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Nitrification of industrial and domestic saline wastewaters in moving bed biofilm reactor and sequencing batch reactor

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ABSTRACT

Nitrification of saline wastewaters was investigated in bench-scale moving-bed biofilm reactors (MBBR). Wastewater from a chemical industry and domestic sewage, both treated by the activated sludge process, were fed to moving-bed reactors. The industrial wastewater contained 8000 mg Cl^-/L and the salinity of the treated sewage was gradually increased until that level. Residual substances present in the treated industrial wastewater had a strong inhibitory effect on the nitrification process. Assays to determine inhibitory effects were performed with the industrial wastewater, which was submitted to ozonation and carbon adsorption pretreatments. The latter treatment was effective for dissolved organic carbon (DOC) removal and improved nitrification efficiency. Nitrification percentage of the treated domestic sewage was higher than 90% for all tested chloride concentrations up to 8000 mg/L. Results obtained in a sequencing batch reactor (SBR) were consistent with those attained in the MBBR systems, allowing tertiary nitrification and providing adequate conditions for adaptation of nitrifying microorganisms even under stressing and inhibitory conditions.

1. Introduction

Nitrification is usually considered a feasible process, since it has been successfully applied to domestic sewage, aquaculture and other easily biodegradable wastewaters. However, the nitrification of wastewaters from chemical industries may be difficult due to their complex composition. Some chemical industries generate wastewaters containing a wide variety of potential toxic compounds [1]. The probable occurrence of antagonistic and synergic effects makes difficult the identification of the causes of process disturbance [2].

Many substances cause nitrification inhibition and some of them are well reported in the literature. A wide range of organic compounds and heavy metals falls within this category [3]. Hockenbury and Grady [4] enumerated 20 compounds, which inhibited ammonia oxidation when found in the medium at a concentration of 100 mg/L. These authors also reported three substances acting as strong inhibitors at low concentrations (<1 mg/L); dodecylamine, aniline and n-methylaniline. Other reported nitrification inhibitors are allylthiourea, 2-chloro-6-(trichloro methyl) pyridine (TCMP), nitrapyrin and allylsulfide [5,6]. Heavy metals species were also reported as inhibitors of nitrifying bacteria [3,7–10]. The extent of the inhibitory effects depends on the agent concentration, but biomass adaptation can, in many cases, overcome the detrimental effect of chemical inhibitors [11].

Nitrification also depends on pH and alkalinity and the complex composition of the industrial wastewaters can pose difficulties to find adequate levels of these parameters. Addition of sodium carbonate and other alkalinity agents may be required to assure the necessary conditions for nitrification.

Chloride salts are often found in wastewaters, but their effect on nitrification seems to be more pronounced at high concentrations (1% m/v, NaCl), as reported by Uygur and Kargi [12]. However, salt itself may cause changes on microbial metabolism, on sludge settleability characteristics and on sludge flocs and biofilms architecture. Salinity also potentially disrupts biofilm composition/extracellular polymeric substances (EPS) and directly affects the oxygen maximum solubility and its transfer rate to the liquid phase [13]. In addition, salts present in complex matrices, like those of industrial waters, can intensify the inhibitory effect of organic substances. Studies regarding the effect of salinity on the nitrification process are difficult to compare and often show contradictory results. The reasons for the contradictions can be explained by the different system configurations and the instability in the experimental conditions, especially related to pH, temperature and presence of inhibitory compounds. Moreover, the way how salt is introduced in the system (as a pulse or by gradual increase), as well as the species present in the reactor (pure or mixed culture, adapted or non-adapted bacteria) can highly influence the results [14]. The comparison of our work to others becomes even more difficult since

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most of the studies in literature describe the effect of salinity on nitrification using synthetic wastewater [14–17] and the ones using real wastewater are really scarce. Moussa et al. [18] investigated the nitrification activity using activated sludge from industrial wastewater treatment plants operated under various salt levels, but they did not evaluate the nitrification performance on-site.

Bioreactor type may also have an important role on the attenuation of nitrification inhibitory effects. The capacity of biomass retention inside the reactor is a key factor to avoid washout or loss of slow-growing bacteria, which can be more resistant to inhibitory substances. The high microbial retention time, typical of some reactors, favors the development of adapted and specialized microbial communities [19]. Moving-bed reactors fulfill the necessary requirements for developing specialized biomass. In addition, they allow raising biomass concentration by increasing the amount of moving supports [20]. This, of course, represents an important advantage compared to the conventional activated sludge system, where the biomass can be easily washed out from the reactor due to a process disturbance, requiring considerable sludge recycling. Compared to other fixed biomass systems, such as submerged biofilters and trickling filters, MBBR systems show no clogging problems and lower head loss. All these special features make easier the management of the moving-bed biofilm reactors.

