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Section III
BIOCORROSION

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Anaerobic SRB Biofilms in Industrial Water Systems: A Process Analysis

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I. INTRODUCTION

Biofilm development in natural and industrial water systems depends on the types and concentrations of electron acceptors (e.g., oxygen, nitrate, and sulfate) and electron donors (organic substrates), as well as on environmental factors including temperature, pH, salinity, and fluid dynamics. Development of sulfate-reducing bacterial (SRB) biofilms can be expected whenever environmental conditions such as redox potential or oxygen tension and nutrients are suitable for SRB growth. For example, SRB biofilms may develop in anaerobic bulk water systems, such as in petroleum-producing facilities, but are also found in aerobic bulk water systems such as cooling water systems. In aerobic bulk water systems, anaerobic microniches and/or anaerobic layers exist in biofilms due to depletion of oxygen by aerobic bacterial activity.[1,2] In all natural and industrial aquatic environments, SRB show a pronounced tendency to adhere to available surfaces and to proliferate to form biofilm.[3–5] Because of this sessile mode of growth, bacteria within these biofilms are often undetected by conventional sampling techniques, which analyze bulk fluid conditions. Nevertheless, it is these biofilm (sessile) SRB that are responsible for much of the anaerobic activity in natural and industrial water systems.

At present, quantitative prediction of SRB activity and growth in industrial water systems is essentially impossible because data on rate

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and extent of SRB growth under relevant environmental conditions are not available. Therefore, it is necessary 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. By comparing data from a variety of environments, a conceptual model is first developed which includes factors such as growth substrate limitation, sulfide inhibition, and the effects of attachment. Translation of the conceptual model into a mathematical form requires much more controlled experimentation to determine kinetic and stoichiometric coefficients (e.g., growth rate, yield) and the specific effects of external factors such as temperature on these coefficients.

As will be shown below, the initial conceptual model must include the following:

- Nutrient availability (including electron donor and acceptor, N, P)
- Effect of temperature
- Sulfide inhibition
- Attachment to surfaces (i.e., biofilm vs. planktonic growth)

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 vs. extracellular products). Temperature can affect both kinetics and stoichiometry via phenomena ranging from thermodynamic activity changes through physical enzyme conformation changes. Product inhibition (sulfide) reduces biochemical activity through numerous mechanisms. Finally, attachment to surfaces has been shown to affect bacterial metabolism in a variety of ways, although it is difficult to specify whether these changes are due to a physiological response to attachment or to an altered extracellular environment resulting from diffusion limitations.[6]

A. Effects of SRB on Industry

SRB are very important microorganisms from an environmental and industrial standpoint. The anaerobic corrosion of metals is enhanced by the activities of SRB and is a universal industrial problem where aqueous process fluids contact equipment. The cost related to corrosion is estimated to be $200 billion per year in the U.S.[7,8] The costs to the U.S. Navy with regard to corrosion has been estimated to be $5 billion per year.[8] Extensive sulfide corrosion problems with concrete sewer pipes and wastewater treatment have also been reported.[9]

In the petroleum industry, SRB cause serious problems including corrosion of equipment, plugging of the petroleum formation, and reservoir souring (contamination of petroleum with H_2S).[4,10–13] Sulfide production by SRB increases the sulfur content of the crude oil, which decreases its value and increases refining costs. Costs for downtime, resulting in loss of production, to clean and replace fouled or corroded equipment easily extend to $10 million per day.[14] Hydrogen sulfide production by SRB leads to the corrosion of down-hole drill strings and casings as well as production facilities.[15] SRB growth in seawater injection systems can lead to corrosion as well as contamination of oil and gas with H_2S and viable SRB. Cord-Ruwisch et al.[16] reported that an increase in H_2S was observed during several years of operation at an oil field in northern Germany, and that H_2S formation resulted in plugging of the injection well by FeS flocs. Comprehensive lists of references regarding SRB causing problems in petroleum industries were reported by Postgate,[17,18] Sanders and Hamilton,[15] and Hamilton.[19] Biofilm accumulation also increases capital costs for equipment in power plants. For example, a nuclear power plant had to replace a condenser after approximately 6 years operation because of severe corrosion attributed partially to microbial activity.[14]

