Pilot scale studies on nitritation-anammox process for mainstream wastewater at low temperature
Karol Trojanowicz, Elzbieta Plaza and Jozef Trela

ABSTRACT
Process of partial nitritation-anammox for mainstream wastewater at low temperature was run in a pilot scale moving bed biofilm reactor (MBBR) system for about 300 days. The biofilm history in the reactor was about 3 years of growth at low temperature (down to 10 °C). The goal of the studies presented in this paper was to achieve effective partial nitritation-anammox process. Influence of nitrogen loading rate, hydraulic retention time, aeration strategy (continuous versus intermittent) and sludge recirculation (integrated fixed-film activated sludge (IFAS) mode) on deammonification process’ efficiency and microbial activity in the examined system was tested. It was found that the sole intermittent aeration strategy is not a sufficient method for successful suppression of nitrite oxidizing bacteria in MBBR. The best performance of the process was achieved in IFAS mode. The highest recorded capacity of ammonia oxidizing bacteria and anammox bacteria in biofilm was 1.4 gN/m²d and 0.5 gN/m²d, respectively, reaching 51% in nitrogen removal efficiency.

Key words | anammox, deammonification, low temperature, mainstream wastewater, nitritation

INTRODUCTION
One stage partial nitritation-anammox process can be an important part of the future – net energy positive – wastewater treatment plant concept. However, it will be possible only if a stable partial nitritation-anammox process can be run at low temperatures and low ammonia nitrogen concentration in the bioreactor. The results obtained by Persson et al. (2014) indicated that the threshold temperature below which capacity of deammonification process significantly decreases is 16 °C. Furthermore, even though activity of anammox was recorded at 5 °C, no adaptation of anammox to low temperature was found. In the study presented by Sultana (2014) upon decreasing ammonia nitrogen concentration to 45 mgN/L, the other factor beside temperature additionally reduced the nitrogen removal rate. This was probably due to the low concentration of process’ substrates (ammonia- and nitrite-nitrogen, inorganic carbon). Furthermore in the course of those experiments nitrite oxidizing bacteria (NOB) outcompeted both ammonia oxidizing bacteria (AOB) and anammox bacteria. Results published in the available literature showed that it is possible to remove nitrogen from mainstream wastewater via partial nitritation-anammox process both in a combined granular-activated sludge system (Wett et al. 2013) and moving bed biofilm reactor (MBBR) (Sultana 2014). Malovanay et al. (2014, 2015) proved the suitability and high capacity of integrated fixed-film activated sludge reactor (IFAS) run at 25 °C for deammonification of mainstream wastewater pretreated in UASB (upflow anaerobic sludge blanket) reactor. It is now commonly agreed that the key factor for effective and efficient mainstream deammonification is the suppression of NOB growth and at the same time keeping an active AOB and anammox biomass (Regmi et al. 2014). It will allow transform capacity of AOB and anammox bacteria into efficiency of nitrogen removal. The scope of the studies presented in this paper was to achieve effective partial nitritation-anammox process in MBBR system with biomass, which was earlier exposed to unfavorable environmental factors (low temperatures) for almost 3 years. Influence of nitrogen loading rate (NLR), hydraulic retention time (HRT), aeration strategy (continuous versus intermittent) and sludge recirculation (IFAS mode) on deammonification process’ efficiency and microbial activity in the examined system was presented.

doi: 10.2166/wst.2015.551
METHODS

The pilot scale MBBR–Hammarby Sjöstadsverk, Stockholm

The pilot scale MBBR of 0.2 m³ was operated at Hammarby Sjöstadsverk, Stockholm, Sweden (research and demonstration facility for innovative water purification) for mainstream deammonification studies. The bioreactor inflow was a low-ammonium diluted supernatant (average concentration of about 45 mgN-NH₄/L), prepared and stored in 1 m³ inlet tank equipped with a mechanical mixer. Detached biomass was separated from treated wastewater in a clarifier. The MBBR was filled with Kaldnes K1 biofilm carriers (500 m²/m³) to 40% of its volume. The biofilm history in the reactor was about 3 years of growth at low temperature (down to 10 °C). It was equipped with on-line sensors for dissolved oxygen (DO), pH, conductivity, redox potential, N-NH₄ and temperature measurements. The temperature in the reactor was kept at two set points (15 or 17 °C) with immerse rod-cooler connected with proportional-integral-derivative (PID) controller. Similarly DO concentration was kept at the set-point with PID controller linked with valves installed in the pipe of pressurized-air supply. All data from the on-line equipment were recorded and collected in a data acquisition system (PC computer) (see Figure 1).

