

# SWEDISH EXPERIENCE WITH DEAMMONIFICATION PROCESS IN BIOFILM SYSTEM

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**Abstract:** Treatment of supernatant from dewatering of digested sludge was performed in a pilot plant scale at Himmerfjärden wastewater treatment plant in the Stockholm region. A moving bed biofilm reactor was used with Kaldnes rings as support material. Deammonification is a two-step process technology in which the first step involves oxidation of part of the ammonium to nitrite followed by oxidation of remaining ammonium with the formed nitrite into nitrogen gas (anammox). The two processes can be performed in two separate reactors or in one-stage biofilm reactor there the outer biofilm layer performs nitrification and an inner layer the anammox reaction. One-stage technology was started by seeding anammox bacteria into a step with partial nitrification and deammonification was rapidly established with an average nitrogen removal between 55 and 88% with influent ammonium concentrations between 350 and 720 g N m<sup>-3</sup>. A removal rate of about 15 g N m<sup>-3</sup> d<sup>-1</sup> could be reached. The process could be monitored by pH and conductivity measurements. Nitrification was the rate limiting step.

**Keywords:** anammox, biofilm, deammonification, digester supernatant, nitrification.

## INTRODUCTION

Deammonification is a recently discovered way to remove nitrogen from ammonia rich streams with low concentration of biodegradable organic materials such as supernatant from digesters and manure and landfill leachate. Full-scale applications with different design and operation modes exist at Rotterdam, the Netherlands, Hattingen, Germany, and Strass, Austria. Although different in design and operation two general principles must be fulfilled. Firstly, partial nitrification must be obtained with oxidation of about 60% of ammonium into nitrite. A special problem is to oxidize ammonium only to nitrite and may be accomplished by use of low oxygen concentration, low pH values, suitable temperature, etc. Secondly, the anammox step should be operated at no or very low oxygen concentrations and nitrite concentrations above about 30 g N m<sup>-3</sup> may inhibit the reaction (Gut, 2006)

It is well-known that many wastewater treatment systems work with aeration/no aeration such as traditional nitrification/denitrification, nitrification/denitrification and biological phosphorus removal and have different requirements such as organic acids in the anaerobic step for biological phosphorus removal and high oxygen concentrations for rapid nitrification in moving bed biofilm reactors. Deammonification should be seen as an additional biological process with oxygen supply/no oxygen supply and general principles from stoichiometry, reaction kinetics, diffusion,

reactor design (suspended growth/fixed film reactors) etc. can be applied for the deammonification process.

Fixed film reactors are commonly used for removal of organic materials, nitrification and denitrification. Only the outer part of the biofilm on the support material is normally active due to diffusion resistance for instance of oxygen. The inner layer may, however, also accomplish different reactions. In nitrifying trickling filters or rotating discs a certain degree of denitrification often occurs in the inner layers of the biofilm. Related to deammonification the CANON process (Completely Autotrophic Nitrogen removal Over Nitrite) as a single autotrophic step was developed in the Netherlands with support material on which formed biofilm had an outer layer for nitrification and an inner layer for anammox (Hao et al., 2002; Sliemers et al., 2003). Intermittent aeration can also be used to secure a suitable ratio of oxygen and oxygen free conditions in the biofilm.

## **THEORETICAL ASPECTS**

### **Rate limitations due to diffusion in one-stage biofilm reactor**

In the moving bed biofilm reactor it is assumed that the Kaldnes rings have an outer layer of nitrification bacteria and an inner layer of anammox bacteria. The limiting factor in diffusion in biofilms is depending on concentration and diffusivity. For nitrification and anammox main components are ammonium (energy source), oxygen (electron acceptor for nitrification) and nitrite (electron acceptor for anammox). Diffusion coefficients are for ammonium, nitrite and oxygen at 25 °C about  $15 - 20 \text{ (cm}^2 \text{ sek}^{-1}) * 10^{-6}$ . If energy source or electron acceptor only partly penetrate the biofilm the process becomes diffusion limited (Harremoës, 1978). As long as ammonium nitrogen concentration in the liquid outside the biofilm is much higher than the oxygen or nitrite nitrogen concentration ammonium diffusion will not limit the process rate. Oxygen concentration will therefore limit the nitrification rate in biofilm reactors. If the produced nitrite is mainly consumed by the inner layer it means that oxygen is the main limiting factor controlling the overall rate of the deammonification process. The value of oxygen concentration outside the biofilm is crucial. A high concentration may inhibit the anammox reaction and increase the oxidation of nitrite to nitrate by bacteria as *Nitrobacter*. A low oxygen concentration means that the nitrification rate is significantly reduced as the oxidation rate is proportional to the square root of the oxygen concentration.

