Determination of the intrinsic kinetic parameters of sulfide-oxidizing autotrophic denitrification in differential reactors containing immobilized biomass

B.S. Moraes *, E. Foresti

Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Av. Trabalhador São-Carlense, 400, Centro, 13566-590 São Carlos, SP, Brazil

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Nitrogen removal coupled with sulfide oxidation has potential for the treatment of effluents from anaerobic reactors because they contain sulfide, which can be used as an endogenous electron donor for denitrification. This work evaluated the intrinsic kinetics of sulfide-oxidizing autotrophic denitrification via nitrate and nitrite in systems containing attached cells. Differential reactors were fed with nitrified synthetic domestic sewage and different sulfide concentrations. The intrinsic kinetic parameters of nitrogen removal were determined when the mass transfer resistance was negligible. This bioprocess could be described by a half-order kinetic model for biofilms. The half-order kinetic coefficients ranged from 0.425 to 0.658 mg N L^{-1/2} h^{-1} for denitrification via nitrite and from 0.190 to 0.609 mg N L^{-1/2} h^{-1} for denitrification via nitrate. In this latter, the lower value was due to the use of electrons donated from intermediary sulfur compounds whose formation and subsequent consumption were detected.

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1. Introduction

Nitrogen removal plays an important role in wastewater treatment because improper disposal of this compound may cause environmental impacts (e.g., eutrophication). Moreover, growing environmental awareness has driven the search for innovative and optimized treatment technologies. In this context, sulfide-oxidizing autotrophic denitrification has arisen as a possible step in the biological nitrogen removal process, especially for the post treatment of effluents from anaerobic reactors that are rich in ammonium, which must be nitrified, as well as soluble and gaseous sulfide. It is noteworthy that the efficient application of this process for the post treatment of effluents from anaerobic reactors depends on further research on new reactor configurations and operation. The reactors have to allow a partial nitrification of the effluent preserving some sulfide for autotrophic denitrification. Autotrophic denitrification coupled with sulfide oxidation has many advantages compared with conventional heterotrophic denitrification. These advantages are due to lower sludge production and the possibility of using an endogenous source of electrons at no cost in the case of the post treatment of effluents from anaerobic reactors.

Sulfide-oxidizing autotrophic denitrification is performed by oxidizing chemolithotrophic sulfur bacteria, which are capable of oxidizing reduced sulfur compounds (\(S^{2-}, S^{0}, S_{2}O^{3-}, SO_{2}^{2-}\)) while reducing oxidized nitrogen compounds (\(NO_{2}^{−}, NO_{3}^{−}\)).

Stoichiometrical reactions for complete autotrophic denitrification via nitrate and nitrite are shown in Eqs. (1) and (2), respectively (Mahmood et al., 2007):

\[
3HS^{-} + 8NO_{3}^{−} + 5H^{+} → 3SO_{4}^{2−} + 4N_{2} + 4H_{2}O \quad (1)
\]

\[
5HS^{-} + 8NO_{3}^{−} + 3H^{+} → 5SO_{4}^{2−} + 4N_{2} + 4H_{2}O \quad (2)
\]

Another economically interesting concept is nitrogen removal via nitrite, called shortcut nitrification–denitrification. This process omits the nitratation step during nitrification and, thus, promotes nitrite accumulation. In this case, denitrification is performed from nitrite, which allows for a reduction of the reaction time. Moreover, there is an economy of alkalinity and energy for aeration in nitrification.

Promising research related to autotrophic denitrification with reduced sulfur compounds has been reported (Kleerebezem and Mendez, 2002; Beristain-Cardoso et al., 2006; Cervantes et al., 2009; Wang et al., 2010; Huang et al., 2011; Wan et al., 2011). However, most of these studies used systems containing suspended cells. There is a lack of literature related to processes containing immobilized biomass. However, there are some studies on denitrifying reactors containing attached cells on elemental sulfur granules, which evaluate the kinetics of autotrophic denitrification using elemental sulfur as the electron donor (Zeng and Zhang, 2005; Moon et al., 2006; Wan et al., 2009).

