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CHAPTER 39

Effects of Heat, Chemicals, and Radiation on Cutting Fluid Flora

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Due to cost and toxicity it is not possible to use optimal levels of inhibitory chemicals for the prevention of cutting fluid biodeterioration. Control of soluble cutting fluid breakdown by mixed aerobic and anaerobic populations has been attempted with low levels of heat (<62 C), radiation (<10 kr), chemicals (between 150-1500 ppm), and selected combinations thereof. Evaluations were made in 5% o/w emulsions, Trypticase Soy broth, and pH 7 phosphate buffer. The radiation source was 10 kr/hr from a ^{137}Cs unit. Activity was determined by 48-hr plate counts in Trypticase Soy agar. Of four chemicals tested in cutting fluid, two produced significant synergism.

INTRODUCTION

Water miscible industrial cutting fluids without controlling measures support large bacterial populations. These bacteria can break down a cutting fluid and cause the loss of its lubricating and rust inhibiting properties. Shortened tool life, machine shutdowns, and the disposal of large amounts of spoiled fluid may result in producing greater cost to the manufacturer than the cost of good preventive measures.

One preventive measure, the use of chemical germicides, depends upon cost, effectiveness over a period of time, toxicity to machine operators, compatibility with the fluid, and corrosiveness. Very few germicides are acceptable from all of these standpoints. If these chemicals are bacterial radiosensitizers, then low doses of radiation could be used with low concentrations of radiosensitizers (Kivel et al., 1966). We used these approaches in cutting fluid systems with pure cultures of *Pseudomonas oleovorans* (ATCC #8062) and anaerobic isolates from spoiled cutting fluid to test for radiosensitization. A chlorinated phenol and hexahydro-1,3,5-tris-2 hydroxy-ethyl-(s) triazine (150 ppm) in both a cutting fluid emulsion and solution showed significant synergism at a dose of 5 kr (Rossmoore and Heinrichs, 1970). Heat is another common microbial control agent. Stehlik and Kaindl (1966) used heat and radiation to synergistically reduce *Saccharomyces cerevisiae* var. *ellipsoideus* populations.

PROCEDURE

In order to use chemicals in combination with radiation, germicide levels were selected which would not produce total kill at low concentrations. The levels ranged from 150-1500 ppm. The radiation source was a ^{137}Cs gamma radiation unit (Model GR-6A—U.S. Nuclear Corp.) with an activity of 10 kr/hr. Aerobic survivors were determined by 48-hr plate counts in Trypticase Soy agar (TSA, Baltimore Biological Lab). Anaerobic counts were made in American Petroleum Institute agar (API, Difco) in screw cap tubes incubated for two weeks at room temperature.

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Evaluation of chemicals and radiation in 5% o/w emulsions simulated industrial conditions. Air was bubbled through 1-liter samples of cutting fluid containing 10 g of cast iron chips plus 10% spoiled cutting fluids as a source of mixed aerobic and anaerobic bacteria. After five days, the air was shut off for two days allowing anaerobiosis to develop. This corresponded to a weekend shutdown period. A portion of each sample was withdrawn at the end of the anaerobic period and fresh fluid added prior to restarting the bubbling. The portions taken were then irradiated for 30 min and plate counts were made.

The effectiveness of chemicals and radiation was also tested in pH 7 phosphate buffer and Trypticase Soy broth (TSB) using *P. oleovorans* as the test organism. Prescription bottles containing 100 ml of either TSB or buffer were supplemented with 150 ppm of the desired chemical and 1 ml of a TSB culture of *P. oleovorans*. These were shaken overnight and then irradiated for 30 min.

The application of heat prior to irridiation was carried out using emulsions from aerobic and anaerobically spoiled cutting fluids. Each was made of 2 gal of 5% o/w emulsion (Sunseco, Sun Oil Co.) with 10% spoiled emulsion added as an inoculum and approximately 100 g of cast iron chips. The aerobic system was continuously circulated for four weeks while the anaerobic system was quiescent. A sample from each system was heated to 63 C and immediately irradiated. Platings were made to determine aerobic and facultatively anaerobic populations.

