STRATEGY FOR THE BIOCONVERSION OF PALM OIL MILL EFFLUENT INTO VOLATILE FATTY ACIDS FOR THE PRODUCTION OF BIODEGRADABLE POLYHYDROXYALKANOATES

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ABSTRACT

The focus of wastewater management has evolved from treatment technology into resource recovery, which permits simultaneous waste minimization and value-added product generation. This study aims to develop a strategy for the biotransformation of the highly polluting palm oil mill effluent (POME) into volatile fatty acids (VFA) for the generation of biodegradable plastics – polyhydroxyalkanoates (PHA).

The influence of solids retention time (SRT; infinite SRT, 9 d and 6 d) and temperature (30°C, 40°C and 55°C) on the production of VFA by acidogenic fermentation of POME was first investigated. Performing acidogenic fermentation at infinite SRT resulted in gradual loss of acidogenic activity with a drop in the degree of acidification (DA) from 50% to 6% progressively. Using 6-d SRT led to higher DA of 48% as compared to 33% achieved at 9-d SRT. On the other hand, the production of VFA at 30°C and 40°C outperformed that at 55°C considerably, with a DA of 48% at both 30°C and 40°C but only 7% at 55°C.

The VFA-rich fermented POME was then utilized as the sole carbon substrate for PHA production by activated sludge. Prior to PHA production, activated sludge was subjected to aerobic dynamic feeding (ADF) process to enhance its PHA storage capacity through cultivation and enrichment of PHA-accumulating organisms. In the ADF process, fermented POME was employed as the sole carbon substrate and supplementary nutrient solution was provided to assist microbial growth. After 74 days of cultivation, the PHA storage capacity of the sludge improved significantly. The cultivated sludge could accumulate 64 wt% PHA per sludge dry weight at the end of the batch PHA production experiment. This was significantly higher than that achieved by the seed sludge at 4 wt%. .

The effect of pH and air supply rate on the production of PHA by activated sludge was subsequently examined. It was found that neutral condition could lead to higher PHA content of 64 wt% PHA per sludge dry weight in comparison with acidic (0.5 wt% at pH 4.5) and alkaline (48 wt% and 32 wt% at pH 8 and pH 9 respectively) conditions. On the other hand, the performance of PHA production improved with the increase in the supply of air to the reactor in a range of 0.2-1.0 vvm. The PHA content attained at 1.0 vvm was 45 wt%, which was approximately 2 times and 3 times higher than that achieved at 0.5 vvm and 0.2 vvm respectively.

The above results have demonstrated the feasibility of converting POME into PHA through a three-stage operating strategy which consists of acidogenic fermentation of POME, cultivation of PHA-accumulating organisms and production of PHA. This bioconversion scheme offers the palm oil industry a more meaningful alternative to manage POME. Unlike the current industrial practice that focuses on treating the pollutants in POME to meet the environmental regulations, the proposed scheme considers the pollutants as valuable feedstock and transforms them into VFA and subsequently into environmental-friendly PHA. Such transformation helps to foster the transition to a more sustainable palm oil industry.

ABSTRAK

Tumpuan pengurusan air sisa telah berubah dari teknologi rawatan ke pemulihan sumber yang membolehkan pengurangan sisa serentak dengan penghasilan produk yang bernilai tinggi. Kajian ini bertujuan untuk membentuk strategi biotransfomasi kumbahan air sisa kilang minyak sawit (POME) kepada asid lemak mudah meruap (VFA) bagi penghasilan bioplastik polihidroksialkanoat (PHA) yang boleh diuraikan oleh mikrooganisma.

Mula-mula, pengaruh masa tahanan pepejal (SRT; SRT tak terhingga, 9 hari dan 6 hari) dan suhu (30°C, 40°C dan 55°C) terhadap penghasilan VFA daripada POME melalui proses penapaian asidogenik dikaji. Proses penapaian asidogenik yang dijalankan pada SRT tak terhingga menunjukkan kehilangan aktiviti mikrob secara beransur-ansur dengan kejatuhan darjah pengasidan (DA) dari 50% ke 6%. Manakala pencapaian DA pada SRT 6 hari (48%) adalah lebih tinggi daripada SRT 9 hari (33%). Prestasi penghasilan VFA pada 30°C dan 40°C adalah jauh lebih baik berbanding dengan prestasi pada 55°C. DA yang tercapai pada 30°C dan 40°C ialah 48% sedangkan hanya 7% tercapai pada 55°C.

Kemudian, hasil penapaian asidogenik POME yang mempunyai kandungan VFA yang tinggi digunakan sebagai substrat karbon yang tunggal dalam penghasilan PHA oleh enapcemar teraktif. Sebelum penghasilan PHA, enapcemar teraktif telah melalui proses pemakanan dinamik aerobik (ADF) untuk mempertingkatkan kapasiti simpanan PHA dengan pemupukan dan pengkayaan mikroorganisma yang boleh menyimpan PHA. Di dalam proses ADF, hasil penapaian asidogenik POME digunakan sebagai substrat karbon yang tunggal dan larutan nutrien sampingan dibekalkan untuk membantu pertumbuhan mikroorganisma. Selepas 74 hari perlaksanaan proses ADF, kapasiti simpanan PHA enapcemar teraktif telah meningkat dengan ketara. Enapcemar teraktif

tersebut dapat menyimpan 64 wt% PHA seunit berat kering enapcemar di akhir ujikaji kelompok penghasilan PHA. Ini lebih tinggi daripada yang dicapai oleh enapcemar asal (4 wt%).

Selepas itu, kesan pH dan kadar bekalan udara terhadap penghasilan PHA oleh enapcemar teraktif dikaji. Kandungan PHA yang lebih tinggi dapat diperolehi dalam keadaan neutral (64 wt% PHA per berat kering enapcemar) berbanding dengan keadaan berasid (0.5 wt% pada pH 4.5) dan beralkali (48 wt% pada pH 8 dan 32 wt% pada pH 9). Manakala pretasi penghasilan PHA bertambah baik dengan peningkatan bekalan udara dari 0.2 vvm ke 1.0 vvm. Kandungan PHA yang diperolehi pada 1.0 vvm ialah 45 wt%, iaitu dua kali ganda dan tiga kali ganda lebih tinggi daripada yang tercapai pada 0.5 vvm and 0.2 vvm masing-masing.

Keputusan di atas telah menunjukkan kebolehlaksanaan penukaran POME kepada PHA melalui strategi operasi tiga peringkat yang terdiri daripada penapaian asidogenik POME, pemupukan mikroorganisma yang boleh menyimpan PHA dan penghasilan PHA. Skim biotransformasi ini menawarkan industri minyak sawit satu pilihan yang lebih bermakna untuk mengurus POME. Berbeza daripada amalan industri sekarang yang tertumpu ke atas rawatan pencemar dalam POME untuk menepati peraturan alam sekitar, skim yang dicadangkan ini menganggap pencemar sebagai bahan mentah yang bernilai dan mentransformasikannya kepada VFA and kemudian kepada PHA yang mesra alam. Transformasi tersebut dapat membantu peralihan kepada industri minyak sawit yang lebih mampan.

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3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
ADF	Aerobic dynamic feeding
AN/AE	Alternate anaerobic and aerobic
COD	Chemical oxygen demand
DA	Degree of acidification (%)
DO	Dissolved oxygen
DOC	Dissolved organic carbon
GAO	Glycogen-accumulating organisms
HCl	Hydrochloric acid
HRT	Hydraulic retention time (d)
$k_L a$	Oxygen mass transfer coefficient (1/h)
NaOH	Sodium hydroxide
OLR	Organic loading rate
P(3HB)	Poly(3-hydroxybutyrate)
P(3HB-co-3HV)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PAO	Polyphosphate-accumulating organisms
PHA	Polyhydroxyalkanoates

$PHA_{initial}$	Concentration of PHA at the beginning of PHA production (mg PHA/L)
PHA_{final}	Concentration of PHA at the end of PHA production (mg PHA/L)
POME	Palm oil mill effluent
q_{PHA}	Specific PHA production rate (mg PHA/mg X/h)
q_{VFA}	Specific VFA consumption rate (mg VFA/mg X/h)
q_X	Specific growth rate (1/h)
SBR	Sequencing batch reactor
sCOD	Soluble chemical oxygen demand (mg COD/L)
$sCOD_{initial}$	Concentration of sCOD at the beginning of fed-batch VFA production (mg COD/L)
$sCOD_{final}$	Concentration of sCOD at the end of fed-batch VFA production (mg COD/L)
SDBS	Sodium dodecylbenzene sulfonate
SRT	Solids retention time (d)
TCOD	Total chemical oxygen demand (mg COD/L)
TOC	Total organic carbon
TSS	Total suspended solids (mg TSS/L)
VFA	Volatile fatty acids (mg VFA/L)

$VFA_{initial}$	Concentration of VFA at the beginning of PHA production (mg VFA/L) or fed-batch VFA production (mg VFA-COD/L)
VFA_{final}	Concentration of VFA at the end of PHA production (mg VFA/L) or fed-batch VFA production (mg VFA-COD/L)
VS	Volatile solids
VSS	Volatile suspended solids (mg VSS/L)
vvm	Gas volume flow per reactor working volume per minute
$X_{initial}$	Concentration of microbial cell at the beginning of PHA production (mg X/L)
X_{final}	Concentration of microbial cell at the end of PHA production (mg X/L)

LIST OF APPENDICES

Chapter 1: Introduction

1.1 Research background

1.1.1 Challenges in the management of palm oil mill effluent

Palm oil is one of the main vegetable oils traded in the global market due to its versatile applications in food, oleochemicals and energy industries. At present, palm oil is produced primarily in Southeast Asia and the leading producers are Malaysia and Indonesia. It is recognized that the palm oil industry has great contribution to the growth of the economy of Malaysia. In year 2010, Malaysia exported 16.7 million tonnes of palm oil and this led to an export earning of RM 44.8 billion (MPOC, 2011). Although palm oil industry is of great economic importance, it is recognized to be highly polluting because of the massive generation of wastewater – known as palm oil mill effluent (POME) – from the palm oil milling process. As illustrated in Figure 1.1, sterilization, oil purification and kernel recovery are the three main processes leading to the generation of POME. It is estimated that one tonne of palm oil production could result in the generation of more than 2.5 tonnes of POME (Ahmad et al., 2003).

POME is an acidic brownish colloidal suspension containing large amount of organic substances with chemical oxygen demand (COD) in a range of 35000-57000 mg/L. Such high COD implies the need of proper POME management to avoid severe environmental pollution. In general, most of the palm oil mills have employed open ponding system for treating POME (Poh & Chong, 2009; Shak & Wu, 2014; Yoochatchaval et al., 2011). Open ponding system, as depicted in Figure 1.2, consists of a series of open ponds whereby POME generated from the mill is first gathered in the collection pond for waste palm oil recovery. After that, POME undergoes primary treatment in the anaerobic pond. It is further treated in either facultative or aerobic pond (with surface aerators) before being discharged into the environment.

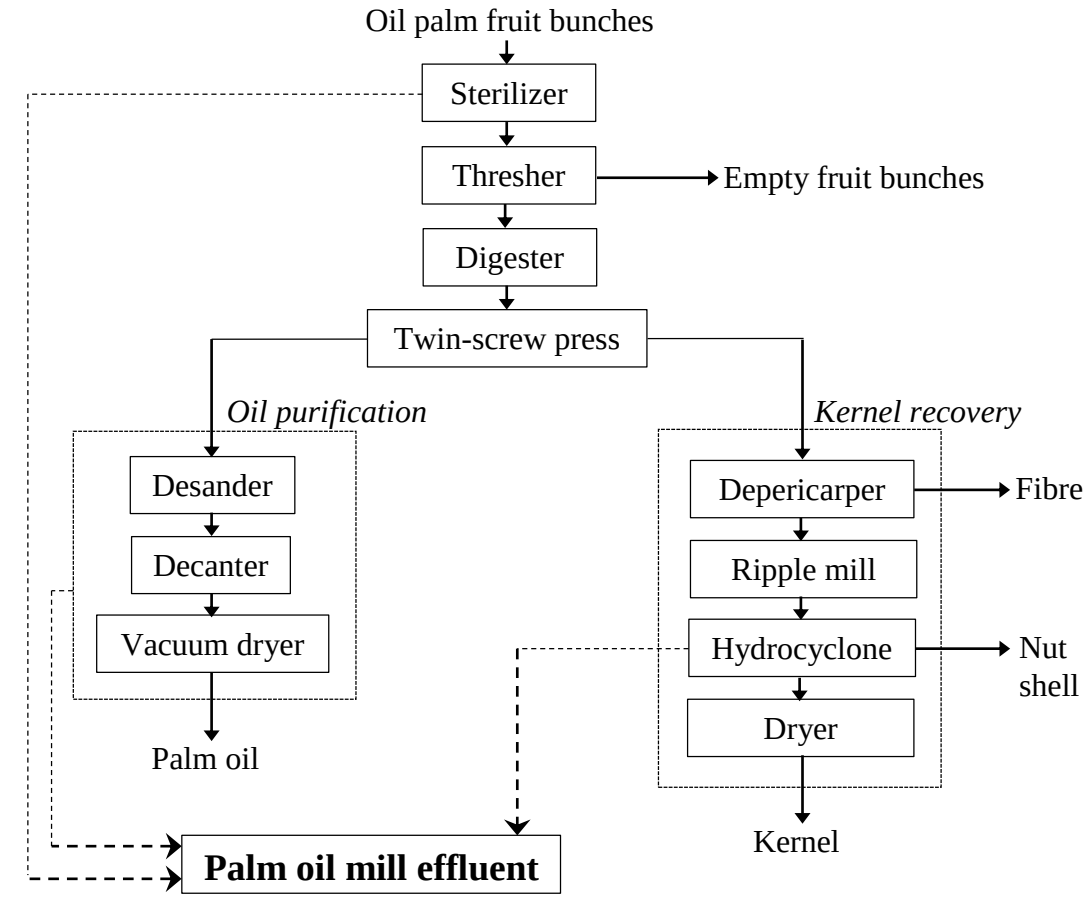


Figure 1.1: Process operation of a typical palm oil mill leading to the generation of POME.

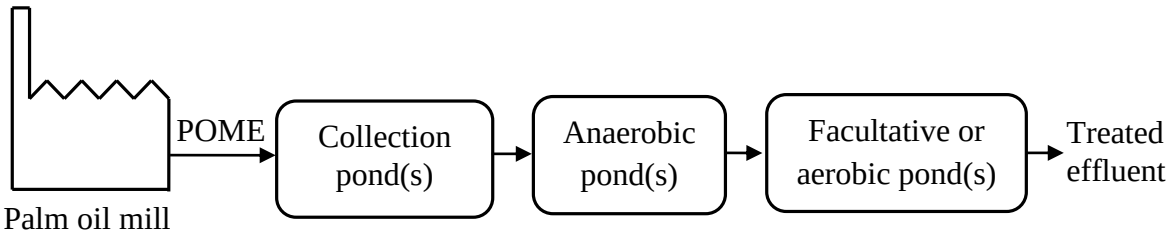


Figure 1.2: Treatment of POME by conventional open ponding system.

However, adopting open ponding system for treating POME has several drawbacks. First of all, the use of anaerobic open ponds causes the release of methane into the atmosphere. Since methane is a green houses gas, long-term operation of the ponding system can contribute substantially to global warming. Secondly, implementation of ponding system requires large area of land because long retention time (20-200 days) is needed in such treatment system (Poh & Chong, 2009). More importantly, this treatment-oriented management approach neglects the potential of POME as a feedstock for the production of various chemicals such as antibiotics, solvents and organic acids (Wu et al., 2009b). Therefore, a more sustainable POME management approach is resource recovery, which allows simultaneous minimization of POME and generation of value-added products. This study adopts the latter approach whereby POME is converted into volatile fatty acids (VFA) which are then utilized for the production of polyhydroxyalkanoates (PHA).

1.1.2 Issues of concern in the production of PHA

PHA are a type of biodegradable plastics which have similar mechanical properties to polyethylene and polypropylene and can be synthesized by microorganisms using renewable resources such as VFA (Salehizadeh & van Loosdrecht, 2004). Although PHA have a broad range of applications in various industries and are environmental friendly (Philip et al., 2007), their substitution over the conventional petrochemical-based plastics is limited by its high production cost. The cost of PHA is about nine times higher than that of the conventional plastics (Mumtaz et al., 2010) and this significantly weakens the competitiveness of PHA in commercial market. One of the main reasons leading to such high cost is the use of expensive and well-defined carbon substrate which contributes to about 31% of the total operating cost (Choi & Lee, 1997). In view of that, low-cost substrate like waste-derived VFA is a promising option. In general, the VFA content in many wastes such as waste activated sludge (Xiong et al.,

2012), dairy wastewater (Mohan et al., 2008) and wood mill effluent (Mato et al., 2010) is inherently low. To increase the VFA content, it is common to convert the organic substances in the waste into VFA via acidogenic fermentation process (Albuquerque et al., 2007; Cavdar et al., 2011; Xiong et al., 2012). Likewise, POME, which contains a large amount of organic substances, can be a potential wastewater to be used for VFA production.

Apart from expensive carbon substrate, the use of pure microbial culture is another factor contributing to high PHA production cost. This is because PHA production by pure microbial culture requires sterile conditions which demands additional energy input and equipment. To eliminate the need of sterilization, open mixed microbial culture such as activated sludge can be employed instead (Reis et al., 2003). The main limitation associated with the use of activated sludge is its lower attainable PHA content as compared to pure microbial culture (Chua et al., 2003). This technical barrier can be resolved by enriching the activated sludge with PHA-accumulating organisms (Albuquerque et al., 2010; Jiang et al., 2012). In addition, the PHA content achieved by activated sludge can be improved via fine-tuning the conditions of PHA production (Jiang et al., 2009; Mohan & Reddy, 2013; Reddy & Mohan, 2012).

1.1.3 A strategy for better POME management and PHA production

In this study, it is proposed to transform the organic-rich POME into VFA which serve as a valuable carbon substrate for economical PHA production by activated sludge. The proposed scheme is depicted in Figure 1.3. First of all, an anaerobic reactor is employed for the production of VFA from POME. Then, a portion of the VFA produced is used to cultivate PHA-accumulating organisms. The remaining VFA is applied to PHA production with activated sludge enriched with PHA-accumulating organisms as the inoculum. The proposed bioconversion scheme kills two birds with one stone. First, it

provides a more useful and valuable approach for managing POME by transforming its organic pollutants into PHA. Second, the implementation of the proposed scheme can also help to reduce the PHA production cost through the use of POME-derived VFA as substrate and activated sludge as inoculum. These could contribute to minimizing environmental degradation and fostering the transition to a more sustainable society.

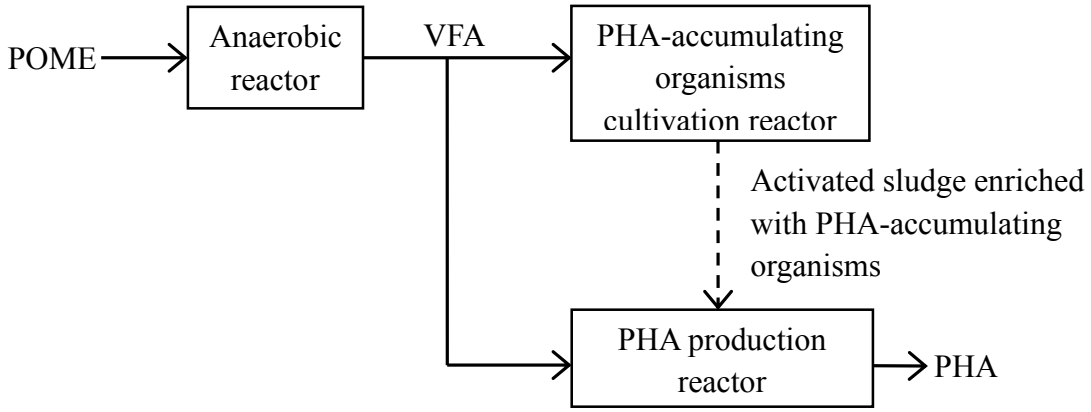


Figure 1.3: Bioconversion of POME into VFA for PHA production by activated sludge.

1.2 Research objectives

This study aims to develop an efficient system for the production of PHA by activated sludge using VFA produced from POME as the feedstock. To assist the development of the above-mentioned novel system, three specific objectives as listed below are set.

Objective 1: To enhance the production of VFA from POME by regulating the solids retention time (SRT) and temperature of the anaerobic reactor

SRT is an important operational parameter as it governs the selection of predominant microbial species in the anaerobic reactor. Long SRT can lead to the dominance of methanogens which consume VFA for methane formation (Miron et al., 2000). Meanwhile, short SRT can cause the wash out of acidogens, thus resulting in poor VFA production.

In addition to SRT, temperature is another parameter of interest due to its significant effect on VFA production (Zhang et al., 2009a; Zhuo et al., 2012). Besides, POME, which is typically discharged at 60-70°C, can potentially be used for VFA production under both mesophilic (20-50°C) and thermophilic (50-60°C) conditions without requiring high heating energy to maintain the temperature of the anaerobic reactor.

Objective 2: To enrich the activated sludge with PHA-accumulating organisms

In practice, wastewater treatment plant is the most common source of activated sludge. Such sludge is essentially good at removing organic pollutants and nutrients, but is not necessarily capable of producing PHA efficiently. Therefore, the production of PHA by activated sludge always suffers from low PHA content (Takabatake et al., 2002). For better PHA production, it is crucial to cultivate PHA-accumulating organisms in activated sludge.

Objective 3: To improve the production of PHA via manipulation of pH and air supply rate in the PHA production reactor

Proper selection of pH is crucial to PHA production because most microorganisms cannot function effectively and/or survive under extremely acidic or alkaline conditions. On the other hand, air supply rate is an important factor because it determines the concentration of dissolved oxygen available for PHA production and it is also closely related to the operating cost. Excessive air supply demands higher energy input whereas insufficient air supply slows the production of PHA.

1.3 Structure of the dissertation

This dissertation consists of seven chapters and the summary of each chapter is provided as follows.

Chapter 1: Introduction provides a brief background on the research topic and explains the motivation behind the research project. It also defines the research objectives and describes the research approaches. At the end of the chapter, it outlines the structure of the dissertation.

Chapter 2: Literature review summarizes and discusses extensively the research findings obtained in the past. It provides a comprehensive overview of the current state of the art in the production of VFA and PHA.

Chapter 3: Materials and methods details the methodology employed for conducting the experiments in line with the research objective. Information about the analytical methods and data analysis is included as well.

Chapters 4-6: Results and discussion consist of three separate individual chapters. *Chapter 4* discusses the influence of SRT and temperature on the production of VFA from POME. On the other hand, *Chapter 5* presents the finding obtained from the cultivation of PHA-accumulating organisms in activated sludge. The effect of pH and air supply rate on the production of PHA by activated sludge is detailed in *Chapter 6*.

Chapter 7: Conclusions and recommendations summarizes and highlights the significance of the key research findings acquired in the research work. It also suggests possible future works for strengthening the knowledge in the related research area.

Chapter 2: Literature Review

2.1 Production of volatile fatty acids from waste

Volatile fatty acids (VFA) are short-chain fatty acids consisting of six or fewer carbon atoms which can be distilled at atmospheric pressure (APHA, 1992). These acids have a wide range of applications such as in the production of bioplastics (Cai et al., 2009; Valentino et al., 2013), bioenergy (Choi et al., 2011; Uyar et al., 2009) and the biological removal of nutrient from wastewater (Ong et al., 2014; Zheng et al., 2010). At present, commercial production of VFA is mostly accomplished by chemical routes (Huang et al., 2002). However, the use of non-renewable petrochemicals as the raw materials and the increasing oil price have renewed the interest in biological routes of VFA production (Akaraonye et al., 2010). In biological VFA production, pure sugars such as glucose and sucrose have been commonly employed as the main carbon substrate (Kondo & Kondo, 1996; Zigová et al., 1999), which raises the ethical concern on the use of food to produce chemicals. This issue can be resolved by utilizing organic-rich wastes for VFA production. Such transformation of waste into VFA also provides an alternative route to reduce the ever increasing amount of waste generated.

In general, the production of VFA from waste is an anaerobic process involving hydrolysis and acidogenesis (the latter is also known as acidogenic fermentation (Bengtsson et al., 2008a) or dark fermentation (Su et al., 2009)), as illustrated in Figure 2.1. In hydrolysis, complex organic polymers in waste are broken down into simpler organic monomers by the enzymes excreted from the hydrolytic microorganisms. Subsequently, acidogens ferment these organic monomers into mainly VFA such as acetic, propionic and butyric acids. Both processes involve a complex consortium of obligate and facultative anaerobes, such as *Bacteriocides*, *Clostridia*, *Bifidobacteria*, *Streptococci* and *Enterobacteriaceae* (Weiland, 2010). However, it is common practice

that the hydrolysis and acidogenesis are conducted simultaneously in a single anaerobic reactor.

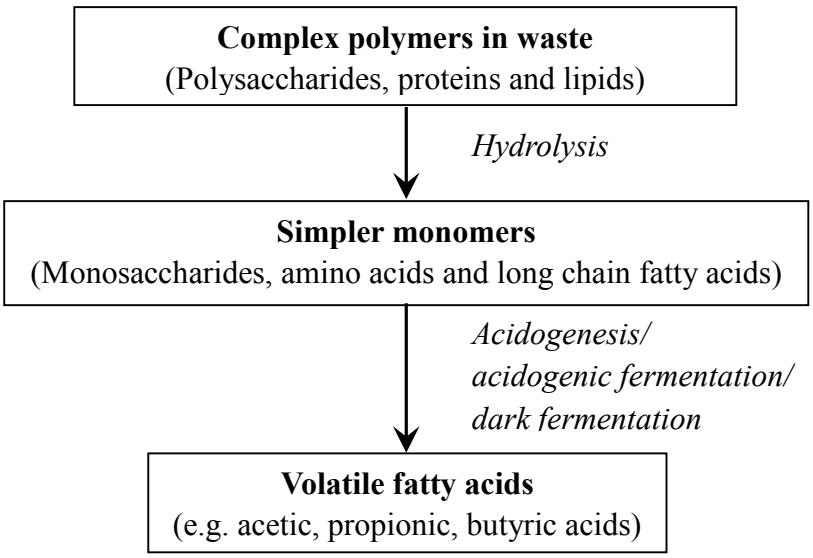


Figure 2.1: Production of VFA from waste (adapted from Angenent et al. (2004) and Weiland (2010)).

A variety of solid and liquid wastes, as presented in Table 2.1 and Table 2.2 respectively, have been studied for their potential to be used for VFA production. Among them, sludge, food waste and organic fraction of municipal solid waste are the three most investigated solid wastes. Meanwhile, wastewaters generated from the agricultural, dairy, pulp and paper industries are the liquid wastes frequently utilized for VFA production. It is difficult to conclude which type of waste is more suitable for VFA production due to the use of different operating conditions and VFA production performance evaluation criteria. However, wastes commonly used for VFA production, in general, are rich in organic substances with COD greater than 4000 mg/L (based on the reported organic content in Table 2.1 and Table 2.2). This could serve as a preliminary guide for waste selection. Besides, the ammonium content of waste should be lower than 5000 mg/L to avoid inhibition of VFA production (Yu & Fang, 2001) though it is an essential nitrogen source for the growth of microorganisms. Apart from

the waste characteristics, the availability and the amount of the waste generated have to be taken into consideration to ensure stable and continuous waste supply for the production of VFA (Salehizadeh & van Loosdrecht, 2004).

2.2 Factors affecting VFA production

The operational pH, temperature, hydraulic retention time, solid retention time and organic loading rate have great effects on the concentration, the yield and the composition of VFA produced from waste. In the literature, most of the researchers examine these factors one at a time and there are only a few works (Hong & Haiyun, 2010; Hu et al., 2006) evaluating their interactive effects. In view of that, the factors affecting VFA production will be discussed individually.

2.2.1 pH

The pH value in the reactor is important to the production of VFA because most of the acidogens cannot survive in extremely acidic (pH 3) or alkaline (pH 12) environments (Liu et al., 2012). The optimal pH values for the production of VFA are mainly in the range of 5.25-11, but the specific ranges are dependent on the type of waste used (Table 2.3). For example, when sludge is used, the optimal pH values are in the range of 8 to 11. The alkaline condition enhances the hydrolysis of sludge through ionization of the charged groups (e.g. carboxylic groups) of the extracellular polymeric substances in the sludge (Nielsen & Jahn, 1999), which are mainly carbohydrate and protein. This causes a strong repulsion between the extracellular polymeric substances (Nielsen & Jahn, 1999), resulting in the release of carbohydrate and protein to the environment. Consequently, more soluble substrates are available for the production of VFA under alkaline conditions (Wu et al., 2009a; Zhang et al., 2009b). Besides, the alkaline environment is not conducive to methanogenesis, thus preventing the consumption of the produced VFA for methane formation (Zhang et al., 2009b). On the other hand, pH

7 was considered optimal for the hydrolysis and acidogenesis of kitchen waste as it led to the highest solubilization percentage of carbohydrate, protein and lipid as well as the highest VFA concentration in comparison with pH 5, 9 and 11 (Zhang et al., 2005). In contrast, the VFA production from wastewater is mostly conducted under acidic condition with optimum pH ranges from 5.25 to 6.0 (Bengtsson et al., 2008a; Oktem et al., 2006). Based on these findings, it seems that alkaline condition favors the production of VFA from sludge whereas neutral and acidic conditions encourage the production of VFA from food waste and wastewater, respectively.

In addition, pH can also affect the type of VFA produced from acidogenic fermentation, particularly acetic, propionic and butyric acids (Bengtsson et al., 2008a; Horiuchi et al., 2002; Horiuchi et al., 1999; Yu & Fang, 2002; Yu & Fang, 2003). The production of propionic acid from dairy wastewater was favored at pH 4-4.5 whereas the acetic and butyric acids were favored at pH 6-6.5 (Yu & Fang, 2002). Similar observation was reported in acidogenesis of gelatin-rich wastewater (Yu & Fang, 2003). On the contrary, when pH increased from 5.25 to 6 in a study using cheese whey (Bengtsson et al., 2008a), the production of propionic acid increased while the production of acetic and butyric acids decreased. In another study, as pH increased from 6 to 8, the main VFA produced from glucose-rich medium changed from butyric acid to acetic and propionic acids, and vice versa (Horiuchi et al., 2002; Horiuchi et al., 1999). This might be caused by the shift in the dominant microbial populations from *Clostridium butyricum* at pH 6 to *Propionibacterium* at pH 8 (Horiuchi et al., 1999). The research findings so far suggest that the optimal pH for the production of a specific VFA is highly dependent on the type of waste used.

