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ABSTRACT

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.

Keywords: MIC, microbiologically-influenced corrosion, Desulfovibrio, sulfate-reducing, photodisinfection

INTRODUCTION

Over 2.3 million miles of pipeline network exists across North America, and many portions of this network 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 forming complex adherent biofilms. 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]. Biofilms have been shown to be 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 [4].

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 [5]. A 2003 study by Zhu *et al.* found a total of 106 different bacterial DNA sequences in samples from standard gas pipelines [6], 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 [7]. Particularly, bacterial strains from the *Desulfovibrio* genus have been identified and implicated in the corrosion of various types of steel and other alloys [8-10].

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 [11, 12]. Other “green” strategies such as the use of plant extracts [13], aerobic biofilms [11], and reduction/oxidation modifiers [11] 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 [14-16]. This technology fundamentally involves the 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 [17-19]. 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 [20]. 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.

EXPERIMENTAL PROCEDURE

Bacterial and Equipment/Reagents

The bacteria used in this study were the sulfate-reducing strains *Desulfovibrio vulgaris* (ATCC#29579) and *Desulfovibrio desulfuricans* (ATCC#14563). Log growth phase bacterial inocula for planktonic experiments were prepared to a concentration of 10^7 colony-forming units per milliliter (CFU/ml). For biofilm growth, bacterial inocula was made to an optical density of 0.150 at 420 nm and diluted in tryptic soy broth. 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) 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 [21]. 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 micron glass fiber-optic cable and terminated at an SMA-type connector. For the broadband illumination experiments, a XD-301 series 150 watt haloid lamp cold light source was used.

Planktonic and Biofilm Photodisinfection Assays

For planktonic exposures, bacterial inocula were added to test solution or water in 96-well plates. 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. Samples were illuminated with an energy dose of 20.6 Joules per square centimeter (340 milliwatts per square centimeter for 60 seconds) 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.

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) [22, 23]. During biofilm growth, 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. 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 seconds as a rinse to remove any planktonic, free-floating bacteria. Pegs were then placed in photosensitizer solution or sterile water (controls) for 30 seconds. The pegs were subsequently either held inverted for 60 seconds in the dark for "no-light" controls, or illuminated for 60 seconds (total energy dose of 13.2 Joules) 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 seconds, followed by 5 minutes of ultrasonication (Model 250HT ultrasonicator, VWR) and a final 10 second 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). Preliminary recovery experiments showed that $10^7 - 10^8$ viable bacteria per ml could be recovered from these biofilms in the absence of any antibacterial treatment. After the growth period, plates were removed from the shaker into an anaerobic cabinet, where all photodisinfection treatments were performed. For photodisinfection treatment, each well was rinsed twice with sterile water to ensure that free-floating planktonic organisms were removed. After the second wash, all liquid was again removed from the well and photosensitizer solution was added for 30 seconds. At the end of this incubation, excess photosensitizer solution was removed from the well and the biofilm was illuminated for 60 seconds using a 150 watt haloid lamp cold light source. Immediately after illumination, recovery media was added to the well. After all treatments were completed, the biofilm plate was placed in an ultrasonicator for 30 minutes to release any surviving organisms into the recovery media. For both biofilm models, samples from the recovery medium after photodisinfection were serially diluted and plated on brucella blood agar supplemented with haemin and vitamin K. Plates were grown anaerobically at 37°C for 72 hours until countable colonies were present in control plates. Controls consisting of photosensitizer alone and illumination alone were run to verify that neither parameter influenced bacterial killing on its own.

Biofilm Exposure to Benzalkonium Chloride

For treatment of biofilms with benzalkonium chloride (BAC), biofilms grown on pegs as described in the first method above were used. 96-peg lids were removed from the bacterial inoculum after 24 hours of incubation and rinsed twice with sterile water to remove any free-floating organisms. Pegs were then placed in aqueous solutions containing either 0% (water only control), 0.01%, 0.1%, or 1% BAC for a period of 60 seconds. After this exposure pegs were immediately placed in recovery media and processed/plated as described above for enumeration of surviving organisms. All BAC exposure conditions were run in triplicate in anaerobic conditions for both strains of bacteria.

