

CHARACTERISATION OF THE MICROBIAL FLORA OF INVERT EMULSION HYDRAULIC FLUIDS

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Summary. The addition of water to certain hydraulic fluids to inhibit combustion has added a new dimension to their deterioration. Microorganisms grow and flourish in this environment. This growth can cause machine shutdown with ultimate loss of production time. This paper deals with methods for detecting and characterizing microorganisms from water containing hydraulic fluids.

Caracterisation de la flore microbienne des fluides hydrauliques à emulsion inverse. L'addition d'eau à certains fluides hydrauliques pour empêcher leur combustion a ajouté une nouvelle dimension à leur détérioration. Des microorganismes se développent et prospèrent dans ce milieu. Cette croissance peut provoquer des rates dans le fonctionnement de la machine entraînant une perte finale du temps de production. Cet article expose les méthodes qui servent à détecter et caractériser les microorganismes rencontrés dans les fluides hydrauliques qui contiennent de l'eau.

Introduction

The passage of U.S. Public Law 91–173 made it desirable if not mandatory that fire resistant hydraulic fluids be used in hydraulic actuated equipment in the United States. A number of fire resistant fluids have been developed all of which include a large percentage of water necessary to ensure fire retardation and prevent combustion.

One major type used extensively incorporates water with oil and a suitable emulsifier to produce an invert emulsion, i.e., a water-in-oil emulsion. In all of those containing water sufficient to prevent combustion, contamination and subsequent deterioration by microorganisms has become a problem which has compelled discontinuance of operation of the equipment involving the hydraulic fluids. Microbiological deterioration of these fluids is not completely equivalent to water based cutting fluid deterioration (Rossmoore & Williams, 1971) since there is less chance for contamination and turnover during use. However, once this semi-closed system becomes contaminated there is little that has been done to prevent the problem from growing in intensity. This our first paper on the microbiology of hydraulic fluids, deals with the retrieval, growth and characterization of microorganisms from selected hydraulic fluids and the development of techniques for so doing. Subsequent papers will deal with the prevention and treatment of microbial contamination in water-in-oil hydraulic fluids.

Mikrobiologie in veränderten Emulsionen hydraulischer Flüssigkeiten. Der Zusatz von Wasser zu einigen hydraulischen Flüssigkeiten zur Verminderung ihrer Brennbarkeit, hat ihre Anfälligkeit gegen biologischen Angriff erhöht. Mikroorganismen wachsen und vermehren sich in diesem Medium. Das kann zu Maschinenschaden und Produktionsausfall führen. Die Arbeit behandelt Verfahren zur Entdeckung und Bestimmung von Mikroorganismen in hydraulischen Flüssigkeiten, welche Wasser enthalten.

Caracterización de la flora microbica de los fluidos hidráulicos de emulsion invertida. El procedimiento de añadir agua a ciertos fluidos hidráulicos para impedir la combustión ha contribuido una nueva dimensión a su deterioración. En tal medio ambiente crecen y florecen los microorganismos. Este crecimiento puede ocasionar la parada de las máquinas con la pérdida consecuente del tiempo de producción. Este documento trata con los métodos de descubrir y caracterizar los microorganismos del agua que contiene fluidos hidráulicos.

Materials and Methods

The sources of organisms reported in this study were from a field sample of hydraulic fluid and several steel filters heavily contaminated. The hydraulic equipment utilizing one of the fluted filters was incapacitated due to malfunction of the filter. It was obvious on examination that this resulted from microbiological growth developing either directly on the filter, or accumulating on it by filtration from the liquid, with consequent reduction of fluid flow (Figure 1).

Characterization of Microbial Species

One of the filters was allowed to dry in a laminar flow hood to facilitate scraping the slime sufficient for recovery of microorganisms. The scrapings were suspended in sterile, distilled water and were inoculated into several types of microbiological media for primary isolation (Tryptic soy broth, Bushnell Haas medium, and Czapek-Dox medium, all from Difco Laboratories, Inc.)