Table 2.1: Various solid wastes used for the production of VFA

Type of wastes	Organic content (mg COD/L)	Reactor type and operating conditions	VFA production performance	References
Waste activated sludge	5470[a]	Batch reactor, pH 11, 60°C, 7 d, 0.02 g SDBS[b]/g VSS	2561 mg TOC/L	(Cai et al., 2009)
	18657	Batch reactor, pH 9, 35°C, 5 d	298 mg COD/g VSS	(Zhang et al., 2009b)
	18657	Batch reactor, pH 8, 55°C, 9 d	368 mg COD/g VSS	(Zhang et al., 2009b)
	14878	Batch reactor, 21°C, 6 d	339 mg COD/L	(Jiang et al., 2007b)
	14890	Batch reactor, 21°C, 6 d	191 mg COD/L	(Jiang et al., 2007a)
Primary sludge	22838	Batch reactor, 21°C, 6 d	85 mg COD/g VSS	(Ji et al., 2010)
	20631	Batch reactor, pH 10, room temp., 5 d	60 mg COD/g VSS/d	(Wu et al., 2009a)
	343[c]	Continuous-flow completely mixed reactor, 25°C, HRT 1.25 d, SRT 10 d	31 mg/g VSS/d	(Maharaj & Elefsiniotis, 2001)
Food waste	Not available	Semi-continuous reactor (once-a-day feeding and draw-off), pH 6, 35°C, HRT 8 d, OLR 9 g/L/d	25000 mg/L	(Lim et al., 2008)
	91900	Batch reactor, 37°C, initial pH 5.5	8950 mg COD/L	(Elbeshbishy et al., 2011)
	146100	Batch reactor, 35°C, 5 d, enzymatic pretreated food waste	5610 mg COD/L	(Kim et al., 2006)
Kitchen waste	166180	Batch reactor, pH 7, 35°C, 4 d	36000 mg/L	(Zhang et al., 2005)
Organic fraction of municipal solid waste	347000[d]	Batch reactor, pH 4-5, 14-22°C, HRT 4-4.5 d	40 mg/g VS fed	(Bolzonella et al., 2005)
	196700[d]	Plug flow reactor, pH 5.7-6.1, 37°C, HRT=SRT 6 d, OLR 38.5 g VS/L/d	23110 mg/L	(Sans et al., 1995b)
	150600[d]	Plug flow reactor, pH 6.6-7.2, 55°C, HRT 6 d, OLR 22.4 g VS/L/d	19581 mg/L	(Sans et al., 1995a)

[a]mg TOC/L; [b]Sodium dodecylbenzene sulfonate; [c]sCOD after dilution; [d]mg COD/kg

Table 2.2: Various wastewaters used for the production of VFA

Type of wastes	Organic content (mg COD/L)	Reactor type and operating conditions	VFA production performance	References
Palm oil mill effluent	88000	Semi-continuous reactor (three times feeding per day), pH 6.5, 30°C, HRT 4 d	15300 mg/L	(Hong et al., 2009)
	30600	Upflow anaerobic sludge blanket reactor, pH 5.2-5.8, 35°C, HRT 0.9 d, OLR 16.6 g COD/L/d	4100 mg/L/d (as acetic acid)	(Borja et al., 1996)
Olive oil mill effluent	70400	Batch reactor, initial pH 6.5, 25°C, 45 d	15600 mg COD/L	(Dionisi et al., 2005b)
	37000	Packed bed biofilm reactor, pH 5.2-5.5, 25°C, HRT 1.4 d, OLR 26 g COD/L/d	10700 mg COD/L	(Beccari et al., 2009)
Wood mill effluent	11110	Continuous stirred-tank reactor, pH 5.5, 30°C, HRT 1.5 d, OLR 2.9 g COD/L/d	42%[a]	(Ben et al., 2011)
	11110	Continuous stirred-tank reactor, pH 5.5, 30°C, HRT 1 d, OLR 6.5 g COD/L/d	37%[a]	(Mato et al., 2010)
Paper mill effluent	7740	Continuous stirred-tank reactor, pH 6, 37°C, HRT 1 d	0.75[b]	(Bengtsson et al., 2008a)
	26300	Batch reactor, 15-25°C, pH 6, 12 d	60%[d]	(Jiang et al., 2012)
	8750[c]	Continuous-flow completely mixed reactor, 30°C, pH 6, HRT 0.67 d	74%[d]	(Bengtsson et al., 2008b)
Dairy wastewater	4420	Continuous flow-completely mixed reactor, pH 6.8-7.2, 35°C, HRT 0.5 d	3100 mg/L/d	(Demirel & Yenigun, 2004)
	4000	Upflow anaerobic sludge blanket reactor, pH 5.5, 55°C, OLR 6 g COD/L/d	1032 mg/L	(Yu & Fang, 2000)
	12000	Upflow anaerobic sludge blanket reactor, pH 5.5, 37°C, HRT 0.5 d, SRT 15 d	2071 mg/L	(Yu & Fang, 2001)
Gelatin-rich wastewater	4000	Upflow anaerobic sludge blanket reactor, pH 6.5, 37°C, HRT 0.5 d	1573 mg/L	(Yu & Fang, 2003)
Pharmaceutical wastewater	40000-60000	Continuous-flow completely mixed reactor, pH 5.5, 35°C, HRT 0.5 d, OLR 13 g COD/L/d	44%[a]	(Oktem et al., 2006)

[a](mg VFA-COD in effluent/mg COD in influent)×100%; [b](VFA-COD/wastewater influent sCOD); [c]sCOD; [d](mg VFA-COD/mg sCOD) ×100%

Table 2.3: Optimal pH for the production of VFA

Type of wastes	pH range studied	Optimal pH (range)	Reactor type and operating conditions	VFA production performance	References
Primary sludge	3-11	10	Batch reactor, room temp.,5 d	60 mg COD/g VSS/d	(Wu et al., 2009a)
Waste activated sludge	4-11	9	Batch reactor, 35°C, 5 d	298 mg COD/g VSS	(Zhang et al., 2009b)
		8	Batch reactor, 55°C, 9 d	368 mg COD/g VSS	
	8-12	11	Batch reactor, 25°C, 4 d	1558 mg COD/L	(Yu et al., 2013a)
	8-11	11	Batch reactor, 60°C, 7 d, 0.02 g SDBS[a]/g VSS	259 mg TOC/g VSS	(Cai et al., 2009)
Kitchen waste	5-11	7	Batch reactor, 35°C, 4 d	36000 mg/L	(Zhang et al., 2005)
Pharmaceutical wastewater	5-6.3	5.5	Continuous-flow completely mixed reactor, 35°C, HRT 0.5 d, OLR 13 g COD/L/d	44%[b]	(Oktem et al., 2006)
Cheese whey	3.5-6	5.25-5.5	Continuous stirred-tank reactor, 37°C, HRT 2 d	0.83[c]	(Bengtsson et al., 2008a)
Paper mill effluent	4.9-6	5.5-6	Continuous stirred-tank reactor, 37°C, HRT 2 d	0.76[c]	(Bengtsson et al., 2008a)

[a]Sodium dodecylbenzene sulfonate; [b][mg $(VFA\text{-}COD)_{effluent}$/mg $(COD)_{influent}$]×100%; [c]VFA-COD/wastewater influent soluble COD

2.2.2 Temperature

The production of VFA from waste had been carried out under different temperature ranges, viz. psychrophilic (4-20°C), mesophilic (20-50°C), thermophilic (50-60°C) and extreme/hyper-thermophilic (60-80°C) conditions (Bolzonella et al., 2007; Bouzas et al., 2002; Cai et al., 2009; Lu & Ahring, 2005; Lu et al., 2008; Maharaj & Elefsiniotis, 2001; Yu et al., 2002; Yu & Fang, 2003; Yu et al., 2013b; Yuan et al., 2011; Zhang et al., 2009a; Zhuo et al., 2012). Increasing the temperature within the psychrophilic and mesophilic temperature ranges is beneficial as it increases the concentration of VFA produced (Yuan et al., 2011; Zhang et al., 2009a), the rate of VFA production (Maharaj & Elefsiniotis, 2001) and the VFA yield (Bouzas et al., 2002). For instance, raising temperature from 10°C to 35°C increased the VFA concentration produced from waste activated sludge by 300% (Zhang et al., 2009a). The increment was due to the presence of greater amount of soluble carbohydrate and protein, which was a result of the improved sludge hydrolysis at higher temperature (Zhang et al., 2009a). Similarly, the VFA production rate increased six-fold as the temperature increased from 8°C to 25°C during the fermentation of primary sludge (Maharaj & Elefsiniotis, 2001).

Further increase in the operating temperature from mesophilic region to thermophilic region and to extreme/hyper-thermophilic region might still improve the VFA production. It had been reported that thermophilic temperature (60°C) could lead to faster biological acclimatization and more active acidogenesis as compared to those at mesophilic temperature (35°C), thus leading to a higher VFA yield (Cai et al., 2009). Meanwhile, the production of VFA at extreme/hyper thermophilic temperatures of 70-80°C had been found to outperform those at thermophilic temperatures of 55-60°C (Lu & Ahring, 2005). Nonetheless, the study of Yu et al. (2013b) showed that temperatures in the range of 45 to 70°C had no effect on the production of VFA. On the contrary, Zhuo et al. (2012) found that the acid-forming enzymes activities at thermophilic

temperature (55°C) were lower than that at mesophilic temperature (37°C). As a consequence, the total VFA concentration achieved at 55°C was 40% lower than that at 37°C (Zhuo et al., 2012). These inconsistent findings were likely caused by the presence of different microbial species in the studies.

If the production of VFA is more favorable at thermophilic or extreme/hyper-thermophilic temperatures, consideration must be given to the trade-off between the magnitude of improved VFA production and the heat requirement to maintain the temperature. A case in point is the acidogenesis of dairy wastewater, which was recommended to be carried out at mesophilic temperature in view of a lower energy demand and a more stable operation, in spite of the slightly higher production rate at thermophilic temperature (Yu et al., 2002). The same recommendation was given to the acidogenesis of gelatin-rich wastewater (Yu & Fang, 2003).

Unlike pH, the influence of temperature on the type of VFA produced is minor. Yuan et al. (2011) performed the fermentation of waste activated sludge in mixed reactors at 4°C, 14°C and 24.6°C. As the temperature increased from 4°C to 14°C, the percentage of acetate reduced from 55% to 43%, but the percentage of propionate and butyrate increased slightly from 20% to 29% and from 11% to 16%, respectively. However, further increase in temperature to 24.6°C did not alter much the VFA composition. Similarly, no significant variation in VFA composition was observed during the acidogenesis of gelatin-rich wastewater from 20°C to 55°C (Yu & Fang, 2003) and in the fermentation of ultrasonic-pretreated waste activated sludge from 10°C to 55°C (Zhuo et al., 2012). Likewise, the composition of the VFA produced from synthetic dairy wastewater at 37°C and 55°C was comparable (Yu et al., 2002). In the acidogenic fermentation of cellulose, acetic acid was the primary VFA produced at 37°C, 55°C and 80°C and butyric acid was the second dominant VFA (Gadow et al., 2013). Propionic acid was detected at 37°C only but its fraction in the VFA was relatively minor. Based

on these results, the effect of temperature on VFA composition seems minor. This observation challenges the common understanding that microbial composition changes with temperature, and has raised the concern whether similar microbial species are present at different temperatures or different microbial species exist but produce similar types of VFA.

2.2.3 Retention time

In acidogenic fermentation of waste for the production of VFA, the retention time of waste and microbial cultures in the anaerobic reactor are critical operational parameters. The former is termed hydraulic retention time (HRT) whereas the latter solids retention time (SRT). Their influences on VFA production will be discussed below.

2.2.3.1 Hydraulic retention time

Applying higher HRT could be advantageous to the production of VFA (Ben et al., 2011; Bengtsson et al., 2008a; Sans et al., 1995a) as the microorganisms have more time to react with the waste. For example, the production of VFA from organic fraction of municipal solid waste increased with HRT in a range of 2-6 d (Sans et al., 1995a). However, prolonged HRT could lead to stagnant VFA production (Fang & Yu, 2000; Lim et al., 2008). Similarly, the production of VFA from dairy wastewater nearly doubled as the HRT increased from 4 h to 12 h, but further increase to 16-24 h only improved the VFA production by 6% (Fang & Yu, 2000). Likewise, the VFA yield and volumetric productivity achieved in acidogenic fermentation of food waste increased as the HRT increased from 96 h to 192 h, but no significant improvement was observed at a HRT of 288 h (Lim et al., 2008). In the co-fermentation of waste activated sludge and fruit/vegetable waste, the concentration of VFA increased from 4400 mg/L to 6100 mg/L as the HRT increased from 1 d to 2 d (Dinsdale et al., 2000). Nonetheless, there

was no significant improvement on the production of VFA at higher HRT of 3 d (6150 mg/L) and 4 d (6620 mg/L) (Dinsdale et al., 2000).

Similar to pH, it was realized that HRT can be used to govern the relative production of propionic and butyric acids from paper mill effluent and whey (Bengtsson et al., 2008a). During the acidogenic fermentation of whey, increasing the HRT from 20 h to 95 h favored the production of propionic acid but it suppressed the formation of butyric acid. Likewise, increasing HRT from 11 h to 24 h also promoted the production of propionic acid from paper mill effluent, but it disfavored the butyric acid production. However, such observation is not universal as HRT, in a range of 1-4 d, did not have significant impact on the composition of VFA in the co-fermentation of waste activated sludge and fruit/vegetable waste (Dinsdale et al., 2000).

2.2.3.2 Solids retention time

In the case of using sludge as waste for VFA production, SRT is equal to HRT because both the waste substrate and microbial cultures are present in same phase. Most of the studies found that lower SRT is beneficial to the production of VFA from sludge (Feng et al., 2009; Miron et al., 2000; Xiong et al., 2012). This is because lower SRT can prevent the dominance of methanogens in the anaerobic reactor as the growth rate of methanogens is lower than that of acidogens (Ferrer et al., 2010). Nevertheless, the SRT should be sufficiently long to promote hydrolysis of the sludge. A case that illustrates this is the acidogenic fermentation of waste activated sludge, in which increasing the SRT from 4 d to 12 d led to 44% higher VFA concentration (Feng et al., 2009) because more soluble substrates resulting from sludge hydrolysis were available. However, further increase in SRT to 16 d resulted in lower VFA concentration although there were even more soluble substrates. It was deduced that the produced VFA was consumed by methanogens. Similarly, it was found that the acidogenic conditions

prevailed at SRT ≤ 8 d whereas methanogenic condition prevailed at SRT ≥ 10 d during the fermentation of primary sludge (Miron et al., 2000).

However, there is very limited number of study on the influence of SRT on the VFA production from wastewater (Salmiati et al., 2007). Salmiati et al. (2007) found that the concentration of VFA produced from POME in an upflow anaerobic reactor increased considerably for SRT of 6 d but reduced for 7 d. Meanwhile, other studies (Yu & Fang, 2002; Yu et al., 2002; Yu & Fang, 2001) normally applied SRT of 15 d in the acidogenic fermentation of wastewater.

The influence of SRT on the VFA composition varies substantially from one study to another. The study of Feng et al. (2009) showed that SRT, in the range of 4 to 16 d, had more influence on the fraction of acetic and propionic acids relative to other higher molecular weight VFA in the acidogenic fermentation of waste activated sludge. Increasing SRT from 4 to 16 d caused the percentage of acetic acid to increase from 32% to 42% but the percentage of propionic acid to decrease from 24% to 14%. However, in another study using waste activated sludge (Yuan et al., 2009), the percentage of acetic acid decreased instead from 66 to 49% with the increase of SRT from 5 d to 10 d whereas the percentage of propionic acid remained nearly constant at 16-18%. The findings were contradictory and it was probably due to the different operating conditions applied in these studies.

2.2.4 Organic loading rate

Organic loading rate (OLR) shows the amount of waste, which can be expressed in terms of COD, VSS, VS or DOC, fed into the reactor daily per unit reactor volume. In the literature, the influence of OLR on VFA production seemed inconsistent but could be rationalized by the presence of an optimum. For example, the VFA concentration produced from starchy wastewater increased linearly with OLR ranging from 1 g

COD/L/d to 32 g COD/L/d (Yu, 2001). Nevertheless, during the acidogenic fermentation of chemical synthesis-based pharmaceutical wastewater (Oktem et al., 2006), the VFA concentration increased with OLR only in the range of 7-13 g COD/L/d. Worse, a slight increase to 14 g COD/L/d caused a drastic drop in the VFA concentration from 3410 mg/L (as acetate) to 1370 mg/L (as acetate) (Oktem et al., 2006). In a fermentation study of two-phase olive oil mill solid residue over an OLR range of 3.2-15.1 g COD/L/d, the maximum VFA concentration was achieved instead at an intermediate value of 12.9 g COD/L/d (Rincón et al., 2008). The VFA concentration produced from food waste (Lim et al., 2008) increased with OLR from 5 g/L/d to 13 g/L/d, but the operation of reactor at 13 g/L/d was unstable because the fermentation broth became very viscous at high loading. These findings suggest that different snapshots of bigger pictures were being observed. The linear dependence range (Yu, 2001) could be interpreted as behavior before the optimum, while the performance deteriorations (Oktem et al., 2006; Rincón et al., 2008) had crossed the optimum. In a case (Lim et al., 2008), rheology and the associated mass transfer implications appeared to be a significant limiting factor outside the traditional operating parameters. Elucidation and moderation of the detrimental effects at high OLR will enable higher rates and intensity of waste treatment, further enhancing the economic feasibility of VFA production.

The OLR applied in the acidogenic fermentation has significant influence on the distribution of the VFA. In the fermentation of synthetic dairy wastewater (Yu et al., 2002), as the OLR increased from 4 g COD/L/d to 24 g COD/L/d, the percentage of acetate declined from 53% to 22% whereas the propionate percentage rose from 13% to 41% under mesophilic condition. Similar trend was observed in the thermophilic operation whereby the percentages of acetate and propionate changed from 44% to 23%, and from 21% to 43%, respectively. In another study on starchy wastewater (Yu,

2001), at a medium OLR of 10 g COD/L/d, propionic acid was the second main acid, but it was substituted by butyric acid at a higher OLR of 26 g COD/L/d. Throughout these ranges, acetic acid remained the primary VFA.

2.3 Treatment of VFA-rich fermented waste for PHA production

The VFA produced from acidogenic fermentation of waste are valuable carbon substrate for microorganisms involved in the production of biodegradable plastics – PHA. It is certainly highly desirable to utilize the VFA-rich fermented waste directly in PHA production. Unfortunately, fermented waste does not contain only VFA though they are often the dominant ones. In some cases, treatment of fermented waste is necessary to minimize the interference of non-VFA compounds on PHA production. The treatment involved is discussed below.

It is essential to regulate the ammonium and phosphorus contents in the fermented waste because excessive nutrients would favor the growth of microorganisms and reduce the conversion of VFA to PHA (Albuquerque et al., 2007). It had been reported that limited nitrogen and phosphorus conditions could lead to higher PHA content and yield (Bengtsson et al., 2008b). Excessive nitrogen and phosphorus, if present in the fermented waste, can be removed simultaneously via struvite precipitation. This technique had been proven to be effective for rapid ammonium and phosphorus removal from fermented waste activated sludge with negligible VFA loss (Cai et al., 2009).

In addition, direct feeding of fermented waste with considerable amount of sludge particles into the PHA production reactor is not advisable as it can lead to the failure of PHA production (Hassan et al., 1997). In general, the fermented waste should be filtered before use (Albuquerque et al., 2007; Yu, 2001). Combined filtration and evaporation system can be considered, if it is desirable to use pure VFA in the production of PHA

(Mumtaz et al., 2008). Nevertheless, pure VFA is rarely needed except in some cases of PHA production by pure microbial culture (Hong et al., 2009).

2.4 Synthesis of PHA

Over the decades, PHA have received great research attention because they are completely biodegradable polymers (Keshavarz & Ray, 2010) and can be produced from renewable resources, e.g. VFA and glucose (Reis et al., 2003). PHA are polyesters of hydroxyalkanoic acids that can be synthesized by a variety of microorganisms such as *Pseudomonas*, *Ralstonia* and *Rhodobacter* (Philip et al., 2007). The synthesis of PHA usually happens under stressed conditions in which nitrogen, phosphorus or oxygen is limited but carbon is available in excess (Keshavarz & Ray, 2010). PHA are synthesized and accumulated in the form of granules inside the microbial cell (Salehizadeh & van Loosdrecht, 2004) and they serve as the intracellular carbon and energy storage compounds (Keshavarz & Ray, 2010).

Two types of PHA commonly synthesized by the microorganisms are poly(3-hydroxybutyrate) (P(3HB)) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) (Albuquerque et al., 2007; Ben et al., 2011; Chua et al., 2003; Reddy & Mohan, 2012). The metabolic pathways involved in the synthesis of these two PHA are presented in Figure 2.2, using acetic and propionic acids as the model carbon substrates. The synthesis of P(3HB) consists of four major steps: (i) transformation of acetic acid into acetyl-CoA, (ii) condensation of acetyl-CoA to acetoacetyl-CoA (catalyzed by β-ketothiolase), (iii) reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA (catalyzed by acetoacetyl-CoA reductase), and (iv) polymerization of (R)-3-hydroxybutyryl-CoA monomers (catalyzed by PHA synthase) (Suriyamongkol et al., 2007).

Unlike P(3HB), the synthesis of P(3HB-co-3HV) involves two precursors namely acetyl-CoA and propionyl-CoA, which eventually are transformed into monomers (R)-3-hydroxybutyryl-CoA and (R)-3-hydroxyvaleryl-CoA, respectively (Hu et al., 2005). Besides, the enzyme responsible for the condensation of acetyl-CoA and propionyl-CoA is 3-ketothiolase, instead of β-ketothiolase as in the case of P(3HB) (Suriyamongkol et al., 2007). These two enzymes have different encoding genes whereby 3-ketothiolase is encoded by *bktB* gene and β-ketothiolase by *phaA* gene (Suriyamongkol et al., 2007).

Apart from the difference in metabolic pathway, P(3HB) and P(3HB-co-3HV) also have distinct chemical structure. As illustrated in Figure 2.2, P(3HB) is a homopolymer of 3HB whereas P(3HB-co-3HV) is a copolymer of 3HB and 3HV. This variance results in different mechanical properties in which P(3HB-co-3HV) is more flexible and tougher than P(3HB). It is reported that the Young's modulus (stiffness) of P(3HB-co-3HV) with 25 mol% 3HV is five times lower than that of P(3HB), but its notched Izodimpact strength (toughness) is eight times higher than that of the homopolymer (Doi, 1990). Therefore, P(3HB-co-3HV) with better mechanical properties has higher market value and is of greater commercial interest.

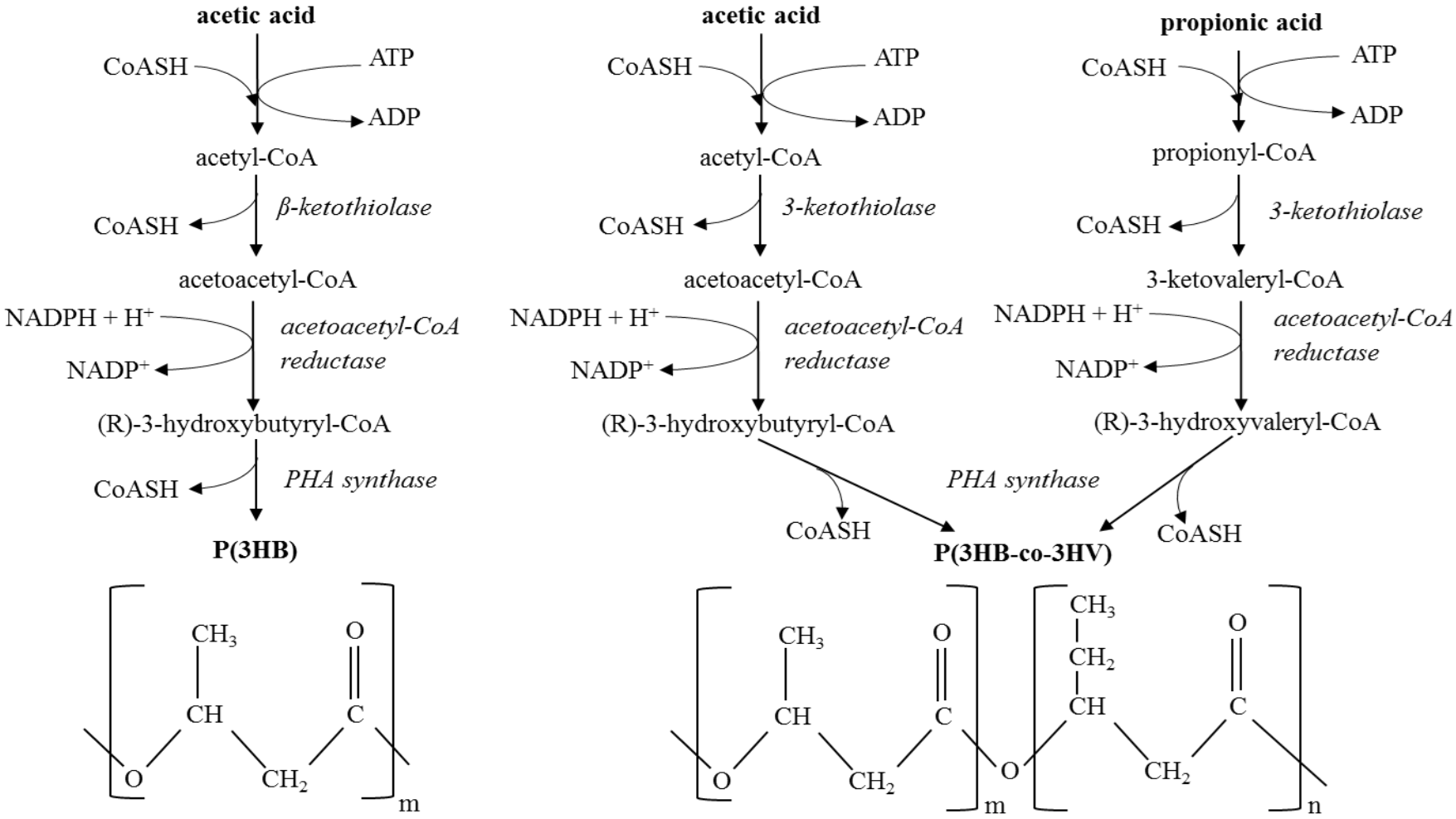


Figure 2.2: Metabolic pathways of P(3HB) and P(3HB-co-3HV) synthesis and their chemical structures (adapted from (Hu et al., 2005; Suriyamongkol et al., 2007)).

2.5 Low-cost production of PHA

Since 1980s, several companies such as ICI, Zeneca Bio Products, Metabolix, Mitsubishi and Zhejiang Tian An have been engaged in the industrial production of PHA (Chen, 2009; Philip et al., 2007). The PHA produced are sold under different trade names in the commercial market. For instance, P(3HB-co-3HV) produced by Zeneca Bio Products is marketed under the trade name of BIOPOL® (Philip et al., 2007). The market price of PHA is approximately $ 4.4-6.0 per kg (Guzman, 2010) which is considerably higher than that of the conventional petrochemical-based plastics at around $ 1.5 per kg (Platts, 2013). Such great difference in price has greatly reduced the competitiveness of PHA in commercial market. Thus, it is crucial to develop low-cost PHA production process to ensure successful commercialization of PHA.

The economic analysis of industrial PHA production revealed that approximately one third of the total operating cost was contributed by the carbon substrate (Choi & Lee, 1997). Therefore, one possible way to reduce the cost of PHA production is by using inexpensive carbon substrate such as VFA produced from the acidogenic fermentation of waste. Besides, industrial PHA production is often carried out under sterile conditions due to the use of pure microbial culture, e.g. *Alcaligenus eutrophus* (Salehizadeh & van Loosdrecht, 2004), as the inoculum. The need to maintain sterile conditions makes PHA production less cost-effective because this requires additional equipment and energy input. Sterilization can be eliminated by employing open mixed microbial culture such as activated sludge (Reis et al., 2003).

The PHA content achieved by activated sludge is lower than pure microbial culture generally (Reis et al., 2003). This aspect can be improved by cultivating PHA-accumulating organisms in the activated sludge. It had been reported that PHA content up to 89 wt% could be achieved by activated sludge subjected to appropriate cultivation

process (Johnson et al., 2009), as will be discussed next in Section 2.6. Apart from cultivating PHA-accumulating organisms, the PHA content obtained from activated sludge can also be enhanced by fine-tuning the PHA production conditions. The pertinent factors affecting PHA production will be discussed in Section 2.7.

2.6 Cultivation of PHA-accumulating organisms

One of the main challenges in PHA production by activated sludge is the low attainable PHA content. It was reported that raw activated sludge collected from municipal wastewater treatment plant was only capable of accumulating 6.0-29.5 wt% PHA per sludge dry weight (Takabatake et al., 2002). Such low PHA content is unattractive for commercial investment. Besides, low PHA content also results in higher downstream PHA extraction cost (Serafim et al., 2008). Hence, numerous efforts have been devoted to increase the production of PHA by activated sludge and one of them is to cultivate PHA-accumulating organisms in the activated sludge. The cultivation of PHA-accumulating organisms can be achieved by subjecting the activated sludge to either the aerobic dynamic feeding (ADF) process or the alternate anaerobic and aerobic (AN/AE) processes. These two cultivation processes will be discussed below.

2.6.1 Aerobic dynamic feeding process

In the ADF process, activated sludge has to undergo successive periods of external carbon substrate availability (feast period) and depletion (famine period), and this results in unbalanced growth (Salehizadeh & van Loosdrecht, 2004). As a countermeasure to survive under the transient conditions, the sludge will increase its growth rate (growth response) and/or store the substrate (storage response) as intracellular carbon and energy storage compounds like PHA whenever external carbon substrate is available (Beccari et al., 1998). Storage response is usually faster than growth response due to the lack of synthesis of enzymes and ribonucleic acids required

for growth in the absence of external carbon substrate for a considerable period of time (Serafim et al., 2008). Thus, in ADF process, PHA-accumulating organisms which are capable to store the external carbon substrate as PHA (internal carbon reserve) in the feast phase have competitive advantage over other microorganisms because they can survive in the famine phase (where there is no external carbon substrate) by consuming the intracellular PHA (Figure 2.3).

As presented in Table 2.4, activated sludge subjected to ADF process can accumulate 45-89 wt% PHA per sludge dry weight. These values are considerably higher than those attained by raw activated sludge in a range of 6.0-29.5 wt% (Takabatake et al., 2002). This confirms the applicability of ADF process to the cultivation of PHA-accumulating organisms. The broad range of PHA content reported (45-89 wt%) is likely due to the use of different cultivation conditions which lead to the enrichment of different kinds of PHA-accumulating organisms. Based on Table 2.4, a wide diversity of microbial species such as *Plasticicumulans acidivorans* (Jiang et al., 2012), *Comomonas* sp. (Villano et al., 2010) and *Thauera* sp. (Villano et al., 2010) had been detected and identified in the cultivation reactor.

To date, the highest PHA content achieved by activated sludge subjected to ADF process is 89 wt% (Johnson et al., 2009). However, expensive pure carbon substrate (sodium acetate) was used in that cultivation process (Johnson et al., 2009) which is in opposition to the purpose of using activated sludge for low-cost PHA production. On the contrary, the best PHA production performance achieved by activated sludge cultivated using waste substrate is 77 wt% so far (Jiang et al., 2012), which is fairly close to that obtained from pure substrate. PHA content of 77 wt% was obtained by Jiang et al. (2012) using fermented paper mill wastewater as the substrate. This encouraging finding implies that fermented wastewater rich in VFA is a highly

promising carbon substrate for low-cost cultivation of PHA-accumulating organisms with high PHA storage capacity.

