SULFUR SOURCES FOR HYDROGEN SULFIDE PRODUCTION IN BIOFILMS FROM SEWER SYSTEMS

Per Halkjær Nielsen

Environmental Engineering Laboratory, University of Aalborg, Sohngaardholmsvej 57, DK-9000 Aalborg, Denmark

ABSTRACT

The relative significance of different inorganic and organic sulfur compounds on the sulfide production in anaerobic biofilms grown on domestic wastewater was investigated. The objective was to improve the understanding of microbial processes in dynamic systems and to evaluate the equations used to predict sulfide formation in pressure mains. Biofilms originally grown on domestic wastewater with sulfate as the only electron acceptor were also able to reduce sulfite and thiosulfate. The bacteria preferred thiosulfate to sulfate if both were present and the sulfide production rates increased with a factor of 1.5. Disproportionation of thiosulfate to equal amounts of sulfide and sulfate was demonstrated to take place in the biofilms but only at low concentrations of organic substrates. Some sulfide production from the organic sulfur compounds cysteine and methionine was observed. The rates were, however, insignificant compared to sulfide production from sulfate reduction in wastewater. Biofilm activity measured as the zero order volume constant \( k_{o} \) was around 0.18 mg SO\(_4\)-S cm\(^{-3}\) h\(^{-1}\) at 20 °C. If the biofilms were grown on domestic wastewater enriched with sulfite or thiosulfate, \( k_{o} \) increased around two times. The sulfide production rate from both sulfite and thiosulfate was found to be considerably higher than the rate from sulfate in these biofilms. The results were modeled using biofilm kinetics which showed that the presence of sulfite or thiosulfate in the wastewater strongly affected the potential sulfide production and could in some cases be a limiting compound besides organic matter. Knowledge about the presence of sulfur compounds other than sulfate in wastewater, e.g. from industrial sources, may therefore be very important to forecast sulfide buildup in sewer systems.

Keywords: Biofilm, sulfide production, thiosulfate, sulfite, disproportionation, cysteine, sewers.

INTRODUCTION

Sulfide production from different oxidized sulfur compounds and organic sulfur compounds is a well known phenomenon in relation to wastewater transportation over long distances in pressure mains (Pomeroy and Bowlus, 1946; Pomeroy and Parkhurst, 1977) and during anaerobic treatment of wastewater (Postgate, 1984). Hydrogen sulfide is a problem due to health hazards, noxious odors and corrosion and it may inhibit biogas production during anaerobic treatment (Postgate, 1984). Several empirical equations are available in order to predict the sulfide production in filled sewer pipes (Thistlethwayte, 1972; Boon and Lister, 1975; Pomeroy and Parkhurst, 1977; Nielsen and Hvitved-Jacobsen, 1988). They are all based on the assumption that the produced sulfide mainly is a result of microbial reduction of sulfate. However, compounds like elemental sulfur (S\(^0\)), sulfite (SO\(_3\)\(^-\)) and thiosulfate (S\(_2\)O\(_3\)\(^-\)) are common in several industrial wastewaters. Sulfite is mainly produced in the pulp and paper industry whereas thiosulfate is produced in photographic processing laboratories and in the pulp and paper industry giving the wastewater concentrations well above 100 mg S l\(^{-1}\) (Milano and Sorber, 1986). Relatively low levels from 0-10 mg S l\(^{-1}\) are reported in wastewater from a potato-starch producing factory (Nanninga and Gottshall, 1986) and in effluents from methane reactors (Kuenen et al., 1984). In most cases, however, the different sulfur compounds probably appear in the wastewaters as a result of sulfide oxidation with dissolved oxygen (Chen and Morris, 1972), for instance in partly filled sewer pipes.
Most sulfate reducers and many other types of bacteria are able to reduce elemental sulfur, sulfite and thiosulfate to sulfide (Postgate, 1984; Barrett and Clark, 1987) but little is known about their preference for the different compounds and the relative substrate uptake rates and growth rates for bacteria growing on the different substrates. Recently it is described that sulfite and thiosulfate not only are reduced to sulfide by sulfate reducers but in some cases also are disproportionated through an inorganic fermentation to sulfide and sulfate (Bak and Pfennig, 1987). It is not yet known if this process may be important in high-rate systems but it seems to be common in natural systems (F. Bak, pers. comm.) and may therefore be of importance also in sewer systems. A more basic knowledge about these phenomena in relation to the sulfide producing bacteria in sewer systems is necessary to improve the use of the equations to predict sulfide formation in sewer systems.

