A one-stage system with partial nitritation and Anammox processes in the moving-bed biofilm reactor

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Abstract The ability of bacterial cultures to create biofilm brings a possibility to enhance biological wastewater treatment efficiency. Moreover, the ability of Anammox and Nitrosomonas species to grow within the same biofilm layer enabled a one-stage system for nitrogen removal to be designed. Such a system, with Kaldnes rings as carriers for biofilm growth, was tested in a technical pilot plant scale (2.1 m^3) at the Himmerfjärden Waste Water Treatment Plant (WWTP) in the Stockholm region. The system was directly supplied with supernatant originating from dewatering of digested sludge containing high ammonium concentrations. Nearly 1-year of operational data showed that during the partial nitritation/Anammox process, alkalinity was utilised parallel to ammonium removal. The process resulted in a small pH drop, and its relationship with conductivity was found. The nitrogen removal rate for the whole period oscillated around 1.5 g N $m^{-2}d^{-1}$ with a maximum value equal to 1.9 g N $m^{-2}d^{-1}$. Parallel to the pilot plant experiment, a series of batch tests were run to investigate the influence on removal rates of different dissolved oxygen conditions and addition of nitrite. The highest nitrogen removal rate (5.2 g N $m^{-2}d^{-1}$) in batch tests was obtained when the Anammox process was stimulated by the addition of nitrite. In the simultaneous partial nitritation and Anammox process, the partial nitritation was the rate-limiting step.

Keywords Anammox; biofilm; digester supernatant; nitrogen removal rate; partial nitritation

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Introduction

Optimisation of volumetric conversion capacity in biofilm systems is governed by a complexity of mixed cultures kinetics. Biofilm wastewater treatment systems provide the basis for optimisation of the volumetric conversion capacity. The condition of the biofilm structure has a significant impact on the stable operation of a treatment plant. Furthermore, the biofilm formation depends on different biological, physical and chemical processes, such as transport of microorganisms, adsorption to the surface, initial adhesion, attachment and detachment.

Biofilm carriers can be used in wastewater treatment for the combination of both nitrifying cultures and Anammox bacteria in one single reactor. Such a system is often called Completely Autotrophic Nitrogen removal Over Nitrite (CANON) (reaction 1) (Keller *et al.*, 1997; Helmer and Kunst, 1998; van Benthum *et al.*, 1998; van Loosdrecht *et al.*, 2000; Third *et al.*, 2001).

$$NH_3 + 0.85O_2 \rightarrow 0.44N_2 + 0.11NO_3^- + 0.14H^+ + 1.42H_2O \qquad \text{(reaction 1)}$$

In a single stage process, ammonium oxidisers in the outer layer of the biofilm can coexist with the Anammox organisms present in the inner layer. In this way, oxygen that inhibits the Anammox process is consumed in the outer layer of the biofilm and Anammox bacteria are protected from oxygen. Aeration devices and reactor configuration determine the transfer of air to the bulk phase. A transfer from the bulk phase over a boundary layer to the biofilm limits oxygen transfer to the bacteria. A second limitation is determined by hydrodynamics conditions (van Hulle *et al.*, 2003).

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According to Hao *et al.* (2002), who modelled the CANON biofilm process, the dissolved oxygen (DO) concentration in the bulk liquid and the nitrogen removal efficiency is proportional to biofilm thickness. It is due to the fact that an anaerobic layer can be easily formed in the thicker biofilm. The absence of substrates such as ammonium or nitrite in the anaerobic layer limits the nitrogen removal. A thicker biofilm is also required to reach the maximum nitrogen removal when the system operates under a higher ammonium surface load.

Biofilm processes are widely studied due to the many possible advantages, such as savings in space, high surface area with relevant organisms, possibilities to obtain high conversion capacity and less biomass production. There are many different biofilm systems that are used in wastewater treatment processes but a moving bed biofilm reactor (MBBR) is one of the few systems that utilise the whole tank volume area for biomass growth without sludge recycling (Ødegaard, 2006). It can be established by the application of carriers for biofilm growth that are suspended in the liquid. It is important to assure the movement of carriers in the whole reactor volume. It can be done by aeration in the case of oxic conditions or by mixers in the case of anaerobic conditions.

