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Simultaneous nitritation–denitritation for the treatment of high-strength nitrogen in hypersaline wastewater by aerobic granular sludge

Santo Fabio Corsino*, Marco Capodici, Claudia Morici, Michele Torregrossa, Gaspare Viviani

Dipartimento di Ingegneria Civile, Ambientale, Aerospaziale, dei Materiali, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy

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A B S T R A C T

Fish processing industries produce wastewater containing high amounts of salt, organic matter and nitrogen. Biological treatment of such wastewaters could be problematic due to inhibitory effects exerted by high salinity levels. In detail, high salt concentrations lead to the accumulation of nitrite due to the inhibition of nitrite-oxidizing bacteria. The feasibility of performing simultaneous nitritation and denitritation in the treatment of fish canning wastewater by aerobic granular sludge was evaluated, and simultaneous nitritation–denitritation was successfully sustained at salinities up to 50 gNaCl L^{-1}, with a yield of over 90%. The total nitrogen concentration in the effluent was less than 10 mg L^{-1} at salinities up to 50 gNaCl L^{-1}. Nitritation collapsed above 50 gNaCl L^{-1}, and then, the only nitrogen removal mechanism was represented by heterotrophic synthesis. In contrast, organic matter removal was not affected by salinity but was instead affected by the organic loading rate (OLR). Both COD and BOD removal efficiencies were over 90%. The COD fractionation analysis indicated that aerobic granules were able to remove more than 95% of the particulate organic matter. Finally, results obtained in this work noted that aerobic granular sludge had an excellent ability to adapt under adverse environmental conditions.

1. Introduction

Fish processing industries require a huge amount of water throughout all production steps, including fish cleaning, cooling, sanitization and floor washing (Cristovao et al., 2015). Depending on the fish to be processed, large amounts of salt in the form of brine are used for obtaining the finished products. Therefore, wastewaters generated by fish factories are characterized by high salt (mainly sodium chloride) concentrations and extreme variability, depending on the process carried out. Nevertheless, fish canning wastewaters are characterized by high suspended solids (up to 5000 mg L^{-1}), organic matter (COD up to 90,000 mg L^{-1}) and nitrogen concentrations (TN up to 3000 mg L^{-1}), especially during periods of fresh fish processing, due to the presence of blood, entrails, oil, flakes and fish tissues (Chowdhury et al., 2010). Due to the high salinity level, their biological treatment would require the application of halophilic microorganisms, which are able to tolerate high salt concentrations. In contrast, non-halophilic bacteria are not suitable for this purpose because of cell disintegration due to the osmotic pressure difference across the cellular membrane that causes the loss of cellular water (plasmolysis) (Dincer and Kargi, 2001). However, the application of non-halophilic microorganisms is possible because a moderate acclimation of activated sludge to high salinity has been tested (Lefebvre and Moletta, 2006; Di Bella et al., 2015).

Currently, aerobic granular sludge is considered to be one of the most promising biological wastewater treatment technologies, thanks to high settling velocity, high biomass retention, simultaneous removal of carbon and nitrogen and their ability to withstand high organic load (Long et al., 2015). For these unique properties, aerobic granular sludge has attracted increasing interest for industrial wastewater treatment (Zhang et al., 2011; Abdullah et al., 2013). Among these, aerobic granular sludge reactors have been successfully used for treating synthetic saline wastewater. Taheri et al. (2012) obtained stable aerobic granules at 10 gNaCl L^{-1}, whereas Wang et al. (2015) successfully adapted aerobic granular sludge at higher salinity levels of up to 80 gNaCl L^{-1}. The authors

* Corresponding author.
E-mail address: santofabio.corsino@unipa.it (S.F. Corsino).

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found that some microorganisms adapting to high salinity gradually became the predominant bacteria. However, all of these studies noted remarkable issues regarding nitrogen removal. Bassin et al. (2011) stated that ammonia-oxidizing bacteria (AOB) could tolerate salt concentrations of up to 33 gNaCl L^{-1}, and Pronk et al. (2014) observed that nitrite-oxidizing bacteria (NOB) showed complete inhibition at 20 gNaCl L^{-1}. Wan et al. (2014) found that under NaCl concentrations of up to 50 gNaCl L^{-1}, full partial nitrification can be achieved, and the nitrite accumulation rate reached nearly 100%. Consequently, at high salt concentrations, the accumulation of either ammonia or nitrite usually occurs when treating saline wastewater. In any case, these studies reported that when treating synthetic saline wastewater, the total nitrogen removal efficiency was moderate and was rarely higher than 70%. Apart from these studies, few experiences with real saline wastewater have been carried out. Figueroa et al. (2008) treated fish canning effluent characterized by salt contents of up to 30 gNaCl L^{-1}. Although organic carbon was completely depleted, nitrogen removal efficiencies were lower than 40%. As in other cases, the main issue was the insufficient nitrogen removal efficiency. Similar results were obtained by Val del Río et al. (2013), who also observed significant disintegration of granules for organic load rates (OLR) higher than 4.4 kgCOD $m^{-3}d^{-1}$. Because high amounts of nitrite accumulation commonly occur in the treatment of saline wastewater, simultaneous nitritation–denitritation processes could be effectively coupled with granular sludge technology to improve nitrogen removal. The simultaneous nitritation–denitritation process has been successfully applied for the treatment of synthetic wastewater by means of aerobic granular sludge (Lochmatter et al., 2014; Li-long et al., 2014). However, to date, this solution has not been applied for treating real wastewater, so it needs to be investigated. Moreover, as reported by De Kreuk et al. (2010), aerobic granular sludge is able to remove particulate substrates. Because the particulate substrate is an important fraction of the organic material in fish canning wastewater (Jemli et al., 2015), aerobic granular sludge reactors could be a good solution for their biological treatment.

