Sulfide removal by moderate oxygenation of anaerobic sludge environments

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Abstract

Introduction of a limited amount of oxygen to anaerobic bioreactors is proposed as a simple technique to lower the level of sulfide in the biogas. This paper presents the results of a bioreactor study and of batch experiments that were performed to obtain better insight into the fate of sulfur compounds and oxygen during micro-aerobic sulfide oxidation. Introduction of a low airflow (0.7–0.9 m³ m⁻³ d⁻¹, corresponding to an O₂/S molar ratio of 8–10) to a fluidized bed reactor fed with low-sulfate vinasse was sufficient to reduce the biogas H₂S-content to an undetectable level. Sulfide was initially oxidized to elemental sulfur, thiosulfate and – most probably – polysulfide. Significant sulfate production did not occur. Bioreactor sludge sampled from the reactor after three weeks' micro-aerobic operation was much faster in oxidizing sulfur than bioreactor sludge sampled during fully anaerobic reactor operation. The reaction proceeded faster with increasing O₂/sulfide ratios.

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1. Introduction

One of the problems associated with anaerobic wastewater treatment is the formation of sulfide upon reduction of sulfate and other sulfur containing compounds (Lens et al., 1998). Release of sulfide is undesirable because of its smell, its toxicity and its corrosive properties. Several different sulfide removal techniques exist (Lens et al., 1998; Burgess et al., 2001), including chemical precipitation as well as gas scrubbing in combination with chemical or biological oxidation processes. A common drawback of these techniques is that they require an extra process unit, implying extra installation and operational costs. As an alternative, introduction of limited quantities of oxygen/air to anaerobic bioreactors can be considered.

Sulfide removal by direct introduction of air into ‘anaerobic’ bioreactor systems has been investigated previously, however in both cases during treatment of sulfate-rich wastewater (Fox and Venkatasubbiah, 1996; Khanal and Huang, 2003). To the authors’ knowledge, moderate aeration to lower the biogas sulfide level during treatment of low-sulfate wastewaters has not been studied before.

Sulfide oxidation proceeds both biologically and chemically. Biological sulfide oxidation in wastewater treatment systems is typically associated with the activity of colourless sulfur bacteria. These bacteria utilize the energy derived from the following overall reactions (Kuenen, 1975):

\[
2\text{HS}^- + \text{O}_2 \rightarrow 2\text{S}^0 + 2\text{OH}^- \quad \Delta G^0 = -169.4 \text{ kJ/mol}
\]

\[
2\text{HS}^- + 4\text{O}_2 \rightarrow 2\text{SO}_4^{2-} + 2\text{H}^+ \quad \Delta G^0 = -732.6 \text{ kJ/mol}
\]

Both biological and chemical sulfide oxidation is believed to start with the formation of polysulfides (\(S_n^-\)), which can be protonated to form elemental sulfur (Brune, 1995;
Further oxidation will give rise to the formation of more oxidized sulfur species such as thiosulfate, sulfite and sulfate (Schlegel, 1993; Steudel, 1996).

In order to obtain feasible sulfide oxidation in micro- aerobically operated wastewater treatment systems, sulfide oxidation should be effective in its competition for oxygen with other oxidative processes such as aerobic oxidation of organic COD. In addition, the sulfide oxidation process should be faster than the re-reduction of oxidized sulfur species.

The research presented in this paper aimed at studying the reaction stoichiometry and kinetics of the micro-aerobic oxidation of sulfide at low concentration levels in oxygen-exposed ‘anaerobic’ granular sludge.

