PRODUCTION OF AEROBIC GRANULAR SLUDGE IN SEQUENCING BATCH REACTOR FOR THE TREATMENT OF PALM OIL MILL EFFLUENT

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DEDICATION

Specially dedicated to my beloved family, my supportive supervisor Dr Adibah, and all my friends.

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ABSTRACT

Malaysia is the world's second largest producer of palm oil. However, production of palm oil results in the generation of large quantities of polluted wastewater known as palm oil mill effluent (POME). It was estimated that for one tonne of crude palm oil (CPO) production, about five to eight tonnes of water are required for processing purposes and around 50% (three to four tonnes) will end up as POME. Direct discharge of raw POME without any treatment will deteriorate the surrounding environment as the organic compounds in the wastewater such as tannins and humic acids, tend to inhibit growth and reduce the rate of photosynthesis of aquatic biota. The unpleasant odour and blackish colour of POME also causes aesthetic problems and raise public concerns. Conventional ponding systems which rely solely on indigenous bacteria often failed to withstand the excessive pollution load and resulted in poor treatment efficiency. Aerobic granular sludge (AGS) produced in sequencing batch reactor (SBR) is a promising alternative to ponding system as it tends to withstand higher organic loadings and greater biomass retention. In this study, AGS was produced in laboratory scale SBR with raw final discharged POME as feed. Three bacterial strains, *Escherichia coli* (AL1), *Bacillus cereus* (AL2) and *Lysinibacillus fusiformis* (ZB2) were added as inoculum into the SBR. These strains were screened in this study based on their abilities to reduce colour intensity and COD level of final discharged POME, while maintaining high viable cell counts when cultivated in POME for five days. The SBR system was operated at the volume exchange ratio (VER) of 50%, hydraulic retention time (HRT) of six hours and organic loading rate (OLR) of 5.0 kg/COD/m3/d for 200 days. The SBR system entered steady state during day-80 of operation period indicated by mixed liquor volatile suspended solids (MLVSS) concentration of above 3,000 mg/L and sludge volume index (SVI) of below 80 mL/g. The SBR system achieved outstanding biomass concentration of 19,200 mg/L, six times higher than normal operating SBR. SVL index as low as 12 mL/g was considered one of the best among similar studies. The microbial communities of AGS were examined at different stages of granulation using Miseq amplicon sequencing system. Results showed the microbial communities of AGS of the age of 20 days, 80 days and 180 days were dominated by phylum Firmicutes and Proteobacteria, whereas the relative abundance of Phyla Actinobacteria and Bacteroidetes reduced as the AGS aged.

ABSTRAK

Malaysia adalah pengeluar minyak sawit kedua terbesar di dunia. Bagaimanapun pengeluaran minyak sawit mengakibatkan penjanaan dalam jumlah yang besar air tercemar yang dikenali sebagai efluen kilang minyak sawit (POME). Dianggarkan untuk menghasilkan satu tan minyak sawit mentah, kira-kira lima hingga lapan tan air diperlukan untuk tujuan pemprosesan dan kira-kira 50% (tiga hingga empat tan) akan berakhir sebagai POME. Pelepasan POME secara langsung tanpa sebarang rawatan akan mencemarkan alam persekitaran kerana sebatian organik di dalam air sisa, seperti tanin dan asid humik, cenderung menghalang pertumbuhan dan mengurangkan kadar fotosintesis biota akuatik. Bau yang tidak menyenangkan dan warna hitam-gelap POME juga menyebabkan masalah estetik dan menimbulkan kebimbangan orang ramai. Sistem kolam konvensional yang hanya bergantung kepada bakteria asal sering gagal menampung beban pencemaran organik POME yang melampau dan mengakibatkan kecekapan rawatan yang rendah. Enapan granular aerobik (AGS) yang dihasilkan dalam reaktor kelompok berjujuk (SBR) dilihat berpotensi sebagai alternatif sistem kolam kerana ia berkebolehan untuk menampung beban organik yang lebih tinggi serta mengekalkan biojisim yang lebih besar. Dalam kajian ini, AGS telah dihasilkan dalam skala makmal SBR dengan menggunakan POME pelepasan terakhir sebagai nutrien. Tiga strain bakteria, *Escherichia coli* (AL1), *Bacillus cereus* (AL2) and *Lysinibacillus fusiformis* (ZB2), telah dimasukkan ke dalam SBR sebagai inokulum. Strain bakteria ini telah disaring dalam kajian ini berdasarkan kepada kebolehan untuk mengurangkan kepekatan warna dan paras COD POME, di samping mengekalkan kiraan sel hidup yang tinggi apabila dikulturkan dalam POME selama lima hari. Sistem SBR ini dikendalikan pada nisbah pertukaran isipadu (VER) sebanyak 50%, masa pembendungan hidraulik (HRT) selama enam jam dan kadar kemasukan organik (OLR) sebanyak 5.0 kg/COD/m3/d selama 200 hari. Sistem SBR didapati memasuki peringkat stabil apabila operasi mencecah hari ke-80, ditunjukkan oleh kepekatan cecair campuran-pepejal terampai meruap (MLVSS) yang melebihi 3,000 mg/L dan indeks isipadu enap cemar (SVI) yang kurang daripada 80 mL/g. Sistem SBR mencapai kepekatan biojisim yang mantap sebanyak 19,200 mg/L, enam kali ganda lebih tinggi daripada sistem SBR biasa. Indeks SVI serendah 12 mL/g juga merupakan pencapaian terbaik untuk sistem ini. Komuniti mikrob AGS telah diperiksa menggunakan penjujukan amplikon Miseq pada masa penggranulan yang berbeza. Keputusan menunjukan komuniti mikrob AGS pada hari ke-20, ke-80 dan ke-180 didominasi oleh filum Firmikutes dan Proteobakteria, manakala kepelbagaian relatif filum-filum Actinobakteria dan Bakteroidetes didapati berkurangan apabila AGS semakin berusia.

