EFFECTS OF BIOCIDES AND A BIOCIDE ENHANCER ON SRB GROWTH

Kaili Zhao, Jie Wen, Tingyue Gu and Srdjan Nesic, Chemical Engineering, Ohio University, Athens, OH 45701

Abstract

Microbiologically influenced corrosion (MIC) has been defined as[1] "An electrochemical process in which the presence of micro-organisms is able to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature." Underground corrosion of pipes was costing the USA 0.5 to 2 billion dollars a year some 25 years ago[2] and is the best know economic disaster induced by sulfate-reducing bacteria (SRB). SRB are among the microorganisms most frequently implicated in MIC of iron, copper and ferrous alloys[3]. To study SRB growth and to find potential mitigation methods for MIC due to SRB, the ATCC 7757 strain of *Desulfovibrio desulfuricans* was used in this research. Currently, mitigation of MIC relies mostly on biocides and biostats. Growing environmental concerns make their uses more restrictive. Lower concentrations and more prolonged effectiveness are desired. In this work, glutaraldehyde and THPS were used as biocides to inhibit planktonic SRB growth. A novel biocide enhancer was used in combination with these two most popular biocides. Experimental results showed that both biocides suppressed the planktonic SRB growth and THPS worked better, and that the biocide enhancer effectively enhanced the biocides in the inhibition of planktonic SRB growth.

Experimental Conditions

In this study, *Desulfovibrio desulfuricans* (ATCC strain 7757) was used. It is a common SRB. Laboratory experiments were carried out in 100ml anaerobic vials. The liquid medium was based on the ATCC 1249 medium (see Table 1) for the growth of *D. desulfuricans* ATCC strain 7757[4]. Ferrous sulfate plus ammonium sulfate were used in all the experiments to replace $Fe(NH_4)(SO_4)_2$ (ferrous ammonium sulfate) because the latter is heat sensitive and needs to be filter sterilized, which is not convenient. Ferrous sulfate and ammonium sulfate are used in the equivalent molar amounts of ferrous, ammonium and sulfate ion in $Fe(NH_4)(SO_4)_2$.

SRB cell numbers were counted under an optical microscope using a hemacytometer (Neubauer chamber, Hausser Scientific) with serial dilutions[5] if needed. All cell growth experiments were carried out in a 37^{o}C incubator.

Tables 2 and 3 show the test matrices for experiments in the vials. The name of the novel biocide enhancer is withheld and it is labeled as "Biocide Enhancer or Enhancer" in this work. Glutaraldehyde (abbreviated as G) and THPS acted as the biocides involved in this study.

Table 1. Composition of ATCC 1249 medium for SRB.

Component I	$MgSO_4$	2.0 g
	Sodium Citrate	5.0 g
	$CaSO_4$	1.0 g
	NH_4Cl	1.0 g
	Distilled water	400 ml
Component II	K_2HPO_4	0.5 g
	Distilled water	200 ml
Component III	Sodium Lactate	3.5 g
	Yeast Extract	1.0 g
	Distilled water	400 ml
Component IV	$FeSO_4$	2.1g
	$(NH_4)_2SO_4$	1.0g
	Distilled water	30ml

Table 2. Test matrix for Glutaraldehyde and Biocide Enhancer on planktonic SRB growth.

Strain	*Desulfovibrio desulfuricans* (ATCC 7757)
Medium	Modified ATCC 1249 liquid medium
Temperature (ºC)	37
pH	7.0±0.1
Total ferrous ion concentration (ppm)	25
Biocide (glutaraldehyde) concentration (ppm)	0; 10; 30; 50
Biocide Enhancer concentration (ppm)	0, 50, 100, 200
Experimental setup	100ml anaerobic vials

Table 3. Test matrix for THPS and Biocide Enhancer on planktonic SRB growth.