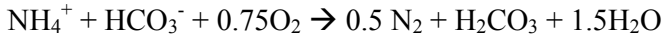
### **Rate limitations due to kinetic factors**

The growth rate of bacteria is influenced by such factors as concentration of energy source and electron acceptor, temperature, pH-value and inhibiting factors. In the nitrification process it is important to avoid further oxidation of nitrite to nitrate. Two important ways are the use of low pH values and oxygen concentrations. However, these two factors limits significantly the nitrification rate. Nitrite concentration is a strong inhibitor for the anammox process. If, however, nitrite is consumed at about the same rate as it is produced this inhibition effect is not of significance.

### **Changes in conductivity, alkalinity and pH values**

Degradation of organic matter in the digester follows the pattern of hydrolysis, acidogenesis and methanogenesis (Söttemann et al., 2005). Neglecting such factors as release of phosphate from the sludge and reactions as reduction of ferric iron to ferrous iron main supply of ions from sludge are ammonium with the counter ions of acetate and hydrogen carbonate. Acetate and hydrogen has about the same equivalent conductance ( $40.9$  and  $44.5 \text{ uS cm}^{-1}$  at infinite dilution and 25 °C, respectively) and therefore ammonium acetate and ammonium hydrogen carbonate will have about

the same conductivity in dilute solutions. As the acetate concentration is low in a well-functioning digester and acetate in a single stage deammonification process will be removed, a reasonable assumption is that main conductivity and pH value changes will depend on changes in ammonium hydrogen carbonate concentration due to the deammonification process (with partial nitrification followed by anammox), This may be written (neglecting cell synthesis):

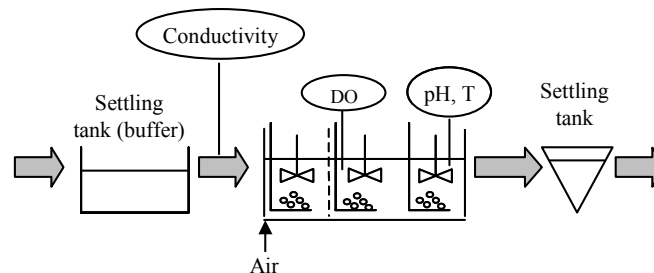


From the formula it should be expected that conductivity decrease mainly is due to removal of ammonium hydrogen carbonate, alkalinity decrease due to removal of hydrogen carbonate and pH drop due to decreasing values of the quotient  $\text{HCO}_3^-/(\text{H}_2\text{CO}_3 + \text{HCO}_3^-)$ . The decrease in these values is depending on the removed amount (conductivity and alkalinity) or removal efficiency (pH value decrease).

## MATERIAL AND METHODS

### Pilot-plant

A pilot plant had been installed at Himmerfjärden wastewater treatment plant southwest of Stockholm. The plant had two separate reactors (each 2.1 m<sup>3</sup>) for treatment of supernatant from dewatering of digested sludge. The reactors may be operated in series or in parallel. The reactors were filled with about 50% of Kaldnes rings with a specific area of 500 m<sup>2</sup> m<sup>-3</sup> and thus an effective specific area of 250 m<sup>2</sup> m<sup>-3</sup>. One of the reactors was mainly studied in the single reactor deammonification process (Figure 1).



**Figure 1** Flow sheet of the pilot-plant with one-stage deammonification processes.

### Analytical and measurement methods

For the studied reactor continuous measurements were made of flow, conductivity, pH-value, temperature and oxygen concentration. Analyses based on grab samples were performed on inorganic nitrogen fractions, COD, organic acids, alkalinity, SS and VSS.

Batch experiments concerning oxygen and nitrite influence of the anammox reaction was performed in a one-liter vessel that was filled with 50% Kaldnes rings from the pilot plant reactor. The vessels could be supplied by air and/or additions of nitrite. Samples were taken out every half hour for measurement of inorganic nitrogen components, pH-value, conductivity, oxygen concentration and temperature.

