PRODUCTION OF BIOGAS AND FATTY ACIDS PHASE BEHAVIOUR FROM PALM OIL MILL EFFLUENT VIA THERMOPHILIC AND MESOPHILIC ANAEROBIC SUSPENDED GROWTH DEGRADATION PROCESS

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by

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NOMENCLATURE

b	Specific biomass decay (l/day)
r_X	Specific substrate utilization rate (g COD/g VSS day)
t	Time (day)
ABSR	Anaerobic Bench Scale Reactor
AF	Anaerobic Filtration
ASGCB	Anaerobic Suspended Growth Closed Bioreactor
Alk	Total Alkalinity
BOD	Biochemical Oxygen Demand (mg/L)
CO_2	Carbon Dioxide Gas
COD	Chemical Oxygen Demand (mg/L)
$COD_{reduction}$	Chemical Oxygen Demand Reduction (%)
CH_4	Methane Gas
CPO	Crude Palm Oil
CSTR	Continue Stirred Treatment Reactor
D	Dilution rate, 1/HRT (1/day)
DNA	Deoxyribonucleic Acid
FFB	Fresh Fruit Bunches
FiT	Feed-in Tariff
HRT	Hydraulic Retention Time (day)
H_2	Hydrogen Gas
H_2S	Hydrogen Sulfide Gas
IAAB	Integrated Anaerobic Aerobic Bioreactor
IAMR	Integrated Anaerobic Membrane Reactor

K_s	Saturation constant for substrate (g COD/L)
L	Liter
MAS	Membrane Anaerobic System
MABR	Modified Anaerobic Baffled Reactor
MFB	Methane-Forming Bacteria
NH_3-N	Ammonia Nitrogen (mg/L)
NJ	Neighbour-Joining
O & G	Oil and Grease (mg/L)
OLR	Organic Loading Rate (g COD / L day)
PCR	Polymerase Chain Reaction
POME	Palm Oil Mill Effluent
Q	Volumetric Flow rate (L/day)
S	Substrate concentration in the reactor (mg/L)
S_1	Influent substrate concentration (mg/L)
S_2	Effluent substrate concentration, (mg/L)
SEDA	Sustainable Energy Development Authority Malaysia
SCOD	Soluble Chemical Oxygen Demand (mg/L)
SRB	Sulfide-Reducing Bacteria
SRT	Solid Retention Time
SS	Suspended Solid (mg/L)
TN	Total Nitrogen (mg/L)
TS	Total Solid (mg/L)
TVS	Total Volatile Solid (mg/L)
UASB	Up flow Anaerobic Sludge Blanket

UASB-HCPB	Up flow Anaerobic Sludge Blanket-Hollow Centred Packed Bed
UASFF	Up flow Anaerobic Sludge Fixed Film
V	Reactor volume (L)
V_{CH4}	Methane Gas Volume (L)
VFA	Volatile Fatty Acid (mg/L)
VOLR	Volumetric Organic Loading Rate (kg COD/m^3day)
VSS	Volatile Suspended Solid (mg/L)
X	Biomass concentration in the reactor, (mg/L)
Y_G	Growth yield (g VSS/g $COD_{removed}$)
Y_{CH4}	Methane yield (L CH_4/g $COD_{removed}$)

Greek letter

μ_{max}	Maximum specific biomass growth rate (1/day)
Θ_c	Critical retention time (day)
μ	Specific biomass growth rate (1/day)

LIST OF PUBLICATIONS

1. Yee-Shian Wong, Tjoon-Tow Teng, Soon-An Ong, Norhashimah Morad, Mohd Rafatullah (2014). Suspended Growth Kinetic Analysis on Biogas Generation from Newly Isolated Anaerobic Bacterial Communities for Palm Oil Mill Effluent at Mesophilic Temperature. RSC Advances. 4: 64659-64667.

2. Yee-Shian Wong, Tjoon-Tow Teng, Soon-An Ong, Norhashimah Morad, Mohd Rafatullah (2014). Identification of Anaerobic Microbial Communities for the Treatment of Palm Oil Mill Wastewater. WIT Transactions on the Built Environment . ISSN: 1743-3509. 156: 353-358.

3. Yee-Shian Wong, Tjoon-Tow Teng, Soon-An Ong, Norhashimah Morad, Mohd Rafatullah (2014). Volatile Fatty Acid and Biogas Profile in the Anaerobic Degradation Process of Palm Oil Mill Wastewater. WIT Transactions on the Built Environment . ISSN: 1743-3509. 156: 633-638.

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PENGELUARAN BIOGAS DAN KELAKUAN ASID LEMAK DARIPADA AIR SISA KILANG KELAPA SAWIT MELALUI PROSES DEGRADASI ANAEROBIK PERTUMBUHAN TERAMPAI TERMOFILIK DAN MESOFILIK

ABSTRAK

Anaerobik proses degradasi air sisa kilang kelapa sawit (POME) telah dilaksanakan pada suhu termofilik (55^0C) dan mesofilik (35^0C) dalam bioreaktor tertutup anaerobik pertumbuhan terampai (ASGCB). Satu siri eksperimen yang berterusan yang menggunakan suapan aliran-kadar pada 0.58, 0.70, 0.88, 1.17 dan 1.75 liter air sisa mentah POME untuk setiap hari kajian telah dikendalikan dalam termofilik dan mesofilik ASGCB. Sehubungan dengan itu, data aliran yang dinyatakan di atas adalah menyerupai masa tahanan hidraul (HRT) bersamaan dengan 24, 20, 16, 12 dan 8 hari. Kecekapan pengurangan COD dalam termofilik dan mesofilik ASGCB adalah dalam lingkungan 90.90% - 83.58% dan 89.66% - 79.83%, antara HRT 8 hari dan 24 hari. Manakala, pH untuk kedua ASGCB adalah dalam lingkungan 8.05-7.74 dan 7.80-7.35. Biogas pada paras yang tinggi dari 19.86 L biogas / hari ke 64.56 L biogas / hari dan 17.79 L biogas / hari ke 46.76 L biogas / hari telah dihasilkan dalam termofilik dan mesofilik ASGCB. Pengeluaran gas metana harian adalah dari 14.92 L CH_4 / hari ke 44.54 L CH_4 / hari dan dari 12.92 L CH_4 / hari ke 30.81 L CH_4 / hari untuk kedua ASGCB. Kandungan gas metana adalah antara 75.11% kepada 68.99% dan 72.50% kepada 65.90% untuk kedua ASGCB. Anaerobik degradasi proses untuk air sisa POME melalui acidogenesis, acetogenesis, methanogenesis dan sulphidogenesis telah berlaku dalam termofilik dan mesofilik ASGCB. Anaerobik degradasi proses melalui acidogenesis dan acetogenesis telah berlaku di peringkat awal dalam anaerobik degradasi air sisa POME. Asid palmitik daripada air sisa POME telah diuraikan kepada rantaian