Temperature Measurements in Planktonic Samples

For planktonic experiments, temperature measurements were taken from within experimental and control sample wells before and immediately after illumination using 0.005

inch diameter fast response type-T thermocouples (Thermalert, Hopkins, MN) and a Thermalert Model TH-8 display (Bailey Instruments Inc, Manchester, UK).

Statistical Analysis

Raw counts for replicates of each experimental condition were averaged and back-calculated given dilution factor to give survival data in CFU/ml. The data were presented as CFU/ml of surviving organisms after treatment, and kill rate was calculated as this value in experimental samples vs. control (no light, no photosensitizer). The final viable bacterial levels after treatments were converted to base-10 logarithms for easy comparison, and experimental results were expressed as both a $\log_{10}$ and percentage reduction in viability. Statistical differences between treatment groups were calculated using one-way ANOVA with *post-hoc* Scheffe's test (SPSS software, Chicago, IL).

RESULTS

Planktonic Kill Assay

Exposures to photodisinfection were run in both anaerobic and aerobic conditions in order to assess the importance of local oxygen during the photoreaction. The results of these treatments are shown in Table 1. Results of exposures of *D. vulgaris* to photodisinfection under aerobic conditions showed a powerful killing effect, with a 4.4 ± 2.3 $\log_{10}$ reduction in viability vs. non-treated controls (>99.99% killing). This effect was significantly reduced in the presence of the reactive oxygen species quencher L-tryptophan, where a reduction of only 1.7 ± 0.6 $\log_{10}$ (~95%) was observed under the same exposure parameters. When photodisinfection was performed under anaerobic conditions the killing effect was also strong, with a 3.5 ± 0.8 $\log_{10}$ reduction vs. non-treated control (>99.9% killing) observed. However, when L-tryptophan was added and photodisinfection was performed in an anaerobic environment, bactericidal activity remained relatively high (2.5 ± 2.1 $\log_{10}$ or >99% reduction from non-treated control). No significant antibacterial action was observed in control conditions consisting of either photosensitizer solution or illumination alone. Because *D. desulfuricans* is an obligate anaerobe, that organism was not used in the aerobic vs. anaerobic photodisinfection experiments. Temperature measurements taken before and immediately after illumination were used to ensure that bulk heating produced in the sample due to absorbed energy did not contribute to bacterial killing. Under the conditions used in this study, sample temperatures after 60 seconds of illumination never exceeded 35˚C, well below that which would adversely affect survival.

Biofilm Kill Assay

Photodisinfection also effectively eradicated biofilms of sulfate-reducing bacteria in a photosensitizer concentration dependent manner. The results of bacterial exposure to various concentrations of methylene blue followed by laser illumination are shown in Table 2. When biofilms were exposed to 0.1% w/v methylene blue followed by non-thermal 670 nm laser illumination, viability of *D. vulgaris* was reduced by 3.2 $\log_{10}$ (>99.9%) vs. non-treated control. Similarly, the viability of *D. desulfuricans* biofilms was reduced by 3.0 $\log_{10}$ (99.9%) vs. non-treated control using the same treatment parameters. Using lower concentrations of methylene blue (0.01% w/v and 0.05% w/v) kills were significantly reduced, with a 0.4 - 0.5 $\log_{10}$ (~50%) reduction vs. non-treated control observed for both organisms after laser illumination. Neither the methylene blue formulation nor the laser light alone led to any significant reduction in biofilm viability for either organism tested. Biofilm exposure to various concentrations of the chemical disinfectant benzalkonium chloride yielded significantly lower kill rates than the 0.1% methylene blue photodisinfection condition (Figure 1). Reductions in viability of *D. vulgaris* biofilms were 0.5 $\log_{10}$ (70%), 1.4 $\log_{10}$ (95%), and 1.5 $\log_{10}$ (96%) vs. non-treated control after 60 second exposure to 0.01%, 0.1%, and 1% BAC, respectively. Reductions in viability of *D. desulfuricans* biofilms were 0.6 $\log_{10}$ (80%), 2.7 $\log_{10}$ (99.7%), and 1.9 $\log_{10}$ (98%) vs. non-treated control after 60 second exposure to 0.01%, 0.1%, and 1% BAC, respectively.

