BITUMEN ATTACK BY MICROORGANISMS

DR. RICHARD W. TRAXLER
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF SOUTHWESTERN LOUISIANA
LAFAYETTE, LOUISIANA

IT IS GENERALLY RECOGNIZED TODAY THAT A VARIETY OF BITUMINOUS MATERIALS ARE SUBJECT TO ATTACK BY MICROORGANISMS (1,2,3). EARLY REPORTS FROM OUR LABORATORY CONFIRM THE FACT THAT ASPHALTS ARE DEGRADED BY A VARIETY OF BACTERIA (4,5). THE PURPOSE OF THIS PAPER IS TO SUMMARIZE THE EXTENT OF OUR RESEARCH INTO THE ATTACK OF BITUMINOUS MATERIALS BY MICROORGANISMS. MOST OF OUR STUDIES DEAL WITH BITUMINOUS MATERIALS OF PETROLEUM ORIGIN AND WILL EMPHASIZE: (A) THE NATURE OF THE ORGANISMS ATTACKING BITUMENS (B) THE NATURE OF THE MICROBIAL ACTION ON BITUMEN AND (C) THE ASPHALTIC CONSTITUENTS UTILIZED BY BACTERIA.

ASPHALT UTILIZING ORGANISMS

A TYPICAL PAVING ASPHALT (135 PENETRATION) DESIGNATED AS "1A" WAS USED FOR ENRICHMENT CULTURE ISOLATION OF BACTERIA CAPABLE OF UTILIZING BITUMEN. THE ORGANISMS OBTAINED WERE SHOWN TO BE MEMBERS OF THE GENERA *Pseudomonas*, *Achromobacter*, *Micrococcus*, *Flavobacterium*, *Bacillus*, *Corynebacterium* AND *Alcaligenes*. LATER IT WAS SHOWN THAT CERTAIN ORGANISMS IN THE GENERA *Mycobacterium* AND *Nocardia* GREW WELL ON ASPHALT SUBSTRATES. THESE RESULTS DEMONSTRATE THE DIVERSITY OF MICROORGANISMS CAPABLE OF UTILIZING A BITUMEN OF THIS NATURE.

A SOIL BURIAL TEST WAS DEVISED USING A VARIETY OF BITUMENS COATED ON BIRCH TONGUE BLADES AND EXPOSED IN SOIL UNDER NATURAL CONDITIONS. ONE SET OF BLADES WAS BURIED IN A SOIL WITH A HIGH MOISTURE CONTENT (16%) AND A DUPLICATE SET IN A SIMILAR SOIL BUT UNDER LOW MOISTURE (6.4%) CONDITIONS. ACTION WAS SCORED BY DETERMINING THE WEIGHT LOSS OF THE BITUMEN COAT FROM

THE TONGUE BLADES AT VARYING TIME INTERVALS. THE RESULT OF ONE SERIES OF EXPERIMENTS IS SHOWN IN FIG. 1.

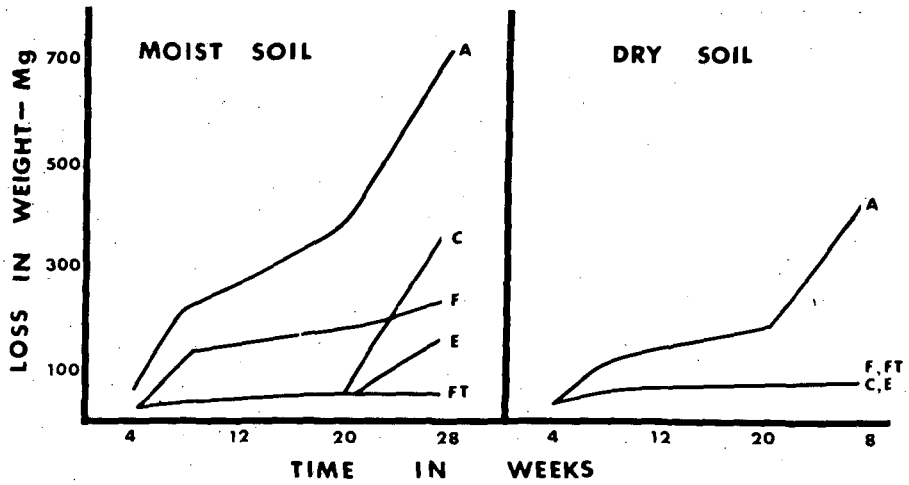


FIG. 1:--EFFECT OF SOIL BURIAL ON WEIGHT LOSS OF BITUMEN COATED TONGUE BLADES.

BITUMEN "A" IS MOST SUSCEPTIBLE TO WEIGHT LOSS IN MOIST SOIL, FOLLOWED IN ORDER BY BITUMENS "C," "F," AND "E." BITUMEN "FT" WAS REPORTED TO BE TREATED WITH A BACTERICIDAL MATERIAL AND DOES NOT SHOW A WEIGHT LOSS EVEN AFTER 28 WEEKS OF EXPOSURE IN MOIST SOIL. IN DRY SOIL APPARENTLY ALL OF THE BITUMENS EXCEPT "A" ARE RESISTANT TO WEIGHT LOSS. IT WOULD APPEAR THAT MOISTURE LEVEL IS INDEED CRITICAL (6) AS POINTED OUT BY HARRIS. ALSO, IT IS OBVIOUS THAT THE BITUMENS DIFFER IN THEIR SUSCEPTIBILITY TO SOIL EFFECTS.

