EVALUATION OF MEMBRANE ANAEROBIC SYSTEM (MAS) FOR PALM OIL MILL EFFLUENT (POME) TREATMENT

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SUPERVISORS' DECLARATION

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I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Special Dedication to Father Lord, my supervisors, my parents,
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ABSTRACT

The direct discharge of Palm Oil Mill Effluent (POME) into river causes serious environmental pollutions due to its high content of organic matter which causes depletion of oxygen in water bodies. The conventional anaerobic POME treatment by using anaerobic ponding system requires longer Hydraulic Retention Time (HRT) and large space. In this study, the efficiency of Membrane Anaerobic System (MAS) in treating POME was investigated. The MAS is a system that consists of an anaerobic digester, a centrifugal pump and Cross-Flow Ultrafiltration (CUF) Membrane with Molecular Weight Cut-off (MWCO) 200 kDa which have been operated at the pressure of 1.5 bars to 2 bars. Three runs of the experiment had been done on three kinds of the POME samples, 50 % diluted raw POME (Run 1), anaerobic digested POME without addition of mixed culture inoculum (Run 2) and anaerobic digested POME with addition of mixed culture inoculum (Run 3). Throughout the study, the MAS system was performing better in treating anaerobic digested POME in term of overall Chemical Oxygen Demand (COD) removal efficiency in Run 2 and Run 3 compared to Run 1, which overall COD removal efficiency were 83.94 % (Run 2) and 77.98 % (Run 3). The overall Total Suspended Solid (TSS) removal efficiencies of MAS on POME were varied between 81.02 % and 99.95 % in three runs of the experiments. The overall Volatile Suspended Solid (VSS) removal efficiency of MAS on POME treatment was varied between 74.66 % and 87.87 %. The methane gas production was 80.79 %, 80 % and 78.26 % for Run 1, 2, 3 respectively. The addition of inoculum did not show any significant effect on methane production in Run 3. The methane gas production in MAS through anaerobic digestion of POME was found to be linearly in relationship with the VSS removal efficiency and organic loading.

ABSTRAK

Pembuangan langsung sisa dari kilang minyak kelapa sawit (POME) ke dalam sungai menyebabkan pencemaran alam sekitar yang serius disebabkan oleh kandungan bahan organik yang menyebabkan kekurangan oksigen dalam sungai. Rawatan POME dengan cara konvensional iaitu, sistem tasik anaerobik memerlukan ruang yang besar dan juga masa penahanan hidraulik (HRT) yang panjang. Dalam kajian ini, kecekapan MAS dalam rawatan POME akan dikaji. MAS adalah satu sistem yang terdiri daripada anaerobik bioreaktor, pam dan juga aliran silang Ultrafiltrasi (CUF) membran dengan pemotongan berat molekul (MWCO) 200 kDa yang beroperasi pada tekanan 1.5 bar hingga 2 bar. Tiga eksperimen telah dijalankan ke atas tiga jenis sampel POME, iaitu POME mentah yang dicairkan 50 % (Eksperimen 1), POME yang telah dirawat secara anaerobik tanpa penambahan bakteria (Eksperimen 2) dan POME yang telah dirawat secara anaerobik dengan penambahan bakteria (Eksperimen 3). Sepanjang kajian ini, sistem MAS didapati lebih cekap dalam penyisihan keperluan oksigen kima (COD) secara keseluruhan dalam Eksperimen 2 dan Eksperimen 3 berbanding dengan Eksperimen 1. Kecekapan penyingkiran COD secara keseluruhan dalam Eksperimen 2 dan 3 adalah sebanyak 83.94 % dan 77.98 % masing-masing. Ketiga-tiga eskperimen dalam kajian ini menunjukan kecekapan MAS dalam merawat POME dengan penyingkiran *jumlah pepejal terampai* (TSS) secara keseluruhan yang tinggi, iaitu di antara 81.02 % dan 99.95 %. Kecekapan MAS dalam penyingkiran jumlah pepejal meruap (VSS) adalah berbeza-beza antara 74.66 % dan 87.87 %. Pengeluaran gas metana dalam Eksperimen 1, 2 dan 3 adalah sebanyak 80.79 %, 80 % dan 78.26 % masing-masing. Dalam Eksperimen 3, penambahan bakteria ke dalam POME tidak menunjukkan sebarang kesan ke atas pengeluaran gas metana. Pengeluaran gas metana melalui pencernaan anaerobik POME dalam MAS adalah didapati berkadar langsung dengan kecekapan penyingkiran VSS dan beban organic dalam sampel POME.

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LIST OF SYMBOLS

%	-	Percentage
^{o}C	-	Degree Celcius
^{o}	-	Degree
cm	-	Centimeter
hr	-	Hours
g	-	Grams
g/L	-	Grams per liters
kDa	-	Kilo Daltons
Kg	-	Kilogram
L	-	Liters
M	-	Molarity (moles/ liters)
mg/L	-	Miligram per liters
mL	-	Mililiters
µL	-	Microliters
µm	-	Micrometer
m^2	-	Meter Squared
m^3	-	Meter Cubed
nm	-	Nanometer
ppm	-	Parts per millions
psi	-	Pound per square inch
rpm	-	Rotation per minute
x g	-	Gravity
v/v	-	Volume per volume

w/w	-	Weight per weight

LIST OF ABBREVIATIONS

ABFFR	-	Anaerobic Baffled-Fixed Film Reactor
AF	-	Anaerobic Filter
APHA	-	American Public Health Association
ATTC	-	Arachem Technical Training Centre
AU	-	Absorbance Unit
BOD	-	Biochemical Oxygen Demand
CER	-	Certified Emission Reduction
CDM	-	Clean Development Mechanism
coA	-	acetyl coenzyme A
COD	-	Chemical Oxygen Demand
CPO	-	Crude Palm Oil
CUF	-	Cross-flow Ultrafiltration Membrane
DGCE	-	Denatured Gradient Gel Electrophoresis
DO	-	Dissolved Oxygen
DOE	-	Department of Environmental
e.g.	-	for example
EPA	-	Environmental Protection Agency
Eq.	-	Equation
EQA	-	Environmental Quality Act
etc.	-	et cetra
FBR	-	Fluidized Bed Reactor
FFB	-	Fresh Fruit Bunch
FISH	-	fluorescent *in situ* hybridization

G	-	Glass
HCOOH	-	Formate
HRT	-	Hydraulic Retention Time
KOH	-	Potassium Hydroxide
MAS	-	Membrane Anaerobic System
MAR	-	Membrane Anaerobic Reactor
MBR	-	Hybrid Membrane Bioreactor
MPOB	-	Malaysia Palm Oil Board
MWCO	-	Molecular Weight Cut Off
NaOH	-	Sodium Hydroxide
n.d.	-	no date
OD	-	Optical Density
OLR	-	Organic Loading Rate
P	-	Plastic
PCR	-	Polymerase Chain Reaction
PES	-	Polyethersulphone
POME	-	Palm Oil Mill Effluent
PTFE	-	Polytetrafluoroethylene
rDNA	-	Recombinant Deoxyribonucleic Acid
RO	-	Reverse Osmosis
SBR	-	Sequencing Batch Bioreactor
spp.	-	species
SRT	-	Solid Retention Time
SS	-	Suspended Solid
TDS	-	Total Dissolved Solid

TN	-	Total Nitrogen
TP	-	Total Phosphorus
TSS	-	Total Suspended Solid
UASB	-	Up-Flow Anaerobic Sludge Blanket
UASFF	-	Up-Flow Anaerobic Sludge-Fixed Film
UF	-	Ultrafiltration
UMP	-	University Malaysia Pahang
U.S.	-	United State
USAFF	-	Up-Flow Anaerobic Sludge Fixed Film
VFA	-	Volatile Fatty Acid
VSS	-	Volatile Suspended Solid
Cr^{3+}	-	Chromium (III) Ions
Cr^{6+}	-	Chromium (VI) Ions
H^{+}	-	Hydrogen Ions
NH_4^{+}	-	Ammonium Ions
NH_4^{+}-N	-	Ammonical Nitrogen
$CH_3CH_2CH_2COOH$	-	Butyrate
CH_3CH_2COOH	-	Propionate
CH_3CH_2OH	-	Ethanol
CH_3COOH	-	Acetate
CH_3OH	-	Methanol
CH_4	-	Methane
CO_2	-	Carbon Dioxide
H_2	-	Hydrogen
H_2O	-	Water

H_2S	-	Hydrogen Sulfide
H_2SO_4	-	Sulfuric Acid
$KC_8H_3O_4$	-	Potassium Hydrogen Phthalate
$K_2Cr_2O_7$	-	Potassium Dichromate
K_2SO_4	-	Potassium Sulfate
Na_2CO_3	-	Sodium Carbonate
NH_3	-	Ammonia

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Malaysia is the world second largest producer of palm oil and palm oil industry is the largest agro industry in Malaysia. According to Ismail (2011) reported in Bloomberg news, the production of the palm oil in Malaysia was estimated to be 18.3 million tonnes this year and it was also estimated to increase 2.2 % to 18.7 million tonnes and flat between 18.6 million to 19 million tonnes in year 2012. Obviously, the world demand and the production of the palm oil have been kept on increasing compared to last year, 2010 which is only 17 million tonnes.

As discussed by Rupani *et al.* (2010), Malaysia contributes 41 % of the world plantation, which is among the largest export of Crude Palm Oil (CPO) ranked after Indonesia. The CPO is a mixture of palm oil and water in the proportion of 35 % to 45 % and 45 % to 55 % respectively, as well as some fibrous materials. Rupani *et al.* (2010) also discussed that large amount of water is needed during the extraction process of CPO, about 7.5 tonnes for each tonne of CPO. Lastly, up to 50 % of water ends up as wastewater, Palm Oil Mill Effluent (POME) with high organic content and also acidic nature. As the world demand of the palm oil is kept on increasing, therefore it indicates that more POME will be produced as the waste from the palm oil mills.

The raw POME is a thick brownish waste that contains high amount of Total Suspended Solids (TSS) 40 500 mg/L, Chemical Oxygen Demand (COD) 50 000 mg/L, Biochemical Oxygen Demand (BOD) 25 000 mg/L (Haris, 2006) and with pH range from 3.8 to 4.5 (Zinatizadeh *et al.*, 2006). The organic content of POME is very high

due to its high BOD and TSS that do not meet the standards of Department of Environment (DOE) to be released into the river. Therefore, POME cannot be discharged into the river directly without treatment as it also depletes the dissolved oxygen content in water bodies and caused aquatic pollutions (Rupani *et al*., 2010). Treatments of POME have to be done to fulfil the requirement of DOE before it has been discharged into river to reduce the pollution to the environment.

Ma *et al.* (1993) mentioned that more than 85 % of the palm oil mills in Malaysia have been using the anaerobic ponding system for biological pond treatment due to its low cost and followed by an open digesting system with aeration in the pond. However, this conventional method of POME treatment does not favour the renewable energy recovery, not economically friendly and also long treatment time needed.

The emergence of membrane separation technology has been recognized to be an efficient alternative in POME treatment (Ahmad and Chan, 2009). By implementing the membrane after the anaerobic digester of the POME treatment enables the zero emission or zero discharge of the POME in the palm oil mill to the environment by recycling back the clean water (permeate after membrane filtration) to the boiler of the CPO processing plant.

Abdurahman *et al.* (2011) proposed that Membrane Anaerobic System (MAS) as an alternative for POME anaerobic treatments with addition of Cross Flow Ultrafiltration (CUF) membrane for physical treatment to an anaerobic digester not only to produce methane gas but also to achieve high treatment efficiency with high removal of COD, TSS and also Volatile Suspended Solid (VSS) in a short treatment period.

This thesis mainly elaborates on the cultivation of anaerobic microorganism which is responsible for the anaerobic digestion process in MAS, evaluation of related parameters of MAS for POME treatment by using three different samples, which are 50 % diluted raw POME, anaerobically digested POME without addition of mixed culture inoculum and anaerobically digested POME with addition of mixed culture inoculum, and the methane gas yield after the POME treatment.

1.2 STATEMENTS OF PROBLEMS

This study consists of three statements of problems that discussed in 1.2.1, 1.2.2 and 1.2.3.

1.2.1 POME causes severe environmental problems

The discharge of POME to the river without treatment causes a serious environmental pollution due to its high organic content and its acidic behaviour. Based on the guidelines set by DOE Malaysia, the BOD limit of the wastewater discharge from industries should not be more than 100 mg/L (Mamun and Idris, 2008). Poh and Chong (2010) mentioned that raw POME having the COD value in the range of 22 660-73 500 mg/L, BOD in the range of 11 730-37 500 mg/L, suspended solid between 7 100 to 24 500 mg/L and pH 4.19 to 5.30. Therefore, the treatment of POME is necessary before it causes pollution to river, brings health hazards and aquatic livings extinction. High organic content of POME causes the depletion of the dissolved oxygen content in river. Yacob *et al.* (2006) stated that 0.5-0.57 tonnes of POME will be released from the palm oil mill while processing a tonne of oil palm fresh fruit bunch (FFB), thus an efficient POME treatment plant has to be established to manage those waste produced.

1.2.2 The disadvantages of the conventional treatment system

The conventional treatment system, anaerobic ponding system followed by open aerated lagoons or facultative ponds and algae ponds requires a huge land. Thus, it is not suitable for the developed countries with limited land. In Malaysia, more than 85 % of the palm oil mills have applied the conventional POME treatment method, anaerobic ponding system due to its inexpensive cost (Ma and Ong, 1985).

The ponding system requires a space about 12 soccer fields and the long Hydraulic Retention Time (HRT) of 45-60 days (Zinatizadeh *et al.*, 2006). In other words, the treatment time for POME treatment until it released into river is about 2 months by using anaerobic ponding system. Ahmad *et al.* (2005) discussed that the conventional treatment system is lack of process efficiency in terms of uncontrolled

production of methane and carbon dioxide in series of open lagoons and the treated POME is not able to comply consistently with the effluent discharge standards once the production of CPO increased.

Another major disadvantage of the anaerobic ponding system is the washout of the biomass responsible for the anaerobic digestion in the pond due to its short Solid Retention Time (SRT) especially when the production of CPO in a large quantity. Although the pond system is cost-effective in operating, it needs a longer HRT, short SRT, high energy consumption for aerated lagoon and high maintenance fees for all ponds. Therefore, the disadvantages of the anaerobic ponding system have offset its operating cost.

With the same concept of recovering wealth from waste, an alternative, MAS is developed to reduce the size of land required, maintenance fees for all ponds with shorter HRT, which indicates more methane gas will be produced, thus reduce the POME treatment cost. Contrary to conventional method, membrane treatment of POME does not require a skilful worker to maintain the system because the system can be highly automated (Ahmad *et al.*, 2003).

1.2.3 Depletion of fossil fuel and clean water resources

Depletion of fossil fuel and clean water resources are two major concerns in future. A lot of efforts have been done on biogas production to replace the non-renewable energy resources such as petrol. According to Bolarinwa and Ugoji (2010), biogas is a renewable fuel produced from anaerobic digestion of organic material, which as the substrate for biomethanation. In addition to that, Nigeria is estimated can produce 6.8 million L of gas daily in terms by anaerobic digestion on starchy wastes which is equal to 3.9 million L of petroleum (Faniran, 1982).

Membrane Anaerobic System (MAS) has not only created an alternative in treating POME, but also biogas production as it works in a closed digester system, unlike conventional anaerobic ponding system and therefore enable methane gas capture easily. To catch in the trend of zero emission in palm oil mills, MAS enables the water

recycling and reduce the usage of water in palm oil mill. The highly efficient CUF membrane in MAS requires only a short treatment period to recover the water from POME and then the clean water recovered can be reused in the steam boiler of the palm oil mills. The water recovered from POME can be used as a drinking water in the future as POME is a non-toxic effluent.

According to Abdurahman *et al.* (2011), the MAS have been proven to obtain a clearer final effluent, a reduction in COD content up to 96.4 % to 98.4 % with HRT of 6.8 days for the POME treatment and also produce biogas, methane with the production rate of 0.25 and 0.57 L/g COD/day. Further development of MAS is necessary as a potential alternative to encounter the problems of fossil fuel and clean water resources depletion in Malaysia.

1.3 RESEARCH OBJECTIVE

The research objective of this study is to evaluate the relevant parameters on the performance of Membrane Anaerobic System (MAS) on POME treatment and methane gas production.

1.4 SCOPES OF STUDY

The scopes of this study can be divided into three perspectives whereby to achieve the objective.

The first scope of the study is to perform a series of experimental works to study the general characteristic of microbial in POME collected from anaerobic pond in Lepar Hilir Palm Oil Factory. Anaerobic microorganism consists of three major groups, acidogens, acetogens and methanogens that are responsible for hydrolysis, acidogenesis, acetogenesis and methanogenesis during the anaerobic digestion. Microorganism is being cultured and isolated by spread plates and streak plates to study its general characteristic and a mixed culture inoculum was prepared to add into the MAS to see its effect on the methane gas production and the POME treatment efficiency.

The second scope of this study is to evaluate the efficiency of MAS on POME treatment based on the relevant parameters. The relevant parameters are COD, TSS, VSS and pH of POME. The comparison of those parameters has been done before and after the MAS treatment for the three different samples of POME, 50 % diluted raw POME (Run 1), anaerobically digested POME without the addition of mixed culture inoculum (Run 2) and also anaerobically digested POME with the addition of mixed culture inoculum (Run 3).

The third scope of this study is to measure the final methane gas production for the POME treatment with three different samples by using MAS and also to see the effects of inoculum added on the methane gas production.

1.5 RATIONALE AND SIGNIFICANCE OF STUDY

This study is seen as significant in three major elements, which are its novelty, applicability and commercialization.

1.5.1 The novelty of the study

The novelty of the study lies in the Membrane Anaerobic System (MAS) which provides an alternative treatment on POME with a higher removal of COD and methane gas production as compared to the conventional anaerobic ponding treatment. Besides, the possibility of recovering drinking water from POME to supply for the residents' use and clean water recovery for the use of the steam boiler in palm oil mill is high by using this system. There is not much studies regarding the microorganism that affects the production of methane gas till now. The MAS model and setup had been patented by University Malaysia Pahang (UMP) in the year of 2010 and being accepted in a high impact journal, Elsevier on 17 August 2010 (Abdurahman *et al.*, 2011).

1.5.2 The applicability of Membrane Anaerobic System (MAS) in this study

The higher production of methane gas from POME with MAS can provide a self-sufficient energy for the palm oil mills to operate daily, which is environmental friendly. The clear permeate of POME obtained from MAS can also be further treated as drinking water and being reuse as the boiler feed water. Those applications not only solve the pollution problems, but also the methane gas production enables the palm oil industries to create the business opportunities or new revenue by taking incentives from Certified Emission Reduction (CER) credits issued by Clean Development Mechanism (CDM) verified by DOE under the Kyoto Protocol. As compared to the conventional anaerobic ponding treatment, MAS requires a smaller space for POME treatment and also less manpower for maintenance due to its highly automatic potency. Hence, it is more worthy and suitable for the developed countries with a limited land area.

1.5.2 The commercialization of Membrane Anaerobic System (MAS) in this study

With the higher production of methane gas in shorter time, clean water recovery and lesser land required in treating POME using MAS, the potential of the system in terms of commercialization is very high. There are 3 companies, Green and Smart Sdn. Bhd., Kuala Lumpur, Hydro K Management (M) Sdn. Bhd., Shah Alam and Esona Environmental Group, Kuala Lumpur, Selangor have been interested in MAS. The system is being looked forward to treat waste other than POME to recover the clean water for the usage of water shortage countries.

Therefore, MAS is a potential system to be commercialised in future as it requires smaller land and shorter HRT to treat POME. Thus, the study of the various parameters effects the anaerobic digestion of POME is important to evaluate the performance of MAS in treating POME, to identify the development on the design of the MAS that can be done in the future and lastly to identify the way of optimise the methane production by biological pretreatment such as addition of inoculum into the system.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The discharged of Palm Oil Mill Effluent (POME) to the environment is causing a lot of pollutions. Therefore, several POME treatments have been developed to increase the efficiency of the treatment systems and also to accommodate the increased demand of palm oil in the world market which indicates more discharge of pollutants. A review of literature was performed to identify the studies that have been done related to this study.

This chapter consists of a literature review of the subtitles which are the history of oil palm and POME, the types of POME treatments, anaerobic digestion, Membrane Anaerobic System (MAS) and kinetic models and factors affecting the anaerobic digestion process. The literature on the POME generally refers to the parameters used to determine its characteristics. Moreover, the aerobic and anaerobic treatments of POME are being reviewed and compared under the subtitle of the types of POME treatments. The type of the POME treatments that being used in this study was MAS, which has been recently developed and designed by Abdurahman *et al.* (2011). Therefore, it is significance to study the relevant parameters so as to evaluate the performance of MAS in treating POME, at the same time, evaluate the production of methane gas.

2.2 OIL PALM AND PALM OIL

As what have been discussed in Chapter 1 (refers to 1.1), oil palm has become the major agriculture commodities in Malaysia that brings large revenue every year. Oil palm is originally from the West and Central Africa and its botanical classification is, *Elaeis guineensis,* Jacq., which is named after in remembrance of Nicholas Jacquin who drew the first illustration in 1763. The oil palm has a life-span of over 200 years (Rupani *et al.*, 2010) and an economic life or commercial value of 25 years (Henderson and Osborne, 2000).

The palm oil is the major revenue earner in ASEAN region (Ng *et al.*, 1987). The Fresh Fruits Bunch (FFB) of the oil palm have been crashed to produce two types of valuable vegetable oils; palm oil and palm kernel oil from its outer flesh (mesocarp) and its nut respectively. Other than that, palm oil has been used in producing soap in the early 1800s. In 19th century, the palm oil has been demanded to produce candles. Palm oil has also been used in food industry to produce margarine and also in chocolate manufacturing industry to replace expensive cocoa butter in 20th century (Henderson and Osborne, 2000).

2.3 THE HISTORY OF OIL PALM AND PALM OIL MILL EFFLUENT (POME) IN MALAYSIA

The oil palm has been introduced to Malaysia in the early 20th century and it has been commercially produced in 1917 (Haris, 2006). Today, Malaysia is the second largest palm oil exporter, ranks after Indonesia which accounts 41 % of world crude palm oil production (MPOB, 2011). As reported by Malaysian Palm Oil Board (MPOB), 11 million tonnes of palm oil have been exported for global food industry and have reached RM 53.9 billion in 2010 (MPOB, 2011) as compared to RM 49.59 billion in 2009 (MPOB, 2010). This statistic again showed that the production of palm oil has been increasing due to the high demand from market. The usage of palm oil now is not only confined to candle and food industry, but also being used in producing environmental friendly energy resources, biodiesel and methane gas to replace petroleum, nonrenewable energy resources.

During the process of extraction of palm oil from the fruits, a large amount of water is used (Zhang *et al.*, 2008). For one tonne of crude palm oil produced, it has been estimated about 5-7.5 tonnes of water are required and more than 50 % of the water will end up as wastewater, that is POME (Wu *et al.*, 2007). As mentioned by Ahmad *et al.* (n.d.), there are more than 500 palm oil mills in Malaysia and 35 million m^3 of POME was being generated annually with reference to 13.9 million tonnes of Crude Palm Oil (CPO) produced. The POME is mainly generated from three main major processing operations in the CPO producing plant, which is sterilizer condensate (36 %), separator sludge (60 %) and hydrocyclone wastewater (4 %) (Borja and Banks, 1994 and Sethupathi, 2004). There are no chemicals being added into the process of CPO production, hence, POME is considered as a non-toxic waste. However, from the high COD and BOD values mentioned above, POME has very high content of organic matter. Therefore, it is still could not be discharged and released into the river directly after the process.

