

Kinetic Investigation of Microbial Souring in Porous Media Using Microbial Consortia from Oil Reservoirs

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Microbial souring (H_2S production) in porous media was investigated in an anaerobic upflow porous media reactor at $60^\circ C$ using microbial consortia obtained from oil reservoirs. Multiple carbon sources (formate, acetate, propionate, iso- and *n*-butyrates) found in reservoir waters as well as sulfate as the electron acceptor were used. Kinetics and rates of souring in the reactor system were analyzed. Higher volumetric substrate consumption rates (organic acids and sulfate) and a higher volumetric H_2S production rate were found at the front part of the reactor column after H_2S production had stabilized. Concentration gradients for the substrates (organic acids and sulfate) and H_2S were generated along the column. Biomass accumulation throughout the entire column was observed. The average specific sulfate reduction rate (H_2S production rate) in the present reactor after H_2S production had stabilized was calculated to be 11.62 ± 2.22 mg sulfate-S/day g biomass. © 1994 John Wiley & Sons, Inc.

Key words: microbial souring • sulfate reduction • porous media • kinetics • biotransformation • oil reservoir

INTRODUCTION

Oil reservoir souring costs oil companies in the United States billions of dollars every year.¹⁸ Reservoir souring refers to the production of hydrogen sulfide (H_2S) in oil reservoirs that have been subjected to water flooding (injection). The process is believed to be mediated by sulfate-reducing bacteria (SRB) present in the reservoirs.^{11,12,16,19,25,35} The bacteria are strict anaerobes, accumulated as biofilms on reservoir porous media, which reduce sulfate from injection water to hydrogen sulfide. Major carbon and energy sources used by SRB in reservoirs are short-chain organic acids obtained from oil partitioning, which are probably the products of incomplete aerobic bacterial oxidation of hydrocarbons.^{2,3,8} An oil-bearing formation process is depicted in Figure 1. It includes water injection, the oil-bearing formation, biotransformation (souring), and separation of the formation mixture (oil + water + gas). The produced water separated from the formation mixture is recycled in some applications for water injection of the oil reservoirs.

Problems associated with H_2S production in oil reservoirs are toxicity of H_2S , corrosion of oil pipelines, and plugging of reservoirs through SRB biomass, which retards the secondary oil recovery. In addition, H_2S production raises the sulfur content in the crude oil and increases oil-refining costs. The problem becomes more serious when sea water, which contains a high concentration of sulfate, is used to inject oil reservoirs.^{14,19,25} Unfortunately, in many oil-bearing formations, sea water is used because of its proximity and availability.

Very little is known about the souring process in an oil formation. Two scenarios have been proposed in the prediction of souring, each of which represents an extreme case. In the first case, souring is assumed to occur in the regions of the formation near the injection well (Fig. 1). The extent of hydrogen sulfide production is assumed to be limited by the injection water composition or by product inhibition.²⁹ In applications where the injection is carried out by a mixture of sea water and produced water, this is a reasonable approach, since the injection water would contain sulfate (electron acceptor) from the sea water and organic acids (carbon and energy sources) from the produced water as well as other essential nutrients (mineral salts). However, this scenario does not explain souring activity when the injection water does not meet all the requirements for SRB growth (e.g., only sea water is used for injection).

The second scenario deals with the cases where the injection water will not support growth of SRB. Souring is assumed to occur in a "mixing zone" between the injection and formation waters immediately behind the water-drive front.¹⁹ Formation water, the water initially present in the reservoir, contains organic acids from degradation of hydrocarbons. In the mixing zone, SRB obtain all required nutrients for growth: sulfate from injection water and organic acids from formation water as well as other nutrients. This scenario is reasonable in that it is again based on the assumption that souring will occur whenever the complete slate of essential nutrients is available. This is consistent to some degree with the ability of oil reservoir consortia to produce hydrogen sulfide over a wide range of temperatures and concentrations^{11,24} and with the presence of SRB throughout the formation.²⁵ However, due to reservoir heterogeneities,⁴ a well-defined

