Corrosion Science 52 (2010) 1447–1452

Contents lists available at ScienceDirect

Corrosion Science

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

Eradication of the corrosion-causing bacterial strains *Desulfovibrio vulgaris* and *Desulfovibrio desulfuricans* in planktonic and biofilm form using photodisinfection

Cale N. Street *, Aaron Gibbs

BioCorrosion Solutions Inc., 5329-156th St SW, Edmonds, Washington, WA 98026, USA

A R T I C L E I N F O

Article history:
Received 25 September 2009
Accepted 16 December 2009
Available online 23 December 2009

Keywords:
A. Steel
C. Microbiological corrosion
C. Oxygen reduction
C. Pitting corrosion

A B S T R A C T

Oil and gas pipelines often fail prematurely due to "microbiologically-influenced corrosion" (MIC). This occurs when free-floating bacteria collect on the inner pipeline surface, eventually forming complex adherent biofilms. Photodisinfection is an effective antimicrobial approach for several biomedical applications. This study evaluated the antibacterial efficacy of photodisinfection against two sulfate-reducing bacterial strains implicated in the process of MIC. Results showed that treatment reduced planktonic bacterial viability by >99.99%. Treatment of biofilms reduced viability by 99.9%, which was greater than the antibacterial effect observed using the biocide benzalkonium chloride under similar exposure parameters. These results suggest that photodisinfection may be useful in addressing MIC in industrial pipelines.
© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

In order to satisfy demand for fossil fuels, over 2.3 million miles of pipeline network exists across North America. Oil and gas pipelines are at risk for premature failure due to internal and external corrosion processes. It has been estimated that 20–50% of internal corrosion is caused by bacteria growing on the inner pipeline surface, a phenomenon known as "microbiologically-influenced corrosion" (MIC) [1,2]. MIC is a complex process that begins with free-floating, planktonic organisms in the transport fluid collecting on the pipe surface and eventually forming complex adherent biofilms. Biofilms, which are extremely durable and resistant to physical removal, are formed when colonizing microbes encapsulate in a slimy, exopolymeric substance composed of secreted polysaccharides, proteins, and nucleic acids. In industrial settings, bacterial biofilms on surfaces result in local microenvironment differences in metabolic processes, pH, and dissolved oxygen, leading to the generation of active pitting corrosion cells [3]. Mature biofilms are usually polymicrobial, composed of anaerobic organisms in the deepest layer (nearest the steel surface) transitioning to aerobic species on the outside at the interface with the surrounding medium [4]. It is well known that bacteria in biofilms behave differently (genetically and physiologically) from their planktonic counterparts [5]. Furthermore, biofilms are much more difficult to eradicate by conventional means (biocides, physical/mechanical scraping) than planktonic bacteria due to strong adherence to surfaces and physical exclusion of antimicrobial substances [6].

Several types of bacteria, known collectively as sulfate-reducers (SRB), are able to reduce sulfate to hydrogen sulfide, a by-product that is highly corrosive to steel [7]. As an initial step in biofilm formation, SRB produce extracellular polymeric substances (EPS) that enable adhesion to steel and subsequent colonization [8]. A 2003 study by Zhu et al. used advanced techniques to characterize the microbial communities harvested from standard gas pipelines [9]. A total of 106 different bacterial DNA sequences were identified in these samples, and among those identified were sulfate-reducers, species that produce nitrates (contribute to metal corrosion), species that are known contributors to biofilm formation, organic acid producers, and hydrogen consuming methanogens. Other groups have reported similar results while characterizing bacterial species isolated from sour gas pipelines [10]. Particularly, bacterial strains from the *Desulfovibrio* genus have been extensively implicated in the corrosion of various types of steel and other alloys [3,4,11–13].