pendek asid lemak seperti asetik, propionik, butyrik dan iso-butyrik melalui proses degradasi acidogenesis. Rantaian pendek asid lemak seperti asetik, propionik, butyrik dan iso-butyrik kemudian diuraikan kepada asid asetik sebagai produk tunggal dalam prosess degradasi acetogenesis. Akhir sekali, asid asetik telah ditukar kepada metana gas dan karbon dioksida gas dalam proses degradasi methanogenesis. Imbangan jisim, persamaan kadar tindak balas dan model Monod telah digunakan dalam termofilik dan mesofilik ASGCB dalam kajian penentuan pekali kinetik. Penilaian pekali kinetik berasaskan termofilik ASGCB telah menghasilkan pekali seperti yang berikut: Y_G (0.364 gVSS / g COD), b (0.103 / hari), μ_{max} (0.58 / hari), k_s (31.90 g COD / L), Θ_c (1.72 sehari) dan Y_{CH4} (0.45 L CH_4 / $COD_{reduction}$). Manakala, penilaian pekali kinetik untuk mesofilik ASGCB menghasilkan pekali seperti yang berikut: Y_G (0.357 gVSS / g COD), b (0.073 / hari), μ_{max} (0.36 / hari), k_s (27.60 g COD / L), Θ_c (2.78 sehari) dan Y_{CH4} (0.34 L CH_4 / $COD_{penurunan}$). Nilai pekali kinetik metana gas pada paras 0.45 L CH_4 / $COD_{penurunan}$ dan 0.34 L CH_4 / $COD_{penurunan}$ daripada termofilik dan mesofilik ASGCB merupakan nilai pekali yang paling tinggi setakat ini dalam anaerobik degradasi proses untuk air sisa POME.

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ABSTRACT

The anaerobic degradation of palm oil mill effluent (POME) was carried out under thermophilic (55^0C) and mesophilic (35^0C) temperature in anaerobic suspended growth closed bioreactors (ASGCB). The termophilic and mesophilic ASGCB were operated by a series of continuous experiments using feed flow-rates of 0.58, 0.70, 0.88, 1.17 and 1.75 liters of raw POME wastewater per day, which correspond to hydraulic retention time (HRT) of 24, 20, 16, 12 and 8 days. The COD reduction efficiency of the thermophilic and mesophilic ASGCB was in the range of 90.90% - 83.58% and 89.66% - 79.83%, respectively, between the HRT 8 days and 24 days. The pH for both ASGCB were in the range of 8.05 to 7.74 and 7.80 to 7.35. High amounts of biogas were produced from 19.86 L biogas/day to 64.56 L biogas/day and 17.79 L biogas/day to 46.76 L biogas/day, respectively. The daily methane gas production were from 14.92 L CH_4/day to 44.54 L CH_4/day and from 12.92 L CH_4/day to 30.81 L CH_4/day, respectively, in the thermophilic and mesophilic ASGCB. The methane gas content ranged from 75.11 % to 68.99 % and 72.50 % to 65.90 % for both ASGCB. The thermophilic and mesophilic ASGCB followed the acidogenesis, acetogenesis, methanogenesis and sulphidogenesis process of degrading the POME wastewater. The acidogenesis and acetogenesis anaerobic degradation occurred in the early stage of anaerobic degradation of POME wastewater. In the acidogenesis stage, the palmitic acids of POME wastewater were degraded to short chain fatty acid such as acetic, propionic butyric and iso-butyric

acids. The acetogenesis anaerobic degradation took place to breakdown the propionic, butyric and iso-butyric acids to the sole end product of acetic acid. Lastly, the acetic acids were converted to methane and carbon dioxide in the methanogenesis process. Mass balance, reaction rate equation and Monod models were followed in both ASGCB in order to determine the kinetic coefficients. The kinetic coefficients for the thermophilic ASGCB produces the following biological kinetic coefficients: Y_G (0.364 gVSS/ g COD), b (0.103/day), μ_{max} (0.58/day), K_s (31.90 g COD/L) , Θ_c (1.72 day) and Y_{CH4} (0.45 L CH_4/ $COD_{reduction}$), respectively. The kinetic coefficients for the mesophilic ASGCB produces the following biological kinetic coefficients: Y_G (0.357 gVSS/ g COD), b (0.073/day), μ_{max} (0.36/day), K_s (27.60 g COD/L) , Θ_c (2.78 day) and Y_{CH4} (0.34 L CH_4/ $COD_{reduction}$), respectively. The value of Y_{CH4} of 0.45 L CH_4/ g $COD_{reduction}$ and 0.34 L CH_4/ g $COD_{reduction}$ for thermophilic and mesophilic ASGCB are so far the highest methane yield for POME wastewater in the anaerobic degradation process.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Palm oil is one of the leading commodities in Malaysia. Currently, Malaysia's palm oil export accounts for 44% of world exports and 39% of world palm oil production in the year 2013 (MPOC,2014). The amount of palm oil production will continuously increase in fulfilling the growing global need for oil and fats products. Although palm oil industry is a major revenue earner for our country but it generates a tremendous amount of wastewater known as palm oil mill effluent (POME). POME is characteristic with high organic content and acidic nature causing environmental problems.

In palm oil industry, POME is mainly produced through the process sterilization of fresh oil palm fruit bunches and also the clarification of crude palm oil (Zinatizadeh et al., 2006). It is estimated that 5-7.5 tonnes of water are used for producing a tonne of crude palm oil and 50% of that water will turn up in POME (Chin et al., 2013). In the year 2011, it was reported that 56 million tonnes of POME were generated from 426 palm oil mills in Malaysia (Chin et al., 2013; MPOB, 2014a). This massive quantity of POME could cause watercourse pollution and create odour problems to the neighbourhood of the palm oil mills.