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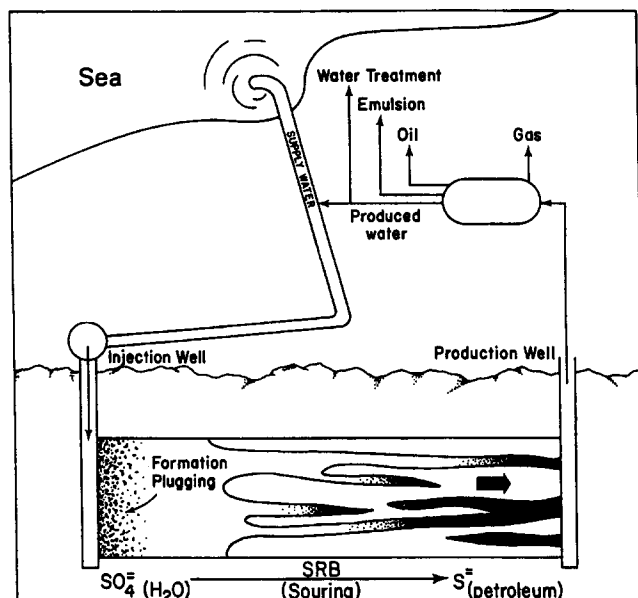


Figure 1. Schematic illustration of an oil-bearing formation process.

mixing zone is hard to obtain. In addition, mixing is far more complex than diffusion across a smooth front, as has been proposed.¹⁹

The prevention and control of souring depends on when and where souring occurs during the formation, on the rate of H_2S production (or sulfate reduction), and on what levels the H_2S reaches. Thus, knowledge of rates and kinetics of souring in porous media is critical for the control of the souring phenomenon. In this study, microbial souring in porous media was investigated using microbial consortia obtained from oil reservoirs. The consortia were grown in an anaerobic upflow porous media reactor operated at a high reservoir temperature using the main organic acids found in reservoir waters as carbon and energy sources.

MATERIALS AND METHODS

Reactor System

An anaerobic upflow porous media reactor was used for the study. A schematic illustration of the reactor system was presented elsewhere.⁹ The reactor was a packed bed (50×5.5 -cm) tubular reactor made of polycarbonate and equipped with four equally spaced sampling ports. Porous media were contained in the reactor by brass gauge mesh over each end of the column. The flow was dispersed by a funnel at each end of the reactor column. A piezometer located near the inlet was used to measure the reduction in intrinsic permeability in the column due to bacterial growth and plugging based on piezometric head change with a constant flow rate. Heavy wall butyl rubber tubing (Cole-Parmer, Masterflex neoprene tubing) was used to minimize oxygen diffusion. 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 . The gas was sterilized by a cotton filter. Volatile H_2S in the effluent was trapped by sodium hydroxide.

Crushed Berea sandstone was used as the porous media. It is a standard material used by many oil companies for in situ investigation of oil recovery.²⁸ The porous media contained sand particles ranging from 35 to $300\ \mu\text{m}$ in diameter with over 50% of particles residing in the $220\text{-}\mu\text{m}$ regime. The ratio of column diameter to grain diameter is greater than 180. According to Schwarz and Smith,²⁶ this is sufficient to ensure that the radial velocity variations due to wall effects are negligible. Sand particles were pretreated by heating at 400 to 500°C for 4 to 5 h to remove organic material and bound water and autoclaved before packing in a column. The sand pretreatment procedure, reactor sterilization, packing of column, and porosity determination were described previously.⁹ The porosity of the sand bed was 0.40 ± 0.02 .

Bacterial Consortia and Cultivation

Produced water from ARCO's Kuparuk (Alaska) fields was used as the microbial consortia. Since production wells are the nearest accessible sites downstream from the point where oil souring occurs, produced waters should contain SRB present in the reservoirs as well as nutrients, sulfate, and sulfide.

Produced waters were concentrated (100 $\times$) on-site, flash-frozen, and stored at -70°C . Previous experiments indicated that the frozen cultures maintained the same microbial activities as on-site produced waters before freezing. The microbial consortia contained mesophilic and thermophilic SRB, as indicated by sulfate reduction and H_2S production in batch cultures of the consortia incubated anaerobically at 35 and 60°C (data not shown). Batch cultures used to inoculate the porous media reactor were prepared by inoculating 1 mL of frozen culture into a 50-mL vial (sealed and filled with purified N_2 gas in the head space) containing 30 mL medium in an anaerobic chamber (model 12356, Coy Laboratory Products, MI). The vials were incubated at 60°C for 7 to 14 days. Three vials with SRB growing in the exponential phase were used to inoculate the porous media reactor at the inlet end.