The simultaneous partial nitritation/Anammox process in the one-stage MBBR for completely autotrophic nitrogen removal was tested in a technical-scale pilot plant, supplied with supernatant from dewatering of digested sludge. The studies were a continuation of previous research concerning the use of two-stage process technology (Gut *et al.*, 2005; Szatkowska *et al.*, 2006). As single-stage technology seemed to have several advantages compared with two-stage technology operational modes based on the use of only one reactor and separation unit, the operation was changed. It was, however, realised that this system with combined partial nitritation and Anammox could be more difficult to control than having the processes in two stages. The main focus was given on the role of oxygen concentration, rate limiting factor (partial nitritation or Anammox) and if the promising control strategies for partial nitritation and Anammox in two-stage technology based on pH and conductivity measurements could also be applied for single-stage technology.

Methodology

Pilot plant

The pilot plant has been operated at the Himmerfjärden Waste Water Treatment Plant (WWTP) in the Stockholm region. The MBBR, with a volume of 2.1 m^3 was supplied with supernatant from sludge dewatering after anaerobic digestion. Kaldnes rings were used as carrier material in the studies to cultivate a biofilm responsible for the partial nitritation and Anammox process. A specific biofilm surface of 250 m^2 per m^3 of reactor volume was provided. Kaldnes carriers filled 50% of the total reactor volume. The Kaldnes rings in the reactor were in motion due to the vertical mixers and air supply from the bottom of the reactor. A detailed description can be found in Cema *et al.* (2006).

To estimate the influence of the nitrogen load on the process performance, the reactor was run at two different hydraulic retention times (HRT), namely 24 and 16 h. The process was monitored by analysis of the inorganic nitrogen forms, alkalinity, COD and total nitrogen. Moreover, manual and on-line measurements of pH value, dissolved oxygen, conductivity and temperature have been carried out in the reactor and also for the influent and effluent. The scheme of the pilot plant is presented in Figure 1.

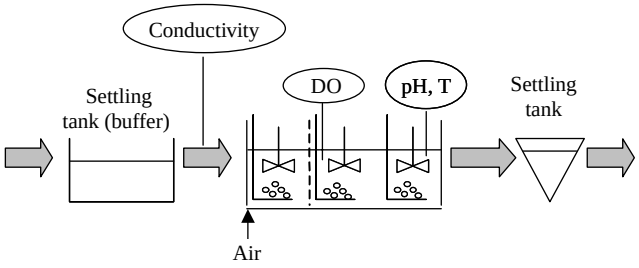

Figure 1 Flow diagram with on-line measurements of the pilot-plant with one-stage partial nitritation and Anammox processes

Batch tests

Batch tests were run in three parallel bottles of each with 1 L working volume. The bottles were filled up to 50% with Kaldnes rings taken from the pilot plant reactor and poured with liquid from the reactor. All tests were performed at a temperature of 25 °C. Magnetic stirrers assured mixing through all tests. In bottle one anaerobic conditions were maintained and NO_2 in the form of $NaNO_2$ solution was added to ensure the Anammox reaction. Air was supplied to the second bottle to keep the DO in the bulk liquid at around 1.4–1.6 g O_2 m^{-3}. The third batch bottle was aerated (1.3–1.8 g O_2 m^{-3}) and additional NO_2 was supplied as $NaNO_2$ solution. Each of the batch tests lasted 4 hours. Every 30 min, 10 mL of sample was collected from the test bottles by a syringe and was filtrated with a pre-filter and a 0.45 μm filter. Samples were analysed for ammonium, nitrite and nitrate with Tecator-Aquatec 5400 Analyzer. Moreover, each test was monitored by measurements of the following parameters: DO, pH, conductivity and temperature.

