Start-up of moving bed biofilm reactors for deammonification: the role of hydraulic retention time, alkalinity and oxygen supply

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Abstract Aerobic and anaerobic ammonium oxidation can be combined in a completely mixed moving bed biofilm reactor, allowing for single-stage ammonium removal from wastewater with low COD/N ratio unsuitable for conventional nitrification/denitrification processes ('deammonification'). Mandatory preconditions are: (a) a low hydraulic retention time to wash out suspended cells competing with mass transfer limited biofilm cells for alkalinity as limiting substrate; and (b) an oxygen flux adapted to the surface loading rate to prevent complete nitrification to nitrate. pH control or 'NH_3 inhibition' of nitrite oxidation are neither useful nor necessary. By this strategy, oxygen limited biofilms with simultaneous presence of NH_4-N and NO_2-N were enriched, which allowed for growth of anaerobic ammonium oxidizers. It could be demonstrated that a deammonifying reactor can be purposefully started up within a reasonable span of time and without prior inoculation, if this explicitly described strategy is applied. Depending on surface loading and air flow rate, N removal rates of 4–5 g N/m^2 d could be achieved at DO concentrations between 1.0 and 4.0 mg/l.

Keywords Anammox; biofilm; canon; deammonification; moving bed; nitrogen removal

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Introduction

For the treatment of wastewater with low COD/N ratio, autotrophic microbial processes offer promising possibilities for N-removal, in particular the *An*aerobic *amm*onium *ox*idation (Anammox) with nitrite as electron acceptor (Strous *et al.*, 1999). If combined with a conventional aerobic ammonium oxidation to supply NO_2-N, a completely autotrophic N-removal is possible, which results in reduced oxygen demand without any need of an additional carbon source. Both processes (aerobic and anaerobic ammonium oxidation) can be combined within stratified biofilms or granules into a single-stage operation step, called 'Deammonification' (Helmer-Madhok *et al.*, 2002) or 'Canon' ('*C*ompletely *a*utotrophic *n*itrogen removal *o*ver *n*itrite', Strous, 2000, Sliekers *et al.*, 2003). In the outer layers, NH_4-N is oxidized aerobically to NO_2-N. In deeper layers without dissolved oxygen, NH_4-N is anaerobically oxidized with NO_2-N to N_2 (Anammox). The entire process thus consists of two combined reactions, carried out by two different groups of organisms which are spatially separated into different habitats (Figure 1).

The process strongly depends on different penetration depths of O_2 and NH_4-N into the biofilm. Furthermore, complete oxidation of nitrite to nitrate has to be prevented, as the latter cannot be utilized by Anammox organisms. 'Deammonification' and 'Canon' have in common that both reactions are combined within a single, completely mixed reactor. 'Deammonification' uses biofilms grown on suspended plastic carriers (moving bed) and was developed from initially observed losses in the nitrogen balance of rotating biological contactor systems, while 'Canon' is based on careful introduction of O_2 into reactors with an enrichment culture of anaerobic ammonium oxidizers. Because of the very low growth rates of Anammox organisms, experiments are cumbersome and time

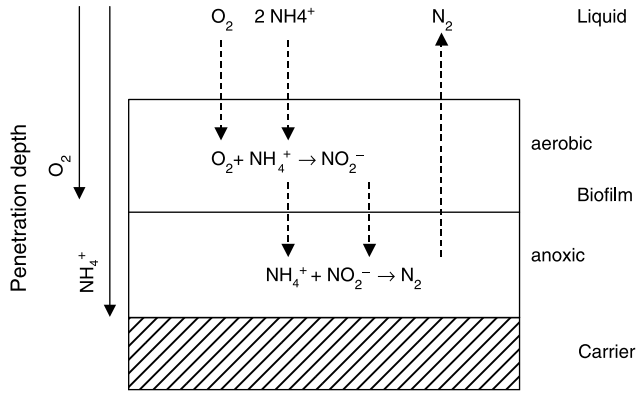

Figure 1 Simplified representation of a deammonifying biofilm coupling aerobic and anaerobic ammonium oxidation

consuming. Consequently, studies of the 'Canon' process were undertaken by simulation (Hao *et al.*, 2001, 2002) or by inoculating new laboratory reactor systems with Anammox biomass from existing enrichment cultures. For the 'Deammonification' process, there has been no experience available on start-up strategies without inoculation within a reasonable span of time. Thus, the present study aimed to determine the operational key factors for purposeful start-up of such a system.

