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Microbial Biopolymerization Production from Palm Oil Mill Effluent (POME)

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1. Introduction

Malaysia is one of the world leaders in the production and export of crude palm oil. In Malaysia, the oil palm industry has contributed vastly towards the country's economic well being. During the economic crisis in the late 1990s, the industry has helped to cushion the impact of the economic downturn through its export-oriented activities, which provided the much needed foreign exchange for the country. Crude palm oil (CPO) production has increased from only 1.3 million tonnes in 1975, to 4.1 million tonnes in 1985 and 7.8 million tonnes in 1995 to 17.56 million tonnes in 2009 (Malaysian Palm Oil Board-MPOB, 2010).

The Malaysian palm oil industrial complex refers to the various direct linkages, processing chains and products created as a consequence of the cultivation of oil palm and the production of the main product (palm oil) and secondary products (palm kernel oil and cake). The oil palm products are employed in numerous food and non-food applications (Butler, 2006). In 2009, there were 418 crude palm oil mills, 59 refineries, 57 downstream industries and 18 oleochemical plants. The Malaysian Palm Oil Board's long-term programme is to establish a biodiesel plant that will produce methyl ester (biodiesel), which can be used to replace petroleum diesel. There were 28 biodiesel plants with a production of 2.7 million tonnes per year methyl ester, respectively (MPOB, 2010). Another potential revenue generator is to convert the large quantity of biomass (13.2 million tonnes dry weight) into added value products (Ming & Chandramohan, 2002). However, this important economic activity generates an enormous amount of liquid effluent from the milling processes (Ahmad et al., 2005). Palm oil mills with the wet milling process accounted for the major production of wastes (Kittikun et al., 2000). Hence, the increase in number of mills will generate more environmental problem.

In general, the palm oil milling process can be categorized into a dry and a wet (standard) process. The wet process of palm oil milling is the most common and typical way of extracting palm oil, especially in Malaysia. According to the industrial standard, the milling process produces wastewater in the range 0.44-1.18 m^3/tonne fresh fruit bunches (FFB) with the average figure of 0.87 m^3/tonne FFB. It is estimated that for each tonne of CPO that is produced, 5–7.5 tonnes of water are required, and more than 50% of this water ends up as palm oil mill effluent (POME) (Ahmad et al., 2003). It has been reported that for every tonne of the CPO produced, about 3.5m^3 of POME is generated, which indicates that with some 500 palm oil mills, more than 17.5 million tonnes of CPO is produced annually. It is

estimated that about 55 million m^3 of POME is generated from the palm oil industry. POME is an oily wastewater generated by palm oil processing mills and consists of various suspended components. On average, for each tonne of FFB processed, a standard palm oil mill generated about 1 tonne of liquid waste with biochemical oxygen demand (BOD) 27 kg, chemical oxygen demand (COD) 62 kg, suspended solids (SS) 35 kg and oil and grease (O&G) 6 kg (Salmiati, 2008).

Despite the fact that the palm oil industry is one of the causes of the environmental pollution, not enough has been done on its improvement. The technology applied in almost all oil palm factories is based on methods developed since the 1970s and 1980s. The major steps in the oil palm processing are as follows:

i. Threshing - Removal of fruits from the bunches. The FFB consists of the fruits that are attached onto the spikelets growing on a main stem. The fruit-laden spikelets are cut from the bunch stem using axe for manual threshing before separating the fruits from spikelets.
ii. Sterilisation - Loose fruits are sterilised in batches using high temperature wet-heat treatment. This is carried out in autoclave by steam application at 120-140 ^{o}C at 3-3.5 bar, for 75 min. Sterilisation prevents fatty acid formations and assists in fruits stripping, as well as preparing the fruit fiber for the next processing steps. Besides, sterilisation fruits stripping can be carried out by cooking in hot water. Cooking breaks down oil-splitting enzyme and stops hydrolysis and autooxidation. The fruits stem is weakened allowing easier removed of fruits from the bunches. Cooking also solidifies protein that dispersed oil-bearing cell microscopically. It weakens the pulp structure, softening it and making it easier to detach the fibrous material and its content during the digestion process. The high heat is enough to partially disrupt the oil-containing cells in the mesocarp and permits oil to be released more readily.
iii. Crushing process - the palm fruits will be passed through shredder and pressing machine to separate oil from fibre and seeds.
iv. Digestion of the fruit - This process releases palm oil in the fruit through cracks the oil-bearing cells. The digestion consists of a steam-heated cylindrical vessel with central rotating shaft that is filled with several beater arms. The fruit is pounded by the rotary beater arms at high temperature, which reduces the oil viscosity. This destroys the exocarp fruits or the outer covering and completes the disruption of the oil cell already begun in the sterilization process. The digestor must be filled to ensure the maximum of storage and the effect of the agitation.
v. Extracting the palm oil – There are two distinct methods of extracting oil from the digested material. One system uses mechanical presses and is called the "dry" method. The other called the "wet" method uses hot water to leach out the oil.
vi. Kernel recovery - The residue from the press consists of a mixture of fibres and palm nuts which are then sorted. The sorted fibres are covered and allowed to heat by own internal exothermic reactions for about two or three days. The fibres are then pressed in spindle press to recover second grade (technical) oil that is used normally in soap-making. The nuts are usually dried and sold to other operators who process them into palm kernel oil.
vii. Refining process - Refining converts crude palm oil (CPO) into refined oil. The refined oil is then processed to segregate fat and obtained refined palm oil. The refined palm olein obtained through fractionation process is used in related industries.

viii. Oil Storage - Palm oil is stored in large steel tanks at 31 to 40°C to keep it in liquid form during bulk transport. The tank headspace is often flushed with CO_2 to prevent oxidation. Higher temperatures are used during filling and draining tanks. Maximum storage time is about 6 months at 31°C.

Governments have made various initiatives to reduce the undesired environmental pollution particularly among major corporations. An efficient treatment system is highly desirable in all palm oil mills in order to control the discharge of effluent to water bodies. The final effluent should meet the standards set by the authority, in the case of Malaysia, Department of the Environment. Alternative treatment with value added package will ensure that the treatment of the effluent will be more efficient, innovative and attractive for the mill's owners. The formulation of the CPO mill effluent discharge standards by phases has brought about a catalytic impact in the development of effluent treatment technology in the form of innovative or newly created technology (Yeoh, 2004; Yusoff, 2006, Ahmad et al., 2008). Some of the POME treatment options are described in Table 1.

