

Kinetic Analysis of Microbial Sulfate Reduction by *Desulfovibrio desulfuricans* in an Anaerobic Upflow Porous Media Biofilm Reactor

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An anaerobic upflow porous media biofilm reactor was designed to study the kinetics and stoichiometry of hydrogen sulfide production by the sulfate-reducing bacterium (SRB) *Desulfovibrio desulfuricans* (ATCC 5575) as the first step for the modeling and control of formation souring (H_2S) in oil field porous media. The reactor was a packed bed (50×5.5 cm) tubular reactor. Sea sand (140 to $375 \mu m$) was used as the porous media. The initial indication of souring was the appearance of well-separated black spots (precipitates of iron sulfide) in the sand bed. The blackened zones expanded radially and upward through the column. New spots also appeared and expanded into the cone shapes. Lactate (substrate) was depleted and hydrogen sulfide appeared in the effluent.

Analysis of the pseudo-steady state column shows that there were concentration gradients for lactate and hydrogen sulfide along the column. The results indicate that most of the lactate was consumed at the front part of the column. Measurements of SRB biomass on the solid phase (sand) and in the liquid phase indicate that the maximum concentration of SRB biomass resided at the front part of the column while the maximum in the liquid phase occurred further downstream. The stoichiometry regarding lactate consumption and hydrogen sulfide production observed in the porous media reactor was different from that in a chemostat. After analyzing the radial dispersion coefficient for the SRB in porous media and kinetics of microbial growth, it was deduced that transport phenomena dominate the souring process in our porous media reactor system. © 1994 John Wiley & Sons, Inc.

Key words: microbial souring • sulfate reduction • porous media • kinetics • stoichiometry • transport phenomena

INTRODUCTION

Microbially induced oil formation souring has caused a serious problem for the petroleum industry in recent years. Oil formulation souring refers to the generation of hydrogen sulfide (H_2S) in oil fields that have been subjected to water flooding (injection). The cause of souring is believed to be mediated by the sulfate-reducing bacteria (SRB) present in oil reservoirs.^{1,4,5,11,13,18,21} The bacteria are strict anaerobes

accumulated as biofilms on oil reservoir porous media, which reduce sulfate from injection water to hydrogen sulfide. The problem becomes more serious when sea water, which contains a high concentration of sulfate, is used to inject oil reservoirs.^{8,11,21} Unfortunately, in many oil-bearing formations, sea water is used because of economy consideration and sea-nearby locations of most oil reservoirs.

Industrial problems associated with hydrogen sulfide production in oil reservoirs are safety (H_2S gas is toxic in low concentrations), corrosion of oil pipes, and plugging of reservoirs through SRB biomass, which retards the secondary oil recovery. Furthermore, hydrogen sulfide production increases the sulfur content of crude oil, which decreases oil quality and increases oil refining costs.¹² Estimated loss related to SRB mediated souring in oil industries is 1 to 2 billion U.S. dollars per year in the United States.¹⁰

Biofilm accumulation in porous media is the net result of microbial cell adsorption, desorption, growth on surfaces, detachment, and filtration.⁶ The SRB attached to a solid surface were entrapped in polysaccharide gels produced by themselves or "slime-forming" bacteria. Within these biofilms the SRB find themselves in a somewhat protected environment in which biocide and oxygen do not penetrate.^{13,22}

Very little is known about the kinetics of microbial sulfate reduction in oil reservoir porous media. The phenomena in a reservoir are complex from a microbial point of view.⁴ The temperature varies from that of the injection water to that of the formation. The concentrations of nutrients vary widely and depend on the composition of the injection water, the composition of the formation water, partition between water, oil, and the rock, and distribution of microorganisms. It has been reported that rock scavenges hydrogen sulfide since many iron-containing minerals are capable of reacting with H_2S forming iron sulfide, pyrite, or pyrrhotite in reservoirs.^{11,14} In addition, the formation of biofilms on reservoir porous media may change the metabolism of microorganisms and makes predictions difficult.

The goal of this souring project is to determine potential mechanisms for the control of microbial souring in oil-bearing formations. Although souring in an oil-bearing

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formation is complex and involves mixed population SRB with multiple substrates, it is informative to start the studies with a single species SRB grown on single substrate in order to investigate transport and chemical processes as well as microbial processes in porous media. The SRB *Desulfovibrio desulfuricans* was used in this study because it was well characterized.^{15,16} In this study, stoichiometry and kinetics of H₂S production by *D. desulfuricans* in sea-sand porous media was analyzed. The microbial dispersion coefficient in the porous media, an important transport parameter, was estimated.

