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Nitrogen removal via short-cut simultaneous nitrification and denitrification in an intermittently aerated moving bed membrane bioreactor

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ABSTRACT

An intermittently aerated moving bed membrane bioreactor (MBMBR) was developed and crucial parameters affecting nitrogen removal from wastewater by simultaneous nitrification and denitrification via nitrite were investigated, without strict control of solids retention time. Changes in the microbiological community and distribution in the reactor were monitored simultaneously. The intermittent-aeration strategy proved effective in achieving nitrition and the chemical oxygen demand (COD) to total nitrogen (TN) ratio was an important factor affecting TN removal. In the MBMBR, the nitrite accumulation rate reached 79.4% and TN removal efficiency averaged at 87.8% with aeration 2 min/mix 4 min and an influent COD/TN ratio of 5. Batch tests indicated that under the intermittently aerated mode, nitrite-oxidizing bacteria (NOB) were not completely washed out from the reactor but NOB activity was inhibited. The intermittently aerated mode had no effect on the activities of ammonia-oxidizing bacteria. Fluorescence in situ hybridizations (FISH) results also suggested that NOBs remained within the system.

1. Introduction

Simultaneous nitrification and denitrification (SND) means that nitrification and denitrification occur concurrently in the same reaction vessel under identical operating conditions. SND has become an attractive technology for nitrogen removal, due to its potential to eliminate the need for separate tanks, required in conventional treatment plants, inducing a simplified and smaller design. The traditional biological nitrogen removal processes involve the oxidation of ammonium (NH_4^+–N) to nitrate (NO_3^-–N) (nitrification) and then reduction with an organic carbon source (chemical oxygen demand, COD) to nitrogen gas (N_2) (denitrification). Both nitrification and denitrification involve nitrite (NO_2^-–N) as an intermediate. Hence, if SND is accompanied by the inhibition of the second step of nitrification (oxidation of nitrite to nitrate), theoretically many advantages over conventional SND could be achieved, including: (1) a 25% reduction in aeration and 40% reduction of COD demand during denitrification, (2) 63% higher rate of denitrification, (3) 300% lower biomass yield during anaerobic growth, and (4) no apparent nitrite toxicity effects for the microorganisms in the reactor [1]. This is of particular interest when biologically removing nitrogen from wastewater with a low COD/TN ratio.

However, the difficulty in removing nitrogen via nitrite lies in achieving specific inhibition or removal of the nitrite oxidizing-bacteria (NOB; those that oxidize nitrite to nitrate) while retaining ammonia oxidizing-bacteria (AOB; those that oxidize ammonia to nitrite), thereby attaining nitrition. At present, most studies achieve nitrition by controlling a number of operational parameters, such as the free ammonia (FA) concentration, the free hydroxylamine (FH) concentration, the pH, the temperature, and the dissolved oxygen (DO) concentration, which have effects on the transient build-up of the nitrite ion [2,3]. Many studies have claimed to achieve nitrition but some crucial problems have not been resolved. For example, (1) the SHARON process was the most mature process for achieving nitrition, but because of its strict operational conditions (30–40 °C, solids retention time (SRT) 1–3 d), it can only be used for a few special wastewater treatments (e.g., sludge-digestion liquid) and is not suitable for municipal wastewater and most industrial wastewater [4]. (2) Low DO concentration would not only affect the rate of nitrification but could also result in sludge bulking [5]. (3) If NOBs cannot be washed out rapidly from the system, they may adapt to the high FA level because of aberrance and, thus, SRT selection remains a problem.

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DO concentration is a key factor affecting nitrition. Under low DO concentration, AOBs have been suggested to out-compete NOBs, based on the higher oxygen affinity of AOBs compared with NOBs [6]. Cecen and Gonenc [7] reported that nitrite accumulation reached a considerable degree at DO to FA concentration ratios lower than 5 during nitrification, and the formation of nitrate was inhibited. No nitrite occurrence was encountered when this ratio exceeded 5, which implies that oxygen limitation leads to nitrite accumulation. Also, some processes allow for simultaneous nitrogen oxidation and reduction, likely to occur via nitrite at low DO concentrations, such as the OLAND process and the CANON process [8,9]. Many factors could affect the selection of DO, such as the ammonium–nitrogen concentration, COD/TN ratio, oxygen mass transfer resistance, amongst others. Too low DO may affect the rate of nitrification, while overly high DO may affect the accumulation of nitrite and result in energy waste.

