# ASPHALT OXIDIZING BACTERIA OF THE SOIL

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THE SOIL MUST BE CONSIDERED AS THE NATURAL HABITAT FOR HYDROCARBON UTILIZING BACTERIA. IN DETERMINING THE POSSIBLE ROLE THAT BACTERIA MIGHT PLAY IN DECOMPOSITION OF ASPHALT OR OTHER COMPLEX ORGANIC MATTER, THE ENVIRONMENT OF THE SOIL MUST RECEIVE CAREFUL ATTENTION.

SOIL CONDITIONS AT THE BOTTOM OF THE BACK-FILLED PIPELINE DITCH HAVE BEEN EXTENSIVELY STUDIED AND HAVE BEEN FOUND TO BE QUITE FAVORABLE FOR MICROBIAL DEVELOPMENT (3). THE DATA INDICATED HIGHER WATER CONTENT AT DITCH BOTTOM WHEN COMPARED WITH LESS COMPACT SOIL OR SEMI-DENSE AREAS IN THE BACK-FILL ABOVE THE PIPELINE, ALLOWING AEROBIC CONDITIONS.

SINCE HYDROCARBON BACTERIA, CAPABLE OF UTILIZING ASPHALT AS THEIR SOLE FOOD ENERGY SOURCE, WERE FOUND IN ALL LOCATIONS WITH WIDELY VARIED GEOGRAPHICAL AND CLIMATIC CONDITIONS (4), THE PRESENCE OF THESE HYDROCARBON UTILIZING TYPES IN NORMAL SOILS RAISES IMPORTANT ECOLOGICAL PROBLEMS AS TO THEIR NICHE IN NATURALLY OCCURRING ORGANIC TRANSFORMATIONS. THE DATA TO BE REPORTED DEAL WITH ASPHALT UTILIZING BACTERIA IN NORMAL SOILS RELATIVE TO THE TOTAL MICROBIAL POPULATION, THEIR NUMBERS AND TYPES IN SOILS WITH ASPHALT PRESENT AND, FINALLY, PROTECTIVE COATING FAILURE DUE TO MICROBIAL ACTIVITY.

## MATERIALS AND METHODS

ASPHALTIC PROTECTIVE COATINGS USED TO STUDY BACTERIAL UTILIZATION WERE ALL COMMERCIALLY-AVAILABLE PRODUCTS.

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PRECAUTIONS IN HEATING AND APPLICATION GIVEN BY MANUFACTURERS WERE OBSERVED IN ALL INSTANCES. ROUTINE BACTERIOLOGICAL EXAMINATIONS FOLLOWED STANDARD LABORATORY PROCEDURES (6) WITH NUTRIENT AGAR, JENSEN'S AGAR, AND MARTIN'S ROSE BENGAL AGAR USED FOR PLATE COUNTS OF "TOTAL" BACTERIA, ACTINOMYCETES, AND FILAMENTOUS FUNGI RESPECTIVELY. INCUBATION WAS FOR 5 DAYS AT 30° C. ESTIMATION OF HYDROCARBON UTILIZING BACTERIA WAS ACCOMPLISHED USING SERIAL DILUTIONS OF SOIL INTO A MINERAL-SALTS DODECANE LIQUID MEDIUM WITH TEN REPLICATES PER DILUTION (4).

OXYGEN UPTAKE BY SOILS FOLLOWED REGULAR WARBURG MANOMETRIC TECHNIQUES (7). FOUR GRAMS OF SOIL WERE USED PER RESPIRATION VESSEL, WITH MOISTURE CONTENT ADJUSTED TO 55% OF FIELD CAPACITY.

