

PARTIAL NITRITATION PROCESS ASSESSMENT

Utvärdering av partiell nitrifikation

by LUIZA GUT, ELŻBIETA PŁAZA, MAJA DE UGOŁĘCKA and BENGT HULTMAN

Department of Land and Water Resources Engineering,
Royal Institute of Technology (KTH), S-100 44 Stockholm, Sweden

e-mail: luiza@kth.se



Abstract

In this study, partial nitrification has the aim to oxidise a fraction of ammonium (about 50–60 %) into nitrite without following oxidation to nitrate. Experiments performed during eight months describe partial nitrification of supernatant from dewatering of digested sludge at Himmerfjärden wastewater treatment plant in the south Stockholm region. The process was the first step (reactor R1) and was followed by a second step (reactor R2) for oxidation of ammonium with nitrite to nitrogen gas. The evaluation was based on time series analysis and results from 15 profiles of inorganic nitrogen compounds and different operational parameters. The effluent Nitrite-to-Ammonium Ratio (NAR) depends on the influent supernatant composition (mainly ammonium concentration and alkalinity) and conditions in R1 (mainly dissolved oxygen concentration and drop in pH value). Modified methodology for biofilm was developed to measure Oxygen Uptake Rate (OUR) of ammonium and nitrite oxidisers.

Key words – Free ammonia, nitrite, nitrite-to-ammonium ratio, oxygen uptake rate (OUR) test, partial nitrification, supernatant.

Sammanfattning

Den partiella nitrifikationen syftar i denna studie till att en viss del av ammonium (ca 50–60 %) oxideras mikrobiellt enbart till nitrit och inte till nitrat. Försök i pilotskala under åtta månader redovisas med partiell nitrifikation av rejektivatten från avvattnings av rötslam vid Himmerfjärdsverket i södra stockholmsområdet. Processen utgjorde ett första steg (reaktor R1) för att följas av oxidation av ammonium med nitrit till kvävgas (anammoxprocessen i reaktor R2). Utvärdering av den partiella nitrifikationen skedde med tidsserieanalys och användning av 15 profilmätningar av oorganiska kvävekomponenter och olika driftparametrar i R1. Utgående kvot mellan nitrit- och ammoniumkväve (NAR) beror på inkommande rejektivattens sammansättning (främst ammoniumhalt och alkalinitet) och processbetingelser i R1 (främst syrehalt och pH-minskning). Modifierad metodik för biofilm utvecklades för att uppmäta bakterieaktiviteten med hjälp av syreupptagningshastighet (OUR) för ammonium- och nitritoxidation.

Introduction

Nowadays, in wastewater treatment plants (WWTP) a nitrification/denitrification process is commonly applied to treat wastewater biologically to remove organic materials and nitrogen. According to the latest findings (van Dongen et al., 2001; Fux et al., 2002), there is a cost-saving alternative to the traditional nitrification/denitrification process that is also based on a two-step approach to nitrogen removal. In the first step, around

55–60 % of the ammonium is converted to nitrite nitrogen by nitrifying aerobic microorganisms in for instance the partial SHARON (Single reactor High activity Ammonia Removal Over Nitrite) process. In the second reactor, an anaerobic ammonium oxidation (Anammox) process is possible in a separate tank and is performed by newly identified Anammox bacteria. Subsequent aerobic and anaerobic conditions in both reactors are obligatory to obtain stable cultures. In the first reactor, nitrification process is ceased at the level of nitrite oxidation (later on

called *partial nitrification*) in order to attain proper influent to Anammox reactor. Anammox bacteria utilize nitrite as electron acceptor and therefore only oxidation from ammonium to nitrite is necessary. Such an approach leads to considerable savings in energy for aeration (37 % of the oxygen consumption for nitrification), and no addition of chemicals is needed. Separate treatment of ammonium-rich wastewater, like supernatant from digested sludge, using partial nitrification/Anammox system has the possibility of substantial economic gains as supernatant can contribute to as much as 20 % of a daily nitrogen load of a WWTP. Moreover, high pH and temperature of digested supernatant can be used to contribute to sustainable wastewater management.

In this paper, a partial nitrification step operated with the purpose of combining with the Anammox process is investigated and evaluated. Special attention is given to development of a new methodology for assessing Oxygen Uptake Rates (OUR) of the biofilm nitrifying bacterial culture. In this contribution, interrelations between operational factors during eight months' successful maintenance of a partial nitrification process at the technical-scale pilot plant (Himmerfjärden WWTP, Grödinge, Sweden), supplied directly with the supernatant from dewatering of digested sludge, are discussed.

