Effects of initial alkalinity and elemental sulfur: dolomitic limestone ratio on autotrophic denitrification of poultry wastewater

Efeito da alcalinidade inicial e proporção de enxofre elemental: calcário dolomítico na desnitrificação autotrófica de efluente avícola

Efecto de la alcalinidad inicial y de la relación azufre elemental: caliza dolomítica en la desnitrificación autótrofa de efluentes avícolas

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ABSTRACT
Autotrophic denitrification from elemental sulfur is an alternative to heterotrophic denitrification for substrates with a low C/N ratio. In this process, nitrogen compounds are reduced from elemental sulfur instead of carbon, as in the conventional process. However, it may be limited by the low concentration of alkalinity in these effluents. Thus, this study evaluated the performance of autotrophic denitrification of nitrified poultry effluent in four fixed bed reactors with a ratio of elemental sulfur/dolomitic limestone (1:0, 3:1, 1:1, 1:3 v/v) under conditions of the decline of initial alkalinity (1000, 800, 600, 400 and 200 mg CaCO₃ L⁻¹). Denitrification efficiencies greater than 84.8% were observed in conditions of initial alkalinity greater than 600 mg CaCO₃ L⁻¹ in the four reactors, associated with maximum yields of 445.1 mg SO₄²⁻ L⁻¹. The slow dissolution of dolomitic limestone may have impaired denitrification efficiency in conditions of initial alkalinity lower than 600 mg CaCO₃ L⁻¹. The autotrophic denitrification process proved viable for the treatment of nitrified effluent, and its efficiency was linked to the food substrate and the dissolution of dolomitic limestone.

Keywords: Biofilm, Nitrate, Poultry Sector, Sodium Bicarbonate, Sulfate, Wastewater Treatment.
autotrófica mostrou-se viável para o tratamento de efluentes nitrificados, e sua eficiência esteve relacionada às características do substrato e à dissolução do calcário dolomítico.

**Palavras-chave:** Biofilme, Nitrato, Setor Avícola, Bicarbonato de Sódio, Sulfato, Tratamento de Águas Residuárias.

**RESUMEN**
La desnitrificación autótrofa a partir de azufre elemental es una alternativa a la desnitrificación heterótrofa para sustratos con una relación C/N baja. En este proceso, los compuestos de nitrógeno se reducen a partir de azufre elemental en lugar de carbono, como en el proceso convencional. Sin embargo, puede verse limitada por la baja concentración de alcalinidad en estos efluentes. Por ello, este estudio evaluó el rendimiento de la desnitrificación autótrofa de efluentes avícolas nitrificados en cuatro reactores de lecho fijo con una relación azufre elemental:caliza dolomítica (1:0, 3:1, 1:1, 1:3 v/v) en condiciones de alcalinidad inicial decreciente (1000, 800, 600, 400 y 200 mg CaCO₃ L⁻¹). Se observaron eficiencias de desnitrificación superiores al 84,8% en condiciones de alcalinidad inicial de más de 600 mg CaCO₃ L⁻¹ en los cuatro reactores, asociadas a rendimientos máximos de 445,1 mg SO₄²⁻ L⁻¹. La lenta disolución de la caliza dolomítica puede haber perjudicado la eficiencia de la desnitrificación en condiciones de alcalinidad inicial inferiores a 600 mg CaCO₃ L⁻¹. El proceso de desnitrificación autótrofa demostró ser viable para el tratamiento de efluentes nitrificados, y su eficiencia estuvo relacionada con las características del sustrato y la disolución de la caliza dolomítica.

**Palabras clave:** Biopelícula, Nitrato, Sector Avícola, Bicarbonato Sódico, Sulfato, Tratamiento de Aguas Residuales.

**1 INTRODUCTION**

Without proper treatment, the release of nitrogenous compounds into receiving waters can trigger a process of eutrophication of these same waters. This process, therefore, poses a potential threat to both the natural environment and human health. In this sense, conventionally, biological nitrogen is removed by autotrophic nitrification followed by heterotrophic denitrification. In these processes, the ammoniacal nitrogen (NH₄⁺) is oxidized to nitrate (NO₃⁻) in the aerobic phase and subsequently reduced to nitrogen gas (N₂) in the anoxic phase. In the effluent from the anaerobic treatment, remaining organic matter, characterized by low degradability, is detected at low concentrations. Therefore, adding exogenous carbon (C) sources, such as electron donors,
is necessary for effective heterotrophic denitrification. Methanol is a commonly used exogenous source of C, which contributes to the increased system operating costs. (Wang et al., 2021; Metcalf & Eddy 2015).

