

CHAPTER 29

Growth of Fungi in Cutting Fluids

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The growth of a *Fusarium* sp. isolated from cutting fluid was observed after the addition of spoiled fluid to simulate residual contamination in sterile fluid, both with and without hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine (T). In systems with no T and no contamination, fungal counts were never above 10^4 colony-forming units/ml. In systems with contaminated fluid and no T, counts reached 10^6 colony-forming units/ml. If 0.05% T was added immediately after inoculation, fungi were controlled; however, 0.2% T did not control previously established populations. The ontogeny of colonial development of a mixed population of *Fusarium* and *Cephalorposium* sp., both originally derived from spoiled fluid, was investigated in a chemical solution fluid.

INTRODUCTION

During recent years several factors have contributed to a change in the microbial populations of water-soluble cutting fluids used in the machine tool industry. Changes in metal-working technology have led to the development of new families of fluids with varying degrees of bioresistance and/or biodegradation. The industry has had to consider the impact of the water pollution control act on permissible effluent levels of waste fluids and of certain biocides judged environmentally toxic (e.g., mercury, phenols). Therein lies the dilemma. The fluid must have maximum biodegradability when it leaves the plant to facilitate treatment before eventual discharge into the ecosystem, but it should also have maximum bioresistance while in use. The simplest solution to this problem has been to ignore bioresistance, concentrate on engineering requirements, and supplement the fluid with a biocide which will effectively prevent growth of microbial species responsible for deterioration. A required feature of such biocides must be biodegradability, so that upon discontinuance and discharge they will cease to be biologically active. In some cases, biocide half-life is shorter than replacement rate *in situ* which can result in sporadic outbreaks of growth of selected species. This factor may represent a significant reason for the shift in microbial populations in cutting fluids.

The pseudomonads were the first group implicated in the deterioration of water-soluble cutting fluids (Lee and Chandler, 1941) and subsequently a case was made for dissimilatory sulfate-reducing bacteria (Bennett, 1957). However, the latter are never seen at the levels noted for pseudomonads, and they can be controlled in the plant by a continuous regimen of aeration, provided that the aerobic species are also controlled by a suitable germicide. More recently, several species of fungi have been implicated as cutting fluid problems (DeMare et al., 1972). The reports indicate that contaminations were of an explosive nature, characterized initially by aesthetic unpleasantness (musty locker-room odor), followed by more gross and visible fouling on machine surfaces. This latter development is often sufficient to produce blockage of filters and orifices. Thus, although

labor and management will unknowingly tolerate much higher bacterial levels, the fungi, by their pervasive odor and appearance, call for obligatory action.

The onset of fungal growth and its visible appearance are sudden and not predictable by routine microbiological procedures. The study presented here is part of a continuing investigation into factors that contribute to the ontogeny of fungal populations in cutting fluids. It is hoped that such information not only will be economically important but may also add to the understanding of microbial succession in oil/water ecosystems.

MATERIALS AND METHODS

Representatives of two widely used cutting-fluid types, a soluble oil and a chemical solution, were selected for the experiments. Two separate sets of experiments were performed to define the problem of fungal growth in cutting fluids. For the first set, some factors were assessed that previous experience suggested contributed to fungal epidemics. This was done in a 5% oil-in-water emulsion varied as described in Table 1. In the second set, some of the data acquired in the emulsion systems were used to follow descriptively the development of fungi in a 4% chemical-solution cutting fluid; this fluid contained no oil, but did contain a nonionic wetting agent, triethanolamine, and an anti-rust agent.

Spoiled, mixed cutting fluid maintained in the laboratory on a continuous basis, and activated to a level of ca. 10^8 bacteria/ml before use, served as an initial source of bacterial inoculum. A *Fusarium* sp., isolated from cutting fluid and not identified further, was carried in the laboratory on Difco Sabouraud Dextrose Agar (SDA). These stocks were added to Sabouraud Dextrose Broth (Difco), and incubated at 25 C with shaking for 5 days. This will be referred to as the *Fusarium* inoculum. In addition, in one experiment a cutting fluid inoculated with the *Fusarium* sp. was used as a fungal inoculum after 3 weeks of incubation.