2. Methods

2.1. Reactor

The reactor system used in this study was the anaerobic fluidized bed reactor described previously (Fdz.-Polanco et al., 2001a,b). The reactor ($V = 1.7 \text{l}$), containing activated carbon and a mixture of granular and disperse sludge ($\sim 30 \text{g VSS l}^{-1}$), treated sulfate-amended vinasse at an organic loading rate of $\sim 6 \text{g COD d}^{-1}$ and a sulfate loading rate of $\sim 1.3 \text{mmol S d}^{-1}$. The hydraulic retention time was 5 days and operational temperature was $33 \pm 2 ^\circ \text{C}$. The sludge bed was fluidized to $\sim 30\%$ sulfide bed expansion by applying a high effluent recycle flow ($\sim 30 \text{h}^{-1}$). After a long period of fairly stable anaerobic reactor operation ($\sim 1 \text{ year}$), oxygen was introduced by leading air (1.2–1.5d$^{-1}$) into the reactor’s inlet tube using a peristaltic pump. Sulfur and COD balances were monitored regularly by measuring the flows of influent and biogas and analysing liquid and gas samples (sulfide, sulfate, thiosulfate, elemental sulfur, biogas composition and COD). Micro-aerobic reactor operation continued for 28 days.

2.2. Batch experiments

Sludge samples from the reactor (before as well as three weeks after air introduction was started) were used to perform batch experiments. The batch experiments were performed in 592-ml glass bottles filled with 300 ml of a 20 mM NaHCO$_3$ solution in basal medium containing (mg $\text{l}^{-1}$) CuCl$_2$ (5.7), K$_2$HPO$_4$ (250), MgCl$_2$·6H$_2$O (82), H$_3$BO$_3$ (0.05), FeCl$_2$·4H$_2$O (2), ZnCl$_2$ (0.05), MnCl$_2$·4H$_2$O (0.5), CuCl$_2$·2H$_2$O (0.04), Na$_2$MoO$_4$·2H$_2$O (0.04), CoCl$_2$·6H$_2$O (1), NiCl$_2$·6H$_2$O (1) and Na$_2$SeO$_3$·5H$_2$O (0.16). Reactor sludge (1.8 g VSS l$^{-1}$) and vinasse ($\sim 4500 \text{mg COD l}^{-1}$, $\sim 0.7 \text{mmol SO}_4^{2-}$-S) were added. Next, the vials were sealed with butyl rubber stoppers and the gas headspace was flushed for 5 min with oxygen-free flush gas (He:CO$_2$, 82:18%). Sulfide was added with a syringe from a Na$_2$S stock solution to obtain an initial total-sulfide concentration of $4.5 \pm 0.4 \text{ mM}$. All vials were incubated at $34 ^\circ \text{C}$ on a rotary shaker at 50 rpm. Oxygen (100%) was added after a preincubation period. Three oxygen levels were applied, corresponding to initial molar O$_2$/sulfide ratios of 0.53 (L), 1.1 (M) and 3.5 (H), respectively. Sulfide, sulfate, thiosulfate, elemental sulfur, biogas composition and COD were measured at selected time intervals. Autoclaved sludge controls and controls without sludge were included, as well as controls with living sludge in the absence of oxygen. All experiments were performed with triplicate batch vials.

2.3. Analyses

Sulfide was determined colorimetrically after reaction with $N,N$-dimethyl-$p$-phenylenediamine oxalate according the method described by Trüper and Schlegel (1964). Anions (sulfate, thiosulfate) were determined by high performance liquid chromatography. The samples were diluted 1:4 with a 10 mM zinc acetate solution, followed by centrifugation (5 min at 16,000g), thus precipitating sulfide as zinc sulfide. The chromatograph was equipped with an anionic column (Waters Millipore IC-pack A-HR, 4.6 $\times$ 75 mm, packed 6 μm) at $20 ^\circ \text{C}$. The carrier liquid, 0.018 M potassium hydrogen phthalate, was pumped at a flow rate of 1000 μl min$^{-1}$. The anions were detected with a conductivity detector (Waters Millipore TCM). Elemental sulfur was analysed by reversed-phase chromatography following the method described by Janssen et al. (1995). Biogas composition (O$_2$, N$_2$, CH$_4$, CO$_2$ and H$_2$S) was determined by gas chromatography, as described by Fdz.-Polanco Iniguez de la Torre (2001). The pH was determined with a WTW pH meter (Berlin, Germany) and a Crison 320 selective electrode. Chemical oxygen demand (COD) and volatile suspended solids (VSS) were determined according to standard methods (APHA, 1995).

