EFFECT OF AMMONIUM AND NITRITE ADDITION ON THE ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUENT

SITI NURUL KHAIRAIMI BINTI IMAS

UNIVERSITI SAINS MALAYSIA

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by

SITI NURUL KHAIRAIMI BINTI IMAS

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LIST OF SYMBOL

Symbol	Description
N	Nitrogen
P	Phosphorus

LIST OF ABBREVIATIONS

CPO	Crude Palm Oil
POME	Palm Oil Mill Effluent
EFB	Empty Fruit Brunch
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
VFA	Volatile Fatty Acid
CPKO	Crude Palm Kernel Oil
FFA	Free Fatty Acid
FFB	Fresh Fruit Brunches
OLR	Organic Loading Rate
HRT	Hydraulic Retention Time
FNA	Free Nitrous Acid
FA	Free Ammonia
SRT	Solid Retention Time
SBR	Sequence Batch Reactor

KESAN PENAMBAHN AMMONIUM DAN NITRIT TERHADAP RAWATAN ANAEROBIK UNTUK EFLUEN KILANG KELAPA SAWIT

ABSTRAK

Kajian tentang kesan penambahan ammonium dan nitrit terhadap rawatan anaerobik untuk efluen kilang kelapa sawit telah dilaporkan. Kandungan nutrien iaitu nisbah COD:N:P yang optimum dalam rawatan anaerobik air sisa yang telah dicadangkan adalah 250: 5: 1. Walau bagaimanapun, kandungan nutrien dalam POME tidak optimum untuk rawatan anaerobik. Nutrien yang mencukupi diperlukan untuk mendapatkan rawatan air sisa yang berkesan. Pengeluaran asid lemak meruap (VFA) yang merupakan produk penting dari rawatan anaerobik mungkin terjejas dengan kandungan nutrien yang tidak mencukupi. Oleh itu, kajian ini mencadangkan untuk menambahkan ammonium dan nitrit bagi meningkatkan kandungan nitrogen dalam POME. Secara teorinya, kehadiran nitrit boleh menyebabkan perencatan methanogenesis dalam rawatan anaerobik. Oleh itu, kesan penambahan ammonium dan nitrit pada rawatan anaerobik POME dengan memasukkan sumber nitrogen yang berlainan disiasat dalam kajian ini. Tiga set eksperimen kumpulan telah dijalankan dengan menggunakan rawatan anaerobik POME dari reaktor utama iaitu SBR dan masing-masing dimasukkan dengan air terion (Reaktor 1), ammonium bikarbonat (Reaktor 2) dan efluen separa nitrifikasi (Reaktor 3). Semua reaktor ini telah beroperasi dengan jumlah isi padu 1 liter dan jumlah nisbah pertukaran 20% dengan 5 hari SRT reaktor. Hasil kajian menunjukkan penambahan nitrit dalam rawatan anaerobik POME telah mengurangkan penyingkiran ammonium dan biomas aktiviti yang seterusnya membawa kepada penurunan penyikiran COD. Penambahan efluen separa nitrifikasi menghalang pertumbuhan mikrob dalam reaktor 3 dan menghasilkan pengeluaran VFA yang rendah. Walau bagaimanapun, penambahan

ammonium dalam rawatan air sisa biologi boleh meningkatkan pertumbuhan biojisim. Pertumbuhan biojisim yang semakin meningkat dalam reaktor 3 akan meningkatkan kecekapan rawatan anaerobik. Oleh itu, penambahan ammonium untuk meningkatkan tahap nitrogen dalam air kumbahan adalah sumber nitrogen sesuai untuk POME tetapi bukan untuk nitrit.

EFFECT OF AMMONIUM AND NITRITE ADDITION ON THE ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUENT

ABSTRACT

A study of effect of nitrite on anaerobic treatment of palm oil mill effluent (POME) was reported. The optimum nutrient content which is COD:N:P ratio in anaerobic treatment of wastewater was suggested to be 250:5:1. However, nutrient content within POME was not optimal for anaerobic treatment. Adequate nutrients are required to get the effective treatment of waste. The production of Volatile Fatty Acid (VFA) which is important product from anaerobic treatment might be affected with the insufficient nutrient content. Hence, this study suggested that to add nitrite and ammonium in order to increase the nitrogen content within the POME. Theoretically, the presence of nitrite can cause the inhibition of methanogenesis in anaerobic treatment. Therefore, effect of ammonium and nitrite addition on the anaerobic treatment of POME by feeding different source of nitrogen was investigated in this research. Three sets of batch reactors experiments were carried out using the anaerobic treatment of POME from parent reactor which is SBR and fed with ionized water (Reactor 1), ammonium bicarbonate (Reactor 2) and partial effluent of nitrification (Reactor 3) respectively. All these reactors were operated at 1 L working volumeand volume exchange ratio of 20% with 5 day SRT of reactors. The results revealed that addition of nitrite in anaerobic treatment of POME reduce the ammonium removal and biomass activity which in turn leads to lower COD removal. Addition of partial effluent of nitrification inhibits the microbial growth in the Reactor 3 and result in low production of VFA. While addition of ammonium in the wastewater biological treatment can enhance the biomass growth. The increasing biomass growth in Reactor 3 will increase the efficiency of anaerobic

treatment. Hence, addition of ammonium in order to increase the nitrogen level in the wastewater is a suitable nitrogen source for POME but not for nitrite.

