Strategy for the biotransformation of fermented palm oil mill effluent into biodegradable polyhydroxyalkanoates by activated sludge

Wee Shen Lee [a], Adeline Seak May Chua [a,*], Hak Koon Yeoh [a], Tadashi Nittami [b], Gek Cheng Ngoh [a]

[a] Department of Chemical Engineering, Faculty of Engineering, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia
[b] Division of Materials Science and Chemical Engineering, Faculty of Engineering, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

HIGHLIGHTS

- Effective production of valuable PHA from fermented POME by activated sludge.
- Successful enrichment of PHA producers via aerobic dynamic feeding process.
- PHA production is best conducted at pH 7.
- Highest PHA content achieved was 64 wt% PHA per sludge dry weight.

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 12 January 2015
Accepted 28 January 2015
Available online 4 February 2015

Keywords:
Aerobic dynamic feeding
Feast and famine
Palm oil mill effluent
Polyhydroxyalkanoates
Resource recovery
Volatile fatty acids

ABSTRACT

Management of wastewater by resource recovery approach allows the transformation of wastewater into valuable resources. This work examines the biotransformation of fermented palm oil mill effluent (POME) into biodegradable plastics – polyhydroxyalkanoates (PHA) – through cultivation and enrichment of PHA-accumulating organisms in activated sludge and the subsequent production of PHA by the cultivated sludge. Enrichment of PHA-accumulating organisms via aerobic dynamic feeding process was effective and had significantly enhanced the PHA storage capacity of the sludge (wt% PHA per sludge dry weight) from 4 wt% (seed sludge) to 40–64 wt% (sludge cultivated for 50 days and more). The cultivated sludge comprised of 42 ± 12% *Betaproteobacteria*, 35 ± 7% *Alphaproteobacteria*, and 13 ± 4% *Gammaproteobacteria*, as estimated by fluorescent *in situ* hybridization. The influence of pH (4.5 in the absence of pH control; and pH 7, 8 and 9) on the production of PHA by the cultivated sludge was subsequently investigated. Neutral pH was the most favorable for PHA production, resulting in a PHA content of 64 wt% in 8 h. The PHA produced was made up of 77 mol% 3-hydroxybutyrate and 23 mol% 3-hydroxyvalerate. These findings signify that the combination of fermented POME and activated sludge offers an alternative to the palm oil and the plastics industries for a more sustainable POME management and an economical PHA production route.

1. Introduction

Palm oil is a vegetable oil with numerous industrial applications. It has been used extensively in food, oleochemical and energy industries for the manufacturing of cooking oil, margarine, soap, biodiesel, etc. Palm oil is produced from oil palm fruit bunches through a series of processes namely sterilization, threshing, digestion, pressing and oil purification. Large volume of high strength wastewater – known as palm oil mill effluent (POME) – is generated from the processing of oil palm fruit bunches. It is estimated that one tonne of palm oil production could result in more than 2.5 tonnes of POME [1].

POME is commonly managed via treatment-oriented approach and most palm oil mills adopt the open ponding system to treat POME [2,3]. This system requires a large footprint due to a long retention time of 20–200 days [2]. Besides, the open emission of methane gas generated from the anaerobic pond contributes substantially to global warming. These drawbacks have prompted the development of better POME management system. One of the options is resource recovery which transforms the organic pollutants in POME into useful resources such as organic acids [4], hydrogen [5] and electricity [6]. Resource recovery management approach allows concurrent minimization of waste and generation of valuable products. We are interested to utilize

* Corresponding author. Tel.: +60 3 79675291; fax: +60 3 79675319.
E-mail address: adeline@um.edu.my (A.S.M. Chua).