Results and discussion

Pilot plant studies

Both the partial nitritation and Anammox processes took place in a single reactor. The system was supplied directly with supernatant that contained high concentrations of ammonium varying from 351–714 g m^{-3} (Table 1) with an average value amounting to 568 g m^{-3}. During the process, part of the ammonium was oxidised to nitrite that reacted

Table 1 Analysis of nitrogen forms, alkalinity, COD, SS, VSS and physical parameters measured in the pilot plant

	NH_4-N (g m^{-3})	NO_2-N (g m^{-3})	NO_3-N (g m^{-3})	HCO_3^- (mmol l^{-1})	COD (g m^{-3})	SS (g m^{-3})	VSS (g m^{-3})	Conductivity (mS cm^{-1})	pH	temp. (°C)	DO (g m^{-3})
Influent											
av.	568			53.86	210	83	66	5.74	7.83		
min	351			39.5	177	50	44	2.74	7.61		
max	714			75.25	271	188	132	6.83	8.30		
st. dev.	62.7			9.1	28.12	44	30	0.58	0.10		
n	52			46	13	9	9	181	182		
Reactor											
av.						142	110	2.86	7.77	25	1.90
min						85	68	1.31	6.90	17	0.5
max						288	208	5.14	8.20	31	5.97
st. dev.						77	51	0.76	0.23	2.39	0.68
n						7	7	181	182	183	183
Effluent											
av.	175	4	37	18.37	160			2.73	7.77		
min	13	1	8	3.93	127			1.31	6.87		
max	369	24	92	33.4	202			4.38	8.19		
st. dev.	90.2	3.8	17.8	7.56	21.40			0.67	0.23		
n	52	52	52	44	11			182	183		

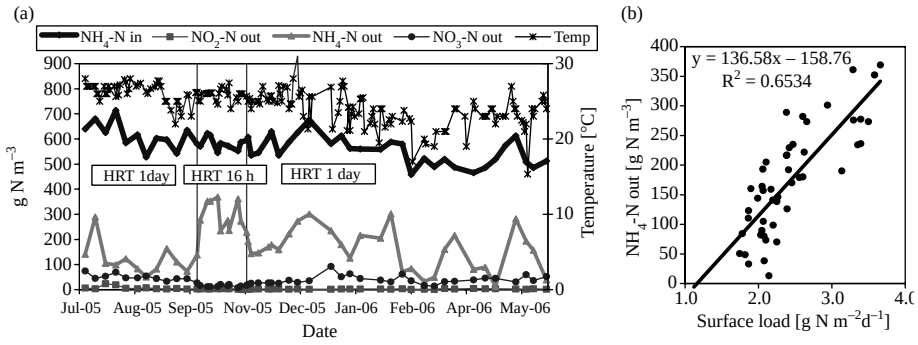

Figure 2 (a) Nitrogen and temperature variations in the partial nitritation/Anammox process; (b) correlation between removed ammonium and removed alkalinity

with remaining ammonium to dinitrogen gas and nitrates (Figure 2a). The total inorganic nitrogen elimination for the whole analysed period was 62.4 ± 13.5% on average. The nitrate nitrogen formation was measured to 6.5% (on average) of the removed ammonium nitrogen as compared with the theoretically expected value of 11%. Differences between experimental data and stoichiometry can result from the presence of heterotrophic denitrifying bacteria. It could be associated with endogenous respiration and decrease of chemical oxygen demand that was registered between influent and effluent values. The average low drop in the COD concentration was equal to 50 g m^{-3}. Suspended and volatile suspended solids (SS and VSS) were also analysed in the supernatant and in the reactor. An insignificant increase in both SS and VSS was noticed probably due to the biomass growth and biofilm detachment during the process. However, the SS and VSS concentrations in the reactor were still low. The VSS/SS ratio amounted to 0.81 ± 0.08 in the supernatant and it remained almost the same in the reactor and amounted to 0.80 ± 0.06.

