CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Industry in Malaysia

2.1.1 History and Development of Palm Oil Industry

Oil palm (*Elaeis guineensis*) is one of the most versatile crops in Malaysia. Oil Palm was first brought into Malaysia in year 1875 as an ornamental plant (DOE, 2009). The growth of the palm oil industry in Malaysia has been phenomenal over the last 4 decades. In order to diminish the country's economic dependence on rubber and tin, the cultivation of oil palm was increased by the government's agricultural diversification programme in early 1960s (MPOC, 2009). In the late 1960s, land settlement schemes established by the government for planting oil palm as a way to eliminate shortage for the landless farmers and smallholders (MPOC, 2009). Today, 4.49 million hectares of land in Malaysia is under oil palm cultivation; producing 2.13 tonnes of palm kernel oil and 17.73 million tonnes of palm oil (MPOC, 2009). Therefore, Malaysia is one the largest producer and exporter of palm oil in the world.

2.1.2 Palm Oil Production Processes

The typical process flow diagram for the extraction of crude palm oil is presented in the appendix B. Crude palm oil (CPO) is extracted from the mesocarp of fresh fruit bunch (FFB). Around 1000 kg of processed FFB can produce 225 kg CPO (DOE, 2009). The

capacity of a large scale mills range from 10 to 60 tonnes FFB/h. After harvest, the FFBs are transported to the mills for processing. These FFBs are sterilized with steam at a temperature of 140 °C and a pressure of 3 bars for 75-90 min. In order to minimise kernel breakage, sterilization helps FFBs for mechanical stripping and preconditioning of the nuts. After sterilization, the FFBs are fed to a rotary drum-stripper where the fruits are stripped from bunches. Under steam heated situation with temperature around 90°C, the fruits are then mashed in the digester. Twin screw presses are normally utilized to press out the oil from the digested mashed fruits under high pressure. Hot water is supplied to raise the flow of the oils. The crude oil slurry is then fed to a clarification system for oil separation and purification. Before storage, the clarified oil is then passed through a high speed centrifuge and vacuum dryer. The press cake discharged from the screw press consists of moisture, oily fibre and nuts, and the cakes are transmitted to a depericarper for nuts and fibres separation. The fibre and nuts are separated by strong air current induced by a suction fan. The nuts are sent to a nut cracker and further to a hydrocyclone to split the shell from the kernel. With the purpose of avoiding the growth of mould for a longer storage time; the kernel is dehydrated to below 7 % moisture.

2.1.3 Palm Oil Mill Effluent (POME)

POME is considered as one of the most polluting agro-industrial residues due to its high organic load. Additionally, POME is in the form of highly concentrated dark brown colloidal slurry of water, oil and fine cellulose materials from sterilisation and clarification stages. From environmental perspective, fresh POME is a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids (40,500 mg/L), O & G (4000 mg/L), COD (50,000 mg/L) and BOD (25,000 mg/L) (Ma, A. N., 2000). Thus, POME has been identified as one of the major sources of aquatic pollution in Malaysia.

The manufacture of palm oil results in the generation of large quantities of contaminated wastewater usually referred to as palm oil mill effluent (POME). Usually, 5-7.5 tonnes of water are required for 1 tonne of CPO production; over 50 % of which ends up as POME (Ma, A.N., 1999). In general, one tonne of POME will be produced from

every 2 tonnes of FFB processed from the mill (Yacob, S. *et al.*, 2005). Moreover, POME is in the form of highly concentrated dark brown colloidal slurry of water, oil and fine cellulose materials from sterilization and clarification stages. It holds a variety of suspended components including an assembly of minor organic and mineral constituents, organelles, short fibres, a range of nitrogenous compounds from proteins to amino acids, a spectrum of carbohydrates ranging from hemicellulose to simple sugars, free organic acids and cell walls (Ugoji, E.O., 1997). Characteristics of POME from literature are shown in the appendix C.

2.2 Palm Oil Mill Effluent Treatment

In Malaysia, the ponding system has been employed by more than 85% of palm oil mills for POME treatment while the rest opted for open digesting tank (Yacob, S. *et al.*, 2005). Ponding system consists of de-oiling tank, acidification ponds, anaerobic ponds and facultative or aerobic ponds (Chan, K.S. and Chooi, C.F., 1984). Usually, long retention time in excess of 20 days is needed for the ponding system and the biogas is released into the atmosphere. Open digester tank and lagoon system can generate 35% and 45% of CH_4 gas respectively (Yacob, S. *et al.*, 2006b). Therefore, the CH_4 emission from the palm oil industry is the largest green house gas (GHG) source in Malaysia.

