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# The influence of nitrate on microbial processes in oil industry production waters

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**Sulfide accumulation due to bacterial sulfate reduction is responsible for a number of serious problems in the oil industry. Among the strategies to control the activity of sulfate-reducing bacteria (SRB) is the use of nitrate, which can exhibit a variety of effects. We investigated the relevance of this approach to souring oil fields in Oklahoma and Alberta in which water flooding is used to enhance oil recovery. SRB and nitrate-reducing bacteria (NRB) were enumerated in produced waters from both oil fields. In the Oklahoma field, the rates of sulfate reduction ranged from 0.05 to 0.16 $\mu$M S day$^{-1}$ at the wellheads, and an order of magnitude higher at the oil–water separator. Sulfide production was greatest in the water storage tanks in the Alberta field. Microbial counts alone did not accurately reflect the potential for microbial activities. The majority of the sulfide production appeared to occur after the oil was pumped aboveground, rather than in the reservoir. Laboratory experiments showed that adding 5 and 10 mM nitrate to produced waters from the Oklahoma and Alberta oil fields, respectively, decreased the sulfide content to negligible levels and increased the numbers of NRB. This work suggests that sulfate reduction control measures can be concentrated on aboveground facilities, which will decrease the amount of sulfide reinjected into reservoirs during the disposal of oil field production waters.** *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 80–86.

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## Introduction

Primary petroleum production typically recovers <30% of the oil in a reservoir, so enhancement methods are frequently used to obtain additional increments. Water flooding is a commonly used enhanced recovery method, but it is often associated with the "souring" of oil fields caused by the increased microbial production of hydrogen sulfide. Hydrogen sulfide is a toxic and corrosive gas responsible for a variety of environmental and economic problems including reservoir souring, contamination of natural gas and oil, corrosion of metal surfaces, and the plugging of reservoirs due to the precipitation of metal sulfides and the consequent reduction in oil recovery. A major source of sulfide in water-flooded oil fields is the result of the metabolic activities of sulfate-reducing bacteria (SRB) [4,5,17,22,24]. These organisms reduce sulfate to sulfide coupled to the oxidation of hydrogen and a wide variety of organic electron donors [29]. Because oil fields are rarely limited in the supply of potential electron donors, the activity of SRB may be limited by the availability of electron acceptors. If sulfate is available, sulfide accumulation can be substantial.

The control of sulfide production is usually attempted through the use of broad-spectrum biocides or inhibitors of sulfate reduction [3,8,28]. However, such strategies are often limited in effectiveness and duration. Biocide use can also pose a substantial environmental hazard [27].

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A multifaceted alternate approach for the control of sulfide accumulation is the use of nitrate, which has the potential to establish competition between two groups of bacteria: the nitrate-reducing bacteria (NRB) and the SRB. Interactions between the nitrogen and sulfur cycles can impact the production, accumulation, and elimination of sulfide in oil field waters. Mechanisms that can influence sulfide concentrations in produced waters are summarized below.

Nitrate and sulfate are terminal electron acceptors for these different groups of bacteria, and competition based on thermodynamics, kinetics, and redox potential is established when both anions are present. The thermodynamic and kinetic effects are difficult to separate. Thermodynamically, the reduction of nitrate to nitrogen or ammonia provides more Gibbs free energy than sulfate reduction [37]. Therefore, in the presence of nitrate, NRB outcompete SRB for available electron donors [19,30]. For example, Hitzman and Sperl [9] demonstrated that NRB outcompeted SRB for volatile fatty acids, which are electron donors commonly found in reservoir water. Biological sulfide production does not occur when the redox potential is above −100 mV [26], and the growth of SRB can be inhibited by elevation of the redox potential. The intermediates of nitrate reduction, nitrous oxide and nitric oxide, increase the ambient redox potential, thus providing prolonged inhibition of sulfide production [13]. It has been demonstrated that the addition of nitrate inhibits sulfide production in many environments and laboratory systems with active nitrate-reducing populations [13,18,22,23,25].