Strain, Medium, Temperature, pH, Ferrous ion concentration and Experimental setup are the same with the above matrix.	
Biocide (THPS) concentration (ppm)	0; 10; 30; 50
Biocide Enhancer concentration (ppm)	0, 50, 100, 200

Results and Discussion

1. Effects of biocides on planktonic SRB growth.

Figures 2 and 3 show that glutaraldehyde alone did not inhibit SRB growth when its concentration was 10ppm and 30ppm; however, glutaraldehyde became effective when its

concentration rose to 50ppm (see Figure 4). In contrast, biocide THPS alone can suppress planktonic SRB growth when its concentration was 30ppm (see Figure 6). This means that THPS has a better effect on inhibition of SRB growth than glutaraldehyde.

2. Effects of Biocide Enhancer on planktonic SRB growth

Figure 1 shows that the Biocide Enhancer did not suppress the planktonic SRB growth in the absence of the biocides and also even in combination with biocides. It did not have a clear-cut suppress of SRB growth when the biocide concentration was low, such as 10ppm (see Figure 2 and Figure 5). The Biocide Enhancer was able to suppress SRB growth when the combined biocides concentration rose above 30ppm (see Figure 3, Figure 4 and Figure 6), and Figure 5 shows that Biocide Enhance combined with biocide THPS 10ppm can enhance the THPS suppression of SRB growth at the beginning of the test.

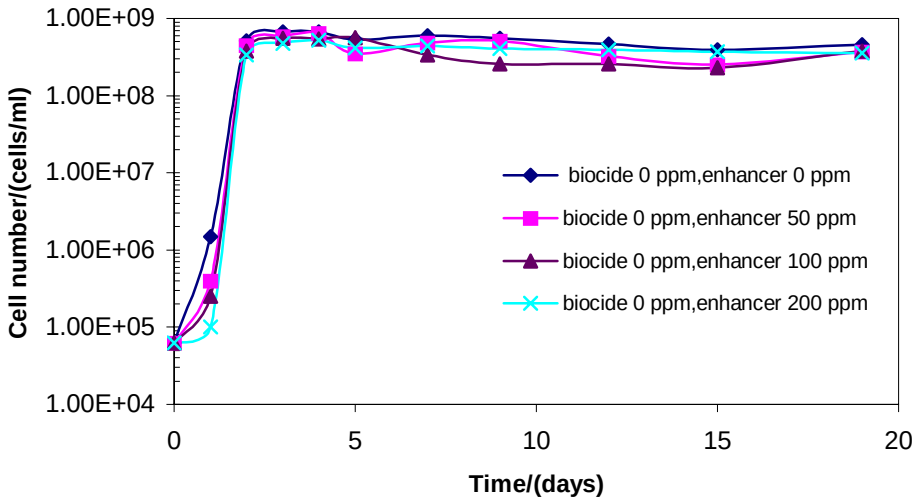

Figure 1. The effect of Biocide Enhancer alone on the planktonic SRB growth.

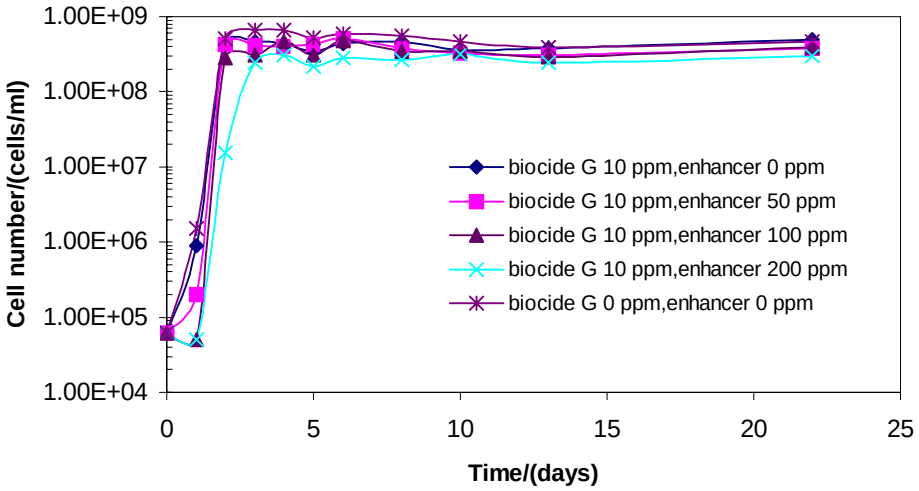

Figure 2. Inhibition of planktonic SRB growth using 10ppm glutaraldehyde with and without Biocide Enhancer

