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Optimization of polyhydroxybutyrate (PHB) production by excess activated sludge and microbial community analysis

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ABSTRACT

In this study, a high value-added and biodegradable thermoplastic, polyhydroxybutyrate (PHB), was produced by excess activated sludge. The effects of the nutritional condition, aeration mode, sodium acetate concentration and initial pH value on PHB accumulation in the activated sludge were investigated. The maximum PHB content and PHB yield of 67.0% (dry cell weight) and 0.740 gCOD gCOD^{-1} (COD: chemical oxygen demand), respectively, were attained by the sludge in the presence of 6.0 g L^{-1} sodium acetate, with an initial pH value of 7.0 and intermittent aeration. The analysis of the polymerase chain reaction (PCR)-denaturing gradient-gel-electrophoresis (DGGE) sequencing indicated that the microbial community of the sludge was significantly different during the process of PHB accumulation. Three PHB-accumulating microorganisms, which were affiliated with the *Thauera*, *Dechloromonas* and *Competibacter* lineages, were found in the excess activated sludge under different operating conditions for PHB accumulation.

1. Introduction

Sewage sludge, which contains pathogens and contaminants, is an inevitable product of biological wastewater treatment processes and can pollute the environment and affect human health if not carefully dealt with. The quantity of sewage sludge that is generated has been continuously increasing each year with the development of urbanization and industrialization. For example, in China, the sewage sludge produced was estimated to be 3.5 million tons (dry weight) in 2005 and will be up to 7.3 million tones (dry weight) in 2010 [1]. Currently, sewage sludge is disposed of by incineration, composting, land application, landfill, ocean dumping, and so forth [2–6]. However, all of these methods have severe limitations, such as the acute and immediate risk of pathogens, endotoxins and contaminants that come from land application sites. In addition, the cost of sewage sludge treatment and disposal can account for up to 60% of the total cost of running a wastewater treatment plant [7]. Hence, the treatment and disposal of sewage sludge has become one of the most critical environmental issues. To date, many studies that are related to the safe handling and disposal of sewage sludge have been reported, in which various cost-effective solutions have been proposed. These include chemical approaches (such as activated sludge ozonation [8] and alkaline thermal sludge hydrolysis [9]) and the production of biodegradable plastics (polyhydroxyalkanoates, PHAs) by the excess sludge's endogenous bacteria [10].

With increasing concern for the environment and high crude oil prices, biopolymers, which are sustainable and environmentally friendly materials, have attracted great interest due to their excellent biodegradability. For example, PHAs (hydroxyalkanoic acid polymers) are synthesized by a wide range of microorganisms to be intracellular carbon and energy reserve materials under nutrient-limiting conditions [11]. The first identified PHA was polyhydroxybutyrate (PHB) from *Bacillus megaterium* [12]. It has been regarded as an environmentally friendly and biocompatible plastic with promising applications in medicine, pharmacy and industry

Abbreviations: AS, acclimated sludge; COD, chemical oxygen demand; DO, dissolved oxygen; MLSS, mixed liquor suspended solids; *MPCC*, maximum PHB concentration; *MPCT*, maximum PHB content; NMDS, non-metric multidimensional scaling; OS, original sludge; PCR-DGGE, polymerase chain reaction-denaturing gradient-gel-electrophoresis; PHAs, polyhydroxyalkanoates; PHB, polyhydroxybutyrate; SBR, sequencing biological reactor; SRT, sludge retention time.

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[13]. To date, the synthesis of commercial PHB at the industrial scale has been based on the use of microbial isolates and pure substrates [14]. However, commercialization of these processes has been limited by their high production costs that are mainly attributed to the costs of the carbon source and polymer recovery [15]. It was reported that the production cost of PHB could be significantly reduced by replacing the pure substrates with activated sludge; the sludge is a well-known mixed culture that is able to accumulate PHB under unsteady-state conditions arising from an intermittent feeding regime and variation in the presence of an electron acceptor [16]. In recent years, studies that concern PHA production by activated sludge have focused on areas such as process modeling and control, bacterial storage mechanisms and polymer characterization [10,13,17–23]. Chua et al. [24] reported that a sludge, which acclimatized with acetate-supplemented municipal wastewater, could accumulate PHA up to 30% of the sludge's dry weight, whereas sludge that acclimatized with municipal wastewater only achieved a 20% PHA content. Chua and Yu [25] achieved a PHA content of 39% of the sludge's dry mass using a chemically processed wastewater that contained xenobiotic organics at a COD/N (COD: chemical oxygen demand) ratio of 140. Kasemsap and Wantawin [26] reported that the highest PHA content of 51% was obtained from a sludge with an 8% polyphosphate content at a pH of 8 using acetic acid as the sole substrate. Rodgers and Wu [27] stated that a 50% PHA content could be obtained using activated sludge that performed enhanced biological phosphorus removal under aerobic or anaerobic–aerobic conditions. Satoh et al. [17] reported that microaerophilic conditions could be used to increase the percent of PHA in activated sludge to achieve contents as high as 62% using sodium acetate as the primary organic substrate. These studies have revealed that the PHA yield in activated sludge is a function of operational parameters and nutritional conditions. However, reports on optimizing the process of PHA synthesis and the variation in microorganisms that correspond to PHA production in activated sludge are scarce to date, and the phylogenetic identities of PHA-accumulating microorganisms in activated sludge have not been well investigated. Therefore, a practical process for PHA production by activated sludge needs to be established to improve PHA production via process optimization and ultimately to control the yield of different PHAs by manipulating the metabolic pathways of these microorganisms.

Therefore, this work aimed to improve PHB production in a residual activated sludge by optimizing the operating conditions of the process. Furthermore, the relationship between PHB accumulation and the microbial community in the sludge was also elucidated. Herein, the process in this work consisted of two stages: the excess sludge was acclimated under aerobic conditions in the first stage, and subsequently, PHB was accumulated by the acclimated activated sludge. PHB accumulation was optimized under different nutritional conditions, aeration modes, carbon source concentrations and pH levels. Moreover, the microbial community structures were characterized by polymerase chain reaction (PCR)-denaturing gradient-gel-electrophoresis (DGGE) sequencing, which has been widely used in environmental microbial studies based on the nucleotide sequences of the species [28].

2. Materials and methods

2.1. Acclimation of excess sludge and culture media

The excess sludge that was used in this work was sourced from the secondary sedimentation tank of a municipal wastewater treatment plant in Xiamen, China. One sequencing biological reactor (SBR) with a working volume of 5 L was used to acclimate the excess sludge without pH control under aerobic conditions. The reactor was operated under a 24-h cycle that consisted of aerobic (23 h), settling (50 min) and withdrawing periods (10 min). The culture medium (3 L) was added at the beginning of the aerobic period, and the effluent was removed at the end of the settling period, which resulted in a hydraulic retention time of 40 h. At the end of each cycle and before settling, a defined volume of biomass was removed to keep the content of the mixed liquor suspended solids (MLSS) around 2 g L^{-1}. In the aerobic period, dissolved oxygen (DO) was supplied through a ceramic membrane disperser by an air compressor and controlled to around 80% of the saturation value. The performance and stability of the acclimation SBR was evaluated regularly before the commencement of the batch tests described below. In each cycle, liquid- and solid-phase samples were taken regularly at the end of the aerobic period to analyze the COD, pH, MLSS and DO using the methods described below. The COD removal was about 96%. The pH in the SBR process was uncontrolled and ranged from 8.9 to 9.5. After the acclimation period, the SBR achieved stability, and the sludge retention time (SRT) of 5 days was maintained. The acclimation was operated at room temperature in the range of 28–31 °C. The culture medium contained the following (mg L^{-1}): CH_3COONa, 1092; KCl, 234; NH_4Cl, 194; $KH_2PO_4 \cdot 3H_2O$, 150; $CaCl_2$, 54; $FeCl_3 \cdot 3H_2O$, 9.8; $MgSO_4 \cdot 7H_2O$, 306; peptone, 334; and yeast extract, 124.

