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POLYHYDROXYALKANOATES (PHAs) PRODUCTION FROM SAPONIFIED SUNFLOWER OIL IN MIXED CULTURES UNDER AEROBIC CONDITION

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Abstract. A laboratory study was conducted to assess the optimal conditions for polyhyroxyalkanoates (PHA) production using saponified sunflower oil (SO). The system was operated under continuous sequential phase of growth and accumulation conditions. The main purpose of using saponified SO was to improve the quality of PHA constituents (e.g. copolymer of hydroxyl-unit) such as hydroxybutyrates (HBs), hydroxyvalerates (HVs) and hydroxyhexanoates (HHs). Typically, SO mainly contains long-chain-fatty-acid (LCFA) with unsaturated fatty acid ($C_{14:1}$ – $C_{18:3}$) and therefore, the selected PHA-producer would be developed during unbalanced growth (feast-famine condition). Operation of the fed-batch for almost 6 months under aerobic dynamic conditions (with various operating conditions) has increased the PHA production up to 33% of dried cell weight. It was also shown that sludge subjected to aerobic condition in mixed cultures could accumulate high amount of PHA by manipulating the cycle length (HRT study). The high specific PHA storage rate (q_p^{feast} = 0.5 C-mol/C-mol. h) as well as the high sludge PHA contents achieved by mixed cultures make this saponified SO competitive with those based on the other vegetable oil (e.g. corn oil, soy oil, etc.)

Keywords: Saponified sunflower oil (SO), polyhydroxyalkanoates (PHAs), feast-famine regimes, mixed cultures

Abstrak. Satu kajian makmal telah dijalankan bagi menentukan keadaan yang optimum bagi penghasilan polihidroksialkanoat (PHA) menggunakan minyak bunga matahari (MB) tersaponifikasi. Sistem ini telah dijalankan semasa keadaan fasa terjujuk berterusan bagi pertumbuhan dan pengumpulan. Tujuan saponifikasi ke atas MB adalah untuk meningkatkan kualiti konstituen PHA (e.g. kopolimer bagi unit hidroksil) seperti hidroksibutirat (HBs), hidroksivalerat (HVs) dan hidroksiheksanoat (HHs). Kandungan utama MB adalah asid lemak rantai panjang (ALRP) di dalam kumpulan asid lemak tak tepu ($C_{14:1}$- $C_{18:3}$) dan penghasil PHA yang terpilih akan dihasilkan semasa pertumbuhan tak seimbang (keadaan *feast-famine*). Operasi

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suap-kelompok selama hampir 6 bulan di bawah keadaan aerobik dinamik (dengan pelbagai keadaan operasi) dapat meningkatkan penghasilan PHA sehingga 33% jisim sel kering. Ia juga menunjukkan bahawa lumpur yang melalui keadaan aerobik di dalam kultur campuran mampu mengumpulkan jumlah PHA yang tinggi melalui manipulasi panjang kitaran (kajian masa penahanan hidrolik). Kadar penyimpanan spesifik PHA yang tinggi (q_p^{feast} = 0.5 C-mol/C-mol. h) serta kandungan lumpur PHA tinggi dicapai dengan menggunakan kultur campuran menjadikan MB tersaponifikasi ini kompetitif berbanding dengan yang dihasilkan dengan menggunakan minyak sayuran yang lain (minyak jagung, kacang soya dan lain-lain).

Kata kunci: Minyak bunga matahari tersaponifikasikan (MB), polihidroksialkanoat (PHA), rejim *feast-famine*, kultur campuran

1.0 INTRODUCTION

The idea of polyhydroxyalkanoates (PHAs) production using mixed culture was ignited owing to PHA role as metabolic intermediate of wastewater treatment and as an alternative for biodegradable plastic. Microorganisms always have a capability of storing and consuming substrate in a more balanced way to generate feast (availability of substrates) and famine (exhausted of substrates) conditions [1].

PHAs are recognized as biodegradable polymer materials because they posses material properties similar to various synthetic thermoplastics and elastomers that are currently used in the market (from polypropylene to synthetic rubber). In addition, upon disposal, they are completely degraded to water and carbon dioxide (and methane under anaerobic conditions) by microorganisms in various environments such as soil, sea and lake water, and sewage. [2, 3].

Since the first finding of PHA by Lemoigne in 1926 [4, 5], more than 100 different monomer units have been identified as constituents of PHA in above 300 different microorganisms including 3-hydroxyalkanotes of 3-12 carbon atoms with large variety of R-pendant groups, 4-hydroxyalkanoates of 4-8 carbon atoms, 5-hydroxypentanoates, 5-hydroxyhexanoate and 6-hydroxydodecanoate. However, only a few of these PHAs have been produced in amounts sufficient to enable the characterization of their material properties and to develop potential applications. Poly(3-hydroxybutyrate) [P(3HB) or PHB] is a homopolymer of 3-hydroxybutyrate and is the most widespread PHAs in natural condition. Some researcher reported that, PHB is a highly crystalline and brittle homopolymer, which restricts its use to a limited range of application. Therefore, it is suggested that poly (3-hydroxybutyrate-co-hydroxyvalerate) [P(3HB-co-3HV) or PHB-co-HV] is better than PHB because it is more flexible and stronger [6, 7].

There are three kinds of polymer produced as intracellular storage, which are glycogen, PHA and polyphosphate. However, only glycogen and PHA are the main reported bacterial storage polymers [1]. The presence of storage compounds such as PHA and glycogen in activated sludge bacteria and mostly in pure culture has

been extensively researched [8-10]. Also, PHA has been reported from several researchers to be the more common storage polymer under conditions of carbon sources excess [11-13].

