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**A Study of the Interactions Between Microorganisms, Microbial
By-Products, and Oil-Bearing Formation Materials**

Final Report

**By
Lewis R. Brown
Azadeh Azadpour
Alex A. Vadie**

December 1992

Performed Under Contract No. AC22-90BC14665

**Mississippi State University
Mississippi State, Mississippi**



**National Petroleum Technology Office
U. S. DEPARTMENT OF ENERGY
Tulsa, Oklahoma**

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Assistant Secretary for Fossil Energy

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EXECUTIVE SUMMARY

The overall purpose of this project was to develop information on the microflora indigenous to subterranean oil reservoirs, with special emphasis on their potential role in microbial enhanced oil recovery (MEOR).

In regard to the microbial residents of subterranean oil reservoirs, it was learned that:

- * significant numbers of microorganisms were present in all 5 reservoirs examined (ranging in depth from 805-14,492 ft)
- * each reservoir had a somewhat distinctive microflora
- * of the 37 pure culture isolates examined, all grew anaerobically and produce one or more products of potential value to MEOR - gas, acid, emulsifiers, polymers, solvents
- * microbial isolates would colonize stratal materials (limestone, sandstone, clay shale) and their growth in sandpacks caused alterations in the flow of water through the sandpack
- * none of the reservoirs contained sulfate-reducing microorganisms
- * ultramicrobacteria were present in one reservoir

The results of this investigation support the concept that microorganisms indigenous to subterranean oil reservoirs are valuable to enhancing oil recovery. Toward this end, studies were conducted to test this concept under the most realistic conditions possible in the laboratory. Specifically, cores from oil reservoirs were employed in core flood experiments. By so doing, the microorganisms, formation materials, oil, and water were in as close to their natural state as possible.

Simulated production water containing supplemental nutrients then were allowed to flow through these cores. No supplemental nutrients were added to the water flowing through the control cores. One test core and one control core were prepared from cores obtained from five different reservoirs and the following results were

obtained when supplemental nitrogen and phosphorus sources were added to the injection water.

- * oil was released from the test cores
- * there was an increase in the number of microorganisms present in the core effluent
- * the production of acid by the microflora resulted in the dissolution of large amounts of carbonate material and the development of new channels
- * some plugging of the more porous zones occurred
- * gas was produced in some cases
- * the addition of trace amounts of ethanol to the injection water greatly enhanced the release of oil and the dissolution of carbonate in the formation material.

In this era of heightened environmental awareness, it is important to note that no adverse environmental effects would occur and the only chemicals involved are common plant nutrients and trace amounts of ethanol, all of which are consumed in the reservoir.

Based on the results of this investigation the following conclusions were drawn:

- * if properly supplied with supplemental nutrients, the microflora in the subterranean oil reservoirs tested, is adequate to enhance oil recovery
- * not only will this microflora reproduce, thus making selective plugging a reality, but also will produce a sufficient quantity of by-products to increase oil recovery
- * the amount of growth and/or by-products can be regulated to prevent the plugging of injection wells
- * desired by-products can be predetermined by selecting the supplemental nutrients
- * no adverse environmental effects will result from using the in situ indigenous microflora of the oil reservoir.

The results from this project form a firm basis for designing specific MEOR processes that will recover more oil from existing fields facing demise in the near future and suggest the possibility of reopening some abandoned fields. Further, if future work confirms the absence of sulfate-reducing microbes in the reservoirs, many of the problems, e. g. corrosion and plugging caused by these microorganisms, could be obviated by modifying current oil field practices.

INTRODUCTION

Microbial enhanced oil recovery (MEOR) is the utilization of microorganisms to increase oil recovery from subterranean reservoirs. The concept of using microorganisms to enhance oil recovery first was proposed in 1926 by Beckman (26); but it was not until the 1940's that any active research was reported, largely in patents and foreign journals (66).

MEOR requires that either (a) microorganisms be introduced into the oil-bearing formation or (b) use be made of microflora indigenous to the formation. The question arises, as to whether microorganisms are present in the formation or were introduced through drilling and production activities. While some skepticism may exist that microorganisms are indigenous to subterranean environments, the body of scientific evidence strongly supports the contention (26, 72, 77) as does an evaluation of reports up to the 1960's (38).

Another factor in MEOR processes is whether oil will be degraded under anaerobic conditions since the preponderance of carbonaceous compounds in reservoirs are hydrocarbons. The issue has become confused by conflicting reports concerning the anaerobic utilization of the oil vs hydrocarbons, since there are many non-hydrocarbon compounds in oil. There are a number of reports in the early literature on the anaerobic decomposition of both oil and hydrocarbons (38), unfortunately, the methodology employed by the earlier workers has been questioned, and thus there was a reluctance on the part of many investigators to believe these reports.

From the above, it is evident that a basic understanding of the interaction between the members of the microbial population indigenous to the reservoir and the interaction between microbial by-products and constituents of the oil-bearing formation is obligatory if MEOR processes are to be useful and yield predictable results.

The overall objective of this research project was to establish that microorganisms are indeed indigenous to oil-bearing formations and to develop basic information on this indigenous microflora.

To accomplish the above stated objective, the following tasks were performed.

TASK 1. Quantitate and characterize the microbial species indigenous to several different oil-bearing formations with special attention being paid to determining if ultramicrobacteria are present.

- TASK 2. Determine the ability of microbial isolates to utilize various carbon and nitrogen sources and identify by-products that may be useful in MEOR processes.
- TASK 3. Determine the interaction between microbial by-products and oil-bearing formation materials.
- TASK 4. Determine the ability of isolates from oil-bearing formations to colonize stratal materials in thin flat sandpicks and to determine if microbial growth in these sandpicks can alter flow patterns during waterflooding operations.
- TASK 5. Determine the ability of in situ indigenous microorganisms in live cores to grow with the addition of supplemental nutrients and determine the impact of this practice on formation materials and oil recovery processes.

Following is a brief Review of Literature and a summary of the more significant overall findings for each task. It was decided that the incorporation of detailed data in these sections would make them cumbersome and consequently, a separate volume containing more comprehensive data has been prepared.

LITERATURE REVIEW

Petroleum microbiology had its beginnings in 1885 with the observations of Miyoshi that a common industrial fungus, Botrytis cinerea, would attack paraffins (37). The pioneering work of Sohngen, as reported in 1913, established the fact that a variety of aerobic microorganisms are capable of growing on paraffins (8). Further, it was shown that soil from the vicinity of active oil wells and soil from oil dumps are reservoirs for hydrocarbon degrading microorganisms (27,29,49,76). Among the most frequently found organisms are members of the genera Mycobacterium and Nocardia.

While the use of microorganisms to enhance oil recovery was first suggested by Beckman (26), it was ZoBell and his co-workers that first undertook serious scientific investigations of this possibility (73,74). ZoBell demonstrated that the sulfate-reducing bacterium Desulfovibrio halohydrocarbonoclasticus, when supplied with petroleum as a source of carbon, was capable of releasing oil from sedimentary materials. ZoBell recognized that microorganisms had the potential capability of enhancing oil recovery through one or more of the following activities:

1. Production of acids that can increase pore space in limestone formations, thereby facilitating the movement of oil.
2. Production of gases that may reduce oil viscosity and effective mobility ratio in waterflooding.
3. Alteration of high molecular weight hydrocarbons, thereby forming more mobile compounds with shorter carbon chains.
4. Production of surface active substances that help to enhance micellar sweeping of oil from the formation. This may be achieved in situ or the materials may be produced and added to EOR processes.
5. Dislodging of oil from the surface of the solid materials in the reservoir due to microbial growth on the surface of the solids.
6. Reduction of the sulfate in gypsum, anhydride, and sulfate minerals causing minerals solubilization and release of oil through the action of sulfate-reducing bacteria.

According to Updergraff (66) two other microbial activities that may play a role in MEOR are: 1. Selective plugging of the more porous oil-bearing formations, thereby

shifting the sweeping agent into smaller but, as yet unswept pores, resulting in increased oil recovery (24) and 2. the increase in viscosity of the water by microbially produced water-soluble polymers.

Active MEOR research led by ZoBell continued from 1940-1955 (70) and included both successful laboratory and field tests. Thorough reviews of the early MEOR patents are given by Davis (27). MEOR's earliest studies (7,40,43,65) were based on ZoBell's methods, his original bacterial cultures, or similar microorganisms. Importantly, many workers that employed sulfate-reducing bacteria (SRB) were unable to demonstrate that these organisms were able to utilize petroleum hydrocarbons (38,65). According to La Riviere (40) SRB grown in culture media rich in an organic carbon source, lactate, were able to release oil from a solid surface by the production of surface active agents. Furthermore, during the anaerobic metabolic activities of SRB, appreciable amounts of hydrogen sulfide are produced that precipitate sulfides of iron and heavy metals that affect permeability and porosity of formation material (38).

Inconsistencies in reported data on release of oil by SRB activities, as well as anaerobic corrosion of iron and steel by SRB caused other microorganisms to be employed in later MEOR experiments. In fact, according to Davis (27) in a successful EOR operation, the activity of SRB should be minimized. Updegraff (67) employed obligate and facultative anaerobes from the Acetobacter, Esherichia, Clostridium, and Bacillus genera to inoculate oil-bearing formations. These bacteria were chosen based on their ability to use water-soluble carbohydrates, including molasses, and to produce large amounts of gases (carbon dioxide and hydrogen). After sugar fermentation, the build up of gases caused substantial increases in the reservoir pressure and helped oil recovery.

In general, there was a lack of interest in MEOR research from mid 1950 to mid 1970 due to the abundance of oil in this country. Renewed research efforts to introduce and control microorganisms in the oil field itself was attributed to higher oil prices and recognition of the importance of MEOR as a viable oil recovery method (66).

When attempts are made to inject normal-sized microorganisms into a formation, as described in some of the early MEOR methods (e.g., 64,73,75), most are unable to penetrate deep into the formation. This problem is addressed in some later MEOR processes, where use of a fracturing medium in conjunction with the microbial inoculum (9) or use of smaller-sized organisms (spores) (36) were proposed. Normal-sized bacteria, even spores, seem to be able to travel through most formations only by way of the large channels between the injection well and the production well. The prospects of obtaining any meaningful penetration of an oil reservoir

seemed rather dim until Lappin-Scott, Cusack, and Costerton (41) suggested the use of newly discovered survival forms of bacteria that have a considerably reduced size and have been reported to penetrate much deeper into sandstone cores than do larger bacterial cells (41,44). These ultramicrobacteria (UMB) are defined as having a diameter of less than 0.3 μm (62). It is noteworthy to point out that, in deep ocean waters and in soil, bacteria may become reduced in size due to a lack of nutrients (5, 20, 42, 62). In an oil reservoir, conditions are conducive to the formation of UMB.

The alternative to injecting microorganisms into the reservoir is to take advantage of (activate) those microbes indigenous to the oil-bearing formation. The question arises, of course, as to whether microorganisms are indeed present in the formation or whether they were introduced through drilling and production activities. While some skepticism may exist as to whether microorganisms are indigenous to subterranean environments, the body of scientific evidence strongly supports the contention (26, 72, 77) and an evaluation of reports up to the 1960's confirms this contention (38).

Irrespective of whether a given MEOR process involves the use of indigenous microorganisms or those injected into the oil-bearing formation, the interaction between the various microorganisms must be taken into account. In mixed microbial populations, the association between members can be symbiotic, antagonistic, commensalistic, or neutralistic and, as has been clearly pointed out, this interaction may be either beneficial or detrimental to the MEOR process (19). For example, the sulfate-reducing bacteria, which are generally regarded as the predominant species in oil reservoirs (3, 6, 38), can cause unwanted plugging. The production of an emulsifier can be beneficial to oil recovery, but another microorganism may destroy the emulsifier or alter the pH of the overall system resulting in a significant reduction in the effectiveness of the emulsifier. Similarly, the beneficial effect of the production of gases, may be negated by the utilization of these gases by another microbial species (16,45,52). Therefore, it is critical to understand the impact of a given MEOR treatment on all members of the microbial population.

Not to be overlooked in considering the activities of a mixed microbial population is the impact of their activity on the strata itself. Acids produced by microorganisms may dissolve certain constituents in the strata or change the charge on the surface of stratal materials. This change in surface charge may alter the effectiveness of an emulsifier or the ability of microorganisms to adhere to the surface.

Another factor that must be taken into account in MEOR processes is whether oil will be degraded under anaerobic conditions. In some respects, the issue has become confused by conflicting reports concerning the anaerobic utilization of

oil vs hydrocarbons, since there are many non-hydrocarbon compounds in oil. There are a number of reports in the early literature on the anaerobic decomposition of both oil and hydrocarbons (38); unfortunately, the methodology employed by the earlier workers has been questioned and thus there was a reluctance on the part of many investigators to believe these reports. As more evidence has been obtained, anaerobic microbial decomposition of oil seems to be firmly established, albeit not all constituents can be metabolized in this fashion. Evidence of indigenous hydrocarbon-utilizing organisms in some reservoirs has been reported (2,34,50). The bacterial catabolism of aromatic compounds (31,53,61) and benzene (60) under anaerobic conditions has been described. The microbial degradation of acenaphthene and naphthalene was shown to take place under denitrifying (anaerobic) conditions (48) and the anaerobic oxidation of crude oil by microorganisms has been described (18, 30). More recent reports on the anaerobic decomposition of hydrocarbons were given at the 1988 annual meeting of the Society for Industrial Microbiology: polycyclic aromatic hydrocarbons (35); homocyclic and heterocyclic aromatic hydrocarbons (33); and cresol isomers (60).

Oil alone, however, is not sufficient to support microbial growth since it is deficient in a source of both nitrogen and phosphorus (11,14,15,21,32,55,68). This deficiency probably accounts for the lack of microbial activity in a reservoir. It has been shown that the addition of a nitrogen and phosphorus source to microorganisms isolated from production water resulted in good microbial growth using oil as the carbon and energy source (10). Therefore, the action of the indigenous microflora and microorganisms injected into the formation on both oil and any exogenous substrates must be understood if MEOR is to yield predicible results (18, 24, 38, 63, 64).

Regardless of whether the microbial population in the reservoir is indigenous or injected, and irrespective of whether the microflora can metabolize oil or exogenously added nutrients, the success or failure of MEOR depends totally upon the ability to regulate the microbial growth. Too much growth can result in plugging of the wells. This problem is addressed adequately in the body of literature that exists on the bacterial plugging of waterflood injection wells (25).

TASK 1

INTRODUCTION

The objective of this Task was to quantitate and characterize the microflora indigenous to several oil-bearing formations, with special attention being paid to the presence of ultramicrobacteria. The geological and petrophysical characterization of the oil-bearing formations from which the cores for this study were obtained is given in Appendix A.

Particular attention was paid to the procedures employed in obtaining and treating cores to be analyzed for microorganisms. In most of the earlier reports on the microflora indigenous to oil reservoirs, the methodology employed in obtaining samples has been questioned. Obviously, proper sampling techniques are central to the question of whether microorganisms are truly indigenous to the reservoirs, and therefore, special emphasis was placed on preventing contamination of the samples with exogenous microorganisms and preventing oxygen from reaching the microorganisms that were present.

MATERIALS AND METHODS

Materials

Cores

Acquisition of Live Cores. Arrangements were made with several oil companies to acquire cores directly from the core barrels immediately as they came from the well.

The cores were obtained from oil-bearing formations with no previous history of enhanced oil recovery activities at all, including waterflooding.

The cores were received as they were pulled from the core barrels (live cores). The cores were broken into 1-ft sections, wiped with 70% ethanol, and immediately placed in BBL® Gaspak® System containers under anaerobic conditions. This procedure was completed within minutes, thus exposure to air was minimal. This procedure was of particular significance for microbiological studies. It also should be pointed out that the pressure in the core tends to force fluid and/or gases outward, thereby reducing further the possibility of exposing the internal section of the core to air. The anaerobic containers were packed in ice, transported to a laboratory, and placed in a refrigerator at 4C until needed.

Preparation of Core Material for Microbiological Analyses. Following removal from the anaerobic jar under an atmosphere of nitrogen, the cores were wiped again with 70% ethanol and cut into four-inch sections using a core saw (Raytech Industries, Stanford Spring, CT). To reach the median of the core, one inch was cut from all sides of the core using a sterile core saw blade. The median part of the core then was placed in a stainless steel core crusher under nitrogen gas, and subjected to 20,000 psi using a hydraulic press. The crushed core was placed in a bacteriological hood containing a nitrogen atmosphere and passed through a sterilized U.S.A. Standard Testing Sieve No 40 (0.0165 opening in inches).

Acquisition of Production Fluids from Cored Wells

Oil and water were received from the cored wells after the wells were put into production. If separators were installed at the site, the fluids were received separately; otherwise, they were shipped to the laboratory unseparated.

In case no production was in progress from the cored wells, fluid samples were taken from the nearest producing well from the same reservoir formation as the cored wells.

Media

All media were prepared using simulated production water with the following inorganic salts per eight liters of distilled water:

NaCl	778.00 g
Na ₂ SO ₄	130.00 g
MgCl ₂ .6H ₂ O	352.00 g
CaCl ₂ .2H ₂ O	36.00 g
KCl	11.00 g
Na ₂ HCO ₃	3.20 g
KBr	1.60 g
SnCl ₂ .6H ₂ O	0.67 g
H ₃ BO ₃	0.41 g
Na ₂ SiO ₃ .9H ₂ O	0.08 g
NaF	0.05 g
NH ₄ NO ₃	0.03 g
FePO ₄ .4H ₂ O	0.02 g

The media employed in this task were:

1. Bacto-Tryptic Soy Agar (TSA) and Bacto-Plate Count Agar (PCA) prepared with simulated production water.
2. Oil agar prepared with simulated production water supplemented with 0.1% KNO₃, 0.37% K₂HPO₄.3H₂O, and 1% filter sterilized crude oil,

and 2% Bacto-Agar. After the agar had been poured and had hardened in the petri plates, a thin overlay was added using oil agar prepared with oil-saturated water, but containing no added oil.

3. Medium for methanogens using formate. The basal medium for methanogens as described by Zeikus (71) was as follows:

KH ₂ PO ₄	0.75 g
K ₂ HPO ₄ ·3H ₂ O	1.45 g
NH ₄ Cl	0.90 g
MgCl ₂ ·H ₂ O	0.20 g
Na ₂ S·9H ₂ O	0.50 g
Trace mineral solution	9 ml
Simulated production H ₂ O	1000 ml

The medium was prepared anaerobically under a 95% N₂-5% CO₂ atmosphere. The pH of the medium was adjusted to 7.4 and 1% sodium formate (w/v) was added prior to autoclaving. Filter-sterilized sodium sulfide solution was added after autoclaving. The trace mineral solution consisted of the following compounds [in grams per liter of distilled water (pH 7.0 with KOH)].

Nitrilotriacetic acid	4.50 g
FeCl ₂ ·4H ₂ O	0.40 g
MnCl ₂ ·4H ₂ O	0.10 g
CoCl ₂ ·6H ₂ O	0.17 g
ZnCl ₂	0.10 g
CaCl ₂	0.02 g
H ₃ BO ₃	0.019g
Na ₂ MoO ₄ ·2H ₂ O	0.01 g

4. Medium for methanogens using CO₂ and H₂. This medium was prepared as described in (3) above with the exception that no formate was added and an atmosphere of 20% CO₂ and 80% H₂ was employed.
5. Bacto-Nitrate Broth prepared with simulated production water.
6. Bacto-Peptone Iron Agar (PIA) prepared with simulated production water
7. Nitrate-reducing, hydrocarbon-utilizing medium. This medium was prepared as described by Rosenfeld (59) but modified by using simulated production water in place of synthetic seawater as follows:

FeSO ₄	0.1 g
K ₂ HPO ₄	0.5 g
KNO ₃	1.0 g
Simulated production H ₂ O	25 ml
Distilled H ₂ O	965 ml

The reaction mixture was adjusted to pH 7.8 prior to the addition of 1% (v/v) crude oil.

8. Sulfate-reducing, hydrocarbon-utilizing medium. This medium was prepared as described by Rosenfeld (59) but modified by using simulated production water in place of synthetic seawater as follows:

Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	0.1 g
K ₂ HPO ₄	0.5 g
(NH ₄) ₂ SO ₄	1.0 g
Simulated production H ₂ O	25 ml
Distilled H ₂ O	965 ml
Crude Oil	10 ml

Chemicals

All organic chemicals used in this research were either reagent or HPLC grade, and all inorganic chemicals were analytical grade. The chemicals were supplied by Sigma Chemical company, St. Louis, MO; and Aldrich, Milwaukee, WI. Bacto-Agar was supplied by Difco Laboratories, Detroit, MI.

Gases

The N₂, H₂, and CO₂ used in these studies were Custom Grade and were supplied by Standard Welders, Columbus, MS.

Methods

Enumeration of Microorganism

Fifty g of sieved core material was mixed with 50 ml of sterile simulated production water that served as the diluent for this and subsequent dilutions. After mixing, 10 ml of the first dilution was transferred to a 90 ml dilution blank. The second dilution was mixed thoroughly before transferring 10 ml to the next dilution blank to make a 1/200 dilution of the original sample. The dilution vessel used was an eight oz prescription bottle. Precautions were taken to insure homogeneity in the suspensions prior to sampling. Thus, particulate matter was present in all samples.

All work was conducted in a bacteriological hood containing a nitrogen atmosphere at constant inlet pressure of 10 psi. To insure that oxygen was absent, samples of the atmosphere in the hood were collected at different intervals

for analysis by gas chromatography (GC).

The conventional spread or pour plate technique was employed in some enumeration procedures while the Most Probable Number (MPN) technique was employed in others. All plate counts were performed in triplicate.

The following groups of microorganisms were enumerated.

Total Heterotrophs were enumerated using the conventional spread plate technique using both TSA and PCA. Plate counts were conducted under both aerobic and anaerobic conditions with the anaerobic cultures incubated in BBL® GasPak® System containers. Plates were incubated at the temperature prevailing in the reservoir from which the samples were obtained.

Oil-utilizing Bacteria were enumerated using the conventional spread plate technique and oil-overlay agar. Incubation was as described for the total heterotrophs.

Two groups of Methane-producing Bacteria were enumerated, both using the Most Probable Number (MPN) technique (three tubes per concentration of sample). One set of tubes was supplemented with 10 g per liter of sodium formate, while the other set of tubes was incubated under an atmosphere of 80% H₂-20% CO₂. Tubes were incubated at the temperature prevailing in the reservoir from which the samples were obtained. All tubes were closed with serum stoppers and methane production determined by GC analysis of the atmosphere in the tube.

Hydrogen Sulfide-producing Bacteria were enumerated using PIA and the conventional pour plate technique. Plates were incubated under both aerobic and anaerobic conditions (using BBL® Gaspack® System containers) at the temperature prevailing in the reservoir from which the samples were obtained.

Denitrifying and Nitrate-reducing Bacteria were enumerated using the Most Probable Number (MPN) technique (three tubes per concentration of sample) and Bacto-Nitrate Broth in test tubes containing Durham fermentation tubes. The tubes were incubated for three weeks at the temperature prevailing in the reservoir from which the samples were obtained. After incubation, tubes showing gas production were recorded as positive for denitrifying bacteria. Spot tests for nitrite were conducted using the sulfanilic acid and α -naphthylamine acetate reagents as described in Standard Methods for the Examination of Water and Wastewater (1). Negative tubes were reexamined after 60 days.

Nitrate-reducing, Hydrocarbon-utilizing Bacteria were enumerated using the the Most Probable Number (MPN) technique (three tubes per concentration of sample) and the nitrate-reducing medium. After three weeks of incubation at the temperature prevailing in the reservoir from which the samples were taken, the presence of nitrite in the tubes was determined as described above. Negative tubes were reexamined after 60 days.

Sulfate-reducing, Hydrocarbon-utilizing Bacteria were enumerated using the Most Probable Number (MPN) technique (three tubes per concentration of sample) and the sulfate-reducing medium. After three weeks of incubation at the temperature prevailing in the reservoir from which the samples were taken, the tubes were examined for the blackening of the agar. Negative tubes were reexamined after 60 days.

Test for Ultramicrobacteria

The original attempt to recover ultramicrobacteria (UMB) was conducted in the following fashion: 50 g of sieved core material was mixed with 50 ml of sterile simulated production water that served as the diluent to make a 1/2 dilution of the original sample. The dilution vessel then was fitted with a serum stopper and the suspension was mixed thoroughly. A sample (20 ml) from the liquid portion of the suspension was carefully removed by means of a sterile 10 ml disposable syringe equipped with a No 21 hypodermic needle. The suspension then was filtered sequentially through membrane filters with 0.8 μm , 0.45 μm , and 0.22 μm pore size in diameter. One ml of filtrate was added to each of the media employed for the enumeration procedures at the final concentration of the one-half strength of the original media. The tubes were reexamined biweekly for growth by staining techniques up to 60 days.

Since original attempts for recovery of UMB failed, all five cores were reexamined as follows: fifty g of crushed core was vigorously mixed with 200 ml of sterile saline solution (2.5% NaCl, w/v) for 10 min using a vortex device. The suspension then was immediately filtered through a Whatman No 1 filter to remove fines which would interfere with the filtration process. The sample then was immediately filtered through a pre-sterilized membrane filter (0.45 μm) to trap normal-sized bacteria. This filter was assembled on a filtration apparatus (Millipore Corp.) after the unit was autoclaved. The filtrate then was refiltered through a pre-sterilized membrane filter (0.22 μm). Five ml of the filtrate then was added aseptically to a test tube with 10 ml Tryptic Soy Broth (TSB) at the final concentration of the one-eighth strength of the original media. The final concentration of NaCl in the tube was 2.5% (w/v). Both the filters and the filtrates were tested for the presence of viable microorganisms weekly.

Main line vacuum was used for the filtration procedure and back contamination was avoided by use of a Swinney holder equipped with a Gelman 13 mm membrane filter (0.45 μm). Controls were established by repeating the filtration procedure by using five ml of a suspension containing E. coli after adjustment to an absorbance of 1.0 at a wavelength of 590 nm on a Coleman Nephelometer.

Isolation of Pure Cultures

Representative colonial types present on agar plates were obtained in pure culture by the conventional streak plate method. In the case of physiological groups enumerated by the MPN technique, streak plates were made from tubes yielding positive results and pure cultures obtained therefrom.

Stock Cultures

Pure culture isolates were grown on agar slants prepared from the same medium from which they were derived. When the isolation medium was a liquid, the medium was solidified with Bacto-Agar (2%). The agar slant then was sealed with a serum stopper and gased with an atmosphere of nitrogen. The vessels were incubated at 32C for two weeks, and then stored at 4C until needed (no longer than three months). All stock cultures were examined for contamination by streaking plates of TSA and then observing the colonial morphology of the resulting colonies using oblique lighting.

Characterization of Microbial Isolates

Each culture was characterized as to cellular morphology, Gram reaction, spore formation, capsule formation, acid-fastness, ability to grow aerobically or anaerobically, and colonial characteristics.

Chemical Analysis of Oil

A profile of the aliphatic and aromatic hydrocarbons in the oil was obtained as follows: A sample of the oil from the core was extracted three times with hexane. The combined extracts were reduced to a small volume and placed on an alumina/silica gel clean-up column. The column was eluted with hexane to collect the base/neutral aliphatics followed by methylene chloride to collect the base/neutral aromatic hydrocarbons. Each fraction was evaporated and then dissolved in the appropriate solvent for analysis. The aliphatic hydrocarbons were analyzed by flame ionization GC (HP 5890) with a non-polar capillary column, 30 m DB5. Oven temperature was at 4.4C initially and at 141C finally. The run length was 59 min.

Chemical Analysis of Water

Samples of production water from the reservoirs were analyzed for total dissolved solids, pH, salinity and hardness as described in Standard Methods for the Examination of Water and Waste Water (1).

The following metals (sodium, potassium, calcium, magnesium, iron, nickel, vanadium, zinc, chromium, copper, and lead) were determined by flame atomic absorption spectroscopy following the 1976 Perkin-Elmer Methods Manual (54). The instrument used was a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer utilizing a 5000 Automatic Burner Control. Light sources were hollow cathode lamps for all analyses except the one for lead which required an electrodeless discharge lamp. For potassium, ionization was suppressed with 1000 ppm lithium. For sodium, ionization was suppressed with 1000 ppm potassium. For calcium and magnesium, interference was controlled with 1000 ppm lanthanum. Vanadium required a nitrous oxide-acetylene flame while all others used an air-acetylene flame. All samples were acidified with nitric acid.

Gas Analysis

Analysis of gaseous compounds was performed on a Fisher Gas Partitioner Model 1200 (dual column, dual detector chromatograph). Column 1 was a 20' X 1/8" aluminum column packed with 37.5% DC-200/500 on 80/100 mesh chromosorb P-AW. Column 2 was a 6' x 3/16" aluminum column packed with 60/80 mesh molecular sieve, 13X. The column temperature was 70C and the injector temperature was 65C. The carrier gas, helium, was employed at a flow rate of 35 ml per min. (back pressure 40 psi). All analyses were performed using a 100 μ l sample. The syringe employed had a gas lock septum (Precision Sampling Corporation, LA). Standard curves were prepared for all gases used throughout these investigations by analyzing various amounts (25 μ l to 100 μ l) of authentic samples and averaging four replications. Identification of gases was achieved by comparison of the retention time of peaks on the chromatogram to the retention times of standard gases. Quantitation was accomplished by comparison of the area under the curve for a given gas to a standard curve prepared with a pure sample of that gas.

RESULTS AND DISCUSSION

Collection and Treatment of Oil Well Cores

To prevent contamination of the cores with extraneous microorganisms and to minimize exposure of the cores to air, special handling procedures were rigorously followed as described in Materials and Methods. These procedures were designed to insure that the results obtained were a true reflection of the microbial population in the oil reservoir,

not contaminants introduced from drilling fluids, and to minimize any alteration in numbers of different types of microorganisms caused by exposure to air.

A total of five cores were obtained for use in this task. The locations from which the cores were obtained are as follows:

Core 1 was cut on June 10, 1990, from Callon Co. Well No 1 Irving Langford in the Langford Field situated in Monroe County, AL. This core was obtained from a depth of 14,492 ft.

Core 2 was cut on July 16, 1990, for Shell Oil Co. Well No Wilmar 133 in the Kern River Field situated in Kern County, CA. The core was obtained from 805 ft.

Core 3 was cut on August 9, 1990, for Texaco Co. Well No 648, JEM "A" NCT-1 in the Mabee Field situated in Andrews County, TX. The core was obtained from 4,725-6 ft.

Core 4 was cut on September 18, 1990, for Oxy USA Inc. Well No Johnson GBSAN No 1809 in the Johnson Field situated in Ector County, TX. The core was obtained from 4,050 ft.

Core 5 was cut on October 16, 1990, for Chevron Co. Well No 679 of the Emsu oil field situated in Lea County, NM. The core was obtained from 4,300 ft.

Of these, Core 1 was used to evaluate the laboratory facilities and procedures to be employed. As a result of this exercise, starting with Core 2, slight modifications were made in the preparation of the cores for analysis from the protocol employed for Core 1. The modified procedure, described in the

Materials and Methods Section, included passage of the crushed core material through a U.S.A. Standard Testing Sieve No 40 (0.0165 opening in inches) prior to examination.

Enumeration of Microorganisms

As indicated in the Materials and Methods, all work was conducted under nitrogen and simulated production water was employed rather than distilled water or artificial seawater in all media. Incubation temperature of the respective reservoirs was employed except for Core 1 where 78C was used instead of 115.6C. During a trial run of the enumeration procedures using Core 1, several difficulties were encountered; namely, a majority of the microorganisms adhered to the stratal materials and the nonuniformity of the material caused wide swings in numbers. Procedures were modified to include the screening of the crushed core material in order to obtain a smaller, more uniform size particles, and care was taken to insure that a uniform amount of suspended matter was

included in each portion employed as an inoculum. Data on the number of anaerobic heterotrophs (as indicated using TSA and PCA) and anaerobic oil-degrading microorganisms in the cores are given in Table 1-1. Even though microorganisms that grew on PIA were present in two cores, none produced H₂S. Companion determinations were made for aerobes and it was found that all cores had organisms that grew aerobically. It was observed that each core contained only a few distinct colonial types and each core seemed to have a slightly different population as indicated by colonial morphology.

The cores also were analyzed for five physiological groups of microorganisms using the MPN technique. Trial experiments had shown that none of the media became turbid and the only visible growth was occurring on the sides of the tubes. As a consequence of this phenomenon, glass beads were added to liquid media in order to increase the surface area available for colonization. Growth in the tubes was confirmed by either the production of CO₂ or by microscopic examination of stains made from the contents of the tubes. The data in Table 1-2 show that all five cores had growth in at least two of the five media employed. Even Core 5 which failed to yield viable microorganisms using the plate count method did demonstrate viable microorganisms using liquid media. It is interesting to note that, while all five cores had microorganisms capable of growing in the hydrocarbon-utilizing, sulfate-reducing medium, no hydrogen sulfide was produced. This finding was particularly surprising in that sulfate-reducing microorganisms have been reported in the literature as the most prevalent organism in oil reservoirs. The reason for the above is not known but it should be noted that none of the cores were from wells showing hydrogen sulfide.

One of the emphases of this task was to determine if ultramicrobacteria (UMB) were present in the cores. Using the procedure described in Materials and Methods, UMB were indeed found in Core 3 which was obtained from a depth of 4,725 ft. Growth in one-eighth strength TSB was obtained from the filtrate passing through the 0.22 μm membrane filter. While growth was evident by the developing turbidity and cells were evident in stains from the broth, the culture did not grow on the solid media (even one-eighth strength). As pointed out in the Materials and Methods, tests were conducted to insure that the 0.22 μm membrane filter was not broken and thus growth resulted from cells passing through the 0.22 μm filter. Following demonstrations that UMB's are indeed present, these cells were adapted to grow in higher concentrations of the TSB as described by MacDonell and Hook (42). Following several transfers to a higher concentration of TSB (from 1/8x to 1x), these cells grew in size (up to 0.75 μm) but still would not grow on solid media. Cells were Gram negative and pleomorphic. By no means does this result indicate that UMB's were absent in the other cores; only that they were not

Table 1-1

Number of Anaerobic Heterotrophs and Anaerobic Oil-degrading Microorganisms in Cores as Determined by the Plate Count Method.

Core No	Total* Heterotrophs on TSA	Heterotrophs on PIA**	Heterotrophs on PCA	Oil-Degrading Microorganisms* on Oil Agar
1	100	<2	<20	240
2	<20	<2	<20	492
3	1500	40	<20	20
4	7200	1600	240	136
5	<20	<2	<20	<20

TSA = Tryptic Soy Agar

PIA = Peptone Iron Agar

PCA = Plate Count Agar

* Numbers expressed as #/g of core

** None of the colonies appearing on the PIA produced H₂S.

Table 1-2

Different Physiological Groups of Microorganisms in Cores as Determined Using the Multiple Tube Fermentation Technique.

Core No	Methanogen ^a	Medium	Hydrocarbon-Utilizing		Nitrate ^c
	CO ₂ :H ₂ Broth	Formate ^b Broth	Nitrate ^c Broth	Sulfate ^d Broth	Broth
1	1	<1	<0.6	240	<1
2	92	<220	19	48	>220
3	>220	19	5	32	6
4	1	2	>220	1	2
5	<1	1	58	>220	1

- a. As determined using MPN Table. Numbers expressed as #/g of core.
 b. Numbers are based on growth in the tubes, not methane production.
 c. Numbers are based on growth, not nitrate reduction.
 d. Numbers based on growth, not sulfate reduction.
 e. Numbers based on tubes showing nitrite production.

detected by the methods employed. To our knowledge this is the first report of finding UMB's in oil reservoirs, although it has been suggested that these microbial forms might be useful in some MEOR processes since their small size would allow greater penetration of the reservoir when injected. Finding UMB's in the cores is not surprising; the starvation conditions in the reservoir could be ideal for their formation. Further, UMB's are known for their ability to survive for extremely long periods of time.

Isolation of Pure Cultures

Predominant colonial types present on agar plates were acquired in pure culture by the conventional streak plate technique. When physiological groups were enumerated by the MPN technique, streak plates were made from tubes containing growth. In both cases, with the help of a dissecting microscope, a colony was picked into a small test tube containing approximately 1.5 ml of sterile sand and water. The tube was vortexed for several minutes, and a loop of the contents streaked onto the surface of another plate containing the appropriate agar. After incubation, the surface of the plate was examined using a dissecting microscope and a well-isolated colony picked onto an agar slant under nitrogen, and after growth was stored at 4C.

The stock cultures were grown on slants of the medium from which they were isolated and numbered with a seven-digit code. In cases where the original medium was liquid, it was solidified by the addition of 2% Bacto-Agar. Details of the culture number are given in Appendix B.

Because of the failure to find sulfate-reducing bacteria initially, (there was growth in the sulfate-reducing, hydrocarbon medium but none of the cultures produced hydrogen sulfide) all isolates were tested for their ability to grow on sulfate-reducing medium prepared in the form of slants using the corresponding oil and production water. All 86 stock cultures were tested for sulfate-reduction but no H₂S production was observed.

Characterization of Microbial Isolates

Of the large number of pure culture isolates obtained from the various samples, 86 anaerobic isolates have been characterized and retained. The number of isolates and the medium from which they were obtained are as follows: 17 from Tryptic Soy Agar; 8 from Plate Count Agar; 1 from Peptone Iron Agar; 30 from Oil Agar; 5 from methane-producing broth with H₂-CO₂ atmosphere; 12 from methane-producing broth with formate; 9 from nitrate broth; 2 from nitrate-reducing, hydrocarbon-utilizing broth; and 2 from sulfate-reducing, hydrocarbon-utilizing broth.

The above isolates were characterized morphologically and culturally and the overall results shown in Figures 1-1 and 1-2. According to the figures, 41 of the isolates were cocci, 40 were bacilli, and 5 were pleomorphic. Gram positive isolates outnumbered Gram negative isolates 52 to 22 with 12 isolate being Gram variable. Also, 29 isolates were Acid Fast, 61 were capsule formers, and 44 were motile. Specifics for each isolate are given in Appendix B.

Chemical and Physical Analyses of Oil

Samples of oil from the cored wells were characterized as to API gravity, viscosity, surface tension and oil-water interfacial tension as shown in Table 1-3.

In addition, aliphatic profiles, and aromatic profiles of the oils were made by the Analytical Support and Food Safety Laboratory at Mississippi State University (MSU).

In evaluating the data obtained from GC chromatograms, it was realized that the aromatic components of the oil samples were present only in trace quantities. In addition, it was notable that oil sample No 2 appeared as a degraded oil, since most of the light aliphatic chains were greatly reduced in concentration. Furthermore, this sample was highly viscous and tar-like in nature while the other samples were less viscous. These characteristics are not surprising in light of the fact that the oil-bearing formation was at a depth of only 805 ft.

Chemical and Physical Analyses of Production Water

Samples of production water from the cored wells were characterized as to density, viscosity, salinity, surface tension, and pH (Table 1-4) and subjected to analysis for some inorganic ions with the aid of Milton Chemical Kits (Table 1-5). Also, the following metals in the production water samples were determined by flame atomic absorption spectroscopy: sodium, potassium, calcium, magnesium, iron, nickel, vanadium, zinc, chromium, copper, and lead (Table 1-6).

SUMMARY

The purpose of this task was to obtain an estimate of the numbers and kinds of microorganisms indigenous to oil reservoirs and to characterize them morphologically and culturally.

1. By using rigid procedures to prevent contamination, it has been shown that viable microorganisms are present in petroleum reservoirs. It should be pointed out that in addition to the five cores employed in this project,

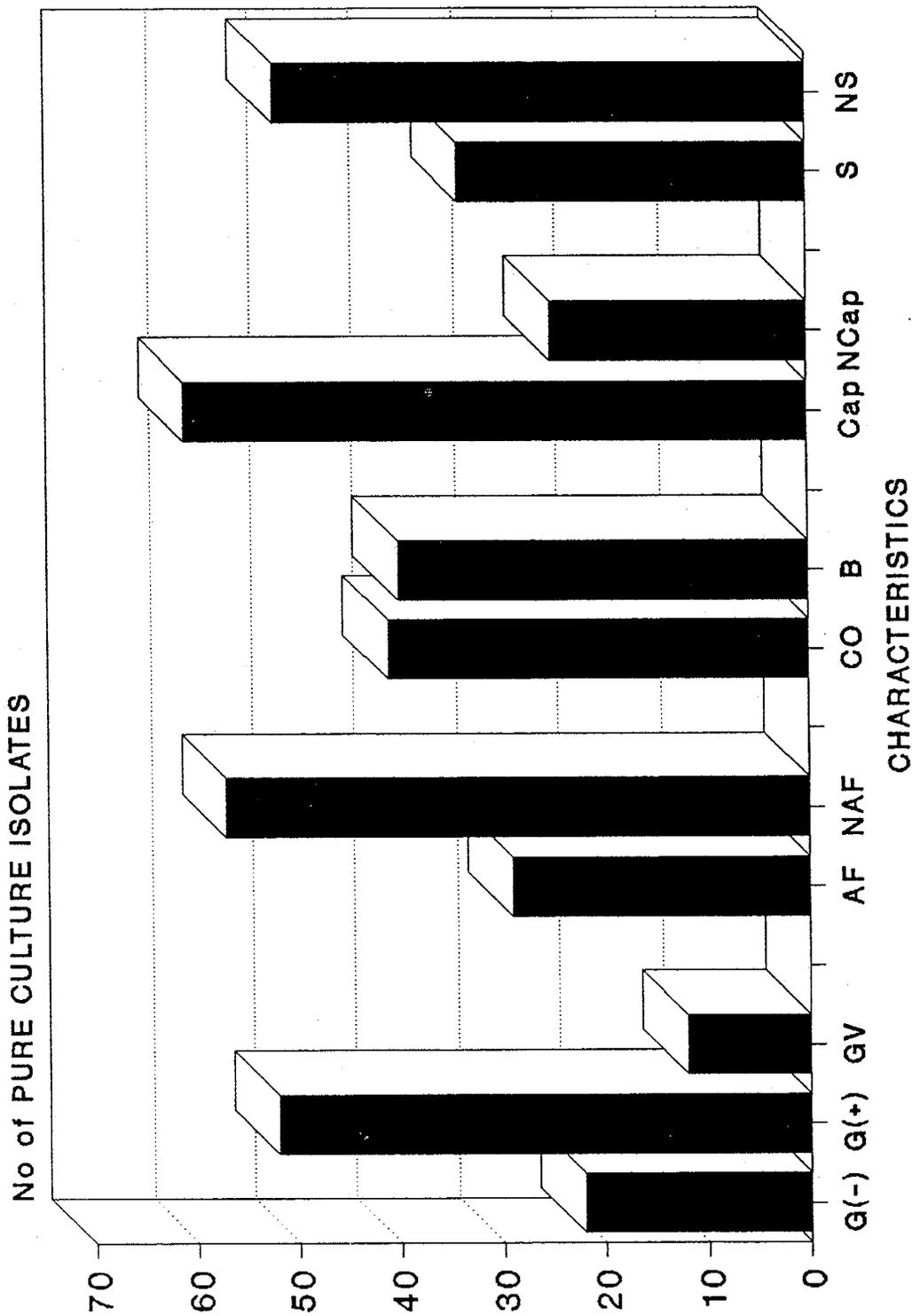


Figure 1-1. Some morphological characteristics of microbial isolates from oil reservoirs
 (G(-), gram negative; G(+), gram positive; GV, gram variable; AF, acid fast; NAF, non-acid fast; CO, cocci; B, bacilli; Cap, capsule; NCap, non-capsule; S, spore former; NS, non-spore former)

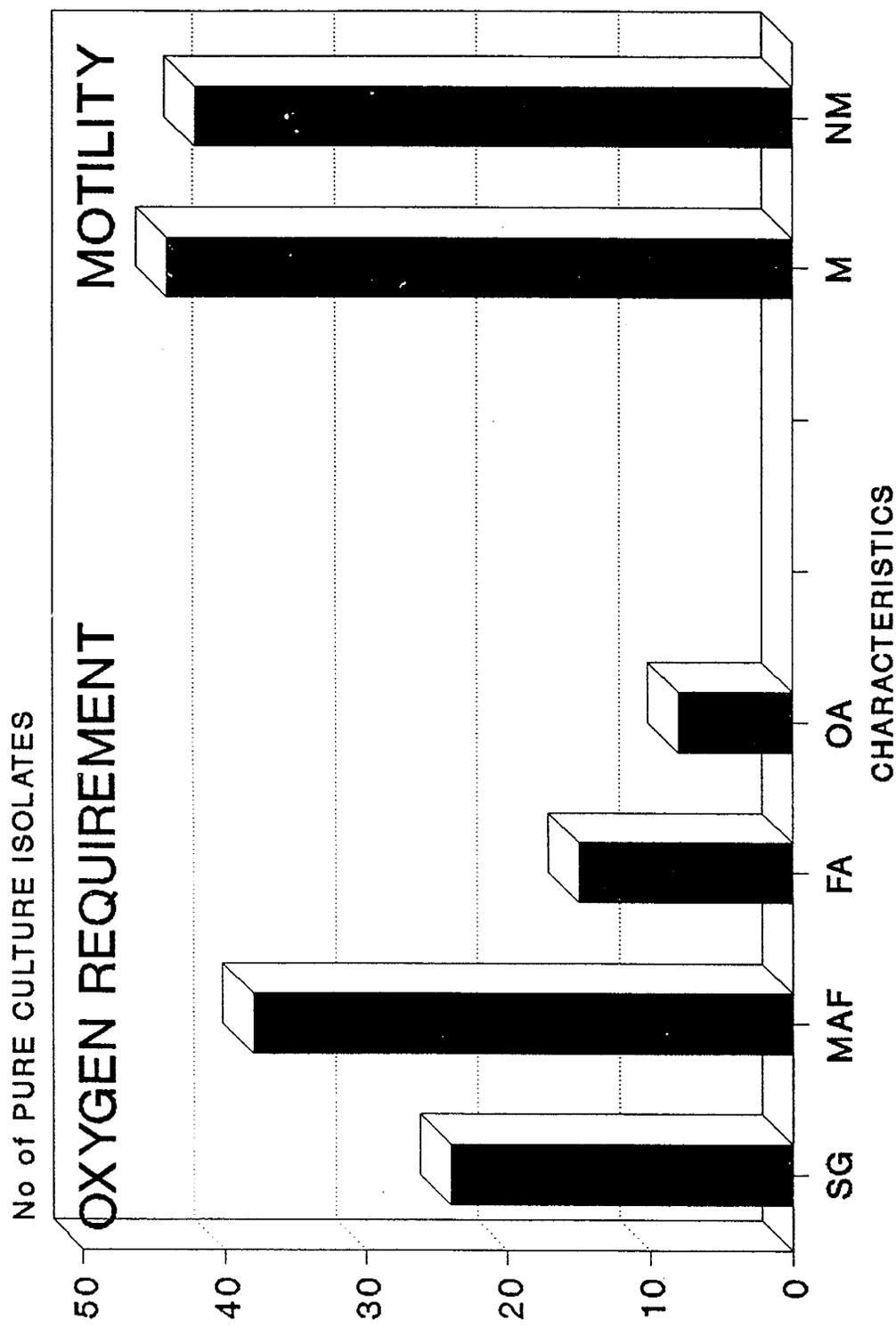


Figure 1-2. Some cultural characteristics of microbial isolates from oil reservoirs
 (SG, surface growth; MAF, microaerophile; FA, facultative anaerobe; OA, obligate anaerobe; M, motile; NM, non-motile)

Table 1-3

Characteristics of Oil from Cored Wells.

