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Biological nitrogen and phenol removal from saline industrial wastewater by submerged fixed-film reactor

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Abstract

In this study a biological nitrogen removal process using a submerged fixed-film reactor was applied to treat industrial wastewater with phenol (1 g/l), a high nitrogen concentration (0.4 g N/l) and high salinity (30 g/l). The process consisted of a pre-denitrification system with a down-flow-up-flow biofilter (two columns, each with an effective volume of 21 l) packed with clayey schists from recycled construction material. The efficiency of the system for reducing COD, phenol concentration and total nitrogen was tested under different running conditions such as influent flow (10, 12 and 15 l/d), air loading (6.8 and 13.6 m^3/m^2 h) and effluent recirculation (300%, 400%, and 600%). The system demonstrated a high capacity for reducing COD concentration (95.75 ± 0.72%), independently of running conditions. The aerobic column eliminated most of the phenol in the influent. Nitrogen removal took place mainly in the anoxic column, and was conditioned by the air loading in the aerated column, owing to the dependence of nitrification on the supply of oxygen. However, this process was not able to achieve a nitrogen oxidation superior to 63%, in spite of a sufficient supply of oxygen and the diluting effect of high recirculation (600%) on the phenol concentration in the influent. In spite of the limitations observed in the process of nitrification, results for the removal of total nitrogen were as high as 83%, owing to a combination of different processes for nitrogen removal.

Keywords: Nitrogen removal; Phenol; Industrial wastewater treatment; Pre-denitrification; Submerged fixed-film reactor

1. Introduction

Discharge of industrial wastewater is the cause of significant deterioration of the environment largely because of the presence of nutrients such as nitrogen. These types of pollutants are responsible for phenomena such as the eutrophication of estuaries and coastal areas [1]. Accordingly, priority is now being given to eliminating nutrients from wastewater before it is discharged offshore.

The biological process of nitrification–denitrifaction is generally used to eliminate nitrogen from wastewater, particularly in the case of urban wastewater [2]. Nevertheless, applying this process to the treatment of industrial wastewater is complicated because of the characteristics of the effluent, which make it extremely difficult to be treated biologically. For this reason, nitrogen compounds have traditionally been eliminated from industrial effluents by means of costly physical–chemical processes [3,4].

Certain chemical-pharmaceutical industries generate wastewater containing an extremely high level of ammonium and phenol, something, which virtually rules out any kind of biological treatment. One possibility is to reduce the concentrations of pollutants by diluting them to levels which, though still high, are low enough to permit the application of biological processes [5,6]. In industries located in coastal areas, sea water can be used as a diluting agent. However, this adds still another factor to be considered, namely the corresponding increase in salinity. Nevertheless, authors such as Glass and Silverstein [7] have successfully used biological technologies for the treatment of effluents with an ionic force of 3.0.

Most biological treatments of nitrogen involve a combination of two separate reactors under aerobic conditions (nitrification) and anoxic conditions (denitrification). Nitrification is the

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autotrophic oxidation of the ammonia, first to nitrite and then to nitrate. Accordingly, denitrification consists of the microbiological eduction of nitrate and nitrite to a gaseous nitrogen compound. The integration of this biological process is possible in two separate configurations: pre-denitrification and post-denitrification. Combination of an anoxic process followed by an aerobic process without the addition of an external carbon source and involving the internal recirculation of the treated effluent (pre-denitrification) is more frequently applied to wastewater with a high organic content [2].

In industrial wastewater treatment, nitrogen removal can be combined with the elimination of toxic substances such as phenol, which in spite of its anti-microbial effect, can be used by various micro-organisms as a carbon source for carrying out heterotrophic denitrification [8]. This combination makes the application of biological processes to the treatment of industrial wastewater doubly interesting.

High salt concentrations have negative effects on organic matter, as well as nitrogen and phosphorous removal [9]. However, several halo-tolerant nitrifying and denitrifying bacteria have been isolated and identified in hypersaline waters [10,11]. Glass and Silverstein [7] provide a good review of previous work in this area, and report on both successful and unsuccessful attempts to acclimate activated sludge for treatment of high-nitrate and high-saline wastewater.

In recent years there have been considerable advances in submerged fixed-film reactor technology for the removal of nutrients, and there are currently different configurations, which may be adapted to the effluents treated [12]. Water treatment by submerged fixed-film filter technology requires the formation of a biofilm around an inert substance. In urban wastewater it is possible to form a biofilm from the influent to be treated, owing to the presence of a high microbial loading [15]. However, with industrial wastewater containing extreme concentrations of pollutants, it is necessary to prepare the biofilm previously, and on occasion to allow a period of time for acclimation of the microbial mass [7]. This is a complicating factor in the biological treatment of industrial wastewater.

This article discusses data collected during the final experimental phase of a study on the nitrogen removal capacity of a lab-scale submerged fixed-film reactor for treating industrial effluent with high phenol concentration and high salinity. Operating with down-flow for denitrification and up-flow for nitrification (pre-denitrification configuration), the submerged fixed-film reactor was packed with clayey schists from recycled construction material. The aim of the experiment was to study the effects of influent flow, air loading and effluent recirculation on the removal of nitrogen, COD and phenol from effluent with high salinity.

