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Modification of nitrifying biofilm into nitritating one by combination of increased free ammonia concentrations, lowered HRT and dissolved oxygen concentration

Ivar Zekker[1,*], Ergo Rikmann[1], Toomas Tenno[1], Anne Menert[2], Vallo Lemmiksoo[1], Alar Saluste[1], Taavo Tenno[1], Martin Tomingas[1]

1. Institute of Chemistry, University of Tartu, 14a Ravila St., 50411 Tartu, Estonia. E-mail: Ivar.Zekker@ut.ee
2. Tallinn University of Technology, 5 Ehitajate St., 19086 Tallinn, Estonia

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Abstract

Nitrifying biomass on ring-shaped carriers was modified to nitritating one in a relatively short period of time (37 days) by limiting the air supply, changing the aeration regime, shortening the hydraulic retention time and increasing free ammonia (FA) concentration in the moving-bed biofilm reactor (MBBR). The most efficient strategy for the development and maintenance of nitritating biofilm was found to be the inhibition of nitrifying activity by higher FA concentrations (up to 6.5 mg/L) in the process. Reject water from sludge treatment from the Tallinn Wastewater Treatment Plant was used as substrate in the MBBR. The performance of high-surfaced biocarriers taken from the nitritating activity MBBR was further studied in batch tests to investigate nitritation and nitrification kinetics with various FA concentrations and temperatures. The maximum nitrite accumulation ratio (96.6%) expressed as the percentage of NO_2^--N/NOx^--N was achieved for FA concentration of 70 mg/L at 36°C. Under the same conditions the specific nitrite oxidation rate achieved was 30 times lower than the specific nitrite formation rate. It was demonstrated that in the biofilm system, inhibition by FA combined with the optimization of the main control parameters is a good strategy to achieve nitritating activity and suppress nitrification.

Key words: moving-bed biofilm reactor; free ammonia; specific nitrite oxidation rate; nitrite accumulation ratio

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Introduction

Ammonium-rich wastewaters (600–2000 mg/L) are generated in wastewater sludge treatment, slaughterhouses, dairies, meat processing, fish canning, yeast factories and in the production of nitrogen fertilizers (Shivaraman, 2003). Even more NH_4^+-N (over 2000 mg/L) may be contained in landfill leachate (Sun et al., 2010). Short-cut nitrogen removal (nitritation combined with denitritation via the Anammox process) is beneficial for the treatment of these wastewaters, where nitrification efficiency is decreased due to a high free ammonia (FA) concentration, but nitritation efficiency is less influenced. The key point in achieving nitrogen removal via nitrite is to oxidize NH_4^+ into NO_2^- instead of NO_3^- and to maintain a stable nitrite accumulation ratio (NO_2^--N/NOx^--N, where NOx^--N = NO_2^--N + NO_3^--N).

In a wastewater treatment plant (WWTP) with anaerobic sludge digestion, the recirculation of reject water from anaerobically digested sludge contributes to 15%–20% of the total influent nitrogen load. Therefore, it is reasonable to treat reject water separately rather than return it to the WWTP inlet for treatment as a part of the main flow (Gut et al., 2006; Aslan and Dahab, 2008). Because of a low carbon to nitrogen (C/N) ratio, nitrogen removal from the anaerobic sludge digester reject water cannot be accomplished easily with traditional biological methods.

The combined nitritation-Anammox process can theoretically save 63% of O_2, 50% of inorganic carbon source (HCO_3^-), and 100% of the organic carbon source demand as compared to the nitrification-denitrification process (Qiao et al., 2009). In terms of microbial consortia, the ammonia-oxidizing bacteria (AOB) have to become the dominant nitrifying bacteria while the nitrite-oxidizing bacteria (NOB) have to be washed out.

Compared to suspended sludge systems in a biofilm system, the achievement a stable nitritation process is different since NOB washout cannot be achieved by a short hydraulic retention time and a low dissolved oxygen (DO) concentration only because of the fixed film biomass with

* Corresponding author. E-mail: Ivar.Zekker@ut.ee

high sludge age.

The main factors that positively affect nitrite build-up include temperatures 35–40°C (Hellinga et al., 1998; Peng and Zhu, 2006), hydraulic retention time (HRT) of 12 hr (Yamamoto et al., 2006), DO concentration between 0.5–1.5 mg/L (Bae et al., 2002; Kim et al., 2003; Ruiz et al., 2006) and FA concentration around 5 mg/L above the inhibition threshold for NOB (Balmelle et al., 1992; Abeling and Seyfried, 1992; Aslan and Dahab, 2008). NOB compete effectively for DO when the nitrite accumulation ratio is higher than 0.75 (Ciudad et al., 2005).

Nitritation can be established by adjusting the pH value, because the balance between ammonium (NH_4^+) and FA concentration is based on the NH_4^+ concentration, temperature and the pH value. As pH value increases, FA concentration will also increase, and NOB such as *Nitrobacter* will become inhibited (Rosenwinkel and Cornelius, 2005). FA concentrations between 10–150 mg/L inhibit ammonium oxidation causing the failure of nitrite build-up. Nitrate build-up would be inhibited between FA concentrations of 1–5 mg/L (Abeling and Seyfried, 1992), and a lower range of inhibitory FA concentration (at 0.1–1.0 mg/L) could also be sufficient for inhibiting nitratation (Anthonisen et al., 1976). However, NOB possess an ability to adapt to FA inhibition, thus the inhibition effect on nitratation may be mitigated on higher FA concentrations. AOB are shown to be more resistant to higher FA concentrations than NOB (Bougard et al., 2006). The FA inhibition by itself, as a sole measure, might not be an effective and sufficient strategy to maintain stable nitrite accumulation in a longer perspective (Rongsayamanont et al., 2010). Therefore, an appropriate combination of several control parameters such as DO and FA concentrations, HRT and temperature is needed.