Attempts to use biofilm kinetics to describe the sulfate reduction in biofilms have been made (Holder et al., 1984; Holder, 1986; Nielsen, 1987; Nielsen and Hvitved-Jacobsen, 1988) in order to improve the models for sulfide formation in sewers and other high-rate systems, but so far no investigations on the reduction kinetics of other important sulfur compounds in these systems have been published.

The objective of this work was to measure some fundamental kinetic parameters and mechanisms for reduction of sulfite and thiosulfate in anaerobic biofilms grown on domestic wastewater in biofilm reactors and to use this information to improve modelling the biofilm kinetics by existing evaluated models (Nielsen, 1987). Important differences in the obtained parameters were found to depend on the conditions under which the biofilms were grown. Biofilm was grown either on wastewater with sulfate as sole electron acceptor or on wastewater with sulfate enriched with thiosulfate or sulfite as electron acceptor. The possible sulfide contribution from sulfur containing organic compounds was investigated as well and the relative significance of all these different sulfide sources was discussed in relation to the prediction of sulfide formation in sewer systems.

MATERIALS AND METHODS

Biofilm reactors. All experiments were performed in annular reactors made of transparent PVC. A magnet at the bottom provided complete mixing with a rotation at around 200 RPM. The inner reactor diameter was 53 mm and the volume around 200 ml. The reactors were operated as batch reactors and were fed 1-4 times a day.

Media and biofilm growth conditions. The biofilms were grown on the supernatant of settled domestic wastewater from the city of Aalborg. They were grown either without any additions (sulfate as electron acceptor, 20-30 mg SO₄⁻⁻⁻⁻ S l⁻¹) or enriched with additions of sulfite (30-40 mg SO₃⁻⁻⁻⁻ S l⁻¹) or thiosulfate (30-50 mg S₂O₃⁻⁺⁻ S l⁻¹) (enriched biofilms). Soluble COD and soluble BOD in the wastewater, determined after filtration through a 0.45 µm filter, were 300-500 and 80-250 mg l⁻¹ respectively. The wastewater was stored under a nitrogen atmosphere at 4°C 1-2 days and the pH was adjusted to 7.1-7.3 prior to use. An inoculum of sulfate reducing bacteria originating from raw wastewater was added to the reactors and the biofilms were grown in the dark at 20°C for 6-8 weeks before the experiments were initiated. The biofilm thickness in the reactors varied between 80-300 µm during the experiments. The thickness was measured microscopically (Nielsen, 1987).

Sulfide production rates. Sulfide production rates were measured by using either wastewater plus 100 mg l⁻¹ yeast extract as organic substrate or by using a mineral salt medium (Nielsen, 1987) with 400 mg l⁻¹ of yeast extract. Yeast extract as organic source has been shown earlier to give the same maximum sulfate reduction rates as the domestic wastewater (Nielsen and Hvitved-Jacobsen, 1988). The sulfide production rates were measured after addition of anoxic sulfate, sulfite, thiosulfate, cysteine and methionine in nonlimiting concentrations. Measurements were performed after the sulfide formation rate in the reactors was observed to be constant (normally after less than 30 minutes). Sulfide was determined photometrically by the methylene blue method (Cline, 1969). However, sulfite and thiosulfate are known to interfere with the sulfide determination (Cline, 1969) and therefore only relatively low concentrations were used. In some experiments the sulfate consumption was measured using radiotracer (³⁵SO₄²⁻), and after the sulfide was precipitated with zinc acetate the radioactivity of the sulfate was measured using a liquid scintillation counter (Packard Instrument, Co., Inc.). Sulfate and thiosulfate were measured using ion chromatography. The sulfur species were separated on a 10 cm anion exchange column (Hamilton PRP X 100), with 7 mM p-hydroxybenzoic acid as eluent at pH 8.4 and flow rate was 2 ml min⁻¹.