These bioprocesses are not completely understood and therefore, further studies are needed before these processes can be fully applied. The present research aimed to investigate fundamental aspects of sulfide-oxidizing autotrophic denitrification via nitrate and nitrite in systems containing immobilized biomass on

* Corresponding author. Tel.: +55 16 3373 8358.
E-mail addresses: bs_moraes@yahoo.com.br (B.S. Moraes),eforesti@sc.usp.br (E. Foresti).
polyurethane foam matrices. Intrinsic kinetic parameters of autotrophic denitrification were determined in differential horizontal reactors fed with nitrified synthetic domestic sewage. Nitrate and nitrite were used as electron acceptors, and dissolved sulfide was used as the electron donor.

2. Fundamentals of the process

The physical and biochemical phenomena involved in systems with attached cells follow the biofilm conceptual model, which has been discussed for decades (Batchelor and Lawrence, 1978; Jansen and Harremoes, 1985; Christiansen et al., 1994; Zeng and Zhang, 2005; Mathioudakis and Aviasidis, 2009). The conceptual model for sulfide-oxidizing autotrophic denitrification in packed-bed reactors can be described as follows: (1) sulfide and nitrate/nitrite are transported from the surface to the inner part of the biofilm by diffusion; (2) a biological reaction of nitrate/nitrite reduction coupled to sulfide oxidation takes place inside the biofilm; and (3) oxidized sulfur compounds and reduced nitrogen compounds — the reaction products — are transported out of the biofilm by diffusion.

Harremoes (1976) initially investigated the significance of pore diffusion to filter denitrification and proposed that half-order reactions could explain this phenomenon. The intrinsic process follows Monod-type kinetics with low Monod constants, i.e., the intrinsic reaction rate could follow a zero-order model (Jansen and Harremoes, 1985). Based on this consideration, the diffusion model was accounted for using molecular diffusion represented by Fick’s Law. Thus, the diffusion phenomenon led to an intrinsic process that could be described by zero- or half-order kinetic models using a simplified pore diffusion model. Eqs. (3) and (4) summarize this model.

Zero-order bulk reaction:

\[ r_a = k_{0a} \text{ valid for } \beta = \frac{2DC}{k_{0a}L} \geq 1 \]  

(3)

Half-order bulk reaction:

\[ r_a = k_{1/2a} \cdot C_i^{1/2} \text{ valid for } \beta < 1 \]  

(4)

where \( r_a \) is the removal rate per unit area of the biofilm surface (g m\(^{-2}\) s\(^{-1}\)); \( k_{0a} \) is the zero-order removal rate per unit of biofilm area (g m\(^{-2}\) s\(^{-1}\)); \( k_{1/2a} \) is the half-order rate constant per unit of biofilm area (g m\(^{-1/2}\) s\(^{-1/2}\)); \( L \) is the thickness of the biofilm (m); \( D \) is the diffusion coefficient of the substrate (m\(^2\) s\(^{-1}\)); \( C_i \) is the bulk substrate concentration at the biofilm surface (g m\(^{-3}\)); and \( \beta \) is the penetration ratio.

According to Harremoes (1976), in systems containing attached cells, such as biological filters, substrates commonly do not reach the interior of the porous biofilm and, thus, the pores become partially effective. Therefore, the penetration efficiency of the substrate in the pore is less than 100%, and the reaction rate becomes dependent on the substrate concentration increase to the half power (Eq. (4)). In this case, a zero-order reaction in a pore that is partially penetrated by the substrate is transformed into a half-order reaction in the exposed area of the biofilm. This model has been applied successfully in research related to autotrophic denitrification with immobilized biomass in sulfur granules (Wang, 1998; Koenig and Liu, 2001; Moon et al., 2004, 2006; Wan et al., 2009).

3. Methods

3.1. Differential horizontal reactors

Five 15-mL differential reactors were used to evaluate the intrinsic kinetic parameters of sulfide-oxidizing autotrophic denitrification. The reactors were made of borosilicate glass, equipped with a Teflon lid, and sealed with rubber O-rings. In this system, the biomass was immobilized in polyurethane foam cubes of 0.5 cm edge, and the substrate was recirculated in a closed circuit.

