BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) USING AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) BIOREACTOR

by

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LIST OF ABBREVIATION

2FI	Two factor interaction
ABR	Anaerobic baffled reactor
AF	Anaerobic filter
Alk	Alkalinity
AMBR	Anaerobic migrating blanket reactor
ANOVA	Analysis of variance
A-PAM	Anionic polyacrylamide
ASBR	Anaerobic sequencing batch reactor
BA	Bicarbonate alkalinity
BOD	Biochemical oxygen demand
CCD	Central composite design
CCFD	Central composite face-centered design
COD	Chemical oxygen demand
COD_{ef}	Effluent chemical oxygen demand
COD_{in}	Influent chemical oxygen demand
C-PAM	Cationic polyacrylamide
CPO	Crude palm oil
CV	Coefficient of variance
DF	Degree of freedom
DOE	Department of environment
DoE	Design of experiment
ECP	Extra cellular polymers
EFB	Empty fruit bunch
FBR	Fluidized-bed reactor
FFB	Fresh fruit bunch

F/M	Food to microorganism
GHG	Green house gases
GSS	Gas solids separator
H_2SO_4	Sulfuric acid
HRT	Hydraulic retention time
ICR	Immobilized cell reactor
MABR	Modified anaerobic baffled reactor
MAS	Membrane anaerobic system
MLVSS	Mixed liquor volatile suspended solids
$NaHCO_3$	Sodium bicarbonate
NTU	Nephlometric turbidity unit
OLR	Organic loading rate
P	Probability of error
p	VFA to alkalinity ratio
PCOD	Particulate chemical oxygen demand
pH	Potential of hydrogen
POME	Palm oil mill effluent
R^2	Coefficient of determination
RBC	Rotating biological contactor
RO	Reverse osmosis
RSM	Response surface methodology
SCOD	Soluble chemical oxygen demand
SD	Standard deviation
SEM	Scanning electron microscopy
SMA	Specific Methanogenic activity
SRF	Solid retention factor

SRT	Solid retention time
SVI	Sludge volume index
TA	Total alkalinity
TCOD	Total chemical oxygen demand
TEM	Transmission electron microscopy
TKN	Total Kjeldahl nitrogen
TSS	Total suspended solids
TVFA	Total volatile fatty acids
UASB	Up-flow anaerobic sludge blanket
UASFF	Up-flow anaerobic sludge fixed film
UF	Ultra-filtration
UFF	Up-flow fixed film
VFA	Volatile fatty acids
VSS	Volatile suspended solids

LIST OF SYMBOLS

		Unit
A	Apparent kinetic constant	(-)
K	Apparent reaction rate constant	(lit CH_4/g COD.d)
k	Transportation rate constant into the granule	(d^{-1})
K_s	Half-velocity constant	(g COD/l)
K_h	Hydrolysis rate constant	(d^{-1})
k_1 and k_2	Reaction rate constants in consecutive kinetic model	(d^{-1})
K_H	Carbonic acid equilibrium constant	mol/l
$K_{a,1}$	Carbonic acid-bicrbonate equilibrium constant	mol/l
$K_{a,2}$	Bicarbonate-carbonate equilibrium constant	mol/l
K_w	Water equilibrium constant	mol/l
L_0	Ultimate BOD (BOD_u)	mg/l
Q	Volumetric feeding flow rate	(l/d)
Q_e	Effluent flow rate	(l/d)
Q_F	Feed flow rate	(l/d)
Q_M	Volume of gas produced per day	(l CH_4/d)
r_M	Methane production rate	(l CH_4/l.d)
S_0	Influent substrate concentration	(g COD/l)
S	Effluent substrate concentration	(g COD/l)
S_g	Hydrolyzed substrate Concentration in the granule	(g COD/l)
S_h	Hydrolyzed substrate Concentration in the reactor	(g COD/l)
t	Hydraulic retention time	(d)
V	Volume of the reactor	(lit)
V_{up}	Up-flow velocity	m/h

X	Biomass concentration	(mg/l)
X_e	Effluent VSS concentration	(mg/l)
X_i	Independent variables / factors	(-)
Y_i	Response	(-)
Y_M	Methane yield constant	(l CH_4/g $COD_{removed}$.d)
Y_x	Growth yield constant	(g VSS/g $COD_{removed}$.d)

Greek symbols

α	Distance from the centre of the design space to axial point	(-)
β_0	Constant coefficient	(-)
β_i	Coefficients for the linear effect	(-)
β_{ii}	Coefficients for the quadratic effect	(-)
β_{ij}	Coefficients for the cross-product effect	(-)
η	Effectiveness factor	(-)
μ	Specific microbial growth rate	(d^{-1})
μ_m	Maximum specific microbial growth rate	(d^{-1})

RAWATAN BIOLOGI KUMBAHAN KILANG KELAPA SAWIT (POME) MENGGUNAKAN BIOREAKTOR ENAPCEMAR ANAEROB ALIRAN-NAIK SAPUT TETAP

ABSTRAK

Reaktor enapcemar anaerob aliran-naik saput tetap (UASFF) adalah satu bioreaktor cipta baru dan digunakan untuk biopenjelmaan cepat bahan organik kepada metana dengan bantuan daripada agregat mikrob berbutir. Satu bioreaktor UASFF berskala makmal dengan satu tangki pengenapan luar telah berjaya direkabentuk dan beroperasi untuk rawatan kumbahan kilang kelapa sawit (POME). Bioreaktor tersebut telah dimajukan untuk memendekkan tempoh pemulaan pada masa penahanan hydraulik (HRT) yang rendah. Bebanan organik ditingkatkan secara beransur dari 2.67 kepada 23.15 g COD/l.hari sepanjang tempoh ini. Enapcemar berbutir didapati terbentuk dengan cepat dalam masa 20 hari dengan saiz berbutir meningkat daripada titik pin pada mulanya sehingga mencapai saiz 2 mm. Pencernaan anaerob untuk POME telah dimodel dan dianalisis dengan dua pembolehubah iaitu HRT dan COD_{in} menggunakan kaedah permukaan sambutan (RSM). Kawasan eksplorasi untuk pencernaan POME telah diambil dari kawasan yang dirangkumi oleh sempadan HRT (1 hingga 6 hari) dan COD_{in} (5260 hingga 34725 mg/l). Peningkatan dalam pembolehubah tersebut mengakibatkan penurunan dalam penyingkiran COD, SRT dan SRF tetapi meningkatkan kadar penyingkiran COD, VFA/Alk, peratusan CO_2 dalam biogas dan kadar penghasilan metana. Persamaan kinetik yang dicadangkan dan satu model Monod yang dipermudahkan telah berjaya digunakan untuk menghuraikan kinetik pencernaan anaerob POME pada kadar bebanan organik antara 0.88 hingga 34.73 g COD/l.hari. Penghasilan metana adalah antara 0.287 hingga 0.348 l

