MICROBIOLOGICAL ACTIVITIES IN PETROLEUM AND PETROLEUM PRODUCTS

(An Annotated Bibliography)

Compiled by
Richard W. H. Lee

July 22, 1963

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Best Available Copy
The biological degradation of individual hydrocarbons found in motor fuels, oils, greases, and in corrosion-preventive and other additive compositions was studied using various species of molds and bacteria that are capable of oxidizing petroleum products. In general, the observed effects of microorganism attack on aircraft engine fuels and lubricants were not of sufficient magnitude to adversely affect their service performance.

For aliphatic hydrocarbons, growth increased as the length of the carbon chain increased. Branched-chain (iso) compounds were more susceptible to attack than were straight-chain compounds. Because the branched chain constituents of gasoline have the highest anti-knock properties, growing bacteria might decrease the octane rating. The growth produced, however, was not appreciable and did not seem to require preventive measures. Unsaturated cyclic compounds did not support growth as well as did saturated cyclic compounds.

Changes in pH caused by mold growth were generally not appreciable, but several samples showed increases or decreases in pH of one unit or more when tested with a specific fungus. These changes were more pronounced under aerobic than under anaerobic conditions. Other noticeable effects of the presence of microorganisms in oil were decrease in densities, discoloring of unsaturated hydrocarbons produced during the oxidative process, and, especially in heavier oils, formation of gummy residues.

There was a definite correlation between the results of individual mold tests carried out under anaerobic conditions in flasks with mineral salts solution and those obtained with the standard tropical aerobic testing technique. Samples that grew mixed molds in the tropical testing chambers also supported growth of individual molds. The growth produced in the flasks, however, was much more pronounced. This indicates that for most organisms the presence of other elements is required before the carbon of the hydrocarbons can be utilized.

Great Britain Department of Scientific and Industrial Research.
1953. 124 p. --

[Petroleum, storage, bacteria, sulfur, Desulfovibrio, growth, culture, corrosion, metals]
Under all practical conditions, inhibited, leaded high-octane aviation gasolines can be stored for several years in steel or concrete containers in the presence of fresh or salt water without effect on gasoline performance. Under severe storage conditions, especially under continuous or prolonged agitation, however, gasolines containing aminophenol type inhibitors show decreases in octane number and lead content and increases in gum formation. The rate of gasoline alteration in the presence of water increases with the intensity of agitation of the two liquid phases and with the ratio of interphase contact area to total gasoline volume.

Plastic or paint coatings containing organic polysulfides, such as thiokol, may release sulfur which renders the gasoline corrosive and lowers its octane number. They are not, therefore, recommended as gasoline tank liners. Certain acid-reacting coatings may change the color of blue gasoline dyes to green. Concrete, even when protected by organic coatings, is frequently attacked by sea water and may form white deposits. Gasoline stored in concrete tanks in the presence of sea water contained an increased amount of residual salts after evaporation.

These experiments were carried out on a small laboratory scale, an intermediate scale, and on a large service scale. 

Knosel, D., and W. Schwartz.
UNTERSUCHUNGEN ZUR ERDOLBAKTERIOLOGIE. II. UBER VORKOMMEN UND VERHALTEN VON MIKROORGANISMEN IN ERDOL [INVESTIGATIONS IN PETROLEUM BACTARIOLOGY. II. OCCURRENCE OF MICROORGANISMS IN PETROLEUM]. Arch. Mikrobiol. 20:362-390 (1954)

[F: Petroleum, bacteria, Desulfovibrio, Mycobacterium, Pseudomonas, Actinomyces, Bacterium, Micrococcus, Bacillus, biomechanism, growth, tests, foreign]

UNTERSUCHUNGEN ZUR ERDOLBAKTERIOLOGIE. III. UBER DAS VERHALTEN VON MIKROORGANISMEN IN ERDOLPRODUKTEN [INVESTIGATIONS IN PETROLEUM BACTARIOLOGY. III. OCCURRENCE OF MICROORGANISMS IN PETROLEUM PRODUCTS]. Z. Hyg. Infectianskrankheiten 140(1):100-126 (1954)

[F: Petroleum, bacteria, Desulfovibrio, Mycobacterium, Pseudomonas, Actinomyces, Bacterium, Micrococcus, Bacillus, biomechanism, growth, tests, foreign]
The multiple-layer bitumen insulation of steel tank interiors in a slime-removal and water-purification plant failed severely after only 2 yr of service. Starting with small holes and blisters, the bituminous coatings developed a porous, spongy structure, followed by complete flaking of large areas and greatly weakened adhesion of the remaining coating. Pores and blisters occurred particularly at the transition zone of the steel walls and the concrete foundation.

This severe damage was not caused by substandard material or by faulty application techniques. A few similar failures of bituminous coatings have been reported from other locations and in the literature. All instances indicated that anaerobic bacteria capable of metabolizing hydrocarbons were the only possible explanation. The probability of bacterial attack is highly increased under the operating conditions prevailing in the water-purification plant, viz., presence of numerous slime-forming microorganisms, including hydrocarbon utilizers, the stagnation of liquid flow in certain areas, which coincided with the areas of greatest damage, and operating temperatures favorable to the growth of most microorganisms, viz., 35-40°C. Where similar conditions prevail, damage to bitumens is to be expected. Thus, oxidative degradation of asphalt roads has been reported near moist, grassy ditches favoring the development of soil bacteria and other microorganisms.

Bacterial attack on hydrocarbons has been shown to proceed preferentially from paraffinic to aromatic to cycloparaffinic compounds. Few data exist, however, on microorganism attack on crude petroleum, particularly on the heavy fractions. Observations were made, therefore, on a Kuwait crude, cut to 272°C and deposited in a 2-cm layer over mud taken from the center and edges of a lake. The mud-water-oil samples were enclosed in 22-mm tubes and observed for periods of 116 (bottom mud) and 125 (edge mud) days.

At the end of 30 days, the edge mud showed few bacteria, most of which were actinomycetes, whereas the
bottom mud, though also poor in population, contained several different types of bacteria. The composition of the crude showed an increase in resins, no change in the asphaltene content, and a decrease in oily constituents from 69.9% to 52.8% with edge mud and to 47.8% with bottom mud. After 60 days, the oily constituents increased at the expense of the resinous and other oxidation products, including asphaltenes, which decreased from 6 to 0.8 and 0.7% in the edge and bottom muds, respectively. This pattern continued after 90 days, when oily constituents had increased to 82.1 and 80.8%, respectively; asphaltenes were also produced during this stage. These changes in the crude were attributed to proliferation of actinomycetes in both media. After 4 mo, the bacterial population diminished, especially in the bottom mud.

In general, the chemical changes in the crude constituents were less appreciable in samples inoculated with bottom than with edge mud in which the cyclic compounds appeared to be attacked first. This change paralleled the increased resin phase described above. In the later stages of attack, the cyclic compounds in the oily fractions increased again.

Bonetti, Elio M.
Ricercne Microbiologiche Sui Terreni Petroliferi E Ossidazione Microbica degli Idrocarburi. (Nota 2) [Microbiological Research on Oilbearing Soils and Microbial Oxidation of Hydrocarbons. (Note 2)].
Ricerca Sci. 25:779-302 (March 1950)

Enrichment cultures were prepared with muds from natural gas and oil wells in the Po Valley and with soils which had come into contact with hydrocarbons and other soils. Forty-eight microorganisms were isolated; twenty of these were able to use phenanthrene and anthracene as their sole source of carbon. Additional tests were made with these twenty using a mineral medium of pH 6.9 to 7.1 to which was added octane, dodecane, hexadecane, phenanthrene, anthracene, or cholesterol. The medium consisted of 1 g ammonium nitrate, 1 g monobasic potassium phosphate, 1 g magnesium sulfate, and 0.2 g calcium carbonate per liter.

Two of the microbial forms were identified as Mycobacterium, two as Acromobacter, one as Nocardia, and two as Flavobacterium. The Mycobacteria utilized both the aliphatic and aromatic hydrocarbons, the others metabolized only the aromatic hydrocarbons. Their behavior on a liquid or agarized medium differed in some cases. For rapid metabolism of phenanthrene and anthracene, a neutral medium is optimal.
Fats such as cod-liver oil and olive oil and fat constituents such as tristearin and glycerol were inoculated with Penicillium glaucum and incubated at 25°C. No growth developed even after 5 mos. However, the addition of a small amount of calcium oleate to the oils and of an inorganic nutrient salt mixture to the fat constituents resulted in mold growth.

Certain oils contain substances which are definitely fungicidal. For example, sorbitol, a normal constituent of fruits, seeds, and oils, will, in dilutions of 1:1000, prevent the growth of Penicillium glaucum on the most favorable medium.

P.D.C. Comment: Contrary to the findings reported in this abstract, other investigators have observed that most fats are attacked by mold.

(1) R. H. Wellman and S. E. A. McCallan found that the addition of castor oil to the plasticizer dioctylphthalate increased its mold susceptibility, particularly in soil burial tests [Monthly O.S.R.D. report on deterioration of plastics by microbiological action. Nov. 1944. - 1,12,12, G-259(3)].

(2) The Electrical Equipment Panel stated that mineral-type oils and greases showed little tendency to grow mold only if free from fatty acids, esters, glycerides or soaps [Tropicproofing of electrical equipment with particular reference to prevention of mould growth in electronic equipment. Jan. 1945. - 1,17,16, WA-3918-3A].

(3) The Controller of Chemical Research and Development remarked that fatty oils are only used in such dilution in mineral oil as not to be prone to attack [Tropicproofing, 46 p. Feb. 1945. - 1,24,21, WA-1337-2A].

(4) Division 19, National Defense Research Committee reported that refined fatty oil from the head of blackfish is susceptible to mold growth [Mould growth; summary of data on materials and treatments. Mar. 1944. - 1,4,8,1, G-125].
Sulfate-reducing bacteria of the Desulfovibrio species catalyze the anaerobic oxidation of a variety of mixed hydrocarbons. This hydrocarbon utilization is apparently associated with a dehydrogenase system. Long-chain aliphatic hydrocarbons are rapidly destroyed by the sulfate reducers, forming transitory intermediates of insoluble fatty acids which, in turn, undergo further degradation.

Substrates of various crude oils and refinery products were emulsified in water with gum arabic, mixed with sterile solutions of mineral salts, inoculated with mixed cultures of sulfate-reducing anaerobes, and incubated in glass-stoppered bottles at 27 C. Hydrocarbon oxidation was accompanied by sulfate reduction which was used as an index of bacterial activity.

Sulfates were not reduced in the presence of gum arabic mixtures containing no hydrocarbon.

ZuBell, Claude E.
ACTION OF MICROORGANISMS ON HYDROCARBONS. (Scripps Institution of Oceanography, La Jolla, Calif. New Series No. 399).

Nearly a hundred species of bacteria, yeast, and molds have been shown to be capable of oxidizing gaseous, liquid, and solid hydrocarbons of the aliphatic, olefinic, aromatic, or naphthenic series. Hydrocarbon-oxidizing microorganisms are widely distributed in soil, water, and recent marine sediments. They are particularly abundant in oil-soaked soil and water over which petroleum products are stored. They grow in simple mineral media enriched with hydrocarbons as the sole source of energy. The presence of free oxygen is generally essential, although nitrate or sulfate serve as hydrogen acceptors for some hydrocarbon oxidizers.

Microbial multiplication, oxygen consumption, nitrate or sulfate reduction, modification of hydrocarbons, and the formation of various metabolic products have been used as criteria of the utilization of hydrocarbons. This activity has been observed at temperatures ranging from 0 to 60 C. Being relatively insoluble and immiscible in water, most hydrocarbons are rendered more susceptible to attack when dispersed throughout mineral media by adsorption on inert solids or by emulsification.

In general, aliphatic hydrocarbons are oxidized more readily than aromatic or naphthenic compounds. Within
certain limits, long-chain hydrocarbons are attacked more readily than similar compounds of small molecular weight. The addition of aliphatic sidechains increases the susceptibility of cyclic compounds to microbial attack. Carbon dioxide is the principal metabolic product; cell constituents, organic acids, alcohols, and unsaturated compounds are among the other substances which have been reported.

Although some crude oils are bacteriostatic, samples of others are destroyed or otherwise modified by microorganisms. Microbial activity is believed to have played an important role in determining the properties of petroleum, and bacteria which oxidize volatile hydrocarbons may provide clues to the location of subterranean deposits of petroleum.

Petroleum products stored in contact with water for prolonged periods may be discolored, emulsified, or otherwise altered by hydrocarbon-oxidizing microorganisms. Coolants and certain kinds of pharmaceuticals containing both hydrocarbons and moisture may be adversely affected. Natural and most synthetic rubbers are susceptible to this type of oxidation.

Microorganisms in soil oxidize methane and other hydrocarbons of biological origin. Applications of crude oil to soil result in increased microbial populations and, in many cases, improve fertility. Even the so-called antiseptic hydrocarbons, such as benzene, toluene, xylene, and naphthalene, are destroyed, and phenol, cresols, naphthols, chlorobenzenes, nitrobenzenes, benzoic acid, salicylic acid, and resorcinol in low concentrations are attacked. The microbial assimilation of phenol in concentrations as high as 0.3% has been demonstrated.
Marked bacteriostatic action may be displayed by all members of a chemical group. The great activity of the nitro compounds probably is not due to a simple oxidation reaction, since it is also found in the aminophenols. Other groups of pronounced general activity were dyes, inorganic compounds, phenol-like compounds, miscellaneous sulfur compounds, halogen carbonic acids, alkaloids, and aldehydes. Lethal activity was also found in carbonic acid anhydrides, aliphatic carbonic acids, ketones, and halogen compounds. Other groups tested were more or less inactive, though occasionally they contained individual compounds of marked activity, e.g., pinene among the hydrocarbons, marfanil among the amines, quinone monoxime among the nitrogen compounds, and picolinic acid in the otherwise inactive group of heterocyclic compounds. The carbohydrates and compounds containing the carbamyl radical demonstrated no bacteriostatic activity.

The aerobic strains were, in general, equally or slightly more resistant than the anaerobic strains. Marked selectivity between the two groups, however, was shown in the action of nitro compounds.

The results of titrations with twenty-five chemical substances on twenty-one aerobic and twenty-seven anaerobic strains were analogous to results obtained from plates in about 75% of the tests. They confirmed the pronounced selectivity of marfanil and of oxidizing substances, such as potassium chlorate, p-nitrobenzyl chloride, iodoform, iodosobenzene, and trinitrophonol, in growth inhibition. The highest titers were obtained with iodosobenzene and p-nitrobenzyl chloride. Selective differences in resistance between various anaerobic strains were not nearly as great as those between the aerobic strains.

Conclusions from the plate tests should be drawn only from definite and consistent bacteriostatic differences between the various bacterial strains, since the deviations between duplicate tests made with anaerobic species ranged from 11 - 1000% in 63 - 75.3% of the cases.

Clostridium tertium was the most resistant of the anaerobic species tested; it gave both the highest percentage of low inhibition and the lowest percentage of high inhibition values. C. perfringens was the most susceptible, with the lowest percentage of low and the highest percentage of medium inhibition values. The largest number of very high inhibition values was found for C. novyi and C. multifermentans.

The species used in the plate tests were C. perfringens, C. tertium, C. novyi, C. multifermentans, Corynbacterium diphtheriae, Staphylococcus aureus, Salmonella paratyphi B, and Streptococcus haemolyticus. A variety of culture media were prepared according to
Zeissler, glucose blood agar being poured into normal Petri dishes, the other media into tubes.

The total amount of inhibition was calculated from the formula \( A = \frac{w}{12(D^2 + Dd + d^2)} \), in which \( d \) is the diameter of the circle representing complete inhibition of growth, and \( D \) is the diameter of a larger concentric circle including partial inhibition of growth. This method is based on the density of inhibition, rather than on the width of the zone of inhibition.

Included in the report is a short resume of work in this field from 1887 to 1947.

PDC Comment: The Prevention of Deterioration Center maintains a card file on bactericides; data from the above report have been transferred to this file.

ENVIRONMENTAL pH AS A FACTOR IN CONTROL OF ANAEROBIC BACTERIAL CORROSION.
Corrosion 4:567-581 (December 1948)

In an investigation of the effect of pH on sulfate reducing bacteria, it was found that active growth could be arrested in media having a pH above 9 - 9.5. This seems to indicate that anaerobic corrosion of pipelines might be inhibited by keeping the alkalinity of the adjacent soil above this bacteriostatic threshold, using either electrical or chemical control. The effectiveness of such measures, however, requires further study.

Sulfate reducing bacteria were cultured from a soil in which anaerobic bacterial activity had been observed. Growth in one medium, containing 2 g magnesium sulfate heptahydrate and 0.1 g calcium chloride dihydrate, in addition to 1 g ammonium chloride, 0.44 g sodium sulfate, 0.5 g dipotassium hydrogen phosphate, 3.5 g sodium lactate, and a trace of Mohr's salt in 1 liter tap water, was far better than in a second, which consisted of 2 g ammonium sulfate, 5 g sodium lactate, 0.5 g dipotassium hydrogen phosphate, and a trace of Mohr's salt per liter of tap water.

The pH values of 7, 8, 9, 10, and 11 were obtained in separate flasks by adding 0.2 N potassium hydroxide to flourishing cultures. Growth at pH 8 - 9 was much greater than at pH 10 - 11. The bacteriostatic rather than bactericidal effect of the higher pH values was evident from the normal growth of the organisms when returned to neutral media after 2-, 7-, and 14-days incubation at the higher pH levels.

The darkening of lead acetate paper caused by the formation of metabolic hydrogen sulfide was taken as the criterion of growth, but the selection of this method was subsequently criticized independently by two other investigators. Another objection regarding these tests was that they are an investigation of the effect of pH on bacterial growth rather than on corrosion.
The stability of cracked gasoline and of pure hydrocarbons was investigated by measuring the rate of gum and peroxide formation on aging. Gasoline gum seems to originate from the oxidation of both reactive hydrocarbons and nonhydrocarbon impurities. Products forming gum were more difficult to inhibit against oxidation than those forming only peroxides.

The gum stability of cracked gasoline was improved by sulfuric acid or similar treatment, by inhibition with 15 - 60 lb antioxidant per 1000 bbl gasoline, or by dilution with straight-run components. The boiling range was important, heavier fractions of inhibited, thermally cracked gasoline being less stable than lighter fractions; this applied to both untreated and treated fractions. Treatment of the heavier fraction consequently improved the stability of the blend more than did treatment of the lighter fraction.

Metal catalysts reduced gum stability but could be completely inactivated by metal deactivators. The latter, which were generally specific in action for only a limited number of metals, had to be present in stoichiometric amounts.

Significantly lowered activation energies of gum-formation reactions were noted in the presence of catalysts or in the absence of antioxidants.

Although these results were obtained with West Coast thermally cracked and straight-run gasolines, they have been confirmed on similar stocks from other sources and may apply also, in part at least, to catalytically cracked fuel.

Most emulsifiable oils, used as coolants and lubricants in cutting, grinding, and forming operations, support bacterial growth when diluted to 1:5 or more. The type of preservative that can be added is determined by its compatibility with and its solubility in the emulsion.