MATERIALS AND METHODS

Porous Media Reactor System

An anaerobic upflow porous media biofilm reactor was designed to study the kinetics of biofilm plugging and sulfide production by the SRB *D. desulfuricans* (ATCC 5575) as the first step for the modeling and control of formation souring in an oil field with microbial consortia (mixed culture). The SRB was very well characterized in chemostat.^{15,16} The reactor was a packed bed (50 × 5.5-cm) tubular reactor made of polycarbonate and equipped with six sampling ports (Fig. 1). Four of them were equally spaced between the inlet and outlet on one side of the column. The other two were located on the other side of the column near the inlet and were equally spaced with the first two sampling ports of the four with respect to the inlet. Porous media were contained in the reactor by brass gauge mesh over each end of the column. The flow was dispersed by a funnel shaped inside the column head (Fig. 1). Heavy wall butyl rubber tubing (Cole-Parmer; Masterflex neoprene tubing) was used to minimize oxygen flux. Sea sand (140 to 380 μm, Fisher) was used as the porous media.

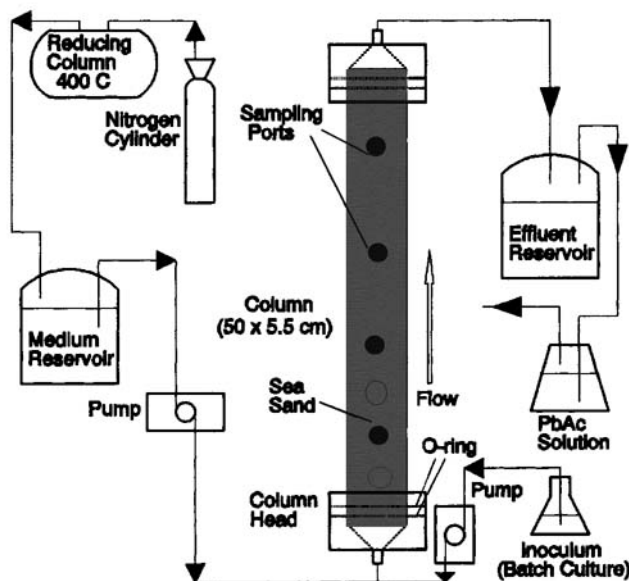


Figure 1. Anaerobic upflow porous media biofilm reactor.

The reactor system before packing with sand particles was sterilized at 121°C (17 psi) for 40 min. Sea sand was pretreated by washing with distilled water, heating at 400 to 500°C for 4 h to remove organic material, and washing with distilled water again before autoclaving. The column was packed with autoclaved sea-sand particles in a laminar flow hood to eliminate microbial contamination. Sand particles were poured (from the top) into the column prefilled with medium. The pumping rate of the medium was controlled such that the liquid level above the sand bed after sand particles settled was 1 to 2 cm. The medium was still pumped through the column even when addition of sand particles was finished (bed height 48 to 49 cm) in order to tighten the bed and condition the column. The final bed height was 47 ± 0.2 cm. The porosity was calculated using the information of final bed volume and the calibrated liquid volume used to pack the bed, which was obtained from the volumetric change of the medium during the packing process. The porosity calculated was 0.37 ± 0.01 .

The medium used was the same as used by Okabe and Characklis¹⁵ with sodium lactate, ammonium chloride, and sodium sulfate as the carbon, nitrogen, and sulfate sources, respectively. The slow continuous nitrogen purge of the medium maintained anaerobic conditions. Traces of oxygen in the nitrogen feed gas were removed by a reducing column containing copper wire maintained at 400°C (Fig. 1). The gas was sterilized by a cotton filter. The temperature was maintained at 25°C. The flow rate of the medium was varied between 45 and 65 mL/h (0.106 to 0.153 h⁻¹ dilution rate with respect to liquid volume) for different runs, which equals 1.89 to 2.74 cm/h of superficial flow velocity. A similar flow regime is used by oil companies for water injection.³ A batch culture of *D. desulfuricans* (log phase) grown in Postgate Medium B¹⁸ with sodium lactate as the carbon source at 35°C was used to inoculate the reactor at the inlet. Volatile H₂S in the effluent was trapped by lead acetate (Fig. 1).

