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 discuss 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.

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
CO₂ Carbon dioxide
CH₃COO⁻ Acetate
H₂ Hydrogen
STP Standard temperature and pressure
CH₄ 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 CO2 and hydrogen by acetogens. Finally the hydrogen is utilized by hydrogenotrophic methanogens while acetic acid and CO2 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 (Demitrel 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 LCFAs are degraded through β-oxidation process (syntrophic acetogenesis) to acetate, H2 and CO2 (Jeris and McCarty 1965; Weng and Jeris 1976) and finally converted to CH4 or CO2 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, LCFAs glycerol undergoes fermentation or acidogenesis. Since LCFAs 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).

Syntrophy is a special case of mutualistic inter-relationship 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
cooperation of fatty acid-oxidizing, fermenting bacteria with hydrogen oxidizing methanogens (McInery et al. 1979) or of phototrophic green sulphur bacteria with chemotrophic sulphur-reducing bacteria (Biebl and Pfening 1978). In most cases of syntrophism the nature of a syntrophic reaction involves H₂ gas being produced by one partner and being consumed by the other.

Oxidation of butyric acid to acetic acid and H₂ 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 H₂ consumer.

Butyrate⁻ + 2H₂O → 2Acetate⁻ + H⁺ + 2H₂ + Energy (+48.2 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 LCFA by the concerted action of proteases, cellulases and lipases respectively. Some researchers suggested

| Parameter  | Mean | Range        | Limits of discharge | Moisture  | Crude protein | Crude lipid |
|------------|------|--------------|---------------------|-----------|---------------|-------------|
| pH         | 4.2  | 3.4–5.2      | 5.0–9.0             | 6.75      | 9.07          | 13.21       |
| Temperature| –    | 80–90        | 45                  |           |               |             |
| Biological Oxygen Demand 3-days 30°Cb | 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         | 150c               |           |               |             |
| Total Nitrogen   | 750    | 180–1400     | 200c               |           |               |             |

*Source*: EQA 1974 (Act 127) and Subsidiary Legislation 2002

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| Characteristics of POME | Parameter limits for wastewater discharge for POME | Major constituents of POME | Quantity (g/g dry sample) |
|-------------------------|--------------------------------------------------|-----------------------------|---------------------------|
| Parametera | Mean | Range  | Limits of dischargea | Moisture  | Crude protein | Crude lipid |
| pH         | 4.2  | 3.4–5.2 | 5.0–9.0             | 6.75      | 9.07          | 13.21       |
| Temperature| –    | 80–90   | 45                  |           |               |             |
| Biological Oxygen Demand 3-days 30°Cb | 25,000 | 10,250–43,750 | 100          | Ash       | 32.12         |             |
| Chemical Oxygen Demand   | 51,000 | 15,000–1,00,000 | –              | Carbohydrate | 20.55        |             |
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| Volatile Solids  | 34,000 | 9,000–72,000  | –                 |           |               |             |
| Oil and Grease   | 6,000  | 130–18,000 | 50                 |           |               |             |
| Ammoniacal Nitrogen | 35    | 4–80      | 150c               |           |               |             |
| Total Nitrogen   | 750    | 180–1400  | 200c               |           |               |             |

