

From: BIODETERIORATION RESEARCH, Vol. I
Edited by Gerald C. Llewellyn and Charles E. O'Rear
(Plenum Publishing Corporation, New York, New York, 1987)

MICROBIAL ECOLOGY OF AN AUTOMOTIVE ENGINE PLANT

H. W. ROSSMOORE, Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA

L. A. ROSSMOORE and C. E. YOUNG, Biosan Laboratories, Inc., Ferndale, MI 48220, USA

INTRODUCTION

The primary concerns for microbiological contamination of industrial fluids, including metalworking fluids, have related to the economic losses resulting from biodeterioration. Despite the isolation of a number of known and putative human pathogens from metalworking fluid, there have been no published reports citing a human infection arising from microorganisms found in industrial fluids. The transient outbreak of a short-term fever and myalgia at Ford Motor Company's Ensite Plant in Windsor, Ontario, during the second week of August 1981 prompted a reexamination of the possibility of infectious disease derived from industrial environmental sources.

The exceptionally high morbidity rate in the affected area of the plant, the clinical symptoms, and the initial direct fluorescent antibody (DFA) findings led to a presumptive diagnosis of Pontiac Fever, a syndrome associated with Legionella spp. infection. There is ample opportunity for aerial transmission of microorganisms from operating metalworking systems (Rossmore et al., 1976; Vedder and Rossmore, 1986). However, the systems with positive DFA for Legionella spp. were both distal from the main working area and, perhaps more importantly, were not producing significant aerosols.

There was minimal confirmation of the original DFA/Legionella spp. findings. Undoubtedly, without the clinical symptoms, the diagnosis of Pontiac Fever would not have been made; surely, there is ample evidence for the environmental ubiquity of Legionella spp. (Fliermans et al., 1979, 1981; Orrison et al., 1981).

Concomitant with the continuing evaluation of the epidemiological studies focused on Legionella spp., a microbiological profile on the

samples collected was established to develop a rationale for a microbial monitoring and control program.

MATERIALS AND METHODS

During the course of 2 weeks, 109 samples were collected for study. These included service water, air washers, production washers, and metalworking fluids. Several systems, especially those in the affected area, were sampled more than once (Figure 1). Samples were retrieved in 100 ml WhirlpakTM bags and maintained and transported at 5-10° C.

When received in the laboratory, the samples were treated to the following regimen:

1. Total colony-forming units (CFU)/ml of aerobic bacteria were carried out in tryptic soy agar (TSA) (DIFCO Laboratories) by standard plate count procedures. Incubation was at 30° C for 48 h.
2. Total yeast and mold counts were done in sabourauds dextrose agar (SDA) (DIFCO Laboratories) supplemented with 50 µg/ml of gentamicin sulfate and incubated as above for 3-5 days.
3. Semi-quantitative determination of sulfate-reducing bacteria with modified API agar (Rossmore et al., 1986).
4. Bacterial and fungal isolates were identified as follows: Isolates were transferred to either TSA or SDA, incubated 24 h, and subsequently identified as to genus or species level. For gram-negative bacteria, API 20E (Ayerst Laboratories, Inc.) was used; for yeasts and molds, API 20C (Ayerst Laboratories, Inc.) and colonial and microscopic morphology were used.
5. All samples were retained and stored at 10° C for future use.

System #s 7 and 200 were of primary interest since they were sources of the Legionella spp. DFA positives reported from a government laboratory. Implicated were System #s 200 for Legionella bozemani and 7 for Legionella pneumophila.

