Microbial Population Control in Emulsion Oil

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In this paper, controlling microbial degradation of water based metalworking system fluid, emulsion oil, is investigated using glutaraldehyde as biocide. Microbial population was determined through colony counting method. Evaluation and results demonstrate that a 15 day treatment by glutaraldehyde results in higher resistance to microbial deterioration. Analysis of variance through multiple comparison tests was conducted and Least Significant Difference (LSD) was determined. An overall observed paired difference between the first and second treatment is significant; therefore, the first treatment, with an addition of glutaraldehyde every 15 days is suggested.

INTRODUCTION

The main purposes of utilizing metalworking fluids are:

- Cooling tools, dies, forms and workpieces,

- Lubricating tools and carry off chips, fines and swarf.

The base of a metalworking fluid is generally non-aqueous with a viscosity greater than water. If an application primarily involves cooling, the metalworking fluid is basically water with similar additives for heavy duty operation and corrosion protection in addition to antimicrobial agents. Addition of water to a metalworking operation creates a favorable environment for a variety of microorganisms which can directly or indirectly cause occupational hazard, economic loss and environmental insult [1-12].

Metalworking fluids become rancid because they contain a rich blend of nutrients that encourage microbial growth. Alkanolamine, hetrocyclic compounds, esters and fatty acids are the major constituents of many water-based metal cutting fluids [13].

The unfavorable effects due to microbial spoilage are: intolerable smell, decrease in pH, change in emulsion stability, increase in skin and respiratory irritation and increase in corrosion rate [2]. These effects never occur prior to diluting oil with water. Contaminants

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may originate in water, or come from other sources including dust, plant dirt and human wastes. Adverse health effects due to long-term exposure to various oil, straight, soluble oil and synthetic metal cutting fluids, as well as environmental concerns, related to cutting fluid disposal, have led to introducing dry metal cutting which eliminates health and safety hazard [14].

Metalworking fluids contain multiple microbial species including bacteria, most probably, Actinobacter, Pseudomonas, Aerobacter and Bacillus and fungi [1-2,15]. Temperature is a factor in substrate utilization and viability by microorganisms. For esters, utilization increases with decreasing temperature, while the reverse is true for acids. The microorganisms were destroyed after exposure to temperatures of 80°C or above for 30 minutes or more, but were able to survive at lesser time or lower temperatures [16].

Preservatives are added to the fluid to provide biocidal and biostatic characteristics in metalworking systems. It should be noted that a preservative will not provide rancidity control for the life of the coolant. As soon as the fluid is placed in use, the preservative level begins to decline as the chemicals react with microorganisms. The preservative is usually the first component to be consumed and this occurs long before the life of the fluid has been exhausted [17]. Therefore, in many cases, preservative degradation does not provide a serious environmental effect [3]. In a few cases, the pH of the discarded fluid should be adjusted to create a favorable environment conducive to biodegradation [4]. It is necessary to add preservatives to a working system at periodic intervals in order to provide maximum lifetime. There exists a wide range of choices of antimicrobial agents [5-

Table 1. Bacterial responses to glutaraldehyde at three different concentrations and treatments.

Treatment	Concentration (ppm)											
	100				200				300			
1	10	10^2	10^3	10^3	10	10^2	10^3	10^2	10	10^2	10^2	10^2
2	10^4	10^5	10^5	10^6	10^3	10^2	10^4	10^5	10^2	10^2	10^2	10^3
3	10^4	10^4	10^6	10^6	10^3	10^4	10^5	10^5	10^2	10^3	10^5	10^5

10]. Due to the extremely diverse composition of cutting fluids, no individual preservative is effective in all coolants. Glutaraldehyde as a new microbiocide has been utilized for metalworking fluids [7,8]. The effect of different glutaraldehyde concentration as a biocide in metalworking fluid has been evaluated in laboratory scale.

