

**ENHANCEMENT OF VOLATILE FATTY ACID
PRODUCTION FROM ANAEROBIC TREATMENT OF
PALM OIL MILL EFFLUENT**

By

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LIST OF ABBREVIATIONS

3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
3R	Reduce, Reuse, Recycle
ABSR	Anaerobic bench scale reactor
ADM1	Anaerobic digestion model No.1
AOP	Advance oxidation process
BNR	Biological nutrient removal
BOD	Biological oxygen demand
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DNA	Deoxyribonucleic acid
FA	Free ammonia
FFB	Fresh fruit bunch
FTIR	Fourier Transformation Infra-Red
GC	Gas chromatography
H ₂	Hydrogen
H ₂ O	Water
HRT	Hydraulic retention time
IWA	International Water Association
LCFA	Long chain fatty acid
MABR	Modified anaerobic baffled reactor
MFC	Microbial fuel cell
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid
MPOB	Malaysia Palm Oil Board

N	Nitrogen
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
OLR	Organic loading rate
OUR	Oxygen uptake rate
P	Phosphorus
PAC	Polyaluminium chloride
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
POME	Palm oil mill effluent
SBR	Sequencing batch reactor
SRT	Solid retention time
SS	Suspended solid
SVI	Sludge volume index
TS	Total solid
UASB	Up-flow anaerobic sludge blanket reactor
UASFF	Up-flow anaerobic sludge fixed-film reactor
UFF	Up-flow fixed film
VFA	Volatile fatty acid
VOA	Volatile organic acid
VSS	Volatile suspended solid

LIST OF SYMBOLS

μ	specific growth rate
μ_{\max}	the maximum specific growth rate
μ_x	specific biomass growth rate
$\mu_{x,VFA}$	specific growth rate of VFA-producing biomass
$\mu_{x,PHA}$	specific growth rate of PHA-producing biomass
b	the specific microorganism decay rate
b_D	decay coefficient of biomass
COD_{effluent}	COD of effluent waste
COD_{influent}	COD of influent feed
COD_R	COD in reactor
f_D	fraction of active biomass contributing to biomass debris
$f_{PHA,VFA}$	fraction of active VFA consuming, PHA-producing biomass
$f_{VFA,COD}$	fraction of active COD consuming, VFA-producing biomass
I	Inhibition factor
K	Chen and Hashimoto dimensionless kinetic constant
K	Process rate coefficient (ADM1)
K_{dec}	Decay rate (ADM1)
K_S	the half saturation constant
$K_{S,COD}$	the half saturation constant, affinity towards COD
$K_{S,VFA}$	the half saturation constant, affinity towards VFA
$K_{S,\max}$	the maximum specific substrate utilisation rate
K_X	Contois kinetic constant
NH_3-N	Ammoniacal nitrogen
OLR	Organic loading rate
OUR_D	Oxygen uptake rate due to biomass decay
OUR_{total}	Overall oxygen uptake rate
Q_{in}	Flow rate of feed into reactor

S	final concentration of substrate
S_0	initial concentration of substrate
S_{COD}	Substrate's COD concentration
$S_{COD,in}$	Initial substrate's COD concentration
S_f	final COD concentration
S_i	initial COD concentration
S_{PHA}	Substrate's PHA concentration
$S_{PHA,in}$	Initial substrate's PHA concentration
S_{VFA}	Substrate's VFA concentration
$S_{VFA,in}$	Initial substrate's VFA concentration
t_{SRT}	the solid retention time
VFA_f	Final concentration of volatile fatty acid in reactor
VFA_i	Initial concentration of volatile fatty acid in reactor
V_R	Volume of reactor
VSS_f	Final concentration of volatile suspended solid in reactor
VSS_i	Initial concentration of volatile suspended solid in reactor
X	the microorganism concentration
X_f	final biomass concentration
$X_{H,0}$	initial reactor MLVSS concentration
$X_{H,t}$	MLVSS concentration at t time
X_i	initial biomass concentration
X_{PHA}	PHA-producing biomass concentration
X_{VFA}	VFA-producing biomass concentration
Y	the growth yield coefficient
ΔS	net change of COD concentration, mg/L
ΔX	net change of biomass concentration
ρ	Process rate (ADM1)

