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RESEARCH ARTICLE

Temperature influence on biological phosphorus removal induced by aerobic/extended-idle regime

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Abstract Previous researches have demonstrated that biological phosphorus removal (BPR) from wastewater could be driven by the aerobic/extended-idle (A/EI) regime. This study further investigated temperature effects on phosphorus removal performance in six A/EI sequencing batch reactors (SBRs) operated at temperatures ranging from 5 to 30 °C. The results showed that phosphorus removal efficiency increased with temperature increasing from 5 to 20 °C but slightly decreased when temperature continually increased to 30 °C. The highest phosphorus removal rate of 97.1 % was obtained at 20 °C. The biomass cultured at 20 °C contained more polyphosphate accumulating organisms (PAO) and less glycogen accumulating organisms (GAO) than that cultured at any other temperatures investigated. The mechanism studies revealed that temperature affected the transformations of glycogen and polyhydroxyalkanoates, and the activities of exopolyphosphatase and polyphosphate kinase activities. In addition, phosphorus removal performances of the A/EI and traditional anaerobic/oxic (A/O) SBRs were compared at 5 and 20 °C, respectively. The results showed the A/EI regime drove better phosphorus removal than the A/O regime at both 5 and 20 °C, and more PAO and less GAO abundances in the biomass might be the principal reason for the higher BPR in the A/EI SBRs as compared with the A/O SBRs.

Keywords Temperature influence · Biological phosphorus removal · Aerobic/extended-idle regime · Sequencing batch reactor · Wastewater treatment

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Introduction

Excess phosphorus (P) in wastewater can result in eutrophication, which has become a severe water pollution problem all around the world. Compared to chemical methods, enhanced biological phosphorus removal (EBPR) is an economical and sustainable process to remove P from wastewater due to its low operation costs (van Loosdrecht et al. 1997). The microorganisms responsible for EBPR processes are known to be polyphosphate accumulating organisms (PAO), which can take up P and accumulate it as intracellular polyphosphate through alternating anaerobic and aerobic conditions (van Loosdrecht et al. 1997). However, such conditions also favor another group of microorganisms known as glycogen accumulating organisms (GAO) which compete with PAO for the available organic substrate without contributing to P removal (Zeng et al. 2002). It has been suggested that EBPR deterioration is often attributed to the presence of GAO (Oehmen et al. 2006).

Temperature is one of the key parameters affecting the performance of EBPR systems due to its impact on the PAO-GAO competition (Oehmen et al. 2007). Early investigators reported partial or complete loss of EBPR functions at low temperatures (Brdjanovic et al. 1997; Beatons et al. 1999). Although most studies agreed that lower temperature caused the decrease in biochemical transformations which was detrimental to EBPR (Brdjanovic et al. 1998), some contradictory results were still documented in the literature. In some studies, low temperatures were found to improve EBPR performance

(Whang and Park 2002; Erdal et al. 2003; Panswad et al. 2003), and the improved performance was proven to be the result of the microbial community shift from GAO to PAO (Erdal et al. 2005). Lopez-Vazquez et al. (2009) reported that PAO was the dominant microorganisms at 10 °C since the metabolism of GAO was inhibited. At 20 °C, the simultaneous presence of acetate and propionate favored the growth of PAO over GAO, while PAO was favored over GAO only at a high pH (7.5) condition when either acetate or propionate was supplied as sole carbon source (Lopez-Vazquez et al. 2009). And at 30 °C, GAO tended to dominate the competition with the higher substrate uptake rates when a single carbon source was provided (Lopez-Vazquez et al. 2009).

It has been proven that BPR from wastewater can be well achieved by the aerobic/extended-idle (A/EI) regime (Wang et al. 2012a). Compared with the current anaerobic/oxic (A/O) process, a strict anaerobic phase was not needed but the idle period was extended to 210–450 min (Wang et al. 2012b). The previous studies upon the A/EI regime mainly focused on the inducing mechanism, external carbon source effects, and optimal control parameters such as initial pH value (Wang et al. 2012a, b, 2013). However, previous studies did not investigate the temperature effect on the PAO-GAO competition in such new phosphorus removal regime, and BPR induced by the A/EI regime under frigid climate conditions, such as 5 °C, has never been reported. In view of the fact that the A/EI regime has no specific anaerobic period but extends the idle phase, the microbial metabolic pathway is different (Wang et al. 2012a), which may cause different behavior in terms of temperature influence on BPR performance as compared with the A/O process. Thus, there is a clear need to study temperature effects on BPR induced by the A/EI regime in order to obtain a better understanding of this new wastewater phosphorus removal regime and to get a better prediction of the process performance.

The purpose of this study was to investigate temperature influence on BPR induced by the A/EI regime. First, BPR performance in six A/EI sequencing batch reactors (SBRs) operated at temperatures ranging from 5 to 30 °C was compared. Then, BPR was linked to PAO-GAO competition, key enzyme activities, and intracellular biochemical transformations to explore the mechanism for temperature effects. Finally, BPR performance between the A/EI and A/O SBRs at the optimal temperature as well as cold temperature (5 °C) were also compared.