2.2. PHB accumulation

The PHB yield was optimized by adjusting the operating parameters in small batch reactors (1-L glass bottles). The effects of nutrient conditions, aeration mode, carbon source concentration and initial pH level on PHB production were investigated. In each batch experiment, 1 L of acclimated sludge at the end of the aerobic period was transferred from the SBR into a smaller reactor. Thereafter, the sludge in the reactor was subjected to continuous aeration or intermittent aeration in the presence of sodium acetate, ammonium chloride or potassium hydrogen phosphate. Alternating aeration and stirring at the same intervals were carried out in the intermittent aeration. A DO of around 80% in the sludge was maintained by aeration through a ceramic membrane disperser with an air compressor. The stirring rate was maintained at 400 ± 5 r min^{-1} using a mechanical agitator. The initial pH value of the sludge was adjusted by adding dilute sulfuric acid or dilute sodium hydroxide. All of the operating conditions that were used in this work are summarized in Table 1. The reactors were operated at room temperature in the range of 28–31 °C.

2.3. Analytical techniques

The PHB in the sludge was determined by the procedures described by Chen and Li [29]. An 8-mL sludge sample was centrifuged at 10,625 × *g* for 5 min; the resulting pellet was lyophilized for 24 h and put into one vial. Then, 2 mL of acidified propanol (20% HCl) that contained benzoic acid as the internal standard and 2 mL of 1,2-dichloroethane were added to the vial. The vial was kept in a heating block at 100 °C for 6 h. After cooling, 1 mL of deionized water was added for extraction. The organic phase was collected and analyzed by gas chromatography (GC9560, China) using a flame ionization detector as well as a 30 m × 0.32 mm Chrompack SE-30 column. Nitrogen was used as the carrier gas. The injection port and the detector were maintained at 200 and 250 °C, respectively. The GC oven was programmed to begin at 100 °C (1 min) and then to increase at a rate of 10 °C min^{-1} to 170 °C, which was maintained for 2 min. The sample injection volume was 1.0 μL. The PHB calibration was performed with a poly(3-hydroxybutyric acid) standard of natural origin (Sigma–Aldrich Chem).

The cell dry weight was quantified as MLSS. Standard methods were used to determine the COD, MLSS, and pH values [30]. The

Table 1
Batch experiment conditions to accumulate PHB from the acclimated sludge.

Batch	Sodium acetate ($g\,L^{-1}$)	Initial pH	Final pH	Aeration mode	Nutrient condition
1	1.5	8.9	9.3	d	NP
2	1.5	8.9	9.3	d	N
3	1.5	8.9	9.3	d	P
4	1.5	8.9	9.3	d	–
5	5.5	8.8	9.4	a	–
6	5.5	8.8	9.4	b	–
7	5.5	8.9	9.4	c	–
8	5.5	8.8	9.4	d	–
9	4.5	8.9	9.4	b	–
10	6.0	8.9	9.5	b	–
11	6.5	8.9	9.5	b	–
12	6.0	5.0	5.6	b	–
13	6.0	5.5	8.1	b	–
14	6.0	6.0	8.7	b	–
15	6.0	7.0	8.9	b	–
16	6.0	8.0	9.3	b	–
Control	–	8.9	9.3	d	–

a: 15-min aeration and 15-min stirring; b: 30-min aeration and 30-min stirring; c: 60-min aeration and 60-min stirring; d: continuous aeration; P: $K_2HPO_4{\cdot}3H_2O$ 59 mg L^{-1}; N: NH_4Cl 153 mg L^{-1}; NP: NH_4Cl 153 mg L^{-1}, $K_2HPO_4{\cdot}3H_2O$ 59 mg L^{-1}.

DO was measured in the bulk with an oxygen electrode (InoLab Oxi Level 2, Germany). The PHB yield (Y_{PHB} in gCOD $gCOD^{-1}$) was calculated according to Chen and Li [29].

2.4. Sample collection and DNA extraction

The sludge samples that accumulated the highest PHB contents from each batch experiment were collected by centrifugation from the reactors and were stored at −20 °C before DNA extraction using the 3S DNA Isolation Kit V2.2 (Shenery Biocolor, China). Approximately 1 g of biomass was weighed into each DNA extraction tube. All of the extraction steps were carried out according to the manufacturer's protocol.

2.5. PCR-DGGE

The PCR was performed in a total volume of 50 μL. Each reaction contained 1 μL of the template, 5 μL of 10× PCR buffer (Mg^+), 2 μL of 10× dNTP, 1 μL of F338GC/R518 (25 pmol/50 μL, F338GC: 5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C ACT CCT ACG GGA GGC AGC AG-3′, R518: 5′-ATT ACC GCG GCT GCT GG-3′), 0.5 μL of Taq polymerase (Takara Ex Taq™, Takara Shuzo, Shiga, Japan), and 39.5 μL of double-distilled water (DDH_2O). According to Murray et al. [31], the thermal cycling program was as follows: initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 45 s, touchdown primer annealing from 63 to 53 °C for 45 s (annealing temperature decreased 0.5 °C for each cycle for the first 20 cycles to a final temperature of 53 °C), annealing at 53 °C for 45 s for the next 15 cycles, and then primer extension at 72 °C for 45 s. The reaction was run for a total of 35 cycles that were followed by a final extension for 10 min at 72 °C. The presence of PCR products was confirmed by analyzing 5 μL of the product on a 1.0% agarose gel, which was stained with ethidium bromide. DGGE was performed with a D-CODE Universal Mutation System (Bio-Rad, Hercules, CA, USA). Here, 20-μL PCR samples were separated on an 8% polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) with a denaturing gradient that ranged from 30% to 60%; a denaturation of 100% corresponds to 7-M urea and 40% formamide. All of the gels were run in 1× TAE buffer (40 mM Tris base, 20 mM sodium acetate, and 1 mM EDTA) at a constant voltage of 150 V at 60 °C. After 5 h of electrophoresis, the gel was stained with SYBR Green at 10,000× dilution in 1× TAE buffer for 30 min. The stained gel was viewed with an ultraviolet transilluminator, and an image was recorded with a CCD camera (Gel Logic 200 KODAK, USA).

2.6. Cloning and DNA sequencing

According to Ahn et al. [32] and Feris et al. [33], seven individual bands were recovered from the DGGE gels for further sequence analysis. Briefly, the bands were transferred to 1.5-mL microcentrifuge tubes and then macerated and mixed with 0.1 mL of Tris–HCl buffer (pH = 8.0). After incubation overnight at 4 °C, the eluted PCR products were again amplified using F338 and R518 primers without GC clamps. Then, they were purified using Qiaquick PCR Clean-up columns (Qiagen, Valencia, CA). The purified products were cloned into the plasmid vector pGEM-T Easy (Promega). Subsequently, the cloned 16S rDNA gene fragments were sequenced by Sangon (Shanghai, China).

