**BIOLOGICAL REMOVAL OF GASEOUS SULFUR DIOXIDE THROUGH THE
REDUCTION TO HYDROGEN SULFIDE BY MEANS OF *Desulfovibrio desulfuricans***

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ABSTRACT

The aim of this paper was to study the biological removal of gaseous sulfur dioxide by using the sulfate reducing bacteria *Desulfovibrio desulfuricans*. In order to achieve contact between the sulfur dioxide and the bacteria, laboratory-scale bioreactors were designed to generate the sulfur dioxide inside of them through a chemical reaction. The bio-reduction occurred by solubilization in the liquid phase of the sulfur dioxide of the gaseous phase, which produced sulfite ions which were used as the final acceptor of electrons by the bacteria suspended in the liquid phase and reduced to sulfide. To evaluate the biological reduction, three kinetics (in triplicate) were taken in batch mode with loads of 15, 20 and 25 m-moles of SO₂ generated in the gas phase per liter of culture medium in the liquid phase. Lactate was used as

substrate (electron donor) and carbon source. The experimental results showed a 100% SO₂ reduction for all the evaluated loads. The tests lasted 24, 72 and 192 hours with lower, intermediate and higher loads, respectively. The total sulfide (S²⁻) produced varied between 75 and 126 mg for the tests with lower and higher load, respectively. These amounts were composed of a fraction in the aqueous phase (46 and 85 mg) and another in the gas phase (29 and 41 mg). These values corresponded to 287 and 533 mg of S²⁻/L in the liquid phase and 1.9x10⁸ and 2.7x10⁸ µg H₂S/m³ in the gas phase. The mass ratio between product formation (sulfide) and electron donor consumption (expressed in COD) ranged from 81% (higher load) to 90% (lower load) of the theoretical value (0.67 mg of sulfide produced per mg of COD consumed).

Keywords: SO₂ removal, hydrogen sulfide, *Desulfovibrio desulfuricans*, batch mode, kinetics.

INTRODUCTION

Numerous industries involve the use of raw materials with sulfur compounds, such as thermoelectric power plants and chemical industries, where large amounts of polluting gases are generated (Li, 2015). One of these polluting gases that entails catastrophic consequences on the environment is sulfur dioxide (SO₂) (Li et al., 2015). Sulfur dioxide, once released into the atmosphere, may react photochemically or catalytically with other atmospheric pollutants to form sulfur trioxide (SO₃), sulfuric acid (H₂SO₄) and various sulfuric acid salts which form the principal constituents of acid precipitation, also known as acid rain (Singh and Agrawal, 2008; Hao et al., 2016). In addition, sulfur dioxide contributes to respiratory diseases, especially in children and the elderly (Chiang et al., 2016; Ghosikali et al., 2016).

There are three basic approaches for reducing SO₂ emissions into the atmosphere, i.e. de-sulfurizing the feedstock before use, conducting the de-sulfurization of the gases while carrying out the combustion or removing SO₂ from the gases generated after combustion (post-combustion de-sulfurization or flue gas de-sulfurization) (Sublette and Dasu, 1993; Bian et al., 2016; De Gisi et al., 2017). The post-combustion de-sulfurization technology most commonly used commercially is the use of disposable sorbents such as limestone (CaCO₃), which have affinity for acid gases such as SO₂ (Srivastava and Jozewicz, 2001; Pandey et al., 2005). These types of processes include physical and chemical methods that can be quite efficient, but generate new wastes and/or require the input of additional chemicals and energy (Philip and Deshusses, 2003; Xie, et al., 2013; Nikolopoulos et al. 2014; Liu et al., 2016; Hao et al., 2017). The costs of chemical and catalytic substances make the de-sulfurization physicochemical processes very expensive, adding to them the costs of disposal of the wastes that are generated in this type of process. . For all these reasons it is necessary to develop new technologies which are low cost and at the same time ecologically friendly to the environment. Since the end of the 80's and 90's, studies have been carried out to develop biological processes to reduce SO₂ (Dasu et al., 1989; Lee et al., 1994; Kaufman, et al., 1997). These processes involve the use of bioreactors (conversions are catalyzed by microorganisms), where large amounts of wastes are not generated and inexpensive raw materials can be used. At present, these biological methods have continued to be evaluated by having in common that gaseous SO₂ in one form or another is introduced into a liquid stream to be reduced in the liquid medium by sulfate reducing bacteria (SRB) (Qian et al., 2015; Fakhari et al., 2015; Duarte et al., 2016; Zhang et al., 2016 and 2017).

