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The nitritation performance of biofilm reactor for treating domestic wastewater under high dissolved oxygen

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ABSTRACT

The objective of this study was to investigate the nitritation performance in a biofilm reactor for treating domestic wastewater. The reactor was operated in continuous feed mode from phases 1 to 3. The dissolved oxygen (DO) was controlled at 3.5–7 mg/L throughout the experiment. The biofilm reactor showed excellent nitritation performance after the inoculation of nitrifying sludge, with the hydraulic retention time being reduced from 24 to 7 hr. Above 90% nitrite accumulation ratio (NAR) was maintained in phase 1. Afterwards, nitratation occurred with the low NH_4^+–N concentration in the reactor. The improvement of NH_4^+–N concentration to 20–35 mg/L had a limited effect on the recovery of nitritation. However, nitritation recovered rapidly when sequencing batch feed mode was adopted in phase 4, with the effluent NH_4^+–N concentration above 7 mg/L. The improvement of ammonia oxidizing bacteria (AOB) activity and the combined inhibition effect of free ammonia (FA) and free nitrous acid (FNA) on the nitrite oxidizing bacteria (NOB) were two key factors for the rapid recovery of nitritation. Sludge activity was obtained in batch tests. The results of batch tests had a good relationship with the long term operation performance of the biofilm reactor.

Introduction

Wastewater that contains a large amount of ammonium will cause a serious eutrophication problem for the receiving water. Biological nitrification–denitrification is commonly used to remove the nitrogen from wastewater. However, these practices usually lead to the need for a large volume reactor and high operating costs. Partial nitritation/anammox (PNA) installations were already successfully operated worldwide in side-stream treatment to reduce aeration energy for nitrogen removal (Gut et al., 2006; Zekker et al., 2013; Lackner et al., 2014). The research focus has now moved to possible applications of PNA in mainstream treatment. Current research suggests that anammox could be achieved at low temperature (about 20°C) and that the biofilm reactor was efficient in the cultivation of anammox bacteria (Zekker et al., 2012b, 2015a; Gilbert et al., 2014). Some measures for the recovery of anammox under adverse conditions were also reported (Jin et al., 2013; Bi et al., 2014; Zekker et al., 2015b). Nevertheless, it is still difficult to achieve nitritation in mainstream wastewater due to the low temperature and low nitrogen concentration. Therefore, it is necessary to investigate the feasibility of partial nitrification measures for treating sewage.

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Nitritation has been achieved by controlling several operational factors, such as low dissolved oxygen (DO) (Blackburne et al., 2008a), high pH (Villaverde et al., 1997), high temperature (Hellinga et al., 1998), and heavy free ammonia (FA) and free nitrous acid (FNA) concentrations (Anthonisen et al., 1976; Vadivelu et al., 2007; Park and Bae, 2009). For low-strength, municipal or domestic wastewater, almost all experiments have been conducted in sequencing batch reactors (SBRs) with an activated sludge system (Blackburne et al., 2008b; Yin et al., 2014). On the other hand, it would be a feasible option to achieve nitritation with low DO. The oxygen saturation coefficients of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are known to be 0.3 and 1.1 mg/L, respectively (Wiesmann, 1994). When oxygen is limiting, AOB were suggested to outcompete NOB (Bernet et al., 2001; Blackburne et al., 2008a). Tokutomi (2004) observed that the growth rate of AOB was 2.6 times faster than that of NOB when the DO was below 1.0 mg/L. However, it was also reported that NOB could be outcompeted at high DO bulk concentrations, since the oxygen supply to the biofilm could be reduced by a thick external boundary layer (Antileo et al., 2007; Brockmann and Morgenroth, 2010; Rathnayake et al., 2013; Zekker et al., 2014). So far, there is little information about the nitritation performance of biofilm reactors for treating domestic wastewater.

It was reported that an alternating aeration strategy was effective in achieving nitritation (Kornaros et al., 2010; Ge et al., 2014). Kornaros et al. (2010) reported that AOB were not affected by anoxic disturbance, while the NOB were seriously inhibited, with a reduced growth rate. Ge et al. (2014) pointed out that NOB adjusted more slowly than AOB to aerobic conditions after anoxic periods. Slow-growing organisms are suggested to grow in the inner part of the biofilm, whereas faster growing organisms are towards to the outer part of the biofilm (Brockmann and Morgenroth, 2010; Rikmann et al., 2012; Zekker et al., 2012a). Moreover, reports have shown that the growth rate of AOB was higher than that of NOB at temperatures above 25°C (Hellinga et al., 1998). Hence, it should be feasible to achieve nitritation rapidly in a biofilm reactor at high temperature.

