acid, valeric acid, iso-valeric acid, and caproic acid), chemical oxygen demand (COD), using the standard protocols [9] and methods as described in [8]. Cumulative methane production data recorded by online data acquisition units of AMPTS-II were analyzed. All those experiments were conducted in triplicate and analysis of variance was used to assess the significance of the results obtained in this work.

Results and Discussion

Influence of biochar addition on VFA degradation during co-digestion.

WSP550℃ biochar's porous structure serves as a good immobilization matrix for enhanced bacterial and methanogens growth in the AD digesters, which leads to higher methane yields. The recent research report WSP550℃ increased the amount of some inorganic salts and metallic oxides, thereby resulting in higher biochar pH and contributed to enhancing buffering capacity upon their addition to AD [10,3]. The presence of oxygen rich functional groups such as C-O and C=O aromatic groups and O/C ratio 0.05 on pyrolysis biochar serves as strong active sites and enhances biochar adsorption capability [6]. All reactors showed a good degradation of organic matter as reflected by increasing sCOD and total VFA increased in day 2-6 (**Fig. 1a**) at which the peak tVFA concentration was reached. In contrast, a 5-fold higher tVFA remained in the control reactor as compared to the concentration in 4.3 and 10.75 g/gVS containing reactor (p-value> 0.005).

As can be seen in **Fig. 1a-b**, the acetic acid (AA) and butyric acid (BA) concentrations remained higher than the control for most of the co-digestion period. Particularly, biochar dosages of 4.3 g/gVS and 10.75 g/gVS had the highest concentrations of these acids. Since these are the most favorable methanogenic substrates, their high concentration supports methane production. The variation of total VFAs a rapid increased was found in acetic acid and butyric acid concentration of the high dosage of biochar addition reactors, followed by a sharp decline (**Fig 1a**). The highest butyric acid concentration was observed in 10g/L and 20 g/L reactors e.g. 18.98, 21.11 g/L during days 3-10 which was 2 fold higher as compared to that of control during this period of co-digestion (**Fig 1b**). Subsequently, a decreased butyric acid and acetic acid was seen at day 22 or biochar amended reactor which would contribute towards a further increase in methane production in this reactor. Accordingly, the pH value of the control group was significantly lower than the biochar addition groups, Zhang et al. (2019) [11] who also found that biochar, can effectively accelerate the degradation of organic acids. This may be due to the fact that biochar has additive contributes to making the surface more alkaline. Propionic acid values decreased with increasing biochar dosage in the co-digestion. It decreased to meagre concentration of 0.45 g/L with 4.3 and 10.75 g/gVS biochar addition while it remained at 3.7 g/L for 1.075 g/gVS biochar reactor and a much higher ~8 g/L in the control. The inhibitory effects of propionic acid on digestion efficiency are known and thus its high degradation was possible with an increasing biochar dosage. Giwa et al. (2019) [12] reported that propionate fermentation was stimulated by biochar addition which resulted in accumulation during initial start-up phase. These results indicated that biochar dosage addition has a significant effect on methane production, shows that, in propionic acid degradation, the acetic acid concentration initially increased but then decreased gradually. In contrast, when addition of biochar was consumed propionic acid concentration declined much faster at lower acetic acid concentration.

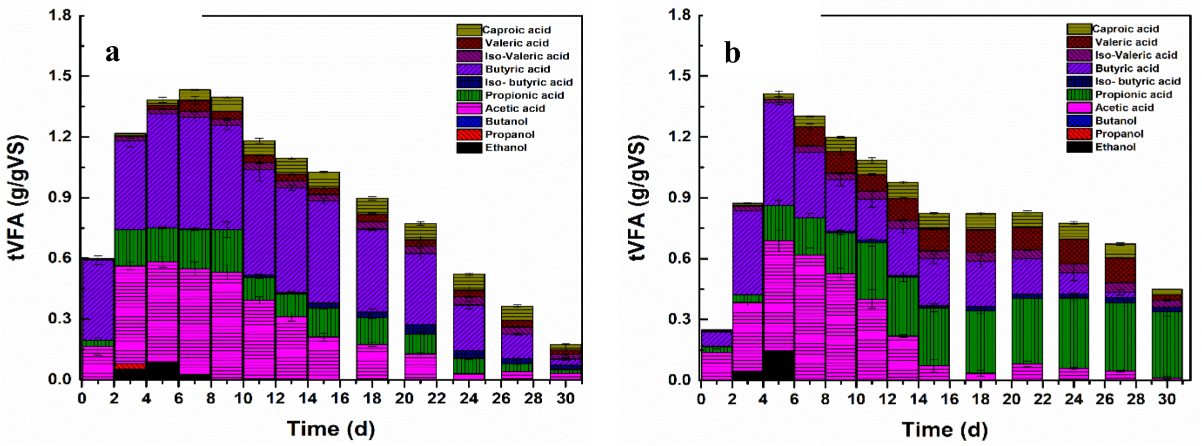

Fig 1. VFA percentage distribution profile for (a) 10g/L WSP550 biochar co-digestion treatment and its comparison with (b) control (no biochar addition).

Performance of biochar amended co-digestion systems in methane production.

Fig. 2 illustrate the effect of different dosage of WSP550℃ addition on cumulative methane production in food waste/sludge co-digestion. The different lag lengths between treatment and control were observed, but their overall cumulative methane production increased positively from 227.3±0.2, (1.075g/gVS), 274.5±1.3 (2.15 g/gVS), 313.9±1.4 (4.30 g/gVS), 299.0±0.8, (10.7 g/gVS), and 223.9±0.6 (control) cumulative methane mL/gVS. Compared with the biochar free group (control), the cumulative methane production increased by 28.6±0.4%, 25.1±0.6%, 18.4±0.3%, and 1.5± 0.04% in biochar addition 2g/L to 20g/L respectively.

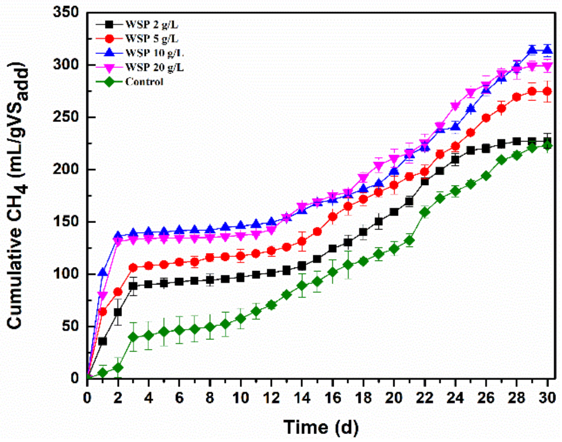

Fig 2. Cumulative methane production in co-digestion with biochar addition at varying dosage. Control is with no biochar addition.

The lag times of the biochar-free control group were 19 days. While that of the 1.075, 2.15, 4.30, and 10.75 g/gVS substrate groups were 15, 14, 12, and 11 days, respectively. Apart from 2.15 g/gVS WSP550℃ addition to reactor, addition of 4.3, 10.75 g/gVS also showed methane yield as a clear trend with biochar dosage was observed and the increase in cumulative methane production (followed by the increase in biochar dosage). However, a further increase in the amount of biochar caused a significant decline in the cumulative methane yield, which was >2% lower than that of the digesters with 10.75 g/gVS. Zhang et al. (2019) [7] suggested that sewage sludge derived biochar to an AD system and the results that biochar addition not only increased the cumulative methane yield considerably but also improved the reaction rate. This result indicated that biochar addition had positive effects on VFA degradation and enhance methane production. **Table 2** summarizes the overall performance of co-digestion with different dosages of biochar addition and its comparison with control. Biochar addition was 1.5-fold higher as compared to control reactors (p-vale <0.05). Overall, from the results of dosage experiments, it follows that similar results are obtained for 4.3 and 10.75 g/gVS biochar addition in terms of VFA degradation and methane production. Considering that biochar addition incurs a cost to the overall digestion process, it would be desirable to use a lower concentration without compromising on the digestion performance. In this case, 4.3 g/gVS biochar addition is proven to provide the desired positive impact on improving the co-digestion efficiency.

Table 1. *Summary of biochar dosage assisted co-digestion relative to control.*

WSP550℃ addition (g/L)	**2 g/L**	**5 g/L**	**10 g/L**	**20 g/L**	**Control** (without biochar)
VS removal efficiency (%)	43.3± 0.05	48.5± 0.02	50.4± 0.01	54.3± 0.02	42.6± 0.04
sCOD$_{max}$ (g/L)	31.4± 0.23	32.4± 0.2	33.5± 0.23	33.6± 0.46	31.6±1.6
Ammonia (mg/L)	1402.2± 6.2	1358.4± 2.2	1354.8± 3.6	1305.5± 1.4	1567.2± 1.5
tVFA$_{max}$ (g/L)	28.05± 1.0	26.89± 0.59	32.8± 0.37	33.3± 1.0	25.54± 0.19
Daily CH$_4$ (mL/gVS$_{add}$)	27.76± 0.2	28.3± 0.4	32.38± 0.2	38.26± 1.1	27.41± 0.3
Cumulative CH$_4$ (mL/g VS$_{added}$)	227.3± 2.4	274.58± 1.3	313.93± 1.4	299.09± 0.8	223.92± 0.6
CH$_4$ production increase with respect to control (%)	1.519	18.44	28.66	25.129	---

Conclusions

Wheat straw pellet biochar promoted higher methane productions as compared to control with no biochar addition, by virtue of their beneficial physicochemical properties. The results demonstrated that with the addition of 4.3 g/gVS biochar the digestive system obtained the highest specific methane yield 28.44± 0.2 % increase with the shortest lag time and can effectively neutralize VFAs, promoting their degradation and production of methane.

Acknowledgements

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EFFECTS OF DIFFERENT CONDUCTIVE MATERIALS ON THE ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE AND FOOD WASTE AND THEIR DIGESTATE DEWATERABILITY

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The addition of conductive materials (i.e. iron-based materials and carbon-based materials) has a positive effect on the anaerobic co-digestion (CAD) process of sewage sludge (SS) and food waste (FW). However, it still remains unknown which conductive materials could ahived a better improvement of CH_4 generation. In addition to the methane production, the digestate dewatering is needed to be considered, which can lower the operational costs of transportation and disposal. However, the digestate dewaterability of different conductive materials is poorly understood. This study therefore investigated and compared the methane production of adding zero-valent iron (Fe^0), magnetite (Fe_3O_4) and biochar (10 g/L) in the CAD process of SS and FW as well as the digestate dewaterability after the CAD process. The results showed that compared to the Fe_3O_4 and biochar groups, the Fe^0 group was more conducive to the decomposition of organic matters, cumulative methane yield, and daily biomethane yield in the CAD process of SS and FW. The highest cumulative methane yield in the CAD process reached 394.0 mL/g volatile solid (17 days) in the Fe^0 group. The digestate dewatering experiments revealed that the total solids content of sludge cake after the addition of Fe^0 and biochar significantly decreased as compared to the Fe_3O_4, indicating higher digestate dewaterability. Overall, Fe^0 could be considered as a better choice in the CAD process of SS and FW taking account of the much higher methane yield and digestate dewaterability.

Keywords: Anaerobic co-digestion, Zero-valent iron, Magnetite, Biochar, Methane.

EFFECT OF BIOCHAR ADDED ON ANAEROBIC DIGESTION OF METHANE FROM MUNICIPAL SLUDGE AND KITCHEN WASTE

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Urban sludge is rich in organic matter and nutrients. Anaerobic digestion of sludge to recover energy has become a global hotspot. Food waste has high organic matter and nitrogen content, and anaerobic digestion alone is easy to acidify and inhibit ammonia. Therefore, the mixed anaerobic digestion of urban sludge and kitchen waste is the future development trend. In the process of mixed anaerobic digestion of municipal sludge and kitchen waste, gas production efficiency is low and energy consumption is high, and urgently needs to develop and improve anaerobic fermentation enhancement technology. Biochar is a kind of material rich in pore structure and large specific surface area, which can provide suitable environment for microbial reproduction and habitat and enhance electron transfer in anaerobic digestion system, thus improving anaerobic digestion efficiency. In this paper, coco-shell biochar (with a specific surface area of 1050 m2·g-1) was added to the mixed anaerobic digestion system of kitchen waste and urban sludge to study the coupling relationship between interspecific hydrogen transfer (IHT) and interspecific electron transfer (DIET) in the mixed anaerobic digestion system and the methane production effect of biochar with different mass concentration gradients (blank group, 1 g·L-1, 3 g·L-1 and 5 g·L-1). The results showed that the methane production in the system with biochar increased compared with the blank group. Among them, the cumulative methane production of the experimental group with the addition of 1 g·L-1 increased most significantly compared with that of the blank group, with a 12.14% increase in methane production. The addition of biochar can better adjust the pH of the system. The pH change of the 1 g·L-1 experimental group has the smallest floating change during the entire anaerobic digestion cycle. The removal rates of SCOD, TS and VS in all the experimental groups with biochar were improved to varying degrees compared with those in the control group, with the greatest improvement in the 1 g·l-1 group, and the degradation rates of SCOD, TS and VS were increased by 48.95%, 23.26% and 10.20% compared with those in the control group. The bacterial flora in the anaerobic digestion system supplemented with biochar is mainly Thermotogae, Firmicutes and Bacteroidetes. All three types of flora play a very important role in the hydrolysis and acidification stage. The main methanogenic flora of the blank group is Methanosarcina. Methanobacterium and Methanolinea were the main methanogenic agents in the experimental system with biochar. These two types of flora are hydrogen-trophic methanogenic archaea, which dominate the entire anaerobic digestion cycle of the biochar experimental group system. During the entire anaerobic digestion cycle, the addition of biochar relieved the acidification of the mixed anaerobic digestion system，reduced the start-up time of the mixed anaerobic digestive system and enhanced methane production, promoted DIET among methanogens, geobacillus, and other methanogenic flora, and enhanced IHT.

Keywords: *Food Waste, Municipal Sludge, Anaerobic digestion, GAC, Biogas production, Microbial community, Electron transfer.*

ANAEROBIC CO-DIGESTION OF COFFEE PULP AND CATTLE MANURE FOR ENHANCED BIOFUEL AND ORGANIC FERTILIZER PRODUCTION

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Coffee is the second-largest agricultural commodity produced in the world, estimating annual production of 9.2 million tons (cherry basis). Through a wet coffee processing method, coffee pulp (CP) represents approximately 42% of the total waste produced. Disposal of CP requires a sustainably efficient method because of the toxic compounds like caffeine, free phenols, and tannins. Anaerobic digestion has been widely used for recovering resources, especially biofuel (biomethane) and organic fertilizer, from various organic waste streams such as animal manure, sewage sludge, crop residues, food wastes etc. However, mono-digestion of CP decreases digester efficiency due to its low pH, high lignin content, and accumulation of volatile fatty acids. Hence, co-digestion of carbon-rich CP waste with nitrogen-rich cattle manure (CM) could be a novel strategy to enhance biomethane generation through nutrient balance and dilution of inhibitory substances, and aid in nutrient recycling via sustainable utilization of digestate as an organic fertilizer.

Biomethane potential tests were carried out to assess the maximal biomethane production at five different co-substrate mixing ratios (0:1, 4:1, 2:1, 4:3, 1:0, on VS basis) in two different inocula, anaerobically digested cattle manure (ADCM) and anaerobically digested waste activated sludge (ADWAS). A series of 250-mL serum bottles, with substrate to inoculum ratio of 1.0, were operated at mesophilic condition for 50 days. Based on the results from ANOVA test followed by Tukey's honestly significant difference (HSD) for multiple comparison test ($p \leq 0.05$), CP:CM of 1:0 resulted in the highest specific methane yield (SMY), 266 ± 6.22 NmL/g VS_{added}, in ADWAS inoculum among all the combinations. Since the studies on long-term operation of co-digestion of CP with CM are limited, a scale-up study comparing mono-digestion and co-digestion of CP:CM at 4:1 is currently being conducted using bench-top semi-continuous digesters. This semi-continuous study will examine the stability and performance at different organic loading rates for both mono-digestion and co-digestion systems. The findings of this study will enhance the resource recovery from the CP and CM waste, in the form of biofuel and organic fertilizer.

Keywords: *Co-digestion, Coffee pulp, Cattle manure, Biomethane potential test.*

Bio-nano Technology for Biowaste Recycling

BIOELECTROCHEMICAL SYSTEMS FOR WASTEWATER TREATMENT AND RESOURCE RECOVERY: FROM LAB TO FIELD APPLICATIONS

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Microbial electrochemical technologies (METs) has made rapid progress in recent years. METs for wastewater treatment, the microbial fuel cells (MFCs) are currently being up scaled for pilot studies. Already a few demonstrations of microbial electrolysis cells (MECs) are operating globally. Electricity driven bioproduction in the form of microbial electrosynthesis (MES) is gaining ground especially for conversion of carbon dioxide into fuels and chemicals. The key advantage foreseen here is the use of excess electricity that is often generated renewably such as from solar cells and wind mills, all of which cannot be utilized immediately and can be fed into a MES to produce chemical compounds. Results with specific bacteria towards bioelectrochemical conversion of CO_2 to organic compounds will be reported. The dissolution of CO_2 and mass-transfer of reducing equivalents/hydrogen are among the limiting factors for this technology. Improvement on the dissolution and mass transfer rate of CO_2 was explored using gas diffusion electrodes (GDEs). VITO has developed GDEs tailored for systems with aqueous electrolytes and a gas-water interface, which are characterized by controllable pore diameters in the polymer-bound active layer, mechanical robustness and low water permeability. These cold-rolled (VITOCORE®) and phase-inversion based (VITO CASE®) electrodes enable reproducible quality in sizes from 10 cm^2 to 1 m^2. Large-scale VITOCORE® air cathodes were recently developed and tested in 85 L and 255 L MFCs to evaluate the impact of the cathode size on MFC performance. In this presentation, a global overview of the most recent developments in METs for resource recovery will be presented with examples from all parts of the world including India on piloting these systems.

Keywords: *Bioelectrochemical systems, Wastewater treatment, Resource recovery: Lab to field applications.*

MICROBIAL-ELECTROCHEMICAL REACTORS FOR THE TREATMENT OF ALKALINE AND SALINE WASTE STREAMS

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ABSTRACT. This study examined the use of bioelectrochemical systems (BES) for treating an alkaline and saline organic synthetic liquor that mimicked oxalate-containing alumina refinery wastewater. Cation exchange membrane-dual chamber BES reactors were constructed and operated at various hydraulic and organic loading rates for performance evaluation. Activated sludge was initially selected as the inoculum, but it was unable to establish the bioanode for oxalate removal due to paucity of oxalotrophic bacteria. Acetate co-addition enabled the start-up of a bioanode under saline and alkaline conditions, and ammonia-nitrogen (N) supplementation could be omitted as the biofilm could satisfy its N requirement presumably via N_2 fixation. A new approach of using graphite granules pre-acclimatised with aerobic oxalotrophic biofilm for start-up of a bioanode was proven effective. Overall, bioelectrochemical treatment is a feasible technology for simultaneously removing organics and recovering caustic soda from alkaline and saline liquor. The technology can be further explored for treating other alkaline and saline waste streams.

Keywords: *Bioelectrochemical systems; Waste treatment; Resource recovery; Haloalkaliphilic microorganisms.*

Introduction

Microbial-electrochemical reactors or bioelectrochemical systems (BES) are being increasingly explored as a versatile and sustainable biotechnology for removing contaminants and recovering resources from waste streams. However, practical application of BES is often hindered by two inherent properties of the waste stream: (1) low ionic strength causing high internal ohmic resistance; and (2) low pH buffering capacity causing drastic acidification hampering electrochemical activity of microbes. Although these constraints can be easily rectified by dosing chemicals, such an approach is impractical, increases operating costs and may cause secondary pollution. Therefore, it seems rational to develop the BES technology for treating waste streams that are intrinsically saline and alkaline.

One possible waste stream could be process water generated from the alumina refining industry. Alumina is aluminium oxide, which is the raw material for producing aluminium (Al). Al is typically extracted from bauxite ores in Bayer processes, where the ores are reacted with caustic liquor (Bayer liquor) under elevated temperature. However, this process also releases organic matter from the ore into the solution. Because the liquor is recycled for multiple times, and caustic soda is regularly replenished, the organics are accumulated over time and the liquor is highly saline. Removal of organics particularly a C2 compound known as sodium oxalate ($Na_2C_2O_4$) from the liquor is very crucial, as if not removed, oxalate would severely reduce the quality and yield of the refining product, as well as increasing caustic consumption. Hence, in this study the use of BES for removing organics (here oxalate) from a saline and alkaline liquor that mimics an alumina refining process liquor was examined. It was anticipated that the process would help recover caustic soda, thereby reducing the industry demand for caustic.

Materials and Methods

Initial testing using activated sludge as microbial inoculum and synthetic media with and without nitrogen supplementation.

The concept was first tested by starting up two cation exchange membrane (CEM)-equipped dual chamber electrochemical cells using activated sludge obtained from a local municipal sewage treatment plant as the microbial inoculum (Fig. 1). Reactor 1 (R1) was fed with an ammonium-N supplemented synthetic Bayer liquor and reactor 2 (R2) was fed with a synthetic Bayer liquor without ammonia-N supplementation. The compositions of the synthetic liquor were designed to mimic the highly saline and alkaline properties of the Bayer process liquor. Unless stated otherwise, sodium oxalate 3.35 g/L (25 mM) and/or sodium acetate (5 mM) were used as the carbon source. Sodium chloride (25 g/L) was added to increase the solution salinity equivalent to that of a typical Bayer liquor [1]. The pH value of the feed solution was maintained at above 10 by adding 2 M NaOH. The synthetic liquor used for R1 consisted of (mg/L): NH_4Cl, 265; $NaHCO_3$, 125; $MgSO_4{\cdot}7H_2O$, 51; $CaCl_2{\cdot}2H_2O$, 15; and $K_2HPO_4{\cdot}3H_2O$, 20.52 and 1.25 mL/L of trace element solution which had the composition of (g/L): $ZnSO_4{\cdot}7H_2O$, 0.43; $FeSO_4{\cdot}7H_2O$, 5; $CoCl_2{\cdot}6H_2O$, 0.24; $MnCl_2{\cdot}4H_2O$, 0.99; $CuSO_4{\cdot}5H_2O$, 0.25; $NaMoO_4{\cdot}2H_2O$, 0.22; $NiCl_2{\cdot}6H_2O$, 0.19; $NaSeO_4{\cdot}10H_2O$, 0.21; ethylenediaminetetraacetic acid (EDTA) 15, H_3BO_3, 0.014; and $NaWO_4{\cdot}2H_2O$, 0.05 [2]. R2 received the same influent as the anolyte, but without NH_4Cl to deprive the microorganisms of nitrogen source. Unless stated otherwise, this medium was used as the anolyte in the anodic chambers of R1 and R2 throughout the entire study. A NaCl solution (25 g/L) was used as the catholyte of both reactors (R1 and R2). Both R1 and R2 were operated with a potentiostat to maintain the anodic potential at defined levels (predominately at +200 mV vs. Ag/AgCl unless stated otherwise). The treatment performances and coulombic efficiencies (CE) were determined at various hydraulic retention time (HRT) and organic loading rates (OLR) over a period of >300 days.

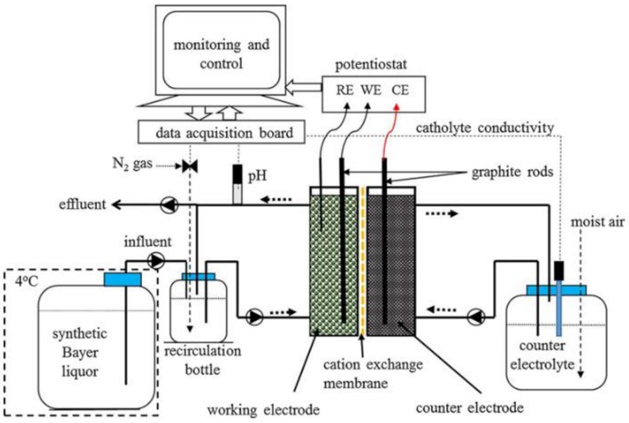


Fig. 1. Schematic diagram of the BES process used in this study.

Start-up of oxalate-degrading bioanode with granular graphite pre-acclimatised with aerobic oxalotrophic biofilm.

The initial experiment suggested that oxalate was only poorly degraded by the anodic microbial community even after a prolonged operation (>300 days), presumably due to a lack of oxalate-degrading microorganisms. To overcome this problem, a different start-up strategy was devised using graphite granules that have been pre-grown with an active aerobic oxalate-degrading biofilm as the BES anode. The aerobic biofilm-granules were established in a separate aerobic pack-bed bioreactor, with the biofilm demonstrated a good oxalate degradation activity of 23.5 kg m^{-3} d^{-1} [3]. The bioactive granules were packed into the BES anodic chamber and the process was initialised with an anodic potential of +200 mV vs. Ag/AgCl (HRT of 1d). The process was subsequently operated in continuous mode at various HRT and OLR, as well as various anodic potentials to determine the ability of the process to degrade oxalate.

Results and Discussion

Activated sludge could not start up a bioanode for oxalate degradation.

The results suggested activated sludge was unable to facilitate anodic oxalate degradation. There was no notable anodic current production over a long period when oxalate was provided as the sole carbon and electron donor. Notable anodic current was recorded only when the influent was supplemented with acetate. With acetate co-addition successfully starting up the bioanode in both the nitrogen supplemented and nitrogen-deficient reactors, the performances of the two BESs at various HRTs and OLRs were determined (Fig. 2).

The results showed that oxalate degradation was low (<10% removal), whereas complete removal of acetate was achieved in both systems (Fig. 2). Nonetheless, good linear correlations (R^2>0.96) between current density and COD removal rate in the two systems were obtained (data not shown), suggesting that the two anodic biofilms could be established under saline and alkaline conditions, and that the anodic biofilm could fulfil its nitrogen requirement without the need to dose nitrogen. This is encouraging from a biotreatment perspective, as it implies that the cost associated with external nutrient addition may be avoided.

The aerobic oxalotrophic biofilm could readily use graphite as the sole electron acceptor.

The inability of the anodic biofilm acclimatised from activated sludge to degrade oxalate was consistent with the results of microbial community analysis, which showed the paucity of bacteria under the family of Oxalobacteraceae, a bacterial group capable of decarboxylating oxalate to formate [6]. Therefore, the concept of using the graphite granules with biofilms of aerobic oxalate-degrading microorganisms as the BES anode for process start-up was tested. It was postulated that the aerobic biofilm should have suitable microorganisms to convert oxalate into formate, enabling a start-up of the bioelectrochemical oxidation of oxalate in the BES.

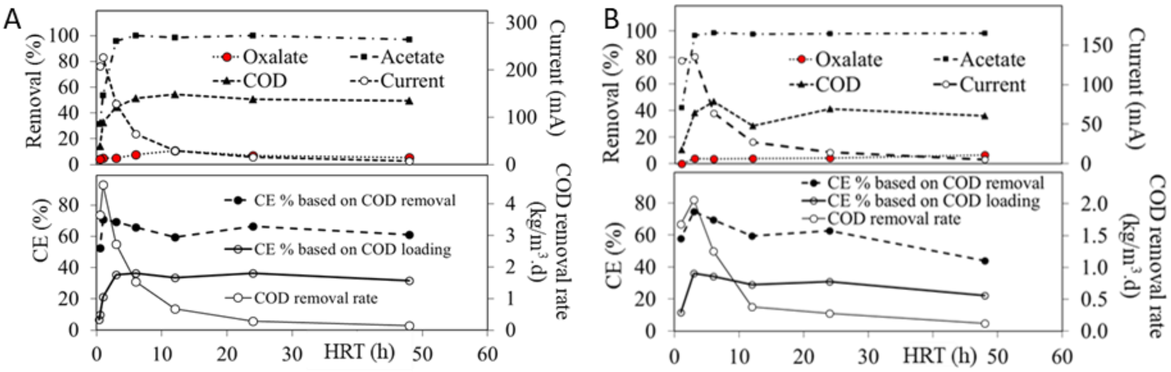
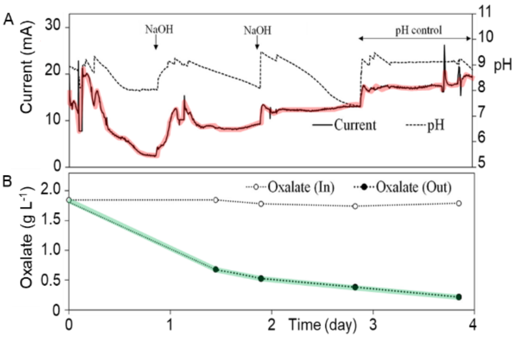


Fig. 2. Performance of (A) nitrogen-supplemented and (B) nitrogen-deficient BES with activated sludge as the microbial inoculum. The figure has been adapted from [3].

The results showed that after the aerobic oxalotrophic biofilm-coated granules were loaded to the BES, anodic current was produced immediately without any lag phase (Fig. 3). The current production also coincided with a slight decrease in the anolyte pH, which was due to anodic oxalate oxidation liberating protons. Increasing the pH to ~9.2 resulted in a sharp increase in current, confirming that the biofilm was alkalophilic (Fig. 3A). This result suggested that the aerobic biofilm almost instantly became anodic, and the biofilm could sustain its ability to degrade oxalate with the graphite instead of oxygen as their electron acceptor (Fig. 3B).

Fig. 3. The aerobic biofilm could readily use graphite as electron acceptor. (A) current and anolyte pH profiles; (B) influent and effluent oxalate concentrations. The anode was poised at +200 mV vs. Ag/AgCl and the BES was operated at an HRT of one day. The synthetic medium was supplemented with ammonia-N. The figure has been adapted from [4].



Further performance evaluation indicated that the established bioanode could facilitate a high current density of 363 A m^{-3} at a HRT of 3 hours (~20 kg oxalate removed m^{-3} d^{-1}). The biofilm also concurrently degraded acetate and oxalate (coulombic efficiency 80%) without apparent preference towards acetate (data not shown). The oxalate removal performance of the BES was comparable with full scale aerobic biotreatment process, with the same order of magnitude in terms of removal rate (Table 1). However, it should be noted that the BES also enables the simultaneous recovery of caustic soda.

Oxalobacteraceae became more abundant in the BES over time.

The microbial communities of the biofilms at the three developmental stages, namely (1) aerobic bioreactor cultivation stage; (2) BES fed with oxalate only; and (3) BES fed with oxalate and acetate were characterised (Fig. 4). Principal coordinate analysis shows that the microbial communities were phylogenetically different among the 3 stages (Fig. 4A), suggesting that the shift in electron acceptor from oxygen to graphite had caused a notable community change, and acetate co-addition further triggered a change in the community composition. The results also suggested that the known oxalotrophic bacterial family, Oxalobacteraceae, became increasingly abundant over time, even more so than the initial aerobic biofilm inoculum (Fig. 4B). Future studies should investigate the ability of the oxalotrophic bacteria to produce current in the BES.

Table 1. Comparison with full scale reactor performance for alumina refinery wastewater treatment.

Process	Electron acceptor	Influent	Reactor size	HRT	Reactor pH	Oxalate removal rate (kg/m^3.d)	Oxalate removal (%)	Recovery of caustic soda	Ref.
Full scale aerobic biofilm reactor	Oxygen	Refinery lake water	450 m^3	15 h	9.7	31	100	No	[7]
Lab scale aerobic biofilm reactor	Oxygen	Synthetic liquor	1.5 L	3.5 h	9.2 ± 3	24	100	No	[5]
BES reactor	Graphite anode	Synthetic liquor	0.5 L	3 h	9.1 ± 4	20	97	Yes	[3]

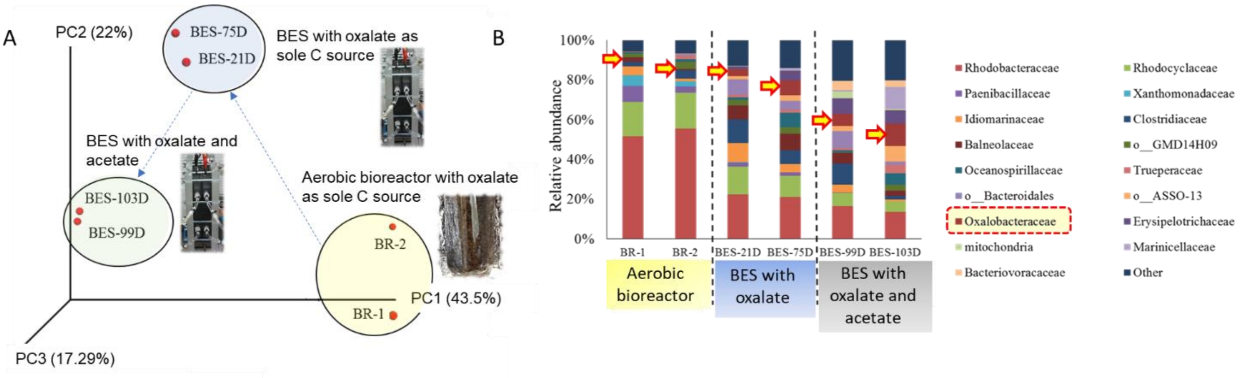


Fig. 4. Microbial community analysis of the biofilms at various stages of the process. (A) principal coordinate analysis; (B) relative abundance at family level. The figure has been adapted from [4].

Conclusions

Bioelectrochemical treatment is a feasible technology for removing organics from alkaline and saline liquors. However, activated sludge was not a suitable inoculum for starting the bioanode for oxalate removal due to paucity of oxalotrophic bacteria. Acetate co-addition enabled the start-up of a bioanode under saline and alkaline conditions, and nitrogen nutrient supplementation could be omitted as the biofilm could satisfy their N requirement presumably via N_2 fixation. The approach of using electrode carriers pre-acclimatised with aerobic oxalotrophic biofilm for starting-up a bioanode was effective and should be explored further. Further development and cost-benefit evaluation of the proposed process are encouraged.

Acknowledgement

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STRUCTURED CARBON MONOLITH FOR GAS ADSORPTION: USING NANO-BIOCHAR AS PRECURSOR

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High efficiency and low-cost adsorption-based gas separation processes have attracted intensive attentions in both industry and research areas. Recently, researchers showed great interest in structured adsorbents due to their significant potential for the improvement of performance and reduction in the cost of cyclic adsorption processes such as pressure swing adsorption. In particularly, by controlling the structure of the adsorbents applied in the separation devices, the parameters dictated by adsorbent loading per unit volume, mass transfer properties, pressure drop, and the thermal management could be optimized, as a result reduce the system cost. In addition, the robust structured adsorbent can significantly reduce the mass loss and minimize the environmental impact. Therefore, developing a structured adsorbent with high mechanical strength and good adsorption properties is highly desired, and have received growing research interest in recent years.

Structured carbon monoliths prepared from carbon-based materials including coal, pitch, CNT, carbon sphere and biomass have been reported with high strength and promising adsorption characteristics display great potential as structured adsorbents for gas storage and separation applications. On the other hand, nano-biochar with its advantages including improved porosity, specific surface area, mobility and other properties as biochar has received much attention recently. Using nanobiochar as precursor for structured carbon monolith will not only take advantages of its excellent performances but also alleviate the controversy about its potential impact on the natural environment.

Keywords: *Carbon monolith, Nano-biochar, Gas adsorption.*

ENHANCEMENT OF FUEL PROPERTIES OF YARD WASTE THROUGH TORREFACTION

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Biomass is seen as a viable source for green, sustainable energy to help meet energy demands in the near future. However, the properties of raw biomass, such as high moisture content, pose challenges for energy extraction processes. To overcome these challenges, the biomass must be pre-treated using processes like torrefaction. Therefore, torrefaction was carried out to investigate the effects of carrier gas and temperature on the properties of yard waste. The yard waste consisted of a 30:70 wt% mixture of fallen twigs and leaves, collected from the University of Nottingham Malaysia. Temperature was varied from 170-300oC, and three carrier gases were used, namely pure nitrogen, pure carbon dioxide and a mixture consisting of a 25:75 vol% mixture of CO_2:N_2 representing flue gas. As temperature was increased, the mass yield of the torrefied yard waste decreased while the HHV increased. The mass yields obtained were in the order of nitrogen<carbon dioxide<flue gas. HHV was in the order of flue gas<nitrogen<carbon dioxide. Energy yield was also calculated to determine losses incurred in the process. Overall, carbon dioxide was shown to be the best carrier gas for energy intensification.

Keywords: *Carbon dioxide torrefaction, Dry torrefaction, Energy yield, Flue gas torrefaction, Yard waste.*

International Conference on Sustainable Biowaste Management 2021

Bio-nano Technology and Application in Waste Recycling

CO_2 CAPTURE AND MICROBIAL CATALYTIC CONVERSION TO BIOENERGY CH_4 IN OIL RESERVOIR SYSTEMS

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Carbon dioxide is a major greenhouse gas contributing to the climate change and its storage in the subsurface petroleum oil reservoir systems is one option for long-term management of this gas from industries for a sustainable development. In the oil reservoir system, due to the indigenous microbiota, the fate of CO_2 in such ecosystems is not known and studies were carried out to investigate the indigenous microbiome in subsurface ecosystem, their metabolic capabilities in transforming both the hydrocarbons and also introduced CO_2 to advance the current knowledge on CO_2 capture in oil reservoirs for permanent storage using Illumina sequencing of genomic DNA and transcribed RNA, detection and identification of transformation metabolites, the genes responsible, and also thermodynamics calculation to find out the best conditions for CO2 conversion to bioenergy as a future technology in energy management. Production water of oil fields in China were used in laboratory-based incubation study for determination of the CO_2 and also the responses of microbial community. In addition, another promising strategy to stimulate and accelerate biological transformation of CO_2 into methane as energy was achieved in this study with ZVI as the alternative electron donor into oil reservoir production waters. Enrichment of *Methanothermobacter* spp. supported its competitive role in biomethanation process *via* CO_2-reducing methanogenesis and formate methanogenesis in ZVI-amended cultures. The detected $FeCO_3$ mineral also presents a potential for immobilization of CO_2 in the presence of ZVI under the anaerobic conditions. Biomethane production with high rates (> 61.67 μmol/(l·d)) amended with ZVI detected in this study provided a potential opportunity for value-added CO_2 management technologies and further bioenergy regeneration from CO_2 in EOR and CCS of oil reservoirs. Stable isotope C-13 labelled CO2 was used to trace the transformation and fate of introduced CO2 in the microcosm systems in this study. Results suggest that biotransformation of CO2 to CH4 and also organic fatty acids occur in production water of oil reservoir and the rate of transformation ca be further accelerated by addition of ZVI as an addition a source of electron donor.

Keywords: Carbon dioxide, Direct microbial transformation, Utilization, Electron donors, Green biotechnology, Value-added products.

RESOURCE EFFICIENT SUSTAINABLE PRODUCTION OF NANOCRYSTALLINE CELLULOSE THROUGH SUGARCANE BAGASSE AND RICE STRAW VALORIZATION

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Agricultural sector generates huge amount of wastes such as bagasse and straw in processing and these underutilized wastes result in rapid increase in greenhouse gases, ultimately leads to climate change. The biochemical composition of the Agri-waste biomass (30-45% cellulose, 20-30% hemicellulose and 15-25% lignin) reflects its potential to cater as ideal substrate for production of value-added chemicals such as reducing sugars, furfurals, ethanol, xylitol, sorbitol, volatile fatty acids, biogas etc. Amongst these derivatives, Nanocrystalline cellulose (NCC) has received much attention in recent years and emerged as a sustainable and assuring nanomaterial, due to its inexhaustible nature and unique properties, namely high specific surface area, low density, thermal stability, high elastic modulus, optical transparency, simple surface functionalization, biodegradability, and biocompatibility. The present study evaluated with an optimized two-step process for the sustainable production NCC using sugarcane bagasse (SCB) and rice straw (RS). Pretreatment of SCB resulted in extraction of cellulose of 0.34g/g followed by NCC production of 0.25g/g SCB with 62.5% yield of NCC from raw SCB. In case of RS, 0.30g/g cellulose was extracted and 0.21g/g of NCC from raw RS, which accounted 60% yield. For both the feedstocks, conversion of cellulose to NCC was critically assessed with XPS, FE-SEM, SEM-EDX, FT-IR, TGA, DTA and DSC analyses. NCC holds various potential applications as engineering and functional material in paper, paints, biomedical devices, electronic sensors, packaging etc. The evaluation of effective method for NCC production using Agri-waste feedstocks accounts an additional benefit to the existing sugarcane industries and waste management methods in the framework of biorefinery and also evokes the biobased economy.

Keywords: *Resource recovery, Agro-industrial waste, Nanocellulose, Sustainability, Bioeconomy*

THE IMPACT OF HEAVY METALS IN THE WASTEWATER SLUDGE ON LIPID ACCUMULATION OF OLEAGINOUS MICROORGANISM

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ABSTRACT. Sustainable development rises new challenge on wastewater sludge management. Sludge management should not only focus on reduction, stabilization, and harmlessness, but also require for resource recycling. Wastewater sludge used for biodiesel production is important way to achieve the goal. However, sludge contains harmful materials such as heavy metals which would impact on lipid accumulation. This study determined the effects of multiple heavy metals in sludge on the growth of microorganisms and lipid accumulation. The results showed that 0.55 mg/L Cd2+ and 50 mg/L Zn2+ had obvious inhibitory effect on the strain. Research on co-fermentation of sludge and oleaginous yeast showed that when the sludge after heavy metals removal was used as the substrate, the lipid accumulation was lower than that of the sludge only after acid treatment (without heavy metals removal).

Keywords: *Heavy metals; Lipid; Sludge; Oleaginous yeast*

Introduction

In the past century, with the world's population has grown, waste production has increased tenfold. China's solid waste production is expected to increase from 520 550 tons/day in 2005 to 1.4 million tons/day in 2025 [1]. Wastewater sludge is one of the fastest growing solid wastes. Study has showed that it can be turned into biofuel through re-fermentation [2-3]. Fuel from biomass resources has become a promising alternative to fossil fuels. However, compared with traditional fossil fuels, there are many uncertainties in the use of biomass to produce biofuels [4]. The oleaginous yeast is an important organism in industry, assimilating carbohydrates into lipids [5]. Municipal sludge generally contains certain heavy metals, which has always been the most important factor restricting the utilization of sludge [6]. There are few studies on the effect of heavy metals on the oleaginous yeast. This study investigated the effects of a variety of common heavy metals on the growth of oleaginous yeast and the ability of producing lipid through fermentation.

Material and Methods

Sludge, microorganism and medium

The sludge used in the experiment was from a local sewage treatment plant in southern China. After 24 h settling at 4 ° C, the supernatant was thrown and the solid part was used in the study. The sludge pH was around 6.8, the mixed liquid suspended solids concentration was 7 g/L, and the initial lipid content of the raw sludge is 13%. The sludge was gone through acid and heat pretreatment before used in lipid production.

The oleaginous microorganism, *Lipomyces starkeyi*, used in the experiment was purchased from the China Industrial Microbial Culture Collection and Management Center. Studies have shown that the bacteria has good oil-producing ability [7-9]. The growth conditions of this oleaginous yeast are shown in Table 1.

The medium used was YPD liquid medium, which contained 20 g/L of glucose, 10 g/L of yeast extract, and 10 g/L bacteriological peptone. The solid medium for cell number determination was prepared by adding 2% agar in the liquid medium.

The fermentation was conducted with shake flasks. The pretreated sludge was first adjusted to pH 6.5 before fermentation and then inoculated with 10% v/v culture, then maintained at 28 ° C and 150-200 rpm. Samples were taken accordingly.

Table 1. Growth conditions of oleaginous yeast

Bacteria	Growth cycle (d)	Growth temperature (℃)	Aerobic type	Growth type
Lipomyces starkeyi	3	28~30	Aerobic	Heterotrophic

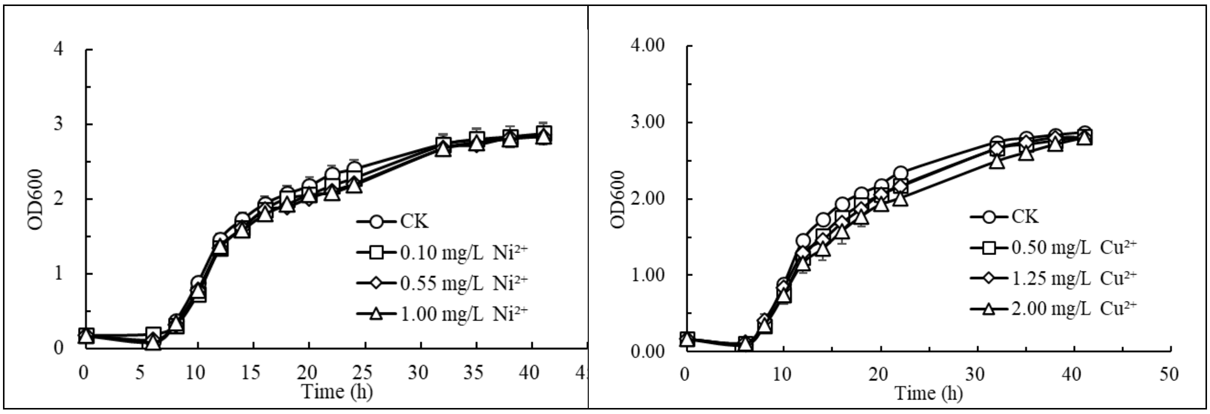
Analytical method

The study separately measured the dissolution of heavy metals (Ni, Cu, Zn, Cr, Cd, Pb) under different acid or heat treatment conditions. This study used ICP-OES (PERKINELMER optima8000, USA) to detect the concentration of heavy metal ions. The growth status of microorganisms in YPD liquid medium containing heavy metals were quantified by OD600. Ultraviolet spectrophotometry (UV-2006) was used to measure the absorbance of the culture medium at a wavelength of 600 nm to indicate the growth of oil-producing microorganisms. In this study, strain biomass determination adopted gravimetric method [2]. The chloroform-methanol method was used for the extraction of microbial oil. Transesterification method was used to prepare biodiesel [3]. Excel 2019 was used for chart drawing and data analysis.

Results and Discussion

The tolerance of microorganisms to heavy metals

According to the dissolution of various heavy metals in the sludge pretreatment process, different investigation ranges were set for the concentration of different heavy metals, and the tolerance of oleaginous yeast to different heavy metals was analyzed. It can be seen from Fig. 1. that the two heavy metal ions, 0.55 mg/L Cd^{2+} and 50 mg/L Zn^{2+} had more significant impacts on the growth of microorganisms. The Pb^{2+} had a slight effect on the biomass of microorganisms in the stable phase.



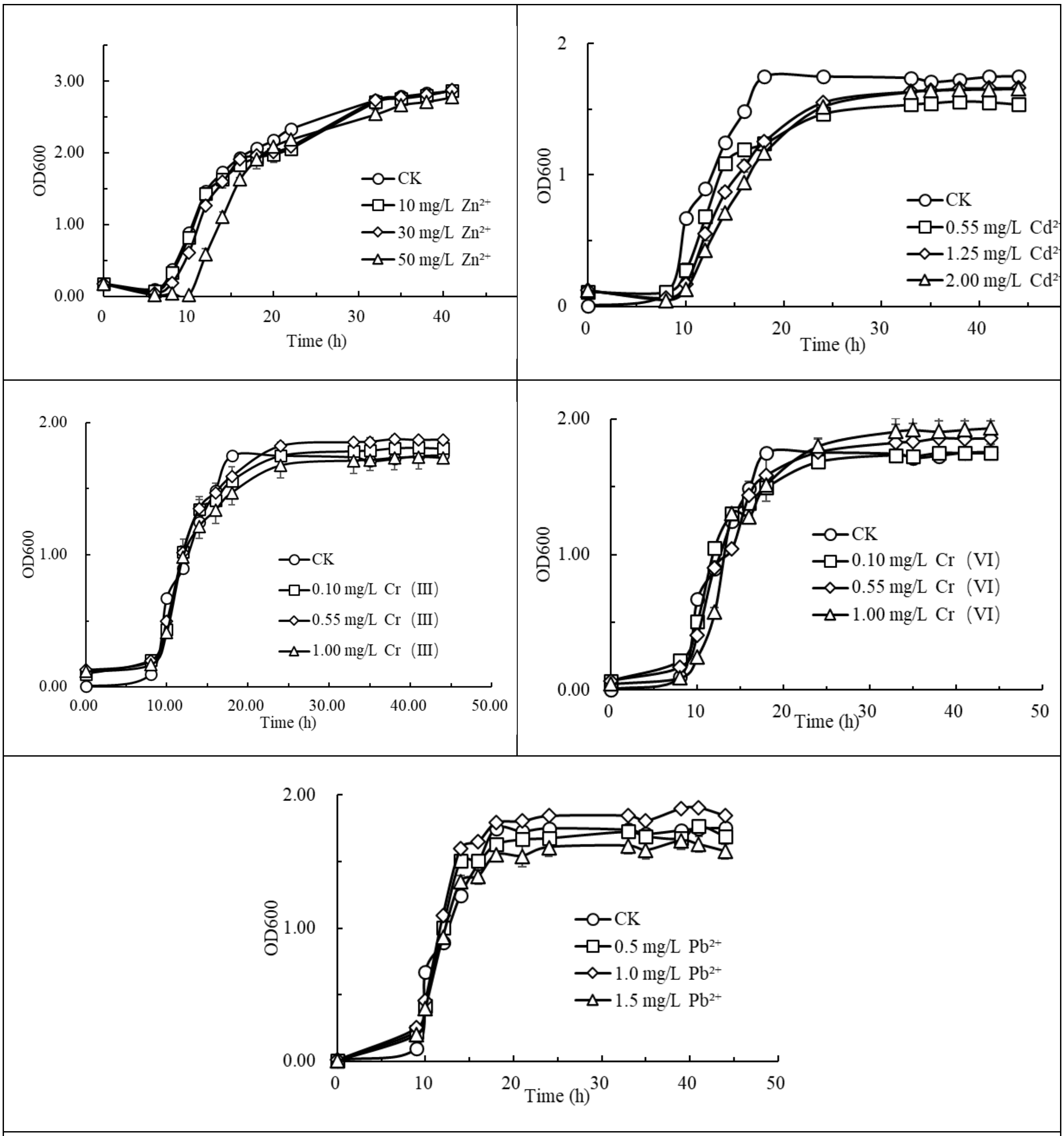


Fig. 1. Growth curve of oleaginous yeast under different heavy metal stress. Ni^{2+} concentration in culture medium=0, 0.10,0.55,1.00 mg/L; Cu^{2+} concentration in culture medium=0,0.50,1.25,2.00 mg/L; c) Zn^{2+} concentration in culture medium=0,10,30,50 mg/L; d) Cd^{2+} concentration in culture medium=0,0.55,1.25,2.00 mg/L; Cr(Ⅲ) ion concentration in culture medium=0,0.10,0.55,1.00 mg/L; Cr(Ⅵ) ion concentration in culture medium=0,0.10,0.55,1.00 mg/L; Pb^{2+} concentration in culture medium=0,0.5,1.0,1.5 mg/L.

Biomass and lipid production of oleaginous yeast in media with different concentrations of Cd^{2+} or Zn^{2+}

The study added a certain concentration of Cd^{2+} (0.55 mg/L) or Zn^{2+} (50 mg/L) to the YPD liquid medium to investigate the impact of these two heavy metal ions on the oil production.

It was found that both the medium with heavy metals and the medium without heavy metals had the highest lipid content up to 50% at 84 h. The lipid content of the medium with Cd^{2+} was 20% lower than the other two groups.

It can be seen from Fig. 2. that after adding Zn^{2+} and Cd^{2+}, the composition of fatty acid methyl esters increased significantly in stearic acid (C18:0) and behenic acid (C22:0).

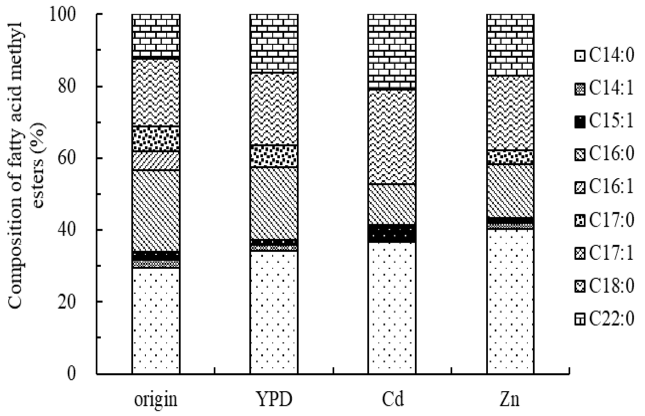


Fig. 2. Fatty acid methyl ester composition

Co-fermentation of sludge and oleaginous yeast to produce Lipid

After the sludge was properly pretreated, the heavy metals in the sludge were leached. At this time, by adjusting the pH and adding ferric chloride, the heavy metals were precipitated and then removed. The oleaginous yeast was respectively inoculated into the unpretreated sludge and the pretreated sludge. The accumulation of lipid under the sludge after heavy metals removal was lower than the sludge only after acid pre-treatment (without heavy metals removal), indicating that the presence of metal ions under this condition was necessary to accumulate microbial lipid.

Conclusions

This study showed that some heavy metals in the sludge will have a certain inhibitory effect on the growth of oleaginous yeast. Certain heavy metals in the sludge may be able to promote the lipid production of oleaginous yeast. This research will provide an idea for the influence of heavy metals on oleaginous yeast. It also reveals that the use of sludge containing heavy metals as a nutrient source for lipid production from oleaginous microbe should conduct pre-determination to understand the possible impact afterwards.

Acknowledgement

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ONE-POT APPROACH CONVERSION OF FRUCTOSE TO 2,5-DIFORMYLFURAN BY CARBON-BASED METAL-FREE CATALYSTS

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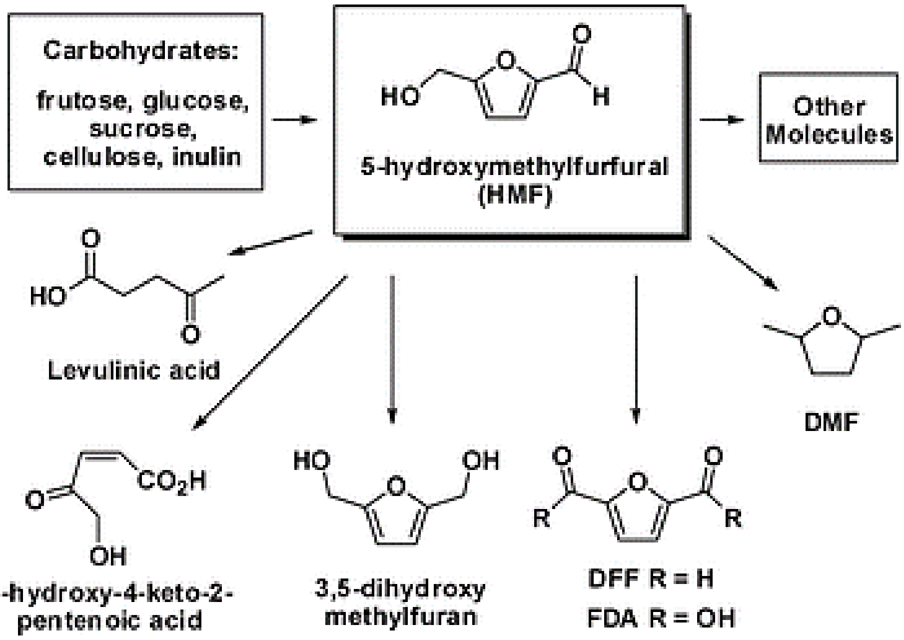
ABSTRACT. Converting biomass into fuels and chemicals to release the energy and resource crisis has attracted lots of attention in recent years, which is also known as biorefinery. A tremendous amount of effort has been devoted to the development of methods for the transformation of carbohydrates to valuable chemicals. 2,5-diformylfuran (DFF), one of the most high-value products in biorefinery, can be used as a raw material for the synthesis of drugs, anti-decomposers, and biological materials. This work aims at the synthesis of DFF using carbohydrate as a raw material by dehydration to 5-Hydroxymethylfurfural (HMF) and in situ selective oxidation of HMF in a one-pot process, to avoid the energy consumption process of HMF separation and purification. The key factor of this process is the excellent catalytic activity and stability of the catalysts. In this study, carbon nanoplatelets were prepared by a facile method and functionalized to be the bifunctional catalysts for the tandem reaction from fructose to DFF. The outstanding catalytic performance of the catalysts was attributed to the rich oxygen-containing groups on the surface of the catalysts. Compared with active carbon, carbon nanotube based catalyst, higher DFF yield can be achieved in a one-pot and one-step conversion of fructose to DFF with oxygen as the only oxidant at atmospheric pressure. The developed catalyst successfully realized the metal-free production of DFF from carbohydrates, and showed no significant loss in reuse and stability test, indicating its great potential in industrial application.

Keywords: *5-Hydroxymethylfurfural; 2,5-diformylfuran; catalyst, oxidation, metal-free*

Introduction

The world is currently facing severe energy shortages and environmental pollution due to the massive consumption of fossil energy. The development and utilization of biomass resources is considered to be one of the most likely important means to replace fossil energy. By converting biomass into fuel, useful chemicals and materials, dependence on fossil resources and carbon emissions can be effectively reduced. In the conversion and utilization of biomass, 5-Hydroxymethylfurfural (HMF) is an important platform molecule and a bridge connecting biomass raw materials and chemical products. As can be seen from Scheme 1, HMF is an important platform molecule that bridges renewable biomass resources and high value-added chemicals.[1] HMF can be obtained from carbohydrates such as cellulose through a series of reactions, including cellulose hydrolysis, glucose isomerization, and fructose dehydration. Afterwards, HMF can undergo oxidation or reduction reactions to produce a series of high-value chemicals. In particular, furan compounds such as 2,5-furandialdehyde (DFF), 2,5-furandicarboxylic acid (FDCA) obtained by selective oxidation of HMF can be used as substitutes for industrially widely used benzene chemicals. This research is aimed at the synthesis of DFF. DFF is one of the most valuable biomass-derived chemicals and has a wide range of applications. It can be used as raw materials to synthesize drugs, antifungal agents, Schiff bases, and biological materials.[2] Therefore, the synthesis of DFF from biomass is one of the research hotspots in recent years. However, the synthesis of 2,5-furandicarbaldehyde has long been based on HMF. Due to the high price of HMF, the production cost of DFF is also very high, which limits its large-scale industrial application. Therefore, if the reaction of HMF oxidation to prepare DFF can be extended one step upstream, that is, HMF is prepared by dehydration of fructose, and then HMF is oxidized to obtain DFF, and these two reactions are carried out in the same reaction system, it will greatly reduce DFF production cost.



Scheme 1. Conversion of biomass into HMF-derived chemicals[1]

The development of the catalytic reaction system and catalyst for preparing DFF directly from fructose is divided into two stages. In the initial stage, researchers use an acidic catalyst and a catalyst that can catalyze the oxidation reaction for the fructose dehydration and HMF oxidation reactions respectively. In this process, the two catalysts need to be added to the reaction system twice, and it is necessary to accurately judge the extent of the reaction to determine the best timing of catalyst addition to ensure the highest possible yield of the target product.[3, 4] If the two catalysts are added at the beginning of the reaction, it will cause the direct degradation of fructose. Such operations are difficult to standardize in actual industrial production. Later, the researchers developed composite catalysts, that is, the acidic group and the oxidizing catalyst active site are integrated to one catalyst, and at the same time, by regulating the activity of the catalyst, the degradation of fructose is avoided.[5] However, the oxidation reaction catalytic center of these catalysts is usually provided by noble metals or transition metal oxides, and there is a problem of metal species leaching in the reaction. Recently, the outstanding performance of graphene oxide in the oxidation of alcohols has inspired us, that is, the preparation of metal-free carbon-based catalysts for the reaction of fructose to DFF.[6] In this work, we use biomass waste as raw materials, use molten salt pyrolysis to obtain carbon materials, and acidify them to obtain bifunctional catalysts that can be used to prepare DFF from fructose.

Material and Methods

Materials

The chemicals including fructose, HMF, DFF, DMSO, cellulose etc used in the experiment were purchased from Sigma-Aldrich and used directly without purification.

Catalysts synthesis

Cellulose was used as the carbon precursor for the synthesis of carbon based catalysts. For a typical process, 1g cellulose was mixed with lithium chloride and potassium chloride at a ratio of 45/55 by weight. The cellulose and salts mixture were uniformly ground and then transferred to a crucible and calcinated at 800 ºC for 5h in nitrogen. After cooling, the black product was dissolved in deionized water with the assistance of sonication. Black carbon materials can be collected after filtrating, washing and drying at a 105 ºC oven for 12 hours, denoted as C-800. Then C-800 was treated with a concentrated nitric acid for 3 hours at 120℃, filtering and washing with sufficient deionized water to remove the redundant nitric acid, the resulting product obtained after overnight dry in an oven was denoted as C-800-A.

Characterizations

The morphology of the catalysts was investigated by field-emission scanning electron microscopy (FESEM; JEOL, JSM-6700F, 5 kV) and transmission electron microscopy (TEM; JEOL, JEM-2100F, 200 kV).

Catalyst reaction and product analysis

The catalytic reaction was carried out in a autoclave reactor with a reaction volume of 25mL. 300mg glucose was dissolved in 15mL DMSO and with the addition of 50mg catalysts. The oxygen pressure was maintained at 10 bar 150℃ for 24h.After the reaction, the catalyst was separated from the reaction mixture by filtration and products were diluted with DI water prior to the analysis by HPLC.
Analyses of fructose, HMF and DFF were conducted on an Agilent 1260 HPLC with a Bio-rad aminex 87H column (300 mm×7.8 mm pre-packed column). The mobile phase was constituted of 5 mM H2SO4 solution at 0.6 mL/min. The column oven temperature was kept at 60 ºC. The amount of each compound was quantified by an external standard calibration curve method, which was constructed based on the pure compound.

Results and Discussion

Fig. 1 shows the morphology of the synthesized carbon material catalyst. After comparison, it is found that the morphology of the C-800-A catalyst treated with nitric acid has changed significantly. The surface of C-800d is relatively smooth, while the surface of C-800-A is rough and shows obvious pore structure. This may be because concentrated nitric acid has strong oxidizing properties, and C-800-A loses part of its instability during the nitric acid treatment of carbon atoms.

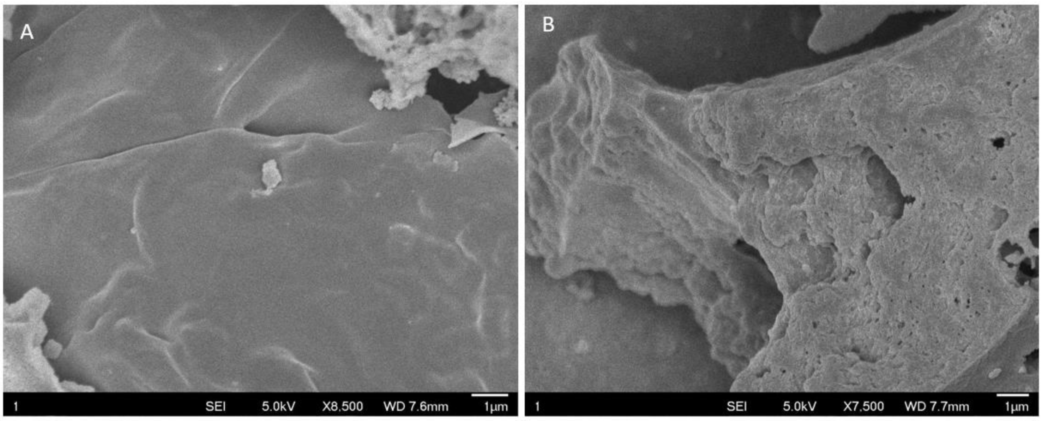


Fig. 1 FESEM images of (A) C-800 and (B) C-800-A.

The catalytic conversion of fructose to DFF was carried out with the two types of catalysts and the results can be seen in Fig.2. Both the two catalysts showed a certain activity in the fructose dehydration and HMF oxidation steps. Fructose dehydration reaction finished in the beginning three hours and then the HMF oxidation reaction became the main reaction. It can be seen that the HMF yield was peaked at 55% and then decrease with the growth of reaction time when C-800 was used as the catalyst. And the DFF yield reached 15% after 24 hours reaction. When C-800-A was used as catalyst, the highest yield of HMF was 75%, and then the HMF can be oxidized to DFF with a final yield of 54% after 24-hour. The difference between the two reaction processes indicates that the acid-treated C-800-A catalyst has increased surface acidity due to the addition of oxygen-containing groups, especially carboxyl groups, thereby showing better performance in the reaction of fructose dehydration to HMF. The yield of HMF determines the theoretical level of DFF yield that can be achieved. In contrast, the catalytic activity of C-800 in the HMF oxidation reaction is not high, while C-800-A shows quite good activity. It can be seen that the effect of acid treatment on improving the catalytic performance is very significant and necessary.

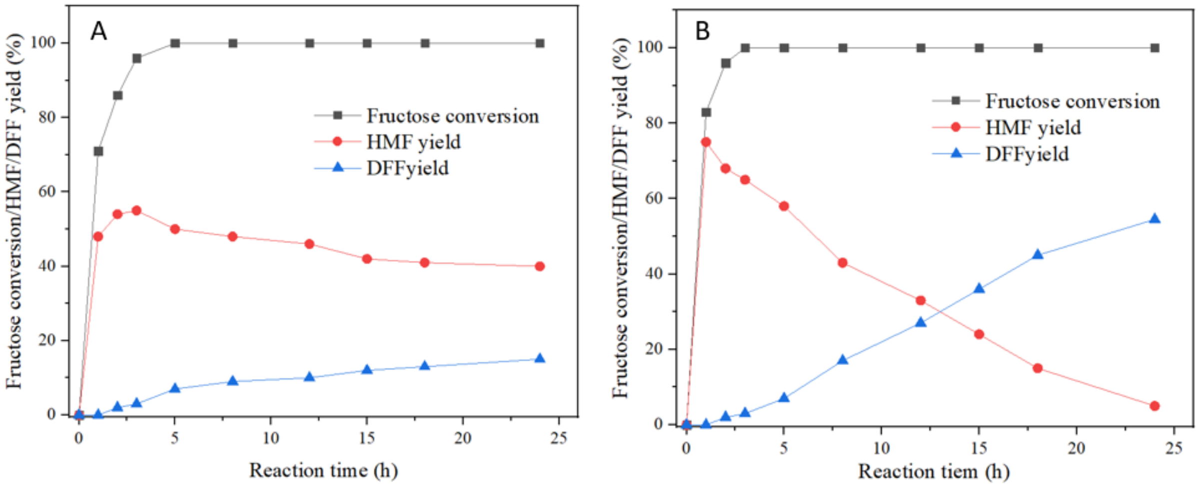


Fig.2. Catalytic conversion of fructose to HMF and DFF by (A) C-800 and (B) C-800-A

Conclusions

Carbon materials bearing rich oxygen-containing groups were synthesized by a facile molten salt method followed by an acid treatment. The catalyst exhibits the catalytic activity of simultaneously catalysing the two-step reaction of fructose dehydration and HMF oxidation [4], which is mainly attributed to the carboxyl groups on the surface of the catalyst. A DFF yield of 53.6% can be achieved by C-800-A in a one-pot and one-step reaction system with atmospheric oxygen as the only oxidant. Using the C-800-A catalyst for the synthesis of DFF from fructose can successfully avoid the difficulties in production separation and activity loss due to metal leaching. Therefore, this work provided a promising strategy for the development of metal-free catalysis in biomass conversion.

Acknowledgement

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DESIGN AND CONSTRUCTION OF NANOBIOCATALYSTS CONSISTING OF IMMOBILIZED LIPASE ON NANOSTRUCTURED CLAY SURFACES FOR CONVERSION OF ORGANIC WASTE TO BIODIESEL

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Lipase (EC 3.1.1.3) is one of the most important industrial enzymes that has been widely applied to produce biodiesel from organic waste, especially grease-containing waste by biocatalytic transesterification mechanism. Despite their excellent catalytic capabilities, the application of natural lipase is often hampered owing to their susceptibility to environmental factors such as high temperature, extreme pH, high salinity, and tolerance towards organic solvents. Enzyme immobilization is an efficient route for solve these problems, thereby permitting the catalysis under harsh reaction conditions at an industrial scale. The nanobiocatalyst is an emerging innovation that synergistically integrates advanced nanotechnology with biotechnology for improving enzyme activity, stability, capability and engineering performances. An important requirement for enzyme immobilization is that the support should provide a biocompatible, modifiable surface, chemical and mechanical stability and it should not interfere with the native structure of the protein, which could compromise its biological activity. Among a various support matrixs, nanostructured clay particles provide an excellent platform for enzyme immobilization, which can improve the efficiency of immobilized enzymes by reducing diffusional limitation as well as by increasing the surface area per mass unit.

In the present study, we aimed at taking advantage of the surface and interface characteristics of clays to develop superior nanobiocatalyst systems via covalently bound lipase on functionalized natural nanostructured clay mineral of montmorillonite(lipase-Mt) and synthetic aminoclay (lipase-AC). Aminopropyl silane (3-aminopropyltriethoxysilane, APTES) was covalently bonded to Mt surfaces by condensation reaction with the surface Si-OH groups. To prevent lipase leaching, the aminopropyl-functionalized Mt derivative or AC were stabilized by cross-linking them with 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC). Immobilization parameters such as enzyme loading amount, EDC concentration, pH, and immobilization time were investigated in detail. The enzymatic activities of the free and immobilized lipase were compared using hydrolysis of p-nitrophenyl palmitate (p-NPP). The lipolytic activity, kinetic characteristics, thermal stability, and storage stability of the Mt-immobilized lipase were also investigated. Experimental results indicated that the specific activity of lipase-Mt and lipase-AC was nearly 5 and 3.4 folds higher than that of free lipase, respectively, due to the hydrophobically interfacial activation of support toward lipase. The maximum reaction rate (V_{max}) for lipase-Mt, lipase-AC and free lipase were 312.5 mM/L·min, 217.3 mM/L·min and 63.7 mM/L·min, respectively. In addition, the lipase-Mt and lipase-AC with hydrophobic property showed higher thermal stability and reusability than free lipase. The present study has provided a promising way for screening, optimizing and rational design of efficient nanomineral-based enzymatic nanobiocatalyst. The present work was financial supported by the National Natural Science Foundation of China (41672039) and Sichuan Science and Technology Program (2019JDJQ0056).

Keywords: *Nanobiocatalyst, Clay minerals, Enzyme immobilization, Lipase, Biodiesel.*

BIODIESEL PRODUCTION FROM MARINE ALGAE NANNOCHLOROPSIS SALINA USING WASTE EGG SHELL AS A NANOCATALYST

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The needs for energy are increased worldwide; in contrast, the resources are getting depleted. Therefore, it leads to the search for bio-fuel as an alternative energy in order to suppress the fuel crisis. Biodiesel is typically made by chemically reacting lipids from a vegetable oil or animal fat with an alcohol producing fatty acid esters, such as methyl or ethyl ester. The present study aims to produce biodiesel from microalgae by the transesterification process using heterogenous catalyst. The initial part of this project was to separate biomass from the algal culture and extract the oil from biomass. Calcium Oxide (CaO) is a potential heterogeneous base catalyst with low cost, high conversion rate, availability and catalyst reusability. The characterization of catalyst was done by SEM analysis FT-IR and XRD analysis. Marine algae *Nannochloropsis salina* was used as feed stock. Algae fatty acid methyl esters (algae FAME) has been obtained from the following reaction optimized conditions; reaction temperature of 65˚C, reaction period of 180 minutes and ratio of oil to methanol at 1:5 with CaO as a catalyst at 4% (wt/v). The confirmation of biodiesel was done by the Ferric chloride test and Hydroxamic acid. The characterization of biodiesel was carried out using GC-MS study.

Keywords: *Nannochloropsis salina, Transesterification, Biodiesel, SEM, FTIR, GC-MS analysis.*

NANOBUBBLE TECHNOLOGY APPLICATION IN AQUAPONICS

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Nanobubbles are an emerging gas delivery technology with potential opportunities in the agricultural field. Due to their several unique properties, such as their high gas solubility, high electrostatic interaction, and their potential formation of reactive oxygen species, nanobubbles can enhance microbial-mediated processes governed by oxygen, especially in aquaculture and aquaponic systems. The perpetual generation of reactive oxygen species could also offer new avenues in pathogen, biofilm, and algal control. With rising population growth and soaring food demand, aquaponics has gained interest in recent years as a sustainable food production system. Aquaponics recycles nutrient-rich aquaculture effluents for vegetables/fruit production. However, in these systems, nitrification is a critical pathway and is often impacted by low dissolved oxygen in the aqueous phase. Low dissolved oxygen levels are also responsible for denitrification, low fish and plant yields, root rot diseases, and the accumulation of toxic forms of nitrogen (e.g., ammonia and nitrite). Due to the limitations of conventional aeration methods to increase the oxygen solubility threshold, applying an innovative nanobubble technology could bring dissolved oxygen levels beyond normal saturation, thus bringing concomitant improvements to fish and plant yields, and overall water quality. The preliminary study presented examines the overall effects of nanobubbles on the growth of butterhead lettuce (*Lactuca sativa* spp.) and tilapia (*Oreochromis aureus* spp.) in floating raft aquaponics, as well as provides insight on several water quality parameters.

Keywords: *Nanobubbles, Dissolved oxygen, Reactive oxygen species, Agriculture, Aquaculture, Aquaponics.*

INNOVATIVE FAST CATALYTIC MICROWAVE-ASSISTED THERMOCHEMICAL CONVERSION OF BIO-WASTES FOR ENERGY AND FUELS PRODUCTION

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Tremendous amount of bio-wastes are generated from various sources each year, causing serious environmental issues, while they could be a valuable source for renewable, abundant, and cheap energy production if managed properly. This transition calls for novel and more efficient conversion technologies to replace the conventional disposal methods such as landfilling and incineration. One of the promising technologies developed in the author's lab is the fast catalytic microwave-assisted thermochemical conversion technology. With the incorporation of microwave absorbents into the microwave heating process, the heating rate, temperature, and energy efficiency can be significantly improved. Another advantage is its versatility, allowing efficient treatment of various types of solid wastes. Recently, this technology has been implemented on several types of solid wastes, including sewage sludge and waste plastics, among others.

Sewage sludge is a major solid waste generated from municipal wastewater treatment process, a large fraction of which is organic materials. Sewage sludge was pyrolyzed in a lab-scale continuous catalytic fast microwave-assisted pyrolysis (fMAP) system, and the optimal pyrolysis temperature was determined to be 500 C, where 87% of energy was recovered from the sewage sludge. Specifically, most of the energy was stored in the liquid (39%) and gas product (47%), which had a notable higher heating value of 20.61 MJ/kg and 22.5 MJ/Nm3, respectively. The high energy content of gas product could be attributed to the absence of carrier gas in the process, which would otherwise be diluted. In addition, the fMAP process gave a highly porous texture to the produced char, making it a potential source of activated carbon for pollutant removal.

Waste plastics, a major component in municipal solid waste, were also investigated as a feedstock for catalytic fast microwave-assisted pyrolysis. Polyolefin-based waste plastics were first pyrolyzed under a temperature of 500 °C with and without catalyst. The liquid yield obtained was merely 22% (wt.), and 53 % remained as solid residue mainly composed of wax material without catalyst. In contrast, the addition of HY zeolite as catalyst boosted the liquid yield to 47% while reducing the solid yield to 0-5%, indicating a remarkable promotion effect of HY zeolite on plastics pyrolysis. In addition, GC-MS analysis of the liquid product showed all the compounds in the liquid were hydrocarbons, and specifically 73% fell into the gasoline range (C6-C12) with 59% being alkenes.

Furthermore, *in-situ* and *ex-situ* catalysis mode were compared during fMAP of a plastic-paper mixture representing another common packaging wastes. While obtaining a liquid yield lower than that with *in-situ* catalysis (33% vs. 44%), *ex-situ* catalysis with HY zeolite generated a liquid product with more alkenes and light components (C6-C7) and less aromatics and heavy compounds, contributing to the lower viscosity of its liquid product. In addition, *ex-situ* catalysis produced more gases (59% vs. 43%) with higher content of high HHV components

such as H2 and C1-C3 hydrocarbons, illustrating an enhanced cracking effect with *ex-situ* catalysis mode. In addition, the *ex-situ* porous SiC foam based structure catalyst seems to have unique advantage in minimizing pressure drop, improving catalytic activity, and prolonging catalyst life.