The electron donors that are oxidized by the SRB are usually organic compounds of low molecular weight and hydrogen. Some widely used substrates are: hydrogen, methanol, ethanol, acetate, lactate, propionate, butyrate, sugar and molasses (Liamleam and

Annachhatre, 2007). Genuses such as *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum* and *Desulfolobus* use lactate, pyruvate, ethanol or certain fatty acids as electron donors (Madigan et al., 2014).

Theoretically, 64 g of COD/mole of sulfate are needed for complete reduction, in the case of sulfite 48 g of COD/mole of sulfite are required. These values indicate that 0.67 or 0.6 mole of COD or electron donor, respectively, are required for bio-converting one mole of sulfate or sulfite, the electron donor being a direct source of carbon and energy.

The growth of mesophilic SRBs is quite a bit slower than that of thermophilic, taking from several days to 2 weeks at 30 °C depending on the species, while the latter at 55 °C generally takes from 12 to 18 hours (Postgate, 1984). One of the causes of the slow growth of these bacteria is the product of their respiration. H₂S decreases growth and in some cases (at high concentrations) reduces it to zero (Postgate, 1984). Generally, acetate-producing bacteria such as *Desulfovibrio* and *Desulfotomaculum* grow faster and appear to be able to grow at times of doubling of between 3 to 6 hours (at 30 °C).

The presence of sulfide inhibits microbial populations, decreasing their activity. On the other hand, sulfide precipitates the iron needed as a micronutrient, decreasing its bioavailability (Postgate, 1984).

In addition to sulfate, many SRB can reduce sulfite, thiosulfate and elemental sulfur to sulfide. Numerous studies have been carried out using sulfate-reducing bacteria for bioremediation of sulfate-contaminated effluents; however, little effort has been made to investigate the gas bio-desulfurization processes with these microorganisms.

The aim of this work was to study the biological reduction of sulfur dioxide to hydrogen sulfide (SO₂ ➔ H₂S) in a single step, through the generation and reduction of sulfite as an intermediate. A direct reduction system of the sulfur dioxide contained in a gaseous stream

would avoid the requirement of a previous absorption step in an aqueous matrix, and then reduce the sulfite and sulfate of the solution in another step. In addition, by directly treating the gaseous stream as a phase above the aqueous phase, the use of pumps to bubble the contaminated gas to the bioreactor is avoided.

Hydrogen sulfide (sulfhydrylic acid) obtained as the final product in the reduction of SO_2 (g) can subsequently be removed by various physicochemical or biological methods already established at an industrial scale (Neculita et al., 2007; Guerrero et al., 2015; Sun et al., 2015; Aguilera and Gutiérrez, 2016; Augelletti, et al., 2017; Meier et al. 2017; Rotunno et al., 2017). Recently, the application of biological micro aeration to the conversion of H_2S to elemental sulfur is predominant and this system completely harmless to the environment (Krayzelova et al., 2014; Ramos et al., 2014; Díaz et al., 2015; Jeniček et al., 2017).

MATERIALS AND METHODS

Experimental set-up

The experimental study for the biological removal of SO_2 was carried in special reactors designed to produce gaseous SO_2 inside. These bioreactors are modified glass flasks of 250 mL of volume which have a glass tube connected on the side, where gaseous SO_2 is produced by a chemical reaction between sodium metabisulfite and sulfuric acid. The tubes have a hole in their upper part, where the gaseous SO_2 can escape and fill the inside of the bioreactors (Figure 1).

The experimental system consisted of a setup in series of 10 bioreactors. 150 mL of culture medium plus 10 mL of SRB inoculum from previous cultures were added to each bioreactor. The bioreactors were placed in an orbital shaker at 150 rpm and 35 °C. Three

kinetics of bio-reduction of SO₂ were performed in batch mode, removing a shaker flask daily and each one being a representative sample in time. In each kinetic study the amount of SO₂ produced inside the flasks was increased to be able to evaluate the biological reduction capacity of the sulfur dioxide in the proposed system. Each experimental run was performed in triplicate. The SO₂ which is produced inside the tubes inserted into the flasks diffuses through the hole in the upper part of each tube towards the inside of the bioreactors and is solubilized in the aqueous phase, producing sulfite ions, according to the following equilibria:

These equations show that per mole of gaseous SO₂ that is dissolved, one mole of SO₃²⁻ in the liquid phase is also formed. The produced sulfite is used as the final acceptor of the electrons and is reduced to sulfide by a culture in suspension of the SRB *Desulfovibrio desulfuricans*. As sulfite is bio-reduced, the chemical equilibrium between the gaseous and aqueous species of SO₂ (SO₂ (g) → SO₂ (aq)) is displaced (to the right) so that the sulfur dioxide continues to be solubilized and therefore continues to add sulfite to the liquid medium. Sodium lactate was used as the source of carbon and energy required for the biological reduction.

