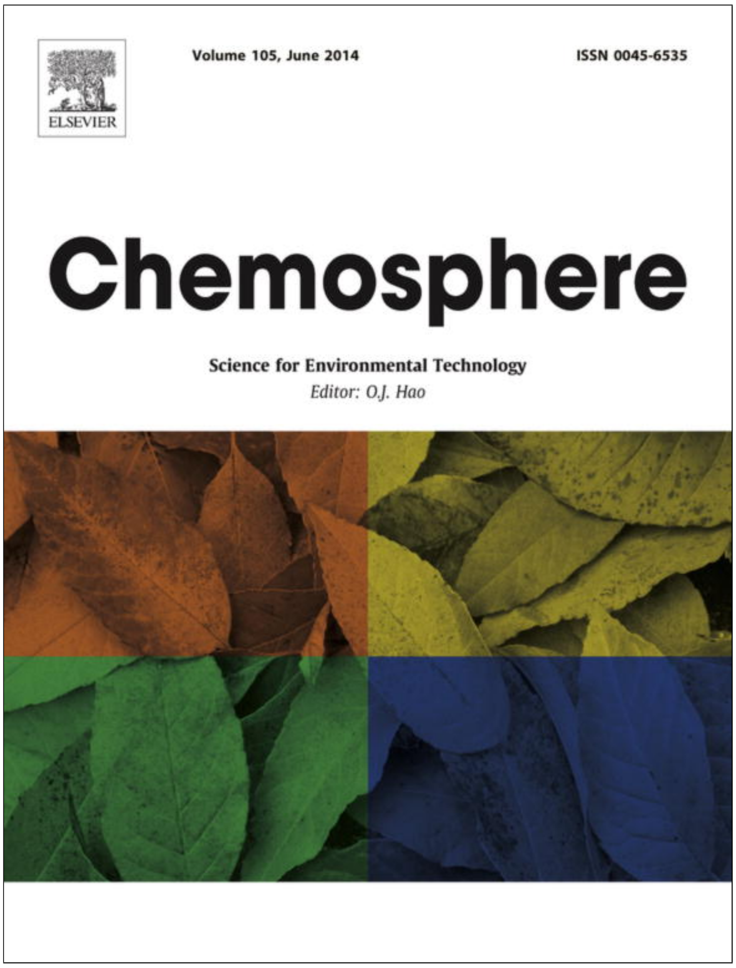

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Biological nutrient removal in a sequencing batch reactor operated as oxic/anoxic/extended-idle regime

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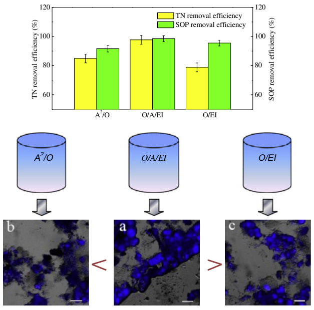
HIGHLIGHTS

- TN and P removal efficiencies in O/A/EI regime were kept above 98% and 99%.
- O/A/EI regime performs better N and P removal than A^2/O and O/EI processes.
- O/A/EI regime provides PAOs advantage over GAOs.

GRAPHICAL ABSTRACT

This paper showed oxic/anoxic/extended-idle (O/A/EI) regime drove superior biological nutrient removal than conventional anaerobic/anoxic/aerobic (A^2/O) and oxic/extended-idle (O/EI) processes.



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ABSTRACT

Previous researches have demonstrated that biological phosphorus removal from wastewater could be induced by oxic/extended-idle (O/EI) regime. In this study, an anoxic period was introduced after the aeration to realize biological nutrient removal. High nitrite accumulation ratio and polyhydroxyalkanoates biosynthesis were obtained in the aeration and biological nutrient removal could be well achieved in oxic/anoxic/extended-idle (O/A/EI) regime for the wastewater used. In addition, nitrogen and phosphorus removal performance in O/A/EI regime was compared with that in conventional anaerobic/anoxic/aerobic (A^2/O) and O/EI processes. The results showed that O/A/EI regime exhibited higher nitrogen and phosphorus removal than A^2/O and O/EI processes. More ammonium oxidizing bacteria and polyphosphate accumulating organisms and less glycogen accumulating organisms containing in the biomass might be the principal reason for the better nitrogen and phosphorus removal in O/A/EI regime. Furthermore, biological nutrient removal with O/A/EI regime was demonstrated with municipal wastewater. The average TN, SOP and COD removal efficiencies were 93%, 95% and 87%, respectively.