Materials and methods

Continously operated moving bed biofilm reactors (MBBR)

Two identical, completely mixed reactors (50 l volume, temperature 30 °C) were operated in parallel. Each reactor was filled to 30% with commercially available carriers made of polyethylene (PE; cylindrical shape, 7.5 mm diameter, 323 m^2/m^3), which were freely suspended by aeration and mechanical stirring. The effective carrier surface area available for biofilm growth was 3.55 m^2 per reactor. Air flow (coarse bubble aeration) was controlled between 0 and 800 l/h. Besides carrier retention by an effluent screen, no biomass retention was applied. At start-up, both reactors were filled with nitrifying/denitrifying activated sludge from a municipal wastewater treatment plant (WWTP) (500,000 PE, sludge age 10–12 d). The reactors only differed in the hydraulic retention time (HRT): reactor 1 was operated at a HRT of 2 days (25 l influent volume per day), reactor 2 at a HRT of 0.5 days (100 l/d).

Wastewater characteristics

The influent wastewater was typical sludge digester supernatant (NH_4-N-rich water from sludge dewatering after anaerobic digestion) from the same WWTP. Before mechanical dewatering by pressing, the anaerobic sludge is thickened using polymeric coagulants. The remaining COD is predominantly non-degradable in WWTPs. To vary influent concentration and thus the surface loading rate, the supernatant was diluted with tap water.

Analytical methods

Ammonium, nitrite, nitrate, ortho-phosphate and COD were determined spectrophotometrically using commercially available ready-to-use test kits (LCK series, Hach Lange GmbH, Duesseldorf, Germany). Kjeldahl-Nitrogen was measured by the Kjeldahl method and alkalinity as HCO_3^- by titration to pH 4.3 with 0.1 M HCl. Dissolved oxygen concentration (DO) and pH were measured with standard probes (WTW, Weinheim, Germany). Influent and effluent were sampled 3–5 times a week (day 1–day 250) or daily (after day

250). Before analyzing for dissolved compounds, samples were filtered using 0.45 μm nylon membrane filters. The actual concentration of free NH_3 was calculated from pH, temperature and NH_4^+ concentration according to Anthonisen *et al.* (1976).

Results and discussion

Biofilm growth and activity

Both reactors were operated for 60 days in a 'complete nitrification' mode, i.e. besides a transient accumulation of NO_2-N for a few days directly after start-up, nitrite concentration was stable below 1 mg/l. As expected, approximately 50% of the influent NH_4-N was oxidized to NO_3-N with alkalinity as limiting parameter (effluent concentration <0.5 mmol/l). However, biofilm growth was very different in both reactors. While there were 0.28 g of biomass attached per m^2 of carrier surface area in reactor 1 (HRT 2 d), there were 3.1 g/m^2 in reactor 2 (HRT 0.5 d). The portion of surface area covered by biomass as well as biofilm depth was also significantly greater in reactor 2 (>90% coverage and approx. 150 μm depth, compared to 50% coverage and less than 50 μm depth in reactor 1). Batch tests with carriers only, bulk liquid from the reactors only, and both combined, showed that the NH_4-N-conversion observed was mainly due to the activity of suspended biomass in reactor 1, but almost completely done by biofilm biomass in reactor 2, although batch tests can only give a qualitative result as they do not allow for a steady state like in the reactors. However, this indicates that a short HRT is necessary for growth of active biofilms by washing out suspended biomass competing for the limiting substrate.

From wastewater composition (Table 1) it is clear that the molar alkalinity/NH_4-N ratio is approx. 1.1. For complete NH_4-N oxidation a ratio of 2 would be required (accounting only for catabolic reactions). Thus, alkalinity is the limiting substrate the organisms compete for.