THE DIFFERENCE IN SUSCEPTIBILITY OF BITUMEN TO BACTERIAL ACTION IS FURTHER SUPPORTED BY LABORATORY EXPERIMENTS (5). IT WAS DEMONSTRATED THAT Bacillus 11-2A ISOLATED BY ENRICHMENT CULTURE WOULD READILY ATTACK ASPHALT 6A, AND UTILIZE BITUMENS "A" AND "E" TO A LESSER EXTENT, BUT WAS UNABLE TO UTILIZE ASPHALT "G." ALL OF THESE MATERIALS WERE DEMONSTRATED TO SERVE AS A GROWTH SUBSTRATE FOR A Flavobacterium. THIS IS FURTHER EVIDENCE FOR THE SPECIFICITY OF ACTION OF A BACTERIUM ON DIFFERENT BITUMENS. SOME OF THE CHARACTERISTICS OF THESE MATERIALS ARE PRESENTED IN TABLE I, FROM WHICH IT IS SEEN THAT THESE MATERIALS VARY IN THEIR PHYSICAL NATURE. IT IS LOGICAL TO CONCLUDE THAT THE MICROBIAL ATTACK ON BITUMENS IS SPECIFIC. THE SPECIFICITY IS DETERMINED NOT ONLY BY THE NATURE OF THE BACTERIUM BUT ALSO BY THE CHEMICAL COMPOSITION OF THE BITUMEN.

TABLE I

PROPERTIES OF BITUMENS USED IN SOIL BURIAL TESTS AND GROWTH STUDIES.

BITUMEN	SOFTENING POINT °F	SPECIFIC GRAVITY 77°F	PENETRATION 77°F/100F/5 SEC MM/10
6A	111	0.985	133
A	195	1.015	48
E	167	1.175	0
G	161	1.269	0
C	214	1.06	3
F	170	1.195	0
FT	173	1.226	0

NATURE OF ACTION

THE RATE OF BITUMEN DETERIORATION UNDER NATURAL CONDITIONS CAN VARY TO A LARGE EXTENT DEPENDING UPON THE MATERIAL AND THE CONDITIONS OF EXPOSURE. IT IS NOT THE PURPOSE OF THIS PAPER TO DWELL UPON THE CONDITIONS OF EXPOSURE. THE WEIGHT LOSS EXPERIMENTS SHOWN IN FIG. 1 MIGHT BE CONSIDERED AN INDEX OF SUSCEPTIBILITY TO THE MICROBIAL POPULATIONS IN THE SOIL. IT IS DOUBTFUL IF THE WEIGHT LOSS IN THESE EXPERIMENTS IS DUE ENTIRELY TO MICROBIAL ACTION. NATURAL WEATHERING EFFECTS PLUS SOIL ABSORPTION COULD ALSO BE INVOLVED IN THIS WEIGHT LOSS. HOWEVER, IT IS LIKELY THAT MICROBIAL ACTION ACCOUNTS FOR THE MAJOR PORTION OF THE LOSS. EARLIER REPORTS (4,5) CITE DEGRADATIVE ACTIVITY OF A NUMBER OF PURE CULTURES ON A SINGLE ASPHALT UNDER LABORATORY CONDITIONS. IN THESE EXPERIMENTS THE RATE OF BREAKDOWN WAS RAPID, 3% TO 25% DEGRADATION OCCURRING WITHIN ONE WEEK OF EXPOSURE UNDER OPTIMUM CONDITIONS OF TEMPERATURE, pH AND AERATION. THERE IS A VAST DIFFERENCE BETWEEN THE LABORATORY CONDITIONS AND THE SOIL BURIAL TEST, IN THAT EXTREMELY THIN FILMS OF ASPHALT WERE USED IN THESE LABORATORY EXPERIMENTS AND RELATIVELY THICK FILMS IN THE SOIL BURIAL EXPERIMENTS. IT IS APPARENT FROM EARLY EXPERIMENTS THAT THE BACTERIAL ATTACK IS ONLY AT THE SURFACE OF THE BITUMEN. WITH THE THIN FILM MOST OF THE BITUMEN IS EXPOSED AS A SURFACE AND THEREFORE AVAILABLE TO THE ORGANISM. IN THE SOIL BURIAL TESTS AND IN-USE THE BITUMEN IS APPLIED AS A RELATIVELY THICK FILM; THEREFORE, ONLY A SMALL OVER-ALL PORTION OF THE BITUMEN IS EXPOSED TO MICROBIAL ACTION.

THE THIN FILM PROVIDES VISUAL EVIDENCE FOR THE MICROBIAL ACTION. FIG. 2 SHOWS A FLASK ON THE LEFT CONTAINING A FILM OF ASPHALT 1A INOCULATED WITH *Mycobacterium ranae* AND ON THE RIGHT AN IDENTICAL FLASK WITHOUT THE TEST ORGANISM. AFTER INCUBATION

FIG. 2:--EFFECT OF *Mycobacterium ranae* ON ASPHALT 1A FILM. TEST FLASK ON LEFT, CONTROL FLASK ON RIGHT. INCUBATION 30°C FOR 1 MONTH.