Evaluations of fluids simulated industrial conditions as described by Heinrichs and Rossmore (1971). Air was bubbled through 1-liter samples of cutting fluid containing 10 g of cast iron chips plus 10% spoiled cutting fluids. After 5 days, the air was shut off for 2 days allowing anaerobiosis to develop. This corresponded to a weekend shutdown period. Water lost by evaporation was replaced with deionized water. A portion of each was withdrawn at the end of the quiescent period and replaced with appropriate fresh fluid prior to reaerating. Aliquots were removed and used for bacterial plate counts in Trypticase Soy Agar (TSA, Baltimore Biological Laboratory) and fungal plate counts on SDA. Incubation of the former was for 48 hr at 30 C and the latter for 5 days at 25 C.

In the emulsion study, three variables: inoculum, germicide (T)¹, and fluid replacement rate (Table 1) were superimposed on the basic regimen described above. This was continued for 8 weeks. After 9 weeks, each system was inoculated with 10^3 cfu (colony-forming units)/ml of the *Fusarium* sp. and maintained for an additional 5 weeks with deionized H₂O added to compensate for evaporation. At this time each of the four systems (2, 4, 6, and 7) was halved and 0.2% germicide was added to one portion. The regimens now labeled 2', 4', 6', and 7' were continued as previously for 4 more weeks.

Chemical solution fluids, a control system, and one containing 0.15% w/v of germicide were inoculated with 50 ml of spoiled fluid and 50 ml of *Fusarium* sp. The fluids were maintained, as described, for 3 weeks.

¹Hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine.

TABLE I. Description of 5% oil/water emulsion systems, weeks 1-8

	System Designation			
	2	4	6	7
Inoculum ^a	—	+	+	+
Germicide (0.1%)	+	+	+	—
Weekly replacement regimen	none	none	25% with germicide	25% without germicide

^a10% by volume of mixed spoiled fluid.

After 3 weeks, another bottle with 0.15% germicide (similar solution) was set up and inoculated with 100 ml from the previous bottle. Prior to initiation of aeration, eight 1 x 3 inch clean glass slides were suspended in the fluid to a depth of 2.5 inches. Individual slides were removed at intervals and examined macro- and microscopically for the onset of fungal colony development.

RESULTS AND DISCUSSION

During the first 8 weeks of study on the emulsion, no fungi were detected in routine platings (Fig. 1-4). In previous studies (Rossmoore et al., 1971), fungi had been detected under similar conditions without advertent inoculation; the source was presumably from the unfiltered laboratory compressed air lines. From the 10th to the 15th weeks, there were variations in trends, with regard to fungal survival, and the onset of bacterial growth in systems that previously had none. By the 15th week, even System #6 which had 25% replacement up to the 8th week had ca. 10^8 bacteria/ml, showing that even an effective germicide has a finite life span.

A comparison of fungal concentrations in bottles exposed to germicide (System #6) and not exposed to germicide (System #7) indicated that after 10 weeks, residual germicide was not effective (Fig. 3a and 4a). The results suggest that the fungal growth in System #6 quite possibly was caused by elimination of bacterial competition due to the germicide's suppression of bacterial growth. The importance of competition can be seen again in Systems #6' and #7' (Fig. 3b and 4b) after the addition of 0.2% biocide. In #6', the biocide reduced the bacterial count to 0 cells/ml and the fungal count to 100 cfu/ml; the fungal count rose almost three logs in 3 weeks while the bacteria remained at zero. The control levels for both microbial groups (bc and fc) remained fairly constant. The significance of microbial dominance is even more evident in #7'. Lines bc and fc which were 16-19 weeks of System #6 show that an established bacterial population at the level of 10^8 cells/ml precludes the subsequent development of fungi. In System #7, that half of the system treated with 0.2% T showed that at low bacterial levels, fungi were not inhibited.

Systems #4 and #6 (Fig. 2a and Fig. 3a) make an interesting comparison. Initially the systems were identical, but #6 received weekly additions of 25% fresh germicide while #4 received only evaporation compensation of deionized water. In #4 the earlier rise of bacterial populations initially inhibited the establishment of fungi, whereas in #6, fungi were able to grow prior to the rise in bacterial levels. A comparison of Systems #2 and #4 demonstrates the effect of initial inoculum on fungal growth in the cutting fluid (Fig. 1a and 2a). System #2 was not exposed to the high bacterial levels in spoiled fluid and consequently contained no metabolic products of prior microbial growth. Bacterial levels

