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AN INNOVATIVE CONTINUOUS FLOW BNR-IC PROCESS FOR NUTRIENTS REMOVAL AND PHOSPHORUS RECOVERY FROM SYNTHETIC AND REAL DOMESTIC WASTEWATER

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Abstract. An innovative continuous flow process linking biological nutrients removal (BNR) with induced crystallization (IC) was used to remove nutrients and recover phosphorus (P) from synthetic and real domestic wastewater. The results showed that a good nutrients removal performance was found regardless of feeding solutions. P recovery efficiency from synthetic wastewater was 70.2% slightly less than that from real domestic sewage (74.2%). Importantly, P recovery can effectively enhance the subsequent biological P removal. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis displayed an obvious shift in microbial community structure when switching feeding synthetic solution to real wastewater. A total of 13 bands were detected in sludge samples using synthetic and real domestic sewage, affiliated with 8 phyla or classes domain Bacteria (*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Flavobacteria, Actinobacteria, Sphingobacteria, Epsilonproteobacteria and Chlorobia*). The results obtained here suggest that the continuous flow BNR-IC process is feasible for nutrients removal and P recovery from domestic sewage and is a promising technology for wastewater treatment combined with recycling of P elements.

Keywords: nutrients removal, phosphorus recovery, induced crystallization, denitrifying polyphosphate accumulating organisms, denaturing gradient gel electrophoresis.

Introduction

Phosphorus (P) is one of critical elements required for biomass growth. According to the Liebig law of the minimum, the biomass (experience formula: $C_{106}H_{263}O_{110}N_{16}P$) (Moheimani *et al.* 2013) growth productivity is mainly determined by the supply of P in water bodies. Nowadays, eutrophication or algae blooming of water bodies is an increasing pollution problem worldwide due to the excessive discharge of P loading into lakes, rivers or reservoirs. Except Oceania and Africa, about 50% of all reservoirs and lakes are subject to eutrophication in all continents (Sengupta, Pandit 2011). In China, lakes of Dianchi, Chaohu and Taihu are eutrophic. Currently, eutrophication is of particular concern in water bodies, since it results in the water environmental degradation, threatens the survival of aquatic species and affects the safety of drinking water. More than 0.02 mg/L of P in lakes can cause eutrophication (Seviour *et al.* 2006). In domestic sewage, the fluctuation of P concentration ranges from 3 mg/L to 10 mg/L, often requiring mandatory removal before discharge into water bodies. Consequently, a strict P limit in effluents from municipal wastewater treatment plants (WWTP) was set in legislation by governments in the world. For instance, there is a compulsory requirement of TP concentration in effluents from WWTP to be less than 0.1 mg/L in North America and 0.5 mg/L in China (Zou *et al.* 2014a). Methods for P removal from domestic wastewater are commonly biological, physical or chemical. Compared with chemical or physical methods, recent advances in enhance biological phosphorus removal (EBPR) have demonstrated that it was regards as an effective and economic technology for P removal from domestic wastewater (Ong *et al.* 2014; Yang *et al.* 2013; Zhang *et al.* 2013).

Today, another a notable aspect of phosphorus problem is that P is a non-renewable mineral resource, mostly obtained from phosphate rock. If current exploitation rate of phosphate rock is continued it is predicted to run out in next 50 years (Gilbert 2009). Thus, it is very urgent to reuse and recycle P from human activities, i.e. phosphorus

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recovery, which has drawn considerable attention in recent years (Acelas *et al.* 2014; Hutnik *et al.* 2013; Qiu, Ting 2014). It is noteworthy that about 3~10 mg/L of P in domestic sewage may provide a valuable potential market for P recovery. Current technology for P recovery from domestic wastewater mainly adopted chemical precipitation and the commonly used processes were the two types of struvite (NH_4MgPO_4, MAP) (Cusick, Logan 2012; Ichihashi, Hirooka 2012) and hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) (Zou *et al.* 2014b), which were regarded as a slow release fertilizer (Yetilmezsoy, Sapci-Zengin 2009). In addition to, Al and Fe ions also have a great affinity for P binding (Westholm 2006). Since phosphate fertilizer accounted for about 80% of used phosphate rock in the world (Guney *et al.* 2008), MAP or HAP (similar to fertilizer component) precipitation should be preferred to recover P element from solution.

