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Pre-processing of raw wastewater in a septic tank leads to phosphorus removal by phosphine production in a sequencing batch biofilm reactor (SBBR)

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ABSTRACT

Two sequencing batch biofilm reactor (SBBRs) were initially inoculated with the same inoculum sludge (dewatered activated sludge), but SBBR2 then was fed with raw wastewater pre-processed in a septic tank containing human faeces. After that, the two SBBRs had been operated under the same condition. The phosphorus removal performance, fate of phosphorus and microbial community diversity in both SBBRs were investigated and compared. The results indicated that the continuous inoculation of SBBR2 with the microfauna of the septic tank transported with the pre-processed influent wastewater had a remarkable effect on the phosphorus removal pathway. In SBBR1 without being pre-processed, the phosphorus was removed by traditional enhanced biological phosphate removal process. On the contrary, the phosphorus removal pathway in the SBBR2 was ascribed to phosphine-based process, with a significant concentration of 3.11 mg PH3 $kgWS^{-1}$ (wet sludge) of matrix-bound phosphine detected. The results of sludge fractionation illustrated that the phosphine-based process mainly included effective decomposition of organic phosphate compounds to generate phosphine. Denaturing gradient gel electrophoresis fingerprints demonstrated that the continuous inoculation of SBBR2 had led to the difference in community compositions.

Keywords: Denaturing gradient gel electrophoresis; Inoculum source; Matrix-bound phosphine; Phosphate reduction; Sequencing batch biofilm reactor

1. Introduction

Traditionally, biological phosphorus removal processes from wastewater can be accomplished by two ways: stoichiometric coupling to microbial growth or enhanced storage in the biomass as polyphosphate (poly-P) [1]. Based on the latter mechanism, the enhanced biological phosphate removal (EBPR) process was developed by engineers for commonly biological phosphorus removal in wastewater treatment

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worldwide. EBPR can be achieved through the activated sludge process by recirculating sludge through anaerobic and aerobic conditions [2]. By this configuration, poly-P-accumulating micro-organisms (PAOs) which accumulate poly-P were selected and grew to dominance in the process. High phosphate removal efficiency can be achieved by withdrawing the excess sludge with high phosphorus content.

Unlike traditional EBPR, the biotic reduction of phosphate to phosphine may become a new pathway for biological phosphorus removal. Based on the mechanism, phosphorus is removed as the release of phosphine, which not only increases the stability of biological phosphorus removal but also simplifies the treatment process.

Phosphine (PH_3), a highly reducing gas, has been proved to be a ubiquitous trace gas in the atmosphere as well as a significant constituent in the phosphorus biogeochemistry cycle [3]. PH_3 exists in the natural environment primarily in two different forms: free gaseous PH_3 and matrix-bound PH_3 (MBP). MBP has been defined as phosphine bound to condensed environmental samples (such as lake sediments, animal manure, human faeces, etc.), which can be liberated into gaseous PH_3 under the effect of acid or alkaline digestion [4–10]. Since gaseous PH_3 produced during the fermentation process is prone to being absorbed by condensed matrix, the actual amount of PH_3 released in free gas form is much less than the total amount of PH_3 produced in soils or sediments [11].

A prominent number of evidences have proved that phosphine is transformed from phosphate biogenetically by phosphate-reducing organisms (PROs). Rutishauser and Bachofen reported that phosphine production from sewage sludge cultures was a microbiologically mediated reaction [12]. Devai et al. detected phosphine in the headspace of an anaerobic bacterium culture in which the peptone and inorganic P-compounds was contained. The P-concentration in the culture medium was reported to decrease from 340 to 198 mg P/L over a period of 56 d [13]. Moreover, the inoculum source is of great significance to the formation of phosphine due to phosphate reduction. Cao et al. performed a lab-scale simulation of converting typical phosphorus-containing substrates into phosphine under anaerobic conditions, and found that chicken manure led to an obvious increment in phosphine emission [14]. Jenkins et al. demonstrated that the generation of phosphine under anaerobic conditions depended upon the inoculum source (animal faeces) and the enrichment culture conditions. Only in culture mediums inoculated with pig and sheep faeces phosphines were detected [15]. Gassmann and Glindemann incubated phosphine-free medium inoculated with human faeces anaerobically and observed a striking increase in phosphine content in the medium [16].