2.7. DGGE patterns and phylogenetic analysis

Digitized DGGE images were analyzed with the Quantity One image analysis software (version 4.0. Bio-Rad USA) [34]; the presence or absence in the DGGE profile was scored with a one or zero, respectively, without considering the band intensity. The Jaccart similarity matrix was generated and analyzed with the nonmetric multidimensional scaling (NMDS) method in the PC-ORD software (Version 5.0, MjM Software, Gleneden, OR, USA) [35]. The sequences that were recovered from excised bands were compared with other sequences previously deposited in GenBank (http://www.ncbi.nlm.nih.gov) using the alignment basic local search tool (BLAST). The sequence alignment and phylogenetic analysis were performed using Mega 4.1 software [36]. The phylogenetic tree was constructed in Mega using the neighbor-joining method.

2.8. Nucleotide sequence accession numbers

The nucleotide sequences that were determined in this study have been deposited in the GenBank database under the accession numbers GQ202681–GQ202687.

3. Results and discussion

3.1. Optimization of PHB synthesis by the activated sludge

3.1.1. Effects of the nitrogen and phosphorous sources

In general, the PHB yield is significantly affected by the nutritional conditions [18,24]. It has been reported that high yield PHB accumulation could be achieved by limiting the nitrogen and/or

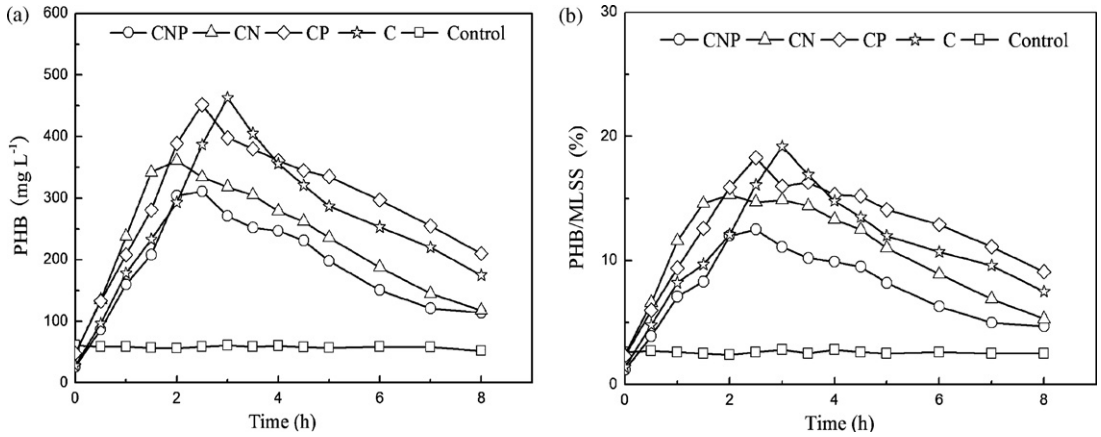

Fig. 1. Effects of nitrogen and phosphorous sources on PHB concentration (a) and content (b). (CNP (Batch 1): CH_3COONa 1500 mg L^{-1}, NH_4Cl 153 mg L^{-1}, $K_2HPO_4{\cdot}3H_2O$ 59 mg L^{-1}; CN (Batch 2): CH_3COONa 1500 mg L^{-1}, NH_4Cl 153 mg L^{-1}; CP (Batch 3): CH_3COONa 1500 mg L^{-1}, $K_2HPO_4{\cdot}3H_2O$ 59 mg L^{-1}; C (Batch 4): CH_3COONa 1500 mg L^{-1}; control (Batch control). The parameters of Batch 1, Batch 2, Batch 3, Batch 4 and Batch control are described in Table 1.)

phosphorous sources [18,21,37]. In this work, given 1.5 g L^{-1} of sodium acetate as the carbon source, variation in the PHB concentration and content over time in the presence/absence of nitrogen and/or phosphorous sources was investigated under continuous aeration. The results are presented in Fig. 1.

As the accumulation proceeded, the PHB concentration and content gradually increased and then decreased over time; however, they remained very low under the circumstances of batch fermentation. Fig. 1 also shows that the maximum PHB concentration (*MPCC*) and content (*MPCT*) in the absence of the nitrogen and/or phosphorous sources were higher than that in the presence of the nitrogen and phosphorous sources. The *MPCC* and *MPCT* values of 463 mg L^{-1} and 19.2%, respectively, were attained in the absence of the nitrogen and phosphorous sources. The above results suggested that in the absence of the nitrogen and/or phosphorous sources, substantial sodium acetate could be transformed into PHB by the microorganisms in the sludge, while some sodium acetate was consumed for microbial metabolism. This confirmed that the nitrogen and phosphorous sources were two important parameters in the production of PHB by the activated sludge.

3.1.2. Effect of the aeration mode

In the absence of the nitrogen and phosphorous sources, the effect of the sodium acetate concentration (1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g L^{-1}) on PHB accumulation was also studied under continuous aeration. The results showed that the highest PHB content of 46.5% was obtained with a sodium acetate concentration of 5.5 g L^{-1} (other data not shown). Thus, the sodium acetate concentration of 5.5 g L^{-1} was used subsequently. Extensive research has been directed towards the generation of PHB under aerobic, anoxic and anaerobic–aerobic conditions [27,38,39]. Therefore, aeration modes are also thought to have a significant effect on the PHB yield.

Fig. 2 displays the variation in the PHB concentration and content over time under different aeration modes. Under three intermittent aeration modes, the DO concentrations increased from around 8% to 80% in 15 min and remained constant prior to the stirring stage in each cycle. In contrast, the DO concentrations decreased immediately until they reached a minimum, i.e., 8%, during the stirring stage. As a result, the microorganisms in the sludge were under facultative anaerobic conditions. As shown in Fig. 2, continuous aeration led to the fastest PHB accumulation during the 7 h of cultivation. This was likely due to the continuous and high concentration of oxygen that was dissolved in the liquid. Fig. 2a and b shows that the *MPCC* and *MPCT* under intermittent aeration were much higher than those under continuous aeration. For example, in comparison with continuous aeration, the *MPCT* value that resulted from intermittent aeration, 30-min aeration and 30-min stirring in each cycle increased by 9% to reach 54.1% of the dry cell mass (Fig. 2b). However, it took much more time for the *MPCC* and *MPCT* to reach their maxima under intermittent aeration than in continuous aeration. After reaching the *MPCC* and *MPCT*, the PHB content and concentration gradually decreased over time. The reason for

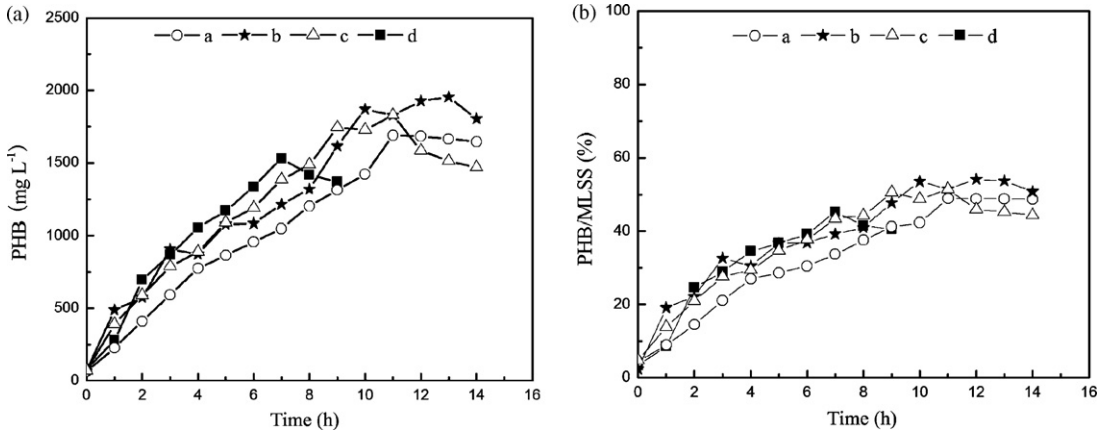

Fig. 2. Effects of aeration modes and accumulation time on PHB concentration (a) and content (b). (a (Batch 5): 15-min aeration and 15-min stirring; b (Batch 6): 30-min aeration and 30-min stirring; c (Batch 7): 60-min aeration and 60-min stirring; d (Batch 8): continuous aeration. The parameters of Batch 5, Batch 6, Batch 7 and Batch 8 are described in Table 1.)

