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Action of Microorganisms on Bituminous Materials

I. Effect of Bacteria on Asphalt Viscosity[1]

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ABSTRACT

TRAXLER, R. W. (University of Southwestern Louisiana, Lafayette), P. R. PROTEAU, AND R. N. TRAXLER. Action of microorganisms on bituminous materials. I. Effect of bacteria on asphalt viscosity. Appl. Microbiol. **13**:838-841. 1965.—Visual effects of *Mycobacterium ranae* on a 135-penetration asphalt (asphalt 1A) are described, which show the texture and rheological characteristics of the asphalt to be modified by microbial action. A bentonite-asphalt emulsion system for asphalts 1A, 3A, and 6A was used to subject these materials to the degradative activity of *M. ranae* and *Nocardia coeliaca* for 4 months at 30 C. *N. coeliaca* caused 1.5-, 3.9-, and 6.8-fold increases in relative viscosity of asphalts 1A, 3A, and 6A, respectively. A similar susceptibility pattern for *M. ranae* was obtained on the same asphalts, but apparently this organism exerted even a greater effect on asphalt 6A since the viscosity of this residue was too hard to be determined satisfactorily. Comparison of these data with analyses of the three asphalts indicates that the organisms probably attack the resin components of the asphalts.

For the past several years, the utilization of bituminous materials by microorganisms has been studied in our laboratory. It is easy to visualize numerous practical applications for these results, and, in addition, considerable information of a fundamental nature may also be obtained from such an investigation. Harris, Kline, and Crumpton (1956) isolated hydrocarbon-utilizing bacteria from the soil-asphalt interface of road mats and further demonstrated the ability of these organisms to degrade asphalts. This work was extended (Harris, 1959) to describe the growth of bacteria on various types of pipeline-coating materials. Harris et al. (1958), using a percolation technique with two different road asphalts and 13 pure cultures of hydrocarbon-utilizing bacteria, made the first attempt to assess microbial effects on asphalts. They scored the action of the bacteria on the basis of changes in the softening point, ductility, and penetration of the asphalts, and they concluded that either a softening or a hardening of the asphalts occurred, depending on the nature of the bacteria present.

Kulman (1958) and Martin (1961) confirmed an effect of microorganisms on various bituminous materials. Their materials were mixtures of bitumen with other ingredients, and it is therefore impossible to truly evaluate the effect of the microorganisms on the bitumen.

This report describes the microbial effect on 135-penetration road-building asphalts as determined by visual observation and viscosity measurements. Viscosity is the physical test with most significance for our purpose. Increase in viscosity can be used as a measure of asphalt hardening, and temperature susceptibility can be evaluated by determining viscosity at different temperatures. Finally, viscosity is related to the chemical makeup and rheological characteristics of the asphalts.

MATERIALS AND METHODS

Organisms. The two organisms used in most of this investigation were *Mycobacterium ranae* and *Norcardia coeliaca.* All organisms were carried as stock cultures on Trypticase Soy Agar (TSA). Inocula were prepared from TSA slants grown at 30 C, washed in saline three times, and suspended to a turbidity of 300 Klett units.

Thin-layer and asphalt-emulsion techniques. The early experiments used a thin layer of asphalt suspended on the mineral salts medium, as described previously (Phillips and Traxler, 1963). This method provided a considerable surface area for microbial action but did not subject much of the asphalt to microbial action unless an extremely thin (100 to 200 μ) film was used. With such thin films, the quantity of asphalt which can be ex-

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posed to microbial action is so low as to limit physical testing.

To avoid this difficulty, an emulsion of asphalt in an aqueous dispersion of bentonite (clay) particles was used to provide the greatest possible surface-to-volume ratio of asphalt for microbial action. A 5% (w/v) suspension of bentonite in warm (80 C) water was prepared and placed in a Waring Blendor. The asphalt to be tested was dissolved in the minimal amount of benzene which allowed the asphalt to be poured, and this asphalt solution was added slowly to the warm bentonite suspension with slow agitation. After the total volume of asphalt had been added, the mixture was agitated at high speed for approximately 30 min, or until a smooth, homogenous emulsion was obtained. The resulting bentonite-asphalt emulsion was autoclaved at 121 C for 30 min, which served to remove all traces of benzene. The product was stable and did not separate upon standing or autoclaving.