CH_4/g $COD_{disingkirkan}$.hari. Pekali biokinetik iaitu pemalar halaju separa ketara (A), pemalar halaju separa (K_S), kadar maksimum pertumbuhan spesifik mikrob (μ_m), pemalar penghasilan metana (Y_M) dan pemalar penghasilan pertumbuhan biojisim (Y_x) juga telah dikira. Pemalar ketara kadar (K), dikira dengan model Monod yang dipermudah adalah dalam lingkungan 2.9 ke 7.4 l CH_4/g COD.hari. Pada kepekatan COD influen yang berbeza, nilai K menunjukkan hubungan lurus dengan perubahan kandungan VSS dalam reaktor. Dalam satu ujikaji berkelompok bagi pencernaan POME, 275 mg $CaCO_3$ kealkalian bikarbonat telah dihasilkan bagi setiap 1000 mg $COD_{disingkirkan}$. Hampir 95 % penyingkiran COD dicapai dalam masa 72 jam dengan kadar penyingkiran COD awal pada 3.5 g COD/l.hari. Model kinetik tindak balas berturutan yang telah digunakan untuk meramal data aktiviti enapcemar semasa ujikaji berkelompok memberikan padanan yang baik dengan keputusan daripada ujikaji ($R^2 > 0.93$). Langkah yang paling perlahan didapati adalah langkah pengasidan dengan pemalar kadar antara 0.015 hingga 0.083 jam^{-1} manakala pemalar kadar bagi langkah metanogen didapati antara 0.218 hingga 0.361 jam^{-1}. Prestasi jangka panjang reaktor UASFF juga telah dikaji dengan POME mentah sebagai suapan pada HRT selama 3 hari dan kepekatan COD influen sebanyak 44300 mg/l. Kaedah pra-rawatan fizik dan kimia juga telah diselidiki. Ujikaji telah dijalankan berdasarkan satu rekaan pusat rencam bermuka tengah (CCFD) dan dimodelkan mengunakan kaedah permukaan sambutan (RSM) dengan dua pembolehubah operasi iaitu kadar aliran suapan (Q_F) dan halaju aliran-naik (V_{up}). Prestasi reaktor dengan suapan POME yang melalui pra-enapan dan pra-rawatan kimia telah dibandingkan. Keadaan optima bagi pencernaan POME secara pra-enapan dan pra-rawatan kimia dengan masing-masing pada 1.65

l/hari Q_F dan 0.6 m/jam V_{up}, dan 2.45 l/hari Q_F dan 0.75 m/jam V_{up}. Dapatan ujikaji adalah berpadanan dengan jangkaan model. Pencirian enapcemar berbutir yang terhasil dalam reaktor UASFF pada pelbagai keadaan operasi menunjukkan ia terdiri terutamanya dari rod berbungkus yang padat (mikroorganisma berupa Methanosaeta) dan micoorganisma berupa cocci (Methanosarsina).

BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) USING AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) BIOREACTOR

ABSTRACT

Up-flow anaerobic sludge fixed film (UASFF) bioreactor is a modern bioreactor and was used for the rapid biotransformation of organic matter to methane with the help of granulated microbial aggregates. A lab scale UASFF bioreactor (3.65 lit) with an external settling tank was successfully designed and operated for palm oil mill effluent (POME) treatment. The bioreactor was developed in order to shorten the start-up period at low hydraulic retention time (HRT). The organic loading was gradually increased from 2.67 to 23.15 g COD/l.d during this period. Granular sludge was found to develop rapidly within 20 days with an increase in size of granules from an initial pinpoint size to about 2 mm. The anaerobic digestion of POME was modeled and analyzed with two variables i.e. HRT and COD_{in} using response surface methodology (RSM). The region of exploration for digestion of POME was taken as the area enclosed by HRT (1 to 6 days) and COD_{in} (5260 to 34725 mg/l) boundaries. An increase in the variables resulted in a decrease in COD removal, SRT and SRF but an increase in COD removal rate, VFA/Alk, CO_2 percentage in biogas and methane production rate. The proposed kinetic equation and a simplified Monod's model were successfully employed to describe the kinetics of POME anaerobic digestion at organic loading rates in the range of 0.88 to 34.73 g COD/l.d. The methane yields obtained were between 0.287 to 0.348 l CH_4/g $COD_{removed}$. Biokinetic coefficients i.e. apparent half-velocity constant (A), half-velocity constant (K_S), maximum specific microbial growth rate (μ_m), methane yield constant (Y_M), and biomass growth yield constant (Y_x) were also evaluated.

The apparent rate constants, K, calculated by simplified Monod model were in the range of 2.9 to 7.4 l CH_4/g COD.d. At different influent COD concentrations, K values showed a linear relationship with variations in VSS content in the reactor. In a batch POME digestion, 275 mg $CaCO_3$ bicarbonate alkalinity was produced per 1000 mg $COD_{removed}$. About 95 % COD removal was achieved within 72 h with an initial COD removal rate of 3.5 g COD/l.d. A consecutive reaction kinetic model employed to simulate the data on sludge activity in batch experiment showed good fit to the experimental results (R^2 > 0.93). The slowest step was modeled to be the acidification step with rate constants between 0.015 to 0.083 h^{-1} while those of the methanogenic step were between 0.218 to 0.361 h^{-1}. Long term performance of the UASFF reactor was investigated with raw POME as feed at a HRT of 3 days and an influent COD concentration of 44300 mg/l. Physical and chemical pretreatment methods were also conducted. Experiments on the pretreated POME digestion were conducted based on a central composite face-centered design (CCFD) and modeled using response surface methodology (RSM) with two operating variables i.e. feed flow rate (Q_F) and superficial up-flow velocity (V_{up}). The performance of the reactor fed with the pre-settled (settling for 3 h) and chemically pretreated (after flocculation) POME was compared. The optimum conditions for the digestion of the pre-settled and chemically pre-treated POME were at Q_F of 1.65 l/d, V_{up} of 0.6 m/h and Q_F of 2.45 l/d and V_{up} of 0.75 m/h, respectively. The experimental findings were in close agreement with the model prediction. The characterization on the granular sludge developed in the UASFF bioreactor at various operating conditions showed that they predominantly consisted of densely packed rod (Methanosaeta-like microganism) and cocci shaped (Methanosarsina) microorganisms.

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CHAPTER 1

INTRODUCTION

1.1 Palm Oil Industry in Malaysia

Oil palm (Elaeis guineensis) is one of the most versatile crops in tropical countries. Palm oil industry is one of the most important contributors to Malaysia economy. Today, Malaysia is the world's largest producer and exporter of palm oil; contributing 49.5 % of world production and 64.5 % of world exports (Malaysian Palm Oil Board, 2004). The total oil palm planted area increased by 4.5 % or 174,000 hectares to 4.0 million hectares in 2005 compared to that in 2004. The production of crude palm oil continued to increase for seven consecutive years reaching 15.0 million tonnes in 2005 from 14.0 million tonnes in the previous year (Malaysian Palm Oil Board, 2005). Therefore, a great action needs to be taken in order to guarantee the sustainable development in palm oil production.