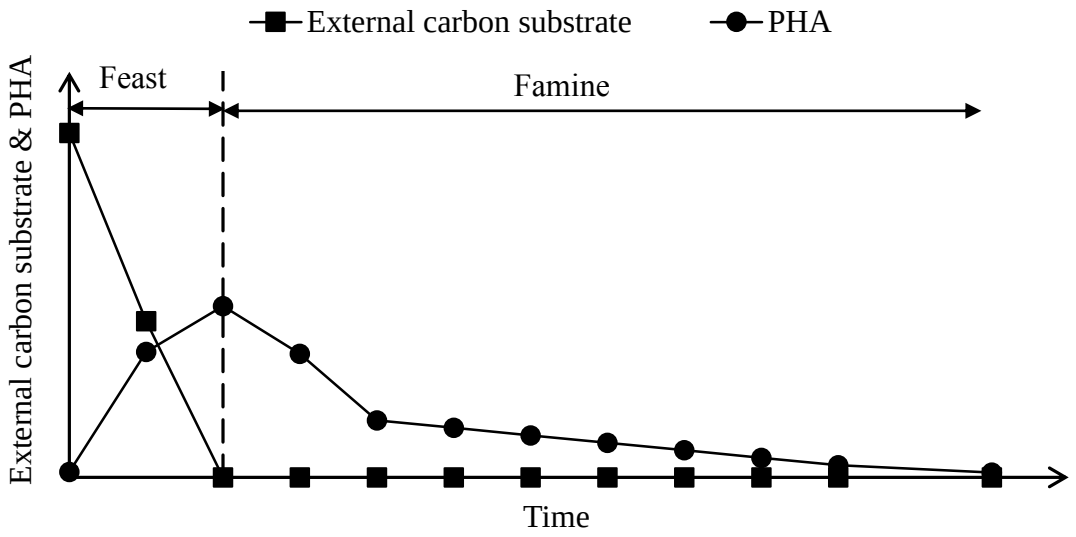


Figure 2.3: Typical profiles of external carbon substrate and PHA in the cultivation reactor of PHA-accumulating organisms operating on ADF process (adapted from Johnson et al. (2009)).

Table 2.4: PHA production performance of activated sludge subjected to ADF cultivation process

Operating conditions of cultivation reactor	Carbon substrate	Microbial population in the cultivation reactor	PHA production performance[a]	References
SBR[d], cycle length 4 h, HRT 8 h, SRT 1 d, pH 7, 20°C, C/N 11 Cmol/Nmol	Sodium acetate	Not available	PHA content[e] = 70%	(Johnson et al., 2010a)
SBR, cycle length 12 h, HRT 1 d, SRT 10 d, 22°C	Sodium acetate	Not available	PHA content = 78.5% PHA storage yield[f] = 0.78[b]	(Serafim et al., 2004)
SBR, cycle length 12 h, HRT=SRT 1 d, pH 7, 30°C, OLR 2.25 Cmmol/L/h	Sodium acetate	*Plasticicumulans acidivorans*	PHA content = > 80%	(Jiang et al., 2011b)
SBR, cycle length 12 h, HRT=SRT 1 d, pH 7, 30°C	Sodium acetate	MCB_clone37 (a member of *Gammaproteobacteria*)	PHA content = 89% PHA storage yield = 0.6[b]	(Johnson et al., 2009)
SBR, cycle length 4 h, HRT 8 h, SRT 1 d, pH 7, 30°C	Sodium acetate	Not available	PHA content = 84%	(Johnson et al., 2010b)
SBR, cycle length 2 h, HRT=SRT 1 d, pH 8.5, 25°C, OLR 8.5 g COD/L/d	Mixture of acetic and propionic acids	*Acinetobacter* sp., *Fusibacter* sp., *Thauera* sp., *Comomonas* sp., *Lampropedia hyalina*, *Exiguobacterium aurantiacum*	PHA content = 46% PHA storage yield = 0.43[c]	(Villano et al., 2010)
SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 20 g COD/L/d	Mixture of acetic, lactic and propionic acids	*Thauera* sp., *Alcaligenes* sp., *Comomonas* sp., *Pseudomonas* sp., *Kluyvera* sp., *Achromobacter* sp., *Xantobacter* sp., *Curtobacterium* sp., *Acinetibacter* sp.	PHA content = 45%[c] PHA storage yield = 0.46[c]	(Dionisi et al., 2006)
SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 12.75 g COD/L/d	Mixture of acetic, lactic and propionic acids	*Methylobacteriaceae* bacterium, *Flavobacterium* sp, Candidatus *Meganema perideroedes*, *Thauera* sp.	PHA content = 61%[c]	(Dionisi et al., 2005a)

Table 2.4 (continued): PHA production performance of activated sludge subjected to ADF cultivation process

Operating conditions of cultivation reactor	Carbon substrate	Microbial population in the cultivation reactor	PHA production performance	References
SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 8.75 g COD/L/d	Mixture of acetic, lactic and propionic acids	Not available	PHA content = 50%[c]	(Dionisi et al., 2004)
SBR, cycle length 4 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 20 g COD/L/d	Mixture of acetic, lactic and propionic acids	*Flavobacterium*	PHA storage yield = 0.55[c]	(Dionisi et al., 2007)
SBR, cycle length 12 h, HRT 1 d, SRT 10 d, feed pH 8, 23-25°C	Fermented sugar cane molasses	Not available	PHA content = 56% PHA storage yield = 0.80[c]	(Albuquerque et al., 2011)
SBR, cycle length 12 h, HRT 1 d, SRT 10 d, feed pH 8, 23-25°C	Fermented sugar cane molasses	Not available	PHA storage yield = 0.59[c]	(Albuquerque et al., 2007)
SBR, cycle length 12 h, HRT 1 d, SRT 10 d, feed pH 8, 23-25°C	Fermented sugar cane molasses	Not available	PHA content = 75% PHA storage yield = 0.81[b]	(Albuquerque et al., 2010)
SBR, cycle length 4 h, SRT 3d, feed pH 7, 21°C	Fermented waste activated sludge	*γ-Proteobacteria* (42%), *α-Proteobacteria* (16.1%), *β-Proteobacteria* (15.2%), *Bacteroidetes* (5.4%), *Chloroflexi* (4.5%), Candidate division *SRI* (4.5%), *Verrucomicrobiae* (3.6%), *Planctomycete* (3.6%), *ε-Proteobacteria* (2.7%), *Sphingobacteria* (1.8%)	PHA content = 72.9%	(Jiang et al., 2009)
Continuous flow reactor, SRT 7 d, pH 7.3, 30°C	Fermented paper mill wastewater	Filamentous bacterium	PHA content = 48% PHA storage yield = 0.66[c]	(Bengtsson et al., 2008b)
SBR, cycle length 24 h, HRT=SRT 2 d, pH 7, 30°C	Fermented paper mill wastewater	*Plasticicumulans acidivorans*	PHA content = 77% PHA storage yield = 0.8[c]	(Jiang et al., 2012)

[a]achieved in PHA production reactor; [b]carbon basis; [c]COD basis; [d]sequencing batch reactor; [e]PHA/sludge dry weight×100%; [f]PHA produced/substrate consumed

2.6.2 Alternate anaerobic and aerobic processes

Alternate anaerobic and aerobic (AN/AE) processes are another technology employed for cultivating PHA-accumulating organisms in activated sludge. Unlike ADF process which is being executed under sole aerobic conditions, AN/AE processes expose activated sludge to anaerobic and aerobic conditions alternately. These alternate conditions lead to the cultivation of two primary groups of PHA-accumulating organisms which are known as polyphosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO) (Serafim et al., 2008). At the beginning of anaerobic conditions, carbon substrate is added into the cultivation reactor and both PAO and GAO will take up the substrate and store it as PHA. Nevertheless, these two organisms utilize different metabolic pathway to obtain the energy required for substrate uptake and PHA storage. GAO gain the energy via glycolysis of intracellular glycogen while PAO acquire the energy through hydrolysis of intracellular polyphosphate which results in the release of phosphate to the external environment (Oehmen et al., 2007). Although their energy sources are different, they rely on the same pathway, i.e. glycolysis of glycogen mainly, to generate reducing equivalents required in the synthesis of PHA (Oehmen et al., 2007). When the condition switches to aerobic and in the absence of carbon substrate, PAO and GAO will utilize the stored PHA for growth, maintenance, and replenishment of glycogen (Oehmen et al., 2007). In addition to these activities, PAO will also take up phosphate from the external environment to replenish polyphosphate (Oehmen et al., 2007). Hence, by observing the phosphate profile (Figure 2.4), one may distinguish the cultivation reactor enriched with PAO from the one dominated by GAO.

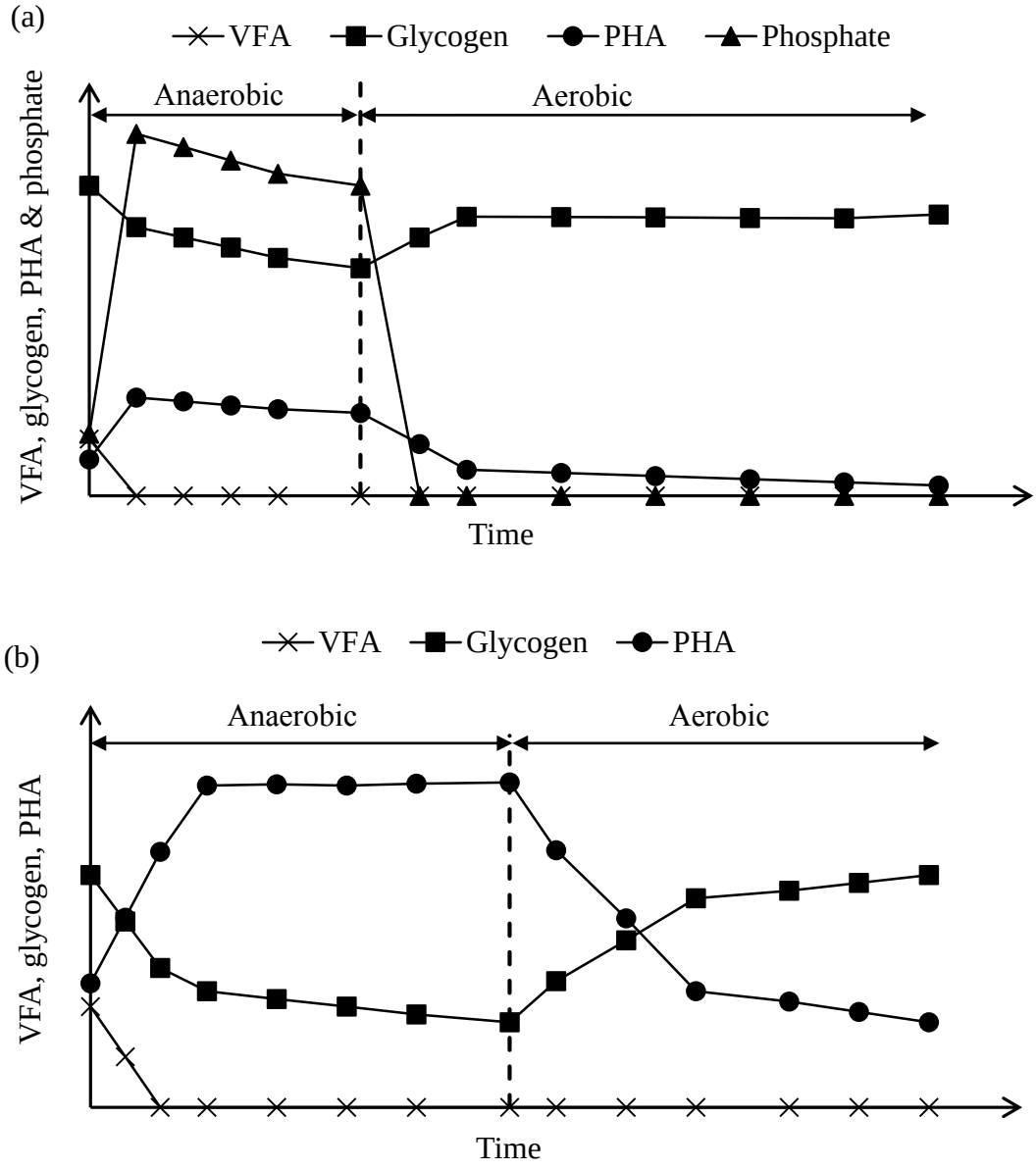


Figure 2.4: Typical profiles of VFA, glycogen, PHA and phosphate in the cultivation reactor of PHA-accumulating organisms operating under AN/AE aerobic conditions. Dominant microbial population in the cultivation reactor: (a) PAO and (b) GAO (adapted from Serafim et al. (2008)).

The PHA production performance of activated sludge subjected to AN/AE cultivation process is shown in Table 2.5. The PHA content achieved is in a range of 20-60 wt%. However, these values were lower than those attained by activated sludge cultivated under ADF conditions in general (by comparing the reported values in Tables 2.4 and 2.5). A modification on the AN/AE cultivation process had been introduced by Satoh et

al. (1998) to improve the PHA storage capacity of the activated sludge. They replaced the anaerobic process with microaerophilic process by introducing a small amount of air in the original anaerobic process. They found that activated sludge subjected to alternate microaerophilic and aerobic processes could accumulate PHA up to 62% of sludge dry weight which was 29% higher than that accumulated by the sludge cultivated using AN/AE processes. The introduction of microaerophilic process allows the activated sludge to gain energy through oxidative degradation of external substrate for PHA accumulation (Satoh et al., 1998). Therefore, PHA-accumulating organisms that cannot store polyphosphate and glycogen can be cultivated as well (Satoh et al., 1998).

Table 2.5: PHA production performance of activated sludge cultivated under AN/AE conditions

Operating conditions of cultivation reactor	Carbon substrates	Microbial population in the cultivation reactor	PHA production performance[a]	References
SBR[b], cycle length 6 h (anaerobic 2 h, aerobic 2.5 h), HRT 1 d, SRT 7 d, pH 7-7.2, 18-22°C	Sodium acetate	*Candidatus* Competibacter phosphatis (50%), *Alphaproteobacteria* (25%)	PHA content[e] = 41% PHA storage yield[f] = 0.75[c]	(Dai et al., 2007)
SBR, cycle length 6 h (anaerobic 2 h, aerobic 2.5 h), HRT 1 d, SRT 7 d, pH 7-7.2, 18-22°C	Sodium acetate	*Candidatus* Competibacter phosphatis (50%), *Alphaproteobacteria* (25%)	PHA content = 41% PHA storage yield = 0.89[c]	(Dai et al., 2008)
SBR, cycle length 6 h (anaerobic 1.75 h, aerobic 3 h), SRT 7.2 d, feed pH 6.8, 20°C	Sodium acetate	Not available	PHA content = 50% PHA storage yield = 0.74[c]	(Rodges & Wu, 2010)
SBR, cycle length 8 h (anaerobic 3 h, aerobic 4.2 h), SRT 10 d, pH 7.7, 30°C, OLR 100 C-mmol/L/d	Sodium acetate	*Candidatus* Competibacter phosphatis (54-70%), *Defluviicoccus vanus*-related Cluster 1 (2-12%), *Defluviicoccus vanus*-related Cluster 2 (<1-2%)	PHA content = 60%	(Bengtsson, 2009)
SBR, cycle length 8 h (anaerobic 2 h, aerobic 4 h), SRT 10 d, pH 7, 25°C	Mixture of sodium acetate, propionic and butyric acids	Not available	PHA content = 20% PHA storage yield = 0.97[d]	(Lemos et al., 1998)
SBR, cycle length 4 h (anaerobic 1 h, aerobic 2 h), HRT 6 h, SRT 5 d, pH 8, 18-25°C	Municipal wastewater	Not available	PHA content = 30%	(Chua et al., 2003)
SBR, cycle length 8 h, (anaerobic 3 h), HRT 1.33 h, SRT 10 d, pH 7.7, 30°C	Fermented sugar cane molasses	*Candidatus* Competibacter phosphatis, *Defluviicoccus* vanus-related organisms	PHA content = 37%	(Bengtsson et al., 2010)
SBR, cycle length 6 h, (anaerobic 1.5 h, aerobic 2.5 h), HRT 12 h, 20°C	Fermented food waste	Not available	PHA content = 60%	(Rhu et al., 2003)

[a]achieved in PHA production reactor; [b]sequencing batch reactor; [c]carbon basis; [c]COD basis; [d]mg polymer/mg COD; [e]PHA/sludge dry weight ×100%; [f]PHA produced/substrate consumed

2.7 Factors affecting PHA production

Functioning differently from the cultivation reactor which provides suitable environment for the growth of the PHA-accumulating organisms in the activated sludge (as discussed in Section 2.6), the PHA production reactor offers conditions favoring the synthesis of PHA with ultimate aim to fully explore and utilize the PHA storage capacity of the activated sludge. Several important factors affecting the achievable PHA content such as oxygen supply, nutrient, pH, and type of VFA will be discussed below. Apart from PHA content, some of these factors also have considerable effects on the composition of the PHA produced.

2.7.1 Oxygen supply

Oxygen supply is one of the most influential parameters affecting the production of PHA. Satoh et al. (1998) reported that the production of PHA increased with oxygen supply, from 20% of the initial sludge dry weight achieved under anaerobic conditions to 50% at an oxygen supply rate of 2.7 mg O_2/min/L. On the other hand, the study of Mohan and Reddy (2013) revealed that microaerophilic conditions (limited oxygen supply) led to higher PHA production than aerobic and anaerobic conditions. In their study, microaerophilic conditions were realized by sparging the air into the closed reactor intermittently, i.e. 4-minute air sparging at every hour. Similar result was obtained in another study of theirs (Reddy & Mohan, 2012) whereby PHA content of 39.6% was achieved under microaerophilic conditions whilst only 35.2% under aerobic conditions. It had been reported that microaerophilic conditions could result in high PHA content of as much as 74% (Din et al., 2012). The enhanced PHA production performance under oxygen-limited conditions is due to better utilization of carbon substrate for PHA production over microbial growth (Third et al., 2003). Nonetheless, operating the reactor at low oxygen supply slows the PHA production process. A

duration of three times longer was required under low oxygen conditions for reaching the same PHA content as achieved under high oxygen conditions (Pratt et al., 2012). Likewise, Third et al. (2003) also found that the PHA production rate at low oxygen supply was around 50% lower than that at high oxygen supply. Therefore, it is important to consider the trade-off between the attainable PHA content and the PHA production rate in deciding the amount of oxygen supplied to the reactor.

2.7.2 Nutrient

Several researchers explored the possibility to enhance the PHA production by limiting the amount of nutrient such as nitrogen and phosphorus (Albuquerque et al., 2007; Basak et al., 2011; Bengtsson et al., 2008b; Serafim et al., 2004; Wang et al., 2007) in the reactor because these elements are required for microbial growth. As such, it can help to reduce the competition of substrate for microbial growth and PHA accumulation. For instance, reducing the ammonia concentration from 2.8 mmol N/L to 0.7 mmol N/L improved the PHA content and PHA storage yield by 14% and 95%, respectively (Albuquerque et al., 2007). The positive effect of lower ammonium concentration on PHA production was observed in these studies (Basak et al., 2011; Serafim et al., 2004; Wang et al., 2007) too. Apart from nitrogen, phosphorus is another nutrient of which its concentration should be regulated properly during PHA production. The PHA content and PHA storage yield attained under phosphorus-limited conditions were, respectively, 1.5 times and 2 times higher than those under nutrient-excessive conditions (Bengtsson et al., 2008b).

2.7.3 pH

In the literature, the production of PHA is usually carried out under neutral and slightly alkaline conditions with pH ranging from 7.0 to 7.7 (Bengtsson, 2009; Bengtsson et al., 2008b; Dionisi et al., 2004; Dionisi et al., 2007; Jiang et al., 2012; Jin et al., 1999;

Johnson et al., 2010b; Lemos et al., 1998). Such prevalence might be attributed to that neutral and alkaline conditions (pH 7-9.5) have been commonly reported as the optimum conditions for PHA production (Chua et al., 2003; Dionisi et al., 2005a; Mohan & Reddy, 2013; Serafim et al., 2004; Villano et al., 2010). Nevertheless, extreme alkaline conditions (pH 10.5) should be avoided because it can completely obstruct microbial PHA production (Dionisi et al., 2005a). On the other hand, acidic conditions do not favor PHA production. As evidence, Chua et al. (2003) only managed to get PHA content of less than 5% at pH 6. Meanwhile, Dionisi et al. (2005a) observed complete inhibition of PHA production at pH 4.5. Besides PHA storage capacity, pH also has substantial influence on the composition of the PHA produced by the activated sludge. When pH increased from 7.5 to 8.5, the percentage of 3HV in the stored PHA increased from 20% to 36% (Villano et al., 2010). Similar trend was observed by Dionisi et al. (2005a) on a broader pH range in which the percentage of 3HV increased from 10% to 30% as the pH increased from 5.5 to 9.5. These findings suggest that one can consider applying alkaline pH to promote the synthesis of 3HV.

2.7.4 Types of VFA

Apart from pH, the types of VFA also have great influence on the composition of PHA produced. A number of studies (Albuquerque et al., 2007; Bengtsson et al., 2010; Jiang et al., 2011a; Lemos et al., 2006; Lemos et al., 1998; Wang et al., 2007) found that acetic and butyric acids favored the production of 3HB whereas propionic and valeric acids promoted the synthesis of 3HV. P(3HB) is brittle and stiff, and thus has limited applications. The incorporation of 3HV into P(3HB) leads to the formation of copolymer P(3HB-*co*-3HV) which is more flexible and tougher (Holmes, 1985). The mechanical properties (stiffness and toughness) of P(3HB-co-3HV) improve with the fraction of 3HV in a range of 0-25 mol% (Doi, 1990). Hence, it is essential to regulate the composition of VFA during acidogenic fermentation to facilitate the production of

PHA with desired properties or alternatively by controlling the pH during PHA production.

2.8 Research needs for the production of PHA from POME

The production of PHA from wastes (e.g. paper mill effluent (Jiang et al., 2012) and sugar cane molasses (Albuquerque et al., 2007)) by activated sludge is commonly accomplished in a three-stage system, i.e. stage (1): acidogenic fermentation of waste to produce VFA, stage (2): cultivation and enrichment of PHA-accumulating organisms in activated sludge and stage (3): production of PHA by the cultivated sludge using VFA-rich fermented waste as the sole substrate. Through such a system, Jiang et al. (2012) obtained a PHA content of 77 wt% using paper mill effluent as feedstock.

In the literature, several studies (Din et al., 2006; Din et al., 2012; Salmiati et al., 2007) had examined the production of PHA from POME by activated sludge. Din et al. (2012) investigated the effect of COD:N:P on PHA production. In their study, the highest PHA content of 44.5 wt% PHA per sludge dry weight was achieved at a COD:N:P ratio of 180:0.7:1. On the other hand, Salmiati et al. (2007) managed to attain a maximum PHA content of 40 wt% by doubling the loading of fermented POME supplied to the reactor. Din et al. [18] achieved a relatively high PHA content of 74 wt% through the application of microaerophilic condition, but the entire PHA production process was fairly long as it took about 40 hours. Hence, there is a need for more studies on the strategy for efficient production of PHA from POME. A shorter PHA production process yielding higher PHA content is highly desirable. This study explores such possibility by adopting the above-mentioned three-stage system for PHA production from POME.

Chapter 3: Materials and Methods

3.1 Overview of three-stage system for the bioconversion of POME into PHA

A three-stage system, as shown in Figure 3.1, is employed for the production of PHA from POME. In the first stage, POME is fermented anaerobically into VFA. Then, a portion of the VFA-rich fermented POME is used in the cultivation and enrichment of PHA-accumulating organisms in the activated sludge. The cultivation step aims to produce an activated sludge of high PHA storage capacity. Finally, the cultivated sludge that is enriched with PHA-accumulating organisms is employed for PHA production using VFA-rich fermented POME as the carbon substrate. Detailed methodology of each stage is explained in Sections 3.2-3.5.

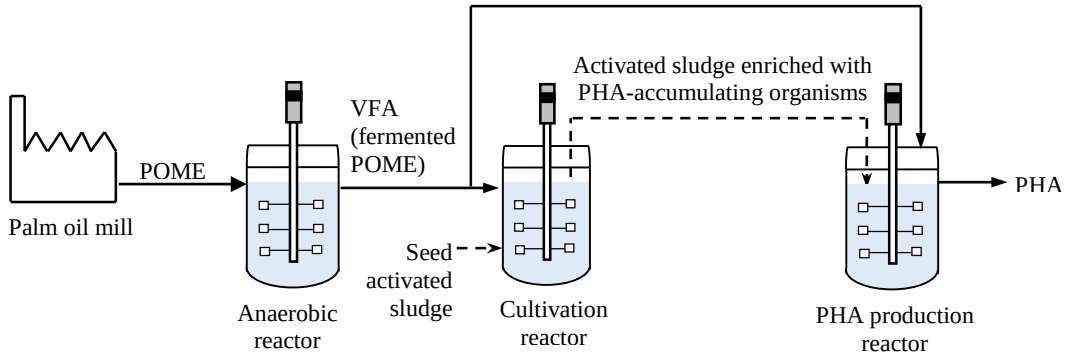


Figure 3.1: Three-stage system for the bioconversion of POME into VFA for the production of PHA by activated sludge.

3.2 Collection and characterization of POME

Raw POME was collected from a local palm oil mill located in the state of Selangor, Malaysia. The POME was allowed to settle at 4°C for 24 h to reduce its solid content. After settling, the supernatant was separated from the settled solid and stored at 4°C for preservation before it was used in acidogenic fermentation. Both the raw POME and the supernatant of the settled POME were characterized for pH, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (sCOD), total suspended solids (TSS), volatile suspended solids (VSS) and VFA.

3.3 Production of VFA by acidogenic fermentation of POME

3.3.1 Operation of the anaerobic reactor at different SRT

Study on the influence of SRT (infinite SRT, 9-d SRT and 6-d SRT) on the production of VFA from POME was conducted using an anaerobic reactor (Figure 3.2) operating at room temperature (30±1°C). Sludge collected from the second anaerobic pond treating POME in the mill was employed as the inoculum. The anaerobic reactor was operated under fed-batch mode with a POME feeding interval of 5 d. The pH in the reactor was not controlled throughout the acidogenic fermentation process.

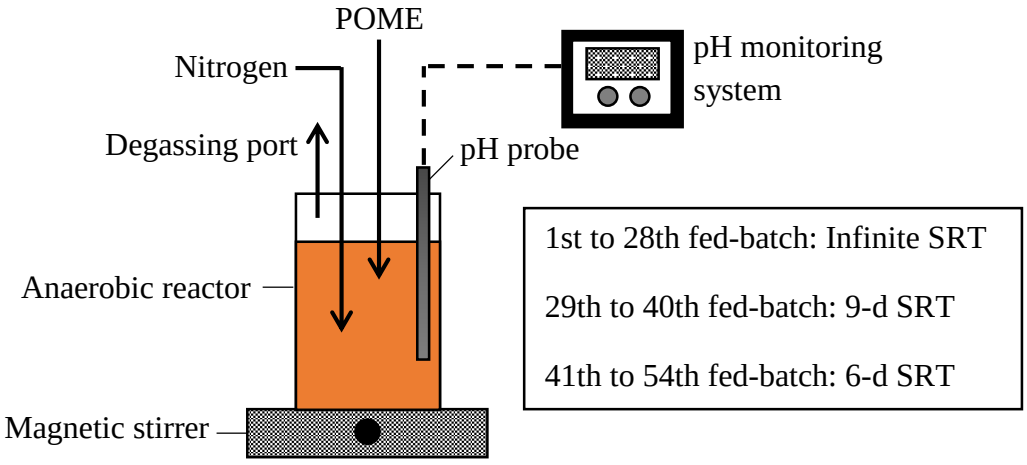


Figure 3.2: Setup of fed-batch anaerobic reactor used for producing VFA from POME at different SRT.

The SRT was reduced along the reactor operation; initially at infinite SRT (1st to 28th fed-batch), then shortening to 9-d SRT (29th to 40th fed-batch) and eventually to 6-d SRT (41th to 54th fed-batch). Infinite SRT was achieved by allowing the mixed liquor in the anaerobic reactor to settle at the end of each fed-batch operation, followed by removing only the supernatant, so that the settled sludge could be retained in the reactor. The operation of the reactor at infinite SRT was terminated at the end of 28th fed-batch due to the extremely poor VFA production performance. At the beginning of 29th fed-batch, seed sludge collected from the second anaerobic pond was added into the reactor

to re-activate and re-initiate the acidogenic fermentation process. Since then, the reactor was operated at 9-d SRT and followed by 6-d SRT.

When the reactor was operated at infinite SRT, the HRT varied with the volume of supernatant withdrawn after settling and was in a range of 9-13 d. When the reactor was operated at SRT of 9 d and 6 d, settling was eliminated from the reactor operation, hence the HRT was equal to the SRT. Anaerobic conditions were created and maintained by introducing nitrogen gas into the reactor at the beginning of each fed-batch operation, during sampling and withdrawal of reactor content. For reactor operating at infinite SRT, purging of nitrogen was not performed during the withdrawal of reactor content to avoid resuspension of the settled sludge.

3.3.2 Operation of the anaerobic reactors at different temperatures

Three 1.5-L anaerobic reactors were used for the production of VFA from POME at room temperature (30±1°C), 40±1°C and 55±1°C. The operating temperatures of 40°C and 55°C were maintained by continuous circulation of hot water (Wisd WiseCircu WCR-P8, witeg Labortechnik GmbH, Germany) through the jacket of the reactors (Figure 3.3).

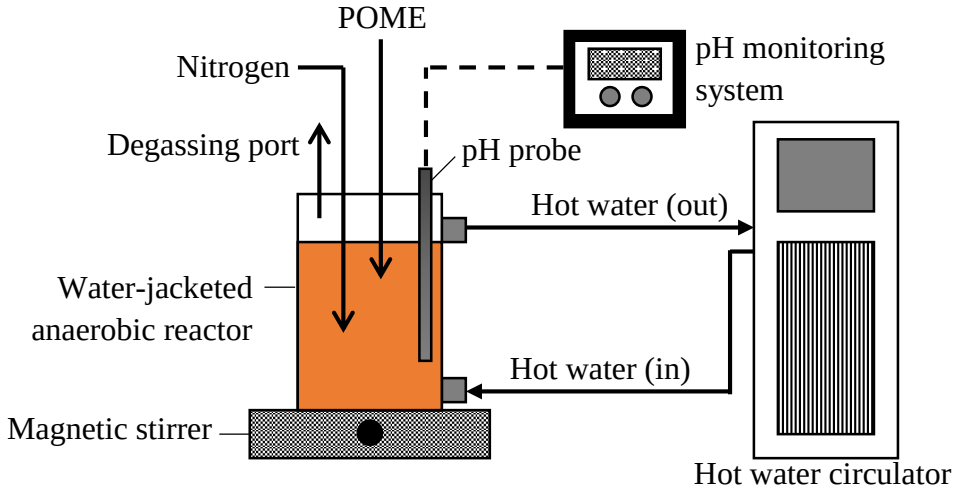


Figure 3.3: Setup of anaerobic reactor used for producing VFA from POME at 40°C and 55°C.