CHAPTER ONE

INTRODUCTION

1.1 Research background

The palm oil industry is one of the key economic drivers of the agriculture sector in Malaysia. This industry has contributed significantly to the country's gross domestic product (GDP) (Sulaiman A., 2010). Palm oil production in Malaysia has increased over the years and it makes Malaysia known as the second largest producer and exporter of palm oil in the world. According to Hassan (2013), the production of crude palm oil (CPO) increased from 1.3 million tonnes in 1975 to 18.6 million tonnes in 2010. The number of processing factory of palm oil in Malaysia kept growing due to the demand for palm oil.

Regardless of the number of processing factory of palm oil increase, the number of waste generated from this process also increased. In 2004, approximately 26.7 million tonnes of solid biomass and about 30 million tonnes of Palm Oil Mill Effluent (POME) are generated from 381 palm oil mill industry in Malaysia (Yacob, 2006). Based on Sulaiman A. (2010), the waste generated from this industry in the form of empty fruit bunch (EFB), oil palm frond (OPF), mesocarp fibre, palm kernel shell (PKS), palm oil mill effluent (POME) and sludge from pond and anaerobic tanks. This study will focus on the more underutilized liquid waste stream, known as POME.

POME is an oily wastewater generated during palm oil processing where CPO is extracted from the palm fruits. Large amount of water are required to extract CPO and about 50% of the water ends up as POME. Ahmad (2005) reported that the composition of POME mainly composed of water (95-96 %), total solids (4-5 %), suspended solids (2-4%), and oil (0.6-0.7 %). POME is an acidic (pH 4-5), hot (80-90 °C), non-toxic because no chemical is added during oil extraction, has high organic content (COD 50,000 mg/L, BOD 25,000 mg/L) and contain appreciable amounts of plant nutrients (Singh, 1999).

POME can be classified as one of the most important source of inland water pollution due to its high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) concentration. POME contains soluble materials with the concentration above the threshold value that are harmful to the environment such as CH_4, SO_2, NH_3, halogens or solids (James R. Pfafflin, 1980). These properties determined that POME is not only most polluting agro-industrial waste , but also very difficult to treat.

Discharging the POME directly into river without proper treatment might deteriote the surrounding environment because it is highly strength wastewater. The oily waste in POME need to be removed to prevent interfaces in water treatment units, avoid problems in the biological treatment stages and comply with water-discharge requirements (Ahmad, 2005). The adverse environmental impact of POME cannot be over emphasized. Thus, there is a need for an efficient and practical approach to conserve the environment and check the deterioration of air and river water quality (Rupaini, 2010).

Currently, there are various known methods available for POME treatment such as membrane treatment system, aerobic treatment, evaporation method and anaerobic treatment (Poh, 2009). Due to high concentration of carbon present in POME, anaerobic treatment has been considered the most suitable method for the treatment of this effluents. Even though the anaerobic treatment have long retention times and long start-up period but this treatment can saves the energy because it does not require energy for aeration and converts organic pollutants into value added product which is methane gas (Poh, 2009).

There are widely types of anaerobic treatment methods such as conventional treatment systems, anaerobic filtration, anaerobic fluidized bed reactor, anaerobic contact digestion, continuous stirred tank reactor (CSTR), up-flow anaerobic sludge blanket (UASB) reactor and up-flow anaerobic sludge fixed-film (UASFF) reactor. The most common method to treat the POME is using a conventional ponding method. Poh (2009) reported that about 85% of the mills practice using ponding system.

1.2 Problem statement

The production of palm oil results in huge amount of polluted wastewater commonly referred to as POME. POME commonly known as high strength wastewater due to its highly polluting properties. Even there are many wastewater treatment system that can be applied to treat the POME, unfortunately the current wastewater treatment system commonly fails to treat the POME effectively to meet the discharged limit that have being proposed by the Department of the Environment (DOE). Before discharge into watercourses, the POME must be treated because if they are untreated effectively, these wastes can cause detrimental effects to environment quality (Gobi, 2013). According to Ammary (2004), the optimum nutrient content which is COD:N:P ratio in anaerobic treatment of wastewater was suggested to be 250:5:1. However, nutrient content within the POME was not optimal for anaerobic treatment. The concentration of nitrogen in POME is lower while COD concentration is higher than what is required by this ratio. COD:N ratio of POME does not meet the recommended ratio for biological treatment, then the anaerobic treatment efficiency could not be optimized. Hence, the possible solution to increase nitrogen content in the wastewater is by added nitrite or ammonium into the wastewater. According to Metcalf (2014), the nitrogen can be in the form of nitrogen gas, ammonia, ammonium ion, nitrite ion and nitrate ion. The presence of nitrite and ammonium in wastewater is known to be toxic to the fish and aquatic life and it also inhibit the microbial activity in the biological treatment. Thus, this experiment is conduct to determine the effect of nitrite and ammonium addition on the anaerobic treatment of POME.

1.3 Research objectives

The overall objective is to study the effect of increasing nitrogen in the wastewater for biological treatment of POME. The specific objectives of this study are:

i. To investigate the effect of ammonium and nitrite addition on anaerobic biomass growth
ii. To determine the effect of ammonium and nitrite addition on anaerobic treatment efficiency
iii. To analyze the Volatile Fatty Acid (VFA) production from POME.

