Microbial Degradation of Asphalt[1]

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ABSTRACT

PHILLIPS, U. A. (University of Southwestern Louisiana, Lafayette) AND R. W. TRAXLER. Microbial degradation of asphalt. Appl. Microbiol. **11**:235–238. 1963.—Organisms of the genera *Pseudomonas*, *Chromobacterium*, and *Bacillus* capable of degrading asphalt were isolated by enrichment cultures. The asphalt degradation by these organisms varied from 3 to 25% after incubation for 1 week. The effects of temperature, pH, and atmosphere of incubation on asphalt degradation were investigated and were shown to vary with different organisms on the same substrate.

There is a considerable volume of literature dealing with microbial growth on pure hydrocarbons, yet little information is available on the action of bacteria on the more complex materials of petroleum origin. Stone, White, and Fenske (1940) reported on microorganisms capable of attacking all fractions of petroleum, including the asphaltic fraction. Harris, Kline, and Crumpton (1956) described the action of microorganisms at the soil-asphalt interface of roadmats, and Harris (1959) described the growth of bacteria on pipeline coatings. Reports by Burgess (1956), Kulman (1958), and Martin (1961) confirmed the action of microorganisms on asphalt and asphaltic products under natural conditions and, to a limited extent, under laboratory conditions. Many of the observations on asphalt degradation by microorganisms have been by the soil burial technique or some modification thereof. This technique is of value but is difficult to assess and yields little information on the mechanisms involved in the microbial action. Most of the laboratory results have confirmed these observations.

This report deals with preliminary laboratory studies with several different bacteria acting on asphalts. It is not intended that this work be applied to the natural conditions under which asphalts and asphaltic products are used, but rather serve as the basis for a fundamental study of the mechanisms of action of microorganisms on bituminous material.

MATERIALS AND METHODS

Asphalt-utilizing organisms were isolated by enrichment culture from soil samples or soil debris from asphalt-shingled roofs. The enrichment medium was the inorganic salts medium described below, with 1% (w/v) asphalt 1-A added as the carbon source. Isolations from the enrichment flasks were made by streaking onto Trypticase Soy Agar (BBL). The organisms isolated were tentatively assigned to a genus by strain characteristics, growth characteristics, and biochemical tests. The strain of *Pseudomonas aeruginosa* used in this study was obtained from the stock culture collection of the Department of Bacteriology, University of Southwestern Louisiana.

Stock cultures of all isolates were carried on both Trypticase Soy Agar slants (BBL) and a sand-asphalt mixture moistened with 2 ml of a broth suspension of the organism. The synthetic salts medium employed contained (per liter of distilled water): NH_4Cl, 0.5 g; NaCl, 4.0 g; KH_2PO_4, 0.5 g; Na_2HPO_4, 1.0 g; $MgSO_4$, 0.5 g; pH 7.0. Asphalt or carbohydrate was added as the carbon source at 1.0 g per liter. This medium was free from precipitate and could be modified by the addition of other salts as needed for specific purposes.

Owing to the hydrophobic nature of asphalt, dispersal into growth medium is difficult. Colloidal suspensions of asphalt are probably the most efficient method for providing surface area for microbial attack but preclude the use of turbidimetric determination of growth. Thin films of asphalt were prepared by dissolving the asphalt in benzene and layering the asphalt-benzene solution onto the surface of the medium. The benzene was removed by evaporation at approximately 80 C, leaving a thin film of asphalt on the surface of the growth medium. In experiments where turbidity measurements were made, the asphalt-benzene solution was used to coat the flask before the addition of sterile basal medium, and the benzene was removed by evaporation.

The inocula were prepared from 24-hr cultures grown on Trypticase Soy Agar (glucose-grown cells) or in a chemically defined medium with 0.1% asphalt 1A (asphalt-grown cells). The cells were collected by centrifugation, washed twice in 0.2 M phosphate buffer (pH 7.0), and resuspended in phosphate buffer to a Klett reading of 100. The inoculum consisted of 1.0 ml of this suspension added to the liquid medium below the asphalt layer or directly to the medium in the coated flasks. The cultures were incubated at 30 C in a New Brunswick model G-26 incubator-shaker at a shaker speed of 180 rev/min or under stationary conditions.

The percentage of asphalt degradation was determined

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from the dry weight of benzene-soluble material recovered after incubation from flasks set up with a known weight of asphalt. Sterile medium treated in the same manner as the test series served as a control for the benzene-extraction procedure used in determinating the percentage of asphalt degradation.