Chemical analyses and basic assays

Concentration of inorganic nitrogen fractions (N-NH₄, N-NO₂, N-NO₃), total nitrogen, chemical oxygen demand (COD) and alkalinity were determined by spectrophotometric method, utilizing Dr Lange cuvette tests and Xion 500 Spectrophotometer (Hach Lange). Sample test solutions were prepared by filtration of grabbed samples of wastewater, taken at the inlet and outlet of pilot scale MBBR, through 0.45 μm filters. Concentration of total-suspended solids and volatile-suspended solids in the bioreactor was determined in accordance with Standard Methods (APHA 1998), utilizing 1.6 μm paper filters (Whatman, grade 40) for sample filtration.

Microbial activity tests

Specific anammox activity was determined with the modified method presented by Dapena-Mora et al. (2007) and Fernandez et al. (2010). Oxygen uptake rate (OUR) batch test was utilized for determining of activity of AOB, NOB and heterotrophs (H). The adapted method of Surmacz-Gorska et al. (1996) and Gut et al. (2005) for biomass growing in a biofilm form was used. As the growth medium in the course of OUR tests a diluted supernatant was used with the final ammonia nitrogen concentration of about 100 mgN/L.

Strategies of the pilot scale MBBR operation

From the MBBR operation period of about 300 days three phases were selected and analyzed in the current article, in which different strategies compliant with the purpose of the studies (achieving efficient mainstream deammonification process at low temperature) were applied. All of the
presented strategies were to have suppressed NOB activity or increased activity of AOB and anammox bacteria. Basic technological parameters of the pilot scale MBBR measured or applied throughout the studies are shown in Table 1. The applied strategies in every phase of the studies were:

- ‘Phase A’—continuous aeration at 15 °C and HRT 20 hours;
- ‘Phase B’—intermittent aeration at 17 °C and HRT 40 hours (variable time intervals and DO concentration);
- ‘Phase C’—transition into IFAS mode of reactor operation (inoculation of activated sludge from conventional sequential batch reactor (SBR)).

RESULTS AND DISCUSSION

Total inorganic nitrogen (TIN) removal efficiency, together with concentration values of nitrogen fractions at the inflow and outflow from MBBR determined in the course of the studies are presented in Figure 2.

<table>
<thead>
<tr>
<th>Process parameter</th>
<th>Phase</th>
<th>Inflow</th>
<th>Outflow</th>
<th>MBBBR</th>
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<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>N-NH₄ [mgN/L]</td>
<td>45.4</td>
<td>35.5</td>
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<td></td>
<td>COD [mgO₂/L]</td>
<td>44.3</td>
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<tr>
<td></td>
<td></td>
<td>44.3</td>
<td>39.1</td>
<td>15.4</td>
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<td></td>
<td>N-NH₄ [mgN/L]</td>
<td>35.5</td>
<td>0.7</td>
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<tr>
<td></td>
<td>N-NO₂ [mgN/L]</td>
<td>4.1</td>
<td>41.1</td>
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<td>N-NO₃ [mgN/L]</td>
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<td>13.4</td>
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<tr>
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<td>pH</td>
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<td>T [°C]</td>
<td>15.2</td>
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<tr>
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<td>ALK [mmolHCO₃/L]</td>
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<td>SS [mg/L]</td>
<td>5.9</td>
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<td>Biofilm [mg/ring]</td>
<td>15.4</td>
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<tr>
<td>E(TIN) %</td>
<td>11.7</td>
<td>25.4</td>
<td>43.9</td>
<td></td>
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<tr>
<td>E(TAN) %</td>
<td>21.0</td>
<td>55.9</td>
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<td>NLR [gN/m²d]</td>
<td>0.19</td>
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<td>NRR [gN/m²d]</td>
<td>0.02</td>
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<td>TAN-RR [mgN/m²d]</td>
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<td>HRT [day]</td>
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<td>Aeration</td>
<td>CA</td>
<td>IA</td>
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</table>