These methods are regarded as conventional POME treatment method whereby long retention time and large treatment areas are required. High-rate anaerobic bioreactors have also been applied in laboratory-scaled POME treatment such as up-flow anaerobic sludge blanket (UASB) reactor (Borja, R. and Banks, C.J., 1994a); up-flow anaerobic filtration (Borja, R. and Banks, C.J., 1994b); fluidized bed reactor (Borja, R. *et al.*, 2001), up-flow anaerobic sludge fixed-film (UASFF) reactor (Najafpour, G.D. *et al.*, 2006), anaerobic contact digester (Ibrahim, A. *et al.*, 1984) and continuous stirred tank reactor (CSTR) (Ugoji, E.O., 1997). Other than anaerobic digestion, membrane technology (Ahmad, A.L. *et*

al., 2007), aerobic activated sludge reactor (Vijayaraghavan, K. *et al*., 2007), and evaporation method (Ma, A.N. *et al*., 1997) have also been used to treat POME.

The anaerobic digestion systems are being used significantly in wastewater treatment especially in agro-industry due to anaerobic digestion which has substantial advantages such as (a) producing CH_4 gas as a valuable end product, (b) low energy requirements (no aeration), (c) generates sludge from process which could be used for land application. In comparison with open digester tank, the anaerobic pond had a higher emission of CH_4 with an average CH_4 composition of 54.4% (Yacob, S. *et al*., 2006a). Furthermore, the CH_4 composition from anaerobic ponds was also found to be more consistent in the gaseous mixture. Therefore, this study focuses on the anaerobic digestion system and the discussion would be limited to this particular treatment system.

2.3 Anaerobic Digestion Processes

Anaerobic digestion is a useful method of treating agricultural, industrial and domestic wastes. In the absence of free oxygen, it is a typical anaerobic ecosystem where complex organic polymeric substances are enzymatically broken down into the final end products of CH_4 and CO_2 by the action of different microbial populations. There are at least four groups of microbial populations responsible for the anaerobic degradation. Basically, the anaerobic digestion process can be divided into 3 main degradation steps: hydrolysis and fermentation, syntrophic acetogenesis and methanogenesis (Harper, S.R. and Pohland, F.G., 1986). The steps in anaerobic digestion involving four groups of bacterial activities are shown in the appendix D.

2.3.1 Hydrolysis and Fermentation (or Acidogenesis)

Hydrolysis is the initial step of the anaerobic degradation for most insoluble organic wastes. In this step, proteins, carbohydrates and lipids in the form of complex suspended

compounds and colloidal matter are converted into their monomer or dimeric components, such as amino acids, single sugars and long chain fatty acids (LCFA). The non-soluble and particulate substrates that are too large to pass through the cell membrane cannot be taken up by organisms and therefore, the extracellular enzymes which are produced by both facultative and strictly anaerobic bacteria carry out the breakdown of organic polymers. This is usually a slow process, which can, in the case of complex substances, be the rate-limiting step in the whole degradation process (Parkin, G.F. and Owen, W.F., 1986). The monomers resulting from hydrolytic bacteria are then fermented to VFAs, alcohols, CO_2, H_2 and some lactic acid (Toerien, D.F. and Hattingh, W.H. J., 1969).

Anaerobic fermentative bacteria which grown on glucose produce VFAs such as acetic, propionic and butyric acids. Acetic acid is the most abundant followed by propionic and butyric acids (Toerien, D.F. and Hattingh, W.H. J., 1969). The conversion reactions are shown as below:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.0)$$

$$3C_6H_{12}O \longrightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O \quad (2.1)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2.2)$$

The first reaction to produce acetic acid from glucose is the preferred reaction under stable conditions (Mosey, F.E., 1983). The second intermediate metabolic product, propionic acid, can also be formed by the following reactions:

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.3)$$

$$3CH_3CHOHCH_2COOH + H_2 \longrightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + 2H_2O \quad (2.4)$$

The second reaction is a result of Propionibacterium consuming lactate, produced by lactic acid bacteria.