The elemental and component analysis of the asphalt 1A used in this study is shown in Table 1. This asphalt is shown to be high in paraffin components.

RESULTS AND DISCUSSION

The organisms isolated by enrichment culture (Table 2) and shown to degrade asphalt 1-A belong to biologically diverse genera. These organisms were not assigned to species in this preliminary investigation, but study of their characteristics demonstrated that *Pseudomonas* 7-2A-B resembled *P. chlororaphis*, and *Pseudomonas* 1-5A-C closely resembled several hydrocarbon-utilizing species described in *Bergey's Manual of Determinative Bacteriology* (6th ed.). The organisms of the genus *Pseudomonas* were selected for this study because of their variations in activity on asphalt and the widely reported activity of pseudomonads on hydrocarbon substrates.

An interesting relationship in the degradative capacity of the different pseudomonads is noted in Table 2. *Pseudomonas* 1-5A-C demonstrated little degradation (3%) after 1 week of incubation but almost complete degradation (90%) after 1 month. *P. aeruginosa*, on the other hand, showed more rapid initial activity (20% in 1 week) with only 49% total degradation at 1 month. This difference in activity could be due to differences in the mechanisms of oxidation. The high rate of degradation observed in this series of experiments cannot easily be compared with those apparently lower rates, observed by other investigators (Kulman, 1958; Martin, 1961), operating under natural conditions. In these laboratory experiments, thin films were used to increase greatly the surface area available to the microorganisms, whereas under natural conditions asphalts are generally applied as thick films which do not provide a great surface area for attack.

Since the physical conditions under which a microorganism acts on a substrate will govern to a considerable extent the speed and efficiency of its action, the effect of temperature, pH, and atmosphere of incubation were investigated in relation to growth of the test organisms on an asphalt substrate and degradation of the substrate. Differences in the response of the various pseudomonads with different conditions of temperature, pH, and atmosphere would also indicate fundamental differences in the mechanisms of asphalt oxidation.

Effect of temperature on substrate utilization. Temperature of incubation will influence the speed of oxidation and also the nature of products from the same substrate. To determine the temperature optima for both growth and degradation, cells grown on asphalt were used for inoculation after washing twice with phosphate buffer (pH 7.0). A sample (1 ml) of the washed-cell suspension (100 Klett units) of each organism was inoculated into 50 ml of basal medium containing 1% asphalt 1-A as the sole carbon source. The flasks were incubated at 20, 25, 30, 37, and 45 C. A turbidity reading (in Klett units) was taken on all flasks after 24 hr; then the flasks were incubated again for 6 days. After 1 week, the percentage of degradation was determined from loss in weight of benzene-soluble material.

TABLE 1. *Analysis of asphalt 1A*

Component	Per cent dry weight
Elemental analysis	
Carbon	85.44
Hydrogen	10.35
Oxygen	1.06
Nitrogen	0.65
Sulfur	2.74
Component analysis	
Asphaltenes	11.0
Resins	21.0
Saturates	51.0
Cyclics	17.0

TABLE 2. *Asphalt 1-A degradation by pure culture at 30 C**

Organism	Degradation	
	1 Week	1 Month
	%	%
Pseudomonas 1-5A-C	3	90
Pseudomonas 7-2A-B	25	—
Pseudomonas 5-3A-B	18	—
Pseudomonas aeruginosa†	20	49
Chromobacterium 6-4A-B	20	—
Bacillus 11-2A-W	5	—

* All flasks were incubated under stationary conditions.

† From stock culture collection, University of Southwestern Louisiana.

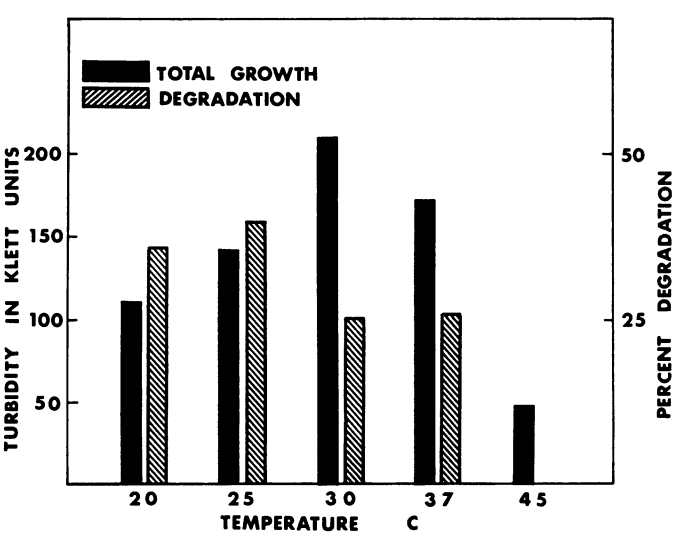

FIG. 1. *Effect of temperature on growth and degradation. Pseudomonas aeruginosa in stationary culture. Total growth after 24 hr, per cent degradation after 1 week.*

