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A LITERATURE SURVEY AND DATA BASE ASSESSMENT: MICROBIAL FATE OF DIESEL FUEL AND FOG OILS

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This report assesses the data base on microbial degradation and microbial concentration of military-relevant fog-generating oils (SGF 1 and 2 and diesel fuel) in soil and freshwater systems. Such data were not found for the SGF oils. For SGF 1, diesel fuel and No. 2 heating oil were considered acceptable models and for SGF 2, light lubricating oils.

Disappearance rates for No. 2 fuel oil have been shown to reach 500 g/m^2/month (690 mg/m^2/hr) in soils during the first few months following

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application. In waters, rates are probably lower, e.g. up to 10 mg/L/day for No. 2 fuel oil. These observations actually represent favorable case situations. Rates vary greatly according to the nature of the material and, for a given material, in response to environmental and operational conditions and the degree of weathering or aging that has occurred.

The data base has been considered critically from the standpoint of its adequacy in meeting the requirement of the US Army for information allowing predictions of the role of biosorption to microorganisms and the rate of biodegradation in soil and freshwater systems, of material deposited from obscurant fogs. It was concluded that to allow such prediction further research would be required in many areas. Toward this end, several research studies on biodegradative processes and biodegradation rates of impacted or settled fog material, with and without prior exposure to sunlight or UV, are recommended and prioritized. These are:

Priority 1

- Change in biodegradation rate with length of environmental exposure
- Effect of deposition in anoxic subsurface zones

Priority 2

- Effect of type of soil, freshwater source, or sediment
- Effect of type and source of oil

Priority 3

- Comparison of biodegradation in oils to that of corresponding settled fog material
- Comparison of low-level or repeated application with high-level application
- Effect of prior exposure to sunlight

Undegraded material remaining after prolonged exposure would be characterized and subjected to further biodegradation studies.

Studies in bioadsorption/bioaccumulation by microbes are not recommended.

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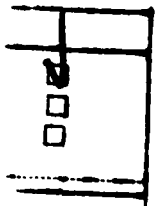

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INTRODUCTION

Certain refined petroleum products have long been used by the US Armed Forces in the production of screening smokes. There are two such oils, smoke generating fog oils (SGF) 1 and 2. Light diesel oil is also used as an obscurant and may replace fog oil for all screening, partly because of the logistic simplification afforded by its use as a motor fuel and partly because smoke formation is possible using the vehicle engine exhaust smoke system (VEESS). Chemically all of these oils are very complex mixtures consisting largely of aliphatic and aromatic hydrocarbons.

Fog generation releases substantial quantities of these materials to the environment, particularly in training areas where repeated use occurs. Exposure of personnel to the fogs is inevitable and under wartime conditions can last for days at a time. A need has thus been recognized for information on both the safety and the environmental acceptability of these oils.

Many aspects of this problem were addressed in a recent (1978) series of Literature Reviews/Problem Definition Studies[1-3] performed for the US Army Medical Research and Development Command by the Franklin Institute. Available information was surveyed in the areas of usage, concentrations encountered in the environment, properties of the oils and the smokes generated from them, human and animal toxicity and occupational health, environmental effects, environmental fate, and methods of sampling and analysis. These surveys indicated a paucity of information in many areas and identified many needs for additional research to achieve a more adequate data base.

Efforts are currently planned or underway to improve the data base in this area. An overall data base assessment on the health and environmental effects of military smokes and obscurants is in progress.[4] The physical and chemical properties of fog oils, diesel fuel, and resultant fogs have been studied,[5,6] as has the inhalation toxicology of diesel fuels.[7-9] Studies on the toxicology of fog oil SGF2 are in progress.[10] Lastly, characterization of oil fogs and their deposition rates and toxic effects are being currently studied by use of a wind tunnel experimental system.[11]

OBJECTIVES

This work was undertaken to perform a search of the literature for information pertinent to the fate of diesel fuel and military-relevant fog oils in the environment. Covered in depth are the biotransformation, biodegradation and bioaccumulation of diesel fuel, fog oils and a range of similar petroleum products in soil and freshwater environments. Literature relating to crude oils, to biodegradation in the marine environment and to chemical pathways of hydrocarbon degradation has been drawn on as needed to enhance understanding and/or prediction of the microbial degradation of middle-weight oils in terrestrial and freshwater ecosystems. The current state of knowledge has been considered critically so as to identify significant data gaps in this area.

APPROACH

Both manual and computer-assisted searches were performed. For the latter the assistance of Scientific and Technical Information Division, US Army Medical Research and Development Command (USAMRDC), was utilized. Descriptor words covering microbial activity in the environment included biodegradation, microbial degradation, biotransformation, bioadsorption, and biodeterioration. These were used as limiters for descriptors related to military fog oils and diesel fuel, to other specific middle-weight petroleum products, and to hydrocarbons and petroleum. Data bases searched are listed in Table 1. Literature available through these data bases as of 1982 was covered.

TABLE 1. DATA BASES SEARCHED

American Petroleum Institute (Appelit)
Biological Abstracts
Chemical Abstracts
CAIN; CABF data bases now AGRICOLA
Enviroline
National Technical Information Service
Orbit IV (Systems Development Corp. International Search Service) (This system also uses the Energy Data Bank [EDB])
Off-line searches from EDB or Dept of Energy Data Bank including Energyline, Petroleum Engineering News, APTIC, Aqualine and Water Research Abstracts
Pollution Abstracts
Smithsonian Scientific Information Service (SSIE)

PROPERTIES OF DIESEL FUEL AND FOG OILS

Properties of diesel fuels were discussed by Liss-Suter and Mason.[1] The fuels of interest are those designed for high-speed engines, such as those of tanks and trucks. For this use three grades are designated in federal specifications,[12] intended respectively for warm weather, winter, and arctic conditions. There are substantial differences in boiling range and viscosity. The specifications are met by a range of products that vary as to crude oil source, proportion of "straight-run" distillate to "cracked" distillate components, and proportion of paraffinic, olefinic, and aromatic fractions.

Diesel fuels belong in the middle distillate range of petroleum fractions, boiling in the range 160° to 371°C. Other middle distillate products include fuel oils No. 1 and 2, kerosene and light gas oil. Molecular size of the substances making up diesel fuel covers approximately the range C_9 to C_{24}. Straight-run diesel fuels tend to be high in paraffinic hydrocarbons (39 to 80%) and in the normal alkane component thereof (Table 2). They are very low in olefins. Cracked diesel fuels are lower in paraffins (35 to 55%), the remainder being largely aromatic hydrocarbons. Small quantities of additives are likely to be present. Those in greatest amount, up to 0.5 percent, may be hexyl or amyl nitrates to improve combustibility.

TABLE 2. HYDROCARBON TYPE ANALYSIS OF MIDDLE DISTILLATES
(From Liss-Suter et al.[2])

Hydrocarbon Type C_{11}-C_{20} Range	Reported Values, wt. %				
	a	b	c	d	e
n- and isoparaffins	40-52	21.4	22.8	43	28
monocycloparaffins	18-25	13.0	10.3	21	12
non-condensed dicycloparaffins	6-12				
non-condensed tricycloparaffins	1-5				
condensed dicycloparaffins	0-2	4.8	3.7	9	7
condensed tricycloparaffins				3	2
alkylbenzenes	3-11	9.8	20.8	5	9
indanes, tetralins	2-7	10.6	8.9	4	7
indenes, dihydronaphthalenes	0-4	3.0	1.9	2	1
naphthalene	3-11	0.2	0.2	7	20
alkylnaphthalenes		18.9	21.1		
acenaphthalenes, other C_nH_{2n-14}	0-3	6.7	4.0	3	6
acenaphthylenes, fluorenes, other C_nH_{2n-16}	0-3	2.8	.8	2	4
tricyclic aromatics, phenanthrenes, C_nH_{2n-16}	0-3	1.2	0.3	1	4
olefins	-	6.6	5.2	-	-

a = Middle distillate, Ehrler et al.;[13]
b = Diesel fuel, Kearns;[14]
c = Fuel oil No. 2, Kearns;[14]
d = Straight-run middle distillate, Fitzgerald et al.;[15]
e = Cracked middle distillate, Fitzgerald et al.[15]

Properties of the fog oils, SGF 1 and 2, were discussed by Liss-Suter and Villaume[2] and Katz et al.[5] Only SGF 2 is being procured at the present time.

