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Monitoring the stability of an Anammox reactor under high salinity conditions

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ABSTRACT

The effects of high salinity conditions (up to 30 g NaCl L^{-1}) on the efficiency and activity of the Anammox process were studied in a sequencing batch reactor. The use of the estimated maximum Specific Anammox Activity (SAA) was evaluated as a monitoring parameter of the performance of the process. The SAA values obtained from biomass adapted under the same salinity conditions and collected from the reactor could be used to calculate the maximum capacity of the system and, therefore, to predict its efficiency at a certain operation condition. Batch assays carried out with non-adapted and adapted Anammox biomass at different salt concentrations indicated a stimulatory effect on the SAA at concentrations up to 6 and 15 g NaCl L^{-1} while higher salt concentrations caused a decrease in the activity. The addition of salt enhanced the aggregation of Anammox biomass in granules with a consequent decrease in the Sludge Volumetric Index from 80 to 25 mL g VSS^{-1}. The system was able to treat a nitrite loading rate around 0.32 g NO_2^--N L^{-1} d^{-1} when salt concentrations of 15 g L^{-1} of NaCl were present in the feeding, with nitrogen removal efficiencies of 99%. The Anammox process exhibited high resistance to the presence of high NaCl concentrations being then recommended to remove nitrogen from effluents with high salt concentrations.

1. Introduction

The combination of partial nitrification and Anammox processes is an attractive alternative to remove nitrogen compounds from high nitrogen loaded wastewater with low organic matter content. The advantages over the traditional combined nitrification/denitrification processes are manifold: less oxygen demand, no carbon addition and less area requirement. However, the application of Anammox may be hindered by the presence of different exogenous compounds for which the effect on the Anammox biomass is still unknown. This is the case of high salinity effluents, as those produced in the fish canning industry due to the use of sea water during the manufacturing processes. These effluents are generally firstly treated via an anaerobic digestion stage, generating an effluent with a low C/N which can be treated by partial nitrification and Anammox processes. Anaerobic digestion has been shown to be an adequate system to treat fish cannery effluents with high salt concentrations [1,2] while other studies indicated that high saline concentrations have negative effects on either partial nitrification [3] or Anammox process [4].

Works carried out to research the effect of exogenous compounds, such as salt, on biological processes are basically focused on the determination of the concentration which causes the destabilization of the system [5,6]. Since these works were performed at different operational conditions (loading rates, biomass concentration) or the biomass used had different characteristics (enrichment degree, adaptation periods), discrepancies between the results obtained are frequently found. Therefore, these factors should be taken into account in the analysis of the effect of exogenous compounds on the efficiency of the process.

Generally the exogenous compounds decrease the maximum specific activity of biomass reducing the loading rate which can be treated by the system. This reduction cannot affect the efficiency of the process if the maximum capacity of the system, calculated as the product of the maximum specific activity of biomass by the biomass concentration, is still higher than the supplied loading rate. However if the capacity turns lower than the loading rate supplied, due to the effect of any toxic compound, the efficiency decreases and the substrate starts to accumulate in the reactor. This fact is critical if the process is inhibited by substrate because its presence causes a loss of efficiency which increases the accumulation of substrate causing a snowballing effect until the system totally loses its efficiency [7]. This effect must be considered in the case of the Anammox process which is inhibited by both ammonia and nitrite, the later being more toxic [8].

Dapena-Mora et al. [9] showed that the determination of the maximum Specific Anammox Activity (SAA) by batch tests could be very useful in order to determine the maximum capacity of the

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system. Nevertheless, when toxicants are present, inhibitory effects found during activity tests cannot be easily extrapolated to predict the efficiency of the system during a continuous operation even when adapted biomass is utilized [4]. This fact could be due to the exposure time or to the adaptation of biomass to the toxicant either by the adaptation of the existing population or by a population shift. Therefore, it should be useful to establish a protocol to determine a value of the maximum specific activity so that it could be used to monitor the operation of the reactor.

For this reason, the aim of the present study was to research the effects of NaCl on the maximum Specific Anammox Activity under different conditions to know which of the obtained values can predict the capacity of the system. On other hand, the effect of salt on physical properties of Anammox biomass will also be studied.

2. Materials and methods

2.1. Anammox reactor

A Sequential Batch Reactor (SBR) with a working volume of 3 L was used to carry out the Anammox process. The SBR was provided with a thermostatic jacket to keep the temperature at 35 °C. Complete mixture was achieved inside the reactor by means of a two-blade mechanical stirrer with a rotating speed of 100 rpm. The pH value was not controlled and ranged from 7.5 to 8.0. Anoxic conditions were kept in the reactor by flushing argon gas.

The SBR was operated in cycles of 6 h distributed in the following periods established according to Dapena-Mora et al. [10]: continuous stirred feeding during 300 min; stirring without feeding during 30 min; settling for 20 min and effluent withdrawal for 10 min. The hydraulic retention time (HRT) was fixed at 1 day.