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LIST OF ABBREVIATIONS

ADMI - American Dye Manufacturers’ Institute
AOPs - Advanced Oxidation Processes
AN Ammoniacal Nitrogen
AGS - Aerobic Granular Sludge
BLASTn - Basic Local Alignment Search Tool of nucleotide
BOD - Biochemical Oxygen Demand
BLASTn - Basic Local Alignment Search Tool of nucleotide
COD - Chemical Oxygen Demand
CTAB - Cetyltrimethylammonium bromide
DNA - Deoxyribonucleic acid
DNS - Dinitrosalicyclic acid
EPS - Extracellular Polymeric Substances
FESEM - Field Emission Scanning Electron Microscopy
g/L - Gram per litre
GCMS - Gas Chromatography–Mass Spectrometry
hr - Hour
HPLC - High-performance Liquid Chromatography
§ - Integrity Coefficient
MBR - Membrane Bioreactor Technology
µL/mg - Micro gram per mili gram
mg/L - Miligram per litre
min - Minute
MLSS - Mixed Liquor Suspended Solids
MLVSS - Mixed Liquor Volatile Suspended Solids
NJ - Neighbour Joining
OLR - Organic Loading Rate
POME - Palm Oil Mill Effluent
rDNA - Ribosomal Deoxyribonucleic acid
rpm - Rotation per minute
rRNA - Ribosomal ribonucleic acid

SBR - Sequencing Batch Reactor

SVI - Sludge Volume Index

NaCI - Sodium Chloride

TAE - *Tris-acetate-EDTA*

TE - *Tris-EDTA*

Tris-HCl - *TRIS hydrochloride*

TOC - Total Organic Carbon

TN - Total Nitrogen

TP - Total Phosphate

TPC - Total Phenolic Compound

v/v - Volume per volume

w/v - Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The oil palm plantation is currently the leading agricultural based industry in Malaysia. In the year of 2012, the rapidly developed oil palm industry is named the fourth largest contributor to the Malaysian economy, providing nearly RM 53 billion of the Gross National Income of the country (Gobi and Vadivelu, 2013). The total plantation area of oil palm during the 60’s is a mere 54 thousand hectares, but has expended exponentially to more than 4.74 million hectares at the year of 2017, with annual crude palm oil (CPO) production of 19.1 million metric tonnes (Varqa, 2017). However, the subsequently by-product generated together with the extraction of cruel palm oil from the palm fruits, is the production of great volumes of high organic load wastewater known as palm oil mill effluent (POME). It was estimated that for 1 tonne of CPO production about 5 – 7.5 tonnes of water is required for processing purposes, and around 50% (2.5 – 3 tonnes) will end up as POME (Choi *et al.*, 2013). Production of palm oil will continue to be increased due to the government’s policy to use them as a source to produce biodiesel ((Lam and Lee, 2011).

