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Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production

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Abstract

In order to assess the applicability at industrial-scale of the anaerobic ammonium oxidation (Anammox) process it is necessary to study the toxic effects on the maximum specific Anammox activity (SAA) of different compounds commonly present in industrial effluents. The present study was focused on the application of batch tests to determine the maximum SAA in different conditions. The batch tests were based on the measurement of nitrogen gas production. The initial conditions for the tests established to obtain the maximum value of the measured SAA were: 30 °C, pH 7.8, shaking speed 150 rpm, and biomass concentration 1 g VSS L^{-1}. The accuracy of the method was evaluated by mass balances of the nitrogen compounds and the obtained errors were smaller than 7%. Neither the initial biomass concentrations tested (0.5–2.0 g VSS L^{-1}) nor the S_0/X_0 ratio between 0.018 and 0.140 g NO_2^--N g VSS^{-1} had significant influence on the estimated SAA. The addition of a second feeding tended to increase it around 20 ± 10%. The developed method is afterwards applied to the study of the inhibition caused on the Anammox process by different compounds (NH_4^+, NO_2^-, NO_3^-, NaCl, SO_4^{2-}, S^{2-}, flocculant, etc.). The effects of chloramphenicol (inhibitor of the denitrifying process) and allylthiourea (inhibitor of the nitrifying process) were tested in order to be used to distinguish between the Anammox activity from nitrifying and denitrifying activities. The developed batch experiments were found suitable to establish not only the maximum SAA of certain sludge but also the inhibitory effects of certain tested compounds.

Keywords: Anammox; Batch test; Inhibition; Specific Anammox activity (SAA)

1. Introduction

The existence of the anaerobic ammonium oxidation process has been initially predicted from calculations based on the Redfield ratio in marine ecosystems and from thermodynamic calculations [1]. Nevertheless, it was not until 1999 that Strous et al. [2] identified the organism involved in the process as a Planctomycete type bacterium. This microorganism combines ammonium and nitrite under anoxic conditions to generate nitrogen gas according to the stoichiometry shown in the following equation:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.256NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Nowadays, bacteria able to carry out the Anammox process are being found in natural water sources and wastewater treatment plants spread all over the world [3]. This process is increasing in importance at global level due to its contribution to oceanic nitrogen loss and its advantages compared to the nitrification and denitrification processes for nutrient removal from wastewater [4]. Its importance in the nitrogen cycle of the oceans has been studied [5] and it has been recently demonstrated that in continental shelf sediments, up to 67% of the nitrogen gas formation is due to anaerobic ammonium oxidation and only 33% due to denitrification. Up to now, the found bacteria performing the Anammox process belong to three main genera: *Candidatus* Brocadia, *Candidatus* Kuenenia and *Candidatus* Scalindua.

From the engineering point of view, its importance is more related to its application for nitrogen removal from wastewaters. It is known that the use of Anammox process combined with partial nitrification would lead to an important reduction of operational costs compared to conventional

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nitrification–denitrification processes [1]. The combined partial nitrification–Anammox process is being currently implemented in the WWTP Rotterdam [1] to treat sludge digester effluents. This process is applicable to wastewaters characterized by low carbon to nitrogen ratio content and high ammonia concentrations [6]. Furthermore, it is common that these streams contain also compounds that inhibit the Anammox process such as chloride, sulphide, sulphate [7–10]. Therefore, studies to test the possible toxicity of the wastewater are necessary to know the feasibility of this treatment.

The influence of toxic compounds on biomass activities is usually researched by means of batch experiments with addition of the studied inhibitory compound, which give important information to be translated to continuous operation. In the case of the Anammox process only a few studies have been focussed on this subject [11]. Further research is needed to develop a simple method to estimate the Anammox activity and establish the effects of different toxic compounds.

