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REVIEWS

Bioenergy from anaerobic degradation of lipids in palm oil mill effluent

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Abstract Fossil fuels are the lifeblood of our society and for many others around the world. The environmental pollution due to the use of fossil fuels as well as their gradual depletion make it necessary to find alternative energy and chemical sources that are environmentally friendly and renewable. Palm oil mill effluent (POME), a strong wastewater from palm oil mills, has been identified as a potential source to generate renewable bioenergies through anaerobic digestion. Thus, it has received considerable attention as feedstock for producing various value added products such as methane gas, bio-plastic, organic acids, bio-compost, activated carbon, and animal feedstock. Lipids are one of the major organic pollutants in POME. Furthermore, waste lipids are ideal potential substrates for biogas production, since theoretically more methane can be produced, when compared with proteins or carbohydrates. The objective of this review paper is to disscuss the microbial communities involved in the anaerobic degradation of long chain fatty acid and bioenergies and by-products from POME. With these options (Renewable and sustainable bioenergies) we can help phase out our dependency on fossil fuels and find clean, efficient, sources of power.

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Keywords Palm oil mill effluent (POME) · Long chain fatty acids (LCFAs) · Green house gas (GHG) · β-oxidation · Lipids

Abbreviations

POME	Palm oil mill effluent
LCFAs	Long chain fatty acids
GHG	Greenhouse gases
COD	Chemical oxygen demand
BOD	Biochemical oxygen demand
CO_2	Carbon dioxide
CH_3COO^-	Acetate
H_2	Hydrogen
STP	Standard temperature and pressure
CH_4	Methane
CSTR	Continuous stirred tank reactor
ABR	Anaerobic baffled reactor
UASB	Up flow anaerobic sludge blanket
EGSB	Expanded granular sludge blanket
DGGE	Denaturating gradient gel electrophoresis
UASFF	Up-flow anaerobic sludge fixed-film reactor
MABR	Modified anaerobic baffled reactor
FBR	Fluidized bed reactor
UV	Ultra-violet
PHA	Poly hydroxy alkanoate
PHB	Poly-β-hydroxy butyric acid
OLR	Organic loading rate
HRT	Hydraulic retention time
VFA	Volatile fatty acid

1 Introduction

Over the last 30 years, Malaysian palm oil industry has grown rapidly and at present it is one of the largest agro-based industries in the world (Wong et al. 2002; Wu et al. 2010). Palm oil mill effluent is considered as one of the most polluting agro-industrial effluent due to its high values of COD and BOD. Today, the percolation of palm oil mill effluents into the waterways and ecosystems, remain a fastidious concern towards the public health and food chain interference (Foo and Hameed 2010). This can cause considerable environmental problems if discharged without effective treatment by polluting land, water and destroying aquatic biota (Cheng et al. 2010; Singh et al. 2011). Therefore, palm oil mills are required to treat their POME prior to discharging it into streams and rivers. In the process of palm oil milling, POME is mainly generated from sterilization and clarification of palm oil in which a large amount of steam and hot water are used (Zinatizadeh et al. 2006; Rupani et al. 2010). POME is acidic (pH 4–5), has discharge temperature of 80–90°C/50–60°C and is non toxic (since no chemicals are added during extraction), (Ahmad et al. 2003). The characteristics and the parameter limits for POME discharge into watercourses in Malaysia are summarized in Table 1. It is rich in organic matter such as proteins, carbohydrates and lipids along with nitrogenous compounds and minerals (Agamuthu and Tan 1985; Habib et al. 1997; Wu et al. 2007). Therefore, it can be reused for biotechnological means. The various effluent treatment schemes, which are currently used by the Malaysian palm oil industry, are listed in descending order: (a) anaerobic/facultative ponds (Rahim and Raj 1982; Wong 1980; Chan and Chooi 1982), (b) tank digestion and mechanical aeration, (c) tank digestion and facultative ponds, (d) decanter and facultative ponds, (e) physico-chemical and biological treatment (Andreasen 1982), and (f) evaporation (Ma 1999a, b) and a clarification coupled with filtration and aeration (UNEP 1994).

In an anaerobic process, the POME is degraded into methane, carbon dioxide and water, and the sequence of reactions included; hydrolysis, acidogenesis (including acetogenesis) and methanogenesis (Bitton 2005; Nwuche and Ugoji 2008; Nwuche and Ugoji 2010). In the process of hydrolysis the complex molecules (i.e. carbohydrates, lipids and proteins) are hydrolyzed into sugars, amino acids and fatty acids etc. by extra cellular enzymes of fermentative bacteria. In acidogenesis, these sugars, fatty acids and amino acids are converted into organic acids by means of acidogenic bacteria. These are further converted to acetate together with CO_2 and hydrogen by acetogens. Finally the hydrogen is utilized by hydrogenotrophic methanogens while acetic acid and CO_2 are utilized by acetoclastic methanogens to methane as a final product. Thus, anaerobic decomposition of organic matter involves the concerted action of several different metabolic groups of microorganisms (Demirel and Scherer 2008; Weiland 2010) to produce biogas that can be used to generate electricity and save fossil energy (Wong et al. 2009).

The compositions and concentrations of proteins, nitrogenous compounds, lipids and minerals found to be present in POME (Habib et al. 1997) are summarized in Table 2. Lipids are one of the major organic pollutants in POME. These compounds are glycerol, bonded to LCFAs, alcohols, and other groups by an ester or ether linkage. During anaerobic treatment, lipids are hydrolyzed into glycerol plus LCFAs by hydrolytic extracellular lipases. Glycerol is further degraded via acidogenesis while $LCFA_S$ are degraded through β-oxidation process (syntrophic acetogenesis) to acetate, H_2 and CO_2 (Jeris and McCarty 1965; Weng and Jeris 1976) and finally converted to CH_4 or CO_2 by methanogenesis (Komatsu et al. 1991; Stryer 1995). Lipid hydrolysis is generally faster than protein or carbohydrate hydrolysis and considered to be a rapid process in anaerobic digestion while subsequent β-oxidation proceeds rather slowly (Pavlostathis and Geraldogomez 1991; Hanaki et al. 1981; Angelidaki and Ahring 1995). After hydrolysis, $LCFA_S$ glycerol undergoes fermentation or acidogenesis. Since LCFA requires an external electron acceptor for oxidation, therefore, its degradation occurs during acetogenesis while mainly saturation or hydrogenation of unsaturated LCFAs takes place in this process. Mainly glycerol is also degraded to acetate, lactate and 1,3-propanediol (Batstone 2000). Proton-reducing acetogens degrade LCFAs in syntrophic association with hydrogen-utilizing methanogens and acetoclastic methanogens (Schink 1997).

Syntrophism is a special case of mutualistic interrelationship between two different microorganisms which together degrade some substances (and conserve energy doing it) which neither could do separately. Thus, this term was coined to describe the close

Table 1 Characteristics and parameter limits for POME discharge into water courses in Malaysia

Characteristics of POME			Parameter limits for watercourse discharge for POME	Major constituents of POME	Quantity (g/g dry sample)
Parameter[a]	Mean	Range	Limits of discharge[a]	Moisture	6.75
pH	4.2	3.4–5.2	5.0–9.0	Crude protein	9.07
Temperature	–	80–90	45	Crude lipid	13.21
Biological Oxygen Demand 3-days 30°C[b]	25,000	10,250–43,750	100	Ash	32.12
Chemical Oxygen Demand	51,000	15,000–1,00,000	–	Carbohydrate	20.55
Total solids	40,000	11,500–79,000	–	Nitrogen-free extract	19.47
Suspended Solids	18,000	5,000–54,000	400	Total carotene	20.07
Volatile Solids	34,000	9,000–72,000	–		
Oil and Grease	6,000	130–18,000	50		
Ammoniacal Nitrogen	35	4–80	150[c]		
Total Nitrogen	750	180–1400	200[c]		
Source: (Ma 1999a, b, 2000)			*Source*: EQA 1974 (Act 127) and Subsidiary Legislation 2002	*Source*: Habib et al. (1997)	

[a] Units in mg/l except pH and Temperature (°C)

[b] The sample for BOD analysis is incubated at 30°C for 3 days

[c] Value of filtered sample

cooperation of fatty acid-oxidizing, fermenting bacteria with hydrogen oxidizing methanogens (McInery et al. 1979) or of phototrophic green sulphur bacteria with chemotropic sulphur-reducing bacteria (Biebl and Pfening 1978). In most cases of syntrophism the nature of a syntrophic reaction involves H_2 gas being produced by one partner and being consumed by the other.

Oxidation of butyric acid to acetic acid and H_2 by the fatty acid-oxidizing Syntrophomonas is a good example of mutual dependence. Syntrophomonas does not grow in a pure culture on butyric acid as the energy released during butyric acid oxidation to acetic acid is highly unfavorable to the bacterium. But, if the hydrogen produced in the reaction is immediately utilized by a syntrophic partner (e.g. methanogen), Syntrophomonas grows luxuriantly in mixed-culture with the H_2 consumer.

$$\text{Butyrate}^- + 2H_2O \rightarrow 2\text{Acetate}^- + H^+ + 2H_2 + \text{Energy}\ (+48.2\ \text{Kj})$$

Wastes or wastewaters with a high lipid-content present an attractive source for methane production because theoretically it has been found that 1.01 L of methane at STP can be produced from 1 g of oleate (unsaturated LCFA, C18:1), whereas only 0.37 L can be produced from 1 g of glucose (Kim et al. 2004). Thus, degradation of lipids produces more biogas with higher methane content as compared to proteins or carbohydrates (Table 3).

This paper will provide a brief overview of the biochemistry, microbiology of anaerobic breakdown of polymeric materials especially of lipids to methane and the roles of the various microorganisms involved along with a brief description of bioenergies and biochemical from POME.

2 Food web of lipid anaerobic process

Anaerobic bioconversion of complex organic matter to methane consists of a series of microbiological processes. As shown in the Fig. 1, first step involves the hydrolysis of complex organic polymers (proteins, carbohydrates and lipids) into monomers by extracellular enzymes that are excreted by fermentative bacteria. Proteins are degraded to amino acids, carbohydrates to soluble sugars and lipids to glycerol and LCFAs by the concerted action of proteases, cellulases and lipases respectively. Some researchers suggested

Table 2 Fatty acids, amino acids and mineral contents of raw POME (*Source*: Habib et al. 1997)

