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SURVEY OF MICROBIAL DEGRADATION OF ASPHALTS WITH NOTES ON RELATIONSHIP TO NUCLEAR WASTE MANAGEMENT*

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ABSTRACT

A survey has been made of the microbial degradation of asphalts. Topics covered include chemical and physical properties of asphalts, their chemical stability, methods of demonstrating their microbial degradation, and environmental extremes for microbial activity based on existing literature. Specific concerns for the use of asphalt in nuclear waste management, plus potential effects and consequences thereof are discussed.

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INTRODUCTION

One purpose of this report is to summarize and document the present knowledge on the composition of asphalts and their susceptibility to microbial attack under various environmental conditions. Although the literature on this subject is rather meager, it seems that virtually all kinds of asphalt are biodegradable by bacteria and fungi at rates varying from extremely slow to fairly rapid. The rates of biodegradation are influenced by the contact of microbial enzyme systems with the asphalt, the chemical composition of the asphalt, its physical state, and environmental conditions.

Asphalt has been used for thousands of years to caulk boats, to cement various building materials, for waterproofing, and for other purposes. Large natural deposits occur in many parts of the world. Such deposits are known variously as bitumen, pitch, asphaltite, gilsonite, grahamite, albertite, wurtzlite, etc. Most, but not all crude oils, contain some asphalt in amounts up to 50 percent or more. Presently in the United States, nearly all commerical asphalt is derived from crude oil. Nearly 75 percent of the asphalt is used for paving, about 15 percent for roofing, and the remainder for a great variety of other purposes, including waterproofing, lining water canals and reservoirs, pipe-coating, insulating, and automobile undercoating. Within the last 10-15 years, primarily in Europe, asphalt has also been used for the encapsulation of low and intermediate-level radioactive wastes (1).

The second purpose of this report is to examine the consequences of microbial degradation of asphalt used to encapsulate radioactive wastes on long-term nuclear waste management. The first asphalt (bitumen) solidification system for radioactive wastes began operation in 1965 at the Commisariate a l'Energie Atomique (CEA) facility in Marcoule, France.

Large-scale asphalt solidification systems have also been put into operation at the Karlsruhe Nuclear Research Center in the Federal Republic of Germany and at the Eurochemic Company in Mol, Belgium. Data and literature pertaining directly to microbial attack of asphalt encapsulated radioactive waste are essentially non-existent. Corresponding research on this topic has not been performed with the same intensity as has related work on leaching, radiolysis, and thermal degradation of asphalt (1,2,3,4).

In this paper, microbial degradation of asphalt used to encapsulate radioactive wastes will be examined primarily from the viewpoint of terminal isolation. This includes deep geologic isolation in a salt mine, e.g., the Waste Isolation Pilot Plant project (5,6) planned in New Mexico, in hard-rock repositories, potentially in deep sea-bed disposal, etc. Waste isolation in near-surface trenches (primarily low-level, non-transuranic wastes) is not emphasized; much of the general microbial degradation information presented herein may, however, be applicable for this mode.

Processing temperatures in the range of 80-195°C are used for the asphalt encapsulation of radioactive wastes (3)--high enough to kill most bacteria and fungi contained in the waste itself. However, bacterial degradation of the asphalt waste from external sources is still quite possible. Asphalt-encapsulated wastes may become contaminated prior to burial in a terminal waste repository or may eventually become contaminated by bacteria contained in other (organic, unprocessed) wastes which may also be buried in the repository. It should also be assumed that any kind of microorganism may find its way into the repository during excavation and emplacement operations. Some fraction of these microorganisms may survive and then adapt to the existing environment, potentially resulting in microbial waste degradation.

POTENTIAL AREAS OF CONCERN

The extent and consequences of bacterial degradation must be rank ordered against other potential problem areas for the utilization of asphalt in long-term nuclear waste management.. These other areas of concern include:

1. Fire in the course of processing, transport, or storage of the radioactive waste form. The possibility that the active asphalt-waste product may burn has always been considered as one of the main disadvantages of asphalt encapsulation. This is the predominant reason that some people prefer other waste treatment techniques (1).
2. Radiolytic degradation of asphalt yielding significant quantities of gas, primarily H_2. Internal irradiation from alpha-emitting transuranic waste is expected to yield greater gas generation than beta and gamma irradiation from the fission and activation products incorporated in low-level wastes. Very few data exist on radiolytic gas generation from asphalt, except from beta and gamma irradiation.
3. Thermal degradation of the asphalt resulting in swelling.
4. Leaching of the active asphalt-waste matrix by ground water or brines in deep geologic isolation. The extent of this concern has been well studied (1,2,3,4) and determined not to be of overriding short-term significance. Potential enchanced migration of radionuclides (due to bacterially produced chelating agents) following leaching has not yet been adequately addressed.
5. Matrix solubilization (7). Many microorganisms have the ability to produce alcohols, esters, ketones, and other fermentation products in which asphalts are soluble, albeit only slightly. Such solubilization may be of negligible significance during a period of

several years, but could result in the perforation or partial matrix solubilization of asphalt encapsulants or coating within decades or centuries. In an environment where water and organic matter (cellulosics, proteins, lipids, etc.--as in unprocessed wastes) are present, along with elements essential for microbial growth, some solubilization of asphalt by fermentation products can be expected.

