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Enhanced phosphorus removal by a humus soil cooperated sequencing batch reactor using acetate as carbon source

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ABSTRACT

The biological phosphorus removal by humus soil sequencing batch reactor process (HS-SBR) and a conventional sequencing batch reactor (cSBR) were compared using acetate as a sole carbon source. The HS-SBR was composed of a humus soil reactor (HSR) and a conventional SBR (designated as hsSBR). The HS-SBR showed a more stable and effective phosphorus removal with the efficiency of 97.3%, while as to the cSBR, it was 80%. However, in the HS-SBR for the removal of COD and nitrogen it was not improved. Moreover, in the anaerobic phase, the hsSBR had greater soluble orthophosphate (SOP) release and poly-β-hydroxybutyrate (PHB) synthesis capacity, but lower poly-β-hydroxyvalerate (PHV) synthesis and glycogen (Gly) degradation capacity than those in the cSBR. In addition, acetate (HAc) uptake rate and SOP release rate in the hsSBR were greater than those in the cSBR. In the aerobic phase, the PHA utilization efficiency for SOP uptake in the hsSBR was higher than the cSBR. All these observations suggested that adding the HSR improved the relative dominance of the phosphate accumulating organisms (PAOs) in the hsSBR.

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1. Introduction

Eutrophication is one of the most important problems associated with water environment worldwide, and phosphorus removal from sewage and industrial wastewaters has been considered to be a key strategy for preventing eutrophication [1]. Enhanced biological phosphorus removal (EBPR) has been proved to be an economic and sustainable method for the phosphorus removal from wastewater [2]. The primary characteristic of EBPR is that the anaerobic and aerobic conditions are alternated so that the growth of polyphosphate accumulating organisms (PAOs) can be incubated. According to the metabolism of phosphorus removal (Fig. 1a), under anaerobic conditions, PAOs can make use of volatile fatty acids (VFAs) as carbon sources by using energy obtained from the hydrolysis of their intracellular poly-phosphate (poly-P) and glycogen (Gly), and accumulate poly-β-hydroxyalkanoates (PHAs) in their cells. During aerobic period, PAOs oxidize PHAs to gain energy for the maintenance of their cells, replenishment of Gly and phosphate uptake [3,4]. Phosphorus removal is ultimately achieved through the wastage of excess sludge containing an elevated amount of phosphorus in the form of accumulated poly-P.

Deterioration of the EBPR has been observed in laboratory-scale systems as well as real scale wastewater treatment plants [3]. The main reason is that glycogen accumulation bacteria (GAOs) are often competed by PAOs for carbon sources under anaerobic condition. The GAOs use VFAs for the synthesis of PHAs by using the energy from the hydrolysis of glycogen in this condition (Fig. 1b) [5,7]. Under aerobic condition, GAOs only accumulate glycogen, without phosphate uptake. As a result, when GAOs accumulated, the performance of EBPR tends to be poor and unstable.

Recent studies have shown that increasing pH [1,6,8] and propionate/acetate ratio [9,10], or decreasing temperature [11,12] could enhance the competitiveness of PAOs, further, the efficiency of phosphorus removal was improved. However, it is not economic and easy to control pH, the concentration of VFAs and temperature in practice. It is urgent to find a practical way to increase the abundance of PAOs in EBPR systems and to ensure a more stable and efficient phosphorus removal.

In recent years, a novel system called humus soil activated sludge process has been used for wastewater treatment [13]. In this process, a humus soil reactor (HSR) is added to a traditional activated sludge process. Part of the sludge returned from the secondary settling tank flows through the HSR, and returns back to the aeration tank. Therefore, the HSR is mainly served as a microorganism cultivation reactor. If the condition of HSR is properly controlled, some favorite bacteria could grow in the reactor, and then enter the main treatment unit, which influence the bacte-