Due to the fact that it was proved that the Anammox process can be operated at a temperature below the range of 30–35 °C (Szatkowska and Plaza, 2006), the process did not require additional heating as the digester supernatant temperature was at 25 ± 2.4 °C (Figure 2a and Table 1). An additional heat was supplied only during the winter period to keep the temperature stable. The average dissolved oxygen concentration measured in the bulk liquid was equal to 1.9 ± 0.7 g m^{-3}. Other researchers who worked with the MBBR technology report a DO concentration of between 0.8 and 2 g m^{-3} (Seyfried *et al.*, 2002). Under such condition it was possible to reach 75 and 71% of nitrogen removal, respectively. At the DO value equal to 5.9 g m^{-3} the nitrogen removal amounted only to 10%. According to Johansson *et al.* (1998) the nitrogen efficiency removal of 60–70% can be obtained under DO concentrations below 1 g m^{-3}. The full-scale MBBR in Germany where the deammonification process was applied gave 80% of nitrogen removal efficiency at DO in the range of 0–4 g m^{-3} (Rosenwinkel and Cornelius, 2005). Oxygen conditions of process performance and removal rates presented in this paper are comparable to the results reported by Seyfried *et al.* (2002). However, the German experiments were run under higher temperatures.

During the first period when the process was operated with an HRT of 1 day, the ammonium nitrogen load was equal to 2 g N $m^{-2}d^{-1}$ and the nitrogen removal efficiency was equal to 70 ± 9.2%. To determine the effect of nitrogen load increase on process performance, the HRT was set at 16 h which resulted in an increase of the nitrogen load to 3.5 g N $m^{-2}d^{-1}$ (Figure 2a). For that period, ammonium in the effluent exceeded 300 g m^{-3} and the average nitrogen efficiency was below 50%. However, the average nitrogen removal rate was insignificantly higher, if compared to the previous period.

It rose from 1.4 g N $m^{-2}d^{-1}$ (value for the first period) to 1.6 g N $m^{-2}d^{-1}$ (the period with HRT = 16 h).

For the period starting 7th November, the HRT was set at 1 day. At that period the effluent ammonium nitrogen concentration was measured above 200 g m^{-3}. It was mainly caused by technical problems, such as no influent supply, ineffective sludge dewatering during centrifugation and consequent pipe clogging both in the pilot plant installation and at WWTP. For that period removal rates oscillated around 1.5 g N $m^{-2}d^{-1}$. For the whole period a relationship was found between nitrogen load and ammonium concentration in the effluent (Figure 2b).

When the deammonifying process was run in two separate steps and the Anammox reactor was operating under the HRT of 3 d the highest recorded removal rate value amounted to 0.9 g N $m^{-2}d^{-1}$ (Szatkowska *et al.*, 2006). Comparing the obtained values of removal rates at two different reactor configurations it can clearly be stated that it is more efficient to perform the process in one stage with combined partial nitration and Anammox processes.

Parallel to the nitrogen utilisation, the removal of alkalinity was estimated and a linear relationship was found between the alkalinity reduction and the ammonium removal (Figure 3b). As given by nitritation process stoichiometry, approximately equal amounts of hydrogen carbonate and ammonium ions are required in order to efficiently perform the partial nitritation process. In this experiment the molar ratio of alkalinity to ammonium for the supernatant stream that supplied the pilot plant amounted to 1.33, while the ratio of removed alkalinity to removed ammonium was 1.16 on average.

During the operational period, a drop in pH value was measured. As ammonium hydrogen carbonate is the main substrate partial nitritation means, a lowering of alkalinity and a pH value decrease, the Anammox reaction to some extent can increase pH value due to cell synthesis. The partial nitritation and Anammox reactions cause a low buffering capacity and therefore pH value measurements should be a valuable parameter for process control. A strong correlation between alkalinity and pH value in the effluent was found (Figure 4a).

The process was also monitored by conductivity measurement, being an excellent indicator for two-stage technology (Szatkowska *et al.*, 2005). The removal of two main ions, ammonium and hydrogen carbonate, resulted in conductivity depletion (Figure 4b). A relationship between conductivity and measured pH values in the effluent was also found although the correlation is not linear as the pH value is a logarithmic function of the hydrogen ion concentration (Figure 4c). Therefore, for a one-stage process both pH and conductivity parameters can be used to access the process performance.