The reactors were fed by continuously pumping the wastewater that was kept in sealed 1-L Duran flasks containing 500 mL of liquid medium. Feeding bottles were placed in a controlled temperature bath at a temperature of 5 °C to minimize the occurrence of biochemical reactions outside of the reactors. The anoxic atmosphere inside the bottles was obtained by flowing N\(_2\) gas in the headspace. The reactors were kept in an incubator under a controlled temperature of 30 °C ± 1 °C.

3.2. Feeding composition and inoculum

The inoculum was obtained from a UASB reactor treating poultry slaughterhouse wastewater in São Paulo State, Brazil. The granular sludge was previously disintegrated in a blender to obtain a homogenous and pasty mixture. Then, the cubes of polyurethane foam were mixed and compressed with a large quantity of sludge. Thereafter, they were kept in contact for at least 2 h, as recommended by Zaiat et al. (1994). After this period, the foam matrices were transferred to a sieve, in which the excess of sludge was removed by frictioning the foam on the screen. Finally, the inoculated foam cubes were placed inside the reactors using tongs, until its complete filling.

The medium simulated nitrified domestic sewage, as used by Callado and Foresti (2000). Some changes were made to the sewage composition according to Moraes et al. (2011). Nitrate and nitrite were used as electron acceptors in separate experiments, and the same nutrient concentration of 20 mg N L\(^{-1}\) was maintained for both assays. The final composition (mg L\(^{-1}\); including the micronutrients present in domestic sewage, was as follows: KNO\(_3\) (144) or NaNO\(_3\) (99), KH\(_2\)PO\(_4\) (36), NH\(_4\)Cl (16), NaHCO\(_3\) (2000), and MgCl\(_2\)-6H\(_2\)O (28), CaCl\(_2\)-2H\(_2\)O (18). Sulfide as a Na\(_2\)S-9H\(_2\)O solution was injected through the rubber sealing on the top of the Duran bottles. Sulfide was supplied in excess (Test I) and at the stoichiometrically required concentration relative to nitrate and nitrite (Test II), based on the biochemical reactions presented in Eqs. (1) and (2). In Test I, total dissolved sulfide (TDS) was applied to the assays with nitrate and nitrite at concentrations of 50 mg TDS L\(^{-1}\) and 30 mg TDS L\(^{-1}\), respectively, resulting in the respective N:S molar ratios of 0.9 and 1.5. In Test II, sulfide concentrations were 30 mg TDS L\(^{-1}\) for nitrate (N:S = 1.6) and 17 mg TDS L\(^{-1}\) for nitrite (N:S = 2,7). A complementary trace elements solution, specific for the enrichment of chemoautotrophic denitrifying culture, was added at 2 mL L\(^{-1}\). It was composed of (g L\(^{-1}\) EDTA (0.50), ZnSO\(_4\)-7H\(_2\)O (0.04), CaCl\(_2\)-2H\(_2\)O (0.07), MnCl\(_2\) (0.03), (NH\(_4\))\(_2\)MoO\(_4\)·4H\(_2\)O (0.01), CuSO\(_4\)-H\(_2\)O (0.02), and CoCl\(_2\)-6H\(_2\)O (0.02) (Beristain-Cardoso et al., 2006).

3.3. Experimental procedure and kinetic analysis

The intrinsic kinetic parameters of autotrophic denitrification via nitrate and nitrite were evaluated in Tests I and II. The methodology for this evaluation was based on Zaiat et al. (1996) and Vieira (1996). Prior to the kinetic assays, the immobilized biomass was adapted to the process in vertical fixed-bed reactors for 30–35 days for each condition analyzed in the kinetic assays (i.e., Tests I and II via nitrate and nitrite). For each condition, five differential reactors were used. Each one was subjected to a different liquid flow rate (Q; cm\(^3\) s\(^{-1}\)). The liquid superficial velocity (\(v_s\); cm s\(^{-1}\)) was calculated based on the flow, as shown in Eq. (5):

\[ v_s = \frac{Q}{e \cdot A} \]  

(5)
where $A$ is the cross-sectional area of the reactor (cm$^2$) and $\varepsilon$ is the bed porosity (0.4).