POME is a thick brownish liquid waste with high content of organic, high temperature, 70-80 ^{o}C (Abdurahman *et al.*, 2011) and acidic nature with pH 3.8 to 4.5 (Zinatizadeh *et al.*, 2006). Due to its polluting nature, it does not meet the discharge standard set by Malaysian Department of Environment (DOE), whereby Biochemical Oxygen Demand (BOD) should not be more than 100 mg/L (Mamun and Idris, 2008); thus, the treatment of POME is needed before it can be discharged, to protect the environment from being polluted (Zhang *et al.*, 2008).

2.4 CHARACTERIZATION OF PALM OIL MILL EFFLUENT (POME)

To determine the characteristics of POME before it can be treated, there are several parameters need to be tested such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Volatile Suspended Solid (VSS) and also pH. The measurements of these parameters are important to evaluate the performance of the type of treatments used to treat POME. Lyberatos and Skiadas (1999) mentioned that the anaerobic digesters will normally exhibit stability problems which only can be avoided by appropriate mathematical models and parameters evaluation. The example of those instabilities that causes the digester failures are a drop in the methane production rate, a drop in the pH, a rise in the volatile fatty acid (VFA) concentration etc.

2.4.1 Biochemical Oxygen Demand (BOD)

BOD test is a significant environmental index to determine the oxygen requirements of wastewaters, effluents and polluted waters (Rastogi *et al*., 2003). In other words, BOD is an indirect method to measure the organic content in the water or wastewater. BOD is measured by oxidizing organics using microorganism at the temperature of 20 ^{o}C in an air incubator and measure the amount of oxygen consumed, expressed in mg/L or parts per million (ppm) in the oxidation process (Mark, 2008).

BOD test is the parameter that usually used to determine the strength of an organic industrial wastewater. BOD is also an indicator of water quality. This test is widely applied to measure the waste loading to treatment plants and the efficiency of the treatment system (Mark, 2008). The high BOD value indicates more microorganisms exist in the wastewaters that use Dissolved Oxygen (DO) in the water to decompose the organic content of the waste and later producing carbon dioxide (CO_2). Therefore, the higher the BOD level means less DO in water or more polluted or contaminated the effluent (Boguski, n.d.). The purpose of testing BOD of the industrial effluent before it discharge into river is to protect the diversity of organisms living in river.

The test of BOD which is according to American Public Health Association (APHA) Standard Methods was known as BOD_5, which is the measurement of oxygen consumed in 5 days test period at 20 ^oC (Eaton *et al.*, 2005). BOD measurement of wastewater during wastewater treatment is important as BOD can be used for process control and to measure the dissolved oxygen concentration in effluent which reviewed the efficiency of the treatment. In wastewater treatment, BOD removal efficiency is being measured as stated in Eq. (2.1).

[(BOD influent – BOD effluent) / BOD influent] x 100 % (2.1)

2.4.2 Chemical Oxygen Demand (COD)

COD is another significant parameter used to determine the organic content of wastewater such as POME (Vyrides and Stuckey, 2008). Mark (2008) mentioned that COD is a test which measures the amount of oxygen required for chemical oxidation of organic matter by strong oxidant in the sample to carbon dioxide (CO_2) and water. The COD value shows the oxygen equivalent of the organic content that can be oxidized by potassium dichromate ($K_2Cr_2O_7$) using silver sulfate as a catalyst under acidic condition (H_2SO_4) (Vyrides and Stuckey, 2008). COD reactions are shown in the Eq. (2.2) below (Arachem Technical Training Centre (ATTC) (M) Sdn. Bhd., 2009).

$$2KC_8H_3O_4 + 10K_2Cr_2O_7 + 41H_2SO_4 \rightarrow 16CO_2 + 46H_2O + 10Cr_2(SO_4)_3 + 11K_2SO_4 \quad (2.2)$$

Eq. (2.2) is refers to the reactions between potassium hydrogen phthalate ($KC_8H_3O_4$), $K_2Cr_2O_7$ and sulfuric acid (H_2SO_4) to produce CO_2, chromic acid and also potassium sulfate (K_2SO_4) during the COD digestion of sample. Organic compounds are oxidized by potassium dichromate and sulfuric acid and the oxidation of organics results in the reduction of Cr^{6+} to Cr^{3+}, which reduce the color of the sample from yellow to green.

There are a few methods that had been used to determine the COD of the wastewater. Among those methods, there are the open reflux method, closed reflux method (Andrew *et al.*, 2005) and also colorimetric measurement by using Hach COD

vials which is the method that have been approved by Environmental Protection Agency (EPA) (ATTC, 2009). In this study, Hach COD method is used to determine the COD values of the POME samples. The COD of the sample was determined using colorimetric measurement with spectrophotometer DR 2400 of Hach after digestion process of 2 hours in COD reactor DR 200. POME sample needed to be tested using High Range COD vial (in the range of 20-1 500 mg/L COD) which is measured increase in green colour at 620 nm. Dilution needed to be done while the COD value is not within the range that could be read by spectrophotometer.

Hanna Instruments Pty Ltd (Hanna) (2008) described that COD removal refers to the percentage of the organic matter purified during the treatment cycle. Therefore, it indicates that the higher the COD removal, the higher the efficiency of the treatment process and less polluted the effluent. Compared to BOD, COD is a faster way of indirect water organic content measurement which requires only 2 hours of digestion with less interference, repeatable, easy and stable to run. COD is more stable than BOD because COD uses potassium dichromate for oxidation whereas BOD is using microorganism for oxidation and microorganism are susceptible to pH, temperature and variables in water. Correlation of COD and BOD is depending on sample and may not be always possible.

2.4.3 Total Suspended Solid (TSS)

Suspended solid is the common parameter that used to define the industrial wastewater. Lenhart and Lehman (2008) mentioned that TSS refers to the total mass of organic matter in wastewater that has retained on a standard glass-fiber filter after evaporation and subsequent drying in an oven until a constant weight. Organic matter in wastewater such as oils, greases that associations with mineral particles such as sand, silts or clay serves as a substrate for bacterial growth.

The sample after the TSS test can be used to be further ignited at high temperature to obtain the Volatile Suspended Solid (VSS) value which can be used to estimate the total biomass concentration in the POME during the treatment. There are three methods to measure the TSS of the water or wastewater sample. The method used

in this study to measure the TSS is APHA: 2540D method that has been approved by EPA found in Andrew *et al.* (2005).

2.4.4 Volatile Suspended Solid (VSS)

VSS refers to a measure of the biodegradable organic matter. VSS is a method used to estimate the total mass of organic matter in a water sample by dry ashing the TSS samples collected (refers 3.4.2) (Lenhart and Lehman, 2008) at 500 ^{o}C $\pm$ 50 ^{o}C in an electric furnace for 15 to 20 minutes (Mark, 2008).

VSS is an important parameter because it indicates the volatile solids removal or reduction in sludge digestion in the settling tank to determine the operational efficiency of certain wastewater treatment units (Mark, 2008).

2.4.5 The definition and control of pH during Palm Oill Mill Effluent (POME) treatment

Frederick (2003) defined pH as the measurement of the hydrogen ion concentration, [H^+] and also stated that pH of waste treatment plant must be within a specific pH range set by the government before it is being discharged. The control of pH in both aerobic and anaerobic POME treatment (refers to 2.5) is important because of little change in pH will affect the methanogenesis process of microorganism, methanogens in the fermenter (Beccari *et al.*, 1996). Ibrahim *et al.* (1984) described methanogenesis as the rate limiting step in anaerobic treatment of POME. Methanogenesis is a process where the methanogens in the fermenter convert the organic content (acetate) of POME into methane gas and carbon dioxide.

Ling (2007) discussed that "the control of reactor pH to obtain interested acids for methane production would improve the treatment performance and stability". Normally, the adjustment of pH during the POME treatment is performed by adding Sodium Hydroxide (NaOH) or Sodium Carbonate (Na_2CO_3) (Chantaraporn *et al.*, 2010). According to Abdurahman *et al.* (2011), the optimum pH range working for the anaerobic digesters in the MAS is between pH 6.7 to 7.8.

2.4.6 Sample preservation techniques based on parameters

Subtopic 2.4.1 to 2.4.5 had described the parameters that would be tested to evaluate the performance of anaerobic digestion during the POME treatment and also the characteristic of POME. Each of the testing described should be analyzed soon as soon as possible after the POME sample collected from the MAS in this study. However, if the testing could not be performed immediately due to the time and physical constraint during the experiment, the POME samples have to be preserved to minimize the changes take place in a sample, either chemically or biologically.

According to ATTC (2009) and United State (U.S.) EPA (1983), it is recommended to perform sample preservation immediately upon the sample collection. Table 2.1 shows the preservation technique needed to be applied to preserved the sample according to each parameter.

Table 2.1: Preservation techniques of samples according to each parameter

Parameters	**Container Plastic (P) Glass (G)**	**Volume required (mL)**	**Preservation**	**Maximum Holding Time**
BOD	P,G	1000	Cool, 4 ^{o}C	48 hours
COD	P,G	50	Cool, 4 ^{o}C Add H_2SO_4 to pH 2	28 days
pH	P,G	None required	None required	Analyze immediately
Temperature	P,G	None required	None required	Analyze immediately
Total Suspended Solid (TSS)	P,G	100	Cool, 4 ^{o}C	7 days
Volatile Suspended Solid (VSS)	P,G	100	Cool, 4 ^{o}C	7 days

Table 2.1 showed the preservation methods of sample based on the parameter once the testing of parameter could not be performed immediately after the sample collection. Based on the Table 2.1, the sample could be stored in Plastic (P) or Glass (G) type container during the preservation for the parameters BOD, COD, TSS, VSS, pH and temperature. For BOD, 1 000 mL samples are recommended to be preserved in 4 ^{o}C chiller for the maximum holding time of two days (48 hours). Whereas for COD, it was recommended to preserve at least 50 mL sample in 4 ^{o}C after the addition of sulfuric acid (H_2SO_4) to pH 2. And the maximum holding time is 28 days. For the pH and temperature of the sample, it is always recommended to test the sample immediately right after the sample collection. For both TSS and VSS, the sample can be preserved up to 7 days by cool them in 4 ^{o}C chiller and the minimum volume which is recommended to preserve is 100 mL for both parameters.

2.4.7 The characteristics of raw Palm Oil Mill Effluent (POME)

As what have been discussed in Chapter 1 (refers 1.2.1), raw POME causes environmental pollution and there is a lot of POME produced as waste daily from the palm oil industries. Therefore, the treatment of POME is necessary to fulfill the standard of industrial wastewater discharged into the river based on Environmental Quality Act (EQA) 1974 (ACT 127) (refers Table 2.3). The parameters discussed (refers 2.4.1 to 2.4.5) are essential to evaluate the effectiveness of the POME treatment. The characteristics of raw POME from the literature have been tabulated in the Table 2.2. The values listed will be taken as references to compare with the characteristics of treated POME by using MAS.

Table 2.2: The typical characteristics of raw Palm Oil Mill Effluent (POME)

Parameters / **References**	**BOD (mg/L)**	**COD (mg/L)**	**TSS (mg/L)**	**VSS (mg/L)**	**pH**
Ahmad *et al.* (2005) in Wong (2007)	25 000	50 000	18 000	34 000	4.7
Mamun and Idris (2008)	31 340	59 700	30 230	25 300	4.5
Zinatizadeh *et al.* (2010)	23 000 - 26 000	42 500 - 55 700	16 500 - 19 500	-	3.8-4.4
Poh and Chong (2010)	11 730 - 37 500	22 660 - 73 500	7 100 - 24 500	-	4.19-5.30

Note:

BOD - Biochemical Oxygen Demand

COD - Chemical Oxygen Demand

TSS - Total Suspended Solid

VSS - Volatile Suspended Solid

According to Table 2.2, BOD of the raw POME is in the range of 11 730 mg/L to 37 500 mg/L (Poh and Chong, 2010), 25 000 mg/L (Ahmad *et al.*, 2005 in Wong, 2007), 31 340 mg/L (Mamun and Idris, 2008) and also 23 000 mg/L to 26 000 mg/L (Zinatizadeh *et al.*, 2010). The COD range of raw POME is also in the range of 22 660 mg/L to 73 500 mg/L as reported by Poh *et al.*, 2010. TSS of raw POME is in the range of 7 100 mg/L to 30 230 mg/L from the literature reviewed. Besides, the VSS value of POME is 25 300 mg/L and 34 000 mg/L as what has been discussed by Mamun and Idris (2008) and Ahmad *et al.* (2005) as cited in Wong (2007) respectively. Basically, as what have been reviewed in Table 2.2, the pH of the raw POME is between 3.8 and 5.3, this shows that POME is acidic.

2.4.8 Parameter limits for discharge of Palm Oil Mill Effluent (POME)

Malaysia government has enforced the rule to limit the discharge of wastewaters by enacted the Environment Quality Act (EQA) in 1974 and also specific regulations for POME in 1977 (Ahmad and Chan, 2009). By comparing Table 2.2 and Table 2.3, it shows that the treatment of POME is mandatory before it can be discharged due to its high polluting nature. Table 2.3 has shown the discharge limits of wastewater according to parameters.

Table 2.3: The discharge limits of wastewaters based on certain parameters

Parameters	**Limits according to period of discharge**		
	1.7.1978 to 30.6.1979	1.7.1981 to 30.6.1982	1.1.1984 and thereafter
pH	5.0-9.0	5.0-9.0	5.0 – 9.0
Temperature (^{o}C)	45	45	45
BOD 3 days, 30 ^{o}C (mg/L)	5,000	500	100
COD (mg/L)	10,000	1,000	-
Total Solids (mg/L)	4,000	1,500	-
Suspended solids (mg/L)	1,200	400	400

Adapted from: Ahmad and Chan (2009)

Note:

BOD - Biochemical Oxygen Demand

COD - Chemical Oxygen Demand

Table 2.3 showed the regulatory discharge limit of POME based on certain parameters. The pH and temperature of the POME before it could be discharged into the river must be between pH 5 to 9 and not above 45 ^{o}C. According to Rupani *et al.* (2010), the temperature of raw POME produced from the CPO processing plant is around 80 to 90 ^{o}C. The BOD (3 days, 30 ^{o}C) of POME must be equal to or below 100 mg/L before discharge into the river, whereas suspended solids of POME must be below or equal to 400 mg/L. For the COD and total solids, there is no discharge standard being set after the year 1984.

2.5 THE TYPES OF BIOLOGICAL PALM OIL MILL EFFLUENT (POME) TREATMENTS

There are several methods used by Malaysia palm oil mills in treating POME. In general, biological POME treatment can be grouped into aerobic POME treatment (refers to 2.5.1), anaerobic POME treatment (refers to 2.5.2) or else combination of both anaerobic and aerobic in sequence (refers to 2.5.3).

2.5.1 Aerobic Palm Oil Mill Effluent (POME) treatments

The aerobic POME treatment process involves the use of free or dissolved oxygen by microorganisms (aerobes) in the conversion of organic wastes to biomass and Carbon Dioxide (CO_2).

According to Chan *et al.* (2009), aerobic biological treatment is normally used for organic wastewaters. This is to achieve a high degree of treatment efficiency where the product of treatment will be discharged into public watercourse (Haris, 2006). Aerobic treatment is not suitable for the POME as the aerobic treatment is more suitable for low organic loading rate condition. POME is normally produced as the waste in large amount. Therefore, POME treatment is preferably using anaerobic treatment

(refers to 2.5.2) because it can sustain a higher organic loading rate compared to the aerobic treatment.

2.5.2 Anaerobic Palm Oil Mill Effluent (POME) treatments

The anaerobic POME treatment process degrades the POME into methane gas (CH_4), CO_2 and water (H_2O) through anaerobic digestion which involves four steps which are hydrolysis, acidogenesis, acetogenesis and methanogenesis in the absence of oxygen (Chan *et al.*, 2009). According to Botheju and Bakke (2011), anaerobic digestion is a biochemical process that converts organic matter using naturally occurring microorganisms under oxygen depleted conditions to produce biogas mainly composed of CH_4 and CO_2.

Contrary to aerobic POME treatment, the anaerobic treatment is mostly applied for waste treatment to recover resources. The anaerobic treatment can be subdivided into two, which are open anaerobic digestion or closed anaerobic digestion. The main difference between open and closed tank digester is the latter enables CH_4 (green house gas) recovery which acts as a cost value. According to Vijayaraghavan *et al.* (2007), open type digesters are recently being converted into closed digesters due to its global warming effects. Therefore, a lot of the closed typed anaerobic digesters have been proposed for POME treatment such as Sequencing Batch Bioreactor (SBR) (Chan *et al.*, 2010 and Fun *et al.*, 2007), Up-Flow Anaerobic Sludge Blanket (UASB) system (Borja and Banks., 1994), Anaerobic baffled-fixed film reactor (ABFFR) (Limkhuansuwan *et al.*, 2009) and also Up Flow Anaerobic Sludge-Fixed Film (UASFF) Bioreactor (Najafpour *et al.*, 2006), Membrane Anaerobic Reactor (MAR) (Abdullah *et al.*, 2005), Membrane Anaerobic System (MAS) (Abdurahman *et al.*, 2011), Hybrid Membrane Bioreactor (MBR) (Ahmad *et al.*, n.d.) which enable the methane recovery during the anaerobic digestion.

The most common methods have been used in Malaysia since 20 years are anaerobic and facultative pond system (Wong *et al.*, 2009). There are more than 85 % of palm oil mills in Malaysia which are applying the conventional anaerobic ponding

system due to its inexpensive costs (Ma and Ong, 1985) while the rest applied open digesting tank (Yacob *et al.*, 2005).

However, both of these conventional methods of POME treatment, anaerobic ponding system and open digestion tank requires long Hydraulic Retention Time (HRT) of one to two months (Abdurahman *et al.*, 2011) and 20 days (Yacob *et al.*, 2005) respectively. As cited in Yacob *et al.* (2005), the ratio of CH_4 production of the anaerobic digestion of POME is 65 % to 35 % of CO_2.

The advantages of anaerobic treatment are less energy consumption, low sludge production, low nutrient requirement of microorganisms and the ability of bioenergy and nutrient recovery, such as CH_4 gas (Chan *et al.*, 2009).

A wide approach of anaerobic POME treatment that had been done has been reviewed from literature. According to Poh and Chong (2008), high-rate anaerobic digesters have been used as part of the POME treatment in a laboratory-scale. For instance, the Up-Flow Anaerobic Sludge Fixed Film (USAFF) bioreactor showed the removal efficiency of COD between 80.6 % and 98.6 %, HRT between one and six days and CH_4 yields obtained were between 0.287 and 0.3481 CH_4/g $COD_{removed}$ per day (Lorestani, 2006).

Furthermore, the two-stage up-flow anaerobic sludge blanket (UASB) reactor has removed COD greater than 90 % and the CH_4 yield is in the range between 0.30-0.331 CH_4/g $COD_{removed}$ per day (Borja *et al.*, 1996). A comparison study has been done by Borja and Banks (1994), showed that both Anaerobic Filter (AF) and Fluidized Bed Reactor (FBR) gave COD removals higher than 90 % at six hour residence times with a loading rate of 10 g COD/ L.day. The FBR gave better performance with higher loading rate which is up to 40 g COD/ L.day. Meanwhile AF only sustained loading rate that is up to 20 g COD/ L.day due to clogging.

2.5.3 Combination of both aerobic and anaerobic Palm Oill Mill Effluent (POME) treatments and other POME treatments

Nowadays, a lot of researchers combined both anaerobic and aerobic treatments to obtain clearer effluent. The review of study on anaerobic-aerobic treatment done by Chan *et al.* (2009) concluded that low energy consumption, low sludge production, low chemical consumption are the advantages of the treatment but it still requires a long HRT and large space.

Apart from aerobic POME or anaerobic POME treatment, Oswal *et al.* (2002) has been proven that POME can be treated by tropical marine yeast named *Yarrowia lipolytica NCIM 3589* and showed a COD reduction about 95 % with HRT of 2 days. Evaporation method which has recovered 85 % of the water from POME can also be used to treat POME (Ma, 2000 as sited in Ahmad *et al*., 2003). However, as mentioned by Ahmad *et al*. (2003), the energy consumption of the evaporation method (with large amount of steam purging) can be a major constraint.

2.5.4 Comparison between aerobic treatment and anaerobic treatment on POME

Aerobic systems are more suitable for the treatment with low strength wastewaters (COD less than 1 000 mg/L) whereby anaerobic systems are suitable for the treatment with high strength type of wastewaters (COD concentration over 4 000 mg/L). Anaerobic treatment of POME also allows the recovery of CH_4 gas as the renewable energy along with Clean Development Mechanism (CDM) under the Kyoto Protocol 1997 (Chan *et al.*, 2009). Hence, compared to aerobic POME treatment, anaerobic POME treatment is still more preferable as generally and was being applied in this study.

Table 2.4 showed the differences between aerobic and anaerobic treatment on POME in terms of organic loading rates and organic removal efficiency, Solid Retention Time (SRT), biomass yield, microbiology and environmental factors, bioenergy and nutrient recovery by referring to Chan *et al.* (2009) and Singh (1999) as cited in Badroldin (2010). Table 2.4 described that both aerobic and anaerobic

treatment provide high organic removal efficiency, but the anaerobic treatment of POME prolongs Solid Retention Time (SRT) to retain slow growing methanogen in the reactor as compared to aerobic treatment that have shorter SRT which essentially mean that biomass washout can be hardly prevented. Compare between aerobic and anaerobic treatment, anaerobic treatment allows high organic loading rate as the specific substrate utilization rate in the reactor is higher than the aerobic treatment. Anaerobic treatment of POME would have the potential of odors problem, however, anaerobic digester of MAS system used in this study is a closed system, therefore the odors problem can be overcame thoroughly.

In terms of energy requirements, aerobic treatment is not economical as high energy consumption is required for aeration to supply enough oxygen for biodegradation process in the reactor. Less nutrients are required for anaerobic treatment, at the same time, it enables bioenergy recovery such as methane gas. Whereas aerobic treatment does not favour the recovery of methane gas as the methanogens which responsible for the methanogenesis phase to produce methane gas are obligate anaerobes. Besides, the anaerobic digestion in the anaerobic treatment was done by a diverse group of microorganism whereby the aerobic treatment is mainly a one-species phenomenon.

Anaerobic treatment process is highly susceptible to the changes in environmental conditions whereas the aerobic treatment is less susceptible to the changes in the environmental changes. Although it is mentioned by Chan *et al.* (2009) that the effluent of the anaerobic digestion treatment on POME is less clear than the aerobic treatment. However, this can be overcame by the MAS in this study which added a Cross Flow Ultrafiltration (CUF) membrane after the anaerobic digester to separate and remove the suspended solid in POME and to produce clearer effluent more efficiently.

Table 2.4: Comparison between anaerobic treatment and aerobic treatment

Features	**Anaerobic treatment**	**Aerobic treatment**
Organic removal efficiency	High	High
Solid Retention Time (SRT)	Longer SRT (enables to retain methanogen in the reactor)	Short SRT (4 to 10 days for activated sludge process)
Organic loading rate	High (10-40kg COD/m^3.day for high-rate reactors)	Moderate (0.5 to 1.5 kg COD/m^3.day for activated sludge process)
Odors	Potential of odors problem	Less odor problems
Start-up time	1-2 months (mesophilic) 2-3 months (thermophilic)	1 week to 4 weeks
Energy requirement	Low	High for aeration
Nutrient requirement	Low	High
Bioenergy and nutrient recovery	Yes	Low
Biomass (sludge) yield	Low biomass yield	High biomass yield
Specific substrate utilization rate	High rate 0.75-1.5 kg COD/ kg VSS.day	Low rate 0.15-0.75 kg COD/ kg VSS.day
Microbiology	Diverse group of microorganisms	Mainly one-species phenomenon
Environmental factors	The process is highly susceptible to changes in environmental conditions	The process is less susceptible to changes in environmental changes
Effluent quality	Moderate to poor	Excellent

Source: Chan *et al.* (2009) and Singh (1999) in Badroldin (2010).

