MECHANISTIC AND KINETICS OF CARBON DIOXIDE FIXATION IN AEROBIC BIOGRANULES DEVELOPED FROM PALM OIL MILL EFFLUENT

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Speciality dedicated to my beloved father and mother,
Abd Rashid Bin Md Noor and Che Yam Binti Sharif

My lovely wife and children,
Thank you so much for your understanding,

My siblings and to all my friend

Thanks for your sacrifices and patience

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ABSTRACT

Palm oil mill effluent (POME) generated from oil palm industries is a major source of water and air pollution. Ineffective wastewater treatment results in excessive production of carbon dioxide (CO_2) that contributes to global warming. In this study, photosynthetic aerobic biogranules (PAG) were develop in a lab-scale phototrophic sequencing batch reactor (PSBR) using POME as substrate. The capability of PAG for CO_2 fixation was determined and the biokinetic parameters were estimated from the chemical oxygen demand (COD) fractionation of POME. The PAG were developed in a four litre column reactor that was operated with four hour per-cycle time for 40 days. After six weeks of development process, it was observed that, compact-structured PAG were formed in sizes between 1.0-3.0 mm and a maximum settling velocity of 97 m/h. The mass liqour suspanded solid (MLSS) and the mass liqour volatile suspanded solid (MLVSS) were found to increased from 2.0-8.0 g/L and 2.5-7.0 g/L, respectively. The field emission scanning electron microscope (FESEM) analysis showed the presence of spherical-shaped bacteria (cocci) with sizes ranging from 1.86-2.57 µm. The removal performance achieved was 55 % for COD, 85 % for total nitrogen (TN) and 72 % for total phosphorus (TP). In additon, the CO_2 fixation was analysed in terms of carbon content of the PAG. The CO_2 fixation rate was 0.0967 g/L/d and for one year application the result was estimated to be 4.178 g/L/d. The stoichiometric and kinetic parameters were determined to describe the bioprocess of PAG development using the PSBR system. The COD fractionation of POME indicated the biodegradable substance was 72.9 % with the largest fraction of 46.8 % for slow biodegradable substances (X_S). The biokinetic parameters for the maximum specific growth rate of heterotrophic biomass (μ_{maxH}) was 3.36 d^{-1} while the half-saturation coefficient for the readily biodegradable substrate (K_s) was 40.1 gm^{-3}. The biokinetic parameters obtained were verified for the development process of PAG in the PSBR system.

ABSTRAK

Efluen kilang minyak sawit (POME) yang terhasil daripada industri minyak sawit merupakan penyumbang kepada pencemaran air dan udara. Sistem rawatan air yang tidak efektif menyebabkan pembebasan gas karbon dioksida (CO_2) berlebihan yang boleh menyebabkan pemanasan global. Dalam kajian ini, fotosintesis biogranul aerobik (PAG) telah dihasilkan dalam sistem reaktor jujukan kelompok (PSBR) berskala makmal dengan menggunakan POME sebagai substrat. Keupayaan PAG untuk penetapan CO_2 telah ditentukan dan parameter biokinetik dianggarkan melalui pemecahan permintaan oksigen kimia (COD) keatas POME. PAG dihasilkan dalam satu turus reaktor empat liter yang beroperasi selama empat jam setiap pusingan selama 40 hari. Selepas enam minggu proses penghasilan, PAG yang terbentuk dalam keadaan berstruktur padat yang bersaiz diantara 1.0-3.0 mm dengan halaju pengenapan maksimum 97 m/h. Pepejal terampai (MLSS) dan pepejal terampai meruap (MLVSS) dijumpai meningkat daripada 2.0-8.0 g/L dan 2.5-7.0 g/L. Analisis mikroskop imbasan elektron (FESEM) telah menunjukkan kehadiran bakteria berbentuk sfera (kokus) yang bersaiz 1.86-2.57 µm. Prestasi penyingkiran yang dicapai adalah 55 % bagi COD, 85 % bagi jumlah nitrogen (TN) dan 72 % bagi jumlah fosforus (TP). Kadar penetapan CO_2 dianalis secara kehadiran kandungan karbon. Nilai penetapan CO_2 yang diperolehi adalah 0.0967 g/L/d dan untuk anggaran penggunaan setahun, nilai yang diperolehi adalah 4.178 g/L/d. Parameter stoikiometri dan kinetik ditentukan bagi menghuraikan bioproses PAG menggunakan sistem PSBR. Pemecahan COD POME menunjukkan sebatian biorosot adalah 72.9 % dengan pembahagian terbesar adalah bahan lambat biorosot (X_S) sebanyak 46.8 %. Parameter biokinetik iaitu kadar pertumbuhan spesifik maksimum untuk jisim heterotrof (μ_{maxH}) adalah 3.36 d^{-1} manakala pekali ketepuan separa yang telah siap terbiodegrasi substrat (K_s) adalah 40.1 gm^{-3}. Parameter biokinetik yang diperolehi telah disahkan bagi proses penghasilan PAG melalui sistem PSBR.