Second-degree polynomial equations were adjusted to each $v_i$ through the 10-h temporal profiles of nitrogen concentration in the bulk liquid ($C_N$, mg N L$^{-1}$), according to Eq. (6):

$$C_N = at^2 + bt + c$$

The observed specific substrate utilization rates ($r_{obs}$), expressed in mg N g VSS$^{-1}$ h$^{-1}$, were calculated as a function of $C_N$ for each applied $v_i$, according to Eq. (7). In this case, differential reactors were treated as batch reactors of constant volume because the experimental system was performed in a closed circuit:

$$r_{obs} = \frac{dC_N}{dt} \big|_{t = t_0} = -2at_i - b$$

Profiles of the observed specific initial substrate utilization rate ($r_{obs} |_{t = 0}$) over $v_i$ were constructed to determine from which $v_i$ value the external mass transfer resistance could be neglected. For this condition, $r_{obs} |_{t = 0}$ must assume a constant value. The dimensionless Biot number ($Bi$) was calculated to confirm the above condition (Eq. (8)). According to Bailey and Ollis (1986), the effect of external mass transfer resistance is not significant for $Bi \gg 100$:

$$Bi = \frac{k_s D_p}{D_e}$$

where $k_s$ is the solid–liquid mass transfer coefficient (cm s$^{-1}$), $D_p$ is the equivalent sphere bioparticle radius (0.5 cm) and $D_e$ is the effective diffusivity in the bioparticle ($1.99 \times 10^{-5}$ cm$^2$ s$^{-1}$) (Perry and Chilton, 1985).

The magnitude of the effects of intraparticle mass transfer resistance in relation to the rates of biochemical reactions were evaluated using the Thiele modulus ($\phi_{obs}$) expressed in Eq. (9) and considering the operation in the absence of external mass transfer resistance. According to Bringi and Dale (1990), if $\phi_{obs} \gg 0.3$, then the internal mass transfer resistance is significant and the diffusion rate is the limiting process:

$$\phi_{obs} = \frac{r_{obs} - R_p^2}{5D_p C_N}$$

where $C_N$ is the specific nitrogen concentration in the bulk liquid (mg N g VSS$^{-1}$ h$^{-1}$) and $r_{obs} = r_{obs} |_{t = 0}$. Intrinsic kinetic parameters were estimated for all trials in which negligible external and internal mass transfer resistances were achieved. Thus, half-order kinetic models were applied to experimental data obtained from temporal profiles of nitrogen removal through the numerical method of Levenberg–Marquardt (Origin 6.0 software). According to this analysis, the result corresponding to $v_i = 0.069$ cm$^3$ s$^{-1}$ was dismissed because it was too disparate in relation to other values (Table 1). It is noteworthy that the biomass concentration (g VSS L$^{-1}$) was much higher when compared with other results. As a consequence, a high value of $r_{obs} |_{t = 0}$ was observed. Considering the analysis for the other results, it was impossible to find a $v_i$ value at which the minimization of external mass transfer resistance began to occur (i.e., when $r_{obs} |_{t = 0}$ remained constant) because these values were very close to each other. Therefore, values of $Bi$ were calculated for all $v_i$. Negligible external mass transfer resistance was found for $v_i$ of 0.054 and 0.075 cm$^3$ s$^{-1}$, corresponding to $Bi$ values of 110.1 and 152.4, respectively.

The same data analysis was conducted for Test II. For this analysis, the result corresponding to $v_i = 0.069$ cm$^3$ s$^{-1}$ was dismissed because it was too disparate in relation to other values (Table 1). It is noteworthy that the biomass concentration (g VSS L$^{-1}$) was much higher when compared with other results. As a consequence, a high value of $r_{obs} |_{t = 0}$ was observed. Considering the analysis for the other results, it was impossible to find a $v_i$ value at which the minimization of external mass transfer resistance began to occur (i.e., when $r_{obs} |_{t = 0}$ remained constant) because these values were very close to each other. Therefore, values of $Bi$ were calculated for all $v_i$. Negligible external mass transfer resistance was found for $v_i$ of 0.054 and 0.075 cm$^3$ s$^{-1}$, corresponding to $Bi$ values of 110.1 and 152.4, respectively.