2.3.2 Syntrophic Acetogenesis

A simpler product such as acetic acid need to be converted from the fermentation products such as propionic and butyric acids as well as ethanol before being utilized by methanogenic bacteria. The bacteria responsible for this conversion are known as acetogenic bacteria (or called H_2 producing bacteria). These reactions are unfavorable under thermodynamic standard conditions, since they exhibit positive Gibbs free energy values ($\Delta G°'$) (Table 2.2). It was concluded that these reactions proceed only if the reaction products (mainly H_2) are sufficiently reduced in concentration to yield a negative net value for the free energy change. This is accomplished by syntrophic association with hydrogen (formate)-utilizing bacteria, usually hydrogenophilic methanogens that serve as hydrogen (formate) scavengers (Boone, D.R. *et al.,* 1989).

Table 2.1: Thermodynamics of some of the reactions involved in syntrophic conversions during methanogenic decomposition.

	$\Delta G°'$(kJ/mol)*
Ethanol + H_2O $\rightarrow$ Acetate$^-$ + $2H_2$	+9.6
Butyrate$^-$ + $2H_2O$ $\rightarrow$ 2Acetate$^-$ + H^+ + $2H_2$	+48.1
Propionate$^-$ + $3H_2O$ $\rightarrow$ Acetate$^-$ + HCO^{3-} + H^+ + $3H_2$	+76.1
HCO^{3-} + $4H_2$ + H^+ $\rightarrow$ CH_4 + $3H_2$	-135.6

Source: Drake, H.L. (1994)
*298K, pH 7, 1M for solutes and 1 atm for gases.

H_2-acerogenic and homoacetogenic bacteria are another group of acetogens which are capable of converting H_2 and CO_2 to acetate, by the following reaction:

$$2CO_2 + 4H_2 \longrightarrow CH_3COOH + 2H_2O \qquad (2.5)$$

Acetobacterium woodee and Clostridium aceticum are bacterial species that are able to carry out the above reaction (Braum, M. and Mayer, F., 1981). and consume other substrates for growth such as fructose, pyruvate, and lactate.

2.3.3 Methanogenesis

Methanogenesis is the last step in anaerobic digestion to produce CH_4 and CO_2 from acetate and H_2 produced in acetogenesis step (Jeris, J. and McCarty, P.L., 1965). Methanogenesis is performed by methanogenic bacteria in all anaerobic ecosystems. In the anaerobic digester ecosystems, these bacteria are the most sensitive bacterial group to oxygen and pH (Barredo, M.S. and Evison, L.M., 1991). Approximately 70 % of the CH_4 is produced by cleavage of acetate (Jeris, J. and McCarty, P.L., 1965), through a reaction below commonly called as aceticlastic (or acetoclastic) methanogenesis.

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad (2.6)$$

Limited number of species isolated in anaerobic conditions that use acetate, belong to Methanosaeta genus (3 species, homotrophic) and Methanosarcina genus (5 species, can also use H_2 and CO_2, methylated C-1 compounds and methanol). Morphologically these two genera are very different: Methanosarcina contains species of cocci, which may occur singly, in packets, or in large pseudoparenchyma whereas Methanosaeta includes large sheathed rods, often forming long filaments and large aggregates (Whitman, W.B. *et al*., 1992).

The uptake of H_2 or formate to produce CH_4 is generally known as hydrogenotrophic methanogenesis (equation 2.6). As referred before, the hydrogenophilic bacteria control the redox pontential of the media, maintaining H_2 concentration at low levels, and thus conditioning syntrophic acetogenesis. This trophic group includes large numbers of species within 5 orders in the archaea (Boone, D.R. *et al*., 1993).

2.4 Acclimatization Phase of Anaerobic Digester

POME is considered as one of the most polluting agro-industrial residues due to its high organic load. Additionally, POME is in the form of highly concentrated dark brown colloidal slurry of water, oil and fine cellulose materials from sterilisation and clarification stages. From environmental perspective, fresh POME is a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids (40,500 mg/L), O & G (4000 mg/L), COD (50,000 mg/L) and BOD (25,000 mg/L) (Ma, A.N., 2000).Thus, POME has been identified as one of the major sources of aquatic pollution in Malaysia.

2. 5 Factors Influencing Anaerobic Digester Performance

Anaerobic digestion particularly the methanogensis process is very sensitive to the operational condition compared with aerobic process. Hence, the process control measures are of critical importance which guard against process instability of anaerobic digestion. A few major factors that greatly influence digester performances in POME treatments are pH, operating temperature, nutrients for bacteria, food to microorganism (F/M) ratio, toxic material, solids and hydraulic retention times and organic loading rates (OLRs) into the digester.