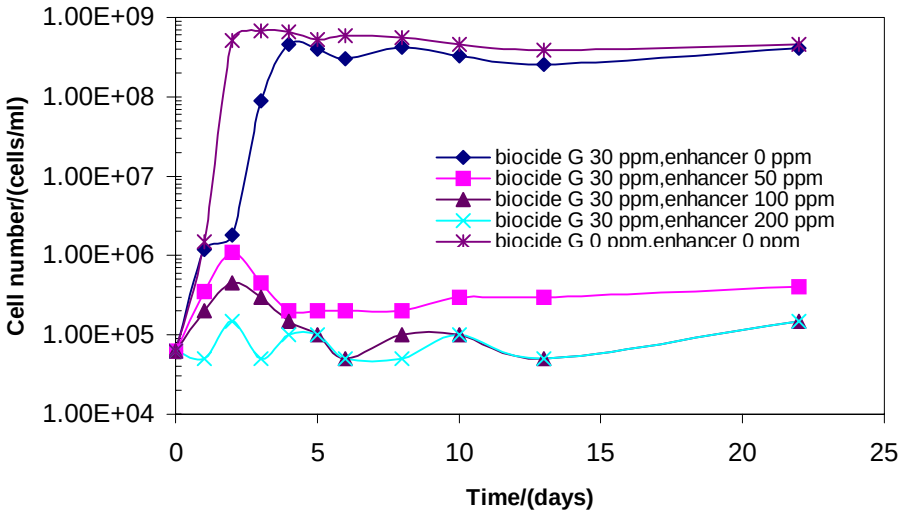

Figure 3. Inhibition of planktonic SRB growth using 30ppm glutaraldehyde with and without Biocide Enhancer

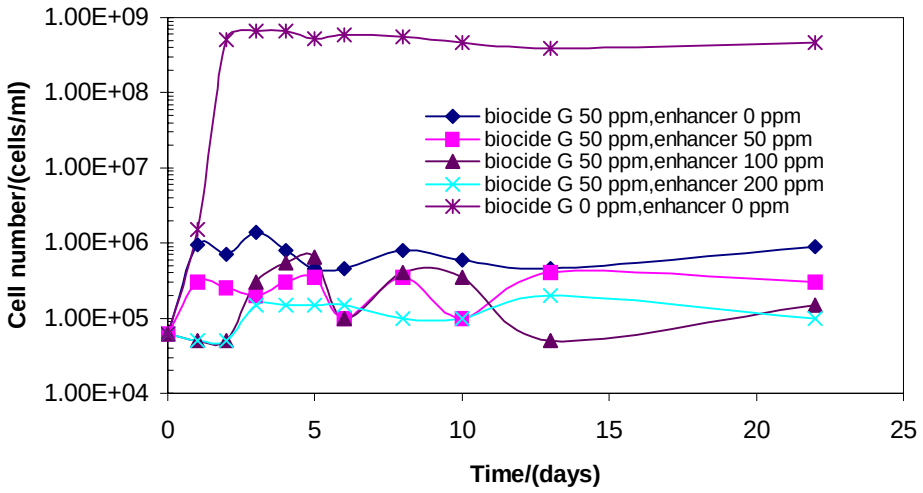

Figure 4. Inhibition of planktonic SRB growth using 50ppm glutaraldehyde with and without Biocide Enhancer