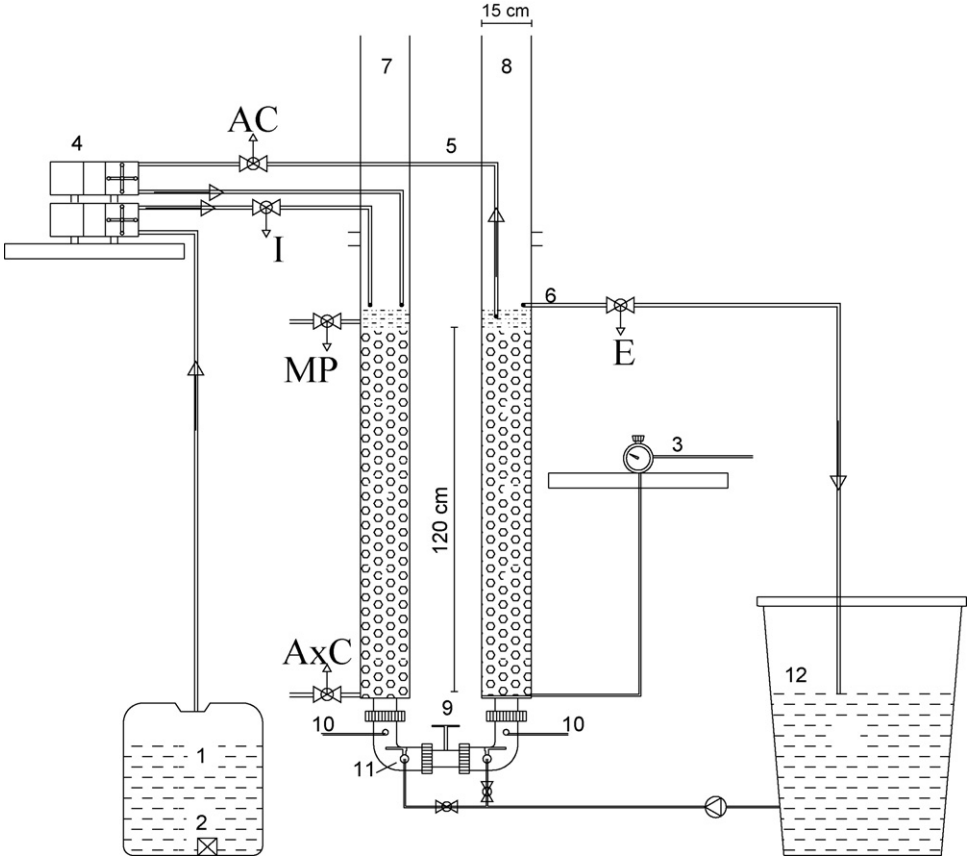

Fig. 1. Lab scale plant process: (1) influent; (2) agitator; (3) air process rotameter; (4) peristaltic pump; (5) recirculation; (6) effluent; (7) anoxic column; (8) aerobic column, (9) valve; (10) inlet air cleaning; (11) inlet water cleaning; (12) outlet water (cleaning water). (I) influent; (MP) influent and effluent recirculation mixed place; (A × C) anoxic column outlet water; (AC) aerobic column outlet water; (E) effluent.

2. Materials and methods

2.1. Lab-scale-plant submerged filter

A lab-scale submerged fixed-film reactor was used in this study (Fig. 1). The lab-scale plant consisted of two identical plexiglass cylindrical columns in series (both 1.4 m high and 15 cm in diameter), operated under conditions of predenitrification. The first biofilter was anoxic and operated downflow, removing organic matter to achieve denitrification (denitrifying column). This column was connected by communicating vessels to an up-flow aerobic filter (nitrifying column) in which nitrogen forms were oxidized to nitrate. The nitrogen oxides formed in this way then passed to the anoxic column by means of internal recirculation to be reduced to nitrogen through denitrification. The system was packed with clayey schists from recycled construction materials up to a height of 1.2 m, reaching an effective volume of 21 l in each column. (Packed material was catalogued as residue by Spanish and European legislation CER 170103) and 3.5 l as headspace. Both columns had systems, which allowed them to be cleaned by both air and water. They were also separated by a valve so that each system was able to clean independently.

2.2. Chemical industrial wastewater

The wastewater used in this experiment was taken from DERETIL S.A., a chemical industrial plant in Almería (Spain). The main activity of this factory is to produce and market antibiotics (β-lactamics). The production processes involved generate diverse types of wastewater of varying composition, but all are characterized by high concentrations of nitrogen and organic material, as well as by a high phenol content. The resulting effluent has a high pollutant loading which complicates the application of biological treatment. Accordingly, the residual effluent was diluted with sea-water, which is readily available because of the coastal location of the factory. The influent treated in our study was obtained in this way. (See Table 1 for characteristics.)

The influent was taken daily from the industrial plant and maintained at 4 °C before its use in the experimental system. Before its treatment, it was tempered for 1 h at 20–25 °C, the average annual temperature at the location of the wastewater-generating plant and at which all of the experiments were performed.