*Source*: (Ma 1999a, b, 2000)
| Fatty acids (g/100 g lipid) | Quantity (g) | Chemical formula of fatty acids | Amino acids (g/100 g protein) | Chemical formula | Quantity (g) | Minerals (mm/g dry sample) | Quantity |
|---------------------------|-------------|---------------------------------|------------------------------|------------------|-------------|----------------------|----------|
| Capric acid (10:0)        | 4.29        | CH₃(CH₂)₄COOH                   | Aspartic acid HOOC–CH₂–CH(NH₂)–COOH | 9.66             | Fe          | 1,311.25             |
| Lauric acid (12:0)        | 9.22        | CH₃(CH₂)₁₀COOH                  | Glutamic acid HOOC–(CH₂)₂–CH(NH₂)–COOH | 10.88            | Zn          | 17.58                |
| Myristic acid (14:0)      | 12.66       | CH₃(CH₂)₁₂COOH                  | Serine HO–CH₂–CH(NH₂)–COOH   | 6.86             | P           | 14,524.52            |
| Palmitic acid (16:0)      | 14.45       | CH₃(CH₂)₁₄COOH                  | Glycine NH₂–CH₂–COOH         | 9.43             | Na          | 102.97               |
| Heptadecanoic acid (17:0) | 1.39        | CH₃(CH₂)₁₅COOH                  | Histidine NH–CH=N–CH=CH₂–CH(NH₂)–COOH | 1.43             | Mg          | 132.94               |
| 10-heptadecanoic acid (17:0) | 1.12   | CH₃(CH₂)₈CH=CH(CH₂)₆COOH        | Arginine HN=C(NH₂)–NH–(CH₂)₃–CH(NH₂)–COOH | 4.15             | Mn          | 27.92                |
| Stearic acid (18:0)       | 11.41       | CH₃(CH₂)₁₆COOH                  | Threonine CH₃(OH)CH(NH₂)–CHCOOH | 2.58             | Ca          | 1,600.59             |
| Oleic acid (18:1n-9)      | 8.54        | CH₃(CH₂)₇CH=CH(CH₂)₇COOH        | Alanine CH₃–CH(NH₂)–COOH     | 7.70             | K           | 1,951.55             |
| Linoleic acid (18:2n-6)   | 4.72        | CH₃(CH₂)₄CH=CH(CH₂)₄COOH        | Proline NH–(CH₂)₃–CH–COOH    | 4.57             | Co          | 0.25                 |
| Linolenic acid (18:3n-3)  | 4.72        | CH₃(CH₂)₂(CH=CHCH₂)₃(CH₂)₆COOH  | Tyrosine HO–Ph–CH₂–CH(NH₂)–COOH | 3.26             | Cr          | 4.02                 |
| Arachidic acid (20:0)     | 7.56        | CH₃(CH₂)₁₈COOH                  | Phenylalanine Ph–CH₂–CH(NH₂)–COOH | 3.20             | Cu          | 11.08                |
| Eicosatrienoic acid (20:3n-6) | 1.49     | CH₃(CH₂)₃CH=CHCH₂(CH₂)₃COOH     | Valine (CH₃)₂–CH–CH(NH₂)–COOH | 3.56             | Ni          | 2.46                 |
| Arachidonic acid (20:4n-6) | 1.12      | CH₃(CH₂)₇CH=CHCH₂(CH₂)₂COOH     | Methionine CH₃–S–(CH₂)₂–CH(NH₂)–COOH | 6.88             | S           | 14.50                |
| Eicosapentaenoic acid (20:5n-3) | 0.36 | CH₃(CH₂)₉CH=CHCH₂(CH₂)₂COOH     | Cystine HS–CH₂–CH(NH₂)–COOH  | 3.37             | Se          | 12.32                |
| Behenic acid (22:0)       | 2.62        | CH₃(CH₂)₂₀COOH                  | Isoleucine CH₃–CH₂–CH(CH₃)–CH(NH₂)–COOH | 4.53             | Si          | 9.62                 |
|                          |             |                                 | Leucine (CH₃)₂–CH=CH–CH(NH₂)–COOH | 6.86             | Al          | 447.01               |
|                          |             |                                 | Lysine H₂N–(CH₂)₃–CH(NH₂)–COOH | 5.66             | B           | 15.77                |
|                          |             |                                 | Tryptophan Ph–NH–CH=CH–CH(NH₂)–COOH | 1.26             | Mo          | 6.07                 |

Source: Habib et al. 1997
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 propanediol (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 substrate with H₂ production is thermodynamically unfavourable unless the partial pressure of H₂ in the medium is kept low by H₂ consuming bacteria such as 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 CH₃COO⁻ and H₂, or in the case of propionate, to CH₃COO⁻, H₂ or CO₂ 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

| Component | Methanogenic reaction | Biogas (l g⁻¹) | CH₄ (%) |
|-----------|-----------------------|----------------|---------|
| Lipids    | C₅₀H₉₀O₆ + 24.5 H₂O → 34.75 CH₄ + 15.25 CO₂ | 1.425 | 69.5 |
| Carbohydrates | C₆H₁₀O₅ + H₂O → 3CH₄ + 3CO₂ | 0.830 | 50.0 |
| Proteins  | C₁₆H₂₄O₅N₄ + 14.5 H₂O → 8.25 CH₄ + 3.75 CO₂ + 4NH₄⁺ + 4HCO₃⁻ | 0.921 | 68.8 |