In this article, we demonstrated an effective modification of nitrifying biofilm into nitritating one in a MBBR system within a short time period. Combination of a low DO concentration (time-based intermittent aeration), an increased FA concentration and higher HRT resulted in a formation of nitritating biofilm with a low nitrification rate.

1 Materials and methods

1.1 Influent

Reject water from anaerobic reactor in Tallinn WWTP (Estonia) was used as a substrate for MBBR operation. It contained sufficient amounts of substrates and microelements for AOB and NOB as shown in Table 1. Prior to feeding into the reactor reject water was diluted with tap water at a volume ratio of 1:9.

1.2 Laboratory-scale moving-bed biofilm reactor (MBBR)

A 20-L (height 70 cm and i.d. 30 cm) laboratory-scale plexiglas reactor with a water-jacket (connected with an Assistant 3180 water bath) was used. The reactor content was mechanically stirred at 100–200 r/min using surface mixing. The influent was fed by using a peristaltic pump (Seko, Italy). A DO controller (Elke Sensor, Estonia) was applied for registrating and maintaining DO concentrations. Aquarium air pump supplied the system with air. Adjustment of pH was done by a chemical dosing pump (Magdos DX-2, Germany), which was connected with a pH controller (Consort, Germany). NaOH solution (4 mol/L) was dosed to the MBBR for increasing FA concentration and the pH of the solution.

Plastic barrels with 100 L volume served as tanks for influent and effluent. The influent was prepared every two days. The biofilm was grown on polyethylene carriers (Bioflow 9, ring-shaped, density 0.95 kg/m^3, specific surface around 800 m^2/m^3) at the temperature of 26.5 ± 0.5°C. The scheme of lab-scale reactor is shown in Fig. 1.

Table 1 Qualities of substrate for MBBR influent

Parameter	Average concentration in Tallinn WWTP reject water
NH_4^+-N	680 ± 76 mg/L
NO_2^--N	1.21 ± 0.5 mg/L
NO_3^--N	0.2 ± 0.2 mg/L
BOD_7	350 ± 90 mg/L
Alkalinity	3950 ± 100 mg/L
pH	7.9 ± 0.3
COD	682 ± 210 mg/L
Phosphate-phosphor	10 ± 1 mg/L
Total sulphide-sulphur (Stot)	1.4 ± 0.4 mg/L
Total suspended solids (TSS)	750 ± 30 mg/L
Ash weight	590 ± 28 mg/L
Volatile suspended solids (VSS)	160 ± 6 mg/L
Mn	0.180 ± 0.004 mg/L
Fe	4.3 ± 0.1 mg/L
Mg	24.0 ± 0.3 mg/L
Ca	30.0 ± 0.7 mg/L
Zn	5.00 ± 0.2 μg/L
B	0.45 ± 0.02 mg/L
Co	9.0 × 0.3 μg/L
Ni	13.0 ± 0.6 μg/L

1.3 Batch tests for determination of nitritation activity and calculations

The ammonium and nitrite oxidation rate and nitrite formation rate on biofilm carrier elements were measured

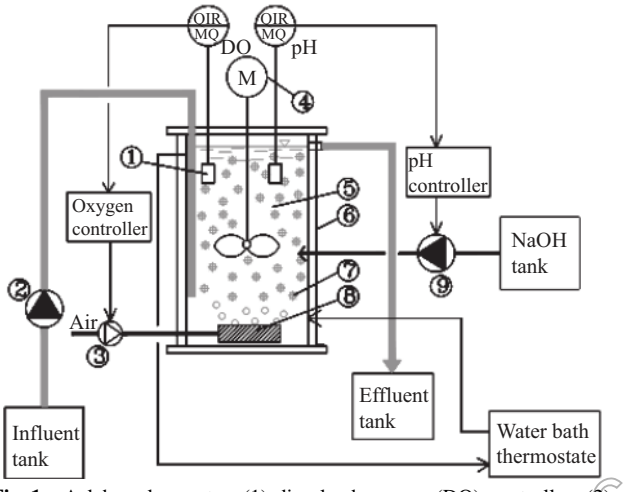

Fig. 1 A lab-scale reactor. (1) dissolved oxygen (DO) controller; (2) influent pump; (3) air pump; (4) mixer; (5) reactor (volume 20 L); (6) water jacket; (7) biocarriers; (8) air sparger; (9) NaOH pump.

in 500-mL Erlenmeyer flasks filled with 55 ring-shaped carriers. For all the batch tests, the average concentration of VSS was 2.75 g VSS/L. The synthetic medium, $(NH_4)_2SO_4$-water solution, was aerated continuously. To avoid DO concentration limitation, the ammonium oxidation test was performed at DO concentration of 6.0 mg/L as described by Pynaert et al. (2003). Temperature, pH, concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were monitored by hourly sampling. Batch tests were performed at various temperatures in the range of 20 to 36°C, maintaining by a thermostated water bath. Batch tests with the biomass were performed between 63–86 days of reactor operation. Before experiment the biomass was pre-aerated to the point of oxygen saturation to reduce the effect of anaerobic ammonia oxidizing bacteria as well as denitrifying bacteria on ammonium and nitrite conversion.