4. Results and discussion

4.1. Denitrification via nitrate

The $C_N$ profiles over time for each applied $v_i$ and for Tests I and II are presented in Fig. 1. Values of $r_{obs}$ were calculated as a function of $C_N$ from polynomial regression analysis and for the measured values of biomass concentration in each differential reactor. The resulting $r_{obs} |_{t = 0}$ values are shown in Table 1.

Profiles of $r_{obs} |_{t = 0}$ over $v_i$ were performed for Tests I and II (Fig. 2). For Test I, $r_{obs} |_{t = 0}$ was kept constant when $v_i \geq 0.069$ cm$^3$ s$^{-1}$. Under such conditions, the external mass transfer resistance was negligible, i.e., the stagnant liquid layer surrounding the bioparticle was minimized. The $Bi$ values obtained for $v_i$ of 0.069 and 0.075 cm$^3$ s$^{-1}$ were 139.4 and 158.5, respectively. These results support the assumption of a non-significant resistance to external mass transfer. The value of $k_s$ for the $Bi$ calculation was estimated using the relationship proposed for the polyurethane foam bed with an $R_p$ of 0.31 cm and a bed porosity of 0.4 for low $v_i$ (Zait et al., 1996) according to Eq. (12):

$$k_s = \frac{0.244 + 0.271 \exp(1.796 - \frac{v_i}{a_i})}{a_i}$$

where $a_i$ is the interfacial area for mass transfer (cm$^2$) determined according to Eq. (13) (Zait et al., 1996):

$$a_i = \frac{n \cdot A_p}{V_L}$$

where $V_L$ is the bulk liquid volume (15 cm$^3$), $A_p$ is the bioparticle area considering an equivalent sphere (0.79 cm$^2$), and $n$ is the number of bioparticles going into the reactor. The counting of particles was carried out at the end of the experiments for each reactor and ranged from 95 to 119 considering all reactors and experiments conducted.

All chemical analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2005). Nitrate (NO$_3^-$-N), nitrite (NO$_2^-$-N) and ammonium (NH$_4^+$-N) were determined by flow injection analysis (FIA). The total dissolved sulfide (TDS) was determined using the methylene blue colorimetric method, and sulfate (SO$_4^{2-}$-S) was measured using the turbidimetric method. Nitrogen and sulfur intermediary compounds were calculated according to principles of mass conservation. Volatile suspended solids (VSSs) and attached solids in polyurethane foam were gravimetrically determined.
Table 1
Equations for the NO$_3$-N concentration as a function of time, the corresponding correlation coefficients ($R^2$) for different applied superficial velocities ($v_s$), and the respective observed specific initial substrate utilization rates for Tests I and II.

| Test | $v_s$ (cm s$^{-1}$) | $C_0$ (mg NO$_3$-N L$^{-1}$) | $R^2$ | $g$ VSS L$^{-1}$ | $r_{obs|t=0}$ (mg N g VSS$^{-1}$ h$^{-1}$) |
|------|------------------|-------------------------------|--------|-----------------|----------------------------------|
| I    | 0.007            | $-0.090x^2 - 0.383x + 21.86$ | 0.981  | 0.424           | 0.903                            |
|      | 0.022            | $-0.079x^2 - 0.953x + 22.10$ | 0.985  | 0.516           | 1.847                            |
|      | 0.054            | $-0.031x^2 - 1.799x + 21.88$ | 0.991  | 0.430           | 4.182                            |
|      | 0.069            | $0.021x^2 - 2.038x + 21.26$  | 0.999  | 0.407           | 5.013                            |
|      | 0.075            | $0.059x^2 - 2.530x + 21.30$  | 0.992  | 0.523           | 4.836                            |
| II   | 0.007            | $-0.045x^2 - 0.314x + 21.85$ | 0.994  | 0.507           | 0.619                            |
|      | 0.022            | $-0.119x^2 - 0.427x + 21.96$ | 0.975  | 0.855           | 0.499                            |
|      | 0.054            | $-0.037x^2 - 0.458x + 21.97$ | 0.983  | 0.606           | 0.757                            |
|      | 0.069            | $-0.057x^2 - 2.555x + 22.54$ | 0.983  | 1.296           | 1.971                            |
|      | 0.075            | $-0.035x^2 - 0.460x + 21.93$ | 0.998  | 0.635           | 0.724                            |

Fig. 1. Temporal profiles for NO$_3$-N concentration obtained for applied $v_s$ of (○) 0.007, (△) 0.022, (□) 0.054, (▼) 0.069, and (□) 0.075 cm s$^{-1}$, as well as the respective adjusted curves from polynomial regression using nitrate ((A) Test I and (B) Test II) and nitrite ((C) Test I and (D) Test II).