On August 27, System #s 7 and 200 were disposed of and recharged, and these were resampled on August 31. Recommendations were to dump, steamclean, and recharge. Steam was not available, and service water was used to flush and clean each system. Maintenance personnel selected a germicide from the chemical crib to compensate for the lack of steam; unfortunately, the antimicrobial agent was one utilized for general sanitizing (quaternary ammonium type) neutralized by soluble

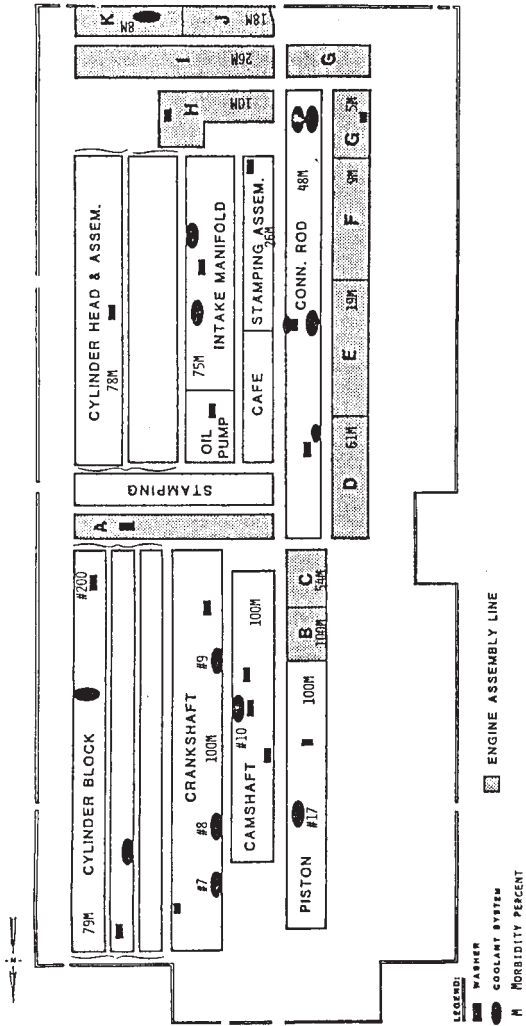


Figure 1. Production and Assembly Departments of the Automotive Engine Plant

oils and phosphates. All three systems had higher counts after disposal, "sanitizing," and recharging. The meaning inherent in these data should serve as an object lesson as to the necessity for improved lines of communication and an improved knowledge base between/among laboratory personnel and machine tool operators with regard to biocide applications.

RESULTS AND DISCUSSION

All of the service water samples tested on September 3, with the exception of one, had excessively high counts (16,000-800,000/ml). It was apparent at this sampling date that the service water could be contributing to the contamination load in functional systems. We learned that service water was a euphemism for marginally chlorinated (0.1 ppm residual) Detroit River water. This water is primarily used (80% of intake) for cooling in the casting plant, a pass-thru function; the remaining 20% is for "fire water," washes, coolants, and sanitary flushes. Comparable City of Windsor water, which is the potable system in the plant, had a residual of 0.7 ppm of chlorine.

On September 15, several key systems were resampled in addition to several air compressor condensate lines and pre- and post-chlorination river water. Raw river water had a fairly modest count (1,500/ml) with a wide variety of species. Post-chlorinated water was collected in thiosulfate to neutralize the residual chlorine. The results suggest that short-term contact (seconds) had no effect on the total count (1,200/ml), although only two species were detected in this sample. A sample of service water was collected from the main header at a point most distal (south) from the intake source. The count was reduced to 21/ml. All the service water systems except one exhibited extremely high values for supposedly uncontaminated water. In addition, sulfate reducers were fairly widespread. Certainly, under-treated river water can only be part of the reason for the contaminated service waters.

On September 15, the #7 main cap bearing system was again sampled, and the total bacterial count on this date was 10^9 /ml. These results are indicative of what happens in an unattended system. On August 27, this system was zero. After dumping, "sanitizing," and recharging, the count on August 31 was 100,000; two weeks later, the same system reached one billion. Undoubtedly, at this level, treatment to control would require some heroic measure -- more than just the recommended dose of biocide by the vendor. None of the other samples collected on

September 15 had a high count, nor did any sample contain an unusual mix of species.

During the course of this study, over 30 species of bacteria, 4 species of filamentous fungi, and 9 species of yeasts were identified (Tables 1 and 2). The organisms, in general, were typical of fresh-water contamination in which some organic nutrients were supplements. The aerobic bacterial population essentially belonged to two major groups: the non-fermenting species, including Pseudomonas, Acinetobacter, Aeromonas, and Moraxella; while the other major group included the facultative fermentative species which includes the enteric bacteria (Enterobacter, Escherichia, Klebsiella, Serratia, Citrobacter, and Providencia) as well as members of the Proteus family which, for purposes of discussion, could be attached to the coliform group.