Table 1
Characteristics of diluted chemical industrial wastewater

Parameters	Concentration (range)
pH	7.32 ± 0.17
Cl^-	25 ± 3 g/l
Salinity	3.4 ± 0.2%
Chemical oxygen demand	2800 ± 360 mg COD/l
Suspended solids	35 ± 30 mg/l
Total nitrogen as N	400 ± 25 mg N/l
Ammonia nitrogen as NH_4^+	340 ± 45 mg N/l
Phenol	1000 ± 150 mg/l

Table 2
Operational parameters and capacity of tested units

Influent flow rate	10, 12 and 15 l/d
Air flow rate	6.8, 13.6 m^3/m^2 h
Effluent recirculation rates (percentaje of the total daily flow rate)	300, 400, 600%
Room temperature	20–25 °C
Column height	2.0 m
Column diameter	15 cm
Bed column height	1.2 m
Column cross-section area	0.0176 m^2
Single bed volume	0.0212 m^3
Support material surface	≈400 m^2/m^3
Support material porosity	0.56
Support material mean diameter	5–7 mm
Support material density	1.75 g/cm^3

2.3. Experimental procedure

Before our study could be carried out, it was necessary for biofilm formation to occur. Accordingly, the influent to be treated was maintained in recirculation at an entry flow rate of 5 l/day, with 100% of the daily total flow rate as internal recirculation and 12 m^3/m^2 h air loading in the aerobic column. The micro-organism growth and biofilm were monitored as well as the concentration of the different pollutants. Once the biofilm had formed and the concentration of pollutants in the effluent was constant (steady state conditions), treatment of the influent was initiated. This process lasted approximately 25 days.

The system was operated under different values with respect to influent flow rate, air loading and recirculation. Running conditions involved a combination of the parameters shown in Table 2. Once steady state conditions had been reached, testing was carried out for a period of 2 weeks for each of the running conditions assayed.

During operation, loading loss was monitored by measurement of water lamina height, the resulting values of which indicated the need for biofilter washing. For this purpose, the packing was loosened by means of water and air in cross-current, first by a constant flow of air (80 m/h) and secondly, by a rising flow of water (30 m/h) and air (60 m/h) for 1 min. Finally, a constant rising flow of water (20 m/h) was applied for 2 min to eliminate the remaining biofilm [13]. The concentration of suspended solids in the discharge from the washing was also monitored in order to determine differences in biomass between the columns.

2.4. Analytical procedures

During the biofilm formation phase, bacterial growth was monitored by measuring the turbidity and total culturable bacteria in the recirculated water. A spectrophotometric method (650 nm) was used to determine turbidity. Total bacteria were counted by means of the dilution plate technique, using trypticase soy agar (TSA, Difco; Franklin Lakes, USA). The inoculated agar plates (three replicates) were incubated at 22 ± 1 °C for 2 days before the colonies were counted.

After inoculation, biofilm formation in the submerged fixed-film reactor was confirmed by scanning electron microscopy. Support material was collected from different points on the column. Next, cells from the biofilm were fixed immediately with 3% glutaraldehyde for 2 h, then rinsed and treated with 1% osmium oxide for 3 h. Subsequent dehydration included rinsing and retention in a graded ethanol series (30%, 50%, 70%, 90% and 100%). Finally, the samples were dried to the critical point and mounted on support stubs for inspection by a Hitachi scanning electron microscope without gold coating.

Every 24 h, water samples of 200 ml were collected from four different locations: (I) influent; (MP) influent and effluent recirculation mixed place; (A × C) anoxic column outlet water; (AC) aerobic column outlet water; and (E) effluent. Fig. 1 shows sample points. All samples were monitored for ammonium, nitrite, nitrate, COD, phenol and total nitrogen concentration.

For ammonium, nitrate and nitrite analyses, water samples were filtered through 0.22 μm membrane filters (HAWP; Millipore Massachusetts, USA) and subsequently measured by an ion-chromatography system using conductivity detection (Dionex® DX-300; Dionex Corporation, Sunnyvale, USA). Before measurement, the filtered samples were diluted to achieve ammonium, nitrate, and nitrite concentrations lower than 10 mg/l. Before IC analysis, samples were again filtered using a silver-impregnated filter (Dionex®, Sunnyvale, CA) to eliminate chloride ion interference with nitrite measurement. To determine total nitrogen, 50 ml of unfiltered diluted sample (1/10) was oxidized at 120 °C for 30 min in the presence of boric acid and perchloric acid. The result of the oxidation was analyzed in a similar fashion to the nitrate analyses. The Chemical Oxygen Demand was measured using the COD closed reflux micro method [14]. Absorbance of the digestate was measured colorimetrically at 600 nm, and the COD concentration was calculated from a calibration curve, prepared with potassium acid phthalate. Chloride interference was avoided by means of silver nitrate. To measure phenol concentration, gas chromatography was used, equipped with a capillary column and a flame ionization detector, using helium as a carrier gas. The pH and dissolved oxygen levels in the effluent were measured using a pH meter and an OXI 921 electrode (Crison®: Crison, Madrid, Spain), respectively.

3. Results and discussion

3.1. Biofilm formation and system start-up

An exponential bacterial growth was observed in the mixture of sea water and recirculating influent. This exponential micro-organism growth was reached in 48 h, resulting in the obtention of a number of bacterial cells up to 10^8 cfu/ml after 72 h of recirculation. This high bacterial loading was sufficient for the formation of a biofilm on the support material, which in addition to facilitating the pre-inoculation phase, provided a clear indication of the possibility of applying a biological process to treat the influent.

