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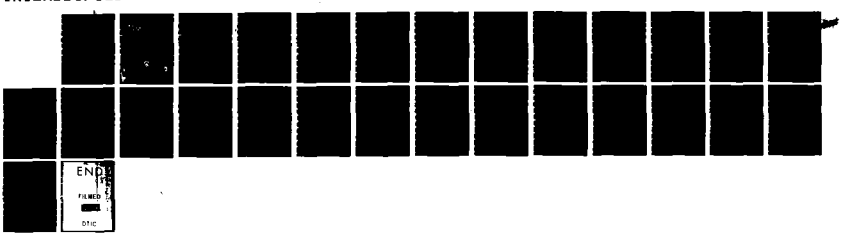

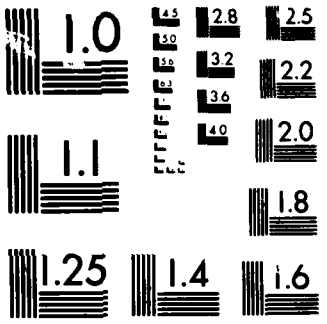

NRL Memorandum Report 5253

# Microbial Deterioration of Hydrocarbon Fuels From Oil Shale, Coal and Petroleum

## III. Inhibition of Fungi by Fuels From Coal

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| JP-5 | Yeast | Microbial contamination |
| Synthetic fuel | *Cladosporium resinae* | Inhibition |

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The anticipated future need for hydrocarbon fuels from sources other than petroleum has impelled a thorough evaluation of the properties of such fuels, including their susceptibility to microbial contamination. The present work confirmed an earlier finding that a JP-5 fuel derived from coal by the Char Oil Energy Development (COED) process was inhibitory to typical fungal contaminants and showed that the inhibition extended to fuels produced by solvent refining as well as to a variety of COED fuels refined in different ways. The inhibition was not due to

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20. ABSTRACT (Continued)

lack of a suitable aliphatic carbon source. Extraction of COED JP-5 fuel with aqueous solutions showed that the inhibitor(s) had a very low water solubility and was not markedly concentrated at the water/fuel interface. An experiment with silica gel as adsorbent indicated that solid adsorbents may furnish a means of removing and concentrating sufficient amounts of the inhibitor for identification. Additional work to identify the source of the fungal inhibition in coal fuel is worthwhile not only because of the pronounced and selective effects produced but because a novel inhibitor may be found which would be useful as a fuel-compatible biocide for controlling microbial contamination in any stored hydrocarbon fuel.

CONTENTS

# MICROBIAL DETERIORATION OF HYDROCARBON FUELS FROM OIL SHALE, COAL AND PETROLEUM

## III. Inhibition of Fungi by Fuels From Coal

## INTRODUCTION

Under the Energy Conversion Synthetic Fuels Program the Naval Air Systems Command is evaluating jet aircraft fuels derived from alternate domestic sources such as coal and oil shale (6). In addition to studies of the physical and chemical properties of these fuels, it has also appeared necessary to assess their susceptibility to contamination by microorganisms because of recurring problems from this source with conventional fuels.

In earlier reports it was shown that an inhibition to growth of typical fungal fuel contaminants by a shale oil-derived fuel was due to nitrogenous constituents which imparted an excessively high pH to the aqueous phase of the test system (4, 5). When the nitrogenous constituents were removed from this fuel by acid extraction, fungal inhibition disappeared. Also a more highly refined shale oil JP-5 containing only 1 ppm nitrogen did not show any microbial inhibition.

Marked inhibition of two principal fuel contaminants, Cladosporium resinae and a yeast (Candida sp.), also occurred with coal-derived fuel produced by the Char Oil Energy Development (COED) process, but certain other microbial genera sometimes found associated with fuels (eg., sulfate-reducing bacteria and a Fusarium fungus) were not significantly affected. In this case, extractive treatments had no effect on the inhibition and it appeared that specific substances in the fuel were responsible. In view of the possibility that there are constituents in low concentration in coal-derived fuels which are compatible with aircraft use and also inhibitory to important fungal contaminants, it was deemed worthwhile to attempt to characterize the source of the inhibition by coal fuel. Experiments carried out for this purpose are described in this report.

## MATERIALS AND METHODS

### Fuels

A petroleum-base fuel, designated Jet-A, was essentially JP-5 without additives. It was the same control fuel as used and described in previous work (4, 5). This fuel was sterilized in all cases by autoclaving 45 min at 121°C.

Crude coal fuels prepared by the COED process were refined by hydrotreatment and distilled to give JP-5 grade fuels. COED-1, 3 and 5 were from Western Kentucky coals and COED-2 and 3 were from Utah coal. Additional descriptions have been given elsewhere (2, 4, 9).

Solvent refined coal fuels (1) were obtained with the help of Mr. Forrest Schaekel (U.S. Army MERADCOM, Fort Belvoir, VA). Descriptive data on these fuels were kindly furnished through the courtesy of Mr. S. J. Lestz and staff of U.S Army Fuels and Lubricants Research laboratory, San Antonio, TX. These samples

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were designated as follows:

AL-11222-F, EDS Distillate, solvent refined coal, mid-distillate.
AL-11189-SP-F, H-Coal, naphtha
#1236, SRC II, naphtha
#1237, SRC II, middle distillate
#1238, SRC II, heavy distillate

Descriptive data on these fuels are given in Table 1. The data in the last three columns may be for different samples of naphtha, middle and heavy distillates than those used in the present work, since the identifying numbers are different. They are included as indications of the properties to be expected.

Coal-derived fuels were usually not sterilized because of the possibility of losing volatile constituents or producing other changes. Filtration through 0.45 μm Millipore filters made no difference in the microbial inhibition seen in test sytems.

