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# Simultaneous partial nitritation and anammox at low temperature with granular sludge



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#### ABSTRACT

Autotrophic nitrogen removal in the main stream appears as a prerequisite for the implementation of energy autarchic wastewater treatment plants. To investigate autotrophic nitrogen removal a lab-scale gas-lift sequencing batch reactor with granular sludge was operated for more than 500 days. The reactor was operated at temperatures between 20 and 10 °C on autotrophic medium with ammonium (60 and 160 mg-N L<sup>-1</sup>) as only nitrogen compound at an HRT of 0.23–0.3 d. The dissolved oxygen (DO) concentration was shown to be an effective control parameter for the suppression of the undesired nitratation process. DO control guaranteed the effective suppression of the nitratation both at 20 and 15 °C, allowing nitrogen removal rates of 0.4 g-N<sub>Tot</sub> L<sup>-1</sup> d<sup>-1</sup> at nitrogen removal efficiencies of 85-75%. Prolonged operation at 10 °C caused a slow but unrestrainable decrease in anammox activity and process efficiency. This study represents a proof of concept for the application of the autotrophic nitrogen removal in a single reactor with granular sludge at main stream conditions.

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#### 1. Introduction

The application of the anammox process for the treatment of concentrated and warm wastewaters characterized by temperatures exceeding 25 °C and influent nitrogen concentrations over 0.1 g-N  $L^{-1}$  (mainly the liquid fraction of the digestate) is nowadays part of the state of the art (van Hulle et al., 2010). The application at lower temperatures and lower nitrogen concentrations would allow to extend the application potential of anammox-related processes to municipal sewage treatment opening new possible scenarios

in designing energy producing wastewater treatment plants (Jetten et al., 1997; Kartal et al., 2010; Siegrist et al., 2008). In order to maximize energy recovery from municipal wastewater removal of the organic carbon as a first step would be most advantageous. This first step could either be a UASB reactor at tropical climates (Aiyuk et al., 2006) or a concentration step at lower temperature regions. This concentration can be performed by physical (e.g. sieving), chemical (e.g. precipitation) or biological (Versprille et al., 1984) methods, or combinations thereof. The concentrated sludge can then subsequently be digested anaerobically resulting in methane containing biogas. The remaining liquid contains ammonium

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that can be removed in an autotrophic process based on anammox (Abma et al., 2010; Jetten et al., 1997; Joss et al., 2009; Kartal et al., 2010; van der Star et al., 2007; Wett et al., 2007). To facilitate autotrophic nitrogen removal on pretreated sewage a process based on prevention of growth of nitrite oxidizing bacteria (NOB) and retaining anaerobic ammonium oxidation (anammox) bacteria in the system in symbiosis with ammonium oxidizing bacteria (AOB) has to be developed. The main challenge for applying anammox in the water line of a WWTP is to achieve a high rate process with good biomass retention and low effluent nitrogen concentration at low water temperatures. A very large decrease in specific anammox activity was reported after lowering the temperature of reactors operated at 30-32 °C (Dosta et al., 2008; Isaka et al., 2008; Vázquez-Padín et al., 2011). Despite this decrease in activity several laboratory studies have reported 1-stage partial nitritation/anammox at lower temperatures ( $\leq$ 25 °C) and reasonable conversion rates (De Clippeleir et al., 2013; Szatkowska et al., 2007; Vázquez-Padín et al., 2011; Winkler et al., 2011; Hu et al., 2013; Lotti et al., 2014a). In natural ecosystems such as Northern European soils and marine sediments, anammox bacteria thrive at low temperatures (<10 °C) and very low ammonium concentrations (µM range) (Hu et al., 2011; Lam and Kuypers, 2011; van de Vossenberg et al., 2008), indicating that there is no fundamental limitation for the anammox process to develop under municipal wastewater conditions. This putative capability was recently confirmed by the study of Lotti et al. (2014a) where anammox bacteria were shown to grow under anoxic conditions in a labscale fluidized bed reactor fed with pretreated municipal wastewater.