Purposive formation of nitrite in biofilms by DO control

After nitrifying biofilms were established in reactor 2, N surface loading rate and air flow rate were varied. Because NO_3-N cannot be utilized by Anammox organisms, nitrification has to be stopped at the NO_2-N-level. Hippen *et al.* (2001) operated deammonifying moving bed pilot plants applying high pH (>8, by adding NaOH) to make use of a suspected inhibition of nitrite oxidation by free ammonia (NH_3). Actually, an inhibition by NH_3 cannot be considered reliable, as it is the aim of the process to remove NH_4-N. At low NH_4-N concentration in a completely mixed reactor, large amounts of base would be neccessary to assure a pH high enough for NH_3 inhibition. The results obtained in the present study show that there is neither a need for elevated pH nor any general influence of NH_3 on nitrite formation in biofilms at N surface loading rates between 1.4 and 11.3 g NH_4-N/m^2 d. At different influent concentrations (i.e. NH_4-N loading rates, because HRT was kept constant at 0.5 d), the ratio of NO_2-N: NO_X-N (with NO_x-N the sum of

Table 1 Average characteristics of undiluted sludge digester supernatant (Average of one year, 60 samples. Mean and standard deviation)

pH	Alkalinity as HCO_3^- (mmol/l)	NH_4-N (mg/l)	PO_4-P (mg/l)	Kjeldahl-N		COD	
				Total (mg/l)	Dissolved (mg/l)	Total (mg/l)	Dissolved (mg/l)
7.7 ± 0.3	67.9 ± 6.0	839 ± 165	31.4 ± 3.5	981 ± 71.4	978 ± 70	516 ± 332	385 ± 76

NO_2-N and NO_3-N) turned out to be only dependent on the oxygen supply to the biofilms (Figure 2).

Figure 2 shows that an inhibition may be effective at 10 mg/l NH_3 or higher. However, there are only few values beyond. On the other hand, almost 100% nitrite possible at NH_3 concentrations of nearly zero. At an influent concentration of 400 mg/l NH_4-N (11.3 g N/m^2 d), the amount of NO_2-N is independent of NH_3. On the contrary, DO concentration showed a much stronger relation at low influent concentration. It is widely accepted that oxygen scarcity suppresses the oxidation of nitrite, as nitrite oxidizers cannot successfully compete with ammonium oxidizers, if O_2 is limiting, because of their lower oxygen affinity (Wiesmann, 1994).

This indicates that alkalinity – which is correlated to the influent N concentration – governed the formation of nitrite at high loading rate, and that oxygen is the additional factor at low loading rate. O_2 limitation in the biofilm will occur if the alkalinity flux into the biofilm is higher than the O_2 flux. If alkalinity is in short supply, ammonium oxidizers will be limited and can no longer compete for oxygen, resulting in NO_3-N formation by nitrite oxidizers (which are catabolically independent of alkalinity). So, if alkalinity is limiting (which is the case at the lowest N loading rate tested here), O_2 supply has to be controlled to ensure O_2 limitation, if nitrite is the desired oxidation product.

In a similar moving bed system, designed to produce a NH_4-N/NO_2-N mixture as influent for a subsequent Anammox reactor in a two-stage process, Fux *et al.* (2004) experienced problems in avoiding further nitrite oxidation in the long term. Indeed, at low surface loading rate it was not possible in the present study to achieve more than 80% NO_2-N, related to total oxidized N. But for a single-stage system, just the presence of nitrite is important for the enrichment of Anammox organisms, rather than avoiding nitrate production completely. Once Anammox organisms are established, they will successfully compete for nitrite due to their high nitrite affinity (Strous, 2000).

