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Performance comparison of two anammox reactors: SBR and UBF

Ren-Cun Jin [a,b], Ping Zheng [a], An-Hui Hu [a], Qaisar Mahmood [a], Bao-Lan Hu [a,*], Ghulam Jilani [c]

[a] *Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China*
[b] *Department of Environmental Science, Hangzhou Normal University, Hangzhou 310016, China*
[c] *Department of Soil Science, University of Arid Agriculture, Rawalpindi, Pakistan*

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Abstract

The performance of two selected high rate anammox reactors, i.e. sequencing batch reactor (SBR) and an upflow biofilter (UBF) were compared operating them simultaneously at 30 °C. The sludge from anaerobic digester of a municipal wastewater treatment plant was used for inoculation and synthetic wastewater was fed to both reactors during the experiment. During start-up, the nitrogen loading rate (NLR) was increased gradually from an initial 140 to 560 mg/l day. The comparison of the performance revealed that the NLRs of SBR (2.7 g/l day) and UBF (2.5 g/l day) were far higher than those of the traditional nitrification/denitrification process. The biomass production of SBR outcompeted that of UBF. SBR took a longer time for start-up, i.e. about 57 days compared with 31 days of UBF. Moreover, SBR tolerated smaller pulse of substrate concentration and hydraulic load, showing a weaker stability compared with UBF.

Keywords: Anammox; Sequencing batch reactor; Upflow biofilter; Biological nitrogen removal; Wastewater treatment

1. Introduction

Nitrogenous compounds like ammonium (NH_4^+) are predominant in many wastewaters and need treatment prior to discharge in order to prevent oxygen depletion and eutrophication of surface water bodies. Nitrogen removal is usually accomplished through sequential nitrification and denitrification processes [1,2]. During such processes, ammonium (NH_4^+) is oxidized to nitrate (NO_3^-) followed by NO_3^- reduction to gaseous nitrogen (N_2). The anammox process is a novel and promising alternative of conventional nitrogen removal systems to treat nitrogenous compounds at lower cost [3,4]. Under anoxic conditions, NH_4^+ is oxidized to gaseous N_2 using nitrite (NO_2^-) as electron acceptor with the production of meager amounts of NO_3^- (Eq. (1)) [5], saving requirements of oxygen and organic matter compared with conventional nitrification/denitrification process. The anammox was discovered in Delft, the Netherlands, and it has been observed in many other places [6–10]. Recently, the reaction has been detected in marine sediments and anoxic water columns [11–13]. It is well established that autotrophic bacteria belonging to the order Planctomycetales carry out anammox reaction [9,14]:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ = 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

A shortcoming in the application of the anammox process is the requirement of a long start-up time, which may be due to slow growth rates of anammox bacteria (the doubling time was reported to be approximately 11 days) [5,15], the reactor carrying out anammox must be efficient in the biomass retention. The investigation on SBR and UBF is extensive and these reactor configurations are assumed as suitable candidates to carry out anammox. The support material provided in UBF promotes the retention of slowly growing biomass [16], while the ideal sludge settlement in SBR is helpful in holding anammox microorganisms [5,17].

The performance comparison is imperative to evaluate different anammox systems, and to offer a guideline for process design. Though, many efforts had been put forth to deal with the performance of anammox systems with different reactor configurations [18–23], limited work is available on the com-

* Corresponding author. Fax: +86 571 86971709.
E-mail address: blhu@zju.edu.cn (B.-L. Hu).

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parison of their performance under the similar prevailing conditions.

Hence, the present work aimed at detailed anammox performance comparison of the two reactor configurations, i.e. SBR and UBF under the similar operating conditions. Their start-up, the maximum NLR, stability and biomass production were compared to provide information on reactor configuration selection.

