Research Article

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Specific ammonium oxidation and denitrification rates in an MBR treating real textile wastewater for simultaneous carbon and nitrogen removal

Tülay Yilmaz,[a,b*] Emir Kasım Demir,[b,c] Senem Teksoy Başaran,[b,c] Emine Ubay Çokgör[a] and Erkan Sahinkaya[b,c*]

Abstract

BACKGROUND: Dissolved oxygen (DO) and aeration on/off time are the determining parameters for simultaneous carbon and nitrogen removal in biological treatment processes. The most effective way to decide the optimum operational conditions, e.g. aeration on/off time or DO concentration during the aeration period, is to determine specific nitrification/denitrification rates. For this purpose, the effects of DO (6 and 3 mg L^{-1}) and aeration on/off time (from 2/2 to 90/360 min) on the specific ammonium oxidation and denitritation/denitrification rates in a membrane bioreactor (MBR) treating real textile wastewater were deeply investigated.

RESULTS: The highest specific ammonium oxidation, denitritation and denitrification rates were obtained as 5.4, 3.8, and 5.3 mg N g^{-1} volatile suspended solids h^{-1}, respectively, at an aeration on/off time of 90/360 min, which corresponded to the increase in specific ammonium oxidation rates by 1.8 and 2.1 times compared to continuous aeration conditions where DO was 6 and 3 mg L^{-1}, respectively.

CONCLUSION: Higher specific ammonium oxidation and denitrification rates can be achieved with the intermittent aeration compared to continuous aeration. Hence existing treatment plants can be retrofitted for higher performance with reduced energy requirements.

Keywords: intermittent aeration; nitrogen removal; specific denitrification rate; specific ammonium oxidation rate; membrane bioreactor

INTRODUCTION

Nitrogen-derived substances in wastewater cause significant problems in the receiving environment, and great efforts are put into nitrogen removal from domestic and industrial effluents. In conventional nitrogen removal processes, organic nitrogen/ammonium (NH_4^+-N) must first be bio-oxidized to nitrite/nitrate under aerobic conditions through the nitrification process, and in the second stage nitrate/nitrate is converted to nitrogen gas under anoxic conditions through denitrification.[1] However, conventional systems involving both processes separately are known for their high operating costs due to high aeration requirements in the nitrification process and high amounts of sludge production during denitrification.[2] Another preferred approach to limit carbon and energy requirements, and hence increase process performance, is the nitritation–denitritation process,[3] where nitrite produced by partial ammonium oxidation is directly converted to N_2 gas during the denitritation process, reducing the carbon and aeration requirement by 40% and 25%, respectively, for nitrogen removal.[4] Hence achieving simultaneous nitrification/nitritation and denitritation/denitrification in a single reactor instead of sequential reactors provides significant operating cost reductions.[5]

Intermittent aeration is a promising process for effective simultaneous carbon and nitrogen removal from wastewater, particularly by creating sequential aerobic and anoxic/anaerobic conditions in a single reactor.[6] In a study by Hasar *et al*., the impact

* *Correspondence to: T Yilmaz, Environmental Engineering Department, Istanbul Technical University, Maslak, 34469 Istanbul, Turkey. E-mail: tulayylmaz@outlook.com; or E Sahinkaya, Department of Bioengineering, Istanbul Medeniyet University, Istanbul, 34700, Turkey. E-mail: erkan.sahinkaya@medeniyet.edu.tr*

a *Environmental Engineering Department, Istanbul Technical University, Istanbul, Turkey*

b *Science and Advanced Technologies Application and Research Center (BILTAM), Istanbul Medeniyet University, Istanbul, Turkey*

c *Department of Bioengineering, Istanbul Medeniyet University, Istanbul, Turkey*

of various aeration strategies (continuous; aeration on/off time of 30/30, 60/120, 60/90, 60/75 min) on the treatment performance of domestic wastewater in a membrane bioreactor (MBR) system was examined. Chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+-N) removal performances were 83.8–99.3%, 87.8–99.1% and 89.4–99.8%, respectively, throughout the study.[7] Habermeyer and Sánchez examined the optimization of the intermittent aeration process in a full-scale biological system treating domestic and industrial wastewater. Three different aeration on/off times of 45/40, 90/80, and 180/160 min were investigated at different DO intervals ranging from 0.7 to 2.5 mg L^{-1}. The optimal operating conditions for COD and nitrogen removal were obtained at an aeration on/off time of 180/160 min with DO of 1.5–1.7 mg L^{-1}.[8]

Optimization of operating conditions such as DO, hydraulic retention time (HRT), carbon/nitrogen (C/N) ratio, and oxic/anoxic cycle time is necessary for effective nitrification and denitrification processes in a single system.[9] The abundance of nitrifying bacteria and the ammonia oxidation rate are highly affected by environmental parameters such as temperature, ammonium, and DO concentrations.[10,11] In particular, the limited DO concentration in the system may affect the reproduction and metabolic activities of nitrifying bacteria.[12] In addition, proportional distributions of ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in biological processes having aerobic and anoxic cycles cause significant differences in nitrification rates.[13] Nitrogen oxides are used as electron acceptors by denitrifying bacteria,[14] and therefore denitrification rates are adversely affected in the presence of oxygen.[15] In batch studies, a decrease of 35% in the specific denitrification rate was observed even in the presence of 0.09 mg L^{-1} oxygen.[16] In general, the effects of nitrate and oxygen concentrations and pH on the denitrification rate are investigated for the control of the denitrification process.[17] In addition, the sizing of the biological pre-denitrification reactor depends on the denitrification rate.[18]