In some cases, when the wastewater to be treated contains a significant amount of refractory contaminants, just the biological treatment is not enough. Even though many organic molecules are readily biodegradable, some are biorecalcitrants [21]. Wastewaters that severely inhibit the nitrification process need to be pretreated chemically or physically before nitrification in order to reduce the concentration of the inhibitory compounds to levels that do not disturb the nitrifying bacteria [22]. In this way, biological and physicochemical processes can be integrated to improve the treatment efficiency. The last processes, either based on oxidizing agents (ozone, hydrogen peroxide) or on the adsorption of pollutants (activated carbon), have been used to remove many organic compounds, functioning as a pretreatment to increase the biodegradability of the wastewater. Among the chemical oxidation processes, ozonation is one of the methods that provide better results in the oxidation of recalcitrant compounds [23]. Due to its physical properties such as micro-porous structure, large surface area and high adsorption capacity, adsorption using granular or powdered activated carbon consists in a well-applied mass transfer method for removing organic and inorganic pollutants from contaminated wastewater [24].

The main purpose of this work was to investigate the nitrification of the wastewater coming from the activated sludge treatment plant of a chemical industry, which contains residual compounds and high salinity content, in a moving-bed biofilm reactor. Experiments were also carried in parallel with treated domestic sewage, in a similar system, acting as control reactor. The effect of pretreatment with ozone and activated carbon on removal of nitrification inhibitors was also investigated.

2. Methods

2.1. Wastewater

Wastewater from a chemical industry (Bayer S.A., Belford Roxo, Rio de Janeiro), which produces polyurethane foams, aniline, paint, varnishes, fungicides, acaricides, herbicides and pesticides, was used in the experiments. Due to the considerable number of products, several compounds are used in the production units and confer high variability to the wastewater in terms of chemical composition. Such substances belong to chemical groups as carbamates, triazoles, triadimenol, imidacloropid, dissulfoton, fonofos, atrazine and others. The variability associated with the recalcitrance of the soluble organic materials and with the high salt content certainly enhanced the wastewater complexity. Samples were collected, every 15 days, after activated sludge treatment and stored under refrigeration until use. The domestic wastewater came from a conventional wastewater treatment plant of Rio de Janeiro. Appropriate amounts of ammonia, as ammonium chloride, were added to both wastewaters in order to achieve NH_4 concentration of approximately 40–50 mg N/L.

2.2. Pretreatment of the industrial wastewater

To remove potential inhibitory substances from the industrial wastewater, two pre-treatment techniques were evaluated (ozonation and carbon adsorption). Ozonation tests were performed in a lab-scale system, consisting of ozonation column (600 mL) and ozonizer (MultiVacuo MV06, Brazil). Ozone concentration in the gas fed to the column was fixed at 7.5 mg/L and ozonation time varied from 10 to 60 min.

Adsorption assays were conducted with powdered activated carbon (PAC) (Norit RBAA-1, USA) to determine best conditions (PAC concentration and time). Carbon concentration varied from 0 to 10 g/L and adsorption experiments were conducted in a Jar-test apparatus at the original pH (7.7) of the industrial wastewater. The performance of both pre-treatments was assessed through the determination of DOC removal efficiency.

2.3. Inhibition assays

To confirm the potential benefit of the pre-treatment techniques for removing inhibitory compounds, inhibition assays were carried out as follows. Biomass was previously acclimated to treated sewage in a batch-reactor and used as inoculum when it was able to achieve high levels of nitrification (>90%). Assays were performed in 100-mL conical flasks in a rotary shaker (150 rpm, 25 °C). Each flask contained 5 mL of inoculum and 35 mL of different media, as follows: (i) industrial wastewater without pretreatment, (ii) pretreated (activated carbon) industrial wastewater, (iii) pretreated (ozone) industrial wastewater, (iv) treated sewage (control experiment). pH adjustment was made when necessary to a value in the range 7–8. No addition of salt was made to the control flasks. Experiments lasted 48 h, but samples were also withdrawn from the flasks after 24 h of incubation. The average percentage of ammonia removal, attained in triplicate experiments, was taken as an indicator of biomass nitrification performance.