Photodisinfection using a broadband light source also achieved significant killing of corrosion causing bacterial biofilms. A summary of these results is shown in Table 3. Biofilms of *D. vulgaris* exposed to 0.1% w/v methylene blue followed by broadband illumination showed a 1.5 $\log_{10}$ (~95%) decrease in viability compared to non-treated control. When exposed to 0.05% w/v toluidine blue O, another phenothiazinium dye photosensitizer, instead of methylene blue prior to illumination *D. vulgaris* biofilm viability was reduced by 1.1 $\log_{10}$ (>90%) compared to non-treated control. Finally, when exposed to a cocktail of photosensitizers consisting of 0.05% w/v of each of methylene blue, toluidine blue O, safranin O, and rose bengal, viability was reduced by 0.8 $\log_{10}$ (~84%) vs. non-treated control. When biofilms of *D. desulfuricans* were tested, exposure to 0.05% methylene blue followed by 60 seconds of broadband illumination resulted in a 0.5 $\log_{10}$ reduction in viability. In the same strain, exposure to the combination formulation of methylene blue, toluidine blue O, safranin O, and rose bengal prior to broadband illumination led to a 0.8 $\log_{10}$ (~80%) reduction in viability from non-treated control. Neither the combination photosensitizer cocktail nor broadband light alone led to any significant reduction in biofilm viability for either organism tested.

DISCUSSION

The prevention of internal pipeline corrosion is a multi-billion dollar industry. While a variety of different bacterial species have been shown to contribute to corrosion of pipeline steel, it is widely accepted that SRB are the most ubiquitous and destructive [24]. Many novel strategies have been shown to inhibit SRB biofilm formation on industrial steel surfaces with varying degrees of success, however a truly useful solution would be: a) highly effective against biofilm-form organisms, b) safe to deploy and non-toxic to humans and the environment, c) relatively inexpensive.

Photodisinfection is an antibacterial technology that is currently used in various biomedical applications [14-16]. Because of it's potent and broad-spectrum activity against both free-floating and biofilm organisms, photodisinfection may also have utility in industrial corrosion applications where bacteria play a major role. In addition, the fact that photodisinfection can easily be delivered to bacteria growing on surfaces suggests that this technology may be ideal for deployment on the inner surface of oil and gas pipelines. In this study, an *in vitro* model was used to show that photodisinfection is highly effective in eradicating several strains of bacteria that are known to cause the corrosion of steel. Exposure to the phenothiazinium photosensitizer methylene blue and non-thermal laser illumination resulted in >99.99% kills of the SRB *D. vulgaris* in planktonic form. As corrosion-causing bacteria residing on the inner surface of pipelines are primarily present in biofilm form, it was also relevant to evaluate the antibacterial efficacy of photodisinfection against biofilms of common SRB's. Experiments using a 670 nm non-thermal laser light source to illuminate biofilms exposed to methylene blue showed a >99.9% eradication of both *D. vulgaris* and *D. desulfuricans*. Higher photosensitizer concentrations produced greater kills due to enhanced penetration of the biofilm ultrastructure.

In industrial applications of photodisinfection, it may be more practical (technically and economically) to use broadband light sources as opposed to focused lasers for illumination of larger surface areas such as the inner surface of pipelines. Thus, we also evaluated broadband illumination using a cocktail of photosensitizer dyes against biofilms of *D. vulgaris* and *D. desulfuricans*. Exposure to this photosensitizer cocktail followed by broadband illumination resulted in 70-80% reductions in viability of both organisms in biofilm form. This was somewhat lower than the efficacy achieved using 670 nm laser illumination and methylene blue. While this study showed that biofilm eradication using photodisinfection can be achieved with a white/broadband light source, further optimization is required to match a photosensitizer or combination of photosensitizers to a specific broadband light source and power density.