The sequencing batch biofilm reactor (SBBR) has provided the advantages of both the biofilm reactor and batch operational mode, and is known to be particularly robust and to withstand extreme conditions. The complex ecosystem in biofilms contains different areas of aerobic, anoxic and anaerobic. Thus, the anaerobic area in the biofilm is able to support a variety of PROs. In the literatures, the successful experiences of the new phosphorus removal pathway based on SBBR were very limited. The objectives of this research were: first, to investigate the availability of treating domestic wastewater for phosphorus removal by phosphine production by pre-processing of raw wastewater in a septic tank; second, to evaluate the change of phosphate in the process of sewage disposal, specifically the removal, transportation, distribution of phosphorus; and finally, to show how the continuous inoculation of SBBR with the microfauna of the septic tank affects the microbial community over time using denaturing gradient gel electrophoresis (DGGE) method.

2. Materials and methods

2.1. Raw wastewater

The raw wastewater in SBBR1, with detailed characteristics shown in Table 1, was collected directly from the sanitary sewer of the student's dormitory. For the other SBBR2, the feed water was pre-processed by a septic tank. To maintain the comparability in raw water quality, glucose ($C_6H_{12}O_6$), NH_4Cl and KH_2PO_4 were then added to the effluent from the septic tank.

2.2. Reactor configurations

As shown in Fig. 1, two SBBRs used in this study were identical, both made of unplasticized polyvinyl chloride, with dimensions of $350 \times 200 \times 250$ mm (L × W × H) and a total effective volume of 10 L. Semi-flexible combined packing materials (polyacrylonitrile disk combined with fibre threads) were chosen as biofilm carriers considering their high specific areas of 350 $m^2\ m^{-3}$ and relatively low costs. The filling ratio of pack materials was about 45%. Oxygen was introduced into the reactor by an air pump. Throughout the duration of experiment, the temperatures in both bioreactors were maintained at 30 ± 0.5℃ with heater. Besides, a timer control system was used as well.

Table 1
Characteristics of the raw wastewater

COD_{cr}/(mg L^{-1})	NH_4^+-N/(mg L^{-1})	T-N/(mg L^{-1})	PO_4^{3-}-P/(mg L^{-1})	T-P/(mg L^{-1})
1,000 ± 150	75 ± 25	130 ± 30	6.0 ± 2.0	7.5 ± 2.0

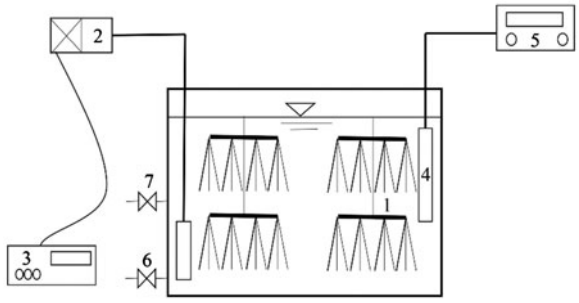

Fig. 1. Schematic diagram of SBBR ((1) Semi-flexible packing materials, (2) air pump, (3) timer, (4) heater, (5) temperature controller, (6) sampling port, (7) emptying pipe).

2.3. Operation procedures

First of all, both reactors were inoculated with 10 g L^{-1} sludge seed by adding them into the two reactors. The sludge seed used in this study was dewatered sludge taken from a sewage wastewater treatment plant in Chongqing, China, which was operated for EBPR process. Then, differently, raw wastewater (SBBR1) and raw wastewater pre-processed in a septic tank containing human faeces (SBBR2) were used as influents, respectively. Finally, both reactors were operated under the same condition: with organic loading of 1.0 kg COD m^{-3} d^{-1}, DO concentration of 4 mg L^{-1} and continuous aeration, running in a cycle at 12 h for 30 d, without discharge of excess sludge. The operation strategies in the two SBBRs were: fill (0.1 h)—aeration phase (11.5 h)—settle (0.2 h)—draw (0.2 h).

