Characterization of Metalworking Fluids as a Function of Time in the System

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ABSTRACT

This report describes the results of a pilot study to examine changes in the biological component of metalworking fluids (MWF) as a function of use. Fluid samples were taken from two newly-charged systems at one-week intervals and characterized with respect to the kinds and numbers of bacteria present and the presence of soluble protein in cell-free supernatants. In addition, pelleted cells were examined by gas chromatography/mass spectroscopy for the kinds and relative amounts of phospholipid fatty acids (PLFA) present. Thirteen different bacterial species were cultured and identified, more than half (8/13) of which were Gram-negative. Total CFU reached levels of 2.2×10^3 / ml in one system and 2.4×10^5 / ml in the other. The most common genus isolated was Pseudomonas. There was no soluble protein detected in any of the samples. Both the numbers and the amounts of PLFA increased as the fluids aged in the systems. In samples from both systems, the chromatograms were dominated by two large peaks that increased with time. These peaks are thought to correspond to unusual fatty acids, not usually associated with pseudomonads.

INTRODUCTION

The health effects of exposure to metalworking fluid (MWF) are not well understood, particularly with respect to the microbiological component of contaminated fluids. Worker complaints range from respiratory irritation to asthma and hypersensitivity pneumonitis, but temporal linkage between exposure and health effects is complicated by changes in the fluid composition that occur with use, time in the tank, the addition of makeup fluids and biocides, the accumulation of hydraulic fluids and lubricants leaking from the machines "tramp oils," and changes in the microbial population due to ecological succession. A better understanding of these changes, particularly those associated with microorganisms, might permit more accurate characterization of the mixed exposures to MWF and their biological contaminants. This report describes a pilot study in which fluid systems from two different metalworking operations were sampled for a period of eight weeks and the biological component of the MWF examined as a function of time in use. The goal of the project was twofold: (1) to design a protocol for sampling and analysis that would permit multiple analyses of the same sample for a better understanding of the changes in the microbial community in the fluids that occur with use; (2) to adapt and develop existing microbiological and biochemical methods specifically for the examination of MWF.

METHODS AND MATERIALS

Two metalworking systems from two different plants were chosen for the collection of field samples: a fifty-gallon system supplying an operation for the turning of steel (BT7415) and a 125-gallon system used in machining cast iron (BT7707). Both systems were settling type systems newly charged with semisynthetic fluids. The formulations of the fluids in the two systems were different, however. Duplicate 500 ml samples were collected from each system weekly, on Monday mornings, August through October 1996. A protocol for splitting the samples for multiple analyses was designed. Additional data concerning the condition of the fluids were collected on site. Some analyses (e.g., plate counts; bacterial culture) were performed immediately. Others (protein assay; phospholipid analysis) were performed later on samples that had been stabilized by freezing or cold storage. Still others (e.g., endotoxin; direct counts using epifluorescence microscopy) await further developments or modification of methods. Culturable bacteria were enumerated using dilution plate counts, and the Biolog microbial identification system (Biolog, Inc., Hayward, CA) was used for the identification of isolates. Community profiles of the microbial populations were generated by examining organic extracts from pelleted cells by solid phase extraction/gas chromatography/mass spectroscopy (SPE/GC/MS) for the kinds and relative amounts of phospholipid fatty acids (PLFA) present.[1] Cell-free supernatant fluids were subjected to ammonium sulfate precipitation (0-80% saturated). Precipitated fractions

were assayed for total protein using the BioRad protein assay (BioRad, Hercules, CA).

RESULTS AND DISCUSSION

Table 1 lists the bacterial species identified from the samples along with Gram stain characteristics and the date of the sample in which the isolate first appeared. Thirteen different bacterial species were cultured and identified using the Biolog system. More than half (8/13) were Gram-negative. In addition, there were several isolates, both Gram-negative and Gram-positive, that have not yet been identified. At least two of the isolates were halophilic (salt-loving) species. Streptococcus pyogenes was the only frank pathogen isolated, although most of the species identified have been associated with opportunistic clinical infections. Total CFU reached levels of 2.2×10^3 / ml from BT7415 and 2.4×10^5 / ml from BT7707. There were no acid-fast bacteria cultured from any of the samples. Although Pseudomonas was the genus most commonly isolated in culture, PLFA analysis suggested that it may not be the most prevalent species present in the fluids.

