

# INFLUENCE OF AERATION STRATEGY ON BEHAVIOUR OF DIFFERENT MICROORGANISMS IN DEAMMONIFICATION PROCESS

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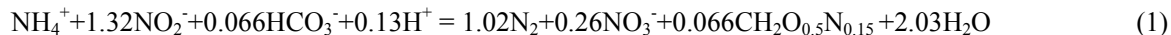
## SUMMARY

In deammonification process applied in a moving bed biofilm reactor (MBBR), oxygen is an important parameter for the process performance and efficiency. The objective of this study was to investigate the influence of aeration strategies on behavior of different microorganisms in deammonification process. Experiments were carried out in MBBR of 200 L volume filled with 40% of Kaldnes biofilm carriers. Activities of different groups of bacteria were studied by Specific Anammox Activity (SAA), Oxygen Uptake Rate (OUR) and Nitrate Utilization Rate (NUR) tests. The results showed that activity of anammox bacteria could be enhanced by introducing non-aerated phase (intermittent aeration) and high nitrogen load. Ammonium oxidizers (AOB) activity in the biofilm was influenced by the oxygen concentration in the bulk liquid. N<sub>2</sub>O production was mainly due to ammonium oxidizers in aerated phase and heterotrophic denitrifiers in non-aerated phase.

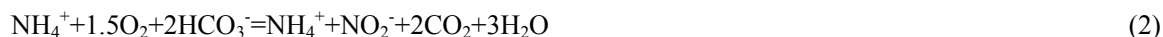
**KEYWORDS:** Deammonification process, moving bed biofilm reactor (MBBR), aeration strategies, microorganism's activity.

## INTRODUCTION

The serious global problem of eutrophication has driven the research on improving nitrogen removal in wastewater treatment process, especially for the highly concentrated ammonium streams, such as landfill leachate and reject water from digester [4, 2]. Many new technologies have been developed based on the anammox (Anaerobic Ammonium Oxidation), which is an effective and low cost process [15]. In anammox reaction (1), ammonium and nitrite are converted into dinitrogen gas under anaerobic condition and produce a small amount of nitrate simultaneously [12].



In order to remove ammonium from wastewater with anammox process, a part of ammonium should be first oxidized to nitrite by ammonium oxidizers (AOB) (2) and the proper ratio between produced nitrite and remaining ammonium is around 1.32.



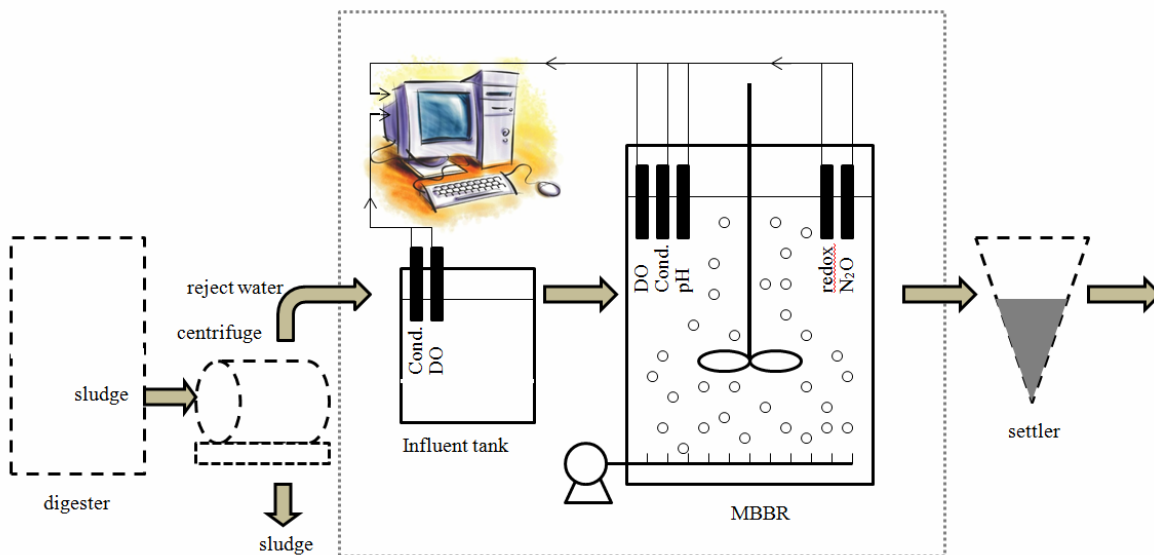
In one stage moving bed biofilm reactor (MBBR) operated with a combination of nitrification and anammox, named deammonification process, two groups of microorganisms, ammonium oxidizers (AOB) and anammox bacteria, are dominating bacteria in the system. However, the presence of the heterotrophic bacteria and nitrite oxidizers (NOB) can not be avoided especially if there is biodegradable carbon source available and high oxygen concentration supplied [20]. Previous studies showed that DO was a significant parameter influencing the nitrogen removal rate and