Many existing full-scale textile wastewater treatment plants are operated under aerobic conditions with a continuous aeration strategy.[19] These plants, having low nitrogen removal performance due to the absence of anoxic conditions, should be retrofitted for higher nitrogen removal. Changing the aeration strategy from continuous to intermittent may easily be applied, and significantly improve the plant performance in addition to decreased energy demand. Although studies have been conducted to elucidate the impact of aeration on/off time on the specific nitrification/denitrification rates of biomass for domestic wastewater treatment,[20,21] there are few, if any, studies on specific nitrification/denitritation/denitrification rates for real-textile wastewater treatment in an intermittently aerated MBR. It would be important for the operators and designers to learn about the effects of intermittent aeration on/off time and nitrite/nitrate accumulation on the specific rates and process efficiency. In the present study, we will provide and discuss the specific ammonium oxidation/denitritation/denitrification rates of the biomass obtained from the intermittently aerated MBR for which long-term performance data were recently published.[22]

MATERIALS AND METHODS

Biomass source

Biomass for batch ammonium oxidation/denitrification studies was obtained from a lab-scale MBR treating real textile wastewater under varying aeration strategies. The MBR achieved COD, color and total nitrogen (TN) removal efficiencies of 84–93%, 40–68% and 25–70%, respectively, depending on the operational conditions.[22] Biomass obtained from the MBR was directly used for the batch studies without making any concentration adjustments, and hence the biomass concentrations (760–3600 mg volatile suspended solids [VSS] L^{-1}) in different batch tests were as illustrated in Table 1.

Batch tests for specific ammonium oxidation rates

In order to determine variations in the specific ammonium oxidation rate, under each tested operational condition (C1–C8) (Table 1), 1 L homogeneous sludge sample was taken from the MBR and continuously aerated in a 2 L glass reactor until a constant DO concentration, which may close to saturation levels, was attained. After reaching stable DO, the tests were started by adding the synthetic concentrated wastewater to obtain initial concentrations of 50 mg NH_4^+-N L^{-1}, 44.6 mg KH_2PO_4 L^{-1} and $NaHCO_3$ as 500 mg $CaCO_3$ L^{-1} (as an alkalinity source) in the aerated batch reactors. The tests were carried out at room temperature (18–25 °C), and variations in NH_4^+-N, nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), COD, and alkalinity were followed by taking samples regularly.

In addition to these batch oxidation tests, the specific ammonium oxidation rates were also measured using a respirometric method based on the oxygen consumption rates of bacteria during the oxidation process. The nitrification reaction according to the United States Environmental Protection Agency is given in Eqn (1):[23]

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (1)$$

Table 1. Operating conditions and biomass concentrations in the tests

Operating conditions in MBR	Biomass (mg VSS L^{-1})		
	In the batch tests for specific ammonium oxidation rates	In the respirometric studies for specific ammonium oxidation rates	In the batch tests for denitritation and denitrification rates
C1 = DO of 6 mg L^{-1}	3310	3480	1655
C2 = DO of 3 mg L^{-1}	3600	2860	2210
C3 = 2 min on/2 min off	3550	3570	1610
C4 = 15 min on/15 min off	3390	2980	1695
C5 = 30 min on/30 min off	3290	3090	1690
C6 = 60 min on/60 min off	2270	2400	1180
C7 = 90 min on/90 min off	2680	2850	1340
C8 = 90 min on/360 min off	1660	1660	760

A BM-Advance respirometer (Surcis, Barcelona, Spain) was used for the measurement of dynamic oxygen uptake rate (R_S) (mg O_2 L^{-1} h^{-1}), specific dynamic oxygen uptake rate (R_{Sp}) (mg O_2 L^{-1} h^{-1}) and consumed oxygen concentration (CO) (mg L^{-1}). The test modes and parameters that can be measured in the BM-Advance respirometer are given by Cristóvão *et al.*[24] Sludge taken from each corresponding MBR operating condition (Table 1) was placed in a respirometric chamber and aerated continuously until a stable DO value was obtained. After reaching stable DO concentration, the respirometric test was initiated with the addition of 50 mL distilled water containing 50 mg NH_4^+-N L^{-1} to determine the R_S, R_{Sp} and CO continuously in the R-mode throughout the test. In the calculation of the theoretical oxygen consumption, biomass growth was excluded, and the oxygen requirements for nitrite and nitrate generation from ammonium were considered as 3.42 mg O_2 mg^{-1} NO_2^--N and 4.57 mg O_2 mg^{-1} NO_3^--N, respectively. The possibility of simultaneous nitrification and denitrification in the calculations was neglected.

Batch tests for specific denitritation/denitrification rates

In order to investigate specific denitritation and denitrification rates, batch reactors (eight batch reactors for each MBR operational condition C1–C8) with an active volume of 100 mL were set up with sludge taken from the MBR. Before starting the tests, the sludge was rinsed with tap water to remove all organic and inorganic matter attached to the biomass and kept in a sealed bottle for 1 day to allow the microorganisms to adapt to the anoxic condition.

