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Bulking sludge for PHA production: Energy saving and comparative storage capacity with well-settled sludge

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Abstract
Two acetate-fed sequencing batch reactors (SBR) were operated under an aerobic dynamic feeding (ADF) model (SBR #2) and with an anaerobic phase before aerobic phase (SBR #1) to select mixed cultures with a high polyhydroxyalkanoates (PHA) storage response. Although kinetic selection based on storage response should bring about a predominance of floc-formers, a bulking sludge with storage response comparable to well-settled sludge was steadily established. An anaerobic phase was introduced before the aerobic phase in the ADF model to improve the sludge settleability (SBR #1), however, due to the consequent increased feast/famine ratio, the performance of SBR #1, in terms of both the maximum PHB (polyhydroxybutyrate) cell content and ∆PHB, was lower than that of SBR #2. SBR #2 gradually reached a steady state while SBR #1 failed suddenly after 50 days of operation. The maximum specific substrate uptake rate and storage rate for the selected bulking sludge were 0.4 Cmol Ac/(Cmol X hr) and 0.18 Cmol Ac/(Cmol PHB-hr), respectively, resulting a yield of 0.45 Cmol PHB/(Cmol Ac) in SBR #2 in the culture enrichment phase. A maximum PHB content of 53% of total suspended solids and PHB storage rate of 1.36 Cmol Ac/(Cmol PHB-hr) was achieved at 10.2 hr in batch accumulation tests under nitrogen starvation. The results indicated that it was feasible to utilize filamentous bacteria to accumulate PHA with a rate comparable to well-settled sludge. Furthermore, the lower dissolved oxygen demand of filamentous bacteria would save energy required for aeration in the culture enrichment stage.

Key words: polyhydroxyalkanoates; mixed cultures; aerobic dynamic feeding; bulking sludge; feast/famine ratio

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Introduction
Polyhydroxyalkanoates (PHA) are a type of polymer that can be accumulated by numerous microorganisms as a carbon and energy storage material. The most common forms of PHA accumulated by microorganisms are polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) (Satoh et al., 1992). PHA are biodegradable and biocompatible (Dias et al., 2006). Plastics that are made from PHA possess physical characteristics similar to petrochemical plastics and are considered to be potential substitutes for these materials to effectively alleviate “white pollution”. PHA products are ten times more expensive to produce than polyethylene products (Reddy et al., 2003), however, due to high production costs, most of which are attributable to operating expenses and substrate costs. Operating expenses include strict sterilization procedures, and the substrate costs are high because of the cost of raw materials such as pure substrate media (Kasemsap and Wantawin, 2007). Alternative substrates derived from waste streams could help to reduce these costs (Braunegg et al., 2004), and the use of PHA production processes based on open mixed microbial cultures (MMCs) rather than pure cultures would be favorable when using waste stream substrates. This is because MMCs can better adapt to changes in substrate supply, and sterilization of the substrate would therefore not be required (Johnson et al., 2010). Storage of PHA by MMCs occurs under transient conditions of carbon supply (Majone et al., 1996), which are commonly referred to as ‘feast-famine’ or aerobic dynamic feeding (ADF). An effective selective pressure for the development of a PHA-producing population is produced by the application of a cyclic feast-famine regime that repeatedly alternates the presence (feast phase) and absence (famine phase) of the substrate. This may be established in a repeated batch cultivation process. During the feast phase, external substrate uptake by organisms is mainly driven toward internal polymer storage. After substrate exhaustion, the accumulated polymer can be used as a carbon and energy source to support cell growth and maintenance. Therefore, microorganisms that can store PHA during the feast phase have a competitive advantage over other organisms, as they can keep growing using stored PHA when no substrate exists (Donisi et al., 2004; Dias et al., 2006; Albuquerque et al., 2007).

