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Development of an obligate anaerobe specific biocide

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SUMMARY

Anaerobic bacteria, such as sulfate-reducing bacteria and clostridia, are capable of generating H_2S and organic acids which corrode metallurgy resulting in millions of dollars of damage to industry annually. The bacteria are obligate anaerobes which grow typically on equipment surfaces under deposits such as biofilms. A successful method of penetrating biofilm and killing the anaerobic bacteria specifically has not been previously presented. We have investigated whether a blend of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole) and a biodispersant would kill *Desulfovibrio*, *Desulfotomaculum*, and *Clostridium* species grown in the laboratory and in field applications. We found the blend significantly reduced the anaerobes in laboratory cultures. However, in a bioreactor designed to induce a high level of biofilm production and enhance underdeposit growth of anaerobic bacteria, a 40–58% increase in the antibiotic-biodispersant blend concentration was required. The metronidazole blend killed obligate anaerobic bacteria specifically but was non-toxic to aerobic bacteria and fungi. These results were confirmed in cooling tower field trial studies.

INTRODUCTION

Metal corrosion in the United States results in billions of dollars in damages annually. While many microorganisms can be involved in microbially-induced corrosion (MIC), the major cause is obligate anaerobic bacteria, particularly the sulfate-reducing bacteria [6]. The mechanism by which sulfate-reducing bacteria (SRB) influence corrosion has been described [6,8]. As a consequence of restricted nutrition, the SRB depend on microbial communities or consortia such as those typically found on industrial metal surfaces. These biofilms [2,4] can provide an anaerobic environment for SRB growth. During growth SRB catabolize sulfur compounds and reduce them to H_2S during respiration [9].

Metronidazole (Fig. 1) has been shown to be active against some anaerobic organisms [1,5,13] and has displayed excellent activity in vitro against the anaerobic bacteria *Clostridium*, *Bacteroides* and *Fusobacterium* [3,7,12]. In this report, we describe the development of a metronidazole test product which was effective in controlling wild type obligate anaerobic bacteria, including SRB, in an industrial cooling tower.

MATERIALS AND METHODS

Microorganisms. Pure strains were obtained from The American Type Culture Collection (Rockville, MD) as *Desulfovibrio desulfuricans* 13541, *Desulfotomaculum nigrificans* 19858, and *Clostridium sporogenes* 17886. Our test culture (described in Table 1) was prepared by composing microorganisms from thirty-two field samples of industrial biofilms. Pure cultures of *D. desulfuricans*, *D. nigrificans*, and *C. sporogenes* were added to the composite to increase the obligate anaerobic SRB level from 2×10^3 cfu to $> 50 \times 10^4$ cfu/g and the number of clostridia from 2 to $> 10^3$ cfu/g.

Media. The tryptic soy broth (TSB), tryptone glucose extract (TGE), potato dextrose agar (PDA), Levine's eosin methylene blue (EMB), Pseudomonas isolation agar (PIA), cetrimide agar, thioglycollate without dextrose and the bacto-agar were purchased from Difco (Detroit, MI). The API medium was obtained in 9 ml vials from VWR Scientific (Chicago, IL). The salts and dextrose were purchased from J.T. Baker (Phillipsburg, NJ), and the sodium lactate and the neomycin sulfate were from Sigma Chemical Co. (St. Louis, MO). The metronidazole was obtained from G.D. Searle (Deerfield, IL). Deeps (10 ml) of SRB medium A (1 g $NaSO_3$, 20 g Bacto-agar, 10 g Bacto-tryptone and 0.0005% ferric citrate per liter) were prepared in 125 mm × 15 mm tubes. Medium B was purchased as sulfite deeps in screw cap vials (68 mm × 16 mm) from Biosan Laboratories (Ferndale, MI). The deeps were overlaid with oil and blanketed with CO_2. The results obtained on medium B were compared with those of SRB medium A.