The direct release of untreated raw POME into the nature water reservoirs such as rivers and streams is strictly prohibited, since POME averagely possessed extremely high chemical oxygen demand (COD) and biological oxygen demand (BOD) concentrations of 50,000 and 25,000 mg/L, respectively (Abdullah *et al.*, 2011). The organic compounds in the wastewater such as tannins and humic acids tend to inhibit the growth or even intoxicate the aquatic biota (Neoh *et al.*, 2013). The unpleasant odour and blackish colour of POME will also cause aesthetic problems and raise public concerns. Majority of the POME treatment schemes being applied by Malaysian palm oil mills are conventional ponding system, tank digestion and mechanical aeration, which have low organic removal efficiencies, extended retention time and are unable to decolourise the brownish colour of POME (Vijayaraghavan *et al.*, 2007). However, more than 85% of Malaysia's palm oil mills still apply these schemes for POME treatment due to its low capital cost (Lam and Lee, 2011).

1.2 Problem Statement

The extreme organic loadings of POME have placed it as one of the most polluted agro-based wastewaters existing in the world. Thereby, effective and eco-friendly disposal of POME is currently at the centre of attention from both the environmental and aesthetic viewpoint. The high suspended solids concentration and dark brownish colour of POME often inhibit the growth of aquatic biota, simply by reducing the absorption of sunlight that set the photosynthetic life to extinction and consequently result in incomplete biogeochemical cycles. The organic compounds within the wastewater also tend to chelate with the metal ions and forming the toxic, stable and recalcitrant ring-structure organometal compounds, thus develop toxicity to the marine biota (Mohan and Karthikeyan, 1997). Humic substances within POME will also react with chlorine during water disinfection treatment to produce carcinogenic byproducts such as trihalomethanes (VukoviĆ *et al.*, 2008). Furthermore, POME possess high organic content with appreciable amount of plant nutrients, and thus can initiate the exponential growth of certain microorganisms in the environment. The erupt growth of these microorganisms can disrupt the balance of the original food chain in the related ecosystem. More attention has been notified from the public related to these issues that shows the necessity to develop some more efficient treatment methods for the treatment of POME.

More than 85% of Malaysia's palm oil mills selected conventional ponding system as the sole treatment system of POME, simply due to its low capital cost and management simplicity. Since conventional ponding system has been established and applied from the very beginning of Malaysia's palm oil industry, it is very difficult to replace it with newer and more efficient treatment processes. Therefore, polishing or tertiary treatment technologies seem very plausible to improve the treatment efficiencies of primary/secondary treatment technologies. Liew *et al.* (2015) reviewed most of the tertiary treatment systems in terms of their operation descriptions and removal efficiencies and revealed that membrane filtration processes (applied under membrane bioreactor technology (MBR)) and advanced oxidation processes were the most effective processes to remediate this high organic load wastewater. However, in reality the applications of these processes in the real oil palm industry is uncommon. This is due the capital investment and operating cost for MBR technology was extremely high, while the application of advanced oxidation processes (AOPs) will also produce highly toxic by-products (Neoh *et al.*, 2016). Biological-based treatment processes with microbial immobilisation such as biofilm formation and granular sludge development do have a great potential in POME remediation due to its low capital cost, more eco-friendly and high versatility. Aerobic granular sludge (AGS) developed in sequencing batch reactor (SBR) has been implemented in the treatment of high strength industrial wastewater, in which simultaneously nutrients removal with high efficiencies under a single reactor sytem (Shaw *et al.*, 2002). AGS is applicable in the treatment of POME with high organic complexity, as it consisted of a microbial community that included aerobic, facultative anaerobic and anaerobic microbes which might able to remove majority of organic pollutants within POME. AGS with the high biomass over volume ratio can ensure good nutrients removal rates during the treatment of POME. Good settleability of the AGS can also ensure good biomass retention within the SBR system. The treatment efficiency of POME can also be increased when strains of bacteria effective in remediate the wastewater are augmented as inoculum during the production of AGS in SBR. However, study in biogranulation of AGS for the bioremediation of raw industrial wastewater is still relatively scarce and requires more findings.

