AD-A097 674 NAVAL RESEARCH LAB WASHINGTON DC F/G 6/13
MICROBIAL DETERIORATION OF MARINE DIESEL FUEL FROM OIL SHALE.(U)
APR 81 M E MAY, R A NEIHOF
UNCLASSIFIED NRL-MR-4489 NL

NRL-MR-4489

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: NRL Memorandum Report 4489
2. GOVT ACCESSION NO.: AD-A097674
3. RECIPIENT'S CATALOG NUMBER:
4. TITLE (and Subtitle): MICROBIAL DETERIORATION OF MARINE DIESEL FUEL FROM OIL SHALE
5. TYPE OF REPORT & PERIOD COVERED: Final Report
6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): M. E. May and R. A. Neihof
8. CONTRACT OR GRANT NUMBER(s):
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Naval Research Laboratory, Washington, DC 20375
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 63724N; WR-N0016[illegible] Z0829 SL; 43-1290; 61-0079-0-1
11. CONTROLLING OFFICE NAME AND ADDRESS: David W. Taylor Naval Ship Research & Development Center, Annapolis, MD 21403
12. REPORT DATE: April [illegible], 1981
13. NUMBER OF PAGES: 16
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office):
15. SECURITY CLASS. (of this report): UNCLASSIFIED
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microbial deterioration	DFM	Cladosporium resinae
Oil shale	Synthetic fuel	Candida sp.
Coal	Fungi	Seawater
Petroleum	Yeast	
Diesel fuel marine	Sulfate-reducing bacteria	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Recurring problems with conventional ship fuels caused by microorganisms have prompted an evaluation of the susceptibility of a recently produced synthetic diesel fuel from oil shale to microbial contamination. The growth of typical microbial contaminants of hydrocarbon fuels has been determined over a four month period in two-phase systems consisting of fresh and sea water media overlaid with fuel. Anaerobic, sulfate-reducing bacteria and a yeast (Candida sp.) grew as well in the synthetic fuel as in fuel derived from petroleum. Growth of certain strains of the fungus, Cladosporium resinae, was initially

(Continues)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

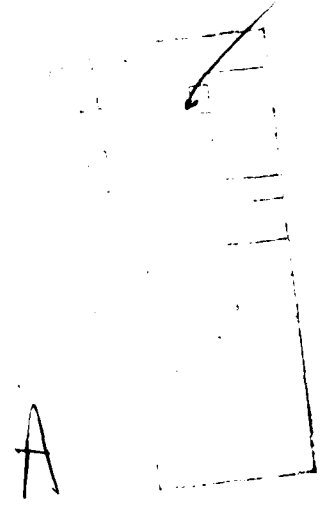
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

251950

20. ABSTRACT (Continued)

delayed in the synthetic diesel fuel but after 8—13 weeks the growth was generally comparable to that in petroleum-derived fuel. This finding indicated that C. resinae may require time for adaptation to constituents in the oil shale fuel. Ultimately, however, it appears that the synthetic diesel fuel is likely to be as susceptible to microbial contamination as conventional diesel fuel has been. Experience acquired with available synthetic fuels shows that their ability to support growth of microbial contaminants varies widely depending on both the source of the crude oil and the refining processes used.

CONTENTS



MICROBIAL DETERIORATION OF MARINE DIESEL FUEL FROM OIL SHALE

INTRODUCTION

The decreasing availability of secure sources of crude petroleum and the almost total dependence of military ships, aircraft and vehicles on liquid hydrocarbon fuels has forced an evaluation of fuels derived from alternate sources under domestic control.

Studies of the physical and chemical properties of fuels from oil shale, coal and tar sands are being made to determine their suitability as replacements or extenders for conventional fuels (1,4,15,16,17). It has also appeared advisable to assess the susceptibility of these new synthetic fuels to microbial contamination, inasmuch as serious problems from this cause have arisen in the past with petroleum-base fuels (13). The possibility that the different chemical composition of the synthetic fuels may favor the growth of microbial contaminants should be explored before these fuels come into wider use.

Problems from microbial fuel contamination generally arise from two distinctly different groups of microorganisms, sulfate-reducing bacteria and fungi (3,8,13). In an oxygen depleted two-phase fuel/water system, the bacteria thrive by the reduction of sulfate to sulfides, which accelerates corrosion in storage tanks and fuel handling systems and generates particulate matter. The fungi, on the other hand, require oxygen, and the most troublesome species tend to form coherent mats at water/fuel interfaces which can clog filters and orifices. The isolation of different species and strains of both groups of microorganisms from conventional fuel systems and from natural sources has been previously described (9,10).

