ACTIVATED SLUDGE AND BIOFILM IN THE ANAMMOX REACTOR – COOPERATION OR COMPETITION?

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ABSTRACT
An anaerobic ammonium oxidation (Anammox) process was established in a moving-bed biofilm reactor. The research question concerned an interrelation between activated sludge and biofilm in the system. Reaction courses in the Anammox reactor were assessed by the use batch tests for nitrogen uptake rates. The tests were done with the aim of recognizing contribution of different bacterial populations in performing nitrogen elimination in the Anammox process. It was demonstrated that activated sludge and biofilm cultures collaborated in the execution of nitrogen removal in the Anammox reactor.

KEYWORDS
Anammox, activated sludge, batch tests, biofilm, moving-bed reactor, oxygen uptake rate

INTRODUCTION

The Moving Bed Biofilm Reactor (MBBR) process is based on the biofilm principle that takes advantage of both the activate sludge process and conventional fixed film systems without theirs disadvantages. Reactor can be operated at very high load and the process is insensitive to load variations and other disturbances (Ødegaard et al., 1994; Dalenfort and Thulin 1996). The Kaldnes suspended carrier system was successful implemented for nitrification process (Hem et al., 1994; Rostron et al., 2001) as well as for denitrification process (Pastorelli et al., 1997). It was also showed that moving-bed biofilm reactor could utilise for deammonification process, which combines partial nitritation and Anammox processes (Seyfried et al., 2001; Rosenwinkel et al., 2005). Research made by Tal et al. (2003) also proved the coexistence of nitrifying and Anammox bacteria on the Kaldnes rings.

In the nitrification process two groups of microorganisms are involved in the aerobic ammonia and nitrite uptake: ammonium oxidizers (reaction 1) and nitrite oxidizers (reaction 2).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad \text{(reaction 1)}$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3 \qquad \text{(reaction 2)}$$

The anaerobic ammonium oxidation is performed by a new group of nitrite-depended Anammox bacteria (Candidatus *Brocadia anammoxidans* and Candidatus *Kuenenia stuttgartiensis*). The overall anaerobic ammonium oxidation can be presented as the reaction (van Dongen et al., 2001):

$$NH_4^+ + 1.32\ NO_2^- + 0.066HCO_3^- + 0.13\ H^+ \rightarrow$$
$$0.26\ NO_3^- + 1.02\ N_2 + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{(reaction 3)}$$

In the two-step deammonification process there is partial nitritation of ammonium to nitrite, to prepare proper influent to the succeeding Anammox reactor. Therefore there are created preferential conditions for growth of ammonium oxidizers and to wash out or to out compete nitrite oxidizers from the system (Jetten et al., 1999; Fux 2003; Wyffels et al., 2004). The goal of partial nitritation is to get stable effluent with appropriate nitrite-to-ammonium ratio of 1:32 according to reaction 3 (Strous et al., 1998; van Dongen et al., 2001). Anammox is an anaerobic process where ammonium reacts with nitrite, which plays a role of an electron acceptor (Strous et al., 1997). Hydroxyloamine and hydrazine were identified as intermediates of this process. Despite of presented above stochiometric ratio for nitrite and ammonium needed for Anammox process, many authors report different results. In the experiments carried out by Depena-Mora et al., (2004) obtained ratios were 1.28 and 1.11 for Gas lift reactor and SBR reactor respectively, additionally in research made by Wyffels et al., (2003) nitrite-to-ammonium ratio was 1.43. So far no one has grown pure cultures of Anammox bacteria (Mohan et al., 2004) and it means that Anammox reactor contain different types of bacteria, which can influence on the reactions rates. Results presented by Schmidt et al., (2002) proved that Brocadia anammoxidans and Nitrosomonas are able to coexist under anoxic conditions and although this two group compete for ammonia, their cooperation also seems to be possible.

In Sweden, the deammonification process was designed as a two step process with partial nitritation as a first step followed by Anaerobic Ammonium Oxidation as a seconds step (Płaza et al., 2002; Trela et al., 2004). The research goal was concentrated on the recognition of influence suspended sludge in the Anammox reactor on the reactions courses and rates. While setting up the moving-bed bioreactor, oxygen-limited environment must be assembled as well but cooperation is viable to obtain when aerobic bacteria grow in suspension and anaerobic culture is in the biofilm.