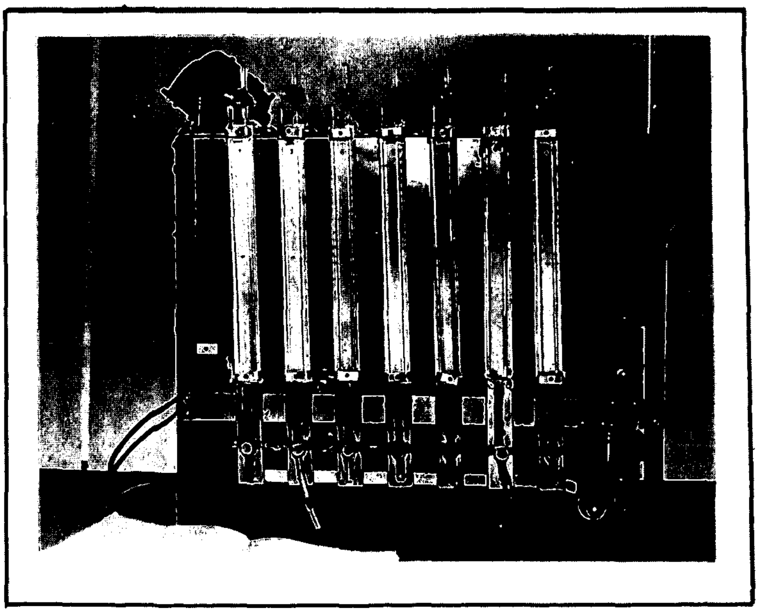

Fig. 1:--The Warburg microrespirometer shown here was used to show rapid oxidation of hydrocarbons by normal soil microorganisms.

## RESULTS

CULTIVATED SOILS AND NON-CULTIVATED SOILS, SUCH AS PASTURES OR FORESTS, HAVE THEIR OWN CHARACTERISTIC MICROBIAL POPULATIONS. WITHOUT RIGOROUS IDENTIFICATION OF PURE CULTURES OR EXTENSIVE USE OF SPECIALIZED TECHNIQUES, SOIL POPULATIONS CAN BE EXPRESSED AS PLATE COUNTS OF BACTERIA, ACTINOMYCETES, AND FUNGI (6). ALTHOUGH THIS PROCEDURE GIVES ONLY RELATIVE INFORMATION, IT DOES GIVE INSIGHT AS TO THE OVER-ALL MICROBIAL ACTIVITY.

TABLE I

PLATE COUNT DATA OF

| SOIL | TOTAL BACTERIA (MILLIONS/GM/SOIL) | ACTINOMYCETES (MILLIONS/GM) | FUNGI (THOUSANDS) | HYDROCARBON BACTERIA (PER GRAM) |
|---|---|---|---|---|
| 1 | 50 | 6 | 390 | 870 |
| 2 | 14 | 2 | 123 | 23,000 |
| 3 | 179 | 45 | 360 | 1,160 |
| 4 | 44 | 11 | 20 | 1,300 |
| 5 | 65 | 4 | 48 | 223 |
| 6 | 24 | 5 | 330 | 5,420 |

PLATE COUNTS OF SIX NORMAL SOILS SHOW HYDROCARBON UTILIZING BACTERIA ARE A SIGNIFICANT PROPORTION OF THE TOTAL MICROBIAL POPULATION.

TABLE I SHOWS PLATE COUNT DATA FOR SIX NORMAL SOILS FROM NORTH CENTRAL KANSAS IN COMPARISON TO THE NUMBERS OF HYDROCARBON UTILIZING BACTERIA. THE COUNTS WERE MADE ON NON-FROZEN SURFACE SOIL IN FEBRUARY AND THUS REPRESENT THE EQUILIBRIUM SITUATION FOR WINTER WEATHER. OVER-ALL POPULATIONS WOULD BE HIGHER IN WARMER SOIL. BACTERIA CAPABLE OF GROWTH ON DECANE WERE PRESENT IN NUMBERS VARYING FROM 223 TO 23,000 PER GRAM

OF SOIL. IT SHOULD BE EMPHASIZED THAT NONE OF THESE SOILS HAD BEEN EXPOSED TO HYDROCARBONS WHICH WOULD CAUSE A SELECTIVE PRESSURE FOR THE DEVELOPMENT OF SPECIALIZED BACTERIA.

KESTER (5) PROPOSED THAT MOST, IF NOT ALL, ACTINOMYCETES CAN UTILIZE HYDROCARBONS. THE HIGH POPULATIONS OF THIS GROUP SHOWN IN TABLE I WOULD INDICATE THESE NORMAL SOILS WOULD HAVE A HIGH CAPACITY FOR THE UTILIZATION OF HYDROCARBONS. THE LIQUID SELECTIVE CULTURE USED TO ESTIMATE BACTERIAL GROWTH ON DODECANE WAS NOT FAVORABLE FOR ACTINOMYCETE DEVELOPMENT.

