

57 (2016) 7378–7386 April



Effect of salinity on nitrogen removal by simultaneous nitrification and denitrification in a sequencing batch biofilm reactor

Zong-Lian She, Xiao-Ling Zhang, Mengchun Gao, Ya-Chan Guo, Lin-Ting Zhao, Yang-Guo Zhao*

Key Laboratory of Marine Environmental Science and Ecology, Ministry of Education, College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China, Tel. +86 532 66786351; email: szlszl@ouc.edu.cn (Z.-L. She), Tel. +86 13589342129; email: 13589342129@139.com (X.-L. Zhang), Tel. +86 532 66781061; email: mengchungao@hotmail.com (M. Gao), Tel. +86 13400112575; email: 770673970@qq.com (Y.-C. Guo), Tel. +86 15725225023; email: 627844424@qq.com (L.-T. Zhao), Tel. +86 532 66786568; Fax: +86 532 66782810; email: ygzhao@ouc.edu.cn (Y.-G. Zhao)

Received 13 August 2014; Accepted 1 February 2015

ABSTRACT

A sequencing batch biofilm reactor (SBBR) was operated at different salinities with focus on reactor performance and nitrogen removal by simultaneous nitrification and denitrification (SND). The SBBR contained suspended-growth sludge and biofilm attached to synthetic fibrous carriers. When salinity increased from 1.4 to 4.2 g NaCl/L, it increased the NH⁺₄-N and total nitrogen removal efficiencies, the nitrification rate (NR), and denitrification rate (DNR). A slight drop in nitrogen removal, NR, and DNR was observed, when the salinity was increased from 4.2 to 9.8 g NaCl/L. Efficient SND occurred in the reactor and the SND efficiency was above 90.7%. Nitrification was the main contribution of the suspended sludge, while the major role of biofilm was denitrification in the SBBR at the salinity of 9.8 g NaCl/L.

Keywords: Salinity; Simultaneous nitrification and denitrification (SND); Sequencing batch biofilm reactor (SBBR); Biofilm; Suspended sludge

1. Introduction

Due to the shortage of freshwater resources, in some coastal cities, for example in Hong Kong, seawater has been used for flushing toilets for about 20 years [1]. In addition, some industries also generate large amounts of saline sewage, such as food-processing, leather, and petroleum industries [2]. These ammonium wastewater containing high levels of salts may affect the nitrogen removal efficiency during biological nitrogen removal (BNR)

*Corresponding author.

processes [3–5]. Numerous studies have reported that salt has adverse effects on BNR performance because salt stress to the involved microbes can result in the inhibition of many enzymes, loss of cellular activities, eventually causing plasmolysis [6,7]. In addition to the direct impact on biomass, salt may also cause changes on the physical and biochemical properties of the activated sludge, such as sludge settleability, sludge bioflocculation, biofilms architecture, and extracellular polymeric substances [8–10]. However, salt inhibition can be reduced significantly after long-time acclimatization of the biomass by increasing salt concentration slowly [11], and the nitrogen

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removal efficiency could be improved when seeded with salt-tolerant bacteria [9].

In conventional BNR processes, nitrogen removal is usually performed through sequential nitrification and denitrification in separate compartments. Studies showed that nitrification and denitrification could occur concurrently in a single reactor under aerobic conditions by controlling the oxygen level, which is often known as simultaneous nitrification and denitrification (SND) [12,13]. Compared with traditional BNR, SND process offers several advantages: (1) it eliminates a second tank (anoxic) or an anoxic zone; (2) constant conditions can be maintained without the complex combination of aerated and anoxic zone, nor measuring and controlling equipment; (3) in sequencing batch reactor (SBR), the SND process reduces the time compared with sequential nitrification and denitrification reactions; and (4) reduction in operation costs due to less carbon source, sludge yield, demand for alkalinity, and aeration requirement [14–16].