activity of different microorganisms in the system [1,7]. DO concentrations should stay at a certain level to allow AOB to produce a sufficient amount of  $\text{NO}_2^-$ -N for anammox reaction but also not too high  $\text{NO}_2^-$ -N level to cause anammox inhibition effect [8] or increasing nitrite oxidizing bacteria (NOB) growth [9]. Intermittent aeration introduces alternating aerated phase (aerobic) and non-aerated phase (anaerobic) inside the system. Two benefits could be obtained from intermittent aeration: a) limited oxygen concentration leads to  $\text{NO}_2^-$  enrichment but less  $\text{NO}_3^-$  production; b) anammox bacteria could decrease the possibility of inhibition from oxygen during non-aerated phase [21].

In this study, an investigation has been carried out to evaluate the activity of different groups of microorganism in the biofilm, such as nitrifiers, heterotrophic bacteria, anammox bacteria and denitrifiers in one stage deammonification moving bed biofilm reactor under different operation conditions, especially under various aeration strategies and different nitrogen loads.

## MATERIALS AND METHODS

**Pilot plant** A moving bed biofilm reactor (MBBR), with working volume of 200 L, was operated at Hammarby Sjöstadsvverk research station for a period of 1.5 years. The reactor was filled with 80 L of Kaldnes biofilm carriers (K1), which are made of polyethylene and have shapes of rings with cross inside. The height of each ring is 7 mm and diameter is 10 mm. The specific surface area of the kaldnes biofilm carrier equals to  $500 \text{ m}^2/\text{m}^3$ . The reactor was continuously fed with reject water from anaerobic digester with ammonium concentration ranging from 800 to 1000  $\text{mg NH}_4^+$ -N/l. Table 1 presents the characterization of the reject water. The temperature inside the reactor was maintained at 25 °C. On-line instruments were installed to measure physical parameters, for instance conductivity, pH, DO and redox potential value. The scheme of the pilot plant is shown in Figure 1. The operation time was divided into 7 periods with varying DO concentrations, aeration strategies and nitrogen loads (Table 2). Concentrations of different nitrogen compounds were analysed each week and Kaldnes biofilm carriers were taken out from the pilot plant reactors for activity tests during the operation time.



**Figure 1.** The scheme of the pilot scale MBBR for deammonification process.

**Table 1.** Characteristics of influent supernatant.

Influent	NH <sub>4</sub> -N (mg/l)	NO <sub>2</sub> -N (mg/l)	NO <sub>3</sub> -N (mg/l)	Alkalinity (mmol/l)	COD <sup>1</sup> (mg O <sub>2</sub> /l)	Conductivity <sup>2</sup> (mS/cm)	pH <sup>2</sup>
av.	977.1	0.04	1.84	77.01	681.17	9.48	8.09
Min.	817.5	0.00	1.32	63.40	390	6.12	7.60
Max.	1110	0.11	2.66	112.00	1280	11.36	8.46
st.dev.	76.0	0.03	0.46	8.86	202.42	1.00	0.25
n	73	11	11	71	49	375	84

<sup>1</sup> COD concentration in the filtrated sample (0.45µm) <sup>2</sup>Daily average value from on-line measurements.

After adaptation time for biomass in period I, different aeration strategies (which included different DO concentrations and the ratio between non - aerated and aerated phase duration R) were introduced into the system from period II to V. The same DO (3 mg/l) but different R (0,1/3) was applied for reactor operation in period II and IV. For period III and IV, the same R (1/3) but different DO (3 mg/l, 3.5 mg/l) was used in the study. A lower nitrogen load, comparing with previous study, was applied in period VI and VII varied between 2.52 and 2.62 gN/ m<sup>2</sup>·d.