The goal of this study is to investigate the potential of vegetable oil in inducing the PHA production. Very little attention has been made in the past on combining fatty acids with mixed culture conditions. To achieve a high productivity of PHA production, fed-batch cultures will be carried out with the control of the nutrient feeding (C/N ratio), DO flowrate, feeding rate and cycle length.

2.0 MATERIAL AND METHODS

2.1 Experiment System

The experiments were performed in a double-jacketed laboratory bioreactor with working volume of 2 litres. Operating conditions were conducted with a different cycle phase as shown in Table 1. The operating principles of a batch activated sludge system are characterized in just three discrete periods: fill, react and draw. In order to control the fast uptake and storage polymer, the system was operated in continuous reaction period, which means no settling or allowing the idle phase (HRT = SRT).

Table 1 Operating phase with SO as substrates

Experiment(s)	Operating phase (h)			
	Aerobic mineral feed	Aerobic feed	Aerobic react	Draw/ Discharge
Growth	5 min	0-1	1–47.9	47.9–48
CN_{so}	No fill	0-1	1–47.8	47.8–48
DO_{so}	No fill	0-1	1–47.8	47.8–48
T_{so}	No fill	0-1	1–47.8	47.8–48
HRT_{so}	No fill	0-1	Up to 95.8	Up to 96

A mixed culture from sewage wastewater and facultative pond were used as inoculums. In the determination of the steady-state period, some parameters were used; total organic carbon (TOC), cell dried weight (CDW) or dissolved oxygen (DO) profiles. During steady state, in one cycle a much higher NH_4^+ (accumulation phase) was added than during normal operation (growth phase). The pH was maintained at 7.00 $\pm$ 0.1 using 2N HCl or 2N NaOH in both growth and accumulation phase.

The temperature was kept constant at 34°C (except for temperature study) by using a thermostat bath. The well-aerated reactors were operated with airflow of 2.39 l/min controlled by a mass-flow controller and stirred with two standard geometry six-blade turbines. Most of the processes were conducted in turbulence regime to ensure the good mass transfer and mixtures of vegetable oil using agitation approximately 1000 rpm.

2.2 Inoculation and Fed-batch Technique

The mixed cultures were first grown in the same batch of SBR mentioned before and cultivated for approximately 36 hr at 30°C. A portion of the preculture medium was transferred to the bioreactor at 10% of the working volume after the cells had reached the late exponential stage. The seed of cultures for fermentation were prepared for at least 24 hours in 2 litres flasks each containing 1.1 litre seed of inoculums, 0.8 litre of mineral solution and 0.1 litre of SO as carbon sources.

In order to measure the SO concentration in the culture broth (as shown in Table 2), 2 ml of the culture broth was adopted to a screw tube and then mixed with 5 ml hexane. After vigorous shaking for 1 min, 1 ml of hexane layer was transferred to a pre-weighted tube, and then dried at 37°C until the hexane phase evaporated. The SO concentration was estimated as the amount of extract by hexane according to the predetermined calibration curve.

Table 2 Typical fatty acids composition in SO

Compound (mol-%)	Omega name	Trivial name	Nomenclature	Group
±6	$C_{16:0}$	Palmitic acid	MCFA	Saturated
±5	$C_{18:0}$	Stearic acid	LCFA	Saturated
±29	$C_{18:1}$	Oleic acid	LCFA	Unsaturated
±58	$C_{18:2}$	Linoleic acid	LCFA	Unsaturated
±0.3	$C_{20:0}$	Arachidic acid	LCFA	Saturated
±0.7	$C_{22:0}$	-	LCFA	Saturated

Note : LCFA : long-chain-fatty-acid
MCFA : medium-chain-fatty-acid

Since the SO is hydrophobic material, the study was performed in saponification method to obtain better mixtures of substrate and medium. It was found that SO solution could only be concentrated up to 120 ml/l by pre-treatment of Triton-X 100. The feeding solution containing 120 ml/l was sufficient to allow high density of cells and relatively high concentration of PHA. About 0.15 g cells (in dry weight) were re-suspended in 100 ml mineral solution and cultivated in a rotary shaker at 200 rpm and 30°C for almost 24 hours. SO was added at the pre-determined concentrations to examine their effect on cell growth and PHA synthesis. About 3.4 g cell mass harvested from the nutrient-rich culture was re-suspended in 1 litre mineral solutions that contained long-chain-fatty-acid (LCFA) of SO. In that case, the initial concentrations of LCFA were controlled at 1.5 – 2.0 g/l. DO concentration was maintained at higher than 25% (during growth stage) and less than 10% (during accumulation stage) of air saturation in most of the study.

2.3 Analytical Procedures

Samples for analysis of ammoniacal-nitrogen (NH_4-N), phosphate-phosporus (PO_4-P), TOC, chemical oxygen demand (COD) and volatile fatty acid (VFA) were immediately centrifuged and filtered using 0.45 μm filters to separate the bacterial cells from the liquid. Then, the supernatant was stored in refrigerator (for TOC, COD and PHA analysis) and in the freezer (for VFA, VSS, CDW, NH_4-N, NO_3^-, PO_4-P and COD). NH_4, VSS, PO_4, NO_3 and COD analyses were done in accordance with Standard Methods. The supernatant of VFAs were measured according to the type of carbon chains. Acetic acid (HAc), propionic acid (HPr), and butyric acid (HBt) were measured using gas chromatography (GC) and a flame ionization detector (FID) by direct injection of acidified aqueous samples (pH 2-3) into a Supelco fused-silica capillary column (ø 0.25 mm x 25 mm). The culture (10 mL) was centrifuged under high rotation (rpm) to ensure the separation between pellet and supernatant. The CDW, volatile suspended solid (VSS) and ash content of the biomass were determined according to Dutch Standard Method [14]. The PHA content of the washed and dried biomass was determined by extraction, hydrolyzation, and esterification in a mixture of hydrochloric acid, 1-propanol, and dichloroethane at 100°C. The resulting organic phase was extracted with water to remove any free acids. The proplyesters were analyzed using GC. Benzoic acid was used as an internal standard throughout the procedure. The PHA content was given as a percentage of the total biomass dry weight (% PHA).