These media were incubated at 30°C for two to seven days at which time isolates were examined microscopically and subsequently transferred to more selective and differential media. 30°C was chosen since the temperature of hydraulic fluids, in practice, ranges from below to above this temperature. Media selected were based on published data from jet fuel studies (Park, 1975; Rogers and Kaplan, 1964) and metal-

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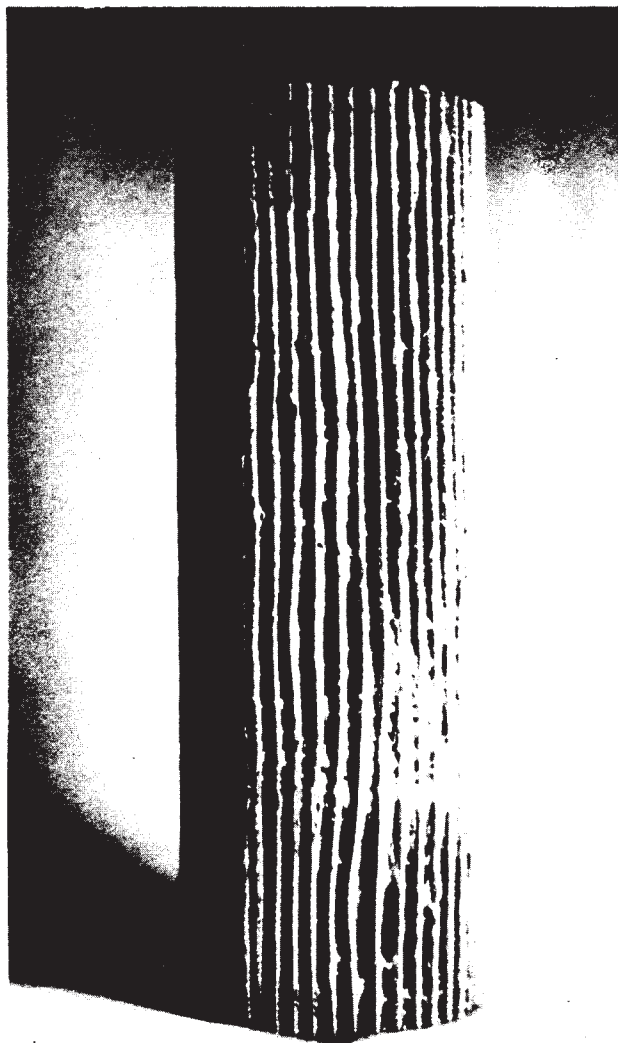


Figure 1. Fluted Steel Filter Used in Hydraulic Actuated Press

working fluids (Rossmoore and Treusch, 1975), Table 3a and 3b. After characterisation on selected media further determination was made either microscopically for fungi or by additional biochemical testing for bacteria. The isolation of organisms and the estimation of numbers from the invert emulsion field samples presented problems because of the external oil phase, since it was impossible to use aliquots for preparing aqueous dilutions. We solubilised the invert emulsions according to a method developed for oil-based cosmetics (McConville, Anger and Anderson, 1974) as follows: 1 ml of invert emulsion was dispersed in 1 ml of Arlacel 80 and the volume was brought to 10 ml with 10% Tween 60 solution. This method of dispersion was used in all subsequent studies involving retrieval and counting of microorganisms from invert emulsions.



Figure 2. Aeration Scheme to Produce Cascading Effect. A corrugated filter is shown in position

Growth Studies in Laboratory Prepared Invert Emulsions

A proprietary emulsifying package was added 7.5% by volume to a paraffinic base oil. The oil-emulsifier mixture was then combined with 40% by volume of sterile tap water in a laboratory blender. This fluid was used for all growth studies. We attempted to contaminate this fluid in several ways:

1. By adding a 10% contaminated fluid to it and aerating the invert emulsion for up to one week. The level of growth produced by this procedure was disappointing and minimal.
2. By adding scrapings from the contaminated filter to the emulsion. This was no more successful than 1, above.
3. A set up that produced maximal results in the laboratory involved the use of the filter described previously. This was placed in a suitable glass container (Figure 2) which allowed both for aeration and for a modified cascading effect of the emulsion. One filter was acid cleaned, washed and sterilized. Another was used as received from the hydraulic equipment. Both were placed into an appropriate size beaker and aerated from compressed air lines in the laboratory for one week. Examinations for microbial types were made subsequent to this and are reported below.