2. Materials and methods

2.1. Synthetic wastewater

Ammonium and nitrite were supplemented to a mineral medium as needed in the form of $(NH_4)_2SO_4$ and $NaNO_2$, respectively. The composition of the mineral medium was (g/l except for trace element solution): $KHCO_3$ 1.25, $NaH_2PO_4{\cdot}2H_2O$ 0.029, $CaCl_2{\cdot}2H_2O$ 0.3, $MgSO_4{\cdot}7H_2O$ 0.2, $FeSO_4$ 0.00625, EDTA 0.00625, and 1.25 ml/l of trace elements solution. The trace element solution contained (g/l) (adapted from van de Graaf et al. [24]): EDTA 15, $ZnSO_4{\cdot}7H_2O$ 0.43, $CoCl_2{\cdot}6H_2O$ 0.24, $MnCl_2{\cdot}4H_2O$ 0.99, $CuSO_4{\cdot}5H_2O$ 0.25, $NaMoO_4{\cdot}2H_2O$ 0.22, $NiCl_2{\cdot}2H_2O$ 0.19, $NaSeO_4{\cdot}10H_2O$ 0.21, H_3BO_4 0.014, and $NaWO_4{\cdot}2H_2O$ 0.050.

2.2. Seed sludge

Activated sludge taken from a mesophilic digester of a municipal wastewater treatment plant served as the inoculum. The seed sludge contained 71.8 g/l suspended solids (SS) and 46.1 g/l volatile suspended solids (VSS).

2.3. Bioreactors

The experiment was conducted in parallel using two plexiglass columns. Each column had a working volume of 1.2 l with an internal diameter of 70 mm and height of 360 mm (Figs. 1a and 2). They were covered with black cloth to avoid the light inhibition and were operated at $30 \pm 1\,^{\circ}C$ and their pH was maintained in range of 7.5–8.0.

The UBF was operated continuously and it was packed with string-shaped three-dimensional-plastic media (Yixing, China) to retain biomass, as illustrated in Fig. 1b. This string consisted of bundles of the soft fibrous media which were evenly spaced at intervals of 30 mm, affixed at the center-line of the column. Its specific surface area was about 400 m^2/m^3.

The anaerobic environment of SBR was maintained by flushing it with argon at the rate of 3 l/min during the experiment. The SBR worked in cycles of 12 h. Each cycle comprised of three phases. The first phase lasted for 11 h during which the reactor was fed with synthetic wastewater and the reaction proceeded. During the second phase (next 0.5 h), the influent and argon supply was terminated and the sludge was allowed to settle, while in the last phase (0.5 h) the supernatant was pumped out of the reactor. The hydraulic retention time (HRT) was adjusted according to the feed volume (between 0.6 and 0.8 l) in each cycle.

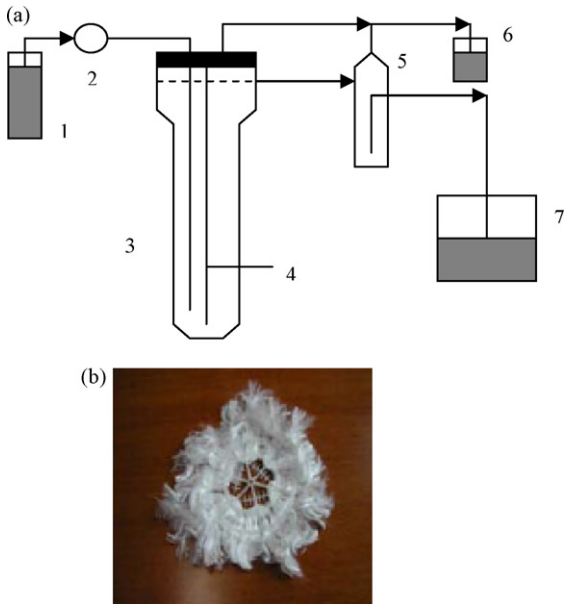

Fig. 1. (a) Schematic diagram of UBF system: (1) feeding tank, (2) peristaltic pump, (3) reactor, (4) media, (5) gas–liquid–solid separator, (6) gas outlet, and (7) effluent collection tank; (b) image of the packing media.

2.4. Analytical methods

The influent and effluent samples were collected on daily basis and were analyzed immediately or stored in a refrigerator at 4 °C until the analyses were carried out. The measurements of NO_3^-–N, NO_2^-–N, NH_4^+–N, pH, SS and VSS were performed according to the standard methods [25].