AT 30°C FOR 1 MONTH THE FILM IN THE TEST FLASK HAS BROKEN AND SETTLED TO THE BOTTOM, WHEREAS THE CONTROL FILM IS UNCHANGED. EVEN MORE SIGNIFICANT IS THE APPEARANCE OF ASPHALT SAMPLES FROM EACH FLASK (FIG. 3). THE TEST ASPHALT IS DULL, ROUGH, CRUMBLY AND HAS LOST ITS USUAL RHEOLOGICAL CHARACTERISTICS, WHEREAS THE SAMPLE FROM THE CONTROL FLASK IS NOT CHANGED IN ITS CHARACTERISTICS.

HARRIS ET AL. (7) PERFORMED A SERIES OF EXPERIMENTS TO DETERMINE THE EFFECT OF VARIOUS BACTERIA ON PAVING ASPHALTS. THEY USED SOFTENING POINT, DUCTIBILITY AND PENETRATION MEASUREMENTS TO DETERMINE THE ACTION OF THE ORGANISMS AND CONCLUDED THAT EITHER A SOFTENING OR HARDENING OF THE ASPHALTS OCCURRED, DEPENDING ON THE NATURE OF THE BACTERIA PRESENT. IN ORDER TO

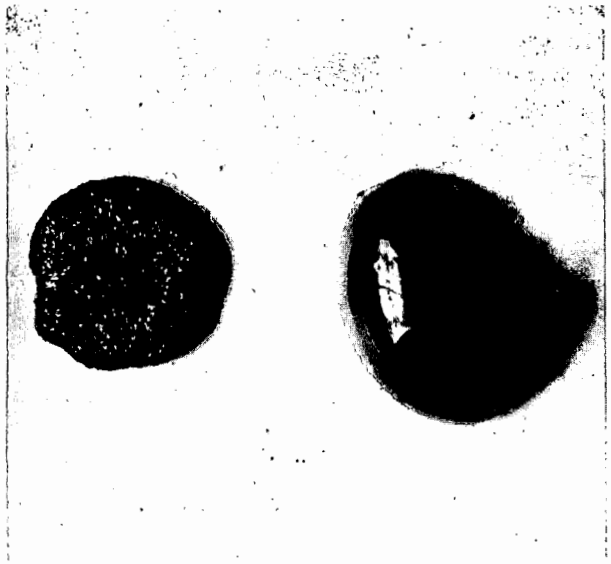


FIG. 3:--ASPHALT SAMPLES COLLECTED FROM CULTURE FLASKS. TEST FLASK ON LEFT, CONTROL FLASK ON RIGHT.

ACCURATELY ASSESS THE EFFECT OF THE ORGANISMS WE MUST ATTEMPT TO EXPOSE THE MAJOR PORTION OF THE ASPHALT TO MICROBIAL ACTION. AN EMULSION SYSTEM WAS USED AND THE ASPHALTS EXPOSED TO *Mycobacterium ranae* AND *Nocardia coeliaca* AT 30°C FOR 4 MONTHS. THE ASPHALTS WERE RECOVERED AND EXAMINED FOR CHANGES IN VISCOSITY. STERILE CONTROLS WERE TREATED IN THE SAME MANNER AS THE TEST FLASKS AND USED TO DETERMINE RELATIVE VISCOSITY OF ASPHALT DEGRADED BY THE MICROORGANISMS. TABLE II SHOWS THAT THE NET EFFECT OF THESE TWO ORGANISMS WAS TO INCREASE THE VISCOSITY OF THE THREE ASPHALTS TESTED. IT IS SIGNIFICANT THAT THE THREE ASPHALTS INVESTIGATED VARY CONSIDERABLY IN THEIR SUSCEPTIBILITY TO MICROBIAL ACTION (AS EVIDENCED BY THE VISCOSITY DATA). IT IS LOGICAL TO ASSUME THAT THIS EFFECT IS

TABLE II

RELATIVE VISCOSITY OF ASPHALTS AFTER MICROBIAL ACTION.

ASPHALT	RELATIVE VISCOSITY*	
	M. ranae	N. coeliaca
1A	2.0	1.4
3A	3.3	3.8
6A	-	6.8

$$*\text{RELATIVE VISCOSITY} = \frac{\text{VISCOSITY DEGRADED ASPHALT}}{\text{VISCOSITY CONTROL ASPHALT}}$$

CONTROL ASPHALTS NOT EXPOSED TO MICROBIAL ACTION. VISCOSITY CALCULATED AT 5×10^{-2} SEC^{-1} RATE OF SHEAR WERE APPROXIMATELY 500,000 POISES AT 25°C.

TIED IN WITH THE ACTION OF THE ORGANISMS ON SOME ASPHALT COMPONENT THAT IS SUSCEPTIBLE TO MICROBIAL ACTION AND IS ALSO RELATED TO THE RHEOLOGICAL CHARACTERISTICS OF THESE ASPHALTS.