Growth in Commercial Invert Emulsions

In order to assess the ability of the isolates to grow actively in commercially available invert emulsions, four fungal, one yeast, three *Pseudomonas* species and one *Proteus* species were grown separately to maximum population density. Equal amounts of each culture were mixed and were added in a mixture at 10% of the volume of the emulsion. The fungi and the *Candida* species were grown in malt yeast peptone glucose broth and the bacteria were grown in trypticase soy broth. However, for maximal growth of *Pseudomonas maltophilia* the broth was enriched with 1 μ M of methionine sulfoxide. The levels of each species at time of inoculation are listed under Results in Table 4. Fifty ml of this mixture was mixed with a sufficient amount of each of the two invert emulsions to make 500 ml of total volume and added to a tall glass beaker containing a clean, sterile cartridge filter, as depicted in Figure 2. These two systems were aerated as described.

Results

In Table 1 are the results of one week of growth in systems described as 1, 2 and 3 in Materials and Methods. Note that only the system with the increased



Figure 3. Close-Up of Filter Surface Showing Slime Formation

surface area (the filter) and the contamination gave significant growth in one week. We have found (Rossmoore and Holtzman, 1974; Rossmoore, Holtzman and Kondek, 1976) that surface texture and area are extremely critical in the ontogeny and extent of microbial growth, especially of fungi.

Of further interest is the fact that qualitatively the filter and contaminated hydraulic fluid had a very similar microbial profile (Table 2). In this regard, the presence of gram negative bacteria and fungi in all three samples is reminiscent of cutting fluid deterioration (Rossmoore and Holtzman, 1974). The specific isolates were characterized from each area and are listed in Table 3a and 3b.

The fungi were identified directly from solid media isolates using the diversity of media mentioned in Table 3a and indirectly by growing them in Bushnell-Haas medium with gentamicin and then subsequently isolating prevalent organisms. The bacteria were similarly identified, either by growing in tryptic soy broth or Bushnell-Haas medium and isolating dominant organisms or by directly growing on agar and selecting different colony types. These were speciated by biochemical reactions on commercially available multimedia strips (Analytical Products, Inc., API, Hoffman LaRoche Enterotubes, or Oxi-Ferm).

In the system involving aeration/cascading with the contaminated filter, slime production with obvious impairment of filter surface characteristics is evident (Figure 3) as is discoloration of the fluid itself (Figure 4).

Those familiar with metalworking fluid microbiology will recognize several ubiquitous types, notably the *Pseudomonas* and *Fusarium* and *Cephalosporium* species. The significance of their presence as well as the other species in terms of biodeterioration cannot be evaluated at the present time.

It can readily be seen from the results with commercial emulsions (Table 4) that the inoculum prepared from the isolates is very active and casual observation of the agar plates indicated that all the species of fungi and the yeast used in the inoculum grew in the emulsion. In addition, the fungal counts only indicate what was detected in the fluid. The filters became continually more covered with what appeared to be fungal slime. This was left undisturbed during the course of the three weeks.

Concomitant with an interest in the biological causes of invert emulsion deterioration should be a concern for its prevention and treatment. The role of water quality on emulsion stability and putative preservative activity must be assessed as has been done with metalworking fluids (Bennett, 1974). Most important in the prophylactic and therapeutic use of antimicrobial agents is a consideration of their affinity for polar and non-

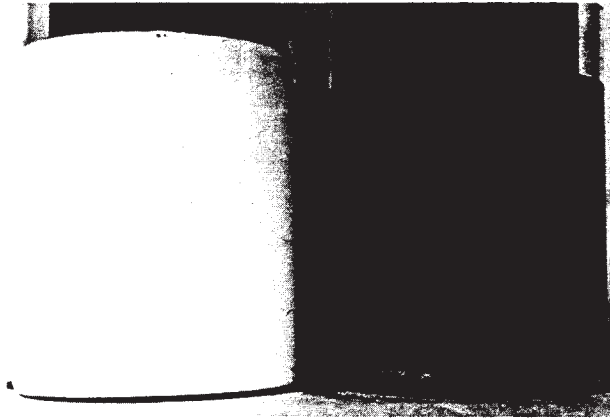


Figure 4. Results of Microbial Growth in Invert Emulsions.