Fatty acids (g/100 g lipid)	Quantity (g)	Chemical formula of fatty acids	Amino acids (g/100 g protein)	Chemical formula	Quantity (g)	Minerals	Quantity (mm/g dry sample)
Capric acid (10:0)	4.29	$CH_3(CH_2)_8COOH$	Aspartic acid	$HOOC–CH_2–CH(NH_2)–COOH$	9.66	Fe	1,311.25
Lauric acid (12:0)	9.22	$CH_3(CH_2)_{10}COOH$	Glutamic acid	$HOOC–(CH_2)_2–CH(NH_2)–COOH$	10.88	Zn	17.58
Myristic acid (14:0)	12.66	$CH_3(CH_2)_{12}COOH$	Serine	$HO–CH_2–CH(NH_2)–COOH$	6.86	P	14,524.52
Palmitic acid (16:0)	14.45	$CH_3(CH_2)_{14}COOH$	Glycine	$NH_2–CH_2–COOH$	9.43	Na	102.97
Heptadecanoic acid (17:0)	1.39	$CH_3(CH_2)_{15}COOH$	Histidine	$NH–CH=N–CH=C–CH_2–CH(NH_2)–COOH$	1.43	Mg	132.94
10-heptadecanoic acid (17:01)	1.12	$CH_3(CH_2)_8CH=CH(CH_2)_5COOH$	Arginine	$HN=C(NH_2)–NH–(CH2)_3–CH(NH_2)–COOH$	4.15	Mn	27.92
Stearic acid (18:0)	11.41	$CH_3(CH_2)_{16}COOH$	Threonine	$CH_3(OH)CH(NH_2)–CHCOOH$	2.58	Ca	1,600.59
Oleic acid (18:1n-9)	8.54	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Alanine	$CH_3–CH(NH_2)–COOH$	7.70	K	1,951.55
Lenoleic acid (18:2n-6)	4.72	$CH_3CH_2(CH=CH.CH_2)_3(CH_2)_6COOH$	Proline	$NH–(CH_2)_3–CH–COOH$	4.57	Co	0.25
Linolenic acid (18:3n-3)	4.72	$CH_3CH_2(CH=CH.CH_2)_3(CH_2)_6COOH$	Tyrosine	$HO–Ph–CH_2–CH(NH_2)–COOH$	3.26	Cr	4.02
Arachidic acid (20:0)	7.56	$CH_3(CH_2)_{18}COOH$	Phenylalanine	$Ph–CH_2–CH(NH_2)–COOH$	3.20	Cu	11.08
Eicosatrienoic acid (20:3n-6)	1.49	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_8COOH$	Valine	$(CH_3)_2–CH–CH(NH_2)–COOH$	3.56	Ni	2.46
Arachidonic acid (20:4n-6)	1.12	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	Methionine	$CH_3–S–(CH_2)_2–CH(NH_2)–COOH$	6.88	S	14.50
Eicosapentaeonic acid (20:5n-3)	0.36	$CH_3CH_2(CH=CHCH_2)_5(CH_2)_2COOH$	Cystine	$HS–CH_2–CH(NH_2)–COOH$	3.37	Se	12.32
Behenic acid (22:0)	2.62	$CH_3(CH_2)_{20}COOH$	Isoleucine	$CH_3–CH_2–CH(CH_3)–CH(NH_2)–COOH$	4.53	Si	9.62
			Leucine	$(CH_3)_2–CH–CH_2–CH(NH_2)–COOH$	6.86	Al	447.01
			Lysine	$H_2N–(CH_2)_4–CH(NH_2)–COOH$	5.66	B	15.77
			Tryptophan	**Ph**–NH–CH=**C**–CH_2–$CH(NH_2)$–COOH	1.26	Mo	6.07
						Sn	33.80
						V	3.08
						As	6.09
						Li	1.44
						Pb	5.15
						Cd	0.44

Table 3 Potential biogas production from different classes of substrates

Component	Methanogenic reaction	Biogas (lg^{-1})	CH_4 (%)
Lipids	$C_{50}H_{90}O_6 + 24.5\ H_2O \rightarrow 34.75\ CH_4 + 15.25\ CO_2$	1.425	69.5
Carbohydrates	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$	0.830	50.0
Proteins	$C_{16}H_{24}O_5N_4 + 14.5\ H_2O \rightarrow 8.25\ CH_4 + 3.75\ CO_2 + 4NH_4^+ + 4HCO_3^-$	0.921	68.8

Source: Alves et al. (2009)

that lipid hydrolysis is generally faster than protein or carbohydrate hydrolysis, and considered to be a rapid process in anaerobic digestion (Pavlostathis and Geraldogomez 1991; Hanaki et al. 1981; Angelidaki and Ahring 1995), while some other researchers reported lipid hydrolysis as a rate limiting step (Beccari et al. 1996). According to some other researchers lipid hydrolysis was not usually the rate limiting step, the overall conversion rate was limited either by degradation of LCFAs or by the physical processes of dissolution and mass transfer of these acids (Rinzema 1993; Hanaki et al. 1987). The apparent discrepancy between the results reported by different researchers may be attributed to differences in the feed concentration and physico-chemical conditions in their experiments.

After hydrolysis, LCFAs and glycerol undergo fermentation or acidogenesis. Although hydrolysis of lipids to glycerol and LCFAs occurs rapidly, subsequent LCFAs degradation via β-oxidation proceeds rather slowly (Pavlostathis and Geraldogomez 1991). Since LCFAs oxidation requires an external electron acceptor, therefore, undergo degradation during acetogenesis while unsaturated LCFAs get saturated in this process and glycerol degraded to acetate, lactate and 1,3 propandiol (Batstone 2000). Weng and Jeris (1976) suggested that degradation of unsaturated LCFAs starts with chain saturation, whereas other authors provide evidence that the direct β-oxidation of unsaturated LCFAs is feasible (Lalman and Bagley 2001; Lalman and Bagley 2000; Roy et al. 1986). However, it is still unknown whether saturated and unsaturated LCFAs are degraded by the same bacteria in methanogenic environments.

During syntrophic acetogenesis the products of acidogenesis are converted into methanogenic substrates i.e. acetate, hydrogen and carbondioxide for methanogenesis. The formation of methanogenic subsrate with H_2 production is thermodynamically unfavourable unless the partial pressure of H_2 in the medium is kept low by H_2 consuming bacteria such as

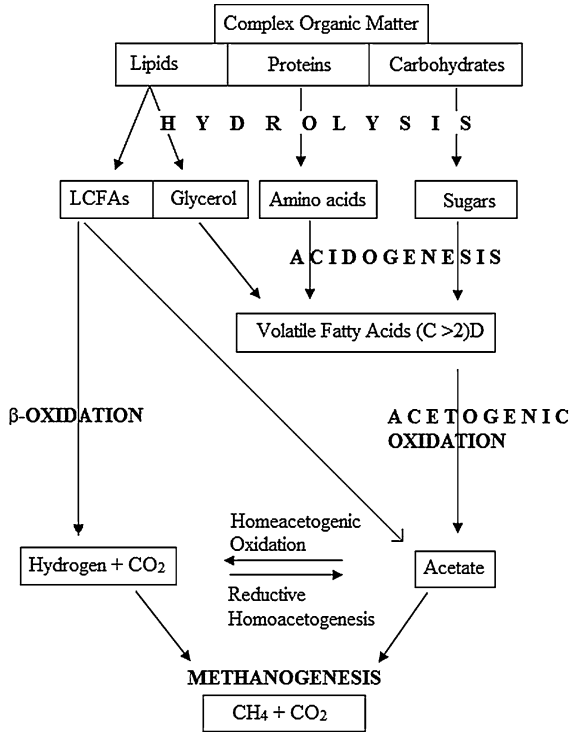


Fig. 1 Food web of methanogenic anaerobic digestion

methanogens (McInerney et al. 1981). Thus, LCFA oxidation to acetate is possible only when the hydrogen partial pressure in the medium is kept low and, therefore, cooperation with hydrogen-consuming microorganisms is necessary (Table 4). For this oxidation, the partial pressure of hydrogen has to be decreased substantially to lower values (<10 Pa) than the ethanol because degradation of fatty acids to CH_3COO^- and H_2, or in the case of propionate, to CH_3COO^-, H_2 or CO_2 is far more endergonic under standard conditions than ethanol oxidation (Table 5). Because of the diverse number of organisms involved in these reactions and their ability to perform other types of metabolisms such as fermentation or sulfate reduction (Schink 1997; McInerney 1992), the

Table 4 Gibbs free energy changes at 25°C for the (possible) reactions involved in syntrophic conversion of oleate and palmitate during methanogenic decomposition (*source*: Sousa et al. 2007a, b, c)

Reaction No.	Process	Reaction	$\Delta G^{0'}$ (KJ reaction^{-1})[b]	$\Delta G^{0'}$ (KJ reaction^{-1})[c]
1	Oleate degradation	$C_{18}H_{33}O_2^{2-} + 16H_2O \rightarrow 9C_2H_3O^{2-} + 15H_2 + 8H^+$	+391	−131
2	Palmitate degradation	$C_{16}H_{31}O_2^{2-} + 14H_2O \rightarrow 8C_2H_3O_2^{2-} + 14H_2 + 7H^+$	+419	−81
3	Hydrogenotrophic methanogens	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−136	−20
4	Acetoclastic methanogens	$C_2H_3O_2^- + H_2O \rightarrow HCO_3^- + CH_4$	−31	−19

Data were obtained or calculated from data reported by Thauer et al. (1977) and Lalman (2000)

[a] Gibbs free energies (at 25°C) calculated under standard conditions (solute concentrations of 1 M and gas partial pressure of 10^5 Pa)

[b] Gibbs free energies (at 25°C) for oleate/palmitate concentration of 1 mM, acetate concentration of 10 mM, and H_2 partial pressure of 1 Pa

organisms that participate at this second step will be called syntrophic metabolizers. Table 5 summarizes some reactions involved in syntrophic metabolism. Normally all LCFAs and ethanol require syntrophic acetogenesis along with two pathways: (1) β-oxidation pathway (as the main pathway) and (2) $\tilde{\omega}$-oxidation pathway by carboxylation of far methyl carbon.

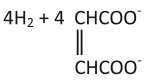
The final step involves two different groups of methanogens, the hydrogenotrophic methanogens which use the H_2 and formate produced by other bacteria to reduce CO_2 to CH_4 and the acetotrophic methanogens which metabolize acetate to CO_2 and CH_4.

3 Microbiology biology of fatty acid degradation

Anaerobic degradation of LCFAs requires syntrophic communities of acetogenic bacteria, performing fatty acid β-oxidation and methanogenic archaea, which consume hydrogen and acetate to low concentrations (Schink 1997). In this syntrophic cooperation, interspecies hydrogen transfer plays a key role. Presently, about 14 species have been identified that are capable of degrading fatty acids in syntrophy with methanogens, all belonging to the families *Syntrophomonadaceae* within the group of low G + C-containing Gram-positive bacteria bacteria (McInerney 1992; Zhao et al. 1993; Wu et al. 2006; Sousa et al. 2007a) and *Syntrophaceae* in the subclass of the *Deltaproteobacteria* (Jackson et al. 1999). Of the 14 species identified so far, only seven are found capable of utilizing LCFAs with more than 12 carbon atoms, including *Syntrophomonas sapovorans* (Roy et al. 1986), *Syntrophomonas saponavida* (Lorowitz et al. 1989), *Syntrophomonas curvata* (Zhang et al. 2004), *Syntrophomonas zehnderi* (Sousa et al. 2007a), *Syntrophomonas palmitatica* (Hatamoto et al. 2007a), *Thermosyntropha lipolytica* (Svetlitshnyi et al. 1996) and *Syntrophus aciditrophicus* (Jackson et al. 1999). Among these microorganisms only four species have the capability of utilizing mono- and/or polyunsaturated LCFAs (with more than 12 carbon atoms): *S. Sapovorans* (Roy et al. 1986), *S. curvata* (Zhang et al. 2004), *T. lipolytica* (Svetlitshnyi et al. 1996) and the recently isolated *S. zehnderi* (Sousa et al. 2007a) (Table 6). The principle pathway of LCFAs degradation is via β-oxidation, but the initial steps involve in the conversion of unsaturated LCFAs are not yet fully understood. According to Weng and Jeris (1976) the degradation of LCFAs starts with the chain saturation, whereas other researchers provide evidence for direct β-oxidation of unsaturated LCFAs (Roy et al. 1986; Lalman and Bagley 2000, 2001). Generally, communities enriched on unsaturated LCFA also degrade saturated fatty acids, but the opposite does not always seem to be the case.