1. Introduction

N and P are the two most important nutrients for organisms, while the excessive accumulation of N and P discharged into water can cause eutrophication which has become a severe water pollution problem all around the world. Almost all wastewater treatment plants (WWTP) worldwide achieve N removal by alternately exposing a population of bacteria including nitrifiers and denitrifiers to oxic environments for nitrification and anoxic conditions for denitrification (Lee et al., 2010). Enhanced biological phosphorus removal (EBPR) processes are commonly conducted to remove P from wastewater by exploiting the ability of polyphosphate accumulating organisms (PAOs) to accumulate P and to store

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it as intracellular polyphosphate (poly-P) under alternating anaerobic/aerobic conditions (Chen et al., 2004; Mullan et al., 2006). To achieve wastewater biological N and P removal, some wastewater treatment processes such as anaerobic/anoxic/aerobic (A^2/O) process have been developed and applied (van Loosdrecht et al., 1998).

In EBPR systems, PAOs store P through sequential anaerobic–aerobic conditions. While another group of microorganisms known as glycogen accumulating organisms (GAOs) are able to compete with PAOs for the available organic substrate without contributing to P removal (Zeng et al., 2002). In many cases, process upsets and P removal deterioration in EBPR plants can be explained by the microbial competition of GAOs with PAOs (Thomas et al., 2003). In nitrification process, ammonium is converted to nitrite by ammonium oxidizing bacteria (AOBs) and then is further oxidized to nitrate by nitrite oxidizing bacteria (NOBs). Then nitrate is reduced into nitrite and then into molecular nitrogen through denitrification (Zhou et al., 2011).

Recently, achieving high wastewater N and P removal efficiency with less energy consumption has become a very urgent great task for WWTP. Though high rates of denitrification can be achieved in pre-anoxic regime under sufficient supply of biodegradable carbon, the energy cost from mixed liquor recycle flows is enormous (Coats et al., 2011). Therefore, many post-anoxic processes have been developed to decrease the energy consumption during wastewater biological nutrient removal (Metcalf and Eddy, 2003). As an example, an innovative static/oxic/anoxic activated sludge process characterized by static phase as a substitute for conventional anaerobic stage was proposed to enhance biological nutrient removal (Xu et al., 2013). Moreover, the anoxic zone is placed before the aerobic zone in pre-anoxic processes. However, the available carbon source in municipal wastewater is mainly in the form of volatile fatty acids (VFAs), which are limited and scarcely satisfy the demand of denitrification and EBPR simultaneously. So far, a novel anaerobic/aerobic/anoxic process has been proposed to realize denitrifying phosphorous removal (Xu et al., 2011). And post-anoxic denitrification driven by polyhydroxyalkanoates (PHAs) and glycogen was achieved within EBPR, which dramatically reduced the need for external carbon augmentation in biological nutrient removal (Coats et al., 2011).

It has been proved that biological P removal can be well achieved by oxic/extended-idle (O/EI) regime (Wang et al., 2008). As compared to conventional EBPR processes, O/EI regime realized biological P removal without experiencing specific anaerobic pools but extending the idle period to 210–450 min (Wang et al., 2012a). Thus, O/EI regime had several advantages such as improved phosphorus removal efficiencies and reduced operational costs (Wang et al., 2012b). However, those previous studies described the general performance in terms of P removal without specifying the importance of improving N removal efficiency. And denitrification capacity of the extended-idle was limited, resulting in the low total nitrogen (TN) removal efficiency. Therefore, it is important to enhance the TN removal of O/EI process through simple strategies.

In the previous study, introducing an anoxic period after the aeration to realize post anoxic denitrification driven by storage compounds was proved to be feasible in an activated sludge process (Chen et al., 2013). However, the accumulation of PHAs was prematurely finished at about the initial 30 min of aeration and the synthesized PHAs were consumed gradually in the remaining oxic stage. It was observed that just half of the synthesized PHAs were left at the beginning of the anoxic phase, which could not provide enough electron donors for denitrification. Though a high P removal efficiency averaging 99% was achieved, TN removal efficiency was only obtained at 83%.

The purpose of this work was to report a new wastewater treatment regime, i.e., oxic/anoxic/extended-idle (O/A/EI) regime, which could achieve excellent wastewater biological nutrient removal in a sequencing batch reactor (SBR). Firstly, the N and P removal performance in O/A/EI regime was compared with that in A^2/O and O/EI processes during the long-term operation. Then, the mechanism for O/A/EI regime driving high N and P removal efficiencies was explored. Finally, the feasibility of using O/A/EI regime to achieve N and P removal was tested in municipal wastewater.

2. Materials and methods

2.1. Sequencing batch reactor setup and operation

Experiments were performed in three lab-scale SBRs each with a working volume of 12 L. Seed sludge was inoculated into the three SBRs to achieve biological N and P removal. Aeration and mixing were supplied through an air diffuser placed in the bottom of the SBRs, and a mechanical stirrer was used to attain sound liquid mixing during anaerobic and anoxic phases. The dissolved oxygen concentration during the oxic phase is about 4 $mg\,L^{-1}$. The pH values were controlled at 7.0 ± 0.2 by dosing 1 M HCl or 1 M NaOH. All SBRs were operated sequentially in 6 h-cycle. One of the SBRs was operated as O/A/EI regime, each cycle of which was consisted of 120 min aeration and 90 min anoxic mix, followed by 55 min settling, 5 min decanting and 90 min idle periods.