Although none of these named groups are considered frank pathogens, many of them are listed as opportunists and have been involved in nosocomial infections and, in some cases, infections of immunologically-compromised individuals. Members of the Pseudomonas genus especially have been associated with these latter infections. In addition, they have been implicated in infections of occluded skin (Hojyo-Tomoka et al., 1973) and from contaminated whirlpool baths (Centers for Disease Control, 1981). Because of its survival capacity in aerosols and its rather broad nutritional niche, Klebsiella species are frequently encountered in environmental situations as well as in upper respiratory infections in the types of situations mentioned previously. Certainly, other members of the groups reported have also been cited clinically, especially Aeromonas and Serratia; however, there have been no reported cases of infectious process among individuals coming in contact with large volumes of fluids containing these organisms. There have been no indications from individuals working in waste treatment facilities who encounter the same species of any greater morbidity than the average individual.

The yeasts and fungi also reflected the same mixed bag of species, some of which have been implicated in the infectious process. None of them is known as a frank pathogen. Fusarium toxicosis is well-known; however, we have no indication that the outbreak had any relationship to trichothocene intoxication. Indeed, the level of Fusarium contamination was fairly low in the whole plant. The same could be said for Cephalosporium and Aspergillus. In addition to Candida albicans, a known member of the microbial flora of man, we frequently

Table 1. Bacterial Species Isolated.

Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas putrefaciens, Pseudomonas stutzeri, Pseudomonas cepacia, Pseudomonas maltophila, Pseudomonas paucimobilis;
Enterobacter aerogenes, Enterobacter cloacae, Enterobacter agglomerans, Citrobacter freundii, Klebsiella pneumoniae, Citrobacter diversa, Serratia liquefaciens, Providencia alkalifaciens, Proteus morgani, Moraxella spp.;
Aeromonas hydrophila, Acinetobacter calcoaceticus var. anitratus and var. lwoffii, Flavobacterium odoratum, Alcaligenes spp.

22 Total

Table 2. Fungal Species Isolated.

Candida albicans, Candida humicola, Candida parasilopsis, Fusarium spp., Cephalosporium spp., Trichoderma spp., Trichosporon beiglii, Trichosporon capitalum, Penicillium spp., Aspergillus spp., Cladosporium spp., Botrytis spp., Saccharomyces spp., Cryptococcus laurentii, Cryptococcus albidus, Cryptococcus burenti.

16 Total

Table 3. System 200 Engine Rinse With Legionella bozemanii DFA Positive.

Date	CFU/ml		Isolated Species	SRB
	Bacteria	Fungi		
8/28	4 x 10 ⁶	0	<u>Klebsiella pneumoniae</u> , <u>Pseudomonas stutzeri</u> , <u>Citrobacter freundii</u> .	0
8/31	1.3 x 10 ⁶	100	<u>Klebsiella pneumoniae</u> , <u>Pseudomonas fluorescens</u> , <u>Pseudomonas paucimobilis</u> , <u>Candida humicola</u> .	0

also found Candida humicola. This organism is a soil yeast and probably has no medical significance (Table 2).

The prevalence of sulfate-reducing bacteria throughout the plant could be more than just a deterioration problem. Certainly, high levels of hydrogen sulfide are not without occupational hazard. A report in the CDC Bulletin cites the maximum level permitted over a work period and also during any one exposure time. The finding in an earlier publication (Porschen and Chan, 1977) suggests that some sort of infectious process is possible with these organisms, although the clinical symptoms do not match all those reported in the outbreak of August 1981. Certainly, the presence of large numbers of sulfate reducers in all the test systems as well as in the service water to those systems should cause concern for the control of this organism.

