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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

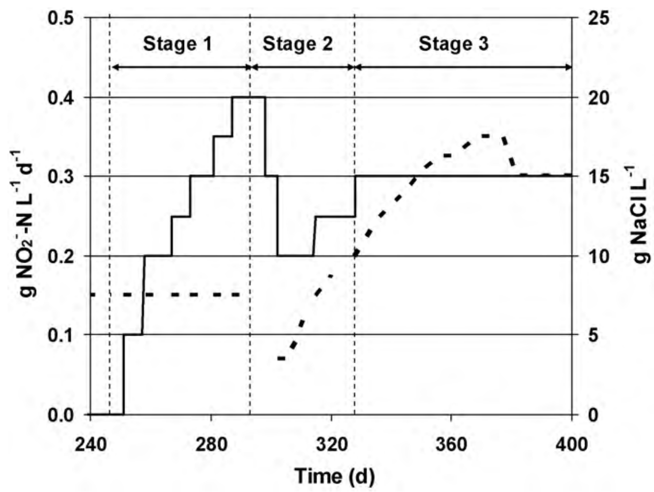

Fig. 1. Nitrite loading rate (NiLR) supplied to the system (- - -) and inlet salt concentration (–) during the whole operational period.

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-

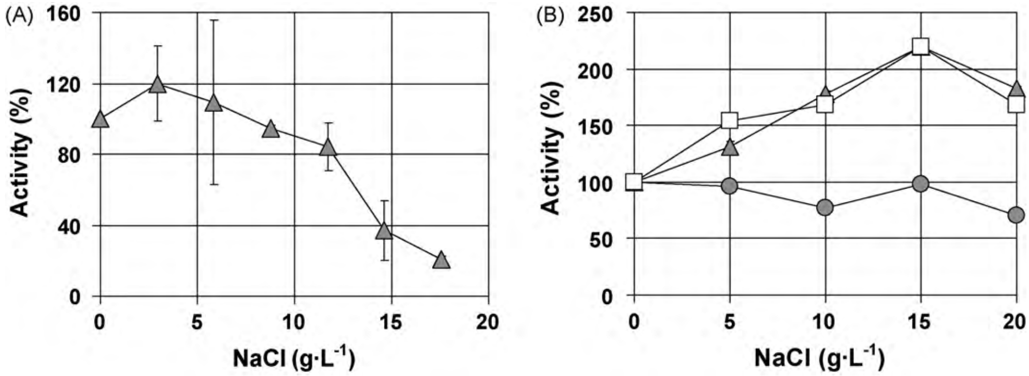

Fig. 2. The SAA values of non-adapted biomass in the presence of different concentrations of salt (2A) and the SAA of the biomass in the reactor operating at the tested NaCl concentration (2B): washing the biomass with standard buffer (SB) (●), washing the biomass with buffer with the same NaCl concentration (SBS) than the present in the reactor (□), without washing the biomass (RB) (▲).

umetric index (SVI) were determined according to the Standard Methods [14].

Morphological studies of the biomass were performed with a scan electron microscope (Digital SEM Leica 440 at 20 kV). The sludge sample was washed three times for 10 min with phosphate buffer 0.05N at pH 7.4 and subsequently fixed with a solution of glutaraldehyde 3% in a phosphate buffer solution over night. After fixation, the sample was dehydrated using ethanol solutions with increasing ethanol concentrations (30%, 50%, 70% and 100%). Later the sample was shaded with gold and observed under the scan electron microscope. Micro-analysis was performed with a scan electron microscope (SEM LEO-435VP; EDX) to research the elemental composition of the biomass samples. FISH tests were carried out according to the methodology of Figueroa et al. [15].

3. Results and discussion

3.1. Effects of the NaCl on the Specific Anammox Activity

The experimental work was carried out in a mature Anammox SBR that had been operated during 240 days before the salt was added in the feeding (data not shown).

The feeding contained, at the beginning of stage I, 300 mg N L^{-1} as ammonia and nitrite respectively. The SAA of non-adapted biomass was estimated from biomass samples collected from the reactor before day 250 (no NaCl was added in the feeding) at different NaCl concentrations (Fig. 2A). The value of the SAA measured in absence of NaCl was of 0.15 g NO_2^--N g VSS^{-1} d^{-1}. A stimulatory effect on the SAA was observed up to 6 g L^{-1} of NaCl while the IC_{50} value (concentration which causes SAA inhibition of 50%) was estimated of 13.5 g L^{-1}. These results are similar to those found by Kartal et al. [4] for an Anammox population of *Candidatus* "Kuenenia stuttgartiensis" although they observed better resistance of their biomass to salinity conditions meaning that Anammox activity was present up to concentration of 90 g L^{-1} of NaCl.