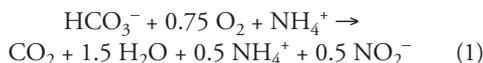
Coupling partial nitrification with the Anammox process

Up until now, the SHARON process is the only fully recognized process in which long-term partial nitrification of ammonium to nitrite is established (Mulder et al., 2001). Similar results have been obtained by treatment of supernatant at Bromma WWTP in Stockholm by the activated sludge process (Tendaj-Xavier, 1985) and by the SBR-technology (Mossakowska, 1994). Suppression of nitrate formation may be based on low pH values (Tendaj-Xavier, 1985), low oxygen values (Mossakowska, 1994) or combination of temperature and residence time in the partial SHARON (van Dongen et al., 2001). Initially, it was designed to couple with heterotrophic denitrification. Recently, a combination of the partial nitrification and Anammox processes has found more support (van Dongen et al., 2001; Fux, 2003). There were also studies concerning nitrite build-up and feasibility of partial nitrification in many types of reactors (Turk and Mavinic, 1989; Bae et al., 2002; Jianlong and Ning, 2003).

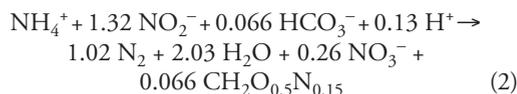
If the partial nitrification process is operated as a preparatory step for achieving efficient nitrogen removal in the Anammox reactor, the quality of effluent from a partial nitrification tank is crucial. Effluent quality can be regulated and monitored by the following factors:

- Nitrite-to-Ammonium Ratio (NAR),
- Alkalinity consumption and pH decrease,
- Conductivity decrease,
- Oxygen supply to nitrification reactor,
- Influent ammonium load,
- Hydraulic retention time (HRT).

In the digested supernatant a quotient $\text{HCO}_3^- : \text{NH}_4\text{-N}$ oscillates around 1.1 (Hellinga et al., 1998). According to the following equation,



the oxidation of 50 to 60 % of ammonium to nitrite is sustained by the buffering capacity of HCO_3^- ions in the supernatant. Further ammonium oxidation leads to the pH drop, shifting equilibrium between ammonium and ammonia towards ammonium (Anthonisen et al., 1976). Consequently, the partial nitrification process is stopped. For the continuous supply of the substrate in combination with proper HRT (1–2 days), a steady state is reached and the required effluent is obtained to perform nitrogen removal in the Anammox reaction:



Due to the above stoichiometric reaction, the NAR should fluctuate theoretically around 1.3 and depends mainly on the oxygen concentration and the needed oxygen supply depends on the influent ammonium load to the partial nitrification step. Furthermore, the level of conductivity is a fast indicator of ammonium concentration in the influent whereas the decrease of conductivity is parallel to alkalinity consumption and subsequent pH drop. Systematic control of the above-enumerated factors gives optimal operation of the partial nitrification process.

Conditions for bacteria involved in partial nitrification process

In nitrification, ammonia is oxidized to nitrate by two different groups of bacteria. The first group, ammonia-oxidizing bacteria (AOB), converts ammonia to nitrite. Then, the second group, nitrite-oxidizing bacteria (NOB), further oxidizes the intermediate product to nitrate. It is recognised that ammonia (NH_3) and not ammonium (NH_4^+) is the substrate for AOB (van Dongen et al., 2001).

Maintenance of the nitrification process requires conditions that enable the NOB to be washed out. However, both AOB and NOB are present simultane-

