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MICROBIAL DETERIORATION OF HYDROCARBON FUELS FROM OIL SHALE, CO--ETC(U)
AUG 80 M E MAY, R A NEIHOF
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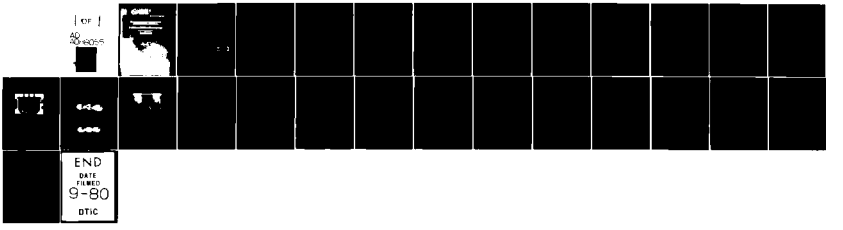

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JP-5	Cladosporium resinae	pH
Synthetic fuel	Candida sp.	Seawater

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

The recurring problems caused by microbial contamination of conventional fuels has led to an evaluation of the susceptibility of aircraft fuels from alternate sources (synthetic fuels). JP-5 fuel from coal and from two different lots of oil shale (Shale I and II) were studied. Shale I differed from Shale II in having a much higher nitrogen content. Anaerobic, sulfate-reducing bacteria were able to grow as well with JP-5 from coal and oil shale as with petroleum. In fungal susceptibility tests with four different strains of Cladosporium resinae and one Candida sp., Shale II supported good fungal growth while Shale I did not; coal was highly inhibitory. The inhibition caused by Shale I was traced to the alkalinity produced by basic, nitrogen-containing extractables in this fuel, but coal contains specific, still unidentified inhibitory constituents. It was also found that the normally high pH of seawater media was sufficient to inhibit C. resinae. Growth which occasionally occurred in seawater/fuel systems (Abstract continues)

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20. (Abstract continued)

was apparently due to establishment of reduced pH in a local microenvironment.

CONTENTS

MICROBIAL DETERIORATION OF HYDROCARBON FUELS FROM OIL SHALE, COAL, AND PETROLEUM. II. GROWTH AND INHIBITION OF BACTERIA AND FUNGI.

INTRODUCTION

As a part of the Energy Conversion Synthetic Fuels Program to decrease United States dependency on crude petroleum from foreign sources, the Naval Air Systems Command has the task of evaluating jet aircraft fuels originating from alternate domestic sources such as coal and oil shale (8). In addition to physical and chemical studies to assess the suitability of these synthetic fuels as replacements for conventional fuels, it has also appeared necessary to assess their susceptibility to contamination by microorganisms because of recurring problems with conventional fuels.

In an earlier report (7) it was shown that many typical microbial contaminants, including fungi, yeast and bacteria, were inhibited in the presence of JP-5 type fuels derived from crudes produced from western Kentucky coal by the Char Oil Energy Development process (COED-5) and from oil shale by the Paraho process (Shale I) (1). An exception was a fungus, Fusarium sp., which grew as well in the synfuels as in petroleum JP-5. Also microbial growth with mixtures of 25% synfuel/75% petroleum fuel was generally as great as with 100% petroleum fuel.

In 1979 fuels from a second shale oil production run at the Toledo refinery of Sohio became available (Shale II) (10). Hydrocracking and acid extraction resulted in a JP-5 fraction with a greatly lowered nitrogen content compared to the fuel from Shale I (9,12). Because the concentration of nitrogen-containing compounds may influence microbial growth (see Reference 7), a major objective of the present investigation was to compare the susceptibility to microbial contamination of Shale II JP-5 with petroleum JP-5 and the synthetic fuels already examined. The microbial species employed has been extended to sulfate-reducing bacteria, a common fuel storage tank contaminant not previously included in this study (7). An effort has also been made to identify the nature of the constituents in coal and Shale I JP-5 which are responsible for fungal inhibition and to assess the reasons for the unfavorable fungal growth in seawater media noted in the preceding report (7).

MATERIALS AND METHODS

Fuels

The petroleum-base fuel, designated Jet-A, was the same as that

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used in previous work (7). It contained no additives and was received in January,1976 from Naval Air Propulsion Test Center, Trenton, NJ.

Coal-derived fuel, also used in the earlier study (7) was produced from Western Kentucky Coal by Sun Oil Co. using high pressure catalytic hydrogenation (4). The finished fuel met most of the requirements for JP-5.

The oil shale products were from crude produced by the Paraho process and subsequently refined to military fuels (2). The JP-5 fraction from the first 10,000 barrel operation (Shale I) had a very high freezing point and a high nitrogen content, 976 ppm, of which 860 ppm was acid extractable (12). JP-5 from the second 73,000 barrel operation (Shale II) was hydrogenated and acid extracted and had only one ppm nitrogen in the finished fuel. Studies of the composition of these fuels appear elsewhere (9,12,13).

