Characterization and kinetics of sulfide-oxidizing autotrophic denitrification in batch reactors containing suspended and immobilized cells

B. S. Moraes, T. S. O. Souza and E. Foresti

ABSTRACT

Sulfide-oxidizing autotrophic denitrification is an advantageous alternative over heterotrophic denitrification, and may have potential for nitrogen removal of low-strength wastewaters, such as anaerobically pre-treated domestic sewage. This study evaluated the fundamentals and kinetics of this process in batch reactors containing suspended and immobilized cells. Batch tests were performed for different \( \text{NO}_x/\text{S}^{2-} \) ratios and using nitrate and nitrite as electron acceptors. Autotrophic denitrification was observed for both electron acceptors, and \( \text{NO}_x/\text{S}^{2-} \) ratios defined whether sulfide oxidation was complete or not. Kinetic parameter values obtained for nitrate were higher than for nitrite as electron acceptor. Zero-order models were better adjusted to profiles obtained for suspended cell reactors, whereas first-order models were more adequate for immobilized cell reactors. However, in the latter, mass transfer physical phenomena had a significant effect on kinetics based on biochemical reactions. Results showed that sulfide-oxidizing autotrophic denitrification can be successfully established for low-strength wastewaters and have potential for nitrogen removal from anaerobically pre-treated domestic sewage.

Key words | autotrophic denitrification, electron donor, immobilized cells, kinetics, sulfide, suspended cells

INTRODUCTION

A wide range of biological processes coexist in the biosphere, maintaining the environmental equilibrium that has sustained life on this planet across history. Nevertheless, many bioprocesses are still unknown or not fully understood by mankind. The study of such processes is crucial, since they may allow the development of new technologies that could lead to viable solutions for issues in many different fields. Such is the case for sulfur-oxidizing autotrophic denitrification, regarding nitrogen removal from wastewaters, which has clear advantages over heterotrophic denitrification, but requires additional studies for full application.

Autotrophic denitrification using reduced sulfur compounds as electron donors is performed by chemolithotrophic microorganisms, capable of reducing oxidized nitrogen compounds (nitrate, nitrite) while oxidizing reduced sulfur compounds (such as sulfide, elemental sulfur, sulfite or thiosulfate) (Batchelor & Lawrence 1978). According to Zhang & Lamp (1999), the process is usually associated with the species \( \text{Thiobacillus denitrificans} \) and \( \text{Thiomicrospira denitrificans} \). When applied to wastewater treatment units for nitrogen removal, autotrophic denitrification is an advantageous alternative over heterotrophic denitrification, since the former depends exclusively on inorganic compounds such as electron donors, which are cheaper than the organic ones. Besides, such inorganic compounds may even be available for denitrification as an endogenous source of electron donors at no cost, in some cases. Other potential advantages are less sludge production and no need for strict control of electron donor dosage, as required when organic compounds are applied for this purpose (Kim et al. 2004).

Beristain-Cardoso et al. (2006) studied autotrophic denitrification using three different reduced sulfur compounds as electron donors: sulfide, elemental sulfur and
thiosulfate. For stoichiometric concentrations of nitrate, higher denitrification rates were obtained for thiosulfate, followed by sulfide and elemental sulfur, indicating that thiosulfate is the most bioavailable electron donor for denitrification. The limited bioavailability of elemental sulfur is probably due to its insolubility characteristic, while sulfide is located in an intermediary bioavailability range. Regarding sulfide as the electron donor, the authors reported that, when nitrate and sulfide were in stoichiometric concentrations, or when there was an excess of nitrate, sulfide was oxidized completely to sulfate in autotrophic denitrification. However, when there was an excess of sulfide, this compound was oxidized partially to elemental sulfur. According to Reyes-Avila et al. (2004) and Mahmood et al. (2007), the stoichiometric reactions for autotrophic denitrification using nitrate and nitrite as electron acceptors, when sulfide is oxidized completely to sulfate, are shown on Equations (1) and (2), respectively.

