EFFECT OF MICROORGANISMS ON BITUMINOUS MATERIALS
- a literature review -

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We regret that some of the pages in the microfiche copy of this report may not be up to the proper legibility standards, even though the best possible copy was used for preparing the master fiche.
A review, with 32 references, is given of the literature on the effect of microorganisms on bitumen. Long-range studies on this problem in relation to the incorporation of radioactive wastes into bitumen are recommended.
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FOREWORD

The application on a large scale of nuclear energy results in an increasing amount of radioactive waste to be treated for permanent safe disposal. Research work on the solidification of these wastes, performed in various countries has resulted in the development of processes for the incorporation into bitumen of radioactive wastes (1).

Although already applied on pilot and industrial scale, the development work on the bituminization of radioactive wastes is going on. One of the aspects being studied is the safety of this method.

Much attention is given to aspects like leaching rate, explosion and fire hazards, self-heating and radiation resistance of the bituminous material used for the incorporation. Several authors consider bitumen as resistant to attack by microorganisms (2, 3), however only one report is known in which microbiological studies on the resistance to microorganisms with (inactive) salt containing bitumen are described (4). Up to now no results of studies on microbiological attack of bitumen, in which radioactive material was incorporated, could be found.

In this publication a review is given of the literature on the effect of microorganisms on bitumen, as published in the non-nuclear literature, with the aim to inform the research workers in the field of the management of radioactive wastes about this problem.
INTRODUCTION

Since the beginning of this century a great number of publications describe the action of various microorganisms on gaseous, liquid and solid hydrocarbons. In 1906 Daha (5) described the utilisation of paraffinic hydrocarbons and in the same year Sühngen (6) published about the isolation of methane-oxidizing bacteria. Beerstecher (7) reviewed in 1954 the subject of hydrocarbon decomposition by microorganisms in a book, dealing specifically with microorganisms in relation to petroleum. The microorganisms involved, theories and biochemical relationships are discussed. Now to-day the microbial decomposition of hydrocarbons becomes more and more of interest in relation to the problem of the pollution of fresh and sea water. Davis (8) in 1956 already proposed the elimination of relatively small concentrations of oil from water by microbial decomposition. ZoBell (9) published in 1963 an extensive survey of the problems associated with oil pollution with 78 references. One of his conclusions was that various kinds of mineral oil might be oxidized by marine bacteria at rates as high as 36-350 g/m³/year. Research in this field is going on (10), but it is not in the scope of this review to go into the details of this work.

The application of bituminous materials is very old. In Gen. XI, 3., reference is made to the use of asphalt as mortar. The recovery of objects from Pre-Biblical times in good condition is one of the reasons why bitumen for many years has been thought to be immune to microbial attack. In 1935 however, Hundeshagen (11) published as one of the first his findings on the action of microorganisms on bitumen. He described how cement slabs covered with asphalt, when stored in moist places, were destroyed by fungus mycelium, which formed a slimy fibrous coating containing crystals of CaCO₃ (the Ca coming from the
surface of the slabs) on the surface. Since this time considerable work has been done by different research groups, resulting in a number of publications, which will be discussed in this review.
REVIEW

From a study of the available literature it became clear that the most studied bacteria in relation to the attack of bitumen are Pseudomonas species. In Table 1 bacteria attacking bitumen are associated. It must however be remarked that in all probability this table is far from complete, but it is only intended to give a general impression of the kind of bacteria.

The growth of these types of bacteria has been studied in soil and in laboratory cultures. Since growth only can occur when an organism is oxidizing the substrate, the growth of the bacterial culture is an index of the bitumen oxidation by microorganisms. When the microorganism grows, it increases in number, and the growth medium becomes more turbid; by measuring the increase of the turbidity, the growth can be followed.

At the University of Southwestern Louisiana, studies have been made on the growth of several genera. An interesting relationship in the degradative capacity of some organisms on asphalt 1-8 is given in Table 2 (22).

