Action of Microorganisms on Petroleum-Asphalt Fractions

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Sometimes while doing the commonplace, one of our senses is momentarily conscious or aware of an intangible thought or reflex. If vivid enough, it might dimly remind us or have a slight connection with something in the past. Failing to recognize or grasp its importance is usually of no great significance. But on rare occasions we stumble upon something of merit. The inception of this study originated in such a manner, and has brought together two sciences which heretofore had been comparative strangers in highway laboratories.

Associating the characteristic odor of butyric acid with the rancid odor of old oil mats aroused the first suspicions of a bacterial action. Previous research into the causes of butterfat rancidity furnished sufficient background of bacteriology to recognize this fermentation in old asphalt mats. In searching for corroboration of this theory in available asphalt literature, none could be found. However, a search in other fields proved very fruitful.

The first attempts to invite bacteria to live on asphalt were crude and, in the light of later developments, were somewhat humorous. At the outset, the idea was contradictory, because it is a generally accepted opinion that bacteria were instrumental in the formation of our underground petroleum pools. It was also difficult to concieve how bacteria could help make it if they destroyed it and how anything would want to eat a substance that is generally accepted as being unpalatable. However, investigation points out few organic substances which are not acceptable sources of food for bacteria or their enzymes.

The first important thing this study brings out is a new conception of asphalt oxidation that has taken place as the result of what now might be called improper design. Secondly, to emphasize asphalt as one of the most durable and versatile materials available, when properly used.

There are sufficient tables and supporting data included for students of bacteriology who might be interested in serious study of the subject. A comprehensive set of tables is included for petroleum engineers interested in the analysis of the asphalts.

The rate of breakdown in the various categories of oils ranging from kerosene to heavy residues is interesting. The prerequisities of microbial action on the asphalt fraction attacked is the major topic.

However, no estimation of how much damage is attributed to microbial action is attempted in the paper. Various types of procedure for laboratory tests are of necessity given in detail to enable those interested to do their own testing. Contrary to the usual rule, there is little value in pictures in this particular instance, but a few were thought necessary to show some of the peculiar results.

Surprising results are found in the extracted asphalt of old oil samples, leading one to believe that there is a great deal that could be done to improve petroleum products by direct treatment with specific types of bacteria.

The paper also points out the reworking of old mats that have had bacterial action in progress on the shoulders in poor practice, because the entire mat becomes contaminated. Chemical inhibitors are also discussed. The paper has little to offer in support of the "syneresis theory" as being a contributing factor in the hardening of asphalt.

● THIS investigation of the action of soil bacteria on asphalt was undertaken for the purpose of proving whether bacteria oxidized asphalt to the extent that bacterial action

TABLE 1

PHYSICAL PROPERTIES OF OIL FRACTIONS STUDIED

Oil No.	Description	Centistokes Viscosity		Estimated Composition[a]			Calc. Mol. Wt.[b]	Est. Ave. No. Rings Per Molecule
		At 210°F.	At 100°F.	%A	%N	%P		
1	Filtered 185 Pa. Neutral	5.93	39.86	9	15	76	407	1.7
2	Portion of (1) Distilled	3.56	17.76	9	16	75	328	1.4
3	Portion of (1) Distilled	5.91	40.37	8	16	76	402	1.7
4	Portion of (1) Distilled	9.66	86.03	9	14	77	504	2.0
5	Portion of (1) Extracted	8.39	120.9	29	13	58	347	2.4
6	Portion of (1) Extracted	4.86	28.19	0	24	76	377	1.5
7	Portion of (1) Extracted	7.00	44.69	0	17	83	459	1.3
8	Penna. Bright Stock	32.91	540	9	13	78	740	2.9
9	Final Raffinate from (8) Extracted	103.8	2321	0	(14)	(86)	1194	(3.1)
10	Portion of Bosco Neutral Extracted	11.1	350.8	54	19	27	270	3.2

[a] Values obtained using method of Vlugter, et al. (1935). % A = % aromatics; % N = % naphthenes; % P = % paraffins.
[b] Obtained using a modified Keith and Roess method (1937).

could be considered one of the main factors of asphalt oxidation. This supposition was against traditional thinking, because it did not conform to the accepted views of petroleum-research chemists on asphalt oxidation.

In less than 2 years, the partial oxidation of asphalt and the oxidation of the oily fraction of asphalt has been definitely accomplished in the laboratory by exposure to soil microorganisms. However, on account of this being a comparatively new field of investigation, much more time and work is needed before this type of oxidation can be properly evaluated. Therefore, this paper is naturally limited and should be considered as a preliminary report.

The asphalt industry has devoted a great deal of work and much thought to the cause of oxidation of certain types of asphalt in our highways, but the question is debatable as to whether or not all the causes have been identified, insofar as being able to duplicate in laboratories the same oxidation which takes the life out of the asphaltic binder on the highway.

The very fact that asphalt mats, in most cases, must be placed on the ground in the enviroment of soil bacteria should have been of more concern in the past, especially where moisture conditions in the interface of the mat and base is ideal for the activity of bacteria.

Less than 100 years ago, chemists, biologists, and plant physiologists were not agreed on the role of soil bacteria, especially the chemists who held experiments by biologists in contempt, since chemists regarded their science as capable of covering all phases of chemical change. Until very recently, the science of bacteriology has been a stranger in a chemistry laboratory. However, it has come to the attention of some petroleum geologists as a possible means of discerning gaseous hydrocarbons in deep wells. Recognition of petroleum microbiology is now becoming highly important to petroleum refining in light of recent investigations in the use of bacterial enzymes as catalysts.

A search of literature has revealed only a fragmentary study of the action of micro-

organisms on petroleum asphalt. Microbiologists evidently shunned these heavier hydrocarbons, because they could not be satisfactorily dispersed in an aqueous solution.

One explanation of why we have not recognized this new concept of oxidation before or why it has not been of general exchange of knowledge is because there has been little or no fratenizing between microbiologists and petroleum engineers. Otherwise, it is difficult to account for the laco of such pertinent information. Bacteriologists were not too concerned with the meaning of damage done to petroleum by bacterial action but, rather, with the questionable effect of the several petroleum products on the growth of bacteria. Had microbiologists realized the important significance of this, they no doubt would have conveyed it to petroleum engineers. It has been the general thought among some petroleum engineers that most any petroleum product would kill bacterial growth, and it would be more than likely that others have not given bacteria a thought.

Excavations of ancient cities of the world have revealed the use of asphalt from natural deposits as bonding mortar for stone and brick work, a water-proofing for stone cisterns, joint filler in stone pavement, etc. The remarkable part that asphalt played in the ecomony of that era is the more remarkable, because it has been found in such a perfect stage of preservation. California has asphalt deposits that yielded the remains of prehistoric animals that existed 200,000 years ago. This should be proof enough of asphalts durability. So why should we question it now? Perhaps we have not always used asphalt to the best advantage, nor have we understood certain limitations of its use, because the asphalt of the Romans that is found in a good state of preservation was used in heavy films of $\frac{1}{4}$ inch or more. It is hoped this study will help to answer some questions on asphalt oxidation.

OCCURRENCE

Fortunately not all oil mats are subject to attack by soil bacteria, mostly because conditions are unfavorable to bacterial activity, such as a lack of moisture at the interface of mat and base, or because of man-made barriers, such as curbing used in street paving preventing the bacterial migration at the edge of the mat. Better design of base course has eliminated moisture entrapment under that mat to a large extent.

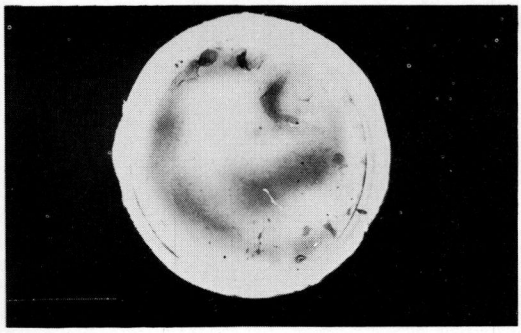
There has been no precedent to follow in this investigation, and some manner of laboratory testing had to be devised to determine whether bacteria or its enzymes con-

TABLE 2

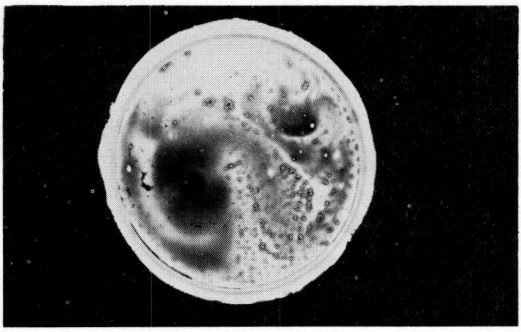
FERMENTATION OF OILS AT ROOM TEMPERATURE

