International Biodeterioration & Biodegradation xxx (2016) 1–4

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Targeted microbial control for hydrocarbon reservoir: Identify new biocide offerings for souring control using thermophile testing capabilities

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ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form
14 July 2016
Accepted 29 July 2016

Keywords:
Thermophilic
Sulfide-producing
Biocide
Souring

ABSTRACT

Mesophilic and thermophilic sulfide-producing microorganisms can thrive in underground environments and cause hydrocarbon reservoir souring during energy recovery operations, and the temperature regime underground can affect the efficacy of biological control programs. In this study, we evaluated the efficacy of selected biocides using a thermophilic and a mesophilic sulfide-producing bacteria. A commonly used oilfield biocide, glutaraldehyde (Glut), and three non-traditional oil&gas field biocides, cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride (CTAC), 4,4-dimethyloxazolidine (DMO), and tris (hydroxymethyl) nitromethane (THNM), were used for the investigation. It was found that Glut was very effective against both mesophilic and thermophilic sulfide-producing bacteria. However, its efficacy persisted for shorter periods at 75° C compared to 35° C. Higher doses of Glut were required for complete bacterial kill over an extended period of time. As traditional preservative biocides, CTAC, DMO and THNM acted slower as compared to Glut. However, their efficacy was enhanced at elevated temperature. CTAC, DMO and THNM all showed improved performance at 75° C versus 35° C, and their efficacy persisted longer than Glut. This study highlights the potential of non-traditional oil&gas field biocides for microbial and souring control in reservoirs with challenging temperature conditions.

1. Introduction

Technologies such as waterflooding and hydraulic fracturing used in hydrocarbon energy recovery involve the injection of a large amount of water into the reservoir, which can stimulate microbial activity and sulfide generation, ultimately leading to reservoir souring (McInerney et al., 1993, McInerney and Sublette, 1997; Youssef et al., 2009). Biofouling causes significant issue in the oil and gas industry. Uncontrolled microbial growth can cause formation damage by bioclogging (Yarwood et al., 2006; Bottero et al., 2010) and the biogeneration of sulfides. Sulfide generation in the reservoir is not only a safety and health concern, but also reduces the economic value of hydrocarbon products, and increases the cost for maintaining asset integrity and product refining.

Biocide technologies have been developed in an attempt to prevent the initiation or mitigate the severity of reservoir souring (Ruseska et al., 1982; Pope et al., 1990; Johnson et al., 2008) but have limited success. The complex and drastic reservoir conditions such as high temperature coupled with the extremophilic organisms that thrive in these environments make reservoir microbial and souring control highly challenging. Subsurface microbiology studies have demonstrated that thermophilic microorganisms thrive in underground extreme environments, which can often be found in hydrocarbon reservoirs (Ghiorse and Wilson, 1988; Lovley and Chapelle, 1995; Onstott et al., 2009; Wang et al., 2013). These thermophiles, particularly sulfide-producing prokaryotes can cause severe reservoir souring during hydrocarbon recovery operations, when water from various sources, particularly sulfate-rich seawater is injected into the reservoir.

Although biocides are routinely used in waterflooding and hydraulic fracturing operations to prevent and mitigate biofouling and biogenic H_2S formation, they have not been extensively studied under reservoir-associated conditions such as testing against thermophilic organisms at elevated temperature (Enzien and Yin, 2011; Enzien et al., 2011). Conventional efficacy studies evaluate biocides against mesophilic bacteria at temperatures much lower

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http://dx.doi.org/10.1016/j.ibiod.2016.07.019
0964-8305/

than actual reservoir temperatures. However, biocides can behave differently at elevated temperatures due to their different thermal degradation profiles and modes of action (McGinley et al., 2011). In addition, mesophiles are not representative microorganisms in hydrocarbon reservoir with elevated temperatures, where thermophiles are often the dominant microbes (Orphan et al., 2000; Bonch-Osmolovskaya et al., 2003; Li et al., 2007). In order to identify new biocide solutions for reservoir microbial and souring control, we benchmarked three non-traditional oil&gas field biocides CTAC, DMO, and THNM against a biocide commonly used in oil and gas fields, Glut. Although CTAC, DMO, and THNM are not traditional oil&gas field biocides, they are well-known preservatives that have been used for a long time in material preservation (Wilfried, 2005). It was also previously discovered that these biocides are synergistic with quick-kill biocides such as Glut (Yin, 2010, 2014). In this study, a method of testing biocide efficacy against thermophiles was used to evaluate the biocidal efficacy of these biocides against thermophilic sulfide-producing *Thermotoga petrophila* at 75 °C. Mesophilic sulfide-producing *Desulfovibrio longus* were tested with the same treatments at 35 °C for comparison to understand the impact of temperature on the efficacy of these biocides. This work was presented at ISMOS 5 conference as a poster presentation.