Organism a) demonstrated little degradation after one week of incubation but almost complete degradation after one month. Contrary here to, (d) showed a more rapid initial activity with only 49% degradation after one month. It is supposed that the difference in activity is due to differences in the mechanisms of oxidation. The rates of degradation are higher than observed during other investigations (Kulman (26), Martin (27)) operating under natural conditions, applying thick films which do not provide a great surface area for attack, like thin films used in laboratory experiments.
Table 1 - Bacteria attacking Bitumen

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas species</td>
<td>12, 13, 15, 16, 19, 21, 22, 24, 25</td>
</tr>
<tr>
<td>Mycobacterium species</td>
<td>14, 15, 23, 24, 25</td>
</tr>
<tr>
<td>Micrococcus species</td>
<td>14, 15, 16, 19</td>
</tr>
<tr>
<td>Bacillus species</td>
<td>19, 21, 22</td>
</tr>
<tr>
<td>Chromobacter species</td>
<td>21, 22</td>
</tr>
<tr>
<td>Nocardia species</td>
<td>23, 24</td>
</tr>
<tr>
<td>Achromobacter species</td>
<td>12</td>
</tr>
<tr>
<td>Sulfomonas Denitrificans</td>
<td>12</td>
</tr>
</tbody>
</table>

References: 15, 16, 19, 21, 22, 23, 24, 25

Table 2 - Asphalt 1-A degradation by pure culture at 30°C

<table>
<thead>
<tr>
<th>Organism</th>
<th>Degradation - % -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Week</td>
</tr>
<tr>
<td>a) Pseudomonas 1-5A-C</td>
<td>3</td>
</tr>
<tr>
<td>b) Pseudomonas 7-2A-B</td>
<td>25</td>
</tr>
<tr>
<td>c) Pseudomonas 5-3A-B</td>
<td>18</td>
</tr>
<tr>
<td>d) Pseudomonas aeruginosa</td>
<td>20</td>
</tr>
<tr>
<td>e) Chromobacterium 6-4A-B</td>
<td>20</td>
</tr>
<tr>
<td>f) Bacillus 11-2A-W</td>
<td>5</td>
</tr>
</tbody>
</table>
Harris described studies on bacterial population of the soil. Data published from soil samples taken along pipelines have dealt mainly with total populations. The pipeline ditch has been shown as favorable for bacterial development (17-18). Hydrocarbon utilizing bacteria found near asphalt coatings in 13 representative locations were compared with bacteria in the same soil, at comparable depth, outside the pipeline ditch in the right-of-way.

Less than 100 bacteria per gram of soil were found away from the line in the right-of-way, while each gram of soil in contact with the asphalt had from 1000 to more than 10000 bacteria capable of using this material as their sole food source. These data follow the expected ecological pattern, because availability of a specialized food causes expansion of populations able to make immediate use of this food. Fig. 1 shows bacterial numbers per gram of soil (dry basis), estimated by serial dilution methods (19). In a more recent publication (20) Harris compares the number of hydrocarbon bacteria per gram of soil present in undisturbed soil at pipeline depth with soil adjacent to asphalt coating. This comparison is given in Table 3, taken from this review.

Traxler (21) studied the effect of the temperature on the growth and asphalt degradation by Pseudomonas aeruginosa in a stationary culture. A turbidity reading was taken after 24 hours. After one week the percentage of degradation was determined from loss in weight of benzene-soluble material. The results of this study are presented in Fig. 2.

The comparison of the optimal temperature for growth on asphalt with the optimal temperature for degradation indicates that degradation is more complete with a temperature about 5°C below the optimal temperature for growth. The explanation of this phenomenon awaits more studies of a fundamental nature on the mode of action of microbial attack on bituminous materials (22).
Fig. 1 - BACTERIAL NUMBERS PER GRAM OF SOIL (DRY BASIS)

Fig. 2 - EFFECT OF TEMPERATURE ON GROWTH AND DEGRADATION
### Table 3 - Population Distributions

<table>
<thead>
<tr>
<th>Hydrocarbon Bacteria per Gram of Soil</th>
<th>Undisturbed Soil in Right-of-Way at Pipeline Depth</th>
<th>Soil Adjacent to Asphalt Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 100</td>
<td>65 %</td>
<td>10 %</td>
</tr>
<tr>
<td>100 to 1,000</td>
<td>21 %</td>
<td>21 %</td>
</tr>
<tr>
<td>1,000 to 10,000</td>
<td>12 %</td>
<td>25 %</td>
</tr>
<tr>
<td>10,000 to 100,000</td>
<td>2 %</td>
<td>34 %</td>
</tr>
<tr>
<td>More than 100,000</td>
<td>0 %</td>
<td>10 %</td>
</tr>
</tbody>
</table>

### Table 4 - Effect of pH on asphalt assimilation

<table>
<thead>
<tr>
<th>Organism</th>
<th>pH</th>
<th>Growth response (Klett units)</th>
<th>Asphalt degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
<td>0.190</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.10</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.42</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.72</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3.15</td>
<td>1.0</td>
</tr>
<tr>
<td>Pseudomonas 7-2A-B</td>
<td>5</td>
<td>1.25</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.92</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.45</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.43</td>
<td>0</td>
</tr>
</tbody>
</table>
Philips and Traxler (22) describe the influence of the pH on asphalt assimilation of two Pseudomonas species. The total growth and degradation were determined after 24 hours of incubation at 30°C. The growth response and degradative capacity of Pseudomonas aeruginosa increased as the pH value increased (see Table 4), whereas Pseudomonas 7-2A-3 showed the inverse effect. The obvious difference in pH optimum for the two organisms would indicate that different mechanisms are involved in the asphalt oxidation by the two organisms.