The discussion above shows that both natural phosphate rock diminution and pollution due to P excessive discharge should be solved quickly. This may lead to a new research on P removal coupled with P recovery from domestic sewage to meet the effluent standards and recycle phosphate rock. Recently, several attempts have been conducted to develop new combination process that remove nutrients and recover P (Ichihashi, Hirooka 2012; Song *et al.* 2014; Yuan *et al.* 2012), in particular to investigate the P recovery from excess sludge. However, most of those reported on the combination process about biological nutrients removal coupled with chemical precipitation. Of them, a well-known wastewater treatment process linking nutrients removal with P recovery is the Phostrip (Yuan *et al.* 2012), adopting biological nutrients removal combined with chemical precipitation, where the P in supernatant generated from excess sludge digestion was recovered by MAP or HAP precipitation. The disadvantages of the process are obvious: (1) increasing cost in excess sludge digestion system; (2) lower P recovery efficiency due to recover P only from excess sludge; (3) chemical precipitation product (such as MAP or HAP) is not directly used to industrial or agricultural activities owe to contain high moisture or impurity content, where the induced crystallization technology may be a preferable alternative to recovery P from solution.

More recently, our research team developed an innovative process of biological nutrients removal (BNR) coupled with induced crystallization (IC), referred herein to as "BNR-IC", responsible for P removal with P recovery (Shi *et al.* 2012; Zou *et al.* 2014a). The process overcame disadvantages of conventional process mentioned above. Although good nutrients removal and P recovery performances were found in our previous results via serial batch experiments (Zou *et al.* 2014c), the behavior of BNR-IC operation in continuous flow is not still unclear, especially for treatment real domestic wastewater rather than synthetic wastewater. Most studies on domestic wastewater treatment were nowadays performed by feeding the artificial wastewater. However, the component of real domestic sewage is more extensive and complex than that of synthetic wastewater, thus resulting in the infeasibility of application of results obtained from synthetic wastewater treatment to practice. Consistently, there is a large discrepancy in results obtained from between batch tests and continuous flow experiments. For this, in the innovative BNR-IC process, real domestic sewage treatment operated by continuous flow has to be performed in the further study.

Therefore, the aim of this study was to investigate whether stable and high nutrients removal and P recovery from synthetic and real domestic wastewater can be obtained in the continuous flow BNR-IC process. The carbon, nitrogen and phosphorus content in the influent and effluent were monitored. Furthermore, the differences of microbial community structure between feeding synthetic wastewater and feeding real domestic sewage were also compared by microbial analysis. The results obtained from here may serve as a good suggestion for application of the novel BNR-IC process to practice.

1. Materials and methods

1.1. Experimental set-up and operation

The BNR-IC process (Fig. 1, left) proposed here consisted of biological nutrients removal (BNR) system and induced crystallization (IC) P recovery column (Fig. 1, right). The BNR system is an anaerobic/anoxic/aerobic process with double-sludge, in which denitrifying polyphosphate accumulating organisms (DPAO) performed denitrifying simultaneous nitrogen and phosphorus removal. The IC column was comprised of crystallization, buffer and settling zones, which was proposed and described by our

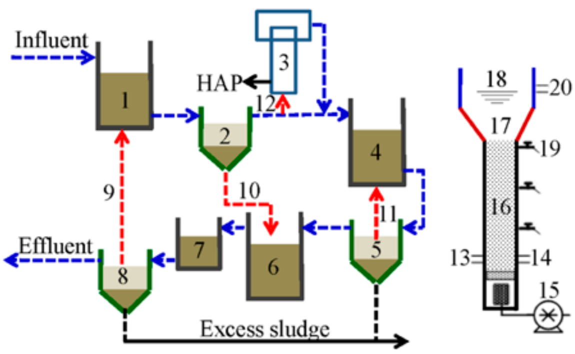

Fig. 1. Schematic diagram of the BNR-IC process.1– anaerobic tank; 2– settling tank; 3– IC column; 4– nitrification tank; 5– settling tank; 6– anoxic tank; 7– post-aeration tank; 8– settling tank; 9– sludge return; 10– sludge bypass return; 11– sludge return; 12– later flow; 13– influent of P-rich solution; 14– feeding CaCl2 solution; 15– aeration; 16– IC reaction zone; 17– buffer zone; 18– settling zone; 19– sample point; 20– effluent

research group (Zou *et al.* 2014b). In the BNR-IC process, the P-rich supernatant in settling tank 2 was partly introduced into IC column to recover P and the sludge from settling tank 2 was led into anoxic tank 6 for denitrifying simultaneous nitrogen and phosphorus removal. The post-aeration tank enhanced the final effluent quality.

