Viability of Selected Microorganisms in Hydrocarbon Fuels

H. G. HEDRICK, M. T. CARROLL, H. P. OWEN, AND D. J. PRITCHARD

Applied Science and Engineering Laboratories, General Dynamics/Fort Worth, Fort Worth, Texas

Received for publication 5 June 1963

ABSTRACT

HEDRICK, H. G. (General Dynamics, Fort Worth, Texas), M. T. CARROLL, H. P. OWEN, AND D. J. PRITCHARD. Viability of selected microorganisms in hydrocarbon fuels. Appl. Microbiol. **11**:472–475. 1963.—A laboratory study of the viability of selected microorganisms in a hydrocarbon fuel medium was carried out on 19 species of microorganisms, representative of the types found as natural contaminants in aircraft fuels. More species remained viable when inoculated in pure cultures than when inoculated in mixed (composite) cultures. Of the 19 species selected, 10 were still viable after 3 months and 5 were viable after 4 months in the pure culture inoculants. In the complete composite culture inoculant, the bacterial species which were viable at the end of 4 months were the same as those found in the pure culture inoculant. No fungi remained viable in the complete composite cultures after a 3-week period. The microorganisms which remain viable in a hydrocarbon fuel medium are considered indicative of a satisfactory inoculum to be used as a test culture in laboratory analysis of mechanical control techniques.

To determine the effectiveness of mechanical methods for killing, removing, or controlling microorganisms in hydrocarbon fuels (i.e., jet aircraft fuel), it is necessary to make a selection of test microorganisms which most nearly represent the natural microflora in contaminated fuel. This selection would constitute an artificial inoculum which could be included in sterility testing of the treated fuel. A question arose as to which species would remain viable when placed in single or mixed cultures in a hydrocarbon fuel medium.

Based on reports (Bakanauskas, 1958; Boeing Airplane Co., 1961; Leonard, 1960; Prince, 1961) stating the possible microorganisms found in naturally contaminated fuels, and on references to hydrocarbon-utilizing microorganisms (Beerstecher, 1954; Davis, 1956; Fuhs, 1961), a group of representative microorganisms was selected for this study. This selection did not necessarily contain all species that have been isolated or identified from naturally contaminated fuel. This group included 19 species considered representative of the general type naturally occurring in aircraft fuel tanks and fuel storage tanks, as well as some which perhaps could be present from various sources of contamination. In addition, some of the microorganisms were selected as good test organisms for physical killing methods, i.e., *Bacillus subtilis*, *Micrococcus radiodurans*, and *Aspergillus niger*.

The purpose of this study was to determine the viability of these selected microorganisms in single and composite cultures in a hydrocarbon fuel-basic salts culturing system.

MATERIALS AND METHODS

The group of microorganisms selected for use in this study are shown in Table 1 with the source for each. The medium for growing the selected microorganisms in single

TABLE 1. *Microorganisms selected for viability studies in hydrocarbon fuels*

Organism	Strain
Bacteria	
Aerobacter aerogenes	GD/FW B-7
Aluminum corrosion 304	Swatek, Long Beach State College, Long Beach, Calif.; Fort Detrick, Frederick, Md.
Aluminum corrosion 308	Swatek, Long Beach State College, Long Beach, Calif.; Fort Detrick, Frederick, Md.
Bacillus cereus	GD/FW B-8
B. subtilus	GD/FW B-9
Clostridium sporogenes	U.S. Air Force Aerospace School of Medicine; ATCC 7955
Desulfovibrio desulfuricans	Prince, ASD; Bennett, University of Houston, Houston, Texas
Flavobacterium arborescens	ATCC 4358
Micrococcus radiodurans	Anderson, Oregon State University, Corvallis
Pseudomonas aeruginosa	GD/FW B-3
P. fluorescens	GD/FW B-12
Sphaerotilus natans	ATCC 11021
Fungi	
Alternaria tenuis	Simmons, Quartermaster Corps, Natick, Mass., 7270
Aspergillus niger	U.S. Department of Agriculture, T.P. 215-4247
Cladosporium resinae	Simmons, Quartermaster Corps, Natick, Mass., 7998
Fusarium roseum	Simmons, Quartermaster Corps, Natick, Mass., 38g
Penicillium ochrochloron	U.S. Department of Agriculture, 1336
Spicaria violacea	Simmons, Quartermaster Corps, Natick, Mass., 1031
Yeast	
Rhodotorula rubra	ATCC 9449

