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Influence of biomass acclimation on the performance of a partial nitritation-anammox reactor treating industrial saline effluents

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HIGHLIGHTS

- Partial nitritation/anammox SBR operated to treat saline canning effluents.
- Intermittent aeration avoids NOB activity at low NaCl and nitrite accumulation.
- Anammox activity loss of 94% after 160 days of salt rise from 2 to 18 g-NaCl L^{-1}.
- Direct exposure to high salt (18 g-NaCl L^{-1}) avoids progressive anammox weakening.
- Partial nitritation/anammox start-up is shortened by direct exposure to high salt.

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ABSTRACT

The performance of the partial nitritation/anammox processes was evaluated for the treatment of fish canning effluents. A sequencing batch reactor (SBR) was fed with industrial wastewater, with variable salt and total ammonium nitrogen (TAN) concentrations in the range of 1.75–18.00 g-NaCl L^{-1} and 112 - 267 mg-TAN L^{-1}. The SBR operation was divided into two experiments: (A) progressive increase of salt concentrations from 1.75 to 18.33 g-NaCl L^{-1}; (B) direct application of high salt concentration (18 g-NaCl L^{-1}). The progressive increase of NaCl concentration provoked the inhibition of the anammox biomass by up to 94% when 18 g-NaCl L^{-1} were added. The stable operation of the processes was achieved after 154 days when the nitrogen removal rate was 0.021 ± 0.007 g N/L·d (corresponding to 30% of removal efficiency). To avoid the development of NOB activity at low salt concentrations and to stabilize the performance of the processes dissolved oxygen was supplied by intermittent aeration. A greater removal rate of 0.029 ± 0.017 g-N L^{-1} d^{-1} was obtained with direct exposure of the inoculum to 18 g-NaCl L^{-1} in less than 40 days. Also, higher specific activities than those from the inoculum were achieved for salt concentrations of 15 and 20 g-NaCl L^{-1} after 39 days of operation. This first study of the performance of the partial nitritation/anammox processes, to treat saline wastewaters, indicates that the acclimation period can be avoided to shorten the start-up period for industrial application purposes. Nevertheless, further experiments are needed in order to improve the efficiency of the processes.
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1. Introduction

The amount of saline wastewater that needs to be treated worldwide is rising due to the increase in industrial activities (petroleum, food, tannery, pharmaceuticals, etc.), the use of seawater for flushing toilets, the infiltrations of seawater in wastewater treatment plants (WWTPs) located in coastal regions, the separation of grey and black wastewaters with decentralized treatment in residential areas, etc.

One of the sectors that generates high salinity wastewaters is the fish canning industry (Cristóvão et al., 2016). This sector is very important in the region of Galicia (northwest Spain), which dominates the national market that places Spain as the leading fish and

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seafood canning county in the European Union (Taboada Gómez et al., 2016). The sector comprises approximately 65 companies distributed mainly along the coast. Similarly, Chile is an important fishing-aquaculture nation in Latin America, generating 48.5% of its aquaculture production and is the fifth country in the world in fishery production (Alfaro and Quintero, 2014). At the present time, the new stringent regulations and the need for preserving the quality of the marine environment in coastal areas make the development of efficient technologies to tackle fish canning wastewater treatment necessary.

According to the concept of "circular economy", wastewater can be considered as a "source of materials" instead of as "waste", to produce for example energy. It is known that the energy contained in the wastewater is enough to compensate for the energy required for its treatment (Gu et al., 2017). One of the alternatives to achieve the energy self-sufficiency of the WWTPs is the application of combined partial nitritation/anammox processes for autotrophic nitrogen removal, which enables the maximization of organic matter valorization via anaerobic digestion for biogas production (Morales et al., 2015b).

In particular, fish canning wastewater treatment normally consists of a physico-chemical process for the removal of solids and fats by dissolved air floatation. In some cases, an additional anaerobic digester is placed subsequently for organic matter valorization as biogas. More rarely, a coupled nitrification-denitrification system is added to remove nitrogen and fulfill the discharge requirements in terms of organic matter and nitrogen removal. However, the nitrification-denitrification processes present two main disadvantages that make the nitrogen removal inefficient: (1) the nitrification step consumes large amounts of oxygen, which involves high energy consumption and (2) the organic matter consumed for the denitrification process cannot be valorized as biogas in the anaerobic digestion step. Therefore, the application of the combined autotrophic partial nitritation/anammox processes is of great interest (Vazquez-Padin et al., 2014). This option allows a 50% saving in the energy needed for aeration, as only half of the ammonium contained in the wastewater is oxidized to nitrite (partial nitritation). Furthermore, all the organic matter previously used in the denitrification process can be used now to produce biogas in the anaerobic digester. This advantage relies on the fact that in the anammox process the ammonium is oxidized to nitrogen gas using the nitrite as an electron acceptor, without the need for an organic source. However, the anammox bacteria are very sensitive to different parameters and compounds, such as salinity (Jin et al., 2012). There are a significant number of studies that report on the inhibition of anammox bacteria by salinity and also their possible adaptation (Scaglione et al., 2017). However, there are no studies available that refer to the performance of the combined partial nitritation/anammox processes for saline industrial wastewater conditions. Furthermore, the research published to date used synthetic saline wastewater (Malovanyy et al., 2015; Wang et al., 2017). Therefore, specific studies with industrial wastewater are necessary to verify if a specific company can shift from an existing nitrification-denitrification treatment system to a partial nitritation/anammmox one.

