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Toluene Depletion in Produced Oil Contributes to Souring Control in a Field Subjected to Nitrate Injection

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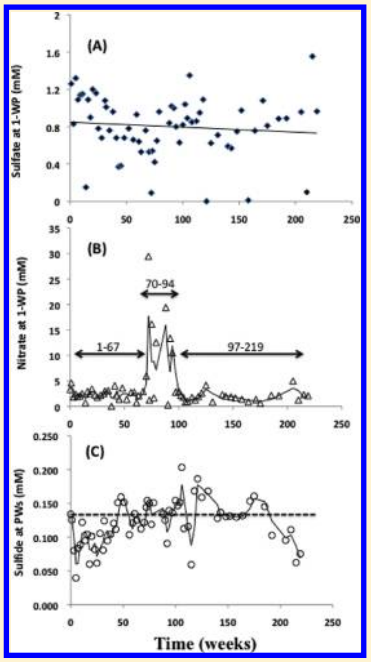
Supporting Information

ABSTRACT: Souring in the Medicine Hat Glauconitic C field, which has a low bottom-hole temperature (30 °C), results from the presence of 0.8 mM sulfate in the injection water. Inclusion of 2 mM nitrate to decrease souring results in zones of nitrate-reduction, sulfate-reduction, and methanogenesis along the injection water flow path. Microbial community analysis by pyrosequencing indicated dominant community members in each of these zones. Nitrate breakthrough was observed in 2-PW, a major water- and sulfide-producing well, after 4 years of injection. Sulfide concentrations at four other production wells (PWs) also reached zero, causing the average sulfide concentration in 14 PWs to decrease significantly. Interestingly, oil produced by 2-PW was depleted of toluene, the preferred electron donor for nitrate reduction. 2-PW and other PWs with zero sulfide produced 95% water and 5% oil. At 2 mM nitrate and 5 mM toluene, respectively, this represents an excess of electron acceptor over electron donor. Hence, continuous nitrate injection can change the composition of produced oil and nitrate breakthrough is expected first in PWs with a low oil to water ratio, because oil from these wells is treated on average with more nitrate than is oil from PWs with a high oil to water ratio.



INTRODUCTION

The Medicine Hat Glauconitic C (MHGC) field, referred to as the Enermark field in a previous paper,[3] is a shallow (850 m), low-temperature (30 °C) field near Medicine Hat, Alberta, from which heavy oil with an American Petroleum Institute (API) gravity of 12−18° is produced by water injection. Souring, the reduction of sulfate to sulfide by sulfate-reducing bacteria (SRB), can be controlled by nitrate injection, but at MHGC application of this technology has some unique challenges. Water-soluble volatile fatty acids (VFA; typically acetate, propionate, and butyrate) often serve as electron donors for sulfate or nitrate reduction in oil fields.[1] However, MHGC field waters have very low VFA concentrations (~0.1 mM) and, instead, alkylbenzenes in oil (toluene and *m*- and *p*-xylene) were shown to serve as electron donor for nitrate reduction.[2] The electron donors used for sulfate reduction have not yet been identified. Second, monitoring of field-wide injection of ~2 mM nitrate indicated a 70% decrease in sulfide concentrations in production wells in the first 6−8 weeks of injection. However, this decrease was followed by a recovery to values observed before nitrate injection was started.[3] This effect was credited to the low temperature of the reservoir and sulfate limitation. When the reservoir is injected with water containing limiting sulfate (0.8 mM), the SRB will live in the near-injection wellbore region (NIWR). Inclusion of 2 mM nitrate in the injection water will stimulate growth of heterotrophic and sulfide-oxidizing and nitrate-reducing bacteria (hNRB and soNRB) in the NIWR, inhibiting the SRB through production of nitrite and other mechanisms, causing the decrease in produced sulfide. However, because of the low reservoir temperature, SRB grow back in a zone deeper in the reservoir, which is free of injected nitrate, restoring produced sulfide concentrations.

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Evidence for this zonation model came from the demonstration that injection of high nitrate pulses at a specific injector gave souring control at a neighboring producer.[3] Hence, it is possible to inject a nitrate dose that exceeds the respiratory capacity of the NIWR. Also, injection of bioreactors with excess VFA electron donor and limiting concentrations of sulfate and nitrate gave a zoned microbial community with the NRB *Arcobacter* present near the inlet and the SRB *Desulfobulbus* present further along the flow path. Pulsed injection of nitrate in this bioreactor system decreased sulfide production, as compared to continuous injection.[4]

Nitrate application to control souring in fields with high bottom-hole temperature is more straightforward,[5−8] possibly because most SRB activity is limited to the NIWR as the bulk of the reservoir is too hot. In contrast, at the MHGC field SRB activity needs to be displaced along the entire flow path from the injector to the producer. Although the prospects of achieving this seem daunting we have continued to monitor the aqueous sulfide concentrations in 15 producing wells for an additional 107 weeks since the previous study,[3] i.e., for a total of 219 weeks. Interestingly, a steep decline in produced sulfide concentrations has been observed recently and the possible reasons for this are analyzed in the current paper.

