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Evaluation of a microbial method to reduce hydrogen sulfide levels in a porous rock biofilm

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SUMMARY

The efficacy of nitrate addition, with and without inoculation with a sulfide-resistant strain of *Thiobacillus denitrificans* (strain F), in reducing sulfide levels in an experimental system using cores and subsurface formation water from a gas storage facility was examined. The addition of nitrate (40 mM) alone to the formation water injected into core systems operated at hydraulic retention times of 3.2 and 16.7 h resulted in lower effluent sulfide concentrations, from an influent concentration of about 170–190 μM to an effluent concentration of 110 and 3 μM, respectively. A reduction in effluent nitrate concentrations in both core systems indicated the presence of indigenous nitrate-using populations. After strain F was inoculated into the core system operated at the shorter retention time, the effluent sulfide concentration decreased from 110 to 16–25 μM. The effluent sulfate concentration increased, and the effluent nitrate concentration decreased concomitant with the presence of high concentrations of denitrifying thiobacilli in the inoculated core system. The denitrifying thiobacilli detected after inoculation were presumed to be strain F since these organisms were not detected in this core system before inoculation, or in any of the samples from the uninoculated core system. These data suggest 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 μM in the core system operated at a short hydraulic retention time.

INTRODUCTION

Hydrogen sulfide is a toxic and corrosive gas that greatly increases the cost of recovery of oil and natural gas. An increase in hydrogen sulfide concentrations is often noted after a petroleum reservoir has been water flooded to improve oil recovery [9,13]. A major mechanism for hydrogen sulfide production in petroleum/gas reservoirs below 80 °C is believed to be microbial metabolism [9]. Since many petroleum reservoirs have environmental conditions favorable for microbial growth [2], and much evidence exists to support the conclusion that these reservoirs contain active microbial populations [1,7], this suggests that souring may be caused by the inadvertent introduction of some limiting nutrient such as a suitable organic electron donor, or a source of nitrogen or phosphorous during waterflood operations [3,6]. Because of their diverse metabolic properties and widespread occurrence, sulfate-reducing bacteria were thought to be the only agents responsible for microbially induced souring. However, sulfate reducers are not the only organisms found in oil/gas reservoirs that produce sulfide [13]. In fact, the most commonly detected sulfide-producing bacteria, such as *Shewanella putrefaciens*, do not use sulfate as an electron acceptor, but use other sulfur oxyanions. Thus, methods to detect or control souring based solely on methods to detect or control sulfate-reducing bacteria may not be effective in actual field situations.

The detrimental activities of sulfide-producing bacteria can be controlled by the effective use of biocides. The use of biocides is most successful in controlling unwanted activities in surface facilities. However, controlling these activities in the reservoir through the use of biocides is often difficult and expensive. Our approach is to manipulate the ecology of the system so that the terminal electron-accepting process is changed from the reduction of sulfur oxyanions to nitrate reduction [5,8]. Thus, even if sulfide producers are present in the reservoir, the accumulation of the unwanted product of their metabolism, sulfide, is prevented. This can be done by adding nitrate and a sulfide-tolerant strain (strain F) of *Thiobacillus denitrificans*.

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T. denitrificans is an obligate autotroph and facultative anaerobe which, under anaerobic conditions, oxidizes sulfide to sulfate by reducing nitrate to nitrogen (N_2) (Eqn. 1).

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

T. denitrificans strain F is not inhibited by inorganic sulfide concentrations in excess of 1000 μM, which inhibit the growth of wild-type strains of *T. denitrificans* [12]. The presence of *T. denitrificans* strain F prevented the accumulation of sulfide by sulfate-reducing bacteria, both in liquid culture and in Berea sandstone cores [8]. The effectiveness of strain F was due to its ability to grow and use sulfide at levels which were inhibitory to the wild-type strain. Strain F readily grows through sandstone cores suggesting that it could be very useful in the control of sulfide accumulation in situ. Because *T. denitrificans* strain F is a chemoautotrophic bacterium, the addition of organic nutrients would not be needed which would limit the growth of indigenous organisms present in the reservoir. The addition of nitrate inhibits sulfide production in many environments [5]. Thus, a combination of strain F and nitrate could effectively control sulfide accumulation in oil and natural gas formations.

