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Microbial Control of Hydrogen Sulfide Production in a Porous Medium

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ABSTRACT

The ability of a sulfide- and glutaraldehyde-tolerant strain of *Thiobacillus denitrificans* (strain F) to control sulfide production in an experimental system of cores and formation water from the Redfield, Iowa natural gas storage facility was investigated. A stable, sulfide-producing biofilm was established in two separate core systems, one of which was inoculated with strain F, and the other core system (control) was treated in an identical manner, but was not inoculated with strain F. When formation water with 10 m*M* acetate and 5 m*M* nitrate was injected into both core systems, the effluent sulfide concentrations in the control core system ranged from 200–460 μ*M*. In the test core system inoculated with strain F, the effluent sulfide concentrations were lower, ranging from 70–110 μ*M*. In order to determine whether strain F could control sulfide production under optimal conditions for sulfate-reducing bacteria, the electron donor was changed to lactate, and inorganic nutrients (nitrogen and phosphate sources) were added to the formation water. When nutrient-supplemented formation water with 3.1 m*M* lactate and 10 m*M* nitrate was used, the effluent sulfide concentrations of the control core system initially increased to about 3800 μ*M*, and then decreased to about 1100 μ*M* after 5 wk. However, in the test core system inoculated with strain F, the effluent sulfide concentrations were much lower, 160–330 μ*M*. Nitrate consumption (5 m*M*) and high concentrations (10^7–10^8 cells/mL) of strain F were detected in the test core system. An accumulation of biomass occurred in the influent lines during 2 mo of continuous operation, but only a small increase in injection pressure was observed. These studies showed that inoculation with strain F was needed for effective control of sulfide production, and that significant plugging or loss of injectivity owing to microbial inoculation did not occur.

Index Entries: *Thiobacillus denitrificans*; sulfide; natural gas; souring; gas storage.

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INTRODUCTION

Microbial sulfide production is probably the major cause of souring of reservoirs with temperatures below 80°C *(1)*. The control of the detrimental activities of sulfate-reducing bacteria in the reservoir through the use of biocides is often difficult and expensive. Our approach is to prevent the accumulation of sulfide by coupling the reoxidation of sulfide to sulfate to the reduction of nitrate using a sulfide-tolerant strain of *Thiobacillus denitrificans*, strain F *(2,3)*.

In a previous paper, the efficacy of nitrate addition, with and without inoculation with a sulfide-tolerant strain of *Thiobacillus denitrificans* (strain F), in reducing sulfide levels in an experimental system using cores and subsurface formation water from the Redfield, Iowa natural gas storage facility was reported *(3)*. The addition of nitrate (40 m*M*) to the formation water injected into core systems resulted in lower effluent sulfide concentrations, from an influent concentration of about 170–190 μ*M* to an effluent concentrations of 110 and 3 μ*M*, for core systems operated at hydraulic retention times of 3.2 and 16.7 h, respectively. Inoculation of strain F into the core system operated at a 3.2 h retention time decreased the effluent sulfide concentration from 110 to 16–25 μ*M*. These data suggested that the efficacy of the nitrate treatment may depend on the residence times of the liquids in the core system, and that inoculation with strain F was required to reduce sulfide levels to <20 μ*M* in the core system operated at the shorter hydraulic retention time.

Although these experiments demonstrated the potential of microbial sulfide control, problems with the experimental system prevented any definitive conclusions regarding the need for strain F inoculation. The small differences between the influent and effluent sulfide concentrations prior to nitrate treatment suggested that the sulfate-reducing biofilm was not very active. The difference in hydraulic retention times between the control core system and the test core system made comparisons impossible. Finally, the duration of the project was too short to determine whether strain F established itself in the rock biofilm.

The specific objectives of this work were to determine the effectiveness of strain F vs nitrate treatment alone in controlling sulfide production by a metabolically active sulfate-reducing biofilm, and to determine if the inoculation and growth of strain F in the core system cause plugging and a loss of injectivity.

MATERIALS AND METHODS

Core System

Core material from the St. Peter formation was provided by Northern Natural Gas Co., Redfield, IA. The core material was cut into cylindrical cores, 5 cm in diameter × 15 cm long. The outside surface of each core was coated with epoxy, wrapped with fiberglass, and then coated with another layer of epoxy. A small portion of each end of the cores was cut off with a diamond saw to provide a smooth, flat surface.