**Table 2.** Operation periods for the pilot plant.

Period	I	II	III	IV	V	VI	VII
Nitrogen load (g N/m <sup>2</sup> ·d)	2.58	3.86	3.59	3.60	3.65	2.52	2.62
Aeration DO (mg/l) <sup>1</sup>	2-2.5	3.0	3.5	3.0	3.5	1.5	1.5
strategies Intermittent aeration	0 <sup>2</sup>	0 <sup>2</sup>	1/3 <sup>3</sup>	1/3 <sup>3</sup>	1 <sup>4</sup>	0 <sup>2</sup>	1/3 <sup>3</sup>
Temperature (°C)	25	25	25	25	25	25	25

<sup>1</sup> Dissolved oxygen concentration during aerated phase <sup>2</sup> Continuous aeration

<sup>3</sup>45 min of aerated phase and 15 min non-aerated phase in each hour

<sup>4</sup> 30 min of aerated phase and 30 min of non-aerated phase in each hour

**Specific Anammox Activity (SAA) tests** The measurement was performed according to the methodology presented in [3] based on the measurement of nitrogen gas production. A vial with total volume of 38 ml was used in the test and temperature was kept at 25°C. 15 Kaldnes biofilm carriers and phosphate buffer were inoculated inside the vial. The vial was then closed with a gas tight coated septum. NH<sub>4</sub>Cl and NaNO<sub>2</sub> solutions were added to reach the substrate concentration of 70 g NH<sub>4</sub><sup>+</sup>-N /l and 70 mg NO<sub>2</sub><sup>-</sup>-N /l. Initial pH was fixed at 7.8. Gas pressure due to nitrogen production by anammox bacteria was checked each 30 min by pressure transducer (Centrepoint Electronics). Specific Anammox Activity was calculated based on the maximum slope of the curve with accumulative pressure along the time.

**Oxygen uptake rate (OUR) tests** The tests aimed to evaluate the activities of heterotrophic bacteria, AOB (mainly *nitrosomonas sp.*) and NOB (mainly *nitrobacter sp.*) based on measurement of the oxidation rate of organic matter content, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>2</sub><sup>-</sup>-N by subsequent addition of selective inhibitors of *nitrobacter sp.* and *nitrosomonas sp.*, which were NaClO<sub>3</sub> and allylthiourea (ATU), respectively, into testing sample [13]. A volume of 100 ml of Kaldnes biofilm carriers were used in the test, which was performed in a 1.56 L 3-neck glass bottle. Diluted reject water was used to have 100 mgNH<sub>4</sub><sup>+</sup>-N/l and COD concentration of 100 mgO<sub>2</sub>/l. The test was performed at 25°C. 17 mM NaClO<sub>3</sub> and 12 mg/l ATU were added, respectively, during the test [5], which was enough for inhibiting nitrosomonas and nitrobacter, respectively. The duration of the test was 15 min and triplicate tests were done for each sample.

**Nitrate utilization rate (NUR) tests** The test was performed to evaluate the heterotrophic denitrifiers activity by the measurement of maximum nitrate utilization rate. A container of 1.5 L was used and was filled with 1 L diluted reject water. Initial  $\text{NO}_3^-$ -N concentration of 100 mg/l was achieved by adding  $\text{NaNO}_3$  solution. Nitrogen gas was supplied to the volume of container during the tests and the duration of each test was 4 hours. Liquid samples were taken each hour and concentration of  $\text{NO}_3^-$ -N was analysed by Dr Lange tests (LCK340) [16].

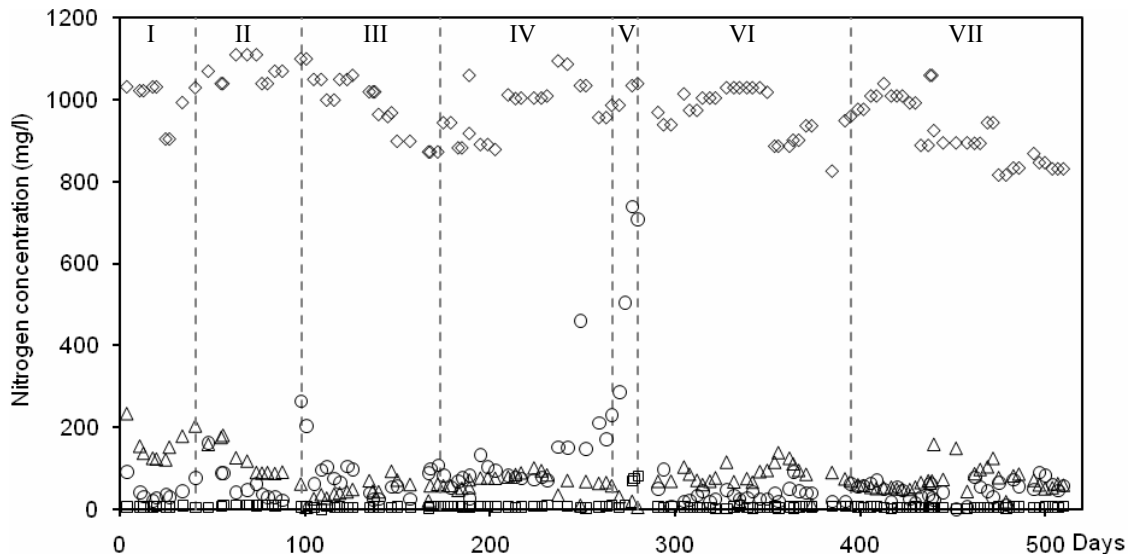
#### ***N<sub>2</sub>O emission measurements***