In the last 20 years several biochemical and molecular methods have been employed to characterize the microbial communities involved in anaerobic degradation especially in anaerobic reactors such as CSTR, ABR, UASBR and EGSBR. The microbial diversity of anaerobic communities that are responsible for degrading LCFAs has not yet been studied extensively. But when the molecular diversity of granular and suspended sludge was assessed during a long term operation of two anaerobic up-flow bioreactors fed with the increasing concentrations of oleate, a physical segregation of the sludge in two fractions

Table 5 Reactions involved in syntrophic metabolism

Reaction input	Reaction output	$\Delta G^{0'}$ [kJ per reaction]
Hydrogen releasing reactions		
Ethanol and lactate		
$CH_3CH_2OH + H_2O$	$CH_3COO^- + H^+ + 2H_2$	+9.6
$CH_3CH(OH)COO^- + 2H_2O$	$CH_3COO^- + HCO_3^- + H^+ + 2H_2$	−4.2
Fatty acids		
$CH_3COO^- + 4H_2O$	$2HCO_3^- + H^+ + 4H_2$	+104.6
$CH_3CH_2COO^- + 3H_2O$	$CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
$CH_3CH_2CH_2COO^- + 2H_2O$	$2CH_3COO^- + H^+ + 2H_2$	+48.3
$CH_3(CH_2)_4COO^- + 3H_2O$	$3CH_3COO^- + 2H^+ + 4H_2$	+96.2
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O$	$3CH_3COO^- + 2H^+ + H_2$	+25.2
Glycolic acid		
$HOCH_2COO^- + H^+ + H_2O$	$2CO_2 + 3H_2$	+19.3
Aromatic compounds		
$C_6H_5COO^- + 7H_2O$	$3CH_3COO^- + HCO_3^- + 3H^+ + 3H_2$	+58.9
$C_6H_5OH + 5H_2O$	$3CH_3COO^- + 3H^+ + 2H_2$	+5.5
$C_6H_4(OH)COO^- + 6H_2O$	$3CH_3COO^- + HCO_3^- + 3H^+ + 2H_2$	+5.2
Amino acids		
$CH_3CH(NH_2)COOH + 3H_2O$	$CH_3COO^- + HCO_3^- + NH_4^+ + H^+ + 2H_2$	+ 7.5
$HOOCCH(NH_2)CHCOO^- + 4H_2O$	$CH_3COO^- + 2HCO_3^- + NH_4^+ + H^+ + 2H_2$	−14.0
$(CH3)_2CHCH_2CH(NH_2)COOH + 3H_2O$	$(CH_3)_2CHCH_2COO^- + HCO_3^- + NH_4^+ + H^+ + 2H_2$	+4.2
$HOOCCH_2CH_2CH(NH_2)COOH + 4H_2O$	$CH_3CH_2COO^- + 2HCO_3^- + NH_4^+ + H^+ + 2H_2$	−5.8
$HOOCCH_2CH_2CH(NH_2)COOH + 7H_2O$	$CH_3COO^- + 3HCO_3^- + 3H^+ + 5H_2$	+70.3
Hydrogen-consuming reactions		
$4H_2 + 2HCO_3^- + H^+$	$CH_3COO^- + 4H_2O$	−104.6
$4H_2 + HCO_3^- + H^+$	$CH_4 + 3H_2O$	−135.6
$4H_2 + 4S^o$	$4HS^- + 4H^+$	−112
$4H_2 + SO_4^{2-} + H^+$	$HS + 4H_2O$	−151.9
$H_2 + H_2C(NH_3^+)COO^-$	$CH_3COO^- + NH4^+$	−78.0
$4H_2 + 4$ 	4 	−344.6
$4H_2 + NO_3^- + 2H^+$	$NH_4^+ + 3H_2O$	−599.6

[a] All the calculations are based on published tables (Thauer et al. 1977; Kaiser and Hanselmann 1982; Dimroth 1983)

was observed. At the top there was a whitish floating fraction and a settled fraction at the bottom (Pereira et al. 2002). This segregation was due to the floatation caused by LCFAs accumulation. Differences between the structures of microbial communities of these two fractions were assessed by comparison of DGGE profiles. The values attained by the similarity indices between bottom and top sludge fractions for the granular and suspended sludge were as low as 56.7 and 29.4% respectively, while at the end of the operation the similarity indices between the original and suspended sludge, and the respective top sludge fractions were 17.3 and 15.2%.

Current developments in biological science using molecular approaches have widened the scope for studies on quantification and identification of the natural ecology of acetogenic bacteria (Hensen et al. 1999; Zhao et al. 1993). Hensen et al. (1999) used new oligonucleotide probes to characterize the phylogenic groups of mesophilic members of the family Syntrophomonadaceae as well as their syntrophic relationship with the hydrogenotrophic methanogens.

Table 6 Characteristics of some syntrophic LCFA-degrading bacteria (*Source*: Alves et al. 2009)

LCFA-degrading bacteria	Morphological characteristics	LCFA utilization range
Syntrophomonas sapovorans[a]	Short curved rods (0.5 × 2.5 μm) Slightly motile Gram-negative Two to four flagella Non-spore forming	Degrades linear saturated fatty acids with 4–18 carbon atoms in co-culture with *Methanospirillum hungatei*. Mono- and di-unsaturated LCFA, such as oleate (C18:1) and linoleate (C18:2), are also oxidized by the co- culture
Syntrophomonas curvata[b]	Slightly curved rods (0.5–0.7 × 2.3–4.0 μm) Motile Gram-negative One or three flagella inserted in both poles Non-spore forming	Degrades linear saturated fatty acids with 4–18 carbon atoms in co-culture with *M. hungatei*. Oleate (C18:1) is also oxidized by the co-culture
Syntrophus aciditrophicus[c]	Rod-shaped cells (0.5–0.7 × 1.0–1.6 μm) Non-motile Gram-negative Non-spore forming	Degrades linear saturated fatty acids with more than four carbon atoms (C4:0 to C8:0, C16:0, C18:0) in co-culture with H2-utilizing*Desulfovibrio* sp. or *Methanospirillumhungatei*
Syntrophomonas zehnderi[d]	Curved rods (approximately 0.4–0.7 × 2.0–4.0 μm) Variable response to Gram staining Slight twitching Motility Spore formation during growth on oleate in co-culture with a methanogen that utilizes hydrogen and formate	Degrades oleate, a mono-unsaturated fatty acid, and straight-chain fatty acids C4:0–C18:0 in syntrophic association with *Methanobacterium formicicum*

[a] Roy et al. (1986)

[b] Zhang et al. (2004)

[c] Jackson et al. (1999)

[d] Sousa et al. (2007a)

Shigematsu et al. (2006) used a 16S rRNA gene approach to study the microbial communities present in a chemostat fed with a mixture of oleic and palmitic acids. However, members belonging to *Syntrophomonadaceae* were detected in the chemostat, but the most predominant microorganisms identified, belonged to the *Bacteroidetes* and *Spirochaetes* phyla and it was suggested by authors that these members could also play a very important role in LCFAs degradation. Microbial diversity of anaerobic sludge after extended contact with long chain fatty acids (LCFAs) was studied, using molecular approaches. Sousa et al. (2007b, c) studied the diversity and dynamics of biomass in reactors treating saturated (palmitate, C16:0) and unsaturated (oleate, C18:1) LCFAs, using 16S ribosomal RNA genetargeted molecular techniques. During this study, it was found that the bacterial communities were dominated by the members of *Clostridiaceae* and *Syntrophomonadaceae* families (Sousa et al. 2007b). A new obligatory syntrophic bacterium, Syntrophomonas zehnderi, was isolated from an oleate-degrading culture and its presence in oleate-degrading sludges detected by 16S rRNA gene cloning and sequencing. Futhermore, presence of LCFA and short chain fatty acid degrading bacteria indicate that the formation of short chain fatty acids takes place by the degradation of LCFAs. However, a significant part of the retrieved bacterial 16S rRNA gene sequences (53%) were most similar to those of yet uncultured microorganisms, with the majority assigned to the phylum *Firmicutes*. Members of *Proteobacteria* and *Bacteroidetes* were also found in the culture.

Furthermore, it has been found that palmitate can be used by an oleate enrichment culture with no changes in the microbial community, while oleate cannot be used by the palmitate enrichment culture under the same condition; it suggests that palmitate is a key intermediate in oleate degradation. Communities enriched on unsaturated LCFAs also degrade saturated LCFAs, but the vice versa doesn't occur in this case. The most likely biochemical degradation of unsaturated fatty acids starts with chain saturation (hydrogenation) followed by β-oxidation. Instead of Obligate hydrogen-producing acetogens (Schink 1997) that degrade unsaturated LCFAs, there is also the existence of microbial communities capable of hydrogenating unsaturated LCFAs to saturated LCFAs (Maia et al. 2007; Paillard et al. 2007). Stable isotope probing with 13C palmitate as a substrate was used by Hatamoto et al. (2007b, c) to find out the microorganisms directly involved in palmitate degradation. Members of the phyla *Bacteroidetes* and *Spirochaetes*, and the family *Syntrophaceae* within the *Deltaproteobacteria*, and members of the family *Syntrophomonadaceae* and genus *Clostridium* within the *Firmicutes* were found in clone libraries from heavy rRNA fractions. Thus, from these results it is evident that under methanogenic condition phylogenitically different groups of bacteria were active in situ in LCFA degradation. Anaerobic bacteria involved in the degradation of LCFAs in the presence of sulfate as electron acceptor, were studied by combined cultivation-dependent and molecular techniques (Sousa et al. 2009). Phylogenetic affiliation of rRNA gene sequences corresponding to predominant DGGE bands demonstrated that members of the *Syntrophomonadaceae*, together with sulfate reducers, mainly belonging to the *Desulfovibrionales* and *Syntrophobacteraceae* groups, were present in the sulfate-reducing enrichment cultures. The results of this study indicate that hydrogen consumption by methanogens is taken over by hydrogen-consuming sulfate reducers, which are known to have a higher affinity for hydrogen than methanogens.

4 Production of bioenergy and biochemicals from POME

Anthropogenic release of greenhouse gases, especially CO_2 and CH_4 has been recognized as one of the main causes of global warming. In Malaysia, the palm oil industry, particularly POME anaerobic treatment, has been identified as an important source of CH_4. Due to increasing awareness of the risk of the environmental pollution and emission of green house gases (GHG), treatment of POME using biological processes in close digesters (UASBR) has gained popularity in the recent years, with over 500 installations worldwide (Latif et al. 2011a, b). POME is an ideal substrate for bioprocessing because it contains high level of degradable organic material which results in a net positive energy or economic balance. During the anaerobic treatment of POME, methane (Yacob et al. 2005, 2006a, b) and hydrogen (O-Thong et al. 2007) are generated, which can reduce the demand on energy resources and dependence on fossil fuels (O-Thong et al. 2008a).

5 Biological methane production

Methane has been categorized as one of the GHG with its global warming potential, 21 times more potent than CO_2 (Ishigaki et al. 2005; Latif et al. 2011a, b). Methane fermentation offers an effective means of pollution reduction, superior to that achieved via conventional aerobic processes. Although practiced for decades, interest in anaerobic fermentation has only recently focused on its use in the economic recovery of fuel gas from industrial and agricultural surpluses. The anaerobic processes has considerable advantages over aerobic active sludge system such as (a) less energy demand (b) minimal sludge formation (c) minimization of unpleasant odour (d) efficient break down of organic substances by anaerobic bacteria to methane. In anaerobic digestion of POME, methanogenesis is the rate limiting step. Since anaerobic conventional digesters require large reactor and long retention time, therefore, high-rate anaerobic reactors have been proposed for POME treatment such as UASBR (Borja and Banks 1994a); upflow anaerobic filtration (Borja and Banks 1994b); fluidized bed reactor (Borja and Banks 1995a, b); and up-flow anaerobic sludge fixed-film reactor (Najafpour et al. 2006); anaerobic contact digester (Ibrahim et al. 1984) and continuous stirred tank reactor (Chin 1981), to reduce the reactor volume, shorten retention time along with the capture of methane gas. Table 7 lists the performance of various anaerobic treatment methods of POME.

Anaerobic treatment of palm oil mill effluent, practiced in Malaysia and Indonesia, results in domination of *Methanosaeta concilii*. It plays an important role in methane production from acetate and the optimum condition for its growth should be considered to harvest biogas as renewable fuel (Tabatabaei et al. 2009). Effective treatment of POME in UASBR is due to its ability to treat wastewater with high suspended solid contents (Fang and Chui 1994; Kalyunzai et al. 1998) that may clog reactors with packing material and also provide higher methane production (Kalyunzai et al. 1996; Stronach et al. 1978). However, this reactor might face long-startup periods if the seeded sludge is not granulated and this problem can be overcome by seeding the reactor with granulated sludge, resulting high performance along with shorter start-up period (Latif et al. 2011a, b).