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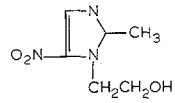

Fig. 1. Chemical structure of metronidazole: 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole.

Growth of microorganisms. The laboratory cultures of field biofilm deposits of aerobic bacteria were mixed in 100 ml TSB using a Waring blender cup. The resuspended cells were diluted 1 : 100 in TSB and grown in 1 l volumes at 32 ± 1 °C. They were subsequently subcultured three times by diluting 1 : 100 into pre-warmed (32 °C) TSB at 24 h intervals. *Desulfovibrio desulfuricans* 13541 and *Desulfotomaculum nigrificans* 17886 were grown and maintained in API medium anaerobically in the presence of H_2 and CO_2 in paraffin sealed vials at 30 ± 1 °C. The SRB were subcultured weekly by diluting 1 : 100 into fresh medium. The *C. sporogenes* 17886 culture was prepared in thioglycollate broth tubes (125 mm × 15 mm), sealed with paraffin plugs, and incubated at 37 °C. The clostridia were then subcultured every 48 h. The clostridia were also verified in thioglycollate with and without neomycin.

The aerobic cells were tested for adhesion capabilities on the biofouling reactor described below. Once attachment was established, the aerobic slime-depositing bacteria were supplemented with the anaerobic strains.

Anaerobic biofouling reactor. The anaerobic biofouling reactor (ABR) is represented schematically in Fig. 1. Its dimensions, the flow rates of the influent, and other parameters are listed in Table 2. Composition of the water and the test conditions are described in Table 3.

The ABR consists of two tubular recycle reactors each with biofouling test sections made of stainless steel core tubes positioned within glass observation tubes (Fig. 2). Test solutions are recirculated in the annular region between the tubes by an Eastern/Iwaki (Hamden, CT) pump driven magnetically by a thermally protected motor. Biofilm attachment and growth occurs on the wetted surface. The biofilm mass is determined gravimetrically after 18 h at 60 °C. Parallel tubes allow biomass determinations before and after treatments.

The ABR was started with a batch induction period. The TSB and dextrose were added at 250 mg/l each to the chemostat and mixed for 5 min. The chemostat was then inoculated with 1 ml of the standard culture CWT3 (Table 2) supplemented with the obligate anaerobes. The

TABLE 1

Composition of field microorganism culture

Type of organisms	cfu/ml
Total aerobic bacteria	1.2×10^8
Enterobacter	8.0×10^6
Pigmented	2.0×10^2
Mucoids	6.0×10^2
Pseudomonas	2.0×10^7
Sporeformers	2.2×10^4
Total anaerobic bacteria	
Sulfate reducers [a]	$> 5.0 \times 10^6$
Clostridia	802
Total fungi	
Molds	< 10
Yeasts	< 10

[a] *Desulfovibrio* and *Desulfotomaculum.*

TABLE 2

Anaerobic biofilm reactor characteristics and dimensions

Reactor	
Recycle flow rate	2 gpm
Recirculation velocity	3 ft/s
Influent flow rate	
(F) Make-up water	24 cm^3/ml
(S) Substrate solution	0.72 cm^3/ml
Volume	1000 cm^3
Hydraulic residence time	42 min
Wetted surface area	1700 cm^2
Biofilm test section	
Number per reactor	2
Tube diameter	
Core tube (Stainless steel)	1.27 cm (0.5 inch) [a]
Observation tube (glass)	1.91 cm (0.75 inch) [b]
Core tube wetted surface area	182.3 cm^2

[a] Outer diameter; [b] inner diameter.