Previous studies of sea water from the area of industrial effluent discharge revealed the presence of micro-organisms capable of degrading phenol and with denitrifying activity [11]. Since the micro-organisms were adapted to the conditions of the influent, there was no need to carry out acclimation processes, although these are normally required in the biological treatment of industrial effluents with characteristics similar to the experimental effluent [7]. Accordingly, it was decided that the inoculant should consist of the industrial wastewater to be treated, diluted with sea water from the area of industrial discharge, which would provide micro-organisms adapted to the pollutants to be eliminated.

Biofilm formation in the submerged fixed-film reactor after inoculation was confirmed by scanning electron microscopy. The results showed that the support material that had been chosen was eminently suitable, and that no pre-inoculation was necessary for biofilm generation. Fig. 2A shows the support material prior to injection and in the absence of biofilm. The clayey material presents a rugged surface ideal for biofilm formation. Fig. 2B and C shows the biofilm developed after inoculation on the support material of the anoxic and aerobic column, respectively. In both cases it is possible to observe the formation of a heterogeneous and porous biofilm, with the characteristic structure of a water channel [16]. The aerobic column biofilm presents greater density. Fig. 2D and E show amplified versions of the images of the biofilms in the anoxic and aerobic column, respectively, allowing us to view the cellular morphology of biofilm components. In both cases rod-shaped forms predominate, although there is greater morphological diversity in the anoxic column. These images confirm the impression of greater bacterial density in the aerobic column.

The different density of the two biofilms was confirmed by analysis of the composition of post-washing discharge. Washing was performed after detection of a loss of loading in the system, which was consistently more accentuated in the aerobic column, with a frequency that was four times greater than that of the anoxic column. The quantities of biomass removed after washing revealed a formation of 2.5 ± 0.72 g/d of biomass in the anoxic column, as opposed to 12.2 ± 1.8 g/d in the aerobic column. The difference in the density and morphology of the biofilms in the anoxic and aerobic columns was motivated by differences in the oxygen and phenol concentrations found in each.

Differences in biofilm composition have been shown to affect the capacity for pollutant elimination [17]. However, such differences may also be due to different conditions prevailing in the medium to which the biofilm adapts without any adverse effect on its de-polluting capacity. Further studies are now being undertaken to determine the relation between biofilm composition and characteristics and the capacity for transformation or elimination of pollutants, as well as to assess the influence of running conditions on the composition and characteristics of biofilms.

3.2. COD and phenol removal

Once the scanning electron microscopy had confirmed biofilm formation, experimental values for influent flow rate, air loading and recirculation were established. Under these condi-