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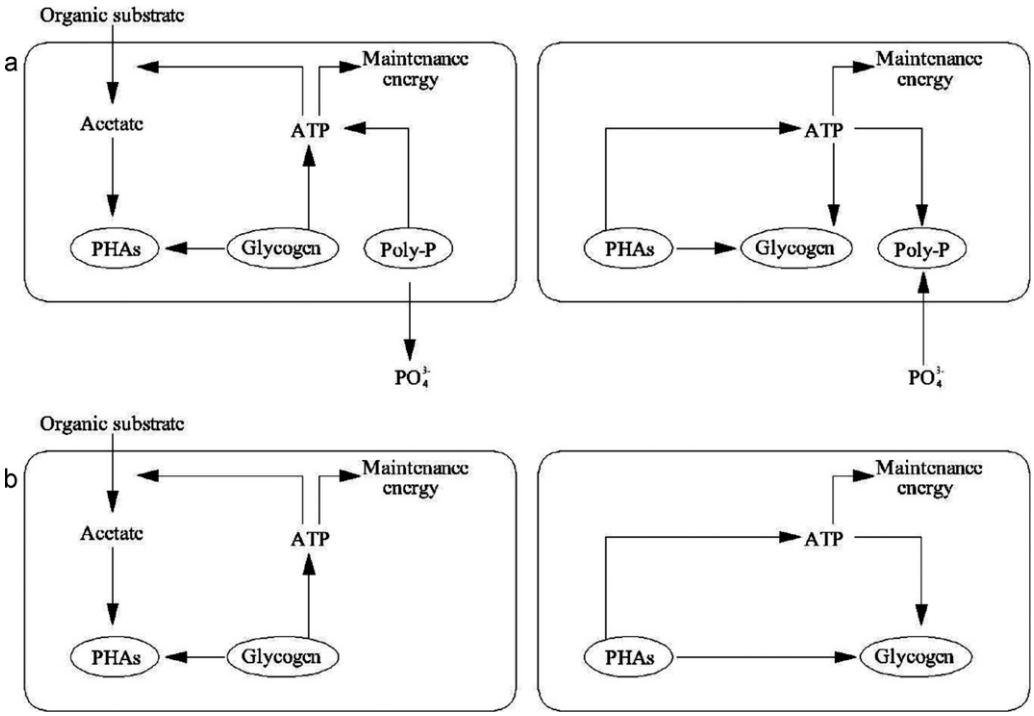

Fig. 1. Schematic diagrams for the mechanisms of the synthesis and catalysis of PHAs, glycogen and poly-p by PAOs (a) and GAOs (b) in anaerobic (left) and aerobic (right) conditions. (ATP, adenosine triphosphate).

rial community significantly. It has been reported that phosphorus removal of this process was above 80% [13].

The previous experimental results showed that a humus soil sequencing batch reactor process (HS-SBR) improved phosphorus removal by 20% when compared with that of a conventional sequencing batch reactor (cSBR) [14]. However, the mechanism of the phosphorus removal in this process is still unclear. Therefore, this study aims to reveal how phosphate release and uptake are correlated with intracellular storage compounds such as PHAs and Gly. In this study, a laboratory scale HS-SBR was operated using synthetic wastewater containing acetate as a sole carbon source and a cSBR was also tested as a parallel comparison.

2. Materials and methods

2.1. Experimental set-up

The study was carried out in a lab-scale HS-SBR process (Fig. 2). The effective volume of the SBR unit (designated as hsSBR to differentiate the cSBR) was 5 L (Ø15 cm × *H* 35 cm). The effective volume of HSR unit was 2 L (Ø10 cm × *H* 30 cm). Eight humus soil columns were packed in the HSR, with a diameter of 3 cm and a height of 7 cm. The hsSBR was operated on two cycles each day with 12 h for each cycle. In each cycle, anaerobic and aerobic operations took 3 h and 5 h respectively, followed by a settling period of 2 h and a decanting of 5 min, and the remaining time used as an idle phase. The dissolved oxygen (DO) concentration was around 6.0 mg/L during the entire aerobic period, except a very short period of time at the beginning in which the DO was near 0. At the end of the aerobic period, part of the mixed liquor was drained from the hsSBR unit to maintain the sludge retention time (SRT) at approximately 10 days. One liter of drained mixed liquor was fed into the HSR, and one liter mixed liquor from the HSR was returned back to the hsSBR unit at the beginning of the anaerobic period. Three liters of the supernatant were decanted after the settling phase was completed, and same amount of a synthetic wastewater was fed into the hsSBR at the beginning of the anaerobic period. The HSR was constantly mixed with a mechanical stirrer and the DO concentration was below 0.1 mg/L. To evaluate the performance of the HS-SBR process, a cSBR was also tested as a comparison in this study. The size and operation of the cSBR were exactly the same as the hsSBR.

