Effect of side-stream phosphorus recovery on biological phosphorus removal performance investigated by chemical and microbial analyses in a novel BNR-IC process

H. M. Zou, X. W. Lu and T. Li

ABSTRACT

The aim of this study was to assess the effect of side-stream ratio (SSR) on performance of phosphorus (P) removal and recovery in a novel process linking biological nutrients removal (BNR) and induced crystallization (IC). Results showed that P removal efficiency was significantly enhanced when given an appropriate SSR, resulting in effluent P concentrations decreasing from 0.75 to 0.39 mg/L with an increase of SSR from 0 to 35%, where a maximum of 7.19 mg/L P recovery amount was obtained at 35% of SSR. Increasing the SSR can favor the P recovery, while an excessively high SSR (more than 35%) would have a negative effect on the subsequent biological P removal in the BNR-IC system. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis showed that in total, 11 DGGE bands of highest species richness were visually detected and significant changes in microbial community structure were found, with SSR variations ranging from 0 to 55%. Moreover, an increase in SSR can cause an increase in microbial community biodiversity; where microbial populations correspond to the 11 bands, they were generally classified into five different phyla or classes (*Beta*-, *Gamma*-, and *Deltaproteobacteria*, as well as *Clostridia* and *Flavobacteria*) based on the evolutionary tree analysis.

Key words | biological nutrients removal, domestic wastewater treatment, induced crystallization, phosphorus recovery

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INTRODUCTION

Phosphorus (P) is a key element in causing the eutrophication of surface waters including lakes, drinking water reservoirs and slow flowing rivers everywhere in the world, which seriously threatens the ecological health of water bodies and the safety of drinking water. A 0.1 mg P/L in freshwater sources may be regarded as the signal of current or future algae blooms (Young *et al.* 1999). P removal from domestic wastewater is commonly recognized as an effective strategy to prevent eutrophication. Therefore, more stringent discharge limits of P concentration in the effluent from wastewater treatment plants are subject to compulsory legislation by governments worldwide (Boltz *et al.* 2012; Choi *et al.* 2012). Conversely, natural phosphate rock reserves available for modern agriculture and the phosphate industry may rapidly deplete due to the fact that P is a non-renewable finite resource, which could be used up in the foreseeable future (probably the next 50–100 years) (Takeda *et al.* 2010; Wong *et al.* 2013), thus highlighting the urgent need to recover P from environments such as domestic wastewater. The combination of P removal with P recovery from sewage is not just a possibility, but an apparent necessity, which would translate into a stronger need for any new process to achieve simultaneous P removal and recovery.

More recently, several attempts have been undertaken to develop an integrated process, in particular to combine the biological and chemical technologies that remove P from domestic sewage and simultaneously recover P in the form of a valuable product such as struvite (MAP, $MgNH_4PO_4 \cdot 6H_2O$) and hydroxyl calcium phosphate (HAP, $Ca_{10}(PO_4)_6(OH)_2$) (Britton *et al.* 2005; Jiang & Wu 2010; Cusick & Logan 2012; Esakki Raj *et al.* 2013; Kodera *et al.* 2013; Wong *et al.* 2013). In the combined process, the biological nutrients removal (BNR) technologies and chemical precipitation were generally adopted for facilitating P removal and recovery from domestic wastewater (Kodera *et al.* 2013), where P in the P-rich solution generated by anaerobic

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sludge digestion was recovered via precipitation of MAP or HAP (Qiu et al. 2012). One large disadvantage of the process is that equipment for the anaerobic digestion of excess activated sludge from the BNR process has to be designed to generate a P-rich supernatant solution, thus most probably leading to an obvious increase in the construction or operation costs. Furthermore, the precipitation product containing P with high moisture content is not easy to use directly in agricultural or industrial activities. One way to overcome these problems may be by using induced crystallization (IC) instead of chemical precipitation for P recovery.