The other two SBR were operated as conventional A^2/O and O/EI regimes. Each cycle of the A^2/O-SBR consisted of 90 min anaerobic, 120 min oxic and 90 min anoxic periods, followed by 55 min for settling and 5 min for decanting. The cycling profile of O/EI-SBR comprised 120 min aeration, followed by 55 min settling, 5 min decanting and 180 min idle periods. 10 L supernatant was discharged at the end of settling phase and was replaced with 10 L synthetic medium. 1.5 L of sludge mixtures from the SBRs was discharged once per day at the end of aerobic zone, resulting in a sludge retention time of 8 d and a hydraulic retention time of 8 h.

2.2. Wastewater and sludge

The synthetic feeding medium used as influent in this study contained 15 mg PO_4^{3-}–P L^{-1}, 40 mg NH_4^+–N L^{-1} and 300 mg L^{-1} of COD. Acetate was selected as the sole carbon source because it was the most common VFAs in domestic wastewaters (Chen et al., 2004). The concentrations of the other nutrients in the synthetic wastewater were listed as follows: 0.005 $g\,L^{-1}$ $CaCl_2$, 0.01 $g\,L^{-1}$ $MgSO_4{\cdot}7H_2O$ and 0.5 $mL\,L^{-1}$ trace element solution which contained ($g\,L^{-1}$): 1.50 $FeCl_3{\cdot}6H_2O$, 0.03 $CuSO_4{\cdot}5H_2O$, 0.12 $ZnSO_4{\cdot}7H_2O$, 0.12 $MnCl_2{\cdot}4H_2O$, 0.06 $Na_2MoO_4{\cdot}2H_2O$, 0.15 $CoCl_2{\cdot}6H_2O$, 0.18 KI, 0.15 H_3BO_3 and 10 EDTA.

The seed sludge was collected from the WWTP in Changsha, P.R. China, which was operated as A^2/O process. The initial concentration of mixed liquor suspended solids (MLSS) was about 4000 $mg\,L^{-1}$.

2.3. Nitrogen and phosphorus removal from municipal wastewater

The investigation was performed in a SBR with a working volume of 12 L. The SBR was operated as O/A/EI regime and was inoculated with seed sludge to achieve biological nutrient removal. Each cycle of the SBR consisted of 210 min aeration and 90 min anoxic mix, followed by 55 min settling, 5 min decanting and 120 min idle periods. The other operational conditions of the two SBRs were the same as that described in Sections 2.1 and 2.2 except that the SBR here received real municipal wastewater, which was collected from the first wastewater treatment plant in Changsha, P.R. China. The main characteristics of the municipal wastewater are presented as below: total phosphate (TP) 6.5–9.7 mg L^{-1}, soluble orthophosphate (SOP) 4.6–7.8 mg PO_4^{3-}–P L^{-1}, TN 32.4–45.8 mg L^{-1}, NH_4^+–N 23.9–36.2 mg L^{-1}, soluble COD 120–216 mg L^{-1}, pH 7.0–7.2.

2.4. Analytical methods

The SBRs were monitored through chemical analytical techniques. TP, SOP, NH_4^+, NO_2^-, NO_3^-, COD, MLSS and mixed liquor volatile suspended solids (MLVSS) were measured according to the standard methods (APHA, 1998). The analysis of ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrate reductase (NR), nitrite reductase (NIR), exopolyphosphatase (PPX) and polyphosphate kinase (PPK) activities was the same as described by Zheng et al. (2011). Sludge glycogen, poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV) and poly-3-hydroxy-2-methylvalerate (PH2MV) were measured according to the methodology described by Wang et al. (2008). The total PHAs was calculated as the sum of measured PHB, PHV and PH2MV.

4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) staining was carried out to analyze the presence of intracellular poly-P granules (Mullan et al., 2006). Sludge samples taken at the end of the aerobic period were used for staining. Fluorescence in situ hybridisation (FISH) technique detailed in the literature (Wong et al., 2005; Carvalho et al., 2007) was used to analyze the microbial composition of the activated sludge. The oligonucleotide probes used for FISH were listed in Fig. SM-1 of Supporting Material (SM). Sludge samples taken from the aerobic period at various times were analyzed by FISH for AOBs, NOBs, PAOs and GAOs. FISH quantification was performed by Image-pro plus 7.0 Software, the relative abundance of interested bacteria was determined as the mean percentage of all bacteria.