TABLE 2. *Viability of selected microorganisms in hydrocarbon fuel medium*

Culture	Presence of growth[a]											
	Incubation time (days)											
	7	14	21	28	35	42	56	70	84	91	105	126
Pure												
Pseudomonas aeruginosa	+	+	+	+	0	+	+	+	+	+	+	+
P. fluorescens	+	+	0	+	0	+	+	+	+	+	+	+
Bacillus subtilis	+	+	0	+	0	+	+	+	+	+	+	+
B. cereus	+	+	0	+	+	+	+	+	+	+	+	−
Aerobacter aerogenes	+	+	0	+	+	+	+	+	+	+	+	+
Clostridium sporogenes	−	+	−	+	−	−	−	−	−	+	−	0
Micrococcus radiodurans	+	−	−	+	−	−	−	−	−	−	−	−
Desulfovibrio desulfuricans	+	+	+	0	+	+	+	+	−	+	−	0
Aluminum corrosion 304	+	+	+	−	−	−	−	−	−	−	−	−
Aluminum corrosion 308	−	−	−	−	0	−	−	−	−	−	−	−
Flavobacterium arborescens	+	−	−	−	−	−	0	−	0	0	0	0
Sphaerotilus natans	+	+	+	+	+	+	0	−	0	0	0	0
Rhodotorula rubra	+	−	−	−	−	−	0	−	0	0	0	0
Cladosporium resinae	+	+	0	+	0	+	+	+	+	+	+	+
Aspergillus niger	+	+	0	+	0	+	−	+	−	+	−	−
Spicaria violacea	−	+	−	−	−	−	−	−	−	−	−	−
Penicillium ochrochloron	+	+	0	−	−	−	−	−	−	−	−	−
Alternaria tenuis	−	−	+	+	+	+	+	−	+	+	+	−
Fusarium roseum	−	−	+	−	−	−	−	−	0	−	−	−
Bacteria composite[b, c]												
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+
P. fluorescens	+	+	+	+	+	+	+	+	+	+	+	+
B. subtilis	+	+	+	+	+	+	+	+	+	+	+	+
B. cereus	+	+	+	+	−	−	−	−	−	+	+	−
Aerobacter aerogenes	+	+	+	−	+	+	+	−	+	+	+	+
M. radiodurans	+	−	−	−	−	−	−	−	−	−	−	−
Aluminum corrosion No. 304	+	+	+	+	+	+	+	+	+	+	+	+
Fungi composite[b, d]												
C. resinae	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	−	−	−	−	−	−	−
Penicillium ochrochloron	+	−	−	−	−	−	−	−	−	−	−	−
Complete composite[b, e]												
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+
P. fluorescens	+	+	+	+	+	+	+	+	+	+	+	+
B. subtilis	+	+	+	+	+	+	+	+	+	+	+	+
B. cereus	+	+	+	+	−	−	−	−	−	+	+	−
Aerobacter aerogenes	+	+	+	+	+	+	+	+	+	+	+	+
M. radiodurans	+	−	−	−	−	−	−	−	−	−	−	−
Aluminum corrosion 304	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	+	+	−	−	−	−	−	−	−	−	−
Penicillium ochrochloron	+	−	−	−	−	−	−	−	−	−	−	−

[a] Symbols: + = typical growth present; − = no typical growth present; 0 = not subcultured.
[b] Microorganisms which were present at least once.
[c] Initially inoculated with all species of bacteria.
[d] Initially inoculated with all species of fungi.
[e] Initially inoculated with all selected microorganisms.

and composite cultures was the standard Bushnell-Haas fuel medium (Bushnell and Haas, 1941) used in the petroleum industry. The culturing system consisted of test tubes containing 10 ml of Bushnell-Haas solution with 5 ml of sterile JP-4 overlay. The JP-4 fuel was sterilized by filtration through a 0.45-μ filter, and sterility was checked by subculturing samples on Potato Infusion Agar and in Thioglycollate broth. The Bushnell-Haas basic salts medium was sterilized at 15 psi and 121 C for 15 min. The tubes of Bushnell-Haas fuel medium were inoculated with cultures of the selected microorganisms taken from stock cultures as: (i) pure inoculants of the bacteria and fungi, (ii) composite inocula of the bacteria, (iii) composite inocula of the fungi, and (iv) composite inocula of the

bacteria and fungi (complete composite). Control tubes of the Bushnell-Haas fuel medium were not inoculated. The tubes were incubated at 30 C.

To check the viability of the microorganisms, transfers were made from the culture tubes to sterile media at weekly incubation periods. Standard streak-plate or broth subcultures were made on the same media on which the stock cultures were carried. The media included nutrient agar, Thioglycollate broth, and nutrient Dextrose Agar for the bacteria and yeast, and Potato Dextrose Agar for the fungi. The subcultures were incubated at 30 C for 3 to 4 days and observed for the typical cultural characteristics of each microorganism. Gram stains were made on the colony growth of the subcultures to determine the cell type and purity of the isolates.

RESULTS AND DISCUSSION

The results of the viability experiment are shown in Table 2.

