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Impact of light oil toxicity on sulfide production by acetate-oxidizing, sulfate-reducing bacteria

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ABSTRACT

Souring, the reduction of sulfate to sulfide by sulfate-reducing bacteria (SRB) is a serious problem for the oil and gas industry. Produced waters from oil fields, injected with seawater, often contain high concentrations of acetate and sulfate, indicating a lack of activity of SRB using acetate as electron donor for sulfate reduction. Experiments with an enrichment of lactate- and sulfate-utilizing SRB from a shale oil field, indicated oxidation of lactate to acetate and subsequent oxidation of acetate in the absence of added shale oil. However, acetate was not oxidized in the presence of shale oil. In view of these results we hypothesized that acetate-utilizing SRB were inhibited by low molecular weight components present in the light shale oil, which had an American Petroleum Institute gravity (API_G) of 38°. Low molecular weight components decrease in concentration with decreasing API_G of the oil. We therefore tested the effects of diluent (83° API_G, consisting predominantly of C5-C7 alkanes), light oils (38, 41 and 46° API_G) and heavy oil (16° API_G), as well as of selected light oil components (toluene, pentane and heptane). Acetate-dependent sulfate reduction by a pure culture of *Desulfobacter postgatei* at 30 °C was inhibited by diluent and light oils, but not by heavy oil. Similar results were obtained with a shale gas field source water enrichment (SWE), which contained *Desulfotomaculum* and *Desulfoarculus* as the most prominent acetate-utilizing SRB. Use of dilutions of oils in heptamethylnonane indicated that diluent inhibited acetate-utilizing SRB in SWE more strongly than light oils. Use of pure oil components indicated toluene to be more inhibitory than either pentane or heptane. GC-MS analysis confirmed that diluent had the highest concentrations of these low molecular weight components, explaining its strong inhibitory effect. Inhibition of acetate-utilizing SRB by light oil components, causing acetate accumulation in produced waters, may contribute to souring control and should be incorporated in souring control models.

1. Introduction

Souring is a major concern to the oil and gas industry and is most commonly caused by sulfate-reducing prokaryotes (SRP), which couple oxidation of organic compounds (including oil components) or hydrogen (H_2) to reduction of sulfate to sulfide (Gittel et al., 2009). SRP include sulfate-reducing bacteria (SRB) and sulfate-reducing archaea (SRA). We will refer to them mostly as SRB for the remainder of this paper, because SRA were not found in the samples used. Sulfate and SRP are often introduced into the reservoir with the water injected for secondary oil recovery (Stetter et al., 1993; Gieg et al., 2011), replacing or complementing indigenous microorganisms, which live in reservoirs with a resident temperature below 85 °C (Magot, 2005).

Metabolically SRB are subdivided into incomplete and complete oxidizers. Incomplete oxidizers will partially oxidize organic compounds like lactate, pyruvate, malate and succinate to acetate and CO_2, whereas complete oxidizers can also oxidize acetate to CO_2 (Muyzer and Stams, 2008; Rabus et al., 2015). Species of the genera *Desulfovibrio* and *Desulfomicrobium* are examples of incomplete oxidizers, whereas all or some species of the genera *Desulfobacter*, *Desulfobacterium*, *Desulfotomaculum*, *Desulfococcus* and *Desulfobacca* are complete oxidizers (Widdel and Pfennig, 1977; Muyzer and Stams, 2008; Rabus et al., 2015). SRB able to use oil components directly as electron donor for sulfate reduction include the

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toluene-oxidizing *Desulfobacula toluolica* (Wöhlbrand et al., 2012) and the alkane-oxidizing *Desulfatibacillum alkenivorans* (Callaghan et al., 2012). Although one might expect such hydrocarbon-degrading SRB to be highly active in oil fields, it has been suggested that anaerobic hydrocarbon degradation involves syntrophs (often *Deltaprotobacteria* or *Firmicutes*), converting hydrocarbons with water to H_2, CO_2 and acetate, which are then converted to methane by hydrogenotrophic and acetotrophic methanogens in the absence of sulfate, or used for production of sulfide by hydrogenotrophic and acetotrophic SRB in the presence of sulfate (Jones et al., 2008; Callbeck et al., 2013; Gieg et al., 2014). Transient accumulation of acetate, observed in oil-containing bioreactors injected with limiting concentrations of sulfate, suggested that hydrogenotrophic SRB were more active than acetotrophic SRB (Callbeck et al., 2013).

Crude oil can be classified as light or heavy based on its American Petroleum Institute gravity (API_G), which is related to its specific gravity (SG in g/cm^3) as API_G = (141.5/SG)-131.5. Light oils with API_G > 31° have high, potentially toxic concentrations of low molecular weight hydrocarbons, including benzene, toluene, ethylbenzene and xylene (BTEX), low chain length alkanes and others, making these oils more inhibitory to oil field microorganisms (Sherry et al., 2014). The substantial solubility of these low molecular weight components in water allows them to easily transfer from oil into the cytoplasmic membrane of microorganisms, which may then loose its integrity by increased permeability of protons and other ions (Sikkema et al., 1995).