Source: Alves et al. (2009)
organisms that participate at this second step will be called syntrophic metabolizers. Table 5 summarizes some reactions involved in syntrophic metabolism. Normally all LCFA-s and ethanol require syntrophic acetogenesis along with two pathways: (1) $\beta$-oxidation pathway (as the main pathway) and (2) $\omega$-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 LCFA-s requires syntrophic communities of acetogenic bacteria, performing fatty acid $\beta$-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 LCFA-s 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 LCFA-s (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 principal pathway of LCFA-s degradation is via $\beta$-oxidation, but the initial steps involve in the conversion of unsaturated LCFA-s are not fully understood. According to Weng and Jeris (1976) the degradation of LCFA-s starts with the chain saturation, whereas other researchers provide evidence for direct $\beta$-oxidation of unsaturated LCFA-s (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 LCFA-s 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

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**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$^{-1}$) | $\Delta G^0$ (KJ reaction$^{-1}$) |
|-------------|---------|----------|---------------------------------|---------------------------------|
| 1           | Oleate degradation | C$_{18}$H$_{33}$O$_2^{2-}$ + 16H$_2$O $\rightarrow$ 9C$_2$H$_4$O$_2^{2-}$ + 15H$_2$ + 8H$^+$ | +391 | −131 |
| 2           | Palmitate degradation | C$_{16}$H$_{31}$O$_2^{2-}$ + 14H$_2$O $\rightarrow$ 8C$_2$H$_4$O$_2^{2-}$ + 14H$_2$ + 7H$^+$ | +419 | −81 |
| 3           | Hydrogenotrophic methanogens | 4H$_2$ + HCO$_3^-$ + H$^+$ $\rightarrow$ CH$_4$ + 3H$_2$O | −136 | −20 |
| 4           | Acetoclastic methanogens | C$_2$H$_4$O$_2^{2-}$ + H$_2$O $\rightarrow$ 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
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 5 Reactions involved in syntrophic metabolism

