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Short- and long-term effects of temperature on the Anammox process

J. Dosta [a], I. Fernández [b,*], J.R. Vázquez-Padín [b], A. Mosquera-Corral [b], J.L. Campos [b], J. Mata-Álvarez [a], R. Méndez [b]

[a] *Department of Chemical Engineering, University of Barcelona, Martí i Franquès, No. 1, 6th Floor, 08028 Barcelona, Spain*
[b] *Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa s/n., E-15782 Santiago de Compostela, Spain*

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Abstract

The application of the Anammox process has been usually focused on the treatment of wastewater with temperatures around 30 °C in order to operate under optimum conditions. In this work, the feasibility of the application of the Anammox process at lower temperatures has been tested. First, the short-term effects of temperature on the Anammox biomass were studied using batch tests. An activation energy of 63 kJ mol^{-1} was calculated and the maximum activity was found at 35–40 °C. Activity tests done at 45 °C showed an irreversible loss of the activity due to the biomass lysis.

A SBR was operated at different temperatures (from 30 to 15 °C) to determine the long-term effects. The system was successfully operated at 18 °C but when temperature was decreased to 15 °C, nitrite started to accumulate and the system lost its stability. Adaptation of biomass to low temperatures was observed when the specific activities obtained during first batch tests are compared to those obtained during the operation of the SBR.

Keywords: Anammox; Sequencing batch reactor (SBR); Specific Anammox activity (SAA); Temperature

1. Introduction

Several kinds of wastewater are characterized by low carbon to nitrogen ratios and very high ammonia concentrations. Some examples are wastewaters coming from fertilizer industry, explosive industry or some pharmaceutical processes [1]. A feasible treatment of this kind of effluents is the combination of a partial nitritation, where 50% of ammonia is oxidized into nitrite in an aerobic reactor, and a subsequent anaerobic ammonium oxidation (Anammox), where ammonia is oxidized by nitrite in a second tank [2–5]. The partial nitritation-Anammox system, compared to the conventional nitrification/denitrification process, avoids the requirement of organic carbon source to denitrify, allows saving over 65% of the oxygen supply and produces a lower amount of sludge [6].

The Sharon process was successfully used to accomplish the partial nitritation of effluents coming from anaerobic sludge digesters [7,8]. However, the application of this process is limited to wastewater with a temperature higher than 30 °C. To achieve the partial nitrification at lower temperatures different approaches were used: inhibition of nitrite oxidizers by free ammonia [9] or operation at low dissolved oxygen (DO) concentrations [10–12]. More recently, Vazquez-Padín et al. [13] also demonstrated that a stable partial nitrification may be achieved in nitrifying granular sludge systems.

Several authors [14–17] found the optimum temperature for the operation of the Anammox process was around 30–40 °C. Perhaps for this reason, most of the works where this process

* Corresponding author. Tel.: +34 981 563 100x16739; fax: +34 981 528 050.
E-mail address: ifernand@usc.es (I. Fernández).

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was applied were carried out at temperature values higher than 30 °C [18–21]. However, recently, Cema et al. [22] proved that a rotating biological contactor (RBC) with the established Anammox process could be successfully operated at temperatures around 20 °C. Similar results were reported by Isaka et al. [23] who operated an anaerobic biological filtrated (ABF) reactor which treated 8.1 g N (L d)$^{-1}$. Moreover, several works done with marine Anammox samples reported measurable activities at low temperatures. Rysgaard et al. [24], working with sediments of the east and west coasts of Greenland, observed Anammox activity between −2 and 30 °C, the optimum temperature being 12 °C. Similar results were found by Dalsgaard and Thamdrup [25] working with marine sediments from the Skagerrak (Baltic-North Sea).

These results indicate that the application of the Anammox process could be not restricted to effluents with temperatures around 30 °C. Therefore, the aim of this work is to evaluate the effects of moderately low temperatures on the stability of this process.

2. Materials and methods

2.1. Experimental set-up

The study of the long-term effects of temperature on the Anammox process was carried out in a lab-scale sequencing batch reactor (SBR) of 1 L. The operating temperature was maintained by means of a temperature controller (PolyScience, USA). pH was not controlled and ranged between 7 and 8. The control of the pumps and the stirrer, according to the different periods of the operational cycle, were performed with a PLC system (CPU224, Siemens). The reactor was 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. [26]. The exchange volume was fixed at 25%, giving a hydraulic retention time (HRT) of 1 day.