THE RELATION BETWEEN ASPHALT COMPOSITION AND HARDENING IS NOT WELL DEFINED BUT SOME INFORMATION IS NOW AVAILABLE (8). CONSIDERABLE EVIDENCE HAS ACCUMULATED OVER THE YEARS WHICH SHOWS THAT ASPHALTS HARDEN WITH AGING AND WHEN SUBJECTED TO HEAT, OXYGEN, OR ACTINIC LIGHT. THIS HARDENING REDUCES THE ADHESIVENESS AND THEREFORE THE IN-SERVICE DURABILITY OF THE MATERIAL. THESE HARDENING EFFECTS CAN TO A LARGE EXTENT BE DUE TO OXIDATION OF CERTAIN ASPHALT COMPONENTS. THE EFFECT OF MICROBIAL ACTION ON THE ASPHALT WOULD BE EXPECTED TO INVOLVE PRIMARILY OXIDATION OF THOSE ASPHALT COMPONENTS UTILIZED FOR GROWTH BY THE MICROORGANISM.

TABLE III

GROWTH OF TEST ORGANISMS ON ASPHALT AND ASPHALT FRACTIONS.

ASSAY MATERIAL	*Ps.* 196Aa	*M. ranae*	*N. coeliaca*
ASPHALT 1A	-	+	+
ASPHALTENES 1A	-	+	+
ACETONE ELUATE 1A	-	+	+
BASE MATERIAL 1A	+	+	*
ASPHALT 3A	-	+	+
ASPHALTENES 3A	-	+	+
ACETONE ELUATE 3A	-	+	-
BASE MATERIAL 3A	+	+	*
ASPHALT 6A	-	+	+
ACETONE ELUATE 6A	-	+	-
BASE MATERIAL 6A	+	+	*

*NOT TESTED

TABLE III SUMMARIZES SOME PRELIMINARY DATA FROM STUDIES IN OUR LABORATORY ON THOSE FRACTIONS FROM THREE ASPHALTS UTILIZED FOR GROWTH BY SELECTED MICROORGANISMS. *Pseudomonas* 196Aa UTILIZES ONLY THE BASE MATERIAL FROM THE THREE ASPHALTS AND WILL NOT GROW ON THE UNFRACTIONATED ASPHALT. *Mycobacterium ranae*, WHICH WAS SHOWN TO DRASTICALLY MODIFY THE ASPHALT (VISCOSITY STUDIES), IS ABLE TO UTILIZE ALL FRACTIONS WHICH HAVE BEEN STUDIED. *Nocardia coeliaca*, WHICH ALSO HAS A DRASTIC EFFECT ON VISCOSITY, WILL UTILIZE ALL FRACTIONS TESTED EXCEPT THE ACETONE ELUATE. THE BASE MATERIAL HAS A CONSIDERABLE ALIPHATIC HYDROCARBON CONTENT, AS WILL BE POINTED OUT LATER. *Nocardia coeliaca* IS KNOWN TO GROW ON MANY OF THE n-ALKANES; THEREFORE IT WILL LIKELY UTILIZE THE BASE MATERIAL.

IN ORDER TO SIMPLIFY THE APPROACH TO THIS PROBLEM THE ASPHALTS WERE FRACTIONATED INTO NARROW FRACTIONS OF SOMEWHAT SIMILAR COMPOUNDS. THE ASPHALTENES (PENTANE INSOLUBLE) WERE SEPARATED FROM THE PETROLENES (PENTANE SOLUBLE) BY TREATMENT WITH N-PENTANE. THE PENTANE SOLUBLE PETROLENES WERE EXTRACTED WITH SULFURIC ACID FOLLOWED BY CHROMATOGRAPHIC FRACTIONATION ON A FLORISIL COLUMN. THE n-PENTANE ELUATE FROM THE FLORISIL COLUMN (BASE MATERIAL) WAS FURTHER FRACTIONATED BY THERMAL DIFFUSION TO PROVIDE NARROW FRACTIONATION OF THE LOWER MOLECULAR WEIGHT AND LESS POLAR PORTIONS OF THE ASPHALTS. ACETONE WAS USED TO FURTHER STRIP THE FLORISIL COLUMN AND REPRESENTS THE ACETONE ELUATE (TABLE III).

THE BASE MATERIAL AND 10 THERMAL DIFFUSION FRACTIONS HAVE RECEIVED THE MOST EXTENSIVE STUDY TO DATE. INFRARED SPECTROPHOTOMETRIC ANALYSIS AND MICROBIAL GROWTH STUDIES HAVE BEEN PERFORMED ON THESE FRACTIONS.

FIGS. 4, 5 AND 6 SHOW THE INFRARED SPECTRA OF THE BASE MATERIAL AND 10 THERMAL DIFFUSION FRACTIONS FROM EACH OF THE THREE ASPHALTS STUDIED (1A, 3A AND 6A). IT SHOULD BE POINTED OUT THAT FRACTION 1 IS FROM THE BOTTOM OF THE COLUMN AND FRACTION 10 FROM THE TOP. ALL BAND POSITIONS ARE REPORTED IN RECIPROCAL CENTIMETERS (CM^{-1}) AND EXPRESSED AS PEAK HEIGHTS RELATIVE TO THOSE INTENSITIES OF FRACTION 1. THE FRACTIONS FROM ASPHALT 1A HAVE CONSIDERABLY MORE ALIPHATIC CHARACTER THAN THOSE FROM THE OTHER TWO ASPHALTS. THERE IS CONSIDERABLE AROMATICITY PRESENT IN THE 1A AND 3A ASPHALT FRACTIONS. IT IS APPARENT EXCEPT FOR FRACTION 10 OF ASPHALT 1A, 3A AND 6A WHICH ARE PREDOMINATELY ALIPHATIC ALL THESE FRACTIONS ARE A MIXTURE OF ALIPHATIC AND AROMATIC COMPOUNDS.