\[
1.25S^{2-} + 2NO_2^- + 2H^+ \rightarrow 1.25SO_4^{2-} + N_2 + H_2O \quad (1)
\]

\[
3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \quad (2)
\]

Autotrophic denitrification has been studied mainly for the removal of nitrate from groundwater, using elemental sulfur as electron donor (Sierra-Alvarez et al. 2007) and for simultaneous removal of sulfide and nitrogen compounds from petrochemical wastewaters (Reyes-Avila et al. 2004). Nonetheless, it could also be applied to anaerobically pre-treated wastewaters. Effluents from anaerobic reactors usually have in their composition ammonium, which can be nitrified, and sulfides, both in dissolved and gaseous form, which could be used as electron donors for autotrophic denitrification (Foresti et al. 2006). The application of such a process could be a viable solution for nitrogen removal from anaerobically pre-treated wastewaters, since their low content of readily biodegradable organic matter cannot be used successfully as an endogenous electron donor source for heterotrophic denitrification. In this way, autotrophic denitrification would make it possible to avoid or reduce the need for exogenous organic compounds addition, lowering post-treatment costs.

The objective of this study was to characterize the autotrophic denitrification process when applied to low-strength wastewaters, using sulfide as electron donor, in order to define its potential for post-treatment of anaerobically pre-treated domestic sewage. For this purpose, batch reactors containing suspended and immobilized cells were operated and monitored using nitrate and nitrite as electron acceptors, and kinetic parameter values were obtained.

**METHODS**

**Batch reactors and inocula**

Tests were performed in triplicate, by using six 1-L glass serum bottles, containing suspended cells (reactors SR1, SR2 and SR3) and 1-cm polyurethane cubes with immobilized cells (reactors IR1, IR2 and IR3). The reactive volume of each reactor was 700 mL, and the bottles were incubated in chambers at 30 ± 1°C (mesophilic conditions), agitated at 150 and 120 rpm, respectively for SR and IR reactors.

Inoculum was obtained from a UASB reactor treating poultry waste, which presented itself in granular form. Hirasawa et al. (2008) studied the characteristics of sludge originating from the same source, and described it as strongly heterotrophic, methanogenic and denitrifying sludge. In this way, the present study aimed to enrich an autotrophic denitrification community out of a heterotrophic sludge, by assuming the presence of autotrophic denitrifiers, even in suppressed conditions. Granular sludge was fragmented for inoculation, and 100 mL were added in each SR reactor. For IR reactors, 100 inoculated polyurethane cubes were added in each reactor, previously weighted without the inoculum. The cubes were inoculated by mixing and compressing them with a large quantity of sludge, and leaving them immersed in it afterwards for 24 h.

**Feeding composition and operational procedures**

Reactors were fed with a synthetic wastewater simulating a typical nitrified effluent from domestic sewage post-treatment systems, as used by Callado & Foresti (2000). Some changes were made to its composition, concerning mainly NaHCO₃ concentrations, which were increased for better buffering and inorganic carbon availability. The final composition was (mg L⁻¹): KNO₃ (144), KH₂PO₄ (36), NH₄Cl (16), NaHCO₃ (2,000), MgCl₂ 6H₂O (28), CaCl₂ 2H₂O (18). When nitrite was studied as electron acceptor, KNO₃ was replaced by NaNO₂, but maintaining the same final nitrogen concentration (20 mg N L⁻¹). Trace elements were supplied by adding 2 mL L⁻¹ of a solution proposed by Beristain-Cardoso et al. (2006), composed of (g L⁻¹):
EDTA (0.50), ZnSO₄·7H₂O (0.04), CaCl₂·2H₂O (0.07), MnCl₂·(0.03), (NH₄)₂Mo₇O₂₄·4H₂O (0.01), CuSO₄·H₂O (0.02), CoCl₂·6H₂O (0.02).