It would appear from studies of Traxier (21) that there is no single mechanism causing bacteria to attack asphalt. If this were the case there would be differences in the specificity of different asphalt degradating organisms for a given asphalt as well as differences in the specificity of a given microorganism for different types of asphalt. It was found that four different organisms which grew at about the same rate on glucose as on the carbon source, had a completely different growth on the same type of asphalt.

On the other hand not all bitumens are attacked equally by a given organism.

Fig. 3 shows that Bacillus will readily attack asphalt 6A and, to a lesser extent, bitumens A and E. Flavobacterium however, will attack all bitumen including type G, which is not attacked by the Bacillus.

Some of these bitumens, together with some other types were used in burial tests (24). Birch tongue blades coated with several types of bitumen were buried in a soil with different moisture conditions. The properties of these bitumens are given in Table 5. Action of the organisms was controlled by determining the weight losses of the bitumen at various intervals. The difference in susceptibility of bitumen to bacterial action can clearly be remarked in Fig. 4.
Fig. 3 - GROWTH: RESPONSE ON DIFFERENT BITUMENS

Table 5 - Properties of Bitumens used in growth studies and burial tests

<table>
<thead>
<tr>
<th>Bitumen</th>
<th>Softening point</th>
<th>Specific Gravity 25°C/77°F</th>
<th>Penetration 25°C/100g/5 sec. 0.1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A</td>
<td>44°C 111°F</td>
<td>0.985</td>
<td>133</td>
</tr>
<tr>
<td>A</td>
<td>195°C 90.5°F</td>
<td>1.015</td>
<td>48</td>
</tr>
<tr>
<td>E</td>
<td>167°C 75°F</td>
<td>1.175</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>161°C 72°F</td>
<td>1.269</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>214°C 101°F</td>
<td>1.060</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>170°C 77°F</td>
<td>1.195</td>
<td>0</td>
</tr>
<tr>
<td>FT</td>
<td>173°C 78.5°F</td>
<td>1.226</td>
<td>0</td>
</tr>
</tbody>
</table>
Traxler (21) describes the effect of aerobic and anaerobic conditions on growth and asphalt degradation by three Pseudomonas species. The results are presented in Fig. 5. It is seen that the efficiency of degradation under aerobic or anaerobic conditions is a matter of organism specificity. Pseudomonas aeruginosa and Pseudomonas 7-A2-3 are more active in growth and asphalt degradation under aerobic conditions. On the other hand Pseudomonas 1-5A-0 can assimilate and degrade asphalt more efficiently under anaerobic conditions.

Little is published on the effect of microorganisms on the physical properties of bitumen. Burgess (28) stated that five years of tests by Montana Highway Department showed attack by bacteria of asphalt surfacing of highways, thereby hardening the asphalt and making it brittle. Harris and his co-workers (29) have done research on this subject. They used a percolation technique with two different road asphalts and more than ten different types of hydrocarbon-utilizing bacteria. They observed changes in the softening point, ductility and penetration and concluded that either softening or hardening of the asphalts occurred depending on the bacteria present. Traxler et al. (23) have studied the influence of microorganisms on the asphalt viscosity. A bentonite-asphalt emulsion system for asphalt 1A, 3A, and 6A was used to subject these materials to the degradative activity of Mycobacterium ranae and Nocardia coeliaca for four months at 30°C.

Nocardia coeliaca caused 1.5, 3.8, and 6-8 fold increases in relative viscosity of 1A, 3A, and 6A, respectively. A similar susceptibility pattern for Mycobacterium ranae was obtained on the same asphalts, but apparently this organism exerted an even greater effect on asphalt 6A since the viscosity of this residue was too hard to be determined satisfactorily. A survey of the results is given in Table 6.
Fig. 4 - EFFECT OF SOIL BURIAL ON THE WEIGHT LOSS OF BITUMEN-COATED TONGUE BLADES

Fig. 5 - OXYGEN EFFECT ON GROWTH AND DEGRADATION BY PSEUDOMONAS SPECIES
Comparison of these data with analysis of the three asphalts indicates that the organisms probably attack the resin components of the asphalts.