Thus, new wastewater treatment methods have been developed, including simultaneous nitrification and denitrification (SND), endogenous denitrification (ED), simultaneous removal of nitrification, denitrification and phosphorus (SNDPR), anaerobic ammonia oxidation (Anammox), nitrification partial/ denitrification (PN/D), autotrophic sulfur denitrification (SADN) and autotrophic hydrogen denitrification (HD). Autotrophic denitrification based on reduced sulfur compounds has been reported as an alternative to heterotrophic denitrification in the treatment of substrates characterized by high nitrate/nitrite concentrations and low organic matter content. This process has been widely used in the treatment of low C/N wastewater such as groundwater, drinking water, urban sewage, aquaculture effluent, and industrial wastewater (Hu et al. 2023; Wang et al. 2022; Tian & Yu 2020; Zhou et al. 2011).

In this process, communities of the genera *Thiobacillus* and *Thermomonas* capable of performing autotrophic denitrification are found, which use inorganic compounds such as sulfide (S$^{2-}$), elemental sulfur (S$^{0}$), and thiosulfate (S$_{2}$O$_{3}$$^{-2}$) as electron donors for denitrification (Pan et al. 2022; Beristain-Cardoso et al. 2006; Wang et al. 2020). Among reduced sulfur compounds, the use of elemental sulfur is prominent because of its low cost relative to commercial organic electron donors and features such as non-toxicity (Park & Yoo 2009; Moon et al. 2006). In reducing nitrate to nitrogen gas, elemental sulfur is oxidized to sulfate through the process shown in Equation 1 (Batchelor & Lawrence 1978) as follows:

\[
1.1S^{0} + NO_{3}^{-} + 0.4CO_{2} + 0.764H_{2}O \rightarrow 0.5N_{2} + 1.1SO_{4}^{2-} + 1.28H^{+} + 0.08C_{5}H_{7}O_{2}N \quad \text{Equation 1}
\]

Compared to heterotrophic denitrification, the primary advantages of autotrophic denitrification include lower sludge production and less rigid dosage control of electron donors (Wang et al. 2020). Biomass yields reported for autotrophic denitrifying microorganisms are 4000-5700 mg volatile suspended solids (VSS) g NO$_{3}^{-1}$. However,
for denitrifying heterotrophic organisms, the results are 8000-12000 mg VSS /mg N-NO$_3^-$ \(^1\) (Oh et al. 2000).

Although autotrophic denitrification has benefits, using elemental sulfur as the electron donor can result in high sulfate production and alkalinity consumption (Tian & Yu 2020; Sahinkayaet al. 2011). It is estimated that at least 45700 mg CaCO$_3$ and 75000 mg SO$_4^{2-}$ are consumed and produced respectively per milligram of reduced nitrate (Sahinkaya et al. 2011; Sahinkaya & Kilic 2014).

Because of the consumption of alkalinity in the process, some researchers have used limestone to provide alkalinity and inorganic carbon for microorganisms (Sahinkaya et al. 2011; Zhou et al. 2011) and to maintain the pH in the desired range of 6.8 to 8.2 (Matsuo et al. 2001). In this application, the availability of alkalinity by the dissolution of limestone may depend on the characteristics of the limestone, the pH of the solution and the initial alkalinity of the substrate.

The autotrophic denitrification in reactors containing elemental sulfur and limestone as the fixed bed has been used effectively to treat synthetic substrates and groundwater contaminated with nitrates (Moon et al. 2006; Sahinkaya et al. 2011; Sahinkaya & Dursun, 2012). However, there are few reports of discussions about the effect of initial alkalinity present in substrates on limestone dissolution and application in real effluents.