2.4. Nitrification in moving-bed reactors

Two identical reactors made of Pexiglas with a useful volume of 5-L were continuously fed, in the first set of experiments, with industrial wastewater and treated sewage, named IND-reactor and C-reactor (acting as a control-reactor), respectively. The IND-reactor and the C-reactor reactor were filled with K3 type Kaldnes support and AMB biomedia, respectively. The amount of support corresponded to a volume fraction of 40%, for both reactors. Fig. 1 shows a schematic representation of one moving-bed reactor system, since the other one had the same configuration.

The chloride content of the industrial wastewater varied from 7500 to 8000 mg/L and the chloride content of the treated sewage was gradually increased up to 8000 mg/L, by adding appropriate amounts of NaCl. After two months of continuous operation, the IND-reactor was fed with industrial wastewater pretreated by activated carbon. When the salinity level of the treated sewage fed to the C-reactor reached the maximum concentration tested (8000 mg Cl^-/L), this reactor was started to be fed with a mixture of treated sewage and pretreated industrial wastewater. The oper-

Table 1
Experimental conditions of the moving-bed reactors.

Experimental run	Reactor	Influent composition		Chloride content (mg/L)	HRT (h)	Time of operation (d)
		SW[a]	IW[b]			
1	IND	0%	100%	8000	24	53
2	IND	0%	100%[c]	8000	48	187
3	C	100%	0%	<30	24	43
4	C	100%	0%	<30	48	9
5	C	100%	0%	1000–2000	48	25
6	C	100%	0%	3000–5000	48	34
7	C	100%	0%	5500–6500	48	43
8	C	100%	0%	8000	48	14
9	C	90–80%	10–20%[c]	8000	48	25
10	C	70–60%	30–40%[c]	8000	48	34
11	C	50–40%	50–60%[c]	8000	48	26
12	C	30–20%	70–80%[c]	8000	48	24
13	C	10–0%	90–100%[c]	8000	48	29

[a] Treated sewage.
[b] Industrial wastewater.
[c] Pretreated (powdered activated carbon – PAC) industrial wastewater.

ation conditions of the moving-bed reactors are summarized in Table 1.

2.5. Nitrification in the sequencing batch reactor (SBR)

Besides acting as a comparative system to the MBBR, a 2-L sequencing batch reactor was operated for a long period of time (around 200 days) to get more information about nitrification inhibition and biomass adaptation to stressing conditions. The start-up conditions of the SBR were: 100% treated sewage, 0% of pre-treated wastewater and 4000 mg Cl^-/L. After that, the percentage of pre-treated industrial wastewater and the salt content were gradually increased in the reactor feed up to 100% (v/v) and 8000 mg Cl^-/L, respectively. The schedule of the SBR operation is commented in more detail in Section 3. This reactor was operated in successive cycles of 48 h. In the beginning of each cycle, 1800 mL of synthetic media were instantaneously added to 200 mL of settled biomass that remained in the reactor. Sedimentation time was 0.5 h and supernatant withdraw lasted 2 min. Biomass concentration in the reactor was kept between 0.5 and 0.7 g/L.

2.6. Analytical procedures and controls

Dissolved organic carbon (DOC) was determined using a Shimadzu Analyzer (model 5000A). Ammonium was determined using a selective electrode, as recommended [6]. Nitrite determination was performed by using the analytical kit provided by Hach Co. Nitrate was determined by ion chromatography, using a Dionex ICS 90 apparatus, and chloride content according to the traditional method of Mohr. Since the concentration of suspended solids in the reactor effluent was small, determination by the conventional gravimetric method was not performed. Polysaccharides in the liquid phase (reactor effluent) were determined according to Cammarota [25]. All experiments were conducted at room temperature (22 ± 2 °C). Dissolved oxygen (DO) in the reactors and pH were frequently monitored, the former was always higher than 3 mg/L and the latter was kept in the range 6.5–8, by adding sodium carbonate to the wastewater providing adequate alkalinity. Atomic absorption spectroscopy was used to determine the concentration of several metals (Zn, Cd, Cu, Ni).