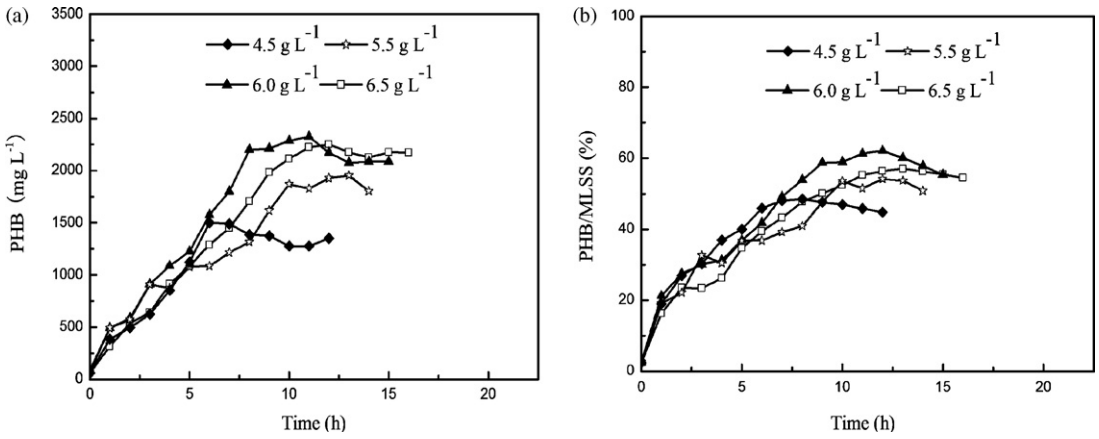

Fig. 3. Effects of carbon source concentration and accumulation time on PHB concentration (a) and content (b). (4.5 g L^{-1} (Batch 9); 5.5 g L^{-1} (Batch 6); 6.0 g L^{-1} (Batch 10); 6.5 g L^{-1} (Batch 11). The parameters of Batch 6, Batch 9, Batch 10 and Batch 11 are described in Table 1.)

this could be the consumption of intracellular PHB as a source of carbon and energy after sodium acetate depletion.

It has been proposed that the stress resistance and survival of sludge microorganisms in an unbalanced environment could be enhanced via PHB production [40]. Herein, the above results also showed that PHB was easily accumulated by microorganisms under such unbalanced growth conditions that combined intermittent aeration and nutrient limitation. Therefore, the intermittent aeration program (30 min of aeration and 30 min of stirring in each cycle) was used subsequently in this work.

3.1.3. Effect of carbon source concentration

To elucidate the relationship between the carbon source concentration and PHB production with a 30-min aeration and a subsequent 30-min stirring in each cycle, the effect of the sodium acetate concentration (varied from 4.5 to 6.5 g L^{-1}) on the PHB concentration and content was investigated. The changes in the PHB concentration and content over time are presented in Fig. 3. As shown here, the *MPCC* and *MPCT* increased as the dose of sodium acetate increased from 4.5 to 6.0 g L^{-1} but decreased at the dose of 6.5 g L^{-1}. This indicated that the PHB production capability could be considerably improved by increasing the dose of sodium acetate. However, the inhibition of PHB production could have resulted from overfeeding sodium acetate, as reported by Serafim et al. [10]. From Fig. 3, the PHB was immediately accumulated after the addition of sodium acetate, and the intercellular PHB content in the sludge increased rapidly in 1 h. Moreover, the PHB content was linearly related to the sodium acetate consumption during the "feast" period (data not shown). The above results revealed that cell growth and PHB accumulation occurred simultaneously in the "feast" phase. It can be seen in Fig. 3a that the PHB concentrations were little affected by variations in the sodium acetate dose, which ranged from 4.5 to 6.5 g L^{-1} before 5 h had passed. However, the PHB concentrations were significantly affected by the dose of sodium acetate in the latter stage of accumulation. Furthermore, the maximum concentration of PHB that was produced in the presence of 6.0 g L^{-1} of sodium acetate was 2325 mg L^{-1}, which was 1.55 times higher than that in the presence of 4.5 g L^{-1} of sodium acetate. Fig. 3b shows that it took 13, 12, 12 and 8 h to reach the *MPCT* values of 57.0%, 62.1%, 54.1% and 48.5% in the presence of sodium acetate concentrations of 6.5, 6.0, 5.5 and 4.5 g L^{-1}, respectively. Thus, the optimized sodium acetate concentration was 6.0 g L^{-1}.

3.1.4. Effect of pH

A high PHB content can be obtained when the pH value of the sludge is adjusted to high pH values (8.0 or 9.0) [18,24]. Therefore, in addition to nutritional conditions and the aeration mode, the pH value of the PHB-accumulating environment might affect the PHB yield. Five pH values were tested to investigate the effect of the pH on PHB accumulation. Fig. 4 indicates the PHB concentrations and contents that were attained at various pH values in batch experiments. As can be seen in Fig. 4, the *MPCC* and *MPCT* values increased as the initial pH value increased from 5.0 to 7.0, whereas they decreased by switching the initial pH value from 7.0 to

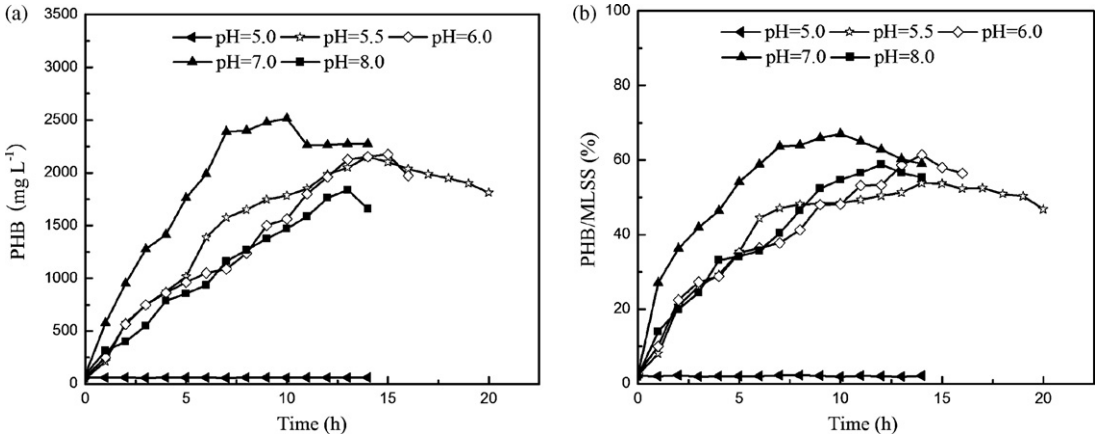

Fig. 4. Effects of pH values and accumulation time on PHB concentration (a) and content (b). (pH = 5.0 (Batch 12); pH = 5.5 (Batch 13); pH = 6.0 (Batch 14); pH = 7.0 (Batch 15); pH = 8.0 (Batch 16). The parameters of Batch 12, Batch 13, Batch 14, Batch 15 and Batch 16 are described in Table 1.)