Selected nitrogen-containing compounds typical of those in Shale I were added to petroleum JP-5 in some of the test systems used here to determine whether the microbial inhibition of Shale I could be attributed to any of them. These were: pyridine (99%), 5-ethyl-2-methyl pyridine (99%), 2,6 lutidine (96%), 2-picoline (98%), 2-ethyl pyridine (98%), 4-tertiary-butyl pyridine (99%), 4-benzyl pyridine (99%) and quinaldine (97%) from Aldrich Chemical Co. Quinoline was reagent grade from Fisher Scientific Co.

Base and Acid Extractions

Base extractions were made by shaking 850 ml portions of fuel and 10 ml of aqueous 0.1 N NaOH in a one-liter separatory funnel for three one-minute intervals separated by one-minute rests. The aqueous layer was drained off and the extraction process repeated an additional two times. The fuel was then washed three times with 100 ml portions of distilled water using the same shaking schedule. Titration of the combined aqueous layers with standard acid showed titratable acidities of 0.44 meq/l for Shale I, 0.18 meq/l for Shale II and 0.068 meq/l for COED #5.

Acid extractions of Shale II and COED JP-5, were made by shaking 850 ml portions of fuel with 10 ml portions of 0.1 N HCl followed by three washes with 100 ml portions of distilled water using the same procedure as for the base extraction. In order to remove all the acid extractables from the Shale I fuel it was necessary to increase the concentration of HCl to 10.0 N; subsequent washing was with seven 100 ml portions of water. Titratable basicities of 56.2 meq/l for Shale I, 0.19 meq/l for Shale II and 0.174 meq/l for coal were obtained. Basic compounds from Shale I were isolated by acid extraction followed by neutralization of the HCl adducts (9).

Anaerobic Bacterial Test Units

The anaerobic inocula, consisting of mixed microbial populations

of sulfate-reducing bacteria and associated bacteria, came originally from three sources:

(1) Laboratory continuous culture - infected Avgas storage tanks of the aircraft carrier, USS YORKTOWN, was the source of the bacteria subsequently maintained as a semi-continuous culture in the laboratory for 10 years (5).

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

(3) Potomac River sediment - near the Blue Plains Sewage Plant.

Small amounts of material from the above three sources were cultured in Sisler and Zobell triple strength medium (Sisler's 3X) (6) 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 N_2. To remove any H_2S present, an additional deaeration of 10 minutes with N_2 was made. The dilution 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 was added. Ten ml of an aqueous medium consisting of a supplemented trypticase soy broth (BBL) (5) was 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 H_2S with ferrous iron in the growth medium to form FeS. The rating system developed by Klemme and Leonard (5) was used where 0 = no blackening, 1 = slightly grey, to 4 which was intense opaque black.

Fungal Test Units

Sources of fungi were as follows:

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

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

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

(4) Cladosporium resinae APPC-11S was isolated from newly creosoted railroad ties near the Alexandria Potomac Power Co., Alexandria, VA. and found to use Shale I JP-5 as a carbon source.

(5) Candida sp. was isolated from water with a skim 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% 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 formulation of Bushnell and Haas (3) with a pH of approximately 6.5 after sterilization (referred to as FW B-H) while the other was that of Klausmeier as modified by Park (11) with a pH of approximately 5.0 after sterilization (referred to as FW K-P).

Fungal test units were also set up with seawater obtained from the Mediterranean Sea (salinity = $37.1\,^0/_{00}$) during a cruise and aged for over a year in the dark at 4°C before use. In all cases the seawater had 0.05% peptone (Difco) and 0.05% yeast (Difco) added. The pH of the seawater peptone-yeast after autoclaving was 8.00 ± 0.02 [SW+PY(8)]. In some cases the seawater medium was adjusted with 1N HCl to 6 [SW+PY(6)].

All test units consisted of 250-ml Erlenmeyer flasks with cotton plugs. Fifty ml of the water phase were dispensed into each flask and autoclaved for 20 minutes at 120°C. Fifty ml of fuel were then added and the unit was allowed to rest overnight. The pH of each flask was readjusted if needed before the addition of 0.5 ml of the inoculum of C. resinae or yeast. 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 visually inspected for growth at appropriate time intervals. The rating system ranged from 0 for no growth, 1 for spore germination to 6 for a mat thicker than 0.5 cm over the entire interface (Figure 1). A study of the dry weight of microbial material corresponding to these visual ratings showed that an increase of one unit in the visual rating corresponded approximately to a doubling of the amount of growth (see Appendix A). At the conclusion of each experiment, viability studies were made on those units showing growth ratings of 0 or 1 by spreading approximately 0.5 ml of 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 without having too wet a surface during incubation. Also at the end of these experiments, pH measurements were made on the water phase in all test units using a glass electrode.