ously in most cultures and it might be difficult to find suitable conditions to favour one over the other (Egli, 2003). In the biofilm cultures, in order to suppress nitrite oxidation by NOB without retarding ammonia oxidation the dissolved oxygen (DO) concentration is controlled around 1.0 mg O₂/l (Plaza et al., 2003; Szatkowska, 2004 a). The NOB have lower affinity for oxygen than AOB (Hellings et al., 1998) and therefore it is possible to obtain stable conversion of ammonium to nitrite by controlling DO concentration. Moreover, researchers found that certain free ammonia (FA) concentration can inhibit nitrite oxidation (Anthonisen et al., 1976; Bae et al., 2002; Jianlong and Ning, 2003). The NOB inhibition occurs at concentrations of 0.1–10 mg NH₃/l (Anthonisen et al., 1976) although there are studies demonstrating the NOB acclimation to FA with time to a concentration of 22 mg NH₃/l (Turk and Mavinic, 1989). Higher FA concentrations in the range 10–150 mg NH₃/l also inhibit the AOB. The inhibition of all the nitrifying microorganisms by free nitrous acid (FNA) is initiated at concentrations ranging from 0.22 to 2.8 mg HNO₂/l (Anthonisen et al., 1976). Nitrite oxidation can also be controlled by other factors, like pH and temperature (Hellings et al., 1998) through regulating the ionisation of ammonia and washing out NOB at higher temperatures (over 30°C), respectively. Careful adjustment of HRT (aiming at 1 day) helps to wash out NOB while retaining AOB. Because the FA concentration increases with the rise of ammonium nitrogen concentration, ammonium-rich wastewaters are especially applicable to shortcut nitrification process at the level of nitrite formation.

Materials and methods

Technical-scale pilot plant

The research is scaled up to a pilot level. The technical-scale pilot plant is run in the continuous mode with a direct supply of supernatant from dewatering of digested sludge at Himmerfjärden WWTP, Grödinge, Sweden (Gut et al., 2005 a, b, Szatkowska et al., 2004 a, b; Trela et al., 2004 a, b, b). It consists of two reactors where subsequently partial nitrification (R1; HRT=1–2 days) and Anammox processes (R2; HRT=3 days) are established as stable. Primary and secondary clarifiers after each of the reactors are set to act as buffers and excess sludge collectors, respectively. The effluent from R1 is diluted in order not to overload R2. Both reactors work as a moving-bed reactor. Each reactor is divided into 3 zones, 45–50 % filled in with Kaldnes rings for fixed biofilm bacterial growth and equipped with mixers, one heater and an online pH meter. Temperature, dissolved oxygen (DO) concentration and pH are measured man-

ually in every zone. NaCO₃/NaHCO₃ solution for pH correction is added in zone 1 of R2 and the correction is steered from the computer by adjusting the optimal range of pH. In order to follow changes through the zones of the reactor, profiles of inorganic nitrogen forms and parameters are fulfilled according to a schedule.

Analytical and sampling procedures

Samples from the technical-scale pilot plant taken according to the schedule were filtrated with a prefilter and 0.45 µm filter and analysed directly. Chemical analysis of NH₄-N, NO₂-N, NO₃-N concentrations, alkalinity, total phosphate phosphorus (PO₄-P), Chemical Oxygen Demand (COD) and organic acid capacity were accomplished by means of the DrLange VIS Spectrophotometer XION 500. DrLange standardized cuvette tests were used. AQUATEC-TECATOR 5400 ANALYZER (flow-injection system based on VIS spectrophotometry) was also used to analyse inorganic nitrogen compounds from samples collected for profile study.

Oxygen Uptake Rate (OUR) test equipment and procedure

A glass bottle with 600 ml working volume was used in the test. A BOD probe YSI 5905 was connected to a DATA LOGGER TESTO® 251 and RECORDER TESTO 0554.0070. To provide proper dosage of chemicals a narrow tube was fixed in the lid and connected with a syringe. The reactor vessel was completely closed, with no remaining air bubbles. To assure proper mixing of the reaction mixture during measurements, a magnetic stirrer was used. The stopper on the tube was fixed to prevent the air bubbles from disturbing the reaction. One-day and 3-hour aeration of the liquor before the test enabled utilization of the organic substrate. Aeration was conducted in the water bath at the same temperature as in the technical-scale pilot plant (32°C). Kaldnes rings with the biofilm culture were taken directly before the test. The liquid contained insignificant amount of suspended biofilm and was neglected in the calculations. The tests lasted up to 10 minutes depending on the course of the reaction.