Thus, the present study focuses on testing the feasibility of implementing a combined partial nitritation/anammox system to treat the effluents produced from an anaerobic digester in operation in a fish canning facility.

2. Materials & methods

2.1. Reactor set-up

A pilot scale sequencing batch reactor (SBR) with a useful volume of 25 L was operated in 6 h cycles comprising: 5 min of feeding, 340 min of reaction with stirring and variable aeration, 5 min of sedimentation and 10 min of withdrawal. The exchange volume was 4.5 ± 0.3 L. The aeration was supplied from the bottom of the reactor by applying a controlled on/off strategy (Table 1) to guarantee an adequate balance between the activities of the partial nitritation and anammox processes. The oxygen concentration ranged between 0.1 and 1.5 mg-O_2 L^{-1} during the whole operational period. The temperature was maintained at 29 ± 1 °C and the hydraulic retention time (HRT) was varied between 1.35 and 2.57 d. A mechanical stirrer (50 rpm) was installed to ensure the mixing of the reactor bulk when the aeration was too low and to favor the mass transfer.

2.2. Industrial fish canning wastewater and SBR operational periods

The SBR was fed with the effluent from an anaerobic digester treating fish canning industrial wastewater. This wastewater was periodically collected at the industry and stored at 4 °C. During the SBR operation, the influent composition varied according to the different collected batches. In this period, different products were processed in the industrial facility (tuna, sardines, mussels, etc.). The main inlet difference in composition corresponded to the NaCl concentration, which increased from 1.75 to approximately 18 g-NaCl L^{-1} in the 6 months of operation. Then, the NaCl concentration of the industrial wastewater was stabilized at 18 g-NaCl L^{-1} when the company processed mussels. Therefore, the SBR was operated in two different experiments: (A) to evaluate the effects of increasing salt concentrations from 1.75 to 18.33 g-NaCl L^{-1}; and (B) to evaluate the effects when a high salt concentration of 18 g-NaCl L^{-1} was directly applied. The main characteristics of the industrial wastewater fed to the SBR in each operational period are summarized in Table 1.

The first experiment (A) lasted 175 days and was divided into four stages according to the aeration strategy applied (Table 1). In Stage A-I the aeration was continuously supplied; in Stage A-II the aeration was intermittent; in Stage A-III the aeration was supplied in continuous mode but an initial anoxic phase of 60 min was included in the operational cycle; finally, in Stage A-IV the previous anoxic phase was maintained and the aeration was intermittent. The second experiment (B) lasted 40 days, after re-inoculation, without modifications in the SBR operational conditions and using the same batch of industrial wastewater.

2.3. Partial nitritation-anammox inoculum

The SBR was inoculated with granular biomass from an ELAN® pilot plant (200 L) performing the combined partial nitritation-anammox processes operated at 30 °C (Morales et al., 2015a). This pilot plant was fed with the effluent from an anaerobic sludge digester containing ammonium and salt concentrations of 500–1000 mg-TAN L^{-1} and 1 - 2 g-NaCl L^{-1}, respectively. The maximum specific anammox activity (SAA) of the inoculated biomass, determined by batch activity tests performed at 30 °C, was 0.562 ± 0.078 and 0.421 ± 0.056 g-N g-VSS^{-1} d^{-1} for experiments (A) and (B), respectively.

2.4. Analytical methods

Analytical determinations of total ammonium nitrogen (TAN = NH_3 + NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), pH, total suspended solids (TSS) and volatile suspended solids (VSS) were carried out according to the standard methods (APHA-AWWA-WPCF, 2005). Total Organic Carbon (TOC) concentration was determined by a Shimadzu analyser (TOC-L, automatic sample injector

Table 1
Characteristics of the fish canning industrial wastewater fed to the partial nitritation/anammox SBR and operating conditions applied during each operational stages.

Stage (range of days)	TAN ($mg\text{-}N\ L^{-1}$)	TOC ($mg\text{-}C\ L^{-1}$)	NaCl ($g\ L^{-1}$)	HRT (d)	Anoxic/aerobic (min/min)	Air on/off (min/min)
A-I (0 - 42)	136 ± 14	48.3 ± 9.4	1.75 ± 0.26	1.41 ± 0.04	0/340	340/0
A-II (43 - 94)	161 ± 25	52.6 ± 9.2	2.38–6.62	1.37 ± 0.04	0/340	2/3 4/2 9/1
A-III (95 - 139)	196 ± 31	29.8 ± 5.8	6.08–10.30	1.35 ± 0.01	60/280	280/0
A-IV (140 - 175)	215 ± 22	36.9 ± 7.1	10.33–18.33	2.57 ± 0.07	60/280	15/45
B (0 - 40)	157 ± 20	35.5 ± 7.4	17.44 ± 1.08	2.01 ± 0.06	0/340	40/20

TAN: total ammonium nitrogen; TOC: total organic carbon.