2.6 ANAEROBIC DIGESTION PROCESS

By referring to 2.5.4, anaerobic treatment is a more preferable treatment on POME as it allows longer SRT, less energy consumption, high substrate utilization and also allows high organic loading which make the POME treatment more feasible than aerobic treatment. Thus, in this study, anaerobic treatment of POME was being selected.

Karakashev *et al.* (2005) mentioned that the anaerobic digestion is a biological process for the treatment of different organic wastes to produce energy in the biogas form and it is also a multistep functioning by different microbial group, such as saccharide and amino acid fermenters, Volatile Fatty Acid (VFA) oxidizers and methanogens.

Poh and Chong (2010) mentioned that a complex mixed culture, whereby more than one species of microbes work together to degrade the organic content of the wastewater. The conversion of organic matter to biogas is mainly involving four stages, hydrolysis, acidogenesis, acetogenesis and methanogenesis by the groups of microorganisms, acidogens, acetogens and also the methanogens respectively (Lyberatos and Skiadas, 1999). Methanogens can be divided into two types whereby hydrogenotrophic methanogen and also acetotrophic methanogen based on the substrate they consume, either is hydrogen with carbon dioxide or acetate respectively.

2.6.1 Hydrolysis

Gerardi (2006) mentioned that the hydrolysis process is the solubilization process of particulate organic compounds and colloidal organic compounds such as proteins into simple soluble compounds by the hydrolytic bacteria. Patil *et al.* (2011) mentioned that hydrolysis involves the transformation of lipids, polysaccharides, proteins, fats, nucleic acids into soluble organic materials or simple organic compounds carried out by strict anaerobes such as *Bactericides, Clostridia* or facultative bacteria such as *Streptococci.* According to Ling (2007), fats are hydrolysed into fatty acids or glycerol; protein is being hydrolysed into amino acids or peptides while the

carbohydrates are being hydrolysed into monosaccharides and dissacharides during the hydrolysis stage in anaerobic digestion.

2.6.2 Acidogenesis

Acidogenesis is the second stage of the anaerobic digestion process by anaerobically converts the hydrolysed products formed in hydrolysis stage by acidogens (fermentative microorganisms) (Patil *et al.,* 2011). According to Lester and Birkett (1999), acidogenesis stage also known as acid-forming stage and the fermentative processes happened during this stage is basically carried by a large diversity of facultative anaerobes and anaerobes. Acidogens converted the hydrolysed products into volatile fatty acids, short chain fatty acids, alcohols, aldehydes, ketones, ammonia, carbon dioxide, sulfide, water, hydrogen and some converted to new cells during the stage of acidogenesis. The VFA that more than four-carbon chain could not be used directly by methanogens and therefore these compounds are further oxidized to acetic acid and hydrogen by hydrogen-producing acetogens through acetogenesis process (Ling, 2007). Table 2.5 below shows that the major acids and alcohols, which produced during acidogenesis stage in anaerobic digestion process that could be used directly or indirectly by methanogen.

Table 2.5: Major acids, alcohols and organic compounds that formed in acidogenesis stage

Substrate formed in acidogenesis stage that used directly by methanogens	**Substrates formed in acidogenesis stage that used indirectly by methanogen**
Acetate (CH_3COOH)	Ethanol (CH_3CH_2OH)
Formate ($HCOOH$)	Butyrate ($CH_3CH_2CH_2COOH$)
Methanol (CH_3OH)	Propionate (CH_3CH_2COOH)

Adapted from: Lester and Birkett (1999)

Based on Table 2.5, the major products of acidogenesis which can be used directly by methanogen to form methane are acetate, formate and methanol. On the other hand, the products that cannot be used directly by methanogens to form methane are ethanol, butyrate and also propionate. Those products can be accumulated during the acidogenesis stage if they are not being degraded by acetogens in the acetogenesis stage (2.6.3). The accumulation of VFA such as butyrate and propionate will later inhibit the production of methane and cause the anaerobic digester failure during the treatment (Işık and Sponza, 2008). Lorestani (2006) reported that strict anaerobic rod shape bacteria such as *Clostridium spp.* is responsible for most of the extra cellular lipase and protease produced and converted the organic molecules into acid products.

2.6.3 Acetogenesis

With reference to Lester and Birkett (1999), those organic acids that cannot be used directly by the methanogen to form methane are ethanol, butyrate and propionate. Acetogenesis stage is very important in anaerobic digestion to convert the butyrate and propionate into acetate, hydrogen and carbon dioxide by preventing VFA accumulation. Acetogens can be divided into two types, hydrogen-utilizing acetogens or hydrogen-producing acetogens. Those microorganism that oxidize organic acids to acetate and hydrogen are basically hydrogen-producing acetogens. The hydrogen-utilizing acetogens are a group of obligatory anaerobic bacteria that utilize the acetyl coenzyme A (coA) to synthesize acetate from C_1 precursors (Lorestani, 2006).

Lorestani (2006) also mentioned that 70 % of the methane formed in the anaerobic digestion is originated from the acetate formed in both acidogenesis and acetogenesis. Hence, acetate is the main precursor for methane gas production during the anaerobic digestion which would mainly involve in two mechanisms. The two mechanisms are pointed to either acetoclastic or two step reaction. The acetoclastic methanogenesis is carried out by *Methanosarcinaceae* or *Methanosaetaceae.* The two step reaction mechanisms means the acetate will first oxidized into hydrogen gas or carbon dioxide and then converted into methane gas, which is performed by acetate-oxidizing bacteria (e.g. *Clostridium spp.*) and also hydrogenotropic methanogens such as *Methanomicrobiales or Methanobacteriales* (Karakashev *et al.*, 2006).

2.6.4 Methanogenesis

During the methanogenesis stage, methane gas is formed from the acetate, carbon dioxide and also hydrogen gas by methanogen. Methanogenesis is the rate limiting step in anaerobic digestion of POME (Ibrahim *et al.*, 1984 in Poh and Chong, 2008). According to Rasche and Ferry (2005), the process of methanogenesis normally employs two different pathways, carbon dioxide reduction with hydrogen by hydrogenotrophic methanogen and fermentation of acetate (the product of acidogenesis and also acetogenesis) to carbon dioxide and methane by acetoclastic methanogens (refers Figure 2.1).

Methanogen, normally with the species of *Methanosaeta concilii* presents with high number in high concentration of acetate which occurred in the bioreactor during POME treatment (Tabatabaei *et al.*, 2010). Therefore, it is believed that *Methanosaeta concilii* is the dominant microorganisms that will be involved in the conversion of acetate to methane gas.

Mladenovska and Ahring (2000) found in Tabatabaei *et al.* (2009) also mentioned that the only acetate-utilizing methanogens identified in anaerobic digestors are from the genera *Methanosarcina and Methanosaeta.* Williams and Crawford (1985) mentioned that methanogen is an obligate anaerobic microorganism which survives only when absence of oxygen (less than 2 ppm) and they metabolize the best in pH range of 6.7 to 8.0.

Compared to acidogens, methanogens grows slower than acidogens. According to Hausrath *et al.* (2007), the autotrophic methanogens which able to produce methane (CH_4) from carbon dioxide (CO_2) and hydrogen (H_2) which involving the two step mechanism mentioned by Karakashev (2006) (refers Eq. (2.3)).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (2.3)$$

Since the methanogenesis is the limiting steps involving the anaerobic digestion, therefore, the culturing more anaerobic methanogen will be an effective way to

complete the methane synthesis pathway. Culturing more anaerobic microorganism may help in accelerating the anaerobic digestion to achieve steady state faster.

The anaerobic digestion process that has been described from 2.6.1 to 2.6.4 can be simplified into the Figure 2.1.

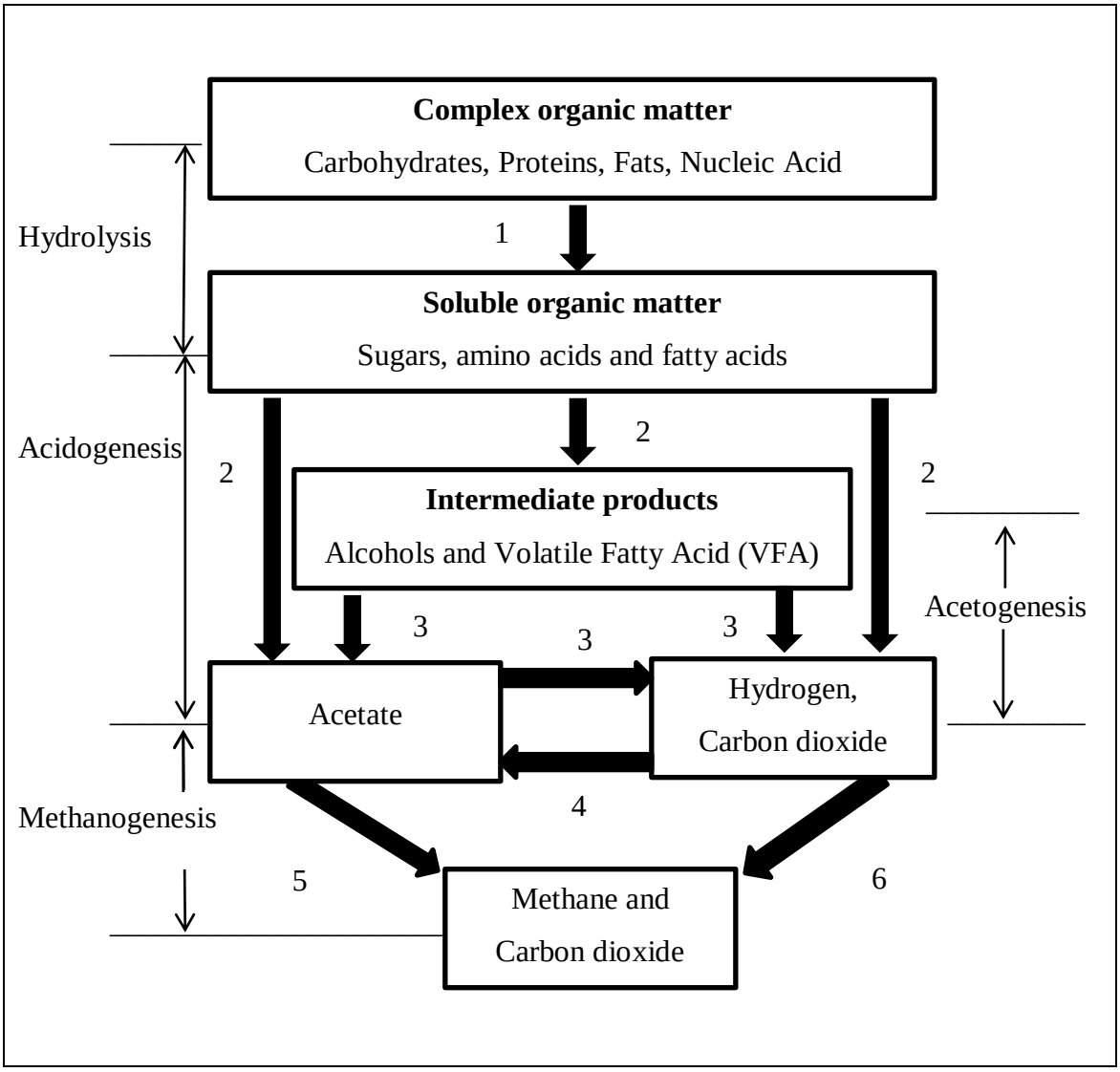

Figure 2.1: Pathway of anaerobic digestion

Source: Lorestani, 2006 and Fang, 2010

Note:

1. Hydrolytic bacteria
2. Acidogens
3. Acetogens (Hydrogen-producing acetogens)
4. Acetogens (Hydrogen-utilizing acetogens)
5. Acetoclastic methanogens
6. Hydrogentrophic methanogens

2.7 MEMBRANE TECHNOLOGIES USED IN PALM OIL MILL EFFLUENT (POME) TREATMENT

Chan *et al.* (2010) mentioned that anaerobic treatment of POME alone can hardly meet the discharge limit requirement of the DOE in Malaysia. Therefore, the conventional anaerobic ponding system for POME treatment was followed by the facultative ponds and algae ponds or mechanical aeration unit so that the POME final discharge will meet the discharge limit of DOE. However, facultative ponds and algae ponds require a large space and mechanical aeration unit is not economical in terms of high energy requirement (Chan *et al.*, 2009). With the emergence of membrane technologies such as microfiltration, ultrafiltration (UF), nanofiltration and Reverse Osmosis (RO) (Ahmad and Chan, 2009), a lot of studies have been done to combine both anaerobic digester and membrane separation technology in POME treatment to replace the conventional methods to achieve high degree of treatment efficiency in shorter time.

According to Ahmad *et al.* (2009), membrane separation technology is recognized as an efficient, economical and reliable technology and applicable in POME treatment. Membrane technology has not just been developed for treating POME but also for the purposes of clean water recovery. Types of the membrane that had been used in the study of Ahmad *et al.* (2009) are RO polymeric, UF polymeric and also ceramic UF membrane. In the study of Ahmad and Chan (2009), UF and RO are suggested to be used to treat the POME at the same time achieving the zero discharge of waste (POME) to the rivers. However, RO membrane still not being preferable to be

used in industrial scale as high pressure is required during the treatment which leads to high energy consumption.

Based on the study done by Wu *et al.* (2007), the treatment of POME using UF showed the reduction of TSS, turbidity, Total Dissolved Solid (TDS) and Chemical Oxygen Demand (COD) up to 97.3 %, 88.2 %, 3.1 % and 46.9 %, respectively by using polysulphone DSS Alfa Laval's flat sheet UF membrane of Molecular Weight Cut Off (MWCO) 20 000 kDa. Three modules of chlorinated polyethylene flat sheet membranes with pore size of 0.4 μm have also been used by Ahmad *et al.* (n.d.) in Hybrid Membrane Bioreactor (MBR) after the process of anaerobic, anoxic and also aerobic treatment for POME. The removal efficiency of COD, Suspended Solid (SS), Total Nitrogen (TN) and Total Phosphorus (TP) achieved by the hybrid MBR were 94 %, 98 %, 83 % and 64 % respectively.

The membrane used in POME treatment usually faces the limitation of fouling which affects the treatment efficiency due to the high TSS content in POME. Therefore, the pretreatment of POME before membrane filtration process is important to be carried out to prevent the clogging of membrane.

Pretreatments of POME such as chemical treatment and activated carbon treatment was done before membrane separation treatment, both Ultrafiltration (UF) and Reverse Osmosis (RO) by Ahmad *et al.* (2003) showed a reduction in turbidity, COD and BOD removal up to 100 %, 98.8 % and 99.4 % respectively with a final pH of 7.

2.8 MEMBRANE ANAEROBIC SYSTEM (MAS)

The Membrane Anaerobic System (MAS) that would be used in this study was developed by Abdurahman *et al.* (2011). It is a system which combined both anaerobic digester (biological treatment) and Polyethersulphone (PES) Cross flow Ultrafiltration (CUF) membrane, which can be used for POME, sewage sludge or slaughterhouse waste treatment. The membrane unit in MAS was designed in the way of cross-flow to make the membrane to be more sustainable to the membrane fouling as compared to the dead-end membrane. Anaerobic digestion of POME takes place in the anaerobic

digester in MAS and membrane functions as a permeable barrier that help in prolong the Solid Retention Time (SRT) in anaerobic digester to allow the POME being fully digested by the microbial community in the digester to produce biogas.

MAS can be used to treat variety of waste besides POME, take for instance, sewage sludge. Study has been conducted on sewage sludge treatment by using MAS and the results of treatment showing the COD removal efficiency of 96.5 % to 99 % with the methane gas yield between 0.19 L/ g COD/ day to 0.54 L/ g COD/ day with the organic loading from 0.1 kg COD/ m^3/ d increased to 10 kg COD/m^3 / d.

MAS has a high efficiency in treating anaerobically digested POME. MAS showed the removal efficiency of COD from 96.6 % to 98.4 % by reducing the Hydraulic Retention Time (HRT) from 600.4 to 6.8 days and the methane gas production range from 0.25 to 0.57 CH_4/ g COD/ day (Abdurahman *et al*., 2011).

2.8.1 Kinetic models applied in Membrane Anaerobic System (MAS)

Kinetic models develop is important for describing the behaviour of the anaerobic digester. The three kinetic models that have been tested in MAS were Monod model, Chen and Hashimoto and Contois model with the efficiency of 99.4 %, 99.7 % and also 99.5 % (Abdurahman *et al.*, 2011).

Table 2.6: The three kinetic models established for substrate utilization in MAS

Kinetic model	Equation	Equation
Monod	$U = \frac{kS}{K_s + S}$	$\frac{1}{U} = \frac{K_s}{K}\left(\frac{1}{S}\right) + \frac{1}{K}$
Contois	$U = \frac{U_{\max}\ x\ X}{Y(B\ x\ X + S)}$	$\frac{1}{U} = \frac{a\ x\ X}{\mu_{\max}\ x\ X} + \frac{Y(1+a)}{\mu_{max}}$
Chen and Hashimoto	$U = \frac{\mu_{\max}\ x\ S}{YKS_o + (1-K)SY}$	$\frac{1}{U} = \frac{Y\ K\ S_o}{\mu_{\max}\ S} + \frac{Y(1-K)^{max}}{\mu_{max}}$

Adapted from: Abdurahman *et al.* (2011)

Based on Table 2.6, MAS is the combination of both anaerobic digester and CUF membrane which show a high COD removal and methane production with shorter HRT. This indicates that MAS is a potential system to be commercialised in future. The study of the kinetic parameters of POME is important to evaluate the performance of MAS in treating POME so as to develop a better system to optimise the methane production.

2.9 FACTORS AFFECTING ANAEROBIC DIGESTION PROCESS AND METHANE GAS PRODUCTION

2.9.1 Factors affecting the anaerobic digestion process

According to McInerney *et al.* (1980) in Ahring *et al.* (1995), anaerobic digestion process is a process consisting a series of microbial reactions which catalyzed by a consortia of different bacteria and therefore the interdependence of bacteria is a key factor of the biogas production. The stability of anaerobic digestion process in anaerobic digesters is very important for biogas production and needed to be controlled from time to time. Those parameters that affect the efficiency of the anaerobic digestion process include temperature, pH, retention time and toxicants such as ammonia, Volatile Fatty Acid (VFA) and hydrogen sulfide.

2.9.2 pH and alkalinity

pH is the most important parameter that affects the growth of the microbial community in the anaerobic digester. Therefore, pH needed to be monitored at its optimum range. Yadvika *et al.* (2004) said that optimum pH range of the anaerobic digester should be within 6.8 to 7.2. As what have been mentioned in 2.6.4, methanogens metabolize the best in the pH range of 6.7 to 8.0. For the pH lower than 6.7, acidogens are more active as compared to methanogens and this will lead to the instability of anaerobic digester as more organic acids will be produced by the anaerobic degradation of organic compounds via acidogenesis phase. Those organic acids accumulated in the digester will lastly inhibit the methanogen activity. Jain and Mattiasson (1998) reviewed in Yadvika *et al.* (2004) found that above pH 5.0, the

efficiency of methane gas production was more than 75 %. pH value of the anaerobic digester also being affected by the carbon dioxide content in the biogas.

Methanogens are very sensitive to the environmental pertubations during the anaerobic treatment. A sudden pH change could lead to an entire anaerobic digester failure (Connaughton *et al.*, 2006 in Wijekoon *et al.*, 2011). Therefore, a certain amount of alkaline, such as sodium bicarbonate and potassium bicarbonate (which are least toxic to bacteria in the digester) needed to be added to the anaerobic digester to adjust the pH in the anaerobic digester. Alkalinity of the anaerobic digester needed to be monitored as high alkalinity in the anaerobic digester serves as a buffer that prevents fluctuations in pH and maintain the digester stability (Gerardi, 2006).

2.9.3 Temperatures

The methane-forming bacteria (methanogens) are active in two temperature ranges, mesophilic range from 30 ^{o}C to 35 ^{o}C and also thermophilic range, 50 ^{o}C to 60 ^{o}C. The methanogens that functioning well in mesophilic condition are known as mesophiles while in the thermophilic condition are known as thermophiles. Vindis *et al.* (2009) mentioned that the anaerobic digestion occurred in mesophilic range is the most widely used as most of the methanogen are mesophiles. Hamilton (n.d.) stated that 35 ^{o}C is the optimal temperature for mesophilic methanogens. Gerardi (2006) mentioned that, at the temperature between 40 ^{o}C and 50 ^{o}C, the methanogen activity is inhibited and 32 ^{o}C is the minimum temperature for anaerobic digestion.

Vindis *et al.* (2009) described that thermophilic anaerobic fermentation reduce the process stability, require a large amount of energy for heating and there is no significant change in the total methane yield from the organic matter for fermentation temperatures ranging from 30 ^{o}C to 60 ^{o}C. In the other hands, Gerardi (2006) claimed that thermophilic digestion has an advantage which is allowing thermal destruction of pathogenic agents with higher temperature and produce an effluent lack of pathogens. He also stated that although higher temperature can increase the volatile solids destruction rate and produce methane gas faster, the thermophilic anaerobes are very sensitive to the rapid change of temperature and this makes the temperature control of

thermophilic digester more difficult than mesophilic digester. This has offset the advantage of thermophilic digester. Low yield of anaerobic thermophiles in thermophilic condition, high endogenous death rates and lack of diversity of these bacteria in thermophilic condition makes the mesophilic anaerobic digestion more preferable in most of the conditions.

2.9.4 Retention Time

In an anaerobic digester, two retention times needed to be monitored, Solid Retention Time (SRT) and Hydraulic Retention Time (HRT). According to Hamilton (n.d.), the SRT is referred to the cell retention time which means the average time of microorganism (solids) in anaerobic digester. In the other hand, HRT means the average time of the wastewater (liquid) remains in the anaerobic digester, calculated by its volume divided by the flow rate of the liquid leaving the digester. According to Gerardi (2006), high SRT value helps the biological acclimation to toxic compounds, as well as maximizing organic removal capacity and providing a buffering capacity for the protection against shock loadings to the digester. To prolong the SRT of the anaerobic digestion, it was recommended to use a fixed-film media, screen or membrane to trap some microorganism to concentrate the microbial community in the anaerobic digester, as the same time, prevent the biomass washout. MAS used in this study is worked based on the above concept.

The time of the suspended cells remain in the reactor equals to HRT. Hamilton (n.d.) discussed that if the HRT of the anaerobic digester is equals to the reproduction time of the organisms living in the reactor, the population of the cells always remains stable as new cells form to replace old cells leaving. Conversely, if the HRT is shorter than the reproduction time of the microorganism and this will cause the washout of the microbial population. Therefore, HRT could be said to control the conversion of volatile solids to gaseous products in an anaerobic digester and affect the rate and extent of methane production.

2.9.5 Effects of the Toxicants

A wide range of the inorganic and organic compounds especially the end products of the anaerobic digestion can cause toxicity in anaerobic digesters. As mentioned by Gerardi (2006), the toxicity in anaerobic digester can be acute or chronic and toxicity levels of those end products needed to be controlled to make sure the methane gas production of the anaerobic digester at its optimum level. In general, those toxicants which are most commonly being reviewed were ammonia, hydrogen sulfide (H_2S) and long chain Volatile Fatty Acids (VFA).