Material and methods

SBR operation at different temperatures

Experiments were performed in six lab-scale SBRs each with a working volume of 2 L. Seed sludge was inoculated into the six SBRs. The temperatures in the six SBRs were respectively kept constant at 5, 10, 15, 20, 25, and 30 °C, by putting them into constant temperature incubators and pumping water with the desired temperatures through tubes. Aeration and mixing were supplied through an air diffuser placed in the bottom of the SBRs at an airflow rate of 2 L/min, which resulted in 4 mg/L of dissolved oxygen at the end of the aerobic phase. The pH value was controlled at 7.0±0.1 by dosing 1 M NaOH or 1 M HCl. All SBRs were operated sequentially in 8-h cycle. The cycling profile comprised 210 min aeration, followed by 55 min settling, 5 min decanting, and 210 min idle periods. Of mixed liquor, 0.25 L was discharged from each reactor daily at the end of aerobic zone but before settling, resulting in the sludge retention time (SRT) of 8 days. The hydraulic retention time (HRT) was 16 h.

The synthetic medium used as influent contained 40 mg ammonia nitrogen (NH_4^+-N)/L, 15 mg PO_4^{3-}-P/L, and 300 mg/L of COD. Acetate was used as the sole carbon source because it was the most common volatile fatty acid (VFA) present in domestic wastewater (Chen et al. 2004). The concentrations of other nutrients in the synthetic medium were presented as below: 0.005 g/L $CaCl_2$, 0.01 g/L $MgSO_4 \cdot 7H_2O$, and 0.5 mL/L trace element solution (Zheng et al. 2011).

Comparison of BPR performance between the A/EI and A/O regimes at 5 and 20 °C

The investigation was carried out in four reproductive SBRs (A/EI-5 °C, A/O-5 °C, A/EI-20 °C, and A/O-20 °C) each with a working volume of 2 L. The biomass was adapted to the A/EI and A/O processes, respectively. Each cycle of the A/O-SBRs consisted of 120 min anaerobic mix and 180 min aeration, followed by 55 min settling, 5 min decanting, and 120 min idle periods according to the literature (Tong and Chen 2009). Of the supernatant, 1 L was discharged from the A/O-SBRs after settling and was replaced with 1 L of the synthetic medium at the end of the idle period. The A/O-SBRs were mixed with a magnetic stirrer during the anaerobic period. During the aerobic time, air was supplied into the A/O-SBRs at a flow rate of 2 L/min. The pH value was controlled at 7.0±0.1 by dosing 1 M NaOH or 1 M HCl. The HRT in the SBRs was 16 h, while the SRT was maintained at 8 days. The synthetic medium and operation of the A/EI-SBRs were the same as that described in Section 2.1. A/EI-5 °C and A/O-5 °C were operated at 5 °C, and temperatures in A/EI-20 °C and A/O-20 °C were controlled at 20 °C. The optimal temperature of 20 °C for the A/EI regime was selected according to the above test, while that for the A/O regime was chosen according to the literature (Brdjanovic et al. 1998).

Analytical methods

Soluble orthophosphate (SOP), NH_4^+-N, nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), acetate, total suspended solids (TSS), and volatile suspended solids (VSS) were measured according to the standard methods (APHA 1998). Glycogen was measured according to the methodology described in the literature (Wang et al. 2012a). One milliliter test mixture and 0.2 ml saturated $HgCl_2$ were added to a heat-resistant tube and heated with 80 °C water for 20 min. The cooled samples were centrifuged at a speed of 2,800 r/min for 20 min, and 1 ml distilled water, 1 ml 5 % phenol, and 5 ml thick sulfuric acid were added in the centrifugal sludge. The samples were measured by spectrophotometry method at 490 nm.

Polyhydroxyalkanoates (PHAs) were measured with an Agilent 7890A Gas Chromatography (GC). Of the sludge samples, 50 mL were mixed with formaldehyde at a ratio of 1 % formaldehyde per sample volume to inhibit biomass activity. The samples were centrifuged and the supernatant was removed. Then, the samples were washed with phosphate buffer solution, recentrifuged, and the supernatant was decanted once more. All samples were lyophilized through a freeze drying unit operated at −58 °C and 0.1 mbar for at least 18 h. Approximately 20 mg of lyophilized sludge samples were added to 2 mL of chloroform and 2 mL of an acidified methanol solution, and the samples were digested in tightly at 100 °C in an oven for 6 h and then cooled to room temperature.

Of the distilled water, 2 mL was mixed with each sample to remove particulate debris from the chloroform phase. Two hours of setting time was allowed to achieve phase separation. The chloroform phase was injected into the GC column, the chromatography was operated with a DB-5 column (30 m length×0.25 mm LD×0.25 μm film), a spilt injection ratio of 1:15 and helium as the carrier gas (1.5 mL·min^{-1}). A flame ionization detection (FID) unit was operated at 300 °C with an injection port temperature of 250 °C. The oven temperature was set to 80 °C for 1 min and increased to 120 °C at 10 °C/min, and then to 270 °C at 45 °C/min and held for 3 min. The PHAs were calculated as the total amount of poly-3-hydroxybutyrate, poly-3-hydroxyvalerate, and poly-3-hydroxy-2-methylvalerate.