Sulfide was supplied as sodium sulfide (Na₂S·9H₂O), and injected into the reactors through the rubber sealing, with a syringe. The same procedure was used for the addition of KNO₃ or NaNO₂, and NaHCO₃. Nitrate and nitrite concentrations were fixed at 20 mg N L⁻¹, while sulfide concentrations varied according to the feeding conditions. Using nitrate as electron acceptor, 30 mg S²⁻ L⁻¹ were supplied in phase I, resulting in a stoichiometric molar NO₃⁻/S²⁻ ratio of 1.6, while in phase II, 60 mg S²⁻ L⁻¹ were supplied, resulting in a molar NO₃⁻/S²⁻ ratio of 0.8, and thus an excess of sulfide as electron donor was present in this phase. Similarly, using nitrite as electron acceptor, 17 and 34 mg S²⁻ L⁻¹ were supplied, resulting in molar NO₂⁻/S²⁻ ratios of 2.7 (stoichiometric, phase III) and 1.4 (excess of electron donors, phase IV), respectively.

The reactors were fed with Na₂S·9H₂O, KNO₃ or NaNO₂, and NaHCO₃ daily, since sulfides and nitrates/nitrates were consumed in less than 24 h. These compounds were added with a syringe separately from the other compounds and trace elements, which was refreshed only twice a week, to prevent excessive cell washout. This peculiar procedure was successful for enriching the biomass and for maintaining it in SR reactors during the whole experiment. When the mineral medium was refreshed, bottles were unsealed and, after a sedimentation period for the SR reactors, the medium was discharged. New medium was then supplied, and sparged with N₂/CO₂ gas for removing residual oxygen and for pH control, which could be maintained between 7.0 and 7.5. pH control was crucial during the experiment, since the addition of sodium sulfide elevated pH values of the mineral medium to harmful levels without buffering. Bottles were then immediately sealed, and the other compounds (Na₂S·9H₂O, KNO₃ or NaNO₂, and NaHCO₃) were added with a syringe, as described above.

Reactors were acclimated during a period of 30 days. After consumption of nitrates and sulfides was detected, the monitoring was performed regularly twice a week. Samples were collected at the beginning and end of cycles for the determination of denitrification performance. During periods of stable and complete removal of nitrate/nitrite, which were only observed in phases II and IV, kinetic parameter values were obtained for nitrate and nitrite as electron acceptors, by collecting timed samples during a single cycle.

Analytical methods

All chemical analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF 2005). Sulfide (S²⁻–S) was determined colorimetrically by the methylene blue method and sulfate (SO₄²⁻–S) was measured by turbidimetry. Nitrate (NO₃⁻–N), nitrite (NO₂⁻–N) and ammonium (NH₄⁺–N) were determined by Flow Injection Analysis (FIA). Volatile suspended solids (VSS), both for suspended cells and cells immobilized in polyurethane cubes, were determined gravimetrically. H₂S in the headspace of reactors and elemental sulfur were not measured, and sulfur mass balance studies were done with sulfide and sulfate data only.

Kinetic analysis

Profiles of specific nitrogen concentration in nitrate and nitrite forms (C_N) over time were performed to determine kinetic parameter values for nitrogen removal. Reaction order was obtained by the integral method, from specific substrate utilization rates (r_N), considering batch reactors at constant volume, according to Equation (3). In this equation, k is the rate, dependent on the reaction order and kinetic model, f(C_N) is the function to be evaluated, consisting of zero and first order reactions, and t is the time. Mathematical models represented by equations were adjusted to the curves obtained experimentally.

\[
(-r_N) = \frac{dC_N}{dt} = k \cdot f(C_N)
\]

(3)

