



## Research article

# Performance and microbial features of the partial nitrification-anammox process treating fish canning wastewater with variable salt concentrations

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## ABSTRACT

The partial nitrification-anammox (PN-AMX) process applied to wastewaters with high NaCl concentration was studied until now using simulated media, without considering the effect of organic matter concentration and the shift in microbial populations. This research work presents results on the application of this process to the treatment of saline industrial wastewater. Obtained results indicated that the PN-AMX process has the capability to recover its initial activity after a sudden/acute salt inhibition event (up to 16 g NaCl/L). With a progressive salt concentration increase for 150 days, the PN-AMX process was able to remove the 80% of the nitrogen at 7–9 g NaCl/L. The microbiological data indicated that NaCl and ammonia concentrations and temperature are important factors shaping PN-AMX communities. Thus, the NOB abundance (*Nitrospira*) decreases with the increase of the salt concentration, while heterotrophic denitrifiers are able to outcompete anammox after a peak of organic matter in the feeding.

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## 1. Introduction

The fish canning industry represents a very important economic sector in the Northwest of Spain (Galicia), with more than 65 factories, located mainly in coastal areas. As a consequence of this industrial activity large wastewater volumes are produced, which contain high solids, organic matter and nitrogen concentrations. Therefore, these effluents need to be efficiently handled previous discharge to avoid pressures over the marine environment. These effluents are normally treated by means of anaerobic treatment technologies, with the main purpose of reducing the chemical oxygen demand (COD) concentration of the wastewater and produce energy as biogas. Nevertheless, the effluents coming from the

anaerobic digester (AD) contain high concentrations of nitrogen, mainly from proteins, which has to be removed in a subsequent step to adjust to disposal limits. Conventionally, the combined nitrification-denitrification process is applied to the removal of nitrogen. This process requires much energy to create aerobic conditions for bacterial nitrification, as well as the availability of organic carbon to remove nitrate by heterotrophic denitrifying organisms (Kartal et al., 2010). If a complete autotrophic process is applied the organic matter can be saved to produce more energy in the AD.

For this reason, most of recent research efforts have been focused on other alternatives for nitrogen removal, such as the combination of partial nitrification and anammox (PN-AMX) processes. Its application is suitable for wastewater streams with high nitrogen but low organic matter concentrations, like the supernatant from anaerobic digesters (Lackner et al., 2014). In the PN-AMX process, half of the ammonium present is oxidized following the nitrification pathway, combined with the subsequent biological reaction of the produced nitrite and the remaining ammonium to produce nitrogen gas according to the anammox reaction. However,

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the presence of certain inhibitory compounds in the wastewater may hinder the application of the anammox based processes at industrial scale (Jin et al., 2012). For example, anammox bacteria are very sensitive to environmental parameters such as salinity (Scaglione et al., 2017), that is one of the main components of the fish canning effluents (Cristóvão et al., 2016).

The occurrence of high saline concentrations in fish canning effluents, which is mainly caused by a high NaCl concentration, may induce salt stress to the microorganisms involved in the biological treatments, with the subsequent inhibition of many enzymes, decrease in the cell activity and eventually plasmolysis (Jin et al., 2012). The salt content is strongly related to the seasonality in the fish canning industry, which may generate sudden saline shock loads or unexpected variations in the salt concentration depending on the changes in the raw material processed in the factory, with concentration that varies from 2 to 35 g NaCl/L (Cristóvão et al., 2016).

Although, there are several research works that report on the effect of salt concentrations on the anammox bacteria activity, most of them show results about batch activity tests and/or the continuous operation of only the anammox process fed with a synthetic medium (Scaglione et al., 2017). Windey et al. (2005) performed the first research work with the PN-AMX process at long term operation increasing the NaCl concentration, although they used a synthetic medium. More recently, Malovany et al. (2015) tackled the adaptation of one-step PN-AMX biomass to increasing salt concentrations from 0 to 10–15 g NaCl/L in 160 days. In their study, the feeding consisted in reject water from an anaerobic sludge digester, while the salinity as NaCl was synthetically added.

As the inoculum available to start-up a PN-AMX process is normally not adapted to salinity, an important aspect to consider is the possible shift in the microbial populations. Despite the advancements of next generation sequencing platforms in microbial ecology, the previous studies about the influence of salinity in the PN-AMX process had paid little attention to this aspect. Moreover, only few studies have analysed the PN-AMX reactor communities using this cutting-edge technology (Agrawal et al., 2017; Wang et al., 2017b). For example, Wang et al. (2017b) studied the microbiological shift in a PN-AMX process with the progressive increase in the salt concentration from 0 to 20 g NaCl/L. However, their research work was performed with a synthetic medium ignoring the microorganisms that can be present in industrial effluents and the perturbations in composition of wastewaters, such as changes in organic matter compounds.