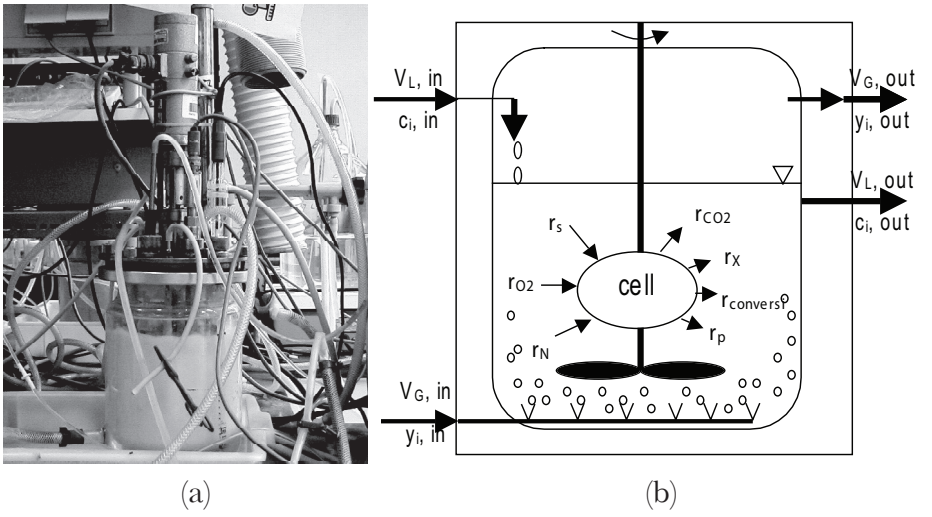


Figure 1 (a) Picture taken for 2 litres of SO as substrate, (b) Layout boundaries on cell accumulation for fed-batch system

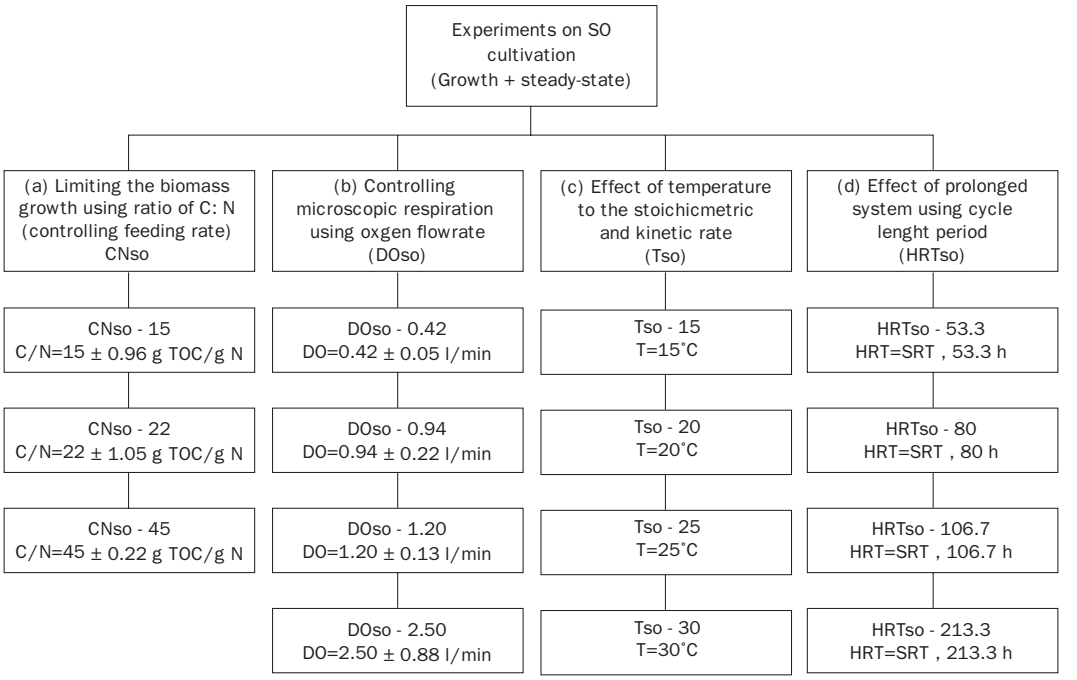


Figure 2 Experimental design in SBR fed-batch system using SO as substrate

3.0 RESULTS AND DISCUSSION

3.1 Introduction

The objectives of this study were to develop both an efficient system and suitable operating procedures that would maximize PHA production in an activated sludge system using selected VFAs (i.e. acetic, propionic and butyric acids) as the primary organic substrates. The goal was to define optimum operating conditions for maximizing the percentage of PHA in the activated sludge biomass, and to characterize the composition of the PHAs, i.e., the PHV, PHB and PHH concentrations as the polymers stored in the cells. A two-stage bioprocess approach that had a growth phase and a subsequent separate PHA accumulation phase was used in all of the experiments as shown in Figure 1. The overall experimental design in SO is summarized in Figure 2.