Two oil-soluble germicidal preservatives, o-phenylphenol and 2,3,4,6-tetrachlorophenol, in amounts of 0.1% of the total weight of the emulsion, proved capable both of keeping hydraulic emulsions free from bacteria and of cleaning bacteria-clogged systems.
RAPID FORMATION OF HIGH CONCENTRATIONS OF HYDROGEN SULFIDE BY SULFATE-REDUCING BACTERIA

Over forty strains of sulfate-reducing bacteria (Desulfovibrio desulfuricans [Beij.] K. and v.N.) were grown in the laboratory to determine the highest concentration of hydrogen sulfide that can be produced by this organism. The strains were obtained from drill-core samples taken from depths of 700-900 ft in sulfur-bearing salt domes, from a marine environment, and from a variety of nonmarine sources. All strains, marine or nonmarine in origin, produced over 2000 mg hydrogen sulfide per liter when grown in suitable media and transferred at frequent intervals, preferably 3-5 days. The production of high concentrations of hydrogen sulfide is associated with rapid rates of formation.

The previously reported maxima of hydrogen sulfide formation, 1468 mg per liter for marine forms and 749 mg per liter for nonmarine forms, was increased to 2000-2500 mg per liter by an improved medium that appears equally suitable for both forms. This medium contains the following salts:

<table>
<thead>
<tr>
<th>Grams per liter</th>
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<tbody>
<tr>
<td>Ammonium chloride</td>
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<tr>
<td>Magnesium sulfate</td>
</tr>
<tr>
<td>Sodium sulfate</td>
</tr>
<tr>
<td>Dibasic potassium phosphate</td>
</tr>
<tr>
<td>Calcium chloride</td>
</tr>
<tr>
<td>Sodium lactate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>Ferrous ammonium sulfate</td>
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</tbody>
</table>

For both marine and nonmarine forms more rapid development occurred in 1% sodium chloride than with no sodium chloride or with 2% or more. The presence of calcium carbonate was important and better results were obtained when this compound was used than when similar pH values were induced by the addition of sodium hydroxide. Larger amounts of sodium sulfate reduced the rate of hydrogen sulfide formation without increasing the total concentration reached.

The high concentration of hydrogen sulfide produced in appropriate media tended to kill contaminating organisms in enrichment cultures; after five or six transfers these organisms were no longer present. Microscopic examination of a limited number of such cultures also indicated the absence of contaminants.
Corrosion of iron by the bacterium Vibrio desulfuricans was studied under anaerobic heterotrophic and autotrophic conditions. According to Kuhr's mechanism the stimulation of corrosion is due to utilization by the bacteria of cathodically produced hydrogen by which the corrosion reaction would otherwise be polarized. Kuhr's equation indicates that if no organic hydrogen donors are present and the hydrogen for sulfate reduction comes solely from iron corrosion, the ratio of total iron corroded to iron present as iron sulfide should have a value of 4. This ratio is closely related to the ratio \( N \) of gram atoms of iron corroded to mol sulfate reduced which should also have a value of 4. Determination of residual sulfate in a culture medium of known sulfate content, in which Armco iron samples had been immersed for 8-56 days, allowed calculation of values of the ratio \( N \). Under heterotrophic conditions these values ranged from 0.9 to 1.5 which, in view of the relatively large amounts of easily oxidizable lactate present, is regarded as in accord with expectations. Under autotrophic conditions, \( N \) values ranged from 5 to 9, indicating that only part of the hydrogen liberated in the corrosion of iron is utilized for sulfate reduction. Mass spectrometer analyses showed that significant amounts of hydrogen gas were evolved.

These results indicate that in autotrophic medium the ratio \( N \) is merely a measure of the extent to which bacteria utilize hydrogen liberated by iron corrosion and that no acceleration of corrosion is produced by such hydrogen utilization. Further support for this view arises
from a study of the influence of inoculation with sulfate-reducing bacteria on the corrosion potential of an iron electrode in air-free autotrophic medium. These measurements revealed no significant cathodic depolarization. Acceleration of corrosion by sulfate reducers in heterotrophic medium is, therefore, considered to be caused not by removal of hydrogen but by some other action of the bacteria, probably their sulfide producing action.

Wormwell, F., and T.W. Farrer. 
ELECTROCHEMICAL STUDIES OF ANAEROBIC CORROSION IN PRESENCE OF SULPHATE-REDUCING BACTERIA. 

[Steel, corrosion, bacteria, Desulfovibrio, polarization measurement, tests]

Stone, Robert W., and Claude E. ZoBell. 
BACTERIAL ASPECTS OF THE ORIGIN OF PETROLEUM. 

[Petroleum, bacteria, biomechanism, Serratia, Mycobacterium, Desulfovibrio]

Sonntag, W. 
BACTARIAL DECOMPOSITION OF SOLUBLE-OIL EMULSIONS. 
Lubrication Eng. 8:234,260 (October 1952) 2 p.

The breakdown of the soluble-oil emulsion in a particular hydraulic press system is described and recommendations are given for prevention of decomposition in this and similar systems.

Whenever circulation was stopped for 24 hr or longer in a 6,350 gal (24 cu m) hydraulic press system, oil separated, black ferrous sulfide scum formed in the accumulator, and hydrogen sulfide odor was noticeable. The water supply for the system was a good tap water with low hardness; however, contaminated drainage water with a high hardness and iron content seeped in through the concrete wall of the cistern and accumulator. The emulsifying agent in the original soluble oil was mainly a mixture of stabilized petroleum sulfonates. When circulation was stopped and oxygen supply was thereby depleted, sulfur bacteria, probably *Sporovibrio desulfuricans* attacked the sulfonates, reducing them to hydrogen sulfide, and released the oil from the broken emulsion.

Recommendations for preventing this type of decomposition are as follows: The entire system should be kept clean and influx of iron-bearing ground water should be
eliminated by proper accumulator insulation. The pH of the press water should be increased to approximately 8.2, because sulfur bacteria require a pH of 7 or lower for development. The system should be thoroughly aerated and good circulation maintained at all times. Adoption of these precautionary measures eliminated all trouble in the hydraulic pressure system described. A germicide can be added if necessary.

Bacteria and molds were found to attack and damage polysulfide polymer linings of concrete underground gasoline storage tanks under tropical conditions. Failures were of two types: (1) In Florida, Thiokol FA sheet-lined tanks operated by water-displacement systems which kept the tanks filled to a constant level showed extensive deterioration in the areas traversed by the gasoline-water interface after 2 yr. (2) Kept in stand-by condition in the tropics, Thiokol MX Latex lined tanks showed deterioration principally on the damp floors, particularly in the areas below the manholes.

The addition of 5% pentachlorophenol to the polysulfide polymers inhibited biological attack for 4 yr, whereas Thiokol linings containing either phenylmercury salicylate or pyridylmercury chloride turned black after 2-mo exposure and showed some mold growth after 6-mo exposure.

The microorganisms attacking the linings were common soil and water bacteria and molds. Specific bacteria isolated and identified included Bacillus megatherium, B. terminalis, Achromobacter liquefaciens, Flavobacterium diffusum, and Sporovibrio desulphuricans. The molds were of the genera Aspergillus, Cladosporium, Alternaria, Fusarium, Trichoderma, and Penicillium.

Using a sulfate-free modification of the medium described in the preceding abstract, the growth of Desulphovibrio desulphuricans, strain 'Hildenborough,' was observed directly. Compounds which were able to replace sulfate for growth included sulfite, thiosulfate, tetra-thionate, metabisulfite, and dithionite. Except for dithionite, which probably decomposed spontaneously to sulfate and sulfur before being reduced, resting cell sus-
pensions reduced these ions, using the theoretical amounts of hydrogen and forming the theoretical amount of sulfide.

The organism was unable to grow with or to reduce di-thionate, perdisulfate, 'formaldehydesulfoxylate,' sulfanate, benzenesulphonate, methanesulfonate, β-hydroxyethanesulfonate, sodium ethylsulfate, dimethylsulfone, or cystine. If purified by redistillation, elementary sulfur was also unattacked. Five other strains of D. desulphuricans, four of them cultivated autotrophically, were also unable to grow with pure elementary sulfur.

Colloidal sulfur permitted slow growth, or slow hydrogen absorption when a resting cell suspension was used. This effect was not due to oxide impurities in the sulfur permitting growth, because an ultrafiltrate of colloidal sulfur had considerably less activity.

A study of hydrogen uptake rates suggested that sulfur, thiosulfate, and tetraethionate were not intermediates in normal sulfate reduction, but that sulfite was.


The growth of bacteria in soluble oil emulsions and methods for determining their number are discussed. Bacteria grow readily in a variety of soluble oils; soil, feces, river water, machine-shop swepings, and pit sludge serve as inocula. Increasing the oil concentration of the emulsion decreases the mean generation time and increases the number of viable bacteria. The addition of peptone to the emulsion increases the number of viable bacteria and results in objectionable odors.

In the enumeration of bacteria in soluble oil emulsions, distilled water or 20 N phosphate buffers at pH 7.0-8.0 appear adequate as diluents, and nutrient agar seems adequate as a plating medium. Diluents containing sodium chloride are undesirable.


Many bacteria grow in soluble oil emulsions causing undesirable conditions such as foul odors and lowered pH with subsequent breaking of emulsion. Because there is little published work on evaluating disinfectants for the emulsions, two test methods—a methylene blue screening test, and a more severe circulation test—were developed.

The methylene blue test is based on the ability of bacteria to lower the oxidation-reduction potential when
actively multiplying in oil emulsions and to decolorize methylene blue thio-cyanate. One part of a sterilized solution containing a 1 g tablet of this compound per 200 ml water is added to 10 parts sterilized 1/4% soluble oil emulsion in water, then 10 ml of this mixture is pipetted into sterilized culture tubes containing 0.3 g iron chips, followed by the addition of disinfectants prepared as 1% stock solutions in oil or ethanol. After standing 1 wk at room temperature, the tubes are inoculated with 0.5 ml of a mixture of heavily contaminated soluble oil emulsions obtained from machine shops and incubated at room temperature.

Disinfectant-free controls reduced methylene blue completely in 36-40 hr. Compounds which inhibited this reduction for 1 wk in 0.1% concentration were considered suitable for the circulation test.

In this test, 3600 ml of a 2% soluble oil emulsion containing the disinfectant is held in a wide-mouthed bottle. The oil is forced by air pressure into a funnel holding 3 g iron chips on a wire screen, and is then returned to the bottle. After 1-wk recirculation the oil is inoculated with 10 ml of a mixed contaminated industrial oil sample; bacterial counts are made after 0, 1, 10, 18, and, if necessary, after 22 and 29 days following reinoculation.

Compounds that passed both tests were 0.010% mercuric chloride, 0.005% o-hydroxyphenylmercury chloride, 0.005% phenylmercury olate, 0.010% azochloramide, 0.10% 2,2'-methylenebis(3,4,6-trichlorophenol), 0.10% hexylresorcinol, and 0.10% octylresorcinol. Sulfanilamide (0.1%) passed the methylene blue test but did not produce sterility in 18 days, although growth was suppressed. Both 0.10% isobutylresorcinol and 0.10% amylresorcinol passed the methylene blue test and sterilized oils in 18 days, but after reinoculation they showed a high bacterial count on the 29th day.

Further testing should be conducted under industrial conditions because rate of flow of the soluble oil through the system, gross contamination with organic matter, method, volume, length of storage, and other factors may effect the efficacy of disinfectants.

G-10165
THE GROWTH OF PATHOGENIC BACTERIA IN SOLUBLE OIL EMULSIONS.

[Mineral oil, emulsion, bacteria, Salmonella, Klebsiella, growth, test]
The more important types of microorganisms associated with oil-field water floods include iron bacteria, sulfate-reducing bacteria, capsule- or slime-forming bacteria, algae, and molds. The biology of these organisms, the difficulties caused by their presence in water floods, and methods of control are discussed. Factors to be considered when choosing a bactericide include toxicity, ease of handling, solubility in oil and water, compatibility with other chemicals, and pH of the system.

Sulfate-reducing bacteria (Desulfovibrio) are important to flood operators because they cause corrosion and, by forming insoluble iron sulfide, plugging of pipes. These organisms are not killed by aeration and their control is a problem in both open and closed systems. Both iron bacteria and capsulated bacteria cause clogging of pipe and plugging of input wells. Capsulated bacteria, however, are of little concern to water flood operators because they form slimes only in waters containing relatively high concentrations of organic matter. Algae cause no problems in closed systems that exclude oxygen and sunlight, but in the presence of sunlight they can form slime masses over the surfaces of exposed water which can result in serious plugging. Molds act similarly to algae but do not require sunlight, only oxygen.

The fresh well water used in the North Burbank flood passes through about 11 miles of pipe, has a pH of 7.2-7.4, and contains 1000 ppm total solids, 0.3 ppm dissolved oxygen, and 0.3 ppm soluble iron. The presence of 30 ppm formaldehyde did not control the sulfate-reducing bacteria or general bacteria. A mixture of trimethyl-'soya' and dimethyl-'dicoco'-ammonium chloride injected upstream of the surge tank in amounts of 7 ppm protected the system for 10 mo from both iron and sulfate-reducing bacteria. A strain of Pseudomonas, however, became resistant and was present in large concentration. An occasional supplemental slug treatment with an amine-type compound may be required to prevent plugging by this organism.

The 'produced' water in this unit comes from oil-water mixtures which enter a heater treater where the two components are separated. The water passes through an anthracite filter, then is mixed with fresh water, and is reinjected into the formation. Quaternaries, primary amines, and mixtures of the two were not satisfactory bactericides for this water since they were
extracted by oil carried over from the heater treater. When 15 ppm sodium tetrachlorophenoxide which has a low oil solubility was injected into the produced fluid upstream of the heater treaters, 3-5 ppm went into the oil phase, and 10-12 ppm into the water leaving the filters. This concentration controls only the sulfate-reducing bacteria. If other types are present a higher concentration is required.

Both the fresh-water and the produced-water systems are operated as closed-type systems.

Thirty different species of bacteria were tested for their survival times in nine different cutting oils. Gram negative bacteria were capable of surviving for considerable periods of time (more than 250 days in some cases) whereas the gram positive bacteria were killed in most instances in less than 1 day. Medium straight and emulsion type cutting oils permitted survival of the bacteria for longer periods than the heavy straight type cutting oils. Survival period was less than 24 hr for the gram positive bacteria in the heavy cutting oil and less than 2 days for the gram negative bacteria.

Results indicate that cutting oils should have bacterial inhibitors added to them. In screening these inhibitors to determine the efficiency of the inhibition and the dilution needed, Pseudomonas aeruginosa, Ps. fluorescens, Proteus vulgaris, and Aerobacter aerogenes are suggested as test organisms since they were the most resistant organisms encountered in this work. The first two organisms were still viable after 250 days in emulsion and medium type cutting oils.

PDC Comment: Thirty strains of enteric pathogens and two strains of Klebsiella pneumoniae were tested for their ability to grow in soluble oil emulsions by H. Pivnic, W.E. Engelhard, and T.L. Thompson. The significance of their results with respect to public health is discussed. [THE GROWTH OF PATHOGENIC BACTERIA IN SOLUBLE OIL EMULSIONS, Applied Microbiol. 2:140-142 (May 1954); PDC G-10165]

Uphoff, D.K., and Gloria E. Wren.
THE RELEASE OF OIL FROM PETROLEUM-MEARING MATERIALS BY SULFATE-REDUCING BACTERIA.

Results of extensive tests indicate that presently known strains of Desulfovibrio probably cannot be applied
successfully in the field for secondary recovery of oil from oil-bearing sands and shales.

In eleven separate experiments involving the use of 103 oil-containing unconsolidated packs of sand or calcareous material and six sandstone core samples, no increase in oil was brought about by the growth of sulfate-reducing bacteria within the porous medium. The thirty-eight enrichment cultures of Desulfovibrio used in these tests were obtained from experiments of Zobell, from oil well brines from Texas, California, and Illinois, and from cores and creek mud from Texas. Oils included five crude oils and one n-cetane--crude oil mixture. Most of the many different aqueous media used resembled sea water in composition but were enriched with phosphate, ammonia, and iron. Packs were saturated by two different methods, and different volumes and methods were used for inoculation under both static and flowing conditions.

Several of the mechanisms postulated for the release of oil depend wholly or partly on the ability of the bacteria to utilize crude oil as an energy source or to modify the crude oil. These studies showed little or no utilization of crude oil and n-hexadecane by any of the Desulfovibrio cultures. It also appears unlikely that these bacteria can modify crude oil sufficiently to affect oil recovery in commercial quantities.

PDC Comment: Related earlier studies on file at the Center include the following:

(1) Claude E. ZoBell, ACTION OF MICROORGANISMS ON HYDROCARBONS, Bact. Revs. 10(1/2):1-49 (March-June 1946) [PDC 5:B 12, G-2114]
(2) Claude E. ZoBell, MICROBIAL TRANSFORMATION OF MOLECULAR HYDROGEN IN MARINE SEDIMENTS ... Bull. Am. Assoc. Petroleum Geol. 31:1709-1751 (October 1947) [PDC G-2298]

G-10941


The effect of reducing agents on the apparent viability of sulfate-reducing bacteria is discussed, and a satisfactory method for determining the number of viable sulfate-reducing bacteria in a culture is described.

Stock cultures of a strain of Desulfovibrio desulfuricans called Hildenborough were subcultured in a medium of glucose, peptone, yeast extract, and sulfate at intervals of not more than 1 wk. A halophilic strain, El Agheila Z, was counted in a medium of lactate, yeast extract, sulfate, and 2.5% sodium chloride. Either medium was supplemented if required with 1.5% agar, 1 μ mol per ml of...
ferrous ammonium sulfate, and various reducing agents such as sodium sulfide, cysteine hydrochloride, or ascorbic acid. The pH was adjusted to 7.4 ± 0.2. Cultures were usually incubated in an anaerobic jar containing hydrogen plus carbon dioxide. Dilution counts in liquid media were made by preparing replicate one-in-ten dilutions in 5 or 10 ml of the test medium and incubating. Colony counts in solid media were made by preparing a single one-in-ten dilution series and adding samples from appropriate dilution levels to replicate tubes of agar. Total counts were determined by diluting a culture in 0.8% saline containing methylene blue and counting in a Thoma hemocytometer of 0.02-mm depth.

Sodium sulfide or cysteine stimulated the growth of the sulfate-reducing bacteria; small populations often did not grow without such supplements. Ascorbic acid, glutathione, or thiolacetic acid had similar properties, but thiolacetic acid was sometimes inhibitory. Dilution counts in liquid media or colony counts in agar did not bear any regular relation to the total count unless one of these supplements was present. With suitable precautions, colony counts reaching 50-60% of the total count were obtained in media incorporating cysteine and a ferrous salt as an indicator of sulfide formation.

Samples of natural origin containing sulfate-reducing bacteria gave greater viable counts in cysteine-iron media than in unsupplemented media. Blackened culture tubes with natural populations were sometimes due to cysteine-decomposing organisms; further examination of positive tubes was therefore necessary.

In the past, dilution in media containing no added reducing agent has frequently been used for the enumeration of D. desulfuricans, and deductions concerning their behavior have been made from such studies. The present work has shown that such dilution procedures give variable results, often unrelated to the total population; figures varying from 1% to 0.0001% of a constant population may be obtained.