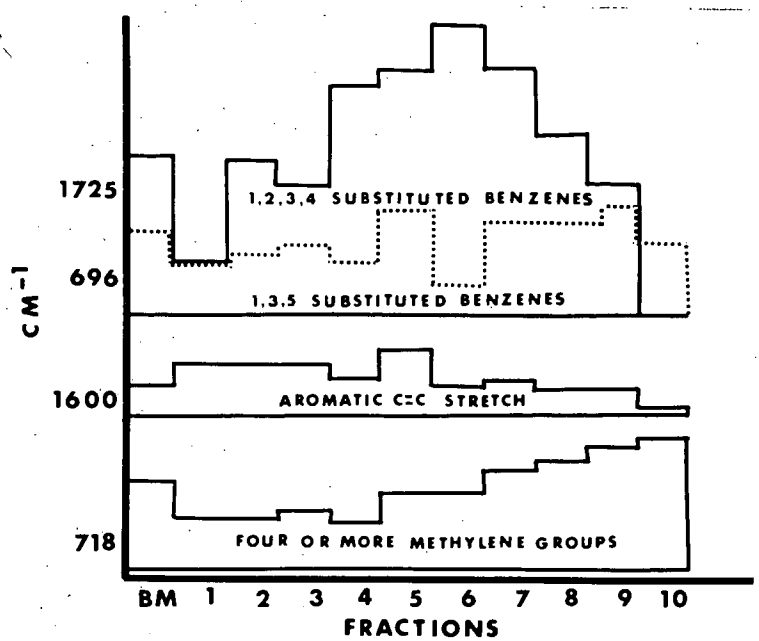
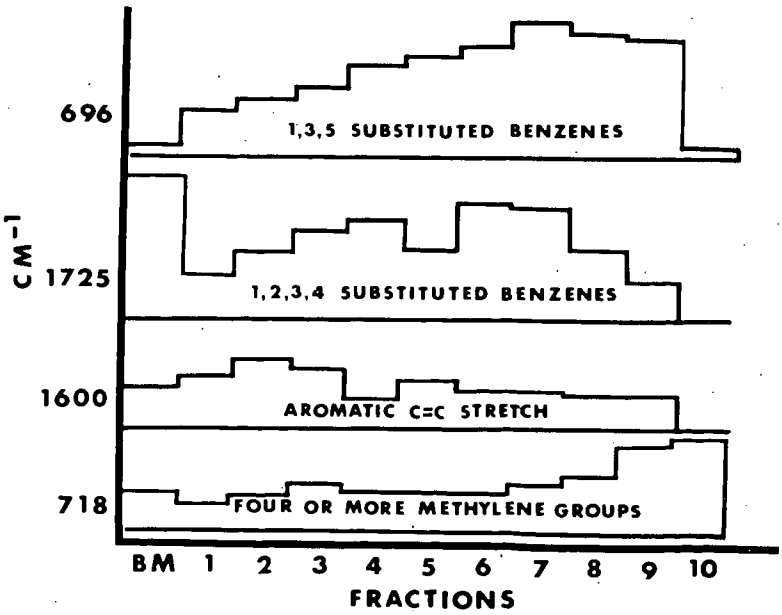


FIG. 4:--

INFRARED SPECTRA OF BASE MATERIAL AND THERMAL DIFFUSION FRACTIONS FROM ASPHALT 1A.

FIG. 5:--

INFRARED SPECTRA OF BASE MATERIAL AND THERMAL DIFFUSION FRACTIONS FROM ASPHALT 3A.



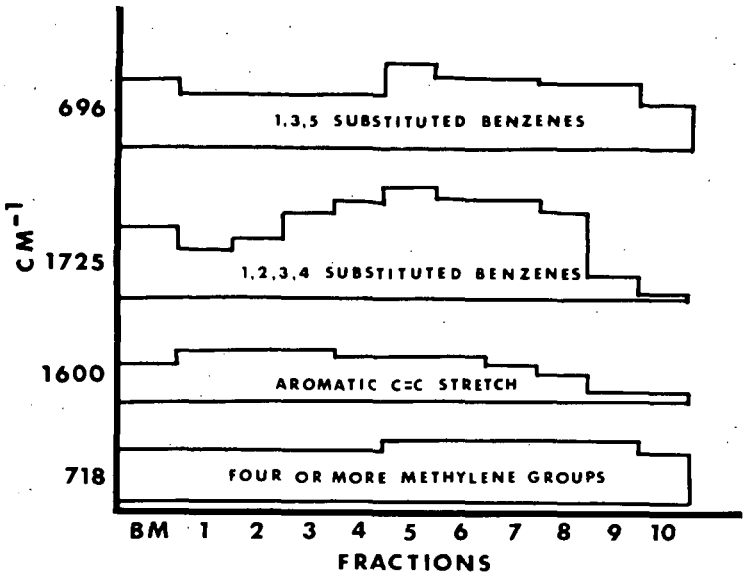


FIG. 6:--INFRARED SPECTRA OF BASE MATERIAL AND THERMAL DIFFUSION FRACTIONS FROM ASPHALT 6A.