B. Control Strategies

1. *Biocides*

Extensive research has been conducted to develop effective biocides with the goal of inhibiting SRB growth and hence sulfide production. For example, in the secondary production of petroleum, injection water used in flooding operations is treated routinely with a biocide (typically glutaraldehyde) to control SRB growth in the injection well, reservoir, and piping.[20] Eagar et al.[21] reported that glutaraldehyde was an effective agent for controlling biofilm growth and activity in test waterflood systems. Also, the results of field study indicated that glutaraldehyde was sufficiently persistent in the distribution system to remain at an efficacious level and to be capable of reducing corrosion to an acceptable rate. Gaylarde and Johnston[22] strongly recommended that biocide test methods for SRB activity should employ mixed sessile SRB in the presence of metal coupons, because sessile SRB on the metal coupon surfaces survived at twice the recommended dose for both biguanide and nitropropanediol.

Biocide addition is often of limited effectiveness, since SRB are associated with other aerobic and anaerobic bacteria in biofilms which coat the surfaces of pipes and other materials. Within these biofilms, SRB are somewhat protected because biocides do not effectively penetrate through the biofilm. All of the reported data have shown that bacteria within biofilms are much more difficult to control with biocides than their planktonic counterparts in these systems.[4,22,23] Thus, biocide treatment may not be an ultimate means to control SRB activity because of rapid microbial regrowth, poor cost effectiveness, and environmental concerns.

The use of ionizing radiation to control SRB activity and growth has recently attracted attention. Ultraviolet radiation was used to kill SRB in injection waters by Ege et al.[23] Gamma radiation was also applied to control SRB at the bottom of the well bores as the water entered the oil reservoir.[24]

2. *Nutrient Removal*

The reduction of the concentration of an essential nutrient (e.g., phosphorous, nitrogen, and/or sulfate) to below the limiting concentration is a potential means of controlling SRB activity, because the essential nutrients control activity and growth of SRB when they become limiting. Maree and Strydom[25] reported the feasibility of microbial sulfate removal from industrial effluent using an upflow packed bed reactor with photosynthetic sulfur oxidation to prevent the emission of sulfide and confirmed the successful performance of the reactor. There is no information in the literature which addresses control of SRB activity and growth by removing required nutrients. Nutrient removal may be a possible means of controlling SRB activity and growth. This would be of benefit both in environmental and economic terms.

3. *Microbial Competition*

Microbial control of sulfide production by SRB using *Thiobacillus denitrificans* has attracted considerable attention lately.[26–28] *T. denitrificans* is an autotroph and a facultative anaerobe which oxidizes sulfide to sulfate using oxygen or nitrate as the electron acceptor. The introduction of viable cells of *T. denitrificans* into environments with SRB has the potential of controlling sulfide production so long as nitrate concentration remains high. The application of this method is an attempt to control sulfide production at or near the water injection well in an oil reservoir. A mutant of *T. denitrificans* (strain F) resistant to glutaraldehyde and sulfide was obtained by McInerney et al.[27] This mutant strain would allow a combined microbial and biocidal (glutaraldehyde) treatment of SRB-contaminated industrial systems. Sublette and Sylvester[29–31] and Sublette[32] have demonstrated that *T. denitrificans* may be readily cultured aerobically and anaerobically in batch and continuous reactors on gaseous H_2S under sulfide-limiting conditions. A microbial process for the removal of H_2S from gases has been proposed based on mixing the gas with a culture of *T. denitrificans*.[29] A practical difficulty is efficiently inoculating *T. denitrificans* into the well-bore area. Further, their activity reproduces sulfate which can then be reduced downgradient. In this case, there would be no net benefit from *T. denitrificans* activity.

The competition for the available electron donors between SRB and methane-producing bacteria (MPB) has also received considerable attention.[33–37] The SRB apparently have a higher affinity (low K_m) for hydrogen and acetate relative to the MPB. Thus, SRB normally dominate both in natural ecosystems, such as freshwater and marine sediments, where methanogenesis was found to be inhibited by the presence of sulfate. Yoda et al.[37] reported that in an anaerobic fluidized bed the methane production rate and MPB biomass decreased after several months of operation at low acetate concentration, whereas sulfate reduction rate increased. On the other hand, MPB were able to form a biofilm faster than SRB at high acetate concentrations, presumably due to the greater ability of MPB to adhere to carrier surfaces than SRB. Hilton and Oleszkiewicz[38] reported that SRB are more sensitive than MPB to the elevated total sulfide concentrations, while both are sensitive to elevated molecular H_2S concentrations. Thus, at high total sulfide concentrations and high pH the MPB should be able to outcompete the SRB for substrate.