Analytical Methods

Lactate concentration was measured using a Sigma enzymatic kit for lactate. Liquid hydrogen sulfide of filtered samples (0.2 μm, Corning, NY) was analyzed by the methylene blue method.¹⁵ Sulfate was measured using a Dionex ion chromatograph (model AI-450; Dionex Co., San Francisco, CA) with a pulse electrochemical detector (model DX 300) using a Dionex Ionpac AS4A-SC column (2 mm). Acetate was measured using the same ion chromatography apparatus with a Dionex Ionpac AS10 column (4 mm). Total bacterial cells in liquid phase were counted by the acridine orange direct count (AODC).

Total biomass in porous media on different sections of the column was measured by slicing the porous media in the column into 14 sections (each section was 3 to 3.5 cm thick) at the end of the experiments. Each section was incubated at 75°C for 4 h to evaporate water and then

heated in an oven at 400 to 500°C for 4 h to remove organic material. The weight loss after removing organic material was used to determine the total biomass. Each section of the sand bed contained biomass attached to the solid phase and bacterial cells in the liquid phase as well as residual substrate as the organic material. Calculations of surface area available for attachment of bacterial cells in the sand bed and of residual substrate indicated that biomass attached to the solid phase (sand) was dominant. Therefore, the measurement represents the solid phase biomass in each section. The biomass in each section was expressed as milligrams of biomass per gram of sand.

RESULTS

A total of three reactor runs were conducted to investigate kinetics and stoichiometry of microbial hydrogen sulfide production in sea-sand porous media. Flow rate, inoculum size, and feed substrate concentration were varied among the three runs (Table I) to evaluate experimental trends and reproducibility of data. It should be noted that in all runs sulfate was in excess (900 mg/L sulfate-S) while substrate (carbon) was limiting (130 to 530 mg/L) in order to obtain interpretable kinetic data of substrate utilization.

Kinetics of Souring in Porous Media

The time course of souring in the porous media reactor is shown in Figures 2a and b. Lactate, sulfate, and hydrogen sulfide levels as well as pH in the effluent were monitored. Lactate was not consumed significantly during the first 3 days after inoculation (Fig. 2a, run 1). Lactate was depleted and hydrogen sulfide appeared in the effluent after 7 days. The pH in the effluent stabilized around 6.6 (feed pH 7.0). A "pseudo" steady state was reached 10 days after inoculation in terms of the chemical species (Fig. 2a). The SRB cell count in the effluent was increasing throughout the time course of the experiments (Fig. 2b; runs 1 and 2). The pseudo-steady state concentrations of lactate, sulfate, and hydrogen sulfide in the effluent for run 1 were around 1, 800, and 57 mg/L, respectively (Fig. 2a).

The initial indication of souring (hydrogen sulfide production) was visually observed as the appearance of well-

separated black spots, precipitates of iron sulfide (medium contained Fe^{2+}), in the sand bed (Fig. 3a, run 1). These initial black spots in the reactor indicate the growth of initially attached microorganisms in the nearby region. After 7 days, the blackened zones expanded radially and upward through the column, indicating the transport of hydrogen sulfide and/or the movement and growth of SRB. New spots also appeared and expanded into cone shapes, indicating the slow development of more attached SRB (Fig. 3b). The black spots expanded (Fig. 3c) and the entire column turned black ultimately.

Analysis of the pseudo-steady state column shows that there were concentration gradients for lactate and hydrogen sulfide along the column (Figs. 4 and 5; run 1). Lactate measurements for two successive days (days 19 and 20) at the pseudo-steady state were made and good agreement was obtained (Fig. 4). Sulfate and H_2S measurements for three successive days (days 18, 19, and 20) were made and the mean and standard deviation for each point were presented (Fig. 5). The results indicate that most of the lactate was consumed at the front part of the column (Fig. 4). Approximately 100 mg/L sulfate-S was consumed while only 57 mg/L H_2S -S was recovered in the effluent (Fig. 5).

Measurements of SRB biomass on the solid phase (sand) and in the liquid phase along the column are presented in Figures 6 and 7, respectively (runs 1 and 3). The measurements indicate that the maximum concentration of SRB biomass in the solid phase resided at the front part of the column while the maximum in the liquid phase occurred only slightly further downstream. This was probably caused by simultaneous attachment and detachment of SRB in the porous media reactor. The SRB biofilm biomass in run 3 was higher than that in run 1 (Fig. 6). This was caused by a higher feed substrate concentration as used in run 3 (Table I).

