Journal of Microbiological Methods 150 (2018) 55–60

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

A semi-continuous system for monitoring microbially influenced corrosion

Mary M. Eid, Kathleen E. Duncan, Ralph S. Tanner*

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, United States

ARTICLE INFO

Keywords:
Microbially influenced corrosion
Biocorrosion
Tween 80
Glutaraldehyde
Acid-producing bacteria
Sulfate-reducing bacteria

ABSTRACT

Microbially influenced corrosion (MIC), also known as biocorrosion, has significant impacts on the environment and economy. Typical systems to study biocorrosion are either dynamic (once-through flow) or static (serum bottle incubations). Dynamic systems can be materials and personnel intensive, while static systems quickly become nutrient limiting and exhibit long incubations. A semi-continuous biocorrosion cell was developed to address these issues. Low carbon shim steel was used as a test surface. Initial results revealed that 50 ppm glutaraldehyde (GLT), a common oil field biocide, in an abiotic cell was 3.6 times more corrosive (24.5×10^{-3} mm/y) than a biocorrosion cell inoculated with a sulfate-reducing bacteria (SRB) enrichment (6.73×10^{-3} mm/y). The SRB inoculated cell treated with GLT (50 ppm) reduced the corrosion rate from 6.73×10^{-3} mm/y to 3.68×10^{-3} mm/y. It was hypothesized that a biocide-surfactant combination would enhance biocide activity, thereby lowering corrosion in a semi-continuous biocorrosion cell. The biocide and surfactant were GLT (30 ppm) and Tween 80 (TW80; 100 ppm). MIC of SRB increased in the presence of a non-inhibitory concentration of GLT (23.4×10^{-3} mm/y), compared to the untreated +SRB condition (8.29×10^{-3} mm/y). The non-ionic surfactant alone reduced MIC (4.57×10^{-3} mm/y) and even more so in combination with GLT (3.69×10^{-3} mm/y). Over 50% of 16S rDNA sequences in the biofilm on the test surface were identified as belonging to the genera *Desulfovibrio* and *Desulfomicrobium*. The utility of a semi-continuous system for MIC studies and biocide testing was demonstrated. The concept of regular partial medium replacement is applicable to different corrosion cell and corrosion coupon geometries. Biocide-surfactant combinations may have the potential to reduce the concentration of biocides used in the field. In addition, a semi-defined medium for enumerating Acid-Producing Bacteria (APB) was developed, resulting in higher recoveries compared to a standard phenol red medium (e.g., 1.1×10^{4} APB/cm^2 vs $< 4 \times 10^{-1}$ APB/cm^2).

1. Introduction

The economic impact of corrosion accounts for 3% of the United States' gross domestic product (Koch et al., 2001). The total cost of corrosion in the oil and gas industry is estimated at $1.37 billion, with 30% of that cost being associated with microbially influence corrosion (MIC; Simmons, 2008). Microorganisms are a leading cause of corrosion in the oil and gas industry, impacting oil infrastructure such as pipelines, storage tanks and separators (Javaherdashti, 2017). MIC, also known as biocorrosion, is corrosion facilitated by the presence of microorganisms making kinetically unfavorable corrosive reactions favorable. Microbes enhance the rate of oxidation of the iron in oil infrastructure (Enning and Garrelfs, 2014; Javaherdashti, 2017). If untreated, holes and cracks can form causing failures, which result in dangerous environmental situations such as that in Prudhoe Bay, Alaska in 2006 (Meggert and Giguere, 2008). Official reports indicate this spill was caused by internal corrosion, or MIC, and a failure to carry out corrosion prevention (Fineburg, 2006).

Oilfield conditions typically select for anaerobic microorganisms. A microbial community in the oil field infrastructure can include *Clostridia*, *Deltaproteobacteria*, *Thermatogae* and *Synergistia* (Duncan et al., 2009; 2014; Liang et al., 2014). Sulfate-reducing bacteria (SRB), thiosulfate-reducing bacteria (TRB), acid-producing bacteria (APB) and iron-reducing bacteria contribute to MIC (Enning and Garrelfs, 2014; Javaherdashti, 2017). Most MIC studies focus on SRB and their role in iron corrosion, oil field souring and reservoir plugging (Beech and Sunner, 2007; Cord-Ruwisch et al., 1987; Enning et al., 2012). SRB belong to two archaeal and five bacterial phylogenetic lineages (Muyzer and Stams, 2008). The most common phylogenetic lineage is the *Deltaproteobacteria* followed by the *Clostridia* and *Thermodesulfobacteria* (Muyzer and Stams, 2008). Thiosulfate-reducers such as *Dethiosulfovibrio* and *Garciella* belong to the class *Clostridia* (Magot et al., 1997; Miranda-Tello et al., 2003). Members within these classes can be acid-producing, also influencing MIC. Field monitoring tests for these