Batch tests were initiated by adding 50 mL sludge to the batch reactors. The batch assays between R1 and R4 were operated in parallel at different nitrite or nitrate concentrations (Table 2). No sludge was added to R5 and R6 to investigate whether the nitrogen removal process was biological. In addition, external organic matter was not added to R7 and R8 in order to determine the nitrate and nitrite removal rates due to endogenous respiration of bacteria, and parallel studies for R5, R6, R7, and R8 were not carried out (a total of 12 batch reactors for each condition). The COD/TN ratio was kept at 6.0 to avoid organic matter limitation in the tests, using acetate as the carbon source in all tests and considering the denitrification/denitritation reactions provided in Eqns 2 and 3:[25]

$$0.25CH_3COO^- + NO_3^- \rightarrow NO_2^- + 0.25HCO_3^- + 0.25H_2O + 0.25CO_2 \quad (2)$$

$$0.375CH_3COO^- + NO_2^- + 0.625H^+ \rightarrow 0.5N_2 + 0.75HCO_3^- + 0.5H_2O \quad (3)$$

Table 2. Operating conditions of the batch reactors for determining specific denitritation and denitrification rates

Reactor No.	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	COD (mg L^{-1})	Biomass (mL)
R1	0	25	150	50
R2	0	50	300	50
R3	25	0	150	50
R4	50	0	300	50
R5	0	25	150	0
R6	25	0	150	0
R7	0	25	0	50
R8	25	0	0	50

In addition, inorganic media given by Sahinkaya and Dilek[26] was also added to the batch reactors in order not to limit the microbial growth.

Denitrification efficiency is affected by many parameters, such as nitrate concentration, temperature, type and concentration of carbon source, and the presence of DO in the system.[15] The sludge was kept sealed for 1 day before the tests to reduce the DO level. All batch tests were conducted at 25 °C in an incubator, and the nitrogen removal rates were determined by monitoring the variations in nitrate, nitrite, and COD concentrations with time.

Analytical methods

COD, alkalinity, suspended solid (SS), and VSS concentrations in the batch tests were followed according to Standard Methods.[27] NO_2^--N and NO_3^--N were measured with a Dionex ICS-5000+ (Thermo Fisher Scientific, Waltham, MA, USA) ion chromatograph equipped with an AS9-HC column. A multimeter (WTW 3420, Xylem Analytics, Weilheim, Germany) was used for DO measurements in the batch reactor. NH_4^+-N concentrations were measured using HACH kits (Hach Company, Loveland, Colorado). Respirometric studies were performed using the BM-Advance respirometer (Surcis). Free ammonia (FA), nitrite accumulation rate, and ammonium oxidation efficiencies were calculated as described by Zhou *et al.*[28] Free nitrous acid (FNA) was determined as given by Asik *et al.*[29]

RESULTS AND DISCUSSIONS

Specific ammonium oxidation rates

It was aimed to determine the effect of different DO values and the aeration on/off time employed in an MBR[22] on the specific ammonium oxidation rate using batch tests (C1–C8) (Fig. 1). The ammonium oxidation efficiencies were above 99% in all batch tests at which DO concentrations were mostly >4 mg L^{-1} in order not to limit the nitrification process.

In the first batch tests conducted with the sludge from the MBR operated at 6 mg L^{-1} DO (C1), the specific ammonium oxidation rate was 3.1 mg N g^{-1} VSS h^{-1}. With a decrease of DO to 3 mg L^{-1} in the MBR (C2), the specific ammonium oxidation rate decreased to 2.6 mg N g^{-1} VSS h^{-1}. No significant changes were observed at the aeration on/off time of 2/2 min (C3) compared to C2, and the specific ammonium oxidation rate was determined as 2.3 mg N g^{-1} VSS h^{-1}. However, by increasing the aeration on/off time to 15/15 min (C4), the specific ammonium oxidation rate decreased to 1.3 mg N g^{-1} VSS h^{-1}. Under the following conditions, the aeration on/off durations were increased, and the specific ammonium oxidation rates were 2.6, 2.1, and 3.2 mg N g^{-1} VSS h^{-1} at the aeration on/off time of 30/30 min (C5), 60/60 min (C6) and 90/90 min (C7), respectively. Further increasing the aeration off time to 360 min at the last run (C8), the highest specific ammonium oxidation rate of 5.4 mg N g^{-1} VSS h^{-1} in the MBR system was obtained. Compared to the batch tests conducted with the sludge obtained from the continuously aerated MBR (conditions: C1 and C2), the batch test carried out with the sludge taken during condition C8, at which the longest aeration off time was performed in the MBR, resulted in approximately two times higher ammonium oxidation rate. These results agree with the literature, reporting that the nitrification rate increases nearly twofold in the case of intermittent aeration compared to continuous aeration due to the dominance of rapid nitrifiers (*Nitrosomonas* and *Nitrobacter*) under intermittent aeration condition rather than the slow nitrifiers (*Nitrosospira* and *Nitrospira*) under continuous-aeration condition.[1] In the two different