The application of autotrophic denitrification using elemental sulfur to nitrified effluents is likely to be favorable because of the low C/N ratio of the effluents. However, the performance of the process may be limited because of low alkalinity. Therefore, the investigation of the use of supplementary sources of alkalinity and the effect of changes in influent alkalinity is necessary. In this context, the purpose of this study is to evaluate the performance of autotrophic denitrification of nitrified poultry wastewater in a fixed-bed reactor of elemental sulfur/dolomitic limestone in ratios of 1:0, 3:1, 1:1, and 1:3, operating under varying conditions of alkalinity, i.e., 1000, 800, 600, 400 and 200 mg CaCO$_3$ L$^{-1}$. 
2 METHODS

2.1 CONFIGURATION OF THE REACTORS AND THE OPERATING CONDITIONS

The experimental setup consisted of four cylindrical fixed-bed reactors constructed of polyvinyl chloride and operated in continuous ascending flow. The volume of each reactor, with an internal diameter of 75 mm and a length of 750 mm, was 1.268 L, distributed in three chambers, the inlet chamber (0.165 L), the reaction bed (0.914 L), and the outlet chamber (0.188 L), as shown in Figure 1.

Elemental sulfur (99% and $d = 2.1 \text{ g cm}^{-3}$) and dolomitic limestone (CaO = 31.8%, MgO = 22.7%, and density $d = 1.8 \text{ g cm}^{-3}$) with particle sizes between 9.5 and 12.5 mm were used as fixed beds, so the porosity of the beds was $47 \pm 3\%$. The volumetric elemental sulfur/dolomitic limestone ratios used in the reactors were 1:0 (R1), 3:1 (R2), 1:1 (R3), and 1:3 (R4) as shown in Table 1.
Table 1. Compositions of the reactor beds

<table>
<thead>
<tr>
<th>Reactor (Elemental sulfur/dolomitic limestone)</th>
<th>R1 (1:0)</th>
<th>R2 (3:1)</th>
<th>R3 (1:1)</th>
<th>R4 (1:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The volume of sulfur (%)</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Elemental sulfur (g)</td>
<td>2.361</td>
<td>1.784</td>
<td>1.149</td>
<td>600.1</td>
</tr>
<tr>
<td>Dolomitic limestone (g)</td>
<td>0</td>
<td>723.3</td>
<td>1.446</td>
<td>2.165</td>
</tr>
</tbody>
</table>

Source: Authors

Peristaltic pumps were used to supply the reactors from a feeding container maintained at 4°C by refrigeration. The reactors were placed in a temperature-controlled chamber at 29 ± 1.2°C with a hydraulic retention time (HRT) of 14 hours and an applied nitrogen load of 114000 mg NO₃⁻ m⁻³ d⁻¹.

Five initial alkalinity conditions were evaluated: 1000 (31 days), 800 (28 days), 600 (32 days), 400 (30 days), and 200 mg CaCO₃ L⁻¹ (29 days). The alkalinity was adjusted by adding sodium bicarbonate (NaHCO₃).

2.2 CHARACTERIZATION OF THE SUBSTRATE

The nitrified effluent from a poultry processing plant was used as the input substrate. Initially, the predominantly ammonified effluent was collected at the output of the first anaerobic lagoon of the plant’s treatment system. The effluent was then nitrified in sequencing batch reactors (SBRs), with useful volumes of 6 L at 28 ± 0.9°C and a cycle time (CT) of 24 hours (inoculation with aerobic sludge present in poultry wastewater pond, with 2.9 g VSS L⁻¹).

The nitrified effluent, which was used to feed the denitrifying reactors, was characterized as follows: pH = 5.1 ± 0.3; 59.3 ± 4.3 mg N-NO₃⁻ L⁻¹; 4.1 ± 3.2 mg N-NH₄⁺ L⁻¹; 3.2 ± 1.2 mg N-NO₂⁻ L⁻¹; 28.1 ± 4.8 mg SO₄²⁻ L⁻¹ and chemical oxygen demand (COD) = 97.8 ± 8.2 mg O₂ L⁻¹. The alkalinity was adjusted by adding NaHCO₃ according to the test parameters.
2.3 INOCULATION AND ACCLIMATIZATION

The inoculum for the autotrophic denitrification was obtained from sludge (14000 mg VSS L\(^{-1}\)) from the anaerobic compartment of an anaerobic-aerobic fixed-bed reactor and acclimatized in an SBR with a useful volume of 4 L operated at 30 ± 1.2°C with a CT of 12 hours.