This study aimed at demonstrating the feasibility of granular sludge-anammox based single stage (aerated system where both AOB and anammox bacteria coexist) autotrophic nitrogen removal on lab-scale at low temperature. A sequencing batch granular sludge based lab-scale reactor (2.7 L) was fed with synthetic medium at conditions relevant for main-stream applications. The ammonium concentration in the reactor was during regular operation always below 70 mg-N  $L^{-1}$  and nitrite below 30 mg-N  $L^{-1}$ . A closely monitored granular sludge process operated in an air-lift sequencing batch reactor (SBAR) was chosen with the objective to achieve adequate biomass retention and high volumetric N-conversion rates as well as to investigate the interactions among different cohabiting bacterial populations. The reactor was operated at decreasing temperatures of 20, 15 and 10 °C. The results obtained during 18 months of operation of the lab-scale reactor are presented.

#### 2. Material and methods

#### 2.1. Long term reactor operation

Experiments were conducted in a lab-scale air-lift reactor (working volume, 2.7 L) with granular biomass operated in a sequencing fed-batch mode (SBAR, sequencing batch air-lift reactor). The reactor was inoculated with granular biomass capable of autotrophic nitrogen removal enriched at room temperature during a previous study (Winkler et al., 2012). The experimental period described in this study was preceded by a long start-up in which the reactor was operated at room temperature ( $22 \pm 3 \,^{\circ}$ C) and fed with ammonium as the only nitrogen source. After eight months of operation at room temperature, the reactor was temperature controlled using a thermostatic bath connected to the double wall of the reactor (referred to as experimental day one). The reactor was operated in five phases as reported in Table 1.

The exchange ratio ( $V_{Effluent}/V_{Reactor}$ ) was fixed at 0.56. The hydraulic retention time (HRT) was fixed at 7.2 h during phase I-IV and it was decreased to 5.4 h in phase V decreasing the cycle length from 4 to 3 h. The total cycle time was 4 h during the first 483 days and 3 h for the last two months of the experimental period. The 4-h cycle consisted of one hour of aerobic feeding phase, about two hours of aerobic reaction phase, one hour of anoxic reaction phase (DO setpoint equal to zero), 4 min of settling phase and 5 min of decant phase. In the 3h cycle the aerobic and anoxic reaction phases were shortened to one hour and 51 min, respectively. In the 4-h (3-h) cycle the ratio between aerobic and anoxic time was 2.85:1 (2.4:1), resulting in the cycle scheme reported in Fig. 1. The settling time of four minutes was chosen such that only particles with a settling velocity larger than 12 m  $h^{-1}$  were effectively retained in the reactor.

The mineral medium consisted of 0.8 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 10 mM KHCO<sub>3</sub>, 0.18 mM K<sub>2</sub>HPO<sub>4</sub>, and 0.22 mM KH<sub>2</sub>PO<sub>4</sub> (8.3 mg-P L<sup>-1</sup>). Ammonium was dosed dissolving NH<sub>4</sub>Cl in the feeding medium to reach the final ammonium concentration reported in Table 1. Trace elements were provided according to Lotti et al. (2014b). The pH was maintained at 7.3  $\pm$  0.2 during the aerobic period by dosage of sulfuric acid (0.2 M) and a solution of sodium carbonate (0.25 M). The dissolved oxygen (DO) automatically concentration was regulated at  $0.5\div 2.5 \text{ mg O}_2 \text{ L}^{-1}$ . The DO was set by recirculating the off-gas and blending with either fresh air or nitrogen gas (N<sub>2</sub>) by means of mass flow controllers (MFCs). In this way the DO could be controlled while a constant biogas flow of 1 L min<sup>-1</sup>

Table 1 – Description of the different operational phases throughout the experimentation.					
Phase	Period	Temperature	$\mathrm{NH}^+_{4_{\mathrm{Influent}}}$	Cycle length	Volumetric N-load
	day	°C	$\overline{\text{mg-N L}^{-1}}$	h	g-NH <sub>4</sub> <sup>+</sup> -N $L^{-1} d^{-1}$
I	1–110	20	160	4	0.53
II	111-425	15	160	4	0.53
III	426440	15÷10	160	4	0.53
IV	440-483	10	130–60	4	0.43-0.27
V	484–541	10	60	3	0.27

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