Based on the above discussion, the aim of the present study was to investigate the nitritation performance in a biofilm reactor. For this purpose, the fast achievement of excellent nitritation performance and the effect of ammonium concentration on the nitritation performance were investigated. Additionally, the effect of the sequencing batch feed mode on the recovery performance of nitritation was also investigated. It is expected that the knowledge obtained in this study will be critical for developing a novel nitritation process and lay the foundation for the application of PNA in mainstream autotrophic nitrogen removal processes.

1. Materials and methods

1.1. Reactor and experimental setup

Fig. 1 shows the reactor configuration scheme of the experimental set-up. A biofilm reactor with a working volume of 89.5 L was used. Dimensions of the unit were: a height of 79 cm and inner diameter of 38 cm. Kaldnes rings (K3 carriers, AnoxKaldnes, Beijing) were used as biomass carriers. The volume of the carriers was 38% of the working volume of the reactor. The carriers had a cylindrical shape (diameter of 25 mm) with a grid of 4 mm. Fig. 6 shows a picture of the carriers. The temperature was controlled at 30°C by three heating rods immersed in the reactor (GM1616, Jiyin, China). No pH adjustment was adopted. The air was supplied by an air diffuser with a constant rate of 500 L/hr at the bottom of the reactor. A programmable logic controller (PCL-812, Advantech, USA) was installed to perform automatic process control. The DO and pH were detected by online instruments.

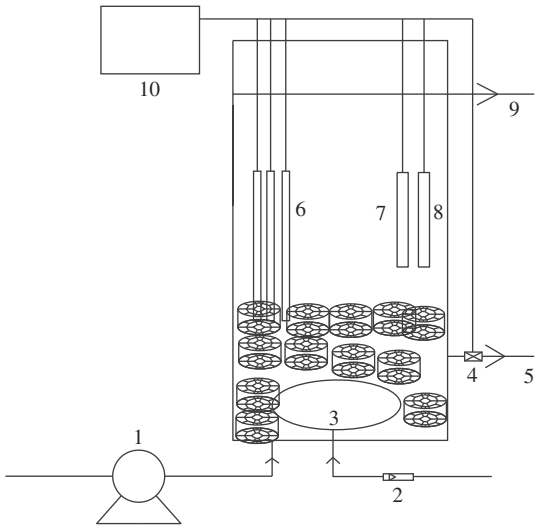

Fig. 1 – Reactor configuration scheme of biofilm reactor. (1) Influent pump; (2) air flowmeter; (3) air diffuser; (4) electromagnetic valve; (5) effluent of sequencing fed batch mode; (6) heating rod; (7) pH electrode; (8) dissolved oxygen (DO) electrode; (9) effluent of continuous fed mode; (10) programmable logic controller (PLC).

1.2. Wastewater and operational conditions

The reactor was operated in four phases. During phases 1 to 3, the reactor was operated in continuous feed mode. The influent was pumped into the bottom and the effluent was discharged at the top of the reactor. In phase 1 (days 1 to 33), the hydraulic retention time (HRT) was shortened from 24 to 7 hr. The HRT in phase 2 (days 34 to 52) and phase 3 (days 53 to 76) was maintained at 7 and 4.6 hr, respectively. In phase 4 (days 77 to 95), the reactor was operated in sequencing batch feed mode with a volume exchange ratio (VER) of 81%. Each cycle contained: feeding (3 min), aerobic reaction (180 min), settling (10 min), decanting (10 min), and idling (1 min). During phase 4, the floc sludge would be withdrawn from the reactor with the effluent since the settling time was 10 min and the VER was 81%. No additional sludge was discharged from the biofilm reactor.

The seeding sludge was obtained from an original SBR in our lab with excellent nitritation performance. The mixed liquor suspended solids (MLSS) and the volume of the seeding sludge were 8000 mg/L and 7 L, respectively. The aerobic NH_4^+–N and NO_2^-–N oxidation activities of the seeding sludge were 0.135 and 0.001 g N/(g VSS · day), respectively (Appendix A Fig. S1). As for

the original SBR reactor, it was fed with the same domestic wastewater as used in this experiment. The temperature was not controlled and the DO concentration was maintained at 2–3 mg/L. The original SBR achieved nitritation by stopping the aeration ahead of ammonium complete-oxidation with an activated sludge system. Domestic wastewater from the residential area nearby the lab was used as feed. The main wastewater characteristics are summarized in Table 1.

1.2.1. Batch tests

Batch tests were performed in serum bottles with a working volume of 500 mL. The composition of synthetic wastewater is described in Table 2. The sludge used in batch tests was prepared as follows: suspended sludge taken from the biofilm reactor was first precipitated in a flask and the supernatant was withdrawn, then the precipitated sludge was concentrated in a centrifuge (4000 r/min × 2 min). The centrifuged sludge was then mixed with distilled water and reconcentrated again to remove the residual nitrogen species.