Core No	Oil				
	API	μ	ST	IFT	T _{res}
1*	38	.87	25.5	- - -	240
2	11.5	.70	35.6	17.62	90
3	29	.75	28.1	14.80	103
4	33.5	.78	26.8	19.30	102
5	33	.83	26.6	3.88	90

* :Well did not produce water

API :Oil gravity, API

μ :Viscosity, cp

ST :Surface tension, dyn/cm

IFT :Interfacial tension, dyne/cm

T_{res} :Reservoir temperature, F

Table 1-4

Characteristics of Production Water from Cored Wells.

Water						
Core No	ρ	μ	SALI	ST	pH	T _{res}
1*	--	--	--	--	--	240
2	1.00	.80	550	45.0	7.7	90
3	1.02	.73	12K	38.4	5.23	103
4	1.04	1.1	27K	69.1	7.98	102
5	1.00	1.2	7.3K	51.4	7.63	90

* : Well did not produce water
 ρ : Density
 μ : Viscosity, cp
 ST : Surface tension, dyn/cm
 SALI : Salinity, ppm
 T_{res} : Reservoir temperature, F

Table 1-5

Analysis of Production Water for the Total Dissolved Solids.

Core No	Cl ⁻	NO ₃ ⁻	NH ₃ ⁻	NO ₂ ⁻	SO ₄ ⁻²	CaCO ₃	OP	TP
2	420	16.50	5.12	4.22	17	120	30.73	2.51
3	-	0.20	8.14	0.00	85	-	21.84	0.00
4	320	5.72	0.33	0.76	164	610	21.59	2.29
5	-	3.12	8.10	0.13	17	3500	31.75	0.95

Data are reported as mg/l of water sample.

OP: Orthophosphate

TP: Total Phosphorus

Table 1-6

Metallic Content of Production Water from Cored Wells.

Core No	Na	K	Ca	Mg	Fe	Ni	V	Zn	Cr	Cu	Pb
2	562	14.1	31.2	3.1	0.1	0.0	1.1	1.81	0.0	0.2	0.0
3	16800	184.0	1630.0	429.1	1.8	1.6	1.5	0.11	0.1	0.1	0.2
4	32200	542.0	267.0	683.2	5.3	4.4	1.5	0.16	0.2	0.2	1.0
5	15900	313.0	1230.0	264.0	1.8	1.6	1.1	0.09	0.1	0.1	0.0

Data are reported as ppm of water sample.

two additional cores were examined and both contained viable microorganisms. Reservoir depths from which the cores were obtained ranged from 805 ft to 14,492 ft. This finding is of particular significance in Microbial Enhanced Oil Recovery (MEOR) since it suggests that there are sufficient microorganisms already present in the reservoir to achieve many of the goals of MEOR.

2. It is considered highly significant that no sulfate-reducing microorganisms were found in either the five cores analyzed in this project or in two additional cores tested. This finding is in contrast to the generally accepted concept that these microorganisms are the most prevalent type found in oil reservoirs. Since only seven cores have been examined it would be presumptuous to assume that all reservoirs are devoid of these organisms but, if subsequently proven to be factual, it suggests that many of the major problems (e.g., corrosion) associated with sulfate-reducing microorganisms could be obviated by modifying procedures for drilling and completing wells.

3. This is the first time ultramicrobacteria (UMB) have been found in this environment, i. e., oil reservoirs.

TASK 2

INTRODUCTION

The objective of this Task was to determine by-product formation by pure and mixed cultures of microorganisms indigenous to oil reservoirs.

MATERIALS AND METHODS

Materials

Microorganisms

The bacteria employed throughout this study were the pure culture isolates obtained in Task 1.

Media

The culture media were prepared using a basal mineral salts solution (MSM) with the following inorganic salts per liter of simulated production water:

$K_2HPO_4 \cdot 3H_2O$	0.50 g
$MgSO_4 \cdot 7H_2O$	0.20 g
$FeCl_3 \cdot 6H_2O$	0.05 g

The pH was adjusted to 7.0 using 10% (v/v) HCl. A precipitate formed in the resulting medium; therefore, the medium was allowed to settle for 24 hrs and only the clear supernate employed. Carbon sources were added in 0.5% (w/v) concentration and nitrogen sources were added in 0.1% (w/v) concentration. When a solid medium was desired Bacto-Agar, was added at a concentration of 20g/l.

For routine cultivation, the incubation vessel employed was a 100 ml serum bottle containing 27 ml of liquid medium. Sterilization was accomplished by autoclaving at 121C (15 psi) for 20 min.

Chemicals

All organic chemicals used in this task were either reagent or HPLC grade, and all inorganic chemicals were

analytical grade. The chemicals were supplied by Sigma Chemical Company, St. Louis, MO; and Aldrich, Milwaukee, WI. Bacto-Agar was supplied by Difco Laboratories, Detroit, MI.

Gases

The nitrogen gas used in these studies was Custom Grade and was supplied by Standard Welders, Columbus, MS.

Methods

Stock Cultures

The morphological and cultural characteristics of the 37 cultures employed in this Task are given in Appendix B.

Cell Concentration Adjustments

Cells were washed from the agar slants with physiological saline (0.85% NaCl, w/v). The harvested cells were centrifuged at 10,000 rpm for 20 min, washed twice with physiological saline, and suspended in a saline (0.425 NaCl, w/v) and potassium phosphate (0.075 M, pH 7.0) solution (1:1 ratio). The cell concentration was kept constant by adjusting the percent light transmission of a 1:10 dilution of the cells on a Coleman Nephelometer to an absorbance of 1.0 at a wavelength of 590 nm and then diluting the original cell suspension appropriately.

The Effect of Carbon and Nitrogen Sources on Growth

The 37 isolates were tested for their ability to utilize oil, *n*-hexadecane, molasses, or acetate as a sole carbon source for growth. Likewise the nitrate ion, the ammonium ion, and urea were tested as sole sources of nitrogen.

The media employed for growth consisted of: the basal mineral salts solution (pH 7) prepared with each of the nitrogen sources at the final concentration of 0.1% with a single carbon source (0.5%). A total of 3.8 ml of the medium was placed in a six ml test tube, sealed with a serum stopper, flushed with nitrogen, and injected with 0.2 ml of the cell suspension. Controls were established for every culture (one without a carbon source, and one without a culture). After one week of incubation, PCA plates were streaked to determine growth. This procedure was repeated weekly. When growth was evident in a tube, one ml of the culture was inoculated into 27 ml of the same medium contained in a 100 ml serum bottle. Prior to injection, the bottle was stoppered with a sterile neoprene serum stopper, secured with an aluminum band and the atmosphere replaced with nitrogen. The vessel then was placed on a shaker (New Brunswick Scientific, New Brunswick, NJ) and oscillated at 100 revolution per min (rpm) during the incubation period (32C for three weeks). Growth was confirmed by removing samples (0.01 ml) of the culture at various times,

streaking plates of PCA, and observing the growth after incubation.

In addition to growth, the cultures were tested for their ability to produce gases, acids, emulsifiers, solvents, and polymers as well as tested for cell surface hydrophobicity.

Gas Production

Analyses for gaseous compounds were performed on a Fisher Gas Partitioner Model 1200 (dual column, dual detector chromatograph). Column 1 was a 20' x 1/8" aluminum column packed with 37.5% DC-200/500 on 80/100 mesh chromosorb P-AW. Column 2 was a 6' x 3/16" aluminum column packed with 60/80 mesh molecular sieve, 13X. The column temperature was 70C and the injector temperature was 65C. The carrier gas, helium, was employed at a flow rate of 35 ml per min. (back pressure 40 psi). All analyses were performed using a 100 μ l sample. The syringe employed had a gas lock septum (Precision Sampling Corporation, LA). Standard curves were prepared for all gases used throughout these investigations by analyzing various amounts (25 μ l to 100 μ l) of authentic samples and averaging four replications. Identification of gases was achieved by comparison of the retention time of peaks on the chromatogram to the retention times of standard gases. Quantitation was accomplished by comparison of the area under the curve for a given gas to a standard curve prepared with a pure sample of that gas.

Acid Production

The initial pH for all media was adjusted to 7.0 using 10% (v/v) HCl or 10% (v/v) KOH. A sample (three ml) of the culture medium from the serum bottle was withdrawn and analyzed using a Fisher Accumet Model 915 pH meter after three weeks of incubation at 25C.

Fatty Acid Production

A sample (0.1 ml) of the culture medium was withdrawn from the serum bottle and streaked on an agar plate containing the same medium as in the serum bottle except for the addition of the 2% (w/v) Bacto-Agar. Anaerobic plates were incubated in BBL® GasPak® System containers for two weeks at 32C. After growth, two loopfuls of bacteria were transferred to five ml of 5% NaOH in 50% aqueous methanol. The resultant sample was heated for 15 min at 100C. The saponified sample was acidified to pH 2 in order to liberate the fatty acids. Following the addition of four ml of boron trifluoride, methanol (BF₃ - CH₃OH, Suppleco, Bellefonte, PA) the mixture was heated at 100C for five min. After cooling, 10 ml of saturated sodium chloride solution was added to the mixture. The fatty acid methyl esters were extracted twice with 1:4

chloroform:hexane. The extracts were combined and were concentrated to 0.5 ml under a nitrogen atmosphere. The fatty acid methyl esters were determined using a GC with a non-polar capillary column (DB5) and a flame ionization detector. Identification of fatty acids was achieved by comparison of the retention times to commercially available known standards of bacterial fatty acids. Quantitation was accomplished by recording the percent area under the curve for each of the fatty acid methyl esters.

Emulsifier Production

A 7.5 ml sample of culture filtrate and 0.1 ml of crude oil were placed on a shaker (Eberbach Corp., Ann Arbor, MI) and agitated at 100 strokes per min for one hr at 25C. Turbidity of the aqueous phase was measured at 540 nm on a B&L Spectronic 20. Quantitation of results was accomplished by comparing the results obtained to a standard curve prepared using the emulsifier [EF-RAG (VET)] produced by Arthrobacter species RAG-1 (ATTC 31012) as described by Rosenberg, et al. (57).

Solvent Production

One ml of CS₂ was combined with nine ml of spent medium. The CS₂ portion was extracted using a Pasteur pipette. To remove impurities, this portion was separated by centrifugation at 5000 x g for 15 min. Solvents were determined using a GC fitted with a carbowax column and a hydrogen flame detector. Identification of solvents was accomplished by comparison of the retention time to control samples.

Polymer Production

Ten ml samples of spent media (three weeks old) were employed for viscosity measurements using a Cannon Fenske Viscometer, SR-100-333, at room temperature. When filter-sterilized crude oil was used as a carbon source (0.5% v/v), a separatory funnel was employed to separate the crude oil from the culture medium before the viscosity measurements.

Cell Surface Hydrophobicity Measurements

The microbial cell suspension grown on the crude oil was placed in separatory funnel. The suspension was allowed to remain quiescent for 30 min for a complete separation of the crude oil from the aqueous phase. The resultant aqueous cell suspension was centrifuged at 10,000 rpm for 20 min, the harvested cells were washed twice and resuspended in Phosphate urea magnesium sulfate (PUM) buffer (pH 7.12, 22.2g K₂HPO₄·3H₂O; 7.26g KH₂PO₄; 1.8g urea; 0.2g MgSO₄·7H₂O; and distilled water to 1000 ml), to an optical density of 0.5 at

550 nm (approximately 2.9×10^8 cfu/ml). Each culture was mixed with 1000 μ l of *n*-hexadecane in a round bottom test tube (10 mm diameter). The tube was preincubated for 10 min at 30C, agitated for 120 sec, and allowed to rest for 15 min to allow the hydrocarbon phase to rise completely. The aqueous phase was transferred to a one ml cuvette using a Pasteur pipette and the light absorbancy was measured at 550 nm on a B&L Spectronic 20.

Physiological Characterization of Mixed Cultures

Six different pure culture isolates were mixed in combinations of two. The carbon source employed was oil (0.5%, v/v) and the nitrogen sources were NO₃ ions, NH₄ ions, or urea. Mineral salt medium (pH 7) was prepared with each of the above nitrogen sources at the final concentration of 0.1%. Twenty seven ml of medium was placed in a 100 ml serum bottle. The bottle was stoppered with a serum stopper secured with an aluminum band, flushed with nitrogen, and inoculated with 1.5 ml of microbial culture.

After growth, the mixed cultures were tested for the production of gases, acids, and emulsifiers according to the protocol described above.

RESULTS AND DISCUSSION

The Effect of Carbon and Nitrogen Sources on Growth

Some MEOR processes are designed to take advantage of the microflora present in the oil reservoir rather than rely on the injection of other microorganisms into the reservoir. In this case, the injection of nutrients for the indigenous microflora is all that is required. At a minimum, a nitrogen source and/or a source of phosphorus will have to be supplied even if oil in the reservoir is to serve as the carbon and energy source. The selection of nutrients to be introduced into a reservoir is critical to success. For example, the activities of the sulfate-reducing bacteria may be deleterious to the activity of other microorganisms or may cause unwanted plugging if allowed to grow uncontrolled. However, if nitrate ions are employed as the nitrogen source at the proper concentration, the sulfate-reducing bacteria should be inhibited (39). Orthophosphate ions would normally be the phosphorus source of choice but, in some reservoirs, it may react chemically with components of the reservoirs and cause plugging or, at least, will limit the distribution of the phosphate in reservoir. Also, if carbonaceous materials are injected into the formation, it must be known which compounds elicit what response from the different microbial residents.

Therefore, based on the above justification, of the 86 pure cultures obtained in Task 1, isolates from each of the following nine isolation media were selected for further testing: 5 from TSA; 5 from PCA; 1 from PIA; 4 from oil agar; 4 from methane-producing (with H₂-CO₂ atmosphere); 6 from methane-producing (with formate); 8 from nitrate broth; 2 from nitrate-reducing, hydrocarbon-consuming broth; and 2 from sulfate-reducing, hydrocarbon-consuming broth.

These isolates were tested for their ability to utilize various nitrogen and carbon sources. The carbon sources employed were oil, *n*-hexadecane, molasses, and acetate. The nitrogen sources were NO₃ ions, NH₄ ions, and urea. Each culture was tested on 12 different media. Each medium consisted of MSM prepared with one of the above mentioned nitrogen sources at a final concentration of 0.1% with a single carbon source (0.5%). The number of isolates that grew in each of the media is shown in Figure 2-1. As the results indicate, all four carbon sources were utilized by one or more of the cultures isolated from the oil reservoirs in the presence of dissimilar nitrogen sources. However, irrespective of the nitrogen source, the largest number of isolates grew in the presence of oil.

Furthermore, these experiments demonstrated that anaerobic microbial growth on crude oil was possible when a nitrogen source (NO₃ ions, NH₄ ions, or urea) and a phosphorus source (orthophosphate present in MSM) were added. The other carbonaceous nutrients selected for these experiments were possible candidates for MEOR processes. Molasses is used widely in anaerobic processes for gas production. *n*-Hexadecane is routinely employed in hydrocarbon studies conducted in laboratories, and acetate is a small organic molecule readily used by many microbial species.

Since it was established that the culture isolates were able to metabolize the substrates employed, it was of interest to determine if they produced by-products that would be useful in recovering oil from subterranean reservoirs.

Physiological Characterization of Pure Culture Isolates

It is a well established fact that in the laboratory some microorganisms produce chemicals such as gas, acids, emulsifiers, solvents, and polymers (4, 12, 13, 17, 22, 23, 69) that would be useful in recovering oil from oil reservoirs. Further, it has been reported that the injection of such microorganisms into subterranean formations can improve oil recovery (19). The use of injected or indigenous microorganisms to enhance oil recovery requires the injection of nutrients for the microbes to produce the desired by-products. Specifically, the physiological characterization of the microorganisms isolated in Task 1 included tests for the

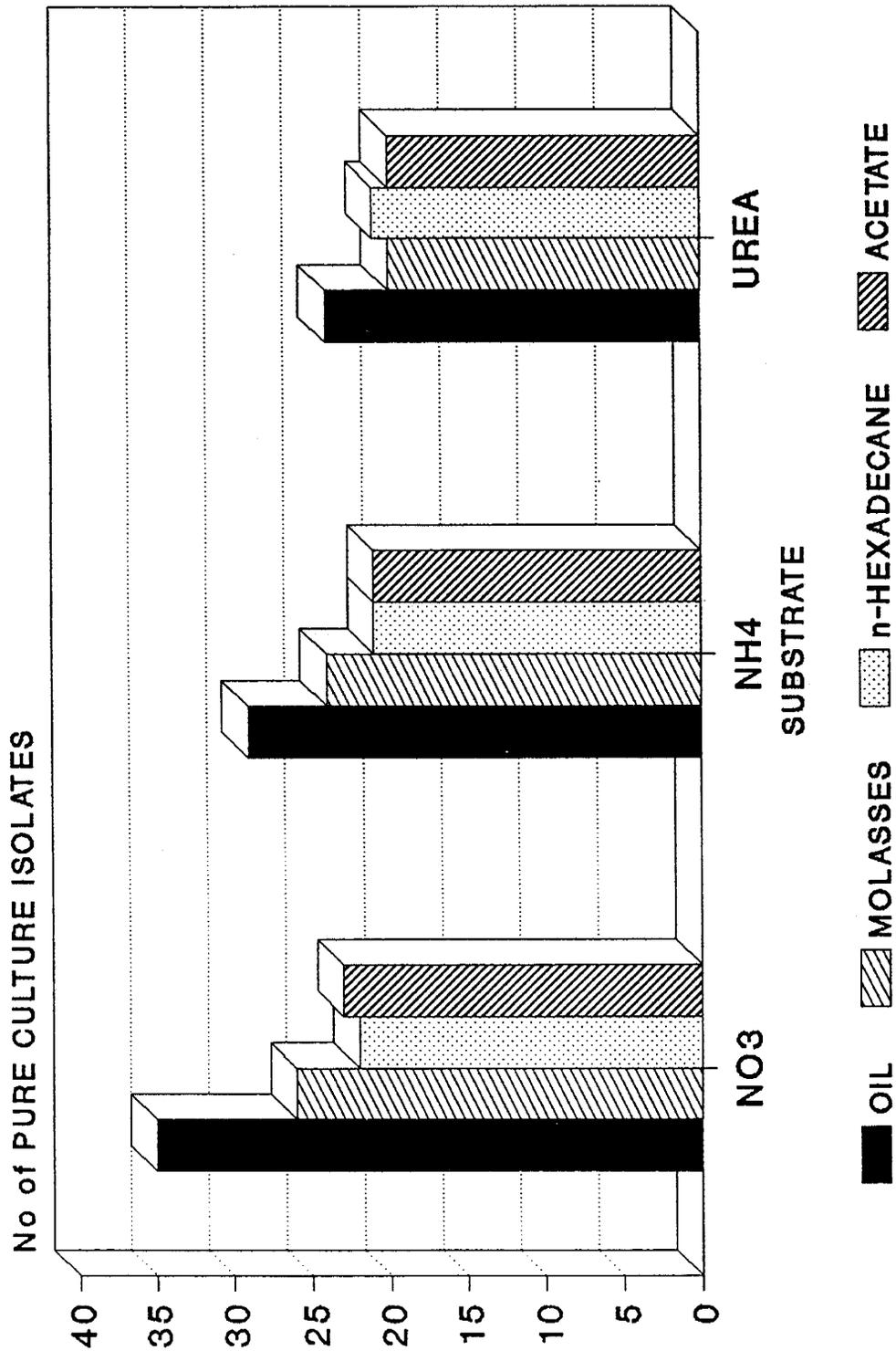


Figure 2-1. The number of pure culture isolates capable of utilizing different carbon and nitrogen sources (37 cultures tested)

production of gases, acids, emulsifiers, solvents, and polymers. In addition, selected cultures, grown on oil, were tested for cell surface hydrophobicity. Thirty-seven pure culture isolates from Task 1 were examined for their ability to yield by-products from crude oil, molasses, *n*-hexadecane, and acetate. The nitrogen sources employed were NO₃ ions, NH₄ ions, and urea.

Gas Production

Tests for the production of gases were performed by growing cultures in closed serum bottles fitted with neoprene stoppers and then analyzing the atmosphere overlaying the cultures. The maximum CO₂ production using four different carbon sources and three different nitrogen sources is shown in Figure 2-2. As may be observed, when molasses was offered as the source of carbon, gas production was dramatically increased when NO₃ ions or NH₄ ions served as the source of nitrogen. With urea as the nitrogen source, gas production was slightly higher for the cultures grown on acetate rather than molasses. Collectively, among the oil-and *n*-hexadecane-grown isolates, the production of CO₂ was reduced significantly below the amount produced when molasses or acetate was offered.

It is of interest that a majority of cultures produced CO₂ from all four carbon sources with all three nitrogen sources. For example, with molasses, 27, 25, and 21 cultures produced CO₂ using NO₃ ions, NH₄ ions, and urea, respectively. Similarly, with oil, 36, 30, and 25 cultures produced CO₂ using NO₃ ions and NH₄ ions, and urea, respectively. With *n*-hexadecane, 22, 22, and 21 cultures produced CO₂ with NO₃ ions, NH₄ ions, and urea, respectively. Finally, with acetate CO₂ was produced by 23, 21, and 20 cultures using NO₃ ions and NH₄ ions and urea, respectively.

Production of Acid

The production of acids was determined by measuring the pH of the culture medium after growth.

The maximum acid production using the twelve different media is depicted in Figure 2-3. As may be observed, the pH of the culture media was lowest when molasses was employed as the carbon source, irrespective of the nitrogen source in the medium. This result would be expected since, generally, one of the end products of sugar fermentation is acid. Many times the anaerobic fermentation of molasses results in the production of large quantities of gas, mainly CO₂. Since a closed system was employed in this study and since CO₂ is soluble in water the subsequent formation of the carbonic acid would contribute to the acidity of the medium.

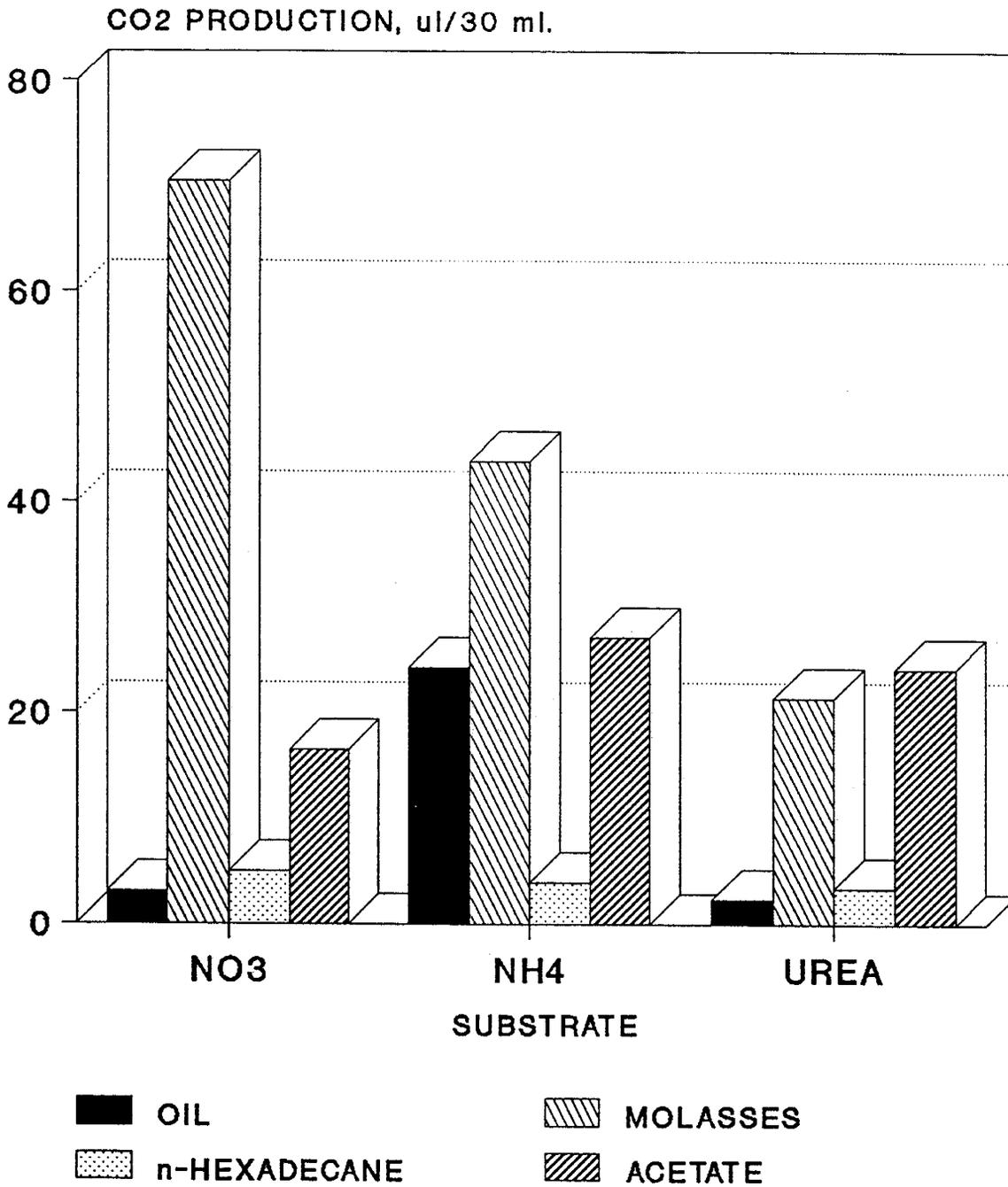


Figure 2-2. Maximum amount of carbon dioxide produced from four different carbon sources using three different nitrogen sources

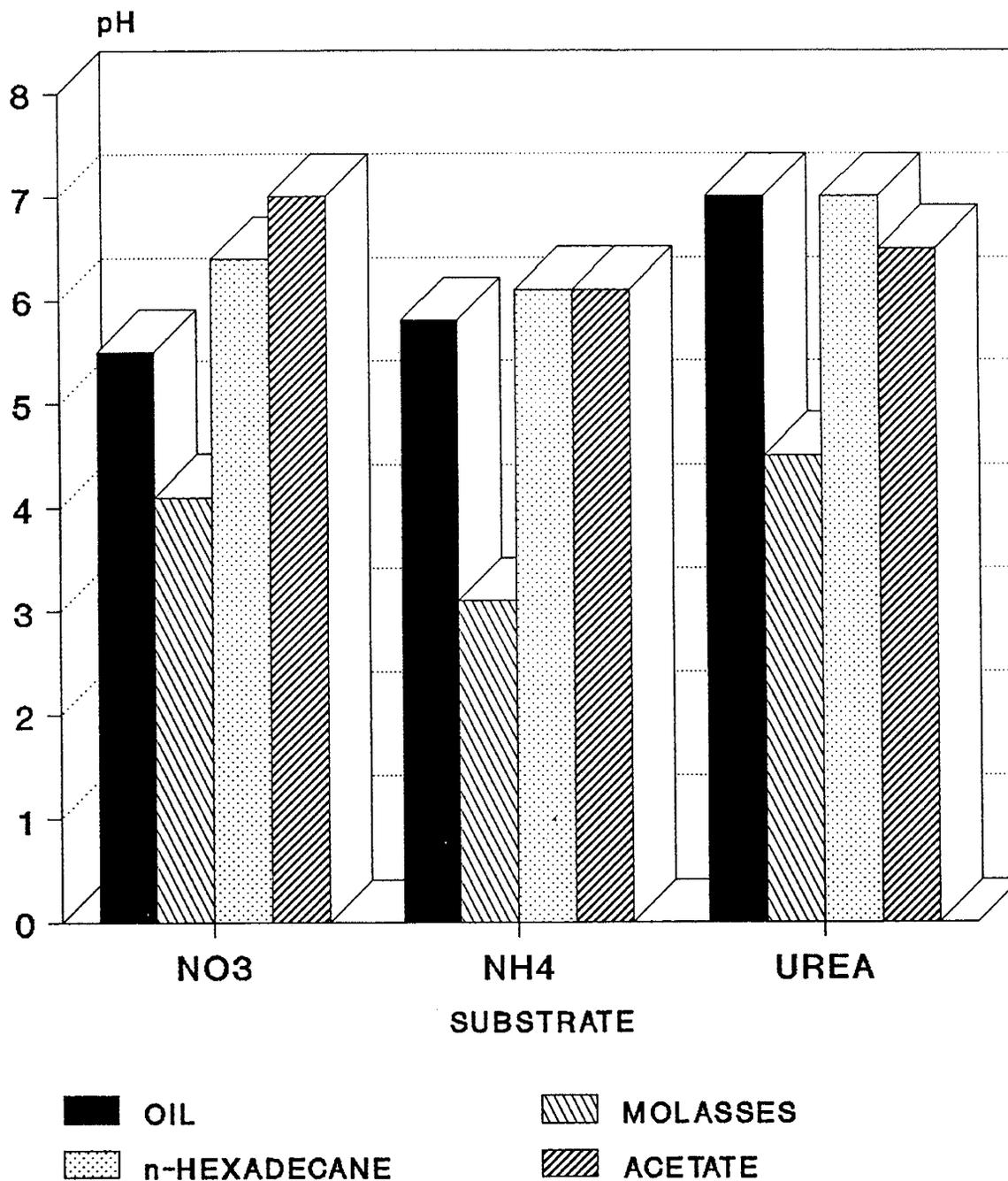


Figure 2-3. The effect of carbon source and nitrogen source on the final pH of culture medium after growth

Fatty Acid Production

In selected cases, the fatty acid content of microbial cultures was determined as described in the Materials and Methods section according to the method described by Moss, et al. (51).

The data in Table 2-1 showed that there was a difference in the types of the individual fatty acids produced by the different cultures. Also, with oil as the substrate, some fatty acids were produced by all cultures, whereas other fatty acids were produced by only a few cultures. The nitrogen source had a minimal impact on the fatty acid profile of the individual cultures with only a few fatty acids being produced with one nitrogen source and not with the other two nitrogen sources. Appendix B contains a summary of the data on the fatty acids produced during the utilization of the selected carbon and nitrogen source for each culture tested.

Emulsifier Production

The ability of the 37 isolates to produce emulsifiers was determined by a modification of the method described by Rosenberg, et al. (57). The maximum values for the emulsifier concentration produced in the different media is shown in Figure 2-4. As may be observed, the concentration of emulsifier ($\mu\text{g/ml}$) was highest for the isolates grown on molasses with NO_3 ions or NH_4 ions with lesser amounts being produced by isolates grown on molasses with urea. For the media prepared with urea, the oil-grown isolates produced 177 $\mu\text{g/ml}$ of emulsifier, while molasses-grown isolates only produced 75 $\mu\text{g/ml}$ of emulsifier. These data indicate that emulsifier was produced from oil- and molasses-isolates in an approximate ratio of 2.3:1. Emulsifier production was always lowest when n-hexadecane and acetate were employed as carbon sources.

The results for n-hexadecane- and acetate-grown isolates were similar in terms of emulsifier production irrespective of the nitrogen sources applied.

Furthermore, when molasses was used as the source of carbon for the 37 cultures, 24 cultures with NO_3 ions, 21 cultures with NH_4 ions, and 20 cultures with urea produced emulsifiers. Likewise, 35, 29, and 25 of the 37 isolates produced emulsifiers in the presence of oil with NO_3 ions, NH_4 ions, and urea, respectively. In the presence of n-hexadecane 14, 14, and 17 of the isolates produced emulsifiers with NO_3 ions, NH_4 ions, and urea, respectively. Also, 22, 15, and 10 of the isolates produced emulsifier in the presence of acetate with NO_3 ions, NH_4 ions, and urea, respectively (Appendix B).

TABLE 2-1

Fatty Acids Produced by Microorganisms Grown on Oil with Three Different Nitrogen Sources.

	Fatty Acid	No* of cultures grown on Oil and:		
		NO ₃ ions	NH ₄ ions	Urea
1.	undecanoic	4	4	7
2.	2-hydroxydecanoic	-	-	2
3.	dodecanoic	14	11	10
4.	tridecanoic	2	1	4
5.	2-hydroxydodecanoic	19	19	15
6.	3-hydroxydodecanoic	15	14	9
7.	tetradecanoic	4	6	10
8.	13-methyltetradecanoic	16	14	10
9.	12-methyltetradecanoic	19	18	8
10.	pentadecanoic	-	2	7
11.	2-hydroxytetradecanoic	17	21	11
12.	3-hydroxytetradecanoic	12	12	12
13.	14-methylpentadecanoic	16	8	6
14.	cis-9-hexadecenoic	2	4	3
15.	hexadecanoic	2	-	4
16.	15-methylhexadecanoic	7	4	10
17.	cis-9,10-methylenehexadecanoic	17	13	6
18.	heptadecanoic	3	6	8
19.	2-hydroxyheptadecanoic	19	18	9
20.	cis-9, 12-octadecadienoic	1	2	5
21.	cis-9-octadecenoic	9	8	5
22.	trans-9-octadecenoic & cis-11-octadecenoic	3	4	7
23.	octadecanoic	19	21	8
24.	cis-9,10 methyleneoctadecanoic	18	20	13
25.	nonadecanoic	1	1	1
26.	eicosanoic	18	19	8

* The total No of tested oil-degradating bacteria were: 21 for NO₃ ions, 21 for NH₄ ions, and 16 for urea.

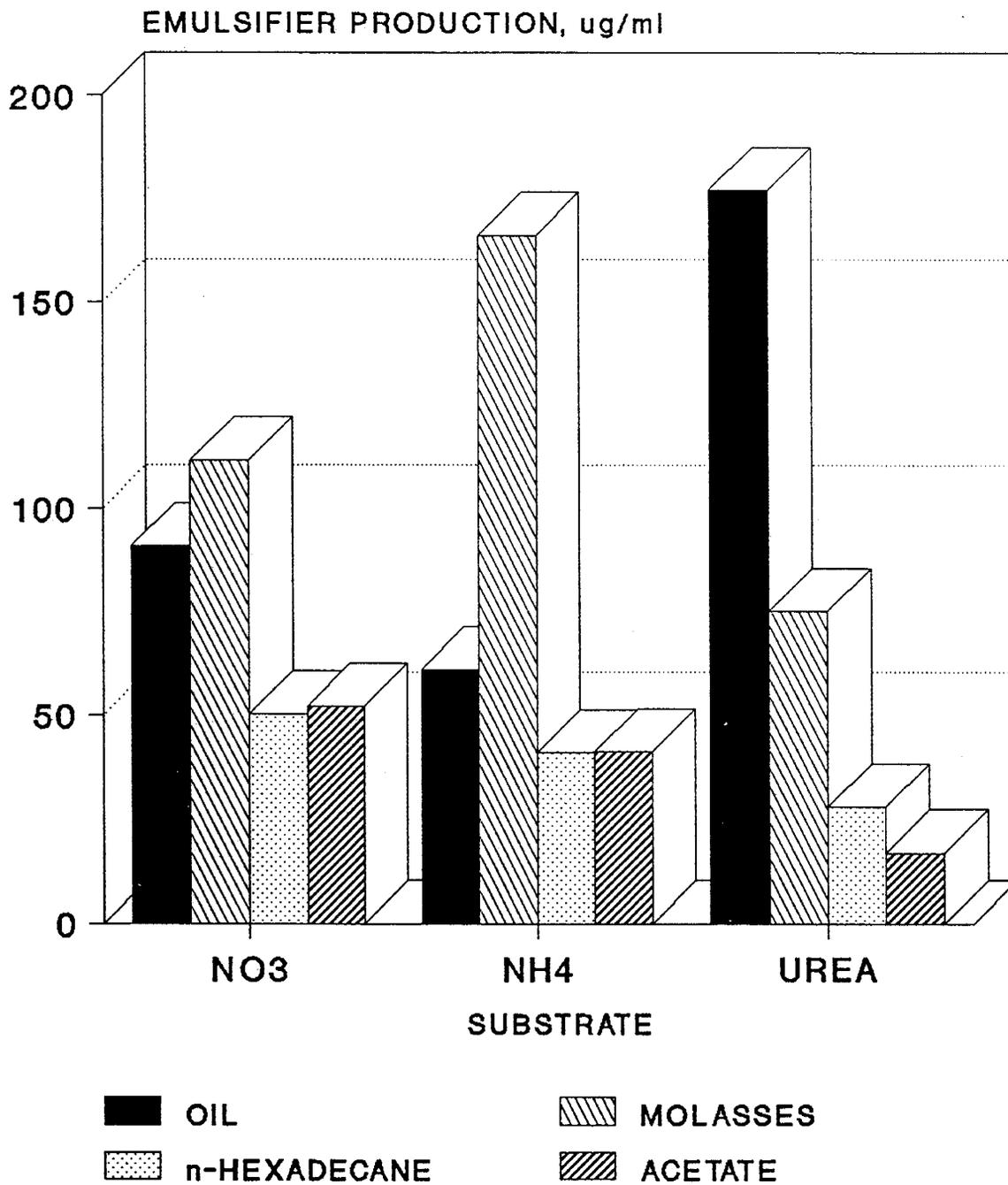


Figure 2-4. Maximum amount of emulsifier produced from four different carbon sources using three different nitrogen sources

Microbially produced emulsifiers can significantly enhance oil recovery since they reduce the interfacial tension between oil, water, and sand surfaces thereby releasing more oil from reservoir pores.

Production of Solvents

The production of volatile solvents that may lower the oil viscosity was determined using carbon disulfide (CS₂) as the organic solvent to extract volatile compounds. The following solvents were determined by GC using a carbowax column with a hydrogen-flame detector: acetaldehyde, ethanol, acetate, methanol, acetone, butanol, benzene, toluene, ethyl acetate, and xylene.

Details of solvent production for the cultures grown on oil, molasses, *n*-hexadecane, and acetate, are given in Appendix B.

According to the data, oil-grown pure culture isolates were able to produce various solvents from oil with NO₃ ions and urea. Among the 37 oil-grown cultures, 14 produced solvents with NO₃ ions, while, only 10 produced solvents with urea. It was notable that none of the cultures grown on oil with NH₄ ions produced detectable amounts of solvent. The following solvents and the number of cultures producing them from oil with NO₃ ions were: acetaldehyde from 11 cultures, ethanol from 11 cultures, acetate from 1 culture, acetone from 5 cultures, butanol from 3 cultures, and toluene from 3 cultures, ethyl-acetate from 1 culture, and xylene from 12 cultures.

Production of Polymer

Production of compounds from crude oil and molasses that increase the viscosity of the aqueous medium was determined by measuring the viscosity of spent culture medium.

The range of polymer production as the result of substrate utilization for the pure culture isolates is shown in Table 2-2. The amount of polymer produced by each culture is given in Appendix B.

These data showed that polymer was produced from oil by 22 of the 37 oil-grown cultures with NO₃ ions. This ability was reduced from 22 cultures to 11 or 7 cultures when oil was utilized with NH₄ ions or urea, respectively.

The highest absolute viscosity was 0.825 cp while the lowest was 1.062 cp for the oil- and molasses-grown pure culture isolates, respectively.

TABLE 2-2

Change in Viscosity* of Media Due to Polymer Production by Microbial Cell Cultures from Molasses and Oil Using Three Different Nitrogen Sources.

Range	Molasses			Oil		
	NO ₃ ions	NH ₄ ions	Urea	NO ₃ ions	NH ₄ ions	Urea
Low	0.962	0.977	1.062	0.825	0.853	0.845
High	0.965	0.996	0.972	0.965	0.887	0.870

*The final absolute viscosity is reported in centipoise at 26 - 27C.

Polymer production by the indigenous microflora may prove useful in future MEOR technology since polymers can increase the viscosity of the water used for waterflooding thereby increasing sweep efficiency.

Cell Surface Hydrophobicity Measurements

The cell surface hydrophobicity of cultures grown on oil was determined by the method of Rosenberg *et al.* (58) and Darnell *et al.* (28). Hydrocarbon adherence was determined by the ability of different bacterial cultures to associate with *n*-hexadecane.

Adherence data showed that the majority of the cultures were hydrophobic when grown on oil; however, some cultures exhibited hydrophilicity by partitioning into the aqueous phase. Interestingly, among the different nitrogen sources tested, all of the cultures that grew with NH_4 ions were hydrophobic whereas cells grown on urea were less hydrophobic (See Appendix B).

Physiological Characterization of Mixed Cultures

Studies involving growth and the production of by-products is relatively straightforward when dealing with pure cultures. With mixed microbial populations, however, determining whether the primary substrate or a by-product from another species is transformed tends to complicate the interpretation of results. Also, the type of association between different species of a mixed culture (symbiotic, antagonistic, commensalistic, or neutralistic) has an impact since the interaction could be either beneficial or detrimental to the MEOR process (19).

Therefore, tests for the production of gases, acids, and emulsifiers, were repeated using mixed bacterial cultures. Six different cultures grown on oil were chosen on the basis of their ability to produce a large amount of a given by-product when grown on oil. These representative cultures were employed in combinations of two. The carbon source employed was oil (0.5%) and the nitrogen sources were NO_3 ions, NH_4 ions, or urea. Mineral salts medium (MSM) was prepared with each of the above nitrogen sources at a final concentration of 0.1%. The range of by-product formation for the mixed cultures is shown in Table 2-3 and cell surface hydrophobicities in Table 2-4. Values for the individual mixtures are given in Appendix C.

Data showed that gas production decreased when cultures were grown in a mixture as compared to that when grown alone. In particular, there was a appreciable reduction in the amount of gas production among the cultures grown in the presence of NH_4 ions. In every case, the average gas production of the

TABLE 2-3

The Ability of Mixed Bacterial Cultures to Produce Gases, Acids, and Emulsifiers.

By-Products	NO ₃ ions		NH ₄ ions		Urea	
	Low	High	Low	High	Low	High
Gas	0.32	2.5	0.52	7.31	0.00	1.81
Acid	6.15	7.4	5.35	6.10	7.16	8.30
Emulsifier	3.18	91.5	1.60	50.49	1.60	177.00

1. CO₂ is reported as μ l of gas produced/30 ml of cell culture.
2. The initial pH for all system was 7.
3. Emulsifier concentration is reported as μ g of emulsifier produced/ml of cell suspension.

TABLE 2-4

Cell Surface Hydrophobicity Measurements of the Mixed Bacterial Cultures Grown on Oil.

	<u>NO₃ ions</u>		<u>NH₄ ions</u>		<u>Urea</u>	
	Low	High	Low	High	Low	High
HC Adherence	-20	67	-4.6	58	-6.2	59

Data are reported as percent decreased in turbidity of cell suspension after mixing with 1000 μ l of n-Hexadecane.

two cultures when grown separately was considerably greater than the amount of gas produced by the corresponding mixed cultures, *i. e.* CO₂ production from culture No 7 and No 37 was 24.29 μ l and 24.03 μ l per 30 ml of cell culture, respectively, when grown individually; but was either reduced to 7.31 μ l of gas produced per 30 ml cell culture when grown in combination.

For acid production, the lowest pH for the mixed cultures was 5.35 and the highest was 8.3 as compared to the values of 5.4 and 8.2 for the corresponding single cultures. Interestingly enough, for the cells grown on oil with NO₃ ions or NH₄ ions, the pH of the media for the mixed cultures was either higher than the pH of the media for the corresponding individual cultures, or higher than the pH of the media for one of the corresponding individual cultures, with the exception of the following combination, No 9+25. However, this trend was not observed among the oil-grown cultures with urea since the results were mixed. In this case, the pH of the media for the mixed cultures was either lower than the pH of the media for the individual cultures or lower than the pH of the media for one of the corresponding individual cultures in six out of the seven combinations.

The range of emulsifier production was essentially the same for both single, and mixed, culture systems. Among the mixed cultures, in one of the seven combinations (No 21 + 33) emulsifier production increased as compared to the values for the corresponding cultures when grown alone irrespective of the nitrogen source. Contrarywise, in three of the seven combinations emulsifier production decreased as compared to the values for the corresponding cultures, or the average of the values for the corresponding cultures when grown separately irrespective of the nitrogen sources; 7+9, 21+33, and 33+37. The result were mixed for three of the seven mixtures; 7+37, 9+25, and 9+37 (Appendix C).

The cell surface hydrophobicity of the oil-grown mixed cultures increased in three of the seven combinations (7+37, 9+25, and 33+37) as compared to the values for the corresponding single cultures irrespective of the nitrogen source. In contrast, in one combination, 21+37, cells grown in the mixture were more hydrophilic as compared to the values for the corresponding single cultures irrespective of the nitrogen source. Finally, the results were mixed for the following three mixtures 7+9, 9+37, and 21+33 as compared to the values when grown alone.

It is notable that the initial cellular concentration of each mixed culture was adjusted to 1:1, but after growth this ratio was altered significantly among some isolates.

SUMMARY

The purpose of this task was to develop information on substrate utilization and by-product formation by the indigenous microorganisms in pure culture using selected nutrients prior to determining by-product formation by mixed microbial populations.

The indigenous microflora of the oil reservoirs can and will grow under anaerobic conditions and do produce substances useful in recovering oil (gases, acids, emulsifiers, solvents, and polymers).

TASK 3

INTRODUCTION

The objective of this Task was to investigate the interaction between by-products from indigenous microorganisms and oil-bearing formation materials.

MATERIALS AND METHODS

Materials

Microorganisms

All cultures employed in this task were pure culture isolates obtained in Task 1 and selected on the basis of their ability to produce large quantities of gas, acid, or emulsifiers.

Media

Mineral Salt Solution. The culture medium consisted of mineral salts solution (MSM) with the following inorganic salts per liter of simulated production water:

KNO ₃ or NH ₄ Cl	1.00 g
K ₂ HPO ₄ .3H ₂ O	0.50 g
MgSO ₄ .7H ₂ O	0.20 g
FeCl ₃ .6H ₂ O	0.05 g

The pH was adjusted to 7.0 using 10% (v/v) HCl. A precipitate formed in the resulting medium; therefore, the medium was allowed to settle for 24 hrs and only the clear supernate employed. When a solid medium was desired, Bacto-Agar was added at a concentration of 20 g/l.