EXPERIMENTAL SECTION

Sampling Site Description. The same 23 sites were sampled as in a previous study.[3] This included 3 water plants (e.g., 1-WP), 2 injection wells (e.g., 14-IW), 14 production wells (e.g., 2-PW), and 3 makeup waters (e.g., 22-MW), as indicated in Figure S1.

Nitrate Injection. A 45% (w/w) calcium nitrate concentrate has been injected into water plants 1-WP and 17-WP since May 7, 2007 (week 1), and in 20-WP since September 15, 2008 (week 71). A constant concentration was injected from weeks 1 to 67 and 97 to 219, whereas nitrate pulses were injected from weeks 70 to 94.[3]

Sample Collection. A total of 64 sampling trips was made throughout the 4-year period. Samples were collected in 1-L Nalgene bottles filled to the brim to exclude air as much as possible. In addition 250-mL Nalgene bottles were filled to the brim and frozen on site with dry ice. Upon arrival in the lab, the 1-L bottles were transferred to an anaerobic hood (10% CO_2, 90% N_2), whereas the 250-mL bottles were stored frozen at −70 °C. Most samples had 5−10% oil, with the balance being produced water. A 5-mL water sample was removed and placed in a 15-mL Falcon tube prior to analysis.

Analytical Determinations. A volume of 125 μL was removed immediately for determination of the aqueous sulfide concentration. The data obtained were in good agreement with those for samples frozen on site.[3] In cases where the 5-mL sample contained some residual oil, 100 μL of dichloromethane was added and the sample was vortexed. Samples were then centrifuged and the cleared supernatant was used to determine the concentrations of sulfate, nitrate, and nitrite, as described elsewhere.[3]

Incubations of Produced Water with Oil and Nitrate or Sulfate. To a set of eight 120-mL serum bottles was added: 70 mL of produced water from 2-PW, 1 mM phosphate and 0.5 mL of MHGC oil; 10 mM nitrate or 20 mM sulfate were then added to duplicate bottles and 10 mM nitrate and 20 mM sulfate were added to four bottles. These incubations were referred to as N, S, and NS respectively. The headspace was 90% (v/v) N_2 and 10% CO_2, and the bottles were closed with butyl rubber stoppers. A sterile control contained 70 mL of autoclaved produced water from 2-PW. Incubations without oil were also done. Concentrations of sulfate, nitrate, nitrite, and aqueous sulfide were monitored in these incubations and were sacrificed when substantial nitrate and sulfate reduction was observed. The aqueous phase was used for community analysis and oil was extracted with dichloromethane according to Gieg et al.[9] The extracts were stored at 4 °C prior to analysis by gas chromatography−mass spectrometry (GC-MS).

Oil Analysis by GC-MS. GC-MS analysis was as described elsewhere.[9] A 1-μL volume of the DCM extract was injected by an autoinjector (7683B series, Agilent Technologies, Santa Clara, CA) into a gas chromatograph (7890N series, Agilent) that was connected to a mass-selective detector (5975C inert XL MSD series, Agilent). The gas chromatograph was equipped with an HP-1 fused silica capillary column (length 50 m, inner diameter 0.32 mm, film thickness 0.52 μm; J&W Scientific) with helium as the carrier gas. Utilization of oil components as a function of time was determined as the decrease in ratio of the peak area for a given component to that of the internal standard. Phytane and *n*-pentadecylbenzene were used as the internal standards for depletion of *n*-alkanes (C6−C24) and alkylbenzenes, respectively. These are not utilized in anaerobic oil-degrading cultures.[9−11] The oil extraction efficiency was determined by comparing the composition of oil, extracted from the sterile control with that of the original MHGC oil.

Microbial Community Analysis. DNA was isolated from 100 mL of field samples and 3 mL of incubations using the Fast DNA Spin Kit for Soil and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA) as per the manufacturer's instructions. The extracted DNA was quantified using a Qubit fluorimeter (Invitrogen) and used for pyrosequencing.

Pyrosequencing. Pyrosequencing of 16S amplicons was done for four incubations and eight field samples. PCR amplification was for 25 cycles with 16S primers 926Fw and 1392R, followed by 10 cycles with FLX titanium primers 454T_RA_X and 454T_FwB, as described elsewhere.[12] Purified 16S amplicons (∼125 ng) were sequenced at Genome Quebec and McGill University Innovation Centre, Montreal, Quebec with a Genome Sequencer FLX Instrument, using a GS FLX Titanium Series Kit XLR70 (Roche Diagnostics Corporation). Data analysis was conducted with Phoenix 2, a 16S rRNA data analysis pipeline, developed in house.[12] High quality sequences, which remained following quality control and chimeric sequence removal, were clustered into operational taxonomic units at 3% distance by using the average linkage algorithm.[13] A taxonomic consensus of all representative sequences from each of these was derived from the recurring species within 5% of the best bitscore from a BLAST search against the SSU Reference data set SILVA102.[14] Amplicon libaries were clustered into a Newick-formatted tree using the UPGMA algorithm with the distance between libraries calculated with the thetaYC coefficient[15] as a measurement of their similarity in the Mothur software package.[16] The Newick format of the sample relation tree was visualized using Dendroscope.[17] The entire set of the raw reads is available from the Sequence Read Archive at NCBI under accession number SRP008318.

