Short and Long Term Effect of Decreasing Temperature on Anammox Activity and Enrichment in Mainstream Granular Sludge Process

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Abstract. This study investigates the impact of lower temperature on short term and long term (down to 10 °C) on a completely anoxic anammox granular sludge process. This is the first time granular sludge Anammox is operated in pure anoxic condition in SBR and at low temperature. Conversion performance, kinetic parameters, sludge characteristics and microbial community were analyzed.

Keywords: Mainstream anammox · Low temperature · Granular sludge

1 Introduction

Partial nitritation/anammox (PN/A) is an autotrophic biological nitrogen removal process which allows to reduce oxygen consumption, remove the need for organic carbon and reduce sludge production. It can thus present a more energy-, carbon- and cost-efficient treatment compared to the conventional nitrification/denitrification process (Vlaeminck et al. 2014). The main roadblocks for PN/A implementation on pretreated sewage, so-called mainstream PN/A, are robust tools to suppress nitrite oxidizing bacteria (NOB) and promote anammox bacteria (AnAOB) under relatively low influent nitrogen concentrations (40–80 mg NH_4^+-N/L), non-negligible amounts of biodegradable organic carbon and relatively low temperatures (10–30 °C). Due to the various potential economic and ecologic advantages to come from applications of anammox in mainstream N-removal processes, increasingly more research is being conducted to adapt anammox biomass to lower temperatures in various set-ups (SBR/MBBR) with flocs, granules or carriers, fed with synthetic or real wastewater.

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2 Materials and Methods

2.1 Short Term Batch Experiments

For the short-term temperature effects, the specific anammox activity (SAA) was determined in 500 mL Schott bottles at five different temperatures (10–30 °C). Four different types (referred to as LISBP, CDA, LSD and LRO) of biomass from anammox-based processes were tested using a synthetic medium with 30 mg N/L (NH_4^+-N/NO_2^--N ratio was 1/1.32), without added COD and incubated under anoxic conditions. The NH_4^+, NO_2^- and NO_3^- concentrations were measured throughout the experiment (which lasted between 9 and 150 h depending on the imposed temperature).

2.2 Long Term Experiments with SBR Reactors

Two anoxic lab-scale SBR reactors have been operated on synthetic influent for over ten months. Both reactors were inoculated with the same biomass and operated identically for the first two months. After this, operating conditions in the reference reactor (SBR_{ref}) were kept constant throughout the entire experiment while in the test reactor (SBR_{test}) operating conditions (temperature and corresponding loading rate) were gradually changed to examine the long term effect of decreasing temperature on its performance. The respective anammox kinetics were determined for each reactor by monitoring the *in situ* NH_4^+, NO_2^- and NO_3^- concentrations all along one reaction phase.

3 Results

3.1 Batch Tests

In the short-term temperature effect experiments, a decrease in temperature resulted in a decrease in specific anammox activity (SAA; expressed in mg NH_4^+-N/g VSS/d) for all four types of biomass, results are shown in Fig. 1. This response was variable depending on the initial biomass and variability was stronger for lower temperatures (15 °C–10 °C). One single temperature coefficient (Arrhenius θ-value) was not sufficient to accurately describe the SAA response for the entire temperature range (30 °C–10 °C). To see if the difference in temperature dependency could be explained by physical or microbiological factors, assays were performed after specific disaggregation and the microbial community composition was analyzed.

3.2 Reactor Performance

For the long-term reactor tests, the volumetric activity of the control reactor SBR_{ref} has continuously increased upon inoculation and reached about six times its initial value up to 11 mg NH_4^+/L/h. In SBR_{test}, the decrease in temperature resulted in a decrease of volumetric activity. This effect was most pronounced when temperature decreased from 30 to 20 °C, resulting in a 3-fold drop in activity (due to biomass

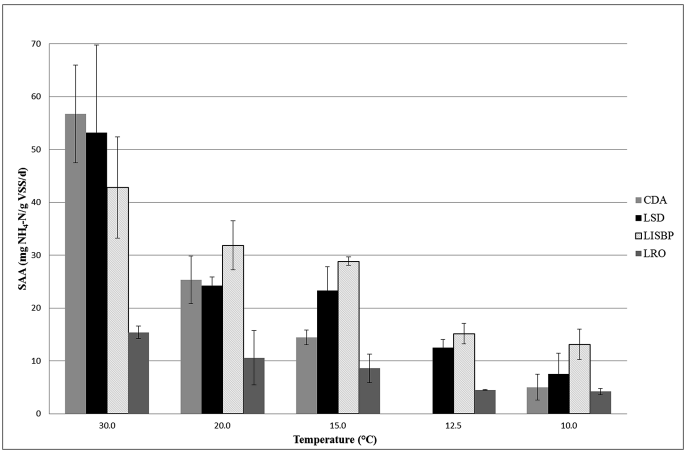

Fig. 1. Specific anammox activities for the different biomasses at different temperatures

selection and sludge loss). Activity recovered throughout the next two months; the temperature was then further decreased. The impact of these decreases in temperature was smaller, the relative loss in activity was lower (20 and 35% for 15 °C and 12.5 °C respectively) and each time activity recovered within the month, even when operated at 10 ° (after a 75% drop in activity). Results from FISH analyses and 16S rRNA amplicon sequencing will be used to compare the evolution in microbial diversity in both reactors and get an indication of the level of anammox enrichment (Fig. 2).