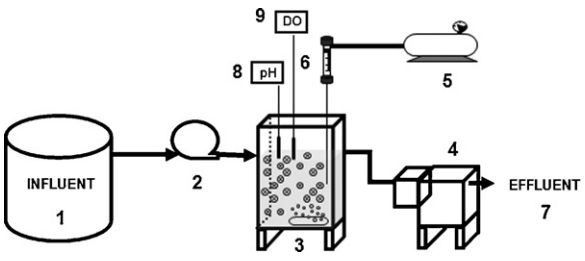

Fig. 1. Moving-bed reactor experimental set up: (1) influent vessel, (2) peristaltic pump, (3) moving-bed biofilm reactor, (4) settler, (5) compressed air line, (6) rotameter, (7) effluent stream, (8) pH electrode, (9) dissolved oxygen probe.

3. Results and discussion

3.1. IND-reactor operation with industrial wastewater

Removal of ammonia was very low in the moving-bed reactor fed with the industrial wastewater. In 52 days of operation, average ammonia removal was 17%, indicating pronounced inhibition of the nitrification process (Fig. 2). Possibly, inhibition was caused by organic compounds present in the industrial wastewater, even at low concentrations. Based on this result, the wastewater was submitted to pretreatment using either ozone or activated carbon in order to remove inhibitory substances. Further, inhibition assays were performed to evaluate the efficacy of each pretreatment.

3.2. Pretreatment results and inhibition assays

Ozonation was not effective to remove wastewater residual organic substances under the tested conditions. DOC removal was negligible. Conversely, activated carbon promoted complete removal of DOC when used in concentrations equal or higher than 2 g/L. Adsorption process was very fast and in half an hour DOC dropped from 16 mg/L to non-detectable levels.

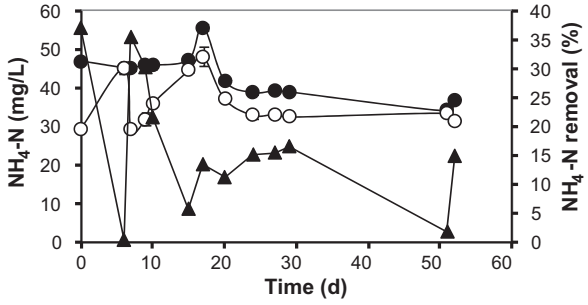

Fig. 2. Ammonia–nitrogen concentration in the influent (●) and effluent (○) of the IND-reactor, ammonia–nitrogen removal (▲) – run no. 1.

Table 2
Inhibition assay results – percentage of ammonia removal.

	24 h	48 h
Industrial wastewater without pretreatment	25 ± 1.5	40 ± 2.0
Pretreated industrial wastewater (ozone)	28 ± 1.6	41 ± 1.1
Pretreated industrial wastewater (activated carbon)	47 ± 5.6	68 ± 5.6
Treated sewage (control)	76 ± 2.3	86 ± 1.2

Results from inhibition assays, shown in Table 2, indicate that pretreatment of the industrial wastewater with activated carbon improved the nitrification levels. Moreover, it can be observed that pretreatment with ozone was not effective, since the ozonated and the industrial wastewater (without pretreatment) results were very close. This fact seems to be related to a poor mineralization during ozonation due to the persistency of intermediate oxidation compounds. Short-chain carboxylic acids (such as pyruvic, glyoxalic or oxalic) are a class of organic compounds persistent to the oxidation by ozone and are normally produced during the ozonation of complex organic molecules. Their refractory properties are responsible for most of the organic content of the ozone-treated wastewaters [26]. Breithaupt et al. [27] used a combined ozone/biological treatment for the mineralization of naphthalene-1,5-disulfonic acid. These authors reported the formation of non-biodegradable intermediates, limiting the efficacy of the combined process. Kuo [28], using a photolytic ozonation process for the treatment of a pesticide wastewater, observed that the COD decreased rapidly in the beginning of the oxidation stage, although no more BOD was produced. This result indicated that the compounds in pesticide wastewater were easily cleaved into smaller molecules by the chemical oxidation process. The authors also mentioned that overexposure of the wastewater to ozone did not generated more biodegradable intermediates, but reduced the BOD_5/COD ratio and produced more toxic compounds with lower half-maximal effective concentration (EC50) in microtoxicity tests. It seems that in our experiment, ozonation of the industrial wastewater had a similar effect of not enhancing the biodegradability of the inhibitory compounds.