About 10 g reconcentrated sludge was then transferred to serum bottles together with 500 mL synthetic wastewater. Meanwhile, about 5 g reconcentrated sludge was processed by the oven and muffle furnace to determine the ratios of dry solids to reconcentrated sludge and volatile solids to reconcentrated sludge. The MLSS and mixed liquor volatile suspended solids (MLVSS) in serum bottles were then calculated according to the former ratios. The aeration was supplied at a flow rate of 250 mL/min to maintain the DO concentration above 5 mg/L in case the activity of sludge was limited by oxygen. Magnetic stirring (200 r/min) was used to keep the biomass in suspension and increase the mass transfer performance. The temperature was controlled at 30°C. Samples were taken at appropriate time intervals.

1.3. Analytical methods

All the samples were filtered with a 0.45 μm filter before analyzing. NH_4^+–N, NO_2^-–N, NO_3^-–N, COD, MLSS, MLVSS and alkalinity were measured according to the standard methods (APHA, 1998). DO, pH, and temperature (*T*) were monitored by a WTW Multi 3420i meter (WTW Company, Germany).

The morphology of the bacteria was observed with a scanning electron microscope (SEM) (FEIQUANTA 200, FEI Company, USA) to acquire more information for better reactor performance. The samples were fixed with 2.5% glutaraldehyde for 3 hr and then rinsed in 0.1 mol/L phosphate buffer solution (PBS, pH 7.2) 3 times. Subsequently, the samples were dehydrated with a series of ethanol–water mixtures (25%, 50%, 75%, 90% and 100% ethanol). The dewatered samples were dried by the critical point method and further sputter-coated with gold for SEM observation.

Table 2 – Composition of synthetic wastewater in batch tests.

Constituents	NH_4^+–N oxidation test	NO_2^-–N oxidation test
NH_4^+–N (mg/L)	70	0
NO_2^-–N (mg/L)	0	70
$NaHCO_3$ (mg/L)	840	0

1.4. Calculation methods

The COD discussed in this study was calculated as Eq. (1) (Liang and Liu, 2007).

$$\mathrm{COD} = \mathrm{CODmeasured} - \frac{8}{7} C_{(NO_2^- - N)\mathrm{effluent}} \quad (1)$$

where, C (mg/L) is the concentration.

The nitrite accumulation ratio (NAR, %) was calculated as Eq. (2) (Liang and Liu, 2007).

$$\mathrm{NAR} = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N} \times 100\%. \quad (2)$$

The FA and FNA concentrations were calculated by the following Eqs. (3) and (4) (Anthonisen et al., 1976):

$$\mathrm{FA} = \frac{17}{14} \times \frac{c(NH_4^+ - N) \times 10^{\mathrm{pH}}}{e^{\left(\frac{6344}{273+T}\right)} + 10^{\mathrm{pH}}} \quad (3)$$

$$\mathrm{FNA} = \frac{46}{14} \times \frac{c(NO_2^- - N)}{e^{\left(\frac{-2300}{273+T}\right)} \times 10^{\mathrm{pH}}} \quad (4)$$

where, *T* (°C) is temperature.

2. Results and discussion

2.1. Nitritation performance

In phase 1, the biofilm reactor started to achieve excellent nitritation performance (days 1–33). Fig. 2 shows the evolution of nitrogen compounds and COD of the biofilm reactor. During days 1–12, the HRT was gradually shortened from 24 to 7 hr. As observed, the ammonium loading rate gradually increased from 0.07 to 0.20 kg N/(m^3 · day) and the COD loading rate increased from 0.24 to 0.69 kg N/(m^3 · day) (Fig. 2d). Correspondingly, the ammonium removal rate rose from 0.06 to 0.17 kg N/(m^3 · day) and the COD removal rate rose from 0.18 to 0.49 kg N/(m^3 · day). On day 12, the effluent NH_4^+–N, NO_2^-–N and NO_3^-–N concentrations were 11.7, 65.5 and 5.5 mg/L with a NAR of 92.2% (Fig. 2a and b), indicating the excellent nitritation performance of the biofilm reactor. During days

Table 1 – Characteristics of domestic wastewater.

Parameters	pH	COD (mg/L)	TN (mg/L)	NH_4^+–N (mg/L)	NO_2^-–N (mg/L)	NO_3^-–N (mg/L)	Alkalinity (as $CaCO_3$) (mg/L)
Range	7.1–8.0	200–320	70–120	60–90	<1	<3	300–400
Mean	7.5	250	90	80	0.15	1.2	320

COD: chemical oxygen demand. TN: the summation of NH_4^+–N, NO_2^-–N, NO_3^-–N and total organic nitrogen.