MARKED SEASONAL VARIATIONS OCCUR WITHIN THE MICROBIAL POPULATIONS IN THE UPPER LAYERS OF SOIL. TOTAL BACTERIA AND HYDROCARBON BACTERIA WERE DETERMINED DURING THE SPRING OF 1964 WHEN SOIL TEMPERATURES WERE GRADUALLY INCREASING, TO LEARN IF HYDROCARBON UTILIZERS COMPRISED A RATHER UNIFORM PERCENTAGE OF THE POPULATION AND WHETHER THIS GROUP RESPONDED TO MORE FAVORABLE GROWTH TEMPERATURES. POPULATION ESTIMATES

TABLE II

PLATE COUNT DATA OF

HC: HYDROCARBON
T: THOUSANDS
M: MILLIONS

| DATE | PASTURE SOIL | | WHEAT FIELD | | GARDEN SOIL | |
|---|---|---|---|---|---|---|
| | BACTERIA (M) | HC TYPES (T) | BACTERIA (M) | HC TYPES (T) | BACTERIA (M) | HC TYPES (T) |
| | PER GRAM | | PER GRAM | | PER GRAM | |
| MAR. 21 | 36 | 10 | 32 | 2 | 19 | 60 |
| " 28 | 58 | 38 | 48 | 6 | 73 | 81 |
| APR. 4 | 76 | 41 | 53 | 18 | 79 | 96 |
| " 18 | 120 | 67 | 98 | 33 | 189 | 256 |
| MAY 16 | 134 | 175 | 89 | 39 | 546 | 976 |
| " 23 | 138 | 162 | 94 | 41 | 518 | 849 |

COMPARISON OF TOTAL BACTERIAL COUNTS WITH HYDROCARBON (HC) BACTERIA IN THREE SOILS AT SIX DATES DURING SPRING 1964.

FOR SIX DATES ARE SHOWN IN TABLE II FOR THREE TYPICAL SOILS. IN EACH SOIL HYDROCARBON TYPES INCREASED AS THE SOIL TEMPERATURES BECAME MORE FAVORABLE FOR GROWTH. THIS INCREASE IN POPULATION DUE TO TEMPERATURE (NOT SELECTIVE FOOD) INDICATES THAT THE ABILITY TO OXIDIZE PARAFFINIC MOLECULES SHOULD BE CONSIDERED A GENERAL RATHER THAN A SPECIALIZED PHYSIOLOGICAL CAPABILITY OF SOIL MICROORGANISMS (3).

FURTHER EVIDENCE THAT THE NORMAL SOIL POPULATION WAS ABLE TO OXIDIZE HYDROCARBONS RAPIDLY AND WITHOUT ADAPTATION CAME FROM SOIL RESPIRATION EXPERIMENTS. OXYGEN UPTAKE RATES WERE MEASURED OVER A 6-HOUR PERIOD IN WARBURG MICRORESPIROMETERS WITH 4 GRAMS OF SOIL TO WHICH WATER ONLY, 1% GLUCOSE, AND 1% DODECANE HAD BEEN ADDED.

COMPARISONS OF OXIDATIVE ACTIVITY ARE SHOWN IN FIG. 2.

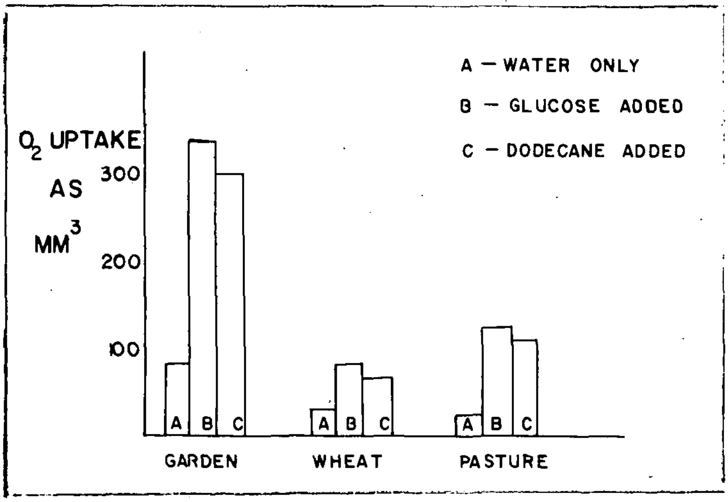


Fig. 2:--One per cent glucose, dodecane, or only water was added to normal soils. Total respiration as $mm^3$ in six-hours interval shows dodecane readily oxidized by the normal soil population.

OXYGEN UPTAKE RATES FOR DODECANE ARE NEARLY AS RAPID AS THOSE FOR GLUCOSE. INDIVIDUAL SOIL DIFFERENCES ARE APPARENT. DATA IN FIG. 2 WERE FROM THE SAME SOIL SAMPLES AS SHOWN FOR MAY 16 IN TABLE II.