Keywords: *Microwave, Thermochemical conversion, Solid waste, Foam structure Catalyst, Energy and fuels.*

FUNCTIONAL AND DYNAMICS OF GENOMIC IN ECO-ELECTROGENIC ENGINEERED SYSTEM DURING AZO DYE WASTEWATER TREATMENT

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Synthetic dyes are major classes of compounds used in textile industry. The effluents released thereof are carcinogenic and mutagenic in nature and pose potential threat to all life forms. Novel and hybrid Eco-electrogenic engineered wetland system (EEWS) was designed with three specific conditions integrated in a sequence to treat azo dye-based textile wastewater. Tank-1 containing the free floating and emergent aquatic macrophytes create anaerobic/anoxic microenvironments and facilitates the reduction of the organic compounds and break down the dye molecules. Tank-2 consists of submerged floating aquatic macrophytes create the aerobic microenvironments which direct the oxidation of intermediate carbon compounds and nitro compounds to nitrification. Tank-3 with free floating macro and micro plants and filter feeders is intended to remove TSS, nitrates, phosphates and pathogens. All the three tanks were integrated with electrode assembly to enhances the dye decolorization as well as reduce retention time of the treatment process. The in-situ potentials help simultaneously to enriches the electroactive bacteria as well as growth of the plants and dye decolorization. Hiseq illumina analysis shows the diverse microbial dynamics with high genus abundance were *Blastochloris, Desulfomonile, Rhodovulum, Oscillochloris*in tank 1 and *Pirellula, Caldilinea, Steroidobacter, Thaumarchaeota* in Tank 2. EEWS have the advantage of no chemical and energy inputs which leads to the reducing the carbon foot prints in dye wastewater treatment.

Keywords: *Hiseq illumina, Aquatic plants, Phytoremediation, Constructed wetland, Microenvironments.*

AN INTEGRATED BIOTECHNOLOGY FOR GOLD RECYCLING FROM E-WASTE USING THIOUREA WITH BACILLUS AND LYSINIBACILLUS SP. (HYBRID) COMBINATION

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E-waste is the toxic legacy of this digital age with the fastest growth rate than any other type of waste (twice the rate of plastic waste). Nearly 43 million tons of e-waste was generated in 2016 and was 8% more than 2014. Due to the good conductivity and less atmospheric oxidation precious metals (PMs) like Au, Ag, Pd and Pt are in good demand in electronics, mostly for making joints/contracts of circuit boards. Gold recovery from waste can be very attractive because natural gold ores are difficult to mine due to its refractory nature and mining can produce toxic compounds such as cyanide. Some gold mining areas fall under geologically unstable regions.

Hybrid bioleaching method, comparatively a new technique entails the complimentary combination of both- chemical and biological leaching for efficient metal selective extraction from e-waste. It promotes using safer chemicals and metal-specific ligands with compatible microbes for better extraction of selective metals.

For bacterial isolation, soil samples were collected from an e-waste dumping and recycling site and study its tolerance and gold leaching performance in the presence of thiourea. Practically, microbial solution was charged with gold chloride (0.02g) and50 mM thiourea. Potentially compatible microbes were selected from the enrichment culture based on their survival using 16r RNA. Bacillus substilis and Lysinibacillus sp. Combination was found to be support in leaching. 90 days leaching pattern was investigated and it was found that hybrid combination increase nearly 48% leaching compared to individual thiourea.

Keywords: *E-waste, Gold, Hybrid, Bacillus, Lysinibacillus, Thiourea.*

STUDIES ON BIOLEACHING AND RECOVERY OF METALS FROM PRINTED CIRCUIT BOARDS USING *ACIDITHIOBACILLUS FERROOXIDANT AND ACIDIPHILIUM ACIDOPHILUM BACTERIA*

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ABSTRACT. In recent years, electronic waste (e-waste) is considered as one of the fastest growing waste streams and causes various environmental impacts and health issues when it is disposed improperly. Therefore, recovery of metals through bioleaching from printed circuit boards (PCBs), a conglomerate part of e-waste using Acidithiobacillus ferrooxidant (A.ferro) and Acidiphilium acidophilum (A.acido) in the study. Parameters like pH, ORP, cell count, PCBs dosages (5-25g), DO content, energy source, etc., were studied to understand their influence on the bioleaching process and recovery of metal (Cu). The metal leaching efficiency of both A.ferro and A.acido for Cu was 91.3% and 91.2% respectively. It can be stated with confidence that the methodology and selected bacteria chosen for the study are not only suitable for the Indian conditions but also, in countries, similar type of 'mesophilic conditions' exist. The method is low cost and environmentally friendly and hence better than its informal counterparts such as: pyrometallurgy and hydrometallurgy. Further, bioleaching has a potential for commercialization and industrial applications.

Keywords: *E-waste, PCBs, Bioleaching, Acidophilic bacteria, Alkaliphile bacteria*.

Introduction

Electronic waste (e-waste) is a popular and informal name for electronic products which are nearing their end of useful life. Further, e-waste is one of the fastest growing waste streams where the volume of the e-waste increases day-by-day exponentially [1]. The printed circuit boards (PCBs), a conglomerate part of e-waste, are the major hazardous waste material which has quite numbers of heavy metals, plastics and resins, which causes various environmental impacts and health issues when it is disposed improperly [2,3]. PCBs are the potential single secondary sources for the recovery of metals. The conventional recovery methods such as pyrometallurgy and hydrometallurgy show many disadvantages than the biohydrometallurgy [4]. Bioleaching in spite of having several advantages, it is found to be at a nascent stage in both national and international level [5].

The present aim of the study is to analyze the bioleaching efficiency of both the selected bacteria under as-is-where-is-basis condition with various cases. In this present paper, only the best results of findings have given for the selected both bacteria.

Material and Methods

Printed Circuit Boards (PCBs)

30 kg of PCBs of various electronic wastes (e-waste) were purchased from a local scrap dealer, available nearer to the study area (Puducherry, India). PBCs were kept in a dry place at 30ºC (room temperature), till they are actually used.

Microorganisms

Acidithiobacillus ferrooxidans was selected for the study, which have been used earlier in the bioleaching process for e-waste. Further, first of its kind, bacteria such as: *Acidiphilium acidophilum*, was also selected for the bioleaching study. All the above bacteria were purchased from well-known culturing / marketing centers.

Acidithiobacillus ferrooxidans (NCIM 5370) and *Acidiphilium acidophilum* (NCIM 5344) were purchased from National Collection of Industrial Microorganisms (NCIM) which is a unit of National Chemical

Laboratory (NCL), Pune, Maharashtra state, India. The above said bacteria were cultured and stored in a deep freezer with their medium and subsequently, maintained by sub-culturing in their respective fresh medium, for every two weeks.

Preparation of PCBs

All the chemicals and reagents used in this study were of analytical grade, unless otherwise stated. All the glassware were cleaned with sulphuric acid solution and was sterilized with an autoclave. At first, PCBs was washed with tap water to remove the dust and other unwanted materials that may be sticking to the surface. Then, the capacitors, wires, bulbs, plastics components which were present in the PCBs were shredded.

Bacterial Culture

Acidithiobacillus ferrooxidans, were cultured in 9K medium as per the standard microbiological culture protocol. *Acidiphilum acidophilum,* were cultured in the following medium which consisted of $(NH_4)_2 SO_4$ – 3.0 g, KH_2PO_4 – 0.5 g, $MgSO_4 . 7H_2O$ – 1.0 g, KCl – 0.1 g, $Ca(NO_3)_2 . 4H_2O$ – 0.01 g, D-Glucose – 10.0 g, distilled water – 1000.0 mL. The pH of the liquid medium was adjusted to 3.5 by the addition of H_2SO_4. The glucose was sterilized and added to the medium separately.

Acclimatization Studies

Two ranges of PCB dosages, such as 'Range-I' and 'Range-II', were considered. In the case of 'Range-I', the PCB dosages considered are: 10g, 30g, 60g, 90g and 120g, whereas, for 'Range-II' the PCB dosages considered are: 10g, 20g, 30g, 40g and 50g. Thus, 'Range-I' is expected to simulate 'shock loading effect' on the cultured medium. In Range-II, the increment in dosage is uniform from the initial to the final stage.

For each bacterium, 150 mL of the cultured medium was taken in a 250 mL of Erlenmeyer flask. After adding each dosage of PCB into the cultured solution, the above flasks were inoculated with the bacterial dosage (BD%) of 10% (V/V%). The above inoculated flask is then incubated at room temperature in a bacteriological incubator. pH, ORP and cell count of the 'inoculated medium' were measured at periodic intervals of 10 days, till 90 days. It was found that there is no bacterial growth beyond 90 days, and hence all the above parameters were monitored only up to 90 days.

Bioleaching Study

Bioleaching study was conducted for both the bacteria in 250 mL Erlenmeyer flasks. The various types of cases and the conditions adopted for the above study were analyzed. The above study is primarily intendent to study the various factors, cases and conditions which influence the bacterial growth and metal leaching efficiency. Comprehensive parameters such as: pH, temperature, oxidation reduction potential (ORP), PCBs dosage, solid/liquid ratios (S/L ratio), bacterial dosage, energy sources dosage, and dissolved oxygen supply, leaching duration, and cell count were considered for the bioleaching study. Further, 10 ml of each sample was drawn periodically for the analysis of various parameters and concentration of metals. All the experiments were repeated twice to check for consistent values.

Analytical Methods and Equipment

The analytical parameters were measured with standard operating protocol to analyze the corresponding parameters with the help of pH meter, humidity meter, dissolved oxygen (DO) meter, digital bacterial counter, vertical safety chamber, bacteriological incubator, inductive coupled plasma optical emission spectroscopy (ICP-OES), UV-Visible spectrophotometer, field emission scanning electron microscopy (FESEM) and fourier-transform infrared spectroscopy (FT-IR).

Results and Discussion

Metal Leaching Efficiency of 'A.ferro'

As the maximum efficiency was obtained for the highest liquid content (250 mL), variation of each metal leached during the bioleaching for various cases (C1, C2, C3 & C4). For PCB dosage of 5g, it is shown in the fig.1 that the metal leaching efficiency for Cu metal is the lowest for C1 and it is the highest for C4. Moreover, the metal leaching efficiency increases from C1 to C4 (continuously) and that the increase is substantial for C3 and C4 (Group 2), when compared to C1 and C2 (Group 1). Based on the highest metal leaching efficiency (%) (i.e. based on C4), the highest metal leaching efficiency is attained for Cu, which is 91.3%, and it is very high. The maximum efficiency exhibited by C4 can be attributed to the maximum cell count / growth attained for C4 and at S/L ratio 5/250. Comparing the metal leaching efficiency (%) obtained in the present study aligning with the results reported by earlier investigators [4 and 6].

Metal Leaching Efficiency of A.acido

For the PCB dosage of 5g, the best results were obtained for C4, and for S/L ratio 5/250, for all the metals considered. Under the above condition, the metal leaching efficiency (%) is obtained for Cu was 91.2% as shown in fig.2. Thus, the ranking of metals based on leaching efficiency of A.acido are the same as that of A.ferro. Comparing the metal leaching efficiency of various metals of C4 and liquid content at 250 mL, and PCB dosage at 5g, it is seen that almost Cu exhibits 'sensitivity'. It should be noted that there is no comparable earlier studies available using the above bacteria. Thus, the results in this study form a first of its kind for the above bacteria.

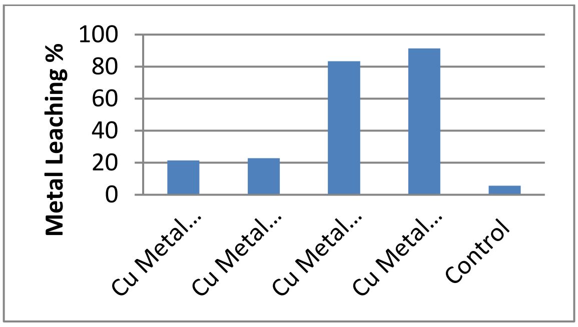

Fig. 1. Copper metal leaching % of 5g of PCBs for various cases and S/L ratios of (5/250) by A.ferro.

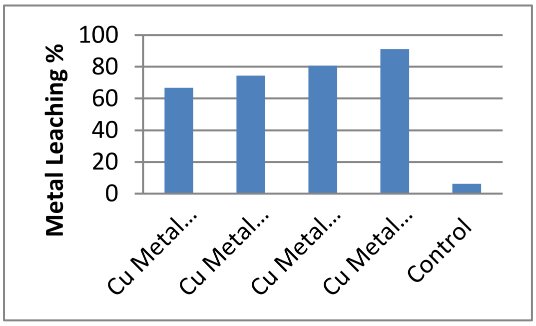

Fig. 2. Copper metal leaching % of 5g of PCBs for various cases and S/L ratios of (5/250) by A.acido.

FTIR Spectroscopic Analysis of Sulphur Oxidizing Bacteria

The selected bacteria in this study are sulphur oxidizing bacteria'. FT-IR analyses of A.ferro, and A.acido are shown in Fig.3 and Fig.4 respectively. The above figures show the presence of bacterially-produced inorganic acid. The transmittance bands observed in the range of 883.58 to 884.94, 1006.94 to 1008.32, 1174.98 to 1177.51 and 1634.87 to 1636.34 cm-1 indicate the presence of HSO^{4-} asymmetric stretch, HSO^{4-} symmetric stretch, SO_4^{2-} stretch and H_2O bending and H_3O^+ stretch, respectively, as reported by [7].

SEM Analysis of PCB before and after bioleaching by bacteria

It is observed from SEM images (Fig.5 and Fig.6) that the surface of PCB before bioleaching exhibits a smooth surface, devoid of cavities and presence of metals, whereas, after bioleaching SEM picture shows rough surfaces, and presence of cavity on the surface of the PCB on the metals, which exhibits the effect of bioleaching.

A very important inference that can be drawn from the above is that the bacteria affect only the metals by bioleaching, but not the PCBs, that is plastic and ceramic parts of PCBs, which is quite different from 'chemical oxidation', which affects the entire PCB, thus causing disposal and environmental problem.

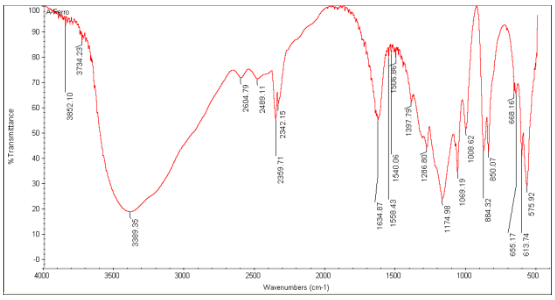

Fig. 3. FT-IR Result for A.ferro.

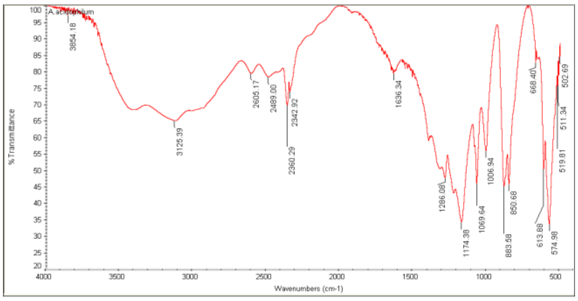

Fig. 4. FT-IR Result for A. acido.

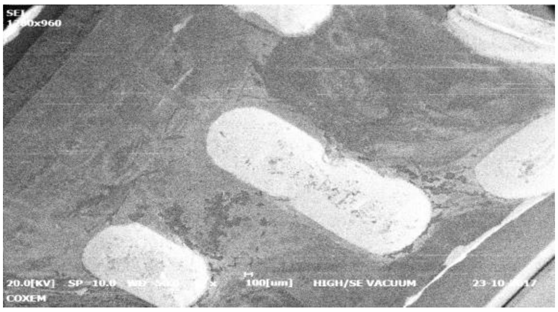

Fig. 5. SEM image of PCB before bioleaching.

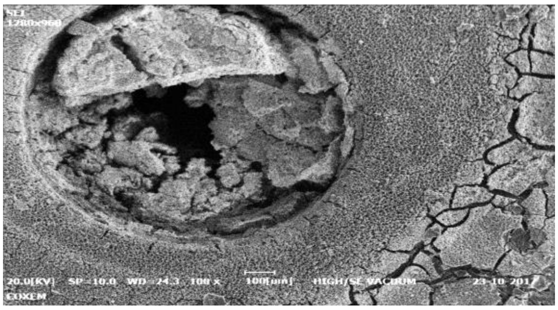

Fig. 6. SEM image of PCB After bioleaching.

Conclusions

The metal leaching efficiency is the lowest for C1 and highest for C4, for the highest liquid content (250 mL). The above phenomenon is found to be best for the PCB dosage 5g, and independent of bacteria. Metal leaching efficiency is the highest in C4 and S/L ratio 5/250, for the metal Cu. However, the actual values will be different for each type of bateria. It can be seen that Cu has attained consistently the high leaching efficiency in both the bacteria.

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BIOSYNTHESIS, ISOLATION AND QUANTIFICATION OF PHYCOBILIPROTEINS BY *DESERTIFILUM* SP. SVMIICT2: EFFECT OF DIFFERENTIAL LIGHT INTENSITIES

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ABSTRACT. Phycobiliproteins are an important group of pigments in cyanobacteria. These pigmented proteins harvests light in cyanobacterial cells and exhibits great potential as biomarkers. In the present study, isolated filamentous cyanobacteria *Desertifilum sp.*SVMIICT2 was studied to evaluate the influence of different light intensities to favor of phycobiliproteins synthesis cultivated in Bolds Basal Media (BBM) for ten days. Phycobiliproteins such C-Phycocyanin (C-PC) and C-Phycoerthryin (C-PE) requires 630 nm and 570 nm respectively. Photosynthetic activity of cyanobacteria at 50 µE/ m^2/sec was measured in terms of Fv/Fm. It measures the photochemical process performance in the Photo system II (PSII). Initial Fv/Fm value of C-PC and C-PE was 0.45 fru (fluorescence relative units). Interestingly during the days progression Fv/Fm decreased in C-PC and increased C-PE. Decreased Fv/Fm favored higher C-PC; purified C-PC is evaluated according to the absorbance ratios at 620 nm and 280 nm. The study achieved C-PC ~ 4 A620/A280 ratios as it is suitable for an analytical grade. Maximum fluorescence (Fm), in C-PC Fm reached on 4th day and in C-PE reached on 8th day. C-Phycocyanin and C-Phycoerythrin have anti-oxidative function, anti-inflammatory activity, anti-cancer function, immune enhancement function, kidney and liver protection pharmacological effects.

Keywords: *C-Phycocyanin, C-Phycoerythrin,* F_V/F_M*, PS-II, Anticancer agents.*

Introduction

Cyanobacteria are photosynthetic prokaryotic oxygen evolving primitive organisms. Photosynthetic process requires chlorophyll a (chl a) and phycobiliproteins which functions as major photosynthetic pigments. These photosynthetic organisms formed in all habitats, naturally and manmade, a basic understanding of the properties, the environmental conditions and the behavior in natural ecosystems, and which support the growth of other organisms. *Desertifilum* sp SVMIICT2 is a prokaryote, filamentous non-nitrogen fixing cyanobacteria, isolated and deposited in the culture collection centre (National collection of industrial microorganisms-NCIM). It is an oxygenic cyanobacteria, light energy converted into chemical energy by utilizing photosynthetic machinery. Along with chlorophyll a primary pigment for photosynthesis cyanobacteria possesses phycobiliproteins (PBPs) such as allophycocyanin (C-APC) phycocyanin (C-PC) and Phycoerythrin (C-PE). These are tetrapyrrole derivatives, able to modulate their content due to light color, intensity, availability of nutrients and environmental factors. In the present study, opted low light intensities 30,50, 70 and 90 µE $m^{-2}s^{-1}$ WW (Orange color:630nm) light for C-Phycocyanin and allphycocyanin (C-PC &C-APC) enrichment and 30,50, 70 and 90 µE m^2/sec BG (Blue green : 570nm) light for C-Phycoerythrin (C-PE).

Materials and methods

Source of the cyanobacteria and nutrient media

Filamentous cyanobacteria isolated from the fresh water source, Nagarjuna sagar project canal. The strain was isolated as described [1], and used cycloheximide as antifungal agent. Morphological and molecular identification of cyanobacteria i.e *Desertifilum* sp SVMIICT2 was confirmed and deposited in CSIR-NCIM. For the Cultivation of cyanobacteria applied 3N BBM [2]. The cultivation of photosynthetic organism completed in eight days of time period.

Multi cultivator

Light experiment was carried out by utilizing Photon System Instruments (PSI, Czech Republic) Multi-

Cultivator MC 1000-OD. Light intensities 30,50, 70 and 90 µE $m^{-2}s^{-1}$ WW (630nm) light for C-Phycocyanin and allphycocyanin (C-PC &C-APC) enrichment and 30,50, 70 and 90 µE $m^{-2}s^{-1}$ BG (570nm) light for C-Phycoerythrin (C-PE) enrichment were chosen for the present study.

Pulse amplitude modulator (PAM)

Pulse amplitude modulator (PAM) instrument is an AquaPen-C AP-C 100 fluorimeter (Photon Systems Instruments, Czech Republic) used to study the chlorophyll fluorescence parameters. Initially, *Desertifilum* sp. SVMIICT1 grown in specified days samples were collected for chlorophyll fluorescence measurements. Fluorimeter AquaPen-C AP-C 100 equipped with blue and red measuring lights, saturating light intensity ranges (0 to 3,000 µmol photons/m^2/s) and actinic light ranges (0 to 1,000 µmol photons/m^2/s) to measure the fluorescence and photosynthetic activity.

Chlorophyll a, b and carotenoids

Total chlorophyll and carotenoids of cyanobacteria were quantified according to the protocol described by [3]. Pigment extraction used 80% Acetone and calculated the concentrations of chlorophyll a, chlorophyll b, and Carotenoids. Chlorophyll a (Ca) at 663.2 nm, chlorophyll b (Cb) 646.8 nm, and Carotenoids (Cx+c) 470nm absorptions were noted at a bandwidth of 0.5nm by using a UV Visible Spectrophotometer. Chlorophyll a, chlorophyll b, and carotenoids were measured according to the following formula.

Carbohydrates and Proteins

Total carbohydrates measured by using phenol sulphuric acid method [4]. Glucose used as a standard and plotted linearity graph. Calculated total carbohydrates present in the *Desertifilum* sp. SVMIICT2 using the standard graph. Absorbance measured at 490nm using UV-Visible spectrophotometer (Spectra Max M series plate Reader). Its cellular protein quantified using bicinchoninic acid (BCA) protein assay kit (Takara-T9300A). Total protein was measured using a standard graph. Water soluble complex absorbance observed at 562 nm with UV Visible spectrophotometer (Spectra Max M series plate Reader).

Lipids

Cyanobacteria cells harvested at the end of the growth phase (8th day) processed for lipid extraction and fatty acid methyl esters (FAME) conversion using opted protocol [5]. FAMEs were estimated by using Gas chromatography-Mass spectrometry (GC-MS) (Agilent 7890B-5977A mass selective detector) by using capillary column (DB-225), and optimized temperature programme. GC-MS operated in selected ion monitoring (SIM) mode, which allows the MS to identify specific compound masses. Helium was the carrier gas used in constant flow mode at a flow rate of 1 mL/min.

Matrix-assisted laser desorption ionization (MALDI)

Cyanobacterial phycobiliproteins were evaluated as specified by using a MALDI-reTOF/TOF UltrafleXtreme (Bruker Daltonics, Bremen, Germany) fixed with pulsed ion extraction (PIE), LIFT devices and reflector lens (re) [6]. Sinapinic acid (SA) is used as matrix, matrix and phycobiliproteins homogenized samples used for analysis. Loaded 1µl of homogenized sample and matrix on 384 well MALDI plate allowed drying at room temperature. After drying observed phycobiliproteins

Results and Discussion

OJIP transient

OJIP transient in cyanobacteri using saturating pulse light, all the antenna pigment complexes coupled with photosystem are assumed to be open (dark adapted) is known to be minimum fluorescence (F_0). Absorption flux (number of photons) per cross section measured by the fluorescence parameters F_0 and F_M, this is the function of chlorophyll content and size of the antenna of the PSII [7]. OJIP transient curve from Origin (O) or F_0 to Peak (P) or F_M is associated with the yield of PSII photochemical reactions and electron transport reactions [8 & 9].

In our study on the 4th and 8th day showed maximum fluorescence (F_m) compared to the other days fluorescence of cyanobacteria grown in light conditions C-PC (50µE). Whereas 8th day showed maximum fluorescence (F_m) compared to the other days fluorescence of cyanobacteria grown in light conditions C-PE (50µE). Comparative analysis of these two light conditions, C-PC is performing low photosynthesis than C-PE. Additionally, *Desertifilum* sp. SVMIICT2 shows lower photosynthetic performance than microalgae.

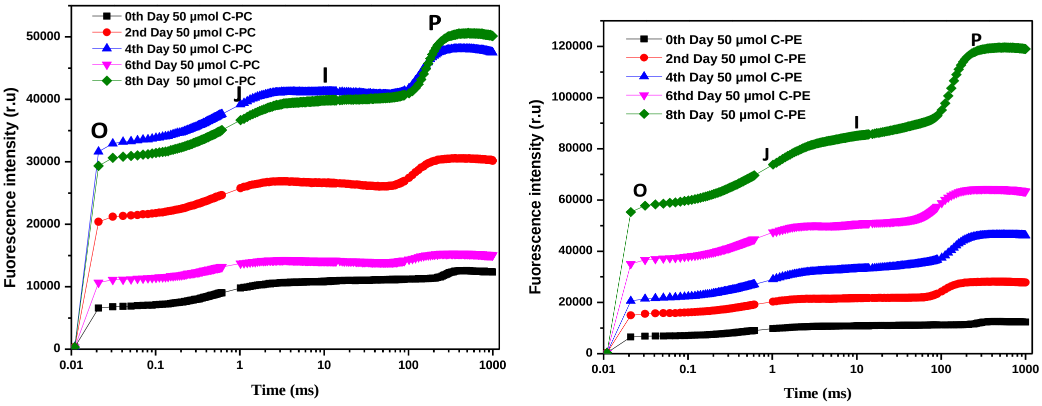

Fig. 1. OJIP graphs of *Desertifilum* sp. SVMIICT2 C-PC and C-PE conditions grown at 50µE/m^2/sec of light intensity.

Fv/Fm

The maximum photochemical yield of PSII is designated as the Fv/Fm ratio. Both the light conditions Fv/Fm values were evaluated using PAM. During days progression in all the light intensities 30 to 90µE/m^2/sec of condition C-PC showed reduction in the Fv/Fm values. It indicates that the PSII activity was decreased in the orange light as well as it favored increment in the water soluble phycobiliproteins. Furthermore, during the cultivation days progression in all the light intensities 30 to 90 50µE/m^2/sec of condition C-PE shown increment in the Fv/Fm values. Here, these conditions shown low phycobiliproteins compared to the C-PC.

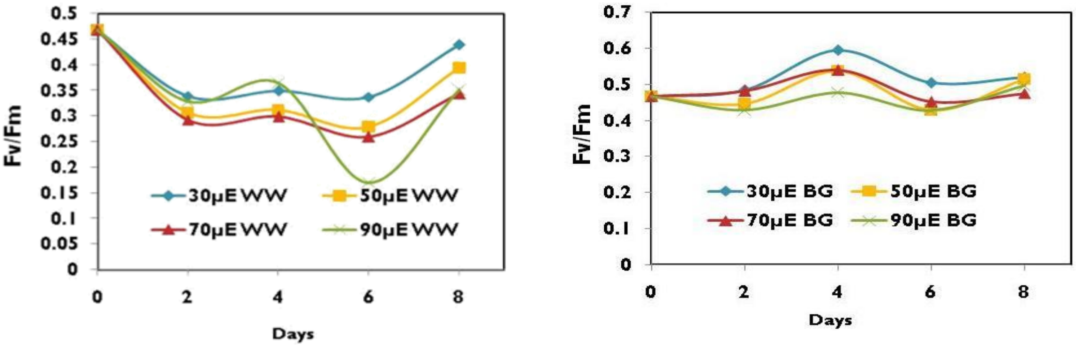

Fig. 2. Changes in maximum quantum yield of photochemistry (Fv/Fm) values of 0-8days cultivation of *Desertifilum* sp. SVMIICT2 with orange (WW) and blue green (BG) varied light intensities.

Lipid profile of cyanobacteria

Total lipid content of *Desertifilum* sp. SVMIICT2 was analyzed with Gas chromatograph – Mass spectrometry (GC-MS) by using optimized Cequier-Sánchez et al., 2008 protocol. Here, we found saturated fatty acids, stearic acid C18:0. Monounsaturated fatty acid oleic acid (C18:1), poly unsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) fatty acids found in the end of the growth phase of cyanobacteria.

MALDI-TOF analysis of phycobiliproteins

MALDI techniques are known as a soft ionization technique here the matrix absorbs laser energy, thereby matrix protects sensitive analyte molecules from thermal decomposition [10]. MALDI-TOF-MS spectrum of 50µE/m^2/sec C-PC conditions showed major peak *m/z* 18kDa, it would be a β –allophycocyanin.

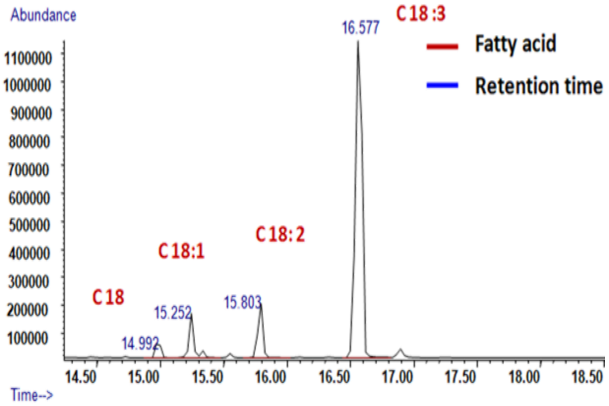

Fig. 3. GC-MS chromatogram of 50µE/m^2/sec C-PC condition of *Desertifilum* sp. SVMIICT2.

Conclusion

The present experimental study revealed, in C-PC condition Fm, reached on 4th and 8th day and in C-PE reached on 8th day. C-PC condition 50µE/m^2/sec was shown higher phycbiliproteins than the C-PE. Because of their low basic maintenance energy and their pigment structure, cyanobacteria favor low light intensity and promote PBP synthesis. As natural colorants, PBPs are becoming more common than synthetic colors because they are environmentally safe, non-carcinogenic and non-toxic. The amounts of tumor necrosis factor in the blood have also been reported with phycocyanin as a protein. Phycocyanin has been linked to the pharmacological properties of PBPs, such as antioxidant, non-inflammatory, and neuroprotective and hepatoprotective function.

Acknowledgement

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DUCKWEED BIOREFINERY WITH DAIRY WASTEWATER TREATMENT

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Duckweed, belongs to Lemnaceae, a potential aquatic plant which is being used as a next generation energy source. Duckweed for the treatment of dairy wastewater (DWW) was studied in an integration with selective fermentation. At the end of DWW treatment the substrate removal was observed to be 74% with simultaneous removal of nitrates (66%) and phosphates (80%) by the end of the cycle (8th day). The increase in duckweed fronds have been noticed with time which increased from 40 g to 50.9 g (wet weight) and from 1.88 g to 3.73 g in terms of dry weight with a frond number (T_i) doubling time of 20% for 8days. The carbohydrates and proteins after treatment of DWW increased to 32% and 10% respectively. The part of duckweed was made into slurry for selective fermentation in which the biomolecules of the duckweed will be fermented on selective basis. The maximum of VFAs of 1120 mg/L, biogas of 275 NmL composition of H_2, CO_2 and CH_4 was obtained. Acid hydrolysed biomass resulted in the reducing sugars of 700 mg/g of biomass which can be used as a substrate for acidogenesis for the production of VFA and biohydrogen.

Keywords: *Selective fermentation, Acidogenesis, Biogas, Bioalcohols.*

International Conference on Sustainable Biowaste Management 2021

Sustainable Bioconversion of Waste to Resource

BIOCONVERSION OF WASTES-TO-RESOURCES: OPPORTUNITIES AND CHALLENGES

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The global population is expected to reach nearly 9.7 billion by 2050 from current population of 7.7 billion. The rising population will significantly increase the demand for resources such as food, feed, chemicals, and fuels. with concomitant generation of large quantity of residues and wastes (water). As per the recent World Bank report, nearly 2,017 million metric tons of waste was generated in 2018 worldwide and is expected to increase to 2,586 million metric tons and 3,401 million metric tons in 2030 and 2050, respectively. Nearly 40-50% of these wastes compose of organic matter. With stringent regulation on disposal of organic wastes in landfill coupled with several environmental concerns such as greenhouse gases emissions, surface water and groundwater contamination, odor emanation, transmission of vectors via birds and insects, there have been significant efforts to eliminate or reduce the disposal of organic wastes into landfill. At the same time large amounts of liquid and gaseous wastes are also generated as a result of our increasing demand for resources. Thus, there is a critical need to valorize these waste streams into plethora of useful resources including food and fuels. This presentation will provide highlights of presenter's on-going research on how anaerobic digestion, fungal biotechnology, biochar, insect farming and aquaponics/bioponics among others can be applied for recovery of resources from diverse waste (water) streams. The presenter will also discuss some of the challenges of waste valorization.

Keywords: *Bioconversion, Wastes-to-resource, Opportunities, challenges.*

INTEGRATED SOIL AND ORGANIC WASTE MANAGEMENT AS A RESOURCE RECOVERY STRATEGY FOR RESILIENT AGRICULTURE IN GUAM

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One of the most threatening factors to soil quality especially in the tropical islands of Micronesia is the low organic matter content. Soil organic matter (SOM) is among the most degradation factor and the cause of poor soil quality in Guam and the other islands of Micronesia. Soil organic matter content are enhanced by effective management activities such as; manipulation of soil environment via conservation tillage and crop rotation practices, and more effectively by land application of composted organic wastes. In our case studies, preliminary results have shown that the application of composted organic wastes increased crop yield and significantly improved the quality of the soils in plots under study. The yield increase was 45%, 38%, and 33% for 30, 60, and 90 tons/ac of application rate as compared to the control (0 tons/ac). Also, the organic matter content of the soils under study increased from 3.4% to 6.3%. In our study, compost was produced from biosolid wastes that otherwise would be discarded in the island's landfills. Compost was produced via mechanical compost turner and applied on soils of agricultural fields as an alternative to commercial fertilizers to provide nutrients and also to enhance the organic carbon content and improve the overall quality of these poor soils of northern Guam. Mature compost was applied on the study plots at the rates of; 0, 30, 60 and 90 tons per acre as soil amendment on the poorly behaved 'Guam soils series' of northern Guam. Corn was planted and monitored for growth performance and yield evaluation. In this presentation, the methodology as well as up-to-date data will be presented to illustrate the effect of land application of composted organic biosolid waste on organic matter content and other soil quality indices as well as crop performance.

Keywords: *Soil organic matter, Biowaste management, Resilient agriculture, Compost and Composting, Guam.*

POTENTIAL FOR INTEGRATED FOOD WASTE TREATMENT WITH WASTEWATER AND SEWAGE SLUDGE IN HONG KONG

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ABSTRACT. Hong Kong is facing an imminent problem of managing its food waste (FW). Relying on landfills, implies the loss of natural resources and contribution to global warming. Diverting FW from the landfills towards wastewater treatment plants (WWTP) could relieve these burdens. This study quantitatively assessed the FW impacts on the operation conditions, greenhouse gas emissions (GHE) and energy balance of an actual WWTP in Hong Kong. Two scenarios i.e., FW co-disposal and treatment with wastewater and anaerobic co-digestion with sewage sludge were investigated by using steady-state modelling and lifecycle assessment (LCA) approaches. Results show that FW addition did not impact adversely on the WWTP's performance. Compared to normal WWTP, the net GHE was improved by ~36% and the energy recovery by ~30-75%. Overall, both scenarios depicted positive prospects compared to landfilling of FW and thus are viable alternative for sustainable FW management.

Keywords: *food waste; co-digestion; food waste disposer; wastewater treatment*

Introduction

Global solid waste generation rate is around 2.0 billion tonnes per year and around 30-60% of it is covered by food waste (FW) [1]. Waste management sector also contributes around 3% in global greenhouse gas emissions (GHEs), attributed mainly to organic waste (~FW) degradation and fossil fuel-based energy consumption. In Hong Kong, approximately 3600 tonnes of FW are generated each day, solely relying on the landfill as the final disposal means [2]. Beside the loss of this valuable natural resource, FW management also consume a large amount of energy, contributes to global warming by direct and indirect GHEs, as well as cause ground water pollution by leachate generation [3]. Therefore, like other developed cities, Hong Kong is also seeking a sustainable solution for FW management, for enhanced resource recovery and least environmental impacts.

Integrated waste management system (IWMS) is regarded as the top solution, where after collection and transportation, instead of simply disposing into the landfills, suitable treatment methods are used to improve the recycling and recovery of resources from the waste. Integration of FW treatment with biological wastewater treatment plants (WWTP) can be a potential option, considering the surplus capacity of activated sludge (AS) and anaerobic digestion (AD) reactors at WWTPs. The diversion of FW towards WWTP can be performed by two ways i.e., by using kitchen food waste disposers (FWD) and co-treating with the wastewater (WW) and co-digestion with sewage sludge (SS) after separate collection and transportation. [4, 5]. Depending on the rate of utilization in the city, the FWD could solve the basic issue of source separation and transportation of FW especially from the domestic sources. FW addition to WWTP could also be positive for biological nitrogen removal process by provision of extra carbon (organic) source [6]. While the co-digestion of FW with SS could also enhance the methane recovery and subsequent energy recovery potential of the WWTPs and can diminish the GHEs from landfilling of diverted FW [7]. However, considering the local conditions, the impacts of FW on the performance of WWTP, final sludge production and treatment, and overall GHE and energy balance from a lifecycle perspective must be evaluated. Therefore, to develop a practical policy and quantify the impacts of these potential integrated scenarios, a rigorous analysis is needed specific to Hong Kong.

In this context, this study performs a case specific lifecycle-based investigation for the evaluation of impacts of FW integration with WWTP. The specific objectives of this study include the development of a case specific plant-wide steady-state model (PWSSM), characterization of wastewater (with/without FW) for the steady-state models, quantitative evaluation of FW impacts on WWTP's performance and capacity, and energy recovery potential of FW co-digestion with SS, as well as evaluation of net GHEs and energy balance of the developed scenarios for combine FW and WW treatment from life-cycle perspective.

Material and Methods

Description of scenarios and research

The main objective of the study is to analyse the practicability of the integration of FW treatment with biological WWTP. Two scenarios considered are co-disposal and treatment with wastewater (CoDT-WW) by using kitchen FWDs and anaerobic co-digestion with sewage sludge (AnCoD-SS) by separate collection and transportation, as shown in Figure 1. Taking the Sha Tin WWTP in Hong Kong as a case-study, the investigation was performed by using mathematical modelling and lifecycle assessment (LCA) procedures. The PWSSM specifically developed for this case-study, uses the kinetic steady-state models of [8] for biological nitrogen removal (BNR) by activated sludge (AS) process, a COD based kinetic model for anaerobic digestion (AD) by [9] and the overall bioprocess stoichiometry of [10] for both processes. The investigation comprises assessment of FW impact on the performance of WWTP, physical capacity, energy demand and sludge output. Then the net energy balance and carbon footprint of the developed scenarios were assessed from lifecycle perspective with discerned contribution by subprocess. The functional unit (FU) opted for this study was treatment of 1 tonne of FW and 1000 m^3 of WW. Local data was used as far as possible, especially for FW and WW characteristics was taken from the previous studies performed on the same WWTP [4, 11, 12]. The boundary settings of the study includes the direct and indirect forms of positive and negative GHEs and direct energy input and recovery, while the emissions from the degradation of organic carbon in FW and WW are considered neutral for being of biogenic origin.

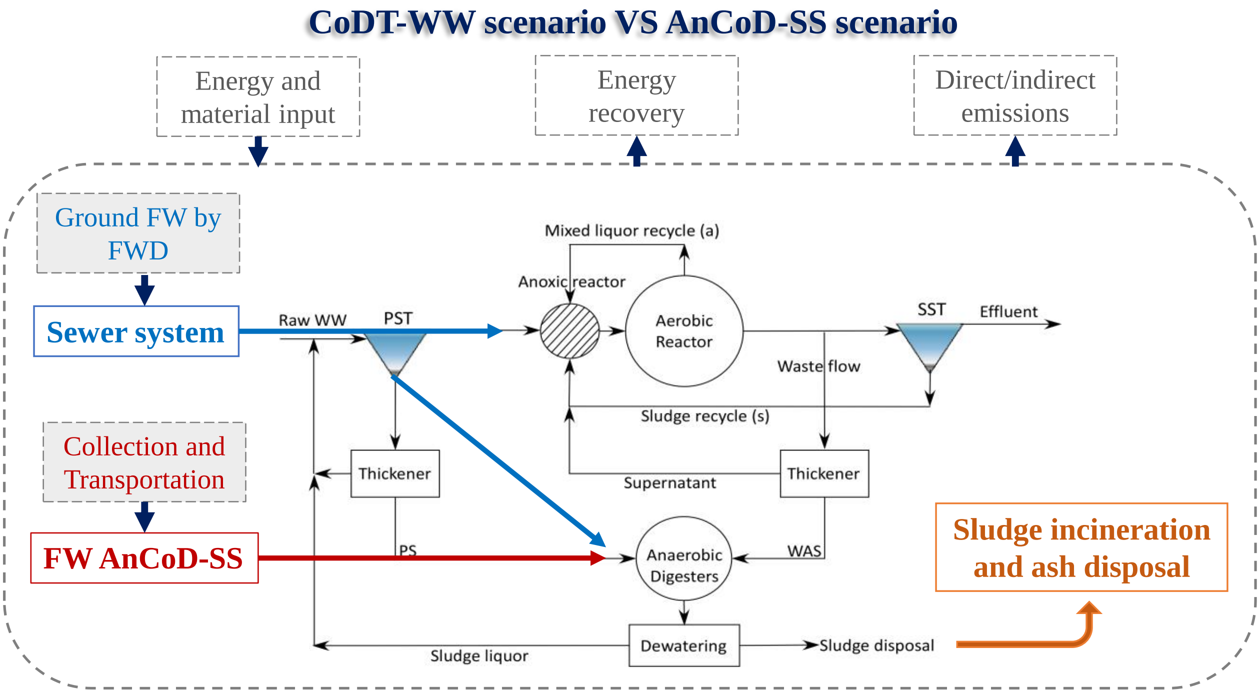

Fig.1. Scenarios for integration of FW with WWTP; co-disposal and treatment with wastewater (CoDT-WW), and anaerobic co-digestion with sewage sludge (AnCoD-SS).

Results and Discussion

Impact on performance of AS process

The performance of BNR (AS) process is only influenced by CoDT-WW scenario, where the impact of FW addition on effluent quality showed slight difference in nitrogen and phosphorus concentrations. The COD concentration was significantly greater but was still under the local effluent discharge standard of 80 mg/l.

The hydraulic capacity of the system was degraded by ~30%, implying that the Sha Tin WWTP has enough physically and biological treatment capacity to handle the extra load by FW. In addition, comparing with the normal WW treatment operation, the overall energy demand of the BNR process was increased by ~24% due to FW addition.

Impact on performance of AD process

To perform a fair comparison, the AnCoD-SS scenario was modelled with same operational parameters as CoDT-WW scenario, especially, the reactor's solids concentration, while the FW to SS ratio was kept as 1:1. These factors define the organic loading rate (OLR) and amount of FW added to the rector in AnCoD-SS scenario. The modelling results of both scenarios show that compared to the normal WW treatment, FW addition increased the methane production by ~73% in CoDT-WW and ~130% in AnCoD-SS scenario, subsequently increasing the energy recovery potential by the same proportion. Whereas the final sludge output was ~33% and ~42% greater in CoDT-WW and AnCoD-SS, respectively.

GHEs and Energy balance of the scenarios

The evaluation and comparison of scenarios from lifecycle perspective was performed for total GHEs and net energy balance. Based on the selected FU, the total positive GHEs were around 22% and 30% greater than normal WW treatment operation in CoDT-WW and AnCoD-SS, respectively, as shown in Figure 2. The contribution in total positive emissions was in order by following processes, organic matter degradation in AS process, material consumption, and the fossil fuel based energy input. While the transportation was the second biggest contributor in AnCoD-SS scenario. However, the greater energy recovery by both scenarios also avoided the greater amount of GHEs, as ~67% by AnCoD-SS and 83% by CoDT-WW. Hence, the net GHEs were almost 36% lesser than the normally operating WW treatment scenario.

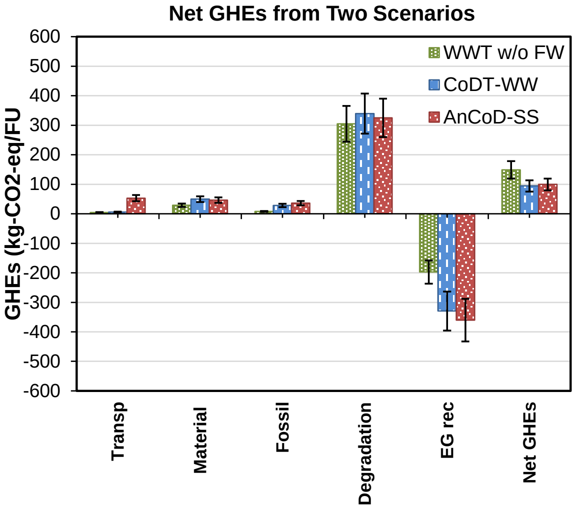

Fig.2. Contribution by subprocesses and total GHEs of three scenarios.

In terms of lifecycle-based energy balance, the contribution in total energy input by subprocesses was in following order, electricity usage by aeration and pumping, heat required by AD, transportation of sludge, and then auxiliary fuel (diesel) consumption at sludge treatment facility. Yet again the collection and transportation of FW in AnCoD-SS scenario was the second largest contributor. The total energy recovery was ~64% greater in CoDT-WW and ~85% in AnCoD-SS scenario compared to the normal WW treatment. Consequently, the net energy balance was negative, providing surplus energy, in CoDT-WW scenario, whereas it was positive in AnCoD-SS and normal WW treatment scenarios, shown in Figure 3. However, excluding the transportation impacts from the evaluation totally changes the ranking of scenarios, making AnCoD-SS scenario the better choice with greater surplus energy than the CoDT-WW scenario. Therefore, it is worth mentioning that for integrated operations at WWTP, the AnCoD-SS scenario is more productive in terms of

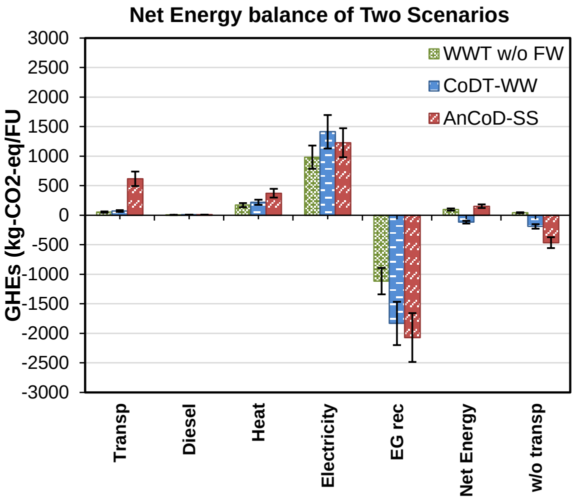

Fig.3. Contribution by subprocesses and net energy balance of three scenarios.

both impact categories, but the transportation is critical for its holistic performance.

Conclusions

The investigation of the integration of FW and WWTP by the CoDT-WW and AnCoD-SS was carried out as a case study in Hong Kong. The energy balance and carbon footprint of the WWTP was even improved compared to the normal operation, owing to the significant energy recovery potential by combine AD of FW and sewage sludge. The sludge output was increased with FW addition but is actually beneficial considering the amount of FW diverted from the landfills and its negative impacts. Therefore, these scenarios offer a feasible and optimistic solution for domestic and commercial FW management.

Acknowledgement

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THE CIRCULAR ECONOMY FOR ORGANICS AS A NEW PARADIGM FOR ADVANCING ORGANICS RECYCLING ACTIVITIES

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‘Sustainability’ is being increasingly replaced by the concept of the circular economy in the realm of waste management and resource recovery. This is a welcome development as the concept of sustainability had lost credibility – everything was sustainable, yet nobody wanted to foot the bill for effecting real change that would bring us closer to being sustainable. The circular economy on the other hand is an economic system aimed at eliminating waste and the continual use of resources, which will function only if all involved parties receive equitable economic or other benefits, and if somebody pays for it**.**

The Circular Economy for Organics links generators, processors and users of organic residues and will be viable in the long-term only if it is driven by demand and economic advantage for all supply chain partners with additional gains and costs being shard in an equitable and mutually acceptable way.

Organics recycling partnerships with agricultural industries offer long-term win-win solutions for both local governments and the farming sector. This can be achieved if farmers are elevated in the organics recycling value chain, for example by contracting them to provide organics recycling services (co-composting / co-digestion), or by establishing long-term compost use partnership agreements with farmers that stipulate quality requirements and a fair price, while on the other hand guaranteeing the beneficial use of all generated compost. Conversely, it is unreasonable to expect farmers to subsidise urban recycling programs through inflated prices for recycled organic products that reflect neither the value farmers can derive from using these products, nor the risk they might be exposed to. The circular economy for organics will make the organics recycling supply and value chain more transparent, and allow for more equitable and acceptable distribution of costs and benefits associated with organics recycling schemes.

Farmers need to be aware of short and long-term monetary values to crop production and soil health through the use of recycled organic products, considering nutrient and non-nutrient benefits gained from applying these products. This requires the availability of user-friendly tools for farmers that predict benefits for different products, soil types, crops, environments, and farming systems. Various projects, such as the development of a nutrient calculator for users of organic soil amendments are currently undertaken in Australia, supporting the establishment of the circular economy for organics.

Keywords: *Circular economy for organics.*

DEVELOPING SENSOR-AIDED COLLECTION OF SOURCE SEPARATED FOOD ORGANICS

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Introducing the collection, processing and beneficial use of source separated organic residues from residential and commercial sources more than 30 years ago revolutionised solid waste management. Today, the segregation at source of garden and/or food organics is an integral part of waste reduction and resource recovery schemes in many countries and municipalities. The success of all source segregation recycling schemes, expressed as diversion and contamination rates, relies to a large extent on the active support and participation of residents and businesses. Recent Australian data encompassing various kerbside food and garden organics collection systems showed impurity levels that averaged 2.6%, representing contamination rates between 0.04% and 17.8% with plastic, metal and containerized food being the most frequently encountered impurities. Information from Hong Kong indicates that contamination levels can be up to 20%. The level of physical impurities in raw materials can be an indicator for chemical contaminants. Reduction of high impurity levels in raw materials will not only reduce the proportion of physical contaminants in the finished compost, but will also likely result in lower concentrations of some chemical contaminants (particularly metals).

This is a major challenge since source segregation schemes for organics are costly to establish and run, and excessive physical contamination levels in raw materials require additional processing steps and end-product quality is deteriorating. Removal of impurities from compost products has its limits, around 95% in the best of cases. This means that raw materials containing 3% impurities can be transformed into screened compost with around 0.45% (by weight) of impurities, in the best of cases. These contaminant levels probably comply with compost quality standards, but initial investigations into microplastics present in recycled organic products and soil environments are likely to force a reduction of acceptable physical contamination levels in raw materials and finished recycled organic products.

This paper will not only present current knowledge concerning physical contaminants in source segregated food/garden organics and generated products (compost/digestate), impurity limits stipulated in end-product quality standards, and the risk of microplastics, but it will also present attempts of reducing impurities in raw materials and hence in recycled organic products.

Experience shows that collection of source segregated food and garden organics can be achieved more easily in rural/regional areas than in densely populated urban areas with many high-rise buildings. Therefore, increased efforts are required to achieve acceptable diversion and contaminant levels for source segregation organics collection schemes in residential high-rise buildings. The development of sensor-aided collection systems might enable the successful collection of source segregated organics also in high rise buildings.

Keywords: *Source Segregation, Organics recycling, Impurities, Microplastics, High-rise buildings.*

IMPROVING WASTE MANAGEMENT APPROACHES FOR SMALL LIVESTOCK FARMS IN VIETNAM

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Agriculture which employs more than half of the population is fading as the most important economic sector in Vietnam. The livestock sector in agriculture plays an important role as it is one of the fastest growing agricultural subsectors. There has been growing public concern over the environmental and human health issues associated with livestock production. Contaminants which include nutrients, pathogens, heavy metals, pharmaceuticals, and natural/synthetic hormones from animal waste generated can be transported into surface and groundwater. This review paper deliveries information from field research and various databases, summarize the current status of waste management approaches in the country. Information on local authorities and national regulatory requirements for waste management approaches was collected and reported. Selected technical data was attached into this paper. In recent years, the livestock sector accounted more than 30 percent of gross agricultural output and growing faster year-by-year. The total amount of animal manure generated in the country is around 80 million tons per year. It is recorded over one-third of livestock manure was directly discharged into the water body without proper treatment which caused of such concerns to water quality as well as aquatic ecosystems. By increasing livestock production, waste management problems will continue to increase and become more serious that needed to improve waste management approach from government. There is a big gap between national standards for livestock wastes discharge at livestock farms realities. For those that have already constructed biogas digesters, there is no monitoring to verify if the biogas effluents meet the standards to discharged into the public environment. Even the biogas effluent could be used as fertilizer for crops, there is no any regulatory to promote this practice. It is needs a long-term strategy for government at all levels to implement effective strategies to manage the livestock waste management in Vietnam.

Keywords: *Environment, Livestock waste, Vietnam, Waste management approach.*

LONG-TERM SAFETY ASSESSMENT OF INDIRECT AGRICULTURAL APPLICATION OF MUNICIPAL SEWAGE SLUDGE THROUGH NET BAGS

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The indirect application (IA) of municipal sewage sludge (MSS) via leachate in agriculture can reduce heavy metal pollution from MSS. To explore the long-term safety of IA, a 10-year field plot experiment was carried out for different patterns of use of sewage sludge. Three treatments were tested: direct application of sewage sludge (SS), application of sewage sludge filled in permeable net bags and stacked into crops (SSN), and application of chemical fertilizer (CF). Banana, papaya, and corn crops were planted in different periods. Heavy metals (Cu, Zn, Pb, and Cd) in the soil, crops, and MSS were monitored and analysed. The results showed that, on a 10-year timescale, the concentrations of all four heavy metals tested in soil samples of the SSN treatment did not exceed the soil standard limits. The yields of fruits and grains in the SSN treatment were not significantly different to that in the CF treatment, and the heavy metal concentrations met the required food standards. In contrast, the SS treatment resulted in soil Cd and Cu concentrations that that did not meet China's soil standards in the 3rd and 4th years of the experiment. After IA (SSN) for 6 months, heavy metal recoveries from sewage sludge were 99.9% for Cu, 94.0% for Zn, 94.9% for Pb, and 94.4% for Cd. Most of the heavy metals were retained in the sewage sludge and were mainly present in the residual fraction. Additionally, 26.5% of organic matter, 64.1% of N, and 83.7% of P were also retained after IA. Based on an estimation of the safe application period (years) for the SS and SSN treatments, it was found that SSN substantially prolonged the safe application years of sewage sludge when compared with SS. In conclusion, the IA of MSS through net bags could considerably reduce the pollution by heavy metals in sewage sludge and could safely supply nutrients for crops.

Keywords: *Municipal sewage sludge, Indirect application, Heavy metals, Long-term safety.*

WASTE FED BIOREFINERIES FOR SUSTAINABLE CHEMICALS AND FUELS

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The rapid growth of the global population has been a major challenge causing a significant threat to the finite resources as well as ecological services. These challenges are prompting to chart a novel path towards adapting sustainable production and consumption strategies. This communication tries to depict the importance of biogenic materials as potential feedstock to be transformed into a source of value through waste fed biorefineries. It also explores the scope of implementing 'circular loops' that strategically directs the flow of resources, their use, extracting value in the form of nutrients, energy and materials post-consumption within. The concept of circular economy (CE) that focuses on the reuse and recycling of materials in technical and biological cycles to reduce waste generation is a critical intervention. As the circular economy is gaining momentum around the globe, the biorefinery will play a key role in the framework of sustainability. However, optimized integrations of unit operations across closed loops are much needed with process intensification strategy in the context of resource recovery efficiency as well as sustainability index.

Keywords: *Biorefineries, Sustainable chemicals Sustainable fuels.*

RECYCLING USE OF ORGANIC WASTES: NEW APPROACH FOR A DEVELOPED CITY IN CHINA

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Globally, the annual organic waste generation is huge, the disposal of organic waste is one of the major challenges that humanity is facing in the twenty-first century. Traditional methods, as incineration and landfill might cause unhygienic conditions and threats to the environment and human beings, and would lose large amounts of recyclable resources if still keep this state until 2100. Here, researchers from the Organic Recycling Research Institute of China Agricultural University (ORRI) discuss the main groups of organic waste and the utilization status of recyclable organic waste resources in the world, and also describe the current status of organic waste utilization at Suzhou, China, and we aim to resolve organic waste pollution especially the ktichen waste in the urban and rural area. At present, three closed organic waste treatment processes have been developed such as biological drying, silo composting and continuous dynamic lane (CDL) aerobic composting, that can be applied to various occasions. Compared with composting reactors in other countries, the self-developed “biological drying + continuous dynamic aerobic composting” reactor has increased processing capacity by 25%, reduced energy consumption by 17%, and effectively reduced greenhouse gas emissions by more than 60%. Through advanced biological drying and aerobic composting technology, various organic wastes can be efficiently converted into biological organic fertilizer and soil conditioner within 7 days. China’s first urban and rural organic waste treatment and utilization demonstration center project was established near Linhu town by ORRI, the products from it will not cause salinization of the soil, and can maintain the yield of rice. This could benefit to link organic waste recycling and ecological agriculture together to feed more people in 2100.

Keywords: *Organic waste, ORRI, Organic recycling, Aerobic composting, Bio-organic fertilizer.*

OPINION OF WASTE MANAGEMENT EXPERTS ON THE IMPLEMENTATION OF SMART WASTE MANAGEMENT IN TEHRAN

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The advent of the fourth industrial revolution “industry 4.0” has given rise to the growth of cyber-physical systems in various fields such as medicine, engineering, management, and manufacturing. The implementation of modern technologies in waste management has brought about the concept of “smart” waste management in so called smart cities. Iran, as a country whose MSW is primarily composed of organic waste, is a candidate for using technologies such as internet of things (IoT) and image processing for better segregation of the waste. In turn, the biodegradable fraction can be used to generate added value, such as in the form of compost or energy. Currently, about 65% of Iran’s MSW is biodegradable but on a national level, only one fourth of this amount is used and the rest is discarded in dumps. In this study, a series of targeted questions have been asked from 27 waste management experts in the country, which include some private company executives, as well as top- and mid-level managers from the municipality and the department of environment. The questions are about the possibility of implementing smart waste management technologies for improving Tehran’s waste management situation. Chi squared distribution as well as Spearman’s coefficient is used to elicit correlations between various responses and their significance. The results show that most experts consider themselves to be aware of the opportunities and challenges of smart waste management. They are generally optimistic about using these technologies, and on average, have mediocre satisfaction of the current waste management practices in the city. As per the experts’ opinions, benefits to the environment such as air pollution and decreased energy use are among the most favorable benefits. Interestingly, the benefits of smart waste management to managing the COVID-19 pandemic did not score highly on the priorities. Overall, interesting trends are observed and it is concluded that according to the experts, smart technologies can be used to improve (bio)waste management in the city.

Keywords: *Waste management, Tehran, Smart cities.*

BIOPONICS – A BIOLOGICAL NUTRIENT RECOVERY TECHNOLOGY IN BIO-CIRCULAR-GREEN ECONOMY

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Bioponics is the combination of hydroponics with the biological recovery of nutrient-rich organic wastes. Organic wastes from several agricultural residues and composts can be used as substrates for organic vegetable productions in bioponics. Bioponics also eliminates agricultural wastewater discharged to the environments as plants are grown in hydroponic grow beds where nutrients are recirculated. Bioponics has a high potential as a sustainable indoor and urban vertical farming. In bioponics, biological processes promote the generations of nutrients from biowastes via anaerobic/aerobic microbial biodegradation, thus allow nutrients assimilation by plants (i.e., vegetables). Nutrient recovery in bioponics relies on the symbiotic relationships among bacteria and plants. However, understanding of ecology and nutrient transformations in bioponics is very limited. Several studies showed that nutrient-rich waste streams such as anaerobic wastewater had a high potential to be used as a nutrient supply in bioponic systems. High acetic acid concentrations were reported increase phosphorus solubilizations of organic wastes. However, no such study has conducted in bioponics.

In this study, chicken manure based-nutrient film technique bioponics was used for growing 14 lettuce heads. Each bioponic system consisted of a grow bed for plant growth, and a biofilter for degradation of chicken manure (organic substrates) and nutrient solubilizations/transformations. Effects of acetic acid addition (17-52 g/L/week) on nitrogen and phosphorus transformations and recovery in bioponics were investigated at a manure loading of 500 g dry wt. per bioponic system. The nitrogen and phosphorus concentrations in bioponics were measured weekly to evaluate the nitrogen and phosphorus concentrations released from chicken manure and microbial degradation on organic nutrients of the chicken manure in the biofilter. In addition, to evaluate the ecology of the systems, 16S rRNA gene sequencing was used to examine the microbial communities in digested chicken manure and plant roots in bioponics at the different acetic acid loads. Microbial samples were taken at the end of each experiment.

Results showed that the acetic loadings (17-52 g/L/week) did not significantly affect the nitrogen and phosphorus concentrations in the bioponics. However, low plant yields were observed at high loads of acetic acid additions. This could be due to the acetic inhibition on plant growth; a slow rate of wastewater containing high acetic concentrations must be considered when integrating to bioponics. Results also showed that several microbial genera were associated with biofilm formation (e.g., *Zooglea* spp.), organic degradation (e.g., *Burkholderiaceae*), nitrification (e.g., *Nitrosomonadaceae* and *Nitrospira* spp.), phosphorus solubilization (e.g., *Pseudomonas* spp.), and plant growth promotion (e.g., *Bacillus* spp.). The results show a symbiosis of organic and nutrient transformations, which was beneficial the bioponic systems. Overall, the results suggest that all bioponics had a high capability for lettuce productions at chicken manure of 500 g dry wt. per bioponic system. However, an intermittent overload of high acetic acids (17-52 g/L/week) must be avoided when used/integrated with bioponics.

Keywords: *Bioponics, Nutrient recovery Bio-circular-green economy.*

URBAN WASTE-BASED BIOREFINERY PROCESSES FOR TRANSITION TO A CIRCULAR (BIO)ECONOMY

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The current global demand of sustainability relies heavily on material and energy efficiency, reduced waste generation and greenhouse gases emissions. This aligns perfectly with the concept of circular economy which has gained momentum in the recent years and is seen more than from a research perspective. Design and adoption of circular economy approaches which account for sourcing, production, consumption, disposal and reuse or recycling of resources is needed to fulfil the criteria of sustainable development. To this end, the biorefinery concept has been envisaged to drive the transition from the current linear economy (extractprocess-consumption-disposal) to a closed loop (bio)economy wherein the value of products, materials and resources is maintained in internal cycles for as long as practically possible. In this regard, bio-based sourcing of high value-added products from non-food and/or waste feedstocks has attracted a great deal of attention in the biorefinery production model. The biowastes generated in urban settings form a great source of such inexpensive and abundant feedstocks which can be valorized to produce valuable bioproducts using microbial cell factories. While fulfilling the criterion of renewability of products, this aspect importantly depends on development of robust production schemes, increased market opportunities, and policies and regulations from government and private institutions. In this talk, the case studies of food waste and textile waste-based biorefinery technologies would be used to present these aspects of a closed-loop waste-based biorefinery model. The conversions of these urban wastes to valuable products such as glycoplipid biosurfactants, microbial enzymes and biocomposites would be discussed.

Keywords: *Waste-based biorefinery, Circular bioeconomy, valuable products.*

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Composting

RESOURCE RECOVERY FROM SOLID ANAEROBIC DIGESTATE: A CRITICAL REVIEW ON CIRCULAR BIO-ECONOMY PERSPECTIVE

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ABSTRACT. Anaerobic digestion of food waste is considered as one of the sustainable solutions to convert food waste to produce bioenergy. However, solid by-product of anaerobic digestion, 'digestate' is becoming serious bottleneck to manage due to its distinctive characteristics. Traditional disposal methods including direct discharge of untreated digestate into land, incineration and landfilling are not favorable in economic and environmental aspects. Several environmental issues such as NH_3 emission, greenhouse gas emission, water pollution, eutrophication, alteration of soil microbiota etc. have been reported from digestate disposal processes. In this review, different types of state-of-the-art technologies available for resource recovery as well as the stabilization of solid fractions of digestate will be discussed. Challenges and opportunities of biological (composting, enzymatic hydrolysis, bio drying), physicochemical (palletization) and thermochemical (pyrolysis, gasification etc.) valorization technologies will be discussed pertaining to sustainability and zero waste concept under circular bio-economy perspective.

Keywords: *Anaerobic digestate; Circular bio-economy; Composting; biomass valorization*

Introduction

Rapid urbanization, industrialization and lifestyle has largely influenced food waste production and the per capita food waste generation is increasing globally. Food waste management has emerged as a high concern service for municipal authorities for its safer disposal. Conventional disposal of food waste into landfill is causing several environmental issues such as water and air pollution along with land acquisition. Anaerobic digestion (AD) technology has been considered as one of the advanced technologies to curb the environmental issues by recycling food waste into bioenergy production [1]. Apart from bioenergy production, the residues of AD process termed as 'food waste digestate (FWD)' has upraised the environmental concerns due to its innate characteristics. The dewatered or solid fractions of FWD comprises of high moisture content (~75%), high ammonium nitrogen (NH_4^+-N), partial organic matter along with microbial biomass and minerals [2].

Scarcity of land and legislative requirements has increased AD plants for food waste treatment in many countries that is resulting in FWD generation in large quantity. For example, the annual FWD production in Germany is 856 kt whereas Italy and China produces 476 kt and 270 kt, respectively [3]. In addition, many Asian countries where the food waste generation is higher have adopted AD process for centralized food waste treatment thereby increasing global FWD production.

Conventional disposal of FWD into land causes 60-70% nitrogen loss majorly through NH_3 emission [4]. FWD could be used as a potential source for resource recovery through several technologies, which could reduce the environmental, and health hazards. Recently, significant growing interest has been shown in digestate research due to increased AD facilities [3].

In this review article, available technologies for FWD valorization into value added products are discussed. The benefits, drawbacks of biological, physicochemical, and thermochemical treatment technologies are outlined.

Food Waste Digestate

European waste catalogue has categorized dewatered digestate as organic waste since it is biologically unstable. The characteristics of FWD depends on AD process, feedstock and dewatering process [2]. The presence of nutrients such as nitrogen (1.1-9.6%), phosphorous (0.1-2.4%) and potassium (0.4-2.3%) makes FWD as a valuable source. The distinct characteristics of FWD from others such as agricultural/ livestock waste digestate are higher NH_4^+-N, higher moisture content and less total solid content. Due to these characteristics, FWD is associated with environmental impacts such as water pollution through leaching, soil contamination, hazardous to human health by pathogens and volatile emissions to air estimated to be 139 g CO_2-eq/ kg digestate. Increased transportation cost has also demanded FWD valorization at the source of its generation [5]. Hence, several resource recovery technologies have been evolved to utilize FWD to produce value added products in an environmentally sustainable manner.

Food Waste Digestate Valorization Technologies

Several technologies have been developed in the past decade for digestate treatment, however, only few of them have been recognized as resource recovery technologies. The basis of resource recovery technologies is to process the FWD to produce value added product that can substitute chemical fertilizer for some extent. Among the developed technologies, composting, drying (biodrying, thermal drying, solar drying) and thermochemical (incineration, pyrolysis, gasification, hydrothermal carbonization) have been elucidated as the promising options for FWD treatment (Fig. 1) [6]. Each of these technologies have their own advantages and limitations. In addition, economic viability needs to be considered in decision making process to ensure long term sustainability.

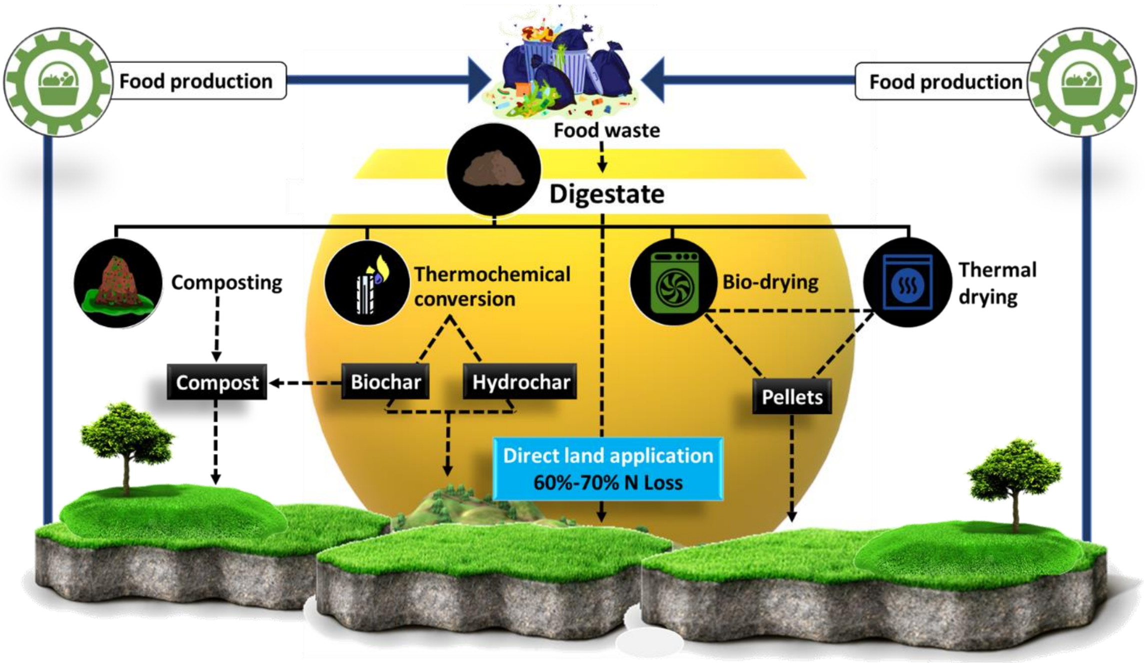

Fig.1. Technologies available for food waste digestate valorization.

Composting

Composting is the microbial biotransformation of organic waste under aerobic conditions to produce stable and mature product call ‘compost’. It is considered as one of the simple and easily operated technology with economic viability. Since FWD comprises of partially degraded organic matter and pathogens, composting can further stabilize the FWD into a safe biofertilizer by reducing its NH4+-N phytotoxicity and by destroying the pathogens. However, the properties of FWD is poor for composting due to high moisture content and low C/N ratio (5-7). Hence, conventional composting of FWD will result in higher nitrogen loss in the form of NH_3 volatilization [3]. Hence, co-composting strategy with high carbonaceous bulking agent such as sawdust is required to condition the FWD properties before composting [7]. Nonetheless, addition of only sawdust in large quantity may hinder the fertilizer value of final compost, hence, alternative additives should be explored to replace some fractions of sawdust. Mature compost with low moisture content can be used as one of the potential substitutes that can also acts as an exogenous microbial inoculum. Physical amendments such as biochar or zeolite have been extensively used in organic waste composting to mitigate nitrogen loss by reducing NH_3 emission. Chemical additives like Mg and P salts have also been proven to be effective in reducing nitrogen loss. Additive strategies could be explored for effective FWD composting to produce nutrient rich compost. Microbial inoculum specifically for enhancing nitrification can further enhance the FWD composting. Extensive research needs to be done in FWD composting to find a possible solution to produce compost with reduced nitrogen loss in an environmental sound manner in a short duration.

Drying

Drying is a conventional process that is aimed at reducing the moisture content of FWD for its further utilization. Dried form of FWD could be easily transported and could be used to produce pelletized form of biofertilizer. However, external energy requirement and possibilities of greenhouse gas (GHG) emission needs to be handled cautiously. Several means of drying such as biodrying, thermal and solar drying have been used previously to dry digestate [6].

The basic principle of biodrying is similar to composting (Fig. 2). In this case, elimination of moisture is set as a priority while maintaining high carbon content. Biodried digestates with 45% carbon, stable and odorless could be valorized in various applications. However, the risk of nitrous oxide emissions is high and special care should be taken [6].

Belt dryer is one of the thermal drying process in which high temperature (85 °C) is used under controlled conditions. Belt dryer achieves 85% TS content in the digestate when high temperature air is fed. Provisions of nitrogen scrubber may reduce the GHG emission. After thermal drying, pelletization of end product becomes easy and can facilitate land application strategies. The limitation of this technology includes additional energy requirements, long drying time, bad odors and gaseous emissions [6].

Solar drying is another option with low capital investment. The evaporation rate during solar drying may range between 0.6 and 3.5tn water/y/m^2. The energy needs for mixing and aeration may rise up to 200kWh/t water resulting in a digestate with 65% dry matter. It's easy operation and use of free solar energy attracts its utilization in FWD drying. However, relatively longer drying time, requirements of larger surface area and its dependency on climate conditions are the bottlenecks of solar drying process [6].

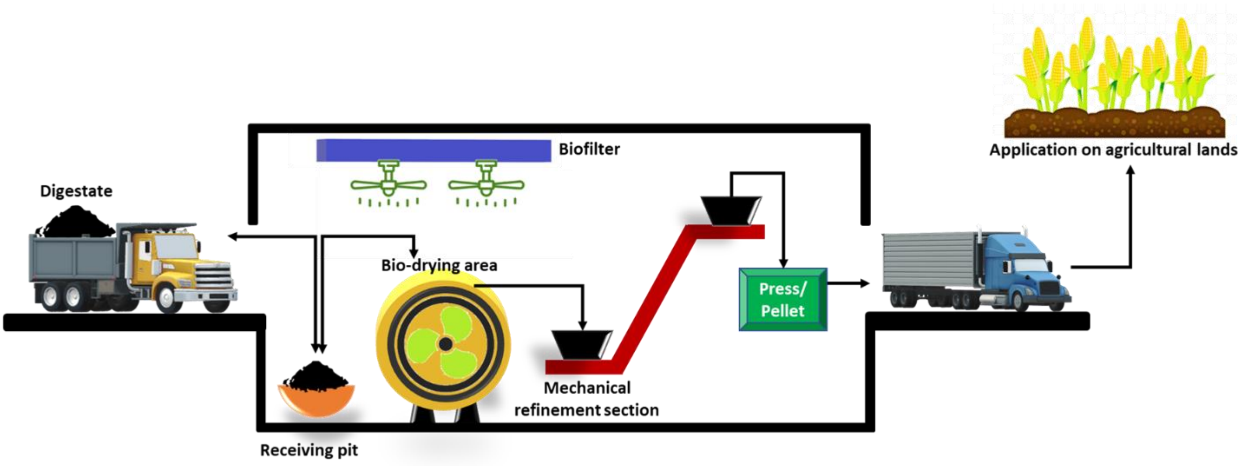

Fig. 2. Schematic representation of bio-drying process

Thermochemical Conversion

Thermochemical technologies are relatively faster conversion processes compared to other valorization technologies (Fig. 3). Incineration of FWD produces ashes that contain up to 20–25% P_2O_5, as well as K, Si, Mg, Al, traces of some heavy metals (e.g. Cd, Zn, Cu). The prospect of recovering nutrients (mostly phosphorous) from ashes has been reported reaching recoveries of 78% in P. However, incineration process is economically not suitable in small scale due to the intensive flue gas cleaning and energy demands. On the other hand, pyrolysis/gasification processes convert FWD into a stable product called 'biochar', which has demonstrated as a value-added product with high nutrient capacity and improved fertilising properties. Complete retention of phosphorous has been demonstrated when digestate was pyrolyzed. However, higher nitrogen loss has been reported during pyrolysis. Hydrothermal carbonization of digestate favors immobilization and retention of nitrogen and potassium over 50% in the liquid phase. Hence, additional treatment step is required to recover the nutrients [6].

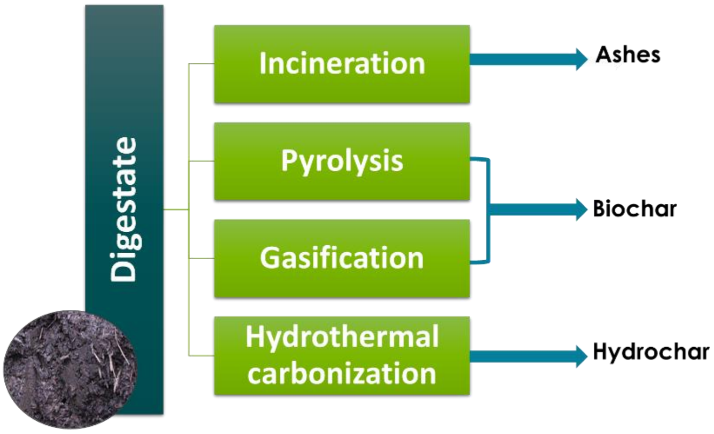

Fig. 3. Thermochemical conversion technologies for digestate treatment

Conclusions

Food waste digestate is a potential source for producing value added products for agricultural applications. Among the available technologies, composting can be used as a simple and low-cost technology for digestate treatment however further research is required to develop strategies for minimizing nitrogen loss and GHG emission. Economic feasibility of different processing technologies for FWD valorization needs to be conducted. The resource recovered output products should be adequately tested in field trials in order to examine the agronomic properties, the effect on the environment and the food safety of digestate products.