RESULTS AND DISCUSSION

Anaerobic studies on the sulfate-reducing bacteria in synthetic fuels. The results of the tests on the sulfate-reducing bacteria after 68 hours incubation are given in Table 1. The sulfate reducers from the USS MERRILL showed the most vigorous growth probably because

they came from a fuel/water system similar to that in the test units and had adapted to it. Inocula from the Potomac River sediment showed the slowest growth, but eventually those units also became intensely black. The sulfate reducers grew very well under all of the fuels.

Fungal growth in synthetic fuels. Tables 2 through 5 show the growth, survival and final aqueous pH for three different strains of C. resinae (DK, DK/adapted, and P-1) and for the yeast, Candida sp. As was found in the preliminary experiments (7), JP-5 made from coal inhibits C. resinae and there is a gradual loss of spore viability, Shale I is also inhibitory but to a lesser extent, and viability was retained almost as well as with petroleum JP-5. Under Shale II there was a slight delay in growth in some C. resinae strains, but generally the growth paralleled that under petroleum JP-5 except for strain P-1 which grew less in the fresh water media under Shale II JP-5.

Candida sp. (Table 5) behaved about the same as in the preliminary experiments (7). It did not grow with Shale I or coal JP-5 except in one case where the seawater pH was lowered to 6 (coal JP-5/SW+PY(6)); and here the growth was atypical consisting only of balls of hyphae at the bottom of the flasks. Candida grew with Shale II JP-5 almost as well as with petroleum JP-5.

Identification of fungal inhibitors in JP-5 from coal and oil shale. It is apparent from the data that growth of the fungal organisms employed here depended not only on the fuel but also on the composition and pH of the aqueous phase. In general, it was noted that the different strains of C. resinae grew better in the fresh water than in the seawater systems while the Candida grew more luxuriously in the seawater media than in either of the two fresh water media. Additional consideration of aqueous effects is postponed until after the main effects produced by the different fuels have been discussed.

Neither acid nor base extractions of Shale II made substantial differences in fungal growth except for the P-1 strain of C. resinae which grew better after both extractions when fresh water media were used (Table 4).

Acid and base extractions of coal JP-5 made no difference in the inhibition which this fuel shows for all C. resinae strains and Candida; also the loss in viability of the inoculum remained high (Tables 2-5).

Base extraction of Shale I did not change its inhibitory qualities. After acid extraction, however, growth was as good with all organisms as under petroleum JP-5 (Tables 2-5 and Figure 2). Removal of a large amount of organic bases would appear to be responsible for this difference. It was surprising, therefore, to find that microbial growth in the presence of petroleum JP-5 with added acid extracted material from Shale I was as good as in neat petroleum JP-5 (Table 6). Possible reasons for this are discussed below.

In additional attempts to identify a particular class of inhibitor in Shale I, non-basic nitrogen residues from a silica gel column (Table 6) and a number of pyridine and quinoline derivatives (Table 7) were added back to petroleum JP-5. No inhibition was observed except with 500 ppm of 4-benzyl pyridine (both DK/adapted and APPC-11S strains) and 500 ppm of 4-tertiary butyl pyridine (APPC-11S strain). It is unlikely that these compounds were present in Shale I in these concentrations although there may be cumulative effects from all nitrogen compounds present and other unidentified inhibitory compounds could play a role.

Aqueous media effects on C. resinae growth. If the initial pH of the seawater media is lowered by the addition of acid (HCl), growth of all strains of C. resinae can occur except under fuel from Coal and Shale I. This is illustrated in Tables 2,3,4 and 6 by comparison of growth in SW + PY (8) and SW + PY (6). The normal high pH of the seawater clearly appears to be inhibitory particularly for the DK, P-1 and APPC-11S strains. Supporting evidence that pH is the crucial variable in C. resinae inhibition in seawater comes from the finding that growth in seawater at an initial pH of 6 (SW + PY (6)) under petroleum, Shale II and acid-extracted Shale I was comparable to growth in fresh water systems (FW B-H, FW K-P).

Although it will be noted that C. resinae did not grow under Shale I fuel in seawater initially adjusted to pH 6, the terminal pH of the aqueous phase in these test units was at least 7.3. This is due to the gradual diffusion of basic materials from the fuel into the water and the poor growth can again be attributed mainly to an unfavorably high pH. If this simple interpretation is valid it follows that the basic materials removed by acid extraction from Shale I should not be inhibitory to C. resinae when neutralized and added back to a system with petroleum JP-5 so long as the pH of the aqueous phase is favorable. This was generally true (cf. Table 6).