4′, 6′-Diamidino-2-phenylindole dihydrochloride (DAPI) staining was carried out to analyze the presence of intracellular polyphosphate (poly-P) granules (Mullan et al. 2006). Sludge samples taken at the end of the aerobic period were used for staining. Fluorescence in situ hybridization (FISH) technique was the same as described by Carvalho et al. (2007), and the analysis of exopolyphosphatase (PPX) and polyphosphate kinase (PPK) activities was the same as described by Zheng et al. (2011).

Results and discussion

Effect of temperature on COD and nitrogen removal in the A/EI regime

It took 40 days for the SBRs to reach steady-state, when COD and total nitrogen (TN) removal efficiencies of all SBRs were above 80 %. COD and TN removal performance of the SBRs operated at different temperatures during steady-state operation was shown in Table 1. Along with temperature increased from 5 to 30 °C, COD and TN removal efficiencies were improved. In this study, 40 mg/L NH_4^+-N was contained in influent, but low levels of effluent NH_4^+-N were detected and low concentrations of NO_2^--N and NO_3^--N were accumulated. The results suggested that nitrogen removal performance was achieved successfully in the A/EI regime, though there was no specific anoxic phase in this wastewater treatment regime.

Effect of temperature on phosphorus removal in A/EI regime

Table 2 summarized the SOP removal performance of the SBRs operated at different temperatures during steady-state operation. The profiles of SOP in the SBRs were shown in Fig. 1. SOP uptake in the oxic stage and SOP release in the extended-idle period were observed in all SBRs, with the highest SOP concentration of mixed liquor during the extended-idle period reaching 12.1 mg/L at 20 °C (Fig. 1). From Table 2, it can be seen that the highest SOP removal efficiency and SOP uptake per gram of VSS were 97.1 % and 0.189±0.017 mmol-P/g-VSS, respectively, both of which were obtained at 20 °C. In contrast, activated sludge exhibited compromised SOP uptake ability at 15 and 25 °C. The data of SOP removal efficiencies showed that BPR performance depended strongly on temperature. The optimum temperature for BPR induced by A/EI regime was proven to be 20 °C.

The effects of temperature on the stoichiometry among P release (P-rel/VFA), PHA syntheses (PHA-syn), and glycogen degradation (Gly-de) in the conventional A/O regime reported by other researchers and the A/EI regime measured in this study were compared in Table 3. It can be seen that temperature obviously affected the cyclic transformations of intracellular storages. For example, with temperature ranging from 5 to 20 °C, glycogen accumulation/VFA in the aerobic stage decreased from 0.52 to 0.26 mmol-C/mmol-C, and glycogen degradation in the idle period decreased from 0.41 to 0.24 mmol-C/g-VSS. In the meanwhile, the aerobic SOP uptake and idle SOP release rates increased from 0.042 and 0.025 to 0.054 and 0.042 mmol-P/g-VSS·h, respectively. When temperature further increased to 30 °C, the data of glycogen accumulation/VFA and glycogen degradation respectively increased to 0.51 mmol-C/mmol-C and 0.43 mmol-C/g-VSS while the aerobic SOP uptake and idle

Table 1 Summary of COD and nitrogen removal performance of the SBRs during steady-state operation (results are averages and their standard deviations in triplicate tests)

Temperature (°C)	*N*				COD	
	Effluent NH_4^+-N (mg/L)	Effluent NO_2^--N (mg/L)	Effluent NO_3^--N (mg/L)	TN removal efficiency (%)	Effluent COD (mg/L)	COD removal efficiency (%)
5	4.16±0.42	1.24±0.12	1.46±0.14	82.9±2.7	40.5±3.7	86.5±3.2
10	3.72±0.28	1.03±0.11	1.42±0.11	84.6±3.1	34.3±2.8	86.6±3.4
15	2.71±0.11	0.64±0.11	1.41±0.16	88.1±2.6	33.7±2.6	88.7±3.7
20	1.68±0.16	0.39±0.08	1.38±0.13	91.4±3.2	31.4±3.1	89.5±4.1
25	1.52±0.19	0.32±0.05	1.29±0.14	92.1±3.5	30.8±2.4	89.7±4.2
30	1.48±0.14	0.37±0.07	1.31±0.16	92.1±2.8	28.7±3.2	90.4±4.3

SOP release rates decreased to 0.49 and 0.036 mmol-P/g-VSS·h, respectively.

Figure 2 showed the DAPI staining results of the sludge samples taken from the SBRs operated at different temperatures. From Fig. 2, more blue areas were found in Fig. 2d than those in other pictures, suggesting that more poly-P granules were accumulated in the 20 °C SBR, which was consistent with the higher SOP release and uptake rates outlined in Table 2.

These results clearly showed that though good BPR performance could be achieved in all SBRs, the BPR efficiency in the SBR operated at 20 °C was the highest. Since temperature influence on BPR in the A/EI regime has never been investigated before, it was necessary to study the reasons for driving higher BPR at 20 °C.