Objectives		**Options technology**	**Impacts**
a) Treatment	Primary treatment	• Waste stabilization ponds (anaerobic, aerobic, facultative)	• odour problems • large footprint • Insufficient effluent quality • colour • low operation and maintenance cost
	Secondary treatment	• Enhanced waste stabilization ponds (aerated lagoon) • Up-flow anaerobic sludge bioreactor (UASB) • Sequencing batch reactor (SBR) • Extended aeration activated sludge	• large variation in effluentquality • insufficient effluent quality • high operation and maintenance cost • high power consumption • insufficient for colour removal
	Advanced treatment	• Membrane bioreactor (MBR) • Membrane filtration • Adding chemical and biochemical produts	• high operation and maintenance cost • need pre-treatment • small area required • good quality for reuse • advanced operation and maintenance
b) Resource recovery	Reuse	• Fertilizer • Animal feed	• Low digestibility of protein • Slow down their composition • High lignin content
	Recovery	• Volatile fatty acid • Polyhydroxyalkanoates • Biogas • Methane	• Produce value-added product • Reduce cost treatment that needs further treatment

Table 1. Options for POME treatment, reuse and recovery

Cleaner production (CP) inventiveness was introduced since 1990s. A study to develop a sustainable CP policy and framework was conducted and implemented to all indigenous industries including palm oil (Ujang, 2008). The cleaner production has been implemented in the crude palm oil industry through the support of government and international donor agencies. The result is an increasing number of mills that implement cleaner technology strategies and approaches. Although there are obvious environmental and often also economic benefits in implementing cleaner production strategies, cleaner production can and often does entail investments.

Currently, recovery of renewable organic-based product is a new approach in managing POME. The technology is aimed to recover by-products such as volatile fatty acid, biogas and polyhydroxyalkanoates to promote sustainability of the palm oil industry. In addition, it is envisaged that POME can be sustainably reused as a fermentation substrate in production of various metabolites through biotechnological advances. In addition, POME consists of high organic acids and is suitable to be used as a carbon source (Alias & Tan, 2005: Md Din et al., 2006a).

2. Sources of POME

The two main wastes resulting from palm oil production in a mill are the solid and liquid wastes. Solid wastes typically consist of palm kernel shells (PKS), mesocarp fruit fibres (MF) and empty fruit bunches (EFB). The liquid waste generated from the extraction of palm oil of wet process comes mainly from oil room after separator or decanter. This liquid waste combined with the wastes from steriliser condensate and cooling water is called palm oil mill effluent (POME). Figure 1 shows the different point sources of waste in palm oil milling.

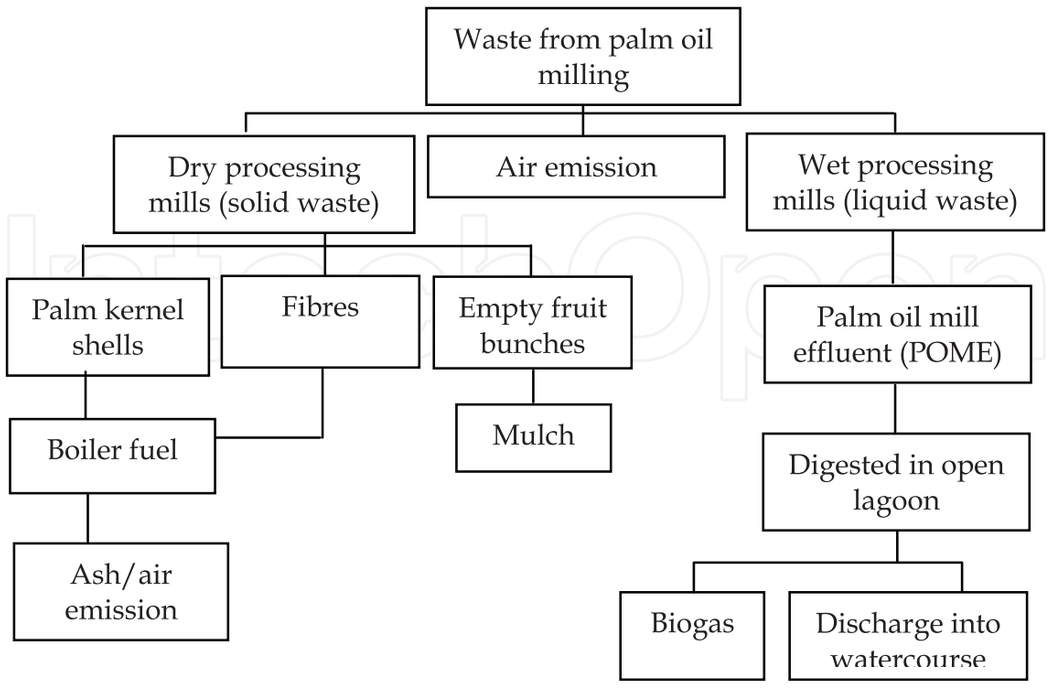

Fig. 1. Sources of waste from palm oil milling

Large quantities of water are used during the crude oil extraction process. Up to about 1.5 cubic meters of water are characteristically used to process one tonne of FFB. From this quantity, about 50% of the water results in the POME, the other 50% being lost as steam, mainly through sterilizer exhaust, piping leakages, as well as wash waters (Anonymous, 1999).

POME comprises a combination of the wastewaters which are principally generated and discharged from the following major processing operations as follows (DOE, 1999; Salmiati, 2008):

- Sterilization of FFB - sterilizer condensate is about 36% of total POME or about 0.9 tonnes POME for each produced tonnes of palm crude palm oil;
- Clarification of the extracted CPO - clarification wastewater is about 60% of total POME (approximately 1.5 tonnes of sludge obtained per tonnes of produced crude palm oil); and
- Hydrocyclone separation of cracked mixture of kernel and shell-hydrocyclone wastewater is about 4% of total POME.

The ratio from the mixture of sterilizer, condensate and separator sludge wastewater is 9:15:1 respectively (Wu et al., 2010). There are other minor sources of relatively clean wastewater that may be included in the combined with POME, which is sent to the wastewater streams. These include turbine cooling water and steam condensates, boiler blow-downs, overflows from the vacuum dryers and some floor washings. The volume of the combined POME discharged depends to a large extent on the milling operations.

3. Characteristic of POME

Palm oil mill effluent (POME) is mainly generated from sterilisation, hydrocyclone and clarification processes in which large amounts of steam and/or hot water are used (Ma, 1999). Distinctive quality characteristics of the individual wastewater streams from the three principal sources of generation are presented in Table 2. POME, when fresh is a thick brownish in colour colloidal slurry of water, oil and fine cellulosic fruit residues. POME is generated from mill operation at a temperature of between 80°C and 90°C and it is slightly acidic with a pH between 4 to 5 (Md Din et al., 2006a). The characteristics of a usual raw combined POME are presented in Table 3 which attests that POME has a very high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), which is 100 times more than the municipal sewage. POME is a non-toxic waste, as no chemical is added during the oil extraction process, but will pose environmental issues due to large oxygen depleting capability in aquatic system due to organic and nutrient contents.

The high organic matter is due to the presence of different sugars such as arabinose, xylose, glucose, galactose and manose at the concentrations of 6.43, 0.44, 0.22, 0.15 and 0.10% dry weight, respectively (Agamuthu & Tan, 1985). The oil residue was in the range of 1-2% which depends very much on the quality of raw material (palm fruits), process control and machine efficiency. The suspended solids in the POME are mainly oil-bearing cellulosic materials from the fruits (Ma, 1999). Since the POME is non-toxic as no chemical is added in the oil extraction process, it is a good source of nutrients for microorganisms.