## RESULTS AND DISCUSSION

### Supernatant composition

Table 1 shows the main parameters of the influent to the deammonification step. For the anammox process the ratio between ammonium and nitrite nitrogen should stoichiometrically be 1.32. The average value of alkalinity/ $\text{NH}_4\text{-N}$  of 1.25 mmol mmol<sup>-1</sup> in the supernatant should therefore be enough for a single stage deammonification process without alkalinity additions as minor contributions to the alkalinity is obtained from cell synthesis by anammox bacteria and a limited heterotrophic denitrification. The stoichiometric use of COD in heterotrophic denitrification reduced to nitrogen gas (neglecting cell synthesis) is 1.72 g COD per g of reduced nitrite nitrogen. In experiments about 20% of COD was removed indicating that less than 5% of nitrogen could have been removed due to heterotrophic denitrification.

**Table 1.** Supernatant composition and parameters (average values).

Parameter	Value	Number of samples
$\text{NH}_4\text{-N}$	589 g/m <sup>3</sup> (42.1 mmol m <sup>-3</sup> )	32
COD	206 g O <sub>2</sub> m <sup>-3</sup>	8
SS	102 g m <sup>-3</sup>	8
VSS	78 g m <sup>-3</sup>	8
Alkalinity	3140 g HCO <sub>3</sub> m <sup>-3</sup> (51.5 mmol m <sup>-3</sup> )	28
Conductivity	5.79 mS cm	115
pH	7.83	cont. measurements
Temperature	26.2 °C (measured in reactor)	cont. measurements
<i>Quotients (samples at same time)</i>		
Alkalinity/ $\text{NH}_4\text{-N}$	1.25 mmol mmol <sup>-1</sup>	28
Conductivity/ $\text{NH}_4\text{-N}$	0.138 mS mmol cm <sup>-1</sup> l <sup>-1</sup>	30
COD/ $\text{NH}_4\text{-N}$	0.34 g O <sub>2</sub> g N <sup>-1</sup>	8
VSS/SS	0.76	8

At infinite diluted solution and at 25 °C the conductivity of a ammonium hydrogen carbonate solution is 0.114 mS mmol cm<sup>-1</sup> l<sup>-1</sup> similar to the value 0.138 in Table 1. Conductivity is a function of the ionic strength and the supernatant contains salts with sources from drinking water and addition of chemical precipitation agents etc. Unpublished studies on conductivity at different steps at Himmerfjärden wastewater treatment plant indicate that up to 90% of the conductivity in supernatant could be attributed to formation of ammonium hydrogen carbonate during digestion.

### Operational modes

The operation as a one-stage mode started at 29 Jun 2005. The hydraulic residence time was 1 day except for the period 4 Oct to Nov 2005 when it was lowered to 16 h. No chemicals were added to the reactor.

Earlier the operation had been two stage mode with the purpose to get partial nitritation in the first reactor followed by anammox in the second reactor. As the anammox reaction also produces nitrate during cell synthesis a possible way to improve the nitrogen removal efficiency is to recycle effluent from the anammox reactor back to the influent to the first reactor to obtain heterotrophic denitrification by remaining organic acids from the anaerobic digestion.