For each asphalt studied, three 1,500-ml flasks, each containing 100 ml of bentonite-asphalt emulsion, were sterilized, and to each flask were added 300 ml of sterile mineral salts medium. One flask of each series was inoculated with 3.0 ml of the washed suspension of *M. ranae*, a second with *N. coeliaca*, and the third flask served as a sterile control. All flasks were then incubated under stationary conditions at 30 C for 4 months.

Asphalt recovery from the emulsion. After incubation, any clear liquid was carefully decanted from the culture. The bentonite-asphalt slurry was transferred to a Waring Blendor and extracted with three to five 150- to 200-ml portions of a 3:1 mixture of benzene and ethyl alcohol. Slow agitation was used to obtain the extraction, since high-speed agitation would cause further emulsification. The benzene-alcohol extractions from each flask were continued until all traces of asphalt had been recovered. With some samples, the bentonite was carried over in the extracts and had to be removed by centrifugation at 2,000 × *g* for 30 min.

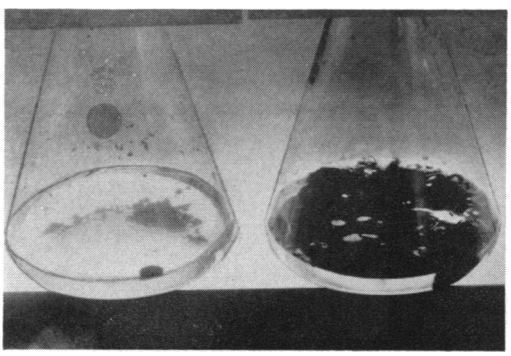

FIG. 1. *Visual effect of Mycobacterium ranae on asphalt 1A film after stationary culture at 30 C for 1 month. Test flask on left, sterile control flask on right.*

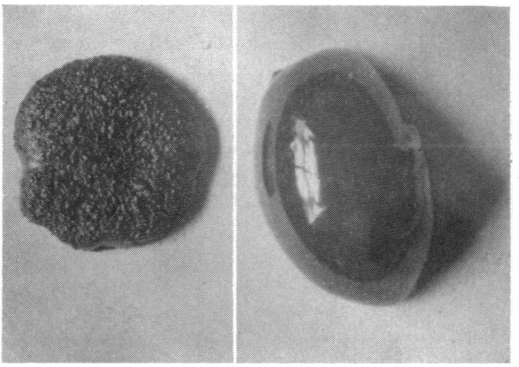

FIG. 2. *Samples of asphalt 1A from test flask (left) and control flask (right).*

The benzene-alcohol was stripped from the extract by vacuum distillation to a volume of 200 to 300 ml. The final solution was then cleared of solvent in a flash evaporator. All samples were then checked for occluded water and dried if necessary.

Testing the asphalts. Viscosity measurements were made at 25 C with a Hallikainen microfilm viscometer (Labout and van Oort, 1956). One advantage of this method is that a small sample (0.2 to 0.5 g) of asphalt is required for the determination. A limitation to our study is the quantity of asphalt that can be exposed to microbial action; therefore, physical tests which require a small quantity of asphalt are necessary.

RESULTS

The early experiments on asphalt degradation (Phillips and Traxler, 1963) used thin films of asphalt on mineral salts medium to obtain degradation values. With the thin-film method, it was possible to determine that bacteria caused changes in the asphalts. Figure 1 shows that the film of asphalt 1A in the flask test (left) was broken and settled to the bottom of the flask, whereas the film on the uninoculated control flask remained intact. It cannot be seen in this photograph, but the medium in the test flask was turbid, indicating growth of *M. ranae*, whereas the medium in the control flask was still clear. Samples from both flasks are shown in Fig. 2. The control asphalt had the typical shiny, homogenous appearance of asphalt 1A and the general physical characteristics of the original material. The sample taken from the test flask (left) was considerably changed in texture and appearance; it was rough, dull, blistered, dry, and crumbly in appearance, and had lost its original adhesiveness and rheological characteristics.

In the initial experiments a 90-penetration asphalt (no. 11) was tested against a number of

enrichment-culture isolates by the thin-film method to determine whether there was a significant change in viscosity (Table 1). These data have little significance as concerns differences in relative viscosity, since the sterile controls showed as much viscosity change as the samples subjected to bacterial action. It was thought that insufficient surface was available to the bacteria to exert a measurable effect on the overall volume of asphalt tested.