A high-salinity anaerobic medium suitable for recovery and growth of marine SRB²¹ was used for the batch vials and the porous media reactor. The composition is listed in Table I. Five short-chain acids (formic acid, acetic acid, propionic acid, *n*-butyric acid, and isobutyric acid) were used as the carbon sources since they were the major carbon sources found in the produced water. A feed concentration of 200 mg/L (varying slightly with each preparation) for each of the organic acids was used. The produced water contained significantly different concentrations (620 mg/L acetate, 74 mg/L propionate, 7 mg/L *n*-butyrate, and 1 mg/L isobutyrate), but this is after the SRB activity has occurred, which would have produced acetate while consuming the other organic acids. The feed

Table I. Medium composition (per liter).

Carbon and energy sources	
Formic acid	200 mg
Acetic acid	200 mg
Propionic acid	200 mg
Isobutyric acid	200 mg
<i>n</i> -Butyric acid	200 mg
Base medium	
Na ₂ SO ₄ -S	300 mg
NaCl	20 g
KCl	0.3 g
NH ₄ Cl	0.3 g
MgCl ₂ · 6H ₂ O	3 g
KH ₂ PO ₄	0.2 g
CaCl ₂ · 2H ₂ O	0.15 g
Trace elements ^a	1 mL
Vitamin solution ^a	1 mL
NaHCO ₃ solution ^a	30 mL
Selenite (Na ₂ SeO ₃) solution ^a	1 mL
Dithionite (Na ₂ S ₂ O ₄) solution ^a	1 mL
Sulfide (Na ₂ S · 9H ₂ O) solution ^a	3 mL

^a From ref. 21.

sulfate concentration was in excess at 300 mg/L (in sulfur) to avoid sulfate limitation. High NaCl and high magnesium concentrations simulated the high-salinity conditions in oil reservoirs. Trace elements and other essential nutrients were also contained in the medium.²¹ Na₂S₂O₄ and Na₂S were added in small amounts in order to maintain anaerobic conditions in addition to purging with purified N₂ gas. The pH of the medium was adjusted to 7.0 (±0.2) by HCl and NaOH. A neutral pH has been found in produced waters of several oil reservoirs.^{1,7}

The porous media reactor was maintained at 60°C by incubating in a 60°C incubator (Stalil-Therm Incubator, Blue M Co., IL). High temperatures of >50°C have been found in ARCO's Kuparuk fields as well as several other oil fields.^{7,25} The flow rate of the medium was maintained at 650 mL/day, which equals a superficial flow velocity of 27.4 cm/day. A similar flow regime is used by oil companies for water injection.⁷

Analytical Methods

Liquid hydrogen sulfide was analyzed by the methylene blue method.¹⁰ 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). Both hydrogen sulfide and sulfate measurements were expressed in terms of sulfur content. Organic acids were measured using the same ion chromatography apparatus with a Dionex Ionpac AS10 column (4 mm). Total bacterial cells in the liquid phase were counted by the acridine orange direct count (AODC).¹⁷

Total biomass in different sections of the porous media column was measured by slicing the porous media in the column into 11 sections (each section 4 to 4.5 cm thick) at the end of the experiment. Each section was incubated at

75°C for 4 h to evaporate water and volatile organic acids and then heated in a 400 to 500°C oven for 4 h to remove all organic material. The weight loss after removing organic material was used to determine the total biomass. The total biomass contained biomass attached to the solid phase and bacterial cells in the liquid phase. However, calculations of surface area available for attachment of bacterial cells in the sand bed indicated that biomass attached to the solid phase (sand) was dominant. Therefore, the measurement represents the solid phase biomass (biofilm) in each section. The biomass in each section was expressed as milligrams of biomass per gram of sand.

RESULTS

Time Course of Souring

The time course of souring in Berea sand porous media is shown in Figures 2 and 3. Effluent concentrations of organic acids, H₂S, sulfate, and cells were monitored. Organic acid consumption was not noted in the effluent until 12 days after inoculation (Fig. 2). The initial indication of souring was the appearance of black spots, precipitates of iron sulfide (FeS; medium contained 0.0075 mM Fe²⁺), at the inlet part of the column. Black spots indicated growth of initially attached SRB in the nearby region and production of H₂S. The black spots expanded upward through the column. The H₂S was detected at upper sampling ports of the column after black spots had expanded upward. Hydrogen sulfide was not detected in the effluent until 23 days after inoculation (Fig. 3), when the entire column had been saturated with H₂S and turned black.