2.9.5.1 Ammonia

Vindis *et al.* (2009) mentioned that free ammonia is generated from the anaerobic degradation of urea and proteins that occurs in the waste during the anaerobic digestion and normally along with high pH values. Ammonia is toxic to methane-forming bacteria. As mentioned by Hamilton (n.d.), ammonia (NH_3) toxicity is highly dependent on pH and ammonia which predominant form at higher pH is more toxic than ammonium ion (NH_4^+). Gerardi (2006) also discussed that the inhibitory level of ammonical nitrogen (NH_4^+-N) and ammonia that cause inhibition onto the anaerobic treatment and anaerobic digesters failure once it exceeds the values 1500 mg/L and above. However, another study done by Steffen *et al.* (1998) mentioned that microbial adaptation is possible towards the high ammonia concentration beyond 2000 mg/L. Basically, the digester failure is caused by an unacclimated populations of methanogen at high ammonia concentrations. Nevertheless, ammonia inhibition could be self-correcting as when the high ammonia concentration inhibits the methanogenesis stage, accumulation of VFA may happen and caused the drop in pH and this phenomenon caused the free ammonia converted to ammonium ions, which is less toxic (Gerardi, 2006).

2.9.5.2 Volatile Fatty Acid and long chain fatty acids

Based on the Hill and Barth (1977) mathematical model found in Lyberatos and Skiadas (1999), the VFA accumulation causes the anaerobic digester failure during the anaerobic digestion. VFA accumulation in the anaerobic digesters was reported to cause the reduction in pH (Bailey and Ollis, 1986 in Špalková *et al.*, 2010) and leading to the failure of the digester but in the study of Prasertsan and Ukita (2001) found that the high-rate anaerobic treatment of POME could tolerate with significant fluctuations of volatile fatty acid concentrations without large changes in pH, especially when the anaerobic digester were in highly buffer condition. Therefore, Špalková *et al.*, 2010 mentioned that the pH can only be used as an indicator of the process stability in wastewaters with low buffering capacity. In addition to that, Ahring *et al.* (1995) mentioned that pH is often too slow for the optimal detection of sudden changes in the anaerobic digester, thus, VFA concentration is one of the most important parameters for the accurate control of anaerobic digestion as uncontrolled level of VFA in anaerobic digester will exert toxicity to methane forming bacteria and acid-forming bacteria.

Long chain fatty acids, such as capric, caprylic, lauric, myristic and oleic acids occur in the digester will inhibit the methane production from acetate as they inhibit the activity of the methanogen. And those long chain fatty acids concentrations should be below 500 g/L before they caused toxicity to the digesters. However, the presence of the excess concentration of volatile acids can be corrected or control by the addition of alkaline compound such as sodium bicarbonate (Gerardi, 2006).

Işık and Sponza (2008) claimed that the accumulation of the VFA would cause inhibition of methane gas production in anaerobic digester and caused the low COD removal efficiencies. Conversely, Ahring *et al.* (1995) mentioned that the high concentration of VFA sometime do not have the adverse effect on the biogas process and different anaerobic system have their own normal levels of VFA depended on the composition of the substrates digested and the operating condition. With reference to Yadvika *et al.* (2004), the concentration of volatile fatty acid, acetic acid in the anaerobic digester should be below 2000 mg/L for an anaerobic fermentation to proceed

normally. In the other hand, Husnul *et al.* (2006) mentioned that a healthy anaerobic digester will normally have VFA concentration of up to 4000 mg/L.

2.9.5.3 Hydrogen sulfide

According to Hamilton (n.d.), sulfate is not a toxicant in anaerobic digester but its presence can reduce the methane gas production as the sulfur reducing bacteria will compete with methanogen for the available hydrogen occur in the anaerobic digester. The genera of the sulfur reducing bacteria are *Desulfuromonas, Desulfovibrio and Desulfomonas*, which are similar in morphology with certain methanogens (Gerardi, 2006). Hilton and Archer (1988) mentioned that the reduced sulfur products of sulfate-reduction such as hydrogen sulfide (H_2S) are inhibitory to methanogenesis. H_2S is one of the most toxic components to anaerobic digesters which lower down the production of biogas in anaerobic digester and this normally occurred during low organic loadings condition. Hamilton (n.d.) has stated that the toxicity of sulfide is dependent on pH; therefore control must be done onto the anaerobic digester to gain a balance between biogas production, source reduction and pH. Nonetheless, Gerardi (2006) suggested a few treatments to reduce the soluble hydrogen sulfide, such as precipitating sulfide as a metal salt, scrubbing and recirculating digester biogas and diluting the sulfides.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter discussed the materials and research methodology that had been used in the study. The process flow diagram of the experimental work (refers Figure 3.1 in 3.1.1), methodology and research instruments would be discussed in this chapter.

Three runs of experimental work (Run 1, Run 2 and Run 3) were conducted in this study for treating different palm oil mill effluent (POME) samples with Membrane Anaerobic System (MAS). The raw POME, which discharged from the crude palm oil processing and some anaerobically digested POME were collected from anaerobic pond Lepar Hilir Palm Oil Factory, Kuantan, Pahang as the samples for this study. The samples were stored in the refrigerator at 4 ^{o}C prior use (refers 3.3.1).

For the microorganisms' general characteristics study, the spread plate dilution and streak plate method (refers 3.3.4 and 3.3.5) were selected in this research to isolate the microorganisms from the sample collected. The growth patterns of microorganism were observed on the agar plates and the one which grown dominantly was further observed under microscope. Gram stain and negative staining (refers 3.3.9.2) were done onto the microorganisms to see the shape and the general morphology of the microorganisms. Then, the inoculum was prepared (refers 3.3.13) from the culture before being added into the anaerobic digester of MAS in Run 3 by tube and peristaltic pump. The growth curve profile of the microorganisms was studied to determine the maximum time needed to culture the microorganisms (refers 3.3.12).

In Run 1, raw POME was diluted 50 % and then was treated with MAS. In Run 2 and Run 3, the samples of the anaerobically digested POME were treated by using MAS without the addition of inoculum or with the addition of inoculum. The inoculum here is referred to the mixed culture inoculum prepared in this study. The setup of the MAS used was discussed in more details at research instruments (refers 3.5). Experiments in three runs of the experiment were run for 5 hours each day for duration of one week. Three samples were taken from MAS for each day of the experiment for the parameter testing. The samples were influent (which taken before the POME sample undergoes anaerobic process in anaerobic digester), retentate (after the anaerobic digestion process) and the permeate. The samples taken were then tested for parameters Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Volatile Suspended Solid (VSS) and pH analysis.

According to Abdurahman *et al.* (2011), methane gas was collected and analyzed by using J tube analyzer (refers 3.4.5). This method assuming that the gases produced in the bioreactor of MAS after the POME treatment were carbon dioxide (CO_2) and methane gas (CH_4).

3.1.1 Process flow diagram

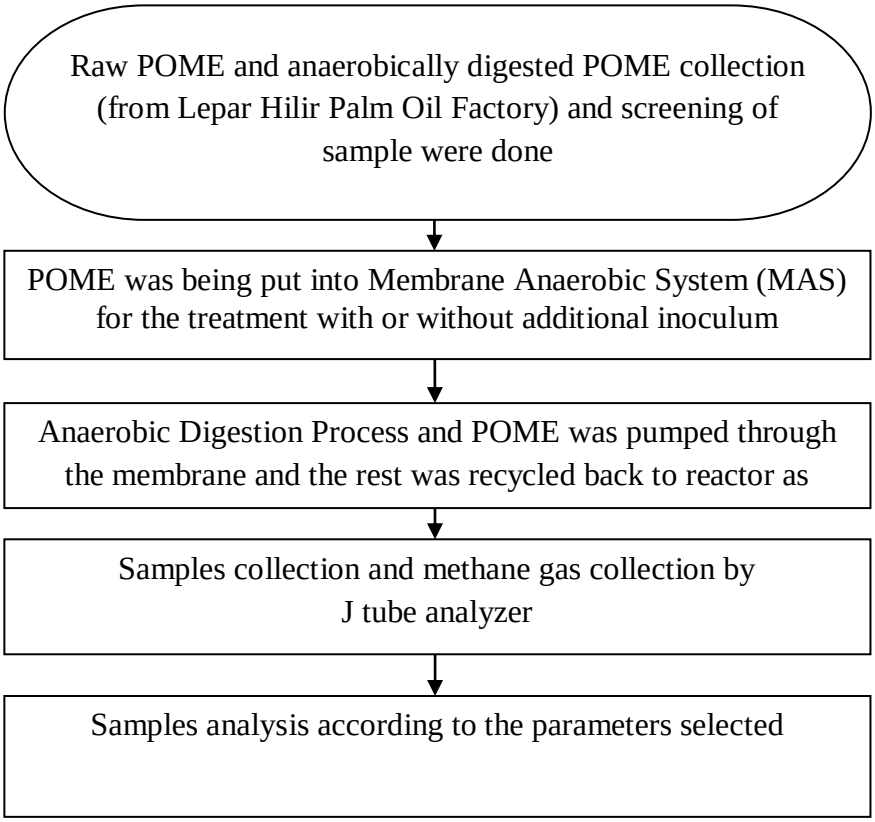

Figure 3.1: The process flow of the experimental works

Figure 3.1 showed the process flow diagram of the experimental works that has been done in this study. Both raw POME and anaerobically digested POME was collected from Lepar Hilir Palm Oil Factory. Three runs of the experiments (Run 1, Run 2 and Run 3) were conducted by using three different types of samples. Run 1 was using 50 % diluted raw POME, Run 2 was using the anaerobic digested POME without the addition of inoculum and Run 3 was using the anaerobic digested POME with the addition of inoculum as the sample. The samples were screened with sieve before putting into MAS to avoid the coarse solid such as soil and colloidal particles from damaging the Cross-flow Ultrafiltration (CUF) membrane of MAS. Table 3.1 and Table 3.2 in 3.2 showed the equipments and materials that were needed during the experimental work.

3.2 EQUIPMENTS AND MATERIALS NEEDED FOR THE EXPERIMENTAL WORK

Table 3.1: Equipment needed for the experimental work

No.	Equipment	Analysis or Functions
1	Microscope	Microorganism general characteristics
2	UV Vis spectrophotometer	Optical density of inoculum
3	Spectrophotometer Hach DR 2400	Chemical Oxygen Demand (COD)
4	COD Reactor DRB 200 Hach	Chemical Oxygen Demand (COD)
5	Buchner flask and funnel	Total Suspended Solid (TSS)
6	pH meter	pH of the samples
7	Laminar Flow Hood	Culturing microorganism
8	J-tube analyzer	Methane gas composition
9	Autoclave	Sterilize apparatus, nutrient broth and agar
10	Furnace	Volatile Suspended Solid (VSS)
11	Magnetic Hot Place	Preparation of nutrient agar (refers 3.3.2)
12	Drying oven	Total Suspended Solid (TSS)

Table 3.2: Materials needed for the experimental work

No.	Materials	Manufacturers or Sources
1	Raw Palm Oil Mill Effluent (POME)	Lepar Hilir Palm Oil Factory, Kuantan
2	Anaerobically digested POME	Lepar Hilir Palm Oil Factory, Kuantan
3	Sodium hydroxide (NaOH)	Sigma Aldrich (M) Sdn Bhd, Selangor
4	Potassium hydroxide (KOH)	Sigma Aldrich (M) Sdn Bhd, Selangor
5	Nutrient Broth	R & M Marketing, Essex, U.K.
6	Nutrient agar	R & M Marketing, Essex, U.K.
7	Disinfectants (70 % ethanol)	Sigma Aldrich (M) Sdn Bhd, Selangor
8	AnaeroGen sachet and anaerobic indicator	Thermo Scientific Microbiology Sdn. Bhd.

3.3 METHODOLOGY

3.3.1 Sampling procedures and sample preparation

The raw material, POME, which was being used in this experiment, was collected from the nearby Lepar Hilir Palm Oil Mill Factory. 25 L of the raw POME after the Crude Palm Oil (CPO) processing (around 80 ^{o}C) (refers Figure 3.2) and 75 liters of anaerobically digested POME from the anaerobic pond (refers Figure 3.3) were collected and put into four 25 liters waste tanks (refers Figure 3.4). The samples were left to cool down before being stored in a cold room at 4 ^{o}C. The screening of raw material was done with sieve before putting into the MAS to prevent the large particles such as soil from damaging the CUF membrane, clogging the pump and also the valves of the MAS system. 35 L of POME was being used for each run of the experiment. Samples were analyzed for COD, TSS, VSS and pH. The experiments were run for three times (Run 1, Run 2 and Run 3) by using three different POME samples. In Run 1, the raw POME was diluted into 50 % (v/v) before used. For Run 2 and Run 3, the anaerobically digested POME from anaerobic pond were being used without or with the additional of inoculum respectively.

Besides the POME samples, 500 mL of the effluent was collected from the anaerobic treatment pond in an autoclaved 500 mL Amber Schott bottle with two tubes (refers Figure 3.5) for the purpose of culturing the microorganisms and microorganisms morphological study. The Schott bottle was tied with a long stick and dipped into the anaerobic treatment pond before it was being put into a tin with a candle lit inside. The purpose of lighting a candle was to create an anaerobic condition for storing the sample all the way transferring it back to UMP. Then, the sample in the Schott Bottle was purged with gas nitrogen for 10 minutes through an autoclaved 0.22 µm Polytetrafluoroethylene (PTFE) membrane air filter to eliminate the oxygen in the headspace of the bottle before tubes were sealed with parafilm and then clipped with aluminum crimps after purging (Cheong and Hansen, 2006). The PTFE membrane air filter was used to eliminate any contamination and the aseptic technique was being applied to ensure the microorganisms that spread on spread plates were all from the

anaerobic pond. Then, the sample was being diluted in serial and spread onto the petri plate and the leftover was being stored in the cold room at 4 ^{o}C.

Figure 3.2: Raw POME sampling after the crude palm oil processing

Figure 3.3: Anaerobic digested POME sampling from the Lepar Hilir Palm Oil Factory anaerobic pond

Figure 3.4: The 25 L tanks used to store the POME sample

Figure 3.5: 500 mL Amber Schott Bottle with two tubes and an aluminum crimps used to collect the effluent for microbial culture

3.3.2 Anaerobic microorganism isolation

Spread plate and streak plate techniques were applied to study the general characteristic and the morphology of microorganism in the anaerobic POME. Modified spread plate and streak plate culture technique was referred to Benson's Microbiological Applications (Brown, 2005), Microbiology: A Laboratory Manual (7^{th} Edition) (Cappuccino and Sherman, 2004) and General Microbiology Laboratory Manual (Siru, 2000).

3.3.3 Preparation of the laminar flow hood, agar plates and agar deep

The preparation method of laminar flow hood and agar plates was modified and adopted from Chua (2011).

The sterile agar plates was prepared as follows: Nutrient agar with formula of Agar, 15 g; Reptic Digest of Animal Tissue, 5.0 g; Sodium Chloride, 5.0 g; Beef Extract, 1.5 g; Yeast Extract; 1.5 g (total 28 g was weighed on an aluminum weighing board on electronic balance) was dissolved into 1 L distilled water with pH 7.4. The nutrient agar was stirred using magnetic stirrer on the magnetic hot plate for 15 minutes before putting into a 1 L Schott Bottle for autoclave at 15 psi, 121 ^{o}C, 20 minutes. The cap of the Schott Bottle was being covered using aluminum foil with a short sterilized indicator tape and loosened before autoclave to avoid the pressure build up in the bottle. The Schott bottle cap was tightened immediately after it had been autoclaved.

The laminar flow hood had to be prepared before preparing the agar plates. The UV light of the laminar flow hood was switched on for at least 30 minutes and then the air flow was on for 10 minutes before used. The working table of the laminar flow hood was swabbed with 70 % alcohol before transferring all the apparatus inside the hood. All the items were being sprayed with 70 % alcohol for decontamination before being brought into the laminar flow hood.

The sterile nutrient agar was then cooled to the temperature around 50 ^{o}C and the mouth of the Scott Bottle will be flamed by using Bunsen Burner before pouring off

approximately 15 mL of sterilize nutrient agar to the pre-sterilized petri plate. The plate was gently rotated to ensure the agar was evenly distributed without splashing any agar medium to the sides. The plate was left to cool before sealed with parafilm to avoid moisture condensed in the plate. To avoid any contamination, the agar plates were arranged in row from the left to the right with their lid half closed in the most internal part of laminar flow hood. The lid was then closed from the right to the left by using the right hand to avoid our hands over the petri plates to avoid the contamination onto them. The agar plates were sealed with parafilm and the excess agar plates were be stored in 4 ^{o}C chiller for the future use.

Agar deeps was prepared by pouring off approximately 20 mL of the nutrient agar into the 28 mL universal bottles and left them to dry. The caps of the universal bottle were sealed with parafilm and stored in 4 ^{o}C fridge for the use of the stab culture storage.

3.3.4 Serial dilution of sample

The effluent taken from the anaerobic pond in Lepar Hilir Palm Oil Mill was diluted into 6 sterile universal bottles (refers Figure 3.6). 9 mL of the sterile distilled water was pipetted into each bottle aseptically and the bottles were then labeled from 1 to 6 as Bottle 1 and thereafter.

1 mL of the POME sample was pipetted into the Bottle 1 by using Eppendorf 1 mL micropipette and the autoclaved pipette tips. Each tip was discarded for each transfer into a beaker which contained 250 mL disinfectants. Then, the total of 10 mL diluted sample was transferred in the Bottle 1 was then mixed well before 1 mL of the diluted sample was then being pipetted aseptically from the Bottle 1 to Bottle 2. These procedures were repeated by pipette 1 mL of the diluted sample from Bottle 2 into Bottle 3 until 6 (Siru, 2000).

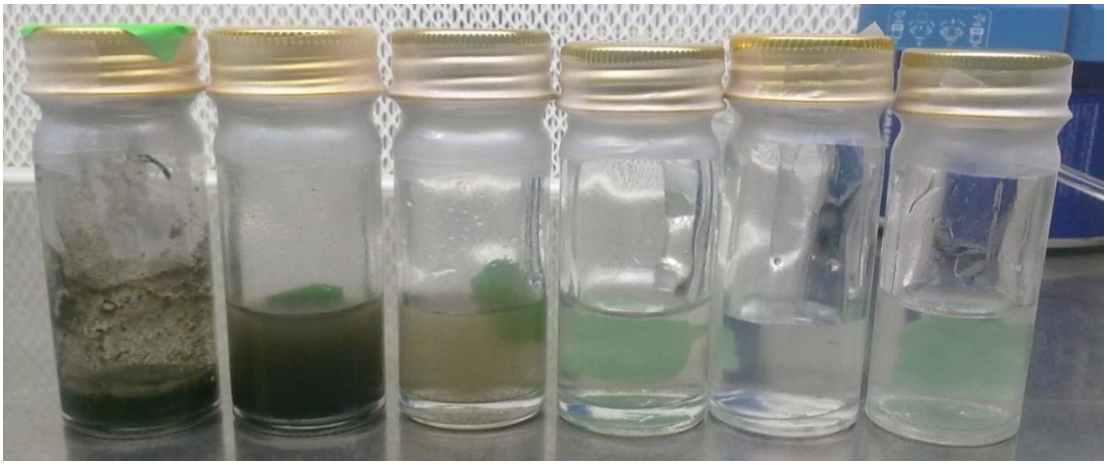

Figure 3.6: Raw sample from anaerobic pond and sample dilution 10^1 to 10^6. From left to right: Bottle 1 to 6.

3.3.5 Spread plate technique

0.1 mL (100 µL) of sample from each dilutions, Bottle 1 to Bottle 6 (refers Figure 3.7), was pipetted using micropipette onto duplicate agar plates (A and B) prepared. To avoid contamination, the glass spreader was dipped into 70 % ethanol, ignited with flame and let to be cooled before spread the 0.1 mL sample by turning 360 degree on the agar plates (Siru, 2000). After the glass spreader was evenly spread over the sample on the agar plates, they were sealed with parafilm, labeled and incubated in an Oxoid AnaeroJar (refers 3.3.7) at 30 ^{o}C for 48 to 72 hours in an inverted position to avoid condensation on the agar surface (Brown, 2005). The colonies that had grown on the agar plate were then further streak on the new agar plates and observed by gram staining procedures (refers 3.3.9) .

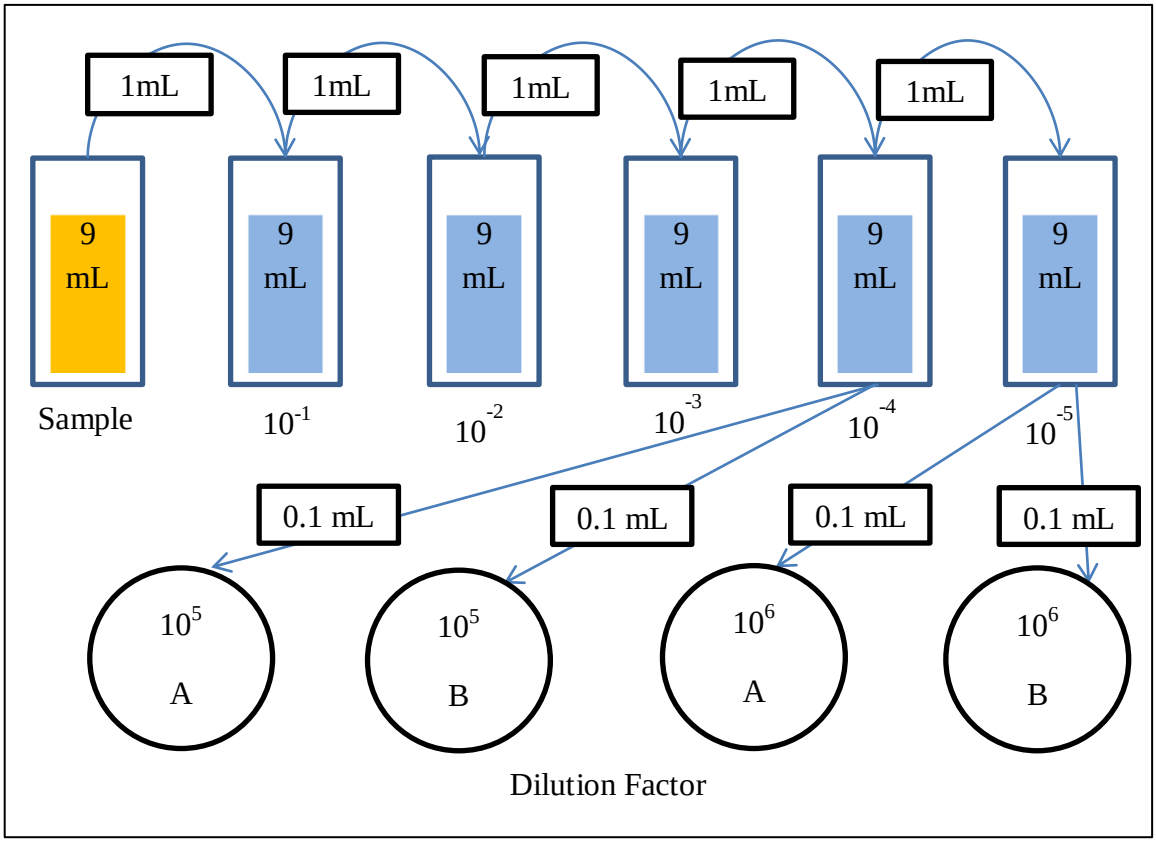

Figure 3.7: Serial of dilution on the agar plates

Figure 3.7 showed the schematic diagram of the serial dilution-agar plate spreading procedure by referring to Cappucinno and Sherman (2004). Figure 3.7 also showed that the Bottle 2 had the dilutions of 10^{-1} and thereafter. The spread plate prepared from the dilutions of 10^{-4} by pipetting 0.1 mL sample was expressed to have a dilution factor of 10^{5} and thereafter. Dilutions factor was defined as the mathematically as the reciprocal of the dilution.