**PENINGKATAN PENGELUARAN ASID LEMAK MUDAH MERUAP
DARIPADA RAWATAN ANAEROBIK AIR SISA KILANG KELAPA SAWIT**

ABSTRAK

Unit rawatan biologi digunakan secara meluas untuk merawat air sisa berkandungan organik tinggi seperti air sisa kilang minyak sawit (POME). Rawatan yang boleh mengguna semula nutrien berguna daripada air sisa diperlukan untuk meningkatkan kelestarian proses rawatan air sisa. Asid lemak mudah meruap (VFA) berpotensi tinggi untuk pelbagai aplikasi misalnya pengeluaran biogas atau plastik boleh dibiodegrasi. Pemulihan nutrien dalam bentuk gas metana (produk akhir pencernaan anaerobik) telah dikaji dengan meluas tetapi kajian tentang peningkatan VFA (produk perantaraan pencernaan anaerobik) masih terhad. Pengeluaran VFA lebih dipilih daripada pengeluaran biogas kerana VFA boleh digunakan untuk menghasilkan produk yang lebih berharga seperti polyhydroxyalkanoate (PHA) (sejenis plastik boleh dibiodegrasi). Peningkatan pengeluaran VFA dicapai melalui penghapusan proses metanogenesis. Dalam kajian ini, reaktor kelompok berjujuk digunakan untuk memperkayakan biomas dalam rawatan anaerobic separa POME untuk meningkatkan pengeluaran VFA. SBR bersaiz 8 L dan masa pengekalan hidraulik adalah selama 5 hari. Kepekatan POME suapan mengandungi kira-kira 33400 mgCOD/L. Reaktor anaerobik berjaya meningkatkan pengumpulan VFA kepada 10500 mg/L VFA (kira-kira 43.8% VFA dalam suapan). Analisis populasi mikrob menunjukkan bahawa pertumbuhan metanogen telah ditindas dan tiada gas metana dibebaskan semasa proses rawatan. Tambahan pula, didapati bahawa peningkatan nitrogen ammonia telah meningkatkan jumlah pengeluaran VFA. Lebih kurang 105.7% pengumpulan VFA dicapai pada nisbah COD: N kira-kira 40: 5. Walau bagaimanapun, perencatan pengeluaran VFA berlaku pada kandungan nitrogen

ammonia yang lebih daripada 4000 mg/L beban kejutan. Selain itu, efluen dari reaktor peningkatan VFA digunakan sebagai suapan untuk penghasilan PHA. Reaktor aerobik pengeluaran PHA (AE1) menggunakan POME yang telah diperkayakan VFA mampu mencapai pengeluaran kepekatan PHA lebih kurang 3773 mgPHA/L (pengeluaran spesifik kira-kira 0.32 mgPHA/mgVSS). Hasil kajian ini menunjukkan bahawa pemulihan VFA berpotensi tinggi untuk menghasilkan plastik boleh dibiodegrasi (PHA). Selain itu, kinetik pertumbuhan mikrob telah dinilai untuk mikroorganisma dalam reaktor peningkatan VFA dan reaktor pengeluaran PHA. Hasil kajian kinetik pertumbuhan digunakan untuk mensimulasikan pengumpulan VFA dan pengeluaran PHA dengan menggunakan perisian MATLAB.

ENHANCEMENT OF VOLATILE FATTY ACID PRODUCTION FROM ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUENT

ABSTRACT

Biological treatment unit is widely used to treat high organic content wastewater such as palm oil mill effluent (POME). A treatment that can salvage the useful nutrient from the wastewater is needed to enhance the sustainability of the wastewater treatment process. Volatile fatty acid (VFA) has high potential to be applied on many application such as production of biogas or biodegradable plastic. The recovery of nutrient in the form of methane gas (end-product of anaerobic digestion) was well researched but the study on the enhancement of volatile fatty acid (VFA) (intermediate-product of anaerobic digestion) was still limited. VFA production is preferred over biogas production because VFA can be used to produce more valuable product such as polyhydroxyalkanoate (PHA) (a type of biodegradable plastic). The enhancement of VFA production was achieved through the elimination of methanogenesis process. In this study, a sequencing batch reactor was used to enrich the biomass in partial anaerobic treatment of POME to enhance the VFA production. The SBR has a working volume of 8 L and hydraulic retention time of 5 days. The influent concentration of POME contains about 33400 mgCOD/L. The anaerobic reactor managed to increase the VFA accumulation to about 10500 mg/L total VFA (about 43.8 % of VFA accumulation in the feed). The microbial population analysis had revealed that the growth of methanogen was suppressed and no methane gas was release during the treatment process. Further, it was discovered that the increase of ammoniacal nitrogen has increased the total VFA production. About 105.7 % VFA accumulation was achieved at COD:N ratio of about 40:5. However, inhibition on the