Military Specification MIL-F-12070A[16] (reprinted in Katz et al.[5]) lists the chemical and physical properties of SGF 1 and 2. The specifications for the fog oils state that they contain no additives such as anti-oxidants, cetane enhancers, preservatives, or corrosion inhibitors. Any petroleum distillate that meets the requirements for viscosity, distillation range, etc. and is free of additives may be supplied as the respective fog oil.[5] SGF 1, the lighter of the two, is a middle distillate product, similar in viscosity to No. 2 fuel oil and to diesel fuel. It is preferred for use in colder climates.

SGF 1 is similar to the diesel fuels in boiling point range (200° to 320°C) and in the probable presence of varying proportions of cracked distillate material. The range of chemical compositions encountered in SGF 1, e.g. proportions of the various hydrocarbon classes, is expected to be similar to the range encountered among diesel fuels, especially as the two are likely to be drawn from the same stocks.

SGF 2 is obtained from the lubricating oil fraction of petroleum and is similar in viscosity to SAE 20 motor oil. This class of materials is obtained as follows: A first distillation volatilizes gases, naphtha, gasoline, and middle distillate fractions. The remaining material is subjected to a second distillation using reduced pressure to obviate the need for extreme temperatures. Materials thus volatilized constitute the lubricating oil fraction.

SGF 2 has a higher viscosity, 100 to 110 Saybolt Universal Units as opposed to about 60 for SGF 1. The boiling range is 370° to 480°C for lubricating oils, and the flash point is a minimum of 160°C. The molecular size range is about C_{20} to C_{50}.[1,2]

In both fog oils, total paraffins and aromatics may be present in roughly equivalent amounts. There is no precisely defined or constant chemical composition for any such petroleum product. Instead, hundreds and probably thousands of chemical species are present. Compounds identified or tentatively identified in SGF-2 by high resolution gas chromatograph (GC)[5] are shown in Table 3. These represent only a small fraction of the total number that were resolved but not identified, or that could not be detected against the massive background of unresolved material. Aliphatic hydrocarbons include normal and branched alkanes, cycloparaffins and olefins. Aromatic hydrocarbons range from one-ring compounds to those with four or more rings. Alkyl aromatics may also be present. The proportions of the various hydrocarbon classes vary widely according to crude oil source and processing technique and even from batch to batch. In addition to hydrocarbons small amounts of polar organic substances (acids, esters, alcohols), organic nitrogen and sulfur compounds, and heavy metals may be present. These latter substances are often partially removed in special treatment steps.

TABLE 3. COMPOUNDS IDENTIFIED IN SGF-2
(ALIPHATICS 43 TO 58%, AROMATICS 40 TO 50%)
BY HIGH RESOLUTION GC OF THE INDICATED FRACTIONS
(from Katz et al., 1980[5])

Aliphatic Fraction

$C_{14}H_{30}$ n-tetradecane
$C_{16}H_{34}$ n-hexadecane
$C_{17}H_{36}$ branched alkane
$C_{17}H_{36}$ n-heptadecane
$C_{18}H_{38}$ n-octadecane
$C_{19}H_{40}$ n-nonadecane
$C_{20}H_{42}$ n-eicosane
$C_{21}H_{44}$ branched alkane
$C_{21}H_{44}$ n-heneicosane
$C_{22}H_{46}$ branched alkane

First Aromatic Fraction

$C_{12}H_{16}$ 2,6-dimethyl-1,2,3,4-tetrahydronaphthalene
$C_{13}H_{14}$ + $C_{14}H_{20}$ + $C_{15}H_{24}$
$C_{13}H_{16}$
$C_{13}H_{16}O$ or $C_{14}H_{20}$
$C_{14}H_{16}$
$C_{15}H_{18}$ dimethylisopropylnaphthalene
$C_{15}H_{22}$
$C_{16}H_{18}$
$C_{20}H_{24}$ 3,6,9,9,10,10-hexamethyl-9,10-dihydrophenanthrene
$C_{20}H_{32}$

Middle Aromatic Fraction

$C_{14}H_{10}$ phenanthrene or anthracene
$C_{14}H_{12}$ methylfluorene
$C_{15}H_{12}$ methylanthracene
$C_{15}H_{12}$ methylphenanthrene
$C_{15}H_{14}$ dimethylfluorene
$C_{15}H_{16}$ dimethylbenzylbenzene plus $C_{14}H_{14}$ dimethylbiphenyl
$C_{15}H_{12}O$ 9-methoxyanthracene
$C_{15}H_{12}O$ 9-methoxyanthracene or $C_{18}H_{16}$
$C_{15}H_{24}O$ methyl-di-tert-butylphenol
$C_{16}H_{34}$ n-hexadecane
$C_{16}H_{14}$ dimethylphenanthrene
$C_{17}H_{16}$ trimethylphenanthrene
$C_{18}H_{8}$ alkylphenanthrene
$C_{18}H_{18}$ 1-methyl-7-isopropylphenanthrene
$C_{20}H_{32}$ n-butyl-n-hexyl-tetrahydronaphthalene

Heavy Aromatic Fraction

$C_{12}H_{12}$ dimethylnaphthalene
$C_{12}H_{8}$ acenaphthalene
$C_{13}H_{14}$ trimethylnaphthalene
$C_{13}H_{10}$ fluorene or phenalene
$C_{14}H_{12}$ 1-methyl fluorene
$C_{14}H_{16}$ C_4-alkylnaphthalene
$C_{14}H_{10}$ anthracene or phenanthrene
$C_{15}H_{14}$ dimethylfluorene
$C_{15}H_{14}$ alkenyl dibenzene
$C_{15}H_{12}$ methylphenanthrene or methylanthracene
$C_{15}H_{12}O$ methoxyanthracene or $C_{16}H_{16}$ isomer
$C_{15}H_{24}O$ 2,6-di-t-butyl-4-methylphenol (ionol)
$C_{16}H_{14}$ dimethylphenanthrene
$C_{16}H_{14}$ ethyl or dimethylanthracene
$C_{18}H_{18}$ C_4-alkylphenanthrene or C_4-alkylanthracene
$C_{19}H_{30}$ 2-n-butyl-5-hexylindan

Nitrogen Base Fraction

$C_{10}H_{9}N$ methylquinoline
$C_{11}H_{11}N$ dimethylquinoline
$C_{12}H_{13}N$ trimethylquinoline
$C_{13}H_{15}N$ tetramethylquinoline (?)
$C_{14}H_{17}N$ pentamethylquinoline (?)
$C_{15}H_{19}N$ hexamethylquinoline (?)
$C_{15}H_{19}N$ C6-alkylquinoline (?)
$C_{15}H_{15}N$ dimethyl-9,10-dimethyl benzoquinoline (?)
$C_{15}H_{13}N$ methylphenylindole or dimethylbenzoquinoline
$C_{16}H_{15}N$ or $C_{15}H_{11}NO$

OIL FOGS: FORMATION, PROPERTIES, AND DEPOSITION

The US Army uses smokes (actually fogs) produced from the fog oils and diesel fuel to provide concealment of troops or installations (FM 3-50). These may be produced from point locations, a series of point locations, or helicopters. Fog generation is discussed in the Franklin Institute surveys.[1,2] Briefly, the oil is introduced into the exhaust system of a gasoline or diesel engine. For fog oils a specially designed fog generating device, such as the gasoline-driven pulse jet generator M3A3 is used. For diesel fuel a large vehicle such as a tank or truck may be used, although newer generators are being designed for diesel fuel. In the exhaust manifold, the oil is vaporized by the high temperature. On release to the atmosphere, the vaporized oil condenses, forming microdroplets. Smoke pots (e.g. NM7 SGF2) are also in the inventory.

Oil consumption by the latest generating device, the gasoline-driven pulse jet generator M3A3, is 150 L fog oil/hr.[2] Up to 240 L/hr of diesel fuel may be used in fog production by a vehicle in the field. Production of oil fog by the exhaust system of the turbine engine of the UH-1 helicopter uses fog oil at a very high rate (57 L/min).[17]

The temperatures to which the oils are exposed in fog generation are given as 540°C for the M3A3[2] and 540° to 620°C for vehicular exhaust manifolds.[1] While ignition of the oil does not occur, some slight thermal decomposition as well as chemical interaction with exhaust gases or oxygen does occur.

The more volatile substances, including n-alkanes up to about C_{11}, remain largely in the vapor state, and evaporative processes proceed during the life of the fog. Other chemical changes occurring between parent oil and fog are small in magnitude, but may include an increase in the percent of aromatic compounds.[5]

Depending on generation time and meteorological conditions, the atmospheric concentration of oil could vary from 13 mg/m^3 to 2,000 mg/m^3 (Liss-Suter, 1978).[3] Aerosol droplet diameter is generally in the range 0.5 to 1.2 μm.[2] A good fog feels dry to the hand, while reducing the disc of the sun to an orange or pink coloration. Physical decay of the fog occurs, partly through agglomeration of droplets, which fosters settling or fallout.[5] Particle concentration change with time is shown in Figure 12 of the report by Katz[5] and an effective agglomeration coefficient can be determined from an equation given there.

