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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 H_2S (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 H_2S 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 $G^{[2,3]}$, including hemi-calcium lactate (L-lactic acid, SIGMA, No.L-2000) as the sole carbon and energy source. Trace elements and vitamins were added[2,3]. A sterile $Na_2S_2O_4$ solution was added as

a reductant until a vigorously growing culture was established. The details of medium preparation, preculture of microorganisms, and chemostat set up protocol have been described elsewhere previously[3].

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[4]. Phosphorous concentration was measured as orthophosphorous using the modified ammonium molybdate–ascorbic acid method described by Harwood et al[5]. Total dissolved sulfide concentration in the liquid phase was measured using the methylene blue method described previously by Cline[6]. 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[11] to cellular carbon using various factors[3]. 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[3].

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 (μ_{max} and K_s) 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 (μ_{max}) 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 μ_{max} was 104 KJ mol^{-1} in the range 12°C–25°C. By comparison, Nielsen[7] 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 (K_{Lac}) for lactate and the cell yield ($Y_{c/Lac}$) were dependent on temperature as shown in Figures 3 and 4. Similar ranges of μ_{max} and K_s obtained in this study have been reported in the literature. Cappenberg[8] reported μ_{max} and K_s 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
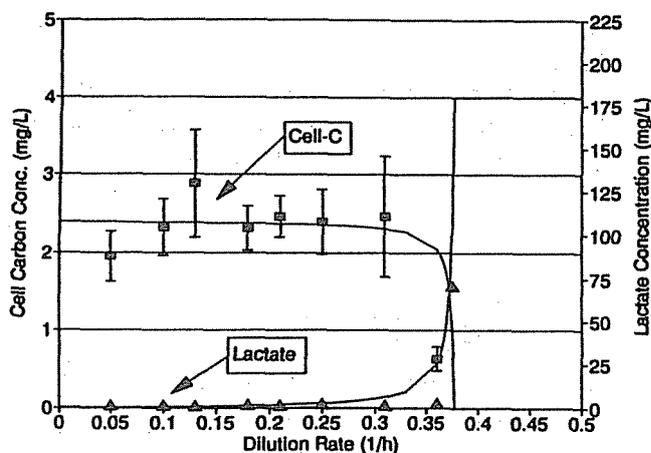

Figure 1. Experimental dependence of the steady state cellular carbon and lactate concentration on the dilution rate at 25°C. The solid lines are drawn using chemostat mass balance equations. The influent lactate concentration was 180 mg/L. Error bars represent the standard deviation of triplicate measurements.

Table 1. Experimentally determined kinetic parameters for *D. desulfuricans* growing on lactate as the sole carbon and energy source (estimated parameter value ±SE).