Modeling biofilm kinetics. The substrate uptake of bacteria is normally described by Michaelis-Menten kinetics and the transportation of substrates by Fick's second law of diffusion (Williamson and McCarty, 1976a). At steady state conditions the mass balance for one limiting substrate, for instance sulfate with no external mass transfer resistance (no hydraulic film layer), can be described by the following equation:

\[ D_f \frac{d^2 S}{dz^2} = \frac{k_{of} S}{K_m + S} \]  

(1)

where \( D_f \) is the diffusion coefficient within the biofilm, \( S \) is the substrate concentration within the biofilm at a distance \( z \) from the biofilm surface, \( K_m \) is the apparent half saturation constant and \( k_{of} \) is the zero order volume constant. This second order, nonlinear differential equation does not have an explicit solution and must be solved by...
numerical methods. The equation describes the concentration profiles through the biofilm from which the surface flux can be calculated. The diffusion coefficient within the biofilm was assumed to be 80% of the value in pure water (Williamson and McCarty, 1976b). The value for sulfate diffusion in pure water of $3.46 \times 10^{-6}$ cm$^2$ s$^{-1}$ at 20 °C (Li and Gregory, 1974) was also chosen for thiosulfate. $K_m$ for sulfate reducers was 45 μg S l$^{-1}$ (Nielsen, 1987) and was assumed to be the same for thiosulfate in this study (90 μg S l$^{-1}$).

RESULTS

The maximum sulfide production rate from different sulfur compounds was determined from biofilms grown on domestic wastewater either with sulfate as the sole electron acceptor or enriched with sulfite or thiosulfate (enriched biofilm). In short term experiments both sulfur compounds and organic substrate were present in nonlimiting concentrations ensuring that the maximum potential sulfide production rate was measured. The experiments demonstrated immediate capability for consumption but nothing about potential growth. The results are shown in fig. 1, table 1 and 2.

Table 1: Maximum relative sulfide production rates from wastewater with various sulfur compounds. The biofilms were grown on wastewater (incl. sulfate) or on wastewater enriched with sulfite or thiosulfate.

<table>
<thead>
<tr>
<th>Biofilms grown on domestic wastewater plus:</th>
<th>SO$_4$</th>
<th>SO$_4$+SO$_3$</th>
<th>SO$_4$+S$_2$O$_3$</th>
<th>SO$_4$+SO$_3$+S$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO$_4$</td>
<td>100</td>
<td>111 ± 5</td>
<td>153 ± 13</td>
<td>163 ± 25</td>
</tr>
<tr>
<td></td>
<td>(n = 7)*</td>
<td>(n = 9)</td>
<td>(n = 6)</td>
<td></td>
</tr>
<tr>
<td>SO$_4$+SO$_3$</td>
<td>100</td>
<td>169 ± 15</td>
<td>243 ± 30</td>
<td>216 ± 4</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>SO$_4$+S$_2$O$_3$</td>
<td>100</td>
<td>100 ± 7</td>
<td>238 ± 28</td>
<td>262 ± 4</td>
</tr>
<tr>
<td></td>
<td>(n = 3)</td>
<td>(n = 6)</td>
<td>(n = 3)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of biofilm reactors

Table 2: Maximum relative sulfide production rates from various sulfur compounds in mineral salt medium with yeast extract as organic source. The biofilms were grown on wastewater (incl. sulfate), or on wastewater enriched with sulfite or thiosulfate.