C. resinae DK/adapted was the only strain to show appreciable growth in normal seawater media (Table 3). This growth was characteristically slow to develop, however, and duplicates did not always grow at the same rate (see Shale II, SW + PW (8)). Growth could start at one place in the interface, usually along the side of the flask as shown in Fig. 3 and from this point the growth could continue over the entire interface. Occasionally the DK strain grew similarily in normal seawater (see Footnote in Table 6). The problem in initiating growth may be in lowering the pH enough in a microenvironment at some point in the interface to allow growth to proceed. Once begun, the considerable capacity of the fungus to produce acid within a colony is sufficient to lower pH in a larger volume and allow growth to spread.

CONCLUSIONS

1. The anaerobic sulfate-reducing bacteria were able to grow as well with all of the synthetic JP-5 fuels as with the petroleum JP-5. Thus

future problems from contamination by sulfate-reducing bacteria of synthetic fuels of the type examined here would be expected to be similar to those experienced in the past with petroleum JP-5.

2. Shale II JP-5 supported good growth of the normal fuel tank contaminating microorganisms. This was in striking contrast to the severe inhibition to fungi seen with Shale I. The difference appears to be due mainly to the additional refining of Shale II fuel that removed basic materials which in Shale I tended to keep the aqueous pH at too high a level for fungal growth.

3. Coal JP-5 was highly inhibitory to all of the yeast and fungal test organisms. The inhibition was not due to acidic or basic extractables or to the lack of a favorable aqueous pH. In view of the possibility that there are constituents in this fuel which are compatible with aircraft use and also inhibitory to fungi at low concentrations, it appears worthwhile to attempt to isolate and characterize these materials.

4. Shale I JP-5 was inhibitory to yeast and fungal test organisms because of the basic extractables in this fuel. However, except for two substituted pyridine compounds at high concentration (500 ppm), the nitrogenous compounds present in Shale I did not cause an inhibition. Most of the inhibition of Shale I appears to be due to the tendency of the nitrogenous constituents to keep the pH of the aqueous phase at too high a level for fungal growth.

5. Different strains of the fuel fungus, C. resinae, showed different preferences for fresh or salt water media but all were inhibited by the normally high pH of seawater. Growth of this organism in seawater may depend on initiation of reduced pH in local microenvironments.

6. In the present stage of synthetic fuel development it is not yet possible to predict with certainty the probable susceptibility of a product to microbial contamination, especially by fungi, from a knowledge of the refining processes used or the conventional properties of the fuel.

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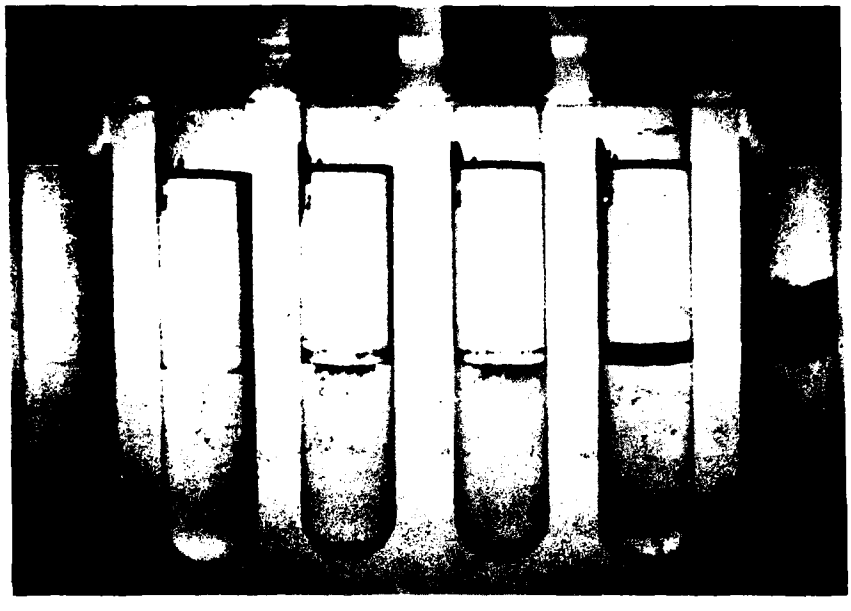

Fig. 1 - Illustration of the rating system for Cladosporium resinae growing at the interface of a two-phase fuel/water system; 0 = no growth to 6 = mat thicker than 0.5 cm.

