Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2016.1163510

57 (2016) 25927–25938
November

Taylor & Francis
Taylor & Francis Group

Maximizing the accumulation of Poly-β-hydroxybutyrate (PHB) in low-carbon urban sewage

Dian Li, Qian Fang*, Dan Yi

Department of Municipal Engineering, College of Civil Engineering, Guangzhou University, Guangzhou, Guangdong 510006, PR China, email: ld9527gz@163.com (D. Li), Tel. +86 013660263796; emails: gz_fq@126.com (Q. Fang), 291826310@qq.com (D. Yi)

Received 4 August 2015; Accepted 26 February 2016

ABSTRACT

This study investigated the influence of different factors in maximizing poly-β-hydroxybutyrate (PHB) accumulation in low-carbon urban sewage. The effects of chemical oxygen demand and total phosphorus (TP) concentrations on PHB accumulation were analyzed using an anaerobic/oxygen sequencing batch reactor. Both the anaerobic phase and the aeration phase were studied. The results showed that maximum PHB accumulations increased as the carbon source increased, growing from 5.45 to 27.51 mg/g, when the carbon source concentration increased from 140 to 400 mg/L, at the end of anaerobic phase. Maximum PHB accumulation decreased from 15.89 to 13.88 mg/g when exposed to influent carbon–phosphorus C/P, suggesting that carbon source is more influential and beneficial for PHB accumulation than TP. With an increase in pre-aeration time from 10 to 50 min, PHB accumulation rose from 21.12 to 31.96 mg/g; this is higher than 15.54 mg/g PHB accumulation without pre-aeration. When the pre-aeration time was fixed at 15 min, but with different pre-aeration fluxes ranging from 20 to 50 L/h, PHB accumulation increased from 23.79 to 30.09 mg/g. These results demonstrate that pre-aeration treatment facilitates PHB accumulation.

Keywords: Low-carbon municipal wastewater; Polyhydroxybutyrate (PHB); Sequencing batch reactor (SBR); Pre-aeration

1. Introduction

Poly-β-hydroxybutyrate (PHB) is a common and well-known PHA, and it is formed by many bacterial species as a result of biosynthesis in non-steady-state conditions. PHB can be used as a source of carbon in the absence of an external carbon source [1]. There is currently great interest in producing PHB from waste-activated sludge from sewage treatment plants. As technology advances, PHB production offers an alternative to petroleum-derived plastics. PHB can be used in agriculture, the food industry, the biomedical field, and in some industrial production settings, and its decomposition products do not pollute the environment [2,3]. PHB also can be an intracellular source of sustained released carbon, and as a carbon source supporting nitrogen and phosphorus removal.

PHB can be accumulated through biosynthesis from bacterial fermentation, genetic engineering, and

*Corresponding author.

activated sludge. However, synthesizing PHB through biosynthesis is 5–10 times more expensive than producing plastic through traditional means [4–6]. To reduce PHB production costs, research has focused on developing inexpensive substrates and new technology. This research collectively suggests that activated sludge could be used to accumulate PHA [1,7]. Research on PHB synthesis using activated sludge has focused on the following categories. First, previous studies have assessed the role of "satiation" and "hunger" in accumulating PHB during alternating activated sludge processes, similar to feast–famine mechanisms in a sequencing batch reactor (SBR). Anaerobic/aerobic-activated sludge is a modified activated sludge process; it both removes organic pollutants and also achieves biological phosphorus removal, recovering phosphorus (phosphate) and PHB (plastic) [8]. PHB accumulation is critical to PHB extraction. Identifying methods to optimize PHB accumulation is of interest because PHB is an excellent biocompatible material and environmentally friendly polymer, offering an alternative to petroleum-derived plastics.

Periodic feeding in an SBR creates cycling between excess and insufficient substrate conditions (called "feast and famine conditions"). These conditions favor the microorganisms most able to quickly store the substrate (during the feast phase) and then reuse it to grow (during the famine phase) [9,10]. The anaerobic conditions for PHB accumulation provide a new potential carbon source, optimizing processes such as carbon denitrification and phosphorus removal. Denitrifying polyphosphate-accumulating organisms (PAOs) can accumulate PHA under anaerobic conditions with the energy supplied from polyphosphate degradation. PAOs can also use PHB to grow and take up phosphorus under aerobic conditions [11]. High PHB accumulation in microorganisms, such as high concentrations of chemical oxygen demand (COD) in sewage, helps mitigate the problem of a low TN or total phosphorus (TP) removal rate. This is a problem caused by having a low carbon source in the anaerobic or aerobic phase [10,12]. Therefore, it is a priority to maximize the use of internal carbon sources, such as PHB, in municipal wastewater treatment to remove phosphorus.

This research used low-carbon urban sewage to study relationship between PHB accumulation, COD, and phosphorus concentrations under different circumstances. We also used the concept of "feast and famine conditions" as an inspiration to assess PHB accumulation during a pre-aeration mode. This paper presents experimental data using several stable operating cycles, demonstrating the test result reproducibility.

2. Materials and methods

2.1. Experimental setup

For this study, a completely automated SBR was operated in the configuration as shown in Fig. 1, with online measurements of dissolved oxygen (DO), pH, and oxidation–reduction potential (ORP). The reactor body was a Plexiglas cylinder (18.0 cm in diameter), with a total flux volume of 12.0 L and a working flux volume of 10.0 L. The reactor was continuously stirred with a paddle during the reaction. Air for the reactor, provided by a vacuum pressure pump, was released through an air stone placed at the bottom of the reactor. One standard SBR cycle (6.0 h) consisted of a rapid fill phase (5 min), a 5 h reaction phase (1 h anaerobic and 4 h aerobic), a settling phase (up to 50 min), and an effluent withdrawal phase (5 min). Time-based changes in the reactor were controlled using an automatic timer. ORP values and pH were collected at 1 min intervals and averaged over 5 min.