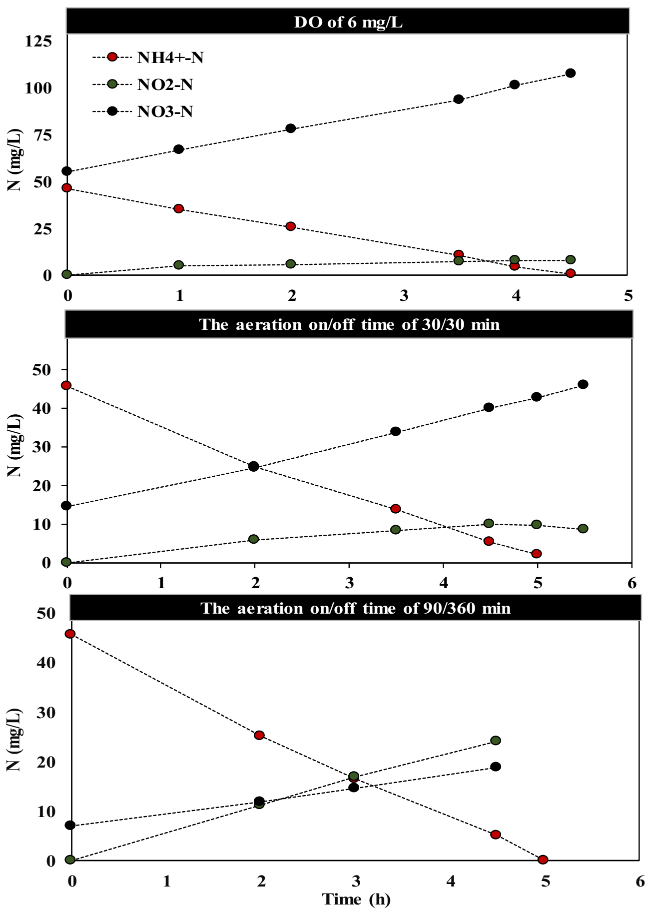

Figure 1. Example results from batch tests for specific ammonium oxidation rates. Condition 1 (upper figure), Condition 5 (middle figure), Condition 8 (bottom figure).

sequencing batch reactors (SBRs) operated under aerobic and alternating anoxic/aerobic conditions, the specific nitrification rates were 2.95 ± 0.26 and 6.16 ± 0.34 mg NH_4^+-N g^{-1} VSS h^{-1} under aerobic and alternating anoxic/aerobic conditions, respectively. One of the most important factors in this increase is that the anoxic cycle and the high nitrite concentrations create favorable conditions for *Nitrosomonas* and *Nitrobacter*, which rapidly grow at high substrate concentrations due to low substrate affinity (high half velocity constant, K_s).[30]

Szpyrkowicz *et al.* reported the nitrification rates as 0.01 and 0.05 mg N mg^{-1} MLSS (mixed liquor suspended solids) d^{-1}, at a solids retention time of 75 and 65 d and dissolved oxygen (DO) of 3.6 and 4.3 mg L^{-1}, respectively, during the treatment of textile dyeing wastewater in a full-scale extended aeration process.[31] The ammonium oxidation rates observed in our study are comparable with the rates reported in the literature for different wastewaters. Gao *et al.* (2004) reported that the specific nitrification rate ranged from 0.27 to 0.56 g NH_4^+-N g^{-1} SS d^{-1} in an aerobic MBR treating synthetic inorganic wastewater.[32] In the other study investigating landfill leachate treatment, the specific nitrification rate was between 0.043 and 0.154 kg NH_3-N kg^{-1} SSV d^{-1} in an SBR.[33] However, in the study by Ganesh *et al.* (2015), the specific nitrification rate was calculated to be high as 6.9 mg g^{-1} MLVSS (mixed liquor volatile suspended solids) h^{-1} during tannery wastewater treatment in the aerobic SBR (cycle and react phase durations were 12 and 10 h, respectively).[34]

The specific nitrate production rates also showed similar trends to the specific ammonium oxidation rate, except for the condition of aeration on/off time 90/360 min. The nitrate production rates were observed to be higher, at 3.5 and 2.3 NO_3^--N g^{-1} VSS h^{-1} at DO 6 and 3 mg L^{-1}, respectively, for the continuously aerated MBR sludge compared to that of intermittently aerated ones as it decreased significantly with increased off time (C3–C8) down to 1.6 NO_3^--N g^{-1} VSS h^{-1} at an aeration on/off time of 90/360 min. Interestingly, the specific ammonium oxidation rate reached its maximum for the tested sludge obtained at the longest aeration-off time (C8). The highest nitrite accumulation ratios observed were 29, 23, 26, and 67% in C4, C5, C6, and C8, respectively, while it was below 1% in C2 with continuous aeration. In the intermittent aeration process, nitrite accumulation occurs commonly due to quickly overcoming ammonium-oxidizing bacteria after a longer anoxic condition.[35,36] Turk and Mavinic observed that with the prolongation of aeration time, the negative effect on nitrite oxidizers was decreased, and the accumulated nitrite was further oxidized.[37] Hence intermittent aeration with long non-aeration times may eliminate nitrite oxidizers, and a partial nitrification process may become dominant.[1]