MATERIAL AND METHODS

Technical-scale pilot plant

The technical-scale pilot plant was designed for studies of the partial nitaritation/Anammox process. In the first reactor, with total working volume of 2.1 m^3, the partial nitritation was carried on, while in the second reactor, (total volume of 2.1 m^3) as a second step, was Anammox process. Both reactors were filled, to 50% of their volume, with Kaldnes ring as a biofilm carrier. The influent supernatant, from dewatering of the digested sludge at the Himmerfjärden WWTP, was continuously pumped to the first reactor and next the Anammox reactor was fed with effluent from the first step. Both reactors were divided into three zones and each zone had a mechanical stirrer. To keep appropriate temperature conditions, the heaters were situated in the first zone of each reactor. Additionally, in the partial nitritation reactor were blowers to ensure aeration for ammonium oxidation. More detailed pilot plant description and supernatant characteristic can be found in earlier papers (Szatkowska et al., 2004; Trela et al., 2004; Gut et al., 2005).

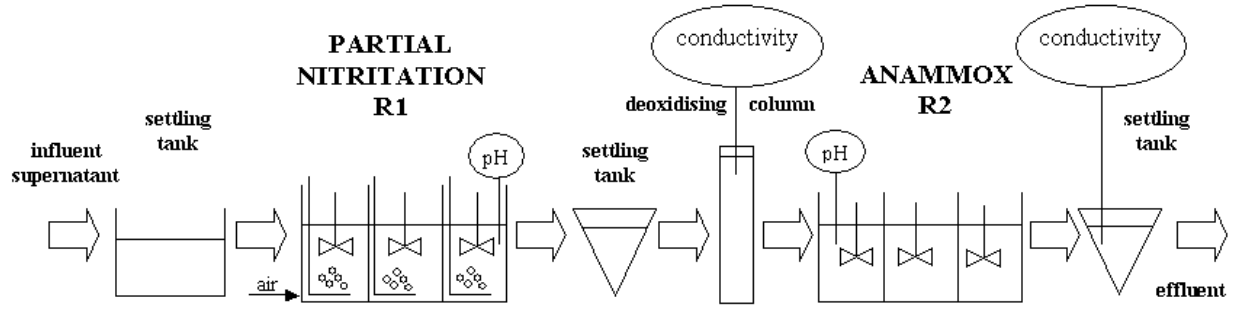

Figure 1. Scheme of the partial nitritation/Anammox system.

Batch tests

During operation of the technical-scale pilot plant, it became possible to perform series of batch tests for studies of nitrogen uptake rates. The tests were made in bottles of 1 litre working volume and the water bath was used to keep the same temperature conditions like in the pilot. These batch tests were divided into four groups (Table 1). In order to recognize significance of the activated sludge and Kaldnes biofilm in each test in one bottle condensed sludge from the Anammox reactor was used and a medium used in the second bottle was different in each group. In the first group, Kaldnes rings and sludge from R2 were used whereas in the second group Kaldnes rings and filtrated supernatant. In the third group, Kaldnes was rinsed out in order to remove sludge covering the rings during pulling out from the reactor. The first time the Kaldnes were rinsed out in tap water and the second time by filtered supernatant (to eliminate negative impact of tap water on biofilm) having the same temperature like in the pilot. Additionally, to confirm previous results, in the fourth group three tests were made: one with activated sludge, second with medium from R2 and the third with rinsed Kaldnes rings and filtered supernatant.

Table 1. Different batch test combinations (K – Kaldnes; S – Sludge, NR-not rinsed; R-rinsed).