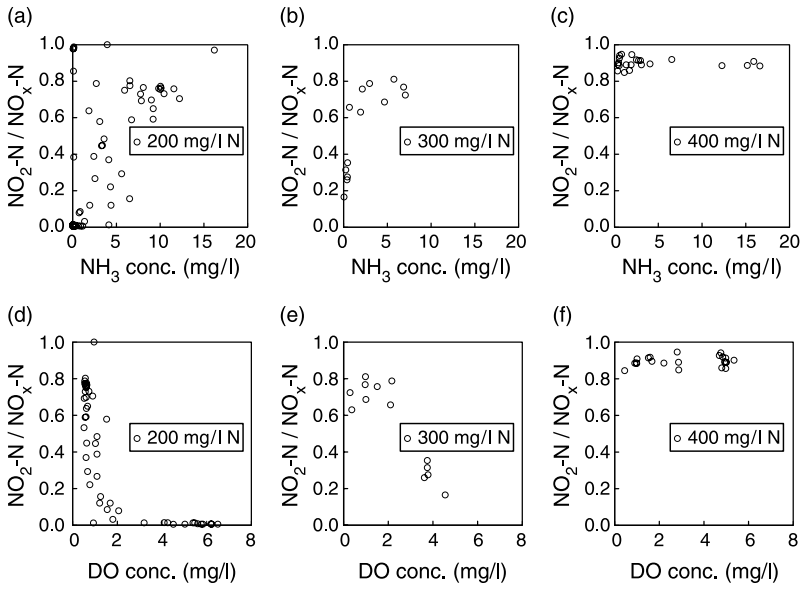

Figure 2 Impact of NH_3 concentration (a–c) and dissolved O_2 (d–e) on nitrite formation by nitrifying biofilms in reactor 2 at different influent concentrations

Deammonification efficiency at different N loading rates and DO concentrations

An oxygen-limited biofilm with NO_2-N production offers a habitat for Anammox organisms: absence of DO and simultaneous presence of NH_4-N and NO_2-N. Deep penetration of the biofilm with NH_4-N is given, as no more than about 50% of the influent ammonium can be oxidized to nitrite due to alkalinity limitation. Consequently, after operating under stable, DO-limited conditions for a few weeks, increasing N-removal could be observed between day 195 and day 265. At the end of this period, 55% of the influent NH_4-N was directly eliminated to N_2 (Figure 3).

An orientating population analysis of the biofilm by fluorescent *in situ* hybridization (FISH) was carried out on day 320 (Schmider, 2004, data not shown). Approximately 25% of the cells detected by a probe mix specific for most Eubacteria, consisting of probes EUB338 and EUB 338II + III (Daims *et al.*, 1999), also hybridized with probe Ban162 (Schmid *et al.*, 2001). This probe is specific for the first Anammox organism described, *Candidatus* 'Brocadia anammoxidans' (Strous *et al.*, 1999). A second Anammox probe specific for *Candidatus* 'Kuenenia stuttgartiensis', Kst157 (Schmid *et al.*, 2001), did not show hybridization. In parallel, conversion rates of N compounds were recorded in anoxic batch tests with original wastewater as NH_4-N source and addition of NO_2-N stock solution (data not shown). The ratio of NH_4-N:NO_2-N consumed