During the past few years, some researchers have investigated factors affecting nitrogen removal via SND. It was found that the ratio of COD/N, temperature, and dissolved oxygen concentration (DO) were important factors influencing the SND process. In a sequencing batch biofilm reactor (SBBR), which is a hybrid reactor filled with carriers in SBR, the optimal COD/N ratio for the simultaneous removal of nitrogen and COD was 12.5, and the SND efficiency reached 98% [17]. SBR can obtain the best SND efficiency when COD/N ratio was adjusted to 11.1 [14]. The effect of temperature on SND via nitrite in a fibrous carrier SBBR was assessed. It was found that the highest total nitrogen (TN) removal efficiency (91.9%) was at 31°C with DO ranged from 3 to 4 mg/L [18]. It was reported that high efficiency of SND via nitrite was achieved in a hybrid sequence biological reactor (HSBR) under limited DO concentration. A DO concentration around 0.5 mg/L was beneficial for the balance of nitrification rate (NR) and denitrification rate (DNR) which could achieve complete SND [19]. Biofilm have great potential for the efficient nitrogen removal via SND process. Biofilm have complex non-uniform structures which can develop favorable anoxic opportunities for denitrifying bacteria. A study showed that the fixed bed SBR had greater nitrogen removal efficiency than SBR reactor due to higher SND efficiency in the fixed bed SBR [13]. The SND efficiency increased with increasing thickness of the biofilm in a SBBR [20]. For SND process in SBBR system, the suspended sludge played a major role in nitrification while denitrification mainly relied on the biofilm [21].

To date, some researchers have investigated nitrogen removal via SND, mainly in COD/N ratio, temperature, DO, and thickness of biofilm. However, the effects of salinity on biological nutrient removal via SND are scarcely examined in a SBBR. In this research, a SBBR filled with fibrous carriers was operated to evaluate nitrogen removal via SND under salt stress. The main objectives were to: (1) assess the performance of the SBBR, (2) examine the effects of different salinity on the nitrogen removal efficiency, ammonia oxygen rate, DNR, and SND efficiency, and (3) study the relative effects of the biofilm and the suspended sludge on nitrogen removal efficiencies at high salinity.

2. Materials and methods

2.1. The SBBR system

The experiment was carried out in a plexiglas SBBR, which contained suspended-growth sludge and biofilm attached to synthetic fibrous material. The SBBR had a working volume of 14.2 L with a cylindrical shape (200 mm in diameter and 600 mm height). Fibrous carriers with a specific surface area of $1,236 \text{ m}^2/\text{m}^3$ were packed in the SBBR. The diameter of each fibrous carrier was 14 cm. Ten units of carrier were attached to a rope with an interval of about 4.5 cm, and the rope was installed vertically in the center of the reactor. Compressed air was supplied via three diffusers at the bottom of the reactor. Mixing was performed through circulating liquid in the reactor by a submersible pump.

The SBBR was operated with three cycles each day. Each cycle (480 min) included five periods: feeding (30 min), aeration (390 min), settling (40 min), decanting (5 min), and idle (15 min). The decanting volume was 5.4 L every cycle. The initial DO concentration was controlled at about 2.5 mg/L at the beginning of the aeration period and mixing was done during the whole aeration period. The pH of the influent varied from 7.05 to 7.45. The reactor was operated with a hydraulic retention time (HRT) of 21 h at room temperature of 20–25 °C. The sludge retention time was about 60 d. The calculation of HRT was in the following:

$$HRT = V/Q \tag{1}$$

where *V* is the working volume of the SBBR, 14.2 L; *Q* is the wastewater quantity treated daily, namely, 5.4 $L/cycle \times 3 cycles/d = 16.2 L/d$.

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2.2. Inoculated sludge and synthetic wastewater

The inoculated sludge was obtained from a fullscale urban wastewater treatment plant (Qingdao, China) which employed a biological nutrient removal process. The sludge was added to the reactor at a concentration of approximate 3,000 mg/L.

In order to control the compositions of the nutrients, synthetic wastewater was used for the experiment. The synthetic wastewater was prepared daily from tap water. The composition of the wastewater was as follows (per liter): glucose, 0.44 g; $(NH_4)_2SO_4$, 0.19 g; KH_2PO_4 , 0.040 g; NaHCO₃, 0.2 g; and NaCl from 1.4 to 9.8 g. The wastewater contained chemical oxygen demand (COD) of about 430 mg/L, ammonia nitrogen (NH_4^+ -N) of 40 mg/L, TN of 45 mg/L, and total phosphorous of 6.5 mg/L.