In recent years, some reports have indicated alternating aerobic and anoxic conditions resulting from intermittent aeration may induce nitrition [10,11]. Yoo et al. [12] achieved nitrogen removal utilizing SND via nitrite in a proposed intermittently aerated cyclic activated – sludge single-reactor process, and suggested some dominative parameters for effective operation. Nowak et al. [13] reported that under anoxic conditions, the decay rate of AOB was zero, while the decay rate of NOB was invariable, almost equaling that under aerobic conditions. In theory, intermittent aeration in the bioreactor has a high probability of resulting in a low DO condition, which would benefit the multiplication of AOB and the accumulation of nitrite. The inhibition of the second step of nitrification (oxidation of nitrite to nitrate) was achieved because of the lag-time in nitration [1]. Also, the anoxic condition caused by mixing time would be beneficial to denitrification via nitrite.

The aim of this study was to achieve nitrogen removal by SND via nitrite in an intermittently aerated moving bed membrane bioreactor (MBMBR), without strict control of SRT. Some dominative parameters for effective operation were selected and the nitrification characteristics investigated. We adopt a two-step nitrification model to evaluate the behavior of both AOB and NOB in the intermittently aerated conditions. Temporal variations in the microbiological community and distribution in the reactor were simultaneously monitored.

2. Materials and methods

2.1. Experimental set-up and operating conditions

Fig. 1 shows a schematic of the experimental apparatus. The reactor was made of plexiglas, with a working volume of 30 L. Temperature was thermostatically controlled at 25 °C. A balance-box with a float-ball valve was used to control the water level. Polypropylene hollow-fiber membranes (Hangzhou Kaihong, China) with a pore size of 0.1 μm and a filtration area of 0.4 m^2 were used. A piece of clapboard with holes was fitted into the MBMBR to divide the reactor into two sections, with a volume ratio of 4:1. Carriers (30% v/v) were placed into the larger section and the membrane module was fixed in the smaller one. A nonwoven carrier was used in the MBMBR. The density of the carriers was 0.27 g/cm^3 and the effective specific surface area was 900 m^2/m^3. The clapboard was added to avoid the suspended carriers accumulating around the membrane module. The intermittently aerated mode was actualized through the modulation of the air pump and the agitator intermittently controlled by a timer-controlled power supply system. During the aerobic phase, the specific aeration demand per membrane area (SADm) was 0.75 m^3/m^2 h.

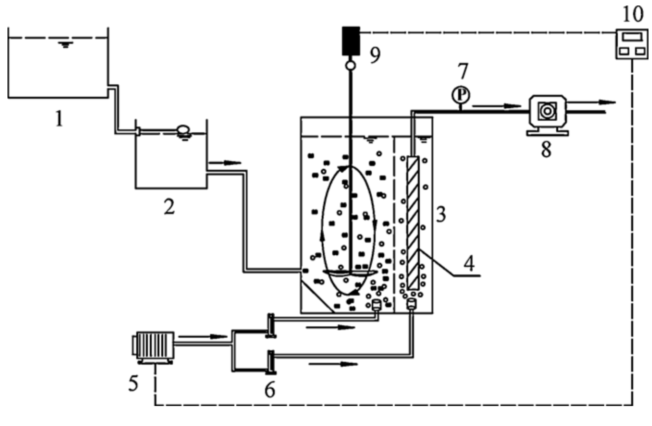

Fig. 1. Schematic of the experimental apparatus. (1) Wastewater reservoir, (2) balance-box, (3) MBMBR, (4) membrane module, (5) air pump, (6) rotameter, (7) vacuum gauge, (8) peristaltic pump, (9) agitator and (10) timer.