Not only can nitrate reduction inhibit sulfide production, but the activities of NRB can remove existing sulfide from oil field waters. For example, nitrate can also serve as an electron acceptor for the reoxidation of sulfide to sulfate [31] or elemental sulfur [33] by

sulfur-oxidizing chemolithotrophic bacteria. The latter reaction is summarized by:

$$5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S^\circ + N_2 + 6H_2O$$

In addition, nitrite produced by NRB can react abiotically with dissolved sulfide to produce elemental sulfur according to the following equation [12]:

$$3HS^- + 2NO_2^- + 5H^+ \rightarrow 3S^\circ + N_2 + 4H_2O$$

Nitrate amendments in the absence of NRB do not produce the desired effect. For example, attempts to reduce sulfide levels in an experimental system using cores and formation water from a gas storage facility failed until an inoculum of *Thiobacillus denitrificans* strain F was used [22,23].

Regardless of the mechanism, the success of nitrate amendments for the control of sulfide production depends on the presence of an active population of NRB. This work describes a reconnaissance carried out to determine the potential for the control of sulfide production with nitrate in two sour oil fields. We evaluated microbial metabolic potentials and observed that the predominant potential for sulfide formation occurred in the aboveground facilities rather than at the wellheads. Further, nitrate was effective in controlling sulfide formation in oil industry production water. Unlike other studies that have focused on controlling sulfide production in the reservoirs or porous medium reactors that simulate reservoirs [7,14,22,23,27], our results indicate that controlling sulfide production in the aboveground facilities may be more appropriate in some souring oil fields.

## Materials and methods

### *Site description and sampling*

We investigated the Bebee-Konawa oil field located near Ada, OK, and the Marion Lake field near Botha, Alberta, Canada. The oil-bearing formation in the former field is the Hunton limestone located at 640–690 m depth. The field has been in operation since 1979 and water-flooded with groundwater from the area. After oil separation, production waters are reinjected into the reservoir. Samples of produced water were taken at the wellhead of two oil wells (wells 1 and 2) and from the oil–water separator where fluids from the two wells were collected (Figure 1A).

The Marion Lake field has been in operation since 1993 and water-flooded since 1995. The oil-bearing layer is in the Upper Mannville formation located at almost 1800 m depth. The field had 28 oil wells and the flows from several wells are combined into a single pipeline in a building referred to as a "satellite." There are a total of five satellites in this field. The combined oil from the satellites flows into two oil–water separators, known as the east and west separators. Figure 1B is a simplified scheme of the flow in part of this oil field and it illustrates that the produced water sampling points were not at the wellheads. Therefore, the oil–water emulsions had been in the pipelines for