At the initial stage of the experiment, the reactor was fed with the wastewater without external salt. After three months, the biofilm grown on the carries became mature and the thickness of the biofilm was around 5 mm. Then the influent with external salt began to be supplied to the reactor. The salt content in the influent was increased gradually from 1.4 to 9.8 g NaCl/L over 178 d and the operation was divided into five stages according to different salinity (As shown in Table 1). Salt concentration was increased after the nitrification efficiency and TN removal had reached stable values for at least 10 d at each salinity level. The mixed liquor suspended solids (MLSS) in the reactor was maintained at 3,500-4,000 mg/L during the stage fed with saline wastewater by wasting of mixed liquor at the end of the aeration period.

2.3. Track studies for each salt content

After the stable performance was obtained at each salinity level, track studies were undertaken. Samples were taken from the reactor every 10 min during the first 0.5 h and every 30 min during the later 6.0 h for measuring the NH_4^+ -N, NO_2^- -N, NO_3^- -N, and COD during the aeration period, allowing the determination of NR, DNR, and SND efficiency. Every test was carried out three times at each salinity studied.

Table 1

Effluent nitrogen and COD concentrations under various salinities at steady state

NR and DNR were determined according to the methods reported by Münch et al. [22]. The SND efficiency was given in Eq. (2).

$$E_{\rm SND} = \left[1 - \left(NO_{\rm x,e}^{-} - NO_{\rm x,i}^{-}\right) / \left(NH_{4,i}^{+} - NH_{4,e}^{+}\right)\right] \times 100\%$$
(2)

where E_{SND} is SND efficiency, $\text{NO}_{x,e}^-$ (N mg/L) is the nitrogen in nitrite and nitrate at the end of aeration, $\text{NO}_{x,i}^-$ (N mg/L) is the nitrogen in nitrite and nitrate nitrogen at the beginning of aeration, $\text{NH}_{4,i}^+$ (N mg/L) is the nitrogen in ammonium and ammonia at the beginning of aeration, and $\text{NH}_{4,e}^+$ (N mg/L) is the nitrogen in ammonium and at the end of aeration.

2.4. Track studies of the biofilm and the suspended sludge

After 178 d of operation, at the salinity of 9.8 g NaCl/L, track studies of the biofilm and the suspended sludge were undertaken. All suspended sludge in the SBBR were removed and transferred to a separate reactor. Thus, one reactor had suspended sludge only, named as suspended sludge system. Another reactor had biofilm only, named as biofilm system. The original SBBR was named as hybrid system. The biofilm and the suspended sludge systems were operated with the same influent strength as the original SBBR. The operation tests in biofilm and the suspended sludge systems were conducted with the same method as the track studies in the original SBBR at each salinity did. Every test was carried out three times for each system.

2.5. Analytic methods

The influent and effluent samples were centrifuged at 5,000 rpm for 10 min to remove micro-organisms. Concentrations of COD, TN, NH_4^+ -N, NO_2^- -N, and NO_3^- -N in the clear samples were measured according to the standard methods [23]. Due to the addition of salt, the standard COD test was modified as described by Yu and Li [24]. MLSS in the reactor was also

NaCl (g/L)	Operating time (d)	NH ₄ ⁺ -N (mg/L)	NO ₂ ⁻ -N (mg/L)	NO ₃ ⁻ -N (mg/L)	TN (mg/L)	COD (mg/L)
1.4	1–73	7.7 ± 1.7	0.5 ± 0.6	3.7 ± 3.4	11.6 ± 2.6	37 ± 7
2.8	74-86	5.3 ± 2.3	2.2 ± 0.7	0.7 ± 0.2	7.7 ± 1.6	48 ± 5
4.2	87-136	1.5 ± 1.9	1.1 ± 0.3	2.8 ± 1.4	5.4 ± 2.0	47 ± 5
5.6	137–153	5.5 ± 2.2	1.0 ± 0.4	1.0 ± 0.8	7.7 ± 2.1	44 ± 5
9.8	154–178	7.0 ± 1.5	1.7 ± 0.4	1.6 ± 0.8	11.8 ± 0.7	47 ± 5

measured according to the standard methods [23]. The pH was tested using pH probes (PHB-4, China). The temperature and DO were measured using a DO detector (Oxi 330i, WTW, Germany).

The experimental data obtained were statistically analyzed by Microsoft Excel[®] software, in which the standard deviations of the monitored parameters were calculated.