Mechanism of driving high phosphorus removal at 20 °C

As usual, microbial competition of GAO against PAO is considered to be mainly responsible for the deterioration of phosphorus removal in EBPR plants (Oehmen et al. 2007). It has been demonstrated that the composition of PAO and GAO in BPR sludge shifted with the variation of temperature (Brdjanovic et al. 1998; Oehmen et al. 2007). For this reason, quantitative analysis of PAO and GAO in activated sludge was carried out via FISH technology (Table 2). The quantitative analysis showed that the abundances of PAO and GAO accounted for 31.7 and 11.5 % of total biomass at 20 °C, respectively, with PAO approximately three times more than GAO. By contrast, the differences between PAO and GAO abundances were relatively smaller in other SBRs. For example, when temperature exceeded 20 °C, GAO tended to become strong competitor against PAO, suggesting the increased GAO proliferation at high temperatures. Now one question may arise as why temperature of 20 °C provided PAO advantages over GAO?

It is well known that BPR is dependent on SOP release and uptake to a great extent, which is directly related to the activities of PPX and PPK, respectively (Mino et al. 1998). Poly-P is built up from ATP by PPK, and poly-P hydrolysis occurs either by the reverse PPK reaction leading to ATP formation from ADP or via hydrolysis by PPX (Lee et al. 2006). Therefore, the variations of PPX and PPK activities will certainly affect BPR performance. The experimental results showed that temperature influenced the activities of enzymes relevant to SOP release and uptake. As shown in Table 4, compared with that in other SBRs, activated sludge in the SBR operated at 20 °C showed higher specific activities of

Table 2 Summary of phosphorus removal performance of the SBRs during steady-state operation (Results are averages and their standard deviations in triplicate tests)

Temperature (°C)	MLSS (mg/L)	MLVSS (mg/L)	P			Bacterial population[a]	
			Effluent SOP (mg/L)	SOP removal efficiency (%)	SOP uptake (mmol/g-VSS)	PAO (%)	GAO (%)
5	2,916±228	2,129±197	2.83±0.15	80.9±2.4	0.147±0.016	20.3±1.6	18.9±1.2
10	3,166±254	2,248±223	1.64±0.13	88.9±2.1	0.134±0.015	24.1±2.1	15.7±1.4
15	3,028±236	2,271±218	0.71±0.11	95.3±2.0	0.179±0.026	25.7±2.3	13.4±1.2
20	3,119±225	2,246±215	0.43±0.07	97.1±1.2	0.189±0.017	31.7±2.6	11.5±1.1
25	2,965±213	2,153±206	0.72±0.09	95.1±1.6	0.186±0.025	28.8±2.4	14.8±1.2
30	3,038±237	2,218±224	1.18±0.19	93.0±2.7	0.172±0.014	21.6±1.3	16.2±1.3

[a] Percentage to all bacteria (EUBmix probe). The values are averages and their standard deviations of three cyclic studies