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LIST OF ABREVATIONS

AGS	-	Aerobic Granular Sludge
ASM	-	Activated Sludge Model
BOD	-	Biochemical Oxygen Demand
CFU	-	Colony Forming Unit
COD	-	Chemical Oxygen Demand
CPO	-	Crude Palm Oil
EBPR	-	Enhanced Biological Phosphorus Removal
EDX	-	Energy Dispersive X-Ray
EFB	-	Empty Fruit Bunches
EPS	-	Extracellular Polysaccharides
FFB	-	Fresh Fruit Bunch
FESEM	-	Field Emission Scanning Electron Microscope
GHG	-	Greenhouse Gases
HRT	-	Hydraulic Retention Time
NGO	-	Non-Governmental Organization
MLSS	-	Mixed Liquor Suspended Solids
MLVSS	-	Mixed Liquor Volatile Suspended Solids
OLR	-	Organic Loading Rate
OPF	-	Oil Palm Fronds
OPT	-	Oil Palm Trunks
OUR	-	Oxygen Uptake Rate

O and G	-	Oil and Grease
PAG	-	Photosynthetic Aerobic Biogranules
POME	-	Palm Oil Mill Effluent
PSBR	-	Phototrophic Sequencing Batch Reactor
SBR	-	Sequencing Batch Reactor
SVI	-	Sludge Volume Index
UAF	-	Upflow Anaerobic Filtration
UASB	-	Upflow Anaerobic Sludge Blanket
TOC	-	Total Organic Carbon
TN	-	Total Nitrogen
TP	-	Total Phosphorus
TS	-	Total Solids
TSS	-	Total Suspended Solids
TVS	-	Total Volatile Solids
NB	-	Nutrient Broth
NA	-	Nutrient Agar

LIST OF SYMBOLS

CH_4	-	Methane
CO_2	-	Carbon dioxide
Chl	-	Chlorophyll
NH_3-N	-	Ammoniacal nitrogen
MT	-	Million tonnes
m^3	-	Meter cube
TKN	-	Total kjeldahl nitrogen
N_2O	-	Nitrous oxide
HFC	-	Hydrofluorocarbon
S_s	-	Readily biodegradable
S_I	-	Inert soluble
X_I	-	Inert particulate
X_s	-	Slowly biodegradable
%	-	Percentage
°C	-	Degree Celsius
t	-	Time
L	-	Litre
mm	-	Millimeter
h	-	Hour
d	-	Day
min	-	Minute

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is rich with natural resources, including crops due to its tropical climate, the most famous of which being oil palm (*Elaeis guineensis*) (Syamsuddin *et al.*, 2016). The ever increasing demand for palm oil has led to the expansion of the land for the oil palm tree plantation, currently occupying the largest farming areas in Malaysia. By 2014, Malaysia already recorded a stunning 5.39 million hectares of oil palm plantations (Awalludin *et al.*, 2015; Rupani *et al.*, 2010).

The oil palm industry has been well recognized for its contributions towards a steady economic growth and a rapid development of the country. However, its huge quantities of by-product waste from the oil extraction process have caused environmental pollutions (Rupani *et al.*, 2010). The waste comprised oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), palm pressed fibres (PPF), palm kernel shells, palm oil mill effluent (POME) and oil palm leaves (Mushtaq *et al.*, 2015).

This study focused on POME since it causes a lot of problems, in terms of water environmental pollution and is the main pollution load to the river (Wu *et al.*, 2009). Generally, POME is generated during the oil extraction process, washing and cleaning that the main content such as fat, grease and cellulosic material (Yacob *et al.*, 2006). In addition, it is also the wastewater discharged from the sterilization process, cracked mixture separation process and crude oil clarification process (Liew *et al.*, 2015).

During the palm oil processing, over 70% of the processed fresh fruit bunch (FFB) was left as oil palm waste (Chiew *et al.*, 2011). It was reported that, in 2004, about 30 million tonnes (MT) of POME was generated while in 2008, the quantities increased to about 44 MT, and the number was predicted to increase further in the subsequent year (Abdullah *et al.*, 2011; Rupani *et al.*, 2010). Each metric ton of crude palm oil (CPO) produced approximately 0.9 – 1.5 m^3 of POME (Yuniarto *et al.*, 2013). Moreover, about 0.5 – 0.7 m^3 of POME is always discharged from every metric tonne of fresh fruit bunches processed (Madaki and Seng, 2013). Thus, it was estimated that in 2014, the total production of crude palm oil was 19.66 million tonnes with approximately 44 million m^3 of POME being generated from palm oil mills in Malaysia (MPOB, 2014).