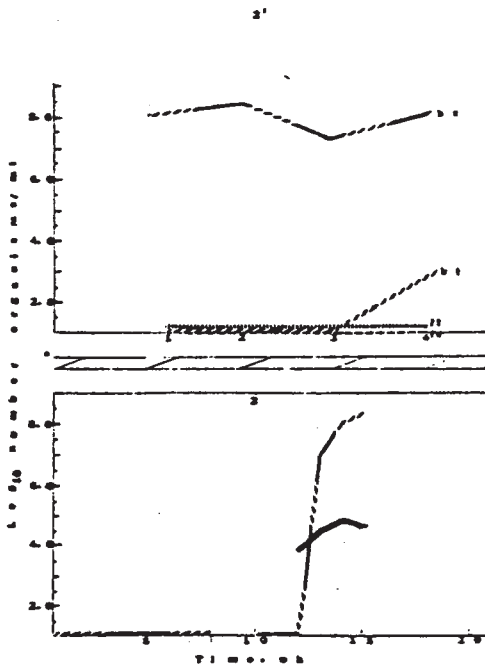


FIG. 1a. System #2 - 5% oil/water emulsion containing 0.1% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine. No inoculum added weeks 1-8. *Fusarium* sp. added at week 9 (10^3 /ml), fungal counts depicted by solid line and bacterial counts by broken line.

FIG. 1b. System #2' - System #2 divided in two, one-half treated with 0.2% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine; where bc = bacterial counts in untreated half, bt = bacterial counts in treated half, fc = fungal counts in untreated half, and ft = fungal counts in treated half.

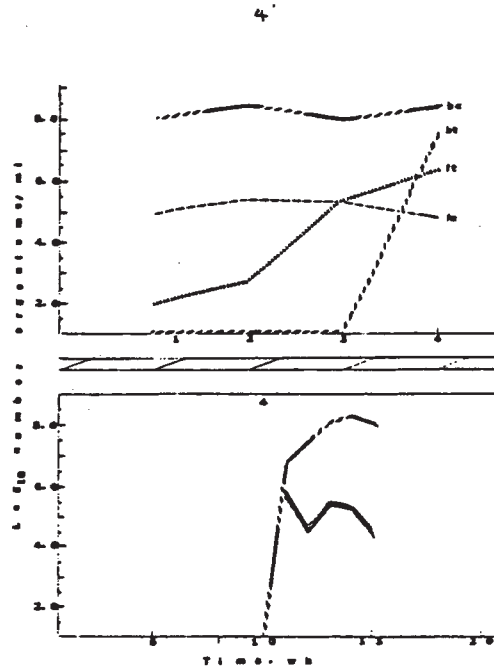


FIG. 2a. System #4 - 5% oil/water emulsion containing 0.1% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine. Inoculum: 10% spoiled fluid, *Fusarium* sp. added at week 9 (10^3 /ml). Solid line represents fungal counts, broken line is for bacterial counts.

FIG. 2b. System #4' - System #4 divided in two, one-half treated with 0.2% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine; where bc = bacterial counts in untreated half, bt = bacterial counts in treated half, fc = fungal counts in untreated half, and ft = fungal counts in treated half.

(Fig. 1a) in #2 after 11 weeks aeration were solely inocula from unfiltered air. The first bacterial growth in this system (2') was apparently insufficient to support the development of fungal growth as in #4' (Fig. 2b).

It appears that the previous history of cutting fluid is extremely critical in the support of fungal growth. If a system is comparatively sanitary, as in #2, and, in addition, has a suitable biocide to prevent bacterial growth, it is doubtful whether fungi will grow well. However, if the level of initial contamination is great (through addition of spoiled fluid) and the germicide is discontinued, as in System #4 or #6, fungal survival and growth is most probable. Two experiments, though incomplete, tend to reinforce these conclusions. Emulsions with varying amounts of spoiled fluid (0-20% by volume) or germicide (0-0.1% by weight with 10% spoiled fluid) were sterilized, then inoculated with *Fusarium* sp. and shaken rather than bubbled. Fungal growth seemed to be proportional to the level of spoiled inoculum added, and in the absence of pre-existing or subsequent bacterial contamination, 0.05% germicide was apparently fungicidal.