4. *Aeration*

Oxygen is the cheapest and most effective inhibitor of SRB activity. If any system can be maintained in an aerated condition, even though the dissolved oxygen concentration is vanishingly small, SRB remain dormant. They are not killed, however, and may become active once anaerobic conditions are reestablished.[39,40] In practice, aeration is of limited effectiveness because SRB are generally associated with other bacteria in biofilms. Oxygen does not effectively penetrate through these biofilms as it is consumed by aerobes.[1] As clearly demonstrated by Lee et al.,[2] SRB activity at the substratum beneath a biofilm can be extensive, even at high dissolved oxygen in the bulk water. Furthermore, introducing oxygen into some industrial water systems increases the extent of pitting corrosion of facilities due to the formation of corrosive compounds such as elemental sulfur and/or pyrite.[2]

II. PROCESS ANALYSIS AND MODELING

SRB biofilm accumulation is a complex phenomenon resulting from several processes occurring in parallel and in series. The rate and extent of these processes, in turn, are influenced by numerous physical, chemical, and biological factors. Thus, a process analysis must be applied to solve biofilm-related problems. The process analysis generally requires (1) development of a conceptual model, (2) development of a mathematical model, and (3) experimental testing, calibration, and validation of the model.

A. Process Analysis

From the viewpoint of a process analysis of a reaction system, the most important components are expressions that quantitatively describe the rate (kinetics) and extent (stoichiometry) of the fundamental processes contributing to biofilm accumulation. Stoichiometry indicates the relationship between the extent of microbial growth and the uptake and production of the chemical species involved. Rate describes how fast the reactions will occur. Both stoichiometry and rate must be known to effectively design and control technical scale processes. The stoichiometric relationships are important since they permit estimation of the rate and extent of biomass and product formation (e.g., hydrogen sulfide) by measuring change in substrate (e.g., sulfate) concentration with time.

A conceptual model describing biofilm accumulation processes would be beneficial in interpreting available historical data and be invaluable in designing future experiments. If the conceptual model could be stated in mathematical terms, a mathematical simulation of biofilm accumulation could be performed on the computer at considerably less expense than laboratory experiments. Furthermore, the expected influence of process variables such as temperature and substrate concentrations could be determined on the computer *prior to* conducting laboratory experiments. The mathematical description of the individual processes could be combined to develop models to extrapolate and generalize experimental results. Many of these fundamental processes have been described mathematically by Characklis and Marshall.[41]

B. Experimental Approach

It is important to proceed in stages, beginning with pure culture work where precisely defined growth conditions and conclusions relevant to those conditions can be made. Understanding of the behavior of single species can then lead to a more rational image of the behavior of a mixed population.

Rate and stoichiometry are often determined in chemostat experiments due to the specified relationships that develop between substrate depletion rate, product formation rate, growth rate, and dilution at steady state. Although batch cultures are sometimes useful for stoichiometric estimates, rate coefficients of microbial sulfate reduction are difficult to measure in a batch culture because pH, sulfide concentration, and limiting substrate are not maintained at the same levels over many generations. On the other hand, chemostat analyses of mixed cultures are made difficult by the tendency of slower-growing organisms to be washed out. Analysis of rate and stoichiometry of processes within a biofilm are frequently complicated by significant mass transfer resistances in the liquid or diffusional resistances within the biofilm.

Nevertheless, biofilm experiments may be necessary in order to understand the changes in stoichiometry and rate which may occur due to physiological changes in the organisms following attachment. Rate and stoichiometry data determined from the pure-culture chemostat experiments (planktonic cells) can be invaluable in designing trials with biofilms (sessile cells) to establish whether or not rate and stoichiometry of the planktonic cells can be used to predict bacterial behavior within the biofilm. Once factors affecting growth and activity of planktonic cells are determined in the chemostat, their quantitative effect on accumulation and activity of biofilms must be determined. Interactions between populations in mixed culture are probably best evaluated at this level as well. Finally, all these data can be incorporated into a model which will permit prediction of SRB behavior in various environments. Sensitivity analyses using the model will identify critical parameters and lead to the development of means to control SRB growth and activity.

