Final products and kinetics of biochemical and chemical sulfide oxidation under microaerobic conditions

Lucie Pokorna-Krayzelova, Dana Vejmelková, Lara Selan, Pavel Jenicek, Eveline I. P. Volcke and Jan Bartacek

ABSTRACT

Hydrogen sulfide is a toxic and usually undesirable by-product of the anaerobic treatment of sulfate-containing wastewater. It can be removed through microaeration, a simple and cost-effective method involving the application of oxygen-limiting conditions (i.e., dissolved oxygen below 0.1 mg L⁻¹). However, the exact transformation pathways of sulfide under microaerobic conditions are still unclear. In this paper, batch experiments were performed to study biochemical and chemical sulfide oxidation under microaerobic conditions. The biochemical experiments were conducted using a strain of Sulfuricurvum kujiense. Under microaerobic conditions, the biochemical sulfide oxidation rate (in mg S L⁻¹ d⁻¹) was approximately 2.5 times faster than the chemical sulfide oxidation rate. Elemental sulfur was the major end-product of both biochemical and chemical sulfide oxidation. During biochemical sulfide oxidation elemental sulfur was in the form of white flakes, while during chemical sulfide oxidation elemental sulfur created a white suspension. Moreover, a mathematical model describing biochemical and chemical sulfide oxidation was developed and calibrated by the experimental results.

Key words | elemental sulfur, mathematical modelling, microaeration, sulfide oxidation, sulfide oxidizing bacteria (SOB), Sulfuricurvum kujiense

INTRODUCTION

Anaerobic treatment of wastewater to convert organic material to biogas, mainly consisting of methane, leads to the simultaneous reduction of sulfate compounds to liquid and gaseous hydrogen sulfide (Ramos et al. 2013). Sulfide in the dissolved form can inhibit methanogenic and acetogenic organisms, may lead to the accumulation of inert material in the sludge (e.g. metal sulfides) and to the deterioration of aerobic post-treatment systems (activated sludge bulking; excessive growth of phototrophs) (Sarti & Zaiat 2011). Gaseous sulfide is toxic, corrosive and flammable and its presence in biogas results in the emission of sulfur dioxide upon combustion (Tang et al. 2009).

Biochemical desulfurization processes are considered to be attractive alternatives to the physical-chemical techniques, because of their lower requirements for energy and chemicals, easy and automated operation, the long life expectancy of system elements, the potential for elemental sulfur recovery and the absence of a solid waste stream (Tang et al. 2009; Díaz et al. 2011; Ramos et al. 2013; Ramos et al. 2014a, 2014b).

Microaeration is a biochemical desulfurization method that is based on the introduction of a small (limited) amount of oxygen into an anaerobic system. This simple sulfide removal technique has already been applied at full scale (Jeníček et al. 2017). Oxygen or air can be dosed directly into the reactor to oxidize sulfide to elemental sulfur, so no additional process units are required (van der Zee et al. 2007; Krayzelova et al. 2014; Krayzelova et al. 2015).

The oxygen availability is the main factor determining the final sulfur products (Janssen et al. 1995). Under oxygen limiting (microaerobic) conditions, i.e. at oxygen concentration below 0.1 mg L⁻¹, sulfur is expected to be the main end product of biological sulfide oxidation (Roosta et al. 2011), with a partial biological oxidation to thiosulfate (van den Ende & van Gemerden 1993). On the other hand, sulfate is the dominant end-product under
higher oxygen availability (Roosta et al. 2011). Chemical oxidation gains importance especially in the systems with higher sulfide concentration (Janssen et al. 1995). Under those conditions, biochemical activity may be limited and sulfide is oxidized chemically, mainly to thiosulfate (Janssen et al. 1995; van der Zee et al. 2007). However, information on the relative importance of biochemical and chemical sulfide oxidation under microaerobic conditions (at oxygen concentration below 0.1 mg L\(^{-1}\)) and on their final products was not found in literature.