In general appearance, POME is a viscous, brownish liquid containing about 95-96% water, 0.6-0.7% oil and 4-5% total solids (including 2-4% SS, mainly debris from fruit). It is acidic (pH 4-5), hot (80-90°C) with an average of chemical oxygen demand (COD) and biological oxygen demand (BOD) values of 50,000 mg/L and 25,000 mg/L, respectively (Najafpour et al., 2006). Thus, there is a need to prevent environmental pollution due to an increase of crude palm oil production. The Malaysian government, therefore, enacted the Environmental Quality Act (EQA 1974) (Prescribed Premises) (Crude Palm Oil) Regulations in 1977 with its amendment in 1982. Sections 18 (1) and 19 of the act that relate to palm oil mill processing industry, has thereafter, set parameter limits for the discharge of POME into the environment as shown in Appendix A.

Biological treatment consisting of anaerobic, facultative and aerobic pond systems has been applied in the palm oil industry since 1982 (Ashhuby et al., 1996). Most of these biological treatments are waste stabilization pond for the treatment of POME. The waste stabilization pond system consists of essentially number of ponds with different functions such as anaerobic, facultative and aerobic process. Anaerobic degradation process is the most effective treatment that has been applied in Malaysia through either pond system or close digesting tank systems for the treatment of highly concentrated POME wastewater. This is because the anaerobic degradation process consists of the following advantages such as (a) less energy consumption, (b) minimum sludge formation, (c) low operation cost, and (d) production of methane gas as energy resource (Rincon et al., 2006).

Anaerobic degradation process is defined as the engineered methanogenic decomposition of organic matter. It is a complex process which involves the decomposition of organic compounds in the absence of molecular oxygen and production of methane (CH_4), hydrogen (H_2) and carbon dioxide (CO_2) gases. The degradation process takes place by different types of anaerobic bacteria. The degradation mechanisms involve hydrolysis, acidogenesis (including acetogenesis) and methanogenesis (Zhang et al., 2010). Hydrolysis is the first conversion reaction where complex molecules such as carbohydrate, lipids and protein are hydrolyzed to sugar, volatile acids, etc. Acidogenic bacteria are then responsible for breaking down the sugar, volatile acids and amino acids in the acidogenesis process. Besides, the methanogenesis process occurs in which hydrogenotrophic methanogens utilize the hydrogen and carbon dioxide gases produced by acetoclastic methanogens from short chain fatty acids to produce beneficial end product of methane gas (Siddique et al., 2011). Therefore, the anaerobic degradation process offers high potential for rapid disintegration of organic matter to produce biogas that can be used to generate electricity and save fossil energy (Linke, 2006).

A significant amount of biogas is produced from the anaerobic treatment of POME wastewater. The concentration of the methane emission may differ depending on the variation in the POME treatment practices. Biogas produced from the anaerobic degradation process of POME wastewater includes methane, carbon dioxide, a small amount of nitrogen, hydrogen and hydrogen sulfide (Basri et al., 2010). Methane gas and carbon dioxide gas are classified as greenhouse gases released to the atmosphere causing global warming. The COD content in POME is allied with the amount of methane gas produced. Literature study shows that 0.238 kg of methane can be produced per kilogram of COD reduction (Yacob, et al, 2006). Therefore, many researches on the POME wastewater towards production of biogas have been carried out.

Currently, the research study on anaerobic degradation of POME wastewater are focusing on the following contents: (a) production of methane gas, (b) production of hydrogen gas, (c) performance of various types of anaerobic bioreactor, (d) kinetic study, (f) temperature effects of thermophilic and mesophilic conditions, and (g) applying known anaerobic bacteria for better outcomes of anaerobic process. Nevertheless, the mechanisms of anaerobic degradation for POME wastewater such as (a) volatile fatty acids profile, (b) biogas production profile and (c) identification of mix culture anaerobic communities involved are not deeply discussed. Hence, it is essential to find out the degradation pathway of volatile fatty acids towards the generation of biogas from POME wastewater by different types of anaerobic bacteria. This new knowledge could be a reference for the palm oil industry to obtain high operational efficiency in their anaerobic treatment of POME wastewater. Besides, common problems such as overload of POME wastewater, inconstantly applied of hydraulic retention time (HRT), low biogas yield and, etc. can be solved with the contribution of that new knowledge. Throughout this research, two laboratory-scaled anaerobic suspended growth bioreactors (ASGBR) were operated at thermophilic and mesophilic temperatures with different hydraulic retention times (HRT) in order to evaluate the volatile fatty acids profile, biogas production profile, bio-kinetics, and to identify the anaerobic microbial communities of anaerobic biodegradation process for POME wastewater.

1.2 Statement of Problems

Currently, 85% of the palm oil industry used the suspended growth anaerobic treatment process either in stabilization pond system or closed digesting tank (Wu et al., 2010; Chin et al., 2013). Hence, the literature study is abound with results of research on attached growth anaerobic treatment such as immobilised cell bioreactor (Borja et al., 1994), two stage up-flow anaerobic sludge blanket (UASB) (Borja et al., 1996), high rate up-flow anaerobic sludge fixed film (Zinatizadeh et al., 2006), and combined high-rate anaerobic reactors (Choi et al., 2013) for the treatment of POME wastewater. Little research has been conducted on the application of anaerobic suspended growth treatment for better control of the POME wastewater anaerobic degradation process. Therefore, this research study using suspended growth anaerobic bioreactor could provide a scientific and engineering basis to be a reference for the palm oil industry.

As mentioned earlier, the large quantity of POME wastewater is generated with the increasing production of crude palm oil. Most of the palm oil industry is facing the common problem of overloading POME wastewater into the anaerobic treatment either in stabilization pond system or closed digesting tank. This scenario has caused poor performance of anaerobic degradation process. Thus, high level of COD and BOD treated POME wastewater is discharged to a nearby watercourse. It could pollutes the aquatic habitats of freshwater, estuarine and marine ecosystems, even affect the aquatic life and adversely impacts human health.

In relation to that, several POME wastewater treatment plants have been successfully operated but majority of the plants are still struggling to observe the Malaysian discharge standards under Environmental Quality Act (EQA 1974) (Prescribed Premises) (Crude Palm Oil) Regulations in 1977. There are little information of having high performance on anaerobic treatment process for POME wastewater that can be referred by them. Therefore, the anaerobic degradation process of POME wastewater always fails in most palm oil factories. An under-designed wastewater plant to cope with ever growing production of crude palm oil and lacking operational control for an anaerobic process are a serious problem to most of them. Therefore, the current study could provide useful information on operational control in the anaerobic degradation process of POME wastewater.