Air was supplied through the air-spargers placed at the bottom of the reaction cell and the airflow was regulated manually by a valve and controlled through measurements of DO concentration by an oxygen meter. The reaction vessel was stirred using a magnetic bar. The pH value was adjusted manually to 8.5 ± 0.2 by dosing 1 mol/L NaOH as it decreased during ammonium oxidation. All solutions consisted of 50 mmol/L HCO_3^- as an inorganic carbon source for AOB and for forming carbonate buffer. Relatively high HCO_3^- concentration was used because of the possible CO_2 stripping (monitored by titrations) and carbon source loss during the kinetic test, also for the prevention of the decrease in ammonium oxidation rate in the last hours of the test. Considering that the augmentation of the biomass was relatively slow during the 6-hr measuring period, the biomass concentration was considered to be constant during the batch experiments.

FA concentrations at different temperatures were calculated using the NH_4^+-N/NH_3-N equilibrium relationship introduced by Anthonisen et al. (1976):

$$\mathrm{FA} = \frac{C_{\mathrm{NH_4^+\text{-}N}} 10^{\mathrm{pH}}}{K_\mathrm{b}/K_\mathrm{w} + 10^{\mathrm{pH}}} \tag{1}$$

$$K_\mathrm{b}/K_\mathrm{w} = \mathrm{e}^{(6344/(273+T))} \tag{2}$$

where, FA (mg NH_3-N/L) is free ammonia concentration, $C_{NH_4^+-N}$ (mg NH_4^+-N/L) is total ammonium nitrogen concentration, and T (°C) is temperature, K_b (mol/L) is basicity dissociation constant of NH_4^+, and K_w (mol/L) is water self-ionization constant.

1.4 Analytical methods

The ammonium content was analyzed using commercial Dr Lange test kits (Hach Lange GmbH, Düsseldorf, Germany) and absorbance was measured on a designated spectrophotometer (Hach Lange Dr. 2800, Germany). Determinations of water-phase nitrate-nitrogen and nitrite-nitrogen were carried out by the colorimetric method. Measurements of COD, total suspended solids (TSS), alkalinity and volatile suspended solids (VSS) were conducted according to standard methods (APHA, 1985).

VSS of the biofilm was determined using 50 carriers from the MBBR tank. The carriers were washed gently with demineralised water to remove residual salts. The carriers were placed in an oven for 24 hr at 105°C. The biofilm was then removed and weighed (APHA et al., 1985; Dupla et al., 2006) and mg VSS per carrier was calculated. The ashes were weighed and mg VSS per carrier was calculated. The standard deviation for the method was 4.9%, which was determined by using three series of 50 ring-shaped biocarriers before, during and after the batch tests.

pH was measured by a portable pH meter (Evikon, Estonia), which was calibrated before the batch experiments. An Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) by designated spectrometer (Varian, Liberty II, Australia) was used for determining the concentrations of microelements in the Central Laboratory of the Estonian Environment Research Ltd., Estonia).

In order to observe the biofilm structure, cross-section micrographs were taken by SEM (Zeiss D940, Germany) equipped with IdFix software and a SAMx 10 mm^2 SDD detector (energy dispersive X-ray detector, based on Silicon Drift technology). The SEM was also connected with active Digital Image Scanning System PE-DISS5+.

The specimens for the SEM were prepared by fixing them with 2.5% formaldehyde in 0.1 mol/L phosphate buffer at pH 7.2 for 1.5 hr. After that the biomass was rinsed in the buffer for 10 min, and in distilled water for 5 min, followed by dehydration with a graded series of ethanol (10%, 30%, 50%, 70%, 90% and 95%), and dried as described previously (Chamchoi and Nitisoravut, 2007; Qiao et al., 2009). Finally, the samples were coated with gold prior to analysis.

1.5 Modification of biofilm from nitrifying to nitritating

About 50% of the reactor volume was filled with biofilm carriers (initial biomass concentration was 2.47 mg VSS per carrier) with a specific nitrite oxidation rate of 284 mg NO_2^--N/(g VSS·day) measured at 15°C. Nitrifying biofilm used as inoculum was received from a fish farm wastewater treatment facility in Saaremaa, Estonia. The NO_3^--producing biofilm was converted to NO_2^--producing one by means of reducing HRT and DO concentrations in the MBBR, and applying pH adjustment to ensure NOB inhibition due to the increased FA concentration (Table 2).

The biofilm modification (total of 106 days) was divided into five phases, as described in Table 2. During the first 10 days (period I) the DO concentration was maintained between 0.3–0.7 mg/L by the DO controller. The HRT was kept 12 hr (influent flow rate 40 L/day) and effluent pH fluctuated between 7.0–7.8 as it was not controlled. FA concentration was between 0.09–0.50 mg/L during the phase.

For the next 4 days (period II) DO concentration was set in the range of 0.2–0.5 mg/L, pH ranged between 7.0–7.4, FA concentration in the liquid phase was 0.08–0.40 mg/L, whereas was lowered to HRT 9 hr (flow rate 55 L/day).