The synthetic wastewater, containing $NaAc{\cdot}3H_2O$ (0.850 g/L), $MgSO_4{\cdot}7H_2O$ (0.090 g/L), $CaCl_2{\cdot}H_2O$ (0.011 g/L), NH_4Cl (0.107 g/L), KCl (0.036 g/L), $NaH_2PO_4{\cdot}2H_2O$ (0.076 g/L), and 0.3 ml/L trace element solution, was prepared daily using tap water. The composition and concentration of the trace-element solution was described by Smolders et al. [15]. The main characteristics of the wastewater influent are shown in Table 1. The nitrate nitrogen (NO_3^--N) detected in influent was probably from the tap water. The ratio between chemical oxygen demand (COD) and soluble orthophosphate (SOP) was 27 mg COD/mg P.

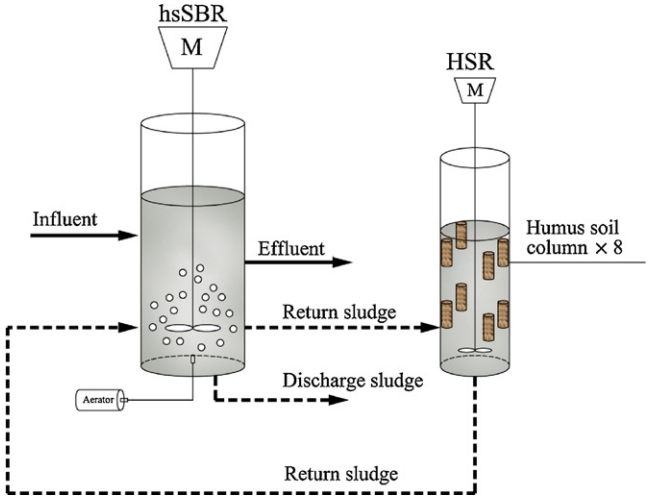

Fig. 2. Schematic diagram of experimental HS-SBR. The mixed liquor drained from the hsSBR at the end of the aeration phase was fed into the HSR; the mixed liquor of the HSR was returned back to the hsSBR at the beginning of the anaerobic phase.

Table 1
Characteristics of the synthetic wastewater.

pH	COD (mg/L)	SOP (mg/L)	NH_4^+-N (mg/L)	NO_3^- (mg/L)
6.92 ± 0.2	400 ± 50	15 ± 0.5	28 ± 3	2.5 ± 0.5

Sewage sludge was collected from a municipal wastewater treatment plant in Shanghai. Humus soil was a commercial product of the W.T.M. Company (Kagawa, Japan) and the corresponding properties are listed in Table 2. The organic matter contains a large amount of humic substances which have carboxyl, alkyl and other organic functional groups.

For the set-up HS-SBR and the cSBR, five liter of sewage sludge (900 mg/L) was added into the reactors. During the first 2 weeks, no mixed liquor was discharged. The mixed liquor of the HSR was returned back to the hsSBR twice a day.

2.2. Analytical methods

The effluent of the hsSBR and cSBR were monitored three times a week for pH, temperature, COD, SOP, NH_4^+-N, NO_3^--N. MLSS and mixed liquor volatile suspended solid (MLVSS) of the two SBRs were measured daily at the end of aeration phase. In a quasi-steady state, an aliquot of mixed liquor was collected from hsSBR and cSBR at 0, 15, 30, 60, 120, 180, 195, 210, 240, 360 and 480 min after the anaerobic stage was started. Some mixed liquor samples were measured for glycogen. A portion of the samples was filtered through a 0.45 μm pore size membrane filter for the analysis of HAc and SOP. The remaining sample was centrifuged at 4000 rpm for 10 min; the pellets were collected and stored in −40 °C freezer for PHAs analyze. The pH and temperature were measured by a pH meter (340i, WTW, Germany). COD was analyzed by using Merck COD reagents according the method recommended by the manufacturer (Merck; Germany). The analysis of SOP, NH_4^+-N, NO_3^--N, MLSS and MLVSS were performed according to standard methods [16]. The concentration of glycogen was measured using the Anthrone method [17].