(a)

(b)

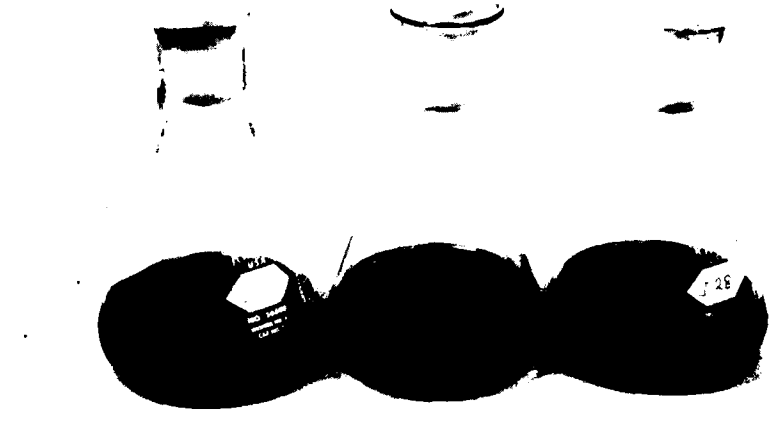

Fig. 2 - (a) Cladosporium resinae DK in a two-phase system of seawater + peptone yeast (pH = 6) under, from the left, Shale I, base extracted and acid extracted Shale I. (b) C. resinae DK/adapted in a two-phase system of fresh water (Klausmeier-Park's mineral salts) under, from the left, Shale I, base and acid extracted Shale I.

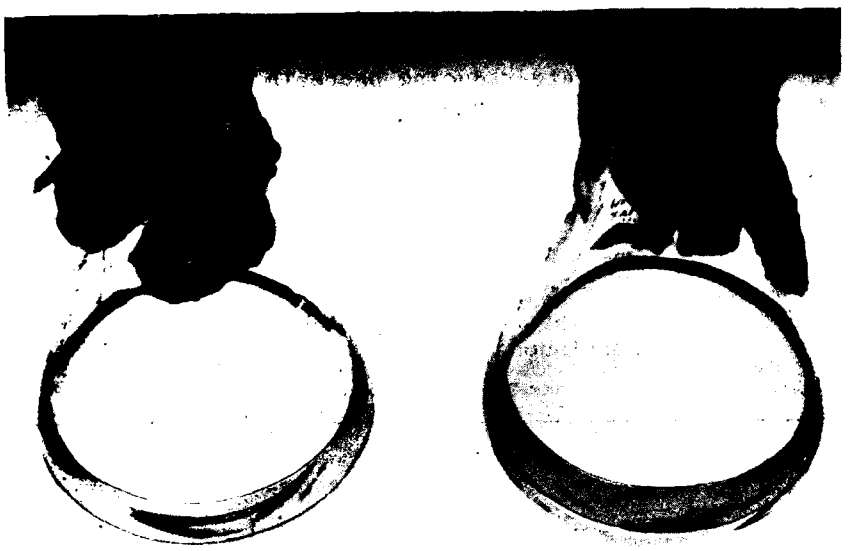

Fig. 3 - Duplicate flasks containing seawater + peptone yeast (pH8) under JP-5 showing Cladosporium resinae growth starting in the right flask on the side at the interface. Growth in this flask eventually covered the interface. The left flask exhibited no growth.

Table 1. Growth of sulfate-reducing bacteria in assay medium under conventional and synthetic jet fuels after 68 hours incubation

Fuel	Source of sulfate-reducing bacteria		
	Laboratory Continuous culture	Potomac River Sediment	Diesel Fuel Tank USS MERRILL
Petroleum JP-5	4	1	5
Coal JP-5	4/5	1	5
Shale I JP-5	2	2	4/5
Shale II JP-5	4/5	2	5
Mineral Oil	4	2	5

Table 2. Growth of C. resinae DK in fuel/water test units; viability on subculture; and final pH of the water phase

JP-5 source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	12	Growth	Viability	pH
Petroleum	FW B-H	2	2½	3	4	4	4	4½	5	5	5	+	4.62/4.69
	FW K-P	2	2½	3	5	5	5	6	6	6	6	+	5.16/5.17
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.83/7.88
	SW+PY (6)	2½	2½	3	3½	4	4	4½	5	5	5	+	3.31/3.36
Coal	FW B-H	0	0	0	0	0	0	0	0	0	0	±	6.44/6.45
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	4.51/4.43
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.81/7.89
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	6.52/6.38
Shale I	FW B-H	1	1	1	1	1	1	1	1	1	1	+	6.88/6.88
	FW K-P	0	1	1	1	1	1	1	1	1	2	+	6.42/6.42
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.89/7.89
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.32/7.42
Shale II	FW B-H	0	1	1½	2½	3	3	4	5	5	5	+	3.77/3.76
	FW K-P	1	1½	2	2½	3	3	3	4½	5	5	+	4.24/4.24
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.94/7.93
	SW+PY (6)	2	2	2½	3	3½	3½	3½	3½	4	4	+	2.82/2.88
Coal-acid extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.58/6.52
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	4.21/4.20
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.79/7.86
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.86/5.94

Table 2. (Continued)