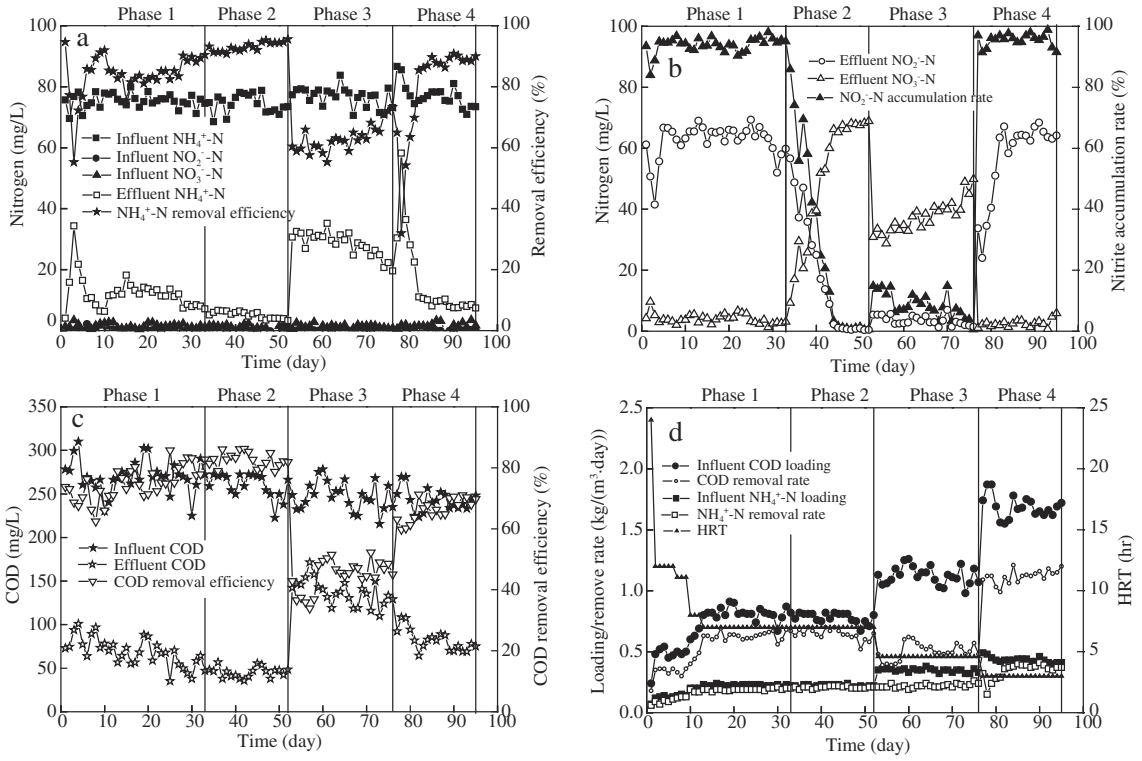

Fig. 2 – Evolution of nitrogen compounds and chemical oxygen demand (COD) during operation: (a) evolution of nitrogen compounds and the NH_4^+–N removal performance; (b) nitrogen compounds and nitrite accumulation ratio (NAR) in the effluent; (c) COD removal performance; (d) influent loading rate, removal rate and hydraulic retention time (HRT) during operation.

13–33, the HRT was maintained at 7 hr, and a stable conversion of ammonium to nitrite was achieved. The effluent NH_4^+–N concentration decreased slightly from 13.1 to 7.1 mg/L and the average NO_2^-–N concentration was 66 mg/L with the average NAR of 94%. Fig. 3 shows the DO, FA and FNA concentrations during phases 1 to 3. In phase 1, the average DO concentration was 5.01 mg/L. The average FA and FNA concentrations were 0.05 and 0.10 mg/L. Fig. 4 shows the sludge activity of different phases in batch tests. In phase 1 (day 33), the aerobic NH_4^+–N and NO_2^-–N oxidation activities of the sludge were 0.366 and 0.005 g N/(g VSS · day), respectively.

