Dynamic Modelling of Biofilm Reactors with Immobilised Sulfate-Reducing Bacteria

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Abstract. This paper presents a simple, reliable and extensible dynamic biofilm model. The model simulates the case of Sulfate-Reducing Bacteria (SRB) grown as biofilm on the surface of an inert support material in a completely mixed bioreactor. The principles of microbial stoichiometry and thermodynamics combined with microbial kinetic equations and chemical reactions have been implemented in AQUASIM software. Simulation results predict the biofilm growth, the reactor bulk volume solute concentrations and their corresponding profiles in the biofilm layer. The interaction of metal ions with biogenic sulfide is also discussed as decontamination means for a wastewater stream containing high metal and sulfate concentrations.

Introduction

Sulfate-Reducing Bacteria (SRB) grow in anoxic environments by catabolising small amounts of simple organic molecules, such as acetate, lactate, ethanol and propionate, as carbon and electron donors. SRB couple this electron transfer by reducing sulfate to sulfide and gaining energy. Metal ions present in the bioreactor feed stream are readily precipitated by the SRB-generated sulfide. These unique metabolic attributes signify the technological importance of these bacteria for potential biological treatment of industrial wastewater streams containing metal ions and high sulfate concentration. Modelling of such processes is essential for understanding the biochemical interactions and importance of the bioreactor operating parameters for successful industrial application.

Model Development

Concept. The biological process simulated by the proposed model is presented in Fig. 1. Wastewater containing sulfate and soluble divalent metal ions is treated in a continuously stirred reactor. Acetate as organic C source and ammonium as N source are present in the feed to support microbial growth. Microbial SRB cells grow as biofilm attached on the surface of an inert material. The metabolic activity of SRB produces alkalinity (HCO$_3^-$) and biogenic HS$^-$. Treated wastewater leaves the reactor containing low concentrations of organic carbon, sulfate, ammonium and metal ions. Metal ions are precipitated as insoluble MeS and retained within the biofilm layer (not shown in picture) as well as in the bulk liquid volume of the reactor.

Biological Processes. The overall formulation of the biochemical activity in the reactor has been developed based on stoichiometry and bacterial energy concepts in the literature [1]. Half reactions for electron donor ($R_d$), electron acceptor ($R_a$) and cell ($C_3H_7O_2N$) synthesis ($R_c$) are represented by (Eq.1), (Eq.2) and (Eq.3), for acetate, sulfate and ammonium as N source respectively.
Figure 1. Conceptual drawing of a completely mixed biofilm reactor with SRB. Biomass grown in biofilm on the surface of a support material comes in contact with the wastewater containing acetate as carbon and electron source, sulfate as electron acceptor and ammonium as nitrogen source. Metal content of the wastewater is sequestered and retained as MeS precipitate.

\[
\frac{1}{8} \text{CO}_2(g) + \frac{1}{8} \text{HCO}_3^{-,(aq)} + \text{H}^+ + e^- \rightarrow \frac{1}{8} \text{CH}_3\text{COO}^- + \frac{3}{8} \text{H}_2\text{O} \quad \Delta G_d^{0'} = +27.41 \text{kJ} / \text{e}^- \text{eq} \quad (\text{Eq.1})
\]

\[
\frac{1}{8} \text{SO}_4^{2-} + \frac{19}{16} \text{H}^+ + e^- \rightarrow \frac{1}{16} \text{H}_2\text{S}_{(aq)} + \frac{1}{16} \text{HS}^- + \frac{1}{2} \text{H}_2\text{O} \quad \Delta G_s^{0'} = +20.85 \text{kJ} / \text{e}^- \text{eq} \quad (\text{Eq.2})
\]

\[
\frac{1}{5} \text{CO}_2(g) + \frac{1}{20} \text{NH}_4^+ + \frac{1}{20} \text{HCO}_3^{-,(aq)} + \text{H}^+ + e^- \rightarrow \frac{1}{20} \text{C}_3\text{H}_7\text{O}_2\text{N} + \frac{9}{20} \text{H}_2\text{O} \quad \Delta G_{pc}^{0'} = +18.80 \text{kJ} / \text{e}^- \text{eq} \quad (\text{Eq.3})
\]