3.2 Specific Rates of PHA Production and Substrate Uptake

In general, the efficiency of PHA production obtained from this study is significantly influenced by the DO condition (%PHA can reach up to $14.43 \pm 2.72\%$) and cycle length (%PHA = $33.77 \pm 3.10\%$) as depicted in Table 4. It is also shown that PHA could be regulated using three limiting conditions: N, P and control of oxygen flowrate. The experiment in controlling C and N has been proven suitable for inducing the PHA production. Similar results were also obtained from several researchers. For example, [15] induced PHA accumulation in activated sludge biomass by controlling the C/N ratios of the feed substrates. They studied four different C/N ratios (24, 48, 96, and 144). Maximum specific polymer yield of 0.374 g polymer/g cell (or 37.4% PHA) was obtained with the highest C/N ratio of 144. However, the results of specific polymer yield in this study could still reach more than 0.6 g polymer/g cell (at C/N = 45 g TOC/g N). However, higher PHA production (only 9% of CDW) was not obtained because the PHA content of the biomass was low compared to the preliminary results (50% of CDW), as shown in Figure 3. This study concluded that temperature did not significantly affected the PHA production if the system was conducted between 20 – 30°C. However, the highest PHA production was obtained at 30°C, which was suitable for tropical operational conditions.

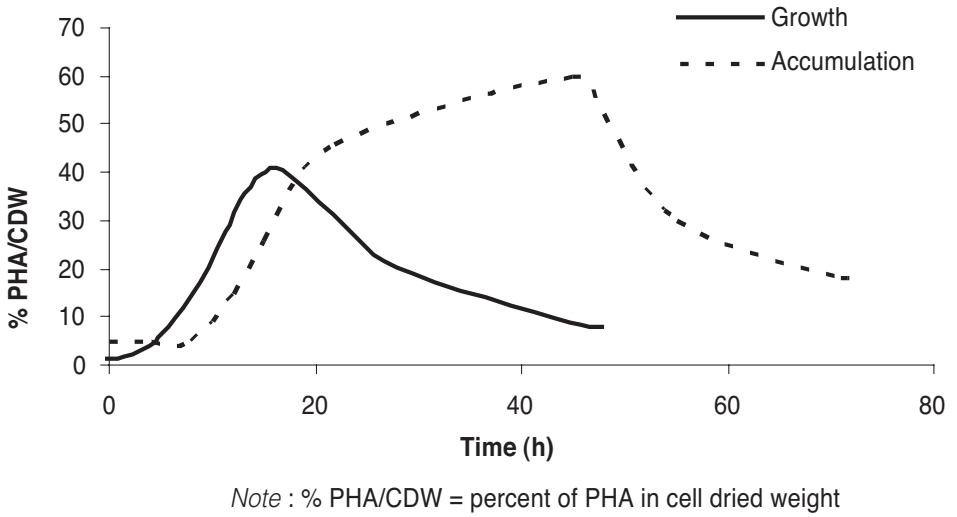
Table 3 Yield of VFAs utilization during growth and accumulation phase

Conditions	HAc			HBt		HPr	
	HAc (mM/h)	$Y^{max}_{PHB/HAc}$ (%)	% HAc/ Total VFA	HBt (mM/h)	% HBt/ Total VFA	HPr (mM/h)	%HPr/ Total VFA
Growth	20	62	84	0.23	11	0.08	4
Acccumulation	64	96	88	0.14	6	0.4	6

Table 4 Specific PHA production and substrate uptake rate at different experimental conditions

Experiment	Cycle length (h)	Temp (°C)	(-) q_s^{feast}, Cmol/ Cmol. h	q_p^{feast}, Cmol/ Cmol.h	$q_p/-q_s^{feast}$ Cmol/ Cmol	%PHA/ CDW (mean value)
CN_{so}-15	24	30	0.135	0.064	0.48	3.33±0.09
CN_{so}-22	24	30	0.409	0.222	0.64	5.39±0.08
CN_{so}-45	24	30	0.661	0.461	0.70	9.06±0.39
DO_{so}-0.42	48	30	0.164	0.094	0.580	14.43±2.72
DO_{so}-0.94	48	30	0.140	0.015	0.110	13.44±1.22
DO_{so}-1.20	48	30	0.089	0.014	0.157	9.54±1.28
DO_{so}-2.50	48	30	0.270	0.030	0.110	9.31±1.40
T_{so}-15	48	15	0.263	0.035	0.130	10.47±0.21
T_{so}-20	48	20	0.545	0.434	0.796	16.08±1.16
T_{so}-25	48	25	0.653	0.121	0.186	18.96±0.64
T_{so}-30	48	30	0.507	0.120	0.236	20.49±1.49
HRT_{so}-53.3	24	30	0.120	0.024	0.203	11.41±0.68
HRT_{so}-80.0	36	30	0.126	0.083	0.660	22.31±1.85
HRT_{so}-106.7	48	30	0.288	0.206	0.720	33.77±3.10
HRT_{so}-213.3	96	30	0.249	0.216	0.870	33.73±2.36

According to Table 5, temperature had a moderate effect on the aerobic stoichiometric coefficients, such as PHA, PO_4 (P) and NH_4 uptake per amount of O_2 consumed. The PHA/oxygen ratio was high in the temperature range of 20 – 25°C. However, at 15°C, the PHA/oxygen ratio was 46% lower than an average in the temperature range of 20 – 25°C. The ratio at 30°C was also low but not more than 25% compared with 20 – 25°C conditions. The NH_4/oxygen ratio shows a consistent trend due to small differences in the measured values. Similarly, as concluded earlier for aerobic kinetics, the coefficient rates, such as P, NH_4 and PHA consumption per active biomass, were strongly influenced by the temperature variations. At 20°C, and especially at 25°C, the overall trend of the oxygen consumption rate sharply changed after 10 hours. This means that during the remaining part of the aerobic phase oxygen was not used for the poly-P formation, but only for the biomass growth and maintenance. At 15 and 30°C, this pattern could not be observed due to incomplete P-uptake in the aerobic phase.