Finally, biofilms of *D. vulgaris* and *D. desulfuricans* were exposed to the chemical disinfectant benzalkonium chloride (BAC) in order to perform a direct comparison with photodisinfection. Concentrations of BAC up to 1% v/v were delivered to bacterial biofilms, resulting in approximately 95% kills of both sulfate-reducing strains. In contrast methylene blue at 1/10 of this concentration with laser illumination produced significantly greater kills of >99.9%. Thus, photodisinfection under optimal parameters has the potential to be more effective than BAC at eradicating corrosion-causing biofilms on surfaces.

The present study addressed many of the issues that would affect the application of photodisinfection to the prevention of MIC in industrial pipelines, including 1) utilizing corrosion-causing organisms in biofilm form, 2) application of the technology in anaerobic conditions, and 3) comparison to existing methods of MIC prevention. The delivery of photosensitizer to biofilms on the inner pipeline surface using existing inhibitor dispersion technology, followed by illumination from a self-powered pigging device would make photodisinfection a feasible option for corrosion control.

CONCLUSIONS

Deployed in industrial applications such as internal pipeline corrosion control, major advantages of photodisinfection include its broad spectrum activity against most known types of bacteria (including those in biofilm form), the ability to eradicate high percentages (>99%) of bacterial populations, lack of contribution to the development of resistant sub-populations, and relatively small environmental impact compared to traditional chemical agents used as industrial biocides. This study demonstrated that photodisinfection is highly effective in eradicating SRB biofilms known to cause MIC in oil and gas pipelines. Future studies will utilize a closed-loop dynamic flow system model and standard corrosion measurement techniques such as scanning electron microscopy and electrochemical impedance spectroscopy to evaluate the ability of photodisinfection to inhibit bacterially-influenced corrosion of steel coupons.

TABLE 1:

Antibacterial activity of photodisinfection against planktonic *D. vulgaris* in aerobic and anaerobic conditions. All photosensitizer concentrations are expressed on a w/v basis.

Test Agent	Illumination	Exposure Environment	Reduction in Viability	
			Log10	Percentage
0.01% MB[a]	60 seconds	Aerobic	4.4±2.3	99.99%
0.01% MB/ 20mM L-tryptophan	60 seconds	Aerobic	1.7±0.6	97.78%
0.01% MB	60 seconds	Anaerobic	3.5±0.8	99.97%
0.01% MB/ 20mM L-tryptophan	60 seconds	Anaerobic	2.5±2.1	99.67%

[a] Methylene blue

TABLE 2:

Antibacterial activity of photodisinfection using methylene blue and laser illumination against *D. vulgaris* and *D. desulfuricans* biofilms in anaerobic conditions. All photosensitizer concentrations are expressed on a w/v basis.

Photosensitizer	Illumination	Organism	Reduction in Viability	
			Log10	Percentage
0.01% MB	60 seconds	D. v.	0.5±0.3	71.7%
0.1% MB	60 seconds	D. v.	3.2±0.9	99.9%
0.05% MB	60 seconds	D. d.	0.4±0.1	63.5%
0.1% MB	60 seconds	D. d.	3.0±0.6	99.9%

TABLE 3:

Antibacterial activity of photodisinfection using various photosensitizers and broadband illumination against *D. vulgaris* and *D. desulfuricans* biofilms in anaerobic conditions. All photosensitizer concentrations are expressed on a w/v basis.

Photosensitizer	Illumination	Organism	Reduction in Viability	
			Log_{10}	Percentage
0.1% MB	60 seconds	D. v.	1.5±0.1	96.8%
0.05% TBO[a]	60 seconds	D. v.	1.1±0.8	92.2%
Cocktail[b]	60 seconds	D. v.	0.8±0.2	83.8%
0.05% MB	60 seconds	D. d.	0.5±0.2	67.6%
Cocktail[b]	60 seconds	D. d.	0.8±0.2	84.1%

[a] Toluidine blue O

[b] Photosensitizer cocktail consisted of 0.05% w/v each of methylene blue, toluidine blue O, safranin O, and rose bengal in ultrapure water