Dodecane (99%) was obtained from Phillips Petroleum Co.

All fuels were stored at 4°C after receipt by the Naval Research Laboratory.

## Microorganisms

The fungus, Cladosporium resinae DK, was isolated from a JP-5 storage tank at a naval air station.

Cladosporium resinae DK/adapted is the above organism after adaptation to growth in seawater.

Candida sp. was a yeast isolated from water with a skim of oil on the surface which had collected in an exposed boiler room of a partially scrapped naval ship.

All organisms were grown on slants of potato-dextrose agar (Difco) 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 approximated $10^7$/ml and inoculation volumes were adjusted to give about $10^6$ colony forming units per test unit.

## Test Units

Test units with freshwater mineral salts media were of two types: a formulation of Bushnell and Haas (3) with a pH of approximately 6.0 after sterilization (referred to as WBH) and another following the formulation of Klausmeier as modified by Park (8) with a pH of approximately 5.0 after sterilization (referred to as FWKP).

Test units with seawater as the aqueous phase were also of two types, differing only in the pH. Aged seawater (salinity, 37.1% ) with additions of 0.05% peptone (Difco) and 0.05 % yeast extract (Difco) was used for both. The pH was unadjusted in one case (pH = 8.0)(SWPY8) and in the other 1 M HCl was added to give a pH of 6.0 (SWPY6).

Test units in most cases were 250 ml Erlenmeyer flasks with cotton plugs. Fifty ml of the water phase were dispensed in each flask and autoclaved for 20 min 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 0.2 to 0.5 ml of the inoculum of fungi 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).

In order to conserve fuel, later experiments were carried out similarly but in 50 ml flasks with 10 ml each of aqueous and fuel phases. Results agreed with those carried out with the larger test units.

Extractions

Equilibration: 150 ml of COED-5 JP-5 were shaken continuously in a 500 ml separatory funnel for 6 hrs with 150 ml FWKP or SWPY8 media. Inhibition of fungal growth was determined in test units with the extracted fuel and fresh FWKP and SWPY6 media. The inhibition of the aqueous extractants was similarly determined with Jet-A as the fuel phase.

Repetitive extraction: 150 ml of COED-5 JP-5 was extracted with ten successive 500 ml portions of FWKP or SWPY8 media diluted to 10% of their normal concentrations. The inhibition of the extracted fuel was then determined in test units with fresh FWKP or SWPY6 media.

Repetitive, prolonged extraction: 800 ml of COED-5 JP-5 fuel was extracted with twelve 100 ml portions of FWKP or SWPY8 media diluted to 10% of their normal concentrations and shaking intermittently for at least one hour for each extraction. The extracted fuel was tested for fungal inhibition in test units with fresh FWKP and SWPY6 media. The aqueous extractants were pooled and concentrated ten-fold in a rotary evaporator. This concentrate and portions of it diluted 1:3 with fresh FWKP and SWPY6 media were examined for fungal inhibition in test units with Jet A fuel. The interfacial material from each extraction was collected, pooled and added to test units with Jet A as the fuel phase.

Acidic and basic extractions: 200 ml quantities of COED-5 JP-5 were extracted with three 15 ml portions of 0.1 M HCl allowing 5 min shaking for each extraction. This was followed by three successive 15 ml extractions with 0.1 M NaOH and three washes with water. The procedure was repeated with the basic extraction preceding the acid extraction. A similar treatment was also carried out using nine successive extractions with distilled water. The interfacial materials from the acidic and basic extractions were combined and tested separately in systems with Jet-A as the fuel.

Silica gel fractionation

One-hundred ml of COED-5 were added to a column (5 cm diameter) of 800 g activated silica gel (Davison Chemical, Grade 923, 100-200 mesh) suspended in pentane. A bed volume (500 ml) of pentane was passed through the column during which time fraction #1 (475 ml) was collected. Then 100 ml of hexane followed by 500 ml hexane/benzene (3:1) and 800 ml methanol were passed through the column. During this time successive fractions of 400 ml (fraction #2) and 450 ml (#3) were collected.

Subsequently an oily, yellow fraction was collected (#4) followed by the methanol washout (#5). The volatile solvents were removed from the fractions by evaporation with gentle heat (40-45° C for #1,2 and 3 and up to 60°C for #4 and #5). The non-volatile liquid remaining in each fraction was: #1, 6.5 ml; #2, 68 ml; #3, 7.5 ml; #4, 15ml; #5, 15ml. Each fraction was divided in half and used to set up duplicate test units with enough FWKP or SWPY6 media and sterile Jet A to make each phase 50 ml. Fractions #1, #2, #3 and #4 were soluble in the fuel phase while #5 was soluble in the water phase.

Estimation of fungal growth

The test units inoculated with fungi or yeast were visually inspected for growth at appropriate time intervals. The rating system ranged from 0 for no growth and 1 for spore germination to 6 for mats thicker than 0.5 cm over the entire interface. A study of the dry weights 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 (5). 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 from the water/fuel interface on potato dextrose agar containing 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, the pH of the water phase of all test units was determined with a glass electrode.

RESULTS AND DISCUSSION

Inhibition by different coal fuels

The inhibition to fungal growth by a number of different coal-derived fuels has been studied to determine the generality of the phenomenon. Tables 2-A and 2-B show the results with COED-1 to 5 and two C. resinae strains. Jet-A petroleum-derived fuel was used as a non-inhibitory control in this and subsequent experiments.

COED-5 was completely inhibitory in most instances and after four months the organisms lost viability in two out of four cases. COED-2 was slightly less inhibitory overall but significantly delayed growth did occur after 15 weeks with C. resinae DK adapted and FWKP media. COED-3 and 4 appear to be least inhibitory especially with fresh water media. This may be associated with the more severe hydrotreatment given to these fuels. It should be pointed out, however, that these COED samples were over five years old and were kept at ambient temperatures for a portion of that time.