2.4. Analytical methods

Water samples were collected once a day at the end of each cycle. During the single operation cycle, samples were taken every hour. Phosphate (PO_4^{3-}-P), and total phosphorus (TP) concentrations were detected by ultraviolet spectrophotometer (HACH, DR 5000) according to Standard Methods [17]. The chemical oxygen demand (COD) was measured following the closed-reflux titration with potassium dichromate according to Standard Methods [17]. Temperature and DO concentration were continuously measured online by a DO detector (HACH LDO, USA).

Sludge samples were collected on the 30th day from the biofilm of both two SBBRs. TP_S concentrations in sludge were measured using potassium sulphate digestion method [12].

With regard to the MBP analysis, "Alkaline digestion—Bromide nitrate (HNO_3–Br_2–H_2O solution) oxidation—Antimony molybdenum spectrophotometry" method was adopted due to the high concentration with an order of magnitude at mgPH_3 kgWS^{-1}. First, 5 mL of sodium hydroxide solution (4.5 mol L^{-1}) was added into reaction flask A in order to transform the MBP in the sludge into gaseous PH_3 under the effect of alkaline digestion, 20 mL of bromide nitrate was added into absorption flask to oxidize the gaseous PH_3 into phosphate and 5 g sludge samples centrifuged for 10 min at 6,000 rpm to remove pore water was placed in reaction flask B for reaction. Second, the system was structured as shown in Fig. 2 and each part of the joints was sealed. The system was purged with high purity nitrogen to remove air and to ensure a reducing environment during the experiment. Third, sodium hydroxide solution in A reaction flask was poured into B reaction flask for reaction. In order to make the reaction rapidly and completely, B reaction

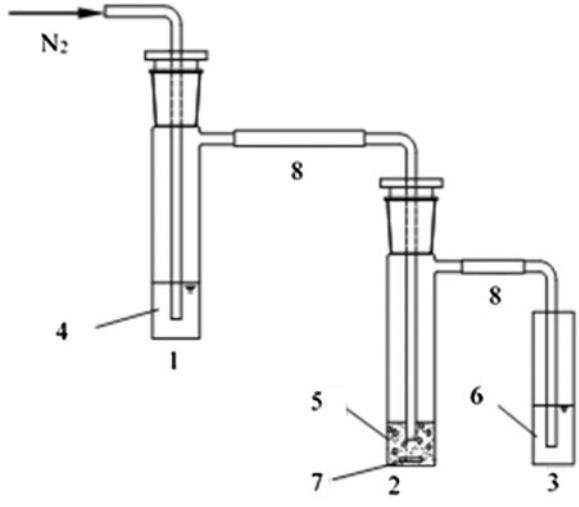

Fig. 2. Schematic diagram of MBP measurement ((1) A reaction flask, (2) B reaction flask, (3) absorption flask, (4) NaOH solution, (5) sludge sample, (6) HNO_3–Br_2–H_2O solution, (7) magnetic stirrer, (8) rubber pipe)).

flask was heated by water bath after it was boiled by alcohol lamp, with magnetic stirring for 10 min. Next, absorption flask was then taken off and the absorption liquid in it was pooled into a beaker. The beaker was heated for a while to oxidize reduced phosphorus completely and to remove both bromine and nitrogen. Finally, the absorption liquid was diluted to 100 mL. Phosphate (PO_4^{3-}-P) concentrations of absorption liquid were measured, and MBP concentrations of sludge were obtained through the conversion.

Sequential extraction steps were performed according to the method of Uhlmann et al. as shown in Table 2 [18]. About 1 g of sludge was placed in a 50 mL acid-washed centrifuge tube for fractionation. The supernatants of each extraction after centrifugation for 12 min at 4,500 rpm were analyzed for molybdate reactive phosphorus. All analyses were done in triplicate, and the MBP results were given on a dry weight (dw) basis.

2.5. Microbial community analysis

The community characteristics of sludge were analyzed using DGGE method.