The present work is focused on the stablishment of the optimal conditions for the determination of the maximum specific Anammox activity (SAA) using batch tests based on the monitoring of nitrogen gas production. Once these conditions were determined, the effects on the maximum SAA of different compounds usually present in wastewaters (NH_4^+, NO_2^-, NO_3^-, NaCl, PO_4^{3-}, SO_4^{2-}, S^{2-}, acetate, flocculants, allylthiurea, chloramphenicol) are studied.

2. Materials and methods

2.1. Batch tests procedure

The assays were performed in vials with a total volume of 38 mL and a volume of liquid of 25 mL, each closed with a gas-tight coated septum capable of withstanding about 2 bars of pressure. The vials were inoculated with Anammox biomass enriched in bacteria belonging to the specie *Candidatus Kuenenia stuttgartiensis* [12], washed and re-suspended in phosphate buffer (0.14 g L^{-1} KH_2PO_4 and 0.75 g L^{-1} K_2HPO_4). The headspace and liquid phase were gasified with argon to remove the oxygen. The initial pH value was fixed at 7.8. The vials were placed in a thermostatic shaker, at 150 rpm and 30 °C until stable conditions were reached. Then the substrates ($(NH_4)_2SO_4$ and $NaNO_2$) were added and pressure was equalized to the atmospheric one. The production of N_2 gas was tracked by measuring the overpressure in the headspace with a time frequency depending on the biomass activity in each vial test. The final pH value was always measured in order to check that it was maintained in the optimal range for the Anammox activity. This methodology has been developed for the determination of denitrifying activities [13]. When an inhibitory compound was tested the corresponding amounts were added together with the substrates [14].

2.2. Performed experiments

A first run of assays, performed in triplicate, was carried out to assess the accuracy of the method to estimate the specific Anammox activity (SAA). In this run, activity tests, using different initial concentrations of substrates of 42, 56 and 70 mg N L^{-1} for both ammonia and nitrite, and an initial biomass concentration of 1 g VSS L^{-1}, were performed to evaluate the consumption and production of nitrogenous compounds in the liquid and gas phases, respectively.

A second run of assays was carried out to study the effects of the initial substrate (NO_2^--N) to biomass concentrations ratio (S_0/X_0) and the initial biomass concentration (X_0) on SAA. To test the effect of the S_0/X_0 ratio, different activity tests with constant biomass concentration of 1 g VSS L^{-1} were performed using several S_0/X_0 ratios between 0.018 and 0.14 g NO_2^--N (g VSS)$^{-1}$. The effect of initial biomass concentration was checked at a constant S_0/X_0 ratio of 0.07 g NO_2^--N (g VSS)$^{-1}$ using several X_0 of 0.25, 0.5, 1.0 and 2.0 g VSS L^{-1}.

A third run of assays, performed in duplicate, was used to determine the influence of a second feeding. In this case initial concentrations of ammonia and nitrite were fixed at 70 mg N L^{-1}, respectively, while the initial biomass concentration was 1 g VSS L^{-1}.

Finally, in a fourth run, the toxic effects of compounds present in some industrial effluents were tested (inhibition assays). The batch experiment procedure was similar to that previously described in the third run, these toxic compounds being added to the vials at different concentrations together with the substrates.

2.3. Calculations

2.3.1. Accuracy of the method

The total amount of N_2 gas produced was calculated from the overpressure measured in the headspace of each vial at the end of the assay by using the ideal gas law equation. The amount of nitrogen removed from the liquid phase was also calculated by measuring the ammonium, nitrite and nitrate concentrations at the beginning and the end of the experiment and taking into account the volume of the liquid phase. The relative error of the method was calculated based on the difference between both amounts.

2.3.2. Specific Anammox activity (SAA)

The N_2 gas production rate was calculated from the maximum slope of the curve describing the pressure increase in the vial along the time (α) (Eq. (2)):

$$\frac{dN_2}{dt} = \alpha \frac{V_G}{RT}, \quad \text{mol N}_2\,\text{min}^{-1} \tag{2}$$

where V_G is the volume of the headspace (L), R the ideal gas coefficient and T the temperature (K).