The nitrite accumulation and activities of AOB and NOB are highly influenced by important factors such as pH, temperature, DO levels, and the presence of FA and FNA.[38,39] A pH between 7.5 and 8.5 was reported as suitable for nitrite accumulation,[40] and the average pH of sludge used in nitrification tests was 8.4 ± 0.2. The DO varied between 4 and 8.8 mg L^{-1} throughout the tests, and nitrite accumulation is generally observed at lower oxygen concentrations.[39] The specific growth rates of AOB and NOB are temperature-dependent, and usually temperatures of 25 °C and above may be favorable for nitrite accumulation due to amplifying the differences in specific growth rates between AOB and NOB.[41,42] AOB and NOB can be affected at an FA range of 10–605 mg N L^{-1} and 0.1–5 mg N L^{-1}, respectively.[43,44] FNA concentration in the range 0.42–1.72 mg HNO_2^-N L^{-1} yielded a 50% decrease in the activity of AOB, whereas NOB are completely inhibited at 0.026–0.22 mg HNO_2^-N L^{-1}.[39] FA concentrations were below 6 mg L^{-1} except for C4 and C6, where maximum FA concentrations of 9 and 12.5 mg L^{-1} were calculated, respectively. FA concentrations were below 0.06 mg N L^{-1} at the end of all the tests. In this study, the FNA ranged from 0 to 0.00029 mg HNO_2^-N L^{-1} in batch tests for determination of the ammonium oxidation rate. Hence, in our study, observing partial nitrification after long non-aeration conditions was mostly due to the slower recovery of nitrite oxidizers from long anoxic conditions compared to ammonium oxidizers.[1] After low DO and a long anoxic period, AOB are less affected due to high oxygen affinity, while the activity of NOB decreases. After the transition from an anoxic condition to an aerobic condition, nitrite accumulation can be observed due to the faster adaptation of AOB to changing conditions and recession in the activities of NOB.[45]

Theoretically, 7.14 mg $CaCO_3$ alkalinity is consumed during the oxidation of 1 mg NH_4^+-N,[46] and the consumed alkalinity in the batch tests was very close to the theoretical alkalinity requirement. For example, the theoretical and the measured alkalinity consumptions were 330 and 300 mg $CaCO_3$ L^{-1}, respectively, at a DO of 6 mg L^{-1}. With an aeration on/off time of 90/90 min, the theoretical and the measured alkalinity consumptions were 373 and 400 mg $CaCO_3$ L^{-1}, respectively. COD removals ranged from 0 to 16% in the batch tests, except for C4 (aeration on/off time of 15/15 min), at which it was 27%. In the batch test of C4, the nitrification efficiency was the lowest in the MBR.[22] Low

Table 3. Specific ammonium oxidation rates in batch tests and respirometric studies

Conditions	Specific ammonium oxidation rate in the batch tests (mg N g^{-1} VSS h^{-1})	R_{Sp} in respirometer (mg O_2 g^{-1} VSS h^{-1})	Consumed oxygen in the respirometer (mg L^{-1})	Consumed oxygen calculated based on nitrite and nitrate produced (mg L^{-1})
C1 = DO of 6 mg L^{-1}	3.1	13.6	363	202
C2 = DO of 3 mg L^{-1}	2.6	11.7	340	222
C3 = 2 min on/2 min off	2.3	6.8	219	200
C4 = 15 min on/15 min off	1.3	6.0	295	269
C5 = 30 min on/30 min off	2.6	11.3	234	200
C6 = 60 min on/60 min off	2.1	13.0	237	235
C7 = 90 min on/90 min off	3.2	16.0	226	239
C8 = 90 min on/360 min off	5.4	8.1	170	181

COD removal in the batch assays is attributed to the non-biodegradable nature of the organics present in the tests, as no external organic matter was added in the batch tests, and the soluble COD came from the MBR liquor together with the biomass. It seems that the COD remaining in the bioreactor after MBR treatment is not further degraded in the batch tests even after long-term aeration. Hence, the MBR may provide high performance and the remaining COD may be regarded as non-biodegradable.[22]

Variations in specific ammonium oxidation rates in respirometric studies

Respirometric studies were performed with sludge from the MBR operated under varying conditions (Table 1). The results of respirometric studies were quite close to the batch tests (Table 3). The maximum R_{Sp} values were 13.6 and 11.7 mg O_2 g^{-1} VSS h^{-1} for a DO of 6 and 3 mg L^{-1}, respectively. In C3 and C4, testing sludge from the intermittent aeration phases, the maximum R_{Sp} decreased to 6.8 and 6.0 mg O_2 g^{-1} VSS h^{-1}, respectively. However, with the increase in aeration-on time, the maximum R_{Sp} also increased, and it was calculated as 11.3, 13.0, and 16.0 mg O_2 g^{-1} VSS h^{-1} for C5, C6, and C7, respectively. Under the last condition (C8) with the longest aeration-off time, the maximum R_{Sp} value decreased compared to previous periods and was 8.1 mg O_2 g^{-1} VSS h^{-1}.

In a study investigating the treatment of real municipal wastewater in an MBR operated under intermittent aeration, it was suggested that there was no clear relationship between the maximum specific oxygen uptake rate (SOUR) (5.7–26.57 mg O_2 g^{-1} VSS h^{-1}) and the ratio of aerobic to anoxic time for heterotrophic biomass, and a reduction in SOUR was reported even at the highest aeration on/off ratio of 0.8.[47] In the study investigating the treatment of simulated textile wastewater containing 1000 mg COD L^{-1} (COD/N/P mass ratio of 100:3.7:30) in an aerobic granular sludge SBR with intermittent aeration, SOUR was measured 40 and 16 mg O_2 g^{-1} MLVSS h^{-1} at the aeration start and end phase, respectively, at an aeration on/off time of 20/30 min (total of six cycles) (without dye feeding). It was reported that SOURs were considerably lower than those measured in previous periods without intermittent aeration.[48]