The OUR test for biofilm cultures had a modified procedure that was proposed by Surmacz-Górska et al. (1996) for the activated sludge. First, the total oxygen uptake was measured. After 1.5–2 minutes, 5 ml of sodium chlorate (NaClO₃) was added to the mixed liquor (final concentration 17 mM/l). Subsequently, after the next 3–4 minutes 5 ml of Allylthiourea (ATU, final concentration 43 µM/l) was added. The values were recorded every five seconds. The test results were presented as the rate of the DO concentration decrease

Table 1. *Influent supernatant – statistical evaluation (September 2003–April 2004).*

Parameter	Average \pm S.D.	No. of samples
Influent NH ₄ -N (mg/l)	612.7 \pm 114.8	46
pH (–)	7.79 \pm 0.11	136
Alkalinity (mmol HCO ₃ [–] /l)	53 \pm 30	23
Alkalinity/Influent NH ₄ -N ratio (mol/mol)	1.1 \pm 0.5	23
Conductivity (mS/cm)	4.84 \pm 0.67	136
COD (mg O ₂ /l)	382 \pm 166	16
Total PO ₄ -P (mg/l)	26 \pm 14	14
Organic acids (mg/l)	87 \pm 70	17
Suspended solids (SS) (mg/l)	172.7 \pm 117.0	7
Volatile suspended solids (VSS) (mg/l)	145.2 \pm 97.4	7

converted to one day and one square meter of the biofilm (g O₂/m²·d). The oxygen uptake rate was calculated from the slope of the linear parts of the resulting oxygen utilization curve by means of linear regression. The oxygen uptake rate due to NO₂-N oxidation (*Nitrite-oxidizing bacteria*, *NOB*) was considered as the difference between the total OUR and the OUR after NaClO₃ addition. The difference between the OUR with NaClO₃ and the OUR with two inhibitors was calculated as the oxygen uptake rate due to NH₄-N oxidation (*Ammonia-oxidizing bacteria*, *AOB*). At the end, the OUR measured in the presence of two chemicals reflected the oxygen consumption of the heterotrophs (*Heterotrophic respiration*, *HT*). By this method, it is not possible to distinguish between the oxygen consumption for substrate oxidation and endogenous respiration of heterotrophic bacteria.

Results and discussion

Influent and effluent characteristics

Partial nitrification process performance in the first reactor (R1) of the technical-scale pilot plant is dependent

on the variability of the influent supernatant composition (Table 1). High deviations from the average values are typical for the influent ammonium nitrogen concentration, alkalinity, conductivity value, Chemical Oxygen Demand (COD) and organic acid concentration. Over the experimental period, an average alkalinity/NH₄-N ratio in the influent was appropriate to attain an effluent NAR ratio between 1.0 and 1.3. Effluent characteristics (Table 2) show proper process operation with respect to the NAR and decrease of alkalinity, pH value and conductivity.

Variations of nitrogen forms

Over the period from September 2003 to April 2004, R1 was supplied directly with the digested supernatant. Influent and effluent values are time-shifted, taking into consideration HRT (Figure 1). Ammonium nitrogen oxidation was successfully suppressed at the level of nitrite formation during the whole period described. Nitrate nitrogen concentration in the effluent was low, with the average value amounting to 16 mg NO₃-N/l. A favourable NAR was maintained for most of the time, with the changes (0.5–1.5) caused by the variability of

Table 2. *Effluent from reactor 1 – statistical evaluation (September 2003–April 2004).*

Parameter	Average \pm S.D.	No. of samples
Effluent N (mg/l)	592.5 \pm 92.2	46
pH (–)	6.56 \pm 0.73	136
Alkalinity (mmol HCO ₃ [–] /l)	5 \pm 6	23
Nitrite-to-ammonium ratio (NAR)	1.17 \pm 0.25	46
Conductivity (mS/cm)	3.38 \pm 0.48	136
COD (mg O ₂ /l)	241 \pm 99	16
Total PO ₄ -P (mg/l)	23 \pm 17	14
Organic acids (mg/l)	77 \pm 29	13
Suspended solids (SS) (mg/l)	230.7 \pm 97.1	17
Volatile suspended solids (VSS) (mg/l)	193.5 \pm 79.3	17

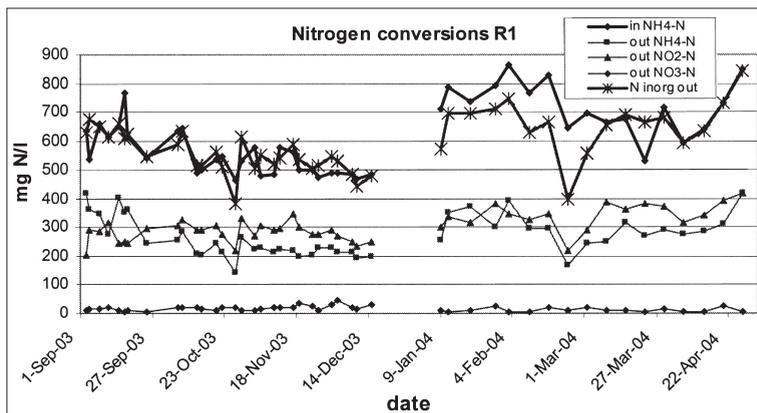


Figure 1. Nitrogen conversions in R1.

the influent ammonium concentration in the range 465–863 mg $\text{NH}_4\text{-N/l}$.