6. Petroleum product utilization. Use of large quantities of asphalt for waste management may conflict with other uses or needs for this product and its derivatives. Since asphalt is not in short supply, however, such utilization is not expected to be of major impact.

Further study is necessary before the relative degree of concern for bacterial degradation of asphalt can be adequately evaluated in relationship to its nuclear waste management use. The possibility exists that none of the stated concerns will be judged (via a cost-risk-benefit analysis) sufficient to hinder the utilization of asphalt for radioactive waste encapsulation.

CHEMICAL PROPERTIES OF ASPHALTS

Asphalts are conglomerates of high molecular-weight hydrocarbons, resins, and asphaltenes, mixed sometimes with up to 27 percent by weight of minerals. After being heated to 100°C to expel water and gases, the mineral content of certain asphalts may be as high as 38 percent. There are many kinds of asphalts, differing widely in chemical composition, depending on sources and treatments.

All asphalts are black or dark brown in color, insoluble in water, and generally solid at temperatures below 70°C. They consist mainly of carbon, hydrogen, sulfur, and oxygen (8). The elementary composition of asphalts free of minerals, water, and gases is as follows:

	Range (%)
Carbon	80-86
Hydrogen	9-11
Oxygen	1-17
Sulfur	1-9
Nitrogen	1
Ash	0.1-1

These elements occur in four major components: saturated hydrocarbons, resins, cyclic hydrocarbons, and asphaltenes in various proportions.

According to Sachanen (9), asphaltenes are dispersed colloidally in petroleum due to peptonization by absorbed resins and heavy polycyclic hydrocarbons, whereas resins form true solutions in petroleum. Asphalts derived by refining petroleum generally have more paraffinic side-chains than natural asphalts such as gilsonite or albertite, for example.

Asphaltenes, which make up from 11 to 32 percent of the bulk of asphalt, are insoluble in nonpolar paraffinic hydrocarbons, soluble in carbon disulfide and chloroform, only slightly soluble in alcohol, ether or low molecular-weight paraffinic hydrocarbons, and insoluble in water. Asphaltenes contain rather high proportions of oxygen (3-11 percent) and sulfur (mostly 7-9 percent). The components are mainly high molecular-weight (> 1500) aromatic and heterocyclic hydrocarbons. The asphaltenes melt in the range of 180 to 280°C. By hydrogenation at moderate temperatures, asphaltenes may be partly converted to resinous materials. Hydrogenation at high temperatures and increased pressures results in a low yield of liquid hydrocarbons.

Resins are amorphous solids that are soluble in most hydrocarbon solvents. The compounds forming resins are structurally similar to those forming asphaltenes. Up to 22 percent, but generally less than 10 percent, of natural asphalts consists of resins. These are mostly napthenic acids of high molecular weight (around 800). Like the asphaltenes, the resins in asphalts contain rather high proportions of sulfur and oxygen.

The paraffinic, naphthenic, and aromatic hydrocarbons in asphalts, along with heterocyclic compounds containing sulfur, nitrogen, oxygen, and small amounts of various metals, have molecular weights ranging from 500 to 5000, although small amounts of lower molecular-weight compounds may be present. An average of 35 percent of the hydrocarbon fraction is saturated; 50 percent or more is aromatic. Sulfur is present primarily in thiophenes and benzthiophenes, with smaller quantities in sulfoxides. Oxygen is present mainly in hydroxyl, carbonyl, and ester groups. Nitrogen is present primarily as pyridines and pyrrole-type structures (10).

PHYSICAL PROPERTIES OF ASPHALTS

At ordinary temperatures, typical asphalts appear to be lyophilic colloids made up of asphaltenes, resins, and hydrocarbons (HC). The term lyophilic denotes a strong affinity between a colloid and solute in which it is dispersed. The same asphaltene dispersed in various HC-resin combinations may yield essentially similar asphalts. Micelles of coal-tar pitches and bitumens consisting of highly cyclic, presumably polyaromatic HC's of high molecular weight, are believed to be surrounded by layers of aromatic compounds grading into naphthenic and paraffinic structures.