For routing cultivation, the incubation vessel used was a 100 ml serum bottle containing 27 ml of MSM. Sterilization was accomplished by autoclaving at 121C (15 psi) for 20 min.

Artificial Seawater. This medium was utilized for the cell reversible sorption studies with the following composition (g/l): NH₄Cl, 0.0007; NaCl, 24; KCl, 0.6; MgSO₄.7H₂O, 5.0; MgCl₂.6H₂O, 3.6; CaCl₂, 0.3; NaNO₃, 0.1; KH₂PO₄, 0.01; FeCl₃, 0.001; tris-HCl, 5.32; tris Base, 1.97; P₁₁ trace metals (below), 10 ml; pH 7.8. The P₁₁ trace metal solution contained (g/l): disodium-ethylenediaminetetracetic acid (EDTA), 1.0; FeCl₃, 0.01; H₃BO₃, 0.2; MnCl₂, 0.04; ZnCl₂, 0.005; CoCl₂, 0.001.

Chemicals

All organic chemicals used in this investigation were reagent grade, and all inorganic chemicals were analytical grade. The chemicals were obtained from Sigma Chemical Company, St. Louis, MO. Bacto-Agar was supplied by Difco Laboratories, Detroit, MI.

Gases

The nitrogen gas used in this study was custom grade and was supplied by Standard Welders, Columbus, MS.

Methods

Cell Concentration Adjustments

Cells were washed from the agar slants with physiological saline (0.85% NaCl, w/v). The harvested cells were centrifuged at 10,000 rpm for 20 min, washed twice with physiological saline, and suspended in a saline (0.425 NaCl, w/v) and potassium phosphate (0.075 M, pH 7.0) solution (1:1 ratio). The cell concentration was kept constant by adjusting the percent light transmission of a 1:10 dilution of the cells on a Coleman Nephelometer to an absorbance of 1.0 at a wavelength of 590 nm and then diluting the original cell suspension appropriately.

Preparation of Cell Suspension in Presence of Formation Material

High purity limestone, sandstone, and clay shale samples were collected, crushed, and pulverized. Prior to sterilization, the pulverized materials were passed through a U.S.A. Standard Testing Sieve No 7 (0.111 opening in inches). Serum bottles were prepared with 25 g of the individual formation material, 27 ml of MSM (pH 7) with NO_3 ions or NH_4 ions, and 5% (v/v) filter-sterilized crude oil. Each bottle was stoppered with a neoprene serum stopper secured with an aluminum band, gased with an atmosphere of nitrogen, and inoculated with three ml of cell suspension (prepared from pure culture isolates as described earlier). The bottles were placed on a New Brunswick rotary shaker at 100 revolutions per min at 32C.

After each week of incubation, a sample was withdrawn from each bottle and streaked on Plate Count Agar to determine if growth had occurred. After growth (usually three weeks), gas, pH, emulsifier, and cell surface hydrophobicity were measured.

Gas Production

Analysis of gaseous compounds was performed using a Fisher Gas Partitioner Model 1200 (dual column, dual detector chromatograph). Column 1 was a 20' x 1/8" aluminum column packed with 37.5% DC-200/500 on 80/100 mesh chromosorb P-AW. Column 2 was a 6' x 3/16"

aluminum column packed with 60/80 mesh molecular sieve, 13X. The column temperature was 70C and the injector temperature was 65C. The carrier gas, helium, was employed at a flow rate of 35 ml per min (back pressure 40 psi). All analyses were performed using a 100 μ l sample. The syringe employed had a gas lock septum (Precision Sampling Corporation, LA). Standard curves were prepared for all gases used throughout these investigations by analyzing various amounts (25 μ l to 100 μ l) of authentic samples and averaging four replications. Identification of gases was achieved by comparison of the retention time of peaks on the chromatogram to the retention times of standard gases. Quantitation was accomplished by comparison of the area under the curve for a given gas to a standard curve prepared with a pure sample of that gas.

pH Measurements of Culture Medium

The initial pH of all media was adjusted to 7.0 using 10% (v/v) HCl or 10% (v/v) KOH. A sample (three ml) of the culture medium from the serum bottle was withdrawn and analyzed using a Fisher Accument Model 915 pH meter after three weeks of incubation at 32C.

pH Measurements of Spent Fermentation Broth

The initial pH of all media was adjusted to 7.0 using 10% (v/v) HCl or 10% (v/v) KOH. A sample (five ml) of the culture medium from the serum bottle was withdrawn and placed into a separatory funnel. To allow for the complete rise of the crude oil, the suspension remained quiescent for 30 min. The resultant culture medium was filtered through Whatman No 1 filter paper to remove particles (limestone, sandstone, or clay shale) and facilitate filtration through a membrane filter. The sample then was passed through a 35 mm Swin - Lok (Nucleopore Corp., Pleasantown, CA) fitted with a 0.45 μ m pore size membrane filter. A (three ml) sample of the resulting filtrate was collected and analyzed using a Fisher Accumet Model 915 pH meter.

Emulsifier Production

A 7.5 ml sample of culture filtrate and 0.1 ml of crude oil were placed on a shaker (Eberbach Corp., Ann Arbor, MI) and agitated at 100 strokes per min for one hr at 32C. Turbidity of the aqueous phase was measured at 540 nm on a B&L Spectronic 20. Quantitation of results was accomplished by comparing the results obtained to a standard curve prepared using the emulsifier [DF-RAG (VET)] produced by Arthrobacter species RAG-1 (ATTC 31012) as described by Rosenberg, et al. (57).

Cell Surface Hydrophobicity

The microbial cell suspension grown on the crude oil was placed in a separatory funnel and allowed to remain quiescent for 30 min to allow for a complete separation of the crude oil from the aqueous phase. The resultant aqueous cell suspension was

centrifuged at 10,000 rpm for 20 min, the harvested cells were washed twice, and resuspended in phosphate urea magnesium sulfate (PUM) buffer (pH 7.12; 22.2g $K_2HPO_4 \cdot 3H_2O$; 7.26g KH_2PO_4 ; 1.8g urea; 0.2g $MgSO_4 \cdot 7H_2O$; and distilled water to 1000 ml), to an optical density of 0.5 at 550 nm (approximately 2.9×10^8 cfu/ml). Each culture was mixed with 1000 μ l of n-hexadecane in a round bottom test tube (10 mm diameter). The tube was preincubated for 10 min at 30C, agitated for 120 sec, and allowed to rest for 15 min to allow the hydrocarbon phase to rise completely. The aqueous phase was transferred to a one ml cuvette using a Pasteur pipette and the light absorbency was measured at 550 nm on a B&L Spectronic 20.

Preparation of Cell Suspensions Without Formation Materials

The culture isolates were grown on oil (0.5% v/v) using either NO_3 ions or NH_4 ions as a nitrogen source. Mineral salts medium (MSM, pH 7) was prepared with each of the above nitrogen sources at the final concentration of 0.1%. Eighty-one ml of the medium was placed in a eight oz prescription bottle fitted with a serum stopper. The bottle was sealed, flushed with nitrogen, and inoculated with nine ml of the pure culture. The vessel then was placed on a New Brunswick rotary shaker at 100 rpm. After one week of incubation (32C), a sample from the bottle was streaked on Plate Count Agar to determine if growth had occurred. This procedure was repeated weekly.

The oil-grown pure cultures were tested for gas, acid, and emulsifier production; cell surface hydrophobicity; and reversible adsorption to solid surfaces. The procedures for the tests were described previously.

Column Assay

Each microbial suspension was divided into three equal portions. Each portion was passed through a column packed with the different formation material. Column eluates were transferred to bottles aseptically.

The columns were prepared as follows: The packed column was constructed of a 12" length of 1/2" glass tubing. The tubing was stoppered at one end with a serum stopper fitted with a 1/8" wide glass tube outlet approximately 1 1/2" in length. The outlet was fitted with a short piece of rubber tubing. A 1/2" plug of glass wool was placed next to the stopper inside the 1/2" tubing. Pulverized limestone, sandstone, or clay shale, that was passed through a U.S.A. Standard Testing Sieve No 7 (0.111 opening in inches) then was added to within 2" of the top of the tubing. The top then was sealed with another serum stopper and the tubes sterilized in an autoclave. The tubes were flushed with nitrogen and 10 ml of simulated production water composed of 1000 ml distilled H_2O containing nine ml of trace mineral solution (71), then closed with a clamp.

Cell Surface Hydrophobicity Measurement. One half of the column eluate was used to measure cell surface hydrophobicity as described in the previous section.

Cell Reversible Sorption to a Solid Surface. The other one half of the column eluate was centrifuged at 10,000 rpm for 20 min. The supernatant was decanted and the cells retained for later use. Samples of individual formation material were suspended in 2.5% NaCl or artificial seawater. The sample was centrifuged for 10 min at 1950 x g, and 20 g of the sample was transferred into nine ml of sterile distilled water. The sample was mixed using a vortex and one ml of a bacterial suspension that was adjusted to an optical density of 0.5 at 550 nm (approximately 2.9×10^8 cfu/ml) was added to the mixture. The sample was incubated for 10 min before centrifugation for five min at 480 x g. The supernatant was decanted, and its volume recorded. In subsequent washing, the formation material was mixed with 10 ml of distilled water, incubated for 10 min, and centrifuged as described above. The number of bacterial cells sorbed onto formation material was determined following desorption by dilution of the sample. To determine the number of cells after each wash, the supernatant was transferred to a one ml cuvette and the light absorbancy measured at 420 nm on a B&L Spectronic 20. The procedure was repeated three times for each sample.

Effect of the Formation Materials on the Spent Fermentation Broth

A sample of the oil-grown cell suspension (prepared as described in Task 2 for mixed cultures) was withdrawn from the serum bottle. The suspension was placed in a separatory funnel and allowed to sit for 30 min for complete separation of the crude oil. The resultant aqueous suspension was filtered through Watman No 1 filter paper then through a 0.45 μ m membrane filter. The culture filtrate then was divided into three equal portions. Each portion was passed through a column packed with a different formation material. The column eluates were collected aseptically.

The spent fermentation broth was then used for pH and emulsifier measurements. These tests were performed according to the procedures described earlier.

RESULTS AND DISCUSSION

Effect of the Formation Materials on Microbial By-products

Reliable information on the interaction between microbial by-products and formation materials, as well as the interaction of microorganisms with formation materials is required to understand some of the mechanisms involved in MEOR. For example, some clay minerals in the rock matrix will absorb emulsifiers, thereby reducing their effectiveness in emulsifying oil. Further, acids

produced by microorganisms will react with carbonaceous cementing materials and thus increase permeability. Microorganisms attach to the rock matrices and alterations of the matrix or environmental conditions can influence the adherence of the microbes to the stratal material. Microbial growth results in a vast array of by-products, not a single product. Consequently, even though the production of an emulsifier is the sought-after effect, the action of all of the other by-products must be known to understand MEOR processes.

Therefore, the approach taken for this task was to grow microbial cultures in the presence of formation materials. The formation materials tested were limestone, sandstone, and clay shale. These materials were selected because they are common materials in oil reservoirs and because of their relative positions at the apices of Hill's proportion triangle for sedimentary rock (63).

Cultures employed in this task were selected on the basis of their ability to produce gas, acid, and/or emulsifiers when grown on oil as determined in Task 2. Eleven cultures from the following groups were used: gas-, acid-, and emulsifier-producers. One-half of the cultures in each group were grown in the presence of NO_3 ions while the other one half had NH_4 ions as the source of nitrogen. Serum bottles were prepared as described earlier. After growth in the presence of the formation materials, tests to determine gas, acid, and emulsifier production were performed.

Based on the data, the formation materials did not prevent the oil-grown culture from producing gas, acid, and emulsifier (Tables 3-1, 3-2, and 3-3 in Appendix D). The maximum values for the test are depicted in Figures 3-1, 3-2, and 3-3. Among the cultures, irrespective of the nitrogen source, the least amount of gas was produced in the presence of the clay shale, while the highest quantity of gas was produced in the presence of sandstone.

In comparing the data for cultures grown in the absence of formation material (Appendix B) to that for cultures grown in the presence of formation material (Appendix D) the following conclusions were drawn. Most cultures produced more gas from oil with NO_3 ions in the presence of formation materials than in the absence of formation materials. Also, one-half of in cultures grown in the presence of formation materials and NH_4 ions produced more gas than they did in the absence of the formation materials.

In terms of acid production, the lowest pH for a culture medium was recorded in the presence of sandstone and NH_4 ions; the highest pH for a culture medium occurred in the presence of clay shale and NO_3 ions (Appendix D). Furthermore, with the exception of culture No 7, the pH of the culture media was reduced for the cultures grown on oil with NO_3 ions or NH_4 ions in the absence of formation materials (Appendix B) as compared to those grown in the presence of formation materials (Appendix D). For culture No 7,

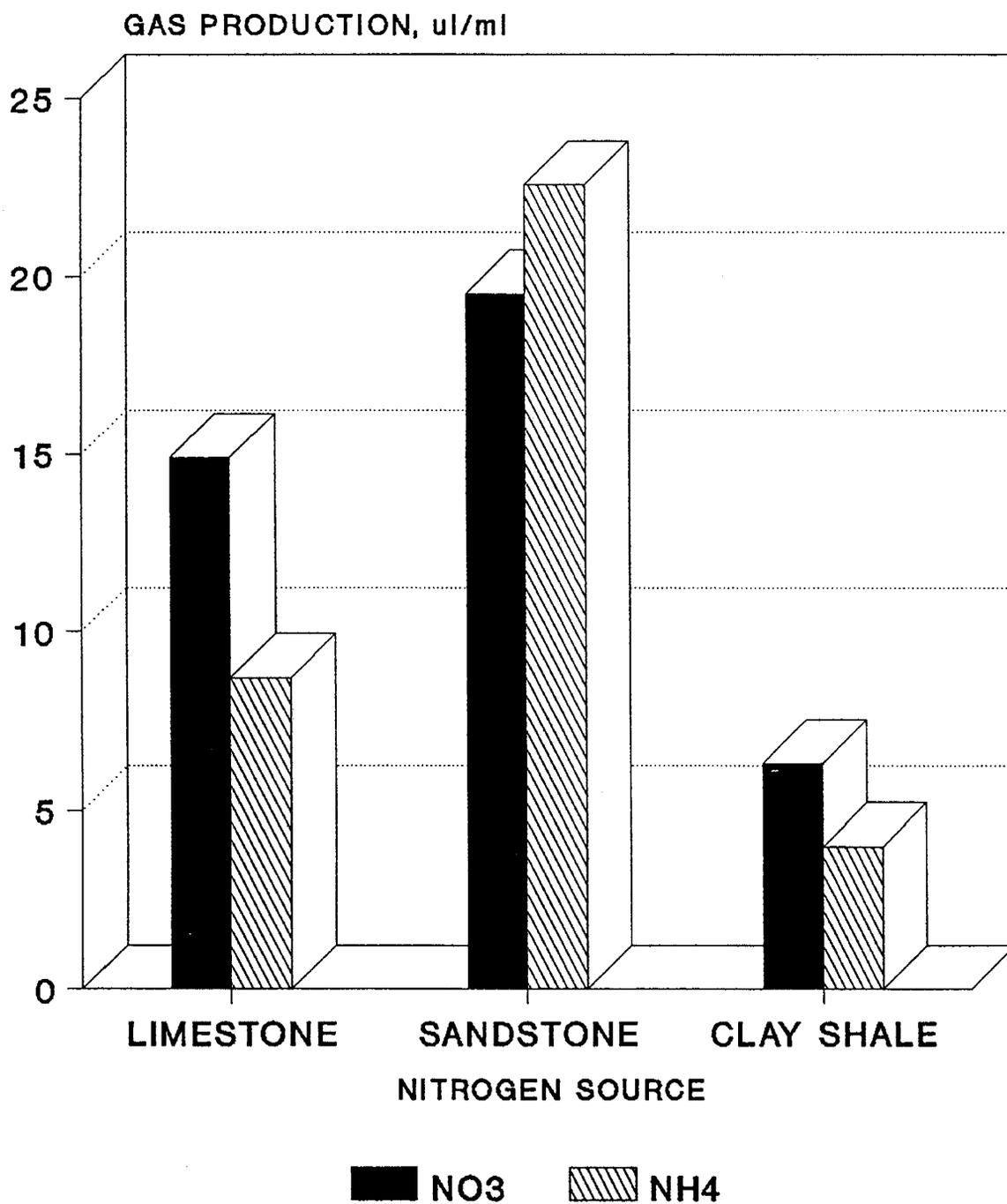


Figure 3-1. The effect of formation materials on microbial gas production

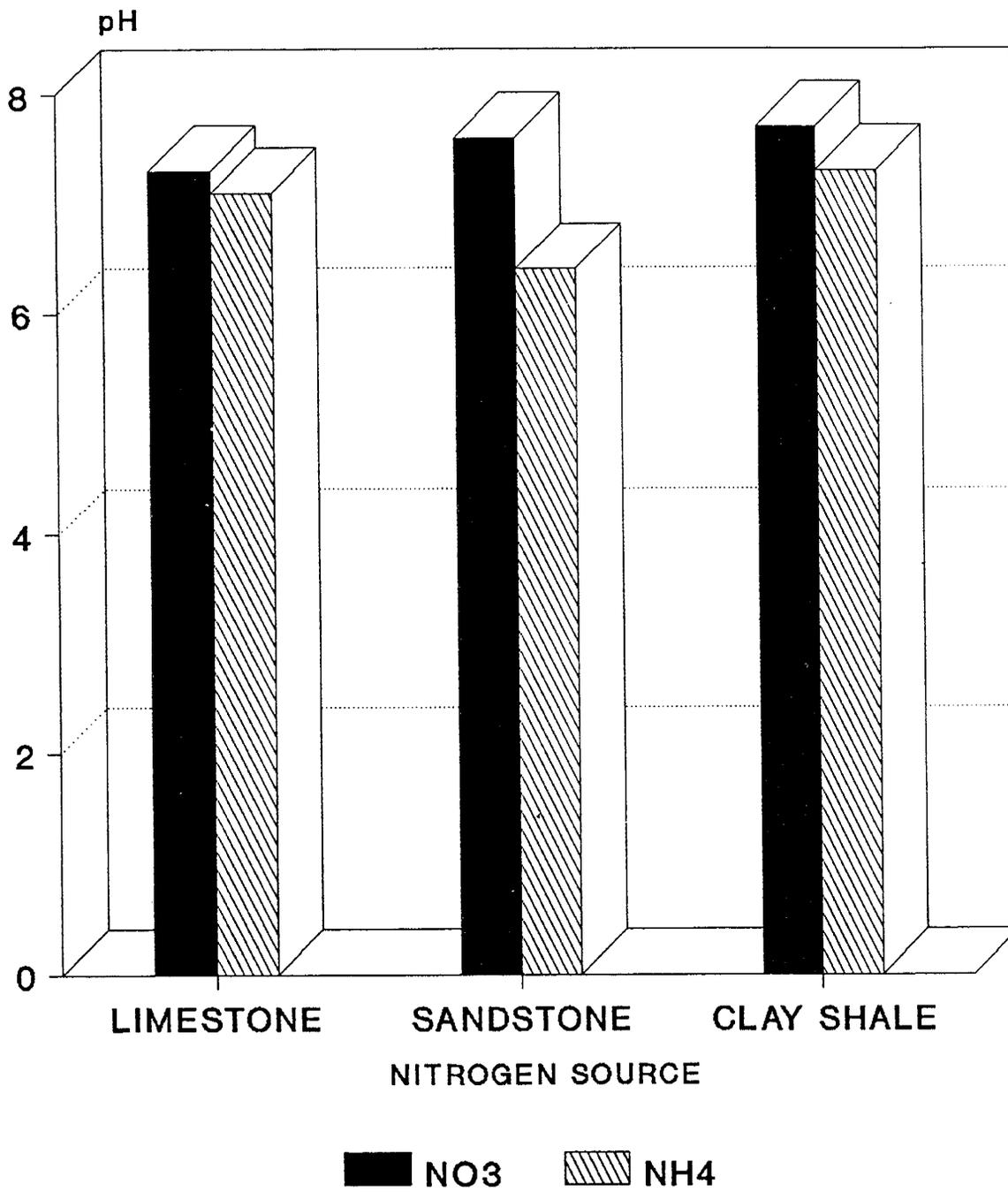


Figure 3-2. The effect of formation materials on microbial acid production

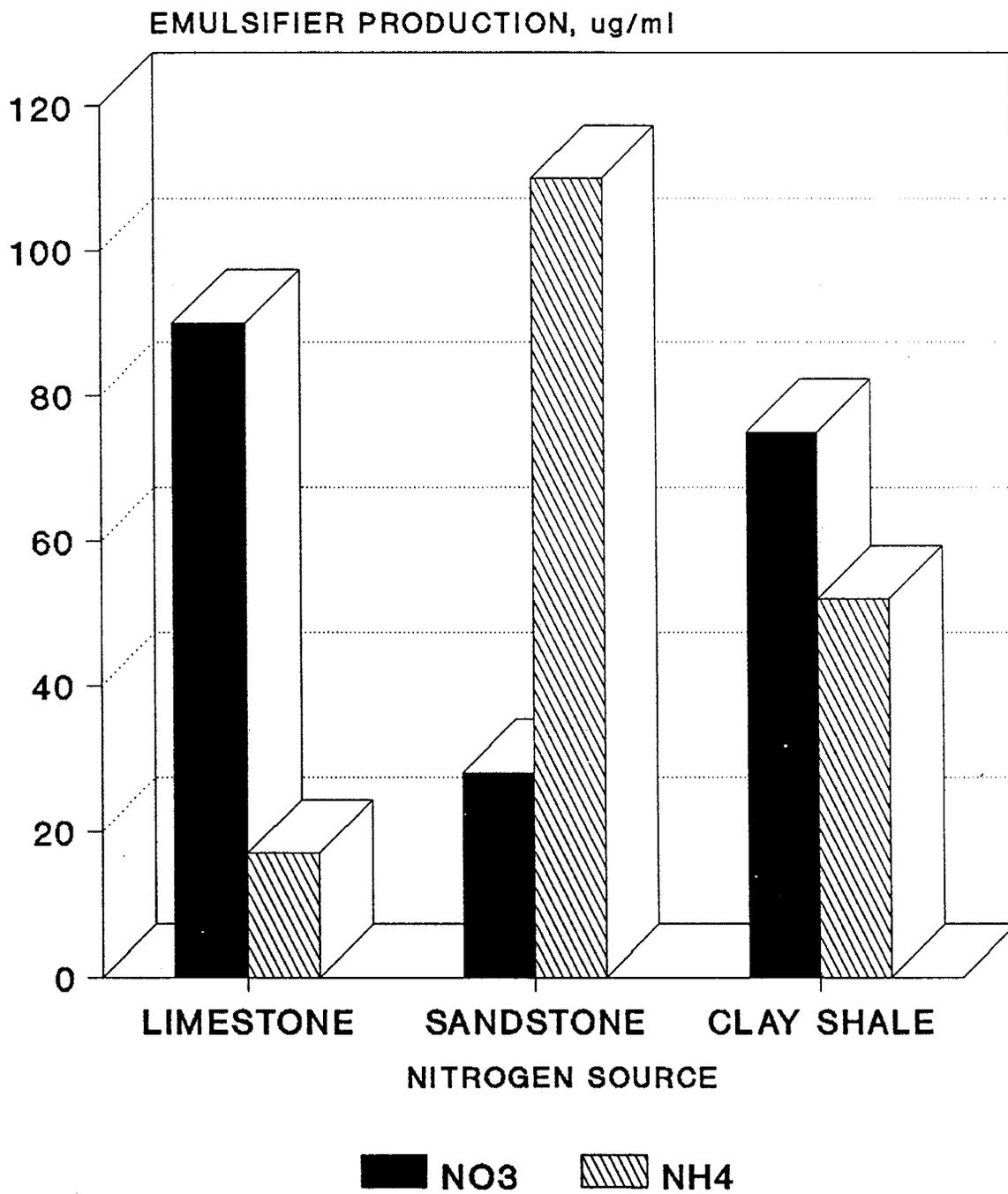


Figure 3-3. The effect of formation materials on microbial emulsifiers

the pH of the medium was reduced as the result of growth on oil with NO_3 in presence of clay shale.

Production of emulsifiers was greatest in the presence of NH_4 ions and sandstone; the least amount of emulsifier was produced in the presence of NH_4 ions and limestone (Appendix D). Once again, comparing the data for Appendix B with that in Appendix D, it may be seen that the production of emulsifiers was decreased for most cultures grown in presence of formation materials and NO_3 ions, while only approximately one-half of the cultures grown in presence of formation materials and NH_4 ions had emulsifier production reduced.

In another series of experiments, selected cultures were grown in combinations of three. Eleven cultures from the following groups were used: gas-, acid-, and emulsifier-producers. The sole source of carbon and energy was crude oil while NO_3 ions or NH_4 ions served as the nitrogen sources. The final pH values obtained from each microbial mixture are given in Table 3-4 (Appendix D). The greatest amount of acid was produced in the presence of sandstone and NO_3 ions or NH_4 ions. It was interesting to note that mixed cultures produced higher amount of acids than did single cultures (see Appendix B and Appendix D).

Interaction of Microbial Cells with Formation Materials

It generally is accepted that the more hydrophobic a cell surface, the greater the tendency of the cell to adhere to a solid surface. The adherence may be permanent, in which case the microbes anchor to the solid surfaces by means of polymer bridging (47). On the other hand, it is thought that in soil environments, reversible sorption is the primary type of adhesion that occurs. Reversible sorption is described as the situation wherein microorganisms are attracted to a surface of unlike charge under conditions where Van der Waals attraction energies exceed the electrical double layer repulsion energies. When microbes are absorbed reversibly to a surface, they are not anchored in any way to the surface. Reversible sorption of microorganisms to surfaces can be overcome by the application of some shear force, by flagellar motion of motile microorganisms, or by reducing the electrolyte concentration to a point where the organisms are repelled from the surface (46). Hydrophobicity of cells is a measure of the degree to which they will be removed from an aqueous medium by a hydrocarbon, such as *n*-hexadecane. In the oil reservoir, both solid surfaces and hydrocarbons are present and the question must be answered as to whether the cells will adhere to the rock surface or migrate with the oil. If cells remain attached to the rock surface, they can act as generators of desired products useful in MEOR; whereas, if they migrate, the probabilities are that they may clog pore throats and thus alter flow patterns.

Therefore, a series of experiments was conducted to study the interaction between microbial cells and the formation materials.

In particular, it was of interest to determine any changes in the cell surface properties including (1) surface charge, and (2) surface adherence. The latter property was detected by alterations in surface hydrophobicity using known hydrophobic cells (as determined in the previous section) from cultures grown on oil in the absence of formation material.

The results for the cell surface hydrophobicity of the cultures grown in the presence of formation materials is shown in Table 3-5, (Appendix D). According to data, cell surface hydrophobicity of the cells was not influenced by the presence of formation materials. This is due to the fact that only one-half of the cell cultures increased in surface hydrophobicity while the other one-half of the cell cultures increased in surface hydrophilicity as compared to the same cultures when grown without formation materials.

Likewise, if the cell surface was altered in regard to the ability of microorganisms to adhere, the Roper and Marshall (56) technique for reversible adsorption was employed. This technique involved determining quantitatively the percent of cells adsorbed to the particular formation material. These results suggest that the desorption of bacterial cells from the formation materials resulted in a pattern similar to that for cells that are adsorbed to the materials at high salinity and are easily desorbed by decreasing the electrolyte concentration. However, reversible adsorption was increased from clay shale to limestone and total recovery was the greatest from sandstone materials.

Interaction of Microbial By-products with Formation Materials

In another series of experiments, the pure culture isolates were grown in the absence of the formation materials. Initially, the cultures were tested for their ability to produce gases, acids, and emulsifiers from crude oil. Also the adherence of microbial cell to the hydrocarbon, and mobilization and movement into the hydrocarbon phase through hydrophobic interaction, were determined (Table 3-6, Appendix D). Then each culture was added to formation materials to determine its reaction, if any, with the formation materials. For this purpose, each microbial suspension was divided into three equal portions. Each portion was passed through a column packed with the different formation material and the column eluates were transferred to bottles aseptically. To determine alterations in cell surface properties, cell surface hydrophobicity measurements were conducted. Finally, to determine if specific compounds are adsorbed by passage through the packed columns, spent fermentation broth was passed through the packed columns. The packed column effluents were tested for changes in acidity and emulsifiers concentration. The results obtained from pH measurements and emulsifiers concentrations are shown in Table 3-7 and 3-8, Appendix D.

Although the highest and the lowest pH of the column eluates were 5.5 and 7.9 irrespective of the formation materials and the nitrogen source, pH alterations by cultures grown without formation materials were 5.5 for the highest and 8.2 for the lowest irrespective of the nitrogen source (Table 3-6).

Additionally, comparisons between the results for the pH of the column eluates (Table 3-7) and the pH of the culture media for the cells grown in presence of formation materials (Table 3-2) indicated that: from limestone columns, 10 of the 11 cultures grown on NO_3 ions had lower pH, while seven of the cultures 11 grown on the NH_4 ions had lower pH; from sandstone columns five of 11 cultures grown on NO_3 ions had lower pH, whereas eight of 11 cultures grown on NH_4 ions had lower pH; and from clay shale columns five of 11 cultures grown on NO_3 ions or NH_4 ions had lower pH.

Emulsifier production increased from 81 $\mu\text{g/ml}$ to 165.69 $\mu\text{g/ml}$ in the presence of NO_3 ions and sandstone, and from 75 $\mu\text{g/ml}$ to 83.09 $\mu\text{g/ml}$ in the presence of NH_4 ions and sandstone when the samples were rinsed through the packed columns as compared to the controls (Table 3-6). However, emulsifier production decreased from 81 $\mu\text{g/ml}$ to 35.69 $\mu\text{g/ml}$ in the presence of NO_3 ions, and from 75 $\mu\text{g/ml}$ to 60 $\mu\text{g/ml}$ in the presence of NH_4 ions when the samples were rinsed through the clay shale packed column as compared to the control (Table 3-6).

Furthermore, according to Table 3-8, among the 11 tested cultures, production of emulsifiers increased in some cases following growth in the absence of formation materials even when the cultures were rinsed through the packed columns as compared to the results for the cultures grown in the presence of formation materials (Table 3-3). Specifically, the following cultures produced higher quantities of emulsifiers: from limestone columns, five cultures were grown on NO_3 ions or seven cultures were grown on NH_4 ions; from sandstone columns, six cultures were grown on NO_3 ions or five cultures were grown on NH_4 ions; and from clay shale columns, four cultures were grown on NO_3 ions or five cultures were grown on NH_4 ions (Table 3-8, Appendix D).

SUMMARY

The purpose of this task was to determine the interaction between microbial cells and/or microbial by-products and the oil-bearing formation materials.

1. The formation materials had virtually no effect on production of gas, acid, and emulsifier.
2. Cell surface hydrophobicity of the cultures, were not influenced by the formation materials.
3. Attached cells were easily detached by reducing the electrolyte concentration.

TASK 4

INTRODUCTION

The objective of this Task was to determine the microbial growth and by-product formation by selected cultures in thin sandpacks. More specifically, the objective was to determine if cultures derived from oil-well cores would colonize stratal material and cause release of oil or alter the flow patterns in the sandpacks subjected to waterflooding.

MATERIALS AND METHODS

Materials

Microorganisms

The six cultures employed in this Task were selected on the basis of their ability to produce gas, acid, and/or emulsifiers when grown on oil in the presence of formation materials as described in Task 3. These cultures are described in Appendix B.

Media

The culture medium used for microbial growth was mineral salts solution (MSM) with the following inorganic salts per liter of simulated production water (described below):

KNO ₃ or NH ₄ Cl	1.00 g
K ₂ HPO ₄ .3H ₂ O	0.50 g
MgSO ₄ .7H ₂ O	0.20 g
FeCl ₃ .6H ₂ O	0.05 g

The pH was adjusted to 7.0 using 10% (v/v) HCl. A precipitate formed in the resulting medium; therefore, the medium was allowed to settle for 24 hrs before use and only the clear supernatant was employed. When a solid medium was desired, Bacto-Agar was added at a concentration of 20 g/l.

For routine cultivation, an eight oz prescription bottle containing 60 ml of MSM was employed. Sterilization was accomplished by autoclaving.

Simulated production water used to prepare the culture medium and for the waterflooding operation was prepared with the following inorganic salts per eight liters of distilled water:

NaCl	778.00 g
Na ₂ SO ₄	130.00 g

MgCl ₂ .6H ₂ O	325.00 g
CaCl ₂ .2H ₂ O	36.00 g
KCl	11.00 g
Na ₂ HCO ₃	3.20 g
KBr	1.60 g
SnCl ₂ .6H ₂ O	0.67 g
H ₃ BO ₃	0.41 g
Na ₂ SiO ₃ .9H ₂ O	0.08 g
NaF	0.05 g
NH ₄ NO ₃	0.03 g
FePO ₄ .4H ₂ O	0.02 g

The pH was adjusted to 7.0 using 10% (v/v) HCl.

This simulated production water was amended in the following ways for use in treating sandpacks.

One solution contained Cl-36 at concentration of 14 μ ci per ml.

One solution contained nitrate ions and glucose. The nitrate stock solution was prepared by dissolving 400 mg of NaNO₃ in 10 ml simulated production water. The working nitrate solution was prepared by mixing 0.5 ml of stock solution with 1,000 ml of the simulated production water. The glucose solution was prepared by adding 0.5 g of glucose to 100 ml of simulated production water.

One solution contained phosphate ions. The phosphate stock solution was prepared by dissolving 20 mg Na₂HPO₄ in 30 ml simulated production water. The phosphate solution for flushing the core was then prepared by mixing eight ml of stock solution with 300 ml simulated production water.

One solution contained P-32 at a concentration of 22 μ ci per ml.

Oil Samples

The cultures were grown on oil from the reservoir of which they were isolated initially. The same oil was employed in preparing the sandpack.

Oil Reservoir Formation Materials

High purity limestone, sandstone, and clay shale samples from unspecified sources were collected, prepared, crushed, pulverized, and passed through a U.S.A. Standard Testing Sieve No 7 (0.111 opening in inches) prior to use.

Sandpack

The configuration and dimension of the sandpacks were as follows:

The plexiglass sandpacks were 6" x 6" by 0.125" and had a total internal volume of 41.49 ml (see Figure 4-1). The sandpack was fitted with a No 17 hypodermic needle in each of two opposite corners to serve as a means of introducing liquids into the sandpack and collecting the effluent. The total liquid volume in the sandpack was approximately 18 ml. On one side of the sandpack 100 holes were drilled, $\frac{1}{4}$ " in diameter. One layer of thin "see through" plastic was placed beneath the side with 100 holes to contain formation materials inside the sandpack. On the opposite side of the sandpack one hole was drilled, 1" in diameter, to be used for filling the sandpacks. The 1" diameter plug was glued on to a $1\frac{1}{2}$ " x $1\frac{1}{2}$ " plexiglass baseplate and, after filling up the sandpack, this hole was closed and then secured with methylene chloride.

Chemicals

All organic chemicals used in this task were either reagent or HPLC grade, and all inorganic chemicals were analytical grade. The chemicals were supplied by Sigma Chemical Company, St. Louis, MO; and Aldrich, Milwaukee, WI. Radiochemicals were purchased from ICN, Irvine, CA. Bacto-Agar was supplied by Difco Laboratories, Detroit, MI.

Methods

Preparation of Microbial Inoculum for Sandpacks

Cells employed in preparation of the sandpacks were washed from agar slants with physiological saline (0.85% NaCl, w/v), centrifuged at 10,000 rpm for 20 min, washed twice with physiological saline, and suspended in a saline (0.425 NaCl, w/v) and potassium phosphate (0.075 M. pH 7.0) solution (1:1 ratio). The cell concentration was kept constant by adjusting the percent light transmission of a 1:10 dilution of the cells on a Coleman Nephelometer to an absorbance of 1.0 at a wavelength of 590 nm and then diluting the original cell suspension appropriately.

Eight oz prescription bottles contained 30 g sterilized formation materials, 60 ml MSM (pH 7) with NO_3^- ions or NH_4^+ ions, and 5% filter-sterilized crude oil. The bottles were sealed, flushed with nitrogen, and inoculated with 5 ml of the pure culture. Microorganisms were given time and opportunity to adhere to the surface of the formation material by placing the bottles on a New Brunswick rotary shaker (100 rpm) at 32C for one week. After incubation, a sample was withdrawn from each bottle and streaked on Plate Count Agar to determine if growth had occurred.

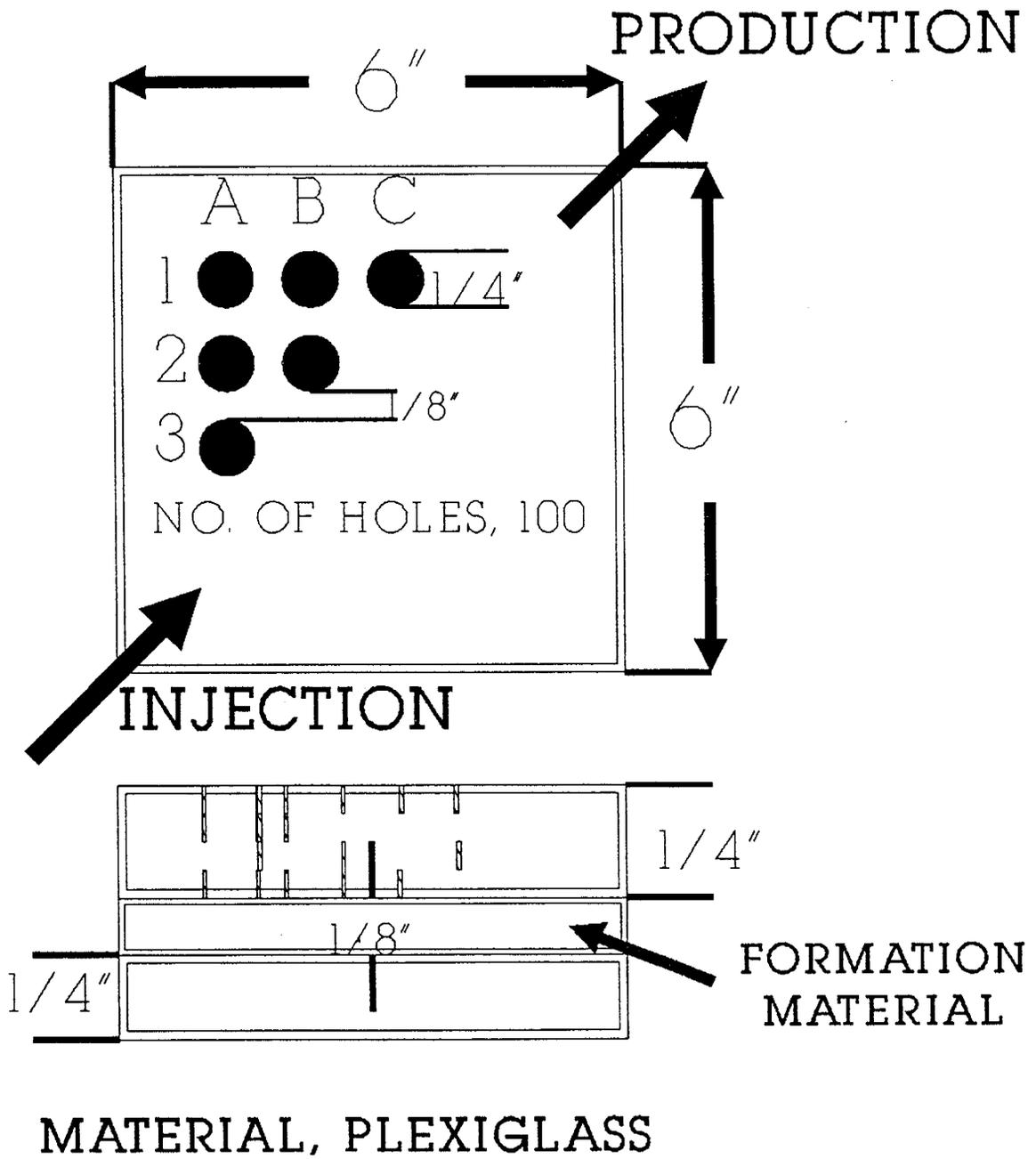


Figure 4-1. Diagrammatic sketch of plexiglass sandpack

In some cases, each pure culture isolate was used individually. In other cases pure culture isolates were grown separately and mixed prior to packing the sandpacks.

Sandpack Preparation

A paste of oil, five ml; simulated production water, 10 ml; microbial inoculum, five ml; formation material 100 ± 10 g; was used to pack the sandpack through the opening provided for this purpose while the sandpack was held on a vortex. The sandpack was packed uniformly and the entry port was closed and sealed.

Simulated production water was pumped slowly (1.4 ml/min) through the sandpack until the effluent no longer contained oil and thus mimicked a depleted oil reservoir. Next, the flow pattern in the sandpack was determined injecting 10 ml of simulated production water containing 140 μ ci Cl-36 at a flow rate of 1.4 ml/min. To determine the location of the injected water, radioactivity measurements were performed at each of the sandpack holes (total of 100 holes/pack) using a Ludlum Model 2200 Scaler Ratemeter. From these measurements, the path of water through the sandpack was projected. The sandpack then was flushed with simulated production water, without Cl-36 until the effluent contained no more radioactivity (See Figure 4-2).

Next, the sandpack was subjected to treatment with simulated production water containing nutrients. This procedure included the addition of nitrate ions and glucose every other day. On alternate days only simulated production water was employed (1.4 ml/min). After one week, the same procedure was repeated using simulated production water containing orthophosphate. Approximately 300 ml of the nitrate and glucose solution or the phosphate solution was allowed to flow through the sandpacks at the rate of 1.4 ml/min. Deviations in flow pattern was assessed by the biweekly addition with Cl-36, (as described above).

In an attempt to identify the areas of the sandpack with the greatest microbial population, orthophosphate labeled with P-32 (injection of 10 ml simulated production water containing 220 μ ci P-32 per sandpack) and nitrate were added to simulated production water and slowly pumped into the sandpack (1.4 ml/min). Following overnight incubation, the sandpack was flushed with simulated production water without P-32. Radioactivity measurements were performed at each of the sandpack holes (total of 100 holes/pack) using a Ludlum Model 2200 Scaler Ratemeter.

Control packs did not receive nitrate ions but did receive Cl-36 and phosphate with P-32 to identify flow patterns and areas of microbial growth.

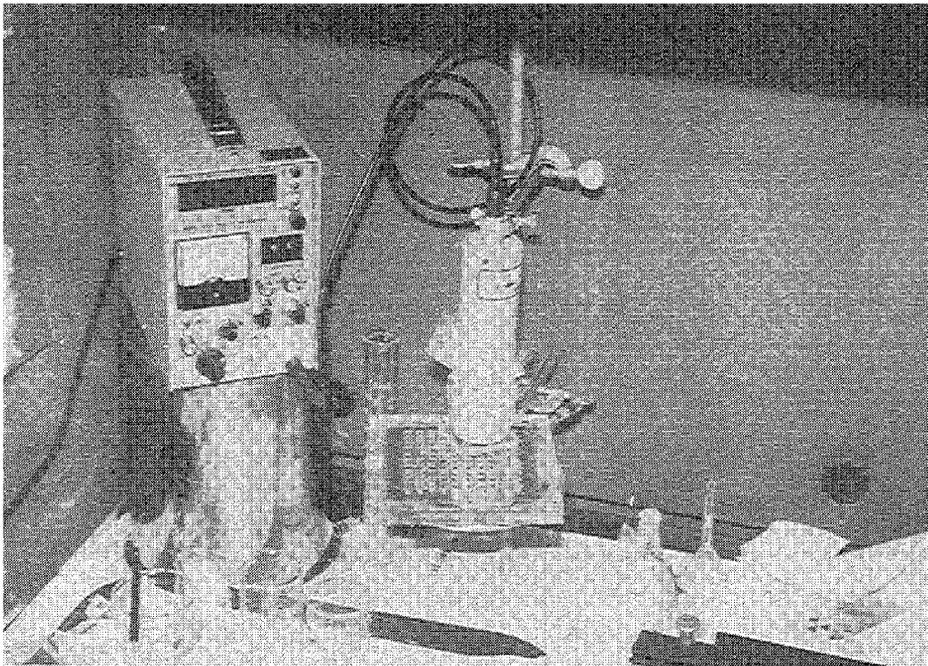


Figure 4-2. Photograph of radioactivity readings being taken on a sandpack.

LET

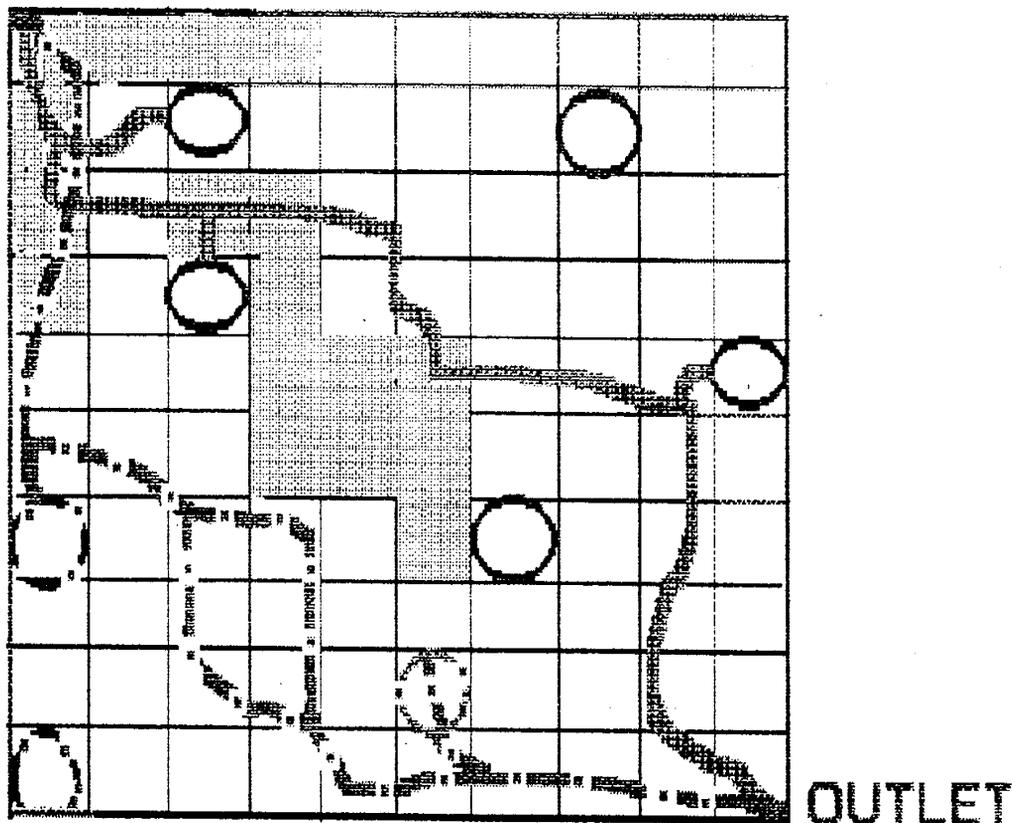


Figure 4-6. Areas of high P-32 content, projected initial flow pattern, and the projected flow pattern of water through a sandpack after treatment of the sandpack contents with supplemental nutrients.