Bacterial plate counts in millions per ml. of one percent oil dispersed in a mineral salt medium

Type of Oil	Time of Permentation in Days							
	0	1	2	4	6	8	10	14
Penna. Crude	13	65	300	380	220	121		
Mich. Crude	8.3	44	420	680	780	290		
Oil No. 1	0.6	35	640	510	380	390		25
Oil No. 2	1.3	65	620	970	720	740		90
Oil No. 3	0.6	88	350	400	540	670		104
Oil No. 4	1.0	47	180	165	210	370		99
Oil No. 5	0.6	121	440	310	300	280		71
Oil No. 6	0.1	270	390	690	140	610		270
Oil No. 7	0.3	14	510	1470	1150	880		39
Oil No. 8	1.2		90	240	91		240	180
Oil No. 9	0.4		6	12	21		51	36
Oil No. 10	1.1		55	87	250		108	157

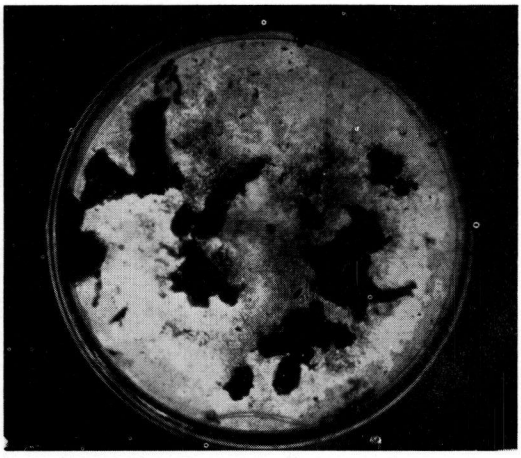


Control No. 1 Extracted Oil From Mexican Straight Run on Sterile Salt Media. Color is greenish orange.



Control No. 2 Extracted Oil From Cracked Asphalt on Sterile Salt Media. Color is greenish orange.

Dark Portion - Remains of Oil Film. The Results of Oil No. 1 on Inoculated Salt Media —Color is gray to Biege Light gray is fatty acid residue.



Dark ragged remains of Oil Film. The Results of Oil No. 2 on Inoculated Salt Media—Color is brown to black. Light gray residue is fatty acid.

Figure 1

tributed either directly or indirectly in the oxidation of petroleum asphalt used for binder in asphalt-aggregate mixes for highway surfacing.

A noticeable odor or rancidity aroused the first suspicions of a bacterial action in oil mat specimens. To date, this has not been duplicated in experiment with oil mat out in the field, but the same rancid odor has been obtained in laboratory tests. Just what particular fatty acid gives off this rancid odor has not been determined and will have to await further investigation.

No tests to date have proven bacterial action on oil mats laid primarily for the specific purpose of proving bacterial attack, because there has not been time to set up experimental work. Nevertheless, laboratory tests have shown a definite bacterial action on asphalt, so it is only a logical deduction that comparative results can be duplicated in the field under favorable conditions. In the following study an attempt will be made to show that laboratory results could have a relationship with field work and are, therefore, an indication of the same oxidation of asphalt that will be found in field tests.

TEST PROCEDURE

It is relatively simple to set up tests for experimenting with bacteria and asphalt to prove the oxidation or decomposition of the oils by bacteria. A source of suitable bacteria can be found in most any garden soil. Ordinary garden soil contains from 50,000

to 200,000 bacteria per gram. Old oil-soaked soil will materially hasten the whole process, because of the relatively large amount of hydrocarbon oxidizers present to clean up the oil.

Dispersion of the asphalt in the media is the toughest problem. Glass cloth is a good vehicle, because it can be weighed before and afterward. Thin films can be made by diluting the asphalt with benzene and then allowing evaporation before covering.

Ottowa sand could be a feasible means of getting a large surface area for oxidation, the whole idea being to expose enough asphalt in order to have a sufficient quantity left for analysis. Using 4 g. of SC-3 on 100 g. was not a sufficient quantity for later analysis. The briquettes lost some stability and appeared dry.

Considerable work has been done on the direct oxidation of asphalt by soil bacteria in this laboratory, but it is little compared to the amount needed before any definite conclusions can be drawn. With all the irrefutable evidence available on the subject, using light hydrocarbons, there is still a lack of substantitating data on the utilization of the very-heavy hydrocarbons by microorganisms.

CYCLE OF PENETRATION

NEW CONSTRUCTION

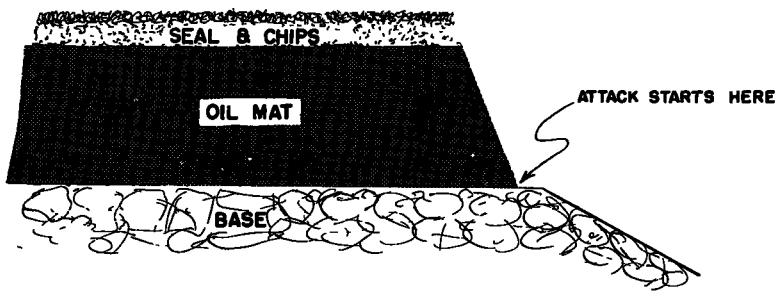


Figure 2

Up to date, the several tests with SC-3 and the extracted oils of asphalt have been conducted, but there has not been time to duplicate these tests, which is essential for verification of data.

Likewise, the field samples which have been studied on the work on these field samples is in the same category. Because field conditions are difficult to control, under the best of circumstances, data from this source are unreliable.

The ordinary oiled road with sweet clover or grasses growing on the shoulder next to the oil mat has all the requisites for microbes to attack its edges. Plant growth indicates cellulose of dead grass roots as nitrogen to furnish the initial energy. The bacteria live on this while adapting the oil fraction of the asphalt as food. The utilization of this oil is slow to begin with, but with favorable temperature and oxygen at the interface of the mat with the base, it flourishes until made dormant by the lack of proper temperature or other unfavorable conditions.

Everything else being equal, the rate of asphalt oxidation by microorganisms appears to be in direct proportion to its viscosity or penetration and is evidently showed as the concentration of the oily medium is reduced, either by refinery distillation or the process of oxidation by microorganisms, to a point where insufficient oil is present for food and energy.

The most-simple and readily understood explanation of the action of microorganisms on the oil components of asphalt is the ability of microbes to utilize the oil as a source of energy and food, their most-outstanding characteristic being their ability to create a favorable enviroment out of one that is hostile to their growth. For instance, a fairly heavy concentration of phenol, which is considered a germicide, can be utilized as a food after alterations.

TABLE 3

OXYGEN UPTAKE AND CARBON DIOXIDE EVOLVED IN OIL DISSIMILATION

Oil	Trial	Time of Incubation Days	Oxygen Uptake	CO_2 Evolved	CO_2 (Corrected)	Ratio CO_2/O_2
			ml.	ml.	ml.	
1	1	6	11.80	7.81	7.42	0.63
	2	5	11.06	7.70	7.31	0.66
	3	5	9.77	6.62	6.29	0.64
5	1	5	6.82	4.52	4.29	0.63
	2	5	7.32	5.14	4.88	0.67
	3	5	7.21	4.77	4.53	0.63
7	1	5	10.89	7.81	7.42	0.68
	2	5	11.23	8.14	7.74	0.69
	3	5	9.77	6.94	6.59	0.67
8	1	8	6.17	2.92	2.77	0.45
	2	8	7.50	3.63	3.45	0.46
	3	8	4.23	2.09	1.99	0.47
9	1	13	2.17	0.42	0.40	0.18
	2	13	3.08	0.24	0.23	0.07
Control (1)	1	5	0.24	0.16	0.15	
	2	5	0.17	0.11	0.11	

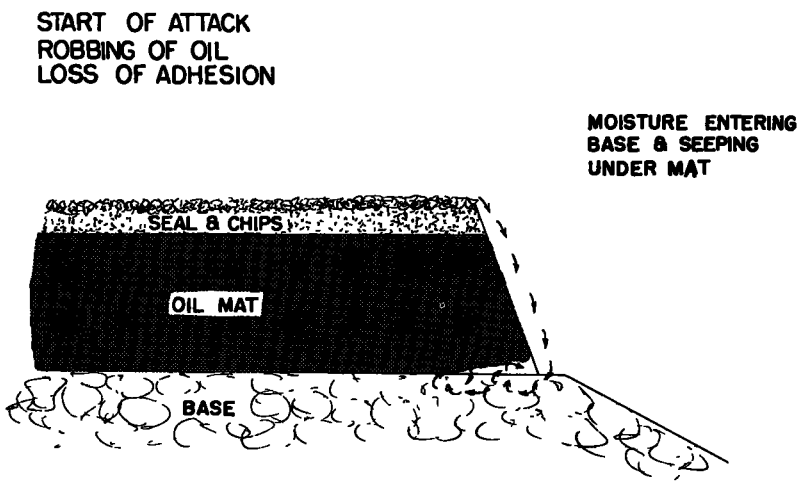


Figure 3.