Fig. 2. Profiles of the observed specific initial substrate utilization rates ($r_{obs|t=0}$) over $v_s$ for (■) Test I and (▲) Test II: (A) denitrification via nitrate and (B) denitrification via nitrite.
The curves that were adjusted to fit the experimental data, corresponding to the half-order model, are shown in Fig. 3. The intrinsic half-order kinetic parameters were estimated for each fitted curve (Table 2).

The higher values of intrinsic parameters for Test I were attributed to the use of different sulfur forms as electron donors during autotrophic denitrification. During Test I, the amount of sulfate formed did not correspond to the amount of sulfide consumed. On the other hand, during Test II the sulfur mass balance was closer, but there were periods in which the low formation of intermediary sulfur compounds was followed by subsequent consumption (Table 3). Thus, the electrons donated to denitrification were also derived from intermediary sulfur compounds, such as elemental sulfur. In addition, the formation of a whitish layer deposited on some polyurethane foam matrices was observed. This is characteristic of elemental sulfur deposition. According to Koenig and Liu (2001), the limiting factor for denitrification using elemental sulfur is the low solubility of this compound in water at room temperature. This electron donor would only be used by microorganisms after solubilization and diffusion into the biomass. Consequently, the biochemical reaction rates of autotrophic denitrification would be affected.

Therefore, the use of elemental sulfur as an electron donor probably caused the lower values for the intrinsic parameters in Test II. In Test I, the sulfur mass balance showed that most of the electrons were donated by partial sulfide oxidation with a higher formation of intermediary sulfur compounds (Table 3). Sulfide is more bioavailable to microorganisms than elemental sulfur; therefore, autotrophic denitrification rates from sulfide oxidation must be greater than from elemental sulfur oxidation.

Half-order parameters were located in an intermediary range compared with values reported by other authors. Koenig and Liu (2001) and Moon et al. (2006) obtained half-order reaction rate constants in denitrifying autotrophic systems with immobilized cells in sulfur granules equal to 3.15 mg N$^{1/2}$ L$^{-1/2}$ h$^{-1}$ and 0.104 mg N$^{1/2}$ L$^{-1/2}$ h$^{-1}$ when the thickness range of the sulfur particles was 2.8–5.6 mm and 2–5 mm, respectively. In both of these
studies, when particle size was increased, the half-order reaction rate constants decreased. Koenig and Liu (2001) reported average values of 1.59 mg N/12 L -1 h -1 (5.6–11.2 mm thickness range) and 1.21 mg N/12 L -1 h -1 (11.2–16 mm thickness range), while Moon et al. (2006) found 0.028 mg N/12 L -1 h -1 for particle sizes larger than 5 mm. In these conditions, the internal mass transfer resistance probably affected the biochemical reactions because the larger the particle size, the more difficult it is to minimize the intraparticle resistance. Therefore, lower constant values were obtained. Moon et al. (2004) also reported different constant values, ranging from 1.90 to 2.18 mg N/12 L -1 h -1, when column reactors packed with 2-mm diameter sulfur granules were used. In addition to particle sizes, the variability in these values can be explained by differences in the inoculum characteristics and enrichment, the reactors configurations and the composition of the treated wastewater.