Liss-Suter[3] suggests 1 hour as the average time particles remain aloft and 1 to 10 km as the average downwind travel. Assuming no evaporation and that all 150 liters of oil are vaporized, a fog produced by 1 hour's use of one fog generator carried 200 m to 1 km downwind with a column spread of 20-150 m would result in an oil fallout concentration of 6 to 60 g/m^2.[3] Note that there would be considerably less oil present on the fallout surfaces than would be found in an oil spill. No actual measurements of deposition rates were found in the literature.

ABIOTIC PROCESSES AFFECTING ENVIRONMENTAL FATE

Deposition of atomized oil materials occurs on soil, water, and vegetation surfaces. The material is then subject to various abiotic processes collectively called weathering. The differential volatilization of low-boiling substances, a process that proceeds with great rapidity in the aerosol state, may continue following deposition. Photooxidation, mainly ultraviolet-induced, begins in that portion of the cloud exposed to sunlight and may continue after deposition.[18] This and other chemical transformations increase the content of oxygen-containing compounds: ketones, alcohols, aldehydes, carboxylic acids and phenolic substances. Photolytic action on aromatic hydrocarbons is a source of insoluble polycyclic aromatic hydrocarbons, which tend to persist in the environment.

Oil that settles on water forms a monolayer or slick initially, with some emulsification resulting from turbulence. Solubilities of diesel fuel and lubricating oil in distilled water are about 40 ppm, with the aromatic fraction contributing the greater part of the dissolved material. Dissolution into the aqueous phase tends to increase greatly over time. This is due in part to adsorption onto particulate material and in part to oxidation reactions, both biotic and abiotic, yielding substances of greater polarity. Oil that attaches to particulates in the water may settle to the bottom. This process too is enhanced by turbidity. Petroleum substances quickly appear in the sediment phase of affected waterways. Here, oxygen tension may be near zero, greatly retarding both biotic and abiotic oxidative changes. Release from sediments tends to occur slowly through biological activity and dissolution, with some material remaining for long periods of time.

The fate of oils impinging on soil surfaces is determined by a complex interplay of factors. Many of these correspond to forces operative in aquatic systems: photolysis, biotic and abiotic oxidative changes, the low solubility of most substances in water, the presence of anoxic zones, and bioemulsification.

The oil droplets that fall out on land become adsorbed to the soil particles. As in the aquatic environment, the more volatile compounds that remain tend to evaporate.

A major difference between aquatic and soil systems lies in the capacity of soil systems to limit oil transport, i.e., to contain the spill. Raymond et al.[19] studied the fate of No. 2 fuel oil applied at the rate of 2.5 kg/m^2 on three differing loamy soils. The oil was readily absorbed, and physical movement from the plots was not detected over a year. Any transport in runoff or percolating water remained in the low parts per million (ppm) range.

The amount of oil held by soil in the absence of water movement, called the residual saturation, ranges from about 1 percent soil weight for gasoline to 20 percent for crude oils and heavy fuel oils. Thus, both lateral and downward movement are severely limited.

With precipitation, some of the remaining oil particles are washed into nearby bodies of water, and some oil is translocated deeper into the soil. The extent of this penetration depends on the soil composition; e.g.; sandy soil offers less resistance to penetration by oil than do clay soils, which are less pervious. There also is a channeling or tunneling effect called the Jamin effect,[20] which retards the movement of oil through the soil. In some spills, groundwater is reached, and here oil movement may occur not only in solution but in bulk as well. This is improbable when dealing with the low application rates represented by fog oil usage.

BIOEMULSIFICATION

As the oil film lies on the water surface undergoing these preliminary changes, the remaining volatile compounds continue to evaporate.

On natural water surfaces, in the absence of extensive wave action, there exists a microlayer of microorganisms in the top 1 μm of surface water with a mixed population of 10^5-10^7 organisms per cm^2.[21-23] Some organisms are encased in a matrix of extracellular polymeric substances (EPS)[24] or algal slime, and this tends to protect them from the xenobiotic materials. The emulsifying action of these EPS-containing biofilms breaks up oily material into increasingly fine droplets and finally into aggregates 10-100 Å in diameter called micelles.[25-28] Extracellular polymeric substances are elaborated by a wide variety of microorganisms in an aquatic environment. These include fungi, algae, bacteria, and the cyanobacteria. Extracellular polymeric substances were also found surrounding bacteria beneath an oil slick.[24]

The soil microorganisms, e.g., Pseudomonas, are very similar to those in the adjacent bodies of water which receive runoff; but there is a higher population of fungi in the soil than in the water microlayer. These microorganisms also seem to be protected by EPS, and also produce emulsifiers, which break down the size of the oil droplets to that of micromicelles.

The organisms producing the emulsification may not be themselves responsible for hydrocarbon breakdown. Yet some organisms that are active in the transformation of hydrocarbons produce emulsifying agents. These include an Arthrobacter studied by Reisfeld et al.[29] and strains of Pseudomonas and Corynebacterium studied by Zajic et al.[30] The emulsifying substances may be long-chain fatty acids or derivatives thereof or more complex polymers. The latter may act also as flocculants.[31] An example of the surfactants produced in the presence of hydrocarbon are the α,α-trehalose-6,6′dicornomycolates (glycolipids produced by n-alkanes in Rhodococcus erythropolis.)[32] This bioemulsification phenomenon plays a role in mobilizing hydrocarbon from oil slicks into the water column and downward in soil systems. Emulsification should enhance biodegradation by increasing the surface area of the oil-water interface. This in turn should make the hydrocarbons more available to microbes and at the same time bring them into better contact with available oxygen and inorganic nutrients. The effects of surfaces on microbial activity were discussed by Marshall, 1978.[33]

BIOCONCENTRATION AND BIOACCUMULATION

Microorganisms may ingest and utilize oil substances without obvious hydrocarbon degradation. The more lipophilic organisms, e.g., mycobacteria, may be preferentially attracted to the oil film, with the more hydrophilic remaining in the microlayer of organisms within the aqueous phase.[34] Small microdroplets (micelles) may then be formed and ingested by the organisms in closer contact with the oil. Microorganisms utilizing hydrocarbons may develop cytoplasmic inclusions containing such substances. Finnerty et al.[35] reported this in yeasts, filamentous fungi, and a strain of Acinetobacter.

The inclusions are visible within the microorganisms as microbodies,[23,36] and in them the oil molecules are in intimate contact with the membrane enzymes. Andrews and Floodgate[37] showed that hydrocarbon accumulation can occur in protozoa. Stirling et al.[38] reported that an apparent Nocardia organism when grown on cyclohexane produced intracytoplasmic membrane structures not found in the same organism grown on succinate. Cold-tolerant hydrocarbon-utilizing bacterial isolates from estuarine waters contaminated by fuel oil spills at Narragansett Bay, RI, and Chedabucto Bay, Nova Scotia, were shown by Traxler and Cundell[39] to develop large inclusions with distortion and elongation of cell shape.

A phenomenon akin to simple bioaccumulation is the utilization of hydrocarbons to form normal microbial cell components with only very limited chemical alteration. This has been reported for a number of microorganisms with regard to their fatty acid composition. Dunlap and Perry[40] found that in a mycobacterium growing on n-alkanes from C_{13} to C_{17}, the major fatty acid in the cells was of the same chain length as the substrate. Acetate incorporation studies suggested that the monoterminally oxidized substrates were utilized without further degradation. Evidence for such utilization by Mycobacterium of 1-alkanes C_{14} to C_{18}, 8-heptadecene and the branched alkanes 2- and 3-methyl octadecane was obtained by King and Perry.[41] Their observation regarding n-alkanes and 1-alkenes was extended to the filamentous fungi Cunninghamella elegans and Penicillium zonatum by Cerniglia and Perry.[42] The phenomenon has been reported also in Acinetobacter[43] and in the yeast Candida.[44]

Accumulation without attendant transformation was reported for a green alga, Enteromorpha, inhabiting a salt marsh ecosystem affected by a spill of No. 2 fuel oil.[45,46] This was considered to represent a possibly significant route of entry for hydrocarbons into the marsh food chain.

Bioaccumulation of petroleum substances may be accompanied by biotransformation, which forms the subject of the next section.