Synthetic wastewater and real domestic sewage were used and compared in this study. The influent flow was 18 L/d, and the bypass sludge flow (10), return sludge flow (5, 8) were all 7.2 L/d. The later flow ratio (12) was 6.3 L/d, i.e. 35% of supernatant from settling tank 2 introduced into the IC column, where 4.5 mM $CaCl_2$ solution was led into the IC column with the flow rate of 0.63 L/d. The aeration flow was 90 L/h both in aerobic tank and post-aeration tank, resulting in the dissolve oxygen (DO) high than 2 mg/L and that was 270 L/h in IC column, for CO_2 stripping and fluidization of seed crystals (calcite used here).

1.2. Synthetic and real domestic wastewater

The synthetic domestic wastewater contained carbon, nitrogen and phosphorus in the typical species of real domestic sewage, specific composition detailed in Table 1. COD, NH_4^+-N, TN and TP concentrations in the influent were 239.2~259.5 mg/L, 38.2~41.8 mg/L, 39.6~43.8 mg/L and 8.72~11.40 mg/L, respectively. Real domestic wastewater was collected from the Southeast University, Wuxi, China and its main characteristics were described in Table 2.

Table 1. Composition of synthetic wastewater used in this study.

Composition of feeds	Concentration, g/L	Composition of nutrient solution	Concentration, g/L
CH_3COONa	0.322	$FeCl_3·6H_2O$	1.50
KH_2PO_4	0.044	H_3BO_3	0.15
$(NH_4)_2SO_4$	0.047	$CuSO_4·5H_2O$	0.03
$CaCl_2$	0.005	KI	0.18
$MgSO_4·7H_2O$	0.05	$MnCl_2·4H_2O$	0.12
Nutrient solution	0.30 mL/L	$Na_2MoO_4·2H_2O$	0.06
		$ZnSO_4·7H_2O$	0.12
		$CoCl_2·6H_2O$	0.15
		EDTA	10.00

Table 2. Characteristics of real domestic sewage used in this study.

Contents	Range	Average
COD (mg/L)	153.71-223.63	188.94 ± 16.26
NH_4^+-N (mg/L)	38.50-55.72	48.26 ± 3.85
TN (mg/L)	50.35-67.28	60.01 ± 3.85
TP (mg/L)	3.76-6.43	5.03 ± 0.67
pH	7.08-7.69	7.36 ± 0.19

1.3. Experimental procedure

Before the continuous flow experiments, the two types of activated sludge responsible for phosphorus removal and nitrification have been cultivated in batch tests (Haiming *et al.* 2014), i.e. the start-up of the BNR-IC system. And then, the operating in continuous flow was conducted and divided into two main phases described as follows: (1) from day 0 to day 30, the BNR-IC was operated by feeding synthetic wastewater; (2) at day 31, the real domestic sewage treatment was started for 60 days. During this period, the mixed liquid suspended solid (MLSS) were 3400~3600 mg/L both in anaerobic and anoxic tank and that was 3000~3200 mg/L in nitrification tank. The solids retention times (SRTs) of nitrification sludge and phosphorus removal sludge were 12 and 16 days, respectively.

1.4. Analytical methods

50 mL of samples collected (once a day) from influent and effluent of the BNR-IC were filtered through 0.45-micron membrane filters using a microfiltration apparatus before analysis. COD and MLSS were monitored in accordance with the standard methods (APHA, 2005). TP, NH_4^+-N and TN were performed by using segmented flow analysis (AutoAnalyzer3, SEAL, UK). DO was determined by a DO meter analyzer (YSI DO200, USA) and pH was assessed by a pH meter analyzer (YSI pH100, USA). Statistical treatment (arithmetic mean and standard deviations) of experimental data was done using ORIGIN software (Microcal Software, Northhampton, MA).