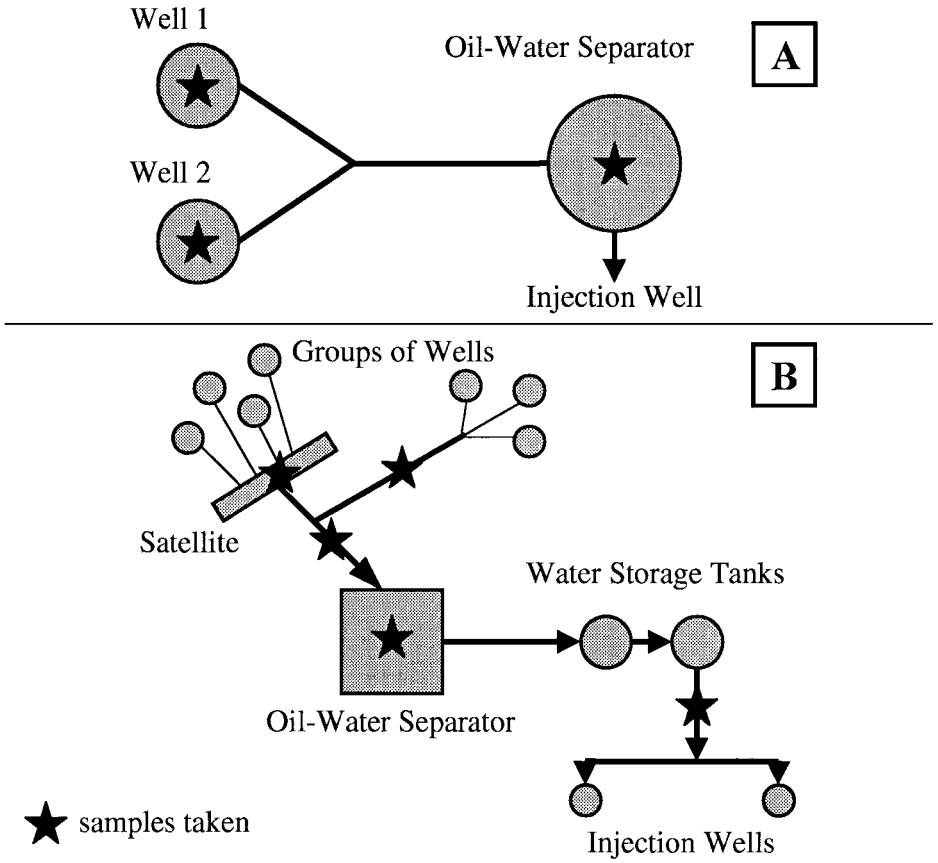


**Figure 1** Simplified schemes of (A) Bebee-Konawa, Oklahoma oil field and (B) Marion Lake, Alberta oil field.

some time before sampling and many of the samples were composites from several wells. Water from the oil–water separator is transferred to water storage tanks, with a total retention time of 2–3 days, before reinjection into the reservoir (Figure 1B). In addition, fresh source water from an aquifer at a depth of 550 m is also used to pressurize the formation. Some parts of the Marion Lake field are treated with the biocide Magnacide 424 (an *N*-coco-1,3-diaminopropane) to control SRB activity.

The Marion Lake field was sampled on three occasions, from 26 different sampling locations during 1999. These samples included the fresh source water, three produced oil–water emulsions (designated samples A, B, and C) from the satellites, produced water from the two oil–water separators, and the stored produced water before reinjection (Figure 1B). The biocide feed was halted for 3 weeks before each sampling trip.

Sampling ports were purged with 5–10 l of produced fluids and samples were collected in sterile glass bottles (2 or 4 l), which were filled completely to prevent contact with air.

### Chemical analysis

Sulfate, nitrate, nitrite, and chloride were determined by ion chromatography using a Dionex DX500 system equipped with an AS4A column, and an ion-suppressed CD20 conductivity detector (Dionex, Sunnyvale, CA). The mobile phase was 1.7 mM sodium bicarbonate–1.8 mM sodium carbonate at a flow rate 2.0 ml $min^{-1}$.

Sulfide concentrations in water samples were determined by colorimetric reaction with dimethyl-*p*-phenylenediamine [34].

### Sulfate-reducing activity

Sulfate-reducing activity was determined by a radiotracer technique [35]. Produced water (10 ml) was dispensed into sterile serum bottles flushed with nitrogen, and supplemented with 2 $\mu$Ci $Na_2^{35}SO_4$ (10 mCi $mmol^{-1}$; Amersham, Arlington Heights, IL) per bottle from an anoxic sterile stock solution. The sulfate amendment did not change measurably the background concentration in the samples. The bottles were incubated for 2 days at room temperature before the pool of total reduced inorganic sulfur compounds was extracted and quantified [35]. Filter (0.22 $\mu$m)-sterilized water samples were used as negative controls.

### Nitrate-reducing activity

The potential for nitrate-reducing activity was determined in laboratory incubations. Water samples (50 ml) were dispensed into sterile serum bottles while inside an anaerobic glove box. The bottles were closed with butyl rubber stoppers, secured with aluminum crimps. Sterile anoxic stock $NaNO_3$ solution was added to give an initial concentration of 5 or 10 mM, and nitrate depletion was monitored.

### Bacterial enumerations

The SRB and NRB were enumerated by a three-tube most probable number (MPN) procedure using 10-fold serial dilutions in selective media. The SRB were enumerated in a medium containing lactate (3.5 g $l^{-1}$) as a growth substrate and 10 mg $l^{-1}$ $FeSO_4$ [36]. Culture tubes were incubated for 4 weeks at 30°C and scored as positive when medium blackening occurred. The NRB were enumerated in a mineral medium (pH 7.2), containing per liter: 0.5 g $NH_4Cl$, 0.3 g $KH_2PO_4$, 0.4 g $MgCl_2{\cdot}6H_2O$, 0.5 g KCl, 0.15 g $CaCl_2{\cdot}2H_2O$, 0.85 g $NaNO_3$, 1 g $Na_2S_2O_3$, 0.1 g yeast extract, 1 mg resazurin, and 10 ml of a trace metal solution [32]. Sodium bicarbonate was added after sterilization to an initial concentration of 5 g $l^{-1}$. The gas phase was $N_2/CO_2$ (80:20). Culture tubes were incubated for 4 weeks at 30°C and were scored positive if they consumed >10% of the available nitrate compared to uninoculated controls. The chloride content of both media was adjusted with NaCl to equal the chloride concentration found in the respective oil fields.