Temperature (°C)	μ_{max} (h^{-1})	K_{Lac} (mg/L)	$Y_{c/Lac}$ (g/g)	m (g/g · h^{-1})
12	0.059 ±0.001	3.7 ±0.75	0.017 ±0.001	−0.35 ±0.54
25 (run 1)	0.38 ±0.002	1.4 ±0.39	0.025 ±0.002	0.23 ±0.36
25 (run 2)	0.41 ±0.001	10.2 ±0.24	0.025 ±0.004	2.12 ±4.13
35 (run 1)	0.37 ±0.004	2.2 ±0.60	0.024 ±0.006	0.45 ±1.08
35 (run 2)	0.46 ±0.11	3.6 ±2.48	0.036 ±0.004	0.82 ±1.14
43	0.55 ±0.003	10.0 ±1.22	0.032 ±0.001	0.28 ±0.0
48	0.115 ±0.001	6.4 ±0.75	0.023 ±0.001	0.27 ±0.47
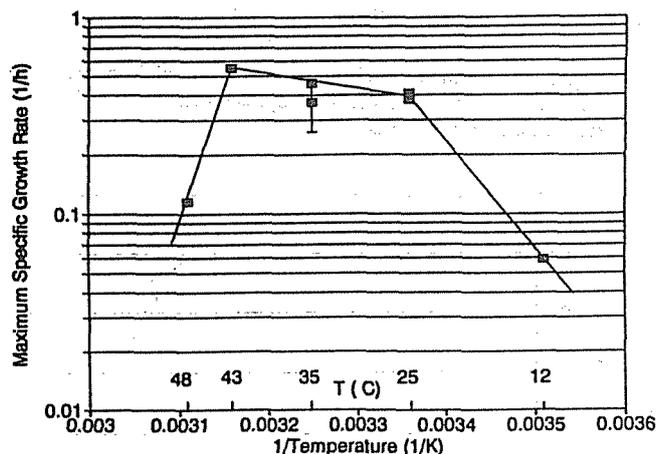
Figure 2. Temperature dependence of the maximum specific growth rate (μ_{max}). The highest μ_{max} of 0.55 h^{-1} was obtained at 43°C. The activation energies for μ_{max} were 14 KJ/mol in the range 25–43°C and 104 KJ/mol below 25°C. Error bars represent the standard error of the estimated μ_{max}. The error bar was not given except one point at 35°C because their standard errors are so small (Table V).
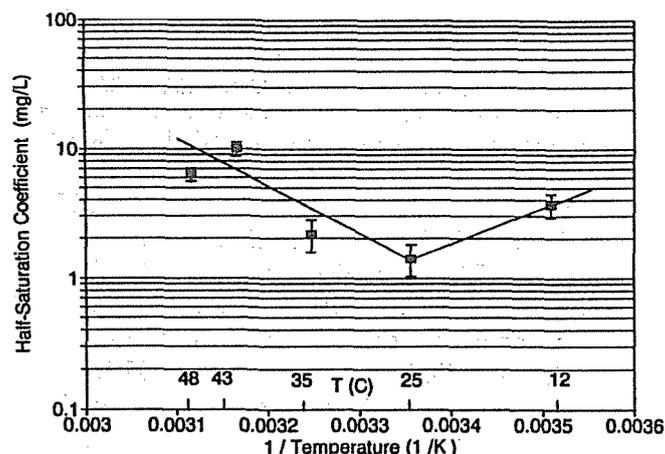
Figure 3. Temperature dependence of the half-saturation coefficient (K_{Lac}). The activation energies were 47 KJ/mol above 25°C and −52 KJ/mol below 25°C. Error bars represent the standard error of the estimated K_s.

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 $[(S_i-S)/S^i]$, to changes in the phosphorous and nitrogen concentrations were presented in Figures 5 and 7. The effluent lactate concentrations increased
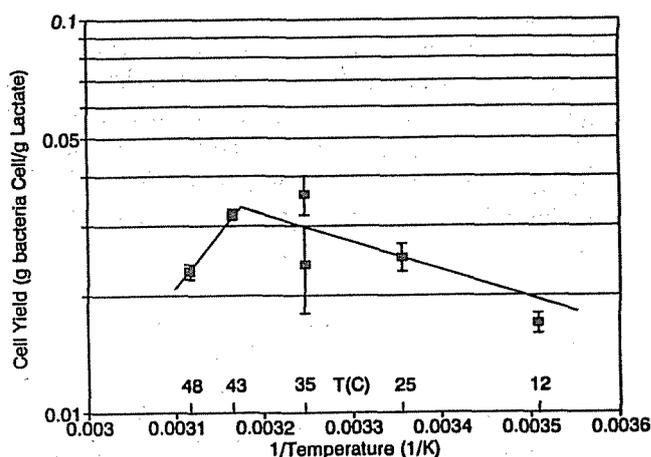

Figure 4. Temperature dependence of the cell yield coefficient ($Y_{c/Lac}$). The highest cellular yield was observed around optimum temperature for growth. Error bars represent the standard error of the estimated $Y_{c/Lac}$.
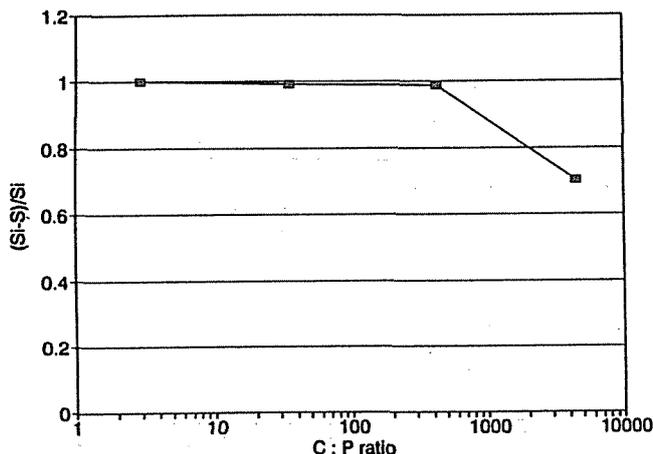
from 0.0 mg–lactate L^{-1} at C:P=3:1 to 113.3 mg–lactate L^{-1} at C:P=4500:1. The effluent phosphorous concentration at C:P=430:1 was still measurable. Thus, at C:P=430:1, medium was low in phosphorous. Above C:P=430:1, the culture shifted from lactate–limited to phosphorous–limited. The fractional lactate utilization rate decreased from 99% at C:N=2.2:1 (w/w) to 85% at C:N=120:1