The bio-reduction of sulfite with lactate as an electron donor for sulfate-reducing bacteria that do not degrade acetate as *D. desulfuricans* is expressed as follows:

The analysis was focused on three kinetic processes in particular, product formation (total dissolved sulfide and H₂S (g)), substrate consumption (lactate) and bacterial growth.

Microorganism used

A culture of the Sulfate-Reducing Bacteria *Desulfovibrio desulfuricans* was obtained from the University of Santiago de Chile. Stock cultures were prepared by incubating the bacteria anaerobically in fully filled test tubes with Postgate B culture medium (Postgate, 1984) at 30 °C. After 1 week the cultures were stored at 4 °C in a refrigerator until use. The composition of the Postgate B culture medium is shown in Table 1.

Ascorbic acid and thioglycolic acid act as reducing agents, so that when cultivating the SRB, the environment has a low re-dox potential (Eh) (from -100 mV) (Postgate, 1984). The culture medium is blackened by the black precipitate formed by the FeS.

Conditioning and acclimation of the biomass for use as inoculum

Sulfate reducing bacteria were cultured in a 1 liter bioreactor in batch mode with 800 mL of Postgate C culture medium (Postgate, 1984), at 35 °C and magnetically agitated. The initial pH of the culture medium was 7-7.5 and was not controlled thereafter. Table 2 shows the composition of the Postgate C culture medium.

Nitrogen was sterilized by filtration (0.2 µm) and pumped into the bioreactor to desorb the sulfide as H₂S (g) and maintain an anaerobic environment. The H₂S (g) was directed to a zinc acetate trap, (Zn(C₂H₃O₂)₂·2H₂O (10 g/L), to precipitate the sulfide as zinc sulfide according to the following reaction:

After 2 days, part of the culture was sub-cultured in the same medium, but this time the sodium sulfate was replaced with sodium sulfite (1.5 g/L sulfite). This is to try to condition

the bacteria to the new electron final acceptor (sulfite ions) prior to the tests. Cells were sub-cultured in this medium every 2 or 3 days for the duration of the experiments (3 months) to have a large amount of biomass (on the order of 1×10^{10} cells/mL) conditioned for the assays. Cell growth was evidenced by direct microscopic counting in the chamber and also by the formation of precipitated sulfide in the trap as an indirect method.

SO₂ generation

Sulfur dioxide was generated by a chemical reaction between sodium metabisulfite and sulfuric acid, inside the glass tubes inserted in the bioreactors. Equation (7) shows this reaction:

To produce the reaction, the metabisulfite was added to the tubes and the sulfuric acid was injected through a septum. Diluted sulfuric acid (one part of concentrated sulfuric acid and 2 parts of distilled water) was injected through the septum using 1 mL syringes with a needle of 13 mm long and 0.3 mm thick (30 gauge) so that when removing the needle from the septum, a hole as small as possible was left so there were no SO₂ leaks. In addition, it was corroborated that SO₂ did not escape by adding a little water with soap and observing that no bubbles were formed, indicating that there was no SO₂ loss.

Chemical analyses

The following chemical analyses were carried out: chemical oxygen demand (COD), suspended biomass, pH, re-dox potential (Eh), sulfite, total dissolved sulfide (S²⁻, HS⁻ and H₂S_(aq)) and gaseous hydrogen sulfide (H₂S_(g)).

The Chemical Oxygen Demand was determined by the closed reflow colorimetric method (APHA, 2012). In this research, the COD measurement was used to show the dynamics of the

organic source (lactate), so the sample was centrifuged (8,000 rpm for 10 minutes at 4 °C) and the supernatant was analyzed. In addition, because sulfide and sulfite increase COD, the sample was acidified (10 drops of concentrated sulfuric acid to 50 mL of sample) and nitrogen was pumped for a few minutes to desorb sulfide and sulfite in the solution as H₂S (g) and SO₂ (g), respectively.

The suspended biomass was determined using the on-camera microscopic counting method (Madigan et al., 2014). Because the sulfite measurement was subjected to error due to the oxidation of sulfite to sulfate, in addition to the interferers in the sample, sulfite was deliberately oxidized to sulfate with hydrogen peroxide and the sulfate concentration was determined. The following reaction shows the oxidation of SO₃²⁻ to SO₄²⁻ with hydrogen peroxide:

Sulfate was determined using the turbidimetric method proposed by Kolmert et al. (2000). For the determination of total dissolved sulfide, the method developed by Cord-Ruwisch (1985) was used.