Limitation effects imposed by external mass transfer resistance on IR reactors were evaluated by Biot number (Bi) (Equation (4)), according to Bailey & Ollis (1986). In this equation, k is the liquid-phase mass transfer coefficient, R_p is the equivalent sphere radius (0.5 cm) and D_e is the effective diffusivity in the bioparticle. This was determined as 80% of the nitrogen diffusion value in water, resulting in 1.59 × 10⁻⁵ cm² s⁻¹ (Perry & Chilton 1985; Droste & Kennedy 1986). The value of k was determined empirically, from correlations that linked physical properties of the liquid, hydrodynamic characteristics of the reactor and geometric characteristics of the bioparticles (Welty et al. 1984; Bailey & Ollis 1986), resulting in 4.32 × 10⁻⁴ cm s⁻¹.

\[
Bi = \frac{k_s \cdot R_p}{D_e}
\]

(4)
In order to verify if the substrate utilization rates were limited by intraparticular diffusion resistance, values of the observed Thiele modulus ($\phi_{\text{obs}}$) were calculated as shown in Equation (5) (Bailey & Ollis 1986). In this equation, $r_{\text{N,obs}}$ is the initial observed specific nitrogen utilization rate.

\[
\phi_{\text{obs}} = \frac{r_{\text{N,obs}}}{g \cdot D_e \cdot C_{N_0}}
\]  

(5)

**RESULTS AND DISCUSSION**

**Batch operations**

**Nitrate as electron acceptor**

The reactors were operated using nitrate as the electron acceptor for approximately 100 days, 50 days for each phase. The results obtained during the monitoring period are presented in Table 1. Values obtained for sulfide concentrations in the beginning of each cycle were always inferior to the theoretical value. This probably was due to the equilibrium between gaseous and dissolved sulfide, governed by Henry’s Law, and also to eventual minor oxidation. Sulfide was usually completely consumed in all phases for both types of reactors. In phase I, sulfate production detected was close to the one expected according to theoretical sulfide addition. On the other hand, in phase II, values obtained were much lower than expected. This implies the formation of intermediary compounds in this phase, such as elemental sulfur. Visual evidence strengthened this fact. The formation of a whitish or yellowish substance in the medium of both reactors and around the polyurethane cubes of the IR reactors, which is a characteristic of elemental sulfur, was observed during phase II. Similar results were obtained by other authors when excess of electron donors was applied (Krishnakumar & Manilal 1999; Beristain-Cardoso et al. 2006).

Nitrate consumption was observed in both phases, as shown on Table 1. Since it was coupled to sulfide oxidation, this indicates the occurrence of autotrophic denitrification in the reactors. Abiotic tests proved such biological activity, as minor or no consumption of sulfide and nitrate were measured in these tests (data not shown). Ammonium remained at low and steady levels during the operation in all phases. In phase I, complete nitrate consumption was detected in the first weeks, but residual nitrate/nitrite was observed afterwards. The unstable nitrate removal coupled to nitrite formation implied that the required molar NO$_3^-$ / S$^{2-}$ ratio (1.6) for complete nitrogen removal from the medium, according to stoichiometry, was not achieved in this phase. This was probably due to the hardships in obtaining precise sulfide concentrations, such as volatilization and oxidation. When reactors were operated in phase II, however, nitrate and nitrite concentrations substantially dropped (Table 1) by the end of cycle. Thus, stable nitrogen removal was only obtained with excess of electron donors, under a molar NO$_3^-$ / S$^{2-}$ ratio (0.8) lower than that predicted by stoichiometry. Other authors adopted the same procedure to establish complete and stable autotrophic denitrification. Kleerebezem & Mendez (2002) obtained an optimum molar NO$_3^-$ / S$^{2-}$ ratio of 1.3, while Beristain-Cardoso et al. (2006) obtained the values 1.45–1.58. Similarly, Campos et al. (2008) and Manconi et al. (2007) obtained the values 1.2 and 0.8–0.9, respectively.