As the temperature of asphalt is increased, e.g., from 250 up to 400-600°C, colloidal and micelle structure is altered, volatile substances are lost, and the fluidity of the residue increases until it flows almost like lubricating oil. When cooled to 25°C, the resulting product is usually less fluid and more prone to be brittle than it was originally. Further cooling causes progressively less fluidity or higher viscosity (fluidity is the reciprocal of viscosity).

(If asphalt encapsulated transuranic radioative wastes are determined to be acceptable for deep geologic waste isolation, the storage temperatures to be expected in the Waste Isolation Pilot Plant would range from 25°C to a maximum of 70°C (5,6)).

The viscosity of asphalts is high, ranging from 10^3 to 10^{20} poises at 0 to 50°C. For comparison, the viscosity of water at 20°C is 0.01005 poises while heavy machine oil has a viscosity of 6.6 poises. The viscosity of a natural pitch is 1.3×10^{10} poises at 15°C, 5.1×10^{11} poises at 0°C. At -77°C (the temperature of a mixture of dry ice and ether) asphalts lose all semblance of fluidity.

The density of asphalts ranges from 1.1 to 1.5 g/cm^3. Most pitches have a density of barely 1.1 as compared with 1.4 to 1.8 for anthracite coal and 0.87 to 0.91 for paraffin wax. Nearly all crude oils are lighter than water, i.e., less than 1 g/cm^3.

CHEMICAL STABILITY

Asphalts are generally resistant to many chemical reagents. As an indication of the oxidation resistance of various components of asphalt, sunlight tends to oxidize:

asphaltenes > resins > aromatic HCs > saturated HCs

(Sunlight, obviously, is not a concern for deep geologic isolation of radioactive wastes. Oxidation is.) Different types of asphalt vary greatly in their susceptibility to solar radiation oxidation. Air-blowing asphalt to increase its hardness increases its resistance to oxidation by sunlight. Air-blown asphalt is produced by blowing air through molten asphalt at 150-260°C (10). During air-blowing, oxygenation and dehydrogenation reactions take place. Nearly one-third of the asphalt produced by industry is air-blown, which converts it into plastic. Many varieties of both blown and distilled asphalts have been utilized for radioactive waste encapsulation.

The microbial oxidation of asphalts has been reported by Tauson (11), Hundeshagen (12), ZoBell (13), Burgess (14), Harris (15), Kulman (16), Traxler et al. (17), and several others.

Asphalt resists attack by dilute sulfuric acid but is attacked by concentrated sulfuric acid and by all concentrations of nitric acid and hydrochloric acid. It does not seem to be attacked by organic acids. Asphalt is not attacked by concentrated alkaline solutions at ordinary temperatures, although dilute alkaline solutions react with acidic asphalt constituents to form salts such as sodium naphthenates which serve as excellent emulsifying agents for asphalt.

Insufficient data exists for the long-term (hundreds to thousands of years) chemical stability of asphalt encapsulated radioactive wastes which may be placed in a deep geologic, terminal waste isolation repository. The chemical durability (i.e., potential leaching by ground waters or brines, interactions with waste containers and adjacent geology) of such wastes would also be potentially compromised by the effects of radiolytic and thermal degradation, as well as by potential microbial attack.

MICROBIAL DEGRADATION OF ASPHALTS (and other organic materials)

The microbial activities of greatest concern for nuclear waste disposal are those involving metal metabolism, particularly metabolism of the heavy metal actinides. There are five major types of metal transformations found in biological systems (7,18,19):

1. Complex and chelate formation of metal ions with organic ligands. Organic acids, hydroxamates, and other microbial metabolic products are very effective chelating compounds (19,20). Of primary concern is the enhanced migration of chelated actinide elements (leached from organic matrix transuranic wastes) moving through the lithosphere (from deep geologic isolation) towards the biosphere. Heterotrophic bacteria and fungi may also degrade metal complexing agents resulting in precipitation of the released ion as water-insoluble hydroxides, oxides, or salts (19,21), thereby retarding nuclide migration.
2. Metabolism which results in oxidation state changes of metals, the metal participating in oxidation reactions which provide energy for microbial growth. It has been experimentally demonstrated that the oxidation states of actinide elements in solution, and changes therein, have a significant effect on migration rates in the lithosphere (22). Microorganisms have been utilized for controlled in situ bacterial leaching of uranium ore (23). Both autotrophic and heterotrophic microorganisms can produce oxidizing and reducing agents, functional in either the solubilization or deposition of uranium (24). Similar leaching of plutonium from transuranic wastes may be extrapolated. Bacterial metabolic action, as well as complex and chelate formation, could have a significant effect on extent of

radionuclide (i.e, uranium and plutonium) migration in a waste repository.