Note : % PHA/CDW = percent of PHA in cell dried weight

Figure 3 Overall percentage of PHA production for every cell acclimatized in growth and accumulation condition

In reference to the results in Table 6, the PHA accumulation inside the biomass components was clearly subjected to specific growth rate (μ). Most of the accumulation of PHA was significant for the specific growth rate at the range of 0.015 were always inconsistent, the study found that the growth during famine could also effect the PHA accumulation. Therefore, the potential of PHA production rate

Table 5 Derivation of stoichiometric and kinetic parameters from single study for aerobic batch experiments at 15, 20, 25 and 30°C and HRT 96.6 hours

Parameter	Unit/°C	15	20	25	30
Stoichiometric parameters					
PHA consumption/O_2 consumption	mg/mg O_2	2.34±1.24	5.06±1.75	4.07±2.09	3.21±0.29
O_2/PO_4 ratio	mg O_2/mg P	0.03±0.01	0.98±0.21	0.87±0.15	0.12±0.05
P-uptake/O_2 consumption	mg P/mg O_2	0.63±0.22	1.45±0.44	1.99±0.11	0.84±0.18
NH_4 consumption/O_2 consumption	mg/mg O_2	0.06±0.17	0.076±0.03	0.12±0.02	0.12±0.03
Kinetic parameters					
P-uptake/Active biomass rate	mg P/mg. h	0.006±0.003	0.023±0.02	0.044±0.04	0.012±0.02
O_2 consumption/ Active biomass rate	mg O_2/mg. h	0.008±0.002	0.013±0.03	0.012±0.01	0.011±0.01
NH_4 consumption/ Active biomass rate	mg/mg. h	0.0001±0.00	0.001±0.03	0.002±0.01	0.002±0.05
PHA consumption/ Active biomass rate	mg/mg. h	0.027±0.08	0.057±0.09	0.064±0.08	0.076±0.08

at famine period (30 percent of overall famine period) was examined. For example, with highest specific growth rate (μ^{famine} = 0.089 h^{-1}), about 0.51 C-mol/C-mol of specific PHA production rate ($q_p^{famine}/-q_s^{famine}$) was achieved (data not shown). The results also showed that the growth rate, substrate uptake rate ($-q_s$), product formation (q_p) and their rates ($q_p/-q_s$) have been increased when the system was exposed to high C:N ratio. However, both temperature (T_{so}) and cycle length (HRT_{so}) studies indicated that the maximum PHA production can be induced up to 620.41 and 387.95 C-mM, respectively. It was concluded that temperature (at 15°C) and prolonged period (more than 2 days) were able to enhance the PHA production. and at specific growth rate as low as 0.02 h^{-1}. However, higher PHA production was not obtained because the PHA content of the cells was low compared to the previous works [16,17] (Table 7).

Table 6 Accumulation of PHA content in various operational conditions under acclimatization of biomass concentration and specific growth rate

Experiment	Variable	Biomass, C_x (C-mM)		Specific growth rate, μ (h^{-1})		PHA^{max} (C-mM)
		Feast	Famine	Feast	Famine	
CN_{so}-15	C/N	352.57	418.00	0.067	0.042	18.50
CN_{so}-22		943.00	1000.57	0.077	0.042	25.10
CN_{so}-45		164.29	481.43	0.136	0.089	31.34
DO_{so}-0.42	DO flowrate	1142.86	1251.43	0.018	0.004	40.36
DO_{so}-0.94		1891.43	3251.43	0.029	0.014	51.81
DO_{so}-1.20		2088.57	2411.43	0.032	0.006	47.21
DO_{so}-2.50		994.29	2171.43	0.066	0.003	29.44
T_{so}-15	Temp	2615.71	311.43	0.026	0.015	620.41
T_{so}-20		2894.29	338.57	0.015	0.021	398.27
T_{so}-25		2517.14	1290.00	0.011	0.016	361.44
T_{so}-30		2595.71	1151.43	0.043	0.019	383.86
HRT_{so}-53.3	Cycle length	1998.57	310.00	0.030	0.042	265.19
HRT_{so}-80.0		1957.14	247.14	0.020	0.005	255.51
HRT_{so}-106.7		1927.14	364.29	0.021	0.015	387.95
HRT_{so}-213.3		1925.71	181.43	0.065	0.006	387.95

Table 7 Comparison study for PHA production at different wastewater and processes