Two core systems were constructed as described in Figure 1. All materials used for the core system construction were noncorrosive. Polypropylene was machined to form end plates for each end of the cores. The end plates and cores were encased in epoxy. Nylon or Tygon tubing was used throughout, and all fittings and valves were composed of nylon, polypropylene, or Teflon™. Gages were mounted on gage guards (Plast-O-matic Valves, Inc., series GGM), which consisted of PVC bodies and Teflon™ diaphragms. Sampling ports contained Teflon™-lined gas chromatography septa. The formation water reservoirs were glass, 2-L bottles.

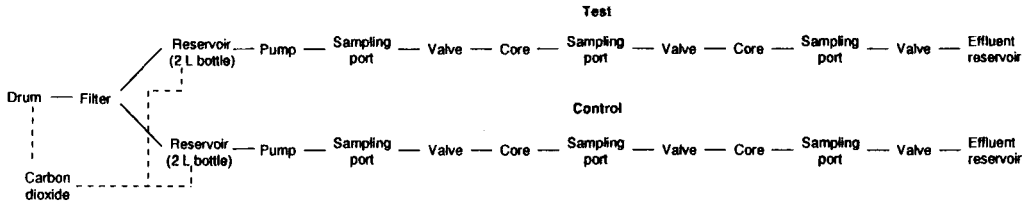

Fig. 1. Core system design.

The core system had a common filter that connected the drum containing formation water to each of the 2-L reservoirs. The 2-L reservoirs and the drum containing formation water were flushed with carbon dioxide for 15 s every min. Two Gilson variable-speed peristaltic pumps were used to supply formation water to the core systems.

Two 55-gal drums of formation water from the Davis #6 well at the Redfield site were delivered to our laboratory by surface freight transportation. The chemical composition of this water has been described previously *(3)*. Acetate (1.8 m*M*) was the only identifiable carbon and energy source for SRB in this water. The drums were stored at room temperature throughout the duration of the study.

The permeability of each core was tested prior to inoculation by connecting individual cores to a flow system that consisted of the peristaltic pump and an inlet pressure gage. A synthetically prepared formation water with the following composition (in g/L) was used: KH_2PO_4, 1.8; $MgSO_4 \cdot 7H_2O$, 0.4; NH_4Cl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.03; $NaHCO_3$, 1.0; and NaCl, 0.8. Three cores had back pressure <2 psig, whereas one core had a back pressure of 20 psig. The latter core was frozen at –20°C overnight to create minute fractures in the rock to increase permeability. After thawing and reconnecting the core to the flow system, it was determined that the back pressure was still high. The core was then flushed with 0.1*N* HCl to dissolve carbonate minerals. This latter treatment reduced the back pressure to <2 psig.

Sulfate-Reducing Enrichment from Formation Water

A mixed population of sulfate-reducing bacteria (SRB) was enriched from the formation water using a medium that contained a bicarbonate buffer, mineral salts solutions, trace metals, 20 m*M* sodium lactate, 40 m*M* sodium acetate, and 20 m*M* sodium sulfate. The composition of the bicarbonate buffer, mineral salts solution, and the trace metals solution are given in Tanner *(4)*. The enrichment medium was prepared anaerobically and dispensed to serum bottles in 20-mL vol. Bottles were autoclaved for 20 min (fast exhaust) before inoculating with 20 mL of formation water. Enrichments were incubated at room temperature or 35°C. The SRB enrichment was transferred to a medium that consisted of Davis #6 formation water with 5 m*M* sodium lactate, and could be repeatedly transferred in this medium without any other nutrient addition. The lactate-supplemented formation water was not prepared by the Hungate technique, but the headspace of the bottle was flushed with N_2. The final concentration of SRB in these enrichment cultures was about 10^7 cells/mL. The final sulfide concentration was about 5 m*M*. The initial sulfide concentration in the formation water was typically 1.2–40 μ*M*. These enrichments were used to inoculate the core system.

