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Storage of oil field-produced waters alters their chemical and microbiological characteristics

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Abstract Many oil fields are in remote locations, and the time required for shipment of produced water samples for microbiological examination may be lengthy. No studies have reported on how storage of oil field waters can change their characteristics. Produced water samples from three Alberta oil fields were collected in sterile, industry-approved 4-l epoxy-lined steel cans, sealed with minimal headspace and stored under anoxic conditions for 14 days at either 4°C or room temperature (ca. 21°C). Storage resulted in significant changes in water chemistry, microbial number estimates and/or community response to amendment with nitrate. During room-temperature storage, activity and growth of sulfate-reducing bacteria (and, to a lesser extent, fermenters and methanogens) in the samples led to significant changes in sulfide, acetate and propionate concentrations as well as a significant increase in most probable number estimates, particularly of sulfate-reducing bacteria. Sulfide production during room-temperature storage was likely to be responsible for the altered response to nitrate amendment observed in microcosms containing sulfidogenic samples. Refrigerated storage suppressed sulfate reduction and growth of sulfate-reducing bacteria. However, declines in sulfide concentrations were observed in two of the three samples stored at 4°C, suggesting abiotic losses of sulfide. In one of the samples stored at room temperature, nitrate amendment led to ammonification. These results demonstrate that storage of oil field water samples for 14 days, such as might occur because of lengthy transport times or delays before analysis in the laboratory, can affect microbial numbers and activity as well as water sample chemistry.

Keywords Ammonification · Nitrate · Sulfate · Sulfate-reducing bacteria · Sulfide

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Introduction

The production of sulfide by sulfate-reducing bacteria (SRB) in oil field waters can lead to corrosion of equipment, impaired oil production because of reservoir plugging, "souring" of produced oil and gas, and health risks to workers [40]. The conventional response to souring is application of biocides, but these are expensive and can be hazardous to human health and the environment [8]. Recent research has focused on the control of sulfide in oil field environments by the addition of nitrate (reviewed by [14]). Among other effects, nitrate may stimulate heterotrophic nitrate-reducing bacteria (HNRB) to compete for nutrients with SRB [19]; enable the oxidation of sulfide by nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) [21]; and lead to the production of nitrite, which inhibits SRB activity [17].

Due to the inherent difficulty of monitoring reservoir behavior directly, samples of produced water collected from the oil field are frequently employed as proxies. Produced water is separated from the oil:water mixture brought to the surface at the oil well head. Using a produced water sample to obtain a reliable assessment of nitrate-mediated control of biogenic sulfide requires that the concentrations of important chemical species, such as sulfide, sulfate and organics, as well as the size and composition of relevant microbial populations, accurately represent the reservoir environment. However, because these

parameters alone are not sufficient to characterize community function or predict the success of nitrate amendment, they are often complemented by batch microcosm studies that permit observation of microbial activities over time in response to various amendments [9, 13, 20, 21]. This technique requires communities of viable cells and is only as accurate as the integrity of the sample. Avoiding contamination is an obvious and recognized issue in oil field sampling [25]; however, no research has yet been directed at changes induced in produced water samples by the commonplace delays resulting from shipping the sample from the field to the laboratory and storage at the laboratory until analysis occurs. As an illustration of transport time, Jenneman et al. [21] wrote that samples "were shipped the day of collection and received, usually, within 1 week." Storage times can also be lengthy, as noted by Gevertz et al. [16], who reported that oil field-produced waters were stored at room temperature (RT) for 3 days to 3 weeks before use in microbiological studies.

The remote location of many oil fields poses a logistical challenge to researchers. Transporting sampling apparatus and personnel to oil fields in order to obtain and immediately process produced water samples is time-consuming and costly, and forfeits a clean and controlled environment for experiment setup. An alternative approach is to supply on-site oil field personnel with sterilized, sealable vessels and detailed handling instructions, and to have the filled vessels shipped to the laboratory where they can be prepared for analysis under controlled conditions. However, the logistical savings of the latter scenario must be weighed against the potential compromise of samples, resulting from the delay in analysis.

Commonly, samples are transported and stored at refrigerated or ambient (room) temperature: ~4 and ~21°C, respectively. Refrigeration at 4°C slows substrate uptake and metabolism by many microorganisms without causing cell death [26], and is a common technique for mitigating distortion of environmental and wastewater samples during short-term storage [24, 34, 37]; however, the effectiveness of refrigeration for preserving oil field-produced water samples has not been documented. To the authors' knowledge, no published studies exist that examine the effects of storage on the anaerobic microbial communities relevant to oil field sampling in general or nitrate amendment studies in particular. Indeed, most available information on the effects of storage on microbial communities in water samples is limited to aerobic or facultative microbes, which typically metabolize and grow more rapidly than their strictly anaerobic counterparts.

Therefore, in this study we compared the water chemistry, microbial complement and response to nitrate amendment of water samples obtained from three different oil fields before and after a 14-day storage period. Samples stored at RT (~21°C) or at 4°C simulated ambient and refrigerated conditions, respectively. Changes in the monitored parameters were used to compare the stored samples to the original water and to make recommendations for oil field produced water sample handling.