The main goal of this study was to analyse the feasibility of treating fish canning wastewater by using aerobic granular sludge in a sequencing batch airlift reactor (SBAR) (Beun et al., 2002). In detail, the study of nitrogen removal by means of nitritation–denitritation processes at high salinity was tested at various salt concentrations. For this purpose, a 78-day experiment was carried out with the goal of finding the best operating conditions for maximizing reactor performance, evaluated in terms of nitrogen and organic carbon removal, at increasing salt concentrations (from 30 gNaCl L^{-1} up to 75 gNaCl L^{-1}). Special attention has been paid to the maximum salt concentration that allows satisfactory nitrogen removal efficiency to be attained.

2. Materials and methods

2.1. Reactor and experimental set-up

The reactor was operating for 78 days divided into 5 periods characterized by different salt concentrations and organic load rates. The reactor was a column-type (100 cm height) with a working volume of 3.5 L (internal diameter of 8.6 cm) and was characterized by an internal riser 50 cm high with an internal diameter of 5.4 cm. Air was introduced via a fine bubble aerator at the base of the reactor at a flow rate of 3 L min^{-1} so that the hydraulic shear forces were approximately 2.4 cm s^{-1}. The filling height was 70 cm, so the height/diameter ratio was equal to 7. The effluent was discharged by a solenoid valve placed at 35 cm from the base of the reactor. Thus, the volumetric exchange ratio (VER) was 50% for each cycle. The experimentation was divided into five periods, each of which was characterized by a different salt concentration. The raw wastewater (salinity equal to 150 gNaCl L^{-1}) was the same for the whole experiment, and it was diluted with tap water to obtain different salt concentrations in each sub-period. From periods I to IVa, the salt concentrations increased from 30 gNaClL^{-1} (I) to 38 gNaCl L^{-1} (II), 50 gNaCl L^{-1} (III) and 75 gNaCl L^{-1} (IVa). From periods I to IVa, the reactor was operating on a 12 h per cycle, included 45 min of influent feeding, 665 min of aeration, 5 min of settling and 5 min of effluent withdrawal. To reduce the organic load rate in period IVb without changing the influent salinity, the cycle length was extended to 24 h, keeping each phase duration constant excepted for the aeration phase. A Programmable Logic Controller (PLC) automatically handled the SBR cycling operations. Due to the long reaction cycle, organic carbon was almost completely degraded before the end of the cycle. Certainly, this limited the availability of the organic substrate for nitrite reduction. Thus, to supply an electron donor for nitrite reduction, a known amount of water spiked sodium acetate (200 mg L^{-1}) was added as an external carbon source from the second part of period II (from the 10th hour of the cycle) to enhance denitrification without interrupting aeration. Due to the simultaneous presence of dissolved oxygen that was preferentially used by heterotrophic bacteria as an electron acceptor for acetate oxidation instead of nitrite, the acetate dose was increased to 400 mgL^{-1} in period III. The sludge retention time was maintained at 45–50 days by daily purging a known amount of mixed liquor volume.

2.2. Wastewater characterization

The fish canning wastewater was collected from a local industry that produces canned anchovies. The fish canning production process begins with the arrival of fresh fish (fish processing phase). Thus, heads and viscera are removed and the fishes are stored in brine. During this process, water is enriched in blood, oil, flakes and salt. Consequently, wastewaters are characterized by very high organic matter content (COD 80000 mg L^{-1}), high total suspended solids concentrations (4621 mg L^{-1}) and high salinity levels (up to 300 gNaCl L^{-1}). After a period of storage (approximately 1–2 months), fish pass to the canning section in which they are washed, preserved in tins and packaged for distribution. The salt and organic matter contents of the process wastewater remain high but are significantly lower than those of the previous phase (COD = 16,000 mg L^{-1} and NaCl = 25 mg L^{-1}). Due to the extreme variability of wastewater composition, in order to analyse the biological performance of wastewaters having nearly the same characteristics, a large volume of raw sewage was collected at one time during the fish-processing phase and stored at 4 °C for the whole experimental period. The main characteristics of the raw wastewater are reported in Table 1.

Hereafter, to obtain the desired salinity, wastewater was diluted with tap water in accordance with a dilution factor ranging from 2 to 5 (v/v). The duration of each phase was not fixed a priori. The phases were changed when steady state conditions, in terms of nutrient removal efficiency, were reached. Each phase lasted at least 15 days. The organic load rate was not controlled, but it was proportional to the dilution factor applied. The main operating conditions are reported in Table 2.

2.3. Analytical methods

All of the chemical–physical analyses (COD, BOD, NH_4–N, NO_3–N, NO_2–N, TSS, and VSS) were performed according to standard methods (APHA, 2005). Soluble COD was determined after filtration through a 0.45 μm membrane; hence, particulate COD

Table 1
Composition of fish canning wastewater before dilution.

$COD_{(TOT)}$	$COD_{(SOL)}$	$COD_{(PART)}$	TOC	BOD	TN	$N{-}NH_4$	TSS	NaCl	Conductivity
[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[g L^{-1}]	[mS cm^{-1}]
16,984	11,362	5622	2108	7060	1152	288	4621	152	160

Table 2
Summary of the main operating conditions.