Group 1		**Group 2**		**Group 3**		**Group 4**		
Test 1 and Test 2		Test 3 and Test 4		Test 5 and Test 6		Test 7		
S	K+S	S	K (NR)	S	K (R)	S	K+S	K (R)

In each test, Kaldnes filled the bottles in 50% of their volume. The initial concentration of NO_2-N and NH_4-N were determined and then $NaNO_2$ and NH_4Cl solutions were added to obtain required initial ammonium and nitrite concentration. The Na_2CO_3/$NaHCO_3$ solution was used for pH correction. The samples were collected every 30 minutes. Suspended Solids (SS) and Volatile Suspended Solids (VSS) were analysed at the beginning of each test. Additionally, temperature, dissolved oxygen (DO) and pH were measured during the batch tests. DrLange VIS Spectrophotometer XION 500 and AQUATEC-TECATOR 5400 ANALYZER (flow-injection system based on VIS spectrophotometry) were used to analyse samples for inorganic nitrogen compounds.

RESULTS AND DISCUSSION

Technical-scale pilot plant

The partial nitritation/Anammox system is designed as a two-step process consisting of an initial partial nitritation (R1) followed by an Anammox process (R2), where the nitrogen removal takes place. The Anammox reactor works as a moving-bed reactor that combines activated sludge and biofilm cultures. The Anammox reactor was operated steadily during the year 2004 and at the

beginning of 2005 obtaining high removal of total inorganic nitrogen with an average value of 254 g N/m^3, which corresponded to an average efficiency of 87% (Figure 2).

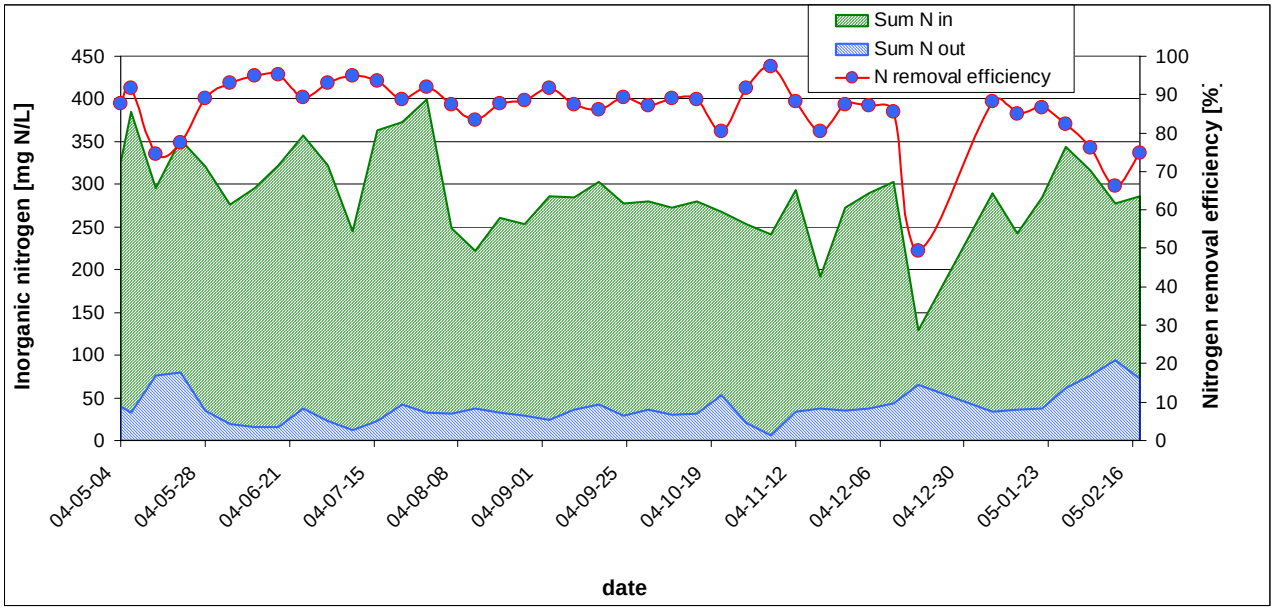

Figure 2. Variations of inorganic nitrogen forms and nitrogen removal efficiency in the Anammox reactor (May 2004 – February 2005).

During the research period concentration of activated sludge in the Anammox reactor was very changeable (Figure 3). The average values were 1.57 g/L and 1.14 g/L for SS and VSS respectively. It was obvious that such high concentration of suspended biomass must have some impact on work of the reactor. Owing to stable results, it seems probable that the activated sludge had rather positive influence on the process.