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DOES THE APPLICATION OF CONDUCTIVE MATERIALS ENHANCE THE BENEFICIAL EFFECT OF ELECTRIC FIELD ON N_2O MITIGATION DURING COMPOSTING?

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ABSTRACT. Different conductive materials were used in the electric-field assisted composting to reduce electric resistance and assess their impacts on nitrous oxide (N_2O) emission and elucidate the underlying mechanism. Results showed that using biochar or Ferric chloride ($FeCl_3$) as conductive materials increased N_2O emissions were increased by 1.1-fold and 4.5-fold respectively. The main mechanism underlying the elevated N_2O emission was attributed to the "dual promotion effect" on nitrifier denitrification: i) the elevated NO_2^- production because ammonia oxidizing bacteria (AOB) gene (bac-amoA) was up-regulated; ii) the enriched abundance of AOB, possibly because oxygen (O_2) availability was elevated after biochar or $FeCl_3$ addition due to the inhibition of bacterial aerobic respiration. Conclusively, the used conductive material should not inhibit biodegradation when used for N_2O mitigation.

Keywords: *Composting, Electric field, Nitrifier Denitrification, Nitrification inhibitor, N_2O mitigation.*

Introduction

Aerobic composting is an effective economic tool for organic solid waste treatment and resource recycling [1]. Nitrous oxide (N_2O) emission represents the main concerns of composting, due to its contribution to the destruction of stratospheric ozone and climate change [2]. N_2O is regulated by a wide variety of microbial communities in multiple biological pathways [3]. Source partitioning of N_2O is a prerequisite for understanding the production mechanism and developing the targeted technique. However, attributing N_2O emission to a specific microbial pathway is difficult due to the complex interaction between N_2O production pathways, as well as the variable temporal and special dynamic. Considering the limitation of single N_2O source partitioning technique, multiple techniques should be combined to differentiate multiple biological pathways for N_2O production.

In essence, aerobic composting is a complex biological redox process, in which electrons are generated and O_2 is used as the terminal electron acceptor [4]. A recent study indicates that strengthening electron transfer through the application of an electric field in composting can lower oxygen (O_2) availability and reduce N_2O emission. Strengthening electron transfer can be achieved by directly enhancing electric potential or reducing electric resistance. Strengthening electron transfer can be achieved by directly enhancing electric potential or reducing electric resistance. Reducing electric resistance value can be achieved by the application of conductive materials, including physical electron shuttle and chemical electrolyte. Specially, biochar has been defined as a physical electron shuttle [5] due to the rich redox-active functional groups (e.g., phenol and quinone) on surface and a graphite-like electrical conductivity structure with a conjugated π-electron system. Meanwhile, Ferric chloride ($FeCl_3$) has been used as a chemical additive for ammonia (NH_3) mitigation [6]. However, $FeCl_3$ as chemical electrolyte may inhibit microbial activity and electron production from biomass biodegradation due to the resulted low pH, and therefore it is not necessary to enhance electron transfer. To date, there is no relevant information regarding whether the application of biochar in electric-field assisted composting would accelerate the electron transfer or not. Here, we hypothesized that the addition of conductive materials would improve O_2 availability although it could accelerate electron transfer, because the addition of conductive materials would minimize electron production from the biological redox process.

The objectives of this study were to evaluate the efficiency and the involved microbial mechanisms of the combined application of an electric field and conductive material on O_2 consumption and N_2O production in composting.

Material and Methods

Composting system, feedstock material and experiment design

This experiment was performed using computer controlled 50-L bench-scale cylindrical composting reactors, as have been previously used and described by a previous study [7]. Fresh poultry manure and sawdust were used as raw materials in this research. There were three treatments with either single or combined applications of the electric field and conductive material in this experiment: i) a direct current voltage of 2V (2V treatment); ii) a direct current voltage of 2V with the addition of 5% w/w biochar (2V+C treatment) and iii) a direct current voltage of 2V with the addition of 5% w/w $FeCl_3$ (analytical reagent) (2V+$FeCl_3$ treatment). A direct-current voltage (2V) was set using a potentiostat (DP305A, MESTEK, China).

Measurement

Exhaust air (cexhaust, mol mol-1) and background air (cbackground, mol mol-1) was collected used for analyzing N_2O concentrations with gas chromatography (Agilent 6890 equipped with ECD detector). Electric resistance was calculated according to Ohm's law, which is equal to the voltage divided by the current. Ammonium (NH_4^+), Nitrite (NO_2^-) and Nitrate (NO_3^-) contents were determined using a segmented flow analyzer (AutoAnalyzer Sanþþ, Skalar, Netherlands). The difference in ^{15}N between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is the so-called site preference (SP), which enabled us to identify the source of N_2O in compost [8]. Compost samples from 6d, 21d, 35d, 42d were selected for functional genes and 16S rRNA gene quantification.

Results and Discussion

The addition of conductive material weakens electron production from bacterial aerobic respiration in spite of accelerating electron transfer

As shown in Figure 1a, the electric current values in treatments 2V+C and 2V+$FeCl_3$ were increased by 123.1% and 69.5% respectively than those in treatment 2V. These results suggests that the addition of biochar or $FeCl_3$ under the electric field can accelerate the electron transfer in compost. Although the electron transfer capacity was enhanced after the addition of conductive materials, O_2 consumption was declined (Figure 1b). This is attributed to the decreasing electron production from bacterial oxidation of organic matters after the addition of biochar and $FeCl_3$ (Figure 1c).

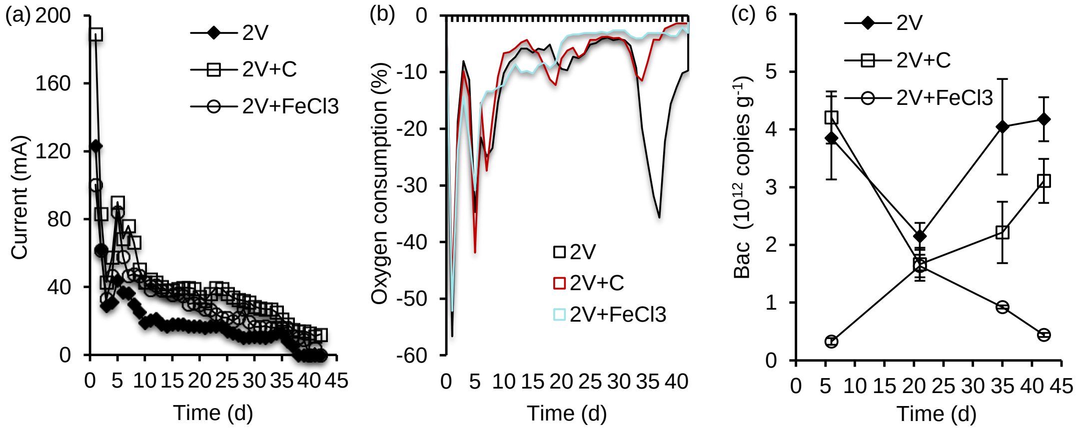

Fig. 1. Changes in current value (a), oxygen consumption (b), bacterial 16S rRNA gene abundance (c) in treatments 2V+C and 2V+FeCl3 compared with treatment 2V.

There are two microbial mechanism underlying O_2 consumption: i) the activity of exoelectrogenic bacteria is enhanced and more electrons flow to extracellular O_2 [4]; ii) more electrons are produced from bacterial aerobic respiration [7]. Provided that the activity of exoelectrogenic bacteria was the major mechanism responsible for O_2 consumption, treatments with conductive material addition, conferring enriched exoelectrogenic bacterial abundance, should have consumed more O_2 than the 2V treatment. These results indicated that bacterial aerobic respiration rather than the activity of exoelectrogenic bacteria is the main mechanism responsible for O_2 consumption, and the promoting effects of an electric field (2V) on bacterial aerobic respiration [7] could be reversed by the opposite effects of conductive materials.

Nitrifier denitrification-derived N_2O is elevated after the addition of conductive material

As shown in Figure 2a, N_2O emissions were increased by 1.1-fold (biochar) and 4.5-fold ($FeCl_3$) respectively after biochar and $FeCl_3$ addition. NO_2^- accumulation were enhanced by the addition of $FeCl_3$ or biochar, and the peak of N_2O emission was synchronized with NO_2^- accumulation (Figure 2b). The N_2O production pathways were further differentiated using isotopomer analysis. As shown in Figure 2c, the SP values of N_2O were low and ranged from -14.9‰ to 8.0‰, indicating that the relative contribution of bacteria denitrification to N_2O production was high [8]. The increase in N_2O emission after the addition of conductive material was mainly attributed to these greater emission rates compared with treatment 2V at the N_2O emission peak, where the abundance of bac-amoA was up-regulated (Figure 2d) and NO_2^- accumulation occurred. Although heterotrophic denitrification also contributed to N_2O production, most was reduced to N_2 (data not shown). These results suggests that nitrifier denitrification plays a more key role in N_2O emission than heterotrophic denitrification under the electric field. It was reported that that nitrifier denitrification (by AOB) is a strong contributor to N_2O emission because genes encoding a N_2O reductase have not been identified in the genomes of any cultured ammonia oxidizer [9].

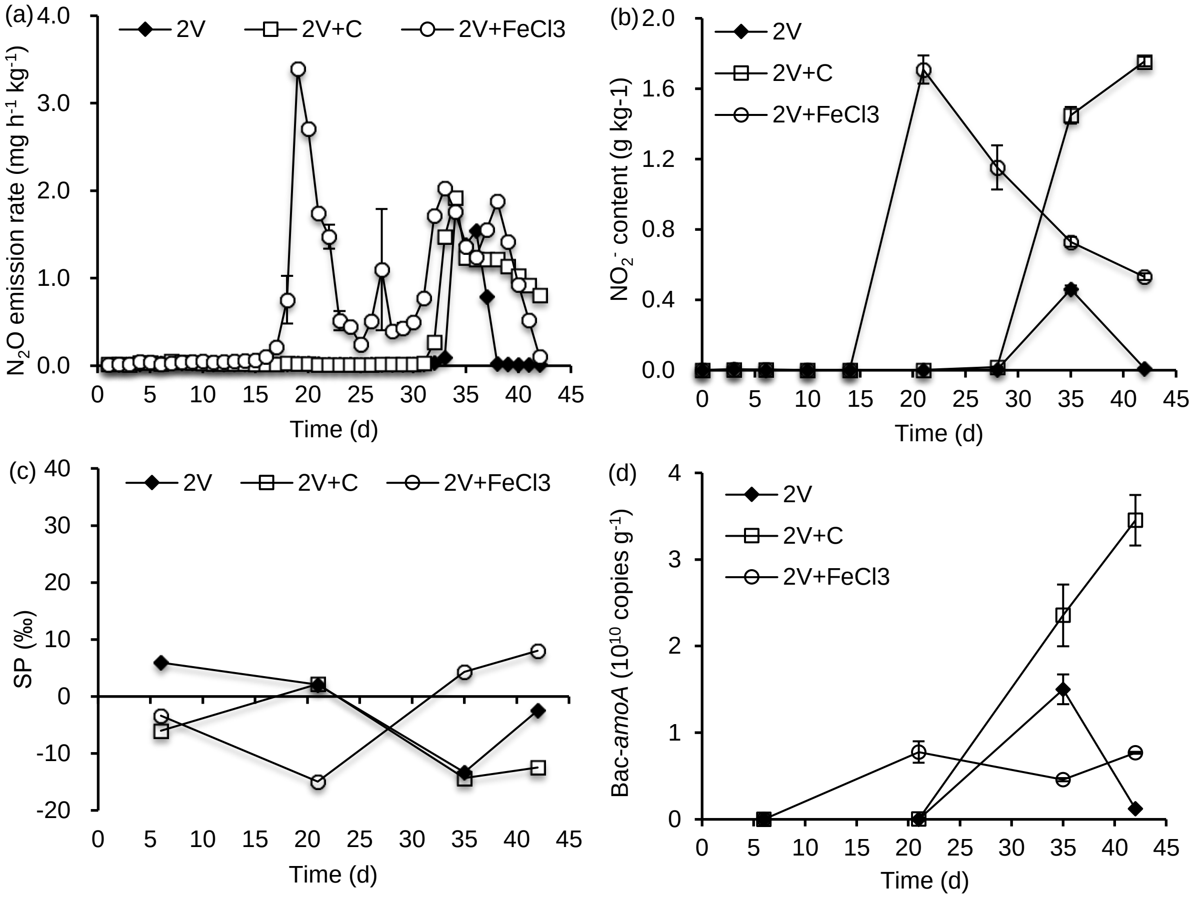

Fig. 2. Changes in emission rate of N_2O (a), NO_2^- content (b), site preference (SP) of N_2O (c) and the gene abundance of ammonia oxidizing bacteria (d) contents during composting.

Conclusions

The application of conductive material increased N_2O emission by 1.1-fold (biochar) and 4.5-fold ($FeCl_3$) respectively. NO_2^- accumulation was aggravated with biochar or $FeCl_3$ addition at the peak of N_2O emission, because more NO_2^--producing but less NO_2^--consuming bacteria. Nitrifier denitrification induced by NO_2^- accumulation contributed to the most importance of N_2O production. The main mechanism underlying the elevated N_2O emission was attributed to the “dual promotion effect” on ND: i) the elevated NO_2^- production; ii) the enriched abundance of AOB Nitrosomonadaceae, which are attributed to the weakened bacterial aerobic respiration resulting in a greater O_2 availability.

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BIOCHAR AS SMART CANDIDATURE TO IMPROVE THE MICROBIAL COMMUNITIES AND MITIGATE THE GREENHOUSE GASES EMISSION DURING POULTRY MANURE COMPOSTING

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This work aimed to evaluate the influence of biochar combined amendment to poultry manure (PM) + wheat straw feed stock was investigated to assess the impact of biochar during the composting. Nitrogen conservation, greenhouse gases (CO_2 CH_4 and N_2O) emissions, nutrient transformation and microbial dynamics affecting these emissions were examined. Six different dosages of biochar were used to compost PM under aerobic conditions in 130L reactor for a 42-d period. A control treatment was also studied using PM without any amendment. The results showed that all of the composts with biochar + zeolite amendment reached the required maturity standard, and gave the highest maturity. In addition maximum microbial community and enzymatic activity were observed in biochar amended treatment. The biochar addition was significantly reduced the ammonia and GHGs emission emissions, but had also impact on reduction of bioavailability of heavy metals. On the first day of PM aerobic incubation, the presence of biochar resulted in increased porosity, due to its higher nano-porosity and surface area. Furthermore, Scanning Electron Microscopy also indicated that in the biochar-amended PM, the dense microstructure on the PM surface disintegrated into fragments with organic fraction degraded and water lost. Overall our results indicate that biochar combined with microbial consortium addition into PM composting was recommended to emit less total greenhouse gas emission and reached maturity on day 35 than control.

Keywords: *Poultry manure, Greenhouse gases, Biochar, Composting.*

COMPOSTING FROM ANAEROBIC DIGESTION IN HONG KONG

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ABSTRACT. Composting with dewatered digestate (DD) after anaerobic digestion from food waste in Hong Kong was studied to improve final compost quality from high ammonia and moisture content dewatered digestate. 4 trials with different mixing formula and operation condition were tested with last trial with addition of sawdust gave close performance to fulfilling Hong Kong Organic Resource Centre (HKORC) Compost and Soil Conditioner Quality Standard (2005). Further trial will be carried out using biochar as one of the input materials.

Keywords: *Anaerobic Digestion; Food waste; Compost; Hong Kong.*

Introduction

Organic Resource Recovery Centre (ORRC phase 1), was commenced in operation since 2018 with daily treatment capacity of 200 tons Source Separated Organic Waste (SSOW) [1]. SSOW are treated with one stage wet anaerobic digestion process. Dewatered digestate are further treated by composting to enhance end-product quality content. However, the high ammonium content and low C:N ratio digestate makes the composting process more challenging and continuing to work on compost quality improvement.

This study focuses on compost quality after different trial of different mixing conditions and operation control, and what would be the next possibility to address remaining issues.

Material and Methods

Input material condition

Dewatered Digestate: This is the target input material to be treated in ORRC1 compost operation. Digestate after anaerobic digestion in ORRC1 has rather low C/N ratio (less than 10) and high ammonium nitrogen (NH4_N) (10,000 – 20,000 mg/kg dry matter). It also contains quite high moisture content of 75%.

Bulking agent (wood chip): Different sizes of wood chips: bigger size wood block (150 mm long x 40 mm wide x 20 mm thick) and small wood chip (40mm x 40 mm x 5), are used in different composting trial in the following study.

Sawdust: Fine powder from wood shredding process from local wood treatment facilities is applied into initial mixing for the purpose of increase C/N ratio and also give a good condition to lower initial moisture content.

Recirculate compost: Small portion of compost produced onsite are recirculated back to first stage composting process, this is aimed to assist in better denitrification in early stage of the process.

Composting trial

Total of 4 testing trials were conducted in the period from July 2019 to August 2020. Different recipes, aeration control practices were tested to optimize performance of composting dewatered digestate into mature compost. A brief summary of different trials is descripted in the following:

Trial 1 and Trial 2: Trial 1 (July 2019) and Trial 2 (September 2019) were conducted with same compost mixing formula given in Table 1. DD ratio was increased from original mixing formula with the purpose to increase treatment amount, and aimed to control initial mixture moisture content down to about 60%. New aeration pattern of 4 hours/ 6 hours cycle was applied with respect to daily pile temperature trend differences.

Table 1. Trial 1 and 2 compost mixing formula.

	Composition	Units	Trial 1 and 2
Bulking agent / Compost / Dewatered Digestate mixture	Bulking agent	w/w	30%
	Compost	w/w	17%
	Dewatered Digestate	w/w	53%

Trial 3 and Trial 4: Sawdust was included in Trial 3 (Dec 2019 – Jan 2020) with formula ratio listed in Table 2. Formula was further finetuned during Trial 4 (Jul – Aug 2020) with result from Trial 3

Table 2. Trial 3 and 4 compost mixing formula.

	Composition	Units	Trial 3	Trial 4
Bulking agent / Compost / Dewatered Digestate mixture	Bulking agent	w/w	20%	25%
	Compost	w/w	17%	10%
	Dewatered Digestate	w/w	53%	50%
	Sawdust	w/w	10%	15%

Results and Discussion

Trial 1 and Trial 2

Ammonium (NH_4^+) content reduced by 950 mg/kg in Trial 1 and 700 mg/kg in Trial 2, which were 25% - 27% (Fig 1a). Seed germination index (SGI) in both trials also could not reach 30% (Fig 1b) while standard required at higher than 80% [2]. NH_4^+ was found reducing greatly but not lower enough to fulfil 700 mg/kg requirement, and SGI also suggesting the compost in both trials were not mature enough. Mixing formula and process should be modified in later trials.

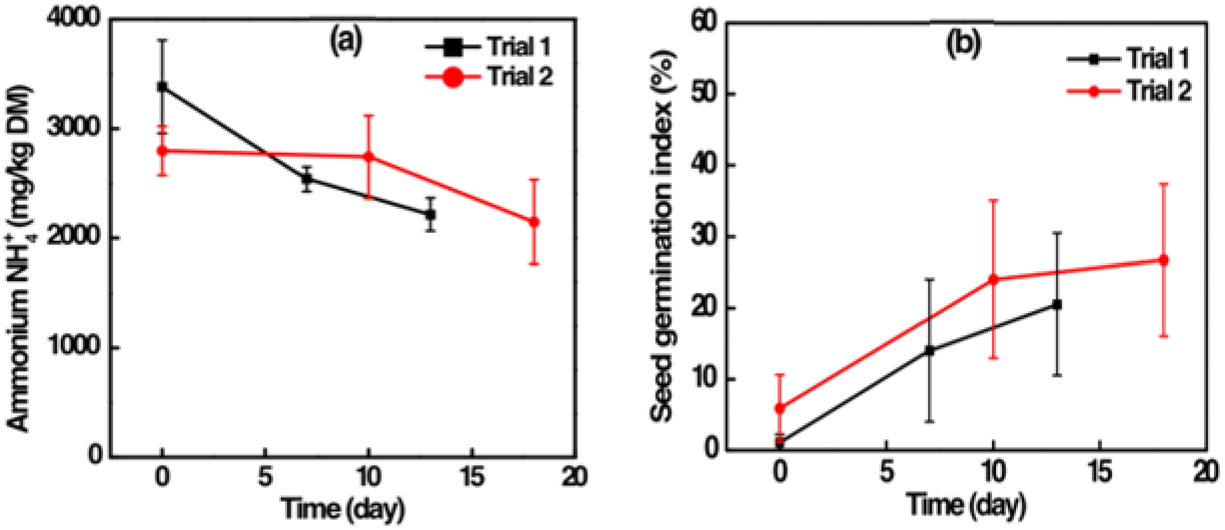

Fig. 1. Ammonium and seed germination index changes in Trial 1 and 2.

Trail 3 and Trial 4

With reference to the trial results from previous trials, modification to add sawdust to the mixing shown in Table 2. Same aeration pattern was adopted according to day-to-day temperature profile changes. SGI was increased to 40% - 45%, but was still underperformance to the Standard requirement.

Recirculating compost and sawdust ratio were further adjusted in Trial 4, and the trial duration was extended for 2 more weeks from 14 days to 28 days to assess decomposition and maturation performance of the compost. Maturity was again evaluated by SGI, good SGI 80% - 90% (Fig 2a) was found after 14 days of composting from three different parts of trial. Comparing to previous trials the SGI was increased significantly gave a good indication that the mixing recipe was more suitable to onsite condition.

While ammonium was still around 2,100 mg/kg at 14 days composting, process was continued and after 14 more days, ammonium content was about 1,000 mg/kg (Fig 2b), which was 68% ammonium removal rate. Although final result was still slightly higher than 700 mg/kg, decomposition of ammonium was shown possible when longer duration could be applied.

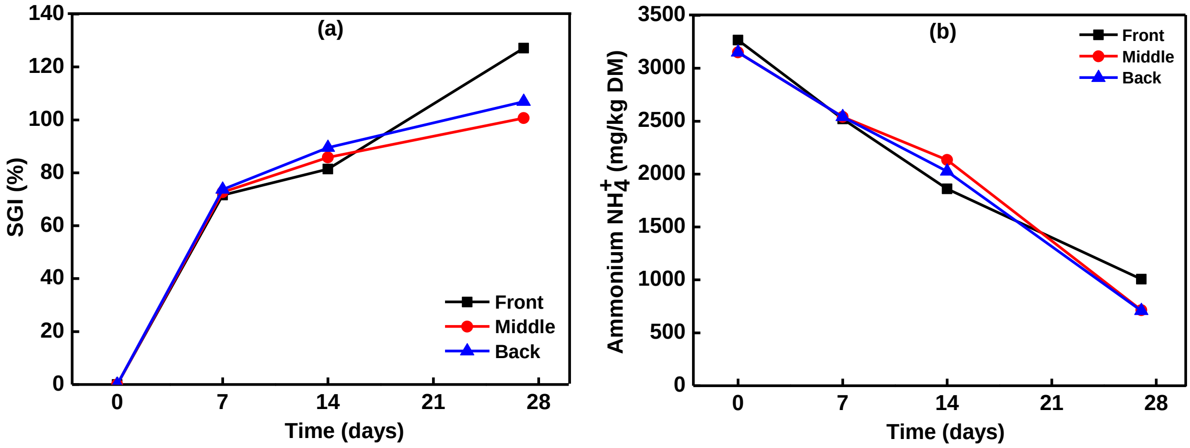

Fig. 2. Ammonium and seed germination index changes in Trial 4.

Conclusions

A mixture of 25% bulking agent, 50% dewatered digestate, 10% compost, and 15% sawdust was used in Trial 4, after 2 weeks processing the compost reached maturity with higher than 80% SGI. With extra 2 weeks, ammonium content further lowered to 800 – 1,000 mg/kg dry matter suggesting compost is close to stable and mature under this condition. This is therefore suggested that the practices adopted in Trial 4 should be effective in treating high ammonium and low C:N ratio digestate. As the ammonium after 2 weeks was still high compared to HKORC standard, where 2 weeks is the ideal compost period to fit in operational flow, finetuning of the formula or improving dewatered digestate condition before composting could be the next step for studies.

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THE PROGRESS OF COMPOSTING TECHNOLOGIES FROM STATIC HEAP TO INTELLIGENT REACTOR: BENEFITS AND LIMITATIONS

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Composting technology is widely used and different technologies have been applied from static heap to windrow composting, and to intelligent reactor composting since the rapid development of landless intensive livestock farms and improved awareness to environmental impacts. However, the lack of a systematic analysis has limited the objective evaluation of these different technologies from the perspective of treatment efficiency, and environmental and economic benefit. This study aimed to use quantitative data from full-scale composting systems (static heap, windrow composting and reactor composting with 8 t treatment capacity per day) installed on a commercial pig farm (6000 head) to compare their treatment efficiency, nutrient losses, product quality and investment and operational costs. The results showed that fresh feedstock entered the thermophilic phase in reactor composting much more quickly (within a few hours) than the other composting methods, and maintained a relatively stable high temperature (55~65°C). This improved the biodegradation process and shortened the composting period. Within the first week, the organic matter degradation in the reactor composting treatment reached 35.7%, significantly greater than for the other treatments. Rapid heating and sustained high temperature promoted the removal rates of the antibiotics tetracycline, doxycycline and sulfamethoxazole. More than 90% of these antibiotics were degraded in the reactor composting treatment, and antibiotic resistance gene abundance was significantly reduced by 79% after composting. Nitrogen loss from the reactor composting was 34% less compared with windrow composting. Although the reactor composting has a higher equipment cost and greater depreciation than the other composting technologies, the total investment and operational costs are comparable to windrow composting. Moreover, with low nitrogen loss, high antibiotic and resistance gene removal rates, reactor composting has benefits regarding nutrient use efficiency and environmental impact.

Keywords: *Reactor composting, Windrow composting, Static heap, Composting efficiency, Environmental and economic benefits.*

BIOCHAR AS SMART CANDIDATE TO REGULATE THE FATE OF HEAVY METALS (CU AND ZN) RESISTANT BACTERIA COMMUNITY DURING THE POULTRY MANURE COMPOSTING

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In this study, the influence of coconut shell biochar addition (CSB) on heavy metals (Cu and Zn) resistance bacterial fate and there correlation with physicochemical parameters were evaluated during poultry manure composting. High-throughput sequencing was carried out on five treatments, namely T1-T5, where T2 to T5 were supplemented with 2.5%, 5%, 7.5% and 10% CSB, while T1 was used as control for the comparison. The results of HMRB indicated that the relative abundance of major potential bacterial host altered were *Firmicutes* (52.88-14.32%), *Actinobacteria* (35.20-4.99%), *Bacteroidetes* (0.05-15.07%) and *Proteobacteria* (0.01-20.28%) with elevated biochar concentration (0%-10%). Beta and alpha diversity as well as network analysis illustrated composting micro-environmental ecology with exogenous additive biochar to remarkably affect the dominant resistant bacterial community distribution by adjusting the interacting between driving environmental parameters with potential host bacterial in composting. Ultimately, the amendment of 7.5% CSB into poultry manure composting was able to significantly reduce the HMRB abundance, improve the composting efficiency and end product quality.

Keywords: *Coconut shell biochar, Poultry manure, Composting, Heavy metals resistant bacterial, Physicochemical properties.*

EFFECT OF BIOCHAR ADDITION ON FOOD WASTE DIGESTATE COMPOSTING AT LOW AND HIGH C/N RATIOS

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ABSTRACT. Food waste digestate (FWD) is generated from the anaerobic digestion process. Post-treatment of FWD in aerobic composting can assure the stability of carbon and produce nutrient rich biofertilizer for agronomic usage. Biochar could improve composting process efficiency due to its high absorption capacity. Previous studies have shown that a C/N ratio of 30 and 10% biochar are considered as favorable for FWD composting. However, composting at a high C/N ratio will increase the demand of bulking agent for adjusting the initial C/N ratio of FWD composting mixture. Hence, in our study, FWD composting at a low C/N ratio with biochar was evaluated to understand compost quality. The effect of the addition of biochar at different ratios (0%, 10%, 15%) on FWD composting at low (15) and high (30) C/N ratio was investigated. The results showed that treatment of 10% biochar with C/N 25 improved composting process and the compost quality (higher seed germination index), while 15% biochar showed negative effects. Among low C/N ratio treatments, 10% biochar showed better performance in reduced phytotoxic nature (NH_4^+-N＜700 mg/kg), and good quality compost (seed gemination index＞80%) in a reasonable duration (28 days). Furthermore, adopting co-composting at low C/N ratios with 10% biochar could reduce the requirement of bulking agent and also produce good quality compost.

Keywords: *Food Waste Digestate, Composting, Biochar, C/N ratio*

Introduction

Recently, anaerobic digestion, a biological process to convert organic waste into bioenergy along with methane production, is widely used in food waste treatment [1]. In addition to biogas, food waste digestate (FWD) is a by-product of anaerobic digestion, consisting of the partially degraded matter, also regarded as a kind of organic waste [2]. Nowadays, as more and more anaerobic digestion plant built to satisfy the increase of food waste resulting in a high density of FWD. FWD is limited application by the presence of the high concentration of nitrogen compounds, mostly in ammonium (NH_4^+) form, and yet it is kinds of the superior organic fertilizers. However, environmental-related problems such as ammonia (NH_3) emission, odour emission, greenhouse gas emission, and phytotoxin occur when FWD is applied directly [3]. So, there is an urgent need to call for suitable environmental and economical technologies for FWD treatment.

Existing research shows that composting is a biological treatment technology suitable for FWD. The metabolism activity of the microbial community during the composting can achieve further decomposition of FWD and carbon stabilization. The mature compost as a final product can be served as a soil conditioner with capabilities of the plant growth enhancement [4]. During the composting, the pH, moisture content, temperature, aeration rate, particle size, and C/N ratios are the critical factors that should be controlled to attain mature compost [5]. The initial C/N ratio is one of the most important factors influencing the composting process and final product quality. The C/N ratio between 25 and 30 is usually recommended as an optimum ratio for the beginning of composting. Sawdust can be used as an ideal bulking agent to adjust the C/N ratio [6 ; 7]. According to our previous study, C/N 25 is the optimum for FWD composting, however, a large amount of the sawdust is required to reach the optimized C/N ratio, due to the high concentration of nitrogen-containing compounds of FWD [8]. A large amount of sawdust regulation were in consequence of a higher operation cost, reduction of the treatment capacity, and the lower quality of the compost because of nutrient dilution [8]. In terms of the cost issue, the low C/N ratio is remaining to be considered to work on the full scale of FWD composting. Recent studies have shown that composting can be implemented effectively at a low C/N ratio of 15, but along with increasing nitrogen loss [9; 10].

Biochar produced from biomass after high-temperature pyrolysis [11]. With high porosity and high surface area, biochar can provide a niche for the microorganism community in composting, thus enhance the overall performance of composting [12]. The biochar dosage is the key factor that directly changes the biodiversity and community of the microorganism of the composting process. Refer to our previous study, 10% biochar is the optimum dosage for the FWD at C/N ratios 25 [8]. As the majority of the studies focus on the application rate between 5% and 10% [12], much less is known about the performance of the high dosage biochar application and it needs more evidence.

However, no study has been designed to address the interaction between biochar dosage and C/N ratio for composting of FWD simultaneously. It is hoped that this research will contribute to a deeper understanding of the FWD composting at a low C/N ratio with biochar addition. Consequently, the research aims to investigate the effect of biochar addition on food waste digestate composting at C/N ratios 15 (low) and 30 (high); 1) to determine the effect of biochar dosage with 10% and 15% (dry wt.); 2) to assess the compost maturity and quality.

Material and methods

Composting material

The food waste digestate and the compost were collected from the O · PARK, Hong Kong. Selected properties of the raw materials are presented in table 1. Biochar was made from coconut shell (pyrolysis at 900 ℃) with in 5 to 10 μm pore size. Particle size was screened between 1mm - 3mm for composting.

Table 1. Selected physicochemical properties of the food waste, compost, sawdust and in the study

Parameters	**Food waste digestate**	**Compost**	**Sawdust**
Moisture content (%)	81.09	39.49	11.12
Total organic carbon (%)	28.31	25.29	43.67
Total Kjeldahl nitrogen (%)	6.3	4.9	0.26
C/N ratio	4.49	5.16	167.96

Composting experimental design

Two groups of different C/N treatment were set for the study. One group was low C/N ratio set at 15, another group was high C/N ratio set at 30. According to the different C/N ratios, the food waste, sawdust and compost were mixed in the ratio of 1:2.4:0.7 for low C/N ratio group, while 1:8:1.2 for high C/N ratio group (dry weight basis, w/w). 0%, 10% (0.32 kg) and 15% (0.48 kg) biochar based on dry weight added into two different C/N ratio groups, respectively. The 0% biochar treatment was severed as the control for each group. 1 kg plastic beads were added in each reactor as bulking agent. The initial moisture content was 60%. About 8 kg of composting mixture was prepared for each treatment for 42 days in 20-bench-scale composting reactors. The description of the composter referred to the previous study [13]. The process of composting was controlled by the programmed system.

Physiochemical analysis

The temperature was continuously monitored by temperature probe while CO_2 emission was tested by WMA-2 CO_2 gas analyser and recorded by the AIDCS System. Moisture content was adjusted to maintain at around 60% before sampling. About 200 g sample was collected from each treatment on day 0, 3, 7, 10, 14, 21, 28, and 42. All the analyses were performed duplicate and followed the standard methods [14]. 20 g of fresh samples were extracted with deionized water at 1:5 ratio in dry weight basis (w/v) for 1 h, then suspensions wil be used to measure pH and electrical conductivity (EC) by using Orion 920 ISE pH meter and Orion 160 conductivity meter, respectively. Then, the suspensions were centrifuged at 13,000 rpm for 10 min and filtered through 0.45 μm membrane filter for the determination of extractable ammonium (NH_4^+-N) and seed germination index (GI). For the test of GI, 5 ml of each testing solution were pipetted to a sterilized petri-dish placed with a Whatman #1 filter paper. Ten cress seeds (*Lepidium sativum.*) were put in each petri-dish and incubated in a dark condition for 72 h at 20-25 ℃. Seed germination index were evaluated by counting the number of germinated seeds and the length of the root radical.

Results and Discussion

Generally, moisture content below 40% causes biologically dormant. Moisture between 40% to 60% is optimum for the composting. According to the table 2, the moisture content of all the treatments were controlled at 40% to 60% moisture content, which were favorable for the composting process.

As a result of biodegradation of organic compounds with heating energy production, the temperature of all treatments increased to 55 ℃ in less than 3 days (data not shown). Moreover, the decrease of the temperature result from a depletion of organic matter. Low C/N ratio treatments showed a longer thermophilic phase than that of the high C/N ratio treatments since the high amount of the digestate constituent required longer decomposed period.15% biochar addition treatments induced the longest thermophilic phases in the same C/N ratios group.

The pH of raw mixture was alkaline because of the presence of the ammonium from the digestate. A reduction of pH in the initial stage and an increase of pH in the later stage were observed in both C/N ratio groups possibly due to the organic matter degradation result in the CO_2 production and protein or organic nitrogen degradation result in NH_3, respectively. Compared with the pH on initial days, pH value in all treatments dropped. The final composts pH was in the range from 7.0 to 8.5, reaching the standard [14].

Electrical conductivity (EC) reflects the salinity of the composting matrix. Compared with high C/N ratio group, low C/N ratio group started with higher EC value. EC values were decreased slower in the later stage as a result of precipitation of mineral salts. The final value of EC was from 5 to 6 showed slightly higher than the salinity tolerant limit set lower than 4 mS cm^{-1} for sensitive crops [15]. As for the high C/N ratios group, with the initial lower EC value, fluctuation in the first 2 weeks were observed then followed by a reduction in the 10% and 15% amendment treatment, yet an increase induced in control treatment, leading a satisfactory EC and being close to 3 mS cm^{-1}. The final value of the control one without biochar amendment was higher than that of the 10% and 15% biochar amendment. It indicated that biochar showed a significant effect with regard to the reduction of salinity in the compost obtained.

The NH_4^+-N concentration decreased dramatically at the thermophilic stage and then decreasing slowly in the late stage. The high content of NH_4^+-N in the thermophilic phase was due to the high concentration of NH_4^+ of FWD. Thanks to the high temperature and high pH, a steep descent of NH_4^+ concentration occurred as an outcome of high amount of NH_3 emission during the thermophilic phase. The later reduction in NH_4^+-N concentration was likely attributed to the ammonia volatilization as well as nitrification and denitrification [16]. The concentration of NH_4^+-N of final product in all treatments met the standards. The control one in the high C/N fell to lowest among each C/N group, followed by 10% and 15% biochar treatments. It suggested that high biochar addition perhaps adsorbed more NH_4^+-N and remained within the compost.

SGI could be one of the reprehensive parameters to show the quality of the compost. After 42 days, all of the compost achieved over 80% SGI, reaching the standard. High C/N ratio treatments with 0% biochar and 10% biochar addition were able to reach the standard requirement on day 7 (data not shown), but the final product the 10% biochar treatment was similar to the 0% biochar treatment. 15% biochar treatment needed 21 days to get mature. Meanwhile, the low C/N ratio treatments with 0% biochar took 21 days to reach over 80% SGI, while it cost 28 days for 10% biochar to reach the standard. And the 15% biochar finally needed 42 days to get mature, which indicated that high biochar dosage may inhibit the seed germination. The SGI result was consistent with other of the NH_4^+-N concentration.

Table 2. The properties of the compost

Treatments		Biochar dosage	Moisture content （%）	pH Value	EC Value (mS cm^{-1})	NH_4^+-N (mg/kg dw)	SGI (%)
Initial day	low C/N ratio	0%	48.23	8.17	7.97	3992.36	1.13
		10%	51.36	8.06	7.97	3437.69	2.12
		15%	45.94	8.51	6.29	3551.34	1.50
	high C/N ratio	0%	45.48	7.56	3.84	1608.41	5.64
		10%	44.57	8.10	3.62	1661.25	6.02
		15%	45.62	8.19	3.14	1419.40	11.28
Final day	low C/N ratio	0%	52.59	7.57	5.38	484.95	144.44
		10%	49.50	7.88	4.26	498.10	165.45
		15%	41.37	7.52	5.02	651.60	88.36
	high C/N ratio	0%	57.86	6.95	4.86	25.09	166.67
		10%	56.54	7.96	3.25	106.20	138.10
		15%	49.52	7.66	2.95	509.30	82.54

Standard values, HKORC [14]	/	25%-35%	5.5 - 8.5	/	< = 700	80.00

Conclusions

The results showed that the treatment of 10% biochar with C/N 30 improved composting process (shortest mature phase) and the compost quality (higher seed germination index), while 15% biochar showed negative effects. Among low C/N ratio treatments, 10% biochar showed better performance in phytotoxic nature reduction (NH_4^+-N＜700mg/kg), and good quality compost (seed gemination index＞80%) in a reasonable duration (28 days). Furthermore, adopting co-composting at low C/N ratios with 10% biochar could reduce the requirement of bulking agent and also produce good quality compost.

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CHARACTERIZATION OF THE DISTILLERY SLUDGE BASED COMPOST AND VERMICOMPOST

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Distillery industrial sludge mixed with cow dung, vegetable waste and saw dust in various proportions was subjected to aerobic composting (with and without earthworms) and vermicomposting (with *Eisenia fetida* and *Eudrillus eugeniae*). It was observed that distillery industrial sludge vermicompost (DSV) and vegetable waste vermicompost (VV) resulted in a reduction in organic carbon and an increase of nitrogen and phosphorous during the vermicomposting process indicating that *Eisenia fetida* and *Eudrillus eugeniae* helped in fast conversion of distillery industry sludge into a value-added product in 120 and 42 days respectively.

Micrococcus sp., Klebsilla sp., Pseudomonas sp., Coryne bacterium sp., Staphylococcus sp., Citrobacter sp., Salmonella sp., Enterobacter sp., and Proteus sp., were identified and their enzymatic activities were recorded. *Pseudomonas* sp, *Klebsilla* sp., and *Micrococcus* sp., exhibited amylase activity, *Coryne bacterium* sp., exhibited protease and lipase activity, Pseudomonas sp., *Klebsilla* sp., *Micrococcus* sp., *Coryne bacterium* sp., *Staphylococcus sp., Citrobacter sp., Salmonella sp., Enterobacter sp., Proteus sp.,* showed cellulase activity. Experiments confirmed that both Distillery sludge (DSV2) and Vegetable waste vermicompost (VV1) were good nutrients for plant when compared with others. The sludge conversion was supported by the addition of vegetable waste and sawdust.

Keywords: *Eisenia fetida, Eudrillus eugeniae, Distillery sludge, Vermicompost.*

PERFORMANCE OF BLACK SOLDIER FLY LARVAE FOR MANURE COMPOSTING

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The increasing number of livestock farms has led to a great deal of manure generation, and its improper treatment results in threats to the environment. Black soldier fly larvae (BSFL) was considered as a promising agricultural generator which outputted protein and fat used in livestock production, BSFL manure in agricultural production. Three types of manure [chicken (T1), pig (T2), and cow (T3)] were inoculated with BSFL (1.2:7 ratio on fresh weight basis), three types without BSFL were used as control (T4, T5, and T6), and both were composted for 9 days. The results showed that the BSFL composting reduced the nitrogen by 6.08-14.37% compared to initial materials, but the value of total phosphorous and potassium were increased greatly by the mass concentration. And BSFL could promote the generation of humic acid and compost maturation, only T1 and T2 treatments enhanced the mobilization of Cu and Zn. BSFL decreased the proteinaceous materials and promoted the aromaticity of manure, T1 and T3 treatments were more prone to humifaction than T2. Meanwhile, BSFL amendment were significantly reduced pathogenic bacteria abundance, especially, the genus of *Bacillus* and *Enterococus*. Therefore, the BSFL could be added as a high-efficiency transformation agent for converting organic manure into stable compost, especially in developing countries.

Keywords: *Black soldier fly larvae, Manure, Composting, Humifaction, Pathogenic, Bacteria.*

COST-EFFICIENT COMPOSTING OF FOOD WASTE AND GARDEN WASTE WITH URBAN HOMOLOGY: ROLE OF MIXING PROPORTIONS AND PROCESS PARAMETERS

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In recent years, rapid economic development and population growth have significantly increased food consumption and thus the generation of food waste in urban areas. As a homologous waste with food waste, urban garden waste has also dramatically produced with the increment of urban green land. Co-composting of these two wastes with same source can efficiently overcome the transportation cost challenging that agricultural wastes, such as cornstalk and spent mushroom substrate, need to experience long-distance transportation from rural to urban before composting with food waste. However, co-composting performance of food waste and garden waste is still known.

This study investigated the effects of mixing proportions and process parameters (i.e. moisture, aeration rate and pH) on composting performance during food waste composting with garden waste as bulking agent. Gaseous emissions and maturity were analyzed to evaluate composting performance. Results show that more than 15% of garden waste (wet weight) added into composting materials could efficiently decline the emission of greenhouse and odours gases and maturity during composting. On the other hand, all process parameters designed in this study could pose notable effects on gaseous emissions. A relatively lower emission of methane and hydrogen sulfide was observed for the treatment with medium aeration intensity (0.24 $L \cdot kg \cdot DM^{-1} \cdot min^{-1}$) than low and high aeration treatments. Moreover, the composting materials with 65% moisture content could be considered as an optimal selection to control gaseous emissions, particularly greenhouse gas. Given the characteristic of low pH for the food waste to postpone the composting progress, optimizing initial pH of composting materials was essential. Our results indicated that enhancing initial pH by calcium oxide (CaO) was significantly efficient to accelerate the achievement of thermophilic stage of composting. More excellently, Addition of 1.5% of CaO (wet weight) into composting materials could considerably alleviate greenhouse gas and ammonia emissions. Results from this study provide unique and valuable insights to the cost-efficient and environmental-friendly management of typical unban wastes that food waste and garden waste.

Keywords: *Composting, Food waste, Garden waste, Mixing Proportions, Process Parameters.*

TOXICITY AND HISTOPATHOLOGICAL EFFECT OF DISTILLERY INDUSTRIAL SLUDGE ON THE EARTHWORM *EUDRILUS EUGENIAE*

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Increasing population accompanied by industrialization has increased the solid waste disposal problem in many of the developing countries like India. It is a well-known fact that the disposal of the waste water and sludge produced by anthropogenic sources is becoming a serious problem of concern. The sludge generated in enormous quantity creates the problem of safe disposal. Sludge is an inevitable, hazardous and odorous by-product from waste-water treatment plants and many industries too. One such industry happens to be the distillery industry producing sludge, which requires safe disposal. But the distillery sludge is reported to contain the nutrients, as it is a by-product of the sugar industry. Though it is accepted to be a suitable soil conditioner, higher concentration of heavy metals limits its continuous use for field crops. Hence, in this study we tested the feasibility of the earthworm *Eudrillus eugeniae, commonly called as African night crawler* to detoxify the distillery sludge through Vermitechnology.

Distillery sludge was subjected to vermicomposting after addition of carbon (cow dung) and nitrogen (saw dust) sources. The concentration of Cd, Zn, Fe, Mn and Cu were checked in all the samples. The sludge exposed earthworm was subjected to metallothionein protein estimation and their histology was also studied.

A reduction in the heavy metal (Cd, Zn, Fe, Mn and Cu) content accompanied with its increase in the earthworm tissues was noticed. Following which the metallothionein content also has shown a considerable increase in the tissues of earthworm which worked on distillery sludge. Further damage to the earthworm tissues was confirmed based on the histology reports. The DSV1 (Distillery Sludge Vermicompost set 1) exposed earthworms were highly affected when compared with earthworms exposed to DSV2 (set 2).

Keywords: *Distillery sludge, Vermicompost, Heavy metal, Histology.*

POTENTIALS FOR MITIGATING GREENHOUSE GAS EMISSIONS THROUGH DIETARY CHANGES AND FOOD WASTE PREVENTION: CASE STUDY MACAU

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Dietary changes and food waste prevention are significant factors in view of mitigating Green House Gas Emissions. They “can reduce demand for land conversion, thereby potentially freeing land" (IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse gas fluxes in Terrestrial Ecosystems Summary for Policymakers Approved Draft Subject to copy edit and layout, 7th August 2019, p.3 and p.20). From 2010 to 2016, global food loss and waste contributed 8-10% of total anthropogenic GHG emissions, and currently, 25-30% of total food produced at the global level is lost or waste (IPCC 2019). Strategies and policies that aim at reducing food loss and waste and influence dietary choices, improve overall food security, serve low emissions trajectories, and thus have the potential to contribute significantly to climate change adaptation and mitigation. Also, the COP 21 Paris Agreement of 2015 aims at reducing per capita global food waste at the retail and consumer levels by 50 % until 2030 (Goal 12.3. https://sustainabledevelopment.un.org/).

The paper investigates the respective potentials of mitigation of GHE by dietary changes and food waste prevention in Macau S.A.R., China. It uses and compares official data regarding consumption and MSW, results of quantitative assessments (e.g. Waste Audit), and systematic qualitative reflection and evaluation in view of mitigation potentials.

Keywords: *Sustainable Development, Dietary Changes, Foodwaste-Prevention, Mitigation*.

ESTIMATION OF GREENHOUSE GAS- N_2O EMISSION VARIATION BY DENITRIFICATION BACTERIA DURING OXYGEN DEPLETION IN BOHAI SEA OF CHINA

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With the extending of coastal and ocean hypoxia area under the impact of anthropogenic activities, the nitrogen cycling relating to the greenhouse gas emission are assumed to be improved under oxygen depletion. Therefore, microbial variations under oxygen depletion is essential for deciphering the related biogeochemical cycling. In this study, a time-scenario sampling was designed along one transect from inshore to offshore in the seasonally-formed oxygen depletion zone of the northwestern Bohai sea from June to August in 2018 at a twenty days interval. Aiming to investigate the N_2O emission process, the diversity and distribution of denitrification bacteria were revealed by high-throughput sequencing, with special focuses on *nos*Z gene in oxygen depletion region. In general, the heterotrophic bacteria became dominant in the bottom water and sediment of Bohai coastal area, where the concentration of nitrate and nitrite were also accumulated. Based on the sampling time scenario, distinct distribution pattern of community composition was observed for June and July, but overlapped in some degree for the two August samples. The vertical pattern displayed a more stable stratification along with the proceeding of oxygen depletion. Environmental parameters including depth, salinity, Chl *a*, nitrite and pH are impacting the distribution significantly, while dissolved oxygen and nitrite are the main impacting factors for the abundance of *nos*Z encoded denitrification bacteria ($p<0.05$), which further confirmed the assumption that the extending of hypoxia will improve the denitrification-related N_2O production. In addition, varied origins of denitrification bacteria were revealed as the OTUs from this study were alignment with OMZ sea water, sediment as well as soil from terrestrial environment displayed by phylogenetic analysis. Our study provides the first inspection into the microbial variations related to N_2O emission under the depletion of dissolved oxygen in Bohai Sea, which may help to understand the consequence of global warming in the ecosystem.

Keywords: *Greenhouse gas, N_2O emission, Denitrification bacteria, Oxygen depletion, Bohai sea.*

BIOCONVERSION OF MUNICIPAL SOLID WASTE TO COMPOST

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Composting is frequently utilized for enhancing the quality of the soil through incorporating organic compounds into the soil. Due to various environmental issues associated with municipal solid waste (MSW) the preference of using municipal solid wastes for the process of composting has received a lot of interest in recent years. The continuous increase in waste will proportionally increase the total volume of waste generated which results into loss in biodiversity and elevation in pollution potential of pollutants. Building awareness among the people for utilizing bio products is a considerable need. In this study, we tested traits of compost which was obtained from municipal solid wastes as a raw material. By employing the standard methods assessment of compost was executed. The organic fraction of MSW consists of 26% vegetables, 23% cooked waste, 15% coconut, 7% paper, 12% wood and 8% leaves. The results also revealed presence of heavy metals such as 8.48 mg/kg lead, 2.71 mg/kg nickel, 179.41 mg/kg zinc in the compost. It showed 0.9860 g/cm^3 bulk density and 2.3 dsm^{-1} electrical conductivity.

Keywords: *Municipal solid waste, Organic fraction, Feedstock, Composting traits, Heavy metals.*

CONVERSION FOOD WASTE AND SAWDUST INTO BIOFERTILIZER EMPLOYING BLACK SOLDIER FLY LARVAE (*DIPTERA: STRATIOMYIDAE*) UNDER THE OPTIMIZED CONDITION

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Food waste (FW) was greatly generated by people consumption, which need an urgent method to recycle. The black soldier fly larvae (BSFL) was a quick waste manager with potentially to decrease the financial cost from FW treatment. To assess the impact of BSFL on conversion FW and sawdust into biofertilizer through the parameters of maturity, nutrient transformation and volatile fatty acids (VFAs). Sawdust was utilized to adjust moisture and make the separation larvae from residue easily. FW and sawdust were employed by BSFL (6.5:0.5:1.2 ratio on fresh weight basis) as T1 and without BSFL called control (T2), while moisture content for FW and sawdust was 86.57% and 5.98%. Results illustrated that BSFL declined the composting time and only 9 days. Compared with initial mixture materials, T1 decreased organic matter (OM), total kjeldahl nitrogen (TKN) and VFAs in 11%, 23% and 46%, which reduced the OM, TKN and VFAs from 97.41 to 85.96%, 23.01 to 17.77 g/kg and 3.12 to 1.69 g/kg. In contrast, the T1 increased the total phosphorous (TP) and total potassium (TK) in 3.8 folds and 5 folds. The value of pH and EC reached at 4.27 and 1100 µS/cm, and the germination index (GI) attained to 70.69%. Therefore, BSFL played a vital role in FW and sawdust recycling, especially reduced conversion time and made the final separation of larvae and substrate easily, saving labor costs.

Keywords: *Black soldier fly larvae, Artificial food waste, Sawdust, Composting, Biofertilizer.*

ELUCIDATING THE OPTIMUM ADDED DOSAGE OF DIATOMITE DURING CO-COMPOSTING OF PIG MANURE AND SAWDUST: CARBON DYNAMICS AND MICROBIAL COMMUNITY

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In order to investigate the optimum added dosage of Diatomite (DM) during co-composting of pig manure and sawdust, six dosages of DM (0%, 2.5%, 5.0%, 10%, 15% and 20%) were added into initial mixtures for 42 days aerobic composting. Results showed that adding DM was beneficial for reducing CH_4 emission and greenhouse gas emission equivalent (GHGE) value by 15.34−23.95% and 14.33−69.08%, respectively. Meanwhile, the main contributor to GHGE value was N_2O (58.76−75.98%), followed by CH_4 (17.22−29.16%) and NH_3 (6.38−13.36%) in order. Moreover, the maximum values in degradation of total organic matter and formation rate of humic substances were 20.39% and 75.11% in 10% DM added treatment (T3), respectively. Furthermore, the increasement of spectral parameters including the specific UV absorbance at 254 nm ($SUVA_{254}$), the specific UV absorbance at 280 nm ($SUVA_{280}$) and relevant parameters of Fourier transform infrared in control were facilitated by DM amendment. Additionally, the higher values in relative abundance of Proteobacteria (50.98%) and Bacteroidetes (12.73%), and related metabolisms like carbohydrate metabolism and amino acid metabolism, as well as lower value of Methane metabolism reported in T3, supported the difference in CH_4 and humification of two treatments. Concluded together, DM was an eco-practical additive to improve quality of end products and reduce potential risks, and the best one was 10% added treatment based on the dry weight in this study.

Keywords: Pig Manure, Sawdust, Co-composting, Diatomite, Microbial Community.

CHARACTERIZATION OF FOOD WASTE FOR VALUE CREATION

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Rapid population growth, combined with increased industrialization, has exacerbated the issue of solid waste generation. Improper management of solid waste has negative environmental consequences, which puts the public health at risk and introduces several other socio-economic problems. Food waste is one of prime solid waste generated. Food waste management is a major concern globally. High moisture content of food waste make it landfill and incineration incompatible, causing secondary ecological footprint and reducing treatment quality. In the effective management of food waste, a set of solutions may be applied and can be evaluated time to time. In a circular economy framework food waste has great potential to be used for value creation such as biofuel, biosurfactants, biopolymers and natural nutrients production. It can be processed through various biological technologies such as anaerobic digestion (AD) and composting. Before using food waste for any process, it is important to characterize it. In this study we have reported characterization of food waste by standard methods for various physiochemical parameters. The food waste showed 83.39% moisture content, 3.07% ash content, 0.31% chloride, 1.03% nitrogen, 7.848% carbon and 0.654% hydrogen. Looking to the results it is concluded that it can be used to prepare compost.

Keywords: *Food waste, Circular economy, Ultimate analysis, Ecological footprint*

THERMOPHILIC DEGRADATION OF VEGETABLE WASTE USING ROTARY DRUM COMPOSTER AND EFFICACY OF ROTARY DRUM FOLLOWED BY VERMICOMPOSTING

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ABSTRACT. Vegetable waste (VW) management is major requirement of all communities. Among all the composting techniques, the rotary drum composting (RDC) requires less time than 20 days for decomposition, and thermophilic degradation was in initial 7 days of the composting. In this study, composting of VW was studied using RDC and RDC followed by vermicomposting (VC). Waste was subjected to RDC for 20 days and in the second run, the RDC was used until day 7 and substrate then fed to VC employing *Eisenia fetida* for 20 days. Temperature rise on the second day was 51.5^{O}C in the RDC. The RDC and combination of VC after the RDC increased the nitrogen content to 2.2 % and 4.15 % respectively from 1.4 %. The volume reduced by 60±5 % on 27th day of RDC followed by VC, which suggests that the technique suits best for urban bodies in management of VW.

Keywords: *Vegetable waste, Rotary drum composting (RDC), Vermicomposting (VC), Bio-waste.*

Introduction

India is the second populous country and the second-largest producer of fruits and vegetables. The average amount of fruits and vegetable wastages is estimated to be 5.8 to 18 percent of total production [1]. The generated waste especially in urban areas was getting dumped into landfills. Several works of literature were on composting but major of them focus on centralized composting and involves windrow and agitated pile composting processes. The duration of composting was nowhere reported as 20 days which is in case rotary drum composting is possible [2,3,4]. Rotary drum composting is one of the promising techniques in terms of the optimized duration of composting. Vermicomposting produces superior quality compost. During vermicomposting, however, earthworms consume organic waste and convert it to vermicast, a humus-like substance that contains N, P, and K in forms that are more readily accessible to plants than those found in the original raw substrate [5,6,7].

It is to be understood that to manage huge community vegitable loads it needs to have an optimized composing duration and promising results. In literature, it has been found that RDC can compost the substrate in 20 days and vermicomposting needs acclimatization of 15 to 20 days and that is the reason VC is time taking process. In this study rotary drum compsting ,and vermi composting were combined to get good quality compost in an optimum duration.

Material and Methods

Substrate preparation

Vegetable waste was collected from the Amingaon market near Campus. For rotary drum composting (RDC) a 550L capacity in-vessel composter and for vermicomposting bamboo baskets of 3 kg capacity were used. The earthworms were cultured in the laboratory and healthy adult earthworms were used for this study. The material was shredded into 2-3cm size and mixed thoroughly with microbial inoculum and bulking agent before feeding to rotary drum composter. The RDC was studied for 20 days and the RDC followed by vermicomposting was studied for 27 days for this study. The initial characterization of the vegetable waste is shown in Table 1.

Fig. 1. The methodology adopted and the steps involving the composting process.

Table 1. Characteristics of the materials used.

Parameters	MC (%)	pH	EC (mS/cm)	VS (%)	C/N ratio
Vegetable waste	90.12 ± 2.22	5.63 ± 0.02	2.35 ± 0.04	77 ± 3.1	16.10 ± 2.3
Cow Dung	80 ± 2	7 ± 0.1	3.3 ± 0.01	91 ± 3	28 ± 0.5
Saw dust	14 ± 1.2	6.7 ± 0.1	4.4 ± 0.1	20 ± 2.2	330 ± 15.5

Experimental setup

In-vessel composter of 550-liter capacity was used for RDC and for VC a bamboo reactor of 5kg capacity was used. The in-vessel composter can be seen in FIG. 1. *Eisenia Fetida* earthworm culture was procured from the Krishi Vigyan Kendra Guwahati and cultured in the laboratory.

Results and Discussion

Physico-chemical analyses

Temperature and moisture content

The Temperature generated due to microbial action on the organic matter is one of the significant indicators of organic matter destruction. The temperature raised to thermophilic range in the initial 24 hours of the composting and was until day 7 of the composting duration. It indicated the active thermophilic bacterial action o substrate and which is the major advantage of the RDC reactor.

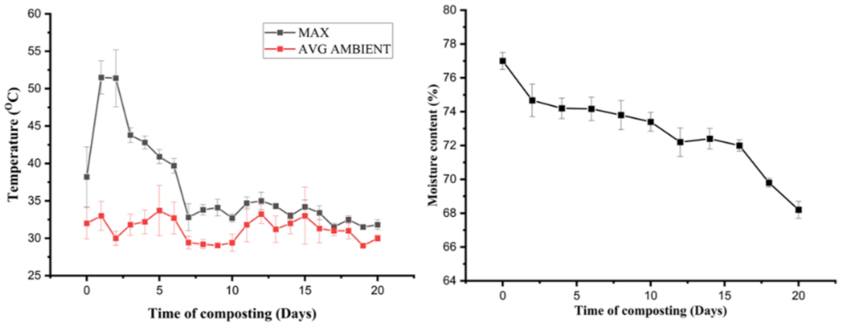

Fig. 2. Variation in the temperature and moisture content of the substrate throughout the composting period.

pH, electrical conductivity (EC), and Ash content

The pH attained towards a basic value by the end of composting. The EC tends to decrease from 8.1 to 6.3 mS/cm. decreasing EC value indicates the conversation of salt concentration of the substrate.

Total solids and Total organic Carbon

The volatile solid destruction explains the degradation of the organic matter which is faster in VC composting post RDC compared to only RDC. The total organi carbon follows the same trend of volatile solids and it is a clear measure of active degradation of organic matter.

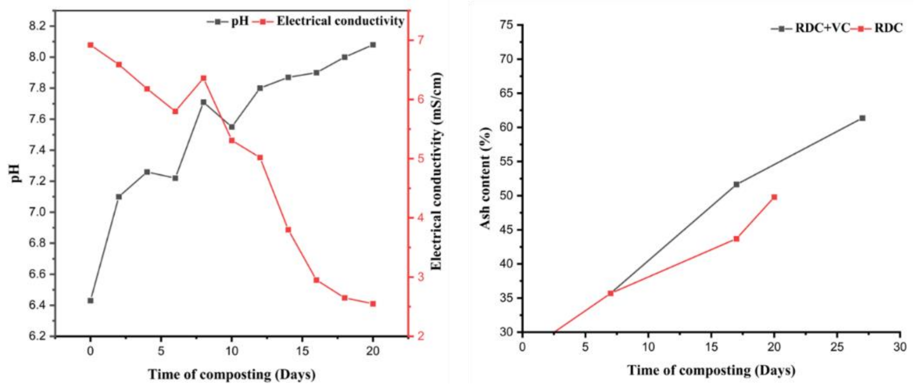

Fig. 3. Variation in a) pH and EC, b) Ash content.

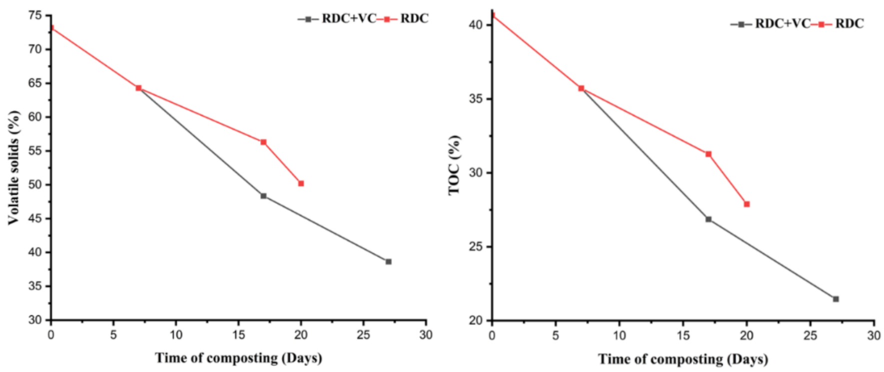

Fig.4. Variation in a) Volatile solids and b) Total organic carbon.

Nutrient Properties

Table 2. Nutrient properties of the compost.

Parameter	Unit	INITIAL	FINAL	
			RDC	RDC+VC
Total Nitrogen	%	1.8 ± 0.1	2.8 ± 0.25	4.15 ± 0.2
Total Phosphorus	g/kg	3.2 ± 0.06	6.65 ± 0.21	15.1 ± 0.25
Potassium	g/kg	12 ± 0.08	24 ± 1.2	30.5 ± 1.25
Sodium	g/kg	1.26 ± 0.12	2.8 ± 0.23	3.3 ± 0.63
Calcium	g/kg	7.15 ± 0.3	10. 5 ± 0.12	14.3 ± 0.25

The composting process makes the substrate gain nitrogen and other nutritional properties. The NPK value of the initial substrate and the final compost produced using RDC alone increased from 1.8 %,3.2 g/kg and 12 g/kg to 2.8 %,6.65 g/kg, and 24 g/kg and VC can further increase NPK to 4.15%, 15 g/kg and 30.5 g/kg accordingly. The final compost produced can be utilized for various purposes.

Conclusions

A maximum temperature of 51^{O}C was seen on the second to the third day of composting, temperature profile after 7th day of the RDC shows that reactor was efficient in the initial 7 days. Higher microbial inoculum helped to achieve active thermophilic degradation in RDC. Combination of RDC and VC was proven best technology in producing nitrogen-rich compost. The *Eisenia fetida earthworm* culture shown high rate of productivity in terms of increase in biomass (18 juveniles and 22 cocoons per 100 gram of final compost). The volume reduction of 40 ± 5 % was noted in both of RDC and RDC followed by VC. Higher volatile solids reduction was observed in combined technique (RDC+VC). The total nitrogen increased from 1.8 % to 4.15 % by the end of VC and it was to be 2.8% by using only RDC.

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TRANSFORMATION OF INTRUSIVE WEED AGERATUM CONYZOIDES INTO A VALUE ADDED PRODUCT THROUGH ROTARY DRUM COMPOSTING

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ABSTRACT. The major constituents on earth surface are plants, terrestrial weeds, animal kingdoms etc. The majority of the problem lies in managing the terrestrial weeds, as they pollute the habitats of plant kingdom by their allellochemical nature. The study aimed at managing the intrusive weed Ageratum conyzoides biologically through 550 L Rotary drum composter (RDC) in the ratio of 1:1 (substrate : cattle manure). In previous literatures vermicomposting of Ageratum conyzoides was cited and it has its own drawbacks in terms of time constraint. The RDC process of A. conyzoides has took place in 20 days with a fine sieve range of 4mm. The produced compost was analyzed with physiochemical and biological parameters with CO2 evaluation and Oxygen uptake rate as the maturity parameters. In order to access the toxicity parameters, phytotoxicity test was performed in four different stages of composting process using Vigna radiata and Allium cepa as the plant models. The results of maturity parameters suggested to do composting process for 20 days, whereas phytotoxicity assay results revealed that the allellochemicals present in the raw A. conyzoides got reduced significantly in the composting process.

Keywords: *Allellochemicals; Ageratum conyzoides; Rotary drum composter;Compost; Phytotoxicity.*

Introduction

A weed is described as a wild plant that grows invisibly and competes with cultivated plants. The dynamic system in which weeds, the climate, and humans interact can be defined as such (Roberts et al., 1982). Furthermore, weeds are uninvited herbs or plants that flourish in unfavourable locations, causing damage to cultivated crops and resulting in significant economic loss. A weed is a plant that is deemed unsuitable for a specific circumstance, or "a plant in the wrong spot." Unwanted plants in human-controlled environments, such as farm fields, gardens, lawns, and parks, are common examples (Janick, 1986). A plant that is a weed in one context is not a weed in another, and where one species of plant is a valuable crop plant, another species in the same genus may be a serious weed, such as a wild bramble growing among cultivated loganberries, according to taxonomy. Volunteer crops (plants) are treated the same way as weeds in a subsequent crop. Furthermore, these weeds have not only harmed biodiversity but have also harmed human health, degraded the environment's ecosystem, and posed a threat to tourism by colonising tourist areas (Kumar and Prasad, 2014).

The invasion of plant species has changed the dynamics of biodiversity and the composition of species. These weeds have a tendency to degrade machine productivity and are adept at colonising new areas (Bajwa et al., 2016). Weed invasions are one of the most serious threats to the global natural environment, and they are also a major threat to the managed ecosystem. Some countries spend billions of dollars per year on weed control in agricultural fields (Pimentel, 2014).

A. conyzoides is a branched annual herb that grows to a height of 0.15 m to 1 m. The plant's stem is coated in fine white hairs, and its leaves are pubescent with long petioles and glandular trichrome on the opposite side. The plant's root system is "tape." The plants have a corymb of 35 to 60 purple or pink flowers that are self-incompatible (Kong et al., 2001).

The utmost objective of the study is the quality of the compost and the toxicity reduction in the compost through germination assay study. The quality of the compost was justified by monitoring the parameters such as potential of hydrogen, Electrical conductivity, Volatile content, ash content, total organic carbon.

Materials and Methods

Materials and Experimental designs

There were so many composting technologies and every technology has its own drawbacks. From the recent years, in vessel composting techniques has become a boon in managing the organic or green waste into a value added products (Kalamdhad et al., 2008). Rotary drum composting (RDC) is one of the best practices in composting techniques (Kalamdhad and Kazmi, 2009). The total capacity of the drum is 550 L. It is balanced on four rubber rollers and rotates mechanically with its handle, which is attached to a metal stand. The central drum module is 1.02 m long with a 0.76 m diameter and a 4 mm thick metal plate. A 40 x 40 mm angle welded longitudinally within the drum provides sufficient mixing, agitation, and aeration of waste during rotation. Anti-corrosive material is used to cover the inside portion of the tubing. The *A. conyzoides* was collected from the vicinities of IIT Guwahati and chopped into 2 – 3 cm using a shredder and was mixed properly with cow dung as an inoculum. The optimized ratio of substrate to the inoculum was fixed to be 1:1 from various literatures (Devi and Khwairakpam, 2020). The major attributes of *A. conyzoides* are pH, 6.15; EC, 5.1 dS/m; moisture content, 74.5%; volatile solids content, 73.6; Total nitrogen, 1.62%; C/N ratio, 25. The attributes of *A. conyzoides* seemed to be so menacing, which founds to be suitable for biological management. The cow dung was obtained from livestock farm of Guwahati, Assam.

The experiments were performed in triplicates and the total volume fed in RDC of 550 litres was 150 kgs. in the ratio of 1:1 and the drum was rotated with a frequency of once per day.

Sampling procedures

The sampling was done from three different locations of the drum in triplicates in alternative days from 0th day to the 20th day of the composting process for performing physio-chemical and biological parameters. 500 g of sample was collected and half of it was kept at 4 °C for analysing biological parameters and remaining half was kept in 105 °C in hot air oven for 24 hours and was crushed and sieved with 0.2 mm sieve and was kept in desiccator for analysing physio-chemical parameters.

Analytical Methods

The temperature was monitored using a digital thermometer in the 6 hours interval from 0th day to 20th day of composting process. Moisture content was calculated by drying the sample at 105 °C in hot air oven for 24 hours. pH and EC were determined by mixing the sample with distilled water in the ratio of 1:10 (Weight/Volume) and was kept for shaking in horizontal mechanical shaker for 2 hours (Jain and Kalamdhad, 2018). The extracts were filtered and kept for analysis. The method used for measuring ammonium nitrogen (NH4-N) was KCL extraction method (Sudharsan Varma and Kalamdhad, 2014). Stannous chloride method was used for analysing Total kjeldahl nitrogen (TKN). Volatile content and ash content was analysed in muffle furnace at 550 °C temperature. The compost's stability was monitored using CO_2 evolution rate and Oxygen uptake rate (OUR) (Jain and Kalamdhad, 2018).

Phytotoxicity assay

The sample preparation for phytotoxicity assay was performed taking 100g of samples mixed with distilled water in the ratio 1:3 (Weight / Volume) and kept for shaking in horizontal mechanical shaker for 12 hours (Haq et al., 2016). After the filtration, the supernatant was taken and stored in 4 °C for phytotoxicity assay. The conditions for phytotoxicity assay was presented in Table 1.

Table 1. Conditions of Phytotoxicity assay (Haq et al., 2016)

Plant Model	Temperature to be set up	Duration of experiment
Vigna radiata	28 ± 1 °C	5 days
Allium cepa	23 ± 1 °C	5 days

Results and Discussions

The microbial diversity of composting process is based on temperature of the composting process. In the present study, the highest temperature peak was found to be 58 °C on 5th day of composting process, which significantly reports the thermophilic activity phase of composting process (Mohee and Mudhoo, 2005). There was increase in temperature from 1st day of composting process to 5th day, which significantly reports that there was a maximum degradation of organic substrates (Mohee and Mudhoo, 2005). The high temperature may not always mentions about the degradation but degradation depends on the organic substrate used in composting process (Hazarika and Khwairakpam, 2018). Circulation and transportation of gases needed for the various metabolic activities of the microorganisms depends on the moisture content (Liang et al., 2003). The moisture content of the final compost should be in the range of 40% to 60% (Bian et al., 2019). All the moisture content pertained during the composting process in triplicates were in significant levels at $P < 0.05$. The results of temperature profile and moisture content are shown in Fig. 1.

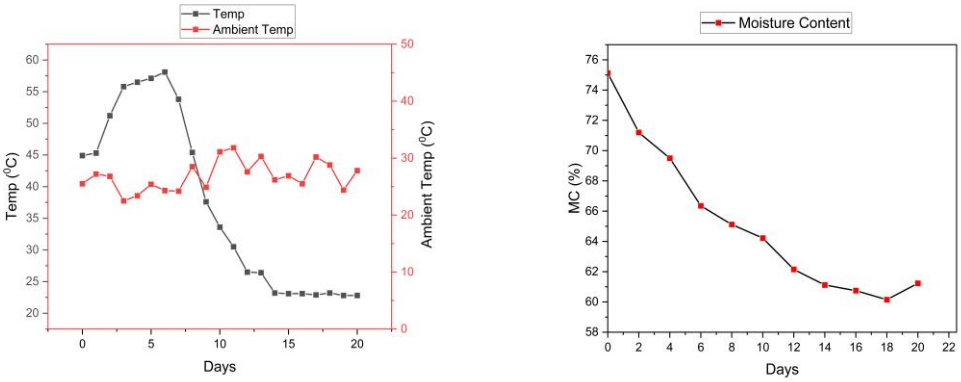


Fig. 1. Variation in temperature and moisture content (Values are mean ± SD of three samples, $P < 0.05$ significant during composting process for temperature and moisture content).

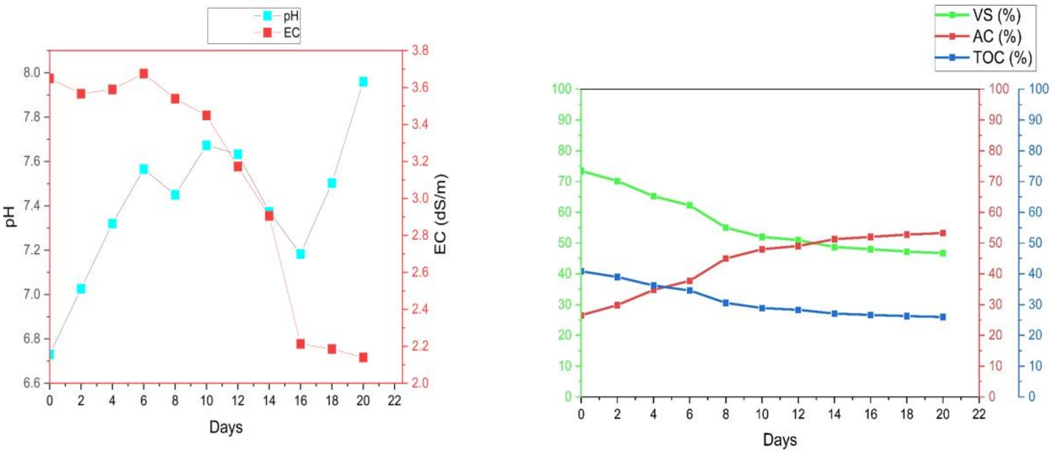
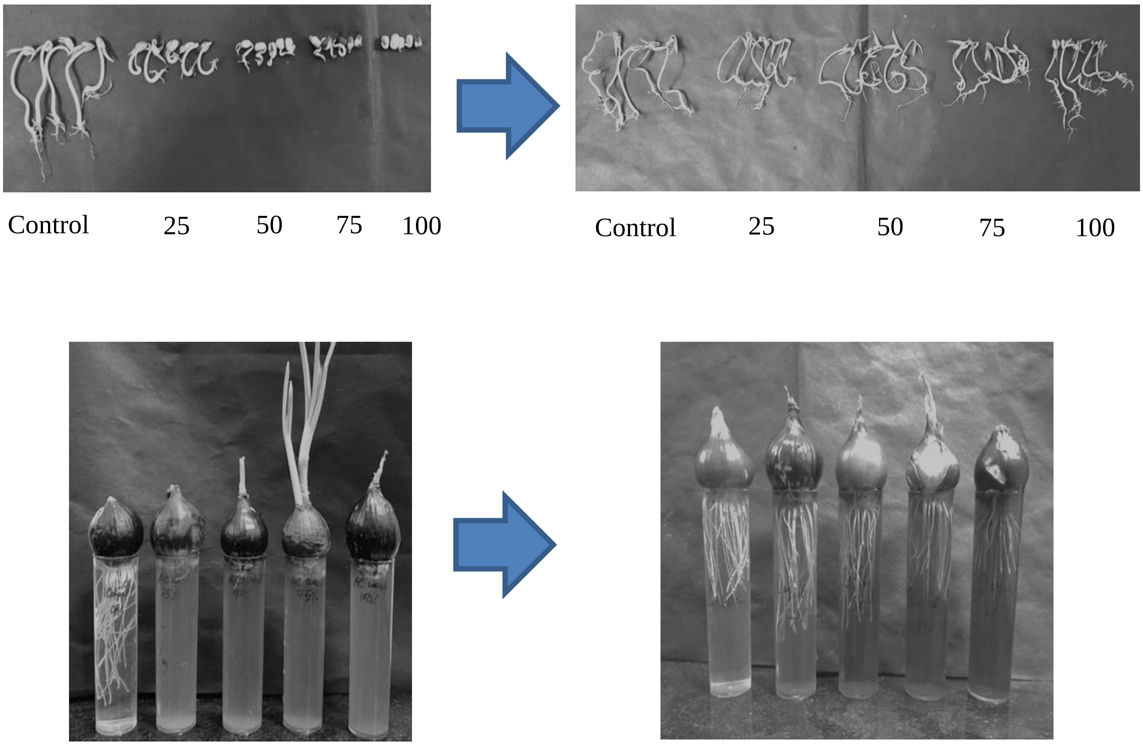


Fig. 2. Variation in a) pH, EC b) volatile solids, total organic carbon (TOC), ash content (Values are mean ± SD of triplicate samples, $P < 0.05$ significant with different mix proportion).

