



Effect of Microbial Growth Products on Biocide Activity in Metalworking Fluids

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ABSTRACT

The effect of bacterial growth products on seven industrial biocides used in metalworking fluids was evaluated. Prior bacterial growth in two typical metalworking fluids resulted in a substrate that uniformly reduced the effectiveness of all biocides when compared to the same unused metalworking fluids. Two selective results of growth in metalworking fluid, lowering of pH and production of sulfide, only affected two of the biocides.

INTRODUCTION

An experience with evaluation of biocidal needs of a field metalworking fluid (MWF) sample (Table 1) led to the study presented in this paper. There are ample published references to the modification of MWF by microbial growth (Almen *et al.*, 1982; Holtzman *et al.*, 1982) but none deals directly with the problem of interaction of microbial growth products with the biocide.

Earlier it was reported that prior growth of aerobes passively or actively encouraged the growth of sulfate reducers in MWF in the laboratory (Isenberg & Bennett, 1959). The growth of fungi in MWF was

TABLE 1
Appropriate Selection of Substrate for Biocide Evaluation for Field Application^a

<i>Biocide</i> ^b	<i>Distant laboratory</i>	<i>Field trial</i>	<i>Local laboratory</i>	
			<i>Without sludge</i>	<i>With sludge</i>
Control	F ^c	F	F	F
MCI 100 ppm	S ^d	F	S	F
HEAT 1500 ppm	S	INC ^e	S	F
HEAT 750 ppm-75 ppm	S	INC	S	F

^aEvaluation was made first in a laboratory distant from the problem site with the recommended fluid, but unused and the testing laboratory's inoculum. Potentially best biocide was added to the manufacturing site. Local laboratory repeated evaluation using spoiled fluid from site, with and without 10% sulfide sludge from bottom of system. System was a 3×10^6 liter central system sump containing 5% soluble oil emulsion feeding a mixed function transfer line mainly cast iron machining.

^bSee Table 2 for full biocide names.

^cF = failure.

^dS = success.

^eINC = inconclusive.

also facilitated by prior growth of an aerobic bacterial population (Rossmoore & Holtzman, 1974). These trends were confirmed for both anaerobic sulfate reducers and fungi in an extensive field study of over 100 MWF systems; the levels of these deteriogens depends on the levels of aerobic bacteria (Rossmoore *et al.*, 1987).

More recently Sondossi *et al.* (1989), showed that pioneer pseudomonads capable of dissimilating hydrocarbons in a soluble oil emulsion were not present in the mixed population at the end of a biocide study, whilst the dominant pseudomonad, recovered at the end of the field study, was unable to grow either on hexadecane or the fresh soluble oil but was resistant to the biocide. However, there appears to be no difficulty in finding MWF isolates capable of growth on hydrocarbons (Foxall-VanAken *et al.*, 1986).

It appears likely that biodeterioration of MWF results in changes in the chemistry of the fluids, positively with respect to survival and growth of some microbial populations (i.e. a friendlier environment has been produced).

Of equal practical consideration to population succession is the question of biocide effectiveness in spoiled fluids. Many test methods have been developed over the past 30 years for the evaluation of biocides in MWF (Pivnick & Fabian, 1953; Carlson & Bennett, 1960; Himmelfarb

& Scott, 1968; Hill, 1969; Rossmore & Williams, 1971; Bennett, 1974; Rogers *et al.*, 1975), some of which use fresh fluids while others utilize the contaminated field sample as the test substrate. There are two consequences of microbial growth in MWFs: (1) the dissimilation of inhibitory ingredients; and (2) the production of stimulating metabolites. Both of these alternatives can affect population succession directly and indirectly by interfering with biocide function. For example, production of nucleophiles, drop in pH, and formation of enzymes related to resistance development all lead to enhanced survival.

MATERIALS AND METHODS

Fluids

Two commercially available MWFs were used in this study, a soluble oil and a synthetic fluid. Each was diluted with tap water to a 5% v/v solution and used in all the MWF studies.