Stoichiometry of Sulfide Production in Porous Media

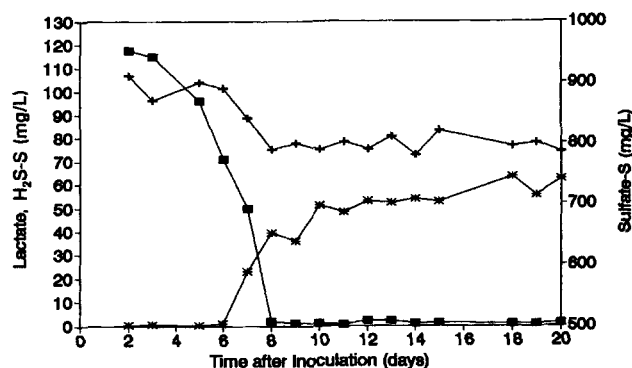
The stoichiometry derived by Okabe and Characklis¹⁵ for hydrogen sulfide production in a chemostat for *D. desulfuricans* (ATCC 5575) with the same ranges of operating conditions shows that approximately 0.5 mol hydrogen

Table I. Stoichiometry of hydrogen sulfide production by *D. desulfuricans* (ATCC 5575) in sea-sand porous media.^a

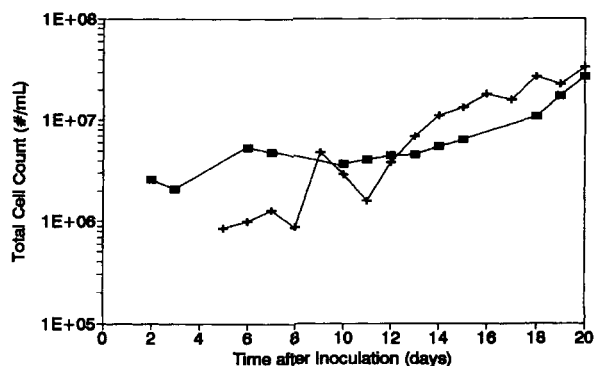
Experiment	Feed lactate concentration (mg/L)	Flow rate (mL/h)	Inoculum size (mL)	Effluent lactate (mg/L)	Effluent H_2S -S (mg/L)	Effluent acetate (mg/L)	Molar ratio	
							Lactate: H_2S	Lactate:acetate
Chemostat ^b							1:0.485 ± 0.015	1:0.89 ± 0.09
Run 1	130	65	200	1.2 ± 0.2	60 ± 3.1	—	1:1.31 ± 0.07	—
Run 2	170	55	50	12.2 ± 0.9	45 ± 1.4	16.2 ± 2.9	1:0.802 ± 0.025	1:0.154 ± 0.028
Run 3	530	45	90	20.5 ± 1.2	146 ± 2.1	—	1:0.806 ± 0.011	—

^a Effluent data are measurements at pseudo-steady state. Means and standard deviations shown for the study represent measurements from 3 successive days ($n = 3$) at pseudo-steady state.

^b Chemostat experiments with the same strain of organism under the same ranges of operating conditions.^{15,16}



(a)



(b)

Figure 2. Time course of souring in sea-sand porous media reactor. (a) Effluent lactate, sulfate and hydrogen sulfide concentrations (run 1: ■, lactate; +, sulfate; *, hydrogen sulfide). (b) Effluent cell concentration (■, run 1; +, run 3).

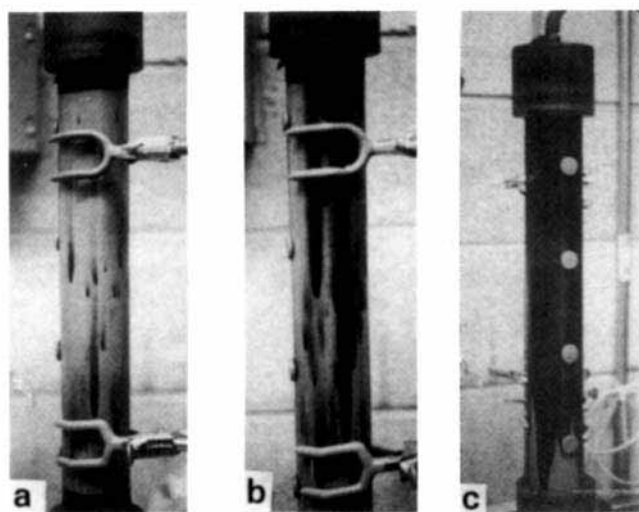


Figure 3. Souring in sea-sand porous media as indicated by appearance of black spots (FeS precipitates, run 1): (a) 4 days after inoculation; (b) 7 days after inoculation; (c) 14 days after inoculation.