The nitrified substrate supplemented with sodium thiosulfate (Na\(_2\)S\(_2\)O\(_3\)) was used to feed the SBR using a method adapted from Moon \textit{et al.} (2004) to establish an autotrophic community from the predominantly heterotrophic sludge. After eight weeks of operation, the enriched inoculum (12,000 mg VSS L\(^{-1}\)) was transferred to the four fixed-bed reactors at a ratio of 32% (v/v). The adherence of microorganisms to the bed was promoted by rest (14 h) and recirculation (24 h) of the substrate. The fixed-bed reactor was started up with the input of the nitrified substrate with initial alkalinity of 1000 mg CaCO\(_3\) L\(^{-1}\). The denitrifying biological activity was considered stable after the fifth week of operation with nitrate removal efficiencies greater than 90%.

2.4 SAMPLING AND ANALYTICAL MONITORING

After achieving the apparent equilibrium state, six samples were collected at each sampling point for each test condition. The temporal profiles were plotted from the samples collected at P1 and P2, i.e., the influent and the effluent samples. The performance indicators were denitrification efficiency (expressed in NO\(_x\) %, being NO\(_x\)=NO\(_3\)-+NO\(_2\)-), denitrification rate (kg NO\(_x\) m\(^3\)-1 d\(^{-1}\)), sulfate production (mg SO\(_4^{2-}\) L\(^{-1}\)), and the increment of alkalinity (mg CaCO\(_3\) L\(^{-1}\)).

The physical and chemical parameters measured were pH (4500 H\(^{+}\)), COD (5220 D), N-NO\(_2\)- (4500 NO\(_2\)- I), N-NO\(_3\)- (4500 NO\(_3\)- I), and N-NH\(_4\)+ (4500 C) following the methods described in the Standard Methods for the Examination of Water and Wastewater (APHA 2005). The concentrations of sulfate (SO\(_4^{2-}\)) (8051) and hydrogen sulfide in an aqueous medium (8131) were determined using Hach test kits and prepared following the recommendations of the APHA (2005). The total alkalinity was measured...
using the method by Ripley et al. (1986). The dolomitic limestone was characterized by X-ray fluorescence spectrometry (XRF) in terms of CaO and MgO at the beginning and end of the experiment when the reactors were opened.

3 RESULTS AND DISCUSSION

3.1 EFFICIENCY OF DENITRIFICATION

The reactors showed similar behavior under all five experimental conditions. The highest average denitrification efficiencies (84.8% to 94.9%) were found under the first three test conditions, showing a denitrification rate of 102000 ± 2000 mg NO₃⁻ m⁻³ d⁻¹ and apparent consumption of alkalinity greater than 244.8 mg CaCO₃ L⁻¹ for the four reactors. The apparent consumption term was used to denote the difference between the initial and final alkalinites; however, this difference cannot be considered the actual consumption because of the possible increase in alkalinity in the system. The denitrification efficiency and alkalinity consumption tended to decrease as the initial alkalinity decreased, starting with the third experimental condition (600 mg CaCO₃ L⁻¹) in R1, R2, and R3 (Figure 2).
Figure 2. (a) NO\textsubscript{x} denitrification efficiency (%) and (b) apparent consumption of alkalinity (mg CaCO\textsubscript{3} L\textsuperscript{-1}) under the five experimental conditions evaluated for R1, R2, R3, and R4.

Under the final test condition (200 mg CaCO\textsubscript{3} L\textsuperscript{-1}), the denitrification rates decreased to 34000 ± 3000, 47000 ± 3000 and 51000 ± 4000 mg NO\textsubscript{x} m\textsuperscript{3} d\textsuperscript{-1} for R2, R3 and R4, respectively. The drop in denitrification efficiency was more marked in R1 because of the absence of dolomitic limestone in the bed, with denitrification rates of 44000 ± 6000 and 34000 ± 3000 mg NO\textsubscript{x} m\textsuperscript{3} d\textsuperscript{-1} under the test conditions of 400 and 200
mg CaCO₃ L⁻¹, respectively. Under these conditions, the pH was 5.0 ± 0.3, thus providing evidence of the increase in alkalinity from the limestone material.

The denitrification efficiencies were higher in conditions of elevation of the initial alkalinity associated with higher proportions of dolomitic limestone, due to the maintenance of pH and the increase of alkalinity.