http://dx.doi.org/10.1016/j.cej.2015.01.103
1385-8947/

List of abbreviations and symbols

3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
ADF	aerobic dynamic feeding
Cy3	sulfoindocyanine dyes
DO	dissolved oxygen
FISH	fluorescence *in situ* hybridization
FLUOS	5(6)-carboxyfluorescein-N-hydroxysuccinimide ester
HRT	hydraulic retention time
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
P(3HB)	poly(3-hydroxybutyrate)
P(3HB-co-3HV)	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PHA	polyhydroxyalkanoates
$PHA_{initial}$	concentration of PHA measured at the beginning of batch PHA production
PHA_{final}	concentration of PHA measured at the end of batch PHA production
POME	palm oil mill effluent
q_{PHA}	specific PHA production rate
q_{VFA}	specific VFA consumption rate
q_X	specific growth rate
sCOD	soluble chemical oxygen demand
SRT	solids retention time
t	duration of batch PHA production
VFA	volatile fatty acids
$VFA_{initial}$	concentration of VFA measured at the beginning of batch PHA production
VFA_{final}	concentration of VFA measured at the end of batch PHA production
VSS	volatile suspended solids
vvm	gas volume flow per reactor working volume per minute
$X_{initial}$	concentration of cell measured at the beginning of batch PHA production
X_{final}	concentration of cell measured at the end of batch PHA production
$Y_{PHA/VFA}$	PHA yield on VFA
$Y_{X/VFA}$	biomass yield on VFA
$Y_{O_2/VFA}$	respiration yield on VFA

fermented POME rich in volatile fatty acids (VFA) for the production of polyhydroxyalkanoates (PHA).

PHA are biodegradable plastics with similar mechanical properties to polyethylene and polypropylene [7]. Commercial PHA production is achieved by pure microbial culture using pure carbon substrate [8]. This results in high PHA production cost which has reduced the competitiveness of PHA in commercial market. The market price of PHA is approximately $ 4.4–6.0 per kg [9] which is considerably higher than that of the conventional petrochemical-based plastics at around $ 1.5 per kg [10]. One of the ways to reduce PHA production cost is by replacing the pure microbial culture with mixed microbial culture such as activated sludge [11]. Such substitution eliminates the need of maintaining high-energy-demanding sterile condition in PHA production by pure microbial culture. The non-sterile condition also permits the use of waste-derived carbon substrate, thus the cost of PHA production can be further reduced as carbon substrate accounts for 31% of the total cost [12].

The production of PHA from wastes (e.g. paper mill effluent and sugar cane molasses [13–15]) by activated sludge is commonly accomplished in a three-step system, as illustrated in Fig. 1. In the first step, waste is converted into VFA – the preferred carbon substrate for PHA production – via acidogenic fermentation process conducted in an anaerobic reactor. Subsequently, a portion of the VFA-rich fermented waste is used in the cultivation and enrichment of PHA-accumulating organisms in activated sludge. The cultivation step aims to produce sludge of high PHA storage capacity. Finally, the cultivated sludge is employed for PHA production with VFA-rich fermented waste as the carbon substrate. Through such a system, Jiang et al. [13] obtained a PHA content of 77 wt% using paper mill effluent as feedstock.

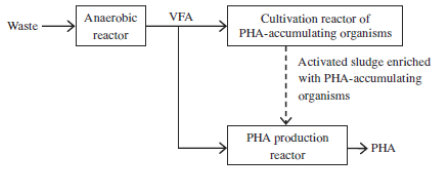

Fig. 1. Biotransformation of waste into VFA for the production of PHA by activated sludge.

In the literature, several studies [16–18] had examined the production of PHA from POME by activated sludge. Din et al. [16] 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 COD:N:P ratio of 180:0.7:1. On the other hand, Salmiati et al. [17] 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 h. 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 adopts the above-mentioned three-step system for PHA production from POME. The first step of acidogenic fermentation of POME had been examined in our recent work [19]. It proved the viability of producing high concentration of VFA from POME. It was also recognized that the fermented POME having a high molar ratio of VFA-C:N:P is a suitable feedstock for PHA production. By using fermented POME as the sole carbon substrate, the present work investigates possible ways for enhancing the performance of PHA production by activated sludge. Two aspects are explored, i.e. enriching the activated sludge with PHA-accumulating organisms and fine-tuning the conditions of PHA production using the cultivated sludge as inoculum.