TABLE 3

Anaerobic biofouling reactor test conditions

Component	Composition
Make-up water	Tap water
	360 ppm Ca
	240 ppm Mg
	pH 8.3
Substrate solution	50 ppm Dextrose
	50 ppm Tryptic Soy Broth
	30 ppm Sodium Sulfite
	50 ppm Sodium Lactate
Temperature	27 °C
Duration	7 days growth
	24–96 h treatment
Inoculum	Field composite and anaerobe culture
Treatment	50–100 ppm Nalco 3WT-138
	(metronidazole + biodispersant)

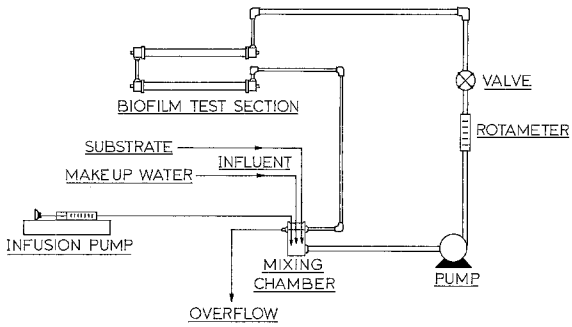

Fig. 2. Schematic of the anaerobic biofouling reactor (ABR).

ABR was allowed to operate in the batch mode for 16 h.

After batch induction fresh broth and make-up water (Table 3) were added to the chemostat (Fig. 2). The chemostat provided a steady source of actively growing cells to the reactor. This was the growth phase. After 7 days a mature biofilm had grown and the system was anaerobic (0.5 ppm O_2). Various dose treatments of biocide were applied for assigned time periods. As a control, 1 g of biofilm was removed from the core tubes at the beginning of the test for complete microbiological analyses. The procedure was repeated after treatment with metronidazole-biodispersant.

Formulation Nalco 7332. Metronidazole was added alone or as a test product, Nalco 7332 (metronidazole-biodispersant). The formulation consisted of 0.9% 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (Fig. 1) active ingredient and 30% proprietary biodispersant. The biodispersant aids the nitroimidazole in penetrating the biofilm. The metronidazole-biodispersant was added to the bench test experiments by standard dilution techniques. To the ABR, the metronidazole-biodispersant was dosed in either a slug concentrate or continuous feed by means of a compact infusion pump from Harvard Apparatus (Millis, MA). The dosages applied were: 58 ppm over a long test period (10 days), 83 ppm over 96 h and 117 ppm for 36 h.

Liquid chromatography. The metronidazole concentration in the product formulation was verified on a Spectra-Physics 8810 liquid chromatograph. The analytical column was a spherisorb-ODS-1, 25 cm × 4.6 mm i.d. The conditions were 10 μl injection size, mobile phase of 10% acetonitrile, 90% water, and detection by UV absorbance at a wavelength of 320 nm, 0.4 AUFS.

Screening experiments. Initially, screening experiments were conducted to obtain the optimum range of metronidazole dosage necessary to reduce the activity of obligate anaerobes with reference to time.

1. 100% kill/inhibition time tests. First, experiments were conducted to determine the dose of biocide for 100% kill. The tests were performed by using a 1 : 100 inoculum of SRB. The metronidazole was added as 9000 ppm to the negative control tube and 1 : 5 serial dilutions of the biocide were made (Table 5).

2. Kill/time tests. The kill study experiments were performed by using the SRB medium without agar. The tubes were inoculated 1 : 100 as above (except controls) and appropriate amounts of metronidazole were added. The tubes were sealed with 1.5 cm liquid paraffin to provide an anaerobic environment. At various time intervals, 1 ml aliquots were withdrawn by syringe. These aliquots were transferred to standard SRB media and incubated at 30 °C.

TABLE 4

Field study system parameters

Calcium	30 ppm
"M" Alkalinity	25–30 ppm
Conductivity	120 μmhos
Cooling water pH	8.0 ± 0.2
Make-up H_2O pH	9.0 ± 0.2
Volume	60 kgal[a]
Water temperature	
Bulk	≤120 °F
Ingot	170–180 °F
Holding time	75 h
Critical casting time	2–2.5 h[b]
Lubricant	50 ppm[c]

[a] 45 kgal actual tower volume + approximately 15 kgal pit levels.
[b] Critical period when equal cooling of ingot surfaces is required. During this time, no plugging can be allowed to interrupt water screen which surrounds the ingot in-process.
[c] An edible oil needed to lubricate the molds used in casting.