Preparation of *Thiobacillus denitrificans* Strain F for Core Inoculation

T. denitrificans strain F had been previously shown to be inhibited by unknown components in formation water from Davis #6 well (*3*). Therefore, *T. denitrificans* strain F was adapted for growth in Davis #6 formation water prior to inoculation of the core system. *T. denitrificans* was anoxically grown in a 2-L fermenter with thiosulfate-mineral-salts medium supplemented with 50 m*M* nitrate to an optical density (460 nm) of 0.8 (*5*). The cells were harvested at 5000*g* at 25°C, and resuspended in the same medium without thiosulfate, and returned to the fermenter. The culture was fed a gas mixture consisting of 1.0% H_2S, 5% CO_2, with the balance being N_2, at a flow rate of 30 mL/min. The culture was incubated at 28°C, and the pH was maintained at 7.0. During this time, all of the H_2S in the feed gas was removed by contact with the culture, and stoichiometric amounts of sulfate accumulated in the culture medium.

After 48 h of operation as described above, 500 mL of the culture were removed and replaced with an equal amount of Davis #6 formation water supplemented with medium components and nitrate, but without thiosulfate. The H_2S-bearing feed gas rate was increased to 45 mL/min. Every 24 h for the next 4 d, an additional 500 mL of the culture were removed and replaced with nutrient-supplemented formation water. The amount of medium removed and replaced each d was then increased to 750 mL until the volume percent of formation water in the reactor reached 93%. Throughout this acclimation period, H_2S continued to be completely removed from the feed gas, and stoichiometric amounts of sulfate accumulated in the culture medium. No elemental sulfur was detected. After a final 24-h period of operation with H_2S gas feed and 93% formation water, cells were harvested by centrifugation at 5000*g* and shipped as a wet cell pellet by overnight delivery to the University of Oklahoma where the core system was operated.

Establishing an Active SRB Population in the Core Systems

Each core system was flushed with 1 mL/min of formation water for 72 h followed by inoculation with 200 mL of an SRB enrichment grown in formation water supplemented with 5 m*M* sodium lactate and 5 m*M* sodium sulfate. Lactate was the carbon and energy source, and sulfate the terminal electron acceptor for the SRB. After 24 h, the tubing in the core systems had a black coating, indicating the production of iron sulfide. A concentrated solution of lactate and sulfate was then injected into each core through the sampling ports. After 48 h, microscopic examination of fluid in the tubing between the cores in each system showed the presence of a mixed microbial population comprised of rod-shaped bacteria of various sizes. None of the organisms were motile. Enumeration studies showed that the liquid in the tubing of each core system contained about 10^{10} SRB/mL. The core systems were then operated at various feed rates of formation water or formation water supplemented with additional acetate (10 m*M*).

Effect of Nitrate on Sulfide Formation

When the test and control core systems had reached steady state with respect to sulfide formation at a feed rate of 0.1 mL/min with formation water supplemented with 10 m*M* acetate, the effect of nitrate on sulfide formation was investigated. Formation water supplemented with 10 m*M* acetate was injected into each core system for 2 wk. This was followed by a 1-wk period with formation water with 10 m*M* acetate and 5 m*M* nitrate.

Effect of *T. denitrificans* Strain F on Net Sulfide Production

The cell pellet of *T. denitrificans* strain F described above was suspended in 75 mL of anoxic formation water (boiled and allowed to cool with an 80%N_2:20%CO_2 gas phase) amended with 10 m*M* sodium acetate and 5 m*M* sodium nitrate. Twenty-five milliliters of this suspension were injected into the test core system via syringe into each of the three sampling ports. The proximal and distal valves of the test core system were closed after inoculation. After 48 h, the flow of formation water with acetate and nitrate was resumed at 0.1 mL/min. Microscopic examination of liquid from the sampling ports showed the presence of gram-negative rods resembling *T. denitrificans* along with other rod-shaped bacteria of various sizes. Enumeration studies of the effluent of the test core system showed that it contained about 10^3 strain F-like cells/mL after 1 wk of operation.

After inoculation, the test core system continued to receive a feed of 0.1 mL/min formation water supplemented with 10 m*M* acetate and 5 m*M* nitrate for 5 wk. The control core system received a feed of 0.1 mL/min formation water with 10 m*M* acetate for 3 wk and then with an additional supplement of 5 m*M* nitrate for 2 wk.