RESULTS AND DISCUSSION

Assessment of the biological reduction of SO₂ of the experimental system

Figures 2A and 2B show the maximum concentration of soluble sulfide and gaseous hydrogen sulfide obtained over time. The trend lines from time 0 show the volumetric productivity indicated by the slope of the equation (expressed as mg/(L h) or g/(m³ h)).

As can be seen, the three amounts of sulfur dioxide produced in the bioreactors were completely reduced to sulfide; however, as the amount of SO₂ generated increased, the longer the time that the biomass present in the cultures took to start to metabolize the sulfite

produced in the medium. This behavior would be expected to be due to substrate inhibition, but when the biomass began to have activity, it was observed that it reduced at a similar rate from the start. If it were a substrate inhibition, the reduction should have started slowly until reaching non-inhibitory levels, in this case of sulfite, and then began to bio-convert at a higher rate. Thus, it appeared that this behavior would not be due to substrate inhibition but rather to a conditioning or acclimation of the biomass to the new environmental conditions. The bioreactors presented a very dynamic environment when the SO_2 was generated inside them, which led to different changes in the microorganism culture medium. When SO_2 was produced, it began to solubilize in the medium, which produced sulfurous acid as an intermediate before reaching the sulfite ion. This caused the pH to decrease greatly. In the experiments, a phosphate buffer was used to maintain the pH close to 7.0, although these environmental changes seemed to affect the present biomass anyway. In addition, in the case of the experiment with a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.4, the re-dox potential rose from -282 mV to -198 mV after 48 hours of the beginning of SO_2 production, and then started to decrease gradually.

Productivity was greater at a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.24, and then decreased as the $\text{SO}_3^{2-}/\text{COD}$ ratio increased and, thus, increased the amount of SO_2 produced in the bioreactors. At a greater amount of SO_2 , a higher the concentration of sulfide was obtained in both phases because productivity is a function of time, and in this case, the reduction in time, or rather the time delay of the biomass in starting to reduce the sulfite, was directly proportional to the amount of SO_2 produced in the bioreactors.

Figure 3 shows the sulfite content in the culture medium as a function of the $\text{SO}_3^{2-}/\text{COD}$ ratio for the three experimental runs carried out. As can be seen in Figure 3, in the case of the $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.24, sulfite was not observed in the medium because the microorganisms reduced all the sulfite generated by the SO_2 in less than 24 h. For this ratio,

the experiments were very stable and the biomass did not have inhibition problems or conditioning problems to the new dynamics of the environment. For the case of the SO_3^{2-} /COD ratio of 0.32, there was about 1000 mg/L of SO_3^{2-} at 24 hours, which is equivalent to 62% of the total sulfite potential present as SO_2 (1600 mg/L). There was a chemical equilibrium between the gaseous SO_2 of the atmosphere of the bioreactors and the SO_3^{2-} produced in the culture medium, which was required to start to consume the latter so that the equilibrium moved to continue solubilizing SO_2 gaseous, thus adding SO_3^{2-} to the medium. At 24 hours the sulfate reducing bacteria began to have activity and for 72 hours, no sulfite was detected in the medium. In the kinetics with the SO_3^{2-} /COD ratio of 0.4, at 24 hours, approximately 1350 mg/L of sulfite were quantified, which meant 67% of the total sulfite potential present as SO_2 (2000 mg/L). As in the previous case, it was required to start the consumption of SO_3^{2-} that was in the culture medium, to shift the equilibrium towards the solubilization of more SO_2 and consequently the formation of more SO_3^{2-} . The SO_3^{2-} remained constant and close to the value previously discussed until it began to be reduced to 144 hours to decrease to 0 at 192 hours. The culture medium was slightly blackened when the biological reduction began, due to the reaction of the sulfide produced with the small amount of iron added to the medium. This reaction served as a qualitative indicator that the reduction had begun.

For the assays carried out with a SO_3^{2-} /COD ratio of 0.24, all the SO_2 generated in the bioreactors was reduced in 24 hours, which is equivalent to 15 m-moles of SO_2 per liter of culture per day ($\approx 1.2 \text{ g SO}_2/(\text{L}\cdot\text{d})$). Dasu and Sublette (1989) obtained a maximum reduction rate of 41 m-moles $\text{SO}_2/(\text{d}\cdot 10^{11} \text{ cells})$, which is equivalent to $2.6 \text{ g SO}_2/(\text{d}\cdot 10^{11} \text{ cells})$. However, these authors did not indicate the volume of the reactor so the volumetric productivity (or volumetric reduction rate) remains unknown. Dasu et al. (1993) obtained a removal rate of 8 m-moles $\text{SO}_2/(\text{L}\cdot\text{d})$ ($0.5 \text{ g SO}_2/(\text{L}\cdot\text{d})$). Selvaraj et al. (1997) reported a