Very recently, our research group from the Institute of Water Pollution Control Engineering of Southeast University, China has developed a novel side-stream process (patent applied for in China, No. 201110431802.4), linking BNR and IC (BNR-IC), which has been found to be effective in P removal and recovery from domestic wastewater (Shi et al. 2012; Zou et al. 2013). Compared with the aforementioned conventional process, the advantages of the new process are: (a) a P-rich supernatant generated by denitrifying polyphosphate accumulating organisms (DPAO) in the anaerobic tank, including the BNR-IC system, omitting the procedure of anaerobic digestion of excess activated sludge; (b) the IC technology was used to recover P in this process, and the crystallization product (mainly HAP) can be dewatered easily and could be potentially used directly as fertilizer or industrial raw materials; (c) the induced HAP crystallization section can greatly reduce the P loading in the anoxic environment (showing excessive P uptake by DPAO), thus resulting in minimal sludge production.

The BNR-IC system (see Figure 1) consisted of a BNR process based on enhanced biological phosphorus removal and an IC column, as shown in Figure S1 (supplementary material, available online at http://www.iwaponline.com/wst/070/351.pdf), achieving not only nutrient removal but also P recovery, which was described in detail in our previous research results (Zou et al. 2014a, b). In this process, P removal from domestic wastewater was achieved by a well known kind of bacteria (DPAO) actively facilitating P removal from sewage by altering the anaerobic and anoxic conditions. According to the DPAO theory (Zhou et al. 2010), DPAO can take up the volatile fatty acids and store them as poly-β-hydroxybutyrate (PHB), and simultaneously release P from the cell into the solution in anaerobic conditions, and then, in the subsequent anoxic environment, PHB is oxidized to generate energy for biomass growth and excessive P uptake by DPAO, i.e., luxury phosphorus uptake (Yao et al. 2013), thus significantly decreasing the P concentration in the solution. With the P taken up into the cells of DPAO, excess activated sludge discharge results in a net P removal from domestic wastewater. Unlike a conventional P recovery process (where the P-rich activated sludge was firstly digested in anaerobic conditions to obtain a concentrated P solution, and then P was recovered by chemical precipitation), in the BNR-IC system, the anaerobic tank was followed by an IC column to directly recover P from the anaerobic supernatant rather than the sludge digestion solution through IC (calcite was used as the seed crystal here). This feature may be highly significant in reducing costs and enabling more convenient management.

Although the BNR-IC process has exhibited a good P removal and recovery performance, the relationship between biological P removal and P recovery by IC is not yet fully understood. The P removal or recovery efficiency heavily depends on the side-stream ratio (SSR), which may determine maximum P removal and recovery efficiencies during the practical operation. Considering the need to achieve good P removal and recovery performance, the determination of optimum SSR is essential. Thus, the aim of this study was to investigate how SSR could affect P removal and recovery performance by chemical analysis, and the change in microbial community structure by microbial analysis (polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used here), in order to better apply the innovative process in practice. The result obtained here by combining chemical analysis with microbial analysis may serve as a new suggestion for application of a combined process for P removal with P recovery from domestic wastewater.

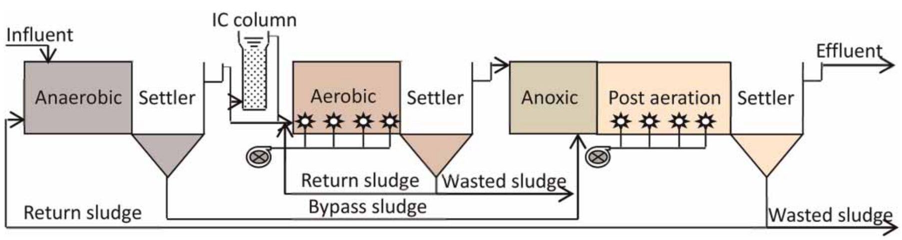

Figure 1 | Schematic diagram of BNR-IC process.

