Dissolved oxygen as a factor influencing nitrogen removal rates in a one-stage system with partial nitritation and Anammox process

G. Cema, E. Płaza, J. Trela and J. Surmacz-Górska

ABSTRACT

A biofilm system with Kaldnes biofilm carrier was used in these studies to cultivate bacteria responsible for both partial nitritation and Anammox processes. Due to co-existence of oxygen and oxygen-free zones within the biofilm depth, both processes can occur in a single reactor. Oxygen that inhibits the Anammox process is consumed in the outer layer of the biofilm and in this way Anammox bacteria are protected from oxygen. The impact of oxygen concentration on nitrogen removal rates was investigated in the pilot plant (2.1 m^3), supplied with reject water from the Himmerfjärden Waste Water Treatment Plant. The results of batch tests showed that the highest nitrogen removal rates were obtained for a dissolved oxygen (DO) concentration around 3 g O_2 m^{-3}. At a DO concentration of 4 g O_2 m^{-3}, an increase of nitrite and nitrate nitrogen concentrations in the batch reactor were observed. The average nitrogen removal rate in the pilot plant during a whole operating period oscillated around 1.3 g N $m^{-2}d^{-1}$ (0.3 $\pm$ 0.1 kg N $m^{-3}d^{-1}$) at the average dissolved oxygen concentration of 2.3 g O_2 m^{-3}. The maximum value of a nitrogen removal rate amounted to 1.9 g N $m^{-2}d^{-1}$ (0.47 kg N $m^{-3}d^{-1}$) and was observed for a DO concentration equal to 2.5 g O_2 m^{-3}. It was observed that increase of biofilm thickness during the operational period, had no influence on nitrogen removal rates in the pilot plant.

Key words | Anammox, biofilm, deammonification, Kaldnes, nitrogen removal, reject water

G. Cema (corresponding author)
E. Płaza
J. Trela
Department of Land and Water Resources Engineering,
Royal Institute of Technology (KTH),
100 44 Stockholm,
Sweden
E-mail: *grzegorz.cema@polsl.pl*

G. Cema
J. Surmacz-Górska
Environmental Biotechnology Department,
Silesian University of Technology,
44 100 Gliwice,
Poland

INTRODUCTION

The biofilm process is one of the biological wastewater treatment processes which purifies wastewater by utilising microbes attached to solid surfaces kept in contact with wastewater (Iwai & Kitao 1994). Biofilm wastewater treatment systems provide the basis for optimisation of the volumetric conversion capacity. Reactors with biofilm assure long biomass residence times even if the hydraulic retention time is low. It makes them especially suitable when treatment requires slow growing organisms with a low maximum growth rate constant like the Anammox bacteria. In the CANON process, aerobic ammonium oxidising bacteria and the Anammox bacteria perform two sequential reactions simultaneously under oxygen-limited conditions (Kartal *et al.* 2007). In the biofilm system both types of bacteria, nitrifiers and Anammox, can co-exist in one reactor due to oxygen and oxygen free zones within the biofilm depth (Sliekers *et al.* 2003). 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 (van Hulle *et al.* 2003). Hao *et al.* (2002a, b) demonstrate, that optimal dissolved oxygen (DO) concentration is related to a certain ammonium surface load. However, this optimum oxygen level also depends on the biofilm thickness and density, boundary layer thickness, the Chemical Oxygen Demand (COD) content of the influent and the temperature (Van Hulle *et al.* 2010). With a defined ammonium surface load, a thicker biofilm is required and a higher dissolved oxygen concentration is necessary at lower temperature in the reactor. A thin biofilm needs a lower dissolved oxygen concentration (Paredes *et al.* 2007). In the Moving Bed Biofilm Reactor (MBBR), the oxygen concentration has a great influence on the nitrification rate when the oxygen is a rate-limiting factor (Hem *et al.* 1994). Additionally, it is proved that nitrite production rate is the rate-limiting step for the Anammox process in a single stage system (Szatkowska *et al.* 2007).