THUS THE RICH GARDEN SOIL WITH HIGHEST POPULATIONS GAVE THE HIGHEST OXYGEN UPTAKE RATES WITH BOTH SUBSTRATES TESTED. LIMITED DATA OF THIS TYPE HAS SHOWN THAT SOIL IN CONTACT WITH FAILING ASPHALT ROADS HAS EXTREMELY HIGH OXIDATIVE RATES (4). FURTHER RESEARCH ON RESPIRATION OF SOIL MAY GIVE VALUABLE INFORMATION REGARDING THE ROLE OF MICROORGANISMS IN DETERIORATION OF COMPLEX ORGANIC MOLECULES.

EARLIER PUBLICATIONS HAVE SHOWN QUITE HIGH POPULATIONS OF BACTERIA ASSOCIATED WITH FAILING ASPHALT ROADS (1) AND HAVE GIVEN EXTENSIVE DATA REPORTING HIGH BACTERIAL COUNTS IN SOIL NEAR PIPELINES (2). FROM FIELD INVESTIGATIONS ALONG OPERATING OIL AND GAS PIPELINES, DATA HAVE BEEN TAKEN FROM ONE HUNDRED BELL-HOLE INSPECTIONS OF ASPHALT COATED LINES. (ABNORMAL OR HIGHLY CORROSIVE SITUATIONS SUCH AS RIVER CROSSINGS, SWAMPS, LEAK SITES OR HIGH SALT SOIL WERE NOT INCLUDED.)

TABLE III COMPARES POPULATIONS OF BACTERIA CAPABLE OF GROWTH ON ASPHALT PRESENT IN SOIL ADJACENT TO THE ASPHALT COATING WITH THE NORMAL POPULATION IN UNDISTURBED VIRGIN SOIL OF THE RIGHT-OF-WAY (APPROXIMATELY 15 FT. TO 20 FT. FROM THE CENTER OF THE BACK-FILLED DITCH) AT THE SAME DEPTH. WITH THE NORMAL SOIL OVER 65% OF THE LOCATIONS HAD LESS THAN 100 HYDROCARBON BACTERIA, ONLY 2% HAD A COUNT OVER 1,000 AND NONE EXCEEDED ONE HUNDRED THOUSAND. WITH AVAILABLE FOOD FROM THE ASPHALT COATING, 34% HAD COUNTS BETWEEN TEN AND ONE HUNDRED THOUSAND, WHILE 10% HAD COUNTS IN EXCESS OF 100,000.

TABLE III

| HYDROCARBON BACTERIA PER GRAM OF SOIL | UNDISTURBED SOIL IN RIGHT-OF-WAY AT PIPELINE DEPTH | SOIL ADJACENT TO ASPHALT COATING |
|---|---|---|
| LESS THAN 100 | 65% | 10% |
| 100 TO 1,000 | 21% | 21% |
| 1,000 TO 10,000 | 12% | 25% |
| 10,000 TO 100,000 | 2% | 34% |
| MORE THAN 100,000 | 0% | 10% |

PERCENTAGES OF ONE HUNDRED ASPHALT COATED PIPELINES SHOWING VARIOUS POPULATION DISTRIBUTIONS OF ASPHALT UTILIZING BACTERIA.

THE KANSAS STATE UNIVERSITY RESEARCH PROJECT, ON WHICH THIS INFORMATION IS BASED, WAS STARTED IN 1956...WITH FIELD TRIPS EACH YEAR SINCE THEN TO EXAMINE MORE THAN 1,000 BELL-HOLE OPENINGS ALONG OPERATING OIL, GAS AND WATER PIPELINES.

THESE BELL-HOLE OPENINGS WERE DUG AND MADE AVAILABLE BY THE PERSONNEL OF THE COOPERATING PIPELINE COMPANIES. SOIL SAMPLES WERE ANALYZED IN THE LABORATORY FOR BACTERIAL AND MOISTURE CONTENT AT DIFFERENT LEVELS OF THE BACK-FILLED DITCH, PLUS pH VALUES, AND THE IDENTIFICATION OF SOILS BY TYPE.