Formate, acetate, propionate and isobutyrate were not consumed when souring was initially observed (12 to 20 days, Fig. 2). *n*-Butyrate was consumed very quickly. Formate, propionate, and isobutyrate were utilized, however, after the *n*-butyrate was depleted. Acetate was produced from longer chain organic acids (propionate, iso- and *n*-butyrate). The level of acetate in the effluent increased as the propionate, isobutyrate, and *n*-butyrate were consumed.

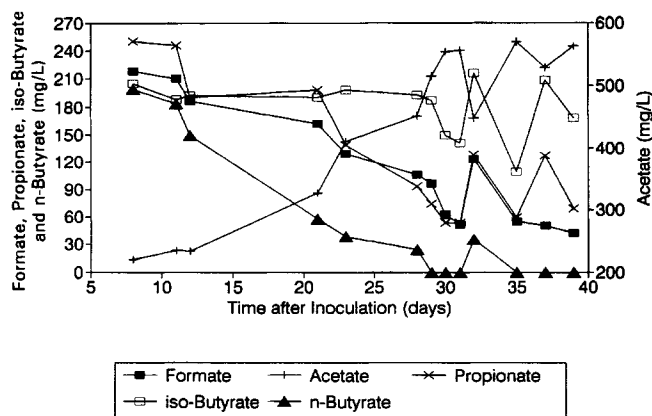


Figure 2. Time course of souring in Berea-sand porous media reactor: changes of effluent carbon source concentrations.

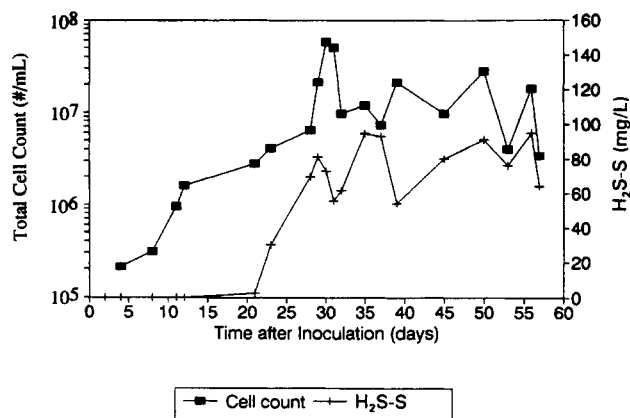


Figure 3. Time course of souring in Berea-sand porous media reactor: changes of effluent H₂S and cell concentrations.

The H₂S was produced throughout this time. The increase in H₂S concentration was accompanied by an increase in cell concentration in the effluent (Fig. 3), indicating active SRB growth in the column because sulfate reduction (H₂S production) is growth associated. The concentration of H₂S in the effluent reached a maximum level around 80 mg/L (in sulfur) 29 days after inoculation (Fig. 3). Concentrations of all measured bioreactor variables (sulfate not shown) became fluctuating after H₂S had reached the maximum level (Figs. 2 and 3). No significant permeability reduction in the column was observed throughout the experiment (57 days), as revealed by the piezometric head change. The pH of the effluent stabilized at 7.0 ± 0.5 throughout the experiment (influent pH 7.0 ± 0.2). Similar results were obtained from a second reactor run with the same consortia under the same operating conditions.

Substrate Utilization and Biomass Accumulation along the Column

Substrate utilization (organic acids and sulfate) and H₂S production along the column were measured by sampling at the various sampling ports in column after H₂S production had "stabilized" (fluctuating within a certain range). A total of three measurements at each sampling port was done by sampling every other day before the end of the experiment. Means and standard deviations ($n = 3$) were reported (Figs. 4 to 6). *n*-Butyrate was utilized very quickly and was not detected at the first sampling port (column distance $z = 10$ cm). Although the standard deviations presented are high, the trends are significant. The results indicate a slow consumption of formate, propionate, and isobutyrate through the column, generating a concentration gradient for each of these organic acids. Relatively higher volumetric organic acid consumption rates (for formate, propionate, and isobutyrate) at $z = 10$ to 30 cm was observed (based on slope of curve). A relatively higher (net) volumetric acetate production rate was observed at the same area (z values of 10 to 30 cm), consistent with consumptions of longer chain organic acids.