As expected, best results from the inhibition assays were achieved in the control flasks, where the nitrification efficiency amounted 86% in a 48-h incubation period. Based in these results, the industrial wastewater was always pretreated with PAC. Adsorption experiments conducted in a Jar-test apparatus showed that use of 2 g/L of PAC for 30 min was sufficient to provide complete DOC removal. Therefore, such conditions were hereinafter used for industrial wastewater pretreatment, which was always performed in a stirred tank.

Nitrification inhibitors seem to be elements or substances which were partially removed by carbon adsorption. Determination of metals (Zn, Cd, Cu, Ni) in the wastewater revealed that their concentrations were fairly below the reported inhibitory levels [3,9,10]. Taking into account the industrial process (products and raw-materials) of the chemical industry which generates the wastewater used in this research (described in Section 2.1), it is likely that the organic compounds containing nitrogen and sulfur, which can still be present after the wastewater pretreatment with powdered activated carbon, are probably within the nitrification inhibitors. All chemical groups involved in the formulation of pesticides, acaricides, herbicides, aniline, varnishes, and polyurethane foams, such as carbamates, triazoles, triadimenol, imidacloropid, disulfoton, fonofos and atrazine, contain either nitrogen or sulfur in their composition. As mentioned before, organic nitrogen compounds such as *N*-dodecylamine, aniline [4], n-methylaniline [4,11], 4-aminophenol and 4-nitrophenol [29] can cause severe inhibition for the nitrifying bacteria even at low concentrations. Hockenbury and Grady [4] mentioned that the inhibitory effect of nitrogen-containing compounds is caused by a competition with ammonia for the active sites on ammonia-oxidizing enzymes, hindering the growth of nitrifying bacteria cells. Nevertheless, no evidence has been provided in the literature. In a similar manner, the same authors pointed out that some compounds similar to nitrite have been hypothesized to be inhibitory because of their competitive effects, although not sufficient explanation was provided. Sulfur-containing compounds are also some of the most powerful inhibitors of the nitrification process; they can act as metal-chelating compounds, inhibiting enzymes that require metals for activation [30]. Some works reported the substantially effect of sulfur compounds on nitrification, like allylsulfide [5], sulfide [31], dimethylsulfide, dimethyldisulfide and ethanethiol [32].

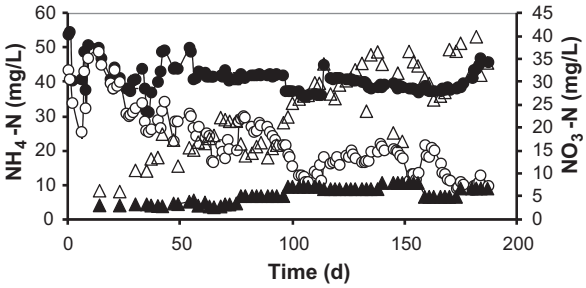

Fig. 3. NH_4–N concentration in the influent (●) and effluent (○), NO_3–N concentration in the influent (▲) and effluent (△) of the IND-reactor – run no. 2.

Unfortunately, up to now, we could not specifically identify the inhibitory substances present in the industrial wastewater. Segregation of the several industrial streams is one of the possibilities to get a deeper insight about what is causing the inhibitory effect on the nitrification process. Screening industrial waste streams can make possible to determine their composition and, if the composition is known, its potential impact on the biological treatment process can be evaluated according to literature data or by using toxicity estimation methods. Even thought results of specific laboratory nitrification inhibition tests are available in literature, it remains unknown how these data can be used for prediction of inhibitory effects in industrial wastewaters treatment plants [33], especially in those receiving several effluent streams with different and variable composition, like the one investigated in this work. The lack of an adequate database and the potential presence of synergistic/antagonistic effects in complex wastewaters limit the application of those inhibition tests.

3.3. IND-reactor operation with pretreated industrial wastewater

Results of the IND-reactor fed with pretreated industrial wastewater are shown in Fig. 3. The behavior of the curves seems to indicate that gradual adaptation of the nitrifying organisms occurred. Despite the intrinsic variability of the wastewater composition and the visible nitrification inhibition in the first days of operation, a clear microbial adaptation trend was observed. Average removal efficiency attained in the last two months of operation was about 60%. The maximum percentage of ammonia removal was about 80%, which was attained in the end of run no. 2. The reason why levels of nitrification higher than 80% were not obtained is likely to be related to some inhibitory compounds that are still present in the wastewater after pretreatment with PAC. Nevertheless, the results showed that acclimation occurred during the continuous exposure of the biomass to inhibitors. Tomlinson et al. [34] reported that the tolerable concentration level of inhibitors for acclimated sludge was several times higher than that for non-acclimated sludge. Basically, acclimation process can be explained by two main different mechanisms. One of them relies