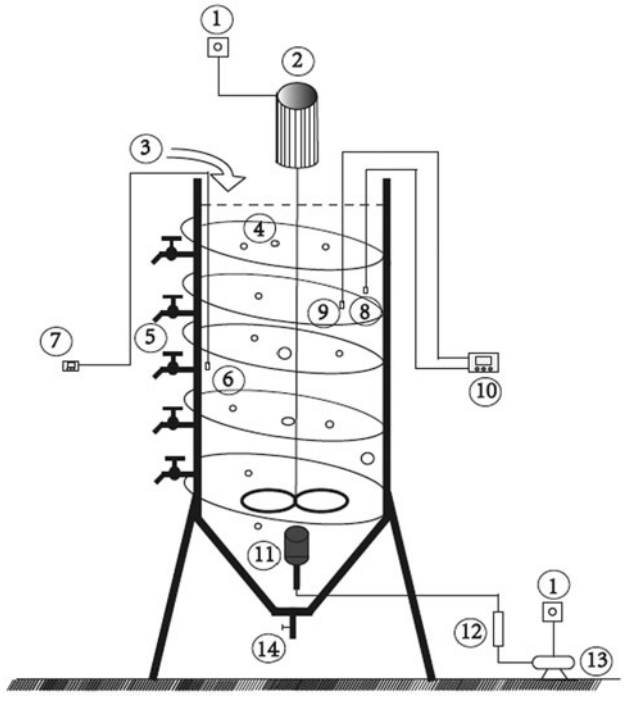

Fig. 1. Schematic diagram of (a) SBR.
Notes: (1) timer, (2) stirrer, (3) influent, (4) SBR reactor, (5) sampling point, (6) temperature sensor, (7) temperature meter, (8) ORP and DO sensor, (9) pH sensor, (10) ORP and pH meter, (11) air stone, (12) air float meter, (13) air pump, (14) discharge sludge and wastewater valve.

2.2. Experimental wastewater

Synthetic wastewater was formulated to simulate municipal wastewater. Trace elements were added based on the method in [13]. The wastewater temperature was maintained at 20 ± –0.5℃ (Table 1).

2.3. Activated sludge

Activated sludge for inoculation in the SBR was collected from an urban wastewater treatment plant in Guangzhou, China. To acclimate the seed sludge, the SBR was operated for two cycles a day in the following cycle sequence: water entering, 1.0 h anaerobic, 4.0 h aerobic, 50 min settlement, 5.0 min decanting. The average sludge age was maintained at 14 d, by regularly draining sludge. After 3–4 weeks, the COD and TP removal fluxes exceeded 90 and 85%, respectively. Mixture liquid suspended sludge (MLSS) was maintained at approximately 4.920 mg/L. As such, the sludge is considered to have an elementary performance level with respect to organic substance and phosphorus removal and the completion of sludge acclimation.

2.4. Experimental design

To maximize PHB accumulation, experimental runs were divided into three groups:

(1) PHB accumulation was studied at different carbon concentrations: 140, 260, and 400 mg/L (influent phosphorus was 6 mg/L). COD, TP, and PHB were measured during the experiment, while phosphorus removal was steady. The pH level was maintained at approximately 7.0 using $NaHCO_3$.
(2) PHB accumulation was studied at different COD/TP ratios: 75, 35, and 21 mg/L (at a constant influent COD of 260 mg/L).
(3) PHB accumulation was assessed during pre-aeration, under the different conditions listed in Figs. 2 and 3. The retention time is used to eliminate the remaining DO from pre-aeration.

Table 1
Characteristics of experimental wastewater

	Synthetic wastewater (mg/L)
COD	140–400
TP	3–12
TN (NH_3-N)	15–25
NO_3-N	0–0.5
NO_2-N	0–0.5

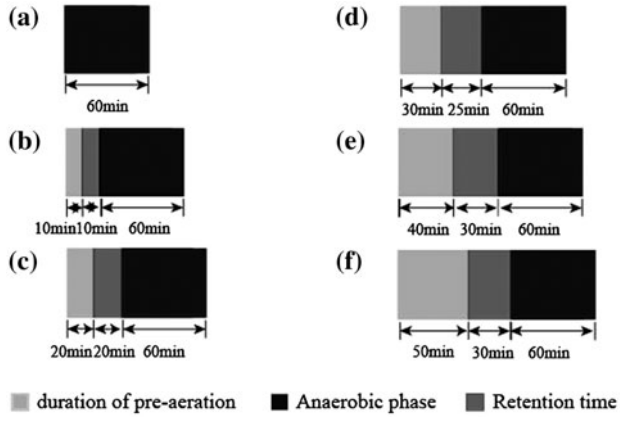

Fig. 2. Operating strategies with different pre-aeration times (the fixed pre-aeration flux is 35 L/h).

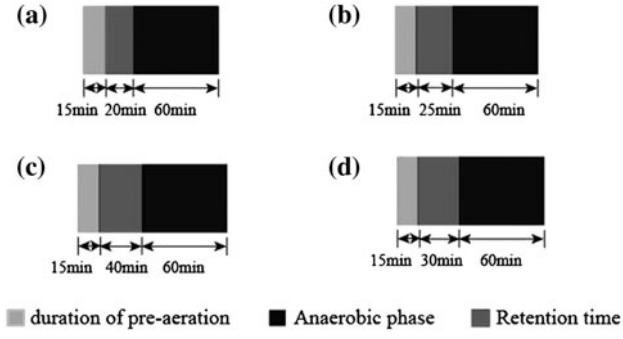

Fig. 3. Operating strategies with different pre-aeration fluxes.

2.5. Analytical methods

CODcr, MLSS, MLVSS, TP, NH_4-N, NO_3-N, and NO_2-N parameters were analyzed using standard methods (APHA, 1995). CODcr was measured using the closed reflux titrimetric method. Samples collected from the reactor to analyze TP, NH_4-N, NO_3-N, NO_2-N, and CODcr were filtered through a 0.45 μm filter, separating the bacterial cells from the liquid. TP, NH_4-N, NO_3-N, and NO_2-N were measured using spectrophotometry with an auto analyzer (Beifen-Ruili UV1600 and UV1200). ORP and pH values were continuously monitored using a WTW Multi 340i meter with an ORP electrode and a pH probe.

Determining the PHB concentration was done using an internal standard method. First, the active sludge was dried. This was followed by the addition of chloroform, methanol, and reacted benzoate ester. Finally, after stratification, the lower organic phase was analyzed using gas chromatography (Agilent, 7890A) [14].