For better control of anaerobic degradation process with high operational efficiency, appropriate loading of POME wastewater is an essential knowledge. Heavy load of organic loading rate (OLR) can cause an up-set or failure to an anaerobic treatment process. It also could lead to an accumulation of volatile fatty acids in the anaerobic degradation process that inhibited the activity of anaerobic bacteria. Thus, it is a need to study the volatile fatty acid profile in order to select suitable OLR for the anaerobic degradation of POME wastewater. With this information, the authorized personnel of palm oil industry can easily operate and control the anaerobic degradation process.

High production rate of biogas from POME wastewater provides potential boost in the renewable energy sector in Malaysia thus encourages the palm oil industry to apply closed anaerobic digesting system. Instability OLR loading of POME wastewater into the anaerobic treatment plant causes low production of biogas. The present study focused on the biogas production profile with different hydraulic retention time to maintain stability of biogas production from anaerobic degradation process. High methane yield can be reached with excellent calorific value for generating renewable energy. Moreover, the amount of hydrogen sulfide gas (H_2S) and carbon dioxide gas (CO_2) can be obtained from the biogas profile study. H_2S and CO_2 have to be removed because sulfuric acid, and carbonic acid can be readily formed when the gases react with water that could corrode or damage the turbine engine of power generation.

Kinetic study is required for a better understanding of the underlying phenomena in the anaerobic digestion process. The kinetic coefficients from the present study could provide valuable knowledge to the palm oil industry for high-quality operational control of the anaerobic process. This is because most of the palm oil mills are facing under design or overload problems in their wastewater treatment plant. The sudden shock load of POME wastewater to the anaerobic process will destabilize, resulting with the wash out of active anaerobic bacteria and a decrease in the performance. Low performance and high generation of sludge were recorded in the anaerobic treatment process. Therefore, there is a need to carry out kinetic study for predicting the compounds produced or consumed at their corresponding rates in the anaerobic treatment. This could avoid the anaerobic degradation of POME wastewater from operating at the critical retention time.

1.3 Objectives of the Study

This research aims to scrutinize the biogas production and volatile fatty acid profiles study for POME wastewater in the anaerobic suspended growth closed bioreactor (ASGCB) at thermophilic and mesophilic temperatures. The following are the specific objectives of the present study:

a) To compare the operational parameters and biogas production between mesophilic and thermophilic anaerobic degradation processes under various hydraulic retention time (HRT).

b) To identify the degradation pathway of anaerobic degradation of POME.

c) To determine the phase behaviour of fatty acids and biogas in anaerobic degradation of POME at mesophilic and thermophilic temperature profiles.

d) To evaluate the kinetic coefficients of anaerobic degradation of POME.

e) To identify the microbial community of anaerobic degradation of POME.

1.4 Scope of the Study

The treatment of POME wastewater is in demand due to the pollution problems created from the huge volume of wastewater generated by the palm oil industry. The anaerobic digestion process is the primary focus in this study. The approach is to treat POME wastewater under various hydraulic retention time (HRT) in two separate anaerobic suspended growth closed bioreactors (ASGCB). The ASGCB was operated based on the anaerobic closed digesting process as the current practise in the most palm oil mills. The ASGCB was operated at two different temperatures of thermophilic (55^{o}C) and mesophilic (35^{o}C) condition. A series of continuous experiments using feed flow-rates of 0.58, 0.7, 0.88, 1.17 and 1.75 liters of raw POME per day, corresponding to Hydraulic Retention Time (HRT) of 24, 20, 16, 12 and 8 days was carried out in the research study. The rate of influent substrate concentration of COD was controlled in the range of 62500-65500 mg/L.

The samples from ASGCB were collected and subjected to the analysis of parameters such as feed and effluent of the total and soluble COD, ASGCB pH, feed and effluent volatile fatty acid (VFA), ASGCB total alkalinity, ASGCB suspended solid (SS) and volatile suspended solid (VSS) for the purpose of operational performance study at each batch of HRT. Therefore, the screening of the best or most suitable HRT can be defined from the operational performance study of ASGCB for the treatment of POME wastewater at two different temperature profiles.

The degradation pathway of the anaerobic degradation of POME wastewater is observed based on the volatile fatty acids and biogas profiles. The volatile fatty acids such as acetic, propionic, butyric and iso-butyric acids were analyzed by using gas chromatography–mass spectrometry (GCMS) analytical method. The biogas such as methane, carbon dioxide, hydrogen and hydrogen sulfide were analyzed by using gas chromatography (GC) analytical method.

Another part of the research involves the evaluation of the kinetic coefficients for the treatment of POME. The kinetic constant of the anaerobic digestion process is a useful tool to describe and to predict the performance of the anaerobic process. In this study, the ASGCB was continuously operated until steady state condition at each batch of HRT was reached in order to determine the kinetic constants. The substrates of COD were selected to analyze the kinetic coefficients of ASGCB at thermophilic and mesophilic temperatures. The kinetic coefficients of ASGCB includes values for growth yield, Y_G, specific biomass decay rate, b, maximum specific biomass growth rate, μ_{max}, saturation constant for substrate, K_s, critical retention time, Θ_c and methane yield, Y_{CH4}. Finally, the identification of anaerobic microbial communities was conducted for both ASGCB by using 16S rDNA analytical method.

1.5 Organization of the thesis

This thesis consists of five chapters. A brief introduction on the status of the palm oil industry; POME wastewater characteristics; regulatory enforcement towards the discharge of effluent; environmental issues of POME wastewater; volatile fatty acids; biogas production; operational control of anaerobic degradation process and kinetic coefficients are given in Chapter one (Introduction). This chapter also includes problem statements that provide some basis and rationale to identify the research direction to be followed in this study. The objectives of the study are stated together with the scope of the research to be covered. The organization of the thesis is also presented in the last section of the chapter.

Chapter two (literature review) covers the review of the history of palm oil industry; processes of crude palm oil production; POME wastewater characteristic and pond treatment system. This chapter also presents the detailed information and specific topics relevant to anaerobic digestion process and kinetic coefficients model development used in this study.