However, using BOD as a characteristic of POME was conventional or old method. Recently, Damayanti et al. (2010) used respirometric test to estimate model parameters for activated sludge modelling of POME. The study found the heterotrophic yield coefficient and some of the COD fractionations of POME are described in Table 4. These coefficients could be serving as basis for design and optimization of a POME treatment process.

Parameters	Sterilizer condensate	Oil clarification wastewater	Hydrocyclone wastewater
pH	5.0	4.5	-
Oil and Grease	4,000	7,000	300
$BOD_{3\text{-}day}$; 30°C	23,000	29,000	5,000
COD	47,000	64,000	15,000
Suspended solid	5,000	23,000	7,000
Dissolved solids	34,000	22,000	100
Ammonical nitrogen	20	40	-
Total nitrogen	500	1,200	100

All units are in mg//L except for pH
Source: DOE (1999)
Table 2. Characteristics of individual wastewater streams

General Parameters	Value	Metals & other Constituents	
		Element	Value
pH	4.2	Phosphorus	180
Oil & Grease	6000	Potassium	2270
BOD_3 @ 30°C	25000	Magnesium	615
COD	50000	Calcium	440
Total Solids (TS)	40500	Boron	7.6
Suspended Solids (SS)	18000	Iron	47
Total Volatile Solids (TVS)	34000	Manganese	2.0
Ammoniacal Nitrogen (AN)	35	Copper	0.9
Total Nitrogen (TN)	750	Zinc	2.3.

Note: All parameter's unit in mg/l except pH
Source: DOE (1999)
Table 3. Characteristics of combined POME

Model coefficient	Values
Total COD (mg/L)	45,000
S_s (mg/L)	50
S_I (mg/L)	16,600
X_s (mg/L)	25,550
X_I (mg/L)	2,800
Y_H (g COD oxidized)$^{-1}$	0.44
Cell COD	14,100
μ_A (/day)	0.76
μ_H (/day)	0.78
K_s (mgCOD/L)	100
b_H (/day)	0.33

Source: Damayanti et al. (2010)
Table 4. Estimated model parameters and state variable of POME

4. The science of biopolymer

The usage of conventional plastics has resulted in environmental degradation (Serafim et al., 2006). The production of biodegradable polymers is seen to be a viable alternative with the increasing environmental pressure to replace conventional plastics. The problem that is faced by industry is the high production costs of biopolymers. Biodegradable polymers derived from polyhydroxyalkanoates (PHAs) are considered to be good candidates for biodegradable plastics due to their large range application and capability of being produced from renewable resources (Dionisi et al., 2005; Bengtsson et al., 2008; Albuquerque et al., 2007). These materials have attracted interest because of their potential use as biodegradable alternatives to petroleum-based synthetic plastics such as polypropylene and polyethylene. Polyhydroxyalkanoates (PHAs) are mainly produced by microbial fermentation processes, and a major challenge is to reduce their production costs (Verlinden et al., 2007). A feasibility study using fermentative volatile fatty acids (VFAs) as carbon source to synthesise PHA by activated sludge was carried out to simultaneously reduce the production cost of PHAs and disposal amount of organic wastes (Salmiati et al., 2010).

Several efforts have been investigated to produce PHAs by microbial fermentation on organic wastes (palm oil mill effluent (POME), olive oil and kitchen wastes). The production of biodegradable polymers from oil palm industry can be seen as beneficial to the environment as well as contributing to sustainable development (Yu, 2003; Salmiati et al, 2007). Until recently, the remaining 90% (empty fruit bunches, fibres, fronds, trunks, kernels, POME) was discharge as waste, and either burned in the open air or left to settle in waste ponds (MPOC, 2007, Salmiati et al., 2010). By utilizing the POME and empty palm oil fibre bunch (EPFB) as carbon source and support matrix, the disposal of POME that needed further treatment could be reduced.

Although POME consists of high organic acids and is suitable to be used as a carbon source, POME is usually present in a complex form that cannot directly be utilised by PHA-producing bacterial species for PHA synthesis. Typically, raw POME is difficult to degrade because it contains significant amounts of oil (tryacylglycerols) and degradative products such as diacylglycerols and monoacylglycerols and fatty acids . (Alias & Tan, 2005; Salmiati et al, 2007). The fatty acids composition (C_{12} - C_{20}) of each of this fraction are different from one another and contribute to a high value of pollution load in POME. Therefore, anaerobic treatment has been proposed to reduce the POME characteristics. It is one of the naturally occurring processes involving decomposition and decay, in which complex organic matters are broken down into their chemical constituents. Hydrolysis and acidogenesis are the first step to convert the wastes to short-chain VFAs (i.e acetic, butyric and propionic acids). After that, the VFAs will be utilised by PHA-producers for PHA production (Bengtsson et al., 2009; Salmiati et al., 2010).

Considering the interest of using fermented substrate as carbon source for production of PHA, therefore, this study is aimed at understanding the mechanism of VFA influences on the yield of polymer production, and to optimise the PHAs production using fermented POME as carbon sources using mixed cultures. An improved system containing two separate bioreactors to satisfy the different physiologies and metabolic activities of the two types of microbes; one for acidogenesis of POME and a second for mixed microbial culture of PHA-producing strains was investigated.

5. Biopolymer production using POME

Ideal life cycle of eco-friendly exposure for PHA bioplastic made from renewable resources likes POME is a closed-loop process (as depicted in Figure 2). The production of bioplastic will subsequently serve as feed to a microbial fermentation process (at the end of cycle), to promote the environmental-friendly effect. Ideally, this process occurs aerobically (in natural and tropical conditions), yielding water (H_2O) and CO_2 in the same proportions that were originally used in photosynthesis. The harmless end products could also be generated from microbial fermentation to produce biofuel energy.

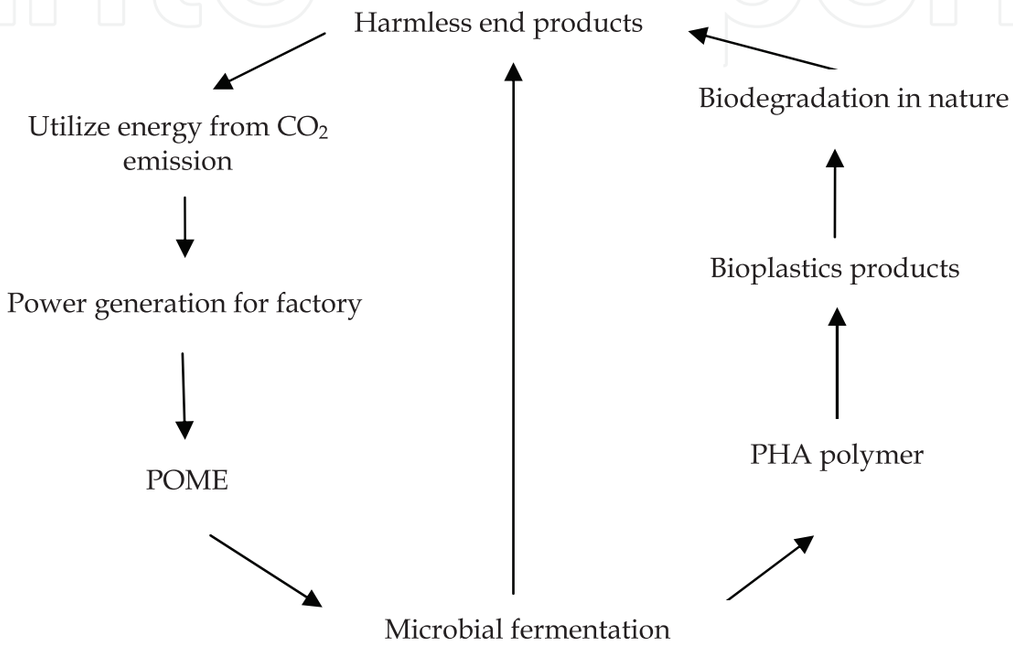

Fig. 2. Proposed cycle loop of regenerating waste from POME to biodegradable plastics, end-up with preventing pollution load to environment

