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Reservoir souring control using benzalkonium chloride and nitrate in bioreactors simulating oil fields of western India

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ABSTRACT

Oilfields located in western India produce crude by injection of large amounts of water during secondary oil recovery. This water is obtained from underground aquifer and contains 5 mM sulfate approximately. Sulfate present in this water is converted in to sulfide by sulfate reducing bacteria (SRB) present in the reservoir. Increased concentration of sulfide in the production fluid negatively impacts oil production and is known as reservoir souring. Most probable number and quantitative PCR enumerated 10^3-10^8 SRB ml^{-1} in various field installations. Isolates from produced water in sulfate containing medium showed similarity with *Desulfotomaculum*, *Desulfomicrobium* and *Thermodesulfobacterium* genera. Similarly, isolates from produced water in nitrate containing medium were related to *Tepidiphilus*, *Paenibacillaceae*, *Kocuria* and *Thauera* genera. In the present study four sand packed bioreactors simulating moderately high temperature oil reservoir were used for different souring control treatments. Results revealed that continuous injection of 4 mM nitrate or 1.5 mM benzalkonium chloride (BAC) was not able to control souring. However, co-injection of 2 mM nitrate and 0.75 mM BAC completely ceased sulfide production and controlled souring.

1. Introduction

Microbial reduction of sulfate to sulfide by sulfate reducing bacteria (SRB) is known as reservoir souring. It is the deleterious process that the oil operator faces during oil production (Gieg et al., 2011). Souring is mainly initiated during the secondary oil recovery process (Voordouw, 2011; Cheng et al., 2018). In secondary oil recovery water is injected in the reservoir to re-pressurize and move the oil towards production well. Due to the scarcity of water for on-shore fields, industries practice produced water re-injection (PWRI). The additional amount of water required for this purpose can be obtained from the nearby wastewater plant, river or underground aquifer etc. (Kumar and Sinha, 2010; Agrawal et al., 2012). It is known as makeup water and usually is a source of sulfate in the reservoir. Presence of sulfate in make-up water leads to souring at the near injection wellbore region (NIWR). Sometime, during PWRI souring starts in above ground installations as soon as makeup-water is mixed with produced water (Voordouw et al., 2011). This is because of the presence of electron donor (residual oil and volatile fatty acids, (VFA)), electron acceptor (sulfate) in the make-up water and favorable growth condition in the above-ground installation (Agrawal et al., 2014). The presence of sulfide in the production fluid imposes a number of serious issues like health risk to the workers, detrimental for field infrastructure (e.g. corrosion) (Li et al., 2018), requires a significant investment on infrastructure, annual gas services (Al-Rasheedi et al., 1999; Hehn et al., 2016) and can lead to the complete or partial shutdown of production facility leading to significant economic loss.

Oil industries generally add biocides in the injection water to kill the microorganisms and control souring. Commonly used biocides in oil industries are Acrolein (Horaska et al., 2012), Glutaraldehyde (Glut) (Crolet, 2005), Tetrakis (hydroxymethyl) phosphonium sulfate (THPS) (Downward and Haack, 1997), Bronopol (Bryce et al., 1978), Benzalkonium chloride (BAC) (Lee et al., 2010) and Cocodiamine (Schaeufele, 1984). Acrolein and Glut are chemically reactive, killing the bacteria by protein or DNA adduction and by reacting with functional groups of protein (amines, amides, and thiol groups) (Gorman et al., 1980; Jones et al., 2010, 2012; Bartlett and Kramer, 2011; McGinley et al., 2011). However, BAC and Cocodiamine are cationic surfactant biocides that function by disruption of the cell membrane. BAC can effectively penetrate microbial biofilms and rupture the microbial cells (Jia et al., 2017). Another property of BAC is that it acts as an excipient (Duncan et al., 1995) allowing another drug to penetrate deeper and as a phase transfer catalyst allowing transferring of the chemical from one phase to another (Tezvergil-Mutluay et al., 2011).

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Although biocides are frequently used by the industries, they have several drawbacks like microbial resistance, health risk to workers and threat to the environment (Kaur et al., 2009; Gieg et al., 2011). In addition to this, biocides unselectively kill both harmful and beneficial microbes in the reservoir (Vance and Thrasher, 2005).

Recently nitrate injection has evolved as a greener alternative to control souring. Nitrate injection has been extensively studied in the laboratory (Myhr et al., 2002; Hubert et al., 2003; Callbeck et al., 2011) and in the field (Sunde et al., 2004; Bødtker et al., 2008; Voordouw and Grigoryan, 2009) during the last two decades. Nitrate injection can control the souring by several mechanisms explained earlier (Gieg et al., 2011; Rellegadla et al., 2017), however it has shown only limited success in real field condition except few examples (Jenneman et al., 1999; Larsen, 2002; Jurelevicius et al., 2008; Lambo et al., 2008; Voordouw and Grigoryan, 2009). The injection point, dose and duration are critical parameters controlling the success of treatment in the field (Kjellerup et al., 2005).

Recently co-injection of nitrate with different biocides (BAC, cocodiamine, THPS and Glut) was evaluated for control of souring. Greene et al. (2006) developed an index for a synergistic effect of nitrite and biocides. The experiments revealed strong synergistic effect with BAC, Glut and bronopol (Greene et al., 2006). Recently, Xue and Voordouw (2015) observed synergy between a long low pulse of BAC or Glut and continuous nitrate injection in controlling souring in sandpack bioreactors simulating low temperature oil fields (Xue and Voordouw, 2015). However, it is important to know, whether similar treatment strategy can also work in moderately high temperature oil fields (*in situ* temperature 55-60 °C).