It is noteworthy that initial nitrate concentration measured for the SR reactors were lower than actually added, as opposed to the IR reactors. In this case, biological reactions might have occurred during the time interval between sampling and analysis, once samples from SR reactors contained suspended biomass. Besides, initial sulfide concentration was always lower than the theoretical value, as reported above. Therefore, there was the probable occurrence of autotrophic denitrification prior to the analysis. However, sulfate concentration data in the beginning of the cycles did not report the occurrence of such a bioprocess. In this way, partial sulfide oxidation may have taken

<table>
<thead>
<tr>
<th>Reactors</th>
<th>Phase</th>
<th>Sulfide (mg S$^{2-}$ L$^{-1}$) t = 0</th>
<th>Sulfide (mg S$^{2-}$ L$^{-1}$) t = 24 h</th>
<th>$\Delta$SO$_4^{2-}$ (mg S L$^{-1}$)</th>
<th>NO$_2^-$ (mg N L$^{-1}$) t = 0</th>
<th>NO$_2^-$ (mg N L$^{-1}$) t = 24 h</th>
<th>NO$_3^-$ (mg N L$^{-1}$) t = 0</th>
<th>NO$_3^-$ (mg N L$^{-1}$) t = 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>I</td>
<td>15.4 ± 3.4</td>
<td>1.2 ± 2.5</td>
<td>23.1 ± 7.0</td>
<td>15.6 ± 0.6</td>
<td>0.6 ± 1.3</td>
<td>0.0 ± 0.0</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>46.7 ± 6.5</td>
<td>3.0 ± 2.2</td>
<td>40.3 ± 15.2</td>
<td>17.5 ± 2.3</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.7</td>
<td>0.4 ± 1.0</td>
</tr>
<tr>
<td>IR</td>
<td>I</td>
<td>20.1 ± 1.4</td>
<td>0.4 ± 0.9</td>
<td>21.9 ± 7.2</td>
<td>19.3 ± 0.2</td>
<td>0.6 ± 1.1</td>
<td>0.4 ± 0.9</td>
<td>4.9 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>49.3 ± 4.5</td>
<td>0.2 ± 0.3</td>
<td>36.2 ± 9.5</td>
<td>20.0 ± 1.0</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>
place. The occurrence of sulfide oxidation in two steps coupled to reduction of oxidized nitrogen compounds has been discussed extensively in the literature; the formation of thiosulfate and elemental sulfur as intermediary sulfur compounds prior to sulfate formation being reported (Reyes-Avila et al. 2004; Cervantes et al. 2009; Gaddekari et al. 2006). According to Reyes-Avila et al. (2004), the step of sulfate formation from intermediary sulfur compounds is slower than the prior partial sulfide oxidation.

The hypothesis of such two-step autotrophic denitrification can also justify the high sulfate values detected (phase II, SR reactors), which were higher than expected considering the nitrate consumption measured, if the final products were only sulfate and nitrogen gas. The possible pathways involved in sulfide-oxidizing autotrophic denitrification lead to different final products, especially regarding sulfur compounds, which vary between sulfate and intermediary compounds. In this way, high standard deviation in the measurements of sulfate implies that the sulfate was not always the only final product.

Nitrite as electron acceptor

Nitrite was added as electron acceptor to the same culture established in the previous tests, during 30 days of operation, 15 days for each phase. Average results obtained are shown on Table 2. Sulfur compounds during phases III (molar NO$_2^-$/S$^{2-}$ ratio of 2.7) and IV (molar NO$_2^-$/S$^{2-}$ ratio of 1.4) had similar behavior when compared to phases I and II, regarding the complete consumption of sulfide in both phases, and partial oxidation when electron donor excess was present in phase IV.

Equivalent results were obtained concerning nitrite as the electron acceptor. Initially, complete nitrite removal was observed, but after the first week nitrite accumulated at the end of cycles. Once again, available sulfide was not enough for achieving the required molar NO$_2^-$/S$^{2-}$ ratio, according to stoichiometry. Complete nitrite removal was immediately observed when the ratio was lowered.

### Kinetic tests

Kinetic parameter values were obtained for both types of reactors during phases II and IV, for nitrate and nitrite as electron acceptors, respectively. In these phases, individual cycles were selected for the performing of kinetic profiles. Results obtained are presented in Figure 1.