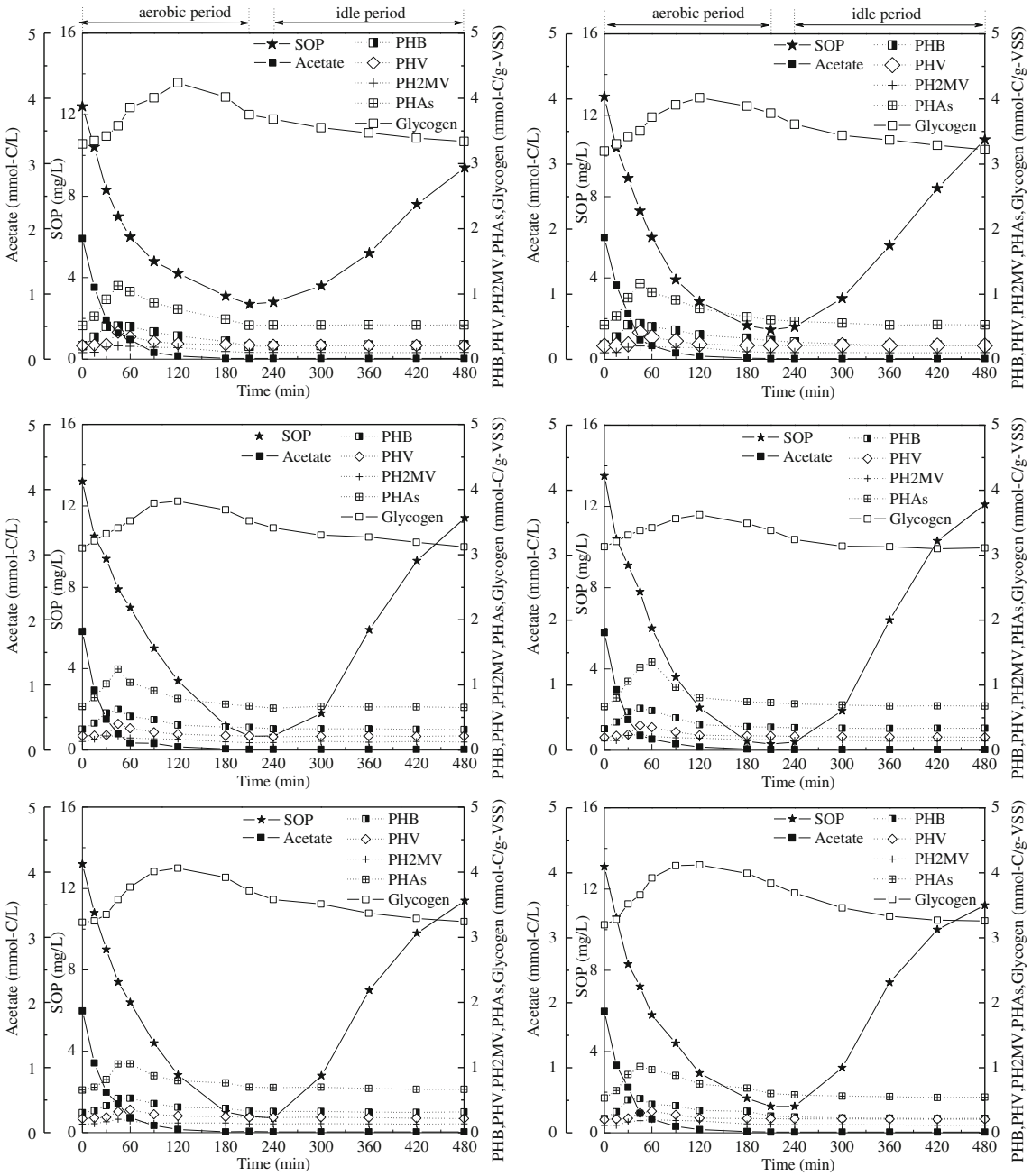

Fig. 1 Variations of SOP, acetate, and intracellular PHAs as well as sludge glycogen during a typical cycle of the SBRs operated at 5 (**a**), 10 (**b**), 15 (**c**), 20 (**d**), 25 (**e**), and 30 °C (**f**). The data reported are the averages and their standard deviations in triplicate tests

PPX and PPK, which was in correspondence with the observed higher SOP release and uptake (Table 2). These results suggested that 20 °C could cause high SOP release and uptake, which thereby resulted in high SOP removal.

Besides, BPR was reported to be closely related to the transformations of intermediate metabolites including intracellular glycogen and PHAs (Mino et al. 1998; Smolders et al. 1994a). From Fig. 1, it can be seen that acetate was fully depleted accompanied by the accumulation of intracellular glycogen and PHAs during 30–60 min of the aeration whereas no obvious SOP release was detected. After acetate was consumed, PHAs degradation and SOP uptake were observed concurrently. However, compared with other SBRs, the SBR at 20 °C showed higher variations of PHAs but lower intracellular glycogen accumulation (the average aerobic glycogen accumulation of the SBRs were 0.51 mmol-C/g-VSS). The transformations of intracellular glycogen and PHAs were found to be associated with PAO and GAO activities, since it was reported that high glycogen accumulation indicated the activated GAO metabolism (Mino et al. 1998). Therefore, the lower endogenous glycogen accumulation measured at 20 °C suggested that the biomass cultured in the activated sludge

Table 3 Comparison of the stoichiometric parameters between AEI and A/O SBRs (this study and previous studies; results are averages and their standard deviations in triplicate tests)

Reference	Temperature (°C)	A/O reactor					A/EI reactor						SRT	Substrate type
		Anaerobic transformations			Aerobic transformations		Aerobic transformations			Idle transformations				
		Gly-de/VFA (mmol-C/mmol-C)	PHA-syn/VFA (mmol-C/mmol-C)	P-rel/VFA (mmol-P/mmol-C)	Gly-syn (mmol-C/g-VSS)	P uptake rate (mmol-P/g-VSS·h)	Gly/VFA (mmol-C/mmol-C)	PHA/VFA (mmol-C/mmol-C)	P uptake rate (mmol-P/g-VSS·h)	Gly-de (mmol-C/g-VSS)	PHA (mmol-C/g-VSS)	P release rate (mmol-P/g-VSS·h)		
This study	5	0.64	1.04	0.14	1.22	0.12	0.52	0.42	0.042	0.41	0.01	0.025	8	Acetate
	10	–	–	–	–	–	0.51	0.31	0.047	0.39	0.04	0.033	8	Acetate
	15	–	–	–	–	–	0.41	0.36	0.051	0.33	0.01	0.038	8	Acetate
	20	0.43	1.51	0.22	1.02	0.19	0.26	0.42	0.054	0.24	0.03	0.042	8	Acetate
	25	–	–	–	–	–	0.46	0.26	0.053	0.34	0.03	0.040	8	Acetate
	30	–	–	–	–	–	0.51	0.34	0.049	0.43	0.03	0.036	8	Acetate
Lopez et al. (2007)[a]	20	1.20	1.97	0.01	1.24	0.20	–	–	–	–	–	–	8	Acetate
Lopez et al. (2009)[a]	10	0.55	1.44	0.56	1.84	0.02	–	–	–	–	–	–	16	Acetate
	15	1.23	1.81	0.03	1.86	0.09	–	–	–	–	–	–	24	Acetate
	20	1.21	1.88	0.03	1.85	0.19	–	–	–	–	–	–	8	Acetate
	30	1.68	2.33	0.01	1.86	0.17	–	–	–	–	–	–	8	Acetate
	35	1.12	1.91	0.01	1.82	0.21	–	–	–	–	–	–	16	Acetate
	40	1.32	2.12	0.00	1.84	0.00	–	–	–	–	–	–	16	Acetate
Lu et al. (2006)	20-24	1.26	0.46	0.62	–	–	–	–	–	–	–	–	8	Acetate/Propionate
Smolders et al. (1994a)	20	1.30	–	0.60	–	–	–	–	–	–	–	–	8	Acetate
Smolders et al. (1994b)	20	0.50	1.33	0.50	–	–	–	–	–	–	–	–	8	Acetate
Zeng et al. (2002)[a]	22	1.12	1.85	0.00	–	–	–	–	–	–	–	–	7	Acetate
Wang et al. (2012a)	20-24	1.16	0.47	0.41	1.83	0.39	0.46	0.52	0.03	0.57	0.38	0.022	13	Propionate