On day 250 the feeding was supplemented with different concentrations of NaCl for 150 days in three operational stages. During stage I, the Anammox SBR was operated for 43 days at a constant NiLR of 0.15 g NO_2^--N L^{-1} d^{-1}, while NaCl concentration was stepwise increased to research its effects on the Anammox process.

The batch experiments carried out using the SB solution gave nearly constant SAA values for all the tested NaCl concentration and a decrease of the SAA was observed only when 20 g NaCl L^{-1} was added (Fig. 2B). This fact could be attributed to a sudden decrease of salt concentration in the reaction media which could reduce the activity of the Anammox biomass [16]. In the experiments performed with the SBS solution, the maximum SAA of the biomass in the reactor was higher than that obtained with the SB solution at the same salt concentration up to 15 g NaCl L^{-1}. When 20 g NaCl L^{-1} were tested a decrease of the SAA occurred. Similar results were obtained when the RB solution was used. Similar results were obtained by Kartal et al. [4] who found an increase of the Anammox activity up to a salt concentration of 30 g NaCl L^{-1} in batch experiments using adapted biomass. However, Windey et al. [5] studying the performance of the OLAND process for the treatment of high salinity wastewater, found that the activity of the salt-adapted Anammox biomass decreased 59% at 30 g NaCl L^{-1}.

It seems clear that the Anammox biomass is highly resistant to the presence of salt concentration (NaCl). Since many Anammox bacteria have been identified in marine ecosystems where the salt concentrations are even higher than those studied in this work, the salt resistance was not an unexpected event [17,18]. The obtained results differ from one work to another indicating that factors such as the kind of enriched Anammox bacteria present and the degree of enrichment in the reactor or even the biomass story are important parameters to take into account.

On the other hand, the results obtained in the present work using adapted and non-adapted biomass showed that when the Anammox biomass is continuously subjected to high NaCl concentrations its resistance to salt clearly increases as was also documented [4,5].

3.2. Monitoring the capacity of the reactor

The efficiency of the Anammox reactor was followed by the depletion of nitrite in the effluent since ammonia was always supplied in excess. During the first and second stages, the removal efficiency of nitrite was around 99%. Nevertheless, during the third period on day 336, nitrite accumulation (up to 82 mg NO_2^--N L^{-1}) appeared in the reactor due to a failure of the electricity supply. The complete efficiency of the system was practically restored despite the continuous increase of the NiLR. Nitrite was also accumulated in the reactor and maintained in a value around 120 mg NO_2^--N L^{-1} when the NiLR was increased from 0.325 to 0.35 g NO_2^--N L^{-1} d^{-1} (day 360). In this case the concentration of nitrite in the influent was lowered to 300 mg NO_2^--N L^{-1} and the high efficiency of the process was restored. The SAA of the biomass measured in batch tests stayed almost constant along this third operational period (around 0.29 g NO_2^--N g VSS^{-1} d^{-1}).

The maximum capacity of the system was estimated as the product of the biomass concentration by the maximum SAA measured in batch tests. As the biomass concentration slightly increased from 0.9 to 1.2 g VSS L^{-1} during the whole operational period, the variations of the system capacity are closely related to the changes of SAA due to the changes of salinity conditions.

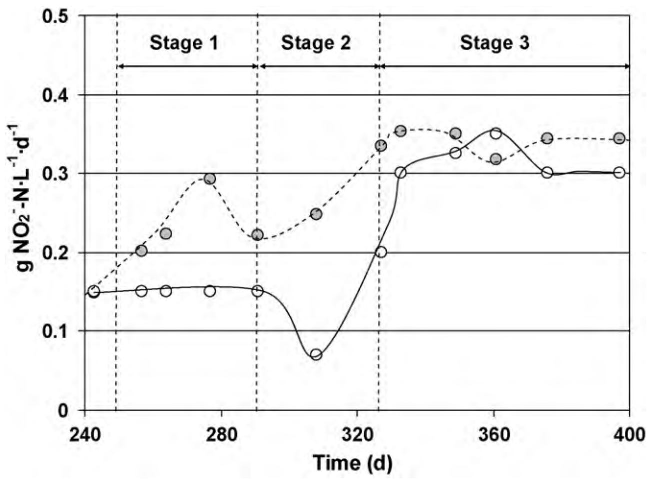

Fig. 3. NiLR supplied (○) and maximum capacity of the system (●) during the whole operational period.