Theoretically, 228.5 mg oxygen is needed for the complete oxidation of 50 mg NH_4^+-N. A small part of the consumed ammonium is used for the synthesis of AOB and NOB, but this part was generally not taken into account in the calculations.[49] However, there is a higher consumption due to endogenous respiration. The amounts of consumed oxygen in our study were approximately 363 and 340 mg L^{-1}, respectively, at a DO of 6 and 3 mg L^{-1}. Higher consumption in these periods may be due to the higher endogenous respiration rates of aerobic heterotrophic bacteria. In C3 (aeration on/off time of 2/2 min), the amount of oxygen consumed was measured as 219 mg L^{-1} – very close to the theoretical value. However, at 15/15 min, the CO increased again and was measured as 295 mg L^{-1}. COs were 234, 237, and 226 mg L^{-1} in C5, C6, and C7, respectively. However, CO decreased to 170 mg L^{-1} at 90/360 min (C8) (Table 3). The reduced oxygen consumption was due to nitrite accumulation, which was evidenced by the batch assays (Fig. 2). The theoretical CO values calculated considering the nitrite and nitrate values produced at the end of the batch tests are given in Table 3. These values were quite close to the data obtained in the respirometric tests, especially when intermittent aeration was applied.

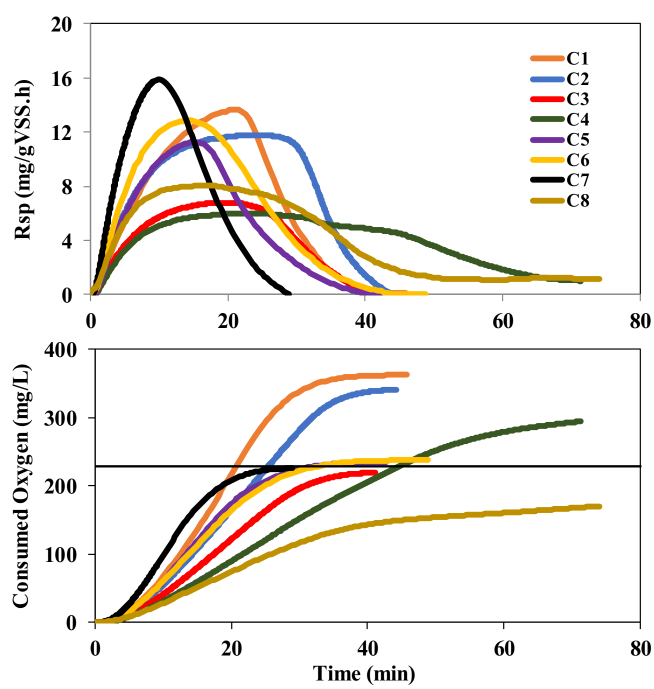

Figure 2. R_{Sp} and CO variations in respirometric studies for specific ammonium oxidation rates. The straight line in the lower figure shows the theoretical oxygen consumption of 228.5 mg L^{-1} O_2).

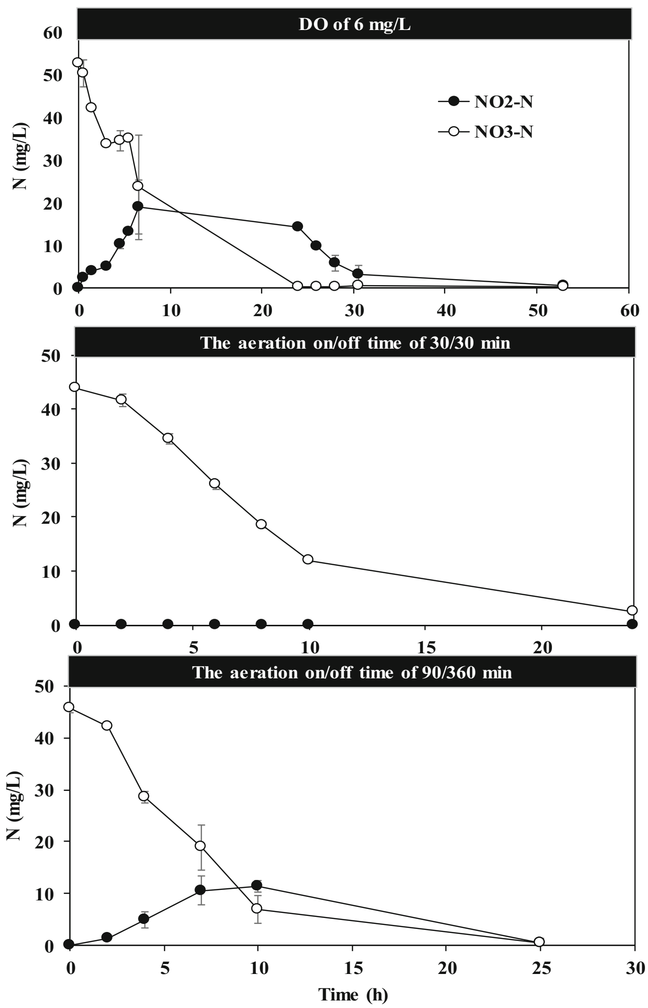

Figure 3. Example results from batch tests for specific denitrification rates for R2. Condition 1 (upper figure), Condition 5 (middle figure), Condition 8 (bottom figure).