THE INFORMATION AND DATA ACCUMULATED DEMONSTRATES THAT BECAUSE OF THE MIXING OF SOILS DURING THE BACK-FILLING OPERATION (AFTER THE PIPE IS LOWERED-IN), THE BACK-FILLED DITCH BECOMES A MAN-MADE ENVIRONMENT, WHICH PROVIDES IDEAL CONDITIONS FOR BACTERIAL DEVELOPMENT AND ALSO FOR THE ACCUMULATION OF SOIL WATER. COMPARING THE ANALYSES OF SOIL SAMPLES TAKEN AT THE SAME DEPTHS IN THE VIRGIN SOIL OF THE RIGHT-OF-WAY AND IN THE BACK-FILLED DITCH PROVES THAT THERE

MUST BE TAKEN INTO CONSIDERATION THE DIFFERENCE IN THE BACTERIAL POPULATIONS AND SOIL WATER CONTENT, BOTH OF WHICH ARE MANY TIMES LESS IN THE RIGHT-OF-WAY THAN IS FOUND IN A PIPELINE DITCH.

SAMPLES OF MANY TYPES OF COATING SYSTEMS WERE REMOVED FROM THE PIPE AND ANALYZED IN THE LABORATORY FOR MOISTURE AND BACTERIAL CONTENT. DATA WAS RECORDED INDICATING THE CONDITION OF THE BOND OF THE COATINGS, CONDITION OF PIPE SURFACE (RE CORROSION, RUSTING AND/OR PITTING), YEARS OF UNDERGROUND SERVICE, USE OF CATHODIC PROTECTION, AND OTHER PERTINENT INFORMATION FOR FINAL EVALUATION PURPOSES.

DURING THE FIELD TRIPS OF THIS COAST-TO-COAST SURVEY IT WAS APPARENT THAT LABORATORY EXAMINATION AND ANALYSES OF THE COATINGS MUST BE MADE TO DETERMINE THE AMOUNT OF BACTERIAL AND MOISTURE PENETRATION IN THE COATINGS, FROM THE SOIL SURROUNDING THE COATED PIPE. DATA HAS BEEN TABULATED (TABLE IV) TO SHOW THE ANALYSES OF THE ASPHALT COATINGS SECURED IN REPRESENTATIVE GEOGRAPHICAL AREAS OF THE UNITED STATES.

FURTHERMORE, THE FIELD DATA, TOGETHER WITH CONSULTATIONS WITH CORROSION ENGINEERS OF THE COOPERATING OIL AND GAS PIPELINES, LED TO THE CONCLUSION THAT BACTERIAL NUMBERS, WATER PENETRATION INTO THE COATING, WATER IN THE SOIL NEXT TO THE PIPE, YEARS OF EXPOSURE TO THE SOIL AND IN DIFFERENT SOIL TYPES MUST BE CONSIDERED. A COMBINATION OF ALL OF THESE IMPORTANT FACTORS DETERMINE COATING PERFORMANCE.

THE DATA IN TABLE IV SHOWS THE BACTERIAL COUNT PER GRAM EACH OF TWO ASPHALT COATING SAMPLES THAT WERE REMOVED FROM OPERATING PIPELINES FOR COMPARATIVE PURPOSES AND SHOWS THE PENETRATION OF THE BACTERIA INTO THE COATING SAMPLES. (SEE THE COUNTS TABULATED UNDER SAMPLE #2.) ALSO INCLUDED IS A

TABULATION OF MOISTURE CONTENT DETERMINED (BY THE DEAN-STARK A.S.T.M. METHOD) ON THE SAME TWO COATING SAMPLES.

TAKING ONLY ONE COATING SAMPLE WOULD NOT REVEAL ANY PENETRATION IN THE PORTION OF THE COATING FILM NEXT TO THE PIPE SURFACE. THEREFORE A TECHNIQUE WAS DEVELOPED FOR TAKING COATING SAMPLE #2, BY REMOVING THE OUTER HALF OF THE COATING.

COATING SAMPLE #1: TOTAL SAMPLES WERE REMOVED, AS FOUND ON THE PIPE WHEN UNCOVERED.

COATING SAMPLE #2: THIS SAMPLE WAS SECURED AFTER APPROXIMATELY ONE-HALF (1/2) OF THE THICKNESS OF THE ORIGINAL COATING SYSTEM WAS REMOVED BY SKIVING (WITH DRAW KNIFE). THEN THIS SAMPLE WAS REMOVED FROM THE PIPE. THIS METHOD WAS DEVELOPED TO ELIMINATE: ANY TRAPPED SOIL OR ORGANIC MATTER IN WRINKLES DUE TO SOIL STRESS, IRREGULAR SURFACES, ETC....ALSO TO ELIMINATE WRAPPING MATERIALS THAT CONTAIN ORGANIC SUBSTANCES WHICH ARE SUBJECT TO MICROBIAL GROWTH.