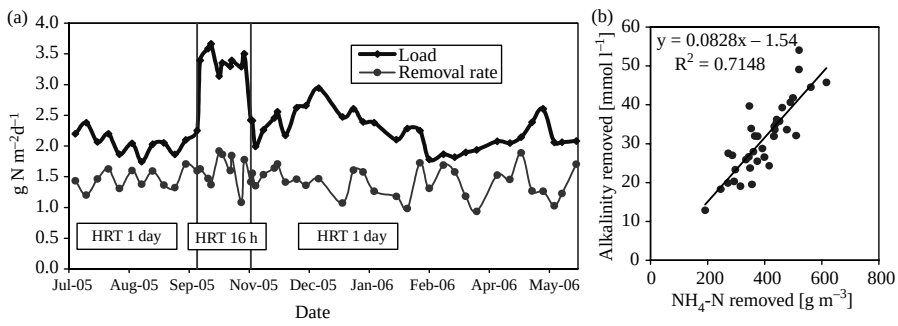

Figure 3 (a) The nitrogen load and removal rate; (b) relation between nitrogen load and ammonium in the effluent

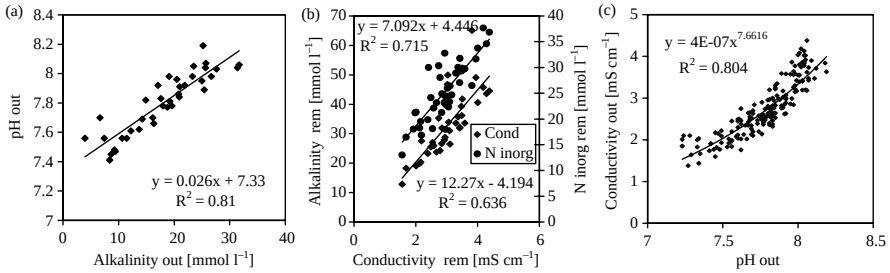

Figure 4 Relation between: (a) alkalinity and pH in the effluent; (b) removed alkalinity and removed nitrogen and conductivity; (c) pH value and conductivity in the effluent

Batch tests

The purpose of the batch tests was to estimate the nitrogen removal rate at oxygen rich and oxygen free conditions and also to investigate the influence of addition of nitrite to the batch volume. Typical nitrogen forms conversion during the batch tests are presented in Figure 5.

The results from batch tests showed that the highest nitrogen removal rates were obtained in the test under anoxic conditions (noDO) where the typical Anammox reaction was simulated. In these tests the nitrite-to-ammonium removal rates ratio of 1.2 on average was close to the stoichiometric value (1.3). The highest measured nitrogen removal rate was equal to 5.2 g N $m^{-2}d^{-1}$. The lowest nitrogen removal rates were observed in tests under aerobic conditions and the average value was 48% of the average of rates obtained in anoxic conditions with nitrite addition (as the highest value set as 100%). In tests with aerobic condition and with the addition of NO_2-N, the average nitrogen removal rate was 85% of this obtained under anoxic conditions (Figure 6a). The highest average percentage of nitrate production (in accordance to removed inorganic nitrogen) was obtained under aerobic conditions and the lowest when no oxygen was supplied (Figure 6b). In tests with DO and NO_2 the removal of nitrite nitrogen was also noticed (Figure 5c), although at lower removal rates than for ammonium and nitrite nitrogen in the test with anoxic conditions (Figure 5a).

The obtained results indicate that the nitrite concentration seems to be the rate-limiting factor for the Anammox reaction in a single stage reactor. The addition of nitrite in the test under aerobic conditions increased the nitrogen removal rates in comparison with tests where ammonium nitrogen was the only substrate. The highest obtained removal rate for simultaneous partial nitritation/Anammox (DO) amounted to 2.96 g N $m^{-2}d^{-1}$.