Field study. An aluminum casting plant having biodeposits 2 inches thick was treated. The biodeposits averaged 1.4 × 10^6 cfu/g SRB and 2 × 10^3 cfu/g clostridia. The total aerobic bacterial count was 6.1 × 10^7 cfu/g with 3 × 10^6 cfu/g *Pseudomonas* species as counted on cetrimide and PIA. The system parameters are listed in Table 4. The treatment consisted of 100 ppm minimum continuous feed of metronidazole-biodispersant. Chlorination maintained the aerobic bacterial counts at 10^4 cfu/ml in the cooling system water (containing 0.2 to 0.4 ppm free residual chlorine). However, the chlorine and/or halogenated biocides did not control the anaerobic bacteria and biodispersant application did not aid in the control of SRB and clostridia.

RESULTS

Screening tests

The results of the initial screening tests are presented in Table 5. The data after 72 h indicated that 72 to

TABLE 5

100% kill of sulfate-reducing bacteria in 72 h in the bench studies using SRB medium A

No.	Dilution	ppm Metronidazole	Kill/growth
Control	None	None	Positive
Control	None	9000	Negative
1	1:5	1800	Negative
2	1:25	360	Negative
3	1:125	72	Negative
4	1:625	14.4	Positive
5	1:3125	2.9	Positive
6	1:15625	0.58	Positive
7	1:78125	0.12	Positive
8	1:390625	0.02	Positive

9000 ppm metronidazole effectively killed or inhibited 100% of the SRB. The minimum lethal dose was between 14.4 and 72 ppm. Growth occurred in the presence of 14.4 ppm metronidazole. When additional testing was carried out extending the time of exposure and dose of metronidazole product, the results obtained could be summarized as in Fig. 3: at 50 to 100 ppm metronidazole, 100% kill was obtained. The 100 ppm dosage provided 100% kill for 7 days. The results summarized in Fig. 3 also indicate that 50 to 72 ppm of metronidazole was needed for 4 to 5.5 days for kill.

The numbers shown in Table 6 are typical of the biofilm sampled off the stainless steel surfaces of the ABR. The numbers essentially remain the same when metronidazole alone is applied to the ABR. The biodispersant alone offers no biocidal effect (U.S. patent pending). When these levels were treated with 83 ppm metronidazole-biodispersant for 96 h, the aerobic bacteria

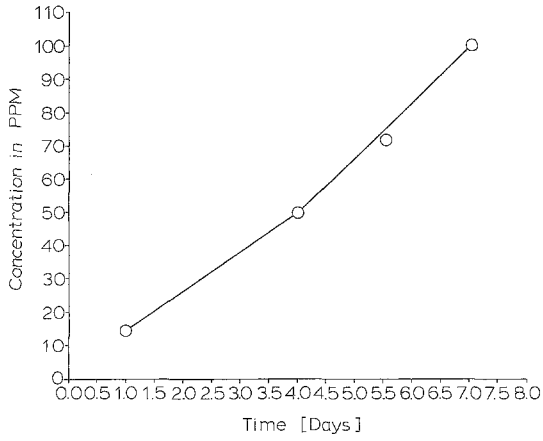

Fig. 3. 100% obligate anaerobe kill by metronidazole over time. The concentration is given in ppm metronidazole (–○–).