3. Results and discussion

3.1. Reactor study

The airflow introduced to the reactor during the micro-aerobic phase was 0.7–0.9 m$^3$ m$^{-3}$ reactor volume d$^{-1}$. This corresponded to an O$_2$/COD ratio of $\sim 0.05$ g O$_2$ per g influent-COD and to an O$_2$/S ratio of 8–10 mol O$_2$ per mol influent-S (i.e. in principle sufficient to oxidize all sulfide formed). Micro-aerobic reactor operation did not lead to a significant change in the COD-balance (with COD removal efficiencies of 86.3 ± 0.7% and 86.6 ± 0.7% for anaerobic and micro-aerobic reactor operation, respectively), because the oxygen load was very low in comparison to the COD load. In contrast, the sulfur balance changed obviously. Table 1 lists the reactor performance data in terms of S-containing compounds during both the anaerobic and the micro-aerobic phases of reactor operation. During both anaerobic and micro-aerobic reactor operation, sulfate was removed almost completely. In the anaerobic phase, 66% of the sulfate-S removed could be recovered as sulfide-S in effluent and biogas. The gap in...
the S-balance (i.e. 34% of the converted sulfate-S was not recovered as sulfide-S) may have been due to limited oxidation with air oxygen entering the top part of the reactor via the not completely air-tight cover, something that was supported by the visual observation of sulfur formation attached to the fixed parts in the top of the reactor.

In the micro-aerobic phase, a smaller fraction (32%) of the removed sulfate-S could be recovered as sulfide-S in the reactor effluent. The biogas H₂S-level often decreased to below the detection threshold of 0.02%, indicating that moderate oxygenation can indeed be applied successfully as a strategy for the removal of sulfate from biogas. The fate of S during micro-aerobic reactor operation is not entirely clear. From the effluent data it is obvious that full re-oxidation to sulfide did not occur and neither did oxidation to thiosulfate. Furthermore, it is clear that at least a fraction left the reactor as elemental sulfur. However, since sulfur tends to attach to surfaces, it was not possible to assess this fraction reliably, as was reflected by the large range of elemental sulfur concentrations detected in the effluent samples (data not shown).

### 3.2. Batch experiments

#### 3.2.1. Reaction stoichiometry

When oxygen was introduced to the batch vials, sulfide disappeared rapidly. Elemental sulfur was formed, as well as thiosulfate (Fig. 1 and Table 2). Substantial formation of sulfate was only observed after the second injection of oxygen in batch vials with living anaerobic sludge in the series with a high oxygen concentration (data not shown).

The total amount of sulfur-, thiosulfate- and sulfate-S formed was mostly less than 50% of the amount of sulfide-S removed. The gap points to the formation of S-containing compounds that were not detected by any of the analytical methods applied. An indication about the nature of these ‘missing’ S-containing compounds can be obtained by considering the molar ratio between the amounts of sulfide-S and oxygen initially removed in batch vials with autoclaved sludge. At all three oxygen levels tested, this ratio amounted to 0.52–0.53 mol O₂ utilized per mol sulfide-S removed. Thus the initial oxidation of sulfide had been mainly the transfer of ~2 electrons from each sulfide molecule, changing the average oxidation state of the sulfur atoms from ~2 to ~0. This suggests elemental sulfur and polysulfides (Sₙ⁻) as the main initial oxidation products, rather than more oxidized sulfur compounds such as sulfite, dithionite and polythionates. According to the mechanism of sulfide oxidation described by Steudel (1996), sulfide is primarily oxidized to polysulfides from which, at pH values near or below 7, elemental sulfur (mostly S₈) can be formed. It can be expected that, at the pH level prevailing in the batch bottles (7.3 ± 0.2), the relative share of polysulfides would be considerable. Therefore, it is reasonable to assume that polysulfide explains the gap between the removed sulfide-S and recovered elemental sulfur and thiosulfate-S. Consequently, the gap values listed in Tables 2 and 3 may be interpreted as indicating (in mol S per mol sulfide-S removed) the concentration of polysulfides formed in the batch vials during sulfide oxidation. However, the lack of an appropriate method to detect polysulfides urges caution with such an interpretation. Moreover, even if elemental sulfur and polysulfides were the only products of sulfide oxidation, putting on a par missing-S and polysulfide would involve the risk of overestimating the actual polysulfide concentration if there were artifacts in the method for elemental sulfur analysis. The latter may especially be relevant if bacteria that intracellularly accumulate elemental sulfur carry out sulfide oxidation.