2. Materials and methods

2.1. Source and preparation of the fermented POME

Fermented POME was obtained from a lab-scale fed-batch anaerobic reactor operating at room temperature (~30 °C). The operational details of the anaerobic reactor could be found in Lee et al. [19]. The characteristics of fermented POME are listed in Table 1.

To maintain a reasonably uniform feed quality, the fermented POME was first filtered (MGC glass microfiber filter, Sartorius) to remove coarse solid particles, diluted with reverse osmosis water, and the pH adjusted (where needed) with 1 M NaOH solution before being fed into the reactors. Unless specified otherwise,

Table 1
Characteristics of the raw, undiluted fermented POME (standard deviations are due to different batches of fermented POME generated from the anaerobic reactor).

Parameters	Concentration
pH	4.5 ± 0.1
sCOD (mg/L)	$(2.0 \pm 0.4) \times 10^4$
VFA (mg/L)	$(8.2 \pm 1.6) \times 10^3$
Composition of VFA	
Formic acid (mg/L)	1.9 ± 5.6
Acetic acid (mg/L)	$(3.1 \pm 0.6) \times 10^3$
Propionic acid (mg/L)	$(2.5 \pm 0.8) \times 10^3$
Butyric acid (mg/L)	$(1.9 \pm 0.4) \times 10^3$
Valeric acid (mg/L)	$(7.0 \pm 1.7) \times 10^2$
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

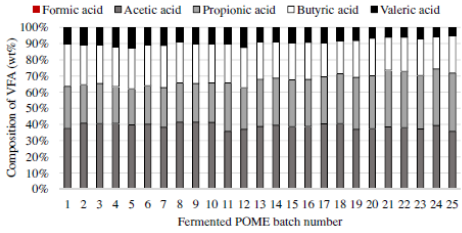

Fig. 2. Compositions of VFA in the fermented POME fed to the cultivation reactor of PHA-accumulating organisms. Formic acid was included for completeness sake, but it was barely noticeable. A total of 25 batches of fermented POME were used throughout the cultivation process.

hereafter "fermented POME" refers to "filtered, diluted, and pH-regulated fermented POME".

2.2. Cultivation of PHA-accumulating organisms via aerobic dynamic feeding (ADF) process

The cultivation of PHA-accumulating organisms was conducted in a 1.2-L sequencing batch reactor (SBR) operating on the ADF process at pH 7 and 28–30 °C. Air was supplied to the reactor at 1.0 vvm without any dissolved oxygen (DO) monitoring or control. 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. The reactor was operated at a cycle length of 24 h. Each cycle consisted of three phases: (a) 6-min simultaneous feeding of 0.3 L fermented POME (carbon substrate) and 0.3 L nutrient solution, respectively, (b) 23.7-h reaction (feast and famine) and (c) 13-min withdrawal of 0.6 L mixed liquor from the reactor. The last step gives a volumetric exchange ratio (total volume of mixed liquor withdrawn from reactor/total working volume of reactor) of 50%. There was no settling phase, resulting in identical hydraulic retention time (HRT) and solids retention time (SRT) of 2 days [13]. The daily loading of fermented POME to the reactor was kept at 360 mg VFA-C/L. The compositions of VFA in the raw, undiluted fermented POME are summarized in Table 1 and detailed in Fig. 2. The latter shows that the VFA fractions in each batch of fermented POME fed into the cultivation reactor remained fairly similar, indicating that these would not affect the microbial selection in our experiments. Nutrient solution was provided to assist microbial growth and it contained ammonium chloride (1070 mg/L), potassium dihydrogen phosphate (1460 mg/L), magnesium sulfate heptahydrate (660 mg/L), N-Allylthiourea (20 mg/L) and a trace element solution (3.33 mL/L) adapted from the work of Ong et al. [20]. N-Allylthiourea was part of the nutrient solution to prevent nitrification [21]. The resulting molar ratio of VFA-C:N:P of the feed was approximately 10:2:1.