Materials and methods

Oil field sample collection and storage

Water samples were freshly collected from three actively producing Alberta oil fields, each of which had been subjected to waterflooding operations for at least 10 years. Oil fields H and D, sampled in November 2007, have been described previously by Eckford and Fedorak [12] who designated them as fields P and N, respectively. Produced water samples from these fields had temperatures of ~30°C. Oil field B, sampled in September 2006, is located near Brooks, Alberta, and has been active since 1993, constantly under waterflood by produced water recirculation [20]. This reservoir has an in situ temperature of ~35°C. Water samples from all oil fields were obtained from free water knockout facilities [12] receiving produced water from souring wellheads. At our request, biocide treatment at oil field D was suspended at least 1 week prior to sampling. This precaution was taken to limit the direct effect of the additive on subsequent microbial activities in the laboratory. As far as could be ascertained by the oil field operators, there had been no biocide programs applied at oil fields B or H since the commencement of their operations.

Native, unstored ("original") water samples were collected from the free water knockout facilities in sterile 4-l polyethylene containers, which were filled to the top to exclude air. Within 30 min of collection, 100-ml portions of samples were transferred from the bottom of the container into sterile borosilicate glass serum bottles, which had been previously flushed with O_2-free N_2. The transfer was done using the hand pump system described previously [20] to minimize exposure to air. At the laboratory, the water from a single serum bottle was used for enumeration of metabolic groups as described below, and measurements of water chemistry were performed on replicate portions from six additional serum bottles. The remaining serum bottles were used to prepare "original" water sample microcosms (i.e., without storage) as described below.

Water samples intended for storage were collected in 4-l gas-tight, screw-cap steel cans lined with an inert mixture of 70% epoxy and 30% phenolic resin (Central Can Co., Chicago, IL). These cans are preferred for the handling and storage of aviation fuel samples according to ASTM standard D 4306 [3]. The can and an outer cardboard box

have Transportation of Dangerous Goods approval for shipment by air transport under the United Nations [39] designation UN 4G/Y4.6/S/09 (D. Wispinski, Fuels & Lubricants Group, Alberta Research Council, personal communication). In preparation for sampling, the cans were washed and sterilized as outlined by Hulecki et al. [20]. In the field, the cans were completely filled with water sample to minimize headspace and capped immediately for transport to the laboratory (<5 h) at ambient temperature. At the laboratory, the cans were stored stationary either at RT or at 4°C for 14 days, at which point the stored samples were subjected to chemical and most probable number (MPN) analysis, and used to prepare microcosms. Under no circumstances did the time required for complete sample processing (chemistry, microbial enumerations and microcosm amendment) exceed 7 h.

Microcosm preparation

Microcosms containing original or stored waters were prepared by aseptically transferring sample into sterile, pre-weighed, N_2-flushed serum bottles fitted with butyl rubber stoppers and aluminum crimps, using the apparatus described by Eckford and Fedorak [12]. Oil field B microcosms were prepared using 125-ml bottles filled with ~100 ml sample; oil field H and D microcosms used 158-ml bottles filled with ~150 ml sample.

The mass of sample in each serum bottle was adjusted to 99 g (oil field B) or 148 g (oil fields H, D) by withdrawing liquid using a sterile syringe. Triplicate microcosms received 1% (v/w) of a sterile brine containing the same approximate chloride concentration as their cognate oil field (oil field B, 7 g NaCl/l; oil fields H and D, 20 g NaCl/l), prepared under a N_2 headspace in boiled, deionized water. These microcosms are designated “unamended” because they received no exogenous nitrate. In contrast, the “amended” microcosms received 1% (v/w) of the same brine containing 1.0 M sodium nitrate (giving a final concentration of 10 mM nitrate amendment). All microcosms were incubated at RT in the dark.

Most probable number enumeration

Microbial counts were estimated using serial 10-fold dilutions and the 3-tube MPN method [2] as adapted to anaerobic enumeration by Eckford and Fedorak [12]. Dilution blanks and enumeration media for each water sample contained the appropriate chloride concentrations, given above. SRB, denitrifying HNRB, fermentative bacteria and methanogens were enumerated using the media and methods described previously [20]. The statistical method of Cochran [7] was used to compare MPN values.

Chemical analyses

At several timepoints during incubation, 1.5-ml liquid samples were aseptically removed from microcosms using sterile syringes. Samples were filtered (0.22-µm pore size; Millipore Canada) into sterile Eppendorf tubes: sulfide was quantified immediately, and the remaining sample was frozen at −20°C pending the other analyses. All analyses were completed within 3 weeks of the sampling date.

Total soluble sulfide was quantified using a colorimetric test kit (CHEMetrics, Calverton, VA). Sulfate, nitrate, nitrite and chloride were quantified using the ion chromatography method described by Eckford and Fedorak [13]. Acetate and propionate were quantified using a direct aqueous injection gas chromatography technique [15] using butyric acid (200 mg/l) as internal standard. Ammonium was analyzed colorimetrically [20].

Results

Changes in water chemistry after 14 days storage

Although all three produced water samples came from oil fields that historically had been souring, only samples from oil fields H and B initially contained detectable concentrations of sulfide (Table 1). Sulfate concentrations were also highest in these two samples. All three water samples contained detectable concentrations of acetate, and samples from oil fields H and D also contained propionate, but butyrate, isobutyrate and valerate were not detected in any of the three original waters. Neither nitrate nor nitrite was detected in any water sample (detection limit = 40 µM).