The SAA is calculated from the N_2 gas production rate divided by the biomass concentration in the vial X (g VSS L^{-1}) (Eq. (3)):

$$\text{SAA} = \frac{dN_2/dt}{XV_L} \frac{28\,\text{g N}}{\text{mol N}_2} \frac{1440\,\text{min}}{d}, \quad \text{g N}_2\text{-N (g VSS)}^{-1}\text{d}^{-1} \tag{3}$$

where V_L is the volume of the liquid phase (L).

Since the values of the affinity constant of the Anammox bacteria for ammonia and nitrite were lower than 10 and 5 μM, respectively [11], it can be considered that the activity measured is the maximum activity for the range of nitrite and ammonia concentrations used.

2.3.3. Activity percentage and IC_{50}

The percentage of activity maintained when inhibitory compounds were tested was calculated as (Eq. (4))

$$\text{SAA}(\%) = \frac{\text{SAA}}{\text{SAA}_0} \times 100 \tag{4}$$

where SAA_0 is the maximum specific activity on the control assay (no presence of toxicants) and SAA the maximum specific activity of the tests with inhibitory compounds.

The IC_{50} is the concentration of the tested compound, which corresponds to a percentage of SAA of 50% compared to that obtained without the presence of this compound.

2.4. Analytical methods

Ammonium was analysed by the phenol–hypochloride method [15]. Nitrite and nitrate were analysed by spectrophotometry [16]. Biomass concentration was measured as volatile suspended solids (g VSS L^{-1}), according to standard methods [16]. The pH value was measured using a selective electrode Ingold model U-455 connected to a pH/mV measurer Crison 506.

The overpressure in the headspace was measured using a differential pressure transducer 0–5 psi, linearity 0.5% of full-scale, Centerpoint Electronics. The biogas composition was analysed with a gas chromatograph Hewlett Packard 5890 Series II.

3. Results and discussion

3.1. Establishment of the specific Anammox activity tests

3.1.1. Accuracy of the method

A first set of assays, to check the accuracy of the method, was carried out according to the procedure described in Section 2. The tested initial concentrations of both ammonia and nitrite in the vials were 42, 56 and 70 mg N L^{-1}. These initial concentrations of substrates were fixed in values as high as possible to decrease the relative error of the measurement coming from the sensitivity level of the pressure transducer (6.67 mV psi^{-1}), but quite below the inhibition values obtained by Strous et al. [11] of 98 mg NO_2^--N L^{-1}. The obtained relative errors were smaller than $7 \pm 4\%$ indicating the accuracy of the batch experiments based on the measurement of gas production for the determination of the maximum SAA. Analysis of the produced biogas composition indicated that more than 99% of the produced gas was N_2.

3.1.2. Effects of the initial substrate to biomass concentrations ratio (S_0/X_0) and of the biomass concentration (X_0)

Several S_0/X_0 ratios between 0.018 and 0.140 g NO_2^--N·(g VSS)$^{-1}$ were tested at a constant biomass concentration of 1 g VSS L^{-1}. The lowest value of S_0/X_0 tested was selected to produce the minimum quantity of nitrogen gas detectable by the pressure transducer. The higher value of S_0/X_0 corresponded to the maximum nitrite concentration tolerated by the biomass, not causing inhibition by substrate of Anammox activity. The results showed small differences between the maximum SAAs obtained (0.26 ± 0.03 g N_2-N (g VSS)$^{-1}$ d^{-1}) for the range of S_0/X_0 ratios assayed.