So far, asphaltenes have not been found to be an acceptable source of hydrocarbon for microbial utilization, and it is doubtful that resins, which are also a polycyclic aromatic compound, can be utilized. Therefore, the oil seems to be the most-attractive portion of asphalt. Once this is consumed to the point where the asphaltenes and resins shield the remaining oil, bacterial growth is halted for lack of a suitable source of hydrocarbon.

The procedure of verifying the action of microorganisms on hydrocarbons is relatively simple and requires a minimum of equipment, care, and time.

The first step is the procurement of a well-inoculated aggregate from under an oil mat or some of the oil mat itself. To the inexperienced operator, this might sound slightly difficult, since the presence of hydrocarbon oxidizers is not visible. So a good rule to follow to find such an aggregate would be the selection of an oil mat, preferably

an SC-3 or MC-3 several years old, which has broken edges. Since moisture is a prerequisite for the growth of microorganisms, it would follow that an oil mat through farm or meadow land would have more than an average bacteria population in the underlying base.

For ease of sampling, pieces of oil mat already cracked or broken off of the shoulder are ideal. It will also be noted that there is a particularly rancid odor to these pieces of broken oil mat when slightly heated.

The next step is to mix up a nutrient balanced salt solution. Put in 200 g. of the above aggregate and allow to stand 48 hours at room temperature or better still at 103 deg.

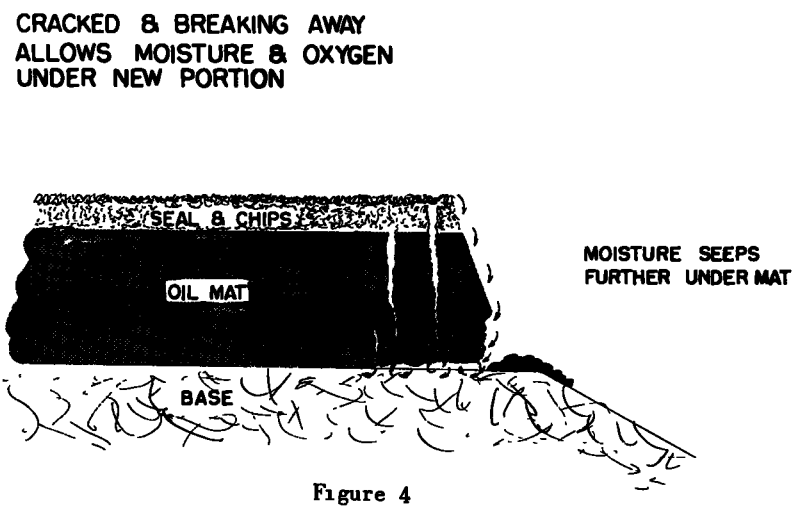


Figure 4

After standing, agitate slightly to get the bacterial slime in suspension; then pour this solution into a large flat container. The container or dish should be covered to prevent evaporation after a thin (this is important) film of asphalt has been poured on the surface of the salt solution. Care must be taken in the selection of the asphalt. A straight-run asphalt has a lighter gravity than the cracked products and will float, being

TABLE 4

ORGANISMS ISOLATED FROM OIL FERMENTATION

Group	Morphology on Agar	No. Isolated	Glucose	Litmus Milk	Nitrate Red'n	Gelatin Liquefaction	Oil Media	Probable Genus
I	Green, flat, spreading	63	A	RP	+	Stratiform	F	Pseudomonas (aeruginosa)
IIa	White, convex, mucoid	46	A	A or —	±	—	A	Achromobacter
IIb	White, convex, mucoid	26	A	R	±	Slow or —	F	Achromobacter
IIIa	White, mucoid, sometimes brownish	28	—	RP	±	—	A	Alcaligenes (radiobacter)
IIIb	White, mucoid, sometimes brownish	52	—	Al	—	—	F	Alcaligenes
Misc.	Reds, yellows, whites, etc.	35						Mixed

TABLE 5
EFFECT OF COMBINED OXYGEN ON BACTERIAL GROWTH IN THE PRESENCE OF AIR

Hydrocarbon	Medium	Corynebacterium Simplex	Proactinomyces Species (17A)	Pseudomonas Strain No. 6	Pseudomonas Strain No. 8	Pseudomonas Pyocyaneus No. 58	"Culture X"
Light oil	Complete	+++	+++	+++	+++	+++	0
Kerosene	Complete	0	0	+++	++++	++++	+++
Light oil	Minus NO_3	+++	+++	+++	+++	+++	0
Kerosene	Minus NO_3	0	0	+++	++++	++++	+++
Light oil	Low PO_4 [a]	+++	+++	++	++	++	0
Kerosene	Low PO_4 [a]	0	0	+	+	++	+
Light oil	Minus SO_4	++++	+++	+++	+++	+++	0
Kerosene	Minus SO_4	0	0	+++	+++	++++	+++
Light oil	Minus all	+++	+++	0	+++	++	0
Kerosene	Minus all	0	0	+++	++++	+++	+++

++++, excellent growth; +++, good growth; ++, moderate growth; +, slight growth.

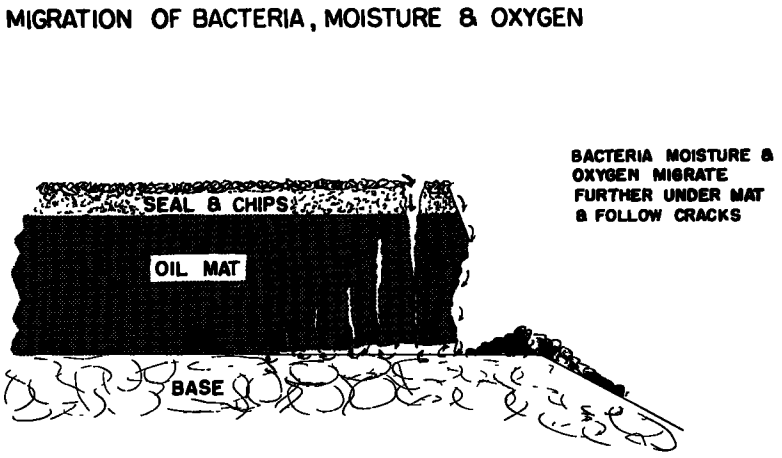


Figure 5.

lighter than water. After a few weeks, the film of asphalt will submerge. If it is desirable to prevent complete submergence of the asphalt film, a piece of plastic screen supported by pieces of glass will retain the film and will aid in removing the asphalt for analysis at the conclusion of the test. The control does not need this precaution.

Several other methods can be suggested, such as impregnating glass cloth with asphalt and burying the cloth in an inoculated soil. The main objection to this is the small amount of asphalt available for final analysis. At room temperature or slightly higher, 50 percent of the asphalt will have disappeared in about 8 weeks. As the asphalt hardens, the action becomes slower until it finally levels off at its equilibrium, providing the same temperature is maintained.

Another visible test is that of identifying the gas given off from a closed container of inoculated oil mix. The gas production of this is measured by the displacement of water in a condenser jacket by the gas given off by the decomposition of the hydrocarbons by the microorganisms. Analysis by an orsat gas analyzer will identify the product as CO_2, CO, etc.

These accelerated tests are suggested as procedures to shorten the time element involved; because if field conditions were simulated in the laboratory, it would take months to obtain the same results which are achieved by acceleration in weeks, as mat temperatures are comparatively low.

TABLE 6

CHANGE IN pH PRODUCED BY CULTURES ON VARIOUS HYDROCARBONS

	In Respirometers				In Flasks	
	Ps. pyocyaneus No. 58	"Culture X"	Pseudomonas strain No. 8	Cory. simplex	Pseudomonas strain No. 8	Cory. simplex
Culture medium alone	6.98	7.10	6.90	6.89		
Control—no hydrocarbon	6.60	N[a]	6.87	6.85	6.87	6.90
Gasoline	6.66	6.82	N	N	N	N
"Skelly-solve"	N[a]	6.85	N	N	N	N
Raw Kerosene	5.65	6.61	6.57	6.81	5.45	6.19
Treated Kerosene	5.81	6.78	6.57	6.65	5.45	5.79
Light oil	5.50	6.88	6.53	6.59	5.92	5.85
Heavy oil	6.52	N	6.70	6.59	6.53	6.26
Paraffin wax	6.15	N	6.69	6.71	6.63	6.33

[a]N = no growth.