Shimazdu ASI-L) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC) concentrations. Cation and anion concentrations were determined by ion chromatography with an Advanced Compact IC system (861, Metrohm). The SAA values were determined by batch assays in vials with a total volume of 38 mL and a liquid volume of 25 mL according to the methodology described by Dapena-Mora et al. (2007).

2.5. Calculations

Free nitrous acid (FNA) and free ammonia (FA) concentrations were calculated according to Anthonisen et al. (1976) from the nitrite and ammonium concentrations, respectively. The operational temperature and the pH values measured in the bulk liquid were considered.

Ammonia and nitrite oxidation rates (AOR and NOR, respectively) as well as nitrogen removal rate by the anammox process (AR) were estimated based on nitrogen balances and the anammox process stoichiometry as g-N L^{-1} d^{-1}, according to Morales et al. (2016). The reactor specific removal rates (AOR/X, NOR/X and AR/X) were determined dividing the oxidation rates by the biomass concentration inside the reactor (X, g-VSS L^{-1}).

The maximum total nitrogen removal percentage by a possible heterotrophic denitrification process ($\%TN_{den}$) was determined based on organic matter balances and according to equation (1).

$$\%TN_{den} = \frac{\left(TOC_{\inf} - TOC_{eff}\right)\cdot 0.933}{TN_{\inf}}\cdot 100 \tag{1}$$

Where TOC_{inf} and TOC_{eff} are the concentrations of total organic carbon (as mg-C L^{-1}) in the influent and in the effluent, respectively; TN_{inf} is the total nitrogen concentration present in the feeding (as mg-N L^{-1}); and 0.933 is the stoichiometric coefficient (as g-NO_3^--N g-C^{-1}) that relates nitrate and organic carbon consumption in the denitrification process, considering acetic acid as a source of carbon. The acetic acid was selected as source of carbon because the measured soluble chemical oxygen demand and TOC ratio (COD_S/TOC) in the feeding was close to it value (average of 1.9 ± 0.5 with maximum value of 2.6 g g^{-1}). Note that part of the organic matter could be consumed aerobically due to the presence of oxygen and/or used for biomass growth, which will decrease the maximum denitrification capacity. Therefore, equation (1) considers only the “maximum potential” value for the heterotrophic denitrification, not the actual value.

The inhibitory effect of NaCl on the anammox activity was expressed as a percentage of activity maintained and calculated according to equation (2):

$$\%SAA = \frac{SAA}{SAA_0}\cdot 100 \tag{2}$$

Where SAA_0 and SAA are the specific anammox activities measured for the inoculum (without salt addition) and with the presence of variable NaCl concentrations (0, 5, 10, 15, 20, 25 and 30 g-NaCl L^{-1}), respectively.

3. Results and discussion

3.1. Partial nitritation-anammox processes performance with progressive NaCl concentration increase

In the first experiment (A) the wastewater collected in the fish cannery was characterized by a progressive and significant increase in the salt concentration, from 1.75 ± 0.26 to 17.40 ± 0.64 g-NaCl L^{-1}, while the nitrogen concentration as ammonium slightly increased from 136 ± 14 to 215 ± 22 mg-TAN L^{-1}. Thus, the fluctuations of the output parameters should be attributable mainly to the NaCl effect on the combined processes (Fig. 1).

In Stage A-I the high specific anammox activity of the inoculated biomass, of 0.562 ± 0.078 g-N g VSS^{-1} d^{-1}, allowed a quick response of the system to the new conditions. During the first 20 d of operation the achieved total ammonium nitrogen removal efficiency (TAR) and total nitrogen removal efficiency (TNR) were approximately 100% and 80%, respectively. In this stage, the aeration was continuously supplied and the dissolved oxygen (DO) concentration was regulated between 0.5 and 1.5 mg-O_2 L^{-1}. However, due to biofouling problems with the oxygen probe which caused underestimated measurements, higher levels of DO concentration may in fact have been achieved during this first stage. Consequently, the nitrate concentration in the effluent was higher than the expected value according to the anammox stoichiometry. In this way, the ratio of (NO_3^--N)-produced to TAN-consumed was 0.30 ± 0.10 g-N g-N^{-1}, which revealed the presence of nitrite oxidizing bacteria (NOB) activity. The TAR was maintained approximately at 100%, but the TNR decreased from 83% on day 11 to 49% on day 37 (Table 2).

From day 43 onwards, an intermittent aeration strategy was used in order to limit the NOB activity (Stage A-II). Different rates of air pulses (on/off) were applied between days 43 and 94 (Table 1). As a consequence, the TAR decreased to an average value of 77% in this stage (Table 2). The total ammonium concentration in the effluent increased from negligible values to concentrations as high as 75 mg-TAN L^{-1} (Fig. 1). However, the TNR was improved from 45% on day 44 to 70% on day 70. This improvement was due to the NOB activity limitation observed by the decrease of the nitrate concentration in the effluent. From the analysis of the results obtained from the monitoring of the operational cycle of day 74 (Fig. 2a) the nitrite concentration inside the reactor was not significant and low nitrate accumulation was measured. These results indicated the good performance of the partial nitritation/anammox processes. However, at the end of Stage A-II the nitrite and TAN started to accumulate, reaching values as high as 50 mg-NO_2^--N L^{-1} and 86.9 mg-TAN L^{-1}, respectively, in the effluent. The nitrite