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A biofilm system, with Kaldnes rings as a carrier material, was used in these studies to cultivate bacteria responsible for both partial nitritation and the Anammox process. Influence of dissolved oxygen (DO) concentrations on the nitrogen removal rates was investigated at a technical-scale pilot plant, supplied with reject water from Himmerfjärden Waste Water Treatment Plant (WWTP).

METHODOLOGY

Pilot plant

The Moving Bed Biofilm Reactor, with total working volume of 2.1 m^3 has been operated at the Himmerfjärden WWTP. The reactor was filled up to 50% of its volume with Kaldnes biofilm carriers with an effective surface area of 250 m^2 m^{-3}. The Kaldnes carriers in the reactor were in a continuous movement caused by vertical mixers and air supply from the bottom of the reactor. The system was continuously supplied with supernatant coming directly from dewatering of digested sludge. During the research period the flow rate to the reactor was equal to 2.1 m^3 d^{-1}.

Previous work in this area (Szatkowska & Płaza 2006), demonstrated that the Anammox process could be operated at a temperature range below 30–35 °C, therefore, the process proceeded at the natural temperature of the incoming supernatant. Only during winter was an additional heater supplied to keep the temperature above 20 °C. The average operational temperature was equal to 24 ± 2.5 °C. The scheme of the pilot plant is presented in Figure 1. Its detailed description and description of the process start-up can be found in Cema *et al.* (2006, 2010).

During the study, samples of both influent and effluent were collected and analysed for inorganic nitrogen forms, alkalinity, total nitrogen and COD. Chemical analyses were conducted using the Dr Lange test tubes. Before analysis all samples were filtrated through a 25 µm prefilter followed by a 0.45 µm filter. Moreover, the process was monitored on a daily basis by manual measurements of the physical parameters (flow rate, pH, temperature, conductivity and DO in the bulk liquid) in the influent, effluent and within the reactor. Additionally, the pilot plant was equipped with on-line devices for monitoring oxygen concentrations (Cerlic BB2), pH (Analon pH 10 Contronic) and temperature. The pilot plant was also provided with two conductivity meters (Dr Lange Analon Cond 10) located in the buffer tank and settling tank, respectively.

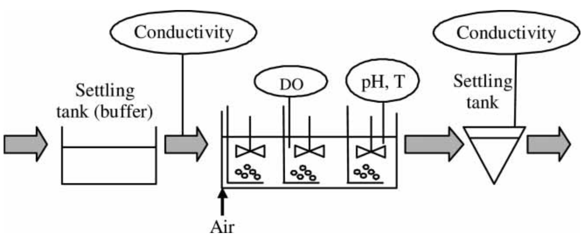

Figure 1 | Flow diagram of the pilot-plant with a one-stage partial nitritation and the Anammox processes.

In order to determine a biofilm thickness, several Kaldnes carriers were randomly collected from the pilot plant and then cut into thin slices to get a proper picture of the biofilm. Then the carriers were scanned with a resolution of 2400 pixels per inch and the biofilm thickness was measured using Gimp2 software (GNU Image Manipulation Program). Twenty different measurements of biofilm thickness were taken for each carrier.

Batch tests

A series of 20 tests was made to study the influence of DO on nitrogen removal rates. Tests were run in four parallel bottles, each of 1 L working volume, filled up to 50% of its volume with the Kaldnes material taken from the reactor. All tests were performed in a water bath to keep temperature stable at 25 °C. Additionally, the magnetic stirrers were used to assure appropriate mixing of medium during the tests. The process was stimulated at DO concentrations ranging from 0.5 to 4 g O_2 m^{-3}. Before the tests, supernatant and Kaldnes carriers from the reactor were stirred for 24 hours without feeding in order to remove substrates and to starve bacteria. After 24 hours, NH_4Cl solution was added to obtain the required initial ammonium concentration. The test duration was 4 hours and the samples were collected every 30 minutes for analysis of inorganic nitrogen forms. In parallel with sampling, DO concentration, pH and conductivity were measured.