In addition to electron donors, elemental sulfur granules served as support material for the biomass. The percentage of the lost mass of elemental sulfur varied between 2 and 4.5%. The fixed bed was associated with low sludge production, preventing the clogging of the bed and the exit of biomass from the system, characteristics that facilitate the maintenance and operation of the system.

3.2 ALKALINITY INCREASE

It observed that the denitrification efficiency was primarily influenced by the availability of alkalinity in the system; however, according to the theoretical stoichiometry of 4.57 mg CaCO₃ mg NO₃⁻¹, approximately 300 mg CaCO₃ L⁻¹ would be needed to remove 66.0 mg NO₃⁻ L⁻¹.

The denitrification rates and, consequently, the removal efficiencies were directly related to the pH. At pH values less than 6.5, the denitrification efficiencies were less than 75%, and the maximum nitrite accumulation was 18 mg NO₂⁻ L⁻¹. According to Oh et al. (2000), the optimum pH range for the occurrence of autotrophic denitrification varies between 7 and 8, with the detection of intermediate products at lower pH values. It should be noted that the lowest pH values were measured at the lowest initial alkalinity conditions (400 and 200 mg CaCO₃ L⁻¹) for all four test reactors (Figure 3a). This may be an indication that the increase in alkalinity provided by the presence of dolomitic limestone may not have been sufficient to buffer the medium.

Although lower denitrification efficiencies were achieved at lower pH values, the alkalinity was higher under those same conditions. The increase in alkalinity was verified by the relationship between the apparently consumed alkalinity and the reduced nitrogen. In R1, where there was no dolomitic limestone in the bed composition, for the five
conditions evaluated, the relationship alkalinity\textit{apparent consumed}:NO\textsubscript{x} reduced \((4.8 \pm 0.1 \text{ mg CaCO}_3 \text{ mg NO}_x ^{-1})\) was above the stoichiometric ratio of 4.57 mg CaCO\textsubscript{3} mg N-NO\textsubscript{3}^{-1}. This finding may be attributed to the use of HCO\textsubscript{3}^{-} ions (NaHCO\textsubscript{3}) as the inorganic carbon source in the metabolism of autotrophic microorganisms (Ghafari \textit{et al.} 2010).

In the other reactors, the difference between the theoretical ratio and the ratio obtained experimentally showed an inverse correlation with the pH (Figure 3), i.e., the lowest pH provided the largest increments in alkalinity, as indicated by lower alkalinity\textit{apparent consumed}:NO\textsubscript{x} reduced ratios.

The greatest increases were found in R3 and R4, under initial alkalinity conditions of 400 mg CaCO\textsubscript{3} L\textsuperscript{-1} \((3.4 \pm 0.7 \text{ and } 3.2 \pm 0.3 \text{ mg CaCO}_3 \text{ mg NO}_x ^{-1})\) and 200 mg CaCO\textsubscript{3} L\textsuperscript{-1} \((3.5 \pm 0.4 \text{ and } 2.9 \pm 0.1 \text{ mg CaCO}_3 \text{ mg NO}_x ^{-1})\).
Figure 3. (a) Final pH and (b) Alkalinity apparent consumed: NO\textsubscript{x} reduced ratio under the five experimental test conditions for R1, R2, R3, and R4.

The increase in the ratios of dolomitic limestone in the composition of the beds resulted in larger alkalinity increases because of the greater surface areas available. However, the increase was similar between R3 and R4 (Figures 3b and 4a), which suggests non-relevant differences in dolomitic limestone ratios above 50%. The supply of alkalinity is not affected by the amount of limestone present in the system but by its
dissolution and by the biological and chemical interactions of the autotrophic
denitrification (Koenig & Liu 2002; Sahinkaya & Dursun 2012). According to Sahinkaya
et al. (2014), limestone dissolution could be limited by a low HRT. However, for this
experiment, an HRT increase of 14 hours increased the formation of hydrogen sulfide.

Therefore, the greater dolomitic limestone dissolution in the system was attributed
to the reduction in pH resulting from the consumption of alkalinity, whose maximum
values were approximately 180 mg CaCO$_3$ L$^{-1}$ (Figure 4b). As shown by this experiment,
pH was one of the primary parameters in the dissolution of calcium-based materials
(Koenig & Liu 2002). However, the increase in alkalinity from the pH decrease was not
sufficient to buffer the system and to maintain microorganism activity, which was thus
partially inhibited.
Figure 4. Behavior of the reactors: R2, R3 and R4 during the experiment. (a) Increase in alkalinity as a function of the composition of the reactor beds and (b) increase in alkalinity as a function of the pH.