Generally, land application is used for the POME disposal alternatives. Releasing the POME on the land brings about stopping up and water logging of the dirt and executes the vegetation on contact. The least expensive method for releasing the POME is to discharge it into the river since POME is a nontoxic oily waste (Rupani *et al.*, 2010). However, the release of wastewater into water bodies causes water exhaustion and results in river contamination (Gan *et al.*, 2012). As such, better alternative treatments must be sought.

Several researchers have studied the various aspects of POME treatment. The typical method available for the treatment of POME in Malaysia is by a biological treatment, consisting of anaerobic, facultative and aerobic pond systems (Abdurahman *et al.,* 2013; Chan *et al.,* 2010). More than 85 % of palm oil processes in Malaysia opt for the ponding system for treatment because of the organic characteristics of POME. These strategies are viewed as a conventional POME treatment technique, including long maintenance times and substantial treatment zones (Poh and Chong, 2009; Perez *et al*., 2001). Meanwhile, combinations of treatments, including tank digestion and facultative ponds, tank digestion and mechanical aeration, decanter and facultative ponds, physic-chemical and biological treatments, were also used for POME treatment (Fairuz, 2012; Vijayaraghavan *et al*., 2007).

In this study, POME wastewater was treated using biogranulation of activated sludge provided with aeration to cause the sludge to become granules, known as an aerobic granulation technology. The aerobic granulation technology provides a new method for a better settle-ability of activated sludge. This is a promising method that can overcome the limitations in the conventional activated sludge system (Dahalan *et al.,* 2015). Most researchers involved in intensive research related to the aerobic granulation technology reported that this technology appeared to be a promising development in biological wastewater treatment (Ni and Yu, 2010; Liu *et al.,* 2009; Chang, *et al.,* 2008; Yilmaz *et al.,* 2008; Adav *et al.,* 2007).

Aerobic granules consist of abundant microorganisms present in the form of biomass aggregates. They are notable for their dense, strong, regular, smooth, and solid structure, as well as rapid settling ability (Dahalan *et al*., 2015; Tay *et al.,* 2001). Aerobic granules also have a high and stable rate of metabolism, are resilient to shocks and toxins due to the protection provided by a matrix of Extracellular Polymeric Substances (EPS) (Adav *et al.,* 2008).

Aerobic granulation technology plays an important role as an innovative technology alternative for an activated sludge process in industrial and municipal wastewater treatments. To facilitate and promote its practical applications in wastewater treatment, researchers worldwide have intensive investigated the fundamentals of aerobic granulations.

1.2 Problem Statement

The study about global warming has expanded enormously, because it is one of the main concerns of humanity in recent times, involving greenhouse gases (GHG). Among all greenhouse gases, more than 60% total emission of carbon dioxide (CO_2) was responsible for the climate change (Anagnostou *et al.,* 2016; Najib *et al.,* 2016; Hosseini *et al*., 2013; Tingem and Rivington, 2009).

It was noted that, an ineffective operation of wastewater treatment results in an excessive release of CO_2 and CH_4 simultaneously, and contributes to global warming and climate change respectively (Dahalan *et al*., 2015). In Malaysia, the ponding system used to treat industrial wastewater, especially palm oil mill effluent (POME), is one of the main sources of GHG emissions. Malaysia's palm oil industry has generated approximately 80 million dry tonnes of solid biomass per annum as the volume is expected to increase to 85–110 million dry tonnes by 2020 (Najib *et al.,* 2016).

A number of researchers highlighted that, without satisfactory treatments of POME in the ponding system, emissions of GHG and environmental degradation can occur (Harsono *et al.,* 2014; Basri *et al.,* 2010; Yacob *et al.,* 2006). The environmental degradation includes much more water resources which are polluted, hence potentially posing threats to irrigated areas. Besides, earlier studies reported that, an approximately 28 m^3 per 1 tonne of POME consisting of 65 % CH_4 and 35 %

CO_2 was also produced as end products of anaerobic digestion from POME waste treatment biogas (Najib *et al.,* 2016; Wicke *et al.,* 2008). In addition, the GHG emissions for one million tonnes of crude palm oil (CPO) from the mills without biogas capture system totalled 987 kgCO_2e, while the mills with biogas capture systems emitted 225 kgCO_2e (Kaewmai *et al.,* 2012).