RESULTS AND DISCUSSION

Both partial nitritation and the Anammox processes occurred in a single reactor. The system was supplied with supernatant containing high concentrations of ammonium nitrogen, varying from 362 to 945 g NH_4-N m^{-3} with the average value amounting to 571.4 ± 96.7 g NH_4-N m^{-3}. The average value of ammonium nitrogen load was equal to 2.3 ± 0.3 g N m^{-2} d^{-1} (0.6 ± 0.1 kg N $m^{-3}d^{-1}$). During the process, part of the ammonium was oxidised to nitrite, which reacted with the remaining ammonium to dinitrogen gas and nitrate was produced by Anammox. The average total inorganic nitrogen reduction for the whole analysed period was 57.5 ± 12.6%. It resulted in nitrogen removal rates reaching a value of 1.3 ± 0.3g N m^{-2} d^{-1} (0.3 ± 0.1 kg N $m^{-3}d^{-1}$) (Figure 2). At the same time the average removal of ammonia nitrogen was 65.5 ± 13.7%, indicating continued ammonia concentration in the effluent of pilot plant. The mean value of ammonium nitrogen in the reactor effluent was 201.7 ± 102.2 g NH_4-N m^{-3}. Such high effluent concentrations of the ammonium nitrogen were mainly caused by technical problems as feed flow failures and ineffective sludge dewatering during centrifugation following pipe clogging both in the pilot plant installation and at the WWTP.

During the operational period, about 41.2 g NO_3-N m^{-3} of nitrate were produced, which corresponded to approximately 11.1% of the removed ammonium nitrogen. These values are very close to a stoichiometric ratio, for the Anammox process (11%) (Strous *et al.* 1998). The presence of heterotrophic denitrifying bacteria in the system could be expected. However, the COD concentration in the reactor influent was low, i.e. the average value equal to 213 g O_2 m^{-3}. What is more, the ratio between COD removal (COD_{rem}) and inorganic nitrogen reduction ($N_{red/inorg}$) (mean $COD_{rem}/N_{red,inorg.} = 0.2$) was very low. The average drop in the COD concentration was equal to 61 g O_2 m^{-3} (only 27% COD reduction). These results indicate that partial nitritation of ammonium to nitrite and the subsequent Anammox process were the main two processes in the reactor.

The DO concentration (Figure 2), as one of the most important parameters influencing the simultaneous partial nitritation/Anammox process, was monitored during the experiment. Since ammonium nitrogen concentration in the bulk liquid is much higher than the oxygen or nitrite nitrogen concentration, ammonium diffusion will not limit the process. Nitrite is produced in an outer layer by nitrifiers but is mainly consumed by anammox bacteria in an inner layer, which means that oxygen is the main limiting factor controlling the overall rate of the partial nitritation/Anammox process in biofilm reactors. The average value of the DO concentration during the whole operational period was 2.3 ± 0.8 g O_2 m^{-3}. Great fluctuations of oxygen concentrations in the reactor (Figure 2) were mainly due to centrifuge breakdowns that resulted in inflow shortages, as well as electricity breakdowns that stopped aeration.

Generally, it can be stated that in a one-step process, the nitrogen removal rate in the Anammox process is dependent on the nitrite production rate during the nitritation process. Nitrification rate in the fixed-film system is often limited by the bulk liquid DO concentration. Using Equation (1) it is possible to determine the DO concentration where oxygen is flux-limited (Iwai & Kitao 1994; Metcalf & Eddy 2004).

$$S_{ba} < \frac{D_{wd} \cdot \nu_a \cdot mw_a}{D_{wa} \cdot \nu_d \cdot mw_d} S_{bd} \qquad (1)$$

where ν_d – molar stoichiometric reaction coefficient for electron donor NH_4-N (moles) = 1, ν_a – molar stoichiometric reaction coefficient for electron acceptor O_2 (moles) = 1.5, S_{ba} – bulk liquid electron acceptor substrate concentration,