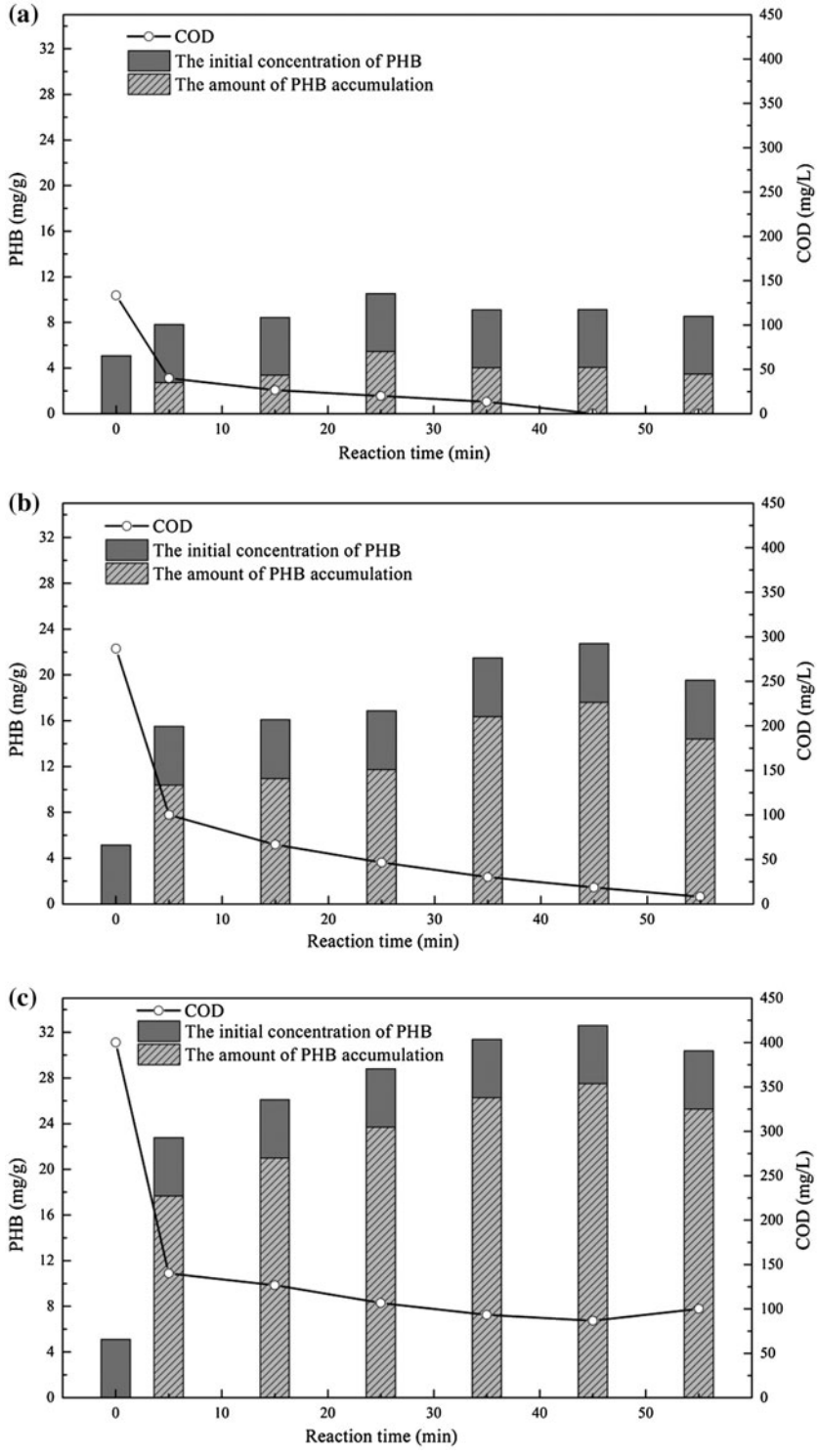

Fig. 4. Changes in PHB and COD profiles at different influent COD concentrations: (a) COD concentration: 133.33 mg/L, (b) COD concentration: 266.67 mg/L, and (c) COD concentration: 400.00 mg/L.

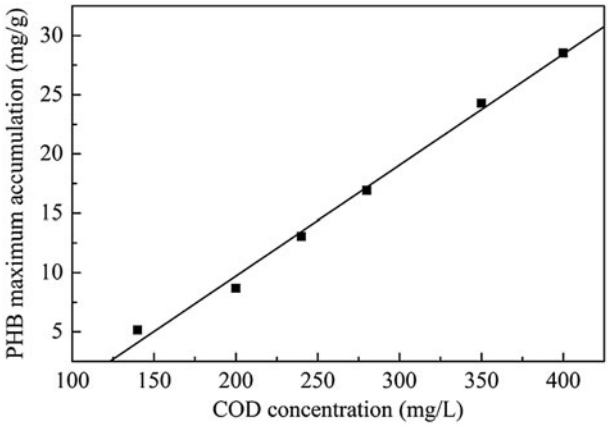

Fig. 5. The linear relationship between COD concentrations and maximum net PHB accumulation.

The suspended solids (SS) concentration was assumed to be composed of active biomass (X) and PHB. The measured PHB concentration of the sludge was expressed as follows:

$$\text{PHB} = \frac{\text{PHB}}{\text{SS}} \times 1000\,(\text{mg/g}) \quad (1)$$

3. Results and discussion

3.1. Effect of influent COD concentration on PHB accumulation performance

Fig. 4 shows the PHB and COD profiles (influent TP of 6 mg/L) under three different influent COD concentrations (133.33, 260.67, and 400.00 mg/L). As COD concentration increased, the maximum accumulation of PHB increased to 5.45, 15.62, and 27.51 mg/g, respectively (Fig. 4(a), (b), and (c)). The fastest and highest proportion of total PHB accumulation occurred in the first 5 min. PHB levels reached 2.73, 10.38, and 17.67 mg/g (with COD concentrations of 133.33, 260.67, and 400.00 mg/L, respectively) in the first 5 min, representing 51, 66, and 64% of the net maximum PHB accumulation. COD degradation was also fastest and highest in the first 5 min, reaching 93.33, 166.67, and 260.00 mg/g. During the anaerobic period, COD consumption aligned with PHB accumulation.