After 5 wk of operation under these conditions, the amount of sulfide production in the control core system was lower than expected based on the amount of sulfate and acetate available in the influent. This suggested that sulfide production may have been nutrient-limited. At this time, the feed to both core systems was changed to formation water supplemented with KH_2PO_4 (0.1 g/L), NH_4Cl (0.5 g/L), and 5 m*M* sodium lactate and 10 m*M* nitrate. After 5 wk of operation with this nutrient-supplemented formation water with lactate, the nitrate concentration was increased to 20 m*M* until the conclusion of testing.

Analytical

Samples for chemical analysis were collected three times a week. Sulfate, nitrate, nitrite, and thiosulfate were measured with an HPLC equipped with a Vydac column (250 × 4.6 mm, phase 3021) and a conductivity detector. The mobile phase consisted of 1 m*M* phthalate, set to pH 4.9 with a 6% boric acid solution. The flow rate was 2 mL/min. The injection volume was 90 µL. Sulfide was measured colorimetrically by a modified version of the methylene blue sulfide assay as described previously *(6)*.

Acetate was measured with a gas chromatograph equipped with a flame ionization detector and a glass column (6 ft, 1/4-in od, 2-mm id) containing GP 10% SP-1200/1% H_3PO_4 matrix. The oven temperature was 130°C, the injector temperature was 170°C and the detector temperature was 175°C. The carrier gas (He) flow rate was 30 mL/min. One-milliliter volume of samples and standards were acidified with 200 µL of a 25% phosphoric acid. Injection volume was 1 µL. All samples were centrifuged for 3 min at 12,000*g* before chromatographic analysis.

ENUMERATION

SRB, acid-producing bacteria (APB), and strain F and strain F-like organisms (denitrifying thiobacilli) were enumerated by the end-point dilution method once a week. Sampling and transfer inoculations were done via syringe. SRB were esti-

mated using (API)-RST medium of Tanner (4). Positive tubes were indicated by the formation of a black precipitate. APB enumerations were done using an anoxic medium containing 0.5% purple broth base (Difco, Detroit, MI) and 1% glucose. Acid production was demonstrated by the change in the pH indicator, bromcresol purple, from purple to yellow. Strain-F was enumerated using the medium of Sublette and Sylvester (5).

RESULTS

Baseline Operation

When formation water without any supplementation was injected into the core systems, the highest effluent sulfide concentration, 185 μ*M*, occurred when the flow rate was 0.1 mL/min. At this flow rate, the concentration of SRB in the effluent was 10^6 cells/mL and the concentration of APB was 10^6–10^7 cells/mL. The differences between the influent and effluent sulfate and acetate concentrations were 0.5 m*M* and 1.5 m*M*, respectively. Faster flow rates (0.2–1 mL/min) gave effluent sulfide concentrations about equal to influent sulfide concentrations (50–100 μ*M*), indicating no net sulfide production. Thus, the system was operated at a flow rate of 0.1 mL/min for the remainder of the study. No organisms capable of growing autotrophically with thiosulfate as the electron donor and nitrate as the electron acceptor were observed in the influent or the effluent of either core system at any of the flow rates used.

Storage of the formation water at room temperature resulted in a decrease in the acetate concentration from 1.8 m*M* to below the detection limit. When this formation water was injected in the core system, little or no sulfide production occurred; the influent and effluent sulfide concentrations were similar, about 90–125 μ*M*. To stimulate sulfide production in the core systems, 10 m*M* sodium acetate was added to the formation water prior to injection into the core. With the addition of 10 m*M* acetate to the formation water, the effluent sulfide concentrations in each core system were similar, and higher than the influent sulfide concentrations (< 90 μ*M*), ranging from 220–270 μ*M* (Table 1). Effluent sulfate concentrations ranged from 4.5 to 5.1 m*M*. Sulfur recoveries were good; 95–107% of the sulfur injected into the core systems as sulfate and sulfide was recovered in the effluents as these two compounds. A 10-fold difference between influent and effluent SRB counts was observed in each core system. Influent and effluent APB counts of both core systems varied from 10^6–10^7 cells/mL. On one occasion, a few organisms (10 cells/mL) capable of growing autotrophically with thiosulfate and nitrate (strain F-like organisms) were detected in the effluent of the control core system.