3.3.6 Streak plate technique

After incubating the agar plates for 72 hours in anaerobic jar (refers 3.3.7), the plates were observed. Different colonies which grow dominantly were selected from the agar plates to be further isolated by streak plate techniques. Inoculating loop was flamed to redness and cooled. Then, a loopful of the culture of the agar plates was obtained aseptically and streak on new agar plates. One edge of the Petri plate cover was lifted

and making as many streaks as possible without overlapping previous streaks onto the agar plate (Siru, 2000). The inoculating loop was flamed again and let cool. The plate was turned to the next sector and streaked it in the same manner until four quadrants as in Figure 3.8. The loop was reflamed before changing direction on the agar plate. The agar plate culture was then sealed with parafilm, labeled and incubated in the anaerobic jar for another 72 hours.

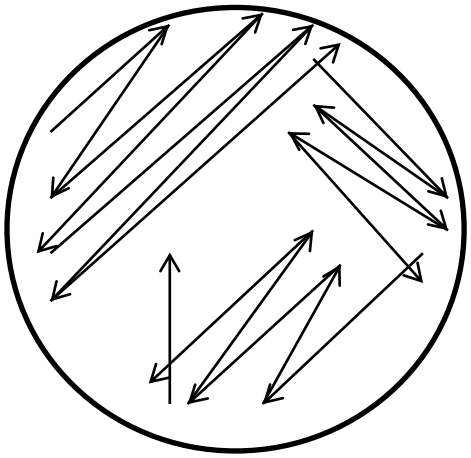

Figure 3.8: The pattern to streak on the agar plate to obtain a single colony

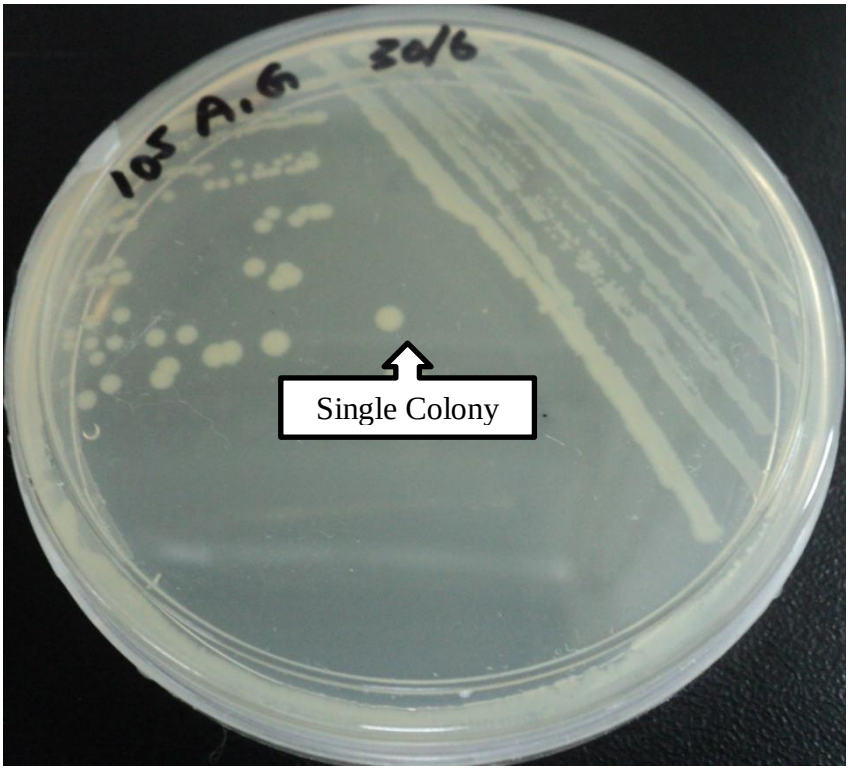

Figure 3.9: Streak Plate was to obtain a single colony.

After 72 hours of incubation, a single colony of the microorganism (refers Figure 3.9) was picked to streak on a fresh agar plate, incubated, sealed and inverted to store at 4 ^{o}C. The colony was being subculture every two weeks on the agar plates to maintain its viability (Baker, 2000). The colony was also kept as a glycerol stock and stab culture (refers 3.3.11) in both -80 ^{o}C fridge and -20 ^{o}C fridge for long term storage for several months (Baker, 2000).

3.3.7 Incubation in Oxoid AnaeroJar

The method of incubating the agar plates anaerobically by using Oxoid AnaeroJar was referred to Boulette and Payne (2007). The 2.5 L Oxoid AnaeroJar (refers Figure 3.10) was used to incubate the spread plate and streak plate. Before using the Anaerobic Jar, the 'O' ring of the jar was ensured to be correctly seated and the vacuum relief screw is in the closed position. The inoculated plates were placed into the plate carrier. Then, the Oxoid Anaerobic Indicator (BR55) was prepared and insert into the smaller, upper clip on the dish carrier. The dish carrier was lowered into the polycarbonate base of the Oxoid AnaeroJar. An AnaeroGen sachet was torn at the tear-nick indicated and immediately placed in the appropriate clip in the plate carrier and secure the four clips of the jar with fingers to properly secure the lid. Then, the AnaeroJar was transferred to put in the incubator at 30 ^{o}C. The anaerobic indicator would change colour from pink to white which indicated the anaerobic condition.

Figure 3.10: Oxoid AnaeroJar

3.3.8 Morphology determination

The microorganism's morphology obtained on the spread plates and streak plate was determined and tabulated in Table 4.1 and Table 4.2 by doing observation based on its colours, form, margin and elevations (refers Table 3.3) according to Cappuccino and Sherman (2004).

Table 3.3: Morphology of colonies on agar plates

Form		
		
Circular	Irregular	Rhizoid
Margins		
		
Entire	Undulate	Filamentous
		
Lobate		
Elevation		
		
Raised	Convex	Flat
		
Umbonate		

3.3.9 Preparation of Smears and Gram Stain

3.3.9.1 Preparation of Smears

Preparation of smears from the solid media was done prior to gram staining (Cappuccinno and Sherman, 2004). A clean glass slide was sprayed with 70 % ethanol and wiped with Kimwipe tissue. By using a loop, one drop of autoclaved water was placed in the centre of the slides. Then, a small amount of the single colony from the petri dish was taken by sterile loop and emulsified or spread in a circular motions in the drop of the water on the slide. Then, the slide was hold at one end; the smear was heat fixed on the slide by passing it over the Bunsen burner two to three times until it was completely dried. Preparation of smears and gram staining were done in the laminar flow hood.

3.3.9.2 Gram stain and negative staining

The procedures of Gram stain were referred to Cappuccinno and Sherman (2004) and Claus (1992). For the Gram stain, the reagents such as crystal violet, gram iodine, 95 % (v/v) ethanol and safranin were used. The slide with the smear preparation was first flooded with crystal violet and let for 1 minute; the slide was gently washed with tap water. Gram's iodine was then gently flooded the slide for 1 minute before washing with tap water. Then, 95 % (v/v) ethanol was added drop by drop until the ethanol runs almost clear to decolorize until a blue tinge being seen. Then, the slide was washed with tap water. Eventually, the safranin was flooded the slide for 45 seconds and then washed the slide with tap water. The slide was dried with blotting paper. Then, slide was examined under the oil immersion with highest power objectives lenses up to 100x lens. Then, the organisms were classified into gram positive and gram negative based on the colour of the stained cells. If the cells are purple stained, they are gram positive and if the cells are pink colour stained, they are gram-negative.

Negative staining was performed by staining with nigrosin after the Gram Stain to confirm the shape of the microorganism. Firstly, a drop of nigrosin stain was placed on one end of a slide. Then, a loop of the culture was placed into the drop of stain and

mixed by using the inoculating loop. Another clean slide was placed against the drop of the mixture of stain and microorganism at a 45 ^{o} angle and allowed the drop to spread along the edge of the slide. Then, the slide was pushed to spread the drop of the suspended organism until a thin smear was formed. Then slide was then observed under the microscope. The shape of the microorganism was recorded and tabulated.

3.3.10 Preparation of glycerol stock and stab agar storage

The glycerol stock storage of the colony was prepared by modifications from the method of Baker (2000) to store the colony for a long period. Single colonies that grew on agar plates were first inoculated into to the 10 mL autoclaved nutrient broth in 12 mL universal bottles and incubated for 2 days in Oxoid AnaeroJar.

Then, 40 % (v/v) of the glycerol solution was prepared by measuring 400 mL of the glycerol with measuring cylinder and poured into the 1L Schott Bottle with 600 mL of distilled water. The Schott Bottle was then autoclaved at 121^{o}C for 20 minutes. Besides the Schott Bottle, 1.5 mL sterilized cryotubes, 12 mL universal bottles and pipette tips were also being autoclaved. The laminar flow hood was prepared as described in 3.3.3.

By using aseptic technique, 5 mL of 40 % glycerol solution and 5 ml of culture broth was transferred into a 12 mL universal bottle and shook gently for well-mixed. Using a clean micropipette tips, 1 mL of the mixture was then being transferred from the universal bottle to a 1.5 mL cryotube. The cryotube was now containing 20 % (v/v) glycerol and culture broth. The culture stock was replicated in 5 cryotubes for future use. The cryotubes were being screwed, labeled and put into the -80 ^{o}C refrigerator for storage.

For the stab agar storage, an inoculating needle was used to transfer a single colony and stabbed into the agar deep prepared (refers 3.3.3). The caps of the universal bottle was screwed and then sealed with parafilm and stored in -20 ^{o}C fridge.

3.3.11 Recovery the microorganism from the glycerol stock and stab agar storage

The glycerol stock was removed from the fridge to laminar flow hood. The inoculating loop was then flamed with Bunsen burner until it turned red hot and cooled. The cryotube was uncapped and the top of the frozen glycerol stock was being scraped off a portion and then inoculated into the 10 mL nutrient broth or streaked onto the agar plates and it was incubated in the AnaeroJar for 72 hours (Baker, 2000).

For the recovery of the stab agar (agar deep) storage, a portion of the culture was scraped off and streaked onto the agar plates or inoculating into the nutrient broth, then the nutrient broth were incubated in AnaeroJar for 72 hours prior used.

3.3.12 Determination of growth curve of the mixed culture

To determine the mixed culture growth profile, both optical density (OD) and also cell dry weight of the mixed culture needed to be determined. The 10 mL culture broth of each type of the microorganism was mixed in a sterilized 1 L Erlenmeyer flask and topped up with the 1 L nutrient broth equally into two 1 L Erlenmeyer flasks and covered with a modified two tubes stopper and purged with nitrogen gas through the autoclaved PTFE syringe filter (refers Figure 3.12). The modified two tubes stopper was then clipped with aluminum crimps. Then, it was also being put in the incubating shaker at 30 ^{o}C and 100 rpm so that the mixed culture could be grown in suspension and acquired the nutrient equally.

The mixed culture growth curve was determined whereby sampling was done in 1 hour interval. 13 mL of the sample was being pipetted and stored in sample vial for optical density reading and cell dry weight determination. The OD of the sample was checked by transferring 3 mL of the sample in cuvette and then checked with UV-Vis spectrophotometer at 660 nm (Chua, 2011) and a clear (fresh) nutrient broth was set as blank. The OD value of the samples was recorded for each hour until it became constant. The same cuvette was being used throughout the experiment. The OD was plotted against the time of culturing the mixed culture.

Then, another 10 mL of the mixed culture sample was then be filled in pre-weighted 15 mL centrifuge tube (refers Figure 3.11) and centrifuged at 7,600xg (10,000 rpm) for 15 min at 5 ^{o}C (Dermlim, 1999 in Kaewchai and Prasertsan, 2002). The supernatant was then removed by using micropipette and then washed with distilled water, the remaining solid was dried on the empty aluminum foil in 105 ^{o}C oven overnight, weight and the reading was recorded until 3 consecutive weights.

Figure 3.11: The ways of arranging the centrifugal tube while centrifugation

Figure 3.12: Purging the mixed culture with nitrogen gas using aseptic technique

3.3.13 Preparation of inoculum

The isolated colonies obtained from the agar plate cultured (refers to 3.3.6) was further used to prepare 3.5 L of the inoculum. 50 mL of the mixed culture from nutrient broth (refers 3.3.11) was inoculated into the 1 L flask containing 500 mL fresh nutrient broth (the ratio of the mixed culture volume to the volume of fresh nutrient broth was 10 % to 90 % (v/v)) (Chua, 2011) aseptically by using a sterile 50 mL measuring cylinder. The flask was incubated at 30 ^{o}C for 48 hours and Optical Density (OD) of the broth was checked with UV-Vis spectrophotometer at 660 nm until it achieved 0.60 absorbance units (AU). Another 4 L nutrient broth was prepared in four Erlenmeyer flasks with modified stopper with two tubes and autoclaved. Transfer 100 mL of prepared nutrient broth to the four 1 L Erlenmeyer flask. Then, the flask was purged with nitrogen (Cheong and Hansen, 2006) before they were incubated at 30 ^{o}C for 48 hours. Then, the 4 L inoculum prepared was transferred into the MAS via peristaltic pump and tube.

3.3.14 Membrane cleaning

The research instrument that would be used in this study was MAS and the setup was described in 3.5. The Cross Flow Ultrafiltation (CUF) membrane unit in the MAS was cleaned before each runs of the experiment to ensure the membrane fouling would not affect the system performance on POME treatment.

Chemical cleaning and physical cleaning had been selected in this study to clean the membrane. The procedures of membrane cleaning was done by disassembled the stainless steel housing of the MAS. The CUF membrane was taken out from the stainless steel housing and then the membrane was soaked in 0.1 M sodium hydroxide (NaOH) solution overnight and a soft brush was being used to brush the outer surface of the CUF membrane gently. The inner part of the membrane was flushed with water before the membrane was assembled back to the stainless steel membrane housing.

3.4 ANALYTICAL METHODS

3.4.1 Chemical Oxygen Demand (COD)

A dichromate oxidation Hach Method 8000 (Hach, 2010) was used for COD analysis. COD of the samples was analyzed using Hach COD digestion reactor, spectrophotometer Hach DR 2400, volumetric pipette 10mL, paper towel and COD digestion reagent High Range (HR) vials. 100 mL of the sample was homogenized for 1 minute in a blender and then COD digestion reactor (refers Figure 3.13) was turned on to pre-heat until 150 ^{o}C. 1 mL of sample was diluted up to 50 mL by using 49 mL of deionized water in Run 1 for the 50 % diluted raw POME. For Run 2 and 3, 1 mL of the sample was diluted up to 20 mL by using 19 mL of deionized water. Then, 2 mL of the sample was pipetted into the HR vial at 45^{o} angle. The vial was heated for two hours in COD digestion reactor. The vial was cooled and the value of COD was measured by spectrophotometer Hach DR 2400. For each run of the experiment, the COD removal efficiency (%) was calculated by using the Eq. (3.1).

[(COD of influent – COD of permeate)/ COD of influent] x 100 % (3.1)

Figure 3.13: COD Digestion Reactor

3.4.2 Total Suspended Solid (TSS)

TSS analysis was followed Henry (1993) method whereby TSS was dried at 103-105 ^{o}C in drying oven. Apparatus needed were glass microfiber filters discs, disposable aluminum dishes, tweezers, Buchner flask and funnel, 70 mm glass microanalysis filter holder, drying oven (103-105 ^{o}C), desiccators and analytical balance. Set up of the Buchner flask with attached funnel, filter pad were shown in the Figure 3.14.

Figure 3.14: Set up of the Buchner flask and funnel with 70 mm glass microanalysis filter holder

For TSS analysis, the 70 mm glass fiber filter disk was inserted onto the base and clamp on funnel. The disk was washed with distilled water and dried in an oven at 103-105 ^{o}C for one hour. The disk was then be desiccated and weighed. Later, the disk was placed on the base and wet with a small volume of distilled water. 10 mL of the sample was pipetted onto the filter and rinsed with water and vacuum was applied. Then, the filter disk was removed carefully from the base and dried at the temperature of 103-105 ^{o}C in an oven for at least 1 hour. The filter disk was weighed after cooling in the desiccators. TSS was calculated by using Eq. (3.2).

Total Suspended Solids (TSS), mg/L = $\frac{(A-B)x\ 1000\ ml/L}{C}$ (3.2)

Where,

A = weight of filter and dish + residue in mg

B = weight of filter and dish in mg

C = volume of sample filtered in ml

3.4.3 Volatile Suspended Solid (VSS)

Alike TSS analysis, VSS analysis was followed modified Henry (1993) method. The sample that was obtained from the TSS analysis was used in VSS analysis. The sample was put in crucible and ignited to the temperature of 550 ^{o}C using furnace for 20 minutes. The sample was cooled, desiccated and weighed. VSS was calculated by using the Eq. (3.3).

Volatile Suspended Solid, VSS = $\frac{(A-B)\ x\ 1000\ ml/L}{C}$ (3.3)

Where,

A = weight of residue + filter and crucible in mg from TSS test (refers 3.4.3)

B = weight of residue + filter and crucible in mg after ignition up to 550 ^{o}C

C = volume of sample filtered in ml

From the total VSS of the sample, the biomass concentration in the reactor was recorded and plotted against Hydraulic Retention Time (HRT) to evaluate the performance of MAS in treating POME.

3.4.4 pH analysis

The pH meter was calibrated by using three standard solutions of pH 4, pH 7 and pH 9.21. After calibration, the pH probe was dipped into the sample to read the pH of the samples and the pH of the sample was recorded.

3.4.5 J-tube gas analyzer

J-tube gas analyzer (Abdullah *et al.*, 2005) was used in collecting the biogas and analysed the methane gas composition. J-tube gas analyzer composed of a glass-tube connected by a flexible hose to a 35 mL syringe, as shown in the Figure 3.15. The amount of methane gas produced was analysed by adding sodium hydroxide (NaOH) solution to absorb Carbon Dioxide (CO_2). 10 mL of 0.5 M of NaOH solution was filled into the J-tube analyzer initially before the gas tube was inserted into the gas line. The biogas was being drawn into the glass tube until certain mark. The end of the glass-tube was immersed into water (Abdurahman *et al.*, 2011). Once the CO_2 has been absorbed into the NaOH solution, a reduction in the length of the biogas was observed. Therefore, the methane in the biogas was calculated using Eq. (3.4).

$$\text{Methane gas volume} = \frac{Final\ length\ of\ gas\ column,cm}{Initial\ length\ of\ gas\ column,cm} \text{ x } 100\ \% \qquad (3.4)$$

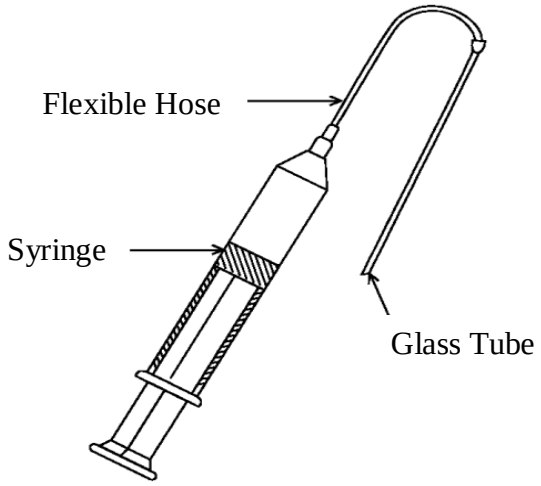

Figure 3.15: J-tube gas analyzer

3.5 RESEARCH INSTRUMENTS

3.5.1 Membrane Anaerobic System (MAS) reactor setup

Laboratory scaled Membrane Anaerobic System (MAS) was used for the experiment. This system consists of a feed tank, four tubular cross flow ultra-filtration membranes (CUF) in a sintered stainless steel holder, a centrifugal pump, and a 50 L anaerobic digester. The CUF membrane used is a type of polyethersulphone (PES) membrane that has a tube diameter of 1.25 cm and an average pore size of 0.1 µm, with molecular weight cut-off (MWCO) of 200 000 Dalton and the length of 30 cm (Abdurahman *et al.*, 2011). The total effective area of the four membranes was 0.048 m^2. The maximum operating pressure on the membrane was 55 bars and the temperature of 70 ^oC, and the pH ranged from 2 to 12. The anaerobic digester is constructed by clear PVC with the total height of 1 m and also the inner diameter of 15 cm. The operating pressure of the pressure gauge was being maintained between 1.5 and 2 bars by adjusting the gate valve at the retentate line. Equipment set up diagram of MAS was shown in Figure 3.16.

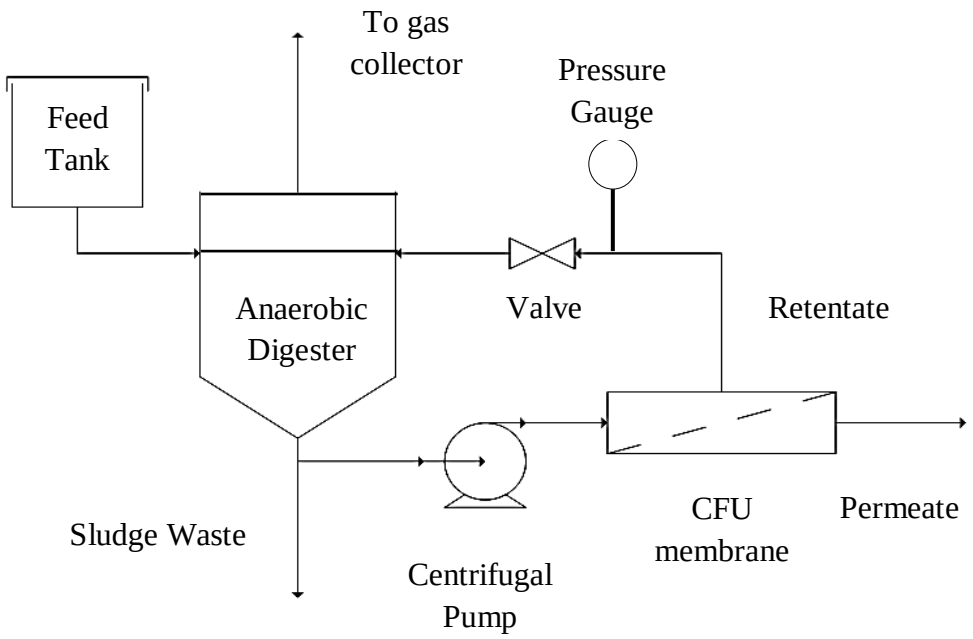

Figure 3.16: Experimental set up diagram of MAS

In conclusion, this chapter discussed about the procedures of the experimental work to prepare the inoculums and test the parameters of the POME before and after it was being treated by MAS. The parameters of COD, TSS, VSS and pH were studied. The performance of MAS in treating POME was then evaluated by the production of methane and also the parameters tested, which were be discussed further in Chapter 4.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

The results obtained from experiment has been tabulated and then plotted into the graphs. The calculation of Chemical Oxygen Demand (COD), pH, Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS) were reflecting the efficiency of the Membrane Anaerobic System (MAS) to treat the Palm Oil Mill Effluent (POME) and the methane gas production. The morphology of microorganism (which was dominant and being selected from the anaerobic pond sample to prepare the mixed culture inoculum for the Run 3) was determined by using spread plate, streaking method and Gram stain. The growth curve of the mixed culture was also determined to obtain the optimum seeding density.