Phase	Day	Dilution factor	Salinity (gNaCl L^{-1})	Conductivity (mS cm^{-1})	OLR (kgCOD $m^{-3}d^{-1}$)
I	0–23	5	30	48.2	3.22 ± 0.064
II	24–39	4	38	52.8	4.11 ± 0.108
III	40–53	3	50	64	5.39 ± 0.067
IVa	51–61	2	75	87	8.02 ± 0.082
IVb	62–78	2	75	87	4.00 ± 0.101

resulted from the difference between total and soluble COD. To accurately examine the composition of influent wastewater, COD fractions were respirometrically evaluated during each experimental phase. Nevertheless, COD fractionation analyses were also performed for the effluent wastewater to identify the removal efficiency of each COD fraction under different operating conditions. The COD fractions, which are classified as soluble readily biodegradable (Ss), soluble inert (S_I), biodegradable and rapidly hydrolysable (Xs), particulate inert (X_I) and active biomass (Xa), were evaluated according to Di Trapani et al. (2011). However, with respect to the latter, activated sludge acclimated to salinity was used to perform respirometric tests for COD fractionation.

EPSs extractions were carried out by the Heating Method described by Le-Clech et al. (2006), and carbohydrate and protein concentrations were determined according to the phenol–sulphuric acid method with glucose as the standard (Dubois et al., 1956) and by the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), respectively.

2.4. Inoculum

The aerobic granular sludge was cultivated with a saline acetate-based wastewater for 6 months. The synthetic wastewater was prepared in accordance with that described by Beun et al. (2002). To obtain the desired salt concentration, a known amount of sodium chloride was added. The activated sludge was acclimated to salinity by gradually increasing the salt concentration in the feed up to 25 g NaCl L^{-1}, with increasing steps of 5 mg L^{-1}. Then, this sludge was used as an inoculum for the SBAR to obtain the aerobic granules. Once aerobic granules were stable at 25 gNaCl L^{-1}, the real wastewater was used as feed. Before these granules were inoculated in the SBAR treating fish canning wastewater, they were fed with a mixture of synthetic and fish canning wastewater for a short period (7 days) to favour a gradual adaptation to the new wastewater.

3. Results and discussions

3.1. Aerobic granules features

The main purpose of this work was to evaluate the biological performance of an aerobic granular sludge reactor for treating fish canning wastewater. Although the evolution of the characteristics of the aerobic granules was not a focus of the present study, a brief resume about some main features, such as the TSS/VSS concentration, the granule size and the EPS content, could help the reader interpret the results that will be discussed in the following sections. The structure of the aerobic granules remained unchanged for the whole experiment. Indeed, their sizes ranged between 1.9 and 2.2 mm. Their morphology gradually evolved from a regular outer shape with a yellow appearance towards an irregular structure with a brownish colour due to the adsorption of inert/particulate material. Although aerobic granules were large and dense enough (256 ± 34 gSST L_f^{-1}), their settling velocity (25 ± 5 m h^{-1}) was slightly lower compared with what was reported in other studies (Winkler et al., 2013). In this case, the bulk viscosity and the buoyancy forces increased with increasing salt concentration, which had a significant effect on the settling behaviour of aerobic granules. The concentration of total suspended solids slightly increased during the first three periods from 4 gTSS L^{-1} to 7 gTSS L^{-1}, whereas the volatile suspended solids concentration remained quite stable at approximately 4.7 gTSS L^{-1}. As a result, the proportion of inorganic material within granules gradually increased. In period IV, a significant TSS increase was observed (up to 18 gTSS L^{-1}), which was mainly due to the large amount of suspended solids of the influent wastewater. Conversely, VSS increased slowly up to 8 gVSS L^{-1}, leading to a significant increase in inert material within granules. Indeed, the VSS/TSS ratio decreased by up to 50% at the end of period IVa, and a massive sludge withdrawal was carried out after that. Then, the TSS concentration was maintained in the vicinity of 12 gTSS L^{-1} and the VSS/TSS ratio was kept near 60%.

EPS analysis revealed that proteins were the predominant fraction. In fact, the average EPS concentrations were 450 mg $gVSS^{-1}$ and 35 mg $gVSS^{-1}$ for proteins and carbohydrates, respectively. Consequently, the ratio between proteins and carbohydrates was 15, on average. As reported in Table 3, it is worth noting that the EPS content increased in each period at increasing salt concentrations. In detail, the results obtained showed that the polysaccharide fraction was almost stable, whereas the proteins gradually increased in size at each increase in salt concentration, confirming what was previously observed by Wan et al. (2014), where microorganisms produced a large amount of EPS, mainly proteins, to face the increasing osmotic pressure. In period IV, the EPS content was lower due to the reduction of the organic load rate. In Table 3, the main features of aerobic granules are summarised.

3.2. Organic carbon removal

BOD and COD (total, soluble and particulate) time courses are shown in Fig. 1. For the whole experiment, BOD (Fig. 1a), total COD and soluble COD (Fig. 1b and c, respectively) showed similar trends.

In detail, concerning both BOD and COD removal efficiency, a progressive increasing trend was observed during period I, mainly

Table 3
Summary of the main characteristics of aerobic granules.