Cl-36,1 [Dense grid pattern]

Cl-36,2 [Sparse grid pattern]

P-32 [Dense grid pattern]

INLET

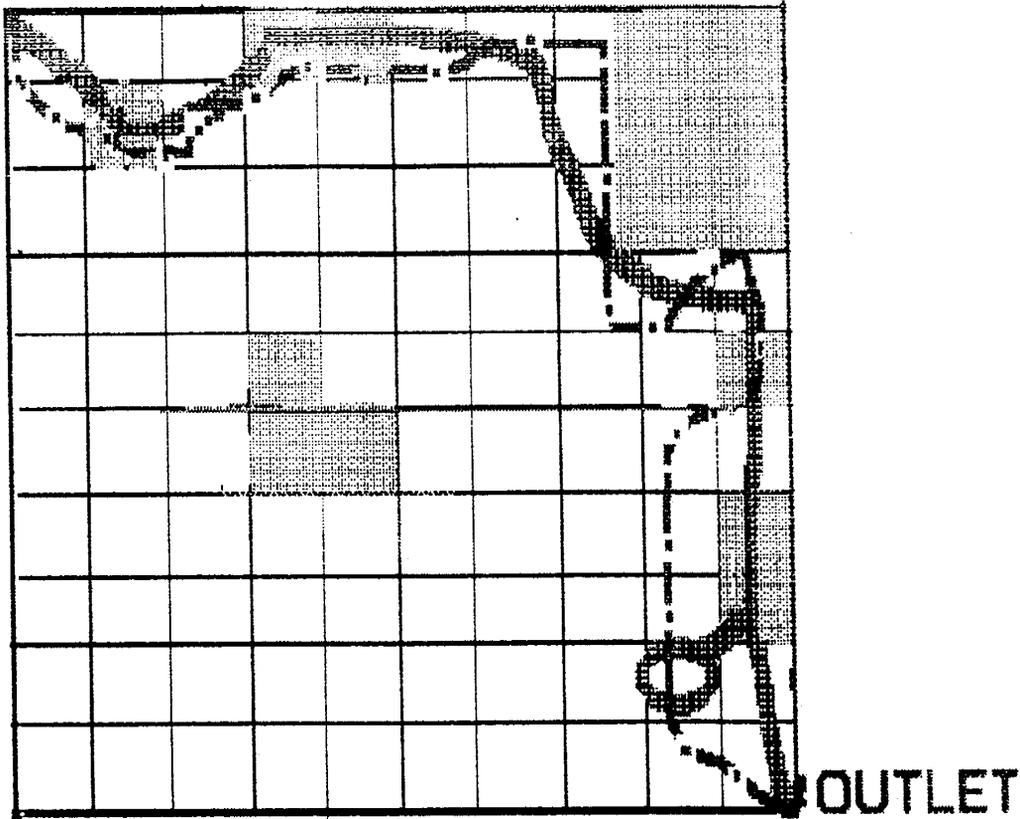


Figure 4-7. Areas of high P-32 content, projected initial flow pattern, and the projected flow pattern of water through a control sandpack.

Cl-36,1

Cl-36,2

P-32

Chemical Analysis of The Sandpack Effluent

A sample (first 10 ml) of the effluent from the sandpack was collected after simulated production water was pumped slowly (1.4 ml/min) through the sandpack. If no oil was visible, the effluent was retained and analyzed as follows: The sample was extracted with an organic solvent, methylene chloride. The extract was reduced to a minute quantity before addition of hexane under an atmosphere of nitrogen. The resultant extract then was concentrated to 200 μ l under a nitrogen atmosphere. The aliphatic profile was determined by GC with a non polar, DB5, capillary column and a flame ionization detector. Oven temperature was at 4.4C initially and at 141C finally. The run length was 59 min.

A second effluent sample was collected from the sandpack for GC analysis after injection of the last simulated production water containing C1-36.

Chemical Analysis of The Sandpack Composite Formation Material

Attempts were made to verify that the area of the sandpack showing high P-32 activity was, indeed, accompanied by areas of high microbial activity. This was accomplished as follows: After addition of simulated production water containing P-32, composite samples from areas of high P-32 activity and low P-32 activity were collected and analyzed by GC for aliphatic profile as described earlier.

RESULTS AND DISCUSSION

Sandpack Study

After establishing that microorganisms are indigenous to oil reservoirs, and that their activities could prove useful to oil recovery, it was shown that these activities are not inhibited by the presence of formation materials. The next logical step was to evaluate these microbial activities in a more realistic setting; e. g., a sandpack. Since it is known that microorganisms form their own microenvironment (the immediate vicinity surrounding the microorganisms) the overall outcome of microbial activity may be different from what might be predicted on the basis of studies on the cultures using conventional microbiological methods. For example, some microorganisms are unable to grow in the presence of another species in the laboratory but can survive and grow quite nicely when afforded an opportunity to develop within their own microenvironment. This is the situation expected in petroleum reservoirs. Another factor impacting on the situation is that some microbes grow at the expense of the by-products of others and, in some cases, this interaction may involve more than two microbial types.

Obviously, if microbes are growing in a reservoir they will increase the space they occupy and, consequently, would alter flow patterns in waterflooding operations. Selective plugging is the basis for at least one MEOR process.

To gain some insight into the activities of selected microbial isolates in a sandpack environment 25 sandpacks were prepared and flushed with simulated production water until the effluent was clear and contained no visible oil. Production water containing Cl-36 was passed into the sandpacks to establish the flow pattern of the water, as illustrated in Figure 4-3. The Cl-36 was flushed from the sandpacks with simulated production water. Twenty three of the sandpacks then were treated with simulated production water containing nitrate ions, orthophosphate ions, and glucose. Two of the packs received only simulated production water and thus served as controls.

After treatment for various lengths of time, the flow patterns were again determined using Cl-36. Figure 4-4 shows both the initial flow pattern and the flow pattern for one of the sandpacks after treatment with supplemental nutrients. Figure 4-5 shows the initial flow pattern and final flow pattern for a control sandpack not supplied with supplemental nutrients. As may be observed, the final flow pattern in the control pattern parallels the initial flow pattern extremely well while the flow pattern in the treated sandpack was altered appreciably.

An attempt was made to identify areas of high microbial activity in the sandpacks by subjecting the sandpacks to P-32 in the simulated production water, flushing with simulated production water without P-32, and then identifying areas of high radioactivity. Figures 4-6 and 4-7 show these areas superimposed on Figures 4-4 and 4-5, respectively. It cannot be stated with certainty that the areas of high P-32 activity actually are areas of high microbial activity. Therefore, samples from areas with high radioactivity and samples from areas with low radioactivity were removed from the sandpacks and the aliphatic profiles of residual oil determined. The data indicated clearly that the residual oil from the areas of high radioactivity had undergone considerably more decomposition than the oil from the areas of low radioactivity even in the control packs.

The initial flow patterns, final flow patterns, and areas of high P-32 concentrations for all 25 sandpacks are given in Appendix E. Even a cursory examination of the flow patterns in these figures reveals that changes in the path of flow were different in all 25 packs. This result was not unexpected in light of the fact that the formation material in the sandpacks was not highly compacted and consequently changes in flow patterns could result from changes in the pressure of the injection water.

Furthermore, changes in the flow pattern in the two control sandpacks was not totally unexpected since the simulated production water did contain small amounts of nitrate ions and orthophosphate ions.

Another difficulty was inherent in the phase of the experiments using P-32 to identify areas of heavy microbial concentration. Here again, the data in the appendix demonstrate the variation in results but the fact remains that there were changes in the aliphatic profile of oil taken from areas of high P-32 activity vs areas of low P-32 activity (Table 4-1, Appendix E).

Certainly, when all of the data are considered, it was reasonable to conclude that microorganisms did indeed grow in the sandpacks and did colonize the stratal material. This conclusion is confirmed in part by the observations noted on many of the figures in the appendix and by virtue of the fact that at the conclusion of the experiment streak plates of the contents of all of the sandpacks yielded viable microorganisms.

SUMMARY

Some difficulty was encountered in evaluating the results of this task due, at least in part, to the lack of compaction of the materials in the sandpacks. Nevertheless, it was shown that the microorganisms did grow in sandpacks and a preponderance of the evidence indicated that they did indeed colonize the stratal material and did cause some alterations in the flow patterns of the injection water.

TASK 5

INTRODUCTION

The objective of this Task was to determine if the treatment of cores from subterranean oil reservoirs with nutrient solutions would activate the in situ indigenous microflora and result in more oil recovery.

The contract called for conducting tests only on two sets (one test and one control) of cores from two different reservoirs. However, with financial support from the University, four additional sets of cores were tested and the results included herein in order to give a more complete picture of the activities of the in situ indigenous microflora.

MATERIALS AND METHODS

Collection of Oil Well Cores

Arrangements were made with several oil companies to acquire cores directly from the core barrels immediately as they came from the well. The cores were obtained from oil-bearing formations with no previous history of enhanced oil recovery activities at all, including waterflooding.

The cores were received as they were pulled out of the core barrels (live cores). The cores were broken into 1-ft sections, wiped off with 70% ethanol, and immediately placed in BBL® GasPak® System containers under anaerobic conditions. This procedure was completed within minutes, thus exposure to air was minimal. This treatment was of particular significance for these microbiological studies. It should be pointed out that the pressure in the core tends to force fluid and/or gases outward, thereby reducing further the possibility of exposing the internal section of the core to air. The anaerobic containers were packed in ice, transported to a laboratory, and placed in a refrigerator at 4C until needed.

Preparation of Cores for Core Flood Experiments

Cores were removed from the GasPak® containers under a nitrogen atmosphere and two adjacent core plugs were cut radially from each core - one to serve as the test core and one as the control core. The plugs were 3-4" long and 1½" in diameter (See Figure 5-1). While still under a nitrogen atmosphere, each plug was inserted immediately into a special heat shrink plastic tube. The plastic wrap shrank as it

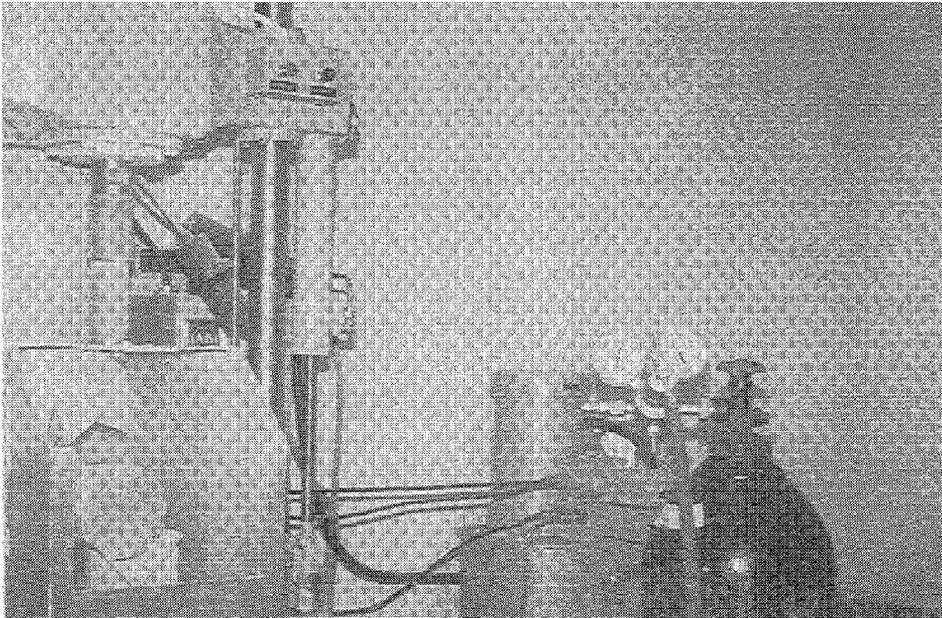


Figure 5-1. Photograph of core cutting machine for making core plugs. (Note tank of nitrogen supplying N_2 atmosphere.)

cooled and wrapped tightly around the core. An entry and an exit port were placed on opposite ends of the core. These ports contained grooves for the reduction of end effects and for more homogeneous distribution of flowing fluids. The entire assembly then was inserted into a thick rubber sleeve (Viton Neoprene Sleeve, 1.5" diameter, with a $\frac{1}{4}$ " wall). The ends of the entry and exist ports were fitted with rubber tubing and clamped shut. Both ends were completely sealed with high strength epoxy glue. The glue was allowed to harden for 24 hrs before the cores were used. Figure 5-2 is a diagrammatic sketch of the assembled core. Figures 5-3 and 5-4 are photographs of the cores.

Treatment of the Cores

Initially, simulated production water contained in a 13½ gal carboy was allowed to flow through the core. The carboy was situated approximately 25" above the core and this hydrostatic head constituted the total pressure applied to the influent. The water was allowed to flow through a core until no oil was visible in the effluent (usually within 24 hrs). After a 24 hr waiting period, experimentation commenced. Control cores received simulated production water only while the test cores received simulated production water containing added nutrients - nitrate as sodium nitrate; orthophosphate as dipotassium hydrogen phosphate; and ethanol.

Simulated production water used for the waterflooding operation was prepared with the following inorganic salts per eight liters of distilled water.

NaCl	778.00 g
Na ₂ SO ₄	130.00 g
MgCl ₂ .6H ₂ O	352.00 g
CaCl ₂ .2H ₂ O	36.00 g
KCl	11.00 g
Na ₂ HCO ₃	3.20 g
KBr	1.60 g
SnCl ₂ .6H ₂ O	0.67 g
H ₃ BO ₃	0.41 g
Na ₂ S ₂ SiO ₃ .9H ₂ O	0.08 g
NaF	0.05 g
NH ₄ NO ₃	0.03 g
FePO ₄ .4H ₂ O	0.02 g

The pH was adjusted to 7.0 using 10% (v/v) HCl.

Analyses of Effluent from Cores

Fluid volume, oil content, and microbial content were measured and recorded periodically for all cores and the final 50 ml of effluent was collected aseptically in a sterile six

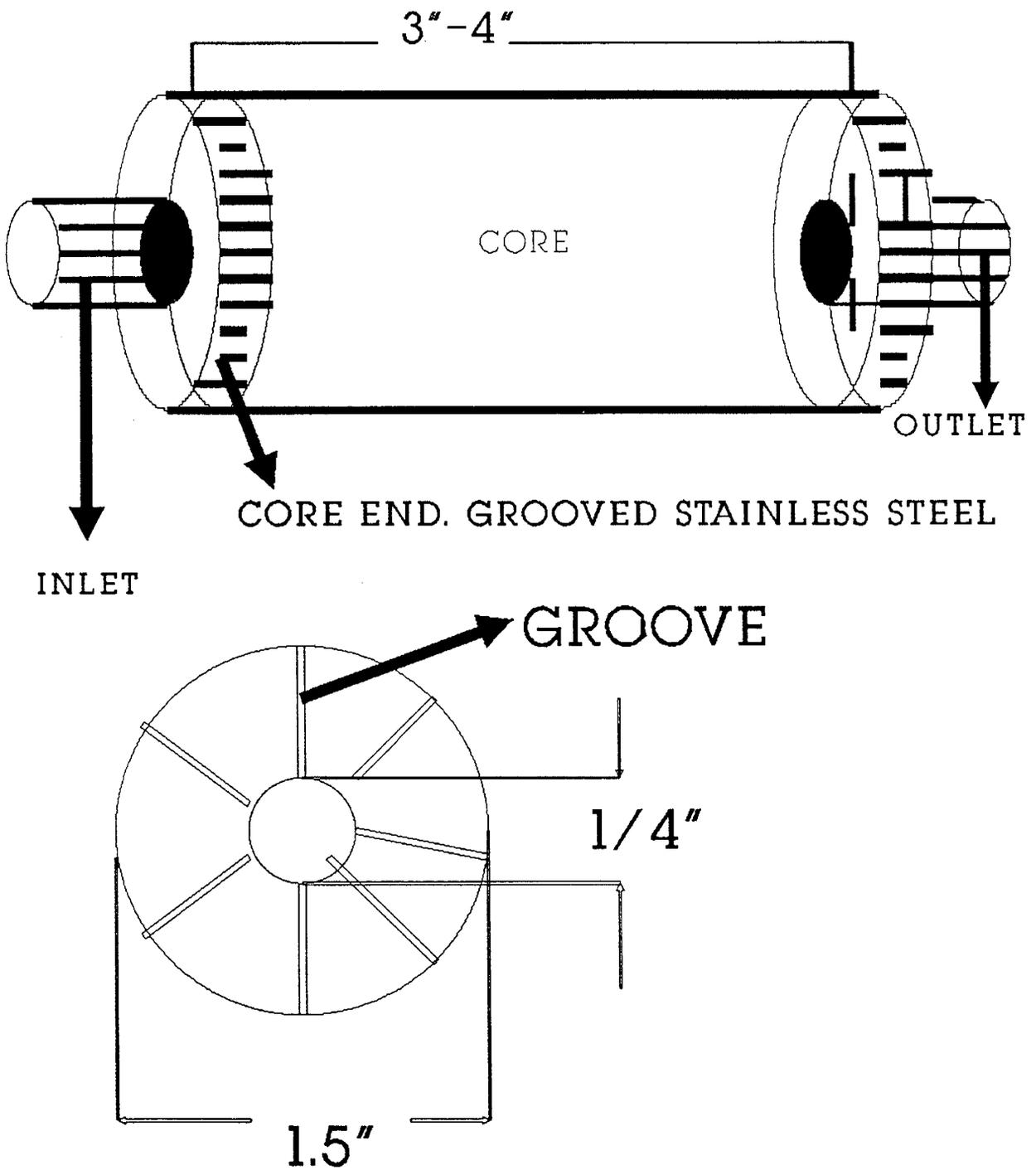


Figure 5-2. Live core assembly.

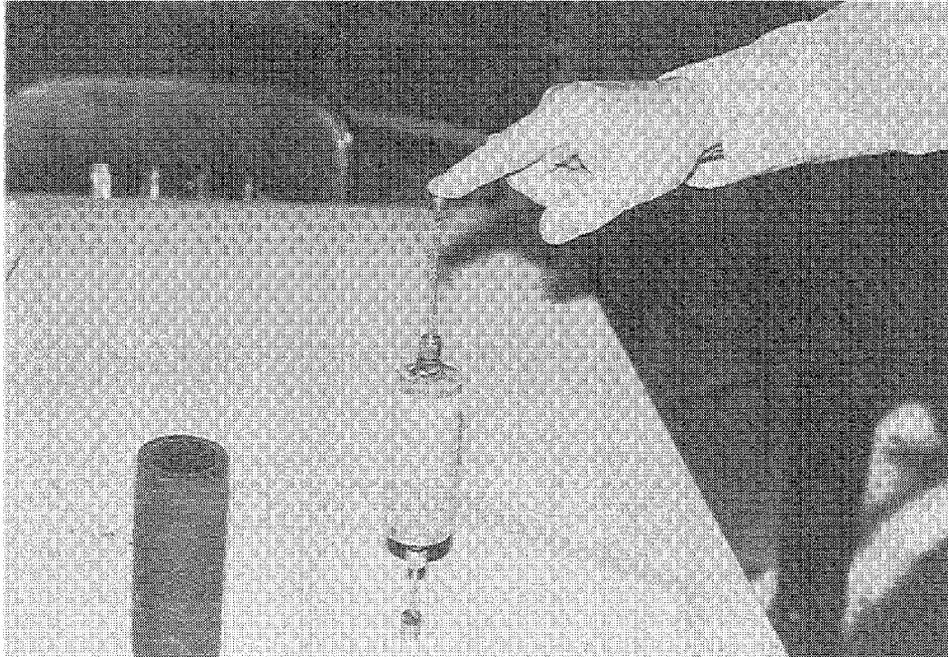


Figure 5-3. Photograph of core in plastic wrap next to the rubber sleeve.

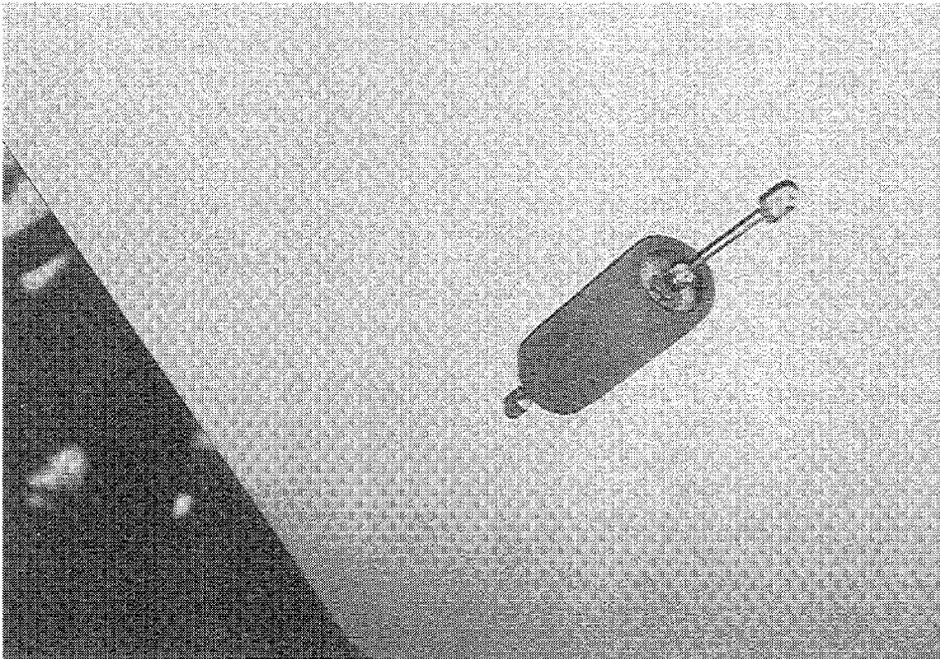


Figure 5-4. Photograph of completely assembled core.

oz prescription bottle. Plate counts were conducted on selected samples using Plate Count Agar prepared using simulated production water. Plates were incubated for 72 hrs under aerobic conditions or two weeks for anaerobic conditions.

RESULTS AND DISCUSSION

General Considerations

The microflora in the oil reservoir undoubtedly exists in numerous loci, each with its own microenvironment. Trying to reproduce such a situation in the laboratory is virtually impossible. Admittedly, the information obtained from Task 4 was helpful, but construction of a realistic model was not possible, at least not within a reasonable period of time and not with all of the heterogeneities of the actual reservoir formation and most importantly, with the microflora in the natural condition. As pointed out by ZoBell (72,76), the microflora in the reservoir exists in dynamic equilibrium with its environment and reconstruction of the total system can not be accomplished. It is known that the microflora can have a major impact on the heterogeneity of the formation - either reducing it or creating it. Studies using Berea Sandstone cores have been helpful but are woefully inadequate for studies involving the in situ indigenous microflora in an oil reservoir. Field studies have been conducted by a number of workers, but the results have been mixed and the contribution of microflora to oil recovery is only speculative. In truth, the heterogeneities of oil reservoirs preclude the conduct of classical scientific experiments wherein the results of a given treatment are compared to a control not receiving the treatment.

Therefore, to study the indigenous microflora in its native microenvironment, live cores were employed so that formation materials, microorganisms, oil, and water were in as near a natural state as possible.

Core History

Information regarding the origin of the cores employed in this task is given in Table 5-1. It may be observed that the information on cores 1-4 already has been given in Task 1 but is included here for convenience. Also given are the petrophysical (Table 5-2) and other characteristics of the cores (Table 5-3).

The Effect of Supplemental Nutrients on the Microflora of Live Cores

Experiments were conducted with cores from five different reservoirs. The complete details of treatment and results are

TABLE 5-1

Location and Depth from Which Cores Were Obtained.

Reservoir No	State	County	Field	Well No	Depth	Cut*	Oil Co
3	TX	Andrews	Mabee	# 648 JEM "A" NCT-1	4,725'	8/9	Texaco
4	TX	Ector	Johnson	J-GBSAN No 1809	4,050'	9/18	Oxy USA
5	NM	Lea	Emsu	679	4,300'	10/16	Chevron
6	TX	Crane	McElroy	JT ME 1156	2,705'	10/14	Pruett
7	WY	Johnson	Cellers Ranch	Texaco No 2	6,568'	7/30	Texaco

* All dates are in 1990.

TABLE 5-2

Grain Density, Permeability and Porosity of Tested Core Plugs

Reservoir No	Formation	ρ_{gr}	K_{gas}	K_{liq}	ϕ
3	Dolomite	2.78	3	1.1	12.8
4	Sndy-Dolom	2.90	2	<1	5.32
5	Dolomite	2.83	20	12	14.6
6	Dolomite	2.78	2.45	2	14.0
7	Sandstone	2.80	3.8	3	13.3

ρ_{gr} : Grain density, gr/cc
 K_{gas} : Gas permeability, md
 K_{liq} : Liquid permeability, md
 ϕ : Porosity, %

TABLE 5-3

Characteristics of Reservoir Fluids.

Reservoir No	GR	μ	ST	ρ	μ	SALI	ST	IFT	T _{res}
3	29	.75	28.1	1.02	.73	12K	38.40	14.80	103
4	33.5	.78	26.8	1.04	1.10	27K	69.10	19.30	102
5	33	.83	26.6	1.00	1.20	7.3K	51.40	3.88	90
6	31	8.00	27.2	1.02	.70	16K	61.40	.91	106
7	18.6	54.00	31.9	1.00	.85	2.4K	7.77	23.96	126

GR : Oil gravity, API
 μ : Viscosity, cp
 ST : Surface tension, dyn/cm
 SALI : Salinity, ppm
 IFT : Interfacial tension, dyne/cm
 T_{res} : Reservoir temperature, °F

given in Appendix G. A brief synopsis of each experiment is given below.

Experiment One

The test core received supplemental nitrogen and phosphorous (as NaNO_3 and K_2HPO_4) while the control core received only simulated production water. The cores employed in this experiment were prepared from a core from Reservoir 3. Over the course of nearly two months the following observations were made:

- The Control Core Effluent: * remained clear
 * had no signs of oil
 * contained approximately
 10^4 microorganisms/ml.
- The Test Core Effluent : * was turbid much of the
 time and contained
 fine particulate matter
- * contained oil
- * contained
 approximately 10^6
 microorganisms/ml
- * became plugged on two
 occasions but flow was
 restarted by applying
 a gentle pressure to
 the influent.

Upon completion of the experiment, the cores were evaluated petrophysically with the following results.

- The Control Core: * had a much darker color due to
 residual oil still in the pores
- * no microchannels were visible
 in the core
- * had a porosity of 12.8%.
- The Test Core : * showed a dissolution of
 carbonate and some microchannels
 were visible
- * was bright in color with only a
 few dark spots due to oil
- * newly formed channels on the
 outside of the sample were
 quite visible

- * chunks of calcite were dissolved causing relatively speaking large cavities along with wide channels
- * wherever there were traces of calcite or in general carbonaceous material, it was dissolved, leaving cavities and channels
- * had a porosity of 17%.

Changes in permeability were minimal due to very tight channels. Appearance of the cores after the tests is shown in Figure 5-5.

Experiment Two

The experimental cores for this experiment were prepared from a core from reservoir 4. The test core received supplemental nitrogen and phosphorous while the control core only received simulated production water. Microorganisms were present in the effluent from the control core but there were no visible signs of oil or particulate matter in the effluent. Contrariwise, not only did the effluent from the test core contain microorganisms, gas was produced and both oil and particulate matter were present. Also, on one occasion, the test core became plugged but, flow was restarted by application of a slightly increased pressure on the influent.

Since the core contained dolomite, and there was evidence of microbially produced acids, it was conjectured that acid production could be enhanced by supplying the microflora with a metabolic precursor of an acid, namely ethanol (15 μ M). The results of this addition were striking in the test core. Within several days, the flow rate increased significantly and large amounts of particulate matter were present in the effluent. While ethanol also was added to the control core, no observable changes occurred indicating that it was not the ethanol per se causing the results observed for the test core. It must be remembered that the control cores did not receive supplemental nitrogen and phosphorus.

Upon completion of the experiment, the cores were subjected to petrophysical analyses with the following results. These cores were highly heterogeneous and consisted of a very tight dolomite. Water would not flow through the cores due to the extremely low permeability and low inlet fluid pressure (hydrostatic head only). Consequently, fluids found their way around the core adjacent to plastic wrap. Therefore, microbial activity occurred only on the surface of the test core with the development of visible cavities and microchannels. No visible changes were evident on the control

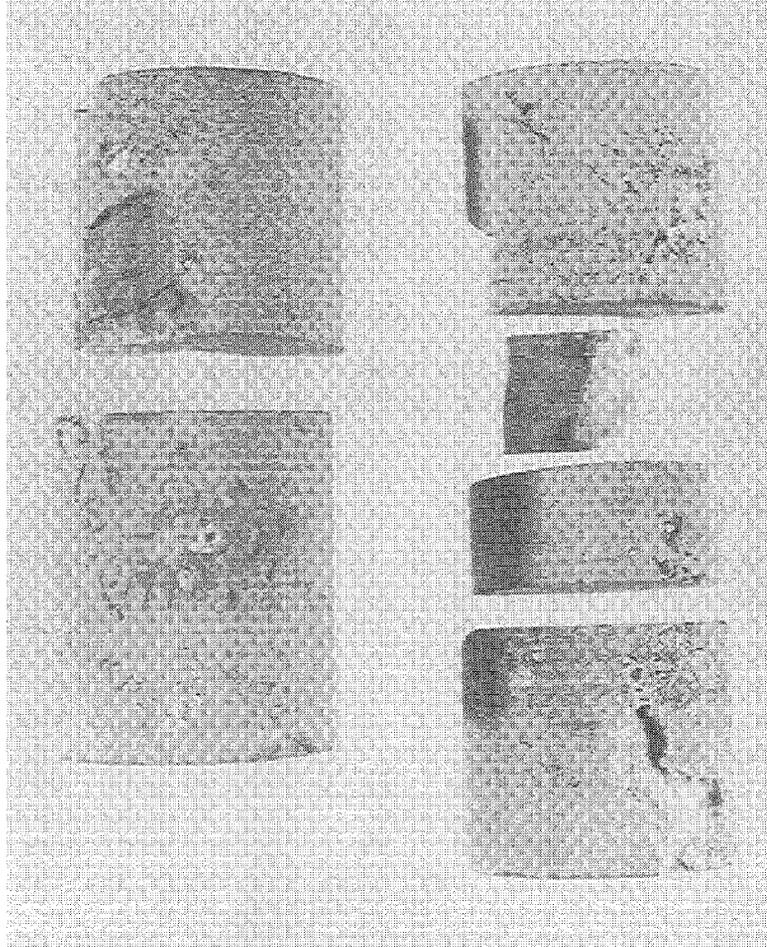


Figure 5-5. Photograph of the control (left) and test cores (right) from reservoir no. 3 after treatment.

core surface. The test core appeared whitish due to removal of oil while the control core was brownish in color from the oil present in it.

The test core porosity was 7.4% while the control core porosity was 5.95%. No data on permeability was collected due to a lack of measurable flow.

Figure 5-6 shows the appearance of the cores after the experiment was completed.

Experiment Three

The protocol for this experiment was the same as for Experiment 2 except that the cores were prepared from a core from reservoir 5.

During treatment, the microbial content of the effluent from the control core was low, there was no particulate matter or oil in the effluent, and the flow rate through the core was very low. After treatment of the test core with nitrogen and phosphorus, there were more microorganisms in the effluent but only a slight trace of oil. After one month of treatment, both the control and test cores were given ethanol and within several days there was a dramatic increase in the flow rate through the test core and particulate matter and oil were present in the effluent.

Petrophysical examination of the cores after completion of the experiment showed that both the test and the control core samples were of a very tight dolomite type. It seemed that whatever channels were available for fluid flow were plugged by formation clay due to waterflooding, some loose fines, and effects of core cutting. Permeability and porosity of the two cores were the same (0.9 - .95 md) and porosity was approximately 11%.

Experiment Four

The cores for this experiment were prepared from a core from reservoir 6 that was predominately dolomite. Once again, flow through the cores was low but after treatment with supplemental nitrogen and phosphorus the effluent from the test core had an increased microbial content, some gas, particulate matter, and oil. The addition of ethanol to the influent of the test core increased the flow through the core significantly while flow through the control remained unchanged.

Petrophysical evaluation of the cores after completion of the experiment showed that both core samples had lost a lot of fines and the samples had become somewhat more porous but differences in porosity were minimal (16.8% for the control and 15% for the test). On the other hand, the permeability of

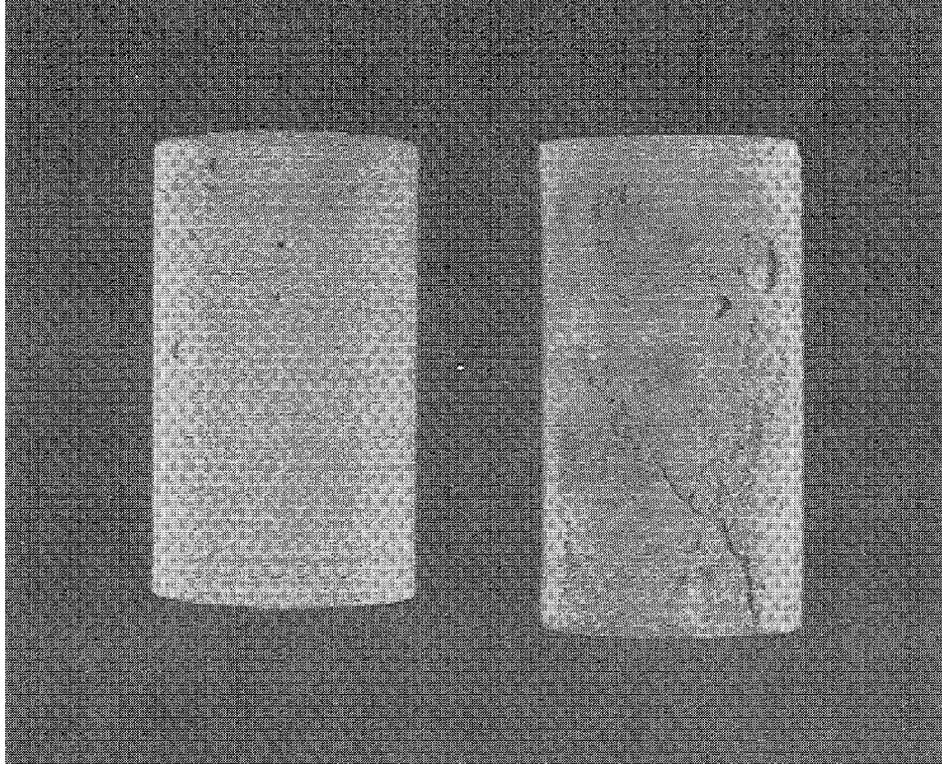


Figure 5-6. Photograph of the control (left) and test cores (right) from reservoir no. 4 after treatment.

the test core increased three-fold (from 3.3 md to 9.3 md) while the permeability of the control core did not change.

Experiment Five

The cores for this experiment were prepared from a core from reservoir 7 that was predominately sandstone. The control core effluent contained approximately 10^4 microorganisms/ml, there was no evident of particulate matter or oil, and the pH only went down to 6.5 even after ethanol was added to the injection water. After treatment of the test core with supplemental nitrogen and phosphorus for only a few days, the effluent showed a 100-fold increase in microbial content, and contained particulate matter and oil. With the addition of ethanol to the test core, flow through the core increased dramatically and the pH fell to 5.0.

Petrophysical examination of the cores showed the following:

- Control Core: * No oil was evident on the heat shrink plastic wrap. The wrap was quite clean and whitish (See Figure 5-7). The core, on the other hand was dark and most of the oil seemed to be in place.
- Test Core : * Sizable amounts of oil b l a c k e n e d the heat shrink plastic wrap (See Figure 5-7). The core was relatively clean and whitish.

The porosity of the test core was 33% higher than that of the control (17.7% vs 13.3%). The permeability of the test core was dramatically higher than the control core (54 md vs 8.6 md). The reduction in oil in the test core vs the control core is shown in Figure 5-8.

SUMMARY

Microorganisms have been shown to be indigenous to subterranean oil reservoirs and have been shown to produce by-products that could assist in oil recovery when supplied with adequate nutrients (Tasks 1-4). The question arises as to whether these microorganisms will perform these same functions in their native habitat thus helping define their value in MEOR processes.

In order to conduct these studies under realistic conditions, actual cores from subterranean reservoirs were

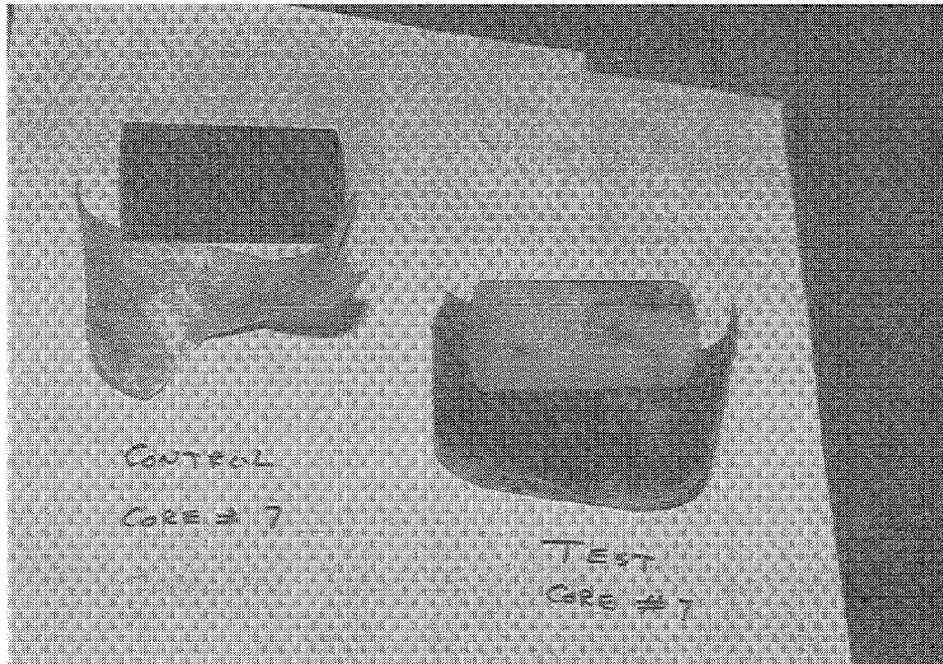


Figure 5-7. Photograph of the control and test cores from reservoir no. 7 after treatment. (Note oil on the wrap of the control core.)

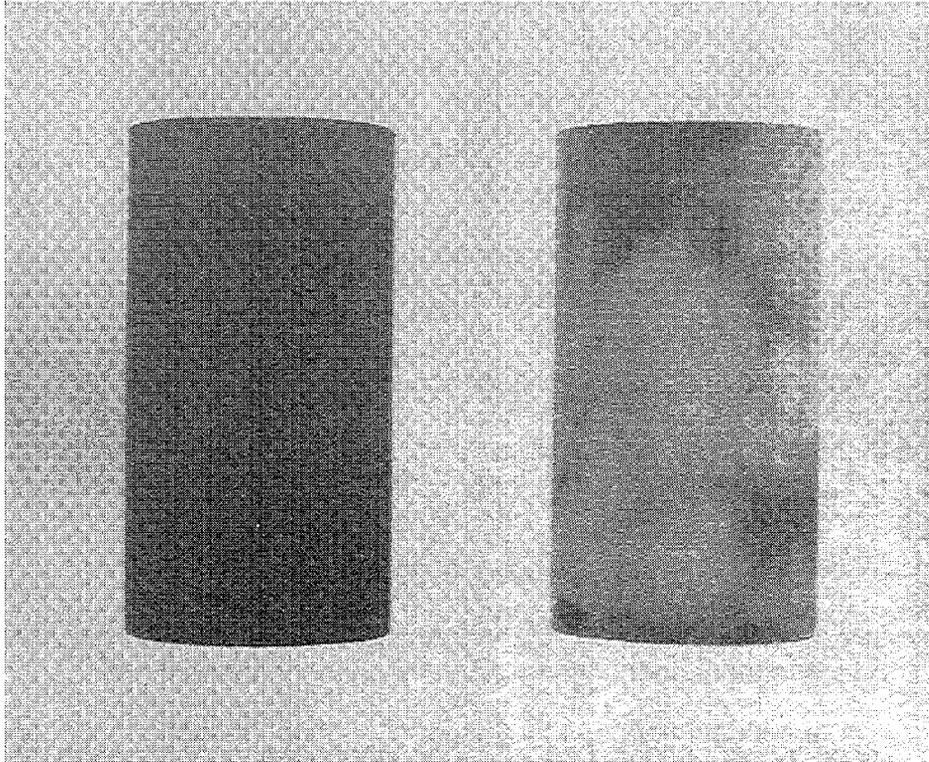


Figure 5-8. Photograph of the control (left) and test cores (right) from reservoir no. 7 after treatment.

employed. Thus, the microbial population in the cores existed in their natural state, *i. e.* attached to the stratal materials and in their own microenvironment. Although the cores used had been obtained a year earlier, they had been kept under a nitrogen atmosphere at 4C.

Obviously, microbes taken from the cores would grow when placed in microbial media but the paramount question was whether the same organisms would grow when small amounts of supplemental nutrients are offered to the cells in their native environment. Further, even if they do take up the supplemental nutrients from the injection water, will they reproduce to an extent that they would be useful as selective plugging tools and/or produce by-products that could assist in oil recovery from the reservoir?

After conducting core flooding experiments using cores obtained from five different subterranean oil reservoirs the following major findings were obtained.

The addition of supplemental nitrate ions and orthophosphate ions to the injection water caused:

- * a 100-fold increase in microorganisms in effluents from the cores
- * a release of fine particles from the cores
- * a dissolution of considerable amount of the stratal material (probably carbonates)
- * a production of gases in some cases
- * a release of more oil

Supplementing the injection water with ethanol in addition to the nitrate ions and orthophosphate ions accelerated most of the above results.

The question may arise as to the cause of the higher than expected numbers of microorganisms in the control cores. The answer to this question may be found by looking at the composition of the simulated production water. It contains small amounts of nitrate ions and orthophosphate ions and, in all probability, enabled the microflora to increase to the levels observed.

Adding micromolar amounts of ethanol to this injection water brought about some rather dramatic results. The fact that the control cores received the same amounts of ethanol as the test cores, but did not exhibit the dramatic increases in flow through the columns, demonstrates that the microflora were converting the ethanol to acid. The acid, in turn, was reacting with the carbonate binding materials in the cores.

The pH dropped appreciably in the core composed primarily of sandstone (Experiment 5) since there was less carbonate to neutralize the acid. The microbial conversion of the ethanol to an acid was expected since the normal pathway of metabolism of hydrocarbons proceeds via the alcohol to the acid.

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APPENDICES

- A. Geological and Petrophysical Characteristics of Reservoirs. (Task 1)
- B. Characteristics of Microbial Isolates from Petroleum Reservoirs. (Task 1)
- C. By-Product Formation by Oil-grown Mixed Cultures. (Task 2)
- D. Effect of Formation Materials on Microbial By-product Formation. (Task 3)
- E. Flow Patterns and other Data on Sandpacks. (Task 4)
- F. Data on Coreflooding Experiments. (Task 5)

Appendix A

Reservoir Characterization

Analytical Processes and Techniques for the Characterization of Cored Reservoir Formations

The following procedures were followed in preparation of core samples for geological studies based on their consolidation status. See diagram.

a- Consolidated Cores

- Core samples were collected from anaerobic containers.
- Hand sample lithological descriptions and photographs of cores were made.
- Cores were cut using a standard dry cutting rock saw into small blocks in preparation to send off for professional thin sectioning.
- Internal structures within the cut sections of rock were studied in detail using a binocular microscope.
- Core cuts were fractured using a hammer to obtain chippings to be studied under the scanning electron microscope [S.E.M.].
- Chippings and cuttings of rocks were repeatedly immersed in toluene, using a soxlet extractor. This process removed all existing hydrocarbons from within the rock. Organic compounds are harmful to S.E.M. machinery.
- Core samples were sent off to Quality Thin Section, Tucson, Arizona, for professional thin sectioning. This company was instructed to:
 - Stain some thin sections to aid in the identification of plagioclase feldspar grains, (using a potassium rhodizonate stain).
 - Stain some thin sections to aid in the identification of dolomite from calcite (using a combined alizarin-red/ferricyanide stain).
 - Impregnate some rock samples prior to thin sectioning using dyed epoxy resin. This was useful in the identification and calculation of pore space.
 - Polish some thin sections with a 0.025 grit finish for B.S.I. study on the S.E.M. (explanation of these techniques is given later).
- Petrography of core samples were described in detail by studying thin sections using a standard petrological microscope. Percentage mineral composition and porosity were statistically calculated using point counts.
- Chipped samples were cleaned with acetone in an ultrasonic bath in preparation for use on the S.E.M.
- Chipped samples were mounted onto S.E.M. stubs using conductive silver paint. Finally these samples were sputter coated (using a Polaron S.E.M. coating system) with either a carbon or a gold-palladium coat for two periods of three minutes each.
- Samples were studied on a JEOL.JSM.35.CF. S.E.M. at the Electron Microscope Center of Mississippi State University. The

S.E.M. was used to identify textural relationships of minerals and factors affecting pore space properties at magnifications and resolutions far exceeding standard petrographic techniques. Three different techniques were used on the S.E.M.:

- Secondary Electron Imagery [S.E.I.] was useful in showing relief of samples often on fractured surfaces.
- Backscattered Electron Imagery (B.E.I) was used on polished thin section samples. Since this technique produces images of contrasting mean atomic number, minerals of different composition could be identified by comparing grey tones in the image against mineral's mean atomic weight.
- Energy Dispersive Spectra (E.D.S.) analysis were used on the S.E.M. to give approximate chemical compositions and were an important aid to mineral identification. A Philip 515 scanning electron microscope equipped with a Kevex 8000 energy dispersive spectrometer with a beryllium window was used. The beryllium window used does not allow elements such as carbon and oxygen to be detected.