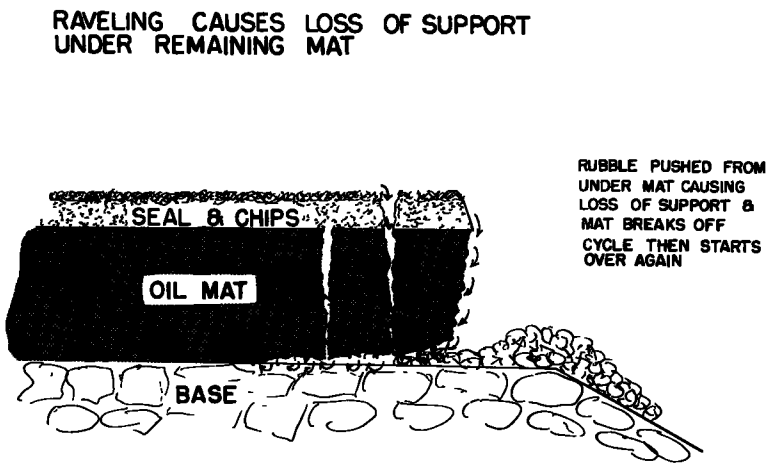


Figure 6.

Those that might undertake these experiments will be surprised how quickly the action of the soil microorganisms effect the appearance of the asphalt film as the oily constituents are dissipated. And it is important to run the controls under sterile conditions, in order to have an accurate comparison. While this study was originated by the writer, there is always reason to believe that others are working with the same idea in mind but, probably, with a different approach and differing procedures.

It should be pointed out that some precaution is necessary in handling these accelerated tests, since the nutrient media and above average temperature can and do produce harmful bacteria, especially if a nutrient agar is employed for making cultures for microscopic examination.

Also, some care is necessary to keep the controls free of contamination; the dishes should be washed thoroughly and kept covered to prevent any soil particles or drops of cultured solution from them.

The first experiment set up consisted of several 2-inch briquettes made of -10 material and oiled with 270-pen, asphalt, immersed in a covered jar which contained soil and water enriched with salts. The soil was taken from the capitol lawn under the grass roots. The gas from the jar was discharged into limewater; the precipitate indicated CO_2. After 6 months, the briquettes were taken out and the condition noted. Three of

TABLE 7

DISTILLATION TESTS ON KEROSENE AFTER BACTERIAL ACTION
(P. pyocyaneus culture No. 58)

Percent Distilled	Control (Aerated)	Control (Not Aerated)	No. 58 (Aerated)	No. 58 (Not Aerated)
	Initial b.p.			
	120°	116°	120°	120°
	b.p.	b.p.	b.p.	b.p.
10	193	190	189	184
20	199	196	195	194
30	202	201	201	202
40	211	205	206	204
50	217	215	213	215
60	223	222	214	221
70	227	228	226	229
80	211	213	232	223
90	225	223	243	243
End point	232	229	257	248

the briquettes had disintegrated, but four were in fair condition, except for a blistering effect on the exterior. The specimens were dried and the asphalt extracted. On examination the asphalt was found to be quite hard (76 pen.). The amount recovered was not sufficient to obtain more data, such as ductility.

Another one of the first set-ups was a platter with a thin film of SC-3 floated on water which had been inoculated with the culture. A control was set up for comparison on sterile salts.

Out of the 35 g., only 13 g. were recovered from the cultured platter. The asphalt had hardened to the extent of having a softening point of 85 F. The control flowed at room temperature, at which 31 g. were recovered. A very-small amount adhered to the platters in both cases.

Care was taken in recovering the asphalt, so there would not be any change due to heat; drying was done at 150 F.; a water bath was used to prevent overheating while distilling of the CCL_4. I should mention that it had been dissolved in CCL_4 and filtered through gooch to remove any soil particles. The control was treated in a similar manner (Table 8).

TABLE 8

ANALYSIS OF THE RECOVERED OILS FROM SC-3

	Control	Bacteria Treated
Insolubles	.05	.08
Asphaltenes	16.97	21.71
Oils	43.95	31.51
Ether Resins	14.98	19.84
Acetone Resins	22.73	25.11
Total	98.68	98.65

CARBON-HYDROGEN RATIO

	Control	Bacteria Treated
Hydrogen	10.25	9.96
Carbon	85.16	84.40
Sulfur	Not run	Not run
	Flows at room temp.	85 deg. softening point

A second set up was run on MC-3 straight run. This time a larger amount of road oil was used in order to have a larger amount left. At the end of 30 days, the control was taken off, likewise the asphalt that had been exposed.

No attempt was made to make a complete analysis of the results, but the asphalt exposed to bacteria had hardened to the consistency of an MC-6 having a penetration of 283 at 77 F. The control was unchanged.

Perhaps the most-instructive project has been the collecting of the CO_2 gas as it is given off at 1,000 g. of oil mix inoculated with culture. A desiccator with clamps was used to contain the oil mix. An outlet at the top is connected to a condenser jacket full of water. As the gases collect, they force out the slightly acidiz-

ed water. This method can show rate of gas production if temperature is fluctuated.

In a recovered asphalt from an old SC-3 mat, the gravity of the asphalt was 1.16 and was 9.64 percent soluble. This is an extremely high gravity, although the ductility and the penetration at 77 F. was 100 and 60, respectively.

OXIDATION OF OILS

The oxidation of the extracted oily component of both straight-run and cracked asphalt by bacteria or its enzymes is relatively fast. Approximately one gram of oil spread as a film on the inoculated salt media shows signs of dissipation in 3 to 6 days. Straight-run oils are oxidized more completely than cracked, as shown by Figure 1.

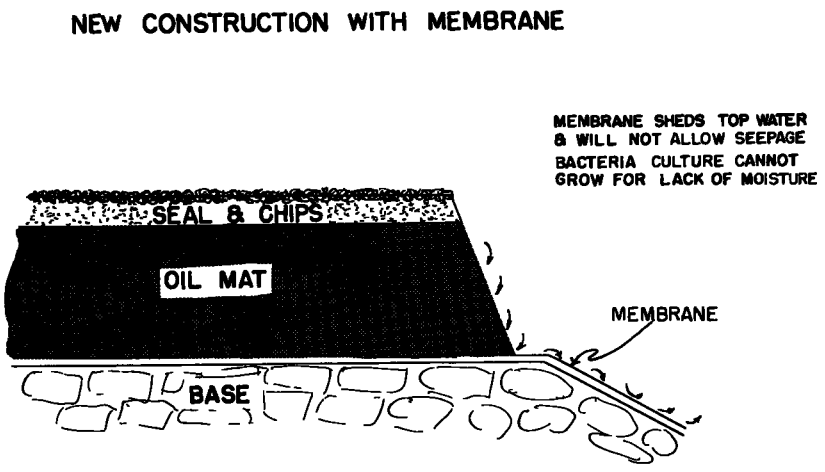


Figure 7

A waxy substance with a melting point of about 140 F. appears on the surface of the media from the straight-run oil in approximately 30 days. After several months this changes to a white substance. The film of slime that submerges gradually disintegrates until it has a ragged appearance. The oils from cracked asphalt retain a much darker color and a heavy body. Speculating on the cause of this will have to suffice until a sufficient quantity of oil is on hand for experiments. Because of the different origin of these oils, their behavior is most important to future study.

MINERAL SALTS MEDIUM

The most-successful method of isolating organisms capable of utilizing hydrocarbons is the use of a mineral-salt hydrocarbon enrichment medium in which the hydrocarbon is the only source used, with minor modifications, by Sohngen (1913), Tausz and Peters (1919), Tauson (1929), Buttner (1926), Haag (1926), Jensen (1934), and Gray and Thornton (1928). The basic-salt medium given in Table 9 proved to be quite satisfactory. The medium is adjusted to ph 7.0 to 7.2 with dilute NaOH. Two percent of washed agar is added whenever a solid medium is needed.

TABLE 9

Water, distilled	1000.0
$MgSO_4$	0.2 g.
$CaCL_2$	0.02g.
KH_2PO_4	1.0 g.
K_2HPO_4	1.0 g.
NH_4NO_3 or $(NH_4)_2 SO_4$	1.0 g.
$FeCL_3$	2 drops conc. sol.

FIELD STUDIES

One of the biggest obstacles in field sampling is reliable data on the road oil used. It is necessary to know the complete analysis of the cutback asphalt. In a great many cases when maintenance crews have worked over an oil mat, there is only a small fraction of the information available. It is not sufficient to find a big difference in the penetration of the extracted

asphalt from samples taken in different sections of the oil mat, such as the big difference between shoulder and centerline samples.

There are so many factors to be taken into consideration that anything definite, in the way of exact duplication, is practically impossible. Therefore, it is safer to approach the study with the idea in mind of duplicating, as nearly as possible, field conditions in the laboratory, where they can be controlled. This eliminates any influence of aggregate absorptions or chemical reaction from the aggregate, unknown pH of moisture, and such variables as extremes of temperature.