High concentrations of acetate in produced waters from oil fields have been found by many authors (Mueller and Nielsen, 1996; Barth, 1991; Beeder et al., 1994; Pham et al., 2009). The presence of high acetate in produced waters with a high concentration of sulfate, as in many seawater-injected fields (Beeder et al., 1994), suggests low activity of acetotrophic SRB. The possibility that acetotrophic SRB are specifically inhibited by low molecular weight hydrocarbons in light oil is investigated in the current paper.

2. Materials and methods

2.1. Incubations with samples used in this study

A survey of samples used is provided in Table 1. All incubations were done in 120 ml serum bottles, containing 70 ml of anoxic CSBK medium (Callbeck et al., 2013) and a headspace of 90% N_2 and 10% CO_2 (N_2-CO_2). Medium was amended by injecting 1 M stock solutions of sodium sulfate, sodium lactate or sodium acetate using a 10 ml syringe. This procedure gave some variation in added concentration. The actual concentrations or concentration changes, as determined by HPLC will therefore be quoted in the text. Oil, a dilution of oil in heptamethylnonane (HMN), or oil components in HMN were added in a volume of 1 ml. Sample (3.5 ml) was used for inoculation and duplicate serum bottles were incubated at 30 °C with shaking (120 rpm) for 21–32 days. The concentrations of sulfate, sulfide, and acetate were always measured as a function of time, whereas that of lactate was measured in experiments with produced water enrichment 3PW_E only.

Produced water sample 3PW from a shale oil field (Table 1) was inoculated into CSBK with 1 M NaCl, 10 mM sulfate and 13 mM lactate. The high concentration of NaCl was added to match the salinity of the sample. The resulting enrichment (3PW_E) was used to inoculate duplicate serum bottles with the same medium in the presence or in the absence of 1 ml of LO_38 shale oil obtained from the same field. A culture of *Desulfobacter postgatei* (Widdel and Pfennig, 1981) was inoculated into 12 serum bottles with CSBK medium with 10 mM sulfate, 20 mM acetate and either no oil or 1 ml of HO_16, LO_38, LO_41 LO_46, or Dil_83 (Table 1). A source water from a shale gas field in northern British Columbia was inoculated in CSBK medium with acetate and sulfate (Table 1: SW enrichment or SWE). SWE was then inoculated into 12 serum bottles with CSBK medium, containing sulfate, acetate and oil as for the incubations with *D. postgatei*.

Incubations with SWE were also conducted with oil diluted with HMN by adding 0, 50, 100, 200, 400, 800 or 1000 μl of oil and 1000, 950, 900, 800, 600, 200 or 0 μl of HMN to a total volume of 1000 μl. Incubations were also done with dilutions of toluene, pentane or heptane in HMN as for oil. Control incubations received either no oil or 1000 μl of HMN without added oil.

2.2. Water chemistry

Sulfate was analyzed by High Performance Liquid Chromatography (HPLC) using a conductivity detector (Waters 423) and an IC-PAK anion column (4 × 150 mm, Waters) with borate/gluconate buffer at a flow rate of 2 ml/min. Lactate and acetate were determined using an HPLC equipped with a UV detector (Waters 2487 Detector) and an organic acids column (Alltech, 4.6 × 50 mm) eluted with 25 mM KH_2PO_4 buffer at pH 2.5. The concentration of aqueous sulfide was measured using the diamine method (Trüper and Schlegel, 1964).

2.3. Microbial community analysis

SWE (70 ml) was centrifuged at 12,000 rpm for 30 min and the pellets were suspended in Phosphate Buffer and mixed with MT Buffer from the Fast DNA® Spin Kit for Soil (MP Biomedicals) as per the manufacturer's instructions. Cells were lysed by the bead-beating method in a FastPrep® Instrument for 60 s at a speed setting of 6.0 in 2 ml Lysing Matrix E tubes (MP Biomedicals, Santa Ana, CA). Following further processing, according to the manufacturer's instructions (MP Biomedicals), DNA was dissolved in 10 mM Tris-Cl, pH 8.5 (Buffer E from the Qiagen QIA Quick kit, Qiagen). The extracted DNA was quantified using a Qubit fluorometer (Invitrogen), using a Quant-iT™ dsDNA HS Assay Kit (Invitrogen).

Table 1
Description of water samples, cultures and oils used in this study.

Name	Description
3PW_E	Enrichment of produced water sample 3PW from a shale oil field in the Bakken formation in Manitoba, Canada, producing LO_38.
Desulfobacter postgatei strain 2ac9	Pure culture of acetotrophic SRB from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, 2034)
SWE	Source water enrichment; sample from a fresh water storage lagoon from a shale gas field in the Montney formation, British Columbia, Canada, was enriched in medium with sulfate and acetate
Dil_83	Diluent with calculated API_G of 83°. Natural gas condensate used to dilute bitumen, consisting mostly of C5-C7 alkanes.
LO_46	Light oil with API_G 46°, produced from a conventional oil field in Papua New Guinea.
LO_41	Light oil with API_G 41° from a shale oil field in the Bakken formation in Saskatchewan, Canada.
LO_38	Light oil with API_G 38° from a shale oil field in the Bakken formation in Manitoba, Canada
HO_16	Heavy oil with API_G 16°, produced from conventional heavy oil field near Medicine Hat, Alberta, Canada.

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