The PHB degraded slightly by the end of the anaerobic phase (Fig. 4). The COD was reduced to a minimum level after only 30 min (Fig. 4(a)), and PHB accumulation was maximized. Without an external carbon source, the PAOs use PHB for endogenous respiration, and PHB degrades. After 45 min, there was still surplus COD (Fig. 4(b) and (c)), and PHB degradation still occurred. This may be due to the disappearance of the microorganism's glycogen [15] or because an electron acceptor, such as NO_3-N and NO_2-N, remained in the system. This may have led to PHB degradation at the end of the anaerobic phase. The COD remained at 0.83, 8.25, and 80.00 at the end of anaerobic phase; the reactor's COD removal efficiency was 99, 96, and 80%. This result points to the need to control the influent COD concentration.

Fig. 5 illustrates a positive correlation between the maximum PHB accumulation and the carbon source concentration. This correlation was calculated as $C_{\text{PHB maximum net accumulation}} = 0.0936 \quad C_{\text{COD}} - 9.012$ ($R^2 = 0.9933$), which is a statistically significant linear correlation.

When the sludge concentration is constant, increasing the carbon concentration requires increased sludge loading. The increase, however, is insufficient to stimulate rapid microbial multiplication, because microbes prefer to store the nutrients, so PHB increases [8]. When the influence of the carbon source exceeds microbe needs for a period of time, the phosphorus removal mechanism is destroyed [16]. As such, COD content was the variable of interest for the next experiment.

3.2. Effect of influent COD/TP ratios on the performance of PHB accumulation

Fig. 6 shows the PHB, COD, and TP profiles under three COD/TP ratios (with an influent COD of 260 mg/L). Maximum PHB levels are 15.97, 15.82, and 14.74 mg/g, at COD/TP ratios of 75, 35, and 21, respectively (Fig. 6(a), (b), and (c)). As the COD/TP ratio decreases, both the maximum net PHB accumulation and the phosphorus levels decrease slightly.

This result can be explained through the following chemical expression [17]:

$$(CH_3COOH)_{1/2} + 0.5(C_5H_{10}O_5)_{1/6} + 0.48HPO_3 + 0.023H_2O \rightarrow 1.33(C_4H_6O_2)_{1/4} + 0.48H_3PO_4 + 0.17CO_2 \quad (2)$$

This expression shows that when there was more phosphorus in the solution, phosphorus release was more difficult and slow. When the influent COD was constant and phosphorus increased, PHB accumulation decreased slightly. The net maximum phosphorus release was 47.85, 45.44, and 42.11 mg/L (Fig. 6), decreasing slightly as the COD/TP ratio decreased. When the COD was consumed, phosphorus accumulated, which corresponds with expression (1) and PHB accumulation.

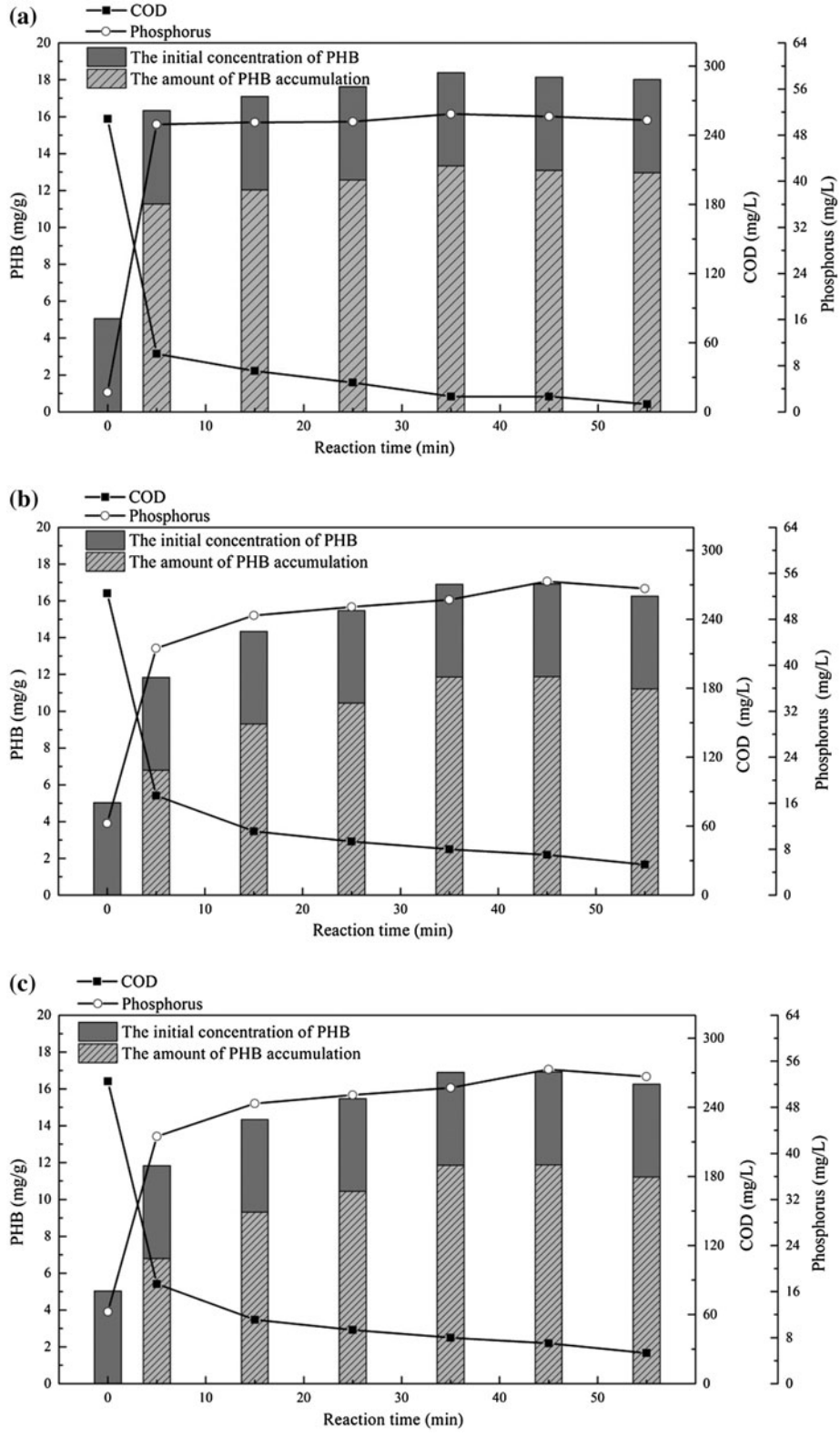

Fig. 6. Changes in PHB and COD profiles at different COD concentrations: (a) COD/TP ratio: 75, (b) COD/TP ratio: 35, and (c) low COD/TP ratio: 21.