3. Results and discussion

3.1. Nitrogen and phosphorus removal in O/A/EI-SBR during the long-term operation

Experiments for wastewater biological nutrient removal were conducted and lasted for 180 d. The data of TN and SOP removal efficiencies during the long-term operation are presented in Fig. 1. As shown in Fig. 1, the efficiencies of TN and SOP removal increased gradually in the first 3 wk, and were kept above 98% and 99% at the influent concentration of 40 mg NH_4^+–N L^{-1} and 15 mg PO_4^{3-}–P L^{-1} after reaching stable state on Day 41. The effluent TN and SOP concentrations during the steady-state operation were lower than 1 and 0.5 mg L^{-1}, respectively, which conformed to the requirements of wastewater discharge standards. Those results proved that biological N and P removal could be well achieved in O/A/EI regime for the wastewater used.

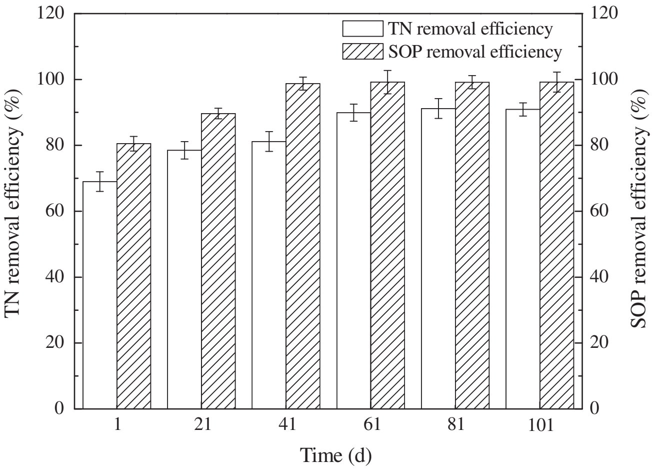

Fig. 1. The efficiencies of TN and SOP removal in O/A/EI-SBR during the long-term operation. The data reported are the averages and their standard deviations in triplicate tests.

3.2. Nitrogen and phosphorus removal in O/A/EI-SBR during one cycle

The cyclic profiles of SOP, NH_4^+, NO_2^- and NO_3^- during the steady-state operation are showed in Fig. 2a. In the initial 15 min of the oxic stage, obvious SOP-P release was observed before substantial SOP-P uptake. The maximum SOP concentration in the mixed liquor achieved 21 mg L^{-1}, about 1.5 times the influent concentration. During the oxic period, almost all NH_4^+ was oxidized to NO_2^- and NO_3^-, resulted in a NO_2^- and NO_3^- accumulation of 4.2 and 3.7 mg N L^{-1}. Besides, nearly all SOP was taken up by microorganisms and a low effluent concentration of 0.29 mg L^{-1} was obtained. The changes of NH_4^+, NO_2^- and NO_3^- in the oxic stage were similar to those observed in the oxic period of O/EI process (Wang et al., 2012a,b). However, the obvious SOP release in this study and our previous study (Chen et al., 2013) was not observed in the above-mentioned studies. The reason for these inconsistent observations was unknown. Aerobic SOP release linked to acetate uptake has been previously described in the literature (Guisasola et al., 2004; Pijuan et al., 2005). According to Pijuan et al. (2005), while acetate was consumed in the feast stage, PHAs were accumulated and SOP was released to the media, and these processes were characteristic of PAOs and it seemed that PAOs behaved in a similar way as under anaerobic conditions. During the following anoxic period, the accumulated NO_2^- and NO_3^- were reduced to free nitrogen, and the TN concentration in the effluent was below 1 mg L^{-1}. In the extended-idle stage, TN concentration did not change but considerable SOP-P release was detected reaching 6.2 mg L^{-1}.

The variations of acetate, glycogen, PHAs, PHB, PHV and PH2MV during one cycle are illustrated in Fig. 2b. With acetate being consumed rapidly in the oxic phase, slight decrease of glycogen and impressive accumulation of PHAs were observed. Apparently, the accumulation of PHB was higher than that of PHV and PH2MV, suggesting that the biosynthesis of PHAs was mainly due to that of PHB but not PHV or PH2MV (Zeng et al., 2002; Carvalho et al., 2007). In the initial aerobic period, carbon source can be directly consumed aerobically, thus accumulation and bacterial growth as well as substrate oxidation might occur simultaneously (Sin et al., 2005). In this study, it was further calculated that the ratio of PHA synthesis/VFA uptake was 0.63, which supported the theory mentioned above and suggested that simultaneous storage and growth processes was occurring in O/A/EI regime. In the subsequent anoxic stage, glycogen and the synthesised PHAs were degraded as carbon and energy sources because acetate was exhausted. The degradation of glycogen was low (about 0.21 mM C L^{-1}), and the decrease of PHAs was mainly due to that of PHB, revealing that the energy need for denitrification was mainly supplied by the consumption of PHB. One thing to be noted was that PHA accumulation was prematurely finished at about 30 min in our previous study (Chen et al., 2013). As a result, the synthesized PHA was consumed gradually in the remaining oxic period, and just one half of the synthesized PHAs were left at the beginning of the anoxic period. In this study, however, sustainable PHA accumulation was realized and PHA content at the end of the aeration was much higher than that in previous study. This might be due to the different operations between the two studies. In our previous study, the SBRs were operated sequentially in 8 h-cycle, the aeration duration was ranged from 1.5 to 4 h with an anoxic phase of 2.5 h was performed, but in the current O/A/EI-SBR, the oxic and anoxic stages were shortened to 2 and 1.5 h, respectively, and the whole cycle time was shortened to 6 h. This difference