Previous study has indicated that biological treatment of POME was still incapable in complying with the standard requirements set by the regulator such as Department of Environment Malaysia (DOE) (Zahrim *et al.*, 2014). In addition, the colour intensity of the effluent was remained high and contained high concentration of recalcitrant organics, based on the high COD/BOD ratio obtained from the effluent. Such limitation was encountered probably due to the widely selection of indigenous microbial community originally from activated sludge as the sole inoculum for biological treatment processes. These indigenous microbial groups may readily adapt to the wastewater; however, it will encounter some difficulties to degrade some toxic and recalcitrant compounds within the wastewater. Therefore, bioaugmentation with selected strains that already been screened for their abilities to treat a certain type of wastewater seem liked a promising option.

1.3 Objectives

The objectives of the study were to:

i. develop aerobic granular sludge (AGS) in sequencing batch reactor (SBR) under aerobic condition.

ii. characterise the AGS formed. Physically characterizations of the AGS and microbial community analysis of the AGS under different age.

1.4 Scope of the Study

The major aim of this study was to develop and characterise AGS by using a laboratory scale of sequential batch reactor (SBR) system under a fixed hydraulic retention time (HRT). Final discharged POME was used as the feeding solution during the development of AGS with addition of simple carbon source to achieve an idea organic loading rate (OLR) in this study. Sludge was collected from an aerobic pond of a local palm oil industry and was used as the seed sludge for the cultivation of AGS. The bacterial inoculum used consists of mixture of three pure cultures of augmented bacteria strains which were isolated from contaminated soil and textile sludge. These strains have been screened for their abilities to grow in POME and removed portion of its COD and colour intensity.

During the 200 days of AGS development period, parameters such as pH, COD, colour intensity and total phenolic compounds were examined to indicate the overall efficiency, effectiveness and stability of the system. Biomass profile such as mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) etc were also monitored during this period. The AGS developed was characterised specifically for their morphology changes, physical strength and settleability. Microstructure observations of AGS under different ages were conducted via field emission scanning electron microscopy (FESEM). The microbial community or metagenomic analysis of the AGS under certain age was identified with the application of amplicon sequencing. Extracellular polymeric substances (EPS) of AGS were extracted and quantified, and the overall contents of EPS were grouped as carbohydrates, proteins, phenolic compounds and DNA. The robustness of AGS in this study was investigated via a series of starvation and organic shock loadings in order to justify the potential application of AGS in real industry conditions where the OLR was always changing. The AGS harvested were applied for treatment test of several high colour intensity and high organic load wastewaters, with certain parameters being closely monitored.

1.5 Significance of the Study

The outcomes of this study will contribute to an improved biological treatment of POME particularly in removing organic pollutants and colour compounds. In comparison with anaerobic biogranules, aerobic biogranules or AGS was more favourable since it does not require specific culture conditions such as anaerobic surroundings for anaerobic biogranules or attaching matrix for biofilm. Instead of using conventional ponding system, SBR system will surely improve the efficiency in POME treatment by means of simultaneous removal of organic pollutants, thus avoiding exposure of high concentration of growth inhibitors and toxic compounds to the microbes that will affect their vulnerability. In order to ensure high cells viability or high biomass retention in the high strength wastewater for better treatment efficiency, AGS that is capable to withstand extreme OLR and high toxicity seems like a very fitting approach. The accomplishment of this research sheds light to a better solution in the biological treatment of high strength wastewater such as POME, leachate, petrochemical wastewater and others.

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LIST OF PUBLICATIONS

1. Neoh, C. H., **Lam, C. Y.**, Lim, C. K., Yahya, A., Bay, H. H., Ibrahim, Z., et al. (2015). Biodecolorization of recalcitrant dye as the sole source of nutrition using *Curvularia clavata* NZ2 and decolorization ability of its crude enzymes. *Environ Sci Pollut Res Int*, 22(15), 11669-11678. (IF: 2.800)

2. Neoh, C. H., **Lam, C. Y.**, Yahya, A., Ware, I., and Ibrahim, Z. (2015). Utilization of Agro-Industrial Residues from Palm Oil Industry for Production of Lignocellulolytic Enzymes by *Curvularia clavata*. *Waste and Biomass Valorization*, 6(3), 385-390. (IF: 1.874)

3. Neoh, C. H., **Lam, C. Y.**, Ghani, S. M., Ware, I., Sarip, S. H., and Ibrahim, Z. (2016). Bioremediation of high-strength agricultural wastewater using *Ochrobactrum sp*. strain SZ1. *3 Biotech*, 6(2), 143. (IF: 1.497)