Correlation between NAR and pH drop

The pH value is an excellent parameter for monitoring of the nitrification process in order to keep the effluent NAR close to 1.3. Figure 2 shows a strong correlation between removed pH (between the inlet and outlet of R1) and the NAR in the effluent. When average pH value in the influent (7.79, Table 1) is decreased by the amount 1.5, the NAR reaches the required value of 1.3. Further decrease of pH (targeting value below 6.5) is not satisfactory, as it leads to inhibition of the nitrification rate.

Profile performance

Over a period of eight months, 15 profiles were performed to study changes of inorganic nitrogen forms and parameters along the zones of R1. The following Figures 3 and 4 represent typical stratification of the inorganic nitrogen forms and parameters (pH, DO and conductivity) between the subsequent zones of R1. Profiles were used as a monitoring tool to determine the contribution of bacterial culture to the nitrification process performance. Profiles represent a transitory state of the nitrification process in R1; therefore, the balance of nitrogen forms is not kept. Profiles, however, provide evidence that nitrification is present mainly in zone 1 and 2 of R1. Minor oxidation of nitrite to nitrate occurs mostly in zone 3. Parallel decrease of pH and conductivity is an expected trend. The DO concentration is stratified between the zones, with the highest values in zone 1 (1–1.5 mg $\text{O}_2\text{/l}$), followed by the DO values in the range 0.5–1.0 mg $\text{O}_2\text{/l}$ in zone 2 and 3.

Free ammonia concentration

Statistical representation of the FA concentration in three zones of the pilot plant (Figure 5) confirms results from the profile performance of the inorganic nitrogen forms (Figure 3). Oxidation of ammonia to nitrite by the AOB is predominantly feasible in zones 1 and 2 due to favourable concentration of FA, which is at the same time inhibitory for the NOB. Significant deviations from the average values provide evidence for a variable composition of the influent supernatant, mainly fluctuating buffering capacity (referring to the pH shift) and temperature changes.

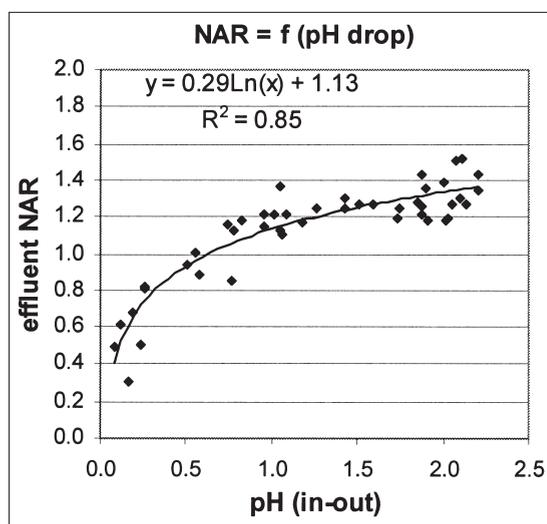


Figure 2. Correlation between the effluent NAR (Nitrite-to-Ammonium Ratio) and the drop of pH value.

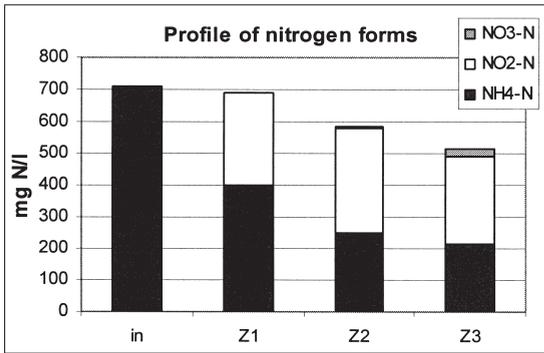


Figure 3. Profile of inorganic nitrogen forms (*in*=influent, *Z*=zone).