Pure culture. It is evident that more species are able to remain viable when present in pure cultures in the Bushnell-Haas fuel medium than in the mixed cultures. Of the 19 species, viable cells of 14 cultures were detected after 7 days, of 10 cultures after 42 days, of 10 cultures after 91 days, and of 5 cultures after the 126-day period. The microorganisms which were viable at all subculturing times included *Pseudomonas aeruginosa*, *P. fluorescens*, *B. subtilis*, *Aerobacter aerogenes*, and *Cladosporium resinae*. Some of the microorganisms in pure culture which were detected sporadically throughout the incubation period were *Clostridium sporogenes*, *M. radiodurans*, *Desulfovibrio desulfuricans*, *A. niger*, *Alternaria tenuis*, and *Fusarium roseum*. It is interesting to note that Aluminum Corrosion 308 was never detected as viable, and that Aluminum Corrosion 304 was viable at 21 days but was not detected again in pure culture. However, this was not the case when these microorganisms were in mixed cultures. *M. radiodurans* was found after 7 days and again at 28 days but did not appear again. Some of the sporadic appearances of some of these microorganisms in the subcultures suggested that possibly the sampling varied, or that these microorganisms have peculiar growth activities in such a nutrient system. It is also evident that a period of adjustment is necessary for some microorganisms (i.e., *A. tenuis* and *F. roseum*) before growth is evident. Some of the microorganisms present did not remain viable as pure cultures for any great length of time. This is indicated by the results obtained with *M. radiodurans*, Aluminum Corrosion 304, *Flavobacterium arborescens*, *Rhodotorula rubra*, *Spicaria violacea*, *Penicillium ochrochloron*, and *F. roseum*.

Bacteria composite. In the bacterial composite, 7 species from a mixture of 12 were viable after 7 days of incubation. Of these seven, five were detected after 42 days; six (*P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *B. cereus*, *A. aerogenes*, and Aluminum Corrosion 304) were viable after 91 days; and five were viable after the 126-day period. *B. cereus* was present at 105 days and *A. aerogenes* at 126 days, but they were not detected at all subculturing times. *M. radiodurans* was lost after 7 days.

Fungi composite. In the fungi composite, three of seven species were viable after 7 days of incubation. Of these three, *C. resinae* was the only microorganism detected at the 126-day period.

Complete composite. In the complete composite, nine species were detected at the 7-day period; this included seven bacteria and two fungi. Five of the nine microorganisms were still viable after the 126-day incubation period. These included *P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *A. aerogenes*, and Aluminum Corrosion 304. *B. cereus* was detected sporadically up to the 105-day period. Of the 19 species making up the complete composite, only 9 species were detected as viable at least once. *P. ochrochloron* and *M. radiodurans* were detected at 7 days but were not subcultured again. *A. niger* was present at 21 days but was not detected after this time. It is significant to observe that the fungus (*C. resinae*) most commonly isolated from naturally contaminated samples was viable in the pure culture test and in the fungus composite but was not detected in the complete composite. The species of yeast (*R. rubra*) included in the microorganisms was not viable in the complete composite.

These results indicate that the selected microorganisms which remain viable for a sufficient period of time in the Bushnell-Haas fuel culturing system would include representative species of bacteria and fungi to serve as inoculum in preparation of artificially contaminated fuel samples.

More species of bacteria in the group of microorganisms in this study were capable of utilizing the nutrients in the Bushnell-Haas fuel medium than were species of fungi. The bacteria also remained viable over a longer period of time than did most of the fungi. This was especially true in the complete composite inoculant. The complete composite inoculant would certainly be more typical of the actual conditions in naturally contaminated fuels. The fact that more bacterial species than fungal species remained viable indicates that this might also be the situation in natural conditions. This assumption has been verified by several people, who have isolated only one or a limited number of bacterial isolates from contaminated fuel samples held over a period of time.

ACKNOWLEDGMENT

This study was done as part of USAF contract AF 33(657)-9181 on "Mechanical techniques for killing, removing, or controlling microorganisms in hydrocarbon fuels" with Charles R. Martel as project monitor.

LITERATURE CITED

BAKANAUSKAS, S. 1958. Bacterial activity in JP-4 fuel. WADC Tech. Rept. 58-32.

BEERSTECHER, E., JR. 1954. Petroleum microbiology. Elsevier Publishing Co., Amsterdam, The Netherlands.

BOEING AIRPLANE CO. 1961. Investigation of corrosion and microbiological degradation in integral fuel tanks. Document no. D3-3629.

BUSHNELL, L. D., AND H. F. HAAS. 1941. The utilization of certain hydrocarbons by microörganisms. J. Bacteriol. **41**:653–673.

DAVIS, J. B. 1956. Microbial decomposition of hydrocarbons. Ind. Eng. Chem. **48**:1445.

FUHS, G. W. 1961. The microbial degradation of hydrocarbons. Arch. Mikrobiol. **39**:374–422.

LEONARD, J. M. 1960. Fuel fungi. Naval Res. Rev.

PRINCE, A. E. 1961. Microbiological sludge in jet aircraft fuel. Develop. Ind. Microbiol. **2**:197–203.