Substrates	Process	PHA (%)	Reference
Activated sludge	Aerobic and anaerobic, fatty acid as carbon source	87	Lemos *et al.* [18]
Activated sludge	Anoxic and aerobic condition, feast-famine, acetate as carbon source	72	Dionisi *et al.* [19]
Activated sludge	Mixed cultures, feast-famine (anoxic-aerobic), acetate	16	Beun *et al.* [20]
Municipal wastes	Growth and accumulation phase with nutrient limitation, aerobic, acetic and propionic acids	70	Punrattanasin [21]
Sewage treatment plant	Feast-famine process, aerobic, mixed cultures	62	Dircks *et al.* [22]
Detergent waste	Batch and fed batch fermentation, nonylphenol ethoxylate (NPE) as carbon source	42	Blaylock, [23]
Activated sludge treating municipal wastewater	Anaerobic-anoxic-oxic condition, mixed cultures, acetate as carbon source	30	Chua *et al.* [24]
Activated sludge biomass	Growth and accumulation phase with P limitation, aerobic, sodium acetate and sodium propionate as carbon source	47	Chinwetkitvaich *et al.* [25]
Food processing wastewater treatment	Growth and accumulation with control C/N ratio, acetic acid as carbon source	33	Khanna and Srivastave. [26]
Saponified fatty acids (SO)	Growth and accumulation, aerobic, feast-famine, (C/N ratio, DO flowrates, temperature effects, cycle length)	34	This study

3.3 Development of PHA Productivity

All of the experiments (C/N, DO, HRT=SRT and temperature variables) have been conducted individually to simulate the "standard" productivity of PHA (Δf_{PHA}) using SO as substrates. The predictive model (Figure 4) has been used to generate the model equation in a single fed-batch culture. Further verification experiment (with this model) was not performed because this formulation was for the scale-down process under the assumed optimized condition. The final optimal formula obtained from different experiment conditions showed that the PHA productivity would increase at high C/N ratio (> 200 g TOC/g N) and HRT=SRT (> 90 h). Unfortunately, the efficiency decreased when exposed to the high air flowrate (> 1l/min) and temperature (> 25^{o}C).

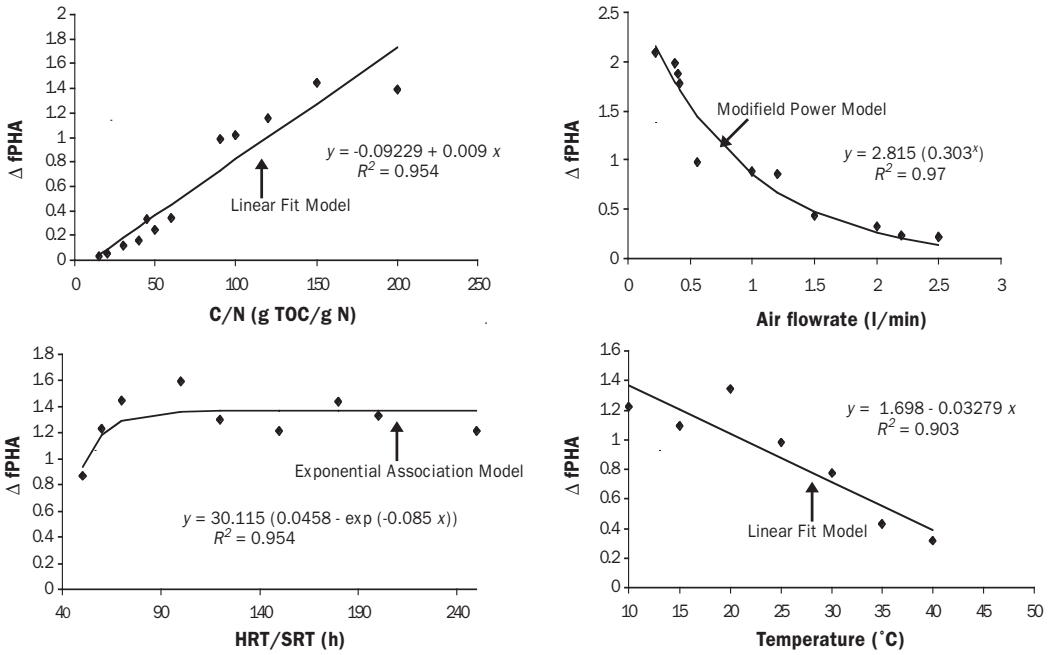


Figure 4 Dependence of the amount of PHA produced on C/N ratio, DO flowrates, HRT = SRT and temperature conditions. (♦) Experiments used for the fitting the points , (—) Model equation developed from fittings

3.4 Fatty Acid Uptakes for PHA Constituents

The patterns of selected fatty acid distributions were shown in Figure 5. In general, the detection of fatty acids was monitored at the early stage of 10 hours, and then depleted afterwards. Propionate was the highest fraction of VFAs followed by butyrate and then acetate. As a consequence, the PHH concentration produced was slightly higher than PHV and PHB. These results confirm that fatty acid constituents is an important parameter to be controlled (i.e. substrate pulses regime) in the processes for PHB production. However, those co-monomers of alkanoic acids (HH and HV) will be useful in restructuring the brittleness of PHB.

From the results obtained, the PHV, PHB and PHH concentrations produced by the activated sludge mixed culture were different under the different operating conditions (i.e. C/N ratios, DO flowrate, temperature and cycle length), even though the organic substrates used were the same. This was expected because during commercial production of PHAs, the substrates are changed when PHAs of different properties are desired. This difference is probably due to the fact that PHA carbon could be recycled multiple times in these continuous experiments unlike the batch experiment of [27] (as shown in Figure 6). For example, it is well

known that the acetic acid results in 3HB but in multiple cycle experiments or because of glycogen carbon, 3HV is also formed to varying degrees [28].