3. Results and discussion

3.1. Effect of salinity on the performance of the SBBR

3.1.1. Organic substance removal

Table 1 and Fig. 1 present the average values of effluent COD concentration and its removal efficiencies of the SBBR during the steady state at different salinities. Although the salinity in the influent ranged from 1.4 to 9.8 g NaCl/L, the reactor still showed high COD removal efficiencies and no significant differences were observed. The COD removal efficiency was maintained at more than 88.3% even after the salt concentration was increased to 9.8 g NaCl/L. Based on the result, it can be concluded that carbon-oxidizing bacteria were not inhibited under the salinity tested. Uygur [9] demonstrated that the specific COD removal rate increased when the salt content increased from 0 to 10 g NaCl/L in a SBR, indicating stimulatory effects of low salt contents on the organisms. Kargi and Dincer [25] also reported that COD removal efficiency was not affected by salinity up to 5 g NaCl/L; however, the efficiency dropped quite significantly with increasing salinity above 10 g NaCl/L.

3.1.2. Nitrogen removal

As shown in Fig. 1, the NH_4^+ -N removal efficiency was higher than 81.0% during the steady state. The



Fig. 1. Removal efficiencies of COD (\Box), NH₄⁺-N (\blacksquare), and TN (\boxtimes) under different salinities in SBBR.

best NH₄⁺-N removal was achieved in the reactor at the salinity of 4.2 g NaCl/L. When the salinity were 1.4, 2.8, and 4.2 g NaCl/L, the respective average NH_4^+ -N removal efficiencies were 81.0, 87.0, and 96.4%. But it decreased again to 87.4 and 83.5% when the salinity increased to 5.6 and 9.8 g NaCl/L. The high NH₄⁺-N removal suggested that biomass acclimation could overcome the detrimental effect of salt and a successful adaptation of nitrifying organisms could be achieved, even at the highest salinity tested. Similar NH_{4}^{+} -N removals were reported in other processes. Bassin et al. [8] observed that NH₄⁺-N removal efficiencies of domestic sewage were generally above 80%, with chloride concentrations ranging from 1,000 mg/L (1.6 g NaCl/L) up to 8,000 mg/L (13.2 g NaCl/L) in a moving bed biofilm reactor (MBBR). It was also reported by Windey et al. [26] that the reactor performance was not negatively affected by exposing the OLAND biofilm at salt concentrations up to 6 g NaCl/L. Uygur and Kargi [4] used a SBR to treat high salinity wastewater. They found that NH₄⁺-N removal was above 80% with salt contents below 10 g NaCl/L, while it decreased to 39% when salt content increased to 60 g NaCl/L.

Just as the NH_4^+ -N removal, the similar tendency was also observed for the TN removal in this study (Fig. 1). The highest TN removal efficiency was obtained when the salinity was 4.2 g NaCl/L. When the influent salinity was as low as 1.4 g NaCl/L, the average effluent NH₄⁺-N concentration was 7.7 mg/L due to incomplete nitrification (Table 1) and the TN removal efficiency was 72.7%. When the influent salinity increased up to 4.2 g NaCl/L, the effluent NH_4^+ -N, NO_2^--N , and NO_3^--N concentrations were low (average 1.5, 1.1, and 2.8 mg/L, respectively) by complete nitrification and efficient denitrification. And so the TN removal efficiency reached 87.9%. With the increase of influent salinity up to 9.8 g NaCl/L, incomplete nitrification occurred again, while complete denitrification was observed. The effluent NH₄⁺-N concentration increased to 7.0 mg/L and the effluent NO₂⁻-N and NO_3^- -N concentrations were 1.7 and 1.6 mg/L. Therefore, the TN removal efficiency reduced to 76.4% by the incomplete nitrification.

It has been reported that salinity can affect the oxygen-transfer rates (OTRs), and then can influence the nitrification, denitrification, and TN removal efficiency. Fast et al. [27] found that OTRs increased obviously with salinity from 0 to 11 g NaCl/L and slightly from 11 to 22 g NaCl/L. Rogers and Fast [28] revealed that OTRs increased 80% at 34 g NaCl/L compared with freshwater. Increased OTRs with increased salinities were attributed to more numerous and smaller bubbles at higher salinities. However, Ruttanagosrigit

et al. [29] indicated that OTRs were not influenced by salinity from 0 to 30 g NaCl/L. In view of these conclusions, further research needs to be done.

