



## Simultaneous nitrification–denitrification and phosphorus removal in a fixed bed sequencing batch reactor (FBSBR)

Yousef Rahimi<sup>a,\*</sup>, Ali Torabian<sup>a,1</sup>, Naser Mehrdadi<sup>a,2</sup>, Behzad Shahmoradi<sup>b,3</sup>

<sup>a</sup> Department of Civil & Environmental Engineering, Graduate Faculty of Environment, University of Tehran, No. 25 Qods St., Enghelab Ave, Tehran, Iran

<sup>b</sup> Department of Environmental Science, University of Mysore, MGM-06 Mysore, India

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### ABSTRACT

Biological nutrient removal (BNR) was investigated in a fixed bed sequencing batch reactor (FBSBR) in which instead of activated sludge polypropylene carriers were used. The FBSBR performance on carbon and nitrogen removal at different loading rates was significant. COD, TN, and phosphorus removal efficiencies were at range of 90–96%, 60–88%, and 76–90% respectively while these values at SBR reactor were 85–95%, 38–60%, and 20–79% respectively. These results show that the simultaneous nitrification–denitrification (SND) is significantly higher than conventional SBR reactor. The higher total phosphorus (TP) removal in FBSBR correlates with oxygen gradient in biofilm layer. The influence of fixed media on biomass production yield was assessed by monitoring the MLSS concentrations versus COD removal for both reactors and results revealed that the sludge production yield ( $Y_{obs}$ ) is significantly less in FBSBR reactors compared with SBR reactor. The FBSBR was more efficient in SND and phosphorus removal. Moreover, it produced less excess sludge but higher in nutrient content and stabilization ratio (less VSS/TSS ratio).

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### 1. Introduction

The adverse environmental impacts associated with ammonia nitrogen include promotion of eutrophication, toxicity to aquatic organisms, and depletion of dissolved oxygen in receiving water bodies because of bacterial oxidation of ammonia to nitrate. Therefore, the removal of nitrogen compounds from wastewater is of increasing importance [1]. In the SBR process, nutrients removal could be accomplished by three methods: (1) biological phosphorous removal and nitrate denitrification by providing anoxic and anaerobic periods, (2) cyclic aeration (on/off) during the reaction period, and (3) operating at low DO concentration to encourage simultaneous nitrification–denitrification (SND) [2]. It is useful to combine both steps of nitrogen removal (nitrification–denitrification) in the same period to reduce the operational requirement for separating processes of nitrification and denitrification in treating municipal wastewater [3].

During the past few years, some researchers have investigated SND in biofilm SBR, whereas most of them have investigated

the integration of moving bed–packing media with SBR process. The nitrogen and phosphorus removal in SBR and moving-bed SBR (MBSBR) with different ammonium loading rates have been investigated [4]. It was found that increasing influent ammonium concentration from 20 mg/L to 80 mg/L resulted in decreasing nitrogen and phosphorus from 77% to 33.3% in SBR, which can be contributed to the effect of incomplete denitrification and pH drop, while the nitrification rate in the aerobic phase did not change remarkably in MBSBR because of occurring SND.

In MBSBR, the efficiency of SND depends on dissolved oxygen, the thickness of the biofilm, and the influent concentration. DO concentration in the reactor is not suggested to be more than 4.0 mg/L. The thicker biofilm is advantageous for SND. TN removal rate by SND could be 74–82% if suitable operational parameters are adopted and also if influent  $\text{NH}_4^+\text{-N}$  is less than 110 mg/L [5]. It was reported that simultaneous removal of phosphorus and nitrogen was possible in the biofilm SBR as indicated by the respective removal ratio of around 90% and 57% at a COD loading of 1.00 kg COD/m<sup>3</sup> per day [6].

Removal of COD from coking-plant wastewater using MBSBR was investigated [7]. In general, the system achieved removal efficiencies of 92.9% at a low organic loading rate (OLR) of 0.449 kg COD/m<sup>3</sup> per day and 70.9% when OLR increased to 2.628 kg COD/m<sup>3</sup> per day. The system had strong tolerance to organic shock loading in this experiment.

