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Short- and long-term effects of ammonium and nitrite on the Anammox process

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ABSTRACT

Autotrophic anaerobic ammonium oxidation (Anammox) is a biological process in which Planctomycete-type bacteria combine ammonium and nitrite to generate nitrogen gas. Both substrates can exert inhibitory effects on the process, causing the decrease of the specific activity of the biomass and the loss of the stable operation of reactors. The aim of the present work is to evaluate these effects in short- and long-term experiments. The short-term effects were carried out with two different types of Anammox biomass, biofilm on inorganic carriers and flocculent sludge. The effects of ammonium on both kinds of biomass were similar. A decrease of the Specific Anammox Activity (SAA) of 50% was observed at concentrations about 38 mg NH_3-N·L^{-1}, while 100 mg NH_3-N·L^{-1} caused an inhibition of 80%. With regards to nitrite, the SAA was not affected at concentrations up to 6.6 μg HNO_2-N·L^{-1} but it suffered a decrease over 50% in the presence of 11 μg HNO_2-N·L^{-1} in the case of the biofilm. The flocculent biomass was much less resistant and its SAA sharply decreased up to 30% of its initial value in the presence of 4.4 μg HNO_2-N·L^{-1}.

The study of the long-term effects was carried out in lab-scale Sequencing Batch Reactors (SBR) inoculated with the biofilm biomass. Concentrations up to 20 mg NH_3-N·L^{-1} showed no effects on either reactor efficiency or biomass activity. However, when free ammonia concentrations reached values between 35 and 40 mg NH_3-N·L^{-1}, the operation turned unstable and the efficiency was totally lost. Nitrous acid concentrations around 1.5 μg HNO_2-N·L^{-1} caused a loss of the efficiency of the treatment and a destabilization of the system. However, a total restoration of the SAA was observed after the stoichiometric feeding was applied to the SBR.

1. Introduction

The Anammox process, in combination with a previous step of partial nitrification, is a suitable alternative in order to treat effluents with high content of ammonia and low carbon to nitrogen (C/N) ratios (Fux et al., 2002). In the partial nitrification step the 50% of the ammonium is oxidized into nitrite. This could be done by means of several different processes like SHARON (Hellinga et al., 1999), oxygen-limited bioreactors (Bernet et al., 2001; Pollice et al., 2002), inhibition of nitrite-oxidizers by free ammonia or free nitrous acid (Anthonisen et al., 1976; Villaverde et al., 1997), or the recent aerobic granular reactors (Vázquez-Padín et al., 2006). In the Anammox process the remaining ammonium is oxidized by autotrophic bacteria using the nitrite as the electron acceptor (Strous et al., 1999). This option allows the reduction of costs compared to the traditional nitrification–denitrification system because less oxygen is required and the addition of organic matter is not necessary. Besides, the low amount of surplus sludge would also lead to a reduction in the operational costs (Jetten et al., 1997).

In order to achieve the successful operation of the Anammox process, the potentially negative effects of the compounds present in the wastewater should be studied. Among these compounds, the substrates were reported to be responsible of losses in the Anammox activity (Strous, 2000; Dapena-Mora et al., 2007; Tang et al., 2009). Taking into account that during start-up or overload periods both substrates could not be completely consumed, their presence in the reaction medium could cause the decrease of biomass activity and the destabilization of the reactor. A new start-up or the recovery of the biomass activity might take long time, especially in the case of industrial-size reactors (van der Star et al., 2007), due to the very slow growth rate of Anammox bacteria (Strous et al., 1999).

Some studies reported data about the inhibitory effect of ammonia and nitrite (Fux et al., 2004; Strous, 2000; Jetten et al., 2005; Dapena-Mora et al., 2007). However, these works were sometimes carried out under very different operational conditions (pH, temperature, continuous/batch tests…) which entails that these results are difficult to extrapolate and to use to design an operational strategy. Moreover,

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the different works about the effects of nitrite did not agree about the concentration threshold that would not be exceeded. In the literature different ranges for this threshold (50–150 mg $N \cdot L^{-1}$, Strous et al., 1999; 30–50 mg $N \cdot L^{-1}$, Fux et al., 2004) can be found. The knowledge about safe levels would be very important for the operators of the wastewater treatment plants with Anammox stages in order to maintain the performance of the system.

Therefore the objective of the present work is to determine the short- and long-term effects of nitrite and ammonium on the Anammox process in order to know the suitable conditions to operate the Anammox reactors. Results will be analyzed in terms of the unionized compounds, which are well known to be responsible for the inhibition of nitrifying bacteria (Anthonisen et al., 1976; Vadivelu et al., 2007) and poly-phosphate accumulating denitrifiers (Zhou et al., 2007).