Fungicidal efficacy of octhilinon preservative in metalworking fluid has been studied in [5]. Octhilinon is utilizable in both synthetic and semi-synthetic metalworking fluids.

The presence of microorganisms in emulsifiable oil systems may be measured through a standard counting method [5-7] or an analytical technique, utilizing High Performance Liquid Chromatography (HPLC), to determine the effect of microbial action on one particular emulsifiable oil system [11].

In this investigation, microbial cutting fluid population in metalworking machine was controlled, using glutaraldehyde as a biocide and a different pattern of treatment was considered. Evaluation of the acceptability of the formulation was determined using multiple comparison tests [18,19].

MATERIALS AND METHODS

The metalworking fluid used in this investigation was petroleum-based, diluted 1:10 with tap water. The investigation was conducted on cutting machines. In all experiments, each cutting machine was filled with 20 liters of fresh emulsion after the system was thoroughly cleaned. Microbial control of the coolant was studied in a separate system at ambient temperature. The biocide utilized in this investigation was a reactive agent, glutaraldehyde $CHO-(CH_2)_3-CHO$, 25%. Different concentrations of glutaraldehyde utilized in the cutting fluid were 100, 200 and 300 ppm, injected into the system at exact intervals of 15, 30 and 45 days. Evaporation losses were restored with tap water or freshly diluted cutting fluid. Bacterial populations in each cutting system were determined by standard pour plating of serial dilutions on plate count agar. Malt agar was utilized for fungal growth. The plates were incubated for 24 to 28 hrs at 25°C for fungal and 37°C for bacterial growth. The number of microorganisms was determined through colony formation units (cfu/ml). Each value is the average of two replicates. Multiple comparison tests were arranged to create statistically homogeneous groups. The grouping of treatment is accomplished through comparing the means of multiple comparison procedures. LSD (Least Significant Difference) test with t-distribution successively judging the significance of pairwise difference must be compared with its LSD. There is a considerable difference at the 1% level of significance [19], since it exceeds the LSD.

RESULTS AND DISCUSSION

Glutaraldehyde treatment was based on the evaluation of glutaraldehyde in an aqueous metalworking system. Three methods of treatment by glutaraldehyde, 15, 30 and 45 days, were applied. Three different concentrations of glutaraldehyde considered were 100, 200 and 300 ppm. Table 1 represents the results of bacterial colony counting at three different biocide concentrations and treatments. The results of fungal count in all tests indicated that the petroleum based fluid is more resistant to fungal spoilage and the fungal count did not exceed 10 cfu/ml, so only bacterial count was evaluated in the cutting system. Therefore, evaluation of the bacterial colony counting has been conducted and numerical values were considered (Table 2).

According to Table 1, three kinds of treatment with different concentrations are conducted. The difference between the treatments is in the time of sampling. It is clear that treatment 1 (15 days biocide addition) reaches an average of about 10^2 cfu/ml, for 200 ppm biocide addition and treatment 2 (30 days biocide addition) results in 10^2 in the case of 300 ppm biocide addition. Treatment 3 (45 days biocide addition) shows higher bacterial counting due to the distance between biocide addition.

Table 2 presents the selection of numerical value (N) for different colony count unit, observed for dif-

Table 2. Numerical value of bacterial colony counting.

Colony Count (cfu/ml)	Numerical Value
10^6	-2
10^5	-1
10^4	0
10^3	+1
10^2	+2
10	+3

Table 3. Microbial responses to glutaraldehyde treatment per 15 days (treatment 1).

Numerical Value (N)	Frequency (F)	$\sum X$ (N*F)	$\sum X^2$ (N²F)
3	3	9	27
2	6	12	24
1	3	3	3
0	0	0	0
-1	0	0	0
-2	0	0	0
sum	12	24	54

Table 4. Microbial responses to glutaraldehyde treatment per 30 days (treatment 2).