Thus, anaerobic digestion process offers great potential for rapid disintegration of organic matter to produce methane that can be used to generate electricity (reaction 3) by burning it in a gas turbine or steam boiler (Linke 2006). Recovery of methane gas, from anaerobic treatment of POME as a renewable energy, represents a more acceptable alternative under the Kyoto Protocol with the objective of reducing the GHG emissions. Methane in the form of compressed natural gas is used as a vehicle fuel, and is claimed to be more environment friendly than other fossil fuels such as gasoline/petrol and diesel (reaction 2). In terms of energy it is comparable to commercially available gas fuels as shown in Table 8.

Research is being conducted by NASA on methane's potential as a rocket fuel (*Lunar Engines*, Aviation Week & Space Technology 2009). One advantage of methane is that it is abundant in many parts of the solar system and it could potentially be harvested in situ (i.e. on the surface of another solar-system body), providing fuel for a return journey (Barry 2007). The potential energy that could be generated from 1 m^3 of biogas is 1.8 k Wh (Ma 1999a, b).

Recently methane emitted from coal mines has also been successfully converted to electricity and other useful products such as methanol for the use in the production of biodiesel e.g. by production of syngas (reaction 1 and 2) in downstream chemical processes (reaction 3). Syngas consists of H_2 and carbon monoxide (CO) and very often some CO_2, and has less than half the energy density of natural gas.

$$CH_4 + H_2O \rightleftarrows CO + 3H_2 \quad (1)$$

$$CO + H_2O \rightleftarrows CO_2 + H_2 \quad (2)$$

$$CH_4(g) + 2O_2(g) \rightleftarrows CO_2(g) + 2H_2O(l) + \text{electricity} \quad (3)$$

6 Biological hydrogen production

Biohydrogen is a promising energy carrier of the future: It is a promising clean fuel as it is ultimately derived from renewable energy sources, environment friendly (Ntaikou et al. 2010), since it burns to water (Guo et al. 2010; Mohammadi et al. 2011), gives high energy yield (142 MJ/kg), and can be produced by less energy-intensive processes (Nielsen et al. 2001; Das and Veziroglu 2001) and has a great potential to be an alternative fuel. Cellulose and starch containing biomass can be used as a reliable and renewable raw material for hydrogen gas production. Due to the nature of POME, with high cellulose and lignocellulosic material, it takes a long time to degrade the organic substances and has a potential as a substrate for generation of hydrogen (Fakhru'l-Razi et al. 2005; Vijayaraghavan and Ahmad 2006). Biohydrogen fermentation can be realized by three main categories: (a) Dark fermentation (b) Photo fermentation (c) dark-photo fermentation (Manish and Banerjee 2008; Tao et al. 2007).

Research in dark fermentation, for hydrogen production, is on the increase in recent years due to its potential importance in our economy (Logan 2002; Yokoi 2002; Yu 2002; Zhang 2003) and has presented a promising route of biohydrogen production compared to photosynthetic routes (Levin et al. 2004). The major advantages of dark fermentation are high rate of cell growth, no light energy requirement, no oxygen limitation problems and ability to run on low capital cost (Levin et al. 2004; Nath and Das 2004; Hallenbeck and Benemann 2002). A variety of bacteria such as *E. coli*, *Enterobacter aerogenes*, and *Clostridium butyricum* are known to ferment sugars and produce hydrogen, using multienzyme systems. Since these "dark fermentation" reactions do not require light energy, so they are capable of constantly producing hydrogen from organic compounds throughout the day and night. Compared with other biological hydrogen-production processes, fermentative bacteria have high evolution rates of hydrogen. However, sugars are

Table 7 Performance of various anaerobic treatment methods on POME treatment (Source: Poh and Chong 2009)

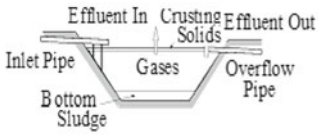
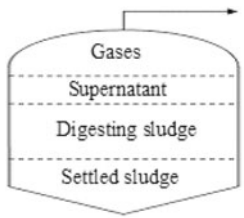
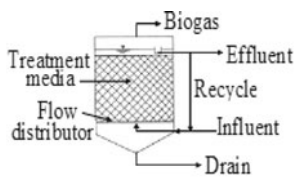
Anaerobic treatment types	*Features*	*OLR ($kgCOD/m^3day$)*	*HRT (days)*	*CH_4 composition (%)*	*COD removal efficiency (%)*	*Reference*
 **Anaerobic pond**	Almost energy free ope-ration, able to tolerate high OLR, recovered sludge cake can be used as a fertilizer but requires large area and long retention times.	1.4	40	54.4	97.8	Yacob et al.(2006a)
 **Anaerobic digester**	Low energy requirements, producing methane gas as a valuable end product, generated sludge can be used for land application but requires long retention time and slow start-up.	2.16	20	36	80.7	Yacob et al.(2005)
 **Anaerobic filtration**	Small reactor volume, producing high quality effluent, short HRT, able to tolerate shock loadings, retains high biomass concentration in the packing but shows instability for high suspended solid (SS) wastewater, lower methane emission, clogging at high OLRs, high media and support cost.	4.5	15	63	94	Borja and Banks (1994b)

Table 7 continued

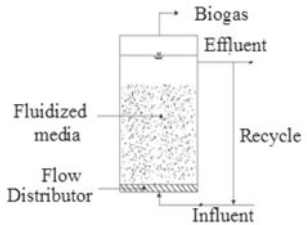
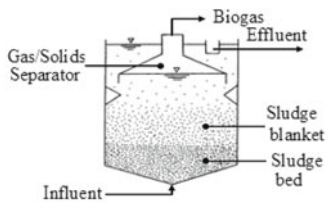
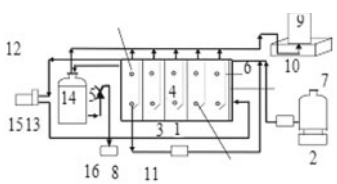
Anaerobic treatment types	*Features*	*OLR (kgCOD/m^{3}day)*	*HRT (days)*	*CH_4 composition (%)*	*COD removal efficiency (%)*	*Reference*
 **FBR**	Most compact of all high-rate processes, very well mixed conditions in the reactor, large surface area for biomass attachment, faster start-up, no channeling, plugging or gas hold-up but requires high power for bed fluidization and high cost of carrier media. Normally does not capture generated biogas and unstable for high SS wastewater.	40.0	0.25	--	78	Borja and Banks (1995b)
 **UASBR**	Low capital cost, good removal efficiency, can handle organic shock loads effectively, useful for the treatment of high SS wastewater, high methane production but longer startup takes before steady state operation, if activated sludge is not sufficiently available, foaming and sludge floatation at high OLRs and performance depends on sludge settleability.	10.63	4.0	54.2	98.4	Borja and Banks (1994c)
 1.Feed Tank 2.Magnetic Stirrer 3.Peristaltic Pump 4. Water Jacket 5.Compartment 6.Biogas Outlet 7. Baffle 8.Peristaltic Pump 9. Gas Collection bottle 10. NaCl solution 11,12 Sampling port 13.Effl.Collection bottle 14. Pipe Y 15. Water Bath 16. Sewer **MABR**	Provide longer solid retention time, suitable for the treatment of high strength SS wastewater, simple and inexpensive, does not require moving part or mechanical mixing device, stable to shock loading and capable of achieving high volumetric rates.	1.60	10	71.2	95.3	Faisel and Unno (2001)

Table 7 continued

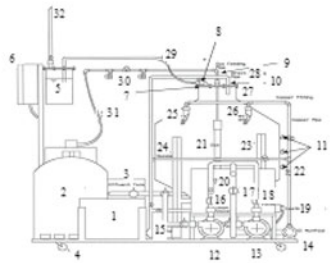
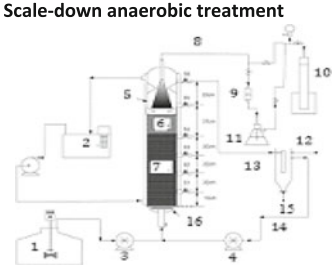
Anaerobic treatment types	*Features*	*OLR (kgCOD/m^{3}day)*	*HRT (days)*	*CH_4 composition (%)*	*COD removal efficiency (%)*	*Reference*
 1. Feeding Tank 2. Gas Cylinder 3. Effluent Tank 4. Lockable Castor 5. Gas Container 6. Switch Box 7. PVC top plate 8. Biogas Outlet 9. Gas recycling 10. POME feeding 11. Sampling ports 12, 13. Feed Pump 14. Steel Angle Bar Platform 15. Recycle Pump 16. Union valve 17, 18. Ball Valve 19. Heating Element 20. Heater Tank 21. PVC Gas Pipe 22. Sampling Points 23. Bioreactor 24. Lower Nozzle 25, 26. Upper Nozzle 27. Flange Cover 28. Direct Feeding Pipe 29. Methane Gas Discharge Valve 30. By-Pass Valve 31. Gas Hose 32. Purge Pipe **Scale-down anaerobic treatment**	Biomass sedimentation condition as applied for the scaled down anaerobic treatment was able to improve anaerobic treatment but relatively low conversion of COD to methane. Produces relatively high effluent quality.	6.0	--	41	>90	Basri et al. (2010)
 1. Feed tank 2. Water Bath 3, 4. Peristaltic Pump 5. Gas solid separator 6. UFF Portion 7. UASB Portion 8. Biogas 9. Gas sampling port 10. Gas holder 11. Water seal 12. Effluent 13. Settling tank 14. Recycle 15. Waste sludge 16. Influent distributor **UASFF bioreactor**	Higher OLR achievable compared to operating UASB or anaerobic filtration alone, problems of clogging eliminated, higher biomass retention, more stable operation, ability to tolerate shock loadings, suitable for diluted wastewater but needs lower OLR when treating suspended solid wastewaters.	11.58	3.0	71.9	97	Najafpour et al. (2006)

Table 7 continued

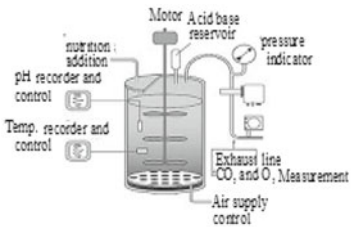
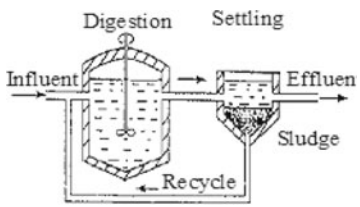
Anaerobic treatment types	*Features*	*OLR ($kgCOD/m^3day$)*	*HRT (days)*	*CH_4 composition (%)*	*COD removal efficiency (%)*	*Reference*
 **CSTR**	Provides more contact of wastewater with biomass through mixing, Increased gas production compared to conventional but less efficient gas production at high treatment volume and less biomass retention.	3.33	18.0	62.5	80	Tong and Jaafar (2006)
 **Anaerobic contact process**[a]	Reaches steady state quickly, short HRT produces relatively high effluent quality butl ess stable due to oxygen transfer in digesting tank and settleability of biomass is critical to successful performance.	3.44	4.7	63	93.3	Ibrahim et al. (1984)

Table 8 Comparison between methane derived from anaerobic digestion of POME and other gas fuels

Chemical properties	Methane	Natural gas	Propane
Gas calorific value (K cal/kg)	4,740–6,150	907	24,000
Specific gravity	0.847–1.002	0.584	1.5
Ignition temperature (°C)	650–750	650–750	450–500
Inflammable limits (%)	7.5–21.0	5–15	2–10
Combustion air required (m^3/m^3)	9.6	9.6	13.8