RESULTS

Sulfate and Nitrate Concentrations in Injection Waters. Oil production in the MHGC field is through produced water reinjection. Injection water is composed of

produced water and makeup water mixed in an average ratio of 2:1 at 1-WP, the main water plant which also provided makeup water for 17-WP (Figure S1). The output from the Medicine Hat sewage treatment plant (Figure S1: 22-MW) was the source of makeup water at 1-WP and the primary input of sulfate in the system. Mixing of 22-MW (average 2.7 mM sulfate; range 1.6−4.5 mM; N = 10) with sulfate-free produced water gave injection water leaving 1-WP with an average sulfate concentration of 0.8 mM (range 0−1.6 mM). This concentration fluctuated depending on system demands for makeup water, but the average showed little change over the 219 week period (Figure 1A).

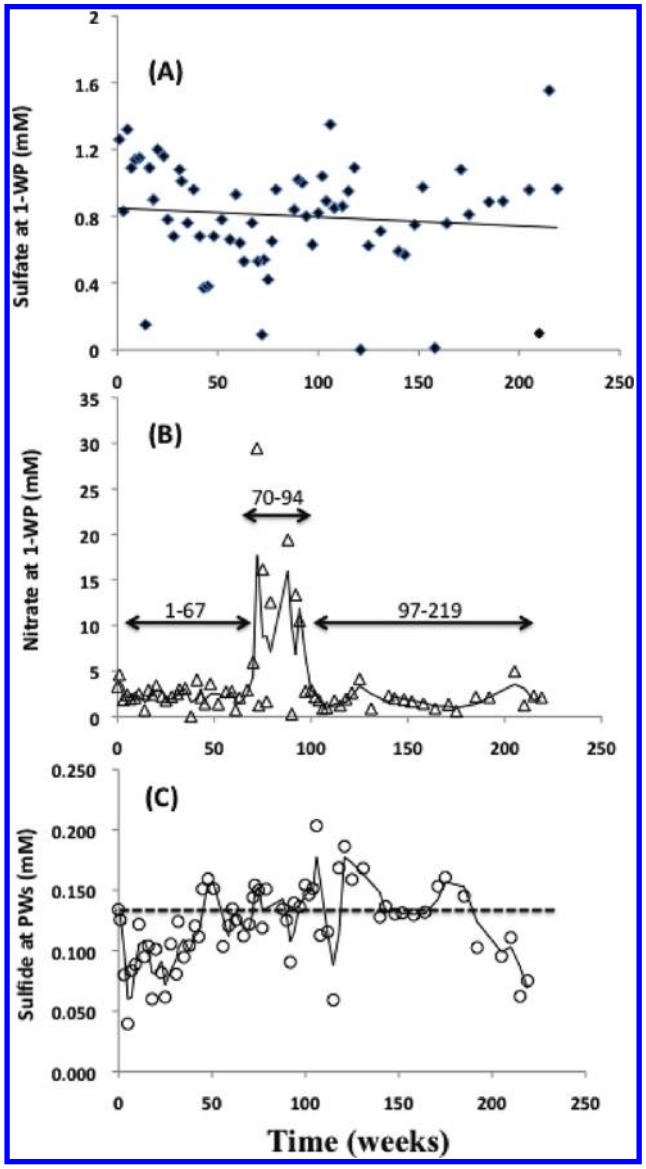

Figure 1. (A) Sulfate and (B) nitrate concentrations at 1-WP, representing the concentrations injected in the field. (C) Volume (V_i) weighted average sulfide concentration ($\Sigma c_i V_i/\Sigma V_i$) for 14 producing wells (i = 1−14) determined over a 4-year period. The dotted line represents the average sulfide concentration prior to nitrate injection. Data for individual wells are shown in Figure S2.

Nitrate was injected continuously at the water plants as a 45% (w/w) calcium nitrate concentrate. Average nitrate concentrations in injection water leaving 1-WP were 2.4 mM (range 0.7−4.6 mM) for weeks 1−67, 11.0 mM (range of 0.3−29.4 mM) for weeks 70−94, and 2.0 mM (range 0.6−5.0 mM) for weeks 97−219 (Figure 1B).