Undoubtedly, photosynthesis process in the terrestrial plant is the most efficient way for carbon dioxide recycling. In another word, the treatment of wastewater by using microalgae has also been highlighted in stabilization ponds. The use of microalgae in wastewater treatments was first proposed by Oswald and Gotaas in 1957 and has received much attention in recent decades (Olguín, 2003; Rawat *et al.,* 2010).

Microalgae are photosynthetic microorganisms which use energy from the sunlight by consuming inorganic nutrients and CO_2 for the development process. However, these technologies have been used mainly in small communities, partly due to the high-cost harvest of algal biomass and an enormous amount of water demanded by the photosynthetic organisms of the water-dependant culture (Gerbens-Leenes *et al*., 2009).

The growing interest on microalgae cultivations is due to the superior productivity and photosynthetic efficiency compared to other terrestrial plants, when they are augmented under proper conditions, such as light, temperature, nutrients, and CO_2 concentration (Hnain *et al.,* 2011; Bibeau, 2009). Although the uses of microalgae are beneficial and have been successful in various carbon sequestration applications, one trivial disadvantage is its incapability to be integrated into aerobic systems (Costa, 2010).

As an alternative, the application of aerobic granulation technology for CO_2 fixation may serve as the limitation for microalgae. This strategy can be applied to reduce the CO_2 emission in the environment (Najib *et al.,* 2016; Dahalan *et al.*, 2015).

The success of aerobic granulation technology in wastewater treatment was obvious in worldwide application. Recent study conducted for aerobic granulation by using synthetic wastewater with the application of photosynthetic bacteria was successfully provide the detail of development process by Dahalan (2012). However, synthetic wastewater is not present the real wastewater condition in the pond or river.

Prior to that, a study has been made by using POME wastewater for the development of aerobic granule by Najib (2017). The study successfully integrated the photosynthetic bacteria analysis for CO_2 reduction. However it appear to limited his study in a modelling study.

Therefore in this study, the development of aerobic granule for CO_2 fixation with modelling study was applied in order to gain better understanding of aerobic granulation process and to support for future experimental and modelling study.

1.3 Aim and Objective of the Study

The aim of this study was to determine the mechanistics and kinetics of CO_2 fixation in photosynthetic aerobic biogranules (PAG). To achieve this, the objectives of this study were as follows:

1. To develop the photosynthetic aerobic biogranules in lab-scale Phototrophic Sequencing Batch Reactor (PSBR) by using palm oil mill effluent (POME) as the substrate.
2. To characterize the photosynthetic aerobic biogranules in terms of physical and chemical factors.
3. To determine the capability of photosynthetic aerobic biogranules for the CO_2 fixation.
4. To estimate biokinetic parameters by using an activated sludge model to describe the biomass performances in the lab-scale PSBR system.

1.4 Scope of Study

This study focused on the development and characterizations of photosynthetic aerobic biogranules developed from sludge. A four litre lab-scale PSBR was used for the aerobic granulation process. The lab-scale PSBR was run in a cycle of four hours per day.

The reactor was equipped with lamps to provide the light source (12 h light/ 12 h dark regime) for creating the photosynthetic conditions. The PAG were developed at room temperature (26.0 ± 2.0 °C). During the development, the morphologies of the PAG were examined using the light microscope. The composition of the PAG was examined by FESEM-EDX, in terms of element composition only, while the detail of bacteria species inside the PAG were not determined in this study.

Furthermore, the photosynthetic aerobic biogranules were characterized for their physical properties, such as settling velocity, sludge volume index (SVI), mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS). For chemical properties, the scope was for the removal performance such as Chemical Oxygen Demand (COD), Total Phosphorus (TP) and Total Nitrogen (TN). The analytical measurements were conducted according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

1.5 Significance of the Study

The aerobic granulation technology has been studied extensively for the improvement of the conventional activated sludge system. Therefore, this study could improve the understanding of the development process for aerobic granulation. Mostly, the aerobic granules were developed by using synthetic wastewater. Therefore, this study can be used as a reference for future studies that would use POME wastewater for granulation.

From the literature review, the development of aerobic biogranules by using lab-scale SBR was studied by Najib (2017) and Dahalan (2012). However the biokinetic studies for the PAG appear to be limited, therefore in this study the biokinetic parameters are need to determine the performance of PAG in the lab-scale PSBR system. In addition, this study evaluated the activated sludge model to obtain the stoichiometric and biokinetic parameters, and the parameters thus obtained could be used to support a more rational design approach for the lab-scale SBR system.

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