AJChE 2004, Vol. 4, No. 1, 11–18

Production of Polyhydroxyalkanoates from Synthetic Wastewater Using Sequencing Batch Reactors in On-Off Sequences

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The biosynthesis of polyhydroxyalkanoates (PHAs) using activated sludge as culture organism from tapioca synthetic wastewater has been studied in a 6L sequencing batch reactor (SBR). It was found that longer aeration time caused greater chemical oxygen demand (COD) reduction but did not achieve the maximum PHA-production rate. The application of on–off sequences, however, gave an acceptable PHA-productivity rate at 0.1033 gPHA L-1 h-1 and a high storage yield of up to 0.32 gPHA/g biomass, although it caused a decrease in pH and affected sludge stability. The on–off variation also produced a higher valerate copolymer content, in the range of 16.44–22.48 % for Run 1 and of 13.09–14.09 % for Run 2, compared to the customary aerated–unaerated variations. The study indicates that the use of on–off SBR sequences may be suitable for obtaining high PHA-storage yields.

Keywords: Activated sludge, aerated–unaerated SBR period, polyhydroxyalkanoates (PHAs), sequencing batch reactor (SBR), *and* synthetic wastewater.

INTRODUCTION

The use of plastic has increased rapidly in the last century, from stationaries to kitchenware to high-tech industrial equipment. The ability of plastic to substitute for other materials has raised its market demand, even yielding the increment of synthetic polymer production such as polypropylene and polystyrene.

Unfortunately, the popularity of plastic has led to serious environmental pollution since its natural decomposition takes a very long time. Non-degradable petrochemical plastics accumulate in the environment at a rate of 25M tonnes/year (Lee et al. 1991).

At present, there are three widely used plastic disposal methods: *incinerating, landfilling,* and *recycling* (Sidikmarsudi 1997). Of these three, *recycling* is considered the best way to dispose of plastic. This method, however, can only handle a very small portion of the total accumulated plastic wastes.

In response to this challenge and the harmful effects of these nondegradable wastes on the environment, there has been a considerable and growing interest in the development of

biodegradable plastic (Leaversuch 1987). Among biodegradable plastics, *polyhydroxyalkanoates* (PHAs) has shown the greatest potential to replace those made of hydrocarbon.

PHAs produce good, fully degradable plastic (Anderson et al. 1990, Salehizadeh et al. 2004) with properties that are similar to those of polyethylene, PE and polypropylene, PP (Lee 1996). Later studies has also proven that the co-polymers of PHA—namely, *poly(hydroxybutyric acid)*, PHB and *poly(hydroxyvaleric acid)*, PHV—are far less permeable to oxygen than either PE or PP, which makes these copolymers more suitable materials for food packaging with the reduced need for the addition of antioxidants (Salehizadeh et al. 2004).

PHA has been widely produced using glucose as raw material via biosynthesis pathway using many photosynthetic and nonphotosynthetic PHA-producing bacteria that have the ability to accumulate PHA in their cells.

However, with the more than 250 microorganisms that synthesize PHA (Choi and Lee 1997), only several species—namely, *Alcaligenes eutrophus* (Kim et al. 1994), *Alcaligenes latus* (Yamane et al. 1996), *Pseudomonas oleovorans* (Brandl et al. 1990), *Pseudomonas putida* (Huijberts and Eggink 1996), and recombinant *Eschericia coli* (Lee and Chang 1994, Lee et al. 1994)—are suitable for the production of PHA both at high concentration and productivity.

Likewise, with the more than 90 different monomer units identified as the constituents of PHA in various bacteria (Steinbuchel and Valentin 1995), only a few PHAs—such as *poly(3–hydroxybutyrate)*, PHB; *poly(3–hydroxybutyrate-co-3–hydrovalerate)*, PHB/V; and *poly(3–hydroxyhexanoate-co-3–hydroxyoctanoate)*, PHHx/O—have been produced to relatively large quantities and are well-characterized (Lee 1996).

The major drawback to the commercialization of PHAs is their much higher price compared with conventional petrochemical-based plastic materials. Much of this price goes to the cost of substrate used in PHA production (Yamane 1993).