Chapter three (Material and methodology) describes in detail the materials and methods used in the present work. This is followed by the detailed experimental setup, sampling procedures, operational processes, biogas and volatile fatty acids profiles study, kinetic coefficients determination, anaerobic bacteria identification and analysis of sample in thermophilic and mesophilic anaerobic suspended growth closed bioreactor (ASGCB).

Chapter four (Results and discussion) presents the results of the five main studies. The acclimatization phase of thermophilic and mesophilic ASGCB was carried out at the beginning of the study. Then, a discussion is presented on the operations of thermophilic and mesophilic ASGCB at different hydraulic retention time (HRT). A comparison of the results obtained with the thermophilic and mesophilic ASGCB in the present study with those obtained in other work is included. The detail of the degradation pathway for the anaerobic degradation process of POME wastewater is further discussed in this chapter. The VFA and biogas profile study of thermophilic and mesophilic ASGCB were also included in this chapter. The kinetic coefficients of both ASGCB are determined from the steady state condition of each batch HRT. Besides, the identification of anaerobic bacteria was conducted in both ASGCB mix sludge samples at the end of each batch operating HRT. Lastly, the outcomes of the present work are applied in the design calculation to the existing industrial scale for the treatment of POME wastewater.

Chapter five (Conclusions and recommendations) states the conclusions and recommendations from the present study. The conclusions are based on the results obtained toward the objectives of this study. This is followed by the recommendations and suggestions for the future studies in this related field.

CHAPTER TWO

LITERATURE REVIEW

2.1 Palm Oil Industry in Malaysia

2.1.1 History and Development of Palm Oil Industry

Oil palm tree (*Elaeis guineensis*) originated from West Africa where it was grown wild and later developed into an agriculture crop. It was first introduced to Malaysia in the early 1870's as an ornamental plant. In the year 1917, the first commercial planting took place at Tennamaran Estate in Selangor, laying the foundation for the vast oil palm plantations and palm oil industry in Malaysia (MPOC, 2014).

Due to the suitable climatic condition, the oil palm plantation develops well in Malaysia. Currently, 5 million hectares of Malaysia land are occupied for palm oil plantation (Ishani and Benjamin, 2014). Malaysia is currently the second largest producer of palm oil in the world after Indonesia with total production of 18 million tonnes of crude palm oil in the year 2013 (MOPB, 2014b). The amount of crude palm oil produced will keep increasing to fulfill the ever growing global market for oils and fats products.

2.1.2 Standard Wet Mill Process and Generation of Residues of Oil Palm

In Malaysia, the wet palm oil milling process is the most common way of extracting crude palm oil (CPO) from fresh fruit bunches (FFB). The Malaysia Palm Oil Board (MPOB) reported that about 426 palm oil mills were in operation with a total capacity of 62.6 million tonnes of FFB in the year of 2013. Generally, 3 tonnes of Palm Oil Mill Effluent (POME) was generated by each ton of CPO produced (MPOB, 2014b).

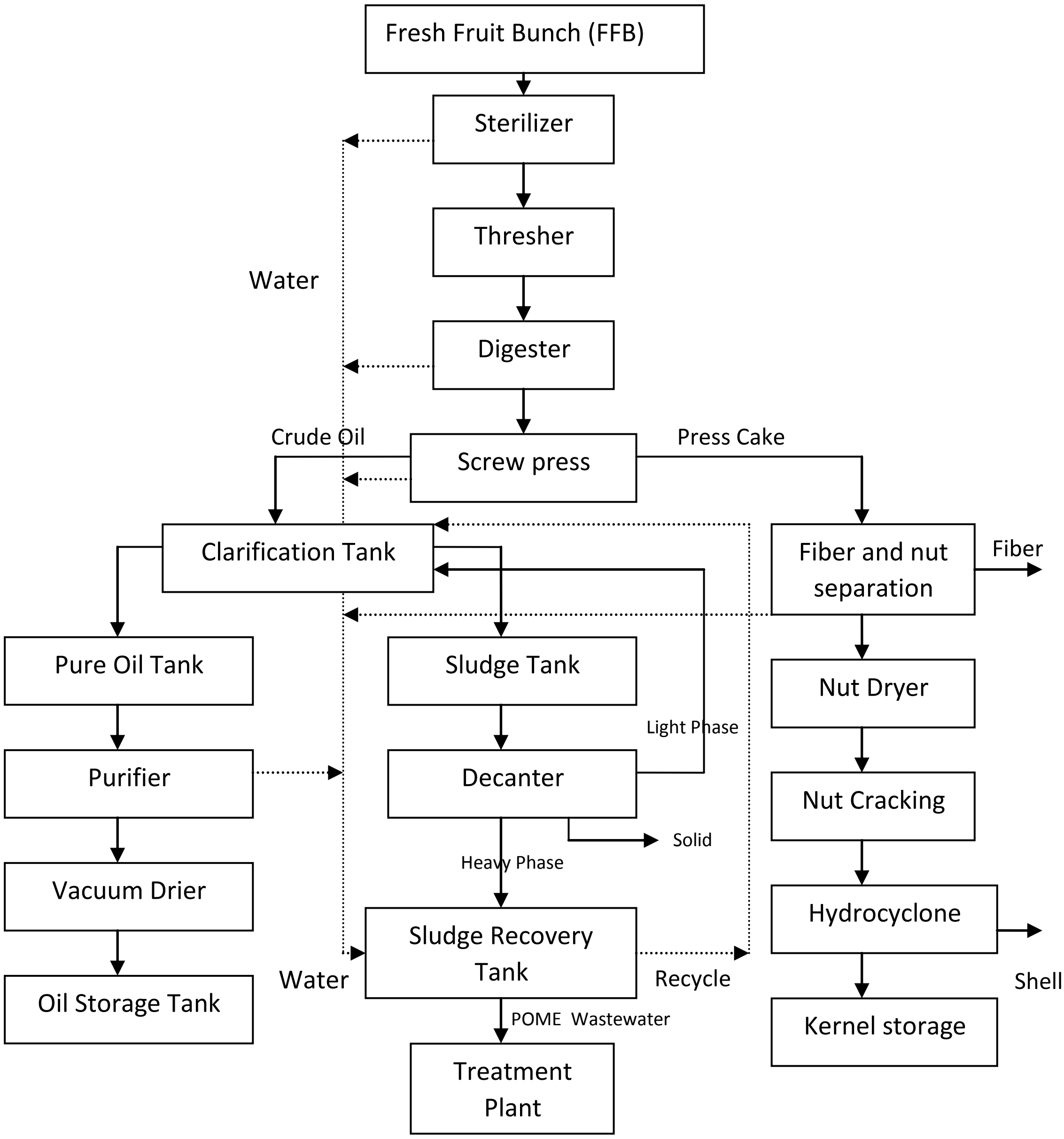
Figure 2.1 illustrates the flow diagram of crude palm oil extraction process and typical production of POME wastewater. Bunches of FFB harvested in the palm oil estate are sent to the palm oil industry for processing. The CPO production capacity of the palm oil industry is in the range between 10 and 60 tonnes FFB/hr. The FFB harvested from the oil palm plantation have to be processed immediately to prevent poor quality of CPO due to the increase of free fatty acid content.