There are currently a limited number of effective strategies to inhibit biofilm growth and MIC inside industrial pipelines. Chemical biocides, consisting of various oxidizing and non-oxidizing agents such as glutaraldehyde and benzalkonium chloride, are most commonly used in practice. However, it has been well documented that these agents do not effectively penetrate established biofilms, and use may lead to the generation of resistant bacterial populations [14,15]. Other "green" strategies such as the use of plant extracts [16], aerobic biofilms [14], and reduction/oxidation modifiers [14] have been proposed, but these remain impractical for widespread commercial use to combat MIC.

Photodisinfection has been demonstrated to be an effective non-antibiotic antimicrobial approach for various biomedical applications [17–19]. This technology fundamentally involves the

* Corresponding author. Tel.: +1 (206) 393 2226; fax: +1 425 489 1101.
E-mail addresses: cstreet@biocorrosionsolutions.com (C.N. Street), aaron.gibbs@gmail.com (A. Gibbs).

0010-938X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.corsci.2009.12.022

use of light energy to activate a photosensitizer molecule, which then either passes its energy on directly to a substrate/target (type I photoreaction), or interacts with molecular oxygen to produce a range of reactive species (type II photoreaction). These reactions lead to the non-specific killing of bacterial cells primarily via lipid peroxidation and membrane damage [20–22]. The photosensitizer molecule used in photodisinfection is usually a dye or stain that absorbs light energy within a given wavelength range. The resulting antimicrobial effect is localized, and has been shown to be highly effective against established biofilms [23]. For this reason, photodisinfection may be useful in the decontamination of biofouled surfaces in industrial settings. This study was designed to evaluate the antibacterial efficacy of photodisinfection against two strains of *Desulfovibrio* genus bacteria known to cause MIC in oil and gas pipelines. The antibacterial effect was also compared with that of the chemical agent benzalkonium chloride, which is one of the most common biocides currently in use in the oil and gas industry.

2. Materials and methods

2.1. Bacterial culture and inocula

The bacteria used in this study were the sulfate-reducing strains *Desulfovibrio vulgaris* (ATCC#29579) and *Desulfovibrio desulfuricans* (ATCC#14563). Both organisms were grown on pre-reduced solid media (brucella blood agar enriched with haemin and vitamin K) at 37 °C under anaerobic conditions (Bactron IV Anaerobic Chamber, Sheldon Manufacturing, Cornelius, OR). Log growth phase bacterial inocula for planktonic experiments were prepared to a concentration of 10^7 colony-forming units per milliliter (CFU/ml) in sterile water using a Genesys 10 spectrophotometer (Thermo-Scientific, Pittsburgh, PA) to measure cell density as a function of absorbance at 420 nm. For biofilm growth, bacterial inocula was made to an optical density of 0.150 at 420 nm and diluted in tryptic soy broth.

2.2. Equipment and reagents

The photosensitizer dyes evaluated were rose bengal (Sigma–Aldrich, St. Louis, MO), safranin O (Sigma–Aldrich), methylene blue (Spectrum Chemical, Gardena, CA), and toluidine blue O (Sigma–Aldrich). All stock and working photosensitizer solutions were prepared in sterile water. Several samples in the planktonic photodisinfection assay were also supplemented with L-tryptophan (Sigma–Aldrich), a known quencher of singlet oxygen and other reactive oxygen species [24]. The media and photosensitizer test solutions used for the anaerobic photodisinfection exposures were pre-reduced under anaerobic conditions to remove all oxygen prior to use. Illumination was performed using a 670 nm non-thermal diode laser system (Ondine Biopharma Corporation, Vancouver, Canada) coupled via 600 μ glass fiber-optic cable and terminated at an SMA-type connector. For the broadband illumination experiments, a XD-301 series 150 W haloid lamp cold light source was used.