Within the following 23 days (period III) when a successful inhibition of nitrification was achieved, the adjustment of pH in the liquid phase of the reactor was performed automatically by using 4 mol/L NaOH, raising

Table 2 Conditions for modification of the biofilm from nitrifying to nitritating

Phase	Days	DO (mg/L)	Aeration regime	pH	HRT (hr)
I	1–10	0.3–0.7	Concentration-based	7.0–7.8	12
II	10–14	0.2–0.5	Concentration-based	7.0–7.4	9
III	14–37	0.1–0.3	Concentration-based	7.5–8.6, pH adjusted to 8.5	7.6
IV	37–86	0–1	Time-based (30 min aerated, 30 min non-aerated)	7.5–8.6, pH adjusted to 8.5	11
V	86–106	0–0.5	Time-based (30 min aerated, 30 min non-aerated)	7.6–8.2	9

HRT: hydraulic retention time.

the pH value back to 8.5. FA concentration in the reactor during the period ranged from 0.7–6.5 mg/L, which in concurrence with other control parameters (Table 2) induced selection mechanisms for disfavouring NOB growth. pH adjustments were performed to increase the FA concentration in the reactor. With increasing pH, FA concentration increases exponentially: $NH_4^+ \leftrightarrow NH_3 + H^+$, $pK_a = 9.25$ (De Boer and Kowalchuk, 2001). HRT was set at 7.6 hr (63 L/day) and DO concentration was between 0.1–0.3 mg/L. Modification of the biofilm to nitritating one was successfully completed in 37 days of operation based on the results of chemical analyses (Fig. 2).

For period IV (between 37–86 days of operation) HRT was increased to 11 hr (flow rate 43 L/day) to sustain a high nitrite accumulation ratio. Intermittent aeration was involved to take advantage of the higher affinity of oxygen of NOB over AOB. DO concentration was set to range from 0 to 1 mg/L by the time-based (30 min aerated, 30 min non-aerated) aeration regime. After 30 min aeration period, DO concentration reached 1 mg/L, then decreased to 0 mg/L by 3 min after the air supply was stopped. The value of pH was automatically adjusted to 8.5 when it decreased below 7.5 (FA concentration between 0.7–5 mg/L).

For period V (86–106 days of operation) HRT was decreased to 9 hr (flow rate 55 L/day). DO concentration was between 0–0.5 mg/L by intermittent aeration. The strategy was chosen to inhibit NOB activity, which showed an increase at the end of the period of 37–86 days. During this phase, pH was in the range of 7.6–8.2 without adjustment. FA concentration was increased (1.4–4.1 mg/L) because of the higher NH_4^+-N concentrations in MBBR.

2 Results and discussion

2.1 Performance of MBBR

The phases of process operation are shown in Fig. 2. During the start-up period in the initial phase the aerobic reactor acted as a nitrification system, but eventually ended up as a partial nitrification system.

For the initial 14 days (phases I and II) the nitrification efficiency of the initial nitrifying biomass showed a continuous increase (Fig. 2). This was observed even at a low DO concentration and a low HRT (Table 2), the latter was set twice lower than needed for the faster propagation of NOB. The initial increase in nitrification activity might occur as a response of the nitrifying biomass to elevated temperature, which it was exposed to after the inoculation compared to the conditions of the fish farm wastewater

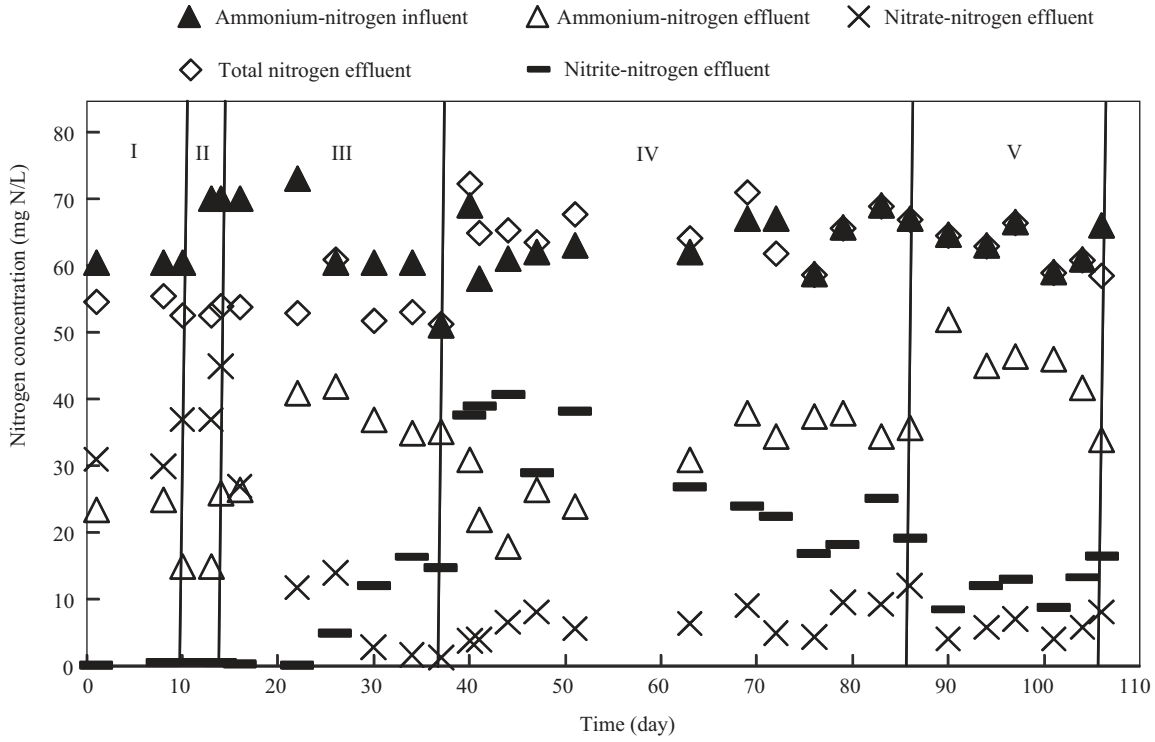

Fig. 2 Performance of the partial nitritation system dependent on aeration regime and other control parameters (pointed out in Table 2).

treatment facility (average temperature 15°C).