Effect of Nitrate Injection on the Aqueous Sulfide Concentration in Produced Water. The volume-weighted, average sulfide concentration for 14 production wells is shown in Figure 1C. As discussed previously,[3] the aqueous sulfide concentration dropped by 70% in the first 6−8 weeks. However, this was followed by a recovery to values that existed prior to nitrate injection after 43 weeks (Figure 1C). Field-wide injection of nitrate pulses from week 70 to 94 (2 weeks at 2 mM, followed by 2 weeks at 15−30 mM) did not lower the average sulfide concentration.[3] However, the return to a continuous injection of 2 mM nitrate from weeks 97 to 219 lowered the average sulfide concentration in produced water significantly from weeks 192 to 219, i.e., after 4 years of nitrate injection.

Effect of Nitrate Injection on Sulfide Production by Individual Wells. The aqueous sulfide concentration (c_i in mM), the volume of produced water (V_i in m^3/day), and the total amount of produced sulfide (c_iV_i in mol/day) are shown as a function of time in Figure S2 for all 14 production wells. Production well 2-PW contributed significantly to the maximum total sulfide production of 250−300 mol/day (Figure S2E) with production of up to 120−160 mol/day (Figure S2A). However, nitrate and nitrite broke through in produced water collected from 2-PW from week 192 onward (Figure 2B). This caused the aqueous sulfide concentration to drop to zero and the sulfate concentration to reach values of 0.3−1.6 mM (Figure 2A), similar to those in injection water (Figure 1A). The elimination of sulfide production from 2-PW, as a result of nitrate injection, is the main reason for the observed drop in average sulfide concentration in produced waters from week 192 onward (Figure 1C). However, several other producing wells (3-PW, 5-PW, 9-PW, 11-PW, and 16-PW) also showed significant declines in c_i (Figure S2). Nitrate breakthrough has not yet been observed in these wells but may be starting in 9-PW and 16-PW (results not shown). Well 7-PW has not produced significant sulfide throughout the 219-week period. On the other hand there were wells with no or only moderate declines in produced sulfide and no nitrate or nitrite breakthrough (4-PW, 10-PW, 12-PW, 13-PW, 15-PW, and 18-PW). Data for 10-PW are shown in Figure 2D and E.

Incubation of MHGC Oil with Nitrate and Sulfate. Toluene and, to a lesser extent, *m*- and *p*-xylene in MHGC oil have been shown to serve as electron donors for the reduction of injected nitrate.[2] However, the oil components used for reduction of injected sulfate have not been identified. Produced water from 2-PW was therefore incubated with MHGC oil, 1 mM phosphate, and either nitrate, nitrate and sulfate, or sulfate only. Addition of phosphate accelerates microbial activity.[2] All added nitrate was reduced within 40 days with formation of nitrite, which was in turn reduced within 90 days (Figure 3A). The main product formed is N_2.[18] When both nitrate and sulfate were present, nitrate reduction preceded sulfate reduction (Figure 3B), whereas when only sulfate was present up to 18 mM sulfate was reduced in 170 days (Figure 3C).

Reduction of nitrate or sulfate through incubation of produced water from 2-PW with MHGC oil resulted in changes in oil composition as compared to a sterile control. Nitrate reduction was coupled to utilization of toluene, *m*- and *p*-xylene, and ethylbenzene (Figure S3-D), in agreement with earlier results.[2,19,20] Estimation of *n*-alkane to phytane peak