CHAPTER TWO

LITERATURE REVIEW

2.1 Palm Oil Mill Effluent (POME)

2.1.1 Palm Oil Tree

Elaeis guineensis which is commonly known as the oil palm is the most important commodity crop for the economy of Malaysia. Currently, Malaysia is the one of largest producer and exports of palm oil in the world. The first development of oil palm as a plantation crop to Malaysia is brought by the British in early 1870's (MPOC, 2012). The average productive life-span of oil palm tree has about 25 to 30 years and an oil palm tree may produce between 8 to 12 bunches of fruit. Figure 2.1 shows the oil palm fruit in spherical shape which consists of a hard seed (kernel) enclosed in a shell (endocarp) which is surrounded by fleshy husk (mesocarp).

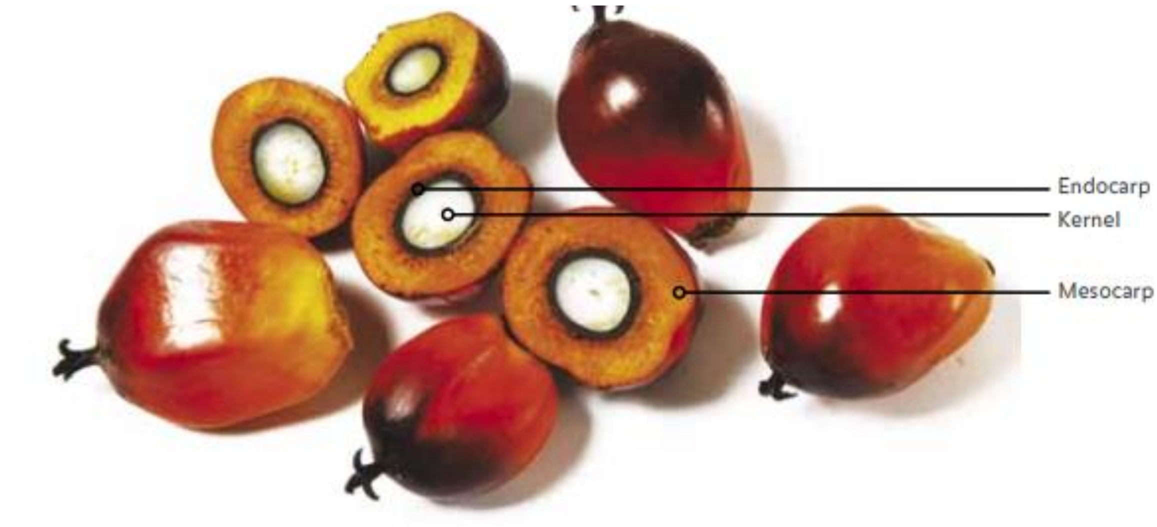

Figure 2.1: Oil palm fruit (MPOC, 2012)

Two different types of oils are produce from oil palm which are crude palm oil (CPO) and crude palm kernel oil (CPKO). The CPO and CPKO undergo refining and fractioning process to produce a variety of edible oils and fats and non-food applications which contributing to the worlds need (MPOB, 2011). Soaps, detergents, toiletries and candles are non-food products of raw material from palm kernel oil production. While, palm oil is used in a wide variety of food products such as cooking oil, shortenings and margarine (Hai, 2002). Although palm oil is primary use for food, it is also increasingly being used as a feedstock for biofuel.

Palm oil is extracted from two types of oils, palm oil from the fibrous mesocarp and lauric oil from the palm kernel. The oil is bright orange-red in its virgin form due to the high amount of beta-carotene (MPOC, 2012). Palm oil has the richest known content of carotenoids which is a rich source of Vitamin A. Palm oil are cholesterol-free, trans fat tree and composed mainly of triglycerides of fatty acid. Composition of fatty acid in palm oil is almost equal between saturated fatty acids and unsaturated fatty acids (Hai, 2002). Palm oil are cholesterol-free, trans fat tree and composed mainly of triglycerides of fatty acid.

2.1.2 Palm Oil Production Processes

Figure 2.2 shows the typical process flow diagram for the extraction of crude palm oil. In order to prevent a rapid rise in free fatty acids (FFA), it is important that the fresh fruit bunches (FFB) are processed after harvesting because it could adversely affect the quality of the crude palm oil (CPO). Firstly, FFB undergo sterilisation process then stripped of the fruitlets in a rotating drum thresher to produce empty fruit bunches (EFB).

The FFB are transported to the plantation for mulching while the fruitlets are conveyed to the press digesters. The fruits are heated in digesters using live steam and continuously stirred to loosen the oil-bearing mesocarp from the nuts as well as to break open the oil cells present in the mesocarp. The digested mash is then pressed to extract the oil (Hai, 2002).

The press cake is then conveyed to the kernel plant where the kernels are recovered. The oil from the press is diluted and pumped to vertical clarifier tanks then undergo purification process to remove dirt and moisture before being dried further in the vacuum dryer. The storage and dispatch is ready for clean and dry oil. The bowl centrifuges is fed with the sludge from the clarifier sediment for further oil recovery and recycled back to the clarifier. The effluent treatment plant (ETP) is used to treat the water/sludge mixture which is referred to as Palm Oil Mill Effluent (POME) (Hai, 2002).

