

Green Biocide Enhancers Enhanced the Biocide Inhibition of the Growth of Sulfate Reducing Bacteria

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Abstract

Billions of dollars are lost each year to various forms of corrosion in many industries. Microbiologically influenced corrosion (MIC) or biocorrosion is a major problem in the oil and gas industry. Sulfate reducing bacteria (SRB) have been most frequently implicated in MIC of steels and other iron containing alloys including carbon steels and even stainless steels. Existing mitigation methods rely mostly on biocides and biostats that either kill planktonic bacteria or inhibit their growth at low concentrations. Much higher concentrations are required to remove established biofilms. Microorganisms are capable of developing resistances after prolonged uses of biocides. Environmental and safety concerns on biocide uses are becoming more restrictive making their field applications more and more costly. Reduced and more effective uses of biocides are becoming highly desirable. A new class of nontoxic and biodegradable biocide enhancers was found to be effective in enhancing glutaraldehyde and Tetrakis Hydroxymethyl Phosphonium Sulfate (THPS) in their inhibition of planktonic and sessile SRB growth. In this study, *Desulfovibrio desulfuricans* (ATCC strain 14563) was used. It is a marine strain SRB. Laboratory experiments were carried out in 100 ml anaerobic vials. Enriched artificial seawater was used as culture medium.

Introduction

Hundreds of billions in US dollars are lost worldwide each year to corrosion. A recent study by CC Technologies and NACE International estimated that the cost of corrosion in the US alone amounts to US\$276B/year (www.corrosioncost.com). Microbiologically influenced corrosion (MIC) or biocorrosion is a major problem in the oil and gas industry. It is claimed that MIC accounts for about 20% of all corrosion of metals and building materials (Flemming, 1996). Sulfate reducing bacteria (SRB) have been most frequently implicated in MIC according to Feio et al. (2000) and Kobrin (1993). Costerton and Boivin (1991) estimated that the MIC damages in production, transport and storage of oil could reach some hundred million dollars in the US every year due to SRB alone, not including costs for lost oil and environmental cleanup. Although some new mitigation methods have emerged, such as adding nitrate to soured reservoirs to promote the growth of nitrate reducers that outcompete with SRB for available nutrients, current mitigation methods rely mostly on biocides and biostats that either kill planktonic bacteria or inhibit their growth at low concentrations. Much higher concentrations are required to treat established biofilms. Increasingly restrictive environmental regulations are making biocide applications more difficult and costly. The cost of treating biofilms runs into billions of dollars each year according to Mitman (2006).

This work presents some preliminary lab data from testing a new class of biocide enhancers that prolong the effectiveness and/or reduce the biocide dosage in the inhibition of planktonic and sessile SRB growth. The project is still ongoing.

Experimental Procedure

The ATCC 14563 strain of *Desulfovibrio desulfuricans* was used in this work. It is a marine strain SRB that favors salty water. Enriched artificial seawater (ASW) was used for SRB growth. Table 1 shows the composition of enriched ASW medium using a salt mix intended for marine aquariums. Table 2 shows the comparison of the Instant Ocean (<http://www.aquacraft.net/>) salt mix with typical natural seawater. Laboratory experiments were carried out in 100 ml anaerobic vials. Nitrogen sparging was used to remove oxygen in water. Disk shaped C1018 coupons were used in the 100 ml vials. Before immersion into vials, the specimen surfaces were polished by sand paper with 200, 400 and 600 grits successively. SRB cell numbers were counted under an optical microscope using a hemacytometer with serial dilutions.