3.1.3. Track studies of nitrogen during aeration period

When the reactor was stable at each salinity level, track studies were carried out to measure the concentration profiles of the nitrogen compounds and COD during the 6.5 h aerated react period, so that the removal of the pollutants could be observed in a greater detail. Fig. 2 shows the profiles of NH_4^+ -N, NO₂⁻N, NO₃⁻N, and COD concentrations in the SBBR. The data in the figure were average values of three tests. The decrease in NH₄⁺-N could be described by one straight line in all the experiments carried out, but the concentrations of NO₂⁻-N and NO₃⁻-N usually decreased in the first part and increased subsequently during the aerated period. To describe the measured data of NO₂⁻-N and NO₃⁻-N, two lines with different slopes had to be used. The DNR was determined separately in the first 1.0 h of aeration and in the following 5.5 h of aeration because the DNR was not constant over the aerated react period. The DNR of an aeration period got through weighted average calculation. Table 2 summarizes the calculated NR, DNR,

and the SND efficiencies under various salinity conditions in this study and compared with reported values. The data in this study were average values and standard deviations of three tests.

In the SBBR, 13.1-30.7 mg/L of NH_4^+-N were removed during 6.5 h at the aeration phase, while only 1.09–3.13 mg/L of NO_2^--N or NO_3^--N appeared. This result could be attributed to efficient SND nitrogen removal during the aeration reaction even at the highest salinity tested. Previous reports [17,22] also showed that an efficient SND process occurred when the NR and DNR were in a balanced equilibrium producing very low amounts of nitrate and nitrite in the system. As shown in Table 2, the SND efficiency was more than 90%, without NO_3^--N or NO_2^--N accumulation, indicating that an efficient SND was achieved in the SBBR in spite of the increase in influent salinity from 1.4 to 9.8 g NaCl/L. The phenomena that the SND efficiency was above 100% at the salinity of 5.6 g NaCl/L could be explained by the reason that both the NO₃⁻-N in influent and the NO₃⁻-N produced from NH⁺₄-N oxidation were reduced to nitrogen gas through denitrification during the aeration period.

As shown in Fig. 2, the main COD uptake activity in the SBBR occurred within the initial 1.0 h, which had also been demonstrated in other studies [17].



Fig. 2. Profiles of NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, and COD concentrations under various salinities.

NaCl (g/L)	NR [mg N/(L h)]	DNR [mg N/(L h)]	$E_{\rm SND}$ (%)	Carbon/nitrogen ratio	Reactor	References
1.4	1.88 ± 0.06	1.67 ± 0.16	90.7 ± 6.4	11.0 ± 2.4	SBBR	This study
2.8	2.26 ± 0.25	2.20 ± 0.16	95.9 ± 9.0	9.9 ± 2.8	SBBR	This study
4.2	3.20 ± 0.18	3.08 ± 0.13	93.7 ± 5.3	9.9 ± 2.4	SBBR	This study
5.6	3.11 ± 0.35	3.40 ± 0.62	108.9 ± 5.4	8.6 ± 2.1	SBBR	This study
9.8	2.86 ± 0.19	2.79 ± 0.17	97.9 ± 3.8	8.9 ± 1.8	SBBR	This study
0.00	4.16	4.07	98.0	12.5	SBBR	[17]
0.00	-	-	83.3	10.0	SCBR ^a	[30]

 Table 2

 Overview of SND efficiencies under different salinity and comparison with reported values

^aSCBR was a suspended carrier biofilm reactor.

After 2 h, COD concentration reduced below 80 mg/L and almost did not vary until the end of aeration reaction.

For salt contents below 4.2 g NaCl/L, the NR and the DNR increased with the increase of salinity, which revealed that both nitrification and denitrification performances were strengthened gradually. The NR and the DNR increased from 1.88 to 3.20 and from 1.67 to 3.08 mg N/(h L), respectively, with the increase of salinity from 1.4 to 4.2 g NaCl/L. Moreover, the relationship between NH⁴₄-N, TN removal, and salinity (Fig. 1) also supported the notion that the increase of salinity (1.4–4.2 g NaCl/L) promoted nitrification and denitrification ability. A stimulation of the microbial activity at low salt concentrations was also reported in other literature. Chen et al. [31] reported that a salt concentration below 4.94 g NaCl/L was in favor of the NR.