To study the range of organisms capable of growing on typical plasticizers and hydrolyzing the fatty materials therein, 358 fungi were grown on coconut oil, methyl acetylic oleate, and dihexyl sebacate. Of the organisms tested 95% grew on coconut oil, 90% on the ricinoleate, and 60% on the sebacate. On the sebacate, 95% of the strains tested showed less than 50 mg growth per flask, or less than 10% conversion of oil to mycelium.
The Aspergilli showed varying activities. A. terreus, A. niger, A. flavus-oryzae series, A. fumigatus, A. clavatus, A. tamarii, A. versicolor, and A. ustus were among the most active; the least active included A. repens and A. wentii. Among the Chaetomium, C. globosum, C. spirale and C. mollipilum were very active; the least active were C. causaeformis and C. velutinum. Cladosporium herbarum isolates had medium to low activity. Insect parasites as a whole appeared to be active oil consumers. Thus Beauveria bassiana, Aspergillus parasiticus, and Aspergillus flavus were very active, whereas the plant parasitic Botrytis cinerea had little activity. The Phycomycetes vary in their oil metabolizing ability, the most active (three species of the genus Cunninghamella) showing mycelial weights equal to 65% of the initial weight of coconut oil and 20-40% of the initial ricinoleate. The Basidiomycetes tested were largely inactive.

Growth was determined by incubating the test organism with 0.5 g of the test material, 50 ml of nutrient solution, and 1000 ml distilled water adjusted to pH 6.4 at 30°C for 7 days and measuring the dry weight of the mycelium after filtering, ethanol extraction of the residual oil, and drying at 70°C.

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G-11713


A sulfate-reducing bacteria, Desulfovibrio desulfuricans (strain El Agheila Z) oxidized malate, fumarate, succinate, lactate, and pyruvate quantitatively to acetate in cultures containing excess sulfate. Polysaccharide accumulated in old cultures.

Cell suspensions harvested from malate media yielded theoretical amounts of sulfide and carbon dioxide from malate, fumarate, or succinate plus excess sulfate. Succinate was formed transiently during malate oxidation. Various inorganic sulfur-containing anions, methylene blue, or oxygen acted as alternative hydrogen acceptors to sulfate for malate oxidation. In the absence of sulfate, malate was metabolized yielding for each 3 mol of malate, two of carbon dioxide and two of succinate plus acetate. In hydrogen, malate or fumarate was partly reduced to succinate. Malate or fumarate accelerated the reduction of sulfate, but not dithionite, in hydrogen. Suspensions treated with cetyltrimethylammonium bromide formed fumarate and lactate plus carbon dioxide from malate. Fumarate was formed via a reversible fumarase; lactate was formed via a decarboxylase system independent of cozymase, Mn++, cocarboxylase, or codecarboxylase.
Cell suspensions harvested from lactate media yielded theoretical amounts of sulfide and carbon dioxide from lactate or pyruvate plus excess sulfate. In the presence of arsenite, pyruvate formation from lactate was demonstrated with both El Aghailela Z and Hildenborough strains.

Suspensions of strain California 43:63 harvested from lactate media quantitatively reduced fumarate in hydrogen to succinate; crotonate, maleate, or acetylene-dicarboxylate were not reduced. Malate was reduced slowly.

The reaction sequence succinate $\rightarrow$ fumarate $\rightarrow$ malate $\rightarrow$ lactate $\rightarrow$ pyruvate $\rightarrow$ acetate probably takes place in these bacteria. A reversible succinate-fumarate system may form a link between sulfate reduction and the oxidation of organic compounds or hydrogen.

Thirty-two cultures of a pink Pseudomonas species, hitherto undescribed in the literature, were isolated from used soluble-oil emulsions obtained from factories in Canada, England, and the U.S.A. The new species, Pseudomonas rubescens n. sp., was not found in all emulsions studied and, where found, represented only 0.1-1.0% of the total microbial population. It predominated only in one emulsion that had been stored 2 yr in a refrigerator.

All cultures were Gram-negative, asporogenic, noncapsulated rods with rounded ends and motile by means of a single polar flagellum. On nutrient agar plates, colonies were circular, smooth, convex, and with an entire edge. After 1 day, they were 0.5-1.0 mm in diameter and colorless, but by 2 days they were 2-3 mm in diameter and pink. Their size increased to 10-15 mm in 10 days, and the color changed to tan or peach after 1 wk.

All but two cultures produced hydrogen sulfide in peptone iron agar shortly after isolation, and one of these had acquired the ability to produce it when restested 6 mo later. In some cultures, hydrogen sulfide was produced abundantly within 24 hr, in others it was detectable only after 14 days' incubation. All cultures reduced litmus milk in 1-4 days, but only two thirds peptonized it in 28 days. Nitrates were reduced to nitrites in 2 days. All cultures contained catalase. Starch was not hydrolyzed, nor was acid produced from glucose, fructose, sucrose, or lactose. All of eight cultures tested utilized lactate, malate, succinate, and fumarate as sole carbon sources, but only half of them used glucose and fructose.
Abundant growth occurred in nutrient broth at 20-30°C, but at 37°C all cultures showed only slight or no growth after 4 days. Noticeable growth occurred at pH 6-10 within 1 day, but at pH 5 or 11 no growth was observed even in 12 days. The new species grows best under aerobic conditions and resembles Pseudomonas putrefaciens except for pigmentation and the ability to ferment sugars and produce odors.

Cowling, J.E.
INERT LININGS FOR HULK FUEL STORAGE TANKS.
BuDocks Tech. Digest No. 1:7-14 (November 1973)
[Petroleum, fuel, storage, tank, concrete, steel, interior, coatings, elastomer, fungi]

Ladd, J.N.
THE OXIDATION OF HYDROCARBONS BY SOIL BACTERIA. I. MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF A SOIL DIPHTHEROID UTILIZING HYDROCARBONS.
Australian J. Biol. Sci. 9:92-104 (February 1956)

A description is given of the morphology and biochemical properties of Corynebacterium sp., a saprophytic soil diphtheroid that is able to oxidize aliphatic hydrocarbons.

The rates of oxidation of n-hexadecane, n-tetradecane, n-decane, n-octane, 1-hexadecene, 1-tetradecene, and 1-dodecene are measured. Respiratory quotient values for the oxidation of n-hexadecane, n-tetradecane, and n-decane indicate their complete oxidation.

Increased dispersion of n-decane by surface active agents does not increase its oxidation rate. An emulsion of n-decane, however, homogenized in a blender and containing only one fifth of the amount of hydrocarbon, is oxidized at the same rate as the nonhomogenized control.

The organism oxidized normal fatty acids (C₄-C₁₀), normal fatty alcohols (C₂-C₁₁), several aliphatic aldehydes, and the higher methyl ketones. It is unable to oxidize the lower ketones or cyclic compounds. Oxidation rates of n-decane with its oxidative derivatives show no summation of the respective individual rates.

At pH 6, oxidation of 0.016 M succinate is inhibited 50% by 0.024 M malonate, but at this pH and concentration malonate had no effect on n-decane oxidation. It is inhibited 75% by 0.004 M fluorooacetate, 100% by 0.008 M cyanide, 90% by 0.12 M azide, 100% by 0.0001 M mercuric ions, and 88% by 0.0008 M iodoacetate. Cysteine (0.008 M) completely reactivates cells inhibited by iodoacetate.

Two nonvolatile acids, chromatographically identical with lactic acid and glutaric acids, are produced during the oxidation of n-decane. No volatile acids were detected.
Emulsion-type oils for high-speed metal cutting are subject to bacterial degradation and may contain from 1 million to over 700 million bacterial organisms per ml. In addition to degrading the oils, these microorganisms also pose industrial health problems.

None of several commercial cutting-oil inhibitors tested prevented bacterial growth for more than a few days. Many inhibited oil samples contained more bacteria than the uninhibited control. Among fifty-one experimental compounds tested as potential bactericides in cutting oils, five compounds used at a concentration of 1000 ppm controlled the growth of bacteria for the full testing time of 60 days under conditions simulating actual cutting-oil service. They included three phenolic compounds, viz., 2,4,5- and 2,4,6-trichlorophenol (Dowicide 2 and 28) and 2,2'-thiobis(3,4,6-trichlorophenol) (Sindar 61). The other two effective compounds were 1,3-dichloro-5,5-dimethylhydantoin (Halane) and tri-(hydroxymethyl)nitromethane. A sixth compound, dialdehyde (glyoxal 30%) was effective for 16 days.

Because compounds similar in constitution to the effective compounds were either totally ineffective or effective for only a short time, a useful two-part test for cutting-oil inhibitors was developed. Compounds which prove effective in a closed-system screening test lasting 7 days are tested in an open system for a longer period of time to assess the effective life of the inhibitor in service.

The susceptibility of five soluble oils and six emulsifiers used in soluble oils to oxidation by Pseudomonas oleovorans or Pseudomonas formiciana was determined. Measurements were made at 30°C in a Warburg respirometer containing 1 ml of washed suspensions of the organisms in the presence of 2.0% soluble oil. These were commercial products and little is known of their composition. Emulsifiers used in concentrations of 0.5% included petroleum sodium sulfonate, naphthenic acids, sodium soap of wood rosin, triethanolamine oleate, and sodium oleate. Tri(polyoxyethylene) sorbitan monooleate was present as a 0.125% solution.
The bacteria varied in their capacity to oxidize individual oils and the oils varied in their susceptibility to oxidation by a given species. Oils containing oleates or fatty materials were rapidly oxidized by both species. The emulsifying agents also varied in oxidizability. Oleates, particularly triethanolamine oleate, were readily oxidized; tria(polyoxyethylene) sorbitan monoleate showed intermediate susceptibility. Naphthenates and wood rosin soaps appeared slightly oxidized, possibly due to contaminants in the sample. Petroleum sulfonates were resistant under the experimental conditions.

PDC Comment: An earlier report by Hilliard Provenick has been abstracted by the Center. [PSEUDOMONAS RUBESCENS, A NEW SPECIES FROM SOLUBLE OIL EMULSIONS, J. Bacteriol. 70:1-6 (July 1955); PDA 13: Biol 24, G-11735].

Anonymous. OIL, EMULSION, CORROSION CONTROL AND PETROLEUM. Corrosion Control. 10:90-98 (August 1960)

[petroleum, crude oil, bacteria, algae, toxiics, corrosion, corrosionproofing]


[Oil, emulsion, bacteria, Achromobacter, Aerobacter, Pseudomonas, Escherichia, Salmonella, Bacillus, Desulfovirbio, yeast, fungi, physiology, taxonomy, review]


Emulsion-type metal-cutting fluids (so-called soluble-oil emulsions) show a bacterial count of many million bacteria per ml after only a few hours of use in open systems. The count can rise to as high as 700 million per ml. Bacteria found in used emulsion oils include both spore-forming and nonspore-forming types; they include Pseudomonas oleovorans and aeruginosa, Escherichia coli, Achromobacter, and many other species.

The sources of bacterial contamination of oils originate largely from contact with the skin, sputum, and
food residues of machine operators and, to a lesser extent, from the water used in making up the emulsion.

Among control measures, frequent pasteurization requires the installation of expensive equipment and does not ensure lasting protection. Chemical inhibitors seem to offer the greatest promise. Compounds found to be successful inhibitors in laboratory tests require further study on the plant scale before their industrial efficiency can be established. Suitable compounds must possess not only germicidal potency, but a low level of human toxicity, high stability and solubility in the oil-water system, compatibility with emulsion components, and reasonable cost.

Commercial application of inhibitors should be combined with good industrial hygiene to prolong the service life of the inhibited emulsion. For increased antibacterial activity, a combination of two or more inhibitors or a periodic change from one compound to another to reduce the development of resistant mutations should be considered.

An extensive listing is included of available published and unpublished information on compounds that have been screened as antibacterial agents in emulsion oils. No indication of effectiveness is given.

THE ISOLATION OF PATHOGENIC BACTERIA FROM USED EMULSION OILS.
Applied Microbiol. 4:332-338 (November 1956)

[Oils, emulsion, petroleum, bacteria, Achromobacter, Aerobacter, Bacillus, Escherichia, Diplococcus, Micrococcus, Paracolobactrum, Flavobacterium, Proteus, Pseudomonas, Sarcina, Shigella, Streptococcus, yeast, collection, isolation, techniques]

THE COEXISTENCE OF PATHOGENS AND PSEUDOMONADS IN SOLUBLE OIL EMULSIONS.
Applied Microbiol. 4:293-299 (November 1956)

[Oils, petroleum, emulsion, bacteria, Pseudomonas, Achromobacter, Escherichia, Aerobacter, Salmonella, Klebsiella, Shigella, culture, biomechanism, technique]

G-13479 Hartsell, S.E.
MICROBIOLOGICAL PROGRESS REPORT. MAINTENANCE OF CULTURES UNDER PARAFFIN OIL.
Applied Microbiol. 4:350-355 (November 1956)

Data obtained in this evaluation and by other investigators show that various strains of bacteria, yeasts,
Basidomycetes, and other fungi survive periods up to 14 yr under paraffin oil. Although in each group some strains suffer loss of viability or loss or gain of characters, it is concluded that both the longevity and the maintenance of cultural characteristics are due to natural biological variation. The strain, the storage medium, the temperature, the subculturing medium, and the frequency and technique of transfer determine the success of the method.

The method can be applied and has its greatest application in small collections of cultures where pumps, special sealers, and complicated suspending menstrua are not available. Recommendation is made that the neutral, white, medicinal-type paraffin oil be sterilized at 121 C for 2 hr, followed by 1 to 2 hr at 170 C. Difficulty from contamination is minimized when dry-heat-sterilized stoppers are used in the flasks containing the oil and in the tubes that receive the medium. Agar slants should be completely covered with oil, otherwise they may act as wicks causing desiccation of the organisms. A high incidence of contaminants on subculturing may imply either poor technique or improper precautions in preparing the receptacles and the oil prior to the storage period. Best results are obtained when giant colonies are used, if a diameter is transected as the inoculum is obtained.

Pivnick, H., M. Fuller, H. Graham and S. Uyeno.
BIOLOGICAL OXIDATION OF SOLUBLE OIL EMULSIONS.
Lubrication Eng. 11:96 (March-April 1955)
[Oils, emulsions, oxidation, bacteria, culture]

Dworkin, Martin, and J.W. Foster.
STUDIES ON PSEUDOMONAS METHANICA (SOHNGEN) NOV. COMB.
J. Bacteriol. 72:646-659 (November 1956)
[Hydrocarbons, Pseudomonas, Methanomonas, physiology, isolation, identification, culture, technique]

Strawinski, Raymond J. (assignor to Texaco Development Corp., New York, N.Y.).
PURIFICATION OF SUBSTANCES BY MICROBIAL ACTION.
U.S. Pat. 2,574,070; November 6, 1951. 3 p.

Complex organic sulfur compounds can be removed from petroleum hydrocarbons by microbial conversion. The compounds are first converted to sulfates by species of Pseudomonas, Alcaligenes, or Bacillus. The sulfates are then reduced to hydrogen sulfide by species of Vibrio desulfuricans, Y. thermodesulfuricans, or Desulfovibrio.
The conversion takes place at 25-30°C at pH 6.5-7.5. The sulfate-reducing culture is preferably added after sulfate formation.

The sulfate-producing bacteria are grown in a mineral salt nutrient medium in the presence of 5-20% of the petroleum hydrocarbon to be treated, and in the absence of inorganic sulfur. A suitable nutrient solution contains 1 g sodium dihydrogen phosphate monohydrate, 2 g dipotassium hydrogen phosphate, 2 g ammonium nitrate, 0.25 g magnesium chloride hexahydrate, and 0.01 g each of manganese chloride tetrahydrate, ferrous chloride, and calcium carbonate in 1 liter distilled water adjusted to pH 7. Accessory growth substances such as vitamins and amino acids, as well as traces of copper, mercury, zinc, bismuth, and iodine can be added to stimulate activity, but more than a trace of these metals can poison the microorganism. A suitable medium for growing the sulfate-reducing bacteria under anaerobic conditions contains, per liter of distilled water, 1 g sodium sulfate, 0.1 g calcium chloride dihydrate, 3.5 g crude oil, and a trace of ferrous ammonium sulfate. After good growth has been obtained, the culture is centrifuged and washed with 0.8% sterile salt solution to remove excess sulfate.

Improved noncorrosive oil compositions with superior wetting action comprise a mineral lubricating oil having a viscosity of 100-125 SUS at 100°F, 0.05-5% preferentially 0.25-2.5% sodium benzoate, and 0.02-20%, preferably 2-5% of a solubilizing agent such as an oil soluble alkali metal petroleum sulfonate. These compositions are useful as rust preventing compositions, emulsifiable cutting oils and textile oils.

The sodium benzoate is preferably formed in situ in the composition, thus, the benzoic acid is dissolved in the lubricating oil, and then the solubilizing agent containing free alkali is added. Preferably the soluble oil contains 0.05-5.0% of a stabilizer such as sodium naphthenate or an aliphatic alcohol having 1-4 carbons.

Antifoaming, oiliness, or extreme pressure agents can be added to the composition. Sodium resinate is included when transparent emulsions are desired. Fungicides such as sodium o-phenylphenoxide can be incor-
Petroleum naphtha or white spirit are suitable solvents for compositions to be applied to metal surfaces. A hard microcrystalline wax is recommended as an additive for rust preventive coating compositions.

In one test, 0.25% sodium benzoate increased the humidity-cabinet life of a rust preventive oil from 12 to 100 hr.

In the tank storage of petroleum and petroleum products a water layer is usually provided in the bottom of the tank to insure against loss by leakage. Microorganisms (bacteria, yeast, and molds) are almost always found in this water, functioning at the interface of the stored material and water. The metabolic processes of these organisms result in the formation of gummy substances, hydrogen sulfide, and peroxides, and in the discoloration of petroleum products. Such undesirable metabolic activity can be prevented by maintaining the water layer at a pH between 9 and 10 and by charging it with a substance capable of being ionized to produce a toxic boron-containing anion. This can be accomplished by two separate agents or by a single agent such as borax.

Tests were conducted with gasoline, naphtha, and crude oil layered over a typical tap water having a pH of 7.4 and inoculated with a solution containing a heavy growth of bacteria. The untreated water samples showed bacterial growth within 24-48 hr, indicating microbial action on the oils. In water treated with sodium hydroxide to give a pH of 9, turbidity was observed in 3 days. No growth or turbidity was evident in water saturated with borax, even after a 2-wk incubation period, nor was there any change in the physical characteristics of the hydrocarbons.
Some of the sludge in storage tanks for JP-4 jet fuel was formed by bacterial action at the water-hydrocarbon interface. Bacterial accumulation occurred only in the water portion. In some cases the fuel was emulsified by bacterial action; in others it was transformed to semi-solid residues. Bacteria unable to utilize the fuel alone were able to utilize 5% solutions of gum inhibitor in JP-4 fuel. Loss of inhibitors resulted in a further increase in gum formation.