In phase II, HRT and DO concentrations were lowered further (Table 2). The nitrate-nitrogen concentration in the effluent increased at the end of the phase (Fig. 2). Thus, the activity of NOB was at least the same as that of AOB, and small amounts of nitrite-nitrogen could be detected (0.14–0.60 mg/L) due to the simultaneous oxidation of nitrite to nitrate at the same time as it was produced by AOB (Fig. 2). The average nitrite accumulation ratio was low (1.4% ± 0.3%).

During phase III (days 14–37), a successful inhibition of NOB, the achievement of high nitrite accumulation ratio was obtained. For days between 14–22 when the HRT and DO concentration range were lowered and pH was adjusted (Table 2) the NO_2^--N content remained unchanged, with an average NO_2^--N/NOx^--N ratio being 1.2% ± 0.1%, while the NO_3^--N concentration decreased about 4 times. The NO_3^--N concentration dropped largely by day 28, indicating a rapid and substantial NOB washout from the biofilm. At the end of the period 57% of NH_4^+-N was oxidized to a state where 90 % of oxidized nitrogen was in the form of NO_2^--N (Fig. 2).

According to Kim et al. (2006), the biofilm reactor has shown 50% of NOB inhibition when FA concentration was as low as 0.7 mg/L. Respiration tests showed that *Nitrobacter* can be inhibited by FA concentration lower than 1.0 mg/L (Vadivelu et al., 2007). NOB can be acclimatized to a low FA concentration when using it as a control parameter without other limiting parameters (HRT, DO concentration) for inhibition.

We assume that the conversion of nitrifying biofilm to nitritating one can be faster under the optimal combination of HRT and the concentrations of DO and FA.

Phase IV (days 37–86) shows a low activity of NOB by the combination of elevated FA concentrations and other control parameters. The average nitrite accumulation ratio during the period was 80% ± 9.7% and the average conversion rate of NH_4^+-N to NO_2^--N between days 37–86 was 46%. Similar results (52% for a swim-bed acrylic fibre carrier and 40% for a swim-bed activated sludge reactor configuration) were achieved by Qiao et al. (2009). However, 57% of the conversion efficiency of NH_4^+-N to NO_2^--N (achieved in phase III) must be ensured on the basis of the NO_2^-/NH_4^+ratio of 1.3 (Strous et al., 1999) for performing a subsequent Anammox process.

In this phase the intermittent aeration regime was used in conjunction with an increased FA concentration (up to 5 mg/L). Increased FA concentration inhibits NOB. At the same time intermittent aeration will lead to an increase in the NO_2^--N/NOx^--N ratio if aeration time is adjusted properly, because after switching on aeration again, *Nitrobacter* has a lag-phase, which is longer than that of *Nitrosomonas* (Rosenwinkel and Cornelius, 2005). In previous publications either intermittent aeration at higher DO concentrations (Katsogiannis et al., 2002) or DO concentrations of 0.2–0.5 mg/L with continuous aeration (Rosenwinkel and Cornelius, 2005; Bernet et al., 2001; Chuang et al., 2007) have been applied. Tokutomi (2004) observed that when the reactor was operated at a DO concentration below 1.0 mg/L, the growth rate of AOB was 2.6 times faster than that of NOB.

As seen in Fig. 3c and d, despite a low DO concentration in the liquid phase of the MBBR, filamentous bacteria were not detected. Moreover, the surface of the biofilm had a relatively even structure. However, the cross-section of the biofilm porous structure inside the biofilm, which probably ensured sufficient mass-transfer of substrates and products of bacterial-mediated reactions between the biofilm and the environment.

During phase V (days 86–106) at low NOB activity without pH adjustment, the average nitrite accumulation ratio of 66% ± 2.6% was mainly retained by non-controlled pH, but just high ammonium ions concentrations and intermittent aeration with low DO concentration in the effluent. Low HRT ensured the optimum ammonium and FA balance for AOB inside the biofilm and ensured a decrease in nitrification efficiency. By the strategy chosen no pH adjustments were needed and a consistently high FA concentration (1.4–4.1 mg/L) inside the system was enough to inhibit most of NOB in the mode with less control parameters.

The effluent nitrate component increased again due to NOB (*Nitrospira*), which had been adapted to the conditions inside the reactor. Occurrence of *Nitrospira* in the biofilm studied was detected by the Polymerase Chain Reaction (PCR) (data not shown). Bacteria representing this genus may have a negative effect on sustaining nitritation, since they can oxidize NO_2^- to NO_3^- even at low DO concentrations (< 1 mg/L) and at higher FA concentrations as their optimal pH is around 8 (Liu et al., 2008; Blackburne et al., 2007; Huang et al., 2010). Activity of *Nitrospira* spp. can be suppressed by lower temperatures (optimum 30–35°C, our MBBR –26°C) or higher NO_2^--N concentrations. *Nitrospira* spp. can be affected by aeration-non-aeration cycles of intermittent aeration (Mota et al., 2005), but the optimal regime of the intermittent aeration suppressing *Nitrospira* spp. activity while sustaining sufficient AOB activity needs further investigation.