METHODS

The BNR-IC operational procedures were as follows: (1) domestic wastewater was introduced into an anaerobic tank, where P was released and chemical oxygen demand (COD) was taken up by DPAO; (2) after settling, the P-rich supernatant is partly led into the IC column to recover P along with effluent from the IC column, which flow into an aerobic tank for nitrification, where the SSR represents the ratio of the flow from the anaerobic tank to the IC column to the total anaerobic flow, which was controlled by varying the speed of the peristaltic pump; (3) sequentially, the clarified supernatant from the nitrification tank is introduced into the anoxic tank for simultaneously denitrifying nitrogen and phosphorus, completed by DPAO, here presenting the excessive P uptake using the nitrate as electron acceptors; and (4) a post-aeration tank is adopted for nitrogen gas stripping and residual nutrient removal in order to ensure or enhance the quality of effluent.

In this study, synthetic domestic wastewater was used; it contained a standard medium for the growth of microorganisms, which consisted of (per L): 322 mg CH_3COONa, 44 mg KH_2PO_4, 47 mg $(NH_4)_2SO_4$, 5 mg $CaCl_2$, 50 mg $MgSO_4 \cdot 7H_2O$ and 3 mL of a nutrient solution (1,500 mg $FeCl_3 \cdot 6H_2O$, 150 mg H_3BO_3, 30 mg $CuSO_4 \cdot 5H_2O$, 180 mg KI, 120 mg $MnCl_2 \cdot 4H_2O$, 60 mg $Na_2MoO_4 \cdot 2H_2O$, 120 mg $ZnSO_4 \cdot 7H_2O$, 150 mg $CoCl_2 \cdot 6H_2O$ and 10,000 mg EDTA). The synthetic domestic wastewater had COD, total nitrogen (TN) and P-PO_4 concentrations of 250 mg/L, 42 mg/L and 10 mg/L, respectively, and the flow rate of influent was controlled at 18 L/d. The mixed liquid suspended solids were maintained at 3.4–3.6 g/L in the anaerobic and anoxic tank, and 3.0–3.2 g/L in the aerobic tank, respectively; the sludge retention time of nitrifying bacteria and DPAO were approximately 16 days and 12 days, respectively.

In this study, the SSRs were set at 0%, 15%, 25%, 35%, 45% and 55%, respectively. With different SSRs, the BNR-IC was continuously operated over 180 days, divided into six stages, with 30 days in each stage.

Soluble samples from effluent were firstly filtered through a 0.45 μm microporous membrane filter to monitor water quality parameters. COD was determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA 2005). PO_4^{3-}-P and TN were analyzed by segmented flow analysis (AutoAnalyzer3, SEAL, UK).

At the end of each stage, 100 mL activated sludge was sampled to analyze the bacterial community by PCR-DGGE according to the following rules: sample pretreatment, DNA extraction and PCR amplification, and DGGE analysis and sequencing of PCR fragments, which were described in detail in our previous research (Zou *et al.* 2014a). The sequencing results obtained here were compared with the closest known relatives using the BLAST program from the National Center for Biotechnology Information database. The phylogenetic tree was carried out using the neighbour-joining method with MEGA version 5.0 software, employing 1,000 bootstrap resampling, and similarities in DGGE profiles were also calculated via Dice coefficient with MEGA.

RESULTS AND DISCUSSION

Effect of SSR on phosphorus removal and recovery performance

The changes in effluent P concentration at different SSR (from 0 to 55%) are shown in Figure 2. It is apparent that as the SSR was increased, P removal efficiencies fluctuated, presenting first a slow increase and then a rapid decrease. Consistently, the P concentration in the effluent decreased from 0.75 to 0.39 mg/L (see Table S1, supplementary material, available online at http://www.iwaponline.com/wst/070/351.pdf) as the SSR increased from 0% to 35% and beyond; SSR = 35%, P removal efficiency decreased. For SSR less than 35%, the increase of P removal efficiency in the BNR-IC system was expected to be due to the enhancement effect of side-stream P recovery on the subsequent biological P removal as a result of a decrease in loading of biological P removal. Reduction in P loading of biological removal meant that less P in solution was removed by functional microorganisms (DPAO) in the process and hence,