3. Substitution of one metal for another within the microbe. This process often occurs with metal containing enzymes. The consequence of the substitution process may be to accumulate and sequester elements within the microbial cell, removing the elements from solution (19). As applied to nuclear waste management, this process yields two possible, opposing consequences (assuming that actinide and/or fission product metals are substituted):
    a) substituted radionuclide aqueous phase migration is significantly retarded due to absorption or precipitation;
    b) fissionable radionuclides (U, Pu) are bioconcentrated to a sufficient extent to result in a criticality incident. (The likelihood of, or consequences of, such a criticality occurrence are not well quantified at this time.)
4. Another potential mechanism of bacterial bioconcentration for radionuclides is the extracellular precipitation of metals in bacterial mucilage, holdfasts, stalks, and sheaths. There are numerous bacteria which produce extracellular polymers which result in the bioconcentration of iron and manganese (25,26). The chemical mechanism involved is presently unknown but does not necessarily involve cell metabolism.
5. Aklylation of metals by biological systems. This is the process involved in the formation of methyl mercury and trimethyl selenide (19). This process is not well understood. From a nuclear waste management viewpoint, it is [illegible] of concern for the potential

volatilization of organo-metal compounds of fission products (e.g., Cs, Sr, I) and actinide elements (e.g., U, Pu) found in the waste.

A laboratory and field investigation program to quantify the rate, extent, and consequences of bacterial degradation (for the topics listed above) of radionuclide-contaminated organic matrix transuranic wastes (including asphalt matrix) on long-term nuclear waste isolation has recently been initiated (7,27) (May, 1978). This study is a segment of the Waste Isolation Pilot Plant (WIPP) Transuranic Wastes Experimental Characterization Program (6). These studies will specifically include biogeochemical mechanisms of radonuclide mobilization and immobilization, microbial production of gases, and microbial transformation rates of gases that were produced abiotically, all under conditions expected in the bedded salt WIPP facility (temperature: 25°-70°C, pressures: 1-150 atmospheres, salt mine geochemistry, radiation fields originating from plutonium contaminated transuranic wastes, and, quite possibly, an anaerobic atmosphere in the long-term). Also included are an assessment of naturally occurring microoganisms found in various existing transuranic waste containers (contemporary to 30 years old) and adjacent soils at the Los Alamos Scientific Laboratory (New Mexico) waste burial sites and the effects of existing microflora under varying conditions of hydration, temperature, salinity, pH, and radiation on transuranic mobility (27).

Microbial degradation data and interpretations thereof will be utilized in overall WIPP safety and consequence assessment studies for determining the long-term environmental safety of isolating transuranic wastes in a bedded-salt, deep geologic repository. Bacterial degradation studies will also be useful in determining which types of organic matrix waste are safe for acceptance in the WIPP; the data will help in the establishment of acceptance criteria relevant to biological modes of degradation and any resultant consequences.

METHODS OF DEMONSTRATING THE MICROBIAL DEGRADATION OF ASPHALTS

As with most particulate and insoluble substances, the rate of attack by microbial enzyme systems is directly proportional to the surface area exposed to oxygenated water and enzymes. Being insoluble in water, asphalts to be tested for microbial degradability are generally specially treated to accelerate the reaction by increasing the surface area without unduly modifying the chemical properties of the asphalt. Unless so treated, it might require months or years to demonstrate appreciable amounts of microbial degradation. Two methods with many modifications have been employed:

1. Chunks of asphalt are crushed or pulverized to give innumerable tiny pieces. Since most asphalts are plastic at ordinary temperatures, they are more susceptible to pulverization at -75°C or colder. The pulverized asphalt is kept frozen until dispersed in nutrient medium, which usually contains sand, diatomaceous earth, bentonite, etc., to hold the asphalt particles apart.
2. Asphalt is dissolved in the minimal amount of benzene or chloroform at 80°C for mixing with inert solids such as sand, clay, crushed glass, etc. After thorough mixing, as in a blender, measured quantities of the mixture are transferred to culture flasks for autoclave sterilization at 121°C, *which drives off all of the* solvent.

Modification of the solvent method includes (a) coating glass microscope slides with extremely thin films of asphalt. After incubation in mineral salts medium, the slides can be examined with a microscope to determine whether bactria have grown on the asphalt or if the asphalt has been perforated by microbial activities (enzymatic); (b) in much the same way, wires can be coated with thin films of asphalt. Coils of such insulated wires can be incubated in mineral salts medium (some inoculated with bacteria and the controls without) and tested periodically for capacitance, conductance, and other properties.