VFA production occurred at ammoniacal nitrogen content of more than 4000 mg/L shock loading. Furthermore, the effluent from the VFA enhancement reactor was used as the feed to produce PHA. The aerobic reactor accumulating PHA using VFA enriched POME (AE1) has achieved output PHA concentration of about 3773 mgPHA/L (specific production of about 0.32 mgPHA/mgVSS). The results shows that the recovery of the VFA has high potential to produce biodegradable plastic (PHA). Additionally, the microbial growth kinetics was evaluated for the microorganism in VFA enhancement reactor and PHA production reactor. The result of the growth kinetics studies was used to simulate the VFA accumulation and the PHA production by using MATLAB software.

CHAPTER 1

INTRODUCTION

1.1 Introduction to palm oil mill effluent in Malaysia

Malaysia is a tropical country produces high quality of agricultural products. It is one of the largest producer and exporter of crude palm oil. In the year 2016, Malaysia yields about 31 % of world palm oil production and about 33 % of the world's total exports of palm oil (MPOB, 2017). Since early 1960s, oil palm cultivation has increased rapidly under the government's programme to diversify agricultural activities. Due to the efforts, currently there are about 5.64 million hectares of land in Malaysia under oil palm cultivation; producing 18.56 million tonnes of crude palm oil and 2.099 million tonnes of palm kernel oil in year 2016.

Considering the high demand for crude palm oil in the world's market, palm oil production has brought much profit to the country. However, the growth in palm oil economy also creates some drawbacks, especially the issue of environmental pollution caused by the wastes generated during the oil extraction processes. The wastes consist of both fibrous waste (such as empty fruit bunch, palm press fibre and palm kernel shell) and less fibrous waste (such as palm kernel cake and liquid discharge). The common practices of the waste management involve the reuse of fibrous material as the boiler's fuel while the wastewater is discharged after treatment. The wastewater originated from palm oil mill is frequently known as palm oil mill effluent (POME).

POME consists of organic matter with high chemical oxygen demand (COD), high biological oxygen demand (BOD), oil and grease, high total solids (TS), and suspended solid (SS) (MPOB, 2014). The discharge of untreated or partially treated POME into receiving water bodies can cause severe environmental problems such as anaerobic condition, odour, clean water scarcity, and endanger aquatic lives. To prevent such problems, the Malaysian's government has enforced the Malaysian Environmental Quality Act 1974 and Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations (1982). POME must comply with the discharge limit set in the regulations (EQA, 2012). Therefore, palm oil mills require efficient management system to treat and dispose the wastes with the aim to reduce the deterioration of air and river quality and conserve the environment.

Most of the common treatment methods of POME are based on 3 major principles namely, the biological, physical-chemical and advance treatments. Dated back to the early 1980s, biological treatment has been used for the treatment of POME; systems such as tank digestion with facultative ponds and tank digestion with mechanical aeration are commonly used (Ma and Ong, 1985). The implementation of other types of reactors (such as up-flow anaerobic sludge blanket and up-flow fixed-film) for biological treatment of POME have also been studied (Borja and Banks, 1994). Yet, the open pond treatment is the most commonly used system to treat POME due to its simplicity and low operating cost. However, it was inevitable that the open ponding methods were generating other problems such as the disposal of bulking sludge and the emission of greenhouse gases.

As time progresses, studies were carried out to search other possible treatment methods which can improve the stability and efficiency of treatment process while reduce problems such as bulking sludge and generation of greenhouse gases. In that sense, physical-chemical method were introduced. Unfortunately, the field of physical-chemical treatment method also encounter some drawbacks. The major obstacle is the cost of materials, the use of materials such as membranes and adsorbent in physical-chemical treatments involves high costing especially in the regeneration of the material. Thus it is impractical to imply them in larger scale. Likewise, advance treatment method such as the application of hydrogen peroxide photolysis also encounter similar halt due to economic viability reason (Liew et al., 2015).