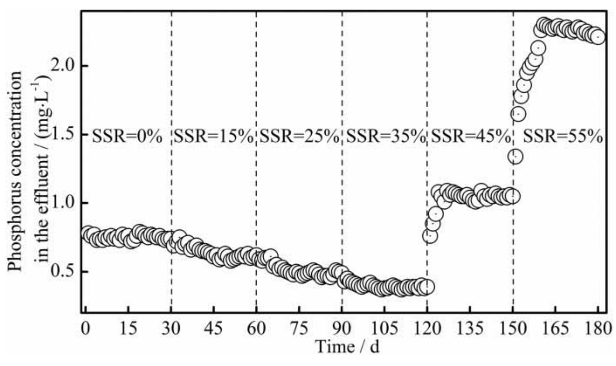

Figure 2 | Variation of phosphorus concentration in the effluent during the experiment over 180 days.

more P was recovered. When the SSR was increased to 45 and 55%, the effluent P concentration started to sharply increase to 1.05 and 2.24 mg/L at 45 and 55%, respectively. Similarly, the P removal efficiency reduced with the SSR variation, which may be mainly due to the fact that excessive SSR (more than 35%) may inhibit DPAO metabolism activity, thereby resulting in the deterioration of releasing P in anaerobic conditions and taking up P in anoxic conditions.

It was also found that when the SSR was increased, ranging from 0 to 55%, the amount of P anaerobic release and anoxic uptake was always decreased, as shown in Figure S2 (supplementary material, available online at http://www.iwaponline.com/wst/070/351.pdf). For P release in anaerobic conditions, the amount decreased sharply from 28.2 to 7.8 mg/L with an increase in SSR, and the value declined rapidly from 35.85 to 6.46 mg/L during the anoxic P uptake. Generally, the ratio of P uptake to release can be used as an assessment of the ability of microorganisms for biological P removal, where excessive P removal from domestic wastewater can occur as the ratio is more than one. With the increase of SSR ranging from 0 to 55%, the ratios of P uptake to release varied from 1.27 to 1.16, 1.09, 1.05, 0.93 and 0.83, respectively. It is noted that the ratio was less than 1 as the SSR was increased up to 45 and 55%, which was consistent with the variation of P removal efficiency described above, also suggesting that higher a SSR would have a negative effect on the subsequent biological P removal in the BNR-IC system.

The experiments in this study were also performed to investigate the effect of SSR on the P recovery efficiency in the IC column (Figure 3). Figure 3 shows that the P recovery efficiency gradually decreased from 79.2 to 55.7% with the increasing SSR ranging from 0 to 55%. However, the total amount of P recovery first increased and then decreased, giving a maximum P recovery amount of 7.19 mg/L at SSR = 35%.

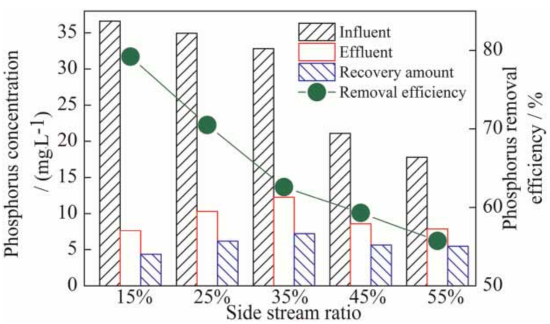

Figure 3 | In IC column, variations of phosphorus concentration in the influent and effluent, phosphorus recovery amount and phosphorus removal efficiency at different SSRs.

The above results in Figures 2 and 3 and Figure S2 show that the enhancement of side-stream P recovery on biological P removal was quite effective, giving a maximum P recovery amount of 7.19 mg/L at an SSR of 35%. Hence, SSR = 35% was taken as an optimum value for P removal and recovery in the BNR-IC system.

Effect of SSR on microbial community structure

The different P removal performances observed in this study could be due to variations in the dominant microorganisms when given with different SSRs, as described earlier. The change of microbial community structure during the experiments following the variation of SSR was investigated using PCR-DGGE analysis of the 16Sr RNA (Figure 4 (left)); lanes comparison and Dice coefficients on the DGGE patterns are shown in Figure 4 (right) and Table S2 (supplementary material, available online at http://www.iwaponline.com/wst/070/351.pdf), respectively. All the major bands and BLAST search results for the DGGE bands are shown in Table S3 (supplementary material, available online at http://www.iwaponline.com/wst/070/351.pdf), and phylogenetic affiliation of the 16S rDNA of the DGGE bands was conducted using MEGA version 5.1 software, as illustrated in Figure 5.