2.2. Feeding composition

The mineral medium fed in this study presents the following composition per litre of demineralised water: 0.707–1.650 g $(NH_4)_2SO_4$, 0.739–1.725 g $NaNO_2$, 0.425 g $NaNO_3$, 1.25 g $KHCO_3$, 0.025 g KH_2PO_4, 0.3 g $CaCl_2$ $2H_2O$, 0.2 g $MgSO_4$ $7H_2O$, 0.00625 g $FeSO_4$, 0.00625 g EDTA, 1.25 mL HCl (1 M) and 1.25 mL trace elements solution [11]. This medium contains ammonia in excess (molar ratio NH_4^+/NO_2^- of 1) to prevent the presence of nitrite in the reactor because of its strong inhibitory effect on the Anammox activity [8]. The concentration of Anammox biomass was of 0.9 gVSS L^{-1} at the beginning of the experiment. This biomass was enriched in bacteria belonging to the species *Candidatus* "Kuenenia stuttgartiensis".

2.3. Operational strategy

The SBR reactor was operated in three different stages (Table 1): (I) the nitrite loading rate (NiLR) was kept constant at 0.15 g N L^{-1} d^{-1} while the concentration of NaCl was stepwise increased from 0 to 20 g NaCl L^{-1} in periods of 6–12 days of duration (the first increase in the NaCl concentration was registered on day 251); (II) this is a transition period meaning that the concentration of NaCl and the NiLR were initially decreased from 20 to 10 g L^{-1} and from 0.15 to 0.07 g N L^{-1} d^{-1}, respectively and afterwards increased up to 15 g L^{-1} of NaCl and 0.20 g NO_2^--N L^{-1} d^{-1}, respectively (days 294–327); in the third stage (days 328–400) the inlet concentration of NaCl was maintained constant at 15 g L^{-1} and the NiLR was stepwise increased from 0.20 to 0.35 g N L^{-1} d^{-1}.

Table 1
Operational strategy.

Stage	Period (d)	NiLR (g N L^{-1} d^{-1})	Salt concentration (g L^{-1})
I	240–293	0.15	0 → 20
II	294–327	0.15 → 0.20	20 → 15
III	328–400	0.20 → 0.30	15

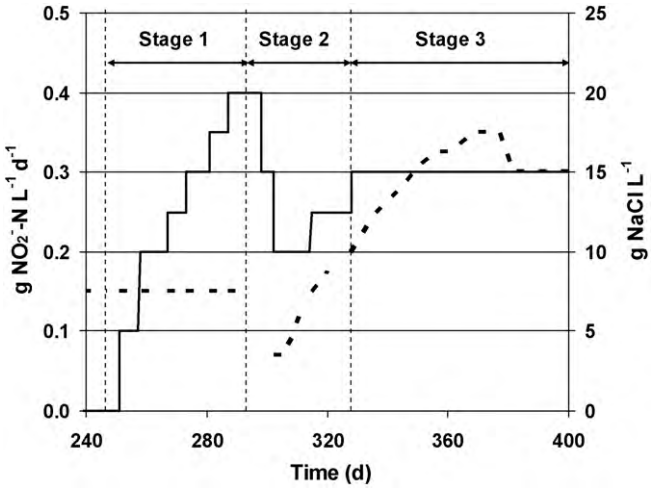

Fig. 1. Nitrite loading rate (NiLR) supplied to the system (- - -) and inlet salt concentration (–) during the whole operational period.

2.4. Measurement of the maximum Specific Anammox Activity (SAA)

The maximum Specific Anammox Activity (SAA) of the biomass from the reactor was measured by means of batch assays described in detail by Buys et al. [12] and modified by Dapena-Mora et al. [8]. Maximum SAA was estimated from the maximum slope of the curve described by the cumulative N_2 production along the time and related to the biomass concentration in the vials. Each test was done in triplicate.

In order to determine the effect of NaCl on a non-adapted biomass, a first series of Anammox activity tests was performed with sludge collected from the 3 L Anammox SBR just before NaCl was added in the feeding. In this case, a standard buffer (SB) containing 0.143 g KH_2PO_4 L^{-1} and 0.747 g K_2HPO_4 L^{-1} was used (Fig. 1).

To evaluate the effect of the salt on adapted biomass a second series of Anammox activity tests was carried out with sludge collected from the Anammox reactor in each operational period, corresponding to the different NaCl concentrations in the feeding. To establish the possible adaptation of biomass to saline conditions, three different buffer solutions were used in parallel for each tested sample:

(1) A standard buffer (SB) containing 0.143 g KH_2PO_4 L^{-1} and 0.747 g K_2HPO_4 L^{-1}.
(2) A standard buffer with the same salt (SBS) concentration as NaCl than that present in the reactor when the biomass was collected.
(3) The own liquid media from the SBR reactor at the corresponding operational conditions (RB).

2.5. Analytical methods

Ammonium was analysed by the phenol-hypochlorite method [13]. Biomass concentration measured as volatile suspended solids (VSS), nitrite and nitrate analysed by spectrophotometry, pH value was measured with a selective electrode Ingold and the sludge vol-