2.5. RTQ-PCR analysis

Sludge samples were analyzed by RTQ-PCR (real-time quantitative PCR) technique [26] to confirm the existence of anammox culture. Based on 16S rRNA gene sequences obtained from the enrichment culture, real-time PCR primer sets were designed for *Candidatus Brocadia anammoxidans* and *Candidatus Kuenenia stuttgartiensis*. The specificity of the primers was checked against the ARB database which indicated that the primers had a high specificity for 16S rRNA gene sequences

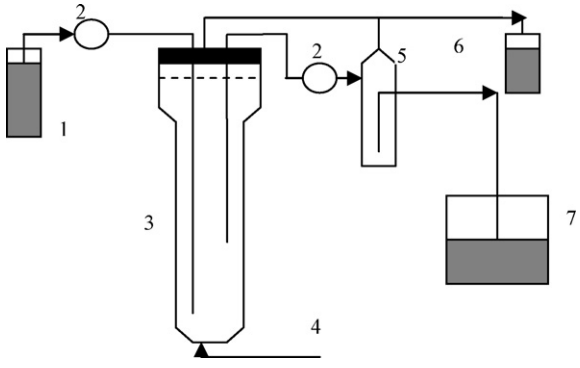

Fig. 2. Schematic diagram of SBR system: (1) feeding tank, (2) peristaltic pump, (3) reactor, (4) argon, (5) gas–liquid–solid separator, (6) gas outlet, and (7) effluent collection tank.

belonging to previously reported anammox bacteria groups [26].

3. Results and discussions

3.1. Start-up

The start-up was accomplished by inoculating both reactors with sludge containing 30 g VSS/l. At 30 °C, both reactors were started at an HRT of 24 h with the influent NO_2^-–N and NH_4^+–N concentration of 70 mg/l each, corresponding to NLR of 140 mg N/l day. The NLR was increased stepwise by raising approximately equimolar concentrations of NO_2^-–N and NH_4^+–N. Previously, no specific standard was available to judge the successful termination of the start-up for anammox process. The nitrogen removal rate (NRR) of 500 mg N/l day was considered as the standard for start-up of anammox process that surpassed the upper limit for nitrification/denitrification process, i.e. 300–500 mg NH_4^+–N/l day [27].

The performance of UBF during start-up period is shown in Table 1. During the initial 6 days, UBF displayed poor performance at influent substrate (NH_4^+–N or NO_2^-–N) concentration of 70 mg/l each resulting in the removal efficiency of 34.8% and 61.0% for NH_4^+–N and NO_2^-–N, respectively. During the following 10 days (days 7–16), the removal efficiency increased gradually, with an ultimate stable NH_4^+–N and NO_2^-–N removal above 90% and 95%, respectively, at constant substrate concentration. From day 17 on, the influent substrate concentration was raised by a step of 28 mg/l upon achieving substrate removal higher than 90%. Till the 31st day, the influent NH_4^+–N and NO_2^-–N concentrations were 272 and 292 mg/l, respectively, with the stable removal efficiencies of higher than 90%. Based on the standard mentioned above, the start-up was finished.

During the subsequent operation, the substrate concentrations were increased further. With the influent concentrations of 301 mg/l (NH_4^+–N) and 315 mg/l (NO_2^-–N), the removal efficiencies sharply declined to 21.7% and 51.5%, respectively. This phenomenon repeated when the influent concentration was lowered to 210 mg/l and raised above 280 mg/l again. Such behavior may be attributed to two factors, i.e. either the NLR had possibly surpassed the reactor potential or the high strength substrate had possibly inhibited anammox. The latter seems more reasonable possibility as Strous et al. [5] observed that the anammox activity was inhibited when NO_2^-–N was higher than 280 mg/l.