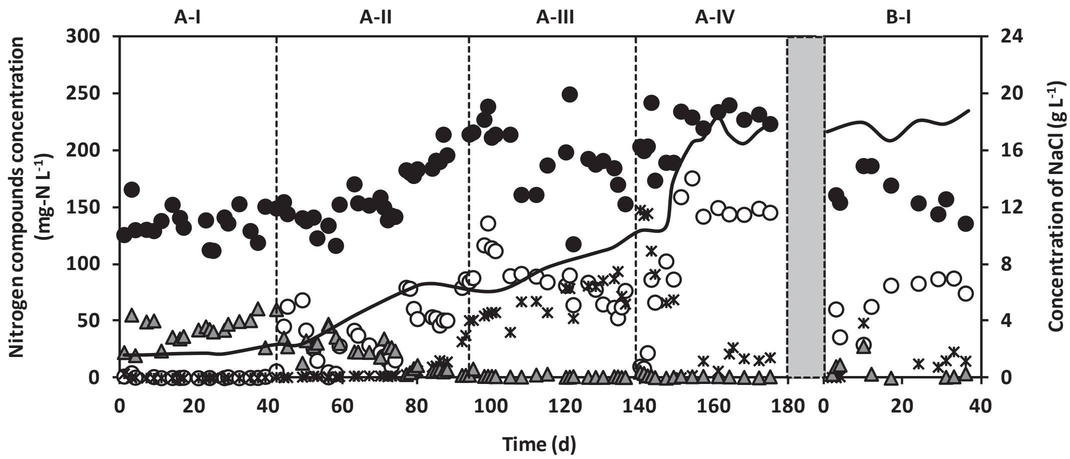

Fig. 1. Profiles of salt and nitrogen compounds concentrations in the different operational stages: NaCl (–), TAN in the influent (●) and TAN (○), NO_2^--N (∗) and NO_3^--N (▲) in the effluent.

Table 2
Operational conditions in the different operational stages with increasing salt concentration.

Operation periods (d)	NaCl ($g\ L^{-1}$)	NRR ($g\text{-}N\ L^{-1}\ d^{-1}$)	TAR (%)	TNR (%)	TN_{den} (%)	FA ($mg\text{-}N\ L^{-1}$)	FNA ($mg\text{-}N\ L^{-1}$)
A-I (0-42)	1.75 ± 0.26	0.067 ± 0.013	99.4 ± 1.0	68.3 ± 9.6	16.7 ± 5.0	$<10^{-4}$	$<10^{-4}$
A-II (43-94)	2.38–6.62	0.197 ± 0.022	76.9 ± 12.3	61.6 ± 7.2	13.0 ± 4.7	0.83–16.80	$<10^{-3}$
A-III (95-139)	6.08–10.30	0.031 ± 0.011	55.0 ± 8.7	19.7 ± 11.0	4.8 ± 1.1	2.66–23.55	<0.01
A-IV (140-175)	10.33–18.33	0.021 ± 0.007	37.7 ± 8.6	26.3 ± 7.4	4.6 ± 2.0	27.86 ± 9.08	<0.01
B (0-40)	17.44 ± 1.08	0.029 ± 0.017	64.1 ± 16.4	46.3 ± 17.1	8.1 ± 2.4	4.86–36.11	$<10^{-3}$

NRR: Nitrogen removal rate; TAR: total ammonium nitrogen removal efficiency; TNR: nitrogen removal efficiency; TN_{den}: total nitrogen denitrified; FA: free ammonia; FNA: free nitrous acid.

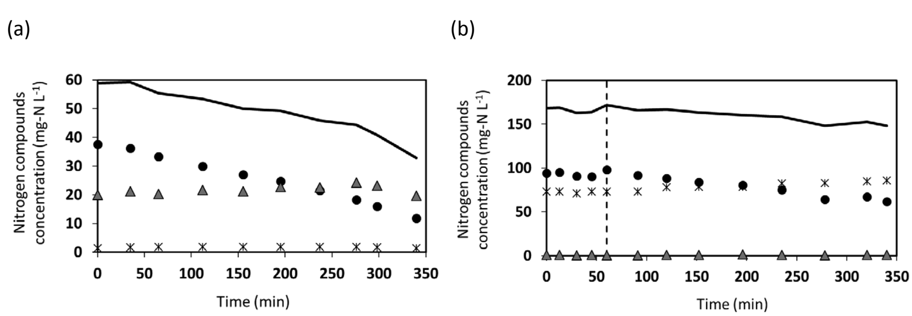

Fig. 2. Evolution of nitrogen compounds concentrations inside the reactor: TAN (●), NO_2^--N (∗), NO_3^--N (▲) and TN (–), for an operational cycle: (a) Stage A-II, day 74 and (b) Stage A-III, day 130.

accumulation inside the SBR was probably due to the combined effect of a decrease in NOB and anammox activities provoked by the increase in NaCl to values of 6–7 g-NaCl L^{-1}. Although the estimated FA concentration also increased to values as high as 16.8 mg-N L^{-1} (Table 2), this concentration was not expected to be inhibitory for granular anammox biomass (Jin et al., 2012), since no effects have been previously detected for FA concentrations up to 20 mg-N L^{-1} (Fernández et al., 2012). The FNA concentration in this stage was lower than 10^{-4} mg-N L^{-1}, thus neither the FNA inhibition effect on anammox (Jin et al., 2012) nor that on the NOB population (Pedrouso et al., 2017) was considered.