reduction rate of 50 m-moles $\text{SO}_2/(\text{L}\cdot\text{d})$ (3.2 g $\text{SO}_2/(\text{L}\cdot\text{d})$). In these investigations the SO_2 was fed to the reactors by bubbling at rates of 1.7 m-moles/(h·10¹¹ cells) (109 mg / (h·10¹¹ cells), 0.33 m-moles/(L·h) (21 mg/(L·h) and 2.1 m-moles/(L·h) (134 mg/(L·h) respectively, while in the present investigation, all SO_2 was generated in the gas phase of the bioreactors from the beginning of the process. On the other hand, in the above-mentioned works, the sulfide produced as H_2S (g) was desorbed with nitrogen. Lens et al. (2003) observed that the sulfide concentration in the reactor liquid of a completely stirred tank reactor rose rapidly to very high values (up to 1.6 g/L in a 10 days period) at a feed rate of 0.5-1.5 g/(L·d). In spite of the aforementioned research works, no paper has been reported in the literature on the biological reduction of gaseous SO_2 in a system similar to that of this research. This makes it difficult to compare the results obtained in this study with other reported studies.

The biomass was conditioned and acclimated for the assays with 1.5 g/L of sulfite without showing inhibitory effects. In this way the biomass strongly grew during this conditioning step. In the assays with higher amounts of SO_2 , a sulfite concentration of 1.35 g/L was detected, which did not generate any inhibitory effects. This further validates the theory that the time that the biomass present in the culture is delayed in starting to reduce sulfite would be due to a conditioning to the new environmental conditions (lag phase of bacterial growth) rather than to inhibition by substrate.

Figure 4 shows the total production of sulfide (sum of the masses of sulfide of the aqueous and gaseous phases) and the total sulfide productivity as a function of the $\text{SO}_3^{2-}/\text{COD}$ ratio. Although sulfide production was observed to grow as the $\text{SO}_3^{2-}/\text{COD}$ ratio increased, the productivity decreased. This shows that sulfide production and productivity were directly and inversely proportional, respectively, to the initial amount of SO_2 gaseous generated in the bioreactors.

As can be seen in Figure 4, by adding more SO_2 , there is more sulfur that would potentially be dissolved as SO_3^{2-} so that the sulfate reducing bacteria could metabolize it and produce sulfide. However, by generating a greater amount of SO_2 , the time it took the biomass to begin to reduce SO_3^{2-} increased and thus decreased productivity.

Figure 5 shows the fractions of the total sulfide found in the liquid and gaseous phases as a function of the $\text{SO}_3^{2-}/\text{COD}$ ratio. The dark color indicates the sulfide dissolved in the culture medium and the light color corresponds to that found in the gaseous phase.

As more sulfide was generated, the fraction in the culture medium (aqueous phase) increased proportionally more than the fraction found in the atmosphere of the reactors (gaseous phase). The results shown in Figures 2A and 2B show the mentioned trends. Figure 2B shows that the increment of gaseous H_2S tended towards a rather asymptote value instead of continuing to grow proportionally in each assay. When sulfate reducing bacteria used SO_3^{2-} in their respiration, they excreted S^{2-} as metabolic waste. The sulfide ion was rapidly hydrolyzed to a mixture of H_2S and HS^- (Postgate, 1984). In addition, H_2S was volatile, whereby one part was maintained in the culture medium as H_2S (aq) and another part was volatilized as H_2S (g). Initially, since there was no hydrogen sulfide in the gas phase, there was a large mass transfer between the two phases, which then decreased as the gaseous hydrogen sulfide accumulated. Bioreactors, by not permitting exchange with the outside and storing everything in them, caused the gaseous hydrogen sulfide to accumulate and thus decreased the exchange between phases. If the system were to constantly remove the sulfide from the gas phase, the mass transfer motive force (differential concentrations between exchange phases) would grow again and the movement of the species from aqueous solution to the gaseous solution would continue. Due to this, the concentration of gaseous sulfide rose quickly at first and then tended towards a stable value. Therefore, as consequence of the phase exchange of the H_2S , in the experimental run corresponding to a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.24, an

amount of 1.6 mg S^{2-} was found in the culture medium per mg of S^{2-} in the gaseous phase as H_2S (g). In the experiments with SO_3^{2-}/COD , ratios of 0.32 and 0.4, 1.8 and 2.1 mg of S^{2-} dissolved in the culture medium, respectively, per mg of S^{2-} in the gaseous phase.