Seventeen different bacteria isolated from contaminated fuel-water-sludge samples were inoculated on Bushnell-Haas medium with or without sterile JP-4 fuel and incubated at about 30°C for 10 days. The fuel was utilized by 11 of the 17 bacteria. No bacterial growth was apparent in tubes containing only Bushnell-Haas medium. The bacteriostatic properties of approved corrosion inhibitors and gum inhibitors also were determined using 5% solutions of these inhibitors with JP-4 fuel as the nutritive solvent. None of these solutions completely inhibited bacterial growth. The corrosion inhibitors tested, in order of diminishing bacteriostatic properties, were: Nosal EDC, Gulf Agent 178, Alox-L-1846, Santolene C, Univor, Kontol 77 M. Of the three gum inhibitors tested, Tenemene 60 showed the highest bacteriostatic activity, followed by 2,6-di-tert-butylphenol, and Paranox 441, respectively.
Sulfate-reducing bacteria were isolated from two y-eight of thirty-three samples of spoiled cutting emulsions. These emulsions represented fifteen different commercial products. Viability of these bacteria proved them to be part of the natural bacterial flora of these emulsions. They caused spoilage by reducing sulfates, sulﬁtes, thiosulfates, tetrathionates, and retails in the emulsion to sulfides. Pure cultures of these sulfate-reducing bacteria could not be obtained. All cultures were contaminated with heterotrophic bacteria capable of growing under aerobic conditions. These heterotrophic organisms may produce conditions that initiate or stimulate growth of sulfate reducers, e.g., anaerobic conditions, decrease in nitrogen content, or production of compounds essential or stimulating for growth of sulfate reducers.

Forced aeration of the emulsion reservoir can effectively eliminate the unpleasant odors, possibly kill the anaerobic sulfate-reducing organisms, and retard the deterioration and spoilage of the emulsions. None of the inhibitors effective against heterotrophic bacteria under aerobic conditions are active against sulfate-reducing bacteria.

Comparative studies of five strains of Clostridium nigrificans, four strains of Sporovibrio desulfuricans, and two strains of Desulfovibrio desulfuricans revealed that the first two organisms are identical and have properties previously described for C. nigrificans and are distinctly different from the third. Since the name C. nigrificans has taxonomic priority, the spore-forming, thermophilic, sulfate reducers should be properly named C. nigrificans and the name Sporovibrio desulfuricans should no longer be used. Both C. nigrificans and S. desulfuricans reduced sulfate, sulﬁte, and thiosulfate under the same conditions. It was also found
that little or no growth occurred in the absence of these sulfur compounds. Glucose, ethanol, lactate, and pyruvate were utilized by all cultures as hydrogen donors for sulfate reduction.

The presence of cytochrome pigments in D. desulfuricans was confirmed and the absence of these pigments in the sulfate reducing bacteria was established.


Current methods for controlling microbial growth in soluble oil emulsions are not so effective. Single germicidal compounds appear to be of limited value although mixtures or successive additions of different compounds, perhaps at monthly intervals, might be helpful. The organic mercurials appear to be fairly effective in controlling bacterial growth, but their toxicity and allergenic characteristics mitigate against their use. Glyoxal, an aldehyde not previously used as a bactericide for cutting oils, shows some promise. In general, the most important prerequisite in a disinfection program is thorough cleaning. Residual dirt may inactivate disinfectants and the larger the number of bacteria, the greater the chance that disinfectant-resistant strains may be present.

The oils tested were a vegetable oil derivative, a mineral oil emulsified with petroleum sulfonate, and an emulsifiable mixture of lard oil and mineral oil. To these were added 18 disinfectants in concentrations of 0.083 to 0.5%, usually 0.1%. Tap water was added in the ratio of 1:10 and 1:100 and the mixture adjusted to pH 7.5. Other oils tested included emulsions containing germicides incorporated by the manufacturer.

In preliminary testing, a mixture of emulsion, iron chips, and disinfectant was shaken for 1 wk at room temperature, inoculated, and shaking resumed for 10 days. The inoculum consisted of bacteria in used oil from 4 machine shops added in amounts to give 1 x 10^7 bacteria per ml of emulsion. Plate counts were made at 1 and 10 days. In a second test emulsions containing disinfectant were circulated for about 8 hr per day for 1 wk, inoculated, and circulation resumed 5 days a wk for 4 wk. Plate counts were made at 1, 2, 4, 7, 14, 21, and 28 days.

All the oils containing disinfectants added by the manufacturer supported large bacterial populations when diluted 1:100; a dilution of 1:10 was required to obtain an appreciable degree of sterilization.
Fourteen cultures of Pseudomonas formicans Crawford were isolated from soluble oil emulsions and compared with Crawford's isolates from sewage. The organisms from the two sources possessed many stable, well-defined characteristics in common, but within the species there were variations in indole production, citrate utilization, and activity in litmus milk.

P. formicans decomposed glucose anaerobically in a manner similar to Escherichia coli except that formate accumulated. Hexokinase and aldolase were demonstrated, but glucose-6-phosphate dehydrogenase was not observed. It was suggested that, for the present, the species be considered as a member of the genus Aeromonas.

The degree of fractionation of the stable sulfur isotopes $^{32}$S and $^{34}$S, resulting from reduction of sulfate to sulfide by Desulfovibrio desulfuricans and oxidation of elemental sulfur to sulfate by Thiobacillus thiooxidans was determined.

During reduction of sulfate to sulfide by D. desulfuricans, no fractionation was observed when the bacteria were cultivated in a stationary medium containing 0.3% magnesium sulfate. In a stationary medium containing 5% of the salt, there was 1.4 to 1.5% fractionation. In a continuously agitated medium, fractionation varied from negligible to 2.7%, depending on the rate of sulfate reduction and concentration. Fractionation was greatest where (1) the reduction rate was exceedingly slow, (2) the amount of sulfate in solution high, (3) only a small portion of the sulfate became reduced, and (4) the medium was kept uniform by stirring. Media containing calcium sulfate exhibited less fractionation than those with magnesium sulfate. Low temperature was an effective means of keeping the meta-
bolic rate slow. At low temperatures, the lag period was shortened by inoculation with a large number of cells. The maximum fractionation of the sulfur isotopes was within the apparent range that occurred during deposition of elemental sulfur in the cap rock of the salt domes of Texas and Louisiana.

No fractionation was noted during oxidation of elemental sulfur to sulfate by T. thiooxidans.

VANDERBILT PETROLEUM ADDITIVES.
April 1957. 5 p.

[Fuels, hydraulic fluids, greases, oils, petroleum products, additives, corrosionproofers, toxics, fungi, bacteria, oxidationproofers, tradenames, tabulation, commercial paper]

Ellis, Lee F., R. Samuel-Maharajah, Laura May Mendelow, Larry Ruth and Hilliard Pivnick.
OXIDATION OF COMPONENTS OF SOLUBLE OILS.
Applied Microbiol. 5:345-348 (November 1957)

[Research, oil, emulsions, oxidation, bacteria, components, Pseudomonas, Flavobacterium]

Twenty compounds used in soluble oils were tested for their susceptibility to oxidation by pure cultures of Pseudomonas 324, P. oleovorans, P. formicans, and Flavobacterium brevis. Compounds resistant to oxidation by all four of the pure cultures were tested with a mixed inoculum in emulsions from four different machine shops.

Only petroleum sodium sulfonate, naphthenic acids, and polyoxyethylated nonylphenol were resistant to all pure cultures and the selected mixtures. The sodium soap of wood rosin was oxidized slightly by the Pseudomonas, but slowly and steadily by the mixed culture. Methylene and ethylene glycol monoethers were quite susceptible to oxidation, while the butyl ether was relatively resistant, being attacked only slowly by P. oleovorans. Fatty materials and their derivatives were readily oxidized by at least one of the pure cultures.

Luchterowa, A.

[Petroleum, geomicrobiology, foreign]
The sludge found in water bottoms of storage tanks for JP-4, a kerosene-type fuel used in jet aircraft, is caused by bacteria and products resulting from bacterial metabolic activity. Using the streak-plate method, 71 bacteria were isolated from sludge samples taken from JP-4 tanks. Genera represented included Pseudomonas, Aerobacter, and Bacterium. Tests carried out on 17 of the bacterial cultures showed that none of the corrosion and gum inhibitor additives in the fuel was satisfactorily bacteriostatic. On the contrary, JP-4 fuel as well as some additives were nutritive. Laboratory evaluations showed that bacteria emulsified the fuel, and produced semisolid gummy residues from it. They formed precipi-
states in amounts and types that could easily affect the filter systems of aircraft. Water soluble, fuel-insoluble bacteriostats were selected for sludge treatment. Preliminary tests showed that 1.5 to 2.0% sodium tetraborate, or 2.0% potassium tetraborate, in the water bottoms of JP-4 storage tank produced bacteriostatic conditions.

Tsunishi, N., and A. Ohtsuki.
A METHOD FOR THE RAPID CULTIVATION OF DESULFOVIBRIO AESTIVARIUS ON FILTER MEMBRANES.
Applied Microbiol. 25:270 (January 1973)

[Research, bacteria, Desulfovibrio, cultures, technique]


[Research, foreign, oil, fractions, Pseudomonas, metabolism, bacteria, sulfate]

A study was made of the effect of bacteria of the Pseudomonas genus on crude oil and its components under anaerobic conditions. The behavior of P. fluorescens denitrificans was observed in five mineral media which contained only one source of carbon: (1) asphaltenes, (2) lubricating oil and petroleum ether tar, (3) an unrefined petroleum fraction boiling above 250 to 300 C, (4) paraffin wax, and (5) crude oil. In other experiments, dehydrated paraffin crude oil samples from the Caucasus were inoculated with either a 4-day old culture of P. fluorescens denitrificans or with a 1:1 mixture of the culture with sulfate reducing bacteria. Air was displaced by nitrogen at 30 to 32 C.

Media with lubricating oil and petroleum ether tar, paraffin wax, and crude oil as the only source of carbon supported the propagation of P. fluorescens denitrificans. Carbon supplied by the other media did not.

The paraffinic oil samples from the Caucasus exhibited compositional changes and signs of active bacterial propagation. P. fluorescens denitrificans propagated alone as well as in conjunction with sulfate reducing bacteria. The asphalt-tar components of all inoculated oil samples sustained carbon and hydrogen losses. Their content of heterocyclic nitrogen-oxygen-sulfur compounds rose from an initial level of 4.07% to 7.02% for components of samples inoculated with the bacterial mixture, and to 6.73% for samples inoculated...
with P. fluorescens denitrificans alone. The naphtenic acid content of the latter cultures was nearly 10 times below that of samples inoculated with the bacterial mixture. Changes in paraffinic components of inoculated samples were in the form of reduced melting points and an increased percentage of methyl groups.

A study was made of the action of 70 strains of denitrifying bacteria and 42 strains of desulfurizing bacteria from oil-well waters on paraffinic and naphthenic oils. Under aerobic conditions, 90% of the strains tested preferentially assimilated paraffinic oil, and 10% of the strains preferentially assimilated naphthenic oil. The amount of growth was inversely proportional to the density of the oil. In both types of oil, the most intensive decomposition of hydrocarbons occurred in hydrocarbons with a low molecular weight. Under anaerobic conditions, the viscosity of the oil diminished; under aerobic conditions, viscosity increased.

The protective current requirements of buried steel pipe are higher in the presence of large quantities of anaerobic sulfate-reducing bacteria, since these organ-
isms reduce both anodic and cathodic polarization values. Depolarizing tests showed that the protective current density calculated on the basis of initial polarization curves was insufficient to guarantee protection in the presence of these bacteria. Almost complete protection (95%) of underground piping in bacterially active soil could be achieved over a 5-mo test period by maintaining a negative protective potential of -0.920 v. In practice, the application of higher than average protective current densities is uneconomical. Instead, the use of bituminous coatings in conjunction with cathodic protection is proposed. Such coatings should contain a bactericide and should be resistant to the increase in pH caused by the applied current.


[Research, Desulfovibrio, growth, sodium chloride, bacteria, sulfate, tolerance]


[Research, bacteria, Thiobacillus, metabolism, sulfites, ores, sulfur]

Two chemosynthetic autotrophic bacteria were isolated from streams leaching exposed ore bodies in Bingham Canyon, Utah. Earlier studies had established that the large amounts of soluble iron and copper in the streams were of biological origin. One of the organisms found is able to oxidize free sulfur, ferrous iron, iron pyrites, molybdenite, and several copper sulfide minerals. This organism is very similar to Thiobacillus ferroxidans. The second organism oxidizes free sulfur, but does not oxidize the sulfide minerals. This one is similar to Thiobacillus thiooxidans. Both organisms are similar to Thiobacillus in tolerating a high acid concentration (pH 2.0 to 3.5).

These chemosynthetic bacteria utilize carbon dioxide as a source of carbon. The addition of organic materials may stop or impede growth. Benzene inhibits pyrite oxidation; acetone has some inhibiting action; kerosene, glucose, and sucrose have little if any effect. Urea and L-cystine may supply the nitrogen requirement; β-alanine and sodium nitrate do not. Optimum urea concentration is 0.05 g per liter. On the basis of contained nitrogen, urea is 10 times more effective than ammonium sulfate as a nitrogen source.
Kuznetsov, S.I., and Z.P. Teleshin.  
[CORE DATA ON THE PHYSIOLOGY OF PROPANE-OXIDIZING BACTERIA].  
Microbiologia 31:1-1 (September/October 1941)  

[Research, foreign, bacteria, respiration, propane, Pseudomonas, 
Mycobacterium, physiology, soil]  

The propane oxidizing ability of three species of 
Mycobacterium and one of Pseudomonas isolated from the 
substrate of Russian petroleum-bearing regions was eval-
uated by a microrespirometric method. The ability of 
the culture to assimilate labelled carbon dioxide in the 
process of chemosynthesis also was tested.  

The endogenous respiration of 100 million bacteria 
amounted to 5 cu mm oxygen per hr. Additions of glucose 
increased oxygen consumption to 7 1/2 to 10 cu mm per hr 
and subsequent additions of propane to 20 to 30 cu mm 
per hr. However, glucose additions which followed those 
of propane failed to increase or even decreased the oxy-
gen consumption rates. This suggests that the bacteria 
are not inclined to use up easily assimilable organic 
compounds in the presence of propane.  

The propane oxidizing bacteria assimilated free car-
don dioxide in the process of chemosynthesis. The 
energy required for the process was released in the 
course of propane oxidation.

Bryner, L.C., and A.K. Jameson.  
MICROORGANISMS IN LEACHING SULFIDE MINERALS.  
Applied Microbiol. 22:1-5 (July 1958)  

[Research, bacteria, Thiobacillus, metabolism, sulfur, w.s. 
sulfur]  

Two chemosynthetic autotrophic bacteria were isolated 
from streams leaching exposed ore bodies in Bingham Can-
yon, Utah. Earlier studies had established that the four 
amounts of soluble iron and copper in the streams were of 
biological origin. One of the organisms could is able to 
oxidize free sulfur, ferrous iron, iron pyrites, pyrite-sulfates, 
and several copper sulfide minerals. This organism is very 
similar to Thiobacillus ferrooxidans. The other organism 
excites free sulfur, but does not oxidize the sulfide 
minerals. This one is similar to Thiobacillus thioketonum. 
Both organisms are similar to Thiobacillus in tolerating 
a high sulf concentration (1% to 1%).  

These chemosynthetic bacteria utilize various sulfur 
as a source of carbon. The addition of organic materials 
may stop or impair growth. Product inhibits pyrite oxida-
tion; acetone has some inhibiting action on the. ...
Microbial attack on linseed oil and linseed oil films was studied under conditions existing on painted surfaces. Test species used included: Pullularia pullulans, Alternaria sp., Aspergillus niger, Cladosporium sp., and one bacterium, Flavobacterium marinum.

Results indicate that linseed oil and linseed oil films can serve at least partially as a source of nutrition for these microorganisms and, in the presence of small amounts of soil or soil extract, they can serve as a complete nutritional medium. All of the microorganisms possess lipolytic enzymes specific for the ester linkage of the triglycerides in linseed oil and oxidative enzymes which catalyze oxidation of both glycerol and fatty acids.

Cleavage of the ester linkages by any single microorganism produced free glycerol and free fatty acids. Subsequent oxidation of both glycerol and fatty acids resulted in the formation of glyceraldehyde and epiphydrinaldehyde. A decrease in viscosity and an increase in water solubility occurred concurrently with these chemical alterations in oil and oil films inoculated with microorganisms. Ultraviolet analysis of uninoculated and inoculated linseed oil films revealed an increased disappearance of both conjugated and nonconjugated material with a concurrent reduction in the number of double bonds as indicated by the decreased iodine value in those films attacked by microorganisms.
'Elcide 75', a proprietary bactericidal mixture composed essentially of Thimerosal (sodium ethylmercur-thiosalicylate) and sodium o-phenylphenoxide, effectively inhibits bacterial growth at very low concentrations in metal-cutting oils. In laboratory tests using a typical mixed bacterial population obtained from spoiled soluble oil emulsions, partial bacterial inhibition was obtained with 12.5 ppm of Thimerosal, and complete inhibition at 25 and 50 ppm. In shop tests, a common soluble oil concentrate, at an emulsion ratio of 40 to 1, was used on various types of machines with and without the addition of Elcide 75. The untreated emulsion had a bacterial population of 15,000,000 per ml after 24 hr, 90,000,000 at the end of 1 wk, and was discarded after 4 wk because of odor. The treated emulsions were completely free of bacteria after 24 hr, and had an average count of 555,000 per ml at the end of 1 wk (a number of them were still sterile). There was no odor or significant emulsion break after 16 wk.

The recommended concentration of Elcide 75 is 1 oz to 4 gal of freshly prepared soluble oil emulsion. Its concentrate form is caustic, corrodes aluminum, copper, and brass, and must be protected from sunlight and high temperatures. After dilution there are no problems as to toxicity, irritation, or health hazard.
The biological reduction of sulfate was studied by means of the spot test reaction for hydrogen sulfide on filter paper impregnated with lead acetate. With the method described, hydrogen sulfide can be estimated in a range of 10 to 170 µg per ml. The evaluation of spots by two workers differed on an average by 8.7%. The method is suggested for the estimation of the number of sulfate reducing bacteria in mineral waters.

Dostalek, Milan and Milos Spurny.
KULTIVACNÍ CHARAKTERISTIKY DESULFURIKACNICH BAKTERII Z NAFTOVÝCH LOZISÍK (CHARACTERISTICS ON CULTURING OF SULPHATE REDUCING BACTERIA FROM OIL DEPOSITS).
Českoslov. Mikrobiol. 1:158-164 (1956)

[Research, foreign, bacteria, oil, sulfate, reduction, culture]

An attempt was made to culture desulfurizing bacteria isolated from the water in oil deposits under laboratory conditions. The mixed cultures contained bacteria of the Desulfovibrio genus together with accompanying microflora not identified in detail. Criteria for the development of the culture were the amount of hydrogen sulfide produced and the length of the lag phase. The initial optimum pH for the culture of Desulfovibrio is 7 to 8. With an initial pH of 7, the optimal initial oxidation-reduction potential of the nutrient solution is within the limits of zero to -40 mv. Good development of the culture occurs, however, when the oxidation-reduction potential is below 150 mv. Hydrogen sulfide production was stimulated by addition of 3 to 10 mg yeast extract per 100 ml nutrient solution. The concentration of sulfate in the nutrient media affected the length of the lag phase and the production of hydrogen sulfide. With low concentrations of sodium sulfate (0.0001 to 0.001 M), there was practically 100% reduction of sulfate to hydrogen sulfide, but the lag phase was prolonged. The optimum concentration is 0.07 to 0.28% sodium sulfate. Under optimal conditions, the lag phase lasts 24 hr, the logarithmic phase a further 48 to 72 hr, and the stationary phase approximately up to the 8th day of culturing. The optimal period of preculturing for further inoculation is 48 to 72 hr. In cultures inoculated with bacteria precultured for a longer period, the lag phase is considerably prolonged and irregular.
A quantitative method for estimating the number of sulfate reducing bacteria in water is based on the use of standard hydrogen sulfide production curves. Curves are obtained from mixed cultures of sulfate reducing bacteria having a known number of organisms per milliliter. A nomogram for evaluating the number of sulfate reducing bacteria in cultures containing between $10^1$ to $10^8$ organisms per ml is given.