Although PHA production by mixed cultures in open
systems has made simple reactor operation and low-cost substrate applications possible, it generates other problems where the predominant culture is easily interchangeable due to the fluctuation of operating conditions including temperature, substrate quality and dissolved oxygen (DO) concentration. It has been well accepted by most researchers that a high substrate gradient, which can introduce a transient condition in a plug flow reactor or a sequencing batch reactor (SBR), can lead to favorable selection of well-settled microorganisms over filamentous ones (Dionisi et al., 2001). This feature provides an explanation of kinetic control of the aerobic bulking exerted by aerobic selectors, plug flow configuration, or intermittent feeding, where a selective pressure is introduced due to the concentration gradient. Although the use of selectors has been successful and has reduced bulking problems in many activated sludge systems, failures are still reported regularly. Recent studies showed that a bulking sludge with even higher PHA storage capacity can be established steadily under such transient conditions (Beccari et al., 1998; Martins et al., 2003a). Martins et al. (2003a, 2003b) made a comprehensive study of sludge settleability under transient conditions. The results showed that the feeding pattern and DO concentration in the feast phase both had a great effect on the proliferation of filamentous microorganisms. A short aerobic fill time ratio (FTROX), which promotes a strong substrate gradient (FTROX < 5.4%), in addition to a pulse feed strategy, resulted in good sludge settleability (Martins et al., 2003a). Furthermore, the results indicated that the maximum acetate uptake rate and PHB production rate of bulking sludge was similar to that of a well-settled sludge. Martins et al. (2003b) found that low DO (≤ 1.1 mg/L) in the feast phase inhibited sludge settleability, leading to the proliferation of filamentous bacteria in the column. Inhibition of settlement occurred more markedly under high substrate loading rate.

The maintenance of high DO under high bulk substrate concentrations in the feast phase is a difficult process due to the high metabolic rate of microorganisms. Maintenance of higher DO levels requires greater electricity consumption for aeration processes. Guo et al. (2010) proposed a strategy to treat wastewater by using limited filamentous bulking sludge under low DO conditions, and conducted tests which showed pollutant removal could be enhanced and energy consumption could be reduced by at least 10% compared to processes using well-settled sludge. Based on the strategy proposed by Guo et al. (2010), a similar strategy may be adopted in PHA production by utilizing bulking sludge to achieve a reduction in energy consumption. Beccari et al. (1998) first reported that filamentous microorganisms can exhibit immediate storage of PHA when subjected to an acetate spike, however up to the present time, few reports have appeared on the utilization of filamentous bacteria for PHA production, nor on long term monitoring of the PHA storage performance of bulking sludge.

The objective of this study was to establish a bulking sludge with high storage response under low DO (around 1.0 mg/L in feast phase) in a SBR under an ADF model. It was also proposed to investigate whether the presence of an anaerobic phase can improve sludge settleability. As such, another SBR was operated in parallel under an anaerobic-aerobic pattern. Selected mixed cultures were withdrawn regularly from both SBRs as a form of excess sludge and batch tests under nutrient-limited conditions were subsequently conducted to evaluate the PHA storage rates, overall yield of the selected bulking sludge and maximum polymer fraction in the biomass. Since acetate was used as the sole carbon source, the PHA produced in this study was purely in the form of PHB.

1 Materials and methods

1.1 SBR for culture selection

Two bench scale SBRs with the same configuration (working volume equal to 4 L) were used for culturing the bulking sludge under periodic conditions. One SBR was operated under ADF conditions (SBR #2) and another SBR was operated with an anaerobic phase before the aerobic phase on the basis of the ADF model (SBR #1). Each reactor was equipped with a stirrer with a two-blade turbine. Air was supplied through ceramic diffusers by two compressed air pumps with a stable flow at 0.25–0.3 m³/hr. The air flow rate was adjusted using two gas rotors flow meters. The reaction temperature was controlled at 20 ± 0.5°C by a glass heater. No attempt was made to control pH in the reactors since pH fluctuation ranged from 8 to 9, which was favorable for the selection of PHB-producing cultures (Chua et al., 2003).

The SBRs were operated under 8 hr cycles which consisted of four discrete periods: fill (10 min); reaction (SBR #1: anaerobiosis 120 min and aerobiosis 270 min; SBR #2: aerobiosis 390 min); settling (70 min) and withdrawal (10 min). At the end of each reaction phase, 130 mL of mixed liquid was withdrawn to maintain the sludge retention time (SRT) of 10 days (the actual value after bulking may be lower than this set value due to the occasional wash-out of the biomass). At the end of each settling phase, 2 L of supernatant was withdrawn using a peristaltic pump to keep the hydraulic retention time (HRT) equal to 16 hr. The PHA production process and the SBR time course applied in the experiment are shown in Fig. 1.