Several other criteria have been used by different investigators to determine whether various asphalts have been degraded by bacteria or fungi:

1. A substantial increase in the number of microbial cells found in mineral salts medium containing asphalt as the only source of energy indicates the microbial utilization of asphalt. Each unit weight of microbial biomass produced expressed as carbon indicates that at least a like amount of carbon has been extracted from the asphalt. The ratio is more likely to be 1:2, because in such energy-yielding reactions, at least half and generally more of the carbon extracted from the substrate is oxidized to CO_2.
2. Measuring the amount of CO_2 produced indicates the amount of asphalt carbon that has been completely oxidized.
3. Oxygen uptake is an indicator of the amount of carbon compound oxidized, assuming that the medium contains no other oxidizable substance. As with other tests, this must be established by the use of appropriate controls.
4. Changes in the appearance of asphalt, its pitting in films or its disappearance provide direct evidence for degradation. The rate of microbial degradation under ordinary conditions is usually so slow that several months may be required to obtain definitive results. Long incubation periods are also required before there are meaningful changes in weight or volume of asphalt, whereas microbial growth, CO_2 production, or O_2 uptake may indicate some asphalt oxidation within a week or two at 25°C.

The mineral salts medium utilized may be seawater for marine bacteria. It may be near-saturated brine if one is concerned with bacteria which may survive and degrade wastes in a bedded salt geologic repository such as the WIPP in southeastern New Mexico.

Composition of brines and groundwater of specific application to WIPP-related studies are listed in the following table (28).

Ion	Solution "A" (mg/liter) (± 3%)	Solution "B" (mg/liter) (± 3%)	Solution "C" (mg/liter) (± 3%)
Na+	42,000	115,000	100
K+	30,000	15	5
Mg++	35,000	10	200
Ca++	600	900	600
Fe+++	2	2	1
Sr++	5	15	15
Li+	20	--	--
Rb+	20	1	1
Cs+	1	1	1
Cl-	190,000	175,000	200
SO_4--	3,500	3,500	1,750
B (BO_3---)	1,200	10	-----
HCO_3-	700	10	100
NO_3-	---	--	20
Br-	400	400	--
I-	10	10	--
pH (adjusted)	6.5	6.5	7.5
specific gravity	1.2	1.2	1.0
total dissolved solids	306 g/l	297 g/l	3.7 g/l

The above solutions are representative of waters which could potentially intrude into the Waste Isolation Pilot Plant. Specified pH values of these solutions are representative of the "as measured" pH's of the naturally occurring solutions on which they are based.

Solutions "A and "B" are near-saturated brines. Solution "A" is based on the analyses of several brines from the Salado region overlying the planned

WIPP facility storage horizon. This is a potash bearing region and consequently is enriched in both potassium and magnesium ions, relative to sodium chloride. Solution "A" is intended to be representative of brine which could intrude into the waste horizon via flow or percolation from above.

Solution "B" is based on the analysis of the brine obtained by dissolving a portion of rock salt core in de-ionized water. The salt core was from the AEC #8 hole, less than four miles from the center of the planned WIPP facility. This analyzed brine has an approximately 98% NaCl content and a total undissolved content (primarily mixed clays, not included in analysis) of less than 1%. Solution "B" is representative of the brine resulting from a conceivable catastrophic scenario at the WIPP mine (flooding, service water line rupture, etc.) whereby water pours in, dissolves the salt, becomes saturated, then begins to attack the waste during the operational phase of the facility. It is also the brine resulting from salt being dissolved with any water released from the waste form itself.

Solution "C" is representative of groundwater pumped from one of the aquifers in the Rustler formation, based on two separate analyses. It consists primarily of a dilute solution of calcium and magnesium sulfates.

A general purpose medium for many soil and fresh-water microbiota has the following composition:

K_2HPO_4	1.0 g
NaCl	2.0 g
$MgSO_4 \cdot 7H_2O$	1.0 g
$(NH_4)_2SO_4$	1.0 g
$CaCO_3$	2.0 g
Trace element solution	5.0 ml (described below)
Distilled water	1000 ml

The trace element solution, prepared in 0.1 N HCl, contains per 5 ml:

$Fe(SO_4)_3.9H_2O$	1.5 mg
$MnCl_2.4H_2O$	0.9 mg
$Co(NO_3)_2.6H_2O$	0.6 mg
$ZnSO_4.7H_2O$	0.2 mg
H_2MoO_4	0.1 mg
$CuSO_4.5H_2O$	5.0 g

The pH of the medium may be adjusted to about 7.0 with dilute NaOH. Should one want to determine whether the microbial degradation of a given asphalt occurs at higher or lower pH values, the pH of the medium should be adjusted accordingly. It is within the realm of possibility that such activity could occur at pH values as low as 1.0 or as high as 10.5. However, most soil and aquatic bacteria, including asphalt oxidizers, grow best within the range of pH 6 and 8.