2.3. Batch PHA production by cultivated sludge

0.6 L of mixed liquor was withdrawn from the cultivation reactor of PHA-accumulating organisms at the end of its cyclic operation. The mixed liquor was centrifuged to separate the sludge from supernatant. Subsequently, the sludge was subjected to batch PHA production to determine the maximum attainable PHA content. In the batch PHA production, fermented POME was employed as the sole carbon substrate. Its pH was adjusted to 7 before being added into the PHA production reactor at concentrations and compositions as shown in Table 2. The concentration of fermented POME was increased with the cultivation days to ensure sufficient amount of VFA was available for PHA production, as the VFA uptake ability of the sludge had improved considerably over 50 days of cultivation. A starting dose of 950 mg VFA-C/L was eventually used as it approached the upper limit of VFA consumable within 8 h. 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. The PHA production was carried out at room temperature of 28–30 °C with an air supply rate of 1.0 vvm. The oxygen mass transfer coefficient was estimated by using the dynamic method as described in the work of Nittami et al. [22]. The oxygen mass transfer coefficient was estimated to be 13.9 h^{-1}, which was reported by Third et al. [23] as an oxygen-limited condition.

For the seed sludge (taken from the municipal wastewater treatment plant) and the activated sludge collected from the culti-

Table 2
Source of the activated sludge and the concentration of the fermented POME added into the batch PHA production reactor.

Source of activated sludge	Description of activated sludge	Concentration of fermented POME added (mgVFA-C/L)	Composition of VFA in fermented POME (wt%)			
			Acetic acid	Propionic acid	Butyric acid	Valeric acid
Municipal wastewater treatment plant	Seed sludge	750 at hour 0	41	25	25	10
PHA-accumulating organisms cultivation reactor	Day 4[a]	750 at hour 0 and 350 at hour 8	42	24	24	10
	Day 14	950 at hour 0 and 550 at hour 8	43	24	23	10
	Day 26	950 at hour 0 and 550 at hour 8	40	23	24	13
	Day 33	950 at hours 0 and 8	40	24	25	11
	Day 50	950 at hours 0 and 8	41	24	25	10
	Day 74	950 at hour 0	39	28	23	10
	Day 131	950 at hour 0	38	32	23	7
	Day 158	950 at hour 0	38	33	23	7
	Day 177	950 at hour 0	38	36	21	6

[a] Cultivation day.

vation reactor of PHA-accumulating organisms between day 4 and day 50, the production of PHA was carried out in a 0.5-L aerobic reactor at an initial pH of 7 for 24 h with feeding of fermented POME at hours 0 and 8. The pH was not controlled throughout the PHA production. For activated sludge taken from the cultivation reactor from day 74 onwards, the production of PHA was conducted in a 1-L aerobic reactor at pH 7 for 8 h with a single feeding of fermented POME at hour 0. The duration of PHA production and the feeding frequency of fermented POME were reduced because the maximum PHA storage capacity of the sludge could be reached in 8 h. This observation is detailed in Section 3.3.

2.4. Batch PHA production at different pH

To fine-tune the batch PHA production condition, the effect of pH was investigated. This operating parameter was chosen due to its great influence on the production of PHA [24]. There was at least one report [25] that PHA producing microbes cultivated at pH = 8.5 performed better at pH = 9.5. It is thus of interest to explore to what extent the microbes could handle production broths at different pH without further acclimatization. Guided by typical ranges used in the literature [24–28], four pH conditions were examined, i.e. without pH control and with pH control at pH 7, 8 and 9. For the PHA production experiment performed without pH control, the pH was found to be 4.5, i.e. the intrinsic pH value of the fermented POME (Table 1), throughout the whole experiment. The production of PHA was carried out in an aerobic reactor for 8 h at room temperature of 28–30 °C. Air supply was maintained at 1.0 vvm. The initial fermented POME loading was kept at 950 mg VFA-C/L. The experiments were conducted by using activated sludge collected from the cultivation reactor of PHA-accumulating organisms on days 72–76 as the inoculum.