2.5.1 Effect of pH

Anaerobic microbial especially the methanogens are sensitive to the acid concentration in the reactor and inhibited by acidic condition. Thus, the microbial community in anaerobic digesters are sensitive to pH changes and methanogens are affected to a greater extend (Leslie Grady Jr. *et al*., 1999). A research done by Beccari *et al*. (1996) confirmed that pH can strongly affect methanogenesis. In general, optimum pH for most

microbial growth is between 6.8 and 7.2 whereas pH lower than 4 and higher than 9.5 are not tolerable (Gerardi, M.H., 2006).

Acetic acids and propionic acids were the main products under the basic condition while butyric acids were formed under acidic and neutral condition. The treatment performance and stability can be improved by the control of reactor pH to obtain interested acids for CH_4 production. This is because low level of propionic acids will minimise the inhibition growth of methane-forming bacteria whereas acetic and butyric acids are favorable substrates for methanogens. Thus, VFA concentration is a significant parameter to monitor reactor performance (Buyukkamaci, N. and Filibeli, A., 2004). Besides that, it was found that digester could tolerate acetic acid concentrations up to 4000 mg/L without inhibition of gas production (Stafford, D.A., 1982). In order to control the level of VFA in the system, alkalinity (Alk) has to be maintained by recirculation of treated effluent (Najafpour, G.D. *et al*., 2006) to the digester or addition of lime and bicarbonate salt (Gerardi, M.H., 2003).

2.5.2 Effect of Operating Temperature

There are three main temperature ranges in the anaerobic digestion processes. Psychrophilic is operated below 25 °C, mesophilic range is between 25 °C to 40 °C and the optimum is at 30 °C to 35 °C. The thermophilic is operated at temperature greater than 45 °C (Hamed, M.E. *et al*., 2004). Normally, POME is discharged at temperatures around 80–90 °C (Zinatizadeh, A.A.L. *et al*., 2006) which actually makes treatment at both mesophilic and thermophilic temperatures feasible especially in tropical countries like Malaysia. However, anaerobic POME treatments in Malaysia are performed only in the mesophilic temperature range.

These studies have reported successful system operation in the thermophilic temperature range, with POME treatment having treatment rate more than four times faster than operation in the mesophilic temperature range (Cail, R.G. and Barford, J.P., 1985). The major contributions of the thermophilic anaerobic process are higher stability for solids reduction, higher biogas production, high resistance to foaming, improvement of the energy

balance of the treatment plant, less odour and high effect of destroying pathogens in the thermophilic digesters (Cuba, V. *et al*., 2003). But, failure to control rising temperature can result in biomass washout (Lau, I.W.C. and Fang, H.H.P., 1997) with accumulation of VFA due to inhibition of methanogenesis. At high temperature, production of VFA is higher compared to mesophilic temperature range (Yu, H.-Q. *et al*., 2002). Therefore, many operators prefer to have digesters operating in mesophilic temperature because of better process stability.

2.5.3 Effect of Nutrient for Bacteria

Methanogenesis is very sensitive to nutrient availability. The essential nutrients comprising of carbon, nitrogen, phosphorus, calcium, magnesium, iron, and trace elements are main ingredients for biomass growth. Moreover, Iron, cobalt, nickel, zinc, copper, manganese, molybdenum, selenium, tungsten and boron are required to stimulate methanogenesis (Speece, R.E., 2006). A rough approximation of minimum nutrient requirements can be based on the stoichiometry of the overall biodegradation process:

$$\text{C-source} + \text{N-source} + O_2 + \text{Minerals} + \text{Nutrients} \rightarrow \text{Cell mass} + CO_2 + H_2O + \text{product} \quad (2.7)$$

Various empirical formulas of bacterial cell mass have been proposed. On the other hand, the most widely accepted are $C_5H_7O_2N$ and $C_{60}H_{87}O_{32}N_{12}P$. In addition, the required ratio of nutrient is C: N: P = 100:10:1 with assumption that half of C is used for cell production and half for energy production by the cells. Bacteria require inorganic nutrients such as ammonium and phosphate to support cell growth and sustain anaerobic digestion processes. Hence ammonium can be used as an indicator of digester performance (Carucci, G. *et al*., 2005). Usually, nitrogen or phosphorus deficiency can be expressed by VFA accumulation (Speece, R.E., 1996).