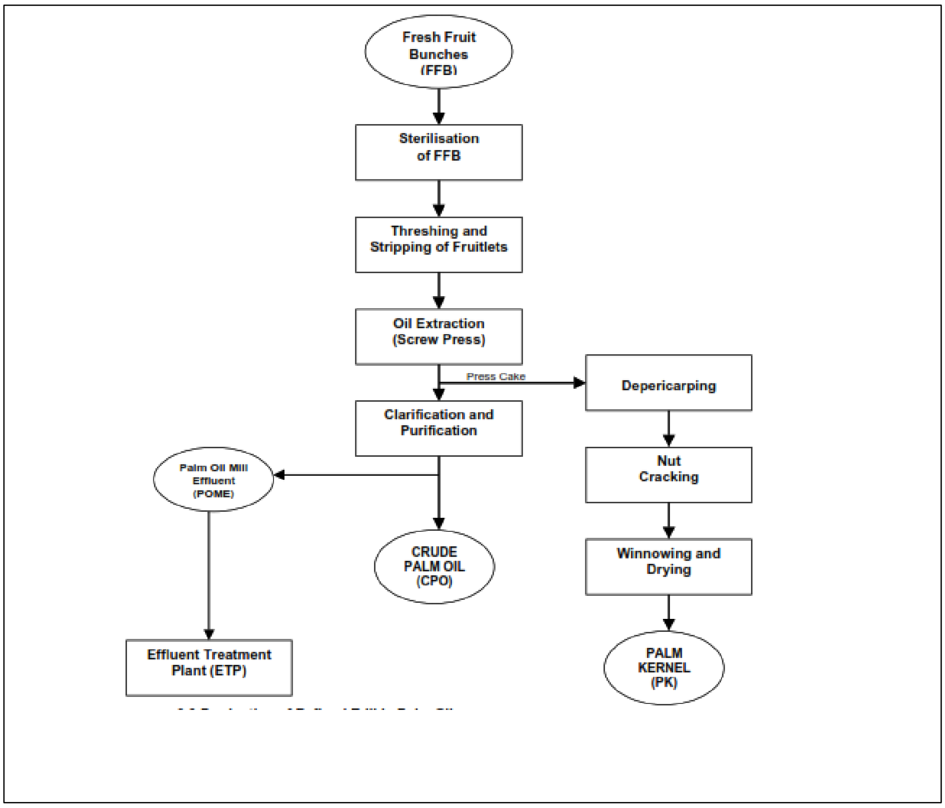

Figure 2.2: Palm oil extraction process and source of waste generation (Hai, 2002)

2.1.3 Characteristics of Palm Oil Mill Effluent

Palm oil mill effluent (POME) is a wastewater produced from sterilization, hydrocyclone waste, and separator sludge in the course of crude palm oil production where separator sludge and sterilizer effluent are the two major sources of POME which contribute to the highly polluting characteristics of the wastewater (Borja, 1995). POME also can be defined as water discharged from industry, which contains soluble materials that are injurious to the environment (James R. Pfafflin, 1980).

POME is generated mainly from oil extraction, washing and cleaning processes in the mill and these contains cellulosic material, fat, oil and grease (Agamuthu, 1995). Three major processing operations responsible for producing the POME are sterilization of FFB, clarification of the extracted CPO, hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone contributes about 36, 60 and 4% of POME respectively in the mills (Sethupathi, 2004).

A lot of water being used during palm oil processing that can generates large quantities of polluted wastewater. During CPO milling, large quantities of steam and hot water are used to clean the fruit and separate the shell and cake from the palm fruit. To produce 1 tonne of crude palm oil, 5-7.5 tonnes of water has been estimated and 50% of the water ends up as POME (Ma, 1999a) (Ma, 1999b). In Malaysia, estimated POME is being produced every year is about 53 million m^3 based on palm oil production in 2005.

Fresh POME is a hot, acidic (pH between 4 and 5), brownish colloidal suspension containing high concentrations of organic matter, high amounts of solids, both suspended solid and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L, oil and grease (4000 mg/L), COD (50,000 mg/L) and BOD (25,000 mg/L) (Ma, 2000). POME is a highly polluted wastewater because contains residual oil. The presence of oil in wastewater can be considered as hazardous pollutants especially in the aquatic environment. The characteristics of typical POME is shown in Table 1:

Table 2.1: Characteristics of Raw POME (Ma, 2000)

Parameters	Value
Temperature (°C)	80-90
pH	4.7
Biochemical Oxygen Demand BOD3; 3days at 30°C	25,000
Chemical Oxygen Demand, COD	50,000
Total Solids (TS)	40,500
Total Suspended Solids (TSS)	18,000
Total Volatile Solids (TVS)	34,000
Oil and Grease (O&G)	4,000
Ammonia-Nitrate (NH_3-N)	35
Total Kjeldahl Nitrogen (TKN)	750

*All values, except pH and temperature, are expressed in mg/L.