1.2 Palm Oil Production Processes

Figure 1.1 presents typical process flow diagram for the extraction of crude palm oil. After harvest, the fresh fruit bunches (FFB) are transported to the mills for processing. Each FFB consists of hundreds of fruits, each of which containing a nut surrounded by a bright orange pericarp which contains the palm oil. These FFBs are sterilized with steam at a pressure of 3 bar and a temperature of 140 °C for 75-90 min. The objectives of this process are to prevent further formation of free fatty acids due to enzyme action, facilitate stripping and prepare the fruit mesocarp for subsequent processing. The steam condensate coming out of the sterilizer constitutes as one of major sources of liquid effluent (Thani *et al.,* 1999).

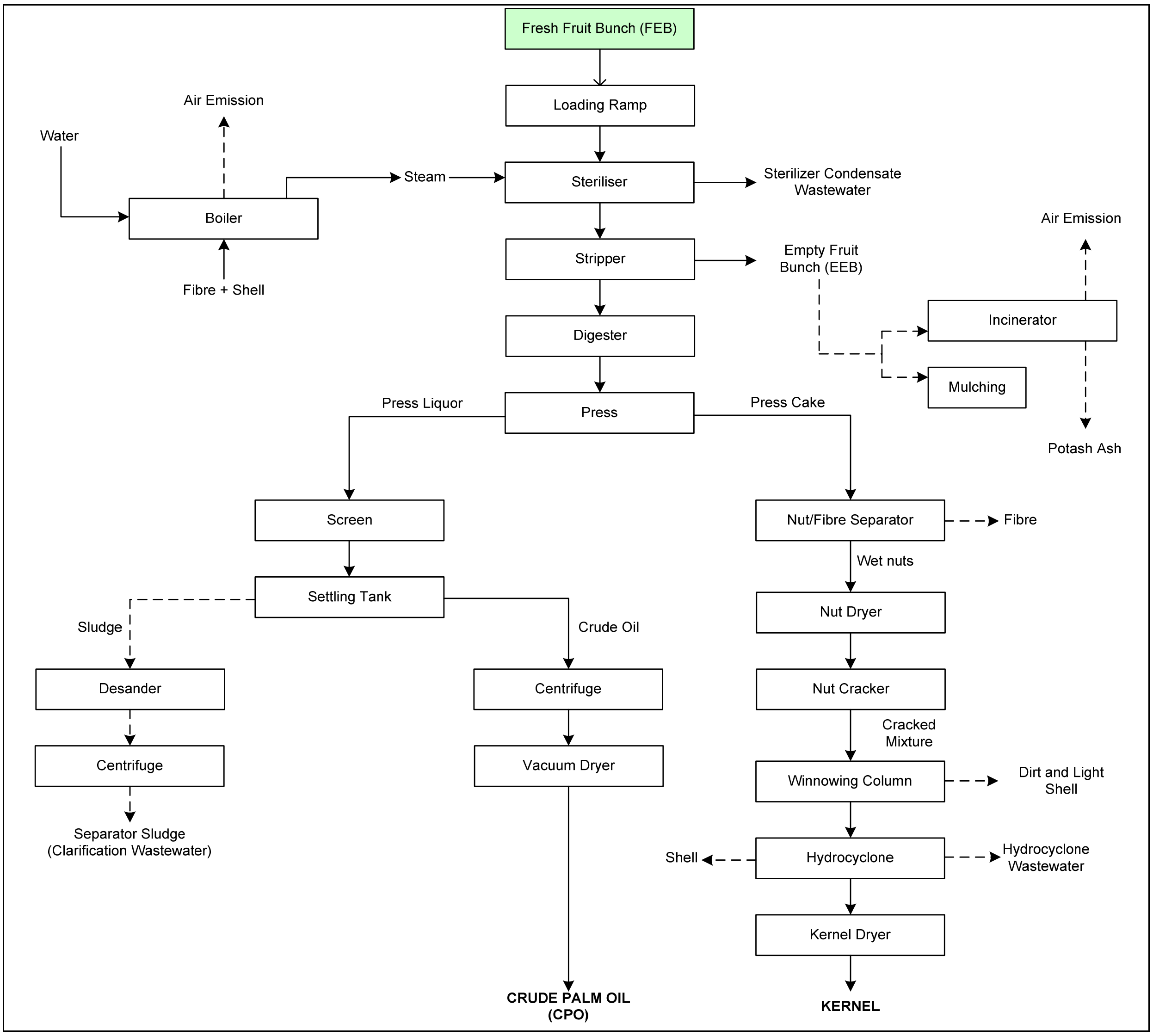


Figure 1.1. Conventional palm oil extraction process and sources of waste generation (Thani *et al.,* 1999).

After sterilization, the FFBs are fed to a rotary drum-stripper where the fruits are stripped from the bunches. The detached fruits are passed through the bar screen of the stripper and are collected below by a bucket conveyor and discharged into a digester. In the digester, the fruits are mashed by the rotating arms. In this stage, the mashing of the fruits under heating breaks the oil-bearing cells of the mesocarp. Twin screw presses are generally used to press out the oil from the digested mash of fruit under high pressure. Hot water is added to enhance the flow of the oils. The crude oil slurry is then fed to a clarification system for oil separation and purification. The fibre and nut (press cake) are conveyed to a depericarper for separation (Thani *et al.,* 1999).

The crude palm oil (CPO) from the screw presses consists of a mixture of palm oil (35-45 %), water (45-55 %) and fibrous materials in varying proportion. It is then pumped to a horizontal or vertical clarification tank for oil separation. In this unit, the clarified oil is continuously skimmed-off from the top of the clarification tank. It is then passed through a high speed centrifuge and a vacuum dryer before sending it to the storage tanks.

The press cake discharged from the screw press consists of moisture, oily fibre and nuts, and the cake are conveyed to a depericarper for nuts and fibres separation. The fibre and nuts are separated by strong air current induced by a suction fan. The fibre is usually sent to boiler house and is used as boiler fuel. Meanwhile, the nuts are sent to a rotating drum where any remaining fibre is removed before they are sent to a nut cracker. Hydrocyclone is commonly used to separate the kernels and shells. The discharge from this process constitutes the last source of wastewater stream (Chow and Ho, 2000). A general mass balance of various products generated from a palm oil mill is shown in Figure 1.2.