Bituminous coatings on iron pipe were investigated with respect to the influence of water, aqueous electrolytes, and electrochemical and bacteriological processes. The influence of ground water on anticorrosive properties was evaluated according to the degree of swelling, leachability, and water permeability.

Two bitumen-coated iron samples were buried in separate waterproof soil boxes; one was wetted by a liquid medium containing a bacterial culture of Desulfovibrio desulfuricans, a sulfur-reducing bacteria. Both boxes were periodically moistened with equal amounts of distilled water for 8 mo. A 0.14% decrease in sulfate ions of the sample wetted with the bacterial culture was observed as compared to an 0.08% decrease in the control. The coating exposed to the bacterial culture lost adhesion. Cathodic protection for 8 mo using a 2 v current suppressed the action of the bacteria, but caused loss of adhesion in bitumen coated iron in both boxes.
The sulfur content of bitumen immersed in a 1 N soda solution for 50 days decreased from 1.05 to 0.70%. In distilled water the final concentration was 0.96%. There was no change in bitumen when immersed in a bacteria-free 3 N salt solution for 20 days at 20 ± 2 °C.

Permeability of bituminous films with thicknesses up to 0.5 mm was 0.5 mg per sq cm after 24 hr in distilled water; films with a thickness of 1 mm were impermeable after 70 days. Two films, one with 15% kaolin filter, with thickness from 0.8 to 3.5 mm were tested in a 10% solution of sodium chloride and 1 N cobalt sulfate solution containing cobalt-60. After 180 to 225 days exposure, no penetration by cobalt and chlorine ions was observed.

PDL-33773 Starkey, Robert L.
THE GENERAL PHYSIOLOGY OF THE SULFATE REDUCING BACTERIA IN RELATION TO CORROSION.
Producers Monthly 22(9):12-30 (June 1958)
[Review, bacteria, sulfur, Desulfovibrio, Clostridium, Desulfotostella, fungi, Schizophyllum, corrosion, ferrous metals, mechanism, theory]

PDL-33875 Littlewood, Dorothy, and J.R. Postgate.
ON THE OSMOTIC BEHAVIOUR OF Desulphovibrio Desulphuricans.
J. Gen. Microbiol. 16:596-603 (June 1957)
[Research, Desulfovibrio, bacteria, sulfate, cell, permeability, biomechanism]

PDL-33927 Tant, C.O., and E.O. Bennett.
THE GROWTH OF AEROBIC BACTERIA IN METAL-CUTTING FLUIDS.
Applied Microbiol. 6:388-391 (November 1958)
[Research, oils, cutting, bacteria, population, Pseudomonas, Proteus vulgaris]

Pseudomonas and Proteus vulgaris are probably the most important organisms involved in the deterioration of metal-cutting fluids. In a series of experiments where fifteen different species of microorganisms were studied for their ability to grow in fresh samples of six commercial metal-cutting fluids, Pseudomonas aeruginosa and P. oleovorans were found to grow rapidly and abundantly in all test fluids and Proteus vulgaris in all but one. Klebsiella pneumoniae, Pseudomonas pyocyanae, and Salmonella typhosa were found abundantly in many products, while Escherichia coli
and *Staphylococcus aureus* showed limited growth in a few products; the growth of these species was dependent upon formulation of the fluid and strain variation of the species. Organisms which did not grow in test fluids include *Achromobacter* sp., *Aerobacter aerogenes*, *Bacillus subtilis*, *Escherichia intermedia*, *Proteus morganii*, α-hemolytic *Streptococcus pyogenes*, and yeast species.

Ishimoto, Makoto, Jiro Koyama, Tatsuhiko Yagi and Masaru Shiraki.

**Biochemical Studies on Sulfate-Reducing Bacteria. 7. Purification of the Cytochrome of Sulfate-Reducing Bacteria and Its Physiological Role.** J. Biochem. (Japan) 44:413-423 (July 1957)

[Research, bacteria, sulfur-reducer, enzymes, physiology, biochemistry, cytochrome]

Anderson, Kenneth E., Regina Lanigan, Francis Liegey, John Worden, Frank Yackovich and Austin Finan.

**The Development of New Bactericides and Flood Water Treatment Based Upon the Physiology of the Sulfate Reducing Bacteria.** Producers Monthly 22(10):10-25 (August 1958)

[Research, Desulfovibrio, bactericide, water, oil well, pH, metabolism, acid, antibiotics, biochemistry]


[A Contribution to the Inhibition of Development of the Sulfate-Reducing Bacteria in the Oil Field of the Kalinovka Layer (in Russian with English summary)]. Mikrobiologiia 26:30-357 (May/June 1977)

[Research, foreign, sulfide, oil wells, bacteria, sulfur, corrosion, bactericide, formaldehyde]

Sulfides and free hydrogen sulfide which often are found in oil field water may cause serious corrosion of oil well equipment. Studies in oil fields of the Kubyshev district indicate that hydrogen sulfide formation
Addition of formaldehyde to oil field water in amounts of 100 mg per liter inhibits development of these bacteria. Pumping 1.5 tons of formaldehyde dissolved in 1200 cu m of water into an oil well killed sulfate reducing bacteria and reduced hydrogen sulfide content in surrounding wells.

Cutting oil emulsions are stabilized against bacterial action by addition of an alkali metal salt of phosphoric, silicic, or boric acid or their mixtures. For example, 2% of common cutting oil, dispersed in an aqueous solution of trisodium phosphate adjusted to pH 8.3 to 9.0 and vigorously stirred with an air current, exhibited no putrefactive smell, hydrogen sulfide development, and color change upon contamination with a 2.5% culture solution.

A new method was developed for defatting petroleum smears in studying microorganisms which take part in the formation, transformation, and destruction of petroleum.
The method consists of washing smears in 1% solution of OP-7 or OP-10 (composition not given) brought to 50 to 60 C, instead of ether, benzene, or gasoline. For good results, slides must be washed three times in three different vessels prior to smearing. Water for the preparation of OP-7 and OP-10 solutions, dissolving dyes, and washing should be distilled and passed twice through a membrane filter to remove microorganisms. Preparations are dried in air for 1 to 2 hr then fixed with a 1:10 alcohol-formalin mixture. Fixation time depends upon smear thickness and may take 3 hr. After fixation, the preparation is dried in air and then between two pieces of filter paper, after which it is transferred to a flat vessel containing 1% OP-7 or OP-10 solution. Various staining preparations may be used depending upon the nature of the investigation. Good results are obtained by staining for 1 to 10 min with a highly diluted magenta red base water-alcohol solution.

Moriyoshi, Seishi.

STUDIES ABOUT THE RELATION OF MICRO-ORGANISMS [sic] WITH FATS AND OILS.

Hyogo Noka Daigaku Kenkyu Hokoku 3(1):13-26 (1957)

[Research, fungi, bacteria, fats, oils, paraffin, metabolism]

Many microorganisms were isolated on agar containing 1% of one of 15 vegetable oils or 4 animal oils as a sole source of carbon. Organisms included Bacillus, Bacterium, Mucor, Rhizopus, Aspergillus, Penicillium, Citromyces, Cladosporium, Verticillium, Oidium, and yeast. Addition of peptone to the media generally promoted growth.

The decomposition of ether-extracted soya bean oil by fungi was studied using 53 species of Penicillium, Aspergillus, and Citromyces. In general, the growth of fungi on the agar plate tended to correspond to the acid number and to the oil decomposition. After 1.5 mo incubation at 30 C with 1% oil, the maximum acid number was 3.14, the minimum 0.47, the average 1.56; acid number of the control was 1.75; the corresponding acid numbers after 2 mo were: 5.25, 0.60, 2.32, and 2.76, respectively. The iodine value generally decreased, indicating attack by microorganisms. When it increased, some unsaturated compounds were produced from glycerol.

A medium consisting of ether-extracted soya bean powder and a nitrogenous salt mixture was most favorable for growth of Aspergillus and Penicillium. The salt mixture and soya bean oil was a less favorable growth medium, and the soya bean oil alone showed an inhibitory effect.
Microorganism behavior is influenced by the state of fats and oils in the medium. Much more oil was decomposed in the presence of emulsifying agents. The influence of fatty acids on microorganisms was tested with 9 saturated and 5 unsaturated fatty acids using Aspergillus oryzae, A. niger, and Penicillium glaucum. Fatty acids of less than 10 carbons such as butyric, caproic, and caprylic acids did not permit the growth of any fungus, but their sodium salts did. Free fatty acids with 10 to 22 carbons were more suitable for fungal growth than their sodium salts.

Verticillium, Oospora, Aspergillus, Endomyces, and Penicillium were isolated from paraffin medium. From 20 to 42% of paraffin in the medium was consumed by these organisms after 2 mo incubation. When added to a salt mixture, paraffin was a less favorable carbon source for fungal growth than fats and oils.

Brittle failure or stress cracking of high-density polyethylene for use as wire and cable insulation can be initiated by one or more of three distinctly different mechanisms, viz., oxidation, environmental stress, and thermal action.

Oxidative embrittlement involves scission of macromolecular chain molecules and gelation, and is a rate reaction. Environmental stress cracking, which is highly dependent on the physical state of the polymer and requires a contact medium, appears to be a substitution of some of the intermolecular forces in the polyethylene by the medium which allows the molecules to separate; the resulting flaw, at which the applied stress concentrates, forms the starting point for destructive crack propagation. The hypothesized mechanism of thermal embrittlement assumes that an applied strain results in an elastic dislocation of crystallites from their lower energy, spherulitic arrangement to an adjacent, higher energy arrangement; the elastic forces of restoration overcome the viscous drag forces and leave points of inherent weakness in the structure which lead to eventual failure. The mechanism is a temperature-time dependent reaction.

Factors affecting the brittle failure of polyethylene include (1) average molecular weight, (2) molecular weight distribution, (3) total crystallinity, (4) degree of branching, and (5) purity. The first two factors predominate in environmental cracking; the third is impor-
tant for thermal embrittlement; and the fourth is decisive in oxidative scission. The last factor, purity, is significant for both oxidative and environmental failures, since the presence of polar groups (e.g., carbonyl) not only encourages oxidation, but also reduces the contact angle between polymer and medium.

High density polyethylenes are more susceptible to thermal embrittlement than low density formulations. Blending the two in suitable proportions decreases this susceptibility, but at the expense of strength, toughness, and other desirable physical properties. Modifications involving copolymerization of ethylene with other monomers offer greater promise. A third possible approach to reducing stress cracking without sacrificing desirable characteristics is the irradiation of polyethylene and other post-forming treatments.

An investigation of the commensal relationship between aerobic and sulfate-reducing bacteria in emulsion oils indicates that the aerobic bacteria oxidize components of the emulsion which inhibit sulfide formation and growth of the sulfate-reducing bacteria. Lowering of the oxidation-reduction potential and production of nutrients by the aerobic flora are possible secondary factors which may stimulate growth of the sulfate-reducing bacteria in these products.

Spoilage of emulsion cutting oils is shown to be the result of the combined activities of two distinct types of microorganisms: anaerobic sulfate-reducing bacteria and a variety of heterotrophic bacteria, the majority of which belong to the genus Pseudomonas. Certain inorganic salts present in the medium used also were necessary or contributory to deterioration. These included potassium monohydrogen phosphate, magnesium sulfate, ammonium sulfate, sodium sulfate, and ferric ammonium sulfate.
When these salts were present, spoilage continued through five subcultures. The organic components of the medium (peptone, yeast extract, ascorbic acid, and calcium lactate) did not support growth of the sulfate-reducing bacteria on subculturing even in the presence of the aerobes.

Ishimoto, Makoto, Jiro Koyama and Yutaka Nagai.
BIOCHEMICAL STUDIES ON SULFATE-REDUCING BACTERIA. 4. SULFATE REDUCTION BY CELL SUSPENSION. 4. THE CYTOCHROMO SYSTEM OF SULFATE-REDUCING BACTERIA.
J. Biochem. (Japan) 41:537-546; 41:763-770 (1954)
[Bacteria, sulfate-reducer, Desulfovibrio, biomechanism]

Ishimoto, Makoto, Jiro Koyama and Yutaka Nagai.
BIOCHEMICAL STUDIES ON SULFATE-REDUCING BACTERIA. 4. REDUCTION OF THIOSULFATE BY CELL-FREE EXTRACT.
J. Biochem. (Japan) 42:41-53 (January 1955)
[Bacteria, sulfate-reducer, thiosulfate, biomechanism, tests]

Guyatts, G.J., and E.O. Bennett.
THE SENSITIVITY OF SULFATE-REDUCING BACTERIA TO ANTIBACTERIAL AGENTS. (THE MERCURIALS).
Producers Monthly 23(1):15-17 (November 1953)
[Research, bacteria, sulfur, toxics, mercury compounds, organic]

Thirty different mercurials were studied for their inhibitory activity against a culture of Desulfovibrio desulfuricans. None of the compounds were effective in concentrations of 50 and 25 ppm. The two most effective, Metaphen and phenylmercury 8-quinolinolate, completely inhibited hydrogen sulfide production for 28 days at a concentration of 100 ppm. Ethylmercurithiosalicylate, methylmercury acetate, and phenylmercury acetate were effective at 250 ppm. Eleven of the compounds were ineffective even at 1000 ppm; nine were effective at 1000 ppm and five at 500 ppm.

Harris, J.O.
MICROBIOLOGICAL STUDIES REVEAL SIGNIFICANT FACTORS IN OIL AND GAS PIPELINE BACK-FILLED DITCHES.
[Bacteria, fungi, yeast, Pseudomonas, Micrococcus, Actinomyces, hortensia, Aspergillus, Botrytis, Penicillium, Encomyces, Thiobacillus, hydrocarbons, coal tar, asphalt, waxes,
Bacterial hydrocarbon oxidation: I. Oxidation of n-hexadecane by a gram-negative coccus. J. Bacteriol. 78:441-444 (September 1959)

PDL-35877


PDL-35878

Strawinski, R.J.


PDL-35882

Hutton, William E., and Claude E. ZoBell.

PDL-35879

Strawinski, R.J., and J.A. Tortorich.


PDL-35880

Davis, John B.


PDL-35881

Stewart, James E., and R.E. Kallio.

Bacterial hydrocarbon oxidation. II. Ester formation from alkanes. J. Bacteriol. 78:726-730 (November 1959)

PDL-35881
PDL-3582
Zobell, Claude E. (assignor to American Petroleum Institute).
Bacteriological Process for Treatment of Fluid-Bearing Earth Formations.
U.S. Pat. 2,413,278; December 24, 1940. 4 p.
[Oil well, recovery system, bacteria, patent]

PDL-3588
Taggart, Millard S., Jr. (assignor to Standard Oil Development Co.).
Oil Prospecting Method.
U.S. Pat. 2,234,637; March 11, 1941. 2 p.
[Petroleum, oil prospecting, bacteria, methodology, patent]

PDL-36034
Mackenzie, K.
The Metabolism of Vibrio Desulfuricans in Anaerobic Petroliferous Formations.
Biochem. J. 51:xxiv-xxv (1952)
[Petroleum, bacteria, sulfur, Desulfovibrio, nutrition, metabolism]

PDL-36138
Bennett, E.O., C.L. Adamson and V.E. Feisal.
Factors Involved in the Control of Microbial Deterioration.
1. Variation in Sensitivity of Different Strains of the Same Species.
Applied Microbiol. 7:368-372 (November 1959)
[Bacteria, toxics, phenols, nitro compounds, resistant, strains]

Fifteen cultures of Pseudomonas aeruginosa were studied to determine their sensitivity to ten phenols in nutrient broth (pH 7.0), to metal cutting fluids (emulsion oil at pH 8.5), and to nine new nitroparaffins in an emulsion oil. Statistical evaluation showed three types of variations in sensitivity: (1) between different strains, (2) between individual strains, and (3) between particular environments in which the inhibitor was studied. Cold storage increased the sensitivity of some strains to certain phenols and increased their resistance to others. Variations were greater with some inhibitors than with others. With phenolic inhibitors, more variation in sensitivity was noted in emulsion oils than in nutrient broth. Variation in sensitivity to nitroparaffin inhibitors was greater than to the phenols.
Effective bacteriostatic agents in petroleum containing lubricants such as cutting oils, hydrocarbon fluids, and core oils have the formula: ROCH(R')C(X)NO₂R where R is methyl or ethyl, R' is an aryl, substituted aryl or heterocyclic group, and X is hydrogen or bromine. Compounds are used in amounts from 0.005 to 10%. Specific compounds cited are 2-nitro-2-bromo-1-propoxy-1-phenyl-propane, 2-bromo-1-(3,4-dichlorophenyl)-1-methoxy-2-nitrobutane, and 1-(2-furyl)-2-bromo-1-methoxy-2-nitropropane.

Jet fuels can support the growth of certain fungi and bacteria. Pellicles and mycelium resulting from such growth can be dislodged, resulting in possible clogging of filters and strainers in aircraft and storage and transport facilities. These organisms and their products may act as stabilizers for fuel-water emulsions. Organisms tend to grow at fuel-water interfaces. Water may be present in fuels due to seepage in storage tanks, and may accumulate in low and inaccessible spots in aircraft. Water also may be introduced deliberately in fuel-transfer systems operating by water displacement. Rigorous exclusion of water in fuel would solve the problem but probably is not feasible. Addition of several chemical inhibitors used in gasoline and compatible with jet fuels is currently being studied. Water soluble-fuel insoluble inhibitors are impractical since large amounts may get into fuels through emulsification in systems operating by water displacement, and inadequate amounts in fuels where water is a variable contaminant.
A FERROUS-ION-OXIDIZING BACTERIUM. 1. ISOLATION AND SOME GENERAL PHYSIOLOGICAL CHARACTERISTICS.
J. Bacteriol. 79:502-509 (April 1960)

[Bacteria, sulfur, iron, Ferrobacillus, Thiobacillus, physiology, growth, isolation, technique]

A NEW SPECIES OF GREEN SULPHUR BACTERIA.

[Bacteria, sulphur, Chloropseudomonas, classification]

MICROBIOLOGY IN SECONDARY RECOVERY SYSTEMS.
Corrosion 16:132-134 (June 1960)

[Bacteria, oil wells, microorganisms, corrosion]

The role of microorganisms in secondary recovery systems, including their relationship to chemical scale and corrosion, is reviewed. Iron bacteria, algae, fungi, slime formers, and corrosive (sulphate reducing) bacteria are considered. Life cycles and nutritional requirements of these organisms are treated with emphasis on the effect of different types of bacteria on each other. Clostridium, a genus of bacteria capable of hydrogen sulfide production and not previously implicated in secondary recovery problems, has been isolated in systems from six states. Clostridia are obligate, anaerobic, sporeforming bacteria of heterotrophic metabolism. Flood water containing them turn black upon standing, produce gas, and the bacteria emit a putrefactive odor when proliferating.
Since the spores are a resting stage, they resist adverse conditions of extreme temperature, pressure, chemicals, and lack of food. They are correspondingly hard to kill. Hydrogen sulfide or iron sulfide produced by them may cause a generalized or slab type corrosion and pitting.