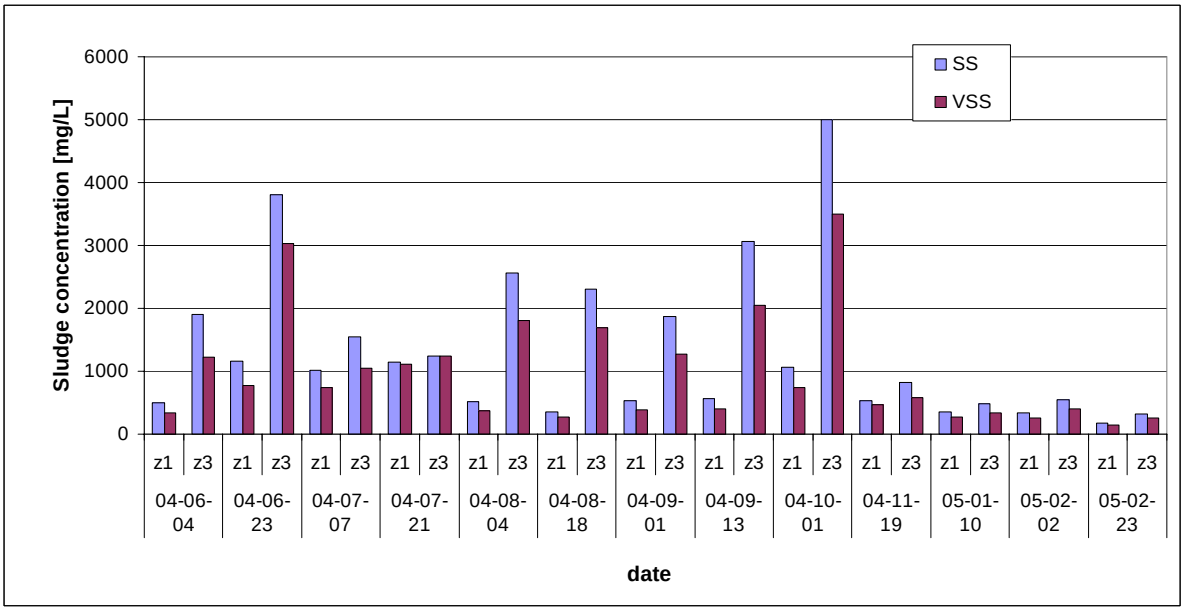

Figure 3. Variations of activated sludge concentration in the Anammox reactor (May 2004 – February 2005).

Batch tests

To confirm the hypothesis of the positive effect of activated sludge and to check that the Anammox process is mainly on biofilm, several batch tests were performed. Tests were divided into four groups. In Table 2 concentrations of SS, VSS and nitrogen removal rates are presented for each batch test.

Table 2. Nitrogen removal rates and sludge concentrations in batch tests (NR-not rinsed; R-rinsed, n/a – not analysed).

		Nitrogen removal rate		Sludge concentration	
		gN/m^3·d	gN/gVSS·d	gSS/L	gVSS/L
Group 1					
Test 1	Sludge	82.8	0.069	3.30	1.20
	K + S	140.6	n/a	0.73	0.37
Test 2	Sludge	283.2	0.102	3.80	2.77
	K + S	197.7	n/a	1.20	0.88
Group 2					
Test 3	Sludge	62.6	0.020	4.50	3.21
	Kaldnes (NR)	77.9	n/a	0.39	0.30
Test 4	Sludge	136.1	0.051	3.77	2.67
	Kaldnes (NR)	76.0	n/a	0.33	0.23
Group 3					
Test 5	Sludge	58.0	0.030	2.60	1.96
	Kaldnes (R)	0	n/a	0.05	0.04
Test 6	Sludge	139.7	0.052	3.90	2.70
	Kaldnes (R)	0	n/a	0.08	0.06
Group 4					
Test 7	Sludge	91.2	0.031	3.86	2.90
	K + S	37.4	n/a	0.31	0.26
	Kaldnes (R)	0	n/a	0.02	0.02