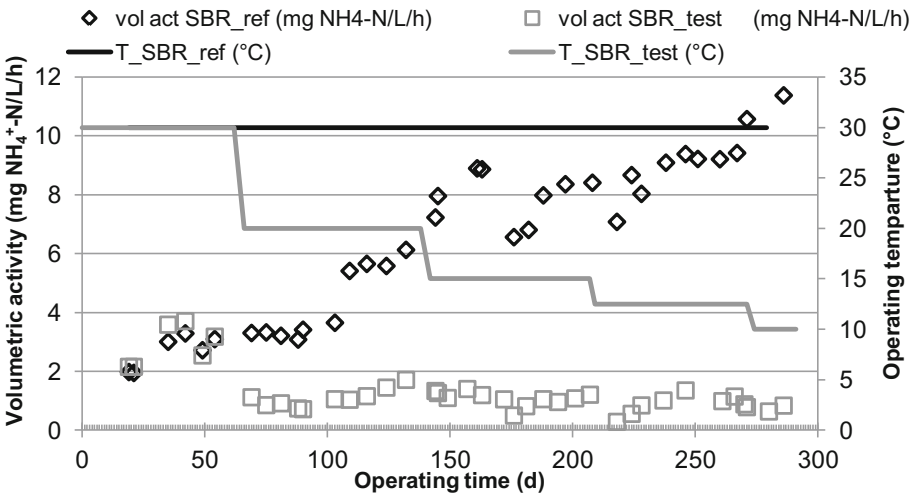

Fig. 2. Volumetric activities related to operating temperature for SBR_ref (black, diamonds) and SBR_test (grey, squares) throughout the experiment

During reactor operation, the biomass evolved from a dark brownish and very heterogeneous, hybrid sludge (the inoculum) containing flocs, granules and suspended biomass to a bright red, predominantly granular sludge. Even at 10 °C, the granules demonstrated very good settling properties due to their size and density. Larger granules seem to be observed (2–6 mm diameter) which could show that lowering the specific growth rate can even improve the granulation process. Analyses of the particle size distribution (PSD) and image analysis are being completed for confirming or informing that observation.

3.3 Long Term vs. Short Term Temperature Effect

The SAA observed at start of the reactor operation (30 °C) was 51.6 mg NH_4^+-N/gVSS/d which was comparable to the activities observed during most of the batch tests at the same temperature (Table 1). Throughout the experiment, SAA increased in both SBR_{ref} (30 °C) and SBR_{test} (10 °C), reaching values of 824.8 and 99.8 mg NH_4^+-N/gVSS/d respectively at day 286. The SAA observed in SBR_{test} at 10 °C was around seven to ten times higher than the values obtained during the short term test at the same temperature. After 286 days, SAA observed at 10 °C was 12% of the activity observed at 30 °C which is comparable to the results found for the CDA and LSD sludge (9 and 14% respectively). The SAA found at 10 °C is the highest value ever reported at that temperature. The observed increases in specific anammox activity during the long term experiment can be correlated to an enrichment and/or adaptation of the anammox sludge. FISH analyses and 16S rRNA amplicon sequencing results will provide more insight. These findings will be useful for the design of future low temperature applications.

Table 1. Comparison of specific anammox activities (expressed in mg NH_4^+-N/gVSS/d) for short and long term experiments

	Short term				Long term		
	CDA	LSD	LRO	LISBP	SBR_{ref} d1	SBR_{ref} d286	SBR_{test} d286
SAA 30 °C	56.8 ± 13.9	53.2 ± 16.5	15.4 ± 1.2	42.8 ± 9.6	51.6	824.8	-
SAA 10 °C	5 ± 2.4	7.5 ± 3.9	4.2 ± 0.6	13.1 ± 2.9	-	-	99.8
Ratio 10 °C/30 °C	9%	14%	27%	31%	-	-	12%[a]

[a]ratio SBR_test/SBR_ref on d286

The SAA values obtained in this study at 10 °C under optimal conditions (purely anoxic, no substrate limitation) are considerably higher than values previously reported in literature, this is illustrated in Table 2.

Table 2. List of specific anammox activities in other low temperature studies

Set-up	Temperature	Specific anammox activity (mg NH_4^+-N/gVSS/d)	Reference
Anoxic batch test (short term)	10 °C	0–11	Lotti et al. 2013
PN/A SBAR	10 °C	22	Lotti et al. 2014
PN/A SBR	10 °C	16	Hu et al. 2013
PN/A gaslift SBR	10 °C	13–19	Hendrickx et al. 2014
Anoxic SBR	10 °C	99.8	This study

4 Conclusions

This study gives new insights concerning Anammox operation at low temperature:

- Long term compared to short term experiments allows much higher SAA at 10 °C, but the decrease in SAA due to temperature reduction was finally in the same order of magnitude.
- Reactor was successfully operated for over 300 days, activity was maintained even at 10 °C
- Long term adaptation/enrichment increased SAA at 10 °C by 7–10 fold compared to batch tests. This is the highest SAA reported at 10 °C for granular sludge corresponding to the optimal conditions for Anammox growth: purely Anoxic and no substrate limitation.
- Granular sludge with excellent settling properties and large granules was formed and maintained at 10 °C indicating that lowering the growth rate can even improve the granulation process

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