Table 2
Comparison of PHA production performances of pure culture microorganisms and activated sludge.

Microorganisms	Carbon substrate	Reaction time (h)	Cell dry weight ($g\,L^{-1}$)	PHA content (%)	Reference
Alkaligenes latus	Sucrose	18	143	50	[42]
Alcaligenes eutrophus	Glucose	50	164	76	[43]
Recombinant*Alcaligenes eutrophus*	Valerate	72	2.0	52	[44]
Recombinant*Alcaligenes eutrophus*	Nonanoate	72	3.1	78	[44]
Recombinant *Ralstonia eutropha*	Fructose	72	1.8	65	[45]
Ralstonia eutropha	Fermented food wastes	73	22.7	73	[46]
Rhodobacter sphaeroides	Glutamate–acetate	60	9.18	95.4	[47]
Activated sludge	Sodium acetate	10	3.7	67	This work

8.0. The order of the *MPCC* values that was associated with the initial pH values was consistent with the following sequence: $MPCC_{pH=7.0} > MPCC_{pH=6.0} > MPCC_{pH=5.5} > MPCC_{pH=8.0} > MPCC_{pH=5.0}$ (Fig. 4a). As shown in Fig. 4b, a PHB content of only about 2% accumulated at an initial pH value of 5.0, and the effect of the pH value on the *MPCT* was slightly different from that on the *MPCC*. The order of the *MPCT* values that was associated with the tested pH values followed the following sequence: $MPCT_{pH=7.0} > MPCT_{pH=6.0} > MPCT_{pH=8.0} > MPCT_{pH=5.5} > MPCT_{pH=5.0}$ (Fig. 4b).The above results confirmed that the pH value is also an important parameter for PHB production, and the PHB production could be significantly improved by controlling the pH. Therefore, we concluded that appropriate adjustment of the initial pH value caused a change in the growth condition of the microorganisms and led to an increase in the polymer storage yield. Chua et al. [24] reported that a low PHB content was obtained at a pH value of 6.0, and the PHB content at a pH value of 8.0 was much higher than that at 7.0. Herein, however, the lowest PHB content (around 2%) was obtained at an initial pH value of 5.0, which is likely due to the presence of undissociated acetic acid [41]. Furthermore, the PHB content at an initial pH value of 5.5 was much higher than that at 5.0. Fleit [41] indicated that at an unsteady state, the undissociated acetic acid could rapidly diffuse into bacterial cells through the cell membrane, which then dissociated and imposed a proton load on the cytosol and subsequently decreased the intracellular pH. As the extracellular pH was adjusted to 5.0, the intracellular pH was likely less than 4.0, which could be detrimental to PHB production. Therefore, the PHB accumulation behavior seemed very sensitive when the initial pH value was increased from 5.0 to 5.5. The pronounced difference between this work and the previous study by Chua et al. [24] might be attributed to the process operating conditions and the characteristics of the sludge.

Moreover, it took 14, 14, 10 and 12 h to attain the maximum PHB contents at the initial pH values of 5.5, 6.0, 7.0 and 8.0, respectively. At an initial pH value of 7.0, the *MPCT*, the PHB production rate and the PHB yield were 67.0%, 125 mg $gSS^{-1}\,h^{-1}$ and 0.740 gCOD $gCOD^{-1}$, respectively. Thus, an initial pH value of 7.0 was selected for subsequent trials.

3.1.5. Assessment of the PHB accumulation

Based on the above results, the PHB content and PHB production rate that were achieved by the activated sludge in this work were compared with those obtained by some pure culture processes (Table 2). The PHB production rate of 125 mg $gSS^{-1}\,h^{-1}$ (or 70 mgC $gSS^{-1}\,h^{-1}$) under the intermittent aeration mode was higher than that of both the 56 mg $gSS^{-1}\,h^{-1}$ reported for glycogen-accumulating organisms [48] and the 58 mg $gSS^{-1}\,h^{-1}$ by activated sludge, which performed enhanced biological phosphorus removal [27]. Furthermore, the PHB production rate that was achieved by the activated sludge in this work was higher than those by pure cultures like *Alcaligenes latus* (31 mgC $gSS^{-1}\,h^{-1}$) [42] or recombinant *Escherichia coli* (42 mgC $gSS^{-1}\,h^{-1}$) [49]. Hence, it is very promising that the production of PHB by the activated sludge appears to have a great potential as a competitive alternative to pure culture practices.

3.2. DGGE band identification and phylogenetic analysis

The PCR-DGGE procedure has been demonstrated to be sensitive for analyzing microbial community compositions in sludge [50]. As shown in Fig. 5, the bacterial community compositions among unacclimated sludge, acclimated sludge and PHB-accumulating sludge were determined. The results indicated that in contrast to the unacclimated sludge sample, the number of bands from the acclimated sludge samples before or after PHB accumulation increased significantly. Seven DGGE bands, which were designated as SP1–SP7, were visually detected and excised from the gel for subsequent sequencing analyses (Fig. 5). The closest matches of the obtained sequences to known species were determined by comparing the sequences with those in the National Center for Biotechnology Information (NCBI) database (Table 3). Furthermore, a phylogenetic analysis of the sequences that were recovered from the DGGE gels (Fig. 6) was performed to determine if there were differences in the distribution of phylogenetic groups among the PHB-accumulating organisms.

As shown in Fig. 5 and Table 3, among these bands, SP1 was closely related (99% similarity) to an uncultured bacterium that was isolated from a reactor by treating it with organic household waste [51]. The presence of the SP4 band, which resulted from the acclimation of the sludge, showed 99% similarity to an uncultured prokaryote from a lake. The SP1 and SP4 bands corresponded to the *γ-proteobacterium Competibacter* (Fig. 6). In the past decade, PHB

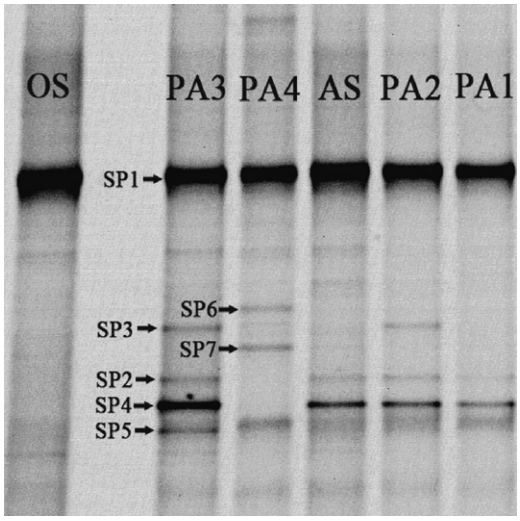

Fig. 5. DGGE separation patterns of PCR-amplified 16S rDNA fragments from sludge samples. (OS: original sludge, AS: acclimated sludge, PA1, PA2, PA3 and PA4, which had attained the maximal PHB content, were from Batch 1, Batch 6, Batch 10 and Batch 15, respectively. The parameters of Batch 1, Batch 6, Batch 10 and Batch 15 are described in Table 1.)