<table>
<thead>
<tr>
<th>Biofilms grown on domestic wastewater plus:</th>
<th>SO$_4$</th>
<th>SO$_3$</th>
<th>S$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO$_4$</td>
<td>100</td>
<td>99 ± 8</td>
<td>183 ± 26</td>
</tr>
<tr>
<td></td>
<td>(n = 8)*</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>SO$_4$+SO$_3$</td>
<td>100</td>
<td>129 ± 14</td>
<td>176 ± 27</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 3)</td>
<td></td>
</tr>
<tr>
<td>SO$_4$+S$_2$O$_3$</td>
<td>100</td>
<td>138 ± 6</td>
<td>189 ± 22</td>
</tr>
<tr>
<td></td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of biofilm reactors
Figure 1: Example on a short term experiment measuring the sulfide production from either sulfate, sulfite or thiosulfate as the sole electron acceptor and yeast extract as organic substrate (see table 2). All substrates were in non-limiting concentrations. The biofilm was grown on domestic wastewater containing sulfate as the only electron acceptor.

**Sulfate.** Sulfate could be used as the sole electron acceptor in all three types of biofilm (table 1). The sulfate reducers in the enriched biofilms (grown with sulfite or thiosulfate) therefore seemed not to be out-competed by other types of bacteria preferentially using sulfite or thiosulfate. The basic biofilm activity measured as the zero order volume constant for sulfate reduction, $k_{ou}$ increased slightly when the biofilms were grown in presence of sulfite or thiosulfate giving a more active sulfate reduction in these biofilms. The increase ranged from $0.18 \pm 0.07$ mg SO$_4$-S cm$^{-3}$ h$^{-1}$ ($n = 5$) to $0.35 \pm 0.12$ mg SO$_4$-S cm$^{-3}$ h$^{-1}$ ($n = 10$) for reactors grown on wastewater enriched with sulfite or thiosulfate.

**Sulfite.** When both sulfite and sulfate were present as potential electron acceptors in the wastewater the sulfide production rate generally was identical with or slightly higher than the rate obtained on sulfate alone. Only when the biofilms were grown on wastewater enriched with sulfite did the rate increase by a factor 1.7 (table 1). Sulfite could be used as sole electron acceptor in all three types of biofilms giving a slightly higher sulfide production rate than sulfate in the enriched biofilms (table 2).

**Thiosulfate.** With both sulfate and thiosulfate as potential electron acceptors the sulfide production rate increased with a factor between 1.5-2.4 mostly in the enriched biofilms (table 1). However, if the experiments were made with limiting concentrations of organic matter (diluted wastewater) the sulfide production from sulfate decreased relatively more than from thiosulfate, and the factor of 1.5-2.4 increased. If thiosulfate was used as sole electron acceptor in the mineral salt medium the sulfide production rate increased with a factor around 1.8 compared to the rates measured with sulfate as the only electron acceptor in all three types of biofilm (table 2). The same relative potential ability of the bacteria to use either sulfate or thiosulfate was independent of the way the biofilms were grown.

**Organic S-compound.** When sulfur-containing organic compounds such as the amino acids cysteine and methionine are degraded by fermentation and sulfate reduction in anaerobic systems, hydrogen sulfide or methane thiol is produced as a side-product. The sulfide production rate from cysteine added to wastewater (wastewater and yeast extract soluble COD 245 mg l$^{-1}$, cysteine COD 55 mg l$^{-1}$ corresponding to 20 mg cysteine-S l$^{-1}$) did not differ significantly from the rate on wastewater (fig. 2) in which only sulfate was responsible for the sulfide production. Methionine did not differ either (results not shown). If methionine or cysteine was added as the only organic substrate to the biofilm without any electron acceptor such as sulfate present, two different patterns for sulfide production were observed (fig. 2). Methionine gave a slow, constant increase in the sulfide concentration. Cysteine was consumed and probably fermented by the bacteria immediately after addition resulting in a very high sulfide production rate (fig. 2). After approximately 20 minutes the sulfide production totally stopped probably due to accumulation of some inhibitory products. This phenomenon was seen many times in several biofilms with different concentrations of cysteine. This inhibition could be overcome if a suitable electron acceptor such as sulfate was added (results not shown).
Sulfur sources for hydrogen sulfide production

Figure 2: Sulfide production from the organic S-compounds cysteine and methionine in a biofilm grown on domestic wastewater. Concentration of cysteine or methionine was 20 mg S l\(^{-1}\). Soluble COD of wastewater plus yeast extract (without cysteine) was 245 mg l\(^{-1}\).