Some authors have reported that the initial S_0/X_0 ratio used in batch experiments influences the obtained values of specific activity in methanogenic [17] and denitrifying biomass [18,19,13]. From other studies in batch experiments it has been found that the higher the S_0/X_0 ratio the higher the specific activity of the biomass [13,17]. Chudoba et al. [18] explained this fact, in the case of aerobic activated sludge exposed to periodical anaerobic conditions, saying that if the initial S_0/X_0 ratio is low ($S_0/X_0 < 2$–4 g COD (g VSS)$^{-1}$) no cell multiplication occurs while substrate is removed but when the S_0/X_0 ratio is high biomass growth is produced and for this reason the substrate removal increases. These authors also observed that, when high initial S_0/X_0 ratios are used, an apparent lag phase on the curves of biomass production is observed due to the time necessary for multiplication of an original small amount of biomass, which causes substrate consumption.

In the case of the Anammox batch experiments, the S_0/X_0 ratios tested did not have a notable effect on the activity and no significant biomass production during the batch test (5 or 6 h) occurred. This is related to the very low growth rate of Anammox biomass. Doubling times between 11 and 19 days for an Anammox culture were reported [20,12]. Therefore, no lag phase and no specific activity increase are expected when the S_0/X_0 ratio is increased.

Initial S_0/X_0 ratios used by other authors measuring SAA ranged from 0.01 g NO_2^--N (g VSS)$^{-1}$ [11] to 0.03 g NO_2^--N (g VSS)$^{-1}$ [21]. The maximum SAAs estimated by these authors were, 1.34 and 0.53 g N_2-N (g VSS)$^{-1}$ d^{-1}, respectively. The differences in the estimated SAA values obtained by the different authors are most probably due to the different conditions of the experiments and the degree of enrichment of the biomass used.

The effects of initial biomass concentration on the estimated SAA were also tested and assays with initial biomass concentrations of 0.25, 0.5, 1.0 and 2.0 g VSS L^{-1} were carried out at a constant S_0/X_0 ratio of 0.07 g NO_2^--N g VSS^{-1}. The maximum SAA was practically constant for these conditions with values of 0.28 ± 0.02 g N_2-N (g VSS)$^{-1}$ d^{-1}.

These observations are different from those found by Sánchez et al. [22] in case of denitrifying activity tests. These authors observed that concentrations of biomass between 0.5 and 1.0 g VSS L^{-1} influenced strongly the specific denitrifying activity but concentrations of 1.5 and 3.0 g VSS L^{-1} did not. However, Chudoba and Pannier [23] found that, for low S_0/X_0, the nitrifying activity of suspended activated sludge did not change for biomass concentrations between 0.5 and 4.0 g VSS L^{-1}. It can be the case that the different observations are due to the different relative conditions used for the different performed activity tests which in the case of Anammox activities is limited by the inhibitory effect of one of the substrates, the nitrite.

3.1.3. Effect of successive feedings

Several assays were performed adding three successive feedings. The second feeding was added to the vials 20 h after the consumption of the substrates in the first feeding (reflected in a constant value of the overpressure measured in the headspace of the vials). Vials were depressurised after the second addition of substrates. The experimental procedure was repeated for the third feeding. The maximum SAA of the biomass increased from 0.25 ± 0.01 (first feeding) to 0.30 ± 0.03 g N_2-N (g VSS)$^{-1}$ d^{-1} (second and third feedings) (Fig. 1). This increase of the SAA in the successive feedings was also observed in the case of den-

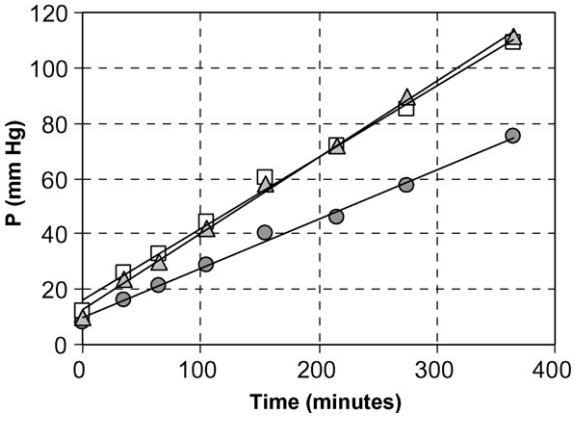

Fig. 1. Increase of the gas pressure along the time during the batch tests with successive feedings: first feeding (●); second feeding (□); third feeding (▲).