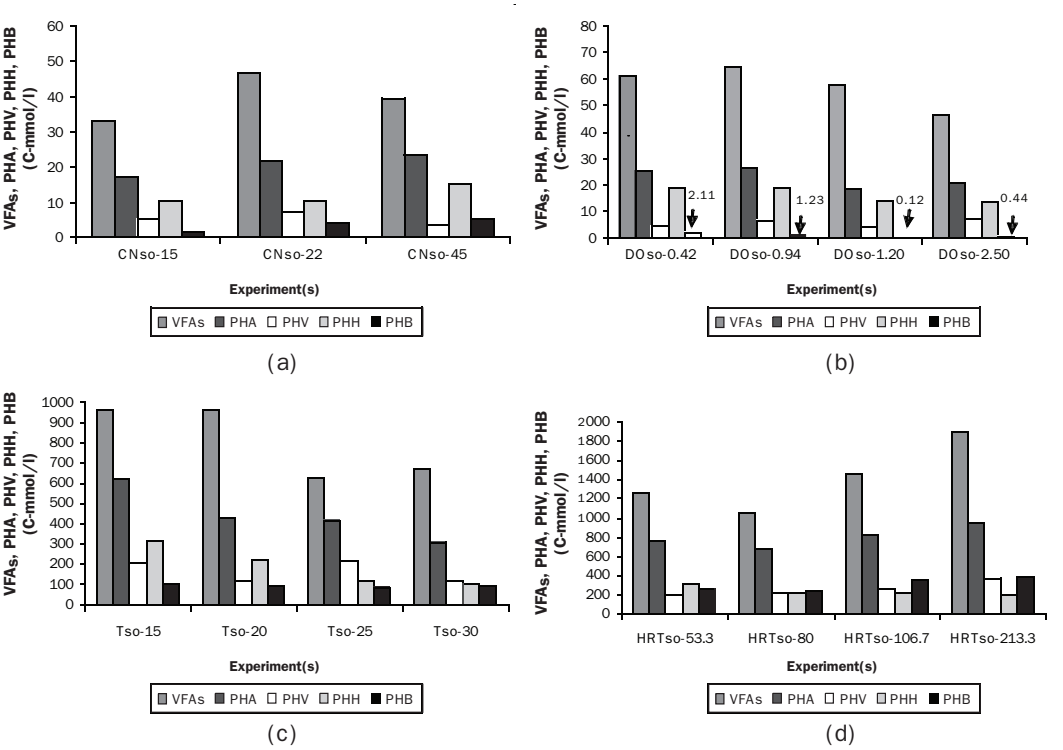


Figure 5 Summary on the VFAs uptake and PHA production at experiments of (a) C/N ratio, (b) DO flowrate, (c) temperature and (d) cycle lengths

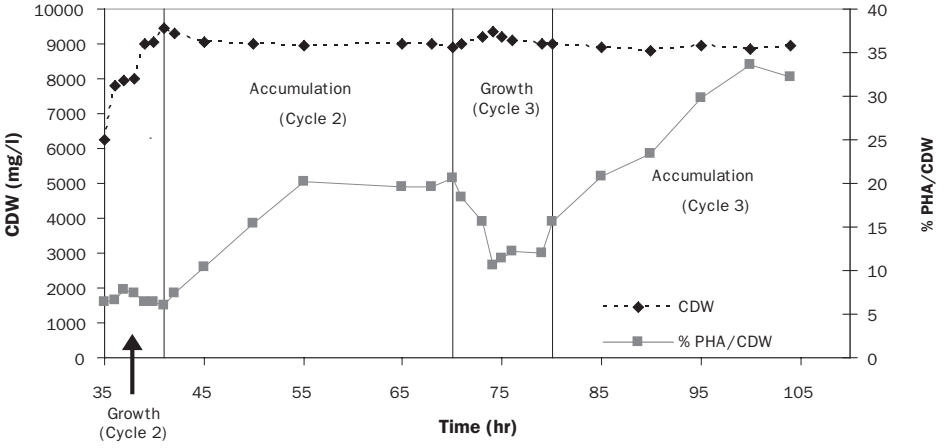
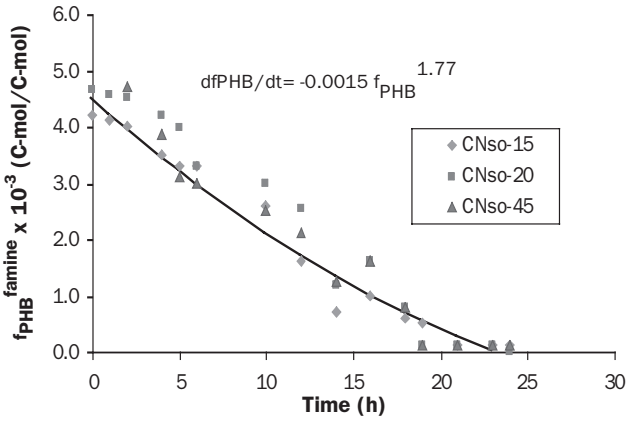
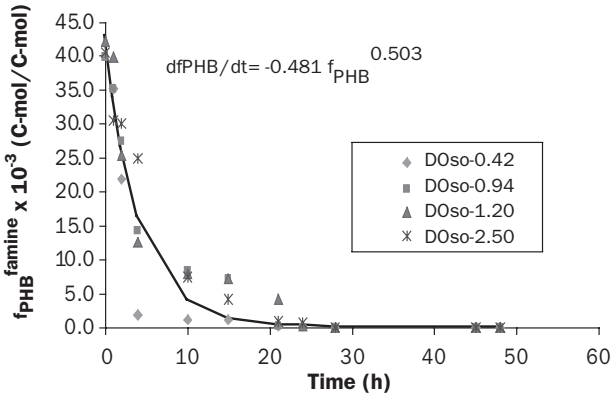