Effect of Nitrate on Sulfide Formation

Formation water with 10 m*M* acetate and 5 m*M* nitrate was injected into each core system. No consumption of nitrate was observed. The influent and effluent sulfide concentrations averaged 60 and 223 μ*M*, respectively, comparable to the concentrations observed prior to nitrate addition (Table 1). Levels of SRB and APB bacteria remained unchanged, and no strain F-like organisms were detected. Nitrate addition also had no effect on influent and effluent sulfate levels. These results indicated that the two core systems did not contain large numbers of microorganisms capable of using nitrate.

Table 1
Effect of Nitrate on Sulfide Production Prior to Inoculation with Strain F

Treatment[a]	Core system	Sample location	Sulfide[b] μM	C.V.[c]	Sulfate mM	%S	Nitrate mM	SRB cells/mL	APB cells/mL	Strain F cells/mL
Acetate	Control	Influent	70	58	4.9		–	10^6	10^7	0
		Effluent	224	8	4.5	97	–	10^7	10^7	0
Acetate and nitrate		Influent	65	7	4.9		5	10^6	10^7	0
		Effluent	229	3	4.8	103	5	10^7	10^6	0
Acetate	Test	Influent	70	34	4.8			10^6	10^6	0
		Effluent	247	11	4.7	106	–	10^7	10^6	0
Acetate and nitrate		Influent	54	22	4.9		5	10^6	10^6	0
		Effluent	216	4	4.8	102	5	10^7	10^7	0

[a]Reservoirs were amended with 10 m*M* sodium acetate or 10 m*M* sodium acetate and 5 m*M* sodium nitrate.
[b]Values reported are means of three samples taken over a 7-d period.
[c]Coefficients of variation.

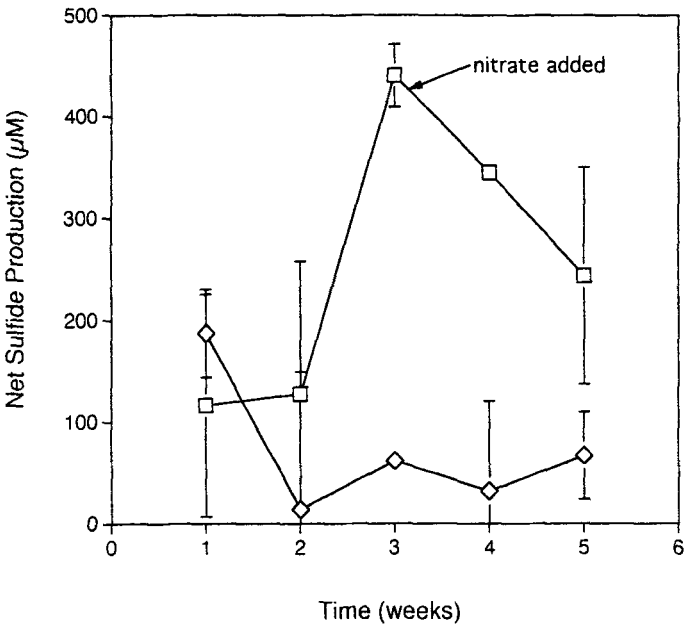

Fig. 2. Effect of strain F inoculation on sulfide production when formation water with 10 m*M* acetate and 5 m*M* nitrate was used. The control core system received acetate-amended formation water without nitrate for the first 3 wk. Control core system, □; test core system, ◇. Standard deviations included.

Effect of *T. denitrificans* Strain F on Net Sulfide Production

After the test core system was inoculated with *T. denitrificans* strain F, the effluent sulfide concentration decreased from 220 μ*M* to an average of about 100 μ*M*, and remained at these levels for several weeks (Fig. 2). The effluent sulfide in the test core system decreased 79% compared to that observed prior to inoculation, and the effluent sulfide concentration was 85% lower than that of the control core system when both received nitrate. During the first 2 wk of injection with formation water without nitrate, the sulfide production in the control core system was about 120–130 μ*M* (Fig. 2). During the third week, sulfide production increased to 440 μ*M*. After nitrate was added to the formation water, the amount of sulfide produced by the control core system decreased to 245 μ*M* by the fifth wk. After nitrate injection, influent and effluent sulfate concentrations in the control core system averaged 4.9 and 4.8 m*M*, respectively, vs 4.9 and 5.1 m*M*, respectively, from the test core system. Sulfur recoveries from both core systems ranged from 95 to 109% during this period, indicating that all of the sulfur injected into the cores as sulfide and sulfate was recovered in the effluent as these two compounds.