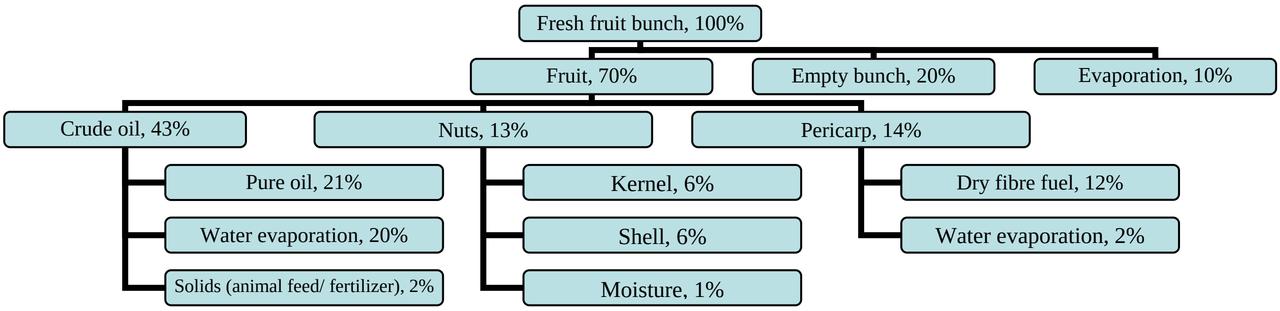


Figure 1.2. Typical fruit and production composition chart of a palm oil mill (Muttamara *et al.,* 1987).

1.3 Wastes Generation in Palm Oil Mills

Beside the main product i.e. the crude palm oil (CPO), the mills also generate many by-products and liquid wastes, which may have a significant impact on the environment if they are not dealt with properly.

1.3.1 Liquid Effluent

The production of palm oil results in the generation of large quantities of polluted wastewater, commonly referred to as palm oil mill effluent (POME). Typically, 1 tonne of crude palm oil production requires 5-7.5 tonnes of water; over 50 % of which ends up as POME (Ma, 1999a). Based on palm oil production in 2005 (14.8 million tonnes), an average of about 53 million m^3 POME is being produced per year in Malaysia (Malaysia Palm Oil Production Council, 2006). The POME comprises a combination of wastewater from three main sources viz. clarification (60 %), sterilization (36 %) and hydrocyclone (4 %) units (Ma, 2000). It contains various suspended components including cell walls, organelles, short fibres, 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).

From environmental perspective, fresh POME is a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids (40,500 mg/l), oil and grease (4000 mg/l), COD (50,000 mg/l) and BOD (25,000 mg/l) (Singh *et al*., 1999; Ma, 2000). POME has been identified as one of the major sources of aquatic pollution in Malaysia. The characteristic of a typical POME is shown in Table 1.1.

Table 1.1. Typical characteristics of POME (Ma, 2000).

Parameter	***Average**	**Metal**	***Average**
pH	4.7	Phosphorous	180
Oil and Grease	4000	Potasium	2270
Biochemical Oxygen Demand (BOD_5)	25000	Magnesium	615
Chemical Oxygen Demand (COD)	50000	Calcium	439
Total Solids	40500	Boron	7.6
Suspended Solids	18000	Iron	46.5
Total Volatile Solids	34000	Manganese	2.0
Ammonical Nitrogen	35	Copper	0.89
Total Nitrogen	750	Zinc	2.3

*All in mg/l except pH.

1.3.2 Solid Wastes

The solid waste materials and by-products generated in the palm oil extraction process are presented as follows:

(1) Empty fruit bunches (EFB) - 23 % of FFB;

(2) Potash – 0.5 % of FFB;

(3) Palm kernel – 6 % of FFB;

(4) Fibre – 13.5 %; and

(5) Shell – 5.5 % of FFB.

The EFB may be incinerated to produce potash which is applied in the plantation as fertilizer by mulching. The fibre and shell materials are used as boiler fuel. The palm kernel is usually sold to palm kernel oil producers for the extraction of the palm kernel oil (Thani *et al.,* 1999).

1.3.3 Gaseous Emission

Palm oil mills are generally self-sufficient in terms of energy requirements due to the availability of adequate quantities of fibre and shell materials that are used as solid fuel in the stream boiler. There are two principle sources of air pollution in the mills viz. the boiler and incinerator that are caused by incomplete combustion of the solid waste materials (waste fibre, shell materials and EFB) (Thani *et al.,* 1999). With regard to that the main practice of treating POME is by using ponding and/or open digesting tank systems (Ma *et al.,* 1999), the emission of green house gases (GHG) (CH_4 and CO_2) from these systems to the atmosphere has been recently reported as a source of air pollution from the palm oil mills (Yacob *et al.,* 2005).

1.4 Environmental Regulations of Effluent Discharge

The environmental control in palm oil industry was decided to be warranted a licensed approach that would permit intimate control of individual factories. It also provides a mechanism for permitting variable effluent standards to be applied based on the demands of prevailing environmental circumstances. The environmental quality regulations for the crude palm oil industry were the first set of regulations promulgated under the Environmental Quality Act (EQA), 1977, for control of industrial pollution sources (Thani *et al.,* 1999).

The Environmental Quality (prescribed Premises) (Crude Palm Oil) Regulations 1977, promulgated under the enabling powers of Section 51 of the EQA, are the governing regulations and contain the effluent discharge standards. Other regulatory requirements are to be imposed on individual palm oil mills through conditions of license (Environmental Quality Act 1974, 2005). The effluent discharge standards ordinarily applicable to crude palm oil mills are presented in Table 1.2.

Table 1.2. Effluent discharge standards for crude palm oil mills (Environmental Quality Act 1974, 2005).

Parameter	unit	Parameter limits (Second schedule)	Remarks
Biochemical Oxygen Demand (BOD; 3-Day, 30 °C)	mg/l	100	
Chemical Oxygen Demand (COD)	mg/l	*	
Total Solids	mg/l	*	
Suspended Solids	mg/l	400	
Oil and Grease	mg/l	50	
Ammoniacal Nitrogen	mg/l	150	Value of filtered sample
Total Nitrogen	mg/l	200	Value of filtered sample
pH	-	5-9	
Temperature	°C	45	

Note: * No discharge standard after 1984.

1.5 Renewable Energy in Malaysia

Due to increasing demand for energy, cost saving and the protection of the environment, anaerobic digestion technology has become a worldwide focus of research. Malaysia's energy sources primarily comprise oil, natural gas, hydropower and coal, although renewable energy (RE) sources such as solar power and biomass are currently being exploited. As presented in Table 1.3, natural gas, hydropower, and biomass energy resources in Malaysia are generally abundant.

Table 1.3. Energy resource potential in Malaysia (ASEAN, 2003).