The price of PHAs is mainly dependent on substrates cost, accounting for about 40% of the total production cost (Md Din et al., 2006a). In the last decade, a variety of low cost carbon substrates (e.g., starch, tapioca hydrolysate, whey and molasses) have been tested for PHA production to reduce the production cost. POME can be considered as an alternative, no cost reusable substrate for PHA production. The production of VFA and PHA using POME as substrates are listed in Table 5. According to Hassan et al., (1997a), with a content of 50% PHA in the dried cells and 2% dissolved in the chloroform, the calculated minimum cost for obtaining PHA from POME is below USD2/kg. By increasing the PHA content in the cell from 50 to 80%, the unit cost of PHA could be slightly reduced; whereas an increase in the amount of PHA dissolved in chloroform from 2% to 5% would result in a remarkable reduction of the PHA cost to less than US1/kg.

Nevertheless, POME is usually presented in complicated forms that cannot be directly reused by PHA-producing species such as *Ralstonia eutropha*, a representative bacterium for PHA synthesis (Salmiati et al., 2007). It was proposed that an anaerobic treatment of POME could be coupled with PHA production using heterotrophic bacteria to reduce PHA

Product	Microorganism, Fermentation medium and Condition based on POME	Maximum production	Reference
VFA	Mixed cultures, POME + palm oil sludge. 30°C, pH was controlled at 7, SBR, 24 h	7.8 g/L	Hassan et al. (1996)
VFA	Mixed cultures, POME + palm oil sludge in the ratio 1:1. 300 rpm, 30°C, pH was controlled at 7, stirred tank bioreactor fermentation. 84h	10 – 14 g/L	Yee et al. (2003)
VFA	Mixed cultures, POME + palm oil sludge, pH was controlled at 7. SBR. 96h	10.27 g/L	Cheong et al. (2004)
PHA	*Rhodobacter sphaeroides* IFO 12203, Synthetic waste based on organic acids profiles obtained during POME treatment. 30°C, pH was controlled at 7, photobioreactor fermentation, ≈ 200h	≈ 4g/L	Hassan et al. (1996)
PHA	*Rhodobacter sphaeroides* IFO 12203, Anaerobically digested, 30°C, pH was controlled at 7, photobioreactor fermenetation.	>2g/L	Hassan et al. (1997a)
PHA	*Alcaligenes eutrophus* H16(ATCC 17699), Standard medium with feeding of acetic acid obtained from anaerobically digested POME. 400 rpm with aeration rate of 0.75L/min. 30°C, pH was controlled at 7, stirred tank bioreactor fermentation, 17h	1.8 g/L	Hassan et al. (1997b)
PHA	*Ralstonia eutropha* ATCC 17699, Concentrated organic acids from anaerobically digested POME (100g/L of total acids with acetic:propionic = 3:1), 400 rpm with aeration rate of 0.75L/min. 30°C, pH was controlled at 7, bioreactor fermentation, ≈65h	≈ 6.25g/L	Hassan et al. (2002)
PHA	Mixed cultures, High concentration of POME with 490 COD/N ratio (g COD/ g N) and 160 COD/P ratio (g COD/ g P), 1000 rpm with aeration rate of 1.5L/min, 30°C. pH was controlled at 7. SBR	24.24 g/L	Md Din et al. (2006b)

Table 5. Various products in bioprocesses using POME as substrates

production costs (Salmiati, 2008). According to Hassan et al. (1996), it was critical to maintain the pH at 7 in the anaerobic treatment of POME by sludge in the first stage of the process, in order for only acetic and propionic acid to be produced and not formic acid and biogas. With increasing concentrations of formic acid (for a pH maintained below 4), the PHA yield and content in *Rhodobacter sphaeroides* IFO 12203 dropped from 0.50 g/g and 67% to 0.21 g/g and 18%, respectively. Hassan et al. (1997b) later found that the presence of sludge in the anaerobically treated POME inhibited PHA accumulation by *R. sphaeroides* IFO 12203. This was attributed to the PHA being produced in a POME without sludge as opposed to a treated POME with sludge. A low concentration of ammonium would accelerate the PHA production in a synthetic waste with an organic acid profile, which was

observed during POME treatment. However, Hassan et al. (1997b) found that addition of ammonium and phosphate to anaerobically treated POME was required to maintain the cell activity and production of PHA since neither ammonium nor phosphate was present in the anaerobically treated POME. In total, the organic acid concentrations obtained from anaerobically treated POME were too low (Hassan et al., 1996; Salmiati et al., 2010) for it to be reused as raw material in the production of PHA on an industrial scale. The underlying reason was that this would require a production reactor with a much larger size than that of a reactor for normal bioplastic production.

The organic acids in the anaerobically digested POME could be concentrated by evaporation for use as substrates in the fed-batch non-sterile PHA fermentation system using *R. eutropha* ATCC 17699. Although the proposed overall zero emission system appeared to be practical, major drawbacks were found, including the rather low yield and productivity of PHA by *R. eutropha* when the concentrated organic acids from POME were used as compared to synthetic organic acids. This could be due to the high presence of ammonium (1.5 g/L) or other compounds in the anaerobically digested POME concentrate (Hassan et al., 2002).

Md Din et al. (2006b) proposed the suitability of using mixed cultures to produce PHA in POME since most prokaryotes are capable of PHA production. The study noted that by using mixed cultures and POME, different types of PHA-constituents could be obtained. The harvesting of these PHA-constituents was more reliable for use as biodegradable plastics material as opposed to a single PHA-constituent. A type of mixed culture was maintained in a SBR, and a high concentration of POME proposed for this system in order to generate autotrophic rather than heterotrophic bacteria in the production of PHA. However, the average PHA production by using POME could only reach 44% of the CDW, indicating that an optimisation of the PHA sludge content must be carried out by varying the oxygen rate, feeding regime or transient conditions.

Acidogenic fermentation POME and conversion of the produced VFAs to PHA by mixed microbial cultures has also been studied (Salmiati et al., 2007). VFA characterisation has been conducted using fermented POME in anaerobically digestion. Earlier studies have shown that the VFAs (acetic, butyric, propionic, etc) have been used as carbon source by bacteria, as individual or mixed of the VFAs (Dionisi et al., 2005; Salmiati, 2008).

