Identification, characterization and application of sulfide-oxidizing bacteria in oil fields

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ABSTRACT

The cycling of sulfur in oil field brines has focused predominantly on bacteria involved in the reduction of sulfur species to sulfides, which are detrimental to the production of these fields. However, recent interest in the addition of nitrates and nitrites to oil field brines for the remediation and control of sulfides has rekindled new interest in sulfide bio-oxidation. Addition of nitrate and phosphates to produced brine from an oil field in Western Canada resulted in the stoichiometric conversion of sulfide to elemental sulfur and nitrate to nitrogen gas. Isolation and characterization of sulfide-oxidizers from this brine revealed the presence of two novel, autotrophic, colorless sulfur bacteria. Culture techniques and reverse sample genome probing were applied to field brines to confirm the identity and role of one of these isolates during a field test. The results suggest that sulfide-oxidizers exist in oil field brines that can be selectively activated *in situ* through the addition of limiting nutrients. However, similar tests applied to oil field brines from West Texas (WT) suggested that heterotrophic sulfide-oxidizers might play a more predominant role in sulfide oxidation in these habitats.

Introduction

The presence of hydrogen sulfide in petroleum reservoirs was recognized prior to 1920, but it was not until 1926 that sulfate-reducing bacteria (SRB) were reportedly isolated from oilfield brines [1,4]. It was later determined that sulfide present in oilfield reservoirs at temperatures below 100^{o} C was likely the result of SRB activity. Ivanov [8] reported convincing evidence that elemental sulfur found in shallow sulfur deposits often associated with oil reservoirs was the result of the activity of the aerobic sulfide-oxidizer, *Thiobacillus thioparus*, oxidizing sulfides previously produced by SRB. It was hypothesized that these thiobacilli were introduced into underground deposits through oxygen-containing waters percolating from the surface. The presence of sulfide-oxidizing bacteria (SOB) has been reported in oilfield brines as well [2,5], but their involvement in the cycling of sulfur in these anoxic habitats has not been established.

Sulfides in oilfield waters are undesirable due to their toxicity, corrosiveness and propensity to form insoluble metal sulfides. Thus, sulfide oxidation to less toxic and less reactive forms of sulfur is desirable. Jenneman et al. [10] reported the ability of nitrate to stimulate the activity of indigenous, anaerobic SOB when amended to sulfide-laden sewage sludges and pond sediments. In 1992, McInerney et al. [13] reported the presence of indigenous, anaerobic sulfide-oxidizing activity in produced brine from a natural gas storage field upon addition of nitrate. Later, McInerney et al. [14] suggested that microbial

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sulfide oxidation was responsible for a 40 to 60% reduction in sulfide at three oil producing wells following the injection of ammonium nitrate as a groundwater tracer into an adjacent water injection well. Since this time, increasing attention has focused on the role of nitrate on the oxidation and control of sulfides by indigenous sulfide-oxidizing bacteria present in oil and gas fields (Table 1). However, surprisingly little attention has been given to the identification and characterization of these sulfide-oxidizing bacteria.

Table 1. Anaerobic sulfide-oxidizing activity by indigenous bacteria found in oil and gas reservoirs.

Reservoir Type	Sample Type	Test location	Temp ^{o}C	Total dissolved solids %	Oxidant	Sulfide-oxidizers identified	Source
Gas storage	Produced brine	Lab	30	0.07	Nitrate	NR	[13]
Oil	Produced brine	Lab/ Field	30	< 0.8	Nitrate	NR	[3]
Gas storage	Produced brine	Lab/ Field	NR	NR	Nitrate	NR	[15]
Oil	Produced brines	Lab	25	2 - 7	Nitrate	1 [a]	[18]
Oil & gas	Produced brines	Lab	40-50	5 - 12.6	Nitrate	NR	[19]
Oil	Produced/ Injected brines	Field	30	< 0.8	Nitrate	NR	[11]
Oil	Produced brine	Field	30	NR	Nitrate	1 [a]	[17]

NR, None reported; [a] tentatively identified as a *Campylobacter sp*. strain CVO

This paper describes efforts being made to identify and characterize sulfide-oxidizing bacteria and explain their role in the cycling of sulfur in shallow oil reservoirs in Western Canada [11] and Western Texas [19]. Also, field tests in these reservoirs of potential applications of anaerobic sulfide oxidation are presented.