The oil field H sample showed evidence of microbial sulfate reduction after 14 days at RT (Table 1). Sulfate depletion (a change of -0.61 ± 0.08 mM, $P < 0.001$) was accompanied by accumulation of sulfide ($+0.47 \pm 0.08$ mM, $P < 0.001$), with concomitant depletion of propionate (-0.42 ± 0.01 mM, $P < 0.001$) and acetate (-0.38 ± 0.29 mM, $P < 0.01$). Assuming stoichiometric ratios of 1 mol acetate to 1 mol sulfate (Eq. 1) and of 1 mol propionate to 1.75 mol sulfate (Eq. 2), the amounts of acetate and propionate consumed over the storage period for oil field H sample at RT was theoretically sufficient to reduce 1.1 ± 0.3 mM sulfate to sulfide. However, only 55% of the theoretical amount of sulfate was reduced, suggesting the presence of an additional sink for acetate and propionate in the water samples. This may be due to the activities of methanogens (Table 1) or the incorporation of carbon into biomass. In contrast to oil field H at RT, sulfate concentrations in oil field H water stored at 4°C were only slightly lower than in the original water (-0.03 ± 0.02 mM, $P < 0.05$; Table 1). Sulfide

Table 1 Selected chemical and microbiological properties of original water samples and samples stored for 14 days at either 4°C or room temperature (RT)

Water sample	Oil field H			Oil field D			Oil field B		
	Original	14 days@ 4°C	14 days @ RT	Original	14 days @ 4°C	14 days @ RT	Original	14 days @ 4°C	14 days @ RT
Analyte concentration (mM)									
HS^-	0.48 ± 0.03[a]	0.37 ± 0.05[b]	0.96 ± 0.05[b]	<0.03	<0.03	<0.03	3.1 ± 0.2	2.8 ± 0.1[b]	3.0 ± 0.1
SO_4^{2-}	0.72 ± 0.01	0.69 ± 0.01[b]	0.11 ± 0.06[b]	0.027 ± 0.001	0.027 ± 0.002	0.002 ± 0.002[b]	0.19 ± 0.01	0.13 ± 0.07	0.17 ± 0.01
CH_3COO^-	5.3 ± 0.2	5.0 ± 0.1[b]	4.9 ± 0.1[b]	2.3 ± 0.1	2.1 ± 0.1[b]	1.8 ± 0.1[b]	0.34 ± 0.03	ND[c]	ND
$CH_3CH_2COO^-$	0.43 ± 0.01	0.42 ± 0.00[b]	0.010 ± 0.001[b]	0.047 ± 0.001	0.042 ± 0.001[b]	0.039 ± 0.006[b]	<0.01	ND	ND
MPN/ml									
SRB	2,300[d]	93,000[b]	9,300,000[b]	230	430,000[b]	1,100,000[b]	4,300	21,000	430,000[b]
	(400–12,000)	(15,000–380,000)	(1,500,000–38,000,000)	(40–1,200)	(70,000–2,100,000)	(150,000–4,800,000)	(700–21,000)	(3,500–47,000)	(70,000–2,100,000)
NR-SOB	15,000	43,000	7,500	75	230,000[b]	750[b]	1,500	21,000[d]	7,500
	(3,000–44,000)	(7,000–210,000)	(1,400–12,000)	(14–230)	(40,000–1,300,000)	(140–2,300)	(300–4,400)	(3,000–47,000)	(1,400–23,000)
HNRB	930	4,300	430	230	930	4,300[b]	240	93	9[b]
	(150–3,800)	(700–21,000)	(70–2,100)	(40–1,200)	(150–3,800)	(700–21,000)	(36–1,300)	(15–380)	(1–36)
Fermenters	2,300	93,000[b]	23,000[b]	23	93	93,000[b]	ND	ND	ND
	(400–12,000)	(15,000–380,000)	(4,000–120,000)	(4–120)	(15–380)	(15,000–380,000)			
Methanogens	230	2,300[b]	1,500	93	150	21,000[b]	ND	ND	ND
	(40–1,200)	(400–12,000)	(4,400–300)	(15–380)	(30–440)	(3,500–47,000)			

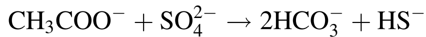
[a] Mean ± standard deviation ($n = 6$)

[b] Significantly different from value in original water ($P < 0.05$)

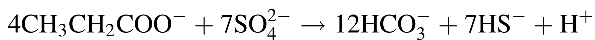
[c] Not determined

[d] MPN estimate with 95% confidence interval in parentheses

concentrations, however, were lower by 23% (a change of -0.11 ± 0.08 mM; $P < 0.01$). Propionate concentrations were virtually unchanged in oil field H water at 4°C relative to the original sample, although the decrease in acetate concentrations (-0.33 ± 0.26 mM, $P < 0.05$) was similar in magnitude to that seen in oil field H sample stored at RT.