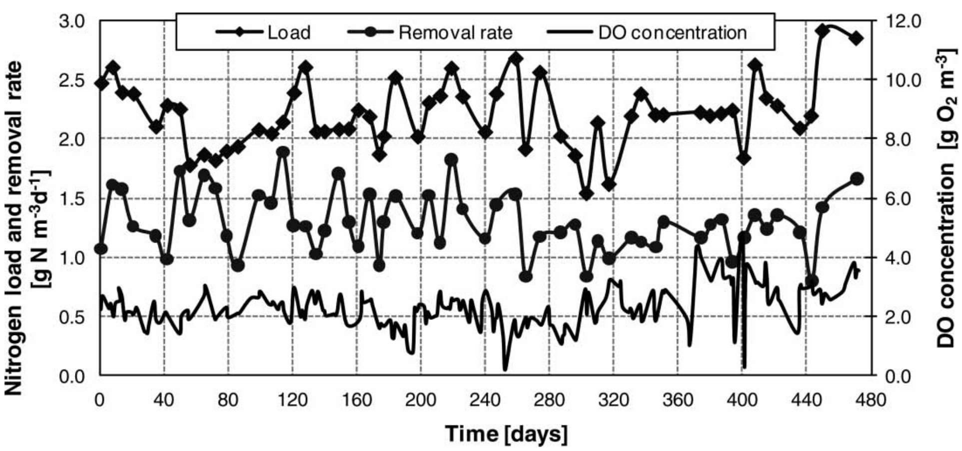

Figure 2 | Nitrogen loads, nitrogen removal rates and DO concentrations in the pilot plant.

S_{bd} – bulk liquid electron donor substrate concentration, D_{wd} – diffusivity coefficient of electron donor in water (NH_4-N) = 1.6 cm^2 d^{-1} (at 20 °C), D_{wa} – diffusivity coefficient of electron acceptor in water (O_2) = 2.6 cm^2 d^{-1} (at 20 °C), mw_a – molecular weight of electron acceptor O_2 = 32; mw_d – molecular weight of electron donor NH_4-N = 14.

By solving this equation for nitritation, it can be stated that when the oxygen to ammonium concentration ratio was below 2.1 g O_2 (g NH_4-N)$^{-1}$, the oxygen concentration was nitrification rate limiting. However, the DO concentration in the reactor was not the only parameter deciding on nitrogen removal rate. Other ones include nitrogen load to the reactor and reactor temperature. In Figure 3(a), the relationship between nitrogen surface loads and nitrogen removal rates is shown. The nitrogen removal rate is increasing along with increase of nitrogen load to the reactor. On the other hand, when nitrogen load exceeded 2.5–3.0 g N m^{-2}d^{-1} the increase of nitrogen removal rate ceased. Additionally, the study performed by Szatkowska & Płaza (2006) showed that the temperature impact on a partial nitritation/Anammox process was significant, especially in the case of a sudden temperature decrease. Probably, the effect of temperature, nitrogen load to the reactor and others factors such as pH-value and NO_2-N concentration in the reactor caused the correlation between oxygen concentrations and nitrogen removal rates in the pilot plant to be insignificant. In Figure 3(b), nitrogen removal rates in the pilot plant for different DO ranges are presented. The best results were obtained for the DO range from 2.6 to 3.0 g O_2 m^{-3} and, surprisingly, for 1–1.5 g O_2 m^{-3}. Hao *et al.* (2002a) assumed that the optimum DO concentration level was related to a specific ammonium surface load in the biofilm. Authors showed that higher ammonium surface loads required higher DO levels in the bulk liquid to achieve the maximum nitrogen removal. In the present study, the ammonium nitrogen load for the DO range from 2.6 to 3.0 g O_2 m^{-3} was 2.31 g N m^{-2}d^{-1}; only slightly higher than for 1–1.5 g O_2 m^{-3} equal to 2.20 g N m^{-2}d^{-1}. Generally, for all DO ranges, ammonium nitrogen load was comparable (Figure 3(b)) and it is difficult to find direct correlation between ammonium surface load and nitrogen removal rates in relation to DO concentration in the bulk liquid.