POME is considered as non-toxic waste because no chemical is added during the oil extraction process. But it will pose environmental issues due to the large oxygen depleting capability in aquatic system due to organic and nutrient contents (Khalid, 1992). It contains high amounts of elements that are vital nutrient for plant growth such as Nitrogen, Phosphorus, Potassium, Magnesium and Calcium (Habib, 1997) (Muhrizal, 2006). POME is a good source of nutrients for microorganisms, since it is non-toxic as no chemical is added in the oil extraction process.

Huge amounts of POME will be discharged from palm oil mill industry. Due to its acidic nature and very high biochemical oxygen demand (BOD), POME cannot be discharged prior to specific treatment. Discharging of POME to the environment cannot be ignoring because it may lead to pollution. POME one of the major contributors to pollution problem and adverse environmental impact. Hence, the palm oil mill wastewater requires an efficient treatment and effective disposal technique before discharged the into environment.

2.2 POME Treatment System

Generally, POME is treated in various ways at industries. POME has been treated using biological, non-biological method and integration of biological and non-biological treatment (Gobi, 2013). These treatment method have the ability to treat POME within safe parameter before being released into environment in order to comply with the demand of the Department of the Environment (DOE). Biological treatment can be divided into anaerobic and aerobic treatment.

2.2.1 Anaerobic Treatment

Anaerobic treatment is time consuming as bacterial consortia responsible for the degradation process requires time to adapt to the new environment before they start to consume on organic matters to grow (Poh, 2009). This treatment is carried out in the absence of molecular oxygen. Anaerobic treatment produces value added product of digestion which is methane gas that can be utilized in the mill to gain more revenue in terms of certified emission reduction (CER) (Poh, 2009).

Three basic reactions occur during anaerobic process, namely, hydrolysis, acidogenesis and methanogenesis (Gerardi, 2003). First basic step is hydrolysis, where insoluble organic materials and higher molecular mass compounds such as carbohydrates, lipids and proteins are converted to soluble organic materials such as sugar and amino acids. These organic material then be hydrolysed further to simple monomers that are used by bacteria (Metcalf, 2014).

The second basic step is acidogenesis, acidogenic bacteria will break down sugar, amino acids and fatty acids and results in production of organic acids (Metcalf, 2014). The last basic step, methanogenesis, is the rate limiting step in anaerobic treatment of POME (Ibrahim, 1984). This reaction is carried out by a group of *Archaea* organisms to split acetate into methane and carbon dioxide and use hydrogen and carbon dioxide to produce methane gas (Metcalf, 2014).

Anaerobic treatment is the most suitable method for the treatment of effluents containing high concentrations of organic carbon (M. Perez, 2001). Anaerobic treatment is commonly chosen because of chemical and physical properties presence in the POME. This treatment has the ability to reduce the COD and the BOD rapidly in the absence of oxygen (Metcalf, 2003). Biodegradable substances undergo degradation process in the presence of microorganisms which in turn reduces the COD of the wastewater (Gobi, 2013).

The advantages and disadvantages of anaerobic treatment are listed in Table 2.2 (Metcalf, 2014). Anaerobic treatment offers a number of attractive advantages which are emission of a value-added product of digestion (methane) as an end-product, required less energy for aeration and produce lower biomass yield. A major disadvantages of anaerobic treatment are long start-up period and retention times.

Table 2.2: Advantages and disadvantages of anaerobic processes (Metcalf, 2014)

Advantages	Disadvantages
Less energy required	Longer start up time to develop necessary biomass inventory
Less biological sludge production	May require alkalinity addition
Less nutrients required	May require further treatment with an aerobic treatment process to meet discharge requirements
Methane production, a potential energy source	Biological nitrogen and phosphorus removal is not possible
Smaller reactor volume required	Much more sensitive to the negative effect of lower temperatures on reaction rates
Elimination of off-gas air pollution	May be more susceptible to upsets due to toxic substances or wide feeding changes
Able to respond quickly to substrate addition after long periods without feeding	Potential for odor production and corrosiveness of gas
Effective pre-treatment process	
Potential for lower carbon footprint	

A large number of anaerobic treatment are available such as conventional treatment systems, anaerobic filtration, anaerobic fluidized bed, up-flow anaerobic sludge blanket (UASB) reactor, up-flow anaerobic sludge fixed-film (UASFF), continuous stirred tank reactor (CSTR) and anaerobic contact process. Table 2.3 shows the advantages and disadvantages for each anaerobic treatment method.

Conventional treatment system is the most common treatment system that is employed in palm oil mills to treat the POME because more economical and have the ability to tolerate big range of organic loading rate (OLR) (Poh, 2009). More than 85%

of the mills have adopted conventional treatment although this treatment required long retention time and large area for digestion compared to other treatments. Chan (1984) reported that ponding system consist of de-oiling tank, acidificationponds, anaerobic ponds and facultative or aerobic ponds.