For the quantification of HAc [1], the filtrate was collected in a 1.5 mL vial, and acidified with 6 mol/L HCl to pH 3.0 before being analyzed in a gas chromatography (GC) (6890N, Agilent) with flame ionization detector (FID) and DB-WAXETR column (length 30 m, internal diameter 0.53 μm, film thickness 1 μm). Nitrogen was used as the carrier gas (25 mL/min). The injection port and the detector were maintained at 220 °C and 250 °C, respectively. The oven temperature was controlled in a program beginning with 110 °C for 2 min, then increasing at a rate of 10 °C/min to 220 °C, and then holding at 220 °C for an additional 2 min.

The analysis of PHAs was conducted according to the method of Liu et al. [1]. Lyophilized sludge samples were digested, methylated, and extracted with chloroform. The extracted methyl esters were analyzed using a GC (Thermo Focus, America) equipped with a HP-5 capillary column (length 30 m, internal diameter 0.32 mm, film thickness 0.25 μm), and a flame ionization detector (FID). Helium gas was used as the carrier gas (30 mL/min). A mixture of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV) (95% and 5% respectively) purchased from Aldrich Chemical Company (USA) was used as standard for calibration. Benzoic acid was used as an internal standard. The sum of measured PHB and PHV was defined as the total PHAs.

The SOP, HAc and PHAs concentrations were the averages of duplicate measurements in one cycle. The Gly were the averages of triplicate measurements in one cycle. There relative standards deviations were below 5% except Gly (10%).

3. Results and discussions

HS-SBR and cSBR took 6 weeks to achieve a quasi-steady state. MLSS in the hsSBR and cSBR were maintained at 1540 ± 150 and 1720 ± 100 mg/L, respectively, in the quasi-steady state (Table 3).

3.1. COD and nitrogen removal in hsSBR and cSBR

The average concentrations of COD, NO_3^--N NH_4^+-N, and SOP in the effluents of the hsSBR and cSBR in a quasi-steady state were showed in Table 3. The average effluent COD and corresponding removal efficiencies were 37 mg/L and 91% in the hsSBR, and 30 mg/L and 93% in the cSBR (Table 3). Clearly, the COD removal efficiencies in both reactors were maintained at a higher level. As a contrast, the COD efficiency in the hsSBR was lower than the cSBR, possibly due to the release of refractory organic matter from humus soil in the HSR.

The NH_4^+-N concentrations in both reactors were not detected (Table 3), indicating that the nitrification was complete. The NO_3-N in the effluent of the hsSBR was approximately 9.0 mg/L, which was higher than that in the effluent of the cSBR (Table 3). At the beginning of anaerobic phase, there was some NO_3^--N residue from last cycle. This amount of NO_3^--N could be reduced rapidly by denitrification. In the hsSBR and cSBR, the HAc required for the denitrification in the anaerobic phase was calculated to be about 9 and 11 mg/L, respectively, accounted for only 4% and 5% of total HAc in the influent. Therefore, NO_3^--N in the influent interference to SOP releasing in anaerobic stage was insignificant and could be neglected (see Section 3.2).

3.2. Phosphorus removal in hsSBR and cSBR

The SOP in the effluent of the hsSBR was only approximately 0.4 mg/L, which was much lower than that of the cSBR with a value of 2.6 mg/L (Table 3); the phosphorus removal in the hsSBR and cSBR reached 97% and 80% respectively. Moreover the phosphorus removal in the hsSBR was very stable (below 1 mg/L), while the removal in cSBR was unstable and tended to be fluctuating (2–5 mg/L). These observations indicated that adding the HSR did significantly improve the phosphorus removal in the hsSBR compared with that in the cSBR.

It is interesting to note that the SOP removals in both hsSBR and cSBR were high (≥80%) even under an operating temperature of 27–31 °C which is more suitable for the growth of GAOs. Panswad et al. [18] reported that GAOs accounted for 64–75% of total MLVSS in an EBPR at 30 °C and the efficiency of phosphorus removal was only 40%. The reason could be that the average pH in the anaerobic stage of the present study was around 7.40 which is in an optimal pH range for PAOs. Lopez-Vazquez et al. [11] suggested that, even at a high temperature, PAOs can be more favored than GAOs when a high pH (7.5) is applied.

With the increased phosphorus removal in the hsSBR, SVI, MLVSS and MLVSS/MLSS decreased for about 6.9%, 18% and

Table 2
Properties of humus soil.