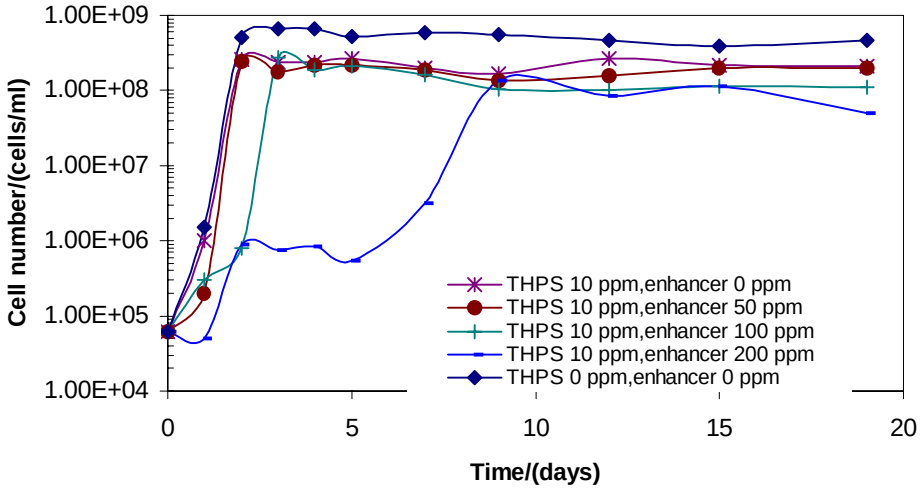

Figure 5. Inhibition of planktonic SRB growth using 10ppm THPS with and without Biocide Enhancer

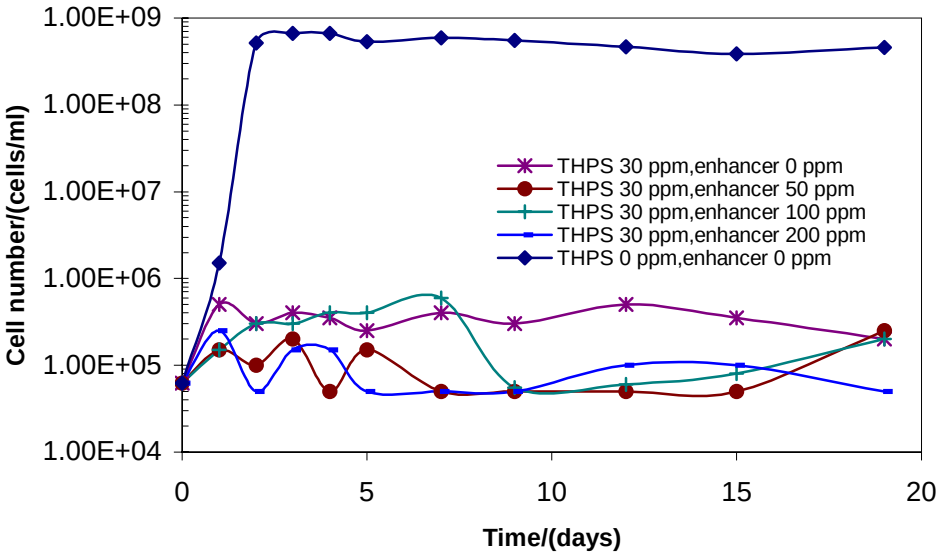

Figure 6. Inhibition of planktonic SRB growth using 30ppm THPS with and without Biocide Enhancer

Conclusions

1. Biocide glutaraldehyde and THPS both can inhibit the SRB growth and THPS is better.
2. The use of the novel biocide enhancer improved the effectiveness of the biocides.

References:

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4. Atlas, R.M., and Parks, L.C. (1997). *Handbook of Microbiological Media*, 2nd ed., CRC Press, Boca Raton.
5. Penn, C.W. (1991). *Handling Laboratory Microorganisms*. Bristol. PA, USA.

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