2.5.4 Effect of Food to Microorganism (F/M) Ratio

In order to monitor the balance between available food materials (BOD or COD) and available organisms (mixed liquor volatile suspended solids, MLVSS), the F/M ratio is a process control calculation utilized in many anaerobic digestion facilities. It also specified the amount of substrate supplied per day per unit amount of biomass in the anaerobic digestion reactor. In order to consume the required number of pounds of waste, the correct number of pounds of bacteria must be supplied. Lower the F/M ratio would result in a greater percentage of the waste being converted to gas.

This parameter is normally applied as a design parameter. A selected F/M value finds out the needed amount of biomass in the reactor and the reactor volume is calculated for a suitable biomass concentration. As F/M ratio cannot be chosen alone from the adopted sludge age, thus this method may not be accurate. The F/M ratio gives the substance removal rate per unit solids in a system as shown in Equation 2.8.

$$\frac{F}{M} = \frac{S_0}{XxHRT} \tag{2.8}$$

2.5.5 Effect of Toxic Material

Toxic material can interrupt the biochemical process in microorganisms employed in the anaerobic digestion treatment system, hence, causing failure of the system. For instance, certain materials, such as ammonia, heavy metals, light metal cations, and sulfide can create unstable conditions within the digester, as their concentration sufficiently increase adequately in anaerobic digesters. High concentrations of potassium and other salts can interrupt cell function (Speece, R.E., 1996) and ammonia is un-ionized above pH 8 which is poisonous to anaerobes. Generally, acetogens are the most sensitive, and concentrations above 3000 mg/L ammonia nitrogen (NH_4-N) are toxic regardless of pH (Calli, B. *et al*., 2005). Besides that, toxic situations also arise because of a sudden change in digester operation, such as overfeeding or excessive addition of chemicals, or because of a shock loading of these materials in the plant influent.

The inhibition of CH_4 formation is the most general effect of excess concentrations of these materials in the digester. This leads to VFA accumulation, pH depression, and digester upset. Thus, general toxicity can be determined by an Anaerobic Toxicity Assay, in which gas production rates are depressed despite abundance of acetate (Carucci, G. *et al*., 2005). Usually, chemical addition can be used to control concentrations of dissolved forms of some of these materials. The most common example of this is control of sulfide using iron salts.

2.5.6 Effect of Solids and Hydraulic Retention Times

The sizing of anaerobic digesters is based on providing adequate retention time in these well-mixed reactors to allow significant volatile solids destruction to happen. Sizing criteria, expressed either as the hydraulic retention time (HRT) (days, the working volume divided by the volume sludge fed to the digester per day) and solids retention time (SRT) (days, calculated as the mass of solids in digester divided by the mass of solids removed per day). The HRT is constant with SRT when there is no change in solids concentration within the digester.

The extent of each of these reactions occurring during anaerobic digestion (hydrolysis, acid formation, and methane formation) and the solids and hydraulic retention times are directly related: a decrease in solids and hydraulic retention time decreases the extent of reaction; an increase in solids and hydraulic retention time increases the extent of each reaction. There is a minimum critical retention time for each reaction, and as this is not offered, the bacteria cannot grow fast enough to remain in the digester, thus, the reaction mediated by these bacteria will stop and the digestion process will be unsuccessful.

2.5.7 Effect of Organic Loading Rates (OLRs)

OLR is a measure of the anaerobic digestion biological conversion capacity. Various studies have confirmed that higher OLRs will decrease COD removal efficiency in wastewater treatment systems (Sanchez, E. *et al.*, 2005). According to Yu and Fang (2003),

carbohydrate degraded at all loading rates but degradation of protein and lipids decreased with an increase in loading. On the other hand, gas production will increase with OLR until a stage where methanogens could not work quick enough to convert acetic acid to CH_4 as a result of accumulation of inhibiting substances like VFAs. At high OLR, methanogenic cannot consume H_2 and resulted in increased of H_2 partial pressure along with decreased of the methane yield (Hardik, P. and Datta, M., 2002).