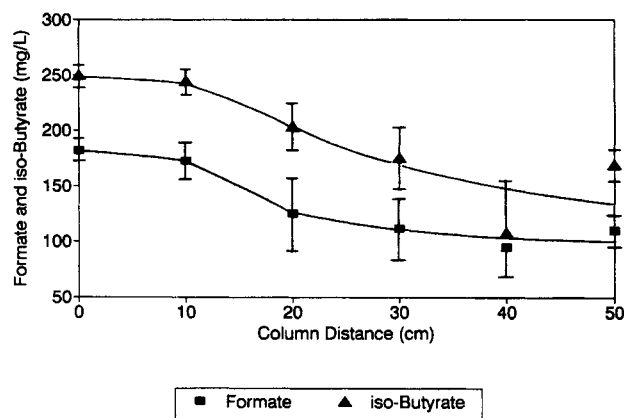


Figure 4. Formate and iso-butyrate concentration gradients in the column after H₂S production had stabilized. Column distances 0 and 50 cm indicate the inlet and the outlet of the column, respectively. Means and standard deviations represent three measurements ($n = 3$) done every other day before the end of the experiment.

Sulfate was reduced and H₂S produced through the column while organic acids were being consumed (Fig. 6). Concentration gradients were generated. It should be pointed out that the reported H₂S concentration represents the liquid-phase measurement. The counterpart of H₂S which precipitated as FeS could not be obtained since the precipitates continuously accumulated in the column; so the actual amount of H₂S produced was higher than measured. A higher volumetric sulfate reduction rate and a higher volumetric H₂S production rate were observed at z values of 0 to 30 cm of the column. This is consistent with the consumption of organic acids observed in this region (*n*-butyrate depleted within the first 10 cm).

Biomass accumulated throughout the column while substrates (organic acids and sulfate) were being consumed (Fig. 7). A high concentration of suspended cells (liquid-phase cells) was found near the outlet end of the column (Fig. 8). This could have been caused by a high detachment of cells from the biofilm nearby (Fig. 7).

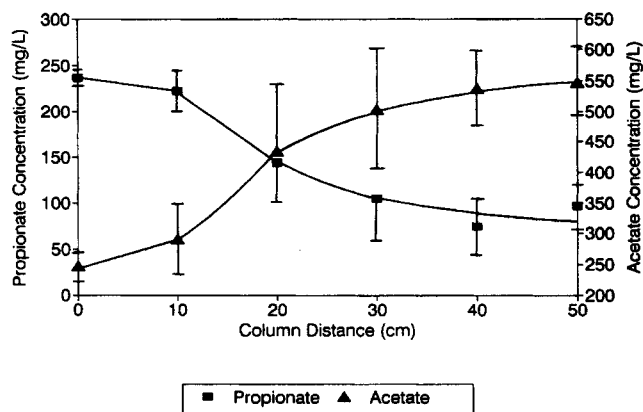


Figure 5. Acetate and propionate concentration gradients in the column after H₂S production had stabilized. Column distances 0 and 50 cm indicate the inlet and the outlet of the column, respectively. Means and standard deviations represent three measurements ($n = 3$) done every other day before the end of the experiment.

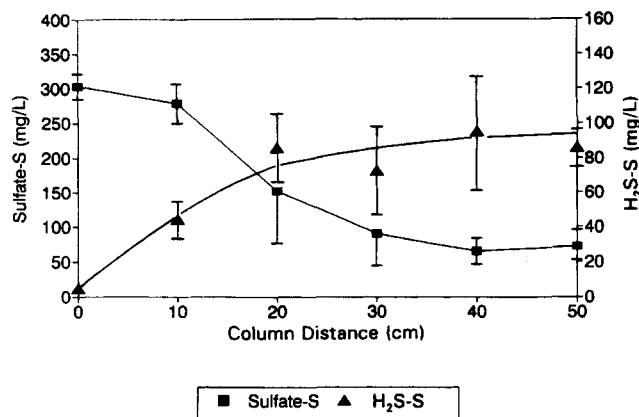


Figure 6. Sulfate and H₂S concentration gradients in the column after H₂S production had stabilized. Column distances 0 and 50 cm indicate the inlet and the outlet of the column, respectively. Means and standard deviations represent three measurements ($n = 3$) done every other day before the end of the experiment.