Preparation of contaminated metalworking fluid

A field sample of spoiled MWF (10 ml) was mixed with 50 ml of fresh MWF and 40 ml of tryptic soy broth (BBL) and incubated for 24 h, with shaking, at room temperature. The bacterial count was 2.3×10^9 CFU/ml. This mixture was added to 900 ml of fresh MWF of the type used in the inoculum. The regimen followed the protocol of ASTM E686.85 (ASTM, 1985) for a period of 8 weeks.

The count at the end of the 8 weeks was 2.1×10^9 CFU/ml for the soluble oil and 1.3×10^9 CFU/ml for the synthetic fluid. A sample of 200 ml of each fluid was set aside to be used as inoculum in biocide evaluation. The remaining 800 ml of each were heated at 60°C for 15 min and then brought to the pH of fresh fluid with 0.1 M NaOH. The count of the heated fluids was <100 CFU/ml.

Evaluation of biocides

Three concentrations of seven biocides commonly used in MWF (Table 2) were added to the following mixtures:

- (1) 45 ml of heated contaminated MWF + 5 ml of the inoculum described above; and
- (2) 45 ml of fresh MWF + 5 ml of inoculum described above.

TABLE 2
Biocides Used in Studies

		<i>Active ingredient (%)</i>
HEAT	1,3,5-tris (2-hydroxyethyl) hexahydrotriazine	78
OXA	4,4-dimethyloxazolidine	77
GLA	Glutaraldehyde	45
MCI	5-chloro-2-methyl-3(2H) isothiazolone + 2-methyl-3(2H) isothiazolone }	14.5
NM	4,4'(2-ethyl-2 nitrotrimethylene) dimorpholine + 4-(2-nitrobutyl) morpholine	90
TN	Tris (hydroxymethyl) nitromethane	50
PCMX	Parachlorometaxylenol	100

The inoculated 100 ml flasks were incubated at room temperature, on a reciprocal shaker, for 72 h.

HPLC regimen

HPLC fingerprints were made on both fluids before and after bacterial growth, according to the following protocol.

The samples were prepared for injection by adding 25% v/v of 6% MgCl₂ solution and extracting with hexane. The samples were then centrifuged for 6 min at 1500 rpm, and the organic layer was separated and discarded. The aqueous layer was then filtered through a 0.22 μm filter, and approximately 1.3 ml portions of this filtrate were put into sample bottles for injection into the HPLC unit.

Chromatography was performed on a Beckman 334 Gradient Liquid Chromatograph using a Whatman 25 cm, 4.6 mm C-18 (octadecylsilane) column. This column has particles of 5 μm size, with a latex-O-Si-O-(CH₂)₁₇-CH₃ configuration. Data were recorded on an Altex C-Ria recorder. The mobile phase was 80% water and 20% methanol. The flow rate was 1 ml/min. Column pressure was approximately 1200 psi.

Samples were injected with an Altex Model 500 Autosampler equipped with a 100 μm sample loop. Detection occurred at 275 nm with a Beckman 165 Variable Wavelength Detector.

MIC study

Subsequent to the study with used MWF, the seven biocides were evaluated to determine the effect of pH and sulfide ion on their minimal

inhibitory concentrations (MICs). These two variables potentially represent growth products that might affect biocide activity.

Inoculum

A strain of *Pseudomonas aeruginosa* isolated from MWF and maintained in the laboratory for 10 years was used in this study.

Medium

Tryptic soy broth (DIFCO Laboratories) at ambient pH 7 and poised at pH 8.7 (pH of MWF) was the base for the MIC study; in addition, one set at both pHs was supplemented with 5 mM Na₂S and contained one of six concentrations of each biocide.

Protocol

A sample (10 ml) of each medium was inoculated with 0.1 ml of a 24 h broth culture of *Ps. aeruginosa*, giving a zero time level of 2×10^7 CFU/ml, and incubated at 30°C for 72 h. Results were recorded as growth or no growth for each concentration of biocide. The net result is reported as MIC in Table 3.