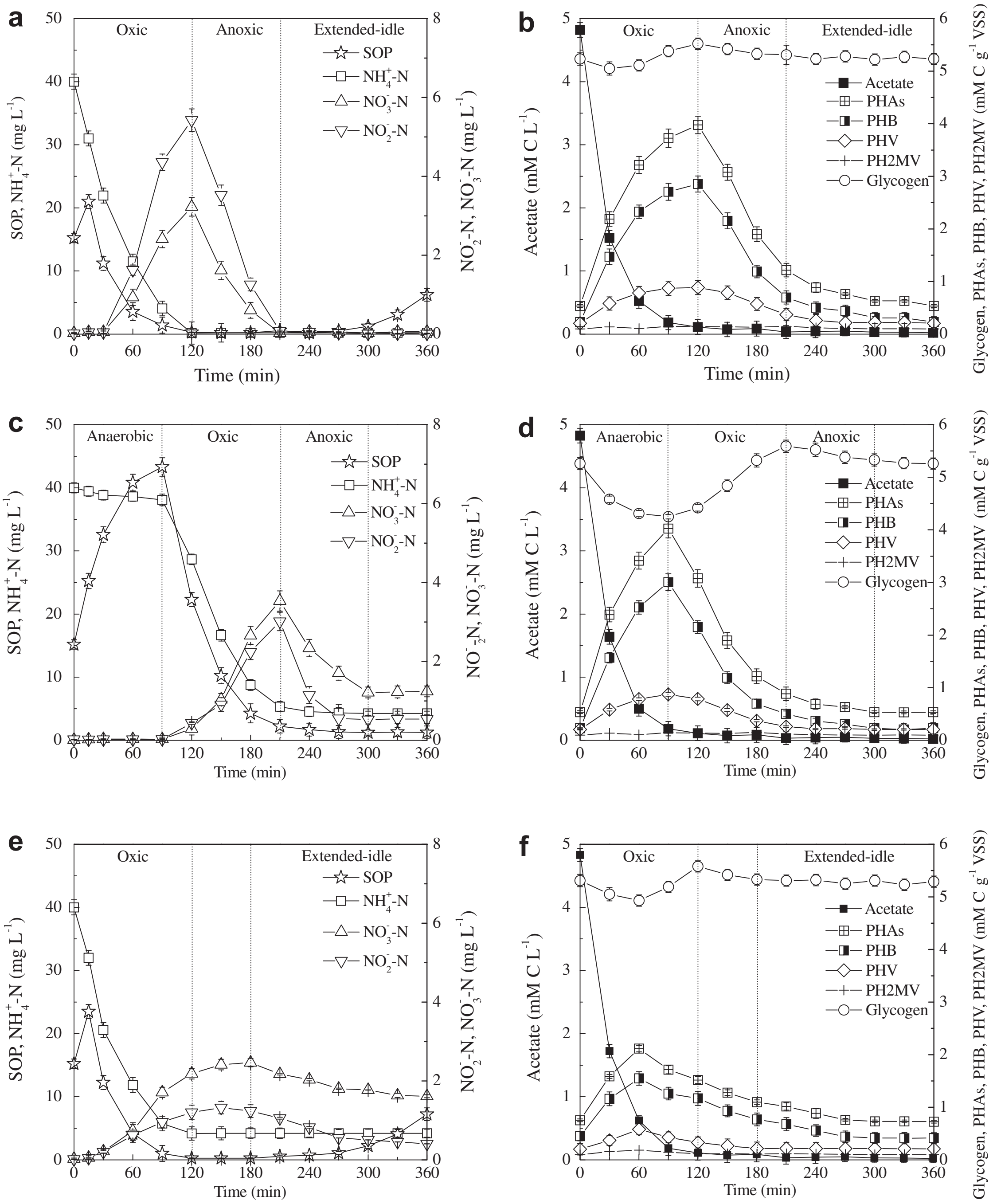

Fig. 2. Variations of SOP, NH_4^+, NO_2^-, NO_3^-, acetate, PHAs and glycogen during a typical cycle of the SBRs (a and b: O/A/EI-SBR; c and d: A^2/O-SBR; e and f: O/EI-SBR). The data reported are the averages and their standard deviations in triplicate tests.

might benefit the metabolism of bacteria capable of producing PHAs.

3.3. Comparison of nitrogen and phosphorus removal performance between O/A/EI and A^2/O as well as O/EI SBRs

Biological nutrient removal performance in A^2/O and O/EI SBRs was also monitored in the long-term operation. Table 1 summarizes the reactor performance of the three SBRs during a 35 d period after reaching steady-state operation.