Approximately 1.2–1.8 m*M* nitrate was consumed by the organisms present in the test core system. No nitrate consumption was observed in the control core system. The amount of nitrate consumption in the test core system was much larger than the expected amount of nitrate consumption (390 μ*M*) based on the difference between the effluent sulfide concentrations of the two core system (210 μ*M*) and the stoichiometry given in Eq. 1.

$$5HS^- + 8NO_3^- + 3H+ \rightarrow 5SO_4^{-2} + 4N_2 + 4H_2O \quad (1)$$

Numbers of SRB in the effluent of the control and test core systems were 10^7 and 10^8 cells/mL, respectively. About 10^7 cells/mL of APB were detected in the effluents of both core systems. The numbers of strain F-like organisms in the effluent of the test core system increased from 10^3 cells/mL immediately after inoculation to 10^4 cells/mL 2 wk after inoculation. No strain F-like organisms were detected in the effluent of the control core system. These data clearly show that the inoculation of strain F was more effective than the addition of nitrate alone in not controlling sulfide production.

Effect of Nutrient Addition on Sulfide Production

The maximum amount of sulfide produced by the control core system (345 μ*M*) was much lower than that expected (5 m*M*) based on the amount of sulfate and acetate present. This suggested that sulfide production in the cores may have been nutrient-limited. To stimulate sulfide production, the formation water with 10 m*M* nitrate was supplemented with inorganic nutrients and lactate was used in place of acetate. Figure 3 shows that these changes greatly stimulated sulfide production in the control core system. Very high effluent sulfide concentrations, ranging from 3060–3780 μ*M*, were observed. In the test core system, which contained strain F, sulfide production remained low with effluent sulfide concentrations <330 μ*M* (Fig. 3). This clearly demonstrates the effectiveness of strain F in controlling sulfide production. Sulfur recoveries from the test core system ranged from 99–109%, but sulfur recoveries from the control cores were high (123–168%) during the first 4 wk with nutrient-supplemented formation water, indicating that more sulfur compounds were produced than were injected into the system.

For 2 wk after the addition of the above nutrients, additional peaks were present that interfered with the detection of nitrate by HPLC, so the amount of nitrate used during this time could not be determined. During the third wk of injection with the nutrient-supplemented formation water, little or no nitrate consumption was detected in the control core system, whereas 4.9 m*M* nitrate was consumed in the test core system. The difference in the amount of sulfide produced by the control core system compared to that of the test core system during this week was 2800 μ*M*. Based on the stoichiometry given in Eq. 1, the amount of nitrate needed to oxidize this amount of sulfide to sulfate is 4.4 m*M*, which is very close to the observed nitrate consumption in the test core.

The concentration of strain F-like organisms detected in the effluent of the test core system increased from 10^4 to 10^7 cells/mL during the 3 wk after nutrient-supplemented formation water was used. Concentrations of SRB and APB in the effluents of the two core systems were 10^8 and 10^7 cells/mL, respectively.

After the third week of injection of nutrient-supplemented formation water, consumption of nitrate (1.3–2.9 m*M*) in the control core system was observed. Concomitant with this was a decrease in effluent sulfide concentration from about 3800 μ*M* to 1100 μ*M* (Fig. 3). This lower effluent sulfide concentration was still four times higher than the effluent sulfide concentration (250 μ*M*) of the test core system at this time. Growth was observed in some of the thiosulfate/nitrate mineral medium dilution bottles inoculated with effluent from the control core system, indicating the presence of 10^3–10^5 nitrate-using bacteria/mL. Microscopic examination showed that the predominant morphology in these bottles was a motile rod distinctly different

