

CHAPTER 1

INTRODUCTION

1.1 Problems and Rationale

In 2010, the Office of Agricultural Economics reported that Thailand's fresh palm production is likely to increase by 15%, from 8.03 million tons in 2009 to 9.2 million tons due to an increase in harvested area. Biodiesel production is the largest driver for crude palm oil (CPO) demand in 2010 as a change in mandatory production from B2 biodiesel to B3 biodiesel (high speed diesel with the 2 and 3% percent of biodiesel supplement, respectively). Based on observation analysis, total CPO production in 2010 found that 23% will be used for industrial consumption of non-biodiesel products, 42% used for biodiesel production, 31% for food use, and the remaining 4% for exports and stocks (Perspectives on the Thai palm oil industry, 2010). The productions of crude palm oil generate the large quantities of polluted wastewater. Typically, 1 ton of crude palm oil production generated wastewater approximately 5.0-7.5 m³, commonly referred to as palm oil mill effluent (POME) (Somporn et al., 2004). Base on palm oil production in 2010 (9.2 million tons of fresh palm), an average of about 16 million m³ POME is being produced per year in Thailand. The POME comprises a combination of wastewater from three main sources of clarification (60 %), sterilization (36 %) and hydrocyclone (4%) units (Ma, 2000). It contains various suspended components including cell walls, organelles, short fibers, a spectrum of carbohydrates ranging from hemicellulose to simple sugars, a range of nitrogenous compounds from proteins to amino acids, free organic acids and an assembly of minor organic and mineral constituents (Ugoji, 1997).

Wastewater from crude palm oil production is an acidic (pH 4–5), brownish liquid containing water about 95–96%, high organic content of 50,000-80,000 mg COD l⁻¹ or 25,000-35,000 mg BOD l⁻¹, total solids content of 5–7% containing 40,000-67,000 mg l⁻¹ dissolved solids and a mixture of various forms of organic suspended solids (SS) (18,000-49,000 mg l⁻¹). It can be noted that POME is a highly polluting wastewater that can deteriorate if discharged directly without any treatment. POME is posing a serious threat to water reservoirs and ecological environment. The POME characters from various sources are shown in Table 1.1. Characteristics of POME reflected the high organic matters with suspended solid (SS) and oil and greases (O&G).

Table 1.1 The characteristics of palm oil mill effluent

Parameter	Ahmad et al., 2006 (Malaysia)		Paepatung et al., 2006 (Thailand)		Yejian et al., 2008 (China)	
	Range	Average	Range	Average	Range	Average
Temperature (°C)	-	-	40-90	65	-	-
pH	-	4.5	4.21-4.86	4.64	-	4.8
Alkalinity (mg l ⁻¹)	-	-	-	-	-	523
VFA (mg l ⁻¹)	-	-	-	-	-	2,287
BOD (mg l ⁻¹)	-	25,000	10,475-56,900	29,170	-	35,430
COD (mg l ⁻¹)	-	50,000	44,053-80,424	62,030	-	79,725
SS (mg l ⁻¹)	-	18,000	11,625-40,725	23,180	-	49,300
TS (mg l ⁻¹)	-	40,500	53,030-97420	65,675	-	67,200
TVSS (mg l ⁻¹)	-	34,000	-	-	-	35,935
TKN (mg l ⁻¹)	-	750	874-1,665	1,124	-	873.6
TP (mg l ⁻¹)	-	-	49-146	106	-	-
Oil and grease (mg l ⁻¹)	4,000-6,000	5,000	1,880-8,580	5,010	-	17,410

According to characteristics of POME, there are several biological treatments such as anaerobic, facultative, and aerobic degradation more widely used in the treatment of POME. However, anaerobic treatment is one of the successful and powerful biological methods for environmental protection (Zhang et al., 2008). Anaerobic digestion methods are mostly regarded to consider because these systems do not require high energy for aeration and allow the recovery of energy in the form of methane in a closed system. Anaerobic digestion has been successfully employed to POME treatment. It is the degradation of complex organic matters under the absence of oxygen. This process is time consuming as bacterial consortia responsible for the degradation process requires time to adapt the new environment before they start to consume on organic matters to grow (Poh and Chong, 2009). POME is high pollution wastewater as characteristics shown in Table 1.1. SS and O&G are main problems in POME treatment system.