2.5. Nile blue A staining, morphology and the relative abundance of bacterial groups

Activated sludge taken from the cultivation reactor of PHA-accumulating organisms when the reactor reached stable operation was examined for the presence of PHA granules and the morphologies of microbes. Nile blue A staining was used to detect the PHA granules stored inside the microbes [13,29–31]. Nile blue A solution was prepared by dissolving 5 mg of Nile blue A in 50 mL of ethanol. 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. After staining, the sludge was examined using a fluorescence microscope (DM2500, Leica, Germany) equipped with filter cube Y3. The morphology study was performed using wet sludge sample and a light microscope (DM2500, Leica, Germany). The relative abundance of *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* was estimated by fluorescent *in situ* hybridization (FISH). The sludge sample collected during the steady state of cultivation reactor was fixed in 4% paraformaldehyde and the FISH was performed according to Daims et al. [32] using the following probes: EUBmix (EUB-338-I, EUB-338-II and EUB-338-III), targeting all bacteria [33,34]; ALF968, targeting *Alphaproteobacteria* [35]; BET42a with the competitor (GAM42a), targeting *Betaproteobacteria* [36]; and GAM42a with the competitor (BET42a), targeting *Gammaproteobacteria* [36]. Oligonucleotide probes were labeled with 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester (FLUOS) or with the sulfoindocyanine dyes (Cy3) supplied by either Rikaken (Nagoya, Japan) or Nippon Gene (Tokyo, Japan), which were applied at the concentration recommended in the publications for each probe. All samples were observed with a BX51 epifluorescence microscope (Olympus, Tokyo, Japan). Note that samples for quantification with FISH were homogenized by ultrasonic treatment before microscopic observation. Biovolume values, which represent the area of cells hybridised with bacterial group probes as a percentage of the total area hybridised with the EUBmix probe, were calculated using the Daime software [37]. The mean values and the standard deviations were calculated on the basis of 20 fields of view.

2.6. Analytical methods

The analyses of VSS, soluble chemical oxygen demand (sCOD), ammonium, phosphate, VFA (formic, acetic, propionic, butyric and valeric acids) and PHA (3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV)) were carried out according to the methods reported by Lee et al. [19].

2.7. Performance evaluation

The PHA storage capacity of the activated sludge was evaluated based on PHA content calculated according to Eq. (1):

$$\text{PHA content} = \frac{\text{PHA}}{\text{Sludge dry weight}} \times 100\% = \frac{\text{PHA}}{\text{Mixed liquor VSS}} \times 100\% \quad (1)$$

Meanwhile, the specific VFA consumption rate, the specific PHA production rate and the specific growth rate were computed using Eqs. (2)–(4). The cell concentration was taken as the difference in concentration between mixed liquor volatile suspended solids (VSS) and PHA [38]:

$$\text{Specific VFA consumption rate } (-q_{\text{VFA}}) = \frac{\text{VFA consumed}}{\text{Initial cell concentration} \times \text{Duration of batch PHA production}} = \frac{\text{VFA}_{\text{initial}} - \text{VFA}_{\text{final}}}{X_{\text{initial}} \times t} \quad (2)$$

$$\text{Specific PHA production rate } (q_{\text{PHA}}) = \frac{\text{PHA produced}}{\text{Initial cell concentration} \times \text{Duration of batch PHA production}} = \frac{\text{PHA}_{\text{final}} - \text{PHA}_{\text{initial}}}{X_{\text{initial}} \times t} \quad (3)$$

$$\text{Specific growth rate } (q_{\text{X}}) = \frac{\text{Cell produced}}{\text{Initial cell concentration} \times \text{Duration of batch PHA production}} = \frac{X_{\text{final}} - X_{\text{initial}}}{X_{\text{initial}} \times t} \quad (4)$$