RESULTS AND DISCUSSION

In every instance (Figs 1-7), the biocides tested were less effective in fluid in which there had been prior bacterial growth, although there were differences between the two fluids and among the seven biocides. These results could be attributable to the production of neutralizing metabolic

TABLE 3
Effect of pH and Sulfide Ion on the Minimal Inhibitory Concentration of Seven Biocides

<i>Biocide (ppm)</i>	<i>Tryptic soy broth</i>		<i>Tryptic soy broth + 5 mM Na₂S</i>	
	<i>pH 7.5</i>	<i>pH 8.7</i>	<i>pH 7.5</i>	<i>pH 8.7</i>
NM	<125	375-500	250-375	375-500
PCMX	>1 000	500-750	>1 000	750-1 000
OXA	>1 000	>1 000	>1 000	>1 000
HEAT	375-500	500-750	250-375	500-750
GLU	>1 000	>1 000	>1 000	>1 000
TN	>1 000	>1 000	>1 000	>1 000
MCI	12.5	12.5	12.5	50-75

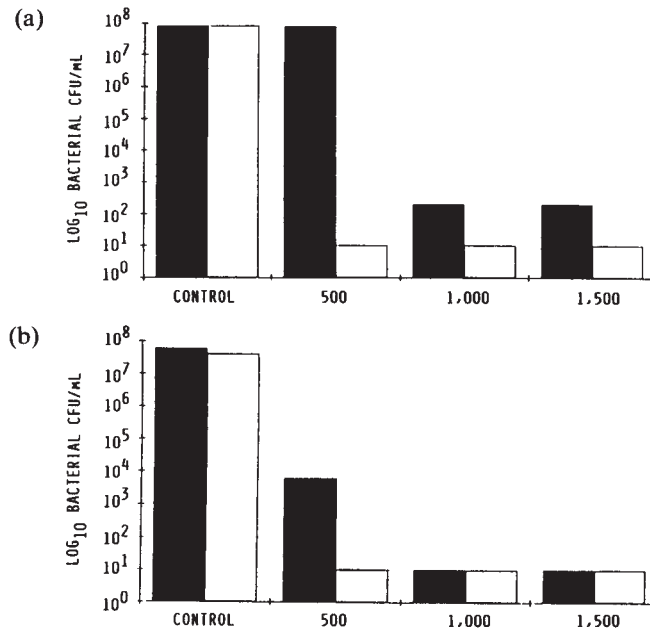


Fig. 1. The effect of bacterial growth in metalworking fluids on the efficacy of GLA: (a) synthetic fluid; (b) soluble oil. ■, Fluid with prior microbial growth; □, freshly prepared metalworking fluid.

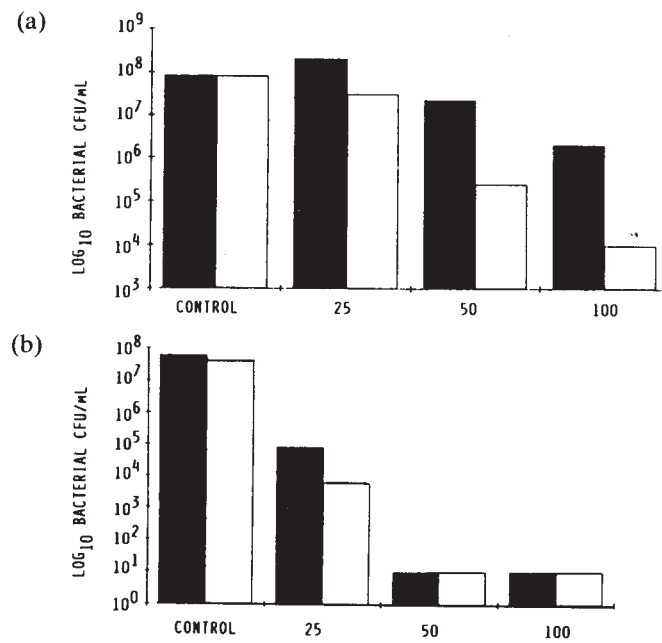


Fig. 2. The effect of bacterial growth in metalworking fluids on the efficacy of MCI: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