Table 3 shows that all of the solvent refined coal liquids were extremely inhibitory and lethal to C. resinae DK. This refining process is so different from the COED process that no claim can be made that the inhibitory mechanism is the same with the two kinds of fuels.

Thus all coal-derived fuels examined so far inhibit fungal

growth but there are differences which may depend on the refining process and coal source.

Stability of coal fuel inhibition

To determine whether the factor in coal fuel causing inhibition of fungal growth was stable, experiments were carried out with COED-5 fuel aged 15 months at room temperature and after autoclaving 45 min at 121°C. Table 4 indicates that neither of these treatments has any effect in alleviating the inhibition of growth of the test organisms.

Experiments with an n-alkane source of carbon added to coal fuel

Inasmuch as the n-alkane content of COED fuels is low, the possibility existed that there was an insufficient concentration of carbon substrate in the fuel to support the growth of fungi. To evaluate this, test units were set up with dodecane additions to COED-5 (Table 5). While growth was rapid and prolific with 100% dodecane, the addition of 20% COED-5 caused a cessation of growth for all organisms and a loss of viability in many cases. A real inhibition and not a lack of suitable carbon source appeared to exist.

This conclusion was further supported by the observation that a zone of inhibition surrounded a small well or pad containing COED-5 in a agar plate whose surface was seeded with fungal spores.

Table 6 shows that although very small amounts of Jet-A or dodecane were sufficient to support considerable growth of fungi and yeast, the same small amounts of COED-5 were completely inhibitory. Small amounts of toxic material in COED-5 were apparently sufficient to produce inhibition.

Mixtures of COED-5 and petroleum JP-5 (Jet-A)

Tables 7-A, B and C summarize results carried out with mixtures of COED-5 in Jet-A. Inhibition was generally nearly complete only at 80% COED-5 except for C. resinae in FWKP where significant growth occurred. The inhibition is obviously more marked with the least favorable aqueous media (SWPY8). It is surprising that Jet-A dilutes out the inhibition of the coal fuel so readily. The result is not in agreement with the experiments with dodecane/COED-5 mixtures where 20% COED-5 was completely inhibitory (Table 5).

Extractions

Because the growth of microorganisms depends on the presence of water, it appears clear that the inhibition to fungal growth by COED-5 fuel is due to some constituent which is able to dissolve in the water media to a small extent or is at least active against organisms growing near the interface of the fuel and water. Thus it appeared reasonable to expect that the inhibitory constituent could be removed and perhaps isolated by aqueous extractions.

COED-5 fuel which was shaken 6 hrs with an equal volume (150 ml) of FWKP or SWPY8 was still inhibitory (Table 8-A). Repetitive extraction of 150 ml COED-5 fuel with ten 500 ml portions of

FWKP or SWPY6 (diluted to 10% of normal concentration) also did not remove inhibition to C. resinae DK (Table 8-A). Extraction of 800 ml of COED-5 fuel twelve times with 100 ml portions of FWKP or SWPY8 diluted to 10% did not alleviate the inhibition in spite of the prolonged shaking time (1 hr or more) allowed for each extraction (Table 8-A). Tables 8-B and C show that the failure to remove COED-5 inhibition to C. resinae DK by extraction extends also to C. resinae DK/adapted and Candida sp. It is interesting but inexplicable that viability of all test organisms is lost in the extracted fuel but not in the COED-5 unextracted control.

In an effort to improve the extraction efficiencies of inhibitory constituents from COED-5, acidic (0.1 M HCl) followed by basic (0.1 M NaOH) extractions (and the inverse order) were made (Tables 9-A and B). Again no reduction in inhibition to the growth of the two C. resinae strains occurred nor were distilled water extractions effective. Collected interfacial material from the acid and base extractions added to Jet-A containing systems gave significant inhibition in FWKP for C. resinae but not in SWPY6. Thus some inhibitory constituent may be present in the interfacial material or there may be enough transfer of COED-5 fuel with the interfacial material to cause the observed inhibition with FWKP.

Although it was not possible to remove enough inhibitory constituent(s) from COED-5 fuel by aqueous extractions to allow growth of fungi, it was still reasonable to examine the aqueous extractant for the presence of inhibitory material in systems containing JET-A as the fuel phase. Table 10 summarizes these experiments. FWKP from the twelve-fold extraction of 800 ml of COED-5 fuel was not inhibitory even when concentrated ten-fold by evaporation. A similar experiment with SWPY6 did show complete inhibition of C. resinae DK for 4 months with the ten-fold concentrated extract. The inhibition was readily removed, however, by a three-fold dilution of the concentrate. The combined interfacial material from these extractions was also not inhibtory with either extractant.

Thus it appears that only with diluted SWPY solution was a significant amount of inhibitor removed from COED-5 by extraction, i.e., sufficient to produce some inhibition when the aqueous phase was tested with Jet-A, but not enough was removed to cause a significant reduction in the inhibition of the extracted COED-5 (Tables 8-A, B, C and 9-A, B).

## Silica Gel Adsorption

None of the fractions from the silica gel column experiment (Table 11) appeared to be markedly inhibitory, which would suggest that some adsorption of inhibitor on the gel is occurring. Fraction 4 may show a slight inhibition that might have been much stronger if it had not been diluted so much by Jet-A in order to carry out the experiments. The experiment should be repeated and refined so that fractions are better defined and the test unit size adjusted so that no dilution of fractions is necessary.