$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$$
$$\Delta G^{\circ\prime} = -47.6 \text{ kJ/reaction} \quad (1)$$



$$4CH_3CH_2COO^- + 7SO_4^{2-} \rightarrow 12HCO_3^- + 7HS^- + H^+$$
$$\Delta G^{\circ\prime} = -341 \text{ kJ/reaction.} \quad (2)$$

RT storage of oil field D water resulted in a significant decline in sulfate concentration after 14 days (Table 1) (-0.025 ± 0.003 mM; $P < 0.001$), although sulfide concentrations remained below the detection limit of the assay (0.03 mM). Acetate concentrations in oil field D water at RT were 0.49 ± 0.23 mM lower than in original oil field D water ($P < 0.01$), a considerable excess over the theoretical amount needed for complete sulfate reduction in that water sample (Eq. 1). Refrigerated oil field D water showed no significant change in sulfate concentration relative to original oil field D water ($P > 0.05$; Table 1); again, sulfide concentrations were below the detection limit. As in oil field D water stored at RT, acetate concentrations were significantly lower in oil field D 4°C than in original oil field D water (-0.23 ± 0.18 mM; $P < 0.01$), suggesting an alternative sink for acetate. Although propionate concentrations declined significantly in both oil field D water stored at RT and 4°C relative to original oil field D water ($P < 0.05$), the magnitude of these changes (-0.008 ± 0.008 mM and -0.004 ± 0.003 mM, respectively) was negligible.

Relative to the original oil field B sample, no significant changes in sulfate concentrations were observed after 14 days storage at RT or 4°C (Table 1). Sulfide concentrations were unchanged in the RT sample (Table 1), but declined by 0.34 ± 0.35 mM, or $11 \pm 11\%$, after refrigerated storage ($P < 0.05$; Table 1). Acetate and propionate were not quantified in stored-water oil field B microcosms.

Changes in MPN estimates after 14 days storage

During the 14-day storage period, SRB MPN increased 4,000-fold in oil field H water at RT ($P < 0.001$; Table 1), providing further support for inference of microbial sulfidogenesis during storage. A 10-fold increase in fermenter MPN was also seen ($P < 0.05$). The 40-fold change ($P < 0.001$) in SRB MPN in oil field H sample at 4°C was significantly smaller than that seen in the actively sulfidogenic oil field H water at RT ($P < 0.001$), and agreed with the consistent sulfate, acetate and propionate concentrations (Table 1). Fermenter and methanogen populations in this 4°C sample were also significantly increased by 40-fold ($P < 0.001$) and 10-fold ($P < 0.05$), respectively.

In oil field D water stored at RT, the 4,800-fold increase in SRB MPN ($P < 0.001$; Table 1) was consistent with the chemical data suggesting active SRB metabolism. All other monitored microbial populations also increased significantly over the 14-day storage of water from oil field D: the largest changes were in fermenter (4,000-fold, $P < 0.001$) and methanogen populations (230-fold, $P < 0.001$), whereas HNRB (19-fold, $P < 0.01$) and NR-SOB population estimates (10-fold, $P < 0.05$) were less affected. Refrigerated storage of the oil field D sample (Table 1) resulted in significant changes only in SRB (1,900-fold increase, $P < 0.001$; not significantly different from change in RT sample, $P < 0.05$) and NR-SOB population estimates (3,100-fold increase, $P < 0.001$). The NR-SOB MPN increase seen after storage at 4°C was significantly greater ($P < 0.001$) than the 10-fold increase seen in oil field D RT.

In oil field B water stored at RT, the SRB MPN increased significantly over 14 days ($P < 0.01$; Table 1), although the 100-fold increase was much less than seen in the analogous samples from oil fields H and D (Table 1). In contrast, the SRB MPN in oil field B water stored at 4°C was not significantly different from the original sample ($P < 0.05$). Refrigerated storage of oil field B water led instead to a 14-fold increase in NR-SOB MPN ($P < 0.05$) and a 27-fold decrease in HNRB ($P < 0.001$). Fermenter and methanogen populations in the oil field B samples were not monitored.

Effects of storage on activity in unamended microcosms

Comparison of unamended microcosms (those devoid of exogenous nitrate) containing original and stored oil field H waters supported chemical data (Table 1) showing that sulfate reduction had occurred during storage at RT but was suppressed during refrigerated storage. Microcosms containing oil field H water that had been refrigerated for 14 days initially contained concentrations of sulfate and sulfide comparable to the original sample (Table 1). They produced 0.76 ± 0.08 mM sulfide from 0.58 ± 0.01 mM consumed sulfate between 5 and 10 days following assembly, very similar behavior to that seen in unamended, original-water microcosms ($+0.59 \pm 0.06$ mM sulfide and -0.64 ± 0.01 mM sulfate, over the same time interval). In contrast, because sulfate reduction in the RT sample had virtually gone to completion over the storage period (Table 1), no appreciable changes in concentrations of sulfate (near zero) or sulfide (near the theoretical maximum

of ∼1.1 mM) were seen in the unamended RT-water microcosms over the 62-day incubation period.

The sulfate concentrations in oil field D water samples were too low (Table 1) to permit accurate measurement of sulfate reduction in unamended microcosms. In unamended microcosms of oil field B, the lack of significant changes in sulfate or sulfide concentrations during incubation ($P < 0.05$ over 49 days) suggests that the microorganisms in this oil field water were unable to reduce sulfate using indigenous electron donors, a property reflected in other work with the sample [20].