## TABLE IV

| ASPHALT COATING | | | | % WATER PENETRATION COATING | | Years Underground Service | % Soil Moisture Next to Pipe |
|---|---|---|---|---|---|---|---|
| Sample No. 1 | | Sample No. 2 | | | | | |
| Aerobes | Anaerobes | Aerobes | Anaerobes | Sample No. 1 | Sample No 2 | | |
| **CENTRAL OHIO** | | | | | | | |
| 32,000 | None | 4,100 | None | 1.6 | 1.6 | 3 | 23.4 |
| 38,000 | 1,100 | 8,600 | +300 | 1.6 | 1.6 | 3 | 28.3 |
| 380,000 | 12,000 | 36,000 | 800 | 1.8 | 1.8 | 3 | 19.4 |
| 420,000 | 17,000 | 19,000 | 11,000 | 1.6 | 1.6 | 3 | 52.1 |
| 18,000 | 2,100 | 2,940* | 350* | 1.8 | 1.8 | 7 | 32.8 |
| **PENNSYLVANIA** | | | | | | | |
| 4,800,000 | 89,000 | 2,600,000* | 42,000* | 7.6 | 5.9 | 6 | 44.7 |
| **CENTRAL KANSAS** | | | | | | | |
| 4,300,000 | 23,000 | 600,000* | 19,000* | 16.0 | 12.1 | 18 | 19.3 |
| 440,000 | 31,000 | 73,000* | 11,000* | 14.9 | 10.9 | 18 | 22.0 |
| **NEBRASKA** | | | | | | | |
| 190,000 | 1,000 | 84,000* | 1,100* | 15.2 | 11.6 | 12 | 33.6 |
| 1,080,000 | 34,000 | 63,000* | 13,000* | 14.6 | 11.9 | 20 | 19.8 |
| 590,000 | 3,000 | 410,000* | +200* | 12.4 | 11.2 | 21 | 31.6 |
| **OKLAHOMA** | | | | | | | |
| 1,500,000 | 400,000 | 3,000* | +100* | 11.2 | 7.1 | 9 | 25.0 |
| 1,250,000 | 280,000 | 78,000 | 19,500 | 12.1 | 9.3 | 9 | 29.0 |
| **MISSISSIPPI** | | | | | | | |
| 2,500 | +300 | 600* | +200* | 1.5 | 1.5 | 5 | 27.2 |
| 5,000 | +100 | 1,700* | +100* | 1.5 | 1.5 | 5 | 15.2 |
| 15,500 | +100 | +200* | +100* | 1.5 | 1.5 | 5 | 26.6 |
| 1,500 | +100 | 1,000* | +100* | 3.0 | 3.0 | 5 | 30.0 |
| **SOUTH TEXAS** | | | | | | | |
| 1,400 | None | 800 | None | 1.8 | 1.8 | 6 | 10.3 |
| 24,000 | 1,000 | 5,000 | 600 | 2.2 | 2.2 | 6 | 23.4 |
| 12,000 | None | 8,000* | None* | 9.3 | 9.2 | 12 | 33.3 |
| **NORTHWEST TEXAS** | | | | | | | |
| 30,000 | 1,300 | 11,000* | 5,000* | 19.5 | 15.7 | 10 | 23.6 |
| 58,000 | 6,000 | 109,000* | 600* | 18.0 | 18.0 | 10 | 29.2 |
| 6,100 | 6,000 | 1,100* | 4,500* | 19.0 | 19.0 | 9 | 32.5 |
| 88,000 | 4,700 | 9,900* | 3,900* | 4.3 | 4.2 | 12 | 11.2 |
| 100,000 | 4,200 | 18,000* | 2,900* | 8.0 | 8.2 | 9 | 22.0 |
| 106,000 | 20,000 | 107,000* | 3,900* | 9.3 | 9.2 | 12 | 27.2 |