Fig. 4(b) shows a C/P ratio of 45, while the carbon concentration is approximately 260 mg/L (Fig. 4(b)), which approaches the level in Fig. 6(b). The maximum PHB accumulation is 15.62 mg/g (Fig. 4(b)), very close to the 15.54 mg/g (Fig. 6(b)) level. Fig. 4(c) shows that the influent C/P ratio is approximately 70 (influent COD is approximately 400 mg/L and phosphorus is about 6 mg/L); the maximum PHB accumulation of 27.51 mg/g is far more than 15.62 mg/g (Fig. 4(b)) and 15.54 mg/g (Fig. 6(b)). The experiment shows that the COD concentration influences PHB accumulation more than phosphorus concentration; as such, influent

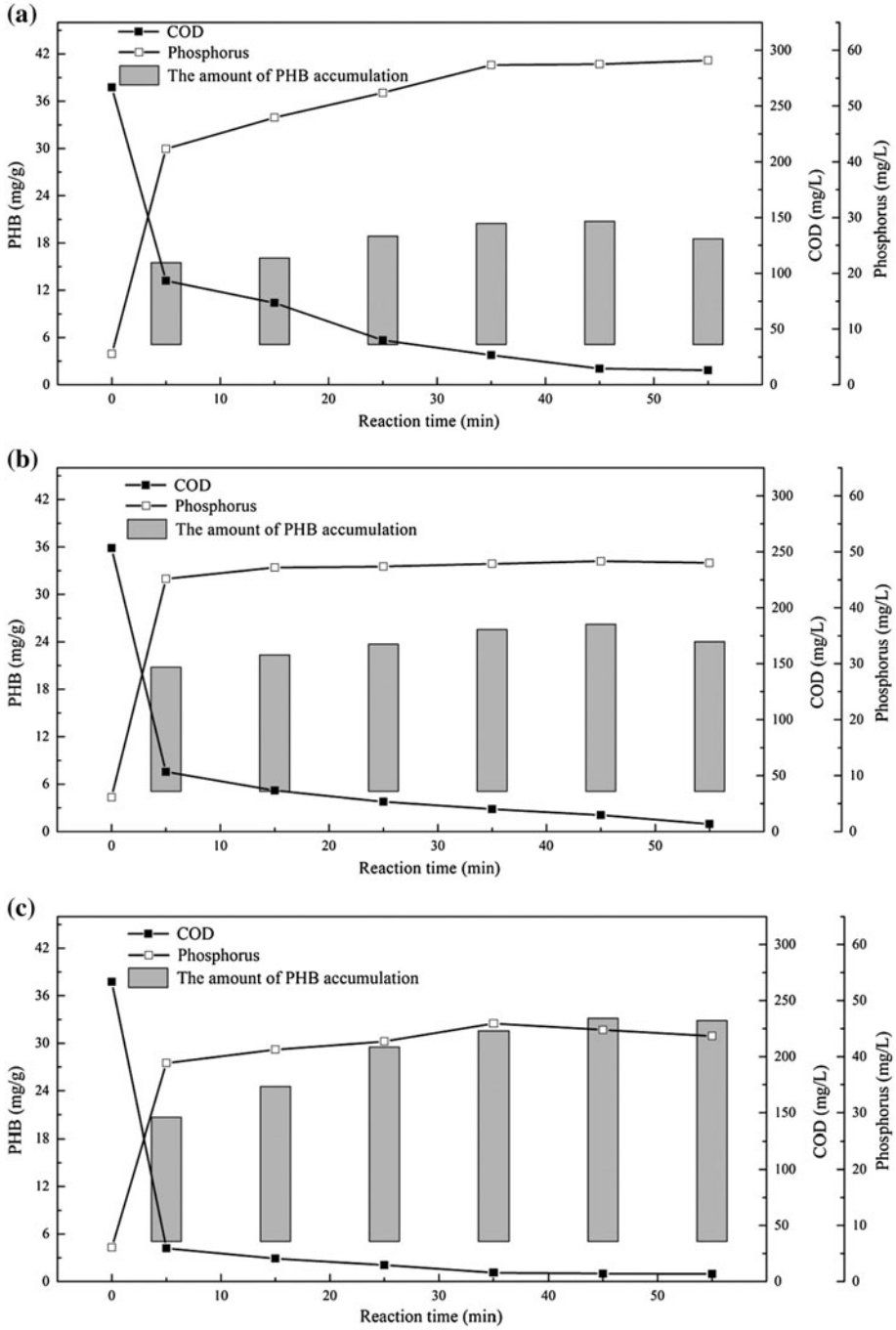

Fig. 7. Changes in PHB, TP, and COD profiles at different pre-aeration time: (a) without pre-aeration, (b) pre-aeration time of 10 min, (c) pre-aeration time of 20 min, (d) pre-aeration time of 30 min, (e) pre-aeration time of 40 min, and (f) pre-aeration time of 50 min.