Growth responses of various microorganisms to jet fuels from oil shale and coal have been studied and shown to be markedly different (9,10). A Fusarium fungus grew as well in all the synthetic fuels as in petroleum JP-5. Sulfate-reducing bacteria were relatively inhibited only in a shale fuel containing considerable concentrations of basic nitrogen compounds. This fuel was also inhibitory to the fungus, Cladosporium resinae, and a yeast (Candida sp.) but no inhibition was noted with another shale oil fuel from which the nitrogen constituents were almost completely removed by hydrogenation and acid extraction. The coal-derived fuels examined were highly inhibitory to fungi. Apparently microbial growth varies with the species of organism, the source of the fuel and the refining processes used.

Manuscript submitted February 12, 1981.

In order to understand the relationship between synthetic fuel composition and the growth response of microbial contaminants in fuel/water systems it is necessary to evaluate each new fuel as it becomes available. In this report we present the results of an investigation of the ability of a diesel fuel refined from an oil shale crude to support growth of typical bacterial and fungal contaminants in fresh and sea water systems.

MATERIALS AND METHODS

Fuels

The petroleum-base fuel used was Diesel Fuel Marine (DFM, MIL-F-16884G) received from the Naval Supply Center, Norfolk, VA in 1973.

The oil shale-derived diesel fuel came from a 73,000 barrel lot of crude produced by the Paraho process (2) and refined into military fuels at the Toledo refinery of Sohio in 1979. It was received in June, 1979 and stored at 4°C. This fuel is designated Shale II DFM in this report. It has been characterized elsewhere (1,15).

Paraffin oil (Fisher), representing an innocuous hydrocarbon, was used in anaerobic test units as an additional control.

Anaerobic Bacterial Test Units

The anaerobic inocula, consisting of mixed microbial populations of sulfate-reducing bacteria and other associated bacteria, came originally from three sources:

(1) Laboratory continuous culture - an infected Avgas storage tank in the aircraft carrier, USS YORKTOWN, was the source of the bacteria subsequently maintained as a semi-continuous culture in the laboratory for 10 years under Avgas (6).

(2) USS MERRILL (DD-976) - diesel fuel tank (12).

(3) Potomac River sediment - near the Blue Plains Sewage Treatment Plant, Washington, D.C.

Small amounts of material from the above three sources were cultured in Sisler and Zobell triple strength medium (Sisler's 3X) (7) and allowed to develop dense populations. A 1:10 dilution was made under n-heptane in filtered seawater (0.45 µm Millipore) that had been previously deaerated with nitrogen. An additional deaeration with nitrogen for 10 minutes was made to remove excess hydrogen sulfide in the diluted inoculum. This inoculum was allowed to rest for 3 to 4 hours before removing aliquots for inoculation of the test units.

The test units consisted of sterile 50 ml screw-top test tubes to which 40 ml of the appropriate fuel were added. Ten ml of an aqueous medium of supplemented trypticase soy broth (BBL) (6) were pipetted under the fuel. One ml of the bacterial inoculum was then added. The tubes were

tightly capped and incubated in the dark at 25°C. All tests were done in duplicate.

The growth of sulfate-reducing bacteria in the anaerobic test units was estimated from the degree of blackening developed from reaction of microbially generated hydrogen sulfide with ferrous iron in the growth medium to form ferrous sulfide. The rating system consisted of 0 = no blackening, 1 = slightly grey, to 5 which was intense, opaque black.

Fungal Test Units

Sources of fungi were as follows:

(1) Cladosporium resinae DK was isolated from a JP-5 storage tank at Naval Air Station, Lemoore, CA.

(2) Cladosporium resinae DK/adapted is the above C. resinae after adaptation to growth in seawater.

(3) Cladosporium resinae P-1 was isolated from sludge from a centrifugal purifier on the USS PETERSON (DD-969).

(4) Candida sp. was isolated from water with a film of oil on the surface which had collected in an exposed boiler room of a naval ship in the process of being scrapped at Curtis Bay, MD.

The fungi and yeast were grown on potato-dextrose agar (Difco) slants with the addition of 0.5% yeast extract (Difco). For inoculation, stock suspensions of the organisms were prepared by dispersing surface growth on a slant in 10 ml of a solution of 0.05% polysorbate 80 (Tween 80) in distilled water. The viable count in these suspensions ranged from 11×10^6 to 56×10^6 colony forming units per ml.