A slight drop in NR and DNR was observed when the salt concentration increased from 4.2 to 9.8 g NaCl/L. It was suggested that the increase of salinity from 4.2 to 9.8 g NaCl/L had almost no influence on the ammonia oxidizers activity and the denitrifier activity. Bassin et al. [8] also reported that salinity did not induce nitrification inhibition to the bacteria by increasing the salt concentration from 1.6 to 13.2 g NaCl/L in a MBBR. Chen et al. [31] stated that the increasing salinity did not affect the specific nitrification rate (SNR) when the salinity was below 1.6 g NaCl/L. However, other previous reports were not in agreement with our results. Yan et al. [6] showed that ammonia oxidizing activity decreased with the increase of NaCl concentrations from 0 to 10 g NaCl/L. Uygur [9] and Panswad and Ahan [32] reported that SNR decreased with the increase of salt content from 0 to 10 g NaCl/L.

3.2. Relative effects of biofilm and suspended sludge

After 178 d of the operation, the relative effects of biofilm and suspended sludge in the SBBR were investigated by track tests at the salinity of 9.8 g NaCl/L. In these tests, the MLSS of the suspended sludge was around 3,900 mg/L (a total 56.50 g in the reactor). The biomass was 8.80 g per piece and 88.03 g in total mass. The ratio of total biomass in suspended sludge system and the biofilm system was 1 to 1.56. The total biomass in the hybrid system was 144.53 g.

3.2.1. Pollutants removal

The removals of nitrogen and organic matter are summarized in Table 3. All three systems obtained above 80% NH_4^+ -N oxidation. The NH_4^+ -N removal efficiency was high at 94.5% in the suspended sludge system, indicating the high nitrification performance of the suspended sludge. The hybrid system had a better TN removal than the biofilm and the suspended sludge did, when they were tested individually. The

Table 3

The removals of nitrogen and organic matter in the biofilm, the suspended sludge, and the hybrid system at the salinity of 9.8 g NaCl/L

Test systems	NH ₄ ⁺ -N removal (%)	TN removal (%)	COD removal (%)	SNR [mg N/(h g)]	SDNR [mg N/(h g)]	E _{SND} (%)
Biofilm	81.9 ± 10.2	76.4 ± 5.8	89.6 ± 3.4	0.30 ± 0.06	0.26 ± 0.09	92.1 ± 3.1
Suspended sludge	94.5 ± 0.4	67.7 ± 1.3	88.5 ± 1.5	0.77 ± 0.14	0.20 ± 0.13	20.0 ± 14.2
Hybrid system	84.0 ± 3.6	76.4 ± 1.8	89.4 ± 1.4	0.28 ± 0.02	0.27 ± 0.02	97.9 ± 3.8

TN removal of the biofilm, suspended sludge, and hybrid systems was around 74.0, 68.0, and 76.0%, respectively. Similar COD removal efficiency was observed in the biofilm, the suspended sludge, and the hybrid systems (averaged 88.5–89.6%).

3.2.2. Ammonia oxidation, nitrite oxidation, and denitrification

Time profiles of nitrogen compounds in the biofilm, the suspended sludge, and the hybrid systems are shown in Fig. 3. The data were average values of three tests. The NRs and DNRs were determined according to the time profiles in the three systems. As the same case in the original hybrid system, the experimental results in biofilm system showed that NO₃⁻-N decreased in the first 1.0 h of the aerated period and then increased. So the DNR was determined separately in the first 1.0 h of aeration and the subsequent 5.5 h of aeration in the biofilm system. For the suspended sludge system, NO2-N increased in the first 3.5 h of the aerated period and then decreased. So the DNR was determined separately in the initial 3.5 h of aeration and the following 3.0 h of aeration in the suspended sludge system. The DNR of an aeration period in both systems was got through weighted average calculation. The SNRs and the specific denitrification

rates (SDNRs) were obtained through dividing NRs and DNRs by the biomass in the systems. The results of the calculated specific rates and SND efficiencies are summarized in Table 3. The data were average values and standard deviations of three tests.

The SNRs and SDNRs for the three systems were significantly different. In the biofilm and the hybrid systems, the ammonia oxidation was relatively slow with the SNR of 0.30 and 0.26 mg N/(hg biomass), even though the variation of DO concentrations during the aeration period in solution were 4.1-7.7 and 2.5-7.0 mg/L, respectively (track study results of DO not shown). This higher DO facilitated nitrite conversion to nitrate. Nitrite build-up did not happen in the two systems and nitrite concentration throughout the tests was lower than 1.8 mg/L. The slow SNR in the biofilm and the hybrid reactors could be attributed to the anaerobic condition in the inner layer of the biofilm. The thickness of the biofilm can hinder the transfer of oxygen. As a result, the inner layer of the biofilm is in an anaerobic condition [33]. The concentration of nitrate was also relatively low during the tests in both biofilm and hybrid systems. This result implied that efficient denitrification occurred during the aeration period even though the DO level was high.