EACH FRACTION WAS ASSAYED FOR GROWTH SUPPORTING ACTIVITY WITH *Mycobacterium ranae* AND *Pseudomonas* 196Aa. TABLE IV SHOWS THE MAXIMUM TURBIDITY DEVELOPING ON EACH FRACTION IN THE *Pseudomonas* 196Aa ASSAYS. *Pseudomonas* WILL GROW ONLY ON THE TOP FRACTIONS WHICH ARE DEMONSTRATED TO BE ALIPHATIC IN NATURE. THE INFRARED DATA, HOWEVER, DEMONSTRATES THE PRESENCE OF ALIPHATIC MATERIALS IN ALL THERMAL DIFFUSION FRACTIONS.

THE HYDROCARBON SPECIFICITY DATA (TABLE V) DEMONSTRATES THAT *Pseudomonas* 196Aa IS LIMITED TO THE UTILIZATION OF n-ALKANES (C-6 TO C-22), ALKENES (C-8, C-10, C-12 AND C-14) AS WELL AS CERTAIN OF THE FATTY ACIDS. THIS ORGANISM WAS INCAPABLE OF

TABLE IV

GROWTH OF *Pseudomonas* 196Aa ON THERMAL DIFFUSION FRACTIONS.

FRACTION SOURCE	MAXIMUM TURBIDITY										
	BM*	1	2	3	4	5	6	7	8	9	10
ASPHALT 1A	64	0	0	0	0	0	0	0	0	25	116
ASPHALT 3A	35	0	0	0	0	0	0	0	0	58	175
ASPHALT 6A	32	0	0	0	0	0	0	0	0	0	122

RESULTS IN KLETT UNITS AFTER 72 HRS. INCUBATION AT 32°C.

*BASE MATERIAL

TABLE V

SUBSTRATE SPECIFICITY OF *Pseudomonas* 196Aa.

n-ALKANES		FATTY ACIDS	
LEXANE	175	LEXANOIC	75
LEPTANE	195	LEPTANOIC	105
OCTANE	200	OCTANOIC	115
NONANE	229	NONANOIC	100
DECANE	200	DECANOIC	95
DODECANE	220	DODECANOIC	183
TRIDECANE	230	-	
TETRADECANE	260	TETRADECANOIC	180
HEXADECANE	260	HEXADECANOIC	190
OCTADECANE	200	OCTADECANOIC	260
EICOSANE	250	-	
DOCOSANE	220	BEHENIC	0
ALKENES		**AROMATIC**	
1-HEPTENE	0	BENZENE	0
1-OCTENE	210	ETHYLBENZENE	0
1-DECENE	178	PROPYLBENZENE	0
1-DODECENE	120	TRIMETHYLBENZENE	0
1-TETRADECENE	300	PENTAMETHYLBENZENE	0
1-HEXADECENE	0	HEXAMETHYLBENZENE	0
		XYLENES (O,M,P)	0
		TOLUENE	0

GROWTH ON ANY OF THE AROMATIC HYDROCARBONS TESTED. THE DECREASED GROWTH RESPONSE OF Pseudomonas 196Aa ON THE BASE MATERIALS FROM THE THREE ASPHALTS AND FRACTION 9 FROM ASPHALTS 1A AND 3A, AS WELL AS THE COMPLETE ABSENCE OF GROWTH ON FRACTION 9 OF ASPHALT 6A, IS DIFFICULT TO UNDERSTAND IF THE ORGANISM IS CAPABLE OF UTILIZING THE ALKANE SHOWN BY INFRARED ANALYSES TO BE PRESENT IN THESE MATERIALS (FIGS. 4, 5 AND 6). IT IS APPARENT THAT THE RATIO OF AROMATIC MATERIAL TO ALIPHATIC MATERIAL IS GREATER IN THOSE FRACTIONS NOT SUPPORTING THE GROWTH OF Pseudomonas 196Aa THAN THE FEW FRACTIONS WHICH DO SUPPORT GROWTH OF THIS ORGANISM. IT WOULD APPEAR, THEREFORE, THAT Pseudomonas 196Aa CAN UTILIZE THE n-ALKANES (OR ALKANE DERIVATIVES) PRESENT IN THE ASPHALT FRACTIONS, BUT THE ORGANISM IS INHIBITED IN THE PRESENCE OF SUBSTITUTED BENZENE COMPOUNDS.