6. Reactor design

In order to investigate the PHA production in POME and mixed culture, two bioreactors have been designed and used to meet metabolic activities in one cycle. The anaerobic part was designed in a cylindrical shape with a working volume of 19 litres, interior diameter 19.5 cm and height 90 cm. The sampling ports were designed along 10 cm height intervals from the bottom. A perforated piping system was used at the bottom of the reactor to ensure homogenous distribution of flow into the reactor and no recirculation of effluent was practiced. Support materials used in the fixed bed, which are: oil palm fibres; spherical in shape with approximately 1 cm diameter (total specific surface area was $0.15 cm^2 m^{-3}$). The acidogenesis process was conducted for 45 days for the inoculation. The reactor was operated at room temperature (28 ± 2^oC) and at varied SRT/HRT. At each HRT, the reactor was operated for six weeks to reach steady state conditions. Steady state conditions were established when the variation in the product concentration were constant (effluent VFAs and COD concentrations). Typically, the reactor was operated without pH control and aeration nor stirring to avoid the acetogenesis process that transforms VFA to other forms.

Acidic slurry produced in anaerobic reactor was then pumped to the aerobic reactor as substrate feeding for the microorganisms that produce PHA. This supply can be varied depending on each SRT/HRT.

The aerobic part was fabricated in two double-jacketed laboratory-scale reactor with six litres effective volume. Figure 3 depicts the schematic drawing of reactor set-up. The operation of aerobic reactor was based on the sequencing batch reactor (SBR) system under feast-famine regime which was conducted on samples taken from fermented POME from anaerobic reactor. The dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% = 9.1 mgL^{-1}). DO concentration and pH were measured continuously. In order to control the oxygen concentration properly, the gas concentrations was controlled using gas flow meter.

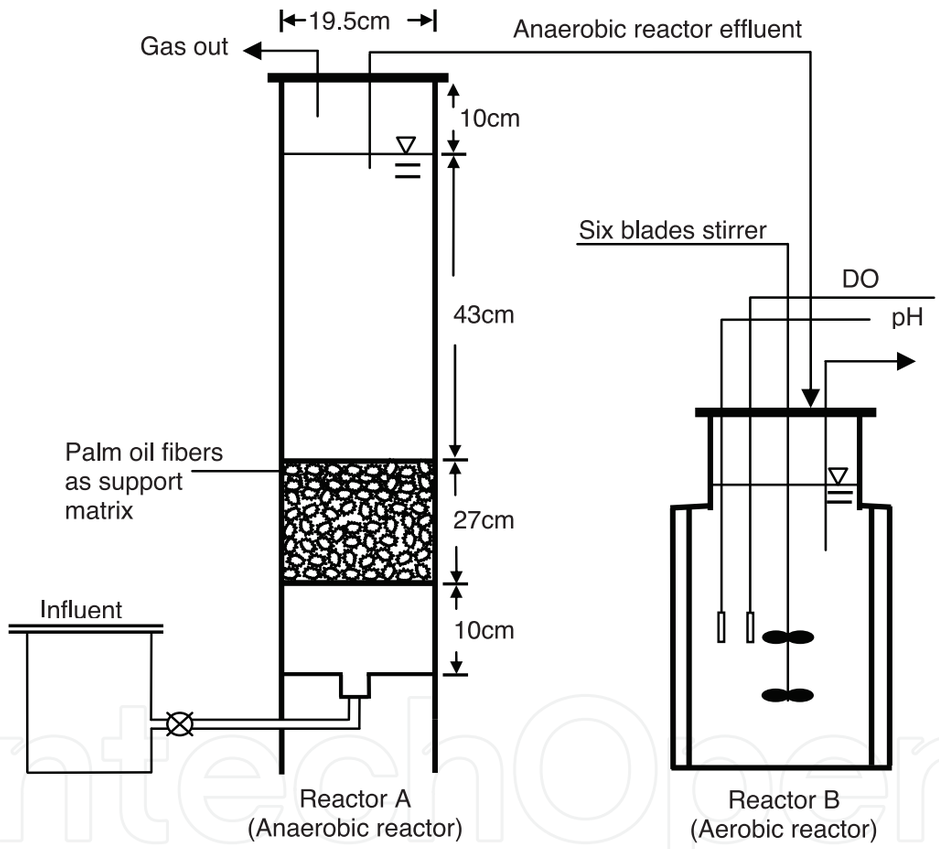

Fig. 3. Anaerobic and aerobic reactors used for the PHA production POME

Raw POME and waste sludge from a third sludge pond was collected from a local palm oil mill wastewater treatment plant. A fresh activated sludge taken from an aeration tank of a local municipal wastewater treatment was used as seed sludge for both of reactor. Waste sludge, POME and sewage sludge were introduced as inoculate to acclimatise the autotrophic and/or heterotrophic bacteria in each reactor. The ratio of the inoculums is 1:2:1. For the anaerobic reactor, the substrates were fed into the reactor at the bottom and the culture medium contained supernatant discharge circulated at the rate of about 40 mL/min through a granulated sludge bed. A perforated piping system was used at the bottom of the reactor to ensure homogenous distribution of flow into the reactor and no recirculation of

effluent was practiced. For the aerobic reactor, at least more than 50% of the working volume must be designed to be discharged as supernatant. The operating principles of a batch activated sludge system are characterised in just three discrete periods: fill, react and draw (discharging). After two to three days, the aerobic system was continuously operated by supplying nutrient adaptation for several weeks, in order to reach a steady-state condition. At steady-state conditions, some parameters has been extensively monitored (e.g. pH, DO, and samples was collected every hours for COD, TOC, NH_4^+-N, P, VFAs CDW and ash constituent analysis). In order to maximise the growth rate and fast substrate uptake rate and storage polymer formation, the system was operated in continuous reaction period, which means no settling or allowing the idle phase (HRT=SRT). The length of HRT and SRT in each treatment depends on the microorganisms' reaction after feeding. The cycle of operation system depends on substrate concentrations. The detailed features of the aerobic are described in Table 6.

Reactor	**Anaerobic reactor**	**Aerobic reactor**
Working volume	19-L (laboratory scale)	6-L (laboratory scale)
Influent feed	Raw POME	VFA (fermented POME)
Temperature	28 ± 2^{o}C	28 ± 2^{o}C
pH	4.0 – 10.0	7 ± 0.1
Aeration and DO	No aeration	1 – 1.7 Lmin^{-1}
HRT /SRT	7-8 hours/ 5-10 days	1 hour/ 4-8 hours
Stirrer	-	300 - 400 rpm

Table 6. Reactor description and condition of each experiments

The acidic supernatant is pumped into the reactor and mixed with biomass that settled during the previous cycle until the time for filling is reached. A mineral solution with composition was added for the growth phase only (Md Din et al., 2006b; Salmiati, 2008). In general, the overall operation period of fermented POME cultivation is shown in Table 7. The filling phase can be mixed in either aerated (oxygen as electron donor) or microaerophilic-aerobic (controlling the oxygen level) conditions. In order to control the oxygen concentration properly, the gas concentrations was controlled using gas flow meter. The reaction phase can also be mixed in similar condition with the common environment needed by bacteria to live. This reactor was equipped with peristaltic pumps for influent feeding and effluent withdrawal, and air compressor was employed for aeration. The procedures of reactor operation, such as feeding, aerating and withdrawing were controlled automatically by timers and temperature was maintained at 28 ± 2^{o}C in a temperature controlled-room. The dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% = 9.1 mgL^{-1}). DO concentration and pH were measured continuously.