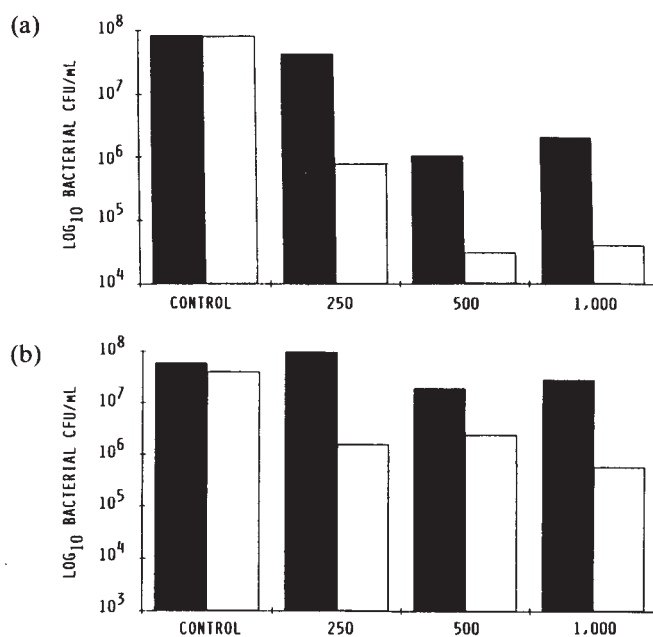


Fig. 3. The effect of bacterial growth in metalworking fluids on the efficacy of NM: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

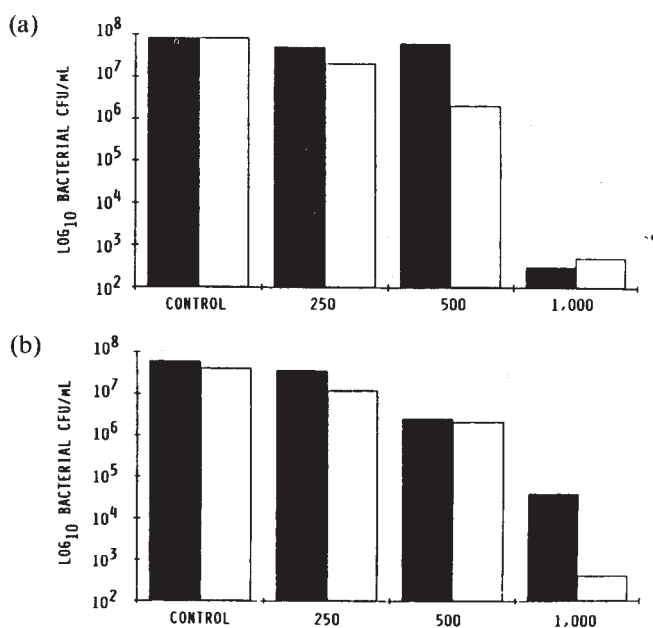


Fig. 4. The effect of bacterial growth in metalworking fluids on the efficacy of OXA: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

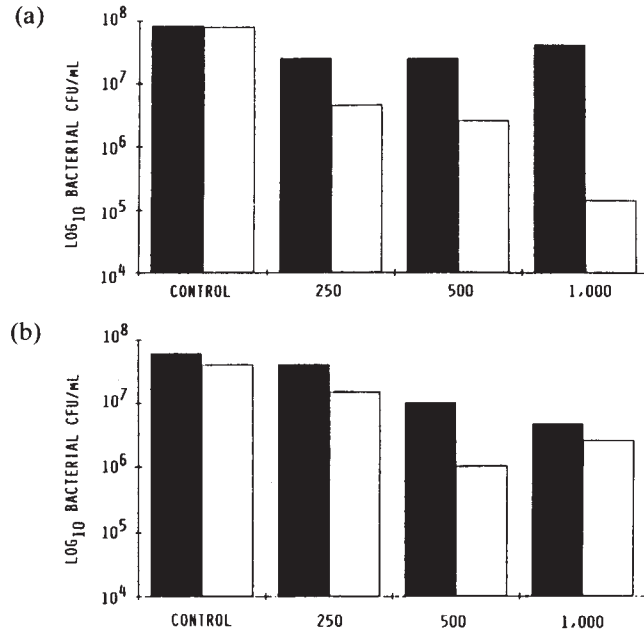


Fig. 5. The effect of bacterial growth in metalworking fluids on the efficacy of PCMX: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