#### 3.2.2. Product formation of chemical sulfide oxidation

In batch series without viable biomass, sulfide can only be oxidized by chemical reactions. Table 2 shows the results of the series without sludge and with autoclaved sludge at the three initial oxygen/sulfide ratios investigated. It is shown that sulfide was mainly oxidized to elemental sulfur and ‘gap-S’ (i.e. presumably polysulfide, see discussion above), whereas the quantity of thiosulfate formed upon sulfide depletion was limited to a maximum of 14%, regardless of the initial oxygen level. Formation of sulfate was only detected at trace levels in the series without sludge at the highest oxygen level. After sulfide depletion some further oxidation to elemental sulfur and thiosulfate was observed. However, even 15 days prolonged incubation with a considerable amount of oxygen still present (series M and H) did not result in further complete oxidation to sulfate.

When comparing the formation of products from chemical sulfide oxidation in the absence of living sludge, it is seen that more elemental sulfur was formed in the series with autoclaved sludge, whereas the gap fraction (presumably polysulfide) was higher in the series without sludge.
The reason for this phenomenon is not known. Possibly, since elemental sulfur formation from polysulfide anions is greatly affected by pH (with lower pH values promoting elemental sulfur formation), this difference may have been due to a slightly lower pH in the series with autoclaved sludge.

3.2.3. Product formation of sulfide oxidation in the presence of living sludge

In the presence of living anaerobic sludge, the product yields of sulfide oxidation (Table 3) were generally fairly similar to those in the absence of living cells (Table 2). There were some differences in elemental sulfur production, i.e. lower elemental sulfur yields in the presence of living anaerobic sludge than in the presence of autoclaved sludge (series L and M only). However, these differences are not necessarily related to biological activity, since they may have been due to small pH differences (see discussion above). Another difference was the higher maximum thiosulfate yield in the series H with living anaerobic sludge than without living sludge.

In the presence of living micro-aerobic sludge, i.e. sludge sampled from the bioreactor after three weeks of micro-aerobic operation, thiosulfate production was always substantial: in the series H more or less similar but in the series L and M significantly higher than in batch vials with living anaerobic sludge. In contrast, the elemental sulfur yields were always lower than in the series with living anaerobic sludge. These observations suggest a role of bacteria in the oxidation of sulfur and the formation of thiosulfate, as well as an increase in the thiosulfate-producing activity of the reactor sludge after the switch from anaerobic to micro-aerobic reactor operation.

3.2.4. Reaction rates

3.2.4.1. Sulfide oxidation. Fig. 2 depicts the pseudo-first-order rate constants that were determined to approximate the sulfide oxidation rates (correlation coefficients always

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Fig. 1. Oxidation of sulfide and product formation upon oxygen addition in batch vials with living anaerobic granular sludge: sulfide (closed triangles), elemental sulfur (closed circles), sulfate (open squares), thiosulfate (open diamonds) and oxygen (dashed line). Left panels: living anaerobic sludge; right panels: living micro-aerobic sludge. Upper panels: low oxygen addition; middle panels: medium oxygen addition; bottom panels: high oxygen addition. The error bars indicate the standard deviations of triplicate batch assays.
higher than 0.95). It is shown that the removal of sulfide in batch vials with living anaerobic sludge proceeded faster with increasing oxygen level. The same trend could be observed in the other batch series, i.e. with micro-aerobic sludge, with autoclaved sludge and without sludge.