Table 3 shows the produced total sulfide, as well as the sulfide normalized taking into account the sulfur added to the system as SO_2 (ratio of final sulfur/initial sulfur). The results of the triplicate values ranged between 0.94-1.03 for the SO_3^{2-}/COD ratio of 0.24, between 0.95-1.06 for the SO_3^{2-}/COD ratio of 0.32 and between 0.95-1.03 for the SO_3^{2-}/COD ratio of 0.4. SO_2 was generated through a chemical reaction between sodium metabisulfite and sulfuric acid, with metabisulfite being the limiting reactant. In the experiments, it was possible to add slightly higher or lower quantities of metabisulfite than the fixed one, which produced these small variations. Also small experimental errors would explain these differences. However, in the three experimental runs the average of the normalized value was very close or equal to 1, which indicates that all the initially fed sulfur was quantified at the end in its reduced form, or in other words that all the SO_2 was reduced to S^{2-} ($S^{4+} \rightarrow S^{2-}$), which was composed of a fraction in the aqueous solution (HS^- (aq) and H_2S (aq)) and another in the gaseous phase (H_2S (g)).

Sulfide is known for its toxic effect, however, it was possible to reduce all the gaseous SO_2 generated in the bioreactors for the three SO_3^{2-}/COD ratios evaluated, reaching concentrations of up to 533 mg/L of sulfide (Figure 2A). At first, it was expected that as there was no exchange of species with the outside and therefore all the sulfide produced was accumulated inside the bioreactors. The experiments with more SO_2 , were inhibited at some time by the sulfide. At 35 °C and pH around 7, about 40% of the total dissolved sulfide was found as H_2S (Okabe et al., 1992), indicating that there were about 200 mg/L of H_2S dissolved in the culture medium in the experiments with a SO_3^{2-}/COD ratio of 0.4. It has been reported that a concentration of H_2S of 547 mg/L completely inhibited the biological activity

of sulfate reduction (Okabe et al., 1995). This would imply that more non-ionized hydrogen sulfide was yet to accumulate to completely stop the biological reduction activity and thereby decrease the ratio of the sulfide produced/sulfur entering the system.

Figure 6 shows the maximum cell concentration quantified in the experiments. While the cell concentration increased as the SO_3^{2-} /COD ratio increased in the experiments, the increase was not proportional, but rather stagnated. It has been reported that from approximately 200 mg/L of sulfide, inhibition in the growth of sulfate-reducing bacteria was observed (Okabe et al., 1992; Okabe et al., 1995). This is in agreement with the results obtained here, since in the experiment with a SO_3^{2-} /COD ratio of 0.24, there were approximately 300 mg/L of sulfide (Figure 2A), and in the following experiments with a higher amount of SO_2 generated in the bioreactors, no growth was observed in proportion to the greater amount of oxidized sulfur that was generated in the system through the SO_2 and that served as electron acceptor for the growth of the bacteria, but rather saw a growth that tended to stagnate.

Figure 7 shows the variation of the normalized sulfide as a function of time for the three experimental runs carried out. As in the previous cases, productivity was higher for the SO_3^{2-} /COD ratio of 0.24, and decreased as this ratio increased; however, this comparison is more fair from the point of view that the values were normalized and no greater amount of sulfide was seen as a result of a higher amount of initial SO_2 . This demonstrates that the decrease in the productivity was due to an increase of the time that the biomass took to reduce the SO_2 .

Table 4 shows the values of COD oxidation (derived from lactate) yield to form sulfide, $Y_{p/s}$, (mg sulfide/mg COD). The theoretical value of this ratio was obtained from equation (5). For bacteria that did not degrade acetate, the yield was 0.67 mg produced sulfide per mg of COD removed as was previously mentioned. In this research the sulfide yields ranged between 0.54 and 0.6. Table 4 summarizes the result obtained. This table also shows the amount of COD removed, as well as the total sulfide quantified. As can be seen, for the SO_3^{2-}

/COD ratio of 0.24, the experimental yield was 90% of the theoretical one, for the SO_3^{2-} /COD ratio of 0.32 the yield was 88% of the theoretical, and for the SO_3^{2-} /COD ratio of 0.4 the obtained yield was 81% of theoretical one.

An analysis of variance was performed on the normalized sulfide and the sulfide/COD yield using the Statgraphics software. The obtained values show that there was no statistically significant difference among the results obtained in the three experimental runs for a significance level of 95%.

Kinetic characterization

The kinetic study was carried out using the results obtained in the experimental run corresponding to a SO_3^{2-} /COD ratio of 0.32. Figure 8 shows the kinetics of production of total soluble sulfide and gaseous hydrogen sulfide in the bioreactors of SO_2 reduction.