Mathematical models are a helpful tool for process understanding and for the simulation of process performance. Pokorna-Krayzelova et al. (2017) presented a model for microaeration in UASB reactor, including several biochemical pathways for sulfate reduction to sulfide and oxidation of sulfide to elemental sulfur. Further biochemical oxidation of elemental sulfur to sulfate was neglected and so was chemical sulfide oxidation. Roosta et al. (2011) estimated kinetics for biochemical sulfide oxidation in a fed batch reactor at dissolved oxygen (DO) concentrations 0.5–6 mg L\(^{-1}\) and Xu et al. (2013) described the kinetics of biochemical sulfide oxidation under DO concentrations from 0.03 to 0.5 mg L\(^{-1}\). However, in all of these studies chemical sulfide oxidation was neglected. A mathematical model describing combined biochemical and chemical sulfide oxidation under microaerobic conditions has not yet been developed.

This study compared chemical and biochemical oxidation of sulfide under microaerobic conditions (DO below 0.1 mg L\(^{-1}\)). Biochemical sulfide oxidation experiments were conducted with a pure culture of *Sulfuricurvum kujiense*, which under microaerobic condition utilizes sulfide and thiosulfate as an electron donor and oxygen as an electron acceptor (Díaz et al. 2011; Ramos et al. 2014a). Kinetic expressions to describe sulfide oxidation under microaerobic conditions were proposed based on the experimental results.

**MATERIALS AND METHODS**

**Experimental set-up**

A batch reactor with a total volume of 2 L was used to study the kinetics of chemical and biochemical sulfide oxidation under microaerobic conditions (Figure 1). The reactor temperature was kept at 35 \(^\circ\)C.

The experiments were conducted in the cultivation medium MBM 1020 (Kodama & Watanabe 2004). The MBM medium consists of (per 1,000 mL): 0.2 g KH\(_2\)PO\(_4\), 0.2 g NH\(_4\)Cl, 0.4 g MgCl\(_2\).6H\(_2\)O, 0.2 g KCl, 0.1 g CaCl\(_2\).2H\(_2\)O, 2.5 g Na\(_2\)S\(_2\)O\(_5\).5H\(_2\)O, 0.1 mg EDTA, 0.2 mg NaNO\(_3\), 0.4 mg FeSO\(_4\).7H\(_2\)O, 0.02 mg ZnSO\(_4\).7H\(_2\)O, 0.006 mg MnCl\(_2\).4H\(_2\)O, 0.06 mg H\(_2\)BO\(_3\), 0.04 mg CoCl\(_2\).6H\(_2\)O, 0.002 mg CuCl\(_2\).2H\(_2\)O, 0.004 mg NiCl\(_2\).6H\(_2\)O, and 0.006 mg Na\(_2\)MoO\(_4\).2H\(_2\)O.

Prior to each experiment, the medium was sparged with nitrogen gas to decrease the oxygen concentration to less than 0.1 mg L\(^{-1}\). The headspace of the reactor was flushed with nitrogen gas from a nitrogen reservoir to remove oxygen traces. The reactor was sealed and samples for initial sulfide, sulfate and thiosulfate concentrations were taken.

The experiments on biochemical and chemical sulfide oxidation (both in triplicates) were initiated by injecting a 10–15 mL of mixed sulfide and thiosulfate stock solution to obtain the initial sulfide concentration from 8 to 10 mg L\(^{-1}\). This concentration was chosen because it is relevant for anaerobic digesters (Krayzelova et al. 2014; Pokorna-Krayzelova et al. 2018). It was nearly impossible to prepare a sulfide stock solution without thiosulfate being present. Thanks to the instability of sulfide and thiosulfate stock solution, the initial concentration of sulfide and thiosulfate slightly varied. The value of pH was kept at 7 ± 1 using 2 M HCl and 0.1 M NaOH solutions. Oxygen concentration was kept below 0.1 mg L\(^{-1}\) with
nitrogen gas and the experiments were stopped when the concentration of oxygen reached that value.

DO concentration, oxidation reduction potential (ORP), pH, and the concentration of sulfide, sulfate and thiosulfate were measured hourly. Sulfide and thiosulfate removal rates were determined as the difference between the initial and final concentration over the measured period of time. The concentration of elemental sulfur formed, $c_{SO}$ (mg S L$^{-1}$), was calculated as the difference between the initial and final concentrations of sulfide, thiosulfate and sulfate (Equation (1)). All concentrations are in mg S L$^{-1}$.

$$c_{SO} = (c_{SO}^{\text{fin}} - c_{SO}^{\text{init}}) - (c_{SO_2}^{\text{fin}} - c_{SO_2}^{\text{init}}) - (c_{SO_4}^{\text{fin}} - c_{SO_4}^{\text{init}})$$ (1)

Biochemical sulfide oxidation

Biochemical sulfide oxidation experiments were conducted with the type strain of *Sulfuricurvum kujiense* (DSM 16994). This strain was obtained from the German Collection of Microorganisms and Cell Cultures and was cultivated according to provided instructions. *S. kujiense* is a facultative anaerobic, chemolithotrophic, sulfur oxidizing bacterium, which under microaerobic conditions utilizes sulfide as an electron donor and oxygen as an electron acceptor (Kodama & Watanabe 2004). The experiments were conducted in triplicates.