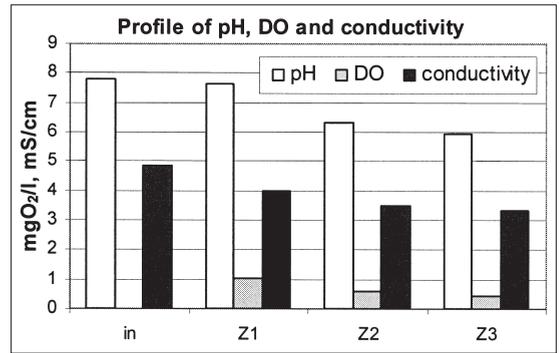


Figure 4. Profile of pH, DO and conductivity (*in*=influent, *Z*=zone).

Oxygen uptake rate tests

Methodology development

The Oxygen Uptake Rate (OUR) tests were based on the methodology applicable for the activated sludge process. The aim of the initial tests (Figure 6) was to adjust a satisfactory amount of the biofilm carriers (Kaldnes rings) to perform a successful OUR test. The carriers and liquid were taken from zone 1 of R1. It was clear that the amount of 100 Kaldnes rings gave the most legible results, with a distinguishable differentiation between the activity of the AOB, NOB and HT. Larger amount of Kaldnes rings was avoided due to total destruction of the biofilm bacterial culture during the test. The inhibitory concentrations based on Surmacz-Górska et al. (1996) were 17 mmol/l of NaClO₃ and 43 μmol/l of ATU; the liquid from zone 1 was aerated for 24 hours before the test.

It was decided to perform subsequent tests with 100 Kaldnes rings in three zones of R1. Further modification was aimed at assessing appropriate inhibitory concentration of NaClO₃ (Figure 7). NaClO₃ suppresses nitrite oxidation and therefore influences results obtained for NOB activity. The lowest values were calculated for concentration of 20 mmol/l. The results with concen-

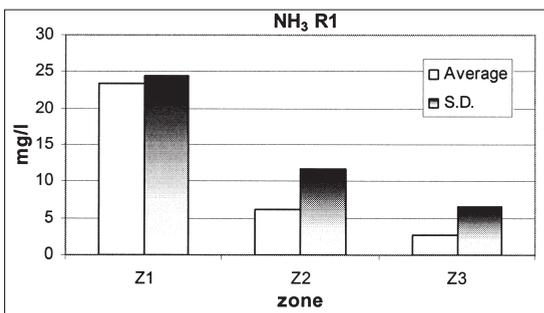


Figure 5. Illustration of free ammonia statistics (*Z*=zone).

trations of NaClO₃ amounting to 25 and 30 mmol/l were rather surprising and therefore these concentrations were excluded as inappropriate for satisfactory test performance. It was decided to continue OUR test performance with NaClO₃ concentration amounting to 17 mmol/l.

Activity of ammonia and nitrite oxidizers over time

In Figure 8 three series of OUR test performed in the period November–December 2003 are shown. The NOB were surprisingly the most active microorganisms, mostly in zone 1 of R1 (average value of 12.6 g O₂/m²·d). It was suspected that due to long 24-hour aeration of the liquid, the NOB were reactivated. It shows that NOB are present in all the zones of R1, although they are suppressed. Consequently, in three series of the OUR tests the potential activity of NOB was measured. The AOB activity was also highest in zone 1 (on average 7.7 g O₂/m²·d). The heterotrophic activity was much lower than for the other groups of bacteria and changed with time and zones of the pilot plant.

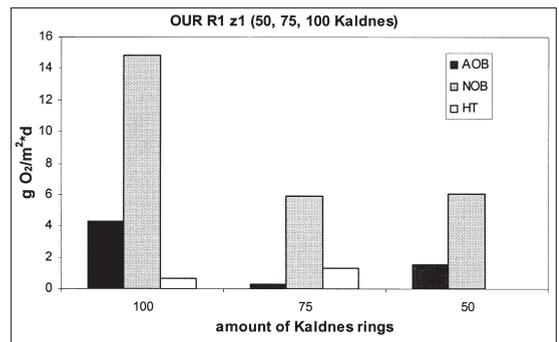


Figure 6. OUR tests with 50, 75 and 100 Kaldnes rings (*z1*=first zone).