2.2 Batch tests with biomass of high nitritating activity from phase IV

2.2.1 Favorable conditions for nitritation, achieving of high nitrite accumulation ratio

The aims of batch assays were to determine the maximum NO_2^--N/NOx^--N ratio, the rates of the conversion of nitrogen compounds by increased FA concentrations and temperatures.

The nitrite accumulation ratio and the rates of the conversion of nitrogen compounds were determined by the slopes of hourly measured concentrations in batch tests and the regression coefficients (R^2) ranged from 0.90 to 0.98. The NO_2^--N/NOx^--N, rates of oxidization of substrate were measured at FA concentrations of 5, 34 and 70 mg/L respectively. The choice of the these concentrations was the following: 5 mg/L as an average concentration in the MBBR, 34 mg/L as an approximate value for FA content in raw reject water (Table 1) at 26°C, and 70 mg/L for receiving the highest NO_2^--N/NOx^--N ratio and as such

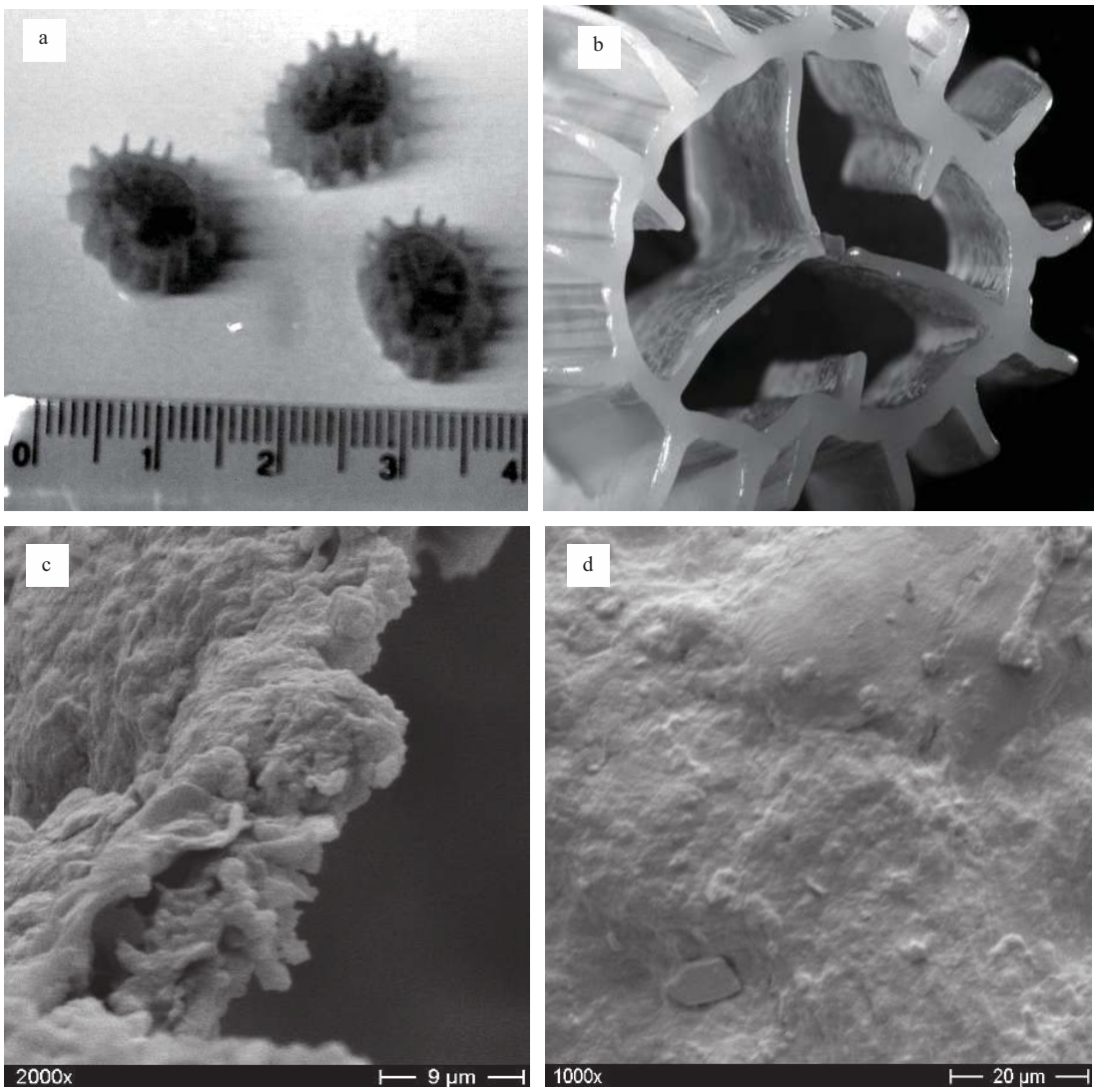

Fig. 3 Blank biofilm carriers, biofilm from the nitritation reactor and the SEM comparison of exterior and interior condition of biofilm taken at day 83. (a) blank Bioflow 9 ring-shaped biocarriers; (b) nitritating biofilm carriers- biofilm mainly on the inner surface of the carrier; (c) the cross-section of biofilm removed from the carrier (magnification of 2000×). Biofilm thickness 10 μm; (d) view of the outer side of the biofilm surface (magnification of 1000×).

high FA content occurs in landfill leachate.