The possibility of 4-chlorophenol (4CP) removal in a MBSBR was studied [8]. The MBSBR showed great robustness against starva-

\* Corresponding author. Tel.: +98 2161113184; fax: +98 2166407719.

E-mail addresses: [you.rahimi@gmail.com](mailto:you.rahimi@gmail.com), [yrahimi@ut.ac.ir](mailto:yrahimi@ut.ac.ir) (Y. Rahimi), [atorabi@ut.ac.ir](mailto:atorabi@ut.ac.ir) (A. Torabian), [mehrdadi@ut.ac.ir](mailto:mehrdadi@ut.ac.ir) (N. Mehrdadi), [bshahmorady@gmail.com](mailto:bshahmorady@gmail.com) (B. Shahmoradi).

<sup>1</sup> Tel.: +98 2161113184; fax: +98 2166407719.

<sup>2</sup> Tel.: +98 216 111 3177; fax: +98 2166407719.

<sup>3</sup> Tel.: +98 9187705355.

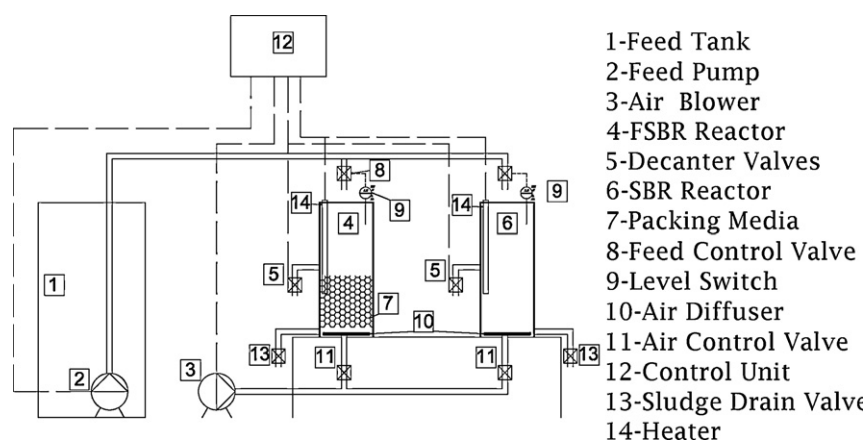


Fig. 1. A schematic diagram of the SBR and FBSBR.

tion periods and shock loads. Suspended biomass presented higher specific degradation rates, but biomass attached did not generate a metabolite that is inhibitory when it accumulates.

In another study for biological removal of phenol from strong wastewaters using MBSBR, the optimum hydraulic retention time (HRT) for the MBSBR was found to be 40 h and the critical phenol-loading rate was 83.4 g phenol/m<sup>3</sup> per hour, which gave a phenol removal efficiency of 99% [9].

A group of researchers [10] investigated the performance of MBSBR and SBR in simultaneous *p*-nitrophenol and nitrogen removal. Their results demonstrate that complete removal of PNP was achievable for the SBR and MBSBR up to loading rate of 0.368 kg/m<sup>3</sup> per day. At this loading rate, the average removal efficiency of ammonia nitrogen for the SBR and MBSBR was 86% and 96% respectively. Based on their results, the performance of the MBSBR was better than that of SBR in PNP and ammonia nitrogen removal.

In all of the aforementioned studies, integrating moving bed with SBR improved nutrient removal and process tolerance to organic shock and toxic loading.

Moreover, a few researchers have investigated SND and sludge quantity and quality in FBSBR, mainly in comparison with conventional SBR reactor in the same conditions [3,11].

The effect of temperature on SND via nitrite in a fibrous carrier FBSBR was assessed. It was found that the highest TN removal rate (91.9%) was at 31 °C with DO ranged 3–4 mg/L [3].

