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Factors affecting microbial sulfate reduction by
Desulfovibrio desulfuricans.

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INTRODUCTION

Sulfate reducing bacteria (SRB) cause serious problems in sanitary sewer systems and industrial
water systems because of production of highly toxic and corrosive hydrogen sulfide gas. The
corrosion of concrete sewers occurs as a result of hydrogen sulfide production from sulfate–rich
sewage by the activities of SRB[1]. Furthermore, problems are well known in the oil industry where
SRB cause serious corrosion of installations, plugging of formations, and contamination of petroleum
with H₂S (souring). In these industries, mathematical models have been developed for predicting and
controlling SRB activity. A range of environmental factors affects the growth and activity of SRB
and other bacteria in these systems, and a more quantitative knowledge of the significance of those
environmental factors is needed to improve the mathematical models. At present, quantitative
prediction of SRB activity and growth in industrial water systems is essentially impossible because
rate and extent of SRB growth under relevant environmental conditions are not available. Therefore,
it is essential to determine effects of environmental factors on the activity and growth of SRB to
develop a comprehensive model and to use this model to predict the SRB behavior in given
environments.

The microbial environment varies widely through the industrial water systems. The system
temperature can have a major influence on SRB activity. Temperature can affect both kinetics and
stoichiometry via phenomena ranging from thermodynamic activity changes through physical enzyme
conformation changes. Nutrient availability can affect both the growth of the organisms (through
energy limitation or through limitations in biosynthetic precursors) as well as the amounts and types
of products (e.g., cell material versus extracellular products). Thus, the reduction of the concentration
of essential nutrients (e.g., N and P) to below the limiting concentration is a possible means of
controlling SRB. Since relatively high sulfide concentrations have been found frequently in these
industrial systems, sulfide product inhibition may be expected to some extent. Product inhibition
(sulfide) reduces biochemical activity through numerous mechanisms. In this manuscript, effects of
temperature, essential nutrients (e.g., N and P), and sulfide concentration on activity and growth of
SRB, here exemplified by Desulfovibrio desulfuricans, were discussed.

EXPERIMENTAL MATERIALS AND METHODS

Experimental System. All experiments were conducted using a chemostat consisting of a
pyrex cylindrical beaker (1.5 L volume). The chemostat was equipped with a butyl rubber biofilm
scraper continuously rotated by a electric motor to remove wall growth. A constant pH and
temperature were maintained using a pH control system with sterile 1.0 N HCl and NaOH solutions
and thermoregulator, respectively. The slow continuous nitrogen purge of the reactor maintained
anaerobic conditions and prevented H₂S accumulation. Traces of oxygen in the nitrogen feed gas were
removed by a reducing column containing copper wire maintained at 400°C.

Microorganisms. Desulfovibrio desulfuricans (ATCC 5575) was grown in Postgate medium
G2, 3), including hemi–calcium lactate (L–lactic acid, SIGMA, No.L–2000) as the sole carbon and
energy source. Trace elements and vitamins were added6 9). A sterile Na₂S₂O₄ solution was added as
Analytical methods. At steady state, effluent samples were obtained for the following analyses: (1) total organic carbon (TOC); (2) soluble organic carbon (SOC); (3) total bacterial counts and cell size; (4) sulfate; (5) sulfide; (6) lactate; (7) acetate; (8) ammonium nitrogen; (9) phosphorous. The samples for SOC, lactate, acetate, ammonium nitrogen, and phosphorous analyses were obtained by filtering an aliquot of the chemostat effluent through 0.22 μm Nuclepore filters. The phenate method was used for ammonium nitrogen analysis. Phosphorous concentration was measured as orthophosphorous using the modified ammonium molybdate–ascorbic acid method described by Harwood et al. Total dissolved sulfide concentration in the liquid phase was measured using the methylene blue method described previously by Cline. The volatile sulfide was measured by trapping the gaseous sulfide in 1.0 N NaOH solution. Cellular carbon concentration was determined converting cell size determined by an epifluorescence technique to cellular carbon using various factors. EPS carbon concentration was calculated by subtracting the calculated cellular carbon from the total biomass carbon (effluent TOC–effluent SOC). The details of the rest of the chemical analytical methods have been described elsewhere previously.

RESULTS AND DISCUSSION

Temperature Effects. Typical behavior was observed for the steady state dependence of cellular carbon and lactate concentration on dilution rates as shown in Figure 1. The EPS carbon concentrations were not significant at any dilution rate for the carbon-limiting experiments. The effluent sulfate concentrations were in the order of 200–300 mg L⁻¹ (depending on dilution rate) so that this chemostat system was limited by the lactate supply. Kinetic constants (μₘₕ and Kₐ) were computed from the effluent lactate concentrations and dilution rates based on the nonlinear regression form of the Monod equation using MSU SAS (statistical software). The resulting kinetic parameters, along with their respective standard error, at each temperature were presented in Table 1. The highest maximum specific growth rate (μₘₕ) of 0.55 h⁻¹ was observed at 43°C. The maximum specific growth rate was relatively constant between 25°C and 43°C and dramatically decreased outside this temperature range with optimum temperature range between 35°C and 43°C as shown in Figure 2. The activation energy for μₘₕ was 104 KJ mol⁻¹ in the range 12°C–25°C. By comparison, Nielsen determined the activation energy for sulfate reduction rate to be 85 KJ mol⁻¹ in a mixed population SRB biofilm. These data suggest that the effect of temperature on microbial sulfate reduction in suspension is not significantly different from the mixed population biofilm.