The SBR was initially flushed with almost pure (99.99%) argon in order to augment the contact between substrate and sludge, and hence to alleviate the substrate inhibition to the anammox bacteria. As illustrated in Table 2, the substrate removal was less than 45% during initial 30 days of start-up. From day 31 on, there was a rapid improvement in the reactor performance, resulting in NH_4^+–N and NO_2^-–N removal efficiencies of 98.4% and 100%, respectively. After an operation of 57 days, the influent NH_4^+–N and NO_2^-–N concentrations were enhanced to 269 and 301 mg/l, respectively, with the stable removal efficiencies above 90%. Hence, the start-up was completed. During the subsequent operation, the influent substrate concentration higher than 280 mg/L was prone to inhibit the anammox activity in SBR.

The results in Tables 1 and 2 show that SBR took comparatively longer start-up period of 57 days compared with 31 days of UBF. The start-up period was shorter than that observed by van de Graaf et al. [24], i.e. approximately 7 months of cultivation using an attached growth system. The excellent start-up in the present study may be attributed to the seed sludge characteristics and/or the operational conditions exercised which are still unclear and are subjected to further clarification.

3.2. Maximum NLR

To determine the maximum treatment capacity of both systems, the NLR was progressively increased by decreasing the operating HRT, while the influent substrate concentration was kept constant at 140 mg/l. Data based on arithmetic means of six or more measurements obtained at pseudo-steady-state are reported. The pseudo-steady-state was indicated by a constant effluent substrate concentration (±5%).

As shown in Table 3, during the HRT test, the HRT of UBF was gradually decreased from 20.7 to 3.02 h, with a concomitant

Table 1
Performance of UBF during start-up

Time (day)	NH_4^+–N			NO_2^-–N			TN
	Influent concentration (mg/l)	Removal efficiency (%)	Removal rate (mg/l day)	Influent concentration (mg/l)	Removal efficiency (%)	Removal rate (mg/l day)	Removal rate (mg/l day)
1st	76.3	27.1	21.7	60.6	60.8	38.6	60.3
4th	60.9	33.8	21.6	65.2	38.3	26.2	47.8
7th	55.4	71.1	41.3	61.2	100	64.1	105
10th	61.9	98.9	64.1	69.6	97.1	70.8	135
13th	67.9	89.6	63.7	67.1	100	70.2	134
16th	77.1	100	80.8	97.2	96.3	98.3	179
19th	109	95.3	109	118	100	124	233
22nd	150	95.5	150	148	98.7	153	303
25th	204	99.3	212	219	100	250	462
28th	244	94.7	232	259	97.3	264	496
31st	265	97.3	256	278	98.5	273	538

Table 2
Performance of SBR during start-up

Time (day)	NH_4^+–N			NO_2^-–N			TN
	Influent concentration (mg/l)	Removal efficiency (%)	Removal rate (mg/l day)	Influent concentration (mg/l)	Removal efficiency (%)	Removal rate (mg/l day)	Removal rate (mg/l day)
3rd	80.2	39.8	35.6	82.7	45.2	41.7	77.3
12th	85.9	50.0	48.0	83.7	55.8	52.1	100
21st	81.8	39.5	35.9	92.7	49.4	51.1	87.0
30th	89.0	48.1	47.7	88.5	47.0	46.3	94.0
33rd	84.7	50.6	47.7	68.2	60.6	46.1	93.8
36th	76.3	84.4	71.7	70.1	87.0	68.5	140
39th	120	98.2	109	112	100	125	234
42nd	159	98.4	151	160	100	176	328
45th	201	90.3	189	189	98.1	207	396
48th	217	97.9	208	207	100	228	436
51st	227	91.1	215	234	96.8	238	454
54th	238	94.3	226	246	99.9	254	480
57th	247	96.5	238	254	98.6	282	520
60th	269	93.8	253	275	97.5	294	548