Methods

Identification

Sulfide-laden, produced brine was collected from the field in sterile, deoxygenated, glass bottles as previously described [3]. The brine was dispensed into sterile, anaerobic, serum bottles and amended with potassium nitrate and sodium phosphate. Oxidation of the sulfide was monitored colorimetrically on subsamples of brine collected periodically.
In some cases, the sulfide-oxidizing activity in field brines could be detected by noting changes in the color and transparency of the brine as sulfide was oxidized to elemental sulfur. Untreated brine was either colorless or black (iron sulfide) when collected, but upon addition of nitrate would shift from a transparent yellow color, indicative of polysulfides, to a turbid, white color as sulfur and calcite crystallized. If the redox indicator resazurin was added to nitrate-amended, sulfide-laden brines, the color change of the brine from colorless to pink (Eh between -110mV and -51 mV) was used as an indicator of the oxidation of sulfide and appearance of nitrite or nitrous oxide [12,16].

Isolates of autotrophic SOB were obtained by plating enrichments on agar medium for the cultivation of *Thiobacillus denitrificans* [7]. Colonies were further purified by serial dilution in media containing sulfide and nitrate. Thus far, two isolates, designated CVO and FWKO B, have been isolated from produced brine at the Coleville (CV) Field in western Saskatchewan, Canada. Examination of their 16S RNA sequences suggests they are members of the delta subdivision of the proteobacteria, which includes other nitrate-reducing SOB such as, *Arcobacter nitrofigilis*, *Campylobacter sp*. and *Sulfurospirillum deleyianum* (personal communication, Gerrit Voordouw). Voordouw et al. [18] using PCR-amplified 16S rRNA genes, were the first to report the presence of *Arcobacter* and *Campylobacter* genotypes, as well as, the sulfide-oxidizer, *Thiomicrospira denitrificans* in oil field brines. However, CVO and FWKO B are the only known pure isolates cultured from oil field brines. No known sulfide-oxidizing bacteria have been isolated from West Texas brines.

Results

Characterization

Sulfide-oxidizing activity in brines from the CV field is believed to be the result of autotrophic SOB since the addition of nitrate and inorganic phosphate alone promotes activity. However, sulfide-oxidizing activity in produced brines collected from several different stratigraphic formations from WT oil fields required the addition of organic acids and yeast extract suggesting oxidation by heterotrophic SOB [19]. Concentrations of sulfide in excess of 5 mM and 14 mM have been completely oxidized by adding nitrate in brine enrichments from the CV field and WT fields, respectively. Furthermore, sulfide-oxidizing activity has been observed over a temperature range from 5^{o} C to 40^{o} C for CV brines and from 40^{o} C to 50^{o} C for WT brines. Activity in brines with a salinity as high as 10 %(wt/wt) has also been demonstrated in either CV or WT brines. Sulfide oxidation rates in excess of 100 mg L^{-1} d^{-1} in batch enrichments of WT brine, and in continuously-fed porous bioreactors inoculated with CV brine have been reported [9]. Stoichiometric conversion of sulfide to elemental sulfur occurs in CV brine enrichments according to the equation [9]:

$$5\ HS^{-} + 2\ NO_3^{-} + 7\ H^{+} \rightarrow 5\ S_o + N_2 + 6\ H_2O$$

Stoichiometry of sulfide oxidation has not been determined for WT brines but elemental sulfur and calcite have been detected as substantial end products.

The following model for the anoxic cycling of sulfur in these shallow oilfield reservoirs is proposed which involves the cycling of sulfide through elemental sulfur by either autotrophic or heterotrophic sulfide-oxidizers (Fig. 1). Sulfide is regenerated by the activity of sulfur reducing bacteria whose activity has been observed in CV brine (unpublished results).