Substantial quantities of NH_4^+ and NO_3^- were present in the effluent of A^2/O and O/EI SBRs, whereas negligible effluent NO_2^- was detected in A^2/O-SBR, indicating that though denitrification occurred to a certain extent in both reactors, the denitrification in A^2/O-SBR was enhanced. In contrast, the effluent SOP concentration in O/EI-SBR was much lower than that in A^2/O-SBR, which suggested that SOP removal in O/EI-SBR was higher than that in A^2/O-SBR.

From Table 1, it could also be observed that the O/A/EI-SBR had a lower TN and SOP concentration in effluent, thus had higher nutrient removal efficiencies than A^2/O and O/EI SBRs. The results clearly illustrated that though biological nutrient removal could be achieved in all SBRs, the O/A/EI-SBR could perform better with the wastewater of this work. TN removal efficiency in O/A/EI-SBR reached 98% during the steady-state operation, higher than the TN removal efficiency averaging 83% in the previous study (Chen

Table 1
Summary of reactor performances of O/A/EI, A^2/O and O/EI SBRs during steady-state operation.[a]

Regime	N				P			
	Effluent NH_4^+–N (mg L^{-1})	Effluent NO_2^-–N (mg L^{-1})	Effluent NO_3^--N (mg L^{-1})	TN removal efficiency (%)	TN uptake rate (mM N g^{-1} VSS h)	Effluent SOP (mg L^{-1})	SOP removal efficiency (%)	SOP uptake rate (mM P g^{-1} VSS h)
O/A/EI	0.82 ± 0.03	0.01 ± 0.01	0.06 ± 0.01	98 ± 1.6	0.31 ± 0.03	0.23 ± 0.02	99 ± 1.3	0.076 ± 0.004
A^2/O	4.26 ± 0.84	0.54 ± 0.12	1.23 ± 0.64	85 ± 2.3	0.28 ± 0.03	1.26 ± 0.26	92 ± 2.4	0.051 ± 0.002
O/EI	4.01 ± 0.51	1.97 ± 0.16	2.49 ± 0.36	79 ± 1.4	0.25 ± 0.02	0.27 ± 0.03	96 ± 1.7	0.057 ± 0.003

[a] Results are averages and their standard deviations.

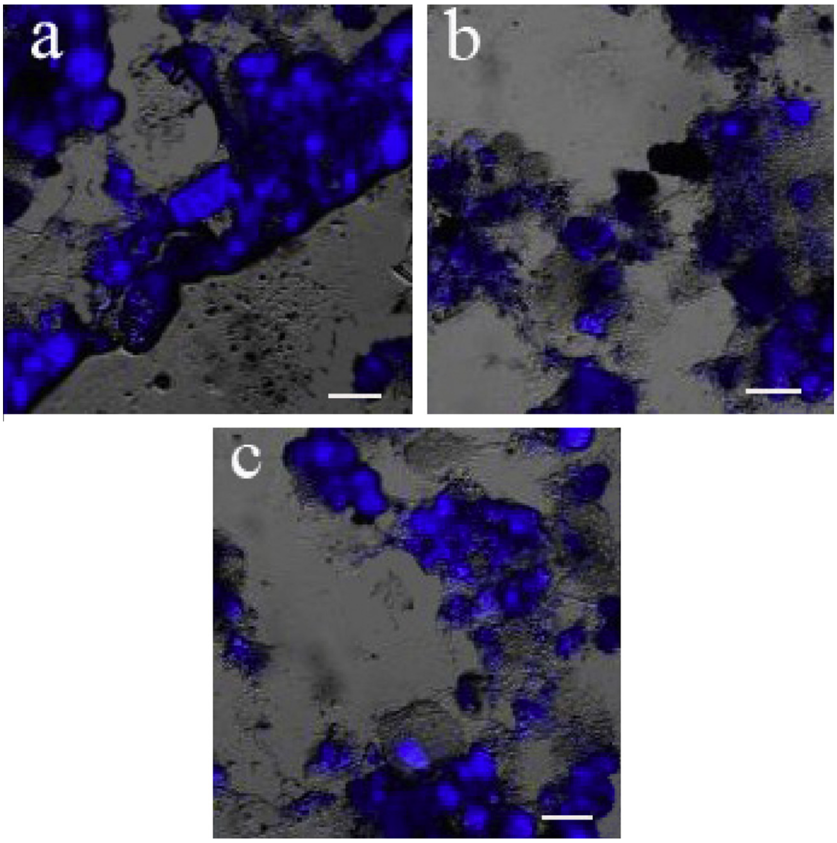

Fig. 3. Micrographs of DAPI staining of the activated sludge samples withdrawn at the end of aeration in the SBRs operated with different regimes (a: O/A/EI-SBR; b: A^2/O-SBR; c: O/EI-SBR). Bar = 5 μm.

et al., 2013). The enhancement of TN removal in this study is due to the higher PHA accumulation, which could enhance denitrification.