Chemical sulfide oxidation

Chemical sulfide oxidation was carried out in the absence of bacteria. To prevent the biological activity during chemical sulfide oxidation, MDM 1020 medium solution was autoclaved prior to use and the batch reactor was washed with ethanol and distilled water. Atmospheric oxygen was used for chemical sulfide oxidation. The experiments were conducted in triplicates.

Analytical methods

The DO concentration and the oxidation reduction potential (ORP) were measured by LD0101 probe (Hach Lange Company, Germany); pH was measured with a SensoLyt probe (WTW s.r.o., Czech Republic). The concentration of sulfide, sulfate and thiosulfate were measured with spectrophotometer DR 3900 (Hach Lange Company, Germany) applying the following protocols: APHA, Standard Methods for the Examination of Water and Wastewater (APHA 2012) for sulfide, sulfate was measured based on the barium sulfate method (Horáková 2007) and thiosulfate concentration as in Nor & Tabatabai (1975). The quantification (concentration in μg mL$^{-1}$) of *Sulfuricurvum kujiense* was measured by Lowry’s method (Waterborg & Matthews 1984).

Sulfur conversion stoichiometry and kinetics

Four main sulfur conversion processes were assumed to take place during biochemical and chemical sulfur oxidation measured in the batch assays: biochemical oxidation of hydrogen sulfide to elemental sulfur by sulfide oxidizing bacteria (SOB) (Table 1, Process 1), biochemical oxidation of elemental sulfur to sulfate by SOB (Table 1, Process 2), chemical oxidation of hydrogen sulfide to thiosulfate (Table 1, Process 3), and thiosulfate disproportionation to elemental sulfur and sulfur dioxide (Table 1, Process 4). The decay of SOB was also incorporated in the model (Table 1, Process 5). The hydrogen sulfide acid-base reaction was considered for reasons of completeness (Table 1, Process 6), even though pH was kept constant in the simulations performed in this study. For each process, the stoichiometric coefficients were calculated from COD and sulfur balances (Table 1). Monod-type equations were used to describe the biological oxidation rates.

The biological conversions were taken up in a model describing the batch reactors, which was implemented in Aquasim 2.0 (Reichert 1998).

Parameter estimation

The maximum H$_2$S uptake rate ($k_{m,H2S,SOB}$), yield ($Y_{SOB}$) and decay rate ($k_{dec}$) of SOB were determined separately from batch experiments with a pure culture of SOB *Sulfuricurvum kujiense* (see Supporting information, available with the online version of this paper).

The maximum uptake rate was determined based on the maximum uptake of sulfide by SOB over a period of time. The cultivation media with the excess of sulfide was prepared. Both the concentration of sulfide and SOB were measured regularly during 4 hours (Supporting information, section S.2). The decay rate constant was calculated based on SOB concentration decrease over time. The cultivation media without sulfide was prepared. The decrease of SOB concentration was measured during 6 hours (Supporting information, section S.3). The biomass yield was determined by relating the growth of SOB to the decrease of sulfide concentration over time. The cultivation media with the excess
Stoichiometric matrix $A_{ij}$ and composition matrix for chemical and biochemical sulfide oxidation

Table 1

<table>
<thead>
<tr>
<th>Components $i$</th>
<th>Processes $j$</th>
<th>Stoichiometric coefficients $A_{ij}$</th>
<th>Composition matrix $S_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>-1</td>
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<td>-1</td>
<td></td>
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<tr>
<td>3</td>
<td>3</td>
<td>-1</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>5</td>
<td>1</td>
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<tr>
<td>6</td>
<td>6</td>
<td>1</td>
<td></td>
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<td>7</td>
<td>7</td>
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<tr>
<td>9</td>
<td>9</td>
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<td></td>
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</tbody>
</table>

Results

Biochemical sulfide oxidation

In the presence of biomass, sulfide was oxidized at an average rate of $29.86 \text{ mg S L}^{-1} \text{ d}^{-1}$ over the time period of 7 hours. The removal of thiosulfate was $5.25 \text{ mg S L}^{-1} \text{ d}^{-1}$ (Figure 2). The concentration of sulfate was stable during the experiments. The oxidation rate of sulfur to sulfate was $0.56 \text{ mg S L}^{-1} \text{ d}^{-1}$. During the experiments, slightly yellowish flakes appeared in the medium.