BIOTRANSFORMATION AND BIODEGRADATION

INTRODUCTION

It has been known for over a century that hydrocarbons are susceptible to microbial degradation. An early review was provided by ZoBell.[47] Progress in this area has been substantial in recent years, and more than 3,000 relevant papers and reports exist. Progress has been spurred by several main developments: (1) newer analytical techniques such as the development of gas chromatography;[48] (2) the opening of new areas in biochemistry such as the discovery of oxygenase enzymes,[49] which incorporate molecular oxygen into organic substrates; (3) interest in using microbes to produce certain desired chemical products from petroleum;[50] and (4) concern over the magnitude and ecological impact of oil pollution. The greater part of the effort in this area has centered on events in the marine environment. The sea is the largest and, for most lost oil, the ultimate receptor system, though an estimate prepared by the National Academy of Sciences[51] ascribes more than one-quarter of oil reaching the oceans to such landward sources as urban runoff and river runoff.

Most reviews dealing with microbial degradation have similarly been concerned with the marine environment.[52-59] Biodegradation in soil and freshwater environments has been reviewed briefly.[60,61] Finally, a number of review papers (next section) have dealt with the biochemical processes and interactions involved in transformation and degradation of hydrocarbons by microorganisms.

CHEMISTRY OF MICROBIAL TRANSFORMATION AND DEGRADATION OF PETROLEUM MATERIALS

Much work has been done on the chemical processes and interactions involved in the action of microorganisms or their enzymes on hydrocarbons or substances produced from hydrocarbons. No attempt will be made to cover this area except very briefly. A recent review has been provided by Higgins and Gilbert[26] and a brief up-to-date summary by Atlas.[61] The earlier literature was reviewed by Van der Linden and Thijsse[62] and that on aromatic compounds by Rogoff.[63] More recent reviews have dealt with aromatics,[64,65] aliphatic hydrocarbons,[66-68] and cyclic hydrocarbons.[69] The biochemical activities of Nocardia, a group of organisms important in hydrocarbon transformation, have been reviewed by Raymond and Jamison.[70] Extracellular metabolic products of hydrocarbon transformation were the subject of a review by Abbott and Gledhill.[71] Finally, the subject of co-oxidation of hydrocarbon substances in the presence of other (utilizable) hydrocarbons was reviewed by Perry[69] and discussed more briefly by Atlas.[61]

Biodegradation of oils is often shown by disappearance of a peak or peaks on a gas chromatogram (GC) or a GC-mass spectrophotometric analysis, yet even when oils are fractionated prior to analysis and fractions are analyzed separately, the sheer number of chemical species present leaves most compounds unresolved and unidentified. For example, $C_{20}H_{42}$ has 366,000 possible isomers.[72] Therefore the degradation or transformation of a single compound (or group of compounds) is often studied to give clues as to how parent oils are degraded or transformed.

Crucial to oxidative attack on hydrocarbons is the activation and incorporation of oxygen from the environment. This requires the action of oxygenase enzymes, which include the following important categories:

(a) α-Oxygenase. This enzyme was reported present only in Arthrobacter simplex,[36] where pentadecane was readily oxidized to pentadecanoic acid. This organism also is able to degrade some fatty acids by oxidative decarboxylation with the evolution of CO_2.

(b) Dioxygenases.[73] Enzymic activation of oxygen from the triplet state to the singlet state is a crucial prerequisite for biological oxidation to occur.[36,74] This is accomplished by dioxygenases, where oxygen is fixed directly into organic compounds, e.g., in the oxidation of benzene to catechol, a process commonly found in bacteria, yeast, and fungi.[75]

(c) Hydroxylases.[36,73,75] These enzymes accomplish the insertion of an oxygen atom and the transfer of electrons, e.g., in the oxidation of acyclic hydrocarbons with alcohols as the intermediate product. These enzymes are also active in the degradation of polycyclic aromatic compounds as mixed-function oxygenases of microsomal origin.

(d) Cytochrome Oxidases. These effect the transfer of electrons in the respiratory chain, usually with the formation of water and an organic acid. Among the cytochrome oxidases is a group termed cytochrome P-450, found in all types of cells including mammalian when the cells are stressed by a soluble hydrocarbon molecule or other xenobiotic.[76]

Important chemical transformations yielding smaller or more degradable oxygen-containing compounds include the following:[36]

(a) Terminal Oxidation. The insertion of activated oxygen usually occurs at the free end of an alkyl hydrocarbon chain, with formation of an alcohol, then an aldehyde, and, with further oxidation, a fatty acid. The fatty acid is then degraded by β-oxidation.

(b) Diterminal Oxidation. The insertion of oxygen at both ends of an alkane (α and ω) to give the dioic acid of the alkane.

(c) β-Oxidation. β-Oxidation is the degradative pathway for fatty acids. The fatty acids are linked to coenzyme A and degraded to the acid with 2 less C atoms and acetyl coenzyme A. ATP is required, and the coenzyme A is recycled.

(d) Subterminal Oxidation. Oxidation of the aliphatic hydrocarbons has been shown to occur also at any C atom in the hydrocarbon chain. This has been demonstrated in some varieties of bacteria but more often with fungi.[36] Secondary alcohols and subsequently ketones are formed.

(e) Oxidation of Double Bonds. Terminal alkenes are readily degraded. These are often attacked at both ends, with the double bond region converted to an epoxide or diol. The utilization of internal alkenes has been reported and can lead to both unsaturated and saturated fatty acids.

(f) Oxidative Cleavage of Aromatic Rings. The dioxygenases insert active oxygen, forming cis-diols of dihydro-benzenoid compounds (trans-diols with eucaryotic organisms). Thus, benzene is converted to catechol, and toluene to catechol or 3-methylcatechol. The next step is usually the formation of an unsaturated acid or dioic acid, by further insertion of oxygen. This breaks the ring structure, and the fatty acids are further oxidized by β-oxidation.

Many studies have indicated that n-alkanes are the most readily degraded class of hydrocarbons, and action on such molecules up to C_{44} has been reported.[77] Complex monooxygenase enzyme systems convert these to the corresponding primary alcohols. Further oxidation proceeds via the aldehyde to the monocarboxylic acid. β-Oxidation may then yield a fatty acid of shorter chain length and acetyl coenzyme A. Other pathways occur, e.g., formation of ketones or alkenes. Omega oxidation has been reported, especially for branched alkanes, which tend to be more resistant.[78]

The "citronellol pathway" occurs in certain Pseudomonas sp. and some Acinetobacter[79] for the degradation of otherwise recalcitrant, branched hydrocarbons. Geranyl-coenzyme A carboxylase is a key enzyme in this pathway, permitting subsequent oxidation to continue by the β-oxidation pathway.

Microbial transformation of aromatic hydrocarbons, including short chain alkylbenzenes, also occurs readily under favorable conditions.[80] If alkyl side chains are present, they are usually oxidized first, and are subject to the reactions described above for n-alkanes. Attack on the benzene ring is typically by the formation of the diacid, with ring cleavage.

This also occurs with polyaromatic hydrocarbons (PAH).[81] These are clusters of benzene-ring structures or -C-C-linked benzene rings e.g., biphenyl. They are less soluble in water than most hydrocarbons and take longer to biodegrade;[82] thus they are highly persistent (unless modified by photooxidation). PAH up to at least four rings, are subject to sequential ring destruction by this general mechanism. Thus, in the degradation of naphthalene, the cis-diol is degraded in several steps through salicylate to catechol.

If side chains are present, oxidation usually occurs at a point next to the ring, but may occur in more than one molecular region. A mixed-function oxygenase is the active enzyme, with the formation of an arene oxide. This oxide can react with glutathione and hydrolyze to a phenolic compound, or, with the addition of water, it can form a diol which can be further oxidized to a diol epoxide. This is a very potent carcinogen, since it binds with DNA.[83] Pseudomonas sp., Beijerinckia, and Nocardia sp. can further degrade these compounds, usually one phenol ring at a time, although the pathways are not clearly defined.

PAH may be associated with heteroaromatics, where a C atom is substituted by a nitrogen, oxygen, or sulfur atom. The sulfur aromatics, e.g., benzothiophenes or naphthobenzothiophenes can be present in fuel oils at up to 2 percent concentration.

A buildup of hydrocarbon substances to larger molecules has also been reported.[25,36,61,84] Very long chain alkanes (waxes) and esters arise during microbial action on at least some oils. These are highly resistant to further

enzymic attack and play a role in the formation of recalcitrant tarry materials.