| Reaction input | Reaction output | $\Delta G^\circ$ [kJ per reaction] |
|----------------|-----------------|----------------------------------|
| **Hydrogen releasing reactions** |
| Ethanol and lactate |
| CH$_3$CH$_2$OH + H$_2$O | CH$_3$COO$^-$ + H$^+$ + 2H$_2$ | +9.6 |
| CH$_3$CH(OH)COO$^-$ + H$_2$O | CH$_3$COO$^-$ + HCO$_3^-$ + H$^+$ + 2H$_2$ | −4.2 |
| Fatty acids |
| CH$_3$COO$^-$ + 4H$_2$O | 2HCO$_3^-$ + H$^+$ + 4H$_2$ | +104.6 |
| CH$_3$CH$_2$COO$^-$ + 3H$_2$O | CH$_3$COO$^-$ + HCO$_3^-$ + H$^+$ + 3H$_2$ | +76.1 |
| CH$_3$CH$_2$CH$_2$COO$^-$ + 2H$_2$O | 2CH$_3$COO$^-$ + H$^+$ + 2H$_2$ | +48.3 |
| CH$_3$(CH$_2$)$_2$COO$^-$ + 3H$_2$O | 3CH$_3$COO$^-$ + 2H$^+$ + 4H$_2$ | +96.2 |
| CH$_3$CH(CH$_3$)CH$_2$COO$^-$ + CO$_2$ + 2H$_2$O | 3CH$_3$COO$^-$ + 2H$^+$ + H$_2$ | +25.2 |
| Glycolic acid |
| HOCH$_2$COO$^-$ + H$^+$ + H$_2$O | 2CO$_2$ + 3H$_2$ | +19.3 |
| Aromatic compounds |
| C$_6$H$_5$COO$^-$ + 7H$_2$O | 3CH$_3$COO$^-$ + HCO$_3^-$ + 3H$^+$ + 3H$_2$ | +58.9 |
| C$_6$H$_5$OH + 5H$_2$O | 3CH$_3$COO$^-$ + 3H$^+$ + 2H$_2$ | +5.5 |
| C$_6$H$_5$(OH)COO$^-$ + 6H$_2$O | 3CH$_3$COO$^-$ + HCO$_3^-$ + 3H$^+$ + 2H$_2$ | +5.2 |
| Amino acids |
| CH$_3$CH(NH$_2$)COOH + 3H$_2$O | CH$_3$COO$^-$ + HCO$_3^-$ + NH$_4^+$ + H$^+$ + 2H$_2$ | +7.5 |
| HOOCC(CH$_2$)(NH$_2$)CHOO$^-$ + 4H$_2$O | CH$_3$COO$^-$ + 2HCO$_3^-$ + NH$_4^+$ + H$^+$ + 2H$_2$ | −14.0 |
| (CH$_3$)$_2$CHCH$_2$(CH(NH$_2$))COOH + 3H$_2$O | (CH$_3$)$_2$CHCH$_2$COO$^-$ + HCO$_3^-$ + NH$_4^+$ + H$^+$ + 2H$_2$ | +4.2 |
| HOOCC(CH$_3$CH(NH$_2$))CHOH + 4H$_2$O | CH$_3$CH$_2$COO$^-$ + 2HCO$_3^-$ + NH$_4^+$ + H$^+$ + 2H$_2$ | −5.8 |
| HOOCC(CH$_3$CH(NH$_2$))CHOH + 7H$_2$O | CH$_3$COO$^-$ + 3HCO$_3^-$ + 3H$^+$ + 5H$_2$ | +70.3 |
| **Hydrogen-consuming reactions** |
| 4H$_2$ + 2HCO$_3^-$ + H$^+$ | CH$_3$COO$^-$ + 4H$_2$O | −104.6 |
| 4H$_2$ + HCO$_3^-$ + H$^+$ | CH$_4$ + 3H$_2$O | −135.6 |
| 4H$_2$ + 4S$^0$ | 4HS$^-$ + 4H$^+$ | −112 |
| 4H$_2$ + SO$_4^{2-}$ + H$^+$ | HS + 4H$_2$O | −151.9 |
| H$_2$ + H$_2$(NH$_3^+$)COO$^-$ | CH$_3$COO$^-$ + NH$_4^+$ | −78.0 |
| 4H$_2$ + 4 CHCOO$^-$ | 4 CH$_2$COO$^-$ | −344.6 |
| 4H$_2$ + NO$_3^-$ + 2H$^+$ | NH$_4^+$ + 3H$_2$O | −599.6 |

*a All the calculations are based on published tables (Thauer et al. 1977; Kaiser and Hanselmann 1982; Dimroth 1983)*
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. Furthermore, presence of LCFAs 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.

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

| LCFA-degrading bacteria | Morphological characteristics | LCFA utilization range |
|-------------------------|-------------------------------|------------------------|
| **Syntrophomonas sapovorans**<sup>a</sup> | Short curved rods (0.5 × 2.5 μm) | Degrades linear saturated fatty acids with 4–18 carbon atoms in co-culture with *Methanospirillum hungatei*. Mono- and di-unsaturated LCFAs, such as oleate (C18:1) and limoleate (C18:2), are also oxidized by the co-culture. |
| | Slightly motile | | |
| | Gram-negative | | |
| | Two to four flagella | | |
| | Non-spore forming | | |
| **Syntrophomonas curvata**<sup>a</sup> | Slightly curved rods (0.5–0.7 × 2.3–4.0 μm) | 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 |
| | Motile | | |
| | Gram-negative | | |
| | One or three flagella inserted in both poles | | |
| | Non-spore forming | | |
| **Syntrophus aciditrophicus**<sup>c</sup> | Rod-shaped cells (0.5–0.7 × 1.0–1.6 μm) | 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 *Methanospirillum hungatei* |
| | Non-motile | | |
| | Gram-negative | | |
| | Non-spore forming | | |
| **Syntrophomonas zehnderi**<sup>d</sup> | Curved rods (approximately 0.4–0.7 × 2.0–4.0 μm) | Degrades oleate, a mono-unsaturated fatty acid, and straight-chain fatty acids C4:0–C18:0 in syntrophic association with *Methanobacterium formicicum* |
| | 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 | | |