Zero-order kinetic models were best adjusted to nitrate and nitrite removal data over time for SR reactors, while first-order kinetic models were more adequate for IR reactors for both electron acceptors. Correlation coefficient ($R^2$) values obtained for first-order adjustments were always high for IR reactors (0.95–0.99). For SR reactors, though, data points were more scattered, and $R^2$ values obtained were lower. For nitrate and nitrite as electron acceptors, respectively, $R^2$ values obtained for zero-order models were in the range of 0.91–0.98 and 0.84–0.94 for SR reactors. Nevertheless, the $R^2$ values obtained for zero-order adjustments were, in general, still higher than first-order for SR reactors, although in some cases they were close. The scattering of data points for SR reactors may be due to the fact that samples collected from those reactors contained suspended biomass that could promote reactions even after the sample was collected, in the short time while it was prepared for analysis. Since samples collected from IR reactors did not contain biomass, this could explain the difference in the quality of results.

Kinetic analysis was made for each individual reactor, since there was a significant variability of kinetic parameter values, and so, average results considering the triplicates would not be a precise approach. Different reactors are influenced by different random events in operation, even when under the same conditions. Each random event may affect the microbial community, defining the future performance of reactors, and increasing variability in results as time passes. Although no major differences in triplicates were observed during monitoring of the reactors, allowing the use of average values, this was not possible for kinetic parameter values, which were considered separately.

### Table 2 | Parameters monitored at the beginning and end of cycles for SR and IR reactors in phases III and IV

<table>
<thead>
<tr>
<th>Reactors</th>
<th>Phase</th>
<th>Sulfide (mg S$^{2-}$ L$^{-1}$)</th>
<th>$\Delta$SO$_{2-}^2$ (mg S L$^{-1}$)</th>
<th>NO$_3^-$ (mg N L$^{-1}$)</th>
<th>NO$_2^-$ (mg N L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t = 0</td>
<td>t = 24 h</td>
<td>t = 0</td>
</tr>
<tr>
<td>SR</td>
<td>III</td>
<td>9.8 ± 0.6</td>
<td>0.9 ± 1.0</td>
<td>15.7 ± 5.4</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>24.3 ± 2.1</td>
<td>0.1 ± 0.1</td>
<td>18.0 ± 3.8</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>IR</td>
<td>III</td>
<td>9.9 ± 0.7</td>
<td>0.1 ± 0.1</td>
<td>7.9 ± 4.7</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>24.3 ± 1.4</td>
<td>0.0 ± 0.0</td>
<td>10.8 ± 3.6</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>
and first-order kinetic parameter values obtained are shown in Table 3.

Maximum zero and first-order kinetic parameter values obtained were 7.05 mg N gVSS\(^{-1}\) h\(^{-1}\) and 1.11 h\(^{-1}\), respectively, for nitrate as electron acceptor. Zero-order parameters were located in an intermediary range, when compared to values reported by other authors. Beristain-Cardoso et al. (2006), Reyes-Avila et al. (2004) and Manconi et al. (2007) obtained denitrification rates in autotrophic systems with suspended cells equal to 2.1, 15.83 and 24 mg N gVSS\(^{-1}\) h\(^{-1}\), respectively. The variability in those values is probably due to differences in inocula characteristics, type and time of enrichment. Concerning nitrite as electron acceptor, maximum parameters obtained were 5.02 mg N gVSS\(^{-1}\) h\(^{-1}\) and 0.37 h\(^{-1}\), respectively for SR and IR reactors. Pérez et al. (2007) obtained a denitrification rate of 32 mg N gVSS\(^{-1}\) h\(^{-1}\) using nitrite as electron acceptor in a batch reactor with intermittent aeration, in which autotrophic denitrification was applied.

Kinetic parameter values for nitrite as electron acceptor were lower than the ones obtained for nitrate as electron acceptor. Cervantes et al. (2009) also observed lower denitrification rates when nitrite was used as electron acceptor, in comparison to nitrate. According to the authors, sulfide had an inhibitory effect on nitrite consumption, since it precipitates trace elements, which are essential to the activity of nitrite-reductase enzymes.