2.3. Planktonic culture photodisinfection assay

For planktonic exposures, bacterial inocula were added to test solution or water (for no treatment controls) in opaque 96-well plates, giving a final sample volume of 200 μl. While all stock bacterial cultures were grown under anaerobic conditions, exposures to photodisinfection were run in both anaerobic and aerobic conditions in order to determine the importance of local oxygen during the photoreaction. Immediately after exposure to the photosensitizer solution, samples were stirred (800 rpm using a magnetic stir bar) and illuminated from above with an energy dose of 20.6 J/cm^2 (340 mW/cm^2 for 60 s) using a non-thermal 670 nm diode laser. The experimental conditions evaluated were the following: (1) 0.01% w/v aqueous methylene blue with illumination in an anaerobic environment, (2) 0.01% w/v aqueous methylene blue/20 mM L-tryptophan with illumination in an anaerobic environment, (3) 0.01% w/v aqueous methylene blue with illumination in an aerobic environment, and (4) 0.01% w/v aqueous methylene blue/20 mM L-tryptophan with illumination in an aerobic environment. In addition, controls consisting of exposure of bacteria to photosensitizer or illumination alone were performed to ensure that neither of these variables contributed to bacterial killing on their own. After illumination, samples were serially diluted and plated on tryptic soy agar solid media for 48 h at 37 °C prior to performing colony counts. The entire experimental protocol was performed in the dark, and all samples were exposed and plated one at a time to limit ambient light exposure and to maintain the exposure time of bacteria to test solution uniform across all replicates. All experimental and control conditions were run in triplicate for each individual experiment, and experiments were performed on three separate occasions to verify reproducibility.

2.4. Biofilm culture photodisinfection assay

Two different biofilm growth/photodisinfection exposure protocols were used in this study. In the first protocol, used for treatment using laser light illumination and exposures to benzalkonium chloride, homogenous biofilms of *D. vulgaris* and *D. desulfuricans* were grown on plastic pegs using a previously published protocol and system (Innovotech, Calgary, Canada) [25,26]. Briefly, bacterial suspensions consisting of 10^8 CFU/ml were prepared in modified Barrs medium for sulfate-reducers (American Type Culture Collection), then diluted 1:40. 200 μl of this working inoculum was added to each well of a 96-well plate, and 96-peg lids were then placed such that one peg was suspended into each well. The plate assembly was then incubated at 37 °C on an Excella E2 gyrorotary shaker (Fisher Scientific, Pittsburgh, PA) at 125 rpm for 24 h. During the incubation period, the visible formation of an insoluble black residue in each well indicated bacterial metabolism, and after incubation a visible biofilm was evident on each peg. Preliminary recovery experiments showed that ~10^6 (*D. vulgaris*) and ~10^5 (*D. desulfuricans*) viable organisms per milliliter could be recovered from these biofilm pegs after 24 h in the absence of any antibacterial treatment. For photodisinfection treatment of biofilm pegs the protocol was as follows: using sterile forceps, pegs were broken away from the lid at the base and placed in sterile water for 60 s as a rinse to remove any planktonic, free-floating bacteria. Pegs were then placed in 300 μl of photosensitizer solution or sterile water (controls) for 30 s. The pegs were subsequently either held inverted for 60 s in the dark for "no-light" controls, or illuminated for 60 s (total energy dose of 13.2 J) using a non-thermal 670 nm diode laser. Immediately after illumination, biofilm pegs were placed in 1 ml of pre-reduced recovery media (PBS/0.5% Tween®-80). Disruption and recovery of surviving organisms from the peg was carried out by vortexing for 10 s, followed by 5 min of ultrasonication (Model 250HT ultrasonicator, VWR) and a final 10 s vortex step.

In the second biofilm growth protocol, used for exposures to photodisinfection under broadband light illumination, biofilms were grown on the inner surface of flat-bottomed 96-well plates (VWR, West Chester, PA). Briefly, 50 μl of bacterial inoculum in log growth phase was added to each well, and plates were incubated (37 °C) on an Excella E2 gyrorotary shaker at 125 rpm for 48 h in order to allow biofilm growth. Preliminary recovery experiments showed that 10^7–10^8 viable bacteria per ml could be recov-

Download English Version:

https://daneshyari.com/en/article/1471016

Download Persian Version:

https://daneshyari.com/article/1471016

Daneshyari.com