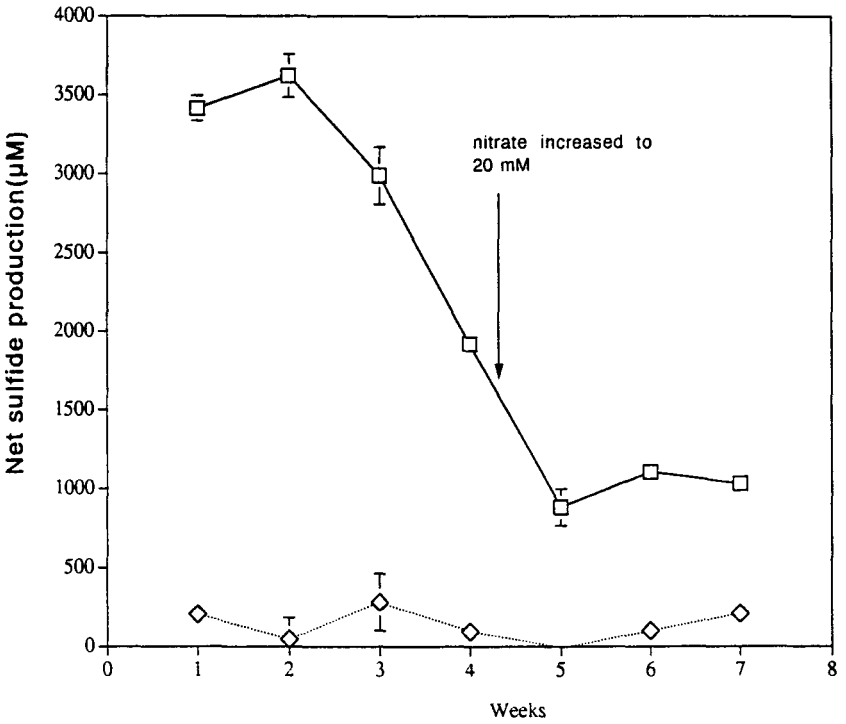

Fig. 3. Effect of strain F inoculation on sulfide production from formation water with lactate, nitrate and inorganic nutrients. Nitrate concentration was increased from 10 to 20 m*M* after 5 wk. Control core system, □; test core system, ◇. Standard deviations included.

from strain F. It should be noted that the numbers of strain F-like organisms detected in effluents of the test core system were always three orders of magnitude greater than the numbers of nitrate users detected in effluents from the control core system.

Increasing the nitrate concentration in the formation water from 10 to 20 m*M* did not markedly affect the sulfide production in either of the two core systems. During this time, the control core system produced about 1000 μ*M* sulfide, whereas the test core system produced 100–200 μ*M* sulfide. Little or no nitrate was consumed in the control core system at this higher nitrate concentration. Also, the numbers of nitrate-using bacteria detected in the effluent of the control core system were very low, about 10 cells/mL. In the test core system, levels of strain F-like organisms remained high, 10^5–10^6 cells/mL, and the amount of nitrate consumed (5 m*M*) was similar to that observed when 10 m*M* nitrate was used. The concentration of APB remained high at about 10^7–10^8 cells/mL in both systems.

The addition of 40 m*M* nitrate to the nutrient-supplemented formation water resulted in high nitrite levels, which interfered with the analysis of sulfide, as was observed in our previous study (*3*). No further work was done at this nitrate concentration.

Plugging

The use of nutrient-supplemented formation water increased the biomass present in the injection lines and on the tubing walls between cores in both core systems. There was an initial increase in the inlet pressure of each core in the two core systems from 1 psig to about 3 psig. However, after several wk of injection of nutrient-

supplemented formation water, the inlet pressures decreased to <2 psig. Increasing the flow rate 20-fold (from 0.1 to 2 mL/min) did not increase the inlet pressure of any of the cores. Thus, little or no plugging occurred during the course of this experiment, even after extended incubations (about 8 mo). The small amount of plugging that was observed was probably owing to the development of the sulfate-reducing biofilm.