Fungal test units with fresh-water mineral salts media were of two types: One followed the formula of Bushnell and Haas (5) and had a pH of approximately 6.5 after sterilization (referred to as FWBH) while the other was that of Klausmeier as modified by Park (14) with a pH of approximately 5.0 after sterilization (referred to as FWKP)

Fungal test units were also set up with seawater obtained from the Mediterranean Sea (salinity = 37.1°/oo) during a cruise and aged for over a year in the dark at 4°C before use. In all cases 0.05% peptone (Difco) and 0.05% yeast (Difco) were added. The pH of the peptone-yeast solution in seawater after autoclaving was 8.00 ± 0.02 (SWPY8). In some cases the seawater medium was adjusted to pH 6 with 1N hydrochloric acid (SWPY6).

Each test unit consisted of a 250-ml Erlenmeyer flask with a cotton plug. Fifty ml of the water phase were dispensed into the flask and autoclaved for 20 minutes at 120°C. Fifty ml of the fuel were then added and the unit was allowed to stand overnight at room temperature. The pH of each flask was readjusted if needed and 0.5 ml of the inoculum of C. resinae or yeast was added. The flask plugs were loosely covered with aluminum foil and the test units were incubated in the dark at room temperature (22°-25°C). All experiments were done in duplicate.

The test units inoculated with fungi and yeast were inspected for growth at appropriate time intervals. The rating system was as follows:

0 = no germination
1 = germination and minute amount of growth, no mat formation
2 = slight interfacial growth, no mat formation
3 = mat formation over one-third of the interface
4 = mat formation over two-thirds of the interface
5 = mat formation over entire interface
6 = mat formation over entire interface with a thickness of 0.5 cm or more.

At the conclusion of each experiment, pH measurements were made on the water phase of each test unit with a glass electrode. A test for viability was made on those test units showing growth ratings of 0 or 1 by spreading approximately 0.5 ml of the liquid taken from the water/fuel interface on potato dextrose agar + 0.5% yeast extract. The agar surface of these plates had previously been allowed to dry so that this large amount of inoculum could be spread and absorbed by the agar without having too wet a surface for discrete colony growth during subsequent incubation.

RESULTS AND DISCUSSION

Anaerobic Studies

The growth of the sulfate-reducing bacteria after 68 hours incubation in test units containing paraffin oil and the two DFM fuels is given in Table 1. The sulfate reducers from all three sources were able to grow with the Shale II DFM as well as with petroleum DFM or paraffin oil. Growth was slower in those test units inoculated with sulfate reducers from the Potomac River sediment, probably because they were not adapted to the fuel/water system used, whereas the organisms from the other two sources were isolated from fuel systems. That an adaption to fuel by the organisms from the river sediment may be occurring is indicated by the observation that after 18 days, growth ratings increased to 3, 5 and 3/5 for petroleum DFM, Shale II DFM and paraffin oil, respectively.

Studies with Fungi

Tables 2-5 show the growth, survival and final aqueous pH for the three different strains of C. resinae (DK, DK/adapted and P-1) and for the yeast (Candida sp.). Where growth in duplicate test units was unequal, ratings for both are shown.

With a few exceptions these organisms had nearly the same growth rating, pH and viability after four months under Shale II DFM as under petroleum DFM. The rates at which the C. resinae strains grew in the early weeks of the experiments tended to be slower in the Shale II DFM. This may signify that a period of adaption of the organisms to the synthetic fuel system exists. No such delay was noted for Candida.

The generally poor and erratic growth of C. resinae in SWPY8 media has been shown elsewhere to be due to the high pH of the seawater (11) (illustrated in Fig. 1). The C. resinae DK strain even lost viability in this medium. The failure of the DK and P-1 strains to grow under Shale II DFM in SWPY8 may be due to the combination of unfavorable pH and the factor in the fuel which caused the retarded growth rate. Decreasing the initial pH of the seawater to six allowed much improved growth of C. resinae in all instances.

It will be noted that with one exception (Candida under Shale II DFM with FWBH media, Table 5) heavy growth of fungal organisms also resulted in a greatly lowered final pH after four months. This supports an hypothesis advanced elsewhere (11) that Candida may promote the growth of C. resinae when they occur together as co-contaminants in seawater/fuel systems by lowering the pH sufficiently, at least in microenvironments, to allow C. resinae to initiate growth and generate its own acidic products. The superior ability of Candida sp. to grow in high pH situations may thus be a major factor in promoting the growth of the more troublesome C. resinae. This situation appears to apply to Shale II DFM as well as to petroleum fuels.