Fast-growing organisms were located towards to the outer part of the biofilm, and AOB grew significantly faster than NOB at temperatures above 25°C (Hellinga et al., 1998;

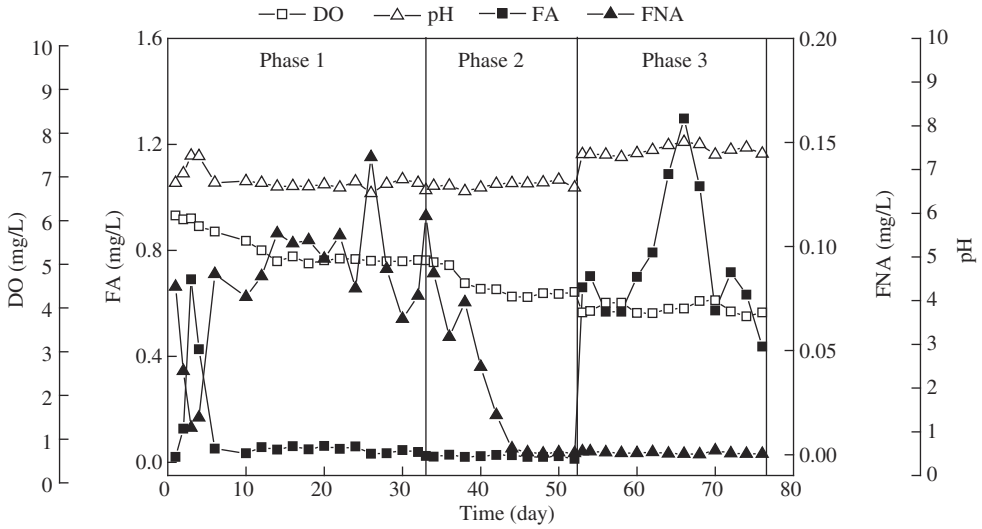

Fig. 3 – The DO, pH, free ammonia (FA) and free nitrous acid (FNA) concentrations observed during phase 1 to phase 3.

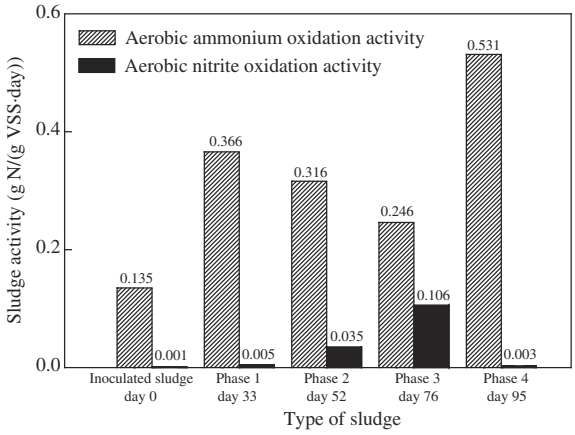

Fig. 4 – The sludge activity of batch tests in different operational phases.

Brockmann and Morgenroth, 2010; Rikmann et al., 2012; Zekker et al., 2012a). In the present study, it was beneficial for AOB to dominate on the surface of the biofilm since Kaldnes rings were used as biomass carriers and the temperature was controlled at 30°C.

It has been reported that the oxygen saturation coefficients for AOB and NOB were 0.3 and 1.1 mg/L, and that AOB and NOB have to compete for oxygen when oxygen is limiting (Wiesmann, 1994). So far, most research achieved partial nitrification under low DO concentrations (Blackburne et al., 2008a). In the present study, high NAR was achieved in phase 1 with the average DO concentration at 5.01 mg/L (Fig. 3). The biofilm has been reported to greatly affect the oxygen mass transfer rate. Antileo et al. (2007) reported that the DO concentration was reduced to 0 and 0.06 mg/L at 30 μm inside the biofilm when the DO concentration in the liquid bulk was 3.3 and 6.8 mg/L, respectively. Subsequently, Rathnayake et al. (2013) pointed out that at 2 mg/L of DO concentration in the liquid bulk, the DO concentration was reduced to 0 mg/L at 100 μm inside the biofilm. As shown in Fig. 6, the thickness of the biofilm on the carriers was 1 mm. Therefore, the DO concentration inside the biofilm might be at low values due to oxygen transfer resistance. The growth of NOB in the inner parts of the biofilm would be largely inhibited. On the other hand, it was pointed out that the maximum growth rate of NOB was at 1.01 day (Wiesmann, 1994). The HRT was less than 24 hr in this phase, equal to the SRT of the floc sludge. Thus, the NOB in floc sludge would be washed out under high DO.

Researchers found that certain concentrations of FA and FNA could inhibit NOB. Anthonisen et al. (1976) reported that the threshold FA concentration for NOB inhibition was between 0.1 and 1 mg/L. Similarly, Park and Bae (2009) pointed out that the threshold FA concentration for NOB inhibition was 0.7 mg/L, while the activity of NOB was inhibited by 50% at FNA concentrations between 0.02 and 0.1 mg/L. Vadivelu et al. (2007) also found that the biosynthesis of *Nitrobacter* was totally stopped at the FNA concentration of 0.023 mg/L. As a result, the FA levels were below the inhibition values for NOB, and the FNA might play a role in the inhibition of NOB.