on the fact that some bacteria acquire the ability to biodegrade inhibitory substances due to long-term exposure [34]. The other one points out that nitrifying bacteria acquire the ability to regenerate new proteins within inactivated cells to recover their activity [35]. According to this last mechanism, the recovery of nitrification activity can proceed even when the bacteria responsible for nitrification are completely inhibited. In this way, an acclimated biomass whose nitrification activity is fully restored can achieve more tolerance against higher concentrations of inhibitory substances, since a considerable fraction of tolerant nitrifying bacteria was generated during the acclimation period. In our case, enhancement of the tolerance against inhibitory compounds may have played an important role in achieving significant nitrification levels. The fact that the compounds present in industrial wastewaters have different biodegradabilities and inhibitory levels makes difficult to predict the necessary period of acclimation for nitrifying bacteria when exposed to them. It also makes the comparison with other literature studies regarding nitrification of industrial wastewaters more complicated.

Fig. 3 also shows that the reactor effluent was enriched on nitrate, indicating that nitrification took place in the reactor. Pretreatment with activated carbon removed not only wastewater residual DOC, but other substances, which possibly affect the activity of nitrifying organisms, even at low concentrations. As mentioned before, nitrogen and sulfur organic compounds are supposed to be the nitrification inhibitors. Thus, a future work in the industry will allow segregating the streams, which are the major source of these compounds.

In this study, it should be remarked that the main purpose of applying physicochemical process (as ozonation and powdered activated carbon adsorption) was to figure out a way to remove the recalcitrant compounds associated with nitrification failure. Even thought the use of PAC was effective in accomplishing that goal, economic balances should be performed to evaluate the feasibility of applying such a process in combination with the biological treatment in full-scale wastewater plant. Obviously in some cases the treatment that should be applied also depends on the local environmental regulations, which can impose strict disposal levels of pollutants. Sometimes, due to the relatively high cost of chemical or physical treatment, one of the first options that should be tried in order to improve the treatment performance is to reduce the effect of the inhibitory substances present in the wastewaters by enhancing the ability of the bacteria to biodegrade them. In the nitrification process, in particular, the increase of the tolerance of nitrifying sludge against inhibition and the improvement of nitrification capacity can be obtained in a long-term acclimation process (as performed in this work and shown in Fig. 3).

Mass balances for nitrogen were performed considering $N–NH_4^+$, $N–NO_2^-$ and $N–NO_3^-$ and nitrogen conservation amounted to 90%, which also confirms that nitrification was really occurring in the reactor.

3.4. C-reactor operation with treated sewage

The C-reactor was fed with treated sewage containing increased concentrations of chloride ion (up to 8000 mg/L) in order to investigate the salinity effect (under the conditions tested) on nitrification process. Fig. 4 shows the ammonia removal efficiency attained in operation runs 3–8. During run no. 3, average nitrification efficiency attained 44% and this period of operation can be considered an adaptation period, which allowed attachment of biomass to the support material and growth of ammonia and nitrite oxidizers organisms. To reach higher nitrification levels, hydraulic residence time (HRT) was increased to 48 h and kept in this value. With the exception of runs 3 and 4, the efficiencies were generally above 80%. This percentage was even higher during runs 8 and 9, reaching 90%. As can be seen, nitrification was substantially affected in the beginning of the runs 6, 7 and 11 due to the new process conditions. Nevertheless, biomass acclimation could overcome the detrimental effect of both salt and industrial wastewater increased concentrations. These results indicated a successful adaptation of nitrifying organisms, even at the highest chloride concentration tested and during operation with pretreated industrial wastewater.

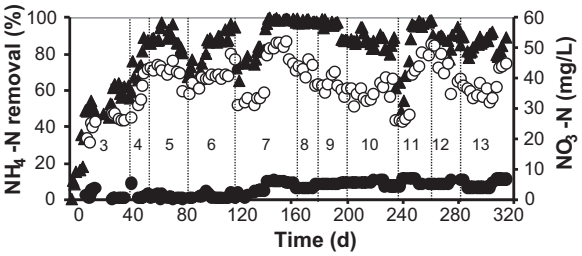

Fig. 4. Ammonia removal (▲) and nitrate concentration in the influent (●) and effluent (○) in the C-reactor – run nos. 3–13.