The half–saturation coefficient (Kₐₗₐc) for lactate and the cell yield (Yₗₐc) were dependent on temperature as shown in Figures 3 and 4. Similar ranges of μₘₕ and Kₐ obtained in this study have been reported in the literature. Cappenberg reported μₘₕ and Kₐ for D. desulfuricans grown in the continuous culture at 30°C were 0.36 h⁻¹ and 4.4 mg–lactate L⁻¹, respectively. The determined cell yield coefficients were in the range between 0.036–0.017 g–cell [g–lactate]⁻¹ in the temperature range 12–48°C. The highest cell yield was observed in the optimum temperature range for growth, 35°C–43°C. The cell yield coefficients obtained in this study were relatively low for at least two
Table 1. Experimentally determined kinetic parameters for D. desulfuricans growing on lactate as the sole carbon and energy source (estimated parameter value ±SE).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$\mu_{\text{max}}$ (h⁻¹)</th>
<th>$K_{\text{Lac}}$ (mg/L)</th>
<th>$Y_{\text{ext, Lac}}$ (g/g)</th>
<th>$m$ (g/g · h⁻¹)</th>
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<tr>
<td>12</td>
<td>0.059 ±0.001</td>
<td>3.7 ±0.75</td>
<td>0.017 ±0.001</td>
<td>-0.35 ±0.54</td>
</tr>
<tr>
<td>25 (run 1)</td>
<td>0.38 ±0.002</td>
<td>1.4 ±0.39</td>
<td>0.025 ±0.002</td>
<td>0.23 ±0.36</td>
</tr>
<tr>
<td>25 (run 2)</td>
<td>0.41 ±0.001</td>
<td>10.2 ±0.24</td>
<td>0.025 ±0.004</td>
<td>2.12 ±4.13</td>
</tr>
<tr>
<td>35 (run 1)</td>
<td>0.37 ±0.004</td>
<td>2.2 ±0.60</td>
<td>0.024 ±0.006</td>
<td>0.45 ±1.08</td>
</tr>
<tr>
<td>35 (run 2)</td>
<td>0.46 ±0.011</td>
<td>3.6 ±2.48</td>
<td>0.036 ±0.004</td>
<td>0.82 ±1.14</td>
</tr>
<tr>
<td>43</td>
<td>0.55 ±0.003</td>
<td>10.0 ±1.22</td>
<td>0.032 ±0.001</td>
<td>0.28 ±0.00</td>
</tr>
<tr>
<td>48</td>
<td>0.115 ±0.001</td>
<td>6.4 ±0.75</td>
<td>0.023 ±0.001</td>
<td>0.27 ±0.47</td>
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Figure 2. Temperature dependence of the maximum specific growth rate ($\mu_{\text{max}}$). The highest $\mu_{\text{max}}$ of 0.55 h⁻¹ was obtained at 43°C. The activation energies for $\mu_{\text{max}}$ were 14 KJ/mol in the range 25–43°C and 104 KJ/mol below 25°C. Error bars represent the standard error of the estimated $\mu_{\text{max}}$. The error bar was not given except one point at 35°C because their standard errors are so small (Table V).

Figure 3. Temperature dependence of the half-saturation coefficient ($K_{\text{Lac}}$). The activation energies were 47 KJ/mol above 25°C and ~52 KJ/mol below 25°C. Error bars represent the standard error of the estimated $K_{\text{Lac}}$.

Figure 4. Temperature dependence of the cell yield coefficient ($Y_{\text{ext, Lac}}$). The highest cellular yield was observed around optimum temperature for growth. Error bars represent the standard error of the estimated $Y_{\text{ext, Lac}}$.

reasons: (1) no yeast extract was used and (2) cell-associated EPS was not considered in yield calculation. However, temperature did not affect stoichiometry of microbial sulfate reduction with exception of the cellular synthesis. These results suggest that a system temperature affects only the rates of microbial reaction but not stoichiometry.