* Corresponding author at: 770 Van Vleet Oval, Norman, OK 73019, United States.
E-mail addresses: mary.eid@ou.edu (M.M. Eid), rtanner@ou.edu (R.S. Tanner).

https://doi.org/10.1016/j.mimet.2018.05.018
Received 14 May 2018; Received in revised form 21 May 2018; Accepted 22 May 2018
Available online 24 May 2018

bacteria are often routine and can detect problematic levels of the bacteria associated with MIC. The accuracy of these methods is important.

Two types of systems, dynamic and static, are commonly used for studying MIC in a laboratory setting (Harris et al., 2010; Liang and Suflita, 2015). An example of a dynamic system is the once-through flow cell (Duncan et al., 2014; Harris et al., 2010; Stipaničev et al., 2013; Tanner et al., 1985). Dynamic systems allow for a more accurate modeling of pipeline corrosion. Due to the constant flow, dynamic systems can be material and personnel intensive. This system can examine multiple test surfaces simultaneously and is constantly flowing with medium and inoculum with a typical incubation time of 30 days. A serum bottle incubation is an example of a static system (Liang and Suflita, 2015). All components are initially added into the bottle with incubations ranging from 90 days to over 300 days (Cote et al., 2014; Liang and Suflita, 2015). In long incubations, once all the nutrients in the incubation vessel have been depleted, bacterial activity within the system is greatly reduced, including MIC. A semi-continuous system for studying MIC was developed to address these issues.

Preventing and treating MIC in the oil and gas industry is achieved with chemical inhibitors, also known as biocides. Glutaraldehyde (GLT) is a common biocide used in the oil and gas industry. GLT is an advantageous biocide due to its broad spectrum of activity, solubility, stability at a wide range of pH and salinities, and biodegradability (Javaherdashti, 2017; Leung, 2001). GLT is inexpensive and used individually and in combination with other compounds such as quaternary amines and nitrites (Greene et al., 2006). A preliminary biocorrosion study, using a low carbon steel test surface, revealed that 50 ppm GLT in an abiotic corrosion assay was 3.6 times more corrosive than those inoculated with a SRB enrichment (Appendix A). Biocides and corrosion inhibitors are generally not thought to cause corrosion but recent studies have focused on the affect biocides have on MIC and corrosion. One study found that most of the corrosion inhibitors tested promoted corrosion despite their ability to inhibit microbial activity (Harris et al., 2010). The inhibitors that were most effective at lowering viable cell counts also had the highest average pitting rate (Harris et al., 2010).

This study examined the use of a biocide-surfactant combination to control MIC. Surfactants, such as Tween 80 (TW80), are compounds used to reduce interfacial and surface tension, typically as emulsifiers, detergents and dispersants commonly used in food, pharmaceutical, environmental, research, cosmetic, and oil and gas industries (Jayashree and Vasudevan, 2007; Torres et al., 2012). In the oil and gas industry, surfactants are used as surface protectants, corrosion inhibitors and oil dispersants (Abdallah and El-Etre, 2003; Hegazy et al., 2012; Javaherdashti, 2017; Place et al., 2016). TW80 is ideal for use in the oil and gas because it does not contribute in galvanic corrosion due to its ion neutrality, is biodegradable and deemed environmentally safe (Banin et al., 2006). Some studies indicated the presence of a surfactant helped enhance the inhibitory activity of biocides (Gopi et al., 2000).

The purpose of this experiment was to determine the utility of a semi-continuous system to study MIC and to determine whether the presence of a surfactant enhanced biocide activity. It was hypothesized that, in a semi-continuous biocorrosion test cell, TW80 would enhance the effect of GLT, resulting in reduced MIC.

Additionally, a semi-defined medium (RST-APB) containing mineral, vitamin and trace metal solutions was developed for enumerating APB (Tanner, 2007). APB are fermentative microbes found in oil field infrastructure, anaerobic digesters, soil, sediment, human microbiota and fermented foods. Enumerating APB is useful to detect a potential MIC problems in oil and gas fields (Javaherdashti, 2017). Commonly used APB media contain peptone and beef extract as major medium components (American Petroleum Institute, 1982; NACE Standard, 2014). The undefined medium components were reduced in the semi-defined APB medium developed here.