TABLE 6

Typical results from biofilm samples drawn from the anaerobic biofouling reactor tubes

	cfu/g
Total aerobic bacteria	1.6×10^9
Enterobacter	$< 1.0 \times 10^3$
Pigmented	$< 1.0 \times 10^3$
Mucoids	$< 1.0 \times 10^3$
Pseudomonas	8.0×10^8
Sporeformers	$< 1.0 \times 10^2$
Total anaerobic bacteria	
Sulfate reducers	4.0×10^3
Clostridia	1.0×10^2
Total fungi	
Molds	< 10
Yeasts	< 10

and the fungi were still unaffected (Fig. 4); however, the SRB were decreased over 3 $\log_{10}$s. When an ABR experiment measured the effect of lower dose over longer time period, 58 ppm metronidazole-biodispersant over 10 days, the results (Table 7) indicated no improvement over the previous findings and no real effect on clostridia. Consequently, the continuous dosage of metronidazole-biodispersant was repeated at 83 ppm and tested over time (at 24, 48, 72 and 96 h). The results summarized in Table 8 indicated very little decrease in total obligate

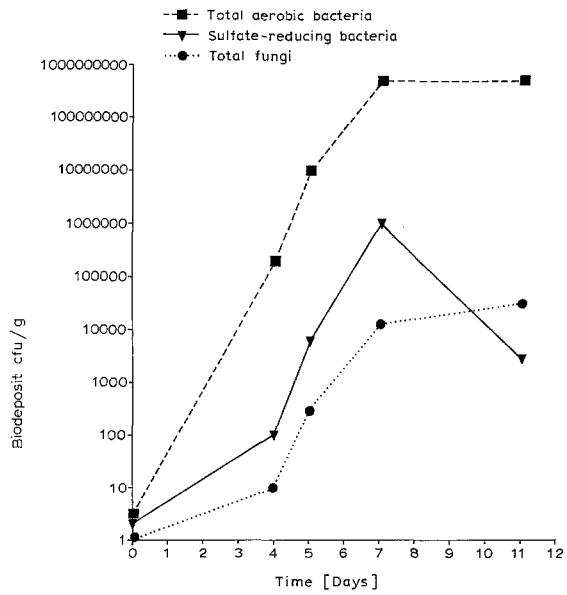

Fig. 4. The effect of 83 ppm metronidazole-biodispersant continuously fed 96 h on 7 day mature biofilm grown from culture CWT3 on the ABR. The SRB (–▼–) were determined in parallel on both SRB media A and B. Total aerobic bacteria (–■–) were counted in TGE; total fungi (–●–) on tartrate-acidified PDA.

TABLE 7

The effects of feeding of 58 ppm metronidazole-biodispersant continuously in the anaerobic fouling reactor for 10 days

Type	Time Days	Total aerobic bacteria	Total fungi	Sulfate reducing bacteria		
				Medium A	Medium B	Clostridia
Bulk H_2O	2	1.8×10^7	<10	2×10^3	$\geq 10^3 - \geq 10^4$ [a]	None [b]
Bulk H_2O	4	6.0×10^6	<10	1.8×10^5	$\geq 10^4$	None [b]
Bulk H_2O	8	1.2×10^7	1×10^1	1.0×10^2	$\geq 10^3$	1×10^0
Deposit-chemostat	8	2.3×10^9	4×10^5	4.0×10^5	$\geq 10^6$	2×10^3
Deposit-tubes	8	1.5×10^9	4×10^2	2.0×10^3	$\geq 10^6$	1×10^2
Bulk H_2O	10	1.6×10^7	<10	1.0×10^3	$\geq 10^3$	None [b]
Deposit-chemostat	10	8.2×10^8	3×10^2	1.0×10^5	$\geq 10^5$	1×10^3
Deposit-tubes	10	1.8×10^9	2×10^4	2.0×10^4	$<10^3$	1×10^2

[a] Range of $\geq 10^3$ to $\geq 10^4$ cfu/g.
[b] None detected on undiluted sample.

anaerobic bacteria in deposit samples at 72 h but a 3 $\log_{10}$ decrease in SRB by 96 h. The decrease in clostridia at 96 h was insignificant.