The pH was increased during the composting process, this was due to the formation ammonium ions and self-buffering capacity due to the increase in calcium carbonate (Pramanik et al., 2007). The electrical conductivity (EC) represents the degree of salinity. During the composting process the EC got decreased to 58.10 % in the final compost. The decreasing trend of EC was due to ammonia volatilization (Wong et al., 1995). The variations of pH and EC were reported in Fig. 2. The organic matter degradation in composting process was assessed by determining the volatile solids content and ash content. Organic waste stabilisation and maturation entail extensive humification, a complex series of processes that convert organic matter into refractory organic compounds similar to those found in natural soils (Sánchez–Monedero et al., 1999). The maximum reduction was observed in thermophilic phase, as thermophilic bacteria putrefies organic substrates extensively. The volatile solid reduction was reduced to 66.1 % in final compost respectively, same was reported in Fig. 2.



Raw

Day 20 (Final Compost)

Fig. 3. Phytotoxicity assay of *Vigna radiata* and *Allium cepa.*

Table 2. GI, Root Length and Biomass of *Allium cepa* in *A. conyzoides* & Day 20 compost extract

Root lengths			Biomass Index	
Concentrations (v/v)	Raw AC	Day 20	Raw AC	Day 20
Control	6.5 ± 0.25	5.9 ± 0.11	1.85 ± 0.01	1.88 ± 0.02
25%	2 ± 0.22	6.1 ± 0.22	1.01 ± 0.01	1.92 ± 0.52
50%	1.1 ± 0.15	6 ± 0.31	0.78 ± 0.01	1.84 ± 0.03
75%	0.5 ± 0.09	5.7 ± 0.25	0.45 ± 0.01	1.68 ± 0.09
100%	0.2 ± 0.02	5.1 ± 0.22	0.21 ± 0.02	1.66 ± 0.22

The Total Nitrogen (TN) of the initial and final day of the compost was 2.38% and 3.5%. The results of phytotoxicity assay of both *Vigna radiata* and *Allium cepa* were reported in Fig. 3.

Conclusions

The volume reduction was found to be 46 ± 6 % to the initial volume. The highest temperature peak was found to be 58 ºC on 5th day and the thermophilic stage was observed between 2nd day to 8th day with a temperature range of 54 ºC to 44 ºC. The maximum degradation was observed in the thermophilic phase of composting process. The total nitrogen increment was recorded as 2.38 % to 3.5 %. The phytotoxicity assay showed a greater Germination index variance from 6.1 to 9 between Raw *A. conyzoides* extract and final compost extract in *Vigna radiata*.

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QUALITY ASSESSMENT OF COMPOST OBTAINED FROM MUNICIPAL SOLID WASTE

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On a global scale, a rise in population density, as well as the migration of the population from rural to urban areas leads to generation of a large amount of municipal solid waste (MSW), which causes serious economic, social and socio-demographic problems. Awareness in public is essential for proper MSW management techniques. Municipal solid waste can be used as feedstock for biofertilizer and biogas production employing different techniques such as composting, vermicomposting and anaerobic digestion etc. Composting is reported as cost-effective technique to treat organic fraction of municipal solid waste. It is biological process in which degradable part of municipal solid waste is transformed to a steady material with excellent characteristics for application in soil. This study was conducted by performing the analysis of physicochemical parameters of compost obtained from municipal solid waste. The compost analysis was carried out using standard method (Fertilizer Control Order (FCO)-1985). Physicochemical parameters of obtained compost were compared with standards. For the studied compost C:N ratio, total potash, total organic carbon, total nitrogen, total phosphates and moisture content were 16:8, 0.94%, 60.05%, 1.00%, 0.94%, and 20.02, respectively.

Keywords: *Municipal Solid Waste, Total nitrogen, Biofertilizer, Compost, Total phosphate.*

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Biochar and its Application

FOOD WASTE HYDROCHAR FOR CATALYTIC DEGRADATION OF ORGANIC CONTAMINANT

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Custom-designed carbonaceous adsorbent/catalyst from ‘green’ sources with desired functionalities under science-informed conditions is indispensable to promote the sustainable industrial wastewater treatment. In this study, we prepared hydrochar by hydrothermal carbonization (HTC) of three types of pre-consumer food waste (*i.e.*, lettuce, taro, and watermelon peel) with different components at various temperatures (*i.e.*, 180−240 °C). The performance of food waste hydrochar was examined through the adsorptive removal and peroxymonosulfate (PMS)-initiated catalytic degradation of a representative, recalcitrant organic contaminant, 2,4-dichlorophenoxy acetic acid (2,4-D). The $LHC_{180–240}$ derived from fibre-rich lettuce manifested a substantial 2,4-D adsorption (77.4−88.4 mg g^{-1}) possibly due to intensive partitioning and/or chemisorption, which were dependent on the mesoporous carbon structure with low aromaticity and abundant C−O functional groups. In comparison, HTC of starch-rich taro at a relatively low temperature (200 °C) produced the THC_{200} that displayed a superior catalytic ability (73.5 mg g^{-1}) probably owing to a highly graphitized C domain with low polarity and enriched ketonic (C=O) functionality, which might facilitate radical/non-radical PMS activation. Interestingly, the $WHC_{180–240}$ produced from watermelon peel with moderate-level carbohydrates and low-fibre content presented an improved structure and functional groups (*i.e.*, C−O and C=O), but inhibited the PMS activation for 2,4-D degradation possibly due to interference by its inherent dissolved organic matter. This study provided insightful guidance for tailoring future design of multifunctional hydrochar adsorbent/catalyst for sustainable remediation.

Keywords: *Food waste hydrochar, Catalytic degradation, Organic contaminant.*

BIOCHARS PRODUCTION USING BIOGAS RESIDUE AND THEIR ADSORPTION OF AMMONIUM-NITROGEN AND CHEMICAL OXYGEN DEMAND IN BIOGAS SLURRY

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ABSTRACT. Modified biochars prepared from straw, pig manure, and chicken manure biogas residues were used to adsorb and remove ammonium-nitrogen (NH_4^+-N) and chemical oxygen demand (COD) in waste water. The results showed that at 400°C－600°C, the adsorption performance of the biogas residue biochars increased with pyrolysis temperature, reaching the maximum at 550°C. Biochar from chicken manure biogas residue exhibited strong NH_4^+-N (20.3%) and COD (28.1%) adsorption capacity. Through $FeCl_3$ modification, the Fe content on the surface of the biochar increased, and the adsorption data of the modified biochar conformed well to the pseudo-second-order kinetics and Langmuir model, and the reaction was dominated by chemical adsorption. Fitting calculation revealed that the FeCl3-modified biochar from chicken manure biogas residue showed maximum NH_4^+-N (55.29 mg/g) and COD (186.24 mg/g) absorption capacity. These results indicated that modified biochar from biogas residue could have potential application for the treatment and recycling of biogas slurry.

Keywords: *Biogas residue; Biochar; Ammonia nitrogen; COD; Adsorption*

Introduction

Biogas produced by anaerobic digestion of organic solid waste has triple strategic significance in terms of energy conservation, emission reduction, and resource utilization. The nontoxic treatment and resource utilization of biogas slurry and biogas residue are directly related to sustainable operation of biogas plants. Biogas slurry is a typical wastewater with high contents of ammonium-nitrogen (NH_4^+-N) and chemical oxygen demand (COD). Biogas residues are complex, and research on the characteristics of biochar residues obtained from biogas plants with different raw materials is still limited. For example, the total C and total N contents vary in different biogas residues, and C and N have a significant influence on the properties of biochar as well as on treatment of pollutants in waste water. However, reports on the preparation of biochar with catalytic activity from biogas residues are scarce. Accordingly, the present study used different biogas residues (straw, pig manure, and chicken manure from biogas plant) as raw materials to produce biochars, and investigated the characteristics of biochars pyrolyzed at different temperatures and activated with different chemical reagents as well as evaluated the efficiency of these biochars in removing NH_4^+-N and COD from waste water.

Material and Methods

Materials

Biogas residues from different fermentation substrates, such as straw biogas residue, pig manure biogas residue, and chicken manure biogas residue, were collected from biogas plants around Nanjing, China. The characteristics of the biogas residues are summarized in Table 1.

Table 1. Basic properties of biogas residue raw materials

Sample	N（%）	C（%）	H（%）	S（%）
Straw biogas residue	1.122±0.15	27.618±0.12	2.407±0.04	0.303±0.05
Chicken manure biogas residue	2.228±0.14	20.331±0.23	1.916±0.05	0.248±0.11
Pig manure biogas residue	1.571±0.27	15.942±0.12	1.697±0.05	0.318±0.08

Carbonization and biochar modification activation methods

The biogas residues were dried and placed in a tube furnace for anaerobic pyrolysis at 400, 500, 550, and 600°C, respectively. By using N_2 as a protective gas (50 mL/min), the heating rate was controlled at 5 °C/min, and the pyrolysis time was 120 min. After cooling, the biochars were repeatedly washed with deionized water at 80°C until neutral, and finally dried, ground, and sieved through 120-mesh sieve.

Subsequently, 50 g of the pretreated biochar (pyrolysis temperature: 550°C, pyrolysis time: 90 min) were taken in a 1-L beaker and modified solution (NaOH, KOH, Na_2CO_3, $FeCl_3$, $FeSO_4 \cdot 7H_2O$, $ZnCl_2$, H_3PO_4, HCl, H_2SO_4, $FeCl_3 \cdot 6H_2O$, and $KMnO_4$ reagents) was added to it at a solid : liquid ratio of 1 : 4 (m/V), mixed, and allowed to stand for 24 h. Then, the supernatant was removed, dried in an oven to constant weight, placed in a clean porcelain boat, and transferred to a tube furnace, with N_2 passed at a constant flow rate of 50 mL/min and constant heating rate of 5 °C/min for 1 h. he biochars modified with different raw materials and different activators were labelled as "S/C/P temperature/reagent + CC."

Waste water adsorption assay

COD and NH_4^+-N adsorption experiment

A simulated biogas slurry with a COD concentration of 500 mg/L was prepared using potassium hydrogen phthalate, Ammonium chloride reagent was used to prepare the simulated waste water with NH_4^+-N concentration of 100 mg/L. A total of 30 mL of the simulated biogas slurry were mixed with 0.03 g of the prepared biochar and incubated at 30°C and 200 r/min for 12 h in a shaker, and the COD and the NH_4^+-N content in the filtrate was measured.

Isothermal adsorption experiment

Based on the results of adsorption kinetics, biochars with high qe values for COD and NH_4^+-N adsorption were selected to study the adsorption and adsorption characteristics of COD and NH_4^+-N at different COD and NH_4^+-N concentrations, respectively. Accordingly, 30, 50, 100, 200, 400, 600, and 800 mg/L COD and NH_4Cl simulated wastewater solutions were respectively prepared with pH adjusted to about 7.0. Then, 0.30 g of biochar was added to 30 mL of simulated wastewater with different COD and NH_4^+-N concentrations, respectively, and incubated at 25°C and 200 r/min in a constant-temperature shaker for 24 h. Subsequently, the concentrations of COD and NH_4^+-N in the simulated wastewater solutions were measured after centrifugation and filtration through a 0.45-μm water filter membrane, and Langmuir and Freundlich model adsorption isotherm fitting was employed using origin 9.0.

Results and Discussion

Influence of different pyrolysis temperatures on the adsorption capacity of biochar

Biochars were prepared by pyrolytic carbonization of three kinds of biogas residues at 400, 500, 550, and 600°C, respectively. Table 2. lists the specific surface area and yield rate (The ratio of the mass of biochar after pyrolysis to the mass before pyrolysis) of biochars obtained at different pyrolysis temperatures. During biochar pyrolysis, the pyrolysis temperature had a significant influence on the adsorption performance of the biochars. The three different raw materials presented similar trends. As the pyrolysis temperature was increased from 400 to 550°C, the specific surface area of the prepared biochar increased, and the adsorption performance improved, reaching a peak at 550°C. The specific surface areas of biochars obtained from straw, pig manure, and chicken manure biogas residues as raw materials were increased from 12.83, 10.37, and 10.74 m^2/g to 36.29, 33.739, and 30.82 m^2/g, respectively.

Table 2. Characteristics of different biochars

Sample	Straw biogas residue biochar		Chicken manure biogas residue biochar		Pig manure biogas residue biochar	
	Yield(%)	BET/($m^2 \cdot g^{-1}$)	Yield(%)	BET/($m^2 \cdot g^{-1}$)	Yield(%)	BET/($m^2 \cdot g^{-1}$)
400°C	86.96±0.06	12.8362	86.73±0.08	10.3792	85.51±0.08	10.7435
500°C	82.23±0.03	27.392	86.61±0.05	24.2949	84.58±0.03	22.6527
550°C	81.37±0.07	36.2896	86.47±0.06	30.8243	84.46±0.01	31.7341
600°C	80.14±0.05	32.9237	86.13±0.06	29.2719	83.93±0.05	29.2335

It can be observed from Table 3 that the adsorption effect of biochar on COD and NH_4^+-N was the best when the pyrolysis temperature was 550°C, and the COD removal rate of C-550-CC was 28.1%. The difference in the removal rate of ammonium nitrogen from the three raw materials of biochars is small, between 16.8% and 20.3%, and the removal rates of COD by S-550-CC and P-550-CC were 26.1% and 20.5%, respectively.

Table 3. COD and NH_4^+-N removal rates of biochars obtained from different raw materials and at different pyrolysis temperatures

Modification method	Straw biogas residue biochar		Chicken manure biogas residue biochar		Pig manure biogas residue biochar	
	COD removal efficiency（%）	NH4+-N removal efficiency（%）	COD removal efficiency（%）	NH4+-N removal efficiency（%）	COD removal efficiency（%）	NH4+-N removal efficiency（%）
400°C	23.8	8.3	18.5	9.1	7.4	8.9
500°C	24.9	14.7	15.6	12.9	14.2	16.7
550°C	26.1	16.8	28.1	20.3	20.5	18.6
600°C	15.1	10.1	24.3	18.8	15.9	12.9

Effects of different activation methods on the adsorption capacity of biochar

Analysis of the adsorption of biochars obtained from biogas residues modified with NaOH, KOH, Na_2CO_3, $FeCl_3$, $FeSO_4 \cdot 7H_2O$, $ZnCl_2$, H_3PO_4, HCl, H_2SO_4, $FeCl_3 \cdot 6H_2O$, and $KMnO_4$ reagents and pyrolyzed at optimal pyrolysis temperature of 550°C revealed that the COD removal efficiencies of C-FeCl3-CC, S-FeCl3-CC, and P-FeCl3-CC biochars were 53.8%, 47.2%, and 49.1%, respectively (the initial solution COD concentration was 500 mg/L). The NH_4^+-N removal rates of S-$FeCl_3$-CC, C-$FeSO_4 \cdot 7H_2O$-CC, and P-$FeCl_3$-CC reached 23.8%, 29.2%, and 26.9%, respectively, (the initial solution NH_4^+-N concentration was 100 mg/L), which were relatively better than those of biochars obtained using other modification methods.

Table 4. COD and NH_4^+-N removal rates of biochar obtained using different modification methods

Modification method	Straw biogas residue biochar		Chicken manure biogas residue biochar		Pig manure biogas residue biochar	
	COD removal efficiency(%)	NH_4^+-N removal efficiency(%)	COD removal efficiency(%)	NH_4^+-N removal efficiency(%)	COD removal efficiency(%)	NH_4^+-N removal efficiency(%)
HCl	25.5	20.2	27.9	16.1	21.3	20.8
H_2SO_4	27.2	11.3	40.2	15.2	38.2	12.6

H_3PO_4	23.9	9.0	25.6	6.8	26.7	10.1
NaOH	12.5	15.5	14.0	16.4	14.0	16.0
KOH	26.2	17.3	32.5	19.6	26.1	17.1
Na_2CO_3	18.9	9.2	21.9	9.4	22.3	9.6
$ZnCl_2$	27.6	14.8	32.1	16.2	27.9	16.9
$FeCl_3$	47.2	23.8	53.8	27.2	49.1	26.9
$Fe_2SO_4·7H_2O$	34.0	22.2	38.6	29.2	27.7	21.1
$Fe^{3+}·Mn^{2+}$	38.6	15.2	27.2	15.4	29.9	15.3

Isothermal adsorption line

The adsorption isotherm model [1,2] describes the relationship between adsorbents and the mass of the adsorbed substance in the solution in the whole adsorption system, and determines the optimal process parameters of the system. The adsorption characteristics of modified biochars in solutions with different COD and NH_4^+-N concentrations were studied, and Langmuir and Freundlich models were fitted. It can be seen from the adsorption isotherm (Fig. 1) that both the Langmuir and Freundlich models of the isotherm adsorption curve can describe the effect of biochar on COD and NH_4^+-N, and the adsorption results of Langmuir model were higher. While the isothermal adsorption curves of biochar to COD and NH_4^+-N presented different fitting conditions, both increased with the initial concentration of the solution. In line with the Langmuir equation, the maximum NH_4^+-N and COD adsorption capacities of C-$FeCl_3$-CC were 55.29 and 186.24 mg/g, respectively.

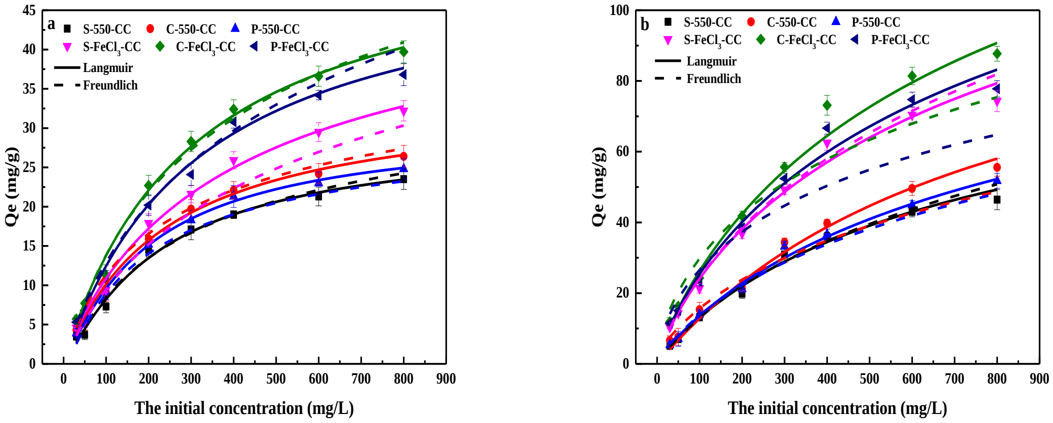

Fig.1. Adsorption isotherm curve of modified biochars. a: S-$FeCl_3$-CC to NH_4^+-N; b: S-$FeCl_3$-CC to COD

Conclusions

The biochar prepared from the three raw materials showed specific adsorption effect on COD and NH_4^+-N. Due to the higher nitrogen content, more abundant functional groups were formed on the biochar, so that the highest COD and NH_4^+-N removal rates of the chicken manure biogas residue biochar reached 28.1% and 20.3%, respectively. The adsorption was observed to conform to the Langmuir model, and was mainly chemical adsorption, forming chemical bonds with pollutants through Fe^{3+}.

Acknowledgement

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SYNTHESIS OF TERNARY MICRO-ELECTROLYTIC FILLERS USING BIOCHAR FROM *LYCIUM BARBARUM* L. BRANCHES AND ITS APPLICATION IN WASTEWATER TREATMENT

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Currently, synthesis of iron carbon (Fe/C) micro-electrolytic fillers are based on traditional electrochemistry technology and it has been successfully applied for treating various refractory organic pollutants available in the wastewaters. In addition, biochar derived from pyrolysis of variety of waste biomass is becoming an emerging carbon material. This is not only an approach to reduce environmental pollution contributed by waste biomass, but it has been also proven to be a cost-effective alternative for high-valued applications of traditional carbon materials via its excellent performance. In this study, a novel micro-electrolytic filler was synthesized using biochar produced from the Lycium barbarum L. branches. The physicochemical properties of these micro-electrolytic fillers were modified by adding copper and iron at a high-temperature anaerobic process. A comparative study was conducted using three different kind of fillers (C, Fe/C, and Fe/C/Cu), determined reaction behavior and was characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). Further the effect of ternary micro-electric fillers was tested on treating the refinery wastewater. The wastewater was treated with different Fe/C ratio fillers, Fe/C/Cu ratio fillers, and initial pH value and collected the treated wastewater and analyzed for COD，TOC, and Gas chromatography–mass spectrometry (GC-MS). Hence arrived the optimum treatment conditions for treating the wastewater with the novel ternary micro-electrolytic fillers.

A reduction of about 80% COD was recorded after 120 minutes of treatment conditions in the wastewater maintained at an aeration rate of 10 mL/min, with an initial pH value of 4.0, Fe/C ratio of 1:1, Fe/C/Cu ratio of 1:1:1. COD removal efficiencies of these three fillers were recorded as 53.01%, 57.83%, and 77.11%, as well as TOC removal efficiencies of 34.71%, 39.44%, and 68.71% , respectively. The SEM images of these three fillers indicated the presence of a number of pore structures, which facilitated the adsorption of organic pollutants. Differently, Fe/C/Cu filler occurred an aggregation of metal atoms, whereas compared with the polymerization of iron atoms in Fe/C filler, Fe/C/Cu filler promoted the reaction by the addition of copper which made the iron atoms more evenly dispersed. XRD analysis revealed that all the three fillers contained Fe and Fe3O4. Only Fe/C/Cu filler contained Cu, which implied that copper oxide was completely converted to copper during high-temperature anaerobic process. The GC-MS analysis identified that some small molecules formed in effluents as intermediates, for example, carboxylic acid, alcohol, alkane, and acetone, which indicated that a large amount of organic pollutants with high molecular compound available in raw torch wastewater, such as 1,3-Adamantanediacetamide, Naphthalene, 2,5,6-Trimethylbenzimidazole, 2-Ethyl-3,5-dimethylpyridine, were degraded during the micro-electrolytic process. Especially, the least residual organic compounds in effluent suggested that Fe/C/Cu filler had the highest degradation capacity.

The outcome of this study confirmed that Fe/C/Cu ternary micro-electrolytic filler is a novel and effective environmentally friendly material and can be utilized for the treatment of refinery wastewater.

Keywords: *Micro-electrolysis, Lycium barbarum L. branches, Ternary micro-electrolytic fillers, COD, Wastewater treatment.*

INFLUENCE OF MODIFIED RICE HUSK BIOCHAR ON GASEOUS EMISSION, NUTRIENT STATUS AND MICROBIAL COMMUNITY DURING SWINE MANURE COMPOSTING PROCESS

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Swine manure is largely composed of abundant organic compounds, which can also cause harm to various aspects of the environment through the release of greenhouse gases or excess nutrients, salt accumulation, growth of pathogenic microorganisms, etc. In this regard, in-vessel composting process with modified black carbon is an effective and economic approach for manure managment. In this study investigated the effect of the modified black carbon (MBC), amendment on swine manure (SM) composting efficiency through physical, physio-chemical, gaseous emissions, microbiological, and phytotoxic analysis during the 50-day process of in-vessel composting. The composting treatments were set-up from three different ratios of black carbon to Swine manure mixed with sawdust (SD)(i.e. SM + SD + 5%MBC (T1), SM:SD + 10 %MBC (T2) and SM:SD + 15 %MBC (T3)), while treatment without black carbon amendment was used as a control, SM:SD (C). The results showed that, compared to the control, biochar amended compost mixtures had significantly reduced ($p \leq 0.05$) C:N ratio, bulk density, organic matter (OM), pathogenic microorganisms and gaseous emissions. On the other hand, biochar amendment mixtures had increased total porosity (TP), water holding capacity (WHC), rapid thermophilic temperature, and nitrate nitrogen. However, with the most prominent effects in terms of nutrient quality and degradation rate of compost mixtures, the amendment of 10% biochar is recommended for poultry manure management through the in -vessel composting process.

Keywords: *Biochar, Swine manure, Nutrient quality, Maturity indices, Phytotoxicity.*

WET AND DRY TORREFACTION OF YARD WASTES FOR BIO-ENERGY APPLICATIONS

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Yard waste includes grass, leaves, and tree and brush trimmings is either dumped or composed as landfills. Since the yard waste is organic in nature, it can be considered as solid fuel for further pyrolysis, combustion or gasification. The calorific value of yard waste can be improved by wet and dry torrefaction techniques. The present research was aimed to determine the energy efficient conditions for both wet and dy torrefaction of yardwastes. In wet torrefaction, the inherent relationship between fuel properties and operating conditions such as temperature, residence time and biomass/water ratio were investigated. Whereas, the operating variables investigated in dry torrefaction were temperature, residence time and type of gases. Wet torrefaction studies were done in a high pressure reactor and dry torrefaction studies were done in a tubular reactor. The fuel properties of resulted torrefied yardwaste were analysed. The mass yield decreased with the increasing reaction temperature thus reaction temperature is a significant parameter in the wet torrefaction process.

Keywords: *Torrefaction, Yard wastes, Bio-energy.*

BIOCHAR INFLUENCES THE IMPACT OF ANTIBIOTIC IN SOIL

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Agricultural lands are frequently subjected to contamination of various antibiotics due to the application of manure into the field. Since 30-90% of the administered antibiotics are excreted via feces and urine, a significant portion reaches the environment. Poultry manure contains high concentration of nitrogen and other essential nutrients. However, its addition to soil is marred by the presence of antibiotics such as enrofloxacin. The presence of ENR at low concentration as 4 mg/kg also inhibits the growth of microbes. Despite the fact that enrofloxacin is a wide spectrum antibiotic, its impact on both Gram positive and Gram negative bacteria. It was hypothesized that addition of biochar to the soil could counteract the magnitude impacts of the antibiotics. In addition, it may contribute additional nutrients including traces of soluble organic carbon and other inorganic nutrients. This present study aimed at investigating the impacts of antibiotics on bacterial population in presence and absence of biochar as a mitigation strategy in an incubation study in the absence of plants. The soil, and soil mixed with poultry manure at 5% were mixed with biochar at 2%, and subsequently spiked with enrofloxacin (ENR) at 10, 50 and 100 mg/kg of soil concentrations along with suitable controls. The flasks were incubated in an oven at mesophilic temperature, and the bacterial and populations were monitored for a period of 4 weeks (1, 3, 7, 14, 21, 28 days). In addition, the physico-chemical properties of the initial soil were analysed.

The addition of biochar indeed negated the effects of antibiotics based on the bacterial population while the effects are pronounced at higher concentrations. Bacterial population increased rapidly while whether the community composition was restored is a critical question to be investigated in the future experiments. As such the impact of antibiotic in manure on the legume-rhizobium symbiosis is virtually untouched necessitating systematic investigation. The physicochemical properties like pH, EC, ammonium-nitrogen, total nitrogen, total organic carbon, available nitrogen and available phosphorus also influenced the bacterial population. After initial inhibition by the antibiotics, the bacterial population increased rapidly while whether the community composition was restored is a critical question to be investigated in the future experiments.

Keywords: *Biochar, Chicken manure, Enrofloxacin, Nitrification, Bacterial population.*

NANO-BIOCHAR PRODUCTION AS A SUPPLEMENTARY SECTOR OF CONVENTIONAL THERMOCHEMICAL BIOREFINERIES

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Conventional thermochemical biorefineries such as torrefaction, fast pyrolysis, and hydrothermal liquefaction lack attraction for large-scale investment for biofuel production mainly due to their relatively low economic competitiveness compared with the production of other sources of fuels. Bringing the conceptions of nano-biomaterial production into conventional biorefineries is potential to enhance the overall economic gain, whist achieving the near-complete valorisation of biomass feedstocks. Biochar is one of the major products during the thermochemical treatment of biomass. Reducing the size of bulk biochar to its nanoscale to produce nano-biochar is considered as a feasible way for high-value valorisation of conventional biorefining. As an emerging area, the preparation, modification and utilization of nano-biochar is still at its infancy. Prior to a successful integrated nano-biochar and biofuel production, this study is conducted to overview recent advances in: (1) methodologies for nano-biochar preparation; (2) compositions and properties of nano-biochar with comparison to bulk biochar; (3) functionalization and applications of nano-biochar; (4) environmental implications. Perspectives on the integration of biofuel and nano-biochar for near-complete valorisation of biomass are given. This study could serve as a guideline for the upcoming researches in nano-biochar.

Keywords: *Thermochemical biorefineries, Biochar, Nano-biomaterials, Nano-biochar, Near-complete valorisation.*

UPGRADE AND TRANSFORMATION FOR BIOGAS PLANTS BASED ON EFFICIENT UTILIZATION OF HEAT ENERGY

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China produces four billion tons livestock and poultry breeding per year, which brings a serious negative effect on environment. Livestock and poultry manure is transformed into biogas with anaerobic fermentation in biogas plants. It is the most common and efficient technique.

Temperature is an important factor affecting the anaerobic digestion process. In order to improve the fermentation temperature, coil heating is extensively applied in the domestic biogas plants. However, it shows low thermal efficiency. To improve the heat-transfer process regarding the slurry, rheological properties were studied, and novel twisted-tube heat exchanger for slurry from biogas plant was invented in our previous work. Here we discussed that the heat energy efficient utilization process of different scale biogas plants. For the biogas plant with less than 1000m³ AD reactor, direct feeding heating method is used. The sensitive analysis of the profit from transformed in biogas plants was carried out. Taking the biogas plant with 300 m^3 AD reactor as an example, on November, December, January, February and March, the surplus biogas produced using the new biogas slurry heat exchanger equals to 3539-14864 m^3 natural gas, and the decreased COD are 1239-5203 kg, which have a significant benefit to the biogas project with scale less than 1000m³.

For the large-scale biogas plants with 10000 m³ AD reactors, biogas boilers are applied in the waste-heat recovery systems. The heat recovered from the biogas slurry is used to compensate the heating load of the front-end regulating pool, which can not only balance the heat demand and emissions of the system, achieve energy self-sufficiency, but also maximize the benefits of the biogas project. The results show that the combination of the biogas boiler and waste-heat recovery can effectively decrease 20-35 % of the consumption of gas in biogas boiler. The biogas saved from the waste-heat recovery can be further used in power generation.

Keywords: *Biogas plants, Heat exchanger, Waste heat recovery, Heat energy utilization.*

THE USE OF COCONUT HUSK AND CATTLE MANURE BIOCHARS IN REMEDIATING MINE-CONTAMINATED SOIL GROWN WITH UPLAND RICE

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Biochar has gained global interest as soil amendment to positively affect properties of soil and subsequently improve crop yields. Numerous research investigations have confirmed that biochars can improve resource use efficiency of the land and remediation protection for any threats. Recently, biochar ability to sequester metals has caught the attention of the mine reclamation sector. Biochar application is promoted to remediate heavy metals in mine-tailing area and improve soil chemical conditions for enhanced plant growth. To study the effect of coconut husk and cattle manure biochar application on the properties of soil from mine tailing site and on the growth and yield of rice grown under upland soil condition a screenhouse pot experiment was conducted. Results showed that adding coconut husk and cattle manure biochar to mine-contaminated soil reduced the concentrations of heavy metals such as copper (Cu) and iron (Fe) in soil and plant tissues such as roots, leaves and stalks, and grains. Cation exchange capacity (CEC), organic carbon (OC), nitrogen (N), and potassium (K) were increased by application of both biochar. Available phosphorus (P) was also increased by applying biochar derived from cattle manure. Plant height, number of tillers, fresh and dry biomass, number of panicles per hill, harvested grain per pot, weight of 1000 grains, percent filled grains, harvest index, and agronomic N, P, and K efficiency were improved by adding coconut husk and cattle manure biochar with the recommended rate of fertilizer.

Keywords: *Cattle manure biochar, Coconut husk biochar, Mine-contaminated soil, Upland rice, Soil degradation, Soil quality.*

NUTRIENT RECOVERY USING BIOCHAR DERIVED FROM AGRICULTURAL WASTE AND ITS ENVIRONMENTALLY-SAFE REUSE

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With the introduction of strategies to improvise global agricultural sector, various challenges faced has to be addressed which includes low productivity (averaging to 60 per cent of the World average), supply chain management and product lifecycle management, and mounting threat to sustainability arising from depleting quality of natural resources, biotic and abiotic stresses, and inefficient use of agricultural wastes. The present research work attempts to narrow down the wide gap existing between the research laboratories and agricultural fields. The release of excess nutrients (nitrogen and phosphorous) through domestic wastewaters and agricultural effluents pollutes the water environment. Thus, not only poses threat to aquatic ecosystem, but also affects human health and other productive activities. The main objective of the present research work was to evolve an appropriate technique not only for the treatment of the wastewater stream but also to ensure recovery of nutrients using biochar and their environmentally-safe reuse.

Biochar as a product has many uses, including water filters and soil improvement properties. Nutrient recovery from agricultural effluents and domestic wastewater using biochar derived from agricultural waste and its reuse in agricultural fields for soil replenishment have the potential to contribute to the better nutrient stewardship and provide some degree of diversification of nutrient supply to help nutrient security in agricultural land. The present research evaluated the performance of utilization of biochar produced from agricultural wastes in the separation of nutrients from synthetic wastewater and actual wastewater (domestic sewage/ agricultural effluent). The characteristics of the biochar derived from agricultural waste before and after the experiments were determined in terms of chemical composition, surface imaging, presence of desirable functional groups, crystallinity due to presence of mineral structures, and the type of micropores. Batch studies using finite volume of mono-nutrient synthetic wastewater and actual realistic wastewater were conducted to study the adsorption behaviour, kinetics and thermodynamics. Further, continuous flow studies were also conducted using mono-nutrient synthetic wastewater and actual realistic wastewater using adsorption columns. The interferences from rest of the pollutants in the wastewater in the adsorptive separation of nutrients were revealed using sophisticated analytical techniques, a supporting mechanism for the adsorptive process was also detected in the case of realistic wastewater. Furthermore, the suitability of the pollutant-laden biochar as environmentally-safe soil replenisher was also evaluated by conducting dynamic leaching tests. A most appropriate reuse or stabilization of the spent adsorbent was also evolved based on the results of dynamic leaching tests.

The results of the research work were highly promising for field application. In addition, the study assessed more accurately the scope for the recovery of nutrients from various wastewater streams for the possible utilization in agricultural fields as soil nutrient replenisher.

Keywords: *Nutrients, Recovery, Wastewater, Biochar, Environmentally-safe reuse.*

BIOCHAR AS AN OXYGEN ACTIVATOR AND BIMETAL DISPERSER FOR THE DEGRADATION OF MULTIPLE ORGANIC POLLUTANTS UNDER OXIC CONDITIONS USING A MICRO-ELECTROLYSIS FILLER

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A micro-electrolysis filler (iron (Fe)-biochar (BC)-copper (Cu)) was synthesized using BC produced from *Lycium barbarum L.* branches, modified by adding Fe and Cu in a high-temperature anaerobic process. To evaluate the applicability of Fe-BC-Cu, the effects of various reaction parameters were systematically investigated. A comparative study was conducted using three different kinds of fillers (BC, Fe-BC, and Fe-BC-Cu) to determine reaction behavior. The results suggested that the addition of Cu could enhance the removal efficiency of chemical oxygen demand, multiple organic pollutants, as well as total organic carbon. The scanning electron microscopy images of Fe-BC-Cu showed that Cu and Fe reunited into loose micro-sized balls rather than dense clusters in the presence of BC, which proved that BC was effective for avoiding bimetallic agglomeration. Meanwhile, under oxic conditions BC played the role of an oxygen (O) activator. Thus, removal by an Fe-BC-Cu micro-electrolysis filler could be explained by the synergistic effects of BC activating O to generate the hydroxyl radical (·OH), Fe/Cu micro-electrolysis and coagulation of iron hydroxide. This study has improved our understanding of the pollutant removal mechanisms in the micro-electrolysis system that occur under oxic conditions. The extraordinary removal performance of multiple organic pollutants has huge potential for the actual industrial application of Fe-BC-Cu micro-electrolysis filler.

Keywords: *Biochar, Micro-electrolysis, Organic pollutants, Bimetal disperser, Oxygen activator.*

INFLUENCE OF BIOCHAR AMENDMENT ON ANTIBIOTIC RESISTANCE GENE ABUNDANCE AND THE BACTERIAL COMMUNITY DURING AEROBIC COMPOSTING OF PIG MANURE

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Livestock manure is often subjected to aerobic composting but little is known about the variation in antibiotic resistance genes (ARGs) during the composting process under different concentrations of antibiotics. This study compared the effects of three different dosage of biochar mixed with bacteria consortium on ARGs and the succession of the bacterial community during composting. Very similar trends were observed in the relative abundances (RAs) of each ARG among the biochar treatments and the control during composting. After composting, the RAs of tetC, tetX, sul1, sul2, and intI1 increased 2–41 times, whereas those of tetQ, tetM, and tetW declined by 48–96%. Biochar addition significantly increased the absolute abundances and RAs of tetC and in tI1, while higher dosage of biochar also enhanced those of tetM, tetQ, and drfA7. The bacterial community could be grouped according to the composting time under different treatments. The highest concentration of biochar had a more persistent effect on the bacterial community. In the present study, the succession of the bacterial community appeared to have a greater influence on the variation of ARGs during composting than the presence of antibiotics. Aerobic composting was not effective in reducing most of the ARGs, and thus the compost product should be considered as an important reservoir for ARGs.

Keywords: *Livestock manure, Antibiotic resistance genes, Biochar, Composting.*

BIOCHAR ACCELERATED THE INITIATION OF HIGH-SOLID ANAEROBIC CO-DIGESTION SYSTEM WITH PIG MANURE AND DEHYDRATED SEWAGE SLUDGE

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The anaerobic digestion (AD) process was traditionally operated at low solids (TS≤8%). Comparatively, high-solid anaerobic digestion (TS ≥15%) has many advantages, such as smaller reactor volume, less energy input for heating, and easy for transportation and land application. Biochar has the characteristics of large specific surface area, rich voids and alkalinity. The use of biochar in AD reactor can alleviate the acidification of the system, increase microbial abundance and improve methane yield at a low cost. However, the dosing method of biochar (i.e. dosage, replenish, and reuse) in high-solid digestion is unclear yet. In this study, the effects of biochar on initiation of high-solid co-digestion with pig manure (PM) and dehydrated sewage sludge (SS) and were investigated.

In this study, the continuous stirred tank reactors (CSTR) under mesophilic condition (35 °C) were fed with SS and PM by gradually increasing TS from 5% to 7%, 10%, and 14%, respectively. The volatile solids (VS) ratio of SS and PM was determined by our previous study and the optimum ratio is 2:5. The TS, VS, soluble chemical oxygen demand (sCOD) of SS were 37.72 ± 0.07%, 16.58 ± 0.06%, 6.30 ± 0.18 mg/g; and PM were 23.11 ± 0.28%, 20.52 ± 0.24%, 8.17 ± 0.07 mg/g. The dosage of biochar in test reactors was set as 4 g/L and the control group without biochar supplementation were also set up. Every two days, 100 mL of effluent was replaced with equal volume of fresh feedstock to realized the o semi-continuous operation. As biochar was discharged with high-solid effluent, the biochar was added along with fresh sample at 4 g/L. The working volume of CSTR was 1.6 L and the hydraulic retention time (HRT) was set as 32 d. Biochar was prepared by using PM as the precursor, which was placed in a hydrothermal reactor at 235 ℃ for 5 h.

At the initial stage of start-up, the system adopted low organic loading rate (OLR) of 1.08 ~ 3.0 VS/(L·d)), volatile fatty acids (VFA) accumulation did not occur and methane production was relatively stable. It might also be attributed to the relatively low dissolved organic matter in dewatered SS. When TS increased to 7%, the methane production of biochar group was 39.38% higher than that of the control group; when TS further increased to 14%, the average methane production and methane production were 496.0 ± 69.2 mL/d and 247.4 ± 35.5 mL/d, respectively. The higher methane yield in biochar supplemented group indicated the promoted microbial stability. The propagation of methanogens and other slow-growing microbial communities might be accelerated by attached growth. Under the condition of high solid content (TS > 8%), the concentration of ammonia in the system presented accumulation trend, which was increased to 716.7 mg/L at TS 14%. When CSTR fed with the TS of 14%, the TS of effluent was reduced to 8.42% and VS/TS is 64.86%, which indicates that there is still some residuary methane production potential in digestate. These findings provide useful guidance for starting high-solid AD system assisted with biochar.

Keywords: *Biochar, Co-digestion, High solid anaerobic digestion, Methanogenesis, Pig manure, Total solids.*

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Bioconversion for Bioproducts

BIO-ELECTROFERMENTATION COUPLED ION SUBSTITUTION ELECTRODIALYSIS FOR IMPROVED CARBON CONVERSION TO CARBOXYLIC ACIDS

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Waste-derived carboxylic acids (CAs) are gaining interest as alternative platform chemicals for the replacement of petroleum-based products. Bio-electrofermentation (BEF) has been used to overcome the thermodynamic limitations of conventional anaerobic fermentation. However, the emission of acidogenic by-products (CO_2) leads to waste of substrate carbon while accumulation of acid products can trigger product inhibition and deteriorate the micro-environment, thus reducing the production rate and yield of CAs. In this study, ion substitution electrodialysis (ISED) is proposed to separate CAs product from the anode of BEF and to reuse acidogenic CO_2 in the meantime. Mitigation of product inhibition was achieved simultaneously with pH adjustment by coupling ISED to BEF. Production of CAs was enhanced significantly after 24 days of semi-continuous operation, which was 1.06 g COD/g VSS, corresponding to 188% increase over the control. The pH in BEF-ISED integrated system was well controlled within a suitable range (c.a. 4.5-7.0) for CAs production. Additionally, integration of ISED with the anode of BEF induced the shift of bacterial communities towards CAs fermentation species of *Wolinella* and *Clostridium sensu tricot-1* by altering the micro-environment. Carbon conversion efficiency of substrate to CAs reached 90.74%, which is the highest value ever reported within the area of anaerobic fermentation.

Keywords: *Carboxylic acids, Bio-electrofermentation, Ion substitution electrodialysis, Carbon conversion, Micro-environment.*

CHARACTERIZATION AND EVALUATION OF A NATURAL DERIVED BACTERIAL CONSORTIUM FOR EFFICIENT LIGNOCELLULOSIC BIOMASS VALORIZATION

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A consortium (HPP) with improved ability in biomass conversion was achieved by adjusting the proportion of *Pseudoxanthomonas taiwanensis* in a natural consortium (HP), but the mechanism behind was unknown. Herein, the diversities of microbial community structure and gene functions of the consortia were analyzed first, and found that HPP had a more balanced microbial structure with enriched gene pathways related to cellular processes, environmental information processing and metabolism. Then, key genes responsible for biomass conversion were further analyzed, finding that their abundance and distribution contributed to HPP's efficient biomass conversion. Finally, consolidated bioprocessing of agricultural wastes by HPP was carried out to verify its enhanced ability, and ethanol with the highest yield that was ever reported was achieved at 0.28 g/g. This is the first study which reported the underlying mechanisms for synergistic effects of microbial consortia, and will guide the artificial construction of complex microbial consortium for specific purpose.

Keywords: *Agricultural residues, Balanced consortium structure, Cellulosic ethanol, Consolidated bioprocessing, Molecular mechanisms.*

INTEGRATED BIOETHANOL AND BIO-CRUDE PRODUCTION THROUGH TWO-STAGE YEAST CO-FERMENTATION AND HYDROTHERMAL LIQUEFACTION

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Industrial symbiosis can nurture eco-revolution via reciprocal money-spinning technological innovations. Conversely, bioethanol refineries are striving to achieve minimal ethanol selling price at par with petro-fuel. This can be unravelled by a) improvising the bioethanol production process i.e., at the level of feedstock input, renewable sugar release and co-fermentation efficiency, and moderate reactor and material input, and b) through transforming the ethanol process spent streams to profitable bio-chemicals/bio-fuels. This research deals with both these aspects wherein biogenic municipal solid waste (BMSW) is employed as a sustainable feed input to produce ethanol, followed by spent stream valorisation via hydrothermal liquefaction (HTL) to produce bio-crude/ bio-oil. Central composite design (CCD) based response surface methodology (RSM) was employed to enhance and optimize bioethanol titre. The refractory architecture of BMSW was cleaved adopting green depolymerising strategy driven by in-house enzymatic concoction. In an effort to involve pentose sugar stream along with typical hexoses for ethanol production, co-fermentation of sugars was conducted using co-culture of *Saccharomyces cerevisiae* NCIM 3594 and *Pichia stipitis* NCIM 3498. Further evoking industrial symbiosis, HTL of spent feed obtained from ethanol fermentation was conducted to upgrade the waste quality through thermal depolymerisation of residual organics of MSW at high temperature and pressure using subcritical water as the solvent system. The resultant is the high energy density bio-crude similar to heavy oil derived from petroleum refining along with other valuable bio-chemicals such as fatty acids, furfurals, reducing sugars, gaseous products and biochar. Thus this study corroborates the prospect for two-stage microbial ethanol fermentation and HTL of BMSW for enhanced energy tapping in form of biofuels/ bio-chemicals that are sustainable alternatives to finite petro-fuels/petro-chemicals.

Keywords: *Industrial symbiosis, Mesophilic yeast, Sugar co-fermentation, Hydrothermal liquefaction, Bioethanol refinery, Waste valorisation*.

SIMULTANEOUS PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) AND EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) FROM PROVIDENCIA SP: OPTIMIZATION, CHARACTERIZATION AND SCALEUP

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Abstract: Owing to their biodegradability and renewability, biopolymers are extensively being employed in industrial and bio-medical sectors as sustainable alternatives to chemically synthesized polymers. In the present study isolated *Providencia sp.* strain depicted an efficient production of both intra and extra cellular biopolymers, Polyhydroxybutyrate (PHB) and extracellular polymeric substances (EPS), respectively. The polymer production process was optimised by varying parameters like carbon load (20, 30 and 40 gL^{-1}) and pH (6, 7 and 8) of the system for enhancing PHB and EPS productivity. Maximum yield of both PHB (2.62 gL^{-1}) and EPS (3.92 gL^{-1}) was observed with carbon load of 30 gL^{-1} at pH 7. Scale-up studies were performed with optimized conditions and extracted EPS and PHB were characterized using FT-IR, FE-SEM-EDX, H^1 NMR, C^{13} NMR, elemental and fluorescence analysis. Thus, the present work opens avenues for production of EPS and PHB that can be potential substitutes to petroleum based polymers.

Keywords: *Biodegradable polymers, Process optimization, Sustainable alternatives, Bioaccumulation, Composites production.*

Introduction

Utilization of crude oil reserves for plastic production and the corresponding adverse effects on the environment have driven significant efforts for developing alternatives to petroleum based plastics [1]. Microbial biopolymers are becoming the centre of attention due to its faster degradability, biological compatibility, non-toxicity, eco-friendly production and low embodied carbon [2-3]. While, few microorganisms can produce polymers outside the cell such as extracellular polysaccharides (EPS), xanthan, alginate, cellulose, etc. others produce them intracellular such as polyhydroxyalkanoates (PHAs), polylactide, capsular polysaccharides, etc. [4]. Among the extracellular polymers, more recently, EPS are emerging as starting materials at industrial scale in nourishments, cosmetic, pharmaceutical, and petroleum industries [5-6]. EPS usually consists of long chains of branched monomeric sugars (C_5 and C_6) or its derivatives and these are accumulated outside the microbial cell membrane [6]. Contemporary research have focused on the bio-engineering of microbial species for improved productivity of specific EPS [7]. On other hand, PHAs are intracellular polymers and being considered as potential renewable materials due to their comparable physical and chemical properties with commercial plastics [2, 4]. In the inadequacy of nitrogen and phosphorous, few bacteria such as *Azotobacter beijerinckii, Pseudomonas sp., Ralstonia eutropha, Bacillus megaterium* etc. accumulate the fermented substrates inside the cell in the form of PHAs and use them for energy through metabolic processes [3, 8-9]. Those accumulated short-chain length (PHA_{scl}) and medium-chain length polyhydroxyalkanoates (PHA_{mcl}) have elastomeric properties with biocompatibility, thus can be applicable in biomedical sector [8-9]. Studies have been carried out to produce both these polymers individually. Considering this, the current work demonstrates the dual production of these polymers using a bacterial strain isolated from bio-hydrogen producing reactor. So far there are no reported information about the dual production (EPS and PHB) capability of these bacteria. However, bacteria belonging to the *Providencia* genus were reported to produce various complex extracellular components like Exopolysaccharides, Xylans and peroxidase [10]. The influence of various process parameters such as pH, carbon concentration and the respective yields were discussed in detailed along with the extraction and characterization of polymers.

Materials and Methods

Chemicals

All the chemicals such as glucose, yeast extract, protease peptone, Luria Bertani (LB) broth etc were procured from Hi-media (India). All the chemicals were used without further purification.

Isolation and Phylogenetic Analysis

Providencia sp. was isolated from a dark fermentation bio-hydrogen producing reactor being operated in lab, by serial dilution method. Pure bacterial isolates were identified and DNA extraction using Machery-Nagel (MN) Soil kit. The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database (Fig 1b). The nucleotide homology and phylogenetic tree illustrates that the isolated strain was genus *Providencia sp.* and submitted to CSIR-NCIM for culture deposition and data to GenBank database for accession number.

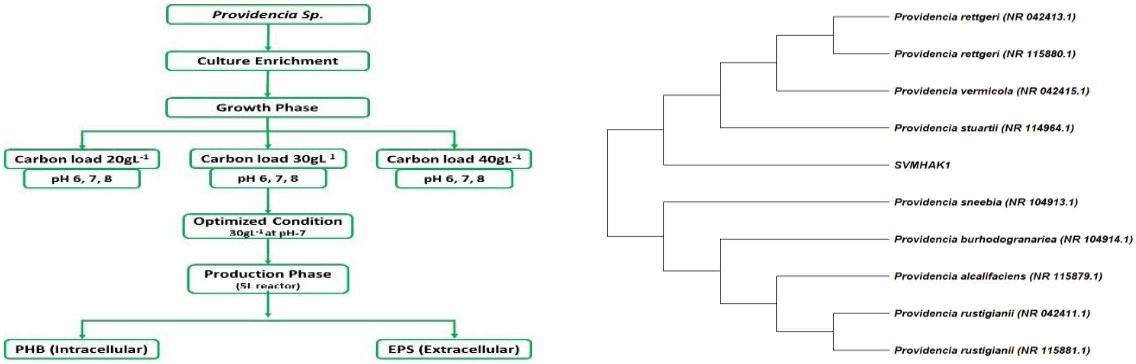

Fig. 1. (a) Schematic representation of experimental methodology for the production of EPS and PHB (b) Phylogenetic tree showing the relationship of 16S rRNA sequences from closely related sequences from GenBank.

Cultivation and analysis

The pure culture was inoculated in the LB broth and nutrient agar using spread plating method and was incubated at 35±2°C for 24 h. The media used for the production process composed of glucose as a substrate (20, 30, 40 gL^{-1}) along with growth supplements (gL^{-1}; Na_2HPO_4 - 5.4; yeast extract- 4; protease peptone- 4; $NaHCO_3$- 1.1). Initial pH of the feed was adjusted to 6, 7 and 8 based on the experimental condition using 0.1 N HCl/NaOH (Fig 1a).

Extraction and characterization

PHB extraction procedure was followed according to the earlier reported method with minor modifications [2], Solvent precipitation method was done for EPS [11]. Characterization was done using FT-IR Spectroscopy of the PHB and EPS samples with Thermo Nicolet Nexus 670 spectrophotometer to identify the functional groups. Both ^{1}H and ^{13}C NMR spectra for PHA were recorded using $CDCl_3$ and Bruker Avance 400 MHz spectrometer for ^{1}H and ^{13}C NMR respectively. The morphological studies of both EPS and PHB biopolymers were examined through the FE-SEM (JEOL, JSM-7610F). The elemental composition was analysed using the Energy Dispersive Spectroscopy (EDS; Oxford X-max). The EPS composition was analysed by Mass-Electron Spray Ionization using Agilent (1290 infinity II; Q-TOF; Mass Hunter software).

Results and Discussion

EPS Production and Characterization

The amount of EPS produced and its functional properties significantly depend on the type of organisms, culturing conditions, media composition and carbon concentration. With time in all the experimental conditions, EPS production was noticed to increase and maximum production was observed at the end of cycle. At 20 gL^{-1} carbon load, EPS production of 0.62 gL^{-1} (24 h), 1.75 gL^{-1} (48 h) and 3.39 gL^{-1} (72 h) was observed. At 30 gL^{-1} carbon load, EPS production was observed to be higher than that observed at 20 gL^{-1} carbon load. EPS production of 1.01 gL^{-1}, 2.01 gL^{-1}, 3.92 gL^{-1} was observed at 24 h, 48 h and 72 h, respectively. However, at 40 gL^{-1} of carbon concentration a decrease in the EPS production was noticed. EPS concentration reduced to 0.41 gL^{-1} at 24 h, 3.01 gL^{-1} at 48 h and 3.61 gL^{-1} at 72 h. At pH 6, EPS yield of 0.9 gL^{-1} at 24 h, 2.1 gL^{-1} at 48 h and 3.41 gL^{-1} at 72 h was noticed. In neutral pH conditions, EPS production of 1.01 gL^{-1} (24h), 2.01 gL^{-1} (48h) and 3.92 gL^{-1} at (72 h) was observed. Slight increase in the pH to 8 limited EPS production and it was found to be 1.02 gL^{-1} at 24 h, 2.14 gL^{-1} at 48 h and 3.3 gL^{-1} at 72 h (Fig a-c). From the FT-IR results of EPS, 3411 cm^{-1} broad peak signifies carbohydrate ring, 2927 cm^{-1} C-H stretching, 1655 cm^{-1} corresponded to C=O stretching vibration of the carboxylic group, 1058 cm^{-1}, of anomeric region that attributes to C-O and C-O-C groups present in polysaccharides (Fig. 4b) [12]. The EPS obtained from different experimental conditions were fractionated using Mass-Electron Spray Ionization analysis and the plots representing EPS composition are depicted monosaccharides contained galactose, arabinose and maltose, which correlated will the standard EPS.

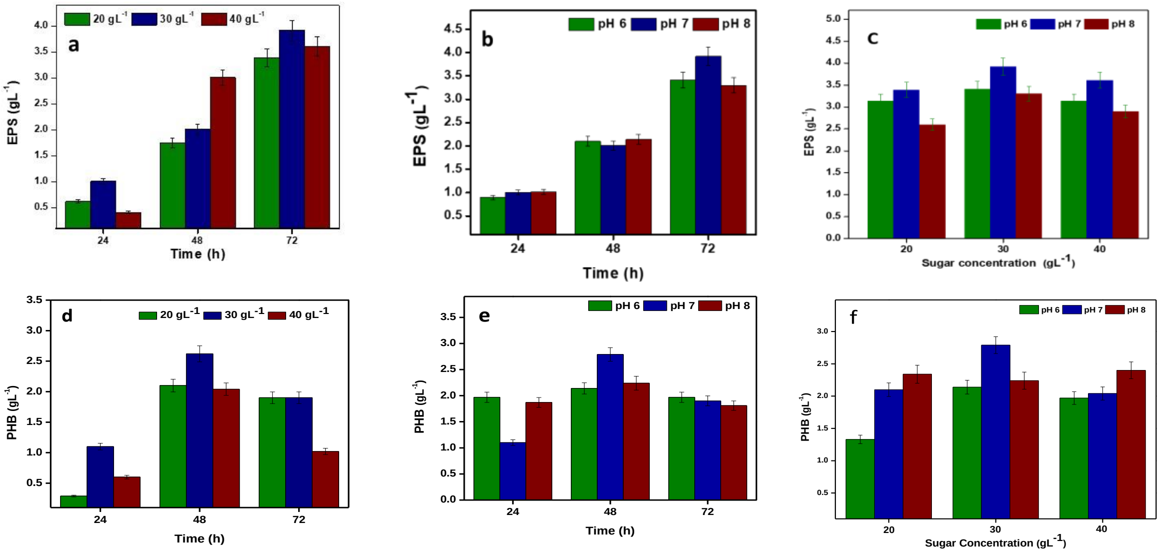

Fig. 2. (a-c) Maximum EPS production 20, 30, 40 gL^{-1} , varying the pH conditions 6, 7, 8 and carbon concentration of 30gL^{-1} (d-f) Maximum PHB production at various carbon concentrations 20, 30, 40 gL^{-1} , pH conditions 6, 7, 8 and carbon loads.

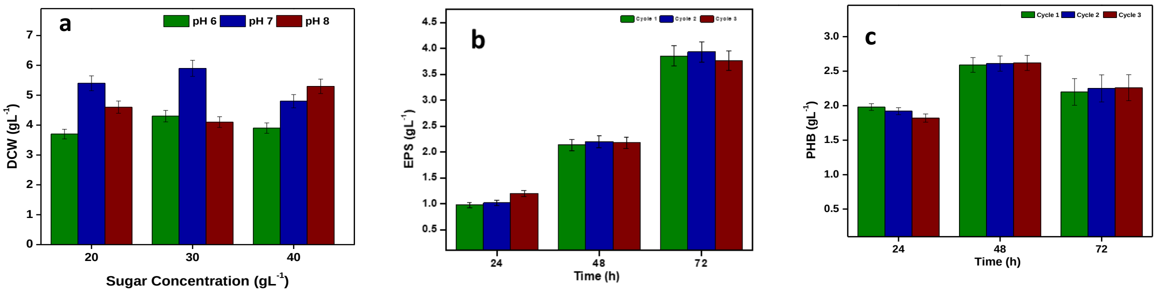

Fig. 3. (a) Sugar concentration *vs* Dry cell weight at different pH conditions (6, 7, 8) Scale up production of (b) EPS and (c) PHB.

PHA Production and Characterization

Initially, 20 gL^{-1} of carbon was supplemented and maximum PHB of 0.29 gL^{-1}, 2.1 gL^{-1} and 1.9 gL^{-1} at 24 h, 48 h and 72 h were observed, respectively. As the carbon concentration was increased to 30 gL^{-1} PHB production of 1.1 gL^{-1} at 24 h, 2.62 gL^{-1} at 48 h and 1.9 gL^{-1} at 72 h was observed. Upon further increasing the carbon load to 40 gL^{-1}, PHB production was observed to be 0.6 gL^{-1} at 24 h, 2.04 gL^{-1} at 48 h and 1.02 gL^{-1} at 72 h. Maximum PHB production of 2.74 gL^{-1} was seen at the carbon load of 30 gL^{-1} at 48 h of operation. The PHB production was studied by varying pH (6, 7, 8) and carbon concentration of 20, 30, 40 gL^{-1}. With pH 6 and 30 gL^{-1} the PHB production was observed to be 2.14gL^{-1} at 48 h, at pH 7, 2.74 gL^{-1} at 48 h, and for pH 8 it was observed to be 2.49 gL^{-1} at 48 h. PHB yield varied with respect to carbon loads and at 20 gL^{-1} (pH 6; 2.1 gL^{-1}; pH 7; 1.9 gL^{-1}, pH 8; 2.32 gL^{-1}) and 40 gL^{-1} (at pH 6; 1.97 gL^{-1}, pH 7.0; 2.04 gL^{-1} and pH 8: 2.1 gL^{-1}). The trend of PHB production varying with carbon concentration was observed to be 30 gL^{-1} >20 gL^{-1} >40 gL^{-1} (Fig 2d-f). FT-IR of PHB contains free -OH stretching at 3422 cm^{-1}. The characteristic 1720 cm^{-1} strong absorption peak represents the ester carbonyl group (C=O), 2924 cm^{-1}, 2853 cm^{-1} and 2956 cm^{-1}, correspond to the CH stretching vibrations of CH_2-CH_2, CH_2-CH_3, and-CH_3 groups (Fig. 4a). ^{1}H NMR spectrum of PHB showed a 1.28 ppm (doublet), 2.5 ppm and 5.26 ppm characteristic signal that corresponds to the methyl, methylene and methyne group. The ^{13}C spectrum of PHB showed four characteristic intense peaks at 169.18 ppm (carbonyl), 19.79 ppm (methylene) (Fig. 4c-d) [13].

Biomass

Growth of bacteria can be quantitatively determined by measuring dry cell weight. Here, glucose with various carbon loads (20, 30 and 40 gL^{-1}) was chosen for growth experiments to monitor the production of both EPS and PHB. Maximum of 5.9 gL^{-1} DCW was achieved at 72 h at 30 gL^{-1} carbon supplementation (Fig. 3a)..

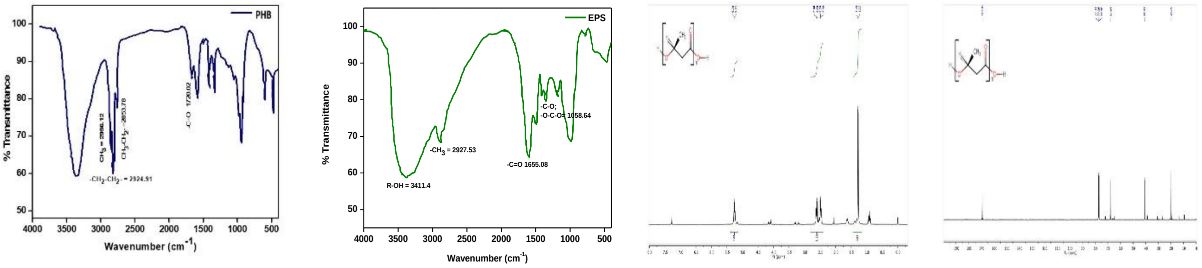

Fig. 4. (a-d) FT-IR, ^{1}H NMR and ^{13}C NMR spectra of extracted PHB and EPS.

Carbon removal

In all the experimental studies, it was found that the substrate consumption pattern showed a similar decreasing trend with an increase in the cycle time, with the highest value corresponding to 63.66%, 50.61%, 60.11% at 20 gL^{-1} with respect to pH 6, 7 and 8 followed by 63.64%, 65.61% and 59.70% at 30 gL^{-1} and with 40 gL^{-1} removal of 61.8%, 60.16% and 48.81% at pH 6, 7 and 8, respectively. Among all the conditions, 30 gL^{-1} at pH 7 showed higher carbon removal efficiency (65.61%) followed by 30 gL^{-1} at pH 6 63.64%. Lower removal efficiency was seen at 40 gL^{-1} with pH 8 (48.81%).

Morphological and EDX analysis

FE-SEM coupled with Energy Dispersive X-ray spectroscopy (EDX) was used to study the surface morphology and elemental composition of the bacterial extracted samples. The elemental spectra shown the composition of 51.0% carbon, 40.1% oxygen, 5.8% nitrogen, 1.7% phosphorus and 1.4% sodium. The elemental composition resulted 67.5% carbon, 28.8% Oxygen, 3.0% Chlorine, 0.3% Sodium, 0.2% Phosphorus and 0.2% Sulphur in the sample which are consistent with their elemental signals.

Scale up Studies

EPS and PHA production at various conditions were optimised and 30 gL^{-1} sugar concentration at pH 7 (5 L fermenter with the working volume of 3.5 L) found EPS yields observed in cycle 1, cycle 2 and cycle 3 were 3.86 gL^{-1}, 3.91 gL^{-1} and 3.88 gL^{-1}, whereas the PHB yields were found to be 2.59 gL^{-1}, 2.61 gL^{-1} and 2.62 gL^{-1} (Fig. 3b-c).

Conclusions

In the present study, dual production of EPS and PHB was carried out using *Providencia sp* and it was clearly seen that EPS and PHB depends on the pH and carbon loads. The mass spectral analysis evidenced the presence of various monomeric components such as C-5 and C-6 carbohydrate moieties. At pH 7.0, 3.92 gL^{-1} of EPS at end of cycle at 72 h and PHB of 2.62 gL^{-1} by end of 48 h were observed. Later the production was scaled up in 5L fermenter and concurrent results were obtained. Both the extracted polymers were confirmed by characterization using FT-IR, FE-SEM, H^1 NMR, C^{13} NMR, elemental analysis. The results shows that integrated dual production process offers new opportunity for large scale EPS and PHB production with low cost sugars in a biorefinery concept.

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EFFECT OF A SUPERSIZED VORTEX FLUIDIC DEVICE ON THE MECHANICAL PROPERTIES AND MICROSTRUCTURE OF A BIODEGRADABLE FILM

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In this study, a process for the production of a biodegradable film with stronger mechanical properties was developed by using shear stress in a thin-film supersized vortex fluidic device (VFD). This allows the formation of a solid brick-style inner microstructure and a smoother surface without porosity in order to improve the mechanical properties, in contrast to the conventional autoclave process, which affords weaker mechanical properties an inner microstructure with cracks, and a rougher surface. Moreover, though the film produced using supersized VFD was stronger, its biodegradability was not compromised, in comparison with the film produced using the conventional autoclave process. Overall, the supersized VFD provides a new, alternative, bottom-up approach for easy, scalable processing of biodegradable films with stronger mechanical properties.

Keywords: *Vortex fluidic device, Biodegradable film.*

CULTIVATION OF MICROALGAE IN A MICROBIAL FUEL CELL FOR ENHANCED BIOELECTRICITY GENERATION TREATING WASTEWATER: A COMPARATIVE STUDY OF *CHLORELLA VULGARIS* AND *SCENEDESMUS QUADRICAUDA*

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ABSTRACT. Microbial fuel cell (MFC) was operated with a micro-algal assisted cathode chamber to supply the oxygen for the cathodic reaction via photosynthesis as a replacement to the traditional mechanical aeration. In this system, both anode and cathode chambers were utilized to treat wastewater with the biological actions of bacteria and microalgae, respectively. The anode chamber was supplied with wastewater containing 1500 mg L^{-1} of COD and the cathode chamber was supplied with wastewater containing 300 mg L^{-1} of NH^{+}_{4}-N. The bioelectricity generation of the cell was compared for two pure culture microalgae strains of *Chlorella vulgaris* and *Scenedesmus quadricauda*. The light was provided with continuous mode and alternative 12/12 hours of light/dark cycles. *Chlorella vulgaris* showed better performance with mean electricity generation of 0.367 (±0.034) compared to *Scenedesmus quadricauda* of 0.197 (±0.029) under continuous light conditions. Higher dissolved oxygen in the cathode chamber up to 13.7 mg L^{-1} was observed with *Chlorella vulgaris*. Under 12/12 hours of light/dark cycles, the electricity generation of MFC fluctuated due to the drop in the dissolved oxygen level in the cathode chamber during dark conditions. The highest biomass productivity and the highest NH^{+}_{4}-N removal efficiency were achieved with *Chlorella vulgaris* under continuous light conditions. In all MFCs, more than 70% of COD removal efficiencies were achieved by anodic bacteria biofilm. *Chlorella vulgaris* showed better performance in MFC applications compared to *Scenedesmus quadricauda* with enhanced bioelectricity generation.

Keywords: *Photosynthetic microbial fuel cell, Bioelectricity, Wastewater treatment, Chlorella vulgaris, Scenedesmus quadricauda.*

Introduction

Microbial fuel cells (MFCs) are bio-electrochemical devices that can convert the chemical energy of organic substances to electricity [1]. A typical two-chambered MFC has an anode chamber and a cathode chamber to accommodate the electrodes. Different types of membranes including proton exchange, anion exchange, cation exchange, and ultrafiltration membranes are used in MFCs to separate the liquid in two chambers while allowing the transportation of proton from anode to cathode [2]. An anode chamber is provided with a liquid medium containing organic compounds. Generally, organic pollutants in wastewater are treated with simultaneous bioelectricity generation. The electrochemically active bacteria attached to the anode and oxidize the organics to produce electrons and protons. A reduction reaction occurs on the cathode. Oxygen is normally used as the electron acceptor in the cathode chamber due to the low cost. Cathode chamber is provided with water or buffer solution and mechanical aeration is widely used to provide aeration. The electrons that transported through an external circuit and protons that passed through the membrane react with oxygen on the cathode to form water [1, 2].

In photosynthetic MFC (P-MFC), the oxygen is supplied by the photosynthetic activities of microalgae to reduce the energy usage of the system in mechanical aeration. Since microalgae assimilate nutrients during photosynthesis, the cathode chamber can be also used to treat the wastewater while generating microalgae biomass. In P-MFCs, microalgae activity plays an important role in providing the oxygen for cathodic reduction reaction. The different microalgae strains can affect the P-MFC performance differently depending on the growth medium. *Chlorella sp*. and *Scenedesmus sp*. are some of the microalgae species that can efficiently treat the wastewater for ammonium contents. In this study, two green microalgae strains of *Chlorella vulgaris* and *Scenedesmus quadricauda* are compared to evaluate the electricity generation and wastewater treatment performances in P-MFC. Light is an important parameter for photosynthesis. Light was provided in continuous mode to accommodate the continuous photosynthesis of microalgae to harvest stable electricity generation. P-MFC was also evaluated under 12/12 h of light/dark conditions in order to simulate the natural solar light conditions.

Material and Methods

Construction of MFC

Two identical replicate reactors (R1 and R2) made of acrylic plates with a working volume of 1 L in both anode and cathode chambers, were employed in this study. A cation-exchange membrane (CMI-7000, Membranes International Inc., New Jersey, USA) having an effective surface area of 200 cm^2 was placed between the chambers. Identical anode and cathode electrodes were made of carbon fiber cloth with an immersed surface area of 204 cm^2. A titanium wire was placed as the core of the electrodes and external circuit components were connected using copper wires.

Microalgae culture

Two pure culture microalgae strains of *Chlorella vulgaris* (TISTR 8580) and *Scenedesmus quadricauda* (TISTR 8610) were purchased from Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand. Cultures were grown in the M8 medium containing (per L) KNO_3 (3000 mg), KH_2PO_4 (740 mg), $Na_2HPO_4{\bullet}2H_2O$ (260 mg), $CaCl_2{\bullet}2H_2O$ (13 mg), FeEDTA (10 mg), $FeSO_4{\bullet}7H_2O$ (130 mg), and $MgSO_4{\bullet}7H_2O$ (400 mg). Artificial lighting was provided by a fluorescent tube lamp.

Acclimatization of anode

Sludge samples were collected from a UASB reactor of Pathum Thani Brewery Co., Ltd., Thailand to provide as bacteria inoculum in the anode chamber. Sludge was inoculated into the anode chamber with a synthetic medium containing acetate during the acclimatization period. The medium contained (per L) $CH_3COONa{\bullet}3H_2O$ (2.72 g), $NaH_2PO_4{\bullet}2H_2O$ (3.30 g), Na_2HPO_4 (4.09 g), and NaCl (1.42 g). Anode chambers were refreshed with the fresh medium when the voltage decreased below 50 mV. The anodic acclimatization was conducted for 5 batches to allow the growth of a mature biofilm.

Operation of P-MFC

Anode chamber was filled with synthetic wastewater containing (g L^{-1}) 3.30 $NaH_2PO_4{\bullet}2H_2O$, 4.09 Na_2HPO_4, 1.42 NaCl, and 10 mL of trace element solution. The composition of the trace element solution was (g L^{-1}) 3.0 $MgSO_4{\bullet}7H_2O$, 0.5 $MnSO_4{\bullet}2H_2O$, 1.0 NaCl, 0.1 $FeSO_4{\bullet}7H_2O$, 0.1 $CaCl_2{\bullet}2H_2O$, 0.1 $ZnSO_4$, 0.01 $CuSO_4{\bullet}5H_2O$, and 0.01 H_3BO_3. Acetate was added as the organic carbon compound to get a chemical oxygen demand (COD) concentration of 1500 mg L^{-1}. During the experiment, the anode chamber was conducted under strictly anaerobic conditions. The cathode chamber was filled with synthetic wastewater containing (mg L^{-1}) 74.7 KH_2PO_4, 90.0 $MgSO_4{\bullet}7H_2O$, 25.0 $CaCl_2{\bullet}2H_2O$, 64.0 NaCl, 5.0 $FeSO_4$, 100.0 $NaHCO_3$, and 1 mL of trace metal mix. The composition of the trace metal mix was (g L^{-1}) 2.86 H_3BO_3, 1.81 $MnCl_2{\bullet}4H_2O$, 220.0 $ZnSO_4{\bullet}7H_2O$, 390.0 $Na_2MoO_4{\bullet}2H_2O$, 79.0 $CuSO_4{\bullet}5H_2O$, and 49 $Co(NO_3)_2{\bullet}6H_2O$. NH_4Cl was added to get an NH_4^+–N concentration of 300 mg L^{-1}. *Chlorella vulgaris* and *Scenedesmus quadricauda* samples harvested from the algal cultures were also inoculated to the cathode chamber to get a biomass concentration of 0.25 g L^{-1} of dry weight. In order to accommodate for microalgae to gain CO_2 from the atmosphere, the cathode chamber was not closed. Light-emitting diode (LED) tube lamps were used to provide the artificial light for the microalgal photosynthesis and the measured intensity was 7000 lx. All experiments were conducted in replicates for *Chlorella vulgaris* (CV-R1 and CV-R2) and *Scenedesmus quadricauda* (SQ-R1 and SQ-R2).

Data acquisition, chemical analysis, and calculations

The voltage across 1000 Ω external resistance was recorded using a voltage data logger (Grant Instruments, Cambridge Co., Ltd). Polarization test was performed at 336 h from the beginning by varying the external resistance from 3300 to 20 Ω. The current density and power density were normalized to the surface area of the cathode. Polarization and power curves were plotted to determine the internal resistance and maximum power density, respectively. The COD in the anodic wastewater and NH_4^+–N in the cathodic wastewater were determined by the standard methods [3]. Dissolved oxygen (DO) was monitored by a DO meter (YSI 550A, Xylem Inc., USA). Statistical significance of the experimental results was measured by the t-tests.

Results and Discussion

Bioelectricity generation of P-MFCs for 460 h (approximately 19 d) of under continuous light conditions is shown in Fig. 1a. *Chlorella vulgaris* showed the highest electricity generation of 0.384 and 0.404 mA in R1 and R2, respectively. However, *Scenedesmus quadricauda* achieved low peak electricity outputs of 0.211 mA and 0.230 mA in R1 and R2, respectively. During the experimental period *Chlorella vulgaris* and *Scenedesmus quadricauda* reached mean electricity generation of 0.367 (±0.034) and 0.197 (±0.029) mA considering both R1 and R2. Differences in the electricity generation with two microalgae strains in the cathode chamber were statistically significant (t-test, $p < 0.05$). The electricity generation was dependent on the DO in the catholyte. *Chlorella vulgaris* generated a higher DO of 10.4-13.7 mg L^{-1} during the experimental period compared to the *Scenedesmus quadricauda* (7.3-9.9 mg L^{-1}).

Under 12/12 h of alternative light/dark regimes, the electricity generation fluctuated as shown in Fig. 1b due to the variations of the DO levels. Due to the photosynthetic activities of microalgae, the electricity generation increased during the light phase. Electricity generation dropped due to the cessation of photosynthesis and the occurrence of microalgal respiration which consumes oxygen during the dark phase [4]. *Chlorella vulgaris* assisted P-MFC reached its peak electricity generation of 0.476 mA in R2, and it was 0.341 mA for *Scenedesmus quadricauda*. However, *Scenedesmus quadricauda* reached a similar peak electricity output in one light cycle only. It was approximately between 0.20-0.25 mA in other light cycles. The electricity generation was maintained to more than 0.2 mA under the dark phase by *Chlorella vulgaris*. However, it dropped to less than 0.05 mA with *Scenedesmus quadricauda*.

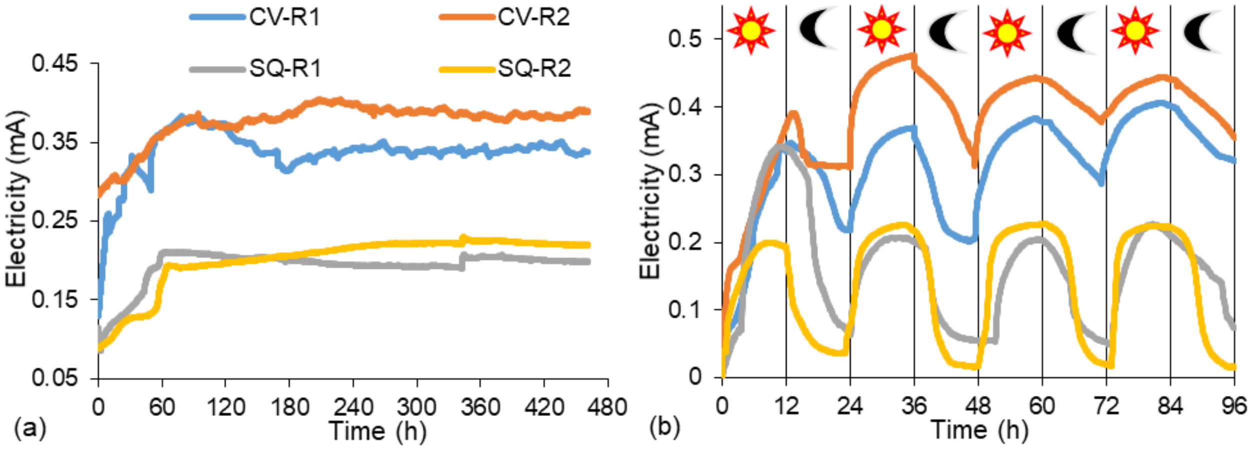

Fig. 1. Electricity generation of P-MFCs under (a) continuous light conditions, and (b) 12/12 h of alternative light/dark conditions.