CRITERIA OF OXIDATION AND OXIDATION RATE

Criteria of hydrocarbon utilization are easily recognized by the production of CO_2; change of pH by acid formation and saponification; change of specific gravity; and loss of quantity. In Table 3 is the oxygen-uptake chart, which shows the oxygen consumed as always being less than the volume of CO_2 produced. Light hydrocarbons use more than the heavy ones. Turbidity is an indication of bacterial action. It is the belief of some investigators that dead cell substance is one of the contributing factors which increase the specific gravity of asphalt that has been worked on by bacteria, acting like a heavy mineral colloid.

In Table 1 is a description of the hydrocarbons used by Stone, Fenske, and White in their work, ranging from light hydrocarbons to fairly heavy residues. The rate of oxidation varies according to the several governing factors, but several workers have given the figure of 0.7 ml. per sq.decimeter in 24 hours on mineral oil.

The paving asphalts seen have a great advantage over the road oils, because the film thickness on the aggregate can be heavier. And since there is no cutback to be lost either by aggregate absorption or evaporation, the initial film is quite permanent. The

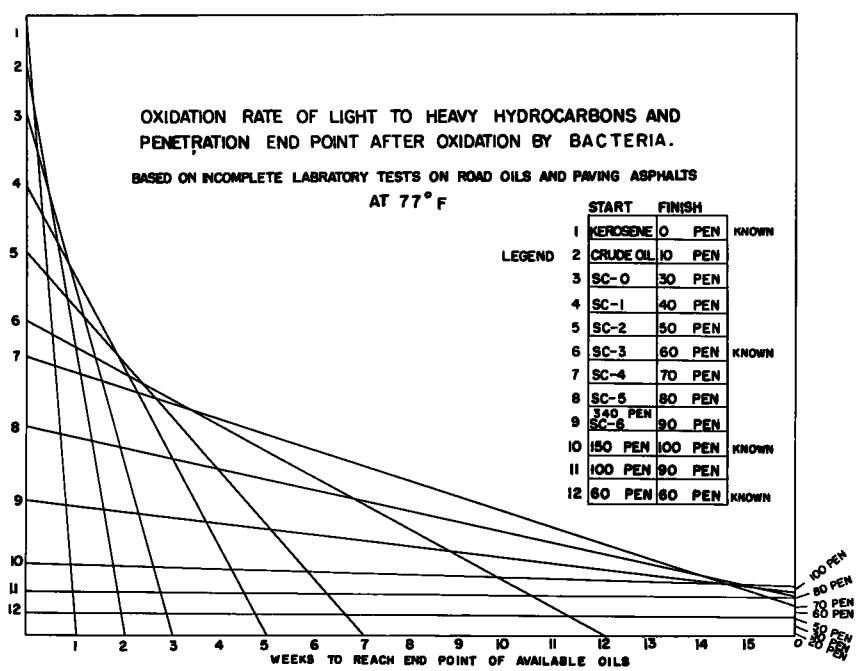


Figure 8

second advantage of paving asphalts is their comparative freedom of light ends that provide initial nutrient to microorganisms.

While this might be the first time that the investigation of bacterial action on road oils and cutback asphalts has been attempted, it is hoped that others will become interested in the potentials of this new concept of oxidation.

Since the rate of bacterial attack depends on the accessibility of oil present, paving

asphalts were not used in this study to any extent due to this hardness and low oil content, rendering them practically immune to microbial attack and no dispersion possibilities in an aqueous media.

There is a tendency to underestimate the importance of film thickness. This is the all-important factor, because all results are dependent on this. While it may not be possible to duplicate film thickness in successive tests, it is essential to obtain a uni-

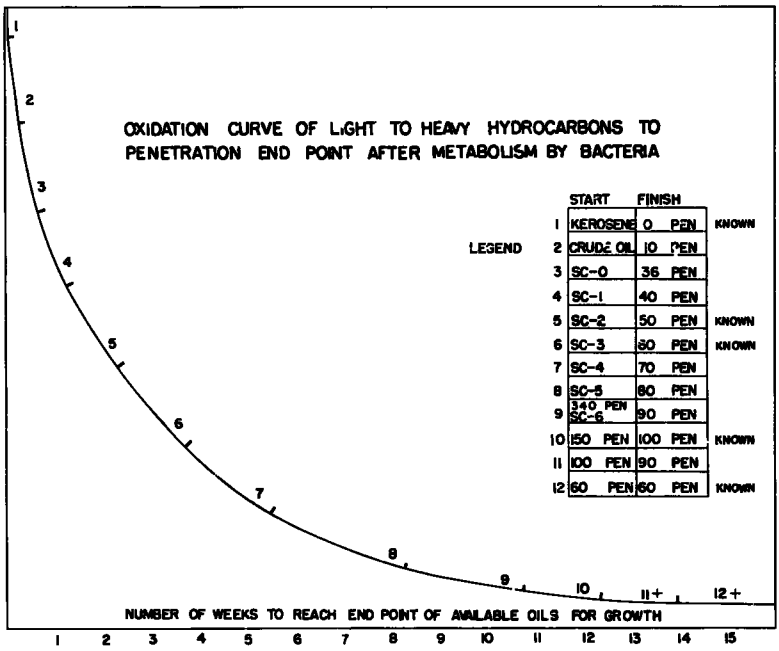


Figure 9

form film. Otherwise the results will not correlate closely.

Temperature is vital to correlating test data. Whatever is found to be the easiest constant temperature to maintain should be used, such as 78 F.; 100F. will accelerate more growth. It is difficult to maintain sufficient moisture in the experiments, due to evaporation at higher temperatures.

Since dispersion is one of the most-difficult problems of using heavy, viscous hydrocarbons, such as asphalts, these materials are difficult to use in bacteriology laboratory experiments. However, in a properly oiled aggregate we find an ideal target for microbial attack, because of the immense surface area of thin oil film presented on the bottom of the mat.

Once underway, this bacterial action migrates upward with capillary moisture as the vehicle. As the oils of the asphalt binder are slowly oxidized, the asphalt becomes progressively harder. The completed action leaves the aggregate with insufficient binder (see Figures 2 and 3). Longitudinal cracks generally appear in the weakened mat through the seal coat after ravelling starts on the bottom of the mat.

Aggregate thus freed from the mat is free to move and is no longer an integral part of the mat. This outside edge of mat, having lost its vertical support, breaks and becomes an easy prey for encroaching traffic to thrust aside. This applies especially to narrow mats. Access is thus gained for further oxidation. Not a very specticular action nor one characterized by sudden results, but nature's methodical process of changing a contaminant back to soil (see Figures 4, 5, 6, and 7).

It is not intended to imply that all the failures in old oil mat shoulders were the results of microbial attack, but, if conditions had been favorable to bacterial action, it could be a contributing factor.

At present it is difficult to distinguish by casual observation the underlying cause of shoulder failure, because so many factors are involved. So how much can be attribut-

ed to microorganisms remains to be discovered by careful sampling of failures and laboratory analysis of the extracted asphalt content remaining in the mat and examination of the base as a cause of the shoulder failure.

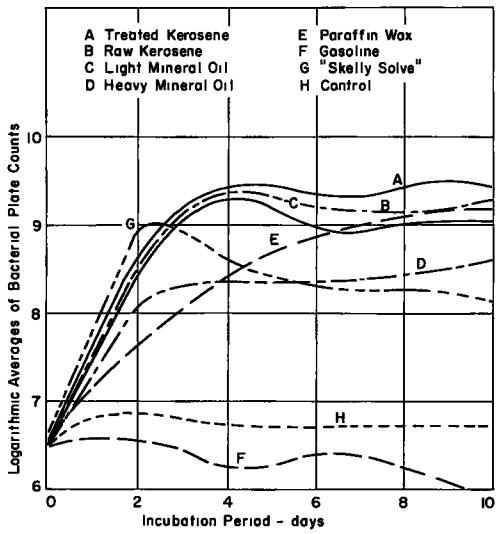


Figure 10. Growth of pseudomenas culture No. 8 on various hydrocarbons. This typical pseudomonas aeruginosa could not utilize gasoline like culture X. But this is significant in the laboratory only since nearly all strains and types are present in natural soils for the destruction of any hydrocarbon that might contaminate the soil.

SUMMARY BY BURGESS

Having established that certain types of asphalt mats are subject to bacterial attack at the shoulder, it is now logical to suggest a practical means to prevent this oxidation in future construction. Inhibiting the oxidation of road oils, used as a binder in asphalt mats, by soil bacteria could be successfully accomplished by undersealing the oil mat. A light ⅛ inch membrane on the outer 18 inches of base before final laydown could provide an adequate underseal protection. This need not extend beyond the anticipated edge of the oil mat, but an extra width would provide additional protection for the base from runoff.