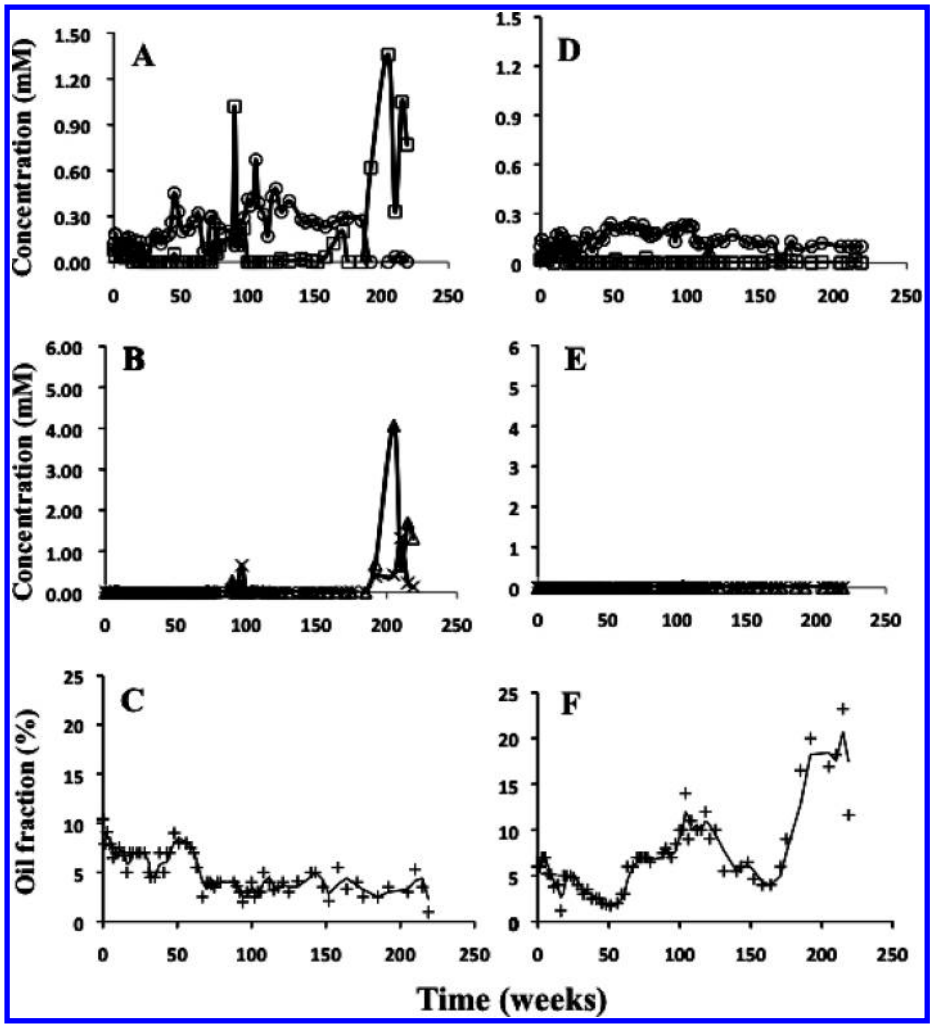

Figure 2. Change in the concentration of (A, D) sulfate (□) and sulfide (○), and of (B, E) nitrate (Δ) and nitrite (×), as well as in (C, F) the percentage fraction of oil (+) in 2-PW (A, B, and C) and 10-PW (D, E, and F) during 219 weeks of nitrate injection. The oil fraction (%) is the volume of produced oil over the sum of the volumes of produced oil and produced water. Producing well 2-PW showed breakthrough of nitrate, nitrite, and sulfate and zero sulfide from week 192 onward. This was not seen in 10-PW.

ratios indicated that C6−C24 *n*-alkanes were not consumed under nitrate-reducing conditions (Figure S3-A). However, sulfate reduction was coupled to utilization of C6−C17 *n*-alkanes. These were significantly depleted in oil extracted from incubations with sulfate after 170 days (Figure S3-C). In addition to alkanes, toluene, xylenes, ethyl-, *n*-propyl-, and higher alkylbenzenes were also used (Figure S3-F). Incubations of MHGC oil with both nitrate and sulfate showed strong depletion of alkylbenzenes and a more limited depletion of *n*-alkanes (Figure S3-B, E), than was observed in incubations with sulfate alone. Overall the data indicate that hNRB use a narrower range of oil components (primarily toluene and some other alkylbenzenes) than SRB, which use both alkylbenzenes and *n*-alkanes.

Analysis of Microbial Community Composition. Comparison of sequences in amplicon libraries for incubations N90, NS120, NS205, and S170 and in amplicon libraries for field samples indicated two main clusters (Figure 4). Cluster I included libraries from incubations N90, NS205, and NS120, as well as water from 20-WP. Cluster II contained libraries from the 7 other field samples. These have high fractions of the methanogens *Methanoculleus* (14−59%), *Methanosaeta* (17−35%), *Methanolinea* (7−18%), and *Methanocalculus* (1−16%). The frequency of taxa represented in these amplicon libraries is listed in Table S1. Due to the presence of a high content of *Pseudomonas*, N90 (66%) and 20-WP (42%) clustered closely together in the phylogenetic tree (Figure 4). Units receiving nitrate or exhibiting nitrate breakthrough had a higher fraction of the hNRB *Thauera*. The values were 7.4% for 14-IW, 4.4% for 2-PW, 2.8% for 20-WP, and 1.1% for 17-WP, whereas for producing wells without current nitrate breakthrough these were 0.8% for 18-PW, 0.6% for 3-PW, 0.4% for 7-PW, and 0% for 4-PW (Table S1). Incubations NS120 and NS205, containing produced water and oil amended with sulfate and nitrate, also had a significant fraction of 1.2−1.4% of *Thauera*. Other hNRB in the communities comprising cluster I included *Azoarcus*, *Arenimonas*, *Comomonas*, and *Rhodocyclaceae*. The sequences in the amplicon library for S170, the incubation with oil and sulfate, were distinct from those in clusters I and II. This community was dominated by *Smithella* (17%) and *Kosmotoga* (10%) and had high fractions of the SRB *Desulfobacterium* (7.5%) and *Desulfovibrio* (4.2%).