## CONCLUSIONS

1. Coal-derived fuels produced by the COED process and by solvent refining were generally inhibitory to growth of the fungus, Cladosporium resinae, and the yeast, Candida sp., although differences appear in COED-5 fuels which may be related to the different refining treatments.

2. Inhibition of fungal growth by COED-5 is due to constituents in the fuel and not to lack of a suitable n-alkane substrate.

3. Inhibitory constituents from COED-5 may adsorb on silica gel, however, considerable additional work is needed to establish the conditions for adsorption and elution in concentrated form. Other adsorbents may be worth investigating.

4. It is axiomatic that the inhibiting constituents in coal fuels must be at least slightly soluble in the aqueous phase to be effective against the microorganisms growing there. However, aqueous extractions were not highly effective in removing inhibition from the fuels. Only with a seawater extractant was any measurable amount of inhibitor removed. Perhaps aqueous extractants containing other solutes would be worth investigating.

5. Additional work to identify the source of fungal inhibition in coal fuels appears worthwhile not only because of the inherent interest in an inhibitor with pronounced selective toxicity at low concentrations for a relatively small group of microorganisms but also because a new fuel-compatible biocide may be made known which could be used to control microbial contamination in any hydrocarbon fuel.

6. In systems containing seawater as the aqueous phase, prolific growth of fungi or yeast tended to lower the pH to a much greater extent than in fresh water systems. This interesting difference should be further investigated in view of its significance for seawater-compensated fuel storage tanks on ships (7).

## ACKNOWLEDGMENTS

This work was supported by the Naval Air Propulsion Center. The authors thank Carole A Komenda for typing tedious tables and Forrest Schaekel and S. J. Lestz for supplying fuel samples and descriptive data.

Table 1. Properties of Solvent Refined Coal Fuels
(U. S. Army Fuels and Lubricants Research Laboratory)

| | AL-11222-F EDS Middle Distillate Coal Derived Donor Solvent* | AL-11189-SP-F H-Coal Naphtha** | AL-9490-SP-F SRC II Naphtha PETC #1785*** | AL-9492-SP-F SRC II Mid. Distillate PETC #1786*** | AL-9491-SP-F SRC II Heavy Distillate PETC #1787*** |
|---|---|---|---|---|---|
| Distillation, °C | | | | | |
| IBP | 202 | 60 | 53 | 186 | 324 |
| 10% | 219 | 96 | 83 | 204 | 334 |
| 50% | 260 | 157 | 110 | 238 | 365 |
| 90% | 357 | 203 | 143 | 273 | + |
| EP | 368 | 235 | 169 | 301 | + |
| Elemental Analysis | | | | | |
| Carbon, wt% | 88.46 | 85.43 | 85.0 | 85.35 | 89.72 |
| Hydrogen, wt% | 10.26 | 12.12 | 13.18 | 9.11 | 7.0 |
| Nitrogen, wt% | 0.12 | 0.28 | 0.40 | 0.94 | 1.23 |
| Oxygen, wt% | 1.2 | 1.83 | 2.14 | 4.82 | 1.8 |
| Sulfur, wt% | 0.090 | 0.22 | 0.30 | 0.30 | 0.61 |
| Existent Gum, mg/100 ml | 478.2 | 146.5 | 9.3 | 17.9 | 20.51 |
| Gravity, Specific | 0.956 | 0.833 | 0.775 | 0.932 | 1.102 |
| Pour Point, °C | -- | -- | -70 | -50 | -1 |
| Stability, Oxidation Accelerated, mg/100 ml | 4.73 | 0.400 | -- | -- | -- |
| Viscosity, @ 40°C | -- | -- | 0.60 | 2.44 | 155.4 |

* Received 12/02/81 from Exxon.
** Received 11/09/81 from Ashland MERADCOM.
*** Received 05/26/80 from DOE, Pittsburgh Energy Technology Center.
-- Not Determined.
\+ Temperature values at 90% and above exceeded the maximum thermometer range.

TABLE 2-A. Growth of Cladosporium resinae DK in two-phase test units using d. rent COED-5 JP-5 fuels and fresh water (FWKP) or seawater (SWPY6) media

| Water phase | Fuel | Ratings of growth after incubation (weeks) 1 | 2 | 3 | 4 | 5 | 7 | 9 | 10 | 14 | 15 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A | 1 | 2 | 2 | 2 | 3 | 4 | 5 | 5 | 5 | 6 | 6 | + | 5.53 |
| | COED-1 | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2[a] | + | 6.34 |
| | COED-2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1[a] | + | 4.31 |
| | COED-3 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | + | 4.41 |
| | COED-4 | 1 | 2 | 3 | 3 | 3 | 3 | 4½ | 5 | 6 | 6 | 6 | + | 4.02 |
| | COED-5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.42 |
| SWPY6 | Jet A | 1 | 2 | 2 | 2 | 2 | 2 | 2½ | 2½ | 3½ | 4 | 4½ | + | 3.70 |
| | COED-1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2[b] | + | 6.71 |
| | COED-2 | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2[a] | + | 6.68 |
| | COED-3 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2[a,b] | + | 6.98 |
| | COED-4 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2[a] | + | 7.16 |
| | COED-5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1[a] | + | 6.36 |

[a] No growth at interface one or two balls of atypical hyphae in water phase.
[b] One small atypical colony at interface.