THE RESULTS OF THE EXPERIMENT SHOWN IN TABLE VI CONFIRM THE INHIBITION OF n-ALKANE OXIDATION BY Pseudomonas 196Aa IN THE PRESENCE OF AROMATIC HYDROCARBONS. THE GREATER THE AROMATICITY OF THE COMPOUND, THE GREATER THE INHIBITION OF n-OCTANE UTILIZATION. BENZENE WAS SHOWN TO BE THE MOST INHIBITORY COMPOUND FOLLOWED BY TOLUENE, PROPYLBENZENE AND FINALLY TRIMETHYLBENZENE, WHICH IS NOT INHIBITORY. TRIMETHYLBENZENE APPARENTLY IS UTILIZED IN THE PRESENCE OF n-OCTANE, BUT NOT ALONE. THIS EFFECT HAS NOT BEEN FULLY INVESTIGATED AT THIS TIME BUT MAY REPRESENT A CO-OXIDATION EFFECT (9).

THE GROWTH RESPONSE PATTERN OF Mycobacterium ranae ON THE THERMAL DIFFUSION FRACTIONS (TABLE VII) IS COMPLETELY DIFFERENT FROM THE Pseudomonas PATTERN. THIS ORGANISM IS CAPABLE OF GROWTH ON ALL OF THE ASPHALT 6A FRACTIONS, AND MOST OF THE 3A AND 1A FRACTIONS. IT WOULD APPEAR THAT Mycobacterium ranae IS NOT AS SELECTIVE AS Pseudomonas 196Aa IN THE MATERIALS THAT IT CAN UTILIZE.

TABLE VI

EFFECT OF AROMATIC HYDROCARBONS ON n-OCTANE UTILIZATION BY *Pseudomonas* 196Aa.

AROMATIC HYDROCARBON	TURBIDITY IN KLETT UNITS 18 HRS.	20 HRS.
CONTROL	55	115
0.2% BENZENE	40	60
1.0% BENZENE	0	0
0.2% TOLUENE	115	160
0.2% PROPYLBENZENE	110	170
1.0% PROPYLBENZENE	5	15
2.0% PROPYLBENZENE	0	0
1.0% TOLUENE	0	0
0.2% TRIMETHYLBENZENE	180	200
1.0% TRIMETHYLBENZENE	115	140
2.0% TRIMETHYLBENZENE	90	115

2% n-OCTANE IN ALL FLASKS

TABLE VII

GROWTH OF *Mycobacterium ranae* ON THERMAL DIFFUSION FRACTIONS.

FRACTION SOURCE	MAXIMUM TURBIDITY										
	BM*	1	2	3	4	5	6	7	8	9	10
ASPHALT 1A	70	0	0	18	95	97	136	154	117	33	0
ASPHALT 3A	182	58	82	85	131	164	185	203	49	0	0
ASPHALT 6A	202	26	48	143	68	87	72	155	128	52	200

RESULTS IN KLETT UNITS AFTER 72 HRS. INCUBATION AT 32°C.

*BASE MATERIAL

EXAMINATION OF THE SPECIFICITY DATA OF THIS ORGANISM FOR PURE HYDROCARBONS (TABLE VIII) INDICATES IT IS NOT ONLY ABLE TO UTILIZE n-ALKANES (C-12 TO C-22) BUT ALSO SOME ALKENES, FATTY ACIDS AND ALL OF THE AROMATIC HYDROCARBON TESTED EXCEPT BENZENE.

TABLE VIII

SUBSTRATE SPECIFICITY OF *Mycobacterium ranae*.

n-ALKANES		FATTY ACIDS	
HEPTANE	0	HEPTANOIC	5
OCTANE	0	OCTANOIC	5
DECANE	0	DECANOIC	5
DODECANE	55	DODECANOIC	80
TRIDECANE	60	-	
TETRADECANE	60	TETRADECANOIC	125
HEXADECANE	60	HEXADECANOIC	65
OCTADECANE	60	OCTADECANOIC	170
EICOSANE	125	-	
DOCOSANE	75	BEHENIC	40
ALKENES		**AROMATIC**	
1-DODECENE	0	BENZENE	0
1-TETRADECENE	40	ETHYLBENZENE	95
1-HEXADECENE	85	PROPYLBENZENE	58
1-OCTADECENE	75	TRIMETHYLBENZENE	112
1-NONADECENE	75	XYLENES (O,M,P)	126-163
		TOLUENE	99

IT IS CONCLUDED FROM THIS DATA THAT *Mycobacterium ranae* IS UTILIZING BOTH AROMATIC AND ALIPHATIC HYDROCARBONS PRESENT IN THE THERMAL DIFFUSION FRACTIONS FROM THESE ASPHALTS, WHEREAS *Pseudomonas* 196Aa IS ABLE TO GROW ON ONLY THE n-ALKANES PRESENT IN THE FRACTIONS. A FURTHER POINT IS THAT *Pseudomonas* 196Aa IS NOT ABLE TO UTILIZE THE n-ALKANE THAT ARE KNOWN TO BE PRESENT (INFRARED DATA) WHEN THE CONCENTRATION OF AROMATIC HYDROCARBON IS HIGH. THIS IN TURN WOULD ACCOUNT FOR THE ABILITY OF

Mycobacterium ranae TO DEGRADE THE UNFRACTIONATED ASPHALT, WHEREAS *Pseudomonas* 196Aa IS UNABLE TO MODIFY THE THREE ASPHALTS. THIS DATA WOULD FURTHER INDICATE THAT *Mycobacterium ranae* MODIFIES THE AROMATIC HYDROCARBON PRESENT IN THE ASPHALTS, WITH BUT MINOR ACTION ON THE SATURATED HYDROCARBONS.