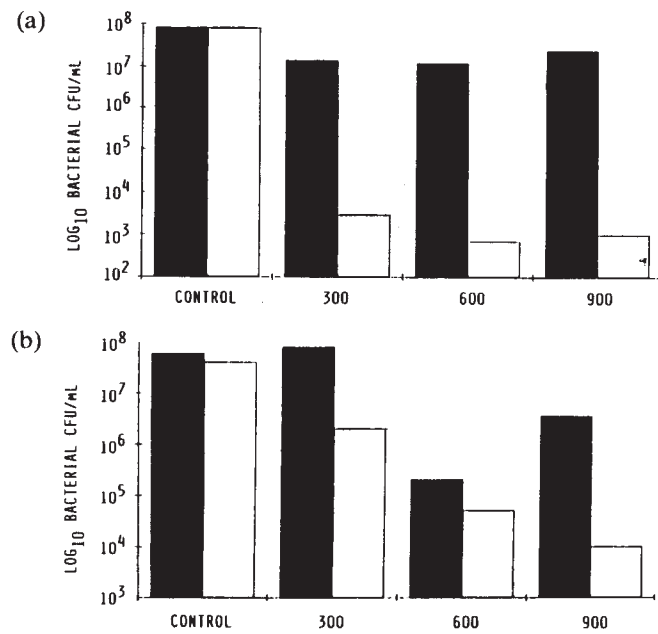


Fig. 6. The effect of bacterial growth in metalworking fluids on the efficacy of TN: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

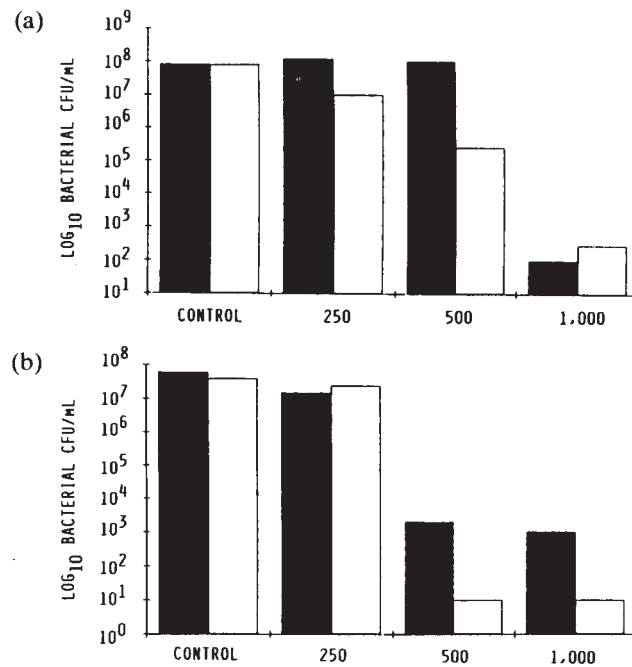


Fig. 7. The effect of bacterial growth in metalworking fluids on the efficacy of HEAT: (a) synthetic fluid; (b) soluble oil. (See Fig. 1 for key.)

products or to the heat-killed biomass or both. The HPLC data for both fluids (Fig. 8) show a reduction in the major peak when comparing fluids before and after bacterial growth.