Various carbon sources—from the more expensive *glucose* (US$1.35/kg PHB) and *acetic acid* (US$1.56/kg PHB) to the relatively inexpensive *cheese whey* (US$0.22/kg PHB) and *hemicellulose hydrozylate* (US$0.34/kg PHB)—have often been studied (Salehizadeh et al. 2004). Although the yields from these different substrates are nearly similar at 0.40 g PHB/g substrate (Salehizadeh et al. 2004), the price of each type of substrate still contributes greatly to the high cost of PHA production.

Indeed, most PHA-production processes are based on pure cultures of particular microorganisms grown on a well-defined nutrition-deficient synthetic media (Dionisi et al. 2004), wherein the selection of the microorganisms and the process itself entail high costs.

Thus, what remains to date a most interesting alternative to the pure cultures utilized in PHA production is the use of mixed cultures, such as activated sludge from wastewater treatment plants. The influent wastewater contains readily biodegradable carbon sources that are often transformed into PHAs by microorganisms and store the PHAs in their cells before being used for their growth (Beun et al. 2002). Hence, activated sludge is presently considered a possible source of biodegradable plastics (Satoh et al. 1998).

The use of mixed cultures, that is *activated sludge*, instead of pure cultures has its advantages. For instance, the process can be simplified because it does not need sterile conditions, and continuous cultures obtained without the risk of culture contamination (Dionisi et al. 2004). Similarly, the cost of the substrate can be extremely decreased by using the wastewater itself as the carbon source. The microorganisms in activated sludge consume carbon from wastewater to grow, causing COD reduction, and start to accumulate PHAs in their cells when the environment changes into a low-dissolved oxygen environment. To support that potential condition, the activated sludge system may be modified in a sequencing batch reactor (SBR), which can easily handle the feeding, aeration, and decanting periods without changing the reactor (Akunna and Jefferies 2000).

Thus, this study aims to find the conditions that not only upheld higher PHA production but also greater COD removal in wastewater.