SUMMARY

WE HAVE DEMONSTRATED THAT THE ORGANISMS CAPABLE OF ATTACKING BITUMENS ARE DIVERSE AND DO NOT FALL INTO A SPECIFIC TAXONOMIC GROUP. THE MICROBIAL ACTION OF BITUMENS SHOWS A SPECIFICITY THAT INVOLVES BOTH THE MICROORGANISM AND THE BITUMINOUS SUBSTRATE. SOIL BURIAL TESTS DEMONSTRATE THE MOISTURE REQUIREMENT FOR MICROBIAL ACTION AS WELL AS DIFFERENCES IN THE SUSCEPTIBILITY OF BITUMINOUS MATERIALS.

IN A LIMITED SERIES OF EXPERIMENTS IT WAS FOUND THAT THE NET EFFECT OF MICROBIAL ACTION ON THREE ASPHALTS WAS AN INCREASE IN THE VISCOSITY OF THE ASPHALTS. IT IS THEORIZED THAT THE INCREASE IN VISCOSITY IS DUE TO THE OXIDATION OF CERTAIN ASPHALT COMPOUNDS WHICH ARE ALSO IMPORTANT IN DETERMINING THE RHEOLOGICAL CHARACTERISTICS OF THE ASPHALTS.

PRELIMINARY STUDIES ON THE COMPONENTS OF ASPHALTS UTILIZED BY MICROORGANISMS INDICATE THAT BOTH THE AROMATIC AND SATURATED HYDROCARBONS ARE UTILIZED BY *Mycobacterium ranae*. THIS ORGANISM CAUSED A DRASTIC MODIFICATION OF THE VISCOSITY OF THREE ASPHALTS. IT WOULD APPEAR THAT THIS MODIFICATION IN RHEOLOGICAL CHARACTERISTICS IS CAUSED BY OXIDATION OF AROMATIC AND SATURATED HYDROCARBONS IN THE PETROLENE (PENTANE SOLUBLE MATERIAL) FRACTION OF THESE ASPHALTS. FURTHER STUDIES ARE TO BE CONDUCTED ON THE ASPHALTENES AND OTHER FRACTIONS FROM THESE THREE ASPHALTS.

ACKNOWLEDGMENTS

THIS WORK WAS SUPPORTED IN PART BY GRANT 907-A2 FROM THE PETROLEUM RESEARCH FUND OF THE AMERICAN CHEMICAL SOCIETY. THE AUTHOR ALSO WISHES TO EXPRESS APPRECIATION TO R. N. TRAXLER, TEXAS TRANSPORTATION INSTITUTE FOR THE ASPHALTS AND ASPHALT FRACTIONS USED IN THIS INVESTIGATION, AND FOR THE INFRARED DATA ON THE THERMAL DIFFUSION FRACTIONS.

REFERENCES

1. HARRIS, J. O. 1959. PIPELINE PROTECTIVE COATING MATERIALS AS GROWTH SUBSTRATES FOR SOIL MICROORGANISMS. TRANS. KANSAS ACAD. SCI., 62: 42-46.

2. BURGESS, S. J. 1956. ACTION OF MICROORGANISMS ON PETROLEUM ASPHALT FRACTIONS. HIGHWAY RES. BOARD BULL. NO. 118.

3. HARRIS, J. O., KLINE, R. M., AND CRUMPTON, C. F. 1956. A STUDY OF THE PRESENCE OF HYDROCARBON UTILIZING BACTERIA AT THE SOIL-ASPHALT INTERFACE OF KANSAS HIGHWAYS. TRANS. KANSAS ACAD. SCI., 59: 495-499.

4. PHILLIPS, U. A. AND TRAXLER, R. W. 1963. MICROBIAL DEGRADATION OF ASPHALT. APPLIED MICROBIOL., 11: 235-238.

5. TRAXLER, R. W. 1962. MICROBIAL DEGRADATION OF ASPHALT. BIOTECH. AND BIOENG. IV: 369-376.

6. HARRIS, J. O. 1960. BACTERIAL ACTIVITY AT THE BOTTOM OF BACK-FILLED PIPELINE DITCHES. CORROSION 16: 131-136.

7. HARRIS, J. O., WORLEY, H. E., MCCASKILL, G. A. AND BALDWIN, L. K. 1958. PRELIMINARY STUDIES OF THE EFFECT OF MICROORGANISMS ON THE PHYSICAL PROPERTIES OF ASPHALT. TRANS. KANSAS ACAD. SCI., 61: 110-113.

8. TRAXLER, R. N. 1961. RELATION BETWEEN ASPHALT COMPOSITION AND HARDENING BY VOLATILIZATION AND OXIDATION. A PAPER PRESENTED AT THE A.A.P.T. MEETING, CHARLESTON, SOUTH CAROLINA, FEBRUARY 6-8.

9. DAVIS, J. B., AND RAYMOND, R. L. 1961. OXIDATION OF ALKYL-SUBSTITUTED CYCLIC HYDROCARBONS BY A *Nocardia* DURING GROWTH ON n-ALKANES. APPLIED MICROBIOL., 9: 383-388.