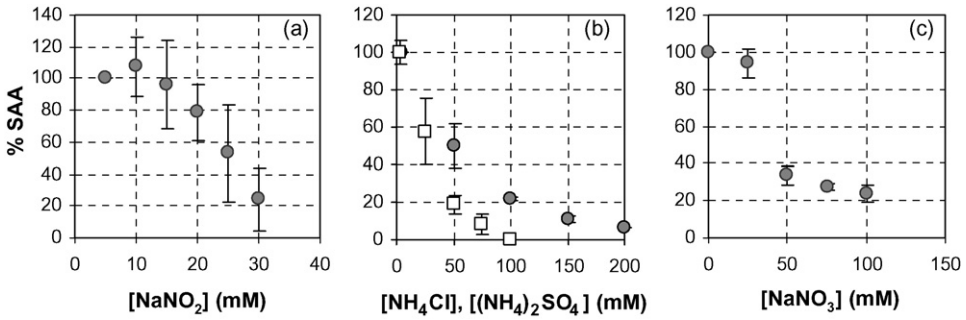

Fig. 2. Inhibition caused by different concentrations of nitrite as $NaNO_2$ (a), ammonium as NH_4Cl (●) and $(NH_4)_2SO_4$ (□) (b) and nitrate as $NaNO_3$ (c).

itrifying and anaerobic biomass [22,24]. This effect could be attributed to the possible presence of the proper conditions (such as presence of some enzymes), which make the process faster than in the first feeding. Biomass increase during the duration of the experiment can be neglected due to the slow Anammox growth rate and its low biomass yield.

Taking into account the results obtained from the previous initial experiments the conditions to estimate the SAA of a sludge were established as follows: temperature 30 °C, pH 7.8, 150 rpm, 1 g VSS/L^{-1}, 70 mg N L^{-1} of ammonium and nitrite, respectively.

3.2. Inhibition tests

3.2.1. Effects of substrates and product

In order to apply the Anammox process for the treatment of industrial wastewaters with high ammonia concentrations it is necessary to know the effect of substrates and product on the SAA. Experiments with different initial concentrations of ammonium, nitrite and nitrate were individually performed at concentrations between 5 and 200 mM (Fig. 2). Nitrite exerted the highest inhibitory effect on the maximum SAA. Concentrations of nitrite of 25 mM corresponded to the 50% inhibition concentration (IC_{50}). This result differs considerably from that obtained by Strous et al. [11] since these authors found that at concentrations of nitrite higher than 7 mM the Anammox activity was completely inhibited (Table 1).

The IC_{50} values for ammonium and nitrate corresponded to concentrations of 55 and 45 mM, respectively. These results partially agree with those found by Strous et al. [11] who exposed the Anammox biomass to concentrations up to 70 mM of ammonium and nitrate in continuous operation in a SBR during 1 week observing no negative effect on the activity. Besides the two different compounds tested containing ammonia presented the same inhibition percentages with respect to the ammonia ion (Fig. 2b). This fact indicates that this is the ion, which is expected to cause the inhibition of the SAA and no specific effect can be attributed to the sulphate or chloride ions at the tested concentrations. From these results, it is suggested that the nitrite concentration in an Anammox reactor must be strictly controlled to avoid inhibition of the process.

Taking into account that substrates concentrations were much higher than the affinity constant values, the obtained results were fitted to several equations describing substrate and product inhibitions (Edwards model, Luong model and classical substrate inhibition equation) but none of these equations was able to describe the kinetics of inhibition adequately. Strous et al. [11] found that their experimental data for ammonia and nitrite inhibition fitted to the Luong model with a correlation coefficient (r^2) of 0.92 for NH_4^+ and 0.84 for NO_2^-.