In this study, we investigated the efficacy of nitrate and *T. denitrificans* strain F in controlling H_2S concentrations in an experimental system using cores and water from an underground gas storage facility. A previous study showed that sulfide levels in the formation increased as groundwater infiltrated the formation after the stored gas had been removed [4].

MATERIALS AND METHODS

Formation water

Formation water, i.e., water produced from the subsurface formation, was collected daily from well Davis-6 of the Northern Natural Gas storage field in Redfield, Iowa. The chemical composition of the water is shown in Table 1.

TABLE 1

Chemical composition of formation water collected from the Davis 6 well in the St. Peter formation

Component	Concentration (ppm)
Iron	0.6
Sulfide	9
Chloride	420
Sulfate	450
Phosphate	1.8
Hardness	960
Total dissolved solids	718
Alkalinity	660

Samples were analyzed by BTI in late March, 1989. The pH of the sample was 7.2.

Core system

The core systems used in these experiments were assembled by Bioindustrial Technologies, Inc. (BTI, Grafton, NY) and located in the laboratory on site [4]. One core system was previously used to test the effectiveness of biocide formulations in controlling sulfide levels in the cores. Following the completion of BTI studies, the core system was flushed with formation water at approx. 75 ml/h for 7 days before the experiments described here were initiated.

The core system contained three cylindrical cores of St. Peter sandstone of about 2.5 cm diameter and 7.6 cm length, each of which was mounted in polyvinyl chloride (PVC) tubing. The cores were connected in series to each other using stainless steel tubing and compression fittings (Fig. 1). The intake line of the core system had a 5-μm pore size membrane filter to remove suspended solids from the fluid before injection into the cores. A sampling port was located at the inlet side of each core and the tubing exiting the last core. The porosity of the St. Peter sandstone was 30%. From the porosity of the cores and the volume of the tubing, the fluid volume of the core system was estimated to be 240 ml. The flow rate of fluids through this core system was 75 ml/h, giving a hydraulic retention time of 3.2 h.

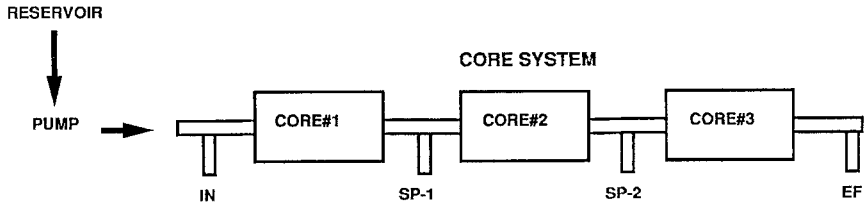

Fig. 1. Schematic representation of the core system located on site at Redfield, IA. IN = influent; SP-1 = sample port 1; SP-2 = sample port 2; EF = effluent.

A second core system constructed in an identical manner as described above was only used to determine the effect of nitrate addition on sulfide production. This core system had not been treated with biocides; because of this, the maximum amount of fluid that could be injected into this core system was lower than that of the first core system. The flow rate of the second core system was 14 ml/h, giving a hydraulic retention time of 16.7 h.

Stock cultures

Stock cultures of *Thiobacillus denitrificans*, strain F, were maintained anaerobically in thiosulfate medium as previously described [11]. In this medium, thiosulfate was the energy source, nitrate was the terminal electron acceptor, bicarbonate was the source of carbon, and ammonium ion was the nitrogen source. Stock cultures were transferred every 30 days and stored at 4 °C until used.

Growth of cells for core injection

T. denitrificans strain F cells were grown in thiosulfate medium in 2-l cultures in a B. Braun Biostat M [10,11]. Temperature was maintained at 30 °C and pH controlled at 7.0 by the addition of 6 M NaOH. The culture received a gas feed consisting of 30 ml/min of a mixture containing 5% CO_2, with the balance being N_2 to ensure that the culture did not become carbon limited. When the OD_{460} of the culture medium reached approx. 1.0 (about 10^9 cells/ml), cells were harvested by centrifugation at 5000 × *g* and 25 °C. Cells were then washed by resuspending the pellet in 15 mM phosphate buffer (pH 7.0), and centrifuging the suspension as above. Cells were shipped as a wet pellet by overnight delivery service to the test site. Sufficient medium was used to resuspend the pellet in a 5-l beaker so that the suspension was slightly turbid. The viable cell concentration of the suspension was estimated by the end-point dilution method using the above medium with thiosulfate. Medium that was injected into the core system was not sterilized.