The two SBRs were fed with the same synthetic medium which consisted of a carbon source and a nutrient source. The carbon source and the nutrient source were made in the experiment are shown in Fig. 1. No. 10 Bulking sludge for PHA production: Energy saving and comparative storage capacity with well-settled sludge
two identical peristaltic pumps to achieve a bulk substrate loading rate of 84 Cmmol/L per day.

The initial inoculation of the two SBRs consisted of concentrated activated sludge taken from the aerobic reactor of the Taiping municipal wastewater treatment plant in Harbin, China. To start the experiment, 2 L of inoculation sludge, with total suspended solids (TSS) of 7600 mg/L, was added simultaneously into each SBR with 1 L of carbon source and 1 L nutrient source.

1.2 PHB batch accumulation assays

Batch tests were carried out for PHB accumulation to assess the maximum PHB accumulation capacity, the PHB storage yield on the substrate and the production rate of the selected cultures enriched in the SBRs. The accumulation assays were carried out in beakers with a working volume of 1 L. One litter of the sludge was taken from the SBRs at the end of a cycle (immediately prior to the new feed). The mixed liquor was then settled and 500 mL of the supernatant was removed. The aeration began at least one hour before feeding to exhaust the residual nutrients in the beakers. To investigate the maximum PHA storage capacity, the substrate was fed in several steps. Each time when substrate was exhausted, 500 mL of supernatant was withdrawn after settling and then 500 mL of the substrate (acetate and nutrients without nitrogen, acetate concentration 80 Cmmol/L) was added. The step-feed tests lasted 10–12 hr. Such a procedure was chosen to achieve a high organic load while maintaining a substrate concentration (Becchi et al., 1998). The acetate concentration and PHB content were measured immediately before and after each spike, and the depletion was indicated by a rapid increase in the DO concentration. Air was supplied through a ceramic diffuser from a compressed-air pump and the temperature was maintained at 20 ± 0.5°C. The pH was not controlled.

1.3 Analytical methods

During the trial, the aerated sludge was sampled at regular intervals for analytical determination. Samples for analysis of acetate, ammonia, phosphate and PHB were first centrifuged in a 5 mL tube with rotation speed 12,000 r/min for 3 min. The ammonia and phosphate concentration of the supernatant and TSS were determined according to the Chinese SEPAC Standard Methods (Chinese SEPAC, 1997). The acetate concentration of the supernatant was measured using a gas chromatograph (GC 7890N/FID, Agilent, USA) with HP-INNOWAX 19095N-123 column. The column temperature was 70°C for the initial 0 min and was increased at 25 to 170°C and maintained for 2 min. The injection temperature was set at 250°C and the flame ionization detector was at 300°C. The determination of PHB content was performed using gas chromatography after methanolytic decomposition as described by Satoh et al. (1996). A gas chromatograph (GC 7890N/FID, Agilent, USA) with an HP-5 column (30 m length, 320 μm internal diameter, 0.25 μm film thickness) was used for all PHB detection. The PHB concentration of the dry sludge was calculated using PHB standards (Sigma, USA).

The DO concentration was measured with a DO meter (WTW, Germany) and pH was monitored with a pH meter (HI8424, HANNA, Italy). Microscopic examination of the activated sludge was carried out at magnifications between 100× and 1000×. Sudan black staining was also applied according to Jenkins et al. (2004).

1.4 Calculations

The PHB content of the sludge was calculated as a percentage of TSS on a mass basis (PHB = (g PHB/g TSS) ×100%). The change in the PHB content over one cycle was determined by subtracting the initial PHB content from the maximum PHB content achieved during the cycle. Active biomass (X) was calculated by subtracting PHB from the volatile suspended solids (VSS). The active biomass concentration was converted from g/L into carbon moles per liter (Cmol/L) under the assumption that the biomass had a general composition of CH₂O₅N₉O₂ with a molecular weight of 25.1 g/Cmol (Beun et al., 2002). PHB concentration was also converted from g/L into Cmol/L according to its monomer formula, C₆H₁₀O₅, with a molecular weight of 215.1 g/Cmol.