Control of biofilm thickness is an important factor in stable operation of biofilm reactors, therefore, the biofilm thickness was also investigated during the study. Three measurements were performed at the beginning of the described research period and then after 7 and 12 months of reactor operation. The increase in biofilm thickness during this time period was observed. At the beginning, the average biofilm thickness was equal to 0.27 mm and it rose to the average of 0.43 mm after 7 months in 2006 and further up to 0.77 mm after one year of investigation. In Figures 4(a), 4(b) and 4(c) changes in biofilm thickness can be observed.

Surprisingly, an increase in biofilm thickness did not result in an increase of nitrogen removal rate or nitrogen removal efficiency. Additionally, a slight decrease in nitrogen removal rates can be observed after one year of rector operation. It can be suggested that other parameters such as nitrogen loading rate, temperature and DO had the main influence on nitrogen removal in the reactor.

The biofilm thickness in a single Kaldnes carrier was not constant and the given values are the average ones. The biofilm thickness values in a single Kaldnes carrier are presented in Figure 5. It can be seen, that the thickness of biofilm varies significantly and that the roughness of the biofilm increases with the biofilm thickness increase.

Batch tests

The purpose of the batch tests was to determine the best oxygen conditions for simultaneous partial nitrification

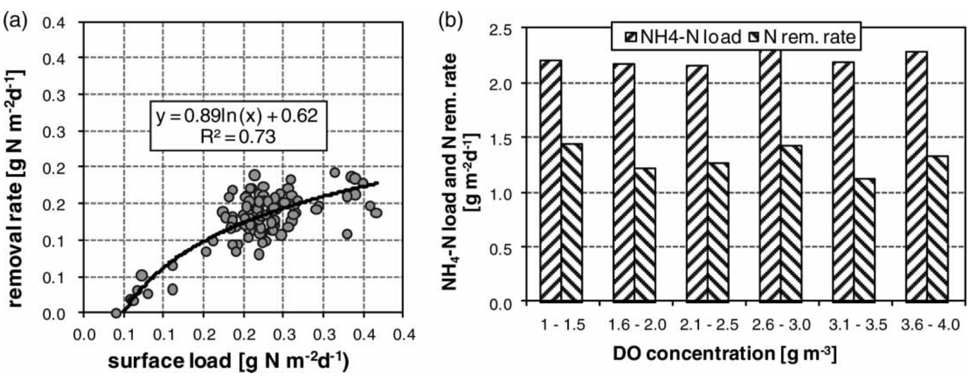

Figure 3 | (a) Relationship between nitrogen load and nitrogen removal rates and (b) nitrogen removal rates in the pilot plant for different DO ranges.

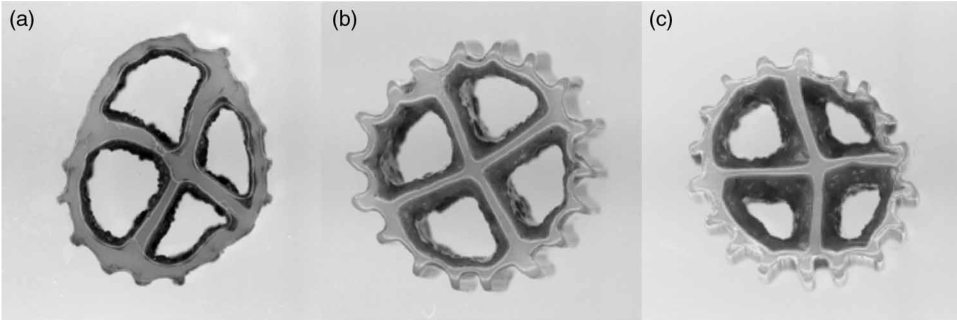

Figure 4 | Changes in biofilm thickness during pilot plant operation: (a) beginning of the described research period, (b) after 7 months and (c) after 12 months.