The dose of metronidazole-biodispersant was increased to 115 to 120 ppm to determine whether a higher concentration would be as effective as the 83 ppm metronidazole-biodispersant in the 24–32 h period. The results at 117 ppm actual feed rate exhibited no reduction in obligate anaerobic bacteria in 24 h but a 4 $\log_{10}$ reduction in SRB had occurred by 32 h (Table 9). No reduction in clostridia was observed in the deposit in the same time frame.

Field test of metronidazole-biodispersant.

In field study conditions where continuous levels of chlorine (0.2–0.5 ppm HOCl) plus biweekly slug additions of a dibrominated aliphatic nonoxidizing biocide had failed to control the SRB population, metronidazole-

TABLE 8

Summary of tests for continuous feeding of 83 ppm metronidazole-biodispersant into the anaerobic biofouling reactor

Type	Time (h)	Total aerobic bacteria [a]	Total fungi [a]	Sulfate reducing bacteria [a]		Clostridia [a]
				Medium A	Medium B	
Chemostat-deposit	0	2.5×10^7	1.2×10^3	>400000	$\geq 10^7$	4×10^3
Tube-deposit	0	8.8×10^7	1.0×10^3	50000	$\geq 10^6$	3×10^3
Chemostat-deposit	24	8.5×10^9	1.3×10^3	>50000	Positive [b]	3×10^3
Tube-deposit	24	2.1×10^{10}	2.0×10^2	>50000	Positive [b]	3×10^3
Chemostat-deposit	0	8.3×10^9	7.0×10^3	100000	$\geq 10^7$	2×10^3
Tube-deposit	0	1.1×10^{10}	1.7×10^3	30000	$\geq 10^7$	3×10^3
Chemostat-deposit	48	1.0×10^{10}	6.0×10^4	30000	$\geq 10^7$	3×10^3
Tube-deposit	48	2.0×10^{10}	6.0×10^4	90000	$>10^5$	2×10^3
Chemostat-deposit	0	2.3×10^9	7.0×10^4	300000	$>10^7$	1×10^3
Tube-deposit	0	1.7×10^{10}	3.0×10^4	>500000	$>10^7$	3×10^3
Chemostat-deposit	72	1.1×10^9	2.1×10^5	400000	$<10^3$	1×10^3
Tube-deposit	72	1.4×10^9	3.6×10^5	50000	$\geq 10^4$	3×10^3
Chemostat-deposit	0	2.5×10^9	2.8×10^4	500000	$\geq 10^5$	6×10^2
Tube-deposit	0	3.7×10^9	1.3×10^4	290000	$\geq 10^6$	1.0×10^3
Chemostat-deposit	96	6.9×10^8	1.1×10^5	1000	$<10^3$	7.0×10^2
Tube-deposit	96	4.5×10^9	3.3×10^4	700	$<10^3$	1.0×10^2

[a] cfu/g.
[b] Positive = growth.

TABLE 9

Reduction of obligate anaerobic bacteria in the anaerobic biofouling reactor using a continuous feed of 117 ppm metronidazole-biodispersant

Type	Time (h)	Metronidazole-biodispersant (ppm)	Total anaerobic bacteria			
			Sulfate reducers		Clostridia	
			cfu/g	% Reduction	cfu/g	% Reduction
Deposit	24	117	10^6–10^6	0	10^3–10^3	0
Deposit	32	117	10^7–10^3	99.9	10^3–10^3	0