4.1.1 Design of the experiment

Experiments had been run three times using three types of Palm Oil Mill Effluent (POME) samples. The sample used for the Run 1 was the raw POME right after the process of Crude Palm Oil (CPO) in the Lepar Hilir Oil Palm Factory which has been diluted to 50 % with distilled water. The samples used for the Run 2 and Run 3 which were the anaerobically digested sample taken from the anaerobic pond of the Lepar Hilir Oil Palm Factory Treatment Plant. Although the Run 2 and Run 3 were using the same sample, the latter one was with the addition of inoculum which indicates biopretreatment of POME. Each run of experiments were conducted for 5 hours subsequently in 7 days. The total operating volume in the reactor for each run was 35 L. The pH, COD, TSS, and VSS were measured every day throughout the experiment for the influent, retentate and permeate before and after the treatment by using MAS.

4.1.2 Morphology identification before inoculum preparation

The POME sample taken from the anaerobic pond was spread onto the agar plate via serial of dilution to determine the morphology of the microorganism in anaerobically digested POME. Each dilution factor was being duplicated into Plates 1 and 2 respectively. The microorganisms were incubated in the anaerobic condition at 30 ^{o}C by using Oxoid AnaeroJar. Figure 4.1 and Figure 4.2 below showed the morphology of the microorganism obtained on Plates 1 and 2 with the dilution factor of 10^{6} using the spread plate method and the dominant colonies chosen to prepare the inoculum were labeled alphabetically according to the species on the plates. The general characteristics of the microorganism based on theirs colours, form, elevation and margin were tabulated in Table 4.1 and Table 4.2 after the observation. Table 4.3 and Table 4.4 showed the shape and Gram stain results of the species chosen from Plates 1 and Plates 2 after streak plates were performed (refers to Appendix A1 and A2).

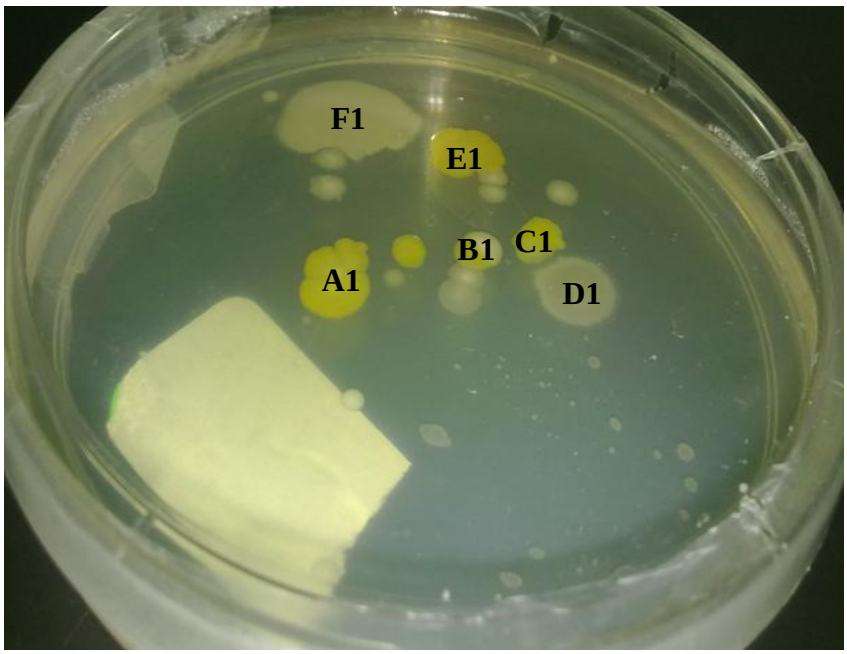

Figure 4.1: Plate 1 with dilution factor of 10^{6}

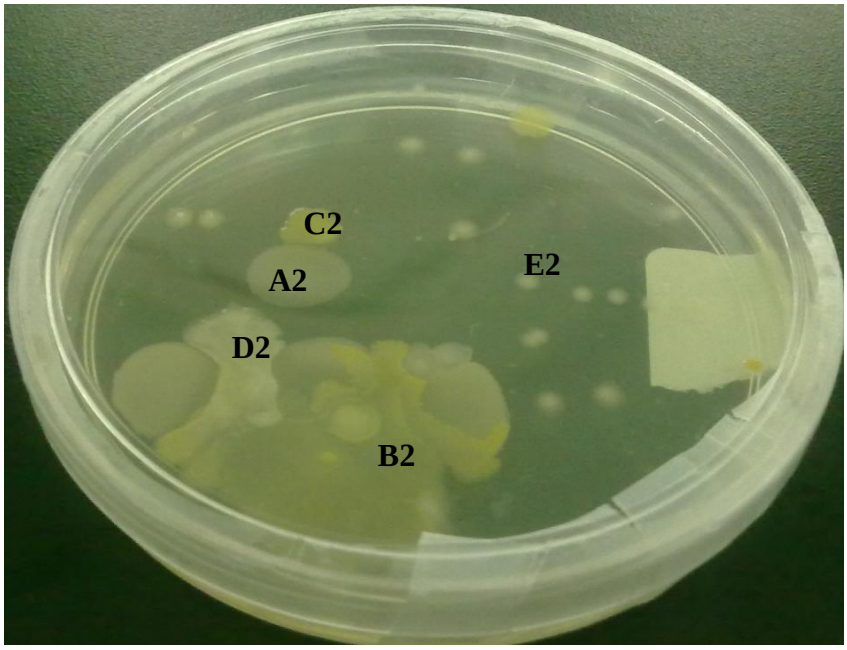

Figure 4.2: Plate 2 with the dilution factor of 10^6

Table 4.1: Microorganism morphology obtained from Plate 1 with dilution factor of 10^6

Colonies	Colour	Form	Elevation	Margin
A1	Yellow	Irregular	Raised	Undulate
B1	White and Yellow	Circular	Raised	Entire
C1	White and Yellow	Irregular	Raised	Undulate
D1	White	Irregular	Raised	Undulate
E1	Yellow	Irregular	Raised	Undulate
F1	White	Irregular	Flat	Undulate

Table 4.2: Microorganism morphology obtained from Plate 2 with dilution factor of 10^6

Colonies	Colour	Form	Elevation	Margin
A2	White	Circular	Flat	Undulate
B2	White and Yellow	Irregular	Flat	Undulate
C2	Yellow	Irregular	Raised	Entire
D2	Milky Yellow	Irregular	Flat	Undulate
E2	White	Circular	Convex	Entire

Table 4.3: Shape and Gram Stain for each colony on Plate 1 under the microscope

Colony	Shape	Gram Stain of Dominant Groups
A1	Rod Shape	Positive
B1	Cocci (Tiny round spot)	Negative
C1	Rod Shape	Positive
D1	Short Rod Shape	Positive
E1	Rod Shape	Positive
F1	Rod Shape	Positive

Table 4.4: Shape and Gram Stain for each colony on Plate 2 under the microscope

Colony	Shape	Gram Stain of Dominant Groups
A2	Rod Shape	Negative
B2	Rod Shape	Positive
C2	Rod Shape	Negative
D2	Short Rod Shape	Positive
E2	Cocci	Positive

According to Table 4.1 and Table 4.2, the colours of the dominant colonies form on the agar plate were white, yellow and milky yellow for both Plates 1 and 2. The microorganism that grew on the plates were believed to belong to the different species as none of them show entirely similarities in terms of colours, form, elevation and margin.

Based on the results in Table 4.1 and Table 4.3, their general characteristics on the agar plates were basically circular and irregular in terms of form. They were showing the elevation of flat (for colonies F1, A2, B2 and D2), raised (for all colonies in Plates 1 except colony F1) and convex (for colony E2). Most of the colonies were showing the margin of undulate except colony B1, C2 and E2.

Table 4.3 and Table 4.4 were showing the shape of a single colony obtained from the streak plate of those colonies taken from Plates 1 and 2 were determined by Gram stain and negative stain by using nigrosin. The results (refer Appendix A1 and A2) showed that they were not yet a pure colony. Hence, the results recorded in Table 4.3 and Table 4.4 was referred to the Gram Stain of the dominant groups in that colony taken. Therefore, it was recommended to streak for more passage number to identify the microorganism or to use the fluorescent *in situ* hybridization (FISH), Denatured Gradient Gel Electrophoresis (DGCE), Polymerase Chain Reaction (PCR) and 16 rDNA cloning in future study to have a better understanding of the microbial population in anaerobically digested POME sample.

Based on the Gram stain results in Table 4.3 and 4.4, most of the shapes of the colonies were in rod shape except colony B1 and E2 which were cocci in shape. The colonies D1 and D2 were showing the short rod shape. As most of the facultative anaerobic or anaerobic species were showing rods and irregular shapes, it was believed that the microorganism obtained belongs to *Archaea* domains instead of *Bacteria* domain according to Garrity (2005). As mentioned by Garrity (2005) in Bergey's Manual of Systematic Bacteriology, the *Archaea* domain may have a diversity of shapes, includes circular, spiral, plate or rod; unicellular and multicellular forms in filaments or aggregate and the colours of the cell masses of the *Archaea group* maybe red, purple, pink, orange-brown, yellow, green, greenish black, gray and white. Therefore, most of

the colonies were found in white and yellow in colours, it was again believed that the colonies cultured were belongs to *Archaea* domain. Besides that, the *Archaea* domain cells stain can be either Gram negative or Gram positive. The major groups of the *Archaea* domain includes the methanogenic *Archaea,* the sulfate-reducing *Archaea*, the extremely halophilic *Archaea* or *Archaea* that lack of cell walls. The microorganisms were then inoculated into nutrient broth to prepare mixed culture inoculum and the cell growth curve of the mixed culture was determined by checking the optical density (OD) versus time to obtain the optimum seeding density before they were entering the stationary phase (refers 4.1.3).

4.1.3 Growth profile of the mixed culture in inoculum

The growth profile of the mixed culture in the inoculum preparation was studied to get the optimum seeding density and also the time of culturing the mixed culture before it was added or seeded into the MAS. Based on the results in Table 4.5, Figure 4.3 and Figure 4.4, the optimum optical density (OD) to reach before its stationary phase was 0.635 at the culturing time of 10 hours.

Table 4.5: Optical density (OD) and Cell Dry Weight of the mixed culture in 12 hours

Hour	Optical Density (OD)	Cell Dry Weight (g/L)
0	0.088	0.2100
1	0.096	0.2500
2	0.132	0.2500
3	0.159	0.2700
5	0.286	0.2300
7	0.534	0.3300
10	0.635	0.5600
11	0.635	0.5800
12	0.646	0.5700

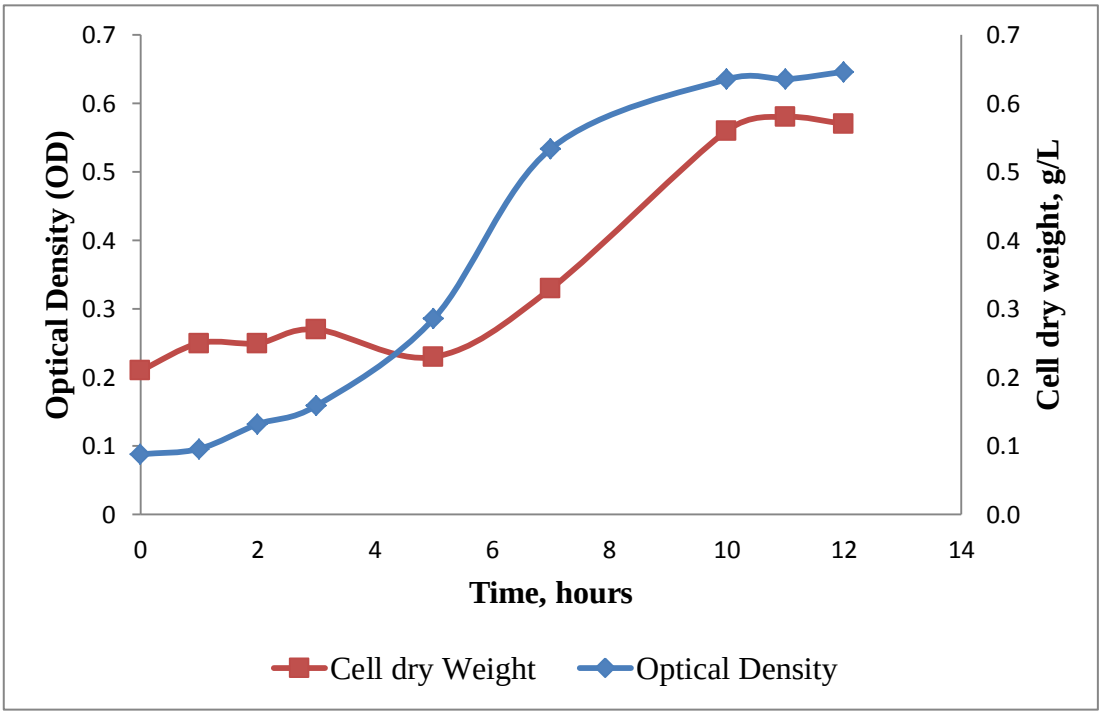

Figure 4.3: The growth profile of the mixed culture

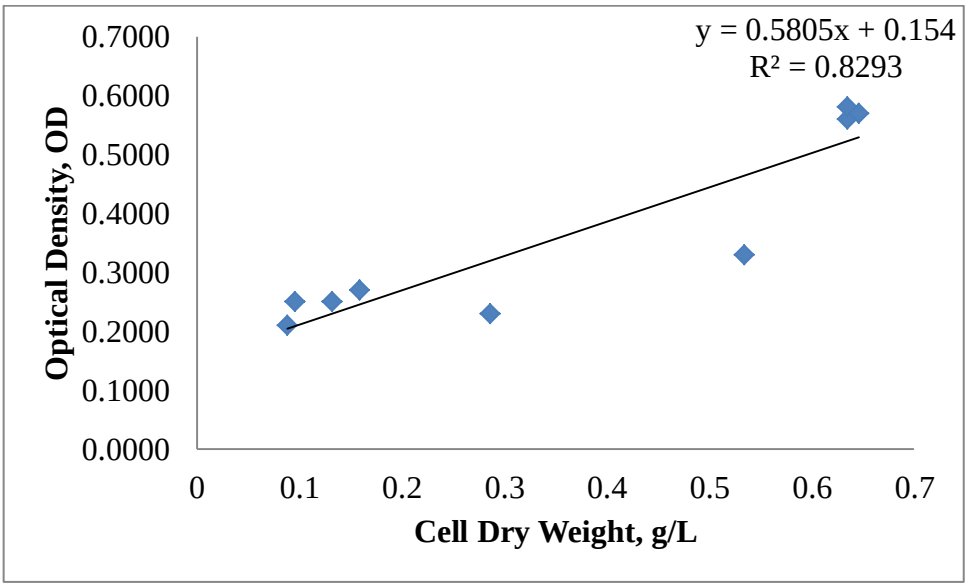

Figure 4.4: The relationship between OD and Cell Dry Weight, g/L

By referring to the Figure 4.3, the mixed culture inoculum was achieving their stationary phase at the culturing time of 10 hours. Table 4.4 showed the Optical Density (OD) of the mixed culture inoculum measured by UV-Vis spectrophotometer and its cell dry weight. OD of the mixed culture inoculum was measured by UV-Vis spectrophotometer dependent of its turbidity. Figure 4.4 showed the linear relationship between the OD measured and the inoculum cell dry weight. Therefore, it indicated that the turbidity of the mixed culture inoculum was depended on the number of cells generated (indicated by the cell dry weight) while there is more cells number, the cloudier the culture broth would be.

The purpose of doing the growth profile study for the mixed culture inoculum in this study was to determine the best inoculum age before it was being seeded into the MAS anaerobic digester. According to Cappucino and Sherman (2004), the stages of a typical growth curve of microorganisms are divided into lag phase, log phase, stationary phase and also death phase. During the lag phase of the cells growth, although the cells are increasing in size, but there is no cell division. At the log phase of the microbial growth profile, the physiologically robust cells would reproduce at a uniform and rapid rate by binary fission under optimum nutritional and physical condition. During the stationary stage, the number of cells undergoing division was equal to the number of the cells that were dying. The depletion of nutrients and buildup of metabolic wastes at the death phase and the cells die at the rapid and uniform rate. Therefore, the best time for the cells to be seeded into the MAS anaerobic digester should be before the mixed culture inoculum entering its stationary phase and at the end of the log phase (Lee, 2006). At that time, the mixed culture was at their highest cell density and optimum microbial activity and this would help in decreasing the lag phase of the cells in the MAS.

Figure 4.4 showed the lag phase, log phase and the stationary phase of the mixed culture inoculum. From Figure 4.3, both cell dry weights and the optical density of the mixed culture inoculum showed that they were finished their lag phase after the culturing time of 4 hours. Figure 4.3 also showed that they were entering stationary phase at the culturing time of 10 hours. In conclusion, the best seeding time for the mixed culture to be seeded into the digester should be at the 10 hours of culturing.

4.2 CHEMICAL OXYGEN DEMAND (COD)

4.2.1 Results on Chemical Oxygen Demand

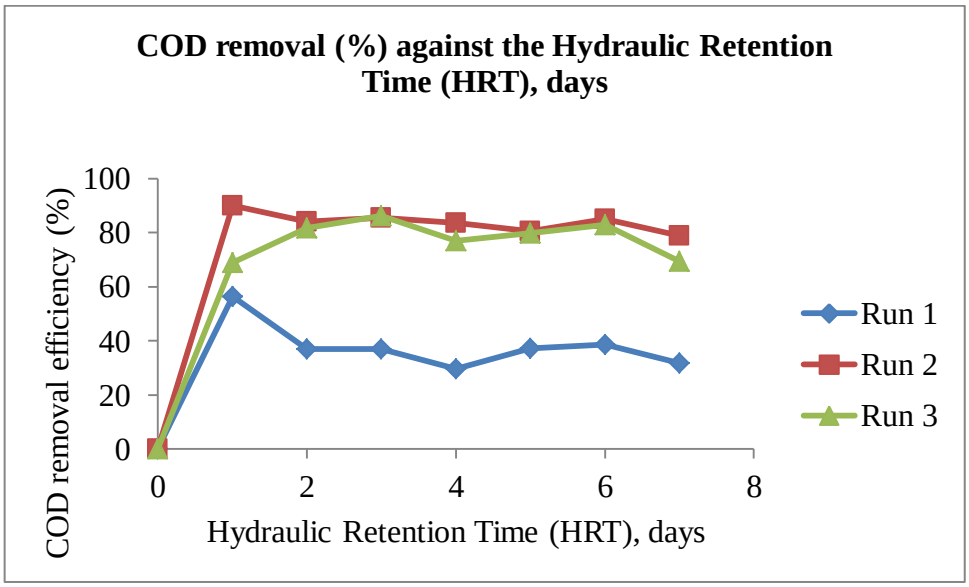

Figure 4.5: COD removal (%) against the Hydraulic Retention Time (days) for all three runs

4.2.1.1 Run 1

Table 4.6: COD results and COD removal percentage (%) of the Run 1

Day	Influent (mg/L)	Retentate (mg/L)	Permeate (mg/L)	COD removal (%)
1	28 467	28 083	12 433	56.32
2	29 267	31 250	18 467	36.90
3	21 350	25 383	13 467	36.92
4	24 883	24 783	17 533	29.54
5	28 583	30 233	17 933	37.26
6	29 100	30 683	17 850	38.66
7	26 967	25 800	18 400	31.77
			Avg	38.20

4.2.1.2 Run 2

Table 4.7: COD results and COD removal percentage (%) of the Run 2

Day	Influent (mg/L)	Retentate (mg/L)	Permeate (mg/L)	COD removal (%)
1	8 827	3 033	880	90.03
2	5 513	3 007	880	84.04
3	5 260	3 360	760	85.55
4	6 887	2 400	1 133	83.54
5	5 740	4 067	1 133	80.60
6	5 793	3 067	873	84.93
7	5 947	1 453	1 253	78.92
			Avg	83.94

4.2.1.3 Run 3

Table 4.8: COD results and COD removal percentage (%) of the Run 3

Day	Influent (mg/L)	Retentate (mg/L)	Permeate (mg/L)	COD Removal (%)
0	7 300	-	-	-
1	5 500	5 030	1 705	69.00
2	7 290	5 360	1 335	81.69
3	7 430	4 100	1 020	86.25
4	6 760	3 470	1 560	76.92
5	6 720	3 000	1 355	79.84
6	6 840	3 410	1 180	82.75
7	4 300	3 690	1 335	69.38
			Avg	77.98

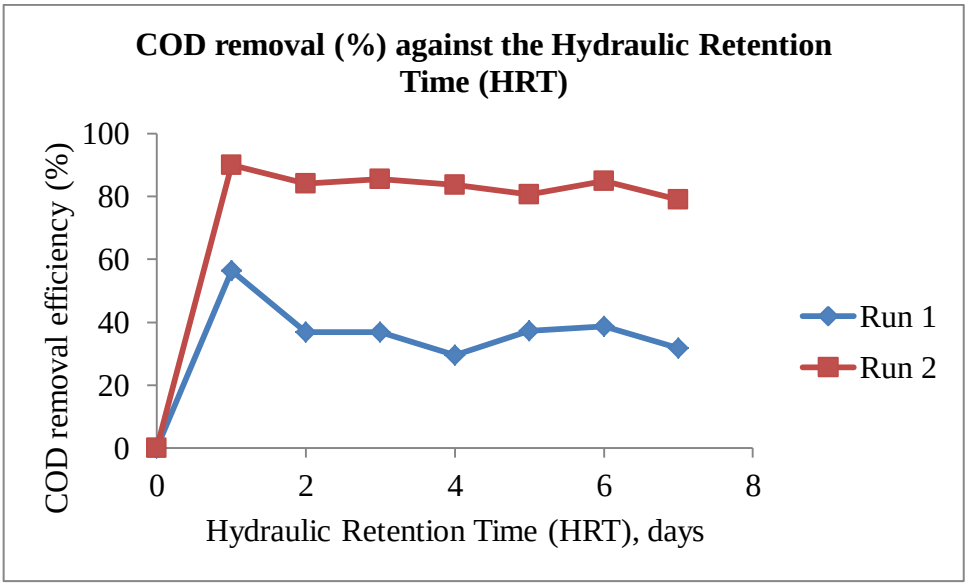

Figure 4.6: Comparison of COD removal efficiency in Run 1 and Run 2

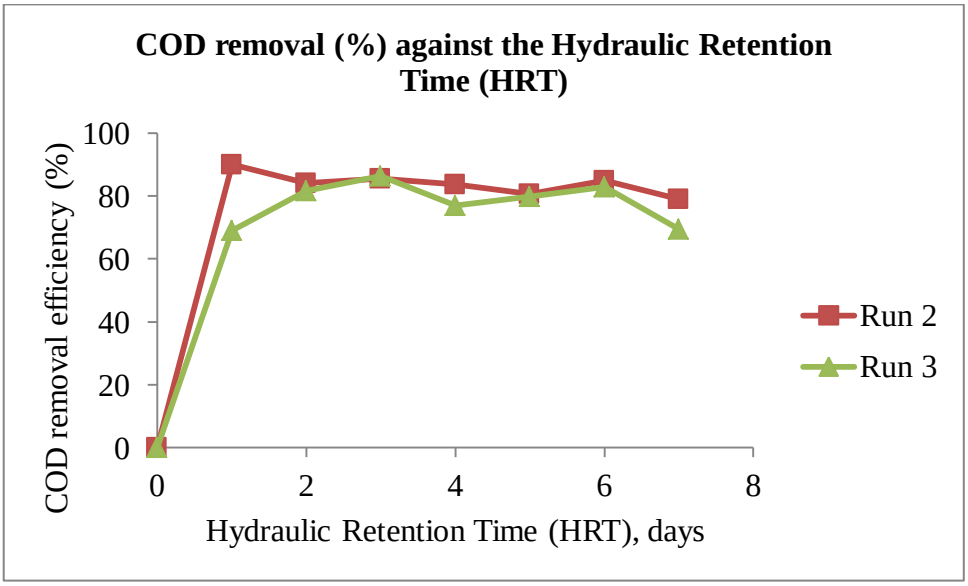

Figure 4.7: Comparison of COD removal efficiency in Run 2 and Run 3

4.2.2 Discussion on Chemical Oxygen Demands

For all the three runs, the start-up of the fermenter taken 4 days for the microbial community in the sample to acclimatize themselves in the new environment and the day to start experiment was considered as the first day.