In order to stop the possible inhibition of anammox bacteria activity by salt and substrates (NH_4^+ and NO_2^-) concentrations, in Stage A-III an anoxic phase of 60 min was implemented at the beginning of the reaction phase. In some cases, sequential oxic/anoxic phases have been implemented in partial nitritation-anammox reactors to separate the two reactions in time. Then, the nitritation occurred during the oxic phase while the anammox reaction took place during the anoxic one (Joss et al., 2009; Abbassi et al., 2014). Taking this into consideration, in the present study the aeration was stopped for 1/6 of the cycle length. The implemented anoxic phase aimed at shortening the reaction time available for aerobic ammonium oxidation and decreasing the nitrite accumulation, while promoting the anammox reaction. Following this strategy, the nitrite concentration inside the reactor diminished. Simultaneously, the decreasing tendency of the TNR stopped and was maintained at approximately 20% during this stage. However, the anammox bacteria were not able to consume the nitrite left from the previous cycle, as indicated by the nitrogen compounds profiles corresponding to the operational cycle measured on day 130 (Fig. 2b). This observation correlates with the low value of the SAA, 0.033 ± 0.025 g-N g-VSS^{-1} d^{-1}, of the biomass inside the reactor on this date, which confirms the 94% decrease in activity in comparison with the inoculum.

As FA concentration started to reach inhibitory values at the end of Stage A-III (up to 23.55 mg L^{-1}), the HRT was doubled to 2.57 d for the next stage, in order to avoid substrate inhibition. Also, intermittent aeration was applied for the next step to promote the nitrite consumption during anoxic steps by anammox reaction.

In Stage A-IV, the combined strategies applied promoted a decrease in the nitrite but an increase in ammonium concentrations in the effluent (Fig. 1). In this case the ammonium oxidizing bacteria (AOB) activity limited the process. As the nitrite concentration was negligible, the TNR increased from values of 20% up to 30%. However, the high NaCl (up to 18.33 g-NaCl L^{-1}) and FA (up to 38 mg-N L^{-1}) concentrations reached in this stage were inhibitory for anammox bacteria. Since a higher HRT could decrease FA concentration but not the NaCl concentration, the decision to stabilize the reactor under the applied operational parameters was taken. FNA concentration was lower than 0.01 mg-N L^{-1} at this stage (Table 2), with maximum values of 0.009 mg-N L^{-1}. Although no inhibition has been reported for anammox biomass at higher FNA values (Jin et al., 2012), Fernández et al. (2012) suggest avoiding concentrations higher than 0.0005 mg-N L^{-1} to maintain a stable anammox operation since inhibitory effects are stronger in long-term experiments. For this reason, the SAA measured at the end of the operation was again 0.035 ± 0.004 g-N g-VSS^{-1} d^{-1}. On the other hand, NOB activity inhibition has been reported for FNA concentrations higher than 0.02 mg-N L^{-1} (Pedrouso et al., 2017). Thus, in the present study, no inhibitory effect was expected on NOB populations at any time during the operation, the NOB decay being attributable mainly to the salt effect.

In fact, the analysis of reactor specific removal rates obtained for each bacterial population at the different salt concentrations tested revealed that the most affected parameter was the NOR/X, associated with NOB activity, suffering a total inhibition at concentrations higher than 4 g-NaCl L^{-1} (Fig. 3). On the other hand, the AOR/X remained stable throughout the operational period up to concentrations of 9.02 g-NaCl L^{-1}. Meanwhile, the decrement of the nitrogen removal in the system was finally due to the decay of the specific nitrogen removal rate (AR/X) associated with the anammox activity decrease, which took place at concentrations over 7.15 g-NaCl L^{-1}.

To the knowledge of the authors the present research seems to be the only experience in which partial nitritation/anammox processes are applied to the treatment of industrial saline wastewater. Although, Dapena-Mora et al. (2006) and Vazquez-Padin et al. (2009) worked with similar substrates, they contained lower salt concentrations than in the present case and operated with a 2-steps SHARON-Anammox process instead of a single partial nitritation-anammox reactor (Table 3). High nitrogen removal rates (NRR) were obtained in both cases due to the high nitrogen loading rates (NLRs) applied to the system, and they also achieved high TNR, probably due to the lower salt concentrations present. The difficulty with obtaining a SHARON effluent with the needed stoichiometric ratio of nitrite/ammonia for the anammox reactor was the main cause of instability and the failure of that process where nitrite accumulation at ratios greater than 1.0–1.3 took place (Dapena-Mora et al., 2007; Vazquez-Padin et al., 2009). The other reported studies performed appropriately (Windey et al., 2005; Malovanyy et al., 2015; Wang et al., 2017) and allowed the achievement of larger NRRs due to the greater NLRs applied, even the highest NRR of 609 mg-N L^{-1} d^{-1} (84% of TNR) was reached at 30 g-NaCl L^{-1} after 172 days of progressive salt increase/acclimation (Windey et al., 2005). These high removal percentages are possible mainly due to the utilization of synthetic substrates, which make it possible to work without the inlet fluctuations of the industrial effluents. Furthermore, the industrial effluents also contain other compounds such as sulfates, phosphates, remains of COD that can interfere with the nitrogen removal process.