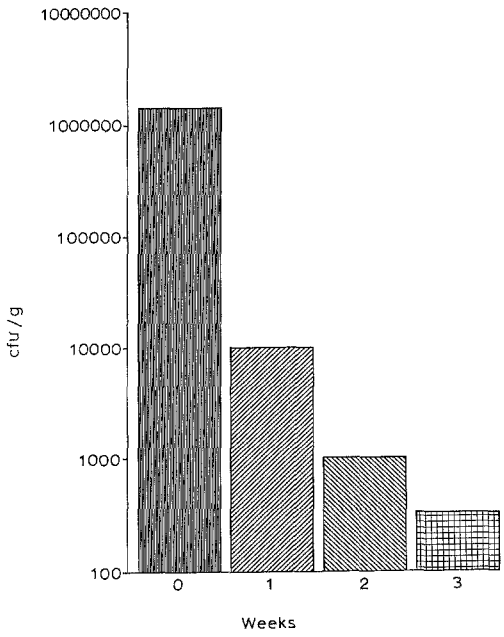

Fig. 5. Sulfate-reducing bacteria levels per gram biodeposit collected from a cooling water system at 0 (control; vertical, 1 (right hatched), 2 (left hatched) and 3 (checkered) weeks after metronidazole-biodispersant treatment (100–120 ppm continuously fed). The counts were run on SRB medium A.

biodispersant was tested. The SRB level was 3 000 000 cfu/g before treatment with metronidazole-biodispersant. The kill rate when 100 to 120 ppm metronidazole-biodispersant was fed continuously (100 ppm was the minimum dose) is shown in Fig. 5. The use of metronidazole-biodispersant resulted in a decrease of 2 $\log_{10}$ in one week with an additional $\log_{10}$ kill after two weeks. Continued usage resulted in further reduction to approximately 300 cfu/g sample. Subsequent decreases (not shown) were to 200 cfu/g sample.

DISCUSSION

The screening experiments for dosage and time indicated that 14.4 to 72 ppm of metronidazole was needed to kill obligate anaerobic bacteria. The metronidazole was needed at 50 to 72 ppm over four to five and one-half days for detectable kill of SRB. Consequently, 50 ppm of metronidazole-biodispersant was used as the minimum dose required to be effective against obligate anaerobes in the dynamic anaerobic biofouling reactor (ABR) tests.

However, when 50 ppm metronidazole was added to the ABR, essentially no effective kill of SRB was measured. The bioreactor was designed to induce a high level of biofilm production and enhance underdeposit growth of anaerobic bacteria. In the ABR, the metronidazole alone was ineffective because it could not penetrate the thick biodeposit of slime-forming bacteria to make contact with the anaerobes. Higher concentrations of metronidazole were not effective and were commercially impractical. However, the metronidazole plus biodispersant blend worked in the ABR against the obligate anaerobes growing beneath the biofilm. When 83 ppm metronidazole-biodispersant was fed continuously into the ABR for 48 h, the product reduced the number of SRB by 2 $\log_{10}$s; for 72 h by 3 $\log_{10}$s; for 96 h by $\geq$3 $\log_{10}$s. Continuous feeding of 117 ppm metronidazole-biodispersant for 36 h decreased the number of SRB by 4 $\log_{10}$s. The use of 117 ppm for 24 h (Table 9) was insufficient for killing obligate anaerobes. The results suggest either that a dose-time relationship which has not yet been defined is involved or that 36 h is the approximate time required for the metronidazole to penetrate the biofilm.

In terms of microbiological results, the field test of the metronidazole-biodispersant was successful; the SRB level was substantially reduced (4 $\log_{10}$s) over the test period. Also, the system pH of the bulk water was found to increase from 6.2 to 8.0, which is the specification pH of the system. Change in the pH was due to killing obligate anaerobic bacteria which had been producing organic

acids and H_2S. The kill of clostridia and SRB allowed the make-up water to reach pH of 8.0. Additional changes in the parameters of the system have been described [15].

Other researchers [10] have shown metronidazole is non-toxic to microorganisms as long as the nitro group (Fig. 1) remains intact. Only obligate anaerobic bacteria are capable of reducing the nitro group due to the low redox potential of their electron transfer proteins flavodoxin and ferredoxin [10]. These facts are important in developing a biocide which first, should be non-toxic to organisms in natural waters where cooling water may be discharged but second, should also be biocidal to specific groups of bacteria which are involved in corroding cooling systems. The results presented in this report indicate that metronidazole-biodispersant was non-toxic to aerobic bacteria, yeasts and molds. But metronidazole was toxic to sulfate-reducing bacteria in both the laboratory and field studies. The effect of metronidazole on clostridia, however, was less than expected from previous reports [5,7,12,13]. Nevertheless, in the field trial metronidazole-biodispersant reduced the clostridial count from 6000 to 200 cfu/g at two weeks. Subsequent decreases were not statistically different. Possibly airborne clostridial spores from soil inoculated the open cooling tower system during the field study so that the level of cells detected remained at 10^2 cfu/g.