[a] Operated with the enriched cultures of GAOs

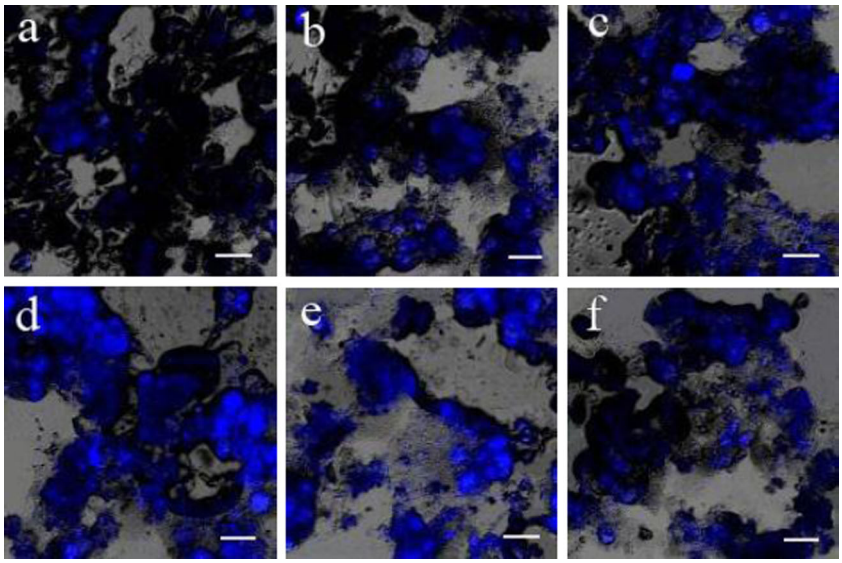

Fig. 2 Micrographs of DAPI staining of the activated sludge samples withdrawn at the end of aeration in the SBRs operated at 5 (**a**), 10 (**b**), 15 (**c**), 20 (**d**), 25 (**e**), and 30 °C (**f**). *Bar* 5 μm

might contain less GAO, which correlated well with the results of FISH analysis (Table 2). Moreover, compared with other SBRs, more SOP was released (about 11.6 mg/L) but less glycogen was degraded (0.27 mmol-C/g-VSS) during the idle stage of the SBR operated at 20 °C. These idle transformations revealed that the energy for bacterial maintenance during the extended-idle period seemed to be mainly provided by poly-P hydrolysis in the SBR at 20 °C, but to be provided by both poly-P hydrolysis and glycogen degradation in other SBRs. The higher energy required from poly-P degradation in the 20 °C SBR would enhance the role of poly-P participating in PAO metabolism. In other words, the intracellular biochemical transformations at 20 °C correlated well with PAO metabolism, which thereby provided PAO advantage over other populations.

According to the results obtained from our current study, it was clear shown that with the increase from 5 to 20 °C an increase of the PAO abundance and decrease of the GAO abundance were observed, which was not consistent with previous studies testing with the A/O regime (Erdal et al. 2003; Lopez-Vazquez et al. 2009). Erdal et al. (2003) reported that in the conventional A/O regime, biomass acclimatized to 5 °C showed higher P removal and lower glycogen transformation as compared to 20 °C, indicating that the low temperatures favored the growth of PAO over GAO. GAO could not compete with PAO at temperatures below 20 °C because of its lower acetate uptake and biomass production rates, but at temperatures above 20 °C GAO competed with PAO due to its higher acetate uptake rate (Lopez-Vazquez et al. 2009). Thus, one might want to know why 20 °C could provide PAO advantages over GAO in the A/EI regime. From the above text, we could conclude that the transformations of intermediate metabolites including poly-P, intracellular PHAs and glycogen were different from the current A/O processes. And the energy for bacterial maintenance during the extended-idle period was mainly provided by poly-P hydrolysis in the SBR at 20 °C, but was provided by both poly-P hydrolysis and glycogen degradation in other SBRs. As a result, a higher aerobic synthesis and idle degradation of glycogen and a lower P uptake rate were detected at low temperatures, which increased GAO abundance and decreased PAO abundance. Furthermore, it can be observed from Table 4 that lower activities of PPX and PPK were obtained at both 5 and 30 °C as compared to 20 °C, and the PPX and PPK activities at low temperature (5 °C) were even lower than that at high temperature (30 °C). With the decrease of PPX and PPK activities, P release and uptake rates also decreased (Table 3). Therefore, the inhibition effect of temperature on enzyme activities might also contribute to the decrease of PAO population.