| 1,330,000 | 2,400 | 72,000* | 1,700* | 19.5 | 15.3 | 11 | 37.0 |
| **SOUTHWEST TEXAS** | | | | | | | |
| 50,000 | 300 | 160,000* | 3,300* | 14.2 | 13.6 | 10 | 41.6 |
| 39,000 | 500 | 43,000* | 200* | 11.6 | 11.6 | 10 | 28.6 |
| 420,000 | 6,000 | 516,000* | 3,000* | 12.8 | 11.8 | 9 | 39.6 |
| 146,000 | 18,000 | 304,000* | 28,000* | 5.2 | 3.2 | 7 | 28.3 |
| 300,000 | 43,000 | 180,000* | 41,000* | 18.0 | 15.8 | 16 | 19.4 |
| **FAR WEST TEXAS** | | | | | | | |
| 290,000 | 12,000 | 180,000* | 9,000* | 18.0 | 18.0 | 12 | 21.0 |
| 267,000 | 69,000 | 210,000* | 51,000* | 19.0 | 19.0 | 11 | 22.0 |
| 4,100,000 | 360,000 | 390,000* | 410,000* | 8.0 | 8.0 | 4 | 14.9 |
| **SOUTHERN CALIFORNIA** | | | | | | | |
| 1,148,000 | 1,300 | 510,000* | 300* | 4.3 | 4.2 | 7 | 9.9• |
| 320,000 | 7,000 | 95,000* | 2,000* | 14.1 | 10.9 | 12 | 21.9 |
| 3,500 | 2,000 | 2,000* | 1,500* | 16.2 | 12.2 | 19 | 25.3 |
| 78,000 | +100 | 15,000* | +100* | 9.8 | 9.9 | 16 | 4.2• |
| 2,800,000 | 17,000 | 2,600,000* | 19,000* | 7.6 | 7.6 | 15 | 11.6• |
| 3,400,000 | 20,000 | 4,800,000* | 18,000* | 9.3 | 9.2 | 7 | 13.7• |
| **CENTRAL CALIFORNIA** | | | | | | | |
| 24,000 | 2,000 | 3,500* | 2,000* | 5.3 | 5.1 | 7 | 5.5 |
| 9,500 | 3,000 | 8,000* | 3,500* | 4.1 | 5.1 | 10 | 2.8• |
| 3,600,000 | 6,000 | 3,000,000* | 6,000* | 3.1 | 3.1 | 9 | 7.0 |
| 4,000,000 | 6,000 | 1,900,000* | 5,000* | 6.1 | 6.1 | 20 | 3.1• |
| 75,000 | 4,000 | 30,000* | 1,000* | 8.1 | 8.1 | 7 | 4.2 |
| 430,000 | 4,000 | 40,000* | 7,000* | 14.3 | 10.7 | 11 | 26.6 |
| **UTAH** | | | | | | | |
| 8,380,000 | 17,000 | 1,740,000* | 21,000* | 6.6 | 6.6 | 7 | 12.4 |
| 1,480,000 | 1,000 | 184,000* | 500* | 11.8 | 11.4 | 18 | 12.8 |
| 58,000 | +100 | 30,000* | +100* | 14.3 | 14.3 | 6 | 9.9 |
| 660,000 | 300 | 54,000* | 300* | 4.1 | 4.1 | 6 | 28.2 |
| **NEW MEXICO** | | | | | | | |
| 260,000 | 17,000 | +200 | 800 | 6.5 | 6.5 | 10 | 8.7• |
| 450,000 | 130,000 | 51,000* | 12,500* | 9.6 | 9.6 | 10 | 18.7• |
| **ARIZONA** | | | | | | | |
| 1,260,000 | 45,000 | 2,000,000* | 60,000* | 8.2 | 8.3 | 10 | 39.2 |
| 2,800,000 | 88,000 | 140,000* | 40,000* | 8.2 | 8.2 | 10 | 28.2 |