4.2. Denitrification via nitrite

As for denitrification via nitrate, the experimental values of CkN over time as a function of vS were subjected to polynomial regression analysis (Fig. 1). The resulting equations for nitrogen concentrations as a function of time and for the respective fobs[0] are shown in Table 4. Profiles of fobs[0] versus vS were obtained to evaluate the absence of the external mass transfer resistance (Fig. 2). For both Tests I and II, the external mass transfer resistance could be neglected for vS of 0.069 cm s -1 and 0.075 cm s -1. The calculated BI values were higher than 100, which confirmed this fact. For Test I, the BI values were 128.7 and 155.1, and for Test II they were 128.7 and 155.4. For these conditions, the evaluation of the effects of the internal mass transfer resistance was performed by calculating fobs, which were much lower than 0.3 for both tests (0.011 to 0.014). Thus, the intrinsic kinetic parameters were estimated for the vS mentioned above, in which negligible external and internal mass transfer resistances were attained.

Half-order models that were fit to the experimental data are presented in Fig. 3, and the respective half-order parameters and the correlation coefficients ( R 2) are shown in Table 5. The values of the half-order coefficients for both tests were close, ranging between 0.425 and 0.657 mg N/12 L -1 h -1. In this case, the sulfur mass balance showed no consumption of intermediary sulfur compounds during temporal profiles (Table 6). Therefore, most of the electrons used for nitrate reduction were donated directly by sulfide during both tests. Throughout the profiles of sulfur compounds, only the generation of intermediary sulfur compounds was detected, and sulfate formation approached the concentration of sulfide oxidized by denitrification of nitrite. Thus, during denitrification via nitrite, the electrons for denitrification were derived mostly from sulfide oxidation, independently of the electron donor (sulfide) concentration. Consequently, the kinetic parameters were similar because most of the electrons were donated by the same sulfur form (i.e., sulfide) in both tests.

Compared with Test I of denitrification via nitrate, in which the same form of sulfur was primarily used as the electron donor (sulfide), the maximum specific nitrogen utilization rate for denitrification via nitrite was slightly lower. Cervantes et al. (2009) also found denitrification rates via nitrite to be lower than those via nitrate (0.25–0.36 mg N g VSS -1 h -1), indicating that nitrite reduction was the limiting step in sulfide-oxidizing autotrophic denitrification. According to the authors, an inhibitory effect of sulfide on nitrite reductases. Therefore, some precipitation of trace metals essential for bacterial metabolism may have occurred. Thus, the slightly lower rates achieved for autotrophic denitrification via nitrite compared with nitrate were probably due to some interaction of sulfide with trace metals, such as copper, which may have impaired the action of

Table 4

<table>
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<tr>
<th>Test</th>
<th>vS (cm s -1)</th>
<th>CkN (mg NO3-N L -1)</th>
<th>R 2</th>
<th>g VSS L -1</th>
<th>fobs[0] (mg N g VSS -1 h -1)</th>
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Table 5

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<th>k1 (mg N/12 L -1 h -1)</th>
<th>R 2</th>
<th>g VSS L -1</th>
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<td>3.776</td>
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<td>0.658 ± 0.031</td>
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Table 6

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<th>TDS (mg L -1)</th>
<th>SO2- S (mg L -1)</th>
<th>Interna (mg L -1)</th>
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4 Internary sulfur compounds.
nitrite reductases. However, for denitrification via nitrate, low nitrite accumulation was observed (<3 mg NO₂⁻-N L⁻¹), which indicates that the nitrite reductases were not as affected in this case. Different types of these enzymes can catalyze the nitrite reduction step, with different structural and functional characteristics, depending on the species of microorganisms involved (Godden et al., 1991; Weeg-Aerssens et al., 1991; Richardson and Watmough, 1999). The possibility of different species acting in denitrification via nitrate and nitrite cannot be ruled out, as well as the distinct types of nitrite reductases used in autotrophic denitrification pathways. Therefore, there is a need of further studies on microbiology and biochemistry to clarify some aspects of the metabolic pathways involved in the autotrophic denitrification using sulfide as electron donor.

5. Conclusions

Intrinsic kinetic parameters of sulfide-oxidizing autotrophic denitrification via nitrate and nitrite were estimated for systems containing immobilized biomass. The experimental results showed that a half-order kinetic model could be successfully applied to describe this bioprocess, providing comparable kinetic parameter values. Half-order kinetic coefficients were affected by the form of the electron donor used; higher parameters were obtained when electrons were donated directly from sulfide oxidation. A higher specific nitrogen utilization rate was achieved in denitrification via nitrate than in denitrification via nitrite.

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References