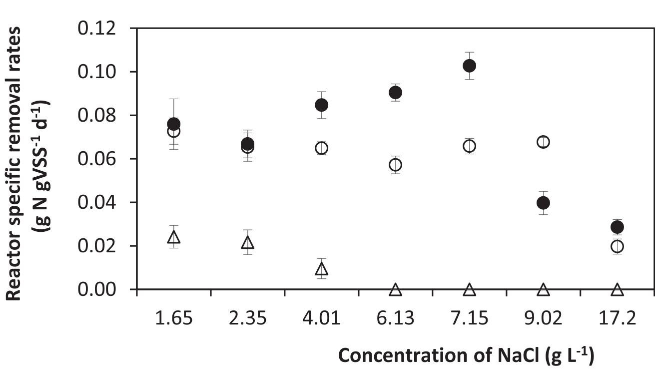

Fig. 3. Reactor specific removal rates for anammox (AR/X) (●), ammonia oxidizing (AOR/X) (○) and nitrite oxidizing (NOR/X) (△) bacteria obtained at different NaCl concentrations during the SBR operation. Specific rates were calculated by dividing removal rates by the biomass concentration inside the reactor (X, g-VSS L^{-1}).

Different causes for process instability have been proposed in these studies. Some of them reported ammonia accumulation and others nitrite accumulation, indicating AOR or AR decrease, respectively. Both phenomena have an explanation based on the degree of acclimation to salt of the biomass in the reactor. In this respect, the community composition (since there are species more tolerant to high salt levels (Gonzalez-Silva et al., 2017; Wang et al., 2017)) and the biomass story (since an adapted biomass increases its resistance to high salt concentrations) are relevant factors (Kartal et al., 2006; Dapena-Mora et al., 2010; Zhang et al., 2010).

Several authors have reported salt inhibition on nitrification (Campos et al., 2002; Moussa et al., 2006; Dapena-Mora et al., 2007) accompanied by nitrite accumulation since the NOB population is more sensitive to NaCl than AOB (Dinçer and Kargi, 2001; Jin et al., 2007). This strategy for NOB inhibition could be useful in partial nitritation/anammox systems, especially when they operate at low nitrogen concentration and temperature, since NOB suppression by salt can improve NRR in these systems (Liu et al., 2008). On the other hand, salt acclimation has been reported for total nitrifying (AOB and NOB) biomass (Panswad and Anan, 1999; Campos et al., 2002), showing higher specific activities for biomass acclimated to high salt levels (13.7–18 g-NaCl L^{-1}) than the non-exposed one. She et al. (2016), also achieved highly efficient nitritation reaction in a nitritation-denitritation reactor after a long-term acclimation period with increasing salt concentration, obtaining high nitrite accumulation percentages (>92%) at concentrations up to 37.7 g-NaCl L^{-1}.

Also, many studies have been focused on determining the anammox tolerance and adaptability to high NaCl concentrations. Dapena-Mora et al. (2007) reported an IC_{50} value of 13.4 g-NaCl L^{-1} for anammox cultures and no inhibitory effect for concentrations below 8.77 g-NaCl L^{-1}, measured in batch activity tests. Nevertheless, good performances of anammox reactors with high salt concentrations using synthetic substrates have been obtained with previous acclimation strategies. Dapena-Mora et al. (2010), measured high SAA of adapted biomass with salt concentrations up to 15 g-NaCl L^{-1} compared to experiments without salt addition. These authors achieved a TNR of 99% and an NRR of 0.32 g-NO_2^--N L^{-1} d^{-1} with 15 g-NaCl L^{-1}. Also, Gonzalez-Silva et al. (2017), Jin et al. (2011), Wei et al. (2016) and Yang et al. (2011) obtained stable anammox reactor performances at 30 g-NaCl L^{-1} by applying long previous acclimation periods, which remained even up to 50 g-NaCl L^{-1} with 85% of TNR, working with marine anammox cultures (Wei et al., 2016). On the other hand, Ma et al. (2012),

Table 3
Different autotrophic nitrogen removal processes with high salt content reported in the literature.