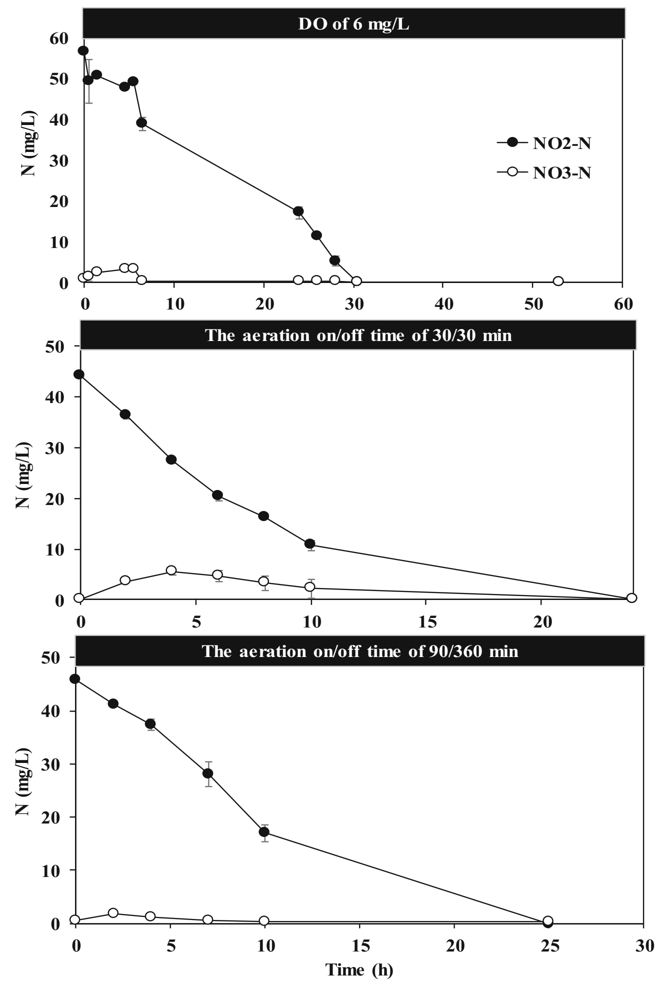

Figure 4. Example results from batch tests for specific denitritation rates for R4. Condition 1 (upper figure), Condition 5 (middle figure), Condition 8 (bottom figure).

Variations in specific denitritation and denitrification rates

In all batch experiments, it was aimed to determine the effects of intermittent aeration on denitritation and denitrification rates. Selected results from the batch tests are presented in Figs 3 and 4. In addition, all the rates calculated for the batch test are given in detail in Table 4. The specific denitritation and denitrification rates were quite low in the tests conducted with continuously aerated sludge at DOs of 6 and 3 mg L^{-1}. The denitrification rate partially increased with increasing the nitrate from 25 to 50 mg NO_3^--N L^{-1} (in R2), and it was 1.2 mg N g^{-1} VSS h^{-1} at a DO of 6 mg L^{-1}. However, the specific denitrification rate was 0.9 mg N g^{-1} VSS h^{-1} at a DO of 3 mg L^{-1} (in R2). Theoretically, the specific denitrification rate in sludge is expected to increase with decreasing DO concentration, although a partial decrease in the denitrification rate was observed. At an aeration on/off time of 2/2 min, the specific denitrification rate partially increased and reached approximately 1.1 mg N g^{-1} VSS h^{-1} (in R2). By increasing the on/off time to 15/15 min, the specific denitrification rate rose to 2 mg N g^{-1} VSS h^{-1}, with a corresponding increase of 46% compared to the highest rate obtained in the previous period. Similar trends were observed in the nitrite reduction rate and in the batch reactors operated with 50 mg L^{-1} NO_2^--N the specific denitritation rates were 1, 0.8, 0.6, and 1.7 mg N g^{-1} VSS h^{-1} in C1, C2, C3, and C4, respectively. By increasing the aeration on/off time to 30/30 min, no significant change in the specific denitrification rate was observed. However, the specific denitritation rate increased to 2 and 2.1 mg N g^{-1} VSS h^{-1} in R3 and R4 tests, respectively, for an on/off time of 30/30 min (C5).

At an aeration on/off time of 60/60 min, the specific denitrification rates increased to 1.5 and 3.3 mg N g^{-1}VSS h^{-1} in R1 and R2, respectively, while the specific denitritation rates were 2.1 and 3.2 mg N g^{-1} VSS h^{-1} in R3 and R4. Although the increase in all rates was expected to continue by increasing the aeration-off time to 90 min, the specific denitrification rates decreased partially, while the specific denitritation rates were similar to the previous tests. Similarly, the TN removal performance also decreased by increasing the on/off time to 90/90 min in the MBR, which was reported in our previous study.[22] The specific denitrification rates increased significantly at an on/off time of 90/360 min, and they were 2.5 and 5.3 mg N g^{-1} VSS h^{-1} in R1 and R2, respectively. However, specific denitritation rates remained unchanged in R3 (50 mg NO_2^--N L^{-1}), while it increased to 3.8 mg N g^{-1} VSS h^{-1} in R4 (50 mg NO_2^--N L^{-1}).