Referring to the Table 4.6, COD removal efficiency in Run 1 kept on fluctuating indicates the instability of the anaerobic digestion in the digester of MAS. As what have been mentioned by Alwari *et al.* (2011) in his study that it was very rare to get precise values for individual measurements on every parameters as the anaerobic digestion was controlled by microorganism in any change in conditions such as temperature, light or accidental movement that may disturb their activity on treatment performance to give a distinct difference in data. Figure 4.5 compared the COD removal efficiencies of three runs, whereas Figure 4.6 and 4.7 were comparing the COD removal efficiencies between Run 1 and Run 2; Run 2 and Run 3.

Based on the Figure 4.5, among the three runs of the experiment, Run 1 showed the lowest treatment efficiency by using the 50 % diluted raw POME, which COD removal efficiency was only in the range of 29.54 % to 56.32 % (refers to Table 4.6). The 50 % diluted raw POME used in the Run 1 was the most concentrated sample in organic matter, with influent COD concentration in the range of 21 350 mg/L to 29 267 mg/L. COD concentration indicates the organic strength of the wastewater and therefore the organic loading in the Run 1 was the highest among the all three runs in this study. The adverse relation between the organic loading and COD removal efficiency corresponded to the results of Alwari *et al.* (2011), Ghani and Idris (2009) and also Vijayaraghavan *et al*. (2007).

The possible reason for the higher COD removal efficiency by using the anaerobically digested POME in Run 2 and Run 3 as compared to Run 1 could be due to the presence of partially degraded organics making them more amenable to further anaerobic digestion (Vijayaraghavan *et al.*, 2007). These results show the similarity in the study performed by Vijayaraghavan *et al.* (2007) who had compared the COD removal efficiency between diluted raw POME (89 %) and anaerobically digested

POME (93 % to 98 %) with aerobic digestion using activated sludge reactor. The diluted raw POME in his study showed lower COD removal efficiency than anaerobically digested POME.

Even though the overall COD removal efficiencies, 38.20 % of Run 1 was lower as compared to Run 2 and Run 3, but the overall removal of COD in concentration by subtracting the influent COD and permeate COD was still the highest. Take for instance, on the first day for each three runs, the COD that has been removed in term of concentration by the MAS in Run 1, Run 2 and Run 3 are 16 034 mg/L, 7 947 mg/L and 3 795 mg/L respectively. This has justified the reason why the methane production of Run 1 was the highest among the three runs, as similar to the study of Ağdağ and Sponza (2007) concluded that the methane production rate was increases linearly with the COD loading rate.

Furthermore, low COD removal efficiency in Run 1 as compared to Run 2 and Run 3 could be due to the accumulation of Volatile Fatty Acid (VFA) because the average pH of the digester in Run 1 was slightly lower than Run 2 and Run 3, which was between the pH ranges of 6.26 to 7.02. Nevertheless, the retentate of Run 1 (refers Table 4.9) did not show a large change in pH because the MAS could be able to tolerate with the fluctuation of VFA concentrations and the pH was always being regulated with the addition of alkaline.

The VFA was also the biodegradable organic matter and therefore the COD in the permeate did not show the trend of decrease in Run 1 (refers Table 4.6). Accumulation of the VFA might also be due to the accelerated hydrolysis of raw POME in Run 1 by the addition of water. However, as what have been mentioned by the Ahring *et al.* (1995) that the accumulation of VFA would not have adverse effect on biogas process if it did not exceed the threshold limit value that reduce the methanogenic activity. Therefore, the methane gas production in Run 1 still remained the highest among the three runs. It was recommended to include the VFA parameter in the future study to determine the stability of the MAS on POME treatment.

Figure 4.5 showed that the overall COD removal efficiency in the Run 2 was the highest among the three runs by using the anaerobically digested POME without addition of inoculum. The COD removal efficiency was showing the trend of decreasing in overall from 90.03 % on Day 1 to 78.92 % on Day 7. This phenomenon could be due to the depletion of substrates necessary for the microbial activity in the digester. Run 2 showed the higher COD removal efficiency as compared to Run 1 might also due to the stable degradation of the substrate in more diluted or low strength sample to stabilize the anaerobic digestion (Ghani and Idris, 2009).

By comparing Run 2 and Run 3 (refers Figure 4.7), the effect of the addition of inoculum did not show significance variation on the COD removal efficiency in general. The COD removal efficiency in Run 3 as shown in Table 4.8 was varied in the range of 69.00 % to 86.25 %. As what have been observed in Run 3, the initial COD of the influent before the addition of inoculum (as biopretreatment) was 7 300 mg/L. The inoculum had been added into the MAS on the last day of the acclimatization process. After the addition of inoculum, COD concentration of the influent was decreased significantly to 5 500 mg/L on the next day. This indicated that the 3.5 L inoculum have accelerated the degradation of the organic acid of the influent into substrate such as VFA, acetate and cause the COD removal efficiency on the Day 1 the lowest among the 7 days in Run 3. The COD removal efficiency in the Run 3 showed an increment on Day 2 might be due to the reason that the substrate forming on Day 1 has been fully degraded into biogas or other metabolites.

In conclusion, MAS was found to perform better treatment on the anaerobically digested POME in terms of the COD removal efficiency as compared to 50 % diluted raw POME. The 50 % diluted raw POME might need a longer HRT to achieve a higher COD removal efficiency. As what have been observed in Run 2 and Run 3, the average COD removal efficiencies for HRT of 7 days were about 83.94 % and 77.98 % respectively. Therefore, the MAS was an applicable alternative for the POME treatment as the CFU in MAS prolonged the Solid Retention Time (SRT) in the digester, at the same time, preventing the biomass washout to decrease the COD concentration in POME. The addition of inoculum in anaerobically digested POME showed a significant decreased in COD removal efficiencies for the first two days of the treatment and this

indicated the acceleration of the acidogenesis and acetogenesis stage of the anaerobic digestion.

4.3 pH AND TEMPERATURE

4.3.1 Results of pH and temperature for each runs

Table 4.9: pH and temperature of the Run 1

Day	pH			Temperature (^{o}C)		
	Influent	Retentate	Permeate	Influent	Retentate	Permeate
1	6.26	6.52	6.72	33.50	42.30	28.80
2	6.48	6.67	7.20	34.60	42.80	30.30
3	7.02	7.12	7.60	33.50	42.50	29.00
4	6.78	6.80	7.43	33.10	41.60	27.70
5	6.76	6.75	7.56	31.80	36.40	28.70
6	6.66	6.66	7.48	31.90	42.00	29.50
7	6.62	6.70	7.52	33.80	42.50	29.70

Table 4.10: pH and temperature of the Run 2

Day	pH			Temperature (^{o}C)		
	Influent	Retentate	Permeate	Influent	Retentate	Permeate
1	7.38	7.51	7.91	29.00	38.50	30.50
2	7.48	7.47	7.94	30.80	38.60	31.00
3	7.50	7.50	8.07	28.10	40.30	30.90
4	7.55	7.58	8.09	32.10	40.60	30.20
5	7.57	7.60	8.09	32.40	38.90	29.30
6	7.14	7.75	8.29	30.90	40.60	29.50
7	7.60	7.51	8.24	30.80	38.01	28.00

Table 4.11: pH and temperature of the Run 3

Day	pH			Temperature (^{o}C)		
	Influent	Retentate	Permeate	Influent	Retentate	Permeate
1	7.18	7.26	7.70	27.60	36.50	29.30
2	7.17	7.21	7.68	28.90	38.10	30.10
3	7.19	7.18	7.70	30.10	37.90	29.20
4	7.27	7.29	7.85	28.90	36.30	28.90
5	7.27	7.32	7.94	29.00	37.60	27.40
6	7.36	7.38	7.97	26.90	37.00	29.10
7	7.27	7.32	7.91	28.60	37.30	27.70

4.3.2 Discussion on the pH and temperature

The pH values of the 50 % diluted POME influent during Run 1 was initially 4.68 and the 5 L of sodium hydroxide was added step wise into the diluted raw POME to adjust the pH of the influent up to pH 6.26 before starting the experiment. For a better monitoring of the pH in the MAS, it was recommended to add in a pH indicator in MAS along with an automated pH controller connected to acid and alkaline in future studies.

The pH values for the anaerobically digested POME sample in Run 2 and Run 3 was not being adjusted as the pH of the sample was within the range of pH 6.7 to 7.8, which was the optimum pH for anaerobic digestion in MAS as mentioned by Abdurahman *et al.* (2011).

As mentioned by Gerardi (2006), methane forming bacteria are active between the pH ranges of 6.8 to 7.2 and decreasing of the pH would cause the methane forming bacteria became less active as compared to the fermentative bacteria which continue to produce fatty acids. And therefore the production of the methane was higher in the Run 1 (refers 4.6) as compared to Run 2 and Run 3 which retentate has exceeded the pH of 7.2. The pH stability of the MAS was also achieved to feed back some portions of the permeate to the digester and hence therefore the pH showed the trend of increasing throughout the experiment time in both retentate and also the permeate in the MAS.

From Table 4.9, 4.10 and also 4.11, the temperature of the retentate were referring to the temperature in MAS during the operation. The retentate temperature in Run 1, Run 2 and Run 3 were varied in the range of 36.40 ^{o}C to 42.80 ^{o}C, 38.01 ^{o}C to 40.60 ^{o}C, and 36.30 ^{o}C to 38.10 ^{o}C respectively. The ranges of temperature for each three runs were in the mesophilic conditions for anaerobic digestion.

The retentate temperature of MAS on Day 5 was the lowest throughout the Run 1 experiment as the unexpected electricity cut off in the middle of the experiment. Overall temperature of the retentate in Run 1 remained at the highest among the three runs probably due to the highest COD concentration (high organic loading) in the 50 % diluted raw POME as compared to anaerobically digested POME used in Run 2 and Run 3. High COD concentration causes more organic molecules breakdown as the microbial has the highest chance to be in contact with the organic molecules in POME to undergo the hydrolysis, acidogenesis and acetogenesis stage to form smaller molecules such as organic acid, alcohol, acetate, carbon dioxide and hydrogen to be used in the methanogenesis stage.

The maximum temperature of the anaerobic digestion process observed in Run 3 was 38.10 ^{o}C (refers to Table 4.11), which was visibly lower than the Run 2 which the maximum temperature of the anaerobic digestion in MAS was 40.60 ^{o}C. It could be due to the effect of the addition of the inoculum as the biopretreatment of the POME in Run 3. The addition of the inoculum had accelerated the degradation of the substrate into formic acid or volatile acids in the POME sample in Run 3. As the reactions of converting the formic acid and volatile acids to the CH_4 and CO_2 are endothermic (Pohland, 1968) and hence the temperature anaerobic digestion in Run 3 was low as compared to Run 2.

Referring to the methane composition accumulated on the last day of the experiment (refers 4.6); it showed that mesophilic population in POME in Run 1 and Run 2 adapt well even the temperature shift to above 42^{o}C and 40^{o}C respectively during the operation of MAS on POME treatment.

4.4 TOTAL SUSPENDED SOLID (TSS)

4.4.1 Results on Total Suspended Solids

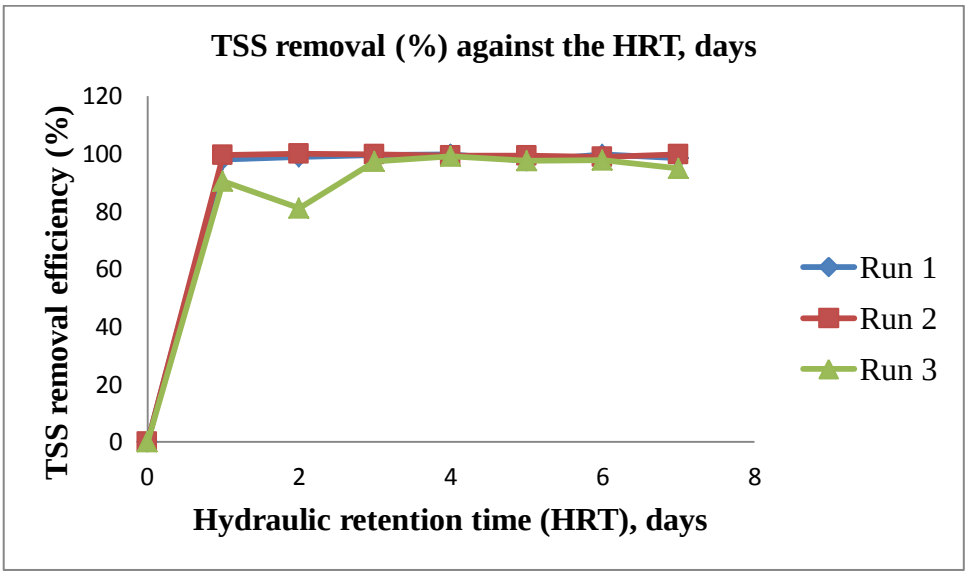

Figure 4.8: TSS removal (%) against the HRT (days) for all the three runs

Table 4.12: TSS results and TSS removal efficiency (%) in Run 1

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	TSS removal (%)
1	4 940	6 170	100	97.98
2	5 610	6 150	60	98.93
3	5 610	5 670	30	99.47
4	5 770	5 680	20	99.65
5	6 070	5 940	150	97.53
6	5 520	3 910	10	99.82
7	4 840	3 390	80	98.35
			Avg	98.82

Table 4.13: TSS results and TSS removal efficiency (%) in Run 2

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	TSS removal (%)
1	7 156.70	880.00	26.70	99.63
2	4 013.80	1 023.30	2.00	99.95
3	7 920.00	793.30	20.00	99.75
4	2 010.00	676.70	13.33	99.34
5	2 173.30	813.30	13.33	99.39
6	3 450.00	1 056.70	40.00	98.84
7	2 813.30	630.00	10.00	99.64
			Avg	99.51

Table 4.14: TSS results and TSS removal efficiency (%) in Run 3

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	TSS removal (%)
0	2 525	-	-	-
1	1 045	1 280	100	90.43
2	2 740	1 570	520	81.02
3	2 680	990	70	97.39
4	3 020	1 055	30	99.01
5	2 700	950	65	97.59
6	5 320	1 350	125	97.65
7	2 440	1 040	125	94.88
			Avg	94.00

4.4.2 Discussion on Total Suspended Solid (TSS) removal efficiency

From the Figure 4.8, the TSS removal efficiencies of three runs were not much in deviation. By referring to Table 4.12, Table 4.13 and Table 4.14, the TSS removal efficiencies by using MAS in POME treatment were varied between 97.53 % to 99.82 %, 98.84 % to 99.95 % and 81.02 % to 99.01 % for Run 1, Run 2 and Run 3 respectively. These results showed that nearly all the suspended solid in the POME had been eliminated by using CUF membrane (polyethersulphone (PES)) in MAS. These results showed the similarity with the results in the study of Shah and Singh (2004), whereby the total removal of suspended solid up to 99.4 % by using PES ultrafiltration membrane.

The reduction of the TSS in permeate observed in those three runs of experiment as compared to the influent and retentate showed the efficiency of CUF membrane used in MAS to prolong the Solid Retention Time (SRT) and prevented the washout of the biomass from the digester (Fakhru'l-Razi, 1994). In overall, the permeate of the POME which undergone the ultrafiltration had reduced the suspended solid content below 400 mg/L and this met the discharge limit of DOE on wastewater to the river. The POME treated by using MAS was safe to be discharged into the river.

4.5 VOLATILE SUSPENDED SOLID (VSS)

4.5.1 Results on Volatile Suspended Solid

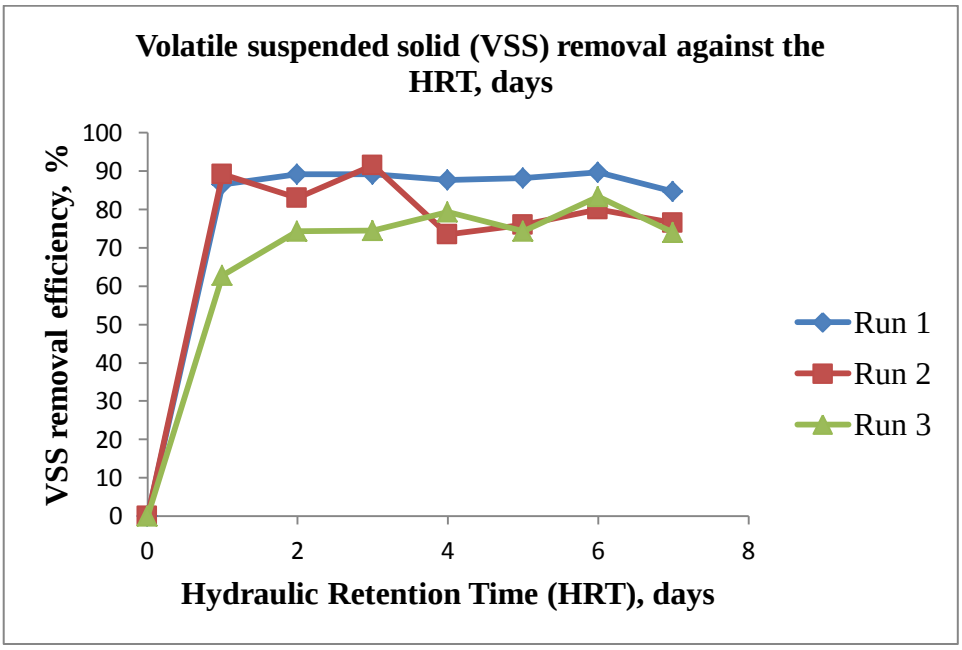

Figure 4.9: VSS removal (%) against the HRT (days) for all the three runs

Table 4.15: VSS results and VSS removal efficiency (%) in Run 1

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	VSS removal (%)
1	4 920	5 700	660	86.59
2	5 460	5 730	590	89.19
3	5 390	5 250	580	89.24
4	5 110	5 340	630	87.67
5	5 920	5 650	700	88.18
6	5 200	3 890	540	89.62
7	4 750	3 420	730	84.63
			Avg	87.87

Table 4.16: VSS results and VSS removal efficiency (%) in Run 2

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	VSS removal (%)
1	5 570.00	1 250.00	600.00	89.23
2	3 490.00	1 306.70	593.30	83.00
3	6 316.70	1 206.70	540.00	91.45
4	1 956.70	993.30	520.00	73.42
5	2 126.70	1 106.70	510.00	76.02
6	3 003.30	1 233.30	600.00	80.02
7	2 586.70	1 100.00	606.70	76.55
			Avg	81.38

Table 4.17: VSS results and VSS removal efficiency (%) in Run 3

Day	Influent, mg/L	Retentate, mg/L	Permeate, mg/L	VSS removal (%)
0	2 465.00	-	-	-
1	1 535.00	1 660.00	570.00	62.87
2	2 850.00	1 525.00	730.00	74.39
3	2 660.00	1 515.00	680.00	74.44
4	3 005.00	1 315.00	620.00	79.37
5	2 720.00	1 330.00	700.00	74.26
6	4 460.00	1 680.00	745.00	83.30
7	2 445.00	1 225.00	635.00	74.03
			Avg	74.66

4.5.2 Discussion on Volatile Suspended Solid (VSS) removal efficiency

VSS refers to the measurement of the biodegradable organic matter. Based on the results on Figure 4.9, the VSS removal efficiency was the highest in Run 1 by using the 50 % diluted raw POME, which has been steadily maintained at the average efficiency of 87.87 % (refers Table 4.15) whereas the average VSS removal efficiency was the lowest in Run 3, 74.66 % (refers Table 4.16). The average volatile suspended solid removal in Run 2 showing the efficiency of 81.38 % (refers Table 4.17). This showed a linear relationship between the compositions of the methane gas which would be discussed later in Figure 4.10.

At the initial stage of the anaerobic fermentation process, the VSS removal efficiency in Run 1 and Run 2 was not much different as shown in Figure 4.9. At the HRT of 4 days, the volatile suspended solid removal efficiency in Run 2 drop significantly from 91.45 % (HRT of 3 days) to 73.42 % and both VSS of influent and retentate were dropping from 6 316.7 mg/L to 1 956.7 mg/L and also from 1 206.7 mg/L to 993.30 mg/L respectively. This indicated that the anaerobic fermentation was entering the phase of methanogenesis and finishing the hydrolysis and acidogenesis phase as majority of the organic matter had been biodegraded by the fermentative microbial community occurs in the system. VSS removal efficiency by using 50 % diluted raw POME in Run 1 showed the stable and highest VSS removal efficiency could be due to the reason of the acceleration in hydrolysis of the POME by the hydrolytic microbial community occurs in the POME. Therefore, the VSS was greatly reduced.

The VSS removal efficiency in Run 2 and Run 3 did not show a significant different on HRT of 4 days and onwards. The reduction of the VSS was great due to the addition of the inoculum in Run 3. The initial VSS of the influent before the addition of inoculum (on the last day of acclimatization period) was 2 465 mg/L and it was discovered that the VSS in the sample was reduced greatly to 1 535 mg/L on the first day of the experiment (refers Table 4.17). This could be the reason why the VSS removal was lowered as compared to the Run 2 in the initial days as the biodegradable organic matter have been degraded by the microorganisms added. The influent VSS

fluctuated higher to 2 850 mg/L on the second day in Run 3 (refers Table 4.17) could be explained by the microorganism well adapt in the system and duplicated themselves as VSS can be also refers to the biomass community in the POME.

