



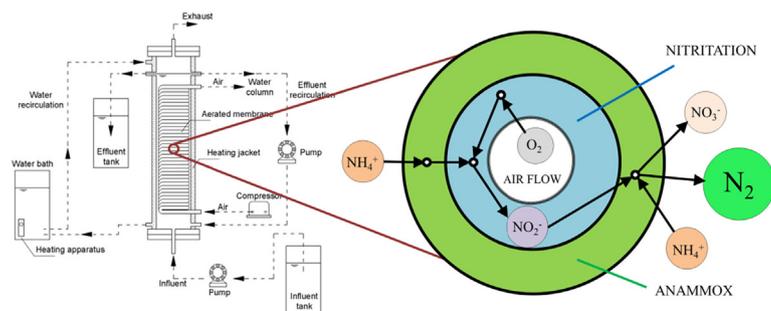
# Fast start-up of the single-stage nitrogen removal using anammox and partial nitritation (SNAP) from conventional activated sludge in a membrane-aerated biofilm reactor

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## GRAPHICAL ABSTRACT



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## ABSTRACT

The single-stage nitrogen removal using anammox and partial nitritation (SNAP) is a promising alternative for low-cost ammonium removal from wastewaters. This study aimed to evaluate the anammox biomass enrichment and SNAP process start-up in a laboratory-scale membrane-aerated biofilm reactor (MABR) at nitrogen loading rates of  $50 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  (period 1) and  $100 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  (period 2). Anammox activity was observed after 48 days, and the SNAP process was stable after 80 days. In period 1, the average total nitrogen (TN) removal was  $78 \pm 6\%$ , and the maximum removal was 84%. In period 2, the average TN removal was  $61 \pm 5\%$ , and the maximum was 69%. Higher dissolved oxygen levels may have caused imbalances in the microbial community in period 2, decreasing the reactor performance. These results demonstrated the potential of the MABR for the fast implementation of the single-stage partial nitritation and anammox processes.

## 1. Introduction

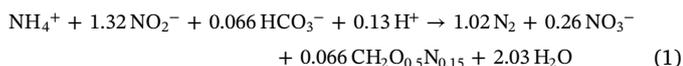
Ammonium ( $\text{NH}_4^+$ ) removal from wastewater through conventional nitrification and heterotrophic denitrification processes requires both extensive energy for aeration and an external carbon source. The

anammox process (anaerobic ammonium oxidation) has emerged as a promising autotrophic alternative especially for ammonium-rich or low C/N ratio wastewaters as mature landfill leachate, sludge digester supernatant or other anaerobic effluents (Mao et al., 2017; Tomaszewski et al., 2017; Van Hulle et al., 2010).

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In the anammox process,  $\text{NH}_4^+$  is oxidized to nitrogen gas ( $\text{N}_2$ ) and a small amount of nitrate ( $\text{NO}_3^-$ ), with nitrite ( $\text{NO}_2^-$ ) as electron acceptor (Eq. (1)) (Strous et al., 1998).



Since anammox bacteria require  $\text{NO}_2^-$  as one of the substrates, the process must be coupled to a partial nitrification (PN) step. This combined partial nitrification-anammox (PN/A) may be performed in one or more stages. The single-stage system is known as Completely Autotrophic Nitrogen removal Over Nitrite (CANON) or Single-stage Nitrogen removal using Anammox and Partial nitrification (SNAP) (Van Hulle et al., 2010; Zhang et al., 2014). According to Lackner et al. (2014), approximately 88% of the PN/A systems are single-stage. This configuration presents some important advantages: lower implementation cost, less operational complexity and greater resistance to substrate inhibition, since the  $\text{NO}_2^-$  produced in the aerobic layer of the biofilm or granule is immediately consumed in the anaerobic layer, avoiding toxic levels in the bulk liquid (Van Haandel and Van Der Lubbe, 2012).

The autotrophic PN/A process results in approximately 60% savings in oxygen demand, 100% in exogenous carbon sources and 80% in excess sludge production (Cao et al., 2017). However, the challenge for large-scale applications of the PN/A process is the low growth rate of the anammox bacteria. The doubling time ranges from 2.1 to 11 days (at 30 °C) which results in a specific growth rate of 0.065–0.334  $\text{d}^{-1}$  (Connan et al., 2016). According to Yin et al. (2016a), doubling time may be even higher, ranging from 11 to 20 days for less favorable conditions. Thus, longer start-up times are required, ranging from 4 months to 1 year when carried out from conventional sludge even under controlled conditions (pH, temperature and dissolved oxygen) and absence of inhibitory substances (Connan et al., 2016).

Membrane-aerated biofilm reactors (MABR) have emerged as a promising alternative for nitrogen removal through the PN/A process. In these systems, membranes are used to permeate gases and to transfer oxygen with higher efficiencies than conventional diffusers (Martin and Nerenberg, 2012). The air diffusion through the membranes does not generate bubbles, reducing the emission of volatile organic compounds (VOCs) and greenhouse gases (methane, nitrous oxide, and others), also allowing the formation of biofilm on their surfaces. The dissolved oxygen (DO) control is more effective, maintaining the microaerobic conditions necessary for the SNAP process (Kinh et al., 2017; Li et al., 2016, 2015; Martin and Nerenberg, 2012; Syron et al., 2015; Syron and Casey, 2008; Wei et al., 2012).