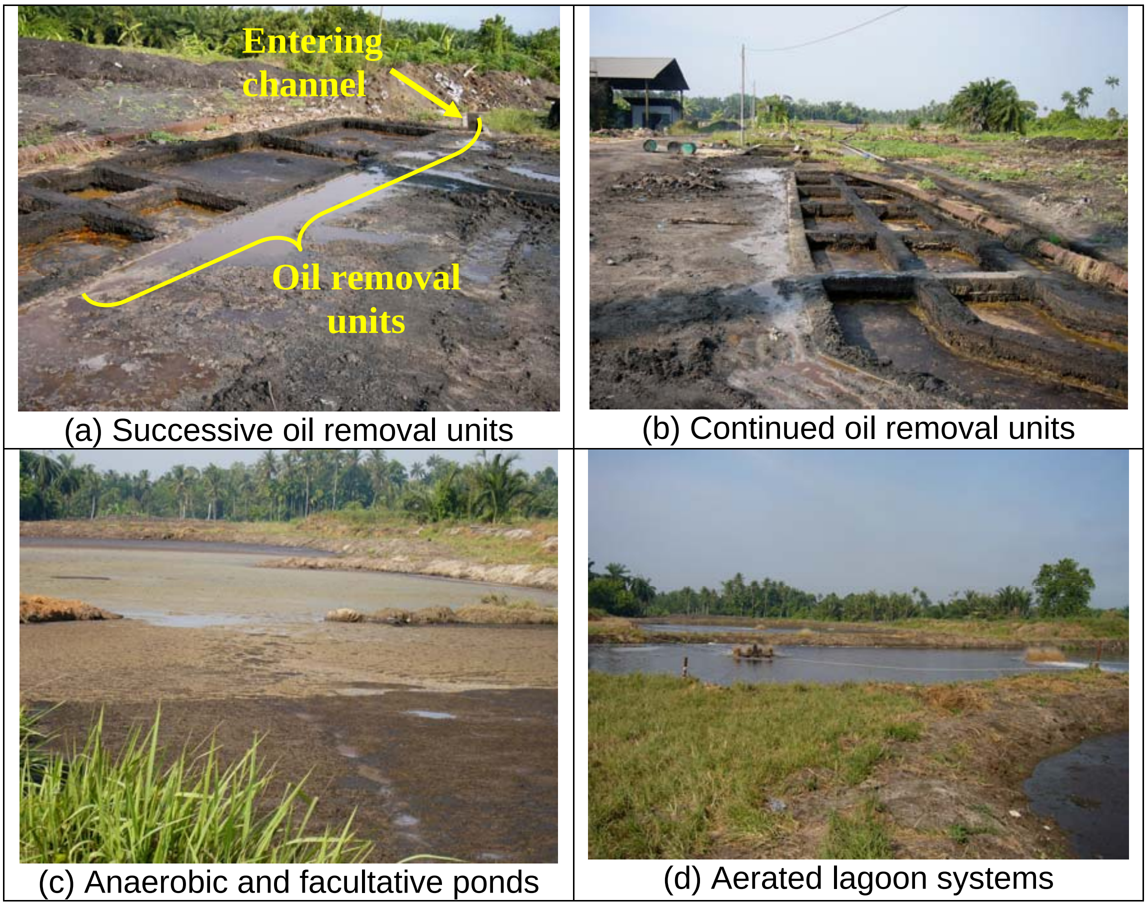
Energy resources	**Amount**	**Unit**
Oil reserve	5.0	Billion barrels
Gas estimate reserve	2402	Billion cubic meters
Coal proven reserve	-	Million tonnes
Hydro power technically feasible	72	Twh/y
Biomass	665	MW
Geothermal potential	-	MW
Wind energy potential	-	MW

The most extensive study on the use of biomass has been on palm oil wastes, which can be utilized to meet the energy requirement of the palm oil mills and the electricity needs of the workers. The total energy potential of the biomass is estimated to be about 5 % of Malaysian electricity demand (EPU, 1999). Therefore, renewable energy has been identified by Malaysian government as the 5^{th} fuel under 'The New Five-Fuel Diversification Strategy' (Energy Commission, 2002; Kannan *et al.,* 2003). Plate 1.1 shows different types of biomass generated by a palm oil mill. From the four biomass sources, three of them (EFB, fibre and shell) can be directly burned as fuel while POME must first be anaerobically converted to methane. Therefore, it is essential for a high rate anaerobic bioreactor to be applied as it can serve dual-function i.e. wastewater treatment and energy generation (organic conversion to methane).

Plate 1.1. Palm oil wastes as renewable energy sources (EPU, 1999).

1.6 Current POME Treatment Systems

Palm oil industries are facing tremendous challenges to meet the increasingly stringent environmental regulations. Over the past decades, several cost-effective treatment technologies comprising anaerobic, aerobic and facultative processes have been developed for the treatment of POME. More than 85 % of palm oil mills use solely ponding systems due to their low costs. It has been reported that only a few mills are equipped with biogas recovery systems (Yeoh, 2004). Plate 1.2 shows a working POME ponding treatment system at a palm oil mill in Nibong Tebal, Penang, which is a common practice in most palm oil mills. Long hydraulic retention times (HRT), low treatment efficiency, high sludge production, extensive land area requirement, emission of large amount of GHG (CO_2 and CH_4) and so on are drawbacks of this conventional POME treatment method. Therefore, the application of an efficient, stable and economic high rate anaerobic treatment system is currently being seriously investigated.



(a) Successive oil removal units
(b) Continued oil removal units
(c) Anaerobic and facultative ponds
(d) Aerated lagoon systems

Plate 1.2. Wastewater treatment system at a palm oil mill in Nibong Tebal, Penang.

1.7 Problem Statement

There are currently about 360 active palm oil mills in Malaysia with a combined annual CPO production capacity of about 15 million tonnes (Malaysian Palm Oil Promotion Council, 2005). On an average, in standard palm oil mills, each tonne of fresh fruit bunch (FFB) processed generates about 0.7 tonne of liquid waste comprising of about 26.3 kg of BOD, 53 kg of COD, 19 kg of suspended solids (SS) and 6 kg of oil and grease. This amounts to a population equivalent of around 60 millions in terms of COD (Thani *et al.,* 1999). Also, palm oil mill wastewater treatment systems are one of the major sources of green house gases in Malaysia due to their biogas emission (36 % CH_4 with a flow rate of 5.4 l/min.m^2) from open digester tanks and/or anaerobic ponds (Yacob *et al.,* 2005). Therefore, palm oil mills in Malaysia face the challenge of balancing environmental protection, their economic viability, and sustainable development after the Department of Environment enforced the regulation for the discharge of effluent from the crude palm oil (CPO) industry, under the Environmental Quality (prescribed premises) (Crude Palm Oil) order and regulations, 1997. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the sustainability of the economy.