4.6 METHANE GAS PRODUCTION IN TERMS OF COMPOSITION

4.6.1 Results on methane gas production

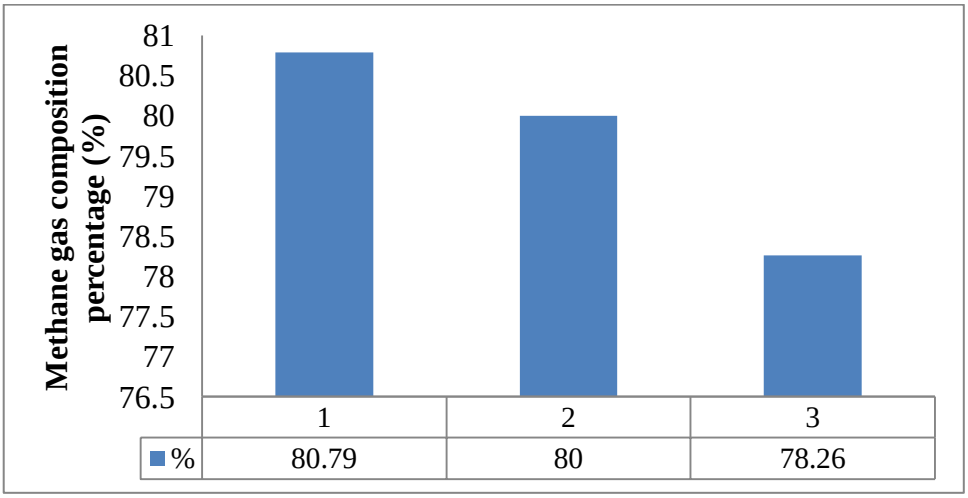

Figure 4.10: Methane gas composition (%) for three runs

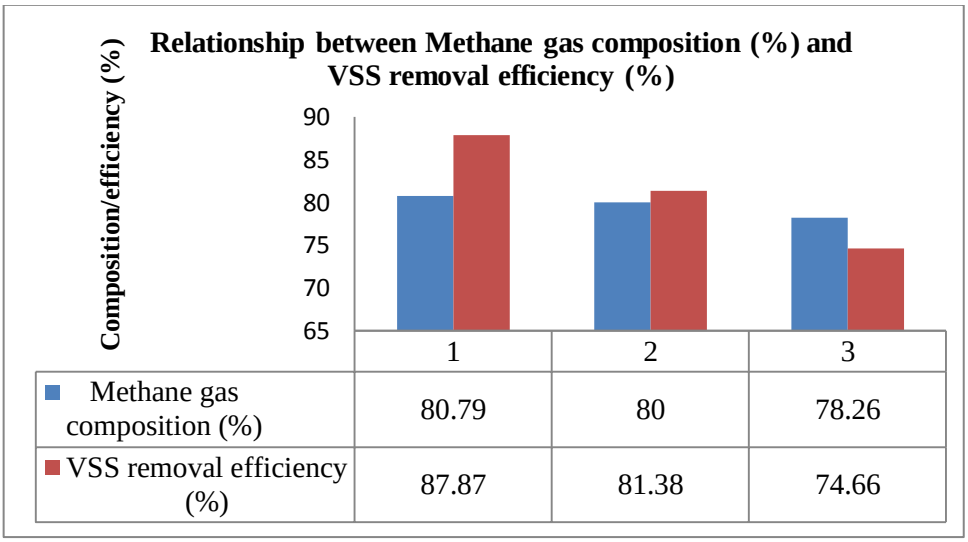

Figure 4.11: Relationship between methane gas compositions (%) and VSS removal efficiency (%)

4.6.2 Discussion on methane gas production

Based on the Figure 4.10 and 4.11, the cumulative methane gas compositions on day 7 of the experiment in Run 1, Run 2 and Run 3 were 80.79 %, 80 % and 78.26 % respectively, tested by using the J tube analyzer. There are many reasons that may inhibit the production of the methane gas such as the overloading of organic, pH, temperature and the competiveness of the microorganism in the system.

By using 50 % diluted POME, the methane gas composition was the highest among the three runs. This had been explained in 4.2.2 which might be due to the reason of high organic loading as compared to the anaerobically digested POME (used in Run 2 and Run 3). The high organic loading stabilized the anaerobic digestion system and hence results in the high methane gas production than another two runs of the experiment.

The gas production of the Run 3 was the lowest with the gas composition of 78.26 % could be due to the reasons of the occurrence of the sulphur reducing bacteria in the sample that competing with the methane forming bacteria that caused the failure of the system to produce high methane gas as the hydrogen sulfide gas formation can partially cause inhibition on methanogenesis process in the anaerobic digester as what been described in Prudence *et al.* (2002). Further biochemical tests needed to be done on the inoculum culture as to eliminate the sulphur reducing bacteria as the morphology of the sulphur reducing bacteria was also in the rod shape and gram negative (Widdel and Pfennig, 1981).

According to Ghani and Idris (2009), methane gas comes from the degradation of VSS. In Figure 4.11, VSS removal efficiencies in each runs of the experiment had showed a linear relationship with the methane gas production.

In conclusion, the MAS could be the alternative for the POME treatment as it could be able to capture the methane gas produced in a close digester with high methane gas composition production, in the range of 78.26 % to 80.79 %.

4.7 EFFICIENCY OF CROSS-FLOW ULTRAFILTRATION (CUF) MEMBRANE ON POME TREATMENT

Table 4.18: Flux of the permeate for the three runs of the experiment

Day	Run 1 $mL/m^2.hr$	Run 2 $mL/m^2.hr$	Run 3 $mL/m^2.hr$
1	3 358.33	7 758.33	4 166.67
2	4 833.33	5 687.50	6 333.33
3	4 900.00	4 333.33	6 250.00
4	4 833.33	4 541.67	5 000.00
5	2 408.33	4 625.00	5 416.67
6	4 166.67	3 750.00	5 791.67
7	3 750.00	3 750.00	6 041.67
Avg	4 035.71	4 920.83	5 571.43

The permeate flux was the indicator that detect the membrane fouling of the POME treatment. The average flux of the permeate in Run 1, Run 2 and Run 3 were 4 035.71 $mL/m^2.hr$, 4 920.83 $mL/m^2.hr$ and also 5 571.43 $mL/m^2.hr$ (refers Table 4.18). With more concentrated POME used in Run 1, the average flux indicated the possibility of membrane fouling as compared to Run 2 and Run 3. The permeate flux on the 5^{th} day of Run 1 was the lowest was due to the unexpected electricity cut off of during the experiment. The permeate flux on the first day of Run 1 and Run 3 was usually lower than the second day of the experiment as the sample would need to take time to fill up the stainless steel housing of the ultrafiltration membrane and therefore the permeate volume was the lowest. However, the permeate flux on the first day of the Run 2 showing the highest flux possibly was due to the error of water remaining in the MAS system after cleaning of the UF membrane. Water was flowed in the MAS to check the leaking of the membrane housing after the membrane cleaning. The error needed to avoid in the future study as this might cause the inaccuracy of the MAS performance evaluation on POME treatment.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Most of the anaerobes microbial found in POME was in irregular form, raised elevation and undulate margin. Colours of the microbial investigated were generally yellow, milky yellow and white. Two kinds of bacteria group found in the agar plate were rod shape with gram positive, short rod shape with gram positive, cocci with both gram negative and gram positive and lastly, cocci shape with both positive and negative gram staining. Those microorganism were believed belong to the *Archaea* domain. The growth profile of the microbial mixed culture showed that the time taken for the mixed culture to enter the stationary phase is 10 hours. To remain the microbial activity of the mixed culture, it was concluded that the maximum time needed to culture the mixed culture were 10 hours before seeding into the MAS.

Based on the results gathered in three runs of the experiment, MAS could be an effective alternative for the POME treatment. MAS gave a better performance to treat the anaerobically digested POME compared to 50 % diluted raw POME. The addition of the inoculum to the anaerobically digested POME in Run 3 did not show positive effect on methane gas production. However, the methane gas production in MAS showed a linear relationship with the VSS removal efficiency and organic loading.

The pH of MAS (refers to the pH of the retentate) was maintained between 6.52 and 7.60 in this study. MAS system was performing well in treating anaerobically digested POME as the average COD removal efficiency was 83.94 % and 77.98 % in both Run 2 and Run 3 respectively. The average flux of the permeate in Run 1, Run 2 and Run 3 were 4 035.71 mL/ m^2.hr, 4 920.83 mL/ m^2.hr and also 5 571.43 mL/ m^2.hr

which indicated the good performance of Cross-flow Ultrafiltration (CUF) membrane in MAS in recovering the water from POME. The addition of inoculum had improved the flux of the permeate through the CUF membrane. The average TSS removal efficiency in Run 1, Run 2 and Run 3 were varied between 97.53 % to 99.82 %, 98.94 % to 99.95 % and 81.02 % to 99.01 %. This results concluded that the CUF membrane in the MAS was performing efficiently in solid liquid separation to reject and retain the solid substrate in the anaerobic digester that required by the anaerobes in the MAS system to produce methane gas. The average VSS removal efficiency in Run 1, Run 2 and Run 3 were 87.87 %, 81.38 % and also 74.66 % respectively showed that the CUF membrane in MAS could prevent the washout of the biomass and therefore higher methane gas could be produced by MAS.

Furthermore, MAS could be an alternative to treat the POME in industry to replace the anaerobic ponding or open digesting system as it enables the methane production in high composition which is in the range of 78.26 % to 80.79 %. The methane gas composition of the Run 1, Run 2 and Run 3 experiment were 80.79 %, 80 % and 78.26 % respectively.

5.2 RECOMMENDATIONS

As referred to the results on the study, the recommendations listed below could be taken into consideration to improve the MAS efficiency and also to enhance the methane gas production in MAS.

i. Study the Solid Retention Time (SRT), Organic Loading Rate (OLR) and Volatile Fatty Acid (VFA) parameters to determine the effect on the MAS stability in treating POME.

ii. Apply a chemical pre-treatment on raw POME such as addition of alum to regulate the pH of the sample before treatment.

iii. Study the method of capturing methane gas and also to determine the biogas composition by using gas chromatography connected to MAS to control the inhibition of methanogen activity.

iv. To modify the design of the stainless steel housing of the CUF membrane to clean the membrane by backflushing method instead of disassembling it each time for cleaning.

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APPENDICES

APPENDIX A1:

GRAM STAIN RESULTS OF COLONIES ON PLATE 1 UNDER LIGHT MICROSCOPE

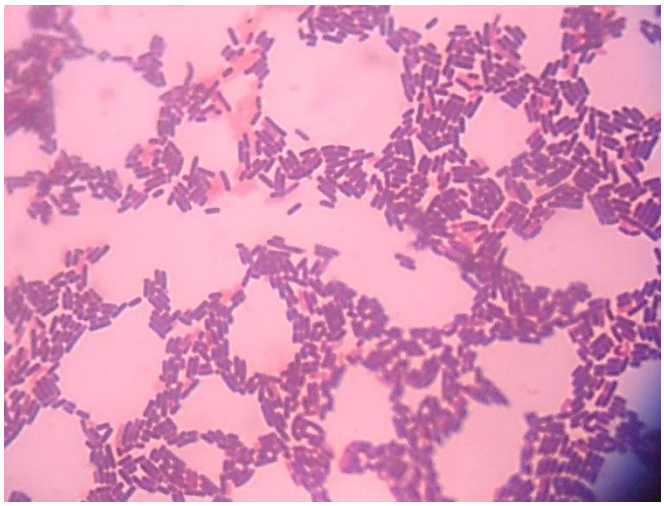

Figure A1.1: Colony A1 on Plate 1

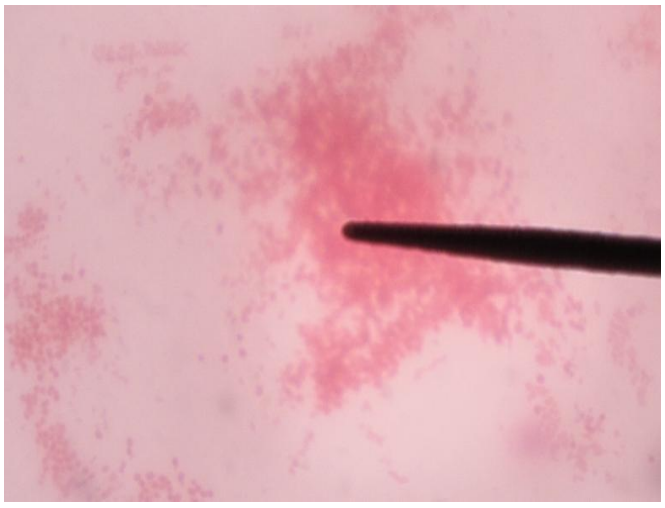

Figure A1.2: Colony B1 on Plate 1

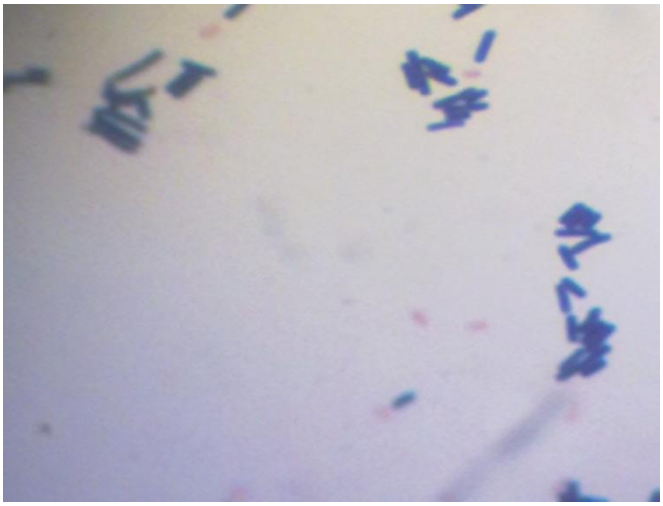

Figure A1.3: Colony C1 on the Plate 1

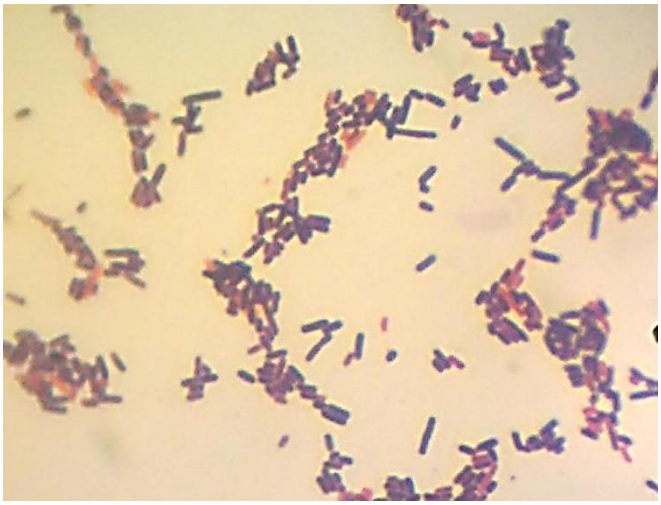

Figure A1.4: Colony D1 on the Plate 1

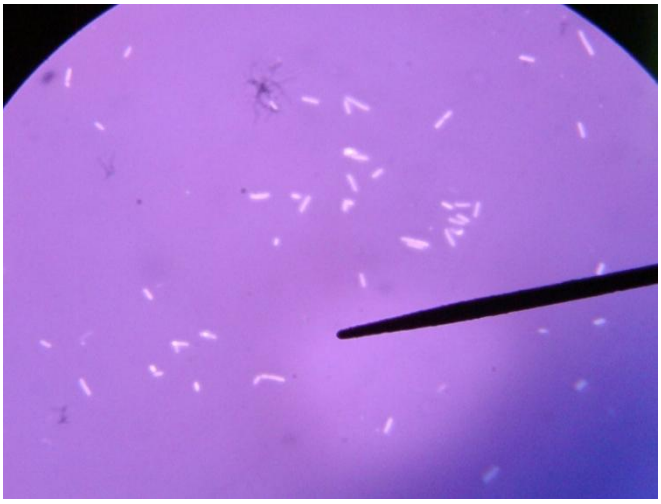

Figure A1.5: Colony E1 on the Plate 1

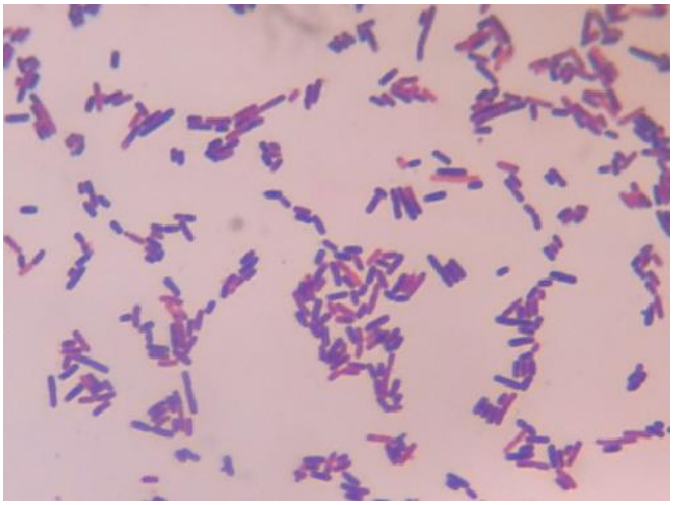

Figure A1.6: Colony F1 on the Plate 1

APPENDIX A2:

GRAM STAIN RESULTS OF COLONIES ON PLATE 2 UNDER LIGHT MICROSCOPE

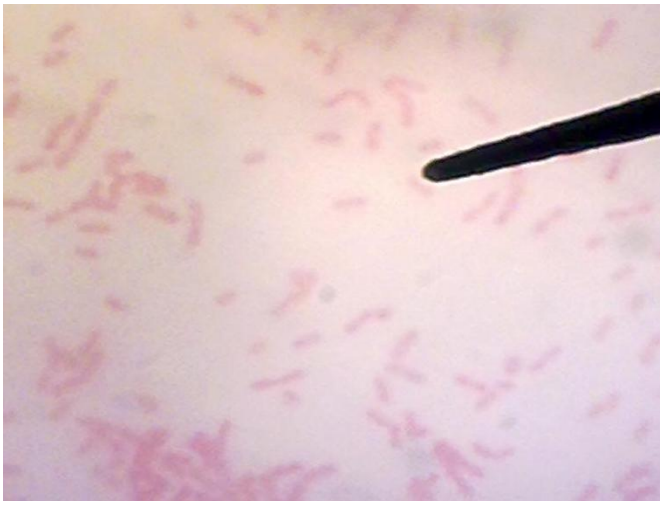

Figure A2.1: Colony A2 on the Plate 2

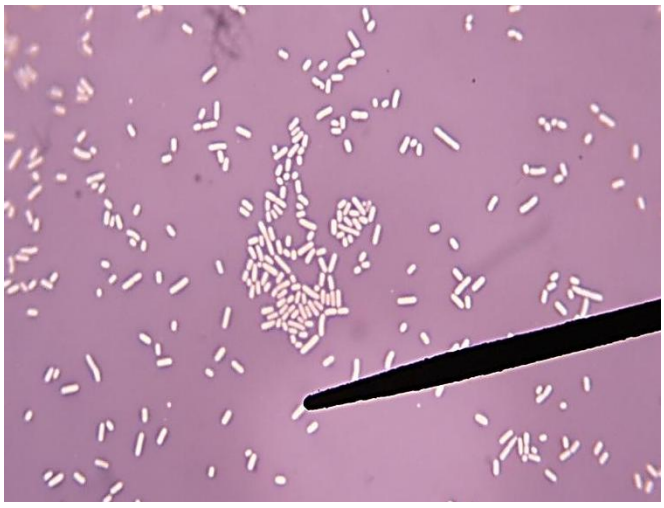

Figure A2.2: Colony B2 on the Plate 2

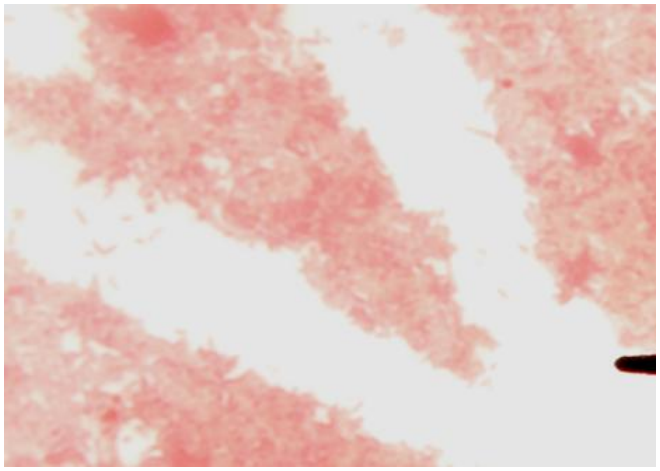

Figure A2.3: Colony C2 on the Plate 2

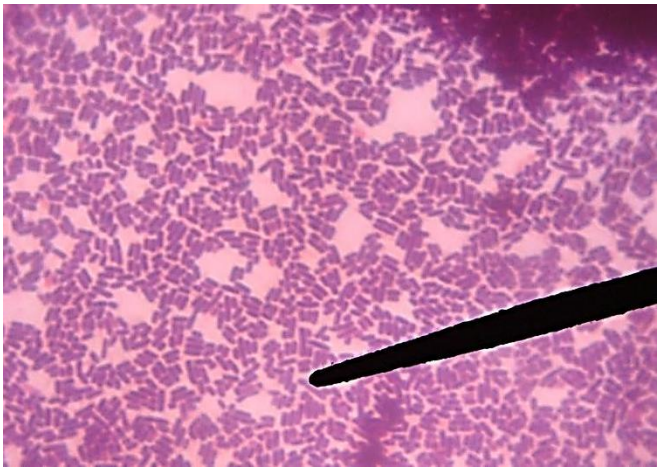

Figure A2.4: Colony D2 on the Plate 2

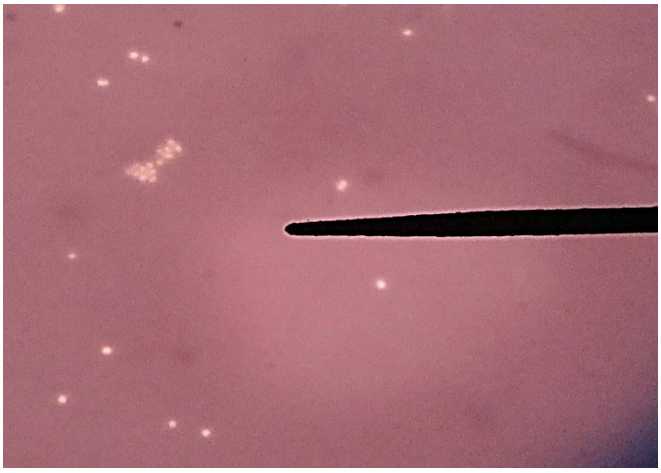

Figure A2.5: Colony E2 on the Plate 2

APPENDIX A3

PHOTOS TAKEN DURING THE EXPERIMENTS

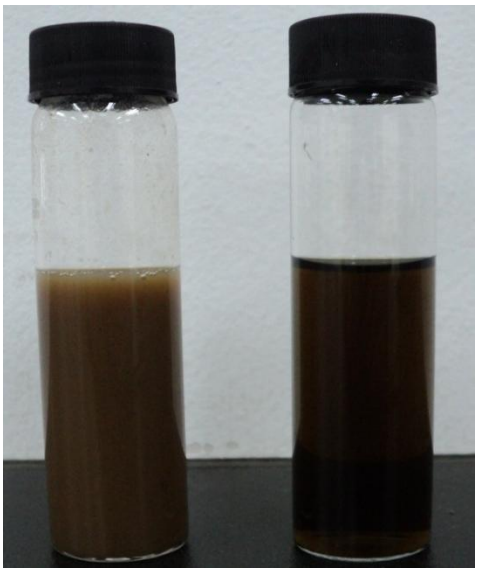

Figure A3.1: 50 % diluted raw POME (left) after treatment (right) in Run 1 by using MAS

Figure A3.2: Anaerobically digested POME (left) after treatment (right) in Run 2 by using MAS

Figure A3.3: Anaerobically digested POME (left) after treatment (right) in Run 3 by using MAS

Figure A3.4: Membrane used (before cleaning) during in the cross-flow ultrafiltration (CFU) MAS

Figure A3.5: MAS used in the study

Figure A3.6: Preparation of the mixed culture inoculum

APPENDIX A4

RAW DATA

Table A4.1: Permeate volume for each runs of the experiments

Day	Run 1 mL	Run 2 mL	Run 3 mL
1	806	1 862	1 000
2	1 160	1 365	1 520
3	1 176	1 040	1 500
4	1 160	1 090	1 200
5	578	1 110	1 300
6	1 000	900	1 390
7	900	900	1 450

Calculation of flux, mL/ m.hr can be done as follow.

The experiment was done 5 hours per day.

Flux = Permeate volumetric flow rate/ effective membrane area

Given the effective area of the membrane: 0.048 m^3

Take for example, for day 1 in the first run,

the volume of permeate collected were 806 mL.

Flux = (806 mL/5 hr) / 0.048 m^2 = 3358.33 $mL/m^2.hr$

The calculation will be performed for the other days.