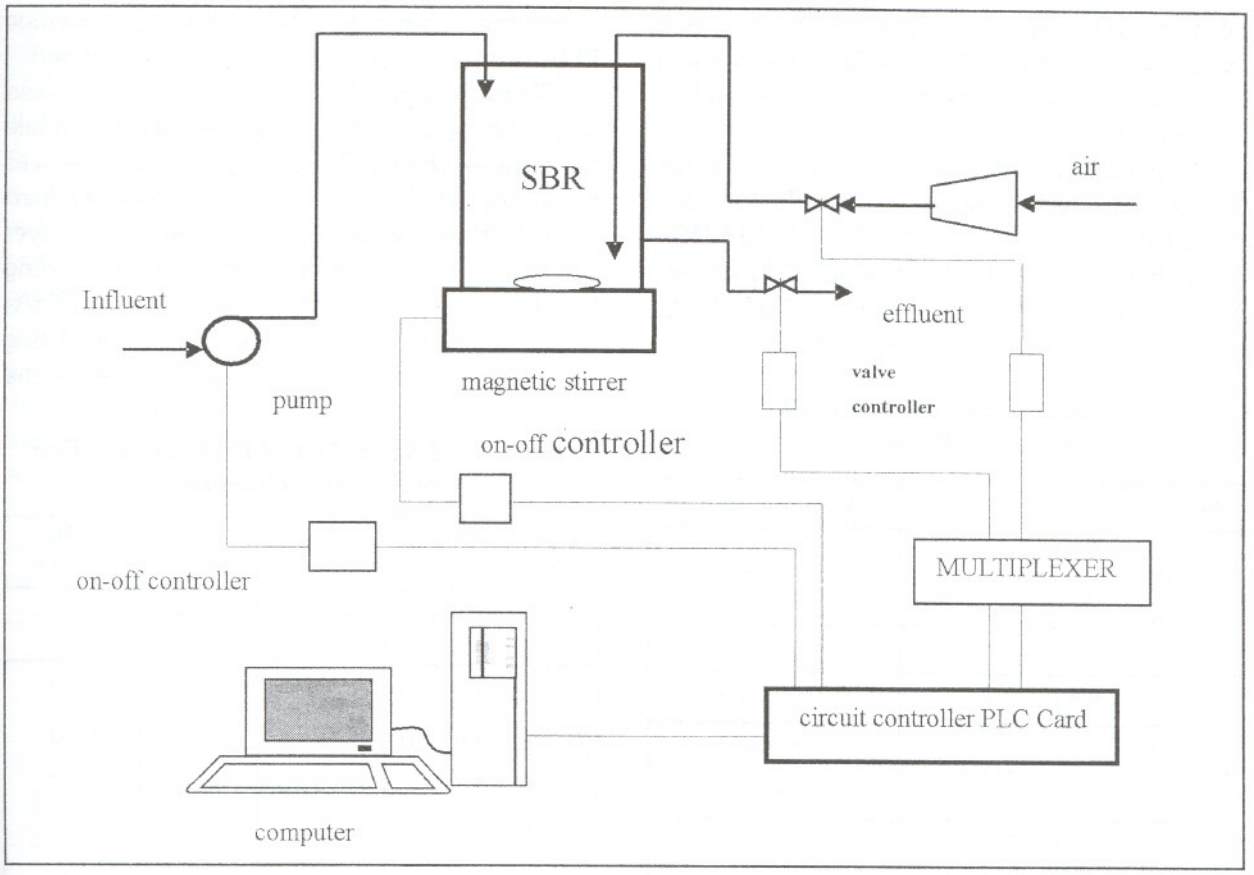

Figure 1. Schematic Diagram of Experimental Setup

EXPERIMENT

This study was carried out using two parallel units of computer-controlled sequencing batch reactors (SBR), each with a capacity of 6 L (see Fig. 1). Modification was introduced in the SBR controlling system so that aeration, feeding, settling, and decanting may be altered at the desired variation. Table 1 shows that the variation was done in the aeration period in one cycle.

By changing the aeration period in one SBR cycle, wastewater treatment and PHA production can be both conducted in the same reactor. In this study, the SBR cycle has been maintained at 12 h, with a 6-h feeding period, and a sludge-retention time of 30 days. Two liters

Table 1. Variation of Aeration Period in One-Cycle SBR

	Time (hours)											
	1	2	3	4	5	6	7	8	9	10	11	12
Run 1												
Run 2												
Run 3												
Run 4												
Run 5												
Run 6												

: aeration on : aeration off, mixing on
: settling : decanting

of activated sludge was retained in each SBR before starting the cycle. The SBR was fed with 3.5 L of synthetic wastewater at a constant flow rate within 6 h.

The SBR was fed 50 g of tapioca starch diluted in 8L water. The feed also contained the following mineral media: $(NH_4)_2SO_4$, KH_2PO_4, $FeCl_3 \cdot 6H_2O$, $MnSO_4 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, and $MgSO_4 \cdot 7H_2O$ (refer to Table 2). NaOH was likewise added to maintain the pH level.

Table 2. Composition of 8L Synthetic Wastewater

No.	Component	Composition
1	Tapioca starch	50.00 *g*
2	$(NH_4)_2SO_4$	3.84 *g*
3	KH_2PO_4	0.0296 *g*
4	$FeCl_3 \cdot 6H_2O$	0.2272 *g*
5	$MnSO_4 \cdot 6H_2O$	0.0024 *g*
6	$CaCl_2 \cdot 2H_2O$	0.32 *g*
7	$MgSO_4 \cdot 7H_2O$	0.16 *g*
8	NaOH (0.1 M)	20.00 *mL*

To maintain solid retention time (SRT) at 30 days, 83 mL of mixed liquor was withdrawn from the reactor at the end of cycle. The amount 83 mL was determined by dividing the 2.5 L of mixed liquor left in the reactor at the end of cycle with the SRT (2,500 mL/30 days = 83 mL/day). This sample would be used for examining MLSS (Mass Liquor Suspended Solid), COD, and PHAs concentration. These analysis methods were described elsewhere (Sidikmarsudi, 1997).