Because of the importance of the outbreak of Pontiac Fever and the concomitant evidence for Legionella spp. in three locations in the affected areas, we evaluated the relationship of these putative legionellae to the other dominant species in those systems. These data are seen in Tables 3, 4, and 5. The Legionella bozemanii and Legionella pneumophila from System #s 200 (Table 3) and 7 (Table 4) were by DFA results only, with no cultural confirmation. Affected individuals had no responsive titers to these species. The accompanying bacteria were not different from the dominant mix seen in other systems in the plant (Tables 6 and 7). The isolation and eventual characterization of Legionella feeleii (Herwaldt et al., 1984) revealed a positive serological response with affected individuals but, again, no correlation with other microbial species (Tables 5, 6, and 7).

The question of preventive maintenance of all systems to prevent both the implication of microbial etiology of an occupational outbreak and also to maintain the functional fluids in better condition would require a change in direction. The aerobic bacterial levels in almost all of the coolant systems were too high to be under control. Mold and yeast involvement is related to aerobic bacterial activity (Figure 2). Except in rare instances, molds and yeasts are only found when there is an established aerobic bacterial population. Occasionally there is a physical sequestering of mold growth so that treatment is difficult and the contamination continues to reappear. The same type of relationship exists between sulfate-reducing bacteria and aerobic bacteria (Figure 3), where the level of aerobic contamination is a prerequisite for sulfate reduction to appear. Again, the location of sources of

Table 4. System #7 Main Cap Bearing Coolant With Legionella pneumophila Sero #1 DFA Positive.

<u>Date</u>	<u>CFU/ml</u>		<u>Isolated Species</u>	<u>SRB</u>
	<u>Bacteria</u>	<u>Fungi</u>		
8/28 [Biocide Added]	0	0	None	0
8/31	1.4×10^5	10^3	<u>Klebsiella pneumoniae</u> , <u>Pseudomonas fluorescens</u> , <u>Candida albicans</u> .	++
9/15	10^9	10^3	<u>Enterobacter agglomerans</u> , <u>Citrobacter freundii</u> , <u>Candida humicola</u> .	++

Table 5. System #17 Coolant/Piston With Legionella feeleii by Isolation on Charcoal Yeast Extract Agar^a.

<u>Date</u>	<u>CFU/ml</u>		<u>Isolated Species</u>	<u>SRB</u>
	<u>Bacteria</u>	<u>Fungi</u>		
8/28	10^8	5	<u>Acinetobacter calcoaceticus</u> , <u>Pseudomonas fluorescens</u> , <u>Aeromonas hydrophila</u> .	++
9/15	10^8	10^3	<u>Citrobacter freundii</u> , <u>Pseudomonas putrefaciens</u> , <u>Candida humicola</u> .	++

^aFrom Herwaldt et al., 1984.

Table 6. Microbial Species in 10% of Isolates.

<u>Species</u>	<u>Total</u>	<u>MWF^a</u>	<u>Wash</u>	<u>Air</u>	<u>Water</u>	
					<u>Raw</u>	<u>Service</u>
<u>Pseudomonas fluorescens</u>	33	15	9	2	0	2
<u>Pseudomonas stutzeri</u>	16	7	5	1	1	1
<u>Citrobacter freundii</u>	39	216	12	2	0	3
<u>Klebsiella pneumoniae</u>	35	16	15	1	1	1
<u>Acinetobacter calcoaceticus</u>	26	11	10	1	1	3
Sulfate Reducers	66	38	17	3	0	5
<u>Candida humicola</u>	29	16	11	1	0	1

^a5% water-in-oil metalworking fluid emulsion

Table 7. Most Frequent Bacterial Couplets Isolated.