For culture enrichment, the average specific substrate...
uptake rate ($-r_5$ in Cmol Ac/(Cmol X-hr)), where Ac represents acetate) and PHB storage rate ($r_p$ in Cmol PHB/(Cmol X-hr)) were calculated using Eqs. (1) and (2):

$$-r_5 = (S_0 - S_e)/X_s/t$$  \hspace{1cm} (1)

$$r_p = (PHB_e - PHB_0)/X_s/t$$  \hspace{1cm} (2)

where, $S_0$ represents the substrate (acetate) concentration after filling and $S_e$ represents the residual substrate concentration. The $S_e$ value always approached 0 when the DO increased to a large extent; PHB$_e$, PHB$_0$ are the PHB concentrations after substrate filling and at the time substrate is exhausted; $X_s$ is the average active biomass concentration in the interval between the end of influent and the time substrate is exhausted; $t$ is the time interval. The substrate transformed into active biomass was calculated from the ammonia uptake on the basis of the average nitrogen content, 11.4% (W/W) of active biomass, assuming the general biomass formula outlined previously. Therefore, the yields of PHB ($Y_{PHB/S}$, Cmol PHB/Cmol Ac) and active biomass ($Y_{XS/S}$, Cmol X/Cmol Ac) as fraction of substrate consumed were calculated by Eqs. (3) and (4):

$$Y_{PHB/S} = (PHB_e - PHB_0)/(S_0 - S_e) = r_p/(-r_5)$$  \hspace{1cm} (3)

$$Y_{XS/S} = (NH_{4,0} - NH_{4,e})/11.4%/25.1/(S_0 - S_e)$$  \hspace{1cm} (4)

where, $NH_{4,0}$ and $NH_{4,e}$ are the ammonia concentration after filling and the residual ammonia concentration when the substrate is exhausted in the reactor.

For PHB accumulation assays, biomass ($X_s$) was evaluated as the average biomass value at the beginning and end of the reaction period. $-r_5$ was calculated by dividing the total substrate consumed in the whole reaction period with $X_s$ and the time used. $r_p$ was calculated by dividing the mass of synthesized PHB by $X_s$ by the time used. $Y_{PHB/S}$ was calculated by dividing the total PHB newly synthesized by total substrate consumed. The increment of biomass was calculated by subtracting the biomass value at the beginning of each batch test from that at the end of each batch. Therefore, $Y_{XS/S}$ was calculated by dividing the increment of biomass by total substrate consumed.

2 Results

2.1 Selection of a bulking sludge under intermittent feeding with low DO

The activated sludge used as the inoculation for both SBRs settled well and the sludge volume index (SVI) of the inoculation was less than 70. There was no aeration during the fill phase, thus little substrate was consumed in the filling phase and a high substrate gradient was ensured. Air was supplied to the mixed liquids immediately after filling and the DO was maintained at approximately 1 mg/L throughout the feast phase. The activated sludge in the two SBRs exhibited a good settleability at the beginning days with a rapid substrate uptake and PHB synthesis. The PHA synthesized in the two SBRs was found to mainly be composed of PHB (data not shown). However, the SVI for the activated sludge rose gradually and reached approximately 200 after 7 days operation, without sludge wash-out. No apparent difference was observed between the appearances of the sludge in the two SBRs, although SBR #1 was operated under anaerobic-aerobic conditions. Filamentous microorganisms were observed in both SBRs from day 4 to day 5 after the inoculation, and sludge wash-out began from around the day 15 in SBR #2 and the day 18 in SBR #1. Despite the occurrence of occasional sludge wash-out, the mixed liquor suspended solids (MLSS) could be maintained between 1.6 to 2.5 g/L through the whole operation term. To avoid intervention, the sludge washed out from the SBRs was not recovered.

Observations using the microscope showed the predominance of filamentous bacteria in the two SBRs after Sudan black staining, as shown in Fig. 2. Filamentous bacteria can extend much longer than their thickness, and curved and grew without branching free in the liquids or as bridges between the flocs, as described by Bengtsson et al. (2008). Large black PHB granules could be observed inside the filaments, especially when the sample was taken at the end of the feast phase. Although filamentous bacteria dominated the reactor, there were a large quantity of flocs, which may have promoted the sludge settling.

