Biocide Usage in Metalworking Fluids: The Effect of Treatment Patterns On Efficacy

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The effects of biocide treatment patterns on antimicrobial efficacy in metalworking fluids was studied. In laboratory experiments, fouled fluids were treated with a commercial biocide at various concentrations and frequencies, while microorganism populations were monitored. For all biocide application rates tested, the efficiency of antimicrobial control was found to vary widely with treatment pattern. Less frequent doses with higher concentrations of biocide were found to be much more effective than low-level, frequent doses. The reasons for this behavior are investigated, and found to be related to biocide residual concentrations, biocide consumption by microorganisms, and changes in the predominant species of bacteria which populated the fluids.

MATERIALS AND METHODS

General

Gluconolactone analysis
Gluconolactone concentrations were determined by gas chromatography, as previously described (6).

Metalworking fluid
The same fluid was used in all of the experiments in this study. It is a soluble oil used in aluminum hot rolling, and contains no biocide.

Microbiological evaluation
Bacteria were counted by standard pour plating of serially diluted samples on nutrient agar. Plates were incubated for 48 hours at 37°C.

Treatment Pattern Studies

Source of microorganisms
The microorganisms used in this study were obtained as a mixed culture from a fouled aluminum hot rolling mill. They were maintained solely on metalworking fluid after leaving the mill, in the following manner:

Stock culture
A fresh sample of metalworking fluid was prepared by dilution of 50 g of the fluid concentrate to a volume of 1 L with sterile deionized water in a sterile culture flask. The mixture was agitated thoroughly, and the flask was covered with a cap which allowed air but not microorganisms to enter. This mixture was inoculated with the microorganisms described above, swirled on a gyrorrotary shaker at 256 rpm and allowed to grow for 4 to 6 weeks at approximately 40°C, allowing the microorganism population to reach a level of approximately 10⁶ colony forming units (cfu/mL). During this period and throughout culture maintenance, 20 percent of the mixture was removed weekly and replaced with much to do with its success as does the choice of specific chemical. For example, how much biocide should be added to a system, and how often? The purpose of this investigation is to explore the relationship of biocide use pattern to efficacy.

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fresh diluted fluid. In addition, evaporative losses were made up with sterile water as needed.

Biocide susceptibility

The stock culture was divided into 50 ml portions and transferred to 250 ml sterile culture flasks. Each flask was capped as described above, and returned to the geyseratory shaker at 40°C. Biocidal expressions (as described active glutaraldehyde) were made periodically, by addition of the appropriate amounts of 45 percent glutaraldehyde, according to the schedules described in the text. Surviving microorganisms were counted immediately before each biocide addition, and at 1, 3, 7, 14, and 24 h thereafter.

RESULTS AND DISCUSSION

The effect of treatment pattern of efficacy

The first series of experiments was designed to examine optimal biocide treatment frequency. The biocide used was glutaraldehyde, a material which is approved by the Environmental Protection Agency for use in metallurgical fluids. A fouled aluminum hot rolling fluid was divided into equal portions, and treated with glutaraldehyde according to the following regimen.

Protocol One: 150 ppm total per week, added as:
1a. 50 ppm, 5 times per week (hereinafter abbreviated 305)
1b. 50 ppm, 3 times per week (503)
1c. 150 ppm once per week (1501)

Protocol Two: 300 ppm total per week, added as:
2a. 60 ppm, 5 times per week (605)
2b. 100 ppm, 3 times per week (1003)
2c. 300 ppm, once per week (3001)

Control: no biocide additions

Treatments were continued for two months, during which time microbial populations were monitored closely.

The results of these experiments are shown in Fig. 1 and 2 for protocols one and two, respectively. In all cases, the mixtures gradually attained a steady level of microbial growth, between 0 and 10^7 cfu/mL. It can be seen in one of the patterns of biocide addition by a striking influence on the level of this steady state.

In protocol one, the addition of 30 ppm of glutaraldehyde every week, however, has virtually no lasting effect on bacterial populations. Similar results were obtained with the addition of 100 ppm times three per week (Monday, Wednesday, and Friday). On the other hand, when the total amount of glutaraldehyde is added in one slug dose of 150 ppm weekly, bacterial populations are reduced by 6 logs during the first week of treatment, and reach undetectable levels (less than 10^5 cfu/mL) after the second week. Bacterial populations remained at undetectable levels for the remainder of the experiment. In this 150/1 protocol, in spite of the fact that, as in all these experiments, the test mixture was insulted weekly with additional inoculum.

The results from protocol two (total 500 ppm per week) were generally similar. In this case, however, the intermediate treatment frequency (100/3) did show some slight improvement in performance over the high frequency treatments, yielding bacterial counts which were consistently, though only modestly, lower than in the 60/5 regimen.

A comparison of protocols one and two serves to point out an even more striking contrast between the various treatment regimens. As noted above, the weekly addition of 150 ppm of glutaraldehyde to the fouled system results in a decrease in bacterial populations to undetectable levels after only the second week of treatment. By contrast, addition of 100 ppm three times per week, twice the total biocide usage, results in only a modest reduction in bacterial levels compared to controls. In fact, the 150/1 treatments proved far superior to even daily additions of 50 ppm of glutaraldehyde (data not shown); the latter resulted in stable bacterial populations of 10^2 to 10^4 cfu/mL. Thus, use of one third the total amount of biocide (i.e., 150 vs. 450 ppm total per week) yielded far better microbial efficacy when applied in the less frequent dosage pattern. The implications of these results for field use of biocides will double as a case of lubrication engineer interested in minimizing biocide costs while maintaining a clean fluid system.