3.2.2. Effects of exogenous compounds

The effects of different compounds, present in many industrial effluents, on the maximum SAA have been tested

Table 1
Effects of different ions present in wastewater on the Anammox activity

Compound	Strous et al. [11][a]		Van de Graaf et al. [26]		Present work
	Concentration (mM)	Effect	Concentration (mM)	Effect	IC_{50} (mM)
Ammonia	70	No effect			55
Nitrite	7	Loss of activity			25
Nitrate	70	No effect			45
Chloride			50	No effect	200
Acetate			1 or 5	Increase	39
Phosphate			1 5 or 50	No effect Loss of activity	21
Sulphide			1 or 5	Increase	0.3

[a] Continuous experiments.

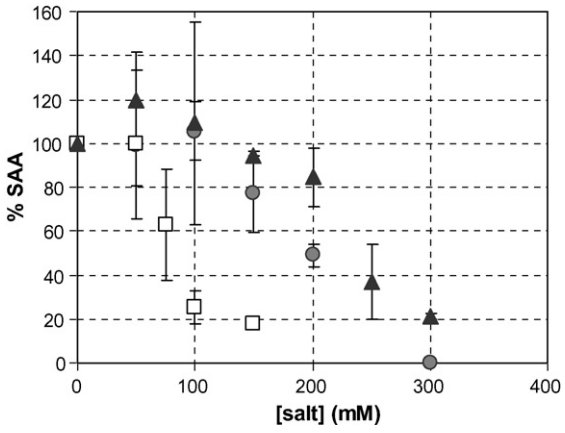

Fig. 3. Effects of KCl (●), NaCl (▲) and Na_2SO_4 (□) on the maximum SAA.

in order to evaluate further industrial applications of the process.

3.2.2.1. Effects of salts. The percentage of the activity maintained at different concentrations of the tested salts is showed in Fig. 3. NaCl concentrations below 150 mM did not affect the Anammox activity while KCl and Na_2SO_4 had effect only at concentrations higher than 100 and 50 mM, respectively.

The IC_{50} observed for Na_2SO_4 (80 mM) was significantly lower than for NaCl and KCl (230 and 200 mM, respectively). These results confirm the findings of the previous section where the inhibitory effect of the two assayed salts (NH_4Cl and $(NH_4)_2SO_4$) was attributed basically to the ion ammonium and not to the anions of the salts. Besides the data obtained with the two salts containing sodium (NaCl and Na_2SO_4) indicate that this ion is responsible for the inhibitory effects at the tested concentrations (Fig. 3). The inhibitory effect showed by Na_2SO_4 could be related to the higher concentration of Na^+ ions in the medium compared to its concentration when NaCl was added at the same molarities. This negative effect of sodium was also observed by Soto et al. [25] to affect methanogenic activity in the anaerobic treatment of mussel processing wastewaters.

These results are similar to those of van de Graaf et al. [26] who observed no effect of KCl on the SAA at concentrations of 50 mM. This decrease of the activity in the presence of salts would be due to an increase of the osmotic pressure in the medium surrounding the cells that affects the transport system through the membrane.

The resistance of the Anammox biomass observed in these assays and taking into account that these microorganisms have been found in different marine environments [5], allows predicting the suitability of this process to be used with effluents containing high salts concentrations.

3.2.2.2. Effect of organic matter, phosphate and sulphide. Acetate and phosphate could be present in concentrations up to 10 and 15 mM, respectively, without causing significant activity decrease. Concentrations of 25 and 50 mM of acetate resulted in 22 and 70% inhibition percentage (Fig. 4).