Water content (%)	pH	Organic matter (%)	Inorganic matter (%)				
			SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO
10	4.0–5.5	≤32	≥50	≤3	≤4.5	≤0.35	≤0.40

Table 3
Characteristics of sludge and effluent in the hsSBR and cSBR.

Process	SVI	MLSS (mg/L)	MLVSS (mg/L)	MLVSS/MLSS (%)	COD (mg/L)	SOP (mg/L)	NO_3^--N (mg/L)	NH_4^+-N (mg/L)
HS-SBR	54 ± 3[a]	1540 ± 150	960 ± 79	62.3 ± 1.2	37 ± 9	0.4 ± 0.3	9.0 ± 1.7	ND[b]
SBR	58 ± 11	1720 ± 100	1181 ± 65	68.5 ± 2.1	30 ± 11	3.0 ± 1.8	6.8 ± 2.1	ND

[a] Values are mean ± standard deviations (n = 10).
[b] ND: not detected.

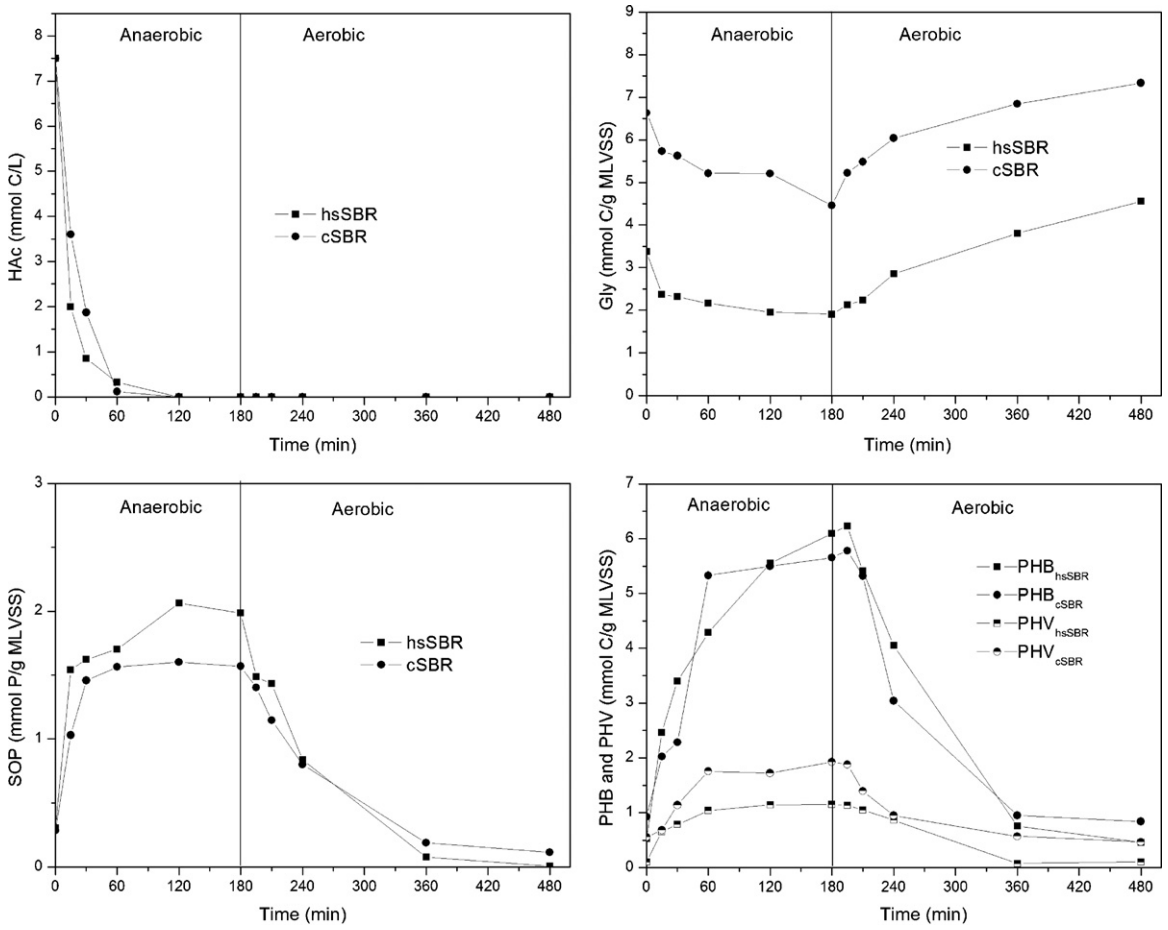

Fig. 3. Variations of HAc, SOP, Gly, PHB and PHV during one cycle in hsSBR and cSBR.