Samples were taken from the reactor with a 60 mL syringe (Syphon, United Kingdom). The syringe was always rinsed with the content of the reactor before sampling. Part of the sample was stored in the refrigerator for analysis. The remaining supernatant was centrifuged at 10,000 rpm for 10 minutes. The centrifugation for separating the debris and supernatant was performed using Sorval RC-5B (Hermmicks, Germany) for 15 minutes at 2000 rpm and 4°C. The supernatant was then filtered by using PVDF-syringe filter. Samples

for analysis of NH_4-N, PO_4-P, TOC and COD and VFA were immediately centrifuged and filtered using 0.45 µm filters to separate the bacterial cells from the liquid, 0.2 µm cone-syringe filters were used for soluble analysis. The supernatant was stored in the refrigerator at -4°C (for PHA analysis) and at -5°C (for VFA, MLSS, MLVSS, CDW, NH_4^+, NO_3^-, PO_4^{2-} and COD). All analytical measurements performed in this study were conducted according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2000).

Experiment (s)	Operating time (min)			
	Aerobic mineral feeding	**Aerobic feeding**	**Aerobic reactor**	**Draw/discharge**
Growth	0 – 60	0 - 60	60 -330	330 - 340
DO (pretreated POME)	No fill	-	0 - 600	-
Mic ae (pretreated POME)	No fill	-	0 - 500	-

Table 7. Operating phase with POME as substrate

The composition and concentration of lipids in POME measured using gas chromatography according to the type of carbon chain. The quantification of TSS performed using volatile suspended solid (VSS) and ash technique according to the Dutch Standard (NNI, NEN) and APHA (2000). VFA (acetic acid [HAc], propionic acid [HPr], *iso*-butyric acid, butyric acid [HBu], *iso*-valeric acid, valeric acid [HVa] and caproic acid) were quantified with a Varian 3400 gas chromatograph after filtration through filters with pore size 1.6 µm (Munktell MGA). The injection volume was 2 µL and the gas chromatograph was equipped with a Chromosorb 101 (80/100 mesh) column (length: 2.5 m, diameter: 2.3 mm) and a flame ionization detector. Nitrogen gas saturated with formic acid was used as carrier gas (30 mL/min). The temperatures of the injector and detector were 240 and 250 °C, respectively. The column temperature was 170 °C for the initial 2 min and was then increased at 10 °C/min to 200 °C and retained for 3 min. PHA determination was conducted according to the Comeou et al. (1988) and PHA concentration was determined according to the method of Braunegg et al. (1998) using benzoic acid as internal standard. CDW was determined as described previously by Wong (2001), and synthesis of PHA was analysis according to the method of Yu & Chen (2006).

7. COD Removal and VFA production from fermented POME

Organic wastes are usually complex in nature, and cannot be directly utilized by PHA-producing microbes for PHA synthesis. POME consists of high organic acids; therefore it is suitable to be used as a carbon source. Typically, raw POME is difficult to degrade because it contains significant amounts of oil (tryacylglycerols) and degradative products such as diacylglycerols and monoacylglycerols and fatty acids (Alias & Tan, 2005; Salmiati et al, 2007). The fatty acids composition (C_{12} - C_{20}) of each of this fraction are different from one another and contribute to a high value of pollution load in POME. The typical characteristics of raw POME are given in Tables 8 and 9 showing the composition of lipids in POME as used in this study. POME is usually present in a complex form that cannot directly be utilized by PHA-producing bacterial species for PHA synthesis. Therefore, anaerobic treatment has been proposed to reduce their POME characteristic. It is one of the naturally

occurring processes involving decomposition and decay, in which complex organic matter is broken down into its chemical constituents (Tay et al., 1996). Hydrolysis and acidogenesis are the first step to convert the wastes to short-chain VFAs (i.e acetic, butyric and propionic acids). After that, the VFAs will be utilized by PHA-producers for PHA production (Lee & Yu, 1997; Yu, 2003).

Parameters	Units	Results
pH	-	4.8 ± 0.21
Total suspended solids	mg/L	35,000 ± 200
Turbidity	NTU	21,000 ± 300
COD	mg/L	65,000 ± 800
BOD_5	mg/L	27,000 ± 800
O&G	mg/L	8,000 ± 300
TOC	mg/L	12,300 ± 570
Phosphorus	mg/L	142 ± 19
Ammonia Nitrogen	mg/L	62 ± 10

Note: values represent means of triplicate determination
Table 7. Characteristics of raw POME obtained from a local palm oil mill factory used in the study

Lipids	Concentrations (%)
Tryglycerides	81.5
Diglycerides	7.0
Monogliycerides	0.5
Free fatty acids	11.0

Table 8. The composition and concentration of lipids in POME used in this study

The fermentation reactor started to produce acids immediately after inoculation. The fermenter biomass consisted of a large number of small free-living bacteria and some small aggregates. The COD removal achieved was as high as 80% in approximate SRT of 6 days, as shown in Figure 4. The COD removal actually occurred during the fermentation process where a high amount of COD removal was used in the generating and synthesizing new bacterial cells for the anabolism route (de la Rubia et al., 2006). This process begins with the hydrolysis of complex organic compounds in the initial POME to more soluble intermediates. Through the process of acidogenesis, these intermediates are broken down primarily into VFAs and other monomer species.
However, the differences in SRT influenced the VFA results; the concentration of the VFA was increased significantly for the SRT of 6 days and the plateau was reached after about 10 days of the fermentation. Then the production rate decreased after 11 days. As shown in Figure 4 where at SRT of 11 days, acidogenic conditions prevail while at SRT ≥ 11 days, methanogenic conditions prevail. It shows that the SRT strongly affects the type and rate of bioconversion process under anaerobic conditions (Miron et al., 2000).
Figure 5 shows the relative distribution of individual acids within VFAs at the end of fermentation. With regard to POME with pretreatment using oil palm fibre, four organic acids were detected as the major fermentative products. Among them, acetic acid was the

main product in the whole range of initial concentrations (50-57%), with lower relative amount of butyric acid (25-33%), propionic acid (4-6%) and valeric acid (1-3%). Isobutyric and lactic acids were in negligible quantities or not produced at all.