In any study of biodegradation of organic material, it is necessary to distinguish between transformation (any chemical alteration), degradation (formation of smaller, simpler molecules) and mineralization (conversion to inorganic materials). The disappearance of hydrocarbon substances may reflect mineralization, uptake of transformation products into protoplasm or even the failure of analytical procedures to recover partially oxidized, polar transformation products. The respiratory quotient (RQ), i.e., moles CO_2 produced/moles O_2 consumed, has been used in assessment of mineralization. Light oils yield RQ values of 0.5 to 0.7, indicating a high rate of mineralization,[85] while heavy oils, with long chain hydrocarbons, may yield lower values.[86]

MICROORGANISMS ACTIVE IN BIOTRANSFORMATION OF HYDROCARBONS

Degradation or transformation of petroleum hydrocarbons is widespread among microorganisms from a taxonomic point of view. Bartha and Atlas[87] listed 22 genera of bacteria, 14 genera of fungi, and 1 algal genus (Prototheca)[88] that were known to contain organisms active in hydrocarbon transformation. The most often reported bacteria active in aquatic environments were listed as Pseudomonas, Achromobacter, Arthrobacter, Micrococcus, Nocardia, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium, Flavobacterium, and Mycobacterium.[57,87] Among yeasts and fungi the most frequently encountered are Candida, Rhodotorula, Sporobolomyces, Cladosporium, Trichosporium, and Torulopsis.[61,87] A survey of organisms from type culture collections was made for the ability to grow on n-alkanes.[89] Between 6 and 20 percent of each group of bacteria, yeasts, and molds tested yielded positive results. The degradation of at least some hydrocarbons is therefore a very widely distributed and common property of microorganisms. Jones and Eddington[90] listed six genera of bacteria and 11 genera of fungi active in an upland moorland soil and its underlying shale. Davies and Westlake[91] found that 28 of 34 fungal isolates isolated by a static enrichment technique from soils of northern Canadian oil-producing areas could grow on one or more of seven crude oils tested. Perry and Scheld[92] found that from 1 to 3 percent of heterotrophic bacteria from soil that grew on nutrient agar would also degrade hydrocarbons. Raymond et al.[19] in their study of artificial oil contamination of three types of loamy soils, observed that hydrocarbon-utilizing microbial isolates were mostly aerobic Nocardia species and fungi. Cladosporium resinae, which has been repeatedly found as a contaminant of jet fuels and has been dubbed the "kerosene fungus," has been isolated from soils as well.[93,94]

Hydrocarbon-utilizing organisms typically constitute a small percentage of the total heterotrophic population in uncontaminated ecosystems. In a study of four freshwater ecosystems in Ohio, Cooney[95] found this to be less than 0.1 percent. This percentage has been repeatedly shown to rise at least temporarily in response to petroleum contamination in various environments. Thus, Mulkins-Phillips and Stewart[96] found percentages of hydrocarbon users generally less than 10 percent in relatively uncontaminated Atlantic waters but ranging up to 100 percent where a history of oil spillage was present. Pinholt et al.[97] found an increase in hydrocarbon-using fungi in soil from 60 to 82 percent and in hydrocarbon-using bacteria from 3 to 50 percent following a fuel oil spill. The prevalence of hydrocarbon degraders is actually a fairly sensitive indicator of such contamination.

Chakrabarty et al.[98] discovered in 1973 that the ability of a strain of Pseudomonas putida to degrade certain hydrocarbons was plasmid-mediated. The first plasmid found directed the degradation of salicylate (SAL plasmid).[99] Camphor (CAM), octane (OCT) (Benson and Shapiro, 1976),[100] naphthalene (NAH), and toluene-xylene (TOL/XYL) plasmids were soon detected. A microorganism with a given plasmid could degrade the corresponding compounds as sole carbon source, but no other hydrocarbon. Using genetic engineering, Chakrabarty[101] was able to cross and recombine the plasmids into one "superbug" that could metabolize several major petroleum hydrocarbons.

The determination of hydrocarbon utilizers has been approached in several ways, and the interpretation of results has sometimes been difficult. One approach is through the use of agar or silica gel media containing hydrocarbons as sole utilizable carbon source. Some important difficulties with this approach are obviated by use of a most-probable-number (MPN) method,[102-104] in which liquid media are used. The presence or absence of turbidity is scored. A variant of the MPN procedure[103] uses radiolabeled hydrocarbons, such as ^{14}C-hexadecane, as an additive to crude oil or other material. Production of $^{14}CO_2$ is then determined, indicating hydrocarbon mineralization.

Individual organisms are not restricted to one oil or a limited range of oil types. Each organism attacks many different oils with comparable facility.[86] However, it is otherwise when individual hydrocarbons or classes of hydrocarbons are considered. The tendency for one organism to attack only certain substances or to accumulate intermediates that it cannot further degrade limits its overall effect on a complex substrate. Thus, a microbial community or consortium of microorganisms is generally necessary to mineralize hydrocarbons.[105] Walker and Colwell[106] reported that combinations of bacteria, yeasts, and fungi provide about twice as much degradation of mixed hydrocarbon substrates as do bacterial or fungal strains individually. In natural systems, mixed microbial populations are almost always encountered.

Finally, although many toxic substances occur in oils or are formed by their chemical alteration, it is doubtful that the diversity of microbial species present in natural systems is adversely affected by oil addition, except perhaps temporarily. Cobet and Guard[107] found no change in species diversity in a beach sand subjected to Bunker C fuel oil. There is a report[108] that species diversity may even be increased by chronic oil pollution.

MECHANISMS AND RATES OF BIOTRANSFORMATION AND BIODEGRADATION

Microorganisms are active mainly at the oil-water interface. Photomicrographs obtained by Gunkel[109] show bacteria lying on the surface of oil droplets. Some organisms seem to exhibit a positive chemotaxis in response to oil droplets.[110,111] Uncertainty exists concerning the mechanism of entry of hydrocarbon substrates of low solubility into microbial cytoplasm. One conjecture is that only molecules in solution in the aqueous phase are taken up. Evidence seems, however, to favor adhesion of organisms to hydrocarbon droplets with uptake of material in the absence of solubilization.[26] Minute aggregates of substrate molecules, called micromicelles, may facilitate the process.[112] Entry into cells is rapid, though evidence for an active transport mechanism has not appeared.

Some products formed during microbial hydrocarbon oxidation are toxic. These include the C_5 to C_9 primary alcohols (Liu, 1973),[113] fatty acids if β-oxidation is lacking (Atlas and Bartha, 1973),[114] and oxidation products of aromatic hydrocarbons (Calder and Lader, 1976).[115] The relevancy of these toxic effects to natural aquatic systems is dubious. The presence of a mixed microbial community would often lead to further breakdown of toxic products, and accumulation might be further limited by dispersion in large quantities of water.

Biotransformation and biodestruction of petroleum hydrocarbons is often subject to an initial lag phase. This may be due in part to the paucity of microorganisms active on such substances, this, in turn, being highly dependent on the recent pollution history of the ecosystem. The lag may result in part from the toxic or bacteriostatic effect of low molecular weight hydrocarbons, an effect that is relieved by their volatilization. Delay before onset of biodegradation may increase with decreasing temperature.[116] The rate at which biodegradation then proceeds varies over several orders of magnitude depending on conditions. The resulting disappearance of the pollutant may be complete for some classes of hydrocarbons, but is usually incomplete for the material as a whole, at least within the time frame of the various reported studies. This follows from the presence, or formation during degradation, of highly resistant material and/or the presence of unfavorable conditions or unfavorable microenvironments.

Attempts have been made in a number of studies to determine the "oil biodegradation potential" of a particular water, soil or sediment. This is a function of both the numbers of organisms active on hydrocarbons and their inherent metabolic capability. The potential may shift, i.e., degradation may not be linear, due to enrichment, diauxic phenomena (see next section), or nutritional limiting factors. Determination of biodegradation potential made under laboratory conditions often yields results quite at variance with natural in situ processes.[87] Thus in marine and coastal environments oil degradation rates may range from 1 to 50 mg/m^3/day while laboratory results using optimized conditions exceed the latter figure by a factor of hundreds or thousands.

In soils, rates of 0.9 to 15 g/m^2/month have been reported[117] for crude oils. Raymond et al.[19] reported rates up to 500 g/m^2/month for a No. 2 fuel oil. There is evidence[118] that frequent small applications are associated with more effective degradation than single large applications. Despite these substantial degradation rates, complete destruction is not readily achieved. Thus in the study of Raymond et al.,[19] oil loss was 46 to 90 percent at the end of 1 year. In a study using laboratory soil columns,[60] 30 percent of a light fuel oil remained after 65 weeks in the upper 10 cm of the column and 70 percent at lower levels.