Process	Substrate	Operation conditions	NLR (mg-$N\ L^{-1}\ d^{-1}$)	OLR (mg-$TOC\ L^{-1}\ d^{-1}$)	NaCl ($g\ L^{-1}$)	TNR (%)	$NRR_{máx}$ (mg-$N\ L^{-1}\ d^{-1}$)	Cause of instability	Reference
SHARON-Anammox	Anaerobic digested fish canning effluents	SBR (Anammox reactor), 3 L, HRT = 1.8 d, 35 °C	300–700	28–67	10	90	460	Nitrite/ammonia ratios larger than 1.0 g N/g N	(Dapena-Mora et al., 2007)
SHARON-Anammox	Anaerobic digested fish canning effluents	SBR (Anammox reactor), 3 L, HRT = 1.8 d, 35 °C	340–670	0–555	5.9	80	300 (average)	High OLR produced nitrite accumulation	(Vazquez-Padin et al., 2009)
Partial nitritation/Anammox	Anaerobic digestion reject water supplemented with NaCl	MBBR reactor, 10 L, HRT = 2.27–4.67 d, 26–27 °C	120–300	na	10	59	78	Ammonia and nitrite accumulation	(Malovanyy et al., 2015)
CANON	Synthetic	SBR, 2 L, HRT = 6 h, 33 °C	800	0	10 15 20	29 24 -	230 190 -	Ammonia accumulation (AOR decreased)	(Wang et al., 2017)
OLAND	Synthetic	RBC reactor, 50 L, HRT = 0.77–0.91 d	525–1100	0	30	84	609	Ammonia accumulation	(Windey et al., 2005)
Partial nitritation/Anammox	Anaerobic digested seafood canning effluents	SBR, 25 L, HRT = 1.35–2.57	78–145	21.5–38.4	18	46	29	Ammonia and nitrite accumulation	This study

na: not available; OLR: organic loading rate; NLR: nitrogen loading rate; RBC: rotating biological contactor; MBBR: moving bed biofilm reactor.

observed decreasing TNR testing NaCl shocks in an anammox reactor for a range of concentrations between 5 and 60 g-NaCl L^{-1}, diminishing with increasing saline concentrations.

Therefore, from these studies it can be concluded that long acclimation periods seem to be the key factor for a stable nitrogen removal process based on anammox and nitrifying microorganisms, in order to select the microbes with high tolerance to salt (Gonzalez-Silva et al., 2017; Wang et al., 2017). In the present research work, the first experiment regarding the performance of partial nitritation/anammox processes for the treatment of fish canning effluents with fluctuating salt concentrations is reported. Stable conditions were established, which made it possible to achieve a TNR of 30% at 18 g-NaCl L^{-1} and strongly inhibited the anammox biomass, which was only 6% of the initial value measured for the anammox biomass used as inoculum.

Besides the salt, the wastewater also contains soluble organic matter at very low concentrations (between 36 and 53 mg-C L^{-1} as TOC, Table 1). Furthermore, the balance between the TOC in the influent and the effluent indicated that the removal efficiency was lower than 50% for the whole operational period, showing a low content of biodegradable organic matter in the influent. Considering a conversion factor of 2.7 g g^{-1} between TOC and COD, the $COD_{biodegradable}/N$ ratio was <0.5 during the whole operational period. Therefore, no significant negative effects on the anammox biomass or the AOB are expected (Mosquera-Corral et al., 2005; Tang et al., 2013). The consumption of small fractions of organic matter in a partial nitritation/anammox one-stage process can take place in aerobic or anaerobic conditions. In the latter case, the organic matter can be used by heterotrophic bacteria during denitrification to consume the produced nitrite or nitrate. Therefore its removal might indicate the presence of active denitrifying bacteria in the system (Wang et al., 2010; Daverey et al., 2012). Although DO levels lower than 0.5 mg-O_2 L^{-1} allow for the simultaneous nitrification and denitrification processes to take place (Hocaoglu et al., 2011), continuous aeration causes a portion of the organic matter to be oxidized by oxygen instead of nitrate. This extra oxygen consumption increases the operational costs of the process. In the present study, the maximum TN removal by a possible denitrification process based on the TOC consumption was determined to be around 15% in Stages A-I and A-II and 5% in Stages A-III and A-IV (Table 2). Furthermore, to determine the contribution of denitrification to the process, a completely anoxic cycle was performed on day 35 (data not shown). In this case only 4 mg-NO_3^--N L^{-1} were reduced at the end of the cycle, while the TOC concentration varied slightly. This experiment confirmed that the denitrification contribution to the total nitrogen removal was negligible. Thus, an organic removal rate of between 4 and 30 mg-TOC L^{-1} d^{-1} was obtained, oxidized mainly by the aerobic route.

3.2. A partial nitritation-anammox process at high NaCl concentration without acclimation

The second experiment (B) lasted 40 days and the industrial wastewater fed had a salt concentration of 17.44 ± 1.08 g-NaCl L^{-1}. The partial nitration/anammox biomass inoculated was directly exposed to this salt concentration without previous acclimation. The NRR was 0.029 ± 0.017 g-N L^{-1} d^{-1} (Table 2). This value is comparable to the NRR in the previous experiment in Stage A-III at moderate salt concentration (6 - 10 g-NaCl L^{-1}), but with progressive acclimation of the biomass for more than 100 days. These preliminary results indicate that, although the anammox bacteria are very sensitive to salt, the acclimation period is not essential and it is therefore feasible to speed up the start-up process for industrial application purposes. In order to clarify this observation, the SAA values obtained for the biomass of this experiment B were