CONCLUSION

In no case was the growth of a microbial fuel contaminant promoted significantly by the presence of a diesel fuel from oil shale as compared with conventional petroleum-derived diesel fuel. Certain of the important fungal contaminants were initially inhibited somewhat by the synthetic diesel fuel which indicated that a period of adaptation was necessary before the growth rate became comparable to that observed in the presence of petroleum fuel. Thus the use of a synthetic diesel fuel like the one evaluated here either alone or in mixtures with conventional fuel is not likely to lead to microbial contamination problems significantly different from those encountered in the past.

REFERENCES

1. Affens, W.A., J.M. Hall, E. Beal, R.N. Hazlett, C.J. Nowack, and C. Speck. Aug. 1980. Relationship between fuel composition and properties. III. Physical properties of U.S. Navy Shale II fuels. Preprints Amer. Chem. Soc., Div. Fuel Chem.

2. Anon. 1977. Energy fact book. Tetra-Tech Report No. TT-A-642-77-306.

3. Bailey, C.A., and M.E. May. 1979. Evaluation of microbiological test kits for hydrocarbon fuel systems. Appl. Environ. Microbiol. 37:871-877.

4. Brinkman, D.W., M.L. Wisman, and J.N. Bowden. 1979. Stability characteristics of hydrocarbon fuels from alternative sources. BETC/RI-78/23. National Technical Information Service, U.S. Dept. of Commerce, Springfield, VA 22161.

5. Bushnell, L.D., and H.F. Haas. 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol. 41:653-673.

6. Klemme, D.E., and J.M. Leonard. 1971. Inhibitors for marine sulfate-reducing bacteria in shipboard fuel storage tanks. Naval Research Laboratory Memo Report 2324.

7. Klemme, D.E., and R.A. Neihof. 1969. Control of marine sulfate-reducing bacteria in water-displaced shipboard fuel storage tanks. Naval Research Laboratory Memo Report 2069.

8. Klemme, D.E., and R.A. Neihof. 1976. An evaluation in large-scale test systems of biocides for control of sulfate-reducing bacteria in shipboard fuel tanks. Naval Research Laboratory Memo Report 3212.

9. May, M.E., and R.A. Neihof. 1979. Microbial deterioration of hydrocarbon fuels from oil shale, coal, and petroleum. I. Exploratory experiments. Naval Research Laboratory Memo Report 4060.

10. May, M.E., and R.A. Neihof. 1980. Microbial deterioration of hydrocarbon fuels from oil shale, coal, and petroleum. II. Growth and inhibition of bacteria and fungi. Naval Research Laboratory Memo Report 4294.

11. May, M.E., and R.A. Neihof. 1981. Growth of Cladosporium resinae in seawater/fuel systems. Dev. Ind. Microbiol., Vol. 22 (in press).

12. Neihof, R.A., and M.E. May. 1979. Microbiological analysis of samples from fuel tanks of USS MERRILL (DD-976). Naval Research Laboratory Letter Report 8353-238. 25 June.

13. Parbery, D.G. 1971. Biological problems in jet aviation fuel and the biology of Amorphotheca resinae. Mater. Organismen 6:161-208.

14. Park, P.B. 1975. Biodeterioration in aircraft fuel systems. Soc. Appl. Bacteriol., Tech. Series 9:105-126.

15. Solash, J., R.N. Hazlett, J.C. Burnett, E. Beal, and J.M. Hall. Aug. 1980. Relation between fuel properties and chemical composition. II. Chemical characterization of U.S. Navy Shale II fuels. Preprints Amer. Chem. Soc.,Div. Fuel Chem.

16. Solash, J., R.N. Hazlett, J.M. Hall, and C.J. Nowack. 1978. Relation between fuel properties and chemical composition. I. Jet fuels from coal, oil shale and tar sands. Fuel 57:521-528.

17. Solash, J., C.J. Nowack, and R.J. Delfosse. 1976. Evaluation of a JP-5 type fuel derived from oil shale. NAPTC-PE-82. Naval Air Propulsion Test Center, Trenton, N.J. 08628.

Table 1. Growth of sulfate-reducing bacteria in aqueous assay medium after 68 hours incubation under conventional and synthetic Diesel Fuel Marine (DFM) and paraffin oil[a]

Fuel	Source of sulfate-reducing bacteria		
	Laboratory Continuous Culture	Potomac River Sediment	Diesel Fuel Tank USS MERRILL
Petroleum DFM	4	2	5
Shale II DFM	5	2	5
Paraffin Oil	4	2	5

[a]Rating system based on blackening in assay medium where 1 = slightly grey, to 5 which was intense opaque black.