Composition of Oil Produced by 2-PW and 10-PW. The reasons for nitrate breakthrough in 2-PW following 4 years of nitrate injection (Figure 2A, B) were investigated in more detail. Because toluene and other short-chain alkylbenzenes are

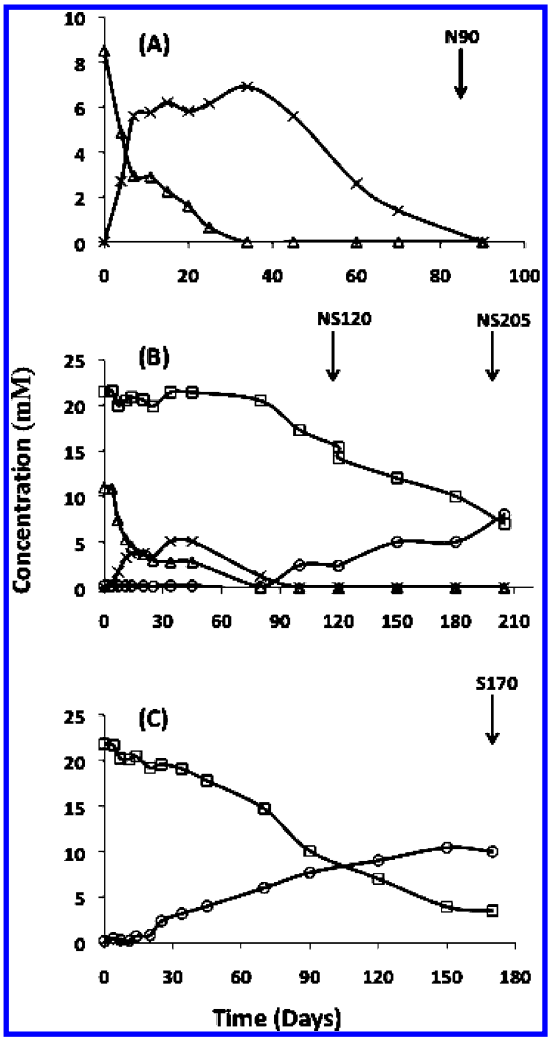

Figure 3. Change in the concentration of nitrate (Δ) and nitrite (×) and of sulfate (□) and sulfide (○) in incubations of produced water from 2-PW and oil with (A) 10 mM nitrate, (B) 10 mM nitrate and 20 mM sulfate, and (C) 20 mM sulfate. Duplicate serum bottles were removed as indicated (↓). These were labeled by the type and length of incubation, i.e., N90 was an incubation with nitrate for 90 days.

a preferred electron donor for the reduction of nitrate, we considered the possibility that nitrate breakthrough is associated with toluene depletion in the produced oil. The oil produced by 2-PW at 192 weeks was compared with that produced by 10-PW, where no nitrate breakthrough and only a limited decrease in sulfide concentration were observed (Figure 2C, D). Relative to the internal standard C15-benzene, oil from 10-PW still had a higher fraction of toluene and ethylbenzene than were present in oil from 2-PW. By drawing on the bank of frozen samples it was established that the oil produced by 2-PW still had appreciable toluene in week 51, i.e., one year after nitrate injection was started, but was depleted of toluene since week 79 (Figure 5B). Increasing, but still only partial depletion of ethylbenzene, *m*-, *p*-, and *o*-xylene, and *n*-propylbenzene, but not of *n*-octylbenzene was also observed in oil from 2-PW. In contrast, the fraction of toluene and other alkylbenzenes did not decrease with time in oil from 10-PW (Figure 5A). Hence, oil produced by 2-PW became increasingly depleted of alkylbenzenes as a function of time.

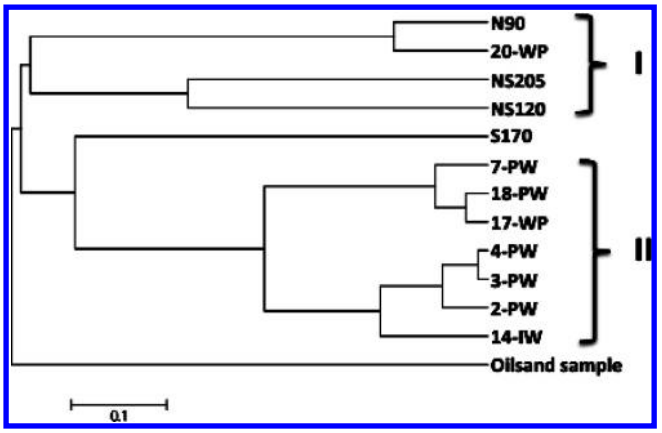

Figure 4. Relational tree for amplicon libraries from incubations of produced water from 2-PW and oil with nitrate (N90), sulfate (S170), or nitrate and sulfate (NS120 and NS205), as well as from field samples collected from 17-WP, 20-WP, 14-IW, 2-PW, 3-PW, 4-PW, 7-PW, and 18-PW. The amplicon library from an oil sands tailings pond served as the outgroup. The tree was generated using the UPGMA algorithm and was visualized with Dendroscope. Bar represents 0.1 substitutions per nucleotide position.