For the suspended sludge system, the SNR average was 0.77 mg N/(h g biomass), which was 2.57 times



Fig. 3. Profiles of NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and TN concentrations in the three systems at the salinity of 9.8 g NaCl/L.

higher than values of the biofilm system. Since the biomass of the biofilm was 1.56 times the biomass of the suspended sludge, the overall contribution of the suspended sludge to ammonia oxidation was 1.64 times (2.57/1.56) higher than that of the biofilm. However, the DO level in suspended sludge (1.0-5.2 mg/L)was lower than the level in the biofilm test. Higher DO value resulted in a higher SNR [22]. That is, if DO conditions in the two reactors were the same, the contribution of the suspended sludge to ammonia oxidation in the hybrid reactor would be greater than 1.64 times compared with the contribution by the biofilm. Thus, the suspended sludge played the major role in ammonia oxidation. Lo et al. [21] also found that suspended sludge achieved 1.8 times overall contribution to ammonia oxidation than that of the biofilm in a HSBR. Nitrate accumulation was found in the suspended sludge (Fig. 3). The highest nitrate concentration accumulated in the bulk solution was 12.8 mg/L (around one fourth of the TN in the feed). The accumulation of nitrate implied that the performance of denitrification was lower than that of nitrification. Nitrate was the major end product of ammonia oxidation in this system. Peaking of NO_2^--N was observed in the reactor. This could be due to nitrite accumulations within the reactor under low DO conditions. Similar observation had been reported in the literatures [22,34].

SDNRs of 0.26, 0.20, and 0.27 mg N/(h g biomass) were observed for the biofilm, the suspended sludge, and the hybrid systems, respectively. The SDNR for the biofilm was 1.30 times more than the rate of the suspended sludge. Considering the total biomass of the biofilm was 1.56 times than that of the suspended sludge, the overall contribution to denitrification from the biofilm in the hybrid reactor was 2.03 times (1.30×1.56) greater than the suspended sludge in the hybrid reactor. Thus, in the hybrid system, the contribution to denitrification to denitrification to denitrification to denitribution to denitrification.

TN of the three systems showed the similar tendency which gradually reduced with time during aeration period. The effluents TN were 11.9, 13.6, and 16.7 mg/L for the hybrid, the biofilm, and the suspended sludge reactors, respectively. Ammonia was the major portion of the effluent TN for the hybrid and the biofilm systems, while nitrate was the major one for the suspended sludge system.

3.2.3. Simultaneous nitrification and denitrification

There were 92.1 and 20.0% SND efficiencies for the biofilm and the suspended sludge reactors at the end of aeration, while the hybrid system had a SND efficiency of 97.9%. The high SND efficiencies achieved in the biofilm and the hybrid systems were due to

oxygen gradient in biofilm, which indicated the important role of biofilm in nitrogen removal efficiency just as observed by others [13,35]. However, the suspended sludge system was detrimental to the SND process. The SND efficiency of the suspended sludge test was low, even though the level of DO was lower than the level in biofilm and hybrid systems. Most of the nitrogen was converted to nitrate at the end of the aeration in the suspended sludge test.

4. Conclusions

The SBBR showed good performance on organic substance and nitrogen removal via SND during the whole experimental period. The average COD removals were above 88.3% at different influent salinity. Even running at high salinity of 9.8 g NaCl/L, NH₄⁺-N and TN removal efficiencies were maintained at 83.5 and 76.3%, respectively. In the SBBR, the suspended sludge played the major role in ammonia and nitrite oxidation and denitrification was the main contribution of the biofilm. A good SND performance was achieved in both the pure biofilm and the hybrid systems. TN removals were 74.0, 68.0, and 76.0% for the biofilm, the suspended sludge, and the hybrid system, respectively. SND efficiencies were 97.9 and 92.1% for the hybrid and the biofilm systems, respectively, but only 20.0% for the suspended sludge system at the salinity of 9.8 g NaCl/L.

Acknowledgments

The authors would like to express their thanks to the Special Grand National Science & Technology Project of China for Water Pollution Control and Treatment (No. 2009ZX07106-003) for financial support.

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