In experiments where heating was employed concurrently with irradiation, *P. oleovorans* and anaerobic cutting fluid isolates in TSB and phosphate buffer were used. Prescription bottles with 100 ml of TSB or buffer, respectively, were inoculated with 1 ml of a *P. oleovorans* TSB culture and shaken overnight. Prior to irradiation, 1 ml of an API broth anaerobic culture was added to each bottle. Three samples, from the broth culture and the buffer culture, were treated with radiation (10 kr), heat (50 C maximum), and a combination of the two. Since the temperature in the center well of the irradiator could not be controlled, the combination of heat and radiation involved immersion of the 10-ml tubed samples into 60 C water in an insulated container. This was placed into the irradiator for the required dose. The heat treatment alone was a duplication of the above, outside of the irradiator. Both samples were heated for the same amount of time. It was determined from prior work that the samples reached a maximum of 50 C. All tubes were placed in ice water following exposure. Platings and anaerobic tubes were prepared.

For the heat after irradiation experiment, spoiled cutting fluid emulsion was irradiated with 10 kr and then placed in a hot water bath. The sample temperature was brought to 50 C, then cooled in ice water. Platings and anaerobic tubes were made.

Results and Discussion

The combined effects of chemicals and radiation plus heat and radiation were calculated by the formula:

$$k = - \frac{d(\log N/No)}{d(\text{rad})}$$

where k = slope, N/No = survival fractions, and rad = dose (kr) (Stehlik and Kaindl, 1966). By comparing the slopes of the controls to the slopes of the experimental combinations, one may determine synergistic effects. Slopes greater than a control slope indicate radiosensitization, while slopes less than a control slope indicate radioprotection. Slopes equalling the control indicate no experimental effects.

As shown in Table 1, dibromopropionic acid and dibromopropanol exhibited significant synergistic effects only at concentrations normally used for microbial control (1500 ppm).

The chlorinated phenol and hexahydro-1,3,5-tris-2 hydroxy-ethyl-(s) triazine were again investigated using *P. oleovorans* in TSB and a pH 7 phosphate buffer system (Table 2). The methyl-n-hexylcarbinol appeared effective only at 5 kr in broth. The apparent loss of radiosensitivity of *P. oleovorans* with the triazine in broth is consistent with findings by Rossmoore and Brazin (1968), who observed that *P. oleovorans* was much less sensitive to gamma radiation in TSB than in cutting fluid emulsion.

The effects of radiation with p-chlorometacresol and 1,3,5-sodium trichlorophenate were also tested using *P. oleovorans* in TSB and pH 7 phosphate buffer (Table 3). Radiosensitization was greatest with 1,3,5-sodium trichlorophenate in both broth and buffer, while the effects due to p-chlorometacresol were minimal.

TABLE 1. *Effect of ^{137}Cs radiation (5 kr) with two brominated aliphatics on aerobic bacteria in a solution type cutting fluid*

	Slope of Survivor Curves Concentration[a]		
	0 ppm[b]	900 ppm	1500 ppm
Dibromopropionic Acid	0.04	0.09	0.16
Dibromopropanol	0.05	0.06	0.12

[a] Average of at least 2–4 trials.
[b] Average starting counts (0 ppm) = 1.1×10^7.

TABLE 2. *Effect of ^{137}Cs radiation with two alcohols (150 ppm) on* Pseudomonas oleovorans *in broth and buffer*

	Slope of Survivor Curves		
Radiation Dose	No Alcohol[b]	Hexahydro-1,3,5-tris-2 hydroxy-ethyl-(s) triazine	Methyl-n-Hexylcarbinol
Broth[a]			
5 kr	0.08	0.08	0.18
10 kr	0.00	0.01	0.02
Buffer[a]			
5 kr	0.17	0.30	0.00
10 kr	0.00	0.45	0.00

[a] Trypticase Soy broth and 1/15 M Sorenson Buffer, pH 7.
[b] Starting counts (no alcohol): broth = 1.6×10^8, buffer = 4.1×10^5.