Specific Sulfate Reduction Rate

The specific sulfate reduction rate in the present reactor system was calculated based on the data obtained from z values of 0 to 30 cm of the column, where most of the biotransformation occurred. The data are:

- Sulfate reduction: 300 ± 15 mg/L – 85 ± 35 mg/L = 215 ± 41 mg/L (in sulfur; Fig. 6).
- Time taken for transport from $z = 0$ to $z = 30$ cm: 25.27 h.
- Void volume: 0.26 L.
- Biomass: 4.57 g (based on Fig. 7).

It is assumed that the amount of sulfate reduced (Fig. 6) was completely used in the biotransformation, as previous experiments indicated that sulfate concentration did not change throughout the column in a sterile reactor system. The specific sulfate reduction rate was calculated to be

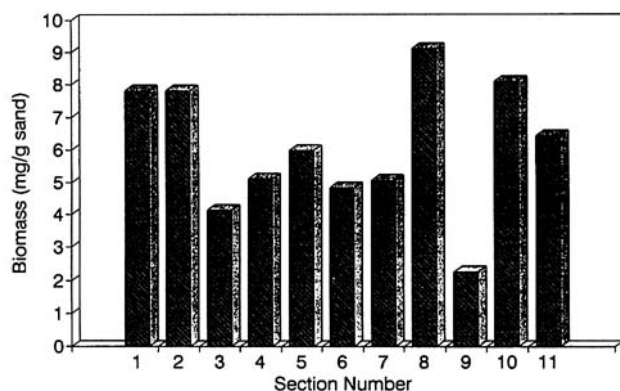


Figure 7. Solid-phase (sand) biomass density along the column. Porous media in the column was sliced into 11 sections (4 to 4.5 cm thick/section; each section contained approximately 120 g sand) after the experiment was done. The biomass density in each section was measured (see Materials and Methods). The density in each section was expressed as mg biomass/g sand. Sections 1 and 11 represent the inlet end and the outlet end, respectively.

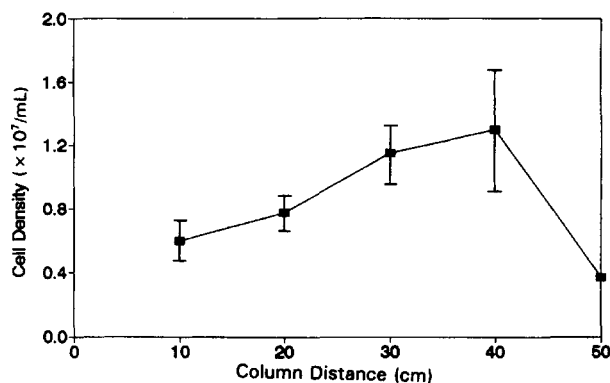


Figure 8. Liquid-phase (suspended) cell density along the column. Means and standard deviations represent three measurements ($n = 3$) done every other day before the end of the experiment.

11.62 ± 2.22 mg sulfate-S/day-g biomass. In the calculation, suspended cells have been neglected since they contributed insignificantly to the biotransformation (to be discussed later).

DISCUSSION

Souring in porous media is a process which combines biotransformation carried out by biofilms (and perhaps suspended cells) and transport phenomena. The accumulation of biofilm in porous media is the net result of microbial cell adsorption, desorption, growth on surfaces, detachment, and filtration.¹³ Calculations were made to approximate the amount of biomass contained in the liquid phase and the amount of biomass attached on the solid phase (sand) for the first six sections of the column (Fig. 7), where the biotransformation primarily occurred. Data from Figures 7 and 8 and a density mean of consortium cells were used for the calculations. The results indicate that the liquid phase in the first six sections contained 7.05×10^{-4} g dry biomass, whereas the corresponding solid phase contained 4.57 g dry biomass (biofilm). The difference is approximately four orders of magnitude. Although biofilm cells have been reported to contain a high amount of extracellular polymeric substance (EPS),^{5,6} the overwhelming difference in biomass indicates the domination of biofilm cells over suspended cells. Hence, the reduction of sulfate to H₂S in porous media is primarily carried out by SRB biofilm cells. A similar conclusion has also been reached in our previous studies on souring using a single-species sulfate-reducing bacterium, *Desulfovibrio desulfuricans* (ATCC 5575), in sea-sand porous media.⁹

n-Butyrate was the best carbon source for the consortia in porous media, since it was the first carbon source consumed during the souring process (Fig. 2). After H₂S production had stabilized, it was found that *n*-butyrate was very quickly depleted in the first 10 cm of the column, whereas the other organic acids had not yet been utilized (Figs. 4 and 5). The other organic acids were utilized when *n*-butyrate was no longer available throughout the rest of the column. Acetate was produced from longer chain acids. The increase in

acetate concentration along the column was concomitant with the consumption of longer chain organic acids (Figs. 4 and 5).