Table 3
Performance of UBF operated at different HRTs

HRT (h)	NH_4^+–N		NO_2^-–N		TN	
	Loading rate (mg/l day)	Removal rate (mg/l day)	Loading rate (mg/l day)	Removal rate (mg/l day)	Loading rate (mg/l day)	Removal rate (mg/l day)
20.7	147	140	154	151	301	291
14.8	225	218	236	236	460	454
13.5	269	260	252	252	521	512
12.4	324	314	305	305	628	619
9.39	391	366	355	322	745	687
7.07	482	459	502	502	984	961
5.70	615	600	628	628	1243	1228
4.60	817	797	841	841	1658	1637
4.00	910	724	906	903	1816	1627
3.38	1110	878	1259	1166	2368	2044
3.02	1230	840	1273	1149	2503	1989

increase in NLR from 301 to 2503 mg/l day. When the HRT was longer than 4.60 h (NLR below 817 mg/l day), the NH_4^+–N removal was always higher than 89%. But the removal efficiency began to drop with decreasing HRT, to reach the minimum value of 64.8% at HRT of 3.02 h. Table 4 shows that both reactors, i.e. SBR and UBF displayed similar performance trends.

The maximum NLR for UBF was 2.5 g/l day, comparable to the value of 2.7 mg/l day gained by SBR, indicating that

Table 4
Performance of SBR operated at different HRTs

HRT (h)	NH_4^+–N		NO_2^-–N		TN	
	Loading rate (mg/l day)	Removal rate (mg/l day)	Loading rate (mg/l day)	Removal rate (mg/l day)	Loading rate (mg/l day)	Removal rate (mg/l day)
20.2	221	211	230	228	451	438
19.0	272	253	303	284	575	538
13.5	332	295	334	302	665	597
11.3	386	386	427	417	813	803
8.72	500	460	604	566	1105	1026
6.89	726	674	749	736	1475	1410
6.10	769	653	657	604	1426	1257
5.11	864	789	883	869	1737	1658
4.21	947	710	954	680	1901	1390
3.85	1163	789	1244	1019	2406	1807
2.43	1292	966	1435	1044	2728	2010

Table 5
Resume of the operation of different anammox reactors

Reactor	Inlet	Support material	NLR (g/l day)	Reference
Fixed bed	Synthetic medium	Glass beads	1.1	[19]
Fixed bed	Sludge dewatering effluent	Soft media	0.6	[20]
Fluidized bed	Synthetic medium	Sand	1.8	[19]
Fluidized bed	Sludge digester effluent	Sand	1.5	[19]
Membrane bioreactor	Synthetic medium	–	0.7	[21]
Granular sludge bed	Synthetic medium	–	2.1	[22]
UASB	Synthetic medium	–	2.5	[23]
SBR	Synthetic medium	–	1.0	[5]
SBR	SHARON effluent	–	2.4	[28]
SBR	Fish canning effluent	–	0.7	[29]
Gas-lift	Synthetic medium	–	8.9	[18]
SBR	Synthetic medium	–	2.7	Present work
UBF	Synthetic medium	Three-dimensional-plastic media	2.5	Present work

both reactors had almost equal capacity for nitrogen removal. The NLRs in present study are comparable to those obtained during previous researches and are summarized in Table 5. Literature survey indicated that NLR values between 1.0 and 2.5 g/l day, except for 8.9 g/l day in a gas-lift [18], could be achieved in anammox reactors, and the NLR were quite high compared to those in conventional nitrification/denitrification systems.

The 16S rRNA gene copy numbers of anammox bacteria in the sludge samples harvested from UBF and SBR during the final phase of loading tests were determined. The results supported the existence of anammox culture in both reactors.

3.3. Stability

In practice, the fluctuations in substrate concentration and flow rate are often encountered during the operation of wastewater treatment plant. After the anammox reactors operation of 1 month under pseudo-steady-state, the influent substrate concentration was increased stepwise (increment of 28 mg/l every 2 days) to test tolerance of bioreactors against substrate concentration shock at fixed HRT.

After the substrate shock test, the substrate concentrations were reduced gradually to the level before shock tests and the reactors were allowed to run under pseudo-steady-state for 2 weeks. Following this, the effect of flow fluctuation on the stability of reactor performance was investigated by shortening HRT by 10% every 2 days, at fixed substrate concentration.

3.3.1. Tolerance to substrate concentration shock

During the substrate shock test, the influent ammonium and nitrite concentrations were changed and the effluent ammonium, nitrite concentrations and substrate removal efficiency were monitored to evaluate the stability of reactor performance. Under each operating conditions, the effluents were sampled every 2 days after complete mixing. The data presented in the paper were the mean values of three observations under specific conditions.