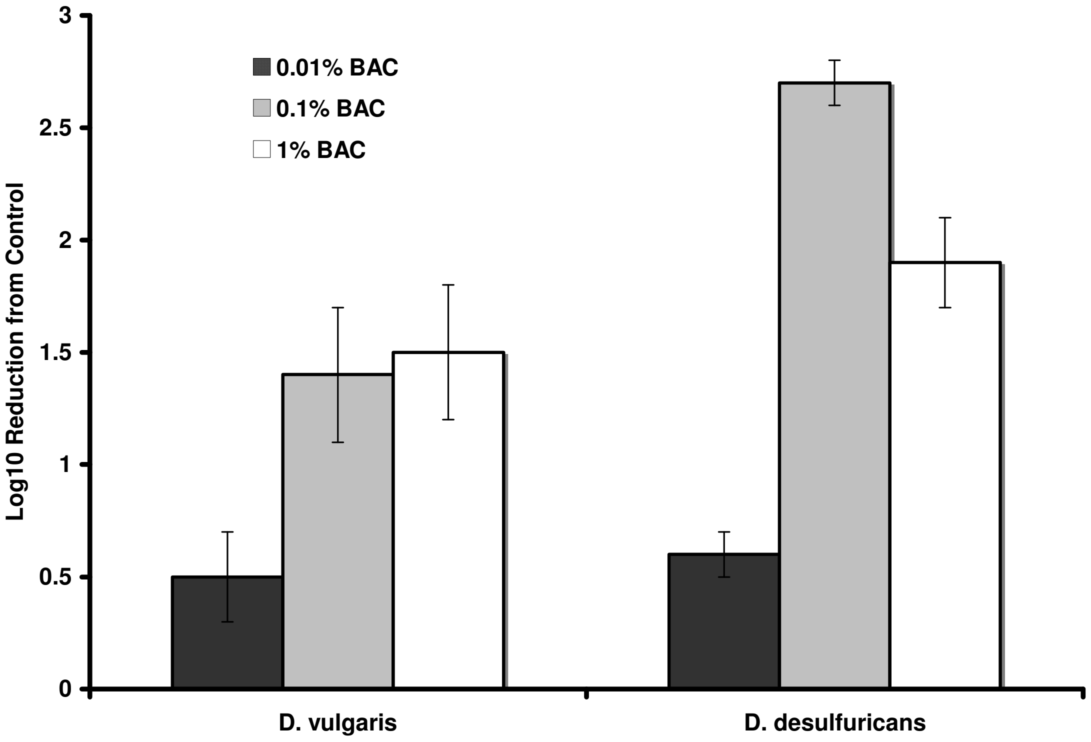

FIGURE 1 – Antibacterial efficacy of various concentrations of benzalkonium chloride (BAC) against biofilms of *D. vulgaris* and *D. desulfuricans* after 60 second exposure. Results are expressed as $\log_{10}$ reduction from non-treated control.

REFERENCES

1. C.G. Peng, J.K. Park, Principal factors affecting microbiologically influenced corrosion of carbon steel, Corrosion 50(9) 1994 669-675.

2. J. Jan-Roblero, J.M. Romero, M. Amaya, S. LeBorgne, Phylogenetic characterization of a corrosive consortium isolated from a sour gas pipeline, Environ. Biotech. 64 (2004) 862-867.

3. S.J. Yuan, S.O. Pehkonen, AFM study of microbial colonization and its deleterious effect on 304 stainless steel by *Pseudomonas* NCIMB 2021 and Desulfovibrio *desulfuricans* in simulated seawater, Corros. Sci. 51 (2009) 1372-1385.

4. T.F. Mah, G.A. O'Toole, Mechanisms of biofilm resistance to antimicrobial agents, Trends Microbiol. 9(1) (2001) 34-39.

5. B. Little, P. Wagner, Quantifying sulfate reducing bacteria in microbiologically influenced corrosion. Technical Report – Naval Research Lab, Monterey, CA (1992).

6. X.Y. Zhu, J. Lubeck, J.J. Kilbane, Characterization of microbial communities in gas industry pipelines, Appl. Environ. Microbiol. 69(9) (2003) 5354-5363.

7. J. Jan-Roblero, J.M. Romero, M. Amaya, S. LeBorgne, Phylogenetic characterization of a corrosive consortium isolated from a sour gas pipeline, Appl. Microbiol. Biotechnol. 64 (2004) 862-867.