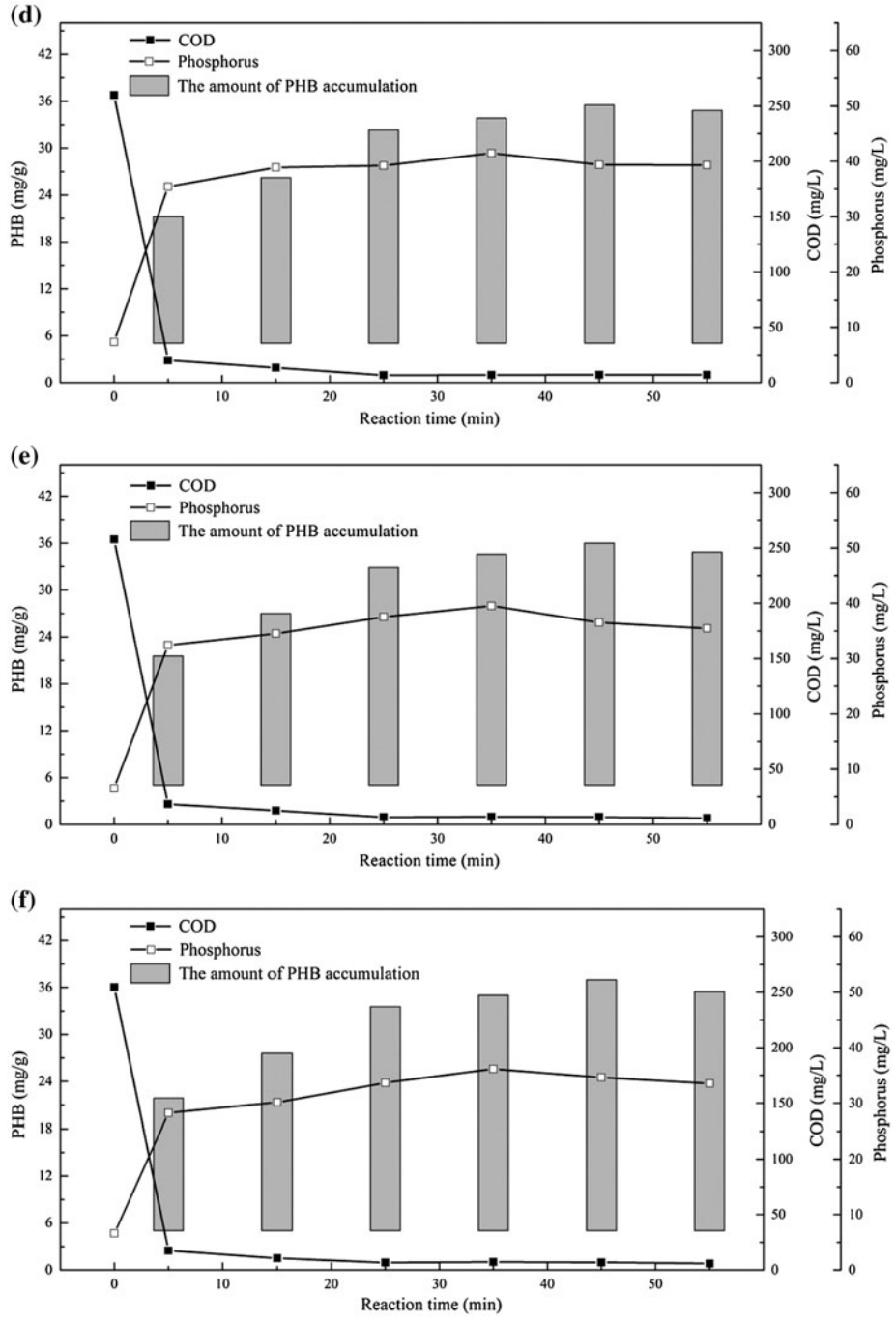

Fig. 7. (*Continued*).

phosphorus content was used as the test variable for the next experiment.

3.3. The role of pre-aeration in PHB accumulation

Pre-aeration involves aeration at different time durations or fluxes before the anaerobic treatment phase, which then exhausts the remaining oxygen from the system.

3.3.1. Different pre-aeration times

Fig. 7 (with an influent COD of approximately 260 mg/L and phosphorus at 6 mg/L) presents PHB

and COD profiles at different pre-aeration time durations of 10, 20, 30, 40, and 50 min, but with a steady aeration flux of 35 L/h (Fig. 2). With the increase in pre-aeration time, the maximum PHB accumulation changed from 21.12 mg/g to 28.06 mg/g to 30.48 mg/g, to 30.95 mg/g and to 31.96 mg/g (Fig. 7(b), (c), (d), and (e)). Without pre-aeration (0 min), the PHB accumulation was only 15.54 mg/g (Fig. 7(a)). With a pre-aeration time of 20 min (Fig. 7(c)), the maximum PHB accumulation was 28.06 mg/g, more than 27.51 mg/g (Fig. 4(c)). This indicates that pre-aeration effectively promotes PHB accumulation. At pre-aeration durations of 0, 10, and 20 min, the maximum PHB accumulation was at approximately 45 min (Fig. 7(a), (b), and (c)). At pre-aeration durations of 30, 40, and 50 min, the maximum PHB accumulation was at approximately 35 min (Fig. 7(d), (e), and (f)). COD degraded to 93.33 mg/L in the first 5 min (Fig. 7(a)); with pre-aeration, COD levels degraded to 53.33, 29.67, 20.00, 18.40, and 17.67 mg/L as duration increased from 10 to 50 min (Fig. 7(b), (c), (d), (e), and (f)). The reactor's efficiency in removing COD was faster and more complete, reaching 64, 79, 88, 92, 93, and 93% over different pre-aeration durations (starting after 5 min). This shows that the pre-aeration mode also speeds up the average reaction flux. In a certain range, the longer the pre-aeration time, the faster the COD degrades and the more PHB accumulates.

Fig. 7(a), (b), and (c) shows a clear increase in PHB accumulation with the increased pre-aeration; this increase was not as obvious with pre-aeration