Moon et al. (2006) evaluated an elemental sulfur fixed-bed reactor (2.5 mm) supplied with synthetic wastewater (60 mg NO₃⁻ L⁻¹) at initial alkalinity of 300, 600, 1200, and 1800 mg CaCO₃ L⁻¹. The kinetic constants of 0.269, 0.976, 3.110, and 2.631 mg NO₃⁻ ½ L⁻¹/2 d⁻¹ showed greater denitrification speed as the alkalinity increased. Complete
removals were observed in alkalinity\textsubscript{initial}:alkalinity\textsubscript{required theoretical} ratios greater than 2. However, signs of alkalinity saturation were reported at ratios greater than 4.

Koenig & Liu (2002) operated fixed-bed reactors with elemental sulfur/limestone ratios of 1:0, 4:1, 2:1, and 1:1 supplied with a synthetic substrate (62-250 mg NO\textsubscript{3}-L\textsuperscript{-1}) and varying initial alkalinitities (622, 476, 231, 168 and 74 mg CaCO\textsubscript{3} L\textsuperscript{-1}). The results suggested that, in wastewater with a maximum concentration of 100 mg NO\textsubscript{3}-L\textsuperscript{-1}, the alkalinity\textsubscript{initial}:alkalinity\textsubscript{required theoretical} ratios for the elemental sulfur/limestone ratios of 1:0, 4:1, 3:1, 2:1, and 1:1 were approximately > 1.7, 1.7 - 1.2, 1.2 - 1.0, 1.0 - 0.5 and < 0.5, respectively, to maintain satisfactory denitrification efficiencies. In addition, maximum increases in alkalinity of approximately 400 mg CaCO\textsubscript{3} L\textsuperscript{-1} were reported.

Because of the low rate of dissolution at pH between 6 and 8 observed in the early experiments, dolomitic limestone was not considered a satisfactory alkalinizing agent to treat wastewater with low alkalinity, as is the case with most nitrified effluents.

Several studies have reported high denitrification efficiencies from the use of limestone in fixed-bed reactors supplied with groundwater or synthetic effluents; however, most of these studies do not specify the type of limestone used. Moon \textit{et al.} (2006) compared the use of different alkalinizing agents in autotrophic denitrification and obtained initial alkalinity dissolution rates of 88, 38, and 14 mg CaCO\textsubscript{3} L\textsuperscript{-1} d\textsuperscript{-1} from oyster shell (CaO = 52.2% and MgO = 0.7%), calcitic limestone (51.0 and 3.2%) and dolomitic limestone (28.6 and 20.8%), respectively. The pH directly influenced the dissolution rates and the use of oyster shells and calcitic limestone provided greater denitrification efficiencies.

The presence of magnesium in the composition of dolomitic limestone has been identified as reducing the reactivity capacity of the rock (Hosten & Gülsün 2004). When the reactors were opened at the end of the experiment, R2 and R3 showed a mass loss of 0.7 ± 0.1% and concentrations of CaO and MgO of 30.5 ± 0.4 and 21.0 ± 0.2%, respectively. The reactor R4, operated for the longest time under different applied nitrogenous loads, showed a mass loss of 5.5%, and concentrations were 27.3% of CaO and 19.2% of MgO.
As support material for biomass, the adherence of the biomass to the dolomitic limestone was better compared to the adherence to elemental sulfur. This was likely because of the rough surface of the granules.