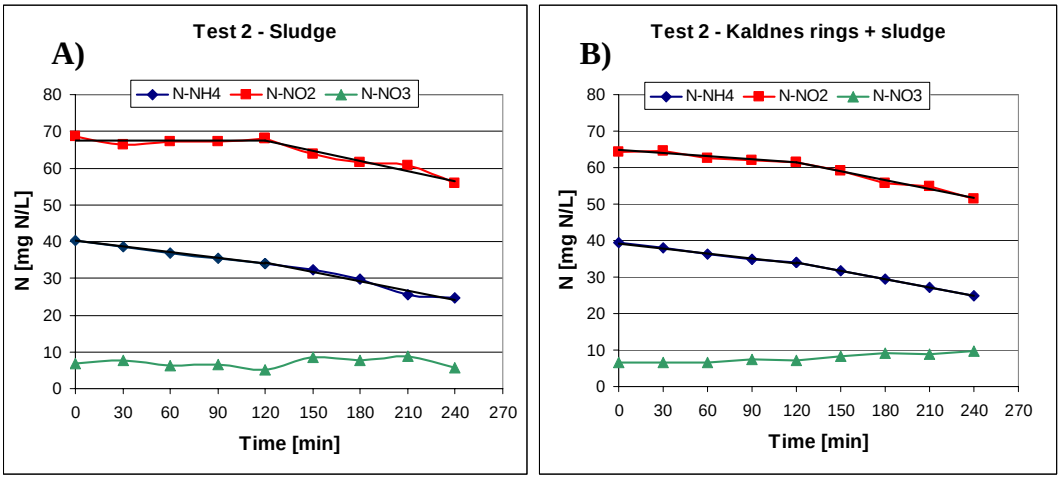

Figure 4. Nitrogen conversions during batch tests no. 2: A) Test 2A for sludge, B) Test 2B for Kaldnes rings and sludge.

In Figure 4 results from test no 2 are shown. Nitrogen removal rate was higher in test with the activated sludge. In these tests it was interesting that at the beginning the ammonium removal rate was much higher than the rate for nitrite both for Test 2A and 2B (Figure 4A and 4B). In the Anammox process it should be the opposite situation according to the stoichiometric reaction. This

phenomenon could be explained by the presence of nitrifiers in the reactor in spite of very low oxygen concentration. Significant drop of pH values from 8.20 to 7.67 could also confirm this hypothesis. Such high activity of nitrifiers might be due to high initial concentration of ammonium and nitrite nitrogen, which is significantly higher than in the reactor and could inhibit the Anammox process temporarily. After two hours, nitrite removal rate exceeded ammonium removal rate in both tests 2A and 2B. However, the nitrification activity prevailed for the test with the activated sludge and, additionally, higher inhibition of the Anammox process at the beginning of Test 2A was obtained. It could be caused by substrate competition between nitrifiers and Anammox bacteria. On the other hand, as it was mentioned before, it could be due to inhibition of the process.

Results from the second group of batch tests are presented in Figure 5. In this group, there was no initial inhibition of nitrogen removal. In the test with the activated sludge, ammonium removal rate was equal to 91.2 gN/m^3·d, which was much higher than nitrite nitrogen removal rate amounting to 50.0 gN/m^3·d (Figure 5A). Also pH drop was measured from 7.95 to 7.46. It confirms the results from previous test and proved that nitrifiers are still present and active. Removal of total inorganic nitrogen indicated that the Anammox bacteria could also live in the suspended biomass. In the second bottle (Figure 5B), where not rinsed out Kaldnes rings and filtrated supernatant were present, VSS concentration was equal 0.23 g/L and was caused by the sludge that covered the rings during pulling them out from the reactor. In this test nitrogen removal rate was lower than in the test with sludge (Table 2). However, removal rate for ammonium was a little bit higher than for nitrite: 39.7 and 32.8 g N/m^3·d, respectively. It suggested that there is also some nitrifiers' contribution in the biofilm population. Basing on calculations of nitrogen removal by activated sludge, and assuming that sludge, in bottle with Kaldnes rings, works in the same way like activated sludge alone, it was proved that mainly biofilm is responsible for nitrogen removal. Results from these calculation shows that in the test with Kaldnes rings and sludge 74% of removed nitrogen is removed by biofilm on the Kaldnes rings.