2.2. Feeding media and operational strategy

The SBR was fed with a synthetic autotrophic medium adapted from Dapena-Mora et al. [26]. The ammonium to nitrite molar ratio in the feeding media was fixed at 1 (150 mg N L^{-1} of each one) to operate in excess of ammonia (Table 1).

The nitrogen loading rate (NLR) applied was kept constant at 0.3 g N (L d)$^{-1}$ and the temperature of the reactor decreased from 30 to 15 °C during the operation (Table 2). However, when the stability of the reactor failed (period VI) the NLR was decreased to 0.05 g N (L d)$^{-1}$ in order to restore the efficiency of the process.

2.3. Specific Anammox activity (SAA) tests

To determine the short-term effects of temperature on the Anammox biomass and to monitor the operation of the SBR, batch activity tests between 10 and 45 °C were performed according to the methodology described by Dapena-Mora et al. [27]. The tests consisted on the measurement of the overpressure generated in closed vials by the nitrogen gas produced. First, the sludge samples were washed with phosphate buffer to provide an optimum pH (7.8). Then, 24 mL of the mixed liquor were inserted in 38 mL vials. The headspace and the liquid phase were flushed with argon to remove the oxygen. Mixing at 150 rpm and control of temperature during the tests were provided by an incubator shaker (New Brunswick Scientific). After 30 min of acclimation in the shaker, the substrates were injected into the vials to reach the initial concentrations of 70 mg NH_4^+-N L^{-1} and 70 mg NO_2^--N L^{-1}. The changes in the pressure were measured and related to the production of nitrogen, according to Eq. (1). In this equation n is the number of moles of nitrogen produced per unit of time (mol N day^{-1}), V_G is the volume of the gas phase (L), R is the ideal gas constant (atm L (mol K)$^{-1}$), T is the temperature (K) and α is the slope of the pressure increase inside the vial versus time (atm day^{-1}).

$$n = \alpha \frac{V_G}{RT} \quad (1)$$

Then, the maximum SAA expressed in g N (g VSS day)$^{-1}$ is assessed according to Eq. (2), where M_{N_2} is the molecular weight of N_2 (g N mol^{-1}), X is the biomass concentration inside the vial (g VSS L^{-1}) and V_L is the volume of the liquid phase (L).

$$\mathrm{SAA} = n \frac{M_{N_2}}{V_L X} \quad (2)$$

Table 1
Feeding and trace solution composition

Feeding composition		Trace solution composition	
Compound	Concentration (mg L^{-1})	Compound	Concentration (g L^{-1})
NH_4^+-N	150	EDTA	15
NO_2^--N	150	$ZnSO_4 \cdot 7H_2O$	0.43
$KHCO_3$	1.25	$CoCl_2 \cdot 6H_2O$	0.24
$CaCl_2$	1.41[a]	$MnCl_2 \cdot 4H_2O$	0.99
KH_2PO_4	50	$CuSO_4 \cdot 5H_2O$	0.25
$MgSO_4$	58.6	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.22
$FeSO_4 \cdot 7H_2O$	9.08	$NiCl_2 \cdot 6H_2O$	0.20
EDTA	6.25	$NaSeO_4 \cdot 10H_2O$	0.20
		H_3BO_3	0.014
Trace solution	1.25 mL L^{-1}	$NaWO_4 \cdot 2H_2O$	0.05

[a] Reduced to 0.07 from period III.

Table 2
Operational periods of the Anammox SBR

Period	Temperature (°C)	Duration (d)
I	30	1–15
II	26	15–29
III	23	29–49
IV	20	49–63
V	18	63–103
VI	15	103–150

2.4. Biomass

The short-term effects of temperature were studied using both biofilm biomass [28] and granular biomass [29] taken from bioreactors operated at around 30 °C ("non-adapted" biomass). All the employed biomass was enriched in bacteria belonging to the specie *Kuenenia stuttgartiensis*. The long-term effects were tested in a SBR reactor inoculated with 7.6 g VSS L^{-1} of the biofilm biomass.