Figure 3: Disproportionation of thiosulfate in a biofilm grown on domestic wastewater with sulfate as the only electron acceptor. A) No disproportionation in the presence of organic substrates. B) Disproportionation to sulfate and sulfide in the absence of organic substrates.
Disproportionation of sulfite and thiosulfate. Biofilms grown on domestic wastewater and never before exposed to thiosulfate were able to disproportionate thiosulfate to sulfide and sulfate. However, no disproportionation of thiosulfate occurred in the presence of organic matter (wastewater) (fig. 3). Normal sulfate reduction took place with a high rate until thiosulfate was added (fig. 3A). Then a shift was observed and thiosulfate was reduced to sulfide with a high rate whereas sulfate disappeared only slowly. The same time course of sulfate in figure 3 and figure 4 in which the sulfate consumption was measured using radiotracer ($^{35}$SO$_4^-$) showed that no or very little disproportionation took place simultaneously. If thiosulfate was added to the same biofilm in the absence of organic matter a disproportionation to nearly equal amounts of sulfate and sulfide was the result (fig. 3B). 24.6 mg S$_2$O$_3$-S l$^{-1}$ was consumed giving...

Figure 4: Preference of thiosulfate to sulfate. Addition of thiosulfate (9.6 mg S l$^{-1}$) to a sulfate reducing biofilm grown on domestic wastewater.

Figure 5: Partial preference of sulfite to sulfate. Addition of sulfite (9.0 mg S l$^{-1}$) to a sulfate reducing biofilm grown on domestic wastewater.
Sulfur sources for hydrogen sulfide production

13.5 mg HS-S \(\cdot \)1\(^{-1}\) and 10.8 mg SO\(_4\)-S \(\cdot \)1\(^{-1}\). It is in fair accordance with Bak and Pfennig (1987) who found the following stoichiometry for disproportionation of thiosulfate in pure cultures of sulfate reducers:

\[
S_2O_3^{2-} + H_2O \rightarrow HS^- + SO_4^{2-} + H^+
\]

Furthermore, the thiosulfate consumption rate decreased to approximately 30 % of the rate in presence of organic matter while the sulfide production rate decreased to around 20 %. Disproportionation of sulfite was also observed in the sulfate grown biofilm. In the enriched biofilms disproportionation of both sulfite and thiosulfate was also found to occur if no organic matter was present (results not shown). The stoichiometry for sulfite disproportionation has not been measured in this study but is according to Bak and Pfennig (1987):

\[
4 SO_3^{2-} + H^+ \rightarrow HS^- + 3 SO_4^{2-}
\]

Preference for specific sulfur compounds. The results shown in figures 3 and 4 indicates that thiosulfate, if present in the wastewater, was preferred to sulfate as electron acceptor. At the same time the sulfide production rate increased in accordance with the results in table 1. Sulfite was to some extent preferred as electron acceptor for sulfite (fig. 5). The sulfate reduction rate decreased with 30-40 % for this biofilm and the sulfide production rate remained almost unchanged.

DISCUSSION

Two typical situations concerning presence of electron acceptors in a dynamic sewer system were investigated. In the first situation the primary electron acceptor was sulfate and only pulses of sulfite or thiosulfate where added and the response was determined in short term experiments. In the second situation sulfite or thiosulfate was primary electron acceptor during growth and the two other compounds occurred only temporarily as primary electron acceptor during the experiments. The results showed that it was important to distinguish between these two biofilm systems due to an increased specific activity \(k_{\text{max}}\) of biofilms grown with sulfite or thiosulfate as the dominating electron acceptor and response in the presence of non-usual sulfur compounds as well.