## Results

### Chemical and biogeochemical characterization of the experimental sites

Produced waters at all the selected locations were devoid of nitrate. When the redox indicator, resazurin, was added to produced water samples, it turned colorless, indicating a low redox potential in the sample (less than −110 mV). In addition, sulfide was detected in most produced waters. These observations indicated the absence of oxygen in the samples. The pH of all produced water samples ranged from 7.5 to 8.5 (Table 1). There was a wide range of temperatures from 8°C to 23°C in the samples obtained from the Marion Lake field (Table 1). In the Marion Lake oil field, the oil–

**Table 1** Geochemical characteristics of samples from the two oil fields

| Oil field | Sample | pH | Temperature (°C) | Chloride (mM) | Sulfide (mM) | Sulfate (mM) |
|---|---|---|---|---|---|---|
| Bebee-Konawa, OK[a] | Well 1 | 7.8 | 25 | 140 | 3.6 | 0.38 |
| | Well 2 | 8.0 | 27 | 95 | 3.6 | 0.52 |
| | Oil–water separator | 7.9 | 23 | 100 | 3.9 | 0.35 |
| Marion Lake, Alberta | Source water | 7.0 | 23 | 250 | b.d.[b] | 0.1 |
| | Produced water A | n.d.[c] | 8 | 390 | b.d. | 8.6 |
| | Produced water B | 7.5 | 23 | 430 | 0.8 | 6.9 |
| | Produced water C | 7.5 | 20 | 520 | 0.5 | 9.0 |
| | East oil–water separator | 7.5 | 44 | 560 | 0.2 | 8.2 |
| | West oil–water separator | 8.0 | 39 | 490 | 0.6 | 8.7 |
| | Produced water after storage | 8.5 | 14 | 510 | 6.2 | 0.95 |

[a]Average data from three separate sampling trips.
[b]Below detection limit.
[c]Not determined.

**Table 2** Biogeochemical characteristics of samples from the two oil fields

| Oil field | Sample | Sulfate-reducing activity ($\mu$M S day$^{-1}$) | Potential nitrate-reducing activity (mM day$^{-1}$) | SRB (MPN ml$^{-1}$) | NRB (MPN ml$^{-1}$) |
|---|---|---|---|---|---|
| Bebee-Konawa, OK | Well 1 | 0.16±0.04 | 0.016±0.004 | $2.5\times10^{3}$ | $2.5\times10^{2}$ |
| | Well 2 | 0.05±0.016 | 0.012±0.0005 | $4.5\times10^{2}$ | $2.5\times10^{5}$ |
| | Oil–water separator | 1.8±0.27 | 0.06±0.02 | $2.5\times10^{3}$ | 15 |
| Marion Lake, Alberta | Source water | 0.06±0.04 | b.d.[a] | 23 | <1 |
| | Produced water A | 0.041±0.03 | 0.07 | 23 | $2.3\times10^{2}$ |
| | Produced water B | n.d.[b] | 0.1 | $2.4\times10^{2}$ | 75 |
| | Produced water C | 0.11±0.17 | b.d. | 43 | 23 |
| | East oil–water separator | 0.68±0.55 | 0.28 | $4.3\times10^{2}$ | $2.3\times10^{2}$ |
| | West oil–water separator | 0.07±0.017 | >0.75 | $9.3\times10^{2}$ | $2.3\times10^{2}$ |
| | Produced water after storage | n.d. | 0.08 | $4.3\times10^{3}$ | $1.5\times10^{2}$ |

[a]Below detection limit.
[b]Not determined.

water separators are heated to hasten the separation process, and the produced water leaving these units was at temperatures of 39–44°C. The water cooled in the storage tanks before reinjection into the reservoir. Temperatures in the Oklahoma field were quite constant, ranging from 23°C to 25°C (Table 1), and heat was not applied to the oil–water separators.