The development of effective and simple methods for treatment of industrial wastewater is a challenging task to environmental engineers and scientists. Considering the high organic character of POME, anaerobic process is the most suitable approach for its treatment. There are several studies on POME treatment which have been carried out using various high rate anaerobic reactors such as anaerobic filter (AF), fluidized bed reactor (FBR), immobilized cell reactor (ICR), up-flow anaerobic sludge blanket (UASB) reactor, anaerobic hybrid digester, membrane anaerobic system (MAS), and modified anaerobic baffled reactor (ABR) (Borja & Banks, 1994a and b, 1995; Fakhrul-Razi & Noor, 1999; Faisal & Unno, 2001). The main advantage of high rate reactors is their ability to retain high biomass concentration in reactor which leads to an increase in rate of waste stabilization in the unit.

Among all the reactors, the most efficient one for POME treatment was found to be anaerobic granular sludge reactor i.e. UASB reactors. The major problems associated with UASB reactors are the long start-up period (2-4 months) and occasional loss of granulation and granules washout at hydraulic stresses, high and very low up-flow velocities. Therefore, modification of the UASB process is required to overcome the existing deficiencies as well as having high-performance methane production from POME. In this study, a modified up-flow anaerobic sludge fixed film (UASFF) bioreactor which is a combination of up-flow anaerobic sludge blanket (UASB) and up-flow fixed film (UFF) section in a single reactor is used.

POME is a high strength wastewater and would result in high organic load even at a low influent flow rate i.e. providing a low up-flow velocity. On the other hand, the up-flow velocity is a critical factor for granule formation in a high rate reactor like UASB reactors. These systems may require effluent recycle to increase the up-flow velocity and promote granulation. However, problems may arise due to the adverse impact of finely dispersed recycled effluent suspended solids (SS) on granule formation and sludge bed stability. In this case, a small settling tank may be provided after the anaerobic reactor to settle out the suspended solids prior to recycling the effluent to the reactor. Therefore, an external settling unit is applied in order to improve performance of the process.

1.8 Research Objectives

The present research has the following objectives:

1. To design, fabricate and perform the start-up of an up-flow anaerobic sludge fixed film (UASFF) reactor rig comprising of a modified UASFF reactor and

an external settling tank. The possibility of shortening the start-up period of the reactor by means of acceleration of the granular sludge formation for POME treatment will be explored.

2. To evaluate the performance of the UASFF reactor in the treatment of POME at wide range of organic loading rate (OLR) and study the interactive effects of hydraulic retention time (HRT) and influent feed concentration (COD_{in}) on the reactor performance.
3. To establish the kinetics of POME digestion reactions and determine the kinetic parameters of the process.
4. To examine biological activity of the granular sludge in batch experiments, including analysis of POME digestion, investigation of the effects of three process variables (COD_{in}, initial bicarbonate alkalinity and biomass concentration) and mass transfer study.
5. To study performance of different pretreatment approaches (physical (primary settling) and chemical (coagulation-flocculation process) methods for raw POME pretreatment.
6. To analyze, model and optimize anaerobic treatment process of physically and chemically pretreated POME in the UASFF bioreactor with respect to the simultaneous effects of two independent operating variables i.e. feed flow rate (Q_F) and up-flow velocity (V_{up}).
7. To evaluate structural and physical properties of the granular sludge developed in the UASFF reactor under different operational regimes of POME treatment.

1.9 Scope of Study

Application of a new design of up-flow anaerobic sludge fixed film (UASFF) bioreactor for the treatment of POME is the main focus of the present study. A lab-scale UASFF bioreactor (3.65 lit) was designed and fabricated to study its feasibility for POME treatment. After reactor start-up, the steady state performance was evaluated under different influent COD concentrations (5260-34725 mg/l) and HRT (1-6 days). In this part of study, in order to study the effect of influent COD concentration, the reactor was fed with a pre-settled POME of different dilutions. The results obtained were used for kinetic study, employing a suitable kinetic model derived from matemathical concepts governing the anaerobic process together with Monod and logistic's equations.

Biological activity of the granular sludge grown in the reactor was evaluated in batch experiment. A consecutive reactions kinetic model was employed to model changes in the process parameters in batch culture and reaction rate constants were determined. Interactive effects of three important process variables on the biological activity of the granular sludge in batch culture were also investigated using response surface methodology (RSM). The variables were the initial COD concentration (COD_{in}) (3000-10000 mg/l), initial bicarbonate alkalinity (BA) (200-2000 mg $CaCO_3$/l), and biomass concentration (2000-6000 mg/l). The substrate mass transfer into granules was also studied in a batch experiment by comparing specific methanogenic activity of disintegrated granules with that of intact granules.

Long term performance of the reactor was evaluated for raw POME treatment with an HRT of 3 days and influent COD of 44300 mg/l. The pretreatment processes studied were chemical pretreatment (coagulation-flocculation process) and physical pretreatment (settling process). The pretreatment processes were aimed at reducing suspended solids (SS) and oil and grease content in POME prior to anaerobic treatment. The anaerobic process treating pretreated POME was modeled using response surface methodology with two operating variables (feed flow rate and up-flow velocity) and twelve responses. The optimum operating conditions were obtained for the digestion of pretreated POME (chemically and physically). The role of the internal packing used as fixed film reactor in the middle part of the UASFF bioreactor was also studied for reactor operation with the chemically pretreated POME.

The reactor was operated at different temperatures (24, 38, 50 and 60 °C) under optimum operating conditions. The reactor stability was evaluated by COD, SS and oil and grease removals, methane yield and VFA/Alk ratio. Physical characteristics of the granular sludge was monitored throughout the study.

1.10 Organization of the Thesis

This thesis consists of six chapters. A brief introduction about the development of palm oil industry in Malaysia, the processes in palm oil mill, wastes generation in the palm oil mill, environmental regulations, sources of renewable energy in Malaysia and current POME treatment systems are given in Chapter 1 (Introduction). This chapter also includes problem statements that provide some basis and rationale to identify the research directions to be followed in this study. Then, the specific objectives of the present study are elaborated in detail together with the scopes of the study to be covered. The organization of the contents of this thesis is also given in the last section of this chapter.

Chapter 2 (Literature Review) discusses technical aspects of anaerobic digestion process, POME treatment methods and pretreatment processes that are related to the present study. Modeling of the anaerobic process using statistical method and kinetics of the process are also discussed in detail.

Chapter 3 (Materials and Methods) presents the detail of the materials and chemicals used in the present study. Then, the overall experimental flowchart is presented. Detail of the experimental set-up is then elaborated in this chapter. This followed by the detail experimental procedures, which include studies of the UASFF bioreactor performance, batch experiments and analytical techniques.