The rate of ammonium oxidation or nitrite formation was divided by 126.2 mg VSS/L as VSS concentration responding to 55 biocarriers (Fig. 3a and b) involved in batch tests and the values obtained were defined as the specific ammonium oxidation rate (SAOR) and the specific nitrite formation rate (SNFR), respectively. Also, the specific nitrite oxidation rate (SNOR) was determined by the same method.

Figure 4 shows the combined effect of an increased FA concentration and temperature on nitrite accumulation ratios. NO_2^--N/NOx^--N for FA concentration of 5 mg/L was 83.5% at 20°C and was 90.3% at 36°C, while the same ratio for FA concentration of 34 mg/L was 85.9% at 20°C and 92.2% at 36°C. The highest relative nitrite accumulation for FA concentration of 70 mg/L was 92% at 20°C and 96.6% at 36°C (Fig. 4). Besides an increased FA concentration, higher achieved nitrite accumulation ratios at elevated temperatures can also be explained by the greater SNFR at above 20°C as compared to SNOR (Fux et al., 2002). Also, at temperatures above 30°C the AOB grow significantly faster than the nitrite oxidizers (Hellinga et al., 1998).

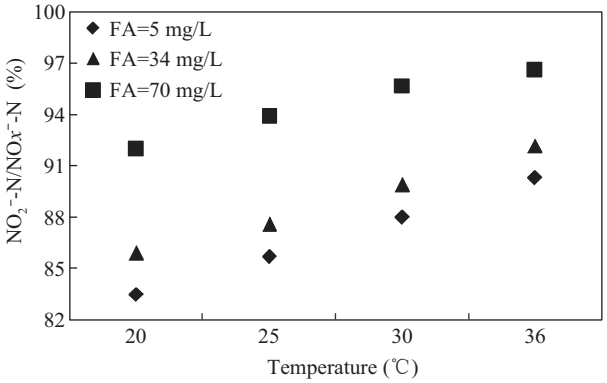

Fig. 4 NO_2^--N/NOx^--N ratios for different FA concentration and temperature combinations.

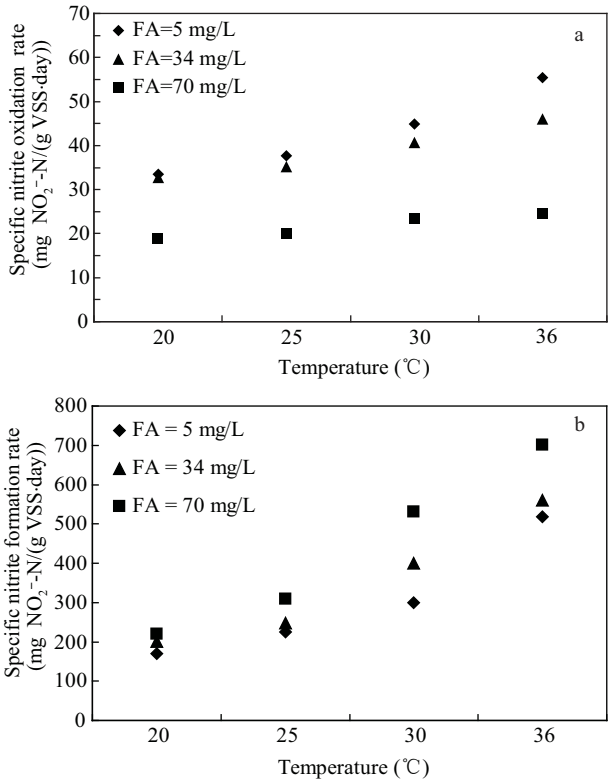

Fig. 5 Specific nitrite oxidation rates (a) and formation rates (b) at various temperatures (20, 25, 30 and 36°C) for FA concentrations of 5; 34 and 70 mg/L.

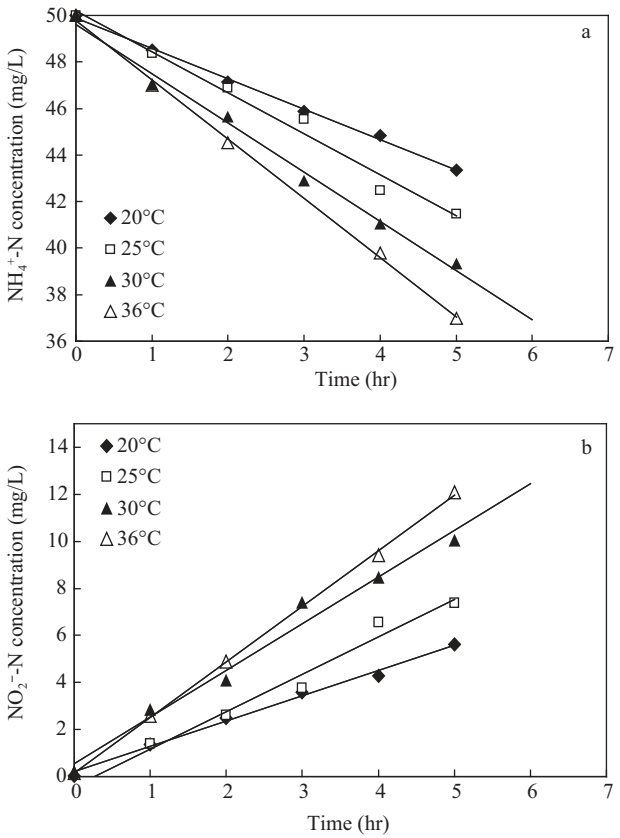

Fig. 6 Time courses of NH_4^+-N (a) and NO_2^--N (b) concentrations at various temperatures (20, 25, 30 and 36°C) for initial ammonium concentration of 50 mg/L.