The MBMBR was maintained in continuous operation for about 6 months. The variations in operational parameters are summarized in Table 1. The SRT was maintained at 15 days by periodically removing sludge mixed liquor.

The MBMBR was inoculated with activated sludge taken from the secondary settling tank of a municipal wastewater treatment plant (Chun-liu, China). Synthetic wastewater fed to the reactor consisted of sodium acetate, NH_4Cl, KH_2PO_4 and mineral solution containing $MgSO_4$ $7H_2O$ (25 mg/L), $CaCl_2{\cdot}2H_2O$ (22 mg/L), $FeSO_4{\cdot}2H_2O$ (20 mg/L) and NaCl (25 mg/L). The initial influent contained 400 mg COD/L, 30 mg $NH_4{}^+$–N/L and 4 mg $PO_4{}^{3+}$–P/L. The pH in the reactor was maintained at 7.6–8.5.

2.2. Analytical methods

COD, ammonia nitrogen ($NH_4{}^+$–N), nitrate nitrogen ($NO_3{}^-$–N), nitrite nitrogen ($NO_2{}^-$–N), mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), and sludge volume index (SVI) were analyzed according to standard methods for the analysis of water and wastewater [14]. A certain amount of carriers were taken out from the bioreactor and placed into a beaker with deionized water of 500 mL. The carriers were then stirred with a magnetic stirrer for 60 min to wash out the biomass fixed within the carriers. The suspension was dried and weighed to calculate the concentration of the biofilm in the MBMBR. DO and pH in the reactor were measured by a DO meter (YSI 55/12 FT, USA) and a pH meter (Sartorius PB-10, Germany), respectively (Aqualytic). TN was determined based on the sum of $NH_4{}^+$–N, $NO_2{}^-$–N and $NO_3{}^-$–N, rather than an independent TN test.

2.3. Fluorescence in situ hybridization

The composition and spatial structure of the microbial community in the reactor (including the biofilm and the suspended biomass) were analyzed by fluorescence in situ hybridizations (FISH). FISH were performed according to the method described by Hibiya et al. [15]. The microbial samples were dispersed into individual cells by ultrasonication, and placed in a hybridization well on a gelatin-coated microscopic slide. NSO190 targeted halophilic and halotolerant β-proteobacterial AOB [16]. Ntspa662 and Nit3 are specifically used to target *Nitrospira* and *Nitrobacter* [17,18]. After hybridization, the microbial samples on the slides were examined using an epifluorescence microscope (OlympusBX51, Japan) together with the standard software package supplied with the instrument (version 4.0).

2.4. Batch tests

A series of batch tests were conducted to assess the nitrification characteristics under the intermittently aerated mode. Here, suspended biomass and biofilm were used for batch tests together, with the ratio of the two fractions adopted according to the state at that time in the bioreactor. The activated sludge sample was centrifuged at 3000 rpm for 10 min, washed three times and then diluted to about 3000 mg/L with deionized water. Sodium acetate, NH_4Cl and KH_2PO_4 were added to the mixed liquor to give the desired concentrations of COD, $NH_4{}^+$–N and $PO_4{}^{3+}$–P. During batch tests, the solution pH was maintained at 7.5–8.5 using $NaHCO_3$. Liquid samples were intermittently removed to analyze COD, $NH_4{}^+$–N, $NO_2{}^-$–N and $NO_3{}^-$–N.

Test a: The initial COD and $NH_4{}^+$–N concentrations were 125 mg/L and 50 mg/L, respectively. An appropriate amount of carbon source was added every 60 min to maintain the COD at about 100 mg/L to explore the characteristics of nitrification with the COD/TN ratio ranging from 2.5 to 5.

Test b1: The initial $NH_4{}^+$–N concentration was 40 mg/L, without carbon source addition. The intermittently aerated mode was adopted during the test in order to compare with test b2.

Test b2: The initial $NH_4{}^+$–N concentration was 40 mg/L without carbon source addition. The continuously aerated mode was adopted during the test.