In a study investigating the treatment of textile wastewater in combined anaerobic and aerobic reactors, the specific denitrification rate in the system ranged from 0.4 to 1.2 mg NO_3^- mg^{-1} VSS d^{-1}.[50] In the study conducted by Cheng and Liu

Table 4. Specific denitrification and denitritation rates in batch tests

Condition	Reactor No.	NO_3^--N (mg L^{-1})	NO_2^--N (mg L^{-1})	Specific denitrification rate (mg N g^{-1} VSS h^{-1})	Specific denitritation rate (mg N g^{-1} VSS h^{-1})
C1	R1	25	—	1.1 ± 0.03	—
	R2	50	—	1.2 ± 0.01	—
	R3	—	25	—	0.5 ± 0.03
	R4	—	50	—	1 ± 0.04
C2	R1	25	—	0.8 ± 0.04	—
	R2	50	—	0.9 ± 0.01	—
	R3	—	25	—	0.4 ± 0.001
	R4	—	50	—	0.8 ± 0.02
C3	R1	25	—	0.5 ± 0.02	—
	R2	50	—	1.1 ± 0.02	—
	R3	—	25	—	0.5 ± 0.01
	R4	—	50	—	0.6 ± 0.003
C4	R1	25	—	0.8 ± 0.01	—
	R2	50	—	2 ± 0.02	—
	R3	—	25	—	1.5 ± 0.01
	R4	—	50	—	1.7 ± 0.05
C5	R1	25	—	1 ± 0.1	—
	R2	50	—	2 ± 0.02	—
	R3	—	25	—	2 ± 0.05
	R4	—	50	—	2.1 ± 0.06
C6	R1	25	—	1.5 ± 0.07	—
	R2	50	—	3.3 ± 0.1	—
	R3	—	25	——	2.1 ± 0.04
	R4	—	50	—	3.2 ± 0.004
C7	R1	25	—	1.2 ± 0.1	—
	R2	50	—	2.8 ± 0.1	—
	R3	—	25	—	2.4 ± 0.2
	R4	—	50	—	3.2 ± 0.2
C8	R1	25	—	2.5 ± 0.02	—
	R2	50	—	5.3 ± 0.46	—
	R3	—	25	—	2.4 ± 0.01
	R4	—	50	—	3.8 ± 0.21

(2001), for anaerobically digested swine wastewater treatment with intermittent aeration, specific nitrification and denitrification rates were 2.79–3.70 and 0.59–1.03 mg NO_3^--N g^{-1} VSS h^{-1}, respectively, at an aeration on/off time of 60/60 min.[51] In an MBR system treating domestic wastewater, the highest denitrification rate was obtained as 2.68 mg NO_3^--N g^{-1} MLVSS h^{-1} at an aeration on/off time of 40/80 min, and it was higher than the relatively shorter aeration-off times.[21] In our study, the highest denitrification rate was 5.3 mg N g^{-1} VSS h^{-1} and was from the sludge obtained from the longest aeration-off time.

In the experiments conducted with acetate as a carbon source in the literature, the denitrification rate generally varied between 3.09 to 10.6 mg N g^{-1} VSS h^{-1}, depending on the operational conditions (reactor type, environmental factors, and sludge properties).[52] The denitrification rate ranged from 0 to 0.7 mg N g^{-1} VSS h^{-1} in batch tests conducted in the absence of organic matter, i.e. acetate. The specific denitrification rate of activated sludge by internal respiration has been reported as 0.2–0.6 mg NO_3^--N g^{-1} VSS h^{-1} in the literature.[53,54]

The highest denitritation rates were 2.4 and 3.8 mg N g^{-1} VSS h^{-1} for R3 and R4 at an aeration on/off time of 90/360 min, respectively. In a study investigating the denitritation performance with different organic carbon sources, the maximum specific nitrite reduction rate was 0.25 g N g^{-1} VSS d^{-1} for glycerol, while it decreased to between 0.13 and 0.17 g N g^{-1} VSS d^{-1} for ethanol, landfill leachate, and fermented primary sludge centrate in the SBR.[55]

In the nitrite-based nitrogen-removing processes, as the nitrite concentration increases the denitritation rate increases, but FNA may form, which inhibits the process.[56] FNA is a product that causes mutagenic effects by reacting with substrates, and its amount in the system increases with the decrease in pH.[57] Abeling and Seyfried reported that the inhibition threshold in the denitrification process of FNA was 0.13 mg HNO_2-N L^{-1}.[44] Ma *et al*. observed that the nitrate-reducing activity of biomass was limited by approximately 60% in an FNA of 0.01–0.025 mg HNO_2-N L^{-1}, while it completely ceased at over 0.2 mg HNO_2-N L^{-1}.[58] In this study, the highest FNA value was 0.00076 mg HNO_2-N L^{-1}, which was considerably lower than the inhibition thresholds given in the literature.

CONCLUSIONS

In this study, specific ammonium oxidation, denitritation, and denitrification rates were investigated in batch assays for which sludge samples were obtained from a lab scale MBR treating textile wastewater at different DO concentrations and aeration on/off time. In the batch tests, the highest specific ammonium oxidation and denitrification rates were obtained at an aeration on/off time of 90/360 min. Compared to the period with continuous aeration, the specific ammonium oxidation, denitritation, and denitrification rates increased by approximately 1.8, 3.7, and 4.3 times, respectively, in the aeration on/off time of 90/360 min. Hence the specific ammonium oxidation, denitritation, and denitrification rates depend significantly on the aeration cycle on/off time, which should be optimized for the bioreactors operated under intermittent aeration conditions for the highest TN removal performance.

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