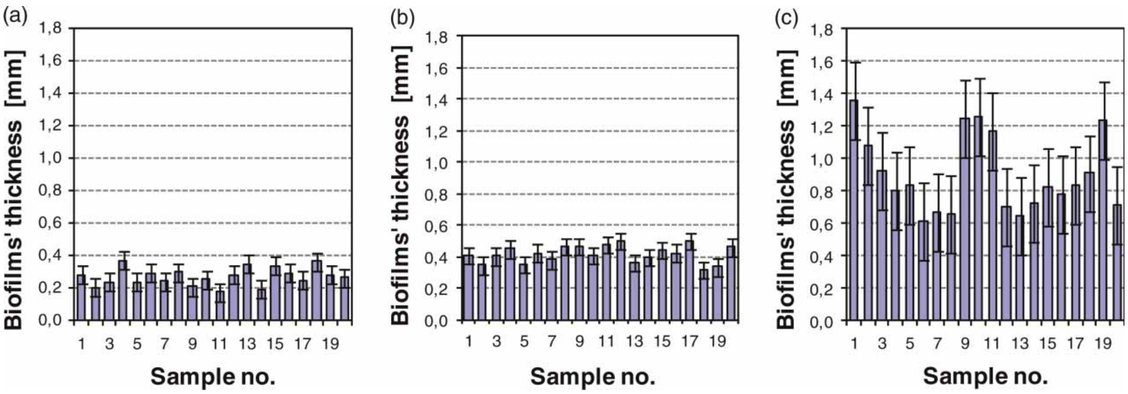

Figure 5 | Biofilm thickness in a single Kaldnes carrier: (a) beginning of the described research period, (b) after 7 months and (c) after 12 months.

and the Anammox processes. Nitrogen conversions in batch tests performed at low and high dissolved oxygen concentration are presented in Figures 6(a) and 6(b). These results clearly indicate that dissolved oxygen concentration in the reactor has a very strong influence on the nitrogen removal rates in the one-stage deammonification process.

The batch tests showed that increase in DO concentration gave higher nitrogen removal rates, but also higher production of nitrate nitrogen (Figures 7(a) and 7(b)). Hem