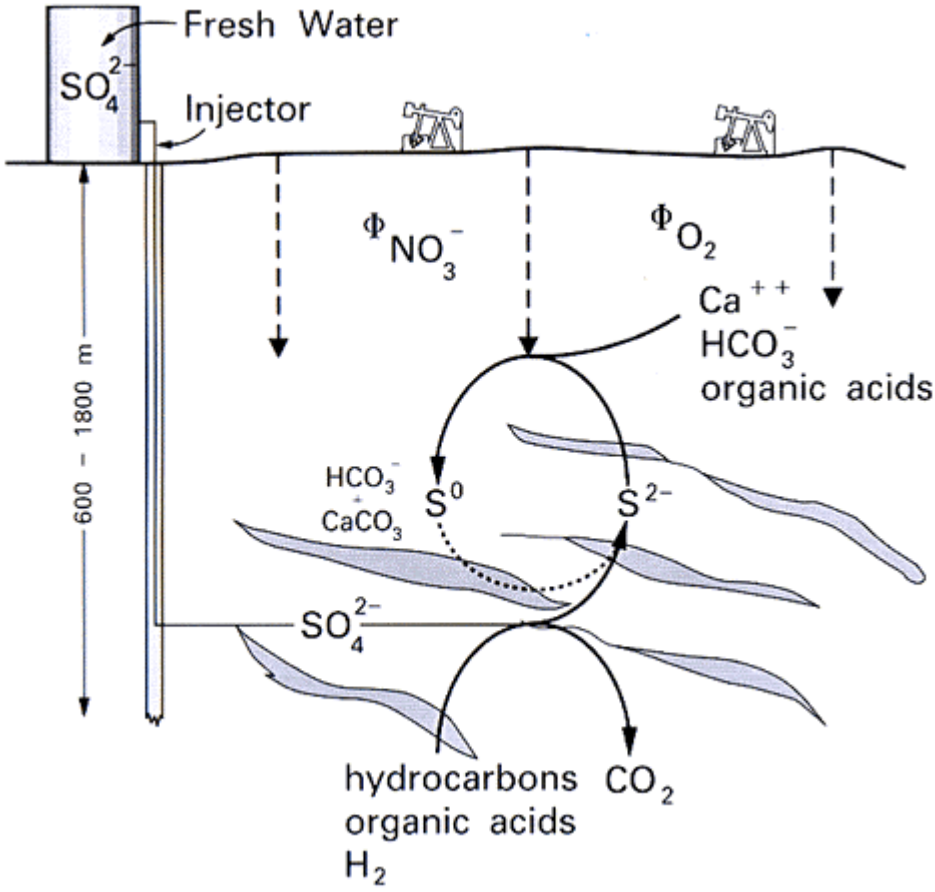

Fig. 1. Sulfate-containing fresh water co-mingles with stratal waters located near hydrocarbon-containing deposits as a result of water injection or aquifer recharge. Sulfide is formed by SRB metabolizing water-soluble organics, hydrocarbons or hydrogen which is then oxidized to elemental sulfur by anaerobic or microaerophilic sulfide-oxidizers (e.g., strain CVO) when exposed to either nitrate or oxygen. Oxidants are introduced into the injected water or are transported from surface sources by the recharge waters. Anaerobic sulfide-oxidizers can be autotrophs or heterotrophs. Calcite ($CaCO_3$) is a by-product of sulfide oxidation presumably due to an increase in pH of the brine resulting from denitrification in these calcium-containing carbonate brines. The cycle is completed by the reduction of elemental sulfur to sulfide by unidentified sulfur reducers.

Discussion

Application

Recently, Jenneman et al. [11] demonstrated in a field test performed at the CV field that injection of nitrate and phosphate for 50 days into two injectors resulted in a 42 to 100% reduction in soluble sulfide and a 50 to 60% reduction in sulfide at two adjacent producing wells. Analysis of SOB and SRB populations at injectors and producers using most-probable-number analysis and reverse sample genome probing of whole communities in both injected and produced brines, revealed no significant increases in SRB and a large increase in SOB, especially the SOB isolate, CVO [17]. This demonstrated that the injection of inexpensive, low toxicity chemicals could selectively stimulate a desired microbial activity resulting in the reduction of an undesirable product, sulfide.

Likewise, Hitzman and Dennis [6] demonstrated that the addition of low concentrations of inorganic nutrients such as nitrate and nitrite to several producing wells in these WT reservoirs resulted in over a 90% drop in wellhead H_2S levels for more than 30 days. This reduction in H_2S was attributed to competition between indigenous nitrate-reducing bacteria (NRB) (e.g., sulfide-oxidizers) and SRB populations for organic acids in the brine, although changes in SRB and NRB were not reported. Again, this demonstrates how the introduction of inexpensive nutrients can selectively alter the reservoir ecology in a beneficial way and suggests a potential role for indigenous SOB.

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