Phase	Salinity (gNaCl L^{-1})	Granules size (mm)	Density (gTSS L_f^{-1})	EPS (mgPN L^{-1})	EPS (mgPS L^{-1})	Settling velocity (m h^{-1})
I	30	1.82	280	357.9	39.9	34
II	38	1.93	271	498.1	44.9	26
III	50	2.01	256	594.3	37.6	24
IVa	75	2.11	241	657.7	41.8	21
IVb	75	2.21	236	316.5	16.3	21

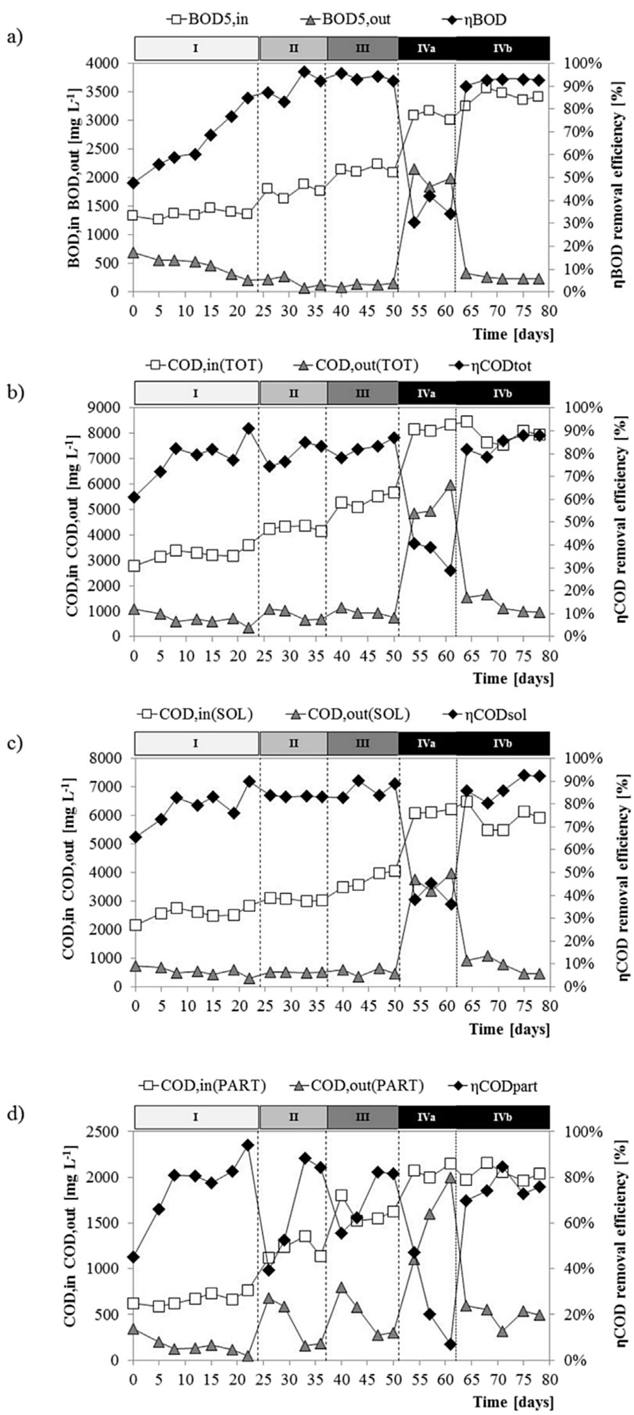

Fig. 1. Influent and effluent concentrations and the removal efficiencies for BOD (a) and total, soluble and particulate COD (b, c, d).

due to the gradual acclimation of biomass to higher salt concentration (30 vs 25 gNaCl L^{-1}) and to the new influent wastewater composition, which was characterized by a lower biodegradability. When steady state conditions were achieved, the total and soluble COD concentrations in the effluent were close to 300 and 280 mg L^{-1}, respectively, whereas the BOD concentration was equal to almost 200 mg L^{-1}. In period II, BOD, total COD, soluble COD and NaCl influent concentration increased according to the lower dilution factor applied, as reported in Table 2. Consequently, the removal efficiency for all three parameters slightly decreased. As reported by Lefebvre and Moletta (2006), a temporary reduction in organic carbon removal could be observed, especially when the changes in salinity are combined with high OLR. However, the removal efficiencies for both rapidly increased to approximately 90% of those of the previous phase. In period III, no significant changes were observed, reflecting that aerobic granular sludge was able to withstand both salt and organic matter load shocks. Taheri et al. (2012) obtained similar results, although at lower salinity levels. The authors attributed the high organic matter removal efficiency to the necessity for microorganisms to adjust their metabolisms to adapt to high osmotic conditions. Taheri et al. (2012) observed that the semisaturation constant (Ks) for the organic substrate increased with increasing salinity. The authors stated that some bacteria have effective transport mechanisms for some osmolytes, and bacteria need more energy when they use this mechanism to adapt to salinity. Thus, more of the carbon source is required, which causes Ks to increase. In period IVa, a significant deterioration of effluent quality was indicated. The organic load rate increased to 8 kgCOD $m^{-3}d^{-1}$, and salinity increased up to 75 gNaCl L^{-1}. For all of period IVa, both the BOD and the COD of the effluent rapidly increased. Consequently, the removal efficiency dropped by up to 38% (BOD and soluble COD) and 28% (total COD) at the end of this period. Similar results were obtained by Val del Río et al. (2013), who observed a deterioration of organic matter removal efficiency for OLR higher than 4.4 kgCOD $m^{-3}d^{-1}$. No other experiences with this salinity level are reported in the literature. Therefore, the cycle length was doubled in period IVb to distinguish the effects of high salinity level and OLR (as mentioned above). As a result, both the influent flow and consequently the OLR decreased by a factor of two. Under these conditions, the organic carbon removal efficiency gradually increased up to 90%, which is in line with the previous periods. In light of these results, it would seem that heterotrophic bacteria are not influenced by salt concentration. Carbon removal efficiency was always approximately 90%, irrespective of the salinity level. Instead, it was harshly affected by the high OLR that characterized period IVa. Indeed, when OLR was reduced (period IVb), the organic carbon removal returned to excellent efficiencies. Under these conditions, heterotrophic bacteria must degrade to a lower substrate amount; in other words, they had a longer time for its degradation. This is particularly important in the case of industrial wastewater treatment in which the presence of slowly biodegradable or particulate organic matter requires a longer time for its hydrolysis and biodegradation.