Core experiments

An experiment was conducted to determine whether indigenous microbial populations capable of oxidizing sulfide and using nitrate as the electron acceptor were present in the core system. Formation water supplemented with 40 mM sodium nitrate was injected into each core system. After each 24-h period, a sample was collected from the sample port located upstream of each core and from the tubing exiting the last core. Samples were analyzed immediately to determine the concentrations of sulfide, sulfate-reducing bacteria, acid-producing bacteria, and strain F. The remainder of each sample was frozen and then analyzed for nitrate, nitrite, sulfate, and sulfite at a later date.

Only the core system with a flow rate of 75 ml/h was used for the remainder of the experiments described below. The *T. denitrificans* growth medium without thiosulfate was injected into this core system for 40 h to determine whether the addition of nutrients would stimulate the production of sulfide in the core system. Samples for chemical and microbiological analyses were taken after 24 and 40 h of medium injection.

The core system was then inoculated with strain F to determine the effectiveness of this organism in controlling sulfide levels in a continuous flow system. A suspension of approx. 10^5 viable cells/ml of strain F in growth medium without thiosulfate was injected into the core system for 6 h (about 0.5 l). This was followed by injection of growth medium without thiosulfate for 24 h. This inoculation procedure was repeated once.

Preliminary studies showed that the formation water contained a substance that was inhibitory to the growth of strain F. In order to acclimate strain F to the formation water, a mixture of growth medium and formation water starting with 90% (v/v) growth medium without thiosulfate and 10% (v/v) formation water with 40 mM sodium nitrate was injected into the core system. Every 12 h, the percent of formation water with nitrate injected into the core system was increased by 10% until only formation water with nitrate was injected into the core system. When the fluid mixture injected into the core was 30, 60 and 80% formation water with nitrate, the core system was again treated with strain F. A cell suspension of strain F, prepared as described above, was injected into the core system for 6 h, followed by a 6-h treatment of the respective mixture of growth medium (without thiosulfate) and formation water with 40 mM sodium nitrate. Samples for chemical and microbiological analyses were taken every 12 h.

During the time that the core system received only formation water with 40 mM nitrate, samples from the core system contained a compound that interfered with the detection of sulfide. This has been previously observed during nutrient-limited growth of strain F and results in incomplete oxidation of sulfide or reduction of nitrate (Sublette, K.L. unpublished data). Because of this problem, the core system was treated with growth medium without thiosulfate for 6 h. For the remainder of the experiment, nutrient-amended formation water with nitrate was injected into the core system. The nutrient-amended formation water with nitrate contained 10 mM $NaNO_3$, and (in g/l) KH_2PO_4 (1.8), $MgSO_4 \cdot 7H_2O$ (0.4), NH_4Cl (0.5), $CaCl_2$ (0.03), $NaHCO_3$ (1.0). Samples for chemical and microbiological analyses were taken after each 24-h period.

Microbiological and chemical analyses

The concentrations of denitrifying thiobacilli, sulfate-reducing bacteria, and acid-producing bacteria were estimated used the end-point dilution method. One milliliter of sample was diluted in the respective growth medium. The inoculated bottles were then incubated at 30 °C and checked for growth on a daily basis.

Denitrifying thiobacilli were enumerated using the growth medium given in [11]. Sulfate-reducing bacteria and acid-producing bacteria were enumerated using BTI-SRB medium and BTI-APB medium (Bioindustrial Technologies, Inc., Grafton, NY).

Samples were analyzed for sulfide immediately by methylene blue method using Hach Chemical (Loveland, CO) field kits. Sulfate, nitrate, and nitrite were determined by high-pressure liquid chromatography (HPLC) using an anion-exchange column and a conductivity detector as previously described [8].