Table 1. Composition of 1 liter enriched artificial seawater

Instant Ocean [®]	Fe(NH ₄) ₂ (SO ₄) ₂	Sodium lactate 60% syrup	Yeast extract
36 g	125 mg	4.5 ml	1 g

Table 2*. Artificial seawater with Instant Ocean[®] salt mix vs. natural seawater

	Salinity (1000ppm)	Na ⁺	Mg ²⁺	Ca ²⁺	K ⁺	Si ²⁺	Cl ⁻	SO ₄ ²⁻	BO ₃ ²⁻	CO ₃ ²⁻ HCO ₃ ⁻
Seawater	35	470	53	10.3	10.2	0.09	550	28	0.42	1.90
Instant Ocean [®]	29.65	462	52	9	9.4	0.19	521	23	0.44	1.90

All measured in millimoles per kilogram. Typical seawater density is 1026 kg/l and pH 8.4.

To study the SRB biofilm on a C1018 steel coupon under scanning electron microscopy (SEM), the coupon's exposed surface was fixed in 2.5% (wt) glutaraldehyde for 8 hours and washed with a graded series (30%, 50%, 70%, 100% by volume) of ethanol for dehydration prior to SEM observations. The samples were subsequently critical point dried and coated with a special gold alloy. For observing the naked metal surface, the coupon surface was cleaned using a HCl solution according to ASTM G1-03 standard.

Results

Two readily biodegradable biocide enhancer candidates were tested experimentally in combination of either glutaraldehyde or THPS, the two most commonly used biocide in the oil and gas industry. The data below were for one of the biocide enhancer in combination with glutaraldehyde.

Figure 1 shows that the biocide enhancer is effective in delaying planktonic SRB growth in enriched artificial seawater. At a high concentration of biocide, SRB growth was even inhibited with the presence of biocide enhancer.

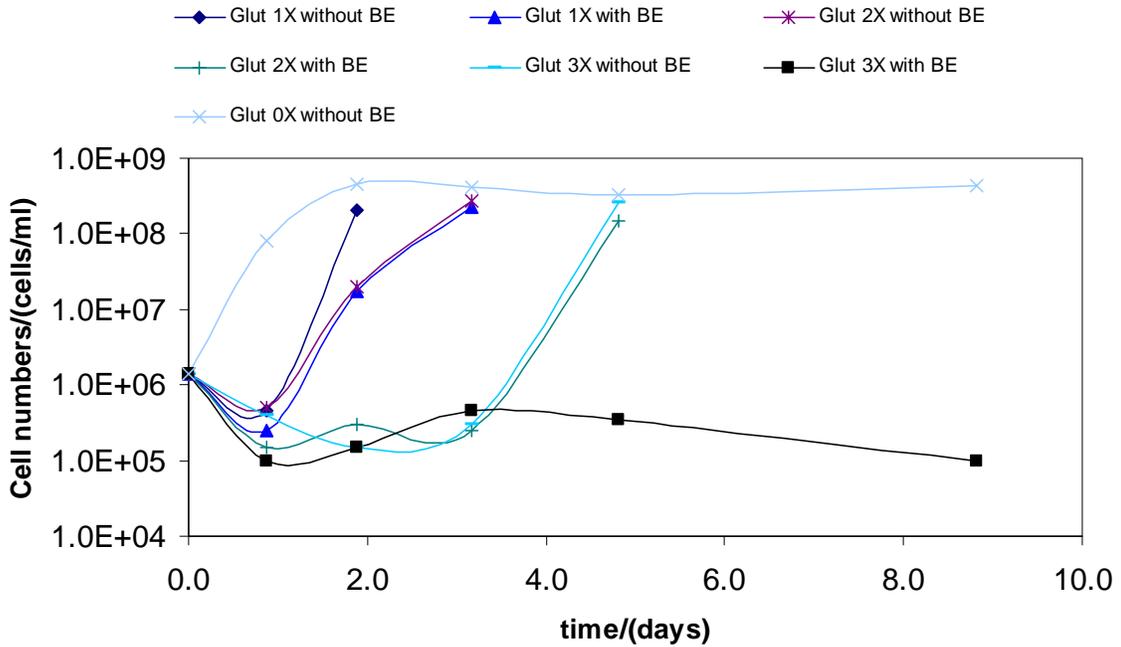


Figure 1. Biocide enhancer (BE) enhanced glutaraldehyde's inhibition of planktonic SRB growth in enriched artificial seawater. Three different biocide concentrations were used.