Polarization and power curves obtained from P-MFCs operated under continuous light is shown in Fig. 2a and 2b, respectively. According to the polarization slope method, the internal resistance of the cell with *Chlorella vulgaris* assisting cathodic reaction was 731.3 and 726.4 Ω. It drastically increased to 1063.3 and 1083.7 Ω with *Scenedesmus quadricauda*. Some of the factors that affect the internal resistance of an MFC reactor are effective surface area of electrodes, catalytic abilities of electrode materials, area of the membrane that contact with liquid, the distance between the two electrodes, and the conductivities of anolyte and catholyte [4, 5]. Since all the experimental parameters were similar except the microalgal inoculum, the increase in the internal resistance could be due to the lower DO levels in the catholyte. When the DO level is higher, it facilitates the cathodic reduction reaction better. The maximum power density obtained from the power curve was 0.00713 and 0.00877 W m^{-2} for *Chlorella vulgaris*, and 0.00249 and 0.00303 W m^{-2} for *Scenedesmus quadricauda* in R1 and R2, respectively.

After 460 h of operation, the COD removal efficiencies reached to 79.8, 75.7, 72.3, and 77.4% in CV-R1, CV-R2, SQ-R1, and SQ-R2, respectively. Anodic conditions are similar in all P-MFCs. Anodic COD removal was independent of the microalgal inoculum in the cathode chamber, thus, the differences in removal efficiencies could be related to the biological activities of bacteria biofilm. The NH_4^+–N removal efficiencies reached to 70.0 and 74.7% in R1 and R2 with *Chlorella vulgaris*, after 460 h under continuous light conditions, respectively. It was 60.2 and 57.4 % for *Scenedesmus quadricauda* in R1 and R2, respectively. These results indicate that *Chlorella vulgaris* can be used to efficiently treat the wastewater that contains NH_4^+ while generating oxygen for the cathodic reaction. The biomass generation was highest with *Chlorella vulgaris* reaching productivities of 0.091 and 0.112 g L^{-1} d^{-1} in R1 and R2 during 460 h under continuous light, respectively. *Scenedesmus quadricauda* reached lower biomass productivities of 0.071 and 0.067 g L^{-1} d^{-1} in R1 and R2, respectively.

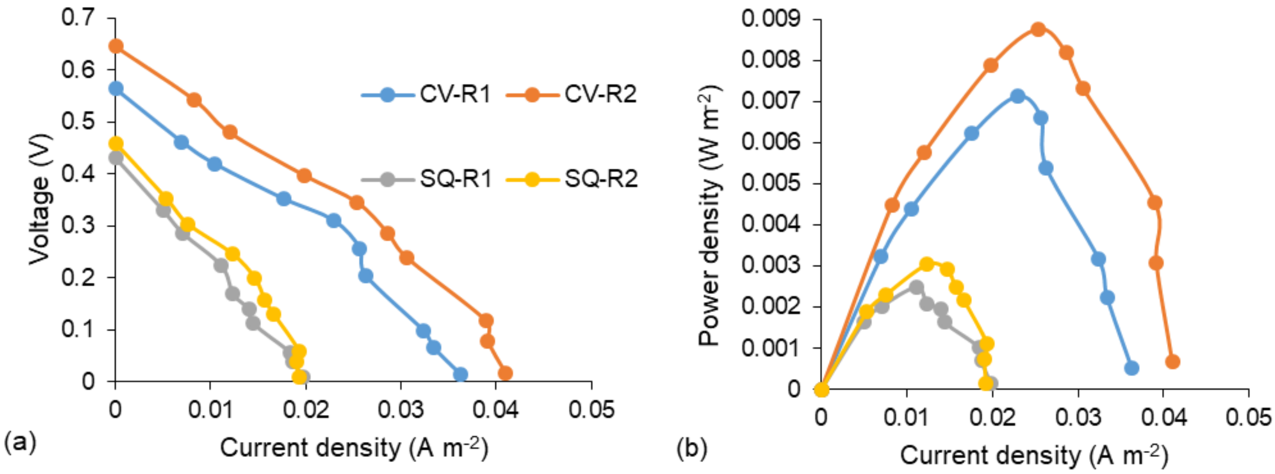

Fig. 2. (a) Polarization, and (b) power curves obtained from the P-MFCs at 336 h.

Conclusions

Chlorella vulgaris assisted P-MFC showed better power generation compared to the *Scenedesmus quadricauda* assisted P-MFC due to the higher photosynthesis of *Chlorella vulgaris* facilitating more DO concentration in the cathode chamber. As a result of the lower internal resistance, the bioelectricity generation of the cell enhanced. Under continuous light conditions, microalgae achieved stable electricity generation in the cell. However, the power generation fluctuated under 12/12 h of light/dark regimes which was conducted to simulate the natural solar light conditions. This could be an obstacle in the practical applications of the systems. Technological approaches to reach the stability of the cell should be investigated in future works.

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DEVELOPMENT AND PROCESS OPTIMIZATION OF REACTIVE EXTRACTION FOR CARBOXYLIC ACID REMOVAL FROM HIGH SOLID LEACH BED REACTOR

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Leached bed reactor (LBR) is a hydrolysis-acidification system is suitable for high solid food waste treatment. In LBR initial rapid mixed culture hydrolysis-acidogenesis process of carbohydrate-rich food waste generates leachate having a wide stream of carboxylic acids with dominant amounts of lactic acid. This leads to decrease in the pH and could alter the free energy change of VFA producing and VFA consuming reactions. Overall inhibitory effects of VFA accumulation could not only delay the acidogenesis and methanogenesis process but also increase the requirements of chemical buffering agents. The strategy of removing volatile fatty acids (VFAs) while they are being produced in LBR could help to alleviate this VFA inhibition and improve the acidogenesis and energy recovery process in LBR.

As a first step, reactive extraction (RE) systems were investigated for the extraction of multiple acids towards developing LBR-VFA extraction system. A wide screening of diluents in combination with extractants was performed and VFA extraction efficiencies for different RE systems were investigated. Synthetic solutions containing equimolar concentrations of individual VFAs were prepared. During physical extraction (PE) experiment, diluents including alkanes, alcohols, esters, and ketones were used alone. For RE, these were combined with extractants such as [Tri-n-octylphosphine oxide (TOPO), tri-n-butyl phosphate (TBP) & Aliquat 336]. Since RE is dependent on extractant & solute concentration selected amine-based RE system (Aliquat 336-Butyl acetate/MIBK) from the first experiment was further evaluated using a range of extractant concentration (0.25, 0.5, 1M) for VFA extraction from LBR leachate samples having different lactate (6-12 g/L) and acetate (0.4-1.25 g/L) concentrations. Extraction was carried out using a different time period (1min, 2.5 min, 5 min, 10 min,16h) to check the efficiency of RE.

P-bonded (TOPO-heptane/Methyl isobutyl ketone (MIBK) and amine-based (Aliquat 336-Butyl acetate/MIBK) RE demonstrated higher distribution coefficients (K) and extraction yield (E %) of the VFAs as compared to the PE process. The off-line RE were promising and extraction efficiencies of 65% (Lactic acid), 75% (Acetic acid), 86.2% (Propionic acid) to almost 100% for long-chain fatty acids (eg. butyric acid, valeric acid and caproic acid) were reached using Aliquat 336 with butyl acetate and MIBK. Results depicted that Aliquat 336-MIBK and Aliquat 336-Butyl acetate performed better than other extractants for lactic acid extraction from synthetic VFA mix. In offline optimization study, an increase in the E % and K was observed with the increase in the extractant (Aliquat 336) and solute (acetate and lactate) concentration and maximum extraction efficiency were observed with 1M of Aliquat 336 concentration. The effects of time on RE depicted that, with the increase in time, E% & K of acetate and lactate was increased. 38.7% acetate & 61.8% lactate was extracted from leachate within 10 min using 1M Aliquat 336-MIBK and 1M Aliquat 336-Butyl acetate respectively. Significant effects of solute concentration on RE was observed as the result shows a linear increasing the trend of K & E% for acetic acid and lactic acid using 1M Aliquat 336-MIBK. However, Aliquat 336-Butyl acetate RE was not effective for acetate extraction as only 18% acetate extraction was achieved within 10 min. For lactic acid, the rate of increase in E% was observed maximum at higher lactate concentration (>10 g/L) for both extraction systems. The increase of acetate and lactate concentration in the leachate had increased the E% of acetic acid and lactic acid by 36% and 51%.

Lactate and other short-chain fatty acid extraction from LBR expected to decrease the requirement of chemical buffers and increase acidogenesis efficiency. Overall, this experiment forms the basis for the development of in situ acidogenesis integrated VFA removal model where energy recovery efficiency can be increased by reducing VFA inhibition and improving leachate characteristics.

Keywords: *Acidogenesis, Leachate, Lactic acid, Reactive extraction, Distribution coefficient*.

HYDROTHERMAL CARBONIZATION OF MANGO SEEDS

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ABSTRACT. Mango which was traditionally eaten fresh has in recent times been turned into a cash crop due to recent advances in processing techniques. Significant quantities of mango fruits are now processed into either dried mango cakes or processed into fruit juice. The current study investigated the carbonization of the seeds as potential energy carriers through hydrothermal carbonization under sub-critical conditions. Keeping the mass fraction and particle size constant, the reaction time was kept at 6 hrs and the reaction temperature was varied between 200 – 240 oC respectively. The results show that the fuel properties of the carbonized mango seeds improved. The thermal heating value increased from 18.70 MJ/kg (untreated seeds) to about 28 MJ/kg and correlated positively with the severity of treatment. However, a negative correlation was observed between process severity and the mass yield. The yield was observed to decrease with increasing severity of treatment. Further analysis of the fixed carbon content, thermal degradation, and interaction between process parameters and response factors are ongoing. Additionally, analysis of the wastewater to determine the usability or otherwise of the HTC process water is ongoing.

Keywords: *Mango seeds; Hydrothermal carbonization; Heating value.*

Introduction

Mangifera indica also referred to as Mango is a tropical fruit enjoyed across the globe because of its sweet juice and health benefits. Production of mangos has seen a surge and is mainly grown in tropical areas, mango production has seen a 75% increase in production from 2003 to nearly 43 million tons in 2016 [1]. Geographically, Asia accounts for more than 72% while Africa accounts for nearly 17% of the world's production. Processing of the mango fruits leaves in its wake a significant portion of the fruit which constitutes the seed. The mango seed shell and kernel accounts for 30 - 45% of the gross weight of the fruit [2]. Thus about 12 million tons of mango seeds residues are generated annually. Due to the high moisture content of the kernel (40%) and mango endocarp (67%) [3] seed and the fibrous mesocarp which may contain high amount of lignin, direct thermal and biological treatment may be limited. The objective of the research was to determine the possibility to energetically valorize the mango seeds through hydrothermal carbonization and the effect of temperature variation on the properties of the hydrochar. This paper only seeks to present preliminary results of the research as more detailed work is ongoing.

Material and Methods

Fresh mango seeds were obtained from a HPW Fresh and Dry fruit processing company in Ghana and transported to Germany within 24 hrs. The seeds were then stored under sub-zero (-18 ºC) temperature conditions until needed. The particle size of the mango seeds was reduced with a laboratory blender Retsch GM 300. The proximate analysis was carried following EN 14744-1 standards. The hydrothermal carbonisation process was carried out in a 1 litre Parr Instrument reactor as shown in Figure 1. A fixed mass (280 g) of the fresh substrate was used for each of the experiments. To maintain a 10% total solid concentration, 520 g of deionised water was added to bring the total mass of substrate in the reactor to 700 g leaving a headspace of about 300 ml. The pH of the sample was measured before and after the carbonization process. After each carbonisation process, before the reactor was opened, the gas in the headspace was evacuated into a gas bag. After opening the reactor, the carbonised sample was separated into solid hydrochar and the hydrolysate with a Buchner funnel attached to a vacuum pump. The wet

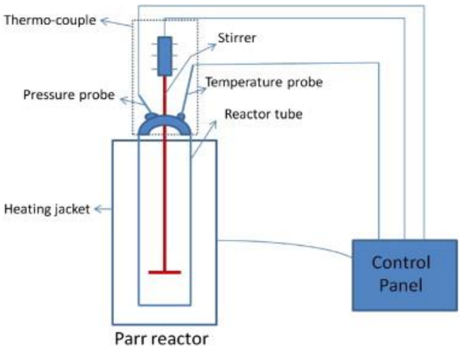

Fig. 4. Setup of hydrothermal carbonization process.

hydrochar was then dried in an oven at 105 ºC. Samples of the dried hydrochar was used to determine the calorific value. The hydrolysate was stored at 4 ºC until needed for further experiments. The calorific value was determined with a Parr Instrument GM 500 bomb calorimeter. All analytical experiments were conducted in triplicates.

Physical characteristics of mango seeds

The mango kernels had a moisture content of 75%, dry matter content of 25%, volatile solids of 98% and ash content of about 2%. The high moisture content of the mango seeds could be due to the presence of some flesh on the seeds due to the processing technique used by the industry in Ghana. This the residual mango flesh was not removed from the kernel prior to the determination of the moisture content and further processing of the seeds. The results of volatile solids and ash content compared favorably with results of [4]. Even though the result moisture content of the fresh mango seeds differed from already reported figures, this could be explained due to the presence of the residual flesh which is high in moisture content as reported by [5]. Further experiment to confirm the moisture content of the dried seeds was found to be 2.5% which is consistent with reported values in literature.

Table 1. Maturity and nutrient properties of the compost obtained from co-composting of abattoir blood meal and horse stable bedding waste.

Parameter	Unit	Fresh mango seed
Moisture content	%	75.00
Dry matter	%	25.00
Heating value	MJ/kg	18.70
Volatile solids	%	98.00
Ash content, %	%	2.00

Hydrochar heating value and yield

The heating value of the hydrochar was also measured. A trend of increasing heating value with increasing process temperature was observed. For instance, the heating value of the mango seeds increased from 19.75 to 22.4 MJ/kg when the seeds were carbonized at a temperature of 200 ºC representing a 20% increase in heating value. A further increase in calorific value to 27.8 MJ/kg was observed as the temperature was increased to 240 ºC. This was expected due in part to the expected carbon enrichment at higher process temperature leading to higher carbonization. The observed trend of increasing heating value as result of increasing process temperature is in agreement with findings of Zhang et al. [6].

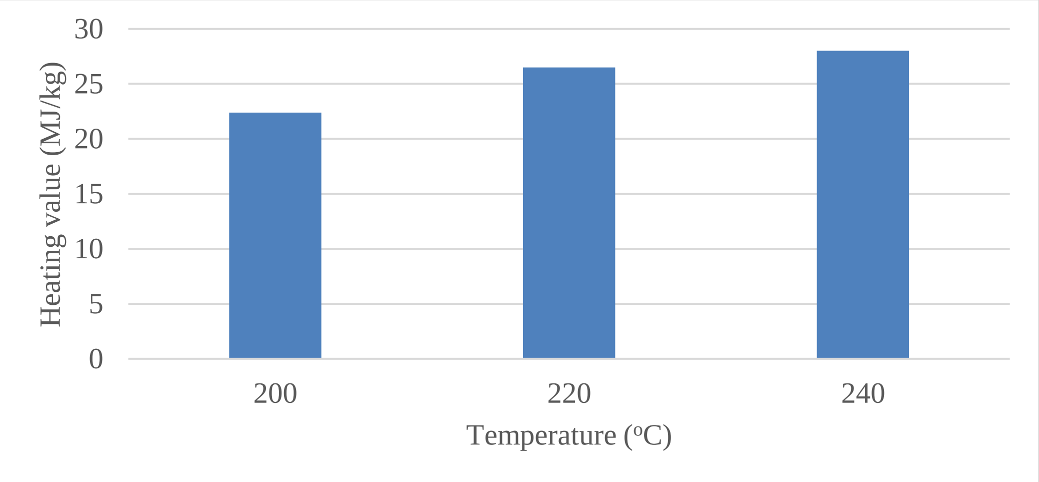

Fig. 5. Heating value of mango seeds hydrochar at different process temperature.

The yield of hydrochar was measured as a percentage of the total solid content of the mango seeds put into the reactor. The yield of hydrochar was found to decrease with increasing process severity. For instance, at process heating temperature of 220 ºC, 50% of the total solids input into the reactor was lost to the hydrolysate and the gaseous phase. The low yield as a result of increasing process severity due to temperature increase, could be due to the increased hydrolysis of the cellulose and hemicellulose as a result of high temperature which could have been triggered by the softening of the lignin. Further, the multiple reaction taking place could have resulted in the conversion of some of the hydrolyzed monomers into methane and hydrogen gas both of which were detected in the gaseous phase but not quantified. Additionally, increased process temperature is reported to increase densification due to carbon enrichment which eventually reduces the molecular weight of the hydrochar. The trend of decreasing yield as a result of increased process temperature is consistent with literature [7].

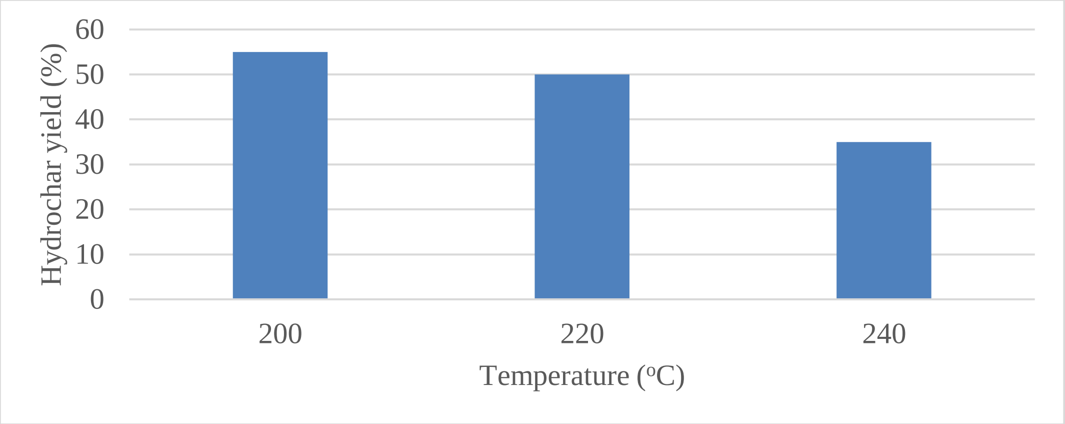

Fig. 6. Yield of mango seeds hydrochar at different process temperature.

Comparison of HTC process with drying or pyrolysis

High moisture content is one of the inhibitory factors that hampers the use if biomass directly as a fuel. The huge thermal load required to dry the biomass to an equilibrium moisture content also negatively affects the heating value of the fuel. As pointed out by Narra [8], the evaporative energy required to reduce the moisture content of the biomass reduces the heating value of fuel significantly. With the fresh mango seeds having a moisture content of 75%, the net heating value is significantly reduced from 18.70 MJ/kg to 4.06 MJ/kg taking into account the effect of the free water. Direct utilization of mango seeds as fuel or in a pyrolysis or gasification plant is thus not prudent unless the energy required drying is free such as from solar or open air drying. One of the key advantages of hydrothermal process is its ability to handle wet biomass. Unlike other thermal process that require the moisture content of the biomass to be reduced significantly, the presence of moisture in both its free and bound state enhances the hydrothermal process. Aside from this, the hydrothermal carbonization process is also a physical and chemical dewatering process. The conversion of the carbon chains through hydrolysis, decarbonylation and decarboxylation reaction results in the removal of carboxyl groups resulting in the lowering of Hydrogen:Carbon and Carbon: Oxygen groups through the production of Carbon dioxide and Hydrogen gases. Further the structural changes resulting in intensive carbonisation leads to the destruction of the cellulose structure thereby leading to the loss of its water affinity property. Lastly the low operating sub critical temperature as compared to pyrolysis and gasification makes it a better bioenergy conversion pathway.

Other Fuel properties

The other fuel properties such as the ash content of the fuel, the fixed organic carbon content, the mineral content of the ash and the thermal degradation characteristics are being analyzed.

Conclusions

Hydrothermal carbonisation of mango seeds can be seen as possible pathway to the energetic valorisation of mango seeds which are otherwise disposed off in environmental unfriendly way. This preliminary results of hydrothermal carbonisation of mango seeds points to significantly improved heating value of the hydrocar when compared with dried uncarbonized mango seeds. The results also confirm the effect temperature has on the heating value and the mass yield of hydrochar produced from mango seeds.

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IMPORTANCE OF SLUDGE CONDITIONING IN ATTENUATING ANTIBIOTIC RESISTANCE: REMOVAL OF ANTIBIOTIC RESISTANCE GENES BY BIOLEACHING CONDITIONING AND SUBSEQUENT COMPOSTING

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Conditioning can drastically improve the dewaterability of sewage sludge and thus it is widely practiced in most wastewater treatment plants (WWTPs). In WWTPs, various antibiotic resistance genes (ARGs) present in sewage are concentrated in the sewage sludge, but the effect of sludge conditioning and subsequent composting on ARGs in sludge remains unclear. Here, we investigated changes in 46 target ARGs and *intI1* during the sludge conditioning treatments and the subsequent composting of dewatered conditioned sludge, to find a useful way to attenuate antibiotic resistance genes in sewage sludge. The effectiveness of different sludge conditioning methods (namely chemical conditioning with PAM, chemical conditioning with Fe[III]/CaO, bioleaching conditioning, and chemical acidification conditioning) in removing ARGs and *intI1* from a municipal sewage sludge was compared by using real-time quantitative PCR, and their impacts on the damage of sludge microbial cells and the sludge bacterial community composition were respectively evaluated by using flow cytometry and illumine sequencing of bacterial 16S rRNA genes. The chemical conditioning with Fe[III]/CaO and bioleaching conditioning drastically reduced both the absolute and relative abundances of most ARGs in the sludge via damaging the bacterial hosts carrying ARGs, which could not be achieved by the other sludge conditioning treatments. In addition, the abundances of ARGs were further reduced after the composting of dewatered bioleached sludge, and the mature and stable compost products with low abundance of ARGs were successfully obtained. Therefore, sludge conditioning can be an important sludge treatment process in attenuating antibiotic resistance in sewage sludge, and the combination of bioleaching conditioning and subsequent composting of dewatered bioleached sludge can be employed as an effective conditioning way to reduce ARGs in sewage sludge, potentially limiting their release to the environment.

Keywords: *Sewage sludge, Antibiotic resistance genes, Removal, Conditioning, Composting.*

BIOPROCESS ROBUSTNESS OF NEWER POLYHYDROXYALKANOATE PRODUCERS AS SUSTAINABLE AND PERSISTENT INDUSTRIAL STRAINS

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ABSTRACT. Polyhydroxyalkanoates (PHAs) are naturally occurring ester-polymers synthesized by several microbes as carbon reserve and protectants against environmental stress. Owing to their exceptional multifaceted properties like insolubility in water, non-toxicity, environmental compatibility, biodegradability, piezoelectricity, and thermoplasticity, these polyesters are gaining huge attention over known biodegradable polymers. However, PHAs are still lagging towards becoming a part of sustainable and circular economy due to 3–4 times higher cost in comparison to conventionally available polymers. The high cost is mainly accredited to the microbial incompetence towards carbonaceous raw materials (>45%) and polymer recovery process (>26%). This can be overcome by identifying efficient PHA producers with elevated substrate utilization capacity and potential to accumulate high amounts of PHAs. We hereby report isolation and potency of a new environmental bacterium for polyhydroxyalkanoate biosynthesis.

Keywords: *Biopolymers; Metabolic optimization; Process behavior; Fermentation,*

Introduction

Polyhydroxyalkanoates (PHAs) are a type of naturally occurring polymers which are synthesized by several microbes for storage of carbon reserve and protectors from environmental stress [1]. These biopolymers comprise of varying length and number of -hydroxyalknoic acids, further segregating into homo or copolymers. This variation usually depends on the type of microbial producer and cultivation conditions. The conditions required by most bacteria for PHA synthesis are defined as feast-famine, where the growth medium is enriched in carbon while being limiting for other nutrients such as phosphorous, nitrogen, or magnesium [2]. This interaction between the producer and the substrate ultimately determines both quality and quantity of the PHA biosynthesis.

The current market for biodegradable PHA based plastics is 25.3 metric-tons per year and is expected to increase fourfold by 2021. This makes them to have the highest relative growth rates among all known biopolymers. The two major reasons for such high growth rates are that they can be produced from renewable sources and their biodegradation is embedded in a closed loop [3]. However, they still haven't been able to fully curb our reliance on conventional petrochemical plastics. This is due to the fact that PHA biopolymers cost is estimated to be 3–4 times higher than conventional polymers, ranging between US$ 4.69–6.06/Kg. Such high costs of PHAs are mainly attributed to the carbonaceous raw materials (>45%) and polymer recovery (>26%) [4]. To overcome these bottlenecks associated with PHAs growth and expansion, major research and development is needed to enhance their commercialization. One of the approaches which can be taken here is through identifying efficient PHA producers with elevated substrate utilization capacity and potential to accumulate high amounts of PHAs.

Various bacteria have been previously reported from various environments, however only a few have been isolated from samples like wastewater and sludge ecosystems. These competitive nutrient limiting environments tend to harbour a huge diversity of microbes with potential polyhydroxyalkanoate synthesis ability [5]. The main focal point of our research here was to isolate and characterize potential PHA producing microbes through a rigorous screening process. The isolated microbes with high potency for PHA synthesis are also identified and optimized for fermentation.

Material and Methods

Isolation and screening

Various environmental samples (sludge, digestate and compost) were collected in sterile glass containers, tapered tightly and stored at 4°C till further analyses. In sterilized water, 1% of sample is dissolved. Then, the sample is serially diluted in sterile distilled water and followed by plating on the carbon rich nutrient agar plate (supplemented with 1% glucose) and incubated at 37 °C for 24 hrs. Morphologically distinct colonies were subculture to obtain pure colonies. The pure isolated colonies were further subjected to 0.02% alcoholic solutions of Sudan Black B. The staining procedure was followed according to Pillai et al. [6]. The isolates were further confirmed for PHA biosynthesis by a qualitative fluorescence-based flow cytometric method using Nile blue A staining [7].

PHA biosynthesis and extraction

For batch shake flask culturing, inoculum was prepared by introducing seed culture of selectively screened isolate in nutrient broth (Beef extract – 3 g/L, Peptone – 5 g/L) and incubated overnight at 37 °C. This seed culture is further used to inoculate Kannan and Rehacek medium with slight modifications by using 2% glucose as the sole carbon source [8]. An inoculum quantity of 10% was used for this study. During fermentation, samples were collected at appropriate intervals and growth pattern was determined by measuring optical density at 600nm. After the fermentation was complete, the culture broth was centrifuged and obtained cells were lyophilized. The lyophilized biomass was subjected to extraction by using chloroform as the organic solvent. Extracted PHA was precipitated by adding chilled methanol. The extracted polymer was subjected to nitrogen evaporation and processed for further analysis.

Production optimization

In order to achieve optimize the synthesis of polyhydroxyalkanoate in the isolated bacterium, different components of the production medium were tested with varying carbon, nitrogen, and salt concentrations. Cultivation of the bacterium was maintained at 37 °C with a shaking speed of 200 rpm. The most efficient categories and concentrations of the production medium were used to further optimize the physiological parameters of fermentation i.e. temperature, pH and inoculum size in batch mode [9]. All the cultures were performed in triplicates.

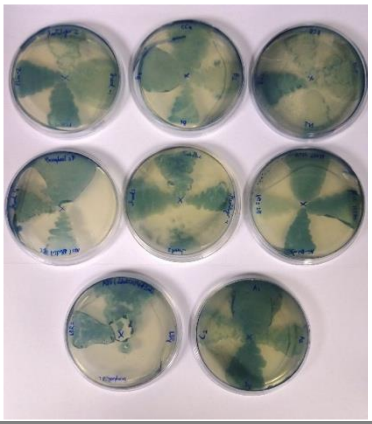
Characterization of polyhydroxyalkanoate

The extracted polyhydroxyalkanoate was analysed by using Gas chromatography - Flame Ionization detection according to the protocol developed by Juengert et al. [10]. The type of PHA was confirmed by comparing it with GC spectra of different pure PHAs.

Results and Discussion

Isolation and screening of PHA producing microbes

Among 60 different isolates which were obtained from different environment samples, one 35 showed positive results for Sudan black B (Fig. 1). This stain acts as a lyophilic dye which stains the intracellular



inclusions as blush black. To further confirm the inclusion bodies to be formed of polyhydroxyalkanoates, Nile blue A fluorescence was tracked using flow cytometer (Fig. 2). The cell population shift on the scattering plot confirmed the presence of PHAs in one of the isolates i.e. IBA1.

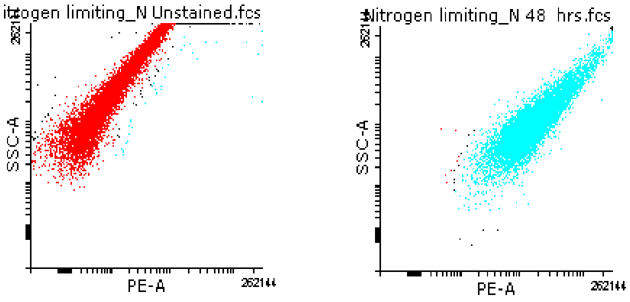


Fig. 1. Sudan Black B staining of potential PHA producers.

Fig. 2. Fluorescence shift in IBA1 before and staining with Nile blue A.

Growth and PHA production

Culture dynamics followed by IBA1 was studies in a semi defined medium using glucose as the sole carbon source. The growth curve observed for this bacterium shows a lag phase till 4thhr followed by the exponential phase till 32ndhr. (Fig. 3). The bacterium then transitions to late exponential phase. Flow cytometric analysis shows cellular PHA accumulation to commence at the early lag phase and gradually increases till the end of exponential phase around 48 hrs which coincides with maximum biomass production (data not shown). The accumulated PHA granules were extracted as biopolymers using chloroform. The accumulated PHA was simultaneously quantified using gas chromatography – flame ionisation detection.

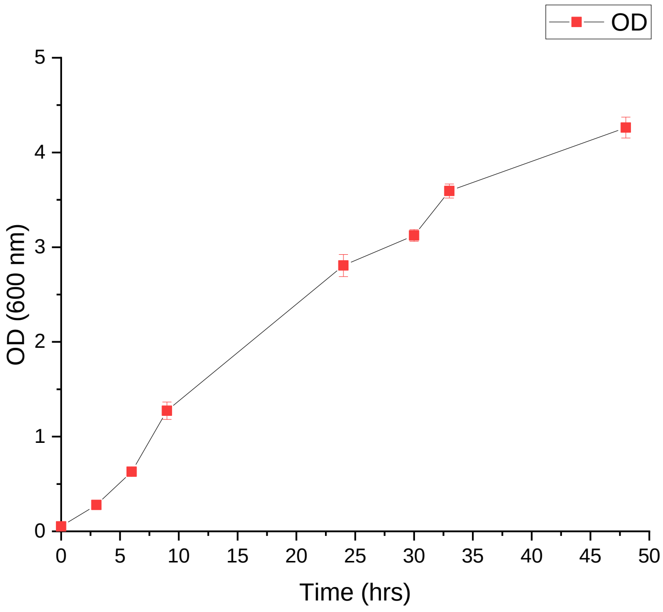


Fig. 3. Growth curve during fermentation of IBA1.

Effect of substrate optimization

To optimize and elevate the PHA production efficiency of different substrate components of the production medium were optimized through one-variable-at-a -time approach in shake flask. The maximum dry cell biomass (DCW) and PHA yield for IBA1 was observed as 9.7 gDCW L^{-1} and 5.7 gPHA L^{-1} respectively. The highest percentage of PHA accumulation was 58.3%.

Characterization of biopolymer

The chemical nature of the extracted polymer was identified as polyhydroxybutyrate. This is the most common type of polyhydroxyalkanoate and finds various applications in biomedical, agricultural and environmental sectors [11].

Conclusions

The present study reports the isolation of a potent polyhydroxyalkanoate synthesizing bacterium i.e. IBA1 using glucose as the sole carbon source. These results open up the potential of environmental microbes as a potential source for PHA biosynthesis. The GC-FID analysis revealed the chemical nature of this biopolymer to be polyhydoxybutyrate. The process of obtaining this biopolymer from IBA1 can enable the development of high productivity process with low-cost nutrients which can be adopted to industrial scale.

Acknowledgement

The authors are thankful to the Department of biology, Hong Kong Baptist University for the support.

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ENVIRONMENTAL AND HEALTH RISK ASSESSMENT OF TECHNIQUES FOR ANAEROBICALLY DIGESTED MANURE CENTRATE: COMPARATIVE INVESTIGATION BETWEEN DENMARK AND CHINA

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Large-scale and intensive livestock industry has globally developed over the last a few decades to satisfy our rising demand on meat, dairy and eggs. This scenario, on the other hand, has resulted in the overwhelming production of livestock wastes, and thus, threatening our sustainable agricultural production. As a well-established technology, anaerobic digestion has been widely implemented for the treatment of livestock wastes with the production of biogas, a source of renewable energy, and digestate, high quality organic fertilizer. In practice, digestate is typically separated into biosolids and highly concentrated liquid, which is also commonly named as digested manure centrate, for effective management. Biosolids are easily handled to produce organic compost, while digested centrate remains challenges, given the imbalance between its continuous production and seasonal agricultural activities.

A series of mature techniques and modes have been applied for the management of digested manure centrate around worlds. These techniques have significant regional features and mainly involve in onsite storage and agricultural spreading, additional concentration for the production of high qualify liquid fertilizer, advanced treatment and clean water reuse or discharge. Thus, this study aims to compare environmental and health risk of techniques and modes that are widely used for the management of digested manure centrate in China and Denmark. Material flow analysis and total life cycle assessment of digested manure centrate from production to application will be performed. Both nutrients and contaminants (i.e. heavy metals and antibiotics) will be included in material flow analysis. Results from this study will provide important advices to advance the management of digested manure centrate depending on local traits.

Keywords: *Livestock wastes, Anaerobic digestion, Digested centrate, Environmental and health risk.*

POLYHYDROXYALKANOATES (PHA) PRODUCTION USING BACTERIAL STRAINS

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ABSTRACT. Enormous usage of plastics in packaging and other products has been creating major problems in their disposal. These non-degradable plastics are accumulating in the environment at a rate of 25 million tons/year. In response to this problem and harmful effects on the environment, considerable interest is developed in producing biodegradable plastics. Bioplastics are a special type of biomaterial produced by a range of microbes, when they cultured under different nutrient and environmental conditions. These polymers are accumulated as storage materials by allowing microbial survival under stress conditions. The search for biodegradable plastics has led to number of partially and completely biodegradable products. Polyhydroxyalkanoates (PHAs) are a group of biologically derived polyesters with diverse applications. PHAs production was evaluated using different bacteria and carbon sources during my research stay in India and Japan. These PHAs are easily biodegradable and can be considered as one of the tools to clean the environment.

Keywords: *Bioplastics; Polyhydroxybutyrate; Bacteria; Pseudomonas; Bacillus.*

Introduction

The world is currently facing the depletion of resources due to the population growth [1]. On the other side, accumulation of plastics and waste materials due to human and industrial activities are creating problems to the environment. A promising approach to solve these problems by the integration of waste treatment with the generation of valuable chemicals, has recently been highlighted [2-4]. A major portion of plastic waste is buried in soil owing to its non-biodegradable nature. Environmentally friendly polyhydroxyalkanoates (PHA), produced by a variety of bacteria, are a substitute for conventional plastics. Poly-β-hydroxybutyrate (PHB) is a kind of PHA stored inside bacterial cells. PHB has several interesting characteristics and can therefore replace synthetic plastics. PHA producing bacteria have been isolated from various sources, and interesting work has been carried out in our laboratory.

Materials and methods

Bacteria

Aerobic consortia treating composite wastewater from domestic and industrial origin was used as biocatalyst for PHAs accumulation. Prior to inoculation, the culture was washed thrice in saline buffer and enriched. Pure bacterial cultures were isolated in our previous studies which are carried out in India and Japan. A loop of each strain was initially inoculated into 90 mL of nutrient broth in a 300 mL Erlenmeyer flask, and kept in a shaking incubator overnight at 180 rpm under dark conditions at 30 °C. The culture was harvested by centrifugation and washed twice with potassium phosphate buffer (pH 7.5) to eliminate nutrient sources. The cell suspension was inoculated into 80 mL of medium and supplemented with specific carbon sources. Medium pH was adjusted to 7 and autoclaved before transfer to flasks or reactors.

PHAs production and extraction

The same conditions which are used for the growth experiments were maintained for the PHAs production experiments by applying nutrient limitation. Bacterial cultures grown in the presence of various substrates were used for PHAs production. Extractions and estimations of PHAs were performed following a previously reported procedure [1, 5, 6]. The biomass was separated by centrifugation and washed with acetone and ethanol. The resultant pellets were mixed with sodium hypochlorite, centrifuged, and then the pellets were dissolved in hot chloroform and filtered. The PHAs weights were calculated gravimetrically. The extracted pure PHAs was analyzed directly by HPLC. A standard curve was prepared using pure PHB (Sigma-Aldrich).

PHAs characterization

The FT-IR spectrum of PHAs produced by bacteria was measured using FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an NMR spectrometer at 20 °C. The NMR sample was dissolved in deuterated chloroform, and impurities were removed by filtration with cotton. TGA was used to determine the decomposition temperature (Td), DSC was used to characterize the melting temperature (Tm) of PHAs.

Results and Discussion

PHAs production using pure substrates

Various bacteria and substrates were used for the production of PHAs. Among them *Hydrogenophaga palleronii* produced highest PHAs (63% DCW) when it was incubated with the carbon source contains the mixture of acetate, propionate and butyrate (Table 1). *Bacillus tequilensis* and *Pseudomonas otitidis* also produced 59% and 58% of PHAs respectively when they incubated with the mixture of acetate, propionate and butyrate. Some of the bacteria produced copolymer, P3(HB-co-HV) and some produced homopolymer, PHB. The copolymer produced from *B. tequilensis* contains 82% HB and 15% HV [7], whereas the copolymer produced from *P. otitidis* contains 91% HB and 5% HV [4].

Table 1. PHAs production using pure substrates.

Bacteria	Substrate	PHAs	Reference
AMBC	Butyrate	33% DCW	[2]
	Acetate	32% DCW	
	Propionate	11% DCW	
	A+P+B	16% DCW	
AMBC	Glucose	54% DCW	[5]
Pseudomonas otitidis	A+P+B	58% DCW	[4]
Bacillus tequilensis	A+P+B	59% DCW	[7]
Serratia ureilytica	A+P+B	51% DCW	[8]
Hydrogenophaga palleronii	A+P+B	63% DCW	[9]
Pseudomonas pseudoflava	A+P+B	57% DCW	[10]
Bacillus sp. CYR1	Acetate	22% DCW	[11]
	Propionate	17% DCW	
	Butyrate	12% DCW	
	Valerate	15% DCW	
	Caproate	9% DCW	
	A+P+B	33% DCW	

AMBC: Aerobic mixed bacterial culture; A+P+B: Mixture of acetate, propionate and butyrate

Table 2. PHAs production using wastewaters.

Bacteria	Wastewater	PHAs	Reference
AMBC	Effluents from dark fermentation	25% DCW	[2]
AMBC	Food waste	35% DCW	[6]
Pseudomonas otitidis	Acidogenic effluents	54% DCW	[4]
Bacillus tequilensis	Acidogenic fermented food waste	36% DCW	[7]
Serratia ureilytica	Effluents from hydrogen producing reactor	31% DCW	[12]
Co-culture of *Bacillus* and *Cupriavidus*	Wastewater from sewage treatment plant	145 mg/l	[3]
Bacillus CYR1	Cheese whey	151 mg/l	
	Wastewater from sewage treatment plant	8 mg/l	

AMBC: Aerobic mixed bacterial culture

PHAs production using wastewater

Various bacteria and wastewaters were used for the production of PHAs. Among them *P. otitidis* produced highest PHAs (54% DCW) when it was incubated with the acidogenic effluents (Table 2). *B. tequilensis* and aerobic mixed bacterial culture also produced 36% and 35% of PHAs respectively when they incubated with food waste. Some of the bacteria produced copolymer, P3(HB-co-HV) and some produced homopolymer, PHB. The copolymer produced from *B. tequilensis* contains 89% HB and 9% HV (Reddy et al., 2014), whereas copolymer produced from *P. otitidis* contains 84% HB and 12% HV (Reddy et al., 2012).

PHAs production using toxic compounds

The bacteria *Cupriavidus* sp. CY-1 and *Bacillus* sp. CYR1 were used for the production of PHAs using toxic compounds. Strain CYR1 produced highest PHAs (51% DCW) when it was incubated with phenol (Table 3). The same strain showed 42% PHAs when it was incubated with naphthalene (Reddy et al., 2015c). Strain CY-1 showed 48%, 43% and 42% of PHAs when it was incubated with phenol, TCE, and naphthalene. Both the bacteria produced homopolymer PHB, and no copolymer was produced (Reddy et al., 2015b, Chang et al., 2021).

Table 3. PHAs production using toxic materials.

Bacteria	Carbon source	PHAs	Reference
Cupriavidus sp. CY-1	Phenol	48% DCW	[13]
	Naphthalene	42% DCW	
	4-t-BP	23% DCW	
	4-CP	13% DCW	
Bacillus sp. CYR1	Phenol	51% DCW	[14]
	Naphthalene	42% DCW	
	4-CP	32% DCW	
	4-NP	29% DCW	
	4-BP	24% DCW	
Cupriavidus sp. CY-1	TCE+phenol	43% DCW	[1]
	DCE+phenol	41% DCW	

Conclusions

PHAs gaining importance as a promising biodegradable plastic, and on the other hand pollution caused by waste materials and toxic compounds is creating many problems to the environment and human health. To fulfill these two objectives, various bacteria were identified with the ability to degrade the toxic compounds/waste materials with simultaneous accumulation of bioplastics. The results from this study showed that bacteria utilized different pure substrates, toxic compounds as a sole carbon and energy source, grew well and converted them into useful bioplastics. Molecular biology studies identified the genes and enzymes which involved in the PHAs production.

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BIOFLOCCULANT PRODUCTION BY NEWLY ISOLATED BACTERIA FROM ACTIVATED SLUDGE USING FISH MARKET WASTE AS A NUTRIENT SOURCE

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ABSTRACT. The fish market waste is one of the serious environmental concerns due to its higher odour and vigorous microbial growth. Hence the bioconversion of fish market waste (FMW) into valuable bio-products will reduce environmental issue and be a sustainable approach. In this study a bioflocculant producing Klebsiella pneumoniae (UKD 24) bacterial strain was cultivated with FMW as the sole nitrogen source to produce the bioflocculant. The optimum pH range of higher bioflocculant production was 6-8. When FMW was supplemented as the nitrogen source, the UKD 24 strain was able to produce ~88.52% flocculation after 48 hours growth. The purified bioflocculant showed the chemical composition of 12.1±0.3% of protein, 24.3±1.5% of polysaccharides and 0.01% of phenolic contents.

Keywords: *Bioflocculant, Fish market waste, Flocculation activity, Activated sludge.*

Introduction

Flocculation is one of the advanced techniques, which has turned the water treatment process more efficient and suitable for the current scenario. During the flocculation, the stable suspended particles are settled as large floccules with the aid of some polymers called flocculants. Presently, the poly acrylamide based ionic polymers have widely occupied the water treatment plants. However, the carcinogenic nature and higher production cost of the currently used synthetic flocculants are causing reluctant to extend their application. Hence the bacterial bioflocculants come to front as a potential alternate for the synthetic flocculants. The bacteria utilize natural organic matter as its substrate for bioflocculant production and it has been proven as it is biodegradable and nontoxic in nature. Moreover in several studies many toxic substances have been converted as the bioflocculants with the help of bacteria [1-3]. Conventionally it is believed that replacing the carbon source with such cheap waste biomass can reduce the cost of the bioflocculant production. However, in deep inside the nitrogen sources such as yeast extract and beef extract are also remarkably contributing the cost of the production media as 21-50% of the cost of carbon source. In this study, in order to replace the expensive nitrogen sources namely yeast extract and beef extract, we supplied the fish market waste (FMV) as the cheap nitrogen source. The production parameters such as glucose level, FMW and inoculum size were optimized with by Box-Behnken design.

Material and Methods

Bacterial strain and chemicals

The *K. pneumoniae* (UKD 24) previously isolated from the activated sludge samples of Ukkadam domestic sewage treatment plant was used for this study. The kaolin clay was purchased from Loba while all other chemicals were purchased from SRL.

Production media

The following media composition was used for the production of the bioflocculant: glucose – 10 g L^{-1}, K_2HPO_4 – 5 g L^{-1}, KH_2PO_4 – 2 g L^{-1}, $MgSO_4$. $7H_2O$ – 0.2 g L^{-1}, NaCl – 0.1 g L^{-1}, nitrogen source – 1 g L^{-1} & pH – 7±0.1.

Determination of flocculating activity

A loop full of bacterial cells was incubated for 24 hours at 28ºC temperature under shaking speed of 180 rpm. 2 mL of the culture broth was centrifuged and subjected to the flocculation test. The flocculation tests were conducted in the standard jar test apparatus (Deep Vision 1926) by using kaolin suspension method [4]. Briefly 2 mL of cell free supernatant was added to the 200 mL of kaolin suspension (5 g L^{-1}) containing

50 mg L^{-1} $CaCl_2$ and the pH was adjusted to 7. This suspension was initially stirred at 200 rpm for 20 minutes and followed by 100 rpm for 3 minutes. After 5 minutes settlement 3 mL of supernatant was collected and observed it's OD at 550 nm under UV-Vis spectrophotometer (Shimadzu UV-1800). Control OD was obtained by repeating the same but replacing the supernatant by sterile uninoculated production media. The flocculation rate was calculated by using following formula:

$$\text{Flocculation } (\%)=(A-B)/A \quad \times 100 \ \ldots\ldots\ldots \ (1)$$

Where A and B are OD550 of the control and sample, respectively.

Extraction of the bioflocculants

A loop full of bacteria was inoculated into 1 L of desired production media and incubated for 48 hours at 28ºC under shaking (180 rpm). The cells were removed by either centrifugation or filtration and the supernatant was transferred to a glass separating funnel followed by 3 volume of ice cold ethanol was added to the supernatant. The funnel was kept at -20ºC for 12 hours to precipitate the bioflocculant and followed by at room temperature for another 12 hours for complete settlement. The supernatant was drained and the ethanol was redistilled. The precipitate was dialyzed (pore size between 12,000 to 14,000 Da) for 12 hours against distilled water and lyophilized. Finally the yield was calculated by weighing.

Characterization of Bioflocculants

The chemical composition of the bioflocculants was probed by different biochemical methods by using desired standards. The total polysaccharide was estimated by phenol sulphuric acid method by using glucose standards [5]. The protein was estimated through Bradford method using bovine serum albumin as the standard [6]. The total uronic acid content was estimated by carbazole-sulphuric acid method with glucoronic acid standards [7]. Folin-ceocaltue method was used to estimate total phenolic content by using gallic acid as the standard [8]. The presenting functional groups of the bioflocculants were predicted by using FT IR spectra. For this, the freeze dried bioflocculants were compressed with the KBr pellets and the infra-red spectrum was recorded in between 400 to 4000 cm-1 wave numbers under transmittance mode (Perkin-Elmer model spectrum RXI). The 3D excitation emission matrix (EEM) was obtained by using spectrofluorometer (Jasco, FP-8200, Japan) from the excitation and emission wavelengths ranging from 200 to 400 and 300 to 550 nm, respectively, with each 5 nm intervals [9].

Statistical analyses

All the experiments were done in triplicates and the analysis of variants was performed by ANOVA. All the graphs were drawn with the help of Originpro 2015 software.

Results and Discussion

The bioflocculant was produced in two different nitrogen sources namely the commercial yeast extract (YE) and the FMW. After 48 hours incubation period the UKD 24 produced 97.7±0.3% of flocculation in the YE supplemented media while it produced 88.5±0.2% of flocculation in FMW supplemented media.

Characterization of the bioflocculants

The polysaccharides, proteins and glycoproteins are thought to be the major backbone of the bacterial bioflocculants. The phenols sulphuric acid assay revealed that the bioflocculant extracted from FMW supplemented media possessed 24.36±1.5% of polysaccharides and the bioflocculant produced from YE supplemented media possessed 56.99±5.3% of polysaccharides. Similarly the bioflocculants revealed the protein levels as 12.15±0.2% in FMW media and 1.54±0.66% in the YE media. The carbazole sulphuric acid test indicated around 6.0±2.8% of uronic acids are presenting in the YE media produced bioflocculant and it was absent in FMW media produced bioflocculant. The phenolic content of the bioflocculants were 0.21% in YE supplied bioflocculant and it was <0.01 in FMW supplied bioflocculant.

The FTIR analysis revealed that the bioflocculant produced from YE media expressed the functional groups namely OH stretch at 3434 cm^{-1}, CH stretch at 2934 cm^{-1}, C=O of aldehydes at 1737 cm^{-1}, bending vibrations of alkanes at 1470 cm^{-1}, methoxy group of sugar derivatives at 1071 cm^{-1}. Similarly the FMW produced bioflocculant revealed the OH, carboxylate, CH bend and methoxyl groups at the corresponding to the wave numbers respectively 3382, 1653, 1470 and 1064 cm^{-1} (Fig. 1). The 3D EEM of the FMW produced bioflocculant revealed distinguised fluorescence signals than YE poduced bioflocculant. It showed a broad and strong fluorophore at λEx/Em 210-230/390-430 nm which represents the humic acids. A broad fluorescent peak with low intensity at λEx/Em 280/350 is corresponding to the tryptophan like aminoacids. The bioflocculant obtaiened from YE suplemented cultrue revealed the fluorescent components namely at λEx/Em 285/330, 230/335, 280/450 and 350/410 nm corresponding to tryptophan like proteins, aromatic amino acids, fulvic acids and humic acids respectively (Fig. 2).

Flocculation activity of the purified bioflocculant

In terms of flocculation activity the YE produced bioflocculant showed good flocculation than FMW produced bioflocculant (98.1±0.7% in YE and 77.6±5.0% in FMW). However, the FMW produced bioflocculant removed 86.15±4.2 of turbidity in the simulated waste water while the YE produced one removed 98.7±0.5% of turbidity.

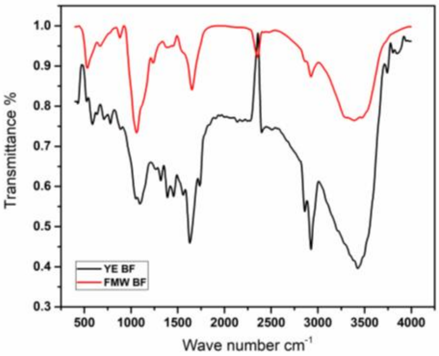

Fig.1. The FTIR spectra of the bioflocculants produced with yeast extract supplementation (YE BF) and fish waste extract (FMM BF).

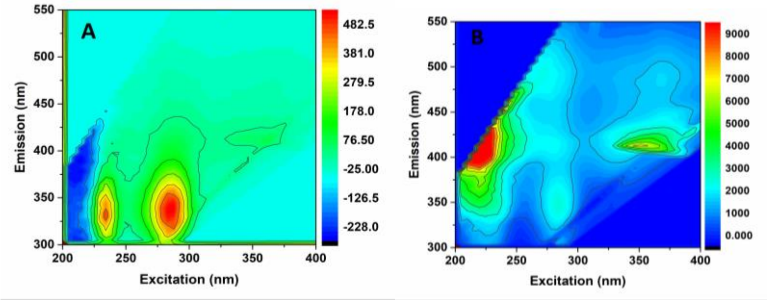

Fig.2. 3D EEM revealed fluorescence peaks of the bioflocculants (A- YE produced bioflocculant, B- FMW produced bioflocculant).

Table 1. Chemical characterization of the FMW.

Parameters	% (w/w)
Total Organic Carbon (TOC)	4.48±0.2
Protein	6.50±0.01
Total Nitrogen	1.8±0.05
Total Ash content	90.80±0.17

Conclusions

In the bioflocculant production media the nitrogen supplements account for ~5.5% of the total media cost. In order to replace the cost effective conventional nitrogen source named yeast extract the cheap fish waste biomass was evaluated. The *K. pneumonia* was able to utilize the fish waste extract as a nitrogen source for the bioflocculant production; however it could not meet the production level of yeast extract. Based on the biochemical tests and spectroscopic analyses it was conformed that the bioflocculants have unique composition and properties while produced by different nitrogen sources. It was also identified that the bioflocculants have unique flocculation properties in the simulated wastewater treatment.

Acknowledgement

This research work was financially supported by the Council of Science and Industrial Research (CSIR) and University Grants Commission (UGC).

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CELL FACTORIES FOR BULK CHEMICAL PRODUCTION FROM INDUSTRIAL SIDE STREAMS

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Carbon efficiency is one of the crucial parameters for the development of sustainable industrial processes. Therefore, any carbon containing side stream of a given process, which is not valorised is problematic. It is not only a waste problem and a potential loss of money, but it also spoils a possibility to increase overall carbon efficiency and thereby compromises efforts to become sustainable.

Microbial processes are one option among others for the valorisation of carbon containing industrial side streams. They offer many advantages as processes involving them are usually environmentally benign and proceed without extreme pressures or temperatures. However, often they suffer from low productivities and low yields. Many industrial side streams are contaminated with substances, which are toxic for microorganisms, aggravating the challenge of low productivities and yield. However, proper purification of the carbon source is costly and therefore most of the times not feasible on economic scale. One process requirement is therefore the identification of microbial cell factories, which can cope with the given constraints.

Lactic acid bacteria are an interesting group of organisms in this context, as they show often a remarkable resistance against inhibiting compounds and conditions. In this contribution we will show case two lactic acid bacterial species – *Enterococcus mundtii* and *Lactobacillus diolivorans,* which have been identified as superior microbial cell factories. One is being established as cell factory for lactic acid production from a wood pulping side stream, the other as base chemical producer from oleochemistry side streams.

Keywords: *Industrial microbiology, Industrial side streams, Lactic acid bacteria, Wood pulping side stream, Spent sulfit liquor, Crude glacerol.*

EXTRACTION OF PECTIN FROM CITRUS LIMETTA PEEL: AN APPROACH TOWARDS WASTE MANAGEMENT

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Pectin is an industrially important compound that is used as a food additive or gelling agent. Extraction of pectin from C*itrus limetta* peel is an economic and eco-friendly approach to obtain pectin while simultaneously reducing the waste management problem. Pectin is a cementing material between plant cell walls, comprising of repeated units of galacturonic acid (GalA). Ultrasound Assisted extraction (UAE) of pectin from *Citrus limetta* peel was investigated and optimised using Response surface methodology (RSM). To optimize the extraction conditions, a five-factorial Box–Behnken design (BBD) was employed with varying process parameters such as pH (1-3), extraction time (5-32 min), duty cycle (5-25 sec), amplitude (40-100), and liquid-solid ratio (14-30). Based on the series of experiments the optimized process parameters for pectin extraction were at pH 1 for 19 min extraction time at amplitude of 70 with a duty cycle of 15:15 and solid liquid ratio of 1:22 g/mL. The maximum yield obtained in the process was 35.582%. The predicted values (27.030%) of BBD were further validated and were found in agreement with experimental yield (25.326%). The moisture content, ash content, and degree of esterification were 8.9%, 2.33%, and 64.03%, respectively. FTIR analysis also confirms the functional groups similarity between commercial and extracted pectin. UAE significantly reduces extraction time, temperatures, and energy consumption of the process. Thus this process can be efficiently employed to meet the global demand of pectin.

Keywords: *Ultrasound; Pectin, Citrus limetta, Valorization, Response surface methodology, Process Parameters.*

IRON NANOPARTICLES (MAGNETITE) -BACILLUS SUBTILIS SYNERGY IN ELECTROFERMENTATION SYSTEM ON PRODUCTION OF BIO-BASED PRODUCTS

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ABSTRACT. Environment conditions can play a major role in controlling the metabolic pathways of bacteria and production of value-added products (VAP). Both the oxidation states of iron [ferrous (Fe^{2+}) and ferric (Fe^{3+})] has the capacity to act as a catalyst in biological systems. The overall objective of this study was to examine the effect of iron nanoparticles on the growth of *Bacillus subtilis* along with the production of VAP in a single chamber Electrofermentation system (EF). The nanomaterials prepared by hydrothermal synthesis was varied from 1 mg/l to 150 mg/l. The results were analysed according to the concentration of the materials and the interaction time between the bacteria at the different concentration. Both positive and negative results were found indicating that iron plays a major role driving the metabolic pathways in bacteria. As the concentration of the materials increased, the growth of *Bacillus* was inhibited as well as biofilm formation reduced. While at low concentration, the production of VAP enhanced signifying iron as a catalyst increasing the electron transport within the bacteria. The diverse functions of iron in bacterial cells namely cell composition, primary and secondary metabolism, catalytic and enzymatic activities is also discussed along with the growth kinetics of bacteria.

Keywords: *Biohydrogen; Platform chemicals; Electron donor; Reactive oxygen species (ROS); fur genes.*

Introduction

Demand for green chemicals and fuels is driving the industry toward greater sustainability in order to increase cost-effectiveness and satisfy consumer demand [1]. *Bacillus subtilis*, a gram-positive bacterium, have several traits, such as the capacity to withstand relatively low pH, high temperature, high sugar, salt and various other harsh conditions, which could be used to develop an advanced biocatalyst and improve the commercial competitiveness of biofuel and volatile acid production. To enhance the production rate, the bacteria was introduced to external electron source in the form of magnetite with respect to electrodes in an EF system. Because of their chemical properties, high surface-to-volume ratio, and special quantum effects, nanoparticles (NPs) also have different properties than their macroscopic counterparts. NPs have previously been shown to have the ability to increase metabolite development in previous studies [2]. The current study is intended to understand the influence of magnetite on the metabolic pathway of *B. subtilis* at different concentrations. Iron's primary functions are catalytic rather than regulatory in nature. Iron is needed for the action of enzymes involved in the tricarboxylic acid cycle's oxidative metabolism, such as aconitase, as well as cytochromes and non-haem iron electron carriers in the electron transport chain [3].

This paper reports a facile hydrothermal method to fabricate magnetite nanoparticles. $FeCl_3.6H_2O$ and NH_4OH were used as precursors to prepare magnetite nanoparticles without any further additives. The prepared nanoparticle was characterised and further added in the fermentation system as well as EF to identify the role of the iron material in the metabolic pathway of *B. subtilis* and further to analyse the product formation. It is already estimated that Iron reduction can deploy an important role in electron transfer in *B. subtilis* [4]. Pyruvate was chosen as a carbon source in the present study, as it is the end product of glycolysis and is a key intermediate molecule required for various metabolic pathways. *B. subtilis* produces lactate, acetate, butanediol, and traces of ethanol from pyruvate [5]. EF was used to resolve the thermodynamic limitations of traditional microbial fermentation and to control metabolism for the production of unique bio-based items [6]. Both traditional fermentation and EF were compared to analyse the synchrony between nanomaterial-microbe-electrode.

Experimental

Materials

All chemicals (AR grade) were used further without any purification. $FeCl_3.6H_2O$, NH_4OH, NH_4Cl, $MgSO_4.7H_2O$, $CuCl_2.2H_2O$, $MnSO_4.H_2O$, $CoCl_2$, $ZnSO_4$, LB broth and Sodium Pyruvate were purchased from Hi Media whereas the Minimal (M9) salt media was purchased from Sigma-Aldrich.

Synthesis of Magnetite (Fe_3O_4) nanomaterial

1.5 g of $FeCl_3.6H_2O$ was dispersed in 50 ml of distilled water and kept under a magnetic stirrer for 15 minutes at 400 rpm. 6mL of NH_4OH was slowly added dropwise followed by a dark brown precipitate. The reaction mixture was then autoclaved for 4 hours at 130°C in a sealed Teflon-lined stainless-steel pressure vessel with a volume of 100 mL with a gauge pressure of 2 bar. A centrifugation-dispersion process was used to collect the black precipitate and purify it with water and ethanol in the ratio 2:1. The reddish black solid powder was collected after 12 h of drying at 65˚ C [7, 8].

Characterisation

XRD was carried out for prepared iron nanomaterial samples using PANalytical Empyrean X-ray diffractometer using Cu Ka (1.54178 Aº) radiation with the X-ray generator operating at 45 kV and 40 mA. The samples were prepared as powder for XRD analysis using sample prepetition kit and back loaded into nickel-coated steel sample holders [9]. Morphological studies of prepared nanomaterials were examined using FE-SEM (JOEL, JSM-7610F) with an increase voltage ranges from 1-15 kV. As the magnetite is high in magnetic energy, the EDAX could not be performed.

Bacillus strain and Growth Condition

Culture was revived from glycerol stock and maintained on Agar plates at aerobic condition. *B. Subtilis* (Genbank accession number: KX470414.1) inoculum for the EF reactor operation was prepared by transferring a single pure colony from Agar plate to nutrient broth (200 mL) and incubating at 37ºC (*optimum temperature for *B. subtilis*) for 10 to 12 h to achieve logarithmic growth of the cells.

Bioreactor

Two sets of design were setup, one with electrodes i.e., Electrofermentation setup and one without electrodes i.e., fermentation system. In both the setups, nine identical bench scale single chambered MEC setup were fabricated using borosilicate glass bottles to have a working volume/total volume of 0.08/0.1 L with headspace of 0.02 L (Fig 1). The electrodes used were graphite electrodes (anode 2 cm,0.5 cm diameter, cathode 4 cm,0.5 cm diameter) and a reference electrode (Ag/AgCl, 3.5 M KCl). Metal solution [NH_4Cl(2g/L),$MgSO_4.7H_2O$(0.01g/L),$CuCl_2.2H_2O$(0.01g/L),$MnSO_4.H_2O$(0.0025g/L), $CoCl_2$(5.6 mg/L), $ZnSO_4$ (28.6 mg/L)], Minimal salt (M9) media (KH_2PO_4,15 g/L; NaCl,2.5 g/L; Na_2HPO_4,33.9 g/L), supplemented with 0.5% (w/v) of pyruvate (in the form of Sodium pyruvate) as carbon source was used as electrolyte in each system. The reactors were inoculated with *Bacillus subtilis* overnight grown in Nutrient Broth. The Magnetite was dispersed in all the reactors with concentration 1 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100mg/L and 150 mg/L respectively with a control.

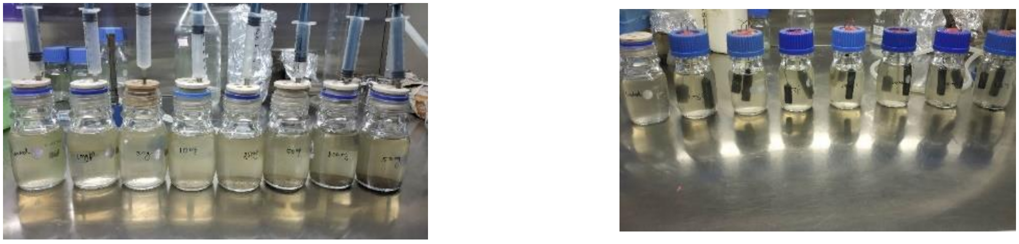

Fig. 1: Reactor setup of Fermentation system and Electrofermentation system respectively.

Analysis

Organic acids (acetate, formate, lactate, succinate etc.) were analysed in HPLC (Shimadzu LC10A) equipped with a UV–VIS detector (210 nm) by injecting 20µL of filtered sample (0.22 µm porosity) into a C18 reverse phase column (250 × 4.6 mm dia; 5 µm particle size, 0.5 mL/h; 210 nm). Acetonitrile was used as mobile phase (40% in 1 N H_2SO_4: pH, 2.5–3.0). Sample peaks were monitored against organic acid standards (all standards were prepared at 1g/L concentration). H_2 gas was analysed using gas chromatography (GC; NUCON 5765) equipped with thermal conductivity detector (TCD; 1/8´´ x 2 m Heysep Q column) with argon as carrier gas. The injector and detector were maintained at 60 ºC each and the oven was operated at 100 ºC isothermally. Growth of *B.subtilis* in EF systems was monitored periodically at 24 h interval using a spectrophotometer (Molecular devises, USA).

Results and Discussion

Characteristics of Magnetite

It can be shown that, the sites and strength of the diffraction peaks are consistent with the normal pattern for Magnetite. The sample show very large peaks (Fig. 2a), indicating the ultra-fine nature and small crystallite size of the particles. The reflection at 2θ values of 23°, 33°, 35°,40°, 50° and 55°corresponds to lattice planes of (220), (311), (400), (422), (511) and (440), respectively for magnetite. An estimation of the magnetite Fe_3O_4 nanoparticles' size was performed using the Scherrer's formula the equation is given below:

$$\mathbf{d=k\ \lambda/\beta\ Cos\ \theta}$$

where d is the particle size of magnetite; k is the shape parameter, also known as the Scherrer constant which is 0.9 for magnetite; λ is the X-ray wavelength (Cu Kα = 1.5418Å); β is the full width at half maximum (FWHM); and θ is the corresponding Bragg diffraction [9]. The particle size was analysed keeping the 2 θ for the highest peak that is 33º for lattice plane (311). The FWHM was analysed and with the equation above, the particle size came 26.6 nm.

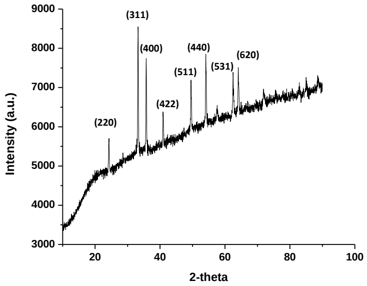

Fig 2a: XRD graph for Magnetite nanoparticle

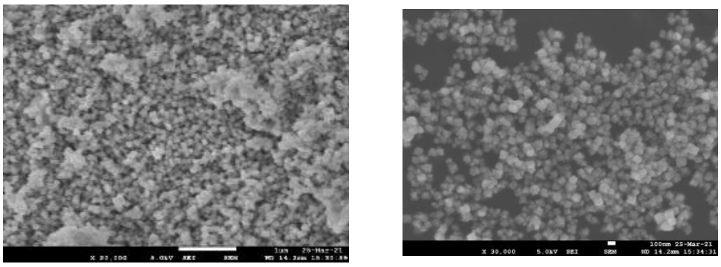

Fig 2b: FE-SEM images of Magnetite nanoparticle depicting cubic spinel structure

The morphology of the magnetite Fe_3O_4 was identified using FESEM. As shown in Fig 2b, the morphology of the non-uniform distributed nanoparticles was observed. The morphology was observed and detected to cubic spinel structure.

Growth of B. subtilis

The growth of *B. subtilis* in fermentation system as well as EF were monitored for 3 days respectively (Fig 3a, 3b). In the fermentation system, the maximum growth was in the reactor consisting 50mg/L iron nanomaterials. The 150mg/L reactor showed a very sharp increase in the 1st day after that the growth was inhibited suggesting that too much iron particles may inhibit the cellular activities of the bacteria. In the EF, the growth was maximum of 25mg/L reactor. All the reactors had a decrease growth rate at 72nd hour unlikely the fermentation system mainly because of the biofilm formation. The declination of the growth after 50mg/L concentration is due to the generation of ROS along with hydroxide and superoxide radicles. ROS may result in oxidative stress, protein degeneration of protein and DNA damage [10].

Organic acids production

The reactors in both the systems including control produced acetate, lactate, succinate and ethanol respectively (Fig. 4a, b). In the fermentation system, the maximum acetate was produced by 10mg/L magnetite concentration reactor, while succinic acid was produced by 50mg/L magnetite concentration reactor. The ethanol and lactic acid production were very low. Similarly, in EF system, the maximum production was ethanol at 25mg/L magnetite concentration reactor. The succinic acid was produced at 10mg/L itself. Generally succinic acid can be produced with CO_2 as a source [11,12] but, when the iron/iron nanoparticles concentration increases up to desired limit, the metabolic pathway favours TCA cycle due to the *fur* regulated genes. Therefore, the production of succinic acid was justified. According to Lin et al., the Iron NPs when subjected in fermentation would result in more amount of acetic acid then ethanol. This matches with results obtained in our experiment as well [13].

Biohydrogen

The metabolic conversion of organic substrates to acid metabolic intermediates in the form of short chain fatty acids or volatile fatty acids leads to the production of H_2 [14,15]. The H_2 production was plotted for both the fermentation and EF as shown in Fig. (5a, b). In the fermentation system, the maximum H_2 production was in 50mg/L added nanomaterial reactor which produced 3 times the bio-H_2 as compared to the control. The EF was more effective as it produced approximately 4 times biohydrogen than the control in 25mg/L reactor. The Iron NPs enhances the activity of ferredoxin oxidoreductase (FdO) due to their increased quantum size and surface area. The activity of FdO leads to the acceleration of hydrogen production [16]. In the control reactor, the Bio-H_2 production is less compared to the reactor with iron NPs because of lower production of the hydrogenase enzyme [17]. Iron ion is an essential component for the formation of hydrogenases and ferredoxin. The iron clusters in the hydrogenase core site serve to transfer electrons between the hydrogen cluster and the external electron carrier (e.g. NADH). Therefore, the addition of iron NPs would facilitate the biosynthesis of hydrogenase and H_2 evolution [18].

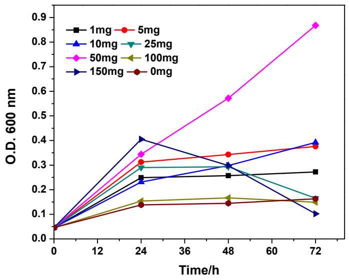

Fig. 3. a: Growth curve determination of *B. subtillis* in Fermentation system

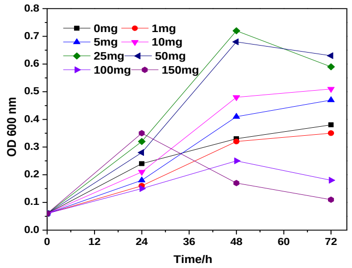

Fig. 3. b: Growth curve determination of *B. subtillis* in Electrofermentation system

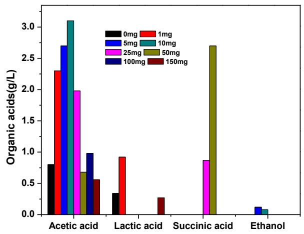

Fig. 4.a: Organic acid production in Fermentation system

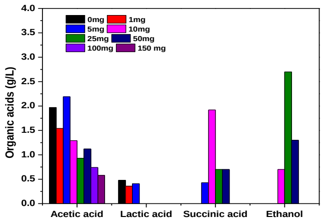

Fig 4: b: Organic acid production in Electrofermentation system

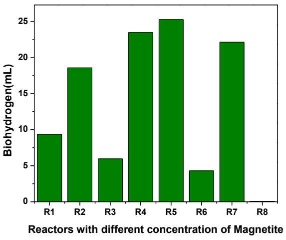

Fig 5. a. H_2 production in the Fermentation system

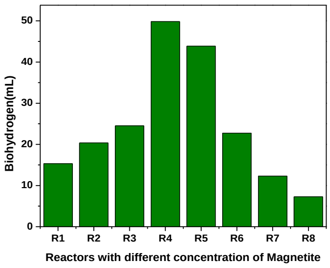

Fig. 5. b: H_2 production in Electrofermentation system

Conclusion

Under the influence of Magnetite, VFA and gaseous end products are produced in elevated concentrations compared to normal control conditions. The increase in the concentration of Magnetite beyond 50 mg/L in both the systems inhibited the *Bacillus* growth as well as production of by-products. In fermentation system, the growth rate was comparatively higher than EF system. The organic acid production was enhanced in both the conditions differing in the by-products received. The Fermentation system produced more amount of acetate, succinate while the EF system produced ethanol, succinate and acetate respectively. The Bio-H_2 was 4 folds more produced in the EF system while 3 folds in the fermentation system compared to the control.

Acknowledgement

The authors wish to thank the Director, CSIR-IICT, Hyderabad for support and encouragement in carrying out this work. Research was supported by DBT-Tata Innovative Fellowship ‘Self-Sustained Design of Photo-Biorefinery for the Closed Loop Production of Fuels and Chemicals’, Department of Biotechnology (DBT; BT/HRD/35/01/02/2018).

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GROUNDNUT OIL CAKE: USEFUL NUTRIENT FOR PULLULAN PRODUCTION BY MICROCOCCUS LUTEUS

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Pullulan is one of the most essential exopolysachharides (EPS) of α(Glucan) units in which most commonly α(1→4) linkage predominate. In the present study, four different agro-industrial wastes namely Groundnut oil cake (GOC), Sun flower oil cake (SuOC), Coconut oil cake (COC) and Mustard oil cake (MOC) were evaluated for pullulan production in solid state fermentation by Micrococcus luteus KX261689. Under the experimental conditions, Groundnut oil cake (GOC) resulted in highest concentration of pullulan (45.26 g/L) among the four solid substrates. Optimum initial pH and moisture content and fermentation time for pullulan production were found out to be 6.5 and 40 %, and 6 days respectively. The present peaks in the FTIR spectrum validated that the obtained purified precipitate was composed of pullulan. XRD analysis showed that the produced pullulan had a fully amorphous structure. A face central composite statistical design was conducted to find out interaction between fermentation time, concentration of GOC, and initial pH value. Results from the study are promising for the economic utilization and value addition of these important agro residues, which are abundantly available in many tropical and subtropical countries.

Keywords: *Pullulan, Micrococcus luteus, Solid state fermentation, Groundnut oil cake, FTIR.*

UNTANGLING THE GENOME OF RARE UNCULTURED BACTERIAL SPECIES FROM PLANT BIOMASS HYDROLYSING MICROBIOME

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Reconstruction of genomes from environmental metagenomes offers great prospect to study the metabolic potential of organisms that are resilient to isolation in the laboratory condition. Here, we were able to assemble 12 high quality draft genomes with an estimated completion of ≥90% from cow (NDC_DR) and buffalo (NDB_DR) rumen metagenomic dataset. By clustering genomes based on average nucleotide identity, majority of these metagenome-assembled genomes (MAGs) were broadly classified as Firmicutes and Bacteroidetes. Comparative analysis reveals that nearly 42% of MAGs represented un-sequenced genus and species, not available in current reference databases. A composite bioinformatic analysis of the MAGs scaffolds using multiple databases (SEED, KEGG, dbCAN) provided a detailed information on the functional attributes of the MAGs. Gene prediction led to the identification of 30,359 protein-encoding genes (PEGs) across 12 MAGs, of which only 44.8% (13,611 PEGs) were annotated against a specific functional attribute. 69,000 were projected to be involved in carbohydrate metabolism. A comparatively higher proportion of carbohydrates associated genes in MAGs, comprising 11.28-22.99% of the total SEED annotated genes indicates relatively high enzymatic activity towards carbohydrate metabolism. Further analysis revealed the presence of 985 carbohydrate active enzymes (CAZymes), covering members from more than 50 glycoside hydrolase family and other CAZymes families. Over 90% of which do not have a good match in the CAZy databases. Additional screens and data mining revealed the presence of an exceptionally high frequency of plant biomass deconstructing gene in Bacteroidetes MAGs (especially B1_2 and C1_22 MAG) as compared to Firmicutes, thus establishing their significant role in plant biomass deconstruction. The results strongly indicate that rumen chamber harbours high numbers of deeply branched and as-yet uncultured microbes that encodes novel CAZymes, candidates for potential use in plant biomass-hydrolyzing and biofuels industries. The inclusion of the 12 MAGs presented here will expand the genomic representation of rumen microbial lineages in the public databases which will further improve the annotation of multi-omics data.

Keywords: *Binning, Genome, Firmicutes, Bacteroidetes, CAZyme, Metagenome-assembled genomes (MAGs).*

DEOILED CAKE AS AN ALTERNATE SUBSTRATE FOR GREEN ENERGY PRODUCTION

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Energy is one of the inevitable and ever demanding need of the world. The world today is going through energy crisis as the fossil fuel are at the brink of extinction. This has led to the search for alternate energy sources. Biohydrogen and Methane are two such sources widely studied. Biohydrogen is carbon neutral fuel which produce only water during combustion and thus is most promising fuel. Methane has an advantage over other fuel as it does not produce NOx, SOx and has lower CO_2 emission which makes it environment friendly. Both hydrogen and methane have high commercial interest with energy density. There are many studies on hydrogen production but are not of commercial interest as high cost of the substrate. The low-cost substrate can give a push to the commercialization of biohydrogen as fuel. Organic waste of oil industry which is the solid residue after the extraction of oil from seeds are deoiled cake. These deoiled cakes can be used as an alternate substrate for biohydrogen and methane production as it has high organic content. In this experimental study anaerobic digestion of ground nut oil cake was carried out for biohydrogen and methane production. Total solid content of Groundnut deoiled cake (GDOC) was 91.9 ± 0.23%. It showed pH 7.2 with 94.99 ± 0.18% presence of volatile solids. The reactor for biohydrogen was inoculated with acidogenic organism and observed for 3 days. The reactor for methane production was established with 8% total solids and 1:1 Inoculum to substrate ratio (ISR) was observed for a 60 days solid retention time (SRT). Cattle dung was used as inoculum. The gas produced was measured daily by liquid displacement method. The content of produced gases (biohydrogen and methane) will be discussed as obtained by Gas chromatography technique.