Energy dispersive spectra (E.D.S.) were obtained from specific areas on fractured scanning electron microscope specimens. The spot from which elemental analysis was obtained is shown by a white cross on the photomicrographs. The spectra show high background counts of gold (Au) and palladium (Pd) due to the Au/Pd coating on the surface of the specimen. Au and Pd peaks were found to mask some elements such as magnesium (Mg) and sodium (Na). This problem was overcome in some cases by using carbon coated specimen. In some cases when peaks of only one or two elements were identified the mineral composition was inferred from the major elements observed, mineral morphology and previous thin section, X-ray diffraction analysis.

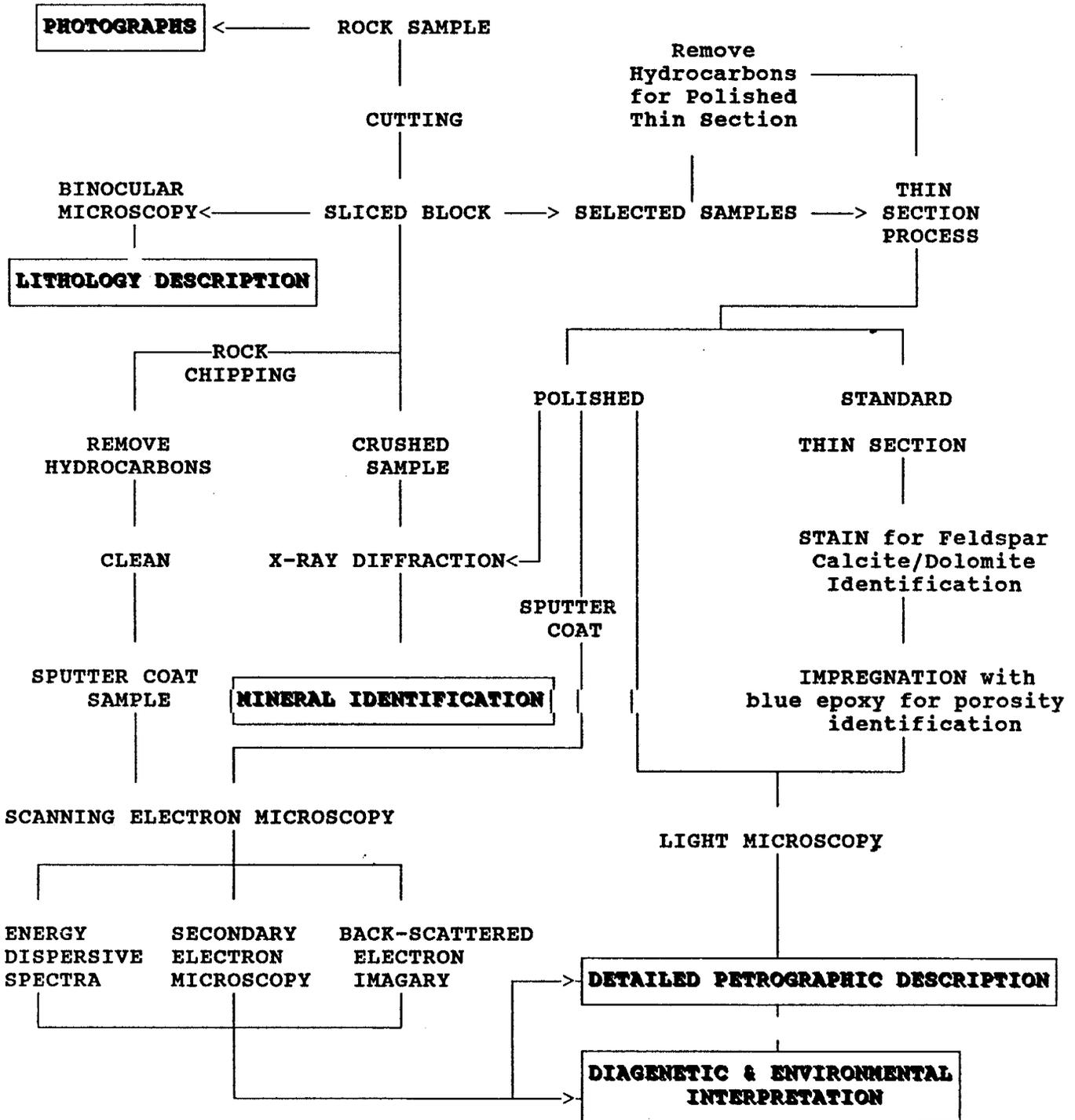
- Samples were crushed into a powder, mounted in a cavity holder and x-rayed. A Scintag, X-ray Diffractometer System (X.D.S.) was used. Minerals were identified by their d-spacings.

b- Unconsolidated Core

- The core sample was collected from an anaerobic container.
- The unconsolidated core was repeatedly immersed in toluene, using a soxhlet extractor. This process removed all existing hydrocarbons from between the grains.
- The resulting dry loose sediment was studied under a binocular microscope. Hand sample lithological descriptions and photographs of cores (loose sediment) were made.
- The loose sediment was sieved for grain size analysis (frequency percentages by weight per grain size).
- The loose sediment was sent off to Quality Thin Section, Tucson, Arizona, for professional thin sectioning. This company was instructed to:
 - Impregnate the loose sediment with a cementing resin before thin sectioning.

- Stain some thin sections to aid in the identification of plagioclase feldspar grains, using a potassium rhodizonate stain.
- Polish some thin sections with a 0.025 grit finish for B.S.I. study on the S.E.M.
- S.E.M. samples were prepared according to different sieved grain sizes. Grains were cleaned using an ultrasonic bath with acetone. Large grains (above 2mm in diameter) were mounted onto S.E.M. stubs using silver paint. Smaller grains were stuck to the stubs using double sided electrically conductive carbon cohesive tape. Very fine grains (<63 micrometers) were mixed with an acetone solution applied to the top of a stub and allowed to desiccate. This was found to sufficiently fix the grains to the stub for S.E.M. observation. Thin sections were mounted on S.E.M. stubs using double sided sticky tape.
- S.E.M. samples were sputter coated (using a Polaron S.E.M. coating system) with either a carbon or a gold-palladium coat for two periods of three minutes each.
- Samples were studied on a JEOL.JSM.35.CF. S.E.M. The S.E.M. was used to identify grain surface textures at magnifications and resolutions far exceeding standard petrographic techniques. Three different techniques were used on the S.E.M., secondary electron imagery, backscattered electron imagery and energy dispersive spectra as described previously for consolidated samples.

FLOW DIAGRAM OF ANALYTICAL PROCESSES AND TECHNIQUES



Reservoir and Geological Characterization of Live Cores

Core No. 1

Oil Company: Callon
Well Name: No.1 Irvin
Field Name: Langford
State: Alabama
County: Monroe
Formation Name: Smackover
Formation Age: Upper Jurassic: Oxfordian
Formation Type: Dolomite/Limestone
Core Depth: 14492 ft.

Core Sample Length: 10 cm.
Core Sample Diameter: 10 cm.

Rock Description

The rock is light to medium gray. There is no indication of hydrocarbons within the rock. The rock consists predominantly of crystalline ferroan dolomite, with minor amounts of detrital grains and some anhydrite or gypsum cement. Detrital grains generally are very fine grained, subrounded to subangular, well sorted, quartz grains. Other grains identified are platy muscovite grains and feldspar grains. The rock contains stylolite structures. No sedimentary structures were identified. See plate 1a, 1b and 1c.

Detailed Petrography

Grain Size: Crystalline dolomite and anhydrite/gypsum.
Detrital grain average diameter is 0.2 mm.

Roundness, Angularity and Detrital Grain Shape: Detrital grains are sub-rounded to sub-angular and spherical in shape. They are occasionally angular in appearance due to overgrowths.

Fabric (Orientation and Packing): Random, no preferred orientation.
Detrital grains are matrix supported.

Porosity: Percentages were measured by point counting thin sections
Porosity = $7.3 \pm 1.8\%$
Most porosity was intercrystalline found existing within a three dimensional network of dolomite crystals. Pore sizes generally do not exceed a maximum of 250 μm . Vugs were present and did not exceed 2 mm in diameter.

Mineral Composition: Percentages were measured by point counting thin sections.

Ferroan Dolomite, $98 \pm 0.8\%$

Quartz (other detrital grains include mica and feldspar),
 $2 \pm 0.6\%$

See Plate 4, the energy dispersive spectra analysis, and Plate 5, x-ray diffraction analysis.

The rock also contains trace amounts of anhydrite and gypsum.

Minor amounts of an authigenic clay mineral, (probably a variety of illite) occur within the rock.

Description of Detrital Grains

Quartz: Detrital grains are monocrystalline and display a conchoidal fracture. Detrital quartz grains also have overgrowths of quartz.

Post-Depositional Minerals

Dolomite occurs as microscopic rhombohedral crystals that vary in size but generally are less than $10 \mu\text{m}$ in diameter. Dolomite rhombs often are zoned with a cloudy center. Occasional anhydrite forms large tabular pore filling crystals with well developed crystal surfaces. Authigenic clay (probably a variety of illite) occurs as a pore-lining and pore-filling authigenic clay. The clay has a crinkly interwoven morphology with rod shaped extensions.

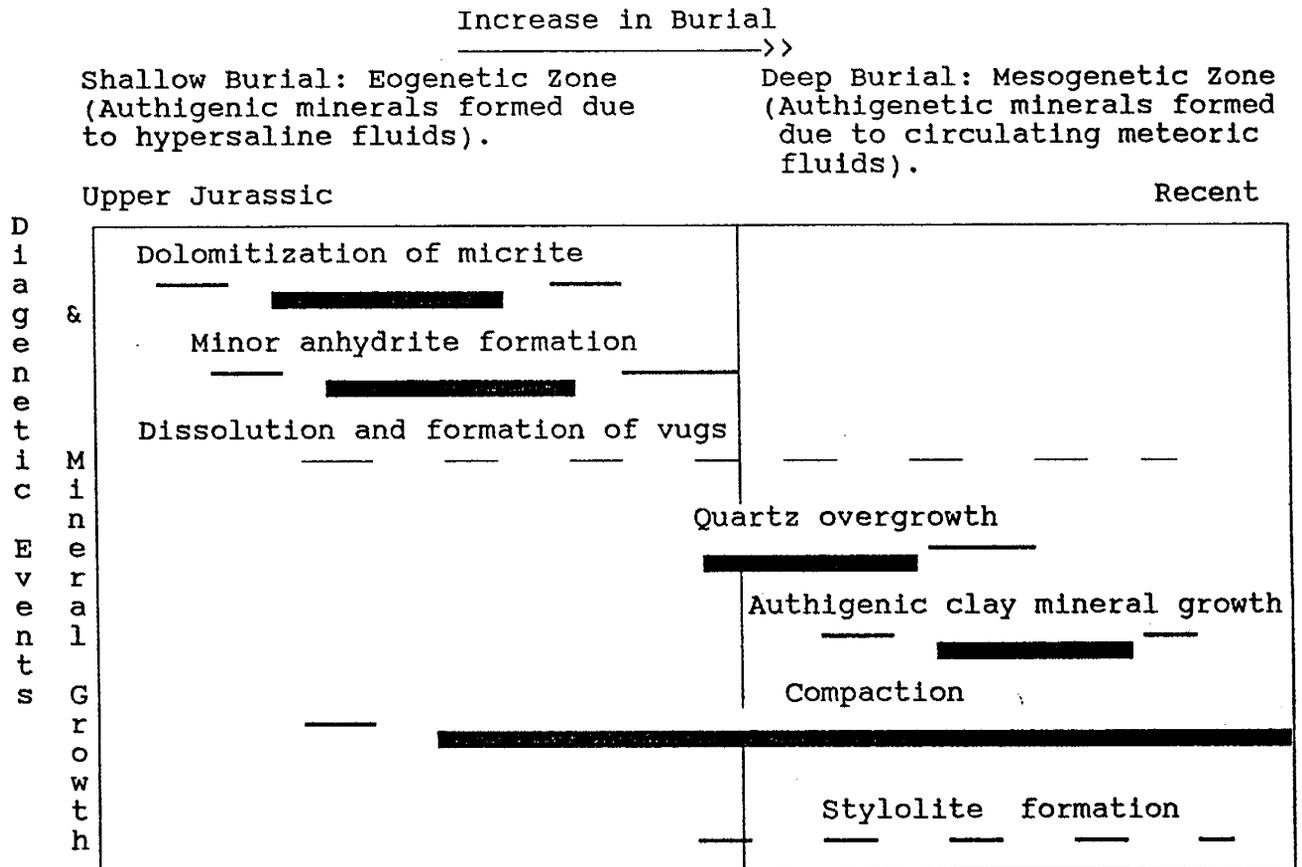
See Plate 2A, 2B, 3A, 3B,3C and 3D.

Diagenesis

List of diagenetic events (the events are not necessarily in the correct chronological order):

1. Dolomitization of calcium carbonate sediment.
2. Formation of anhydrite cement.
3. Dissolution of dolomite and formation of vugs.
4. Formation of quartz overgrowths.
5. Growth of pore-filling authigenic clay.
6. Compaction and formation of stylolites.

Relative Sequence of Diagenetic events for Core No.1



Key: ■ Relative timing of diagenetic event is certain

— — — — — Dissolution, timing uncertain

— Relative timing of diagenetic event is uncertain

Note: Sequence of diagenetic events were identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No. 1 was cored from the Jurassic, Smackover Formation of the Langford Field, southwest Alabama. The Smackover Formation has been described in detail by Mancini and Benson (1980). They divided the formation into the Upper and Lower Smackover. The Lower Smackover is composed of a low energy, dense, organic rich, laminated mudstone, packstone and wackestone carbonate. The Upper Smackover is dominated by moderate to high energy, shallow subtidal cross bedded oolitic and oncolitic packstones and grainstones, often interbedded with intertidal, low energy lagoonal peloidal mudstones and wackestones. The Langford Field most likely is a stratigraphic trap formed due to the disappearance of porosity by massive beds and infilling evaporates found at the top of the formation. Porosity usually disappears updip to the north.

The 10 cm length of studied core was a microcrystalline dolostone located from the Upper Jurassic. The studied core was found to be of a homogeneous lithology. It is important to note that the microcrystalline dolostones, similar to the studied core sample are interbedded with very fine grained dolomitic sandstones.

Depositional Environment

During the Jurassic Period, southwest Alabama was a carbonate ramp that was modified by paleotopographic highs (Mancini and Benson, 1980) into three depositional basins, the eastern extension of the Mississippi Interior Salt Basin, the Manila Embayment, and the Conecuh Embayment. These basins were bound to the north by a terrestrial clastic source area and separated by paleotopographic highs trending north to south. The Langford Field is located on a paleotopographic high between the Manila Embayment and the Conecuh Embayment. In this area open shallow marine, moderate to high energy conditions prevailed.

Locally permeable lithofacies found on paleotopographic highs between basins are believed to have been sites of preferential dolomitization. Dolomitization probably took place when normal seawater and freshwater mixing occurred in the subsurface creating magnesium rich brines (Mancini and Benson, 1980). Siliclastic sediments were deposited intermittently in a shallow, subtidal, high energy environment above wavebase. Detrital grains within the core sample may be eolian or fluvial in origin.

Description of Plates

- Plate 1: The core sample studied
1a and 1b show the complete length of the slabbed core.
1c shows an end view of the core.
- Plate 2: Photomicrographs of thin sections.
2a. Cross polarized light view of green/brown, high relief crystalline dolomite. Small white, low relief areas are detrital quartz grains. Black areas are pore space.
2b. Plane polarized light view of green/brown, high relief crystalline dolomite. The black area in the photograph is organic matter that has been concentrated along a stylolite.
- Plate 3: Scanning electron micrographs of core No.1
3a. Bar is 100 microns x 200.
Crystalline intergrown rhombohedral dolomite crystals. Voids (intercrystalline pore space) are seen in the center of the photomicrograph.
3b. Bar is 10 microns x 200.
Possible quartz overgrowth (QO) development on a detrital quartz grain. Quartz overgrowths have well developed crystal surfaces.
3c. Bar is 10 microns x 200.
Dolomite rhombs (D) and well developed hexagonal quartz crystal (Q). The irregular crinkly interwoven substance seen between dolomite rhombs is an authigenic clay.
3d. Bar is 10 microns x 3600.
Authigenic pore-filling clay. The clay has a crinkly, interwoven morphology being made up of many small rod shaped extensions.
- Plate 4. Energy dispersive spectra analysis
4a. An Iron(Fe) rich dolomite, $\text{Ca,Mg}(\text{CO}_3)_2$.
4b. Fe,K,Ca clay. Probably a variety of illite.
4c. Fe,K,Ca clay. Probably a variety of illite.
- Plate 5. X-Ray diffraction analysis.
5a. D: Ferroan dolomite (mineral comparison card is shown)
5b. D: Dolomite (mineral comparison card is shown)
5c. D: Dolomite (mineral comparison card is shown)

Plate 1a

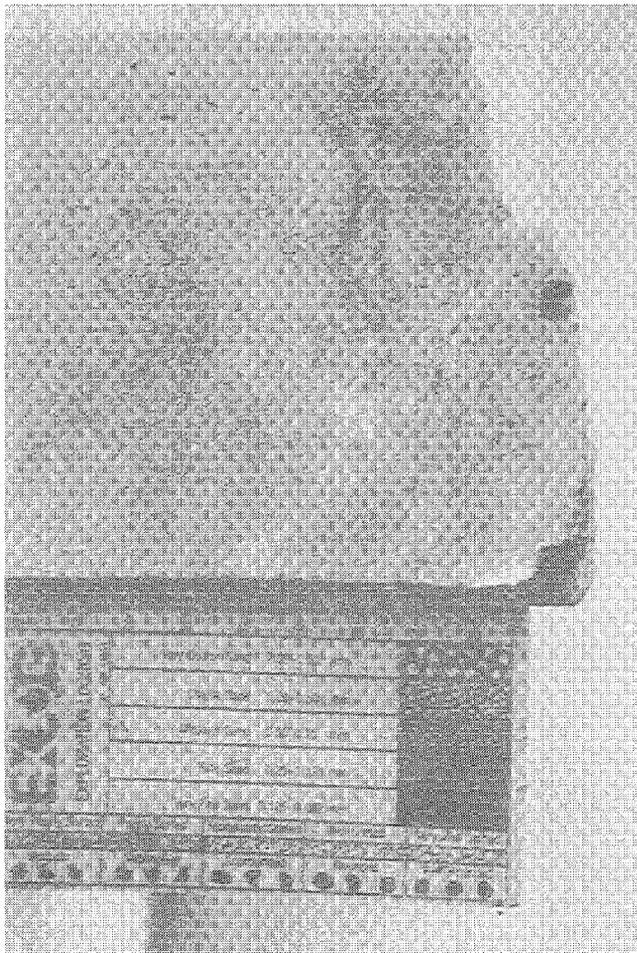
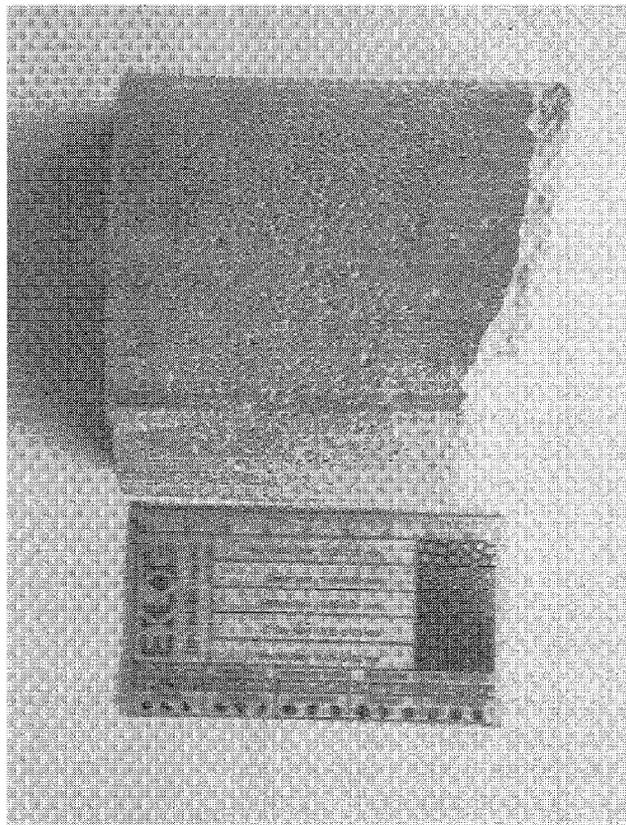


Plate 1b

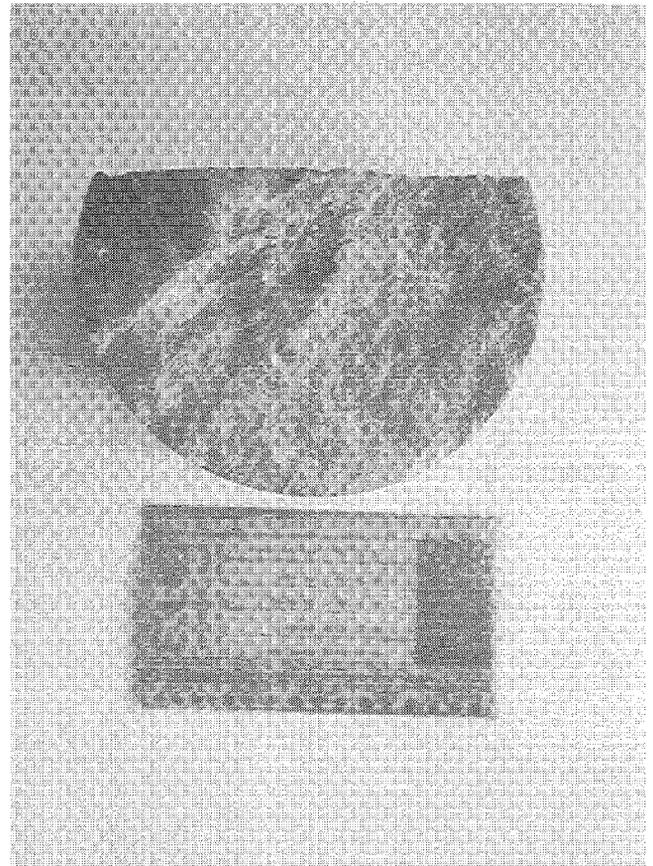


Plate 1c

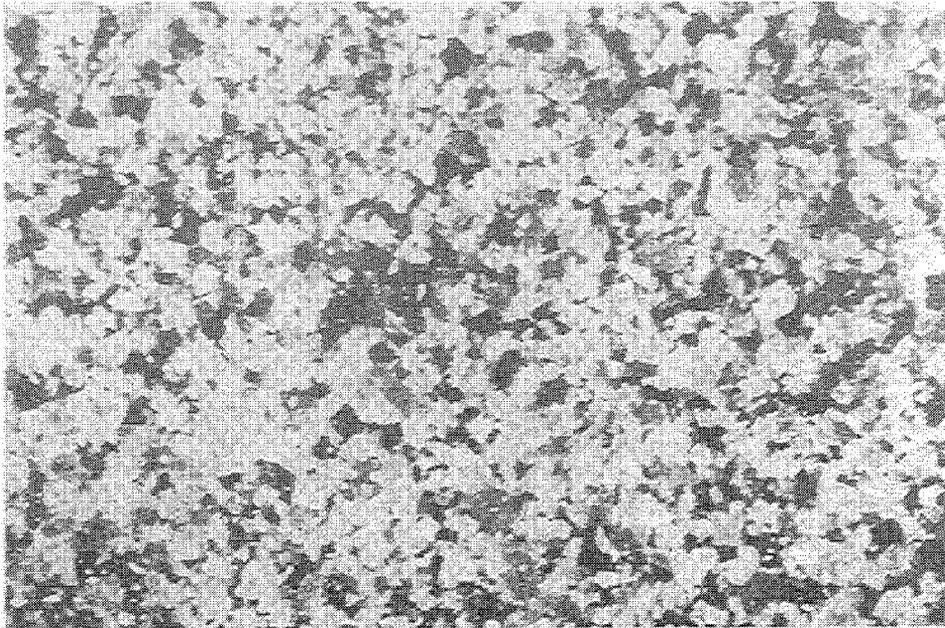


Plate 2a

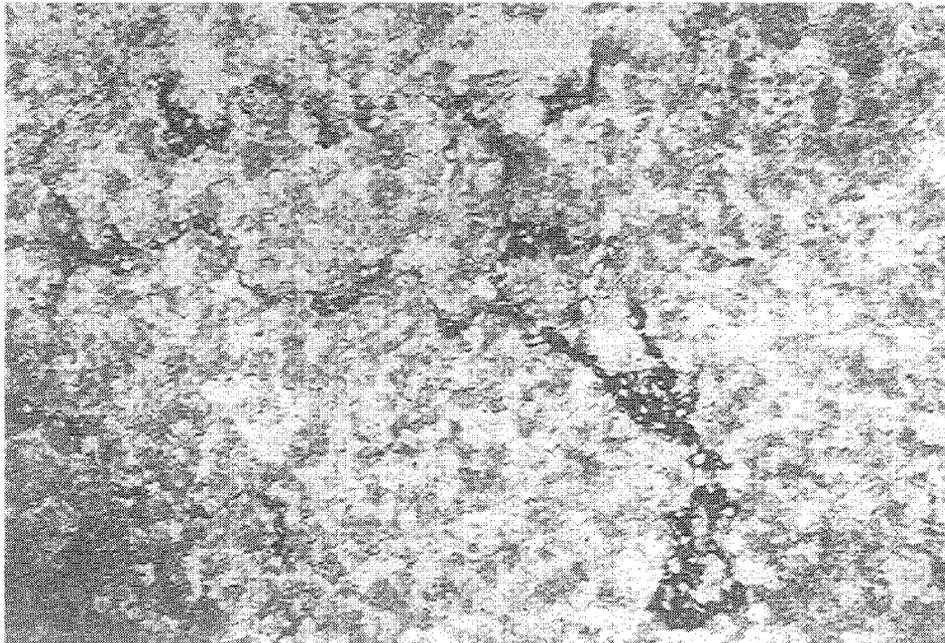


Plate 2b

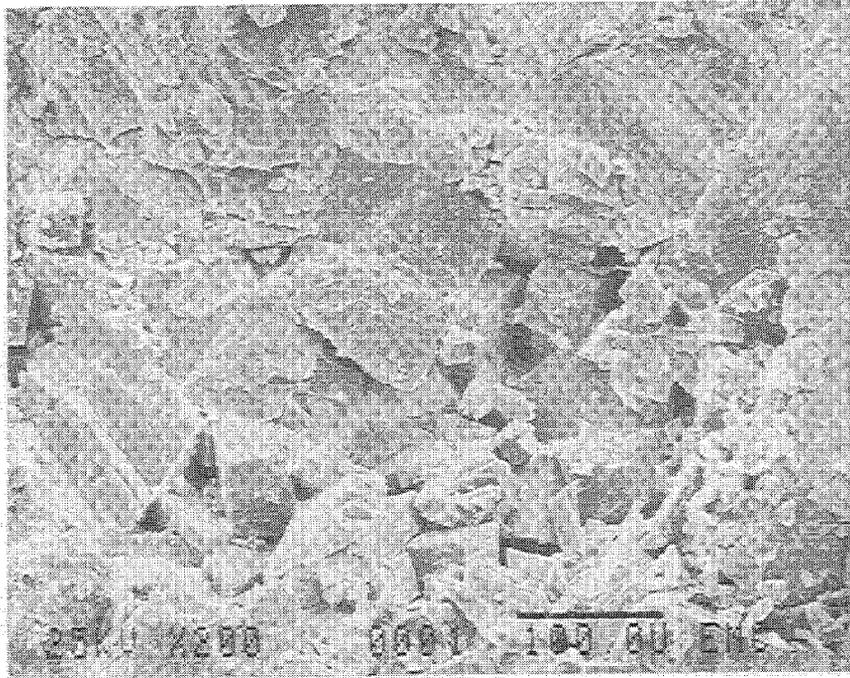


Plate 3a

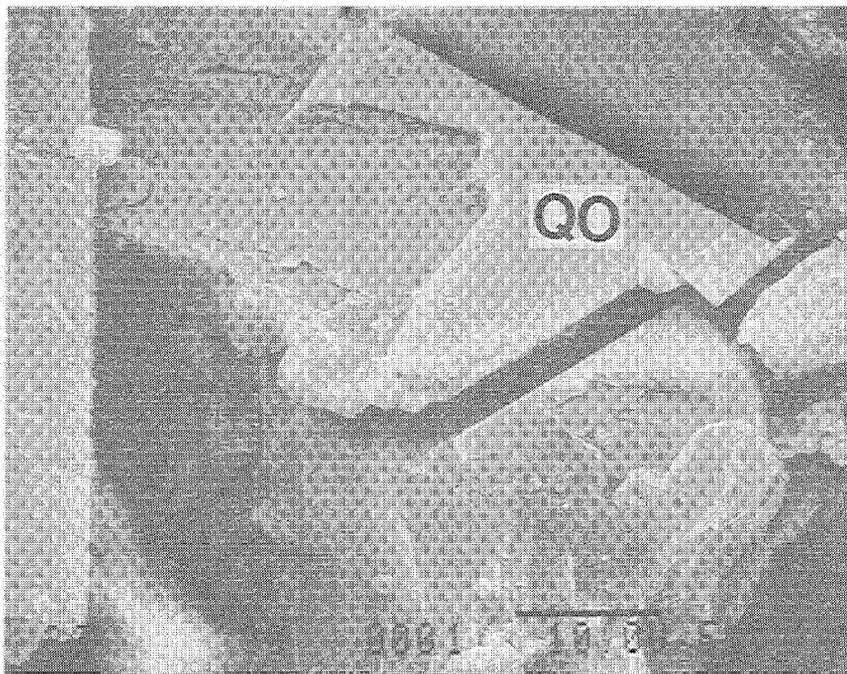


Plate 3b

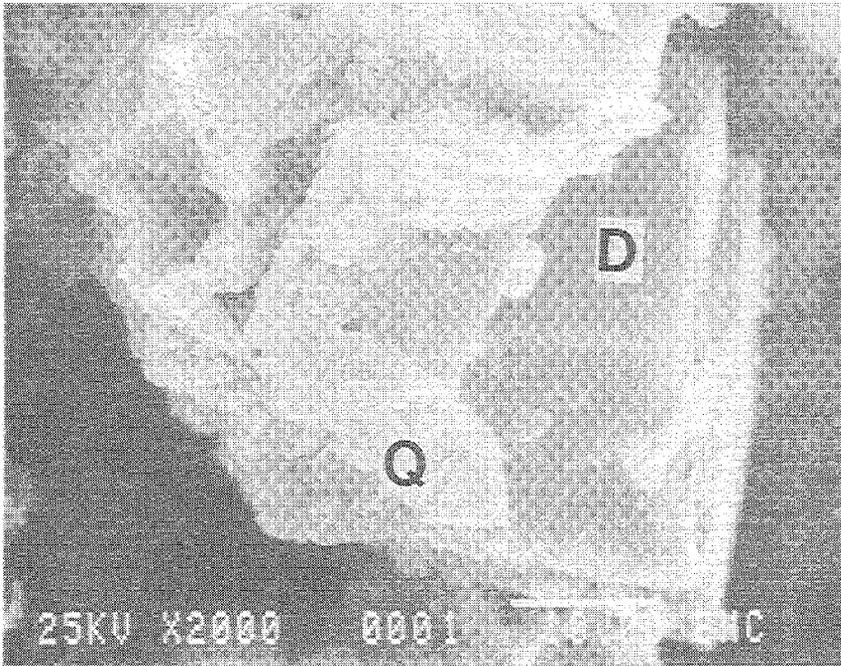


Plate 3c

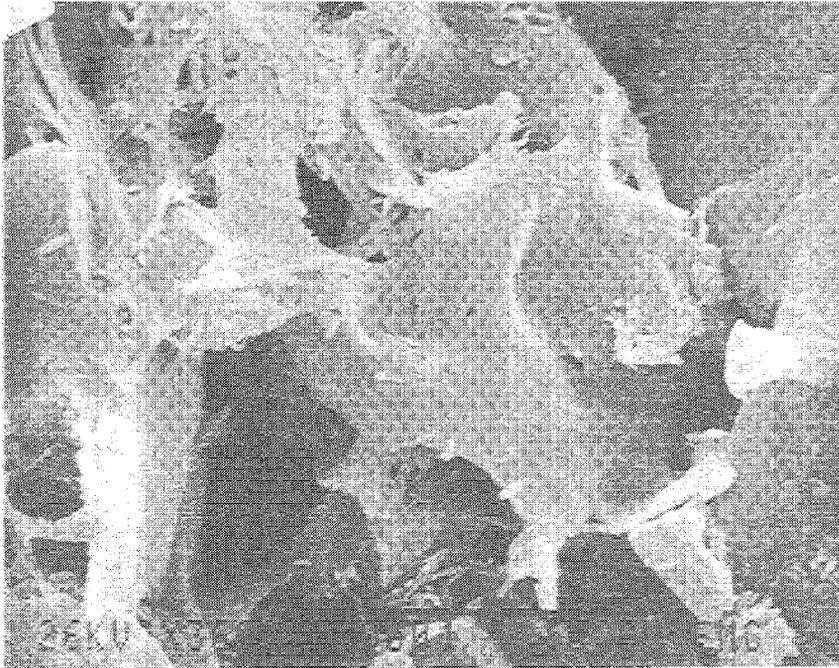


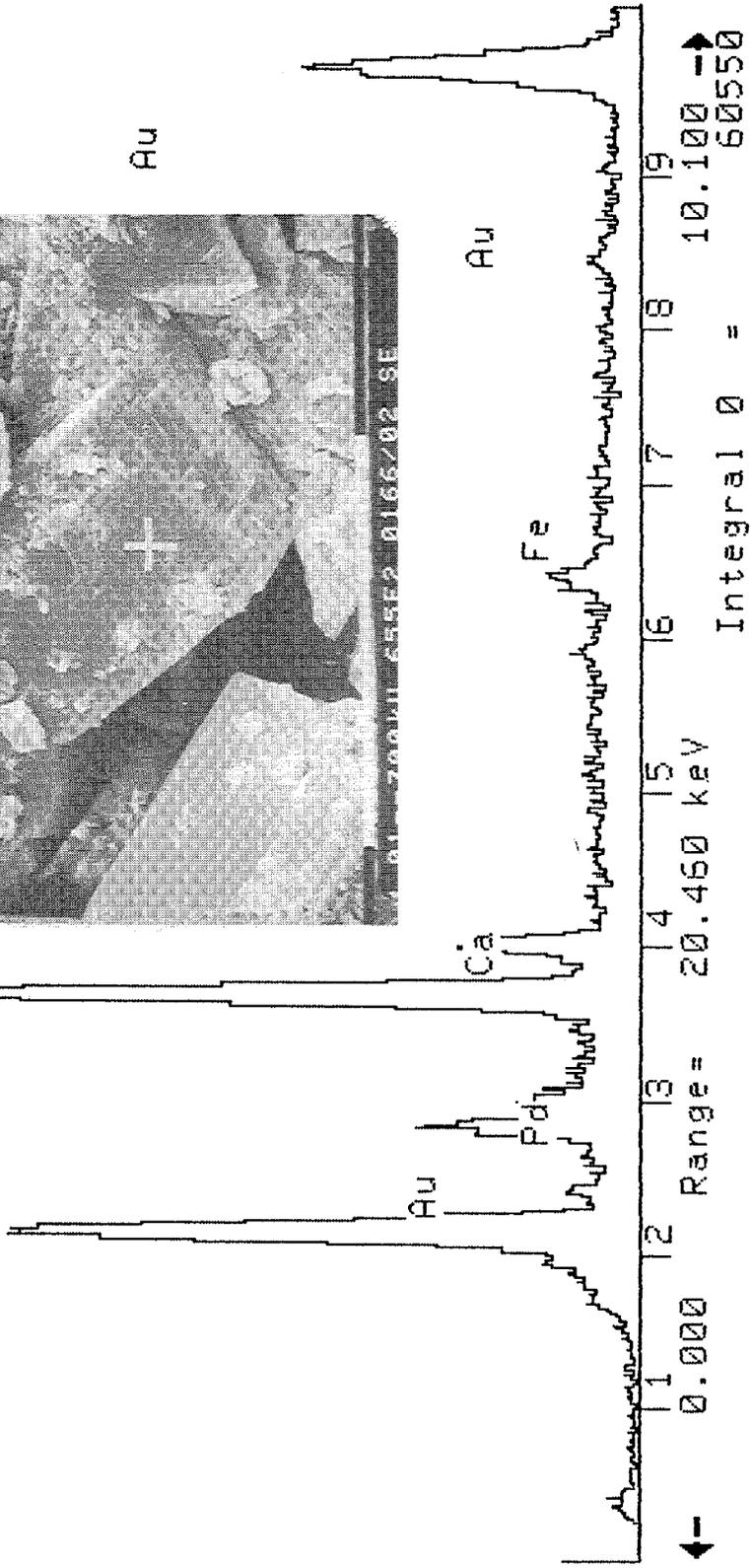
Plate 3d

30-Oct-1991 16:29:18

AV1 Dolomite
Vert= 1229 counts
Quantex >

Preset= 100 secs
Elapsed= 100 secs

Disp= 1



← 0.000 Range= 20.460 keV Integral 0 = 10.100 →
60550

Plate 4a. Core No.1 An Iron(Fe) rich dolomite $\text{Ca,Mg}(\text{CO}_3)_2$.

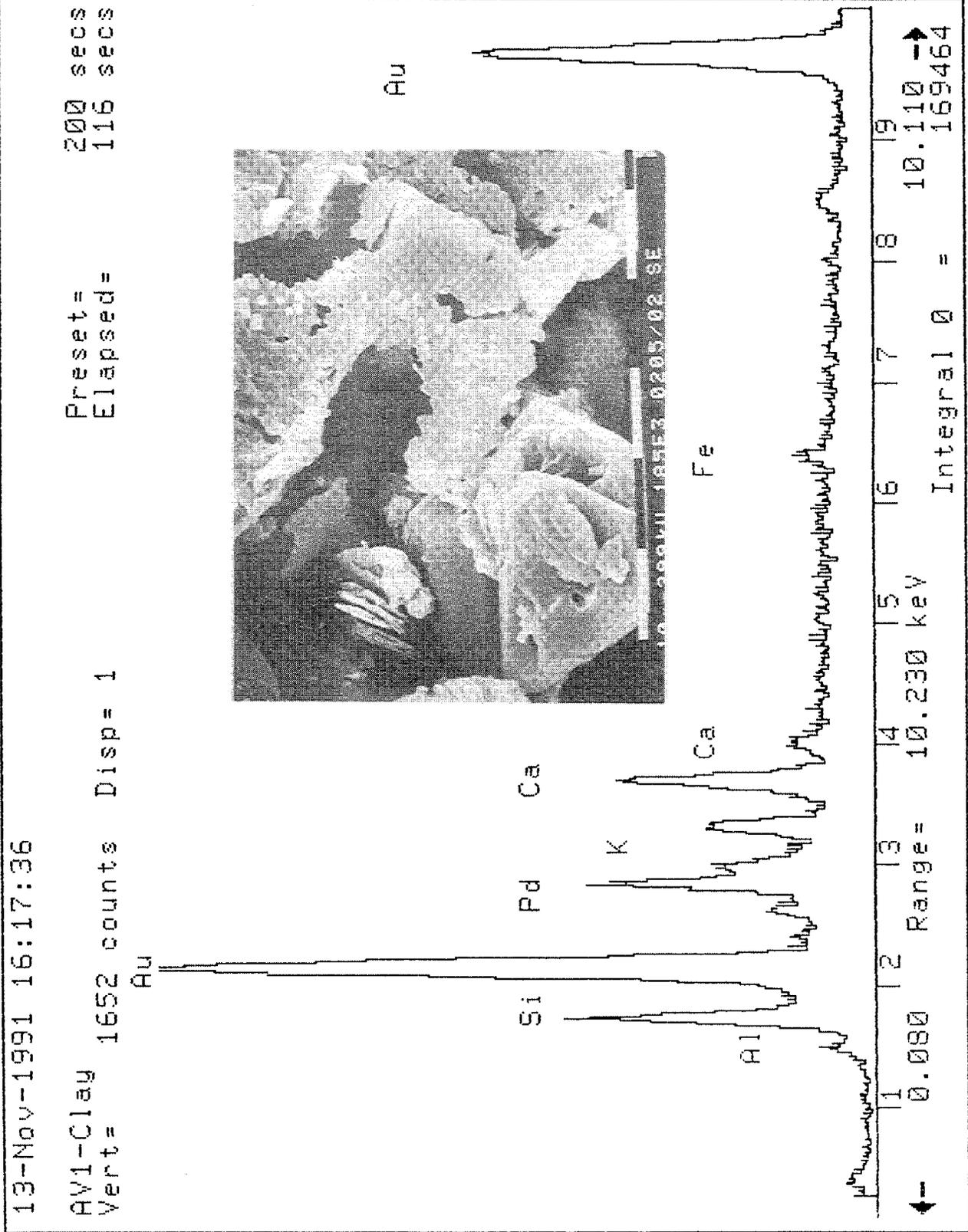


Plate 4b. Core No.1 Fe, K, Ca, clay. Probably a variety of illite.

13-Nov-1991 16:03:11

AV1-Clay

Vert=

613 counts

Disp= 1

Preset=

200 secs
148 secs

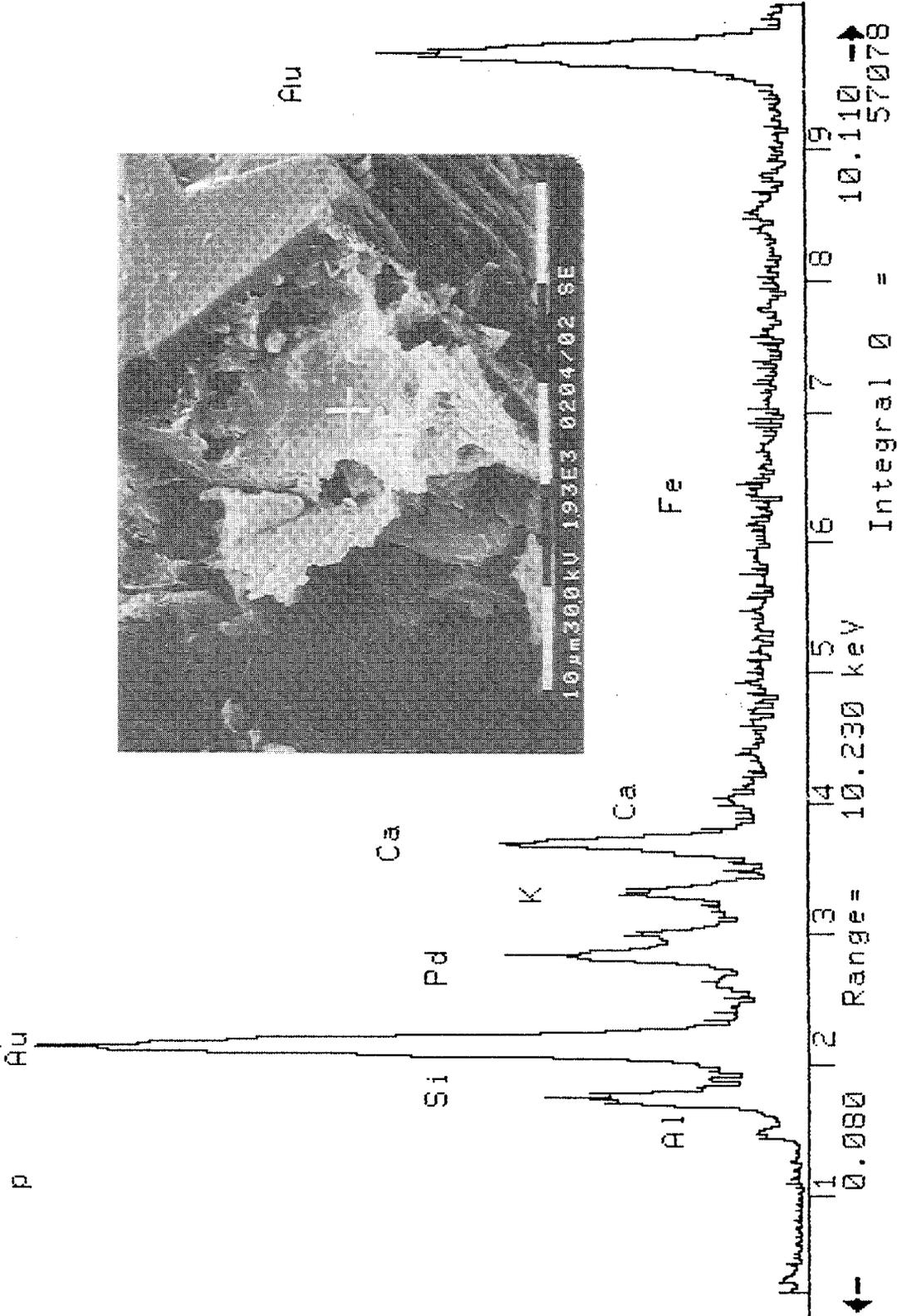


Plate 4c. Core No.1 Fe, K, Ca, clay. Probably a variety of illite.