2.2. Effect of low NH_4^+–N concentration on the robustness of nitritation

In phase 2, in order to investigate the robustness of nitritation at low NH_4^+–N concentration, the HRT was controlled at 7 hr (days 34–52). Unexpectedly, nitritation was totally destroyed during this phase. As Fig. 3 shows, the average DO concentration was 4.09 mg/L, while the average FA and FNA concentrations were 0.03 and 0.02 mg/L. The average FA and FNA concentrations were below the threshold of NOB inhibition. The effluent NH_4^+–N concentration was below 7 mg/L (Fig. 2a). As a result, the effluent NO_2^-–N concentration decreased from 56.6 to 0.42 mg/L, while the effluent NO_3^-–N concentration increased from 9.2 to 68.8 mg/L (Fig. 2b). Meanwhile, the NAR decreased from 95.0% to 0.62%. The aerobic NH_4^+–N and NO_2^-–N oxidation activities of the sludge in phase 2 (day 52) were 0.316 and 0.035 g N/(g VSS · day), respectively (Fig. 4).

The aerobic NH_4^+–N oxidation activity of sludge in phase 2 decreased by 13.7% compared to that of phase 1. This result indicated that the sludge activity of AOB was limited in this phase. Im et al. (2014) reported that the AOB concentration played an important role in the accumulation of nitrite. Previous studies reported that the ammonium affinity constant was below 1 mg/L (Wyffels et al., 2004; Van Hulle et al., 2007). Due to the substrate mass transfer resistance, the ammonium in the inner part of the biofilm might be far less than 1 mg/L when the NH_4^+–N concentration was below 7 mg/L in the reactor. As a result, the AOB activity would be limited inside the biofilm, and the consumption of DO on the surface of the biofilm would decrease. Hence, the NOB on the biofilm might be able to gain enough DO for the oxidation of nitrite. Additionally, Fux et al. (2004) reported that NOB could accumulate after 11 months of stable nitritation in the MBBR. Similar results were reported, since the biofilm improved the retention of slow growth bacteria (Blackburne et al., 2008b; Brockmann and Morgenroth, 2010).

2.3. Effect of improving NH_4^+–N concentration on the recovery of nitritation

In order to investigate the effect of improving NH_4^+–N concentration on the recoverability of nitritation, the HRT was shortened to 4.6 hr in phase 3 (days 53–76). As Fig. 3 shows, the average DO concentration was 3.8 mg/L, while the average FA and FNA concentrations were 0.79 and 0.005 mg/L. The effluent NH_4^+–N concentration maintained at 20–35 mg/L (Fig. 2a). However, NO_3^-–N still dominated in the effluent. The effluent NO_2^-–N concentration was below 5 mg/L while the effluent NO_3^-–N concentration was above 30 mg/L (Fig. 2b). The aerobic NH_4^+–N and NO_2^-–N oxidation activities of the sludge in phase 3 (day 76) were 0.246 and 0.106 g N/(g VSS · day), respectively (Fig. 4). The aerobic NH_4^+–N oxidation activity of sludge in phase 3 decreased by 22.2% compared to that of phase 2. The reason for this was related to the lower HRT in phase 3. With a shorter HRT, the NH_4^+–N concentration was improved, which was beneficial for the increase of aerobic NH_4^+–N oxidation activity. However, a large amount of AOB would be washed out, resulting in the decrease of aerobic NH_4^+–N oxidation activity. As for the NOB, the aerobic NO_2^-–N

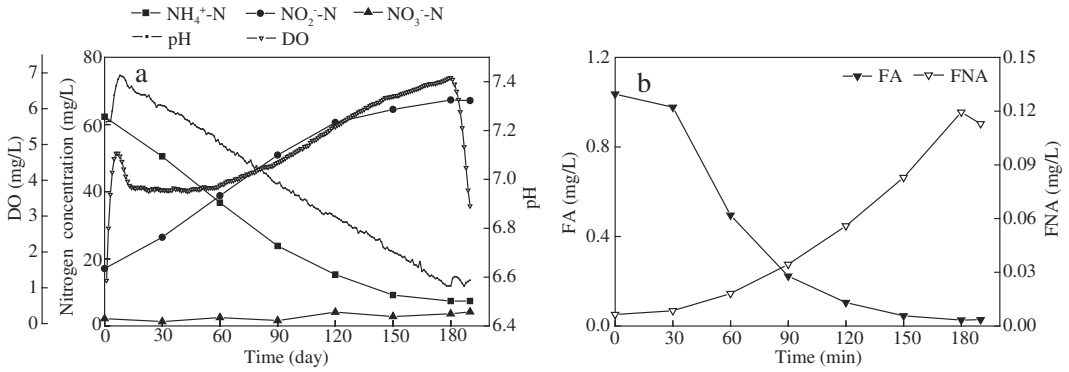

Fig. 5 – Measurements obtained during one cycle of operation: (a) evolution of nitrogen compounds; (b) FA and FNA concentrations.