<sup>a</sup> Roy et al. (1986)
<sup>b</sup> Zhang et al. (2004)
<sup>c</sup> Jackson et al. (1999)
<sup>d</sup> Sousa et al. (2007a)
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 phylogenetically 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 Desulfovibrioales 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 CO₂ and CH₄, 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 CH₄. 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 CO₂ (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 m³ 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 H₂ and carbon monoxide (CO) and very often some CO₂, and has less than half the energy density of natural gas.

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 Vezirgolu 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

\[
\begin{align*}
\text{CH}_4 + \text{H}_2\text{O} & \rightleftharpoons \text{CO} + 3\text{H}_2 \\
\text{CO} + \text{H}_2\text{O} & \rightleftharpoons \text{CO}_2 + \text{H}_2 \\
\text{CH}_4(g) + 2\text{O}_2(g) & \rightleftharpoons \text{CO}_2(g) + 2\text{H}_2\text{O(l)} + \text{electricity}
\end{align*}
\]
| Anaerobic treatment types | Features | OLR (kgCOD/m² day) | HRT (days) | CH4 composition (%) | COD removal efficiency (%) | Reference |
|---------------------------|----------|--------------------|------------|---------------------|---------------------------|-----------|
| Anaerobic pond            | Almost energy free operation, 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/m²·day) | HRT (days) | CH₄ 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 flotation at high OLRs and performance depends on sludge settleability. | 10.63      | 4.0        | 54.2                | 98.4                      | Borja and Banks (1994c) |
| 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                      | Faisal and Unno (2001)  |

1. Feed Tank 9. Gas Collection bottle
2. Magnetic Stirrer 10. NaCl solution
3. Peristaltic Pump 11,12 Sampling port
4. Water Jacket 13. Effluent Collection bottle
5. Compartment 14. Pipe Y
6. Biogas Outlet 15. Water Bath
7. Baffle 16. Sewer
8. Peristaltic Pump
Table 7  continued

| Anaerobic treatment types | Features | OLR (kgCOD/m³day) | HRT (days) | CH₄ composition (%) | COD removal efficiency (%) | Reference |
|---------------------------|----------|-------------------|------------|---------------------|----------------------------|-----------|
|                           |          |                   |            |                     |                            | Basri et al. (2010) |
| 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                 |                            |           |

Scale-down anaerobic treatment

| Feature     | Details      |
|-------------|--------------|
| 1. Feed tank|              |
| 2. Water Bath|             |
| 3, 4. Peristaltic Pump| 12. Effluent |
| 5. Gas solid separator| 13. Settling tank |
| 6. UASB Portion| 14. Recycle |
| 7. UASB Portion| 15. Waste sludge |
| 8. Biogas| 16. Influent distributor |
| 9. Gas sampling port|        |

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.

| Feature     | Details      | OLR (kgCOD/m³day) | HRT (days) | CH₄ composition (%) | COD removal efficiency (%) | Reference |
|-------------|--------------|-------------------|------------|---------------------|----------------------------|-----------|
| UASBF bioreactor |              | 11.58             | 3.0        | 71.9                | 97                         | Najafpour et al. (2006) |
| Anaerobic treatment types | Features | OLR (kgCOD/m³/day) | HRT (days) | CH₄ 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 Jaafer (2006) |
| Anaerobic contact process* | Reaches steady state quickly, short HRT produces relatively high effluent quality but less 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) |
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\textsubscript{2} and O\textsubscript{2} in the presence of light energy. (2) Photo-heterotrophic bacteria (\textit{Rhodobacter} sp.) utilize organic acids (98–99\%) such as acetic, lactic and butyric acids to produce H\textsubscript{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).