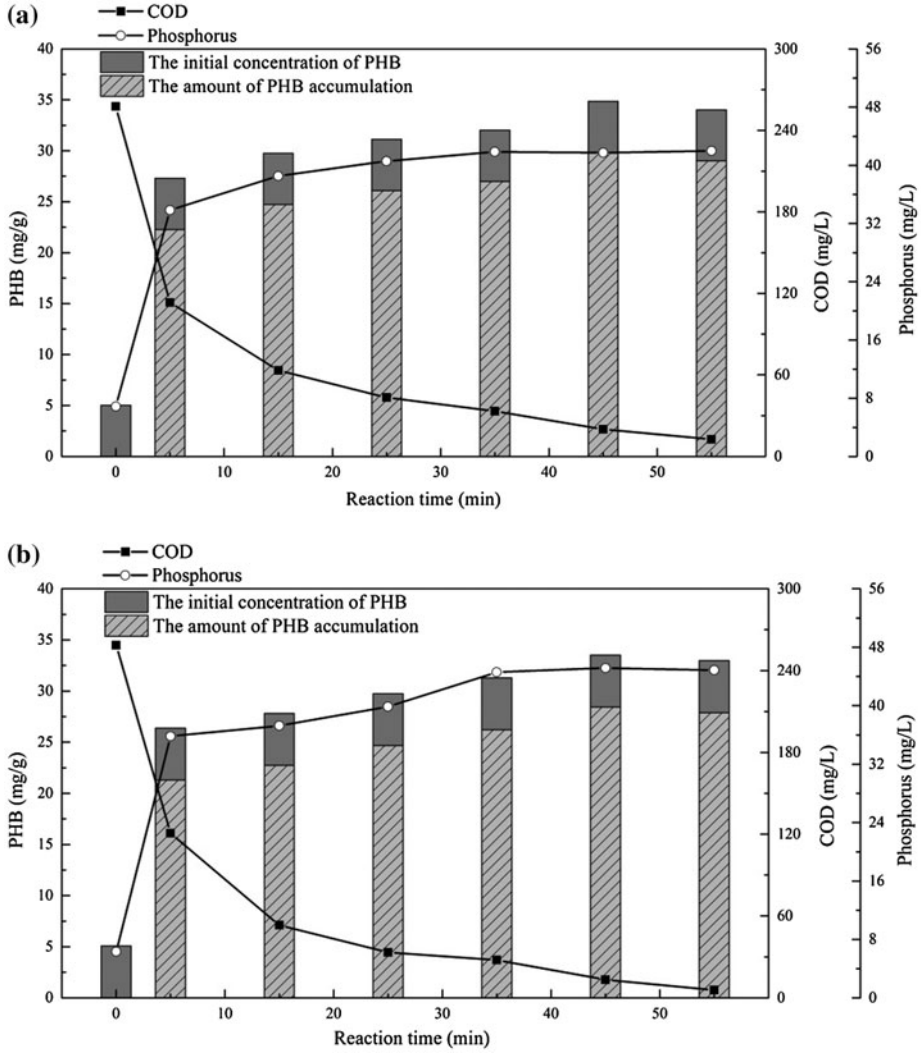

Fig. 8. Changes in PHB, TP and COD profiles at different pre-aeration fluxes: (a) Pre-aeration flux of 20 L/h, (b) Pre-aeration flux of 30 L/h, (c) Pre-aeration flux of 40 L/h, and (d) Pre-aeration flux of 50 L/h.

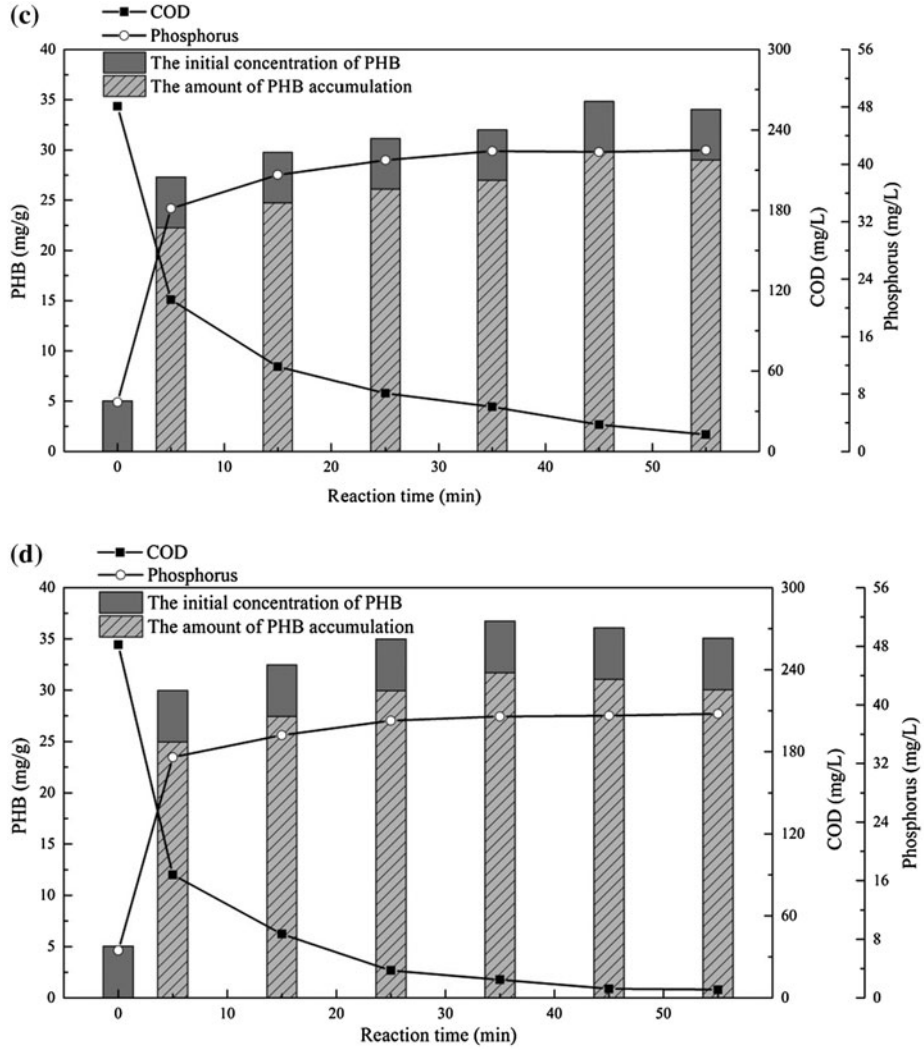

Fig. 8. (*Continued*).

durations of 30, 40, and 50 mins (Fig. 7(d), (e), and (f)). Results show that when the pre-aeration flux was 35 L/h, a 20-min pre-aeration time was sufficient. Controlling the pre-aeration time saves energy.

The phosphorus release trend was synchronized with PHB accumulation, and the maximum phosphorus concentration in bulk reached 41.90, 37.59, 32.01, 28.94, and 26.96 mg/L with increased pre-aeration time (Fig. 7(b), (c), (d), (e), and (f)); the phosphorus release without pre-aeration was 47.48 mg/L (Fig. 7(a)). Phosphorus release results directly result phosphorus uptake and also may lead the phosphorus removal system. Pre-aeration led to a decrease in phosphorus concentration. Similar to PHB accumulation, when the pre-aeration flux was 35 L/h, a 20-min pre-aeration time was sufficient. As such, for a stable phosphorus removal system, a controlled pre-aeration time is needed.