Sludge samples were collected by scraping the sludge from the surface of the biofilm carriers in two SBBRs weeks after the start of the period of adaptation and from inoculum sludge and septic tank. All samples were kept at −20℃ until used for DNA extraction.

The total DNA of sludge samples was extracted with a bead beater and three freeze–thaw cycles in boiling water and liquid nitrogen [19]. Finally, detect the extraction effect by 0.8% agarose gel electrophoresis. DNA was stored at −20℃ until analyzed.

PCR amplification of sludge sample DNA. The samples were subjected to PCR using universal primers targeting all bacteria: F357GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and 518R (5′-ATT ACC GCG GCT GCT GG-3′), the F357GC primer containing a GC clamp (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGGG). The PCR condition was performed using an initial denaturation step at 94℃ for 5 min and 30 cycles of denaturing (45 s at 94℃), annealing (45 s at 60℃) and extension (90 s at 72℃); and the final extension for 10 min at 72℃. The PCR products was verified by agarose gel electrophoresis (2.0% agarose, 1 × Tris-acetate-EDTA) followed by ethidium bromide staining to confirm the product size.

PCR products were analyzed by DGGE with D-code universal mutation detection system (Bio-Rad laboratories, USA). Twenty-five microlitres of each PCR product was loaded onto 8% (w/v) polyacrylamide gel (containing 37.5:1 of acrylamide to bis-acrylamide) with a linear denaturant gradient ranging from 37.5 to 55% (of urea, w/v and formamide, v/v). The electrophoresis was performed at 60℃, initially at 200 V (10 min) and then at 80 V (900 min). After the electrophoresis, the gel was stained for 25 min with ethidium bromide and immediately photographed under UV transillumination using BIO-RAD Versa Doc.

Gel images were analyzed using quantitation software version 4.6.2 (Bio-Rad Laboratories, USA). DNA bands were automatically detected and the similarities in band patterns were measured as Dice coefficients (unweighted data based on band presence or absence).

3. Results and discussion

3.1. Effect of the pre-processing of the raw wastewater on the performance of SBBRs in phosphorus removal

The SBBR1 was fed with raw wastewater, while SBBR2 was fed with raw wastewater pre-processed in

Table 2
Extraction scheme and fractional composition of reactive phosphorus

Extraction medium	Fraction acronym	Main species
H_2O (for 10 min)	H_2O-RP	Easily extractable (washable) phosphorus
BD (0.11 mol L^{-1} buffered sodium dithionite for 30 min at 40℃)	BD-RP	Reductant soluble phosphorus
NaOH (1.0 mol L^{-1} sodium hydroxide for 16 h at room temperature)	NaOH-RP	Iron- and Aluminium-bound P (Fe, Al–P)
HCl (0.5 mol L^{-1} hydrochloric acid for 24 h at room temperature)	HCl-RP	Calcium- and magnesium-bound P (Ca, Mg–P)
NaOH (1.0 mol L^{-1} sodium hydroxide for 24 h at 85℃)	NaOH85-RP	Refractory P, inorganic or organic polyphosphates

a septic tank containing human faeces. The performances of the two SBBRs were investigated and the results are shown in Fig. 3. As for SBBR1, the TP removal efficiencies gradually increased during the first 13 d. On the 13th day, the effluent TP concentration was reduced to 0.72 mg L^{-1} with the removal efficiency of 90.9%. TP removal efficiencies were 85–94% from day 13 to day 17 after acclimation, while TP removal efficiencies decreased from day 17. On the 29th, the influent TP concentration was 8.80 mg $^{-1}$L, while the TP concentration in the effluent was as high as 9.80 mg L^{-1}, indicating that an apparent phosphorus release was probably due to the prolonged operation. For SBBR2, the increase of TP removal efficiencies was observed during the first seven days. On the seventh day, the effluent TP concentration was 0.90 mg L^{-1} with the removal efficiency of 90.5%. TP removal efficiencies remained stable at 82–92% in following long-term operation (Fig. 3 shows only the first 30 d) after acclimation, with the residual TP concentration below 1.3 mg L^{-1}. Phosphorus release did not occur in the SBBR2.