Biocide consumption in a fouled system

In considering the mechanism which might result in the aforementioned effects, we wondered whether biocidal de- piction by microorganisms (4) might be responsible for the observed microbial response. To investigate this possibility, glutaraldehyde was monitored over time in the test solutions. The contaminated fluid was treated with the various concentrations of glutaraldehyde.

When the contaminated fluid was treated with 50, 30, or 10 ppm of glutaraldehyde, essentially all of the biocide was depleted within 5 hours (Fig. 3). Treatment with 100 ppm resulted in loss of 70 percent of the added glutaraldehyde within a grid count of the remainder over the course of a week. By contrast, when 150 or 300 ppm of glutaraldehyde was added to the fouled fluid, biocidal concentrations decreased rapidly for the first few hours, but then stabilized at a level only a few more ppm in the next 7 days. Control experiments showed that virtually no glutaraldehyde was lost under similar conditions in fluid.

When the glutaraldehyde residual concentrations are compared with bacterial growth in these fluids, an explanation for the greater efficiency of large slug doses may be discerned. Glutaraldehyde is known to exert its antimicrobial activity by chemical reaction with the external surface of cells, resulting in the loss of glutaraldehyde as the cells are killed (6) and references cited therein. In these experiments, the lower doses of glutaraldehyde (30 through 100 ppm) resulted in an initial decrease in bacterial populations, followed by a rebound after the biocide was depleted by reaction with the microorganisms. On the other hand, in cases in which the larger doses were applied (150 to 300 ppm), bacterial populations were reduced to virtually zero, so that glutaraldehyde loss slowed drastically within several hours after treatment. Since no viable cells remained, the biocide was monitored at constant levels, and bacterial regrowth was prevented even when the fluids were re inoculated with fresh inoculum. In support of this explanation, it should be noted that total glutaraldehyde loss was virtually complete in one week when up to 100 ppm was added in the single slug dose. By contrast, only 40 and 50 ppm were consumed by the 150 and 300 ppm doses, respectively.

Because of this biocide usage by bacteria in a fouled system, actual concentrations in a fluid may be much lower than would be expected based on the amount of biocide added. For this reason, it is desirable to monitor actual biocide concentrations in a system whenever possible, since this will give a rapid indication of potential effectiveness. By comparing biocide stability with bacterial counts (as indicated by dipstick of other means) over a period of time, certain trends may be observed. Based on such trends, the need for further biocide additions may be judged in a much more immediate fashion than by anodated plate count or dipsticks, which require 25 to 48 hours for analysis.

Bacterial selection

During these experiments, an interesting effect was observed when the fouled fluid was treated with subidal doses
of biocide. In one experiment, the fouled fluid was divided into equal portions, and each was treated with daily doses of glutaraldehyde ranging from 20 to 100 ppm. Microbiological response is shown in Fig. 4. As expected, larger doses of biocide initially yielded larger population reductions. However, in all cases, repeated addition of doses which were insufficient to kill all of the bacteria in the mixtures led to an antimicrobial response which was of decreasing magnitude.

We initially ascribed the apparent decreasing effectiveness of the biocides to development of resistance by the bacteria (9). However, as we have not observed this behavior in response to glutaraldehyde in the past, we decided to investigate the matter further. The principal strains of bacteria were isolated from the untreated fouled fluid and characterized. One species, Pseudomonas fluorescens, was found to predominate greatly, and was accompanied by lesser amounts of Pseudomonas stutzeri and another unidentified Pseudomonas. The Pseudomonas fluorescens was isolated and used to inoculate a sterile sample of fluid, which was allowed to grow to a cell density of approximately 10^5 cfu/ml. This mixture was then treated with successive doses of glutaraldehyde, as described above for the mixed culture. In this case, however, the microbiological response to the treatments was completely different (Fig. 5). After successive additions of 100 ppm of glutaraldehyde, equivalent or slightly increasing bacterial reductions were observed, reducing the populations to zero after the fourth addition.

The implication of these results is that resistance was not induced in individual strains of bacteria after repeated glutaraldehyde treatments. If such were the case, the pure strains would be expected to behave similarly to the mixed strains, and give gradually decreasing response to the biocide treatments. Rather, in the case of the mixed culture, a selection process appears to have occurred: the predominate strains, which were most susceptible to the biocide, were killed off rapidly by the first several biocide additions. The less susceptible strains, normally present at only low levels, then grew up to take their place; their growth resulted in the relative insensitivity to biocide exhibited by the treated mixed cultures. In support of this explanation, the bacteria remaining after repeated biocide treatments were identified, and proved to be a different species (CDC IV C-2) from those which were identified in the untreated mixtures.

CONCLUSIONS
1. Microbiologically fouled fluid systems should be treated with less frequent, larger doses of biocide, rather than more often with smaller doses.
2. A relationship exists between biocide stability and microbial populations. High bacterial loads cause increased biocide usage.
3. Maintenance of systems with near-zero microbial populations is desirable, since this precludes establishment of species which are resistant to the biocide used. Use of lower biocide levels may allow selection of resistant microbial strains, and thereby result in higher overall biocide needs. The phenomenon of biocide depletion by microorganisms may be more or less pronounced with other chemical microbicides. Studies are continuing to investigate the dynamics of microorganism interactions with other antimicrobial agents.

REFERENCES