As can be seen in Figure 8 sulfide production was not detected during the first 24 hours of the experiment. However, at 48 hours the culture medium had slightly darkened by the reaction of the sulfide produced with the iron of the medium, indicating that the biological reduction had begun. At that time, the total dissolved sulfur concentration determined in the culture medium was 200 mg/L and the concentration of hydrogen sulfide gas in the headspace of the reactors was $1.8 \times 10^8 \mu\text{g}/\text{m}^3$. At 72 hours, the concentration of both compounds increased up to 414 mg S^{2-} /L and $2.4 \times 10^8 \mu\text{g H}_2\text{S (g)}/\text{m}^3$, respectively. The next measurements showed that the sulfide concentrations did not continue to increase, indicating that the reduction kinetics had ended at 72 hours. In addition, at 72 hours no dissolved sulfite was detected in the culture medium, concluding that all SO_2 had been reduced.

Figure 9 shows the dynamics of pH and re-dox potential in the bioreactors. The culture medium started with a pH of 7.7 and a Eh of -266 mV. At 24 hours after the experiment was started, the pH dropped to 7.06 due to the dissolution of SO_2 in the culture medium, but still

remained above 7 due to the action of the phosphate buffer included in the medium. On the other hand, the Eh decreased slightly in that period. At 48 hours the reduction had started, which caused the pH to rise slightly and the Eh decreased from -278 to -391 mV. At 72 hours the pH was kept constant and the Eh decreased to -414 mV and thereafter the reading stabilized near that value. The pH rose again a little in the next hours and like the Eh, then it remained constant. By crossing the results, it can be observed that the change in environmental conditions was due to the dissolution of SO₂, which acidified the medium, and to the production of sulfide, which alkalized the pH that is due to the biochemical reactions of sulfate reducing bacteria (proton consumption). A strong decrease in the Eh of the culture medium was also observed due to the sulfite produced, which is a strongly reducing species.

Figure 10 shows the kinetics of the biomass present in the bioreactors and of the COD of culture medium. At time 0 there were 7×10^7 cells per mL of culture medium and 5034 mg/L of COD. At 24 hours the COD remained stable, however, it was observed that the biomass grew up to 4×10^8 cells/mL even when there was no generation of S²⁻ (therefore, no SO₃²⁻ reduction). This increase in cell concentration was attributed to the fact that the cultured cells were ready to duplicate, since the inoculum used in the experiments was in full growth, and therefore, microbial growth was observed from the beginning. The same behavior and trends were observed in the experimental run corresponding to a SO₃²⁻/COD ratio of 0.4 (data not shown).

It can be observed in Figure 10 that the times in which the sulfide was produced and the lactate (COD) was consumed coincide with the time in which the biomass grew. The same happened with the pH and the re-dox potential, while the biochemical reactions take place, the environment inside the reactors was very dynamic, then stabilized when arriving at a steady state. This demonstrated the correlation between the dynamics of the compounds analyzed and the microbial activity. Biomass used lactate as a source of carbon and energy and sulfite

as the final acceptor of electrons in its respiration and produced sulfide that excreted into the medium as metabolic waste. As the bacteria consumed lactate and sulfite, they produced sulfide and the biomass increased, and then stopped when the limiting substrate ran out (sulfite produced from SO_2).

From the experimental data plotted in Figure 10, the maximum specific growth rate of the microorganisms ($\mu_{\max}$) was calculated as well as the cells/COD ratio (number of cells produced/mg COD consumed). These values were found to be 0.03 h^{-1} and 1,417,827,225, respectively.

Dutta et al. (2007) reported a maximum specific growth rate of 0.05 h^{-1} using lactate as the source of carbon and energy and sulfite as the electron final acceptor for the sulfate reducing bacteria *Desulfotomaculum nigrificans*. The $\mu_{\max}$ value obtained in the present work was very similar to that reported by Herrera et al. (1991) (0.04 h^{-1}), which was also obtained for *Desulfovibrio desulfuricans* using lactate as a source of carbon and energy. Moreover, it has been reported that the weight of this bacterium should be approximately $3.5 \times 10^{-13} \text{ g/cell}$ (Herrera et al., 1993). Using this value, a yield of 0.5 g cells/g COD consumed was obtained. As 1 g of lactate is equivalent to 1.0787 g COD, therefore the yield would be 0.54 g cells/g lactate consumed, which is a value of the same order of magnitude as that reported by Herrera et al. (1991) of 0.86 g cells/g lactate consumed.