Chemical sulfide oxidation

In the absence of biomass, sulfide was oxidized at an average rate of $12.06 \text{ mg S L}^{-1} \text{ d}^{-1}$ over a 7-hour time period. The removal of thiosulfate was $6.83 \text{ mg S L}^{-1} \text{ d}^{-1}$ (Figure 3). The concentration of sulfate was stable ($7.48 \pm 0.44 \text{ mg S L}^{-1}$) during the experiments. The oxidation rate of elemental sulfur to sulfate was only $0.03 \text{ mg S L}^{-1} \text{ d}^{-1}$. During the experiments, the colour of the medium changed from colourless to slightly yellowish.
Kinetic parameter estimation

The maximum H$_2$S uptake rate, decay rate, and the yield coefficient were determined by the experiments with the pure culture of *Sulfuricurvum kujiense* (data in Supporting information, available with the online version of this paper). The maximum H$_2$S uptake rate, $k_{m,H2S,SOB}$, was 482 mmol S mg COD$^{-1}$ h$^{-1}$, the decay rate, $k_{dec,xSOB}$, was 0.24 h$^{-1}$ and the yield coefficient, $Y_{SOB}$, was 10.37 mg COD mmol$^{-1}$ S.

The remaining parameters were estimated by fitting simulated data to the experimental results for biochemical oxidation (Figure 2). Table 2 summarizes the kinetic parameter values obtained.

The simulated concentrations of sulfide, thiosulfate, and DO showed a good fit with the experimentally measured data (Figure 4), corresponding with a root-mean-square error of 0.065 mg S L$^{-1}$ for DO concentration, 0.192 mg S L$^{-1}$ for thiosulfate concentration, and 0.738 mg S L$^{-1}$ for sulfide concentration. The root-mean-square error of sulfate was 1.034 mg S L$^{-1}$. The total sulfur concentration in the model compare to the experiment was almost the same (21.33 mg S L$^{-1}$ for experiments compared to 21.34 mg S L$^{-1}$ for the model at the end).

### Table 2: Model parameters and their values as calculated (1) (Supporting information, available with the online version of this paper) or obtained through model calibration (2) (Figure 4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Reaction order with respect to H$_2$S</td>
<td>–</td>
<td>1.1 (2)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Reaction order with respect to O$_2$</td>
<td>–</td>
<td>0.9 (2)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Reaction order with respect to S$_2$O$_3^{2-}$</td>
<td>–</td>
<td>0.5 (2)</td>
</tr>
<tr>
<td>$k_{dec,xSOB}$</td>
<td>Decay rate</td>
<td>h$^{-1}$</td>
<td>0.24 (1)</td>
</tr>
<tr>
<td>$k_{m,H2S,SOB}$</td>
<td>Maximum H$_2$S uptake rate</td>
<td>mmol S mg COD$^{-1}$ h$^{-1}$</td>
<td>482 (1)</td>
</tr>
<tr>
<td>$k_{m,S,SOB}$</td>
<td>Maximum S$^0$ uptake rate</td>
<td>mmol S mg COD$^{-1}$ h$^{-1}$</td>
<td>0.001 (2)</td>
</tr>
<tr>
<td>$K_{s,H2S}$</td>
<td>Half saturation constant for H$_2$S</td>
<td>mmol L$^{-1}$</td>
<td>0.001 (2)</td>
</tr>
<tr>
<td>$K_{s,O2}$</td>
<td>Half saturation constant for O$_2$</td>
<td>mmol L$^{-1}$</td>
<td>0.1 (2)</td>
</tr>
<tr>
<td>$K_{s,S}$</td>
<td>Half saturation constant for S$^0$</td>
<td>mmol L$^{-1}$</td>
<td>0.1 (2)</td>
</tr>
<tr>
<td>$k_{H2S,chemo}$</td>
<td>Chemical H$_2$S oxidation rate</td>
<td>h$^{-1}$</td>
<td>0.001 (2)</td>
</tr>
<tr>
<td>$k_{S2O3^{2-},disp}$</td>
<td>S$_2$O$_3^{2-}$ disproportionation rate</td>
<td>h$^{-1}$</td>
<td>10 (2)</td>
</tr>
<tr>
<td>$Y_{SOB}$</td>
<td>Biomass yield</td>
<td>mg COD mmol S$^{-1}$</td>
<td>10.37 (1)</td>
</tr>
</tbody>
</table>
DISCUSSION