Figure 2 below shows biofilm formation on the coupon surface when only glutaraldehyde was used. The peanut-shaped sessile SRB cells were clearly visible in a biofilm. In comparison, Figure 3 shows no visible SRB cells when glutaraldehyde was used with the biocide enhancer.

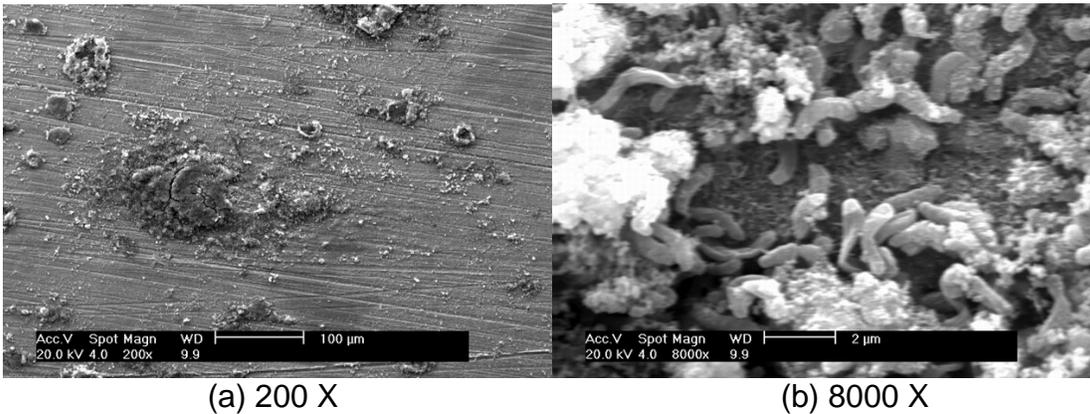


Figure 2. Coupon surface images for biocide without enhancer.

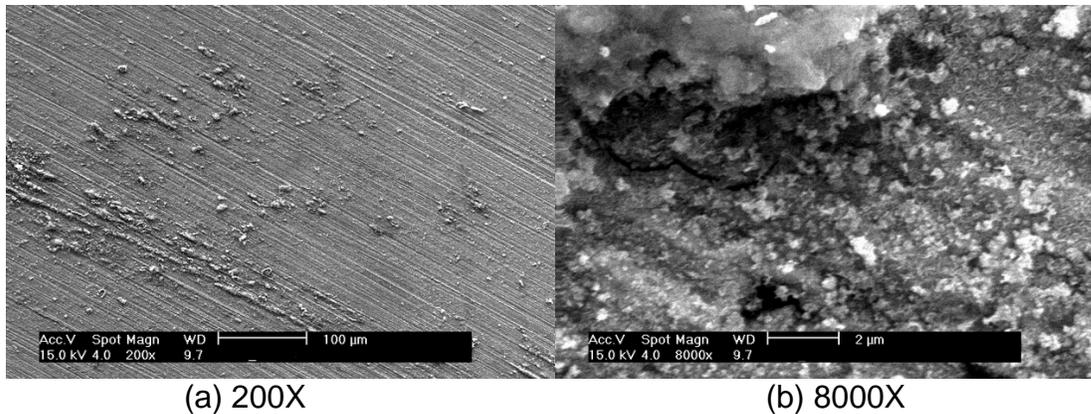


Figure 3. Coupon surface images for biocide with biocide enhancer.

Conclusions

The biocide enhancer showed enhancement of glutaraldehyde in its inhibition of planktonic SRB growth. When glutaraldehyde was used without a biocide enhancer at a low concentration, sessile SRB cells in a biofilm were clearly visible. With the biocide enhancer, sessile cells were hardly noticeable. This shows the enhancement of glutaraldehyde in its inhibition of the establishment of SRB biofilm by glutaraldehyde. Experiments are being conducted to test the efficacy of the biocide enhancer in the treatment of established biofilms.

Acknowledgement

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