The present research was done with the aim to design effective souring control strategy for moderately high temperature oil fields of western India. In this study, we enumerated oil field microbes including SRB and isolated the SRB and NRB from produced water. Further, we evaluated the effectiveness of biocide, nitrate, or combination of nitrate and biocide injection as different souring control strategies.

2. Materials and methods

2.1. Site description and samples collection

The western Indian oil fields are located in the state of Rajasthan and spread over 3111 km^2. These oil fields started producing in 2000 and showed the first instance of souring in 2009. Oil produced from these fields are high in paraffinic content and therefore the company operates its all facilities at moderately high temperature (Bhaumik and Mohanram, 2014) like heated pipelines and water injection. These fields consist of 27 zones and 1000 wells, out of which 200 wells are currently producing. Oil is produced by PWRI, for which makeup water is collected from an underground aquifer (T-NR) (Kothiyal et al., 2012). Acrolein is injected every fifth day in source water, storage tank and PWRI facilities as a measure to control souring. However, inconsistent number of SRB and high sulfide concentration in the field samples indicate ineffective souring control.

Samples were collected from three oil fields, named as Raj-M, Raj-B and Raj-A. Water movement in this oil field is shown in Fig. 1. Source water from wells 1-TSW, 2-TSW, 3-TSW, 4-TSW and 14-TSW move to common point 5-TSW. From 5-TSW water flows in to open pond 6-MSW. From 6-MSW water is transported to storage tanks (8-MIW and 9-MIW) via common inlet 7-MIW. Produced water from various fields also gets mixed with the source water in the storage tanks. This water in the storage tanks is heated at 55-60 °C and supplied to injection wells in all the three oil fields via common outlet 10-MIW. Samples were collected from all the above-mentioned points and a injection wells and production wells from all the three fields in January and February 2015. Samples were collected in 1 L sterile plastic bottles, filled up to the brim. Chemical analysis of the samples includes determination of the concentration of dissolved sulfide by diamine method (Trüper and Schlegel, 1964), sulfate by barium chloride method and nitrate & nitrite by sulphanilamide method (Ridnour et al., 2000).

2.2. Isolation of various microbes from produced water sample

Produced water from 16-MPW was inoculated in Coleville Synthetic Brine-K (CSBK) medium (Hubert et al., 2003) with 3 mM VFA (mixture of acetate, propionate and butyrate, Table S-1) and 10 mM sulfate for enrichment of SRB, 3 mM VFA and 10 mM nitrate for enrichment of heterotrophic nitrate reducing bacteria (hNRB) and 10 mM nitrate and 5 mM sulfide to enrich sulfide oxidizing nitrate reducing bacteria (soNRB) at 45 and 60 °C (sodium salts). Isolation of bacteria was done by roll tube method in serum bottles mentioned in Experiment S-1 (Hungate and Macy, 1973). When the colonies appeared (after 10-15 day) in serum bottles each colony was transferred to the respective liquid medium. After growth in liquid medium, DNA was isolated by MP Biomedical DNA Extraction kit (MP biomedical, Canada) as per the manufacturer protocol. 16S rRNA gene was amplified with 926F and 1392R primers and sequenced by Sanger sequencing method with 926F primer. Sequences were submitted to NCBI GenBank database with accession number KY419355 to KY419365.

2.3. Enumeration of SRB by most probable number (MPN) and quantitative PCR (qPCR)

MPN technique is the simplest and most frequently used cultivation-based method to enumerate SRB in the oil field. In this procedure, samples were serially diluted tenfold in API RP-38 (American Petroleum Institute Recommended Practice 38) medium. Incubation was done at 60 °C for 28 days. Growth was observed as blackening of the medium. The highest dilution at which growth has been observed, was used to estimate the number of bacteria per ml in the sample. MPN was done in triplicate for all the samples.

Enumeration of SRB and total bacteria was also done by qPCR. For this DNA was isolated from 200 ml of the field samples using MP Biomedical Soil DNA Extraction kit (MP biomedical, Canada) as per the manufacturer protocol. The qPCR was done as described earlier (Agrawal and Lal, 2009) with primers set DSRp 2060F (5′CAACATCGTYCAYACCCAGGG3′) and DSR4R (5′GTGTAGCAGTTACCGCA3′) for *dsr*B gene quantification of SRB and EUB341F (5′CCTACGGGAGGCAGCAG3′) and EUB534R (5′ATTACCGCGGCTGCTGCTGGC3′) for 16S rRNA gene quantification.

The qPCR analysis was performed in Lightcycler® 96 (Roche, Switzerland). Reaction volume is same for both the reactions (20 µl), which includes 10 µl 2× SYBR master mix, 0.2 µl of each primer, 8.6 µl molecular grade water and 1 µl DNA sample. The thermocycling program for *dsr*B gene qPCR was as follows: initial denaturation or pre incubation at 94 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 20 s and extension at 72 °C for 30 s. The amplification condition for 16S rRNA gene qPCR was initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s and extension at 72 °C for 20 s. Data acquisition was performed using SYBR green Type 1 detection channel after the PCR extension step.

For *dsr*B and 16S rRNA gene standard curve, triplicate dilution series of extracted plasmids pJET 1.2, were used (Agrawal and Lal, 2009). The linearity and reproducibility of the standard curves were tested.

2.4. Establishment of souring in bioreactors

Up-flow sand packed bioreactors were set up to simulate oil reservoir conditions. These bioreactors were designed with 60 ml plastic luer lock syringes with a thin layer of glass wool at the inlet port and packed with sterile sand (Sigma, 50-70 mesh size) (Callbeck et al., 2011; Xue and Voordouw, 2015). The outlet of the bioreactors was