2.5. Analytical methods

Ammonium was analyzed by the phenol–hypochloride method [30]. Nitrite and nitrate were analyzed by spectrophotometry and by capillary electrophoresis [31]. The concentrations of solids, determined as total suspended solids (TSS), the fraction corresponding to the biomass as volatile suspended solids (VSS), and the sludge volumetric index (SVI) of the sludge were determined according to Standard Methods [31]. The pH value was measured using a selective electrode Ingold model U-455 connected to a pH/mV measurer Crison 506.

The distribution of particle size was measured using an image analysis procedure [32,33]. Images of the granules were taken with a digital camera (Coolsnap, Roper Scientific Photometrics) combined with a stereomicroscope (Stemi 2000-C (Zeiss)). For the digital image analysis, the programme Image ProPlus was used.

Fluorescence *in situ* hybridization (FISH) technique was employed for the selective detection of particular organisms inside the reactor [34,35]. The oligonucleotide probes used were PLA46, specific for *Planctomycetes* [36], AMX820 for Anammox bacteria [37], BAN162, specific for *Brocadia anammoxidans* [37] and KST1275, specific for *K. stuttgartiensis* [38].

3. Results and discussion

3.1. Short-term effects of temperature on specific Anammox activity

The maximum SAA of both granular and biofilm Anammox biomasses were measured in batch tests by triplicate at temperatures between 10 and 45 °C. The temperature dependency profiles obtained for both biomasses (Fig. 1) were very similar and consistent with the results obtained by other authors [15,39]. An exponential increase of the SAA was observed for temperatures up to 40 °C, while assays carried out at 45 °C showed a negative effect of the temperature on the activity. An activation energy of 63 kJ mol^{-1} was calculated for both Anammox populations according to the modified Arrhenius model [40]. Strous et al. [14] obtained a similar value (70 kJ mol^{-1}) for Anammox biomass cultivated at 30 °C, while Dalsgaard and Thamdrup [25] and Rysgaard et al. [24] reported values of 61 and 51 kJ mol^{-1}, respectively, for Anammox biomass from marine sediments.

When assays were done at 45 °C the liquid phase acquired an orange coloration, which could indicate the biomass lysis. In order to confirm this fact, a second feeding was added inside the vials, after substrates of first feeding were consumed, and almost

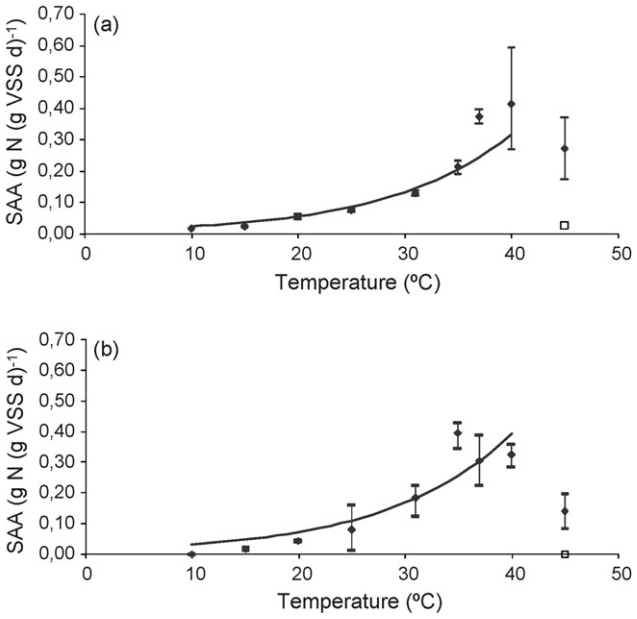

Fig. 1. Temperature dependency profiles for granular (a) and biofilm (b) Anammox biomass (experimental SAA (♦); experimental SAA with the second injection of substrate (□); modified Arrhenius model (—)).