III. FACTORS AFFECTING SRB ACTIVITY AND GROWTH

In this section, major environmental factors affecting SRB activity and growth are discussed, with emphasis on rate and stoichiometry.

A. Temperature Effects

Temperature is a major process variable in microbial reactions. Temperature affects the rate of growth and substrate utilization in two ways: (1) the rates of biochemical reaction and (2) the transport rate of substrate into microorganisms. In chemostat studies, the maximum specific growth rate (μ_{max}) of *Desulfovibrio desulfuricans* was relatively constant between 25 and 43°C and dramatically decreased outside this temperature range, with the optimum temperature range between 35 and 43°C.[42] The activation energy for μ_{max} for planktonic cells was 104 kJ mol^{-1} in the range 12 to 25°C. By comparison, Nielsen[43] 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 suspended pure-culture systems is not significantly different from the mixed-population biofilm. At higher temperatures, thermophilic SRB with temperature optima in the 55 to 70°C range would be expected to proliferate. Over a broad range of temperatures, significant population shifts might be expected.

The half-saturation coefficient (K_{Lac}) for lactate and the cell yield ($Y_{c/Lac}$) are dependent on temperature.[42] Because cell yields are low, however (see below), the stoichiometry between lactate uptake and sulfate uptake almost entirely reflects the catabolic activity of the cells. In *D. desulfuricans* suspended culture, this stoichiometry was relatively unaffected by changes in temperature.[42] This latter observation is not surprising in light of the redox stoichiometry (electrons accepted by sulfate, donated by lactate) dictated by catabolism. Thus, with the exception of cellular synthesis, temperature primarily affects the rates of SRB-mediated reactions, but not their stoichiometry.

B. Nutrient Requirements

By operating a chemostat under nitrogen- and phosphorous-limiting conditions, stoichiometric limiting C:P and C:N ratios (w/w) for *D. desulfuricans* were determined to be 400:1 to 800:1 and 45:1 to 120:1, respectively.[42,44] These stoichiometric limiting ratios were much higher than typical aerobic population values, because oxidation of lactate to acetate with sulfate as an electron acceptor yields about only 6% of the theoretical free energy of complete oxidation with oxygen as an electron acceptor, as described below:

$$CH_3CHOCOO^- + 0.5\,SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + 0.5\,HS^- + 0.5\,H^+ \qquad \Delta G° = -14\ kJ$$

$$CH_3CHOCOO^- + 3\,O_2 \rightarrow 3\,HCO_3^- + 2\,H^+ \qquad \Delta G° = -247 kJ$$

Since the biomass yield from lactate with sulfate (Y_{c/Lac^-} = 0.03 [g cell] [g lactate]$^{-1}$)[42] is approximately 6% of an aerobic system ($Y_{x/s}$ = 0.50 [g cell][g lactate]$^{-1}$),[41] phosphorous and nitrogen requirements are expected to be concomitantly reduced. Postgate[18] reported that SRB have the same cell elemental composition as most other bacteria. Typical analytical data for *D. vulgaris* from continuous culture are C, 46.5%; H, 7.2%; N, 12.5%; S, 1.3%; and P, 0.23%. Using these data and the cell yield of 0.03 (g cell)(g lactate)$^{-1}$, limiting C:P and C:N ratios would be 15,000:1 and 270:1, respectively, based on total amounts required for balanced growth. In comparison with the measured ratios above, observed growth limitation occurs at C:N and C:P ratios well below those required merely for balanced growth. It should be noted, however, that

TABLE 1. Stoichiometries of Microbial Sulfate Reduction by a Mixed-Population Biofilm Containing SRB and Planktonic *D. desulfuricans* in Pure Culture

Mixed-population biofilm[7]
$CH_3CHOHCOO^- + 0.48\ SO_4^{2-} \rightarrow 0.24\ CH_{1.4}N_{0.2}O_{0.4} + 0.33\ CH_3COO^- + 0.70^{a}\ S^{-2} +$ 2.1 Carbon products

Planktonic population of *D. desulfuricans*[42]
$CH_3CHOHCOO^- + 0.46\ SO_4^{2-} \rightarrow 0.098\ CH_{1.4}N_{0.2}O_{0.4} + 0.98\ CH_3COO^- + 0.50\ S^{-2} + 0.99\ CO_2$

[a] Sulfide measurements compromised due to FeS accumulation in biofilm.

energy requirements for uptake would be expected to increase when concentrations of these nutrients become extremely low.