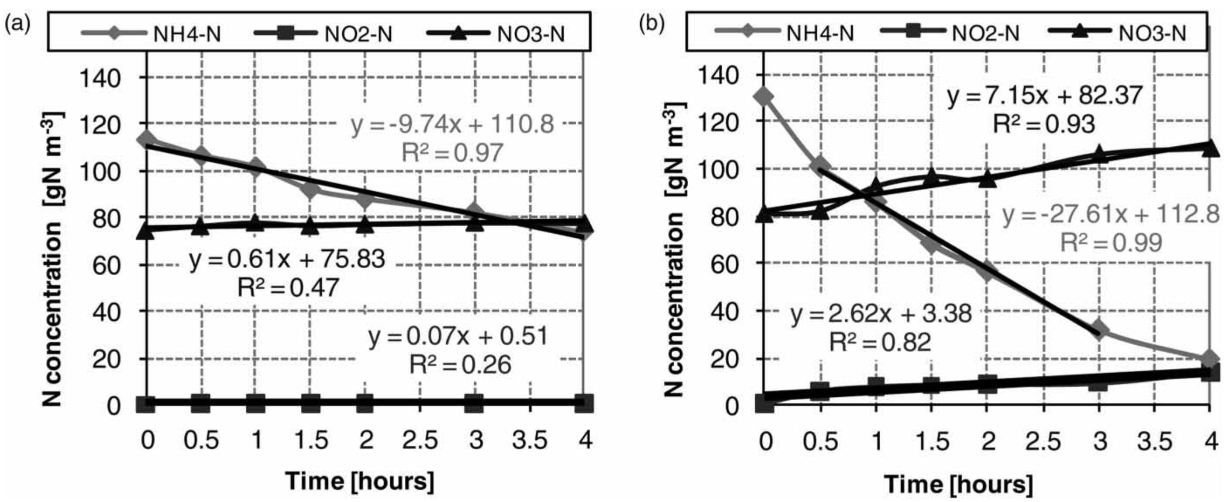

Figure 6 | Nitrogen conversions during batch tests with medium from the deammonification reactor: (a) low DO concentration 0.55 g O_2 m^{-3} and (b) high DO concentration 4.27 g O_2 m^{-3}.

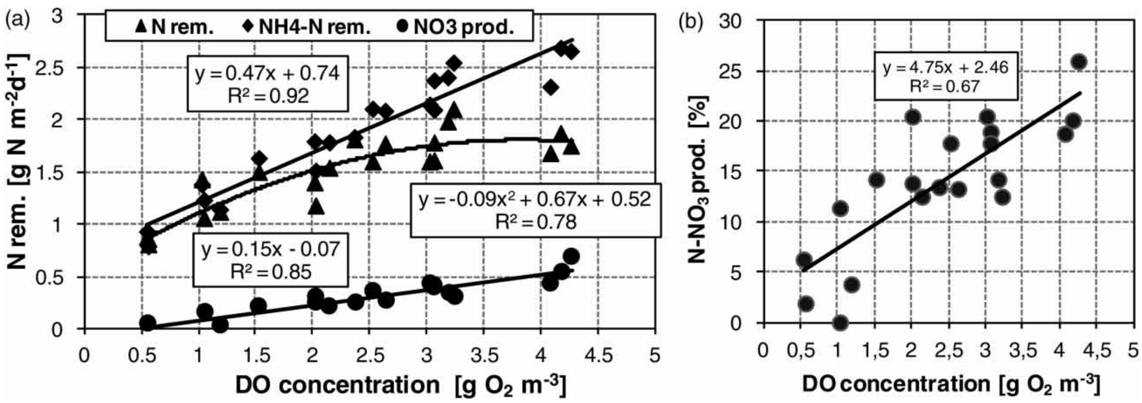

Figure 7 | (a) Nitrogen removal rates in batch tests at different DO concentrations and (b) nitrate production during batch tests at different DO concentrations.

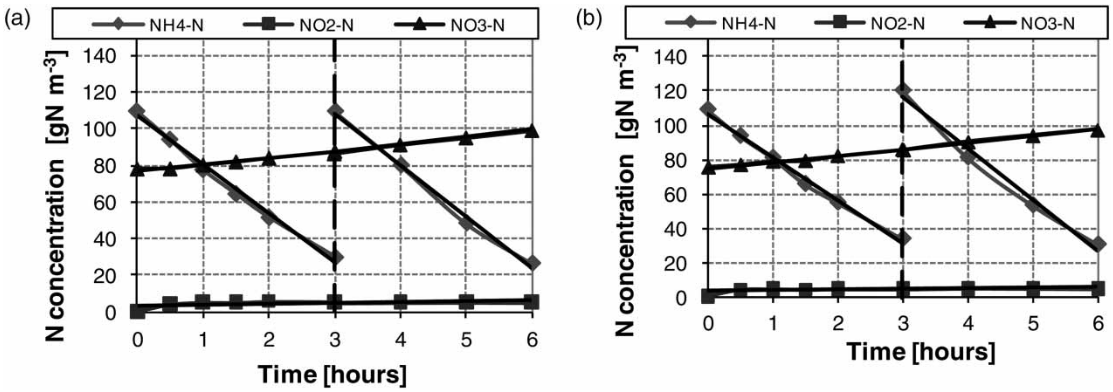

Figure 8 | (a) and (b) Nitrogen profiles during batch tests with a supplementary addition of artificial ammonium nitrogen.