In the present research work the combined PN-AMX process is applied for the treatment of industrial saline wastewater produced in a fish cannery. The effect of salt concentrations on the performance of a PN-AMX granular sludge reactor was evaluated as: (1)

sudden salt shock loads, up to 16 g NaCl/L, and (2) progressive adaptation to concentrations up to 10 g NaCl/L. The evolution of the main microbial populations present in the biomass from the reactor was determined to prove the effects of salinity and organic matter concentration on the PN-AMX performance.

## 2. Materials and methods

### 2.1. Experimental set-up

A laboratory sequencing batch reactor (SBR) with a working volume of 1.5 L was used for the experiments. The aeration system consisted in a diaphragm pump (Laboport N86, KNF) for the air supply and an air diffuser located at the bottom of the reactor. This system provided good mixture inside the reactor and the dissolved oxygen (DO) concentration necessary for the partial nitrification process. The DO concentration was periodically measured with a DO probe (Hach Lange, model HQ40d Portable Meter) and was manually regulated by changing the closing degree of an air valve located in the gas inlet conduction.

The operational cycles were of 180 min and distributed as follows: 5 min of batch feeding, 160 min of aeration, 10 min of settling and 5 min of effluent withdrawal. The hydraulic retention time (HRT) varied between 0.9 and 1.3 days for all the operational period (Table 1). The industrial wastewater was periodically collected (every 1–2 months) after the AD in operation in a fish canning industry (Catoira, Pontevedra) and stored at 4 °C. The industrial wastewater was characterized by a high variability in its composition, especially in terms of NaCl and organic matter concentrations (Table 1). The fluctuations on the industrial wastewater composition could not be controlled or predicted, and they were mainly due to changes in the processed product at the industrial facility (tuna, mussels, sardines, etc.), as well as the performance of the anaerobic digester, which worsened when the industrial wastewater contained high salt concentrations.

### 2.2. Operational conditions

Two different experiments were assessed to study the influence of salinity over the PN-AMX process treating industrial wastewater.

The first experiment served to test the performance of the PN-AMX process at moderate salt concentrations and its posterior recovery after a shock of salinity took place. It lasted 150 days and the SBR was operated at laboratory room temperature ( $24 \pm 2$  °C). The operation was divided in three stages according to the different salt concentration of the fed industrial wastewater (Table 1). The wastewater produced by the industry in this period was characterized by a moderate salt concentration (between 1.7 and 4.3 g

**Table 1**

Operational conditions and characteristics of the wastewater fed to the reactor in the different operational stages.

	First experiment			Second experiment		
	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Days	0–73	74–127	128–155	0–64	65–220	221–445
Temperature (°C)	24.0 ± 0.7	23.7 ± 1.1	25.6 ± 1.3	29.3 ± 0.9	30.3 ± 1.5	31.0 ± 2.1
HRT (d)	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
DO (mg O <sub>2</sub> /L)	2.7 ± 0.5	3.1 ± 1.1	4.0 ± 0.3	1.4 ± 0.5	1.1 ± 0.4	0.5–3.5
NaCl (g/L)	3.5 ± 0.2 (15.4 ± 0.8 <sup>a</sup> )	1.7 ± 0.3	4.3 ± 0.4	3.4–12.2	2.8–8.3	8.6 ± 0.9
NH <sub>4</sub> <sup>+</sup> (mg N/L)	291 ± 51	206 ± 22	216 ± 15	262 ± 29	250 ± 44	220 ± 35
TOC (mg C/L)	54 ± 10	47 ± 10	49 ± 7	50 ± 9	50 ± 9	45–200 <sup>b</sup>
IC (mg C/L)	345 ± 30	285 ± 35	349 ± 16	324 ± 38	299 ± 33	350 ± 63
PO <sub>4</sub> <sup>3-</sup> (mg/L)	124 ± 16	97 ± 21	48 ± 12	17–85	96 ± 14	13–704
SO <sub>4</sub> <sup>2-</sup> (mg/L)	82 ± 45	76 ± 18	156 ± 46	89 ± 46	111 ± 27	0–284
pH	7.8 ± 0.1	7.6 ± 0.1	7.5 ± 0.2	7.6 ± 0.2	7.6 ± 0.1	7.6 ± 0.3

<sup>a</sup> Punctual salt peak in days 32–36.

<sup>b</sup> Total organic carbon (TOC) concentration very variable.

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