Above 20°C, acceleration of nitritating activity was observed together with an activation of nitrification at different FA concentrations. SNOR at 36°C, for FA concentration of 5 mg/L was 1.7 times as high as that at 20°C (Fig. 5). When then temperature increase from 20–36°C , the SNFR increased at FA concentration of 5 mg/L about 3 times (Fig. 6). The increase was much higher as compared to SNOR. In comparison, Kim et al. (2008) reported an increase in SNOR by a factor of 2.6 with an increase in temperature (10–30°C).

For FA concentration of 70 mg/L, the SNOR at 20°C was 1.3 times lower than that at 36°C. The SNFR increased 3.2 times between temperatures of 20 and 36°C. The SNFR increased by a higher factor compared to the SNOR in this case also. At 20°C the SNOR for FA concentration of 5 mg/L was 1.8 times higher than that for FA of 70 mg/L. At 36°C the SNOR was 2.3 times higher for FA concentration of 5 mg/L compared to FA concentration of 70 mg/L. However, at 20°C the SNFR for FA concentration of 5 mg/L was 1.3 times lower than that for 70 mg/L. The SNFRs differed 1.35 times at 36°C between FA concentrations of 5 and 70 mg/L (Fig. 5a and b).

For FA concentrations from 4.9 to 71.9 mg/L, 50%–60% reduction in SNOR was observed by the respirometric method (Hawkins et al., 2010). Our experiments confirmed that the reduction of the SNOR at the same temperature (30°C) for FA concentrations ranges between 5 and 70 mg/L was 52% (Fig. 5). Hawkins et al. (2010) reported the major inhibition of FA concentration to occur at pH 8.0 while in our study the same inhibition was detected at pH 8.5; they also noted a sharp increase in the inhibition of NOB (between 30% and 75%), when pH was increased from 7.8 to 8.5.

It can be clearly observed that the SNFR and the SNOR were increased by temperature. The SNFR increased by an increase in FA concentration whereas the SNOR decreased (Fig. 5a and b). Therefore, with high FA concentrations the NO_3^- production can be avoided.

2.2.2 Specific ammonium oxidation rates and specific nitrite formation rates for the same initial substrate concentrations

The changes in NH_4^+-N and NO_2^--N concentrations at the same initial NH_4^+-N concentration (50 mg/L) were measured at different temperatures. FA concentrations for the experiments illustrated in Fig. 6 were 5.1, 5.9, 10.3, and 13.8 mg/L at 20, 25, 30, and 36°C, respectively.

Increase in the SAOR along with an increase in temperature is shown in Table 3. The SAOR in a suspended growth type system can achieve high values ranging from 170 to 290 mg NH_4^+-N/(g VSS·day) with a negligible nitrate production (Tokutomi, 2004; Yang et al., 2003; Bae et al., 2002). Experiments with initial NH_4^+-N concentration of 50 mg/L exhibited the highest SAOR of 484 mg NH_4^+-N/(g VSS·day) with significantly high SNFR (Table 3). The results emphasize a great potential for the elimination of nitrogen from wastewater via short-cut nitrification when the nitrate production is held at a low level.

Table 3 Specific ammonium oxidation rate (SAOR) and specific nitrite formation rate (SNFR) at varied FA concentrations and temperatures

Temperature (°C)	FA (mg/L)	SAOR (mg NH_4^+-N/(g VSS·day))	R^2 of NH_4^+-N	SNFR (mg NO_2^--N/(g VSS·day))	R^2 of NO_2^--N
20	5.1	248	0.99	205	0.99
25	5.9	335	0.98	302	0.97
30	10.3	403	0.99	377	0.98
36	13.8	484	1.00	460	1.00

R^2 is regression coefficients of hourly measured concentrations.

3 Conclusions

(1) The laboratory-scale experiments conducted with reject water showed that it is possible to convert the nitrifying biofilm to a nitritating one in 37 days by a combination of low HRT, time controlled intermittent aeration and a low DO concentration and, furthermore, by increased FA concentrations. (2) The main challenge encountered while sustaining nitritation after conversion of biofilm from nitrifying into nitritating was achieved was the activity of NOB representing genus *Nitrospira*. These bacteria are tolerant to low DO concentrations and showed adaption to FA concentrations present in MBBR. (3) At 36°C for FA concentration of 5 mg/L the specific nitrite oxidation rate achieved was about 10 times lower than the specific nitrite formation rate. At the same temperature for FA concentration of 70 mg/L the specific nitrite oxidation rate achieved was about 30 times lower than the specific nitrite formation rate, showing significant in NOB activity by increasing FA concentrations. (4) Nitrite accumulation ratio (maximum 96.6%) and the specific nitrite formation rate correlated positively with the increased temperature and FA concentrations during the batch experiments.

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