Table 2.3 Advantages and disadvantages of various anaerobic treatment methods (Poh, 2009)

	Advantages	Drawbacks
Conventional anaerobic digestion	Low capital cost	Large volume for digestion
	Low operating and maintenance cost	Long retention times
	Able to tolerate big range of OLR (pond) thus can easily cope POME discharge during high crop season	No facilities to capture biogas
	Recovered sludge cake from pond can be sold as fertilizer	Lower methane emission
Anaerobic filtration	Small reactor volume	Clogging at high OLRs
	Producing high quality effluent	High media and support cost
	Short hydraulic retention times	Unsuitable for high suspended solid wastewater
	Able to tolerate shock loadings	
	Retains high biomass concentration in the packing	
Fluidized bed	Most compact of all high-rate processes	High power requirements for bed fluidization
	Very well mixed conditions in the reactor	High cost of carrier media
	Large surface area for biomass attachment	Not suitable for high suspended solid wastewaters
	No channeling, plugging or gas hold-up	Normally does not capture generated biogas

	Faster start-up	
UASB	Useful for treatment of high suspended solid wastewater	Performance dependent on sludge settleability
	Producing high quality effluent	Foaming and sludge floatation at high OLRs
	No media required (less cost)	Long start-up period if granulated seed sludge is not used
	High concentration of biomass retained in the reactor	Granulation inhibition at high volatile fatty acid concentration
	High methane production	
UASFF	Higher OLR achievable compared to operating UASB or anaerobic filtration	Lower OLR when treating suspended solid
	Problems of clogging eliminated	
	Higher biomass retention	
	More stable operation	
	Ability to tolerate shock loadings	
	Suitable for diluted wastewater	
CSTR	Provides more contact of wastewater with biomass through mixing	Less efficient gas production at high treatment volume
	Increased gas production compared to conventional method	Less biomass retention
Anaerobic contact process	Reaches steady state quickly	Less stable due to oxygen transfer in digesting tank
	Short hydraulic retention time	Settleability of biomass is critical to successful performance
	Produces relatively high effluent	

2.2.2 Factors Affecting Anaerobic Treatment Performances

Anaerobic treatment influences on few major factors for an optimum performance. This treatment involves microorganisms, so suitable conditions have to be established to keep all the microorganisms in balance. The few major factors are pH, mixing, temperature, nutrients for bacteria and organic loading rates into the digester (Poh, 2009).

2.2.2 (a) pH

Methanogenesis in anaerobic treatment is strongly sensitive to pH changes (Beccari, 1996). When the pH in digester deviates from the optimum value, methanogenic activity will decrease (Poh, 2009). Gerardi (2006) proved that optimum pH for most microbial growth is between 6.8-7.2 while pH lower than 4 and higher than 9.5 are not tolerable. A neutral pH is favourable for biogas production, since most of the methanogens grow at the pH range of 6.7-7.5. High production of volatile fatty acid concentration cause a drop in pH which inhibited methanogenesis (Patel, 2002). A high alkalinity is needed to assure pH near the neutrality. Recirculation of treated effluent to the digester (Najafpour, 2006) or addition of lime and bicarbonate salt (Gerardi, 2003) are necessary to maintained the alkalinity.

2.2.2 (b) Mixing

Mixing is an essential parameter in anaerobic treatment because it provides good contact between microbes and substrates, reduces resistance to mass transfer, minimizes build-up of inhibitory intermediates and stabilizes environmental conditions (Leslie, 1999). Karim (2005) found that mixing can improved the performance of digesters treating waste with higher concentration. But, Gerardi (2003) not encouraged for rapid mixing because methanogens can be less efficient. Without mixing, foaming will occurs but too mixing will stresses the microorganisms.

2.2.2 (c) Temperature

Temperature is also an important factor because fresh POME discharged at temperature around 80-90°C which makes it is possible to carried out at both mesophilic and thermophilic temperatures. In Malaysia, anaerobic treatments of POME are conducted in the mesophilic temperature range (30-38°C) (Jeremiah David Bala, 2014). Actually there are various study have been conducted to investigate the anaerobic treatment in thermophilic temperature range (50-60°C) and it reported successful operation in this temperature range. POME treatment rate in thermophilic condition is more than four times faster than operation in the mesophilic temperature range (Cail, 1985). Yu (2002) reported that substrate degradation rate and biogas production rate at 55 °C was higher that operation at 37 °C.

2.2.2 (d) Organic Loading Rate

Torkian (2003) reported that high OLRs has the ability to reduce COD removal efficiency in wastewater treatment systems. Generally, OLR is related to substrate concentration and hydraulic retention time (HRT), hence, in order to obtain good digester operation, these two parameters must be in good balance relation.

2.3 Effect of Nitrite and Ammonia on Microbes

Ammary (2004) suggested that the optimum nutrient content which COD:N:P for anaerobic treatment of wastewater is 250:5:1. However, nitrogen content within the POME is lower for effective anaerobic treatment. Hence, the possible solution to increase nitrogen content in the wastewater is by added nitrite or ammonium into the wastewater.

2.3.1 Nitrite

Nitrite, NO_2^- consist of a nitrogen atom with two oxygen atom which one of the oxygen atoms carries a negative charge. Nitrite is easily oxidized to the nitrate form because it is relatively unstable molecule. Nitrite is a fairly strong acid and when the pH drops below 4, nitrite becomes protonated to nitrous acid, HNO_2. It is reported that high concentration of nitrite in wastewater can be severe inhibitor on a wide range of microorganisms.

The inhibition effect of nitrite can slow, or even completely discontinue microbial activities and reconfigure the microbial community structure. This effect depend on concentration and operating pH and temperature (Gobi, 2013). A few research have been reported that the presence of nitrite in wastewater can affect the microbial such as anammox bacteria, methanogenic bacteria and pathogens and yeast.