Table 2-B. Growth of Cladosporium resinae DK/adapted in two-phase tests units using different COED-5 JP-5 fuels and fresh water (FWJKP) or seawater (SWPY6) media.

| Water phase | Fuel | Ratings of growth after incubation (weeks) 1 | 2 | 3 | 4 | 5 | 7 | 9 | 10 | 14 | 15 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A | 1 | 3 | 4 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | + | 6.76 |
| | COED-1 | 0 | 0 | 0 | 1 | 2 | 2 | 2½ | 2½ | 3 | 3 | 3 | + | 4.10 |
| | COED-2 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 4 | 4 | + | 4.47 |
| | COED-3 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 4 | + | 4.04 |
| | COED-4 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 4 | 5 | + | 4.32 |
| | COED-5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.40 |
| SWPY6 | Jet A | 2 | 2½ | 3 | 3 | 4½ | 5 | 5 | 5 | 5 | 5 | 5 | + | 2.97 |
| | COED-1 | 1 | 2 | 2 | 2 | 2 | 2 | 2½ | 2½ | 2½ | 2½ | 2½ | + | 8.12 |
| | COED-2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.74 |
| | COED-3 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2[a] | + | 7.04 |
| | COED-4 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2½ | + | 5.82 |
| | COED-5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.93 |

[a] One small atypical colony at interface.

Table 3. Growth and viability of Cladospor[illegible] resinae DK after three months in two-phase test units using different coal-derived, solvent-refined fuels and fresh water medium (FWKP).

| Fuel | Growth | Viability |
|---|---|---|
| Jet A control - no inoculum | 0 | - |
| Jet A control with inoculum | 6 | + |
| EDS Middle Distillate | 0 | - |
| H-Coal, Naphtha | 0 | - |
| SRC II, Naphtha | 0 | - |
| SCR II, Middle Distillate | 0 | - |
| SCR II, Heavy Distillate[a] | 0 | - |

[a] Density greater than water. Test units consisted of fuel on bottom and FWKP on top.

Table 4. Studies on growth and viability of fungi in aged and heat treated COED-5 JP-5/seawater (SWPY6) and freshwater (FWKP) systems after three months incubation.

| Fuel | C. resinae DK | | | | C. resinae DK/adapted | | | | Candida sp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SWPY 6 | | FWKP | | SWPY6 | | FWKP | | SWPY6 | | FWKP | |
| | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable |
| Jet A | 4½ | + | 6 | + | 5 | + | 6 | + | 5 | + | 3 | + |
| COED-5 JP-5: | | | | | | | | | | | | |
| Aged 15 months room temperature | 0 | - | 0 | + | 0 | - | 0 | + | 0/1 | - | 0/1 | - |
| Water extracted, aged 15 months | 0 | - | 1 | + | 0 | - | 0/1 | + | 1 | - | 0 | - |
| Autoclaved: 45 min. at 121°C | 0 | - | 0 | - | 0 | - | 0 | - | 1 | - | 1 | - |

Table 5. Growth and viability of two Cladosporium resinae strains and Candida sp. in two-phase test units (50-ml flasks) with seawater (SWPY6) and fresh water (FWKP) media and COED-5/dodecane mixtures after three months incubation.

| Fuel Mixtures (ml/ml) | C. resinae DK | | | | C. resinae DK/adapted | | | | Candida sp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SWPY6 | | FWKP | | SWPY6 | | FWKP | | SWPY6 | | FWKP | |
| | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable |
| COED-5 JP-5 Dodecane | | | | | | | | | | | | |
| 10/0 | 0 | - | 0 | - | 0 | - | 0 | - | 1 | - | 0 | - |
| 9/1 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| 8/2 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| 7/3 | 0 | - | 0 | - | 0 | - | 0 | + | 0 | - | 0 | - |
| 5/5 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| 2/8 | 0 | - | 0[a] | + | 0 | - | 0 | + | 0 | - | 0 | - |
| 0/10 | 5 | + | 6 | + | 4 | + | 6 | + | 5 | + | 3½ | + |
| Petroleum Jet-A/Dodecane | | | | | | | | | | | | |
| 0/0.06 | 3 | + | 4 | + | 2 | + | 4 | + | 5 | + | 4 | + |
| 0/0.2 | 4½ | + | 5 | + | 4/3 | + | 5 | + | 5 | + | 4 | + |
| 0.03/0.03 | 3 | + | 3½ | + | 2½ | + | 3½ | + | 4½ | + | 4 | + |
| 0.06/0 | 2½ | + | 2½ | + | 2½ | + | 3 | + | 4 | + | 4 | + |
| 0.1/0.1 | 3½ | + | 4½ | | 3½ | + | 4½ | + | 5 | + | 4 | + |
| 0.2/0 | 3 | + | 3½ | + | 3 | + | 4 | + | 4½ | + | 4 | + |

[a] Growth (rating of 2) occurred in these flasks after 6 months.

Table 6. Growth and viability of two strains of Cladosporium resinae and Candida sp. in two-phase test units (50-ml flasks) with varying volumes of COED-5 fuel and seawater (SWPY6) or freshwater (FWKP) media.

| Fuel Mixtures (mL) | C. resinae DK | | | | C. resinae DK/adapted | | | | Candida sp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SWPY6 | | FWKP | | SWPY6 | | FWKP | | SWPY6 | | FWKP | |
| | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable | Growth | Viable |
| Petroleum Jet A | | | | | | | | | | | | |
| 10.0 | 4½ | + | 6 | + | 5 | + | 6 | + | 5 | + | 3 | + |
| COED-5 JP-5 | | | | | | | | | | | | |
| 0.06 | 1/2 | + | 0 | - | 1/0 | + | 2 | + | 0 | N/D | 0 | N/D |
| 0.2 | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D |
| 0.4 | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D | 0 | N/D |
| 1.0 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| 2.0 | 0 | - | 0 | - | 0 | - | 0 | - | 1[a] | - | 1[a]/0 | - |
| 6.0 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 1[a] | - |
| 10.0 | 0 | - | 0 | - | 0 | - | 0 | - | 1[a] | - | 1[a]/0 | - |

[a] One small ball of hyphae.