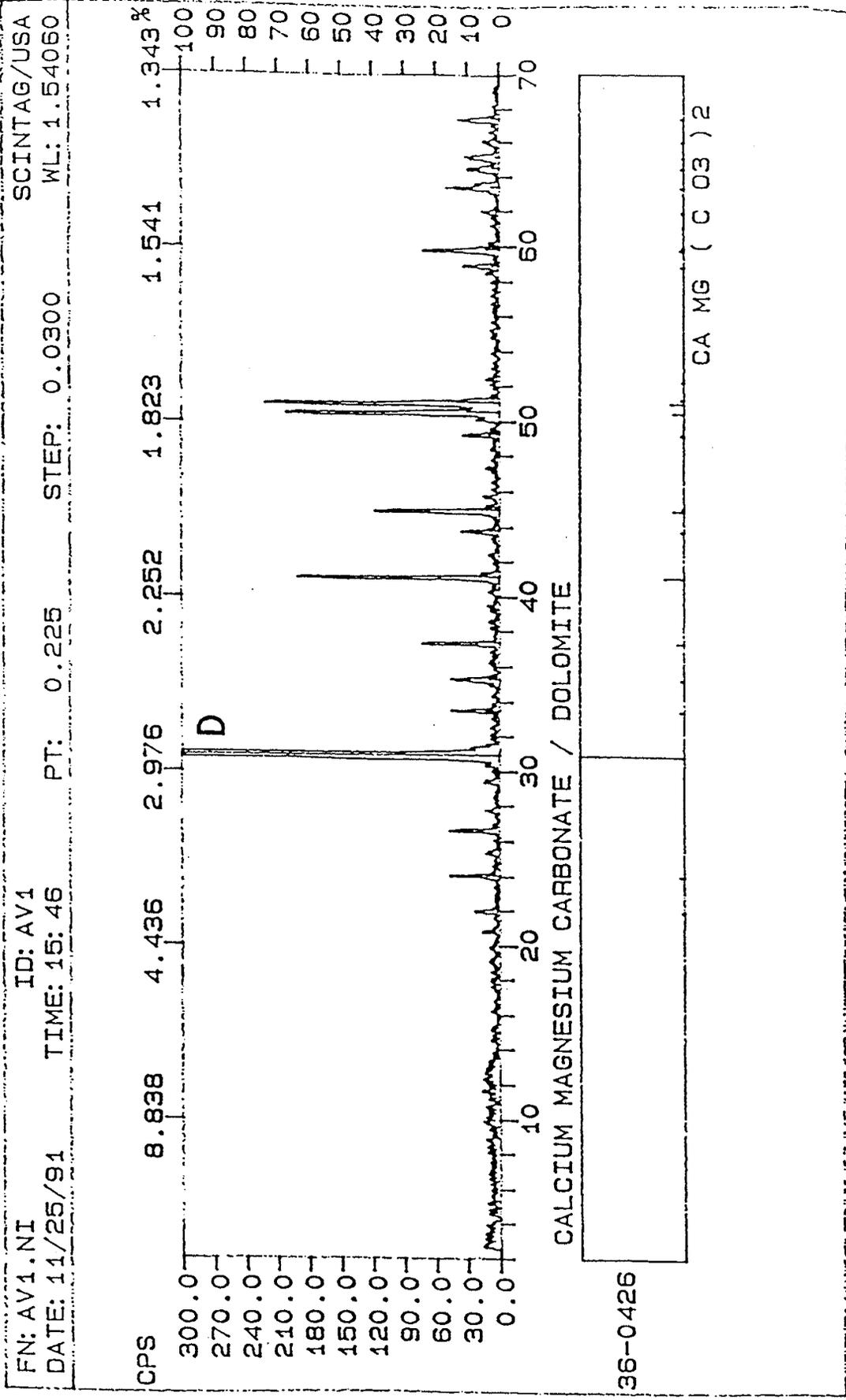


Plate 5a- D: Ferroan dolomite (Mineral Comparison Card is shown)

FN: AV1B.NI ID: AV1 B SCINTAG/USA
 DATE: 11/25/91 TIME: 16: 04 WL: 1.54060
 PT: 0.225 STEP: 0.0300

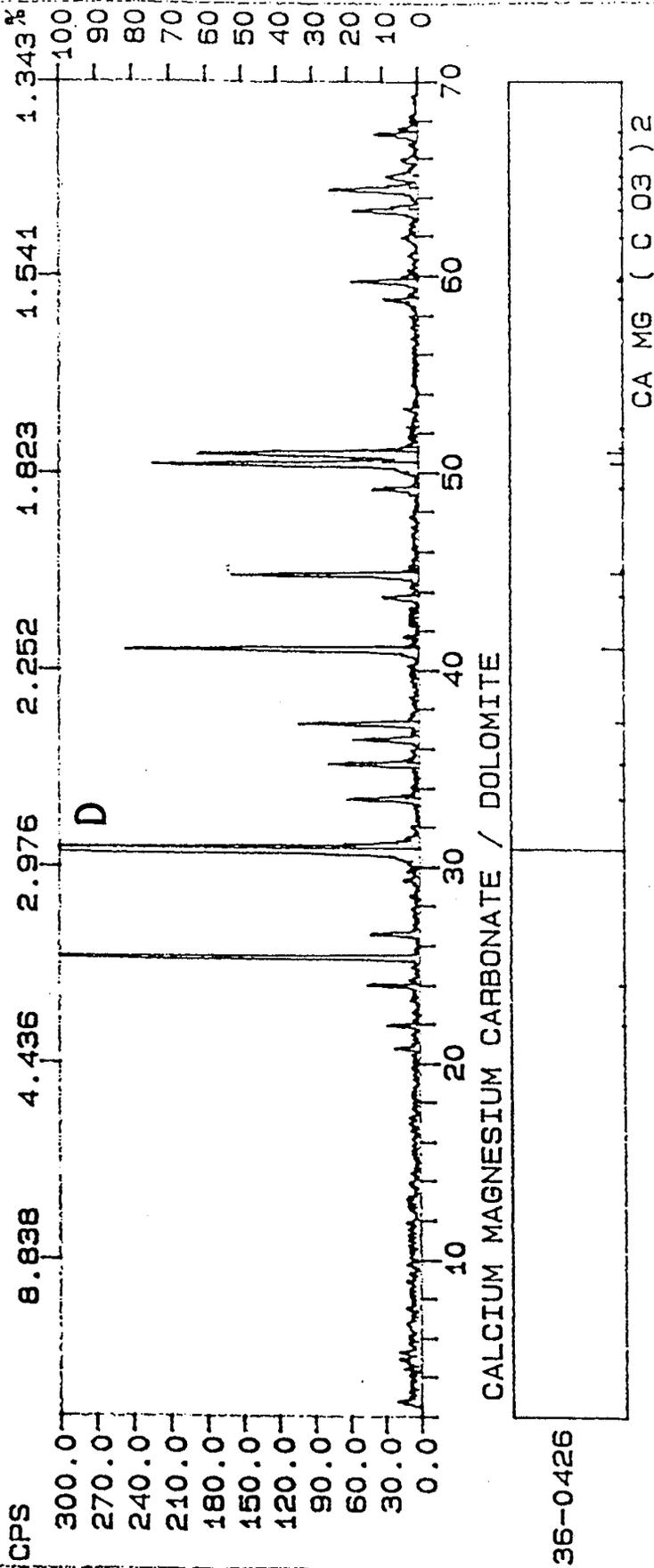


Plate 5b- D: Dolomite (Mineral comparison card is shown)

FN: AV1TS.NI ID: AV1 THIN SECTION SCINTAG/USA
 DATE: 11/25/91 TIME: 15:04 PT: 0.225 STEP: 0.0300 WL: 1.54060

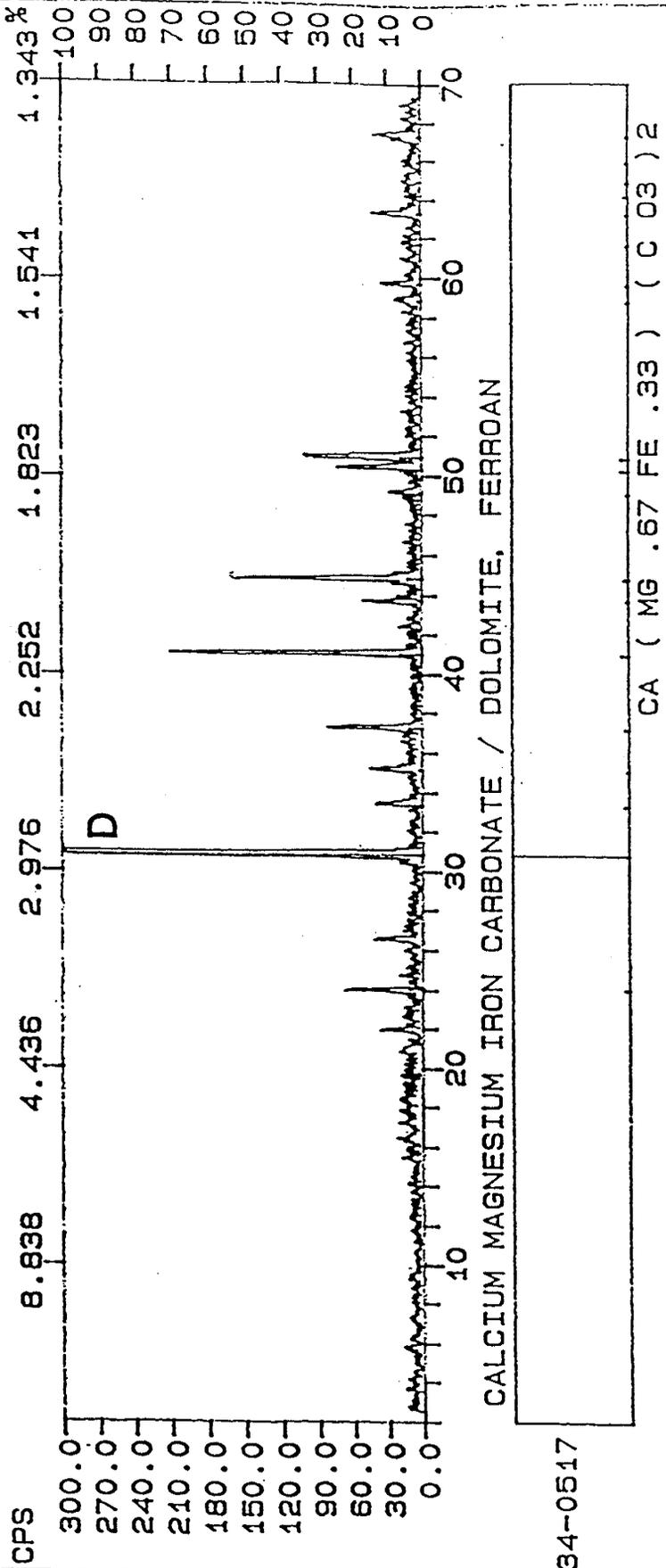


Plate 5c- D: Dolomite (Mineral comparison card is shown)

Core No. 2

Oil Company: Shell
Well Name: Wilmar 133
Field Name: Kern River
State: California
County: Kern
Formation Name: Kern River Series Sand
Formation Age: Plio-Pleistocene
Formation Type: Clay Sand Sediment
Cored depth: 805-806 ft.

Loose sediment.

Rock Description

The loose sediment is light gray or black to dark gray because of oil staining. It is a poorly sorted lithic arenite consisting of sub-rounded to sub-angular grains. Sieve analysis shows a mean grain size between 1000-500 μm (coarse sand grain size). Grains are formed of quartz, plagioclase feldspars, biotite, hornblende, and lithic fragments, that are granitic in composition. Sedimentary structures could not be identified due to the nature of the loose sediment.

Detailed Petrography

Grain Size: Range from a maximum of 2 cm to clay size.
Most common grain size was between 1000-500 μm .
The rock is poorly sorted.

Roundness, Angularity and Detrital Grain Shape: Detrital grains are sub-rounded to sub-angular and occasionally angular.
They are sub-spherical in shape.

Fabric (Orientation and packing): It was not possible to determine any preferred orientation from the loose sediment sample. The sample is a loose, grain supported sediment.

Porosity: Interparticle; not possible to measure from thin sections of a loose sediment sample.

Mineral Composition: Percentages measured by point counting thin sections.

Lithics, 43 \pm 5.7%
Quartz, 31 \pm 5.3 %
Plagioclase Feldspar, 18 \pm 4.1%
Biotite, 4 \pm 0.3 %
Hornblende, 2 \pm 0.1 %

The rock also contains minor amounts of authigenic clay (probably a variety of smectite).

Description of Detrital Grains

Lithics: Predominantly igneous lithic grains, occasionally metamorphic and possibly some sedimentary.

Igneous: Granite to granodiorite in composition, containing 35% quartz, 5% orthoclase feldspar, 55% plagioclase feldspar, 5% biotite plus minor amounts of hornblende and sphene. Also contains local fine-grained volcanic rock fragments.

Metamorphic: Polycrystalline elongate crystals of strained quartz and micas. Possibly a schist rock type.

Quartz: Translucent, milky occasionally light orange in color. Sub-rounded to sub-angular, locally angular grains displaying a conchoidal fracture. Commonly the grains are monocrystalline or polycrystalline, equigranular plutonic quartz. Quartz grain surfaces show "en echelon" 'V' shaped patterns.

Plagioclase feldspar: White, sub-rounded grains most commonly orthoclase feldspar. Feldspars show evidence of alteration to a clay mineral.

Post-Depositional Minerals

Grains are coated with a 1 μm thick layer of authigenic clay, probably smectite. This authigenic clay has a honeycomb morphology and infills pits and irregularities on the surface of detrital grains.

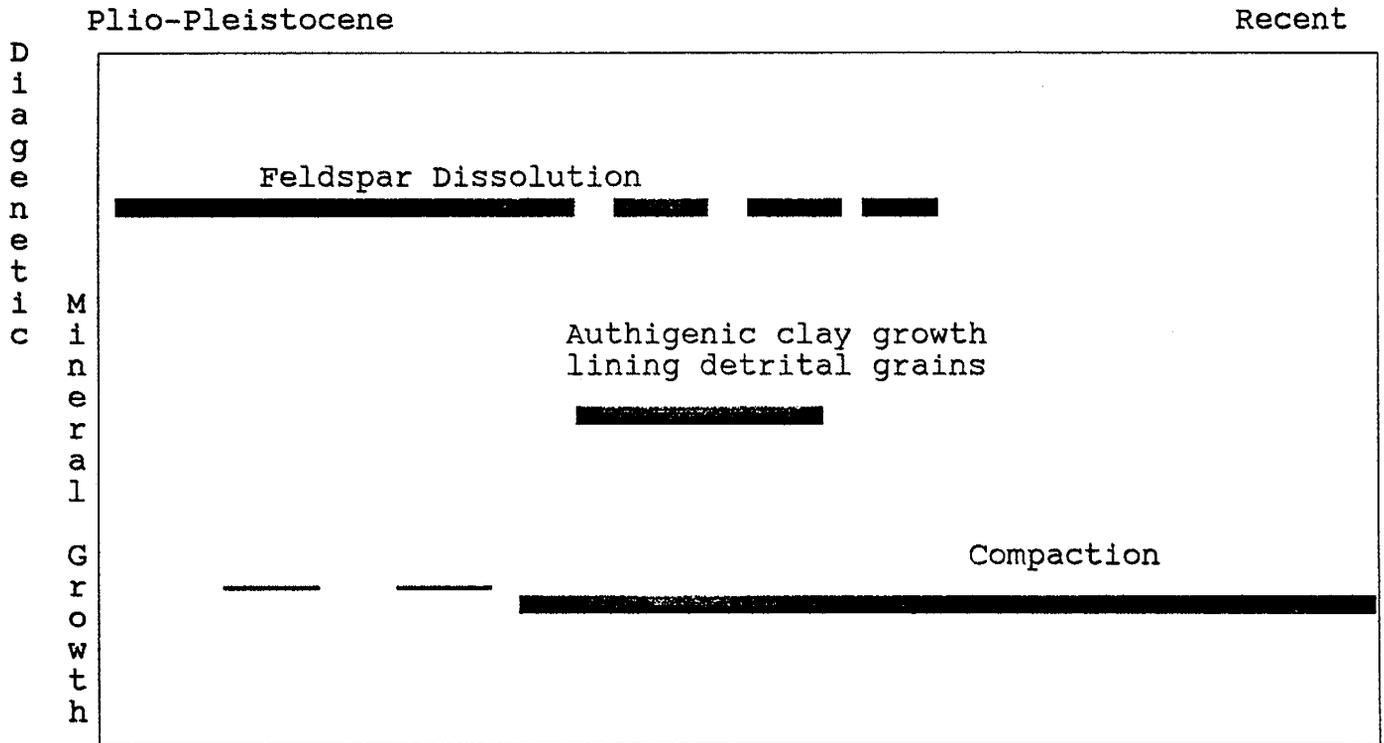
Diagenesis

List of diagenetic events (the events are not necessarily in the correct chronological order):

1. Compaction
2. Feldspar alteration and dissolution.
3. Authigenic clay mineral growth lining detrital grains.

Relative Sequence of Diagenetic events for Core No.2

Increase in Burial
 _____>>
 Shallow Burial: Eogenetic Zone



Key: ■ Dissolution
 ■ ■ Dissolution, timing uncertain
 ——— Relative timing of diagenetic event is certain
 ■ ■ ■ Relative timing of diagenetic event is uncertain

Note: Sequence of diagenetic events are identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No.2 was cored from the Miocene-Pleistocene sands, Kern River Formation of the Kern River field on the eastern side of the San Joaquin Valley in California. The Kern River Formation has been described by Kodl and others (1990). The formation is over 1000 ft (about 300 meters) thick consisting of unconsolidated gravel and sand interbedded with various non-reservoir deposits such as silt and clay. Authigenic pore-filling clay is an important factor in locally reducing productivity. The gross producing interval is highly stratified, often containing ten or more separate sand layers within a 200 to 300 ft (60 to 90 meters) interval. Individual sand bodies generally vary from a few feet to 40 ft (1 to 13 meters) in thickness. The core sample was taken from one of these porous sand layers. The studied sample was found to be of a homogeneous lithology. The sand units or packages (Kodl *et al.*, 1990) are lenticular in shape. Original oil entrapment was controlled largely by updip sand pinchouts possibly in combination with the groundwater table and possibly hydrodynamic flow.

Depositional Environment

During the Miocene-Pleistocene, high energy alluvial channel sand complexes were deposited in braided stream channels. Unstable tectonic conditions persisted in this area of California during the accumulation of non-marine sediments. The overall depositional pattern of the productive interval resembles the form of an alluvial/ fluvial deposystem (Kodl *et al.*, 1990). Cross bedding within the sands indicate a source area to the east.

Pore-lining authigenic clay probably has been deposited by near-surface water percolating downward below the water/sediment interface of the stream bottom. Stream beds were the principal areas of recharge for the groundwater regime (Kodl *et al.*, 1990). Some chemical elements such as K, Al, Mg, Fe, Si required for authigenic clay mineral growth may have been derived from the dissolution of other grains within the rock.

Description of Plates

Plate 1: The loose sediment sample studied.

- 1a. The loose sediment before removal of hydrocarbons.
- 1b. The loose sediment sample after removal of hydrocarbons.

Plate 2: Photomicrographs of thin sections.

- 2a. Plane polarized light view of sub-angular to sub-rounded detrital grains. Clear, low relief grains are quartz and high relief, opaque grains are lithic fragments or feldspar.
- 2b. Cross polarized light view of clear, low birefringence quartz, grey feldspars and lithic grains.
- 2c. Cross polarized light view of quartz, and lithic grains. The fine grained green/brown mottled fragment probably is an igneous rock fragment. A small mica fragment is located within the center of the photomicrograph.

Plate 3: Scanning electron micrographs of core No.2

- 3a. Bar is 10 microns x 1300.
Fracture surface of a detrital quartz grain. The fracture is conchoidal in places.
- 3b. Bar is 10 microns x 1100.
Surface of a feldspar grain. The delicate surface features of the grain indicating dissolution.
- 3c. Bar is 10 microns x 1600.
Surface of detrital quartz grain (Q). An Authigenic clay (AC) with a honeycomb morphology infills irregularities on the surface of the grain.
- 3d. Bar is 1 micron x 5400.
Magnified view of authigenic clay. The clay forms a 1 micron layer on the surface of the detrital grain and is composed of many interconnecting plates.
- 3e. Bar is 1 micron x 4800.
In this area the authigenic clay is lined with a layer of horizontal plates, forming a smooth surface. The honeycomb morphology of the clay is seen breaking through in the middle of the photomicrograph.
- 3f. Bar is 10 microns x 2000.
Feldspar (F) surface displaying dissolutional features. The surface of the detrital grain is covered in authigenic clay (AC).
- 3g. Bar is 10 microns x 3000
Authigenic clay (AC) infilling dissolutional features within a feldspar grain (F).

Plate 4- Energy dispersive spectra analysis

- 4a. Detrital quartz, SiO_2 grain
- 4b. Ca rich plagioclase feldspar, anorthite $\text{CaAl}_2\text{Si}_2\text{O}_8$.

- 4d. Biotite $K_2(Mg,Fe)6-2(Fe,Al,Ti)O-2[Si_{6.5}Al_{2.3}O_{20}](OH,F)_4$
4c. Ca,Fe clay. Probably a variety of smectite.

Plate 5- X-Ray diffraction Analysis.

- 5a. Q: Quartz
P: Plagioclase feldspar
5b. Q: Quartz
P: Plagioclase feldspar
5c. Q: Quartz

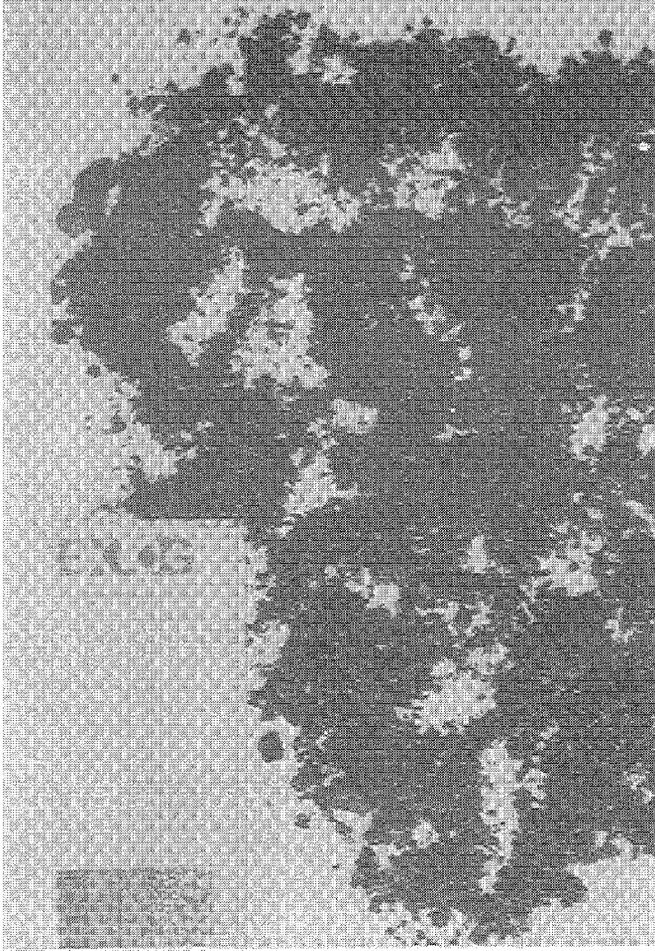


Plate 1a

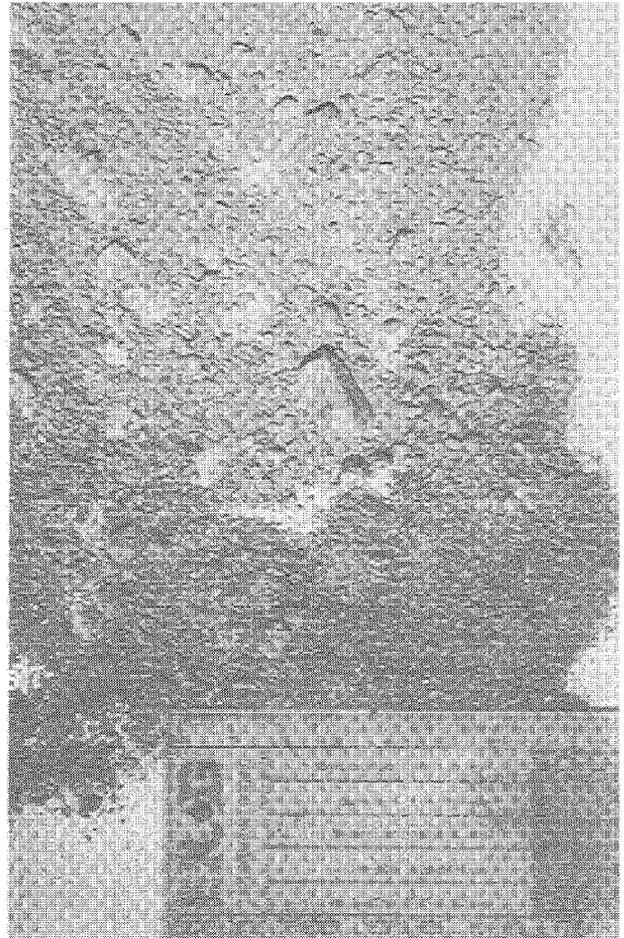


Plate 1b

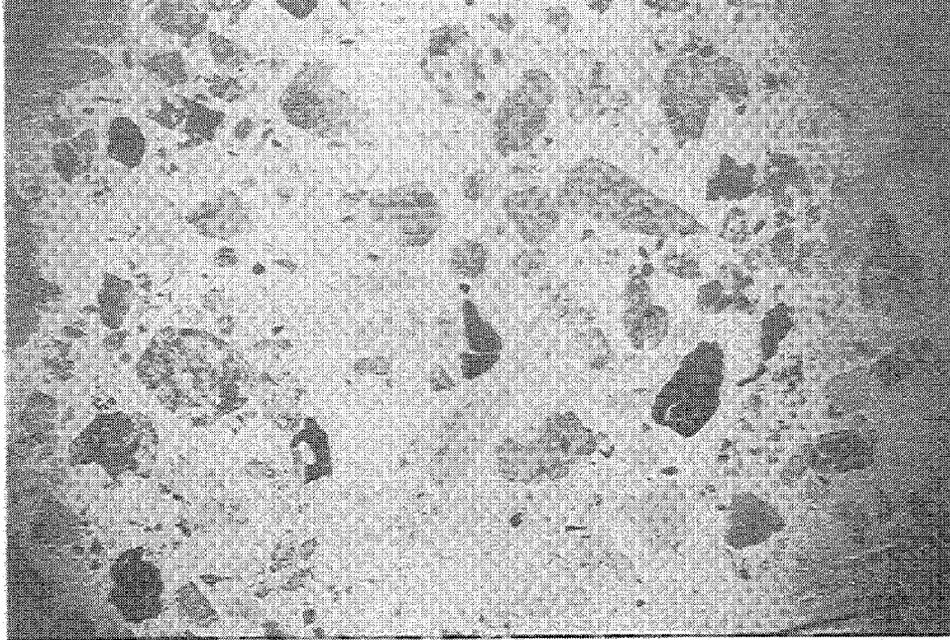


Plate 2a

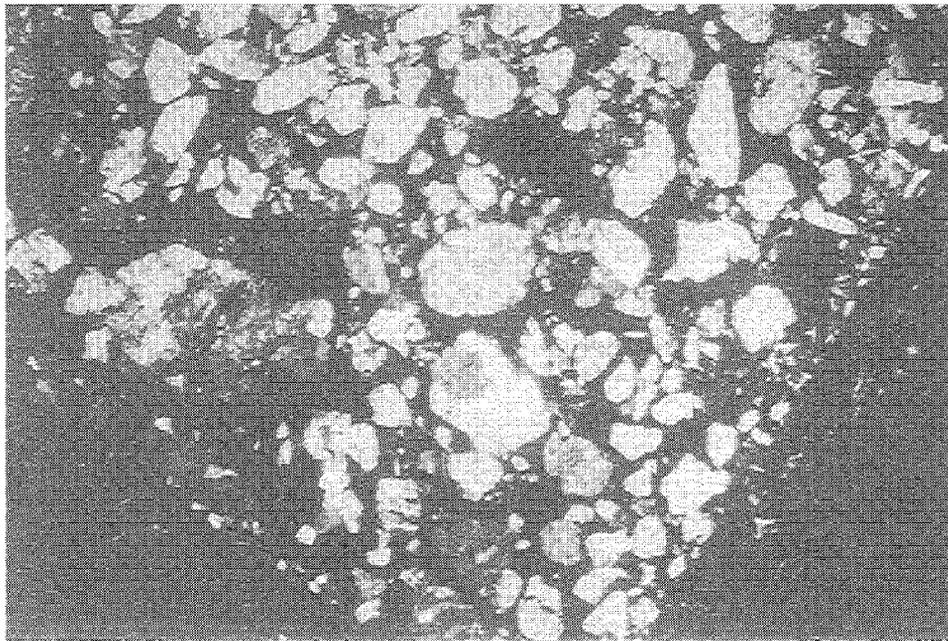


Plate 2b

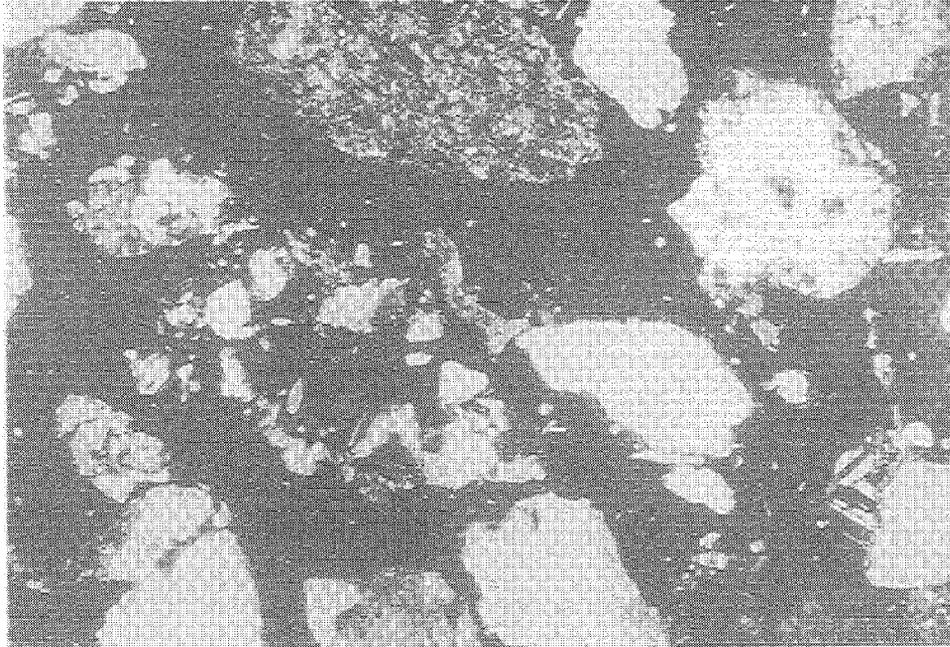


Plate 2c

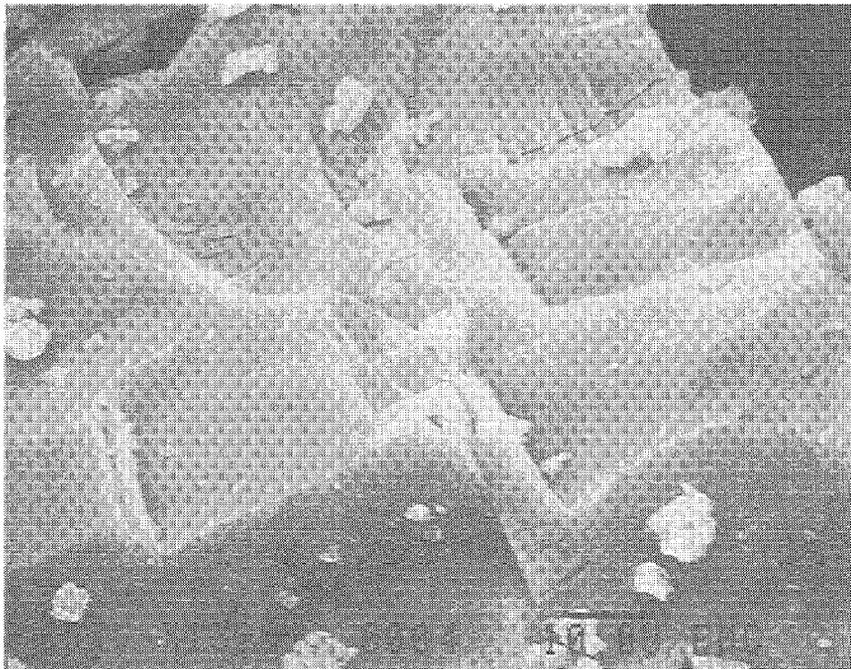


Plate 3a

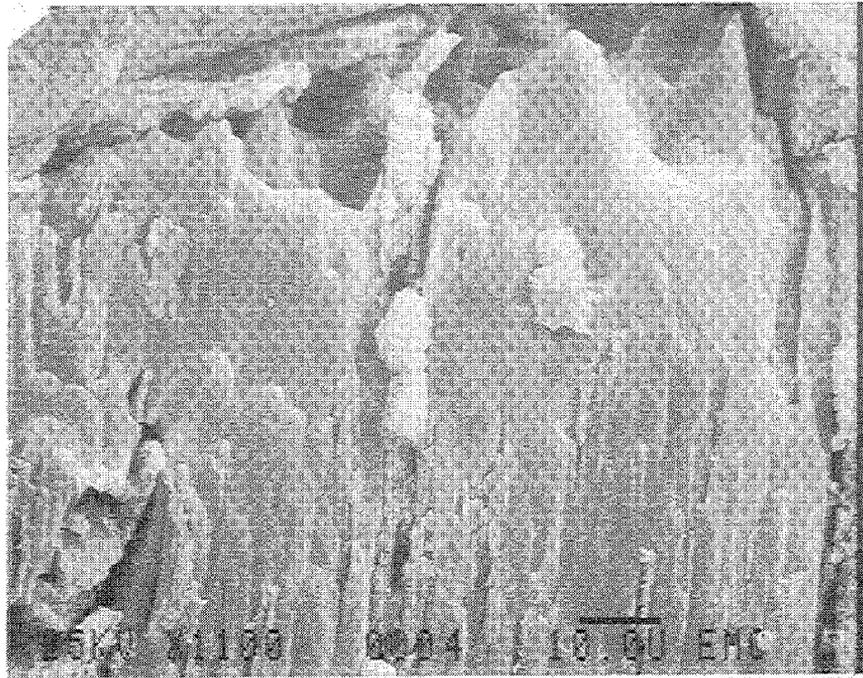


Plate 3b

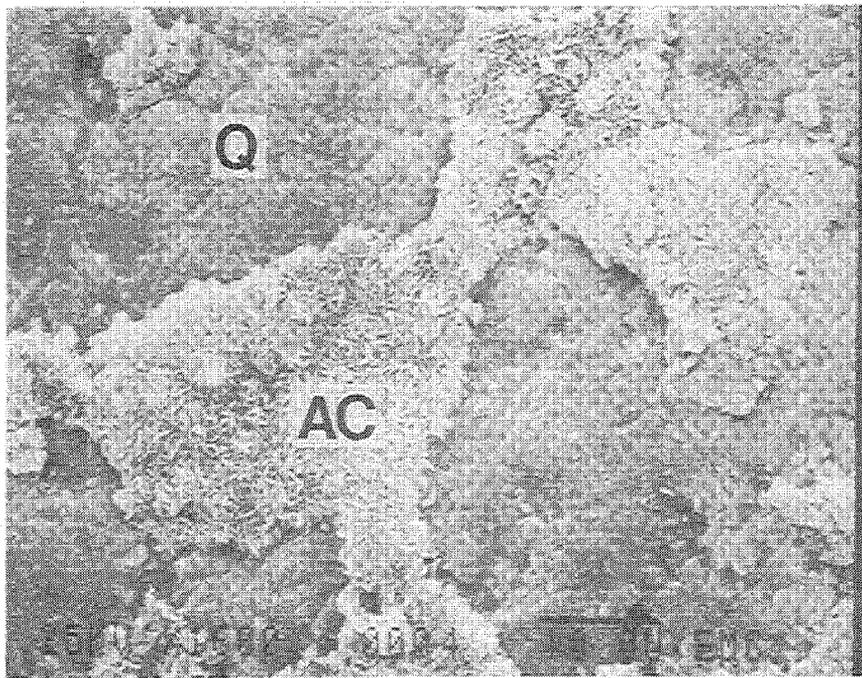


Plate 3c

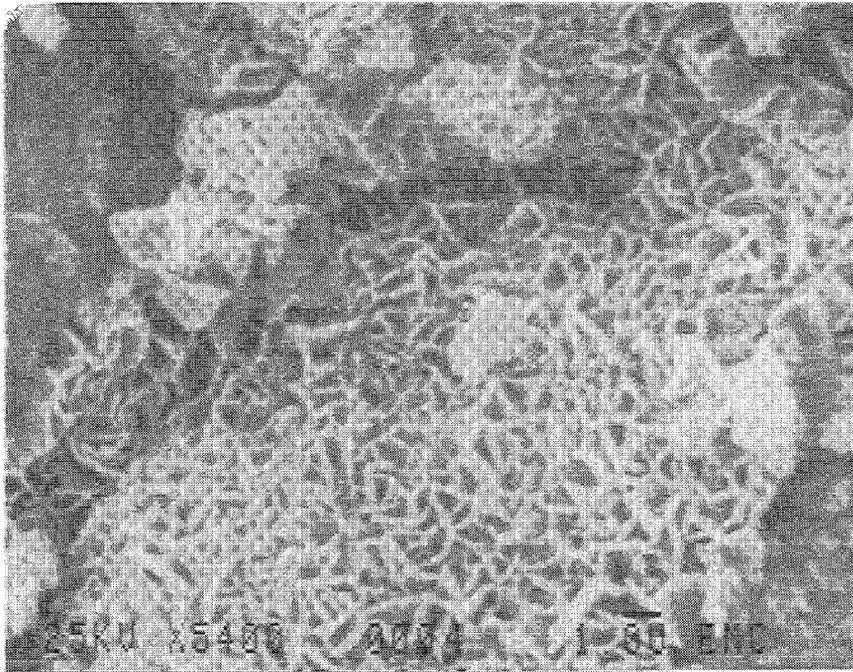


Plate 3d

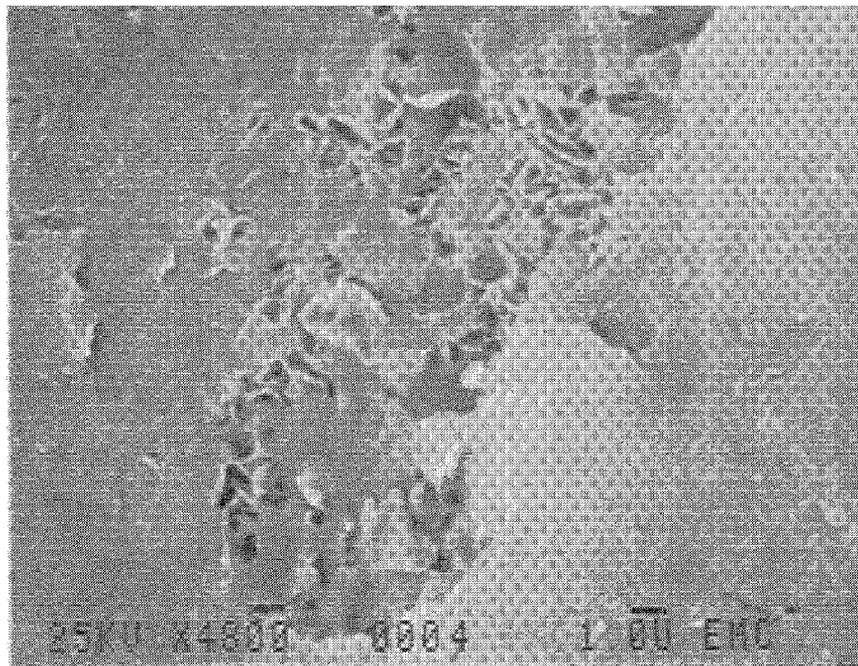


Plate 3e

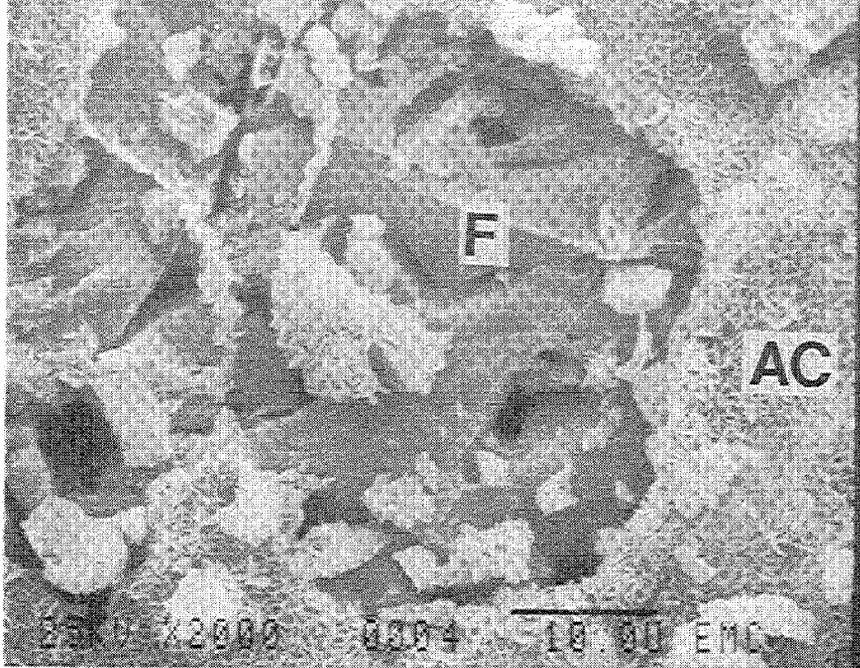


Plate 3f

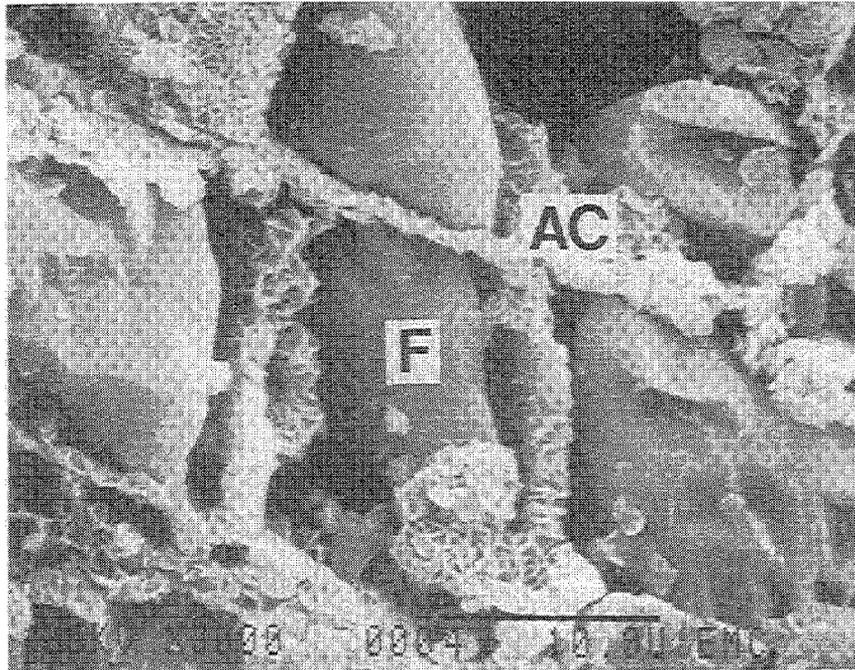


Plate 3g

30-Oct-1991 14:02:00

Quartz 613 counts Disp= 1
Preset= 100 secs
Elapsed= 100 secs

Vert= 613 counts Disp= 1

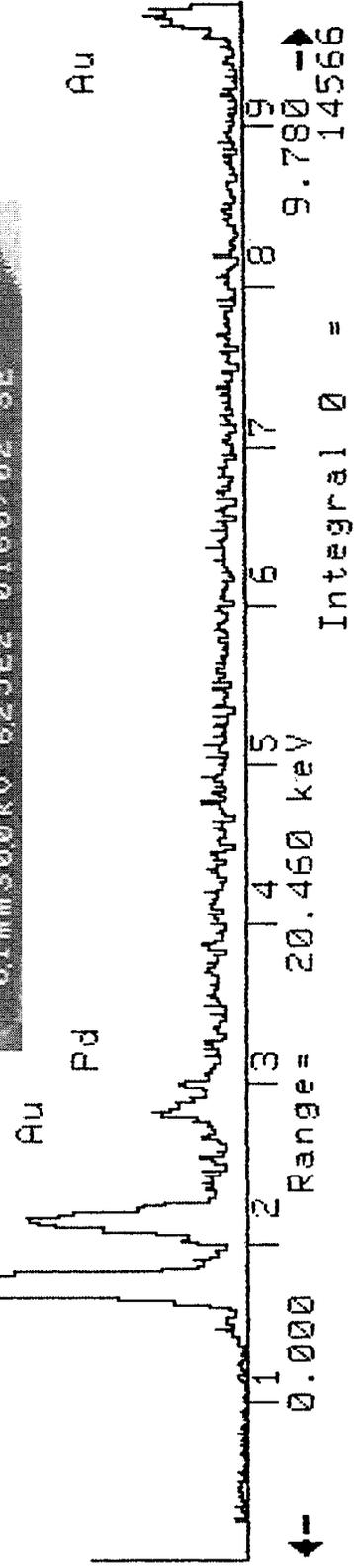
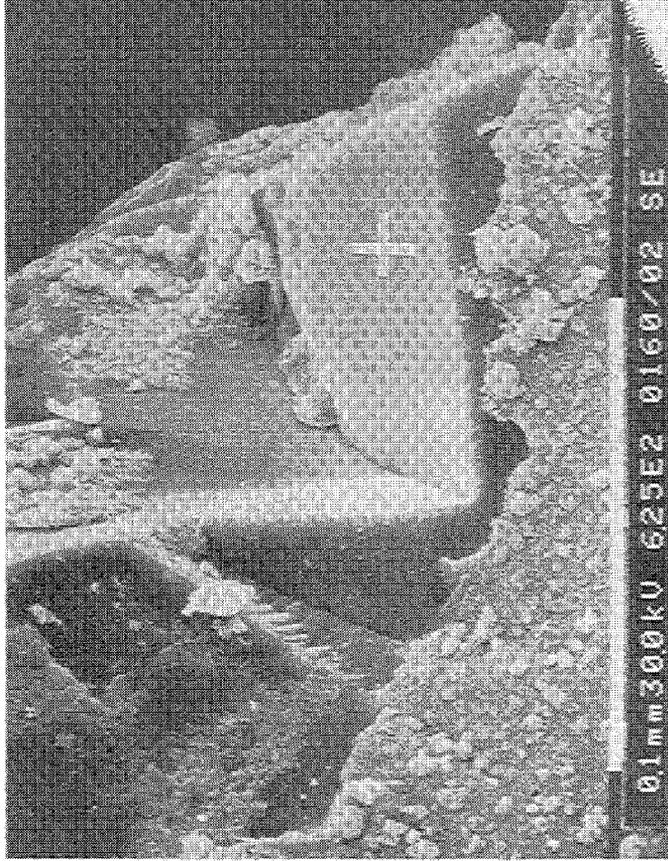


Plate 4a. Detrital Quartz, SiO₂ grain.