The production of extracellular polymeric substances (EPS) by *D. desulfuricans* increased when phosphorous and nitrogen concentrations became limiting.[42,44] As a result of increased EPS production, cell production decreased by about 50% when both N and P became limiting. In biofilm systems, increased EPS production may offset any advantage gained by reducing the biomass production rate. Increased EPS production may influence injection well plugging of oil reservoirs as described by Lappan and Fogler.[45] An 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.[4,46]

The specific growth rate of planktonic *D. desulfuricans* cells was shown to be a function of both sulfate and lactate concentrations in the bulk water.[42,44] The specific growth rate was influenced by the sulfate and lactate concentrations below about 10 and 6 mg l^{-1}, respectively. When sulfate became limiting, the observed specific growth rate and cell yield decreased due to an increase in the maintenance energy requirement. The increase in maintenance energy requirement was attributed to the fact that *D. desulfuricans* might ferment lactate at low levels of sulfate.

When SRB are part of mixed-population biofilms, their activity may become limited by the transport of substrate; SRB rate and stoichiometry may not be accurately reflected by measurements of bulk solution concentrations.[43] Lee[7] reported the stoichiometry of microbial sulfate reduction in a mixed-population anaerobic biofilm containing SRB (Table 1). The stoichiometric coefficient for bacterial cells (0.240) was relatively high compared to that obtained from planktonic monopopulation SRB (0.098). Further, the mixed population system produced only a third of the acetate produced by the monopopulation system. The reduction of net acetate production as well as the increase in biomass production may be attributed to acetate uptake by general anaerobic bacteria. These results are consistent in that a mixed culture would be expected to make better use of available substrate than a pure culture. What is surprising is that the stoichiometric ratio of lactate: SO_4^{2-} was 2:1 in both systems. The stoichiometric ratio between lactate and sulfate may thus be applicable to a variety of mixed-population SRB biofilm systems. Because community interaction influences the fate of carbon sources, however, stoichiometric coefficients based solely on cell yield per unit of organic substrate are not expected to remain constant between systems.

C. Sulfide Inhibition

Sulfate reduction generates hydrogen sulfide that may be inhibitory to the organisms that produce it. Okabe et al.[44] reported that 50% inhibition of lactate utilization by *D. desulfuricans* occurred at approximately 500 mg total sulfide per liter, but that the effect was largely reversible. Reis et al.[47] reported that a batch culture of the genus *Desulfovibrio* was directly inhibited by the H_2S produced and that complete inhibition occurred at approximately 550 mg l^{-1} H_2S concentration. Shimada[48] reported that 100 mg l^{-1} of H_2S inhibited the growth of a mixed SRB population in batch cultures and that no SRB growth was observed at 500 mg l^{-1} of H_2S. The overall effect of sulfide on the microbial sulfate reduction is qualitatively described by some authors.[18,38,49–51] Sulfide toxicity is strongly dependent on pH because molecular H_2S can pass through the bacterial cell membrane.[52] Reis et al.[47] reported an inhibitory effect of sulfide on the specific growth rate of *Desulfovibrio* and found that H_2S is the most inhibitory form of sulfide. Collectively, these results suggest that analysis and prediction of SRB growth and activity should account quantitatively for inhibition and toxicity effects of sulfide, particularly in the nonionized form of H_2S.