Concerning particulate COD (Fig. 1d), a significant constant increase was observed during period I. Microorganisms gradually started to adapt to a new organic substrate that was characterized by a significantly lower biodegradability compared with the synthetic wastewater. Therefore, the bacteria slowly began to hydrolyse and degrade the slowly biodegradable and particulate substrate of the influent wastewater. De Kreuk et al. (2010) observed that the particulate substrate is removed by adsorption at the granule surface, after which it is hydrolysed and bacteria degrade the hydrolysis products. It worth noticing that, in each period change, particulate COD removal rapidly decreased but gradually recovered up to a steady state value. Several explanations for these results can be made. First, due to the lower dilution factor, the particulate COD load increased in periods II and III. Consequently, the bacteria needed more time to adapt to the new conditions so that they could hydrolyse and biodegrade a higher amount of particulate matter. In the second instance, as will be discussed below, high effluent solid concentrations were measured at the beginning of a new period. This was partly due to a slight detachment of aerobic granules. In fact, as previously observed by Di Bella and Torregrossa (2013), the increase of OLR initially caused a partial disintegration of granules that led to the increase in particulate COD in the effluent. As previously observed for total and soluble COD, particulate COD removal efficiency dramatically dropped in period IVa, whereas it rapidly increased in the last period (IVb). This confirms that a longer reaction time is necessary for the hydrolysis and the subsequent oxidation of particulate matter. It is conceivable that the gradual adsorption of particulate and inert material on the surfaces of granules reduced their adsorption capacities in the long run (this aspect will be better discussed in the following paragraph). To improve the particulate removal, a selective discharge of the exhausted granules could be beneficial. These granules likely become heavier than the others, so a selective withdrawal could be easily done.

3.3. COD fractionation results

COD is probably the most used parameter for quantifying the amount of organic matter in wastewater. In the literature, in most cases, data of total COD are reported. However, especially for industrial wastewater, the organic matter could be present in soluble form in addition to particulate. The knowledge of the COD fractions in the industrial wastewater could be useful because the removal of each fraction requires appropriate processes.

The results of COD fractionation are shown in Fig. 2. As could be seen in Fig. 2a, the predominant fraction of the influent COD was the soluble inert fraction (50% on average), and the proportions of the soluble readily biodegradable, slowly biodegradable and readily hydrolysable COD, inert particulate, and active biomass fractions were on average 20%, 18%, 12%, and 4%, respectively. COD fractionation in the effluent (Fig. 2b) was carried out at the end of each period, when steady state conditions in terms of organic carbon removal were achieved.

In periods I and II, the effluent was mainly constituted by the soluble inert and active biomass fractions (88–92%). The percentages of the slowly biodegradable substrate and the soluble readily biodegradable fraction were very low. In periods III and IVa, the particulate inert fraction increased to 9% and 14%, respectively, confirming the reduction of adsorption capacity of the aerobic granules in the long period. Similarly, the proportions of the soluble readily biodegradable and slowly biodegradable substrate fractions increased. This could be related to the biomass ageing, as at the end of period IVa where the ratio between the volatile and total suspended solids was lower than 50%. At the beginning of period IVb, 500 mL of mixed liquor was purged. Consequently, both the soluble readily biodegradable and slowly biodegradable substrate fractions in the effluent decreased.

At the same time (as mentioned above), the purging of mixed liquor allowed the discharge of the exhausted granules; therefore, the adsorption capability was restored. As a result, the particulate inert fraction in the effluent significantly decreased from 14% to 4%. However, the role played by the contextual increasing of hydraulic retention time from 24 to 48 h is not to be excluded; indeed, bacteria had more time to hydrolyse and thus to remove the inert particulate fraction under those conditions. Furthermore, the removal of the particulate fraction could be related to the EPS content of aerobic granules. Indeed, the amount of particulate matter removed, evaluated as the difference between the sum of the particulate slowly biodegradable and particulate inert substrates in the influent and effluent wastewaters, was well related to the EPS-bound protein fraction (Fig. 3). The authors indicated that the EPS analysis is not the main purpose of this work. However, with the aim of identifying a possible mechanism of the removal of inert/particulate material, a brief mention of the EPS content of the aerobic granules is necessary.

As could be seen in Fig. 3, the increase in the content of EPS-bound proteins caused a linear increase of particulate matter removal. Proteins are hydrophobic substances forming a dense and sticky structure that is the basis of the granulation process (Zhu et al., 2012). EPS can form hydrogen and ionic bounds and can establish a special gelatinous structure that is able to trap (or bridge) inert particles. Because of these properties, EPS could be considered a natural clotting agent (Sheng et al., 2010). Therefore, in addition to the adsorption phenomena observed by De Kreuk et al. (2010), the role of EPS in the removal of organic particulate matter must be taken into account.

