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The effect of nitrite inhibition on the anammox process

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ABSTRACT

The negative effect of nitrite on anammox activity has been reported widely during the past decade. Although the adverse effect is clear, conflicting reports exist on the level at which it occurs and its reversible/irreversible nature. An in depth study on nitrite inhibition therefore was performed in which the influence of environmental factors was evaluated. Anammox activity was measured in anammox granules by continuously monitored standardized manometric batch tests extending the interpretation by evaluation of lag times, maximum conversion rates during the tests and substrates/product conversion ratios. The granules where obtained from a one-stage anammox reactor, the dominant anammox organisms belonged to the *Brocadia* type. The observed 50% activity inhibition for nitrite (IC_{50}) was 0.4 g N L^{-1}. The activity recovered fully after removal of the nitrite. Conversion in fresh medium after exposure to up to 6 g NO_2^-–N L^{-1} for 24 h showed less then 60% loss of activity. Presence of ammonium during nitrite (2 g N L^{-1}) exposure resulted in a stronger loss of activity after nitrite exposure (50% and 30% in presence and absence of ammonium respectively). Presence of oxygen during nitrite incubation led to a maximum activity reduction of 32%. The recovery after exposure indicates that the adverse effect of nitrite is reversible and thus inhibitory rather than toxic in nature. Similarities between exposure at three different pH-values indicate that nitrite rather than nitrous acid is the actual inhibiting compound.

1. Introduction

The anaerobic ammonium oxidation (anammox) process represents a cost-effective nitrogen removal process for treatment of ammonium-rich wastewater (Fux and Siegrist, 2004; Van Dongen et al., 2001), getting rapidly introduced in practice worldwide (van der Star et al., 2007). The responsible microorganisms grow on ammonium with nitrite as electron acceptor resulting in production of dinitrogen gas. The anaerobic and autotrophic nature of these organisms permits significant savings in aeration energy, no need for organic carbon and a lower sludge production. The bacteria performing the anammox process form the order "Brocadiales" within the phylum *Planctomycetales* (Jetten et al., 2010) of which the two "candidatus" genera *Brocadia* and *Kuenenia* are the most relevant for wastewater treatment.

One of the most critical aspects in the anammox process stability is nitrite, since it is the electron acceptor in the process and converted by anammox bacteria, but also a potential inhibiting compound. Nitrite concentrations as low as 5 and 40 mg N L^{-1} have been reported as strongly inhibitive (Wett (2007) and Fux (2003), respectively). Strous et al. (1999), who first reported the adverse effect of nitrite, found a complete but reversible, inhibition of the process at

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100 mg N L^{-1}. Other authors reported similar concentrations as detrimental for the anammox process, but indicating the nitrite inhibition either as reversible or irreversible (e.g. Fux et al., 2004; Jetten et al., 2005; López et al., 2008; Van Dongen et al., 2001). A few reports indicate even higher nitrite tolerance (Cho et al., 2010; Dapena-Mora et al., 2007; Egli et al., 2001; Fernández et al., 2012) with the highest reported non-inhibitory value reported by Kimura et al. (2010) (toxicity threshold higher than 300 mg N L^{-1}).

The wide range of observations regarding nitrite which was observed makes it difficult to predict, model or design anammox-based technologies. Therefore an in depth study of nitrite inhibition to anammox bacteria was made. Particular emphasis was given to the recovery of the anammox bacteria after exposure to nitrite. We differentiate between inhibition, defined as a phenomenon which is reversible and depending on the time of exposure and the concentration of inhibiting compound, and toxicity, that is defined as the irreversible process of activity loss depending on the time of exposure and the concentration of the toxicant.

The use of standardized manometric batch tests was first proposed by Dapena-Mora et al. (2007). This method was modified to increase accuracy and reliability, and used it as a reproducible methodology for our research. The standard evaluation of maximum conversion rates was extended by evaluating also the duration of lag times and substrates/products conversion ratios.

2. Materials and methods

2.1. Manometric test equipment

The assays were performed in closed bottles equipped with manometric sensors including a data storage system for 360 datapoints (OxiTop Control AN6 (WTW, Weilheim, Germany)). The system was used previously for evaluation of anammox activity (Scaglione et al., 2009). The manometric devices consisted of 340 mL vials provided with a measuring head with a pressure transducer (sensitivity level 1 hPa). Each vial had two lateral holes closed with a puncturable rubber septum for substrate injections and sampling.