DGGE analysis of the six activated sludge samples from SSR 0 to 55% indicated the diversity of microbial ecologies during the experiment. In total, 11 DGGE bands (from band 1 to band 11) of highest species richness were visually detected and excised from the gel for subsequent sequencing analysis, as shown in Figure 4 (left). The microbial populations corresponding to the 11 bands were classified into five different phyla or classes including, *Beta-*, *Gamma-*, and *Deltaproteobacteria*, as well as *Clostridia and Flavobacteria* (see Table S3 and Figure 5). Among these bands, six bands (1, 2, 4, 5, 6 and 9) were found in all lanes, which most closely related to *Ferribacterium* spp., *Thiothrix* spp., *Acidovorax* spp., *Acinetobacter* spp., *Simplicispira* spp. and *Comamonas* spp., respectively. This indicated that the microorganisms corresponding to the six bands may be responsible for P removal in anaerobic-anoxic conditions, i.e., DPAO. The difference in intensities among the six bands may result from the influence of side-stream P recovery since P concentration in the anoxic tank may determine the growth of microorganisms responsible for P removal to some extents based on the metabolic theory of DPAO (Podedworna & Żubrowska-Sudoł 2012; Lanham *et al.* 2013). Band 7 and 8 (most closely related to *Bdellovibrio* spp. and *Rhodocyclus* spp., respectively) were found in other lanes except in lane 1, suggesting that side-stream P

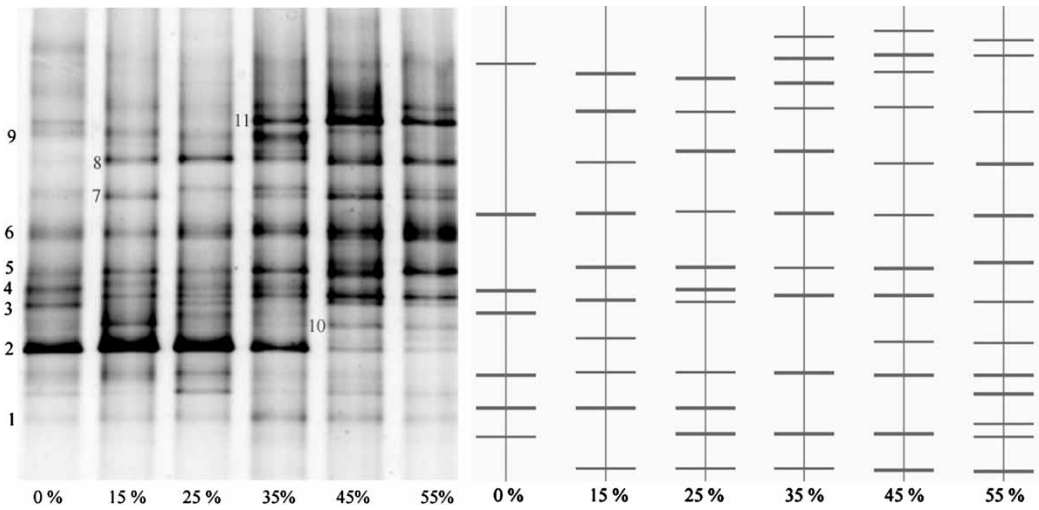

Figure 4 | DGGE fingerprint of 16Sr DNA fragments (left) and lanes comparison (right) generated from samples given different SSRs.