et al. (1994) reported that in the moving bed biofilm reactor, the oxygen concentration has great influence on the nitrification rate if oxygen is a rate limiting factor. In that situation, the nitrification rate was close to a first-order function of the oxygen concentration, indicating liquid film diffusion to be the important rate limiting mechanism. In the one stage partial nitritation/Anammox process, the nitritation rate is a bottleneck of the overall nitrogen removal rate. On the other hand, oxygen also inhibits the Anammox process. An increase of aeration efficiency and oxygen concentrations in the bulk liquid causes a decrease of the anaerobic layer of biofilm, followed by the Anammox efficiency decrease.

In a series of batch tests, the highest nitrogen removal rates were obtained for DO equal to 3 and 4 g O_2 m^{-3}. In tests carried out at the mean DO concentration of 3.06 ± 0.6 g O_2 m^{-3}, the average nitrogen removal rates were 1.8 ± 0.22 g N $m^{-2}d^{-1}$, ammonium removal was 2.31 ± 0.19 g NH_4-N m^{-2} d^{-1} while nitrate production was 21.7 ± 5.2%. When DO was increased to 4.18 ± 0.09 g O_2 m^{-3} removal rates for nitrogen and ammonium were 1.76 ± 0.1 g N $m^{-2}d^{-1}$ and 2.55 ± 0.21 g NH_4-N $m^{-2}d^{-1}$, respectively and nitrate production was 31.5 ± 7.3%. Moreover, a nitrite production rate of 0.22 ± 0.1 g NO_2-N m^{-2} d^{-1} was observed as well, which resulted in increase of nitrite concentration in the batch reactor. These results suggested that increase of dissolved oxygen concentration above 3 g O_2 m^{-3} would not result in increase of nitrogen removal rates.

To examine the influence of a longer aeration time on the nitrogen removal rate, two additional tests were performed. A NH_4Cl solution was added during the tests after 3 hours of performance, when the ammonium concentration decreased below 40 g m^{-3}. The solution was added in order to extend the test duration. The DO concentrations during these test were around 3 g O_2 m^{-3} and this value was selected as the one for which the best nitrogen removal rates were achieved. The results are presented in Figures 8(a) and 8(b).

The addition of ammonium nitrogen after 3 hours of test and additional extension of the test did not affect the nitrogen elimination. The nitrogen removal rates after ammonium addition at the third hour of the test, were comparable with those achieved at the beginning of the test (Figures 8(a) and 8(b)). These results proved that the DO concentration of 3 g O_2 m^{-3} does not inhibit nitrogen removal rates during a long term operation.

CONCLUSIONS

- The maximum nitrogen removal rate was 1.9 g N m^{-2} d^{-1}, while the average removal value was 1.31 ± 0.31 g N m^{-2} d^{-1}, during the study at the pilot plant.
- It was shown that the best results were achieved for oxygen concentrations around 3 g O_2 m^{-3} with the average nitrogen removal rates of 1.8 ± 0.31 g N m^{-2} d^{-1}. The highest nitrogen removal rates, 2.09 g N $m^{-2}d^{-1}$, were observed at the DO concentration of 3.15 g O_2 m^{-3}, in batch tests.
- Increase of a nitrite concentration in the batch reactor as well as decrease of inorganic nitrogen removal rates were observed at the DO concentration of 4 g O_2 m^{-3}. It can lead to nitrite accumulation in the reactor and in consequence to a loss of the Anammox process.
- Increase in a biofilm thickness had no influence on nitrogen removal rates in the pilot plant.
- The process with partial nitritation and the Anammox process running in parallel can be applied as a separate treatment of supernatant, which would finally lead to nitrogen load reduction in the WWTP's effluent.

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