Unisense microelectrode and Teledyne analytical instruments (Model GFC-7002E) were used to measure the  $\text{N}_2\text{O}$  gas concentration in the liquid and gas phase, respectively.

## **RESULTS AND DISCUSSION**

### ***Nitrogen removal from MBBR with deammonification process***

Values of different nitrogen compounds concentration in the influent and effluent are shown in Figure 2. The first period was the adaptation period for the biomass and nitrogen load was  $2.58 \text{ gN/m}^2\cdot\text{d}$ . During this period, around 80% of nitrogen was removed. According to anammox stoichiometric reaction, approximately 12% of nitrogen in the influent would convert to  $\text{NO}_3^-$ -N. A high concentration of  $\text{NO}_3^-$ -N, which was 160 mg/l, was obtained in the system, which was higher than the expected value of 120 mg/l. It meant that  $\text{NO}_3^-$ -N was not only produced by anammox bacteria but also by NOB. From period II to V, the nitrogen load was increased beyond  $3.5 \text{ gN/m}^2\cdot\text{d}$  (Table 3). During these periods, different aeration strategies were introduced into the system and results showed that the highest nitrogen removal efficiency of 88 % and nitrogen removal rate of  $3.11 \text{ g N /m}^2\cdot\text{d}$  were obtained at period III when  $R = 1/3$  and  $\text{DO} = 3.5 \text{ mg/l}$  (Table 3). When aeration strategy with  $\text{DO} = 3.5\text{mg/l}$  and  $R=1$  were introduced into the system in period V, the process efficiency decreased as expected, with a high  $\text{NH}_4^+$ -N concentration in effluent, which was around 600 mg/l, and pH inside the reactor increased beyond 8. To avoid free ammonia inhibition on the biomass, the liquid inside the reactor was manually removed away. From period VI to VII, the nitrogen load inside the reactor was decreased to  $2.5 \text{ gN/m}^2\cdot\text{d}$ . The process efficiency was back to 80% of nitrogen removal efficiency. Average nitrogen removal rate of  $2.52 \text{ gN/m}^2\cdot\text{d}$  was obtained from the whole operation period of deammonification process at the pilot plant, which was higher than the value of  $1.9 \text{ gN/m}^2\cdot\text{d}$  from the full scale deammonification plant [10].



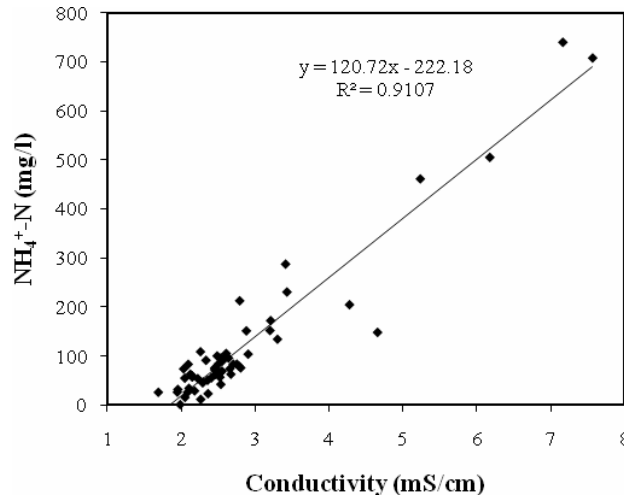
**Figure 2.** Concentrations of different nitrogen compounds in influent and effluent. ( $\diamond$   $\text{NH}_4^+$ -N in influent;  $\circ$   $\text{NH}_4^+$ -N in effluent;  $\Delta$   $\text{NO}_3^-$ -N in effluent;  $\square$   $\text{NO}_2^-$ -N in effluent).