* Unbonded

• Surface Soil Moisture <1.0%

ELECTRON MICROGRAPHS, FIGS. 3 AND 4, ILLUSTRATE THE TYPICAL MICROSCOPIC APPEARANCE OF TWO MICROORGANISMS COMMONLY FOUND IN NORMAL UNDISTURBED SOILS IN CONTINENTAL NORTH AMERICA. ALSO LARGE NUMBERS HAVE BEEN CULTURED FROM ASPHALT COATINGS AND FROM THE SOIL SURROUNDING COATED PIPELINES.

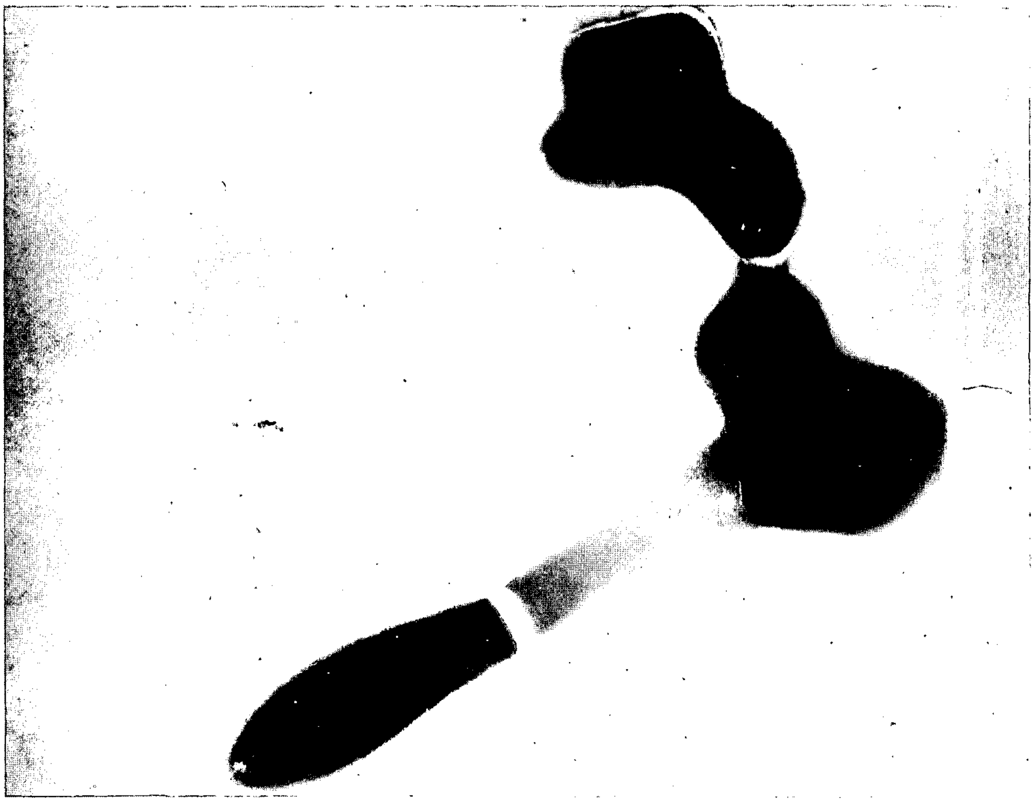

Fig. 3:--53,200 magnification. Pleomorphic rod-shaped cells, commonly seen in species of Nocardia, a common type of actinomycete isolated from asphalt coatings. Moisture under the coating -- some pitting -- anaerobic corrosion on the pipe -- no bond of coating -- 10 years' underground service. In these bacteria, branch forms may be seen, with swollen and club-shaped rods.

BOTH TYPES OF BACTERIA GREW WELL IN PURE CULTURES IN THE LABORATORY WHEN ASPHALT, OR A WIDE VARIETY OF LIQUID OR SOLID HYDROCARBONS WERE THE SOLE SOURCE OF ENERGY AND FOOD FOR THE DEVELOPING CELLS.

Fig. 4:--16,500 magnification. Medium-size rods, members of genus *Pseudomonas*, isolated from both asphalt coating and soil surrounding the pipe. Coating separated from pipe at several locations -- severe pitting -- 14 years' underground service -- coating sample this location, 370,000 aerobes, 560,000 anaerobes, moisture content 16.4%.

SUMMARY

BACTERIA CAPABLE OF GROWTH ON PARAFFINIC HYDROCARBONS ARE NORMAL INHABITANTS OF THE SOIL. POPULATIONS OF BACTERIA WITH THESE CAPABILITIES MAY VARY FROM SEVERAL HUNDRED TO THOUSANDS PER GRAM OF SOIL. ACTINOMYCETES COMMONLY ATTAIN COUNTS OF MILLIONS PER GRAM. RESPIRATION MEASUREMENTS SHOWED DODECANE IMMEDIATELY AND RAPIDLY OXIDIZED BY "NORMAL" SOIL, INDICATING LARGE POPULATIONS WITH THIS PHYSIOLOGICAL ABILITY. COUNTS OF ASPHALT-UTILIZING BACTERIA SHOWED MUCH HIGHER POPULATIONS IN SOIL ADJACENT TO COATED PIPELINES THAN IN UNDISTURBED SOIL OF THE RIGHT-OF-WAY AT THE SAME DEPTH.

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