The active biomass fraction in the effluent could be assumed to be a good indicator of biomass synthesis in the system. As observed in Fig. 2b, its fraction in effluent wastewater gradually decreased with increasing salt concentration. In detail, the ratio between the amount of active biomass (mgXa L^{-1}) and the biodegradable COD removed (sum of particulate slowly biodegradable and soluble readily biodegradable) resulted in an excellent correlation with the salt concentration (Fig. 4). The data of period IVb were not taken into account because the cycle length was different. This observation was interpreted as the amount of synthesized new biomass per unit of organic matter removed, which gradually decreased with increasing salt concentration. Taheri et al. (2012) stated that the main biomass kinetics coefficients were not affected by salinity. However, in the present study, the results obtained revealed a slight decrease in biomass activity when salt concentration increased. The reason for this disagreement could be related to the higher salt concentration of wastewater in this work.

Nevertheless, it is interesting to note that the synthesis of new biomass occurred despite the high salt concentration. This means that specialized bacteria, probably halophilic or halo-tolerant strains, developed within reactors and in some way colonised aerobic granules, promoting their gradual acclimation to extreme salinity levels. Moreover, the correlation of the exponential decrease indicated that the synthesis of new biomass clearly decreased with increasing of salt concentration, but it did not cease. These results suggest that the system could tolerate higher salinity as long as a longer acclimation period is guaranteed for the achievement of steady-state operating conditions.

Furthermore, the results presented so far confirmed that aerobic granular sludge is a very good solution for the treatment of wastewater with high particulate matter content because of its ability to remove the particulate and inert fractions. Heterotrophic bacteria were not affected by high salt concentrations. However, high OLR reduced the organic removal efficiency, mainly due to the poor influent biodegradability level that requires longer reaction periods for its complete degradation.

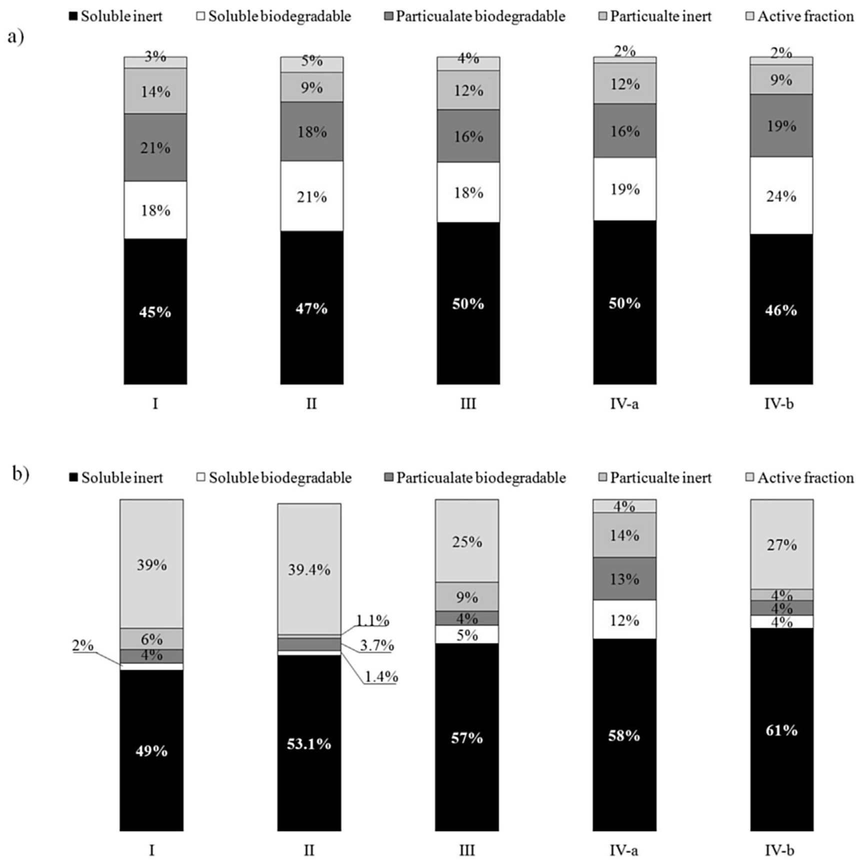

Fig. 2. Results of the COD fractionation carried out for influent (a) and effluent (b) wastewater in each experimental period.

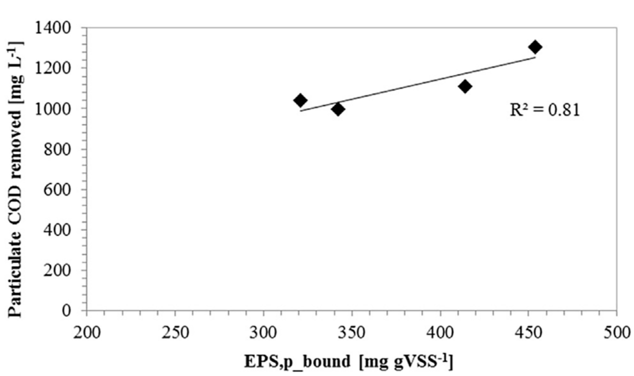

Fig. 3. Correlation between the COD particulate fraction removed and the specific protein fraction of EPS in aerobic granular sludge.