In another research, FBSBR was applied for treatment of milk industry wastewater [11]. The COD, BOD<sub>5</sub>, TKN, oil and grease removal efficiencies of the FBSBR system, under a high organic loading of 1340 g BOD<sub>5</sub>/m<sup>3</sup> per day, were 89.3 ± 0.1, 83 ± 0.2, 59.4 ± 0.8, and 82.4 ± 0.4% respectively, while their removal under the same organic loading conditions using conventional SBR system was 87.0 ± 0.2, 79.9 ± 0.3, 48.7 ± 1.7, and 79.3 ± 10% respectively.

In the present study, two parallel reactors (conventional SBR and FBSBR) were operated at the same conditions to determine the effects of fixed bed on biological nitrogen and phosphorous removal, to improve sludge quality, and to reduce sludge production yield.

## 2. Materials and methods

### 2.1. Experimental set-up and operating conditions

The experiment was carried out in two parallel reactors (SBR and FBSBR) with a working volume of 7 L, a diameter of 0.2 m, and a height of 0.3 m (Fig. 1). In FBSBR system, plastic media (polypropylene, SANCO-Iran) with a specific surface area of 350 m<sup>2</sup> m<sup>-3</sup> and

total volume of 2.3 L (Fig. 2) was installed on the bottom of the reactor. The exchangeable volume of each reactor was 2.6 L. The reactors were operated at a fixed temperature of 20 ± 2 °C using a thermostatic heater. The SBR was operated in cycles of 4, 6, and 8 h. System was controlled using timer switches (theben-Germany). Each cycle was comprised of four phases: during the first phase, the reactor was continuously fed for 15 min; in the second phase, the reactor was aerated for 165, 285, and 405 min depending upon the cycle duration; the third phase of settling lasted 45 min; and finally in the fourth phase, effluent withdrawal was applied for 15 min. The operational pH ranged between 6.5 and 7.5 without control.

The experiments were conducted using a synthetic wastewater to avoid any fluctuation in the feed concentration, to provide a continuous source of biodegradable organic pollutants, and to simulate domestic wastewater (variable from low strength to very high strength). The constituents of synthetic wastewater are given in Table 1. The reactors were acclimatized for about 30 days prior to monitoring. The seed sludge was 7500 mg MLSS/L collected from the return activated sludge of a conventional activated sludge process of a local wastewater treatment plant (West township in Tehran, Iran).

In both reactors (SBR/FBSBR), synthetic wastewater was fed to reactor with a pump and its flow was controlled with an electrode level switch. The decanting was carried out using electric valves to remove supernatant. Air was supplied using an electromagnetic blower (Resun model ACO-018-China) and air diffusers were controlled by a DO meter (MI-65, Martini Instruments).

Table 1  
Constituents of the synthetic wastewater used in this study.

Compounds	Concentration range (mg L <sup>-1</sup> )
Organics and nutrients	
Sodium acetate (NaCOOH)	100–200
Glucose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	100–200
Sucrose	100–200
Starch	100–200
Milk powder	100–200
Ammonium sulfate ((NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> )	150–300
Potassium phosphate (KH <sub>2</sub> PO <sub>4</sub> )	140–150
Trace nutrients	
Calcium chloride (CaCl <sub>2</sub> ·2H <sub>2</sub> O)	0.37
Magnesium sulfate (MgSO <sub>4</sub> ·7H <sub>2</sub> O)	5
Manganese chloride (MnCl <sub>2</sub> ·4H <sub>2</sub> O)	0.28
Zinc sulfate (ZnSO <sub>4</sub> ·7H <sub>2</sub> O)	0.45
Ferric chloride anhydrous (FeCl <sub>3</sub> )	1.45
Cupric sulfate (CuSO <sub>4</sub> ·5H <sub>2</sub> O)	0.4
Cobalt chloride (CoCl <sub>2</sub> ·6H <sub>2</sub> O)	0.4
Sodium molybdate dihydrate (Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O)	1.25