Optimal growth occurred at 30 C (Fig. 1) for *P. aeruginosa*, and optimal degradation at 25 C. The other two pseudomonads showed the same temperature optima.

Comparing the optimal temperature for growth on asphalt with the optimal temperature for degradation of asphalt indicates that degradation is more complete with a temperature about 5 C below the optimal temperature for growth. This phenomenon raises the question of whether the organisms actually assimilate the asphalt more rapidly at the lower temperature or the organisms are more efficient in solubilization of the asphalt at the lower temperature. The answer to this question awaits more studies of a fundamental nature on the mode of action of microbial attack on asphalt.

Effect of pH on substrate utilization. Asphalt-grown inocula cells of *P. aeruginosa* and *Pseudomonas* 7-2A-B were used to determine the effect of pH on growth of the organisms and degradation of asphalt. The organisms were inoculated into asphalt media buffered at pH 5, 6, 7, 8, and 9. Total growth and degradation was determined after 24 hr of incubation at 30 C. The growth response and degradative capacity of *P. aeruginosa* increased as the pH value increased (Table 3), whereas *Pseudomonas* 7-2A-B showed an inverse effect (that is, better growth and degradation at more acidic values). The obvious difference in pH optima for the two organisms would indicate that different mechanisms are involved in the asphalt oxidation by the two organisms.

Effect of oxygen on substrate utilization. Pseudomonads are facultative in their oxygen requirements and therefore could assimilate asphalt by either an aerobic or an anaerobic pathway. Glucose-grown suspensions of *P. aeruginosa*, *Pseudomonas* 7-2A-B, and *Pseudomonas* 1-5A-C were inoculated into duplicate flasks (50 ml) of basal medium with asphalt 1A as the sole carbon source. One series of flasks was incubated aerobically as stationary cultures while the second series was incubated in Brewer anaerobic jars. After 24 hr of incubation at 30 C, turbidity readings were taken on all flasks. The flasks were incubated again under the original conditions for an additional 6 days, at which time the percentage of asphalt degradation was determined.

Both *P. aeruginosa* and *Pseudomonas* 7-2A-B showed maximal growth and asphalt degradation under aerobic conditions (Fig. 2). *Pseudomonas* 1-5A-C demonstrated maximal activity under anaerobic conditions. The apparent anaerobic degradation by *Pseudomonas* 1-5A-C could account for the low initial degradation and high final degradation by this organism, as mentioned previously (Table 2). When grown in stationary culture, the medium would be aerobic but, after continued incubation without agitation, the depths of the culture flask should be under essentially anaerobic conditions. *P. aeruginosa* showed growth and degradative capacity under both aerobic and anaerobic conditions, the aerobic activity being the greater. The growth response of *Pseudomonas* 7-2A-B, however, was extremely sparse under anaerobic

TABLE 3. *Effect of pH on asphalt assimilation**

Organism	pH	Growth response (Klett units)	Asphalt degradation
			%
Pseudomonas aeruginosa	5	190	0
	6	210	0.5
	7	242	1.2
	8	272	2.0
	9	315	1.0
Pseudomonas 7-2A-B	5	125	2.7
	6	92	1.0
	7	50	0
	8	45	0
	9	43	0

* Determinations were made after 24 hr of incubation at 30 C.