Both the numbers and the amounts of PLFA increased as the fluids aged in the systems. In samples from both systems, the chromatograms were dominated by two large peaks that increased with time. These peaks are thought to correspond to unusual fatty acids, not usually associated with pseudomonads. Investigations to identify the microbial constituent(s) associated with the peaks are continuing, as are experiments to measure soluble endotoxin in the fluids.

Ordinarily, when bacteria are grown in liquid media, enzymatic and other proteins accumulate in the supernatants. Some bacteria, including the pseudomonads, may also secrete molecules that are classified as exotoxins. Analysis of cell-free supernatants of these fluids demonstrated no detectable protein in any of the samples. This may have been due to the high pH (8.7-9.3) and the presence of metal salts, which could have resulted in the rapid unfolding and denaturation of secreted proteins. However, there may be antigenic determinants present on protein fragments in the fluids capable of initiating an immunological reaction in susceptible individuals.

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REFERENCES

1. **Findlay, Robert**: The use of phospholipid fatty acids to determine microbial community structure. In Molecular Ecology Manual 4.1.4. The Netherlands. Kluwer Academic Publishers. 1996. pp. 1-28.

Table 1. Bacteria cultured from semisynthetic fluid mixtures from two metalworking operations

Species	Gram Stain	System	Sample Date*	Notes
Ochrobactrum anthropi	negative	BT-7415	8-8-96	
CDC Group B-I/B-3	negative	BT-7415	8-8-96	unnamed species
Alcaligenes faecalis ss faecalis	negative	BT-7415	8-8-96	common in feces, soil, water
Tetragenococcus halophilus	positive	BT-7415	8-8-96	tolerates high salt
Pseudomonas glathei	negative	BT-7415	8-8-96	
Streptococcus pneumoniae	positive	BT-7415	8-9-96	human pathogen
Staphylococcus hominis	positive	BT-7415	8-8-96	human commensal (skin)
Corynebacterium halophilus	positive	BT-7415	8-8-96	tolerates high salt
Pseudomonas pseudoalcaligenes	negative	BT-7415 BT-7707	9-16-96 8-26-96	
Alcaligenes xylosidans	negative	7415	9-16-96	
Unidentified Gram negative	negative	7415	9-16-96	
Staphylococcus auricularis	positive	7415	9-30-96	human commensal (ear)
Unidentified Gram positive	positive	7415	9-30-96	
Unidentified Gram negative	negative	7707	8-26-96	
Serpens flexibilis	negative	7707	9-5-96	often found in pond sediment
Pseudomonas dimuta	negative	7707	9-30-96	
Unidentified Gram negative	negative	7707	9-30-96	
(additional isolates still unidentified)				

*Date of sample in which isolate was first identified.

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II

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The reader will notice a variety of nomenclature differences among authors when referring to these fluids which were the subject of the Symposium and of this volume. Indeed, even the Symposium title reflected some of this variation: "*Metalworking* Fluids Symposium II," and "The Industrial Metalworking Environment: Assessment and Control of *Metal Removal* Fluids." Lest we add to the confusion, our use of the term *metalworking* in the title "Metalworking Fluids Symposium II" was a conscious decision based on nothing more than to maintain continuity with the title from the first Symposium. It was for that reason that "Assessment and Control of Metal Removal Fluids" was added in recognition of, and to call attention to the fact that the vast majority of research and data to date has been generated on a subset or class of metalworking fluids known as **metal removal fluids**. In addition to metal removal fluids, the very general term 'metalworking' fluids also encompasses the large and general classes of *metal protecting* fluids, *metal forming* fluids, and *metal treating* fluids. Besides functional differences between metalworking fluid classes, there are substantial compositional differences both between and within classes. So while it is somewhat sloppy though quite common and generally harmless to use generic terms such as metalworking fluids, or machining fluids, or coolants, the reader should be well aware of these important distinctions and that in virtually all instances where there is a connection with purported health effects, the person is really referring to that subclass of metalworking fluids known as *metal removal fluids*.

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