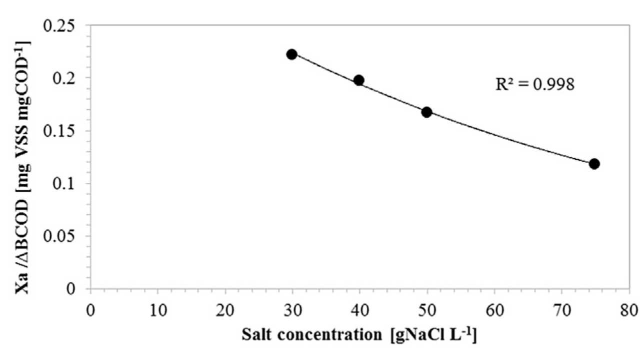

Fig. 4. Relationship between the effluent active biomass/biodegradable COD removal ratio and the influent salt concentration.

3.4. Nitrogen removal

Results concerning nitrogen removal are shown in Fig. 5. In detail, Fig. 5a reports the influent and effluent total nitrogen concentrations and the removal efficiency for the whole experiment. In Fig. 5b, the concentrations of nitrogen compounds in the effluent are reported. Fig. 5c illustrates the nitrogen mass balance: the area between the curve of total nitrogen effluent concentration and total nitrogen influent represents the net nitrogen removed due to heterotrophic bacteria growth (evaluated as the 5% of BOD removed), which supplies useful information regarding the nitrogen removed by the nitritation–denitritation process in the absence of direct nitrogen measurement in the off-gases.

In the earlier days of period I, nitrogen removal efficiency showed that a slightly decreasing trend might be due to the complexity of the new nitrogen substrate, which is mainly present in organic form (Fig. 5a). Thus, the removal efficiency gradually increased up to 70% at the end of this period. The main effluent nitrogen compound was nitrite (Fig. 5b), in concentrations ranging from 50 mg L^{-1} to 70 mg L^{-1}. Both ammonium–nitrogen and nitrate concentrations in the effluent wastewater were very low. The former confirms that ammonia oxidizing bacteria (AOB) are not affected by salt concentrations up to 30 gNaCl L^{-1} as also reported by Bassin et al. (2011), whereas the latter demonstrates that nitrite oxidizing bacteria (NOB) are completely inhibited at this salinity level (Pronk et al., 2014). The total nitrogen removed by simultaneous nitritation–denitritation was almost 37%. Previous studies

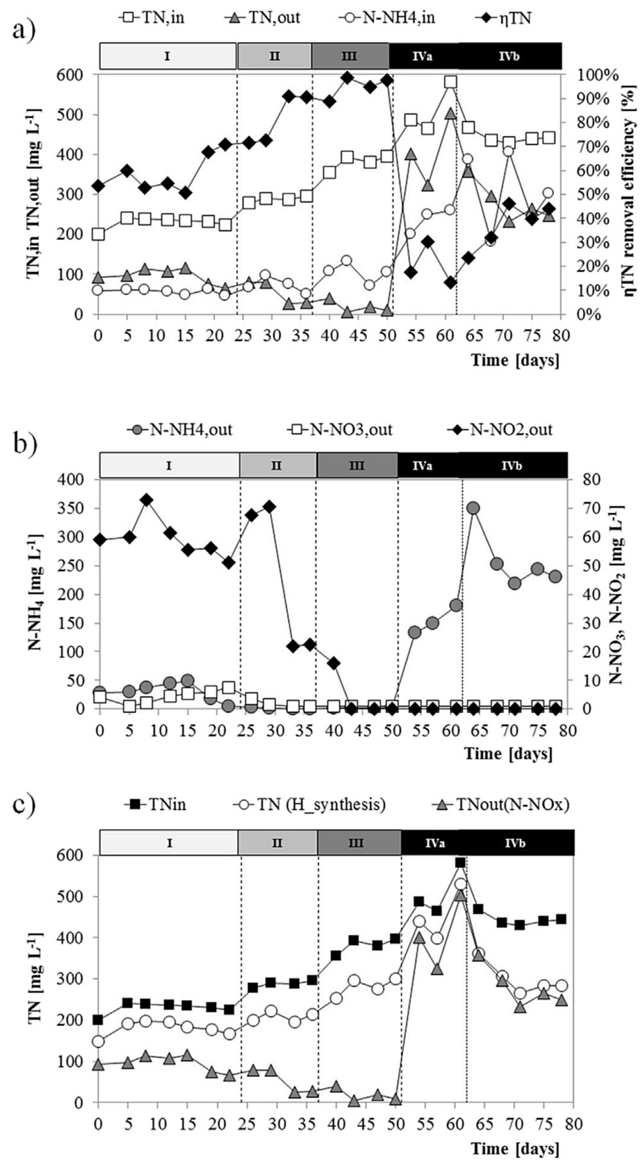

Fig. 5. Influent and effluent concentrations and removal efficiency for TN (a); concentrations of nitrogen compounds in the effluent (b); nitrogen mass balance (c).

demonstrated that ammonium oxidation in aerobic granular sludge reactors begins when the main part of organic carbon has been degraded, whereas reduction of nitrite or nitrate begins simultaneously with ammonium–nitrogen oxidation in the inner layers of aerobic granules (Mosquera-Corral et al., 2005). Furthermore, denitrification occurs at the beginning of the reaction cycle (feast phase) because the concomitant oxygen consumption in the outer layers of granules is due to degradation of the organic substrate, and the presence of oxidised forms of nitrogen derived from the previous cycle creates anoxic conditions in the inner layers of granules (Di Bella and Torregrossa, 2013).