2.5. Two-step nitrification model

A two-step nitrification model describing the batch tests (test b1 and test b2), with and without aerobic mode control, was adopted to evaluate the nitrition experiments [19]. Nitrification was split into two sub-processes: ammonium oxidation and nitrite oxidation, which is a different approach from other models (e.g., ASM [20]), where nitrification is considered as a single-step process. We use the model equations as follows:

Monod-based growth kinetics for AOBs:

$$\mu_{AOB} = \mu_{AOB}^{MAX} \frac{S_{NH_4}}{K_{s,NH_4} + S_{NH_4}} \frac{S_o}{K_{o,AOB} + S_o} \qquad (a)$$

Monod-based growth kinetics for NOBs:

$$\mu_{NOB} = \mu_{NOB}^{MAX} \frac{S_{NO_2}}{K_{s,NO_2} + S_{NO_2}} \frac{S_o}{K_{o,NOB} + S_o} \qquad (b)$$

where μ_{AOB}, μ_{NOB} represent growth rate of AOB and NOB, respectively (d^{-1}); S_{NH_4} represents ammonium–nitrogen concentration (mg N/); S_{NO_2} represents nitrite–nitrogen concentration (mg N/L); K_{S,NH_4} and K_{S,NO_2} represent substrate half saturation constants with respect to ammonium and nitrite, respectively (mg N/L); $K_{o,AOB}$ and $K_{o,NOB}$ represent oxygen half saturation constants of AOB and NOB, respectively (mg O_2/L); S_O represents oxygen concentration (mg O_2/L).

The key assumptions in the model include:

(1) Denitrification was not considered in the model because no carbon source was added during the test.
(2) Ammonification was not included in the model; only inorganic nitrogen was considered.
(3) The assimilation of ammonium for cellular growth was not included in the model because the growth of heterotrophic bacteria was not considered in the model and the influence of cellular assimilation of ammonium would be the same for both nitrifying bacterial groups, hence not favoring either group.
(4) Decay or lysis of bacteria was not considered in the model because the decay rates of AOB and NOB were assumed to be identical, thus not favoring either group predicted by this model.

The default model parameters are shown in Table 2. According to Blackburne et al. [21], in the model, the higher mass transfer affected the K_s value used for AOBs compared with the K_s value

Table 1
Operational parameters in the experiment.[a]

Phase	Operational days (day)	COD_{in} (mg/L)	TN_{in} (mg/L)	COD/TN	HRT (h)	Biofilm (mg/L)	Suspended MLSS (mg/L)	MLVSS/MLSS	Cycle time (min)	
									Aerobic	Anaerobic
I	1–19	235.8 (27.2)	42.2 (10.2)	5.6	16	886	3080	0.86	Continuous	–
II	20–40	229.7 (36.3)	42.4 (9.4)	5.4	16	935	3174	0.90	2	2
III	41–127	215.2 (34.5)	42.8 (7.5)	5.0	16	1062	3236	0.90	2	4
IV	128–175	214.2 (26.7)	57.5 (7.9)	3.7	16	1125	3428	0.92	2	4

[a] Standard deviation is given between parentheses; COD_{in}, influent COD concentration; TN_{in}, influent TN concentration.

Table 2
Default model parameters.

Model parameter	Value	Reference
$K_{s,AOB}$	0.75 mg N/L	[22]
$K_{s,NOB}$	0.15 mg N/L	[23]
$K_{o,AOB}$	0.03 mg O_2/L	[19]
$K_{o,NOB}$	0.4 mg O_2/L	[19]
μ_{AOB}^{MAX}	0.65 days^{-1}	[21]
μ_{NOB}^{MAX}	0.65 days^{-1}	[21]

for NOBs, which was chosen as a value corresponding to the situation where mass transfer effects are largely ignored. Ammonium must diffuse from bulk liquid into flocs before being used by AOBs, whereas, nitrite is produced within flocs and therefore has a much smaller diffusion distance before reaching the NOBs.