**Table 3.** Nitrogen removal from MBBR with deammonification process.

Period	I	II	III	IV	V	VI	VII
Nitrogen load ( $\text{gN}/\text{m}^2 \cdot \text{d}$ )	2.58	3.84	3.57	3.67	3.71	2.52	2.62
Nitrogen removal rate ( $\text{gN}/\text{m}^2 \cdot \text{d}$ )	2.02	3.22	3.11	3.02	1.35	2.20	2.26
Nitrogen removal efficiency (%)	79	84	88	82	40	87.0	86.5
$\text{NH}_4^+$ -N removal efficiency (%)	96	94	93	91	39	96	95

**Monitoring the process by on-line measurements**

Table 4 presents the daily average value of on-line measurements in MBBR. The change of pH value during deammonification process is caused by nitrification, anammox and denitrification reactions. A drop of pH is expected in nitrification due to production of  $\text{H}^+$  from the reaction while a possible increase of pH will happen if there are only anammox and denitrification reaction occurring under low acid capacity situation. Redox potential (reduction oxidation) value, which is a measurement of system oxidization capacity, was also measured during the pilot plant operation.



**Figure 3.** Correlation between concentration of  $\text{NH}_4^+\text{-N}$  and conductivity (daily average from on-line data) during intermittent aeration.

A high redox potential value indicates a large oxidation capacity and a low redox potential value means a large reduction capacity in the system. Previous study [14] found out that the conductivity has a linear correlation with  $\text{NH}_4^+\text{-N}$  concentration. The changes of conductivity value corresponded to the changes of  $\text{NH}_4^+\text{-N}$  concentration. A similar result was found also in this study (Figure 3).

In the first period high value of oxygen supplied with continuous aeration and concentration of DO in the reactor equaled to 2.5 mg/l (as average value) increased the redox potential value to 117 mV and decreased the pH value from 8.02 to 6.7. Conductivity value was 1.5 mS/cm and a low effluent concentration of  $\text{NH}_4^+\text{-N}$  was observed. Most of the  $\text{NH}_4^+\text{-N}$  was oxidized to  $\text{NO}_2^-\text{-N}$  or  $\text{NO}_3^-\text{-N}$ . From period II to V, oxygen supply was decreased gradually under the same nitrogen load condition which caused decrease of the redox potential value. The observation of increased conductivity value gave information of increase concentration of  $\text{NH}_4^+\text{-N}$ . In the last two periods, a lower nitrogen load was used compared with the previous study. A similar result was derived compared with the results gained from period II to V; with intermittent aeration and less oxygen supplied, pH value and conductivity increased while redox potential value decreased.

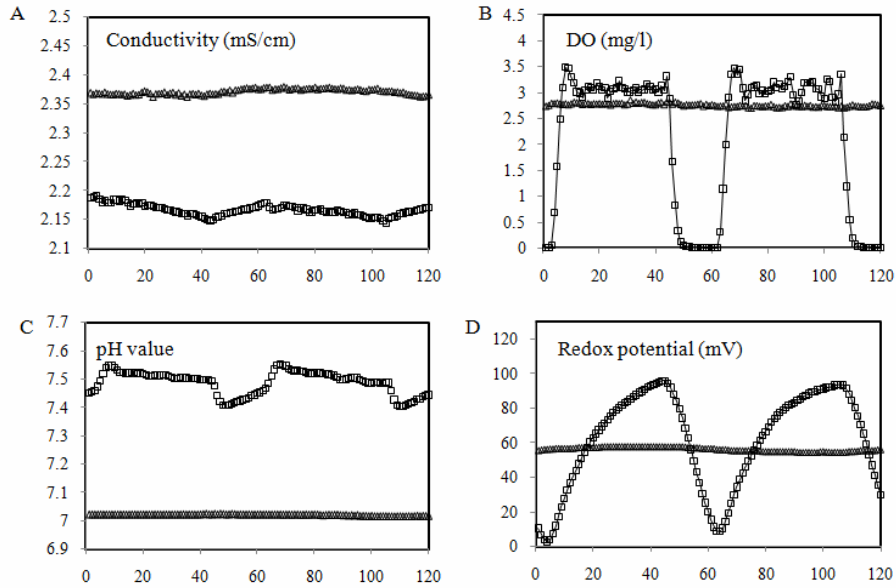
**Table 4.** Results from pH, redox potential and conductivity measurements.