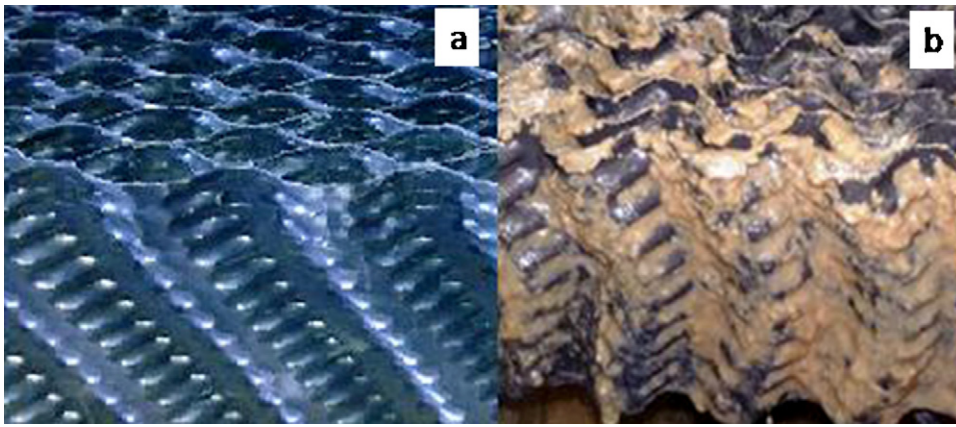


Fig. 2. Biofilm modules used in the FBSBR system, (a) new, (b) after biofilm produced.

Table 2

Statistical comparison of two reactors.

Parameter	SVI	Yield	TP removal	Nitrogen content	Phosphorus content	TN removal	VSS/TSS
Sig. (2-tailed)	0.654	0.047 <sup>a</sup>	0.003 <sup>b</sup>	0.521	0.002 <sup>b</sup>	0.002 <sup>b</sup>	0.021 <sup>a</sup>

<sup>a</sup> Correlation is significant at the 0.05 level (2-tailed).

<sup>b</sup> Correlation is significant at the 0.01 level (2-tailed).

## 2.2. Analytical methods

All the results were obtained from the bioreactor at steady state. Supernatant from one entire cycle was collected in a container, and the mixed liquor was sampled at the end of aeration time. The DO concentration was measured using a DO meter (MI-65 Martini Instruments), and the pH value was measured using a pH meter (HACH-Germany). The measurement of COD, MLSS, total nitrogen (TN), oxidized nitrogen ( $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N),  $\text{NH}_4^+$ -N, orthophosphate concentration (ortho-P) and TP was carried out using a spectrophotometer (DR-5000 HACH-Germany). In TN analysis, an alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm. Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 420 nm [12]. The MLSS content was analysed according to standard methods [13].

Nitrogen removal through the biological assimilation was calculated using biomass total Kjeldahl nitrogen (TKN) content of  $7.5\% \pm 0.3$  by weight of MLVSS, the biomass yield, and average COD removed. Removal efficiencies ( $R$ ) were calculated using following equations [14]:

$$R_{\text{total}} = \frac{\text{TN}_i - \text{TN}_e}{\text{TN}_i} \times 100 \quad (1)$$

$$R_{\text{ass}} = \frac{Y_{\text{obs}} \times \Delta\text{COD} \times 0.0705}{\text{TN}_i - \text{TN}_e} \times R_{\text{total}} \quad (2)$$

$$R_{\text{SND}} = \frac{\text{TN}_i - \text{TN}_e (Y_{\text{obs}} \times \Delta\text{COD} \times 0.0705)}{\text{TN}_i - \text{TN}_e} \times R_{\text{total}} \quad (3)$$

where  $R_{\text{total}}$  = total nitrogen removal efficiency,  $R_{\text{ass}}$  = nitrogen removal efficiency by assimilation and  $R_{\text{SND}}$  = nitrogen removal efficiency by SND,  $\text{TN}_i$  = total nitrogen concentration in influent,  $\text{TN}_e$  = total nitrogen concentration in effluent,  $Y_{\text{obs}}$  = observed

biomass production rate,  $\Delta\text{COD}$  = COD concentration in influent – COD concentration in effluent.

## 3. Results and discussion

### 3.1. Statistical analysis

A nonparametric test (Mann–Whitney  $U$  test) was used to identify the relationships between two reactors (Table 2).