Chloride concentrations of the produced waters from the Bebee-Konawa field ranged between 95 and 140 mM (Table 1). Produced waters from the Marion Lake field contained much higher chloride concentrations, ranging from 390 to 560 mM.

The source water for the Marion Lake field flooding contained no detectable sulfide, very little sulfate (0.1 mM), and about half the chloride concentration found in the produced waters (Table 1). Thus, the sulfate found in the produced waters from the Marion Lake field originated from the petroleum reservoir. The Bebee-Konawa field exhibited relatively high sulfide concentrations (≥3.6 mM), whereas sulfate concentrations were relatively low (0.58 mM) (Table 1). These chemical characteristics were consistent with the prospect of sulfate reduction occurring in the formation.

The produced waters from the Marion Lake field had much higher sulfate concentrations, between 6.9 and 9.0 mM, and lower sulfide concentrations ranging up to only 0.8 mM. The most notable exception was the sample taken after the produced water had flowed through the water storage tanks at the Marion Lake site. Passage through these storage tanks decreased the sulfate concentration to 0.95 mM (Table 1) and increased the sulfide concentration to 6.2 mM. These changes suggested that SRB were active in the storage tanks.

In consistent fashion, we detected sulfate reduction in samples from the Oklahoma field wellheads and from the oil–water separator. The rates of sulfate reduction varied from 0.05 to 0.16 $\mu$M S day$^{-1}$ at the wellheads, but were about an order of magnitude higher in the sample from the oil–water separator (Table 2). These data led to the hypothesis that the majority of the sulfide produced at this facility occurred after the oil was pumped above ground.

The produced waters from the Marion Lake oil field exhibited comparable rates of sulfate reduction to those measured for the Oklahoma samples. Increased rates of sulfate reduction were detected in samples from the east oil–water separator, but because of the high degree of variability among replicates, it is not clear whether microbial activity in the separator was significantly different from other samples from this field (Table 2). However, the high sulfide concentration in the produced water after storage (6.8 mM, Table 1) again suggested that sulfate reduction is important in aboveground facilities at the Marion Lake oil field.

For both oil fields, the potential for nitrate-reducing activity was higher in samples taken from oil–water separators than from other produced waters (Table 2). For example, in the Oklahoma field, the nitrate-reducing activities in the wellhead samples were 0.016 and 0.012 mM day$^{-1}$, whereas the activity was 0.06 mM day$^{-1}$ in the sample from the oil–water separator. Similarly, nitrate-reducing activities in samples from the oil–water separators in the Marion Lake field were higher that in the other produced water samples. In the case of the west oil–water separator, all nitrate was consumed within 1 week, which was the first time the incubations were sampled to measure nitrate depletion. These observations suggest that because these waters have a potential for microbial nitrate reduction, nitrate treatment could effectively control sulfide production in the aboveground facilities without inoculation with exogenous NRB as proposed by others [11].

The numbers of NRB and SRB detected in samples collected from the two oil fields were not particularly revealing (Table 2). There was a wide range in the number of NRB detected in the Oklahoma field, ranging from 15 to $2.5\times10^{5}$ cells ml$^{-1}$. Only the source water at the Marion Lake field failed to reveal a detectable population of NRB, but the other counts ranged from 23 to $2.3\times10^{2}$ cells ml$^{-1}$. The numbers of SRB in the Bebee-Konawa field clustered in a relatively narrow range ($4.5\times10^{2}$ to $2.5\times10^{3}$ ml$^{-1}$), whereas a slightly broader range was detected in samples from the Marion Lake field (Table 2). In the Marion Lake field, the produced waters from the pipelines and the oil–water separator had numbers of SRB up to $9.3\times10^{2}$ ml$^{-1}$, whereas the source water contained only 23 ml$^{-1}$ (Table 2). The highest number of SRB was found in the produced water after storage ($4.3\times10^{3}$ ml$^{-1}$), consistent with the sharp decrease in sulfate and increase in sulfide content that occurred in the storage tank (Table 1).