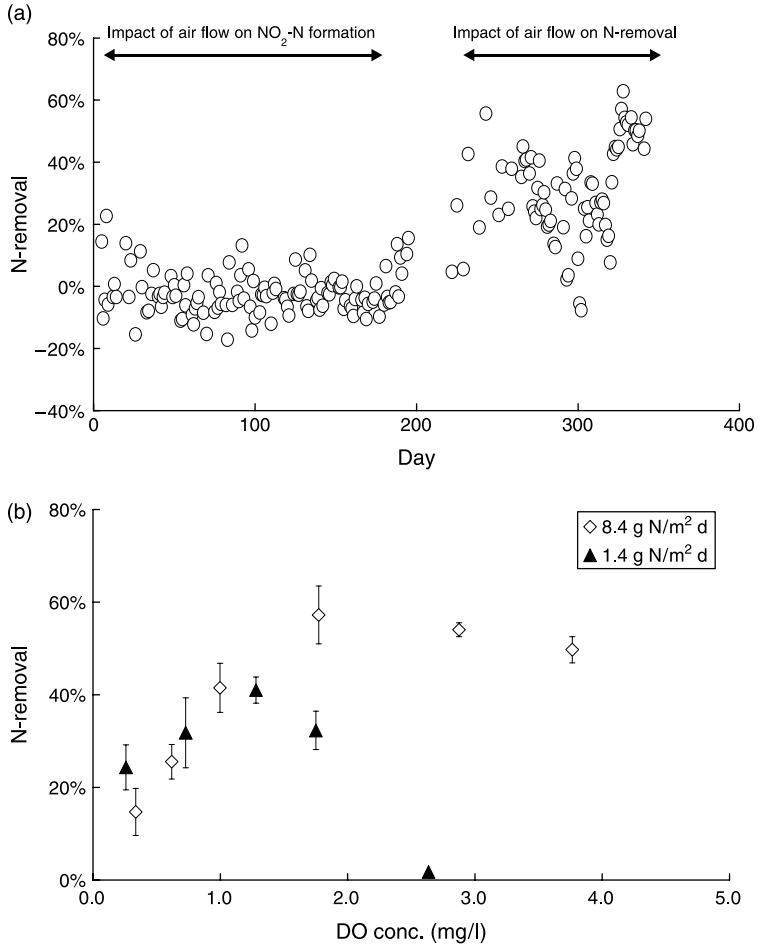

Figure 3 (a) Experimental phases and N-removal in reactor 2, (b) Deammonification efficiency depending on N-loading rate and oxygen concentration (mean and standard deviation)

and NO_3-N produced was repeatedly found to be -1.00: -1.30: $+0.26$, which is identical with the stoichiometry of the Anammox reaction as described by Van de Graaf *et al.* (1997). These results suggest that at least one type of Anammox organism was not only present in the biofilm, but also actively metabolizing. Possible competing conversions such as denitrification of nitrite were below the detection limit.

A test series with low (1.4 g N/m^2 d) and high (8.4 g N/m^2 d) N loading rate (equal to an influent concentration of 50 mg/l and 300 mg/l NH_4-N, respectively) showed a specific optimum of oxygen supply for each surface loading rate (Figure 3B), which is in good accordance with model predictions given by Hao *et al.* (2001) for the 'Canon' process. Note that DO *concentration* is depicted on the x-axis. Actually, the respective optima represent large differences in air flow rate: at 1.4 g N/m^2 d, the apparent optimum is 41% removal at a DO of 1.3 mg/l (resulting from an air flow of 60 l/h), while at 8.4 g N/m^2 d, 60% removal was reached at 1.8 mg/l O_2 (air flow: 300 l/h). Even at an air flow rate of 800 l/h (DO concentration of 4.0 mg/l), still 50% nitrogen elimination was observed. This strongly reflects the crucial role of the *flux* of the limiting substrates O_2 and alkalinity into the biofilm, rather than their concentration in the bulk liquid.

Since a loading rate of 1.4 g N/m^2 d in these experiments was equal to an influent concentration of 50 mg/l NH_4-N (which is in the range of municipal wastewater), it is also demonstrated that deammonification is not restricted to wastewater with high N-concentration.

Conclusions

For purposeful growth of biofilms a short HRT is mandatory, ensuring the wash-out of suspended organisms. Otherwise the reactor will work as a chemostat, and growth of biofilm organisms will be limited by mass transfer compared to suspended cells. In particular, this applies for deammonification reactors, where O_2 consumption by aerobic ammonium oxidizers in outer biofilm layers is necessary to provide an anoxic environment for Anammox organisms in deeper layers. Oxidation of NO_2-N as necessary electron acceptor for the Anammox reaction can be prevented by controlling the oxygen supply, depending on the N-load. Alkalinity, which is stoichiometrically related to NH_4-N in certain wastewaters, is the limiting substrate besides O_2. At (realistic) NH_4-N surface loading rates between 1.4 and 11.3 g N/m^2 d, there is no need for pH control for 'NH_3 inhibition' of nitrite oxidation. Autotrophic, single-stage N-removal from wastewater with low C/N ratio by a completely mixed moving bed biofilm system is possible, if aeration is adapted to the wastewater load. There is also no need for 'low' DO (< 1 mg/l): the ratio of alkalinity- to O_2-flux into the biofilm is crucial. In this study, 4–5 g N/m^2 d could be eliminated at DO concentrations between 2 and 4 mg/l. It could be shown that such a moving bed-based system can be purposefully started up from scratch within a relatively short time without inoculating with Anammox-biomass, if proper conditions are applied.

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