9.1% respectively (Table 3). It indicated that phosphorus removal increased the settleability of the sludge, reduced the sludge concentration in the mixed liquor, and COD reduction and nitrogen removal. Similar observations were also reported by Schuler and Jang [19] and Wang et al. [4]. Schuler and Jang found that EBPR had better settleability than non-EBPR plants. Wang et al. reported that the lower MLVSS/MLSS indicated that higher phosphorus content in biomass.

3.3. Anaerobic transformations comparison between hsSBR and cSBR

To reveal the difference of SOP removal performance between hsSBR and cSBR, HAc, SOP, Gly and PHAs were analyzed periodically to determine the major anaerobic and aerobic transformations occurred in both reactors. The variations of the concentrations of these compounds prolonged with time during a quasi-steady cycle in the hsSBR and cSBR were shown in Fig. 3. The net increase or decrease of SOP, Gly and PHAs were also calculated and presented in Tables 4 and 5, respectively.

As shown in Fig. 3, in the anaerobic phase, HAc in the hsSBR and cSBR was generally consumed very fast, from 7.5 mmol C/L at the beginning to near 0 at 60 min. Corresponding to the rapid consumption of HAc, the SOP was released rapidly in the initial period of time in both reactors. Overall, SOP release in the hsSBR and cSBR was respectively 1.68 mmol P/g MLVSS and 1.28 mmol P/g MLVSS (Table 4). The hsSBR releasing SOP was higher than the cSBR by 24%.

The Gly in both reactors decreased in the same fashion as the HAc and SOP, rapid in the 60 min then slow down in the later hours. Overall, Gly in the hsSBR was lower than that in the cSBR

Table 4
The anaerobic transformations during one cycle in hsSBR and cSBR.

Item	hsSBR	cSBR
SOP release (mmol P/g MLVSS)	1.68	1.28
Gly degradation (mmol C/g MLVSS)	1.47	2.17
PHB synthesis (mmol C/g MLVSS)	5.56	4.73
PHV synthesis (mmol C/g MLVSS)	1.05	1.37
Gly-degradation/VFA uptake (mol C/mol C)	0.20	0.38
SOP release/PHA synthesis (mol P/mol C)	0.25	0.21

Table 5
The aerobic transformations during one cycle in hsSBR and cSBR.

Item	hsSBR	cSBR
SOP uptake (mmol P/g MLVSS)	1.98	1.45
Gly synthesis (mmol C/g MLVSS)	2.65	2.88
PHB degradation (mmol C/g MLVSS)	5.64	4.81
PHV degradation (mmol C/g MLVSS)	1.04	1.46
Gly synthesis/PHA degradation (mol C/mol C)	0.40	0.46
SOP uptake/PHA degradation (mol P/mol C)	0.30	0.23

for about 2.5 folds. The Gly degraded in the hsSBR and cSBR was respectively 1.47 mmol C/g MLVSS and 2.17 mmol C/g MLVSS. Also, PHB and PHV changed rapidly in the first 60 min and stabilized in the later. Overall, PHB in the two reactors were quite similar, although the total PHB synthesized in the hsSBR was more than that in the cSBR (5.56 and 4.73 mmol C/g MLVSS) (Table 4). Unlike PHB, PHV in the hsSBR and cSBR reactors was very low; it only represented 16% and 22.5% of total PHAs in the hsSBR and cSBR, respectively. This finding is supported by the observation of Lemos et al. [20]. It should be note that the total PHV synthesized in the hsSBR was 1.05 mmol C/g MLVSS, which was less than that in the cSBR with the value of 1.37 mmol C/g MLVSS (Table 4). According to Zeng et al. [7], the anaerobic SOP release and the subsequent aerobic SOP uptake are the indicators for the presence of PAOs, while the presence of PHV in the cycle could be an indicator for the presence and functioning of GAOs. Hence, these results indicate that PAOs were dominant organism in both hsSBR and cSBR. However, the Gly degradation/HAc uptake ratio in the hsSBR was 47% lower than in the cSBR, and the corresponding SOP release/PHA synthesis ratio in the hsSBR was 19% greater than in the cSBR (Table 4). It is well known that GAOs degrade only cellular Glycogen to supply both ATP and reducing equivalents for converting HAc to PHA, but not degrade poly-P for PHA synthesis. Therefore, a lower Gly degradation/VFA uptake ratio and a grater SOP release/PHA synthesis ratio indicated that there were relatively more PAOs and less GAOs in the hsSBR.