3. Results and discussion

3.1. Performance of the MBMBR

3.1.1. Organic substance removal

Fig. 2 presents the variations in COD concentration and its removal efficiencies in the intermittently aerated MBMBR for the entire experimental period. The bioreactor performed well on organic carbon removal. The effluent COD concentration averaged 14.9 mg/L and COD removal efficiencies averaged 93.2%. The results indicate that changes in aerobic duration and COD/TN ratio in the influent exhibited virtually no influence on COD removal.

3.1.2. Nitrogen removal

The long term operational period for the two systems comprised two steps. The first step was the start-up period, with the MBMBR aerated continuously to evaluate the system performance in complete aerobic condition (phase I). During the second step, the intermittently aerated mode was adopted to investigate the effect of aerobic duration/anoxic duration on NH_4^+–N and TN removal efficiencies (phases II–IV). In order to elucidate the extent of short-cut nitrification, the nitrite accumulation rate (NAR) was defined as follows:

$$\mathrm{NAR} = \frac{\text{effluent } NO_2{}^-\text{–N}}{\text{effluent } NO_2{}^-\text{–N} + \text{effluent } NO_3{}^-\text{–N}} \times 100\% \qquad (c)$$

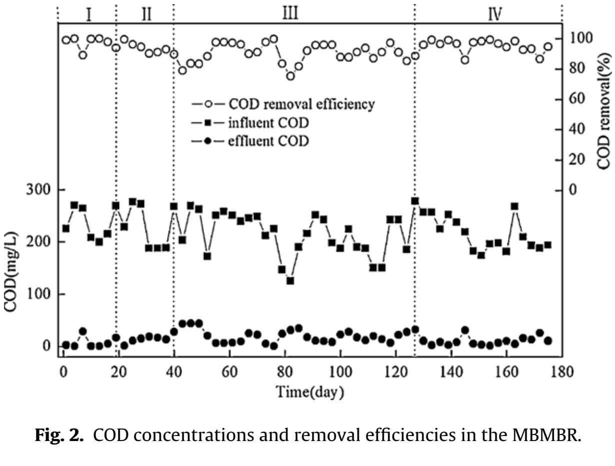

Fig. 2. COD concentrations and removal efficiencies in the MBMBR.

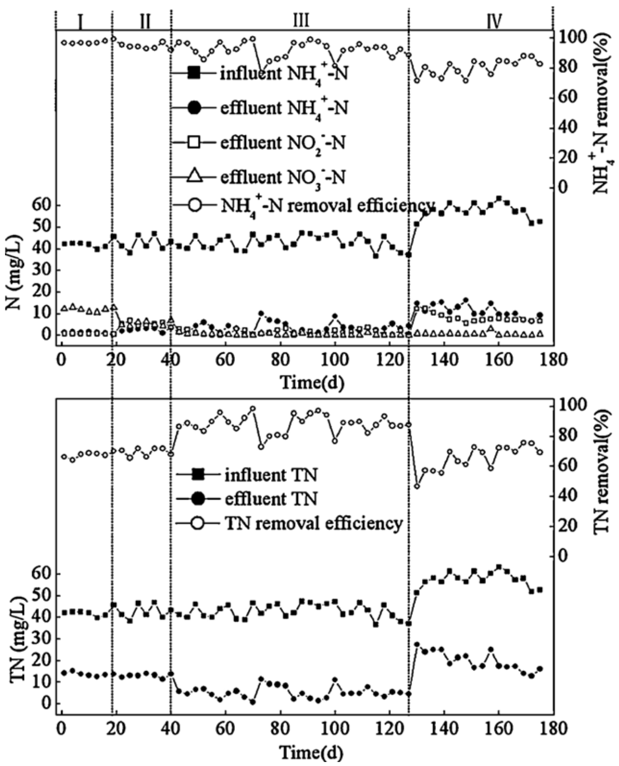

Fig. 3. NH_4^+–N, NO_2^-–N, NO_3^-–N, TN concentrations and removal efficiencies in the MBMBR.