### 3.2. Sludge quantity and quality

#### 3.2.1. Sludge production yield

The variation of sludge production yield versus organic loading rates in FBSBR and SBR reactors has been presented in Fig. 3. Statistical analysis (Table 2) revealed that  $Y_{\text{obs}}$  in FBSBR was significantly less than SBR reactor ( $\alpha < 0.01$ ). The  $Y_{\text{obs}}$  values were varied from 0.43–0.28 g SS/g COD in FBSBR and 0.56–0.35 g SS/g COD in SBR

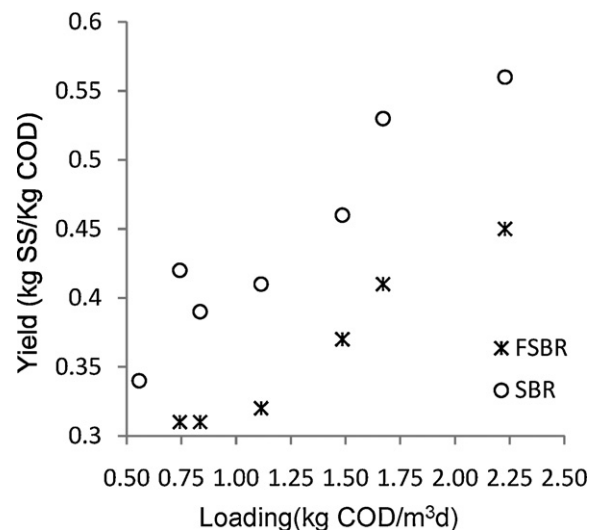


Fig. 3. Sludge production rate in various organic loading rates.

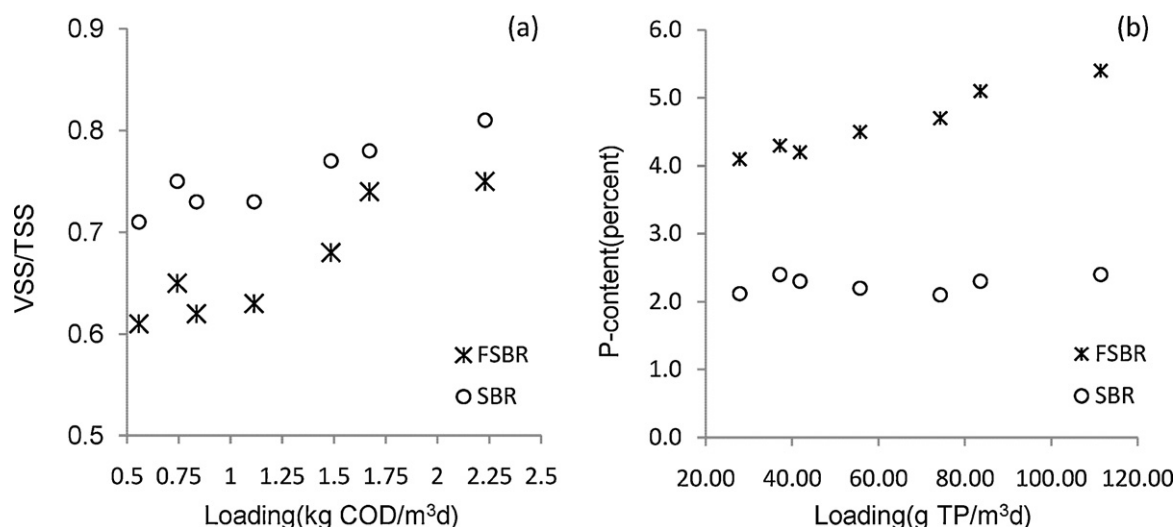


Fig. 4. Sludge characteristics in two reactors, (a) sludge stabilization ratio (VSS/TSS) and (b) phosphorus concentration in dry solids.

reactor. Therefore, the sludge production rate was less in FBSBR compared with SBR reactor. This can be attributed to high cell retention time in biofilm and also to occurring the dissolved oxygen and substrate gradient in the biofilm layer that leads to endogenous respiration [15,16].