Period	I	II	III	IV	V	VI	VII
pH*	6.7±0.5	7.3±0.3	7.6±0.2	7.7±0.3	8.2±0.1	7.4±0.4	7.6±0.2
Redox* (mV)	117±57	62±34	58±43	-1±39	-44±17	20±43	-10±51
Conductivity* (ms/cm)	1.5±0.5	2.1±0.6	2.4±0.5	2.5±0.5	6.7±0.8	2.3±0.3	2.9±1.5

\*Daily average value from on-line instruments

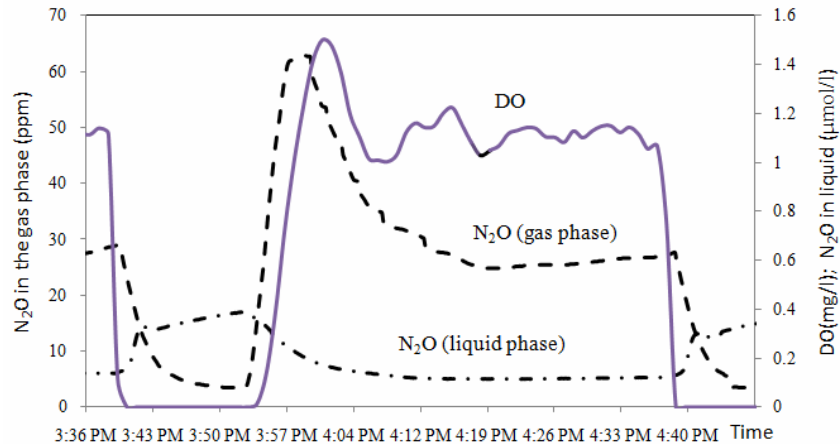
Examples of on-line measurement were chosen from period II and IV to illustrate the variation of different parameters under continuous and intermittent aeration, respectively (Figure 4). During the period of continuous aeration, pH, redox potential and conductivity showed a stable value with a constant concentration of DO. When intermittent aeration was introduced into the system, an increase of DO concentration led to an increase of redox potential value. However, when DO concentration was stable and the redox potential value was still increasing, with a smaller increase rate. Redox potential value dropped when oxygen supply stopped. Changes of pH had

another pattern. When there was oxygen supplied, pH inside the reactor decreased slightly. During non-aerated phase with DO inside the reactor of approximately 0 mg/l, pH was increasing due to continuously feeding and anammox reaction. The changes of conductivity had a similarity with pH variation. A decreased conductivity value due to nitrification process was observed when oxygen was supplied while increased conductivity was observed in anaerobic phase due to continuous feeding of  $\text{NH}_4^+\text{-N}$ .



**Figure 4.** Example of on-line measurements from 2 hours during the different aeration strategies. □ DO = 3.5 mg/l, R= 1/3 (intermittent aeration); Δ DO = 3 mg/l, R=0 (continuous aeration); A) conductivity; B) DO ; C) pH value; D) redox potential value.

Concentrations of  $\text{N}_2\text{O}$  in both gas and liquid phase changed with aeration (Figure 5). When aeration stopped, concentration of  $\text{N}_2\text{O}$  in the gas phase decreased. However, the concentration of  $\text{N}_2\text{O}$  in the gas phase never reached zero. It is suspected that during the non-aerated phase, a little amount of  $\text{N}_2\text{O}$ , produced inside the reactor, could be transferred into the gas phase. At the same time, most of the produced  $\text{N}_2\text{O}$  stayed in the liquid phase during non-aerated phase and an increase of  $\text{N}_2\text{O}$  concentration could be observed. When aeration started, a peak value of  $\text{N}_2\text{O}$  concentration in the gas phase was detected. The reason could be that a large amount of  $\text{N}_2\text{O}$  in the liquid phase was stripped out by the air and causing an increase of  $\text{N}_2\text{O}$  concentration in the gas phase. After the peak,  $\text{N}_2\text{O}$  in the gas phase reached a stable value of 30 ppm (in aeration stream). In the aerated phase, concentration in the liquid phase was almost stable and around  $0.1 \mu\text{mol/l}$ .