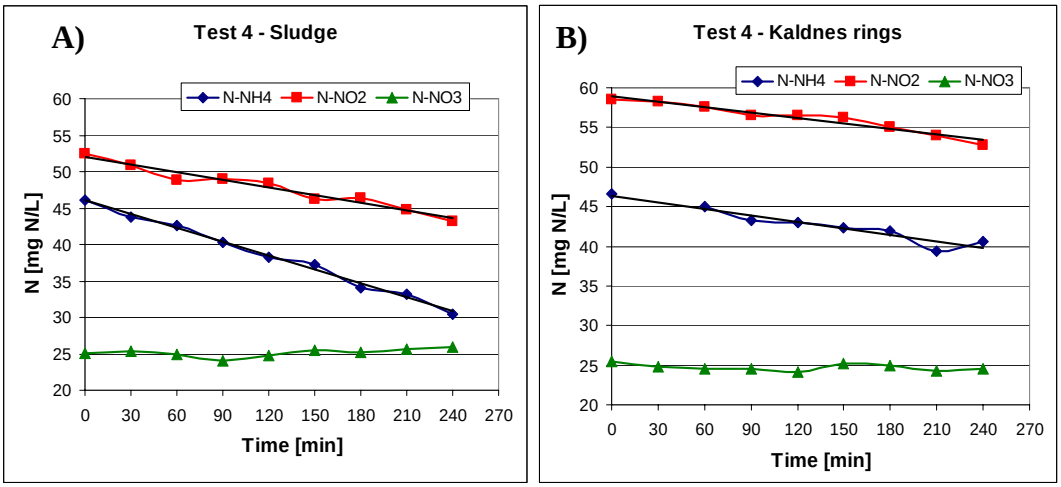

Figure 5. Nitrogen conversions during batch tests no 4: A) Test 4A for sludge, B) Test 4B for Kaldnes rings (without rinsing out) and filtrated supernatant.

Very interesting results were obtained from the third group of batch tests (Figure 6). In this series of tests, results from the bottle with the activated sludge (Figure 6A) were similar to the previous test (Figure 4A). However, the results for Kaldnes rings (Figure 6B) were unexpected, because nitrogen removal was not detected. Instead, minor oxidation of ammonium to nitrite was noticed. High oxygen concentration during the test could be the main reason of this situation, which could inhibit the Anammox process. This problem took place before the test, in spite of the fact the oxygen was removed from the bottle by using nitrogen gas to obtain oxygen free condition. However, during the

test oxygen concentration exceeded 0.5 mg O_2/L. In the same time, in the bottle with activated sludge such problems were not detected and oxygen concentration was below 0.1 mg O_2/L. It seems most probable that nitrifiers, which are present mostly in activated sludge, play a role of oxygen removers. The amount of nitrifiers in the biofilm on Kaldnes rings is insufficient to perform deoxidation.