Chapter 4 (Results and discussion) which is the main part of this thesis is outlined by ten main studies. In first section, characteristics of POME is analyzed in detail followed by the second section that elaborates the performance of the UASFF bioreactor. Then, kinetic study of POME digestion in the reactor is discussed in the third section. In the following section, the biological activity of the granular sludge in batch experiments is analyzed. The performance of the reactor with raw POME is investigated in the fifth section. In section 6, the performance of the reactor when fed with POME pretreated with two pretreatment processes are discussed. Then, detail of the process modeling and optimization for digestion of the two pretreated POME in the UASFF reactor is elucidated. Detail information on the role of the internal packing, effect of temperature on the reactor performance and physical characteristics of the granular sludge are also studied and presented in last three sections of this chapter.

Chapter 5 (Conclusions) concludes the findings from the current studies. To avoid confusion, contents of this chapter are arranged according to the sequence of their appearance in Chapter 4.

Chapter 6 deals with recommendations for future studies in the related field made from the understanding and information generated in the present study. These recommendations are given due to their significance and importance to be further investigated and explored by future research work in this area.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

This chapter provides a brief review on the general concepts of anaerobic wastewater treatment processes. The review covers the mechanism of anaerobic digestion, various anaerobic treatment processes, factors affecting anaerobic process, an overview of various POME treatment processes and options to remove POME solids and oil & grease as pretreatment process. The design of experiment using response surface methodology which was applied in this research to model and optimize the process is also elaborated. Finally, a brief review on model development in anaerobic wastewater treatment processes will provide basic knowledge for the kinetic modeling addressed in this study.

2.1 Anaerobic Digestion

Biological treatment processes are cost effective processes that utilize microbial communities of varying degrees of diversity that interact in a multitude of ways to mediate a myriad of biological reactions (Wise, 1987, Jans and Man, 1988). Anaerobic digestion has been widely accepted as an effective alternative for wastewater treatment and simultaneous fuel gas production. Its successful application arises from the development of new and innovative reactor designs (Surampalli and Tyagi, 2004).

Compared to conventional aerobic methods of wastewater treatment, the anaerobic wastewater treatment concept indeed offers fundamental benefits such as low costs, energy production, relatively small space requirement of modern anaerobic wastewater treatment systems, very low sludge production (10-20 % of COD removed) with very high dewaterability, stabilized sludge and high tolerance to unfed conditions (Lettinga, 1995; Droste 1997; Metcalf and Eddy, 2003).

Previously, perceived drawbacks of anaerobic treatment systems such as high susceptibility of microbes (in particular methanogens) to a variety of xenobiotic compounds, low stability of the process and long start-up period, could be attributed to lack of knowledge of the basic principles of the process. As a matter of fact, the anaerobic digestion process is highly stable, provided the system is operated in the proper conditions. It may be needed that optimum operational conditions to be determined for each particular type of wastewater and more importantly, the process must be sufficiently understood by engineers and operators (Lettinga, 1995).

2.1.1 Microbiology and Biochemistry of Anaerobic Digestion

In anaerobic digestion, organic matters are degraded to methane and carbon dioxide in discrete steps by the concerted action of several different metabolite groups of microorganism. The main pathways of anaerobic digestion are shown in Figure 2.1 (Pavlostathis and Giraldo-Gomez, 1991). The salient features of those bacteria involved in the stabilization process are as follows:

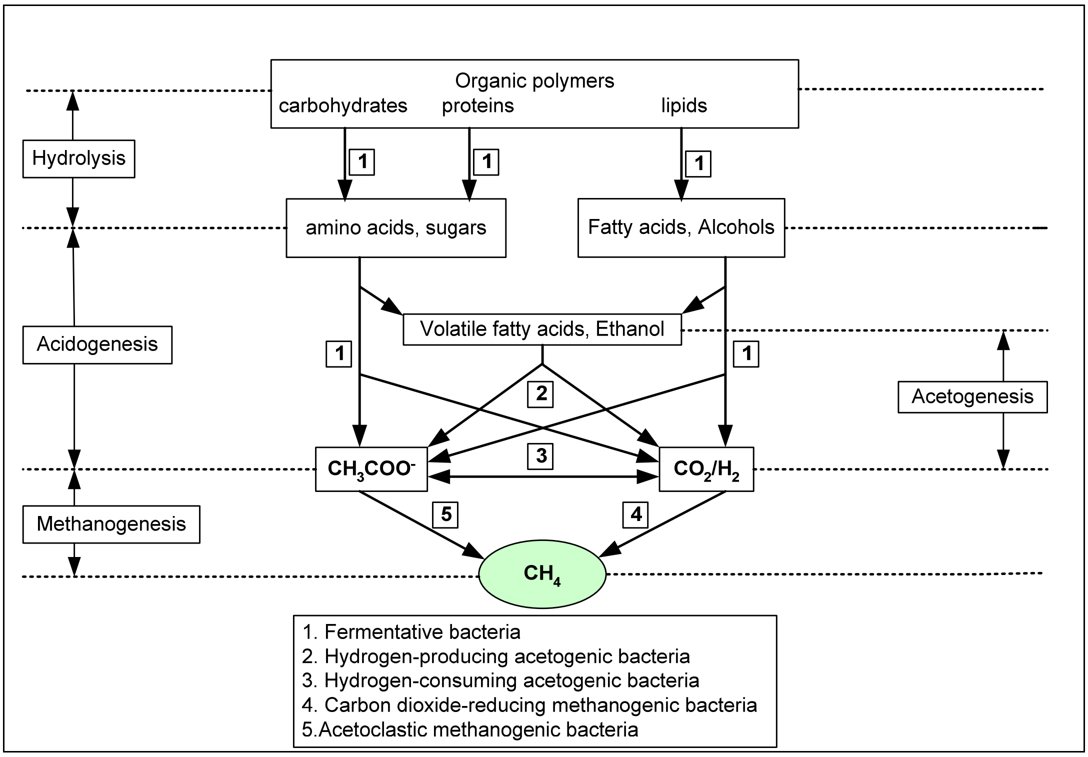


Figure 2.1. Anaerobic conversion of organic matter to methane, (Pavlostathis and Giraldo-Gomez, 1991).

2.1.1(a) Hydrolysis

The first step for most digestion process is hydrolysis during which, particulate matters are converted to soluble compounds that can be hydrolyzed further to simple monomers to be subsequently utilized by fermentative bacteria. The group of nonmethanogenic microorganisms responsible for the fermentation process consists of facultative and obligate anaerobic bacteria (Metcalf & Eddy, 2003). Extra cellular enzymes excreted by the fermentative bacteria catalyze the hydrolysis reactions. As no mineralization of organics is involved, this conversion results in no reduction in COD (Eckenfelder, 2000). Although most of biopolymers are readily degradable, the cellulose of highly lignified plant material (straw, wood, etc.) has been shown to be resistant to hydrolysis (Lynd *et al.*, 2002). The rate of hydrolysis is a function of factors such as pH, temperature, composition and particle size of the substrate (Veeken *et al.,* 2000, Paramsothy *et al.,* 2004). Volatile fatty acids production from the hydrolysis-acidification of the coffee pulp was investigated by Houbroun and his coworkers (2003) and 23 % (COD based) hydrolysis was achieved at an organic loading rate (OLR) of 5 g COD/l.d.