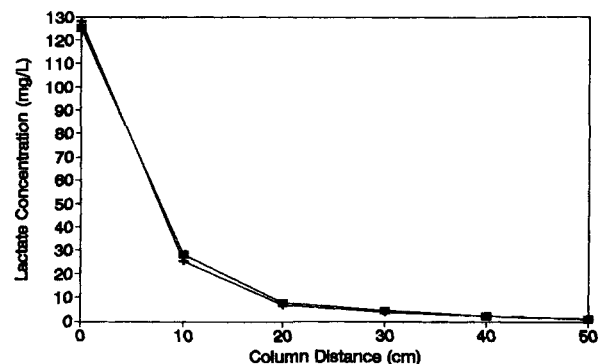
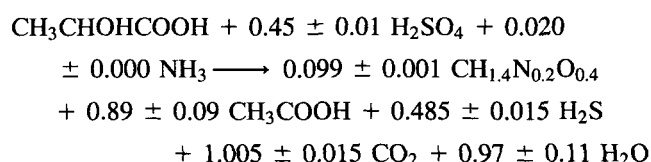


Figure 4. Lactate concentration gradient along sea-sand porous media reactor at pseudo-steady state (run 1: ■, day 19; +, day 20). Column distances 0 cm and 50 cm indicate the inlet and the outlet of the column, respectively.

sulfide and 0.9 mol acetate are produced from 1 mol lactate consumed, as



where $\text{CH}_{1.4}\text{N}_{0.2}\text{O}_{0.4}$ indicates bacterial cells. The stoichiometry measured in the porous media for the three runs is shown in Table I. The calculation was based on liquid-phase measurements. The counterpart of H_2S precipitated as FeS could not be obtained since FeS continuously accumulated throughout the column. Variations of flow rate and feed substrate in the range tested did not affect the overall stoichiometry. A high inoculum size appeared to increase the hydrogen sulfide production with a higher molar ratio of hydrogen sulfide to lactate obtained.

The results show that the molar ratios of lactate consumed to hydrogen sulfide produced were $1:1.31(\pm 0.07)$, $1:0.802(\pm 0.025)$, and $1:0.806(\pm 0.011)$ for runs 1, 2, and 3, respectively (Table I). Since hydrogen sulfide precipitated

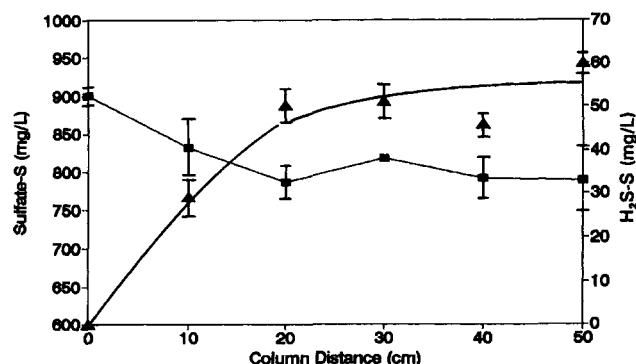


Figure 5. Sulfate and hydrogen sulfide concentration changes along sea-sand porous media reactor at pseudo-steady state (run 1: ■, sulfate; ▲, hydrogen sulfide). Means and standard deviations represent measurements from 3 successive days ($n = 3$) at pseudo-steady state. Column distances 0 cm and 50 cm indicate the inlet and the outlet of the column, respectively.