Fig. 3 illustrates the variations in NH_4^+–N and TN concentrations as well as their removal efficiencies in the MBMBR throughout the experiment. It can be seen that in phase I, the NH_4^+–N removal efficiency averaged 97.0% and the average effluent NH_4^+–N concentration was 1.25 mg/L. The influent NH_4^+–N was almost completely removed, whereas the average TN removal efficiency was only 67.6% and the effluent TN concentration averaged 13.67 mg/L. The NAR was only 4.5%, which indicates that the nitrification was full-range nitrification and the main product was nitrate. In phase II, the intermittently aerated mode (aeration 2 min/mix 2 min) was adopted, the effluent NH_4^+–N concentration showed a slight increase and averaged 2.53 mg/L, while the average NH_4^+–N removal efficiency decreased to 94.0%. The average TN removal efficiency increased to 69.5% and the NAR increased to 49.1%. Although the TN removal was not significantly improved, the nitrite accumulation was achieved gradually under the intermittently aerated mode, which indicates that the intermittently aerated mode was an effective approach to controlling the nitrification to stop at nitrition. With the adjustment of the intermittent time, for phase III (aeration 2 min/mix 4 min), the TN removal efficiency averaged 87.8% and the average effluent TN concentration was 5.43 mg/L, which indicates that lengthening the mixing time is effective in improving TN removal. The NAR increased to 79.4% and the average effluent NO_3^-–N concentration was only 0.35 mg/L. At the same time, the change in anoxic duration did not have an obvious influence on NH_4^+–N removal, with the NH_4^+–N removal efficiency averaging 91.8% and the average effluent NH_4^+–N concentration being 3.49 mg/L. In order to explore the performance of the short-cut nitrification at a lower COD/TN ratio, the influent NH_4^+–N concentration was increased to 57 mg/L during phase IV. The NH_4^+–N removal efficiency decreased to 80.3% and the average effluent NH_4^+–N concentration was 11.32 mg/L. In this phase, aeration 2 min/mix 4 min could not supply sufficient DO concentration for NH_4^+–N removal. Simultaneously, the TN removal efficiency deceased to 65.5%, with an average effluent TN concentration of 19.8 mg/L, which indicates that the carbon source was insufficient for denitrification at a low COD/TN ratio of 3.8. The NAR increased to 93.3% due to the high nitrogen load. It can be concluded that the intermittent aeration time is an important factor for achieving short-cut nitrification and that the COD/TN ratio is another key factor for achieving TN removal. In the MBMBR, aeration 2 min/mix

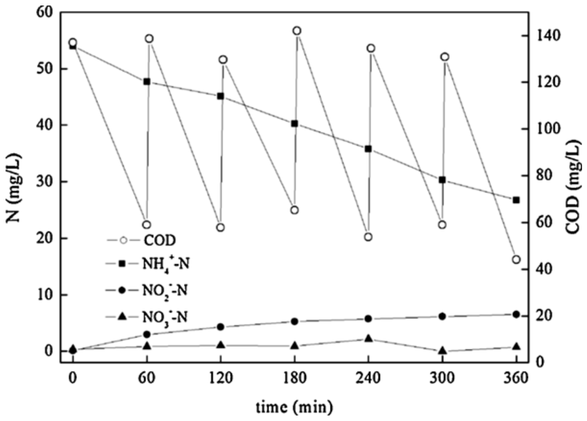

Fig. 4. NH_4^+–N, NO_2^-–N, NO_3^-–N and COD profiles during test a.

4 min is a suitable duration for simultaneous COD and TN removal at an influent COD/TN ratio of 5.