RESULTS

The addition of nitrate alone to the formation water which was injected into the core system operated at a hydraulic retention time of 3.2 h resulted in lower effluent sulfide levels (Table 2). Concomitant with the reduction of sulfide was the decrease in nitrate concentrations in the core effluent, suggesting the presence of indigenous microbial populations capable of oxidizing sulfide using nitrate as the electron acceptor. However, no growth was observed in the medium used to enumerate denitrifying thiobacilli, either prior to or after treatment of the core system with nitrate alone, indicating that the core system did not contain chemoautotrophic denitrifiers similar to thiobacilli. The addition of nitrate did not affect the numbers of sulfate-reducing bacteria and acid-producing bacteria. Interestingly, the sulfide levels in the influent and the effluent before the treatments began were similar. This sug-

TABLE 2

The effect of nitrate and inoculation with *Thiobacillus denitrificans* (strain F) on the concentration of sulfide in the core system with a hydraulic retention time of 3.2 h[a]

Additions to formation water[b]	Sample location	Sulfide (μM)	Sulfate (mM)	Nitrate (mM)	SRB (cells/ml)	APB (cells/ml)	Strain F (cells/ml)
None	IN	170	4.8	0.5	10^5	10^3	<10
	EF	160	4.0	0.5	10^5	10^5	<10
Nitrate							
1st treatment	IN	170	1.4	39.0	10^5	10^3	
	SP-1	ND	1.4	28.5	10^7	10^7	
	SP-2	ND	1.4	19.3	10^5	10^5	
	EF	110	1.4	19.2	10^5	10^5	
2nd treatment	IN	190	4.8	39.5	10^7	10^7	<10
	SP-1	ND	1.1	36.1	10^7	10^7	<10
	SP-2	ND	2.5	30.1	10^5	10^5	<10
	EF	110	7.8	21.9	10^5	10^7	<10
Strain F + nitrate + nutrients							
1st treatment	IN	160	2.6	10.6	10^7	10^7	10^7
	SP-1	140	3.5	8.4	10^7	10^7	10^7
	SP-2	75	5.2	6.8	10^7	10^5	10^7
	EF	25	5.8	3.5	10^7	10^7	10^7
2nd treatment	IN	150	2.3	9.2	10^7	10^7	10^5
	SP-1	47	3.3	7.8	10^7	10^7	10^7
	SP-2	25	3.7	5.2	10^7	10^7	10^7
	EF	16	4.7	4.7	10^7	10^7	10^7

[a] Abbreviations: SRB, sulfate-reducing bacteria; APB, acid-producing bacteria; ND, not determined; IN, influent; SP-1, sample port-1;SP-2, sample port-2; EF, effluent (see Fig. 1 for sampling locations).

[b] Formation water was amended with sodium nitrate, certain inorganic nutrients, and inoculated with strain F as described in Materials and Methods.

gested that little or no sulfide production occurred within the core system when organic nutrients were not added to the formation water to support the growth of sulfate-reducing bacteria.

In the core system operated at a hydraulic retention time of 16.7 h, the effluent sulfide concentration was 60 μM, even though the influent sulfide concentration was the same as that injected into the other core system (170 μM). The reason for the decreased effluent sulfide concentration in this core system was not determined. After nitrate treatment, the effluent sulfide concentration of this core system decreased to 3 μM, supporting the conclusion that addition of nitrate is useful in controlling sulfide levels.

Injection of nutrients into the core system operated at a hydraulic retention time of 3.2 h did not stimulate sulfide production. The influent and the effluent sulfide levels were low (1.3 and 1.6 μM, respectively) when only *T. denitrificans* growth medium without thiosulfate was injected into the core system. The numbers of sulfate-reducing bacteria were not affected (data not shown). This again suggested that little or no sulfide production occurred within the core system. After inoculation with strain F and injection of the growth medium without thiosulfate for 24 h, 10^7, 10^5, and 10 cells/ml of denitrifying thiobacilli were detected at sampling locations, SP-1, SP-2, and EF (Fig. 1), respectively. The number of denitrifying thiobacilli increased to 10^5 cells/ml at location EF after the subsequent treatment with cells followed by medium injection. These bacteria were presumed to be *T. denitrificans* strain F since no denitrifying thiobacilli were detected prior to inoculation. Thus, strain F was maintained in the core system when growth medium without thiosulfate was used. Only low levels of sulfide (1 to 2 μM) were detected during these treatments.