### 3.2.2. Sludge volume index (SVI)

Both FBSBR and SBR showed good settling characteristics and statistical analysis (Table 2) showed no significant difference between two reactors in terms of SVI ( $\alpha \gg 0.05$ ). The SVI in FBSBR and SBR reactors were 98–130 and 87–140 respectively.

### 3.2.3. Sludge stabilization ratio (VSS/TSS)

The variations of VSS/TSS with loading rates are shown in Fig. 4a. It can be seen that the biofilm plays an important role on the sludge stabilization ratio. Statistical analysis showed that VSS/TSS in FBSBR was significantly less than SBR reactor ( $\alpha < 0.05$ ). The lower VSS/TSS in FBSBR can be attributed to the higher solid retention rather than SBR. The effect of SRT on sludge stabilization has been proved previously and VSS/TSS relates with SRT inversely [2,17,18].

### 3.2.4. Nutrients content in sludge

Fig. 4b indicates that sludge TP content was less in SBR reactor compared with FBSBR reactor, but contrary to more nitrogen removal in FBSBR reactor, no significant difference was found in TN content of sludge in both reactors (Table 2). These can be related to the biological phosphorous and nitrogen removal mechanisms. Biological nitrification and denitrification together make up the most useful process to remove nitrogen. During nitrification, ammonium is first oxidized to nitrite or nitrate by aerobic chemolitho-autotrophic bacteria. Nitrite and nitrate are then reduced to  $N_2$  gas in the denitrification process by chemoorgano-heterotrophic denitrifying bacteria under anoxic conditions. During these processes, most of the nitrogen removed finally emits as  $N_2$  gas and does not store in biomass. On the other hand, phosphorous removal occurs by storing in biomass via luxury uptake phenomena [16].

### 3.3. COD removal rate versus COD loading

Both reactors showed high COD removal efficiencies under stable operation conditions throughout the study period (Fig. 5). However, in lower organic loading rates no significant differences were observed but the FBSBR reactor exhibited higher COD removal rate at higher loading rates. The FBSBR showed the best perfor-

mance at loadings in the range of 0.5–1.5 Kg COD/m<sup>3</sup> per day. It indicates that microbes in attached-growth biofilm combined with suspended growth sludge in the FBSBR have a higher ability to remove organic carbon and a better endurance of shock loading than single suspended growth sludge in the SBR. Similar findings on biofilm application in various processes such as moving bed bioreactor (MBBR) process, integrated fixed film activated sludge (IFAS) process, biofilm membrane bioreactor (MBR) and Linpore<sup>®</sup> process have been reported [2,8,19–21].

### 3.4. Nitrogen and phosphorous removal

For highlighting the SND in reactors, no anaerobic and anoxic periods were anticipated. The relationship between ammonia-N loading and nitrogen removal rate and TP loading and removal rate were examined, and the results are shown in Fig. 6.

Fig. 6a reveals that total-N removal rates increased with the decrease of the ammonia-N loading. When nitrogen loading was 55 g/m<sup>3</sup> per day, TN removal rates in SBR and FBSBR reactors could reach 38% and 88% respectively. As ammonia nitrogen loading was increased to 222 g/m<sup>3</sup> per day, TN removal rate was dropped in both SBR and FBSBR reactors to 44% and 70% respectively. It is very

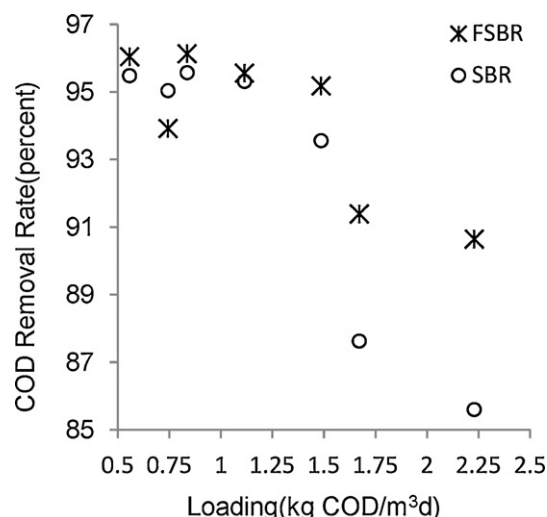


Fig. 5. COD removal rate in various organic loading rates.