oxidation activity of the sludge in phase 3 was 2 times higher than that of phase 2. A large amount of NOB would also be washed out, resulting in the decrease of the aerobic NO_2^-–N oxidation activity. Nevertheless, as the aerobic NH_4^+–N oxidation activity of sludge decreased, the NOB on the biofilm might show stronger competitive advantage in terms of DO. Therefore, the sludge activity of NOB in phase 3 was stronger than that of phase 2.

In phase 3, the FA concentrations were in the range of inhibition values for NOB, while the FNA concentrations were below the inhibition level. However, nitratation was not inhibited at the average FA concentration of 0.79 mg/L. Previous studies reported that the acclimation phenomenon took place after NOB were exposed to high FA for long periods of time. Fux et al. (2004) pointed out that NOB were not inhibited at high FA concentrations between 20 and 25 mg/L after a long time of acclimation. Afterwards, Villaverde et al. (2000) also reported that the threshold of specific FA inhibition on NOB increased from 0.2 to 0.7 mg NH_3–N/g VSS after four more months of operation. Thus, high FA acclimation of NOB might contribute to the high aerobic NO_2^-–N oxidation activity in this phase.

2.4. Effect of sequencing batch feed mode on the recovery of nitritation

In phase 4, the biofilm reactor was operated in sequencing batch feed mode to recover nitritation (days 77–95). As a result, the biofilm reactor recovered nitritation performance rapidly. The NH_4^+–N conversion rate gradually increased and the effluent NH_4^+–N concentration decreased to 7.4 mg/L on day 95 (Fig. 2a). As observed in Fig. 2b, the effluent NO_2^-–N concentration increased from 33.7 to 63.5 mg/L during days 77–82 and then stabilized at 64 mg/L. The effluent NO_3^-–N concentration was below 6 mg/L in this period. From day 77 on, the NAR reached 97% and remained at 95% in the following days. The aerobic NH_4^+–N and NO_2^-–N oxidation activities of the sludge in phase 4 (day 95) were 0.531 and 0.003 g N/(g VSS · day), respectively (Fig. 4). As the reactor was operated with a short settling time and a high VER, the MLSS was constant at about 1000 mg/L. The suspended sludge in the reactor mainly consisted of the detached sludge from the biofilm. A certain amount of floc sludge was withdrawn from the reactor with the effluent. This was beneficial for the rapid washout of NOB from the reactor.

Fig. 5 shows the performance of the SBR during one cycle on day 95. The aeration was supplied at t = 0 min after the influent was completely fed into the reactor. During 0–190 min, the NH_4^+–N concentration decreased from 62.3 to 7.4 mg/L, while the NO_2^-–N concentration increased from 17.1 to 67.1 mg/L with the NO_3^-–N concentration below 4.2 mg/L. The NH_4^+–N removal efficiency and NAR were 88.1% and 94.1% in the effluent. The DO concentration was above 3.35 mg/L from 3 to 190 min. During 0–190 min, the FA concentration gradually reduced from 1.03 to 0.03 mg/L and the FNA concentration increased from 0.006 to 0.119 mg/L, respectively. That is, the FA concentration was above 0.22 mg/L during 0–90 min while the FNA concentration was above 0.03 mg/L during 90–180 min.

Nitritation recovered rapidly in phase 4. As observed in Fig. 5, the FA during 0–90 min and the FNA during 90–180 min were in the range of inhibition values for NOB. The phenomenon of FNA following the disappearance of FA in inhibiting the NOB activity has been reported by other researchers under different operational conditions (Liu et al., 2008; Wei et al., 2014). Additionally, the aerobic NH_4^+–N oxidation activity of the sludge in phase 4 was 1.1 times higher than that of phase 3. The reason for this higher activity was related to the

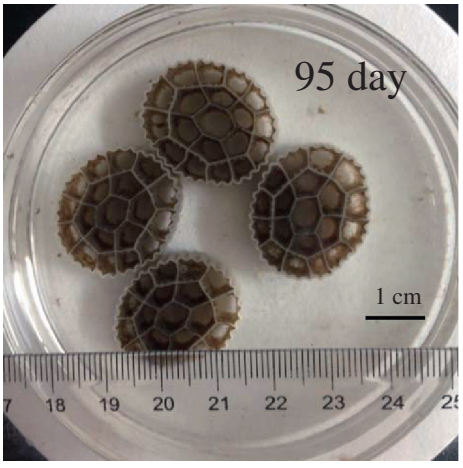

Fig. 6 – The photo of Kaldnes ring on day 95.