DISCUSSION

In our previous work, the efficacy of *T. denitrificans* strain F in controlling sulfide production in the core system at the Redfield site was studied *(3)*. However, several factors complicated the interpretation of our data, and prevented us from obtaining data that clearly showed that inoculation of *T. denitrificans* strain F was required. The two core systems that were operative at the Redfield site at the time of our previous study had different hydraulic retention times, which made direct comparisons between the test (inoculated) and control (uninoculated) systems impossible. A decrease in sulfide concentration from an influent concentration of 170 μ*M* to an effluent concentration of 60 μ*M* in the control core system prior to nitrate addition indicated that a sulfide removal mechanism independent of nitrate addition was operative. Finally, neither of the two core systems had a microbial population that was actively making sulfide. The two core systems used in the current work had excellent injectivity, which allowed both systems to be operated over a range of identical hydraulic retention times, and the current core systems actively produced sulfide. When formation water supplemented with only 10 m*M* acetate was used, 200–300 μ*M* sulfide were produced. When the formation water was supplemented with lactate, ammonium nitrogen, and a phosphorous source, large amounts of sulfide, in excess of 3000 μ*M*, were produced. This provided an ideal experimental system to test the effectiveness of strain F under conditions that simulated actual field conditions as closely as possible.

The data clearly show that inoculation of *T. denitrificans* strain F was needed to control sulfide production effectively, and that strain F was effective in systems that had the potential to produce large amounts of sulfide. When formation water with acetate and nitrate was used, a 78% reduction in effluent sulfide concentration was observed in the test core system inoculated with strain F compared to the control core system. When formation water supplemented with lactate, ammonium nitrogen, phosphorous, and nitrate was used, the effluent sulfide concentration of the test core system was 92% less than that of the control core system. A reduction in effluent sulfide concentration was observed after extended operation (5 wk) in the control core system when nutrient-supplemented formation water and 10 m*M* nitrate were used. However, the effluent sulfide concentrations in the control core system were still very high, 1000 μ*M*, indicating that the addition of nitrate alone was not very effective in controlling sulfide production. Nitrate consumption was detected in the control core effluents, indicating that some nitrate-using bacteria were present. Most likely, these bacteria were SRB capable of using either sulfate or nitrate as the electron acceptor *(7)*, since the predominant organisms in enumeration bottles were morphologically similar to several types of sulfate reducers and the cells were much larger than strain F. The numbers of these nitrate-using bacteria in the control core system effluents decreased to low levels after 20 m*M* nitrate were used.

Our study shows that strain F can be maintained in the system for long periods of time without reinoculation. High concentrations of strain F were detected 3–4 mo after inoculation at the two sampling ports downstream from each core in the test

core system. Growth of strain F in the core system did not lead to additional plugging of the core. The small increase in the inlet pressure that occurred after nutrient-supplemented formation water was used also occurred in the control core system, and, thus, was not a result of the growth of strain F in the core system. The presence of high levels of strain F at the same time that a decrease in the effluent concentrations of sulfide and nitrate was observed indicated that strain F was present and metabolically active.

Our previous study found that, in the core system treated with strain F and nitrate, the effluent sulfate concentration was almost twofold higher than the influent concentration, and that this difference was much greater than the amount expected from the oxidation of the small amount of sulfide present *(1)*. This suggested that sulfur-containing compounds had accumulated within the test core system and were being oxidized to sulfate. In the current study, the amount of sulfur entering the core systems as sulfate and sulfide was recovered as these two compounds in the effluent, except when the control core was initially treated with nutrient-supplemented formation water with 10 m*M* nitrate. This was also the time when high concentrations of sulfide were detected in the effluent, suggesting a period of very active metabolism. The core material used (St. Peter sandstone) is a carbonaceous sandstone that effervesces when strong acid is added to the material (V. K. Bhupathiraju, and M. J. McInerney, personal observation). It is possible that the increased metabolic activity resulted in acid production in the core system, which leached sulfate from the core material. Another possibility is that the microbial population used the sulfate present both in the influent and in the core material for sulfide production. When effluent sulfide concentrations decreased to 1000 μ*M*, close to 100% of the sulfur injected into the core system as sulfide and sulfate were recovered in the effluent as these two compounds.

Some residual sulfide was present in the effluent of the test core system even in the presence of excess amounts of nitrate. There are several possible explanations for this. The residence time of the system may have prevented the complete utilization of sulfide before it left the system. The sulfide detected in the effluent may have been produced at a site near the distal end of the core system where levels of strain F may have been low, or the apparent affinity for nitrate by strain F in biofilms may have been low. Further work would be needed to determine the reason for residual sulfide levels in the effluent.

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