DISCUSSION

Souring control with nitrate has been especially successful for high-temperature reservoirs injected with seawater. Souring in these reservoirs is caused by the preferential growth of SRB in the NIWR[5] and is relatively easily controlled by nitrate, because of the high temperature elsewhere in the reservoir. Although the presence of thermophilic sulfate- or sulfur-reducers has been demonstrated,[21,22] their contribution to the overall souring activity remains to be quantified.

The sulfate and nitrate, injected in the low-temperature field studied here, were completely reduced along the flow path indicating, in sequential order, a zone of nitrate reduction, a zone of sulfate reduction, and a zone of methanogenesis.[4] Nitrate breakthrough at 2-PW did not decrease the fraction of methanogenic genera (Table S1), although it should be pointed out the sample was collected in week 192, when nitrate breakthrough was first observed (Figure 2B).

Comparison of microbial community compositions indicated separation according to the electron acceptor used (Figure 4). Cluster I consists of communities primarily engaged in nitrate reduction with oil, whereas cluster II is dominated by methanogens. The community in S170, where sulfate was the sole electron acceptor, was distinct from both of these. The high fraction of sequences for methanogens in 17-WP and 14-IW reflects the fact that these receive approximately 70% methanogen-rich produced water and 30% makeup water. Exposure to injected nitrate was, apparently, too short to change the planktonic community composition. Water plant 20-WP was exceptional in its high content of *Pseudomonas* (42%), similar to that found in N90 (66%), and its near-absence of methanogens. *Pseudomonas* species are capable of reducing nitrate and this genus has been observed before in enrichments with nitrate and VFA.[23] All units receiving nitrate (17-WP, 14-IW, and 20-WP) or exhibiting nitrate breakthrough (2-PW) had a higher fraction of *Thauera* (Table S1), a betaproteobacterium that can use toluene or VFA as electron donor for nitrate reduction.[24] In view of this it is likely that *Thauera* is a major component of the nitrate-reducing zone in the reservoir.

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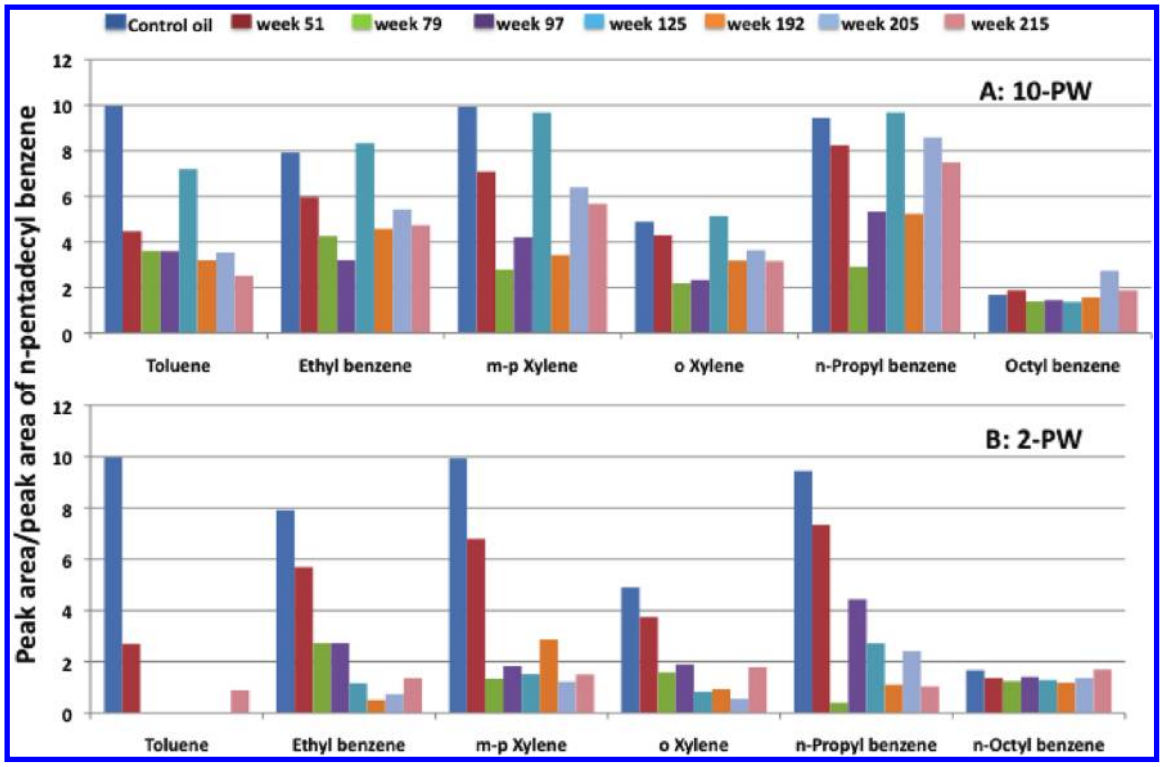

Figure 5. Depletion of alkylbenzenes in oil extracted from produced waters collected from (A) 10-PW and (B) 2-PW as a function of time. The presence of alkylbenzenes is represented as the ratio of peak areas of alkylbenzene versus pentadecyl benzene.