<u>Couplet</u>	<u>#</u>	<u>%</u>
<u>Citrobacter freundii</u> <u>Klebsiella pneumoniae</u>	17	15
<u>Citrobacter freundii</u> <u>Pseudomonas fluorescens</u>	9	8
<u>Citrobacter freundii</u> <u>Acinetobacter calcoaceticus</u>	9	8
<u>Klebsiella pneumoniae</u> <u>Pseudomonas fluorescens</u>	16	14

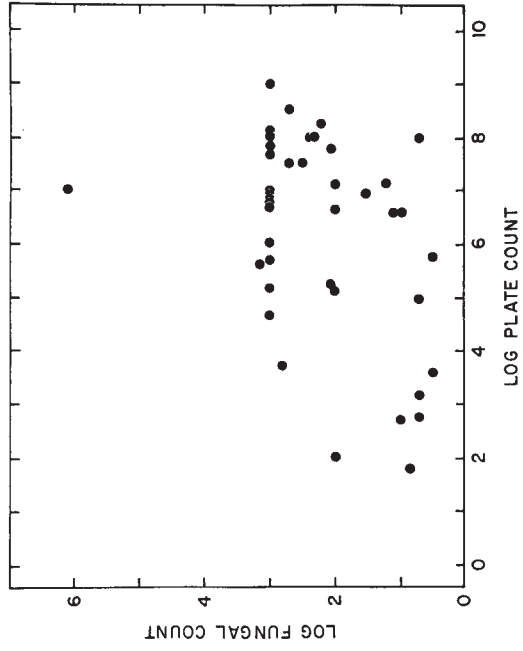
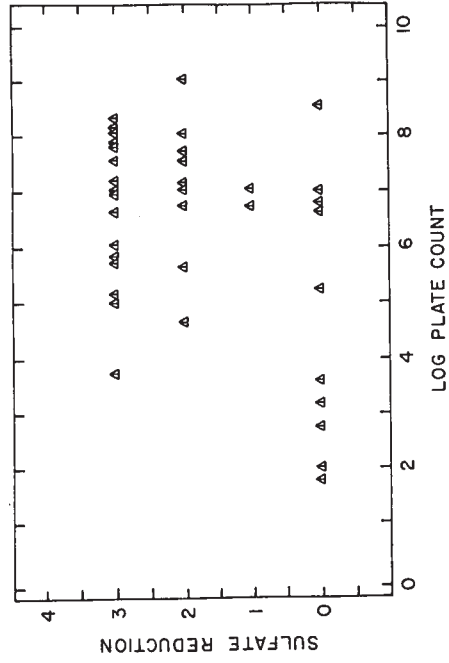


Figure 2. The Relationship Between Aerobic Bacterial Count and Fungal Count



contamination for sulfate reducers in bottom sludge makes treatment difficult once systems become contaminated with these organisms. Throughout the manufacturing area, overflow sumps served as a source of foul odors and contamination and apparently served no useful function.

The selection and use of antimicrobial agents has been a rather haphazard affair throughout the automotive industry. In most cases, the biocide selected is one of several approved by the company, the approval being based as much on historical background as on efficacy. In addition, there is a great deal of reliance on vendor advice, both vendors of the specific biocides and vendors of metalworking fluids who may be using one or more approved biocides in their product. Currently, there are no more than 10 biocide packages approved by the EPA (either in the U.S. or Canada) for use in metalworking fluids. This approval implies registration which requires a battery of toxicologic tests, environmental impact studies, and available efficacy data (Rossmore, 1986).

Some biocides can be incorporated in metalworking fluid concentrates and have proven stability during storage. When diluted to appropriate levels, the biocide is available for use in the metalworking fluid. More often than not, biocide additions must be made directly into the use system since it is difficult to predict the rate of turnover of pre-treated metalworking fluid. The longevity of the fluid may exceed the usable life of the biocide. In other cases, biocides not compatible with fluid concentrates are added directly to the system. Some chemical species are incompatible with amines; some are incompatible with sulfides; some are sequestered by non-polar systems and removed from activity.

From the available list of biocides, it should be possible to find the most effective combination for situations in any one plant. This means evaluation. Currently, there are two ASTM documents for this type of evaluation (American Society for Testing and Materials, 1985a, 1985b). Because a rational method for evaluation should include the microbial population from the site, the metalworking fluid used, and the metal being worked, it is impossible for either the vendor of biocides or the vendor of metalworking fluids to have answers for every use situation. Biocides should never be used much below recommended dose levels since with a number of them, this is the equivalent of selecting for resistant organisms and, in fact, may be worse than nothing. Combinations should be looked at to improve efficacy, essentially to search for synergism.