There are many technologies were applied to study the pretreatment methods in the removal of SS and residual O&G content in POME but it has limited (Tay, 1991). Suspended and colloidal components in the form of fat, protein, and cellulose in POME have adverse impact on bioreactors performance and can cause deterioration of microbial activities and washout of active biomass. Moreover, the high SS content can prevent the system from operating at high organic loading rates (OLR) in various types of bioreactor (Borja and Banks, 1994). Besides, it is highly composed of oil and dispersedly greases on surfaces and its content is about 5,000-17,000 mg l⁻¹. This property makes it not only highly polluting but also extremely difficult to treat by conventional methods (Ahmad et al., 2003; Fadzilah and Mashitah, 2010). Approximately 2000 mg l⁻¹ of O&G presents in an emulsified form in the supernatant of POME together with low pH and dissolved oxygen contents, and high

organic loads are the most toxic for microbial populations by inhibiting the growth especially on seed germination and methanogen (Ahmad et al., 2006). Even though, SS and O&G are the major problems of POME treatment but they have high potential for gas production (Poh and Chong, 2009).

Anaerobic biological systems offer more potential with high performance the treatment of POME and methane gas production via microorganism under suitable conditions. Nevertheless, conventional anaerobic POME treatment method takes long retention times and large treatment areas are required. Due to this, high rate anaerobic bioreactors have been proposed and developed to reduce this problem by shorter hydraulic retention times (HRT), smaller reactor volume and low cost of investment. Granulated seed sludge (Ahmad et al., 2003), utilizing seed sludge from same process (McHugh et al., 2003), or maintaining suitable pH and temperature in the high-rate anaerobic bioreactor for growth of bacteria consortia in POME treatment system (Yacob et al., 2005) has been used to reduce the retention time.

The examples of high rate anaerobic bioreactors are anaerobic filtration, fluidized bed reactor, upflow anaerobic sludge blanket (UASB) reactor with investigation by several researches (Borja and Banks, 1994; Liu et al, 2002; Najafpour et al, 2006). POME treatment has been successful with these reactors by achieving COD removal efficiency up to 90% with the high operating OLR as shows in Table 1.2.

Table 1.2 Performances of various anaerobic treatment methods on POME treatment

Type	OLR (kg COD m ⁻³ d ⁻¹)	HRT (d)	Methane composition (%)	COD removal efficiency (%)	References
Anaerobic pond	1.4	40	54.4	97.8	Yacob et al., 2006
Anaerobic digester	2.16	20	36	80.7	Yacob et al., 2005
CSTR	3.33	18	62.5	80	Tong and Ja, 2006
Anaerobic contact digestion	3.44	4.7	63	93.3	Ibrahim, 1984
Upflow anaerobic filtration	4.5	15	63	94	Borja and Banks, 1994b
UASB	10.63	4	54.2	98.4	Borja and Banks, 1994
UASFF	11.58	3	71.9	97	Najafpour et al., 2006

One of the interesting high rate anaerobic bioreactors is upflow anaerobic sludge fixed-film (UASFF) reactor. The UASFF reactor is a hybrid reactor with an upflow fixed film (UFF) part over an upflow anaerobic sludge blanket (UASB) section (Borja and Banks, 1994). It is one of the granular

sludge combining with attached biofilm bioreactors that are used for the rapid biotransformation of organic matter to methane with protection of granulated microbial washout. Moreover, it has been used successfully for the treatment of POME because this reactor was a good strategy to accelerate anaerobic granulation and to achieve high COD removal efficiency in a short period of time at high organic loading rate (Najafpour et al., 2006).

The anaerobic digestion is a biological process in which a complex community of microorganisms. Performance of anaerobic bio-treatment was depended on the population, activity profiles and performance of four groups of microorganism; hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria and methanogens, in anaerobic digestion system. One of the significant drawbacks in anaerobic treatment of wastewater is the difficulty of start-up procedures, which can be time-consuming and unproductive. Major problems lie in the inconsistency of effluent and the establishment of the most suitable microbial population for the waste to be treated. Adequate seeding of the bioreactor is critical, as the growth rates of anaerobic microorganisms tend to be slow, especially methanogens.

In anaerobic POME treatment system, methanogenesis is the rate limiting step in anaerobic digestion of POME (Ibrahim, 1984). Moreover, because of POME consists of various suspended components including cell walls, organelles, short fibers, a spectrum of carbohydrates ranging from hemicellulose to simple sugars, a range of nitrogenous compounds from proteins to amino-acids, free organic acids and an assembly of minor organic and mineral constituents (Ugoji, 1997). POME has special characteristics as high SS and O&G which difficult to biodegradation and need specific microbial degradation. Therefore, those suspended solid hydrolysis are also limiting step in biodegradation of POME.

Various strategies have been applied for improving degradation from fermentative processes, such as the careful bioreactor with nutrients. The main reason is towards enhancing and speeding the microbial activity, increasing of the bacterial diversity and enhancing its degradation potential (Lee, 1993). SS is organic matter that will be hydrolyzed by hydrolytic bacteria. Then, organic acid formed in the hydrolysis and fermentation stages are converted by acetogenic bacteria to acetic acid, after that methanogen plays a further role in conversion of organic acid to methane gas. Whereas, microbial lipase producer is microorganism produce lipase enzymes that they are often useful to digest O&G in bioreactor. *Bacillus* species have been found to produce lipase and also other enzyme such as cellulase, amylase and xylanase (Yacob et al., 2006b). Therefore, understanding the microbial community responsible for organic degradation is important to bioreactor operation and control.