Preliminary studies suggested that the formation water contained a compound inhibitory to the growth of strain F (data not shown). Therefore, the fraction of formation water injected into the core was increased in steps in order to acclimate strain F. Relatively high concentrations (10^5 to 10^7 cells/ml) of strain F were detected at locations SP-1, SP-2, and EF during the course of this treatment, suggesting that strain F was active and growing in the core system. Throughout this period, the concentration of sulfide in the effluent of the core system was 70 to 89% lower than the influent concentration. There was also a reduction in the nitrate concentrations in the core system, suggesting that these two processes were linked. The sulfate concentrations in the effluent relative to the influent concentration of the core system increased from 2.6 to 4.2 mM after strain F inoculation, which was much higher than that expected from the oxidation of influent sulfide alone (see below).

When the influent was shifted completely to formation water with 40 mM, the concentration of strain F in the core system decreased from 10^7 cells/ml to 10^3 cells/ml, and interferences in effluent sulfide analyses were observed. This suggested that some essential nutrient may limit the growth of strain F which would result in incomplete oxidation of sulfide or in reduction of nitrate. In subsequent treatments, the concentration of nitrate was decreased from 40 mM to 10 mM and nutrients were added to the formation water.

The treatment of the test core system with strain F and the subsequent injection of formation water with reduced nitrate concentrations and nutrient amendments resulted in the reestablishment of strain F in the core system (Table 2). Concomitant with the increase in strain F was the disappearance of the interfering substance from the effluent of the core system. After the population of strain F was reestablished, an 84 to 99% decrease in sulfide concentration in the effluent compared to the influent was observed. There was a substantial reduction in the levels of nitrate and a substantial increase in the levels of sulfate in the effluent compared to the influent of the core system. However, the amount of sulfate detected in the effluent of the test core system was much higher than that expected if strain F completely oxidized only the sulfide present in the formation water.

DISCUSSION

The addition of nitrate alone to the formation water did result in the reduction of sulfide in the two core systems. This was most pronounced in the core operated at a hydraulic retention time of 16.7 h where little or no sulfide was detected in the effluent after nitrate treatment. Addition of nitrate alone was not as effective in reducing sulfide concentrations in the other core system operated at a shorter retention time, where the effluent sulfide concentration decreased only 40% after nitrate treatment. This indicates that the efficacy of nitrate treatment depends on the retention time of liquids in the system. The different treatment histories of the two core systems prior to the initiation of this work may have markedly altered microbial populations, making it difficult to determine whether other factors also contributed to the differences in the efficacy of the nitrate treatment between the two core systems. However, it is clear that the strain F treatment was more beneficial than nitrate alone in reducing sulfide concentrations in the core system operated at the shorter retention time. After strain F was added, the effluent concentration of this core system was 84 to 90% lower than when the core system was treated only with formation water plus 40 mM nitrate (Table 2).

High concentrations of strain F were observed at each sampling port in the core system operated at the shorter hydraulic retention time after inoculation and injection of nutrient-supplemented formation water with 10 mM nitrate. Thus, strain F was able to colonize the core system and successfully coexist with the indigenous microbial populations. Growth of strain F in the core system did not result in significant increase in the pressure drop through the system. The presence of high levels of strain F at the time when effluent concentrations of sulfide and nitrate decreased, and sulfate increased suggests not only that strain F was maintained in the system, but that it was metabolically active. Strain F-like organisms were not detected in samples from the core system before inoculation with strain F. Thus, the further reduction in effluent sulfide levels observed after inoculation were likely due to the activity of strain F.