These three reactors were seeded with sludge collected from different POME treatment ponds with temperatures closest to the operating temperatures of the reactors. The seed sludge for reactor operating at 55°C was sampled from a POME collection pond with a temperature of 53-63°C. On the other hand, the reactor operating at 40°C was inoculated with a mixture of sludge taken from the first and the second anaerobic ponds with temperatures of 45°C and 33°C, respectively. Meanwhile, the sludge seeded into the reactor operating at 30°C was collected from the aforementioned second anaerobic pond.

Except operating temperature, all three reactors were operated under similar fed-batch mode with a feeding interval of 5 d. The HRT and SRT were maintained at about 6 d. The pH in all reactors was not controlled. To create and maintain the anaerobic conditions, nitrogen gas was introduced into the reactor at the beginning of each fed-batch operation, and during sampling and mixed liquor withdrawal. All three reactors were operated for 60 d, corresponding to a total of 12 fed-batch operations. Table 3.1 summarizes the conditions of the reactors.

Table 3.1: Operating parameters investigated in the fed-batch production of VFA by acidogenic fermentation of POME

Investigated operating parameter	Fed-batch number	SRT (d)	Temperature (°C)
SRT (series investigation)	1-28	Infinity	30
	29-40	9	30
	41-54	6	30
Temperature (parallel investigation)	12	6	30
	12	6	40
	12	6	55

3.3.3 Evaluation of VFA production performance

Degree of acidification (DA), as originally defined by Equation (1), is a parameter commonly used to assess the performance of VFA production from wastewater (Oktem

et al., 2006). DA signifies the percentage conversion of soluble organic substrates available in wastewater into VFA.

$$\mathrm{DA} = \frac{\text{VFA produced}}{\text{Initial substrate}} \times 100\% \quad = \frac{\mathrm{VFA}_{\text{final}}}{\mathrm{sCOD}_{\text{initial}}} \times 100\% \tag{1}$$

In this work, to account for the existence of VFA in POME prior to acidogenic fermentation, DA was computed differently as in Equation (2).

$$\mathrm{DA} = \frac{\text{VFA produced}}{\text{Initial substrate}} \times 100\% \quad = \frac{\mathrm{VFA}_{\text{final}} - \mathrm{VFA}_{\text{initial}}}{(\mathrm{sCOD} - \mathrm{VFA})_{\text{initial}}} \times 100\% \tag{2}$$

Here it was recognized that VFA would contribute to the sCOD measured, so its concentration was deducted from $\mathrm{sCOD}_{\text{initial}}$ to avoid overestimating the availability of fermentable soluble organic substrates.

For the same reason, the percentage of substrate consumption in acidogenic fermentation of POME was calculated according to Equation (3).

$$\%\ \text{substrate consumption} = \frac{\text{Substrate consumed}}{\text{Initial substrate}} \times 100\%$$

$$= \frac{(\mathrm{sCOD} - \mathrm{VFA})_{\text{initial}} - (\mathrm{sCOD} - \mathrm{VFA})_{\text{final}}}{(\mathrm{sCOD} - \mathrm{VFA})_{\text{initial}}} \times 100\% \tag{3}$$

On the other hand, the percentage of COD reduction was calculated using Equation (4).

$$\%\,\mathrm{COD\ reduction} = \frac{\mathrm{sCOD}_{\text{initial}} - \mathrm{sCOD}_{\text{final}}}{\mathrm{sCOD}_{\text{initial}}} \times 100\% \tag{4}$$

The equivalent COD concentration of VFA was used in the calculation of DA and percentage of substrate consumption for consistency of units. The equivalent COD concentration was obtained by multiplying the absolute VFA concentration with their

respective conversion factor as listed in Table 3.2. The conversion factor was adapted from the work of Alexiou et al. (1994).

Table 3.2: Conversion factor used to calculate the equivalent COD and carbon concentrations of VFA

Type of VFA	Conversion factor for calculating	
	Equivalent COD concentration	Equivalent carbon concentration
Formic acid	0.348	0.261
Acetic acid	1.066	0.400
Propionic acid	1.512	0.486
Butyric acid	1.816	0.545
Valeric acid	2.036	0.588

3.4 Operation of the cultivation reactor of PHA-accumulating organisms

The cultivation of PHA-accumulating organisms was carried out in a 1.2-L aerobic reactor (Figure 3.4) operating under aerobic dynamic feeding conditions at 28-30°C. The aerobic conditions were achieved by supplying air at around 1 vvm (gas volume flow per reactor working volume per minute) to the reactor. The cultivation reactor was inoculated with activated sludge collected from a local municipal wastewater treatment plant located in the federal territory of Kuala Lumpur, Malaysia. It was operated in sequencing batch mode with a cycle length of 24 h. Each cycle consisted of three phases: (a) 6-min feeding of 0.3 L carbon substrate and 0.3 L nutrient solution, (b) 23.7-h reaction (feast and famine) and (c) 13-min withdrawal of 0.6 L mixed liquor from the reactor. There was no settling phase, thus the HRT was equal to the SRT at 2 d (Jiang et al., 2012). The pH in the cultivation reactor was maintained at 7.0 ± 0.2 (Jiang et al., 2012; Johnson et al., 2009) by adding either 1 mol/L hydrochloric acid (HCl) or 1 mol/L sodium hydroxide (NaOH) solution.

The VFA-rich fermented POME obtained from the anaerobic reactor operating at 30°C was used as the sole carbon substrate. Prior to feeding, the fermented POME was filtered through 1.2 µm filter paper (MGC glass microfiber filter, Sartorius) to remove coarse solid particles. Then, it was diluted with reverse osmosis water (Arium® 61316,

Sartorius) to a concentration of 1440 mg VFA-C/L. On the other hand, the nutrient solution consisted of 1070 mg/L ammonium chloride, 1460 mg/L potassium dihydrogen phosphate, 660 mg/L magnesium sulfate heptahydrate, 20 mg/L N-Allylthiourea and 3.33 mL/L trace element solution was provided to assist microbial growth. N-Allylthiourea was added to prevent nitrification (Johnson et al., 2010a). The composition of the nutrient solution was adapted from the work of Jiang et al. (2012). Meanwhile, the composition of the trace element solution was adapted from the work of Ong et al. (2012) and was presented in Table 3.3.

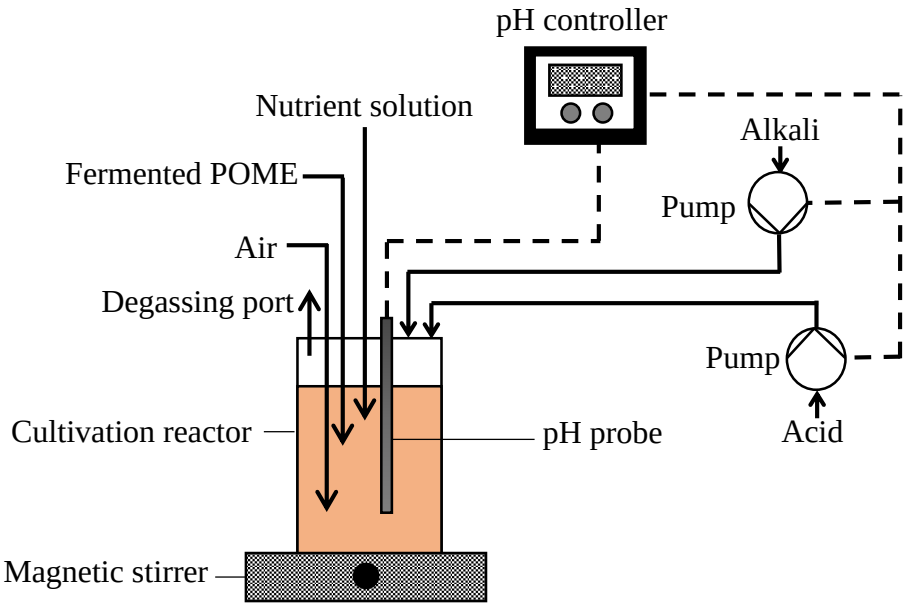


Figure 3.4: Setup of the cultivation reactor of PHA-accumulating organisms.

Table 3.3: Composition of trace element solution

Chemical	Concentration (mg/L)
Ethylenediamine tetraacetic acid	14400
Iron (III) chloride hexahydrate	2160
Potassium iodide	260
Boric acid	220
Cobalt (II) chloride hexahydrate	220
Manganese (II) chloride tetrahydrate	170
Zinc sulfate heptahydrate	170
Sodium molybdate dehydrate	80
Copper (II) sulfate pentahydrate	50

3.5 Batch PHA production by activated sludge

To evaluate the PHA storage capacity of the activated sludge cultivated under ADF conditions, 0.6 L of mixed liquor was withdrawn at the end of the cyclic operation of the cultivation reactor from time to time and subjected to PHA production. For sludge collected between day 4 and day 50, the production of PHA was carried out in a 0.5-L aerobic reactor at 28-30°C for 24 h. The aerobic conditions were achieved by supplying air at 1 vvm to the reactor. The pH was not controlled throughout the whole PHA production process. Fermented POME obtained from the anaerobic reactor operating at 30°C was employed as the sole carbon substrate. It was filtered and its pH was adjusted to 7 using 1 mol/L NaOH before being added into the reactor at concentration as shown in Table 3.4. It could be seen that the concentration of fermented POME increased with the operating days of the cultivation reactor. Such increment was to ensure sufficient amount of VFA was available for PHA production because the VFA uptake ability of the activated sludge had improved considerably over the 50 d of cultivation. Unlike the cultivation reactor, no nutrient solution was provided to the PHA production reactor in order to suppress microbial growth and to promote PHA production.

Table 3.4: Source of activated sludge and concentration of fermented POME added into the PHA production reactor

Source of activated sludge	Description of activated sludge	Concentration of fermented POME added into the reactor (mg VFA-C/L)
Municipal wastewater treatment plant	Seed sludge	750 at hour 0
Cultivation reactor of PHA-accumulating organisms	Day 4[a]	750 at hour 0 and 350 at hour 8
	Day 14	950 at hour 0 and 550 at hour 8
	Day 26	950 at hour 0 and 550 at hour 8
	Day 33	950 at hour 0 and hour 8
	Day 50	950 at hour 0 and hour 8
	> Day 75	950 at hour 0 only

[a]Cultivation day

For sludge taken from day 75 onwards, the PHA production was carried out in a 1-L aerobic reactor at pH 7 and 28-30°C for 8 h. Air was supplied at 1 vvm to the reactor.

Fermented POME (after filtration and pH adjustment as described in the paragraph above) was added into the reactor at a concentration of 950 mg VFA-C/L only at hour 0. Again, no nutrient solution was provided. The duration of PHA production and the feeding frequency of fermented POME were reduced because the maximum PHA storage capacity of the activated sludge could be reached in 8 h. This observation is detailed in Section 6.2.

3.5.1 Batch PHA production at different pH values

The production of PHA at different pH values (Table 3.5) was carried out in a 1-L aerobic reactor. Four different pH conditions were investigated, i.e. no pH control and with the control of pH at 7, 8 and 9 (±0.2). The pH was maintained at desired value by dosing either 1 mol/L HCl or 1 mol/L NaOH solution into the reactor. The dosing frequency was determined by a pH control system (αlpha pH 800 pH controller & EC-ARTSO05B pH probe, Eutech Instruments, Singapore). Other operational details of the PHA production reactor were similar to those mentioned in the paragraph above. Activated sludge collected from the cultivation reactor of PHA-accumulating organisms on days 72-76 was used as the inoculum in the pH study.

Table 3.5: Conditions of pH and air supply rate applied to the batch production of PHA by activated sludge originated from the cultivation reactor of PHA-accumulating organisms

Study	pH	Air supply rate (vvm)	Concentration of fermented POME added into the reactor (mg VFA-C/L)
Effect of pH	No pH control (~4.5)	1.0	950 at hour 0
	7	1.0	950 at hour 0
	8	1.0	950 at hour 0
	9	1.0	950 at hour 0
Effect of air supply rate	7	1.0	950 at hour 0
	7	0.5	950 at hour 0
	7	0.2	950 at hour 0

3.5.2 Batch PHA production at different air supply rates

In the literature (Din et al., 2012; Mohan and Reddy, 2013; Reddy & Mohan, 2012; Third et al., 2003), it is often reported that oxygen-limited condition (also known as microaerophilic condition) could enhance the production of PHA. Therefore, the PHA production test was conducted at reduced air supply rate, from 1 vvm to 0.2 vvm, to limit the oxygen availability in the PHA production reactor for possible improved PHA production performance. Experiments on the influence of air supply rates on PHA production were conducted under conditions similar to those described in Section 3.5.1, except the pH was kept at 7 and air was supplied to the reactor at three different rates of 0.2, 0.5 and 1.0 vvm (Table 3.5). Air supply rate was regulated at the desired value by using an air flow meter (RZ-32900, Cole-Parmer, United States of America). Activated sludge used in the air supply study was taken from the cultivation reactor of PHA-accumulating organisms on days 177-179.

The oxygen mass transfer coefficient ($k_L a$) at each air supply rate was estimated by using dynamic method as described in the work of Nittami et al. (2013). The estimation was carried out in the PHA production reactor containing mixed liquor taken from the cultivation reactor of PHA-accumulating organisms and reverse osmosis water. Fermented POME (the substrate) was not added because its presence would lead to strong consumption of dissolved oxygen (DO) by the sludge, making re-oxygenation to saturation impossible during the estimation of $k_L a$. The concentration of DO in the reactor was measured using YSI 550A DO meter (YSI, United States of America).

3.5.3 Evaluation of PHA production performance

The PHA storage capacity of the activated sludge was evaluated by using PHA content calculated according to Equation (5).

$$\text{PHA content} = \frac{\text{PHA}}{\text{Sludge dry weight}} \times 100\% = \frac{\text{PHA}}{\text{mixed liqour VSS}} \times 100\% \quad (5)$$

Meanwhile, the specific VFA consumption rate, specific PHA production rate and specific growth rate were computed using Equations (6-8). The cell concentration was taken as the difference in concentration between mixed liquor VSS and PHA (Albuquerque et al., 2011).

Specific VFA consumption rate ($-q_{VFA}$)

$$= \frac{\text{VFA consumed}}{\text{Initial cell concentration} \times \text{Duration of experiment}} = \frac{VFA_{initial} - VFA_{final}}{X_{initial} \times time} \quad (6)$$

Specific PHA production rate (q_{PHA})

$$= \frac{\text{PHA produced}}{\text{Initial cell concentration} \times \text{Duration of experiment}} = \frac{PHA_{final} - PHA_{initial}}{X_{initial} \times time} \quad (7)$$

Specific growth rate (q_X)

$$= \frac{\text{Cell produced}}{\text{Initial cell concentration} \times \text{Duration of experiment}} = \frac{X_{final} - X_{initial}}{X_{initial} \times time} \quad (8)$$

3.6 Analytical methods

3.6.1 Chemical analysis

TSS and VSS were determined according to the standard methods (APHA, 1992). In TSS analysis, the sample was dried in an oven at 103 ± 2°C for 1 h. On the other hand, VSS analysis was carried out in a furnace operating at 500 ± 50°C for 15-20 min. The COD of was determined in accordance with HACH method 8000 whereby 2 mL of sample was added into the COD digestion vial (HR, HACH, United States of America) and heated at 150°C for 2 h. After that, the vial was allowed to cool to room

temperature and the COD was measured by using a colorimeter (DR/890, HACH, United States of America).

The concentrations of VFA (formate, acetate, propionate, butyrate and valerate), ammonium and phosphate were measured by using ion chromatography (861 Advanced Compact IC, Metrohm, Switzerland) equipped with conductivity detector. Each of these analyses was carried out using different column, eluent, suppression solution and regeneration solution, as detailed in Table 3.6. Standard solutions used in VFA analysis were prepared from concentrated formic (≥98%, Merck), acetic (99.8%, Riedel-de-Haën), propionic (>99%, Merck), butyric (≥99%, Merck) and valeric acids (≥98%, Merck). Ammonium and phosphate standard solutions were prepared by dissolving ammonium chloride (≥99.8%, Merck) and potassium dihydrogen phosphate (99.5%, Merck) in ultra pure water (Arium® 611UF, Sartorius) respectively.

Table 3.6: Column, eluent, suppression solution and regeneration solution used in the analyses of VFA, phosphate and ammonium by ion chromatography

Type of compounds	Column	Eluent (flow rate)	Suppression solution	Regeneration solution
VFA (formate, acetate, butyrate, valerate, propionate)	Metrosep Organic Acids – 250/7.8 column	0.5 mmol/L sulfuric acid (0.5 mL/min)	50 mmol/L lithium chloride	Ultra pure water
Phosphate	Metrosep A Supp 5 – 150/4 column	339 mg/L sodium carbonate & 84 mg/L sodium hydrogen carbonate (0.7 mL/min)	100 mmol/L sulfuric acid	Ultra pure water
Ammonium	Metrosep C 4 – 150/4 column	117 mg/L pyridine-2,6-dicarboxylic acid & 110 µL/L 65% nitric acid (0.9 mL/min)	Nil	Nil

The PHA was extracted from the sludge according to the method described by Satoh et al. (1996). First of all, the PHA-containing sludge sample was lyophilized in a freeze-dryer (FDU-1100, EYELA, Japan) for 24 h. Then, 2 mL of chloroform and 2 mL of

acidified methanol containing 10% v/v sulfuric acid and 20% w/v benzoic acid were added to the dried sample. The mixture was then heated in an oven at 100°C for 24 h. After cooling down to room temperature, it was added with 1 mL of 14% aqueous ammonia solution. Then, the mixture was subjected to centrifugation to separate it into two distinct liquid layers. The bottom layer, which contained chloroform and PHA, was withdrawn and transferred into a vial. After that, the vial was added with ultra pure water. The mixture was centrifuged and the bottom layer was withdrawn and used for PHA analysis.

The PHA analysis was carried out using gas chromatography (GC2010, Shimadzu, Japan) equipped with capillary column (J&W, DB-Wax) and flame ionization detector. Nitrogen was used as the carrier gas. During the analysis, the temperature of the column changed with time in the following sequences: (a) maintained at 90°C for 2 min, (b) increased from 90°C to 120°C at a rate of 25°C/min, (c) increased from 120°C to 138°C at a rate of 2°C/min, (d) increased from 138°C to 160°C at a rate of 8°C/min, (e) increased from 160°C to 180°C at a rate of 50°C/min and (f) kept at 180°C for 3 min. The whole PHA analysis took about 18 min. Two types of PHA were quantified in this study namely 3HB and 3HV by employing poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (Natural origin, PHV content 12 wt%, Sigma-Aldrich) as the standard.

3.6.2 Microscopic observation

Activated sludge taken from the cultivation reactor of PHA-accumulating organisms was subjected to various microscopic examinations, i.e. morphology study, identification of Gram-positive and Gram-negative bacteria and observation of PHA accumulated inside the sludge. The study of morphology was performed using wet sludge sample and a light microscope (DM2500, Leica, Germany). For the identification

of Gram-positive and Gram-negative bacteria, the sludge was subjected to Gram staining in accordance with the protocol provided by Merck which was the supplier of all the chemical solutions used in Gram staining. The air-dried sludge was first stained with crystal violet solution for 1 min. Then, it was rinsed briefly using Lugol's solution. After that, the sludge was stained with Lugol's solution for 1 min, followed by rinsing with ultra pure water. Next, it was washed with decolorizing solution until a clear stream leaving the slide was observed. The decolorizing step took 10-15 sec. The sludge was then rinsed with ultra pure water and stained with safranine solution for 1 min. Finally, the sample was rinsed with ultra pure water to remove excess safranine solution. Under the observation of a light microscope (DM2500, Leica, Germany), Gram-positive bacteria would appeared in blue/violet color while Gram-negative bacteria in pink/red color. On the other hand, Nile blue A staining was used to identify the PHA stored inside the sludge. This staining method has long been employed by many researchers (Jiang et al., 2012; Kitamura & Doi, 1994; Oshiki et al., 2011; Ostle & Holt, 1982) to detect the presence of PHA. Prior to staining, the sludge sample was air-dried on a glass slide. It was then stained with Nile blue A solution for 15 min at room temperature. Nile blue A solution was prepared by dissolving 5 mg of Nile blue A in 50 mL of ethanol. After staining, the sludge was examined using a fluorescence microscope (DM2500, Leica, Germany) equipped with filter cube Y3. The PHA stained with Nile blue A solution would emit bright orange color.

Chapter 4: Influence of SRT and Temperature on the Production of VFA from POME

4.1 Characteristics of POME

The characteristics of raw POME and supernatant of settled POME are compared in Table 4.1. It could be seen that raw POME was an acidic, organic-rich wastewater containing a lot of suspended solids. To reduce its solid content, raw POME was allowed to settle for 24 h. It was found that about 89% of the TSS in raw POME could be removed via this simple solid separation technique, resulting in a final TSS concentration of 2400 mg/L.

Table 4.1: Characteristics of raw POME and supernatant of settled POME recovered after 24 h gravitational settling (standard deviations are due to different batches of POME collected from the mill)

Parameters	Raw POME	Supernatant of settled POME
pH	4.7 ± 0.3	4.6 ± 0.4
TSS (mg/L)	21000 ± 2000	2400 ± 800
VSS (mg/L)	20000 ± 2000	2200 ± 700
TCOD (mg/L)	55000 ± 10000	27000 ± 6000
sCOD (mg/L)	24000 ± 5000	24000 ± 5000
VFA (mg/L)	2700 ± 800	2700 ± 900
sCOD/TCOD	0.43 ± 0.03	0.90 ± 0.04
VFA/sCOD	0.15 ± 0.05	0.15 ± 0.06

The removal of such a great amount of TSS had resulted in a lower TCOD concentration. However, the supernatant of settled POME was still considered a suitable feedstock for VFA production because it remained considerably rich in organic matter with TCOD at 27000 mg/L. More importantly, most of the organic matter is soluble as indicated by the high ratio of sCOD/TCOD at 0.9. This ratio was double that of the raw POME at 0.43. The higher fraction of soluble organic matter in the supernatant of settled POME is advantageous to microorganisms as they could more easily utilize the organic matter for VFA production.

According to Table 4.1, VFA were detected in the supernatant of settled POME with an average concentration of 2700 mg/L. Nonetheless, they were not the main fraction of the soluble organic matter in POME, as indicated by the low ratio of VFA to sCOD at 0.15. Consequently, there is room to maximize the utilization of POME by further converting the remaining organic matter into VFA via acidogenic fermentation.

Another piece of useful information derived from the characterization study was that pH control might not be required during the acidogenic fermentation of POME. This is because the acidic nature of POME (pH 4.6) could suppress the activity of methanogens which are active within a narrow pH range of 6.4-7.8 (Grady et al., 2011). Methanogens, if present in the reactor, could consume the VFA for methane formation. Without pH control, VFA can be produced at lower cost in line with the economic advantage of using waste for the production of a value-added product. Collectively, the results obtained from the characterization study suggest that POME is a suitable feedstock for VFA production.

4.2 Influence of SRT on the production of VFA

Fed-batch production of VFA from POME was carried out in an anaerobic reactor at 30°C. The reactor was operated at reduced SRT; starting from infinite SRT, then decreasing to 9-d SRT and subsequently to 6-d SRT. The DA profile corresponding to each SRT is depicted in Figure 4.1(a). It was observed that the performance of VFA production at infinite SRT deteriorated gradually in which the DA fell from 50% to less than 10% in the first 17 fed-batch operations. Thereafter, the VFA production performance remained poor, achieving an average DA of 6 ± 2% only in the subsequent 11 fed-batches. In literature, poor VFA production at high SRT is often related to the presence of slow-growing methanogens which consume the VFA for methane formation (Feng et al., 2009). However, in this study, there is no obvious sign of active

methanogenic activity, as indicated by the fairly low COD reduction at 4 ± 3% on average (Figure 4.2).

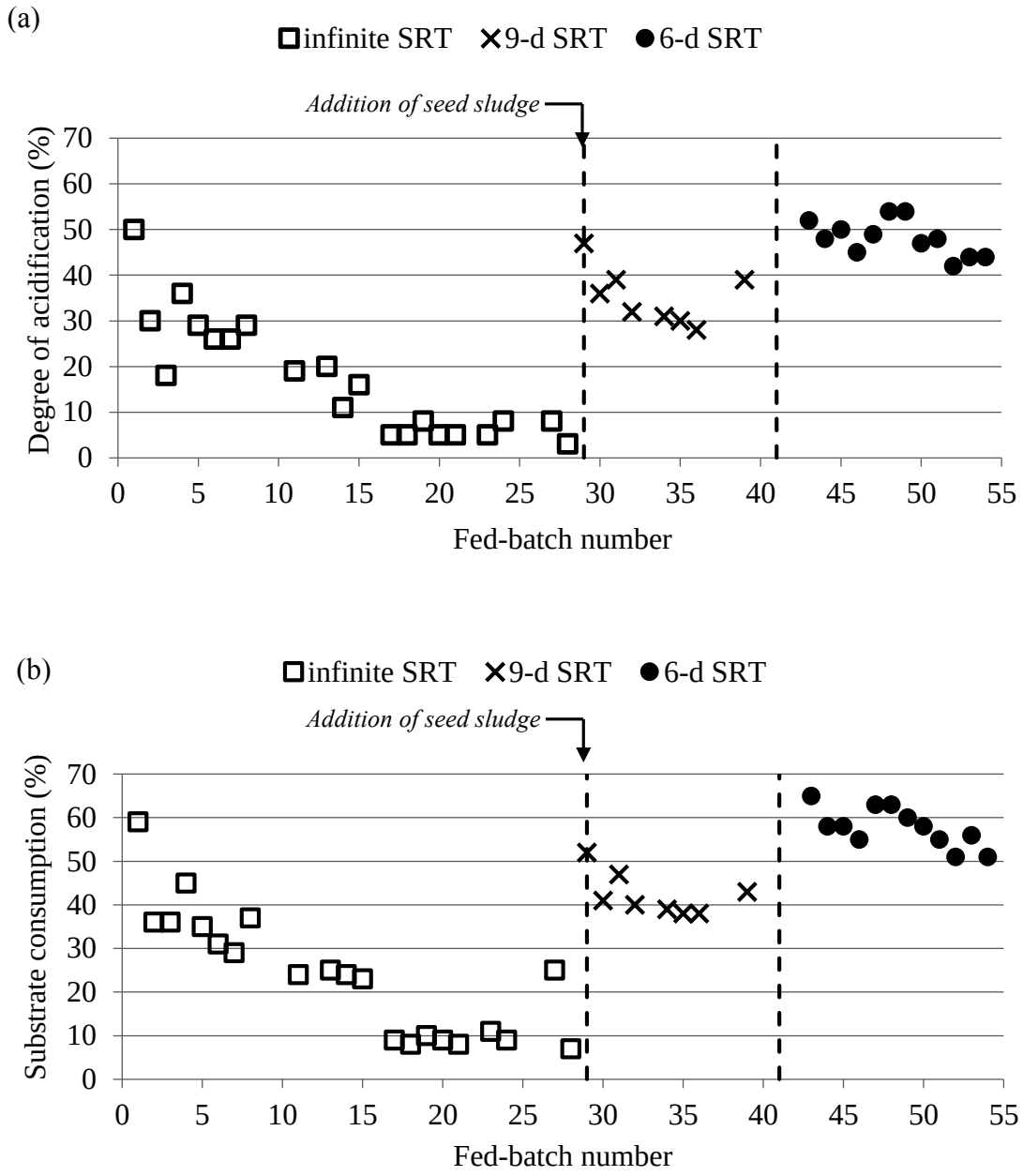


Figure 4.1: (a) Degree of acidification and (b) percentage of substrate consumption in the acidogenic fermentation of POME at infinite SRT, 9-d SRT and 6-d SRT. The vertical dashed lines represent the changeover of SRT.

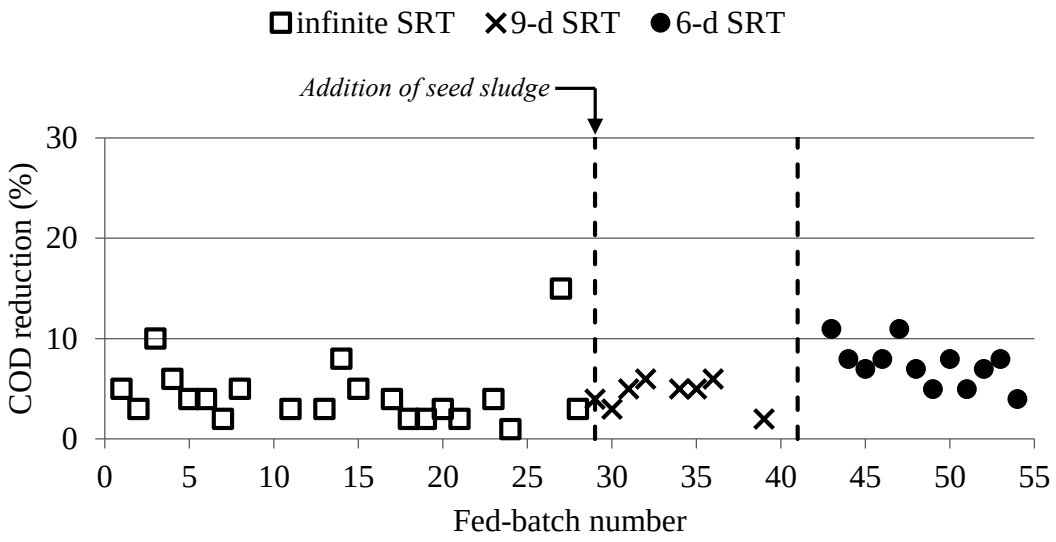


Figure 4.2: Percentage of COD reduction in the acidogenic fermentation of POME at infinite SRT, 9-d SRT and 6-d SRT. The vertical dashed lines represent the changeover of SRT.

Such contradictory observation is likely due to the inhibition of methanogenesis by the acidic conditions in the anaerobic reactor (pH 4.2-5.0) because methanogens are normally active under neutral conditions (pH 6.4-7.8) (Grady et al., 2011). Judging from Figure 4.1(b), the main reason behind the poor VFA production at infinite SRT seems to be reduced microbial activity as the percentage of substrate consumption dropped progressively over 28 fed-batches of acidogenic fermentation.

An attempt to recover the VFA production performance was made by adding seed sludge collected from the anaerobic POME treatment pond into the reactor. Accordingly, the performance of VFA production bounced back, reaching a DA of 47%. This encouraging result implies that the deterioration in VFA production performance as a result of old sludge age can be resolved by providing additional seed sludge to the reactor. The anaerobic reactor was then operated at 9-d SRT to prevent the loss of microbial activity. The operation of reactor at 9-d SRT lasted for 12 fed-batches, leading to an average DA and substrate consumption of 33 ± 5% and 41 ± 4%, respectively. From 41th fed-batch onwards, the SRT of the anaerobic reactor was

shortened to 6 d. The acidogenic activity could still be sustained at this lower SRT, and interestingly it led to higher DA of 48 ± 4% on average. The better VFA production performance was due to higher microbial activity, as indicated by the higher percentage of substrate consumption of 58 ± 4% at SRT of 6 d.