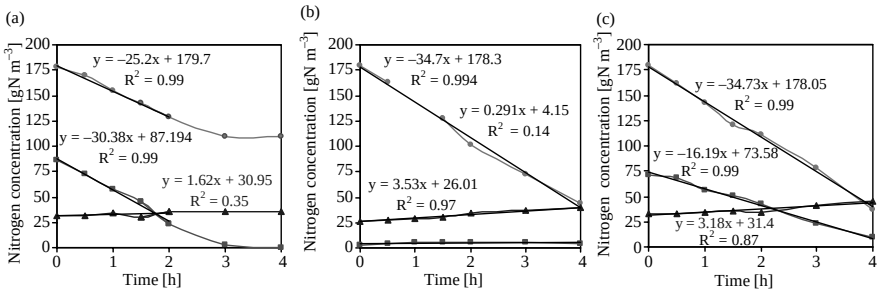

Figure 5 Nitrogen conversions in the batch tests: (a) anoxic conditions (noDO); (b) aerobic conditions (DO); (c) aerobic conditions with addition of an $NaNO_2$ solution (DO + NO_2-N) -●- NH4-N; -■- NO_2-N; -▲- NO_3-N

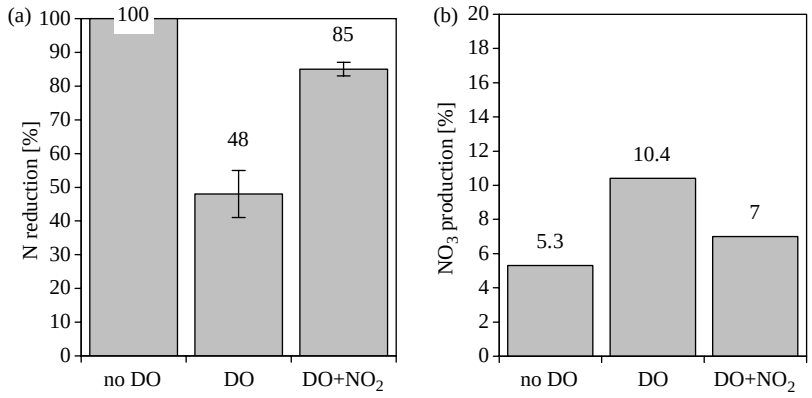

Figure 6 (a) An average percentage nitrogen removal in anoxic (no DO), aerobic (DO) and aerobic conditions with external nitrites supply (DO + NO_2); (b) average percentage production of nitrates

The starting concentrations of nitrite nitrogen were comparable to tests under anoxic and aerobic conditions with external nitrites supply. However, the nitrogen removal rates were higher under anoxic conditions. It could be explained by two phenomena, competition of aerobic and anaerobic ammonium oxidisers within the biofilm or by partial penetration of the oxygen into Anammox layer of the biofilm in aerobic condition and thereby lowering the reaction rate due to inhibition of the Anammox bacteria.

Conclusions

- The biofilm bacterial culture was able to perform simultaneously two processes, Anammox and partial nitritation in a single-stage reactor at technical-scale pilot plant.
- The maximum nitrogen removal rate obtained for the pilot plant during 1-year experimental period at temperature of approximately 25 °C amounted to 1.92 g N $m^{-2}d^{-1}$ (1.45 g N $m^{-2}d^{-1}$ on average).
- A strong correlation was found between removed alkalinity and removed ammonium during the process.
- Conductivity and pH turned out to be suitable tools for process monitoring. A strong relation was found between removed nitrogen, removed alkalinity, conductivity and pH values in the effluent.
- The highest nitrogen removal rate in the batch test simulating the simultaneous partial nitritation/Anammox process at oxygen-rich conditions amounted to 2.96 g N $m^{-2}d^{-1}$, for simulation of the Anammox process only under oxygen free conditions it was equal to 5.2 g N $m^{-2}d^{-1}$.
- The batch tests showed that in a single-stage process for nitrogen removal, the nitrite production during the partial nitritation seems to be the rate-limiting step for further Anammox reaction.

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