A Fischer–John melting point apparatus was used to determine the melting point of the PHAs produced, based on previous research (Yu et al. 1999). This study assumed that polymers produced by activated sludge bacteria were either poly(3–hydroxybutirate), PHB or its copolymer poly(3–hydroxybutyrate-co-3–hydrovalerate), PHB/V. With the standard PHB-melting point at 177°C, which for a standard PHV is at 100°C (Sidikmarsudi 1997), the PHA-melting point obtained fell at the middle of both points. It was assumed that the polymers produced contained both PHB and PHV and had been formed as a copolymer. Using this assumption, the average PHA production per sequence can be defined.

To form a plastic sheet, the 2.5 L of mixed liquor was separated by gravitational force. While the filtrate—*the COD-reduced wastewater*—was being disposed of, the sludge—*the cells obtained at the bottom*—were collected in an Erlenmeyer flask. The cells were then broken down by adding NaOCl (3 mL/10 mL mixed liquor). The PHAs withdrawn from the cells had to be extracted using chloroform (at the same amount as that of the

Table 3. MLSS and COD-Reduction at End of Transient Condition

Run No.	Ratio	Days to Achieve	MLSS	COD Removal
	Aerated: Unaerated	Steady State	*mg/L*	%
1	5:4 (on–off)	25	5,000	53.74
2	4:5 (on–off)	20	3,400	51.02
3	3:6	9	8,700	68.97
4	5:4	9	6,200	66.85
5	6:3	9	4,600	70.59
6	8:1	9	3,200	73.28

Table 3 shows that runs 1 and 2 took a longer time to achieve steady state condition. This could be attributed to the unaerated condition in between aerobic conditions. When aeration was stopped, anaerobic microorganisms in the activated sludge colonies started to degrade organic matters into volatile fatty acids through the acidogenic process. These volatile fatty acids could be used by methanogenic bacteria to produce methane in the anaerobic condition (Banister and Pretorius, 1998). However, in runs 1 and 2, methanogenic process did not lead to the acidogenic process because the anaerobic condition was always followed by an aeration stage. After steady state conditions achieved, these sequences dropped the pH up to 4.00 (Figure 2), while the activated sludge microbes had an optimum pH of 6 to 8. This condition decelerated the COD consumption in runs 1 and 2. This condition, however, did not occur in other sequences. The pH level in runs 3 to 6 remained invariable at 6.

Table 3 also explains that longer aeration time caused higher COD removal. The maximum COD removal was achieved at Run 6 (73.28%) with 8 hours aeration. Longer aeration gave the sludge more time to decompose organic matters in the wastewater. Thus, the sludge would have contained some anaerobic microbes, which were not as fast as the aerobic ones in degrading organic matters.

In order to study the effect of longer aeration period to the PHAs productivity, we investigated the PHAs production per hour in one SBR cycle (runs 3 to 6). PHAs production reached its peak after the aerated period ended, as shown in Figure 3. In Run 3, for example, when aeration stopped after 3 hours, PHAs production would rapidly increase. When oxygen was available, the TCA-cycle, which led to cell growth, occurred. The TCA-cycle was blocked when aeration stopped; then acetoacetyl co-A would be produced and PHAs would be accumulated.

During the last three hours in each variation, PHAs concentration decreased. It was thought that if the unaerated period was maintained too long, the PHAs accumulated in the cells would be used as reserve food. Runs 3 and 4 show that the peak PHAs accumulation was reached 4 hours after the aerated period ends. On the other hand, in the variations with a longer aerated period, it was considered that carbon sources fed into the SBR were used mostly for cells growth. Because the carbon entered into the PHAs metabolism was small, PHAs production in a longer aerated period would be less than that produced in a longer unaerated condition.

Thus, in order to gain optimum condition, both aerated and unaerated conditions cannot be maintained too long. Therefore, we introduced the on–off sequence in order to accumulate more PHAs in the cells.