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     |
| Ignitability temperature (°C)            | 650–750  | 650–750     | 450–500 |
| Inflammable limits (%)                   | 7.5–21.0 | 5–15        | 2–10    |
| Combustion air required (m\textsuperscript{3}/m\textsuperscript{3}) | 9.6     | 9.6         | 13.8    |

Source: Ma (1999a, b)

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\textsubscript{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\textsubscript{2}/L POME) | Reference               |
|-----|-----------------------------|-----|------------------|-----------------------------------------------|-------------------------|
| Dark fermentation                      |     |                  |                                              |                         |
| 1   | Mixed culture               |      | 60               | 23.82 mmol H\textsubscript{2}/(l-medium)      | Morimoto et al. (2004)  |
| 2   | Mixed culture               | 5.5  | 60               | 4.708 ml H\textsubscript{2}/(l-medium)        | Atif et al. (2005)      |
| 3   | Mixed culture               | 5.0  | –                | 0.42 l biogas/g COD\textsubscript{destroyed} with 57% hydrogen content | Vijayaraghavan and Ahmad (2006) |
| 4   | \textit{Thermoanaerobacterium} | 5.5  | 60               | 4.4 l H\textsubscript{2}/(l-medium) per day    | O-Thong et al. (2007)   |
| 5   | \textit{Thermoanaerobacterium} | 5.5  | 60               | 6.1 l H\textsubscript{2}/(l-medium per day)   | O-Thong et al. (2007)   |
| 6   | \textit{Thermoanaerobacterium} | 5.5  | 60               | 6.5                                           | O-Thong et al. (2008a, b) |
| 7   | \textit{Clostridium butyricum} | 5.7  | 36               | 6.9                                           | Chong et al. (2009b)    |
| 8   | \textit{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  | \textit{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), \( \text{H}_2 \) and \( \text{CO}_2 \) by acetogenic-anaerobic bacteria. Usually photo-heterotrophic bacteria (\textit{Rhodobacter} sp.) are used for photo-fermentation of VFAs to \( \text{CO}_2 \) and \( \text{H}_2 \) (Kapdan and Kargi 2006; Manish and Banerjee 2008). Fang et al. (2005) reported that a maximum hydrogen yield, 2.5 mol-\( \text{H}_2 \)/mol-acetate and 3.7 mol-\( \text{H}_2 \)/mol-butyrate could be achieved by \textit{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-
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
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. 2002; Mumtaz et al. 2008). During anaerobic treatment of POME, the organic acids are produced 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 transplantsations, 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 10 PHA production from POME

| Product | Microorganism           | Temp (°C) | pH | Reactor                  | Maximum production g/l | Reference                   |
|---------|-------------------------|-----------|----|--------------------------|-------------------------|-----------------------------|
| PHA     | *Rhodobacter spheroids* | 30        | 7.0| Photo-bioreactor         | ≥4                      | Hassan et al. (1996)        |
|         | IFO 12203               |           |    |                          |                         |                             |
| PHA     | *Rhodobacter spheroids* | 30        | 7.0| Photo-bioreactor         | >2                      | Hassan et al. (1997b)       |
|         | IFO 12203               |           |    |                          |                         |                             |
| PHA     | *Alcaligenes eutrophus* | 30        | 7.0| Stirred tank             | 1.8                     | Hassan et al. (1997c)       |
|         | H16 (ATCC 17699)        |           |    |                          |                         |                             |
| PHA     | *Ralstonia eutropha*    | 30        | 7.0| Bioreactor fermentation | ≥6.25                   | Hassan et al. (2002)        |
|         | ATCC 17699              |           |    |                          |                         |                             |
| PHA     | Mixed cultures          | 30        | 7.0| Sequencin-g batch reactor | 24.24                  | Md Din et al. (2006b)       |
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.

| 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 fermentation  | 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 | –  | 35        | Flask fermentation         | 120               | 0.079                   | Wu et al. (2005)   |

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 biogas 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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