Usually, the wastewater treatment facility does not generate revenue to a company. Hence, a wastewater treatment system which can generate income is a surplus to the industry. A complete anaerobic treatment of wastewater can produce methane gas as end product (Ohimain and Izah, 2017). The methane gas produced can be captured as biogas to produce heat and electricity. The generation of electricity can be sold to the electricity provider for additional income. Nevertheless, methane is a greenhouse gas and it is an air pollutant if leakage occurs. Meanwhile, production of PHA (biodegradable plastic) from waste treatment had gained more attraction due to higher profitability, reduce the production of greenhouse gas, and to reduce the dependency on non-biodegradable plastic. With proper assumption and generalisation, Kleerebezem et al. (2015) had estimated that the revenue of methane and PHA production from VFA was about 3600 €/day (RM 16820/day) and 20200 €/day (RM 94390/day). Therefore, it is better to adopting strategy to produce PHA rather than methane through VFA in anaerobic treatment.

1.2 Problem statement

Traditionally, the treatment of wastewater has been focusing on the removal of nutrient, organic content and harmful substances from the wastewater. In the recent years, the focus has been shifted towards the recovery of nutrient and valuable by-products during the wastewater treatment process. Many researchers managed to utilise the organic content of wastewater for the production of polyhydroxyalkanoates (PHA) and biogas during wastewater treatment (Ali Hassan et al., 1997, Chua et al., 2003, Salehizadeh and Van Loosdrecht, 2004, Parawira et al., 2008, Wang et al., 2009, Jiang et al., 2012, Wong et al., 2014, Ohimain and Izah, 2017). Wastewater of high COD especially agricultural wastewater such as POME has high potential for the mass production of PHA and biogas due to the abundance of carbon content (Md. Din et al., 2012, Chin et al., 2013, Ahmed et al., 2015).

The typical biological wastewater treatment system includes both anaerobic and aerobic systems. Wastewater with high COD content undergo anaerobic treatment to partially reduce the COD content before channelling into aerobic treatment system. Anaerobic treatment is more energy efficient and it does not require equipment for aeration. In anaerobic treatment, a sequence of biochemical process occurs, namely, hydrolysis, acidogenesis, and methanogenesis (Tchobanoglous et al., 2004, Grady et al., 2011). The first stage of anaerobic treatment is hydrolysis, at this stage the complex organic contents (carbohydrates, proteins, lipids) are broken down into smaller constituents such as simple sugar, amino acids and long chain fatty acids (LCFA). Followed by the acidogenesis stage where the products of hydrolysis are utilised by acidogens and converts into VFAs, alcohols, and carbon dioxide. Further, in methanogenesis, the products of acidogenesis are used to produce methane, carbon dioxide and hydrogen. In sub-sequent aerobic treatment, the aerobic microorganisms

utilise oxygen to further oxidise the remaining organic content of wastewater carried over from anaerobic treatment. Generally, the whole anaerobic and aerobic process could take up about 45 – 60 days to complete. Additionally, the ponding system treatment is taking up a lot of land area. Hence, modification on the treatment system could help to reduce these drawbacks and improve the recovery of nutrients. Further, it can also reduce the release of greenhouse gases such as carbon dioxide and methane into atmosphere.

One of the valuable intermediate products of anaerobic treatment system is VFA (Lee et al., 2014). VFA is produced in the acidogenesis stage. However, these VFA will be converted into methane gas in the subsequent methanogenesis phase. Although methane gas is an energy source which can be converted into electricity, but not all wastewater treatment plants have the facility to capture and convert the released methane into electricity (Chin et al., 2013) thus the uncaptured methane is released as greenhouse gas. Alternatively, if the anaerobic process could be stopped at the acidogenesis stage, there is no generation of greenhouse gas and it can accumulate VFA at the same time. Additionally, VFA can be used as the substrate to produce PHA. PHA is biodegradable plastic monomer accumulated naturally inside microorganism in wastewater treatment. Hence, it is important to modify the treatment system in order to prevent methanogenesis, stop anaerobic process at acidogenesis stage and enhance VFA accumulation.

The operating condition plays important role in eliminating methanogenesis to optimise the production of VFA. Among the parameters proven to have effect on VFA accumulation are hydraulic retention time (HRT), solid retention time (SRT), temperature, pH, and the availability of micronutrient in the treatment system (Lee et al., 2014). Results from earlier studies manage to give a rough idea on how to construct