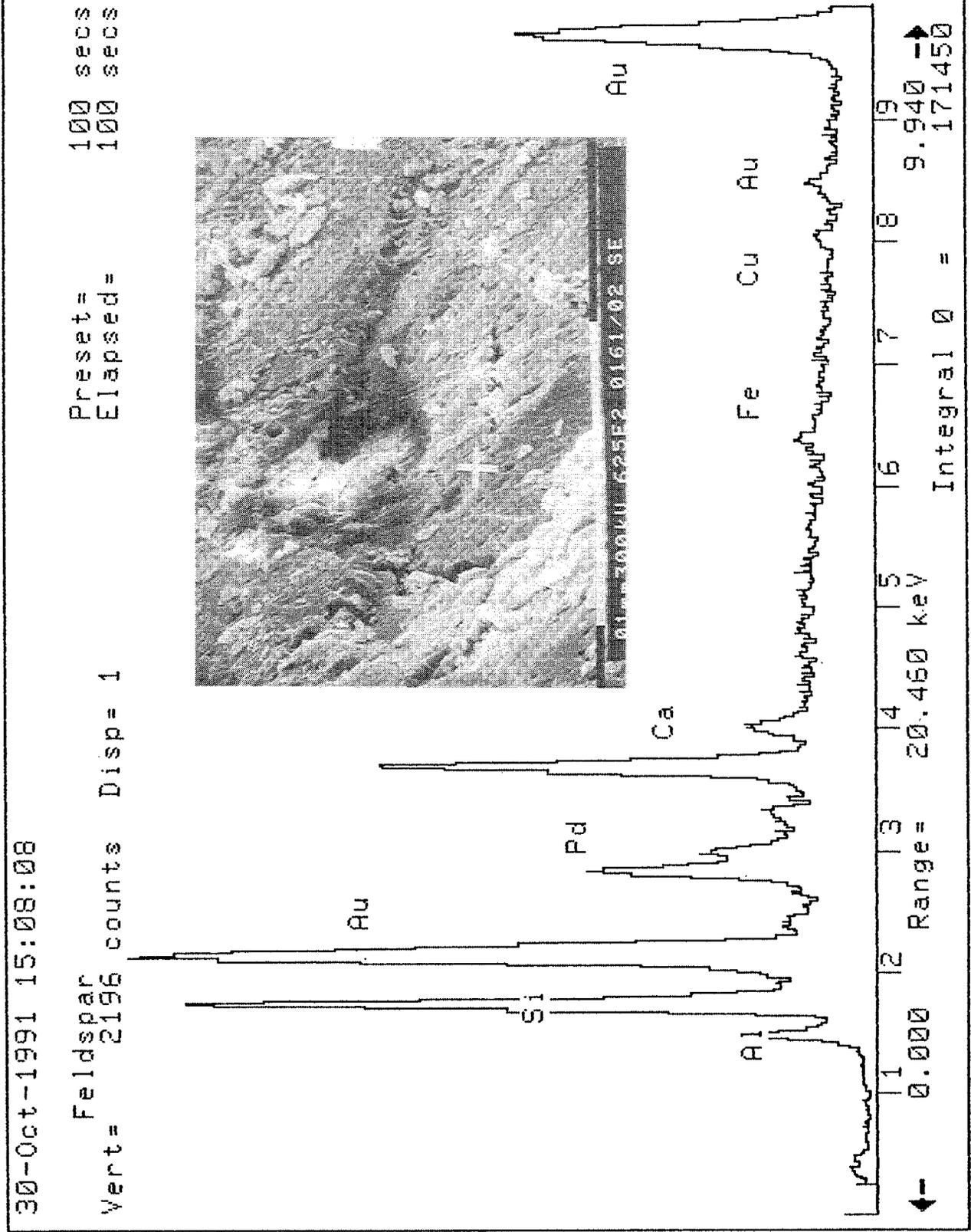


Plate 4b. Ca rich plagioclase feldspar, anorthite $\text{CaAl}_2\text{Si}_2\text{O}_8$. The feldspar also contains minor amounts of Fe.

30-Oct-1991 15:22:37

Vert= 546 counts
Feldspar
Disp= 1
Preset= 100 secs
Elapsed= 100 secs

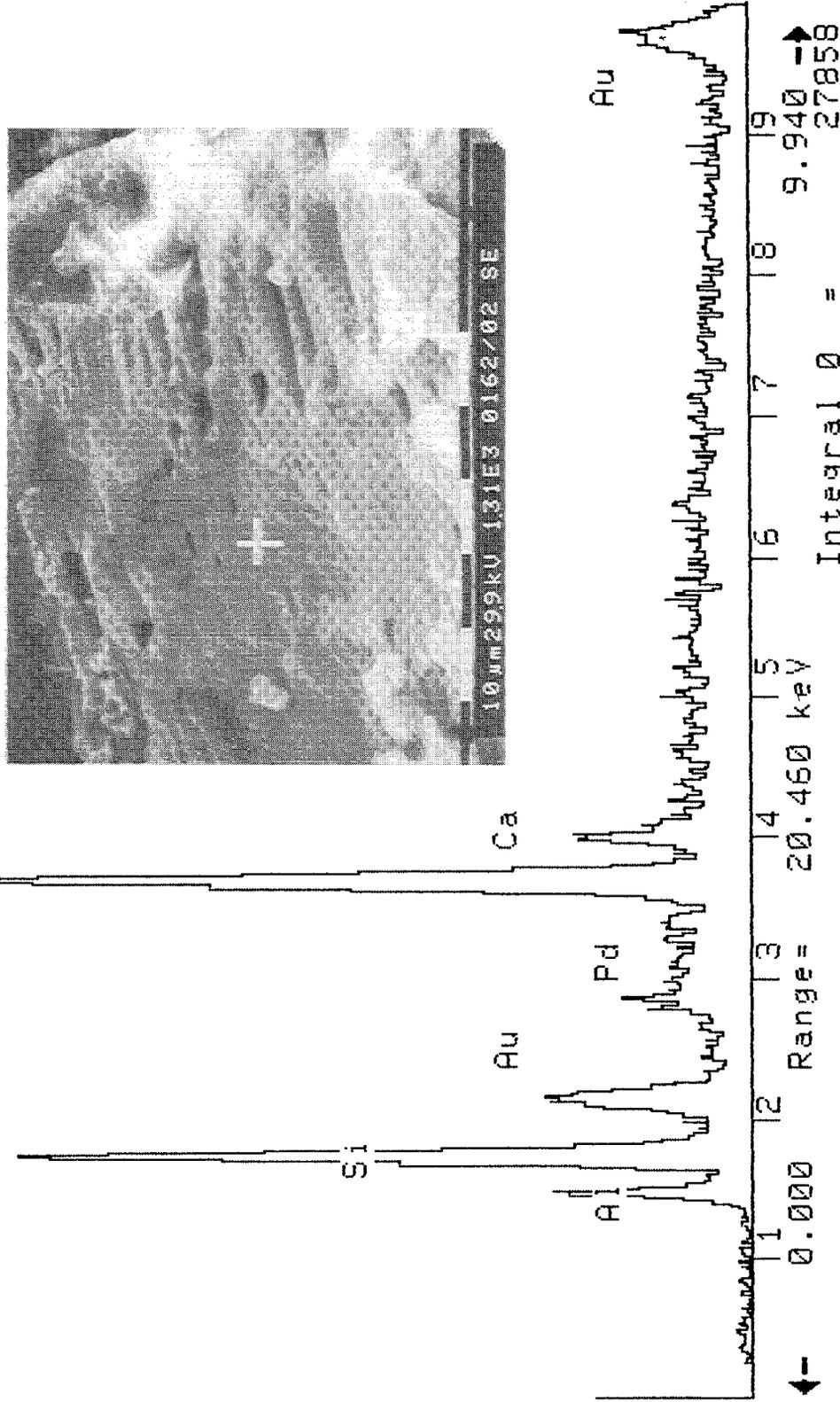
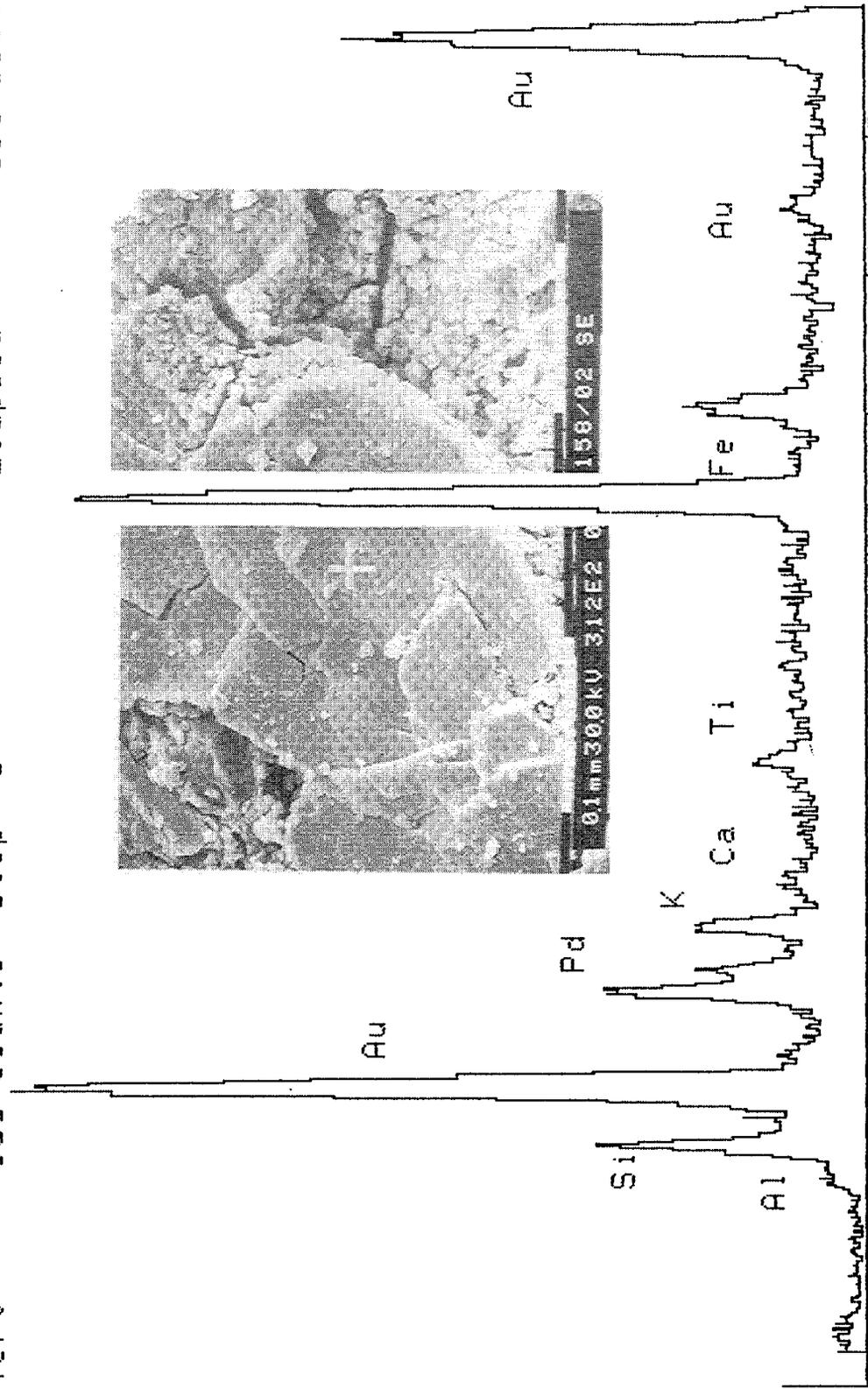


Plate 4c. Ca rich plagioclase feldspar, anorthite $\text{CaAl}_2\text{Si}_2\text{O}_8$.

30-Oct-1991 12:22:48

Vert= Biotite 988 counts Disp= 1
Preset= 100 secs
Elapsed= 100 secs



← 0.000 Range= 20.460 keV Integral 0 = 9.940 →
76751

Plate 4d. Biotite $K_2(Mg,Fe)_6-2(Fe,Al,Ti)O-2[Si_6-5Al_{2-3}O_{20}](OH,F)_4$.

30-Oct-1991 11:57:21

Vert= 3407 counts Disp= 1 Preset= 100 secs
Elapsed= 100 secs

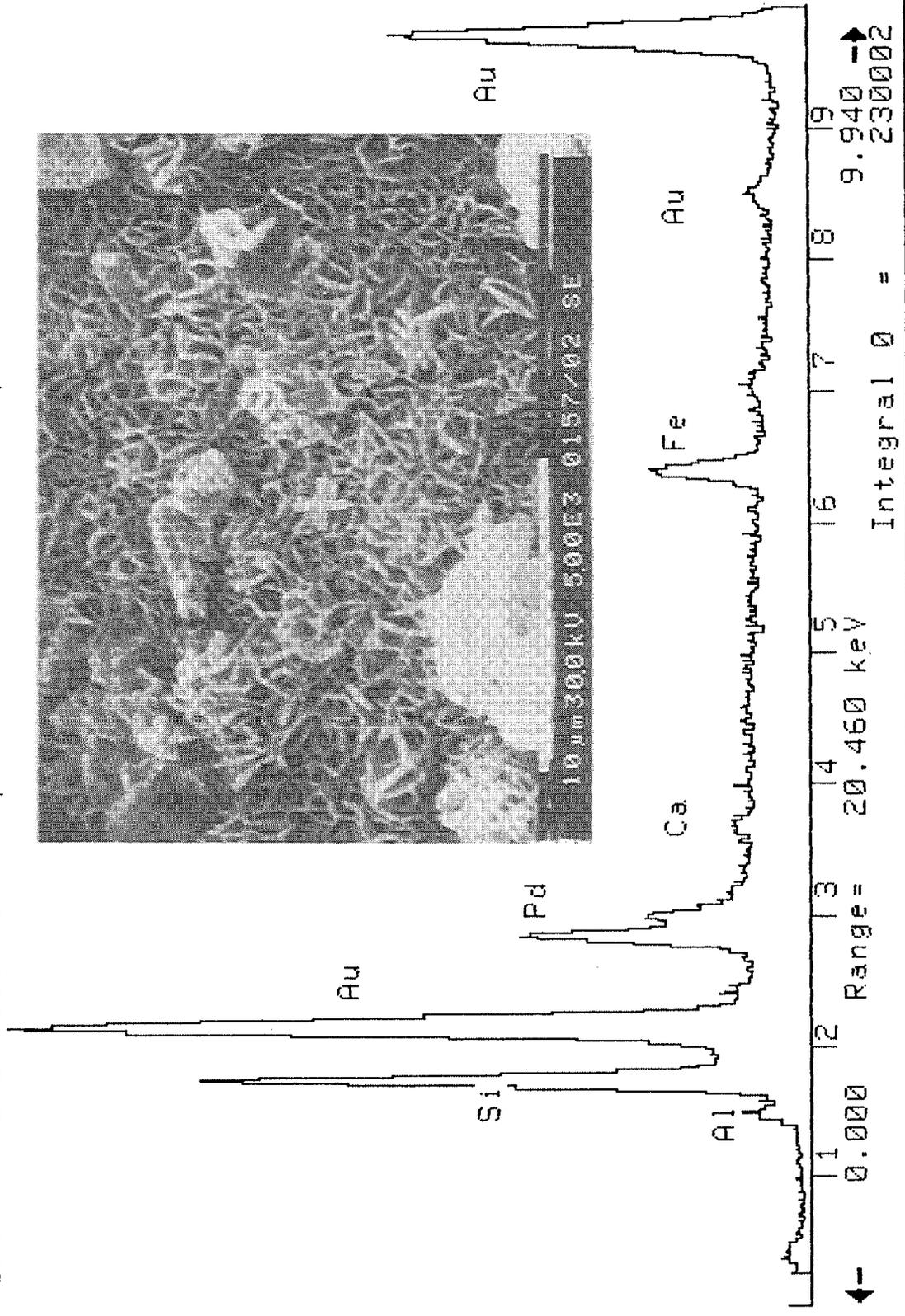


Plate 4e. Ca, Fe, clay. Probably a variety of smectite.

FN: AV4.NI
DATE: 08/19/91

ID: LOOSE SAND
TIME: 09: 48

PT: 0.225
STEP: 0.0300

SCINTAG/USA
WL: 1.54060

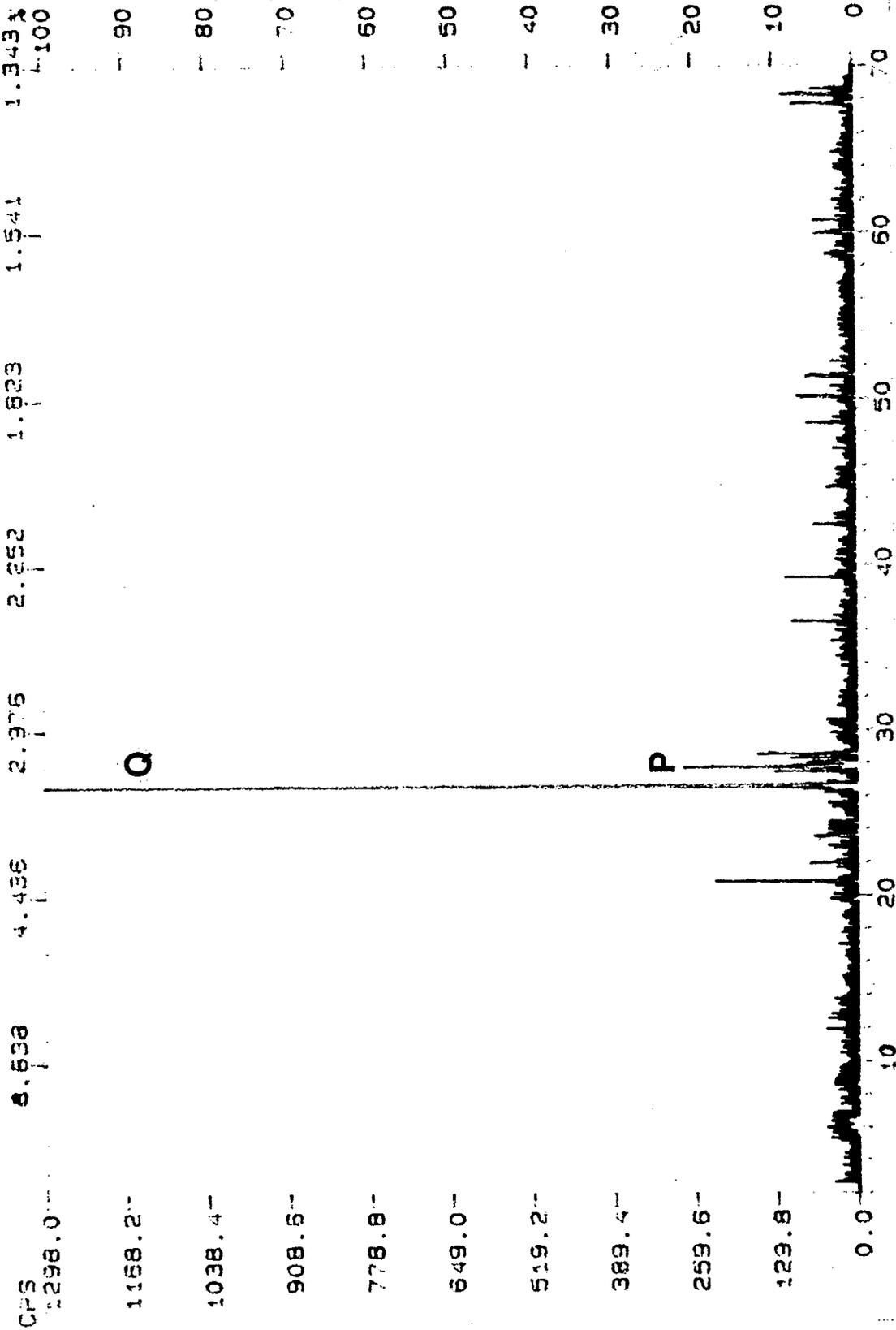


Plate 5a. Q: Quartz P: Plagioclase feldspar

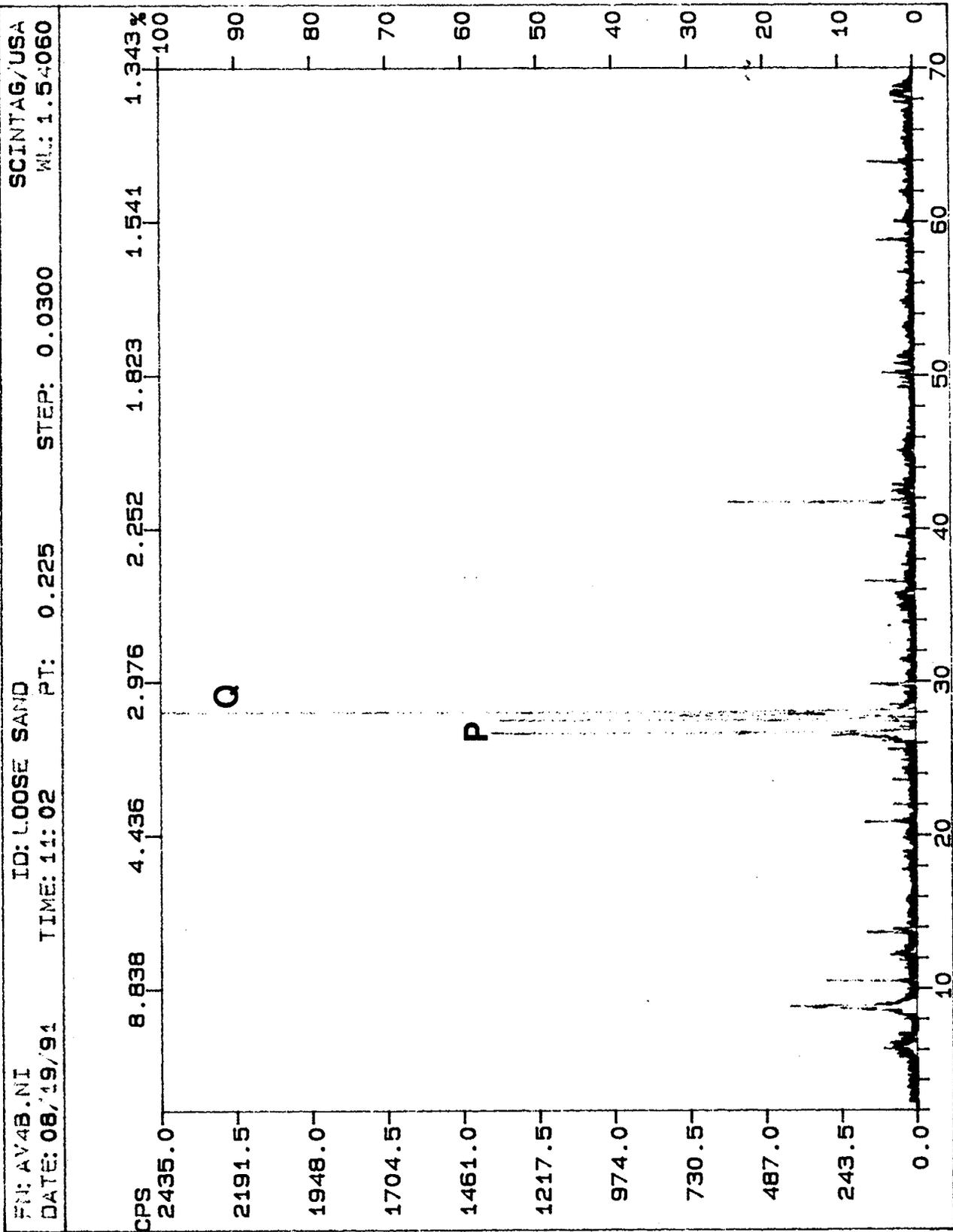


Plate 5b. Q: Quartz P: Plagioclasse feldspar

FN: AV4TS.NI ID: AV4 THIN SECTION SCINTAG, USA
 DATE: 08/19/91 TIME: 11:18 PT: 0.225 STEP: 0.0300 WL: 1.54060

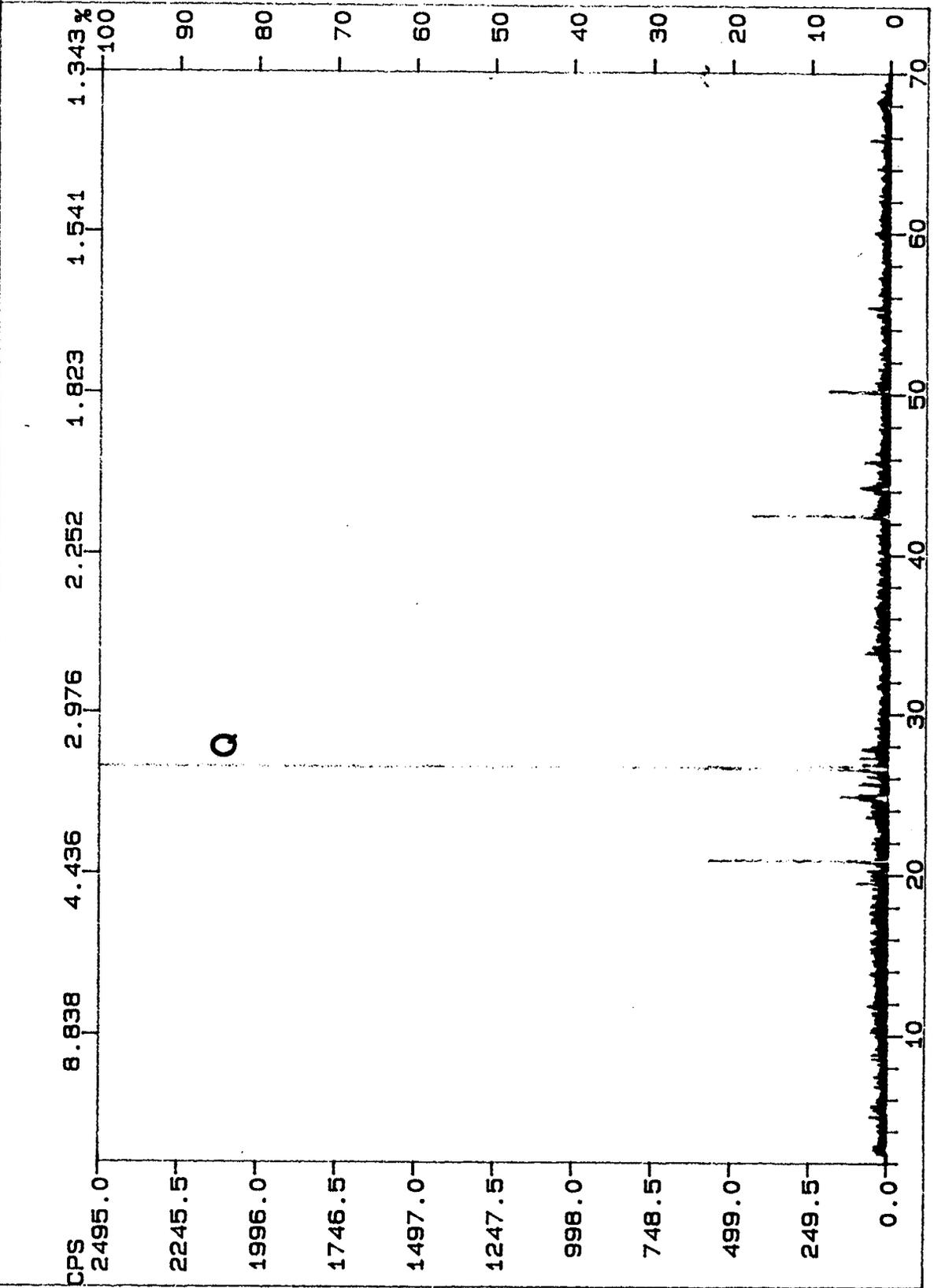


Plate 5c. Q:Quartz

Core No.3

Oil Company: Texaco
Well Name: #684, JEM"A"NCT-1
Field Name: MABEE
State: Texas
County: Andrews
Formation Name: San Andres
Formation Age: Upper Permian; Guadalupian Section
Formation Type: Dolomite
Core Depth: 4725.5 - 4726.5 ft.

Core Length: 8 cm.
Core Diameter: 8.5 cm.

Rock Description: A buff to light brown (light gray in color when the hydrocarbon content is removed) dolostone consisting predominantly of crystalline dolomite and anhydrite. White crystalline anhydrite occurs as a patchy cement and as large nodules, occasionally 2.5 cm in diameter. The rock has a lack of layering and contains no sedimentary structures.

Detailed Petrography

Grain Size Crystalline dolomite and anhydrite matrix. Fine dolomite consisting of rhombohedral crystals generally < 10 μ m.

Roundness, Angularity and Detrital Grain Shape
No detrital grains were identified.

Fabric (Orientation and Packing)
Random, no preferred orientation.

Porosity Percentages were measured by point counting thin sections
Porosity = 2.9 +/- 1.5 %
Porosity was found mainly to exist within a three dimensional intercrystalline network of dolomite crystals. Since intercrystalline porosity is difficult to measure in thin section the true porosity is probably greater than the figure given above. Pore throats average 10 μ m in diameter. In some areas small vugs (<0.1 mm) were found.

Mineral Composition Percentages were measured by point counting thin sections

Dolomite	92.0 +/- 2.7 %
Anhydrite	7.0 +/- 2.3 %
Gypsum	1.0 +/- 0 %

The rock also contains minor amounts kaolinite.

Detrital grains
Not present.

Post Depositional Minerals

Dolomite occurs as microscopic (10 μm in diameter) often poorly shaped rhombohedral crystals.

Anhydrite and gypsum forms large tabular, orthorhombic crystals with well developed flat crystal surfaces. Anhydrite and gypsum often occurs together within nodules, up to 2.5 cm in diameter. Anhydrite displays possible replacement textures of gypsum. Anhydrite crystals (0.25-1.0 mm in diameter) in some areas also have a poikilotopic texture which encapsulate dolomite crystals and infill pores. Anhydrite crystals, 5 μm in diameter were seen nucleating on the surface of some dolomite crystals.

Authigenic kaolinite clay occurs as pore-filling, stacked pseudo hexagonal plates, 5 μm in diameter.

Diagenesis

List of diagenetic events [these events are not necessarily in the correct chronological order].

1. Early dolomitization of calcium carbonate.
2. Gypsum cement formation.
3. Growth of poikilotopic and pore-filling and dolomite enclosing anhydrite cement (including the possible replacement of earlier formed gypsum).
4. Growth of pore-filling kaolinite.

RELATIVE SEQUENCE OF DIAGENETIC EVENTS FOR CORE NO. 3

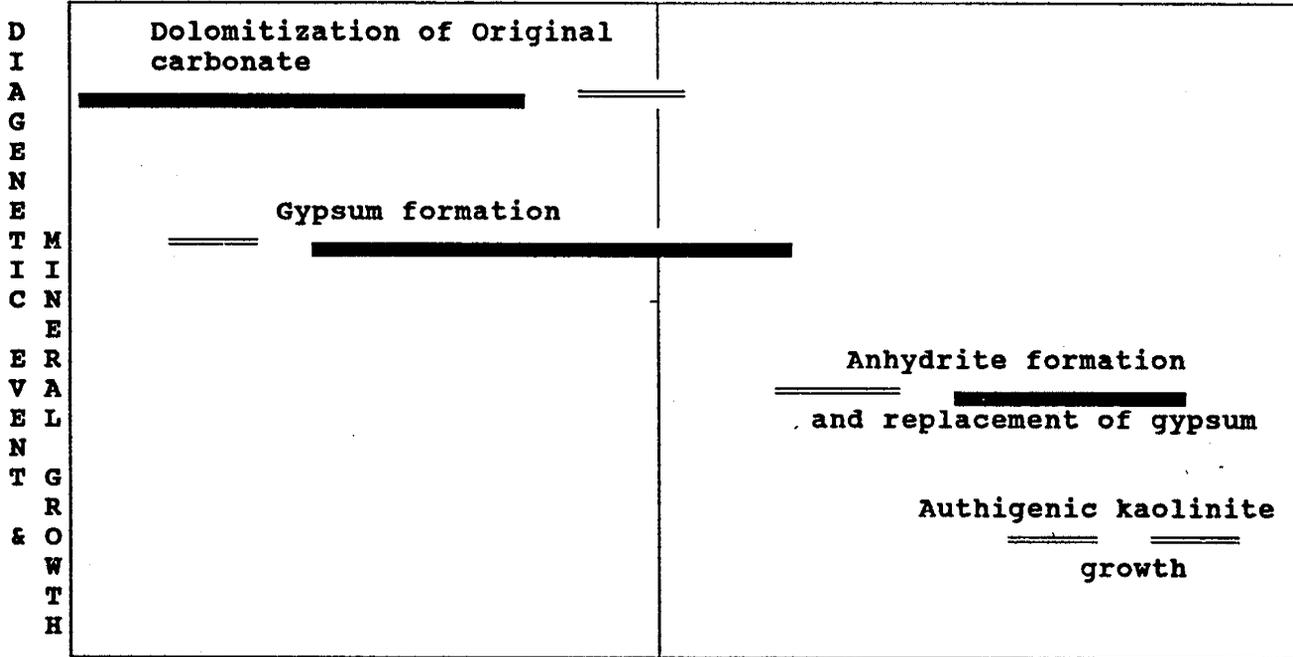
Increase in Burial >>>

Shallow Burial: Eogenetic Zone
(Authigenetic minerals formed due to hypersaline fluids)

Deep Burial: Mesogenetic Zone
(Authigenetic minerals formed due to circulating meteoric fluids)

Middle Permian

Recent



Key: ■ Relative timing of diagenetic event is certain.
 ══ Relative timing of diagenetic event is uncertain.

Note: Sequence of diagenetic events are identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No.3 was cored from the Permian, San Andres Formation of the Mabee Field, west Texas. The reservoir consists of more than 160 feet of dolomite. The reservoir has been described in detail by Urshel (1986). The 8 cm length of core studied generally was found to be of a homogeneous rock type. The complete reservoir section consists of variable lithologies. At the base of the succession silty dolomicrites are overlain by nearly 80 feet of a dolomitized oolitic facies, containing varying amounts of terrigenous detrital quartz. The majority of detrital quartz is found within this facies at the base of the reservoir. The studied core, a crystalline dolostone, was taken from this interval but does not contain any clastic component. The clastic component was derived from highlands to the northwest and interpreted as being eolian in origin (Urshel, 1986). The overlying facies is fine to medium grained crystalline dolomite with anhydrite nodules and cement that effectively destroys all porosity, near the top.

Depositional Environment

At the base of the sequence the rocks indicate a change from low energy shelf to a moderate to high energy, oolitic shoals. The succession suggests upward shallowing from intertidal-tidal flat to supratidal sabkha environment characterized by anhydrite deposits. During the Permian Period, the west Texas area was a topographic elongated basin trending north-northwest to south-southeast. This basin, known as the Midland Basin, was bordered by the Central Basin Platform to the west and an Eastern Shelf to the north and east. The San Andres Formation represents a regressive, upward shoaling sequence which changes from low energy, marine conditions to tidal flat and supratidal sabkha setting.

Description of Plates

Plate 1. The core sample studied.

- 1a and 1b show end views of the core.
- 1c shows the complete length of the core.

Plate 2. Photomicrographs of thin sections

- 2a. Cross polarized light view of patchy anhydrite with 2nd to low 3rd order birefringence. The surrounding brown areas are crystalline dolomite. The frame is 3 mm cross.
- 2b. Cross polarized light view of an anhydrite nodule. Note the possible replacement of lower birefringence gypsum crystals by anhydrite. The frame is 2 mm cross.

Plate 3. Scanning electron micrographs

- 3a. Bar is $10\mu\text{m}$ x 720
Poorly shaped dolomite crystals (D) and a large anhydrite crystal (A).
- 3b. Bar is $10\mu\text{m}$ x 1500
Pore-filling authigenic kaolinite occurring as stacked pseudo hexagonal plates.
- 3c. Bar is $10\mu\text{m}$ x 2000
An anhydrite crystal (A) growing around a well formed dolomite crystal (D).
- 3d. Bar is $1\mu\text{m}$ x 5400
Very small euhedral orthorhombic crystals of anhydrite (A) growing on poorly shaped dolomite (D).
- 3e. Bar is $10\mu\text{m}$ x 2000
Poorly formed crystals of dolomite (D). Note some well formed crystal faces of, possibly a second stage of dolomite growth.
- 3f. Bar is $10\mu\text{m}$ x 1100
Anhydrite replacing gypsum (G). Gypsum has smooth crystal surfaces indicating possible replacement.

Plate 4. Energy dispersive spectra analysis

- 4a. Poorly shaped dolomite, $\text{CaMg}(\text{CO}_3)_2$ rhomb.
- 4b. Anhydrite CaSO_4 (Note: Sulfur is masked by the Au peak)
- 4c. Orthorhombic anhydrite crystals growing on dolomite

Plate 5. X-ray diffraction analysis

- 5a. D: Dolomite, A: Anhydrite
- 5b. D: Dolomite, A: Anhydrite
- 5c. D: Dolomite, A: Anhydrite

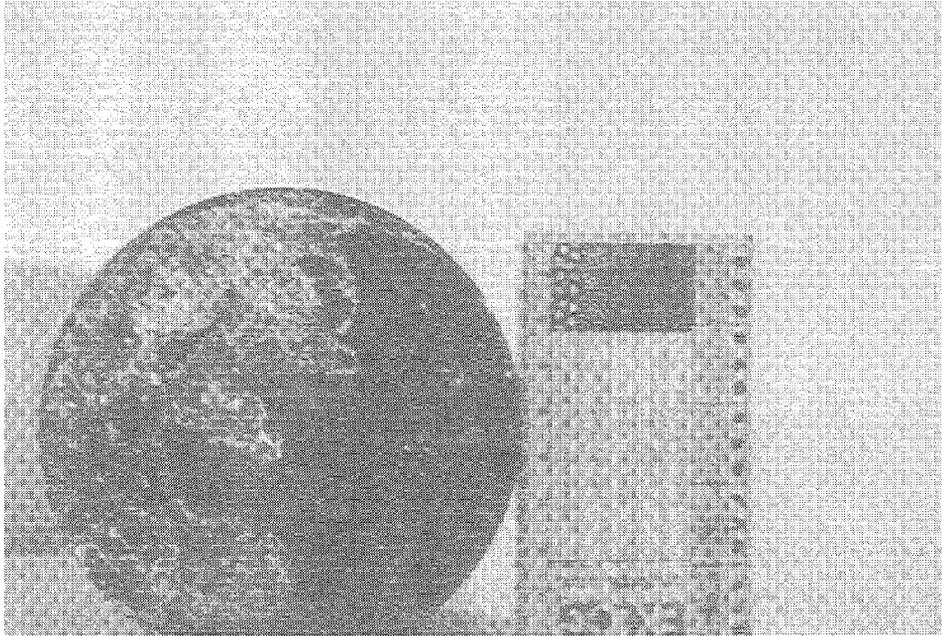


Plate 1a



Plate 1b

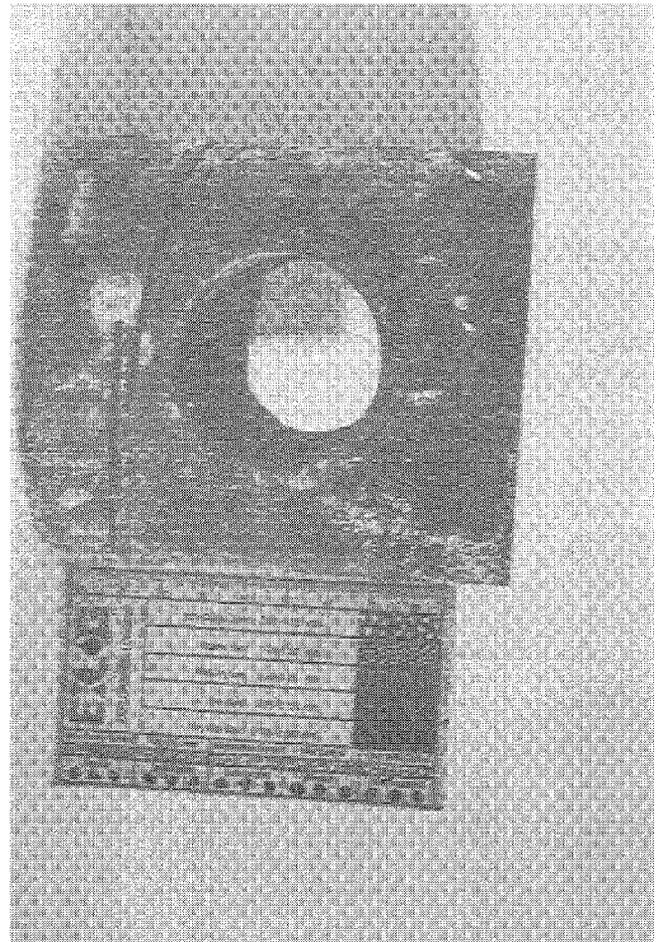


Plate 1c

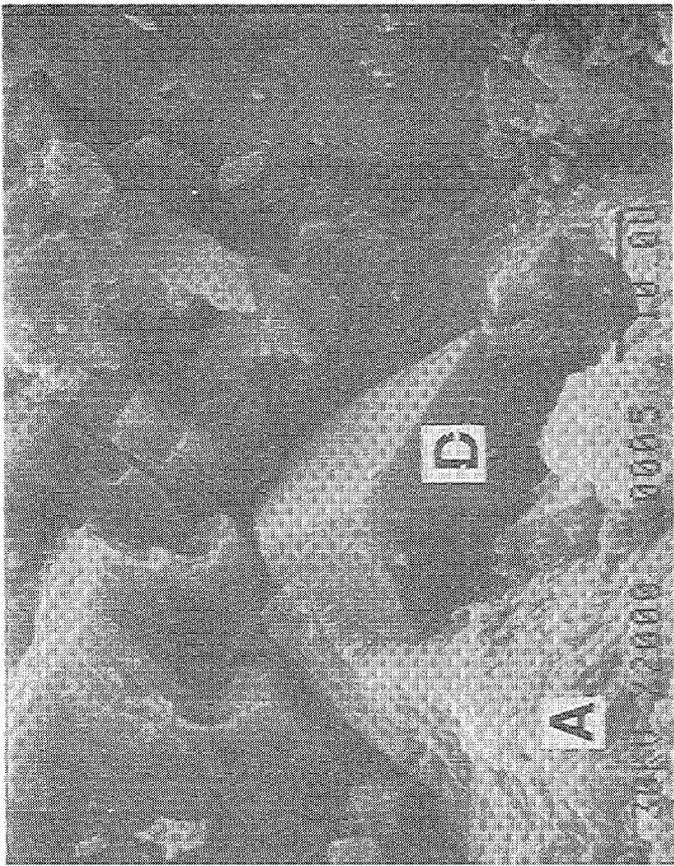
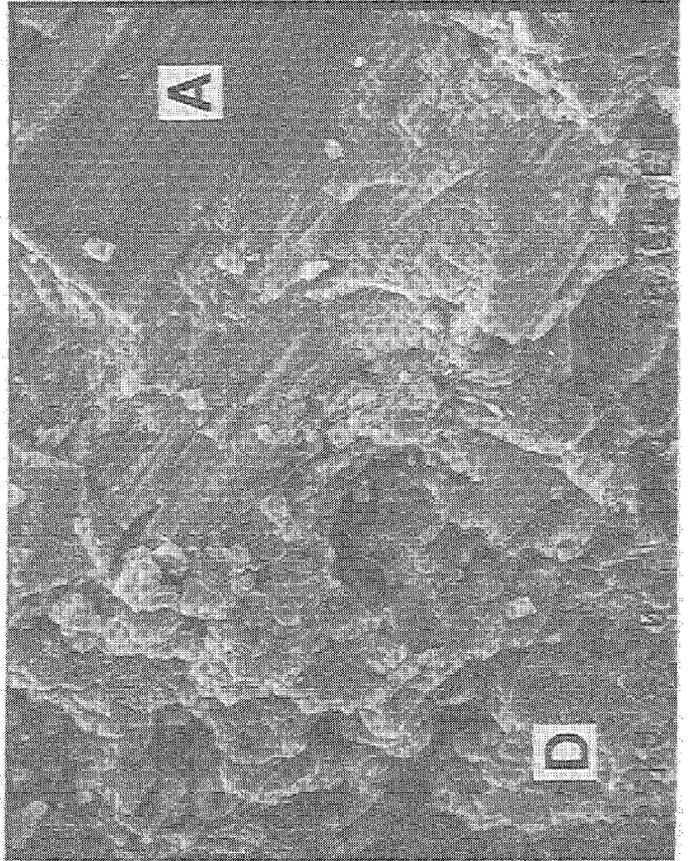