First-order kinetic parameter values obtained for IR reactors could not be compared to other values, since

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>(k_0) (mg N gVSS(^{-1}) h(^{-1}))</th>
<th>(k_1) (h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (NO(_3^-))</td>
<td>4.23</td>
<td>6.76</td>
</tr>
<tr>
<td>Nitrite (NO(_2^-))</td>
<td>2.37</td>
<td>3.90</td>
</tr>
</tbody>
</table>
there are no reports of such a reaction order for autotrophic denitrification systems using sulfide as electron donor. Nevertheless, kinetic modeling on the biochemical reactions for IR reactors was influenced by mass transfer phenomena. According to Bailey & Ollis (1986), external and internal mass transfer resistances are significant for \( Bi < 100 \) and \( \phi_{obs} > 0.3 \), respectively. In this work, the average value for \( Bi \) was \( 13.57 \pm 0.00 \) for both electron acceptors and the average values for \( \phi_{obs} \) were \( 0.500 \pm 0.07 \) and \( 0.363 \pm 0.02 \) for nitrate and nitrite, respectively (Table 4). Therefore, the specific nitrogen utilization rates were limited effectively by the stagnant liquid layer around the bioparticles and by intraparticular diffusion. In this way, the model and kinetic parameter values obtained were apparent, including not only the biochemical reactions, but the mass transfer physical phenomena. Besides, complete substrate consumption does not occur for first-order exponential decay models applied to biochemical reactions, as it was observed, being peculiar to zero-order models. Additionally, Garbossa (2006) obtained a zero-order kinetic model for nitrogen removal of synthetic nitrified domestic sewage using sulfide as electron donor in batch assays with immobilized cells. The reactor was maintained at agitation of 150 rpm to reduce mass transfer resistances. Thus, there is evidence that, if the mass transfer resistances had been minimized in the present tests, the kinetic model for nitrogen removal could have been a zero-order model, as occurred in SR reactors. In this case, the biochemical reaction rates would not have been affected by substrate concentrations.

**CONCLUSIONS**

Autotrophic denitrification was observed in reactors with suspended and immobilized cells, and could be enriched from a heterotrophic inoculum. Therefore, the environment provided by the synthetic wastewater simulating a nitrified domestic sewage effluent allowed the development of autotrophic denitrifiers. In this way, the technical viability of applying sulfide-oxidizing autotrophic denitrification was verified in this case.

Sulfide oxidation can be directed either to sulfate or intermediary compounds, according to \( \text{NO}_3^- / S^{2-} \) ratios, and kinetic parameter values obtained for nitrate as electron acceptor were higher than the ones obtained for nitrite.

For reactors containing suspended cells, zero-order models were better adjusted, while for reactors containing immobilized cells, first-order models were more adequate. In the latter, the observed Thiele modulus and Biot number values revealed the significant effects of external and internal mass transfer resistance on kinetic modeling based on biochemical reactions. Thus, the models and kinetic parameter values obtained were apparent, including not only the biochemical reactions, but the mass transfer physical phenomena.

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**Table 4** Average values for dimensionless parameters used on evaluation of mass transfer phenomena in IR reactors

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>Reactors</th>
<th>( Bi )</th>
<th>( \phi_{obs} )</th>
<th>( Bi )</th>
<th>( \phi_{obs} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (( \text{NO}_3^- ))</td>
<td>IR1</td>
<td>13.57</td>
<td>0.580</td>
<td>13.57</td>
<td>0.397</td>
</tr>
<tr>
<td></td>
<td>IR2</td>
<td>13.57</td>
<td>0.496</td>
<td>13.57</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>IR3</td>
<td>13.57</td>
<td>0.425</td>
<td>13.57</td>
<td>0.363</td>
</tr>
</tbody>
</table>

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