### *Microbial processes in laboratory incubations*

Laboratory experiments focused on the produced waters as possible targets for nitrate amendment to control sulfide production aboveground. Figure 2A shows the sulfide concentrations in laboratory incubations that contained produced water from the oil–water separator in the Bebee-Konawa field. This water contained a relatively low sulfate concentration (0.35 mM, Table 1), and little

sulfide production occurred in the unsupplemented sample. Substantially more sulfide was evident when 5 mM sulfate was added to the incubation mixture, which was further increased when sulfate and oil were added together (Figure 2A). These results suggested that the activity of SRB was limited, in part, by the availability of both a terminal electron acceptor and suitable electron donors.

Produced water from the oil–water separator at the Oklahoma field was used to determine nitrate-reducing potentials in the presence and absence of the Bebee-Konawa oil. In both cases, the potential was 0.06 mM day$^{-1}$. Thus, the presence of a separate oil phase did not stimulate the rate of nitrate reduction, and dissolved components in the produced water provided an adequate supply of electron donors for this process.

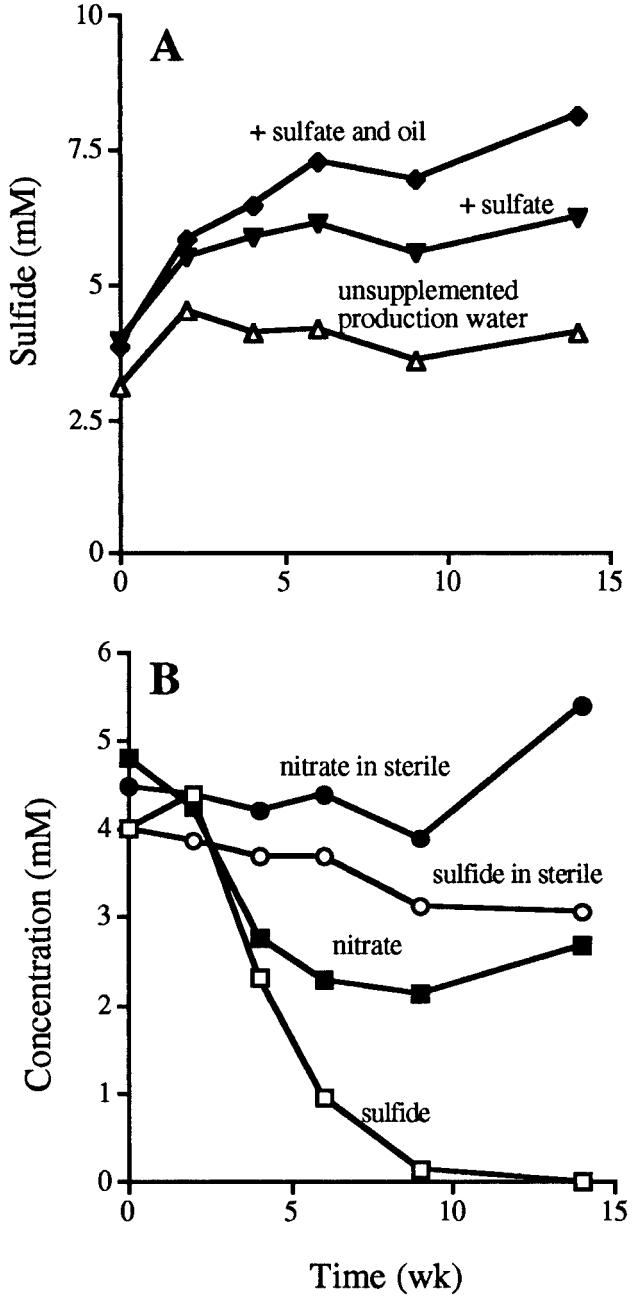


**Figure 2** Sulfide and nitrate concentrations in laboratory incubations that contained produced water from the oil–water separator at the Bebee-Konawa field. (A) No nitrate added; (B) amended with 5 mM nitrate.