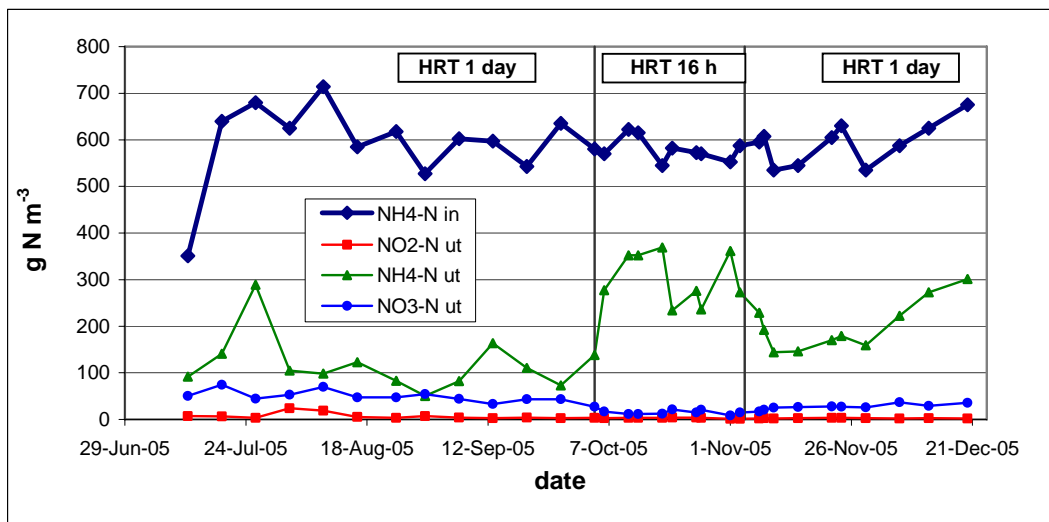
Recirculation of effluent from reactor 2 (anammox reactor) initiated a significant reduction of nitrogen in reactor 1 that could not be explained by heterotrophic denitrification estimated to be less than 5%. An increase of nitrogen removal was accompanied by an increase of conductivity

reduction but also of a decrease of alkalinity reduction (related to minor heterotrophic denitrification and cell synthesis in the anammox reaction). The nitrate concentration increased from values between 2.5 to 30 g N m<sup>-3</sup> in earlier period with nitrification process to values between 33 and 123 g N m<sup>-3</sup>, also indicating a significant anammox reaction.

As the anammox process due to seeding from the second anammox step was established in the first reactor it was decided to operate the removal in deammonification as a single stage process from 29<sup>th</sup> June (2005). Main results up to 22<sup>nd</sup> Dec. are shown in Table 2 and in Figures 2 and 3.

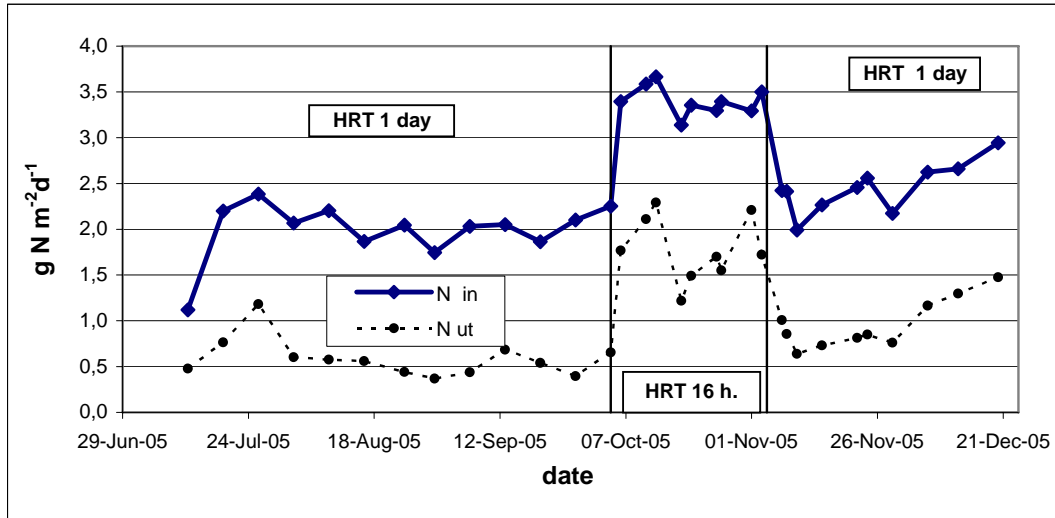
**Table 2.** Main results related to single stage deammonification operation during 29<sup>th</sup> June and 22<sup>nd</sup> Dec (average values).