RATE OF DEGRADATION

Even under the most favorable conditions for the microbial degradation of asphalts, the rate is generally very slow. It usually requires several days to a few weeks to demonstrate the microbial degradation of various asphalts. The rate is influenced primarily by the chemical composition of the asphalt and particularly its physical site. The rate of its degradation appears to be directly proportional to the surface area exposed to oxygenated water per unit weight of asphalt. Most natural soils seem to contain enough moisture to satisfy the water requirements of bacteria (29). Certain oil-oxidizing bacteria may contain enough water to enable them to oxidize oil, resulting in the formation of enough water to keep them growing (30):

$$C_{15}H_{32} + 23\ O_2 \rightarrow 15CO_2 + 16H_2O$$

Determination of the minimum amount of water necessary for microbial activity is an area that requires further study. The amount of water available in a deep geologic waste respoitory is not yet well quantified, but is expected to be extremely small (in a bedded salt repository, about 0.5 weight percent) or not available. This may be a significant limiting factor to microbial action, mitigated, of course, by the amount of moisture available in an individual waste package.

By increasing the contact between enzymes and substrate, certain detergents tend to increase the rate of oil or asphalt oxidation. A good many species of bacteria produce surfactants (31,32). The microbial production, properties, and functions of surfactants, with particular reference to petroleum, have been reviewed by Zajic and Panchal (33). The biodegradation of asphaltenes and other hydrocarbons by a Pseudomonas species was found (34) to be promoted by bio-emulsifiers or surfactants.

Corynebacterium lepus and Pseudomonas asphaltenicus are new species of bacteria recently isolated (35) from asphalt deposits in Ontario, Canada. Both species attack higher hydrocarbons and produce surface-active substances that substantially reduce the interfacial tension of mineral salts solution overlayered with kerosene. Although its optimal growth was on n-decane, P. asphaltenicus was shown to utilize a wide range of hydrocarbons, including asphaltenes which make up an appreciable part of most asphalts.

By virtue of its increased viscosity at higher temperatures, asphalt is more susceptible to microbial degradation at increased temperatures. Microbial reaction rates are also accelerated by higher temperatures to the extent that the system is thermostable. However, the highest temperature at which microbial activity occurs appears to be in the neighborhood of 100°C, significantly above the 70°C expected in a low-level transuranic waste repository (6).

ENVIRONMENTAL EXTREMES FOR MICROBIAL ACTIVITY

Relatively few microbial species survive prolonged exposure at temperatures higher than 55 to 60°C. However, several species of thermophilic algae, bacteria, and fungi thrive at temperatures ranging from 45 to 70°C (36). A few species grow in thermal waters at 70 to 95°C. Bacteria have been found growing profusely on glass slides submerged in hot springs at 93.5 to 95.5°C (37). Sulfate-reducing bacteria were recovered from oil-well cores drilled at 2,000 to 4,000 m, where the ambient temperature was 60 to 105°C (38). Cultures were grown at 70°C at 1 atm. One culture grew at 85°C when compressed to 400 atm. At a pressure of 1,000 atm, it reproduced at 104°C.

Although the microbial degradation of oil is generally more rapid in the range of 25 to 45°C than at lower temperatures, crude oil has been shown to be oxidized at temperatures as low as -1.1°C (39). Bacterial growth in glycerol medium at -10°C has also been demonstrated (40).

A good many microbial species survive prolonged compression to 1,000 atm at around 3°C (deep-sea temperature and pressure). However, only a few species, called barophiles, are able to grow under these conditions. Most bacteria are physiologically active at hydrostatic pressures ranging from 100 to 400 atm (41). Hyperbaric oxygen, CO_2, and certain other gases are bacteriostatic or bactericidal at pressures of only 5 to 10 atm. Again, this may be of considerable significance in a deep geologic isolation facility, due to rock overburden (lithostatic) confining pressures (150 atmospheres in the WIPP).

Within the range of 0.1 and 30 mg/liter, the concentration of dissolved oxygen has no discernible effect on the rate of hydrocarbon oxidation by microorganisms (13). However, the rate of such oxidation is appreciably slower in the absence of free oxygen than when some oxygen is present. The microbial oxidation of hydrocarbons under strictly anaerobic conditions has been reported by Tauson and Aleshina (11), Novelli and ZoBell (42), Traxler and Bernard (43), Ward and Brock (44), and several others. Ward and Brock (44) demonstrated the transfer of ^{14}C from hexadecane to bacterial biomass and CO_2 under anaerobic conditions.