Table 2. Growth of Cladosporium resinae DK in marine diesel fuel (DFM)/water test units; viability on subculture; and final pH of the water phase.

DFM source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Petroleum	FWBH	2½	3	3½	4½	5	5	5	6	6	6	+	4.84/4.81
	FWKP	3	3	4	5	5	5	6	6	6	6	+	4.47/4.45
	SWPY8	0	0	0	0	0	0	0	0	0	0	-	7.77/7.82
	SWPY6	2½	2½	3	3½	4	4½	5	5	5	5	+	3.70/3.64
Shale II	FWBH	1	1½	2½	3	4	4½	5	5	5	5	+	4.78/4.76
	FWKP	2	2	2½	4½	5	5	5	6	6	6	+	4.73/4.64
	SWPY8	0	0	0	1	1	1	1	1	1	1	-	7.86/7.88
	SWPY6	2	2	2½	3	3	3½	3½	4	4½	5	+	3.71/3.76

Table 3. Growth of Cladosporium resinae DK/adapted in marine diesel fuel (DFM)/water test units; viability on subculture; and final pH of the water phase.

DFM source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Petroleum	FWBH	4	5	5	5	5	5	6	6	6	6	+	4.67/4.63
	FWKP	4	5	5	6	6	6	6	6	6	6	+	4.13/4.09
	SWPY8	2	2½	2½	3	3	3/3½	3½/4	3½/4	4½/5	5	+	3.57/3.44
	SWPY6	4	4	4½	5	5	5	5	5	5	5	+	2.97/3.01
Shale II	FWBH	1/0	1	1	2½	2½	3	3½/4	4½	4½/5	5	+	4.63/4.85
	FWKP	1	2	2	3	4½	5	5	5	6	6	+	4.77/4.65
	SWPY8	0	0	0	0	0	0	0	0	0	0	-	7.80/7.94
	SWPY6	2	2	2½	3	3	3	3½	4½	5	5	+	3.18/3.10

Table 4. Growth of Cladosporium resinae P-1 in marine diesel fuel (DFM)/water test units; viability on subculture; and final pH of the water phase.

DFM source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Petroleum	FWBH	3	3½	3½	4	4	4½	4½	5	5	6	+	4.27/4.28
	FWKP	3	3	3½	4½	5	5	5	6	6	6	+	4.16/4.14
	SWPY8	2	2	2	2½	2½	2½	2½	2½	3	3	+	4.24/4.41
	SWPY6	3	3	3½	3½	4/3½	4	4½/4	5/4½	5/4½	5/4½	+	3.26/3.42
Shale II	FWBH	0	1	1	2	2½	2½	2½/3	3	3	4½/5	+	4.22/4.09
	FWKP	1	2	2	3	3½	4½	5	6	6	6	+	4.38/4.18
	SWPY8	0	0	0	0	0	0	0	0	0	0	+	8.00/8.01
	SWPY6	2	3	3	3	3	3	3	3	3	3	+	6.60/5.71

Table 5. Growth of Candida sp. in marine diesel fuel (DFM)/water test units; viability on subculture; and final pH of the water phase.

DFM source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Petroleum	FWBH	4	4½	5	5	5	5	5	5	5	6	+	2.84/2.78
	FWKP	3	3½	4/3½	5	5	5	5	5	5	5	+	2.61/2.56
	SWPY8	3	3	4	5	5	5	5	5	5	5	+	4.29/4.31
	SWPY6	4/3	4½/3½	5/4½	5	5	5	5	5	5	6	+	3.66/3.70
Shale II	FWBH	4	4	4	4	4	4	4	5	5	5	+	6.18/6.23
	FWKP	3	4	4½	5	5	5	5	5	5	5	+	2.47/2.45
	SWPY8	3/4	3/4	4/4½	4½/5	5	5	5	5	5	5	+	4.28/4.24
	SWPY6	3/4	4	4½	5	5	5	5	5	6	6	+	3.49/3.56

Fig. 1 - Growth of Cladosporium resinae DK in two-phase water/Shale II DFM fuel systems. There are four different water phases, starting from the left: Bushnell-Haas mineral salts (FWBH), Klausmeier-Park mineral salts (FWKP), seawater + peptone yeast pH 8 (SWPY8) and seawater + peptone yeast pH 6 (SWPY6).

END

DATE
FILMED

5 81

DTIC