The membrane can be made of either hot-applied, 60-penetration, cat-blown asphalt or any 60-pen. asphalt of equal ductility at 30 F. or quick-breaking emulsion using 80-pen. base stock could be substituted, providing, it also had good or equal ductility.

The ultimate goal of this project is the elimination of shoulder maintenance due to this type of oxidation. Several maintenance foremen estimate 60 percent of oil-mat maintenance is shoulder replacement.

Syneresis or migration of the oil component of road oils as a factor contributing to the hardening of road oils is not compatible with known aggregate absorption as found in "Scoria" by the writer. This is not to be confused with evaporation of light ends or cutback from MC or RC road oils. But it is known that certain asphalts harden to some extent in tight containers. The cause of this is not clearly understood, although it is probably polymerization.

A high softening point (175 F.) is essential for an asphalt that is to be used for membrane.

The possibility of using chlorinated asphalt to inhibit bacterial activity is being explored.

INVESTIGATION BY STONE, FENSKE AND WHITE

The conditions necessary for attack on oils by microorganisms have been summarized by Tausson (1928) as follows: (1) presence of water with mineral salts; (2) a nitrogen source, such as the ammonium or nitrate ion; (3) free access of oxygen; (4) a neutral reaction and a buffer such as $CaCO_3$ to maintain it.

However, there has been little attention given as to what hydrocarbons are most subject to attack and the mechanism of their breakdown, although Tauson and co-workers (1934) have shown that some acids and unsaturation are produced in the bacterial dissimilation of crude and lubricating oils.

The purpose of this work was to determine how wide a range of petroleum actions could be readily attacked, to attempt to find which of several representative petroleum fractions were most subject to attack, and to isolate and characterize a number of organisms able to develop on a hydrocarbon medium.

DEVELOPMENT OF CULTURES ON OIL

Erlenmeyer flasks were prepared containing 0.5 g. oil, 50 ml. H_2O, 0.25 g. $CACO_3$, 0.25 percent NH_4NO_3, 0.1 percent Na_2HPO_4, 0.05 percent KH_2PO_4, 0.05 percent $MgSO_4$, 0.02 percent $MnCl_2$, and traces of Ca, Fe and Zn. The flasks were inoculated with one gram of garden soil, incubated for a period of from 10 to 20 days and shaken twice daily. When considerable decomposition appeared as indicated by the emulsification of the oil and increased turbidity of the medium, 1 ml. of the mixture was transferred to another flask containing all the above ingredients except soil.

After two to three transfers the breakdown of the oil proceeded faster and the period of incubation was shortened accordingly. Flasks were incubated at 20 deg. room temperature (23-26 deg.), 30 and 37 deg. C.

After at least six successive transfers from the original flask containing soil inoculum, bacterial counts were made on the medium by plating on standard nutrient agar. A mineral-salt oil agar was also used, but it was observed that nutrient agar gave slightly higher counts. Furthermore, in all cases tested the organisms which appeared on the mineral-salt oil medium grew when transplanted to the nutrient agar. As continued cultivation on nutrient agar caused a marked decrease in the ability of the cultures to attack hydrocarbons, cultures were picked from the plates in proportion to the numbers present and kept on the mineral-salt oil medium.

The materials used in this study included several crude oils, heavy oil residues such as petrolatum (Paraffinic) and asphaltic tar (aromatic) and the various oils given in Table 1. The filtered 185 Pennsylvania neutral was a conventionally refined 10-W grade of motor oil. This oil is typical of light oils or neutral made in the Pennsylvania area, and is a relatively homogeneous mixture of hydrocarbons. The distillation fractions of this oil, designated as Oils 2, 3, and 4 in Table 1, were prepared by high vacuum fractional distillation and differ from each other essentially in molecular weight. Each oil is a narrow boiling fraction with a very small molecular weight range.

Oils 5, 6, and 7 of Table 1 were prepared by solvent-extraction of oil with acetone (Hersh 1938). Thus, these fractions represent hydrocarbons differing essentially in molecular structure, whereas those prepared by distillation represent fractions differing essentially in molecular size or weight.

Oil 8 is a typical heavy grade oil with a higher molecular weight and more heterogeneous composition than Oil 1. Oil 9 resulted from exhaustive acetone extraction of Oil 8 and may be considered to be a very high molecular weight, highly paraffinic hydrocarbon mixture.

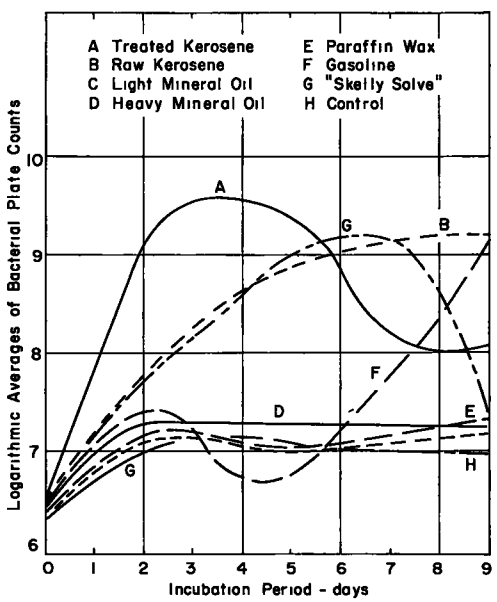


Figure 11. Growth of pseudomenas culture X. on various hydrocarbons. This culture which was isolated from water taken from the bottom of distillate storage tank had the ability to utilize "Skelly-Solve" which is a hydrocarbon fraction containing a high percentage of hexane, which was readily utilized, but cyclic compounds such as beneze and light mineral oil were not utilized.

Oil 10, an aromatic extract from a Louisiana light distillate oil, represents a relatively low molecular weight aromatic-type hydrocarbon oil.

Mixed cultures are developed from soil capable of attacking each oil or oil fraction studied as well as the crudes, petrolatum and asphalt. After several transfers the medium weight and crude oils were found to be quite rapidly broken down and usually com-

pletely emulsified in three to five days at room temperature. The heavier oils were more slowly dissimilated, the emulsification process requiring as long as three weeks. Bacterial counts were made on the various fermentations after six to ten consecutive transfers. Petrolatum and asphalt supported the growth of large numbers of organisms concentrated at the oil-water interface of the separate globules, but owing to the difficulties of dispersion, no counts were made on these materials.

Typical counts of the mixed flora found in the breakdown of crude oil and certain fractions are shown in Table 2. In the majority of cases the peak in bacterial flora occurred between the third and sixth day of fermentation. At this time there was usually one type of colony predominating in each type of oil. All the organisms examined from the various oils at the different temperatures appeared to be short, motile, gram-negative rods. In the crude oils, pseudomonas seemed to be the outstanding type. White opaque colonies appeared most frequently in the light oils. White and mucoid colonies were predominant in many of the heavy oils.

In most cases pigmented forms other than pseudomonas did not appear until after a peak in total count was reached. There was little indication of the specificity of a certain organism for a particular type of oil. However, if a mixed culture from a rapidly fermenting oil of highly aromatic nature was transferred to a paraffinic type (or vice versa) the growth lagged for several days. The culture was usually able to adapt itself to its environment and become active in the new oil within one transfer.

It was apparent that the lighter weight oils were more easily attacked than the heavier fractions. This is evident from the counts given in Table 2, if counts of Oils 2 and 4 are compared, or of Oils 2 and 8, or Oils 8 and 9. In the fermentation of mixtures containing as much as five to ten percent of Pennsylvania 185 neutral oil measurements of the unfermented oil showed an increase in viscosity, indicating a preference of the organisms for the lighter fractions. Also, it could be readily observed that the light oils disappeared from the surface of the medium much more quickly than the heavy ones. The crudes supported growth in a degree comparable to the light oils and were subject to rapid emulsification although globules of the heavy fractions could still be discerned on the surface after two weeks' fermentation.

The bacterial counts also indicate that the paraffinic oils were attacked more rapidly than the aromatic types as can be seen by comparing aromatic oils with paraffinic Oil 7. Oil 6, which contains a relatively high percent of naphthene compounds occupied an intermediate position with respect to growth supported. The slower attack of aromatic oils can also be shown by comparing Oil 10 (strongly aromatic) with Oil 7 or the light neutrals 1, 2, and 3. Although Oil 10 is more viscous than the light oils it has a smaller average molecular weight.

MANOMETRIC STUDIES

Several representative oils were chosen for determination of the oxygen required for their dissimilation by mixed cultures. The studies were carried out with a Warburg manometric apparatus immersed in a constant temperature bath and shaken continously by motor. The technique and apparatus used was similar to that described by Dixon (1934). The cultures were inoculated and cultivated in standard manometric flasks and respiration measured over a period of several days at 30 C. In each flask was placed 3.8 ml.of mineral salt solution, 50 mg. of the desired oil and 0.2 ml. of the corresponding mixed culture inoculated. The mineral salt solution was of the same composition as that described previously except that $(NH_4)_2SO_4$ was substituted for NH_4NO_3. Readings were taken twice daily for intervals of two hours, these figures were plotted and from the area subtended by the curve the total respiration during the entire time was calculated.