Other hNRB in cluster I (Figure 5) included *Azoarcus*, *Arenimonas*, and *Rhodocyclaceae*. *Azoarcus* has previously been detected in samples from wells in the same field that showed nitrate breakthrough.[18] *Azoarcus* is a nitrate-reducing, alkylbenzene degrader,[19,20] whereas the nitrate-reducing *Arenimonas* has been isolated from oil-contaminated soil.[25]

Incubation of produced water with oil and sulfate gave a community dominated by *Smithella*, a syntrophic deltaproteobacterium that metabolizes propionate through interspecies hydrogen transfer to methanogens,[26] and *Kosmotoga*, an anaerobic heterotroph capable of degrading oil organics.[27] *Smithella* may conceivably also partner with *Desulfovibrio*, which has a limited capacity to degrade oil organics but is great at capturing H_2. *Desulfobacterium*, which couples complete oxidation of oil organics to CO_2 to the reduction of sulfate,[28] was found only in incubation S170 (Table S1).

Hence pyrosequencing indicated the microorganisms that may be present in the zones of nitrate reduction, sulfate reduction, and methanogenesis thought to exist in the MGHC field. Toluene and some other alkylbenzenes serve as the electron donor for nitrate reduction, whereas a wider variety of oil components is used for sulfate reduction (Figure S3). Although injection of high nitrate pulses at a single injector has controlled the sulfide output of a neighboring producer for the duration of the pulsed injection (Figure S2D), field-wide application of this strategy has not yet been successful.[3] However, four years of continuous injection has led to control of sulfide production, coupled to breakthrough of sulfate, nitrate, and nitrite at 2-PW (Figure 2A, B). The sulfide concentration in producers 3-PW, 5-PW, 9-PW, and 16-PW has also reached zero (Figure S2). As a result, the average sulfide concentration for all 14 producers that were monitored has decreased significantly since week 192 (Figure 1C).

In explaining these observations we note that the fraction F (%) of produced oil, shown for 2-PW and 10-PW as a function of time in Figure 2C and F, when averaged for the 4-year period was 4−5% for 2-PW, 3-PW, 9-PW, and 5-PW, 6−8% for 7-PW, 10-PW, 12-PW, and 13-PW, 10−12% for 4-PW, 15-PW, 16-PW, and 18-PW, 17% for 19-PW, and 22% for 11-PW. Hence, zero sulfide production with (2-PW) or without (9-PW, 3-PW, and 5-PW) nitrate breakthrough correlated with a low fraction of produced oil. Exceptions were 7-PW (8% of oil), which has not produced significant sulfide throughout the 219-week period, and 16-PW (12% of oil), which produced a higher fraction of oil and also had near-zero sulfide. A possible reason for this correlation is that the nitrate in water to the toluene in oil ratio is higher for wells with a low, as compared to wells with a high, F value. The concentration of toluene in MHGC oil has been estimated at 5 mM.[2] Contacting this oil with 20 volumes of 2 mM nitrate could thus represent an excess of nitrate electron acceptor over toluene electron donor in view of the following equation:[29]

$$C_7H_8 + 7.2NO_3^- + 0.2H^+ \rightarrow 7HCO_3^- + 3.6N_2 + 0.6H_2O$$

This analysis does not take into account the fact that other alkylbenzenes are also partially oxidized, and that injected nitrate may contact more oil than what is being produced on its travel from injection to producing wells. We thus assume that the hNRB in the reservoir predominantly use electron donors in oil that is being produced for reduction of nitrate. Given this assumption, we expect nitrate breakthrough (Figure 2B) and production of toluene-depleted oil (Figure 5B) once the fraction of produced oil drops to 5%, when injecting 2 mM nitrate. In view of this, nitrate breakthrough in more wells with low F may be anticipated. Unfortunately, because we do not

have estimates for breakthrough times based on data with injected tracers, we cannot be sure when this will happen.

From this analysis it follows that the nitrate dose that should be applied to achieve souring control cannot be stated unequivocally. If a constant concentration of 10 mM had been applied, we might have reached zero sulfide in most of the producing wells analyzed in this study by this time. It also appears that the zones of nitrate reduction, sulfate reduction, and methanogenesis present along flow lines are not static, but move from injectors to producers with continued nitrate injection.

■ ASSOCIATED CONTENT

Supporting Information

Three figures with information on sampling sites in the MHGC field, flow of produced water, sulfide concentrations in 14 producing wells, and the utilization of alkanes and alkylbenzenes in incubations of produced water and oil; one table showing the distribution of pyrosequencing reads into taxa. This material is available free of charge via the Internet at http://pubs.acs.org/.

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