3.5. C-reactor operation with the mixture of treated sewage and pretreated industrial wastewater

The objective of this study, in particular, was to investigate the influence of gradual increase of pretreated industrial wastewater percentage in the reactor feeding. During run nos. 9–13, the percentage of pretreated industrial wastewater added to the treated sewage was increased from 10 to 100%. Although was observed some fluctuations in the reactor performance, the adaptation capacity of biomass to the submitted conditions was clearly perceptible, reaching more than 90% of NH_4–N removal efficiency in all mentioned runs (Fig. 4).

Evidence of nitrification was assured by nitrate production and nitrogen mass balance. Fig. 4 shows nitrate concentration profiles in influent and effluent, during experimental run nos. 3–13. According to the nitrogen mass balances performed for each run, conversion of nitrogen species in the nitrification process was close to the theoretical value (100%). The small differences between the theoretical value and the obtained values can be attributed to other nitrogen forms, which were not quantified (hydroxylamine, nitrous oxides and others) or aerobic deamonification [36]. Stripping of ammonia can be considered negligible since the pH values were always below 8.

Nitrification levels observed in the operation of the C-reactor during runs 3–8 indicate that chloride ion, in the range investigated, was not responsible for the nitrification inhibition noticed in the IND-reactor, as could be expected. Thus, we can infer that nitrification inhibition was caused by residual substances present in the treated industrial wastewater. Furthermore, the moving-bed reactor allowed a relatively fast adaptation of nitrifying organisms to growing salinity levels. The results obtained when the C-reactor was submitted to increased dosages of pretreated industrial wastewater (no. 9–13), headlined the importance of adopting a strategy of gradual adaptation of the microorganisms, especially when working with complex and poor biodegradable effluents containing inhibitory substances.

Although salinity did not induce nitrification inhibition to the bacteria in the C-reactor for the range of chloride concentration tested (1000–8000 mg/L), results obtained with additional experiments, conducted in a sequencing batch reactor, which was submitted to a salinity shock load (50,000 mg NaCl/L), showed that these large salt concentrations were able to provoke irreversible effect in the nitrifying microorganisms population, as well as observed by Moussa et al. [14]. The nitrification efficiency attained

Table 3
Soluble polysaccharide contents for some experimental runs.

Run no. (wastewater)	Soluble polysaccharide content (mg/L)	
	Influent	Effluent
2 (IND-reactor)	1.3 ± 0.2	7.8 ± 0.2
6 (C-reactor)	1.8 ± 0.2	12.0 ± 0.5
7 (C-reactor)	1.5 ± 0.2	11.1 ± 0.4
8 (C-reactor)	1.0 ± 0.2	12.0 ± 0.1
9 (C-reactor)	1.3 ± 0.1	15.8 ± 0.3
10 (C-reactor)	0.7 ± 0.3	13.9 ± 0.4
11 (C-reactor)	0.9 ± 0.3	12.7 ± 0.2
12 (C-reactor)	1.0 ± 0.2	15.5 ± 0.2
13 (C-reactor)	2.2 ± 0.3	18.1 ± 0.5

in the SBR before the shock was above 90%, decreasing to about 40–50% in the post-shock period.

Monitoring of soluble polysaccharides in moving-bed reactors is an important feature, since operation conditions may lead to an excessive production of such substances. An event of polysaccharides overproduction was reported by Reis [1] in a moving-bed applied to the secondary treatment of a synthetic wastewater. A step of coagulation/flocculation downstream the moving-bed reactor has been proposed [37] for removal of solids and colloidal substances. Although the concern about soluble polysaccharides is related to the treatment of organic loaded wastewaters and heterotrophic communities, monitoring of these substances was performed in our work to verify their production during nitrification. Table 3 shows the results of polysaccharide contents for experimental runs 2 and 6–13.

The soluble polysaccharide content in the influent of both moving-bed reactors was low but substantial higher values were noticed, in the effluent, as shown in Table 3. The increased polysaccharides content in the effluent is directly related to the increased amount of biomass in the reactor outflow. The presence of biomass in the effluent is mainly caused by biofilm detachment. Nevertheless, biomass growing in suspension, although present in small quantities in moving-bed biofilm reactors, also contributed to increase biomass concentration in the effluent.