Source: Ma (1999a, b)

relatively expensive substrates that are not available in sufficient quantities to support hydrogen production at a scale required to meet energy demand. Photosynthetic hydrogen production basically involves two pathways. (1) Algae (Cyanobacteria) break down water to H_2 and O_2 in the presence of light energy. (2) Photo-heterotrophic bacteria ((*Rhodobacter* sp.) utilize organic acids (98–99%) such as acetic, lactic and butyric acids to produce H_2 (Tao et al. 2007). Photo-fermentation requires light in the ultra-violet range up to 400 nm (Kahn and Durako 2009) and the UV light has the potential to eliminate foreign microorganisms and to prevent contamination but the production rates with photo-fermentation is not as high as with dark-fermentation. However, various researchers reported that anaerobic microflora found in POME was able to produce hydrogen (Chong et al. 2009a, b, c) and methane gas was not observed in the evolved gas (Morimoto et al. 2004; Atif et al. 2005). Vijayaraghavan and Ahmad (2006) isolated hydrogen generating microflora from the cow dung in POME, based on pH adjustment (pH 5) coupled with heat treatment proved to be promising candidate towards hydrogen generation. Hydrogen production and growth of hydrogen producing bacteria were increased by nutrient (N, P and Fe) supplementation (O-Thong et al. 2007, 2008a, b). Furthermore, from the Table 9, it is evident that dark- fermentation can produce hydrogen more efficiently than photo-fermentation. The major advantages of the 2nd pathway of photo-fermentation is more favorable process economy, reduced operating problems and higher rate of H_2 gas production. Organic acids produced in the acidogenic phase of anaerobic digestion of organic materials may be used as the substrate for production of hydrogen by the photo-heterotrophic organisms. Therefore, the hydrogen yield may be improved by using a two-stage process such as dark and photo-fermentations or

Table 9 Yield of biohydrogen production from POME using dark and photo-fermentation technique

No.	Microorganism	pH	Temperature (°C)	Biohydrogen yield (L H_2/L POME)	Reference
Dark fermentation					
1	Mixed culture	uncont rolled	60	23.82 mmol H_2/(l-medium)	Morimoto et al. (2004)
2	Mixed culture	5.5	60	4.708 ml H_2/(l-medium)	Atif et al. (2005)
3	Mixed culture	5.0	–	0.42 l biogas/g $COD_{destroyed}$ with 57% hydrogen content	Vijayaraghavan and Ahmad (2006)
4	*Thermoanaerobacterium*	5.5	60	4.4 l H_2/(l-medium) per day	O-Thong et al. (2007)
5	*Thermoanaerobacterium*	5.5	60	6.1 l H_2/(l-medium per day)	O-Thong et al. (2007)
6	*Thermoanaerobacterium*	5.5	60	6.5	O-Thong et al. (2008a, b)
7	*Clostridium butyricum*	5.7	36	6.9	Chong et al. (2009b)
8	*Clostridium butyricum*	5.5	37	3.4	Chong et al. (2009c)
9	Mixed culture	5.5	23–25	1.2	Yusoff et al. (2009)
Photofermentation					
10	*Rhodopseudomonas palustris*	6.0	–	1.1	Jamil et al. (2009)

by their combinations. Hydrolysis (acid or enzymatic) of starch/cellulose to highly concentrated sugar solution is the first step in fermentative hydrogen production from waste biomass followed by dark fermentation of resulting carbohydrates to volatile fatty acids (VFA), H_2 and CO_2 by acetogenic-anaerobic bacteria. Usually photo-heterotrophic bacteria (*Rhodobacter* sp.) are used for photo-fermentation of VFAs to CO_2 and H_2 (Kapdan and Kargi 2006; Manish and Banerjee 2008). Fang et al. (2005) reported that a maximum hydrogen yield, 2.5 mol-H_2/mol-acetate and 3.7 mol-H_2/mol-butyrate could be achieved by *Rhodobacter capsulatus*. Thus, the effluents of dark-fermentation, which are mainly composed of organic acids, could be used as the substrate of photosynthetic non sulphur bacteria to produce hydrogen. Redwood and Macaskie (2006), used glucose and Tao et al. (2007) used sucrose as the substrate in the two-step fermentation process, and found remarkably improved compared with that of dark-fermentation. A combined biohydrogen and biomethane generation process has been proposed for organic solid wastes (Ueno et al. 2007), food wastes (Han and Shin 2004), cheese whey (Antonopoulou et al. 2008), olive mill wastewater (Koutrouli et al. 2009) and wastewater sludge (Ting and Lee 2007). As for as POME is concerned, neither dark-photofermentation nor dark-methanogenic process has yet been investigated. Therefore, biological and engineering studies need to concentrate on these issues for commercial viability and sustainable energy production from POME.

7 Production of bioplastics (PHA)

PHA (Fig. 2) is a group of biodegradable polymers, with properties similar to polypropylene and polyethylene (Salehizadeh and Van Loosdrecht 2004). Unlike normal plastics, which are non-biodegradable, these bioplastics biodegrade naturally to carbon dioxide and water within a few weeks upon disposal in the soil or the environment. PHA is an energy and carbon storage molecule produced in bacteria during growth limiting conditions (Lee 1996; Salehizadeh and Van Loosdrecht 2004). Due to the problems and harmful effects of conventional plastics on the environment, there has been a considerable interest in the development of biodegradable plastics for the last few decades (Lee and Yu 1997; Chua et al. 2003; Kumar et al. 2004). PHB and its copolymer, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3-HV)] are the most widespread PHAs, although other forms are also possible. However, the current pure culture production is expensive. The high cost of PHA has so far caused it to play a marginal role in the world's plastic market [120]. To decrease the production cost of these biopolymers, open mixed cultures can be used for PHA production (Albuquerque et al. 2007). Several workers have studied PHA production by mixed cultures using synthetic organic acids (Dionisi et al. 2005a, b; Lemos et al. 2006; Bengtsson et al. 2008). In recent years, to reduce the cost price considerably, POME has gained great attention by the research institutions and industrial sectors due to its potential as sources of carbon and nitrogen for microbial growth (Hassan et al. 1997a, b, c, 2002; Zakaria et al. 2008). A suggested three step process for PHA production in open mixed cultures, consists of: (1) acidogenic fermentation of the waste stream to produce VFAs, (2) selection of PHA storing organisms, (3) accumulation of PHA in the selected biomass (Dionisi et al. 2005b).

Since, most prokaryotes are capable of PHA production (Chua et al. 2003), therefore, it was possible to use the mixed cultures to produce PHA in POME (Din et al. 2006a, b). The initial mixed culture was developed by using 10% activated sludge from the sewage treatment plant and 90% from palm oil mill effluent (POME). It was observed that by using mixed cultures and POME, different kinds of PHA-constituent could be obtained and its harvesting was more reliable for the use of biodegradable plastics than a single PHA-constituent. The cultivation was maintained in a single batch-fed reactor and operated in two steps: First the system was allowed for an extensive growth (using a nutrient medium), and then a feed with limited nutrients (no nutrient medium adaptation) will be introduced in the next step. The average production of PHA, by using POME, could only reach up to 44% of the cell by dry weight. However, the favourable factors (e.g. temperature and harvesting time) have been made in the next stage to induce the PHA production (Din et al. 2006a, b). Table 10 shows the production of PHA from POME.

A new isolate, designated as strain EB172, was isolated from a digester tank, treating palm oil mill effluent and was investigated by polyphasic taxonomic approaches (Zakaria et al. 2010). The cells were rod-

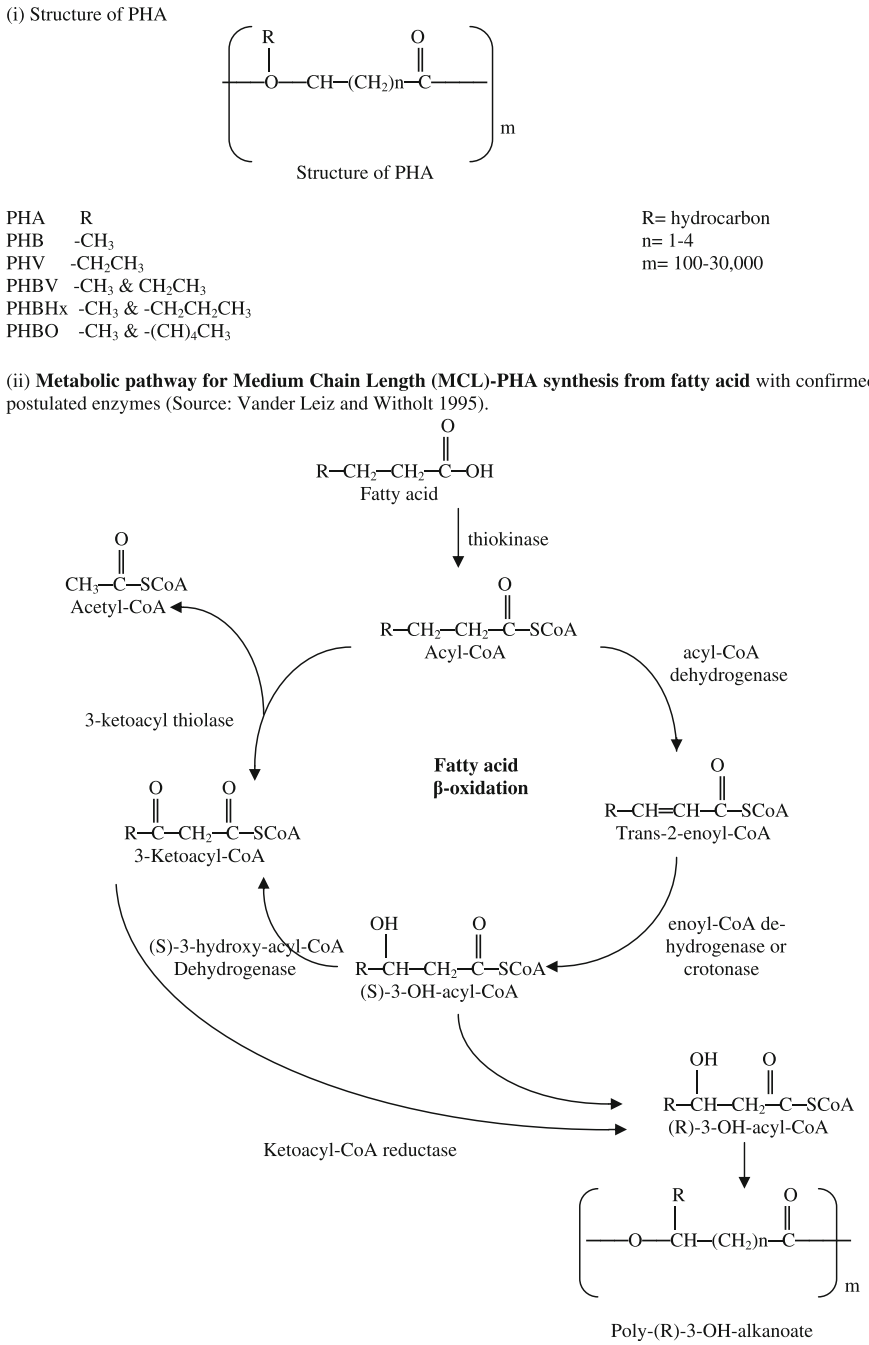


Fig. 2 (i) Structure of PHA. (ii) Metabolic pathway for Medium Chain Length (MCL)-PHA synthesis from fatty acid with confirmed or postulated enzymes (*Source*: Van der Leij and Witholt 1995)

shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Biochemical test, microscopic observation, cellular fatty acids analysis and 16S rRNA gene sequence analyses revealed that this strain was placed in the cluster of genus Comamonas and differed from the existing Comamonas species. Furthermore, the strain was capable of accumulating PHA up to 59% of CDW in fed-batch cultivation process with pH–stat continuous feeding of mixed organic acids derived from anaerobically treated POME (Zakaria et al. 2010).