Biochemical versus chemical sulfide oxidation

Under oxygen limiting (microaerobic) conditions, at oxygen concentrations below 0.1 mg L\(^{-1}\), elemental sulfur was the major end product of both chemical and biochemical sulfide oxidation. The biochemical and chemical sulfide oxidation rates were 29.9 and 12.0 mg S L\(^{-1}\) d\(^{-1}\), respectively. That is, sulfide oxidation in the presence of biomass was about 2.5 times faster. Assuming that the rate of chemical sulfide oxidation was independent of the presence of bacteria, approximately 60% of the elemental sulfur was formed through biochemical oxidation of sulfide and 40% through the chemical pathway. Alcántara et al. (2004) reported that the activity of SOB severely decreased at oxygen to sulfi concentration below 0.1 mg L\(^{-1}\). Under oxygen limiting (microaerobic) conditions, at oxygen measurement uncertainty range, Munz was observed. These differences are likely to fall within the uncertainty range. The predictions of the model presented in this paper correlated well with the experimental data. The concentration of DO, sulfide and thiosulfate showed a good fit (Figure 4). The concentration of sulfate was little overestimated in the simulations. However, the lower concentration of sulfate obtained during experiments could be caused by the uncertainties accompanied with the measurements.

Kinetic parameter estimation

The predictions of the model presented in this paper correlated well with the experimental data. The concentration of DO, sulfide and thiosulfate showed a good fit (Figure 4). The concentration of sulfate was little overestimated in the simulations. However, the lower concentration of sulfate obtained during experiments could be caused by the uncertainties accompanied with the measurements.

The kinetic parameters of chemical sulfide oxidation obtained in this study were compared with literature (Table 3). The chemical H\(_2\)S oxidation rate, \(k_{\text{H}_2\text{S}, \text{chemox}} = 0.06 \text{ min}^{-1}\), was about the same as determined by Wilmot et al. (1988) (0.055 min\(^{-1}\)). However, the reaction orders, \(\alpha\) and \(\beta\), were different. The concentration of sulfide was comparable, but the concentration of oxygen was 53 to 207 times lower in the present study. The reaction orders, \(\alpha\) and \(\beta\), in this study (1.1 and 0.9, respectively) were similar to the results of O’Brien & Birkner (1977) (1.02 and 0.8, respectively). However, the chemical H\(_2\)S oxidation rate was different. Again, it could be caused by the different oxygen concentration (70–367 times lower in this study).

As a direct comparison of kinetic parameter values available in the literature with the ones obtained in this study (Table 3) is hampered by varying experimental conditions, the resulting sulfide oxidation rates were compared instead (Figure 5), for a range of sulfide (1–19 mg S\(^2\) L\(^{-1}\), x-axis) and oxygen (0.01–0.20 mg O\(_2\) L\(^{-1}\), y-axis) concentrations.

The dependency of chemical sulfide oxidation rate on the sulfide concentration differs across various literature sources. The kinetic parameters of chemical sulfide oxidation are presented in Table 3.