JP-5 source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (4 months)		
		1	2	3	5	6	7	8	10	12	Growth	Viability	pH
Coal - base extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.50/6.50
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.43/4.50
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.93/7.94
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.90/5.81
Shale I acid extracted	FW B-H	2½	3	3	3½	4	4½	4½	4½	5	5	+	4.70/4.70
	FW K-P	3	4	4½	5	5	5	5	6	6	6	+	5.63/5.57
	SW+PY (8)	0	1/0	1/0	1/0	1½/0	1½/0	2/0	2/0	2½/0	3/0	+/-	6.53/7.88
	SW+PY (6)	2½	2½	3	3	3	3	3½	4	4	4½	+	3.44/3.44
Shale I base extracted	FW B-H	0	0	0	1	1½	1½	1½	2	2	2	+	6.95/6.91
	FW K-P	0	0	0	1	1	1	1½	2	2	2	+	6.50/6.44
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.95/7.94
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.35/7.42
Shale II acid extracted	FW B-H	1	2	2½	3	4	4½	4½	5	5	6	+	4.47/4.55
	FW K-P	1	1½	2½	3½	4½	5	5	5	6	6	+	5.20/4.94
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.76/7.84
	SW+PY (6)	1	2	2½	3	3	3½	4	4½	4½	5	+	3.38/3.33
Shale II base extracted	FW B-H	0	1	2	3	3	3½	4	5	5	5	+	4.40/4.73
	FW K-P	0	1	2	3	4	4½	4½	5	5	6	+	5.29/5.15
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.86/7.93
	SW+PY (6)	0	1	1½	2½	2½	2½	3	4	4½	5	+	3.45/3.57

Table 3. Growth of C. resinae DK/adapted to fuel/water test units; viability on subculture; and final pH of the water phase

JP-5 source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (5 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Petroleum	FW B-H	1	2	2½	3½	4	4½	4½	4½	5	5	+	4.45/4.50
	FW K-P	3	4½	5	5	6	6	6	6	6	6	+	4.47/4.50
	SW+PY (8)	0	1	2	2	2½	2½	2½	2½	3	5	+	3.36/3.32
	SW+PY (6)	2	2½	3	3½	4	4½	4½	4½	5	5	+	2.96/2.92
Coal	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.49/6.48
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.49.4.47
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.86/7.88
	SW+PY (6)	0	0	0	0	0	0	0	0	0/1	0/1	-/+	6.35/6.56
Shale I	FW B-H	1	1	1	1	1	1	1	1	1	1	+	6.85/6.86
	FW K-P	0	1	1	1	1	1	2	2	2	2½	+	6.48/6.48
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.73/7.88
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.23/7.35
Shale II	FW B-H	1	1	1	2	2	2½	3	3½	4½	5	+	4.24/4.25
	FW K-P	1	2	2	2½	3	3½	3½	4/3½	5/4½	6/5	+	4.53/4.59
	SW+PY (8)	0	0	1/0	1/0	2/1	2/1	2/1	2½/2	4/2	5/3	+	3.19/4.32
	SW+PY (6)	2	3	3	3½	3½	4	4	4	4	4	+	2.97/2.96
Coal - acid extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.53/6.50
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.43/4.37
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.90/7.89
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.65/5.87

Table 3. (Continued)

JP-5 source	Water phase	Ratings of growth after incubation (weeks)									Terminal readings (5 months)		
		1	2	3	5	6	7	8	10	13	Growth	Viability	pH
Coal - base extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.53/6.47
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.50/4.41
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.88/7.91
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.81/5.77
Shale I acid extracted	FW B-H	2	2½	3	4	4	4½	4½	4½	5	5	+	4.59/4.44
	FW K-P	4	4½	5	5	5	6	6	6	6	6	+	4.53/4.39
	SW+PY (8)	0	0/1	1	2	2	2½	2½	2½	2½/3	3/3½	+	4.72/4.03
	SW+PY (6)	2½	2½	2½	3	3	3½	3½	3½	4	4	+	3.03/3.08
Shale I base extracted	FW B-H	1	1	1	1	1	1/2	1½/2	1½/2	2/2½	2/2½	+	6.85/6.76
	FW K-P	0	1	1	1	1	2	2	2	2½	2½	+	6.19/6.21
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	±	7.92/7.94
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.33/7.38
Shale II acid extracted	FW B-H	0	0	1	2	2½	2½	2½	3½	4½	5	+	4.49/4.46
	FW K-P	1	2	2½	3½	4	4½	5	5	6	6	+	4.40/4.37
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	+	7.77/7.89
	SW+PY (6)	1	2	2	2	2½	2½	2½	3	4	4½	+	3.05/3.16
Shale II base extracted	FW B-H	0	0	0	1	1	2	2	2½	3	5	+	4.65/4.61
	FW K-P	0	2	2	2½	3	4	4½	5	6	6	+	4.48/4.38
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	+	7.76/7.93
	SW+PY (6)	1	1	1	1/2	1/2½	1/2½	1/2½	1/3	1/3	2/4½	+	7.48/3.35