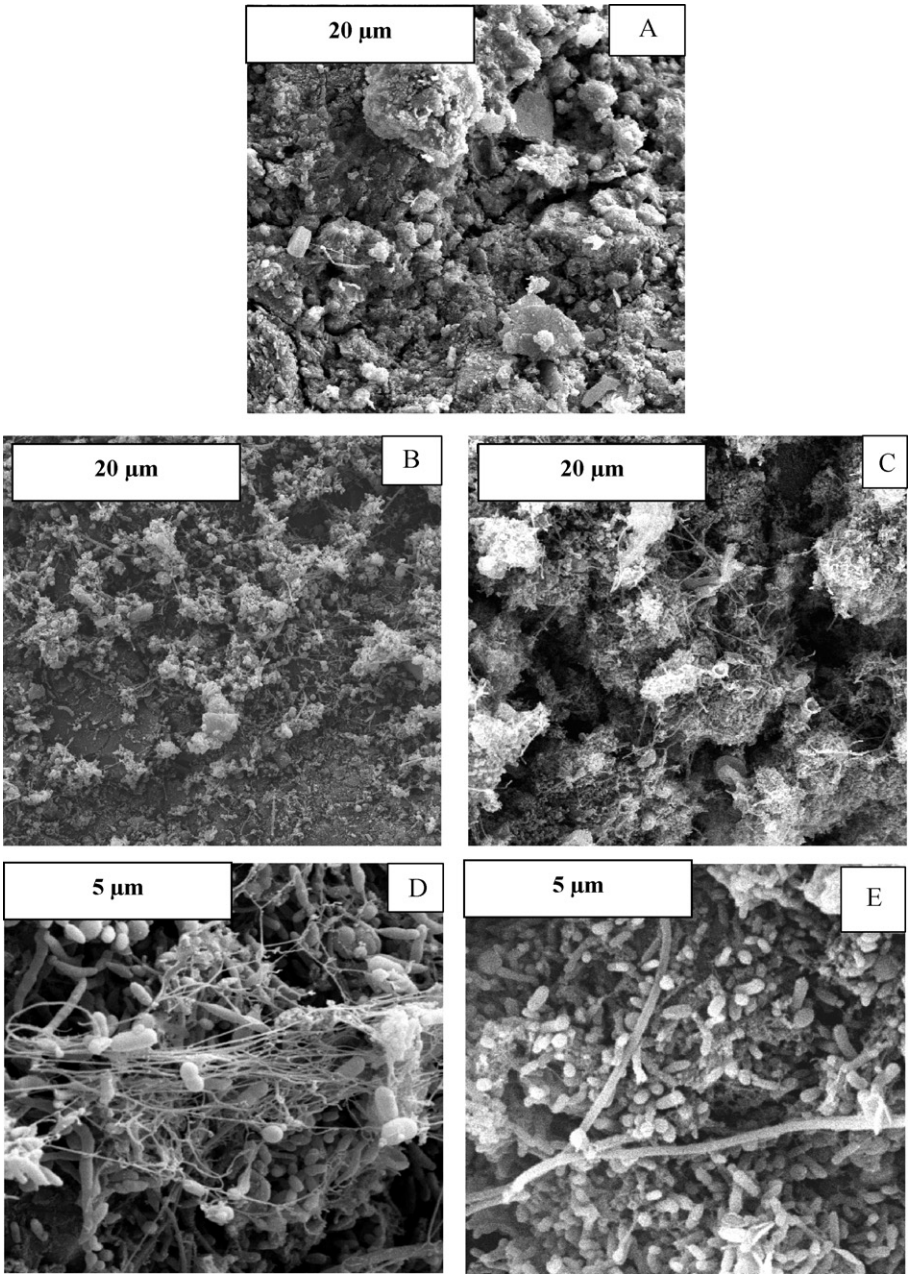

Fig. 2. Scanning electron microscopy: (A) support material before inoculation; (B) and (D) anoxic column support material with biofilm formed after inoculation; (C) and (E) aerobic column support material with biofilm formed after inoculation.

tions, concentrations of COD, phenol and the different forms of nitrogen (ammonium, nitrite and nitrate) were monitored at the established sample points. When it was observed that concentrations had stabilized, study of the system was initiated. Stable state conditions were obtained similarly for all test achieved (about 6–7 days) with no statistical significant differences between the different experimental conditions. This procedure was repeated for each of the tests undertaken.

Submerged filters have been shown to be highly efficient at removing organic material from wastewater [18]. The present system was no exception, presenting an average reduction of COD concentration in the effluent with respect to the influent of $95.75 \pm 0.72\%$.

Fig. 3 shows balances for COD (g/d in each sample point) for each of the tests undertaken. COD loading increases with the increase in influent flow rate, but the system is shown to be capable of absorbing organic material, with average removal values ranging from 25.0 g COD/d for a flow rate of 10 l/d to 43.12 g COD/d for a flow rate of 15 l/d. There were no statistically significant differences between final effluent concentration for tests with different influent flow rates and air loadings ($p = 0.2457$ and 0.3214, respectively). However, there were statistically significant differences ($p = 0.0044$) between effluent COD concentration achieved with different recirculations. Our results show that there was a decrease in the final concentration as recirculation increased. COD is formed mainly by phenol

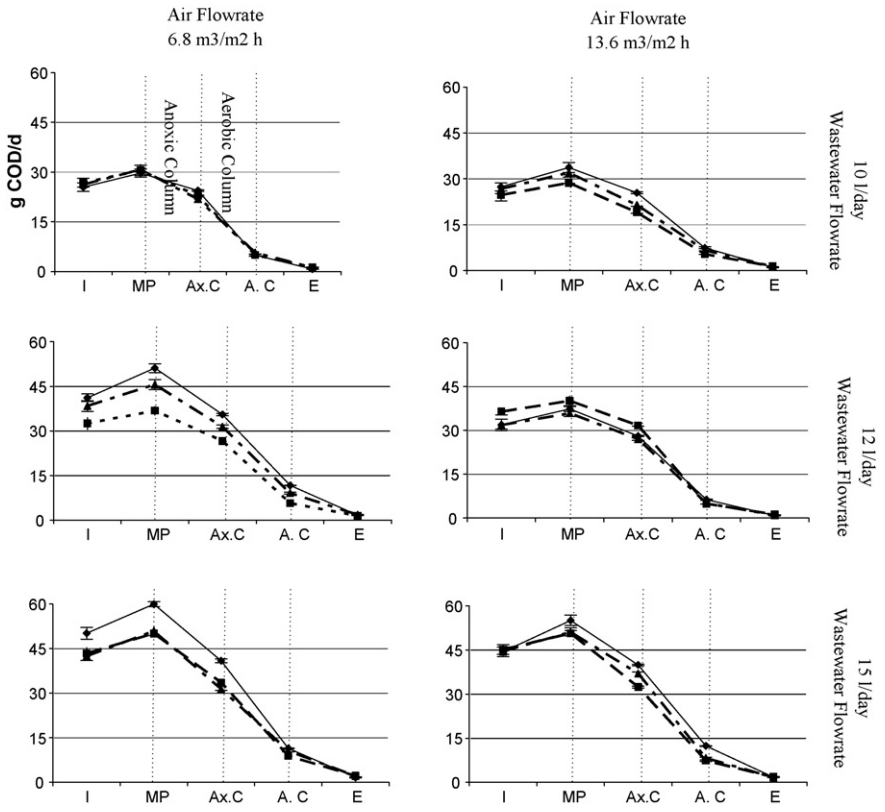

Fig. 3. COD Balances for different experimental running conditions in different points of the column: (I) influent; (MP) influent and effluent recirculation mixed place; (A × C) anoxic column outlet water; (AC) aerobic column outlet water; (E) effluent. (■) 300%, (▲) 400%, (♦) 600% of recirculation rate.

compounds in the industrial wastewater under study (Table 1). Experimentally, we determined that 1 mg/l of phenol corresponded to 2.21 mg COD/l, which is in consonance with values observed by Fang et al. [19]. This indicates that between 70 and 80% of the influent COD concentration corresponds to phenol. The results obtained for phenol concentration reveal a great capacity for eliminating this compound, similar to those for COD. The system is shown to adapt to the increases in pollutants resulting from the increase in influent flow rate, with an average elimination performance of 97.2% ± 1.23%.