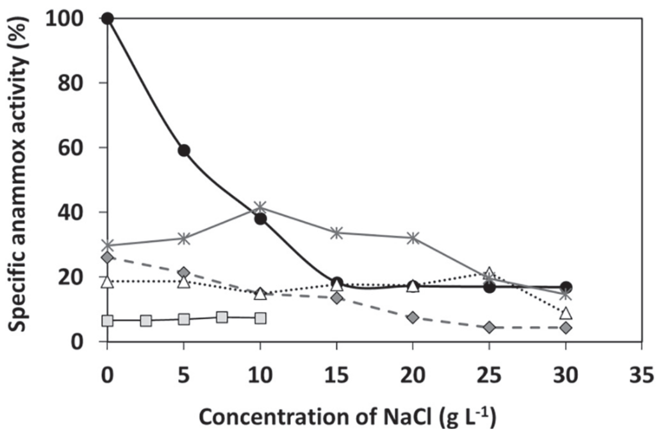

Fig. 4. Percentage of remaining SAA in the assays with the salt concentration for the SBR biomass on different operational days: inoculum (●), Stage A-IV on day 142 (■), Stage-B on days 11 (◆), 31 (△) and 39 (✻). The values correspond to average values of the experiments performed in triplicate (standard deviation lower than 10%).

compared with those from the previous one and are discussed in the following section.

3.3. *Effects of NaCl concentration and acclimation on the specific anammox activity (SAA)*

The SAA, using a medium supplied with different NaCl concentrations, was evaluated for the biomass used as an inoculum and for the biomass of the first experiment (A) on day 142 and of the second experiment (B) on days 11, 32 and 39 (Fig. 4). The anammox activity of the inoculum was strongly inhibited at concentrations of 15 g-NaCl L^{-1}; between 15 and 30 g-NaCl L^{-1} the percentage of SAA remaining being approximately 17% with respect to the initial values. After 142 days of continuous operation with increasing salt concentrations (experiment A) the percentage of SAA remaining was only 7% at all the NaCl concentrations tested. However, this low SAA cannot be attributed only to the effect of high NaCl concentrations, as the use of industrial wastewater contains different compounds at different concentrations which might also affect the correct performance of the process, as mentioned before.

The start-up periods of a partial nitritation-anammox reactor located in an industrial facility are prolonged due to the acclimation procedure needed by the biomass to reach maximum capacity, which in many cases requires the use of dilution water to adjust the applied loads. Furthermore, the possible loss of anammox activity in the acclimation period must be considered too. Thus, in practice the acclimation period in full scale systems should be shortened to attain stable conditions as soon as possible. For this reason, in the case of the fish canning industry, which produces high saline effluents, the possibility of applying the partial nitritation/anammox process without previous acclimation is of interest. Considering this aspect, experience B was performed to understand the direct effect of high saline industrial wastewater (18 g-NaCl L^{-1}) on the SAA of the inoculated biomass. The results showed that a start-up of the partial nitritation/anammox process directly fed with high saline industrial wastewater is possible. After 11 days of continuous operation at 18 g-NaCl L^{-1} the SAA values worsened in comparison with those of the inoculum at almost all the NaCl concentrations tested (Fig. 4). However, on day 31 the SAA values were similar to those on day 11, and a slight improvement between 15 and 25 g-NaCl L^{-1} was even observed. The results obtained on day 39 for the SAA values showed a clear recovery of anammox activity in comparison with day 11. Furthermore, the activity of the biomass at 15 and 20 g-NaCl L^{-1} improved in relation to that of the inoculum. To date, long acclimation periods with small salt concentration increments have been successfully tested in partial nitritation-anammox reactors (Windey et al., 2005; Malovanyy et al., 2015; Wang et al., 2017), but no comparison with direct biomass exposure to salt has been made. In this research better results were obtained with direct exposure with regard to nitrogen removal efficiencies obtained, lower anammox inhibition and faster recovery from the initial salt shock.

4. Conclusions

The removal of nitrogen from fish canning effluents with high salt content was accomplished in a partial nitritation/anammox reactor.

Percentages of 61.6 ± 7.2% of nitrogen removal efficiencies were obtained with effluents containing concentrations of up to 6.6 g-NaCl L^{-1}; nitrite accumulation and the instability of the process were observed at higher salt levels.

Stable conditions were reached in the partial nitritation-anammox SBR after 154 days of progressive increase in salt concentration up to 18 g-NaCl L^{-1}. In these conditions, the nitrogen removal efficiency was 30% (0.021 ± 0.007 g-N L^{-1} d^{-1}) which corresponded to a remaining SAA of 6% compared to that of the inoculum.

Intermittent aeration was more effective than continuous mode, allowing decreasing NOB activity when low salt concentrations were present (approximately 2–6 g-NaCl L^{-1}), and it stabilized the process performance in the other assayed conditions.

Better results were obtained without progressive acclimation to salt than with acclimated biomass. The nitrogen removal rate obtained after 40 days of inoculation with non-adapted biomass was 0.029 ± 0.017 g-N L^{-1} d^{-1} (46.3% of efficiency) at 17.44 ± 1.08 g-NaCl L^{-1}. Furthermore, the specific anammox activities of the biomass were higher after 39 days of operation with salt concentrations of 15 and 20 g L^{-1}.

These results confirm that the acclimation of anammox biomass to high salt concentrations can be done without applying long start-up periods where the salt concentration is progressively increased. In this way, the time required for the start-up of industrial reactors might be significantly shortened, although this strategy is only possible if there is enough inoculum available to couple with the initial strong decrease in anammox activity. Further experiments with industrial saline wastewaters are needed to achieve stable operation under fluctuating salt concentrations, which are very common in these kinds of effluents.

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