2.3.1 (a) Anammox bacteria

Nitrite inhibition was independent of pH with a narrow range of 7.0-7.8 (Strous, 1999). At the same time, Egli (2001) reported FNA was the inhibitor because the anammox bacteria was completely inhibited at pH 6.0-6.5 and optimal at higher pH 7.5-8.0 with a constant nitrite feeding level. Nitrite/FNA can be inhibitor to anammox bacteria even at very low concentration. Strous (1999) has proven that even at a very low concentration of 6.0×10^{-3} mg HNO_2-N/L, it can completely inhibit the activity of an enriched anammox culture containing Candidatus *Brocadia anammoxidans*.

2.3.1 (b) Methanogenic bacteria

Kluber (1998) reported that nitrite was the strongest inhibitor on methanogenic bacteria. During biochemical steps in anaerobic treatment, acetic acid, hydrogen and carbon dioxide are the only end-products that can be converted directly into methane gas by methanogenic bacteria. The methanogenesis stage is totally dependent on the production of acetic acid from the acidogenesis stage (Gray, 2004). Even in low nitrite concentrations, that were sufficient enough to completely inhibit methanogenesis (Kluber, 1998). Tugtas (2007) reported that at a nitrite concentration of 50 mg NO_2^- mg/L, it can inhibit 80 % of methane production and the recovery of methanogenesis only occurred once exposed to 250 mg/L of nitrite concentrations. This recovery take occur after complete nitrite reduction which is 7 days.

2.3.1 (c) Pathogens and yeast

Numerous studies has shown that FNA has inhibitory effect on pathogens and yeast. Carlsson (2002) reported that FNA inhibited urinary pathogens while Mortensen (2008) showed that FNA can inhibit the yeasts, which could cause sludge bulking problem in wastewater treatment plants.

2.3.2 Ammonium

Total ammonia nitrogen (TAN) has two principal forms which are ammonium ion (NH_4^+) and free ammonia (FA) (NH_3). FA has been suggested to be main cause of inhibition. There are four types of anaerobic microorganisms in anaerobic treatment. Among them, methanogens are found to be least tolerant and stop the growth due to ammonia inhibition (Kayhanian, 1994). Xuchuan Shi (2017) reported methanogenesis can inhibited at high concentration of ammonia. High concentration of ammonia will cause the accumulation of volatile fatty acid (VFA) and low methane yield.

After considering the previous study, this study will focus on how to increase the nitrogen content in order for anaerobic treatment can work effectively to treat the wastewater. The source of total nitrrogen that can be used are organicnitrogen, ammonia, nitrate and nitrite. But in this study we only investigated effect of ammonium and nitrite addition on the anaerobic treatment of POME.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample collection

The sample of raw palm oil mill effluent (POME) was collected from the palm oil mill at Tali Ayer Palm Oil Bagan Serai, Perak in a container and brought back to the laboratory. The sample was obtained from anaerobic pond. POME sample taken was stored in the cooler room at 4°C to the wastewater from undergoing biodegradation due to microbial action.

3.2 Experimental procedures

Biomass from parent SBR was used for this study. This study was carried out in three sets of batch reactor with 1 L of working volume and 20% volume exchange ratio at room temperature. Each batch reactor was prepared by added 50% volume of batch reactor with anaerobic POME which consist biomass from SBR and mixed them with 50% volume with different sources of nitrogen. Control reactor was set for Reactor 1 without addition of ammonium or nitrite. While for Reactor 2, ammonium bicarbonate is added and Reactor 3 is added with effluent of partial nitrification.

This experiment was conducted for 10 days with 5 days of SRT. Every 2 days, the samples from each reactor was taken for the analysis to check the concentration of MLSS, MLVSS, nitrite, COD, VFA. Before that, the samples were centrifuged for 15 minutes at 14 rpm and they are kept at fridge for 2 hours. After that, the samples were centrifuged again before being analysed for VFA, COD, nitrite and ammonium. Every day, 200 mL of the sample was decanted from each reactor and then 200 mL of new feed was added into the reactor.

3.2.1 Preparation of new feed

Three set of new feed were prepared with 1 L of working volume in three separate reactor i.e. fresh POME with the addition of deionized water (Reactor 1A), fresh POME with the addition of ammonium bicarbonate (Reactor 2B) and fresh POME with the addition of effluent of partial nitrification (Reactor 3C). These reactors were stored in cooler room at 4°C. Every day, 200 mL of new feed for each reactor was added into the Reactor 1, Reactor 2 and Reactor 3.

3.2.2 Sample analysis

3.2.2 (a) Total Suspended Solid Analysis

A set of Buchner flask was used to determine the amount of TSS in the treated samples. Filter papers were dried in oven at 103°C for an hour before it can be used to filter the sample. Then, 20 ml of the sample was used to be sucked using the pump through the Buchner flask. Steps applied for all samples. After that, the filters were dried again in the oven at the same temperature of 103 °C for an hour. The initial and after filtration weight of filter papers were recorded in order to determine the concentration of TSS.

3.2.2 (b) Volatile Suspended Solid Analysis

The filter papers from total suspended solid was heated to 550°C in a muffle furnace. Dwell the filters for 30 minutes. Then, let the filters cool and weight the filters.