KINETICS

The kinetics of alteration of organic compounds by microorganisms is related by the Monod equations to the concentration of the compounds (C) at time t. The pseudo-first order constant, k(X), can be determined from the regression curves using the equation $-dC/dt = k(X)(C)$. With hydrocarbons, however, the limited water solubility of these compounds causes considerable problems. When considering a complex mixture such as lubricating oil,

consisting of many compounds with varying solubilities, this needs to be considered. In general, the solubility of each individual component varies with the octanol/water coefficient.[119] The given formula for log substrate concentration (log S) includes the fugacity ratio (rate of evaporation at a given temperature and pressure). Thus, the oil could act as a film with a given depth (in μm) and behave according to a zero-order reaction or half-order reaction as in biofilm kinetics.[120] No references were found on these reactions with any petroleum products. If the oil is photooxidized into epoxides or other compounds, this needs to be considered, since epoxides, in general, are more water soluble than the parent compounds. The type of surfactant produced by the microbial biomass also needs to be considered since each type of surfactant has its own critical micelle concentration[28,32] and its own surface tension properties.

Second-order rate constants (k) are determined by dividing the pseudo-first-order rate constant by the concentration of microorganisms (X). To do this it is necessary to count a multitude of different types of microorganisms found in an ecosystem. The methods used in the literature are: Total or hydrocarbonoclastic plate counts; Most Probable Numbers (both total and hydrocarbonoclastic); INT numbers, using [2-(p-nitrophenyl)-5-phenyl tetrazolium] reduction for detection of metabolizing organisms, where the tetrazolium salt acts as an electron acceptor; or direct count by acridine orange[121] staining for DNA.[122] Algae and phytoplankton are assayed by their chlorophyll content[123] or in special counting chambers. All of these methods have their own peculiar advantages,[124,125] but most investigators are now using the acridine orange direct count method.[122]

A diauxic phenomenon was reported[126] for the multiply branched alkane pristane. The substance was not degraded while in the presence of hexadecane. Sparing can also be caused by nonhydrocarbon nutrient materials. The utilization of hexadecane was reduced by acetate.[127] Degradation of oil can be inhibited or delayed by the presence of peptone.[109,128,129] Ward and Brock[130] reported that glucose addition to lake water repressed hexadecane utilization in a diauxic manner. Sparing effects play a significant role in determining rates of oxidation of various components and thus affect the overall weathering process in complex mixtures.[61]

Some enzymes involved in alkane catabolism are subject to inhibition by common metabolites. The oxygenases required for the initial attack on hydrocarbons are typically inducible enzymes, though induction is sometimes accomplished by molecules other than the substrates being oxidized.[128,131,132] Glucose metabolism is subject to inhibition or repression by hydrocarbon in a Candida strain[133] and Cladosporium resinae.[134,135] Microbial population changes occur as oil composition changes during degradation because the products formed by certain organisms serve as substrates for others.

Degradation of a compound may be facilitated by the presence of a chemically related, biodegradable compound. The compound in question does not induce the necessary enzymes but "fits" enzymes induced by the other substance. This is called co-oxidation or co-metabolism and has been reviewed by Perry.[136] Thus, Pirnik et al.[126] found that a Brevibacterium could use pristane and other branched alkanes only in the presence of n-alkanes. The co-oxidized material is not necessarily broken down further or utilized by the

co-oxidizing organism. Thus, co-oxidation tends to produce a spectrum of partially oxidized substances, thereby increasing the "envelope" of unresolved molecular species. Nevertheless, the partial oxidation effected may help to make these poorly soluble substances susceptible to further attack by other organisms. The role of co-oxidation in relation to recalcitrant substances has been reviewed by Horvath.[137]

Rates of biodegradation vary enormously between the various classes of substances present in petroleum. The following generalizations are possible.[36,61,62,87,95] The n-alkanes from C_{10} to C_{25} are the most readily degraded and utilized, a wide range of microorganisms being involved. Shorter alkanes are quite volatile. As chain length increases above C_9 the yield of oxidized material increases, while the rate of oxidation decreases. Saturated compounds are degraded more readily than unsaturated ones and straight chain compounds more readily than branched ones, especially where branching is extensive or creates quaternary carbons. If the alkanes are branched at the β-position, β-oxidation is usually blocked.[61] Branched-chain alkanes and alkenes and cycloalkanes are attacked by a very limited range of organisms. Aromatic compounds are partially oxidized by many but are assimilated by few organisms. Polynuclear aromatics, while less toxic than simpler aromatics, are metabolized by fewer organisms and at low rates. Cycloalkanes are fairly toxic, and the initial degradative attack is generally accomplished through co-metabolism. Degradation rates are low. The most resistant classes seem to be: (1) polynuclear aromatics (PAH), (2) such alicyclic substances as the tripentacyclics (hopanes), and (3) very long chain aliphatics that seem to be largely a product of the environmental alteration (union) of shorter chain molecules. Herbes and Schwall[138] gave turnover times in oil-contaminated sediment as 7 hr for naphthalene, 400 hr for anthracene and 30,000 hr for benz(a)pyrene.

Studies on biodegradation of individual compounds are of value, e.g. in elucidating chemical pathways or in establishing whether mineralization occurs. Such studies are not necessarily a guide to degradation rates occurring with mixed substrates, since the presence of a given substance may produce either an enhancing or a sparing effect on the degradation of other substances.

The overall nature of a hydrocarbon mixture influences the degradability of individual components. Crude oils of high saturated hydrocarbon and low sulfur content were found more readily degradable than the converse.[139] Destruction rates for various crudes varied over a factor of 40 in studies using soil and marine bacteria.[117] In oils of high paraffinic content, the middle fractions: kerosene, light fuel oils and light to medium lubricating oils are the most readily degraded.[86] Walker et al.,[139] using a mixed bacterial population from oil-contaminated sediment in Baltimore harbor, reported that a light fuel oil (No. 2) was more readily degraded than a heavy fuel oil (No. 6 Bunker C). Percents degradation in 7 weeks were 55 percent and 11 percent, respectively.

EFFECT OF NUTRITIONAL AND ENVIRONMENTAL FACTORS

Rates of oil degradation are highly dependent on water quality or soil type, availability of oxygen and inorganic nutrients and temperature. In soils, microbial activity toward hydrocarbons decreases with soil depth,[60,140] and is influenced by pH,[141-143] clay content and other factors.

Like other biological processes, biodegradation of hydrocarbons is greatly retarded by low temperatures. This heightens concern regarding petroleum spillage on soils or aquatic systems in arctic regions. In a study by ZoBell,[144] degradation was tenfold faster at 25°C than at 5°C. Gibbs et al.[145,146] reported an average Q_{10} value of 2.7 (2.7-fold increase in degradation rate in response to a 10°C increase in temperature) for the degradation of oil in beach gravel over the range 6 to 21°C, using a seawater laboratory system.

Cook and Westlake[147] reported that a shift in temperature produced changes in the prevailing microbial forms active on Canadian and Alaskan crude oils. In a seawater system, Walker and Colwell[106] reported that only bacteria were active at 0° and 5°C, though yeasts and fungi participated at 10°C. Numbers of hydrocarbon users were reported to be lower in winter than in summer in Raritan Bay[148] and Chesapeake Bay.[149]

Relative rates of loss in the various hydrocarbon classes may be altered as well. In a study using seven oils of differing weight, greater degradation of the paraffin fraction was observed at 10°C, while the aromatic fraction was more readily degraded at 20°C.[150]

A number of studies have reported that hydrocarbon degradation is correlated with season, being lower in winter. In the Arctic, degradation essentially ceased when tundra soils were frozen.[151-153] Ludzack and Kinkead[154] found a strong seasonal effect on oil disappearance in river water. Dibble and Bartha found rates of oil degradation in a kerosene-contaminated field in New Jersey to be correlated with mean monthly temperature.[155] The effect of cold conditions on biodegradation may be somewhat tempered by a selective shift to cold-adapted (psychrophilic or facultative psychrophilic) organisms. Atlas and Bartha[148] found that hydrocarbon mineralization at 5°C was greater in Raritan Bay water samples collected in winter than in summer samples.

Lastly, temperature may exert an inverse effect on the degree of degradation achieved. Colwell et al.[156] reported 21 percent of Metula crude oil hydrocarbons degraded at 22°C but 48 percent at 3°C. In both cases mixed beach sand populations were used that were previously adapted to the experimental temperature.