As a whole, this study has demonstrated the importance of regulating SRT in the production of VFA from POME. Based on the average DA achieved at the investigated SRT, it is recommended to conduct the acidogenic fermentation of POME at 6-d SRT. The recommended value is within the range of SRT (6-12 d) suggested by other researchers (Feng et al., 2009; Miron et al., 2000; Salmiati et al., 2007) for efficient production of VFA from waste.

Apart from DA, SRT also has considerable effect on the final VFA composition obtained in the acidogenic fermentation of POME. As shown in Figure 4.3, the percentage of acetic acid in the VFA produced at infinite SRT was significantly higher than that at 9-d SRT and 6-d SRT. Meanwhile, the fraction of propionic, butyric and valeric acids at infinite SRT was relatively lower. Formic acid was detected only at infinite SRT, but its fraction was trivial in relative to other type of VFA. On the contrary, the VFA composition obtained at 9-d SRT and 6-d SRT was alike in which acetic acid was the dominant VFA, followed by butyric, propionic and valeric acids.

The VFA composition is important to PHA production because it can affect the types of PHA produced and hence their mechanical properties. It had been reported that acetic and butyric acids favored the formation of 3HB meanwhile propionic and valeric acids promoted the generation of 3HV (Albuquerque et al., 2007; Lemos et al., 2006). Since copolymer of P(3HB-co-3HV) is more flexible and tougher than homopolymer P(3HB) (Holmes, 1985), it is thus preferred to use the fermented POME obtained at 9-d SRT or

6-d SRT for PHA production because of their higher percentage of propionic and valeric acids.

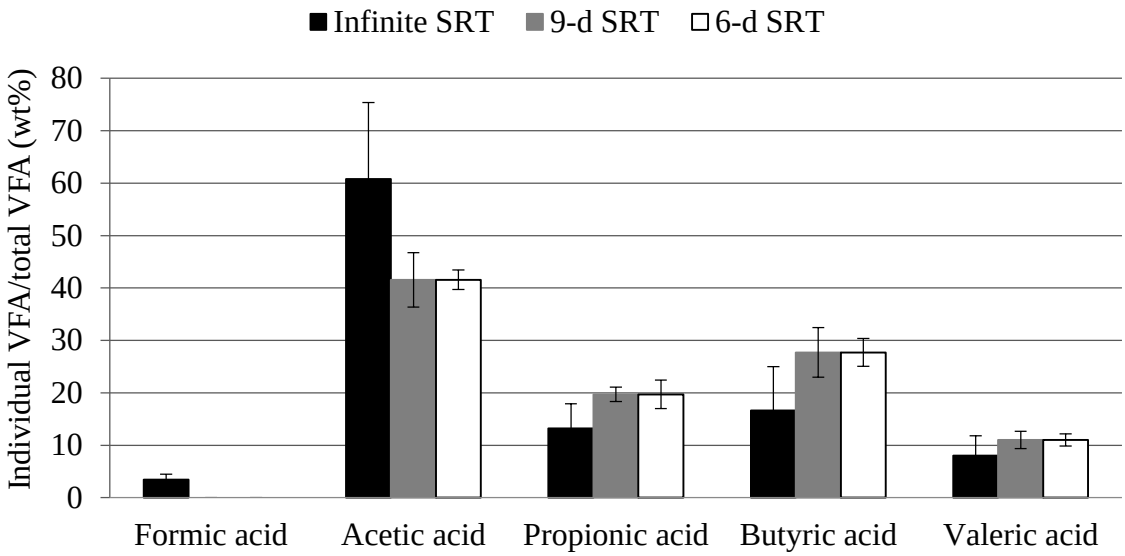


Figure 4.3: Average composition of VFA obtained at the end of fed-batch acidogenic fermentation of POME at infinite SRT, 9-d SRT and 6-d SRT.

4.3 Influence of temperature on the production of VFA

A total of 12 fed-batches of acidogenic fermentation of POME were executed at 30°C, 40°C and 55°C and their respective DA profiles are depicted in Figure 4.4(a). These fed-batch fermentations were conducted at SRT of 6 d. According to Figure 4.4(a), there was not much difference in the performance of VFA production conducted at 30°C and 40°C, as indicated by the fairly similar DA obtained at these two temperatures. On average, the DA attained at 30°C and 40°C was 48 ± 4% and 48 ± 5% respectively. However, these values were about six times higher than that achieved at 55°C at 7 ± 6%. The poorer VFA production at 55°C was caused by the lower microbial activity in the reactor, as the percentage of substrate consumption at 55°C was also approximately six times lower than those at 30°C and 40°C (Figure 4.4(b)). This reduction in microbial activity might be resulting from either poorer substrate uptake ability of the thermophilic VFA-forming microorganisms or relatively fewer such microbes in the

reactor. A similar finding had been reported by Zhuo et al. (2012) whereby the consumption of substrate decreased with the increase in temperature from 37°C to 55°C during the acidogenic fermentation of ultrasonic-pretreated waste activated sludge, leading to lower VFA production at 55°C. Active methanogenesis was not detected at all three temperatures investigated, as indicated by the low COD reduction which was always less than 11% (Figure 4.5).

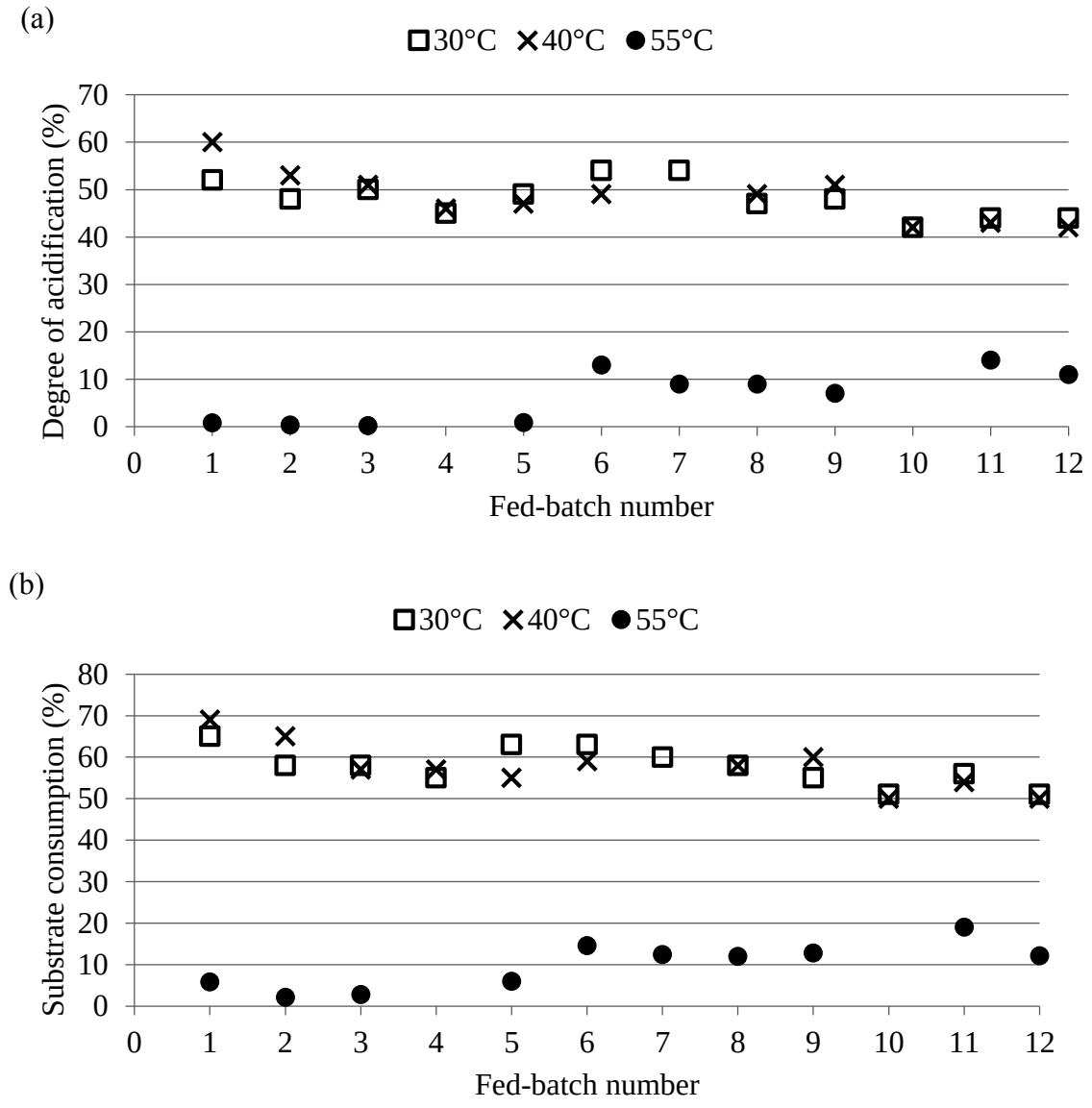


Figure 4.4: (a) Degree of acidification and (b) percentage of substrate consumption in the acidogenic fermentation of POME at 30°C, 40°C and 55°C.

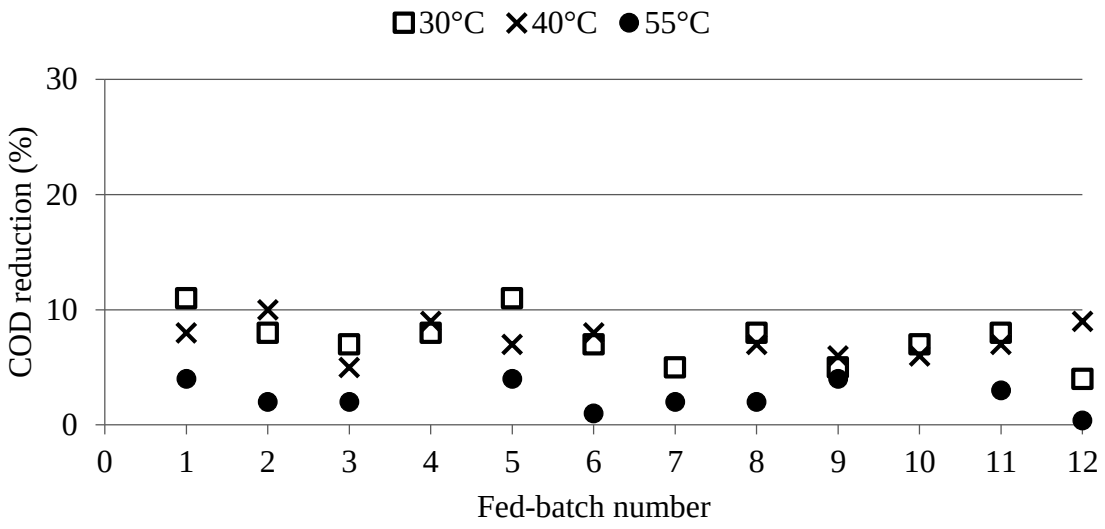


Figure 4.5: Percentage of COD reduction in the acidogenic fermentation of POME at 30°C, 40°C and 55°C.

Based on this study, it is obvious that mesophilic temperatures of 30°C and 40°C favor the production of VFA from POME as compared to thermophilic temperature of 55°C. Furthermore, the comparable VFA production at 30°C and 40°C suggests that temperature control will not be required in actual industrial application because most of the palm oil producers like Indonesia, Malaysia and Thailand are tropical countries with ambient temperatures at around 25-32°C. Nevertheless, POME, which is commonly discharged at 60-70°C, has to be cooled to 40°C before use in acidogenic fermentation. Such cooling of POME is presently accomplished in palm oil mills by using one or two open ponds in series, hence no additional cooling facilities are necessary to retrofit the mills for VFA production.

The influence of temperature on the composition of VFA obtained at the end of acidogenic fermentation of POME is illustrated in Figure 4.6. Regardless of the operating temperature, acetic acid was the primary VFA in the fermented POME. Furthermore, it was observed that the relative abundance of propionic, butyric and valeric acids obtained at 30°C and 40°C did not differ much. However, at 55°C, the percentage of propionic and valeric acids in the fermented POME was comparatively

lower. Formic acid was detected at 55°C but its percentage was insignificant as compared to the other VFA.

As mentioned in Section 4.2, the composition of VFA can affect the types of PHA produced whereby acetic and butyric acids favored the formation of 3HB whereas propionic and valeric acids encouraged the generation of 3HV (Albuquerque et al., 2007; Lemos et al., 2006). Having considered copolymer of P(3HB-co-3HV) has better mechanical properties as compared to homopolymer P(3HB) (Holmes, 1985), fermented POME obtained at 30°C or 40°C, which consists of higher fraction of propionic and valeric acids, is the preferred carbon substrate for PHA production.

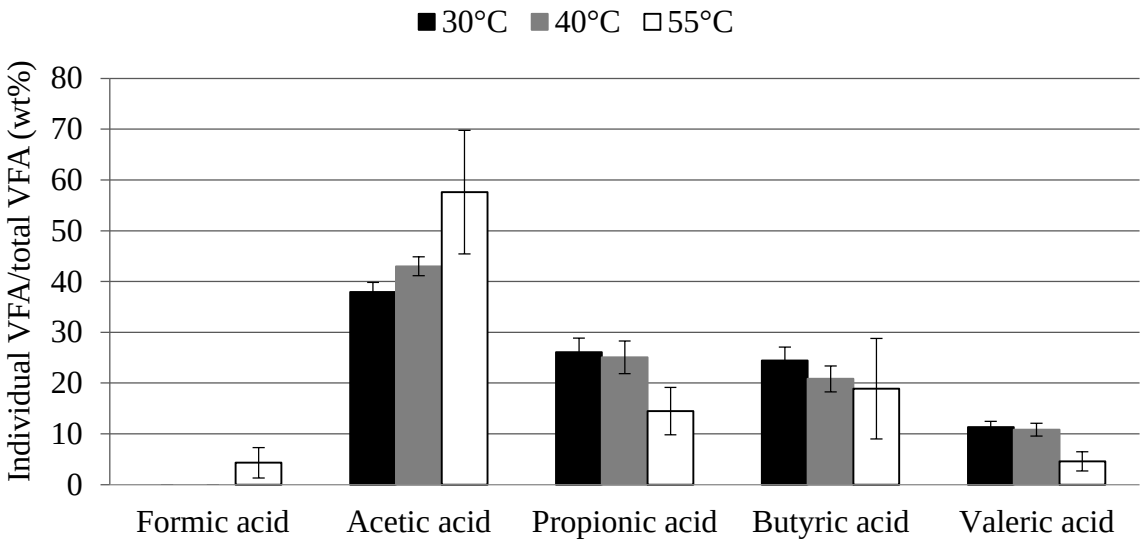


Figure 4.6: Average composition of VFA obtained at the end of 12 fed-batches of acidogenic fermentation of POME at 30°C, 40°C and 55°C.

4.4 Long-term stability study on the production of VFA

Fed-batch acidogenic fermentation of POME was carried out at 6-d SRT and 30°C for 9 months. The stability of VFA production within this long period of time was monitored, evaluated and presented in Figure 4.7(a). It was observed that the performance of VFA production in the first 13 fed-batches was fairly stable with DA averaging out at 48 ±

4%. However, there was a sharp decline in DA to 23 ± 6% (on average) in between 14th and 29th fed-batches. Unlike in the cases of infinite SRT and 55°C, this reduction in DA was not due to reduced microbial activity, though lower substrate consumption was observed in Figure 4.7(b). Here, the drop in DA and percentage of substrate consumption was a result of the lower amount of fermentable organic substrates available in the POME fed into the reactor.

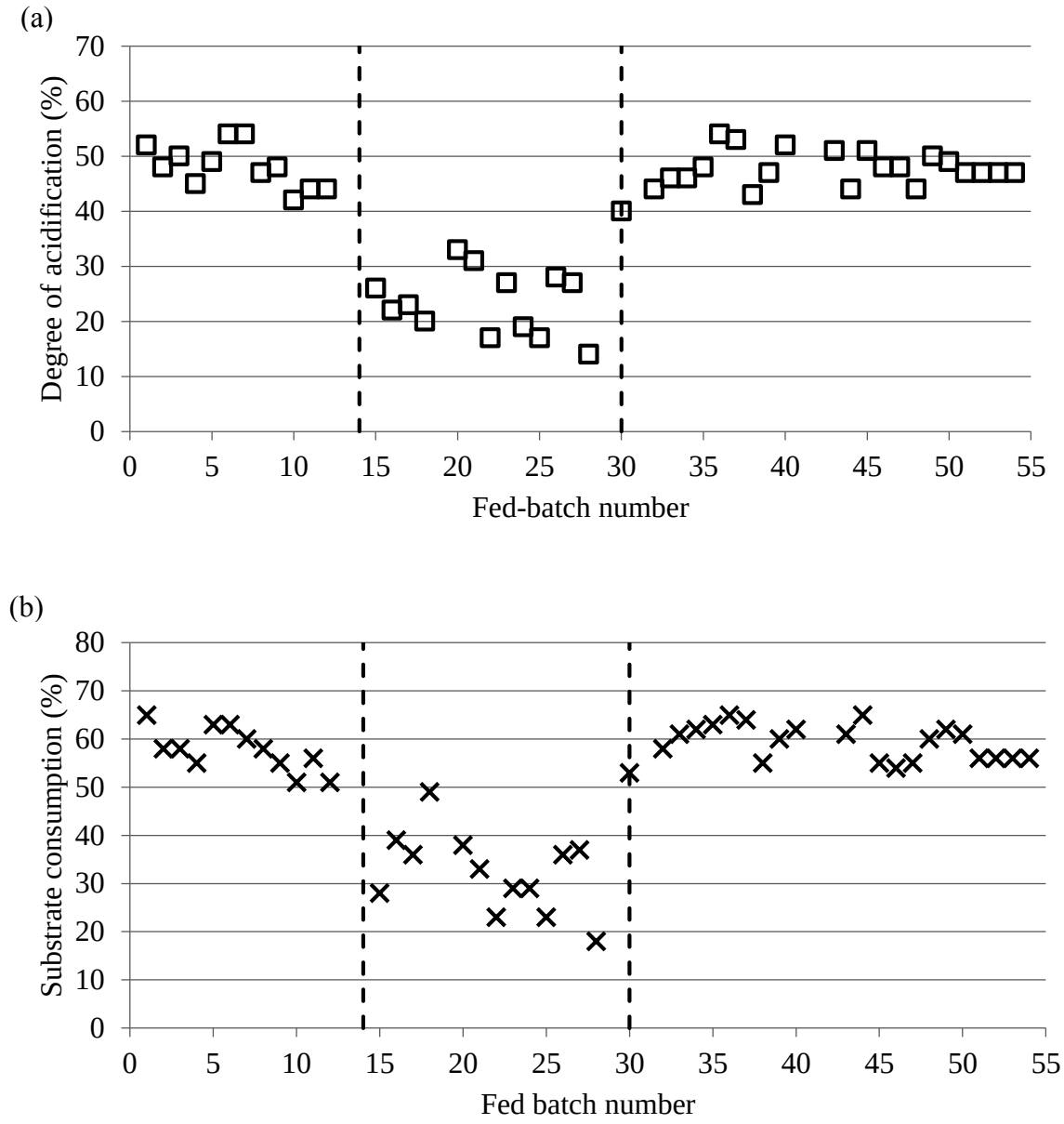


Figure 4.7: (a) Degree of acidification and (b) percentage of substrate consumption in long-term acidogenic fermentation of POME at 6-d SRT and 30°C. The vertical dashed lines represent the changeover of type of POME fed into the reactor. The characteristics of each type of POME are presented in Table 4.2.

Based on Table 4.2, the POME used in fed-batch numbers 14-29 has a higher ratio of VFA to sCOD of 0.47 as compared to that used in the fed-batch numbers 1-13 at 0.15. This implies that the former contains less organic substrates because 47% of the sCOD is VFA. Besides, as shown in Table 4.3, only 17% of the remaining sCOD is fermentable. The percentage of fermentable soluble organic substrate in the POME used in fed-batch numbers 14-29 (17%) is much lower than that employed in fed-batch numbers 1-13 (49%) and 30-54 (57%). The presence of a lower fraction of fermentable organic substrates in POME would result in lower substrate uptake during acidogenic fermentation and subsequently reduce the conversion of substrate into VFA. This explains the lower DA and percentage of substrate consumption observed in fed-batch numbers 14-29. The lower amount of fermentable organic substrates available in the POME used in the 14th-29th fed-batches is likely owing to the occurrence of acidogenic fermentation in the POME collection pond in the mill, as hinted by the relatively higher VFA concentration of 5400 mg/L (Table 4.2). The POME used in this study was collected at the outlet of the collection pond.

Table 4.2: Characteristics of supernatant of settled POME used in the production of VFA at 6-d SRT and 30°C (standard deviations are due to different batches of POME collected from the mill)

Fed-batch	1-13	14-29	30-54
Source of POME			
Inlet/outlet of POME collection pond	Outlet	Outlet	Inlet
Characteristics of POME			
pH	4.6 ± 0.4	4.6 ± 0.1	4.7 ± 0.1
TSS (mg/L)	2500 ± 1000	1100 ± 500	2200 ± 1100
VSS (mg/L)	2200 ± 900	900 ± 500	2000 ± 1000
TCOD (mg/L)	23000 ± 4000	17000 ± 1000	27000 ± 5000
sCOD (mg/L)	21000 ± 3000	15000 ± 900	24000 ± 4000
VFA (mg/L)	2300 ± 600	5400 ± 800	730 ± 90
sCOD/TCOD	0.90 ± 0.05	0.87 ± 0.03	0.89 ± 0.04
VFA/sCOD	0.15 ± 0.07	0.47 ± 0.02	0.03 ± 0.01

To verify the abovementioned hypothesis, POME was sampled at the pond inlet and its characteristics are presented in Table 4.2. It has lower ratio of VFA to sCOD (0.03) and

VFA concentration (730 mg/L) as compared to the POME collected at the outlet of the pond. This comparison suggests that acidogenic fermentation did take place in the POME collection pond because of the increase in the fraction of sCOD as VFA and the concentration of VFA.

When the POME collected at the pond inlet was fed into the anaerobic reactor, the performance of VFA production bounced back and remained quite stable in the subsequent 25 fed-batch operations, achieving an average DA of 48 ± 3%. This result also affirms that the previous fall in DA was due to the low availability of the fermentable organic substrates in POME instead of the reduced microbial activity.

Table 4.3: Compositional analysis on the soluble organic compounds in POME

Type of soluble organic compounds[a] in POME		Equation	Fed-batch		
			1-13	14-29	30-54
[1]	VFA (%)	VFA/sCOD in POME × 100%	15	47	3
[2]	Total soluble organic substrates[b] (%)	100% – [1]	85	53	97
[2A]	Fermentable soluble organic substrates[c] (%)	[2] × average percentage of substrate consumption	49	17	57
[2B]	Non-fermentable soluble organic substrates[d] (%)	[2] – [2A]	36	36	40

[a]represented by sCOD; [b]defined as non-VFA organic compounds; [c]consumable by the sludge during acidogenic fermentation; [d]inconsumable by the sludge during acidogenic fermentation

On the other hand, there is no sign of active methanogenesis throughout the 9-month acidogenic fermentation process, as indicated by the low percentage of COD reduction (Figure 4.8). Collectively, these results show that it is highly feasible to achieve and maintain fairly steady and stable production of VFA by acidogenic fermentation of POME at 30°C and 6-d SRT in long run. Besides, it is notable that the DA achieved in this study (48%) is comparable to that attained in the acidogenic fermentation of wood mill effluent (Ben et al., 2011), pharmaceutical wastewater (Oktem et al., 2006) and dairy wastewater (Demirel & Yenigun, 2004) at 42%, 44% and 56% respectively. This

implies that POME is another promising wastewater for VFA production with par performance.

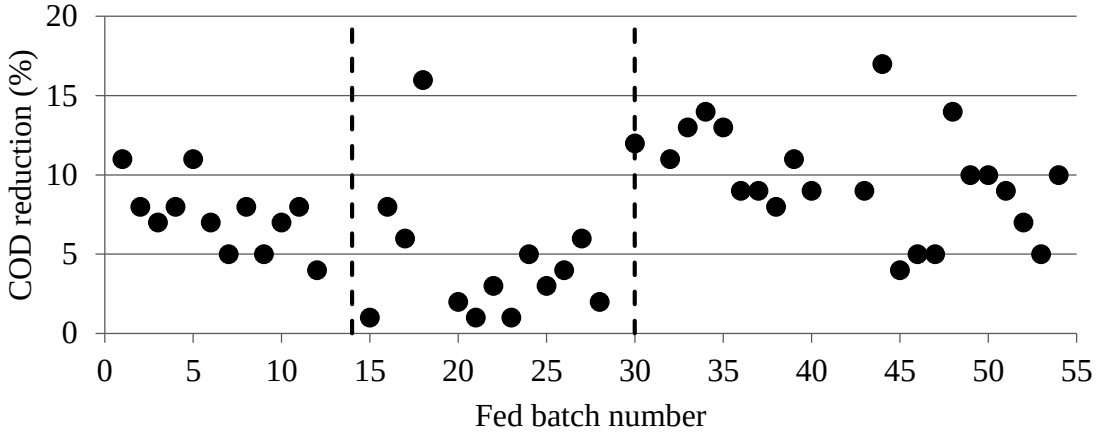


Figure 4.8: Percentage of COD reduction in the acidogenic fermentation of POME in long-term acidogenic fermentation of POME at 6-d SRT and 30°C. The vertical dashed lines represent the changeover of the type of POME fed into the reactor. The characteristics of each type of POME are presented in Table 4.2.

Chapter 5: Enriching the Activated Sludge with PHA-accumulating Organisms

5.1 PHA storage capacity of the raw activated sludge treating municipal wastewater

In this study, raw activated sludge sampled from a local municipal wastewater treatment plant was subjected to PHA production test to evaluate its PHA storage capacity. As illustrated in Figure 5.1, the PHA storage capacity of the raw activated sludge was extremely low as it could accumulate only about 9 wt% PHA per sludge dry weight in 24 h. This result was similar to that obtained by Takabatake et al. (2002) which was in a range of 6.0-29.5 wt%. Such low PHA storage capacity implies that the direct use of activated sludge treating municipal wastewater for PHA production is ineffective and inappropriate. Instead, the sludge should be subjected to proper cultivation process such as the ADF process to enrich with PHA-accumulating organisms with high PHA storage capacity.

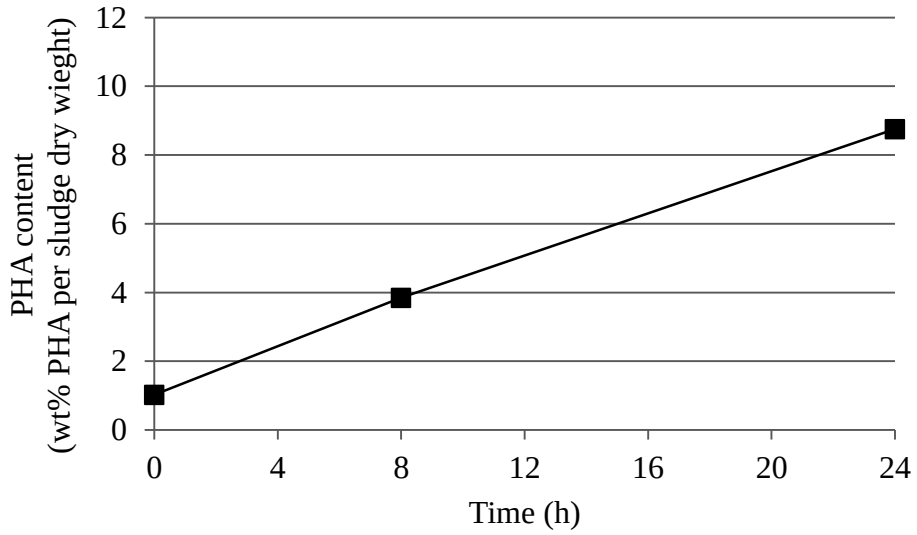


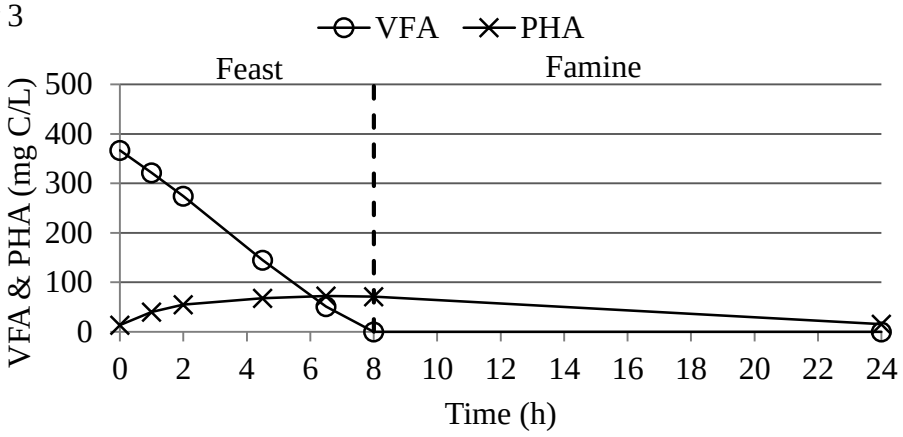
Figure 5.1: PHA production performance of the raw activated sludge taken from the municipal wastewater treatment plant.

5.2 Cultivation of PHA-accumulating organisms via the ADF process

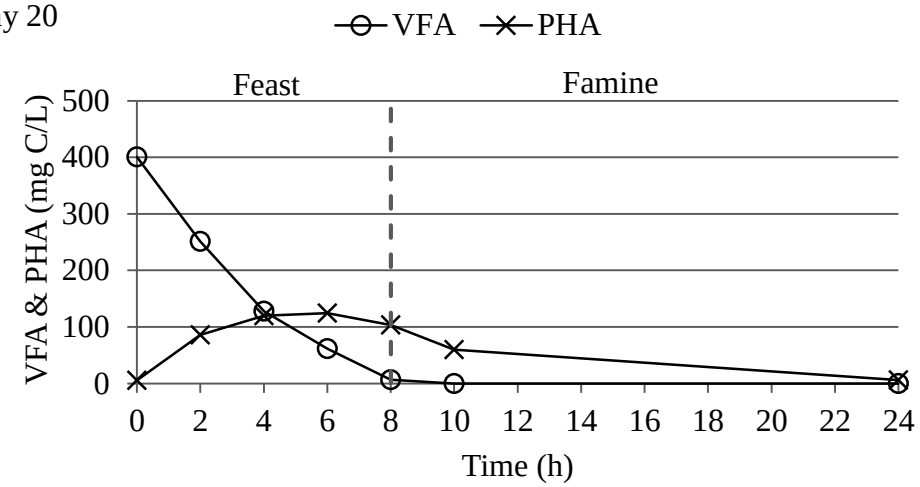
Based on the result discussed in Section 5.1, a reactor operating on ADF process was established to cultivate PHA-accumulating organisms. The reactor was seeded with activated sludge collected from municipal wastewater treatment plant at a concentration of 2650 mg VSS/L. VFA-rich fermented POME was used as the sole carbon source. Figure 5.2 shows the concentration profiles of VFA, PHA and sCOD in the cultivation reactor obtained on day 3, day 20, day 49, day 85 and day 126. It is apparent from Figure 5.2 that the sludge was exposed to transient availability of substrates (also known as feast and famine) repetitively. As evidenced, VFA, which were the dominant organic substrates in the fermented POME, were available only for 4-8 h, but one complete cyclic reactor operation took 24 h. Although the depletion of VFA did not result in zero sCOD, the residual organic compounds did not save the sludge in the cultivation reactor from starvation. These residual organic compounds were barely "edible" as indicated by the minor reduction in the sCOD concentration after the exhaustion of VFA. This observation implies that the sludge has to undergo a long period of famine once VFA are no longer available in the cultivation reactor.