Process performance at temperature of 15 °C and short HRT

In the first period (phase A), the NLR was set at about 0.2 mgN/m²d with HRT of around 0.8 day, and temperature was kept near 15 °C. The continuous aeration mode was applied with steady DO concentration value of 0.5 mgO₂/L. The low efficiency of both TIN and ammonia-nitrogen removal were observed during this phase (11.7% and 21.0%, respectively). The highest activity of all microbial fractions was determined for NOB (which resulted in corresponding bioreactor capacity of nitrite-nitrogen oxidation of about 32 gN/d). Nitrogen load in the same period was about 10.3 gN/d. Low rate of ammonium oxidation resulted from relatively low oxygen supply throughout that phase and at the same time competition of ammonium oxidizers with NOB bacteria for oxygen. In a dedicated batch experiment it was proved that if only DO is not a limiting factor, the ultimate ammonium oxidation is possible by AOB community growing in the pilot scale reactor. The ammonium oxidation rate must have been affected also by low free ammonia (FA).
concentration in the reactor, which is the real substrate for ammonium-oxidizers growth. Concentration of FA is the function of total ammonia (TAN) concentration, pH and temperature in the reactor (Anthonisen et al. 1976). In the period being described, under applied operational conditions in the pilot scale MBBR, average concentration of FA was 0.2 mgN-NH3/L. This value is below affinity constant of AOB (KS) for this substrate (0.3 mgN-NH3/L) (Tora et al. 2010). Average value of TIN removal was about 0.8 gN/d. It covered about 26% of the reactor capacity for nitrogen removal through anammox route. The difference between amount of ammonia utilized and TIN removal was the load of nitrite-nitrogen oxidized by NOB to nitrates. As it was emphasized earlier, the key factor for efficient mainstream deammonification is the suppression of NOB growth and at the same time keeping an active AOB and anammox bacteria. This becomes even more challenging at low temperature of wastewater as NOB are less temperature sensitive than AOB and anammox (lower activation energy of NOB). Furthermore, taking into account basic kinetic parameters of bacterial growth (maximum growth rate (υmax) and yield coefficient (Y)), the theoretical NOB potential of nitrite oxidation (and resulting substrate utilization rates/substrate fluxes in the reactor) is one order of magnitude higher than for AOB and two orders of magnitude higher than for anammox. This implies that even low amounts of NOB in biofilm can successfully compete with (or even outcompete) AOB and anammox. The phenomena of generating high substrate fluxes by NOB will have a higher influence at lower temperatures and under conditions of absence of inhibitors of NOB growth (like FA or free nitrous acid (FNA)). Lack of those inhibiting factors is certain in a low strength mainstream wastewater. In the ‘phase A’, the average concentrations of FA and FNA were 0.2 mgN/L and 1.3·10⁻⁴ mgN/L, respectively. The corresponding inhibition constants of NOB for those factors are 0.1 and 0.2 mgN/L (Van Hulle 2005). Although concentration of FA was about two times higher than inhibition constant, a significant suppression of NOB growth was not noticed. Possibly the decrease of nitrogen flux (induced by NOB) through inhibition by FA was compensated by other metabolic features of this group of autotrophs (expressed as ratio between maximum growth rate and yield coefficient), as mentioned earlier. The temperature affects the microorganisms of deammonification processes directly (by influencing maximum growth rate of bacteria) and indirectly (by changing concentrations of some substrates and inhibitors, examples: FA, FNA).

Based on this knowledge, the scope of presented research was to find an effective and feasible control method of NOB growth at low temperatures and low nitrogen concentrations in the reactor. Therefore, different intermittent aeration strategies were tested in the second stage of the presented studies (phase B).

### Influence of intermittent aeration strategy on process performance

In the course of the ‘phase B’ of MBBR operation, an intermittent aeration strategy was applied in order to suppress
NOB biomass in the biofilm. It was justified by findings of Regmi et al. (2013, 2014) and Al-Omari et al. (2013) concerning differences in values of half saturation coefficients for oxygen ($K_{O2}$) of AOB and NOB bacteria ($K_{O2}^{AOB}$, $K_{O2}^{NOB}$ are 0.1 mgO$_2$/L and 0.6 mgO$_2$/L, respectively). It was also known that maximum growth rate of AOB is higher than NOB (0.8 d$^{-1}$ and 0.6 d$^{-1}$, respectively) (Van Hulle 2005; Kaelin et al. 2009). It supported the concept of aeration strategy with high DO concentration (higher than 1 mgO$_2$/L) and intermittent anoxic conditions. The proposed mechanism of NOB growth suppression consists of depriving nitrite oxidizers from one of the substrates (oxygen in a non-aerated phase or nitrates during aeration). Due to this, an aerated phase is usually short and non-aerated periods are prolonged. However, quoted studies regarded AOB and NOB biomass growing in a suspended form. In the case of biofilm it was unknown whether it would be a successful strategy. In the biofilm a gradual decrease of DO and any other substrates’ concentration takes place. As the result of biofilm’s heterogeneity we could also expect that NOB bacteria can find a niche inside a biofilm, where growth conditions are optimal. The results obtained by Person et al. (2014) indicated that the threshold temperature below which capacity of denitrification process significantly decreases is 16°C. Because of this in this phase temperature in the pilot scale bioreactor was increased from 15 to 17°C – above the determined earlier threshold level. The low efficiency of nitrogen removal in ‘phase A’ was caused by overloading the bioreactor with nitrogen and keeping very low temperature in the reactor (NLR was higher than estimated bioreactor capacity of nitrogen removal via nitritation-anammox process). Due to this it was decided to increase HRT from about 21 hours to about 40 hours. As a result the nitrogen load was lowered from 10 to 5 gN/day. The MBBR was intermittently aerated with different length of aerated (‘AERon’) and non-aerated (‘AEROff’) phases and variable set points of DO concentrations in the reactor (see Table 1). Throughout the first 40 days a positive trend took place in the system. Under stable NLR, the gradual decrease of ammonia-nitrogen concentration occurred. At the same time nitrate-nitrogen concentration were kept below 10 mgN/L. The continuing decrease of NOBs and at the same time a substantial increase of AOB activity took place throughout that time, as well (see Figure 3). Also activity of anammox bacteria increased from about 0.1 to 0.3 gN/m$^3$d. However, from the time when ammonia-nitrogen concentration decreased to about 20 mgN/L, that advantageous trend stopped. The increase of nitrate-nitrogen concentration together with NOB activity was recorded during that interval. The stable efficiency of TIN and TAN removal of around 27% (maximum 31%) and 65%, respectively, were kept during that phase. However, no significant suppression of NOB bacteria occurred regardless of the applied intermittent aeration strategy. Their activity was still the major limiting factor of the process. It was shown very well in Figure 2 that higher ammonia-nitrogen removal rate in the reactor always resulted in higher nitrates concentration at the outlet.