Period	29 <sup>th</sup> June-4 <sup>th</sup> Oct	4 <sup>th</sup> Oct-4 <sup>th</sup> Nov	4 <sup>th</sup> Nov-22 <sup>nd</sup> Dec
Hydraulic residence time, d	1.2	0.7	1.0
Temperature, °C	26.3	25.8	25.4
Oxygen concentration, g m <sup>-3</sup>	1.51	1.65	1.83
Influent pH-value	7.81	7.85	7.86
Effluent pH-value	7.54	8.0	7.87
Influent alkalinity, mmol l <sup>-1</sup>	55	49	53
Effluent alkalinity, mmol l <sup>-1</sup>	11	27	22
Influent conductivity, mS cm <sup>-1</sup>	5.6	5.8	6.0
Conductivity reduction, %	60	40	50
Influent inorganic nitrogen, g N m <sup>-3</sup>	592	580	594
Effluent ammonium, N/m <sup>-3</sup>	119	285	202
Effluent nitrite, g N/m <sup>-3</sup>	7	2.8	2.5
Effluent nitrate, g N m <sup>-3</sup>	49	15.5	27.2
Removal of inorganic nitrogen, %	70	48	61
Influent inorganic nitrogen load, g N m <sup>-2</sup> d <sup>-1</sup>	2.0	3.4	2.45
Removed inorganic nitrogen, g N m <sup>-2</sup> d <sup>-1</sup>	1.40	1.62	1.49



**Figure 2** Nitrogen in one-stage deammonification process.

As expected a higher removal efficiency of nitrogen leads to lower effluent alkalinity and pH values. Based on the experimental data a lot of correlations were estimated for relations between influent, effluent, and removed parameter values. A compilation is given in Table 3.



**Figure 3** Removed nitrogen.

**Table 3.** Compilation of different relationships based on linear regression of  $y = f(x)$ .

Parameter on y axis	Parameter on x-axis	Relationship	(No)	R <sup>2</sup>
Infl. inorg. N (mM l <sup>-1</sup> )	Infl. conductivity mS cm <sup>-1</sup>	$y = 7.90 - 4.23$	(1)	0.85
Effl. inorg. N (mM l <sup>-1</sup> )	Effl. conductivity mS cm <sup>-1</sup>	$y = 7.40 - 4.87$	(2)	0.96
Rem. inorg. N (mM l <sup>-1</sup> )	Rem. conductivity mS cm <sup>-1</sup>	$y = 7.77 + 2.54$	(3)	0.93
Infl. alkalinity (mM l <sup>-1</sup> )	Infl. inorg N (mM l <sup>-1</sup> )	$y = 0.904 + 12.4$	(4)	0.45
Effl. alkalinity (mM l <sup>-1</sup> )	Effl. inorg. N (mM l <sup>-1</sup> )	$y = 1.23 - 0.81$	(5)	0.83
Infl. alkalinity (mM l <sup>-1</sup> )	Infl. conductivity mS cm <sup>-1</sup>	$y = 9.96 - 8.22$	(6)	0.60
Effl. alkalinity (mM l <sup>-1</sup> )	Effl. conductivity mS cm <sup>-1</sup>	$y = 9.78 - 8.59$	(7)	0.94
Effl. conductivity mS cm <sup>-1</sup>	Effl. pH-value	$y = 2.72 - 18.3$	(8)	0.81
Effl. pH value	Effl. alkalinity mM l <sup>-1</sup>	$y = 0.0265 + 7.27$	(9)	0.93

The first three relationships reflect the concentration or removed ammonium hydrogen carbonate. At infinite dilution and at 25 °C the conductivity of one mmol of ammonium hydrogen carbonate is approximately 11.8 mS/cm. At a concentration of 40 mmol l<sup>-1</sup> of ammonium hydrogen carbonate (560 g N m<sup>-3</sup>) the conductivity value of 1 mmol ammonium hydrogen carbonate (due to effects of ionic strength) is approximately 8.3 mS/cm or similar to the slope of relationships (1) – (3).

The correlation coefficient in relation (4) is rather low although the slope is in general agreement with production of one mM alkalinity per released mM of ammonium. The quotient increased somewhat for the effluent (relationship 5). Influent and effluent alkalinity were linear functions of the conductivity (relationships 6 and 7). The table also shows that the pH-value in the effluent can be predicted for a given value of the effluent conductivity or alkalinity or that the effluent value of conductivity and alkalinity can be predicted based on pH measurements and thereby also the effluent concentration of inorganic nitrogen.

Conductivity and pH measurements are simple to do and can also be performed continuously. Due to the high correlation coefficients in Table 3 for the relationship most of control and monitoring may be based on conductivity and pH measurements while grab samples for measurement of inorganic nitrogen fractions and alkalinity may be seen as valuable supplements to follow up the deammonification process.