Left Filter System: No Growth.

Right Filter System: Bacterial and Fungal Growth. Notice the discoloration in the contaminated system.

polar solvents. It has been established (Rossmoore and Moore, 1966) that the efficacy of an antimicrobial agent in a two-phase system (e.g., emulsion) is dependent on both its major solubility and time of dissolution, whether in the finished emulsion or in the water or oil prior to emulsification. These are crucial factors in the selection of treatments for either preservation of new emulsions or discontinuance of old emulsions.

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TABLE 1

Estimation of Microbial Growth in Invert Emulsions

Systems*	Bacteria/ ml	Fungi/ ml
(1) Emulsion plus 10% contaminated hydraulic fluid	< 30	< 30
(2) Emulsion plus 1% by weight of dried filter scrapings	< 30	< 30
(3) Emulsion plus contaminated filter	3×10^4	3×10^3
(4) Emulsion plus sterile filter	< 30	< 30

* All systems above treated to a regimen of aeration for one week from a laboratory compressed air line.

TABLE 2

Types of Organisms from Invert Emulsions

	Field Hydraulic Slime	Filter Slime	Filter-Grown Laboratory System
Bacteria			
Gram negative	+	+	+
Gram positive	+	+	–
Yeast	–	+	+
Fungi	+	+	+

TABLE 3a

Fungal and Yeast Species Identified From Invert Emulsions

<i>Fungal Type</i>	<i>Times Isolated</i>
<i>Aspergillus sp.</i> (3)	5
<i>Penicillium sp.</i>	2
<i>Fusarium sp.</i>	9
<i>Ambylosporium sp.</i>	2
<i>Cephalosporium sp.</i>	5
<i>Candida sp.</i>	1

TABLE 3b

Bacterial Species Isolated from Invert Emulsions

<i>Bacterial Type</i>	<i>Times Isolated</i>
<i>Bacillus sp.</i>	2
<i>Bacillus sp.</i>	4
<i>Pseudomonas maltophilia</i>	2
<i>Pseudomonas cepatia</i>	2
<i>Proteus mirabilis</i>	1

Media Used: (1) Potato Dextrose Agar, (2) Cooke Rose Bengal Agar, (3) Sabourauds Glucose Agar, (4) Czapek Dox Medium plus Yeast Extract, (5) Maltose Yeast Extract Agar, (6) Biggy's Agar, (7) 1+ Gentamicin, 20 µgm/ml, (8) 2+ Gentamicin, 20 µgm/ml, (9) 3+ Yeast Extract and Gentamicin, 200µgm/ml (10) Bushnell and Haas Medium and Gentamicin, 20 µgm/ml.

Media Used: (1) Tryptic Soy Agar, (2) Bushnell Haas Medium, (3) Tryptic Soy Agar, (4) Oxi-Ferm Tubes (Roche Diagnostics), (5) Enterotubes (Roche Diagnostics), (6) API Profile (Analytab Products, Inc.).

TABLE 4

Growth of Microbial Isolates as Mixed Inoculum in Commercial Invert Emulsion

	0 Time		1 Week		2 Weeks		3 Weeks	
	B/ml	F/ml	B/ml	F/ml	B/ml	F/ml	B/ml	F/ml
Emulsion 36182	2.1x10 ⁶	2.5x10 ⁶	1.2x10 ⁷	3.6x10 ⁴	3.2x10 ⁸	3.3x10 ⁴	1.2x10 ⁸	8 x10 ⁴
Emulsion 44803	5.8x10 ⁶	2.8x10 ⁴	x10 ⁷	5.2x10 ²	1.4x10 ⁸	1.9x10 ³	1.5x10 ⁸	1.6x10 ⁴

B = Bacteria F = Fungi and Candida

Inoculum:

Proteus mirabilis 10 ⁸	Aspergillus sp. 3.6 x 10 ⁶
Pseudomonas cepatia 7 x 10 ⁸	Cephalosporium sp. 3.8 x 10 ⁵
Ps. fluorescens 1.2 x 10 ⁸	Fusarium sp. 2.4 x 10 ⁵
Ps. maltophilia 5.7 x 10 ⁸	Penicillium sp. x 2.6 x 10 ⁶
	Candida sp. 6 x 10 ⁶