Table 4 Activities of the key enzymes related to BPR in the SBRs (the data reported are the averages and their standard deviations in triplicate tests)

Temperature	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
PPX[a]	0.009±0.003	0.014±0.004	0.020±0.003	0.022±0.002	0.018±0.003	0.012±0.003
PPK[b]	0.113±0.004	0.168±0.005	0.279±0.003	0.289±0.005	0.283±0.003	0.219±0.003

[a] The unit is micromole pnitrophenol/(per minutes per milligram protein)

[b] The unit is micromole NADPH/(per minutes per milligram protein)

Table 5 Summary of reactor performances and bacterial population in of A/EI and A/O SBRs during steady-state operation (results are averages and their standard deviations)

Temperature (°C)	Regime	P			N					Bacterial population[a]	
		Effluent SOP (mg/L)	SOP removal efficiency (%)	SOP uptake rate (mmol-P/g-VSS·h)	Effluent NH_4^+-N (mg/L)	Effluent NO_2^--N (mg/L)	Effluent NO_3^--N (mg/L)	TN removal efficiency (%)	TN uptake rate (mmol-N/g-VSS·h)	PAO (%)	GAO (%)
5	A/EI	2.74±0.19	81.5±2.1	0.052±0.007	4.01±0.36	1.11±0.13	1.66±0.13	83.1±1.6	0.32±0.03	21.4±1.2	17.1±1.3
5	A/O	5.27±0.32	64.4±2.7	0.043±0.005	4.23±0.41	1.73±0.17	2.23±0.15	79.5±1.2	0.31±0.05	18.1±1.1	17.7±1.2
20	A/EI	0.41±0.07	97.2±1.1	0.061±0.006	1.49±0.12	0.29±0.08	1.39±0.12	92.1±1.0	0.34±0.02	32.7±2.4	11.2±1.1
20	A/O	1.01±0.16	93.2±1.3	0.059±0.005	2.24±0.23	0.24±0.13	1.24±0.13	90.7±1.6	0.34±0.03	25.4±1.3	19.8±1.2

[a] Percentage to all bacteria (EUBmix probe). The values are average and standard deviation

Comparison of BPR performance between the A/EI and A/O regimes at 5 and 20 °C

According to the above studies, the optimal temperature for BPR in the A/EI regime was demonstrated to be 20 °C, which was also reported to be the optimal temperature for EBPR induced by the A/O regime in some literatures (Panswad et al. 2003; Lopez-Vazquez et al. 2009). For this reason, it was interesting to compare the BPR performance between the A/EI and A/O regimes under their optimal temperature. Furthermore, successful EBPR operation has been observed at very low temperatures, even 5 °C (Brdjanovic et al. 1998; Erdal et al. 2003). A higher phosphorus, removal capacity and a corresponding decrease in glycogen transformations were found from biomass acclimatized to 5 °C as compared to 20 °C (Erdal et al. 2003). Therefore, it is necessary to compare