Transient availability of substrates in the cultivation rector would induce a selection pressure on PHA-accumulating organisms because these organisms manage to store the substrate as intracellular PHA and utilize the PHA to survive the long famine (no external substrate). As depicted in Figure 5.2, the profiles VFA and PHA in the cultivation reactor were in agreement with the metabolic behavior of the PHA-accumulating organisms. Simultaneous uptake of VFA and storage of PHA was observed in the feast phase. Meanwhile, in the famine phase, consumption of PHA was noted after the depletion of VFA. Such observation indicates successful cultivation of PHA-accumulating organisms through the ADF process.

(a) Day 3



(b) Day 20



(c) Day 49

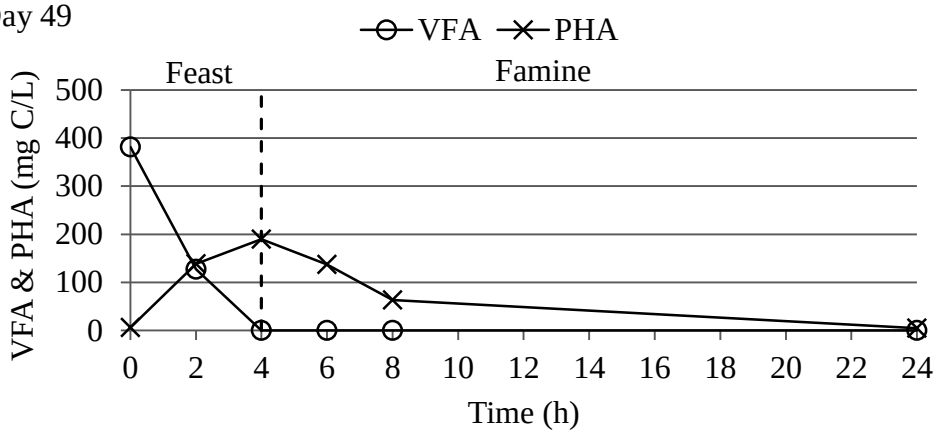
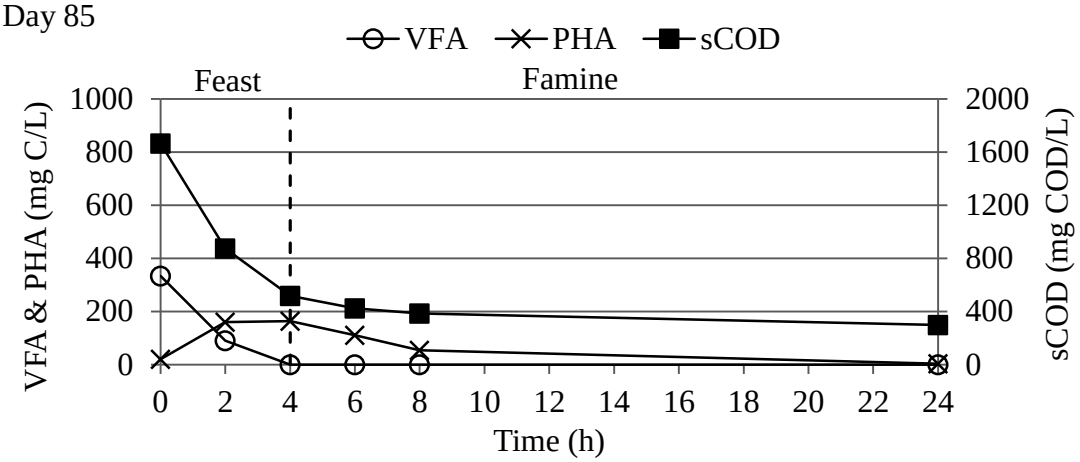


Figure 5.2: Concentration profiles of VFA, PHA and sCOD in the cultivation reactor of PHA-accumulating organisms monitored on (a) day 3, (b) day 20, (c) day 49, (d) day 85 and (e) day 126. The vertical dashed lines represent the changeover from feast phase to famine phase. The concentration of sCOD was not measured on days 3, 20 and 49.

(d) Day 85



(e) Day 126

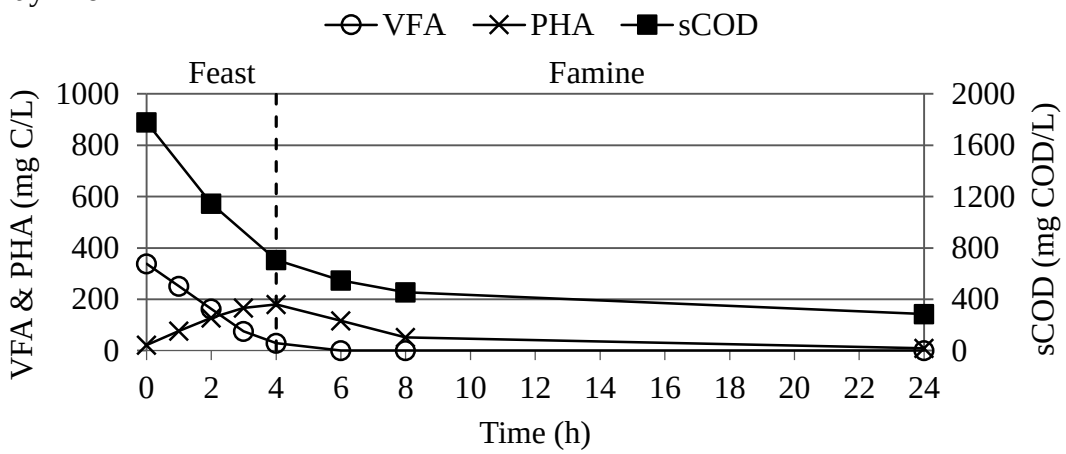
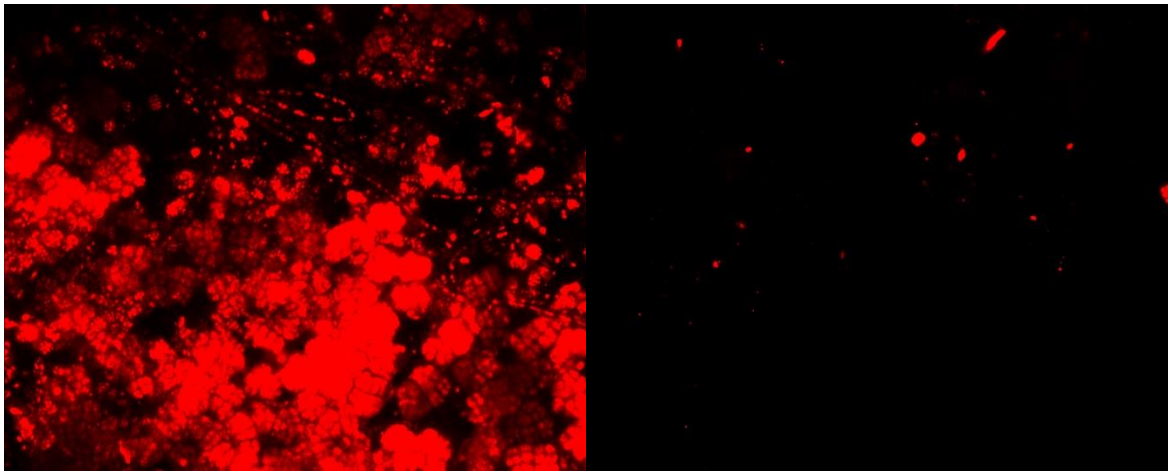


Figure 5.2 (continued): Concentration profiles of VFA, PHA and sCOD in the cultivation reactor of PHA-accumulating organisms monitored on (a) day 3, (b) day 20, (c) day 49, (d) day 85 and (e) day 126. Vertical dash line represents the changeover from feast phase to famine phase. The concentration of sCOD was not measured on days 3, 20 and 49.

The results obtained from the microscopic observation (Figure 5.3) were also consistent with the data acquired from the chemical analyses (Figure 5.2). Figures 5.3(a) and 5.3(b) show the PHA storage condition inside the sludge at the end of feast and famine phases, respectively. The presence of PHA was indicated by the bright orange color. It was observed that the number of spots with bright orange color in Figure 5.3(a) was far more than that in Figure 5.3(b). This was because PHA storage took place in the feast phase, so abundant PHA was observed in Figure 5.3(a). On the other hand, PHA was

consumed by the sludge in the famine phase, thus little PHA was detected in Figure 5.3(b).



(a) (b)

Figure 5.3: Microscopic examination of the PHA stored inside the sludge. Sludge was collected from the cultivation reactor of PHA-accumulating organisms (day 212) at the (a) end of feast phase and (b) end of famine phase. Bright orange color indicates the presence of PHA.

To evaluate the efficiency of ADF process in cultivating PHA-accumulating organisms, activated sludge was withdrawn from the cultivation reactor on different cultivation days and subjected to PHA production test. The efficiency of cultivation was assessed in terms of the maximum PHA content that could be attained by the cultivated sludge during the test.

Figure 5.4 presents the maximum PHA content achieved by the activated sludge cultivated between day 0 and day 177. It was realized that the seed sludge (day 0) could accumulate merely 4 wt% PHA per sludge dry weight in 8 h. Such low PHA content indicates that the PHA-accumulating organisms compose only a minor fraction of the total microbial species present in the seed sludge. After 4 days of cultivation, the PHA storage capacity of the sludge improved tremendously to 29 wt%. This improvement confirms the role of ADF process in enriching the sludge with PHA-accumulating organisms. From day 14 to day 33, the PHA content was in a range of 18-25 wt%. The

fairly stagnant low PHA content obtained within this period of time implies that PHA-accumulating organisms have yet become the dominant microbial species. Other microorganisms that are not capable of storing PHA coexist with them in the sludge. It seems that a longer cultivation period is necessary to eliminate the former from the cultivation reactor.

To test this hypothesis, the operation of the cultivation reactor was extended. On day 50, the PHA storage capacity of the sludge enhanced remarkably, achieving PHA content of 40 wt%. Since then, the PHA content was maintained at a higher range of 45-64 wt%. These positive results confirm that longer cultivation period is beneficial to the enrichment of PHA-accumulating organisms.

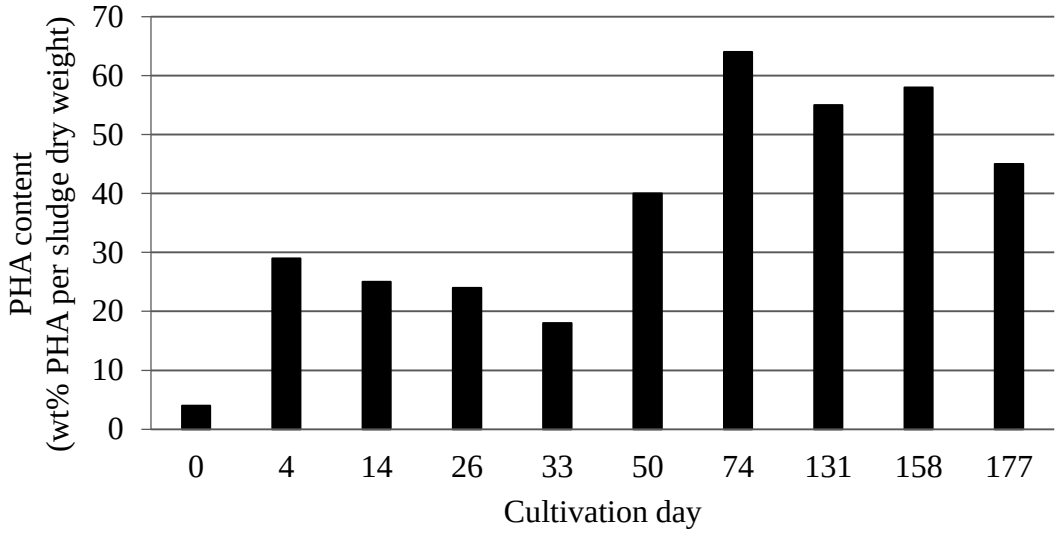


Figure 5.4: PHA content of the activated sludge achieved at hour 8 in the PHA production test. The sludge was taken from the cultivation reactor of PHA-accumulating organisms on different cultivation days.

Apart from the long cultivation period, the long famine phase experienced by the sludge during ADF process also plays a significant role in the enrichment of PHA-accumulating organisms. According to Figure 5.5, the length of famine phase was in between 14 h and 20 h, which accounted for 58-83% of the total cycle length. In literature, the length of famine phase was typically in a range of 46-92% of the total

cycle length (Dionisi et al., 2007; Villano et al., 2010). The long famine phase could result in the death of non-PHA-accumulating organisms because they could not survive in the long absence of external substrates. Consequently, these organisms would be eliminated from the cultivation reactor. This in turn promotes the domination of PHA-accumulating organisms as they could endure the long famine by utilizing the PHA stored during the feast phase for metabolism.

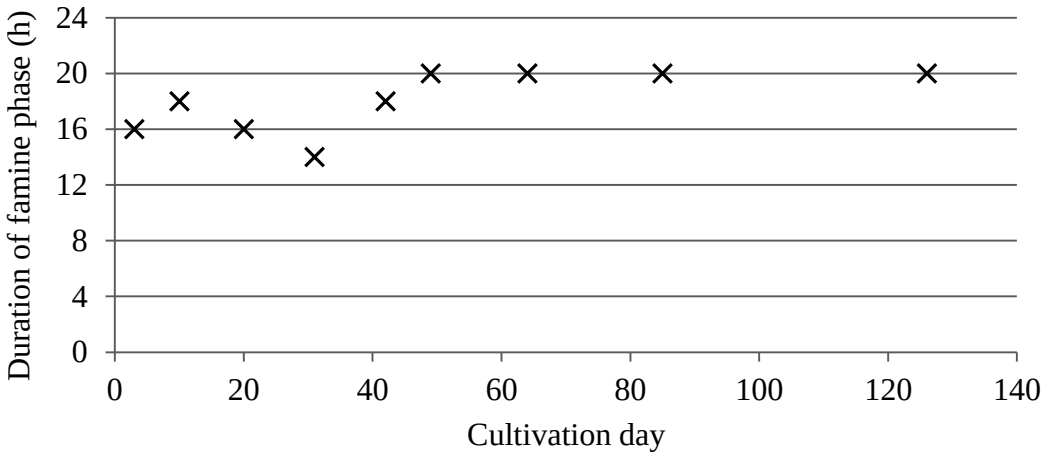


Figure 5.5: Duration of the famine phase in the 24-h cyclic operation of the cultivation reactor of PHA-accumulating organisms.

Another factor leading to the enrichment is the operation of cultivation reactor at short SRT, i.e. 2 d as in this study. Short SRT reduces the number of microbial species that could survive in the reactor, thus resulting in the greater elimination of the non-PHA-accumulating organisms. This helps the domination of PHA-accumulating organisms in the sludge. Chua et al. (2003) found that the PHA storage capacity of the sludge cultivated at SRT of 3 d was 10% higher than that cultivated at SRT of 10 d. Similar result was obtained by Coats et al. (2007) in which reducing the SRT from 6 d to 4 d improved the PHA content by 12%.

In this study, the highest PHA content achieved was 64 wt% PHA per sludge dry weight, which is in the middle ranking among those reported in the literature (Table 5.1). To date, the highest PHA content achieved by the activated sludge cultivated via

ADF process was 89 wt% (Johnson et al., 2009). The high PHA content was partially attributed to the long sludge cultivation period of 16 months (this has not taken into account that the seed sludge had been subjected to another ADF process for 28 months before being used in the study) (Johnson et al., 2009). On the other hand, it was realized that the PHA storage capacity of the sludge cultivated using fermented POME as carbon substrate (64 wt%) was better than those using pure substrate (46-50 wt%) (Dionisi et al., 2004; Dionisi et al., 2006; Villano et al., 2010), fermented paper mill wastewater (48 wt%) (Bengtsson et al., 2008b) and fermented sugar cane molasses (56 wt%) (Albuquerque et al., 2011). This implies that fermented POME is another promising carbon substrate for low-cost cultivation of PHA-accumulating organism via the ADF process.

Table 5.1: Maximum PHA content achieved by the activated sludge cultivated via the ADF process

Maximum PHA content (wt%)	Carbon source	Cultivation conditions of activated sludge	References
46	Mixture of acetic and propionic acids	SBR, cycle length 2 h, HRT=SRT 1 d, pH 8.5, 25°C, OLR 8.5 g COD/L/d	(Villano et al., 2010)
48	Fermented paper mill wastewater	Continuous flow reactor, SRT 7 d, pH 7.3, 30°C	(Bengtsson et al., 2008b)
50[a]	Mixture of acetic, lactic and propionic acids	SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 8.75 g COD/L/d	(Dionisi et al., 2004)
50[a]	Mixture of acetic, lactic and propionic acids	SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 8.5 g COD/L/d	(Dionisi et al., 2006)
56	Fermented sugar cane molasses	SBR, cycle length 12 h, HRT 1 d, SRT 10 d, feed pH 8, 23-25°C	(Albuquerque et al., 2011)
64[a]	Mixture of acetic, lactic and propionic acids	SBR, cycle length 2 h, HRT=SRT 1 d, pH 7.5, 25°C, OLR 12.75 g COD/L/d	(Dionisi et al., 2005a)
64	***Fermented palm oil mill effluent***	***SBR, cycle length 24 h, HRT=SRT 2 d, pH 7, 28-30°C***	***This study***
72.9	Fermented waste activated sludge	SBR, cycle length 4 h, SRT 3d, feed pH 7, 21°C	(Jiang et al., 2009)
75	Fermented sugar cane molasses	SBR, cycle length 12 h, HRT 1 d, SRT 10 d, feed pH 8, 23-25°C	(Albuquerque et al., 2010)
77	Fermented paper mill wastewater	SBR, cycle length 24 h, HRT=SRT 2 d, pH 7, 30°C	(Jiang et al., 2012)
78.5	Sodium acetate	SBR, cycle length 12 h, HRT 1 d, SRT 10 d, 22°C	(Serafim et al., 2004)
> 80	Sodium acetate	SBR, cycle length 12 h, HRT=SRT 1 d, pH 7, 30°C, OLR 2.25 Cmmol/L/h	(Jiang et al., 2011b)
84	Sodium acetate	SBR, cycle length 4 h, HRT 8 h, SRT 1 d, pH 7, 30°C	(Johnson et al., 2010b)
89	Sodium acetate	SBR, cycle length 12 h, HRT=SRT 1 d, pH 7, 30°C	(Johnson et al., 2009)

[a]COD basis

5.3 Microscopic observation of the activated sludge sampled from the cultivation reactor of PHA-accumulating organisms

Activated sludge taken from the cultivation reactor of PHA-accumulating organisms was examined under light microscope for morphology study. As depicted in Figure 5.6, most of the microorganisms in the sludge had morphology similar to cocci (some in tetrad arrangement), coccobacilli and filamentous bacteria. The sludge was also subjected to Gram staining for the identification of Gram-positive and Gram-negative bacteria. Based on Figure 5.7, the sludge contained both Gram-positive (blue/violet color) and Gram-negative (pink/red color) bacteria. Specifically, it seemed that the filamentous bacteria were Gram-positive while the cocci and coccobacilli were made up of a mixture of Gram-positive and Gram-negative bacteria.

To identify the PHA-accumulating organisms in the sludge, Nile blue A staining was performed and the result was presented in Figure 5.8. PHA stained with Nile blue A would emit bright orange color under fluorescent light. It was noticed that the bright orange color in Figure 5.8(b) coincided with the location of cocci, coccobacilli and filamentous bacteria in Figure 5.8(a). This observation suggests that these three types of bacteria are capable of accumulating PHA. PHA-accumulating organisms with similar morphological structures had been observed in the studies of Jiang et al. (2012) (cocci) and Bengtsson et al. (2008b) (filamentous bacteria) as well.

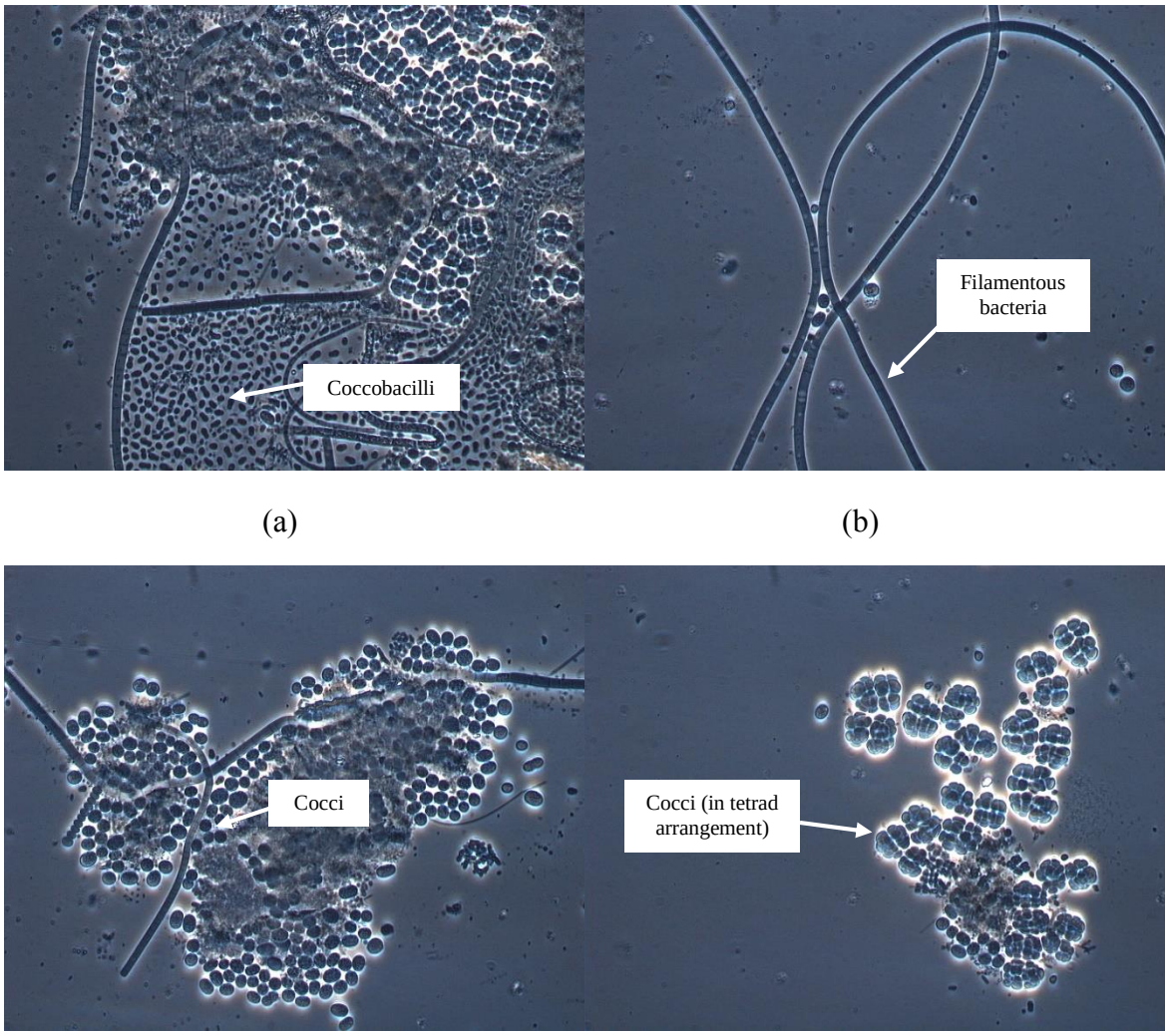


Figure 5.6: Microscopic images showing the morphologies of the microorganisms in the activated sludge taken from the cultivation reactor of PHA-accumulating organisms: (a) coccobacilli, (b) filamentous bacteria, (c) cocci and (d) cocci in tetrad arrangement.

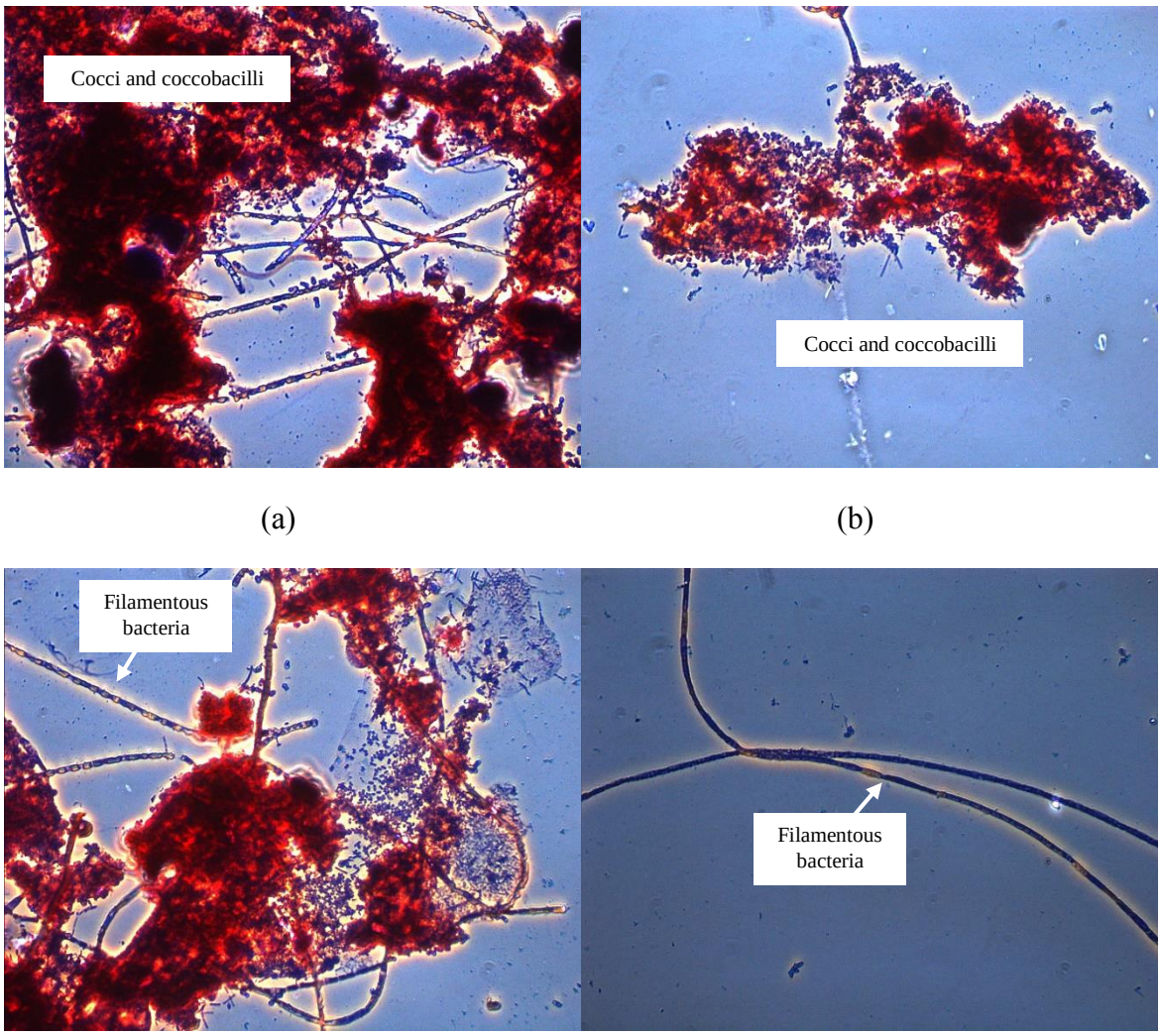
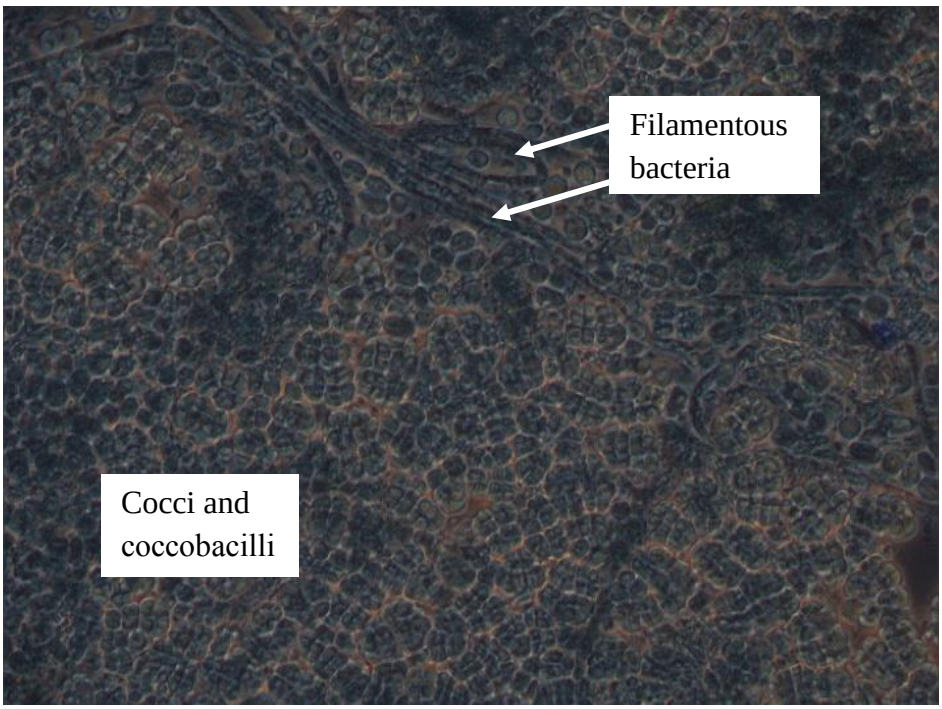
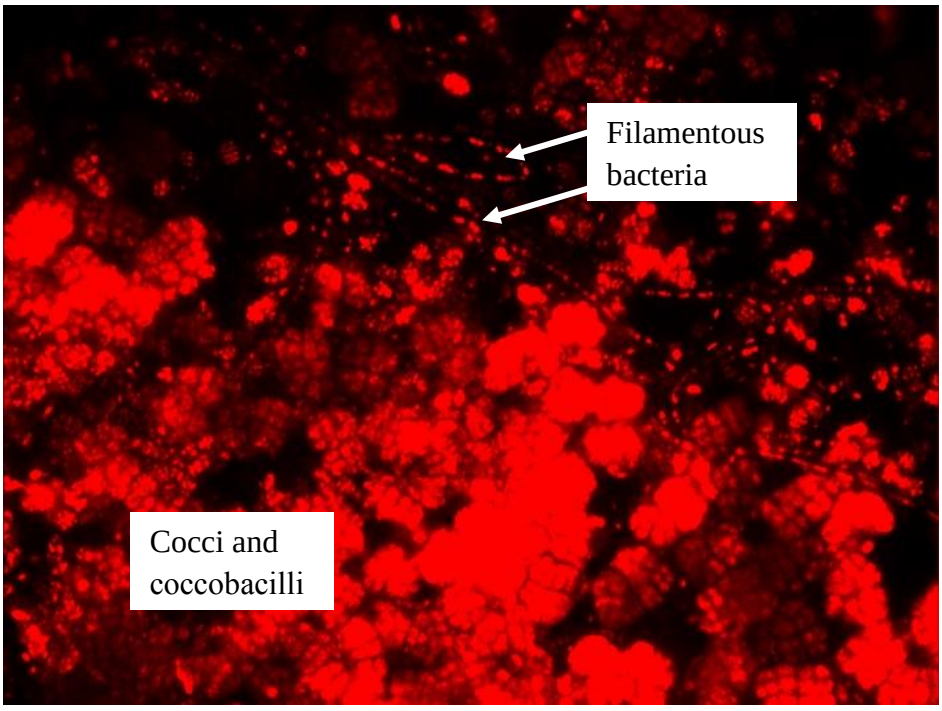


Figure 5.7: Microscopic images of the sludge subjected to Gram staining. Blue/violet color indicates Gram-positive bacteria while pink/red color denotes Gram-negative bacteria. (a-b) Cocci and coccobacilli were made up of a mixture of Gram-positive and Gram-negative bacteria whereas (c-d) filamentous bacteria were Gram-positive.