From the PHAs production data shown in Table 4, it is obvious that on–off variation (aerated–unaerated–aerated–unaerated), i.e. runs 1 and 2, increased the PHAs accumulated in the cells. Both runs 1 and 2 produced PHAs up to 2.47 gram per 2.5 L mixed liquor (0.99 g/L), while runs 3 to 6 only produced PHAs from 1.95 to 2.07 gram per 2.5 L mixed liquor (0.78–0.81 g/L). On–off variation could have forced PHAs production to happen consecutively with cell

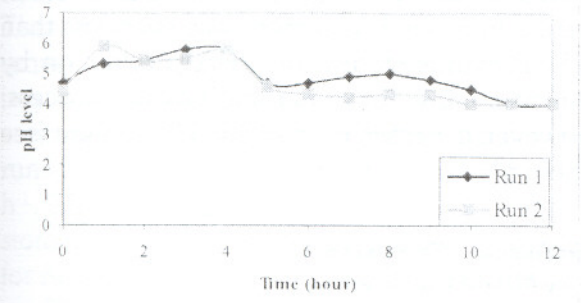

Figure 2. The pH Level of the Activated Sludge/Hour in One Steady-State SBR Cycle (The pH Level of Fresh Feed is Maintained at 6–7)

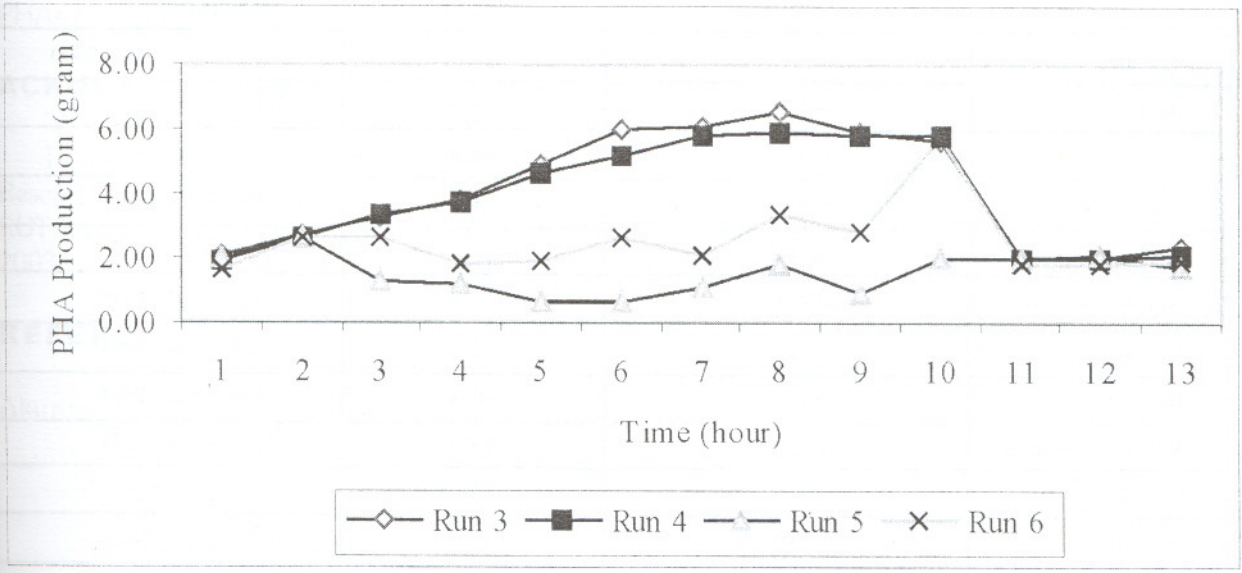

Figure 3. PHA Production/Hour in One SBR Cycle

growth. The cells reproduced in the aeration phase and then PHAs accumulated in the unaerated phase. In aerated-unaerated variation (runs 3 to 6), the higher value of average production was accomplished at Run 3. This condition resulted from a longer anaerobic phase, which might have extended PHAs accumulation in the cells.