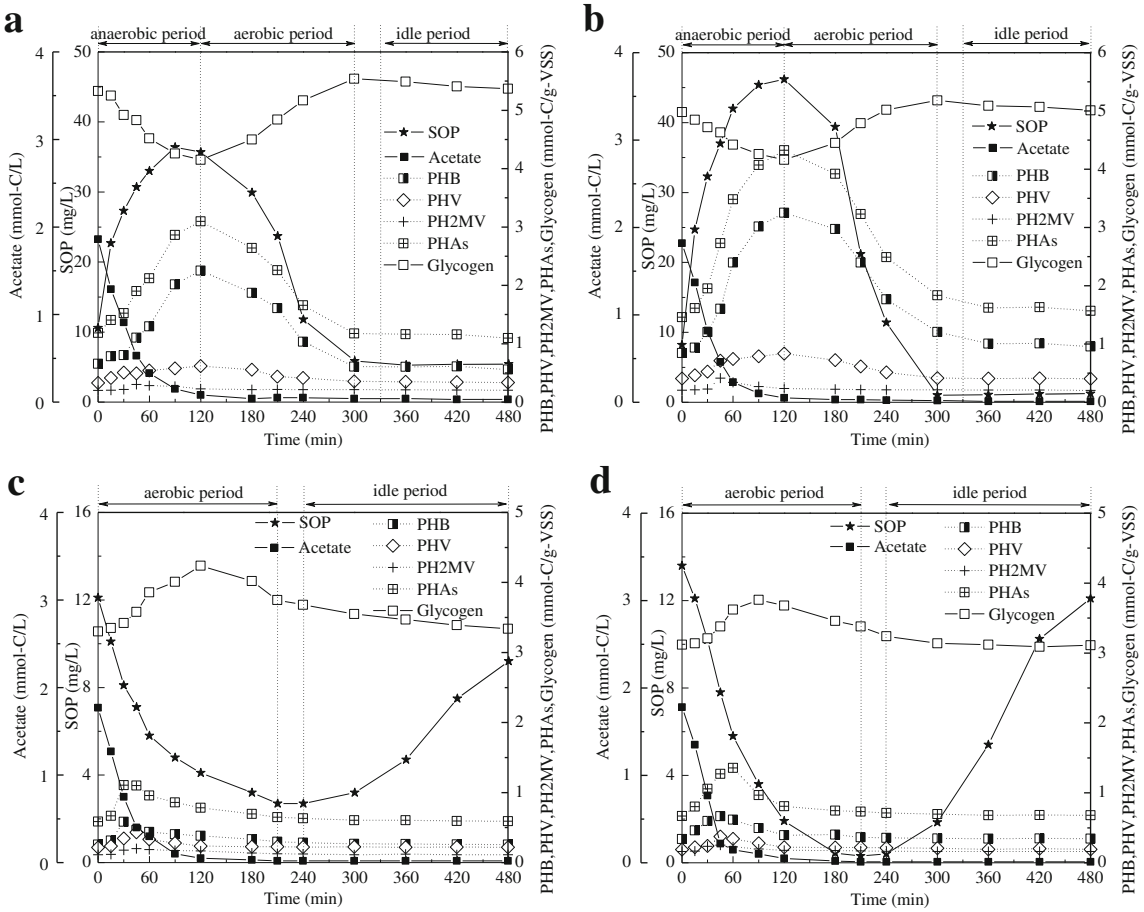

Fig. 3 Variations of SOP, acetate, and intracellular PHAs as well as sludge glycogen during a typical cycle of the SBRs operated with the A/O (**a** and **b**) and A/EI regimes (**c** and **d**) at 5 and 20 °C, respectively

the BPR performance between the A/EI and A/O regimes under low temperatures (i.e., 5 °C).

The comparison results of the BPR performance between the A/EI and A/O SBRs were summarized in Table 5. It could be seen that effluent SOP was lower in the A/EI SBRs as compared with that in the A/O SBRs at the same temperature, suggesting that higher BPR efficiencies were achieved in the A/EI SBRs. Especially at 5 °C, SOP removal efficiencies in the A/EI and A/O SBRs were respectively 81.5±2.1 and 64.4 ±2.7 %, with A/EI SBR 17.1 % higher than the A/O SBR. The results showed that the A/EI regime drove better BPR than the A/O regime at both 20 and 5 °C.

The cyclic variations of acetate, SOP, PHAs, and glycogen in the A/EI and A/O SBRs are presented in Fig. 3. The consumption of acetate was accomplished in the anaerobic phase in the A/O regime but completed aerobically in the A/EI regime, which signified a substantial difference in metabolic mechanism. Compared with that in the A/O SBRs, the PHAs accumulations measured in the A/EI SBRs were much lower. For example, the synthesized PHAs were 1.9 and 0.5 mmol-C/g-VSS in the A/O and A/EI SBRs at 5 °C, respectively. It is reported that acetate uptake and the subsequent PHAs accumulation require ATP and NADPH, which are usually provided respectively by poly-P hydrolysis and glycogen degradation in EBPR (Smolders et al. 1994a). The TCA cycle is naturally more active aerobically, since the cells are capable of oxidizing the NADPH produced by the TCA to ATP via oxidative phosphorylation (Smolders et al. 1994a; Oehmen et al. 2007). In this study, the TCA cycle seems to have supplied both ATP and NADPH for PHAs synthesize in the A/EI SBRs due to the negligible SOP release and glycogen consumption during PHAs accumulation, indicating that some of the acetate was consumed to provide ATP via TCA cycle. As a result, less PHAs was accumulated in the A/EI SBRs as compared with the A/O SBRs. Moreover, it is well-known that a part of glycogen degradation will be used for PHAs accumulation in the A/O regime, which is another reason for the higher PHA accumulation in the A/O SBRs.

The bacterial populations in the A/EI and A/O SBRs at 5 and 20 °C are summarized in Table 5. It can be seen from Table 5 that the samples of activated sludge from the A/EI SBRs contained more PAO and less GAO than those from the A/O SBRs at both 5 and 20 °C, which was consistent with the BPR efficiencies in Table 5 and seemed to be a major reason for the A/EI regime showing better BPR performance.

In addition, from Table 5, it can be also seen that substantial quantities of effluent nitrate were detected in all SBRs. According to the literature, the appearance of nitrate in anaerobic pool would easily deteriorate EBPR due to the restrain to anaerobic phosphate release (Guerrero et al. 2011). Under anaerobic conditions, PAO uses the energy yielded from poly-P hydrolysis to transport VFA through their cell membranes and produce intracellular PHAs. The denitrification compromises EBPR since the denitrifiers compete the substrate against PAO, which reduces VFA transported to PAO and thus decreases the energy required from poly-P hydrolysis (Wilderer et al. 2001). Therefore, the transfer of nitrate into the anaerobic zone inhibits phosphate release and impairs the phosphorus removal in the system (Kuba et al. 1994). As compared with the A/O process, poly-P hydrolysis in the A/EI regime occurred in the idle period, during which no substrate was left and no VFA needed to be transported. Hence, the inhibitory effects of nitrate on SOP release in the A/EI regime might be much slighter than that in the A/O regime. In our recent studies, it was proven that the AEI regime could bear higher level of nitrate (Wang et al. 2012a). Consequently, the higher tolerance for nitrate of the A/EI regime might be another reason for the A/EI SBRs exhibiting better SOP removal than the A/O SBRs.

Conclusions

The efficiency of BPR induced by the A/EI regime depended strongly on temperature and the highest SOP removal rate of 97.1 % was obtained at 20 °C. Temperature affected the transformations of PHAs and glycogen, and the activities of PPX and PPK. The biomass cultured at 20 °C contained more PAO and less GAO, which might be a major reason for the high BPR efficiency. Moreover, the A/EI regime drove higher BPR at the optimal temperature and had a better tolerance for cold temperatures as compared with the A/O process.

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