Considerable work has been done on the biodegradation of hydrocarbons under anaerobic conditions. Because metabolism of these substances must begin with an oxidation step, an alternate electron acceptor is needed. Nitrate and sulfate have received consideration. Nitrate tends to be depleted in anaerobic environments by denitrifying organisms. Ward and Brock[157] calculated that hexadecane oxidation coupled with sulfate reduction is probably not sufficiently exergonic (energy-releasing) to support microbial metabolism. These workers studied ^{14}C-hexadecane biodegradation in the

anaerobic hypolimnion of a Wisconsin lake. No significant breakdown was detected with or without added nitrate or sulfate, though degradation did occur if oxygen was permitted to enter the system.

The evidence favors the conclusion that anaerobic biodegradation at the expense of sulfate and perhaps nitrate does occur, but only at very low rates. It should not be an important factor in natural ecosystems,[61,158] including soils.[60]

Substantial biodegradation does occur under conditions where oxygen tension is very low or where the influx of oxygen occurs at a very low rate. The K_m of many oxygenases (oxygen concentration allowing the enzyme activity to proceed at 50 percent of maximal rate) is extremely low, and even oxygen levels that are undetectable chemically can seriously alter experimental outcomes. Molecular oxygen, which is soluble in oils, penetrates oil-contaminated soils and sediments to a degree that depends on depth, the concentration of oil, the presence of cracks and fissures in soils or of burrowing worms in sediments,[159,160] and other factors. Typically, a gradient occurs in which biodegradation shows a strong negative relation to depth. Blakebrough,[60] in his laboratory soil column experiments, found detectable degradation of a light fuel oil to a depth of 45 cm.

The metabolic utilization of hydrocarbon materials requires substantial amounts of inorganic nitrogen and phosphorus as well as smaller amounts of sulfur, iron, and other elements. Dibble and Bartha,[118] in a study of the land disposal of waste oil, gave optimal ratios as 60:1 for C/N and 800:1 for C/P. Where N and P are in short supply, as in most marine waters, oligotrophic (nutrient-limited) lakes and some soils, and where large quantities of oil impact on a limited area, the resulting relative nutrient deficiency may become the limiting factor in biodegradation. This situation may encourage nitrogen-fixing activity,[161] but reports of enhancement of biodegradation through fertilization have generally indicated a requirement for both N and P.

Ward and Brock[130] reported an increase of oil oxidation in oligotrophic freshwater lakes up to 20-fold by addition of 300 µg N and 100 µg P per liter. Bergstein and Vestal[162] obtained enhanced biodegradation of crude oil in Arctic tundra ponds through the use of an oleophilic fertilizer consisting of paraffinized urea and octyl phosphate. Nutrient deficiency may be a limiting factor in soils as well, since some studies have shown enhancement of biodegradation on addition of N and P.[19,147,163-166]

BIODEGRADATION OF DIESEL FUEL, FOG OILS AND CLOSELY RELATED MATERIALS IN SOIL AND FRESHWATER

SOIL SYSTEMS

Raymond et al.[19] studied the degradation and transport of oils applied to small, level, 1.7 x 3 m replicate soil test plots with no history of previous oil contamination. The soils were a silt loam (pH 5.4) at Marcus Hook, PA., a sandy loam (pH 6.3) at Tulsa, OK, and a clay loam (pH 7.6) at Corpus Christi, TX. Oils tested included home heating oil No. 2, residual heating oil No. 6,

and two used crankcase oils. All oils were tested at all study sites, and, for each, oil plots with and without added N and P fertilizer were studied. In the late fall each plot received a single application of 2.5 kg oil/m^2, and mixing of oil and fertilizer (if used) with the soil was accomplished by rototilling to a depth of 10 to 15 cm. An increase in hydrocarbon-utilizing microorganisms was noted in all cases, and this persisted throughout the course of the 1-year study. There was little evidence of degradation at the Pennsylvania or Oklahoma sites during the winter months. The highest rates of oil disappearance, in the April-June period at Tulsa, were about 500 g/m^2mo. Overall degradation during the course of the study ranged from 48.5 to 90 percent, the highest values pertaining to No. 2 fuel oil. A positive effect of fertilizer addition was largely restricted to the Pennsylvania site. Here added N and P seemed to gain importance only after about 50 percent of the oil had been degraded. There was no rise in detectable organic acids and aldehydes associated with the reduction in hydrocarbon material, and significant increases in hydrocarbons or other organics in leachate or runoff were not found. Paraffinic and aromatic materials were reduced to about the same degree.

A higher rate of degradation was reported by Francke and Clarke,[167] who studied oils spread on 360 m^2 cultivated and fertilized field test plots at the Oak Ridge, TN, Y-12 plant. The oils used were crankcase oil and vacuum pump oils. Rates of oil loss over the brief 3-month test period were about 2,500 g/m^2 mo. This represented about 40 percent of the applied carbon. The brevity of the study did not permit evaluation of possible residue levels.

Blakebrough et al.[60] studied the persistence and movement of a light home heating oil in laboratory soil columns. The columns were 50.5 mm in diameter and 1,450 mm in height and were packed with a mixture of one part light sandy loam garden soil to nine parts white sand. A single surface application of oil was made at 1.25 L/m^2. Regimens of simulated regular rainfall were carried out. At 65 weeks, about 60 percent loss of oil had occurred in the uppermost 10 mm of the soil column. The extent of degradation increased with time and decreased with depth. Downward movement of the oil produced 2.5 percent saturation at a depth of 600 mm with a fairly sharp cutoff at 680 mm. The greatest depth at which detectable hydrocarbon degradation occurred was only about 45 mm at 65 weeks but was still increasing. About 30 percent of oil held within the upper 45 mm had been metabolized at 65 weeks. Hydrocarbon degradation was less at lower pH values.

Percent loss in both the n-paraffin fraction and the total aromatic fraction exceeded the overall percent loss. The RQ values were somewhat low, indicating incomplete oxidation of the degraded hydrocarbon, and infrared spectrophotometry showed the presence of partially oxygenated products. An interesting finding of the respirometry studies was that an initial sharp rise in oxygen uptake occurred in the absence of bio-oxidation of hydrocarbon. The explanation may lie in the lysis of some organisms, due to toxic effects, with subsequent enhanced metabolism on the part of other organisms.

Johnson et al.[168] studied the effect of low-level chronic hydrocarbon input on the hydrocarbon-degrading potential of soils. Soil samples were obtained close to roadways and from points not exposed to highway runoff. The mixed microbial populations in soils exposed to highway runoff and in wet soils possessed a significantly greater potential for degradation of diesel

fuels, kerosene, and hexadecane than those from dry soils or soils not exposed to highway runoff.

FRESHWATER SYSTEMS

Pritchard et al.[169] studied the biodegradation of a light diesel oil in a laboratory continuous culture system. Conditions were chosen to approximate those of Lake Ontario. Under quiescent conditions, mini-oil spills followed a consistent pattern of slow emulsification, dispersion, and chemical alteration. It was shown that the variety of products formed by biotransformation included some that were more resistant to degradation than the original oil as well as some that were more toxic to fish.

Ludzack and Kinkead[154] reported that the half-life of commercial motor oil in sewage-inoculated BOD dilution water at 20°C was about 1 week. Biodegradation rates were lower, though still comparable, when Ohio river silt was employed as inoculum. Most of the lost hydrocarbon was accounted for by CO_2 production. Over a period of 6 weeks, rates of hydrocarbon disappearance declined. An important mechanism was that biological stabilization was associated with sedimentation of the emulsified material. Degradation rates declined sharply with reduction in temperature. At 10°C, 20 to 30 percent was degraded per week, while at 4°C no apparent degradation occurred.

The changes occurring in crude and refined petroleum products in both fresh and brackish water were studied by Brown and Tischer.[170] Marked physical changes occurred under both aerobic and anaerobic conditions, though biodegradation was more rapid under aerobic conditions. RQ values ranged from 0.4 to 0.6, indicating complete oxidation of most material. Microbial activity was enhanced by addition of a nitrogen source and inorganic phosphate.

Effects of No. 2 fuel oil on various components of laboratory microcosms, simulating lake or pond environments, were studied by Johnson and Romanenko.[171] Ecological effects included a 1,000-fold increase in oil-degrading bacteria.

CONCLUSIONS

A very substantial amount of work has been done on the complex and many-faceted problem of the biological degradation of petroleum and petroleum-derived materials in natural ecosystems. Predominant have been short-term studies measuring decreases in total oil burden, disappearance of materials from chromatograms or degradation of pure compounds or known mixtures. Little work has been done on the more recalcitrant materials, such as the polynuclear aromatics, or on the heteroaromatic or nitrogen-base compounds. This report provides a brief overview of the principles that form a part of our present understanding of petroleum oil biotransformation and biodegradation.