Table 7-A. Growth of *Cladosporium resinae* DK in two-phase test units using different fresh water and seawater media and different concentrations of COED-5 JP-5 in Jet A.

| Water phase | % COED-5 in Jet-A | Ratings of growth after incubation (weeks) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 11 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FWBH | 0 | 2 | 3 | 3 | 3 | 4 | 4½ | 4½ | 5 | 5 | 6 | + | 4.34/4.35 |
| | 10 | 2 | 3 | 3 | 3/4 | 4/5 | 5 | 5 | 5 | 5 | 6 | + | 4.41/4.50 |
| | 20 | 1½ | 2½ | 3 | 3 | 4 | 4 | 4½ | 5 | 5 | 5 | + | 4.39/4.33 |
| | 40 | 1 | 2 | 2½ | 2½ | 3 | 3 | 3 | 4 | 5 | 5 | + | 4.44/4.39 |
| | 60 | 0 | 1 | 2 | 2 | 2½ | 2½ | 2½ | 3 | 4 | 5 | + | 4.44/4.34 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | + | 5.98/6.17 |
| FWKP | 0 | 2 | 3/4 | 3½/4 | 4½ | 5 | 5 | 6/5 | 6/5 | 6/5 | 6/5 | + | 4.79/3.41 |
| | 10 | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | 6 | 6 | + | 4.71/4.82 |
| | 20 | 2 | 2½ | 3 | 4 | 4½ | 5 | 5 | 6 | 6 | 6 | + | 4.84/4.75 |
| | 40 | 1 | 2 | 2½ | 3 | 3½ | 4 | 5/4 | 5 | 6 | 6 | + | 4.72/4.74 |
| | 60 | 1 | 1 | 2 | 2 | 2½ | 2½/3 | 2½/3 | 4½ | 5 | 6 | + | 4.60/4.68 |
| | 80 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2½ | 2½ | 4 | + | 4.26/4.30 |
| SWPY6 | 0 | 2 | 4 | 4 | 4½ | 5 | 5 | 5 | 5 | 5 | 5 | + | 3.41/3.43 |
| | 10 | 2 | 3/2 | 3/2½ | 4/2½ | 4½/3 | 4½/3 | 5/3 | 5/3½ | 5/4 | 5/4 | + | 3/49/3.63 |
| | 20 | 1 | 2 | 2½ | 3 | 4 | 4 | 4½ | 4½ | 5 | 5 | | 3.51 |
| | 40 | 1 | 2 | 2 | 2½ | 3 | 3 | 3 | 4½ | 4½ | 5 | + | 3.62/3.59 |
| | 60 | 1 | 1 | 1 | 2 | 2 | 2 | 2½ | 3 | 3 | 4 | + | 3.80/3.82 |
| | 80 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | + | 6.67/7.05 |
| SWPY8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.08/8.09 |
| | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.06/8.07 |
| | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.07/8.07 |
| | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.07/8.07 |
| | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.07/8.05 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.06/8.06 |

Table 7-B. Growth of *Cladosporium resinae* DK in two-phase test units using different fresh water and seawater media and different concentrations of COED-5 JP-5 in Jet A.

| Water phase | % COED-5 Jet-A | Ratings of growth after incubation (weeks) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 11 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FWBH | 0 | 1 | 1 | 2 | 3 | 3½ | 4 | 4½ | 5 | 5 | 5 | + | 4.35/4.42 |
| | 10 | 1 | 1 | 2 | 2 | 3/3½ | 3½ | 4/4½ | 5 | 5 | 5 | + | 4.54/4.45 |
| | 20 | 1 | 1 | 2 | 2 | 2½/3 | 3/3½ | 3/4 | 3½/5 | 4/5 | 4½/5 | + | 4.48/4.47 |
| | 40 | 1 | 1 | 1 | 2 | 2½ | 3 | 4 | 5/4½ | 5 | 5 | + | 4.43/4.50 |
| | 60 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 4 | 5 | + | 4.44/4.35 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.38/6.32 |
| FWKP | 0 | 1 | 2 | 4½ | 5 | 5 | 5 | 6 | 6 | 6 | 6 | + | 4.40/4.45 |
| | 10 | 1 | 2 | 3 | 4½ | 5 | 5 | 5 | 6 | 6 | 6 | + | 4.52/4.49 |
| | 20 | 1 | 1 | 3 | 4 | 5 | 5 | 5 | 6 | 6 | 6 | + | 4.56/4.53 |
| | 40 | 1 | 1 | 2 | 3½ | 4½ | 5 | 5 | 5 | 6 | 6 | + | 4.54/4.50 |
| | 60 | 1 | 1 | 1 | 2 | 2 | 4 | 4½ | 5 | 5 | 5 | + | 4.54/4.53 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | + | 4.28/4.25 |
| SWPY6 | 0 | 2 | 2 | 2 | 3 | 3½ | 4 | 4½ | 5 | 5 | 5 | + | 3.17/3.33 |
| | 10 | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4½ | 5 | 5 | + | 3.48/3.46 |
| | 20 | 1 | 1 | 1 | 2 | 3 | 3 | 3½ | 4½ | 5 | 5 | + | 3.57/3.43 |
| | 40 | 1 | 1 | 1 | 2 | 2 | 2½ | 3 | 4 | 4½ | 5 | + | 3.71/3.73 |
| | 60 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2½ | 3 | 4½ | + | 4.06/4.03 |
| | 80 | 0 | 0 | 0 | 0 | 1/0 | 1/0 | 1/0 | 1/0 | 1/0 | 1/0 | + | 6.45/6.77 |
| SWPY8 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2½ | 3 | 5 | + | 3.52/3.94 |
| | 10 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 3/2½ | + | 4.96/7.37 |
| | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | + | 8.01/7.81 |
| | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 8.06/8.05 |
| | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | +/- | 8.04/8.08 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.05/8.05 |