**Table 3**  
Loadings and performance of FBSBR reactor.

Loading (kg COD/m <sup>3</sup> .d)	Loading (g NH <sub>4</sub> -N/m <sup>3</sup> .d)	Loading (g TP/m <sup>3</sup> .d)	Y <sub>obs</sub> (kg SS/kg COD)	R <sub>COD</sub> <sup>a</sup> (%)	R <sub>TN</sub> <sup>b</sup> (%)	R <sub>assim</sub> <sup>c</sup> (%)	R <sub>SND</sub> <sup>d</sup> (%)	R <sub>TP</sub> <sup>e</sup> (%)
0.56	55.71	27.86	0.28	96.04	88.48	29.25	59.23	79.60
0.84	83.57	41.79	0.31	96.13	81.33	30.57	50.77	86.40
1.11	111.43	55.71	0.32	95.56	73.68	33.09	40.60	86.52
0.74	74.29	37.14	0.30	93.92	73.60	31.28	42.32	76.88
1.49	148.57	74.29	0.37	95.18	72.80	34.55	38.25	88.44
1.67	167.14	83.57	0.41	91.39	60.00	36.05	23.95	86.88
2.23	222.86	111.43	0.43	90.64	70.00	38.88	31.12	90.16

<sup>a</sup> COD removal rate.

<sup>b</sup> Total nitrogen removal rate.

<sup>c</sup> Total nitrogen removal rate by assimilation.

<sup>d</sup> Total nitrogen removal rate by simultaneous nitrification–denitrification.

<sup>e</sup> Total phosphorus removal rate.

**Table 4**  
Loadings and performance of SBR reactor.

Loading (kg COD/m <sup>3</sup> .d)	Loading (g NH <sub>4</sub> -N/m <sup>3</sup> .d)	Loading (g TP/m <sup>3</sup> .d)	Y <sub>obs</sub> (kg SS/kg COD)	R <sub>COD</sub> <sup>a</sup> (%)	R <sub>TN</sub> <sup>b</sup> (%)	R <sub>assim</sub> <sup>c</sup> (%)	R <sub>SND</sub> <sup>d</sup> (%)	R <sub>TP</sub> <sup>e</sup> (%)
0.56	55.71	27.86	0.35	95.48	38	32.65	5.347	79.2
0.84	83.57	41.79	0.39	95.57	58.67	34.27	24.4	39.31
1.11	111.43	55.71	0.41	95.31	54.13	38.91	15.22	31.99
0.74	74.29	37.14	0.37	95.04	53.6	36.78	16.82	20
1.49	148.57	74.29	0.46	93.56	59.8	40	19.8	22
1.67	167.14	83.57	0.53	87.63	51.73	39.56	12.17	36.53
2.23	222.86	111.43	0.56	85.6	43.8	41.34	2.457	51.6

<sup>a</sup> COD removal rate.

<sup>b</sup> Total nitrogen removal rate.

<sup>c</sup> Total nitrogen removal rate by assimilation.

<sup>d</sup> Total nitrogen removal rate by simultaneous nitrification–denitrification.