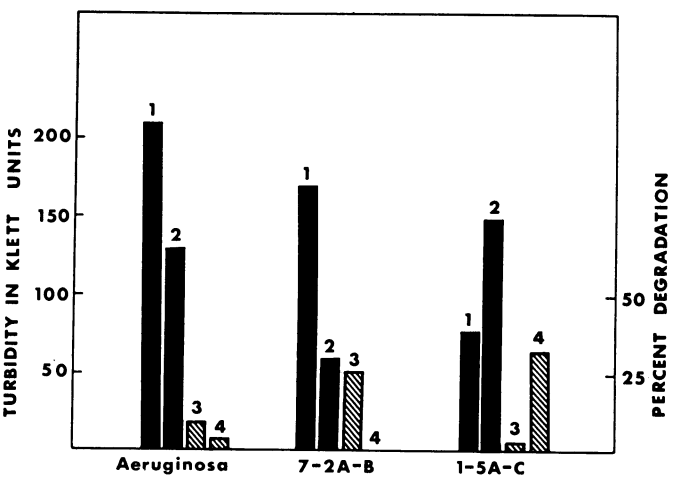

FIG. 2. *Oxygen effect on growth and degradation by Pseudomonas species. Symbols: 1, aerobic growth; 2, anaerobic growth; 3, aerobic degradation; 4, anaerobic degradation.*

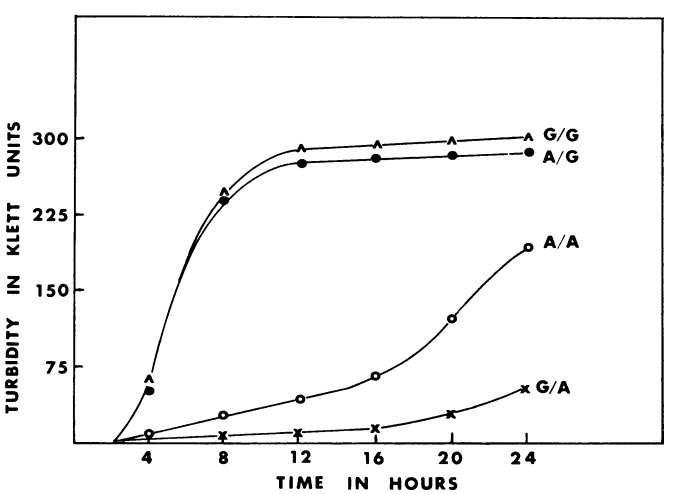

FIG. 3. *Growth response of Pseudomonas aeruginosa on glucose and asphalt. G/G, glucose-grown cells on glucose; A/G, asphalt-grown cells on glucose; A/A, asphalt-grown cells on asphalt; G/A, glucose-grown cells on asphalt.*

conditions, and no measureable degradation occurred in the absence of oxygen. *Pseudomonas* 1-5A-C apparently degraded asphalt under anaerobic conditions more efficiently than under aerobic conditions. Logically, one would expect anaerobic metabolism to convert more substrate for an equivalent amount of growth than aerobic metabolism.

Effect of inoculum origin on substrate utilization. There is an obvious difference in the growth response of *P. aeruginosa* on asphalt 1-A, dependent upon the substrate used to grow the inoculum cells. Cells grown for 24 hr at 30 C on basal medium with glucose as the carbon source were compared with cells grown on basal medium with asphalt as the carbon source. Both types of cells were inoculated into basal medium with glucose and basal medium with asphalt 1-A, and readings were made at 2-hr intervals for 24 hr. Figure 3 shows that either glucose- or asphalt-grown cells of *P. aeruginosa* give approximately the same growth response on glucose, even when conditioned by growth on the asphalt substrate. Asphalt-grown cells did give a better response on the asphalt substrate than glucose-grown cells. The cells grown on asphalt showed a longer lag phase, lower logarithmic growth rate, and less total growth than cells grown on glucose.

The data that have been presented indicate that organisms of the genus *Pseudomonas* vary to a greater or lesser extent in the mechanisms by which they oxidize an asphalt substrate. All these studies were made with a single asphalt cement (135 penetration; American Society for Testing Materials) designated asphalt 1-A. The use of a single asphalt rules out the possibility that differences observed were due to differences in the chemical nature of the substrate.

Chemically, asphalt is an aggregation of complex organic compounds. Varying amounts of paraffinic and aromatic hydrocarbons (Traxler, 1961) are present. The assumption that pure hydrocarbons predominate in asphalt has been proven erroneous. In addition to carbon and hydrogen, relatively small amounts of nitrogen, oxygen, and sulfur are present. These elements are present as one or two atoms in large molecules and, thus, the percentage of nonhydrocarbon molecules is considerable.

Temperature, pH, and oxygen tension optima are all basic characteristics of organisms and are directly concerned with the function of the enzyme systems of the organism. Since changing these variables in relation to the oxidative capacity of an organism for asphalt obviously affects organisms to differing degrees, it is safe to assume differences in the oxidative mechanisms of the various organisms studied in this investigation.

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