If \(f_s\) is the fraction of electrons used for cell synthesis and \(f_e\) the fraction of electrons used for sulfate reduction, then the overall biochemical reaction \(R\) can be written as follows [2]:

\[
R = f_sR_s + f_eR_e - R_d \quad (\text{Eq.4}) \quad \text{where} \quad f_s + f_e = 1 \quad (\text{Eq.5})
\]

By combining equations (Eq.1) - (Eq.5), the overall biochemical reaction is written as follows:

\[
\frac{1}{8} \text{CH}_3\text{COO}^- + \left(1 - f_s\right) \frac{1}{8} \text{SO}_4^{2-} + \frac{f_s}{20} \text{NH}_4^+ + \frac{f_s}{40} \text{H}^+ \rightarrow \frac{f}{20} \text{C}_3\text{H}_7\text{O}_2\text{N} + \left(1 - \frac{f_s}{4}\right) \text{HCO}_3^- + \frac{3f_s}{8} \text{HS}^- + \frac{3f_s}{20} \text{H}_2\text{O} \quad (\text{Eq.6})
\]

Ignoring biomass formation (i.e. \(f_s=0\)) (Eq.6) leads to the simple chemical reaction:

\[
\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{HS}^- \quad (\text{Eq.7})
\]

In the absence of experimental data, maximum values of \(f_s\) denoted as \(f_s^{0'}\) can be estimated by thermodynamic analysis [1]. For acetate metabolism (\(\Delta G_s^{0'} = +27.41 \text{kJ} / \text{e}^- \text{eq}\)), with sulfate as electron acceptor (\(\Delta G_s^{0'} = +20.85 \text{kJ} / \text{e}^- \text{eq}\)) and SRB biomass formation with ammonium as nitrogen source (\(\Delta G_{pc}^{0'} = +18.80 \text{kJ} / \text{e}^- \text{eq}\)), assuming overall electron transfer efficiency (\(\varepsilon=0.6\) and \(n=1\)), a value of \(A\) is calculated as follows [1].

\[
A = -\frac{\Delta G_p + \Delta G_{pc}^{0'} + \varepsilon \Delta G_s^{0'}}{\varepsilon \Delta G_s^{0'}} = -\frac{35.09 - 27.41 + 18.80}{0.6 (20.85 - 27.41)} = 11.21
\]

\[
f_s^{0'} = \frac{1}{1 + A} = \frac{1}{1 + 11.21} = 0.082, \quad f_e^{0'} = 1 - f_s^{0'} = 1 - 0.082 = 0.918 \quad (\text{Eq.8})
\]

Assuming that \(f_e\) is 60% of \(f_s^{0'}\) [1], (Eq.6) can be written as:
0.125CH₃COO⁻ + 0.11885SO₄²⁻ + 0.00246NH₄⁺ + 0.00369H⁺ →  
0.00246C₅H₇O₂N⁺ + 0.2377HCO₃⁻ + 0.11885HS⁻ + 0.00738H₂O

(Eq.10)

**Microbial Kinetics.** Growth of SRB is assumed to proceed according to a mixed Monod type of reaction summarised by (Eq.11) and death of SRB according to (Eq.12), followed by lysis of dead cells according to (Eq.13).

\[
\begin{align*}
    r_{p,SRB-NH_4} &= \mu_{\text{max},SRB} \left( \frac{C_s}{K_s + C_s} \right) \left( \frac{C_{SO_4}}{K_{SO_4} + C_{SO_4}} \right) \left( \frac{C_{NH_4}}{K_{NH_4} + C_{NH_4}} \right) X_{SRB} \\
    r_{\text{death,SRB}} &= k_{\text{death,SRB}} \left[ 1 - \left( \frac{C_s}{K_{d,s} + C_s} \right) \left( \frac{C_{SO_4}}{K_{d,SO_4} + C_{SO_4}} \right) \right] X_{SRB} \\
    r_{\text{lysis,SRB}} &= k_{\text{lysis}} X_{\text{inert,SRB}}
\end{align*}
\]

(Eq.11)

(Eq.12)

(Eq.13)

**Chemical Reactions.** In the simplified form of the model presented in this paper, only the fast abiotic chemical precipitation of Me²⁺ with HS⁻ is considered. This reaction is considered rapid and in equilibrium with typical solubility product value K_{sp} = 10^{-30} for MeS.