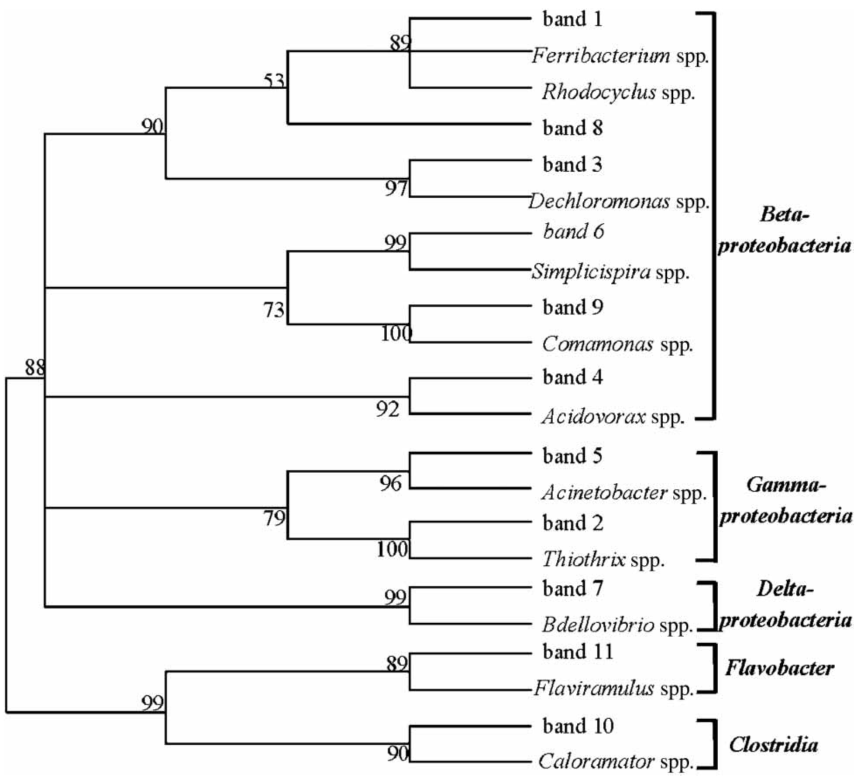

Figure 5 | Phylogenetic tree (carried out in MEGA version 5.1 software), of 16SrDNA excised from DGGE showing the phylogenetic affiliations between strains obtained in activated sludge studied here and their closest relatives derived from GenBank.

recovery does indeed influence the biological P removal observed from the chemical and microbial analyses.

Compared with lanes 1, 2, 3 and 4 representing SSRs of 0%, 15%, 25% and 35%, respectively, lanes 5 and 6 corresponding to SSRs of 45 and 55% had a higher diversity, probably indicating the negative effect of a higher SSR on DPAO growth. This may result from the P concentration in anoxic tank decreasing when given a higher SSR in the BNR-IC system, thus probably supporting the growth of other microorganisms. Moreover, microbial community similarities in six lanes were analyzed via Dice coefficients, as shown in Table S2, where the Dice coefficients of 34.1% and 40.3 between lanes 1, 5 and 6 were relatively low, suggesting that large bacterial community changes occurred when also given a higher SSR (more than 35% here). This may be attributed to the change of P concentration in the anoxic tank, as per with the discussion mentioned above.

CONCLUSIONS

The effect of side-stream phosphorus recovery on biological phosphorus removal was investigated to assess the performance of phosphorus removal and recovery in an integrated process linking BNR and IC, by using chemical analysis and microbial analysis (PCR-DGGE). In the BNR-IC system, phosphorus removal efficiency was significantly enhanced when given an appropriate SSR for phosphorus recovery, showing that the effluent phosphorus concentration decreased from 0.75 to 0.39 mg/L with an increase of SSR from 0 to 35%, where a maximum of 7.19 mg/L phosphorus recovery amount was obtained at an SSR of 35%. However, excessively high SSR (more than 35%) would have a negative effect on subsequent biological P removal in the BNR-IC system. Moreover, increasing the SSR can cause an increase in microbial community biodiversity, and significant changes of microbial community structure were found by using PCR-DGGE analysis with the SSR variations ranging from 0 to 55%, where in total 11 DGGE bands of highest species richness were visually detected and microbial populations corresponding to those bands were generally classified into five different phyla or classes (*Beta*-, *Gamma*-, and *Deltaproteobacteria*, as well as *Clostridia* and *Flavobacteria*) based on the evolutionary tree analysis. The result obtained here may serve as a new suggestion for application of a combined process for P removal with P recovery from domestic wastewater.

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