These results indicated that the TP removal pathway in the two SBBRs were different. The TP removal from wastewater in the SBBR1 was done by traditional PROs (PAOs). The complex ecosystem in biofilms contained different combinations of the following microbial processes: aerobic oxidation, nitrification, denitrification, phosphorus accumulation and methanogenesis [20–22]. Consequently, in the study, the stratification in aerobic–anoxic–anaerobic biofilms had ensured the phosphorus removal in SBBR1 under aeration in the bulk liquid. Phosphates were first released by PAOs activities under anaerobic conditions and then excessive took by PAOs activities under aerobic conditions [23]. The TP removal would be achieved if the excess sludge with high phosphorus content was discharged [24]. The phosphorus release occurred on the 29th day because the excess sludge was retained in the reactor, while the TP removal pathway in the SBBR2 was based on the phosphine-related process. Due to the conversion of dissolved phosphate to phosphine (both free gaseous PH_3 and MBP), no phosphorus release occurred. The concentrations of MBP in the sludge from both SBBRs were detected on the 30th day. No MBP was detected in inoculum sludge or in the sludge of SBBR1, while a significant MBP concentration of 3.11 mgPH_3 kgWS^{-1} (wet sludge) was detected in the sludge of SBBR2. From the engineering perspective, the phosphine-based process was meaningful for wastewater treatment because phosphorus was removed with high efficiency as the form of phosphine, which can overcome the two main drawbacks of traditional EBPR: the phosphorus release and the disposal of excess sludge containing high concentration phosphorus.

3.2. The fate of phosphate in single operation cycle of the SBBRs

As shown in Fig. 4, during the first 30 min of the single operation cycle, TP concentration in the SBBR1 increased and then sharply decreased to a low level, due to the phosphorus uptake by PAOs. The TP removal behaviour in the SBBR1 was similar to the conventional EBPR process in which phosphorus was significantly released by PAOs during anaerobic phase while rapidly absorbed in aerobic phase for PAOs growth and intracellular poly-P formation [25]. While

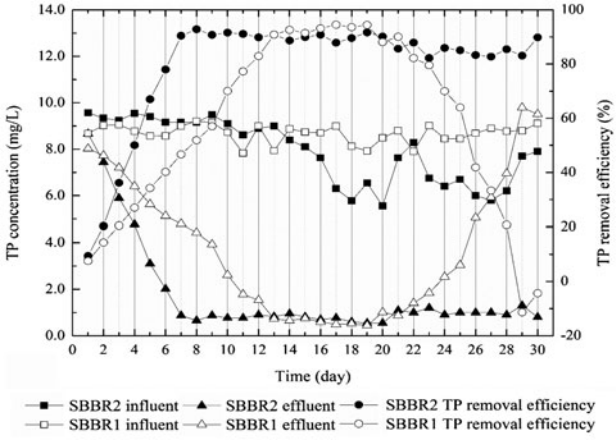

Fig. 3. Phosphorus removals in two SBBRs during 30 d operation period.

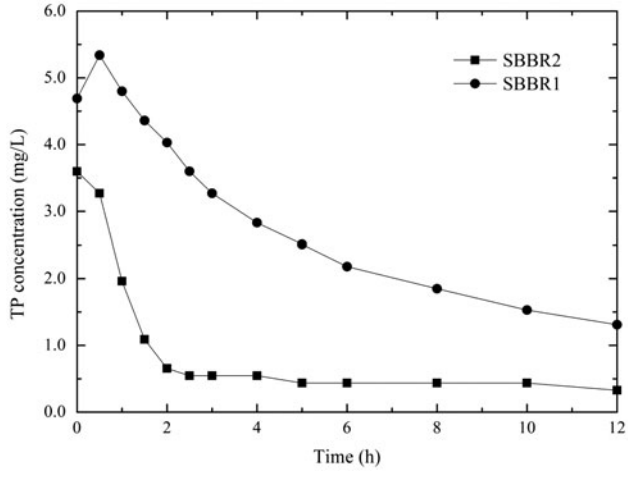

Fig. 4. Variations of TP in the single operation cycle the 14th day.

for SBBR2, TP concentration kept decreasing throughout the operation cycle, which was completely different from traditional phosphorus removal process in single operation.