On arrival, the FFB are subjected to steam-heat treatment in the horizontal sterilized for around 1 hour at the temperature of about 140ºC and the pressure at 40 N/m^2. The purpose of the sterilization process is to soften the oil palm fruits so that it can easily be detached from the stalk while threshing. The duration, temperature and pressure control of sterilization are dependent on the age and growth of the FFB. The detached fruits are further softened with steam in the digesters. The digester mashes the fruits and then is passed through the screw press where the oil and the juice from the fruits are extracted. Here, the compressed crude palm oil may contain approximately 48% oil, 45% water and 7% solids (Chow and Ho, 2000).

After the screw press process, the crude oil flows to the clarification tank while the press cake is sent to the fiber and nut separation process. The fiber is used as a source of fuel for the boiler process that generates superheated steam in the digester process. The nut is dried off in the nut dryer. Then, it is cracked off in the hydro-cyclone process to separate the kernel and shell. The kernel is collected and stored in the kernel storage while the shell is burned to generate heat for boiler process. The boiler is an important equipment for generating heat and steam for the sterilizer, thresher and digester process. The steam condensate from the sterilizer, thresher and digester process are further injected into the respective machinery to maintain a high temperature required throughout the milling process.

In the clarification tank, hot water is added to the crude palm oil (CPO) to reduce the viscosity so that CPO can float on the surface. The upper layer of crude palm oil is channelled to the pure oil tank. The lower section of the clarification tank is channelled to the sludge tank. A decanter process is applied after the sludge tank for settled and removed of solids and water contain. The light phase containing CPO from the decanter is pumped back to the clarification tank. The raffinate of the decanter process is pumped to the sludge recovery tank. The remaining solids are removed from the decanter process. The hot water from the sterilizer and the digester process is channelled into the sludge recovery tank.

Two layers of oil phase and water phase are formed in the sludge recovery tank. The lighter phase from the sludge recovery tank, which consists of oil is recycled to the clarification tank. The heavier aqueous phase is discharged to the wastewater treatment plant and is known as POME wastewater. Finally, the CPO is skimmed off the surface of clarification tank. It will be purified, vacuum dried and stored in the CPO storage tank until it is transported to the refinery industry for further processing (Chow and Ho, 2000).



Sources: MALPOM Industries Sdn Bhd, (2014)

Figure 2.1: Schematic diagram of palm oil extraction process

2.1.3 Palm Oil Mill Effluent (POME)

Through the wet extraction process of crude palm oil, a large amount of wastewater is produced. The wastewater is known as Palm Oil Mill Effluent (POME). POME is considered as one of the most polluting agro-industrial residues due to its high organic load. It is in the form of highly concentrated dark brown colloidal slurry of water, oil and fine cellulosic materials, at 80°C and 90°C from sterilisation and clarification stages (Ahmad et al., 2005). For every ton of oil palm fresh fruit bunch, it is estimated that 0.5-0.75 tons of POME will be released (Yacob et al., 2006). POME is a colloidal suspension, containing 95 – 96% of water, 0.6 – 0.7% of oil and grease and 4 – 5% of total solids (Ma, 2000). This highly polluting wastewater can create odour problems to the neighbourhood of the mills, a nuisance to the passers-by or residents and river pollution. Table 2.1 shows the refined characteristic of POME from the literature.

Table 2.1: Characteristics of POME wastewater

Parameter	Unit	Range
pH[a,b,c]	-	4-5
Biochemical oxygen demand (BOD)[a,b,c]	mg/L	25,000 - 65,000
Chemical oxygen demand (COD)[a,b,c]	mg/L	44,500 - 85,000
Total Solids (TS)[a,c]	mg/L	30,500 - 55,000
Suspended Solids (SS)[a,c]	mg/L	18,000 - 36,500
Volatile Solids (VS)[a,c]	mg/L	34,000 - 49,500
Oil and grease (O and G)[a,b,c]	mg/L	4000 - 9000
Ammonia Nitrogen (NH_3 -N)[a]	mg/L	35 -105
Total Nitrogen[a]	mg/L	750 - 770
Volatile fatty acids (VFA)[a,c]	mg/L	1500 - 7500
Palmitic Acid[c]	mg/L	7400 - 9500
Acetic Acid[a,c]	mg/L	1900 - 3200
Carbon[b]	%	40 -4 8
Hydrogen[b]	%	5.3 - 9.1
Nitrogen[b]	%	0.9 - 2.7
Sulfur[b]	%	0.5 - 1.3
Oxygen[b]	%	21 - 55

Source: ([a] Zinatizadeh et al.,2006; [b] Jeong et al., 2014; [c] Chin et al., 2014)

2.2 Palm Oil Mill Effluent Wastewater Treatment

The treatment employed for POME wastewater in Malaysia follows to a great extent the principles of biochemical operations. Three different types of biochemical treatment operations are used. These include: a) open tank digester and extended aeration system (Lim et al., 1984), b) closed anaerobic digester and land application system (Ma, 1999), and c) pond treatment system (Lim et al., 1984). The choice of treatment system depends to a large extent on the company's preference, location of the mill and availability of usable land. However, the pond treatment system is the most popular as it is adopted by more than 85% of the mills in Malaysia nowadays (Ma, 1999; Chin et al., 2013).