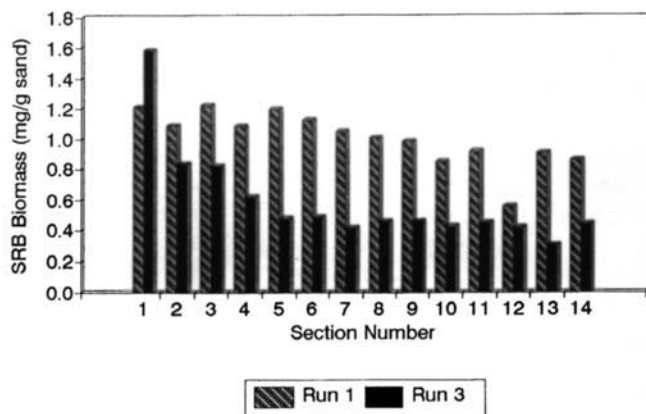


Figure 6. Solid-phase (sand) SRB biomass distribution along the column (runs 1 and 3). Porous media in column was sliced into 14 sections (3 to 3.5 cm thick/section) after experiments were done. Section 1 and section 14 represent the inlet end and the outlet end, respectively.

was not taken into account, the actual stoichiometry regarding hydrogen sulfide production could have deviated more from that in a chemostat. The molar ratio of lactate consumed to acetate produced in run 2 was $1:0.154(\pm 0.028)$. The results indicate a marked deviation of the stoichiometry in porous media from that found by others in a chemostat.

Radial Dispersion Coefficient of SRB in Porous Media

Souring in porous media is a process combining SRB growth and transport phenomena, especially the transport of microorganisms which reduce sulfate to hydrogen sulfide. Therefore, the radial dispersion coefficient for the SRB in porous media was investigated. It was found that radial transport in the upflow tubular reactor dominates in terms of transport phenomena, as can be seen from Figure 3, which shows a slow radial dispersion process (slow process is limiting).

The order of magnitude of the radial dispersion coefficient for the SRB can be estimated from the radial

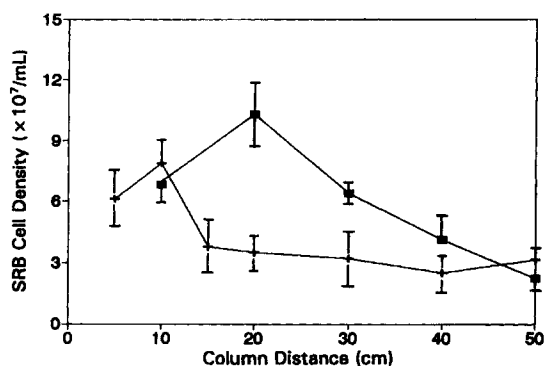


Figure 7. Liquid-phase SRB cell density along the column (■, run 1; +, run 3). Means and standard deviations represent measurements from 3 successive days ($n = 3$) before end of experiments (pseudo-steady state). Column distances 0 cm and 50 cm indicate the inlet and the outlet of the column, respectively.

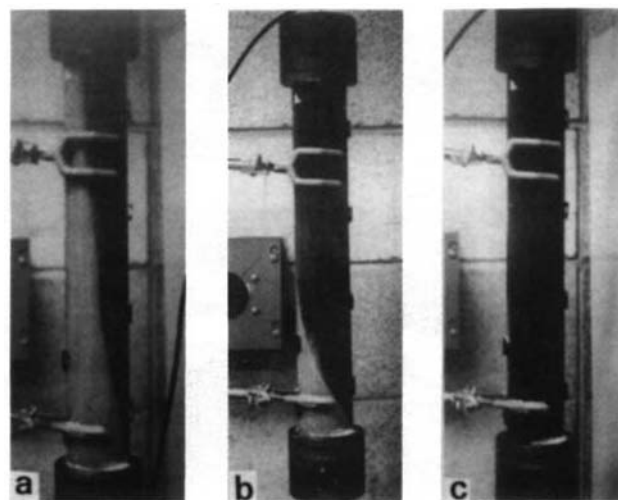


Figure 8. Radial expansion of growth of SRB in sea-sand porous media (run 2): (a) 16 days after inoculation; (b) 21 days after inoculation; (c) 27 days after inoculation.

expansion of growth in porous media (Fig. 8, run 2). The porous media reactor was inoculated such that the inoculum concentrated on one side of the inlet and entered the reactor (Fig. 8). The radial expansion of the black area (FeS) indicates the growth of SRB. No bacterial cell was detected from the "white" areas in the column, as indicated by sampling from the nearby sampling ports (bottom of column; Fig. 8). As SRB continuously grew and the black zone expanded, bacterial cells and consumption of substrate were detected at the sampling ports. Montgomery et al.¹³ also demonstrated that the expansion of black spots in Berea sandcores indicated the dispersion of SRB in the porous media.