The result also indicated that the phosphorus removal pathway in SBBR2 wasnot the traditional one, and the phosphorus was transformed by phosphine-based process into phosphine (both free gaseous PH_3 and MBP).

3.3. Mass balances of the phosphorus in the 30-d operation

Mass balances of phosphorus in the both SBBRs were further calculated in 30-d operation and shown in Tables 3 and 4. It can be observed that a decrease of 1,330 mg phosphorus in the liquid was accompanied with an increase of 660 mg phosphorus in the solid, indicating that a TP loss of 670 mg in the system over 30-d operation in SBBR2. Differently, in terms of SBBR2, a total of 1,192 mg phosphorus in the liquid decreased. A significant increase in the number of phosphorus in the solid was witnessed with 1,041 mg. Consequently, only 151 mg phosphorus lost in the system during the same period.

The huge gap in the amount of phosphorus loss in the system between the two SBBRs indicated that the TP removal pathway in the two SBBRs varied. As SBBR1 was traditional EBPR process, slight phosphorus loss (151 mg) in the system was as a result of biomass growth. The majority of phosphorus removal from wastewater was accomplished by poly-P storage in the sludge (1,041 mg). Conversely, with regard to SBBR2, the large phosphorus loss in the system (670 mg) could be resulted from the emission of gaseous PH_3. Phosphorus was mainly removed from wastewater due to gaseous PH3. This result in SBBR2 was in agreement with the observation of Devai et al., who suggested that 30–45% loss of the TP in open-air sewage treatment plants could be attributed to the release of PH_3 into the atmosphere [13].

3.4. The distribution of phosphorus during wastewater treatment

Results of phosphorus fractionation of sludge are shown in Table 5. The concentration of TP_S in SBBR2 and SBBR1 declined by 3.91 mg P gDS^{-1} (dry sludge) and 0.58 mg P gDS^{-1}, respectively, compared to inoculum sludge. The dramatic decrease of TP_S in SBBR2 indicated that the high MBP contents in the sludge, which released in gaseous phosphine under circumstance conditions, played an important role in it. It was also founded that the main decrease of TP_S in the SBBR2 was Org-P (organic P = TP_S—Inorganic P) with a decreased amount of 0.22 mg P $gDS^{-1.}$

The results indicated that phosphine-based process included effective decomposition of organic phosphate compounds to generate phosphine. Yu and Song reported that phosphine in the sediments of Jiao Zhou Harbor were mostly resulted from micro-biotic decomposing of organic phosphate under anaerobic condition [26]. Liu et al. proved that the content of phosphine was correlated relatively more with the presence of organic phosphate, with a correlation

Table 3
Mass balances of the phosphorus in SBBR2

Phosphorus in the liquid	Influent TP	6.5 ± 0.5 mg L^{-1}
	Effluent TP	1 ± 0.2 mg L^{-1}
	TP removal efficiencies	85%
	TP loss	5.5 ± 0.2 mg L^{-1}
	Treating wastewater per day	8 ± 0.3 L d^{-1}
	TP loss in total	$1{,}326 \pm 4.5$ mg
Phosphorus in the sludge	Inoculum sludge	
	Sludge amount	100 ± 1.0 g
	TP concentration in sludge	16.9 ± 0.5 mgP gDS^{-1}
	Biofilm	
	Sludge amount	104.3 ± 1.0 g
	TP concentration in sludge	18.1 ± 0.7 mgP gDS^{-1}
	Sediment	
	Sludge amount	19.4 ± 0.4 g
	TP concentration in sludge	23.6 ± 0.5 mgP gDS^{-1}
	TP increase in total	656 ± 4.0 mg
TP loss in the system	$\Delta = 1326–656$ mg = 670 ± 4.5 mg	