negligible activity was observed (Fig. 1). UV–vis absorption spectra of the liquid phase from the SAA batch tests done at 35, 40 and 45 °C were analyzed (Fig. 2). For the test done at 45 °C, a maximum peak between 400 and 410 nm and a smaller one between 515 and 550 nm were observed. Cirpus et al. [41] analyzed the UV–vis spectrum of a 10 kDa cytochrome *c* present in cell extracts from a culture of *K. stuttgartiensis*. They observed a maximum absorption at 410 nm for the oxidized form of the protein. Huston et al. [42] also observed a maximum peak around 410 nm and two smaller peaks at 520 and 550 nm. Therefore, the orange colour of the liquid phase at the end of the assay could be attributed to the segregation of cytochrome *c*, which causes an irreversible loss of the activity. The negative effect of high temperature was also found by Toh et al. [17], which tried to select and enrich an Anammox consortium from sludge of a municipal treatment plant at 37 and 55 °C. These authors obtained Anammox activity at a mesophilic range but they could not select thermophilic Anammox organisms at 55 °C.

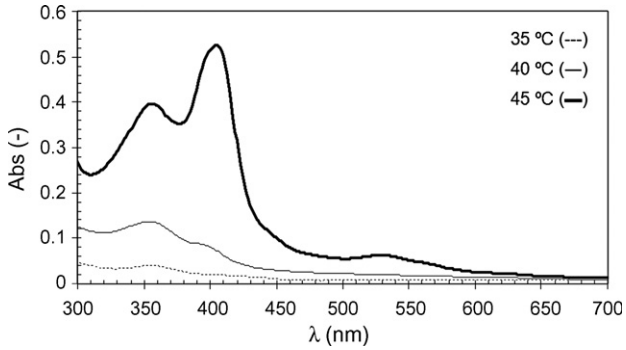

Fig. 2. Absorbance profiles of the supernatant of the SAA tests at 35, 40 and 45 °C.

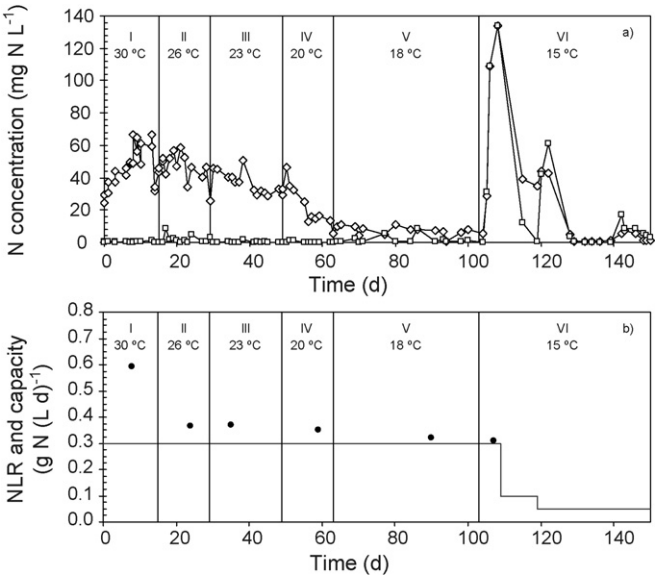

Fig. 3. (a) NH_4^+-N (◊) and NO_2^--N (□) concentrations in the effluent and (b) nitrogen loading rate (—) and maximum nitrogen removal capacity (●).

3.2. Long-term effects of temperature on the Anammox process

The biomass was progressively adapted to lower operating temperatures since a drastic change in the operational conditions could lead to a destabilization of the biological system [43]. Fig. 3a shows the NH_4^+-N and NO_2^--N concentrations in the effluent. Nitrite (the limiting substrate) was totally depleted until the operating temperature was decreased from 18 to 15 °C. At 15 °C the system was not able to remove all the nitrite applied and, therefore, this compound was accumulated in the reactor. As nitrite is inhibitory for the Anammox process, even at moderate concentrations [44,45], this accumulation caused a decrease of the capacity of the system, which led to a higher nitrite accumulation, which provoked a snowballing effect, the system losing totally its efficiency. The complete efficiency of the system was only restored when the NLR was decreased to 0.05 g N (L d)$^{-1}$. However, the SAA was not recovered after 1 month of operation at this low NLR (data not shown). Then, the operating temperature was increased to 30 °C in order to restore the capacity of the system. This strategy was not able to improve the mentioned capacity after two and a half months of additional operation. At the end of the experiment the SAA remained below 0.02 g N (g VSS d)$^{-1}$. This fact would indicate an irreversible loss of the activity that could be caused by the mixed effect of the low temperature and the presence of nitrite [4,14].