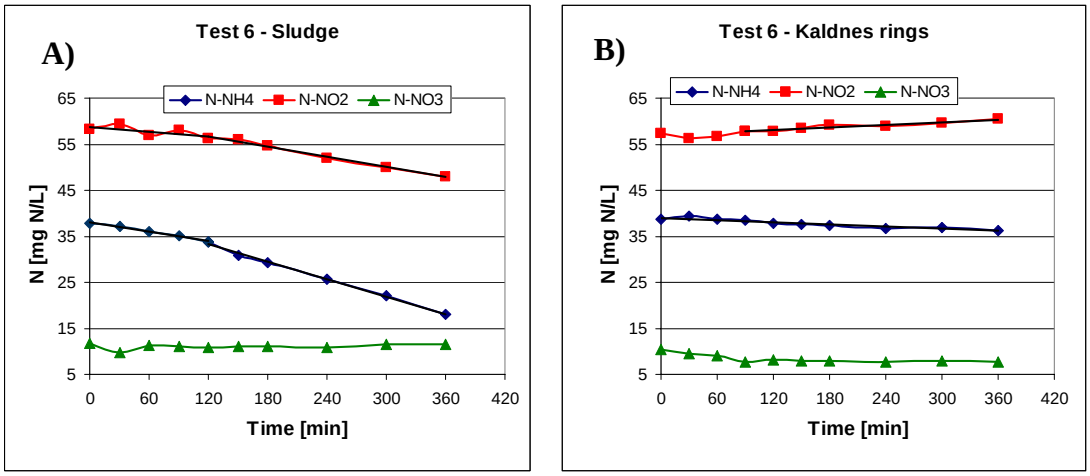

Figure 6. Nitrogen conversions during batch tests no. 6: A) Test 6A for sludge, B) Test 6B for Kaldnes rings (rinsed-out) and filtrated supernatant.

Last tests (no. 7) were performed to confirm previous experiments. Obtained results showed the same problems with oxygen (inhibiting parameter for the Anammox process) in the bottle with rinsed-out Kaldnes rings and filtered supernatant. To compare the tests with the activated sludge and medium from the Anammox reactor, higher nitrogen removal rate was obtained for the sludge. What was interesting, in the bottle with the activated sludge, dissolved oxygen concentration was always a little bit lower than in the bottle with the mixture of Kaldnes rings and sludge. It could have some influence on the results.

Both in the mixture of Kaldnes rings and sludge and in the tests with the activated sludge, it could be observed that along with the increase of VSS concentration, also nitrogen removal rate was increasing (Figure 7A and 7B). However, much smaller concentration of VSS in the tests performed with combined biofilm and activated sludge is related to much higher nitrogen removal than that observed in the test with activated sludge only. It can indicate two hypotheses: firstly, the Anammox bacteria are present in higher percentage in biocenosis of biofilm than in the biocenosis of activated sludge and secondly, it is possible that nitrifiers, present in the sludge, create favourable conditions for Anammox bacteria on the biofilm by consumption of oxygen that can diffuse into the liquid.

In Figure 7C and 7D, relationships between nitrogen to VSS ratio and nitrogen removal rates are presented for the tests performed only with the concentrated sludge. The decrease of nitrogen removal rate along with the increase of nitrogen to VSS ratio was observed in different tests. Because in neither of the tests with the activated sludge there were no problems with oxygen, it seems most probable that it is mainly due to increase nitrogen load in the tests. It is clearly seen that along with the increase in nitrogen load, the nitrogen removal rates were decreasing. It could be a little surprising that at such low nitrogen load during the test the efficiency in nitrogen removal deteriorated. Explanation of this could be that in the batch tests very high concentration of nitrogen forms are reached at the beginning of the test, which is significantly higher than in the system with continuous flow.