A piperazine derivative, 1-(5-nitro-2-thiazolyl)-4-acetyl piperazine is a specific inhibitor of anaerobic organisms including Gram positive and Gram negative bacteria, actinomyces and protozoa, but not their aerobic counterparts. The minimum inhibitory concentration for anaerobic organisms ranged from 0.7 to 1 \( \mu \text{g/ml} \), for Trichomonas vaginalis to 250 \( \mu \text{g/ml} \) for Clostridium perfringens. The aerobic and facultative organisms were approximately 300 to 1000 times more resistant than obligate anaerobes, regardless of taxonomic status.

The effect of the compound was microbiocidal, since exposed populations were rendered sterile within 24 hr at the minimum stated inhibitory concentration. The activity against susceptible anaerobes was independent of pH, exogenous protein, and could not be reversed by 20 times the concentration, on a molar basis, of such substances as thiamine, riboflavin, folic acid and adenine.

The selective inhibition of obligate anaerobes was not related to anaerobiosis per se, since facultative organisms under anaerobic conditions showed their normal resistance to the piperazine. Blocking haem pigments of facultative organisms with potassium cyanide did not induce sensitivity to the drug. Desulphovibrio desulphuricans, which possesses a reversibly oxidizable pigment similar to cytochrome \( c_3 \), was as sensitive as anaerobes such as Clostridium novyi and Trichomonas vaginalis, which are totally devoid of such pigments.
Growth of certain bacteria and fungi in water phases in conjunction with jet fuels decreases filterability and causes malfunctioning of fuel systems.

Twenty substances known to be toxic to microorganisms were studied in an environment of JP-5 fuel and aqueous salts solution; the fuel was the sole source of carbon. The test bacteria were 17 cultures obtained from bottom sludge in JP-4 fuel storage tanks; all were checked for ability to grow in the presence of JP-5 fuel. The toxicants included mercaptobenzothiazole compounds, a chlorinated thiobisphenol, several quaternary compounds, sodium tetraborate, silver, tetraethyllead antiknock mixture and its two principle constituents, pure tetraethyllead, and ethylene dibromide.

Four n-alkyl quaternary ammonium chlorides and an n-alkyl isoquinolinium chloride prevented bacterial growth when incorporated in the solution at 50 or 100 ppm, but not at lower concentrations. Only the isoquinolinium substances caused no turbidity in either the fuel or the water phase. Although none of the quaternary compounds were inhibitory at concentrations as low as desired, some may be useful in keeping microbial contamination to a minimum in such areas as storage tanks. Silver metal, toxic at exceedingly low concentrations, was bactericidal in smaller amounts than required for fungicidal activity. Tetraethyllead and ethylene dibromide incorporated individually in JP-5 at roughly 0.13 and 0.5 ml per gallon, respectively, were inhibitory to bacteria. In combination in the antiknock preparation, however, these compounds exhibited a lesser degree of toxicity. Work on the problem is proceeding.

Bennett, E.O., and R.H. Bauerle.
The sensitivities of mixed populations of bacteria to inhibitors.

[Mercury compounds, nitroparaffins, phenols, bacteria, toxics, sulfur]

The sensitivities to three classes of inhibitors of pure and mixed cultures of Pseudomonas aeruginosa and Desulfovibrio desulfuricans were determined. Five phenols, six mercurials and six nitroparaffins were used, and the
inhibitory concentration in parts per million of each compound is given for both pure and mixed cultures. In addition, the sensitivity of one culture in the presence of dead cells of the other to one compound from each class of the inhibitors was determined.

The presence of *P. aeruginosa* produced in some cases an increase in resistance of *D. desulfuricans* to substituted phenols a consistent increase to nitroparaffins, but no marked response to mercurials. The sulfate reducing bacteria produced a significant increase in resistance of the pseudomonas to mercurials but an increase in sensitivity to phenols.

Dead *P. aeruginosa* cells had no effect on the sensitivity of sulfate reducing bacteria to phenols, mercurials or nitroparaffins. Dead sulfate reducing bacteria produced an increase in resistance of the pseudomonads to mercurials and nitroparaffins, but did not increase the sensitivity of the pseudomonas to phenols. The significance of these results is discussed.

DeGray, R.J., and L.N. Killian.
BACTERIAL SLIMF AND CORROSION IN PETROLEUM PRODUCT STORAGE. Ind. Eng. Chem. 52:74A-76A (December 1960)

[Gasoline, petroleum products, storage, bacteria, toxic, corrosion]

Samples from water-petroleum interfaces in refinery and bulk terminal tanks contain a variety of bacteria, all prevalent in soil and ground waters. *Bacillus* sp. predominate. Some species utilize hydrocarbons; others, nutrients in the water bottoms. Highest bacterial populations occur at pH 6.96. Roiling of a working tank promotes growth. Agitation, colloids (especially at the interface), and certain surfactant rust inhibitors (normally used at 25 to 50 ppm in gasoline and kerosine) all foster bacteria by increasing the availability of hydrocarbons. Bacteria promote iron corrosion and slime formation. Slime holds rust and other sediment in suspension at the interface, encouraging microorganism growth. Introduction of a boron compound (either by direct addition to the water bottom or as boron-gasoline) to a level equivalent to 0.05% elemental boron prevents or reduces bacterial contamination of gasoline. The relative efficacy of a boron compound depends on its structure; in trialkyl borates, chain length seems significant.
Sixteen nitroparaffins were studied for their ability to inhibit microbial growth in six different major metal cutting fluids. Tris(hydroxymethyl)nitromethane, 2-nitro-2-ethyl-1,3-propanediol dipropionate, 2-bromo-2-nitropropyl acetate and 2-nitro-2-ethyl-1,3-propanediol exhibited superior activity against both aerobic and anaerobic flora commonly found in these products. Some of the nitroparaffins were superior to over 100 phenolic compounds previously studied; tests with aerobic bacteria were carried out at 1000 ppm, and at 800 ppm with anaerobic sulfate reducing bacteria (Desulfovibrio desulfuricans). The inhibitory activity of tris(hydroxymethyl)nitromethane is believed to be due in part to its ability to release formaldehyde slowly in alkaline environment. Nitroparaffins which do not release formaldehyde are added to undiluted products by the manufacturers. In general, the nitroparaffins are cheap, relatively nontoxic, and readily soluble in the various cutting fluids. They have considerable potential use in other types of industrial deterioration problems.
In the procedure used, each test unit was reinoculated with a mixture of bacteria once a week. This is a more effective procedure than the use of a single inoculation at the beginning of the experiment. For example, with a single inoculum, 2-nitro-1-butanol was completely inhibitory for 101 days in a concentration of 500 ppm. When fresh inoculum was added weekly, the same compound was effective for 78 days in a concentration of 1000 ppm.

Anonymous.

Hitzman, Donald O., and Ralph P. Schneider (assignor to Phillips Petroleum Co.).
MICROBIOLOGICAL CORROSION PROTECTION BY GERMICIDAL ZONE AND PROTECTIVE COATING.

[Buried and partially buried objects are protected against microbiological corrosion by: (1) applying a protective coating material containing 5 to 500 ppm (usually 50 to 100 ppm) of a suitable germicide to the object; and (2) treating the 1 to 5-in. thick contiguous layer of fill to a similar level with a different germicide—providing a double defense against environmental adjustment by bacteria.]

The coating may be any of the regular commercial types, e.g., bitumen-impregnated wrapping or poly (vinyl chloride). A wide range of germicides are suitable, selection of type and level depending on such factors as nature of soil and microorganisms, and working characteristics of coatings. Fill additives are preferably water-insoluble. Typically, sulfate-reducing bacteria are combated with arsenicals or chlorophenols; sulfur-oxidizing, nitrosifying, and nitrifying types, with phenolics or cresols. Other factors influencing selection include toxicity, ease of handling and cost. Objects which can be protected include pipes, pipelines, tanks and posts.
Finely divided copper 8-quinolinolate, p-toluene-sulfonamide, or trinitrobenzene may be incorporated as a fungicide in Type 53 lining (a blend of polysulfide latex and saran latex, NAVDOCKS Spec. 47Yb). Apart from copper 8-quinolinolate, which may be added several days before use, the agents are admixed as dry powders not more than 24 hr before application of the latex.

In view of the relative immunity of Type 53 lining to fungal attack, fungicide levels should not exceed 1.0% of the weight of resin solids pending determination of the effects, if any, of the additives on fuels. The fungicidal/bactericidal efficacy of additions of 1 oz finely divided metallic silver to 100 gal Type 53 latex is under study.

Bacterial attack on petroleum products in storage or transit occurs mainly at oil-water interfaces. Water is essential for bacterial growth, but large percentages of organisms may migrate to the oil phase, and activity is known to occur in clear oil layers. Rate of consumption is proportional to interfacial area and may amount to 100 gal/yr in 55,000-gal tanks; emulsification increases area and rate as much as 10 times. Most of the hydrocarbon utilizing bacteria are ubiquitous, and active at 32 to 140 F. Aerobes, the more common problem, use atmospheric oxygen; anaerobes reduce sulfates and nitrates.

Many species consume methane. Aliphatic compounds are more susceptible to mixed cultures than aromatics or naphthenics; olefins more than paraffins; and long-chain hydrocarbons more than similar compounds of low molecular weight. Gasoline, kerosine, lubricating oil, and paraffin wax are oxidized in this ascending order of ease.
Coloration or discoloration may result, but fuels may remain clear despite abundance of filter-clogging organisms. Bacteria may destroy or seriously impair the efficacy of rust inhibitors. One type badly corrodes brass, forming a heavy gel. Hydrogen sulfide produced by anaerobic reduction of sulfate in tank water bottoms and oxidized by air to elementary sulfur can render fuels unusable. Centrifugal pumping of the heavy scum of fuel, rust, and biological matter usually present at oil-water interfaces invariably produces tolerable emulsions. Deterioration of transformer oils and lubricants and diesel fuel in laid-up engines has been attributed to oxidizing bacteria. Cutting oil emulsions require bactericides to prevent break-down.

Filters are commonly clogged by invisible biological slimes. Insoluble impurities in oils include the products and by-products of petroleum consumption by >100 biological species of about 40 genera. Degradation of stored oils is commonly marked by an odor of hydrogen sulfide, rancid fat, ammonia, or mildew. Bacteria may lower the octane rating of gasoline stored over water either by preferential attack on branched-chain hydrocarbons or by promoting the precipitation or degradation of tetraethyllead; significantly, all commercial gasoline inhibitors are bactericides. Substantial water tolerance variations in refinery stocks may be attributable to biological creation of polar molecules in either crude or distillates; clear stored fuels can form interfacial scums when shaken with water.

Anaerobes accelerate internal pitting of tank bottoms; coating insulating is broken down. In working tanks and tanker holds, they may oxidize themselves under rust blisters; aerobes cooperate by quickly removing free oxygen. Consumption of petroleum spilled on soil is rapid. Such contamination may reduce soil fertility during the first year; eventually, the activity of oil-consuming bacteria may increase fertility. Subsequent spillage on the same surface is consumed even more quickly by the heightened concentration of bacteria. Agricultural oil sprays nourish bacterial populations, so that subsequent applications disappear more rapidly. Asphalt slowly decomposes when mixed with normal soil, but not in sterile soil. Bacteria control oil pollution of water both by oxidation and by causing the oil to sink.

Biological activity may develop explosive concentrations of methane in tanks, and cause oxygen depletion in inactive tanks. Oil products are heat-sterilized in refining. Reinfection can be controlled by: (1) cleanliness of containers and pipes; (2) elimination of water bottoms; (3) substitution of distilled or rain water (low in essential nutrient mineral salts) for less pure waters; and (4) use of suitable bactericides.
In the Yareg oil field a reduction of viscosity, specific gravity, and sulfur content of petroleum was observed over a period of several years. Laboratory studies revealed the presence in the petroleum strata of sulfate-reducing and denitrifying organisms, and also other, unidentified bacteria. Microbiological change occurring in petroleum fractions under laboratory conditions depended on the composition of the fractions, culture conditions, etc. For example, in the 250 to 400°C fraction, the action of microorganisms resulted in an increase of naphthenes and a reduction of methane components. The mazut (petroleum residue) fraction showed the highest reduction of specific gravity and viscosity. An analysis of petroleum water showed the absence of nitrates, sulfates, and phosphates, i.e., conditions of "nitrogen hunger," which evidently stimulate the utilization by bacteria of the heavy, nitrogen-containing petroleum fractions. This was confirmed in laboratory experiments. In the absence of nitrogen in the culture media, the heavy fractions were utilized to a large extent, whereas they remained unchanged in media containing nitrogen. Cultivation of bacteria on the light boiler fuel fraction in the presence of adequate nutrients resulted in an increase of tar and sulfur contents and specific gravity. These experiments indicate the possibility of improving the flow of petroleum by artificially creating a "nitrogen hunger," and thus promoting the degradation of the heavy petroleum fractions.
Electrochemical model tests were carried out with the purpose of detecting the exact mechanism of anaerobic microbiological corrosion of iron and steel caused by sulfate reducing bacteria. The experimental results show that the rapid corrosion taking place in the presence of these bacteria (Sporovibrio desulfuricans) can be attributed to strong anodic and cathodic depolarization. Changes in the slope and relative position of anodic and cathodic polarization curves, observed in different phases of the experiments, are interpreted on
the basis of bacterial activity and the place of precipitation of corrosion products. Correlations between results obtained in the laboratory and those observed in practice are discussed in connection with the possibility of mitigating anaerobic microbiological corrosion.

A method of preventing formation of biological gels in distillate fuel oils comprising at least two fuel oil fractions boiling in the No. 2 fuel oil range is presented. At least a portion of one of the fractions with a total acid number of > 0.1 is treated with 1 to 20 vol % of a 2 to 40° Bé, aqueous caustic soda solution. The neutralized oil is then separated from the partially spent solution and washed with 10 to 200 vol % water containing 0.001 to 0.1% of an oil insoluble microicide, e.g., the sodium salt of 2,4,5-trichlorophenol, an alkylolylmethyltrimethylammonium chloride whose alkyl group contains 9 to 15 carbon atoms, or an alkyldimethylbenzylammonium chloride whose alkyl group contains 8 to 18 carbon atoms. After separation from the wash water, the neutralized oil is blended with the other fraction or fractions to form a fuel having an acid number < 0.1 and resistance to microbial gelation.

A total of 184 microorganisms were isolated from jet fuel-water bottom samples collected from storage tanks at nine Air Force bases. These isolates included 75 fungi, 104 bacteria, and 5 nonclassical iron-depositing bacteria; 61% of the total isolates remained viable for 20 days in jet fuel-mineral salts substrates. These may
be considered to have the ability to attack the hydrocarbons and form sludge. The viable isolates appeared to fall into five fungal and three bacterial groups. The most predominant and durable fungal group were the brown fungi, with 87% surviving the 20 day viability test. Among the bacterial groups, the more homogeneous and transparent bacterial species predominated although the heterogeneous and opaque bacteria had the greatest survival rate.

Of 178 water-soluble materials evaluated as potential sludge inhibitors, alkyl quaternary ammonium acetate \((C_{17}H_{37}O_2N)\), dimethylamine borane, and tri-n-butyl borate were effective microbicides and are recommended for trial in bulk storage tanks. Several other compounds controlled microbial growth satisfactorily, but are not recommended because of the presence of elements such as halogens or heavy metals potentially deleterious to fuel properties and fuel system materials.

A 90-day storage test showed that except for an increase in dielectric constant, microorganisms did not appreciably affect properties of jet fuel.

Submers, Claude R., Jr. (assignor to Gulf Oil Corp., Pittsburgh, Pa.).
FUEL OIL COMPOSITIONS CONTAINING ANTIMICROBIAL AGENTS.
U.S. Pat. 2,975,042; March 14, 1961. 5 p.

[Oil, fuels, fungi, bacteria, toxic, patent]

Microbial gel deposition in petroleum distillate fuels in contact with an aqueous phase can be controlled by addition of oil-soluble materials such as a 40% solution of formaldehyde in n-propyl alcohol, Dowicide 1 (o-phenylphenol) or n-propyl alcohol or by water-soluble, oil-insoluble compounds such as mixed alkyltolylmethyltrimethylammonium chlorides containing alkyl groups with 9 to 15 carbons or mixed alkylidimethylbenzylammonium chlorides having alkyl groups with 8 to 18 carbons. The water soluble compounds are dissolved either in the bottom water or incorporated in the oil using dispersing or blending agents or a mutual solvent such as acetone, alcohol or glycerol. Effective concentrations in oil or bottom water range from 0.01 to 1.0 vol %; 0.1% usually suffices. Oil may be treated at any point between refinery and burner or after infection.
A simple, fast, inexpensive and clean method of cultivating anaerobes is described. An inoculated plate and Eh indicator (B.B.L. thioglycollate-resazurin broth incorporating 1.5% agar) in a 5 ml glass cup are inserted in a 7 by 6 in. polyethylene bag with one side 1/4 to 1/2 in. shorter than the other. To a 35 mm, fine porosity Seitz filter disc placed in a plastic or metal screw cap are added 0.6 to 1.0 gm dry powdered pyrogallic acid and 2 ml of a 50% aqueous solution of anhydrous sodium carbonate. The cap is inserted in the polyethylene bag and the bag immediately sealed with 3/4 in. cellulose tape and incubated.

The method produced good growth of Clostridium perfringens, C. tetani, C. sporogenes, and anaerobic streptococci within 18 to 24 hr incubation. The resazurin indicator changed from pink to yellow in about 4 hr indicating the anaerobic state of the environment. A similar technique using sodium carbonate and a weak solution of hydrochloric acid provides a carbon dioxide environment.
(15 amino acids in all) was found in Proactinium corallinus, which, along with fungi, formed a thick pellicle on petroleum. However, only slight growth was observed and no amino acids were found in the culture fluid of this organism on the same medium but without the addition of petroleum.

Comparison with the amino acids found in ozokerite-like bitumens shows that the amino acid composition of the petroleum contains all the amino acids present in the bitumen and more. Amino acids in the bitumen were detected only after hydrolysis with 25% sulfuric acid and only in the more altered fraction of dark paraffins. Data obtained suggest that the process of enrichment of the original material by amino acids can occur under conditions of aerobic utilization of paraffin-base petroleum by microorganisms. The amino acid-containing complexes found in bitumens apparently originated as the result of the death of microbial cells growing at the expense of petroleum, and are included in the composition of the bitumen in the form of the autolytic products of protein. Individual free amino acids can be further assimilated by other microorganisms.

Lyalikova, N.N.
THE PHYSIOLOGY AND ECOLOGY OF THIOBACILLUS FERROOXIDANS IN RELATION TO ITS ROLE IN THE OXIDATION OF SULFIDE ORES (REVIEW).
Microbiology 29:556-560 (March-April 1961)
[Bacteria, iron, Thiobacillus, physiology, biomechanism, oxidation, review]

Skalon, I.S.
A NEW METHOD FOR SEPARATING AEROBIC AND ANAEROBIC SPECIES OF MICROORGANISMS.
Microbiology 29:657-658 (May-June 1961)
[Fungi, bacteria, enzyme, isolation, analysis, technique]

MICROORGANISMES RESPONSABLES DE L'ALTERATION DE L'HUILE DE PALME PENDANT LE STOCKAGE [MICROORGANISMS RESPONSIBLE FOR THE ALTERATION OF PALM OIL DURING STORAGE].
Oleagineaux 16:227-233 (April 1961)
[Oil, Palm oil, storage, fungi, decomposition, Aspergillus, Mucor, Rhizopus, Penicillium, foreign]

A mycological study was made of 202 samples of oil which were collected at various production and storage
sites in Nigeria, 18 collected in Ghana, 6 in Sierra Leone and 6 in South Cameroun. Numerous lipolytic fungi and yeast were isolated from these samples on an agar medium containing oil, Tween, and Nile Blue pH indicator. The frequency of occurrence of the fungal species isolated varied within wide limits. Thus, Paecilomyces varioti was found in 53 samples, Aspergillus gigantea in 40, A. niger in 33, and Rhizopus nigricans in 31. Among species found in a few samples only were: A. tamarii, Monilia aceronomia, Penicillium lapiduosum, and Mucor corticulus.