The order of magnitude of the radial dispersion coefficient for the SRB was determined using the radial transport equation

$$\frac{\partial X}{\partial t} = D_m \frac{\partial^2 X}{\partial r^2} + \frac{D_m}{r} \frac{\partial X}{\partial r} \quad (1)$$

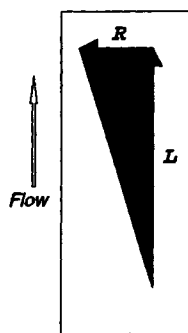
where X , r , and D_m represent concentration of the SRB, the radial coordinate, and the radial dispersion coefficient for the SRB in porous media, respectively. Orders of magnitude of the terms in Eq. (1) are

$$\frac{\partial X}{\partial t} = O \left[\frac{\Delta X}{t^*} \right] \quad (2)$$

$$D_m \frac{\partial^2 X}{\partial r^2} = O \left[D_m \frac{\Delta X}{R^2} \right] \quad (3)$$

$$\frac{D_m}{r} \frac{\partial X}{\partial r} = O \left[D_m \frac{\Delta X}{R^2} \right] \quad (4)$$

where O represents order of magnitude and t^* is the process time taken to reach the radial dispersion of distance R . In the illustration of Figure 9, $t^* = L/\text{superficial flow velocity}$. Substituting Eqs. (2) to (4) into Eq. (1) yields the order of magnitude of radial dispersion coefficient (D_m) for



$$t^* = L/(\text{superficial flow velocity})$$

$$D_m = O[R^2/t^*]$$

Figure 9. Calculation of the radial microbial dispersion coefficient in porous media (D_m) based on radial expansion of microbial growth.

the SRB in porous media as

$$D_m = O \left[\frac{R^2}{t^*} \right] \quad (5)$$

In Figure 8a, microorganisms radially diffused an average distance of 1.5 cm (R) measured at the outlet end when fluid moved from the inlet to the outlet, which took 20.8 h (t^*). The order of magnitude of the radial dispersion coefficient for the SRB in porous media was calculated to be 2.6 cm²/day. The order of magnitude was confirmed by run 1 in which the radial expansion of several black spots (Fig. 3) was monitored and the order of magnitude of D_m was calculated.

DISCUSSION

Porous Media Kinetics

Formation souring in an oil reservoir is a complex process. Transport and chemical processes (reaction, adsorption, etc.) as well as microbial processes are involved. Material balance calculations using data from Figs. 6 and 7 for run 1 and data for the cell size and density of *D. desulfuricans*¹⁵ indicate that each milliliter of liquid phase in the first three sections of the column contained approximately 9.151×10^{-6} g dry biomass, whereas the corresponding solid phase covered by the 1 mL liquid contained a dry biomass of 4.899×10^{-3} g. Hence, the reduction of sulfate to hydrogen sulfide in porous media is primarily carried out by SRB biofilms on porous media.

Due to a higher substrate concentration, cells attached to the front part of the column grew faster than those in the other parts and a higher buildup of biomass was observed in the area (Fig. 6). Most of the substrate was depleted at the front part of the column (Fig. 4). The observations are consistent with results from several other investigators. In their studies of microbially enhanced oil recovery using *Bacillus* strain 47 and a Berea core reactor, Raiders et al.²⁰ found that the plugging was localized at the inlet and outlet

faces of the cores and was attributed to microbial biomass production at the inlet face and biogas accumulation at the outlet face. Taylor and Jaffé²³⁻²⁵ and Taylor et al.²⁶ investigated the permeability reduction in a sand porous medium (ASTM C-190, Soil Test, Inc.), which was caused by growth of sewage and sludge bacteria. They reported that most of the substrate was consumed at the front part of the sand reactor, where biomass plugging occurred. They attributed the high permeability reduction and a thicker biofilm found at the front part of the sand column to high substrate utilization and biofilm growth in the region. The way biofilm plugs up in porous media will affect the method to be used to mitigate the biofouling problem. For example, biocide can be applied through drilling bores in a water injection process if front plugging were the case.