Although phenol removal took place in both the anoxic and the aerated columns, in most cases the aerated column presented removal values 2.5 times greater than those of the anoxic column. In both columns phenol removal increased in accordance with the increase in influent flow rate. This demonstrates the adaptation of the system to increases in phenol loading; moreover, no toxic effect was shown in the activity of the biofilm with highest quantities of phenol in the system. Phenol compounds in the influent were rapidly degraded, eliminated or transformed into new biomass. Such rapid transformation was due to the fact that the types of micro-organisms forming the biofilm were taken from a medium perfectly adapted to phenol [11].

Transformation of phenol is possible in a denitrifying system in which the compound may act as an electron donor [8], although transformation is usually affected by the hydraulic retention time of the process [20]. Jou and Huang [21], reported that the elimination of phenol from industrial wastewater required the maintenance of high biomass activity, and this would seem to be achieved by the application of a submerged filter system with clayey schists with a granulometry of 5–7 mm.

COD concentration in the treated effluent presented an average concentration of 117 ± 34 mg COD/l, while average concentration of phenol was 30.4 ± 6.5 mg/l (67.2 ± 14.4 mg COD/l), with varying values principally dependent on recirculation. This indicates a phenol percentage lower than that observed in the influent (57%). Sá and Boaventura [22] found that during the process of biodegradation of phenol by *Pseudomana putida*, various metabolites highly resistant to degradation were produced. This would explain the variation of COD composition in the treated effluent, which may present a higher percentage of compounds of low biodegradability.

3.3. Nitrogen removal

In contrast to the results for the removal of organic materials, significant statistical differences ($p = 0.001$) were observed for total nitrogen removal under the different running conditions assayed. It may thus be concluded that the different variables considered in this study affect the capacity for nitrogen removal from industrial wastewater.

Fig. 4 shows the results obtained for nitrogen with an air loading of 6.8 m^3/m^2 h. In all cases, nitrification is shown to be deficient, with a maximum nitrogen oxidation of 26% for tests

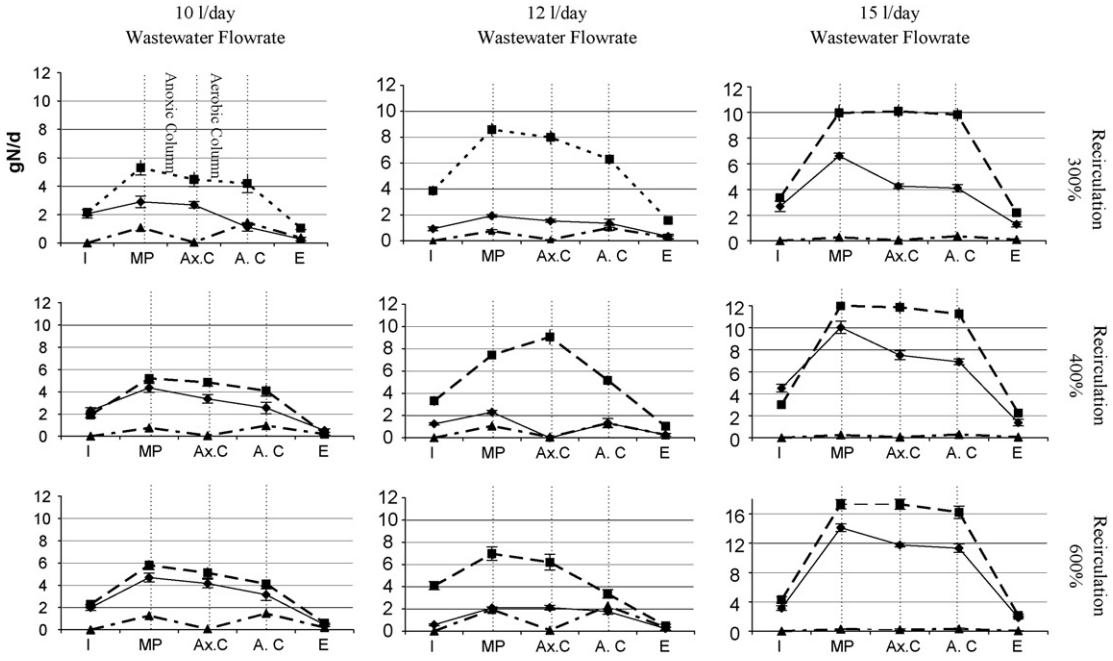

Fig. 4. Balances for the different forms of nitrogen: (♦), N-organic nitrogen; (■) $N–NH_4^+$, (▲) $N–NO_3^-$ for an air loading of 6.8 m^3/m^2 h, corresponding to the different influent flow rates and recirculation rates assayed in different points of the column: (I) influent; (MP) influent and effluent recirculation mixed place; (A × C) anoxic column outlet water; (AC) aerobic column outlet water; (E) effluent.

with an influent flow rate of 12 l/d and 600% recirculation, and minimum nitrogen oxidation of 1% for tests with an influent flow rate of 15 l/d. Consequently, there was a low transfer of nitrate to the anoxic column.