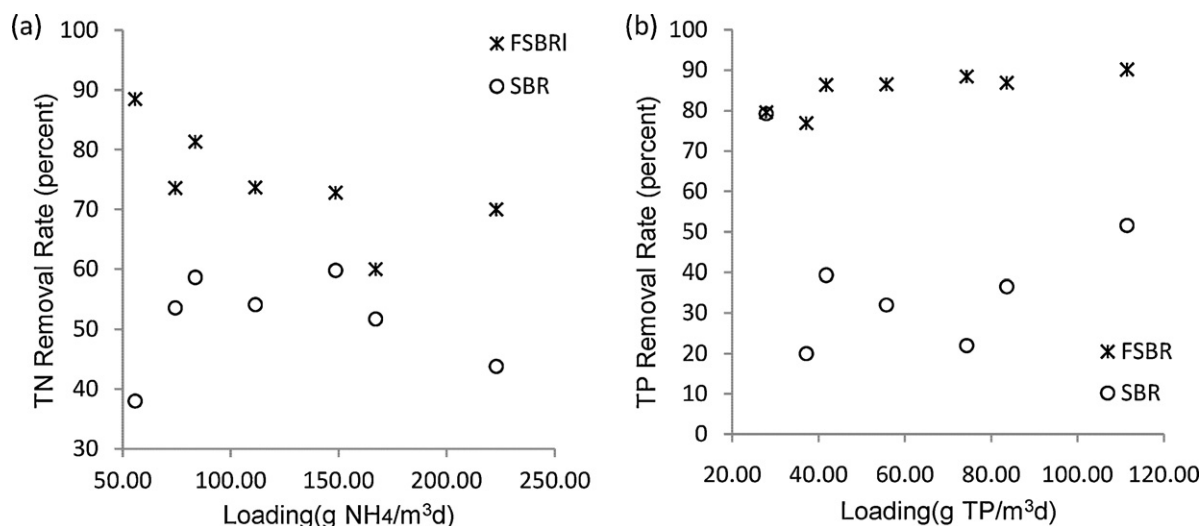
<sup>e</sup> Total phosphorus removal rate.

interesting that although the reactors were operating without any anoxic or anaerobic periods in the operational cycles, concentration of total nitrogen in the effluent of FBSBR reactor was low and also much lower than SBR reactor ( $\alpha < 0.01$ ). This fact could be explained by the reason that there was effective SND during the reaction phases. The SND rates in FBSBR reactor were significantly higher than SND rates in SBR reactor ( $\alpha < 0.01$ ). Nevertheless, the nitrogen removal rate by assimilation in SBR reactor was so higher than FBSBR reactor that could be attributed to high sludge production rate in SBR reactor.

Fig. 6b illustrates the profile of TP removal efficiencies in the two systems during the whole experiment. As Fig. 6b indicates, biofilm had a significant influence on the phosphorous removal.

The TP removal rates in FBSBR and SBR were 77–90% and 20–79% respectively.

Tables 3 and 4 illustrate the COD removal, TN removal, nitrogen removal by assimilation, nitrogen removal by SND and TP removal rates in the two systems during the whole experiment. In SBR reactor, assimilation plays the main role in nitrogen removal and there is a linear correlation between nitrogen removal by assimilation and organic loading rate. On the other hand, in FBSBR reactor, the main role in the removal of nitrogen is played by SND instead of assimilation. The sludge yield in FBSBR was lower than SBR reactor. Moreover, SND in FBSBR was higher than SBR reactor because of oxygen gradient in biofilm, which indicates the important role of biofilm in nitrogen removal rate as was observed by others [22,23].



**Fig. 6.** Nutrient removal rates in the reactors, (a) nitrogen removal rate, (b) phosphorus removal rate.

#### 4. Conclusion

Both FBSBR and SBR showed excellent performance on organic substance removal for OLR of 1.5 kg COD per day; however, the FBSBR was more efficient than SBR reactor at higher OLRs. The nitrogen removal rate in FBSBR reactor was higher than SBR reactor, which could be attributed to SND in FBSBR reactor. The FBSBR reactor showed higher phosphorus removal than SBR reactor that could be related to occurring oxygen gradient in biofilm layers.

The sludge production rate in FBSBR was lower (25–30%) than conventional SBR system and its excess sludge was more stabilized containing more phosphorus; therefore, the excess sludge of FBSBR has higher fertilizing value.

For highlighting the SND in reactors, no anaerobic and anoxic periods anticipated. TP and TN removal efficiency could be increased significantly by optimizing the effective parameters illustrated.

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