Table 4. PHA Production Data in Steady-State Condition

Run No.	Average Melting Point °C	Average PHA Production *gPHA L^{-1}*	Average Productivity Rate *gPHA L^{-1} h^{-1}*	Storage Yield *gPHA/g biomass*
1	135	0.93	0.1033	0.27
2	140	0.93	0.1033	0.32
3	161	0.79	0.0878	0.11
4	164	0.78	0.0867	0.14
5	157	0.76	0.0844	0.23
6	157	0.74	0.0822	0.26

In terms of productivity rate, which is calculated based on 9 hours reaction time, both runs 1 and 2 gave the highest rate at 0.1033 gPHA L^{-1} h^{-1} (Table 4). This is, however, smaller compared to previous work, which achieved up to 0.14 gPHA L^{-1} h^{-1} (Dionisi et al., 2003). On the other hand, the storage yield gained in both runs 1 and 2 were much higher compared to other similar works (Table 5). This result proves that conducting on–off sequences would increase the amount of PHAs stored in the biomass cells.

The melting point of the PHAs accumulated tended to decrease toward the drop of pH, which indicated the appearance of valerate as copolymer. From the melting point data, it was calculated that Run 1 had hydroxyvalerate (HV) content of 16.44 to 22.48%. A similar PHAs production was obtained for Run 2; however, with a lower HV content at 13.09–14.09 %. For runs 3 to 6, the HV content was even less than 5%. Previous studies on PHA production by mixed cultures in anaerobic/aerobic process, however, generally gave higher HV content (see Table 5).

Table 5. Different Anaerobic/Aerobic Processes Used in PHA Production by Mixed Cultures

Carbon Source	Storage Yield *gPHA/g biomass*	HV Content%	Reference
Acetate	0.167	10.00	Smolders et al., 1994
Volatile fatty acids	0.230	38.00	Levantesi et al., 2002
Acetate	-	12.00	Liu et al., 1997
Acetate	-	11.00	Hesselmann et al., 2000
Acetate	0.026	31.00	Satoh et al., 1992
Propionate	0.025	59.00	Satoh et al., 1992
Malate	0.009	75.00	Satoh et al., 1992
Lactate	0.018	76.00	Satoh et al., 1992
Acetate	0.201	25.00	Lemos et al., 1998
Propionate	0.128	72.00	Lemos et al., 1998
Butyrate	0.131	40.00	Lemos et al., 1998
Volatile fatty acids	0.121	45.00	Lemos et al., 1998
Acetate	0.164	10.00	Satoh et al., 1996
Propionate	0.267	45.00	Satoh et al., 1996
Volatile fatty acids	0.283	51.00	Satoh et al., 1996

Even though this study shows that on–off sequences induce high storage yield, further study has to be conducted in order to gain high PHAs productivity rate. A system, which consists an on–off sequence paralleled with a common aerobic–anaerobic SBR sequence might also be investigated for obtaining higher COD consumption rate. However, it is also important to conduct further research using actual industrial wastewater with more methodical PHAs analysis.

CONCLUSIONS

An aerated period in the SBR cycle resulted in greater COD reduction but would not give great contribution in accumulating PHAs. An unaerated period in the cycle, on the other hand, promoted the production of PHAs either as poly(3-hydroxybutyrate) (PHB) or its copolymer poly(3-hydroxybutyrate-co-3-hydrovalerate) (PHB/V). Therefore, the application of on–off aerated sequences (runs 1 and 2), which reduced pH level but gave higher PHB and P(3HB-co-3HV) concentration. The average PHB productivity for runs 1 and 2 was the same at 0.1033 gPHA L^{-1} h^{-1}. These sequences also gave high average storage yield at 0.27 and 0.32 gPHA/g biomass for runs 1 and 2 respectively.

This investigation itself is only preliminary study in order to find the optimum SBR cycle that can accumulate PHAs as well as reduce COD level in acceptable amount. Further study using actual industrial wastewater with more thorough PHAs analysis should be conducted.

ACKNOWLEDGMENT

This study was funded by The Ministry of Research and Technology, Indonesia, through the RUT X project with Contract No. 14.28/SK/RUT/2003.

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