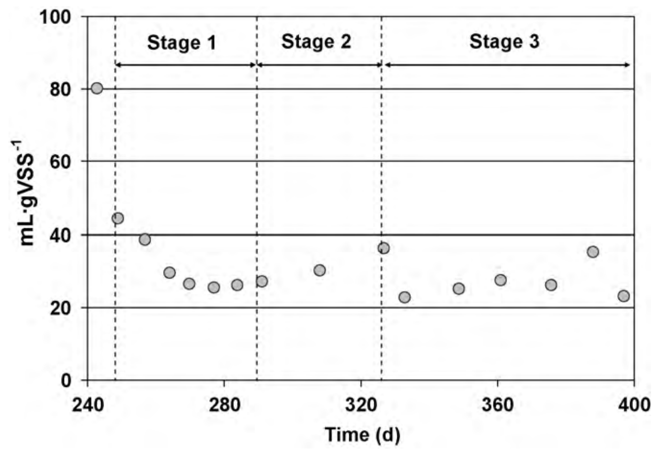

Fig. 4. Change of the SVI during the operation.

The maximum capacity of the system was estimated during the whole operational period using the SAA values measured using non-adapted and adapted biomass. When the SAA values obtained with non-adapted biomass are extrapolated according to the operational conditions of the reactor, the capacities estimated are lower than those estimated from the substrates consumption in the SBR for each NaCl concentration. Similar results are found when the SAA values measured with adapted biomass using SB solution are employed. Nevertheless, if the capacity of the system is calculated on basis of the SAA obtained with adapted biomass under the same salinity conditions of the reactor (SBS and RB), the results obtained can perfectly explain the efficiency of the reactor (Fig. 3). During the first and second periods, the capacity was always higher than the NiLR supplied which justifies the high efficiency observed. During the third period, the capacity of the system was almost constant at a value around 0.34 g NO_2^--N L^{-1} d^{-1}, therefore, when NiLR supplied exceeded this value, nitrite started to accumulate in the system (day 360). A decrease of NiLR supplied from 0.35 to 0.30 g NO_2^--N L^{-1} d^{-1} restored the nitrite removal efficiency.

These results showed that the measurement of the maximum SAA under similar salinity conditions of the reactor can be used to know the maximum NiLR which can be supplied to the system maintaining a high efficiency. This is useful to operate the system close to its maximum capacity and to get shorter start-up. However, Kartal et al. [4] found a total loss of the Anammox activity when 45 g NaCl L^{-1} were introduced continuously while the same salt-adapted biomass maintained 90% of its maximum activity in batch tests at the same salt concentration. This fact could be explained by a mixture inhibition of salt and nitrite during the continuous operation due to the nitrite concentration used during continuous assays was higher than during batch tests.

Windley et al. [5], using batch tests, observed specific activities of 46 and 32 mg NH_4^+-N g VSS^{-1} d^{-1} for nitrifying and Anammox biomasses, respectively, at 30 g NaCl L^{-1}. However, the limiting step of the OLAND process operated continuously in the presence of the same salt concentration was the nitrifying process. In this case, the discrepancy between results of batch and continuous assays could be related to the different dissolved oxygen levels used during continuous operation (lower than 1 mg O_2 L^{-1}) and nitrifying batch tests (above 3.3 mg O_2 L^{-1}). For this reason, specific nitrifying activity in the reactor is lower than the activity measured in discontinuous assays. This fact remarks that it is important to carry out batch assays under similar operational conditions to the reactor in order to extrapolate the results.