**Table 3** Microbial counts in incubations containing produced water B from the Marion Lake oil field

| Microbial types | Initial counts (MPN ml$^{-1}$) | Counts after 7 weeks of incubation (MPN ml$^{-1}$) | |
|---|---|---|---|
| | | Nitrate amended | Unamended |
| NRB | $7.5\times10^{1}$ | $4.3\times10^{4}$ | $9.3\times10^{2}$ |
| SRB | $2.4\times10^{2}$ | $2.4\times10^{2}$ | $2.4\times10^{6}$ |

When produced water samples from the oil–water separator in the Bebee-Konawa field were amended with nitrate (5 mM), approximately half of the amendment was consumed during a 14-week incubation (Figure 2B). Over the same period, the sulfide concentration was reduced to nearly undetectable levels in the nonsterile incubations. There was little change in these components in the sterile controls (Figure 2B).

Lower initial nitrate concentrations (1 or 2 mM) in the Oklahoma produced waters yielded only a temporary effect. That is, sulfide was incompletely removed and sulfate reduction resumed after nitrate was depleted (data not shown).

Experiments were done with produced water B from the Marion Lake field to determine if nitrate amendment could stimulate NRB growth and control sulfide production. The sample contained 1 ml of oil from this field to serve as a potential electron donor for SRB and NRB. The produced water contained an ample supply of sulfate (6.9 mM, Table 1) for the SRB. Some of the incubations were amended with 10 mM nitrate, while others were not.

Without the nitrate amendment, sulfide accumulated to about 5 mM during the 9-week incubation, whereas in the nitrate-amended incubations, nitrate was consumed and the sulfide concentration never exceeded 0.3 mM (the initial sulfide concentration). The numbers of NRB and SRB were determined at the beginning of this experiment, and after a 7-week incubation (Table 3). The addition of nitrate to the Marion Lake produced water stimulated a 570-fold increase in the number of NRB, resulting in $4.3\times10^{4}$ ml$^{-1}$ at the later sampling time. In contrast, the number of SRB after 7 weeks was the same as that measured at the start of the incubation. Without nitrate amendment, the number of SRB was 10,000-fold greater after 7 weeks than at the beginning of the experiment, whereas only a slight (12-fold) increase in the number of NRB was seen. These changes in the bacterial numbers are consistent with the amounts of sulfide measured in the experiment.

## Discussion

There is little correlation between bacterial counts and the specific reducing activities presented in Table 2. For example, the numbers of SRB in samples from well 1 and the oil–water separator from the Oklahoma field were both $2.5\times10^{3}$ ml$^{-1}$, but the sulfate-reducing activity in the latter sample was an order of magnitude higher than that of the former sample. Data from the Marion Lake field showed that the number of SRB in the west oil–water separator was about 40 times greater than that of the produced water A, yet the sulfate-reducing activities differed by less than a factor of 2.

Similarly, there was no correlation between the number of NRB and nitrate-reducing activity (Table 2). The highest number of NRB in the Bebee-Konawa field was from well 2, which was 1000-fold greater than well 1. However, nitrate-reducing activities in these samples were very similar at 0.012 and 0.016 mM day$^{-1}$, respectively. Three samples from the Marion Lake field had the same number of NRB ($2.3\times10^2$ ml$^{-1}$), but the nitrate-reducing activity was dramatically different in produced water A and the east and west oil–water separators (0.070, 0.28, and >0.75 mM day$^{-1}$, respectively) (Table 2). Enumeration was performed in a selective NRB medium, containing yeast extract and thiosulfate. The potential nitrate-reducing activity was measured by adding only nitrate to the produced water. This approach was necessary to demonstrate that the potential for nitrate reduction existed, but the results cannot be compared directly to the enumeration results. Such findings indicate that a survey of microbial counts in samples from oil fields may not accurately reflect the potential for microbial activities in the samples. This discrepancy between counts and activity is a well-known phenomenon observed in other environmental studies [2,15,21].