Table 4. Growth of C. resinae P-1 in fuel/water test units; viability on subculture; and final pH of the water phase

JP-5 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	FW B-H	2	2	2½	3	3	3½	4	4½	4½	5	+	4.21/4.11
	FW K-P	2	2½	2½	3	3½	4	4½	5	5	5	+	4.80/4.65
	SW+PY (8)	0	0	0	0	1	1	1	1	1	1	±	8.05/8.05
	SW+PY (6)	1	2	2	2	2	2	2	2	2	2	+	6.98/7.23
Coal	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.45/6.44
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	4.62/4.53
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.97/8.01
	SW+PY (6)	0	0	0	0	0	0	0	0	1/0	1/0	+	6.46/6.42
Shale I	FW B-H	1	1	1	1	1	1	1	1	1	1	-	6.88/6.89
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	6.41/6.42
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	±	7.99/8.03
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.29/7.45
Shale II	FW B-H	0	0	0	0	0	1	1	1	1	2	+	6.32/6.13
	FW K-P	0	1	1	1	1	1	1	1	1	2	+	3.68/3.58
	SW+PY (8)	0	0	0	0	0	0	1	1	1	2/1	+	4.87/7.80
	SW+PY (6)	3	3	3	3	3	3	3	3	3	3	+	3.52/3.53
Coal - acid extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.47/6.47
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.43/4.32
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.90/7.99
	SW+PY (6)	0	0	0	0	1	1	1	1	1	1	+	6.49/6.71

Table 4. (Continued)

JP-5 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
Coal - base extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.50/6.49
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	4.47/4.45
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.98/8.00
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.86/5.82
Shale I acid extracted	FW B-H	2	2½	2½	3	3½	4	4	4½	5	5	+	4.08/4.09
	FW K-P	2	2½	2½	2½	3	3½	3½	4½	5	6	+	5.08/4.80
	SW+PY (8)	0	1	1	1	2	2	2	2	2	2	+	7.91/7.92
	SW+PY (6)	2	3	3	3	3	3	3	3	3	3	+	7.59/7.18
Shale I base extracted	FW B-H	0	1	1	1	1	1	1	1	1	1	+	7.01/6.97
	FW K-P	0	0	1	1	2	2	2	2	2	2	+	6.38/6.38
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.91/8.00
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	±	7.40/7.49
Shale II acid extracted	FW B-H	0	0	1	1	1	2	2	2	2	3	+	5.65/4.32
	FW K-P	0	1	1	2½	2½	2½	3	4	5	6	+	4.28/4.28
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	±	7.99/8.01
	SW+PY (6)	0	0	0	0	0/1	1	1	1	1	1	+	7.11/7.00
Shale II base extracted	FW B-H	0	0	0	0	1/0	1	1	2	2	3	+	6.41/6.02
	FW K-P	0	0	1	2	2½	2½	2½	3	3	6	+	4.25/4.00
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	±	7.95/7.94
	SW+PY (6)	0	0	0	0	0	0	1	1	1	1	+	6.72/7.02

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

JP-5 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	FW B-H	2	2	2½	2½	3½	4	4	4½	4½	4½	+	5.34/5.64
	FW K-P	2	2	2½	2½	3½	4	4½	5	5	5	+	3.14/3.09
	SW+PY (8)	3	3½	3½	4	4½	4½	4½	5	5	5	+	3.76/3.76
	SW+PY (6)	3	4	4½	5	5	5	5	5	5	5	+	3.27/3.28
Coal	FW B-H	0	0	0	0	0	0	0	0	0	0	+	6.43/6.41
	FW K-P	0	0	0	0	0	0	0	0	0	0	-	4.35/4.36
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.93/7.94
	SW+PY (6)	0	1	1	2	2	2	2	2	2	2	+	7.59/7.66
Shale I	FW B-H	0	0	0	0	0	0	0	0	0	0	+	6.87/6.86
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	6.45/6.41
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	±	7.82/7.88
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.46/7.58
Shale II	FW B-H	1	2	2	2½	3	3½	4	4½	5	5	+	3.37/3.33
	FW K-P	2	2½	2½	2½	3½	4	4	4½	4½	5	+	3.06/2.89
	SW+PY (8)	3	3	3	4	4	4½	4½	4½	4½	4½	+	3.04/3.02
	SW+PY (6)	3	3½	3½	4	4	4½	4½	4½	4½	4½	+	2.85/2.86
Coal - acid extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.47/6.46
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.22/4.22
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.90/7.92
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.83/5.88

Table 5. (Continued)