Numerous environmental and operational parameters, such as pH, type of volatile fatty acids (VFAs) in the influent, SOP to VFA ratio, and temperature, have been identified as key factors to leverage the competition between PAOs and GAOs. In this study, the variations of pH in a cycle were ranged 7.12–8.16 in the hsSBR, for the cSBR, the pH varied between 7.14 and 8.19. The initial pH in anaerobic phase in the hsSBR had a slightly lower value than that in the cSBR. It is due to the fact that the pH of the return sludge from HSR was lower. However, the cSBR and hsSBR had almost the same pH value at the end of anaerobic phase. At any given time, the differences in pH values between two reactors were less than 0.2. Thus the pH should not be the main reason for the difference in PAOs and GAOs between the hsSBR and cSBR. The type of VFA in the influent, and temperature should not be the reason, because they were the same in these two reactors. Therefore, the difference in fractions of PAOs and GAOs in the two processes should only be explained by the effect of the HSR. It is highly possible that the environment of the HSR was favored by the PAOs, therefore the percentage of PAOs in the HSR was increased. As a result, by returning the mixed liquor of the HSR back to the hsSBR, the PAOs in the hsSBR was enriched. Liu et al. [21] has been reported that *Rhodocyclus*, a known PAO, was a dominant bacterium in humus soil activated sludge process.

As shown in Fig. 4, SOP release rate in the first 30 min in the hsSBR was higher than that in the cSBR. This was consistent with HAc uptake and PHB synthesis rates in the stage were high in the hsSBR compared with that in the cSBR. This confirmed that PAOs percentage in the hsSBR was higher than the cSBR. It has been reported that the maximum HAc uptake rate of PAOs (i.e. *Accumulibacter*) was higher than GAOs (i.e. *Competibacter*) [12], more PAOs and less GAOs in the hsSBR improved these rates. In the following 30 min, SOP release rate and PHB synthesis rate were higher in the cSBR.

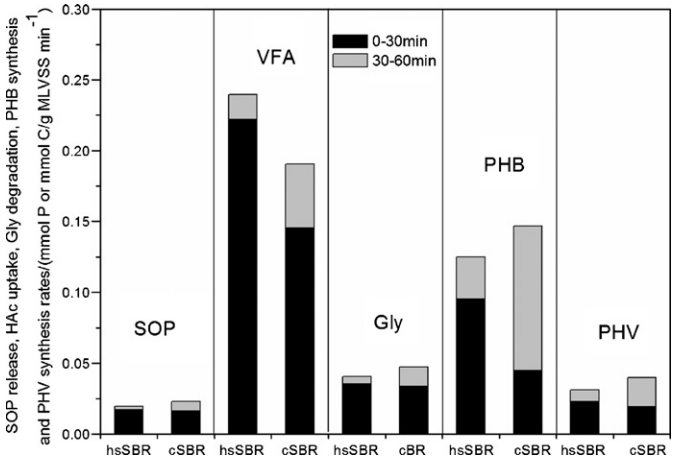

Fig. 4. SOP release, HAc uptake, Gly degradation, PHB and PHV synthesis rates in the initial anaerobic time. Since these major components changed dramatically only in the initial 60 min (Fig. 3), all these rates were calculated based on this period of time of anaerobic phase.