The PHA, biomass and respiration yields on the VFA consumed were calculated using Eqs. (5)–(7).

$$\text{PHA yield on VFA } (Y_{\text{PHA/VFA}}) = \frac{\text{PHA produced}}{\text{VFA consumed}} = \frac{\text{PHA}_{\text{final}} - \text{PHA}_{\text{initial}}}{\text{VFA}_{\text{initial}} - \text{VFA}_{\text{final}}} \quad (5)$$

$$\text{Biomass yield on VFA } (Y_{\text{X/VFA}}) = \frac{\text{Cell produced}}{\text{VFA consumed}} = \frac{X_{\text{final}} - X_{\text{initial}}}{\text{VFA}_{\text{initial}} - \text{VFA}_{\text{final}}} \quad (6)$$

$$\text{Respiration yield on VFA } (Y_{\text{O}_2\text{/VFA}}) = 1 - Y_{\text{PHA/VFA}} - Y_{\text{X/VFA}} \quad (7)$$

3. Results and discussion

3.1. Feasibility of direct application of activated sludge for PHA production

In this work, seed sludge employed for cultivating PHA-accumulating organisms was collected from a municipal wastewater treatment plant. Prior to cultivation, the sludge was subjected to batch PHA production test to evaluate its PHA storage capacity. It was found that the PHA storage capacity of the 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. [39]. They evaluated the PHA storage capacity of 18 activated sludge samples taken from 4 different municipal wastewater treatment plants and realized that those sludge were capable of accumulating 6.0–29.5 wt% PHA per sludge dry weight. The low PHA content obtained in this work implies that the direct use of raw activated sludge employed in our work for PHA production is ineffective. Instead, the sludge should be subjected to a proper cultivation process to enhance its PHA storage capacity through the enrichment of PHA-accumulating organisms.

3.2. Cultivation of PHA-accumulating organisms via the ADF process

To enrich the activated sludge with PHA-accumulating organisms, a cultivation reactor operating on the ADF process was established. Fig. 3(a–e) show the concentration profiles of VFA, PHA and sCOD in the cultivation reactor obtained on days 3, 20, 49, 85 and 126. As seen in Fig. 3(a–e), the sludge was exposed to transient availability of substrate (also known as feast and famine) repetitively in the ADF process. It was observed that VFA were available only for 4–8 h in the 24-h cyclic operation of the cultivation reactor. VFA were the dominant organic substrate in fermented POME (62% of the sCOD was VFA; Table 1) and the preferred carbon substrate for PHA production. After the depletion of VFA, other organic substrates were still present in the cultivation reactor, as indicated by the residual sCOD concentration. Nevertheless, the rate of sCOD uptake was low in the famine phase (Fig. 3(d and e)), implying that the sludge had to endure a long period of famine once VFA were no longer available in the cultivation reactor.

Transient availability of the preferred substrate in the cultivation rector would favor the selection of PHA-accumulating organisms because these organisms manage to store the substrate as intracellular PHA and utilize the PHA to survive the long famine (i.e. no external substrate). As depicted in Fig. 3(a–e), the profiles of 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 famine phase, consumption of PHA was noted after the depletion of VFA. This observation signifies that the ADF process is capable of promoting the cultivation of PHA-accumulating organisms in activated sludge.