Therefore, denitrification processes prevalently occur at the beginning of the SBR cycle and during the reaction cycle simultaneously with ammonium oxidation. In the first case, heterotrophic bacteria uses the organic matter coming from the influent wastewater, whereas they use storage products in the second case because most of the organic carbon, especially the readily biodegradable fraction, has been removed. The COD fractionation demonstrated that the amount of the biodegradable fraction in the effluent in period I was very low. Therefore, it is possible that the lack of organic carbon resulted in the failure to complete the simultaneous nitritation–denitritation process. To enhance the nitrite reduction, a known amount of sodium acetate was added as an external carbon source on the 28th day (half of period II). The addition of a readily biodegradable substrate created a new metabolic condition, as usually occurs at the beginning of the reaction cycle (De Kreuk et al., 2005). In this way, oxygen consumption was due to heterotrophic bacteria for acetate oxidation in the outer layers of aerobic granules. Consequently, the oxygen concentrations in the inner layers were very low, favouring anoxic conditions. Here, heterotrophic bacteria used nitrite as an electron acceptor for oxidation of the external carbon source. To permit the formation of a sufficiently large anoxic layer, the oxygen concentration within the reactor was not controlled because the aerobic granules were larger than 2 mm (Di Bella and Torregrossa, 2013). Under these conditions, at the end of period II, total nitrogen was removed with a yield of approximately 90%. The effluent nitrite concentration was slightly higher than 20 mg L^{-1}, and the nitrogen removed by nitritation–denitritation increased up to 64%. As mentioned above, an unknown amount of sodium acetate was degraded by direct oxidation; consequently, the organic substrate was not fully utilized for denitritation reactions. To improve the total nitrogen removal, the dose of sodium acetate was doubled at the beginning of period III. Immediately, nitrogen concentrations in the effluent were lower than 5 mg L^{-1}, and the total nitrogen removal efficiency was close to 98%. At the beginning of period IVa, the concentrations of both total nitrogen and salt in the influent increased according to the lower dilution factor. The nitrogen removal efficiency dramatically dropped to less than 20% in a few days, indicating the complete inhibition of AOB strains. The sodium acetate dosage was interrupted in this period. In period IVb, nitrogen removal efficiency gradually recovered up to 45% on average. However, as shown in Fig. 5c, the main mechanism of nitrogen removal was heterotrophic synthesis. Therefore, a longer reaction cycle did not produce important enhancements in ammonium–nitrogen oxidation.

There are two possible explanations for these results. The former is certainly related to the high salinity level. Among the findings reported in the literature, Wan et al. (2014) did not observe AOB inhibition up to 50 gNaCl L^{-1}, whereas Wang et al. (2015) observed a severe AOB inhibition at salinities over 8% (approximately 75 gNaCl L^{-1}). Therefore, it is possible that AOB strains could tolerate salinity up to an undefined level between 50 and 75 gNaCl L^{-1}.

Furthermore, in period IVa, the ammonium–nitrogen concentration in the influent wastewater significantly increased from less than 100 mg L^{-1} up to 250 mg L^{-1}, representing more than half of the total nitrogen concentration. This led to a significant increase in free ammonia concentration up to approximately 40 mg L^{-1}, which could be detrimental to the AOB community (Yang et al., 2004). In the previous periods, the proportion of ammonium–nitrogen to total nitrogen was lower (25% on average). Following the ammonification process within the reactor, the ammonium–nitrogen concentration gradually increased, but its oxidation probably occurred, avoiding the accumulation of large amounts of free ammonia.

Nevertheless, these results indicated that at salinities up to 50 gNaCl L^{-1}, nitrogen can be efficiently removed, despite very high nitrogen influent concentrations (400 mgTN L^{-1}) and the adverse environmental conditions associated with the high salt content. A gradual acclimation strategy of autotrophic bacteria to high salinity level is possible and allows avoiding their inhibition. It is also possible that, as indicated by the fractionation tests, an autochthonous biomass developed within the bioreactor and gradually colonized the granules. To understand whether the excellent nutrient removal efficiencies were due to the acclimation of originally acclimatised bacteria or to the autochthonous biomass, further in-depth investigation by means of specific microbiological analyses (e.g., FISH) are necessary to discern their roles in the nitrogen removal process. In conclusion, the combination of partial nitrification and nitrite–denitrification processes is a successful strategy for enhancing nitrogen removal in the treatment of hypersaline wastewater.

4. Conclusions

In this work, the treatment of fish canning wastewater by aerobic granular sludge was investigated. The aerobic granules adapted to increasing salt concentrations, and no significant deterioration in their structure was indicated. Excellent performance in terms of carbon removal was obtained (over 90%). In detail, aerobic granules simultaneously favoured the adsorption and clotting of particulate organic matter that was mostly hydrolysed and then oxidised by microorganisms. Therefore, salinity did not affect the organic carbon removal. Instead, it was mainly influenced by OLR. Despite the high salinity, nitrogen was fully removed by the nitritation–denitritation process, with a maximum efficiency of 98% at 50 gNaCl L^{-1}. The maximum salt concentration at which aerobic granular sludge could support the process without significant loss of efficiency was 50 gNaCl L^{-1}. However, it is possible that at salt concentrations higher than 50 gNaCl L^{-1}, a lower salt gradient could be applied to avoid the inhibition of autotrophic bacteria. For instance, incremental steps in salinity of 1 or 2 gNaCl L^{-1} should be tested in future experiments.

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