3.4. Aerobic transformations comparison between hsSBR and cSBR

As shown in Fig. 3, HAc in both reactors were near zero in the entire aerobic phase. The SOP concentration in both reactors decreased steadily until being stabilized to a low level at around 180 min. It indicated that the SOP was taken up effectively by the PAOs in both reactors. The aerobic SOP uptake in the hsSBR and cSBR was 4.09 and 3.00 mmol P/g MLVSS, respectively (Table 5). The Gly concentration in both reactors increased sharply in the first 60 min and then gradually increased until the end of the aerobic phase. The Gly synthesized in the hsSBR in this phase was less than that in the cSBR (2.65 mmol C/g MLVSS versus 2.88 mmol C/g MLVSS) (Table 5). The PHAs (PHB and PHV) in both reactors decreased steadily to a low level at approximately 180 min. However, the degradation of PHB was much greater than PHV in both reactors. The amounts of PHB degraded in these two reactors were 5.64 and 4.81 mmol C/g MLVSS, respectively, and the amounts of PHV degraded were 1.05 and 1.46 mmol C/g MLVSS respectively (Table 5). These results suggested that aerobic SOP uptake was more in correspondence with PHB degradation. Filipe and Daigger [22] reported that high SOP uptake in the aerobic stage was related to high PHA degradation. Randall and Liu [23] also reported that SOP uptake was influenced by the PHAs composition at the beginning of the aerobic phase, and the higher PHB percentages imply the higher SOP uptake.

The PHA utilization efficiency for SOP uptake and Gly synthesis during aerobic phase could be further expressed by the ratio of SOP uptake to PHA degradation [24] and the ratio of Gly synthesis to PHA degradation. The SOP uptake/PHA degradation in the hsSBR was 27% greater than that in the cSBR, and the corresponding Gly synthesis/PHA degradation ratio in the hsSBR was 13.3% lower than that in the cSBR. It indicated that the microorganism in the hsSBR had a higher PHA utilization efficiency for SOP uptake and lower PHA utilization efficiency for Gly synthesis than those in the cSBR.

As shown in Fig. 5, the hsSBR had a higher SOP uptake rate than cSBR in both 0–30 min period and 30–60 min period. The corresponding Gly synthesis rate and PHAs degradation rates in the hsSBR were lower than those in the cSBR in both time zones, except the PHB synthesis rate in 0–30 min period. This observation indicated that the high SOP uptake rate was favored or companied with the low rates of Gly synthesis and PHAs degradation. As mentioned

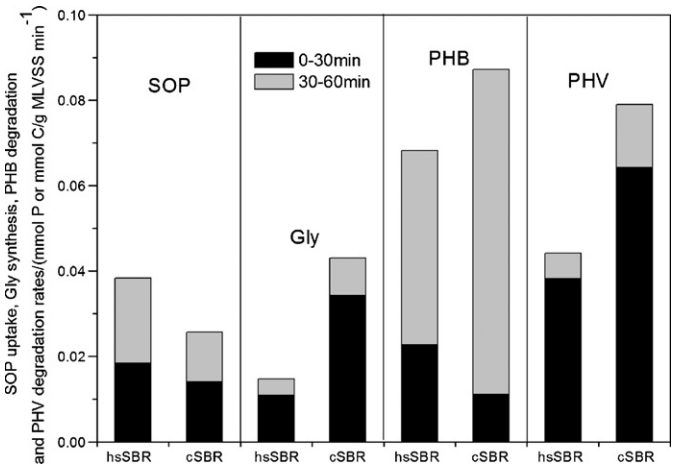

Fig. 5. SOP uptake, Gly synthesis, PHB and PHV degradation rates in the initial aerobic time. Since these major components changed dramatically only in the initial 60 min (Fig. 3), all these rates were calculated based on this period of time of aerobic phase.

above, the other conditions such as pH, temperature and characteristic of the influent were similar, thus the discrepancy in SOP uptake rate between the two reactors must be only reflected by the abundance of PAOs.

4. Conclusion

The efficiency of SOP removal in the hsSBR was 97.3% compared with the removal efficiency of 80% in the cSBR. During the anaerobic phase, the hsSBR showed greater SOP release and PHB synthesis capacity, but lower PHV synthesis and Gly degradation capacity than the cSBR. In addition, HAc uptake rate and SOP release rate in the hsSBR were greater than those in the cSBR. In the aerobic phase, the hsSBR showed greater SOP uptake and lower Gly synthesis capacity than the cSBR. Further, the PHA utilization efficiency for SOP uptake in the hsSBR was higher. All these observations suggested that adding the HSR improved the relative dominance of the PAOs in the hsSBR.

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