3.2. Specific nitrification rate test

In order to explore the characteristics of nitrification and TN removal under the intermittently aerated mode, batch test a was carried out on day 137. It can be seen from Fig. 4 that the profile of NH_4^+–N declined progressively with time during the whole test and the average nitrification rate was 4.55 mg/L h. At the end of the test, the nitrite was 6.53 mg/L but nitrate was only 0.71 mg/L, which indicates that intermittent aeration is an effective approach for achieving nitrition. It is also noted that from 0 to 60 min, as the COD/TN ratio changed from 2.54 to 1.24, nitrite rapidly increased to 2.95 mg/L. At the 61st min, the COD/TN increased to 2.91 as carbon source was added. From 61 to 120 min, the COD/TN changed from 2.91 to 1.28 and the nitrite concentration increased to 4.28 mg/L. In the second 60 min reaction, the accumulated nitrite concentration was 1.33 mg/L, which was less than that during the initial 60 min reaction (2.95 mg/L). A similar situation could also be observed in the subsequent reaction. In other words, with a gradual decrease in the NH_4^+–N concentration, the average COD/TN ratio increased and most of the nitrification product (nitrite) could be simultaneously removed. This observation suggests that under intermittent aeration, the COD/TN ratio remains an important factor affecting TN removal.

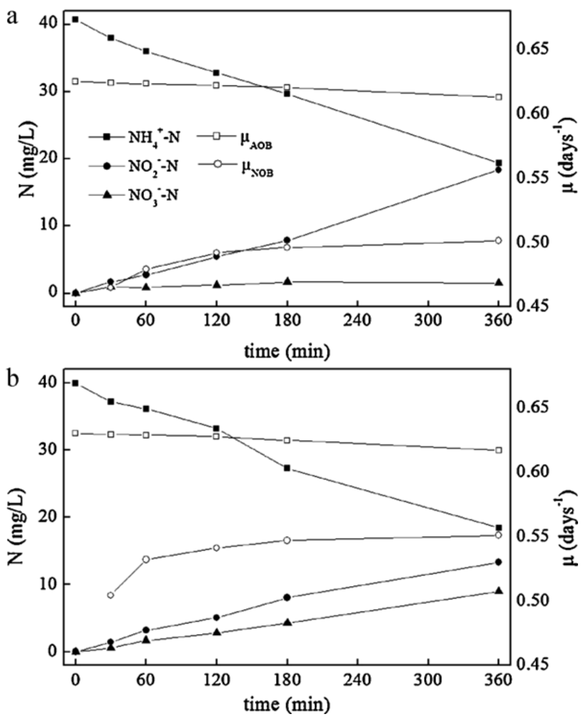

Fig. 5. NH_4^+–N, NO_2^-–N, NO_3^-–N, COD, μ_{AOB} and μ_{NOB} profiles for test b1 (a) and test b2 (b).

3.3. Characteristics of nitrification with intermittent aeration

To explore the cause for nitrite accumulation under intermittent aeration, two parallel batch experiments were carried out on day 141. Test b1 was operated under the intermittently aerated mode and test b2 was operated under the continuously aerated mode. It can be seen from Fig. 5 that for test b1, NO_3^-–N was very low throughout the entire test and NO_2^-–N accumulated gradually. At the end of the test, the NAR had increased to 92.2%. On the other hand, for test b2, the NO_3^-–N remained below 2 mg/L during the first 120 min, then NO_3^-–N increased gradually during the subsequent 240 min. At the end of the test the NAR was reduced to 59.6%. This observation indicates that NOBs had not been washed out from the reactor but remained in the system. However, the activities of the NOBs were inhibited under the intermittently aerated mode.