TABLE 3. *Effect of ^{137}Cs radiation with two phenols (150 ppm) on* Pseudomonas oleovorans *in broth and buffer*

	Slope of Survivor Curves		
Radiation Dose	No Phenol[b]	p-Chloro-metacresol	1,3,5-Sodium Trichlorophenate
Broth[a]			
5 kr	0.02	0.03	0.18
10 kr	0.00	0.07	0.24
Buffer[a]			
5 kr	0.02	0.04	1.00
10 kr	0.23	0.22	0.97

[a] Trypticase Soy broth and 1/15 M Sorenson Buffer, pH 7.
[b] Starting counts (no phenol): broth = 4.3×10^7, buffer = 1.2×10^7.

TABLE 4. *Effect of aeration on sensitivity of aerobes and facultative anaerobes to heat and ^{137}Cs radiation in cutting fluid emulsion*

Radiation Dose	Slope of Survivor Curves: Aerated[a] Unheated	Aerated[a] Heated[b]	Stagnant[a] Unheated	Stagnant[a] Heated[b]
2.5 kr	0.14	0.20	0.10	0.11
5.0 kr	0.04	0.10	0.04	0.10

[a] Starting counts: aerated = 2×10^8, stagnant = 6.5×10^6.
[b] Samples were heated to 62 C and immediately irradiated.

TABLE 5. *Effect of heat and ^{137}Cs radiation (5 kr) on* Pseudomonas oleovorans *and* Desulfovibrio *sp. in broth and buffer*

	Slope of Survivor Curves[b,d]: *P. oleovorans* Heat[a]	*P. oleovorans* Rad.	*P. oleovorans* Heat[a] and Radiation	*Desulfovibrio* sp. Heat[a]	*Desulfovibrio* sp. Rad.	*Desulfovibrio* sp. Heat[a] and Radiation
Broth[c]	0.54	0.09	0.44	0.45	0.04	0.52
Buffer[c]	0.61	0.45	0.64	0.30	0.28	0.50

[a] Samples were heated to a maximal 50 C in a water bath while being irradiated.
[b] Average of four trials.
[c] Trypticase Soy broth and 1/15 M Sorenson Buffer, pH 7.
[d] Average starting counts: *P. oleovorans*: broth = 3.2×10^8, buffer = 2.3×10^7; *Desulfovibrio* sp.: broth = 4.2×10^3, buffer = 4.2×10^2.

Stehlik and Kaindl (1966) used heat before, during, and following radiation in their experiments and found that the greatest effect was from heat applied during radiation. We adapted these approaches for use on cutting fluids. With heat applied before radiation (Table 4), the aerated flora seemed more sensitive not only to the lower dose of radiation but also to heat alone. Heating to 63 C gave a slope of 0.06 for the aerated system, while a slope of 0.03 resulted for the stagnant system. Conversely, the facultative anaerobes in the stagnant system were more resistant to heating and subsequent irradiation.

Heating concurrent with radiation (Table 5) did not follow the results of Stehlik and Kaindl (1966). In fact, no synergistic effects were noted. Obviously, the same procedure should be repeated using a cutting fluid system if the finding that *P. oleovorans* is more sensitive in such an environment is consistent.

The results of heat following radiation were consistent with those of Stehlik and Kaindl (1966) in that no synergistic effects occurred. The temperature (50 C) used in this and the previous experiment was intended to test for radiosensitization without producing massive kill by itself. It is entirely possible that a temperature range exists in which maximal sensitization could occur. We did not seem to reach this range but more work at higher temperatures might yield the desired results.

From all the data obtained, the combination of selected chemicals and gamma radiation show immediate potential as an effective and efficient method for controlling cutting fluid flora. Heat and radiation may also prove effective in the future. Not only is gamma radiation of use in the control of cutting fluid flora but it is also a specialized tool or biophysical probe for studying interactions on a biochemical level. By noting the conditions under which certain effects take place during irradiation, we may gain a better understanding into the molecular alterations produced, including possible explanations for radiation effects in the living cell. Such information is the goal of our future work.

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