Since POME contains high organic content, almost being non-toxic, can be suitable for PHA production. VFAs can be stored as PHA by bacteria in the opened

Table 10 PHA production from POME

Product	Microorganism	Temp (°C)	pH	Reactor	Maximum production g/l	Reference
PHA	*Rhodobactor spheroids* IFO 12203	30	7.0	Photo-bioreactor	≈4	Hassan et al. (1996)
	Rhodobactor spheroids IFO 12203	30	7.0	Photo-bioreactor	>2	Hassan et al. (1997b)
	Alcaligenes eutrophus H 16 (ATCC 17699)	30	7.0	Stirred tank	1.8	Hassan et al. (1997c)
	Ralstonia eutropha ATCC 17699	30	7.0	Bioreactor fermentati-on	≈6.25	Hassan et al. (2002)
	Mixed cultures	30	7.0	Sequencin-g batch reactor	24.24	Md Din et al. (2006b)

mixed cultures. For the selection of PHA storing organisms, the feast-famine approach is most commonly used (Dionisi et al. 2005a; Albuquerque et al. 2007; Bengtsson et al. 2008). In the feast-famine selection, the substrate is fed in pulses, whose concentration alternates from high (feast) to low (famine) (Dionisi et al. 2004). A final accumulation step is used to saturate the biomass with PHA (Dionisi et al. 2004).

8 Production of organic acids

Besides, biogas/methane generation from anaerobic treatment of POME [64], that can also be used to generate organic acids, which are effectively used in a variety of industrial processes (Hassan et al. 2002; Mumtaz et al. 2008). During anaerobic treatment of POME, the organic acids are produced as an intermediate which can be used as raw material for PHA production (Hassan et al. 1996, 1997b, c, 2002). Biotransformation of hexoses to gluconic, itaconic, citric and lactic acids is being carried out on a large scale to produce basic ingredients for laundry detergents, glues, preservatives and polylactides. Plastic materials, made from polylactides, are used in medicines related to transplantations, due to its excellent compatibility with tissues and skin. The production of mixed organic acids, from anaerobicallay treated palm oil mill effluent, has been shown to be a renewable and cheaper carbon sources of PHAs production (Hassan et al. 1997a, b, c, 2002; Zakaria et al. 2008). Hassan et al. (1996) reported that more than 70% of the BOD sources in POME, were converted to organic acids by *R. spheroids* IFO 12203 and a 50% PHAs yield from organic acids achieved. The predominant organic acids, at a higher and lower pH, were acetic acid and formic acid respectively. Freezing and thawing of treated POME was successfully applied to separate sludge solids in conjunction with sludge recycle system, so that continuous anaerobic treatment retention time can be shortened to 3.5 days (Phang et al. 2002, 2003). The performance of the anaerobic treatment of palm oil mill effluent for the production of organic acids at a short retention time of less than 5 days, was carried out by Lee et al. (2003) by incorporating a sludge recycle system without pH control and calcium carbonate. They showed that by incorporating a sludge recycle system with the freezing-thawing method, the retention time for the treatment could be reduced to 3.5 days without affecting the generation of organic acids.

The production of itaconic acid by *Aspergillus terreus* IMI 282743 from filtered Palm Oil Mill Effluent (POME), as an added supplement, was investigated by Jamaliah et al. Jamaliah et al. (2006). Itaconic acid (methylene butanedioic acid; common synonyms: methylene succinic acid, 3-carboxy-3-butanoic acid, propylenedicarboxylic acid) is one of the promising substances within the group of organic acids. Jamal et al. (2005) used POME and wheat flour as a medium and screened potential microorganisms for citric acid production and showed that *Aspergillus* (A 103) produced the highest concentration of citric acid after 2 days of fermentation.

Alam et al. (2008) investigated the bioconversion of POME, by adding co-substrate (glucose and wheat flour and nitrogen source–ammonium nitrate), for

Table 11 Organic acid produced from POME

Product	Microorganisms	pH	Temp (°C)	Reactor	Fermentation time	Maximum production g/l	Reference
Organic acids	Mixed cultures	7	30	Bioreactor fermentation	24	7.8	Hassan et al. (1996)
Organic acids	Mixed cultures	5	30	Stirred tank	84	10–14	Yee et al. (2003)
Citric acid	*Aspergillus* (A103)	$3_{(initial)}$	27–30	Flask fermentation	48	0.28	Jamal et al. (2005)
Citric acid	*Aspergillus niger* (A103)	$5_{(initial)}$	32	Flask fermentation	168	5.2	Alam et al. (2008)
Itaconic acid	*Aspergillus terreus* IMI 282743	–	35	Flask fermentation	120	0.079	Wu et al. (2005)

citric acid production under optimal conditions and observed higher removal of chemical oxygen demand (82%) with the production of citric acid (5.2 g/l) on the final day of fermentation process (7 days). Mumtaz et al. (2008) generated organic acids with low molecular weight such as acetic acid, propionic acid and butyric acids from partial anaerobic treatment of palm oil mill effluent (POME), using pilot scale filtration and evaporation system. The recovery of organic acids has a significant and economical impact, since around 50% cost of PHA production is believed to be associated with the substrate itself. Thus, the organic acid generated during acid-phase anaerobic digestion of POME can be converted into value-added products and fine chemicals and may serve as a renewable feed stock for biosynthesis of PHA. Table 11 summarizes production of organic acid from POME.

9 Conclusion

Renewable energy has been identified globally as a key driver to achieve economic growth while ensuring minimal environmental harm. Simultaneously, the current development of green technology and its related policies have enhanced the growth of renewable energy in the country.

The use of POME as a renewable energy resource can improve energy security while reducing the environmental burdens of waste disposal. The Malaysian palm oil industry, with 4.69 million hectares of planted land has a tremendous opportunity in supplying renewable energy. Energy from wastewater therefore facilitates the integration of water, waste and energy management within a model of sustainable development. Presently, renewable energy represents 5% of all prime energy use, but by the year 2060, it is strongly predicted that it will reach 70%. It is estimated that these palm based materials could generate up to 1,260 MW of energy. This amounts to nearly 10% of the maximum energy demand of electricity in Malaysia. Therefore, oil palm-based biomass can be expected to play a prominent role in the future when the demand for renewable energy is expected to increase rapidly.

In Malaysia, 5% of the electricity generated by renewable energy can easily be met by renewable sources. POME can be treated anaerobically to breakdown organic matters while releasing biomethane and sometimes, biohydrogen. Lipids are suitable substrates for high-rate anaerobic wastewater treatment and are also ideal co-substrates for AD plants. By utilizing appropriate technology and following the right feeding strategy, lipids can be effectively converted to methane by syntrophic consortia of acetogenic bacteria and methanogenic archaea. Application of cultivation and molecular techniques to the study of microbial composition of LCFA-degrading sludges provided important insight into the communities involved in the degradation of these compounds.

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References

Agamuthu P, Tan EL (1985) Digestion of dried palm oil mill effluent by *Cellulomonas* sp. Microbiol Lett 30:109–113

Ahmad AD, Ismail S, Bhatia S (2003) Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination 157:87–95

Alam MZ, Jamal P, Nadzir MM (2008) Bioconversion of palm oil mill effluent for citric acid production: statistical optimization of fermentation media and time by central composite design. World J Microbiol Biotechnol 24: 1177–1185

Albuquerque MGE, Eiroa M, Torres C, Nunes BR, Reis MAM (2007) Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. J Biotechnol 130:411–421

Alves MM, Pereira MA, Sousa DZ, Cavaleiro AJ, Picavet M, Smidt H, Stams AJM (2009) Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA). Microbial Biotechnol 2(5):538–550

Andreasen T (1982) The AMINODAN system for treatment of palm oil mill effluent. In: Proceeding of regional workshop on palm oil mill technology and effluent treatment. PORIM, Malaysia, pp 213–215

Angelidaki I, Ahring BK (1995) Establishment and characterization of anaerobic thermophilic (55°C) enrichment culture degrading long chain fatty acids. App Environ Microbiol 61(6):2442–2445

Antonopoulou G, Stamatelatou K, Venetsaneas N, Kornaros M, Lyberatos G (2008) Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. Ind Eng Chem Res 47:5227–5233

Atif AAY, Fakhru'a-Razi A, Ngan MA, Morimoto M, Iyuke SE, Vesiroglu NT (2005) Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. Int J Hydrogen Energy 30:1393–1397

Barry P (2007) Methane Blast. NASA. May 4, 2007

Basri MF, Yacob S, Hassan MA, Shirai Y, Wakisaka M, Zakaria MR, Phang LY (2010) Improved biogas production from palm oil mill effluent by a scaled-down anaerobic treatment process. World J Microbiol Biotechnol 26:505–514

Batstone DJ (2000) High rate anaerobic treatment of complex waste water. PhD Thesis, Queensland University, Australia

Beccari M, Bonemazzi F, Majone M, Reccardi C (1996) Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Water Res 30(1):183–189

Bengtsson S, Werker A, Christensson M, Welander T (2008) Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. Bioresour Technol 99:509–516

Biebl H, Pfening N (1978) Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Arch Microbiol 117:9–16

Bitton B (2005) Wastewater microbiology, 3rd Ed. Wiley, Hoboken, New Jersey, Chap 13, 2005 pp 345–369

Borja R, Banks CJ (1994a) Anaerobic digestion of palm oil mill effluent using an up-flow anaerobic sludge blanket (UASB) reactor. Biomass Bio 6:381–389

Borja R, Banks CJ (1994b) Kinetic of methane production from palm oil mill effluent in an immobilized cell bioreactor using saponite as support medium. Biores Technol 48:209–214

Borja R, Banks CJ (1994c) Treatment of palm oil mill effluent by upflow anaerobic filtration. J Chem Technol Biotechnol 61:103–109

Borja R, Banks CJ (1995a) Response of an anaerobic fluidized bed reactor treating ice-cream wastewater to organic, hydraulic, temperature and pH shocks. J Biotechnol 39:251–259

Borja R, Banks CJ (1995b) Comparison of an anaerobic filter and an anaerobic fluidized bed reactor treating palm oil mill effluent. Process Biochem 30:511–521

Chan KS, Chooi CF (1982) Ponding system for palm oil mill effluent. In: PORIM proceedings of regional on palm oil mill technology and effluent treatment, pp 185–192

Cheng J, Zhu X, Borthwick A (2010) Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresour Technol 101:2729–2734

Chin KK (1981) Anaerobic treatment kinetics of palm oil sludge. Water Res 15:199–202

Chong ML, Sabaratnam V, Shirai Y, Hassan MA (2009a) Biohydrogen production from biomass and industrial wastes by dark fermentation. Int J Hydrogen Energy 34:3 277–3287

Chong ML, Abdul Rahman NA, Rahim RA, Aziz SA, Shirai Y, Hassan MA (2009b) Optimization of biohydrogen production by *Clostridium butyricum* EB6 from palm oil mill effluent using response surface methodology. Int J Hydrogen Energy 34:7475–7482

Chong ML, Rahim RA, Shirai Y, Hassan MA (2009c) Biohydrogen production by *Clostridium butyricum* EB6 from palm oil mill effluent. Int J Hydrogen Energy 34:764–771

Chua ASM, Takabatake H, Satoh H, Mino T (2003) Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT) and acetate concentration in influent. Water Res 37:3602–3611

Das D, Veziroglu TN (2001) Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 26:13–28

Demirel B, Scherer P (2008) The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane a review. Rev Environ Sci Biotechnol 7:173–190

Dimroth K (1983) Thermochemische daten organischer verbindungen. In D'ans-Lax Taschenbuch für Chemiker und Physiker, Bd. 2. Springer, Berlin, pp 997–1083

Din MF, Ujang Z, Muhd Yunus S, Van Loosdrecht MCM (2006) Storage of polyhydroxyalkanoates (PHA) in fed-batch

mixed cultures. Fourth Seminar on Water Management (JSPS-VCC). Johor, Malaysia July 11–13

Din MF, Ujang Z, van Loosdrecht MCM, Ahmad A, Sairan MF (2006b) Optimization of nitrogen and phosphorus limitation for better biodegradable plastic production and organic removal using single fed-batch mixed cultures and renewable resources. Water Sci Technol 53:15–20