During shock tests, the influent NH_4^+–N concentration in the UBF was increased from 345 to 519 mg/l, while increase in NO_2^-–N ranged from 443 to 676 mg/l at 8.69 ± 0.58 h HRT. Accordingly, total NLR was enhanced from 2367 to 3559 mg/l day. The substrate removal efficiency declined from 77.3% in the beginning to 41.1% in the end (Table 6).

In SBR, at fixed HRT of 21.8 ± 0.5 h, the influent NH_4^+–N was increased from 351 to 515 mg/l, and NO_2^-–N from 467 to 640 mg/l. Accordingly, NLR increased from 879 to 1294 mg/l day. However, the substrate removal efficiency declined from 92.0% to 49.4% towards the end (Table 7). Because the substrate removal efficiency of SBR changed more obviously than that of UBF, the UBF was considered more stable to substrate concentration shock.

3.3.2. Tolerance to hydraulic shock

Within HRT range of 7.1–4.9 h in UBF, the ammonium and nitrite removal efficiencies were stable at about 80% and 85%, respectively. However, upon further decrease in HRT to 3.7 h, the removal efficiencies dropped to 67% and 71%, respectively (Fig. 3a).

Table 6
Performance of UBF at different substrate concentrations under substrate shock

NH_4^+–N				NO_2^-–N			
Influent concentration (mg/l)	Removal efficiency (%)	Loading rate (mg/l day)	Removal rate (mg/l day)	Influent concentration (mg/l)	Removal efficiency (%)	Loading rate (mg/l day)	Removal rate (mg/l day)
345	81.5	1037	845	443	74.1	1330	985
398	66.2	1075	711	557	57.1	1501	857
432	61.7	1099	678	601	53.1	1532	814
500	56.1	1282	719	639	46.6	1638	763
519	51.9	1547	803	676	32.8	2012	660

Table 7
Performance of SBR at different substrate concentrations under substrate shock

NH_4^+–N				NO_2^-–N			
Influent concentration (mg/l)	Removal efficiency (%)	Loading rate (mg/l day)	Removal rate (mg/l day)	Influent concentration (mg/l)	Removal efficiency (%)	Loading rate (mg/l day)	Removal rate (mg/l day)
351	97.5	377	368	467	87.9	502	441
390	94.1	426	401	505	84.0	552	463
423	85.3	485	414	550	75.3	631	475
495	64.1	544	348	621	54.2	681	369
515	48.1	577	277	640	50.5	717	362

Ammonium and nitrite removal efficiencies in the SBR at HRT 10.1–16.4 h during initial stages were comparable with the UBF, which stabilized at about 81% and 95%, respectively. As HRT was decreased to 6.2 h, the removal efficiencies dropped to 69% and 81%, respectively (Fig. 3b). These results suggested that relatively shorter HRT could be more applicable in the UBF than in the SBR for the purpose of achieving the same nitrogen removal efficiency. Thus, the UBF is relatively inert to the hydraulic shock. After comprehensive comparison of the stability against substrate concentration and hydraulic shocks, it could be proposed that the UBF exceeded the SBR in stability maintenance.