Table 3
Phylogenetic affiliation of the 16S rRNA gene sequence obtained from DGGE bands.

Band	Closely related sequence	GenBank accession no.	Similarity (%)
SP1	*Uncultured bacterium clone OT90-1_org* (FJ945735)	GQ202681	99
SP2	*Uncultured beta proteobacterium* (AB276369)	GQ202682	90
	Uncultured bacterium (AB292428)		90
SP3	*Uncultured Thauera* sp. clone REV_R1PII_10B (FJ933477)	GQ202683	89
SP4	*Uncultured prokaryote clone Se1-4* (GU208342)	GQ202684	99
SP5	*Uncultured bacterium* (FJ444783)	GQ202685	91
SP6	*Klebsiella pneumoniae* strain HPC758 (AY838331)	GQ202686	80
SP7	*Uncultured Thauera* sp. clone PI5B (FJ439023)	GQ202687	83
	Thauera aminoaromatica strain NS2 (FJ609688)		82

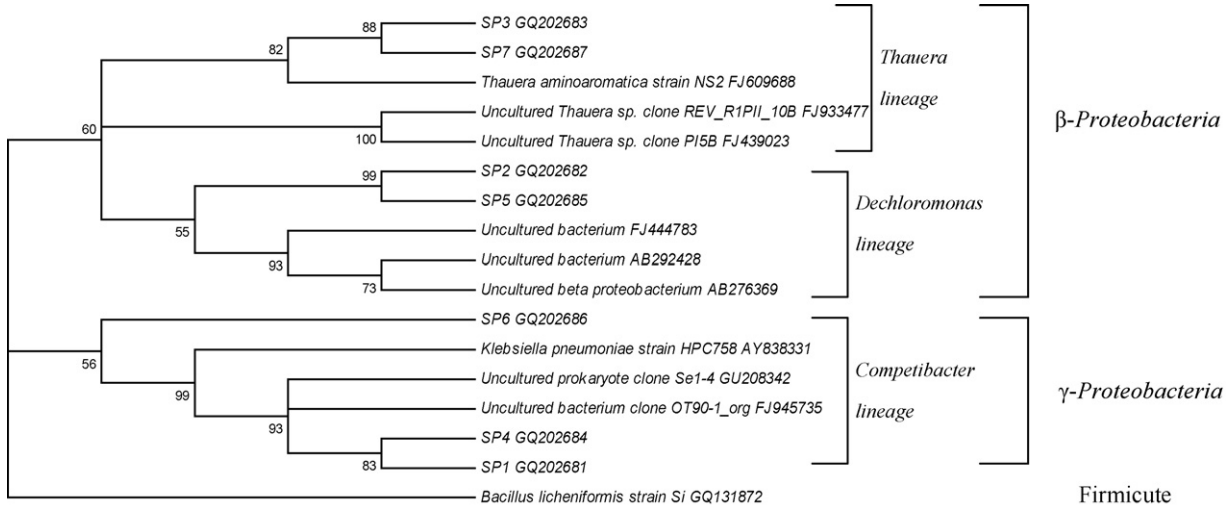

Fig. 6. Phylogenetic tree of partial 16S rRNA gene sequence amplified with the F338 and R518. Bootstrap support values with 1000 replicates are given along the branches.

accumulation by the *Competibacter* lineage has been extensively studied [52,53]. Therefore, the microorganisms corresponding to SP1 and SP4 were assumed to play important roles in PHB production by the sludge. In the AS, PA1, PA2 and PA3 (Fig. 5), SP2 showed 90% similarity to an uncultured bacterium and an uncultured beta *proteobacterium*. The former bacterium has been found in full-scale activated sludge processes and has been used to clarify the phylogenetic affiliations of polyhydroxyalkanoate (PHA)-accumulating microorganisms [50]. The latter was a perfect match for the PAOmix probes [54], and they corresponded to the *Dechloromonas* lineage, which has been reported in PHB production [54]. This revealed that the presence of the SP2 band, which was found in the acclimated sludge, was likely to be responsible for PHB accumulation. The SP5 band was also the result of sludge acclimation and showed 91% similarity to an uncultured bacterium from a PHA-producing mixed microbial culture (unpublished data). This fact indicated that the microorganism, which corresponds to SP5, might be responsible for PHB accumulation. Hence, the microorganisms corresponding to SP2 and SP5 could be identified as belonging to the *Dechloromonas* lineage (Fig. 6). The SP3 band that is found in PA2, PA3 and PA4 (which resulted from the intermittent aeration condition) showed 89% similarity to an uncultured *Thauera* sp. found in a membrane bioreactor and in conventional activated sludge processes from petroleum refineries (unpublished data). Therefore, the microbial community might have changed with facultative bacteria dominating under the stirring phase in this study. The SP7 band, which was only found in PA4, showed similarities of 83% and 82% to an uncultured *Thauera* sp. and *Thauera aminoaromatica*, respectively. It has been reported that the *Thauera* strains have the ability to accumulate PHA [50]. Therefore, the microorganisms that corresponded to SP3 and SP7 could be identified as belonging to the *Thauera* lineage (Fig. 6). SP6, which was only found in PA4, could be identified with the *Competibacter* lineage (Fig. 6) and showed 80% similarity to a *Klebsiella pneumoniae* strain that was isolated from a common effluent treatment plant [55]. Correspondingly, a PHB-producing bacterium that was isolated from activated sludge was identified as *K. pneumoniae* and employed to produce bioplastics using synthetic media, malt and soy wastes [56]. Thus, the enrichment of the microorganism that corresponded to SP6 resulted from adjusting the pH during PHB accumulation; this bacterium may participate in PHB accumulation.

Based on the above analysis, the recovered sequences were most closely related to three bacterial groups in the *β-proteobacteria* and *γ-proteobacteria*: *Thauera*, *Dechloromonas* and *Competibacter*. The DGGE results showed that different process operating conditions led to different microbial community structures, which might be due to the different metabolic pathways of the microorganisms under different operating conditions. The results of cloning and DNA sequencing confirmed that there were some known, specific PHB-accumulating microorganisms present during PHB accumulation.

3.3. Changes in microbial community structure

Nonmetric multidimensional scaling (NMDS) has been used to visualize changes in the microbial community's structure [34,35]. The NMDS map that was obtained by analyzing the DGGE profiles showed an uneven variation in the community structure (Fig. 7a). The profile of the microbial community's structure at AS was more closely related to the profiles at PA1, PA2, PA3 and PA4 than to that at OS. Furthermore, the profile of the microbial community's structure at PA2 was closer to that at PA3 than those at PA1 and