3.3.2. Different pre-aeration fluxes

Fig. 8 shows PHB accumulation, phosphorus accumulation, and COD degradation profiles in the anaerobic phase of a 15-min pre-aeration period (Fig. 3). As the pre-aeration flux increased from 20 to 40 L/h, the PHB accumulation reached 23.79, 28.43, 29.82, and 30.07, respectively (Fig. 8). Conversely, PHB accumulation was only 15.54 mg/g without pre-aeration (Fig. 8(a)). When the pre-aeration flux was 50 L/h, the PHB accumulation reached its maximum level at approximately 35 min (Fig. 8(d)). When the pre-aeration flux was 20, 30, and 40 L/h, the

PHB accumulation reached its maximum level at approximately 45 min (Fig. 8(a), (b), and (c)). The phosphorus release trend was aligned with PHB. The maximum phosphorus concentration decreased from 41.54, 38.54, 35.13, and 32.21 mg/L at the different flux (Fig. 8), while the phosphorus release without pre-aeration was higher, at 47.48 mg/L (Fig. 7(a)).

When comparing the effects of a longer pre-aeration time (Fig. 7(e) and (f)) against a maximum pre-aeration flux (Fig. 8(d)) on PHB accumulation and the stability of the phosphorus removal system, the length of time has a larger effect than the flux. As such, to increase PHB concentrations, it is most effective to add a short (15 or 20 min) pre-aeration time and a moderate pre-aeration flux (35 or 40 L/h).

Pre-aeration leads to microbe hunger; microbes consume the PHB and also consume ATP and release phosphorus. The influent phosphorus concentration is almost the same at the beginning of the experiment; pre-aeration leads to a decrease in sludge ATP; phosphorus release decreases slightly during the anaerobic phase. After the low COD is added to the SBR, microbial use of external carbon is very short; bacteria with the ability to store PHB quickly take advantage of the external carbon and become the dominant bacteria. In systems without an external carbon source, microbes can decompose PHB to obtain energy. Without extracellular polymeric substances, such as PHB, there is endogenous respiration when the substrate is exhausted, slowing growth. For microbial systems operating in a "survival of the fittest" mode, when the best competitor has the capacity to store PHB, PHB will accumulate.

4. Conclusions

This study highlighted PHB accumulation dynamics under the different COD, COD/TP ratios, and the mode of pre-aeration, showing that:

(1) The influent COD concentration was positively correlated with maximum PHB accumulation under limited or low influent COD, fixed biomass, and TP concentration.
(2) When the influent COD was constant, as the COD/TP ratio decreased, the PHB accumulation was more difficult and slower. When comparing the effects of COD and phosphorus concentration on PHB accumulation, the effect of COD on PHB accumulation is larger than phosphorus.
(3) Adding a short pre-aeration time and a moderate pre-aeration volume effectively promotes PHB accumulation. Conversely, a long pre-aeration time or high pre-aeration volume does not benefit PHB accumulation. It is important to properly control pre-aeration of the PHB to maximize accumulation.

Acknowledgments

The authors thank Guangzhou University for its support. This project was financially supported by the National Natural Science Foundation of China (Grant no. 21207023); the Science and Technology Program of Guangdong, China (Grant no. 2012B010500029); and the Science and Technology Program of Guangzhou, China (Grant no. 1563000640).

References

[1] H. Satoh, T. Mino, T. Matsuo, PHA production by activated sludge, Int. J. Biol. Macromol. 25 (1999) 105–109.
[2] A.L. Xu, Y.Y. Lao, Q. Zhang, J.L. Xia, Extraction and characterization of PHB from acidiphilium cryptum DX1-1, J. Wuhan Univ. Technol. Mater. Sci. Ed. 25 (2010) 938–943.
[3] T. Zhang, M.T. Chaudhry, Z. Liu, Genetic and biochemical characterization of poly 3-hydroxybutyrate depolymerase from Diaphorobacter sp. PCA039, World J. Microbiol. Biotechnol. 26 (2010) 1803–1811.
[4] S.N. Mudliar, A.N. Vaidya, M. Suresh Kumar, S. Dahikar, T. Chakrabarti, Techno-economic evaluation of PHB production from activated sludge, Clean Technol. Environ. Policy 10 (2008) 255–262.
[5] R. Perez-Feito, D.R. Noguera, Recovery of polyhydroxyalkanoate from activated sludge in an enhanced biological phosphorus removal bench-scale reactor, Water Environ. Res. 78 (2006) 770–775.
[6] S. Khanna, A.K. Srivastava, Recent advances in microbial polyhydroxyalkanoates, Process Biochem. 40 (2005) 607–619.
[7] Y.I.H. Satoh, T. Mino, Activated sludge as a possible source of biodegradable plastic, Water Sci. Technol. 38 (1998) 103–108.
[8] C. Wei, C. Zhiqiang, W. Qinxue, Effect of COD concentration on synthesis of poly-hydroxyalkanoates by activated sludge, China Water Wastewater 21 (2010) 148–151 (in Chinese).
[9] M. Majone, K. Dircks, J.J. Beun, Aerobic storage under dynamic conditions in activated sludge processes. The state of the art, Water Sci. Technol. 39 (1999) 61–73.
[10] J.J. Beun, K. Dircks, M.C.M. Van Loosdrecht, J.J. Heijnen, Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures, Water Res. 36 (2002) 1167–1180.

[11] 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.
[12] K.A. Third, B. Gibbs, M. Newland, R. Cord-Ruwisch, Long-term aeration management for improved N-removal via SND in a sequencing batch reactor, Water Res. 39 (2005) 3523–3530.
[13] R. Barat, T. Montoya, L. Borrás, J. Ferrer, A. Seco, Interactions between calcium precipitation and the polyphosphate-accumulating bacteria metabolism, Water Res. 42 (2008) 3415–3424.
[14] J. Wang, J. Yu, Kinetic analysis on formation of poly (3-hydroxybutyrate) from acetic acid by Ralstonia eutropha under chemically defined conditions, J. Ind. Microbiol. Biotechnol. 26 (2001) 121–126.
[15] D. Brdjanovic, M.C.M. van Loosdrecht, C.M. Hooijmans, T. Mino, G.J. Alaerts, J.J. Heijnen, Innovative methods for sludge characterization in biological phosphorus removal systems, Water Sci. Technol. 39 (1999) 37–43.
[16] C.W. RandaII, R.W. Chapin, Acetic acid inhibition of biological phosphorus removal, water Environ. Res. 169 (1997) 955–960.
[17] G.J. Smolders, J. van der Meij, M.C. van Loosdrecht, J.J. Heijnen, Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence, Biotechnol. Bioeng. 43 (1994) 461–470.