Effects of storage on activity in nitrate-amended microcosms

The 14-day storage period affected the response of microcosms amended with nitrate. For each of the three produced water samples, the nature and magnitude of these changes were different depending on storage temperature.

The nitrate-amended microcosms prepared from the refrigerated oil field H sample (Fig. 1b) were most similar to the original-water oil field H microcosms (Fig. 1a) regarding nitrate, sulfide and acetate consumption. In both these triplicate series, nitrate was converted nearly quantitatively but transiently to nitrite, which subsequently appeared to be reduced along with consumption of acetate. Some minor differences were observed, most notably the production of slightly more ammonium in the refrigerated microcosms (6.6 ± 1.0% of consumed nitrate) than in the original microcosms (1.4 ± 1.9%; $P < 0.05$). Because ammonium was a minor product in these amended microcosms prepared from original and 4°C-stored oil field H water, denitrification was presumed to be the dominant pathway for nitrite depletion in these two sets of microcosms.

The activities in the triplicate microcosms prepared from RT-stored oil field H water were not reproducible. One microcosm (Fig. 1c) exhibited a pattern of nitrate reduction coupled to sulfide and acetate consumption similar to that seen in original oil field H microcosms (Fig. 1a), albeit considerably slower. Ammonification was also more apparent in this microcosm than in original oil field H microcosms, with ammonium accounting for 24% of the nitrate consumed during 62-day incubation. However, the other two microcosms exhibited a different response to nitrate amendment (Fig. 1d): ammonification dominated, with 98 and 85% of consumed nitrate being converted to ammonium by the end of the incubation and less nitrite accumulating transiently. Sulfide concentrations decreased slowly over the first 8 days following nitrate amendment, but regained their initial levels by 12 days, and subsequently declined to zero by the end of incubation. This pattern of changes in sulfide and nitrate chemistry was reminiscent of that found by Hulecki et al. [20] to be associated with nitrate-mediated sulfur cycling.

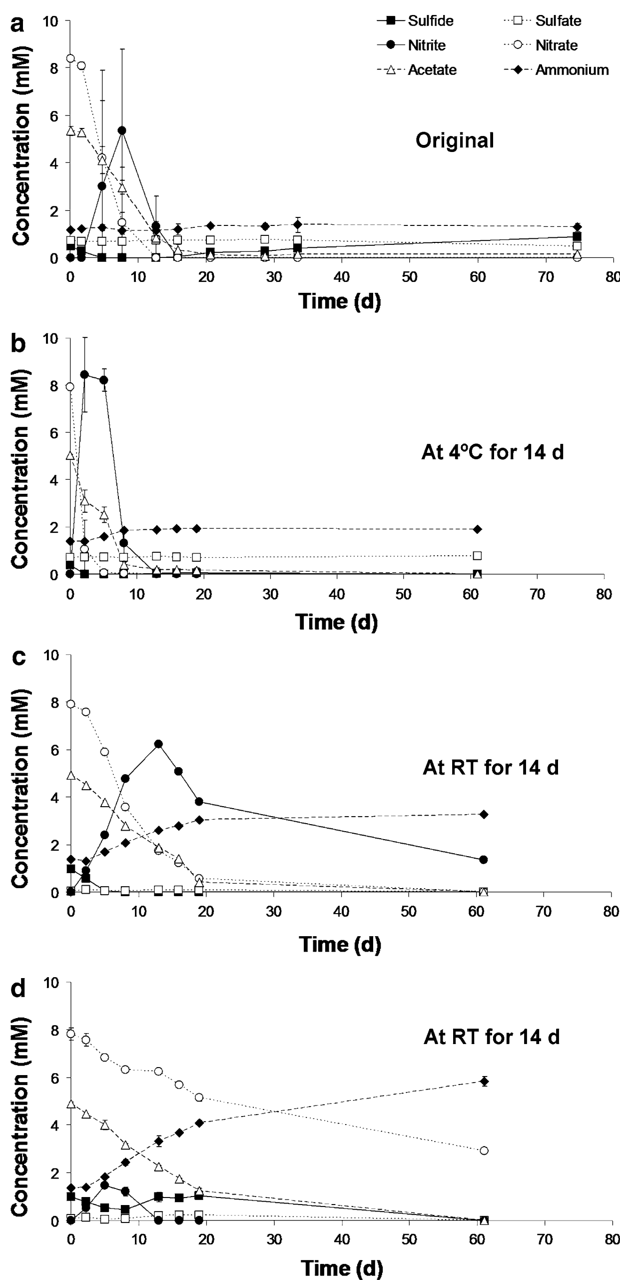


Fig. 1 Chemical analyses of nitrate-amended microcosms containing water from oil field H: original (**a**); stored at 4°C (**b**); stored at RT (one of three replicates) (**c**); stored at RT (other two replicates) (**d**). *Points* in (**a**) and (**b**) represent means of triplicate microcosms and in (**d**) the mean of two replicates. *Error bars*, where visible, show ± 1 standard deviation

Chemical changes in nitrate-amended microcosms prepared from refrigerated oil field D water (Fig. 2b) were only slightly different from those seen in original-water microcosms (Fig. 2a). Nitrate metabolism in the refrigerated-water microcosms commenced after a shorter lag time (<2 days) than the original microcosms (between 2 and