2.2.1 Pond Treatment System

Ponds have been widely used as a method of sewage disposal since the ancient times (Gray, 1992). Most of the pond systems that have been applied for the treatment of POME wastewater in Malaysia are classified as waste stabilization pond. According to Arceivala (1998), stabilization pond is similar to an activated sludge process but differs in the following ways: Stabilization ponds have i) long retention period, ii) low loading rate, iii) less active microbial biomass, and iv) less mixing and agitation where the particulate solids settle and form sludge layer in which the anaerobic process breakdown occurs. The configuration of the pond system consists of mostly a number of ponds of different functions such as anaerobic, facultative and aerobic ponds, which are made up of earthen structures with no lining (Ma, 1999).

2.2.2 Anaerobic ponds

Anaerobic ponds for POME wastewater treatment consist of at least two ponds connected in series to other ponds. The raw POME wastewater is directly channelled into the anaerobic pond from the sludge recovery tank. Anaerobic pond system is very effective in the treatment of effluents with high strength; biodegradable organic contents (BOD>500) generated in large quantities from agricultural and food industries (Gray, 1992). Anaerobic ponds are usually designed with deeper basins than the other ponds in order to reduce the surface area to volume ratio thereby minimizing re-aeration (since oxygen transfer through the air-water interface is undesirable) and heat loss (Gray, 1992). The anaerobic ponds for POME wastewater treatment in Malaysia are usually 5-7 meters in depth (Chooi, 1984). Three zones can be identified in the pond. They include: the scum layer, the supernatant layer and the sludge layer (Kosaric, 1992). Anaerobic reaction takes place in the sediment include solubilization of biodegradable particulate matter followed by hydrolysis, acidogenesis, acetogenesis and methanogenesis process (Rajbhandari and Annachhatre, 2004).

Anaerobic ponds are used as preliminary treatment for high strength organic wastes, and for partial stabilization of the waste before secondary treatment takes place. Organic loading is considerably reduced, and the retention time needed is long (Ashhuby et al., 1996). The organic loading for POME treatment varies from 0.2-0.35 kg BOD/m3/day with a minimum of 30 days HRT (Ma, 1999; Poh and Chong, 2009; Mumtaz et al., 2010).

2.2.3 Facultative Ponds

Facultative ponds are characterized by having an upper zone of aerobic and a lower zone of anaerobic process occurring simultaneously (Gray, 1992). As the digested effluent enters the basin from the anaerobic pond, the settleable and flocculated colloidal matter settle to the bottom to form a sludge layer where organic matter decompose anaerobically (Rajbhandari and Annachhatre, 2004). The remainder of the organic matter either soluble or suspended, passes into the body of the water where decomposition is mainly aerobic or facultative and rarely anaerobic (Gray, 1992). Aerobic and facultative bacteria are the primary decomposers although fungi may be present in the pond system. The fungi is present because of the high pH caused by the photosynthetic activity of the algae (Arrceivala, 1998). The dominant bacteria are genera Pseudomonas, Achromobacter and Flavobacterium. The soluble degradation products such as ammonia, organic acids, and inorganic nutrients are also released and subsequently oxidized aerobically in the water layer. Facultative ponds are much shallower than the anaerobic ponds, and are usually 1-1.5 meters in the depth in order to maintain dissolved oxygen in the basin. Depth above the upper limit may cause some odour problem, while depth below 0.7 m may encourage growth of rooted aquatic weeds, which may not only damage the lining of the pond and hinder circulation but also attract mosquitoes and other flies (Gray, 1992). The retention time of facultative ponds depends on load, depth, evaporation rate, and loss by seepage, but are shorter than that of anaerobic ponds. The HRT for POME wastewater treatment is between 8-16 days (Ashhuby et al., 1996; Lam and Lee, 2011).

2.2.4 Aerated Ponds

Aerated ponds are applied in tertiary treatment process after facultative process to increase the effluent quality (Grady et al., 1999). The effluent quality is improved by reducing the concentration of ammonia, nitrate, phosphate and suspended solids that remain untreated from the secondary treatment process of anaerobic and facultative process. Two types of aerated pond system, namely the aerobic pond and the aerobic-anaerobic pond processes are currently applied for waste stabilization. Total concentration of suspended solids is equal in the aerobic pond. In aerobic-anaerobic pond, aeration happens at the top layer of the aerobic pond. Not all the suspended solids concentration maintains in the aerobic-anaerobic pond process. Hence, the insufficient aerated suspended solids sink to the bottom of the pond. The bottom layer of the aerated pond undergoes for the anaerobic process. As a result, de-sludging practice is always carried out for the aerobic-anaerobic pond process because it contains high concentration of suspended solids. About 70% to 90% of BOD reduction efficiency can be achieved in aerobic pond system, but the effluent may contain relatively high concentration of suspended solids that gave the turbid appearance. Therefore, installation of settling tank or shallow pond for removal of solids should be carried out after aerobic pond process.

The aerated pond used in the treatment of POME wastewater in Malaysia can be described as aerobic-anaerobic pond. The current aerated pond contains high concentration of suspended solids, and highly turbid effluent from the pond has to be channelled to the settling pond before final discharge. Some aerobic ponds of POME wastewater treatment are equipped with mechanical surface aerators for oxygen supply. The hydraulic retention times for recovery tank, acidification, anaerobic, facultative and aerobic ponds are 1, 4, 45, 8 and 8 days, respectively (Ashhuby et al., 1996; Ma, 1999). For the purpose of cost minimization, many palm oil mills in Malaysia do not apply aerated pond because of energy consumption in operating the aeration pump. In these cases, the HRT for the facultative pond system should be increased to 16 days. The pond system has however been reported to be reliable, stable and capable of producing good final quality effluent (Chan and Chooi, 1982; Chooi, 1984; Poh and Chong, 2009; Mumtaz et al., 2010).

2.3 Anaerobic Digestion Process

The anaerobic digestion process stabilizes a wide variety of organic materials and concurrently produces methane from the digestion process. Various kinds of organic materials in sewage sludge, municipal solid waste, industrial wastewater and agriculture waste are degraded and ultimately converted into methane (CH_4) and carbon dioxide (CO_2) gases in the anaerobic digestion process (Annachhatre, 1996). Microorganisms are used under anaerobic condition (absent of oxygen) in the anaerobic digestion to degrade organic matters (Raymond, 1974).