In spite of the fact that no infectious disease has been reported from metalworking fluids, it would be imprudent to allow systems to develop strong odors and obnoxious growths just because these may not constitute a health hazard. The judicious selection and use of biocides should preclude the development of microbiological problems.

REFERENCES

- American Society for Testing and Materials (ASTM) (1985a). Standard method for evaluating the bioresistance of water-soluble metalworking fluids. ASTM Designation D 3946-80. In: 1985 Annual Book of ASTM Standards. ASTM, Philadelphia.
- American Society for Testing and Materials (1985b). Standard method for evaluation of antimicrobial agents in aqueous metal-working fluids. ASTM Designation E 686-80. In: 1985 Annual Book of ASTM Standards. ASTM, Philadelphia.
- Centers for Disease Control, U.S. Dept. of Health and Human Services/ Public Health Service (1981). Outbreak of Pseudomonas aeruginosa serotype 0:9 associated with a whirlpool. Centers for Disease Control Morbidity and Mortality Weekly Report (MMWR), 30(27), 329-331.
- Fliermans, C.B., Cherry, W.B., Orrison, L.H., Smith, S.J., Tison, D.L., and Pope, D.H. (1981). Ecological distribution of Legionella pneumophila. Appl. Environ. Microbiol., 41, 9-16.
- Fliermans, C.B., Cherry, W.B., Orrison, L. H., and Thacker, L. (1979). Isolation of Legionella pneumophila from non-epidemic related aquatic habitats. Appl. Environ. Microbiol., 37, 1239-1242.
- Herwaldt, L.A., Gorman, G.W., McGrath, T., Toma, S., Brake, B., Hightower, A.W., Jones, J., Reingold, A.L., Boxer, P.A., Tang, P.W., Moss, C.W., Wilkinson, H., Brenner, D.J., Steigerwalt, A.G., and Broome, C.V. (1984). A new Legionella species, Legionella feeleeii species nova, causes Pontiac Fever in automobile plant. Am. Intern. Med., 100, 333-338.
- Hojyo, T., Theresa, M., Marples, R.R., and Kligman, A.M. (1973). Pseudomonas infection in superhydrated skin. Arch. Dermat., 107, 723-727.
- Orrison, L.H., Cherry, W.B., and Milan, D. (1981). Isolation of Legionella pneumophila from cooling tower water by filtration. Appl. Environ. Microbiol., 41(5), 1202-1205.

- Porschen, R.K., and Chan, P. (1977). Anerobic vibrio-like organisms cultured from blood: Desulfovibrio desulfuricans and Succinivibrio species. J. Clin. Microbiol., 5(4), 444-447.
- Rossmore, H.W. (1986). Microbial degradation of water-based metalworking fluids. Chapter 14. In: Comprehensive Biotechnology, Vol. 3, pp. 249-269 (M. Moo-Young, C.L. Cooney, and A.E. Humphrey, eds.), Pergamon Press, New York.
- Rossmore, H.W., Holtzman, G.H., and Kondek, L. (1976). Microbial ecology with a cutting edge. In: Proceedings of the Third International Biodegradation Symposium, pp. 221-232 (J.M. Sharpley and A.M. Kaplan, eds.), Appl. Science Publishers Ltd., London.
- Rossmore, L.A., Wireman, J.W., and Rossmore, H.W. (1986). Rapid field method for the detection and enumeration of sulfate reducers. In: Biodeterioration 6, Proceedings of the Sixth International Biodeterioration Symposium, pp. 413-419 (S. Barry and D.R. Houghton, eds.), C.A.B. Intl. Mycological Inst., The Biodetn. Soc., United Kingdom.
- Vedder, K.W. and Rossmore, H.W. (1986). The detection of airborne sulfate-reducing bacteria from metalworking fluids. In: Biodeterioration 6, Proceedings of the Sixth International Biodeterioration Symposium, pp. 453-459 (S. Barry and D.R. Houghton, eds.), C.A.B. Intl. Mycological Inst., The Biodetn. Soc., United Kingdom.