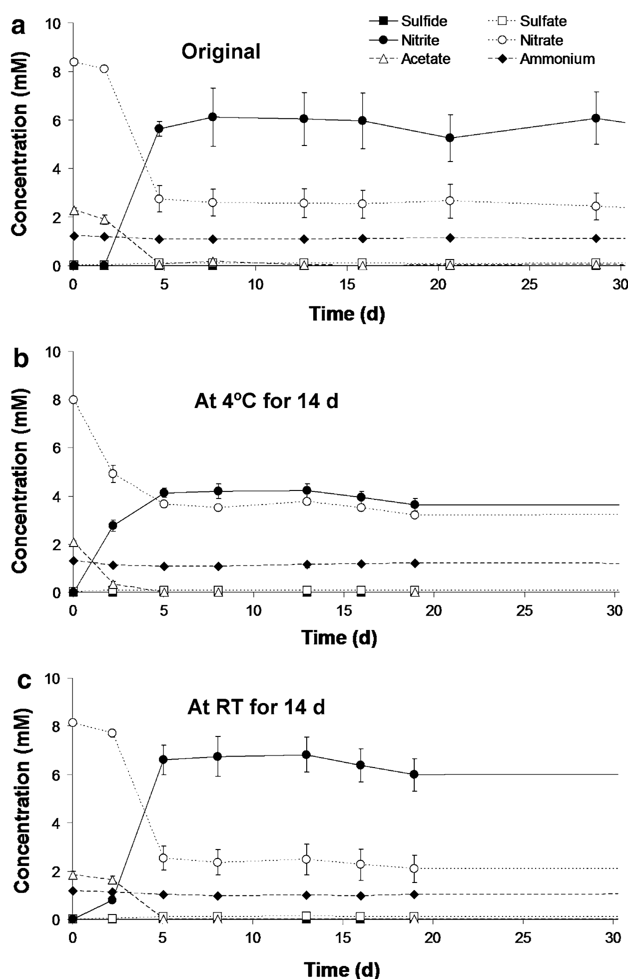


Fig. 2 Chemical analyses of nitrate-amended microcosms containing water from oil field D (original) (**a**); stored at 4°C (**b**); and stored at RT (**c**). *Points* represent means of triplicate microcosms; *error bars*, where visible, show ± 1 standard deviation. Analyte concentrations did not change significantly between the samplings at 19 and 61 days ($P > 0.05$); data between these timepoints are omitted for sake of clarity

5 days), and significantly less nitrate was consumed in the former than in the latter ($P < 0.05$; Table 2), despite complete consumption of acetate in both series.

Nitrate-amended microcosms prepared from oil field D water stored at RT (Fig. 2c) showed no significant difference in lag time or extent of nitrate consumption ($P < 0.05$) (Table 2) compared to the original water microcosms. However, microcosms containing RT-stored water accumulated a higher relative concentration of nitrite (1.00 ± 0.03 mol/mol nitrate) than those containing either original oil field D sample or the refrigerated sample ($P < 0.05$; Table 2). In contrast to stored-water oil field H microcosms, in which ammonification of nitrate was significantly more prevalent relative to original oil field H microcosms, ammonium production in all nitrate-amended oil field D microcosms was effectively nil (Table 2).

Sulfide consumption (Fig. 3a) and nitrate consumption (Fig. 3b) in nitrate-amended oil field B microcosms were comparable regardless of storage, with a slightly shorter (but significant; $P < 0.05$) lag time for sulfide removal from RT-stored water.

Discussion

Changes in water chemistry and sulfate reduction activity after 14 days of storage

No studies have addressed the effects of storage on the chemistry and microbiology of anaerobic, sulfidogenic produced water samples from oil fields. However, several studies do note the effects of storage temperature on the preservation of sulfide concentrations. In a study of pulp and paper mill process liquids, Douek et al. [11] found that chemical changes in soluble sulfur species, occurring at RT, could be mitigated by refrigeration or freezing. Putz and Strauβ [31] found that refrigerated storage minimized generation of H_2S and organic acids in wood pulp samples, but they nonetheless recommended the immediate measurement of redox potential and organic acid concentrations in such samples.

The results we obtained from stored oil field-produced waters agree with these findings. Sulfate reduction during storage at RT was an apparent source of chemical change in water samples from oil fields H and D water: significant sulfate consumption was detected in both samples after the 14-day RT storage (Table 1), and the metabolic product, sulfide, was also detected in the oil field H sample. Oil field B water did not show significant change in sulfate or sulfide concentrations at RT (Table 1) because of the inability of its microflora to reduce sulfate under the experimental conditions. Sulfate reduction appeared to be effectively suppressed by refrigeration, as demonstrated by the lack of change in sulfate concentrations in oil field H and D water samples stored at 4°C (Table 1). Refrigeration even preserved the state of sulfidogenesis to the extent that the lag time preceding sulfate reduction in unamended microcosms prepared from refrigerated oil field H water was the same as in the original unamended oil field H microcosms.