D. Effects of Attachment on Activity and Growth

Attachment of SRB to surfaces may result in observed changes in activity and/or growth. It is often unclear whether this change is due to changes in the local environment of the cell or a more fundamental change in cell metabolism. There is some experimental evidence of altered metabolism of aerobic cells in biofilms,[53] but little evidence is available for SRB. For instance, Fukui and Takai[54] reported that free-living *D. desulfuricans* cells produced colonies more rapidly than particle-associated ones, suggesting an attachment-mediated physiological change. For more mature biofilms, the distinction becomes much less clear, as H_2S may accumulate due to mass transfer limitations.[43] The resulting inhibition may produce effects which are indistinguishable from physiological response to attachment, such as development of maintenance requirements (increased activity) and retardation of growth. Thus, an accurate description of the effects of growth on a surface requires a distinction between growth and activity responses. Further study is needed to distinguish between *a priori* physiological response to attachment and responses to microenvironmental changes.

IV. SRB BIOFILMS

A. SRB Biofilm Accumulation Models

There is very little quantitative information available related to the rate and extent of SRB biofilm accumulation. Nielsen[43] studied a mixed-population biofilm containing SRB in an annular biofilm reactor. He reported that the biofilm thicknesses reached 300 to 400 μm, but that biofilm was no longer fully penetrated by sulfate at concentrations less than 100 mg l^{-1}. Typical zero-order volumetric rate constants for sulfate reduction in a biofilm without sulfate limitation were determined to be 5.4 to 8.9 mg SO_4^{2-} cm^{-3} h^{-1} at 20°C. The sulfide production from biofilms grown on domestic wastewater was modeled using biofilm kinetics and found to agree with experimental results.[55] Sulfate limitation in a typical sewer biofilm, with a thickness of 200 to 300 μm, was shown to occur around sulfate concentrations of 3 to 5 mg SO_4–S l^{-1}, but zero-order rate constants for sulfate reduction were also much lower: 0.12 to 0.17 mg SO_4^{2-} cm^{-3} h^{-1} at 20°C. These results are consistent in that diffusion limitations become less important as the reaction rate decreases.

Lee[7] reported that accumulation of a mixed-population biofilm containing SRB was strongly dependent on substrate loading rate. The biofilm thickness easily reached about 1000 μm at high substrate loading (100 mg C m^{-2} h^{-1}), whereas the thickness was about 5 μm at low substrate loading rate (5.4 mg C m^{-2} h^{-1}). Under both loadings, the biofilm was uniformly distributed and very rigid.

Empirical models for the prediction of sulfide production from sewer systems have been published.[55–57] However, the biofilm kinetics and effects of nutritional and physical factors are not taken into account in these models. More quantitative and comprehensive prediction models for sulfide production are necessary for more accurate prediction. To design new wastewater treatment systems to minimize sulfide production or to efficiently control sulfide production in industrial water systems, a reliable method that predicts the sulfide production rate is needed.

V. CONTROL STRATEGIES BASED ON NUTRIENT REQUIREMENT

As all SRB require sources of N, P, and S to support and maintain growth, reduction of one or all of these nutrients to below limiting levels may provide a means to control SRB activity. Laboratory experimental results indicate that the removal of sulfate from industrial water has the greatest potential to control SRB activity. Conversely, phosphorous and nitrogen requirements for SRB growth were so small (below a few mg l^{-1}) that effective removal by existing biological and/or chemical treatments becomes prohibitively costly. In petroleum reservoirs, for example, formation waters contain sufficient quantities of measurable ammonium ions to support balanced growth on the available carbon. Ammonium ion levels as high as 250 mg l^{-1} have been measured.[58] Ammonium levels up to 87 mg l^{-1} were measured in formation waters in the North Sea oil fields operated by Shell Expro.[58] The major source of nutrients in the oil reservoir is likely to be the formation water itself. Thus, removal of nitrogen from the injection water may not be a feasible means to control SRB activity in the oil field. Ironically, scale- and corrosion-inhibitor chemicals typically added to the injection water can enrich the system in C, N, and P.

Sulfate is sometimes absent in the oil reservoir *prior to* introduction of seawater in secondary recovery operations. Removing sulfate from the injection water may therefore be a feasible way to control SRB activity in oil reservoirs. However, biological processing for sulfate removal from seawater or produced water would be hindered by the same operating difficulties that recovery systems currently experience: high sulfide concentrations, a need for a treatment method for the produced sulfide, and high corrosion rates. Further technology development is needed in order to make such a system economically feasible.

VI. SUMMARY

Most of 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 physicochemical factors on SRB growth and activity are understood and quantified, a more rational image of SRB behavior in environments such as oil reservoirs during waterflooding 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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