Table 4
Mass balances of the phosphorus in SBBR1

TP loss in the liquid	1,192 ± 3.4 mg	
Phosphorus in the sludge	Inoculum sludge	
	Sludge amount	100 ± 1.0 g
	TP concentration in sludge	16.9 ± 0.5 mgP gDS^{-1}
	Biofilm	
	Sludge amount	108.4 ± 1.1 g
	TP concentration in sludge	20.3 ± 0.6 mgP gDS^{-1}
	Sediment	
	Sludge amount	21.6 ± 0.6 g
	TP concentration in sludge	24.6 ± 0.5 mgP gDS^{-1}
	TP increase in total	1,041 ± 3.0 mg
TP loss in the system	Δ = 1,192–1,041 mg = 151 ± 3.4 mg	

Table 5
Phosphate fractions of sludge (mg g^{-1} dry sludge)

Sample	TP_S	Iorg-p	Org-P	H_2O-P	BD-P	NaOH-P	HCl-P	NaOH (85)-P
Inoculum sludge	17.99	8.96	9.03	1.42	2.58	3.58	0.91	0.46
SBBR1	17.41	9.18	8.23	1.03	2.86	3.08	1.08	1.14
SBBR2	14.08	7.97	6.11	0.54	2.24	3.24	1.54	0.41

parameter up to 0.81 [27]. Han et al. found an increment of both gaseous PH_3 and MBP when the soil sample was incorporated with C–P bond organic phosphate [28].

Inorganic phosphorus (Iorg-P) in this study consists of five fractions (H_2O-P, BD-P, NaOH-P, HCl-P and NaOH (85)-P). The comparison of different Iorg-P species in three sludge samples showed that the fraction of sedimentary P (NaOH-P), which refers to P bound to metal (hydr)oxides, mainly of Fe and Al and reductant soluble phosphate (BD-P), which refers to P bound to ferric hydroxide, was the most important mass fractions of TPs in sludge.

The results indicated that chemical precipitation and adsorption play a critical role in the removal of phosphorus from the solution. Other three species were a minor portion of Iorg-P in the sludge, indicating its minimal effect on the P removal.

3.5. Microbial community structure

The DGGE fingerprinting of PCR-amplified 16S rDNA was shown in Fig. 5. From a qualitative perspective, the community fingerprints of the four samples followed different patterns. After the start of the period of adaptation, the sudden appearance of several bacterial species (additional bands) in two SBBRs appeared in comparison to inoculum sludge. The dominant strains (bold bands) in two SBBRs were different.

This indicated that a significant shift in microbial community from inoculum sludge was operated when the wastewater was present in two SBBRs. Fig. 5 also showed that the continuous inoculation of SBBR2 with pre-processed wastewater transporting the microfauna of the sceptic tank led to a significant change in the distribution of band migration between two SBBRs.

The similarities between the different DGGE patterns were determined by calculating similarity indices (expressed as percentages) based on the Dice similarity coefficient. The Dice coefficient is commonly used to compare species composition of different ecosystems. Dice similarity matrix (Table 6) of DGGE fingerprints showed that SBBR1 and human faeces had the highest similarity (56.2%), while SBBR2 and inoculum sludge (43.6%) bore the highest resemblance.

This indicated that the continuous inoculation of SBBR2 with pre-processed wastewater transporting the microfauna of the sceptic tank could have led to the difference in community compositions. The SBBR2 was phosphine-based process, in which microbial community was different from traditional EBPR process of SBBR1. The continuous inoculation served as a decisive factor for phosphine-based process developing during biological wastewater treatment.