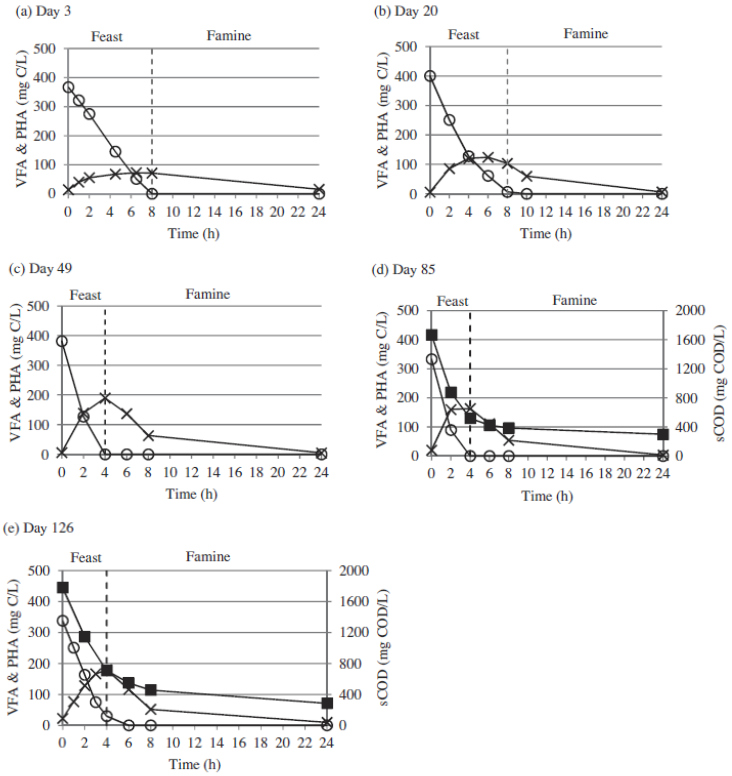

Fig. 3. Concentration profiles of VFA (○), PHA (×) and sCOD (■) in the cultivation reactor of PHA-accumulating organisms monitored on (a) day 3, (b) day 20, (c) day 49, (d) day 85 and (e) day 126. The vertical dash line represents the changeover from the feast phase to the famine phase. The concentrations of sCOD were not measured on days 3, 20 and 49.

An increase in the PHA content was observed at the end of feast phase during the cultivation process. On day 3, the PHA content was 8 wt%. It increased to 22 wt% on day 20, and varied in between 22 and 33 wt% thereafter. The increment in the PHA content signifies the success in the enrichment of the population of PHA-accumulating organisms in the activated sludge through the ADF process.

Results of Nile blue A staining (Supplementary Fig. 1(a–d)) were consistent with the data acquired from the chemical analyses. Supplementary Fig. 1 (a-b) and (c-d) correspond to the sludge collected at the end of the feast and the famine phases, respectively. The orange fluorescence signals in Supplementary Fig. 1(b) and (d) indicate the presence of PHA granules. It can be seen that there are more signals in Supplementary Fig. 1(b) than in Supplementary Fig. 1(d) because PHA was accumulated in the feast phase while PHA was consumed in the famine phase, resulting in little PHA being detected. Supplementary Fig. 1(a and b) show that PHA granules were mostly observed in floc-forming bacteria while filamentous bacteria stored PHA to a lesser degree.

3.3. PHA production by cultivated sludge

Although the cultivation reactor was operated at a low SRT of 2 days, the sludge was still capable to grow and sustain itself, allowing it to be harvested for PHA production. As illustrated in Fig. 4, the MLVSS concentration in the cultivation reactor dropped progressively from 2650 mg/L to 633 mg/L in the first 31 days of the reactor operation but was thereafter maintained in a range of 548–653 mg/L (day 31–85), followed by a higher range of 908–966 mg/L (day 126–173).

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

Fig. 5 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 concomitantly with the consumption of VFA, but at a decreasing rate. After that, VFA were replenished through the addition of fermented POME into the reactor. Due to challenges in prolonged data collection, and considering the typical duration of a work shift in industrial production, it was decided to conduct and compare the PHA production for 8 h only.