2. Materials and methods

2.1. Inoculum and bacterial enumeration

SRB were enriched in 50 ml of SRB medium (Tanner, 1989; Tanner, 1995) in 125 ml serum seal bottles containing 1 g of powdered iron, prepared anaerobically under N_2 (Balch and Wolfe, 1976). Enrichments were inoculated with 0.5 ml of sediment collected from the Duck Pond located at the University of Oklahoma in Norman, OK. Enrichments were incubated for two weeks at 38 °C.

Three tube most probable number (MPN) technique was used to determine planktonic and biofilm counts for SRB and General Heterotrophic Bacteria (GHB; Banwart, 1981). MPN medium was SRB medium and the GHB MPN medium was 1/3 strength Tryptic Soy Broth (no. 211825, Becton Dickinson and Co., Sparks, MD). Medium was prepared anaerobically in Hungate type tubes (no. 2047-16125, Bellco Glass, Inc., Vineland, NJ). Enumerations were incubated for 28 days before a final count was taken.

2.2. A semi-continuous biocorrosion testing system

Low carbon steel shim (no. 16130, Precision Brand Products, Inc., Downers Grove, IL) was used as the test surface. Test surfaces were handled on a metal surface, such as clean aluminum foil, using forceps and gloves and cut into appropriate size (10 cm × 1.5 cm). The test surface was rinsed and cleaned with acetone twice and dried for 10 min in a drying oven at 100 °C. Initial weights were recorded and test surfaces were place inside a 25 mm Pyrex screw cap tubes (no. S76112G, Fisher Science Education) and taken into an anaerobic chamber (Coy Laboratories Products, Inc., Grass Lake, MI). Tubes were sealed with a no. 2 rubber stopper (no. 14-30D, Fisher Science Education) and removed from the chamber. Stoppers were cut flush with the tube top and secured with screw caps containing pre-drilled holes. Corrosion cells were gas exchanged under N_2 (Balch and Wolfe, 1976) and sterilized.

The medium used in the corrosion cells was a modified SRB medium (Tanner, 1995) designed to promote biofilm formation (/liter): 50 ml SRB feeding mineral solution; 5 ml vitamin solution (Tanner, 2007); 3 ml trace metal solution (Tanner, 2007); 1 g TES; 1.5 ml 60% sodium lactate; 0.5 g yeast extract (no. 212750, Becton Dickinson and Co.); pH 7.3–7.5. SRB feeding mineral solution contained (/liter): 90 g NaCl; 10 g Na_2SO_4; 5 g $(NH_4)_2SO_4$; 4 g $MgSO_4{\cdot}7H_2O$; 6 g KH_2PO_4; 0.5 g $CaCl_2{\cdot}2H_2O$. Forty ml of sterile SRB feeding medium was aseptically added to each corrosion cell. One ml of inoculum from the SRB enrichment was added to each biotic corrosion cell. Glass syringes were used (no. 5292, 5293 and 5294, Becton Dickinson and Co., Rutherford, NJ). Corrosion cells were incubated at 30 °C for seven weeks. Five ml was removed from each biocorrosion cell daily and replaced with 5 ml of SRB feeding medium.

Treatments began one week after the start of incubation, during which biofilms developed in the inoculated biocorrosion cells. The biocide was 30 ppm glutaraldehyde (GLT; Sigma-Aldrich, Co., St. Louis, MO) and the surfactant was 100 ppm TW80 (National Biochemical Corp., Twinsburg, OH). Treatment conditions included: untreated; +GLT; +TW80; +GLT + TW80 with and without SRB inoculum. The negative controls were the uninoculated tubes with no biocide or surfactant addition. The positive controls were inoculated cells with no biocide or surfactant addition. Treatments were performed in triplicate. Biocide and surfactant amendments adjusted 30 and 100 ppm final concentrations, respectively, at each feeding.

2.3. Final processing of corrosion cells and corrosion evaluation

Corrosion cells were disassembled inside an anaerobic chamber after final planktonic counts were taken. The test surface was suspended in 20 ml of sterile, anoxic 1% NaCl. Biofilm was mechanically removed from the test surface into a sterile reservoir (no. 13-681-502,