Nutrient Requirements. D. desulfuricans was cultured at a constant dilution rate of 0.2 h⁻¹ at 35°C and at various ammonium nitrogen and phosphorous concentrations to determine the effects of those concentrations on activity and growth. Responses of fractional lactate utilization rate, expressed as [(Sᵢ−Sᵢ+Sᵢ)], to changes in the phosphorous and nitrogen concentrations were presented in Figures 5 and 7. The effluent lactate concentrations increased from 0.0 mg–lactate L⁻¹ at C:P=3:1 to 113.3 mg–lactate L⁻¹ at C:P=4500:1. The effluent phosphorous concentration at C:P=430:1 was still measurable. Thus, at C:P=430:1, medium was low in phosphorous. Above C:P=430:1, the culture shifted from lactate–limited to phosphorous–limited. The fractional lactate utilization rate decreased from 99% at C:N=2.2:1 (w/w) to 85% at C:N=120:1.
and then decreased to 40% at C:N = 230:1 (Figure 7). The effluent nitrogen concentration at C:N = 45:1 was 0.29 mg-N L⁻¹. Thus, the medium at C:N = 45:1 was low in nitrogen. The metabolism shifted from lactate-limited to nitrogen-limited above C:N = 45:1. The metabolism shifted from lactate-limited to phosphorous-limited and nitrogen-limited as evidenced by: (1) significant amounts of lactate in the effluent and (2) lower cell yield. Limiting stoichiometric C : P and C : N ratios (w/w) for D. desulfuricans determined were 400:1–800:1 and 45:1–120:1, respectively. These stoichiometric limiting ratios were much higher than typical aerobic population values, because D. desulfuricans partially oxidizes lactate to acetate and CO₂. Thus, cell production from substrate is approximately 10 times less than in aerobic systems and phosphorous and nitrogen requirements are 10 times less also. Therefore, reduction of SRB activity in an oil field by removing nitrogen and phosphorous may not be reasonable.

The production of EPS by D. desulfuricans increased when phosphorous and nitrogen concentrations became limiting. As a result of increasing in EPS production, cell production decreased by about 50% when both became limiting as shown in Figures 6 and 8. The increase in EPS production may influence injection well plugging of oil reservoir described by Lappan and Fogler⁹. The increase in EPS production also influences biocide performance in industrial water systems because the effectiveness of the biocide treatment is significantly reduced by the protective and highly adsorptive nature of the EPS¹⁰.

Figure 5. Response of lactate oxidation ([S - S]/S) by D. desulfuricans to change in the phosphorous concentration: D = 0.2 h⁻¹, temperature 35°C. The limiting C : P ratio is in the range 400:1 to 800:1.

Figure 6. Change in cell and EPS carbon yield coefficients with changing C : P ratio. Error bars represent the standard deviation triplicate measurements.

Figure 7. Response of lactate oxidation ([S - S]/S) to change in the carbon (C): nitrogen (N) ratio: D = 0.2 h⁻¹, temperature = 35°C. The limiting C : N ratio (w/w) is in the range 45:1–120:1. Error bars represent the standard error of measurement (n = 2). The error bar is not presented except for two points at 120:1 and 230:1 because the other standard errors are so small (Table IV).

Figure 8. Change in cell and EPS yield coefficients with changing C : N ratio. Error bars represent the standard errors of measurement (n = 2).
**Sulfide Inhibition.** *D. desulfuricans* growing at a constant dilution rate of 0.2 h⁻¹ and at 35°C was exposed to various sulfide concentrations. The pH was strictly maintained at 7.0. Exposure to 150 mg–S L⁻¹ slightly decreased fractional lactate utilization and cellular production as shown in Figures 9 and 10. Sulfide concentration of 280 mg–S L⁻¹ dramatically decreased cellular production and increased EPS production. Cellular carbon production was reduced in half by a sulfide concentration of about 230 mg–S L⁻¹. The fraction of EPS carbon increased with increasing total sulfide concentration. After exposure to 600 mg–S L⁻¹, 50 mL of culture medium was transferred to 450 mL of the fresh culture medium without sulfide, and the cell numbers were monitored to examine the recovery from sulfide inhibition. Cell numbers slowly increased without a lag phase and reached the same cell number as the control. The doubling time of the sulfide–treated culture was approximately one-third of the control. The experimental result indicate that under high sulfide concentrations, SRB activity is strongly inhibited, even though other nutritional and physical conditions are suitable for SRB growth. Also sulfide precipitates Fe, so that Fe may become the limiting nutrient for growth. These results suggest that sulfide effect on SRB growth and activity must be quantitatively determined and incorporated into a comprehensive SRB biofilm model to accurately predict SRB behavior.

**SUMMARY**

The kinetic and stoichiometric data reported above were obtained from pure cultures under well–controlled laboratory conditions. Real environments are much more complex and dynamic; many interactions between SRB and other components(the substratum, other bacterial species, and metabolic products) occur. Future investigations must be conducted with mixed populations containing SRB and in environments which are representative of industrial and natural systems. Comparison with pure culture data will provide information critical to further model development and calibration.

When the nutritional requirements of SRB and the effects of the physico–chemical factors on SRB growth and activity are understood and quantified, a more rational image of SRB behavior in environments can be obtained. This approach will eventually lead to the development of a comprehensive model that will permit more accurate prediction of SRB behavior. It will also permit means of controlling SRB growth and activity in industrial water systems where SRB cause many problems.
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