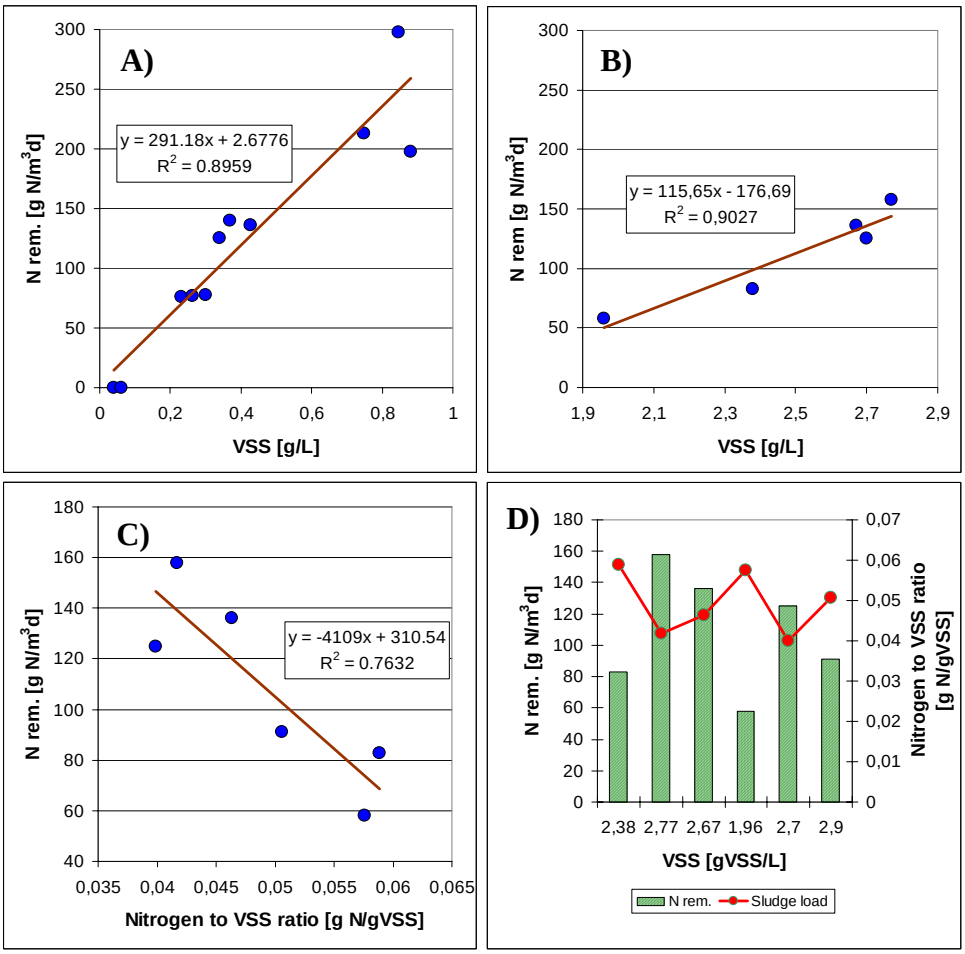

Figure 7. A) Correlation between VSS concentration and average nitrogen removal rate in the tests with mixture of Kaldnes rings and sludge. B) Correlation between VSS concentration and average nitrogen removal rate in the tests with concentrated activated sludge. C) Relationship between nitrogen to VSS ratio and nitrogen removal rate – test with activated sludge. D) Relationship between VSS concentration, nitrogen removal rate and nitrogen to VSS ratio in the test with activated sludge.

Cooperation or competition?

Performed batch tests proved that in the Anammox reactor nitrifiers are present. It seems probable that it is mainly due to the seeding of nitrifying bacteria from the partial nitritation reactor to the second reactor. Obtained results show that nitrifiers are mostly in the activated sludge and their amount on biofilm is insignificant. Some substrate competition between nitrifiers and Anammox bacteria could be possible. On the other hand, the tests showed that nitrifiers are responsible for oxygen consumption. Oxygen diffusion into mixed liquor can inhibit the nitrogen removal. It appears that it is rather some cooperation of different type of bacteria than competition, what was proven by the performed batch tests. Fact that under the period of tests' execution nitrogen removal process efficiency in the pilot plant operated at Himmerfjärden WWTP was on average 84% also confirms this hypothesis.

The moving-bed system is adequate to gain cooperation of many bacterial cultures in removing nitrogen. The results from this study on the Anammox reactor demonstrated that there is the nitrifying activity present in the Anammox reactor and it is concentrated chiefly in the activated

sludge. The cooperation of activated sludge and Kaldnes is responsible for total effect of nitrogen removal but Anammox activity focuses on biofilm Kaldnes rings.

CONCLUSIONS

- To answer the question stated in the title, there is a cooperation of both bacterial cultures in activated sludge and biofilm in the nitrogen conversions and the Anammox process is established successfully due to the fact that nitrifiers play role of oxygen removers.
- In the batch test with both Kaldnes and sludge, the nitrogen removal capacity is much higher than in the tests with concentrated sludge only. The contribution of biofilm on Kaldnes rings made up the efficient Anammox performance.
- It is interesting to notice that nitrifiers are outcompeted by Anammox bacteria in the biofilm so that their population is very small, but still active.
- Big variety in the reaction rates and bacterial activities is related to changes in the influent characteristics to the Anammox reactor and changeable activity of different bacterial populations.

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