#### Rate limiting factors in single-stage Deammonification

In Table 2 it is seen that the effluent values of nitrite nitrogen are small (between 2.5 and 7 g N m<sup>-3</sup>) compared with effluent ammonium concentrations (between 119 and 285 g N m<sup>-3</sup>). The effluent nitrate concentrations are between 15.5 and 49 g N m<sup>-3</sup>. Nitrate may either be obtained through the cell synthesis of anammox bacteria or oxidation of nitrite to nitrate by bacteria as Nitrobacter. The quotient between nitrate nitrogen formation and removed inorganic nitrogen was 0.104, 0.053, and 0.069 for the periods 29<sup>th</sup> June – 4<sup>th</sup> Oct, 4<sup>th</sup> – 4<sup>th</sup> Nov, and 4<sup>th</sup> Nov – 22<sup>nd</sup> Dec, respectively. The anammox reaction including cell synthesis should produce 0.126 g nitrate nitrogen per amount of removed inorganic nitrogen. This calculated value is less than observed values indicating that some heterotrophic denitrification has occurred and that the oxidation of nitrite to nitrate by Nitrobacter is small.

Results from Table 2 indicate that at the used oxygen concentration between 1.51 and 1.85 g O<sub>2</sub> m<sup>-3</sup> access to nitrite for the anammox process is the limiting factor. Increased nitrite concentration may be obtained by increasing the oxygen concentration to enhance nitrification rate or supply of nitrite.

Batch tests were performed to evaluate effects of oxygen and nitrite supply to the anammox rate by use of existing Kaldnes rings from the reactor (Trela et al., 2006, Szatkowska et al., 2006). Obtained removal rate of nitrogen is shown in Table 4.

**Table 4.** Removal rate of nitrogen at different conditions of supply of oxygen and nitrite

Removal rate, g N m <sup>-2</sup> d <sup>-1</sup>	Conditions (No)	2 <sup>nd</sup> Nov	9 <sup>th</sup> Nov	12 <sup>th</sup> Nov	15 <sup>th</sup> Dec
N removal	No oxygen supply; nitrite addition (1)	4.32	5.18	4.88	4.18
NH <sub>4</sub> -N removal		1.94	2.42	2.54	2.0
NO <sub>2</sub> -N removal		2.45	2.92	2.83	2.4
Quotient NH <sub>4</sub> -N/ NO <sub>2</sub> -N removal		1.26	1.21	1.11	1.2
N removal	Oxygen supply; nitrite addition (2)	3.58	4.58	4.2	3.51
NH <sub>4</sub> -N removal		3.3	3.3	2.97	2.85
NO <sub>2</sub> -N removal		0.37	1.55	1.61	1.04
Quotient NH <sub>4</sub> -N + NO <sub>2</sub> -N removal/ N removal		1.03	1.06	1.09	1.11
N removal	Oxygen supply; no nitrite addition (3)	2.05	2.96	1.97	1.98
NH <sub>4</sub> -N removal		2.33	3.3	2.19	2.28
NO <sub>2</sub> -N removal		-	-	-	-
Quotient NH <sub>4</sub> -N Removal/N removal		1.14	1.11	1.11	1.15
Quotients	N removal (2)/(1)	0.83	0.88	0.86	0.81
	N removal (3)/(1)	0.47	0.65	0.40	0.47

The experiments were performed at 25.3-26 °C and at four occasions between 2<sup>nd</sup> Nov and 15<sup>th</sup> Dec. Studies without oxygen supply (mode 1) had an oxygen concentration between 0.11 and 0.22 g O<sub>2</sub> m<sup>-3</sup> and during oxygen supply (mode 2 and 3) the concentration varied between 1.34 and 1.80 g O<sub>2</sub> m<sup>-3</sup>. The initial ammonium concentration varied between 130 and 355 g N m<sup>-3</sup>. At no nitrite addition the initial concentration was between 1.3 and 3.8 g N m<sup>-3</sup> and the initial concentration at nitrite addition was between 71 and 79 g N m<sup>-3</sup>.