**Transition of the reactor into an IFAS mode**

It has been recently proved by Malovanyy et al. (2014) that running partial-nitritation/anammox process in a hybrid MBBR activated sludge system (integrated fixed-film activated sludge – IFAS) improves significantly both capacity and efficiency of the process. Furthermore intermittent aeration strategy applied for suppressing of NOB bacteria while they grow in the suspended form seems to be more effective than in case of the biofilm (Al-Omari et al. 2013; Regmi et al. 2014). Therefore in the last phase of the research, a transition from the MBBR to IFAS mode of reactor operation has been started and studied. MBBR was equipped with additional sedimentation tank with volume of about 80 liters and recirculation pump (see Figure 1). A volume of about 70 liters of conventional activated sludge was sampled from SBR operated at Hammarby Sjöstadsverk Stockholm. The equivalent amount of biomass in that volume of activated sludge was about 200 g. The sampled sludge was decanted and transferred into the sedimentation tank and started being recirculated into MBBR. The concentration of activated sludge in the reactor upon activated sludge addition was in the range from 100 to 500 mgSS/L (in comparison to about 6 mgSS/L in previous stages of the bioreactor’s operation). Bioreactor was aerated intermittently with 15 minutes and 60 minutes of aerated and non-aerated phases, respectively. DO concentration was 1.5 mgO$_2$/L. At the beginning of that phase of reactor operation the activity of AOB, NOB and H bacteria (growing in a suspended form) was about 0.2 gO$_2$/gSS.d. The high activity of both NOB and H, at the beginning of that phase was rational since activated sludge came from conventional wastewater treatment system (see Figure 3). However just after two weeks a positive trend occurred of enriching activated sludge with AOB bacteria and eliminating NOB and H from that compartment of the system. Out-selection of NOB was probably caused by intermittent aeration strategy and heterotrophic bacteria.
started being removed from the suspended biomass also due to relatively low ratio of COD to N-NH$_4$ in the influent. Because of higher nitrites flux, the activity of anammox biomass (growing in biofilm) was also at a relatively high level of 0.5 gN/m$^2$d. As the result of those advantageous changes the efficiency of the system
increased from 23 to 44% (maximum 51%) - see Table 1 and Figure 2. Because IFAS seems to give the best opportunities for successful process running and NOB out-selection, this phase of the studies should be continued for a longer period, so we can formulate a final conclusion as to whether this system can be a solution for efficient mainstream deammonification.

CONCLUSIONS

On the basis of the discussed results the following key findings can be presented:

- Intermittent aeration strategy is not sufficient as a NOB growth’s control tool in MBBR systems for one-stage autotrophic deammonification at low temperature and low N-NH₄ concentration.
- NOB are well protected inside a biofilm and cannot be irreversible inhibited by high FA concentration.
- IFAS mode of plant operation gave the promising results with inoculation of biomass growing in a conventional SBR.
- IFAS mode suppression of NOB growing in a suspended form is probably easier to be achieved than in MBBR system.

ACKNOWLEDGEMENTS

Karol Trojanowicz was supported with a post-doctoral fellowship from the Swedish Institute (SI) within the Visby Program. The research work was financed by Swedish Water Development (SVU), Swedish Environmental Research Institute (IVL), the Royal Institute of Technology (KTH). The experimental work was performed at Hammarby Sjöstadsværk, Stockholm, Sweden (Swedish Water Innovation Center). Karol Trojanowicz’s participation in the conference ‘IWA Nutrient Removal and Recovery 2015: moving innovation into practice’ was sponsored by St. Pigeon Krosno State College, Poland.

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First received 12 August 2015; accepted in revised form 12 October 2015. Available online 26 October 2015