(a)



(b)

Figure 5.8: Microscopic examination of the sludge subjected to Nile blue A staining. Sludge was taken at the end of the feast phase. (a) is phase contrast image whereas (b) is fluorescence microscopic image. These two images were captured at the same location.

Chapter 6: Effect of Fermented POME Characteristics and Operating Conditions on the Production of PHA

6.1 Characteristics of fermented POME

The characteristics of fermented POME generated from the acidogenic fermentation process conducted at 30°C and 6-d SRT were presented in Table 6.1. The fermented POME had high VFA content in which 62% of the sCOD was VFA on average. This result was comparable to that reported by Bengtsson et al. (2008b) whereby VFA accounted for 74% of the sCOD in the fermented paper mill wastewater used for PHA production. The high VFA content obtained in this study is advantageous because VFA have been identified as the main carbon substrates leading to the formation of PHA.

As shown in Table 6.1, the fermented POME also had a high molar ratio of VFA-C:N:P at 130:2:1, indicating that it was rich in carbon but deficient in nutrients. This characteristic is considered beneficial to PHA production as it would favor the utilization of carbon for PHA production over microbial growth (Bengtsson et al., 2008b). These two positive characteristics imply that fermented POME is a potential feedstock for PHA production.

Table 6.1: Characteristics of fermented POME (standard deviations are due to different batches of fermented POME generated from acidogenic fermentation)

Parameters	Concentration
sCOD (mg/L)	20000 ± 4000
VFA (mg/L)	8200 ± 1600
NH_4^+-N (mg/L)	70 ± 16
PO_4^{3-}-P (mg/L)	80 ± 16
VFA/sCOD (%)	62 ± 4
VFA-C:N:P (on molar basis)	130 ± 19 : 2 ± 0.5 : 1

6.2 Effect of duration in the production of PHA

Figure 6.1 shows the concentration profiles of VFA and PHA in the 24-h PHA production test. In the first 8 h, it was observed that the PHA concentration increased along with the consumption of VFA. This affirms the viability of using fermented POME for PHA production. After that, despite the replenishment of VFA through the addition of fermented POME into the reactor, the PHA concentration reached a plateau even though there was continuous uptake of VFA. The maximum PHA production capacity by the activated sludge seemed to have been reached. Based on this observation, it is recommended to conduct the PHA production for 8 h only to avoid unnecessary waste of substrate and time. This could help to improve the economy of PHA production process as well.

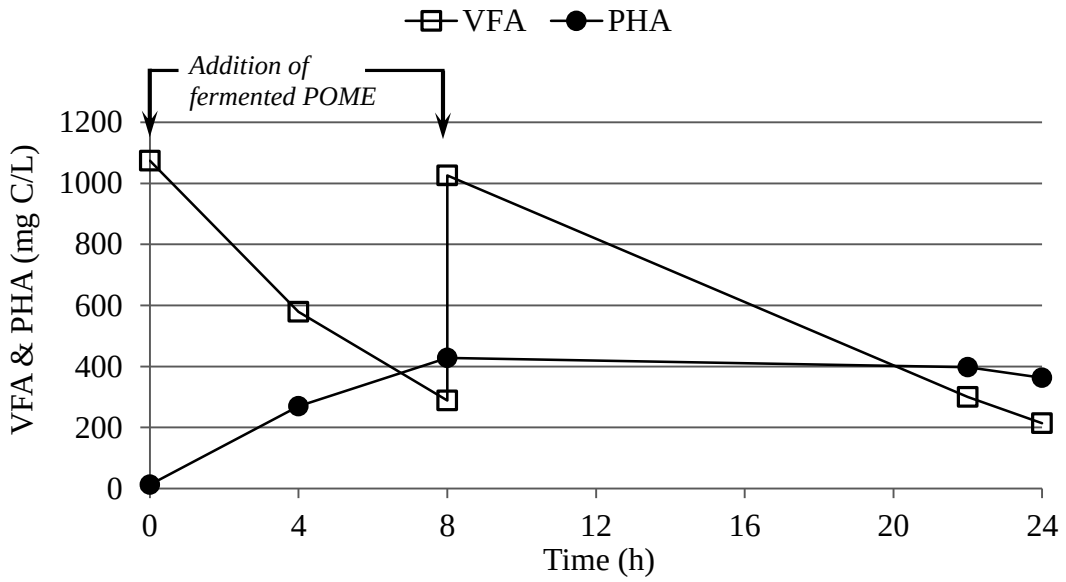


Figure 6.1: Concentration profiles of VFA and PHA in PHA production using activated sludge taken from the cultivation reactor of PHA-accumulating organisms on day 50.

6.3 Effect of pH on the production of PHA

The production of PHA was carried out under different pH conditions, viz. without pH control and with pH control at pH 7, 8 and 9 respectively at air supply rate of 1.0 vvm. The corresponding PHA content achieved is depicted in Figure 6.2. In the absence of pH control (the pH in the reactor was 4.5 throughout the entire experiment), the resulted PHA content was the lowest at 0.5 wt%. This extremely poor result was due to the low microbial activity in the PHA production reactor, as indicated by the negligible VFA uptake during the experiment (Figure 6.3). Inactive sludge was likely caused by the strong acidic conditions (pH = 4.5) inside the reactor. This pH was the intrinsic pH value of the fermented POME produced from the acidogenic fermentation process. A plausible cause of the poor PHA production at acidic pH is the toxic effect of non-dissociated VFA exerted on the PHA-accumulating organisms. Under acidic conditions, most of the VFA would present in non-dissociated form because the degree of dissociation of VFA is reduced by the increase in the concentration of hydrogen ion. Since non-dissociated VFA could penetrate the cell membrane and enter into the cell body (Fleit, 1995), this could lead to significant drop in intracellular pH which is detrimental to PHA production (Chua et al., 2003). It had been reported that pH 4.5 could lead to complete inhibition of PHA production (Dionisi et al., 2005a).

This result shows that the direct use of inherently acidic fermented POME for PHA production is not workable. Hence, the production of PHA was conducted under neutral and alkaline conditions (pH 7-9). Based on Figure 6.2, pH 7 led to the highest PHA content of 64 wt% PHA per sludge dry weight. However, increasing the pH to 8 and 9 resulted in lower PHA content of 48 wt% and 32 wt% respectively. These findings suggest that neutral pH is more advantageous to PHA production as compared to acidic and alkaline pH. Similar result was obtained by Mohan and Reddy (2013) in which pH 7 led to higher PHA accumulation as compared to pH 6 and 8.

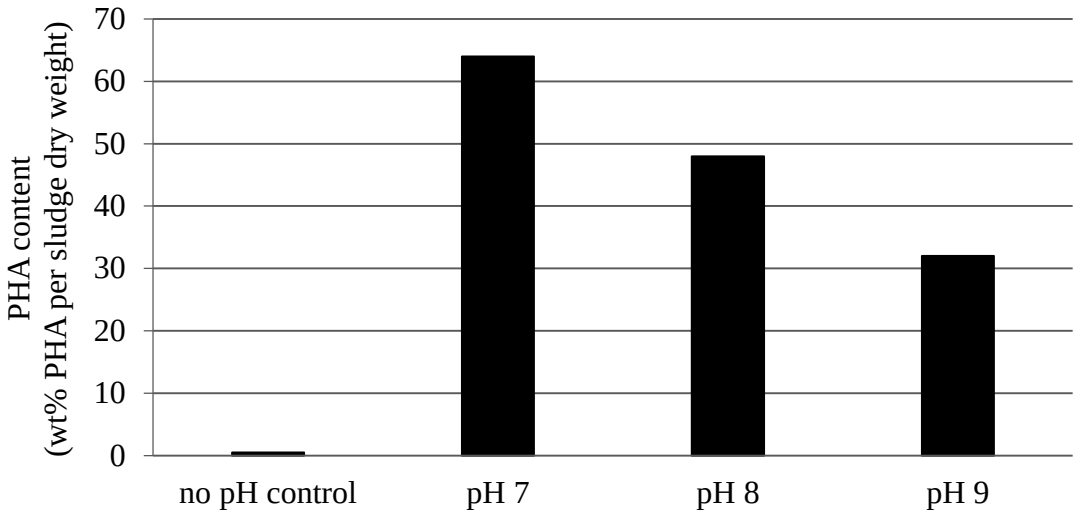


Figure 6.2: Final PHA content achieved in the production of PHA at pH 7, 8 and 9, and under the condition of no pH control. The PHA production was conducted at an air supply rate of 1.0 vvm for 8 h using activated sludge collected from the cultivation reactor of PHA-accumulating organisms on days 72-76 as inoculum.

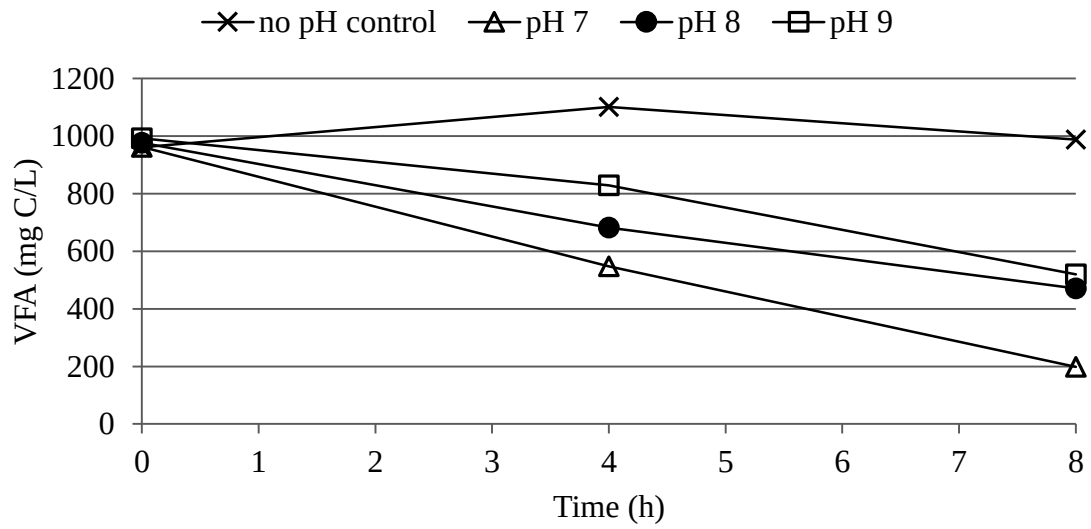


Figure 6.3: Concentration profile of VFA in the production of PHA at pH 7, 8 and 9, and under the condition of no pH control.

In this study, the higher PHA content attained at the neutral pH was attributed to higher microbial activity, as indicated by the increase in the specific VFA consumption rate with the reduction in pH from 9 to 7 (Table 6.2). The specific PHA production rate at pH 7 was the highest among the pH values investigated. Furthermore, lower microbial growth at pH 7 also contributed to higher PHA content per unit biomass. Based on Table 6.2, the specific growth rate achieved at pH 7 was four times lower than that at pH 8. The lowest specific growth rate was obtained at pH 9, but this did not lead to a higher PHA content than that at pH 7 because the advantage of low microbial growth diminished against its exceptionally low specific PHA production rate.

Table 6.2: Specific VFA consumption, PHA production and microbial growth rates during PHA production at air supply rate of 1.0 vvm and pH 7-9

pH	$-q_{VFA}$ (mg VFA/mg X/h)	q_{PHA} (mg PHA/mg X/h)	q_X (1/h)
7	0.47	0.24	0.014
8	0.36	0.17	0.060
9	0.26	0.06	0.009

Unlike its great influence on the PHA content, pH, in a range of 7-9, has insignificant effect on the composition of PHA. As illustrated in Figure 6.4, the fraction of 3HB and 3HV was nearly the same under these three pH conditions in which 3HB accounted for 76-79 mol% of the total PHA whilst 3HV contributed to 21-24 mol%. This finding is in contrast with the previous studies (Dionisi et al., 2005a; Villano et al., 2010) which found that increasing pH from 7.5 to 9.5 could lead to 13-16 mol% higher of 3HV. Such discrepancy was likely due to the presence of different type of PHA-accumulating organisms in the sludge which responded differently toward the change in pH. As a whole, this study had demonstrated the necessity of regulating the pH in the production of PHA. It is recommended to conduct the PHA production at pH 7 as it led to the highest PHA content and the formation of copolymer P(3HB-co-3HV).

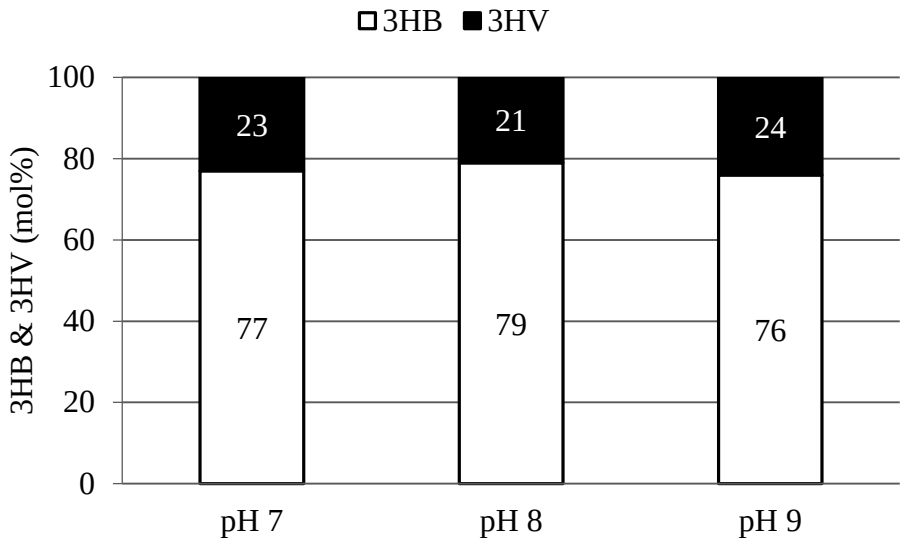


Figure 6.4: Percentage of 3HB and 3HV in the PHA obtained at the end of PHA production at pH 7, 8 and 9.

6.4 Effect of air supply rate on the production of PHA

The influence of air supply rate of 0.2, 0.5 and 1.0 vvm on the final PHA content achieved by the activated sludge at pH 7 is presented in Figure 6.5. It was observed that the PHA content increased with the air supply rate. When the air was supplied at 0.2 vvm, the PHA content attained was rather low at 15 wt% PHA per sludge dry weight. As the supply of air increased to 0.5 and 1.0 vvm, there was improvement on the PHA production, resulting in PHA content of 24 wt% and 45 wt% respectively. As shown in Table 6.3, higher air supply rate gave rise to larger oxygen mass transfer coefficient. The oxygen mass transfer coefficient at air supply rate of 1.0 vvm was 4.8 times larger than that at 0.2 vvm. With the presence of higher concentration of oxygen, the sludge could generate more energy through oxidative degradation of carbon substrates. The availability of greater amount of energy would allow the sludge to produce more PHA. As evidenced, both specific VFA consumption rate and specific PHA production rate increased with the air supply rate (Table 6.3). The study of Satoh et al. (1998) also showed that the performance of PHA production could be enhanced by increasing the supply of oxygen. In their study, the percentage of PHA per initial sludge dry weight

raised by 2.5 times as the oxygen supply increased from 0.3 mg O_2/min/L to 2.7 mg O_2/min/L.

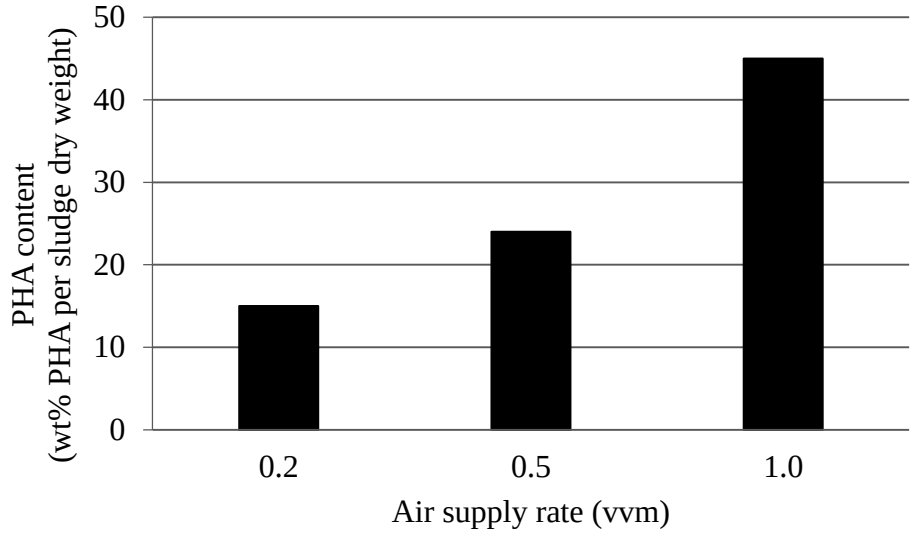


Figure 6.5: Final PHA content achieved in the production of PHA at air supply rate of 0.2, 0.5 and 1.0 vvm. The PHA production was carried out at pH 7 for 8 h using activated sludge collected from the cultivation reactor of PHA-accumulating organisms on days 177-179 as inoculum.

Table 6.3: Specific VFA consumption, PHA production and microbial growth rates during PHA production at pH 7 and air supply rate of 0.2-1.0 vvm

Air supply rate (vvm)	k_La (1/h)	$-q_{VFA}$ (mg VFA/mg X/h)	q_{PHA} (mg PHA/mg X/h)	q_X (1/h)
0.2	2.9	0.10	0.03	0.05
0.5	5.5	0.17	0.06	0.07
1.0	13.9	0.44	0.18	0.10

By comparing Tables 6.2 and 6.3, it was noted that different specific VFA consumption, PHA production and microbial growth rates were obtained in the pH and the air supply studies conducted at pH 7 and air supply rate of 1.0 vvm. The variation was likely caused by the activated sludge employed in the respective study. In pH study, the activated sludge was collected from the cultivation reactor of PHA-accumulating organisms on days 72-76 whereas in the air supply study, the sludge was taken on days 177-179. With a cultivation interval of approximately 100 days, there might be a change in the population of the microbial community in the activated sludge, hence resulting in

different microbial responses even though the sludge was subjected to similar conditions for PHA production.

As compared to PHA content, the influence of air supply rate on the percentage of 3HB and 3HV in the PHA was relatively minor. As shown in Figure 6.6, regardless of the air supply rate, the primary PHA produced was 3HB with percentage in a range of 65-78 mol% whereas the percentage of 3HV ranged from 22 mol% to 35 mol%. Based on the results obtained in this study, air supply rate of 1.0 vvm was found to be the most favorable for PHA production.

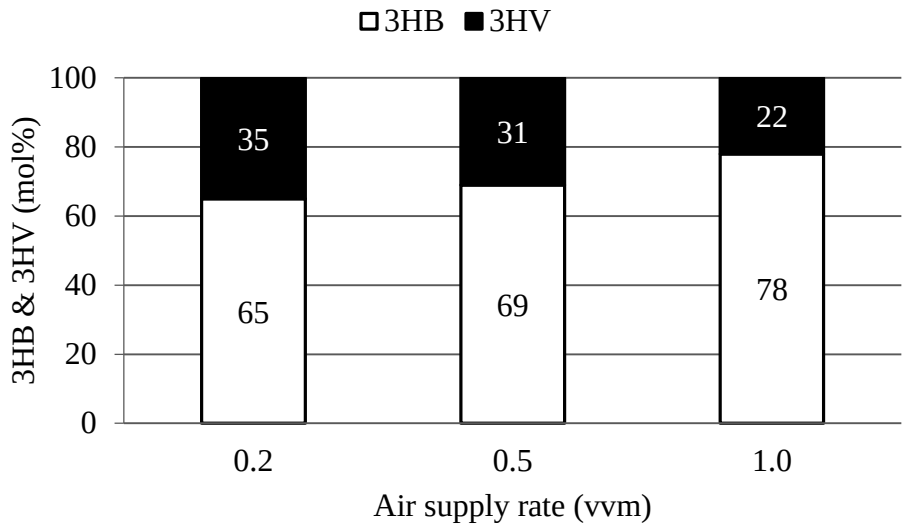


Figure 6.6: Composition of PHA obtained at the end of PHA production at air supply rate of 0.2, 0.5 and 1.0 vvm.

Chapter 7: Conclusions and Recommendations

7.1 Conclusions

This research work has examined the viability of using POME for the production of VFA and their subsequent application to the generation of eco-friendly PHA. Based on the results obtained from different sets of experiments, it can be concluded that

(i) POME is a promising wastewater for the production of VFA due to its high content of soluble and fermentable organic substrates.

(ii) Operating the anaerobic reactor fermenting POME at 6-d SRT led to better VFA production in comparison with infinite SRT and 9-d SRT. Besides, it could prevent the loss of acidogenic activity and avoid the dominance of methanogens which could consume VFA for methane formation. On the other hand, performing acidogenic fermentation of POME at mesophilic temperatures of 30°C and 40°C resulted in better VFA production as compared to thermophilic temperature of 55°C. The comparable VFA production performance at both 30°C and 40°C suggests the possibility to conduct the fermentation at ambient temperatures of 25-32°C and without temperature control.

(iii) The application of the ADF process successfully enriched the activated sludge with PHA-accumulating organisms. This improves the overall PHA storage capacity of the activated sludge; from 4 w% of PHA per sludge dry weight (seed sludge) to a maximum of 64 wt% PHA per sludge dry weight (sludge cultivated for 74 days via ADF process).

(iv) Fermented POME, which has high VFA content and high molar ratio of VFA-C:N:P, is a suitable feedstock for the production of PHA by activated sludge.

(v) Direct application of the inherently acidic fermented POME to PHA production is not recommended since acidic condition resulted in lower PHA content as compared to neutral and alkaline conditions. Neutral pH is preferable as it led to the highest PHA

content. Supplying air at 1 vvm to PHA production reactor is recommended as it provided higher concentration of oxygen to activated sludge for energy generation, thus leading to superior PHA production performance than those at 0.2 vvm and 0.5 vvm.

7.2 Implications of this work

The findings of this research work provide valuable information to the current society, notably the palm oil and plastics industries. It offers a "greener" alternative strategy for POME management and provides the investor with low-cost PHA production route. The implementation of the proposed bioconversion system could help the transition to a more environmental-friendly and sustainable society. The implications of this work are listed as follows.

(i) The bioconversion of POME into PHA offers an option for the replacement of the conventional open ponding system used to treat POME. This can eliminate the open emission of methane gas from the anaerobic open pond, thus reducing the carbon footprint of the palm oil industry.

(ii) The utilization of POME for PHA production also enables space savings. Smaller land area is required for implementing the whole PHA production system in comparison with the current POME treatment system (open ponding system) as the retention time has been reduced greatly from 20-200 d (Poh & Chong, 2009) to approximately 7 d. Acidogenic fermentation of POME requires a retention time of 6 d whereas PHA production needs 8 h to complete. The retention time of the cultivation reactor of PHA-accumulating organisms is not taken into account here as it can be operated in parallel with the anaerobic reactor fermenting POME.

(iii) The utilization of VFA generated from POME and activated sludge allows the production of PHA at low cost. The former reduces the substrate cost while the latter

saves the equipment and energy costs by eliminating the need of sterile condition which is mandatory for PHA production by pure microbial culture.

(iv) Using POME generated from plant source (oil palm fruit bunches) for PHA production can reduce the dependency of current society on petrochemicals.

(v) The use of completely degradable PHA in current society can help to resolve the problem associated with the disposal of conventional plastic wastes which are known for their low biodegradability.

7.3 Recommendations for future works

This section provides several suggestions for future studies.

(i) Fine-tuning the operating conditions (e.g. temperature and SRT) of the cultivation reactor of PHA-accumulating organisms could be pursued to improve the overall PHA storage capacity of the activated sludge as different conditions could lead to the enrichment of different types of PHA-accumulating organisms in the sludge.

(ii) Characterization of the microbial community in the anaerobic reactor fermenting POME and that in the cultivation reactor of PHA-accumulating organisms can help to reveal and identify the key microbial population involved in the production of VFA and PHA. By studying and understanding their metabolic behavior, one can apply more suitable environment conditions for VFA-producing organisms and PHA-accumulating organisms to grow and function, thus leading to better production of VFA and PHA.

(iii) Characterization of the structure and properties of PHA produced by activated sludge can provide information on the potential uses and applications of PHA in commercial market. Besides, it is crucial to examine downstream processes involved in the recovery of PHA, e.g. extraction and purification of intracellular PHA.

(iv) Pilot-scale study is required to determine the transferability of the technology from the laboratory to the commercial market. During pilot-scale study, it is important not only to fine-tune the technical aspects, but also to scrutinize the economics.

REFERENCES

Ahmad, A. L., Ismail, S., & Bhatia, S. (2003). Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination, 157*, 87-95.

Akaraonye, E., Keshavarz, T., & Roy, I. (2010). Production of polyhydroxyalkanoates: the future green materials of choice. *Journal of Chemical Technology and Biotechnology, 85*, 732-743.

Albuquerque, M. G. E., Eiroa, M., Torres, C., Nunes, B. R., & Reis, M. A. M. (2007). Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. *Journal of Biotechnology, 130*, 411-421.

Albuquerque, M. G. E., Martino, V., Pollet, E., Avérous, L., & Reis, M. A. M. (2011). Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: Effect of substrate composition and feeding regime on PHA productivity. composition and properties. *Journal of Biotechnology, 151*, 66-76.

Albuquerque, M. G. E., Torres, C. A. V., & Reis, M. A. M. (2010). Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection. *Water Research, 44*, 3419-3433.

Alexiou, I. E., Anderson, G. K., & Evison, L. M. (1994). Design of pre-acidification reactors for the anaerobic treatment of industrial wastewaters. *Water Science and Technology, 29*(9), 199-204.

Angenent, L. T., Karim, K., Al-Dahhan, M. H., Wrenn, B. A., & Domíguez-Espinosa, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology, 22*(9), 477-485.

APHA. (1992). *Standard methods for the examination of water and wastewater* (18th ed.). United States of America: APHA.

Basak, B., Ince, O., Artan, N., Yagci, N., & Ince, B. K. (2011). Effect of nitrogen limitation on enrichement of activated sludge for PHA production. *Bioprocess and Biosystems Engineering, 34*, 1007-1016.

Beccari, M., Bertin, L., Dionisi, D., Fava, F., Lampis, S., Majone, M., Valentino, F., Vallini, G., & Villano, M. (2009). Exploiting olive oil mill effluents as a renewable resource for production of biodegradable polymers through a combined anaerobic-aerobic process. *Journal of Chemical Technology and Biotechnology, 84*, 901-908.

Beccari, M., Majone, M., Massanisso, P., & Ramadori, R. (1998). A bulking sludge with high storage response selected under intermittent feeding. *Water Research, 32*(11), 3403-3413.

Ben, M., Mato, T., Lopez, A., Vila, M., Kennes, C., & Veiga, M. C. (2011). Bioplastic production using wood mill effluents as feedstock. *Water Science and Technology, 63*(6), 1196-1202.

Bengtsson, S. (2009). The utilization of glycogen accumulating organisms for mixed culture production of polyhydroxyalkanoates. *Biotechnology and Bioengineering, 104*(4), 698-708.

Bengtsson, S., Hallquist, J., Werker, A., & Welander, T. (2008a). Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production. *Biochemical Engineering Journal, 40*, 492-499.

Bengtsson, S., Pisco, A. R., Reis, M. A. M., & Lemos, P. C. (2010). Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. *Journal of Biotechnology, 145*, 253-263.

Bengtsson, S., Werker, A., Christensson, M., & Welander, T. (2008b). Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresource Technology, 99*, 509-516.

Bolzonella, D., Fatone, F., Pavan, P., & Cecchi, F. (2005). Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds. *Industrial & Engineering Chemistry Research, 44*, 3412-3418.

Bolzonella, D., Pavan, P., Zanette, M., & Cecchi, F. (2007). Two-phase anaerobic digestion of waste activated sludge: Effect of an extreme thermophilic prefermentation. *Industrial & Engineering Chemistry Research, 46*, 6650-6655.

Borja, R., Banks, C. J., & Sáncherz, E. (1996). Anaerobic treatment of palm oil mill effluent in a two-stage up-flow anaerobic sludge blanket (UASB) system. *Journal of Biotechnology, 45*, 125-135.

Bouzas, A., Gabaldón, C., Marzal, P., Penya-roja, J. M., & Seco, A. (2002). Fermentation of municipal primary sludge: Effect of SRT and solids concentration on volatile fatty acid production. *Environmental Technology, 23*(8), 863-875.

Cai, M., Chua, H., Zhao, Q., Sin, N. S., & Ren, J. (2009). Optimal production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation. *Bioresource Technology, 100*, 1399-1405.

Cavdar, P., Yilmaz, E., Tugtas, A. E., & Calli, B. (2011). Acidogenic fermentation of municipal solid waste and its application to bio-electricity production via microbial fuel cells (MFCs). *Water Science and Technology, 64*(4), 789-795.

Chen, G. (2009). Industrial production of PHA. In G. Chen (Ed.), *Plastics from bacteria: Natural functions and applications* (pp. 121-132): Springer.

