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# Stable partial nitrification for low-strength wastewater at low temperature in an aerobic granular reactor

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## ABSTRACT

Partial nitrification for a low-strength wastewater at low temperature was stably achieved in an aerobic granular reactor. A bench-scale granular sludge bioreactor was operated in continuous mode treating an influent of  $70 \text{ mg N-NH}_4^+ \text{ L}^{-1}$  to mimic pretreated municipal nitrogenous wastewater and the temperature was progressively decreased from 30 to 12.5 °C. A suitable effluent nitrite to ammonium concentrations ratio to a subsequent anammox reactor was maintained stable during 300 days at 12.5 °C. The average applied nitrogen loading rate at 12.5 °C was  $0.7 \pm 0.3 \text{ g N L}^{-1} \text{ d}^{-1}$ , with an effluent nitrate concentration of only  $2.5 \pm 0.7 \text{ mg N-NO}_3^- \text{ L}^{-1}$ . The biomass fraction of nitrite-oxidizing bacteria (NOB) in the granular sludge decreased from 19% to only 1% in 6 months of reactor operation at 12.5 °C. *Nitrobacter* spp. were found as the dominant NOB population, whereas *Nitrospira* spp. were not detected. Simulations indicated that: (i) NOB would only be effectively repressed when their oxygen half-saturation coefficient was higher than that of ammonia-oxidizing bacteria; and (ii) a lower specific growth rate of NOB was maintained at any point in the biofilm (even at 12.5 °C) due to the bulk ammonium concentration imposed through the control strategy.

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## 1. Introduction

For the achievement of sustainable (energy-neutral or even energy-positive) wastewater treatment plants the use of anammox for sewage treatment has been proposed (Kartal et al., 2010). The performance of one-stage nitrogen (N) removal of pretreated municipal nitrogenous wastewater has been tested with sequencing batch reactors (SBR) as a first approach (Winkler et al., 2011; Hu et al., 2013). In many of the studies, the known weak point of those trials is that nitrite-

oxidizing bacteria (NOB) developed in the long-term operation, triggering the production of nitrate, and decreasing importantly the N-removal performance with anammox (Winkler et al., 2011; De Clippeleir et al., 2013). Even in the treatment of the sidestream (reject water), with more advantageous conditions for anammox-based N-removal, the development of NOB in one-stage granular reactors was a problematic issue during the maintenance routines, like short-term aeration pulses, which are thought to lead to healthy NOB population (Joss et al., 2011).

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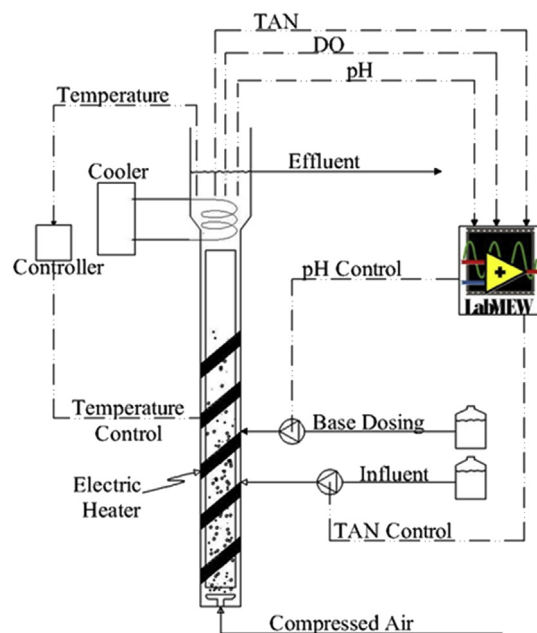
A two-stage N-removal system operating in continuous mode could be thought also as an appealing solution for sewage treatment (Regmi et al., 2014). In fact, a two-stage N-removal system was proposed as a potential alternative (nitrification with activated sludge, (Ma et al., 2011); nitrification with granular reactor, (Torà et al., 2013)). In the past, poor results were reported, and little attention had been paid to partial nitrification with biofilm reactors either because such a process was thought difficult to be maintained in the long-term (Garrido et al., 1997; Fux et al., 2004) or because trials yielded not the expected results (Bernet et al., 2005). However, stable nitrification in biofilm reactors operating in continuous mode have been reported for the specific treatment of the sidestream (Torà et al., 2013) and other types of rich ammonium wastewaters (Tokutomi, 2004; Bougard et al., 2006; Bartrolí et al., 2010) at temperatures over 20 °C. Also the process has been deeply studied through mathematical modeling (Pérez et al., 2009; Brockmann et al., 2010; Jemaat et al., 2013). The success of such a treatment relies in the use of a control strategy to maintain the adequate ratio between oxygen and ammonium concentrations in the reactor bulk liquid, as to repress NOB activity in the biofilm (automatic control for partial nitrification to nitrite in biofilm reactors, ANFIBIO) (Bartrolí et al., 2010; Jemaat et al., 2013). However, when applying such a strategy to the mainstream, two different challenges could be outlined: (i) the partial nitrification reactor would need to produce the adequate ratio between ammonium and nitrite concentrations as to feed a subsequent anammox reactor and (ii) to the best of our knowledge, a stable partial nitrification reactor (achieving an effluent with a nitrite/ammonium ratio of 1), with floccular, attached or granular biomass, at temperatures lower than 15 °C and treating low-strength ammonium wastewaters has not been reported. Only some studies achieved stable full nitrification in SBRs at these conditions but treating low nitrogen loading rates, NLR: 0.05–0.10 g N L<sup>-1</sup> d<sup>-1</sup> (Yuan and Oleszkiewicz, 2011; Gu et al., 2012). Other reactors operated at higher NLRs showed sudden deterioration of the nitrification at temperatures lower than 15 °C (Yamamoto et al., 2006).