Operational mode 1 with no oxygen supply and nitrite addition gave the highest removal rates (in average 4.6 g N m<sup>-2</sup> d<sup>-1</sup>). If oxygen was supplied in addition to nitrite (operational mode 2) the rate was about 0.85 of that of operational mode 1. If only oxygen was supplied (operational mode 3) the reaction rate was about 50% of that at operational mode 1 (in average 2.2 g N m<sup>-2</sup> d<sup>-1</sup>).

The removal rate according to batch operation (3) may be compared with results from the single stage pilot plant deammonification reactor in Table 2 with an average removal rate for the different periods between 1.4 – 1.6 g N m<sup>-2</sup> d<sup>-1</sup> at about the same oxygen concentrations (average values for the periods 1.5 – 1.8) and similar to used oxygen concentration values in batch tests with oxygen supply (1.3 – 1.8 g O<sub>2</sub> m<sup>-3</sup>). Removal rates according to pilot plant results are therefore in average about 68% of removal rate results in the four batch tests and are in general agreement due to the large variation in oxygen concentration during the pilot plant operation and possible disturbances in operation or due to influent composition with possible inhibitory substances, effects of pH-values etc. Present operational mode of the single stage deammonification process indicate that a removal rate of ammonium of 1.5 g N m<sup>-2</sup> d<sup>-1</sup> can be obtained although that possibilities seem to exist based on batch tests to increase the removal rate under optimal conditions with a factor up to three.

For the value of 1.5 g N m<sup>-2</sup> d<sup>-1</sup> and a specific area of 250 m<sup>2</sup> m<sup>-3</sup> the corresponding value for the removal rate of nitrogen is equal to 400 g N m<sup>-3</sup> d<sup>-1</sup> (or 17 g N m<sup>-3</sup> h<sup>-1</sup>). For a treatment plant with a removal of 34 g N m<sup>-3</sup> the anammox process (with its special conditions and today mainly applicable in wastewater treatment plants for supernatant) would only take 2 h in residence time and thus much less than traditional nitrification/denitrification. To remove a certain amount of nitrogen the anammox process seems to require less volume than traditional nitrification/denitrification. The low operational costs in deammonification and simple control methods (as conductivity and pH) makes deammonification in a single stage as a very attractive possibility.

In a fixed film reactor (as the use of Kaldnes material) the rate limiting step is oxidation of ammonium to nitrite. This reaction requires a certain volume. As the anammox process proceeds in an inner layer of the carrier material and gets supply of nitrite from an outer layer of nitrification bacteria the volume need for only nitrification or for combined nitrification and anammox is about the same (if carrier materials are used). In that respect single-stage deammonification has an advantage compared with two-stage technology with one stage for nitrification followed by one stage for anammox.

## **CONCLUSIONS**

Experiments were performed of a one-stage system with partial nitrification and anammox process with a moving-bed biofilm reactor at Himmerfjärden treatment plant during the last half year of 2005. Obtained results were compared and evaluated according to some basic theoretical concepts.

- (1) Diffusion limitations should be expected for oxygen in the outer layer of nitrification bacteria and of nitrite in the inner layer of anammox bacteria.



- (2) Low concentrations of oxygen will due to reaction kinetics and diffusion resistance diminish significantly the nitrification rate.
- (3) Influent salts in the supernatant are dominated by ammonium hydrogen carbonate. Influent concentrations and nitrification and anammox reactions can therefore be reasonably well predicted by conductivity measurements.
- (4) The pH value in the effluent was a linear function both alkalinity and conductivity in the effluent.
- (5) The quotient of removed nitrite nitrogen and ammonium nitrogen was quite close to expected quotient for the anammox reaction based on stoichiometry.
- (6) The volume need for combined nitrification and anammox and only nitrification is about the same by use of carrier materials due to nitrification being the rate limiting step. This gives single-stage technology a potential advantage compared with use of separate nitrification and anammox steps.

Single stage deammonification can be reasonably well understood and evaluated based on different theoretical concepts. For supernatant removal rates of nitrogen was measured to about  $1.5 \text{ g N m}^{-2} \text{ d}^{-1}$  (corresponding to  $400 \text{ g N m}^{-3} \text{ d}^{-1}$ ) indicating that deammonification needs less volume than traditional nitrification/denitrification. The much lower energy costs and no need for chemical additions makes deammonification in a single-stage a very attractive process alternative.

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