These 8 species and two species frequently occurring in fruits of the palm tree (Cunninghamella elegans and a Sclerotium species) were inoculated on sterile samples of palm oil, containing 0.5, 1, 2, 4, and 8% water, and incubated at about 25°C for 8 wk. Weekly determinations were made of the "lipolytic effect" of the fungi, i.e., of the difference between free acidity of the inoculated samples and that of sterile control samples. Preliminary tests showed that the increase of acidity upon storage at 25°C of sterile oil is practically independent of its water content, and averages 0.3% per mo. The tabulated data for the 10 organisms tested show that, with the exception of Mucor corticulus, all fungi accelerated the rate of palm oil hydrolysis. A niger, A. tamarii, Paecilomyces varioti, Rhizopus nigricans, and Sclerotium are classified as strongly lipolytic, causing after 8 wk a lipolytic effect of 1.5-3.3 even at low water concentrations. The remaining 4 species were weakly (0.5-0.9) lipolytic. For most species the lipolytic effect was somewhat higher at high water contents. Since the lipolytic activity of fungi is significant at water concentrations below the permissible maximum for export (2%), the lipolytic microorganisms can be considered as the main cause of palm oil deterioration during storage at temperatures at which the autocatalytic hydrolysis process is very slow.
Hydrazine derivatives of the general formula \(XH(R)N(R')Y\) are highly active against a wide spectrum of bacteria and fungi including plant pathogens. \(R\) and \(R'\) represent a carboxylic acyl group with 1 to 18 carbons; \(X\) and \(Y\) are preferably \(\text{SCCl}_3\) groups but one or the other may be hydrogen. Compounds are prepared by reacting an alkali metal salt of the diacyl hydrazide with perchloromethyl mercaptan. They may be used in solid or liquid form in the manufacture of leather, paper, felt, glue, size and aqueous cutting oils to protect against damage by fungi and bacteria.

The following preparations used in the petroleum industry for control of biological corrosion were tested against Desulfovibrio desulfuricans:

1. an alkylaminopropylene amine (16-18 carbons);
2. acetate of primary copramine;
3. dimethylbenzyldecylammonium chloride;
4. dimethyldicoprammonium chloride;
5. dodecyldimethylammonium oxide or hydroxide;
6. sodium...
alkylaminopropionate (alkyl derived from copra fatty acids); (7) sodium alkylaminoacetate (alkyl from copra fatty acids); (8) octadecyldipolyglycolbenzylammonium chloride; (9) a 1:1 mixture of 3 and 4; (10) a 1:1 mixture of 3 and 2; (11) a 1:1 mixture of 3 and 6; (12) a complex of copper alkylaminopropionate; (13) a complex of copper dodecylaminoacetate; (14) formaldehyde (40% aqueous solution).

Preparations 2 and 14 were bacteriostatic in a concentration of 10 ppm; 10 and 13 were active at 30 ppm; 3 and 9 at 30 ppm; 11 at 40 ppm. Preparations 1, 4, 5 and 12 were bacteriostatic at 50 ppm, compounds 6, 7 and 8 at 80 to 100 ppm.

Preparations 1, 2, 3, 4, 8, 9 and 13 had a strong bactericidal action, killing 100% of bacterial cells within 15 min at a concentration of 5 ppm. At the same concentration, preparations 5 and 10 showed a slow but satisfactory killing action, whereas 6, 7, 11, 12 and 14 gave unsatisfactory results (6 to 30% surviving cells after 1 hr contact).

Generally the bactericidal paralleled bacteriostatic action; preparations 8 and 14 were exceptions. The former had a weak bacteriostatic action but was strongly bactericidal; the latter a strong bacteriostatic but a slow killing action. Compounds containing the dodecyl radical were particularly effective bactericides.


Lubricating oils for optical instruments under conditions of high temperature and humidity (such as in a tropical climate) are protected against fungal attack for 1 to 1.5 yr by adding 1% by wt of powdered hexylresorcinol to the oil heated to 90 to 95 C. The lubricant can be a mixture of an oil and ceresin with or without the addition of small quantities of bitumen, petrolatum, rosin and slaked lime.
A one-day accelerated test for distillate fuels of widely varying composition is described. One-liter oil samples which have been filtered through filter paper are placed in a 0.5 gal bottle with four freshly sand-blasted strips of 5 x 3 x 1/16 in. SEA 1020 steel. The oil is purged with oxygen for 5 min, then the bottle is sealed, oven heated for 24 hr at 212 ±2 F, cooled at room temperature for 0.5 hr and in cold tap water for another 0.5 hr. Solids formed are determined by suction-filtering the oil through a weighed gooch crucible. Results correlate well with sediment formation in a 2-yr field storage test and in long-term laboratory drum and bottle tests. The test can be used to predict the effect on stability of manufacturing variables such as caustic washing, inhibitors and hydrogenation. The One-Day Iron Stability Test gives reasonable agreement with a 9-mo field test in 100 barrel tanks. The test is useful for screening sediment-inhibiting additives but does not pinpoint small differences in effectiveness of additives. It will not determine actual amount of color darkening in field storage but gives an indication of the relative color stability of a group of base oils without additives. It should be used only for predicting the stability of freshly manufactured oils (not older than 2 wk) because storage time under air increases sediment formation.
The oil-water ratio in soluble oil emulsions may have a profound effect on activity of added antibacterial agents. In tests with nineteen compounds with oil-water ratios varied from 1:10 to 1:100, using Pseudomonas aeruginosa as the test organism, a 1:40 dilution usually was most difficult to inhibit. Inhibitory concentration generally rose sharply as dilution increased from 1:10 to 1:40, dropped slightly to a 1:70 dilution and then fell to a 1:90 dilution. There was no significant difference in the amount required for a 1:100 dilution.

Only one compound, 1-bromo-1-nitropropane, was effective in the same concentration (100 ppm) in all oil-water ratios; 2,4,6-trichlorophenol was most markedly affected by dilution (50, 1100 and 400 ppm being required at 1:10; 1:40 and 1:90 dilutions, respectively). Outstanding nitroparaffins were 2-nitro-2-methyl-1,3-propanediol, 2-nitro-2-ethyl-1,3-propanediol and 2-nitro-2-ethyl-1,3-propanediol dipropionate. These were effective at 40, 50, 40; 20, 40, 40 and 50, 100, 50 ppm at dilutions of 1:10, 1:40 and 1:90, respectively. In general, sodium ethylmercurithiosalicylate was more active than any of the nitroparaffins; it was effective at 5, 75 and 5 ppm at dilutions of 1:10, 1:40 and 1:90, respectively.
Sixty-three phenolic compounds were studied for their inhibitory activity against Desulfovibrio desulfuricans. A concentration of 25 ppm of 2-bromo-4-phenylphenol, 4-chloro-2-cyclohexylphenol and 2-chloro-4-nitrophenol completely inhibited hydrogen sulfide production for 28 days. Eleven compounds were ineffective at 1000 ppm; thirty were inhibitory at 1000 ppm and the remaining nineteen were inhibitory in concentrations from 50 to 500 ppm.

Inhibitory activity of approximately 200 nitroparaffins against the sulfate reducing bacterium, Desulfovibrio desulfuricans, was determined. The following 7 compounds at a concentration of 25 ppm completely inhibited for 28 days production of hydrogen sulfide (used as a criterion of growth): 3-chloro-3-nitro-2-butanol, 2-chloro-2-nitro-1-butanol stearate, 2-chloro-2-nitrobutyl acetate, 4-chloro-4-nitro-3-hexanol, 1-chloro-1-nitro- and 3-chloro-3-nitro-2-pentanol and 2-chloro-2-nitro-1-propanol. Forty-one compounds were ineffective at concentrations of 1000 ppm; the remaining compounds were inhibitory at concentrations from 50 to 1000 ppm. Sulfur bacteria were more sensitive to many of these compounds than to mercurials or phenols. The effect of molecular substitution appears somewhat similar to that observed with other compounds such as phenols. Nitro are more effective than amino groups and chlorine more than bromine groups.
BENZIN, PETROLEUM, PARAFFIN OIL AND PARAFFIN AS CARBON- AND ENERGY SOURCE FOR MICROBES.


[PETROLEUM, gasoline, paraffins, hydrocarbons, bacteria, Mycobacterium, Micrococcus, Actinomyces, decomposition, nutrition, growth, tests, foreign]

Munz, E.
ZUR PHYSIOLOGIE DER METHANBAKTERIEN [ON THE PHYSIOLOGY OF METHANE BACTERIA].

[Bacteria, methane, physiology, foreign]

Tausz, Jeno, and Marta Peter.
NEUE METHODE DER KOHLENWASSERSTOFFANALYSE MIT HILFE VON BAKTERIEN [NEW METHOD OF HYDROCARBON ANALYSIS WITH THE AID OF BACTERIA].

[Hydrocarbon, analysis, bacteria, technique, foreign]

Allen, Fraser H.
THE MICROBIOLOGICAL ASPECTS OF GASOLINE INHIBITORS.

[Petroleum products, gasoline, bacteria, toxics, phenols, storage, stabilizers]

Tausson, W.O.
UBER DIE OXYDATION DER BENZOLKOHLENWASSERSTOFFE DURCH BAKTERIEN [THE OXIDATION OF BENZENE HYDROCARBONS BY BACTERIA].
Planta 7:735-758 (May 1929)

[Hydrocarbon, benzenes, oxidation, bacteria, foreign]

Lipman, C.B., and L. Greenberg.
A NEW AUTOTROPHIC BACTERIUM WHICH OXIDISES AMMONIA DIRECTLY TO NITRATE AND DECOMPOSES PETROLEUM.
Nature 129:204-205 (February 1932)

[Petroleum, ammonia, decomposition, oxidized, bacteria, Bacillus]
THE ROLE OF ANAEROBIC \( \text{-PHOSPHOSULFATE } \) IN THE REDUCTION OF 
SULFATE TO SULFIDE BY \text{DESULFOVIBRIO } \text{DESULFURIANS}. 
J. Biol. Chem. 131:651-666 (January 1942)

[Fungi, Desulfovibrio, biodegradation, culture]

Selit' r, I.L., and I.V. Dotrovol' skaya. 
PROLONGED HIDATION BY BACTERIA OF THE ABILITY TO DECOMPOSE FAT. 
Microbiology 30:58-64 (July-August 1961)

[Fat, decomposition, bacteria, Bacterium, Pseudomonas, biodegradation]

U.S. Armed Services Technical Information Agency (Ruth B. Henery). 
COMPIIATION OF REFERENCES ON MICROBIOLOGICAL CONTAMINATION 
OF FUELS. 

[Fuel, petroleum, bacteria, fungi, research programs, tests, 
standards, bibliography, abstracts]

MICROORGANISMS ATTACKING PETROLEUM AND PETROLEUM FRACTIONS. 
J. Bacteriol. 39:91 (1940)

[Petroleum, bacteria, culture]

Bushnell, L.D. and H.F. Haas. 
THE UTILIZATION OF CERTAIN HYDROCARBONS BY MICROORGANISMS. 
J. Bacteriol. 41:651-673 (1941)

[Hydrocarbons, petroleum products, gasoline, kerosene, 
mineral oil, paraffin, decomposition, bacteria, Pseudomonas, 
Corynebacterium, Mycobacterium, Proteus, Mycopluja, Escherichia, 
Staphylococcus, Azotobacter, Serratia, Aerobacter., Rhizobium, 
Lactobacillus, Bacillus, Sarcina, Spirillum, Eberthella, 
Shigella, Salmonella, growth, culture, tests]

Hopkins, Sydney John and Albert Charles Chibnall. 
GROWTH OF ASPERGILLUS VERSICOLOR ON HIGHER PARAFFINS. 
Biochem. J. 26:113-142 (1932)

[Paraffins, fungi, Aspergillus, growth]
This report concerns the microbial flora found throughout the surface facilities of six water-injection systems in Texas and Oklahoma. Each system is described in detail and water quality data are presented when available.

Desulfovibrio, Pseudomonads, Sphaerotilus and Bacillus were predominant genera. Achromobacter, Micrococcus, Clostridium, Flavobacterium and Sarcina were also isolated in significant numbers. Molds, iron bacteria, sulfur bacteria and soil bacteria occurred less frequently. Pseudomonads and Desulfovibrio were found in all systems. There was no relationship between age of the system and type of flora, nor were there specific differences in flora of untreated systems and those treated with a microbicide. The greatest number of different species (per sample) occurred in two systems handling produced brine containing appreciable quantities of entrained oil. Problems due to microorganisms were apparent in only one system. Membrane filter tests indicated that bedding filters generally do not improve water quality. They do not remove fine solids, and filters and other surface vessels are excellent environments for microbial growth.
Muraoka, James S.

THE EFFECTS OF MARINE ORGANISMS ON ENGINEERING MATERIALS FOR DEEP-OCEAN USE.


[Bacteria, sulfur, fungi, foulers, borers, sea water, iron, steel, nonferrous metals, concrete, polymers, elastomers, cordage, Proactinomyces, Actinomyces, Pseudomonas, Micromonaspora, Thiobacillus, Desulfovibrio, stone, wood, bibliography, review]
Barclay, T., and P. Spencer.  
THE EFFECT OF DULCINATE ASSIMILATION ON THE INDUCTION OF  
ARGININIC ACID SYNTHESIS IN FUNGI.  
[Fungi, arginine, culture, biosynthesis]  

Romnenko, V.I.  
USE OF THE AUTORADIOGRAPHIC METHOD FOR A QUANTITATIVE ASSAY  
OF METHANE-OXIDIZING BACTERIA.  
Microbiology 30:292-293 (September-October 1961)  
[Methane, oxidation, bacteria, assay, technique]  

Arnshtein, A.M.  
ROLE OF AZOTOBACTER IN BIOLOGICAL PURIFICATION OF PETROLEUM  
WASTE WATERS.  
Microbiology 30:269-271 (September-October 1961)  
[Petroleum, waste water, decontamination, bacteria, Azotobacter]  

Bogdanova, V.M.  
UTILIZATION OF NITROGENOUS PETROLEUM COMPOUNDS BY MICROORGANISMS  
UNDER ANAEROBIC CONDITIONS.  
Microbiology 30:265-266 (September-October 1961)  
[Petroleum, nitrogen, bacteria, decomposition, analysis]  

Kellogg (M.S.) Company, New York, N.Y. (Walter D. Schmidt  
and Warren C. Shreiner).  
PRELIMINARY DESIGN STUDY FOR A JET FUEL PURIFICATION CENTER  
FOR AIRCRAFT CARRIERS.  
[Fuel, jet, contaminants, purification, filtration, ship]  

Purolator Products, Inc., Rahway, N.J. (Richard G. Seed and  
Arthur A. Fowle).  
A GENERAL STUDY OF DIVERSE FILTRATION PHENOMENA WITH POSSIBLEL  
APPLICATIONS TO AIRCRAFT FUEL FILTRATION.  
U.S. Wright Air Development Center. Technical Report 54-181;  
... ASTIA Doc. 33464 (November 1952) 58 p.  
[Fuel, jet, contaminant, inc, filtration, applications, review]
BIBLIOGRAPHY ON MICROORGANISMS AFFECTING PETROLEUM AND PETROLEUM PRODUCTS INCLUDING REPORTS ON SULFATE-REDUCING BACTERIA (REVISED).


1. Bennett, E.O.
   THE DETERIORATION OF PETROLEUM PRODUCTS BY BACTERIA.

2. Yeager, Charles C.
   MICROBIAL PROBLEMS IN THE REFINING OF OIL.

3. Lada, Arnola.
   THE ROLE OF MICROORGANISMS IN SECONDARY OIL RECOVERY.

   STUDIES ON INDUCTION AND REPRESSION IN ACTIVATED SLUDGE SYSTEMS.
   Applied Microbiol. 10:264-271 (May 1962)
Cladding, J., A. Arndt, and D. Ghezzi. 
ANALYSIS OF POLARIZATION OF \( \text{H}-5\text{-HET}-1\text{-ANE} \) FROM \( \text{\textit{H}}\)-HEPTANE BY RASTING 
THY OF \( \text{HYDROCARBON~AERUGINOSA} \). 

[bacteria, \textit{Pseudomonas}, hydrocarbon, heptane, biocatalysis]

Center (Walter M. Rejuki). 
CONFERENCE MINUTES; INTERAGENCY BASIC RESEARCH PLANNING 
HYDROCARBON JET FUELS AND FUEL SYSTEMS MICROBIOLOGY. 
August 1977. 70 p.

[Fuels, jet, bacteria, fungi, contaminants, conference]

Forget, A., and V. Fredette. 
SODIUM AZIDE SELECTIVE MEDIUM FOR THE PRIMARY ISOLATION OF 
ANAEROBIC BACTERIA. 
J. Bacteriol. 133: 1217-1222 (June 1978)

[bacteria, aerobes, anaerobes, isolation, technique]

Rogoff, Martin H. 
CHEMISTRY OF OXIDATION OF POLYCYCLIC AROMATIC HYDROCARBONS 
BY SOIL PSEUDOMONADS. 

[Hydrocarbons (aromatic), oxidation, bacteria, \textit{Pseudomonas}, biocatalysis]

Isoro, Masao, and Takeo Abe. 
MICROBIAL TRANSFORMATION OF STEROIDS. PART I. 1-DEHYDROGENATION 
OF HYDROCORTISONE BY BACTERIA (in Japanese). 

[Steroids, oxidation, bacteria, \textit{Pseudomonas}, \textit{Acetobacter}, 
\textit{Xanthomonas}, \textit{Micrococcus}, \textit{Vitriol}, \textit{Sarcina}, \textit{Lactobacillus}, 
\textit{Acetobacter}, \textit{Flavobacterium}, \textit{Escherichia}, \textit{Aerobacter}, 
\textit{Serratia}, \textit{Bacterium}, facultative, culture, technique, foreign]

Isoro, Masao, and Takeo Abe. 
MICROBIAL TRANSFORMATION OF STEROIDS. PART II. OXIDATION 
OF HYDROCORTISONE BY THE MIXED CULTURE OF DIFFERENT BACTERIA. 

[Steroids, oxidation, bacteria, \textit{Pseudomonas}, \textit{Acetobacter}, 
\textit{Xanthomonas}, \textit{Micrococcus}, \textit{Vitriol}, \textit{Sarcina}, \textit{Lactobacillus}, 
\textit{Acetobacter}, \textit{Flavobacterium}, \textit{Escherichia}, \textit{Aerobacter}, 
\textit{Serratia}, \textit{Bacterium}, facultative, culture, technique, foreign]
Corrosion of aircraft turbine fuel tanks whose surfaces are coated with Suna-N phenolic resin is directly related to the location and concentration of contaminants: corrosion appears as small discolored areas, mainly where contaminants settle by gravity. Pitting to 0.030 in. can affect 30% of a surface within 4 mo. Intergranular attack is mainly subsurface and proceeds along elevated grain boundaries. Contaminants in fuel supplies include saline or brackish water, surfactants originating as refinery by-products or oxidation products, iron rust, and microorganisms. These contaminants may interrelate as follows. Water, rust, and microorganisms enter the tanks through surfactant-contaminated fuel or deteriorated filter-separators. Rust holds the water, providing a bed for the growth of bacteria in contact with the topcoating. Bacterial acid products react with the rust which penetrates the topcoating; the organic acids then attack the aluminum.