This quantitative information is only indicative of a reduction in UV absorbing materials, although it does reflect degradation. Degradation was reported for soluble oil emulsion based on IR and GC (Holtzman *et al.*, 1982), and HPLC was used to monitor microbial activity in aluminium can forming fluid (Almen *et al.*, 1982). The results (Table 3) with the MIC study were not too revealing; in only two instances did either pH or sulfide ion have an effect, and only the isothiazolone was negatively affected by sulfide while the nitromorpholine was actually more active at the lower pH. This latter finding, although not especially helpful in controlling MWF bacteria, is not entirely unexpected. Antimicrobial agents vary in their response to pH change; halogens, organic acids and phenols are all more active at lower pH, while glutaraldehyde and isothiazolones appear to be more active at higher pHs.

In a related finding, a formaldehyde donor biocide protected isothiazolone from nucleophilic attack at pH 7 but not at pH 9

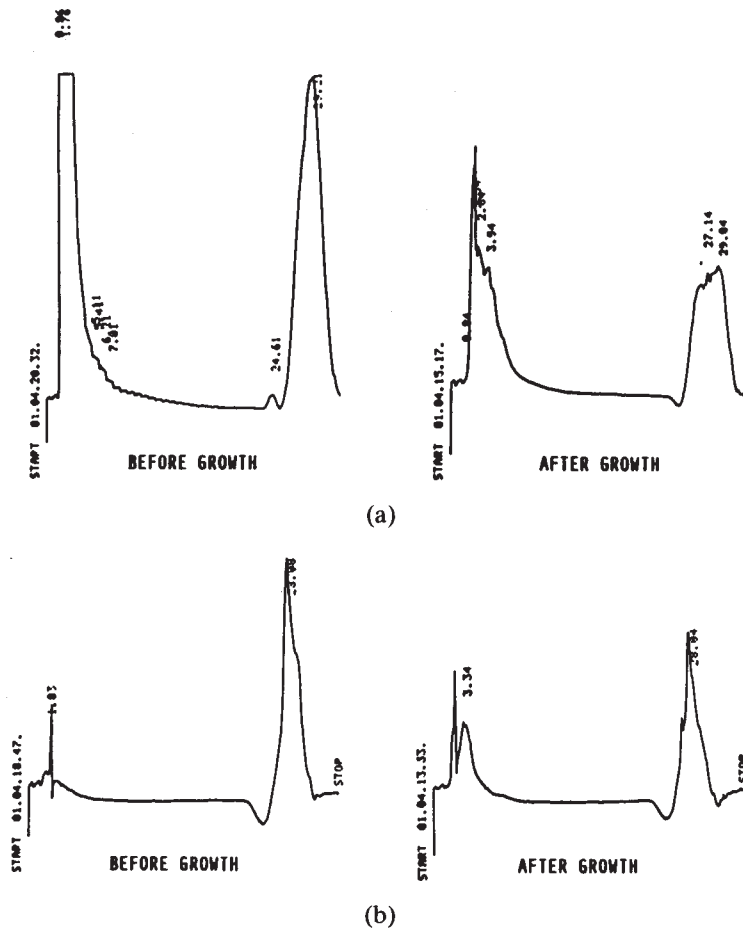


Fig. 8. HPLC scans before and after bacterial growth in metalworking fluids. The complete protocol is described in the text: (a) 5% soluble oil fluid; (b) 5% synthetic fluid.

(unpublished data). Douglas *et al.* (1990) showed that formaldehyde-based biocides reacted equally with free endotoxin as well as cell-bound endotoxin when measured by the *Limulus amoebacyte lysate* (LAL) test. Earlier it was found (unpublished data) that cell populations based on LAL extrapolations were 10 times higher than viable cell counts in MWF; this has recently been verified by Mattsby-Baltzer *et al.* (1989). This reactive biomass must be considered in the dosing of any contaminated MWF. Judging the impact of microbial growth products in an actual system is often complicated by two additional related factors; the chemistry of the fluid (Sondossi *et al.*, 1985) and the potential for resistance in the resident microbial population (Sondossi *et al.*, 1989).

Certainly, this interrelated trinity must be considered in evaluating the specific role of microbial growth products on biocide efficacy.

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