Although refrigerated storage clearly mitigated sulfate reduction, it also resulted in a net loss of sulfide from the only two samples in which sulfide was detectable, oil fields H and B (Table 1). These declines in sulfide concentration may have been due to a combination of removal by the lining of the storage cans, and chemical or microbial oxidation of sulfide as a result of contact with contaminating air. Although the resin mixture used to line the storage vessels used in this experiment is considered suitable for the handling and storage of fuel samples, no information exists with respect to its reactivity or permeability to

Table 2 Effects of storage and storage temperature on the activities of oil field D produced water in nitrate-amended microcosms

Water sample used in microcosms	Change in NO_3^- concentration over incubation period (mM)	Maximum molar ratio of NO_2^- produced to NO_3^- consumed	Molar ratio of NH_4^+ produced to NO_3^- consumed
Original water	−6.4 ± 0.5[a]	0.78 ± 0.07	−0.01 ± 0.01
Stored at RT for 14 days	−6.0 ± 0.5	1.00 ± 0.03[b]	−0.01 ± 0.01
Stored at 4°C for 14 days	−4.7 ± 0.1[b]	0.77 ± 0.06	−0.03 ± 0.01

[a] Mean ± 1 standard deviation ($n = 3$ for all series)

[b] Denotes significant difference from other values in column ($P < 0.05$)

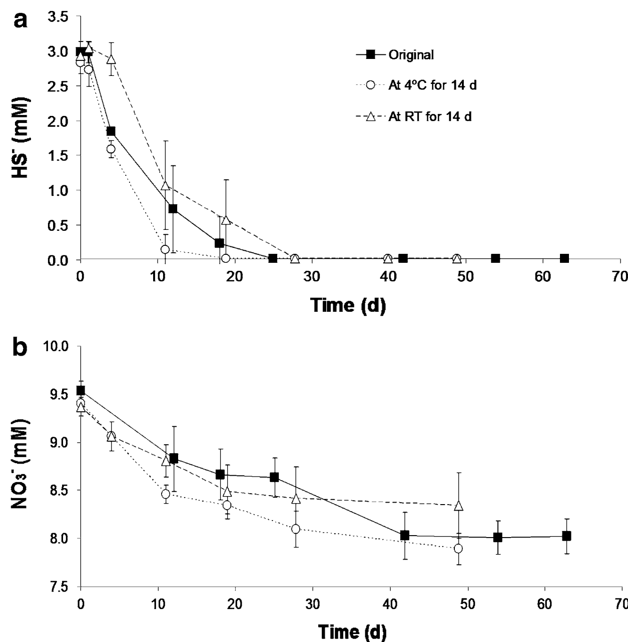


Fig. 3 Sulfide (**a**) and nitrate (**b**) concentrations in nitrate-amended microcosms prepared from original and stored oil field B water samples. *Points* represent means ± 1 standard deviation, among triplicate microcosms

sulfide. Loss of H_2S and other volatile sulfur compounds to other "inert" enclosure materials, including glass, Teflon and polycarbonate, has been demonstrated [23].

O_2 contamination could lead to the loss of sulfide through chemical oxidation [10, 38] or microbial oxidation by chemolithotrophic microorganisms [33]. The increased MPN estimates of facultative chemolithotrophic NR-SOB in all three refrigerated samples (Table 1), despite the absence of nitrate, may reflect the presence of O_2. Assuming stoichiometry of 1 to 2 mol O_2 per mol of S(-II) oxidized chemically [18, 27], the sulfide removed from the oil field H and B samples stored at RT would require between 48 and 96 ml, and between 150 and 300 ml, respectively, of air (21% O_2 v/v). The presence of this much air in the 4-l cans is quite unlikely, given that the sampling containers were filled to the top. In addition, the survival of O_2-sensitive methanogens in the stored samples (Table 1) argues against significant O_2 contamination.

However, if a small amount of O_2 had been introduced during sampling, under these O_2-limiting storage conditions, some chemolithotrophic bacteria may have oxidized some sulfide to elemental sulfur ($2H_2S + O_2 \rightarrow 2S^0 + 2H_2O$) or to thiosulfate ($2H_2S + 2O_2 \rightarrow H_2S_2O_3 + H_2O$) [41]. We did not analyze for these two potential sulfide oxidation products in the stored water samples.

Despite the possibility of these proposed sulfide removal mechanisms at both storage temperatures, decreased sulfide concentrations were seen only at 4°C. Any sulfide removal occurring in the samples from oil fields H and B stored at RT was probably masked by concomitant generation of sulfide from sulfate or other substrates. Refrigeration inhibited sulfidogenesis (Table 1), removing this masking effect.

Unlike the wood pulp samples studied by Putz and Strauβ [31], significant generation of acetate or propionate was not observed in any stored produced water samples: this was probably due to the lack of suitable organic substrates (such as plant biomass) for fermentation. Instead, acetate and propionate concentrations declined in samples stored at either temperature, likely to be a result of microbial metabolism (respiration, fermentation or biomass production), although the possibility of removal by abiotic processes, such as sorption to the container lining, cannot be excluded from consideration. Combined, stoichiometrically more acetate and propionate were consumed than would have been necessary to account for reduction of the observed quantity of sulfate. Excess acetate and propionate may have been consumed in biomass formation, or by microorganisms performing metabolisms other than sulfate reduction: notably, fermenter and methanogen MPN were significantly increased in the oil field D water sample stored at RT relative to original oil field D water (Table 1).