Although radiolytic degradation of water can yield hydrogen plus oxygen, studies on the radiolytic degradation of cellulosic transuranic contaminated waste materials (45,46) have indicated that oxygen (in air) originally contained in the waste package is depleted in the formation of CO and CO_2.

It has not been determined whether asphalt is attacked by microorganisms in the absence of free oxygen. If certain anaerobes attack asphalt, they do so at a rate that is much slower than aerobic degradation.

Various kinds of microorganisms differ greatly in their osmotic pressure or salinity requirements. Most grow best in the neighborhood of 3 to 30 atm. Organisms that thrive at appreciably higher osmotic pressures characteristic of brines are described as being osmophilic or halophilic. Those that grow in saturated salt solutions are called extreme halophiles. The osmotic pressure of an aqueous solution saturated with NaCl is about 210 atm. Extreme halophiles reproduce and are otherwise physiologically active in concentrated brines (47), the Dead Sea (48), solar salts (49), rock salt deposits (50), and Great Salt Lake (51). Smith and ZoBell (52) demonstrated the growth of several types of bacteria in Great Salt Lake, the salt content of which ranges from 300 to 336 g/liter. Much of the extensive literature on halophilic bacteria has been reviewed by Flannery (53), Brown (54), and Kushner (55).

Microoganisms grow in natural conditions and in the laboratory at redox potentials ranging from Eh -450 to +850 mv (56,57,58). The activity of iron-oxidizing bacteria are normally found in an environment with an Eh range limited to +60 to 850 mv at one exteme; sulfate-reducing bacteria are active in the range of Eh -450 to +50 mv.

Bacteria that oxidize sulfur to sulfate have been shown to do so in medium that is as acidic as pH 0.5 (59). Starkey and Waksman (60) reported the growth of _Acontium velatum_ and other fungus that degrades cellulose in medium as acidic as pH 0.1. Of the 82 species of bacteria listed by Altman and Dittmer (61), 8 are documented as being able to grow below pH 2.5 or above pH 10.0. _Bacillus circulans_ and _Rhizobium leguminosarum_ grew at pH 11.0. _Streptococcus faecalis_ grew at pH 11.1. All 51 strains of _Agrobacterium radiobacter_ studied by Hofer (62) were said to grow well in media at pH values as high as 11.5.

REVIEW OF LITERATURE

Although the existing literature available on the bacterial degradation of asphalts may not be directly applicable for nuclear waste management purposes, a review is presented as background material, for potential applicability.

Pitch of vegetable origin consisting largely of resins was reported (63) to be oxidized by various soil bacteria. Such bacteria were shown to be widespread in nature, being found in garden, forest, peat, and oil-field soils. The rate of bacterial decomposition reached 0.4 g of pitch per dm^2 ($dm^2 = 100\ cm^2 = 15.5\ in^2$) of soil in 2-1/2 months. The ability of bacteria to degrade pitch was closely related to their ability to oxidize aromatic hydrocarbons, phenols, and phenomethanes.

After finding large populations of asphalt-oxidizing bacteria in soil underlying defective spots in old asphalt-paved highways, it was suggested (13) that microbial activity might contribute to the deterioration of such highways. An examination of 38 different asphalts provided by the American Petroleum Institute revealed that all were susceptible to microbial oxidation in the presence of moisture, minerals, and free oxygen. Big differences were observed in the biodegradability of the asphalts. The degradation rates were generally very slow. Judging from the amount of O_2 consumed, asphalt dispersed in medium on the surface of glass was estimated to be oxidized at rates ranging from about 5 to 75 g per cm^2 during eight weeks' incubation at 25^oC (13).

Microbial activity was reported (14) to be responsible for the deterioration of asphalt roads in Montana. Burgess (14) suggested the application of more effective underseals and better road-grading procedures to prevent soil moisture from contacting the asphalt. He also proposed investigating the feasibility of employing chlorinated asphalts to inhibit microbial growth.

Similar observations were made by Harris et al. (64) who observed the utilization of asphalt by bacteria associated with asphalt-paved highways in

Kansas. The bacteria included species of Mycobacterium, Flavobacterium, Pseudomonas, and Micrococcus. Harris (65) isolated numerous pure cultures of bacteria from soil at the soil-asphalt interface of Kansas highways. Most of the cultures grew in mineral salts medium containing asphalt as the sole source of energy. The asphalt was dispersed on quartz sand by melting it at increased temperature, which probably expelled some of the more volatile components. Certain physical properties of paving asphalts were shown to be altered by microbial activity at ambient temperatures within a few weeks.

Later, bacteria were incriminated (66) in the degradation of asphaltic coatings on pipe buried in soil. Many of the asphalt-utilizing bacteria were similar to those found by Harris et al. (15) at the soil-asphalt interface of Kansas highways. Harris (67) noted a steady increase in the microbial population in soil surrounding asphalt coatings on buried pipes and essentially no microbial growth around coal-tar coatings. Mainly pseudomonads, corynebacteria, and actinomycetes were involved in the destruction of asphalt.