8. A.K. Lee, M.G. Buehler, D.K. Newman, Influence of a dual species biofilm on the corrosion of mild steel, Corros. Sci. 48 (2006) 165-178.

9. T.S. Rao, A.J. Kora, B. Anupkumar, S.V. Narasimhan, R. Feser, Pitting corrosion of titanium by a freshwater strain of sulphate reducing bacteria (*Desulfovibrio vulgaris*), Corros. Sci. 47 (2005) 1071-1084.

10. G.G. Geesey, R.J. Gillis, R. Avci, D. Daly, M. Hamilton, P. Shope, G. Harkin, The influence of surface features on bacterial colonization and subsequent substratum chemical changes of 316L stainless steel, Corros. Sci. 38(1) (1996) 73-95.

11. B. Little, J. Lee, R. Ray, A review of 'green' strategies to prevent or mitigate microbiologically influenced corrosion, Biofouling 23 (2007) 87-97.

12. H.F. Ridgway, C.A. Justice, C. Whittaker, D.G. Argo, B.H. Olson, Biofilm fouling of RO membranes – its nature and effect on treatment of water for reuse, J. Am. Water Works Ass. 76 (1984) 94-102.

13. L.R. Chauhan, G. Gunasekaran, Corrosion inhibition of mild steel by plant extract in dilute HCL medium, Corros. Sci. 49(3) (2007) 1143-1161.

14. G. Jori, C. Fabris, M. Soncin, S. Ferro, O. Coppellotti, D. Dei, L. Fantetti, G. Chiti, G. Roncucci, Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications, Lasers Surg. Med. 38 (2006) 468-481.

15. M. Wainwright, Photodynamic antimicrobial chemotherapy (PACT), J. Antimicrob. Chemother. 42 (1998) 12-28.

16. M. Wilson, T. Burns, J. Pratten, G.J. Pearson, Bacteria in supragingival plaque samples can be killed by a low-power laser light in the presence of a photosensitizer, J. Appl. Bacteriol. 78 (1995) 569-574.

17. W.P. Thorpe, M. Toner, R.M. Ezzel, R.G. Tompkins, M.L. Yarmush, Dynamics of photoinduced cell plasma membrane injury, Biophys. J. 68 (1995) 2198-2206.

18. M. Bhatti, A. MacRobert, B. Henderson, M. Wilson, Exposure of *Porphyromonas gingivalis* to red light in the presence of the light activated antimicrobial agent toluidine blue O decreases membrane fluidity, Curr. Microbiol. 45 (1998) 118-122.

19. M. Bhatti, S.P. Nair, A. MacRobert, B. Henderson, P. Shepherd, J. Cridland, M. Wilson, Identification of photolabile outer membrane proteins of *Porphyromonas gingivalis*, Curr. Microbiol. 43 (2001) 96-99.

20. C.N. Street, A. Gibbs, L. Pedigo, D. Andersen, N.G. Loebel, *In vitro* photodynamic eradication of *Pseudomonas aeruginosa* in planktonic and biofilm culture, Photochem. Photobiol. 85 (2009) 137-143.

21. M. Bhatti, A. MacRobert, S. Meghji, B. Henderson, M. Wilson, A study of the uptake of toluidine blue O by Porphyromonas gingivalis and the mechanism of lethal photosensitization, Photochem. Photobiol. 68(3) (1998) 370-376.

22. L. Lagace, M. Jacques, A.A. Mafu, D. Roy, Biofilm formation and biocides sensitivity of *Pseudomonas marginalis* isolated from a maple sap collection system, J. Food. Prot. 69(10) (2006) 2411-2416.

23. J.A. Sandoe, J. Wysome, A.P. West, J. Heritage, M.H. Wilcox, Measurement of ampicillin, vancomycin, linezolid, and gentamycin activity against *enterococcal* biofilms, J. Antimicrob. Chemother. 57(4) (2006) 767-770.

24. W. Lee, Z. Lewandowski, P.H. Nielson, W.A. Hamilton, Role of sulfate reducing bacteria in corrosion of mild steel: A review, Biofouling 8(3) (1995) 165-194.