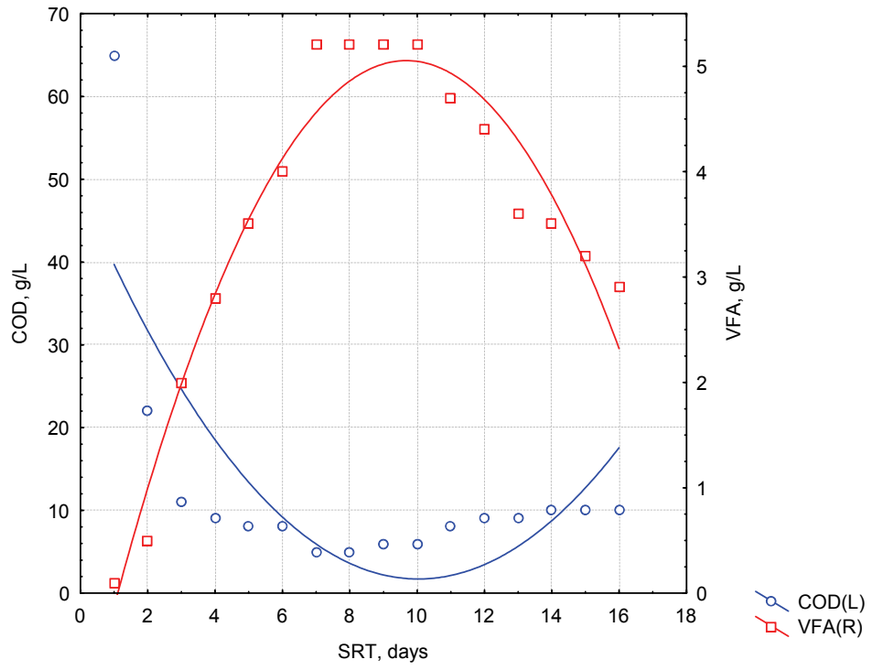

Fig. 4. Profile of VFA and COD concentration using anaerobic process at room temperature

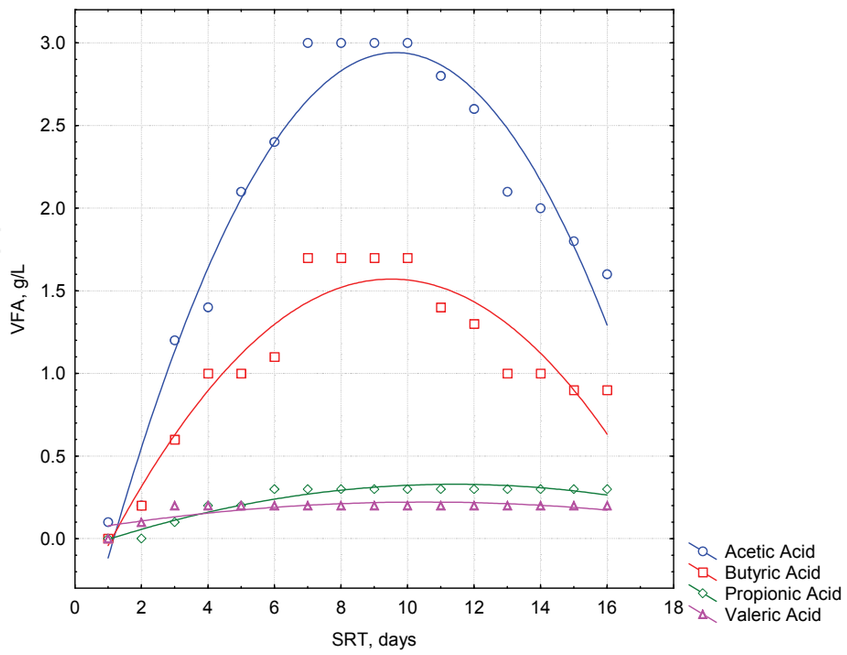

Fig. 5. The time courses of acidification of POME under anaerobic conditions

The VFAs in the anaerobic reactor are shown in Figure 5. The predominant VFA products in the effluent of this anaerobic reactor are acetic and butyric acids and their concentrations reached 3.0 g/L and 1.7 g/L starting after six days of the fermentation process and decreased after 12 days. About 0.3 g/L of propionic acid and 0.2 g/L of valeric acid were accumulated in anaerobic digestion and these are kept at a constant level for most of the time. The concentrations of propionic and valeric acids were relatively low in the effluent. The result indicated that even-numbered carbon fatty acids degraded more easily than odd-numbered carbon fatty acids. In addition, the degradation of the even-numbered fatty acids was not a rate-limiting step (Shin et al., 2001).

The different VFA distribution can have a great influence on the composition of the polymer produced in the subsequent steps, namely the percentage of HV monomers within the copolymer P(HB-HV). Indeed, VFAs containing an even number of carbon atoms (i.e. acetic and butyric acids) mostly lead to formation of HB monomers, whereas VFAs containing an odd number of carbon atoms (i.e. propionic and valeric acids) mostly lead to the formation of HV monomers (Yu, 2003; Dionisi et al., 2005). As an increase of HV content within the copolymer generally improves its thermal and mechanical properties, the negative effect of centrifugation on propionic and valeric acids production has to be considered along with the important beneficial effects discussed above (Dionisi et al., 2005).

VFA characterisation has been conducted using fermented POME in anaerobic reactor. Table 10 shows the composition of VFAs found in this study. Fermented POME composition constitute up to 50% of acetic acid, 30% of butyric acid, and 10% of propionic and lactic acids. Earlier studies have shown that the VFAs (acetic, butyric, propionic, etc) have been used as carbon source by bacteria, as individual or mixed or of the VFAs (Yu, 2003; Dionisi et al., 2005; Bengtsson et al., 2008).

Systematic name	**Trivial name**	**Compound**	**Values**
Ethanoic Acid	Acetic acid	CH_3COOH	57
Butanoic Acid	Butyric acid	C_3H_7COOH	33
Propanoic Acid	Propionic acid	C_2H_5COOH	6
Pentanoic Acid	Valeric acid	C_4H_9COOH	4

Table 10. VFAs composition (%) of fermented POME in Anaerobic reactor

For the cultivated fermented POME composition, an overall effect on growth and accumulation factor has been proposed, as shown in Figure 6. The concentration of PHA biomass component was determined as PHB and the value obtained (gPHB/gVSS) from the system could reach up to 4.0 x 10^{-2} g/gVSS. Under this situation, the cell growth (active biomass) increased with the initial feed of VFAs and reached steady after five hours, ranging from 2 to 5 g/L. Immediately, the PHB content increased to the maximum levels up to 4.20 x 10^{-2} g/g VSS, before sharply decreasing to 2.0 x 10^{-2} g/g VSS.

The low concentration of PHB during this preliminary experiment may be affected by low concentration of VFAs in the medium. However, the type of polymer was homopolymer (PHB). This suggests that the mixture of acetic acid and butyric acid is a good carbon source for PHA synthesis, but unlike propionic acid, valeric acid cannot be used for synthesis of hydroxyvalerate (HV) monomer, even though both acids have three carbons.