Keywords: *Deoiled cake, Anaerobic digestion, Biohydrogen, Biogas, Gas chromatography.*

International Conference on Sustainable Biowaste Management 2021

Bioconversion for Biofuels

BIOTRANSFORMATION OF ORGANIC SUBSTRATES TO BIOFUEL AND BIOPRODUCTS BY *THERMOTOGA NEAPOLITANA*

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The environmental impact of excessive exploitation of fossil fuel reserves has inspired several sustainable neo-carbon-neutral technologies. To that end, biological processes like dark fermentation may be leveraged to bioconvert carbohydrate-rich feedstocks to fuels like hydrogen (H_2) or commercially valuable organic acids like lactic acid. Capnophilic lactic fermentation (CLF) is a novel anaplerotic pathway able to convert acetic acid to lactic acid using CO_2 as a carbon enhancer in the hyperthermophilic bacterium *Thermotoga neapolitana*. In this study, we investigated the effect of temperature (50-90 ^oC), salinity level (0-60 g NaCl/L), buffering agent (bicarbonate, phosphate, MOPS, TRIS, and HEPES), and carbon source (arabinose, xylose, glucose, fructose, lactose, sucrose, starch, carboxymethyl cellulose, and laminarin) on the simultaneous production of H_2 and L-lactic acid under CLF conditions. Batch experiments were performed either in 120 mL serum bottles or with a 3.0 L pH-controlled continuous stirred-tank reactors (CSTR) system. The experimental data showed that *T. neapolitana* grew well at 70 and 80 ^oC whereas no growth was observed below 60 and above 80 ^oC. The largest H_2 and acetic acid production were between 10 and 30 g/L of NaCl, whereas the lactic acid production increased up to 50 g/L NaCl. All buffeting agents showed similar metabolic profiles under CLF conditions except for the experiment without any buffering agent. The study reveals the robustness and flexibility of the CLF-based technology using *T. neapolitana* fermentation under various operating environmental conditions. In this context, the application of microorganisms for the biotransformation of organic waste to renewable energy and valuable chemicals would increase resource recycling and support the circular economy.

Keywords: *Fermentation, Thermotoga neapolitana, Organic waste, Biofuel, L-lactic acid.*

IMPROVEMENT OF SLUDGE DEWATERABILITY BY ANAEROBIC DIGESTION AND MECHANISM ANALYSIS BASED ON MOISTURE DISTRIBUTION

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Anaerobic digestion (AD) has been the main technology of realizing sludge stabilization and reclamation. The degradation process of organic matter limited by the degree to which the moisture combines organic fractions in sludge variation, sequentially influenced the moisture distribution in the sludge, consequently having an effect on sludge dewaterability. The objective of this study was to investigate sludge dewaterability in terms of moisture distribution in sludge and bond energy under anaerobic digestion, and the mechanism of AD enhanced sludge dewaterability. Moisture distribution and bond energy were used to evaluate sludge dewaterability undergoing AD. The impact of moisture distribution and organic fractions in sludge dewatering during an AD process was analyzed. Results indicated that AD altered mechanically bound moisture (MBM) transformed into free moisture (FM); thereby improving sludge dewaterability. The relationship between FM and MBM was studied using low field ^{1}H nuclear magnetic resonance, with statistically significant results (R=-0.912, p<0.01). Correlations between FM/MBM with extracellular protein (extra-PN) were also significant (R=-0.861, p<0.01/ R=0.869, p<0.01). Results presented will provide a better understanding towards having better control of the reduction of sludge.

Keywords: Moisture distribution, Anaerobic digestion, Dewaterability.

PRODUCTION OF XYLITOL BY IMMOBILIZED *CANDIDA TROPICALIS* EBL-X39 CELLS FROM RICE STRAW HYDROLYSATE

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Xylitol, a rare pentose sugar alcohol, has received wide global demand due to its unique properties like insulin independent metabolism, anticariogenicity, laxative nature, sweetening power similar to sucrose, low-calories and absence of Maillard reaction. It is approved for usage in food, health and pharmaceutical products in many countries. Currently, most of the xylitol is produced by catalytic reduction of D-xylose in the presence of catalyst Raney nickel under elevated pressure and temperature conditions at industrial scale but this chemical process is complex, laborious, cost and energy intensive. Alternatively, two biotechnological approaches for xylitol production i.e. fermentation process (using whole cells) and enzymatic approach (using xylose reductase) are more promising. The use of whole microbial cells has many advantages over other processes such as low production cost due to absence of purification steps and requirement of moderate conditions. D-xylose, one of the most abundant pentose sugars, is predominantly present in the hemicellulosic sugar of hardwoods and agricultural residues. The abundance and ease of isolation of D-xylose from agricultural residues make it a potential feedstock for the production of xylitol. Keeping in view the above facts and applications of xylitol in addition to abundance of agricultural waste as raw material, the present study has been conducted for the production of xylitol from rice straw hydrolysate using whole cells of an isolated strain of *Candida tropicalis* EBL-X39 immobilized in sodium alginate beads. Various immobilization parameters were optimized to obtain efficient and stable beads for maximum production of xylitol. The maximum utilization of xylose of 68.7% was obtained with xylitol yield of 0.54 g/g (of xylose) after 100 h of incubation under optimized immobilization conditions at pH 6.5 at 30°C. The maximum xylitol yield of 0.72 g/g and 0.54 g/g was achieved when tested under agitation (100 rpm) and stationary conditions, respectively, after 80h and 100 h of incubation. The reusability of the prepared beads was also tested for production of xylitol in batch system using rice straw hydrolysate (obtained after acid hydrolysis using concentrated sulphuric acid). The xylose utilization of 71.4% with yield of 0.63 g/g was obtained in the first cycle and xylose utilization was reduced to 14% with xylitol production of 0.1 g/g during 7th cycle. The ability of immobilized cells for xylitol production was also tested by successfully operating the continuous system in the form of packed bed reactor system for 7 days using rice straw hydrolysate. It is concluded from these studies that agricultural residues like rice straw can be used as raw material for bioproduction of xylitol using immobilized yeast cells.

Keywords: *Xylose, Xylitol, Rice straw, Candida, Bioconversion.*

EFFECTS OF BIOCHAR AND MAIZE STOVER MULCH ON THE PHYSICAL PROPERTIES OF A SANDY LOAM SOIL AND MAIZE YIELD

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ABSTRACT. Field studies on modification of physical properties of a sandy loam soil by adding charcoal (CH), maize stover biochar (CMS) and maize stover mulch (UNMS) was conducted. The objective was to improve the soils' productivity and increase maize yield by improving the physical environment. We hypothesized that: a) the UNMS, CH and CMS would improve maize yield by improving the soil physical conditions, b) the CMS would give a better nutrient and environmental management option than the feedstock (UNCMS). The treatments were; 5 t ha^{-1} (CMS); 14.6 t ha^{-1} (UNMS); 5 t ha^{-1} CH and control, (CTR); replicated three times on a randomized complete block design. Soil moisture and temperature data were collected from Data logger connected to sensors installed at 10 cm soil depth. A roving moisture data was also measured. The data were analyzed and results showed a more favourable soil environment under UNMS treatment. This resulted in increase of 19.14 % fresh stover (5.29 t ha^{-1}); 22.89 % dry cob (9.61 t ha^{-1}); 11.54 % de-husked cob (6.38 t ha^{-1}) and 10.83 % grain yield (5.22 t ha^{-1}), compared to the same yield parameters of CHMS and other treatments.

Keywords: *Charred maize stover; Feedstock; Soil moisture; Soil temperature.*

Introduction

The use of charred biomass (which is popularly referred to as biochar) as soil amendment has been suggested as a way to improve the soils' productivity by improving its physical, biological and chemical properties [1]. Most studies however, have focused on the modifications to the soils' chemical and biological properties, but limited quantitative studies exist on modification to the physical properties and subsequent translation to affect crop yield. Moreover, any benefit that could otherwise have been obtained from the uncharred biomass (feedstock) when used as soil amendment is masked by what the biochar could afford, and hardly compared in studies to make any informed choice between them. Field studies was conducted on a sandy loam soil to ascertain the mechanism underpinning soils' productivity improvement as the physical environment is modified by adding biochar and feedstock used as mulch, as well as increase maize yield. Prior to starting the study, we had hypothesized that biochar, including charcoal, would improve maize yield by improving the soil's physical environment (mainly moisture and temperature) that affect crop performance, as opposed to those achieved through chemical or biological means. We had also hypothesized that the charred maize stover would give a better nutrient and environmental management option than the feedstock used as stubble mulch.

Methodology

The experiment involved field application of locally manufactured charcoal, maize stover biochar and maize stover mulch on a sandy loam soil (Chromic Lixisol – [2]). Prior to planting, soil samples were taken at 15 cm depth for lab analysis for nutrient status. Fertilizers were applied at the rate of 60 kg ha^{-1} N, P, and K, to ensure that none of the major nutrients were limiting. The maize stover was charred at a low heating rate of 0.1 ºC/second to 420 ºC, using muffle furnace. Four treatments; 5 t ha^{-1} CHMS; 14.6 t ha^{-1} UNMS; 5 t ha^{-1} CH and a control CTR were imposed on a randomized complete block design with three replicates. Each plot size measured 7.2 m^2 (3 m x 2.4 m). A distance of 2 m was left between plots of adjacent blocks. Sowing was done ensuring 12 plants m^{-2}, with 60 cm between rows and 40 cm between plants. Data logger DL2e was used to automatically record and store soil moisture and soil temperature (half-hourly averages of 30 seconds). There were 36 sensors i.e. 12 ThetaProbes (T_p = 12), 12 thermistors (T_m = 12), and 12 thermocouples (T_c = 12) to measure soil moisture, temperature and surface temperatures respectively with each type allotted to one of the four treatments in triplicates. The T_c, T_m and T_p were installed at 2 cm, 10 cm and 10 cm depth respectively. The stored data were downloaded by serial interface (RS 232) via a laptop for analysis. The output of the T_p was converted to a volumetric soil moisture unit (θ_v %) using the formula:

$$\theta_v = \frac{\left(1.07 + 6.4V - 6.4V^2 + 4.7V^3\right) - 1.6}{8.4} \quad (1)$$

where θ_v = Volumetric moisture content (θv %) V = moisture reading from the data logger (millivolts).

A roving moisture data was taken at 10 cm depth, using hand-held ThetaProbe connected to moisture meter HH2. Ten volumetric moisture status (θ_v %) were read off, from the meter, per plot and recorded in two days. The high number was to cater for the small scale variability in transpiration and evaporation losses, soil composition, density etc. However, the assumption was that the soil on each plot was homogeneous irrespective of the treatment. Before using the parametric statistical tests ANOVA from GenStat™ software, data sets were tested for normality.

Results and Discussions

Figure 1a presents the observed moisture content recorded by the data logger for all treatments, from 12th to 21st October, 2010. At most times, the moisture content was highest for UNMS than all other treatments, this was followed by CH. This suggests that applying dry biomass to soil in its raw state as mulch/organic matter does not only improve the soil's moisture content, it does so better than when it's converted into biochar/charred (Figure 1b). The macro porous nature of UNMS, its binding property resulting in soil's aggregates stability as affected by the organic material, is the reason for this [3]. Consistent with biochar-induced increase in soil moisture reported by others [4, 5], we observe higher moisture content in the charcoal amended plots compared to the charred maize stover and the control plots. This was the case even after rainfalls, suggesting that more water was absorbed and stored in the charcoal (predominantly μm sized pores [8]) whenever it rained. This, according to [6, 7 and 8] was the result of the often macro porous nature of the charcoal, reflecting the cellular structure in the feedstock from which it was produced. The CHMS, however, could not mimic this characteristic of the CH and thus did not increase soil moisture to a point where it was statistically significant compared to the CTR. The observations were generally consolidated by the two-day roving soil moisture data presented in Table 1. Mirroring the diurnal moisture variations in the graph, UNMS showed higher moisture content than all treatments. CH also showed higher ($p = 0.028$ and 0.009 for the two days respectively) moisture content compared to the CTR plot. However, even though there was no significant difference between CH and CHMS from the Table, there was, when the diurnal variation was averaged for the period (CH = 0.3308 $cm^3 \, cm^{-3}$; CHMS = 0.2995 $cm^3 \, cm^{-3}$ [$p < 0.001$]), indicating higher moisture content for CH amended plot than CHMS. The average moisture content for CTR reported for the period was 0.2971 $cm^3 \, cm^{-3}$, which was not statistically different ($P = 0.081$) from CHMS and as also shown in Table 1.

Changes in soil temperatures in response to UNMS, CH and CHMS application to the experimental plots and control soil are shown in Figures 2. UNMS did show distinct differences in most part of the day, i.e. lowest maximum day temperature for most days than others. This is the result of reduction in the soil's thermal conductivity by the maize stover biomass, absorbing less heat during the day. However, the CTR soil cools more rapidly than UNMS, so that by middle of night the minimum soil temperature of CTR was 1.99°C lower ($P < 0.001$) than minimum soil temperatures under UNMS. The UNMS soil temperature, markedly rose more rapidly, reaching a peak that was on average 0.83°C cooler ($P < 0.001$) compared to the CTR. The differences in soil temperatures under all the treatments were most obvious around noon and about 4 am at down, as differences in the partitioning of energy by them became more apparent during those times. The order of day temperature variation is UNMS<CHMS<CH<CTR. Thermal conductivity is also affected by moisture content [9], as observed in Figure 1, which is highest in the UNMS treatment plot. The net radiation is also affected (not measured). Although the soil temperature patterns were similar, the control soil experienced greater temperature extremes than others.

As observed and explained in Figure 1, UNMS conserved soil moisture better than CHMS, and thus, so affected the soil's radiative and thermal properties. The mulch decreased the net radiation reaching the soil and thus prevented direct heating of the soil particles, thereby reducing the energy available for warming the soil during the daytime (Fig 2 a). Besides, incorporation of the UNMS into the soil reduces the top soil's bulk density, which reduced the thermal conductivity of the soil [10], because air has a much lower thermal conductivity than soil particles. In the night, however, the mulch conserved and retarded heat loss. These characteristics offset any advantage of the black colour CHMS may have (comparing CHMS and UNMS,

Figure 2 b), because black objects are widely known to absorb heat. Thus, we observed a warmer night for UNMS and a warmer day for CHMS. CH was warmer during the day than CHMS, even though both are black. The higher moisture content and subsequent reduction in radiative and thermal properties, were masked by its darker colour compared to the CHMS, warming the soil during the day time than the CHMS.

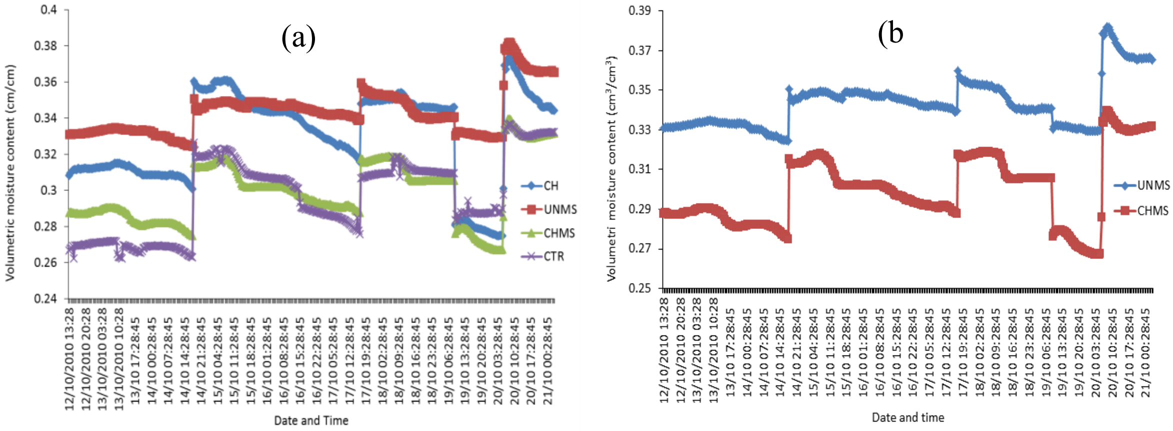

Fig. 1. (a): Diurnal variation of soil moisture of CHMS, CH, UNMS and CTR plots. Figure 1(b): Diurnal variation of soil moisture of CHMS and UNMS amended plots.

Table 1. Soil moisture content taken at two different days using handheld moisture meter.

Treatment	Moisture content (%)*	
	22/09/2010	13/10/2010
CTR	26.31 (a)	27.75 (a)
CHMS	27.6 (ab)	28.29 (ab)
CH	28.97 (b)	29.45 (b)
UNMS	30.90 (c)	29.5 (c)

* *These averages are based on 10 observations in triplicates.*

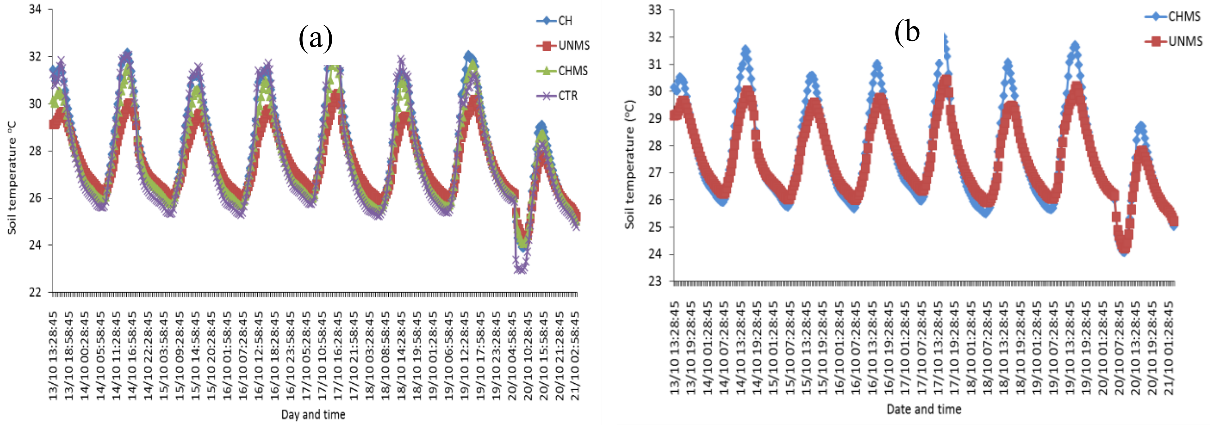

Fig. 2. (a) Diurnal variation of soil temperature of CHMS, CH, UNMS and CTR plots. Figure 2 (b): Diurnal variation of soil temperature of CHMS and UNMS amended plots.

Conclusion

In the end, the more favourable soil environment under maize stover mulch treatment resulted in increase in 19.14 % fresh stover (5.29 t ha^{-1}); 22.89 % dry cob (9.61 t ha^{-1}); 11.54 % de-husked cob (6.38 t ha^{-1}) and 10.83 % grain yield (5.22 t ha^{-1}), compared to the same yield parameters of CHMS in Table 2 and other treatments. Maize stover mulch thus becomes the preferred option to a poor farmer whose ultimate goal is to maximize yield and water use efficiency.

Table 4. Mean yield parameters of maize and Duncan's multiple comparisons of the means.

Treatment	Yield (tha^{-1})			
	Fresh stover	Cob	De-husked cob	Grain yield
CTR	4.28 (a)	7.16 (a)	5.56 (a)	4.43 (a)
CHMS	4.44 (a)	7.82 (b)	5.72 (a)	4.71 (b)
CH	4.79 (ab)	9.05 (c)	5.94 (b)	4.93 (c)
UNMS	5.29(b)	9.61 (c)	6.38 (c)	5.22 (d)

Acknowledgment

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STUDY ON THE MIGRATION BEHAVIOR OF HEAVY METALS AND CHARACTERISTICS OF PHOSPHORUS-ENRICHED BIOCHAR PREPARED BY MICROWAVE PYROLYSIS OF MUNICIPAL SEWAGE SLUDGE

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Municipal sewage sludge is a kind of typical biomass waste, its output is increasing year by year and the phosphorus content is abundant. It could not only realize the efficient collaborative treatment of municipal sewage sludge, but also realize the recovery and utilization of phosphorus to convert municipal sewage sludge into phosphorus-enriched biochar, which is in line with the sustainable development concept of waste management. However, the heavy metals restrict the utilization of phosphorus-enriched biochar, therefore, it is significant and necessary to clarify the migration behaviour of heavy metals.

In this paper, Microwave Pyrolysis was adopted to produce the phosphorus-enriched biochar. On the basis of exploring the effects of microwave power, temperature and additives on the characteristics of phosphorus-enriched biochar, the influence of individual and interaction effects of different additives on the migration and transformation of phosphorus were deeply studied. In addition, the leaching characteristics, occurrence forms and fixation mechanism of heavy metals were investigated. Finally, the safety of phosphorus-enriched biochar was evaluated via the methods of OPTI and RAC risk assessment.

The results showed that the pH, BET and total phosphorus (TP) contents of phosphorus-enriched biochar increased with the increase of microwave power and temperature, and the maximum TP contents was 42.7 g / kg. ID / IG increased first and then decreased, it reached the maximum when the power was 1600 W and the temperature was 500 °C, respectively. It was found that temperature was an important factor affecting the conversion of amorphous carbon to graphene like structure. In addition, the addition of CaO could effectively promote the transformation of non-apatite inorganic phosphorus (NAIP) to apatite inorganic phosphorous (AP) (mainly $Ca_9Al(PO_4)_7$, $Ca_3(PO_4)_2$, $Ca_3Mg_3(PO_4)_4$, $Ca_{10}(PO_4)_6(OH)_2$). The addition of $MgCl_2$ promoted the conversion of AP to NAIP (mainly $AlPO_4$). When CaO and $MgCl_2$ were added at the same time, they showed antagonistic effect, and CaO played a leading role. Microwave pyrolysis had a good passivation effect on heavy metals in phosphorus-enriched biochar. Under some conditions, the leaching amount of heavy metals was zero. It was found that the increase of aromatic structures, the enhancement of polarity and alkalinity, the formation of crystal structures and complexation reactions, the strengthening of pore structures, and the densification process of phosphorus-enriched biochar surface were the main mechanisms for the immobilization of heavy metals in municipal sewage sludge during microwave pyrolysis.

Keywords: *Municipal sewage sludge, Microwave pyrolysis, Phosphorus-enriched biochar, Heavy metals.*

ASSESSING BIOCHARS AS BIO-PRODUCTS FROM SLOW PYROLYSIS OF DIFFERENT ORGANIC RESOURCES AND EVALUATE PROPERTIES AS SOIL CONDITIONERS

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ABSTRACT. Biochar is an alternative and beneficial strategy in managing plant residues and animal manures. A series of comprehensive studies were conducted to produce and characterize biochars as bio-products using slow pyrolysis biochar-producing stove and evaluate the properties of biochars as soil conditioners. Different organic resources (plant and animal wastes) were collected and prepared for thermal treatments at temperatures ranging from 300 to 650°C. This paper discusses the results of using material imaging techniques (FE-TEM and FE-SEM) and BET physisorption system to elucidate the chemistry of biochars and the adsorbed nutrients onto them. Comparison among the variety of origins, apparent differences were observed between biochars on the domination of nutrients retained. The porosity of all biochars was highly variable. The physical and chemical characteristics of biochars influence their effectiveness in controlling the retention/adsorption of nutrients. Similarly, the HAABF and SEM images of biochars revealed that the properties of raw feedstocks and pyrolysis process influenced substantial changes in their surface morphology and chemistry. Results such as these concurred with the pyrolytic conditions of biochar production which properties and mineral agglomerates substantiate that biochars can be used as soil conditioner in the improvement of soil quality.

Keywords: *Organic wastes, Slow pyrolysis, Soil amendment, Soil degradation, Soil fertility.*

Introduction

Biochar application is a rapidly emerging innovative technology to improve the health of intensively cultivated soils. It is also an alternative and beneficial strategy in managing animal manures and plant residues. Biochar is a carbon-rich form of charcoal produced through the thermal pyrolysis process of biomass wastes of plant and animal origin with little or absence of oxygen. It is a by-product of the transformation of biomass into bioenergy that can be applied onto the agricultural land as soil conditioner because of their potential for improving soil quality. Biochar properties are highly influenced by pyrolysis conditions and original characteristics of organic materials if used in improving soil conditions. This study aims to characterize the biochars as bio-product of slow pyrolysis derived from different plant and animal biomasses and evaluate their properties for their use as soil conditioners.

Material and Methods

Collection of Raw Organic Wastes and Preparation of biochar using the Biochar-producing Stove

The plant biomass wastes were collected from the residues of previous experiments at UP Los Baños. The animal wastes (carabao, cattle and swine) were collected from the nearby farms of the campus. The water hyacinth (*Eichhornia crassipes*) plants were obtained from a freshwater lake at Los Baños, Laguna. All raw materials were first cleaned from foreign objects and air-dried. Subsamples of raw materials were taken and dried at 105°C for 24 hours for the characterization of raw samples. Accordingly, remaining raw materials were cut into small pieces (4-6 cm) and oven-dried at 60°C before pyrolysis. Chars of P1 (rice straw), P2 (rice hull), P3 (corn husk), P4 (corn cobs), P5 (corn leaves), P6 (coconut husk), P7 (coconut coir dust), P8 (water hyacinth), P9 (mahogany flower receptacle), M1 (cattle manure), and M2 (swine manure) were collected and assessed as by-products of slow pyrolysis in a prototype (biochar-producing stove) developed from a NRCP-DOST funded-project); with heating temperature ranging from 300°C to 650°C at different residence time.

Characterization of Biochars

The elemental analyses of biochars were carried out using the conventional procedures. The physical properties were analyzed using Quanta Chrome Nova e2200 Brunauer-Emmett-Teller Automated Nitrogen Multilayer Physisorption system at Nanotechnology Laboratory, U.P. Los Baños. The surface morphological changes of biochar samples were taken at different magnifications by using the JEOL JEM-2100F Field Emission-Transmission Electron Microscope (FE-TEM) at Materials Science Division, Industrial Technology Development Institute-Department of Science and Technology, Philippines. Scanning Tunneling Electron Microscope analysis was performed in obtaining High Angle Annular Bright Field (HAABF) images. Energy Dispersive X-ray analysis was made on the areas of interest of each biochar samples. FE-SEM Imaging with EDX mapping was made possible using Dual Beam Helios Nanolab 600i for surface morphology analysis.

Results and Discussions

Characterization of Biochars

Biochar is a by-product of the transformation of biomass into bioenergy in thermochemical processes [1] and is one of the methods more accepted by both legislation and industry to reduce waste and obtain valuable products [2]. The production of biochars from slow pyrolysis of different organic resources showed varying level of nutrient elements (Figure 1). Biochar is mainly produced from plant residues or animal manures, which are pyrolyzed in a low- or zero-oxygen environment as reported previously [3]. Recent reviews on biochar properties are reported to have high pH. In the present study, the pH of the P2 biochar is 7.3 while the others ranged from pH 8.21 to 10.4. The amount of nitrogen (N) in the biochars derived from different biomass wastes ranges from 0.50 to 1.5%. The P9, only biochar that originates from woody organic resource, had the least amount of major nutrients N, phosphorus (P), potassium (K) but displayed the highest level of organic carbon (24% OC) and recorded a strongly alkaline pH (10.4). It also gave low levels of micronutrients: Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn). Contrariwise, P8 had the highest amount of N, K, Ca and Mg when compared with other biochars derived from light materials. Biochars derived from animal manures (M1 and M2) demonstrated highest concentration of P and high amounts of Fe, Cu, Zn and Mn. The pyrolysis process converts any organic matter into a highly stable form of carbon which is perfectly used as a soil additive to improve nutrient retention and carbon storage in soil [4].

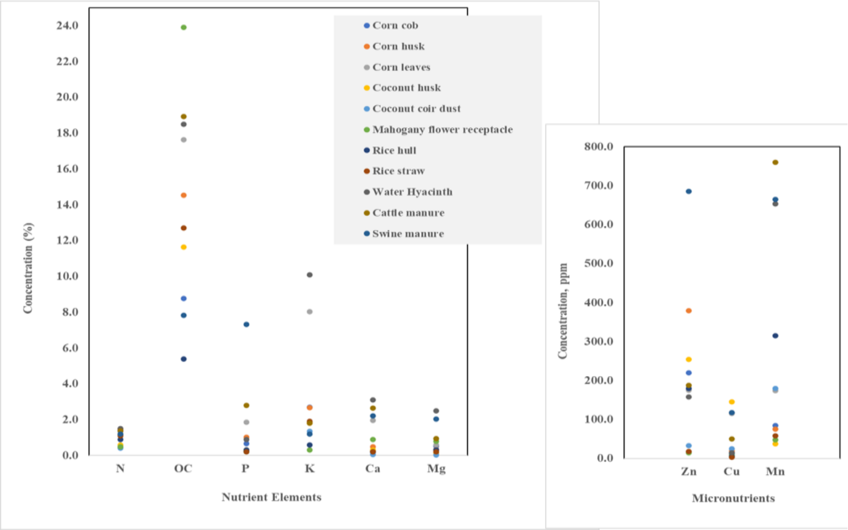

Fig. 1. Chemical properties of biochars derived from different organic biomass wastes.

Surface morphology TEM-EDX and SEM-EDX

The TEM photographs of biochars at varying magnifications is shown in Figure 2. The High Angle Annular Bright Field (HAABF) images, spectrum and elemental X-ray maps of all biochars revealed areas high in C and other minerals rich in K, Cl, Ca, Mg, Si, and Al. As previously known, C concentration is an important criterion for characterizing biochar and it also reflects the biochar's stability in the soil. The utility of biochar for a specific application depends on its inherent properties. The organo-mineral particles previously defined [5] as organic material with a high mineral contribution. Organo-minerals usually have a positive effect on soil nutrient transformation and adsorption.

The images through SEM morphological analysis that display the changing trends in biochar structures are presented in Figure 3. These images of biochars indicate that the properties of raw feedstocks and pyrolysis process strongly influenced substantial changes in their surface morphology. Studies of [6] and [7] mentioned that SEM can produce different pictures of biochars with different feedstocks.

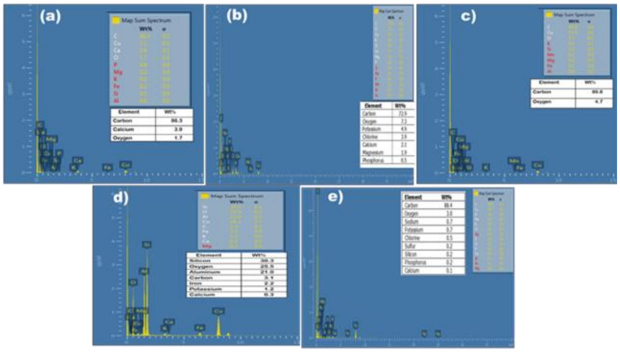

Fig. 2. HAABF images and spectrum on the areas of interest of each biochar samples (a) mahogany flower receptacle (b) water hyacinth (c) rice straw (d) rice hull (e) corn leaves.

Biochars originating from plant wastes showed completely different appearance and disordered structures. It may be assumed that these biochar samples retained the original macrocellular morphology of the original materials. These images are likely due to the lignin component undergoing only partial degradation, causing a rough texture, so that the original shape of the particles was largely preserved [8]. The SEM images of biochars derived from animal manures exhibited many pores and cracks on the surface and profile. The micropores, which have large surface area, are a preferred habitat for microbial communities in utilizing the soil. The macropores present on biochar may provide suitable dimensions for cluster of micro-organisms to grow and reproduce and the micro and meso porosity of biochars may also allow it to retain more moisture in soil and increase the water holding capacity [9].

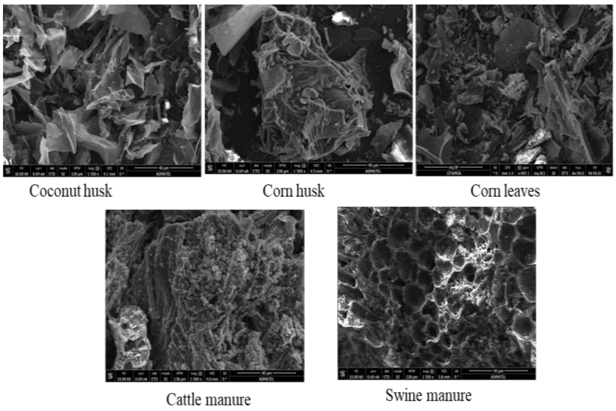
Coconut husk
Corn husk
Corn leaves
Cattle manure
Swine manure

Fig. 3. FE-SEM images taken at 1,500X magnification (a) coconut husk (b) corn husk (c) corn leaves (d) cattle manure (e) swine manure.

Surface area analysis BET

As is known, porosity creates during the pyrolysis process is an important physical feature of biochar in soil processes and its behavior.

Table 1. Summary of Surface Area and Porosity of the Biochar Samples.

Biochar(s)	Average Surface Area (m^2/g)	Average Pore Size/Radius (nm)	Average Pore Volume (cm^3/g)
Rice Straw (P1)	45.511 ± 5.23	1.923 ± 0.39	3.5E-02
Rice Hull (P2)	78.208 ± 4.46	1.926 ± 0.21	1.1E-02
Water Hyacinth (P3)	9.845 ± 2.13	1.917 ± 0.12	1.7E-02
Corn Husk (P4)	0.622 ± 5.51	1.927 ± 0.40	7.0E-03
Corn Cob (P5)	10.416 ± 0.57	2.150 ± 0.61	1.2E-02
Corn Leaves (P6)	3.445 ± 0.76	1.918 ± 0.39	ND
Coconut Husk (P7)	107.186 ± 11.08	31.542 ± 4.55	3.0E-03
Coconut Coir Dust (P8)	15.968 ± 2.31	1.918 ± 0.73	1.2E-02
Mahogany Flower Receptacle	0.473 ± 0.41	1.707 ± 0.88	4.0E-03
Cattle manure (M1)	68.035 ± 5.12	1.913 ± 0.61	4.5E-02
Swine manure (M2)	14.890 ± 3.41	1.927 ± 0.17	2.9E-02

Mean ± Standard deviation (n=2)

Table 1 shows BET surface area and total pore volume for all biochar samples. Results showed that the properties of raw organic biomass wastes influenced the surface area, pore volume and radius of biochars. Among them, the BET surface area and pore size were extremely high in coconut husk 107.19 m^2/g and 31.54 nm, respectively. These properties were found drastically low in mahogany flower receptacle with

0.47 m^2/g and 1.91nm, respectively. The higher surface area is preferable because it helps to improve the soil structure and increase total water retention in soil [10]. The average pore volume reported the lowest value of $3.0x10^{-3}$ cm^3/g and closely followed by $4.0x10^{-3}$ cm^3/g for coconut husk and MFR, respectively. The highest average pore volume was found in cattle manure, rice straw and swine manure with $4.5x10^{-2}$, $3.5x10^{-2}$ and $2.9x10^{-2}$, respectively. Average pore diameter denoted the pores are mostly mesopores and macropores. Mesopores and macropores are useful for liquid-solid adsorption [10]. In soil environments, biochars with small particle size and high internal porosity are expected to contribute most to the enhancement of soil quality. Because of their relatively low surface areas and the coarse particle size distributions, these biochars may not be ideal for soil quality enhancement. On the other hand, the large particle size should enhance the stability of the biochar C in soil environments [11].

Conclusions

The data presented in this work showed that the type of feedstock and pyrolysis conditions strongly affect the physical and chemical properties of the biochars produced from slow pyrolysis of plant and animal wastes. The surface morphology properties of the biochars were also remarkably influenced by the same factors. In conclusion, biochar is a highly potential material that can be used as soil conditioner in many types of soil to improve crop production. The abundant pores and minerals in the biochars are helpful for ameliorating soil through the supplies of micro spaces and mineral nutrition.

Acknowledgement

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MULTI-STAGE COMPUTER AIDED-MOLECULAR DESIGN (CAMD) APPROACH IN BIO-OIL SOLVENT DESIGN TO UPGRADE BIO-OIL QUALITY

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As one of the world's largest palm oil producer, a vast amount of oil palm biomass wastes were generated daily in Malaysia, which leads to disposal problem. With fast pyrolysis technology, these biomass wastes can be liquefied into bio-oil through a simple thermochemical process. However, direct application of fast pyrolysis bio-oil as a biofuel is limited due to its undesirable properties such as low heating value, high viscosity, high corrosiveness and storage instability. Solvent addition is a simple way of upgrading bio-oil to improve some properties of bio-oil, which helps for further processing and storage. Current work aims to design optimal solvents that improve the properties of bio-oil while displaying desirable targets properties and minimal environmental impact. Thus, a novel multi-stage computer-aided molecular design (CAMD) approach is proposed. The procedure started with definition of the molecular design problem where the product needs were determined accordingly based on the requirements from regulations and specifications. The identified product requirements are translated into measurable quantitative target properties such as density, viscosity, higher heating value and etc. In this work, ASTM D 975 and EN:590 standards of diesel fuel are taken into account while defining the property targets. Following on, suitable property prediction models are selected to estimate the targeted physicochemical and environmental properties of solvents. However, the property prediction models were expressed in the form of different indexes, especially for environmental properties. Thus, the molecular signature descriptor is introduced and served as a common platform to solve the CAMD problem simultaneously. Due to the combinatorial nature of higher-order signatures, the complexity of CAMD problem increases as the height of signature increases. For this reason, a multi-stage framework is developed to reduce the size of CAMD problem by only generating higher-order signatures that are consistent with each other. After determining all possible solvents, the thermodynamic property, Gibbs free energy of mixing was conducted to evaluate the stability and miscibility of the solvent-oil blend.

Keywords: *Fast pyrolysis bio oil, CAMD, Solvent.*

REFUSE DERIVED FUEL AS A SOURCE OF ENERGY PRODUCTION: A WAY TOWARDS SUSTAINABILITY

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One of the world's most pressing concerns has been the energy crisis. As the country strives to become an industrialized nation, the Municipal Solid Waste amount is expected to go up significantly in the near future. It is desirable that the waste can be used as an advantage to humankind. RDF (Refuse Derived Fuel) is a renewable energy source produced from combustible components of Municipal Solid Waste. RDF possess high calorific value. RDF-derived energy can benefit both the economy and the environment. The RDF can be made using a variety of organic waste present in the MSW. In the present study refuse derived fuel was subjected to laboratory analysis to determine its properties, including its calorific value. The analysis of the samples was carried out and the moisture, volatile matter and gross calorific value were found to be 24.60 ± 5.19%, 50.29 ± 8.24%, 4389 ± 496.39 Kcal/kg. The performed study led to the conclusion that the studied RDF can be used as a source for energy generation.

Keywords: *Refuse derived fuel, Energy and Environment, Proximate Analysis, Municipal Solid Waste.*

ANAEROBIC DIGESTION, VOLATILE FATTY ACIDS AND MEMBRANE BIOREACTORS

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Anaerobic digestion (AD) is a well-known technology that is used internationally for biogas or biomethane production from e.g. manure, sludge or food wastes. Biogas is normally used for heating, cooking, electricity and car fuels and the residuals have common applications as fertilizers. Although an established industry worldwide, the low market value of biogas and fertilizer, and the challenges with storage and transportation, has made biogas production economically unappealing. The biology of AD shows symbiosis of four categories of bacteria and archaea including hydrolytic organisms, acidogens, acetogens and methanogens. The result of the activity of the first three groups of these microorganisms is the conversion of wastes and feedstocks to volatile fatty acids (VFAs) mainly acetic, propionic, butyric, valeric and caproic acids and hydrogen, while the methanogens convert these intermediate products to methane and carbon dioxide. VFAs have numerous applications as mixed or individual acids, supporting from food and pharmaceuticals industries to wastewater treatment.

Boosting VFAs production by AD and simultaneously inhibiting methanogens result in accumulation of VFAs in the AD culture. There are several factors contributing to this accumulation, where low pH (e.g. lower than 6.0) or high pH (e.g. higher than 10) is among the most important ones. In addition, other factors including inoculum pretreatment (thermal or chemical), feedstock type and loading rate and presence of oxygen that affect VFAs accumulation. We have examined VFAs production from various wastes and residuals such as food wastes, citrus wastes, wastewater treatment sludge, cow manure, chicken manure etc. All these materials can be used to produce VFAs and not methane.

Another challenge to address is removing VFAs from AD culture without disturbing the stability of the biological system. We were successful in in situ recovery of VFAs solution using membrane bioreactors (MBRs). The MBRs were developed using flat sheet membranes in form of immersed Integrated Permeate Channels (IPC) and also external tubular membranes. This presentation covers the results of many years of development of our group in this field.

Keywords: *Anaerobic digestions, Wastes, Biogas, Volatile fatty acids, Membrane bioreactors.*

IMPROVING THE ASH REMOVAL EFFICIENCY OF AGRICULTURAL RESIDUES BY TRADITIONAL WATER LEACHING AND MICROWAVE-ASSIST LEACHING

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Feedstock quality and pelleting parameters will affect the quality (mechanical durability) of pellets if the residues are densified. Agricultural residues are ideal alternative materials to replace the reducing fossil fuel. However, most agricultural residues have high ash content which will reduce the quality of densified pellets and cause corrosion, slagging and fouling in the furnace.

Water leaching is defined as the removal of water soluble and ion-exchangeable inorganic constituents from solid substrate using water. Water leaching is reported as a low-cost pre-treatment to reduce inorganic constituents in the biomass content. Water leaching is effective for removing water-soluble elements, but the traditional water leaching by low temperature water bath is not effective enough because it always takes a long time to obtain an ideal result. In this study, traditional water leaching by water bath and microwave-assist water leaching were applied to detect the effect of ash removal efficiency of agricultural residues.

Size reduction affects pellet durability as finer particles generally produce pellets that are more durable. Size fractionation will be the first step of the whole pretreatment process. Samples will be grinded by using a hammer mill. Several screen sizes will be tested to determine the size distribution. And the ash content of each size will be test to detect if there are any significant differences in the resulting particle size distribution and hence the ash content of each size fraction. The size of which does not meet the ash content standard will be carried out by the water leaching as the next step of ash reduction treatment.

The wheat straw and corn stalk were used in this study. For each washing, 5g samples were used with the biomass/water ratio of 1:20 and 1:30 respectively. For traditional water bath leaching test, the duration will be set 6 hours and 12 hours and the temperature will be 25 and 45. For microwave-assist water leaching test, three power levels will be applied. The duration will be set as 2min and 3min. All samples will be put inside digestion containers which will make sure the experimental condition is closed.

All biomass samples will be analyzed for the physical and chemical properties, including ash content and element composition, moisture content and heating value. The inorganic composition will be analyzed for a range of elements before and after leaching. The influence of traditional water leaching and microwave-assist water leaching will be compared by the results.

Keywords: *Ash removal, Agricultural residues, Traditional water leaching, Microwave-Assist Leaching.*

INTEGRATED BIOHYTHANE PRODUCTION FROM FOOD WASTE – INFLUENCE OF INCREASING ORGANIC LOADS

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ABSTRACT. The present study was focused on the effect of organic loading rate (OLR) was evaluated for biohythane production though anaerobic digestion (AD). In AD food waste as substrate and municipal solid waste as source of inoculums employed of the biohythane production. The two reactors were operated at different hydraulic retention time (HRT) of 144h (6 days) for H2 production and 432h (18 days). Methane production optimized OLRs 30, 40, and 50g COD/L. To clearly understand that the integrated of PT-system (H2) with UT-system (Likewise: D_1+D_6; D_2+D_9; D_3+D_{12}; D_4+D_{15} and D_5+D_{18}) in anaerobic digestion at neutral redox condition. Experimental data showed the results at OLR 50g COD/L, the methanogenic activity was conventional and biohythane production of 17.91L from integration of PT-40 with UT-50 was seen in the system. Moreover, the higher specific composition of biohythane (H_2+CH_4) in the system showed 0.27 (H_2/(H_2+CH_4)), compared to other combination of reactors. The biohythane composition (H_2/(H_2+CH_4)) varied from 0.50 to 0.20 during stabilization. The result confirmed that methanogenic dominance has a positive effect in the UT-7 reactor performance when food waste is supplemented at an OLR of 30, 40, and 50g COD/L. Finally, the major developments of improving biohythane yield from combined reactors at different OLRs.

Keywords: *Acidogenic Fermentation; Biohythane; Bio-H_2; Bio-CH_4; Volatile Fatty Acids (VFA)*

Introduction

Food waste is the most common component of urban solid waste, and its production is rapidly growing. In India, 4.19 million tonnes of food waste were landfilled in 2018, accounting for 31% of overall MSW. Due to the environmental concerns of greenhouse gas (GHG) emissions and the chance of groundwater leakage, landfilling of food waste may not a safe process. Treatment of food waste using anaerobic digestion (AD) has become more common in recent years as a way to generate bioenergy in the form of methane, which can then be used to generate heat and electricity (Van Fan et al., 2018). However, owing to the high biodegradability of food waste, which induces rapid acidification, food waste AD is a dynamic biological mechanism that can result in low operational efficiencies or even system failure. To potentially address the problems faced in food waste mono-digestion, co-digesting food waste with other feedstocks such as sewage sludge, compost, and so on has been used (Mata-Alvarez et al., 2011). Food waste and sludge co-digestion systems recover more methane than mono-digestion systems, also demonstrate a high concentration of intermediate metabolites such as volatile fatty acids (VFA) such as acetate, butyrate, and propionate, as well as long-chain acids in co-digestion processes (Chakraborty et al., 2018; Kaur et al., 2019). In co-digestion, such an aggregation prevents methane production. Therefore, the aims of this study, the effects of the OLR and HRT in the conventional anaerobic digestion system using the municipal solid waste (MSW) and the food waste (FW). These two different reactors with extended HRT at various OLRs are among the most common waste streams that are currently used for biohythane production, which principally relies on the systems.

Materials and Methods

Characterization of the food waste and Inoculum

The food waste used in this study was collected from CSIR-IICT institute canteen, used as substrate for production of biohythane (10-20% H2, 80-90% methane). The collected food waste sample contained cooked meals, boiled vegetables, bones, leafy vegetables, egg shells, plastics, tissue paper and rotten fruits.

From which the fermentable fraction was separated manually and used for the experiment. The segregated food waste was then blended into slurry using electronic blender and then filtered through a stainless-steel sieve (300 µm) to avoid problems of pipe clogging in feed recirculation operation. The homogenized food waste was sealed in plastic bags, stored at 4ºC to ensure the compositional quality and the same food waste was used in all the experimental conditions.

Experimental Procedure

The hydrogen and methane fermentation reactors HRT of 6days and 18days operated at mesophilic condition with a loading rate of 30, 40, and 50g COD/L. Samples were collected every 24h for analysis from the digested system. Since the experiments were designed to operate at pH7, prior to the start of each cycle, the pH of two reactors were adjusted to neutral by using 0.1 N NaOH (alkali) solutions. The N_2 gas was sparged into the reactors for 5 minutes after every feeding and sampling operation to maintain the anaerobic condition. Pre-treated culture denoted as PT-30, PT-40, PT-50 whereas untreated culture was labelled as UT-30, UT-40, UT-50. PT sludge and UT sludge were integrated for bio-hythane production based on their ORL 30,40 and 50. The integrated reactor setups (System I to system IX) were represented as follows: System-I (PT-30+UT-30), System-II (PT-30+UT-40), system-III (PT-30+UT-50), system-IV (PT-40+UT-30), system-V (PT-40+UT-40), system-VI (PT-40+UT-50), system-VII (PT-50+UT-30), system-VIII (PT-50+UT-40), and system-IX (PT-50+UT-50). A total of nine experiments were designed to understand the ORLs influence on biohythane production.

Analytical Procedure

The hydrogen produced in the reactors was estimated with the help of a microprocessor based pre-calibrated H_2 sensor (ATMI GmBH Inc.). Chemical oxygen demand (COD-closed refluxing titrimetric method), Volatile Fatty acids (VFAs) estimation, and pH were performed as per the standard methods (APHA, 1998). The composition of the biogas was monitored with Gas Chromatography (GC; Agilent-7890B) using thermal conductivity detector (TCD) equipped with HayeSep-Q (80/100, 2m, OD:1/8, ID: 2mm SS column) employing argon as carrier gas. The injector and detector were maintained at 80º C each and the oven was operated isothermally at 100ºC. The VFA composition was analysed using high performance liquid chromatography (HPLC; Shimadzu LC10A) employing UV-Visible detector (210 nm) and C18 reverse phase column (250 × 4.6 mm diameter; 5 µm particle size, wave length: 210 nm). Mobile phase was prepared using 40% acetonitrile (Pump-A) and 0.1M H_2SO_4 (Pump-B) and flow rate was maintained of 0.5 mL/min.

Results and Discussion

Biohydrogen production

The AD integrated systems were evaluated for biohythane production from food waste with OLRs of 30, 40, and 50g COD/L. Figure-1 depicts the biohydrogen production profile from the pretreated culture reactors. The results showed that, the production of hydrogen started from 4h and continued up to 120h and the stability of biohydrogen output rate (L/h) was seems to be proportional with respect to time (144h). However, it was observed that the H_2 production was considerably steady state at the end of the cycle (120h). After the end of the cycle, due to dominance of methanogenic activity over acidogenesis has led to the formation of CH_4. The cumulative maximum H_2 production (CHP) and H_2 production rate (HPR) were monitored in the anaerobic digestion with increasing OLR and HRT. Maximum production of H_2 was observed at 120h in PT-40 (6L) followed by PT-30 (4.5L) and PT-50 (4.40L). During the operation at 30g COD/L, the observed maximum H_2 production of 4.49L (Day-5; 120h) followed by 4.44L (Day-4; 96h), 3.66L (Day-3; 72h), 3.22L (Day-2; 48h) and 2.51L (Day-1; 24h) and hydrogen production rate was observed 37.4mL/h followed by 37mL/h, 30.5mL/h, 26.83mL/h and 20.9mL/h.

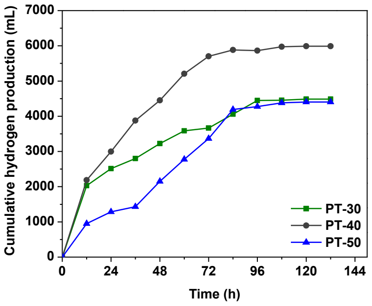

Figure 1: Biohydrogen (mL) production observed at different organic load in pretreated and untreated culture and operated at uncontrolled pH 7

Biomethane Production

The cumulative methane production (CMP) and methane production rate (MPR) at various OLRs in the systems were shown in the Fig-2. The maximum methane production was noticed in the untreated culture (UT-7) of 1.2L at 30g COD/L, 2.0L at 40g COD/L and 1.3L at 50g COD/L, respectively. The cumulative methane production (CMP) and mthane production rate (MPR) were observed in the anaerobic digestion with increasing OLR of food waste. Maximum production of CH_4 was observed at 432h in UT-50 (11.92L) followed by UT-40 (7.92L) and UT-30 (6.01L). During the operation at 30g COD/L, the observed maximum CH_4 production of 6.01L (Day-15; 360h) followed by 5.42L (Day-12; 288h), 3.03L (Day-9; 216h), 1.23L (Day-6; 144h) and 0.61L (Day-3; 72h) and methane production rate was observed 16.7mL/h, respectively. As a result, the microbial activity had a long lag time that may be due to bacteria exposure to food waste or a low organic concentration gradient in the substrate to the micro biota. Further, the OLR was increased to 40g COD/L, where the maximum CH_4 production of 7.92L (Day-15; 360h) followed by 4.76L (Day-12; 288h), 2.16L (Day-9; 216h), 0.89L (Day-6; 144h) and 0.39L (Day-3; 72h) and methane production rate was observed 22.0mL/h, respectively.

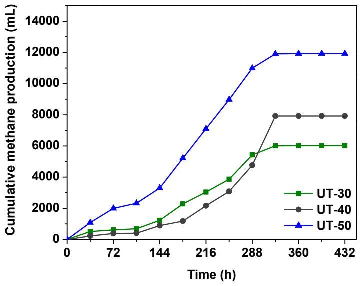

Figure-2: Cumulative Methane Production (CMP) was observed at pH-7 during at various OLRs

Biohythane Production

Organic Loading Rate (OLR) represents the amount of food waste fed into the reactor per unit of working volume for biohythane production. Three different OLRs 30, 40 and 50g COD/L were used in the anaerobic digestion at mesophilic condition. The two reactors were integrated of PT-system (Bio-H_2) for 144h with UT-system (Bio-CH_4) for 432h in anaerobic digestion. The two different outcomes biohythane (HCNG) production and composition of biohythane (HCNG) were achieved in which exclusively produce bio-H_2 and bio-CH_4 can be combined for HCNG formation. The biohythane production and composition of biohythane were evaluated in integrated of two reactors of results as bio-H_2 (PT) and CH_4 (UT) in anaerobic digestion from food waste shown in Figure-3. By the end of cycle (144h for H_2; 432h for CH_4), more biohythane production was observed in the system, when the integration PT-system (bio-H_2) with UT-system (bio-CH_4) because of formation of good production and composition from low bio-H_2 production from PT-7 with relatively more CH_4 formation from UT-7. The maximum cumulative biohythane production of 17.91L at Day-5 (H2-5.99L; PT-40) and Day-18 (CH4-11.92L; UT-50) with the percentage of H_2 content and CH_4 content were 33% and 67%, and the higher production rate was 41.46mL/h, respectively. Based on the results, PT-system integrated with UT-system for the production of biohythane and for good composition of biohythane in anaerobic digestion system.

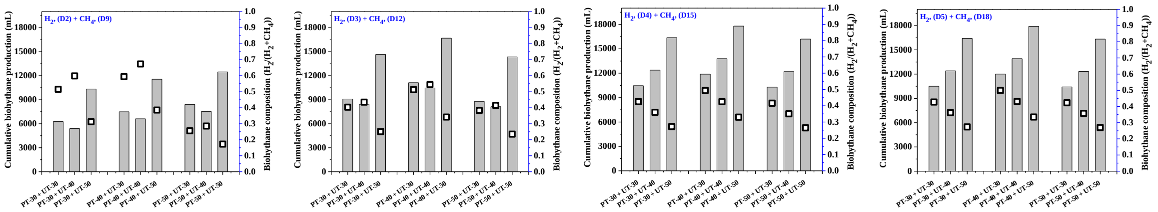

Figure-3: Biohythane production and composition observed at different OLRs in AD

Conclusion

The effect of the three various OLRs and two different HRT of two separate reactors in the anaerobic digestion using the food waste and municipal solid waste was evaluated. The two separate reactors process could at best handle an OLR of 40g COD/L (PT; 144h) and OLR of 50g COD/L (UT; 432h) for biohythane production. A more stable operation could be achieved with integration of PT-40 (H2; Day-4) and UT-50 (CH4; Day-18) of 17.91L and composition of 0.33 was observed. However, the suitable biohythane production and composition was also noticed in the system integration of PT-30 (H2) with UT-50 (CH4) showed 16.41L and 0.27 during the anaerobic digestion operation. The present study illustrated that the feasibility of integration of two reactors at different OLR and HRT using food waste and municipal solid waste in anaerobic fermentation.

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COMPARATIVE STUDY ON THE BIOMETHANE POTENTIAL OF TERRESTRIAL AND AQUATIC WEEDS

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Extensive usage of cash crops for biogas production followed the food crop crisis in many parts of the world and led to seek substitutes for the feedstocks. A vast portion of weed biomasses are rich in cellulose and hemicellulose and can be a suitable solution. These lignocellulosic weed biomasses are known to cause ecological imbalances, and they need to be managed. These weeds can broadly be classified as terrestrial and aquatic. Their availability in abundance through rapid invasive growth and the need to be controlled makes it a suitable substitute for feedstock. However, due to high lignin content, different pretreatment strategies are employed for better biogas production and efficient methane yield. This study aims to conduct a comparative analysis between the biomethane potential of terrestrial and aquatic weed biomasses. The two most common and aggressive invasive weeds Ageratum conyzoids and Hydrilla verticillata were selected as substrates, representing terrestrial and aquatic weeds, respectively. Four of these weeds are notorious for their invasive nature and the problems that they cause on the native ecosystem. Biomethane Potential Test (BMP) was conducted as a tool to check the feasibility and optimize the Food to Microorganism (F/M) ratio with cow dung as the inoculum. BMP assay revealed that F/M ratio 2 acquired maximum biogas production for terrestrial weed A. conyzoids, whereas for H. verticillata, it was F/M ratio 2.5. The highest methane production was achieved on the 25th day around 205 ± 10 mL CH4/g VS (volatile solids) for A. conyzoids, and cumulative biogas production reached up to 4994 ± 25 mL. For H. verticillata, maximum methane production was observed on 33rd day (180±20 mL CH4/ g VS), and cumulative biogas production reached up to 4857 ± 25 mL.

Keywords: *Anaerobic Digestion, Biogas, Lignocellulosic biomass, Food to microorganism (F/M) ratio, Biochemical methane potential.*

VAPORIZING SECONDARY SLUDGE OF DAIRY INDUSTRY FOR BIOHYTHANE PRODUCTION

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The over exploitation of fossil fuel for energy production has compelled researchers to search for an alternative of conventional fossil fuels. Hydrogen and methane are two widely studied alternate energy sources. The biohydrogen studies has been extensively conducted on simple sugars which imposes cost on production. The search for cheap and unconventional sources can certainly give a force for commercialization of biohydrogen production. The conversion of waste into energy can solve two burning issues: (a) environmental problem and (b) energy crisis. The dairy industry secondary solid waste which is increasing day by day is land spread and the energy therein is wasted. In this context dairy secondary sludge was used for biohydrogen and methane (hythane) production in a two-stage process (first biohydrogen and then biomethane production). In this research the valorization of the sludge has been carried out for energy production. The sludge when collected has 2.00 ± 0.05 % Total Solids, and 7.0 pH. The biohydrogen producing reactor was established using acidogenic bacteria as inoculum. The gas was measured for biohydrogen production and then the spent was used for biomethane production in which cattle dung was used as inoculum and with 8% TS. The results of COD, BOD, electrical conductivity and the amount of gas produced, and its Gas chromatography studies would be discussed.

Keywords: *Biohydrogen, Methane, Hythane, Dairy Sludge, Energy crisis, Bio-valorization.*

COMPARISON OF DIFFERENT ADSORBENTS FOR IODATE REMOVAL IN WATER ENVIRONMENT

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ABSTRACT. Iodine-131 (I-131) has been a problem. When exposed to a high oxygenated water environment, the radioactive iodine will change to iodate (IO_3^-), which is fatal to human and other living things. The challenge in removing IO_3^- is to find an adsorbent which are environmentally friendly and low cost. This study employed different kinds of adsorbents to remove IO_3^- from water. The selected adsorbents were further investigated in order to determine the optimum condition for the adsorption process in a batch mode. Maximum adsorption capacities (qmax) of 10.4 and 32.3 mg/g for anion-exchange resin and pomegranate skin, respectively was obtained through Langmuir isotherms. Adsorption increased until 120 min contact time and then became stable. With increasing temperature anion-exchange resin showed increase in adsorption while pomegranate skin showed highest adsorption at 25°C. Results show that pomegranate skin as low-cost waste can be used for iodate adsorption with minimal processing.

Keywords: *Iodate; Radioactive iodine; Anion-exchange resin; Pomegranate Skin; Adsorption.*

Introduction

Since the early 20th century, electricity was generated by nuclear power plant [1]. However, nowadays people are generally concerned about the safety of this energy alternative. Since the accident of Fukushima and Chernobyl, radioactive iodine has been leaked into the environment and into the food chain. Iodine-131 (I-131) is of concern due to its half-life of 8 days and high specific energy of 0.13 MBq/g [2].

Iodine-131 when released into a high oxygenated water environment will oxidize and take a form of IO_3^-. The study about the removal of IO_3^- from a solution is still limited. Most common method that has shown significant removal is the removal by adsorption process. Iron oxides could adsorb iodine up to 48% after 200 days [3]. However, the comparison between synthesized and fully biobased adsorbent has not been studied. In this research, a screening test was done for several adsorbents. Only pomegranate skin and the anion-exchange resin exhibited satisfactory IO_3^- adsorption efficiency. Both adsorbents were explored to determine the optimal condition for IO_3^- removal in batch mode. They were further characterized to understand the mechanism behind the IO_3^- adsorption.

Material and Methods

Reagents

IO_3^- stock solution was prepared from 99.5% potassium iodate (KIO_3). Sulfuric acid (H_2SO_4), potassium iodide (KI), potassium hydroxide (KOH), and hydrochloric acid (HCl) were purchased and used as received for IO_3^- quantification.

Adsorbent preparation

The pomegranate skins and mangosteen pericarps were obtained from a local market. They were washed with deionized water (DI), roughly cut into small pieces, and dried at 50°C for 24 h.

The anion-exchange resins and granular activated carbon (800 iodine number) were bought and used without any further modifications. The activated carbon was cleaned with DI water.

Adsorption Experiment

The quantification of IO_3^- was a colorimetric method and was adopted from a literature [4]. The absorbance at 428 nm was performed with UV Visible Spectrophotometer (Genesys 10).

For the initial screening test, an adsorbent dose of 0.5 g/L were added to an IO_3^- of initial concentration 5 mg/L. The concentration was chosen based on the amount of I-131 (in the form of I^-) that can transform into IO_3^- in the ocean. The flasks were shaken in an orbital shaker for 3 h at 150 rpm at room temperature.

Further experimentations were conducted with pomegranate skins and anion-exchange resins due to their screening test high efficiency. The dose of adsorbents were 0.5 g/L and 1 g/L in 5 mg/L IO_3^- with a contact time of 2 h at room temperature. After that, the contact time was varied from 0 to 700 min. Temperature was investigated as well with 5, 25, 45, and 60°C, using a shaking incubator. Effect of pH was also investigated by varying the pH from 3 to 11. The pH level was adjusted using 1 M KOH and 1 M HCl.

After the kinetic experiment, the adsorption capacities (q) were calculated from Equation 1and the results were fitted with linearized isotherm equations. The linear equations used were Langmuir isotherm model and Freundlich isotherm model as shown by Equations 2 and 3, respectively:

$$q = (C_i\text{-}C_e)/(m/V) \tag{1}$$

$$C_e/q_e = 1/K_L q_{max} + C_e/q_{max} \tag{2}$$

$$\ln q = (1/n)\ln C_e + \ln K_F \tag{3}$$

where C_i and C_e are the initial and final IO_3^- concentration, respectively (mg/L). q_{max} is the IO_3^- adsorption capacity predicted by the Langmuir model (mg/g). K_L and K_F are the Langmuir (L/g) and Freundlich constant (L/g), respectively. n represents the degree of free energy and sorption intensity.

Characterization of adsorption

The selected adsorbents were characterized to understand possible adsorption mechanisms. Fourier Transform Infrared spectroscopy (FTIR) was used to identify the functional group on both virgin adsorbent and adsorbent that underwent IO_3^- adsorption.

Results and Discussion

Screening test

The results for the screening test are shown in Figure 1. Both anion-exchange resin and pomegranate skin had higher % IO_3^- removal compared to other adsorbents as 31.7% and 63.4%, respectively. Therefore, these adsorbents were selected for further experimentation. Mangosteen pericarps may contain IO_3^- adsorption functional group but the adsorbent efficiency was still below 30%. Activated carbon showed the lowest adsorption efficiency (<10%).

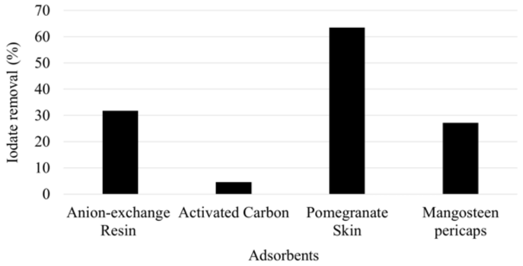

Fig. 1. Screening test of adsorbents for iodate removal (5 mg/L initial IO_3^- concentration).

Effect of adsorbent dosage

The result of the IO_3^- removal from pomegranate skin and anion-exchange resin with different dose of adsorbents are displayed in Figure 2a. The optimal adsorbent dosage for pomegranate skin and anion-exchange resin are 0.5 g/L and 1 g/L, respectively at 5 mg/L initial IO_3^- concentration.

Effect of contact time

The results for the effect of contact time on the adsorption is shown in Figure 2b. For pomegranate skin, the results revealed the optimal adsorption time to be at 2 h with 55% adsorption. For anion-exchange resin, the results revealed adsorption time at 3 h yielded the most IO_3^- removed with the value of 54.6% IO_3^- removed.

Effect of concentration of iodate

The adsorption capacity (q) [calculated from Equation 1] over final I^- concentration (C_e) plot is shown in Figure 2c. Pomegranate skin and anion-exchange resin had the maximum experimental q values of about 32 and 16 mg/g, respectively.

Effect of temperature

The results for the effect of temperature are shown in Figure 2d. For pomegranate, the temperature that yielded the most IO_3^- adsorbed was 25°C. For anion-exchange resin, the optimal temperature for adsorption was 60°C. A higher adsorption capacity at higher temperature may indicate an endothermic reaction.

Effect of pH

The results for the effect of pH are shown in Figure 2e. Optimal pH for pomegranate to adsorb the most IO_3^- was with the pH of 3. For anion-exchange resin, the optimal pH for adsorption was 7.

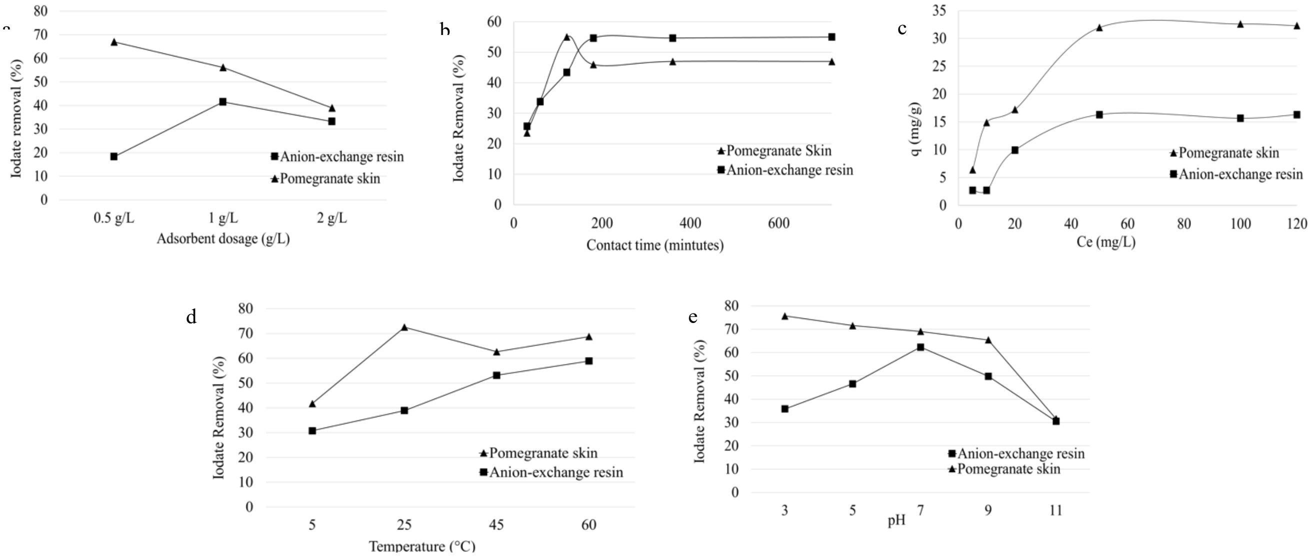

Fig. 2. Adsorption experiment results (a) Effect of adsorbent dose, (b) Effect of contact time, (c) Adsorption capacity over final I^- concentration, (d) Effect of temperature, (e) Effect of pH.

FTIR of pomegranate skin

The FTIR result for pomegranate skin is show in Figure 3. The assigned characteristics of adsorption to the peaks which were shifted were at the wavenumber of 2918.74 – 2849.31, 1729.84 – 1607.38, 1462.74, and 1027.87 corresponded to C-H (stretching), C=C (aromatic), and C-O-C, respectively. These functional groups are responsible for the IO_3^- adsorption process for pomegranate skin and are related to anthocyanin. Thus, anthocyanin played important role in the adsorption of IO_3^- for the pomegranate skin.

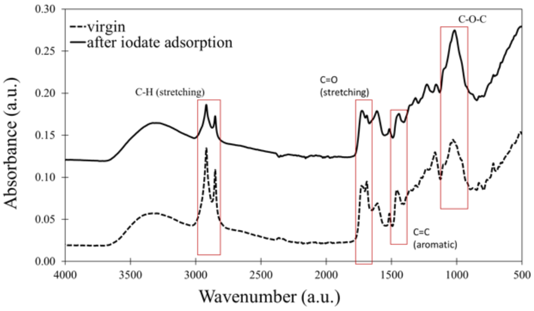

Fig. 3. FTIR spctra pomegranate skin.

FTIR of anion-exchange resin

The FTIR result for anion-exchange resin is shown in Figure 4. The assigned characteristics of adsorption to the peaks which were shifted were at the wavenumber of 3343.96, 1614.13, and 1475.28 corresponded to O-H (stretching), C=C , and C=O (stretching), respectively. These functional groups might not be directly responsible for the IO_3^- adsorption. They were shifted due to the electrostatic attraction created by quaternary ammonium cations presented in the anion-exchange resin.

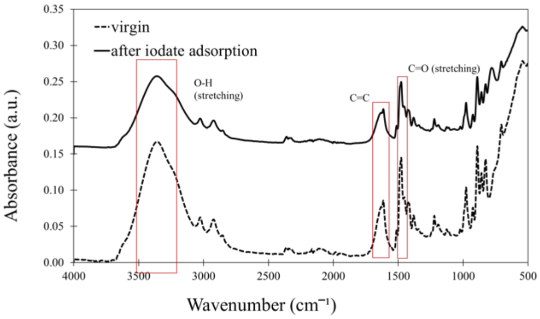

Fig. 4. FTIR spectra anion-exchange resin.

Langmuir isotherm

The linear fits of the Langmuir model (calculated from Equation 2) are shown in Figure 5. Both adsorbents have the R^2 values of 0.787 and 0.969 for anion-exchange resin and pomegranate skin, respectively. With this information, the adsorption process can be predicted with a monolayer adsorption process. The calculation with Langmuir model yielded maximum adsorption capacities (q_{max}) of 10.4 and 32.3 mg/g for anion-exchange resin and pomegranate skin, respectively. The value of q_{max} calculated from the Langmuir model is similar to the experimental q for pomegranate, but not quite similar for anion-exchange resin.

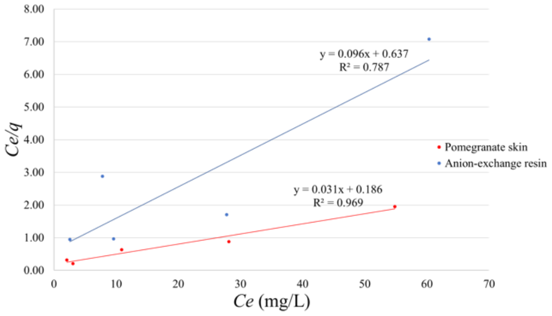

Fig. 5. Langmuir isothermal model.

Freundlich isotherm

The linear fits of the Freundlich model (calculated from Equation 3) are shown in Figure 6. Both adsorbents have the R^2 values of 0.523 and 0.803 for anion-exchange resin and pomegranate skin, respectively.

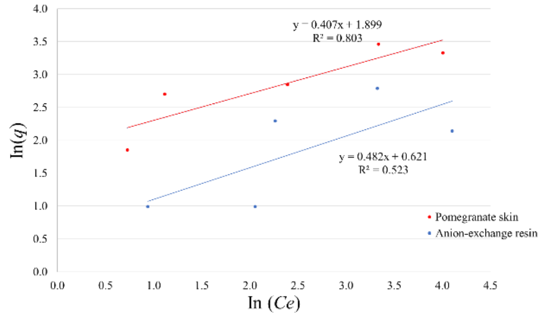

Fig. 6. Freundlich isothermal model.

Conclusions

The initial screening test showed that only the pomegranate skin and anion-exchange resin showed higher % IO_3^- removal compared to other adsorbents. For pomegranate, with 2 hours, it yielded 55% IO_3^- removed in 10 mg/L initial concentration. For anion-exchange resin, with 3 hours, it yielded 54.6% IO_3^- removed in 5 mg/L initial concentration. Both adsorbents fitted better with the Langmuir isotherm model with maximum adsorption capacities (q_{max}) of 10.4 and 32.3 mg/g for anion-exchange resin and pomegranate skin, respectively. FTIR results has shown that the functional groups involved in the adsorption process are C=C, C=O, and C-O-C.

Acknowledgement

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DEVELOPMENT OF SUSTAINABLE BIODIESEL PRODUCTION FROM *MADHUCA INDICA* USING GREEN CHEMISTRY PRINCIPLES AND TECHNO-ECONOMIC ANALYSIS

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The rapid industrialization and increase in the population have led to great energy demand in recent days. Various alternative sources of energy have been explored by researchers. Biodiesel is reported as the best alternative renewable bioenergy. In the present work, Mahua oil is a non-edible source used for effective biodiesel production with green chemistry principles and techno-economic analysis. The manganese doped zinc oxide is used as a heterogeneous catalyst for the production of biodiesel from Mahua oil. The main focus of this study was to reduce the reaction energy consumption and waste generation with the help of green process. The response surface methodology was used for process optimization. The central composite design was performed to analysis three responses: conversion, energy consumption and green chemistry balance. In green chemistry balance consist of five parameters like carbon efficiency, atom economy, reaction mass efficiency, stoichiometric factor and environmental factor. The green chemistry balance was confirmed the biodiesel was highly eco-friendly. Techno-economic analysis of large scale production of 10,000 L of biodiesel from madhuca indica was studied. The economic analysis disclosed that it is possible to produce biodiesel from the modeled plant more profitably.

Keywords: *Biodiesel, Madhuca indica, Green chemistry, Techno-economic analysis.*

International Conference on Sustainable Biowaste Management 2021

Emerging Pollutants and Fate During Resource Management

CONFIRMATION OF LANDFILL GASES OXIDATION IN PHYTOCAPPING SYSTEMS IN INDIA USING COMPUTATIONAL BIOLOGICAL TOOLS

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Landfill gas (LFG) containing is generally produced by anaerobic degradation of organic waste, which can sometimes lead to poor environmental, degraded hygiene and aesthetics due to the risk of fire and explosion, gas migration in the subsurface soils thus affecting the overall environmental health of surrounding area. This problem can be addressed by alternative landfill covers like phytocaps which allows the landfill gas to oxidize into carbon dioxide with the help of increased bio-availability of oxygen, however due to the low permeability of the landfill cover, no aerobic oxidation occurs inside the landfills. Anaerobic oxidation increases the overall possibility of LFG oxidation thus rejuvenating the site to its natural ecosystem. Plants hold up the soil and moisture in the top cover of landfill cover and thereby alter the permeability of the soil and reduce the emissions of LFGs into the environment. Though plant-soil based phytocap enhances the LFG oxidation, the microbes played a vital role in the process. The microbes growing in synergy with the plants are contributing to the actual LFG oxidation in the phytocaps. Upon the oxidation of the LFGs, occurrence and activity of the microbes must be confirmed. A laboratory-scale phytocapping system is observed for the reduction of LFGs by 86%. The present study focuses on the confirmation of the role of microbes in LFG oxidation using the Computational Biological Tools. The isolated microbes from the various depths of the landfilling systems were sequenced (16S and ITS). After BLAST analysis microbes were identified as *Klebsiella*. The evolutionary analysis of the isolated bacteria and reported microbes will be done by the phylogenetic analysis. Further study of activity of microbes and kinetics of gas mitigation will help in designing appropriate phytocapping cover under Indian climatic condtions.