In spite of the occurrence of unstable periods, the system maintained its good biomass retention capacity. The solids retention time, calculated as the ratio between total biomass inside the reactor (g VSS) and the biomass wash-out rate in the effluent (g VSS d^{-1}), remained around 150 days along the whole experiment.

The maximum capacity of the system during each operational period was calculated as the product of biomass concentration inside the system and the maximum SAA measured in batch tests done at the operating temperature. As it can be seen in Fig. 3b, the maximum capacity of the reactor was decreasing along the operational period, although that decrease was soft in the change from 20 to 18 °C. Taking account that the biomass concentration was almost constant, the loss of capacity is directly related to the decrease of the SAA.

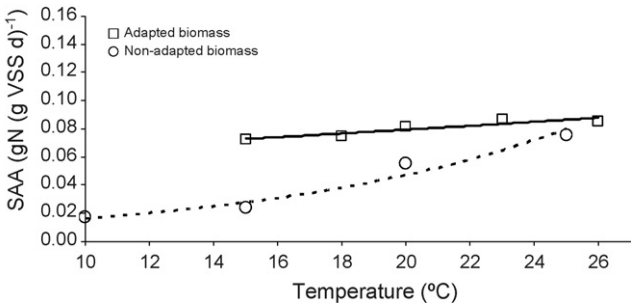

Fig. 4. SAA of non-adapted (◯) and adapted (□) Anammox biofilm biomass.

The nitrogen removal rate treated at 20 °C was much lower than that reported by Isaka et al. [23], which could be probably explained by the high biomass concentration of their system (20 g SS L^{-1}). Cema et al. [22] operated a RBC reactor at 17 °C and obtained an average inorganic nitrogen removal rate of 0.5 g N (L d)$^{-1}$ which is in the range of that obtained in the present work at 18 °C.

Fig. 4 shows the SAA values obtained with no-acclimated biofilm biomass at different temperatures (Section 3.1) and those values determined during the operation of the reactor. Not only the values of SAA for adapted biomass are higher, but also the diminishing tendency is softer than in the case of non-adapted biomass. Therefore, the slow adaptation of the Anammox sludge seems a key factor in order to operate an Anammox reactor at low temperatures. Taking account of the very slow growth rate of the Anammox biomass [14], an advisable start up strategy to operate a system at low temperatures could have two steps. The first one would be the production of the required amount of biomass, working in a separate reactor at a temperature close to the optimum. Then, the second step would be the slow adaptation of the biomass to low temperatures in the same reactor and, finally, the inoculation of the low-temperature reactor could be carried out.

In period I, the ratio NO_2^--N to NH_4^+-N consumed was 1.38 ± 0.10, which agrees with the value of 1.32, commonly assumed in literature [14,46]. However the ammonium concentration in the effluent decreased along the operation of the reactor (Fig. 3a) and, therefore, the cited ratio decreased to 1.05 ± 0.01 when the reactor was operated at 18 °C (period V). Dalsgaard and Thamdrup [25] also observed a ratio of 1 for Anammox biomass coming from marine sediments in anoxic incubations at 15 °C. This change of the observed stoichiometry could be related to a change of the Anammox bacteria metabolism in response to environmental stress [47–49].

FISH analysis showed no qualitative changes in the bacterial populations present in the sludge during the whole experiment and the physical characteristics of Anammox biomass remained

almost constant (SVI of 58 mL g VSS^{-1} and mean diameter of 1.35 mm).

4. Conclusions

The maximum activity of non-adapted Anammox biomass was observed between 35 and 40 °C, while a temperature of 45 °C caused an irreversible decrease of the Anammox activity due to biomass lysis.

By decreasing gradually the temperature, an Anammox SBR was successfully operated at 18 °C. When temperature was decreased to 15 °C, the maximum capacity of the reactor also decreased and the system turned unstable due to nitrite accumulation.

Adaptation of the biomass to low temperatures was observed. However, neither changes on the physical properties of sludge nor qualitative changes on the bacterial populations were found during the operation of the reactor.

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