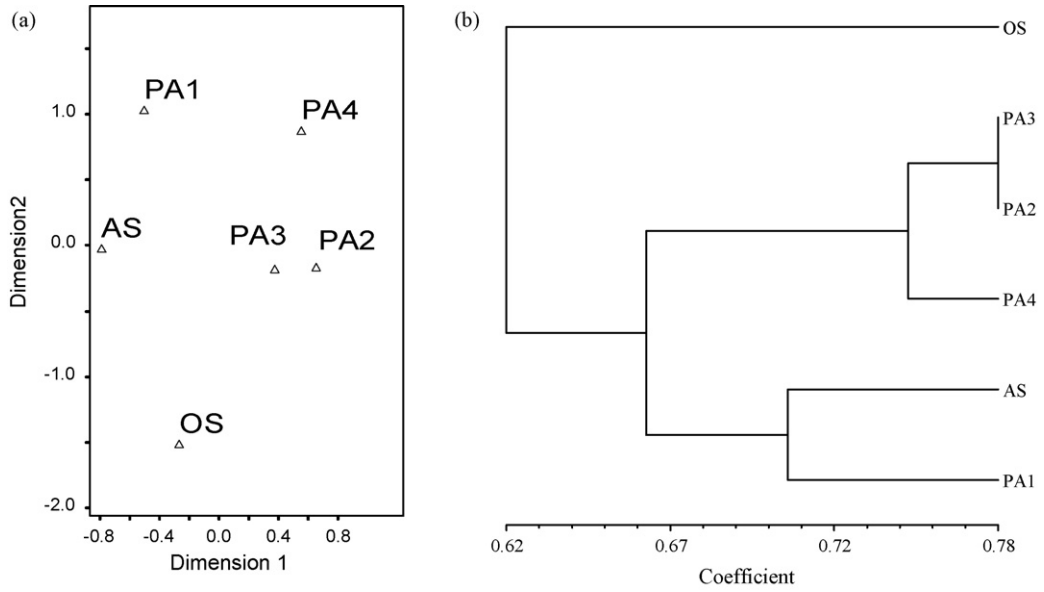

Fig. 7. NMDS map (a) and clustering tree (b) from the analysis of DGGE profile based on the 16S rRNA gene.

PA4. In addition, the community-structure profiles of acclimated sludge and PHB accumulated sludge were similar and located at the top of the map, while the profile of the original sludge stands at the bottom of the map. These trends were also supported by the clustering analysis that is shown in Fig. 7b.

The NMDS result reflected the appearances and disappearances of some of the DGGE bands (Figs. 5 and 7). Some specific bands were detected in the lanes at different operating conditions with PHB accumulation by the sludge. For example, SP6 and SP7 were only apparent in the lane from PA4, whereas SP3 was detected in the lanes from PA2, PA3, and PA4. The above results confirmed that the microbial community's structure has a close connection with the process operating conditions in PHB accumulation from activated sludge. Furthermore, it can be deduced from the DGGE bands that the microorganisms exhibited a strong PHB-accumulating ability.

4. Conclusions

In this work, optimization of the PHB production process by excess activated sludge was investigated, and the microbial community was analyzed. The maximum PHB content, which accounts for 67.0% (PHB/MLSS) of the dry cell mass, was attained by the sludge in the presence of 6.0 g L^{-1} sodium acetate with an initial pH value of 7.0 and intermittent aeration. The microbial community structure was closely associated with the process operating conditions for PHB accumulation. Three PHB-accumulating microorganisms (which were affiliated with the *Thauera*, *Dechloromonas* and *Competibacter* lineages) were found in the activated sludge samples.

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References

[1] http://www.sdpc.gov.cn/fzgh/ghwb/115zxgh/P020080407602927680747.pdf.
[2] M. Khwairakpam, R. Bhargava, Vermitechnology for sewage sludge recycling, J. Hazard. Mater. 161 (2009) 948–954.
[3] K. Hara, T. Mino, Environmental assessment of sewage sludge recycling options and treatment processes in Tokyo, Waste Manage. 28 (2008) 2645–2652.
[4] M. Scholz, Wetland Systems to Control Urban Runoff, Elsevier Science Publishers, Amsterdam, 2006, pp. 163–174.
[5] S. Renou, J.G. Givaudan, S. Poulain, F. Dirassouyan, P. Moulin, Landfill leachate treatment: review and opportunity, J. Hazard. Mater. 150 (2008) 468–493.
[6] X. Wang, T. Chen, Y. Ge, Y. Jia, Studies on land application of sewage sludge and its limiting factors, J. Hazard. Mater. 160 (2008) 554–558.
[7] Y. Wei, R.T. Van Houten, A.R. Borger, D.H. Eikelboom, Y. Fan, Minimization of excess sludge production for biological wastewater treatment, Water Res. 18 (2003) 4453–4467.
[8] S.-B. He, G. Xue, B.-Z. Wang, Activated sludge ozonation to reduce sludge production in membrane bioreactor (MBR), J. Hazard. Mater. 135 (2006) 406–411.
[9] E. Neyens, J. Baeyens, C. Creemers, Alkaline thermal sludge hydrolysis, J. Hazard. Mater. 97 (2003) 295–314.
[10] L.S. Serafim, P.C. Lemos, R. Oliveira, M.A.M. Reis, Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions, Biotechnol. Bioeng. 87 (2004) 145–160.
[11] A.J. Anderson, E.A. Dawes, Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates, Microbiol. Rev. 54 (1990) 450–472.
[12] M. Lemoigne, Produits de déshydration et de polymerization de l'acide β-oxybutyrique, Bull. Soc. Chim. Biol. 8 (1926) 770–782.
[13] S. Philip, T. Keshavarz, I. Roy, Polyhydroxyalkanoates: biodegradable polymers with a range of applications, J. Chem. Technol. Biotechnol. 82 (2007) 233–247.
[14] L.R. Castilho, D.A. Mitchell, D.M.G. Freire, Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation, Bioresour. Technol. 100 (2009) 5996–6009.
[15] J. Choi, S.Y. Lee, Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation, Bioprocess Biosyst. Eng. 17 (1997) 335–342.
[16] H. Salehizadeh, M.C.M. Van Loosdrecht, Production of polydroxyalkanoates by mixed culture: recent trends and biotechnological importance, Biotechnol. Adv. 22 (2004) 261–279.
[17] H. Satoh, Y. Iwamoto, T. Mino, T. Matsuo, Activated sludge as a possible source of biodegradable plastic, Water Sci. Technol. 38 (1998) 103–108.
[18] K. Johnson, Y. Jiang, R. Kleerebezem, G. Muyzer, M.C.M. van Loosdrecht, Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity, Biomacromolecules 10 (2009) 670–676.
[19] M. Beccari, L. Bertin, D. Dionisi, F. Fava, S. Lampis, M. Majone, F. Valentino, G. Vallini, M. Villano, Exploiting olive oil mill effluents as a renewable resource for production of biodegradable polymers through a combined anaerobic–aerobic process, J. Chem. Technol. Biotechnol. 84 (2009) 901–908.
[20] M.G.E. Albuquerque, M. Eiroa, C. Torres, B.R. Nunes, M.A.M. Reis, Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses, J. Biotechnol. 130 (2007) 411–421.
[21] K. Bernat, I. Wojnowska-Baryła, A. Dobrzyńska, Denitrification with endogenous carbon source at low C/N and its effect on P(3HB) accumulation, Bioresour. Technol. 99 (2008) 2410–2418.