Different sulfide removal rates were observed between the different batch series at each oxygen level. It is shown that the reaction proceeded much faster in batch vials with micro-aerobic sludge than in those with anaerobic sludge, which was probably a result of the changed biomass composition, i.e. growth of (poly)sulfide-oxidizing bacteria, upon micro-aerobic reactor operation.

Fig. 2 furthermore shows slightly higher sulfide removal rates in batch vials with autoclaved sludge than in batch vials with living sludge. The removal of sulfide in batch vials with living anaerobic sludge proceeded faster with increasing oxygen level. The same trend could be observed in the other batch series, i.e. with micro-aerobic sludge, with autoclaved sludge and without sludge.

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Table 2
Oxygen utilization and product formation upon sulfide oxidation in the absence of living sludge

<table>
<thead>
<tr>
<th>Sludge</th>
<th>O2-level</th>
<th>mol O2 removed per mol sulfide removed</th>
<th>Sulfur compound production and gap in S-balance (all values in mol S per mol initial sulfide-S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>L</td>
<td>0.51</td>
<td>0.13 0.14 0.73</td>
</tr>
<tr>
<td>1-aut.</td>
<td>L</td>
<td>0.52 ± 0.01</td>
<td>0.42 ± 0.02 0.08 ± 0.06 0.58</td>
</tr>
<tr>
<td>0</td>
<td>M</td>
<td>0.49 ± 0.12</td>
<td>0.11 0.12 0.77</td>
</tr>
<tr>
<td>1-aut.</td>
<td>M</td>
<td>0.53 ± 0.02</td>
<td>0.34 ± 0.03 0.14 ± 0.05 0.52 ± 0.08</td>
</tr>
<tr>
<td>0</td>
<td>H</td>
<td>0.53 ± 0.10</td>
<td>0.28 ± 0.06 0.14 ± 0.03 0.55 ± 0.09</td>
</tr>
</tbody>
</table>

a Not included are data from the oxygen-free controls (no formation of elemental sulfur, thiosulfate or sulfate).

b Values printed in regular font refer to the concentrations measured immediately after sulfide depletion; values printed in italics refer to the maximum concentrations measured.

c Sludge types: 0 – no sludge; 1-aut. – autoclaved anaerobic sludge.

d Oxygen levels: L – low (0.53 mol O2 per mol sulfide); M – medium (1.1 mol O2 per mol sulfide); H – high (3.5 mol O2 per mol sulfide).

e Fraction of the initial sulfide concentration that could not be retrieved as elemental sulfur, thiosulfate or sulfate.

f Value probably overestimated.

Table 3
Oxygen utilization and product formation upon sulfide oxidation in the presence of living sludge

<table>
<thead>
<tr>
<th>Sludge</th>
<th>O2-level</th>
<th>mol O2 removed per mol sulfide removed</th>
<th>Sulfur compound production and gap in S-balance (all values in mol S per mol initial sulfide-S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>0.67 ± 0.07f</td>
<td>0.23 ± 0.05f 0.10 ± 0.01f 0f NAf</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>0.51</td>
<td>0.10 0.25 0.52</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>0.53 ± 0.02</td>
<td>0.19 ± 0.02 0.10 ± 0.01 0.71 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>0.58 ± 0.31</td>
<td>0.13 ± 0.05 0.23 ± 0.05 0.49 ± 0.09</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>0.71 ± 0.10</td>
<td>0.28 ± 0.03 0.10 ± 0.00 0.62 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>0.65</td>
<td>0.17 ± 0.01 0.35 ± 0.01 0.39</td>
</tr>
</tbody>
</table>

a Not included are data from the oxygen-free controls (no formation of elemental sulfur, thiosulfate or sulfate).

b Values printed in regular font refer to the concentrations measured immediately after sulfide depletion; values printed in italics refer to the maximum concentrations measured.

c Sludge types: 1 – anaerobic sludge; 2 – micro-aerobic sludge.

d Oxygen levels: L – low (0.53 mol O2 per mol sulfide); M – medium (1.1 mol O2 per mol sulfide); H – high (3.5 mol O2 per mol sulfide).