Plugging will ultimately reduce porosity^{19,20,28} and decrease hydraulic conductivity.²³⁻²⁶ In the present experiments, however, no reduction in flow rates was observed. This was probably due to the short-term operation (20 to 30 days) with a high permeability porous media column (porosity 0.37), as compared to the low permeability cores^{19,20,27,28} and long-term operations (up to 365 days)²³⁻²⁶ used by the other investigators. It should be noted that biotransformation in porous media is a dynamic process. In the present experiments, only "pseudo" steady state was reached; the cell count in the effluent was increasing throughout the experiments. This indicates that biofilms were still growing and cells were detaching from the biofilm. The extent of biomass buildup at the front would have been intensified and the gradients of the substrate, sulfate, and sulfide concentrations along the column would have become steeper if the experiments had been continued.

Cells in liquid phase in a section of the porous media reactor consist of detached biofilm cells in the section and growth of detached biofilm cells from the previous section(s). The slight lag of the maximum concentration of SRB in the liquid phase behind the maximum in the solid phase in the column (Figs. 6 and 7) indicates a detachment phenomenon. Similar results were also obtained by Taylor and Jaffé^{23,24} in their studies on the growth of sewage and sludge bacteria in ASTM C-190 sand porous media.

An imbalance between the amount of sulfate-S consumed (100 mg/L) and the amount of H₂S-S produced (57 mg/L, effluent) was observed (Fig. 5). The solubility of H₂S in water is about 60 mg/L,¹⁸ which is similar to the column effluent H₂S concentration. It is therefore possible that H₂S has accumulated as a gas phase in the column. Part of the difference could be explained by scavenging of H₂S as FeS. Since the amount of substrate used (129 mg/L, run 1) can at most produce 80 mg/L of H₂S-S (based on electron balance) if all the substrate has been metabolized to CO₂, the discrepancy is also explained by reactions of sulfate with porous media (possibly carbonate to immobilize sulfate through the formation of calcium sulfate) through microbial processes. Sulfate and lactate did not react with

porous media without microorganisms, which was revealed by the result of incubation of the influent with porous media (data not shown).

Stoichiometry

The stoichiometry in porous media as reported was different from that in a chemostat using the same strain of microorganism under the same ranges of operating conditions. More hydrogen sulfide and less acetate were produced in porous media than that in a chemostat (Table I). No contamination was found when testing for fermentative microorganisms on R2A agar aerobically, and examining for contaminants microscopically for samples from the various sampling ports and the effluent.

It has been reported that immobilization of cells, whether artificial (e.g., entrapment of cells in a gel) or natural (formation of biofilm), changes cell metabolism.^{2,7,9,17,29,30} Zhang et al.³⁰ reported that immobilization of *Escherichia coli* (strain K-12, wild type) cells in alginate substantially altered growth, substrate consumption, β -galactosidase induction, and byproduct formation in both aerobic and anaerobic conditions. In a chemostat, *D. desulfuricans* produced acetate,^{15,16} which cannot be used by the organism.^{5,18} The concentration of acetate measured in the present experiments was much lower than that in a chemostat (Table I). The metabolism in *D. desulfuricans* might have been changed in porous media through the formation of biofilm and might have been switched to produce other metabolite(s) which can be used by the organism to reduce sulfate.

Transport

The order of magnitude of the radial dispersion coefficient for *D. desulfuricans* in porous media, an important transport parameter, was calculated to be 2.6 cm²/day, which was compared to microbial growth parameters. The growth of the organism follows Monod kinetics.¹⁵ The maximum specific growth rate ($\mu_{\max}$) and half-saturation constant (K_S , for lactate) for the organism under the operating conditions were determined to be 0.37 h⁻¹ and 1.4 mg/L, respectively.¹⁵ Clearly, the time scale for microbial transport is days and that for growth is hours. In a porous media reactor of the present size, the transport process is limiting. The analysis is consistent with our experimental observations (Fig. 3). Therefore, transport phenomena dominate the souring process in our reactor system.

CONCLUSIONS

The study reveals complex kinetic behavior of souring in sea-sand porous media, caused by *D. desulfuricans*, which serves as a basis for studies of biotransformations in porous media including souring in an oil reservoir with microbial consortia. The following are concluded: (1) Most of the substrate was consumed at the front part of the column,

where SRB biomass accumulated. Concentration gradients along the column were obtained for the substrate and hydrogen sulfide. (2) The stoichiometry of hydrogen sulfide production in sea-sand porous media was different from that in a chemostat under the same operating conditions. (3) Transport phenomena dominate the souring process in our reactor system.

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