In the case of experiments with low acetate concentrations, an increase of the gas production was observed. These results could be due to the recently found capacity of the Anammox bacteria to carry out propionate oxidation simultaneously with anaerobic ammonium oxidation [27]. Although this observation requires more research because in the present case acetate was the organic compound. In previous studies van de Graaf et al. [26] observed also an increase of the Anammox activity in batch tests by adding acetate. These authors carried their assays using ammonia and nitrate as substrates and they claimed that the acetate was used for the nitrate reduction to nitrite, which was found to be the real substrate of the Anammox bacteria and thus the latter could perform their activity.

In the case of phosphate, the IC_{50} value of 20 mM is above the range of phosphate concentrations usually found in industrial wastewaters. This value is also higher than the one found by van de Graaf et al. [26] who observed a total inhibition at 5 mM.

On the other hand, the effect of sulphide on SAA was tested because SO_4^{2-} reduction takes place quite often in anaerobic digesters, mainly transformed into H_2S. Concentrations of sulphide between 1 and 2 mM caused a decrease of 60% of SAA. The SAA was absent at concentrations of sulphide above 5 mM. These results disagree with those of van de Graaf et al. [26] who found stimulation of the activity in both batch and continuous assays. These authors used nitrate as electron donor for the Anammox biomass and explained their results considering that sulphide could reduce nitrate to nitrite, which is the authentic electron donor of the process.

These results suggest that an effective operation of the previous treatment stage is necessary to cause the oxidation of organic

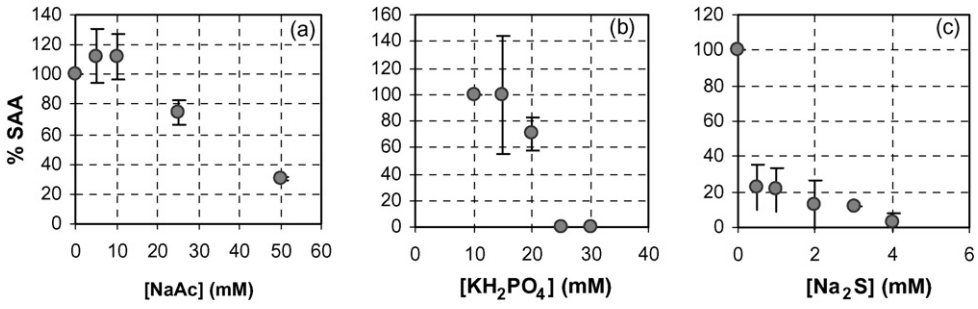

Fig. 4. Effects of NaAc, KH_2PO_4 and Na_2S on the maximum SAA.

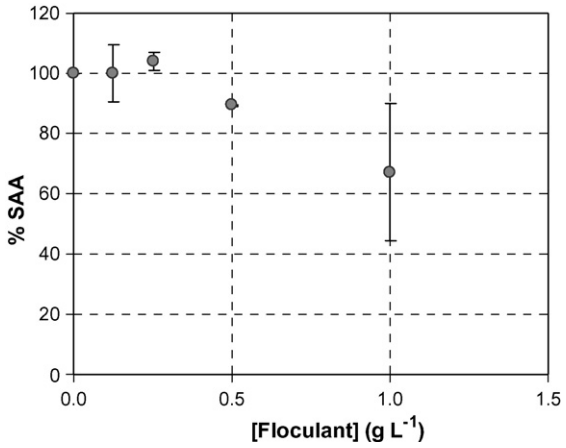

Fig. 5. Effects of flocculant on the maximum SAA.