e Fraction of the initial sulfide concentration that could not be retrieved as elemental sulfur, thiosulfate or sulfate. Not applicable (NA) in the series where sulfide was not completely removed (series 1-L).

f Sulfide was not completely removed, values refer to the moment of oxygen (instead of) sulfide depletion.
vials with anaerobic sludge or without sludge. This may point to catalysis of chemical sulfide oxidation by compounds, e.g. redox-mediating enzyme cofactors, released from the cells by cell lysis during autoclaving, in analogy with sulfide oxidation catalysis by quinones (Steudel, 1996) and with autoclaved sludge catalysis of azo dye reduction by sulfide (van der Zee et al., 2003).

3.2.4.2. Oxygen removal. The initial profiles of the oxygen vs. time plots follow the same pattern as the sulfide vs. time plot. The initial molar ratios of oxygen utilized vs. sulfide removed are listed in Table 2, second column. It is shown that the ratios were more or less similar for most situations, which indicates that the consumption of oxygen in these series was mainly linked to sulfide oxidation and not to other oxygen-consuming processes.

After depletion of sulfide in both series with living sludge at high oxygen level, the oxygen level remained constant for an extended period before further decrease. This apparent ‘lag phase’ before utilization of oxygen for processes other than sulfide oxidation, e.g. vinasse biodegradation, lasted almost one day in the series anaerobic sludge and was much shorter (a few hours) in the series with micro-aerobic sludge.

3.2.4.3. Sulfide reappearance. The reappearance of sulfide was observed, albeit after longer incubation (~1 day) and at a lower rate. The same phenomenon could be observed in the batch vials at higher initial oxygen levels: at medium oxygen level, sulfide reappeared after 0.4 days (series with micro-aerobic sludge) and after 1.7 days (series with anaerobic sludge), and at high oxygen level, sulfide reappeared after 1 day (micro-aerobic sludge) and after more than 2 days (series with anaerobic sludge). This shorter delay of sulfide reappearance in the batch series with micro-aerobic sludge was at least partly due to the faster consumption of excess oxygen, which might have been due to the presence of O₂-respiring bacteria in micro-aerobic sludge. Moreover, higher sulfide reappearance rates were observed in the series with micro-aerobic sludge (Fig. 3), indicating an increased population of polysulfide, sulfur and thiosulfate-reducing bacteria in micro-aerobic sludge compared to anaerobic sludge. Sulfide reappearance was, however, always slower than sulfide oxidation.

4. Conclusions

Introduction of a limited airflow (0.7–0.9 m³ m⁻³ d⁻¹, corresponding to an O₂/S molar ratio of 8–10) to a vinasse-treating anaerobic fluidized bed reactor resulted in a lowering of the biogas H₂S concentration, often to below the detection level (0.02%). Introduction of air did not raise the effluent sulfate concentration, which indicated that sulfate reduction continued to occur and that there was no substantial re-oxidation of reduced sulfur species to sulfate. This situation remained fairly stable for the entire period (28 days) of micro-aerobic reactor operation, indicating that sulfide oxidation effectively competed for oxygen with other oxidative processes such as aerobic biodegradation of organic COD.
Supporting batch experiments showed that sulfide was oxidized mainly to species with an average oxidation state of the sulfur atoms around zero, i.e. elemental sulfur and presumably polysulfide. It was demonstrated, furthermore, that micro-aerobic sludge (i.e. reactor sludge sampled after 3 weeks of micro-aerobic operation) had a higher sulfide oxidizing activity than the original anaerobic reactor sludge. In addition, the reappearance of sulfide, i.e. re-reduction of oxidized sulfur species, was also faster with micro-aerobic sludge. This reappearance only occurred after complete depletion of oxygen and at a lower rate than that of sulfide removal.

The observation that sulfide concentrations in the reactor’s effluent and biogas did not increase with time during micro-aerobic reactor operation demonstrated that sulfide oxidation in the reactor was faster than re-reduction of oxidized sulfur species, thereby indicating that moderate oxygenation of anaerobic bioreactors can be a useful means of lowering the level of sulfide in the biogas.

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