Recently developed detection methods and an improved quality control procedure are described.
designer and the electrical system control specialist to insure more practical servo valve and hydraulic system requirements. Some contamination will always prevail and must be designed for. The controls and hydraulics industry should sharply reappraise the impractical fluid cleanliness standards now imposed on it.

Jet fuel contamination and resultant tank corrosion have caused aircraft accidents. The problem is reviewed. Complete analysis of unusual materials is imperative to prevent unexpected and intolerable contamination; fuels generally contain traces of soluble or insoluble matter which do not require complete removal. Distribution systems effectively remove solids and water by filter coalescer equipment. Surfactants, particularly those extracted by water, impair coalescer operation and may contribute to microbial growth and tank corrosion. The CRC (Coordinating Research Council, Inc.) Water Separometer test is useful in detecting surfactant contaminants. In dispensing fuel, proper procedures must be followed rigorously to eliminate free water and tramp solids. The relative efficacy of anti-icing additives in fuel can be measured by low-temperature pumpability criteria. Laboratory techniques investigated serve to measure the effects of additives on organic tank coatings and of fuel-additive combinations on filter-separators.

Samples of used cutting fluids were collected weekly for 10 wk from five central sumps selected to include three major types of cutting fluids: straight soluble oil, heavy duty soluble oil and oil-free synthetic solutions. Germicides used were o-phenylphenol in three sumps, tris(hydroxymethyl)methane, and a mixture of 2-mercaptobenzothiazole and sodium dimethyldithiocarbamate. Samples were cultured separately for total counts, coliform counts and anaerobic sulfate reducers. In-plant conditions were checked with biological results.

Correlation between coliform number and oil deterioration was low. Conversely, sulfate reducing bacteria were much in evidence in spoiled and malodorous emulsions. Storage at 10 C for 4 wk greatly reduced total and coliform counts but levels of sulfate reducers represented by hydrogen sulfide were maintained. Other factors which related to coolant life included heat stability, tramp oil acceptance, fine retention and corrosivity.
Eight of ten samples from storage and aircraft fuel tanks at Ramy and Eglin AFB contained tan or black sediments and/or slimy gelatinous sludge. Motile and nonmotile bacteria predominated; fungi filaments were found in three, with no growth in the substrates. Brown fungi were present in two storage tanks and black fungi in a KC-135 tank. Transparent bacteria occurred in eight samples, opaque bacteria in six, and mucoid bacteria in one. No chromogenic, sulfate-reducing, sulfur-depositing or iron-oxidizing bacteria were found. Sulfur-oxidizing bacteria may have been present in one wet sludge sample from a corroded wing tank. Iron-depositing bacteria were present in eight samples and occurred throughout Ramy AFB. Analysis of dry and wet sludge from an aircraft tank at Ramy AFB showed a high content of ash oxide, nitrogen and sulfur, indicating presence of large amounts of inorganic salts and organic sediment. Spectrographic analysis showed aluminum as the main constituent, with minor amounts of silicon, sodium, zinc, magnesium, iron and calcium. The wet sludge contained 2,400 ppm chloride, indicating a high salt water content.
Oil/water interfacial samples from the AVCAT stowage tanks of H.M.S. "Victorious" contained *Hormodendrum* sp., probably a strain of *Hormodendrum* Nordoi Bruhne or an allied species. No evidence of *Nocardia* was found. Of 12 fungitoxics tested, 8-quinolinol sulfate was the most lethal (at 1:20,000 against cultures) and the only one meeting other requirements of the application. These included solubility in water but not in fuel, effectiveness at very low concentrations, low cost, nontoxicity, nonprecipitating in sea water, nonfoaming, nonemulsifying, and having no metal radicals.

Growths from the hydraulic system of H.M.S. Centaur consisted of the hyphal stages of *Aspergillus* spp. The hydraulic fluid is a 40/60 glycerol-water mixture containing disodium hydrogen phosphate and sodium nitrite as corrosion inhibitors. The system should be thoroughly cleansed using an aqueous solution of 0.1% 'Panacide' sodium and then filled with the hydraulic fluid containing a similar amount of the fungicide. The fluid should be changed every 3 mo.

Bacterial spoilage of cutting oil emulsions is prevented by addition of 25 to 2500 ppm (usually 100 ppm) of a compound of the type RNHgCO₂R' where R is an alkyl or alkoxyl alkyl with 1 to 4 carbons and R' is an alkyl with 1 to 3 carbons. Methylmercury acetate is claimed.
A study was made of Thiobacillus ferrooxidans isolated from the acid drainage of some northern West Virginia coal mines. Cultures isolated from ferrous iron agar, then subcultured for 85 consecutive transfers in thiosulfate broth during a period of 14 mo, were able to use ferrous iron as their energy source when returned to ferrous iron broth. Thiosulfate utilization was quantitatively followed by iodine titration and pH change; ferrous iron oxidation was quantitatively determined by use of 1,10-phenanthroline. Characteristic colonial forms were produced by T. ferrooxidans growing on thiosulfate agar irrespective of whether the inoculum was from iron broth or thiosulfate broth.
A new approach to the control of fungi and bacteria which contaminate aircraft fuel is the accumulation of information on the chemical make-up of the cell walls of four of these organisms. Particular emphasis is on the resistant wall materials, chitin and cellulose.

Cytochemical analyses of Aspergillus niger ATCC 6275 reveal the presence of chitin in the longitudinal and cross walls of the hyphae, and in the primary lamellae; both chitin and cellulose occur in the secondary lamellae of the walls of conidiophores, vesicles and conidia. Similar analyses show the presence of chitin alone in the walls of the corresponding cells of Myrothecium verrucaria ATCC 9095. Adaptations of standard cytochemical procedures to identification of cell wall materials in Pseudomonas aeruginosa ATCC 13388 indicate the probable presence of chitin in the primary and of cellulose in the secondary wall lamellae. Attempts to identify cell wall materials in Escherichia coli K12 10798 show definite differences in the make-up of the primary and secondary wall lamellae and the possible presence of cellulose in the secondary deposits.

Maruzzella, Jasper C., Saul Reine, Harold Solat and Arthur Zeitlin.
THE ACTION OF ESSENTIAL OILS ON PHYTOPATHOGENIC BACTERIA.
Plant Disease Rept. 47:23-26 (January 1963)

At concentrations from 1:1000 to 1:10,000, in vitro activity of 123 essential oils was tested on growing cultures of Corynebacterium michiganense ATCC 10202, Pseudomonas glycinea NRRL B-838, P. striafaciens NRRL B-864, and Erwinia carotovora ATCC 5011. Seventy-eight oils inhibited ≥ 1 organism at concentrations ≤ 1:4000. Organum red (40 to 45% phenols), redistilled Madagascar clove leaf, Zanzibar clove stem, redistilled Cassia cinnamon, Zanzibar clove, northern wintergreen leaf, Ceylon cinnamon bark and pimento leaf were highly inhibitory against all organisms. C. michiganense was the most and P. glycinea the least sensitive organism.
Sulfate-reducing bacteria are widely distributed in oil producing and water injection systems. Frequently they cause plugging of injection wells and a severe type of pitting corrosion. They enter water injection systems via contaminated drilling muds, supply water or produced fluids. Suspect situations include: 'black water' or water containing suspended iron sulfide particles; sour gas (hydrogen sulfide) injection systems; sour oil, gas or water wells; and pitting corrosion, particularly where tubercles and iron sulfide are evident. Positive identification is described in API RP 38. Each water system has its own tolerable 'critical number' of these bacteria. Control is by choice of equipment and by effective bactericides. Regular bacterial analyses, judiciously interpreted, are the best guide to adequate control.
TSF-6201 also supported a slower rate of growth than did similar fractions of TSF-6203, a fuel that consistently showed a high growth rate. The growth-retardant components were not isolated.

Of forty-four hydrocarbons (forty-two saturated naphthenes and two straight-chain paraffins) screened, only dodecane and cetane, the only straight-chain hydrocarbons, were attacked. Urea clathrate formation disclosed that TSF-6201 contained less n-alkanes than TSF-6203. On incubation of n-alkane fraction of TSF-6201 the growth rate was greater or at least equal to that of TSF-6203. These results indicate that microorganisms in the NRL mixture attack n-alkanes preferentially. This is confirmed from the slow rate of growth of RP-1 fuel which contains mostly saturated naphthenes with only a low percentage of n-alkanes.

PDL-47137

BACTERIAL OXIDATION OF GASEOUS ALKANES.
Arch. Mikrobiol. 35:92-104 (1960)

PDL-47197

General Dynamics, Convair, Fort Worth, Texas.
MICROBIOLOGICAL CORROSIVE EFFECTS ON STRUCTURAL MATERIALS USED IN AIRCRAFT FUEL TANKS.

Microbiologically corroded integral fuel tank specimens from B-520, KC-135 and F-102 aircraft were cut into sizes suitable for microscopic examination and identity of the aluminum alloy was verified by emission spectrograph. Types of corrosion were determined by visual and metallographic examination of specimens sectioned through typical areas of attack. Intergranular corrosion predominated in the cratered and exfoliated surfaces of 7178 and 7075 alloys, and pitting corrosion with some intergranular attack on 2024 alloy. Corrosion varied from small isolated pits, 0.001 in. deep, to large areas of exfoliation 0.0224 in. deep. Screening tests on mechanical, chemical and electrochemical methods for removing sealants, coatings and corrosion products established that chemical techniques using a mixture of nitric and hydrofluoric acids were most suitable. Suitable methods were established for measuring depth of attack and for classifying corrosion. These employ an optical micrometer, an optical comparator, a stereomicroscope and a metallograph. Additional work is required in measurement and classification of surface areas and volume of attack.

Screening tests were initiated to select microbiological and chemical corrosion systems capable of duplicating the corrosion on fuel tank specimens. The microbiological system includes natural inoculum from Ramey AFB and single and composite cultures of specified bacteria and fungi in deionised water/fuel, Bushnell-Haas/fuel, Bushnell-Haas-cystine/fuel and seawater/fuel
media. Chemical investigations include a variety of systems designed to produce concentration cells, galvanic cells, chemical attack, etc., in various standard and modified environments.

Future plans include tensile and fatigue testing of specimens of the following alloys, 2024-T351, 7075-T651, 7079-T651 and 7178-T651, after being corroded by both chemical and microbial means.

PDL-47445
Baumgartner, A.W.
MICROBIOLOGICAL CORROSION—WHAT CAUSES IT AND HOW IT CAN BE CONTROLLED.
[Oil wells, ferrous, corrosion, mechanism, bacteria, sulfur, Desulfovibrio, water systems, preventive treatments]

PDL-47478
Von Riesen, V. Lyle.
HYDROGEN SULFIDE PRODUCTION BY PSEUDOMONAS AERUGINOSA. II. QUALITATIVE SUBSTRATE STUDY.
[Bacteria, sulfur, Pseudomonas, biomechanism]

PDL-47479
U.S. Quartermaster Corp. Quartermaster Research and Engineering Center, Natick, Mass.
MINUTES OF ELEVENTH CONFERENCE PREVENTION OF MICROBIOLOGICAL DETERIORATION OF MILITARY MATERIEL.
November 1962. 76 p.

PDL-47517
Schwitzer, M.K., and Achaz V. Saldern.
SCHUTZ GEGEN KORROSION UND MIKROORGANISMEN IN PRIMAREN OLSONDEN UND FLUTUNGAANEN MIT HILFE VON KATIONOGENEN OBERFLACHENAKTIVEN SUBSTANZEN [PROTECTION FROM CORROSION AND BACTERIA IN PRIMARY AND SECONDARY OIL PRODUCTION BY USING CATIONIC SURFACE ACTIVE AGENTS].
Erdol u. Kohle 15:974-977 (December 1962)
[Oil wells, oil fields, bacteria, toxic, corrosionproofer, amines, surfactants, foreign]

PDL-47565
Ishimoto, Makoto, and Daisaburo Fujimoto.
BIOCHEMICAL STUDIES ON SULFATE-REDUCING BACTERIA. X. ADENOSINE-5'-PHOSPHOSULFATE REDUCTASE.
J. Biochem. (Japan) 50:299-304 (October 1961)
[Bacteria, sulfur, Desulfovibrio, enzymes, culture, techniques]
Wilcockson, George W. (assignor to U.S. Borax and Chemical Corp., Los Angeles, Calif.).

METHOD FOR INHIBITING BACTERIAL ACTIVITY IN PETROLEUM.

[Petroleum products, storage, bacteria, toxics, boron compounds (organic), patent]

With or without a water bottom, petroleum and its fractions and other hydrocarbons in storage are protected from bacteria by adding an alkali metal borate glycol and an alkyylene group of 2 to 6 carbons in length and containing a total of 2 to 20 carbons. Claimed compounds are sodium or potassium ethylene (or diethylene) glycol borate, sodium or potassium 1,2-propanediol borate, sodium or potassium 2,3 (or 1, 4)-butanediol borate, or sodium triethylene glycol borate. Compounds are formed by heating 6 to 20 moles diol to 1 mol alkali metal tetraborate at 75 to 110 °C for a sufficient time to drive out water. Liquid condensation product is added in sufficient quantity to cover the tank bottom.


SOME EFFECTS OF MICROBIAL GROWTHS ON SURFACANT PROPERTIES OF FUELS.
Biotechnology and Bioengineering 4(4):357-367 (December 1962)

[Fuels, jet, bacteria, fungi, surface effects]

Fungi and bacteria from fuels (JP-4, JP-5 and an alkylate bottom fraction) were grown in jet fuel (or hydrocarbon)—salt solution mixtures, and the layers were analysed for surfactant properties. Controlled surface activity was imparted to some samples by adding minute quantities (1000 ppm) of either petroleum sulfonates or petroleum naphthenates. Analyses after growth periods of 12 to 14 wk showed either little change or slight reduction in the surface active properties of any sample. Examination of the aqueous bottoms revealed that microbial growths did effect increases in their surface active properties.

Traxler, R.W.

MICROBIAL DEGRADATION OF ASPHALT.
Biotechnology and Bioengineering 4(4):369-376 (December 1962)

[Asphalt, decomposition, bacteria, fungi]
In flooding oil-bearing strata for petroleum recovery, anaerobic sulfate-reducing bacteria are controlled by injecting at an effective level (normally 10 to 15 ppm) an aqueous solution of reaction products (at 100 to 300°C for 1 to 4 hr) of an oxirate-containing compound and an oxyalkylation-susceptible nitrogen-containing compounds on mixtures thereof. The product may be formed from: (1) epoxidized soybean oil reacted with ethylenediamine or a mixture of diethylenetriamine, triethylenetetramine and tetraethylenepentamine; (2) epoxybutyl stearate reacted with hydrazine or the above mixture of amines; or (3) an epoxidized alcohol derived from soybean oil reacted with the amine mixture.

During flooding of oil-bearing strata with water, sulfate-reducing bacteria are controlled by adding 2 to 100 ppm of certain nitroamines. Claimed compounds include 2-nitro-2-ethyl-1,3-bis(dimethylamino)propane, 2-nitro-2-methyl-1,3-bis(dibutylamino)propane, 2-nitro-2-methyl-1,3-dimorpholinopropane, 2-nitro-2-methyl-1,3-bis(2-hydroxypropyl)aminopropane and 2-nitro-methyl-1,3-dipiperidylpropane.
Organic contaminants containing oxygen, nitrogen or sulfur from corrosive sludge in liquid petroleum hydrocarbons, particularly jet fuels in contact with sea water, are reduced by introduction of strains of microorganisms (Bacillus, Achromobacter, Pseudomonas, Thiobacteria or mixtures thereof) which metabolize one or more of the contaminants. Organisms for use in the liquids hydrocarbons are obtained from sludge formed at the interface of a crude oil and salt water. They are cultured in sea water containing as an added nutrient a compound with at least one of the elements, oxygen, nitrogen or sulfur, present in the contaminant. Cultures are added to contaminated fuels containing sea water and incubated.
One hundred and twelve strains of bacteria isolated from soils were cultured in media containing 5% kerosene as carbon source, 2% agar, 0.25% K$_2$HPO$_4$, 0.1% MgSO$_4$ 7H$_2$O, and 0.5% NH$_4$NO$_3$ or 0.3% urea. In 4 days at 26.5 C, 47 strains produced amino acids; 17 strains produced ultraviolet absorbers. Amino acids were identified by paper chromatography as alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, serine, tyrosine and valine. One strain growing in the kerosene—mineral salts—urea medium attained a dry cell yield of 0.8 gm/100 ml after 3 days, of which 5% was ribonucleic acid.

A 14-mo field study evaluated an organo-boron microbicidal fuel additive, Biobor RD, in a storage tank containing 1 million gal diesel fuel and in the operation of 5 diesel locomotives. The following conclusions are drawn: Microorganisms suspend water and other fuel contaminants and induce filter clogging. At 20 ppm boron, the microbicide sterilizes the fuel, the interface, water bottoms and sludge. Fuel contaminated with microorganisms can clog locomotive filters in < 1 mo; sterilization with the organo-boron additive permits 6 mo filter life. The microbicide substantially reduces injector tip deposits and engine wear; it does not affect the physical or chemical properties of the engine oil. There is no increase in carbon, varnish or sludge on any engine part. Spectroscopic oil analysis techniques are not affected. The microbicide stops production of harmful sulfides.
THE OCCURRENCE OF MOLDS IN MODERN INDUSTRIAL CUTTING FLUIDS.
Lubrication Eng. 19:110-11? (March 1963)
[Cutting fluids, fungi, growth]

Churchill, A.V.
MICROBIAL FUEL TANK CORROSION: MECHANISMS AND CONTRIBUTORY FACTORS.
Materials Protection 2:18-20,22-23 (June 1963)
[Fuel, jet, tanks, corrosion, bacteria, fungi, contaminants, water, surfactants, toxins, chromium compounds, Aspergillus, Penicillium, Spicaria, Fusarium, Hormodendrium, Cladosporium, review]

McGregor, James M.
MICROORGANISMS FAIL TO GROW IN FURANE LINED JET FUEL STORAGE TANKS.
Materials Protection 2:24-26,28 (June 1963)
[Fuel, jet, tanks, bacteria, corrosion, linings, coatings (organic), furane resins]

Ward, C.B.
CORROSION RESULTING FROM MICROBIAL FUEL TANK CONTAMINATION.
Materials Protection 2:10-12,14,16 (June 1963)
[Fuel, jet, tanks, corrosion, bacteria, fungi]

Imai, Morieko.
STUDIES ON CEROPHILIC GROWTH OF MOULDS ON WAX PARAFFIN. II.
COMPARATIVE STUDIES ON CEROPHILIC AND TONOPHILIC GROWTH OF MOULDS.
[Paraffin, wax, fungi, Acrotheca, Aspergillus, Penicillium, Cladosporium, Alternaria, Curvularia, Monascus, growth, culture, comparison]
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