OLR is related to substrate concentration and HRT, therefore a good balance between these two parameters have to be gained for excellent digester operation. Short HRT will reduce the time of contact between substrate and biomass. As the HRT and influent waste concentration are known, the OLR can be calculated using the equation 2.9.

$$OLR = \left(\frac{1}{HRT}\right)(C_1) \tag{2.9}$$

2.6 Biogas

Biological decomposition of organic matter by anaerobic digestion results in the production of particularly useful effluent called biogas. Biogas is primarily mixture of methane (CH_4), carbon dioxide (CO_2) and minute traces of hydrogen (H_2), hydrogen sulphide (H_2S), nitrogen (N_2), ammonia (NH_3) and sulfur dioxide (SO_2). CH_4 is the only constituent of biogas with significant fuel value. The inert diluents of CO_2 and N_2 reduce the calorific content of the gas, while H_2S, corrosive nature wears away the anaerobic digester and pipes involved in the gas distribution. The CH_4 fraction can range from 40 to 85 % based on methanogen vitality, substrate quality and reactor type (Yacob, S. *et al*., 2006b). Being mostly CH_4, biogas can be collected and burned, like natural gas to produce thermal or electric energy. But a large percentage of the CO_2 remains in solution (Shelton, D.R. and Tiedje, J.M., 1984). Since CH_4, CO_2 and H_2 are the main constituents of anaerobic

biogas, thus this research study focused mainly on CH_4, CO_2 and H_2. The varying percentage constituents of biogas according to literature are shown in the appendix E.

2.6.1 Carbon Dioxide (CO_2)

CO_2 is a chemical compound consists of two oxygen atoms covalently bonded to a single carbon atom. For many years, carbon dioxide emission has been the most essential issue of the environment, thus we have to limit CO_2 emission in order to put a stop to climate change. Fossil fuels which produce energy and released CO_2 upon combustion are limited resources. Therefore, the solution for both issues is renewable energy options with CO_2 as the renewable energy as it is compatible with the environment. If locally produced, it definitely will be a major bonus.

Radwan A. and Constant M. G. van den Berg (1999) showed that the CO_2 content of biogas from anaerobic digesters ranged from 9-40 % by volume. Like CH_4, CO_2 compositions of biogas are good parameters for monitoring digester stability (Mathiot, S. *et al*., 1992) although others suggested that the variations in its composition become visible only when the digesters changed to advanced stage of imbalance (Frigon, J. C. and Guiot, S.R., 1995).

2.6.2 Hydrogen (H_2)

H_2 gas is a clean and ideal alternative energy for the future, therefore H_2 gas production and utilization have been highly investigated (Penner, S.S., 2006). Biohydrogen generation from renewable biomass would reduce dependence on fossil fuel, decrease the CO_2 emissions and produce usable bioenergy. The major advantage of energy from H_2 is the zero carbon emissions, since the utilization of H_2, either via combustion or via fuel cells, results in pure water (Claassen, P.A.M. *et al*., 1999).

In nature, biohydrogen is produced during acidogenic wastewater treatment process where acid forming bacteria produces organic acid compound, H_2 and CO_2 (Angenent, L.T. *et al*., 2004). However, no H_2 is bubbling out due to the coexisting bacteria that readily consume H_2 as a source of reducing power. Therefore, a specific environment needs to be created to support the growth of H_2 producer and reduce the number of H_2 consumer. For this aim, pretreatment on sludge sample by heat and chemical method could be carried out to eliminate the presence of methanogens as most of the H_2 producer could produce endospore that survive in harsh environment (Zhang, Y.F. *et al.*, 2005).

2.6.3 Methane (CH_4)

CH_4 is a chemical compound with the chemical formula CH_4. It is the main component of biogas. Biogas contains about 60 % CH_4 that can be utilized to generate electricity or utilized as heat or fuel for vehicles. Several engine types are modified to run on biogas, and emerging technology in high temperature fuel cells can convert and store energy from biogas (Sorge, G., 2006). However, POME can produce CH_4 during anaerobic digestion. The survey from literature and from biogas facilities in the European Community published by Radwan A. and Constant M. G. van den Berg (1999) showed that the CH_4 content of biogas from anaerobic digesters ranged from 52 to 95 % by volume. In addition, CH_4 content of biogas is regarded as the best indicator of overall digester performance whereby low CH_4 content of biogas could represent inhibition of methanogenic bacteria (Fannin, K.F., 1987).