JP-5 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
Coal - base extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	-	6.53/6.55
	FW K-P	0	0	0	0	0	0	0	0	0	0	±	4.52/4.39
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	-	7.77/7.87
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	-	5.87/5.91
Shale I acid extracted	FW B-H	3	3	3½	3½	3½	4	4	4	4½	4½	+	3.85/4.31
	FW K-P	3	3	3½	3½	4	4	4	4	4½	4½	+	2.97/2.90
	SW+PY (8)	2½	2½	3	4	4	4½	4½	4½	5	5	+	3.72/3.72
	SW+PY (6)	3	3	3½	4½	4½	4½	4½	5	5	5	+	3.36/3.34
Shale I base extracted	FW B-H	0	0	0	0	0	0	0	0	0	0	+	6.80/6.82
	FW K-P	0	0	0	0	0	0	0	0	0	0	+	6.37/6.36
	SW+PY (8)	0	0	0	0	0	0	0	0	0	0	+	7.97/7.98
	SW+PY (6)	0	0	0	0	0	0	0	0	0	0	+	7.56/7.65
Shale II	FW B-H	2	3	3	3½	3½	3½	4	4	4½	4½	+	6.05/5.97
	FW K-P	2	2½	2½	3	3	3	3½	4	4½	4½	+	3.30/3.23
	SW+PY (8)	3	3	4	4½	5	5	5	5	5	5	+	3.71/3.71
	SW+PY (6)	4	4½	4½	5	5	5	5	5	5	5	+	3.25/3.26
Shale II base extracted	FW B-H	2	2½	3	3½	3½	3½	3½	4	4½	4½	+	6.00/5.91
	FW K-P	2	2½	3	3½	3½	4	4	4½	5	5	+	3.31/3.28
	SW+PY (8)	3	3	3½	4½	5	5	5	5	5	5	+	3.90/3.82
	SW+PY (6)	3	3½	4	5	5	5	5	5	5	5	+	3.28/3.26

Table 6. Growth of different strains of C. resinae after 6 months incubation in fuel/water test units using synthetic fuels and adding extracts of Shale I to the water phase under petroleum JP-5 as compared to growth under petroleum JP-5

Organism	Fuel phase additive	Additive to water phase (ppm)											
		FW K-P				SW+PY (8)				SW+PY (6)			
		0	20	100	500	0	20	100	500	0	20	100	500
C. resinae DK	Petroleum	6				0				5			
	Shale I	2½				0				2			
	Petroleum/acid extract of Shale I		6	6	5		0	0/4[a]	0		5	5	5
	Petroleum/nonbasic nitrogen plus residues from silica gel column		6	6	6		1	0	0		5	5	5
C. resinae DK/adapted	Petroleum	6				5				5			
	Shale I	3/2				0				2			
	Petroleum/acid extract of Shale I		6	6	6		5	5	2		5	5	5
	Petroleum/nonbasic nitrogen plus residues from silica gel column		6	6	6		2	5	5		5	5	5
C. resinae APPC-11S	Petroleum	6				0				2½			
	Shale I	4				0				2			
	Petroleum/acid extract of Shale I		6	6	6		0	0	0		2½	2½	2
	Petroleum/nonbasic nitrogen plus residues from silica gel column		6	6	6		0	1	0		2½	2	2

[a]See text (p. 6)

Table 7. Growth of two strains of Cladosporium resinae in fresh water (K-P) under petroleum JP-5 with additives of nitrogenous constituents of Shale I.

Fuel phase/additive	DK/adapted Additive to aqueous phase (ppm)				APPC-11S Additive to aqueous phase (ppm)			
	0	20	100	500	0	20	100	500
Petroleum JP-5	5				5			
Shale I JP-5	2				2			
Pyridine		5	5	5		5	5	4
5-ethyl-2-methyl pyridine		5	5	5		5	5	4
2,6 lutidine		5	5	5		5	5	4
2-picoline		5	5	5		5	4	4
2-ethyl pyridine		5	5	5		5	5	4
4-tertiary-butyl pyridine		5	5	5		5	5	2
4-benzyl pyridine		5	5	2		5	4	2
Quinoline		5	5	5		5	5	5
Quinaldine		5	5	5		5	5	4

APPENDIX A

Key to the rating system for growth in two-phase fuel/water systems including approximate dry weights

Rating	Dry Weight (mg)	Description
0	0.34 ± 0.12[a]	No germination, growth or mat formation detected.
1	0.34 ± 0.23	Germination and minute amount of growth detected, no mat formation.
2	1.5 ± 0.52	Slight growth at interface.
3	9.7 ± 3.5	Mat formation over 1/3 of interface.
4	26.0 ± 0.3	Mat formation over 2/3 of interface.
5	64.0 ±17.9	Mat formation over entire interface.
6	115.0 ±41.0	Mat formation over entire interface with a thickness of at least 0.5 cm.

[a]Mean values ± standard deviation.

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