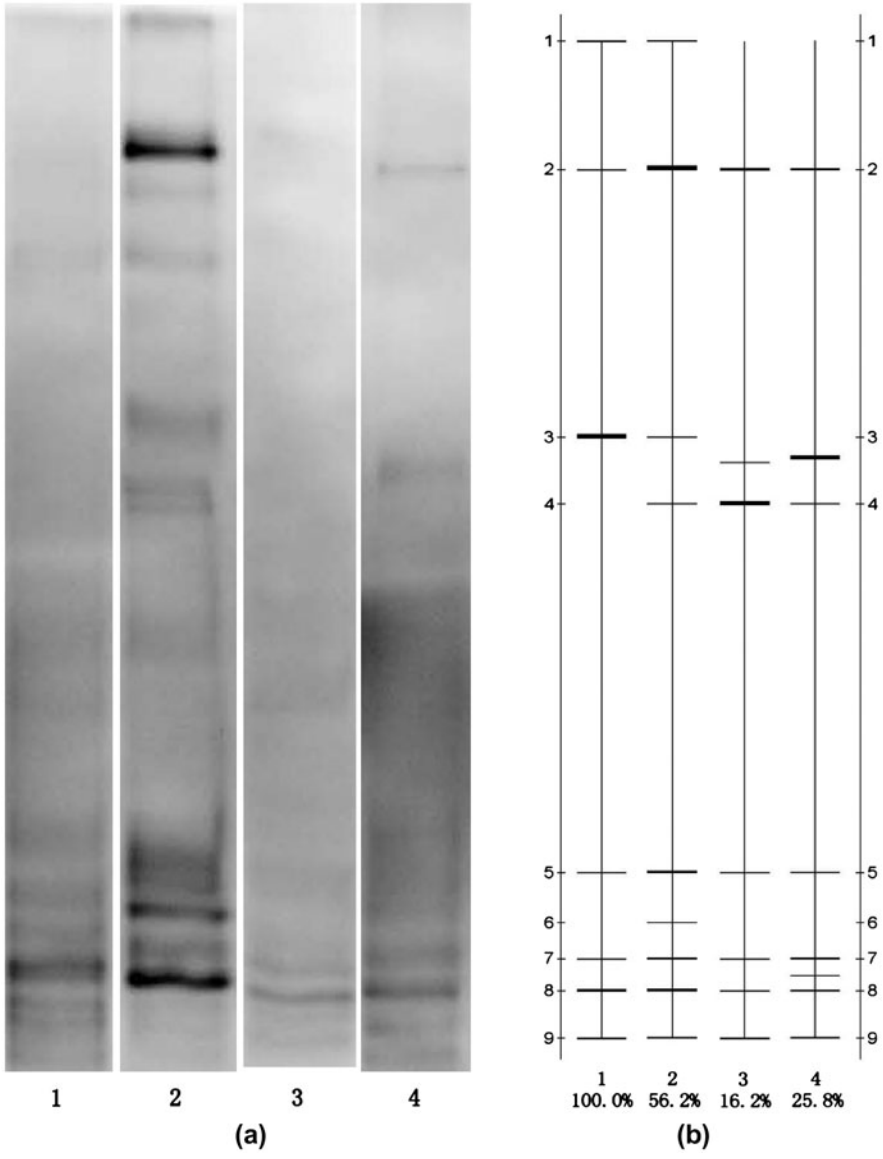

Fig. 5. DGGE analysis of the three sludge samples. (a) DGGE images of the PCR products. Numbered gel lanes contain PCR-amplified 16S rDNA gene fragments from reactors: (1) human faeces, (2) inoculum sludge, (3) SBBR1, (4) SBBR2. (b) A schematic representation of overall DGGE banding patterns showing band number (side bars) and generated from quantity one which is based on bands presence or absence.

Table 6
Dice coefficients (Cs) comparing the similarities of PCR-DGGE fingerprints (Fig. 5)

Similarity (%)	Human faeces	Inoculum sludge	SBBR1	SBBR2
Human faeces	100.0	25.8	33.4	43.6
Inoculum Sludge	25.8	100.0	56.2	16.2
SBBR1	33.4	56.2	100.0	35.9
SBBR2	43.6	16.2	35.9	100.0

4. Conclusions

The pre-processing of the raw wastewater in a septic tank containing human faeces had led to phosphorus removal by phosphine production in SBBR. Compared to SBBR1, SBBR2 was inoculated with the microfauna of the septic tank transported with the pre-processed influent wastewater in which the phosphorus removal pathway was phosphine-based process other than traditional EBPR process. The removal efficiency of TP remained stable and high (85–94%) throughout the long-term operation without phosphorus release. Results of phosphorus fractionation of sludge revealed that this phosphine-based process mainly included effective decomposition of organic phosphate compounds to generate phosphine. What's more, DGGE fingerprints indicated that the continuous inoculation of SBBR2 had led to the difference in community compositions.

Acknowledgement

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