Figure 5. Response of lactate oxidation $((S_i - S)/S_i)$ 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 wi changing C:P ratio. Error bars represent the standard deviation triplicate measurements.

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 CO_2. 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 Fogler[9]. 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 EPS[10].
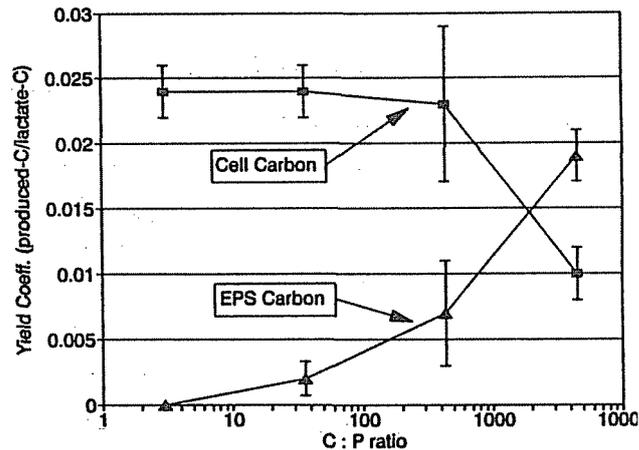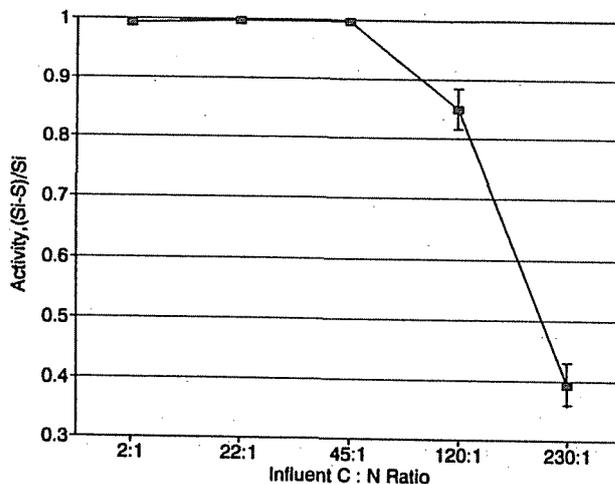

Figure 7. Response of lactate oxidation $[(S_i - S)/S_i]$ to change in the carbon (C):nitrogen (N) ratio: $D = 0.2\ h^{-1}$, temperature = 35°C. The limiting C:N ratio (w/w) is in the range 45:1–120:1. Error bars represent the standard error of measurement ($n = 2$). The error bar is not presented except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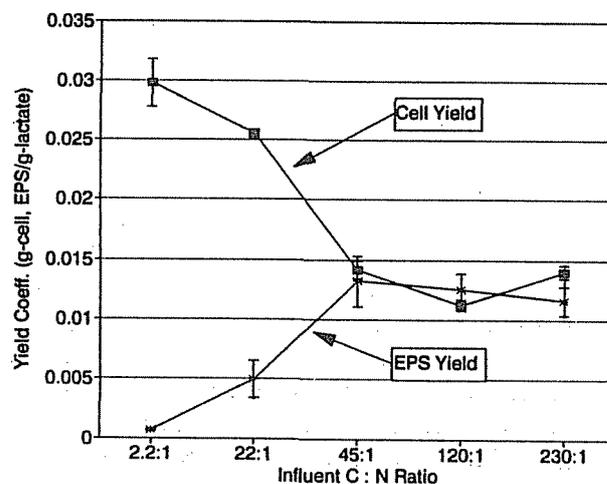