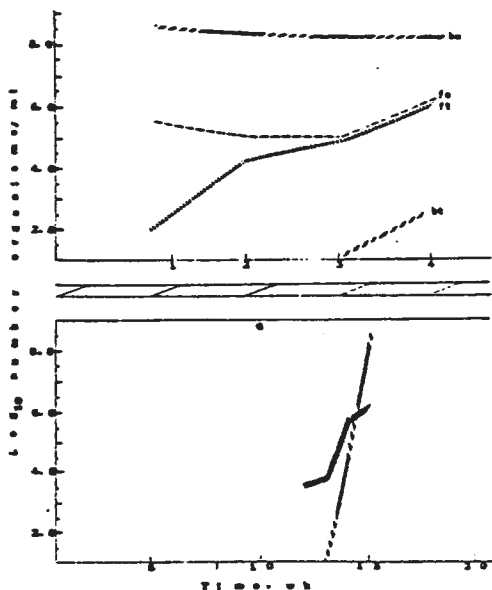


FIG. 3a. System #6 — 5% oil/water emulsion containing 0.1% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine. Inoculum: 10% spoiled fluid, 25% replacement of original fluid weekly. *Fusarium* sp. added at week 9 (10^3 /ml), fungal counts depicted by solid line and bacterial counts by broken line.

FIG. 3b. System #6' — System #6 divided in two, one-half treated with 0.2% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine; where bc = bacterial counts in untreated half, bt = bacterial counts in treated half, fc = fungal counts in untreated half, and ft = fungal counts in treated half.

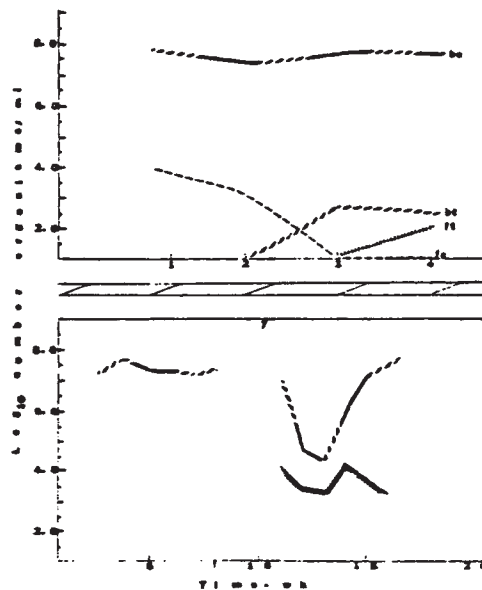


FIG. 4a. System #7 — 5% oil/water emulsion without germicide. Inoculum: 10% spoiled fluid; 25% replacement of original fluid weekly. *Fusarium* sp. added at week 9 (10^3 /ml), fungal counts depicted by solid line and bacterial counts by broken line.

FIG. 4b. System #7' — System #2 divided in two, one-half treated with 0.2% hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine; where bc = bacterial counts in untreated half, bt = bacterial counts in treated half, fc = fungal counts in untreated half, and ft = fungal counts in treated half.

TABLE 2. Microbial growth in chemical solution cutting fluid

	Week (colonies/ml)		
	1	2	3
Bacteria^a			
No germicide	1.4×10^8	6.7×10^7	2.8×10^8
Germicide (0.15%)	36	0	3×10^6
Fungi^b			
No germicide	4.4×10^3	8.2×10^3	2.5×10^4
Germicide (0.15%)	0	17	7.9×10^5

^aAll samples were inoculated with 50 ml spoiled fluid which had a count of 10^9 /ml, initial samples contained 5×10^7 cells/ml.

^bAll samples were inoculated with 50 ml of a *Fusarium* culture in Sabouraud Dextrose Broth. The inoculum contained 2.4×10^5 fungal colonies/ml, initial samples contained 1.2×10^4 cfu/ml.



FIG. 5. Fungal colonies on bottle surface of chemical solution cutting fluid.

The results of the study with chemical-solution coolant (Table 2) demonstrated the explosive nature of fungal growth. After 2 weeks the fungal count was 17 cfu/ml; there was no gross observation of fungal growth at that time, but within 3 days the inside of the bottle became covered with fungal colonies (Fig. 5). It appeared from these data that plate counts were not true indicators or predictors of the actual quantitative level in the fluid. This observation might explain an anomaly often seen in the plant where large central systems reek of mustiness but where plate counts are extremely low. Careful and painstaking examination of machines and sumps usually revealed tenacious clumps of fungal growth.