Table 3 | The kinetic parameters of chemical sulfide oxidation

<table>
<thead>
<tr>
<th>(k_{\text{H}_2\text{S}, \text{chemox}} \text{ min}^{-1})</th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(c(\text{S}^2^-)) mg S(^2) L(^{-1})</th>
<th>(c(\text{O}_2)) mg O(_2) L(^{-1})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>1.1</td>
<td>0.9</td>
<td>8.64</td>
<td>&lt;0.096</td>
<td>This paper</td>
</tr>
<tr>
<td>0.57</td>
<td>0.41</td>
<td>0.39</td>
<td>5.12–300</td>
<td>0.1–8.5</td>
<td>Buisman et al. (1990)</td>
</tr>
<tr>
<td>0.055</td>
<td>0.38</td>
<td>0.21</td>
<td>2.88–9.6</td>
<td>5.1–19.8</td>
<td>Wilmot et al. (1988)</td>
</tr>
<tr>
<td>67.6</td>
<td>1.15</td>
<td>0.69</td>
<td>1.6–6.4</td>
<td>19.2</td>
<td>Jolley &amp; Forster (1989)</td>
</tr>
<tr>
<td>1.44</td>
<td>1.02</td>
<td>0.80</td>
<td>0.64–38.7</td>
<td>6.7–35.2</td>
<td>O’Brien &amp; Birkner (1977)</td>
</tr>
<tr>
<td>a</td>
<td>0.81–0.99</td>
<td>0.19–0.16</td>
<td>0–8</td>
<td>0–4.2</td>
<td>Nielsen et al. (2004)</td>
</tr>
<tr>
<td>a</td>
<td>1.34</td>
<td>0.56</td>
<td>1.6–6.4</td>
<td>2.7–5.1</td>
<td>Chen &amp; Morris (1972)</td>
</tr>
</tbody>
</table>

\(a\) \(k_{\text{H}_2\text{S}, \text{chemox}}\) depended on the pH value and varied from 11.8 to 16.38 M\(^{-1}\) h\(^{-1}\).
sources (Figure 5). While for Wilmot et al. (1988) and the present paper the rate was almost independent of the sulfide concentration, for Jolley & Foster (1985) and O’Brien & Birkner (1977) chemical sulfide oxidation rate strongly depended on the concentration of sulfide. In the study of Buisman et al. (1990) it depended on the concentration of oxygen. Higher oxygen concentration implied a lower dependency of the sulfide concentration on the chemical sulfide oxidation rate.

For oxygen concentration, the trend was the same for all sources: the higher the concentration of oxygen, the higher the chemical sulfide oxidation rate. However, the actual values were different. In the present paper, the chemical sulfide oxidation rate ranged between 0.001 and 0.36 mg L$^{-1}$ min$^{-1}$. The closest values were obtained by Wilmot et al. (1988) (0.02–0.12 mg L$^{-1}$ min$^{-1}$) and Buisman et al. (1990) (0.09–1.02 mg L$^{-1}$ min$^{-1}$). Buisman et al. (1990) estimated the kinetic parameters for low oxygen concentration (starting at 0.1 mg O$_2$ L$^{-1}$). However, the concentration of sulfide was very high (up to 300 mg S$^2-$ L$^{-1}$) compared to the present study. In the case of Wilmot et al. (1988), it was the other way round: the sulfide concentration was similar.
Flakes of elemental sulfur appeared (up to 9.6 mg S$^{2-} \text{L}^{-1}$), while oxygen was too high (up to 19.8 mg O$_2$ L$^{-1}$). The experiments of Jolley & Forster (1985) and O’Brien & Birkner (1977) were made with too high oxygen concentration (19.2 and up to 35.2 mg O$_2$ L$^{-1}$, respectively), resulting in too high chemical sulfide oxidation rate (2.8–658.0 mg L$^{-1}$ min$^{-1}$ and 0.04–8.0 mg L$^{-1}$ min$^{-1}$, respectively).

Table 4 summarizes the results of the kinetics of biochemical sulfide oxidation to elemental sulfur found in the literature and compares it with this study. It clearly shows that few authors have quantified the kinetics of chemical and biochemical sulfide oxidation in one oxygen-limited system. Moreover, the public data so far are not consistent and more dedicated measurements should still be performed to allow independent calibration of mathematical models.

### CONCLUSIONS

- Under microaerobic conditions (DO below 0.1 mg L$^{-1}$), elemental sulfur was the major end-product of both biochemical and chemical sulfide oxidation.
- In the presence of bacteria, approximately 60% of the elemental sulfur was formed through biochemical oxidation of sulfide and 40% through the chemical pathway.
- The volumetric biochemical sulfide oxidation rate was approximately 2.5 times faster than the chemical sulfide oxidation rate.
- Flakes of elemental sulfur appeared during biochemical oxidation, while suspended elemental sulfur was formed during chemical oxidation.

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