Table 7-C. Growth of Candida sp/ in two-phase units using different fresh water and seawater media and different concentrations of COED-5 JP-5 Jet A.

| Water phase | % COED-5 Jet-A | Ratings of growth after incubation (weeks) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 11 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | 0 | 2 | 2 | 3 | 3 | 3 | 3 | 3½ | 4 | 4 | 4 | + | 5.59/5.52 |
| | 10 | 1 | 2 | 2½ | 3 | 3 | 3 | 3 | 3½ | 4 | 4 | + | 5.74/5.67 |
| | 20 | 1 | 2 | 2½ | 3 | 3 | 3 | 3/3½ | 3½/4 | 4 | 4 | + | 5.61/4.21 |
| | 40 | 0 | 1 | 2 | 2 | 3/2½ | 3 | 3 | 4 | 4½/4 | 5 | + | 5.75/5.53 |
| | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2/1 | 4½/2 | 5 | + | 4.87/5.84 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.35/6.36 |
| FWKP | 0 | 2 | 2 | 2½ | 3 | 4 | 4 | 4½ | 4½ | 5 | 5 | + | 3.16/3.11 |
| | 10 | 1 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4½ | 4½ | + | 3.28/3.06 |
| | 20 | 1 | 2 | 2½ | 3 | 3 | 4 | 4 | 4 | 4½ | 4½ | + | 3.18/3.33 |
| | 40 | 0 | 1 | 1 | 2 | 2½ | 3½ | 4 | 4½ | 5 | 5 | + | 3.22/3.37 |
| | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 4½ | + | 3.45/3.47 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.45/4.39 |
| SWPY6 | 0 | 2 | 3 | 3 | 4 | 4 | 4 | 4½/4 | 4½ | 4½ | 4½ | + | 3.53/3.53 |
| | 10 | 2 | 3 | 4 | 4 | 4 | 4½ | 4½ | 4½ | 5 | 5 | + | 3.55/3.56 |
| | 20 | 2 | 2 | 3 | 3½ | 4 | 4½ | 4½ | 4½ | 5 | 5 | + | 3.59/3.56 |
| | 40 | 2 | 2 | 2 | 2 | 2 | 2½ | 3 | 3½ | 4 | 4½ | + | 3.56/3.55 |
| | 60 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | + | 7.03/7.85 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.05/6.23 |
| SWPY8 | 0 | 2 | 3 | 4 | 4 | 4½ | 4½ | 4½ | 5 | 5 | 5 | + | 3.82/3.87 |
| | 10 | 2 | 2 | 2 | 3 | 4 | 4½ | 4½ | 4½ | 4½/5 | 5 | + | 3.82/3.86 |
| | 20 | 0 | 1 | 2 | 2 | 2 | 2 | 2½ | 3 | 3 | 4½ | + | 4.02/3.97 |
| | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 8.07/8.12 |
| | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -/+ | 8.09/8.09 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 8.05/8.08 |

Table 8-A. Growth of Cladosporium resinae DK in two phase systems with fresh water (FWKP) or seawater (SWPY6) media and COED-5 extracted with FWKP and SWPY 6 solutions.

| Water phase | Fuel Phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 6 | 9 | 13 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A (control) | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | + | 5.37/5.39 |
| FWKP | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.52/4.40 |
| FWKP | COED-5 extracted once with FWKP (150:150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.27 |
| FWKP | COED-5 extracted 10x with diluted FWKP (500:150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.42 |
| FWKP | COED-5 extracted 12x with diluted FWKP (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.16/4.17 |
| SWPY6 | Jet A (control) | 2 | 3 | 3 | 3 | 4 | 4½ | 5 | 5 | + | 3.22/3.25 |
| SWPY6 | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0/1 | -/+[a] | 5.67/6.19 |
| SWPY6 | COED-5 extracted once with SWPY6 (150:150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.80 |
| SWPY6 | COED-5 extracted 10x with diluted SWPY6 (150:500) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | ±[a] | 6.46 |
| SWPY6 | COED-5 extracted 12x with diluted SWPY6 (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.45/5.41 |

[a] Small, hard sclerotia-like balls on bottom of flasks constitute only viable forms present.

Table 8-B. Growth of *Cladosporium resinae* DK/Adapted in two-phase systems with fresh water (FWKP) or seawater (SWPY6 media and COED-5 extracted with FWKP and SWPY6 solutions.

| Water phase | Fuel Phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 6 | 9 | 13 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | JET A (control) | 2 | 3 | 4 | 5 | 5 | 5 | 6 | 6 | + | 4.60/4.74 |
| FWKP | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -/+ | 4.48/4.39 |
| FWKP | COED-5 extracted 12x with diluted FWKP (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.30/4.21 |
| SWPY6 | Jet A (control) | 2 | 2 | 2½/3 | 3 | 4/3½ | 4½ | 5 | 5 | + | 3.03/2.99 |
| SWPY6 | COED-5 (control) | 0 | 0 | 0 | 0 | 0/1 | 0/1 | 0/1 | 0/1 | -/+[a] | 5.80/6.57 |
| SWPY6 | COED-5 extracted 12x with diluted SWPY6 (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.54/5.52 |

[a] Small, hard sclerotia-like balls on bottom of flasks constitute only viable forms present.