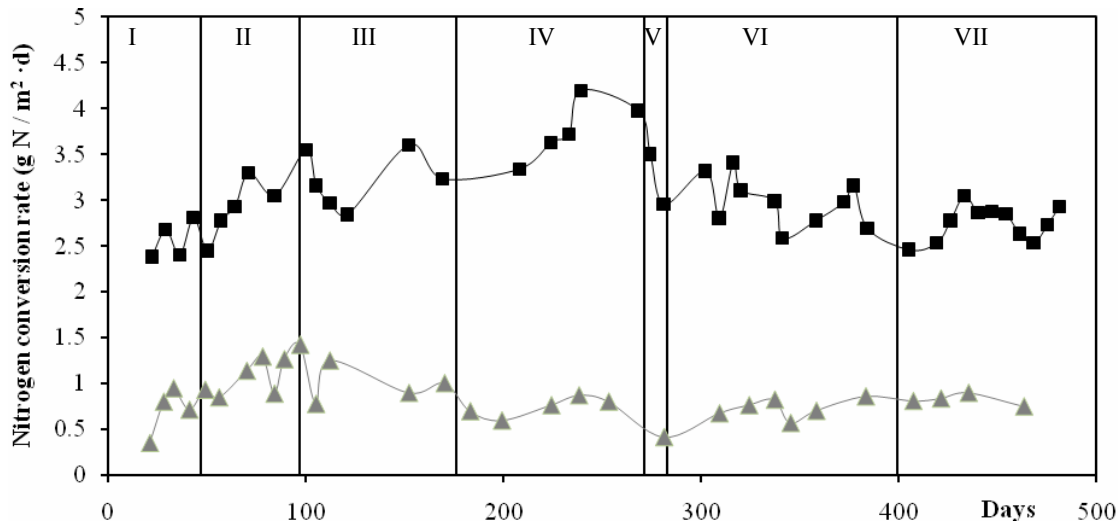
**Figure 5.** Variation of  $N_2O$  production in liquid phase and gas phase during intermittent aeration.   
 - · -  $N_2O$  concentration in the liquid phase; — DO concentrations; - -  $N_2O$  concentration in the gas phase.

In previous study [11], it was proved that Anammox bacteria did not produce  $N_2O$  gas. During the non-aerated phase, most of  $N_2O$  was probably produced by heterotrophic denitrifiers while during the aerated phase, a limited DO concentration was the reason for ammonium oxidizers producing  $N_2O$ .

#### ***Evaluation of different microorganisms' activity***

Figure 6 presents the results of the anammox bacteria and denitrifiers activity measurements. The average value of SAA during the whole operation period was  $3.01 \text{ gN/m}^2 \cdot \text{d}$  with the maximum value of  $4.3 \text{ gN/m}^2 \cdot \text{d}$ , which is comparable to the value of  $4.45 \text{ gN/m}^2 \cdot \text{d}$  obtained in another study [17]. The average value for denitrifiers activity was  $0.85 \text{ gN/m}^2 \cdot \text{d}$ . From period I to IV, the activity of anammox bacteria increased due to three reasons: a) an increased nitrogen load allowed the biofilm to meet more available substrate; b) high oxygen concentration in aerated phase made AOB possible to produce more nitrite; c) continuous aeration switched to intermittent aeration provided anammox bacteria a better anaerobic conditions and anammox bacteria could avoid inhibition from oxygen. Anammox activity dropped down during period V because of a high concentration of free ammonia inside the system, which was around  $120 \text{ mg/l}$ . Although nitrogen removal rate inside the reactor decreased below  $1.0 \text{ gN/m}^2 \cdot \text{d}$ , batch test for anammox activity (SAA) showed that anammox bacteria did not lose all their activity. From period VI to VII nitrogen load was decreased, anammox bacteria had a stable activity value around  $2.8 \text{ gN/m}^2 \cdot \text{d}$ . Denitrifiers showed a relatively stable activity and varied between  $0.4$  and  $1.5 \text{ gN/m}^2 \cdot \text{d}$  for the whole studied period. The denitrification process is very hard to avoid in the deammonification biofilm system when even small amount of organic carbon is available. In this study COD concentration of around  $680 \text{ mgO}_2/\text{l}$  and acetate acid concentration of  $120 \text{ mg/l}$  was in the influent. Probably most of the biodegradable organic carbon was consumed by heterotrophic bacteria in the flocculent sludge, however there was still some organic carbon either diffused into the biofilm or stored inside the body of the cells.