2.2. Origin of the biomass

The biomass used originates from the full-scale anammox reactor of Dokhaven-Sluisjesdijk wastewater treatment plant. The reactor contains granular anammox sludge and treats reject water after partial nitritation in a SHARON reactor. The size distribution of the granules was determined with the aid of image analysis (Tijhuis et al., 1994). 94% of the granules analyzed presented a diameter of 1.1 ± 0.2 mm. During the experimental period, the anammox reactor was operated at the design volumetric load of 7.1 kg N m^{-3} d^{-1} (van der Star et al., 2007). During 2010 the average reactor conditions where: temperature 34 ± 2.5 °C, pH 7.2 ± 0.4 and concentrations of nitrogen in effluent were 50 ± 20 mg NH_4^+–N L^{-1}, 15 ± 15 mg NO_2^-–N L^{-1}, 95 ± 20 mg NO_3^-–N L^{-1} (de Kreuk, 2011). The biomass was confirmed to consist of a "*Brocadia*" enrichment during the period of the tests by *fluorescence in situ hybridization* (FISH), the sludge hybridized with AMX820 and not with KST157 probes (Schmid et al., 2001).

2.3. General procedure for manometric tests

After sampling the granular sludge was brought under non-aerated conditions to the laboratory (30 min travel time) and directly used for the tests. The biomass was washed and re-suspended in a *washing medium*: a medium containing the microelements needed to avoid nutrient limitation (Van De Graaf et al., 1996) as well as 25 mM HEPES (N-2-hydroxyethyl-piperazine-N′-2-ethane sulfonic acid) buffer. The pH value of the medium was set to 7.5 with 0.1 M NaOH or H_2SO_4. After this, the headspace and liquid phase (200 mL) were sparged with nitrogen gas to obtain anoxic conditions. The bottles were placed in a thermostatic shaker, at 170 rpm and 30 °C until the headspace pressure (rising as a result of the temperature change) had stabilized. Then overpressure was released (by inserting a needle connected to a water-filled vessel to act as a water-lock) and substrates were injected. The *injected solutions* contained $NaNO_2$, $(NH_4)_2SO_4$ and NH_4HCO_3 dissolved in high purity water obtained through a milli-Q™system. The initial concentration of ammonium and nitrite was 50 mg N L^{-1} unless mentioned otherwise. To avoid inorganic carbon limitation the initial bicarbonate concentration was set to 32.7 mg L^{-1} while taking into account that part of the inorganic carbon partitioned to the headspace during equilibration. The pressure increase caused by the nitrogen gas production and accumulation in the headspace was automatically measured and recorded during the entire test for subsequent processing. Once the pressure reached a constant value (and all nitrite was assumed to be converted), a liquid sample was taken for chemical analysis (pH, ammonium, nitrite and nitrate).

2.4. Preliminary tests

To assess the accuracy and reliability of the method for measurement of the maximum specific anammox activity (MSAA) a set of preliminary assays was performed in duplicate according to par.2.3. The same test runs were performed with different batches of biomass during the entire experimental period to exclude any effect on changes in biomass composition in the full-scale reactor.

- *Ammonium and nitrite level*: Starting concentrations of 40, 50, 60, 70 and 80 mg N L^{-1} of ammonium and nitrite were tested. The initial biomass concentration was 1.0 g VSS L^{-1}. The test was also used to evaluate the nitrogen balance for each experiment.
- *Biomass level*: Varying levels of biomass (0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 g VSS L^{-1}) were used for tests with 40 mg NH_4^-–N L^{-1} and 40 mg NO_2^-–N L^{-1} as well as 60 mg NH_4^-–N L^{-1} and 60 mg NO_2^-–N L^{-1}.
- *Effect of buffer solution*: Biomass was suspended in 5.3 mM phosphate buffer (Dapena-Mora et al., 2007), HEPES (25 mM) buffer or non-buffered medium (the rest of the medium composition was standard as described above). The experiments were performed in triplicate.