In the succeeding experiment the sequence of development of fungal colonies was demonstrated utilizing the submerged slides (Figs. 6a, b, c). Although the original inoculum was a pure culture of *Fusarium*, the first indication of fungal activity on the glass slide did not appear to be *Fusarium* (Fig. 6b) but proved to be *Cephalosporium* conidia. The developing colonies consisted of two species growing together. Since no attempt was made to maintain sterility or to filter the air used for bubbling, *Cephalosporium* could have been an adventitious contaminant. We have isolated this group before from laboratory samples as well as in the field. The physiological significance of this relationship is not known at this time. Possibly the development of holdfasts and subsequent slime masses are dependent upon the interaction of the two species. This is now being investigated under controlled conditions.

The total significance of fungal growth in cutting fluids should not be minimized without further study since *Fusarium* species can metabolize hydrocarbons similar to

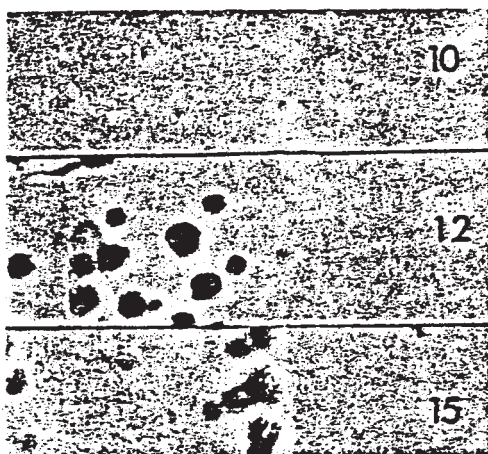


FIG. 6a. Glass slides withdrawn from chemical solution cutting fluid at 10, 12, and 15 days after inoculation. Notice colonies barely visible at 10 days, distinct at 12 days, and becoming confluent at 15 days.

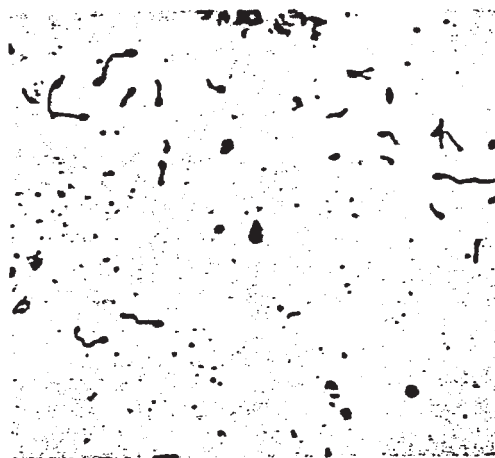


FIG. 6b. Conidia (*Cephalosporium*) most with germination tubes. Glass slide submerged 10 days in fluid. Magnification is about 100X.

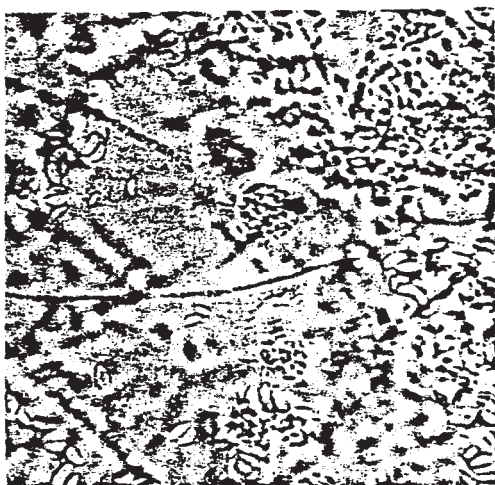


FIG. 6c. Typical conidiophore with conidia cluster of *Cephalosporium*. Glass slide submerged 12 days. Magnification is about 450X.



FIG. 6d. Typical septate conidia of *Fusarium*. Glass slide submerged 12 days. Magnification is about 450X.

those found in cutting fluids (Flippin et al., 1963), and at least one species, *Fusarium moniliforme*, was reported recently to produce a mycotoxin having vertebrate toxicity (Cole et al., 1973). It is clear that any attempt to control microbial growth in cutting fluids must also consider fungal growth as a separate though related control problem.

ACKNOWLEDGMENTS

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