2.2 SBR performance with a bulking sludge

Figure 3 shows the variation in the operational parameters of the reaction regime in one typical cycle of the two SBRs on day 19 after the inoculation. In spite of the anaerobic phase in SBR #1, the substrate was quickly consumed when oxygen was supplied to the two SBRs. The end of the feast phase was clearly and directly observed by a rapid increase in DO when all acetate was consumed. The remainder of the reaction regime was regarded as the famine phase. Although different operational conditions were employed in the two SBRs, the performance of the two reactors showed similar characteristics in the aerobic phase: the acetate supplied at the beginning of the cycle was rapidly taken up and used both for cell growth and PHB accumulation. Ammonia and PHB concentrations slowly decreased in the famine period, which indicated that the previously stored PHB was used to continue cell growth in this phase. A C/N ratio which ensured the presence of ammonia throughout the full feast and famine cycle was used. The behavior observed was very similar to previously reported experiments with acetate-fed SBRs.

![Fig. 2](https://example.com/figure2.png)
After more than 3 months of SBR operation, the best PHB production capacity of the SBRs bulking sludge, which the PHB producing capacity gradually increased. Although under a bulking state, the microorganisms within the two SBRs did not lose PHB storage capacity. An immediate PHB storage response was observed when aeration was supplied with acetate present. The maximum specific substrate uptake rate and the storage rate were 0.4 Cmol Ac/(Cmol X-hr) and 0.18 Cmol PHB/(Cmol X-hr), respectively, in the enrichment phase. A total yield of 0.45 Cmol PHB/Cmol Ac was determined.

Some differences were found to occur between the two conditions within the SBRs during operation. Due to the addition of the anaerobic phase in SBR #1, the time period of the aerobic phase was reduced, which subsequently reduced the time for sludge proliferation. This was as indicated by the ammonia concentration in the effluent, which in SBR #1 was always higher than in SBR #2 (Fig. 2). Moreover, the addition of the anaerobic phase to the cycle increased the feast/famine (F/F) ratio in SBR #1. This was expected to lead directly to the decrease of the ΔPHB (PHB<sub>i</sub>-PHB<sub>0</sub>) content as there was not enough time for PHB consumption in a shorter famine phase. However, the sludge concentration of SBR #1 decreased within 2–3 days after 50 days operation without any sign of failure. This result was not anticipated because the anaerobic phase was considered to improve the sludge settleability and system stability. It was therefore difficult to draw conclusions on whether the existence of the anaerobic phase gave rise to this rapid failure of SBR #1 as, during the 50 days operation period, the sludge in SBR #1 showed a PHB accumulation performance similar to that in SBR #2. SBR #2 operated stably for more than 3 months during which the PHB producing capacity gradually increased.

2.3 Batch tests on SBRs bulking sludge

The PHB production capacity of the SBRs bulking sludge, enriched under low DO conditions, was investigated in fed-batch experiments without nitrogen addition designed to maximize the cellular PHB content. Figure 4 shows the maximum PHB content achieved in each batch test after more than 3 months of SBR operation. The best PHB batch accumulation performance, in terms of PHB sludge content (53%), polymer storage yield (0.77 Cmol PHB/Cmol Ac) and average specific PHB storage rate (1.36 Cmol PHB/(Cmol X-hr)) was achieved with the sludge from SBR #2 after 102 days operation and 10 hr aeration, as shown in Fig. 5.

The sludge settleability improved markedly after PHB accumulation in the batch experiments. The SVI of the bulking sludge after PHB accumulation decreased to less than 100, but was always more than 300 when the sludge was originally removed from the SBRs. This phenomenon represents a positive outcome for subsequent PHB extraction and may help in the separation of sludge from mixed liquids.

3 Discussion

3.1 Sludge bulking under dynamic feeding with low DO

It is well accepted that a steep substrate gradient occurs in highly dynamic activated sludge systems where biomass experiences alternately high and low substrate concentration (feast and famine). Examples include SBRs or contact tank systems, induced storage and regeneration phenomena, and this can be used for kinetic control of
bulking, since filamentous microorganisms were thought to have a limited storage ability compared with floc-forming bacteria (Majone et al., 1996; Krishna and van Loosdrecht, 1999). Based on this theory, a biomass selector has often been employed as an engineering tool to control sludge bulking in practice (Nakhla and Lugowski, 2003; Al-Mutairi, 2009). An alternating feast-famine condition stimulates a high readily biodegradable COD (RBCOD) uptake rate and those bacteria which can store substrate at a high rate in the selector do not need to adapt their growth rate to the low ambient dissolved substrate concentrations, thus have an advantage over the low food-to-microorganism (F/M) filaments. Although bio-selectors have successfully reduced bulking problems in many activated sludge systems, there are still regular reports of failure (Ekama et al., 1996). Martins et al. (2003b) extended the hypothesis to state that the reduced steep concentration gradients of oxygen and organic substrate in a sludge floc induces the selection of filamentous bacteria. They suggested increasing the DO alone, or adopting both low DO and low organic substrate concentrations as a strategy to improve settleability. However, due to high respiration rates under the feast phase, it is difficult to maintain a high DO concentration. In this study, the DO concentration reached up to approximately 8.0 mg/L in the famine phase, and decreased to approximately 1.0 mg/L in the feast phase. Thus, a bulking sludge was established in just two weeks. Although the bulking sludge had high SVI (more than 300), this sludge settled better in reactors than in test tubes and sludge wash-out was not severe enough to destroy the SBR operations. The filamentous bulking under high substrate gradient in our experiment was in accordance with the studies reported by Beccari et al. (1998) and Martins et al. (2003a), which suggested that a steep substrate gradient for kinetic control of filamentous bulking should not be considered as an absolute rule.