Moreover, TN and SOP uptake rates in O/A/EI-SBR were higher than those in A^2/O and O/EI SBRs, which was consistent with the comparisons of SOP removal efficiencies and suggested the better stability of TN and SOP removal in O/A/EI-SBR as compared to A^2/O and O/EI SBRs.

Fig. 3 shows the DAPI staining results of the sludge samples taken from the SBRs. More blue areas in Fig. 3a were found than the other two pictures, suggesting that more poly-P granules were accumulated in O/A/EI-SBR than in A^2/O and O/EI SBRs, which was consistent with the higher SOP-P release and uptake rates detected in O/A/EI-SBR.

3.4. Mechanism of O/A/EI regime driving higher nitrogen and phosphorus removal than A^2/O and O/EI processes

Table 2
Bacterial population in A/EI and A/O SBRs.[a]

Regime	AOBs (%)	NOBs (%)	PAOs (%)	GAOs (%)
O/A/EI	25 ± 3.2	21 ± 3.1	28 ± 3.2	11 ± 2.7
A^2/O	21 ± 2.6	21 ± 3.3	19 ± 2.3	14 ± 3.1
O/EI	15 ± 1.3	11 ± 1.4	23 ± 3.1	14 ± 2.3

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

Despite the fact that biological nutrient removal were well achieved in all SBRs, the highest TN and SOP removal efficiencies were obtained in O/A/EI-SBR. The average efficiencies of TN and SOP removal in O/A/EI-SBR achieved 98% and 99%, respectively, which were higher than those in A^2/O and O/EI SBRs and the results reported in reference (Wang et al., 2012a). Therefore, it was necessary to study the reasons for the O/A/EI-SBR driving higher TN and SOP removal than the A^2/O and O/EI SBRs.

As usually, the accumulation of NO_2^- and NO_3^- in nitrification was related to AOB and NOB abundance (Ma et al., 2009), and

Table 3
Activities of the key enzymes related to biological nutrient removal in the SBRs.[a]

Regime	AMO[b]	NOR[b]	NR[b]	NIR[b]	PPX[c]	PPK[d]
O/A/EI	0.031 ± 0.004	0.076 ± 0.003	0.042 ± 0.004	0.269 ± 0.003	0.028 ± 0.003	0.269 ± 0.017
A^2/O	0.026 ± 0.003	0.075 ± 0.005	0.041 ± 0.003	0.237 ± 0.004	0.023 ± 0.002	0.237 ± 0.008
O/EI	0.023 ± 0.005	0.053 ± 0.003	0.037 ± 0.003	0.214 ± 0.002	0.026 ± 0.002	0.264 ± 0.012

[a] The data reported are the averages and their standard deviations in triplicate tests.
[b] The unit is μmol nitrite/(min mg protein).
[c] The unit is μmol pnitrophenol/(min mg protein).
[d] The unit is μmol NADPH/(min mg protein).

microbial competition of GAOs with PAOs was considered to be responsible for EBPR deterioration (Oehmen et al., 2005). For this reason, quantitative analysis of AOBs, NOBs, PAOs and GAOs in activated sludge was carried out via FISH technology (Table 2). It was found that AOB abundance in O/A/EI-SBR was higher than that in A^2/O and O/EI SBRs, while the abundance of NOBs in O/A/EI-SBR was similar to that in A^2/O-SBR. This result was in accordance with the high NO_2^- accumulation in O/A/EI-SBR. Moreover, the quantitative analysis shows that PAOs and GAOs respectively accounted for 28% and 11% of total biomass in O/A/EI-SBR, with PAOs 17% more than GAOs. By contrast, the differences between PAO and GAO abundance were relatively small in A^2/O and O/EI SBRs. The FISH analysis suggested that the O/A/EI regime can provide PAOs advantage in the competition with GAOs, which seemed to be the major reason for the higher SOP removal in O/A/EI-SBR as compared to that in A^2/O and O/EI SBRs.

It was reported that biological nutrient removal is closely related to intracellular PHAs and glycogen transformations (Mino et al., 1998). As shown in Fig. 2, acetate was rapidly depleted accompanied by the accumulation of intracellular PHAs and glycogen in the oxic period. After acetate was completely consumed, the accumulated PHAs were progressively degraded. Nevertheless, the O/A/EI-SBR showed lower glycogen variations as compared to the A^2/O and O/EI SBRs (Fig. 2). The transformation of glycogen was related to GAO activity, with high glycogen accumulation usually indicated high GAO activity (Mino et al., 1998). Therefore, the lower glycogen accumulation detected in O/A/EI-SBR suggested that the biomass cultured in the activated sludge contained less GAO activity, which was correlated well with the results of abundance analysis (Table 2). Moreover, obvious SOP-P release was observed (about 6.2 mg P L^{-1}) but little glycogen was consumed during the extended-idle stage of O/A/EI-SBR. The idle transformations revealed that the energy for bacterial maintenance during the extended-idle period seemed to be provided by poly-P hydrolysis in O/A/EI-SBR, but was provided mainly by poly-P hydrolysis and glycogen degradation in A^2/O and O/EI SBRs. The lower energy required from poly-P degradation in O/A/EI-SBR enhanced the role of poly-P participating in PAO metabolism. That is to say, the intracellular biochemical transformations in O/A/EI-SBR correlated well with the metabolism of PAOs, which provided PAOs with a selective advantage over GAOs in PAO-GAO competition.