In spite of the somewhat poor performance for nitrification, the system demonstrated significant total nitrogen removal, with average levels varying between a minimum of 41% for tests with an influent flow rate of 15 l/d and 300% and a maximun of 77% of the total amount of nitrogen in the system for tests with an influent flow rate of 12 l/d and 600%. Removal took place in both columns, although the anoxic column presented a superior performance with reductions between 1.63 and 2.82 g N/d, as opposed to between 0.12 and 1.2 g N/d in the aerated column.

When air loading was increased to 13.6 m^3/m^2 h a significant improvement in nitrification was observed (Fig. 5), with a minimum nitrogen oxidation of 10% for the tests with an influent flow rate of 15 l/d and 300% recirculation, and maximum nitro-

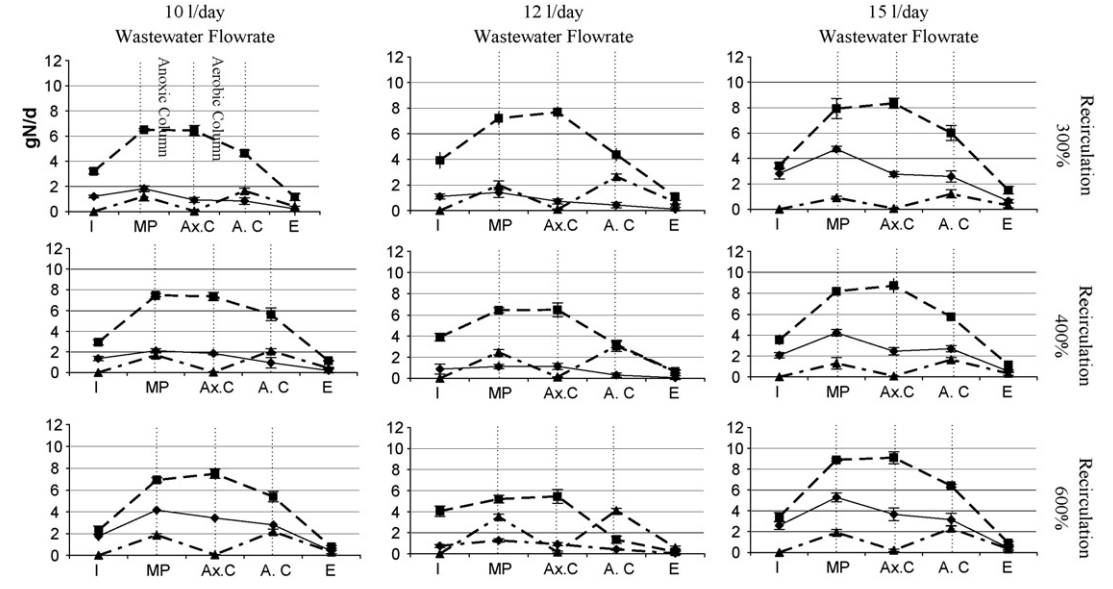

Fig. 5. Balances for the different forms of nitrogen: (♦), N-organic nitrogen; (■) $N–NH_4^+$, (▲) $N–NO_3^-$ for an air loading of 13.6 m^3/m^2 h, corresponding to the different influent flow rates and recirculation rates assayed in different points of the column: (I) influent; (MP) influent and effluent recirculation mixed place; (A × C) anoxic column outlet water; (AC) aerobic column outlet water; (E) effluent.

gen oxidation of 63% for the tests with an influent flow rate of 12 l/d and 600% recirculation. In this conditions, a higher transfer of nitrate to the anoxic column was observed, where it was completely removed from the system.

As a consequence of the higher nitrification, total nitrogen removal was also higher, with a minimum values of 58% for tests with an influent flow rate of 10 l/d and 300% recirculation and a maximum of 83% of the total amount of nitrogen in the system for tests with an influent flow rate of 12 l/d and 600% recirculation. Again, elimination was more significant in the anoxic column, where values ranged from 2 to 3.45 g N/d, while in the aerobic column values were similar to those obtained with the lesser air loading, ranging from 0.3 to 1.38 g N/d.

Due to the dependence of nitrification on dissolved oxygen concentration [23], increasing the system air loading led to a corresponding increase in the elimination of total nitrogen, given that in our system increases in air loading signified a direct increase in the amount of oxygen. This naturally meant a corresponding increase in the transfer of nitrate to the anoxic column, where there was higher nitrogen removal. For this reason, statistically significant differences ($p = 0.001$) were found in nitrogen removal, depending on the air loading. In approximate terms, the levels required for nitrifying the total nitrogen in the influent were half those required for oxidizing the total COD. An air loading of 6.8 m^3/m^2 h provided sufficient oxygen for the oxidation of organic material, but not for nitrogen oxidation. However, when the air flow rate was doubled, nitrification reached a level of only 63%, indicating that other factors must affect the process. Both salinity [9] and the presence of phenol [24] have been shown to be inhibitors of nitrification, and this may explain why the process was limited in our system.

In pre-denitrifying systems, internal recirculation represents a physical limitation to the overall performance of the process. Thus for a recirculation of 300%, the best denitrification result was 75%, while for a recirculation of 600% the best result was 85.7%. This might encourage the application of higher recirculation values with a view to obtaining better levels of performance. However, a limiting factor is posed by the greater energy consumption involved in higher recirculation. Moreover, the quantities of residual oxygen entering the anoxic column by means of the recirculated water may lead to an inhibition of the denitrifying activity.