When the fermentations were carried out for longer than seven days, a large fluctuation in results was noted. Allowing for small errors in technique and the relatively large variation to be expected from mixed culture studies, the results obtained during the first five days of incubation were fairly consistent. In practically every case the peak of respiratory activity came in this interval. However, it was noted that the actual amounts of oxygen consumed and carbon dioxide liberated varied according to the rate

the temperature of incubation and the age of the inoculum. Some typical results are shown in Table 3. About 10 ml.of oxygen were taken up in the fermentation of Oils 1 and 7 containing predominatly paraffin hydrocarbons. In Oil 5 (aromatic) the uptake was slower. The breakdown of Oil 8, which has an average molecular weight of nearly two times the light oils and is more viscous, consumed only about half as much oxygen during the longer time interval. Oil 9 which is still heavier and composed of larger molecules had still less oxygen uptake.

As a check on the respiration to be expected from the cells alone, a control is shown based on 0.2 ml.of inoculum from oil fermentation suspended in buffer with no oil added. The amount of respiration is negligible and in comparison with the growing culture.

The carbon dioxide values are subject to an error as a small amount of gas would originate from the $CaCO_3$ buffer, not only by the action of organic acids that may have been formed during fermentation but also from free sulfuric acid produced by the utilization of nitrogen from the $(NH_4)_2SO_4$. The latter can be roughly estimated at between 0.08 and 0.1 ml.CO_2 for ml.of medium on an active culture producing nearly a gram of moist cells per liter, assuming that the cells contained 2.5 percent nitrogen on a wet basis. As large scale fermentations carried out under similar conditions indicate that usually about 80 percent of the original oil can be recovered, only about 0.20 to 0.30 ml. of CO_2 could be liberated by mono-carboxylic acids formed from 0.050 g. of the oil having a ml.wt.in the range of 400. The total correction amounts to an approximate reduction of only 5 percent for the CO_2 evolution of the light oils. The heavy oils have been corrected accordingly.

Figure 12

The CO_2/O_2 ratios using the corrected values are subject to the errors present in the CO_2 determination but show fairly consistent values for the different oils. It is noteworthy that in the case of the light oils, the ratios are high for incomplete oxidation of the hydrocarbons. The theoretical respiratory quotient for complete oxidation of a long-chain paraffin hydrocarbons with the formula $CH_3(CH_2)_nCH_3$ is approximately 0.67.

$$(CH_2)_n + 1.5nO_2 \text{ -- } nCO_2 + nH_2O$$

$$CO_2/O_2 - n/1.5 \text{ X } n - 0.67$$

The respiratory quotients of the light oils are in the neighborhood of 0.65.

If the hydrocarbon molecules were oxidized without decarboxylation considerable loss of CO_2 the gas ratios should be much lower. The rest indicate that a large percentage of the molecules attacked were completely oxidized to CO_2.

The fermentation of the heavier oils containing longer molecules not only did not produce as much CO_2 but furthermore gave a much lower CO_2 to O_2 ratio. In the case of Oil 9 the CO_2 evolution was practically negligible, indicating a much less complete oxidation of the heavy oils.

COMMENT

It is evident from the studies presented here together with those of other investigators, that under favorable conditions microorganisms can be found that are capable of attacking practically any hydrocarbon from methane up to the heaviest paraffinic or asphaltic residues. However, the heavier oils become more difficult to attack as the viscosity and molecular weight increase. This is due in part to the fact that the more viscous oils are harder to disperse in a liquid medium and hence there is less surface exposed to the growth of microorganisms. But the difficulty of attack is probably also attributable to the larger molecule. Strawinski and Stone (1940) have found that compound the range of 10 or 16 carbon atoms are attacked more readily than those smaller molecular weight.

Observations by the authors as well as Bushnell and Haas (1940) on the fermentation of gasoline and kerosene indicate that, although both substances are quickly attacked, the kerosene is more subject to breakdown than the gasoline with bacterial counts up to a billion per ml. or comparable to counts observed in light oils. From these results, it is evident that in oils of a predominatly paraffinic nature the fractions from kerosene up to medium weight lubricating oils include the range most easily attacked by bacteria.

Both bacterial counts and manometric studies indicate that paraffinic oils are more easily broken down than corresponding oils of aromatic nature. However, it must be emphasized that the predominatly aromatic fractions proved to be very acceptable carbon and energy sources. The most unexpected observation in this connection was the apparent lack of specificity of one culture for any certain type of oil. Even when such studies are extended to pure hydrocarbons it is possible to find that an organism which is adapted to grow on a purely paraffinic source, such as cetane, will begin to grow immediately when transferred to a strictly aromatic compound such as naphthalene.

The organisms capable of attacking oils appear to be present wherever samples were taken. Only gram-negative rods were found, whether the enrichment cultures were incubated at 20C., 30 C., or 37 C. These findings are not entirely in accord with Solingen (1913) who observed mycobacteria at 37 C. nor with Bushnell and Haas (1941). One reason for the absence of acid-fast bacteria in the present work is that the cultures were transferred at intervals of 7 to 14 days and probably there was not sufficient time for the development of these slower-growing organisms.

It is evident, particularly among the non-pigmented forms, that there is no sharp line of demarcation between the different groups. The adaptability of these bacteria is further realized when it is considered that in most soil samples there is little chance of long chain hydrocarbons having been present for unnumbered bacterial generations. It is obvious that there is no specialized group of organisms here but that we are dealing with the common soil forms which possess the ability to adapt themselves to an infinite variety of organic compounds.

SUMMARY BY STONE, FENSKE AND WHITE

1. Cultures capable of attacking crude oil, lubricating oils, vaseline, asphalt, and all other petroleum fractions used were obtained from garden soil.

2. It was found that the light to medium-weight fractions are more subject to attack than the heavy viscous portions and that the paraffinic fractions are more readily broken down than the aromatic types.

3. The breakdown of oil is an oxidative change characterized by a high bacterial

count, emulsification and sometimes a decrease in pH.

4. There was much less oxygen uptake in fermentations of heavy oils compared to similar lighter fractions. Likewise aromatic fractions utilized less oxygen than the paraffinic types.

5. The CO_2 to O_2 ratio for the dissimilation of light oils is in the neighborhood of 0.65. In heavy oils the ratio drops to a much lower figure.

6. The organisms were all motile gram-negative rods including Pseudomonas, and many white-mucoid types. They were obtained from all soil samples tested and appear to be of common occurrence.

7. The cultures did not exhibit a specific ability to attack one type of oil but rather a capacity to adapt themselves, according to conditions, to attack the particular oil that was present.

SUMMARY OF STUDY BY BUSHNELL AND H. F. HAAS

Cultures of organisms capable of using petroleum fractions such as "Skelly-solve," gasoline, kerosene, light and heavy mineral oils, and paraffin wax as the source of carbon and energy for their metabolism were isolated from various petroleum storage tanks; they were found to be good sources for the isolation of organisms of this type. Organisms possessing this ability are not necessarily confined to such habitats, since practically all the pseudomona cultures, regardless of their origin, were capable of utilizing kerosene. This was demonstrated by the fact that cultures isolated from various sources, such as abscesses, mastitis, water, and fecal matter of animals, were all able to utilize it.

Bacteria of other genera were also found capable of this activity, including certain species of the micrococci, corynebacteria, and Culture X exhibited a preference for paraffinic to the naphthenic or cyclic hydrocarbons. Many of the cultures were able to withstand as high as 10 to 15 transfers under kerosene without diminution in growth, thereby indicating that accessory growth factors are not needed, or that the organisms were able to synthesize these substances.

Respiration studies indicated that the hydrocarbons were oxidized largely to carbon dioxide and water. The respiratory quotients of various bacterial cultures on different hydrocarbons varied from 0.30 to 0.70. No direct correlation between the respiratory quotient and the nature of the hydrocarbon was observed.

Some evidence was obtained to show that long-chain organic acids and unsaturated hydrocarbons were formed during the bacterial decomposition of the hydrocarbon fractions. The formation of organic acids was indicated by small changes in the pH of the medium and by the increased ease of formation of emulsions of oil and water.

The bacterial production of unsaturated hydrocarbons was indicated by changes in the distillation temperatures of the kerosene. The boiling point of the last 20 percent of kerosene distilled was higher, probably indicating that polymers with a higher boiling point were formed from the unsaturated hydrocarbons during the process of distillation.