Sludge samples (100 mL) were collected at day 30 and 90 for Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analysis, described in our previous report (Zou *et al.* 2014c), mainly including sludge sample pretreatment, total DNA extraction, PCR amplification, DGGE analysis and sequencing of PCR-amplified 16S ribosomal (r) DNA.

2. Results and discussion

2.1. Removal of nutrients from synthetic and real domestic wastewater

Figures 2 and 3 and Table 3 show the carbon, nitrogen and phosphorus concentrations of influent and effluent in the BNR-IC process, using synthetic and real domestic wastewater. In the final periods of operation, a similar nutrients removal behavior was found in the system at day 30 and day 90, respectively. At the stable state, COD, NH_4^+-N, TN and TP concentrations of effluent remained at a low level and were on average 18.75±3.50 mg/L, 5.64±0.26 mg/L, 8.99±0.97 mg/L and 0.42±0.03 mg/L in synthetic wastewater and 25.35±9.72 mg/L, 4.35±0.52 mg/L, 11.89±1.54 mg/L and 0.46±0.02 mg/L in real domestic sewage, respectively, meeting the discharge standard of

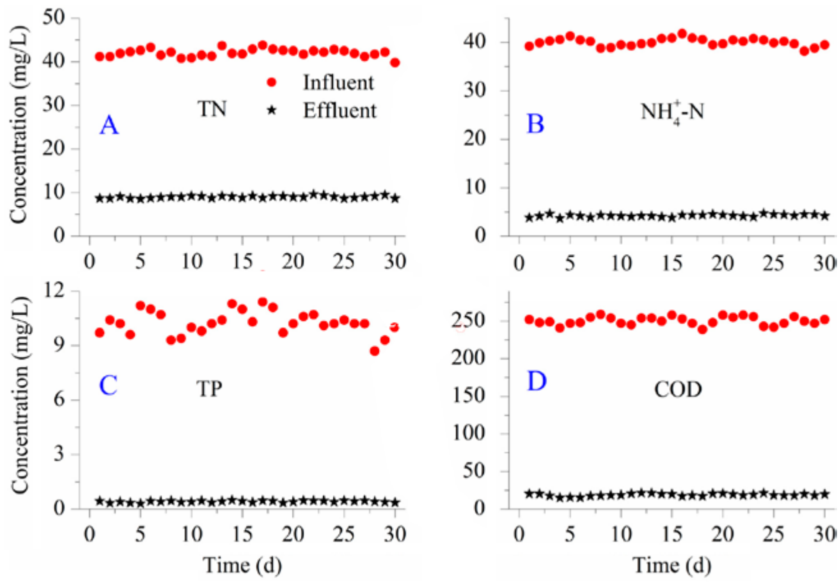

Fig. 2. Variations of COD, NH4-N, TN and TP in the influent and effluent and removal efficiency during synthetic wastewater treatment

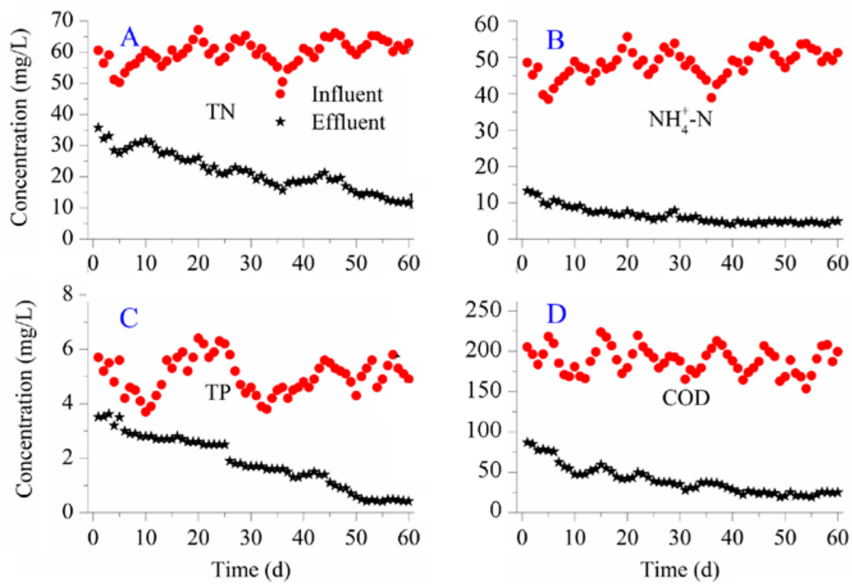

Fig. 3. Variations of COD, NH4-N, TN and TP in the influent and effluent and removal efficiency during real domestic sewage treatment