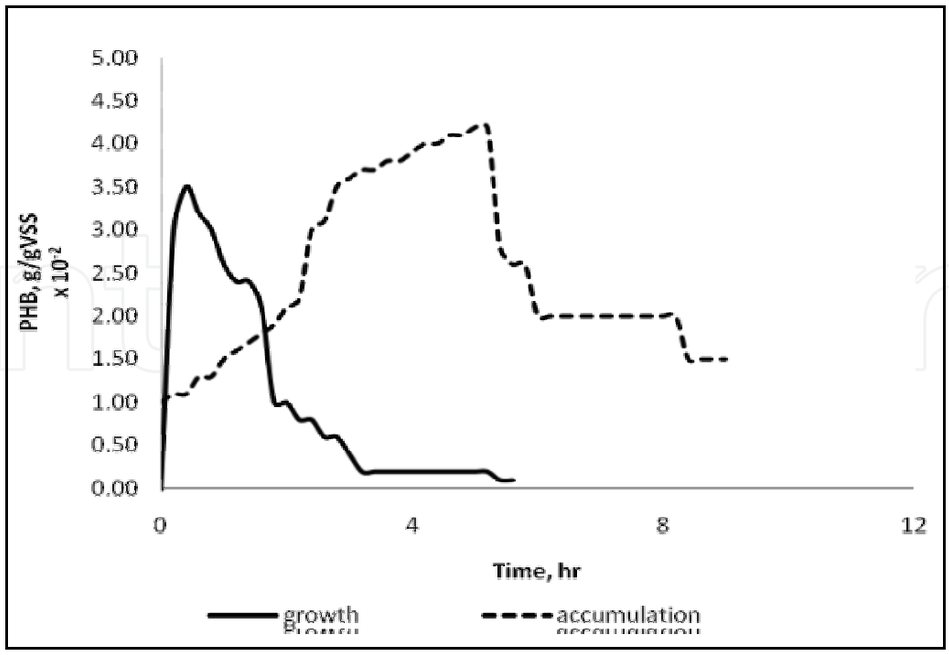

Fig. 6. PHB production during pre-determined growth and accumulation conditions

8. Substrates concentration

With current biological PHA production practice, the feedstock is estimated at 30 to 50% of the total production costs (Braunegg et al., 2002). These estimates vary somewhat with the microbial species utilized, carbon source, PHA yield and PHA production capacity (Gerngross & Slater, 2000). The quantity and form of the carbon substrate dictates the polymeric structure and yield of the PHA (Coats, 2005).

In this study, for 50% feeding (second phase of feeding), the biomass content is obviously higher than for 25% feeding (first phase) as shown in Table 11. The concentration of biomass produced increased directly with substrate volumes. In addition, the amount of polymer produced per substrate consumed increased concurrently with VFA production rate.

Parameters	Anaerobic (Stage A)		Aerobic (Stage B)	
	Influent	Effluent	25% substrate	50% substrate
COD(g/L)	65	8	25	32
COD removal (%)	-	80	45	60
VFA (gCOD/L)	NA	10.1	4.2	12.2
Cell dried weight (CDW) (%)	-	-	13	30
PHA (% CDW)	-	-	12	40

Table 10. The concentration of parameters for the two step process

The concentration of a substrate supplied affects the amount of polymer produced (Serafim et al., 2006). In other words, the amount of PHA in the biomass will be accumulated and increased directly in proportion to the amount of the initial carbon source for about 40% from cell dried weight as in Table 11. Besides, the volume of substrates influenced the time

of accumulation. The length of the feast-famine period depends on the amount /volume of substrate. If the substrate fed was in smaller volume, then the usage of air for oxidizing and for substrate storage into microorganism becomes shorter as shown in Table 11.

9. Molecular weight of PHA

The original molecular size of PHA is usually very large (1,000-2,000 kDa) compared to those of synthetic polyesters (100-200 kDa). Reduction of the molecular weight to some extent does not affect the mechanical properties of the biopolymers. The bioplastics, however, do become brittle or less ductile when the molecular weight is greatly reduced (e.g., <100-200 kDa) (Yu & Chen, 2006). PHA recovered by NPCM or dispersion digestion can change the molecular weight (M_w) of PHA. PHA samples were measured to evaluate the effect of M_w on this digestion. Cells were digested with different ratios of $CHCl_3$: $NaOCl_2$ for 3 hours at 35^{o}C. Table 11 shows the M_w of PHA recovered. The number of weight (M_n) and average molecular weight (M_w) of intact PHA recovered by chloroform extraction were 9 x10^5 and 22 x10^5 respectively. The M_w of PHA recovery at the 0.5 ratio was 18 x10^5. At both ratios 1 and 2 the weights (20 x10^5) were similar. This suggests that there is a negligible degradation of PHA when sodium hypochlorite is used as extraction. The digestion at low temperature and low concentration of sodium hypochlorite is found to give negligible degradation of PHA (Choi & Lee, 1999; Salmiati, 2008).

Treatment	Number average molecular weight (Mn) (10^5)	Weighted average molecular weight (Mw) (10^5)	Polydispersity Index (PI)
Intact cell by chloroform	9.0	22	2.5
0.5	7.0	18	2.6
1	7.1	20	2.8
2	6.9	20	2.9

Table 11. Molecular weight of PHA recovered by $CHCl_3$ and $NaOCl_2$ for 3 hours at 35°C

The polydispersity index (PI) (weighted average molecular weight/number average molecular weight), reflects the structural degradation of PHA. Table 11 is the PI of the PHA recovered by chloroform and sodium hypochlorite extraction. As the sodium hypochlorite concentration increases, the PI increases significantly for mixed culture biomass. There has been a number of reports by Choi & Lee (1999); Wong (2001) and Hu (2004) in which the authors stated that bacteria PHA granules were in a mobile amorphous state, which is consistent with this result. On the basis of these findings, the patterns of the PI that occurred as the hypochlorite ratios increased were analyzed. The PHA in the bacteria was protected from hypochlorite digestion by its crystalline morphology. When PHA was recovered by using the dispersion technique, however, the crystalline morphology could not protect the molecule because the PHB is soluble in chloroform.

10. Conclusions

Palm oil mill effluent (POME) is to be managed under cleaner production framework, no longer end-of-pipe-engineering solutions. Thus, on top of pollution control, it must be create

high value added to the investment. Therefore, this study has successfully shown the possibility of PHA production from POME in a two-stage-process consisting of anaerobic acidogenic fermentation and step aerobics processes. The reactor consisted of two components, anaerobic and aerobic systems. The experiment has demonstrated that POME is a potential feedstock substrate for the production of PHA from high storage capacity mixed cultures under microaerophilic-aerobic and feast–famine conditions. The cycle of microaerophilic-aerobic condition influences the PHA production.

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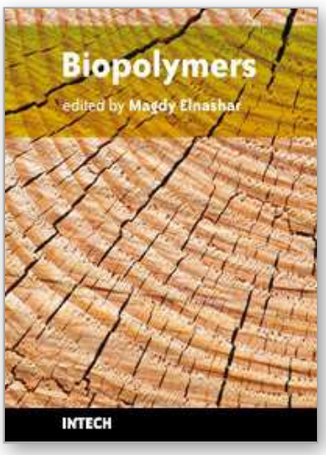

Biopolymers

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Biopolymers are polymers produced by living organisms. Cellulose, starch, chitin, proteins, peptides, DNA and RNA are all examples of biopolymers. This book comprehensively reviews and compiles information on biopolymers in 30 chapters. The book covers occurrence, synthesis, isolation and production, properties and applications, modification, and the relevant analysis methods to reveal the structures and properties of some biopolymers. This book will hopefully be of help to many scientists, physicians, pharmacists, engineers and other experts in a variety of disciplines, both academic and industrial. It may not only support research and development, but be suitable for teaching as well.

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