In a conventional biofilm, both the electron donor and acceptor are provided by the bulk liquid external to the biofilm; the diffusion pathway for both substrates is thus the same, characterizing a co-diffusional biofilm. In an MABR system, the oxygen (final electron acceptor) is supplied by the membrane, while the electron donor is supplied by the bulk liquid; hence, the diffusion of the substrates occurs in opposite directions in the biofilm, resulting in a counter-diffusion biofilm. Thus, in an MABR, the ammonia-oxidizing bacteria (AOBs) are established in the zones closer to the membrane, rich in oxygen, and the anammox bacteria occupy the anaerobic or anoxic zones on the biofilm-liquid boundary, forming a stratification arrangement opposite to that found in conventional biofilm (Kinh et al., 2017; Li et al., 2016; Lin et al., 2015).

Therefore, this study aimed to evaluate the anammox biomass enrichment and SNAP process start-up in a membrane-aerated biofilm reactor (MABR). The reactor was inoculated with conventional activated sludge to evaluate the system performance in obtaining the SNAP process from a general sludge.

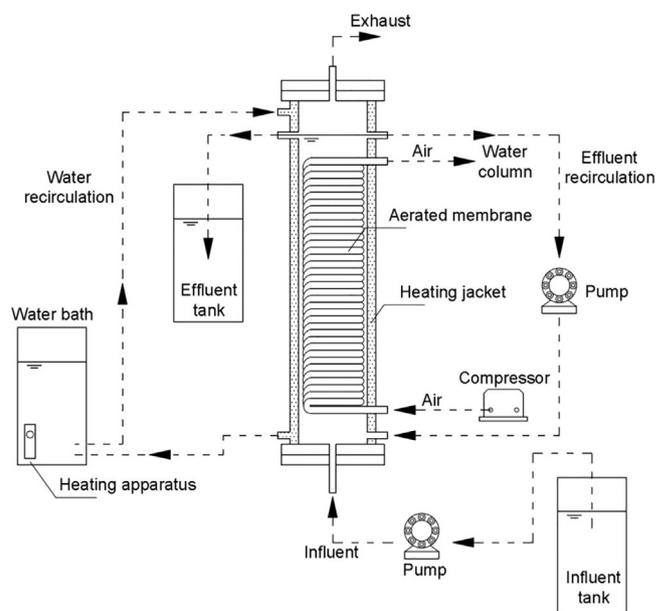


Fig. 1. Schematic representation of the MABR.

## 2. Materials and Methods

### 2.1. MABR setup

The MABR consisted of a continuous and upflow reactor made of acrylic with an inner diameter of 9.5 cm, a height of 50 cm and a total volume of 3.2 L (2.7 L of working volume) (Fig. 1). The MABR internal temperature was maintained at  $(31.3 \pm 0.7)$  °C through a thermostatic water jacket. Recirculation of the effluent with the same feed flow was applied.

The air diffusion was performed through a helical-shaped silicone tube (Perfimed®, Brazil) with a 6.3-m length, 10-mm external diameter, 5-mm internal diameter, 2.5-mm wall thickness and 4-cm<sup>2</sup>/cm<sup>3</sup> specific area. One end of the silicone membrane was connected to an air compressor, and the other was immersed in a water column, resulting in an internal pressure that ranged from 70 to 130 mbar. The system was aerated continuously.

### 2.2. Oxygen transfer tests in the MABR

To evaluate the microaeration potential of the silicone tubular membrane, oxygen transfer tests were performed at three air pressure levels (70, 100 and 130 mbar) and three air flow levels (1, 2 and 3 L·min<sup>-1</sup>). The dynamic method was used, which consists in removing DO of clean water, by adding sodium sulfite, and further oxygen addition until medium saturation (Garcia-Ochoa and Gomez, 2009).

DO concentrations were monitored every 30 s, using a Hach® HQ430D portable meter equipped with a luminescent dissolved oxygen sensor (LDO101), and volumetric oxygen transfer coefficients ( $K_L a$ ) were determined through linear regression. The temperature was maintained at  $(30 \pm 1)$  °C. The experimental  $K_L a$  data were analyzed using a two-way ANOVA statistical analysis to evaluate air pressure and air flow influences, considering a significance level of 0.05.

### 2.3. Synthetic wastewater and inoculum sludge

The synthetic wastewater used in the experiment was adapted from Van de Graaf et al. (1996). This medium contained (per L of demineralized water):  $\text{NH}_4\text{Cl}$ , variable;  $\text{NaHCO}_3$ , 2 g;  $\text{KH}_2\text{PO}_4$ , 0.0272 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.3 g;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.18 g; and 1 mL of trace elements solutions I and II. Trace solution I contained (per L of demineralized

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