Dionisi D, Majone M, Papa V, Beccari M (2004) Biodegradable polymers from organic acids by using activated sludge enriched by aerobic periodic feeding. Biotech Bioeng 85(6):569–579

Dionisi D, Beccari M, Di Gregorio S, Majone M, Petrangeli Papini M, Vallini G (2005a) Storage of biodegradable polymers by an enriched microbial community in a sequencing batch reactor operated at high organic load rate. J Chem Technol Biotechnol 80:1306–1318

Dionisi D, Carucci G, Papini MP, Riccardi C, Majone M, Carrasco F (2005b) Olive oil mill effluents as a feedstock for production of biodegradable polymers. Water Res 39: 2076–2084

Faisel M, Unno H (2001) Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. Biochem Eng J 9:25–31

Fakhru'l-Razi A, Yassin AAA, Lyuke SE, Ngan MA, Morimoto M (2005) Biohydrogen synthesis from wastewater by anaerobic fermentation using microflora. Int J Green Energy 2:387–396

Fang HHP, Chui HK (1994) Comparison of startup performance of four anaerobic reactors for the treatment of high-strength wastewater. Resour Conserv Recycling 11:123–138

Fang HHP, Liu H, Zhang T (2005) Phototrophic hydrogen production from acetate and butyrate in wastewater. Int J Hydrogen Energy 30(7):785–793

Federal Subsidiary Legislation (1974) Federal subsidiary legislation: environmental quality act 1974 [ACT 127], environmental quality (Sewage and Industrial Effluents) regulation 1979. Available: http://www.doe.gov.my (29/2/2008)

Foo KY, Hameed BH (2010) Insight into the applications of palm oil mill effluent: a renewable utilization of the industrial agricultural waste. Renew Sustain Energy Rev 14:1445–1452

Guo XM, Trably E, Latrille E, Carrère H, Steyer JP (2010) Hydrogen production from agricultural waste by dark fermentation: a review. Int J Hydrogen Energy doi: 10.1016/j.ijhydene.2010.03.008

Habib MAB, Yusoff FM, Phang SM, Ang KJ, Mohamed S (1997) Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. Aquaculture 158:95–105

Hallenbeck PC, Benemann JR (2002) Biological hydrogen production: fundamentals and limiting processes. Int J Hydrogen Energy 27:1185–1193

Han SK, Shin HS (2004) Performance of an innovative two-stage process converting food waste to hydrogen and methane. J Air Waste Manage Assoc 54:242–249

Hanaki K, Nagase M, Matsuo T (1981) Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. Biotechnol Bioeng 23:1591–1610

Hanaki K, Matsuo T, Nagase M, Tabata Y (1987) Evaluation of effectiveness of two-phase anaerobic digestion process degrading complex substrate. Water Sci Technol 19:311–322

Hassan MA, Shirai Y, Kusubayashi N, Karim MIA, Nakanishi K, Hashimoto K (1996) Effect of organic acid profiles during anaerobic treatment of palm oil mill effluent on the production of polyhydroxyalkanoates by Rhodobacter sphaeroides. J Ferment Bioeng 82:151–156

Hassan MA, Shirai Y, Umeki H, Yamazumi H, Jin S, Yamamoto S, Abdul Karim MI, Nakanishi I, Hashimoto K (1997a) Acetic acid separation from anaerobically treated pal oil mill effluent by ion exchange resins for the production of polyhydroxyalkanoate by Alcaligens eutrophus. Biosci Biotechnol Biochem 61(9):1465–1468

Hassan MA, Shirai Y, Kusubayashi N, Karim MIA, Nakanishi K, Hashimoto K (1997b) The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by Rhodobacter sphaeroides. J Ferment Bioeng 83: 485–488

Hassan MA, Shirai Y, Umeki H, Yamazumi H, Jin S, Yamamoto S (1997c) Acetic acid separation from anaerobically treated palm oil mill effluent by ion exchange resins for the production of polyhydroxyalkanoate by Alcaligenes eutrophus. Biosci Biotechnol Biochem 61: 1465–1468

Hassan MA, Nawata O, Shirai Y, Rahman NAA, Yee PL, Ariff A (2002) A proposal for zero emission from palm oil industry incorporating the production of polyhydroxyalkanoates from palm oil mill effluent. J Chem Eng Jpn 35:9–14

Hatamoto M, Imachi H, Yashiro Y, Fukayo S, Ohashi A, Harada H (2007a) *Syntrophomonas palmitatica* sp nov., an anaerobic, syntrophic, long-chain fatty-acidoxidizing bacterium isolated from methanogenic sludge. Int J Syst Evol Microbiol 57:2137–2142

Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2007b) Diversity of anaerobic microorganisms involved in long-chain fatty acid degradation in methanogenic sludges as revealed by RNA-based stable isotope probing. Appl Environ Microbiol 73:4119–4127

Hatamoto M, Imachi H, Ohashi A, Harada H (2007c) Identification and cultivation of anaerobic, syntrophic longchain fatty acid-degrading microbes from mesophilic and thermophilic methanogenic sludges. Appl Environ Microbiol 73:1332–1340

Hensen KH, Ahring BK, Raskin L (1999) Quantification of syntrophic fatty acid-beta-oxidizing bacteria in a mesophilic biogas reactor by oligonucleotide probe hybridization. Appl Environ Microbiol 65:4767–4774

Ibrahim A, Yeoh BG, Cheah SC, Ma AN, Ahmad S, Chew TY, Raj R, Wahid MJA (1984) Thermophilic anaerobic contact digestion of palm oil mill effluent. Water Sci Technol 17:155–166

Ishigaki T, Yamada M, Nagamori M, Ono Y, Inoue Y (2005) Estimation of methane emission from whole waste landfill site using correlation between flux and ground temperature. Environ Geol 48:845–853

Jackson BE, Bhupathiraju VK, Tanner RS, Woese CR, McInerney MJ (1999) *Syntrophus aciditrophicus* sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. Arch Microbiol 171: 107–114

Jamal P, Alam MZ, Salleh MRM, Nadzir MM (2005) Screening of microorganisms for citric acid production from palm oil mill effluent. Biotechnology 4:275–278

Jamaliah MJ, Muhammad NIS, Yeong WT (2006) Factor analysis in itaconic acid fermentation using filtered POME by *Aspergillus terreus* IMI 282743. J Kejurutraan 18:39–48

Jamil Z, Mohamad Annuar MS, Ibrahim S, Vikineswary S (2009) Optimization of phototrophic hydrogen production by *Rhodopseudomonas palustris* PBUM001 via statistical experimental design. Int J Hydrogen Energy 34:7502–7512

Jeris JS, McCarty M (1965) The biochemistry of methane fermentation using 14C tracers. J Water Pollut Control Fed 37:178–192

Kahn AE, Durako MJ (2009) Wavelength-specific photo-synthetic responses of Halophila johnsonii from marine-influenced versus river-influenced habitats. Aquat Bot 91(3):245–249

Kaiser JP, Hanselmann KW (1982) Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. Arch Microbiol 133: 185–194

Kalyunzai SV, Skylar VI, Davlyatshina MA, Parshina SN, Simankova MV, Kostrikina NA, Nozhevnikova AN (1996) Organic removal and microbiological features of UASB-reactor under various organic loading rates. Biores Technol 55:47–54

Kalyunzai S, de los Santos LE, Martinez JR (1998) Anaerobic treatment of raw and preclarified potato-maize wastewater in a UASB reactor. Biores Technol 66:198–199

Kapdan IK, Kargi F (2006) Biohydrogen production from waste materials. Enzym Microb Technol 38:569–582

Kim SH, Han SK, Shin HS (2004) Two-phase anaerobic treatment system for fat-containing wastewater. J Chem Technol Biotechnol 79:63–71

Komatsu T, Hanaki K, Matsuo T (1991) Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. Water Sci Technol 23:1189–1200

Koutrouli EC, Kalfas H, Gavala HN, Skiadas IV, Stamatelatou K, Lyberatos G (2009) Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. Bioresour Technol 100:3718–3723

Kumar S, Tamura K, Nei M (2004) MEGA 30: Integrated software of molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5(2):150–163

Lalman JA (2000) Anaerobic degradation of linoleic (C18:2), oleic (C18:1) and stearic (C18:0) acids and their inhibitory effects on acidogens, acetogens and methanogens. Ph.D. thesis, University of Toronto: Toronto, Canada

Lalman JA, Bagley DM (2000) Anaerobic degradation and inhibitory effects of linoleic acid. Water Res 34:4220–4228

Lalman JA, Bagley DM (2001) Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. Water Res 35:2975–2983

Latif MA, Ahmad A, Ghufran R, Wahid ZA, (2011) Effect of temperature and organic loading rate on upflow anaerobic sludge blanket reactor and CH_4 production by treating liquidized food waste. Environmental Prog & Sustain Ener doi:10.1002/ep.10540

Latif MA, Ghufran R, Wahid ZA, Ahmad A (2011) Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. Water Res 05–049

Lee SY (1996) Bacterial polyhydroxyalkanoates. Biotechnol Bioeng 49:1–14

Lee S, Yu J (1997) Production of biodegradable thermoplastics from municipal sludge by two-stage bioprocess. Resour Conserv Recycling 19:151–164

Lee HJ, Lee SC, Kim JD, Oh YG, Kim BK, Kim CW, Kim KJ (2003) Methane production potential of feed ingredients as measured by in vitro gas test. Asian-Aust J Anim Sci 16(8):1143–1150

Lemos PC, Serafim LS, Reis MAM (2006) Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding. J Biotechnol 122:226–238

Levin DB, Pitt L, Love M (2004) Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy 29:173–185

Linke B (2006) Kinetic study of thermophilic anaerobic digestion of solid wastes from potato processing. Biomass Bioenergy 30:892–896

Logan BE (2002) Biological hydrogen production measured in batch anaerobic respirometers. Environ Sci Technol 36:2530–2535

Lorowitz WH, Zhao HX, Bryant MP (1989) *Syntrophomonas wolfei* subsp. *saponavida* subsp. nov., a long chain fatty-acid degrading, anaerobic, syntrophic bacterium; *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov.; and emended descriptions of the genus and species. Int J Syst Bacteriol 39:122–126

Ma AN (1999a) Treatment of palm oil mill effluent. Oil palm and environment: malaysia perspective. Malaysia Oil Palm Growers'Council, pp 277

Ma AN (1999b) Innovations in management of palm oil mill effluent. The Planter Kuala Lumpur 75(881):381–389

Ma AN (2000) Environmentalmanagement for the palm oil industry. PalmOil Dev 30:1–9

Maia MRG, Chaudhary LC, Figueres L, Wallace RJ (2007) Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek 91:303–314

Manish SR, Banerjee R (2008) Comparison of biohydrogen production processes. Int J Hydrogen Energy 33:279–286

McInerney MJ (1992) The genus *Syntrophomonas*, and other syntrophic bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer, New York, pp 2048–2057

McInerney MJ, Bryant MP, Costerton JW (1981) *Syntrophomonas wolfei* gen. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. AppI Environ Microbiol 41:1029–1039

McInery MJ, Bryant MP, Pfennig N (1979) Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch Microbiol 122:129–135

Mohammadi P, Ibrahim S, Annuar MSM, Ghafari S, Vikineswary S, Zinatizadeh AA (2011) Influences of environmental and operational factors on dark fermentive hydrogen production from wastes: a review. World Appl Sci J 13(2):188–199

Morimoto M, Atsuko M, Atif AAY, Ngan MA, Fakhru'l-Razi A, Iyuke SE (2004) Biological production of hydrogen from glucose by natural anaerobic microflora. Int J Hydrogen Energy 29:709–713

Mumtaz T, Abd-Azizl S, Rahman NAA, Yee PL, Shirai Y, Hassan MA (2008) Pilot-scale recovery of low molecular weight organic acids from anaerobically treated palm oil mill effluent (POME) with energy integrated system. Afr J Biotechnol 7(21):3900–3905