Figure 8. Change in cell and EPS yield coefficients with changing C:N ratio. Error bars represent the standard errors of measurement ($n = 2$).
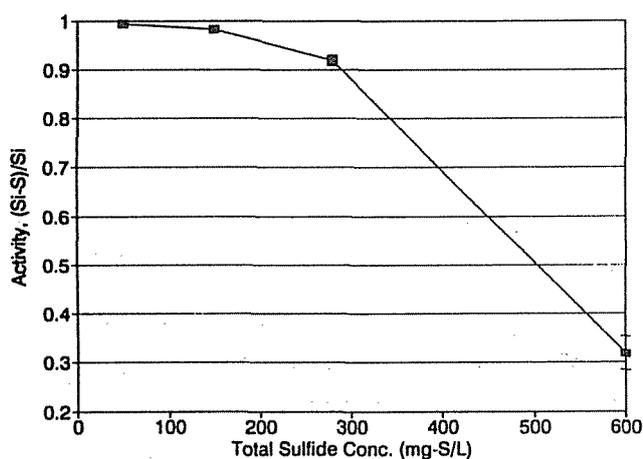
Figure 9. Response of lactate oxidation $[(S_i - S)/S_i]$ to change in the total sulfide concentration: $D = 0.2$ h^{-1}, temperature 35°C, pH 7.0. Na$_2$S · 9H$_2$O was added to obtained desired total sulfide concentration. Error bars represent the standard error of measurement ($n = 2$). The error bar is not presented except for 600 mg TS/L because the other standard errors are so small (Table V).
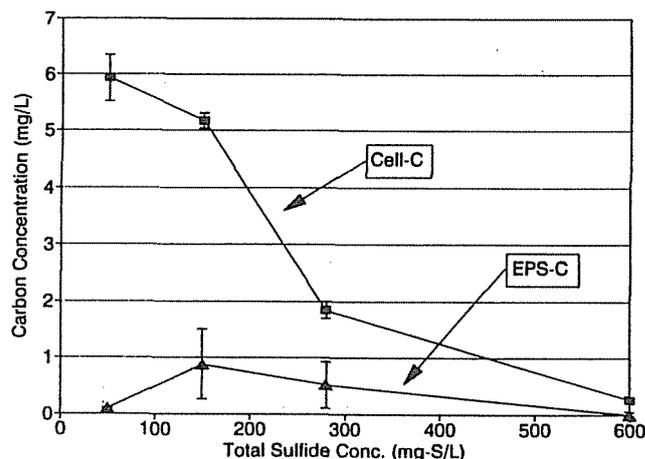
Figure 10. Change in cellular and EPS carbon concentrations with changing total sulfide concentration. Error bars represent the standard errors of measurement ($n = 2$).

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.

REFERENCES

1. Mori, T., T. Nonaka, K. Tazaki, M. Koga, Y. Hikosaka, and S. Noda. 1992. Interactions of nutrients, moisture, and pH on microbial corrosion of concrete sewer pipes. *Wat. Res.* **26**: 29–37.

2. Postgate, J. R. 1984. In: *Sulfate–Reducing Bacteria*, 2nd ed. Cambridge Univ. Press, Cambridge.

3. Okabe, S. and W. G. Characklis. 1992. Effects of temperature and phosphorous concentration on microbial sulfate reduction by *Desulfovibrio desulfuricans*. *Biotechnol. Bioeng.* **39**: 1031–1042.

4. APHA, AWWA, WPCF. 1989. Standard methods for the examination of water and waste water, 1989. 17th ed. Washington, DC.

5. Harwood, J. E., Van Steenderen, R. A. and Kuhn, A. L. 1969. A rapid method for orthophosphate analysis at high concentrations in water. *Water res.*, **3**, 417–423.

6. Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanog.* **14**: 454–458.

7. Nielsen, P. H. 1987. Biofilm dynamics and kinetics during high–rate sulfate reduction under anaerobic conditions. *Appl. Environ. Microbiol.* **53**: 27–32.

8. Cappenberg,Th. E. 1975. A study of mixed continuous cultures of sulfate–reducing and methane–producing bacteria. *Microbiol. Ecol.* **2**: 60–72.

9. Lappan, R. E. and Fogler, H. S. 1990. The effects of bacterial polysaccharide production on formation damage. *Society of Petroleum Engineers*. SPE 19418: 165–172.

10. Ruseska, I., Robbins, J., Costerton, J. W., and Lashen, E. S. 1982. Biocide testing against corrosion–causing oil–field bacteria helps control plugging. *Oil and Gas Journal*. **80**: 253–264.

11. Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.