The concentrations of organic acids were fluctuating when H₂S in the effluent reached a maximum level (Figs. 2 and 3). Attachment and detachment of microorganisms in porous media as well as bacterial competition for substrates in the mixed culture system may have resulted in various interactions. This may be reflected by the high standard deviations for the substrates (organic acids and sulfate) and H₂S measured along the column (Figs. 4 to 6) and for the data fluctuation at steady state (Figs. 2 and 3).

Higher volumetric substrate consumption rates (organic acids and sulfate) were found at the front part (*z* values of 0 to 30 cm) of the column (Figs. 4 to 6). This is consistent with results of our previous studies on *D. desulfuricans* and observations from several other investigators. Our studies on souring induced by *D. desulfuricans* in sea-sand porous media indicated that most of the substrate (lactate) was consumed in the first 20 cm of the 50-cm column with the highest volumetric consumption rate observed within the first 10 cm.⁹ Taylor and Jaffe³⁰⁻³² and Taylor et al.³³ investigated the permeability reduction in a sand porous medium (ASTM C-190, Soil Test), caused by growth of sewage and sludge bacteria. They reported that most of the substrate (methanol) 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.

Permeability reductions ranging from 80 to 99.9% for different porous media reactor systems, nutrient feeds, and microbial compositions have been reported by numerous investigators.^{15,20,22,23,25,27,31,33} However, no significant permeability reduction in the column was found in our experiments. A possible explanation is the very low specific growth rate of the consortia, which might have resulted in low cell density in the column during the experimental period. The maximum specific growth rate ($\mu_{\max}$) of the consortia was only $0.04 \pm 0.01 \text{ h}^{-1}$ measured in a batch system at 60°C with the same carbon sources.¹ Another possibility is that a short-term operation (<60 days) and a small flooding volume of medium (<100 pore volumes) had been used in our experiments, as compared to the long-term operation (up to 356 days) and high flooding volumes (>2000 pore volumes) used by these investigators.

Biomass accumulation throughout the entire column was observed (Fig. 7). This is different from our previous results on *D. desulfuricans*⁹ and the observations of several other investigators,^{15,20,22,23,27,30-34} in which biomass plugging at the front part of the column had been reported. An interpretation is the utilization of multiple substrates in our systems. Though a favorable substrate (*n*-butyrate) may have been depleted in the front part of the column, the other substrates were still available throughout the column for growth of organisms (Figs. 4 to 6). This is different from the studies done by the other investigators

and our investigation of *D. desulfuricans*, in which only one substrate was used. Due to a higher substrate concentration, cells attached to the front part of the column grew faster and plugged up the reactor at the front part.

The average specific sulfate reduction rate of SRB in Berea sand porous media after H₂S production had stabilized under the operating conditions was estimated to be $11.62 \pm 2.22 \text{ mg sulfate-S/day g biomass}$. It should be noted that biofilm may still be growing in the reactor column even though the concentration of H₂S had stabilized. The increase in biomass or change in diffusion limitation within the biofilm due to growth may alter the estimation. The value may simply represent an order-of-magnitude estimate in the present reactor system.

CONCLUSIONS

The study investigated microbial souring in porous media under simulated oil reservoir conditions (microbial consortia, carbon sources, nutrients, and temperature). A reservoir is more complex than the present reactor system. However, key issues (rate, kinetics, etc.) which impact the souring process in porous media can be identified with the present reactor system. Relatively higher volumetric substrate consumption rates (organic acids and sulfate) and a relatively higher volumetric H₂S production rate were found at the front part of the reactor column. Concentration gradients of substrates (organic acids and sulfate) as well as H₂S were generated along the column. Biomass accumulated throughout the column presumably due to the availability of substrates in the entire column.

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