The corrosion of metal pipes and cables coated with asphalt has been attributed (16) to the microbial deterioration of the asphalt. An abundance of asphalt-degrading bacteria were found in the soil surrounding the conduits.

When bituminous roofing material was buried in moist soil, it was found (68) to give rise to large populations of asphalt-oxidizing bacteria. The microbial destruction of the asphalt was caused by oxidizing bacteria. This microbial destruction was believed to leave the residual materials more susceptible to abiogenic oxidation and other kinds of weathering.

An abundance of asphalt-oxidizing bacteria, expecially species of Pseudomonas, Chromobacterium, and Bacillus, were generated in soil in which

Phillips and Traxler (69) buried asphalt shingles. Certain strains, notably P. aeruginosa, which originally could not utilize asphalt, acquired the ability to do so after being cultivated for a few generations in glucose medium containing asphalt. Enrichment cultures degraded from 3 to 25 percent of the asphalt within a week when the walls of culture flasks were coated with a thin film of asphalt by dissolving it in benzene. After introduction of 100 ml of mineral salt medium into each 1500 ml flask and autoclaving at 121oC to evaporate all of the benzene, a film of asphalt was layered on the surface of the medium as well as on the flask sides and bottom. The asphalt was described as being rich in paraffins.

This raises the question of whether only certain fractions of asphalt are attacked by bacteria. From the extensive literature on the microbial utilization of petroleum hydrocarbons and related products, it is well established that certain fractions of crude oil and products refined therefrom are more readily utilized than other fractions. But under favorable conditions, virtually all kinds of hydrocarbons and most, if not all, heterocyclic and resinous compounds appear to be susceptible to microbial attack. (See reviews by Andelman and Suess (70), Atlas and Bartha (71), Beerstecher (72), Dagley (73), Davis (74), Floodgate (75), Foster (79), Fuhs (77), Gibson (78), Rogoff (79), Treccani (80), and ZoBell (13,81,82.)

Depending on the kinds of bacteria present and various environmental conditions, the paraffin fractions of asphalts seems to be attacked more rapidly than the heterocyclic compounds of asphaltenes. If so, destruction of the paraffins may render the remaining fractions more vulnerable to enzymatic and chemical action. The complete disappearance of the last traces of certain asphalts subjected to bacterial action for several months is evidence enough that all components are susceptible to microbial degradation. More information is needed on which asphalts are least susceptible to such degradation, and what can be done to make them even less vulnerable to microbial attack. Preliminary work at Eurochemic (4) indicated that blown asphalts (used for radioactive waste encapsulation) are less attacked than distilled asphalts; bituminous coal tar showed the highest comparative resistance to microbial attack.

Resins of four different kinds of asphalts were shown by Traxler et al. (17) to be attacked by soil bacteria. Mycobacterium ranae was shown to modify the rheological properties of asphalt within four months at 30oC. M. ranae and Nocardia coeliaca caused 1.5-to 6.8-fold increases in the relative viscosity of different asphalts.

SUMMARY

The chemical and physical properties of asphalts that influence their biodegradability have been outlined. Many of the components of asphalts, such as asphaltenes, resins, and paraffinic, napthenic, and aromatic hydrocarbons are more resistant to chemical reagents, such as strong acids or alkalines, than to microbial enzyme systems. Several different methods for demonstrating the microbial degradation of asphalts have also been described. Since 1934, nearly a hundred different kinds of asphalts have been shown by various investigators to be degraded by bacteria or related microorganisms. Although only a few species attack asphalt, such species appear to be widely distributed in soil. They are most abundant in soil or bottom sediments that have been in contact with crude oil or asphalt. Asphalt-oxidizing bacteria have been incriminated in the deterioration of asphalt-paved streets and asphalt coatings on buried pipes or other steel structures. This suggests that under certain conditions of storage where microorganisms, minerals essential for their growth, and a little moisture are present, bituminous coatings might be breached by bacteria within a few decades.

The bacterial degradation of asphalt used for radioactive waste encapsulation has in the past received almost negligible study. The resultant effects and consequences of such degradation, particularly of metal (radionuclide) metabilization and potential enhanced radionuclide migration, could have a significant impact on the acceptability of such waste forms for long-term, deep geological isolation and environmental safety. The specific concerns for use of asphalt (and other organic matrix materials) for nuclear waste management have, therefore, been related to the existing literature on microbial degradation of asphalts. A laboratory and field sampling program to address these concerns has been recently initiated and was briefly described.

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