The higher \(k_{\text{max}}\) value in the presence of sulfite or thiosulfate was probably caused by a higher density and/or a higher specific activity \(V_{\text{max}}\) of the sulfate reducers in the biofilm. This can be explained partly by the enhanced growth yield reported for sulfate reducers growing on these compounds. From pure cultures of sulfate reducers it is known that sulfite or thiosulfate generally gives a growth yield around 2-3 times higher than the yield of sulfate (Widdel and Pfennig, 1981; Nethe-Jaenchen and Thauer, 1984; Cypionka and Pfennig, 1986). Both compounds can enter the biochemical pathway directly for dissimilar sulfate reduction in the cells without the energy consuming reactions as needed to utilize sulfate, as sulfite and maybe thiosulfate are intermediate compounds in the pathway (Postgate, 1984).

Pomeroy and Bowlus (1946) have observed that sulfide production in sewer systems increased in the presence of sulfite or thiosulfate but it was only observed in long term experiments with growth as an important factor. In this study short term addition of sulfite to a biofilm grown on wastewater with sulfate as the usual electron acceptor increased the sulfide production rate only slightly although the bacteria to some extent preferred this compound to sulfate. Thiosulfate was strongly preferred to sulfate and the sulfide production increased immediately after addition with a factor of 1.5. The factor was even larger at low concentrations of organic matter. This observation is important to describe and predict turnover of sulfur compounds in dynamic systems with low, varying concentrations of thiosulfate and organic matter.

Many sulfur compounds (except sulfate) are reduced by bacteria outside the sulfate-reducing group (Barrett and Clark, 1987) and these bacterial groups are in some anoxic environments more numerous than the sulfate reducers (Obuekwe et al., 1983). Therefore, as observed (table 1), a relatively higher sulfide production rate from sulfite and thiosulfate compared to that of sulfate could be expected from biofilms grown on wastewater enriched with sulfite or thiosulfate. On the other hand, the sulfate reducing bacteria in the enriched biofilms seemed not to be out-competed by other bacteria only able to reduce thiosulfate because the relative sulfide production rate from thiosulfate as sole electron acceptor compared to sulfate was identical in all three types of biofilm (1:1:8, table 2). The reason for this discrepancy is not known, but the presence of more than one electron acceptor and use of wastewater (plus some yeast extract to secure non-limiting concentration) as organic source might be important.

The study demonstrated that disproportionation of thiosulfate and sulfite could occur in high-rate systems. Thiosulfate was found to disproportionate to nearly equal amounts of sulfate and sulfide in accordance with the results from pure culture of sulfate reducers (Bak and Pfennig, 1987). An important key parameter seemed to be the presence of organic substrate as the disproportionation never took place at high levels of organic substrates. The energy yield from oxidation of organic matter with thiosulfate as electron acceptor is higher than the yield obtained from disproportionation of thiosulfate (Widdel, 1988). This might be the reason for the bacteria to change their metabolic strategy. Some experiments showed, however, that disproportionation could take place even at low levels of organic.
matter. This was probably caused by a lack of suitable substrates or different conditions in the inner and outer part of the biofilm. This phenomenon may therefore be important in sewer systems transporting low strength wastewater, for instance in combined sewers.

The degradation of sulfur-containing organic compounds is not assumed to play any significant role in the sulfide production in sewer systems (Pomeroy and Bowlus, 1946). Although cysteine and methionine could contribute to the sulfide production in biofilms grown on domestic wastewater the rate was very low compared to sulfate reduction in the present study. Sulfide seems to be produced directly during the degradation of cysteine while mainly methanethiol and dimethylsulfide are produced from methionine (Kadota and Ishida, 1972). These methylated, reduced sulfur compounds are probably converted to methane and sulfide by methanogenic bacteria (Kiene et al., 1986). There are probably some methane producers in the biofilm reactors and they may be responsible for the observed sulfide production. Sulfide production from cysteine may only be significant if a major part of the wastewater consists of cysteine which probably seldom is the case. If cysteine was added as sole substrate an inhibition of sulfide production was observed after a short time. Cysteine is assumed to be fermented to acetate and hydrogen via pyruvate (McInerney, 1988). So far, however, there is no explanation for this inhibition phenomenon.