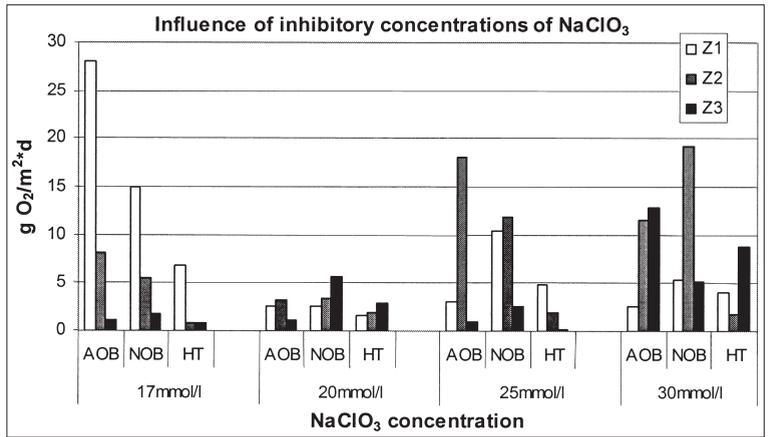


Figure 7. Influence of inhibitory concentrations of NaClO₃ (Z=zone).

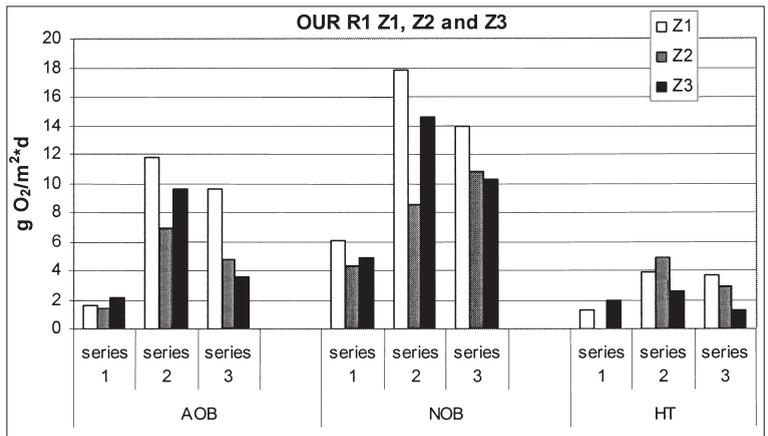


Figure 8. Activity comparison of AOB, NOB and HT between the zones (Z) of R1.

3-hour aeration of the liquid before the test was proposed and gave results presented in Figure 9. The AOB were most active in zone 1, as was expected from the profile performance of the inorganic nitrogen forms (Figure 5). Similarly, the highest activity of NOB was calculated for zone 1 but it was always 1.7 times lower than the AOB activity. There was a decrease of activity of all the microorganisms' activity along the zones. The heterotrophic respiration decreased with subsequent zones of R1 as well but it was insignificantly small and oscillated around 2.9 g O₂/m²·d. The results are in agreement with the results from profile performance (Figure 3).

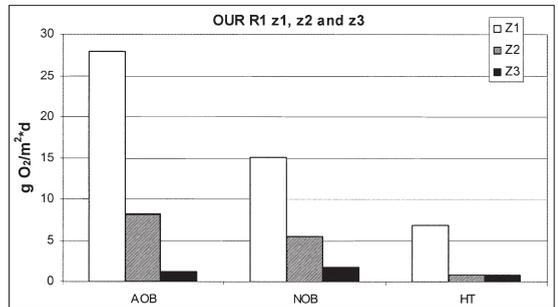


Figure 9. The OUR test with 3-hour aeration before the test (Z=zone).

Conclusions

- A sustained nitrite-to-ammonium ratio without significant formation of nitrate can be obtained in the partial nitritation process in a moving-bed biofilm reactor. It is especially suitable as a preparatory step for nitrogen removal in an Anammox reactor.

- The ammonia oxidation with the purpose of obtaining proper effluent nitrite-to-ammonium ratio around 1.3 in the partial nitritation process in R1 of the pilot plant is influenced by the pH value, DO level in the zones of R1, and the availability of ammonia for ammonia-oxidizing bacteria.

- Variable influent nitrogen concentration as well as ammonia oxidation in the reactor can be monitored by measurements of conductivity. Profiles of inorganic nitrogen forms and parameters along the zones of partial nitrification reactor are a fast indicator of the reaction route.
- Nitrite-oxidizing bacteria can be outcompeted by regulating DO and FA concentration, together with maintaining high temperatures (over 30°C) and appropriate HRT.
- The OUR test can be used efficiently for monitoring purposes in the treatment systems for nitrogen removal. Further development of the test methodology for biofilm cultures is necessary.

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