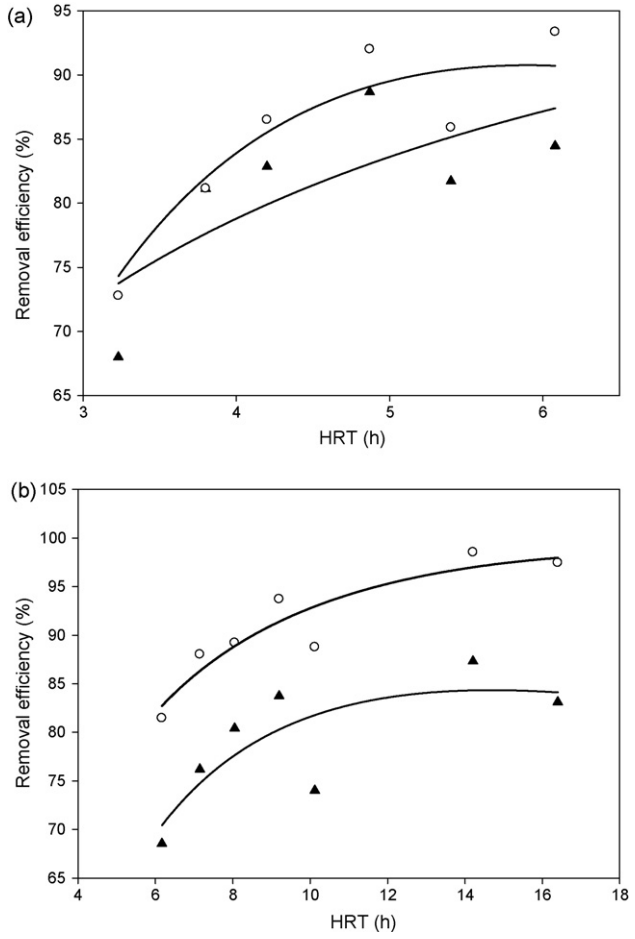

Fig. 3. Effect of HRT on NH_4^+–N and NO_2^-–N removal of UBF (a) and SBR (b) under hydraulic shocks. (▲) NH_4^+–N and (◯) NO_2^-–N.

3.4. Biomass production

Owing to very slow growth rates of anammox bacteria, it is difficult to directly determine the biomass production in the reactors. The physiological basis of nitrate production from nitrite during anammox process is unclear and the related enzyme is also uncertain hitherto. According to the anammox reaction model developed by Strous et al. [5], reductive force is produced from oxidation of nitrite to nitrate for cell synthesis. Based on the stoichiometry of the anammox reaction (Eq. (1)), 3.1 mg nitrate is produced and 26 mg dry cellular materials are synthesized when 280 mg ammonium is utilized, which was confirmed by the experimental value of 0.0714 mg dry cellular materials per milligram of ammonium [5]. Moreover, until now no other reactions producing nitrate have been discovered in anammox systems. Thus, the output of anammox organisms in the reactors can be estimated indirectly by the amount of nitrate produced. Fig. 4 shows the effluent nitrate concentration in both reactors under pseudo-steady-state. With the influent ammonium concentration higher than 336 mg/l, the effluent nitrate concentration in UBF generally decreased with increasing ammonium concentrations during the present study. However, the effluent nitrate concentration in SBR was stable and always higher than the corresponding value in UBF at any supplied feed. The results suggested that the SBR was more suitable reactor configuration for growth and accumulation of anammox organisms than UBF when exposed to high strength substrate.

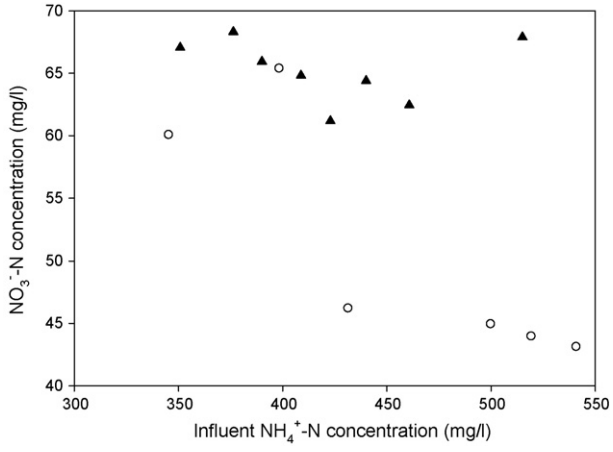

Fig. 4. Relationship between effluent nitrate concentration and influent ammonium concentration in UBF and SBR. (▲) SBR and (◯) UBF.

4. Conclusions

The performance of two different types of anammox reactors—upflow biofilter and sequencing batch reactor was compared in the present work. The maximum NLR of UBF and SBR were as high as 2.5 and 2.7 g/l day, respectively. The UBF proved to be better reactor configuration than SBR in terms of start-up time and stability against loading shocks, but inferior to SBR in terms of biomass production.

Acknowledgements

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