3.3 SULFATE PRODUCTION

The production of $\text{SO}_4^{2-}$ indicated the onset of autotrophic denitrification. Concerning the nitrogen removal efficiency, the amount of $\text{SO}_4^{2-}$ produced tended to increase as the proportion of dolomitic limestone in the beds and initial alkalinity increased (Figure 5a).
The production of SO$_4^{2-}$ tended to stabilize (407.5 and 445.1 mg SO$_4^{2-}$ L$^{-1}$) starting at the initial alkalinity of 600 mg CaCO$_3$ L$^{-1}$, in which the denitrification efficiencies remained constant. There was no evidence of a decrease in process performance from the SO$_4^{2-}$ concentration. According to Campos et al. (2008), partial and total inhibition can be found at concentrations greater than 500 and 6,400 mg SO$_4^{2-}$ L$^{-1}$, respectively.
The mean SO$_4^{2-}$-produced:NO$_x$-reduced ratios ranged from 6.3 and 8.0 mg SO$_4^{2-}$:mg NO$_x$ (Figure 5b) and tended to decrease as the pH increased, showing the consumption of SO$_4^{2-}$. High concentrations of SO$_4^{2-}$ (Sahinkaya 2009) associated with pH greater than 6.5 may have favored the establishment of sulfate-reducing bacteria (SRB) from inoculation by mixed sludge, resulting in coefficients less than the stoichiometric ratio of 7.5 mg SO$_4^{2-}$:mg N.

Although the fraction of remaining COD (97.8 ± 8.2 mg O$_2$ L$^{-1}$) from the nitrified substrate is not readily biodegradable (removals below 10%), the SRB can use organic carbon supplied by endogenous respiration or decomposition of autotrophic biomass (Sahinkaya 2009) to obtain hydrogen sulfide (H$_2$S) as an end product. In addition, hydrogen sulfide can be detected when an excess of sulfur exists from the depletion of electron receptors, according to Equation 2 below (Sierra-Alvarez et al. 2007). Consequently, low applied nitrogenous loads can promote excess sulfur (Luna-Velasco et al. 2010; Capua et al. 2015) in fixed beds containing elemental sulfur.

$$4S^0 + 4H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+$$  \hspace{1cm} \text{Equation 2}

The concentrations of hydrogen sulfide dissolved in the medium were less than 3 mg L$^{-1}$ under all conditions tested. However, hydrogen sulfide produced inside the reactors could be present in different forms (H$_2$S, HS$^-$ and S$^2-$), with a predominance of H$_2$S at pH values less than 7.0. Therefore, the reported H$_2$S concentration may not explain the total concentration of sulfide produced.

3.4 SPATIAL DISTRIBUTION

The NO$_x$ and sulfate concentrations along the reactor height are shown in Figure 6. It was observed that N removals and SO$_4^{2-}$ productions were more evident in the first 270 mm traveled, in the four reactors, and tended to increase according to the initial alkalinity conditions (600>400>200 mg CaCO$_3$ L$^{-1}$) (Figure 6), possibly due to the solubility and availability of NaHCO$_3$ (Sun & Nemati 2012).
Figure 6. Spatial profile of concentrations of $\text{SO}_4^{2-}$ e $\text{NO}_x$ ($\text{NO}_3^- + \text{NO}_2^-$) under the initial alkalinity conditions of 200, 400 e 600 mg de $\text{CaCO}_3$ $\text{L}^{-1}$ to (a) R1, (b) R2, (c) R3 and (d) R4.
In R1, concentrations tended to stabilize from the second collection point (P2). In the other reactors, from this point on, the removal of N and the production of $\text{SO}_4^{2-}$ tended to increase according to the proportion of dolomitic limestone in the composition of the beds, probably due to the increase in alkalinity. Hydrogen sulfide concentrations were detected from P3 (470 mm).
4 CONCLUSIONS

The use of elemental sulfur as the electron donor was shown to be effective in autotrophic denitrification, with high nitrogen removal efficiencies. Because of its low solubility, the amounts of elemental sulfur in the bed composition favored the mass transfer in the process. The dissolution of dolomitic limestone was greater under conditions of pH less than 6. However, because of its slow rate of dissolution, the use of dolomitic limestone as the only source of alkalinity cannot both buffer the medium and maintain satisfactory denitrification efficiencies.

Experiments showed that at the elemental sulfur/dolomitic limestone ratios of 1:0 and 3:1, the required alkalinity ratio was at least twice the theoretical need. At the ratios of 1:1 and 3:1, this ratio was 1.5. Autotrophic denitrification by elemental sulfur was found to be effective in the treatment of nitrified poultry wastewater. The efficiency of the process is related to the initial substrate alkalinity and the rate of dissolution of dolomitic limestone. Therefore, its application to effluents characterized by low alkalinity is not recommended.

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