With respect to obligate anaerobes causing corrosion, continuous bacterial culture studies have been described recently [14]. We are currently investigating underdeposit corrosion by both SRB and clostridia to determine whether metronidazole-biodispersant kill rates and cessation of underdeposit corrosion correlate over time. In the field trial discussed here, corrosion problems were not observed on mild steel coupons after metronidazole-biodispersant treatment whereas pitting in the metal was found prior to treatment.

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REFERENCES

1 Brook, I. 1989. Pediatric Anaerobic Infection: Diagnosis and Management. p. 352. The C.V. Mosby Co. St. Louis.
2 Characklis, W.G. and K.E. Cooksey. 1983. Biofilms and microbial fouling. Adv. Appl. Microbiol. 136: 93–138.
3 Chow, A.W., V. Patten, and L.B. Guze. 1975. Susceptibility of anaerobic bacteria to metronidazole: relative resistance of non-spore forming gram positive bacilli. J. Infect. Dis. 131: 182–187.
4 Costerton, J.W., R.T. Irvin, and K-J. Cheng. 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. 35: 299–324.
5 Edwards, D.I., M. Dye, and H. Carne. 1973. The selective toxicity of antimicrobial nitroheterocyclic drugs. J. Gen. Microbiol. 76: 135–145.
6 Hamilton, W.A. 1985. Sulfate-reducing bacteria and anaerobic corrosion. Annu. Rev. Microbiol. 39: 195–217.
7 Jones, B.M., I. Geary, M.E. Lee, and B.I. Duerden. 1989. Comparison of the in vitro activities of fenticonazole, other imidazoles, metronidazole, and tetracycline against organisms associated with bacterial vaginosis and skin infections. J. Antimicrob. Chemother. 33: 970–972.
8 King, R.A. and J.D.A. Miller. 1971. Corrosion of the sulfate-reducing bacteria. Nature (Lond.) 233: 491–492.
9 LeGall, J. and G. Fauque. 1988. Dissimilatory reduction of sulfur compounds. In: Biology of Anaerobic Microorganisms (Zehnder, J.B., ed.), pp. 587–640, John Wiley and Sons, New York.
10 Muller, M., D.G. Lindmark, and J. McLaughlin. 1979. Mode of action of metronidazole on anaerobic microorganisms. Int. Congr. Symp. Ser. R. Soc. Med. 18: 223–228.
11 Muller, M., D.G. Lindmark, and J. McLaughlin. 1976. Mode of action of nitroimidazoles on trichomonads. In: Biochemistry of Parasites and Host-Parasite Relationships (Van den Bossche, H., ed.), pp. 537–544, North-Holland, Amsterdam.
12 Sutter, V.L. and S.M. Finegold. 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. Antimicrob. Agents Chemother. 10: 736–742.
13 Tally, F.P., V.L. Sutter and S.M. Finehold. 1972. Metronidazole versus anaerobes. Calif. Med. 117: 22–26.
14 Weimer, P.J., M.J. Van Kavelaar, C.B. Michel, and T.K. NG. 1988. Effect of phosphate on the corrosion of carbon steel and on the composition of corrosion products in two-stage continuous cultures of *Desulfovibrio desulfuricans*. Appl. Environ. Microbiol. 54: 386–396.
15 Wiatr, C.L., C.R. Smyrniotis, E.R. Brundage. 1990. The importance of microbiological control in casting. Light Metals 1990. Amer. Instit. Mining, Metallurgical and Petroleum Eng., pp. 911–916.