3.2 SBRs performance over the time of operation with bulking sludge

The performance of the two SBRs showed a similar trend in terms of the maximum PHB cell content and PHB content before the failure of SBR #1, as shown in Fig. 6. Both of the SBRs showed an unstable performance in the initial 30 days after the inoculation, with the PHB cell content and PHB content fluctuating considerably during operation. After 30 days, SBR #2 gradually reached a steady state while SBR #1 failed suddenly after 50 days operation. Though the reason for the failure could not be determined, the performance of SBR #1 in terms of both the maximum PHB cell content and ΔPHB was lower than that of SBR #2 throughout the operating period. This may be because the existence of the anaerobiosis resulted in an increase in the F/F ratio, which subsequently decreased the pressure favoring a PHB storage response by the culture. Beccari et al. (1998) and Dionisi et al. (2006) described the mechanism by which the F/F ratio to which a culture is exposed affects the selective pressure for PHB-storing organisms. The selective pressure is possibly related to the decrease of the internal growth limitation that induces a PHB storage response in the subsequent SBR cycle. If the famine phase is not long enough to ensure internal limitation, the culture will shift to growth when an external carbon source is supplied, and therefore accumulate less PHB.

The kinetic parameters of SBR #2 over the time of operation are shown in Table 1. SBR #1 is not shown due to the failure at day 50. Table 1 and Fig. 6 indicate that a stable state was gradually achieved after 40–50 days operation. The F/F ratio was maintained at about 0.23, which was in the range of 0.20–0.60, a range proposed by many researchers as the best regime for the enrichment of PHB-producing cultures (Doinisi et al., 2006; Albuquerque et al., 2010). The organic loading rate of 84 Cmmol/L per day in this study was close to 90 Cmmol/L per day (the value was calculated on the basis of reported profiles) which was proposed as the best loading rate in the ADF model (Albuquerque et al. 2010). However, the PHB content remaining at the end of each operation cycle in this study (7%–9%) was higher than the result reported by Albuquerque et al. (2010) of between 2% and 4%. A cycle of 12 hr with an aerobic reaction duration of 11 hr was applied in Albuquerque’s study, while a much shorter cycle of 8 and 6.5 hr anaerobiosis was used in our study. As discussed above, the length of this cycle under a fixed

![Fig. 5 Profiles of substrate, biomass and the maximum PHB content achieved in batch tests carried out under nitrogen starvation with the bulking sludge selected from SBR #2 at day 102 after inoculation.](image-url)
organic loading rate might have an influence on the length of the famine phase. If the famine phase is not long enough to ensure a complete consumption of PHB, it may lead to a decreased internal growth limitation. PHB storage performance would be restrained in the subsequent feast phase. This may explain the lower value of $-\tau_S$, $r_P$ and corresponding $Y_{\text{PHB}/X}$ resulting from this study.