Keywords: *Landfill, LFG, Phytocapping, Microbial activity, Phylogenetic analysis.*

STRENGTHENING ELECTRON TRANSFER THROUGH SUPPLEMENTARY ELECTRIC FIELD COULD REDUCE THE POTENTIAL ENVIRONMENTAL RISK OF HEAVY METALS AND ANTIBIOTIC RESISTANCE GENES IN AEROBIC COMPOSTING

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ABSTRACT. Heavy metals (HMs) and antibiotic resistance genes (ARGs) are considered as primary pollutants in livestock manure due to their non-degradability and diffusion. The existing of HMs proved might induce microbial HMs resistance that will further affect the abundance of ARGs due to the co-resistance and cross-resistance mechanism. The application of supplementary electric field (E) or biochar (B) has been reported could strengthening electron transfer in manure composting process that will stimulate microbial activity and affecting HMs speciation and antibiotic degradation. However, the synergistic effect of strengthening electron transfer on HMs redox and ARGs removal and their interaction have not been clarified. The aim of this study was to investigate the effect of applying E and B on the fate of ARGs and HMs and their interaction during aerobic composting. To achieve it, HMs sequential extraction, quantitative PCR, and Illumina MiSeq sequencing were used. Results showed that applying E and B enhanced the microbial activity, especially the relative abundance of electroactive bacteria enhanced by 73% and 34% respectively. Compared to control treatment, treatment E reduced the bioavailable factors (BF) of Cu, Cr and As by 18.52-38.34%, meanwhile the relative abundances of *tet* and *sul* resistance genes were reduced by 26.99% and 66.68%, respectively. Network analysis showed that BF was a considerable factor that affected the transfer of ARGs, of which BF of Cu was closely related to the potential hosts of *tetW*, BFs of Cr and As were closely related to the potential hosts of *tetC*, *tetG*, *sul1* and *sul2*. Most of these potential hosts were from *Firmicutes* and *Proteobacteria* on phylum level, the abundance of the two phyla increased and decreased respectively under electric field. Redundancy analysis indicated that mobile genetic element *int1* also played an important role in ARGs diffusing, which can function as a medium mediating the effect of HMs on the transfer of ARGs, and its relative abundance reduced by applying electric field. These findings suggest that the applying E is a prospective way that could potentially lower the environmental risk of ARGs and HMs and mediate their interaction.

Keywords: *Electric field, Heavy metals, Antibiotic resistance genes, Composting.*

Introduction

The manure from animal husbandry and poultry breeding is a crucial reservoir for antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs). Composting is a practical and effective way to turn manure into organic fertilizer, which can not only improve soil fertility, but also can modify soil as amendments. However, previous studies have shown the complex behaviors of ARGs that not always been effectively removed after composting[1]. Thus, it’s urgent to find an effective way to hinder the ARGs enrichment during composting. Heavy metals (HMs) have been proved as a major factor affecting ARGs abundance due to the existing cross-resistance and co-resistance mechanisms[2]. Most of the HMs used as additives can’t be metabolized by animals and then are excreted via manure and urine[2], which could impose permanent selective pressure on ARGs. Many studies reported that biochar addition can reduce HMs bioavailability[3] and ARGs abundance[1]. And biochar was described as electron shuttle because of those functional groups which can be both electronic donor and acceptor[4]. In addition to biochar, electric field is another way to facilitate electron transfer, and its effect on ARGs removal in wastewater treatment has been found in some researches[5].

We hypothesized that electric field may stimulate electron transfer more effectively than biochar owing to the directional movement of electrons. Therefore, microbial activity could be stimulated during composting process, and then HM passivation and ARGs removal could be accelerated under electric field. We applied electric field and biochar in this study to (1) investigate the fate of ARGs and HM speciation with supplementary of electric field and biochar; (2) characterize the effect of electric field and biochar on bacterial community structure during chicken manure composting; (3) evaluate the correlations between ARGs, bioavailable HMs and bacteria.

Material and Methods

Composting setting

Compost mixtures, prepared by mixing chicken manure and sawdust (3.5:1), were adjusted to a C/N of ~20 and water content of ~65%. 18.5 kg mixtures were transferred to 50L cylindrical stainless steel composting reactor. Graphite columns (height 50 cm, diameter 6 cm) was placed in center of each reactor as anode, while inner stainless steel wall acted as cathode. The DC power (0-30V) was connected to the anode and cathode by wires to provide a stable DC voltage for composting system. Three treatments were setup as follows: 1) CK: no supplementary was provided; 2) E: 5V voltage was provided; 3) B: 5% biochar was added (dry weight basis). Air flowed through the bottom of the reactor by using compressor, and the ventilation rate was adjusted to make sure that the ventilation rate through the compost was 0.2 L/min/kg (dry weight). The composting biomass were turned over and sampled on day 3, 6, 10, 14, 21, 35 and 42. The compost temperature and current were automatically recorded every hour.

Sequential HM extraction

Four different fractions of HMs (Cu, Zn, Cd, As and Cr) was determined by inductively coupled plasma mass spectrometry (ICP-MS) after extraction, based on the modified BCR (European Community Bureau of Reference) sequential extraction procedure[6]. The bioavailability factor (BF) was calculated by the ratio of the content of Exc and Red to total metal content and used to present the biotoxicity of HMs.

DNA extraction, qPCR and High-throughput sequencing

Total DNA was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, US) according to the manufacturer's instructions. Four tetracyline resistance genes (*tetC*, *tetG*, *tetL*, *tetW*), two sulfonamide resistance genes (*sul1*, *sul2*), one MGE (*intI1*), and 16S rRNA gene were quantified using quantitative polymerase chain reaction (qPCR). The relative abundance (RA) was calculated as the ratio of garget gene copy number to 16S rRNA gene copy number. Bacterial community structure was analyzed through sequencing of the 16S rRNA gene (Shanghai Majorbio Bio-pharm Technology Co. Ltd).

Results and Discussion

The effect of treatments on HMs distribution and ARGs relative abundance in composting

Applying electric field can reduce biotoxicity through enhancing the passivation of heavy metals (Cu, As and Cr) and decreasing the exchangeable fraction (Zn and Cd) (Fig.1). Supplementary electric field could inhibit the enrichment of ARGs (*sul1*, *sul2* and *tetG*) and mobile genetic element (*intI1*), Biochar presented negative effects on removal ARGs in this study (Fig.2).

Potential hosts of ARGs and correlation between ARGs, MGE and BF of HM

The potential hosts of ARGs were identified through network analysis ($P < 0.01$, $r > 0.6$)(Fig.3). *tetW* had 14 potential hosts, eight of them belonged to *Firmicutes* and all of them distinctly decreased after composting, which may explain the significant decrease of *tetW* at the end of composting. Furthermore, all of these 14 potential hosts genera correlated with Cu_BF representing the selective pressure of Cu, indicating that the abundance of *tetW* may be affected by Cu through the selective pressure on the potential hosts. BF of As and Cr was highly correlated with potential hosts of *tetC*, *tetG*, *sul1*, *sul2*, *intI1*. These results suggest that the selective pressure applied by HMs could facilitate the transfer of ARGs in compost, supported by Li et al.[7]that the presence of HMs accelerated the spread of ARGs, especially *tetG* and *sul1* increased 2.67- and 3.86-fold, respectively.

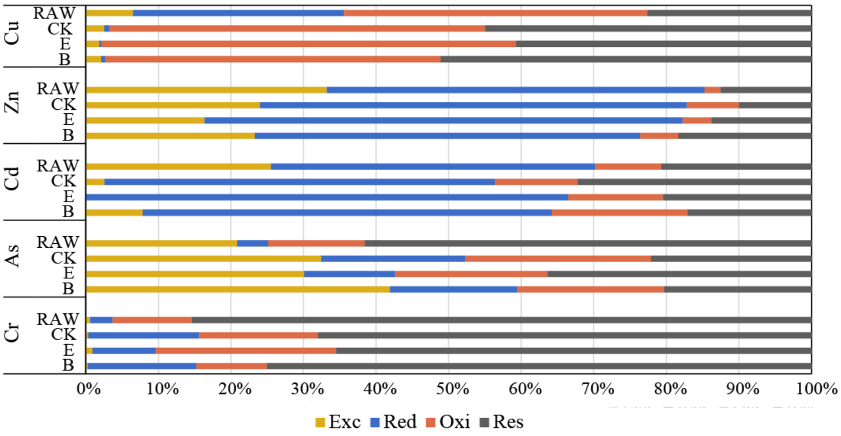

Fig. 1. Distribution of Cu, Zn, Cd, As and Cr in raw material and compost products. Exc, Red, Oxi and Res represented exchangeable, reducible, oxidizable and residual fraction, respectively. RAW represented raw material.

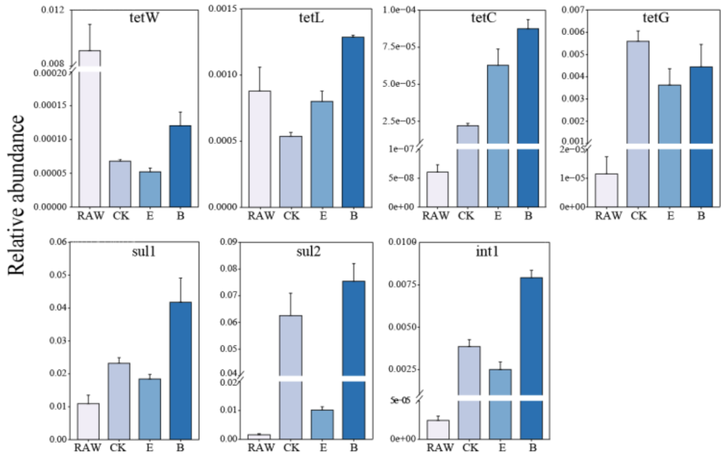

Fig. 2. Relative abundances of ARGs and MGE in raw material and compost products. RAW represented raw material.

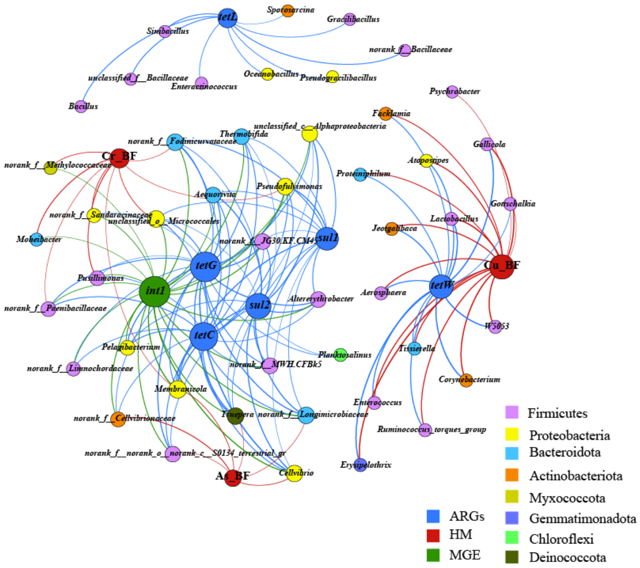

Fig. 3. Network analysis shows the potential hosts (top 50 genera) of antibiotic resistance genes. Connections indicate significant positive correlations ($p < 0.01$, $r > 0.6$) based on the Spearman's correlation coefficients.

Conclusions

The supplementary electric field seems to be a promising strategy for effectively reduce the potential environmental risk of HMs and ARGs in compost. The selective pressure imposed by HMs (Cu, As and Cr) could greatly affect the fate of *tetW*, *tetC, tetG, sul1* and *sul2*. In addition, *intI1* and potential host bacteria were important as a medium mediating in the HGT of heavy metals and antibiotic resistance genes.

Acknowledgement

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BIO-WASTE RECYCLING AND GREENHOUSE GAS EMISSION REDUCTION

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Municipal solid waste (MSW) generation, transport and management is one of the largest sources for greenhouse gas (GHG) such as methane (CH_4) and CO_2 emissions in the USA. As of 2018, approximately 292.84 million tons (MT) of MSW are generated i.e., 4.9 pound per day per person. In which, 50% of waste is landfilled (~146 MT) and nearly 50% recycled through different routes e.g., composting, anaerobic digestion, thermal energy recovery, animal feed and direct material recycling, etc. Through the different MSW management approaches, the total GHG emission reduction achieved in 2018 was estimated as 193 million metric tons of carbon dioxide equivalent (MMTCO_2eq.), in which 80% reduction (155 MMTCO_2eq.) was succeeded only through paper and cardboard recycling. Even though food waste produced to the equal percentage that of paper and cardboards, they are less recycled and more than ~35 MT of food waste ended-up in landfills. Across the United States more than 2629 of landfills are operated that are accounted for ~ 110.6 MMT CO_2Eq of CH_4 emissions in 2018. Food waste is the major bio-waste in landfills that contributing to GHG emissions through anaerobic degradation. Therefore, diverting food waste from landfill could be a possible approach to reducing the GHG emissions and thereby percentage contributions in the national GHG emission sectors. However, there are challenges in storing the food waste for long term or disposal through conventional processes such as anaerobic digestion/composting/thermal conversion methods. In addition, transportation costs and GHG emissions are high for a bio-waste with the high moisture contents.

In this paper, we have proposed a decentralized approach to convert food waste into various end products (e.g., organic acid, hydrogen, compost, etc) through integrated bioprocess technologies. The three different process integration scenarios are developed, techno-economics are calculated and compared. The total GHG emissions for proposed integrations were also calculated in terms of net energy balance calculations, while compared with the food waste landfilling and GHG emissions as base case scenario. In specific, Scenario 1 included a hydrolysis process followed separation of liquid and solids for anaerobic digestion (CH_4) and composting, respectively. In Scenario 2, hydrolysis process followed by aerobic fermentation process (for H_2) and composting of liquid and solid fractions, respectively. In Scenario 3, the hydrolysed food waste used for growing the algae through heterotrophic bacterial-algal polyculture systems and solid residue is used for composting process. The produced bacterial-algal poly cultures are potential source for biofuel production. From the results, integration of bacterial-algal technology (Scenario 3) found to be more promising in processing of food waste in terms of techno-economics and net GHG reductions. However, proposed process integration needs to be tested at lab and pilot scale before commercialization was concluded.

Keywords: *Municipal solid waste, Composting, Anaerobic digestion, Energy Recovery, GHG emission, Techno-economics.*

DEVELOPMENT OF NUTRIENT MANAGEMENT TECHNOLOGIES FOR SUSTAINABLE RICE FARMING FOR MITIGATING WATER AND ATMOSPHERIC POLLUTION

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ABSTRACT. Rice is the staple food of Sri Lankans. In Sri Lanka, out of the total rice production, a considerable amount of rice straw and rice husk goes wasted. Hence, there is a great potential for the production of quality compost and rice husk charcoal. The main objective of the study was to design a lysimeter simulation of a landfill bioreactor to determine possible avenues to produce biogas from agricultural wastes. Secondary objectives were to produce rice husk charcoal coated urea as a slow releasing fertilizer and to compare the leaching losses of nitrogen, phosphorus and potassium using leaching columns and to evaluate rice husk charcoal coated urea developed in the study as a slow releasing fertilizer and compare the total N, P, K in soil and yield of rice production. After 200 days of operation of lysimeter, nitrate nitrogen and phosphate of the permeate were 1.23 mg/l and 0.03 mg/l, respectively. BOD increased during the first 14 days and it gradually decreased after 200 days. The quality of the compost complied with the SLS guidelines. The study shows that there is a high potential of producing compost from landfill bioreactor using rice straw. Rice husk charcoal coated urea can potentially be used as a slow-releasing nitrogen fertilizer which reduces leaching losses of urea. Besides, the coating is less costly and helps reduce two-third of urea usage, and saves 70% fertilizer cost.

Keywords: *Nutrient management; Sustainable rice farming; Mitigating water; Atmospheric pollution.*

Introduction

Rice is the staple food for Sri Lankans. Rice cultivation is the major agricultural activity of the country since ancient times. The country produces nearly 30.7 MMT of rice in 2019 [1]. This yields almost the same amount of straw. Rice straw and husk are renewable energy sources and a highly valued organic fertilizer. Intensive agriculture is associated with increased fertilizer and pesticide use [2]. The application of inorganic fertilizer has become a burden to the country. The excessive use of organic and chemical fertilizers leads to deterioration of the quality of water. Coated fertilizers are used to improve the efficiency of fertilizer application. Biochar is a form of charcoal produced through the heating of natural organic materials under low oxygen conditions [3]. It gradually releases nitrogenous compounds thus enhances plant growth. At present, urea is the most widely used form of nitrogen fertilizer (60-70%) in the country. Mixing both urea and rice husk charcoal in soils produces a slow-releasing fertilizer [4]. A significant amount of rice straw and rice husks remain unused and burned in open fields. Producing biochar using rice husk and rice straw compost is practicable in Sri Lanka.

Methodology

The experiments were conducted at Meewathura University Farm, University of Peradeniya, Sri Lanka. Laboratory testing was carried out at the Agricultural Biotechnology Center and the Department of Agricultural Engineering, University of Peradeniya, Sri Lanka.

Field Experiment

An experiment was conducted during *Maha* seasons. The location selected for the experiment was in Megoda Kalugamuwa, Kandy, Sri Lanka. Bund clearing, first ploughing, second ploughing, plastering bunds, puddling, leveling were carried out for land preparation. The field was divided into three blocks into 20 m x 5 m) and each block was subdivided into four plots 5 m x 5 m) (RCBD). Addition of inorganic and organic fertilizers to the plots (each replicated 3 times. The soil sampling was done once a week for 14 weeks period and plants height were also measured Treatment 1 application of inorganic fertilizer; Treatment 2 application of urea coated charcoal; Treatment 3 application of inorganic fertilizer and rice straw compost; Treatment 4 application of rice straw compost and urea coated charcoal.

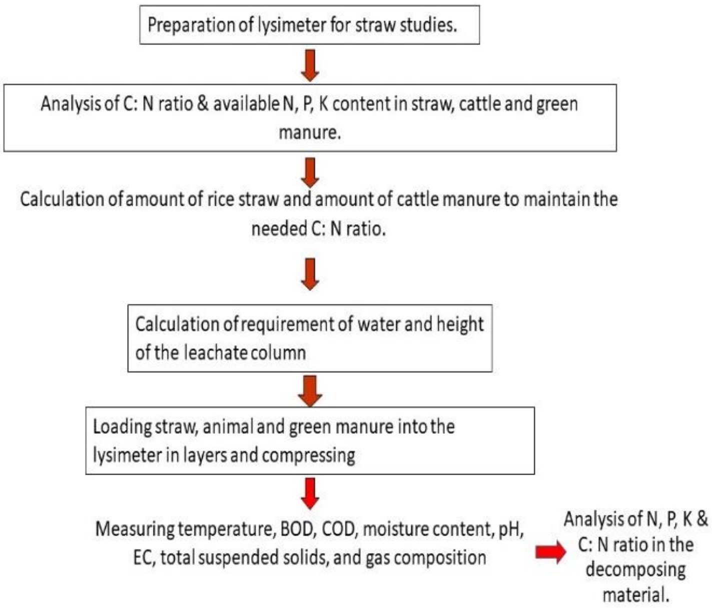

Fig.1. Lysimeter studies.

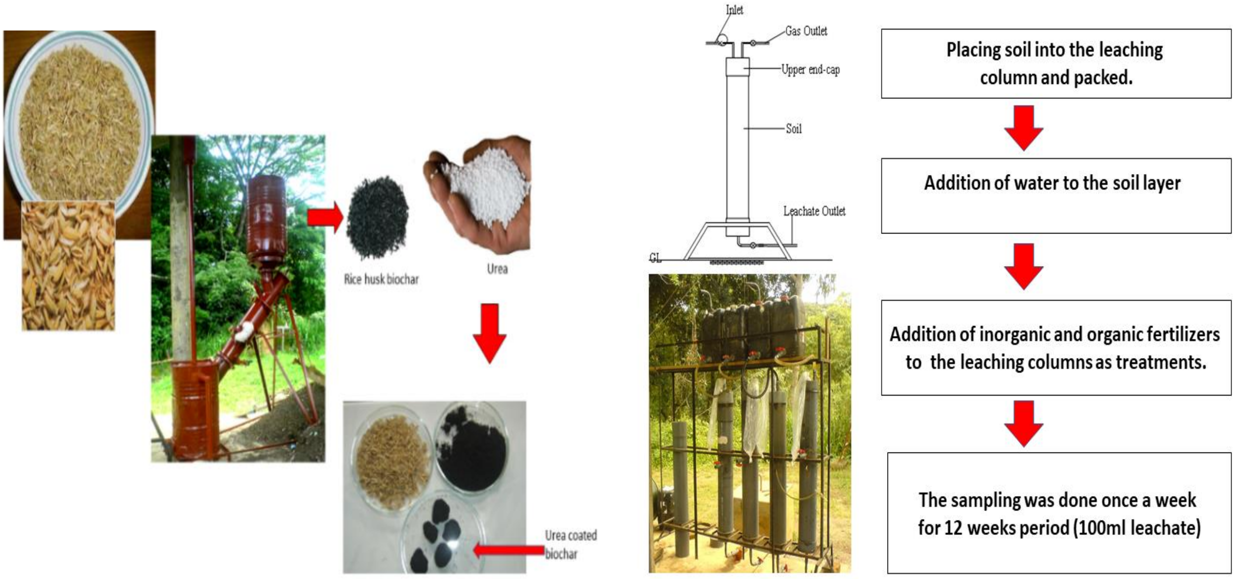

Fig.2. Leaching column studies.

Results and Discussion

Lysimeter studies

Nitrate nitrogen and phosphate of permeate were less than permissible limits. The ratio of BOD/COD indicated that the biodegradation of leachate was high. The quality of compost is tallied with the Sri Lankan Standards (SLS) guidelines. There was a high potential of producing compost from landfill bioreactor using rice straw. After 200 days of operation, NO_3^--N and PO_4^{3-}-P of the permeate were 1.23 mg/l and 0.03 mg/l, respectively.

Leaching column studies

Table 1. Leaching losses of the nutrients.

Nutrient	Treatment 1	Treatment 2	Treatment 3	Treatment 4

NO_3-N (kg/ha)	$30.10^a \pm (0.31)$	$21.64^b \pm (0.51)$	$16.64^c \pm (0.21)$	$9.26^d \pm (0.11)$
PO_4-P (kg/ha)	$0.549^a \pm (0.21)$	$0.419^b \pm (0.45)$	$0.435^b \pm (0.16)$	$0.360^b \pm (0.39)$
K (kg/ha)	$4.357^a \pm (0.95)$	$3.294^b \pm (0.32)$	3.709b$^c \pm (0.21)$	$2.915^c \pm (0.98)$

Treatment 1-inorganic fertilizer only, Treatment 2- rice husk charcoal coated urea only, Treatment 3- rice straw compost prepared from anaerobic digestion, and Treatment 4- no fertilizer as a control. Mean followed by the same letter at each column are not significantly different ($p=0.05$), each value represents the mean of two replicates. SD is given in parenthesis.

Treatment 1 has the highest value of NO_3^--N leaching and 2, 3, and 4 come in descending order. Treatment 1 has the highest value of P leaching and 2, 3, and 4 come in descending order. Using charcoal as a coating material of urea also can save the reduction loss of P. Treatment 1 has the highest value of K leaching and 2, 3, and 4 come in descending order. Using charcoal as a coating material of urea also saves the reduction loss of K. Charcoal coated urea and rice straw compost have potential in terms of reducing groundwater pollution of N, P, and K due to leaching compared to the treatment 1.

Results of the field experiment

When considering the rice grain yield in *Maha* season, treatment 3 ($2664^b \pm 12$) was significantly different from treatment 1 ($2664^b \pm 12$), 2 ($2776^b \pm 4$), and 4 ($2444^b \pm 4$) kg/ha. One-third of urea from the recommended fertilizer was used for treatments 2 and 4, thus reduced two-third of urea usage and saved 70% fertilizer cost for treatments 2 and 4. Compost was used on three separate applications for treatments 3 and 4 only.

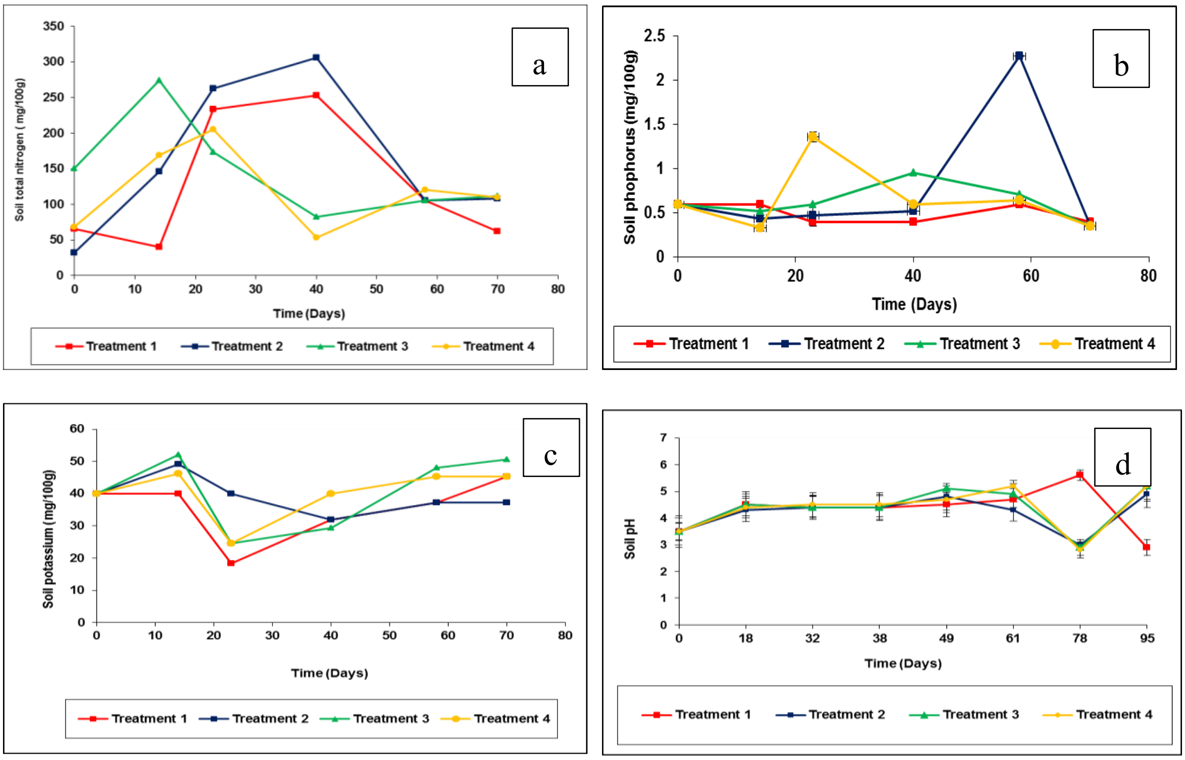

Fig. 3. Relationship between the (a) nitrate-nitrogen (b) phosphorus (c) potassium and (d) pH in soil with different treatments-*Maha.*

Conclusions

There is a high potential of producing compost from landfill bioreactor using rice straw. After 200 days of operation, nitrate nitrogen and phosphate of the permeate were 1.23 mg/l and 0.03 mg/l, respectively. BOD increased during the first 14 days and it gradually decreased after 200 days. Rice husk charcoal coated urea can be used as a slow-releasing nitrogen fertilizer which reduces leaching losses of urea. It also helps reduce phosphate and potassium leaching. The coating is less costly and helps to reduce the fertilizer cost (70% of urea cost).

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INTELLECTUAL PROPERTY RIGHTS IN E-WASTE MANAGEMENT: WHY AND HOW?

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The present century is a century of knowledge indeed a century of mind. The respect and protection of individual property is one of the main fundamental principles of free and just societies. Without the protection of property, a modern society would most probably end in anarchy and distress. Granting and protecting property rights to intellectual achievements is interpreted as a kind of contract between innovators and society. Thus, the temporary exclusive right to market the innovation is an incentive for the innovators to expose the ideas and make it available to others. The disclosure of new ideas enables other individuals or groups to further innovate upon the basis of this new knowledge, which may lead to other innovations. Eventually this guarantees early and broad access to knowledge and innovations and increases the world's knowledge base and therefore innovative capacity.

Electronic and Electrical waste, popularly known as e -waste products, do not decompose or rot away. The information and communication technology (ICT) sector in the last twenty years or so in India has revolutionized life of one and all, ratcheting a viral effect on electronic manufacturing industries leading to phenomenal growth in terms of both, volume and applications. The booming usage of electronic and electrical equipments has created a new but very dangerous stream of waste, called "electronic-waste", or simply known as **e-waste**. Electronic waste or e-waste is one of the rapidly growing problems of the world. E-waste comprises of a multitude of components, some containing toxic substances that can have an adverse impact on human health and the environment if not handled properly. In India, e-waste management assumes greater significance not only due to the generation of its own e-waste but also because of the dumping of e-waste from developed countries. This is coupled with India's lack of appropriate infrastructure and procedures for its disposal and recycling. E-waste is much more hazardous than many other municipal wastes because electronic gadgets contain thousands of components made of deadly chemicals and metals like lead, cadmium, chromium, mercury, polyvinyl chlorides (PVC), brominated flame retardants, beryllium, antimony and phthalates. Long-term exposure to these substances damages the nervous systems, kidney and bones, and the reproductive and endocrine systems, and some of them are carcinogenic and neurotoxic.

The environmental protection can improve by promoting innovation in beneficial environmental technology through application and reformation of intellectual property laws. However, there is no need to grasp all the details of these legal areas; an understanding of fundamental principles will suffice. intellectual property law applicable to environmental technology touches on issues of special concern to legal practitioners, regulators, and managers who need to assess their companies' proprietary rights in environmental technology. Trade secrets, patents, and trademarks are considered as international aspects of protecting environmental technology. Voluntary efforts to protect biodiversity are also considered as an example of how proprietary rights can serve the ends of conservation.

The Indian firms account for lesser number of patents for their innovations as compared to their foreign counterparts. The reason is because the investment in R & D activity is small due to lack of capital for trials in innovating product and establishing product in market. There exists a lack of continuity in patenting activity particularly in e-waste.

Keywords: *E-waste, Innovation, Protection.*

NUTRIENT REMEDIATION BY *MONORAPHIDIUM NEGLECTUM* AND *MESSASTRUM GRACILE*- A COMPREHENSIVE STUDY

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Two microalgae strains *Monoraphidium neglectum* and *Messastrum gracile* belonging to Selenastraceae family was isolated from wastewater. The isolated strains were cultivated under mixotrophic condition to compare its wastewater treatment efficiency and its concomitant effect on nutrient uptake, biomass, photosynthetic activity and biochemical constituents. Several photochemical parameters involving electron transport rate (ETR), Non photochemical quenching (NPQ), quantum yield (Y(II)) and Fv/Fm using Pulse Amplitude Modulator (PAM) were measured for evaluating microalgae photosynthetic performance with respect to light response curves. Comparison and performance evaluation of the two strains with respect to soil fertility and plant growth was studied.

Keywords: *Synthetic dairy wastewater, Fatty acid profile, FAME characterization, Photosynthetic properties, Pyrolysis.*

AN ASSESSMENT ON OPPORTUNITIES OF SEWAGE FED AQUACULTURE PRACTICES IN BANGLADESH: CHALLENGES AND WAY FORWARD

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Bangladesh is one of the world's leading fish producing countries with a total production of 410.34 million MT, where aquaculture contributes 56.44 percent of the total production. Fishes meet the demand of 60 percent animal protein of mass people in this country. Fresh water body like ponds, river, lake and wetland are the main sources of aquatic animals which is the main mean of livelihood for about 1.2 million people. Fishes grow up in this water body naturally. However, fish production in this sweet water decreased as an alarming rate as the volume of such water body declining due to shifting agricultural, industrial and residential infrastructure in Bangladesh. Consequently, the lower income people specially the fishermen of Bangladesh are suffering a lot due to scarcity of fishes for time being. Most of them are intend to change their profession. Therefore, artificial aquaculture become popular and it reached to 56 percent whereas capture fish is 28 percent and marine is 16 percent in Bangladesh. Thus, a crying need appear regarding the supplying of fed for aquaculture. Gradually the sewage fed aquaculture become popular in Bangladesh. This paper aims to assess the opportunities of sewage fed culture which will find out the challenges and recommend the way forward. Both primary and secondary data will be collected. Experience of fishermen who are practicing fed aquaculture will be covered through FGD. Expert opinion will be collected through KII to justify the socio- economic viability of fed aquaculture. As a good number of fishermen around in Bangladesh developed a technique of using domestic sewage for fish culture and this technique is widely used to meet the growing demand for fish in this thickly populated country. The technique is considered to be unique and is the largest operational system in the Asian sub continent to convert waste in to consumable products.

Keywords: *Domestic sewage, Employment generation, Poverty alleviation, Resource.*

EFFECTS OF LEAD ON PETROLEUM DEGRADING BACTERIA ISOLATED FROM CONTAMINATED SOIL IN ZHUHAI, GUANGDONG, CHINA

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ABSTRACT. Soil pollution, particularly of petroleum hydrocarbons and lead contamination, has become increasingly concerned due to rapid urbanization and industrial development. Bioremediation of petroleum hydrocarbon pollution using microorganisms is a promising solution due to the absence of secondary contamination. In this study, two bacterial isolates (B-7 and B-10), with distinctive colony characteristics, were screened from petroleum contaminated soil collected in Zhuhai, Guangdong Province, China. Using 16S rRNA sequencing technique and biochemical analyses, B-7 and B-10 were identified to be closely related to *Achromobacter denitrificans*, and *Mycolicibacterium phocaicum* N4, respectively. Petrol degrading rates of the two isolates were determined by UV-visible spectrophotometer in this study. With consideration of recovery rate and background evaporation rate, in the absence of lead, in a five-day interval study, the petrol degradation rate of B-7 and B-10 were observed at approximately 2.4 g petrol/L for both isolates. Co-contamination with lead at 1, 5, 10 and 20ppm significantly inhibited petrol degrading potential of both isolates, with B-10 demonstrated significantly higher lead tolerance. Future studies are needed to evaluate the effects of other abiotic factors, such as pH, temperature, nutrient contents and concomitant exposure to other pollutants and biotic factors, such as microbial community, on the petroleum hydrocarbons degrading efficiency of these isolates.

Keywords: *Bioremediation, Lead Contamination, Petroleum hydrocarbons.*

Introduction

Petroleum hydrocarbons and heavy metals contamination is a widespread environmental issue in China and across the world. Due to the high toxicity of petroleum hydrocarbons, remediation of oil polluted environment is of imminent need. Since the use of petrol and petroleum-relevant products are inevitable due to economic development, while physical and chemical remediation approaches usually come with high cost and potential threats in secondary contamination [1], expanding the currently limited understandings in bioremediation using native petroleum degrading microorganisms, together with high tolerance to heavy metals, will have high ecological significance. As no research has considered the joint pollution scenario caused by lead and petrol while the scenario is likely to be occurring in the environment due to rapid urbanization, evaluating the feasibility on applying petroleum degrading and lead resistant soil microorganisms can have a real-world significance.

Material and Methods

Soil Sample Collection

Soil samples were collected in selected contaminated sites in Zhuhai, Guangdong, China. In brief, approximately 200g of topsoil samples were collected in depth between 2 to 15cm from four selected locations in Zhuhai with known history of petrol pollution. Soil samples were brought back to the laboratory within 4 hours from collection and sieved by 2mm pore size sieves. Sieved samples were collected in aseptic bags and stored at 4°C for further analysis.

Enrichment, Isolation, and Identification

5g soil samples were incubated with 250mL minimal salt media (MSM) containing 2g of sterilized filtered petrol as the sole carbon source and incubated with shaking at 200 rpm for 10 days at room temperature. Spread plating technique was applied to further inoculate the isolates onto MSM plates with petrol as the sole source of carbon and energy. By collecting the single colonies and inoculating on nutrient agar, the bacteria was isolated and characterized. Selected strains with different colony morphology were characterized by biochemical analyses and were subsequently sent to Shenzhen National Genebank for 16S rRNA gene sequencing.

Biodegradation of Petrol

$1*10^9$ cells of each strain were transferred to 100mL MSM supplemented with 2g sterilized petrol as the sole carbon and energy source to determine the petrol degrading rate in a five-day study period. In addition, cell growth was determined by OD600 at every 24 hours. After 5 days of incubation, the remaining petrol in MSM was extracted by petroleum ether and the absorbance of extracted petrol was determined under a UV-visible spectrophotometer at optimal wavelength. The degradation rate of bacterial isolate was determined by Formula 1:

$$R_i = [M_0 - (M_E - M_i) * r]/ M_O * 100\% \qquad (1)$$

Where R_i represented the degrading rate of the i^{th} bacteria; M_E represented the actual remaining petrol determined in evaporation group; M_i represented the measured remaining petrol in i^{th} bacteria media; r represented the recovery rate; M_0 represented the initial weight of the petrol in MSM.

Biodegradation of Petrol with Lead Contamination

The effects of lead on bacterial growth and petrol degrading rates were determined by adding $1*10^9$ cells to the petrol containing MSM supplemented with 1mg/L, 5mg/L, 10mg/L, or 20mg/L Pb^{2+} and using the same method as mentioned above.

Results and Discussion

Identification of the Screened Bacteria

After screening and isolation, two bacterial strains with distinct colony morphology were selected, namely B-7 and B-10. B-7 was observed to be a Gram-negative rod-shape bacillus, while B-10 was a Gram-positive coccobacillus. By 16S rRNA sequencing and biochemical analyses, B-7 was found to show the highest homology to *Achromobacter denitrificans* while B-10 was found to have 99.71% sequence homology to *Mycolicibacterium phocaicum* N4. Similar strains of *A. denitrificans*, such as *A. denitrificans* strain SP1, *A. denitrificans* ASU-035, *A. xylosoxidans* sub. *denitrificans* strain EST4002 and strain TB, have recently been identified to be able to degrade di(2-ethylhexyl)phthalate, pyrene, 2, 4-dichlorophenoxyacetic acid and reduce nitric oxide, respectively [2-5]. Although the genera *Mycolicibacterium*, such as *M. mucogenicum, M. frederiksbergense* IN53, *M. vanbaalenii*, have been reported to be able to degrade methy t-butyl ether, polychlorinated biphenyls and petroleum hydrocarbons [6-8], the strain *M. phocaicum N4*, which shows 99.71% sequence homology to the isolated B-10 strain in this study via 16S rRNA sequencing technique, has recently been isolated from human bronchial aspirate from patients with tuberculosis symptoms [9].

Effects of Lead on Petrol Degradation Rates

After considering the amount of background evaporation and recovery rate, the degradation rates of 2g petrol with lead concentration ranging from 0 to 20ppm were determined on B-7 and B-10 in 100 mL MSM culture medium. Over a 5-day study period, in the absence of lead, both strains demonstrated similar petrol degradation rate of approximately 12% (~2.4 g petrol/L/5day). Under lead exposure, strain B-10 demonstrated higher tolerance to lead as significant inhibition in petrol degradation rate was only observed when lead was present at 5ppm or at higher concentration, while significant inhibition in petrol degradation rate was observed in B-7 exposed to 1 ppm lead in MSM culture medium (Figure 1).

Effects of Lead on Growth Response Curves

Higher tolerance of B-10 to lead was also observed in the growth response curve study. Exposure to lead at 1ppm resulted in over 80% reduction of cell density in the B-7 culture while less than 30% reduction in cell density was observed in the B-10 culture (Figure 2). Cell growth was completely abolished at lead concentration of 5ppm for B-7 and 10ppm for B-10. Although Shi et al. [10] reported that the average lead concentrations in agricultural soil in China was around 50 ppm, while significant inhibition in growth and petrol degradation rate was observed at 5 ppm in this study, however, since the physico-chemical properties of soil is very different from that of liquid culture medium, particularly in the accessibility and availability of lead ions, further studies should be carried out in simulated soil environment for more accurate and environmentally realistic results.

(a) (b)

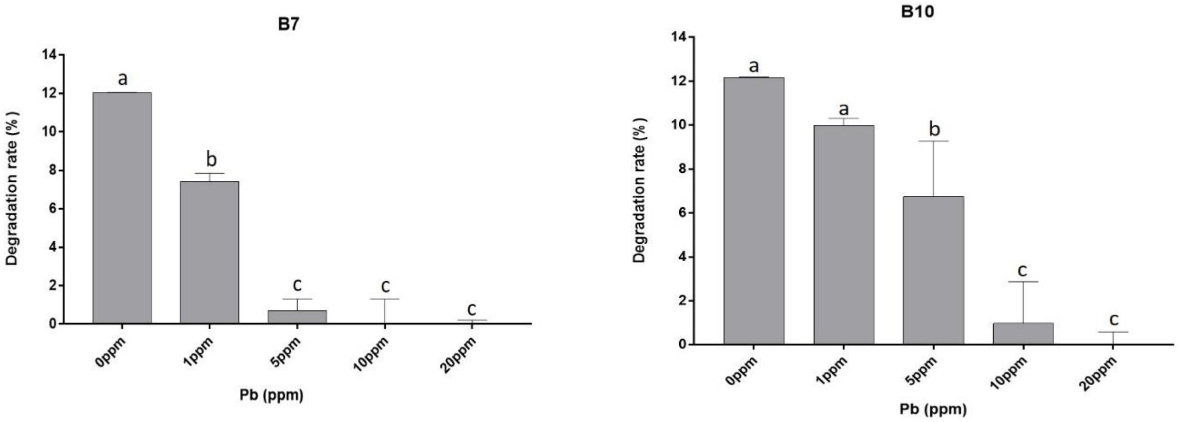

Fig. 1. Petroleum degrading rates of bacterial isolate (a) B-7 and (b) B-10 upon exposure to 0, 1, 5, 10 and 20ppm Pb for 5 days. Data are expressed as mean ± S.D. (n=3). Differences detected between different treatment groups marked with the same English letter are not significantly different from each other ($p > 0.05$).

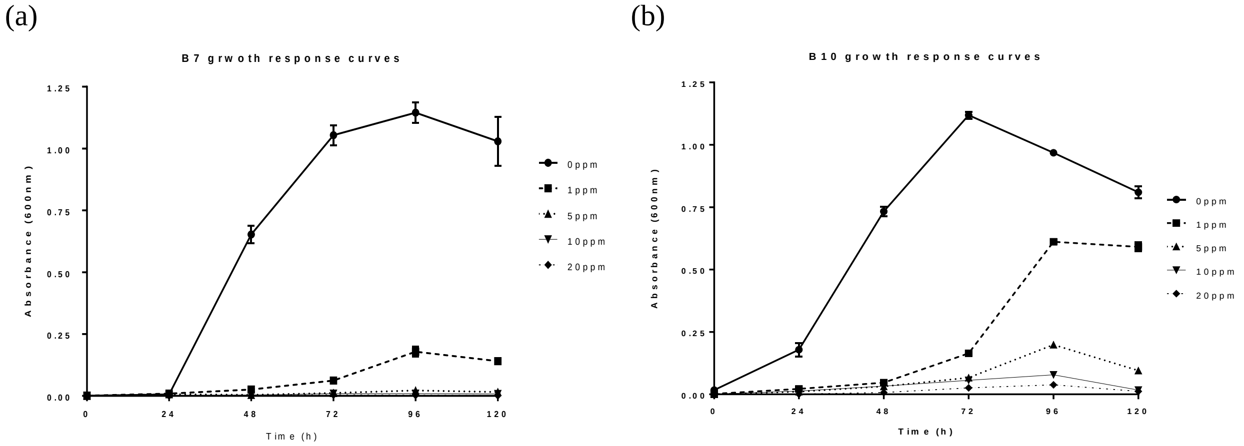

Fig. 2. Growth response curves of (a) B-7 and (b) B-10 upon exposure to 0, 1, 5, 10 and 20ppm Pb for 5 days. (n=3)

Conclusions

Two petroleum degrading bacteria, B-7 and B-10 were screened and identified in this study. The bacterial isolate, B-7, showed high similarity to *Achromobacter denitrificans*, while B-10 showed 99.7% sequence homology to *Mycolicibacterium phocaicum* N4. Both strains exhibited petrol degradation rate of 2.4 g/L during a 5-day study period, with B-10 exhibited higher level of tolerance to lead exposure. Unfortunately, since *M. phocaicum* N4 may be a potential human pathogen, cautions should be taken in future studies when considering B-10 as potential candidate for petrol remediation. Due to the wide range of organic pollutants that *A. denitrificans* can biodegrade and its absence of pathogenicity, B-7 is a more promising candidate for the remediation of soil polluted by organic pollutants. Further studies should be conducted to improve the resistance of lead on B-7, i.e., by continuous cultivation/inoculation of B-7 in the presence of lead ions in MSM with petrol as its sole source of carbon and energy. In addition, future experiments should be carried out in simulated soil environment as the complexity of soil may directly affect heavy metals bioavailability. Investigations on the effects of abiotic factors, such as pH, temperature, moisture contents, nutrient level and co-contamination with other pollutants on the petrol degradation efficiency of B-7 and B-10 should also be conducted. Finally, microbial ecological studies should also be performed in the future to evaluate the effects of microbial consortium on petrol degradation to provide more comprehensive and environmentally realistic information for future *in situ* applications of these bacterial isolates in contaminated sites.

Acknowledgement

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PRODUCTION, CHARACTERISATION AND APPLICATIONS OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) USING ACTIVATED SLUDGE FORTIFIED WITH CRUDE GLYCEROL

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Extracellular polymeric substances (EPS) are eco-friendly and economical biopolymers with significant role in pollutant removal, bio-flocculation, settling and dewatering of activated sludge. In this study, EPS production by an EPS-producing microbial strain (isolated from wastewater sludge) was studied using activated sludge fortified with glycerol. Crude glycerol characteristics changes with the process of biodiesel production (use of type of oil, type of catalyst, operating conditions and biodiesel purification process). Therefore, the study compared crude glycerol samples as carbon sources from different biodiesel companies (*BIO-LIQ, BIOCARDEL, ROTHSAY)* in Canada and their effect on EPS production and characterisation was observed. The maximum slime EPS (S-EPS) concentration (12.34 g/L) was produced when sludge fortified with *BIO-LIQ* crude glycerol was used, higher than pure glycerol (10 g/L) at 72 h. The S-EPS was enhanced (16 g/L) when purified *BIO-LIQ* glycerol (by acid treatment) was used. It was also observed that more S-EPS was produced when sludge fortified with glycerol was used as compared to only glycerol (no sludge). EPS produced from $Ca(OH)_2$-treated sludge fortified with glycerol was found to be better in terms of protein content, flocculation activity and dewaterability. Structural composition analysis using Fourier Transform Infrared Spectroscopy (FT-IR) revealed the presence of distinct functional groups in the produced S-EPS. The role of produced EPS in the removal of COD, heavy metals and other impurities from landfill leachate was also investigated which depicted its potential in various environmental applications.

Keywords: *Exopolysaccharides; Bacterial polymers; Slime EPS; Crude glycerol; Activated sludge; Impurities.*

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Microplastics in the Environment

EFFECT OF MICROPLASTICS ON THE GREENHOUSE GASEOUS AND AMMONIA EMISSIONS DURING ORGANIC WASTE COMPOSTING

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Microplastic as an emerging pollutant have been widely observed and detected in the organic waste such as sewage sludge, municipal organic waste and manures. Composting and anaerobic digestion accepted as effective means to dispose and recycle the organic waste. Recently, many researches indicated that the microplastic could affect the anaerobic digestion such as carbon and nitrogen transformation. However, the influence of microplastic on the composting process have not been studied. The aim of this study was to investigate the effect of different microplastics on the greenhouse gases and ammonia emission during cow manure composting. Different kinds of microplastics (0.5%PE, 0.5%PVC and 0.5%PHA) were mixed with the initial feedstock materials and composted for 60 days, the mixture without microplastic regarded as control. The temperature, pH, greenhouse gases and ammonia emissions were detected during the composting process. The results showed that the different microplastics presented the various effect on the temperature, pH, greenhouse gases and ammonia emissions. The PVC and PHA could apparently prolong the thermophilic phase (7-10 days) as compared to the control (4 days), while the PE have no obvious effect on the temperature variation. Compared to the control, the PVC decreased the 7.42% CH_4 emission, while the PE and PHA improved the CH_4 emissions by 7.79% and 8.15%, respectively. For N_2O emission, the existing of PE could significantly improve 62.67% emission, while the PHA and PVC decreased 12.93% and 0.71% N_2O emissions. Meanwhile, the PHA and PE obviously promoted the NH_3 volatilization (by 20.87% and 33.90% respectively.), but the PVC decrease the 30.44% NH_3 emission. Overall, the current study indicated that the PVC presented in organic waste could mitigate the greenhouse gases (CH_4 and N_2O) and ammonia emissions, while the PE and PHA could enhance the secondary pollution during composting process.

Keywords: *Organic waste, Composting, Microplastics, Greenhouse gases, Ammonia.*

OCCURRENCE OF MICROPLASTICS IN COMMERCIALLY HARVESTED BLOOD COCKLES IN THAILAND

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ABSTRACT. Recent studies have indicated that the bivalves are bioindicators for microplastic pollution, since they are filter feeders, and accumulate microplastics during their feeding process. This study focused on the investigation of microplastics in blood cockles (Tegillarca granosa) from an aquaculture farm and the Khlong Dan market in Samut Prakan, Thailand. The abundance of microplastics was identified by fluorescence microscope with Nile Red tagging. The dominant microplastics are fragments and fibers which are 0.05-0.3 mm in size. The average concentration of microplastic in commercial market samples and aquaculture samples were 10.90 particles/individual and 6.10 particles/individual, respectively. The majority of polymers that were found are Ethylene/propylene copolymer, polypropylene, polyethylene, and low-density polyethylene. The findings showed that microplastics in commercial cockles are much higher than in aquaculture cockles. The accumulation of microplastics in marine organisms can lead to the biomagnification of microplastics along the food chain and impact food safety and human health.

Keywords: *Microplastic, Blood cockle, Aquaculture, Market, Human health.*

Introduction

Plastics have become omni present in our lives, due to their properties of lightweight, durable, and inexpensive. The global plastic production was 360 million tons in 2018, resulting in a huge generation of plastic waste[1]. These plastic wastes can be degraded into small micron-sized particles (<5 mm), called microplastics (MPs)[2]. Since the size of microplastics is very small, they spread easily in the environment. Studies have reported presence of MPs in freshwater, marine environment and also in the air.

Microplastic pollution in the marine environment has become a global concern because of their small sizes and their potential impacts on living organisms. The effects of microplastic on organisms are both of a chemical or physical nature. When ingested, microplastics can occlude the digestive tract of organisms and also act as a vector to transfer organic pollutants and metals into bodies of organisms[3].

Bivalves turn out to be a bioindicator for microplastic pollution as they are filter feeder organisms, which in their feeding process can accumulate the microplastics. When microplastics in seawater encounter the surface of the gills, they can be caught and trapped into mucus and then absorbed with the gill epithelium or carried into the mouth and digestive system[4]. A study from South Korea, investigated microplastics in four popular bivalve species, including mussel (*Mytilus edulis*), oyster (*Crassostrea gigas*), Manila clam (*Tapes philippinarum*), and scallop (*Patinopecten yessoensis*)[5]. The mean concentration of microplastics in these four species was 0.97 ± 0.74 particles/individual. Another study on microplastics in blood cockles (*Tegillarca granosa*) was conducted in 3 different markets of Semarang, Indonesia[6]. They found 9.87 ± 4.76 particles/individual in cockles from Karang Ayu market, 20.93 ± 9.80 particles/individual from Peterongan market, and 23.17 ± 13.02 particles/individual from Bulu market.

Tegillarca granosa is harvested commercially for food in Thailand. As a bivalve, *Tegillarca granosa* are used as bioindicators as they filter water by eating the suspended organic material in water that needs to be treated. On the other hand, contaminants such as microplastics or heavy metals can accumulate in the species. This causes biomagnification along the food chain and make the chance of microplastics consumption by humans subsequently increased.

Thailand is the sixth-largest plastic waste-producing country in the world[7]. Plastic wastes degrade in the environment to form microplastics and are found in air, freshwater, and marine environments. At present, studies on microplastics in Thailand are insufficient to understand the uptake of microplastic by marine organisms. Therefore, this research aims to investigate the microplastics contamination in blood cockles to understand the sizes and types of microplastics that can be uptaken by this filter feeders which may cause potential impact to human health when consumed as seafood.

Material and Methods

Sampling

Samples were collected from commercial market and aquaculture farm. The blood cockles were harvested from a farm in Bang Bo district, Samut Prakan Province, Thailand in the wet season. Blood cockles were also collected from Khlong Dan market, Bang Bo district in Samut Prakan Province, Thailand. One kilogram of blood cockles was collected from each location. Fresh blood cockles were covered in an aluminium foil and then preserved at -18°C in the laboratory refrigerator for further analysis.

Sample analysis

Five bivalve soft tissues were detached from the shells into separate beakers from both market and aquaculture farm. Experiments were conducted in duplicate. The wet soft tissues were weighted and then dried in an incubator at 60°C for 48 hours. To digest the organic matter of the soft tissue, 30% hydrogen peroxide and iron (II) were added to each beaker. The beakers were set on a hot plate for 24 hours. After digestion, all tissues were separated by sieving through the 0.053 mm stainless steel sieve. The light plastics with particles density of less than 1.4 g/cm^3 were isolated using sodium iodide with a density of 1.5 g/cm3. The floating solids were filtered on the cellulose nitrate membrane by vacuum filtration and dried in the incubator. After drying, solid particles were quantified by using Nile Red tagging with fluorescence microscopy (DeltaVision^{TM} Elite Cell Imaging System).

Characterization

Microplastics (MPs) were inspected by micro-Fourier Transform Infrared Spectroscopy (micro-FTIR) (Nicolet iN10 MX). A total of 10 particles of each sample were scanned by micro-FTIR. Spectra were matched with a standard series of polymers and plasticizers databases. A hit index of 70% match was employed to confirm the polymer types.

Results and Discussion

Number of microplastics

Results of microplastics from the aquaculture farm and the wholesale market in Samut Prakarn Province, Thailand are presented in Table 1. As shown in Table 1, the average number of MP particles found in the commercial cockles (10.90 particles/individual) is much higher than the blood cockles from the agriculture farm (6.10 particles/individual). The higher number of MPs particles in the commercial cockles may be due to packaging and transportation activities that used a lot of plastics[8].

Table 1. Average number and concentration of MPs from two sampling area.

Sampling Area	Number of MPs		Weight of organisms (g)		Average concentration	
	Sample-1	Sample-2	Sample-1	Sample-2	particles/10 g	particles/individual
Aquaculture cockles	27	34	27.49	30.02	9.41	6.10
Commercial market cockles	72	37	40.49	20.16	18.07	10.90

The result of this study is lower than some of the previous studies. At Jambi, Indonesia, MP pollution in blood cockles were investigated at 3 different locations in a mangrove forest area of Pangkul Babu[9]. They found 434 ± 97.05 particles/individual. Their study includes MPs size lower than 0.1806 mm thus this could be the reason for the higher number of MPs compared to this study. The most abundant size fraction is 0.1356 - 0.1806 mm, which is similar to our results where predominant sizes are in 0.05-0.3 mm range. Another study by Suprayogi, Ivana (2018)[6], investigated MPs in blood cockles at 3 different markets in Semarang, Indonesia. They found 9.87 ± 4.76 particles/individual in cockles from Karang Ayu market, 20.93 ± 9.80 particles/individual from Peterongan market, and 23.17 ± 13.02 particles/individual from Bulu market. Their result from Karang Ayu market is similar to our commercial cockle result (10.90 particles/ individual). The higher average concentration of the results from both mentioned studies may be due to the high population near the sampling area.

Whereas, the result of this study is slightly higher than the result from the study of MPs concentration in Channel coastlines (France)[10]. In this study, MPs were found in cockles with the concentration ranged from 2.46 ± 1.16 particles/individual. Moreover, their study found that most MPs were 0.1356 to 0.1806 mm which is similar to our results.

Figure 1 (A,B) shows images of fragment and fiber after Nile Red tagging under fluorescence microscopy. The dominant MPs shapes found in this study are fragments and fibers. This implies that the majority types of MPs found in blood cockles is a secondary MPs which are derived from a larger plastic product such as plastic bag or plastic bottle. Various studies also found fragments and fibers as dominant MPs shape in their studies, which correlate to the result of this study[11,12].

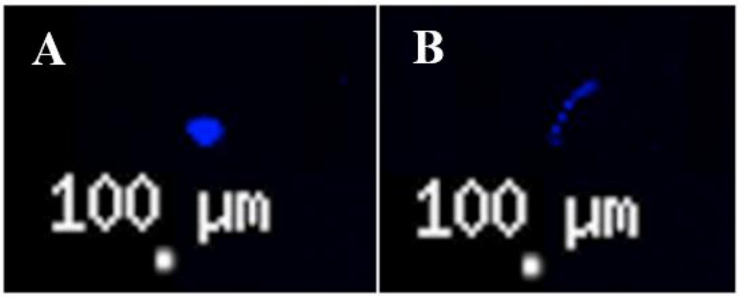

Fig. 1. (A) Images of fragment and (B) fiber after nile red tagging under fluorescence microscope.

Characteristic of microplastics

Micro-FTIR results demonstrate four main polymer types in the cockle samples. Percentages of the polymer types are shown in Figure 2 Ethylene/Propylene copolymer (EPR), polypropylene (PP), polyethylene (PE), and low-density polyethylene (LDPE) were abundant polymer types in all samples. A study in Semarang in Indonesia reported MPs in commercial sea-foods from the area predominantly as PE and EPR[13]. The result of this study is also comparable to characteristics of MPs in market bivalves from South Korea[5]. In this study, PE and PP were also the major polymer types found in the popular bivalve species which are in line with the current study.

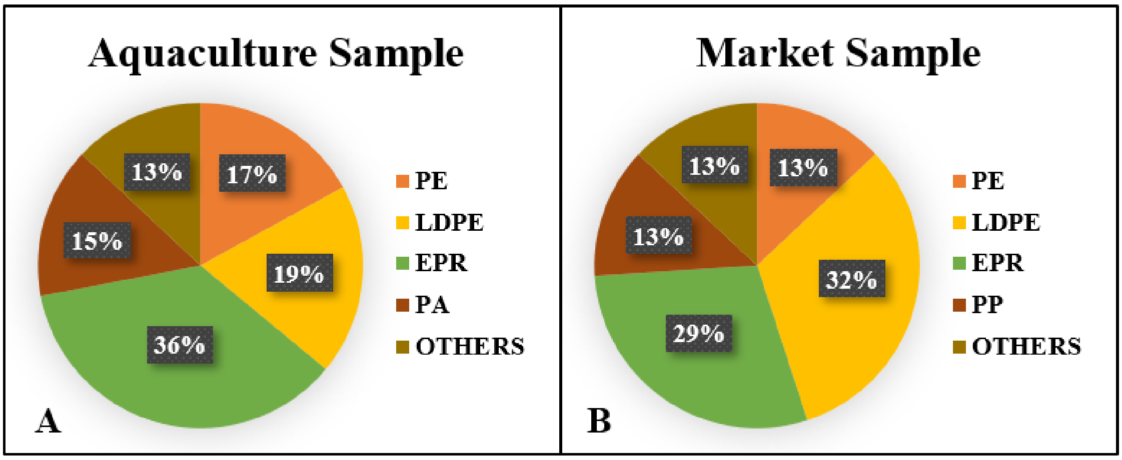

Fig. 2. Percentage of polymer type (A): Aquaculture sample, (B): Market sample.

The potential sources of MPs may come from urban and industrial activities around the study area. Plastics such as EPR, PP, and PE were widely used in the cosmetics industry and in daily life products[14], such as hand cleansers, facial scrubs, toothpastes, plastic bags, disposable plastic cups, etc. Since these polymers have low densities, they can float on the water surface of the ocean and are ingested by marine organisms. PE and LDPE can derive from grocery bags and drinking containers. PP can derive from household products and rigid packaging[15]. EPR can derive from cosmetic goods. Other MPs such as tributyl phosphate, diisodecyl phthalate, diisononyl phthalate and hydrous magnesium silicate were discovered, but in small quantities relative to the EPR, PP, PE, and LDPE.

Conclusions

MPs were found in both commercial and aquaculture blood cockles. The number of MPs in commercial cockles is much higher than in aquaculture cockles. The dominant microplastics are fragments and fibers which are 0.05-0.3 mm in size. The majority types of the polymer found in samples are EPR, PP, PE, and LDPE. These plastics may come from plastic packaging and household products. Currently, the number of studies in Thailand is insufficient to explain the marine organism's behavior on the uptake of MPs. Thus, this study provides background data for further studies about MPs in marine organisms in Thailand.

Acknowledgement

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BIODEGRADABLE PLASTICS - PROS AND CONS BIOPLASTICS

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ABSTRACT. Due to many good (but also bad) characteristics and flexible workability, plastics are an important part of our everyday life. Bioplastics are regarded as a possible alternative to conventional plastics. Currently, bioplastics account for less than 2% of global plastics production. A further increase is expected in the future. However, biodegradable plastics and their waste are controversially discussed. For example, biodegradability is seen as an opportunity in the fight against littering of the environment, but also as a risk for the increased input of plastics into the environment. As a result, there are different views on the ecological benefits of biodegradable plastics and different approaches to dealing with such waste. Against this background, different strategies and disposal concepts for biodegradable plastics will be compared. Bioplastics are biodegradable, bio-based or both. The term biodegradable describes a chemical process in which microorganisms present in the environment convert the material into natural substances such as water, carbon dioxide and compost. According to European Standard EN 13432, in order to be described as "biodegradable", no more than 10% of the test materials used should be found as residue after a good 12 weeks of composting and subsequent sieving through a 2 mm sieve. In this experiment, this means that the mass loss of sample material after 12 weeks must be at least 90%. In Germany, the use of bioplastics is a controversial issue. It does not always make sense to replace conventional plastics. Bioplastics are mainly discussed as an alternative material for single use plastics. Even then, the question remains whether it makes sense to use bioplastics only once and whether they should be disposed of correctly - as a plastic together with all other plastics or as a biodegradable material together with the biowaste? Of course, bioplastics can be used in many different ways. Meaningful and less meaningful applications are presented and evaluated. Own tests on bioplastics on a laboratory scale (0.8 m^3 reactors) and in composting plants are presented and recommendations for practical and sensible use are given. Regional, political and practical differences in different countries will be presented.

Keywords: *Biodegradable plastics, Bioplastics, Composting, Bio-waste.*

Introduction

Bioplastics is an incomprehensible and misleading term for many people. The term bio-based plastics describes these plastics much better. This is not about oxo-degradable plastics that are not compostable and break down into microplastics! However, biodegradable plastics and their waste are controversially discussed. For example, biodegradability is seen as an opportunity in the fight against littering of the environment, but also as a risk for the increased input of plastics into the environment. As a result, there are different views on the ecological benefits of biodegradable plastics and different approaches to dealing with such waste. Against this background, different strategies and disposal concepts for biodegradable plastics will be compared.

Was sind "Biokunststoffe"?

Bio-based plastics are made entirely or partly from renewable biological resources. The properties of bio-based plastics can vary significantly from one material to the next. Biobased or partially biobased non-biodegradable plastics (PE, PET or PVC) have identical properties compared to their conventional versions. These bio-based plastics cannot be distinguished from conventional plastics except by scientific analysis. Bio-based plastics are mostly used in packaging applications. They are also used in fibres in the textile industry.

Biodegradable plastics are plastics that are degraded by microorganisms under certain conditions to water, carbon dioxide (methane) and biomass. Biodegradable plastics can be bio-based (made from renewable raw materials or wastes), made from fossil carbon carriers or mixtures of both.

To support consumers in their decision-making and give them confidence in the degradability of plastics, universal standards have been implemented in the EU, new materials have been developed and a logo of degradability has been introduced. Consumers are concerned with biodegradability, assuming that these plastics are also bio-based.

There are therefore two classes of biodegradable plastics:

- Biobased bioplastics whose components are made from renewable raw materials (or wastes).
- Plastics made from fossil carbon sources (petroleum, coal) in combination with biodegradable additives that enable biodegradation.
-

The main benefit of bioplastics is said to be the positive influence on the environmental impact of a product (reduction of greenhouse gas emissions, conservation of fossil resources, use of local resources, possibly recycling of by-products). Bioplastics are also of socio-economic interest, they are perceived positively by consumers. Consumer demand for sustainable products is satisfied. The company that uses bioplastics gets a positive image.

All further considerations refer to biodegradable plastics; deviations are noted.

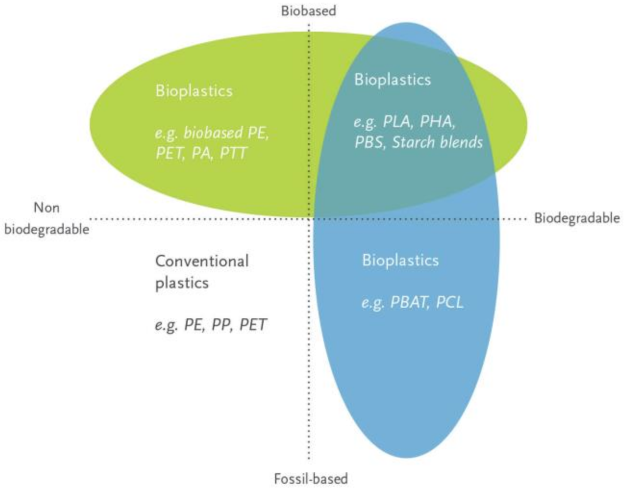

Fig. 1. Material coordinate system of bioplastics[11] (PE: polyethy- lene, PET: polyethylene terephthalate, PA: polyamide, PTT: Polytrimethylene terephthalate, PLA: Polylactic acid, PHA: Polyhydroxyalkanoates, PBS: Polybutylene succinate, PBAT: Polybutylene adipate terephthalate, PCL: Polycaprolactone, PP: Polypropylene).

Current situation of knowledge and recycling of bioplastics

Many European countries consider biodegradable plastics useful for single-use or short-term use. In Germany, there are sometimes other recommendations.

Single-use products are short-lived and generate unnecessary waste, whether biodegradable or not. If biodegradable disposable items are disposed of in the environment (littering), this has similar negative consequences as the input of conventional plastics, as the items hardly degrade in nature and only very slowly. The degradability of degradable plastics is often overestimated[5]. Biobased plastics (biodegradable or not) can only reduce the littering of the aquatic environment to a limited extent. The microplastic problem can also only be reduced to a limited extent. References to biodegradability may even encourage careless and inconsiderate handling of waste. The result could be increased littering of the environment, higher demand for cleaning measures and misdirected waste in bio-waste bins. Reusable is almost always the better choice!

In Germany, there has been a discussion for years about the sense of using biodegradable plastics. Biobased plastics are supposed to contribute to the de-carbonisation of the economy.

Degradable plastics are new to the German biowaste recovery and recycling systems for conventional plastic packaging. Biodegradable waste collection bags for separately collected biowaste are often sorted out in composting plants (and fermentation plants) due to technological reasons. In both systems, the biodegradable plastics are collected as a disturbance fraction, separated and thermally treated. Biodegradable plastics are biodegradable, but need time and appropriate environmental conditions to do so. According to DIN EN 13432, biodegradability means that a material must be more than 90 per cent degraded (particles larger than 2 mm) by microorganisms or fungi after three months under defined temperature, oxygen and humidity conditions.

In some European countries, biodegradable plastics are used to reduce littering problems. If discarded products and packaging are biodegradable, it could be good for the environment.

Biodegradable plastics need to be integrated into the systems of sorting and recycling plastic packaging. For this, sorting facilities would have to be upgraded accordingly and the recycling of new these plastics would have to be organised.

Biodegradable plastic bags encourage citizens to better bio-waste collection, but they can only degrade in composting plants and anaerobic digestion facilities if the appropriate technologies are prepared for them. Rotting times of at least 3 months must be guaranteed. It must be remembered that rotting time costs money. Only composting systems that return the screen overflow to the fresh biowaste in an internal cycle after intensive rotting achieve more than 90 days of rotting time for this screen overflow and a degradation of the biodegradable collection bags.

German composting plants fear that people do not distinguish between biodegradable and traditional collection bags and that the input of plastics (contaminants) into organic waste will increase as a result. The technology-related sorting out and disposal of the degradable bags (together with other impurities) also cause additional costs for the composting plants. Other packaging or single-use products made of degradable plastics (cups, cutlery, plates) do not belong in the bio-waste collection. They also need at least 90 days to degrade. In addition, the greater wall thickness can delay degradation. In home composting, biodegradable plastic products need an even longer time.

In anaerobic digestion plants, collection bags or other packaging made of degradable plastics can hardly contribute to biogas formation due to their low percentage by mass[13], if they are degradable at all in the relatively short treatment time in anaerobic digestion plants. Pre-treatment by changing the pH value can make the bioplastics more fermentable[1]. The discussion about adjusting the conditions for biodegradation hardly considers the needs of plant operators who want to treat biowaste and have to earn money.

In Germany, the limits for impurities in composts and fermentation residues have been tightened. The Biowaste Ordinance[4] limits the proportion of foreign substances. The proportion of impurities in composts and fermentation residues is limited even more by the Fertiliser Ordinance[7]. Since 2017, a distinction has been made between deformable plastics (film components), limit value 0.1 % DM, and all other impurities (hard plastics, glass, metal, etc.), limit value 0.4 % DM. The pollutant content of fermentation residues and composts has been reduced over the last 30 years. There are problems with the content of impurities, especially plastics. Foreign substances in compost endanger its utilisation in agriculture. The Federal Compost Quality Association[3] has further tightened the limits for impurities in composts. The following applies: area sum of the sorted out deformable plastics over 2 mm maximum 10 cm^2/l FS! The impurities in biowaste composts are mainly plastic film residues, also from biodegradable collection bags, which have not yet been degraded.

Good experiences with the use of biodegradable plastics have been made with stand-alone solutions. At events where there is no possibility of packaging brought to the event by the visitors being introduced, the food waste can be composted together with the degradable packaging, bowls and cups. The composting company can then treat this fraction separately. Large sporting events can thus be organised with almost no waste if all cups and bowls are biodegradable.

The German Federal Environment Agency considers bio-based plastics to be rather unsustainable than conventional plastics[16]. The production of conventional fossil-based plastics causes more climate-impacting CO_2. Bio-based plastics have a higher acidification and eutrophication potential. Their raw materials come from agricultural production. There may be competition for land. The carbon footprint of bio-based plastics depends on a large number of framework conditions. Land use, agricultural market and international trade can be influencing factors and affect the climate balance in both directions[9]. If plants that are food crops are used as raw materials, the carbon footprint can even be negative[8].

The biodegradability of some bio- and petroleum-based plastics may help to reduce marine pollution. However, this hardly improves the GHG balance, as biodegradability ultimately releases CO_2. Biodegradable plastic is not always completely bio-based. Biodegradability depends on many factors, e.g. time and various (environmental) conditions (moisture, nutrients, oxygen supply, temperature, material thickness). In soil and water bodies, degradation can therefore be very limited[12, 13, 14]. So limited, in fact, that there is little advantage over non-degradable plastics[15]. Whether biobased or not hardly matters.

The recycling of bioplastics could generate additional emission savings at the end of the plastic's life. For recycling, the material composition is essential. Plastics that are similar in chemical structure to fossil-based plastics can be recycled just like them. For example, PET plastic bottles with a biobased content or other biobased variants of PE, PP and PET. In contrast, plastics with a different chemical structure, including biodegradable plastics, are sorted out in recycling plants. These bioplastic products must be thermally treated. In this case, the waste management benefit of biobased plastics is currently largely reduced to their greenhouse gas neutrality in the incineration processes. In the target fractions of the plastics sorting plants, bioplastics do not yet interfere; their share is still too low[2].

In terms of pollutant release from the products, biobased plastics appear to release similar pollutants as fossil-based plastics[17].

Summary

Plastics could become more ecological through bioplastics if residual materials are used. Bioplastics can then contribute to climate-friendly de-carbonisation. Biodegradable plastics need to be fitted into an existing waste management system or the introduction of biodegradable plastics is directly linked to the establishment of a corresponding system. The problem of littering can also only be solved to a limited extent by biodegradable plastics, as the degradation of these plastics in nature is very slow!

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COMPOSTING AS A SUSTAINABLE TECHNOLOGY FOR CONVERSION OF MUNICIPAL SOLID WASTE TO BIOFERTILIZERS: ROAD BLOCKS AND PERSPECTIVES

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A huge amount of solid waste is produced through domestic, agricultural and industrial activities which results in giving rise to atmospheric diversities and contamination which sooner or later disrupts the whole environmental structure. Municipal solid waste is one of the major concern of researchers globally. In recent years, because of adaption of different lifestyles and speedy development in economy, immense growth in urbanization and population growth took place. Enhancement in waste production results into high quantity of wastes, high amount of pollution and loss of various species. Due to variety of environmental impacts the utilization of bio-based products has received a high amount of attention all around the world. Bioconversion of wastes into bioproducts such as biofertilizers has been into demand. It is essential to raise social consciousness about the advantages of using bioproducts. There are a lot of methods used for synthesizing the biofertilizers from solid waste such as composting, vermicomposting, and anaerobic digestion. Composting is a natural procedure for degradation of raw materials such as yard wastes, food wastes, plants, manure and urban wastes to organic materials. Composting is considered as a primeval method which can be executed at small scale as well as on a large scale. Compost was made from municipal solid waste. In laboratory assessment compost characteristics were analysed. The compost examination was carried out by using existing protocol of Fertilizer Control Order (FCO -1985). The compost contained 1.24% total nitrogen, 1.08% total phosphate, 15.84% total organic carbon, and 0.99% of total potash. Final results of compost characteristics were equivalent to the recommended criteria of Fertilizer Control Order. The obtained results represent that the prepared compost can be used as fertilizer.

Keywords: *Biofertilizer; Bioconversion; Sustainability; FCO -1985; Bio-products.*

International Conference on Sustainable Biowaste Management 2021

Alternative Biowaste Utilization

IMPROVEMENT OF MECHANICAL, THERMAL, AND BARRIER PROPERTIES OF CASSAVA STARCH-BASED CAST FILMS USING NATURAL FIBRES

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ABSTRACT. Cassava (Manihot esculenta) is a tropical starchy root crop mainly used in food applications, but its high starch content and compatibility with other polymeric material make it a useful feedstock for high-value applications such as plastics and composites. To overcome these drawbacks, cassava starch was formulated with a range of additives to prepare neat polymers. Also, natural fibers were included in the polymer matrix to improve the mechanical and thermal properties of the cassava starch-based plastic films. Various combinations of chitosan, polyvinyl alcohol (PVA), gelatine, nanocellulose, and cinnamon essential oil were used as additives in the preparation of cast films. The films formulated with chitosan showed an increase in tensile strength and thermal stability. Cinnamon essential oil and chitosan helped significantly reduction of microbial activity of the films, but the mechanical properties were negatively impacted by the inclusion of essential oil. Cassava starch-based fully biodegradable polymers have a great potential for applications in agriculture and environmental fields.

Keywords: *Cassava starch, Cast films, Natural fibres, Bio-based plastics.*

Introduction

Cassava is a robust productive starchy root crop major source of carbohydrates that is grown chiefly as a food. Since starch is attributed to offering a favorable combination of cost, availability and performance are said to be highly effective and valuable as compared to other polymers [1,2]. A significant component of cassava root is starch which can be around 80% of the total dry weight [2]. The quality of starch in cassava is highly responsible for the determination of the quality of cassava itself. The biopolymers of cassava starch have shown excellent properties in film formation for obtaining flexible and extensible materials of homogeneous and smooth surfaces [3]. However, these films are relatively brittle materials that do not favor mechanical properties and have a high sensitivity to humidity, restricting their use especially in humid environments [4]. To overcome these drawbacks, cassava starch was formulated with a range of additives to prepare neat polymers. Also, natural fibers were included in the polymer matrix to improve the mechanical and thermal properties of the cassava starch-based plastic films. Various combinations of chitosan, polyvinyl alcohol (PVA), gelatine, nanocellulose, and cinnamon essential oil were used as additives in the preparation of cast films. Such composites are completely biodegradable and compostable and therefore considered “environmentally friendly” since, at the end of their useful life, they can be discarded without causing any damage to the environment [5]. The objectives of this research were to evaluate the feasibility of applying natural fibers with cassava starch and improve the properties of the composites. Furthermore, to evaluate properties of cassava starch with various combinations of chitosan, polyvinyl alcohol (PVA), gelatine, nanocellulose, and cinnamon essential oil were used as additives in the preparation of cast films.

Materials and Methods

Extraction of the cassava starch

Cassava roots were peeled, washed, and disintegrated into 1cm cubes. Then the cassava cubes were pulverized in a high-speed blender for 5 mins. The resulted pulp was suspended ten times its volume of water and stirred for 5 mins. Pulp was filtered using a double-fold cotton cloth. The resulted filtrate was allowed to stand for 2 hrs for the starch to settle, and the top liquid was decanted and discarded. Water was added to the sediment, and the mixture was stirred again for 5 mins. Extracted cassava starch was dried at 65°C for 3 hrs.

Determination of the composition of the extracted cassava starch

Moisture content, crude fiber, protein, fat, and ash content of the cassava starch were determined using AOAC 925.40 (2000), AOAC 962.09(2005), AOAC 984.13 (2000), AOAC 948.22 and AOAC 923.03 (2000) standards, respectively. All the analyses were carried out in triplicate.

Cassava bagasse, White peel, and brown peel preparation

Cassava bagasse, white peels, and brown peels were dried at 50° C to constant weight, shredded, ground, and sifted through a 100 sieve, and were used as a filler.