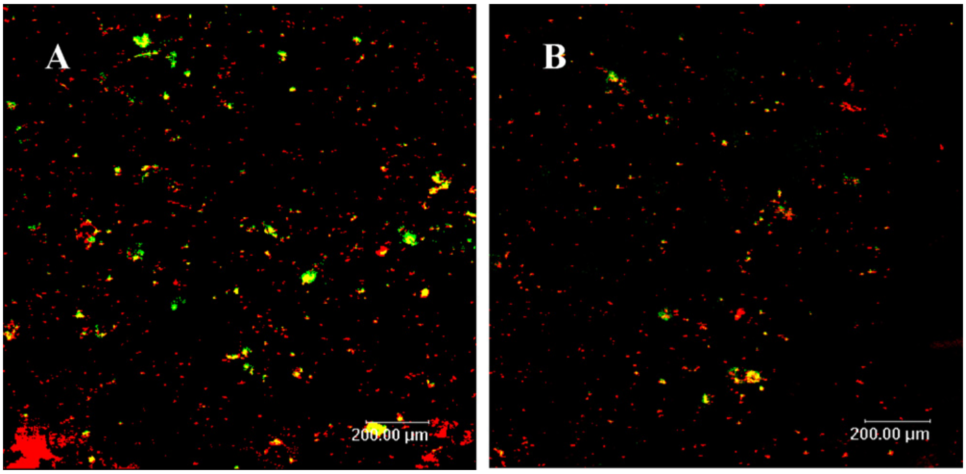

Fig. 6. FISH micrographs of microbial samples, with a CY3-labeled NSO190 (red) probe, a FITC-labeled Ntspa662 (green) probe and a FITC-labeled Nit3 (green) probe. (A) Suspended biomass sample taken on day 12 and (B) suspended biomass sample taken on day 137. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Hence, at the beginning of test b2, the NO_3^-–N remained very low but with continuous aeration, the activities of the NOBs recovered gradually and the NO_3^-–N concentration increased.

The values of μ_{AOB} and μ_{NOB} during the batch tests are also presented in Fig. 5. It can be seen that in test b1, μ_{NOB} slightly increased during the initial 120 min but it remained below 0.50. The NO_3^-–N concentration was very low during the test b1. For test b2, during the initial 30 min, the value of μ_{NOB} was below 0.50 and NO_3^-–N did not accumulate. After about 60 min, the value of μ_{NOB} increased to 0.53 and the NO_3^-–N concentration began to accumulate simultaneously. Then the value of μ_{NOB} increased smoothly and reached 0.55 by the end of the experiment. The value of μ_{NOB} in test b2 was clearly higher than that in test b1, implying that the activities of the NOBs recovered under continuous aeration. In contrast, the values of μ_{AOB} were almost constant during the whole experiment period for both tests, suggesting that the change in aeration could not affect the activities of the AOBs. Kornaros et al. [24] also reported that the AOBs did not exhibit any impact following the anoxic disturbance, while the NOBs were seriously inhibited.

3.4. The microbiological community and distribution in MBMBR

Suspended biomass samples were taken from the reactor on days 12 (Fig. 6A) and 137 (Fig. 6B). To assess the composition of the biofilm cultured on the non-woven materials in steady state, FISH was performed with the 16S rRNA targeting oligonucleotide probes NSO190, Ntspa665 and Nit3. NSO190 targeted halophilic and halotolerant β-proteobacterial AOB, Ntspa665 and Nit3 targeted *Nitrospira* and *Nitrobacter*, respectively. At the beginning of the experimental period, without control of aeration, the suspended biomass consisted of AOB reacting with NSO190, and NOB reacting with Ntspa665 and Nit3. AOB and NOB accounted for 54 ± 5% and 48 ± 5% of the total biomass, respectively. After long term control of intermittent aeration, the NOB percentage was significantly reduced, accounting for about 26 ± 5% of the total biomass (Fig. 6B). The results indicate that under short SRT conditions, some NOBs could be washed out from the system but they could not be completely eliminated, as a small amount could be observed. Combined with the results described in Section 3.3, one may conclude that under the intermittently aerated mode, shortcut nitrification was achieved by inhibiting the activities of the NOBs, not by their removal.

4. Conclusion

An intermittently aerated MBMBR was investigated to achieve SND via nitrite. Results demonstrated that intermittent aeration was an effective approach to achieve nitrition and the COD/TN ratio is another key factor affecting TN removal. Batch tests indicated that under the intermittently aerated mode, NOBs were not completely washed out from the reactor but remained in the system. However, the activities of NOBs were inhibited and their activities could recover under subsequent continuous aeration. The changes in aeration had no effect on the activities of AOBs. FISH results proved that NOBs could also be observed in the intermittently aerated bioreactor.

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