As sulfide in the effluent decreased nitrate and sulfate increased in the core system inoculated with strain F, suggesting that as sulfide was used, nitrate was reduced, and sulfate was produced as predicted from Eqn. 1. However, the effluent sulfate concentration of the core system after inoculation was almost twice the influent concentration, and this difference was much greater than that expected if the only source of sulfate was the oxidation of sulfide in the influent. This suggests that sulfur-containing compounds had accumulated within the test core system and were being oxidized to sulfate. This would explain why such a large decrease was observed in the effluent concentrations of nitrate after strain F treatment. One source of endogenous sulfur compounds may be iron sulfide precipitates since these materials were clearly visible in the formation water and the tubing entering the core systems. Montgomery et al. [8] showed that strain F can metabolize sulfide in the form of iron sulfide resulting in clearing of the medium and removal of blackened areas in sandstone cores. Iron sulfide precipitates that form as a consequence of sulfide production can plug pores in porous rock and lead to the loss of injectivity. The fact that strain F is able to use these precipitates should increase the permeability and injectivity of oil and gas wells.

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REFERENCES

1 Beeman, R.E. and J.M. Suflita. 1990. A microbiological examination of a fresh sedimentary core taken from a discovery oil well. Abstr. Annual Meeting American Society for Microbiology Q-221, p. 366. American Society for Microbiology, Washington, DC.
2 Clark, J.B., D.M. Munnecke and G.E. Jenneman. 1981. In situ microbial enhancement of oil recovery. Dev. Ind. Microbiol. 22: 695–701.
3 Cochrane, W.J., P.S. Jones, P.F. Sanders, D.M. Holt and M.J. Mosley. 1988. Studies on the thermophilic sulfate-reducing bacteria from a souring North Sea field. In: Proceedings of the Society of Petroleum Engineers, European Petroleum Conference, London, UK, pp. 301–316, Society of Petroleum Engineers, Dallas, TX (SPE No. 18368).
4 Dziewulski, D.M., S.P. Fraleigh, D.H. Pope, S. Thomas, P. Puente and G. Annexstad. 1990. Microbial production of hydrogen sulfide in gas storage and production fields: field studies, preliminary modeling and control. In: Corrosion 90, pp. 35/1–35/20. National Association of Corrosion Engineers, Houston, TX.
5 Jenneman, G.E., M.J. McInerney and R.M. Knapp. 1986. Effect of nitrate on biogenic sulfide production. Appl. Environ. Microbiol. 51: 1205–1211.
6 Knapp, R.M., M.J. McInerney, D.E. Menzie and R.A. Raiders. 1989. Microbial field pilot study, Final report for period Dec. 15, 1986 to March 31, 1988. Department of Energy DOE/BC/14084-6. National Technical Information Service, Springfield, VA.
7 McInerney, M.J. and D.W.S. Westlake. 1990. Microbial enhancement of oil recovery. In: Microbial Mineral Recovery (Erlich, H.L. and Brierley C.L., eds.), pp. 409–445. McGraw-Hill Publication Co., New York.
8 Montgomery, A.D., M.J. McInerney and K. Sublette. 1990. Microbial control of the production of hydrogen sulfide by sulfate reducing bacteria. Biotech. Bioeng. 35: 533–539.
9 Orr, W.L. 1977. Geologic and geochemical controls on the distribution of hydrogen sulfide in natural gas. In: Advances in Organic Geochemistry 1975 (Campos, R. and J. Goni, eds.), pp. 571–597, Enadimsa, Madrid, Spain.
10 Sublette, K. 1987. Aerobic oxidation of hydrogen sulfide by *Thiobacillus denitrificans*. Biotech. Bioeng. 29: 650–659.
11 Sublette, K. and N.D. Sylvester. 1987a. Oxidation of hydrogen sulfide by *Thiobacillus denitrificans*. Biotech. Bioeng. 29: 245–257.
12 Sublette, K. and M.E. Woolsey. 1989. Sulfide and glutaraldehyde resistant strains of *Thiobacillus denitrificans*. Biotech. Bioeng. 34: 565–569.
13 Westlake, D.W.S. 1991. Microbial ecology of corrosion and reservoir souring. In: Microbial Enhancement of Oil Recovery – Recent Advances (Donaldson, E.C., ed.), pp. 257–263, Elsevier, Amsterdam.