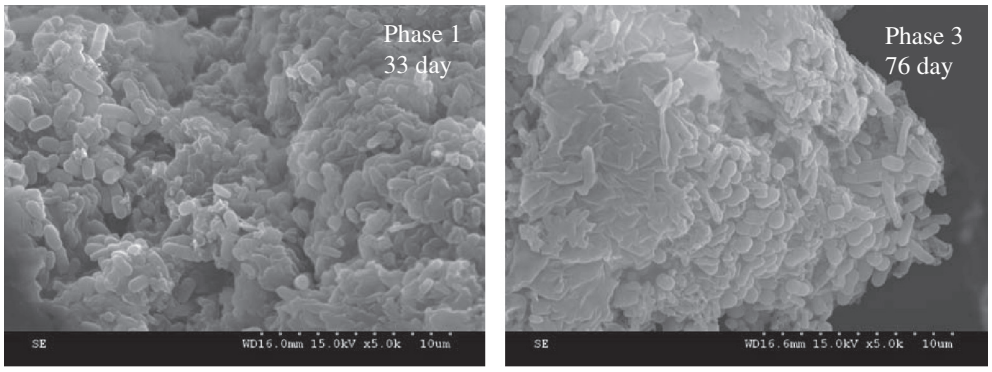

Fig. 7 – The scanning electron microscope (SEM) images of sludge during operation.

excellent retention performance of sludge in SBR running mode. As a result, the DO would be reduced inside the biofilm due to the greater consumption of DO on the surface. It was beneficial for the AOB to outcompete NOB. Previous studies reported that the alternating aeration strategy was considered to be effective in achieving nitritation (Kornaros et al., 2010; Ge et al., 2014). In the present study, as observed in Fig. 5, the aeration was stopped during the filling and drainage period, for 16 min, and the aeration time in one cycle was 177 min. The DO concentration was above 3.35 mg/L from 3–190 min. Therefore, it was long enough for the NOB to recover activity after anoxic conditions. The anoxic period in sequencing batch feed mode might have had a limited effect on the inhibition of NOB in this phase. Since studies have reported that NOB could accumulate after a long time of stable nitritation in the biofilm reactor (Fux et al., 2004; Blackburne et al., 2008b; Brockmann and Morgenroth, 2010), the stability of nitritation in SBR still needs further investigation.

2.5. *Morphology observation*

A photo of the ring-shaped carriers is shown in Fig. 6. There was an obvious increase in the thickness of the biofilm after 95 day operation. Recently, Gilbert et al. (2014) achieved nitritation under low DO (0.1–0.5 mg/L) for low-strength wastewater in a biofilm reactor with Kaldnes rings as carriers. The morphology of the sludge in different phases was observed by a SEM (Fig. 7). It can be seen that spherical bacteria dominated in phase 3, while short rod-shaped bacteria dominated in phases 1 and 4. Several studies reported that the morphology of the dominant bacteria was mainly short rod-shaped in the nitritation phase (Guo et al., 2009; Shen et al., 2014). However, Xu et al. (2012) found that the dominant bacteria in nitrifying granules were mainly cocci and bacilli. It was difficult to identify these clusters of bacteria by SEM viewing alone. Therefore, FISH analysis and quantitative real-time polymerase chain reaction (qPCR) should be performed for further study.

3. Conclusions

The nitritation performance in treating domestic wastewater was studied under high DO conditions in a biofilm reactor. The recovery performance of nitritation was also studied. The biofilm reactor achieved excellent nitritation performance, with the HRT reducing from 24 to 7 hr within 33 days. The fact that faster-growing organisms were expected to be dominant on the surface of the biofim and the mass transfer effect of DO in the biofilm played important roles in the good nitritation performance in phase 1. Nitritation was destroyed when the NH_4^+–N concentration was below 7 mg/L. The high DO concentration combined with the reduced AOB activity under low ammonium concentration led to the damage of nitritation. The improvement of the NH_4^+–N concentration to 20–35 mg/L had a limited effect on the recovery of nitritation. However, nitritation recovered rapidly after changing the continuous feed mode to sequencing batch feed mode. The improvement of AOB activity and the combined inhibition effect of FA and FNA on the NOB were two key factors on the fast recovery of nitritation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.09.006.

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