2.1.1(b) Acidogenesis

In the acidogenesis step, the hydrolysis products are absorbed by the cells of fermentative bacteria to be fermented or anaerobically converted into compounds such as alcohols, short-chain fatty acids, formic acid, carbon dioxide, hydrogen, ammonia and sulfide. The organic substrates serve as both the electron donors and acceptors. The final products of the metabolic activities of these bacteria depend upon the initial substrate (Figure 2.1) as well as the environmental conditions. As an example, consider the following reactions of glucose metabolism (Mosey, 1983).

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{2.1}$$

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_2COOH + 2H_2O \tag{2.2}$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \tag{2.3}$$

The first reaction is the most preferred. It produces acetic acid which is the major precursor of CH_4. The other two reactions occur when there is an accumulation of H_2 in the system. In Equation 2.2, there is a clear utilization of H_2 while in Equation 2.3, there is also hydrogen production but of lesser quantity (two molecules against four in the first reaction).The increase in the acid load of the system is also lower (one mole butyric acid against two moles acetic acid in the first reaction).

Many hydrolyzing microorganisms and acidogens can coexist in anaerobic methanogenic biofilms but little information is available on the characterization of the bacteria involved in the acidogenic phase (Zellner *et al.*, 1999; Bramucci and Nagarajan, 2000). Miyamoto (1997) reported that bacteria belonging to *Clostridium sp*. have been isolated from different types of anaerobic digesters but without specifying the effluent type treated. *Clostridium sp*. is responsible for most of the extra cellular lipase and protease produced, and convert the metabolites into acid products. These strict anaerobic microorganisms are rod-shaped, 2.8-3.0 mm long and 0.5-0.6 mm wide. The optimal growth temperature and pH vary between 35-37 ^{o}C and 4.5-7.0, respectively (Zigová *et al.*, 1999).

2.1.1(a)(i) Acetogens, Hydrogen-Producing Bacteria

Propionate and butyrate are thought to be converted to acetate only by syntrophic acetogens in concert with hydrogen-utilizing methanogens (Lowe *et al.*, 1993). *Syntrophobacter wolinii* was the first syntrophic propionate-degrading culture isolated from methanogenic enrichments from an anaerobic municipal sewage digester in association with hydrogen-utilizing bacteria (Lowe *et al.*, 1993). Propionate-oxidizing *Syntrophobacter*-like bacteria have been identified in microcolonies in intimate association with methanogens (De Bok *et al.*, 2004).

These bacteria are responsible for converting organic products of fermentative bacterial activity such as alcohols, propionic acid and butyric acid into acetic acid, CO_2 and H_2O as follows (Rittmann and McCarty, 2001):

$$CH_3CH_2OH + H_2O \longrightarrow CH_3COO^- + H^+ + 2H_2 \quad \Delta G^0 = +9.6kJ/mol \tag{2.4}$$

$$CH_3CH_2COO^- + H_2O \longrightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \quad \Delta G^0 = +76.1kJ/mol \tag{2.5}$$

$$CH_3CH_2CH_2COO^- + 2H_2O \longrightarrow 2CH_3COO^- + H^+ + 2H_2 \quad \Delta G^0 = +48.1kJ/mol \tag{2.6}$$

Acetate is the major intermediate in the bioconversion of organic matter to methane and carbon dioxide. About 70 % of the total methane produced in anaerobic digestion originates from acetate. Thus, the production of methane from acetate is an important step in the anaerobic digestion process (Rittmann and McCarty, 2001). A peculiar characteristic of these reactions is that they remain thermodynamically unfavorable (ΔG^0 =+ve) unless the H_2 produced is constantly removed from the system. The utilization of the hydrogen produced by the acidogens and other anaerobes by the methanogens is termed interspecies hydrogen transfer (Metcalf and Eddy, 2003).

2.1.1(b)(ii) Acetogens, Hydrogen-Utilizing Bacteria

The H_2-utilizing or homoacetogenic bacteria are a group of obligatory anaerobic bacteria that utilize the acetyl coenzyme A (CoA) pathway to synthesize acetate from C_1 precursors. These bacteria grow autotrophically on H_2 and CO_2 and/or heterotrophically on a variety of organic compounds, with mixotrophic growth on H_2 and a suitable organic substrate being observed in some species (Breznak and Kane, 1990; Wood and Ljungdahl, 1991). These bacteria also contribute towards the acetic acid pool in anaerobic digestion for subsequent conversion to methane. They are thermodynamically highly efficient because they do not produce H_2 and CO_2 during growth on multi-carbon compounds (Zeikus, 1981) including glucose, fructose, lactose, pyruvate, etc. The reaction is presented as follows:

$$4H_2 + 2CO_2 \longrightarrow CH_3COOH + 2H_2O \qquad (2.7)$$

2.1.1(c) Methanogenesis

Methanogenic bacteria (as obligate anaerobes) have a limited substrate spectrum which includes formate, alcohols (2-propanol/CO_2, 2-butanol/CO_2), methyl group compounds (methanol, methylamine), acetate and H_2 and CO_2. The conversion of these compounds to CH_4 can be represented as (Speece, 1985; MetCalf and Eddy, 2003):

$$4HCOOH + H_2O \longrightarrow CH_4 + 3HCO_3^- + 3H^+ \qquad (2.8)$$
$$\Delta G^0 = -36.1 kJ/mol$$

$$4CH_3OH \longrightarrow 3CH_4 + HCO3^- + H^+ + H_2O \qquad (2.9)$$
$$\Delta G^0 = -79.9 kJ/mol$$

$$4CH_3NH_3^+ + 3H_2O \longrightarrow 3CH_4 + HCO_3^- + 4NH_4^+ + H^+ \qquad (2.10)$$
$$\Delta G^0 = -57.4 kJ/mol$$

$$2(CH_3)_2NH_2^+ + 3H_2O \longrightarrow 3CH_4 + HCO_3^- + 2NH_4^+ + H^+ \qquad (2.11)$$
$$\Delta G^0 = -112.2 kJ/mol$$

$$4(CH_3)_3NH^+ + 9H_2O \longrightarrow 9CH_4 + 3HCO_3^- + 4NH_4^+ + 3H^+ \qquad (2.12)$$
$$\Delta G^0 = -170.8 kJ/mol$$

$$CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3^- \qquad (2.13)$$
$$\Delta G^0 = -37.0 kJ/mol$$

$$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + +3H_2O \qquad (2.14)$$
$$\Delta G^0 = -135.5 kJ/mol$$