Figure 6 The PHA production and CDW concentration during reproducibility phase

3.5 Kinetic Rates of PHA Degradation

Figures 7 and 8 show the best fit for all data sets (CN_{so}-fit, DO_{so}-fit, T_{so}-fit and HRT_{so}-fit) which were calculated based on the famine degradation of PHB fraction. Because of different f_{PHB} values for each data set at the start of the famine period, the independent data sets were shifted in time so that they overlapped and could be shown in Figure 6. According to this result, the fast PHB degradation occurred under different DO flowrate with k = -0.48 h^{-1} and n = 0.503. At the same time, the slowest PHB degradation appeared under temperature effects with k = 0.00016 h^{-1} and n = 0.07. Due to this, the PHB degradation was determined from fastest to slowest reaction as follows: DO_{so}-fit > HRT_{so}-fit > CN_{so}-fit > T_{so}-fit. It is clear that PHB degradation occurred at massively significant rate under DO effects because the cells become more efficient in balancing the growth and storage under feast/famine period as compared to the others.





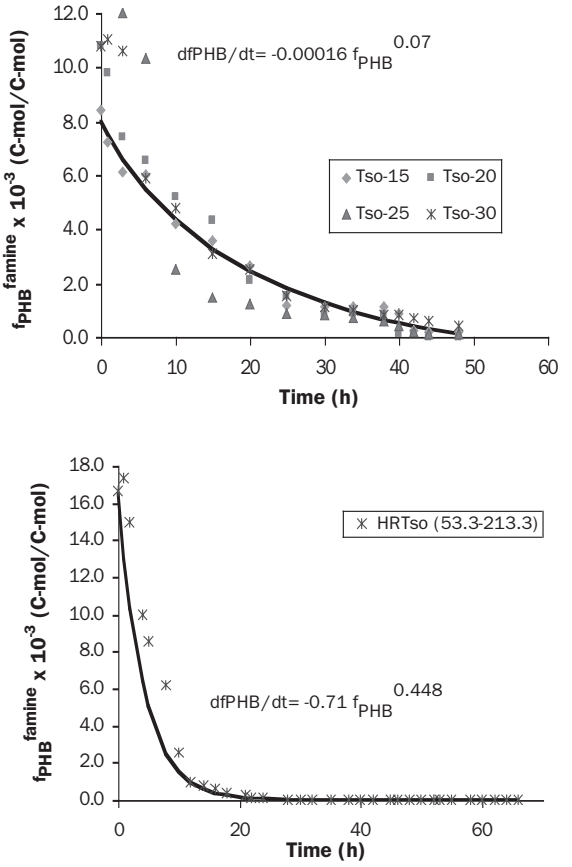


Figure 7 Curve estimate of PHB degradation using differential method

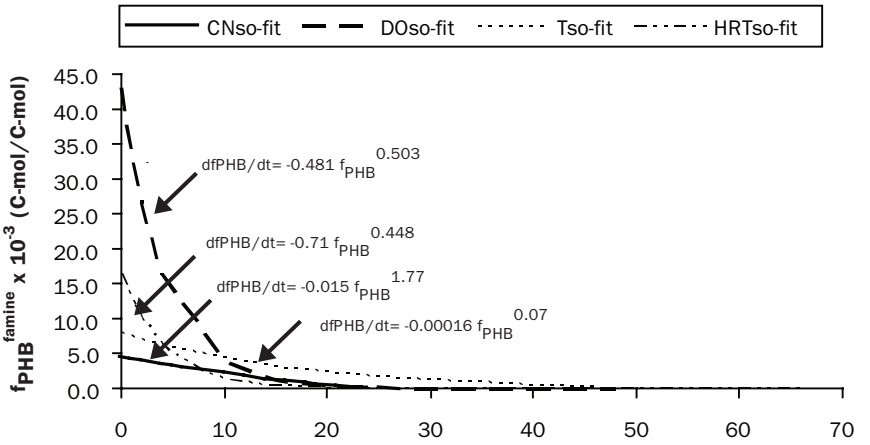


Figure 8 Degradation of PHB under different fitted conditions

4.0 CONCLUSION

The following conclusions were revised from the study of the cultivation of saponified fatty acid (SO), which are:

(i) The higher C/N (more than 45% of carbon feeding) ratio accumulated in the system, the slower the readily biodegradable matter formation (1 to 1.5 times). Then, the biomass concentration will slow-down, resulting in low PHA formation in a longer period (i.e. more than 24 hour),

(ii) The accumulation of storage polymers is independent of temperature, with no high PHA formation (11–20% of CDW) when the process was operated at range 15 – 30°C. However, exposure of the microorganisms responsible for cultivation to temperature changes from 15 to 30°C showed that the stoichiometry of the aerobic processes was sensitive towards temperature changes. At the same time, the correlation between fatty acid conversions (i.e. VFAs) and the production of PHA is obvious, indicating that cold temperature (less than 20°C) will lead to the increment of VFAs (two times higher than warmer conditions) and then benefits for polymer storage,

(iii) At specific growth rates in the feast period ($\mu^{feast} < 0.066\ h^{-1}$), the ratio of PHA over substrate consumption ($q_p/-q_s$) was experimentally determined at 0.6 - 0.7 C-mol/C-mol which can be concluded as high value. At this specific growth rates, the ratio of $q_p/-q_s$ depends strongly on the $-q_s$ and μ in the feast period,

(iv) Degradation of internal stored PHB can be described as a whole degradation of PHA contents in the cells and considered as first order rate. PHB degradation occurred at a significant rate under DO effects ($k = -0.48\ h^{-1}$) because the cells become more efficient in balancing the growth and storage under feast/famine period as compared to the others.

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