[22] P.C. Lemos, Y. Dai, Z. Yuan, J. Keller, H. Santos, M.A.M. Reis, Elucidation of metabolic pathways in glycogen-accumulating organisms with in vivo ^{13}C nuclear magnetic resonance, Environ. Microbiol. 9 (2007) 2694–2706.
[23] Y. Dai, Z.G. Yuan, K. Jack, J. Keller, Production of targeted poly(3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source, J. Biotechnol. 129 (2007) 489–497.
[24] A.S.M. Chua, H. Takabatake, H. Satoh, T. Mino, Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent, Water Res. 37 (2003) 3602–3611.
[25] H. Chua, P.H.F. Yu, Production of biodegradable plastics from chemical wastewater—a novel method to reduce excess activated sludge generated from industrial wastewater treatment, Water Sci. Technol. 39 (1999) 273–280.
[26] C. Kasemsap, C. Wantawin, Batch production of polyhydroxyalkanoate by low-polyphosphate-content activated sludge at varying pH, Bioresour. Technol. 98 (2007) 1020–1027.
[27] M. Rodgers, G. Wu, Production of polyhydroxybutyrate by activated sludge performing enhanced biological phosphorus removal, Bioresour. Technol. 101 (2010) 1049–1053.
[28] Y. Liu, T. Zhang, H.H.P. Fang, Microbial community analysis and performance of a phosphate-removing activated sludge, Bioresour. Technol. 96 (2005) 1205–1214.
[29] H. Chen, X. Li, Effect of static magnetic field on synthesis of polyhydroxyalkanoates from different short-chain fatty acids by activated sludge, Bioresour. Technol. 99 (2008) 5538–5544.
[30] APHA, Stand Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, USA, 1995.
[31] A.E. Murray, J.T. Hollibaugh, C. Orrego, Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments, Appl. Environ. Microbiol. 62 (1996) 2676–2680.
[32] J. Ahn, T. Daidou, S. Tsuneda, A. Hirata, Characterization of denitrifying phosphate-accumulating organisms cultivated under different electron acceptor conditions using polymerase chain reaction-denaturing gradient gel electrophoresis assay, Water Res. 36 (2002) 403–412.
[33] K. Feris, P. Ramsey, C. Frazar, J.N. Moore, J.E. Gannon, W.E. Holbert, Differences in hyporheic-zone microbial community structure along a heavy-metal contamination gradient, Appl. Environ. Microbiol. 69 (2003) 5563–5573.
[34] Y.P. Wang, Q.B. Li, J.Y. Shi, Q. Lin, X.C. Chen, W.X. Wu, Y.X. Chen, Assessment of microbial activity and bacterial community composition in the rhizosphere of a copper accumulator and a non-accumulator, Soil Biol. Biochem. 40 (2008) 1167–1177.
[35] C. Lee, J. Kim, H. Do, S. Hwang, Monitoring thiocyanate-degrading microbial community in relation to changes in process performance in mixed culture systems near washout, Water Res. 42 (2008) 1254–1262.
[36] S. Moormann, F. Seehusen, D. Reckling, J. Kilwinski, C. Puff, M. Elhensheri, P. Wohlsein, M. Peters, Systemic adenovirus infection in bearded dragons (*Pogona vitticeps*): histological, ultrastructural and molecular findings, J. Comp. Pathol. 141 (2009) 78–83.
[37] M.F. Md Din, Z. Ujang, M.C.M. van Loosdrecht, A. Ahmad, M.F. Sairan, Optimization of nitrogen and phosphorus limitation for better biodegradable plastic production and organic removal using single fed-batch mixed cultures and renewable resources, Water Sci. Technol. 53 (2006) 15–20.
[38] J.J. Beun, F. Paletta, M.C.M. van Loosdrecht, J.J. Heijnen, Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures, Biotechnol. Bioeng. 67 (2000) 379–389.
[39] A.S. Çığgın, Ö. Karahan, D. Orhon, Effect of high nitrate concentration on PHB storage in sequencing batch reactor under anoxic conditions, Bioresour. Technol. 100 (2009) 1376–1382.
[40] J.A. Ruiz, N.I. López, R.O. Fernández, B.S. Méndez, Polyhydroxyalkanoate degradation is associated with nucleotide accumulation and enhances stress resistance and survival of *Pseudomonas oleovorans* in natural water microcosms, Appl. Environ. Microbiol. 67 (2001) 225–230.
[41] E. Fleit, Intracellular pH regulation in biological excess phosphorous removal systems, Water Res. 29 (1995) 1787–1792.
[42] T. Yamane, M. Fukunage, Y.W. Lee, Increased PHB productivity by high-cell-density fed-batch culture of *Alcaligenes latus*, a growth-associated PHB producer, Biotechnol. Bioeng. 50 (1996) 197–202.
[43] B.S. Kim, S.C. Lee, S.Y. Lee, H.N. Chang, Y.K. Chang, S.I. Woo, Production of poly(3-hydroxybutyric acid) by fed-batch culture of *Alcaligenes eutrophus* with glucose concentration control, Biotechnol. Bioeng. 43 (1994) 892–898.
[44] T. Fukui, T. Kichise, Y. Yoshida, Y. Doi, Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyheptanoate) terpolymers by recombinant *Alcaligenes eutrophus*, Biotechnol. Lett. 19 (1997) 1093–1097.
[45] S. Taguchi, H. Nakamura, T. Kichise, T. Tsuge, I. Yamato, Y. Doi, Production of polyhydroxyalkanoate (PHA) from renewable carbon sources in recombinant *Ralstonia eutropha* using mutants of original PHA synthase, Biochem. Eng. J. 3725 (2003) 1–7.
[46] D.G. Cheng, Y. Jian, C. Jian, Coupling system for food wastes anaerobic digestion and polyhydroxyalkanoates production with *Ralstonia eutropha*, Chin. J. Process Eng. 2 (2002) 161–167.
[47] K. Sangkharak, P. Prasertsan, Optimization of polyhydroxybutyrate production from a wild type and two mutant strains of *Rhodobacter sphaeroides* using statistical method, J. Biotechnol. 132 (2007) 331–340.
[48] S. Bengtsson, A. Werker, T. Welander, Production of polyhydroxyalkanoates by glycogen accumulating organisms treating a paper mill wastewater, Water Sci. Technol. 58 (2008) 323–330.
[49] S.Y. Lee, H.N. Chang, Effect of complex nitrogen source on the synthesis and accumulation of poly(3-hydroxybutyric acid) by recombinant *Escherichia coli* in flask and fedbatch cultures, J. Environ. Polym. Degrad. 2 (1994) 169–176.
[50] M. Oshiki, T. Mino, H. Satoh, M. Onuki, PHA-accumulating microorganisms in full-scale wastewater treatment plants, Water Sci. Technol. 58 (2008) 13–20.
[51] J. Cardinali-Rezende, R.B. Debarry, L.F.D.B. Colturato, E.V. Carneiro, E. Chartone-Souza, A.M.A. Nascimento, Molecular identification and dynamics of microbial communities in reactor treating organic household waste, Appl. Microbiol. Biotechnol. 84 (2009) 777–789.
[52] G.R. Crocetti, J.F. Banfield, J. Keller, P.L. Bond, L.L. Blackall, Glycogen-accumulating organisms in laboratory-scale and full-scale wastewater treatment processes, Microbiology (SGM) 148 (2002) 3353–3364.
[53] A. Oehmena, A.M. Saunders, M.T. Vives, Z. Yuan, J. Keller, Competition between polyphosphate and glycogen accumulating organisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources, J. Biotechnol. 123 (2006) 22–32.
[54] J. Ahn, S. Schroeder, M. Beer, S. McIlroy, R.C. Bayly, J.W. May, G. Vasiliadis, R.J. Seviour, Ecology of the microbial community removing phosphate from wastewater under continuously aerobic conditions in a sequencing batch reactor, Appl. Environ. Microbiol. 73 (2007) 2257–2270.
[55] A. Kapley, T. De Baere, H.J. Purohit, Eubacterial diversity of activated biomass from a common effluent treatment plant, Res. Microbiol. 158 (2007) 494–500.
[56] A.L. Wong, H. Chua, W.H. Lo, P.H.F. Yu, Synthesis of bioplastics from food industry wastes with activated sludge biomass, Water Sci. Technol. 41 (2000) 55–59.