The amount of polysaccharide in the IND-reactor effluent was almost half of the corresponding values observed in the C-reactor. Although sewage salinity varied from 3 to 8 g Cl^-/L (runs 6–8), and the proportion of pretreated industrial wastewater in the reactor feeding was increased from 10 to 100% (runs 9–13), no significant variation on soluble polysaccharide content was observed in the C-reactor effluent. Even though effluent polysaccharides contents absolute values are low, comparison with nitrification moving-bed reactors data is not possible due to the absence of published information on this subject.

3.6. Biomass characteristics in the moving-bed reactors

Observations of the biofilm removed from the supporting carry media by optical microscopy revealed the occurrence of dense agglomerates and protozoa. For the C-reactor, the increase on salinity enhanced biofilm fragmentation and reduced the number of mobile protozoa. Floc fragmentation and reduction on protozoa abundance were already reported by Freire et al. [38], treating saline wastewater in a sequencing batch reactor. Micrography of the biomass attached to the supports obtained by optical microscopy revealed the presence of a diversified microflora in both C-reactor and IND-reactor, although less protozoa abundance and diversity were observed in the last reactor. Such characteristic was probably related to the complex composition of the industrial wastewater fed to the IND-reactor.

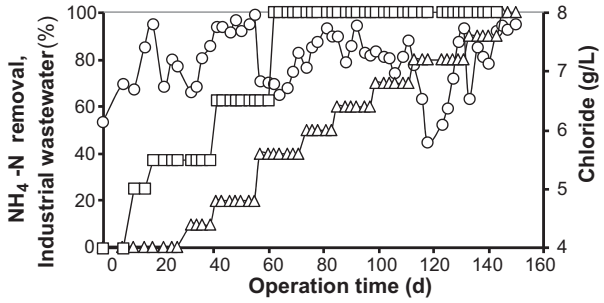

Fig. 5. SBR response to gradual increase on salt content and pretreated industrial wastewater percentage in the feed stream. Ammonia removal efficiency (○), industrial pretreated wastewater percentage (△) and chloride concentration (□).

3.7. Sequencing batch reactor results

Operation of the reactor started with treated sewage containing a chloride concentration of 4 g/L because, at that time, the C-reactor was being operated with that concentration. Also, it was our intent to promote a salt shock load, intensifying suspended biomass adaptation. This way of operation (shock loads) can allow the development of a resistant and adapted biomass, as commented by Panswad and Anan [16]. The SBR was gradually fed with a higher salt concentration up to 5.5 g/L and then an increasing proportion of pretreated industrial wastewater (activated carbon) was added to the treated sewage. Fig. 5 shows the percentage of ammonia removal during the operation of the SBR. Results show that gradual adaptation of the biomass occurred. It seems that the adaptation procedure adopted was able to develop a nitrifying consortium acclimated to the stressing conditions imposed, which promoted more than 90% of ammonia removal even when the SBR was fed with 100% of pretreated industrial wastewater. This result also emphasizes the relevance of performing gradual adaptation, using a less recalcitrant influent.

4. Conclusion

Nitrification of the wastewater from a chemical industry, previously treated by activated sludge, was strongly inhibited. Two pretreatment techniques were tested to remove inhibitors: ozonation and activated carbon adsorption. The latter was much more effective under the investigated conditions and was selected and applied upstream the nitrification step. Salinity was not responsible for the low or moderate nitrification levels observed, since high levels of ammonia oxidation were achieved at the highest chloride concentration tested (8 g/L) in the C-moving-bed reactor. Biomass adaptation was a key factor to improve nitrification performance. Operation of the IND-reactor for a long period (100-d) allowed reaching 70% of ammonia removal. Also, the relevance of microbial adaptation was highlighted in tests with the sequencing batch reactor, which attained ammonia removal efficiencies higher than 80% when fed with an influent containing the highest chloride concentration and 100% (v/v) of pretreated industrial wastewater.

The moving-bed reactor due to its capacity of biomass retention showed to be very effective to perform nitrification of treated sewage and industrial wastewater. Suspended solids content and soluble polysaccharides concentrations in the reactor effluent were very low, dispensing further treatments. Salinity increase promoted fragmentation of biomass agglomerates and reduced the abundance and diversity of protozoa.

In the near future, investigation of the industrial streams composition will allow identification of the specific nitrification inhibitors.

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