SRB have been the focus of many studies in the petroleum industry (for reviews, see Refs. [4,20]), but few studies have enumerated NRB in oil field waters, and all used procedures employing different media and are not entirely comparable. Adkins *et al* [1] used both a molasses- and sucrose-based medium to enumerate heterotrophic NRB in four carbonate petroleum reservoirs. These samples yielded very low numbers of NRB, with the highest MPN count being 4 ml$^{-1}$. Jenneman *et al* [14] monitored the increase in NRB during the injection of nitrate into the reservoir at the Coleville field in Saskatchewan, Canada. For their MPN procedure, they supplemented filter-sterilized brine from this field with nitrate, phosphate, and the redox indicator resazurin. The brine contained about 3 mM sulfide that served as the electron donor, and the counting procedure was selective for sulfide-oxidizing NRB. The numbers of NRB were $10^5$ cells ml$^{-1}$ before nitrate injection and $10^8$ cells ml$^{-1}$ during nitrate injection. Telang *et al* [33] used a defined medium, with the same salt composition as the Coleville brine, with both sulfide and acetate as potential electron donors to determine the numbers of NRB in five oil fields. They reported MPN values of $10^6$ cells ml$^{-1}$ in three of these samples, and $10^2$ cells ml$^{-1}$ in the other two samples. The major electron donor in our assay was thiosulfate because it is less toxic than sulfide and can be used by the sulfide-oxidizing NRB, *T. denitrificans* [16]. The medium also contained a small amount of yeast extract, which may have stimulated some heterotrophic growth. The numbers of NRB that we observed (Table 2) were typically about $10^2$ cells ml$^{-1}$, and comparable to the lower cell numbers observed by Telang *et al* [33].

All produced water samples we examined harbored NRB (Table 2). Thus, nitrate treatment in these two oil fields would be expected to stimulate nitrate-reducing activity and the use of an inoculant would be unnecessary. However, it is not known if these findings will prove general for other oil field waters.

Batch culture incubations of produced waters from two oil fields indicated that different concentrations of nitrate were required to control sulfide production. The amendment of 5 mM nitrate stopped sulfide production in the Bebee-Konawa samples, whereas 10 mM nitrate was required for the Marion Lake produced water samples. These concentrations are similar to those reported for other laboratory investigations in which indigenous NRB were stimulated to control sulfide production. In their study of oily waste sludges, Londry and Suflita [18] observed that 16 mM nitrate prevented sulfide accumulation. Gevertz *et al* [6] observed that the addition of 5 mM nitrate promoted the depletion of all of the sulfide (3.8 mM) in produced waters from the Coleville field in Saskatchewan. Each oil field should be assessed to determine whether NRB are present and the nitrate concentration required to suppress sulfide production. Of course, the actual concentration required in the water-handling facilities may be higher due to the presence of biofilms on oil equipment. Typically, higher concentrations of biocides are required to control microbial activities in biofilms relative to planktonic microorganisms [28] and the same is likely true for nitrate treatment.

Experiments showed that nitrate amendment of produced water from the Marion Lake facility controlled sulfide production. The bacterial counts in Table 3 confirmed that the number of NRB increased markedly with nitrate amendment. Their numbers increased 570-fold, which is comparable to the 1000-fold increase observed by Jenneman *et al* [14] in field trials with nitrate injection into the Coleville reservoir.

Petroleum industry efforts to control sulfide production using either biocides [10] or nitrate injection [14] are largely focused on injecting these control agents into a suitable reservoir. However, this study suggests that reservoirs may not be the only, or even the major, source of sulfide production problems in oil field operations. In this survey of only two oil fields, the majority of sulfide production and sulfate-reducing activity were evident in aboveground facilities. Enumeration data and activity profiles from these two oil fields showed that aboveground facilities could be effective targets for sulfide control measures with nitrate, rather than treating the reservoir which has been the focus of other studies [7,14].

We did not observe a dramatic increase in SRB numbers in the storage tanks relative to the numbers detected at the wellhead. Presumably, this is because only planktonic bacteria were enumerated. It is well established that vast numbers of bacteria can reside in oil field biofilms [28], but these organisms were not assayed in our survey. However, the survey of sulfate-reducing activity certainly suggested that the aboveground conditions are conducive for the biological production of sulfide.

The sulfide-enriched produced waters are routinely injected back into the reservoir to repressurize the formation (Figure 1). Clearly, the sulfide generated aboveground can then contribute to the overall souring of the oil field. For example, at the Marion Lake facility, the daily injection of produced water from the storage tanks, containing about 6.2 mM sulfide (Table 1), is 400 m$^3$. This corresponds to the injection of 80 kg of sulfide into the reservoir each day. Thus, controlling sulfide production by treating the water-handling system with nitrate would greatly reduce the quantity of this material being reinjected into the formation. On the basis of these findings, the oil companies operating these two fields are planning to implement field trials of nitrate injection to control sulfide production and souring.

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