Table 3. Averaged concentrations of COD, NH_4-N, TN and TP in the influent and effluent and those averaged removal efficiencies when synthetic or real domestic wastewater

Contents (mg/L)	Synthetic domestic wastewater			Real domestic wastewater		
	Influent	Effluent	RE (%)	Influent	Effluent	RE (%)
COD	250.00 ± 2.30	18.75 ± 3.50	92.51 ± 1.41	188.94 ± 16.26	25.35 ± 9.72	87.33 ± 2.05
NH_4^+-N	40.00± 0.82	5.64 ± 0.26	85.93 ± 1.28	48.26 ± 3.85	4.35 ± 0.52	90.21 ± 1.08
TN	42.00 ± 1.21	8.99 ± 0.97	78.62 ± 1.36	60.01 ± 3.85	11.89 ± 1.54	79.66 ± 2.09
TP	10.00 ± 0.46	0.42 ± 0.03	95.20 ± 0.99	5.03 ± 0.67	0.46 ± 0.02	90.67 ± 1.18

municipal domestic sewage treatment (GB 18918-2012) formulated by Chinese government. The low P content in effluent was attributed to the joint action of induced crystallization P recovery and biological phosphorus removal by DPAO. In conventional wastewater treatment process, P content of effluent was hard to meet the stringent discharge standard due to the lack of carbon in real domestic sewage, i.e. low C/N. However, in the BNR-IC system, although the C/N ratios (5.9±0.8 in synthetic wastewater and 3.2±0.3 in real domestic sewage) were still low, P concentrations of effluent were both less than 0.5 mg/L, this suggesting that P recovery effectively enhance the subsequent biological P removal by DPAO due to the decrease at P loading in solution. Another reason may be that DPAO mainly performed the denitrifying simultaneous nitrogen and phosphorus removal in anoxic tank, considerably saving the consumption of carbon source (Lv *et al.* 2015).

During the treatment of synthetic wastewater (30 days), the COD, NH_4^+-N, TN and TP removal performances (Fig. 2) were still constant stable and more than 90% of C and P and around 80% of N were removed from synthetic solution, where those removal efficiencies were 92.51±1.41%, 85.93±1.28%, 78.62±1.36% and 95.20±0.99%, respectively. This is mainly due to the fact that two types of activated sludge responsible for P removal and nitrification were cultured by feeding synthetic wastewater.

For real domestic sewage treatment, as was expected, the nutrients removal efficiencies were extremely low and fluctuant during the beginning period (Fig. 3). With the operation in continuous flow, carbon, nitrogen and phosphorus concentrations in effluent decreased gradually and those removal efficiencies finally remained stable and high after approximately 55 days. The COD, NH_4^+-N, TN and TP removal efficiencies were 87.33±2.05%, 90.21±1.08%, 79.66±2.09% and 90.67±1.18%, respectively, in which, compared with synthetic wastewater treatment, both COD and P removal rates were slightly lower while the N removals including NH_4^+-N and TN were somewhat higher. The lower COD and P removal performances probably result from a decrease in DPAO when treating real domestic sewage. A high content of microorganisms responsible for only denitrifying rather than denitrifying simultaneous nitrogen and phosphorus in domestic wastewater may lead to the high nitrogen removal performance.