To confirm this explanation, an asphalt-emulsion system was used which would provide considerably more surface area available for attack with an equivalent volume of asphalt. The results of this system with the use of two test organisms known to be good asphalt degraders and three different 135-penetration asphalts are shown in Table 2. All flasks were incubated at 30 C for 4 months under stationary conditions. The effect of the microorganisms on the asphalts was to cause a significant increase in the viscosity of the asphalts. Further, the three asphalts varied in their susceptibility to microbial action as evidenced by the viscosity data.

We were unable to determine the viscosity at 25 C of asphalt 6A which had been acted upon by *M. ranae*. The residue from this test was very sensitive to temperature change. It was extremely fluid at temperatures used to prepare films for the viscosity test, preventing the preparation of satisfactory films. At 25 C (standard temperature of measurement), the viscosity was too high to determine by the microfilm technique.

TABLE 1. *Effect of bacteria on viscosity of 90-penetration asphalt no. 11*

Organism	Incubation time	Viscosity (poises at 25 C)	Relative viscosity*
	weeks		
Original sample	0	2.0×10^6	—
Sterile control	6	3.3×10^6	1.00
B-1-1	2	3.5×10^6	1.06
1-B-D	6	3.0×10^6	0.91
4A-L-1	6	2.8×10^6	0.85
4-B-RTL-9	6	2.7×10^6	0.82

* Relative viscosity = viscosity test asphalt at 25 C/viscosity control asphalt at 25 C.

The viscosity results obtained with *M. ranae* and *N. coeliaca* on the three asphalts are similar in that both organisms caused a hardening of the asphalts tested. The order of susceptibility to microbial action of asphalts 1A and 3A was about the same for both organisms. The greatest action by *N. coeliaca* was on asphalt 6A, the same material which was so drastically modified by *M. ranae* that we were unable to measure its viscosity.

DISCUSSION

It is logical to assume that the increased viscosity of the degraded asphalts is due to the action of the organisms on some asphalt component susceptible to microbial action and related to the rheological characteristics of the asphalts. An examination of the analyses of the three asphalts may give some insight as to the component(s) likely to be responsible for the observed effect on viscosity (Traxler, 1963).

The low asphaltene content (pentane-insoluble material) of asphalt 6A, which was most readily attacked by the test organism, would indicate that the organisms derive their carbon requirement from some portion of the pentane-soluble components of low molecular weight in these asphalts. It is possible that asphaltenes in higher concentration are inhibitory. However, unpublished data do not support this hypothesis. The resin content of the asphalts increases as the susceptibility to viscosity change by microbial action increases, whereas the amount of oils (saturate and cyclic components) decreases in the asphalts most susceptible to microbial action. On this basis, it appears that the resin com-

TABLE 2. *Changes in viscosity[a] of asphalts by microbial action[b]*

Asphalt	Viscosity at 25 C (poises)[c]				Relative viscosity[d] of asphalt subjected to	
	Original asphalt	Asphalt from control test	Asphalt subjected to			
			Mycobacterium ranae	*Nocardia coeliaca*	*M. ranae*	*N. coeliaca*
1A	0.520×10^6	0.90×10^6	1.80×10^6	1.30×10^6	2.0	1.45
3A	0.526×10^6	0.62×10^6	2.06×10^6	2.40×10^6	3.3	3.80
6A	0.360×10^6	0.38×10^6	—	2.60×10^6	—	6.80

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

ponents of the three asphalts are most susceptible to degradation by *M. ranae* and *N. coeliaca*.

As can be seen from the data in Table 2, there was an increase in viscosity of the control asphalts (uninoculated) over the untreated original samples. This viscosity increase was probably caused by traces of bentonite not removed by the extraction procedure, loss of oils from the asphalts due to their adsorption by bentonite, or the lengthy air exposure and mild heating used in the procedure. There is no reason to expect that the magnitude of these effects in the control flasks would be significantly different from the magnitude of the same effects in the test flasks. To cancel the observed increased viscosity effect, we used the control asphalt as our basis for determining relative viscosity, rather than the viscosity of the original material.

Considerable work will be required to determine the exact chemical identity of the asphaltic components subject to microbial degradation. Aside from the obvious applied aspects of this problem, there exists the wealth of fundamental data to be obtained which will be of value in determining at least partially the chemical nature of these bituminous materials.

ACKNOWLEDGMENTS

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