Najafpour GD, Zinatizadeh AAL, Mohamed AR, Isa MH, Nasrollahzadeh H (2006) High-rate anaerobic digestion of palm oil mill effluent in an upflow sludge-fixed film bioreactor. Process Biochem 41:370–379

Nath K, Das D (2004) Improvement of fermentative hydrogen production: various approaches. Appl Microbiol Biotechnol 65:520–529

Nielsen AT, Amandusson H, Bjorklund R, Dannetum H, Ejlertsson J, Ekedahl LG (2001) Hydrogen production from organic wastes. Int J Hydrogen Energy 26:547–550

Ntaikou G, Antonopoulou, Lyberatos G (2010) Biohydrogen production from biomass and wastes via dark fermentation: a review. Waste Biomass Valorization pp 1–19

Nwuche CO, Ugoji EO (2008) Effects of heavy metal pollution on the soil microbial activity. Int J Environ Sci Technol 5(3):409–414

Nwuche CO, Ugoji EO (2010) Effect of co-existing plant species on soil microbial activity under heavy metal stress. Int J Environ Sci Technol 7(4):697–704

O-Thong S, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland NK (2007) Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. Enzyme Microb Technol 41:583–590

O-Thong S, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland NK (2008a) Optimization of simultaneous thermophilic fermentative hydrogen production and COD reduction from palm oil mill effluent by Thermoanaerobacterium-rich sludge. Int J Hydrogen Energy 33:1221–1231

O-Thong S, Prasertsan P, Karakashev D, Angelidaki I (2008b) Thermophilic fermentative hydrogen production by the newly isolated Thermoanaerobacterium thermosaccharolyticum PSU-2. Int J Hydrogen Energy 33:1204–1214

Paillard D, McKain N, Chaudhary LC, Walker ND, Pizette F, Koppova I (2007) Relation between phylogenetic position, lipid metabolism and butyrate production by different *Butyrivibrio*-like bacteria from the rumen. Antonie Van Leeuwenhoek 91:417–422

Pavlostathis SG, Geraldogomez E (1991) Kinetics of anaerobic treatment. Water Sci Technol 24(8):35–59

Pereira MA, Roest K, Stams AJM, Mota M, Alves MM, Akkermans A (2002) Molecular monitoring of microbial diversity in expanded granular sludge bed (EGSB) reactors treating oleic acid. FEMS Microbiol Ecol 41:95–103

Phang LY, Wakisaka M, Shirai Y, Hassan MA (2002) Freezing and thawing technique for the removal of suspended solids and concentration of palm oil mill effluent (POME). J Chem Eng Jpn 35(10):1017–1019

Phang LY, Hassan MA, Shirai Y, Wakisaka M, Abdul Karim MI (2003) Continuous production of organic acids from palm oil mill effluent with sludge recycle by the freezing-thawing method. J Chem Eng Jpn 36(6): 707–710

Poh PE, Chong MF (2009) Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Bioresour Technol 100:1–9

Rahim BA, Raj R (1982) Pilot plant study of a biological treatment system for palm oil mill effluent. In: Proceedings of regional workshop on palm oil mill technology and effluent treatment. *PORIM*, Malaysia, pp 163–170

Redwood MD, Macaskie LE (2006) A two-stage, two-organism process for biohydrogen from glucose. Int J Hydrogen Energy 31(11):1514–1521

Rinzema A (1993) Anaerobic digestion of long chain fatty acids in UASB and expanded granular sludge bed reactors. Process Biochem 28:527–537

Roy F, Samain E, Dubourguier HC, Albagnac G (1986) *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long-chain fatty acids. Arch Microbiol 145:142–147

Rupani PF, Singh RP, Ibrahim MH, Esa N (2010) Review of current palm oil mill effluent (POME) treatment methods vermicomposting as a sustainable practice. World Appl Sci J 11(1):70–81

Salehizadeh H, Van Loosdrecht MCM (2004) Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnical importance. Biotech Adv 22:261–279

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280

Shigematsu T, Tang Y, Mizuno Y, Kawaguchi H, Norimvra S, Kida K (2006) Microbial diversity of mesophilic methanogenic consortium that degrade longchain fatty acids in chemostat cultivation. J Biosci Bioeng 102:535–544

Singh RP, Embrandiri A, Ibrahim MH, Esa N (2011) Management of biomass residues generated from palm oil mill vermicomposting a sustainable option, resources. Conserv Recycling 55:423–434

Sousa DZ, Smidt H, Alves MM, Stams AJM (2007a) *Syntrophomonas zehnderi* sp. nov., an anaerobe that degrades long chain fatty acids in co-culture with *Methanobacterium formicicum*. Int J Syst Evol Microbiol 57:609–615

Sousa DZ, Pereira MA, Smidt H, Stams AJM, Alves MM (2007b) Molecular assessment of complex microbial communities degrading long chain fatty acids in methanogenic bioreactors. FEMS Microbiol Ecol 60:252–265

Sousa DZ, Pereira MA, Stams AJM, Alves MM, Smidt H (2007c) Microbial communities involved in anaerobic degradation of unsaturated or saturated long chain fatty acids. Appl Environ Microbiol 73:1054–1064

Sousa DZ, Alves JI, Alves MM, Smidt H, Stams AJM (2009) Effect of sulfate on methanogenic communities that degrade unsaturated and saturated long-chain fatty acids (LCFA). Environ Microbiol 11:68–80

Stronach SM, Rudd T, Lester JN (1978) Start-up of anaerobic bioreactors on high strength industrial wastes. Biomass 13:173–197

Stryer L (1995) Fatty acid metabolism, 4th edn. Biochemistry W.H. Freeman and Co., New York, pp 603–628

Svetlitshnyi V, Rainey F, Wiegel J (1996) *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. Int J Syst Bacteriol 46:1131–1137

Tabatabaei M, Zakaria MR, Rahim RA, Wright ADG, Shirai Y, Abdullah N, Sakai K, Ikeno S, Mori M, Kazunori M, Sulaiman A, Hassan MA (2009) Electron J Biotechnol 12(3):ISSN:0717-3458

Tao YZ, Chen Y, Wu YQ, He YL, Zhou ZH (2007) High hydrogen yield from a two-step process of dark- and photo-fermentation of sucrose. Int J Hydrogen Energy 32(2): 200–206

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotropic anaerobic bacteria. Bacteriol Rev 41:100–180

Ting CH, Lee DJ (2007) Production of hydrogen and methane from wastewater sludge using anaerobic fermentation. Int J Hydrogen Energy 32:677–682

Tong SL, Jaafar AB (2006) POME Biogas capture, upgrading and utilization. Palm Oil Eng Bull 78:11–17

Ueno Y, Tatara M, Fukui H, Makiuchi TT, Goto M, Sode K (2007) Production of hydrogen and methane from organic solid wastes by phase-separation of anaerobic process. Bioresour Technol 98:1861–1865

UNEP (1994) Treatment wastewater in the rubber industry. In: Cleaner production in the Asia-Pacific economic cooperation region. United Nations Environment Programme, Paris, pp 21–30

Van der Leij FR, Witholt B (1995) Strategies for the suitable production of new biodegradable polyesters in plants: a review. Can J Microbiol 41(Suppl 1):222–238

Vijayaraghavan K, Ahmad D (2006) Biohydrogen generation from palm oil mill effluent using anaerobic contact filter. Int J Hydrogen Energy 31:1284–1291

Weiland P (2010) Biogas production current state and perspectives. Appl Microbiol Biotechnol 85:849–860

Weng C, Jeris JS (1976) Biochemical mechanisms in methane fermentation of glutamic and oleic acids. Water Res 10:9–18

Wong FM (1980) A review on the progress of compliance with the palm oil control regulations. Seminar on advances in palm oil effluent control technology, Kuala Lumpur, pp 142–149

Wong PW, Nik MS, Nachiappa M, Balaraman V (2002) Pretreatment and membrane ultra filtration using treated palm oil mill effluent (POME). Songklanakarin J Sci Tech 24(suppl):891–898

Wong YS, Kadir MOAB, Teng TT (2009) Biological kinetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent. Bioresour Technol 100(21):4969–4975

Wu TY, Mohammad AW, Md Jahim J, Anuar N (2005) Penghasilan asid itakonik oleh Aspergillus terreus IMI 282743 dalam pelbagai jenis medium penghasilan. Pascasidang Kolokium Jabatan Kejuruteraan Kimia dan Proses. Malaysia, pp 147–151

Wu C, Liu X, Dong X (2006) *Syntrophomonas erecta* subsp. *Sporosyntropha* subsp. nov., a spore-forming bacterium that degrades short chain fatty acids in co-culture with methanogens. Syst Appl Microbiol 29:457–462

Wu TY, Mohammad AW, Jahim JMd, Anuar N (2007) Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochem Eng J 35:309–317

Wu TY, Mohammad AW, Jahim JM, Anuar N (2010) Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. J Environ Manag 91:1467–1490

Lunar Engines (2009) Aerojet has completed assembly of a 5,500-pound-thrust liquid oxygen/liquid methane rocket engine: a propulsion technology under consideration as the way off the Moon for human explorers. Aviat Week Space Technol 171(2):16

Yacob S, Hassan MA, Shirai Y, Wakisaka M, Subash S (2005) Baseline study of methane emission from open digesting tanks of palm oil mill effluent treatment. Chemosphere 59:1575–1581

Yacob S, Shirai Y, Hassan MA, Wakisaka M, Subash S (2006a) Startup operation of semi-commercial closed anaerobic digester for palm oil mill effluent treatment. Process Biochem 41:962–964

Yacob S, Shirai Y, Hassan MA, Wakisaka M, Subash S (2006b) Baseline study of methane emission from anaerobic pond of palm oil mill effluent treatment. Sci Total Environ 336:187–196

Yee PL, Hassan MA, Shirai Y, Wakisaka M, Karim MIA (2003) Continuous production of organic acids from palm oil mill effluent with sludge recycle by the freezing–thawing method. J Chem Eng Jpn 36:707–710

Yokoi H (2002) Microbial production of hydrogen from starch manufacturing wastes. Biomass Bioenergy 22:389–395

Yu H (2002) Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures. Int J Hydrogen Energy 27:1359–1365

Yusoff MZM, Hassan MA, Abd-Aziz S, Rahman NAA (2009) Start-Up of biohydrogen production from palm oil mill effluent under non-sterile condition in 50 L continuous stirred tank reactor. Int J Agric Res 4:163–168

Zakaria MR, Abd-Aziz S, Ariffin H, Rahman NAA, Yee PL, Hassan MA (2008) *Comamonas* sp. EB172 isolated from digester treating palm oil mill effluent as potential polyhydroxyalkanoate (PHA) producer. Afr J Biotechnol 7(22):4118–4121

Zakaria MR, Tabatabaei M, Ghazali FM, Aziz SA, Shirai Y, Hassan MA (2010) Polyhydroxyalkanoates production from anaerobically trated palm oil mill effluent by new bacterial satrain *Comamonas* sp. EB172. World J Microbiol Biotechnol 26(5):767–774

Zhang T (2003) Biohydrogen production from starch in wastewater under thermophilic condition. J Environ Manage 69:149–156

Zhang CY, Liu XL, Dong XZ (2004) *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. Int J Syst Evol Microbiol 54:969–973

Zhao H, Yang D, Woese CR, Bryant MP (1993) Assignment of fattyacid-beta-oxidizing syntrophic bacteria to *Syntrophomonadaceae* fam. nov. on the basis of 16S rRNA sequence analysis. Int J Syst Bacteriol 43:278–286

Zinatizadeh AAL, Mohamed AR, Abdullah AZ, Mashitah MD, Husnain Isa M, Najafpour GD (2006) Process modeling and analysis of palm oil mill effluent treatment in an upflow anaerobic sludge fixed film bioreactor using response surface methodology (RSM). Water Res 40:3193–3208