**Model Parameters.** Acetate, sulfate and ammonium concentrations are represented in the model by the variables C_s, C_{SO_4}, and C_{NH_4} in mol/L. For active and inert biomass, X_{SRB} and X_{\text{inert,SRB}} are used in mol/L. Typical values of microbial kinetic parameters used in the model are listed in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum specific growth rate</td>
<td>(\mu_{\text{max},SRB})</td>
<td>0.2 day⁻¹</td>
</tr>
<tr>
<td>Substrate half saturation coefficient</td>
<td>(K_s)</td>
<td>90×10⁻⁶ mol/L</td>
</tr>
<tr>
<td>Sulfate half saturation coefficient</td>
<td>(K_{SO_4})</td>
<td>300×10⁻⁶ mol/L</td>
</tr>
<tr>
<td>Ammonium half saturation coefficient</td>
<td>(K_{NH_4})</td>
<td>2.8×10⁻⁶ mol/L</td>
</tr>
<tr>
<td>Death rate constant</td>
<td>(k_{\text{death,SRB}})</td>
<td>0.02 day⁻¹</td>
</tr>
<tr>
<td>Substrate half saturation coefficient in death rate</td>
<td>(K_{d,s})</td>
<td>8×10⁻⁶ mol/L</td>
</tr>
<tr>
<td>Sulfate half saturation coefficient in death rate</td>
<td>(K_{d,SO_4})</td>
<td>30×10⁻⁶ mol/L</td>
</tr>
<tr>
<td>Cell lysis rate constant</td>
<td>(k_{\text{lysis}})</td>
<td>0.1 day⁻¹</td>
</tr>
</tbody>
</table>

**Reactor Operating Conditions – Simulation Software.** For the purpose of the model, a total volume of 10 L has been selected. The bulk liquid phase of the reactor is considered as 80% of the total volume while 20% is filled with the biomass support material. Under these conditions, biofilm specific surface area is estimated at 0.6 m²/L reactor. For the initially established biofilm, a thickness of 100 µm is assumed. Wastewater at a volumetric flow rate of 50 L/day, containing acetate (1,000 mg/L), sulfate (1,000 mg/L), ammonium (100 mg/L N-NH₄⁺) and metal ions (10 mg/L) is assumed to be treated in the reactor. Soluble species are diffused in the biofilm layer through a liquid mass transfer boundary layer of 50 µm. Diffusion coefficients have been calculated at 25 °C [2]. The well documented biofilm compartment model of the simulation software AQUASIM has been used for model implementation [3].

**Results and Discussion**

Typical simulation results for the SRB bioreactor are presented in Fig. 2. Fig. 2(a) depicts biofilm growth vs. time starting from an initially established thickness of 100 µm. Biofilm reaches a
dynamic steady state condition after a period of 200 days of continuous operation because of sulfate depletion in the deepest layer of biofilm (Fig. 2c, day 200). Acetate and sulfate profiles in the biofilm layer are presented in Fig. 2(b) and 2(c) respectively. Under the selected conditions, sulfate is the rate limiting component after 200 days. Fig. 2(d) shows bicarbonate profiles, which are typically concave downward and typically decrease in concentration as a component produced in the biofilm. Concentration of soluble metal at the exit of the reactor is at sub ppb levels as it precipitates in the biofilm and bulk reactor volume as insoluble MeS [2].

Figure 2. Simulation results for (a) biofilm growth, (b) acetate (c) sulfate and (d) bicarbonate profiles in the biofilm layer. Circles represent the corresponding concentrations in bulk liquid.

Conclusions

The microbial dynamics of a biofilm reactor can be studied by the modelling tool presented. Simulation results present favourable conditions for metal sequestering by biogenic HS⁻ production. This process can be applied for the treatment of metal-laden industrial wastewater.

References