Moreover, CH_4 is a green house gas that has 21 times the heating effect compare with CO_2. At the same time, biogas CH_4 is renewable unlike natural gas which is mined from underground wells and is a non-renewable fossil fuel. Therefore, we have to use CH_4 as an energy source in order to protect the environment by removing the CH_4 from atmosphere and replacing the use of non-renewable fossil fuels with renewable energy.

2.6.3.1 Methane Yield (Y_{CH4})

The methane yield is a vital economic factor in the anaerobic digestion of palm oil mill effluent (POME) and the result of the balance between the flows of organic carbon to catabolism and anabolism in methanogenic ecosystems. Usually, estimating the potential methane yield normally needs lengthy laboratory testing.

The maximum theoretical yield can be determined from the COD of the biomass or from an ultimate analysis. According to literature, the theoretical relationship between COD removal and CH_4 production is 2 mol COD removal results in the production of 1 mol CH_4 (John, E.C., 1999). At standard conditions, that yield is 0.35 m^3 CH_4 per kilogram $COD_{removal}$ (Jerry, L.H. and Douglas, D.B., 1998).

Buswell and Muller develop Equation 2.10 for predicting stoichiometric methane yield from the compositional data of biomass (Francis, J.P., 1998).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad (2.10)$$

The upper theoretical methane yield for most biomass species is about 0.47 m^3/kg VS (Sorge, G., 2006).

In most cases, the methane yield coefficient, Y_{CH4} is determined by experimental data and equation 2.11. As the volume of gas produced per day, $V_{methane}$ (L CH_4/d), is assumed to be proportional to the amount of substrate consumed (Wheatley, A., 1990), then

$$V_{methane} = Y_{CH_4} q(S_0 - S) \qquad (2.11)$$

Where,

S_0 = the substrate concentration (gCOD/L) at the digester inlet,

S = the substrate concentration (gCOD/L) at the digester effluent,

q = the feed flowrate.

2.7 Volatile Fatty Acids (VFAs) of POME

In the anaerobic digestion of POME, complex organic materials are first hydrolyzed and fermented by rapidly growing and pH-insensitive acidogenic bacteria into VFAs (Siegrist, H. *et al*., 1993). The slowly growing acetogenic bacteria then oxidize the VFAs into acetate, H_2, and CO_2 that are appropriate as substrates for the methanogenic bacteria (Ozturk, M., 1993).

It is well-known that VFAs can cause microbial stress if present in high concentrations, resulting in a depletion of buffering capacity and a depression of pH to levels that also inhibit the hydrolysis and acidogenesis phase (Palmisano, A.C. and Barlaz, M.A., 1971). It has been reported that even when process pH is optimal, the accumulation of VFAs may contribute to a reduced rate of hydrolysis of the solid organic substrate (Banks, C.J. and Wang, Z., 1999), or even to inhibition at extremely high levels (>10 g L^{-1}) (Palmisano, A.C. and Barlaz, M.A., 1971). Moreover, inhibition of the fermentative bacterial population by its main product VFAs when using glucose as the main substrate (Van den Heuvel, J.C. *et al*., 1992) has also been observed. Besides that, they also affect the loading, efficiency and running stability of the methanogenesis phase (Ren, N.Q. and Wang, B.Z., 1994). Thus, the terminal fermentation products produced in the acidogenesis phase are very important for the entire system performance. Meanwhile, the conversion rate from volatile fatty acids (VFAs) to acetic acid will affect the methanogenic bacteria quantity, and subsequently affect the degradation rate of acetic acid and methane yield.

In addition, it is widely known and accepted that the occurrence of VFAs in anaerobic sludge decrease if the chain length increase. As shown in many studies, the conversion rates of VFAs to CH_4 differ in the order of acetic acid, ethanol, butyric acid and propionic acid (Ren, N.Q. *et al*., 2003). All VFAs are first degraded to acetic acid before

being degraded to methane, and their conversion rates also differ in the order of ethanol, butyric acid and propionic acid. In general, lactic acid, which has the potential to be converted to propionic acid, is an undesirable terminal fermentation product. Thus, accumulation of propionic acid always results in failure of methanogenesis (Ren, N.Q. *et al*., 2005). In addition, one of the most main approaches of maintaining the carbon and electron flow during anaerobic digestion is to control the organic loading to the system, constructing a favorable environment for the mixed culture of microorganisms, to ensure VFAs production and utilisation rates are balanced (Bhattacharya, S.K. *et al*., 1996).