Existing data on the biodegradation or biotransformation of military-relevant fog generating oils (SGF 1 and 2 and diesel fuel) in soil and freshwater systems were presented where available. Such data were not found for

the SGF oils. For SGF 1, diesel fuel and No. 2 heating oil were considered acceptable biodegradation models and for SGF 2, light lubricating oils.

The data base has been considered critically from the standpoint of its adequacy in meeting the requirement of the US Army for information allowing predictions of the role of biosorption to microorganisms and the rate of biodegradation in soil and freshwater systems, of material deposited from obscurant fogs.

Disappearance rates for No. 2 fuel oil have been shown to reach 500 g/m^2/month (690 mg/m^2/hr) in soils during the first few months following application.[19] In waters, rates are probably lower, e.g. up to 10 mg/L/day for No. 2 fuel oil (Johnson and Romanenko, 1983).[171] These observations actually represent favorable case situations. Rates vary greatly according to the nature of the material and, for a given material, in response to environmental and operational conditions and the degree of weathering or aging that has occurred.

EFFECT OF NATURE OF MATERIAL ON BIODEGRADATION RATES

Rates of hydrocarbon disappearance decline greatly with time,[154] being far lower in the 10 to 50 percent of the original material that persists after months of exposure. Existing data are not adequate to assess these lower rates or the nature and degree of recalcitrance of the well-aged residual material.

Quantitative differences in biodegradation rate are anticipated between the types of military-relevant fog-generating oils and between oils of the same type that differ as to supplier, batch, crude oil source, or refining process. No studies were found that provided such comparisons between different oil types or stocks. There are differences between the liquid oils used in reported research and material subjected to vaporization in an engine exhaust system. The latter is known to sustain some pyrolytic alteration and has opportunity for chemical interaction, at high temperature, with substances present in the exhaust gases.

No microbiological fate studies were found using material deposited or collected from actual oil fogs; nor was there work on the products of photooxidation/photolysis of oils or oil fogs.

EFFECT OF ENVIRONMENTAL CONDITIONS ON BIODEGRADATION RATES

Disappearance rates for hydrocarbons are near zero in anaerobic microenvironments and are very low in materials that reach depths greater than 10 to 35 cm in the soil column[60] or that are deposited in aquatic sediments. Existing data do not permit assessment of disappearance rates under these conditions.

Biodegradation rates are greatly depressed by low temperature[154] or freezing conditions, an obvious consideration for arctic waters and regions with long winters. Some data exist on this temperature/seasonal effect for No. 2 fuel oil[19] as well as other petroleum oils.

Limited availability of nitrogen and/or phosphorus in the receiving soil or water can significantly retard biodegradation.[19] For this to become a limiting factor available nutrients must be swamped by input carbon, creating an unfavorable balance. At the calculated input of 6 to 60 g oil/m^2/hr^3, this cannot be ruled out for situations where nutrient-limited soils or small lakes are exposed repeatedly to fogs generated for training purposes. Raymond et al.[19] found an effect of added N and P on one soil but not others, using No. 2 fuel oil.

No data were found on the effect of soil type, type of sediment, or water quality on biodegradation rates of fog-generating or model oils.

EFFECT OF OPERATIONAL FACTORS ON BIODEGRADATION RATES

Soils subjected to repeated or chronic application of hydrocarbon show increased biodegradation potential for diesel fuel.[168] The depression of biodegradation rate that arises from inadequacy of available nutrients is also dependent on application rate. Available information does not, however, allow quantitative prediction of these effects.

CHEMICAL CHANGES IN BIODEGRADATION OF SGF, DIESEL AND MODEL OILS

Accumulation of oxygen-containing intermediates (acids, aldehydes, esters) was reported by Blakebrough et al.[60] but was not detected by Raymond et al.[19] Ludzack and Kinkead[154] reported that most lost carbon appeared as CO_2. We may conclude that with time most applied hydrocarbon undergoes mineralization. Different classes of hydrocarbon are degraded at comparable though unequal rates.[60]

BIOSORPTION/BIOACCUMULATION

Data on biosorption to microorganisms or bioaccumulation of the SGF oils, diesel fuel, and model oils were not encountered.

RECOMMENDATIONS

Several kinds of studies are recommended to define the rates and degree of completeness of the microbial degradation of material deposited from military-relevant oil fogs (SGF-2 and diesel fuel only).

It is recommended that these studies utilize the whole, unfractionated parent oils or unfractionated material collected from actual fogs. Parts of the work might be done using the parent oils, e.g., preliminary experiments or tests comparing parent oils to settled or impacted fog material. However, because of substantial change from parent oils in relative amounts of volatiles and perhaps aromatics,[5] and the possibility of other alterations induced by thermal, photochemical or other processes, it is recommended that the definitive observations, insofar as possible, be made on material collected from actual oil fogs, probably in a laboratory setting.[5] Such fogs should be produced by devices comparable to the M3A3 or VEESS system in actual field use.

Furthermore, the questions raised in what follows must ultimately be answered for oil that has been exposed to sunlight or simulated sunlight. This is because oil used as an obscurant in the field will undergo considerable photochemical change both before and after deposition on soil or water.

In the experiments, oil would be placed in the presence of natural, mixed microbial communities in natural soil, water, or aquatic sediment. They should provide quantitative information on the following, with suggested priority levels 1 (the highest) through 3, as indicated:

Priority 1. Rates of biodegradation of hydrocarbon materials change greatly with time, because of weathering, volatilization, and the disappearance of the more degradable substances. A second factor exerting an enormous effect on biodegradation rates is oxygen availability, which in turn is highly dependent on location, especially depth within the soil or sediment. Biodegradation achievable over weeks or months, or in anoxic situations, cannot be predicted from short-term observations in well-oxygenated material. Therefore, the following should be investigated:

a. The change in biodegradation rate with aging (time of exposure). Both rate of disappearance and rate of mineralization (CO_2 production) should be quantitated as a function of time and percent applied hydrocarbon remaining.

b. The effect of subsurface soil deposition or deposition in aquatic sediment. Rate of disappearance should be quantitated as a function of depth in soil or sediment and of sediment vs. overlying water. Comparisons of the percent hydrocarbon remaining after stated intervals of time should be made. Determinations of oxygen tension should be made at various points in both time and space.

Priority 2. It is probable that considerable variability in biodegradation will follow from varying either the test article (settled fog material) or the nature or source of the environmental substratum (water, soil, aquatic sediment). Biodegradation rates may not be affected by these variables as profoundly as by the factors described under Priority 1. Nevertheless, in the following the test article and the environmental situation are varied within altogether realistic limits.

a. The variability in biodegradation rates encountered when natural soils, waters, and sediments that differ as to type, source, quality and prior exposure are tested.

b. The variability in biodegradation rates encountered when SGF-2 and light diesel fuel samples that represent different batches, suppliers, or parent crude oils are tested.

Priority 3. Here (b,c) a realistic test article is compared with an approximation thereof. In a, below, two kinds of application scenarios are compared. It is thought that,[168] because of differences in impact on microbial flora and interaction with nutrient supplies, differences in application rate may lead to important differences in biodegradation response.

a. Comparison of biodegradation rates under conditions of low-level or chronic application of oil vs. one-time higher-level application.

b. Comparison of biodegradation rates of oils and of settled or impacted fog material from the same oil(s).

c. Effect of prior exposure to sunlight, or simulated sunlight, on biodegradation rates, comparison of exposed to non-exposed.

It is anticipated that gas chromatographic (GC) techniques will constitute the primary tool for monitoring hydrocarbon disappearance or transformation in these studies and that the resolving power of such techniques can be increased by prior fractionation of sample materials. This will yield much information on the reduction or disappearance of specific peaks and classes of substances. To serve the goal of defining the rates and completeness of biodegradation of military-relevant fog oils it is recommended that GC and GC-mass spectroscopy analysis be designed to include the following:

1. Estimation of total remaining material, including the more polar substances arising from partial oxidation, during progress of the above-described studies.

2. Estimation and characterization of residual material remaining after prolonged exposure to microbial communities. This material should be considered for further work in biodegradation, including studies with specific microbial isolates.

The experiments described above would be undertaken in a laboratory setting. A subsequent phase would seek to narrow the gap between laboratory conditions and complex field situations. To do so, the experimental questions addressed in the laboratory would be approached in carefully designed studies using small field plots, microcosms or outdoor experimental channels. Both soil and freshwater systems would be included. The possibility of developing mathematical simulation models for the description and prediction of some of the events under study should be explored.

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