As shown in Table 1, the specific substrate (acetate) uptake rate ($-\tau_S$) and corresponding PHB storage rate ($r_P$) stabilized at 0.4 Cmol Ac/(Cmol X-hr) and 0.18 Cmol PHB/(Cmol X-hr), respectively, resulting in a yield of 0.45 Cmol PHB/Cmol Ac. These dynamic parameters varied widely in different studies. The specific acetate uptake rate in this study (0.4 Cmol Ac/(Cmol X-hr)) was equal to that reported by Beun et al. (2000), but lower than that obtained by Dionisi et al. (2001) (0.5–0.9 Cmol/(Cmol-hr)). The PHB storage rate of 0.18 Cmol PHB/(Cmol X-hr) was less due to the high PHB content remaining at the end of the famine phase in this study. Despite this, the PHB storage rate found was comparable to that obtained by Dionisi et al. (2004), who reported a PHB storage rate of 0.17 Cmol/(Cmol-hr) 80 days after inoculation. The highest PHB storage rate of 2.1 Cmol/(Cmol-hr) and yield of 0.65 Cmol/(Cmol-hr) was reported by Johnson et al. (2009) in a SBR after more than 2 years operation after inoculation. There are few available studies which report performance comparable with these values. They suggested that a combination of factors led to this extraordinary performance, including long SBR cycles (12 hr), short SRT (1 day), carbon limiting conditions and a high operating temperature (30°C).

### 3.3 PHB accumulation under nitrogen starvation

The results of batch tests for PHB accumulation are shown in Figs. 4 and 5. There was no obvious improvement in the maximum PHB cell content between day 21 and day 58 after the inoculation, which indicated that the reactor was undergoing an unstable transitional state at that time. A slight sludge wash-out might be responsible for the slow effect of enrichment as no recovery was applied. The PHB storage capacity increase was proportional to the operation time of the SBR, which proved the hypotheses that the PHB storage response is affected by the acclimatization time (Albuquerque et al., 2010).

The duration of one of the batch tests is shown in Fig. 5, as a maximum PHB content was achieved with the sludge selected in SBR #2 when it was operated 102 days after the inoculation. The maximum PHB cell content of 53% of TSS was achieved eventually at 10.2 hr under complete nitrogen starvation. A storage yield of 0.77 Cmol PHB/Cmol Ac was achieved with an average specific PHB storage rate of 1.36 Cmol PHB/(Cmol X-hr).

These two important values were comparable to many studies reported (Table 2), however, the acclimatization time was only 102 days in this study. According to the literature reported, an acetate-fed SBR was mostly used to enrich PHB producing MMCs, however, the researchers only provided the best results they achieved after a long-term operation, most of which were no less than one year. There was limited study focused on the sludge settleability and SBR performance in the initial several months after the inoculation. The PHB storage performance obtained with MMCs with the feeding of either acetate or fermentation liquids of actual wastes in some recent studies are listed.

### Table 1 Kinetic parameters of SBR #2 over time of operation under ADF model

<table>
<thead>
<tr>
<th>Operation time after inoculation (day)</th>
<th>$Y_{\text{PHB}/X}$ (Cmol PHB/Cmol Ac)</th>
<th>$Y_{\text{XS}/X}$ (Cmol X/Cmol Ac)</th>
<th>$-\tau_S$ (Cmol Ac)/(Cmol X-hr))</th>
<th>$r_P$ (Cmol PHB/(Cmol X-hr))</th>
<th>$F/F$ (min/min)</th>
<th>TSS (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.4</td>
<td>0.17</td>
<td>0.24</td>
<td>0.094</td>
<td>0.176</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>0.38</td>
<td>0.17</td>
<td>0.41</td>
<td>0.14</td>
<td>0.206</td>
<td>2.1</td>
</tr>
<tr>
<td>19</td>
<td>0.26</td>
<td>0.22</td>
<td>0.31</td>
<td>0.080</td>
<td>0.194</td>
<td>2.6</td>
</tr>
<tr>
<td>27</td>
<td>0.26</td>
<td>0.16</td>
<td>0.47</td>
<td>0.093</td>
<td>0.321</td>
<td>1.4</td>
</tr>
<tr>
<td>47</td>
<td>0.28</td>
<td>0.26</td>
<td>0.43</td>
<td>0.16</td>
<td>0.294</td>
<td>1.6</td>
</tr>
<tr>
<td>58</td>
<td>0.46</td>
<td>0.23</td>
<td>0.39</td>
<td>0.18</td>
<td>0.254</td>
<td>2.1</td>
</tr>
<tr>
<td>79</td>
<td>0.45</td>
<td>0.21</td>
<td>0.40</td>
<td>0.18</td>
<td>0.233</td>
<td>2.3</td>
</tr>
<tr>
<td>101</td>
<td>0.46</td>
<td>0.22</td>
<td>0.41</td>
<td>0.19</td>
<td>0.226</td>
<td>2.4</td>
</tr>
</tbody>
</table>