The weight after drying at 103°C and 550°C of filter papers were recorded in order to determine the concentration of VSS.

3.2.2 (c) Volatile Fatty Acid

After centrifuge for 15 minutes, the samples were filtered using to remove any residue present in samples. 1.5 mL of each sample was put in the small bottle. The bottles were kept at fridge. The balance of the remaining samples were used to analyse nitrite, ammonium and COD. Then, the concentration of VFA was analysed by using an Agilent 7890A GC, with a flame ionization detector (FID) and a 30 m x 530 µm Agilent DB-WAXetr column.

3.2.2 (d) Nitrite

The balance samples from VFA was diluted with ionized water with the ratio 1:14. 10 mL of the diluted sample was pipetted into the screw cap test tubes and it is called as prepared sample. Meanwhile, another test tube was added with 10 mL of deionized water and act as blank. The nitrite reagent was added into the test tubes and the test tubes are invert gently to mix. Then, let the test tubes for 5 minutes before the nitrite concentration can be read using HACH 2400 spectrophotometer.

3.2.2 (e) Ammonium

0.1 mL of the diluted sample was pipetted into the test tubes and it is called as prepared sample. Meanwhile, another test tube was added with 0.1 mL of deionized water and act as blank. The ammonium salicylate and ammonium cyanurate reagents were added into the test tubes and the test tubes are invert gently to mix. Then, let the test tubes for 20 minutes before the ammonium concentration can be read using HACH 2400 spectrophotometer.

3.2.2 (f) Chemical Oxygen Demand Analysis

MD 200 COD VARIO Photometer was used to analyze the concentration of COD of the treated sample. The range for the spectrophotometer is 1500 mg/L hence every sample needs to be diluted before they can be analyzed. The high range COD vial was used in this analysis because the concentration of COD of POME was very high and need to be diluted. Hach DBR 200 COD digestion reactor was preheated to 150 °C before the vial can be placed in it. 2 mL of diluted sample was pipetted into the COD vial and it was called as prepared sample. Meanwhile, another vial was added with 2 mL of deionized water and act as blank. Both of the vials are cap tightly and rinse with deionized water and wipe with clean paper towel. The vials are invert gently to mix, placed in the reactor and heated for 2 hours. Then, the vials were cooled to room temperature before the COD concentration can be read using MD 200 COD VARIO Photometer.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Performance of Parent SBR

In this experiment, sequencing batch reactor (SBR) act as a parent reactor for anaerobic treatment of POME. Commonly SBR systems have 5 steps, which are carried out in sequences as follows: filling, reaction, settling, decanting and idle. But in this experiment, the cycle of SBR only contains three steps which are filling (15 min), reaction (23.5 hour) and decanting (15 min).POME is added into the SBR during the filling operation. During the reaction period, the biomass consumes the substrate under anaerobic condition. During decanting operation, treated effluent is removed. The SBR operates in a 24 hour cycle with solid retention time (SRT) of 5 days. In this case, the HRT is equal to SRT since there is no recycling step. The pH in SBR was not controlled. The working volume of SBR was 8L and kept at room temperature (30 ± 3°). POME was the sole feed for the SBR. The initial COD concentration of raw POME was 28800 mg/L.

Figure 4.1 shows the cycle study of POME in SBR for 24 hours. The initial COD concentration in SBR was 25150 mg/L. After 24 hours, the concentration of COD decrease to 24180 mg/L. The percentage of COD removal in parent reactor is 69.62%. Biomass in SBR oxidize the COD in SBR to obtain energy. This oxidation process actually help in COD removal. Heterotrophic bacteria obtain their energy from the oxidation of organic carbon. Hence, COD which is a measure of available electrons, is a convenient way in which to express the concentration of organic matter in the wastewater. Consequently, COD is also a convenient technique for expressing the concentration of biomass (Grady, 1999).

Decreasing of COD concentration due to the evolution of MLVSS in SBR. This is because biomass present in SBR need energy for growth. Hence, biomass will oxidize chemicals to obtain the energy. This can be proven by looking the MLVSS concentration of the reactor in Figure 4.1 keep increasing from 1080 mg/L to 1290 mg/L. The increasing of MLVSS concentration indicates that the population of biomass in the parent SBR keep growing as much as 19.44%.

pH has a significant impact on the performance of anaerobic processes. Methanogens activity in anaerobic treatment are affected because of decreasing pH compared to other microorganisms (Grady, 1999). Effect of pH decreasing in the activity of acidogenic bacteria is less significant. A decrease in pH will increase the production of VFA. From Figure 4.1, it shows that VFA production is increasing with the decreasing of pH from 5.1 to 5.0. The decreased pH will reduce the activity of the methanogens, thereby increasing their use of acetic acid and hydrogen. This will cause a further accumulation of VFA and a further decrease in the pH.

From Figure 4.1, the concentration of VFA after feeding POME in the SBR was 7440 mg/L. At the end of cycle, the VFA production was increasing to 10240 mg/L. It shows that, the rate of increasing VFA in SBR was 37.63%. The increasing of VFA production is due to the decreasing of pH value and increasing of biomass growth in anaerobic treatment. Anaerobic processes are operated to convert biodegradable particulate organic matter into VFA by biomass (Grady, 1999).