2. Materials and methods

2.1. Biocides

All four biocides are products of The Dow Chemical Company (Table 1).

2.2. Bacterial culture and culture media

Cultures of a thermophilic bacterium, *Thermotoga petrophila* ATCC BAA-488, and a mesophilic bacterium *Desulfovibrio longus* ATCC 51456, were purchased from American Type Culture Collection (ATCC). Both are sulfide-producing bacteria. ATCC recommended culture media for each of these two bacterial strains were used for culturing these bacteria.

2.3. Matrices

For efficacy testing against both *T. petrophila* and *D. longus*, sterile sea salt based synthetic water was used as the testing matrix, which contained (per liter): 18 g of sea salt (Instant Ocean), 0.02 g of sodium lactate, 0.01 g of sodium acetate, and 0.1 g of reducing agent sodium thioglycolate at pH7. After autoclave, the matrices were cooled down and stored inside an anaerobic chamber with a 95% N_2 and 5% H_2 gas environment.

2.4. Efficacy test

The efficacy test was conducted inside a Bactron anearobic chamber with a 95% N_2 and 5% H_2 gas environment. Test matrix was inoculated with *T. petrophila* or *D. longus*, at a final concentration of ~10^7 cells per mL (determined by measuring the optical density of inoculums suspension at 620 wavelength, which was pre-correlated with viable cell counts enumerated via serial dilution culture method). One mL aliquots of this contaminated matrix were then treated with 20 μl of each of the biocides at 8 different active concentrations (200 mg/L, 133 mg/L, 89 mg/L, 59 mg/L, 40 mg/L, 26 mg/L, 18 mg/L, 12 mg/L). These treated samples and non-biocide controls were then incubated at 75° C (for *T. petrophila*) or 35° C (for *D. longus*) for 16 days under anaerobic conditions. During the incubation, aliquots (20 μl) of each treated and biocide treated sample were aseptically collected in triplicate at different time points (2 h, 24 h, 72 h, 168 h, 240 h, 384 h) and added to growth media (500 μl) and incubated at 75° C (for *T. petrophila*) or 35° C (for *D. longus*) for 7 days to check bacterial viability. If no growth happened after the incubation period, it was considered that a complete kill had achieved. Also, each sample was re-inoculated with 10 μl of about 10^7 cells per mL of *T. petrophila* or *D. longus* suspension after each sampling, except the 2hr time point. Biocidal efficacy was determined on the basis of the biocide concentrations required for complete kill (no growth in viability testing) of the test bacteria.

3. Results and discussion

3.1. Biocidal efficacy against mesophilic sulfide-producing *Desulfovibrio longus* at 35 °C

Most traditionally used oil and gas field biocides are those well-known disinfectant products such as Glut, quaternary ammonium compounds, THPS. CTAC, DMO and THNM are traditionally used as preservative biocides in many industry applications (Wilfried, 2005) and were not used in oil and gas field until recent years (Enzien et al., 2011; Raymond et al., 2014).

In this study Glut, CTAC, DMO and THNM were first tested against mesophilic sulfide-producing bacteria *D. longus*, at the optimum growth temperature of this bacterial strain, 35 °C. Glut has been known for its fast bacterial kill activity and has been used as disinfectant in industry and medical fields (McGinley et al., 2011; Venkatesh et al., 2014). It was demonstrated in this study that Glut was highly effective against *D. longus* for the entire testing period and was the most effective biocide among the four. (Table 2). As traditional preservative biocides, CTAC, DMO, and THNM had much slower killing action as compared to Glut, which reached its highest efficacy of this study in 2 h. DMO did not reach the highest efficacy after 24 h while CTAC and THNM did not reach the highest efficacy

Table 2
Biocidal efficacy against mesophilic sulfide-producing *Desulfovibrio longus*.

Biocide	Biocide concentration (mg/L A.I.) required for complete *D. longus* kill as determined in the viability tests[1]					
	2 hr	24 hr	72 hr	168 hr	240 hr	384 hr
Glut	12	12	12	12	12	18
CTAC	>200	200	89	39.5	89	133
DMO	>200	133	89	89	133	200
THNM	>200	>200	>200	133	133	200

[1]Non-biocide controls showed good growth in the study. Triplicates were used to determine complete control.

Table 1
Biocides tested in this study.

Product name	Active component
AQUCAR GA 50	Glutaraldehyde (Glut)
AQUCAR TA 64	cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride (CTAC)
AQUCAR A 78	4,4-dimethyloxazolidine (DMO)
AQUCAR TN 50	tris (hydroxymethyl) nitromethane (THNM)

Please cite this article in press as: Yin, B., et al., Targeted microbial control for hydrocarbon reservoir: Identify new biocide offerings for souring control using thermophile testing capabilities, International Biodeterioration & Biodegradation (2016), http://dx.doi.org/10.1016/j.ibiod.2016.07.019