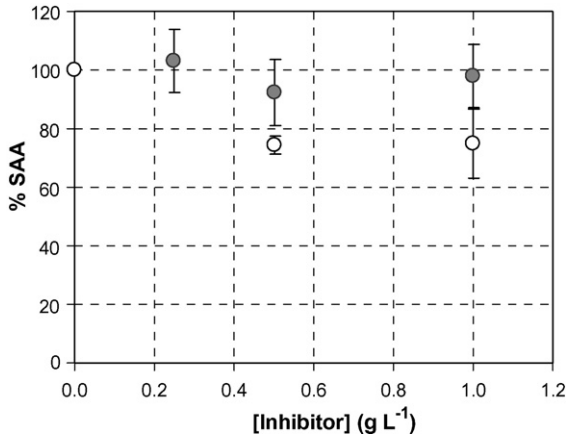

Fig. 6. Effects of allylthiourea (◯) and chloramphenicol (●) on the maximum SAA.

matter and sulphide, preventing their entry into the Anammox reactor.

3.2.2.3. Effect of flocculant. A physical–chemical process-like flocculation can be used to remove colloidal organic and inorganic substances from wastewater previous to the Anammox process. Therefore, the effect of flocculant on this process must be tested.

Concentrations between 0 and 1 g L^{-1} of flocculant (Floculex CS-49) were added to the batch tests as representative values usually applied in the treatment of industrial wastewaters (Fig. 5). Both the IC_{50} and IC_{100} (100% inhibition concentration) were found to be higher than the assayed concentrations. The flocculant used was a polymerising positively charged compound and caused the formation of conglomerates of biomass (2–3 mm) during the test and an immediate biogas production. No physical detrimental effect was caused on the Anammox activity.

3.2.2.4. Effect of allylthiourea and chloramphenicol. Anammox bacteria have never been found isolated but in communities with other microorganisms. The difficulty to obtain pure cultures of Anammox bacteria can be given by the necessity of the presence of other bacteria to remove toxic products or to provide some essential nutrient. This makes necessary to test some specific inhibitors to distinguish Anammox activity from other activities of the nitrogen cycle (nitrifiers and denitrifiers), which use common substrates.

Allylthiourea is a very specific inhibitor of the nitrification process, causing a 50% inhibition in a mixed culture at a concentration of 1.4 mg L^{-1} [28]. Concentrations up to 1 g L^{-1} of allylthiourea caused no significant decrease of the maximum SAA (Fig. 6), which agrees with van de Graaf et al. [26]. So this compound can be used to distinguish Anammox and nitrifying activities in batch tests.

On the other hand, chloramphenicol acts inhibiting the formation of the peptidic bonds for the proteins synthesis. This antibiotic is usually used against the infections provoked by Gram-negative bacteria, but it can inhibit eukaryotic cells as well as prokaryotic ones. Despite this fact, it did not cause inhibition on the Anammox activity in the batch tests using concentrations up to 1 g L^{-1} (Fig. 6). van de Graaf et al. [26] found that the effect of chloramphenicol on the Anammox activity depended on the enrichment degree of the culture.

This can be, therefore, a useful method to distinguish Anammox activity from the activity of other microorganisms that are inhibited by chloramphenicol. For example, Okpokwasili and Eleke [29] reported the inhibition of pure cultures of *Nitrosomonas* and *Nitrobacter* by chloramphenicol at concentrations of 13.3 mg L^{-1} in batch assays. Most interesting application is based in the total inhibition of denitrification, at a concentration of 300 mg L^{-1} of this compound, found by Brooks et al. [30].

4. Conclusions

The conditions for a quick and simple method for the determination of the maximum SAA were established as follows: temperature 30 °C, pH 7.8, 150 rpm, 1 g VSS L^{-1}, 70 mg N L^{-1} of ammonium and nitrite, respectively. The average errors estimated from the balances were lower than 7% indicating the accuracy of the method.

Results from the batch tests indicate that when the Anammox sludge is enriched the maximum quantities of substrates susceptible to be treated can be easily estimated knowing the SAA of the sludge.

To guarantee the proper operation of the Anammox process the concentrations of nitrite must be controlled under the inhibitory levels and the organic matter and sulphide must be oxidized during a previous stage. The presence of salts, phosphate or flocculant at relatively high concentrations does not represent a problem for the process.

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