As a result of this investigation, it has been established that the bacterial utilization of hydrocarbons is a characteristic common to many types of microorganisms and that in nature this process probably occurs to a greater extent than is generally recognized. The oxidation of hydrocarbons was found to occur on simple media; in fact, ordinary well water at the bottom of a distillate tank was able to support a bacterial count of approximately 900,000 organisms per milliliter.

The respiration studies indicated that the oxidation of hydrocarbons is similar to the oxidation of other organic compounds and that such end products as carbon dioxide, water organic acids, and unsaturated hydrocarbons are produced.

LITERATURE SEARCH BY BITUMINOUS DIVISION OF BUREAU OF RECLAMATION

A literature search was undertaken in conjunction with the soil burial tests to attempt to uncover work of any bearing on service life of bituminous materials used for buried mem-

branes which may be subjected to conditions of service favorable to attack by microorganisms. Considerable literature was found descriptive of the attack of microorganisms on a large variety of petroleum derived hydrocarbons and related compounds, but very little specifically on attack upon asphalt. However, since even the most durable rocks, and everything else, slowly yield to the succession of diversified attacks by species of fungi, bacteria, yeasts, molds, and powerful enzymes produced by them, it was thought wise to investigate the degree of susceptibility of asphalt to microorganism attack.

The ability of microorganisms to utilize petroleum hydrocarbons and related materials has been recognized for a long time. For instance, as long ago as 1906, Rohn wrote concerning the utilization of paraffin by fungi. Again, in 1913, Sohngen recorded the utilization of paraffins by certain fungi.

In 1917, P. L. Gaines, wrote concerning the effect of paraffin on the accumulation of amonia and nitrates in the soil (J. Agr. Research, Vol.10: 355-364).

In 1928, P. H. H. Gray and H. G. Thornton, in "Soil Bacteria that Decompose Certain Aromatic Compounds," Zentr. Bakt. Parasitenk. Abt. II, 73: 74-96, say that bacteria which are widely distributed in soil are able to oxidize substances which are usually thought to be potent bactericides. When soil was teated with hexane benzene, toluene, xylene, naphthalene phenol, cresol, resorcinol, phlorglucinol, pseudocumene, mesitylene, cymene and pinene; these genera were capable of utilizing one or more of the above compounds; micrococcus, Mycobacterium, Bacterium, Bacillus and Spirillum.

Vo. O. Tauson, in "The Oxidation of Benzene Hydrocarbons by Bacteria." Planta, 7: 735-757 (Chem. Abs. Vol. 23: 3945, 1929) found a great variety of microorganisms able to utilize hydrocarbons, and isolated three of the species. Bacterium naphthalinicus B. naphthalinicus (liquefaciens) and Bacterium naphthalinicus B. (non-liquefaciens) which oxidized hydrocarbons.

Zo Bell, Grant, and Haas observed the growth of water bacteria in the presence of 1 percent phenol and a like concentration of emulsified tri-cresol. (Zo Bell, C. E., Grant, C. W., and Haas, H. F., "Marine Microorganisms Which Oxidize Petroleum Hydrocarbons." Bul. Am. Assn., Petrol. Geol., Vol. 27: 1175 (1943).

The bacteria did very well despite the presence of these strong chemicals which are normally considered good bactericides. They also found marine bacteria capable of utilizing petroleum ether, gasoline, kerosene, lubricating oil, crude oils, petroleum, paraffin and microcrystalline waxes, mineral oil, methane, pentane, hexane decane, trimethylpentane, tetratriacontane, benzene, toluene, xylene, cyclohexane, anthracene, naphthalene, pyridine, natural rubber, isoprene, neoprene, and other synthetic rubbers. The aromatic and cyclic hydrocarbons were very slowly utilized.

They state that most of the bacteria oxidize hydrocarbons only in the presence of free oxygen although some of them can utilize nitrate as a hydrogen acceptor and possibly some of them can activate sulfate as a hydrogen acceptor which allows the bacteria to utilize these compounds as food. (In the latter case hydrogen sulphide would be produced which inhibits bacterial oxidation in concentrations exceeding 0.0001 ml. per liter and would have to be removed from the system in some way for disintegration to progress). They found Proactinomyces, Actinomyces, Pseudomonas, Microspora, Mycobacterium and possibly other genera are able to oxidize hydrocarbons.

CONCLUSION BY BITUMINOUS SECTION, BUREAU OF RECLAMATION

The ultimate effect of the attack of microorganisms should not be underrated. The most durable materials of construction and even the most resistant rocks eventually crumble before the successive attacks of soil bacteria and fungi, or the enzymes and decomposition products produced by them. This is the ordinary process of soil formation. However, the rate of attack on asphalt of the proper hardness is so slow as to be of negligible effect on membranes where the temperature does not exceed 70 F. for those on the order of ¼ inch thick as used in Bureau of Reclamation construction.

Writer's note: These bureau tests were run with above normal ground temperatures in compost beds. Once again this points to the most important controlling factor; temperature. Moisture is important and a prerequisite, but the amount above minimum requirements is unimportant, likewise, oxygen and a favorable pH range. As temper-

atures decrease, bacterial activity slows until it becomes practically dormant at 50 F.

The natural temperature of the soil, four to six inches below the surface, is quite constant in the summer months and under these conditions the normal activity of soil bacteria and their catalytic enzymes is characterized by the orderly procedure found in the decomposition of dead organic material (asphalt not excluded) into plant nutrients and soil. But as the temperature is raised, the activity increases far beyond the normal rate (see Figure 12).

Figure 2 depicts new construction with conventional oil mat with top seal coat on a select borrow base course. Under normal conditions this base course is comparatively free of any soil bacteria because, very little, if any surface soil is present since the top soil with the overburden is removed in a new pit. Therefore, new construction is made with comparatively sterile soil which would remain sterile if protected from the migration of soil bacteria.

Figure 3 shows runoff percolating into the base at the interface of the mat with the base. Under normal conditions it takes two or three years for soil bacteria to slowly migrate across the borrow pit to the edge of the mat. This interval of time is sufficient to furnish the necessary moisture under the edge of the mat for the propogation of the hydrocarbon oxidizers which have migrated there.

Figure 4 demonstrates initial ravelling on the underside of the mat as the bacterial enzymes attack the oils in the asphalt. Ravelling on the bottom of the mat is a slow insiduous process involving each particle of the aggregate individually. The thin film of asphalt surrounding and covering large and small particles is very thin, especially in open graded aggregates, and the thinner the film the more vulnerable it is. It is fortunate indeed that mat temperatures are well below the minimum for the growth of bacteria 90% of the time. Otherwise the process of oxidation on road oils would be very rapid; such as found in the tropics on native resins used for soil binder (Jones, London University 1954). Even the most durable asphalts used for protective membranes are not immune to bacterial attack when a constant temperature of 104 F is maintained with hydrocarbon oxidizing bacteria present; such as found in the methane gas towers in a sewage disposal system in Koln Germany. This asphalt was of high quality with a softening point of 185 F. It had a thickness of one-fifth of an inch and in five months was practically useless as a protective membrane for the steel as seen by Picture No. 5.

Figure 5 shows the seal coat cracking which allows the first pieces to break away from the edge of the mat, which in turn allows moisture and oxygen to seep farther under the mat. The loss of vertical support coupled with a loss of strength makes the edge of the mat an easy prey to break down with traffic.

Figure 6 demonstrates the progressive ravelling caused by further inroads of oxidation on the bottom of the mat. This ravelled portion of the oil mat should be taken into consideration when determining asphalt content because this ravelled aggregate is generally compacted into the softened base where it loses its former identity.

Figure 7 depicts new construction which is designed to prevent bacterial attack by the use of an asphalt membrane, and also provides a happy medium to protect the base from runoff at the edge of the mat. It is highly probable, in the not too distant future, that considerable new construction will embody the use of asphalt membrane for enveloping the entire base either for retaining a prescribed amount of moisture in the base or protecting the base from excessive moisture and alleviation of frost boil damage by preventing ice lenses forming in the base from underground springs.

Figure 12 showing oxidation of asphalt membrane lining, as the result of bacterial attack on the interior of steel tank used for production of methane gas from sewage. This extreme oxidation is the result of preheating the sewage with steam to 104 F., which accelerates the activity of the bacteria. The membrane was approximately $^{3}/_{16}$ inch thick and a softening point of 185 F. The bubbles in the picture are the result of gas production in the asphalt proper from pinpoint intrusion or residual bacterial enzymes left on the surface after cleaning the tanks. Three weeks after this picture was taken, the asphalt was completely oxidized. It is highly probable that a highly chlorinated asphalt would resist oxidation even when subjected to these extreme conditions. It is unlikely that comparable results would be found in an asphalt mat where temperatures are at a minimum. Were it not for the enzymes-

catalyzed chemical reactions which help the bacterial utilization of hydrocarbons for energy or cell structure material, asphalt oil fractions would be comparatively immune to attack at normal temperatures.