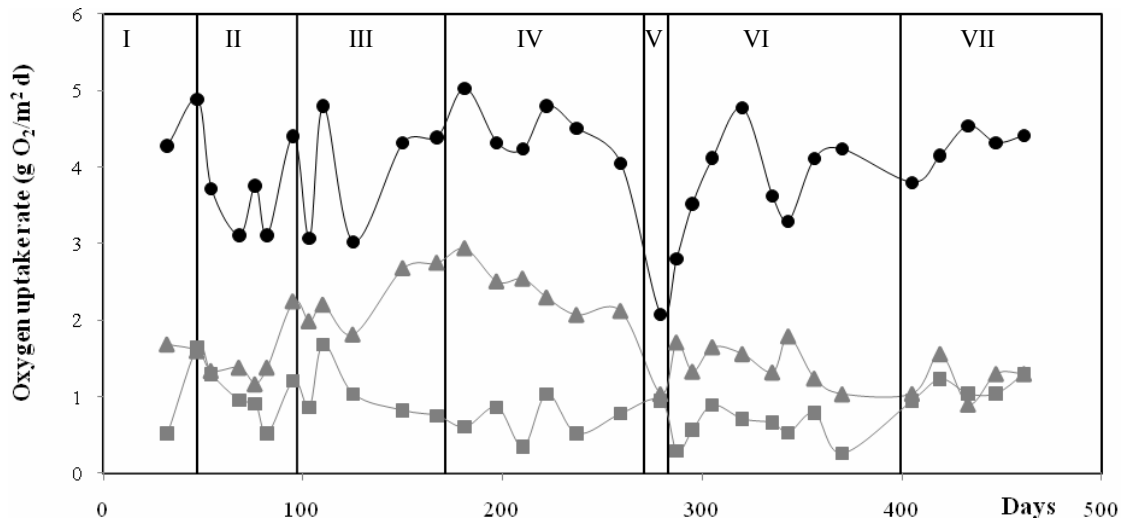




**Figure 6.** Variation of specific anammox activity (SAA) ■, denitrifiers activity (NUR) ▲.

OUR tests results showed that an average value of 4.00, 1.74 and 0.86  $\text{gO}_2/\text{m}^2\cdot\text{d}$  for AOBs, heterotrophic bacteria and NOBs activity, respectively. Although different aeration strategies were introduced inside the system during the whole operation period, AOBs activity was relatively high and played the dominating role in the oxygen consumption. It is known that in deammonification biofilm system, nitrifiers, and heterotrophic bacteria are in the outer layer while anammox and denitrifiers are in the inner layer of the biofilm. It is assumed that the amount of oxygen consumers is depended on the substrate surface load (organic matter and nitrogen load), and the thickness of the biofilm. Compared with anammox bacteria, nitrifiers and heterotrophic bacteria are faster growing bacteria. They would be pushed towards out of biofilm and stay inside the reactor as flocculent sludge [19]. In this case, the activity of AOBs in the biofilm cannot reach very high value due to the limitation of biofilm thickness. Similar study was performed [18] and results showed that AOBs activity was  $2.8 \text{ gO}_2/\text{m}^2\cdot\text{d}$  and NOBs activity was  $1.82 \text{ gO}_2/\text{m}^2\cdot\text{d}$ .

In this study, intermittent aeration did not decrease the activity of AOBs while in some extent, it limited the NOBs growth. A similar result was obtained in another study with intermittent aeration, where NOB growth was limited by the low oxygen concentration [6]. In period V, AOBs activity decreased as expected due to less oxygen supplied. However, it recovered very soon after the restart of the reactor.



**Figure 7.** Variation of AOB (●), NOB (■) and heterotrophic bacteria (▲) activity.

#### Conclusions

- Oxygen concentration (DO) together with ratio between non-aerated phase and aerated phase (R) are two parameters influencing performance and efficiency in one-stage deammonification process. The highest average nitrogen removal rate and efficiency equal to 3.11 g N/m<sup>2</sup>·d and 88 %, respectively were achieved at DO = 3.5 mg/l and R = 1/3.
- In moving bed biofilm reactor with one stage deammonification process, anammox and AOBs play the dominating roles in the biofilm. The average and maximum values of specific anammox activity (SAA) were 3.01 gN/m<sup>2</sup>·d and 4.3 gN/m<sup>2</sup>·d, respectively. The Oxygen uptake rate for AOBs was 4.0 gO<sub>2</sub>/m<sup>2</sup>·d as average value and with maximum value of 5.1 gO<sub>2</sub>/m<sup>2</sup>·d.
- Activity of anammox bacteria was influenced by the nitrogen load and anaerobic conditions. Denitrifiers were presented in the system due to available organic carbon source.
- A proper choice of intermittent aeration could limit NOBs activity efficiently.
- N<sub>2</sub>O production was mainly due to AOB in aerated phase and heterotrophic bacteria in non aerated phase.
- Choice of proper aeration strategy could decrease air supply and energy could be saved without any loss of process efficiency.

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