The practical implications of the results presented here may be demonstrated with thiosulfate as an example where the biofilm kinetics for a typical biofilm in a pressure main is modeled. In sewer systems where both sulfate and thiosulfate are present both compounds may contribute to the sulfide formation from the biofilms. Generally it is assumed that the sulfide production rate depends only on concentration and quality of the organic matter in the wastewater and not on the sulfate concentration which is assumed to be present in high nonlimiting quantities (Nielsen and Hvitved-Jacobsen, 1988). If the biofilms are grown on domestic wastewater with sulfate as the sole electron acceptor a relative low activity \(k_{of}\) is obtained. If a pulse of thiosulfate in nonlimiting concentration is added to the wastewater it immediately replaces sulfate as the electron acceptor. Furthermore, it results in an increased sulfide production rate at approximately 50% (no disproportionation) which is significantly higher than the sulfide production rate normally expected from sulfate (Figure 6A). At lower concentrations (below 7-8 mg S\(_2\)O\(_3\) S\(^{-1}\)) the total sulfide production rate arises from a reduction of thiosulfate in the outer part of the biofilm and a reduction of sulfate in the inner part. Thus, in this case sulfide production from thiosulfate is limited by the availability of thiosulfate and not as normally expected by the organic matter. Sulfate only limits the sulfide production in a biofilm at bulk water concentrations below 3-4 mg SO\(_4\) S\(^{-1}\) which is far below the sulfate concentration in most wastewater.

Figure 6: Biofilm kinetics for a typical biofilm from a pressure main with a thickness of 300 \(\mu\)m grown on domestic wastewater (A) or wastewater enriched with thiosulfate (B). Equation 1 was solved by a numerical method with the parameters mentioned in the text.
If the biofilms are grown on wastewater containing thiosulfate as primary electron acceptor a higher sulfide production rate is expected in a full penetrated biofilm due to the higher $K_d$ value. The ratio of 1:2.4 for sulfide production from sulfate and thiosulfate is also expected. In this case the potential maximum sulfide production rates are significantly higher and the limiting concentrations of sulfate and thiosulfate would also be considerably higher (Figure 6B). In such wastewater systems the sulfide production rate may be highly affected by variations in the thiosulfate concentration, and knowledge of these phenomena is therefore essential to predict the sulfide buildup.

CONCLUSION

Hydrogen sulfide formation from sulfite and thiosulfate is of potential importance in biofilms from sewer systems. Depending on the growth conditions of the biofilms the sulfide production rate from sulfite was 1-1.7 times the rate from sulfate. The rate from thiosulfate was increased by a factor of 1.5-2.4 compared to sulfate.

The organic sulfur compounds cysteine and methionine gave some hydrogen sulfide production when added as organic source to the wastewater. However, the formation rate was insignificant compared to sulfide formation from sulfate reduction.

Thiosulfate and sulfite were observed to be disproportionated to sulfate and sulfide in the biofilms at low concentrations of organic matter. This may be important to predict sulfide formation in diluted wastewater.

Sulfide production from sulfate, sulfite and thiosulfate in wastewater was modeled using biofilm kinetics. It was shown that the presence of several oxidized sulfur compounds in the wastewater is important because they might influence the sulfide production differently.

ACKNOWLEDGMENT

I thank T. Hvitved-Jacobsen and N. Iversen for helpful discussions and critical review of the manuscript and A. Tjørnand for technical assistance. This study was supported in part by the Danish National Science Foundation grant no. 16-3677.H-747 and the Danish Biotechnology Program.

REFERENCES