CONCLUSIONS

The design of the bio-reduction system allowed to generate the established amount of sulfur dioxide and to reach a 100% reduction in batch mode for all evaluated loads. The flexibility of the design can be used for other studies of gases (produced through a chemical reaction between a solid and a liquid) in microcosms. At higher SO_2 loading, the longer the system takes to start the biological reduction, the lower the productivity. In the range of 15 to

25 m-moles of SO₂ generated in the gas phase per liter of culture medium of the batch-operated bioreactors, a 100% biological reduction is achieved, after 192 hours for the maximum load studied. Based on the results it is concluded that in the case of operating a reactor in continuous mode, SO₂ should be fed at a rate of 15 m-moles/(L·d) and, then, the load should be increased until the system begins to accumulate sulfite in the liquid medium, indicating that the maximum activity of the biomass present in the reactor is being exceeded. From the results obtained it is concluded that the sulfite is used as final acceptor of electrons in this system, since the increase of the sulfide concentration coincides with the exponential growth of the biomass and the consumption of lactate as a source of carbon and energy.

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Figure captions

Fig. 1. Soluble sulfide (1A) and gaseous hydrogen sulfide (1B) produced in the three experimental runs as a function of time: Ratio $\text{SO}_3^{2-}/\text{COD}$ 0.24; Ratio $\text{SO}_3^{2-}/\text{COD}$ 0.32; Ratio $\text{SO}_3^{2-}/\text{COD}$ 0.4.

Fig. 2. Sulfite content in the culture medium as a function of the $\text{SO}_3^{2-}/\text{COD}$ ratio for the three experimental runs carried out.

Fig. 3. Variation of the total production of sulfide (sum of the sulfide masses of the aqueous and gaseous phases) and the total sulfide productivity as a function of the $\text{SO}_3^{2-}/\text{COD}$ ratio.

Fig. 4. Variation of normalized sulfide with time for the three experimental runs carried out: $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.24, $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.32, $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.4.

Fig. 5. Evolution of total soluble sulphide and gaseous hydrogen concentrations with time in the experimental run corresponding to a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.32.

Fig. 6. Evolution of pH and redox potential, Eh (mV), in the experimental run corresponding to a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.32.

Fig. 7. Evolution of sulfate reducing bacteria biomass concentration and COD in the experimental run corresponding to a $\text{SO}_3^{2-}/\text{COD}$ ratio of 0.32.

Table 1. Composition of the Postgate B culture medium.*

Name	Formula	(g/L)
Potassium dihydrogen phosphate	KH_2PO_4	0.5
Ammonium chloride	NH_4Cl	1
Calcium sulfate	CaSO_4	1
Magnesium sulfate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2
Sodium lactate	$\text{NaC}_3\text{H}_5\text{O}_3$	3.5
Yeast extract	N.D.	1
Ascorbic acid	$\text{C}_6\text{H}_8\text{O}_6$	0.1
Thioglycolic acid	$\text{C}_2\text{H}_4\text{O}_2\text{S}$	0,1
Iron Sulfate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.5

*Adjust to pH 7.0-7.5

N.D.: non-determined

Table 2. Composition of the Postgate C culture medium.*

Name	Formula	(g/L)
Potassium dihydrogen phosphate	KH_2PO_4	0.5
Ammonium chloride	NH_4Cl	1
Sodium sulfate	NaSO_4	4.5
Calcium chloride	$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	0.06
Magnesium sulfate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.06
Sodium lactate	$\text{NaC}_3\text{H}_5\text{O}_3$	6
Yeast extract	N.D.	1
Iron Sulfate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.004
Sodium citrate	$\text{C}_6\text{H}_5\text{O}_7\text{Na}_3 \cdot 2\text{H}_2\text{O}$	0.3

*Adjust to pH 7.0-7.5

N.D.: non-determined

Table 3. Initial sulfur, produced sulfide and ratio of final sulfide/initial sulfur for each $\text{SO}_3^{2-}/\text{COD}$ ratio tested.

$\text{SO}_3^{2-}/\text{COD}$ ratio	Initial Sulfur added as SO_2 (mg S^{4+})	Total sulfide quantified (mg S^{2-})	Ratio Sulfide _{final} /Sulfur _{initial}
0.24	77	75	0.97
0.32	102	103	1.01
0.4	128	126	0.98

Table 4. COD removed, produced total sulfide and sulfide/COD yield ($Y_{p/s}$)

SO₃²⁻/COD ratio	COD removed (mg COD)	Total sulfide quantified (mg S²⁻)	$Y_{p/s}$ (mg sulfide/mg COD)
0.24	125	75	0.60 ± 0.05
0.32	176	103	0.59 ± 0.04
0.4	232	126	0.54 ± 0.09

Figure 1

a)

b)

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7