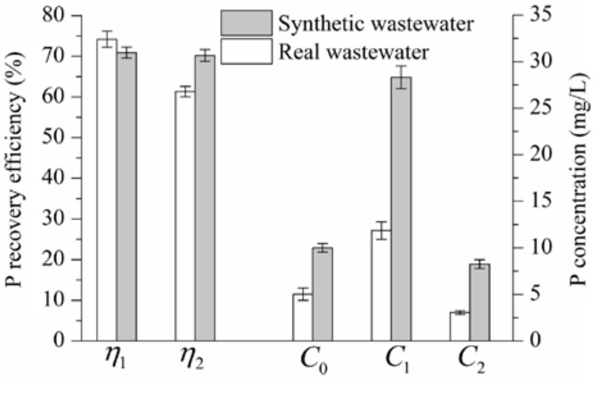

Fig. 4. P concentrations in the influent and effluent of IC column and its recovery efficiencies from synthetic and real wastewater

2.2. Recovery of phosphorus from synthetic and real domestic wastewater

This study introduces an innovative technology and approach to recover P from effluent of anaerobic phase by induced crystallization in the BNR-IC system. The P removal efficiency in IC column and recovery efficiency per liter of wastewater were calculated with the following equations (1) and (2), respectively.

$$\eta_1 = \frac{C_1 - C_2}{C_1} \times 100\% ; \quad (1)$$

$$\eta_2 = \frac{0.35\,(C_1 - C_2)}{C_0} \times 100\% , \quad (2)$$

where: C_0 is the P concentration in synthetic or real domestic wastewater (mg/L); C_1 is the P concentration in the effluent of settling tank 2 (mg/L); C_2 is the P concentration of IC column effluent (mg/L); 0.35 represents the later flow ratio.

Figure 4 presents the influent and effluent characteristics including C_0, C_1 and C_2, P removal efficiency in IC column (η_1) and P recovery efficiency in BNR-IC (η_2), calculated according to Eqs. (1) and (2), during treatment of synthetic and real wastewater.

As can be seen from Figure 4, the thickener P-rich supernatant was obtained in anaerobic tank (C_1) and partly sent to the induced crystallization column for recovering P. During the crystallization process, P content in the solutions fed to the IC column gradually decreased ranging from 28.31 to 8.35 mg/L for synthetic wastewater and 11.85 to 3.06 mg/L for real domestic wastewater, displaying a good P removal performance during induced crystallization process. A specific P concentration of 10 mg/L in the synthetic wastewater was assumed, with a resulting 70.2% of P recovery efficiency. Nevertheless, when P concentration was 5.03 mg/L in real domestic sewage, 74.2% of P recovery efficiency was found. This suggests that lower P concentration in wastewater may lead to a higher P recovery efficiency.

2.3. Change of microbial community structure with feeding synthetic and real domestic wastewater

A total of 13 bands with strong intensity were observed in the two DGGE band patterns, as given in Figure 5. Although there was no significant difference on nutrients

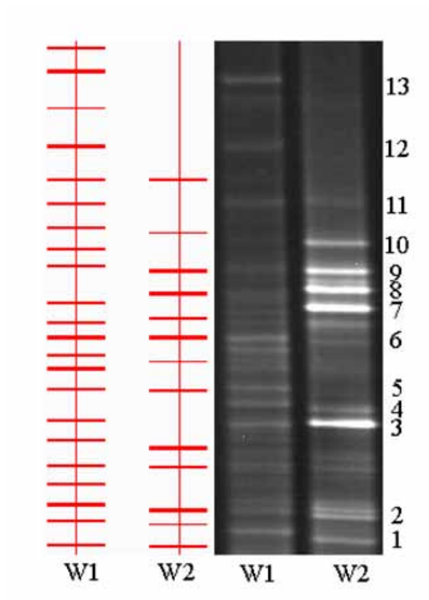

Fig. 5. DGGE fingerprint of 16Sr DNA fragments (right) and lanes comparison (left). W1-real domestic wastewater; W2-synthetic domestic wastewater

removal efficiencies between using synthetic and real domestic wastewater, sludge sample with real domestic sewage displayed a larger diversity of microbial community structure than that with synthetic wastewater. This suggests that influent composition appears to have an influence on the microbial populations, which was consistent with the report (Boelee *et al.* 2011). This may be due to more complex compositions in real domestic sewage than that in synthetic wastewater, probably supporting other bacterial growth. Moreover, real domestic wastewater may be introduced in various microorganisms when passing through sewage conduits (Thai *et al.* 2014). Here, Dice coefficient, comparing the similarities of DGGE fingerprints from different feeding, was 0.42, also suggesting a large shift of microbial community structure when switching feeding synthetic wastewater to real domestic wastewater.