3.3. Biomass characteristics

The effect of salt on the physical properties of biomass is very important in order to maintain a good retention of the biomass inside the system. In this case, when NaCl was supplied to the feeding media physical properties of the sludge changed from flocculent to granular, the SVI significantly decreased from 80 to 25 mL g VSS^{-1} (Fig. 4). A decrease in the SVI of Anammox biomass from 120 to 50 mL g VSS^{-1} was also reported by Fernández et al. [19] when adding NaCl to a synthetic media with concentrations up to 10 g NaCl L^{-1}. Improvement of the SVI of the biomass from 42 to 11 mL g VSS^{-1} after 150 days of operation has been observed also by Campos et al. [6] in a nitrifying reactor treating 4 g NH_4^+-N L^{-1} d^{-1} and containing in the feeding media concentrations of a mixture of salts (NH_4Cl, $(NH_4)_2SO_4$, KH_2PO_4 and NaCl) up to 525 mM. Dahl et al. [20], treating in a nitrifying system wastewater with high salinity (20 g Cl^- L^{-1}) and ammonia concentration of 120 mg NH_4^+-N L^{-1}, obtained a sludge with a SVI of 32 mL g VSS^{-1}. In other cases a negative effect was observed like in the case of Moon et al. [21]. These authors found floc size decreased and settling properties deteriorated with increasing salt concentration when they treated seafood wastewater with a SBR at NaCl concentrations up to 10 g L^{-1}.

The VSS/TSS ratio of the sludge decreased from 0.85 to 0.70 indicating the accumulation of inorganic matter in the granular biomass. This was confirmed from observations of the biomass by electron microscopy. A microanalysis of the precipitates indicated that the precipitate formed was mainly $Ca_3(PO_4)_2$. The ions Ca^{2+} and PO_4^{3-} were supplemented with the feeding mineral medium in the form of $CaCl_2$ and KH_2PO_4, respectively. In some cases it was observed under the stereomicroscope the granulation of the biomass around these precipitation nuclides, what could explain the high decrease in the SVI because of the addition of salt to the system. These results agree with the inert nuclei model proposed by Hulshoff Pol et al. [22]. In this model, the presence of inert microparticles, anaerobic bacteria could attach to the particle surfaces to form the initial biofilms, and the mature granules can be further developed through the growth of these attached bacteria under given operation conditions. In this case, the precipitates of $Ca_3(PO_4)_2$ could serve as the inert nuclei or micro-size biocarrier for bacterial attachment and further granulation.

van Langerak et al. [23] studied the development of anaerobic granular sludge in UASB reactors using different calcium concentrations in the influent. These authors found that a low Ca/Na ratio, a small granule diameter and a high crystallization rate constant (in that case, low $CaCO_3$ growth inhibition) stimulate precipitation in

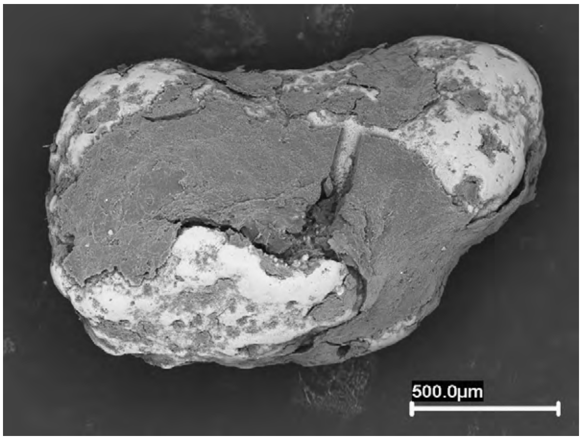

Fig. 5. Granule formed by Anammox biomass (dark colour) and $Ca_3(PO_4)_2$ precipitate (white colour).

the bulk solution, and are considered to be favourable parameters for the development of sludge with a high ash content and a good quality.

The granulation of the biomass is also related to the change of its aspect from disperse flocs to granules. The colour of the biomass also changed to a more intense red as the granule density increased probably due to the more compact aggregation of the Anammox bacteria around the precipitates (Fig. 5).

4. Conclusions

(1) The presence of NaCl in the media causes an increase of the SAA for both adapted and non-adapted biomass at concentrations between 3 and 15 g NaCl L^{-1}, respectively.
(2) Anammox biomass is able to cope with different NaCl concentrations with no detrimental effect on the SAA up to concentrations of 15 g NaCl L^{-1}.
(3) Batch activity assays carried out under similar operational conditions to the reactor can be used to estimate the maximum capacity of the system and to avoid overloads when the effect of the presence of an inhibitory compound is studied.
(4) High salt concentrations did not have long-term detrimental effects on the physical properties of sludge and even promote the aggregation in the form of granules of the Anammox biomass.

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