Numerical Value (N)	Frequency (F)	$\sum X$ (N*F)	$\sum X^2$ (N²F)
3	0	0	0
2	4	8	16
1	2	2	2
0	3	0	0
-1	2	-2	2
-2	1	-2	4
sum	12	6	24

ferent treatments. Tables 3 to 5 demonstrate the statistical determinations of glutaraldehyde treatment at each 15, 30 and 45 day intervals in a metalworking system. Results of metalworking treatment are evaluated through statistical method with multiple comparison tests [19]. Frequency (F) is the number of observations for each numerical value (N).

STATISTICAL CALCULATIONS

From Tables 1 to 4, total responses, n, and other statistical parameters are:

$n = 12*3 = 36$,

$\sum \sum X = 26$,

$\sum \sum X^2 = 96$,

$CF =$ coefficient of variation,

$CF = \sum X^2 / n = 18.77$.

The total sum of squares is denoted by SS_{to} and is defined as:

$$SS_{to} = \sum \sum X_{ij}^2 \cdots - CF = 77.23.$$

The treatment sum is defined as:

$$SS_{tr} = \sum n_i X_i^2 - CF = 33.63,$$

X is the mean.

The sum of squares due to error is:

$$SS_e = SS_{to} - SS_{tr} = 43.6.$$

Table 5. Microbial responses to glutaraldehyde treatment per 45 days (treatment 3).

Numerical Value (N)	Frequency (F)	$\sum X$ (N*F)	$\sum X^2$ (N²F)
3	0	0	0
2	1	2	4
1	2	2	2
0	3	0	0
-1	4	-4	4
-2	2	-4	8
sum	12	-4	18

MS_e is the mean square obtained as the result of dividing SS_e by its DF (Degree of Freedom, 33 in this investigation),

$$MS_e = 1.32.$$

LEAST SIGNIFICANCE DIFFERENCE

Least Significance Difference test (LSD) procedure uses the t-distribution successively to judge the significance of pairwise comparison. An observed paired difference, d_{ij}, is compared with its LSD defined as:

$$LSD = t_{(1\alpha/2),v(S_{d_{ij}})},$$

$$S_{dij} = \sqrt{MS_e(1/n_i + 1/n_j)},$$

$$n_i = n_j = n,$$

$$S_{dij} = \sqrt{2MS_e/n} = 0.469.$$

d_{ij} for treatments 1 and 2 is 2-0.5=1.5.

The difference of the average for treatments 1 and 2 is $d_{ij} = 1.5$, however, the results for treatment 3 (45 days) is not acceptable for comparison tests. It is lower than the LSD.

The t-distribution at %1 significance with DF value of 33 is 2.72 [19, Table A4] and so LSD is 1.275. Multiple comparison test for treatments 1 to 3 was conducted and as mentioned previously, treatment 3 is not acceptable; therefore, d_{ij} for only treatments 1 and 2 is comparable with LSD.

An observed paired difference, d_{ij}, is compared with LSD, since $d_{ij} = 1.5$ exceeds the LSD value of 1.275. Treatments 1 and 2 are significantly different. Treatment 1 (15 day treatment) for controlling microbial metalworking at three different concentrations, 100, 200 and 300 ppm is more acceptable. Furthermore, 100 ppm treatment with a 15 day interval is economically proposed, because during the manipulation in metalworking system one, colony counting data is lower than 10^3 cfu per ml.

CONCLUSION

Microbial contamination of a petroleum-based cutting fluid is significantly controlled in a metalworking system (cutting machine), using glutaraldehyde as biocide at three different concentrations: 100. 200 and 300 ppm. Three methods of treatment were examined, where biocide was added after 15, 30 and 45 day treatment intervals, respectively. Bacterial colony counting method was used for determining spoilage of the system. Experimental observation indicated that treatment 1 is more effective in controlling microbial population in cutting fluids.

Statistical method, using Least Significance Difference (LSD) as a method for pairwise comparison, indicated that treatment 1 is significantly different from the two other treatments; therefore, treatment 1 with 100 ppm biocide addition is suggested.

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