Anaerobic digestion is the primary biological treatment process for high organic strength wastewater, since it produces less sludge compared to aerobic process. The anaerobic digestion of organic waste has been performed for about a century and has advantages over aerobic treatment process because of its high organic removal rates, low energy requirement, low sludge and energy production (Angenent et al., 2004). Fig 2.2 illustrates the scheme of anaerobic degradation process for POME wastewater. At first, the complex materials such as polysaccharides, proteins and neutral fats are hydrolyzed into the component monomers of monosaccharides, amino acids and long chain fatty acids by the extracellular enzymes. These monomers are then fermented into intermediates such as volatile fatty acids as acetate, propionate and butyrate acids and also hydrogen gas. These intermediates are ultimately converted to methane gas (Gee and Chua, 1994; Toprak, 1994: Guerrero et al., 1999).

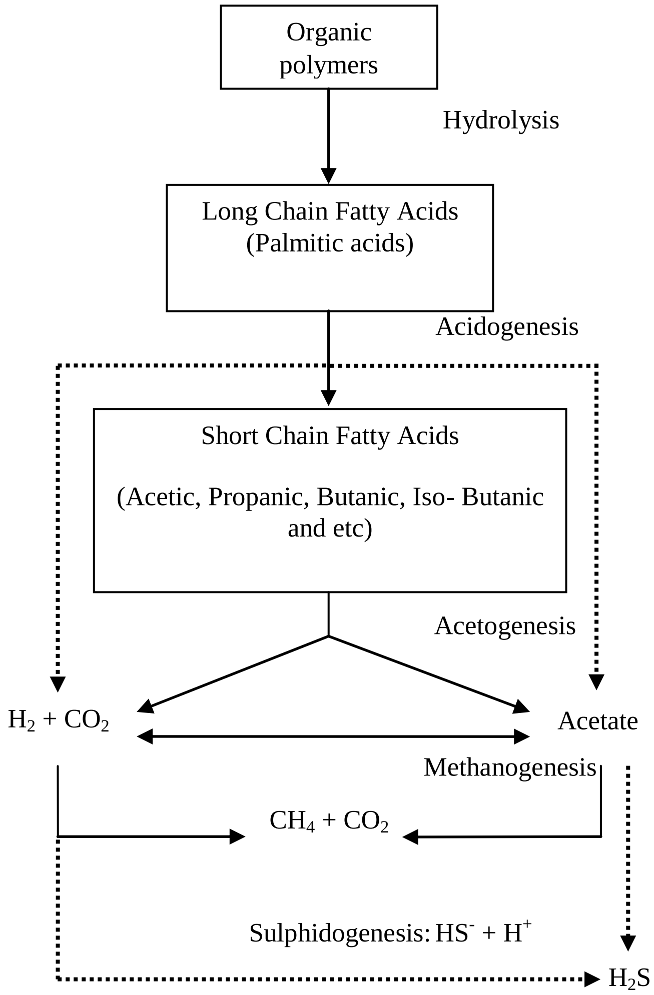


Figure 2.2: Scheme of the anaerobic degradation process for POME wastewater.

The hydrolysis phase is sometimes regarded as a part of the acidogenesis phase (Rajbhandari and Annachhatre, 2004). Toerien and Hattingh (1969) named hydrolysis and acidogenesis phase as non-methanogenic phase because the hydrogen gas as compound other than acids are formed at this stage. Speece (1996) named hydrolysis and acidogenesis phase as constant BOD phase and methanogenesis phase as BOD reduction phase because only methane formation occurred. Throughout anaerobic digestion, organic materials in the solids state are liquefied in hydrolysis process. Besides, the soluble organic matters are gasified at methanogenesis process. Various types of microbial communities are involved in the anaerobic degradation process. The reaction in the acidogenesis phase occurs by a group of bacteria called acidogenic bacteria while the methanogenic bacteria and hydrogen producing acidogenic bacteria are responsible for the methanogenesis phase (Chynoweth et al., 1999).

2.3.1. Hydrolysis

The first phase in anaerobic digestion process is hydrolysis in which complex substrates such as carbohydrates, proteins, and lipids are converted into their respective smaller atomic mass soluble organic monomers such as sugar, alcohol, amino acids and volatile fatty acids (Reeta et al., 2013). Hydrolysis is a slow and rate-limiting stage in the acid forming phase (Speece, 1996). The smaller soluble organic matters are converted immediately to short chain volatile fatty acids along with carbon dioxide and hydrogen gases (Turovskiy and Mathai, 2006; Reeta et al., 2013). This is accomplished by several hydrolytic enzymes such as cellulases, cellobiose, xylanase, amylase, lipase and protease secreted by hydrolytic microbes (Lam and Lee, 2011). Equation 2.1 shows an example of a hydrolysis reaction converting an organic waste into a simple sugar (Speece, 1996):

$$\mathbf{C_nH_{4n}O_{2n}} \rightarrow \frac{\mathbf{n}}{6}\mathbf{C_6H_{12}O_6} + \mathbf{nH_2O} \qquad (2.1)$$

2.3.2 Acidogenesis (Fermentation)

Acidogenesis phase is also referred to as fermentation occurred with acidogenic bacteria to break down sugar, fatty acids and amino acid produced at the hydrolysis stage into simpler organic compounds. Acetic, propionic, butyric, and lactic acids are the most frequently generated end-products at this stage. These fatty acids are produced at the amount of 87%, 67%, 10% and 70%, respectively by the acid-forming bacteria (Gray, 2004). Overall, the acidogenic bacteria or acid forming bacteria convert the end products of hydrolysis stage into the following: (1) volatile acids such as acetate, butyrate, formate, lactate and succinate, (2) alcohol such as ethanol and methanol, (3) acetone, and (4) carbon dioxide, hydrogen and water (Gerardi, 2006). Final products of fermentation eventually become precursors of methane production (Lam and Lee, 2011). The conversion reaction in acidogenesis phase can be represented by:

$$\mathbf{C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2} \qquad (2.2)$$

In fermentative pathways, hydrogen (H_2) is produced. The production of hydrogen gas is necessary for an anaerobic digester where hydrogen is a significant substrate for the production of methane (CH_4). The level of hydrogen must be kept low because high pressure of hydrogen can inhibit the acetogenic bacteria in the anaerobic degradation process (Bruce and Perry, 2001; Gerardi, 2006;). Besides that, in an anaerobic digestion system, acidogenesis phase and methanogenesis are in dynamic equilibrium. After the conversion of organic matters into volatile acids and hydrogen, the latter are converted to methane and carbon dioxide (Speece, 1996).