Table 8-C. Growth of *Candida* sp. in two-phase systems with fresh water (FWKP) or seawater (SWPY6) media and COED-5 extracted with diluted FWKP and SWPY6.

| Water phase | Fuel Phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 6 | 9 | 13 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A (control) | —— contaminated —— | | | | | | | | | |
| FWKP | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.39/4.54 |
| FWKP | COED-5 extracted 12x with diluted FWKP (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.17/4.19 |
| SWPY6 | Jet A (control) | 2 | 3 | 3 | 3½ | 4 | 4½ | 5 | 5 | + | 3.30/3.33 |
| SWPY6 | COED-5 (control) | 1/0 | 1/0 | 1/0 | 1/0 | 1 | 1 | 2 | 2 | +[a] | 7.47/7/51 |
| SWPY6 | COED-5 extracted 12x with diluted FWKP (100:800) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.56/5.54 |

[a] Small balls of mycelium on bottom of flask constitute only viable form present.

Table 9-A. Growth of Cladosporium resinae DK in two-phase systems with COED-5 fuel extracted with acidic and basic solutions.

| Water phase | Fuel Phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 7 | 9 | 14 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A (control) | 1 | 2 | 2 | 2 | 4 | 5 | 5 | 6 | + | 5.53 |
| FWKP | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.42 |
| FWKP | COED-5 extracted with acid-base | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.29 |
| FWKP | COED-5 extracted with base-acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | 4.29 |
| FWKP | COED-5 extracted with water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.17 |
| FWKP | Jet A + Interfacial material from COED-5 extractions | 0 | 0 | 1 | 1 | 2 | 3 | 4½ | 4½ | + | 5/27 |
| SWPY6 | Jet A (control) | 1 | 2 | 2 | 2 | 2 | 2½ | 3½ | 4½ | + | 3.68 |
| SWPY6 | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | ±[a] | 6.36 |
| SWPY6 | COED-5 extracted with acid-base | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | ±[a] | 6.22 |
| SWPY6 | COED-5 extracted with base-acide | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | ±[a] | 6.34 |
| SWPY6 | COED-5 extracted with water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.14 |
| SWPY6 | Jet A + interfacial material from COED-5 extractions | 1 | 2 | 2 | 2 | 2 | 2½ | 3½ | 4½ | + | 3.71 |

[a] Small balls of hyphae on bottom of flasks constitutes only growth form.

Table 9-B. Growth of Cladosporium resinae DF adapted in two-phase systems with COED-5 fuel extracted with acidic and basic solutions.

| Water phase | Fuel Phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 7 | 9 | 13 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FWKP | Jet A (control) | 1 | 3 | 4 | 5 | 5 | 6 | 6 | 6 | ± | 4.76 |
| FWKP | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.40 |
| FWKP | COED-5 extracted with acid-base | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.30 |
| FWKP | COED-5 extracted with base-acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 4.42 |
| FWKP | COED-5 extracted with water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 4.23 |
| SWPY6 | Jet A (control) | 2 | 2½ | 3 | 3 | 5 | 5 | 5 | 5 | + | 2.97 |
| SWPH6 | COED-5 (control) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.93 |
| SWPY6 | COED-5 extracted with acid-base | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.02 |
| SWPY6 | COED-5 extracted with base-acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 6.07 |
| SWPY6 | COED-5 extracted with water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 5.19 |

Table 10. Growth of *Cladosporium resinae* DK in two-phase systems with Jet A fuel and fresh water (FWKP) or seawater (SWPY6) media used for extraction of COED-5.

| Water phase | Growth ratings after incubation (weeks) 1 | 2 | 3 | 4 | 7 | 9 | 13 | Terminal readings (4 months) Growth | Viability | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| FWKP (control) | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | + | 5.38 |
| FWKP from one 6-hr extraction of COED-5 | 2 | 3 | 3 | 4 | 5 | 6 | 6 | 6 | + | 5.47 |
| FWKP from 12x extraction of COED-5 concentrated: | | | | | | | | | | |
| 0-fold | 3 | 4½ | 5 | 5 | 5 | 5 | 6 | 6 | + | 5.37 |
| 3-fold | 2 | 3 | 5 | 5 | 5 | 5 | 6 | 6 | + | 5.34 |
| 10-fold | 2 | 3 | 4 | 4½ | 5 | 5 | 5 | 6 | + | 5.29 |
| FWKP + Interfacial material from 12x extraction of COED-5 | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | + | 5.43 |
| SWPY6 (control) | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | + | 3.23 |
| SWPY6 from one 6-hr extraction of COED-5 | 1 | 2 | 2 | 2 | 2 | 3 | 5 | 5 | + | 3.84 |
| 1 SWPY6 from 12x extraction of COED-5 concentrated COED-5 concentrated: | | | | | | | | | | |
| 0-fold | 2 | 2 | 2½ | 2½ | 4 | 4½ | 5 | 5 | + | 3.41 |
| 3-fold | 1 | 2 | 2 | 2 | 3 | 4½ | 5 | 5 | + | 3.47 |
| 10-fold | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 7.54 |
| SWPY6 + interfacial material from 12x COED-5 extraction | 2 | 3 | 3 | 3 | 4 | 4½ | 5 | 5 | + | 3.34 |

Table 11. Growth of Cladosporium resinae DK in two-phase systems with FW aqueous medium and fractions of COED-5 from a silica gel column.

| COED-5 Fraction | Growth ratings after incubation (weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 2 | 3 | 4 | 4½ | 5 | 5 | 5 |
| 2 | 2 | 2 | 2 | 2½ | 3 | 4 | 5 |
| 3 | 2 | 3 | 4 | 4½ | 5 | 5 | 6 |
| 4 | 1 | 2 | 2½ | 3 | 4 | 4½ | 4½ |
| 5 | 1 | 1½ | 2½ | 3 | 5 | 5 | 5 |
| Jet A fuel (control) | 2 | 3 | 4 | 4½ | 5 | 5 | 6/5 |

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