Changes in MPN estimates of microbial population sizes

Studies of coliforms and aerobic pathogens in environmental and effluent water samples [24, 30, 34, 37] have confirmed that refrigerated storage temporarily preserves the integrity of estimates of these communities over a short

storage period. However, the anaerobic nature of the produced water samples and microbial populations considered in this study precludes direct comparison with these studies, and necessitates some important qualifications. Apart from the slower cell growth associated with anaerobic metabolism, the presence of sulfide or sulfidogenic microbial populations in particular has implications for microbial enumeration. Sulfide is a reactive chemical that can exert significant influence over the microflora resident in a sample. Many anaerobic microorganisms present in oil field samples, including methanogens and SRB, require the reducing conditions provided by sulfide in order to respire. At the same time, sulfide is a toxicant to many microbes [6], including some methanogens [28] and even certain SRB [29, 32], and has been shown to modulate or completely inhibit nitrate metabolism by NRB [1, 5, 35, 36]. The size and activity of these microbial communities may therefore be affected by storage-related changes to sulfide concentration, discussed above.

The five anaerobic microbial metabolic groups monitored in this study exhibited different responses to storage and storage temperature. With respect to SRB, increased enumerations in samples from oil fields H and D stored at RT coincided with detectable sulfate reduction (Table 1). In contrast, the water from oil field B, which did not convert sulfate to sulfide, showed a relatively small SRB MPN increase after 14 days at RT. Suppression of sulfidogenesis during refrigerated storage was associated with smaller increases in SRB numbers in all three samples.

Significant increases in fermenter and methanogen MPN in oil field D water stored at RT (Table 1) and the consumption of more acetate and propionate than was required to account for reduced sulfate suggest that these communities were also active. The relatively small fold increases in fermenter and methanogen MPN seen in oil field H water stored at RT could be due to toxicant effects exerted by the increased sulfide concentration in that sample (Table 1).

Estimations of nitrate-reducing microbial counts after storage at either temperature were relatively stable, with the exception of NR-SOB MPN in refrigerated oil field D water (a 3,100-fold increase, Table 1). Given the absence of both nitrate and detectable sulfide in the oil field D water sample, this observation is not readily explicable in terms of cell growth, but may instead reflect an increase in viable particle concentration due to other means, such as dispersion of cell aggregates.

Effects of storage on microbiological responses to nitrate amendment

Although the effects of refrigerated storage on nitrate metabolism were minor relative to RT storage, storage at 4°C resulted in a small but consistent shift in microbial metabolic activity in the two sulfidogenic waters from oil fields H and D. Accelerated nitrate consumption was seen in microcosms prepared from both refrigerated samples (Figs. 1b, 2b), despite a lack of significant changes in HNRB numbers ($P > 0.05$; Table 1). This may have been linked to storage-related declines in sulfide concentration due to suppression of sulfate reduction in refrigerated samples. Sulfide concentrations as low as 0.03 mM have been shown to affect NRB metabolism [35], so potentially critical accumulations of sulfide, in oil field D water especially, may have gone undetected by our quantification method (lower detection limit of 0.03 mM).

RT storage of oil field H water increased the preponderance of ammonification (Fig. 1c, d) compared to original-water microcosms (Fig. 1a), likely as a result of the increased sulfide concentration resulting from RT storage, from 0.51 ± 0.03 mM to 0.96 ± 0.05 mM (Table 1). Sulfide is known to inhibit denitrification-specific enzymes in some NRB [36] and to induce denitrifying NRB capable of ammonification to adopt the latter pathway [5, 35]. Furthermore, the persistence of sulfide between 8 and 20 days (Fig. 1d) in the microcosms containing RT oil field H water represents an equilibrium shift in the sulfur cycle between sulfidogenic microbes and sulfide-oxidizing NR-SOB [20], possibly due to toxigenic effects of increased sulfide concentrations on NR-SOB [4, 22].

Consumption of sulfide and nitrate in oil field B microcosms was largely unaffected by storage at either temperature (Fig. 3). This correlates with the inability of the oil field B microflora to reduce sulfate under native conditions, relatively stable sulfide concentrations and thus less scope for sulfide-induced changes in nitrate metabolism as seen in the samples from the other two oil fields.

Conclusions

This is the first study of its kind to assess the effects of oil field-produced water storage. Here we collected waters from three oil fields with temperatures of ~30°C, and we demonstrated that storage of these samples causes changes in the chemical content and microbial communities in the samples. These changes are dependent both on storage temperature and sample source. In agreement with the extant literature regarding storage of environmental water samples, refrigeration is clearly superior to RT storage, although significant changes were seen after 14 days (possibly a worst case scenario for storage time) at both temperatures. Sulfide concentration appeared to be especially sensitive to storage. To ensure that data obtained from these produced water samples are representative of in situ water to the greatest possible extent, therefore, it is

advisable to avoid sample storage wherever possible and to refrigerate samples when storage is unavoidable. However, environmental parameters, such as salinity, pressure, temperature, pH and petroleum types, differ greatly in various oil reservoirs. Because of this variability, no standard set of optimal conditions for storing produced water samples during shipping may exist.

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