Choi, J., Chang, H. N., & Han, J. (2011). Performance of microbial fuel cell with volatile fatty acids from food wastes. *Biotechnology Letters, 33*, 705-714.

Choi, J., & Lee, S. Y. (1997). Process analysis and economic evaluation for Poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Engineering, 17*, 335-342.

Chua, A. S. M., Takabatake, H., Satoh, H., & Mino, T. (2003). Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. *Water Research, 37*, 3602-3611.

Coats, E. R., Loge, F. J., Smith, W. A., Thompson, D. N., & Wolcott, M. P. (2007). Functional stability of a mixed microbial consortium producing PHA from waste carbon sources. *Applied Biochemistry and Biotechnology, 136-140*, 909-925.

Dai, Y., Lambert, L., Yuan, Z., & Keller, J. (2008). Microstructure of copolymers of polyhydroxyalkanoates producd by glycogen accumulating organisms with acetate as the sole cabron substrate. *Process Biochemistry, 43*, 968-977.

Dai, Y., Yuan, Z., Jack, K., & Keller, J. (2007). Production of targeted poly(3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source. *Journal of Biotechnology, 129*, 489-497.

Demirel, B., & Yenigun, O. (2004). Anaerobic acidogenesis of dairy wastewater: the effects of variations in hydraulic retention time with no pH control. *Journal of Chemical Technology and Biotechnology, 79*, 755-760.

Din, M. F. M., Mohanadoss, P., Ujang, Z., van Loosdrecht, M. C. M., Yunus, S. M., Chelliapan, S., Zambare, V., & Olsson, G. (2012). Development of Bio-PORec® system for polyhydroxyalkanoates (PHA) production and its storage in mixed cultures of palm oil mill effluent (POME). *Bioresource Technology, 124*, 208-216.

Din, M. F. M., Ujang, Z., van Loosdrecht, M. C. M., Ahamd, A., & Sairan, M. F. (2006). Optimization of nitrogen and phosphorus limitation for better biodegradable plastic production and organic removal using single fed-batch mixed cultures and renewable resources. *Water Science and Technology, 53*(6), 15-20.

Dinsdale, R. M., Premier, G. C., Hawkes, F. R., & Hawkes, D. L. (2000). Two-stage anaerobic co-digestion of waste activated sludge and fruit/vegetable waste using inclinded tubular digesters. *Bioresource Technology, 72*, 159-168.

Dionisi, D., Beccari, M., Gregorio, S. D., Majone, M., Papini, M. P., & Vallini, G. (2005a). Storage of biodegradable polymers by an enriched batch reactor operated at high organic load rate. *Journal of Chemical Technology and Biotechnology, 80*, 1306-1318.

Dionisi, D., Carucci, G., Papini, M. P., Riccardi, C., Majone, M., & Carrasco, F. (2005b). Olive oil mill effluents as a feedstock for production of biodegradable polymers. *Water Research, 39*, 2076-2084.

Dionisi, D., Majone, M., Papa, V., & Beccari, M. (2004). Biodegradable polymers from organic acids by using activated sludge enriched by aerobic periodic feeding. *Biotechnology and Bioengineering, 85*(6), 569-579.

Dionisi, D., Majone, M., Vallini, G., Gregorio, S. D., & Beccari, M. (2006). Effect of the applied organic load rate on biodegradable polymer production by mixed microbial cultures in a sequencing batch reactor. *Biotechnology and Bioengineering, 93*(1), 76-88.

Dionisi, D., Majone, M., Vallini, G., Gregorio, S. D., & Beccari, M. (2007). Effect of the length of the cycle on biodegradable polymer production and microbial community selection in a sequencing batch reactor. *Biotechnology Progress, 23*, 1064-1073.

Doi, Y. (1990). *Microbial polyesters*. United States of America: VCH Publishers.

Elbeshbishy, E., Hafez, H., Dhar, B. R., & Nakhla, G. (2011). Single and combined effect of various pretreatment methods for biohydrogen production from food waste. *International Journal of Hydrogen Energy, 36*, 11379-11387.

Fang, H. H. P., & Yu, H. Q. (2000). Effect of HRT on mesophilic acidogenesis of dairy wastewater. *Journal of Environmental Engineering, 126*, 1145-1148.

Feng, L., Wang, H., Chen, Y., & Wang, Q. (2009). Effect of solids retention time and temperature on waste activated sludge hydrolysis and short-chain fatty acids accumulation under alkaline conditions in continuous-flow reactors. *Bioresource Technology, 100*, 44-49.

Ferrer, I., Vázquez, F., & Font, X. (2010). Long term operation of a thermophilic anaerobic reactor: Process stability and efficiency at decreading sludge retention time. *Bioresource Technology, 101*, 2972-2980.

Fleit, E. (1995). Intracellular pH regulation in biological excess phosphorus removal systems. *Water Research, 29*(7), 1787-1792.

Gadow, S. I., Jiang, H., Watanabe, R., & Li, Y. (2013). Effect of temperature and temperature shock on the stability of continuous cellulosic-hydrogen fermentation. *Bioresource Technology, 142*, 304-311.

Grady, C. P. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). *Biological wastewater treatment* (3rd ed.). Boca Rotan: CRC Press.

Guzman, D. (2010). Bioplastic development increases with new applications. Retrieved March 19, 2014, from http://www.icis.com/resources/news/2010/10/25/9402443/bioplas tic-development-increases-with-new-applications/

Hassan, M. A., Shirai, Y., Kusubayashi, N., Karim, M. I. A., Nakanishi, K., & Hashimoto, K. (1997). The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by *Rhodobacter sphaeroides*. *Journal of Fermentation and Bioengineering, 83*(5), 485-488.

Holmes, P. A. (1985). Applications of PHB - a microbially produced biodegradable thermoplastic. *Physics in Technology*, *16*, 32-36.

Hong, C., & Haiyun, W. (2010). Optimization of volatile fatty acid production with co-substrate of food wastes and dewatered excess sludge using response surface methodology. *Bioresource Technology*, *101*, 5487-5493.

Hong, S. K., Shirai, Y., Aini, A. R. N., & Hassan, M. A. (2009). Semi-continuous anaerobic treatment of palm oil mill effluent for the production of organic acids and polyhydroxyalkanoates. *Research Journal of Environmental Sciences, 3*(5), 552-559.

Horiuchi, J.-I., Shimizu, T., Tada, K., Kanno, T., & Kobayashi, M. (2002). Selective production of organic acids in anaerobic acid reactor by pH control. *Bioresource Technology, 82*, 209-213.

Horiuchi, J., Shimizu, T., Kanno, T., & Kobayashi, M. (1999). Dynamic behavior in response to pH shift during anaerobic acidogenesis with a chemostat culture. *Biotechnology Techniques*, *13*, 155-157.

Hu, W. F., Sin, S. N., Chua, H., & Yu, P. H. F. (2005). Synthesis of polyhydroxyalkanoate (PHA) from excess activated sludge under various oxidation-reduction potentials (ORP) by using acetate and propionate as carbon sources. *Applied Biochemistry and Biotechnology, 121*(1-3), 289-301.

Hu, Z., Yu, H., & Zheng, J. (2006). Application of response surface methodology for optimization of acidogenesis of cattail by rumen cultures. *Bioresource Technology, 97*, 2103-2109.

Huang, Y. L., Wu, Z., Zhang, L., Cheung, C. M., & Yang, S. (2002). Production of carboxylic acids from hydrolyzed corn meal by immobilized cell fermentation in a fibrous-bed bioreactor. *Bioresource Technology, 82*, 51-59.

Ji, Z., Chen, G., & Chen, Y. (2010). Effects of waste activated sludge and surfactant addition on primary sludge hydrolysis and short-chain fatty acids accumulation. *Bioresource Technology, 101*, 3457-3462.

Jiang, S., Chen, Y., & Zhou, Q. (2007a). Effect of sodium dodecyl sulfate on waste activated sludge hydrolysis and acidification. *Chemical Engineering Journal, 132*, 311-317.

Jiang, S., Chen, Y., Zhou, Q., & Gu, G. (2007b). Biological short-chain fatty acids (SCFAs) production from waste-activated sludge affected by surfactant. *Water Research, 41*, 3112-3120.

Jiang, Y., Chen, Y., & Zheng, X. (2009). Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by acitvated sludge submitted to the aerobic feeding and discharge process. *Environmental Science & Technology, 43*, 7734-7741.

Jiang, Y., Hebly, M., Kleerebezem, R., Muyzer, G., & Van Loosdrecht, M. C. M. (2011a). Metabolic modeling of mixed substrate uptake for polyhydroxyalkanoate (PHA) production. *Water Research, 45*, 1309-1321.

Jiang, Y., Marang, L., Kleerebezem, R., Muyzer, G., & van Loosdrecht, M. C. M. (2011b). Effect of temperature and cycle length on microbial competition in PHB-producing sequencing batch reactor. *ISME Journal, 5*, 896-907.

Jiang, Y., Marang, L., Tamis, J., Van Loosdrecht, M. C. M., Dijkman, H., & Kleerebezem, R. (2012). Waste to resource: Converting paper mill wastewater to bioplastic. *Water Research, 46*, 5517-5530.

Jin, D., Chen, J., & Lun, S. (1999). Production of poly(hydroxyalkanoate) by a composite anaerobic acidification-fermentation system. *Process Biochemistry, 34*, 829-833.

Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G., & van Loosdrecht, M. C. M. (2009). Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. *Biomacromolecules, 290*(10), 670-676.

Johnson, K., Kleerebezem, R., & van Loosdrecht, M. C. M. (2010a). Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs. *Water Research, 44*, 2141-2152.

Johnson, K., van Geest, J., Kleerebezem, R., & van Loosdrecht, M. C. M. (2010b). Short- and long-term temperature effects on aerobic polyhydroxybutyrate producing mixed cultures. *Water Research, 44*, 1689-1700.

Keshavarz, T., & Ray, I. (2010). Polyhydroxyalkanoates: bioplastics with a green agends. *Current Opinion in Microbiology, 13*, 321-326.

Kim, H. J., Kim, S. H., Choi, Y. G., Kim, G. D., & Chung, T. H. (2006). Effect of enzymatic pretreatment on acid fermentation of food waste. *Journal of Chemical Technology and Biotechnology, 81*, 974-980.

Kitamura, S., & Doi, Y. (1994). Staining method of poly(3-hydroxyalkanoic acids) producing bacteria by nile blue. *Biotechnology Techniques, 8*(5), 345-350.

Kondo, T., & Kondo, M. (1996). Efficient production of acetic acid from glucose in a mixed culture of *Zymomonas mobilis* and *Acetobacter* sp. *Journal of Fermentation adn Bioengineering, 81*(1), 42-46.

Lemos, P. C., Serafim, L. S., & Reis, M. A. M. (2006). Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding. *Journal of Biotechnology, 122*, 226-238.

Lemos, P. C., Viana, C., Salgueiro, E. N., Ramos, A. M., Crespo, J. P. S. G., & Reis, M. A. M. (1998). Effect of carbon source on the formation of polyhyroxyalkanoates (PHA) by a phosphate-accumulating mixed culture. *Enzyme and Microbial Technology, 22*, 662-671.

Lim, S., Kim, B. J., Jeong, C., Choi, J., Ahn, Y. H., & Chang, H. N. (2008). Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. *Bioresource Technology, 99*, 7866-7874.

Liu, H., Wang, J., Liu, X., Fu, B., Chen, J., & Yu, H. (2012). Acidogenic fermentation of proteinaceous sewage sludge: Effect of pH. *Water Research, 46*, 799-807.

Lu, J., & Ahring, B. K. (2005). *Effects of temperature and hydraulic retention time on thermophilic anaerobic pretreatment of sewage sludge*. Paper presented at the 4th International Symposium on Anaerobic Digestion of Solid Waste, Copenhagen.

Lu, J., Gavala, H. N., Skiadas, I. V., Mladenovska, Z., & Ahring, B. K. (2008). Improving anaerobic sewage sludge digestion by implementation of a hyper-thermophilic prehydrolysis step. *Journal of Environmental Management, 88*, 881-889.

Maharaj, I., & Elefsiniotis, P. (2001). The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters. *Bioresource Technology, 76*, 191-197.

Mato, T., Ben, M., Kennes, C., & Veiga, M. C. (2010). Valuable product production from wood mill effluents. *Water Science and Technology, 62*(10), 2294-2300.

Miron, Y., Zeeman, G., Lier, J. B. V., & Lettinga, G. (2000). The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. *Water Research, 34*(5), 1705-1713.

Mohan, S. V., Babu, V. L., & Sarma, P. N. (2008). Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. *Bioresource Technology, 99*, 59-67.

Mohan, S. V., & Reddy, M. V. (2013). Optimization of critical factors to enhance polyhydroxyalkanoates (PHA) synthesis by mixed culture using Taguchi design of experimental methodology. *Bioresource Technology, 128*, 409-416.

MPOC. (2011). Annual report 2010 - leveraging on sustainability. Retrieved September 26, 2011, from http://www.mpoc.org.my/pubs_view.aspx?id=3135953f-8771-436b-9dab-899773b2dcob

Mumtaz, T., Abd-Aziz, S., Rahman, N. A. A., Yee, P. L., Shirai, Y., & Hassan, M. A. (2008). Pilot-scale recovery of low molecular weight organic acids from anaerobically treated palm oil mill effluent (POME) with energy integrated system. *African Journal of Biotechnology, 7*(21), 3900-3905.

Mumtaz, T., Yahaya, N. A., Abd-Aziz, S., Rahman, N. A. A., Yee, P. L., Shirai, Y., & Hassan, M. A. (2010). Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent - a Malaysian perspective. *Journal of Cleaner Production, 18*, 1393-1402.

Nielsen, P. H., & Jahn, A. (1999). Extraction of EPS. In J. Wingender, T. R. Neu & H. C. Flemming (Eds.), *Microbial extracellular polymeric substances: Characterization, structure and function* (pp. 58). Berlin: Springer.

Nittami, T., Katoh, T., & Matsumoto, K. (2013). Modification of oxygen transfer rates in activated sludge with its characteristic changes by the addition of organic polyelectrolyte. *Chemical Engineering Journal, 225*, 673-678.

Oehmen, A., Lemos, P. C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L. L., & Reis, M. A. M. (2007). Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research, 41*, 2271-2300.

Oktem, Y. A., Ince, O., Donnelly, T., Sallis, P., & Ince, B. K. (2006). Determination of optimum operating conditions of an acidification reactor treating a chemical synthesis-based pharmaceutical wastewater. *Process Biochemistry, 41*, 2258-2263.

Ong, Y. H., Chua, A. S. M., Fukushima, T., Ngoh, G. C., Shoji, T., & Michinaka, A. (2014). High-temperature EBPR process: The performance, analysis of PAOs and GAOs and the fine-scale population study of Candidatus "Accumulibacter phosphatis". *Water Research, 64*, 102-112.

Ong, Y. H., Chua, A. S. M., Lee, B. P., Ngoh, G. C., & Hashim, M. A. (2012). An observation on sludge granulation in an enhanced biological phosphorus removal process. *Water Environment Research, 84*(1), 3-8.

Oshiki, M., Satoh, H., & Mino, T. (2011). Rapid quantification of polyhydroxyalkanoates (PHA) concentration in activated sludge with the fluorescent dye Nile blue A. *Water Science and Technology, 64*(3), 747-753.

Ostle, A. G., & Holt, J. G. (1982). Nile blue A as a fluorescent stain for poly-beta-hydroxybutyrate. *Applied and Environmental Microbiology, 44*(1), 238-241.

Philip, S., Keshavarz, T., & Roy, I. (2007). Polyhydroxyalkanoates: biodegradable polymers with a range of applications. *Journal of Chemical Technology and Biotechnology, 82*, 233-247.

Platts. (2013). Petrochemical prices slip 2% in November on weakened demand. Retrieved March 19, 2014, from http://www.platts.com/news-feature/2012/pgpi/index

Poh, P. E., & Chong, M. F. (2009). Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. *Bioresource Technology, 100*, 1-9.

Pratt, S., Werker, A., Morgan-Sagastume, F., & Lant, P. (2012). Microaerophilic conditions support elevated mixed culture polyhydroxyalkanoate (PHA) yields, but result in decreased PHA production rates. *Water Science and Technology, 65*(2), 243-246.

Reddy, M. V., & Mohan, S. V. (2012). Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. *Bioresource Technology, 103*, 313-321.

Reis, M. A. M., Serafim, L. S., Lemos, P. C., Ramos, A. M., Aguiar, F. R., & Van Loosdrecht, M. C. M. (2003). Production of polyhydroxyalkanoates by mixed microbial cultures. *Bioprocess and Biosystems Engineering, 25*(6), 337-385.

Rhu, D. H., Lee, W. H., Kim, J. Y., & Choi, E. (2003). Polyhydroxyalkanoate (PHA) production from waste. *Water Science and Technology, 48*(8), 221-228.

Rincón, B., Sánchez, E., Raposo, F., Borja, R., Travieso, L., Martín, M. A., & Martín, A. (2008). Effect of the organic loading rate on the performance of anaerobic acidogenic fermentation of two-phase olive mill solid residue. *Waste Management, 28*, 870-877.

Rodges, M., & Wu, G. (2010). Production of polyhydroxybutyrate by activated sludge performing enhanced biological phosphorus removal. *Bioresource Technology, 101*, 1049-1053.

Salehizadeh, H., & van Loosdrecht, M. C. M. (2004). Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. *Biotechnology Advances, 22*, 261-279.

Salmiati, Ujang, Z., Salim, M. R., Din, M. F. M., & Ahmad, M. A. (2007). Intracellular biopolymer productions using mixed microbial cultures from fermented POME. *Water Science and Technology, 56*(8), 179-185.

Sans, C., Mata-Alvarez, J., Cecchi, F., Pavan, P., & Bassetti, A. (1995a). Acidogenic fermentation of organic urban wastes in a plug-flow reactor under thermophilic conditions. *Bioresource Technology, 54*, 105-110.

Sans, C., Mata-Alvarez, J., Cecchi, F., Pavan, P., & Bassetti, A. (1995b). Volatile fatty acids production by mesophilic fermentation of mechanically-sorted urban organic wastes in a plug-flow reactor. *Bioresource Technology, 51*, 89-96.

Satoh, H., Iwamoto, Y., Mino, T., & Matsuo, T. (1998). Activated sludge as a possible source of biodegradable plastic. *Water Science and Technology, 38*, 103-109.

Satoh, H., Ramey, W. D., Koch, F. A., Oldha, W. K., Mino, T., & Matsuo, T. (1996). Anaerobic substrate uptake by the enhanced biological phosphorus removal activated sludge treating real sewage. *Water Science and Technology, 34*, 9-16.

Serafim, L. S., Lemos, P. C., Albuquerque, M. G. E., & Reis, M. A. M. (2008). Strategies for PHA production by mixed cultures and renewable waste materials. *Applied Microbiology and Biotechnology, 81*, 615-628.

Serafim, L. S., Lemos, P. C., Oliveira, R., & Reis, M. A. M. (2004). Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. *Biotechnology and Bioengineering, 87*(2), 145-160.

Shak, K. P. Y., & Wu, T. Y. (2014). Coagulation-flocculation treatment of high-strength agro-industrial wastewater using natural *Cassia obtusifolia* seed gum: Treatment efficiencies and flocs characterization. *Chemical Engineering Journal, 256*, 293-305.

Su, H., Cheng, J., Zhou, J., Song, W., & Cen, K. (2009). Improving hydrogen production from cassava starch by combination of dark and photo fermentation. *International Journal of Hydrogen Energy, 34*, 1780-1786.

Suriyamongkol, P., Weselake, R., Narine, S., Moloney, M., & Shah, S. (2007). Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants - A review. *Biotechnology Advances, 25*, 148-175.

Takabatake, H., Satoh, H., Mino, T., & Matsuo, T. (2002). PHA (polyhydroxyalkanoate) production potential of activated sludge treating wastewater. *Water Science and Technology, 45*(12), 119-126.

Third, K. A., Newland, M., & Cord-Ruwisch, R. (2003). The effect of dissolved oxygen on PHB accumulation in activated sludge cultures. *Biotechnology and Bioengineering, 82*, 238-250.

Uyar, B., Eroglu, I., Yücel, M., & Gündüz, U. (2009). Photofermentative hydrogen production from volatile fatty acids present in dark fermentation effluents. *International Journal of Hydrogen Energy, 34*, 4517-4523.

Valentino, F., Brusca, A. A., Beccari, M., Nuzzo, A., Zanaroli, G., & Majone, M. (2013). Start up of biological sequencing batch reactor (SBR) and short-term biomass acclimation for polyhydroxyalkanoates production. *Journal of Chemical Technology and Biotechnology, 88*, 261-270.

Villano, M., Beccari, M., Dionisi, D., Lampis, S., Miccheli, A., Vallini, G., & Majone, M. (2010). Effect of pH on the production of bacterial polyhydroxyalkanoates by mixed cultures enriched under periodic feeding. *Process Biochemistry, 45*, 714-723.

Wang, J. Y., Hua, F. L. T., Y. F., Chan, S. Y., Sin, S. N., Chua, H., Yu, P. H. F., & Ren, N. Q. (2007). Synthesis of PHAs from waster under various C:N ratios. *Bioresource Technology, 98*, 1690-1693.

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology, 85*, 849-860.

Wu, H., Yang, D., Zhou, Q., & Song, Z. (2009a). The effect of pH on anaerobic fermentation of primary sludge at room temperature. *Journal of Hazardous Materials, 172*, 196-201.

Wu, T. Y., Mohammad, A. W., M., J. J., & Anuar, N. (2009b). A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnology Advances, 27*, 40-52.

Xiong, H., Chen, J., Wang, H., & Shi, H. (2012). Influence of volatile solid concentration, temperature and solid retention time for the hydrolysis of waste activated sludge to recover volatile fatty acids. *Bioresource Technology, 119*, 285-292.

Yoochatchaval, W., Kumakura, S., Tanikawa, D., Yamaguchi, T., Yunus, M. F. M., Chen, S. S., Kubota, K., Harada, H., & Syutsubo, K. (2011). Anaerobic degradation of palm oil mill effluent (POME). *Water Science and Technology, 64*(10), 2001-2008.

Yu, H.-Q., & Fang, H. H. P. (2002). Acidogenesis of dairy wastewater at various pH levels. *Water Science and Technology, 45*(10), 201-206.

Yu, H., Fang, H. H. P., & Gu, G. (2002). Comparative performance of mesophilic and thermophilic acidogenic upflow reacctors. *Process Biochemistry, 38*, 447-454.

Yu, H., Wang, Z., Wang, Q., Wu, Z., & Ma, J. (2013a). Disintegration and acidification of MBR sludge under alkaline conditions. *Chemical Engineering Journal, 231*, 206-213.

Yu, H. Q., & Fang, H. H. P. (2000). Thermophilic acidification of dairy wastewater. *Applied Microbiology and Biotechnology, 54*, 439-444.

Yu, H. Q., & Fang, H. H. P. (2001). Acidification of mid- and high-strength dairy wastewaters. *Water Research, 35*(15), 3697-3705.

Yu, H. Q., & Fang, H. H. P. (2003). Acidogenesis of gelatin-rich waastewater in an upflow anaerobic reactor: influence of pH and temperature. *Water Research, 37*, 55-66.

Yu, J. (2001). Production of PHA from starchy wastewater via organic acids. *Journal of Biotechnology, 86*, 105-112.

Yu, J., Zheng, M., Tao, T., Zuo, J., & Wang, K. (2013b). Waste activated sludge treatment based on temperature staged and biologically phased anaerobic digestion system. *Journal of Environmental Sciences, 25*(10), 2056-2064.

Yuan, Q., Sparling, R., & Oleszkiewicz, J. A. (2009). Waste activated sludge fermentation: Effect of solids retention time and biomass concentration. *Water Research, 43*, 5180-5186.

Yuan, Q., Sparling, R., & Oleszkiewicz, J. A. (2011). VFA generation from waste activated sludge: Effect of temperature and mixing. *Chemosphere, 82*, 603-607.

Zhang, B., Zhang, L.-L., Zhang, S.-C., Shi, H.-Z., & Cai, W.-M. (2005). The influence of pH on hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion. *Environmental Technology, 26*(3), 329-340.

Zhang, P., Chen, Y., Huang, T., & Zhou, Q. (2009a). Waste activated sludge hydrolysis and short-chain fatty acids accumulation in the presence of SDBS in semi-continuous flow reactors: Effect of solids retention time and temperature. *Chemical Engineering Journal, 148*, 348-353.

Zhang, P., Chen, Y., & Zhou, Q. (2009b). Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH. *Water Research, 43*, 3735-3742.

Zheng, X., Chen, Y., & Liu, C. (2010). Waste activated sludge alkaline fermentation liquids as carbon source for biological nutrients removal in anaerobic followed by alternating aerobic-anoxic sequencing batch reactors. *Chinese Journal of Chemical Engineering, 18*(3), 478-485.

Zhuo, G., Yan, Y., Tan, X., Dai, X., & Zhou, Q. (2012). Ultrasonic-pretreated waste activated sludge hydrolysis and volatile fatty acid accumulation under alkaline conditions: Effect of temperature. *Journal of Biotechnology, 159*, 27-31.

Zigová, J., Šturdík, E., Vandák, D., & Schlosser, Š. (1999). Butyric acid production by *Clostridium butyricum* with integrated extraction and pertraction. *Process Biochemistry, 34*, 835-843.

LIST OF PUBLICATIONS

Journal articles

Research article

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2014). Influence of temperature on the bioconversion of palm oil mill effluent into volatile fatty acids as precursor to the production of polyhydroxyalkanoates. *Journal of Chemical Technology and Biotechnology*, *89*(7), 1038-1043. (*ISI-cited publication; Tier 1 in the category of Chemical Engineering; Impact factor 2.504*)

Review article

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2014). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal*, *235*, 83-99. (*ISI-cited publication; Tier 1 in the category of Chemical Engineering; Impact factor 3.473*)

Chapter in book

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2013). Mesophilic and thermophilic production of volatile fatty acids from palm oil mill effluent for polyhydroxyalkanoates generation. In K. Yamamoto, H. Furumai, H. Katayama, C. Chiemchaisri, U. Puetpaiboon, C. Visvanathan & H. Satoh (Eds.), *Southeast Asian Water Environment 5*. United Kingdom: IWA Publishing.

Conference proceedings

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2013). *Bioconversion of palm oil mill effluent into volatile fatty acids as precursor to the production of*

polyhydroxyalkanoates. Paper presented at the 5th IWA –ASPIRE Conference and Exhibition, Daejeon Convention Center, Daejeon, Korea.

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2012). *Mesophilic and thermophilic production of volatile fatty acids from palm oil mill effluent for polyhydroxyalkanoates generation*. Paper presented at the 10th International Symposium on Southeast Asian Water Environment, Hilton Hanoi Opera Hotel, Hanoi, Vietnam.

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2011). *Profiling the production of acetic and propionic acids in acidogenic fermentation of palm oil mill effluent – a preliminary study*. Paper presented at the 9th International Symposium on Southeast Asian Water Environment, The Emerald Hotel, Bangkok, Thailand.

Appendix A: Operation of Anaerobic Reactor in SRT study

Table A.1 shows the volume of POME fed into the anaerobic reactor and the volume of sludge withdrawn from the anaerobic reactor in the SRT study. The anaerobic reactor was operated under fed-batch mode with a POME feeding interval of 5 d.

Table A.1: Details of the operation of fed-batch anaerobic reactor fermenting POME into VFA in the SRT study

Fed-batch	Working volume of reactor (L)	Volume of POME fed (L)	Volume of sludge (mixed liquor) withdrawn (L)	SRT (d)	HRT (d)
1-28	0.8-1.8	0.3-1.0	No	∞	9-13
29-40	1.5	0.8	0.8	9	9
41-54	1.5	1.2	1.2	6	6

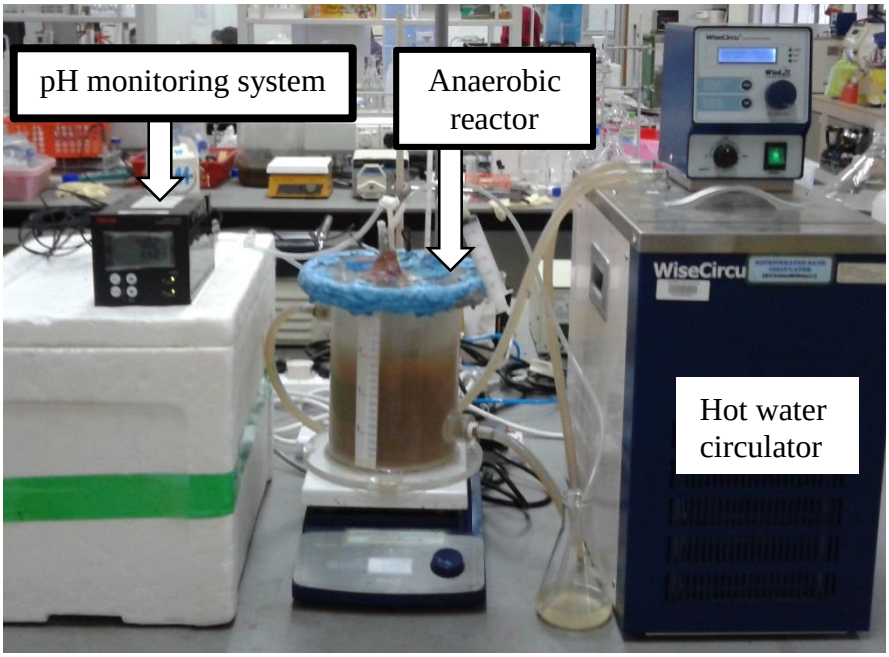
There was no sludge withdrawal from the anaerobic reactor in the 1st-28th fed-batch operations. The sludge was retained in the anaerobic reactor by allowing the mixed liquor in the reactor to settle at the end of fed-batch operation, followed by removing only the supernatant. For the 29th-54th fed-batch operations, the anaerobic reactor was operated in the absence of a settling phase. This led to simultaneous withdrawal of fermented POME and sludge (in the form of mixed liquor) from the reactor at the end of the fed-batch operation, resulting in the same HRT and SRT.

Below shows the calculations of SRT and HRT for 29th fed-batch operation using the data presented in Table A.1.

$$\mathrm{SRT} = \frac{\text{Working volume of reactor}}{\dfrac{\text{Volume of sludge (mixed liquor) withdrawn from the reactor}}{\text{Sludge (mixed liquor) withdrawal frequency}}} = \frac{1.5\,\mathrm{L}}{\dfrac{0.8\,\mathrm{L}}{5\,\mathrm{d}}} = \sim 9\,\mathrm{d}$$

$$\mathrm{HRT} = \frac{\text{Working volume of reactor}}{\dfrac{\text{Volume of POME fed into the reactor}}{\text{POME feeding frequency}}} = \frac{1.5\,\mathrm{L}}{\dfrac{0.8\,\mathrm{L}}{5\,\mathrm{d}}} = \sim 9\,\mathrm{d}$$

Appendix B: Setup of Anaerobic Reactor Used for Producing VFA from POME at 55°C



Appendix C: Setup of the Cultivation Reactor of PHA-accumulating Organisms