Fig. 6 presents the final PHA content achieved by the cultivated sludge (cultivation days 0–177) in the 8-h batch PHA production test. The seed sludge (day 0) could accumulate 4 wt% PHA per sludge dry weight. Such low PHA content indicates that the PHA-accumulating organisms composed only a minor fraction of the total microbial species in the seed sludge. After 4 days of cultivation, the PHA storage capacity of the sludge improved tremendously to 29 wt%. From day 14 to day 33, the PHA content dropped to a range of 18–25 wt%. The decrease in the PHA production capacity of the cultivated sludge was chiefly due to the cultivation reactor being still in the process of reaching the steady state. The MLVSS fell sharply from day 0 to day 31 (Fig. 4), as the short SRT of 2 days would wash out the slow growers. The fairly low PHA content obtained within this period of time implies that PHA-accumulating organisms were not the dominant microbial species. A longer cultivation period might be necessary to attain steady-state condition and to further eliminate the non-PHA-accumulating organisms from the cultivation reactor. Indeed, the MLVSS became stabilized at around 548–637 mg/L on days 42–85. The PHA storage capacity of the sludge also enhanced remarkably, achieving a PHA content of 40–64 wt%. The MLVSS later stabilized at 908–966 mg/L on days 126–173. The PHA content attained within this period of time was in a similar range of 45–58 wt%, suggesting that PHA-accumulating microbes had increased in numbers while maintaining their storage capacity. It will be of interest to study the microbial population in future to cross-check and ascertain the nature of this shift in MLVSS. Nevertheless, the great improvement in the PHA content achieved after day 33 confirms the enrichment of PHA-accumulating organisms in the sludge. However, for better process control, it would be highly preferable that the range of variability in the PHA content be narrower. For this, the dynamics and the stability of the mixed microbial system require further exploration.

The long famine phase experienced by the sludge in the cultivation reactor plays a significant role in the enrichment of PHA-accumulating organisms. In this study, the duration of famine phase was in between 14 h and 20 h, which accounted for 58–83% of the total cycle length. This is akin to the typical length of famine

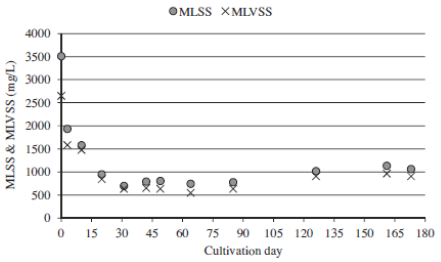

Fig. 4. Profiles of MLSS and MLVSS in the cultivation reactor of PHA-accumulating organisms. These were measured at the end of the cyclic operation, except for day 0 which was at the beginning of the operation to determine the inoculum concentration.

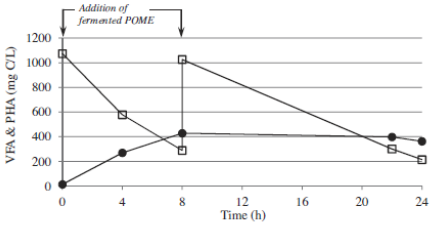

Fig. 5. Concentration profiles of VFA (□) and PHA (●) in PHA production using activated sludge taken from the cultivation reactor of PHA-accumulating organisms on day 50. A similar profile was obtained on day 74.

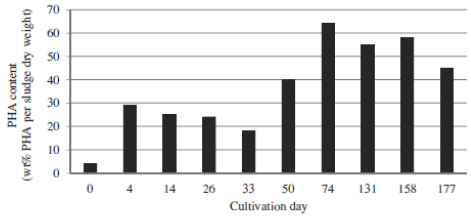

Fig. 6. PHA content of the activated sludge achieved after 8 h in the batch PHA production experiment. The sludge was taken from the cultivation reactor of PHA-accumulating organisms on different cultivation days.

Link to Full-Text Articles :

http://www.sciencedirect.com/science/article/pii/S1385894715001400