Of these 13 bands, 6 (band 1, 2, 3, 4, 6 and 11) bands were common to both the synthetic wastewater sludge and real domestic sewage sludge. The rest, 3 (band 5, 12 and 13) bands were only found in real domestic sewage sludge and another 4 (7, 8, 9 and 10) only presented in synthetic wastewater sludge. As can be seen in Table 4 and Figure 5, 13 distinct DGGE bands revealed that microbial species were affiliated with 8 phyla or classes domain Bacteria: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*, *Actinobacteria*, *Sphingobacteria*, *Epsilonproteobacteria* and *Chlorobia*.

16S rRNA fragments of bands 3, 7, 8, 9, 10 and 11 belong to genus *Flavobacterium* spp., *Pseudomonas* spp., *Terrimonas* spp., *Thermomonas spp.*, *Simplicispira* spp. and *Arcobacter spp.* Of them, *Pseudomonas* spp., *Terrimonas* spp. and *Thermomonas spp.* with high content in the

Table 4. NCBI BLAST search results of sequences from DGGE bands.

Band	Closest match	Similarity %	Phylogenetic affiliation
1	*Acinetobacter spp.*	98	*Gammaproteobacteria*
2	*Rhodocyclus spp.*	96	*Betaproteobacteria*
3	*Flavobacterium spp.*	100	*Flavobacteria*
4	*Brachymonas spp.*	98	*Betaproteobacteria*
5	*Tetrasphaera spp.*	99	*Actinobacteria*
6	*Dechlorosoma spp.*	95	*Betaproteobacteria*
7	*Pseudomonas spp.*	100	*Gammaproteobacteria*
8	*Terrimonas spp.*	100	*Sphingobacteria*
9	*Thermomonas spp.*	100	*Gammaproteobacteria*
10	*Simplicispira spp.*	100	*Betaproteobacteria*
11	*Arcobacter spp.*	100	*Epsilonproteobacteria*
12	*Chlorobaculum spp.*	87	*Chlorobia*
13	*Methylocystis spp.*	98	*Alphaproteobacteria*

sludge were only found as feeding synthetic wastewater, probably responsible for P removal. *Tetrasphaera spp.* (band 5), *Chlorobaculum spp.* (band 12) and *Methylocystis spp.* (band 13) were only detected within the DGGE patterns of feeding real domestic sewage. *Tetrasphaera spp.* was regarded as a PAO in wastewater treatment system (Kristiansen *et al.* 2013). It is probably that the corresponding organism of band 12, *Chlorobaculum* spp., is responsible for a sulfate reduction and was often found in secondary sedimentation tanks (Zhang *et al.* 2012). It was introduced into the BNR-IC system possibly from real domestic wastewater. In addition to, the band 2 corresponding to *Rhodocyclus spp.* is a typical DPAO capable of simultaneous nitrogen and phosphorus removal and was often found in biological enhanced phosphorus removal system (Huang *et al.* 2015; Lv *et al.* 2015; Zengin *et al.* 2011).

Conclusions

This study showed that effective nutrients removal and phosphorus recovery were achieved in the novel BNR-IC process regardless of feeding types (synthetic or real domestic wastewater). The major phosphorus recovery mechanism was induced crystallization of HAP. When the BNR-IC system achieved the stable state, the COD, NH_4^+-N, TN and TP concentrations of effluent remained at a low level both for treating real domestic sewage and synthetic wastewater, meeting the discharge standard of municipal domestic sewage treatment (GB 18918-2012) formulated by Chinese government. Phosphorus recovery efficiency from synthetic wastewater was 70.2% slightly less than that from real domestic sewage (74.2%). Importantly, phosphorus recovery effectively enhanced the subsequent biological phosphorus removal. It was found that an obvious shift in microbial community structure was observed by PCR-DGGE, and diversity of sludge using real domestic sewage was higher than that with synthetic wastewater. The results of this study suggest that the BNR-IC operated in continuous flow is feasible for nutrients removal and phosphorus recovery from domestic sewage.

Acknowledgements

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