The activities of enzymes relevant to N and P removal in the SBRs are shown in Table 3. It is well known that nitrification is relevant to AMO and NOR (Juliette et al., 1993), and NR and NIR are two key enzymes in denitrification (Kristjansson and Hollocher, 1980), whereas SOP release and uptake are directly related to PPX and PPK activities, respectively (Mino et al., 1998). As shown in Table 3, though activated sludge in the O/A/EI-SBR showed higher specific AMO, NOR, NR, NIR, PPX and PPK activities as compared to A^2/O and O/EI SBRs, the ratio enzyme activity/Bacterial population was constant in the SBRs. For example, PPX activities in O/A/EI and O/EI SBRs were 0.028 ± 0.003 and 0.026 ± 0.002 μmol NADPH/(min mg protein). The ratio enzyme activity/bacterial population was calculated using the formula enzyme activity/(FISH% × MLVSS). The PAOs population and MLVSS in O/A/EI and O/EI SBRs were 28 ± 3.2% and 23 ± 3.1% and 2578 and 2513 mg L^{-1}, respectively. Therefore, the ratios enzyme activity/bacterial population in the two SBRs were almost equal. These results confirmed that the higher enzyme activities measured in O/A/EI-SBR resulted from the different microbial communities in the SBRs.

Table 4
Summary of the performance parameters in the reactor during the 150 d operation.[a]

Item	Ave	Max	Min
Influent SOP-P (mg L^{-1})	5.54	7.8	4.6
Effluent SOP-P (mg L^{-1})	0.23	0.49	0.07
SOP-P removal efficiency (%)	95	98	88
Influent NH_4^+–N (mg L^{-1})	36.8	36.2	23.9
Effluent NH_4^+–N (mg L^{-1})	1.12	2.96	0.17
NH_4^+–N removal efficiency (%)	98	99	92
Influent NO_2^-–N (mg L^{-1})	0.04	0.06	0.01
Effluent NO_2^-–N (mg L^{-1})	0.11	0.17	0.03
Influent NO_3^-–N (mg L^{-1})	0.09	0.14	0.01
Effluent NO_3^-–N (mg L^{-1})	0.13	0.21	0.06
Influent TN (mg L^{-1})	40.7	45.8	32.4
Effluent TN (mg L^{-1})	2.94	3.62	0.37
TN removal efficiency (%)	93	97	89
Influent COD (mg L^{-1})	187	216	120
Effluent COD (mg L^{-1})	23.4	38.3	8.65
COD removal efficiency (%)	87	96	74

[a] The values are average and standard deviation.

3.5. Biological nutrient removal from municipal wastewater with O/A/EI regime

It took about 21 d before effluent TN and SOP became stable. Table 4 shows the COD, NH_4^+, NO_2^-, NO_3^-, TN and SOP concentration in influent and effluent as well as removal efficiencies during the 150 d long-term operation. From Table 4, it can be seen that the influent NH_4^+ and SOP concentration ranged from 23.9–36.2 and 4.6–7.8 mg L^{-1} with the effluent NH_4^+ and SOP concentration lower than 5 and 0.5 mg L^{-1}, respectively, resulted in an average NH_4^+ and SOP removal efficiency of 98% and 95%. Moreover, the average effluent NO_2^- and NO_3^- concentration were 0.11 and 0.13 mg NO_x–N L^{-1}, and the average TN removal efficiency reached 93%. The COD concentration in effluent was kept lower than 50 mg L^{-1} under the influent concentration of 120–216 mg L^{-1}. Those results suggested that biological nutrient removal from municipal wastewater could be well achieved by O/A/EI regime.

4. Conclusions

Biological nutrient removal was well achieved in O/A/EI regime with the wastewater composition of this work, and the TN and SOP removal efficiencies in O/A/EI regime were higher than those in A^2/O and O/EI processes. The O/A/EI regime had higher PHA transformation and lower glycogen transformations compared to O/EI process, resulted in more AOBs and PAOs and less GAO activities in the biomass, which was the major reason for the high TN and SOP removal efficiencies in O/A/EI-SBR. In addition, biological nutrient removal with O/A/EI regime was demonstrated in municipal wastewater. The average efficiencies of TN, SOP and COD removal were 93%, 95% and 87%, respectively.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.12.043.

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