Here, we would like to demonstrate the feasibility of partial nitrification in a granular reactor operating in continuous mode at low temperatures, treating a wastewater with low N concentrations. Microbiological analyses and mathematical modeling tools will be used to better understand the experimental results.

## 2. Materials and methods

### 2.1. Reactor set-up, inoculum and wastewater

An airlift reactor with working volume of 2.5 L was used; see a detailed diagram in Fig. 1. Compressed air was supplied through an air diffuser placed at the bottom of the reactor and manually manipulated to maintain the dissolved oxygen (DO) concentration in the bulk liquid in the range 1–5 mg O<sub>2</sub> L<sup>-1</sup>. There was not a closed loop DO control and the air flow rate was weekly selected to maintain the DO level inside the mentioned range. The DO concentration in the bulk liquid was measured on-line by means of a DO electrode (DO 60-50,



**Fig. 1 – Schematic diagram of the reactor set-up showing the peripheral instrumentation and control loops. DO: dissolved oxygen; TAN: total ammonia nitrogen ( $\text{TAN} = \text{N-NH}_4^+ + \text{N-NH}_3$ ).**

Crison Instruments, Spain). The pH was measured online with a pH probe (pH 52-10, Crison Instruments, Spain) and automatically controlled at  $8.0 \pm 0.1$  by dosing a Na<sub>2</sub>CO<sub>3</sub> 0.5 M solution. Temperature was controlled at different values in the experiments,  $30.0 \pm 0.1$ ,  $20.0 \pm 0.1$ ,  $15.0 \pm 0.1$  and  $12.5 \pm 0.1$  °C by means of a cooling system (E100, LAUDA, Germany) and an electric heater (HBSI 0.8 m, HORST, Germany) connected to a temperature controller (BS-2400, Desin Instruments, Spain). The total ammonia nitrogen ( $\text{TAN} = \text{N-NH}_4^+ + \text{N-NH}_3$ ) in the bulk liquid was controlled varying the inflow rate. Before day 71, the TAN control was made manually based on the off-line bulk liquid TAN concentration measurement. From day 71 to day 250, the TAN control was automated by using an on-line TAN probe (NH4Dsc probe with a Cartridge cartridge, Hach Lange, Germany) using a proportional controller. From day 250 onwards, the TAN control was again made manually to check the feasibility of implementing this technology without a TAN on-line sensor.

The airlift reactor was inoculated with a nitrifying granular sludge. The origin of the inoculum was a granular sludge reactor treating real reject water, which was inoculated with activated sludge (Torà et al., 2013). *Nitrobacter* spp. was quantified through Fluorescence in situ hybridization (FISH) technique as the dominant NOB population in the inoculum, but small amounts of *Nitrospira* spp. could be expected, although were not quantified through FISH. The nitrifying granules were stored at 4 °C for 7 months after collection from the pilot reactor treating the reject water. The nitrifying granules had an average particle size of 0.5 mm (Torà et al., 2013).

The biofilm airlift reactor was fed with a synthetic influent mimicking the pretreated municipal wastewater from an anammox-based WWTP (Kartal et al., 2010). The pretreatment

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