Roediger (30) (Cited in ref. 13) attributes the hardening action of the microbial attack to one or more of at least three cases:

1. Loss of the more susceptible, lower molecular weight oils which are effective solvents and plasticizers.
2. Conversions of the lower molecular weight hydrocarbons to high molecular oxygenated compounds.
3. Polymerization of unsaturated compounds that result from microbial attack.

Very little is published about the use and effectiveness of products that inhibit microbial action. Burgess (28) proposed a treatment of highway asphalt with pentachlorophenol. Asphalt products have been treated with copper pentachlorophenate (27) and in more recent publication of Vaeser (31) microbiological attack is said to be inhibited by additives with phenolic characteristics or the "usual Cu or Hg salts". With present knowledge it is not yet possible to select an inhibitor that will be active against all types of microorganisms. Besides this, the question arises as to the extent an inhibitor will lose its effectiveness in course of time, due to chemical changes or to the fact that an inhibitor-sensitive microorganism can mutate to an inhibitor-resistant one.
Table 6 - Changes in viscosity\(^a\) of asphalts by microbial action\(^b\)

<table>
<thead>
<tr>
<th>Asphalt</th>
<th>Viscosity at 25 C (poises)(^c)</th>
<th>Relative viscosity(^d) of asphalt subjected to</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original asphalt</td>
<td>Asphalt subjected to</td>
<td>M. ranae</td>
</tr>
<tr>
<td></td>
<td>from control test</td>
<td>Mycobacter. ranae</td>
<td>Nocardia coeliaca</td>
</tr>
<tr>
<td>1A</td>
<td>0.520 x 10(^6)</td>
<td>1.80 x 10(^6)</td>
<td>1.30 x 10(^6)</td>
</tr>
<tr>
<td>3A</td>
<td>0.526 x 10(^6)</td>
<td>2.06 x 10(^6)</td>
<td>2.40 x 10(^6)</td>
</tr>
<tr>
<td>6A</td>
<td>0.360 x 10(^6)</td>
<td>0.38 x 10(^c)</td>
<td>2.60 x 10(^6)</td>
</tr>
</tbody>
</table>

\(\text{a - Viscosity approximately 500,000 poises at 25 C.}\)
\(\text{b - Bacterial action on a clay emulsion of these asphalts.}\)
\(\text{c - Viscosities were calculated at 5 x 10}^2\ \text{sec}^{-1} \text{rate of shear.}\)
\(\text{d - Viscosity at 25 C of asphalt subjected to bacterial action/viscosity at 25 C of asphalt from control.}\)
CONCLUSION

From the literature survey it can be concluded that microorganisms capable of attacking hydrocarbons, including bitumen, under aerobic and anaerobic conditions, are widely present in nature.

Conditions like pH, temperature, oxygen present, moisture content, influence the growth and degradation, in which connection it should be remarked that optimum conditions for these parameters can vary, depending on the type of organism and type of bitumen studied. In general, Martin (27) found microbiological attack to be fastest for steam-refined asphalts, followed by air-blown asphalts and finally coal tar pitches.

It is clear that laboratory tests, executed under optimum conditions, result in a faster growth and greater degradation than under realistic conditions. Thick coatings are, even under laboratory conditions, subject only to superficial attack.

Unfortunately no publications on the microbial attack could be found of more recent date than 1966. The very interesting work of Traxler and his group was not continued (32).

In relation to the use of bitumen for the incorporation of radioactive wastes, it should be remarked that the storage of these incorporated wastes will be for very long periods.

The studies done for the French Commissariat à l'Energie Atomique as described in a report of Rodier et al. (4), lasted only 6 to 7 months. Nevertheless an abnormally high presence of microorganisms of Pseudomonas species was reported in the water present in the container, in which an asphalt-CaCO₃ mixture was submerged in river water; soil tests resulted in a granular outside layer with a lower bitumen content. It was found that one kind of bitumen was clearly more resistant to microbial attack than another one.
In view of these facts, it seems that long-range and intensive studies on the problem of microbial attack of bitumen into which radioactive wastes are incorporated should be recommended, taking in consideration the intermediate and final storage conditions of these wastes. Together with this, the influence of radiation on microorganisms should be studied. The question how far microorganisms are radio-resistant or radiation-sensitive organisms mutate to radiation-resistant ones, and how far radiation influences the growth, and consequently degradation, has to be answered.
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