### Table 2 Results of recent studies on PHA production by mixed cultures

<table>
<thead>
<tr>
<th>Substrate (process)</th>
<th>Max-PHA (% of TSS)</th>
<th>$Y_{\text{PHA}/X}$ (Cmol PHA/Cmol Ac)</th>
<th>Max-PHA (% of TSS)</th>
<th>$Y_{\text{PHA}/X}$ (Cmol PHA/Cmol Ac)</th>
<th>Accumulation time (hr)</th>
<th>Enrichment time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>53.0%</td>
<td>0.66</td>
<td>79%</td>
<td>0.60</td>
<td>6</td>
<td>2 years</td>
<td>Johnson et al., 2009</td>
</tr>
<tr>
<td>Acetate/lactic/ propionic VFAs</td>
<td>10.8% of VSS</td>
<td>0.32*</td>
<td>38% of VSS</td>
<td>0.38*</td>
<td>1.5</td>
<td>80 days</td>
<td>Dionisi et al., 2004</td>
</tr>
<tr>
<td>VFAs</td>
<td>25%</td>
<td>0.70</td>
<td>75%</td>
<td>0.81</td>
<td>NA</td>
<td>3 years</td>
<td>Albuquerque et al., 2010</td>
</tr>
<tr>
<td>Acetate (continuous process)</td>
<td>30%–40% of VSS</td>
<td>0.56</td>
<td>67.2%</td>
<td>0.70</td>
<td>7</td>
<td>18 months</td>
<td>Serafim et al., 2004</td>
</tr>
<tr>
<td>VFAs</td>
<td>11%</td>
<td>0.53</td>
<td>48%</td>
<td>0.28</td>
<td>24 hr with</td>
<td>250 days</td>
<td>Bengtsson et al., 2008</td>
</tr>
<tr>
<td>Acetate (CSTR)</td>
<td>NA</td>
<td>0.45</td>
<td>47% of TSS</td>
<td>0.69b</td>
<td>4–5 hr</td>
<td>NA</td>
<td>Beccari et al., 1998</td>
</tr>
<tr>
<td>Acetate</td>
<td>24%</td>
<td>0.45</td>
<td>53% of TSS</td>
<td>0.77</td>
<td>10.2 hr</td>
<td>102 days</td>
<td>This study</td>
</tr>
</tbody>
</table>

NA: not available; *data calculated according to figures and profiles presented in corresponding articles; b similar bulking sludge was used.
in Table 2. Comparable results were obtained with the bulking sludge, in both the culture enrichment phase and PHB production phase, to the results obtained by Dionisi et al. (2004), Serafim et al. (2004) and Bengtsson et al. (2008), a shorter acclimatization time and much lower DO concentration were applied in this study in SBR operation. Moreover, the increase of biomass was just 72 mg/L (2.87 Cmmol/L) (Fig. 5), which indicated a nearly zero growth under nitrogen starvation.

The most unexpected phenomenon was that the settleability of the bulking sludge used in the batch tests became better after several hours aeration under nitrogen starvation. There were no flocs present when the sludge was originally transferred from the SBRs to the batch tests, however, dense sludge flocs was formed after the PHB accumulation in all batch tests. The SVI value after PHB accumulation under nitrogen starvation was always less than 100 while the original SVI value was more than 300. This phenomenon was not reported by any of the other publications. The improvement of the sludge settleability might due to the high PHB content formed inside the microorganisms and the exact mechanism should be explored more in future study.

4 Conclusions

In this study, a stable bulking sludge was established in a SBR which operated with an ADF model under low DO conditions. The selected bulking sludge showed a high PHB storage rate and yield in the subsequent batch accumulation tests under nitrogen starvation that were comparable to those obtained by well-settled sludge according to reported studies. The bulking sludge settled quite well after the PHB accumulation experiment, which is favorable for the subsequent PHB extraction procedure and encourages the utilization of filamentous bacteria to accumulate PHA. If a bulking sludge with comparable PHA storage capacity could be selected steadily under low DO, it would enable an energy-saving PHA production method with MMCs. Another SBR operated in parallel under anaerobic-aerobic conditions failed after 50 days operation, which may suggest that conditions become unstable with the addition of an anaerobic phase.

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References