There have been many research studies on the microbial community investigation in various types of wastewater but not much in POME anaerobic treatment study. The objectives aimed to facilitate and understanding of microbial behavior and reactions in the anaerobic digestion process

which could potentially be applied to POME treatment. Rincon et al. (2008) studied microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue. The results showed that eubacteria mostly represented by the genus *Clostridium* as a predominant bacteria at low OLR, whereas other bacterial communities such as *γ-proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Deferribacteres* are abundant bacteria at high OLR in the range of 1.5 - 9.2 g COD l⁻¹ d⁻¹. The archaeal community was mainly represented by four phylotypes belonging to the genus *Methanosaeta* independently of the OLR (Rincon et al., 2008). It can be noted that, microbial community study is an important indicator for monitoring the performance and stability of wastewater treatment system. Understanding the structure and function of microbial complex communities would be very useful for improving operating condition and performance of wastewater process.

Since POME characteristics from various sources show the fluctuations in SS and O&G concentrations and both these parameters notice as one major parameter on the process performance and stability of anaerobic reactor. The effect of SS and O&G concentrations on anaerobic reactor was attractive in this study. So far, anaerobic hybrid reactor (AHR) combining of sludge bed and fixed film reactor was applied in this study to treat and produce biogas from POME. The understanding of microbial role, function and structure in sludge and packed zone in AHR can explain how SS and O&G concentrations impact both zones of AHR. These knowledge will be useful for improving the process strategy of operating condition, modified microbial consortium and performance of AHR. Therefore, process performance including stability, microbial characteristics in terms of quality (activity) and quantity, and microbial diversity were studied in sludge and packed zones of AHR in this study.

1.2 Objectives

- 1.2.1 To study the effect of SS and O&G concentrations on process performance and stability in sludge and packed zones of AHR
- 1.2.2 To study SS and O&G concentrations on microbial characteristics in term of microbial community, population as well as microbial activity in sludge and packed zones of AHR

1.3 Scopes of research work

1.3.1 Raw POME was directly collected from crude palm oil plant and was studied characteristics. It was analyzed physical and chemical characteristics such as pH, BOD, TCOD, SCOD, SS, O&G, TVA and alkalinity according to standard method (APHA, 2005). Before reactor operation,

influent was prepared by adjusting SS concentration of POME at 5, 7, 10, 11 and 12 g l⁻¹, respectively, that it also changed in other characteristics according to increasing of SS concentrations.

1.3.2 Sources of inoculum seed were selected by regarding similar type of feedstock to reduce acclimatization in startup period. Therefore, inoculum seed was collected from POME treatment system and analyzed its quantity and quality via volatile suspended solid (VSS) and specific methanogenic activity (SMA) analysis, respectively.

1.3.3 Anaerobic hybrid reactor (AHR) was made up from acrylic column with working volume 5,000 ml. The upper part of AHR was fitted by nylon with specific surface area of 2 m²m⁻³ which this zone is called packed zone while bottom part was sludge zone.

1.3.4 AHR was operated under ambient temperature (~35-37°C). The operation condition starts with low SS concentration of 5 g l⁻¹ and HRT of 5 d. When the system ran to steady state, it was continued operated at least 3-4 HRTs to ensure the system. During this period, overall process performance and stability were routine monitored including biogas production. Microbial population and community in both sludge and packed zones were analyzed using molecular techniques as quantitative polymerase chain reaction (qPCR) and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Moreover, microbial activity in sludge and packed zones during anaerobic POME digestion was determined. The reactor was operated with step-wise increasing of SS concentrations with constant HRT at 5 d. At each steady state, process performance and stability included microbial characteristics were studied. Finally, all results of reactor operations at various SS concentrations were analyzed to identify the optimum SS concentration of POME treatment to obtain high performance and stability as well as understanding of microbial community dynamic in the system.

1.4 Expectations of Research work

Knowing and understanding the capacity of POME treatment at high organic load with SS and O&G by anaerobic hybrid reactor will be very useful for good managing and improving reactor performance. Moreover, understanding of the structure and function of microbial complex communities can be help to improving the operation and control of POME treatment system and maintain the optimum condition for microbial activity in the system.

The operation cost may be reduced. In additional, not only high quality of treated wastewater which low pollution from organic contents will be released to environment but also the high yield of biogas production will be getting form suspended solid and oil and greases because it is high potential substances for biogas production.