Recirculation was the variable with greatest influence on the process of nitrogen removal, with a linear relation between increase in recirculation and total nitrogen removal in the system, independently of the influent flow rate and air loading. This influence was also observed in nitrification, particularly in tests with an influent flow rate of 12 l/d and higher air loading, where the accumulation of nitrate in the aerobic column increased in accordance with the increase in recirculation. Dilution of phenol concentration achieved by recirculation reduces the toxic effect of this compound on nitrification, explaining the better performance obtained for nitrification when recirculation is increased.

Increases in recirculation also promoted the transfer of nitrate to the anoxic column, and with it the nitrogen removal in this column, whose greater effectiveness was directly linked to the elimination of nitrate. In this way, the influence of recirculation was reflected both in the performance and in the net removal of total nitrogen. This demonstrates the importance of internal recirculation in a pre-nitrifying system, in which the nitrate formed in the aerated column has to re-circulate to the anoxic column for transformation by means of denitrification, while at the same time the influent contaminant load is diluted.

Under these running conditions, residual oxygen, which accumulates as air loading and recirculation are increased, did not represent a limitation in our system, even when operating with maximum air loading and recirculation. Similarly, authors such as Punzava et al. [25], who combined processes of nitrification and denitrification in the same biofilm reactor, observed that denitrification occurred in spite of the presence of oxygen.

The system presented greater elimination of nitrogen in the anoxic column, to which the nitrate formed in the aerobic column is transferred. For tests with an influent flow rate of 12 l/d and an air loading of 13.6 m^3/m^2 h, nearly all the nitrogen eliminated in the anoxic column was due to the elimination of nitrate. In turn, it is reasonable to suppose that the nitrate elimination is mainly due to heterotrophic denitrification. This seems likely bearing in mind that the anoxic column eliminated phenol in quantities superior to the stoichiometric quantities required for the disassimilatory reduction of nitrate using phenol as an electron donor [8], while the presence of residual oxygen is of little significance given the dimensions of the column. In contrast, most of the nitrogen eliminated in the aerobic column is due to assimilation, since the high quantity of oxygen constantly provided by the air loading would limit processes of nitrogen removal such as denitrification.

For the remaining tests in which nitrification was less significant, nitrogen was eliminated in the anoxic column to a greater extent than can be explained by denitrification. Part of this nitrogen removal may be due to the assimilation of nitrogen in the biomass. However, production of biomass in this column was markedly inferior to that of the aerobic column (five times less), suggesting that the quantities of assimilated nitrogen must be less. Such mismatches in the balances are more pronounced in tests in which nitrification was very low, in spite of which the anoxic column maintains a significant nitrogen removal.

These results led us to suspect that other mechanisms were responsible for nitrogen removal in the anoxic column. As a result of recirculation, the column receives a constant supply of residual oxygen, which does not affect the supposed denitrification. At the same time, ammonium supplied by the influent is also present. Considering these conditions, partial nitrification may take place, with the formation of nitrite in conditions of limited oxygen [26]. Nitrite was not detected in significant quantities in either of the two columns, suggesting that the mechanism involved may be anaerobic ammonium oxidation (Anammox) as described by Jetten et al. [27]. This may also be the mechanism for eliminating nitrate [28].

Our studies at lab-scale give useful information for planning a new treatment plant at full scale. Biological treatment, without previous inoculation or acclimation phase, is possible for industrial wastewater with a high concentration of phenol and high salinity. Optimal operational conditions and the maximun COD,

phenol and nitrogen removal is given together with information about microbial biofilm formation. However test conditions at lab-scale are ideal (stable influent, high dissolvel oxigen level, etc.) compared with full scale [29]. So, other studies at pilot-scale, working with wastewater directly from the production processes, are necessary for giving the achieved design data more reliability.

4. Conclusions

The data obtained in our study shows that submerged biofilter technology is an efficient system for treating industrial wastewater with high nitrogen content, high salinity and high phenol concentrations. As such, it offers an interesting alternative to other types of treatment, such as physical–chemical and activated sludge processes. Specifically, the following conclusions were reached in the course of the experiment:

1. Treatment of industrial wastewater with a high concentration of phenol and high salinity may be undertaken using biofilm systems without any need for previous inoculation or acclimation. Dilution of the industrial wastewater with sea-water from the discharge area facilitates the rapid formation of an active biofilm on the influent.
2. The system demonstrated excellent capacity for eliminating the high concentration of phenol (which also implies high COD elimination) from industrial effluent with high salinity. Under the COD loading conditions of the experiment, a 6.8 m^3/m^2 h air flow rate and 300% recirculation are sufficient for COD and phenol removal.
3. Nitrification was shown to be a limiting process for the selection of system running conditions. Nitrification was affected principally by air loading, given the greater need for oxygen, and by system recirculation because of the dilution of pollutant concentrations such as phenol.
4. Nitrogen removal from the experimental industrial wastewater by means of submerged filter-bed technology combined with a pre-denitrifying system can be explained mainly by processes of nitrification heterotrophic–denitrification and nitrogen assimilation. However, both processes are not sufficient in themselves to explain the nitrogen removal shown in the measurements of the system.

In this type of system, microbial fixation and biofilm formation on the support surface are two of the most important factors, since they affect the levels of elimination of every pollutant. It is also important to use an inoculant appropriate for biofilm formation, which should be stable and adapted to the characteristics of the influent to be treated.

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