2. Materials and methods

2.1. Specific Anammox activity (SAA) tests

To determine the short-term effects of ammonia and nitrite on the Anammox biomass, batch experiments were performed according to the methodology described by Dapena-Mora et al. (2007). The tests consisted of the measurement along the time of the overpressure generated in closed vials by the produced nitrogen gas. They were performed at least by triplicate and with an initial pH value of 7.8 provided by the use of phosphate buffer (0.14 g L^{-1} KH_2PO_4 and 0.75 g L^{-1} K_2HPO_4), which also prevented significant pH variations during the tests. In order to assess the short-term effects of ammonium, batch assays were performed with a fixed initial nitrite concentration of 70 mg NO_2^--$N \cdot L^{-1}$ and ammonium concentrations of 70, 700, 1400, 2100 and 2800 mg NH_4^+-$N \cdot L^{-1}$. To evaluate the effects of nitrite, the tests were performed with a fixed initial ammonium concentration of 70 mg NH_4^+-$N \cdot L^{-1}$ and nitrite concentrations of 70, 140, 210, 280, 350 and 420 mg NO_2^--$N \cdot L^{-1}$.

The biofilm biomass employed in the tests was taken from the SBR systems used to study the long-term effects of ammonia and nitrite. Flocculent biomass was also tested in order to assess the influence of the biomass type and it was collected from an Anammox reactor used for Anammox biomass enrichment (Dapena-Mora et al., 2004a). The short-term tests performed with both types of biomass were done under similar conditions.

SAA tests were also used in order to monitor the reactor by assessing the maximum removal capacity. This variable was calculated by multiplying the maximum SAA obtained in batch tests and the concentration of biomass in the system.

2.2. Experimental set-up

Long-term experiments were carried out in two Sequencing Batch Reactors of 5 L (SBR1) and 3 L (SBR2) of useful volumes, respectively. Temperature was controlled at 33 °C (SBR1) and 30 °C (SBR2) by using thermostatic jackets. The complete mixture inside both reactors was achieved using mechanical stirrers with rotating speed of 150 rpm. The control of the pumps and different periods of the operational cycles was performed with a PLC system (CPU224, Siemens). Both reactors were operated in cycles of 6 h distributed in four periods: mixed fill (300 min), mix (30 min), settle (15 min) and draw (15 min) according to Dapena-Mora et al. (2004b). The exchange volume was fixed at 25% and the Hydraulic Retention Time (HRT) was maintained at 1 day in both cases.

To prevent the oxidation of the excess of ammonium or nitrite, the headspace of both reactors was continuously flushed with 200 mL min^{-1} of Ar.

Table 1
Operational strategy.

Reactor	Periods	Days	NH_4^+-N_{inf} (mg L^{-1})	NO_2^--N_{inf} (mg L^{-1})
SBR1	I	0–25	180	250
	II	26–39	250	250
	III	40–74	375	250
	IV	75–103	425	250
	V	104–172	500	250
	VI	173–200	750	400
SBR2	I	0–48	150	185
	II	49–69	150	200
	III	70–95	150	220
	IV	96–118	150	240
		119–132	150	200
	V	133–160	150	240
		161–166	150	280
		167–221	150	200
	VI	222–257	150	300
	VII	258–281	150	400

Note: Shaded cells correspond to recovery periods.

2.3. Feeding media and operational strategy

Both reactors were fed with a synthetic autotrophic medium described by Dapena-Mora et al. (2004a). The operational strategy in both cases was fixing an initial ammonium to nitrite molar ratio approximately at the stoichiometric value (1.32 NO_2^--N/NH_4^+-N according to Strous (2000)). Then the concentrations of ammonium (SBR1) or nitrite (SBR2) were stepwisely increased (Table 1), while the limiting substrate concentration (nitrite for SBR1 and ammonium for SBR2) was not changed. This strategy allowed maintaining the effective nitrogen loading rate applied constant. The effective nitrogen loading rate was calculated as follows: the sum of the concentration of limiting substrate in the feeding plus the stoichiometric concentration of the substrate in excess, divided by the hydraulic retention time.

2.4. Inocula

SBR1 was inoculated with enriched Anammox biofilm biomass from a laboratory scale SBR operated at the University of Santiago of Compostela (Fernández et al., 2008). The support material was natural zeolite. This zeolite was clinoptilolite (ZeoCat, Spain) with particle size between 0.5 and 1.0 mm. The initial average particle (biofilm plus support) size was 1.4 mm. SBR2 was inoculated with biomass taken from SBR1 at the end of its operation. The average particle size at the inoculation of SBR2 was 1.1 mm. The initial concentrations of biomass were 1.2 and 2.0 g $VSS \cdot L^{-1}$ for SBR1 and SBR2, respectively. The initial SAA values were 0.49 g N $(g\ VSS \cdot d)^{-1}$ and 0.21 g N $(g\ VSS \cdot d)^{-1}$ for the biomass of SBR1 and SBR2, respectively.

2.5. Analytical methods

Ammonium was determined spectrophotometrically (APHA, 1998) while nitrate and nitrite were determined by capillary electrophoresis (Waters Capillary Ion Analyzer). The concentrations of solids, determined as Total Suspended Solids (TSS), the fraction corresponding to the biomass as Volatile Suspended Solids (VSS), were determined according to the Standard Methods (APHA, 1998). As zeolite particles were not affected by calcination at 550 °C, the concentration of biomass in the biofilm was assessed as VSS determined according to the Standard Methods (APHA, 1998).

The distribution of particle size was measured using an Image Analysis procedure (Tijhuis et al., 1994; Jeison and Chamy, 1998). Images of the granules were taken with a digital camera (Coolsnap,