Cassava starch-based thin film preparation

Cassava starch films were prepared using a casting method. Film solution (5 wt% dry basis) was prepared by dissolving cassava (5% dry basis) in hot water (80° C), containing cassava bagasse, white peel, and brown peel suspension of 5% (dry basis) and 1.5% (dry basis) Glycerol as the plasticizer. Same way chitosan/gelatine/cinnamon oil/ PVA (5% dry basis) was prepared. The suspension was mixed directly together with a hot plate magnetic stirrer for 15 min. Thus, the prepared solution was poured onto the glass plate. Water s evaporated from the petri dish in a conventional oven at 50ºC for 12h. Dried films were put in an airtight container and stored at ambient temperature.

Cellulose and nanocellulose preparation from bagasse/ white peel/ brown peel

The alkali treatment was performed to purify the cellulose by removing lignin and hemicellulose from cassava bagasse fibers. The ground cassava bagasse (passed through100 mesh) was treated with an alkali solution (4 wt% NaOH). The mixture was transferred into a round bottom flask and treatment performed at 80oC for 1 h. The solid was then filtered and washed several times using distilled water. This treatment was performed twice, before and after the bleaching process. Following alkali treatment, the bleaching process was completed by adding a buffer solution of acetic acid, aqueous Sodium chlorite (4 wt%), and distilled water at 70ºC for 1 h. The mixture was allowed to cool and was filtered using excess distilled water. The bleaching process is repeated two times. The acid hydrolysis treatment was conducted on the fibers after alkali treatment and bleaching at a temperature of 60ºC using 40% of sulphuric acid for 1 h under continuous stirring. The hydrolyzed material is washed by centrifugation at 10,000 rpm for 10 min followed by sonication. This centrifugation step was repeated several times by adding distilled water until the constant pH of pulp in the range of 5–6 was reached.

Film Characteristics

The thickness of the films (manual Thickness Gauge -Te Clock Dial, SM - 528); Mechanical Test (ASTM D 882 – 02. The tensile strength test was performed using Instron 3365 Universal Testing machine -Instron Ltd, Buckinghamshire); Water absorption of the thin films (ASTM D570-98); Biodegradability test (aerobic compost environment test) and FTIR spectrums (FTIR spectra of samples were obtained in transmittance mode using a Perkin Elmer Spectrum) were measured.

Results/Observations/Outputs

Composition of cassava starch and fibers

Cassava starch with fibers thin films

The thickness of the films was measured at tenth different places using manual Thickness Gauge. The thickness of the films ranged from 150 to 200 μm for cassava starch-based films. The band at 3500-3200cm^{-1} O-H groups of free hydroxyl groups and bonded O-H in polymeric compounds such as alcohol, phenols, and carboxylic acids. The band at 3000-2850cm^{-1} symmetric or asymmetric stretching of CH, CH_2 of aliphatic acids. The bands at 1750-1680cm^{-1} stretching vibration of C=O bond of carboxyl groups. The bands at 1375-1000cm^{-1} C-O stretching of COOH and the bands at 1515cm^{-1} C=C lignin aromatic vibration. The band at 1100–1152 cm−1 is for C-O, C-C, and C-O-H stretching. The band at 1100–900 cm^{-1} corresponding to the C-O-H bending vibrations. The tensile strength values of the cassava starch with white peel films 8.21MPa have the highest value followed by bagasse and brown peel films. Considering the elongation at break values of the cassava starch with bagasse films have the highest value 38% followed by white peel and brown peel films. Starch thin film tensile strength 5.05MPa and tensile strain at break 44%. The highest amount of water uptake was found after 24h from cassava starch with white peel (15.22±0.071) followed by Brown peel and bagasse. All the films showed significant degradation at 15 days. The highest biodegradability showed cassava starch with Brown peel followed by white peel, bagasse, and starch neat films.

Table 1. Composition of the extracted cassava starch and fibers.

Components	Cassava Starch	Bagasse	White peel	Brown peel
Dry Matter (%)	88.08 ± 0.043	91.89 ± 0.447	93.25 ± 0.204	90.41 ± 0.217
Ash (%)	0.16 ± 0.008	0.68 ± 0.007	3.64 ± 0.020	9.79 ± 0.049
Crude Protein (%)	0.65 ± 0.012	0.83 ± 0.016	5.85 ± 0.040	4.56 ± 0.065
Crude fiber (%)	0	20 ± 0.294	13 ± 0.205	50 ± 0.849
Fat (%)	0.199 ± 0.007	0.237± 0.001	0.079 ± 0.330	0.382 ± 0.012

Data are means of three replicates
Cassava white peel % = 12; Cassava brown peel %= 3; Whole peel (%)= 15

Cassava starch with chitosan, PVA and gelatin thin films

The gelatin, cassava starch and chitosan spectra were (1333 cm^{-1}). The bands at 3384–3422 cm^{-1} OH and/or NH stretching. The band at 2930–2939 cm^{-1} C–H stretching. The band at 1630–1660 and 1540–1570 cm^{-1} C=O stretching (amide I) and NH bending (amide II). The band at 1740 cm^{-1} carbonyl group in the starch and chitosan films This result indicated that interactions were present between the hydroxyl groups of cassava starch and the amino groups of gelatin and chitosan. Cassava/chitosan films presented the lower elastic modulus, i.e., lower rigidity, and a higher percentage of elongation. Tensile strength and 3.2MPa±0.1 and 146% ±6 tensile strain at break. The tensile strength of cassava/PVA film was 48.2MPa ±0.65 and 8.12% ±0.25 tensile strain at break. Cinnamon essential oil and chitosan helped significant reduction of microbial activity of the films, but the mechanical properties were negatively impacted by the inclusion of essential oil (Tensile strength 1.36 MPa ±0.14 and 264% ±20.15 tensile strain at break).

Conclusions

Films containing fibers show higher tensile strength. Water absorption capacity and biodegradability were improved with the cassava starch with fibers. The films formulated with chitosan showed an increase in tensile strength cinnamon essential oil and chitosan reduced microbial activity of the films, however, mechanical properties were negatively affected. Mechanical properties of cast films could be improved with the use of chitosan, PVA, gelatine, and nanocellulose with cassava starch combinations. Glass transition temperature and the melting point could be improved with the addition of natural fibers. Cassava starch-based fully biodegradable polymers have a great potential for applications in agriculture and environmental fields.

Acknowledgment

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APPLICATION OF COFFEE HULL FIBER IN THERMOPLASTIC COMPOSITES

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At present, more and more attention has been paid to the use of wood fiber from crop straw to replace the scarce wood fiber to strengthen biological composites. As a solid waste, coffee shell can be used to reduce environmental pollution. In this paper the potential application of coffee hull (CH) of the reinforced polyethylene (PE) matrix composites was studied for the first time. The mechanical properties, hygroscopicity, thermogravimetric analysis, fiber treatment and microstructure of CH reinforced composites were studied. The results show that incorporation of coffee hull markedly improved the mechanical properties of the reinforced high density polyethylene (HDPE) matrix composites. Micrographs show a strong interfacial adhesion between the CH fiber particles. This property may be the main reason for the stability between composites. At the same time, the effects of different treatment methods on the mechanical properties and water absorption of the composites were studied. Calcium hydroxide ($Ca(OH)_2$), silane coupling agent (SCA), maleic anhydride grafted polypropylene (ma-g-pp), stearic acid (SA), ethylene bisstearamide (EBS) and their mixtures (ma-g-pp, SA, EBS) were used to treat the fiber surface. The results show that ($Ca(OH)_2$) treatment is the best way to improve its properties. It may be due to the removal of surface active functional groups (-OH) and the induction of hydrophobicity of CH fiber, which improves the compatibility with polymer matrix. Therefore, the application of coffee shell in composite materials has important industrial significance.

Keywords: *Coffee hull Fiber, HDPE, Thermoplastic Composite, Mechanical Properties, Fiber Treatment.*

ENVIRONMENTAL ASSESSMENT OF THE TRANSFORMATION OF FOOD WASTE TO ANIMAL FEED VIA A SOLAR DRYING UNIT IN GREECE

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ABSTRACT. The goal of the manuscript is to present the life cycle environmental impact assessment of the infrastructure required to transform the separated food wastes into animal feed utilizing an altered solar drying process. The scope of the study includes the infrastructure of the pilot drying unit. The analysis indicates that the construction of the floor that the pilot plant will be based on, is the dominant factor in the overall impact of the infrastructure. Moreover, the results presented indicate that the major environmental impacts of the solar drying unit are generated by the operation of the solar drying unit, due to the usage of electricity.

Keywords: *Life cycle assessment, Food waste, Solar drying, Animal feed, Hospitality.*

Introduction

In the EU, it is estimated that 90 million tonnes of food waste is produced every year, equivalent to 180 kg per person [1]. In some EU countries, especially those in the south, the majority of food waste ends up in landfill. In Greece, for example, more than 95% of food waste ended up in landfill in 2013, either directly or indirectly.

Food waste from the hospitality sector is a key waste stream which causes policy implications in connection with the EU Landfill Directive (1999/31/EC). In order to tackle the aforementioned issue, an EU based partnership has been formed in order to implement the Life+ F4F (Food for Feed) project [2]. The main aim of the project is to evaluate, through a pilot-scale demonstration, an innovative and simple technology, and a low-emission process that enables the safe transformation of food waste, mainly from hotels (and more generally from the hospitality industry and restaurants), into animal feed. This reuse process, in line with the circular economy concept, will transform a waste management process into a feed producing one. Since it utilizes solar power (directly and indirectly), it is also a low energy and low carbon emission process. The project aims to influence EU legislation on waste, the Circular Economy Package and the Roadmap to a Resource Efficient Europe.

Fig. 1. Outline of the solar drying pilot plant process.

The first step of the process will be the collection from selected hotels of separated food waste. The collected food waste will be transferred to the pre-fabricated unit, where hand sorting and pre-treatment takes place, where non-food materials will be removed via hand sorting (see Fig. 1). Then, the preselected food waste will be shredded and then pulverized and transferred forward via a pump to one of two drying channels of the solar drying greenhouse. The dried product will be the raw material for the production of animal feed.
The solar drying unit is essentially a greenhouse, covered by polycarbonate, windows are covered with insects' net. Roof based fans are used to extract moisture from the sun drying hall, connected with the meteorological station and the roof windows, in order to reduce the internal humidity of the drying unit. The main aim is to accelerate the material drying rate. It consists of two drying halls, covered by stainless steel. The floor of each drying hall (15m long and 5m wide, with 0.80m high reinforced concrete side walls, also covered by stainless steel), is covered with an extensive network of pipelines connected with solar panels and a heat pump in order hot water to accelerate the drying rate. On the top of the pipelines, a high-quality stainless still is covering the drying hall surface, where the pulverized food waste is in contact with. Each

drying tank has a different type of drying turner (a horizontal and a vertical turner are being used). The turners are a prototype system custom-made for the process. They have several motors and sensors for a variety of moves: a) moving in the drying hall corridor using wheels rolling on the sidewalls, in various speeds and both directions (front and backwards), b) increasing and decreasing the height of the turner's drum, c) turning the drum both directions and in various and control speeds, e) estimating its position from the ends of the corridor at all times, and f) including a series of safety operation mechanisms (e.g., emergency stop).

Material and Methods

Life Cycle Assessment (LCA) is a reliable and widely used method to address the environmental aspects and potential environmental impacts throughout a product's lifecycle. The principles, procedures and methods of LCA are presented based on the terminology and structure of the ISO 14040:2006 and 14044:2006. According to those guidelines, LCA is composed of four phases (ISO 14040:2006):

- Goal definition and scope phase
- Inventory analysis phase
- Impact Assessment phase
- Interpretation phase

The goal of the study

The goal of the present study is the environmental impact assessment via means of LCA of the construction and operation of the solar drying unit designed, constructed and operating in the framework of the LIFE+ F4F project.

The scope of the study

The scope of the present study includes all infrastructure of the pilot solar drying unit. More specifically, it includes the landscaping of the surrounding space, the necessary excavations for the construction of the pilot unit, the construction of the flooring and the underground wastewater collection tank, the construction of the pre-sorting unit (within the pre-sorting unit, hand sorting of the collected food waste from the hotels is taking place).

Infrastructure inventory

The infrastructure of the pilot unit includes the metallic solar drying greenhouse, with metallic frame and polycarbonate cover, with its doors and roof windows. Moreover, it includes the metallic structure, the electromechanical equipment of the pre-sorting unit (a PVC curtain, a conveyor belt for waste hand sorting, a chipper/crusher shredder and a pulveriser, an INOX bowl with an Archimedes screw and a feeding pump, a submerged wastewater pump and the electrical equipment control panel).

It also includes the polycarbonic sheets for the covering of the greenhouse, the underfloor heating system of the greenhouse, the feeding system pipeline, two inverter units for the cooling of the pre-sorting unit, the insect-proof net of the greenhouse, the hydraulic and electrical infrastructure of the solar drying unit.

The key components were extracted from the master plan of the pilot plant. These are:

- materials (e.g. reinforced concrete and asphalt) and operations (e.g. excavation) for landscaping and floor construction;
- metallic structures (presorting unit and solar drying greenhouse);
- water supply and drainage infrastructure (e.g. excavation and pipes);
- and electrical infrastructure (e.g. cables).

Results and Discussion

The results presented are those of Life Cycle Impact Assessment, the third phase of the LCA. The purpose of LCIA is to provide additional information to help assess a product system's impact and to better understand their environmental significance. This phase includes accounting, evaluating, and explanation the potential environmental impacts generated by the product through categorization and characterization of the flows. The CML2 baseline 2000 method was selected for the assessment of the impacts.

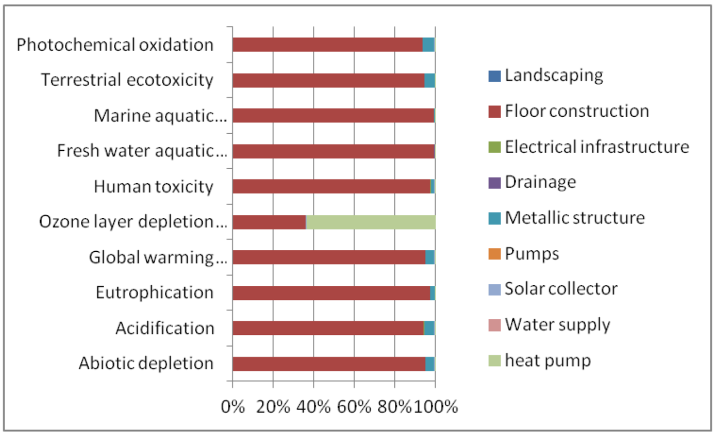

Fig. 2. % contribution to the impact categories of different materials used in the infrastructure of the solar drying unit.

The relative impact assessment characterisation results per impact are presented in Fig. 2. The construction of the pilot unit floor is the process that dominates the environmental impacts, followed by the metallic structure of the plant, which is also present in key impact categories (abiotic depletion, global warming, photochemical oxidation, acidification and eutrophication).

So far, the results of the impact assessment of the infrastructure have been presented. In the next lines the contribution of the operation of the solar drying unit will be examined. The % contribution of the infrastructure and operation components are also presented in Fig. 3. The results presented in Fig. 3 indicate that the operation (red color) contributes the most to all impact categories, except freshwater aquatic ecotoxicity and eutrophication.

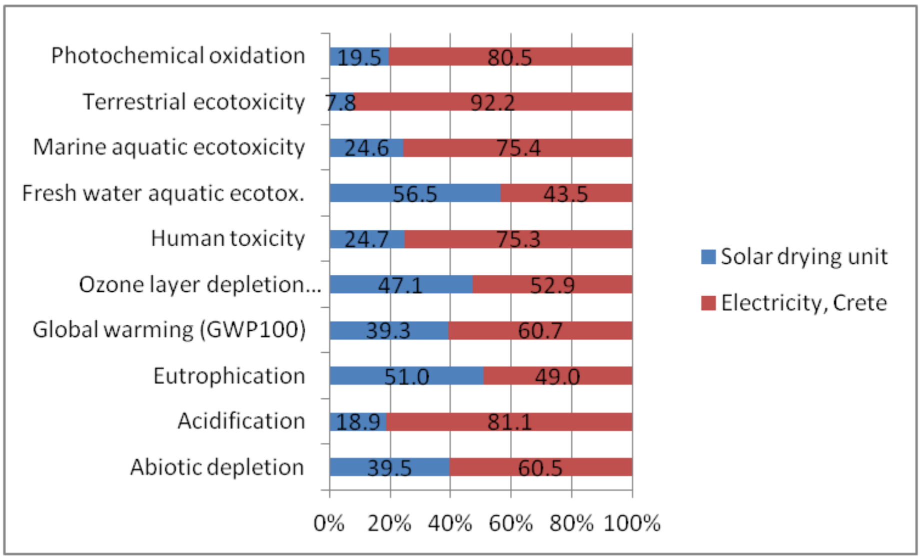

Fig. 3. % contribution to the impact categories of the infrastructure and operation of the solar drying unit.

Conclusions

A solar-driven pilot plant for the transformation to animal feed of food waste collected from hotels was constructed and is operating. An analysis has been performed for the environmental impact assessment of the required infrastructure. The analysis indicates that the construction of the floor that the pilot plant will be based on, is the dominant factor in the overall impact of the infrastructure. Moreover, the results presented indicate that the major environmental impacts of the solar drying unit are generated by the operation of the solar drying unit, due to the usage of electricity.

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MATERIAL UTILIZATION OF BIOMASS AND THE DEVELOPMENT OF STRAW/PLASTIC RATTAN COMPOSITES (SPRC)

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Innovation in bioresources, which includes biomass resources, genetic resources and also bioinformation resources, is currently one of the most active areas in China. Bioresource science has undergone tremendous growth in recent years as the search for environmentally safe and renewable raw materials to replace petroleum for the production of energy, chemicals and other products continues. Bioresource science and engineering has become a true multidisciplinary field combining traditional areas of chemistry, biology, chemical and biochemical engineering, agricultural sciences and other fields with new developments in biotechnology, genetics and bioengineering. New innovations in both materials and their applications have been a driving force as the engineering of bioresources strives to serve industry through use of cheap, sustainable, safe and renewable raw materials. Herein, some examples of the application and industrialization of bioresources are presented. The development of straw biomass into novel materials for industrial use is of huge, and in fact, limitless, potential. For example, over 700 million tons of straw can be collected each year from main crops in China.Industrial utilization is the most reasonable way to solve the straw problem as an agricultural by-product. New materials and new products made from straw have led to the formation of new industries and huge markets, and form a new industrial model, the basis of which is ‘not making waste translate to other waste’. Straw biomass from agricultural sources contains a considerable amount of natural plant fiber, and it has high strength and modulus. As a type of natural polymer reinforcing material, straw fiber has a number of advantages, including better characters, being cheaper, easily acquired, widely sourced, green or environmentally benign, and renewable.

A novel imitation rattan was firstly developed, and it was found that this new rattan has the characteristics of durability, mold-proof and moth-proof, anti-aging and green environmental protection. Straw-polyethylene rattan (SPER) with modified wheat straw powder was fabricated and tested for its mechanical properties. The composite with 5% modified fiber content with tensile strength (15.72MPa) was about the same as that of pure polyethylene (15.25MPa), while the elongation at break value(612.80%) was better than that of pure plastic rattan (573.53%). The physical appearance of the composite rattan resembles natural rattan, with better apparent quality and texture. Thus provides powerful evidence for the potential industrial use of SPER. As a promising mean of biomass management, a wide range of biomass, including food processing by-products, wooden packing items, and even coffee residue could be utilized for making a wade range of new materials.

Keywords: *Bioresource Innovation, Straw biomass, New material, Imitation rattan.*

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Advance Bioconversion

EXPLORATION ON THE BEST PREPARATION SCHEME OF ACTIVATED CARBON FROM SOLID WASTE IN SUGAR REFINERY

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ABSTRACT. In this paper, the potential of using solid waste from sugar refinery to prepare activated carbon was investigated, and the preparation conditions were optimized. Two activators were compared, and the preparation conditions were preliminarily selected according to the results of TG analysis. The experimental data shows that the AC prepared by $ZnCl_2$ are superior to use KOH samples in carbon yield and methylene blue adsorption capacity. Therefore, the suitable conditions of prepare BAC active by $ZnCl_2$ were further discussion. The response surface methodology (RSM) was used to design the experiment and fit the mathematical model according to the experimental data. The variance analysis was performed to investigate the reliability of the fitting equation. Finally, the optimal conditions for the preparation of BAC by $ZnCl_2$ were determined as follows: impregnation ratio of 1:2, carbonization temperature of 500℃, AC yield of the product 53.698%, methylene blue adsorption value of 335.408mg/g.

Keywords: Activated carbon; Preparation conditions; Optimize; RSM.

Introduction

Activated carbon is an excellent functional material with strong adsorption capacity, it has good mechanical and chemical stabilities, and regeneration. The traditional carbon material is made of coal, oil and so on, the raw material through a series of processing to get a non-metallic material. Due to the deterioration of the environment and the lack of coal resources, some clean materials used to replace the traditional materials for the preparation of activated carbon. And the cheap, high carbon content, renewable biomass materials (such as coconut husk, rice husk, corn cob, bagasse) can meet the requirements. Bagasse [1] is a kind of biomass raw material and a by-product of sugar industry. There is no significant difference in the main composition of bagasse among different varieties. The mass fraction of cellulose is about 43.8%, the mass fraction of hemicellulose is about 28.6%, the mass fraction of lignin is about 23.5%, and the carbon content is 44.17% [2-3].

In this paper, the preparation process of activated carbon from bagasse, the method of optimizing experimental design [4-5] and the effect of activated carbon on the adsorption of methylene blue under different conditions were described.

Material and Methods

Raw materials and reagents

Sugarcane waste residue (BR) is provided by Guangxi COFCO Jiangzhong Sugar Industry Co. Ltd. Methylene blue (AR), $ZnCl_2$ (AR) and potassium hydroxide (AR) were provided by Tianjin Fengchuan Chemical Reagent Co.Ltd. Nitrogen and air are provided by Tianjin Liufang Gas Chemical Reagent Co. Ltd. Deionized water was prepared in the laboratory.

Preparation of activated carbon

Activated carbon from sugarcane residue was prepared by chemical activation using $ZnCl_2$ and KOH as activators. The impregnation ratio was the mass ratio of sugarcane waste residue to chemical activators. In each experiment, 2.5g of sugarcane residue and activator were fully mixed by stirring with impregnation ratios of 1:0.5, 1:1, 1:1.5, 1:2 and 1:2.5 as experimental points. After impregnation for a period of time, in the presence of nitrogen, the temperature range was set from 400°C to 700°C, and the carbonization time was kept for 60min.The sample was cleaned with 1.0 mol·L-1 hydrochloric acid solution for 30min until the filtrate was neutral. Activated carbon from bagasse was obtained after filtration and drying.

XACs characterization

The pore structure of activated carbon was characterized by thermogravimetric analysis, scanning electron microscopy (SEM), nitrogen adsorption and desorption, etc. The yield of carbon and the adsorption amount of methylene blue were taken as the basis for the evaluation of the quality of activated carbon

Results and Discussion

Thermogravimetric analysis

According to thermogravimetric analysis, the weight loss below 400 ℃ is mainly due to water evaporation and the pyrolysis of cellulose, hemicellulose and lignin. As can be seen from the TG curve, the quality decreases very slowly with the increase of pyrolysis temperature above 400 ° C. Therefore, in the following experiments, 400 ° C is set as the lowest carbonization temperature. The influence of preparation conditions on performance of bagasse activated carbon. Firstly, single-factor experiments were conducted to explore the influence trends of carbonization temperature and impregnation ratio on the performance of bagasse activated carbon (Fig. 1). As can be seen from the figure, when the carbonization temperature is 500℃, the adsorption capacity of methylene blue and the carbon yield are higher than other temperatures. When the impregnation ratio is 1:2, the adsorption capacity of methylene blue has reached 335.408 mg/g, and the yield of carbon is 53.698%

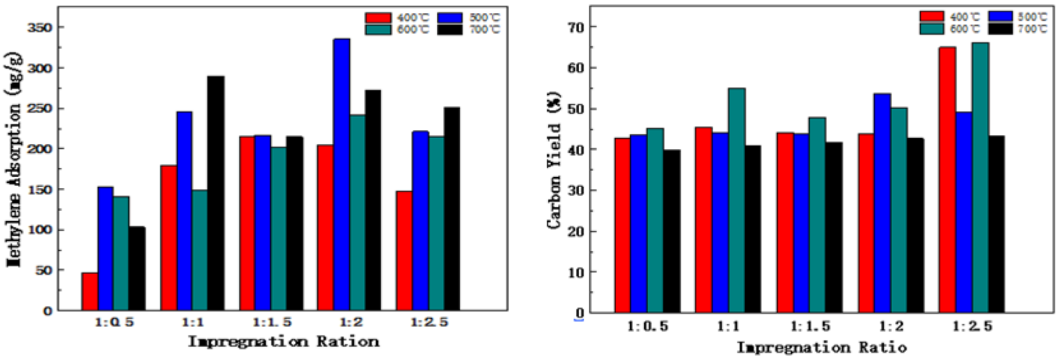

Fig. 1. Influence of carbonization temperature on AC yield and adsorption capacity of methylene blue.

Characterization of activated carbon

In order to further verify the rationality and reliability of the results, nitrogen adsorption and desorption were used to characterize the activated carbon prepared under the above conditions, and the surface area, pore volume, pore radius and BJH cumulative specific surface area data were obtained (Table 1). When the carbonization temperature is 500℃, the specific surface area of different impregnation ratios are as follows:1:2>1:1>1:2.5>1:0.5. When the impregnation ratio is 1:2, the effect of different carbonization temperature on the surface area is 500℃>600℃>700℃.

Table 1. BJH adsorption-desorption capacity and Single point hole radius.

Impregnation Ratio	Activation Temperature(°C)	Specific surface area（m2·g-1）			Pore volume(cc/g)		
		S_{BET}(㎡/g)	S_{μ}(㎡/g)	Sm (㎡/g)	V_T (cc/g)	V_{μ}(cc/g)	Vm (cc/g)
1：0.5	500	17.438	−3.013	20.451	0.093	0.006	0.087
1：1	500	729.802	249.112	480.690	0.478	0.327	0.149
1：1.5	500	175.310	147.267	28.043	0.131	0.082	0.049
1：2	500	1169.097	822.210	346.887	0.724	0.527	0.197
1：2.5	500	457.754	375.114	82.640	0.273	0.216	0.057
1：2	600	622.796	572.974	49.822	0.362	0.312	0.049
1：2	700	25.281	12.876	12.405	0.045	0.012	0.033

SEM images

SEM (Fig. 2) diagram of bagasse activated carbon obtained at impregnation ratios of 1:1,1:2,1:2.5, and carbonization temperatures of 400℃, 500℃ and 600℃.The surface morphology of activated carbon can be seen clearly. There is no obvious structural collapse area on the surface of bagasse. When the impregnation ratio is 1:2 and the carbonization temperature is 500℃, the surface morphology of activated carbon prepared is irregular connected pore structure, with a large number of micropores, mesopore and a few macropores.

This kind of pore structure distribution is conducive to the adsorption of different particle sizes of activated carbon, which provides a larger specific surface area for activated carbon.

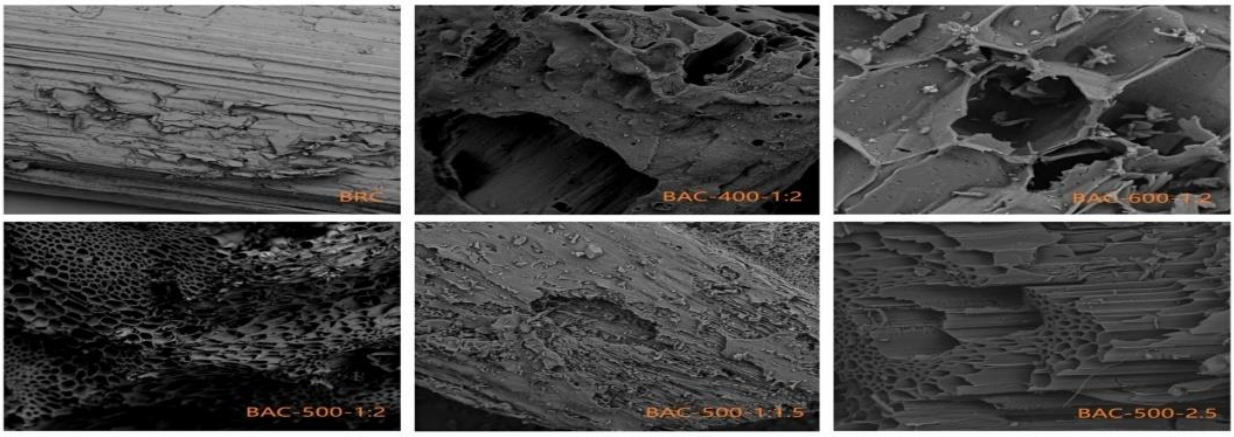

Fig. 2. SEM images.

Mechanism Analysis
According to the analysis of activation mechanism, the activated carbon prepared under the condition of carbonization temperature of 500℃ and impregnation ratio of 1:0.5, because the content of $ZnCl_2$ is too little, the contact between $ZnCl_2$ and raw material is not sufficient. When the impregnation ratio was 1:2 and the carbonization temperature was 700℃, the activator accelerated the activation of bagasse intramolecular fibre layer due to the high temperature, resulting in the collapse of bagasse cellulose channel and the collapse of micropores into mesopore and macropore, and the pore volume decreased rapidly. When the impregnation ratio was 1:2 and the carbonization temperature was 500℃, the specific surface area of activated carbon reached the maximum of 1169.097 m^2/g, the cumulative specific surface area of BJH adsorption was 346.887 m^2/g, the cumulative specific surface area of BJH desorption was 407.571 m^2/g, and the average pore diameter of single point was 12.4A. According to the analysis of the pore structure, it can be inferred that the adsorption capacity of methylene blue is the largest at this time, which is consistent with the experimental data.

Response surface test design and results

Experimental design and data analysis
With impregnation ratio and carbonization temperature as variables, the model regression fitting of linear model and quadratic polynomial model for the carbon yield of activated carbon and the adsorption value of methylene blue was carried out by using Design-Expert 8.0 software, and the quadratic polynomial fitting equation was obtained:

$$\text{Yield}(Y1) = 335.41 + 44.19A - 6.87B + 20.43AB - 87.68A^2 - 47.78B^2$$

$$\text{Adsorption capacity}(Y2) = 53.7 - 0.34A + 4.89B - 0.67AB - 1.39A^2 - 2.10B^2$$

In equation 4-1: Y1 is the carbon yield; Y2 is the adsorption capacity of methylene blue; A is carbonization temperature ℃; B is the impregnation ratio. The absolute value of each coefficient in the formula indicates the influence degree of each factor on the yield of activated carbon and the adsorption value of methylene blue. The positive and negative of the coefficient indicates the influence direction of each factor on the iodine value.

For the design of the experimental scheme and the comparison between the experimental value and the equation fitting value (Table 2). Design-Expert 8.0 was used to optimize the carbon yield and the adsorption capacity of methylene blue. When the impregnation ratio was 1:2 and the carbonization temperature was 500℃, the expected parameters had the maximum value. Under these conditions, the verification test was repeated for 5 times, and the predicted adsorption value of methylene blue was 335.41 mg/g and the predicted carbon yield was 53.70%, as shown in Table 5. The actual adsorption value of methylene blue was 335.4081mg/g, and the predicted carbon yield was 53.70%. The predicted value is basically consistent with the actual value, which indicates that the optimal process conditions obtained by response surface optimization are reliable and the model is reliable

Table 2. Central Composite DesignC Experimental design and results.

Run Order	Argument		Response Value Y1/%		Response Value Y2/mg·g-1	
	Impregnation Ratio	Activation Temperature	Actual Value	Predicted Value	Actual Value	Predicted Value
1	1:2.5	500	50.65	50.04	183.91	162.45
2	1:2	700	42.66	45.97	271.52	213.45
3	1:2	500	53.70	53.70	335.41	335.41
4	1:2	500	53.70	53.70	335.41	335.41
5	1:2	500	48.10	47.56	59.07	105.11
6	1:1.5	600	47.88	45.72	201.29	234.79
7	1:2	600	50.04	46.70	204.23	256.38
8	1:2	500	53.70	53.70	335.41	335.41
9	1:1.5	400	44.05	44.62	215.62	175.50
10	1:2	500	53.70	53.70	335.41	335.41
11	1:3	500	46.81	49.03	277.51	258.30
12	1:2	400	43.96	44.51	245.10	252.27
13	1:2	500	53.70	53.70	335.41	335.41

Response surface analysis

As shown in Fig. 3, it can be seen from Fig. (a) and (b) that under the interaction of impregnation ratio and carbonization temperature, the influence of impregnation ratio on carbon yield and methylene blue adsorption capacity. With the increase of carbonization temperature, the saturated adsorption amount of methylene blue on activated carbon increased first and then decreased. When the carbonization temperature was higher than 550℃, the saturated adsorption amount of activated carbon decreased with the extension of carbonization time, which was related to the pore enlargement caused by the violent reaction at high temperature. With the increase of impregnation ratio, the saturated carbon yield also increased first and then decreased. The contour line of the saturated carbon yield was elliptical, which indicated that the interaction between carbonization temperature and carbonization time is significant.

Parameter optimization

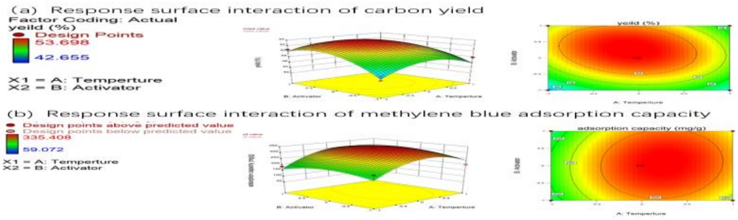

Fig. 3. Response surface interaction of carbon yield and adsorption capacity of methylene blue capacity.

Conclusions

Activated carbon was prepared from bagasse which used $ZnCl_2$ and potassium hydroxide as activators. The effects of impregnation ratio and carbonization temperature on the adsorption performance of activated carbon were investigated by response surface test based on the adsorption capacity of methylene blue and carbon yield. The optimal preparation conditions were as follow: $ZnCl_2$ as activator, impregnation ratio of 1:2, carbonization temperature of 500℃. Under these conditions, the SEM was used to characterize the activated carbon. The activated carbon was characterized by a large number of micropores and mesopores with pores of different sizes and strong adsorption capacity. The specific surface area of the sample was $12.44m^2/g$ (BET multi-point method), the adsorption capacity of methylene blue was 335.408 mg/g, and the carbon yield was 53.698 mg/g.

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SELECTIVE OXIDATION OF 5-HYDROXYMETHYL-2-FURFURAL USING VANADIUM DOPED CARBON NITRIDE

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ABSTRACT. 5-Hydroxymethyl-2-furfural (HMF) is a versatile platform chemical derived from cellulose. It is one of the most coveted chemical building blocks due to the presence of alcohol and aldehyde functional groups on the furan ring. Doped graphitic carbon nitride (g-C_3N_4) is a promising catalyst for the oxidation of HMF. In this study, the efficacy of vanadium doped C_3N_4 for the conversion of HMF to 5-formylfurancarboxylic acid (FFCA) and 2,5-diformylfuran (DFF) was explored using photocatalysis and thermal catalysis. HMF conversion of 54% and 86% was observed with photocatalysis and thermal catalysis, respectively. With photocatalysis DFF was the major product with 37% yield and 71% selectivity whereas, HMF resulted in the formation of two products, DFF and FFCA with thermal catalysis. At the end of 4 h reaction 30% DFF with 37% selectivity and 36% FFCA yield with 31% selectivity was obtained by thermal oxidation of HMF.

Keywords: *Photocatalysis; carbon nitride; diformylfuran; hydroxymethylfuran*

Introduction

Lignocellulosic biomass has emerged as a promising renewable feedstock for the production of fuels and valuable chemicals. Heterogeneous photocatalysis is an environmentally benign technique for valorisation of organic compounds. Although photocatalysis has been widely used for degradation of an array of environmental pollutants in the past, the selective transformation of organic compounds to value added products is yet to be entirely explored. Achieving high conversion of the reactant along with targeted selectivity requires careful design and development of photocatalyst. 5-Hydroxymethyl-2-furfural (HMF) is a versatile platform chemical derived from biomass. Isomerization of glucose results in production of fructose which forms HMF upon dehydration [1]. The presence of alcohol and aldehyde functional groups on the furan ring makes it a highly sought-after chemical. This structure can act as a precursor for several high-value chemicals for the polymer industry such as 2,5-furandicarboxaldehyde (FDC), 5-formylfurancarboxylic acid (FFCA), 2,5-diformylfuran (DFF), and 2,5-furandicarboxylic acid (FDCA) [1, 2]. These partially oxidized products can be used as a monomer to synthesize heterocyclic ligands, polymers, adhesives, binders, antifungal agents, and resins [1]. Currently, the manufacturing of these products is deleterious to the environment as it requires the use of toxic chemicals and results in generation of massive amounts of hazardous waste. Selective oxidation of functionalities while keeping the furan ring intact poses a major challenge. Catalytic oxidation of HMF has been conducted by many researchers but the desirable conversion and selectivity is achieved only under high temperature of reaction, use of organic solvent, alkaline environment and expensive noble metals as catalyst.

Graphitic carbon nitride (g-C_3N_4) has emerged as a promising catalyst for the conversion of HMF to beneficial products owing to its valence band potential which restricts the formation of hydroxyl radicals due to water oxidation [3, 4]. The presence of hydroxyl radicals in the reaction system is undesirable as it catalyses unselective reactions. C_3N_4 has some limitations such as, rapid recombination rate of photoinduced electron–hole pairs, poor quantum efficiency, and few adsorption and active sites which limits its photocatalytic efficiency [4]. Several efforts have been made to manipulate its bandgap and light absorption ability for effective charge transfer of electrons and holes such as doping with precious and non-precious metals, semiconductors and defect engineering [3].

C_3N_4 was prepared by a facile calcination method. Vanadium was incorporated by calcination of C_3N_4 and ammonium monovanadate. The physical and chemical characterization confirmed the successful incorporation of vanadium into C_3N_4 structure. The photocatalyst improved the light absorption property and increased the charge separation and transfer, resulting in enhanced photo-oxidation efficiency. Photocatalytic studies demonstrated that optimized V-doped C_3N_4 showed high HMF conversion with desirable DFF yield and selectivity. This was also compared with thermal catalysis and the catalyst was found to active in the production of DFF and FFCA in the same reaction time.

Material and Methods

Catalyst preparation

Initially C_3N_4 was synthesized using melamine as precursor and heated at 550 °C in a crucible for 2 hours at the rate of 2.3 °C/min in static air. The resulting yellow agglomerates were milled into powders, washed thoroughly with dilute hydrochloric acid and deionized water to remove the unreacted melamine. The resultant C_3N_4 (1 g) was added to 150 mL distilled water with 200 g of ammonium monovandate and thoroughly mixed on a stirrer at 100 °C. The mixture was completely dried at 60 °C for 6 h prior to calcination at 140 °C for 2 h at a ramp of 2.3 °C/min. The resultant product was designated as vanadium doped C_3N_4 (V-CN). The catalyst was used as such for photocatalysis of HMF. V-CN was further calcined in a tube furnace in the presence of nitrogen at 700 °C for 2 h at a rate of 2.3 °C/min for thermal catalysis. The calcined catalyst was designated as V-CN-700.

Photocatalysis of HMF

Photocatalytic oxidation was conducted in a glass photoreactor with an aqueous solution of HMF (initial concentration = 5mM) using 0.05 g of V-CN. The photoreactor was equipped with a lamp holder, condenser for temperature control and thermal sensors. The total reaction volume was 100 mL. The reaction was initially conducted in dark for 1 h to establish adsorption-desorption equilibrium. Subsequently, the reaction was carried out under 300 W UV/vis lamp for a period of 4 h. Hydrogen peroxide (2 mL) was added to the reactor every two h to facilitate the reaction. Aliquots (1 mL) were sampled every hour to assess the HMF conversion and potential oxidation products. The samples were analysed using HPLC (Waters 2695 HPLC with DAD & FLD). Dark controls were conducted under the same reaction conditions. All the reactions were conducted at room temperature.

Thermal catalysis of HMF

Thermal catalysis was conducted in a 3-neck round bottom flask with a reaction volume of 5 mL and catalyst loading of 0.05 g. The initial HMF concentration was 100 mM. The reaction was conducted in dimethyl sulfoxide (DMSO) at 140 °C in the presence of oxygen. The total reaction time was 4 h. Similar to photocatalysis, the samples were analysed every hour. Samples drawn from the reaction mixture were centrifuged to remove the catalyst and diluted in water and filtered before analysis in HPLC. The reaction was conducted in duplicates.

Results and Discussion

Characterization of vanadium doped carbon nitride

V-CN was successfully synthesized and SEM micrographs reveal slab-like structures (Fig 1a). The slabs of V-CN appear to be tightly stacked together with well-defined edges. These micron -sized particles appeared to have a rough surface and were observed to be unevenly distributed throughout the sample. V-CN-700 displayed smaller particle size with aggregation (Fig. 1b). In contrast to V-CN, these particles displayed random shapes. Amorphous nature of the catalyst V-CN-700 was revealed through XRD spectra with diffraction peaks at 2θ values of 37.6°, 43.7°, 63.5°, 76.3° and 81.2° corresponding to the typical stoichiometric face-centered cell as depicted in Fig. 1c. Similar XRD pattern is reported by Wu et al. [6]. The low angle peak at 14.1° and the maximum intensity peak characteristic of for C_3N_4 at 27.6° was completely masked in the XRD spectra indicating doping of vanadium and change of crystal structure in the catalyst. The absence of low angle peak maybe attributed to the decrease in the planar size of C_3N_4 layers [5]. The miller indices for the various peaks for V-CN-700 have been indicated in the Fig. 1c. These correspond well with vanadium doped CN reported in literature.

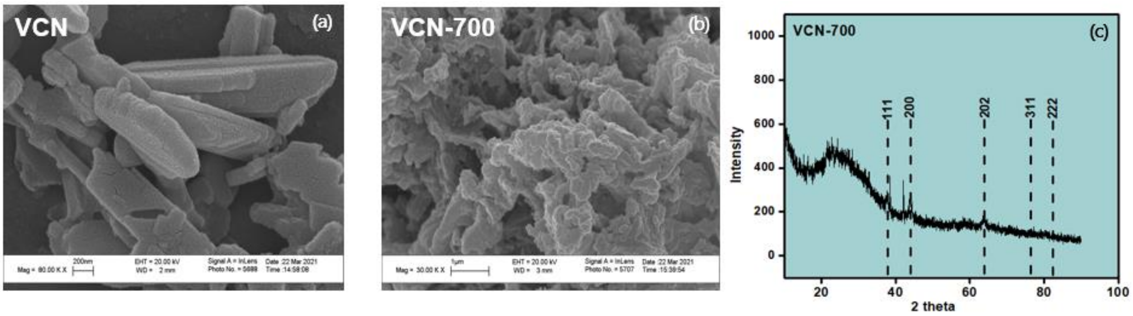

Fig.1. SEM micrographs of (a) V-CN, (b) V-CN-700 and XRD spectra of V-CN-700

Selective oxidation of HMF

Vanadium doped C_3N_4 was observed to successfully convert HMF to value added oxidation products under both the processes, photocatalysis and thermal catalysis. Photocatalytic oxidation of HMF showed that with only 4 h reaction time 54% HMF was converted. The main product of HMF photooxidation using V-CN was DFF with 37% yield and 71% selectivity as depicted in Fig. 2. This reaction was observed to proceed through selective oxidation of the hydroxyl functionality in HMF. The selective oxidation of HMF can be attributed to the generation of superoxide radicals in the solution [4]. In the absence of light or catalyst, no DFF was obtained, suggesting that both are necessary for this photocatalytic oxidation reaction. It was also observed that DFF production was influenced by the incident light as the yield and selectivity for DFF was low when the experiment was conducted under UV irradiation. Similar findings were reported by Wu et al. [3]. These results suggest that doping vanadium into C_3N_4 enhances the photocatalytic properties of the latter by reducing the recombination rates of photoexcited electrons thereby assisting in selective oxidation of HMF. Moreover, photocatalysis offers a green conversion technology for HMF conversion.

Thermal oxidation of HMF resulted in the formation of two products, DFF and FFCA. At the end of 4 h reaction 30% DFF with 37% selectivity and 36% FFCA yield with 31% selectivity was obtained with 86% HMF conversion as depicted in Fig. 2. It has previously been reported that pristine C_3N_4 has inferior HMF conversion activity [7]. Reported studies with vanadium doped C_3N_4 have shown high activity with DFF as the main product whereas, synthesized V-CN-700 shows HMF conversion with two valuable product formation within a short reaction time. Prolongation of the reaction time may lead to increase in yields and selectivity.

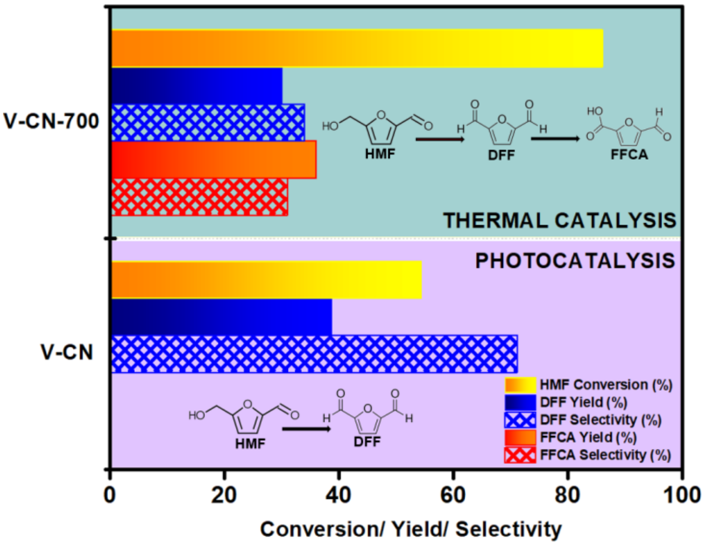

Fig.2. Comparison of thermal catalysis and photocatalysis for HMF conversion

Conclusions

As synthesized vanadium doped C_3N_4 catalyst that can be used for conversion of HMF to valuable products with both photocatalysis and thermal catalysis. Both processes displayed comparable DFF yield at the end of 4 h reaction time. Photocatalytic oxidation of HMF in water can provide an environmentally benign method to produce DFF. Furthermore, this study indicates that photocatalytic synthesis of organics can be explored as it prevents the use of strong chemical oxidants, toxic solvents, high temperatures, and pressures.

Acknowledgement

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SELECTIVE OXIDATION OF 5-HYDROXYMETHYLFURFURAL GENERATED FROM FRUCTOSE DEHYDRATION OVER MOLYBDENUM CARBIDE CATALYST

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ABSTRACT. Developing high-efficiency and low-cost photocatalysts by avoiding expensive noble metals, yet remarkably improving direct synthesis of 2,5-diformylfuran (DFF) from fructose yield, is a great challenge. The unique molybdenum carbide nanodots were prepared through a facile method and applied as a catalyst for the direct synthesis of 2,5-diformylfuran (DFF) from fructose. The molybdenum carbide structure and surface group are the key factor for the catalytic performance in fructose to DFF transformation. Herein, the well synthesized molybdenum carbide nanodots catalyst was calcine again in different atmosphere (H_2, CO_2, O_2), it is still kept good activity in both the dehydration of fructose to 5-hydroxymethylfurfural (HMF) and the aerobic oxidation of HMF to DFF. Under the reaction conditions, DFF yield of 70% could be achieved. In addition, the molybdenum carbide catalyst can be easily separated from the reaction solution and reused without significant loss in activity. The excellent catalytic performance of the molybdenum carbide was attributed to the abundant active sites and rich oxygen-containing groups on the surface of the catalysts. Thus, this research provided an efficient method for the transformation of fructose to DFF in tandem reactions of dehydration and oxidation.

Keywords: *Fructose, 5-Hydroxymethylfurfural, 2,5-Diformylfuran, molybdenum carbide, biomass*

Introduction

An important approach to meet the current needs of energy and chemicals is biorefinery, which may replace human's dependence on fossil fuel resources. **[1]** So, it is of great significance to develop efficient catalysts to transfer the biomass which abound in nature to industrial chemicals. Among the variety of chemicals, 5-hydroxymethylfurfural (HMF) is considered an important intermediate due to its rich chemistry and potential availability from carbohydrates such as fructose, glucose, sucrose, cellulose and inulin. The selective oxidation of HMF can produce several kinds of furan chemicals, such as DFF, 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), 5-formyl-2furancarboxylic acid (FFCA) and 2,5-furandicarboxylic acid. **[3]** Therefore, various conventional oxidants such as NaOCl, $BaMnO_4$, pyridinium chlorochromate, oxalyl chloride, and 2, 2, 6, 6-tetramethylpiperidine-1-oxide have been reported for oxidation of HMF to DFF, FFCA or HMFCA. **[4, 5]**. To date, the noble metal or their compound material such as Au, Pt/C and Pd/C are used in the selective oxidation of HMF to DFF as an efficient reaction process **[6-8]**. However, the scarcity and high cost of these noble metals lead to their limitation in practical applications. Therefore, it is significantly important to explore alternative low-cost elements rich in nature as the catalysts, which is still a great challenge.

Transition-metal carbides such as molybdenum carbide have been proposed as functional materials. For instance, always used as electrode or photocatalytic materials due to their excellent electrical conductivity and mechanical and chemical stability. **[9, 10]** However, no reports can be found in literature about the activity of molybdenum carbide for selective oxidation of HMF to DFF. Considering that molybdenum carbide has showed tunable nanostructures, abundant surface functional groups and outstanding density of surface reactive sites may be a candidate for oxidation of HMF to DFF.

Inspired by these studies, we reported a new approach to convert fructose to DFF in a one-pot and one-step reaction process using molybdenum carbide nanodot as a catalyst. A series of molybdenum carbide catalysts at different reaction conditions were prepared and tested for the fructose to-DFF transformation. The catalyst showed excellent catalytic performance, and DFF yield as high as 80% was achieved under the optimized reaction conditions with molecular oxygen as the oxidant. Also, the as-prepared samples were characterized by X-ray diffraction, transmission electron microscopy (TEM) and EDX-mapping.

Material and Methods

Materials

$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (99.95%, Aladdin), melamine (98%, Sigma), Fructose (98%, Sigma), 5-hydroxymethylfurfural (99%, Sigma) and other materials were obtained from commercial suppliers and used without further purification.

Catalyst preparation

Molybdenum carbides synthesized via simple mixing melamine and ammonia molybdate, followed by a high-temperature treatment. Further treatment by calcination in different gas atmosphere is applied to change the chemical and physical properties of the molybdenum carbides

Catalyst characterizations

Powder X-ray diffraction (XRD) was performed on a Bruker Advance 8 X-ray diffractometer (Cu Kα radiation λ = 0.154 nm). Raman spectra were collected on a Renishaw 1000 Raman spectrometer with a 514 nm HeNe laser, of which the detection depth is ~150 nm. transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HR-TEM) images of the samples were obtained by the field emission scanning electron microscopy (FE-SEM, JSM-7001F, Japan). X-ray photoelectron spectroscopy (XPS) spectra were recorded on an AXIS HIS 165 spectrometer (Kratos Analytical) with a monochromatized Al Kα X-ray source.

Catalytic reaction

One-pot and one-step synthesis of DFF from fructose. In a typical run, the direct conversion of fructose to DFF was as follows: fructose (200 mg), catalyst (20 mg), and DMSO (5 mL) were added into a three-necked, round-bottomed flask equipped with a magnetic stirrer and condenser. The reaction was heated in an oil bath, and oxygen was bubbled through the reaction mixture at a flow rate of 20 mL/min. After the reaction, the catalyst was separated from the reaction mixture by filtration and products were diluted with DI water prior to analysis by HPLC. The reactor diagram for synthesis of DFF from fructose is showed in Fig. 1.

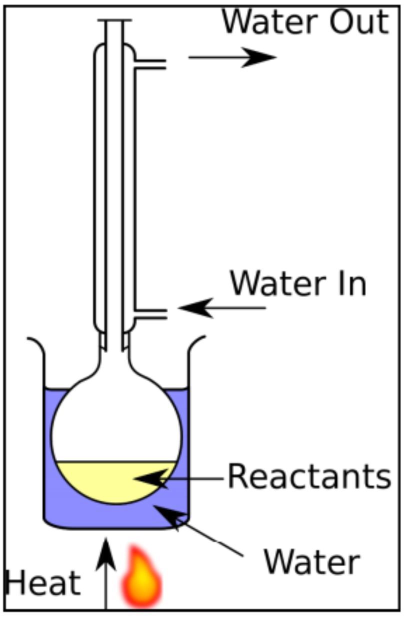


Fig. 1. Reactor for one-pot and one-step synthesis of DFF from fructose

Results and Discussion

XRD and Raman analysis

The crystalline phase compositions of molybdenum carbide with different Mo mass ratios were performed by XRD. As shown in Fig. 2a, four main peaks at 38.03°, 39.52°, 61.43°, and 74.80°can be observed, respectively, corresponding to the (2 0 0), (1 0 2), (0 4 0) and (2 2 3) diffraction peaks of (JCPDS PDF #15-0457), which demonstrates the successful fabrication of pure molybdenum carbide. In addition, the peak pattern did not change with different Mo mass ratios. Fig. 2b shows the Raman spectra of molybdenum carbide. Two distinct Raman peaks at around1340 and 1576 cm^{-1} are corresponding to the D-band and G-band of the carbon-based matrix, respectively.

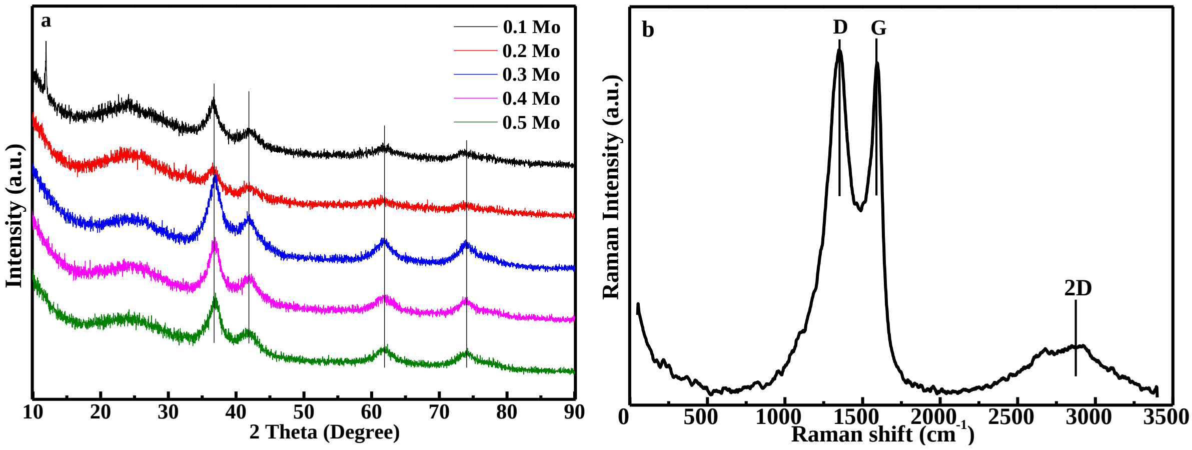
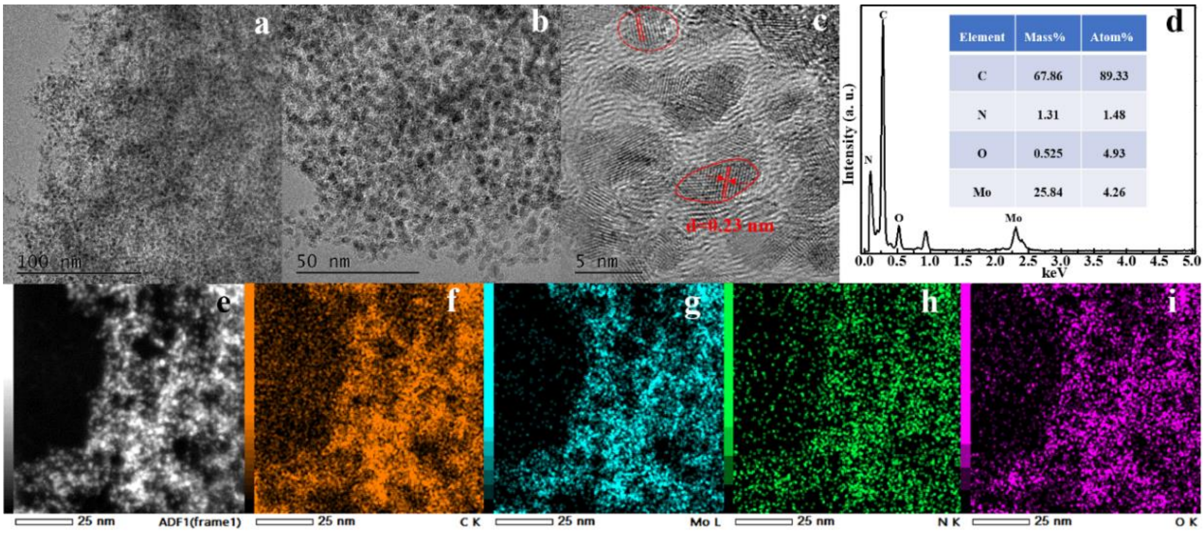


Fig. 2. a) XRD patterns of molybdenum carbide with different Mo mass ratios. b) Raman spectra of molybdenum carbide

TEM, HR-TEM, and EDX-mapping analysis

The morphology of as-prepared samples was investigated by TEM. The TEM images show the nanodots morphology of samples (Fig. 3a), with 6-8 nm diameter (Fig. 3b) can be seen. In Fig. 3c, an HR-TEM image reveals that the lattice spacings of 0.23 correspond to the (101) plane of molybdenum carbide (JCPDS 35-0787). The nanodots and dispersion will provide rapid electron transport, thereby increasing the HMF oxidation reaction. Fig. 3d is the energy-dispersive X-ray (EDX) spectroscopy and element content analyses, the Mo, C, O and N elements are detected, but the content and atom atomic ratio of O and N is quite low, it means that the molybdenum carbide is catalyst. The (EDX) mapping was performed to further elucidate the structure of molybdenum carbide and revealed a homogeneous distribution of Mo, C, N, and O in the hybrid.



Element	Mass%	Atom%
C	67.86	89.33
N	1.31	1.48
O	0.525	4.93
Mo	25.84	4.26

Fig. 3 a, b) TEM image of molybdenum carbide. c) HRTEM image with the inset showing the (101) plane of b-Mo2C. d) EDX spectroscopy and element content. e-i) Corresponding EDS mapping revealing the different elements.

Catalytic activity of fructose conversion to DFF

The molybdenum carbide was treated under different atmosphere conditions (O_2, CO_2, H_2, and air), the performances of the of DFF from fructose are further investigated. The molybdenum carbide still showed excellent activity in the oxidation of HMF generated from fructose to DFF, which showed in Fig. 4.

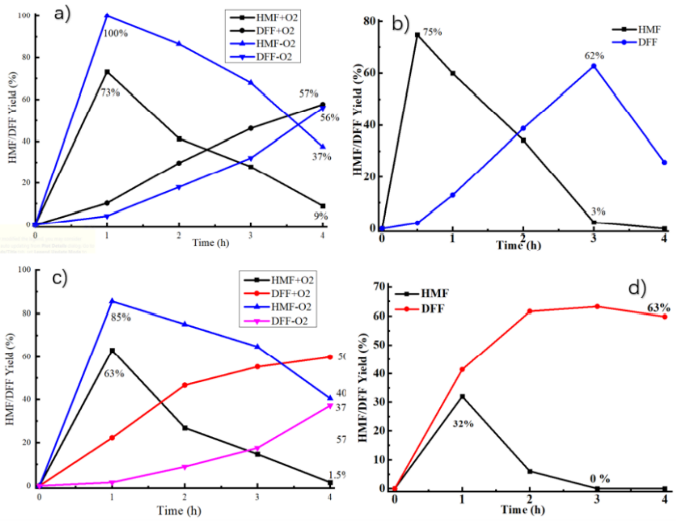


Fig. 4 Oxidation of HMF generated from fructose by molybdenum carbide treated by a) CO_2, b) H_2, c) O_2, d) Air

Conclusions

In summary, we have developed molybdenum carbides as a catalyst for one-step synthesis of DFF from fructose. Experimental results indicate that a DFF selectivity of more than 70% can be achieved by converting fructose to DFF under atmospheric conditions. The excellent catalytic performance of the molybdenum carbide was attributed to the abundant active sites and rich oxygen-containing groups on the surface of the catalysts.

Acknowledgement

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CATALYTIC FAST PYROLYSIS OF RICE HUSK FOR THE HIGH QUALITY LIQUID FUELS PRODUCTION

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ABSTRACT. A lab-scale catalytic pyrolysis unit with the feeding rate of 100-500 g h^{-1} was designed and manufactured with a fluidized bed reactor. The effects of reaction temperature, catalyst on the properties of pyrolysis products were investigated. Subsequently, the effects of catalyst weight and position on the properties of biochar was studied. The mechanism of biomass pyrolysis was investigated. The highest bio-oil yield was 46.9 wt% with bio-char and non-condensable gas yields of 30.0 wt% and 23.1 wt%, respectively, at 500°C in non-catalytic pyrolysis process. In the process of biomass fast pyrolysis, the cellulose was pyrolyzed to monomers. Then some chemicals were produced from these monomers through a series of reactions: isomerization, dehydration, esterification and so on.

Keywords: Rice husk; fluidized bed reactor; catalytic fixed bed reactor; ZSM-5; pyrolysis mechanism

Introduction

Bio-oil derived from biomass fast pyrolysis can be used as a candidate to replace the fossil fuels or feedstock to produce some renewable chemicals [1]. The applications of this technology is handicapped, due to the low quality of bio-oil, such as high water content and viscosity, low pH value and heating value, and thermal instability [2]. Catalytic fast pyrolysis is a promising and attractive approach for the production of renewable aromatic compounds with highly deoxygenated molecules to improve the bio-oil quality and stability in a single-step process [3]. ZSM-5, a solid acid catalyst with the key feature of shape selectivity, is the most investigated and effective catalyst for a higher yield of aromatics [4].

Catalytic fast pyrolysis of biomass has been investigated by several researchers with zeolites. Carlson et al. [5] studied catalytic fast pyrolysis of solid biomass derived compounds for green gasoline production and found that the active site and pore structure of the ZSM-5 catalyst can affect the product selectivity. In the research of Cheng et al., [6] ZSM-5 modified by Ga was used as the catalyst to produce aromatic compounds with the feedstock of biomass, where the reactions of decarbonylation, olefin aromatization, oligomerization, cracking, diels-alder condensation, alkylation and dehydration were found in the process of catalytic fast pyrolysis. Paasikallio et al. [7] studied the catalytic fast pyrolysis of pine sawdust based on a circulating fluidized-bed reactor with HZSM-5 catalyst and found that 24% of the feedstock carbon was obtained in the bio-oil, which contained a high content of lignin derived water-insoluble chemicals. Zhang et al. [8] studied the catalytic fast pyrolysis of biomass with the assistance of microwave for bio-oil production and found that the external acid sites of HZSM-5 catalyst decreased with the increase of the SiO_2 deposited amount.

To date, though many impressive researches focus on the catalytic fast pyrolysis of biomass, the studies on the effects of operation condition and catalyst on the products (like biochar) characteristics were not sufficient and there is a clear need to investigate the mechanism in catalytic fast pyrolysis. In this study, a lab-scale catalytic pyrolysis unit with the feeding rate of 100-500 g h^{-1} was designed and manufactured with a fluidized bed reactor and a catalytic fixed bed reactor. The effects of reaction temperature, catalyst weight and the catalyst location on the pyrolysis products were investigated. Subsequently, the mechanism of biomass pyrolysis was studied through Py-GC/MS analysis. In addition, speculative pyrolysis pathway of cellulose was discussed by the results of GC/MS and Py-GC/MS analysis. This work could provide a valid reference and technical support for biomass fast pyrolysis and bio-oil potential utilization.

Material and Methods

Material

Rice husk was used as the raw material in this work with the particle size of 80 mesh (<0.18 mm) and bulk density of 340 kg m^{-3}. Proximate analysis was performed according to ASTM E 1756-08, ASTM E 1755-01 and ASTM E872-82 for moisture content, ash content and volatile matter, respectively. Fixed carbon content was calculated by difference.   Before the experiments, rice husk was put into an oven to reduce the water content at 105°C for 12 h. The ZSM-5 zeolite catalyst was brought from Nankai University Catalyst Co., Ltd. Si/Al: 38; type: NKF-5D-38-1-C; density: 0.6-0.75 g cm^{-3}.

Lab-scale catalytic pyrolysis system

The schematic diagram of the lab-scale catalytic fast pyrolysis system is shown in Fig. 1. The pyrolysis system can be divided into three parts: the feeding system, the reactor system and the condensation system. The feeding system mainly consists of hopper, feeding screw I, feeding screw II and cooling water jacket. Biomass is transported into the system by a two-stage screw feeder and carrier gas (N_2). Cooling water jacket around the feeding screw II prevents the tube blocking caused by biomass pyrolysis in the feeding system. The reactor system mainly consists of gas preheater, gas buffer, reactor I (fluidized bed reactor), cyclone, char pot and reactor II (catalytic fixed bed reactor). The fluidized bed reactor was made of 306 stainless-steel pipe, and its internal diameter and height were 40 and 550 mm, respectively. The temperature of fast pyrolysis system is monitored by dozens of thermocouples and regulated by a PID controller. The pyrolysis vapor from biomass pyrolysis passes through three stage condensation, which is made of quartz glass. The condensation system mainly consists of condenser I (water cooling), electrostatic generators, condenser II (electrostatic precipitator), condenser III (dry ice and acetone condenser), cotton wool filter and gas meter. Bio-oil I was collected in bio-oil pot I which is from condenser I and II. Bio-oil II is collected in bio-oil pot II which is from condenser III. So two bio-oil samples are obtained in each experimental condition.

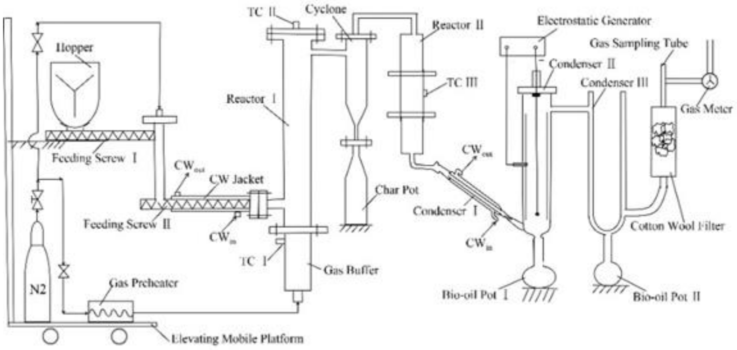

Fig. 1 Schematic diagram of the lab-scale catalytic fast pyrolysis system

Results and Discussion

Effects of the pyrolysis conditions on the bio-oil properties

In the fluidized bed reactor, 150 g of sand with the particle size of 0.28-0.45 mm was used as bed material. When the catalyst was put into the fluidized bed, the weight of the sand decreased in accordance with the catalyst weight in order that the total weight of fluidized bed materials (sand and catalyst) is 150 g. In Run 1-4, the pyrolysis temperature was varied from 400°C to 550°C to investigate the effect of temperature on the physicochemical properties of products. In Run 3, Run 5 and Run 6, the effect of the catalyst weight in fluidized bed was studied, while the pyrolysis temperature was fixed at 500°C. In Run 5 and Run 7, the effect of catalyst location was investigated, while the pyrolysis temperature and catalyst weight were fixed at 500°C and 2 g.

Table 1 shows the mass balance analysis in each pyrolysis condition. According to Table 1, the bio-oil yield was increased with the temperature changes from 400°C to 500°C in non-catalytic pyrolysis researches; then the yield of bio-oil was decreased with the increased temperature. The highest bio-oil yield was 46.9 wt%

with bio-char and non-condensable gas yields of 30.0 wt% and 23.1 wt%, respectively, at 500°C in non-catalytic pyrolysis process. The catalyst ZSM-5 negatively affects the production of bio-oil. When the catalyst was transferred from the fluidized bed to the catalytic fixed bed, the bio-oil yield increased. In the research of Cai et al., [9] the yields of the bio-oil, biochar and non-condensable gases were 47.7±2.7, 30.6±2.0 and 21.8±2.8 wt%, respectively, when the rice husk was pyrolyzed in a commercial-scale plant based on fluidized bed reactor. Zhang et al. [10] studied the rice husk pyrolysis, and the yields of bio-oil, biochar and non-condensable gases were 40.2%, 32.1% and 27.7%, respectively.

Table 1 Mass balance analysis

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7
Bio-oil yield (wt%)	23.2	39.3	46.9	40.0	33.5	31.3	40.3
Biochar yield (wt%)	42.9	34.0	30.0	34.3	37.0	42.2	46.3
Non-condensable gas yield a (wt%)	33.9	26.7	23.1	25.7	29.6	26.6	13.4

Characteristics of biochar

The effects of catalyst weight and position on the properties of biochar was studied. In proximate analysis, the content of moisture and volatile matter decreased with the increase of catalyst weight. When the catalyst was placed in the catalytic fixed bed, the content of moisture, ash and fixed carbon increased while the volatile matter decreased. In thermal characteristics analysis, with the increasing of catalyst weight, specific heat capacity increased while the thermal diffusivity decreased. When the catalyst was placed in the catalytic fixed bed, thermal resistance and thermal diffusivity increased while thermal conductivity and specific heat capacity decreased.

In proximate analysis, moisture content decreased with the increase of catalyst weight. When 4 g catalyst was placed in the fluidized bed, the minimum moisture content was obtained (0.60±0.12 wt. %). When 2 g catalyst was placed in the catalytic fixed bed, moisture content increased 53.2% compared with 2 g catalyst placed in the fluidized bed. The maximum ash content was obtained in Run 6 when 4 g catalyst was placed in the fluidized bed, reaching a value of 63.89±0.96 wt. %. The maximum fixed carbon content was 44.2 wt. % when 2 g catalyst was placed in the catalytic fixed bed (Run 7). The content of volatile matter decreased with the increase of catalyst weight. The minimum volatile matter content was obtained in Run 6 when 4 g catalyst was placed in the fluidized bed, reaching a value of 12.40±0.51 wt. %. When the catalyst was transferred from fluidized bed to catalytic fixed bed, the volatile matter content decreased 10.2%. In the process of fast pyrolysis, most volatile matter becomes pyrolytic vapor, which will generate bio-oil when the vapor is quenched in the condensation system. So the volatile matter content in biochar can measure the extent the fast pyrolysis reaction. The catalyst favors the occurrence of thermal cracking reaction. The promoting effect of the catalyst increased with its weight. What's more, when the catalyst was placed in the catalytic fixed bed, the promoting effect will be enlarged. The highest heating value was obtained in Run 7, reaching a value of 17.529±1.263 MJ kg^{-1}.

Fast pyrolysis mechanism

The mechanism of biomass fast pyrolysis is very complicated mainly for the following three reasons: 1) there are many kinds of biomass such as rice husk, wood, grass and so on. The composition and structure of biomass varies greatly from different sources; 2) Hundreds of chemicals were detected in the pyrolytic vapor or bio-oil, and the contents of those substances were mostly very low; 3) those chemicals can react with each other in the fast pyrolysis process or in the transportation and storage period, which involves series of reactions.

The speculative cellulose pyrolysis are as follows: Firstly, cellulose is converted to glucose by depolymerization. Glucose can be converted to D-allose and mannose by the isomerization reaction. Levoglucosan is produced through the dehydration reaction from glucose. By retro-grade aldol condensation reaction, glyceraldehyde and 1,3-dihydroxyacetone are produced from glucose and the two isomers can be interconverted by isomerization reaction. Glyceraldehyde can be converted to glycerol by the reduction reaction. Then glycerol is dehydrated to hydroxyacetone. Aldehyde and methanal are produced from glyceraldehyde by retro-grade aldol condensation reaction. Glycolaldehyde and acetic acid are isomers, which can be interconverted by isomerization reaction. Methanol can be produced from methanal by reduction reaction. Acetic acid and methanol are esterified to form methyl acetate. Carbon dioxide and ethanol can be produced from glyceraldehyde through decarbonylation. Zhou et al. [11] investigated the fast pyrolysis mechanisms of glucose-based carbohydrates (glucose, cellobiose, maltohexaose and cellulose) and found lots of reactions, such as thermohydrolysis, dehydration, retro-aldol reaction and isomerization, involve in the fast pyrolysis process based on experimental findings. Wu et al. [12] studied the pyrolytic characteristics of glyceraldehyde, a model compound of cellulose, by TG-FTIR and Py-GC/MS and found that light fragments (such as CO, CO_2, H_2O and glycolaldehyde) can be produced from glyceraldehyde direct decomposition.

Conclusions

A lab-scale catalytic pyrolysis unit was designed and manufactured with a fluidized bed reactor and a catalytic fixed bed. The highest bio-oil yield was 46.9 wt% with bio-char and non-condensable gas yields of 30.0 wt% and 23.1 wt%, respectively, at 500°C in non-catalytic pyrolysis process. The catalyst promotes the biomass pyrolysis for the volatile matter content of biochar decreased in the catalytic pyrolysis experiments. In the fast pyrolysis process, cellulose is firstly pyrolyzed to monomers. Then some chemicals are produced from these monomers through a series of reactions.

Acknowledgements

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REGULATORY INFLUENCE OF CONDUCTIVE MATERIALS ON INTERSPECIES ELECTRON TRANSFER AND CARBON FLUX DURING ELECTROMETHANOGENESIS

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Single chambered Electromethanogenesis (EM) systems (Total/Working volume-0.5L/0.4L) were operated using graphite electrodes and designed synthetic wastewater (20g/l; 48h) to comparatively analyze the effect of conductive materials (CM; Activated charcoal (AC) and Algae biomass char (AB)) under both closed circuit (CC; 100Ω) and applied potential (AP; -0.8V) to analyze their role as intermediates for electron transfer during microbe-electrode interactions towards enhancing methanogenic activity. Three phase experiments were performed, control (without electrodes, CC/AP and AB/AC), CM control (with electrodes with CC/AP) and CM system (with electrodes, CC/AP and AC/AB). Comparatively, results showed higher biogas/methane production of 7.35/4.4 L with conversion efficiency of 60% under influence of conductive materials. The study provides a specific strategy for increasing the overall biogas/methane yields with influence of conductive materials on bacterial electron transfer rates, while driving towards overcoming the limitations of methanogenesis process.

Keywords: *Microbial-electrocatalyzed system, Biogas upgradation, Oxidation-reduction reactions, Biofilm, Renewable energy, Direct interspecies electron transfer (DIET).*

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有機資源回收中心第二期
Organic Waste Treatment Facilities, Phase II

項目簡介

有機資源回收中心第二期(O·Park2)是香港特別行政區政府環境保護署轄下的一個包括設計、建造及營運(DBO)的項目，並由Agrivert、怡和機器、愛銘建築(國際)有限公司(中國建築全資子公司)組成AJA聯營。有機資源回收中心第二期預計將於2023年完工，並開始投入營運15年。

香港堆填區每日處理3,700噸有機廢物，其中1,000噸由工、商行業產生。將這類可生物降解的廢物丟棄到堆填區並不符合可持續發展原則，更會進一步減少原已緊拙的堆填區空位，並增加長期的環境負擔。

有機資源回收中心第二期選址原上水沙嶺禽畜廢物處理中心位置，面積2.5公頃。

本項目採用厭氧消化技術，將有機廢物轉化為生物氣以用作再生能源及粒狀堆肥作商業用途。本項目預計每天可收集並處理300噸已作源頭分類的有機廢物，並期望可減輕堆填區負擔。

另外，本項目採用設計、建造及營運(DBO)，令承辦商可以從設計開始使用創新技術，建造一個嶄新的標誌性環保設施。

PROJECT OUTLINE

Organic Waste Treatment Facilities, Phase II, known as O·Park2, is designated project of the Environmental Protection Department of the Hong Kong SAR in contract form of Design, Building and Operate (DBO). The project was awarded to Agrivert Ltd jointly with Jardine Engineering Corporation and Alchmex International Construction Ltd. (a wholly-owned subsidiaries of CSHK), namely AJA JV. O·Park2 targets to be commenced in 2023 and AJA JV will still undertake the operation for 15 years.

Hong Kong disposes about 3,700 tonnes of organic waste to the landfill each day, of which about 1,000 tonnes were from the Commercial and Industrial source. The disposal of such biodegradable waste to landfills is not sustainable as it leads to depletion of limited landfill space, and bring landfill gas and leachate that impose long-term environmental burden. O·Park2 situates at former site of Sha Ling Livestock Waste Composting Plant at Northern New Territories, in size of 2.5 hectares. It adopts anaerobic digestion technology to turn the organic waste into biogas for energy recovery and granular for commercial fertilizer. The project is capable of receiving and processing approximately 300 tonnes source-separated organic waste each day to alleviate the saturation of landfills.

The DBO contract provide us the allowance for innovative design and cutting-edge technologies, which enable us to build up a new benchmark for environmental infrastructure.