Plate 3a

Plate 3b

Plate 3c



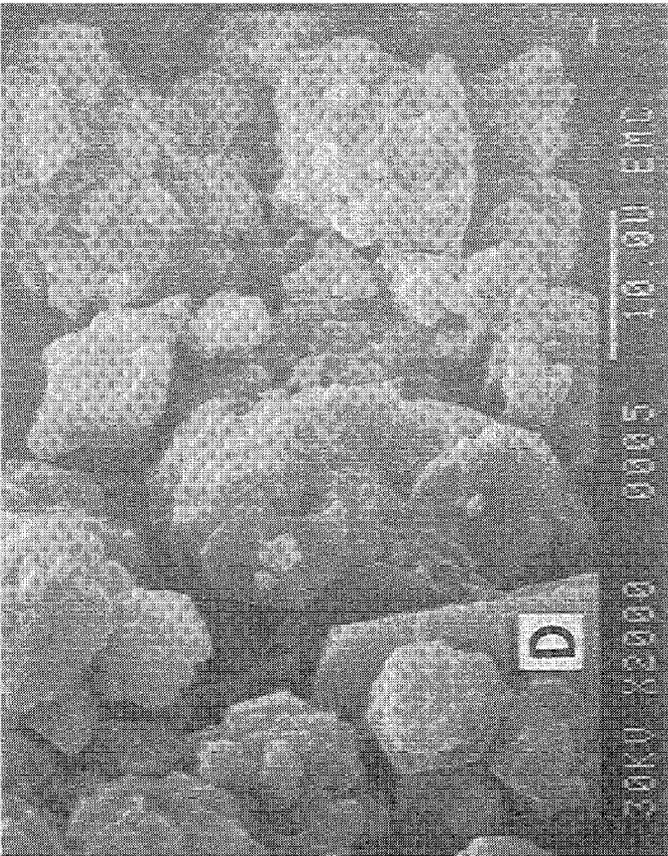


Plate 3e

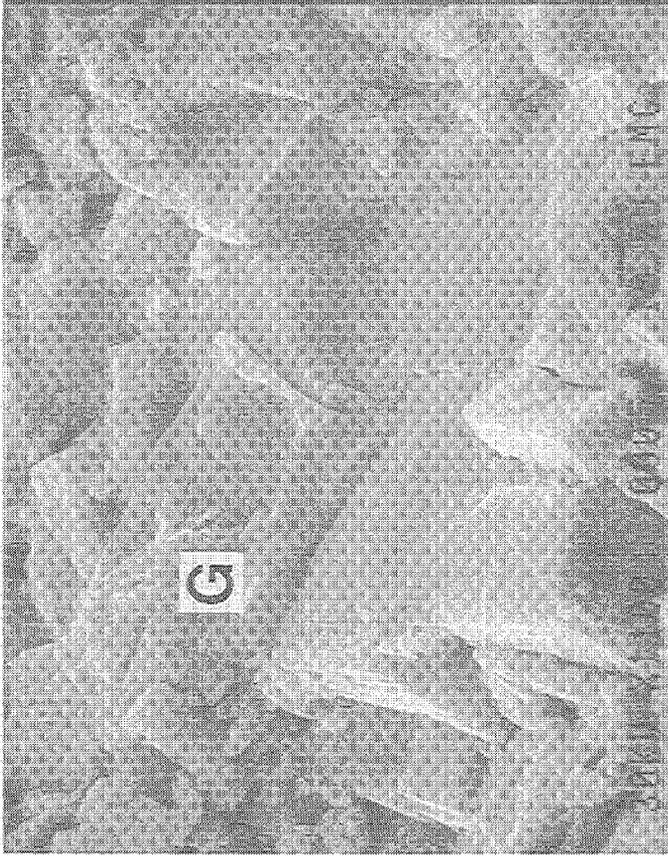


Plate 3f

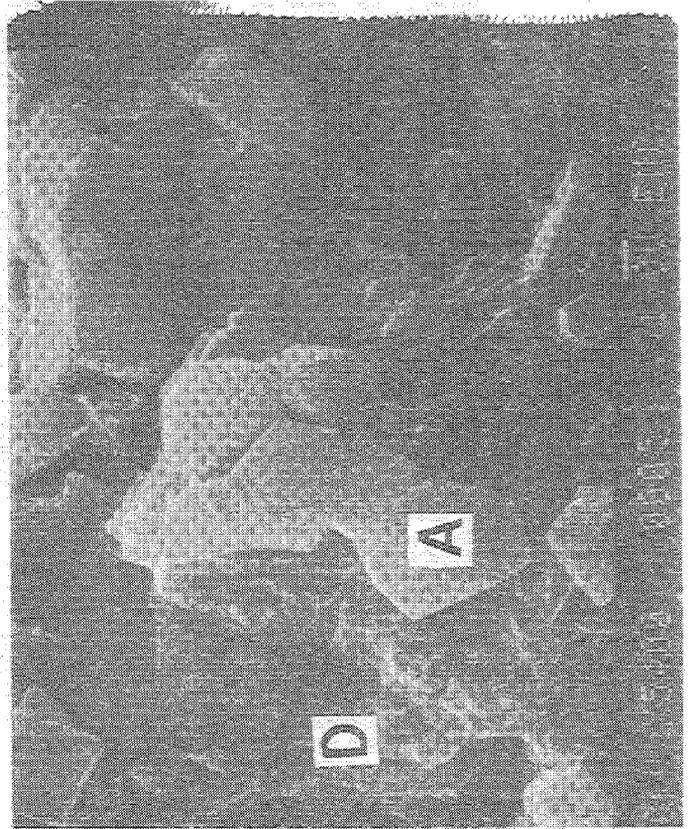


Plate 3d

14-Jul-1992 15:53:42
 Execution time = 4 seconds
 Vert= 4595 counts Disp= 1
 Preset= 100 secs
 Elapsed= 100 secs

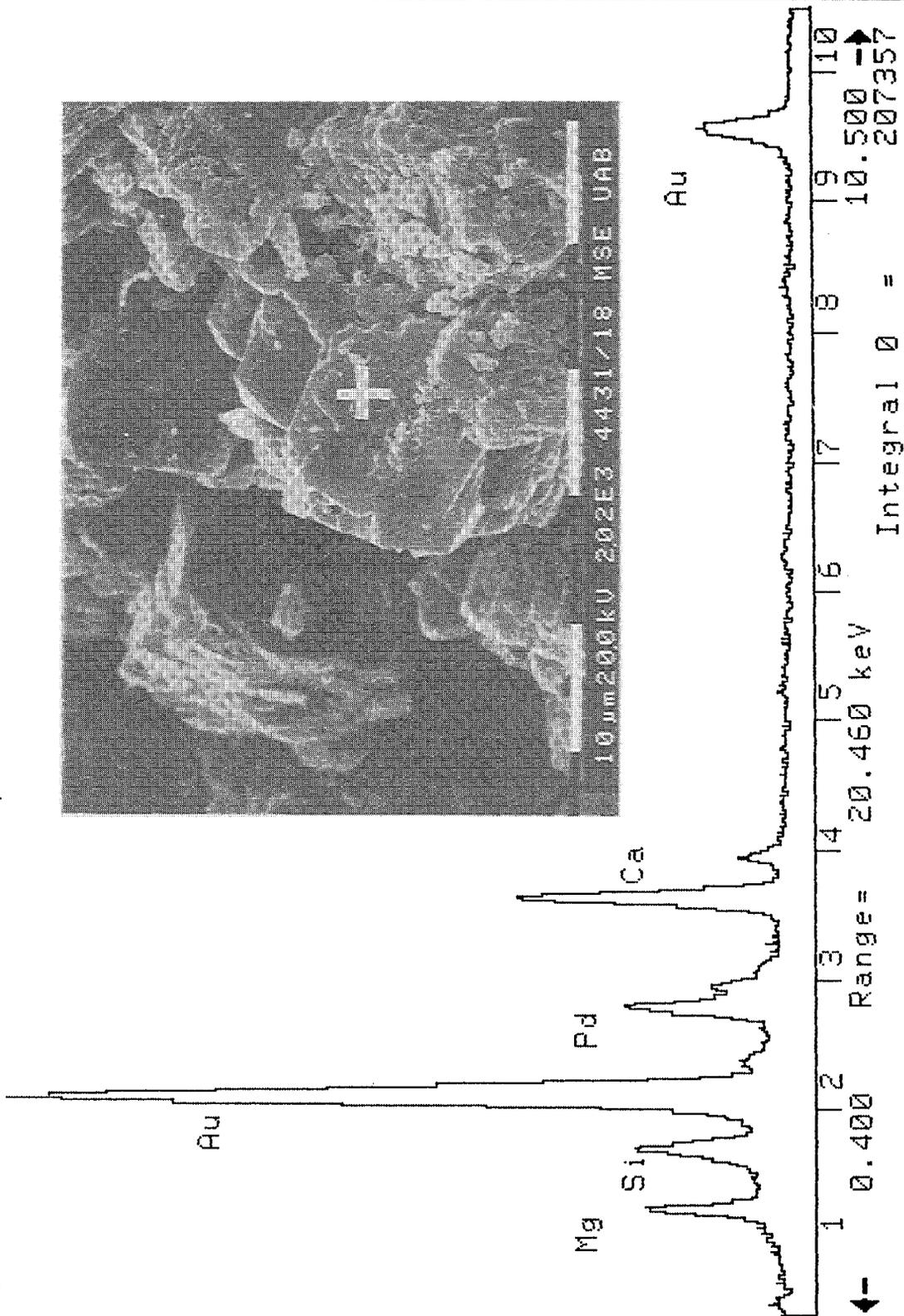
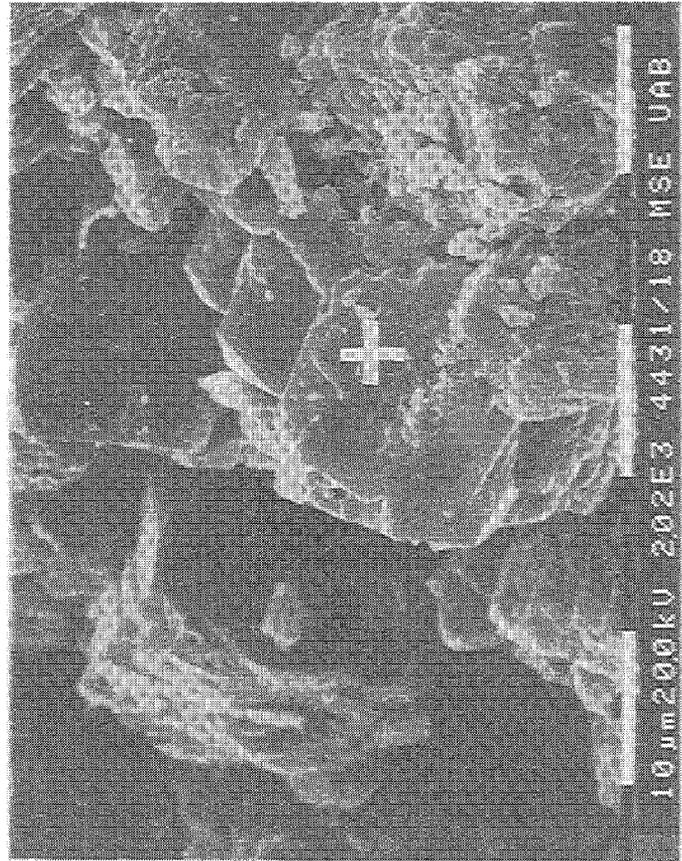


Plate 4a. Poorly shaped dolomite, $\text{CaMg}(\text{CO}_3)_2$.

14-Jul-1992 15:55:40
 Execution time = 4 seconds
 anhydrite03
 Vert= 4408 counts Disp= 1

Preset= 100 secs
 Elapsed= 100 secs

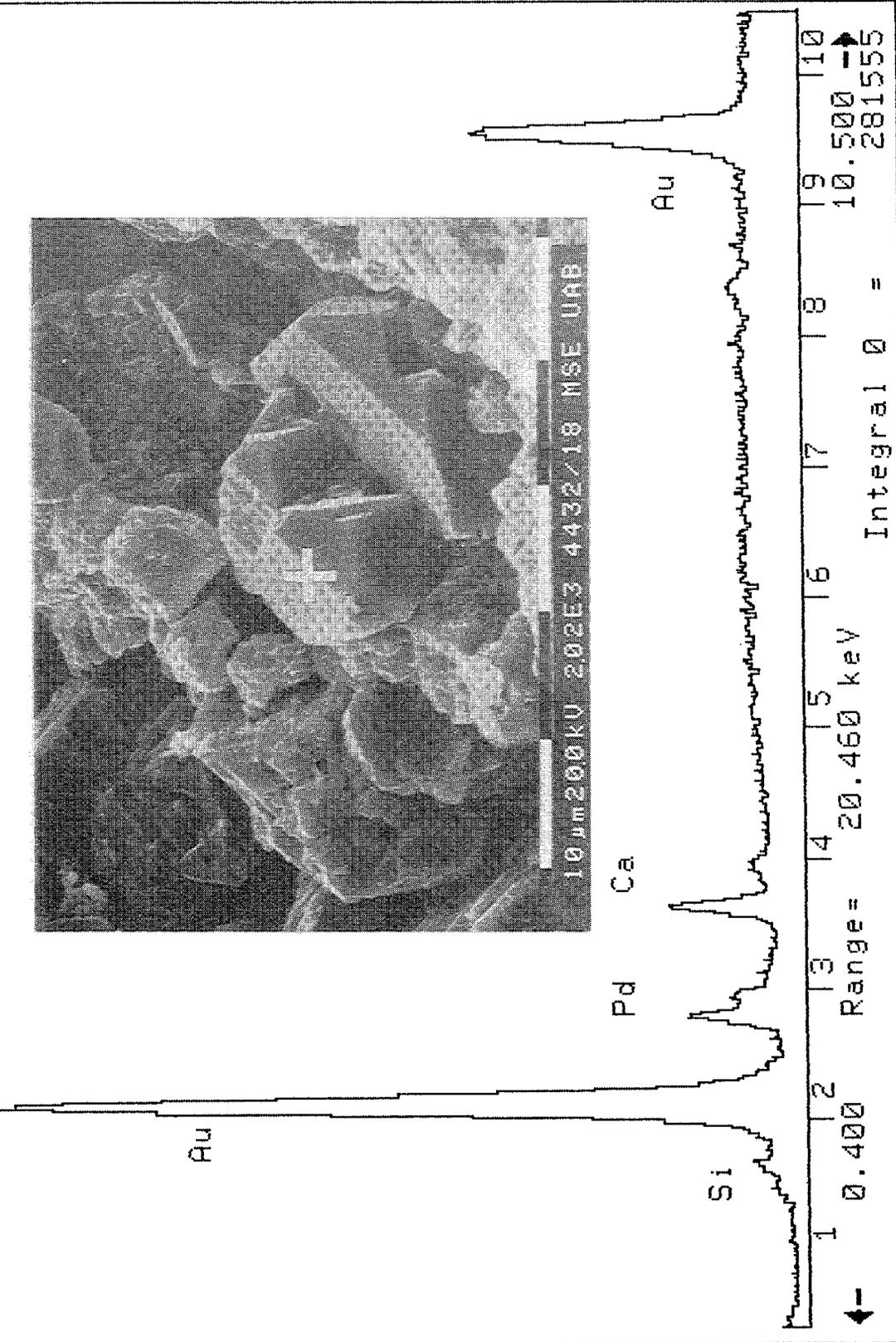
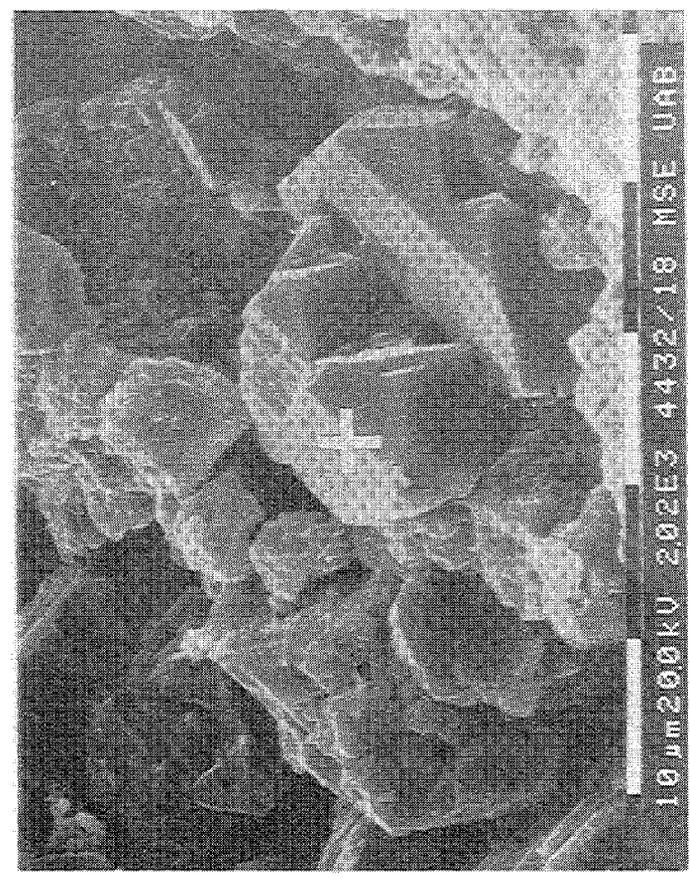


Plate 4b. Anhydrite CaSO₄

14-Jul-1992 16:00:22
 Execution time = 4 seconds
 anhydrite04
 Vert= 2953 counts Disp= 1
 Preset= 100 secs
 Elapsed= 100 secs

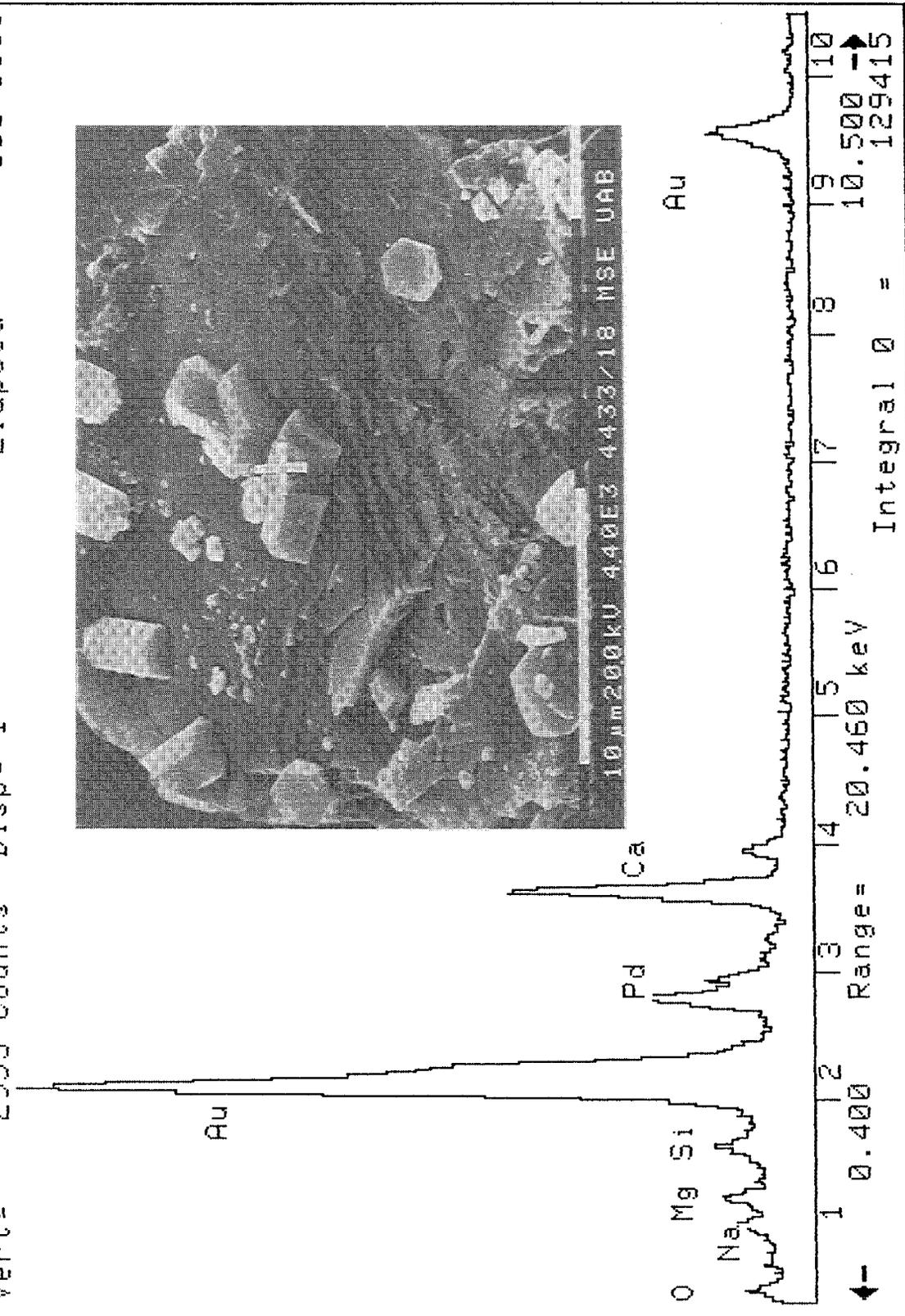
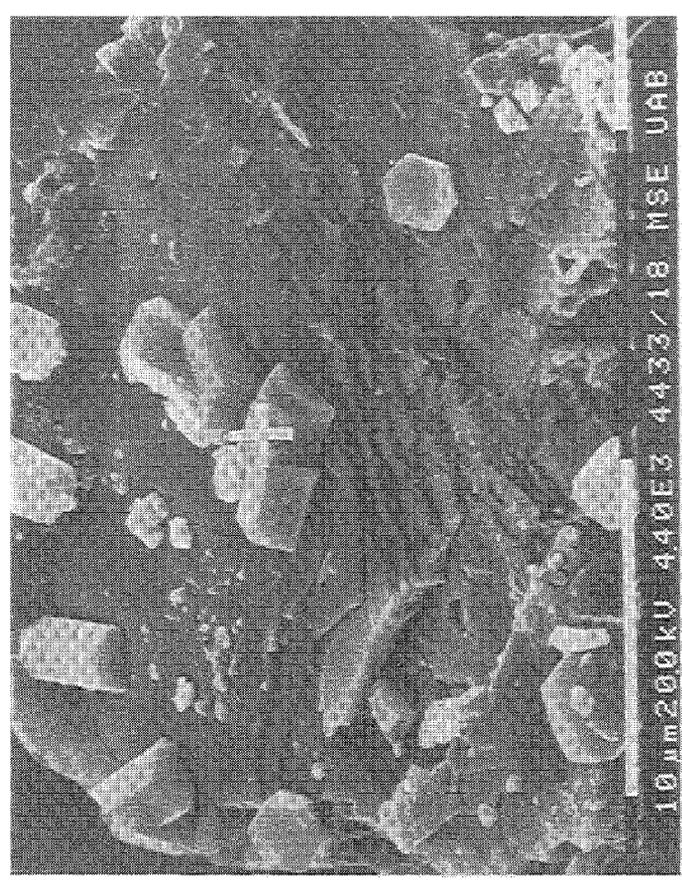


Plate 4c. Orthorhombic anhydrite crystals growing on dolomite.

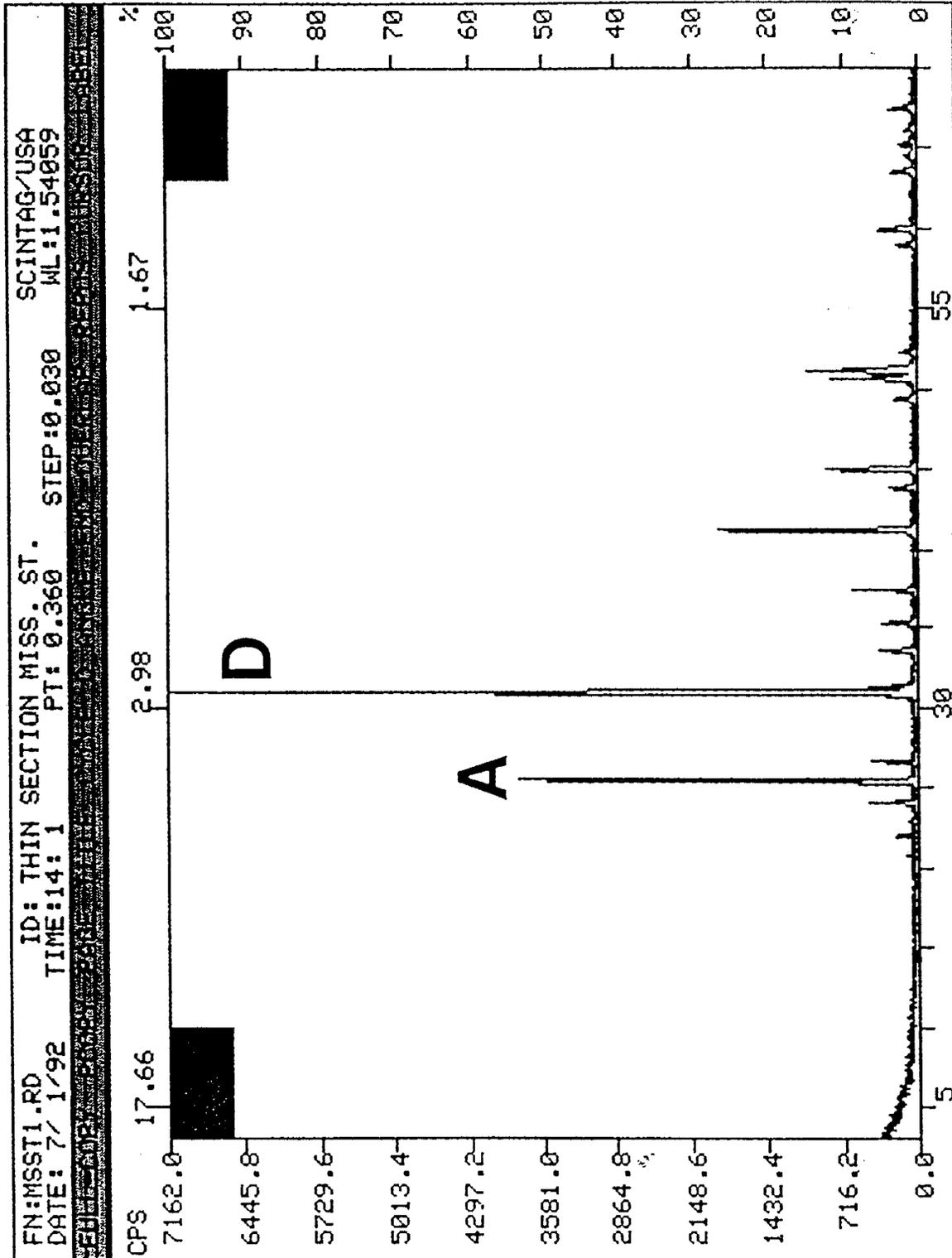


Plate 5a. D: Dolomite, A: Anhydrite

FN:MSST2.RD ID: SAMPLE 1 POWDER SCINTAG/USA
 DATE: 7/ 1/92 TIME:14:16 PT: 0.360 STEP:0.030 WL:1.54059
 5737.0 5163.3 4589.6 4015.9 3442.2 2868.5 2294.8 1721.1 1147.4 573.7 0.0

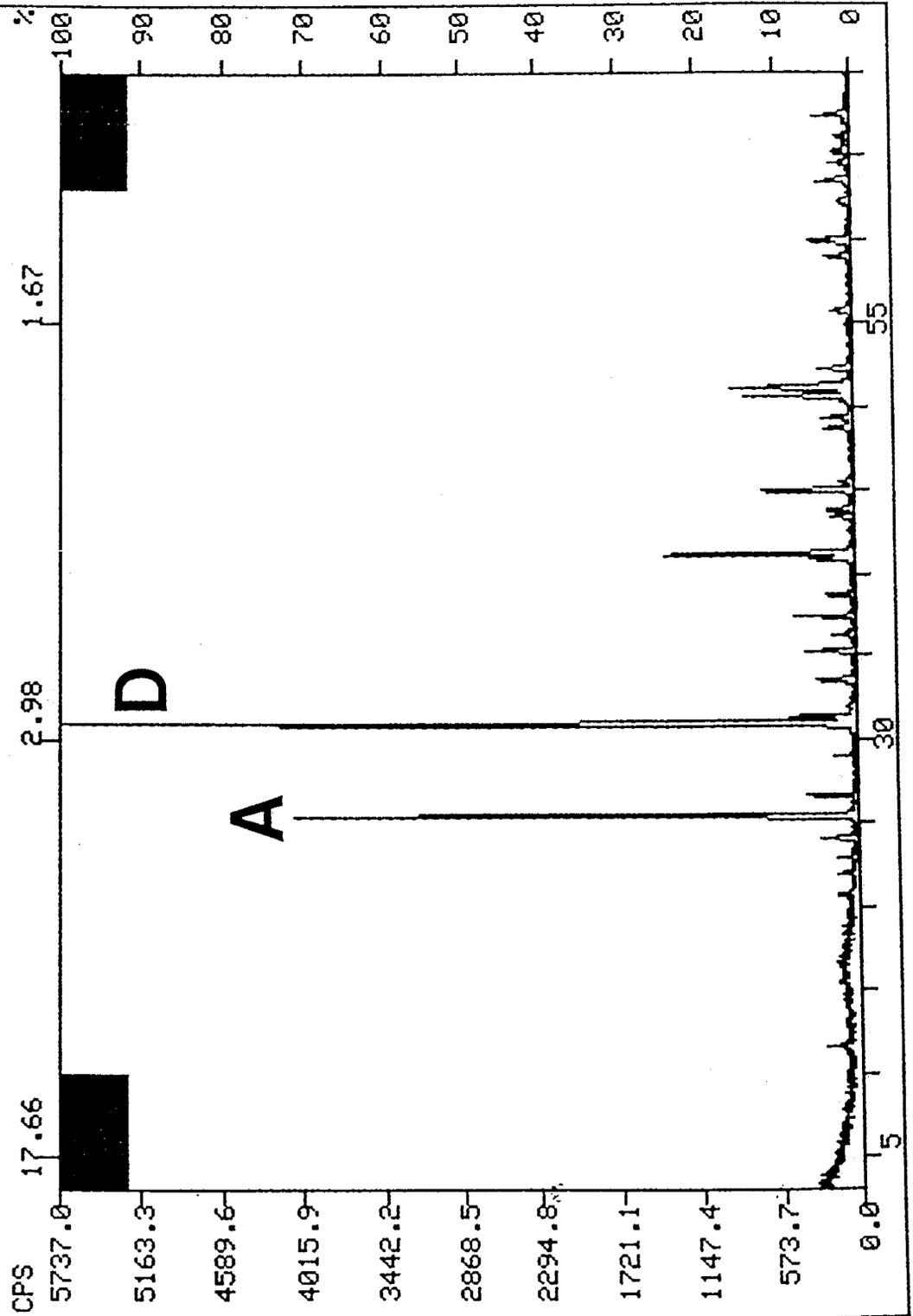
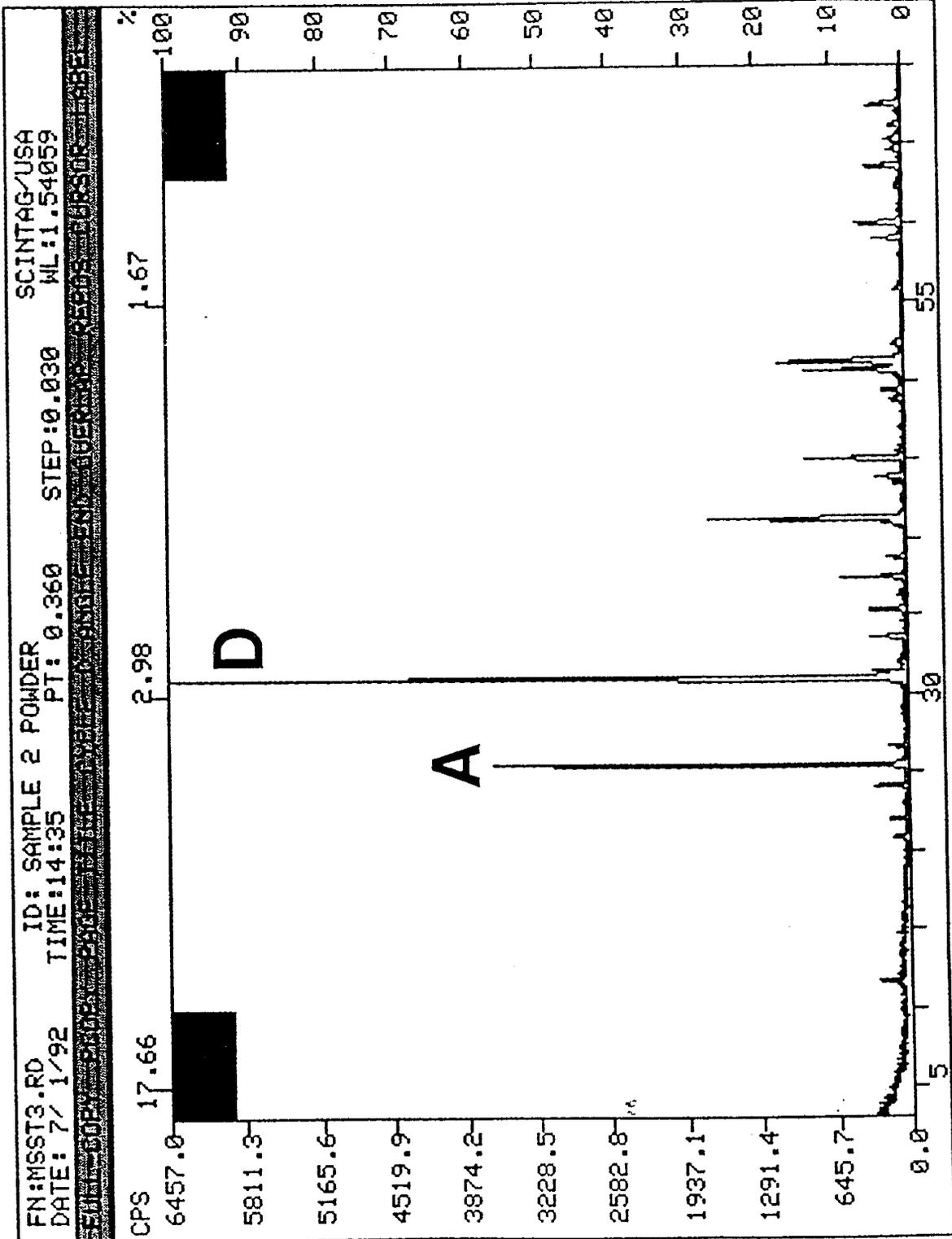


Plate 5b. D: Dolomite, A: Anhydrite



A: Anhydrite

Plate 5c. D: Dolomite,

Core No. 4

Oil Company: OXY. U.S.A. Inc.
Well Name: JOHNSON GBSAN, No. 1809
Field Name: JOHNSON
State: Texas
County: ECTOR
Formation Name: GRAYBURG
Formation Age: Upper Permian; Guadalupian Section
Formation Type: Sand-Dolomite
Core Depth: 4050 ft.

Core Length: 13.0 cm.
Core Diameter: 10.0 cm.

Rock Description

A bluish to light gray dolostone containing dark gray to brown patches due to the presence of hydrocarbons. The rock is a wackestone, composed of dolomitic micrite and dolomitic peloids. Peloids vary in size from 10 mm to less than 0.25 mm (averaging around 0.25mm) in diameter and they are both rounded and elliptical. They are matrix supported and show little deformation due to compaction. The rock is cemented by gypsum and anhydrite. Nodular textures are present but limited in size (less than 10 mm) and abundance. The rock has a lack of layering and no sedimentary structures were identified.

Detailed Petrography

Grain Size: Peloid allochems composed of a fine dolomitic micrite range from 10 mm to less than 0.25 mm. average 0.25 mm. Very fine dolomite micrite matrix, varying in size from less than 1 μ m to 0.05 mm. Coarser crystals were found in the center of some peloids. Pore-filling crystalline gypsum and anhydrite.

Roundness, Angularity and Detrital Grain Shape: Peloid allochems are both spherical and elliptical. Peloids display little deformation.

Fabric (orientation and packing): Random, no preferred orientation. Peloids are matrix supported.

Porosity: Percentages measured by point counting thin sections - Porosity = 2.5 ± 1.3 %
Vugs are common within peloids and the dolomitic matrix. Large vugs generally may be leached peloids. Vugs were less than 0.25mm in diameter. The porosity percentage above does not include intercrystalline porosity. Much intercrystalline porosity exists between dolomite rhombs.

Mineral Composition: Percentages measured by point counting thin sections.

DOLOMITE	63 ± 2.8 %
ANHYDRITE	25 ± 2.5 %
GYPSUM	12 ± 2.1 %

Opagues present in small amounts.

Detrital Grains: Not present.

Post-Depositional Minerals

Dolomite often occurs as microscopic rhombohedral crystals. Rhombohedral crystals occasionally are hollow. Vugs are lined with coarse euhedral dolomite rhombs.

Gypsum forms radiating, a circular crystals locally 1 mm in length. Nodular textures are present but limited in size (less than 10 mm) and in abundance.

Anhydrite forms large tabular, equant crystals occasionally less than 1 mm in size. In localized areas anhydrite forms delicate needle like projections about 5 μ m in length and less than 1 μ m in width. These crystals are pore filling. Anhydrite crystals have a poikilotopic texture that encapsulates peloid allochems. Anhydrite crystals display replacement textures of gypsum. Both gypsum and anhydrite show evidence of encapsulating and replacing dolomite.

Diagenesis

List of diagenetic events (the events are not necessarily in the correct chronological order):

1. Dissolution of micrite and peloids forming vugs.
2. Dolomitization of carbonate micrite and peloids.
3. Growth of coarse vug-lining dolomite rhombohedral crystals.
4. Growth of pore filling gypsum and anhydrite cements.
5. Replacement of dolomite micrite and peloids by gypsum.
6. Replacement of dolomite micrite and peloids by anhydrite.
7. Replacement of gypsum by anhydrite.

See chart.

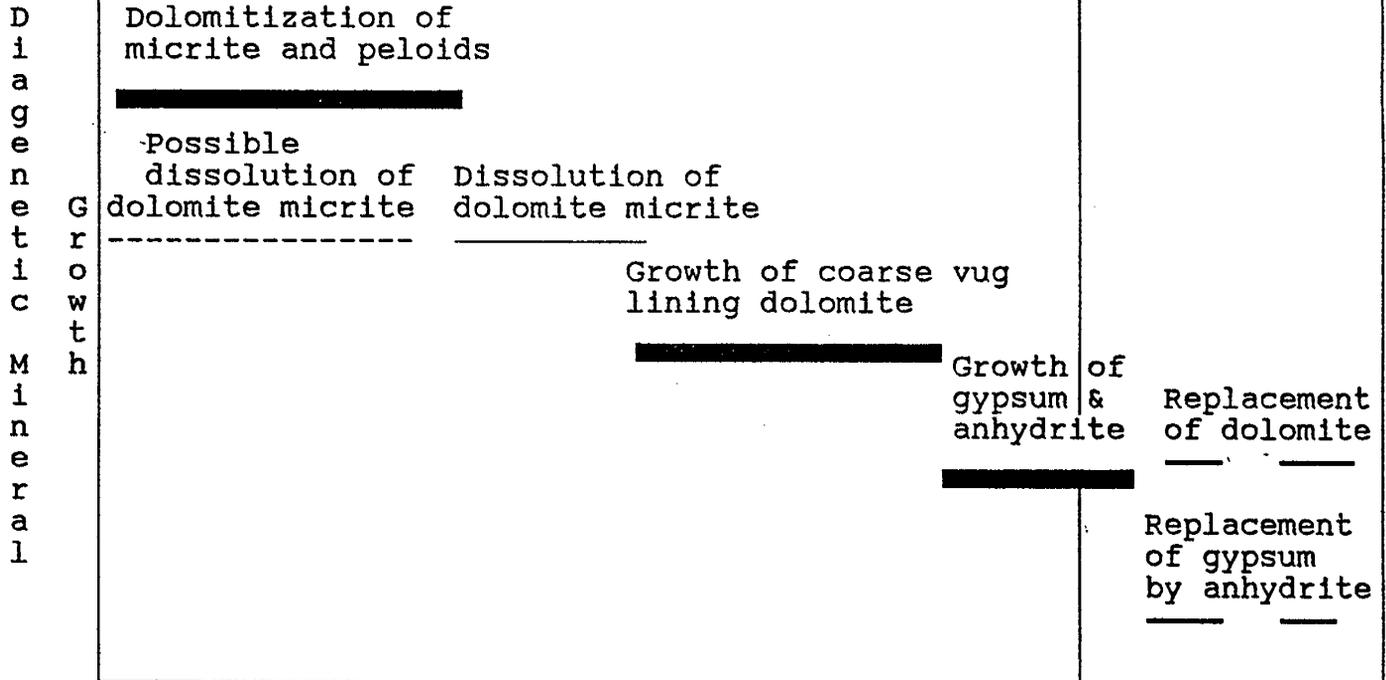
Relative Sequence of Diagenetic events for Core No.4

Increase in Burial
—————>>

Shallow Burial: Eogenetic Zone
(Authigenic minerals formed due to hypersaline fluids).

Deep Burial
Mesogenetic
Zone

Middle Permian



Key: — Dissolution
 ----- Dissolution, timing uncertain
————— Relative timing of diagenetic event is certain
————— Relative timing of diagenetic event is uncertain

Note: Sequence of diagenetic events are identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No.4 was cored from the Permian, Grayburg Formation of the Johnson Field, west Texas. The Grayburg Formation has been described in detail by Bebout et al. (1987). Bebout divided the formation into 3 units of sand or silt beds selected by logged gamma ray markers. A lower unit comprises of 80 ft of fusulinid wackestone. A middle unit consists of a crinoid packstone/grainstone and vertically structured facies (vertically aligned structures believed to be algal in origin, (Bebout et al., 1987)). The upper unit consists of a fusulinid wackestone at the base, pellet and ooid grainstone near the top and pisolite grainstone and anhydrite at the top.

The studied core was a peloid wackestone, dolostone from the upper unit of the formation. The studied core sample was found to be generally of a homogeneous lithology. It is important to note that the peloid dolostone lithology studied was not the predominant lithology within the reservoir.

Depositional Environment

During the Permian Period, the west Texas area was a broad shallow basin trending north-northwest to south-southeast on an intracratonic platform known as the Central Basin platform. The Central Basin was bordered by deeper basins. To the east by the Midland Basin and to the west by the Delaware Basin. Within the Central Basin platform interior, low-energy carbonates and evaporative deposition dominated. Peloidal carbonates indicate a broad, low-energy shelf environment with water depths generally below normal wave base (Bebout et al., 1987; Ward et al., 1986). Peloids probably are altered fecal pellets. Dolomitization probably took place in the shallow subsurface (Bebout and et al., 1987). This occurred when hypersaline waters flowed downward from arid, supratidal, sabkha settings originating through the evaporation of seawater. These fluids also likely are to have emplaced anhydrite and gypsum cements (Bebout and et al., 1987).

Description of Plates

- Plate 1. The core samples studied.
1a, shows side views of the studied length of core.
1b and 1c shows end views of the core.
- Plate 2. Photomicrographs of thin sections.
2a: Cross polarized light view of green-grey, fine micritic peloidal dolomite. Allochems within this photomicrograph mainly are peloids. White and areas of high birefringence are anhydrite crystals.
2b: Cross polarized light view of green-grey peloids. High birefringence crystals are anhydrite while the lower birefringence crystals probably are gypsum.
2c: Cross polarized light view of peloidal dolostone with anhydrite and gypsum crystals. Note that some of the peloids have been partially replaced by anhydrite.
2d: Cross polarized light view of peloidal dolostone with anhydrite and gypsum crystals. Vugs are seen within the dolomite micrite in the center of the photomicrograph.
- Plate 3. Scanning electron micrographs of core No.4
3a: Bar is 100 μm x 150.
Dolomitic matrix, composed of microscopic rhombohedral crystals of dolomite. Larger dolomite crystals can be seen developed within the pore spaces.
3b: Bar is 10 μm , x 150.
Rounded dolomite rhombohedral crystals.
3c: Bar is 10 μm x 400.
Large well developed rhombohedral dolomite crystals that line a vug.
3d: Bar is 10 μm x 2000.
Anhydrite crystal (A) growing around coarse vug lining dolomite rhombohedral crystals (D).
3e: Bar is 1.0 μm x 6000.
Rounded dolomite crystal (D) encapsulated within an anhydrite crystal (A). The rounded edges of the dolomite crystal may indicate replacement.
3f: Bar is 1.0 μm x 6000.
Needle like crystal growths, protruding from an anhydrite or gypsum crystal.
- Plate 4. Energy dispersive spectra analysis
4a. Dolomite $\text{Ca, Mg}(\text{CO}_3)_2$
4b. Dolomite $\text{Ca, Mg}(\text{CO}_3)_2$
4c. Vug-lining dolomite crystals $\text{Ca, Mg}(\text{CO}_3)_2$
4d. Anhydrite crystal, CaSO_4
4e. Needle like crystals of anhydrite, CaSO_4
- Plate 5. X-Ray diffraction analysis
5a. D: Dolomite, A: Anhydrite
5b. D: Dolomite A: Anhydrite
5c. D: Dolomite A: Anhydrite G: Gypsum?

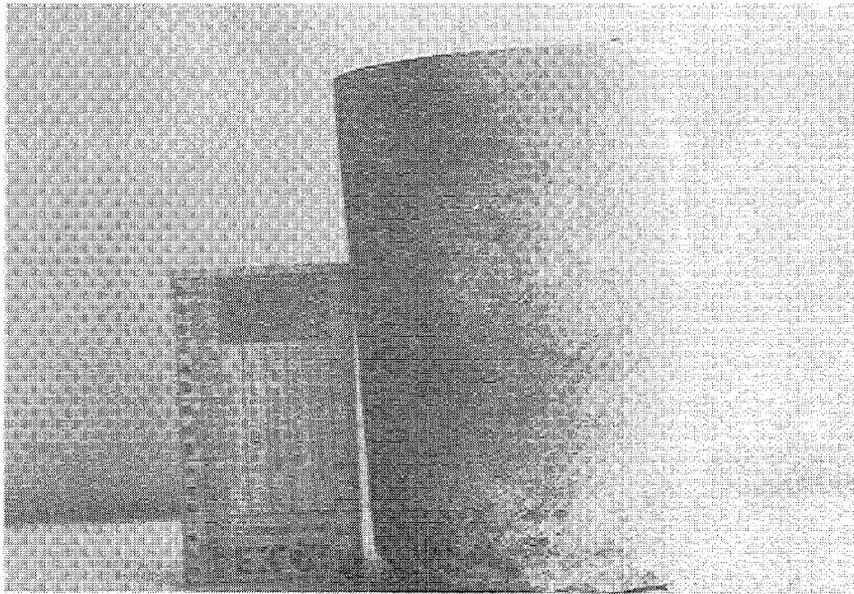


Plate 1a

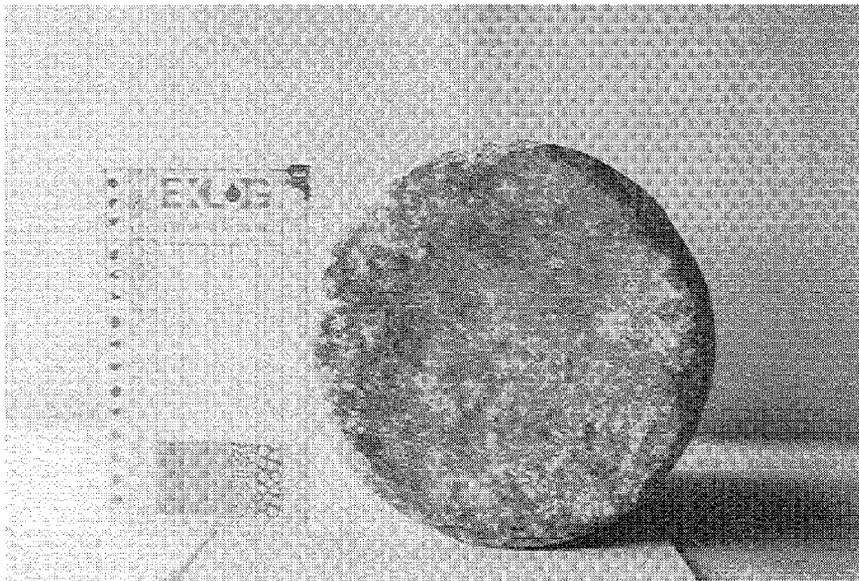


Plate 1b

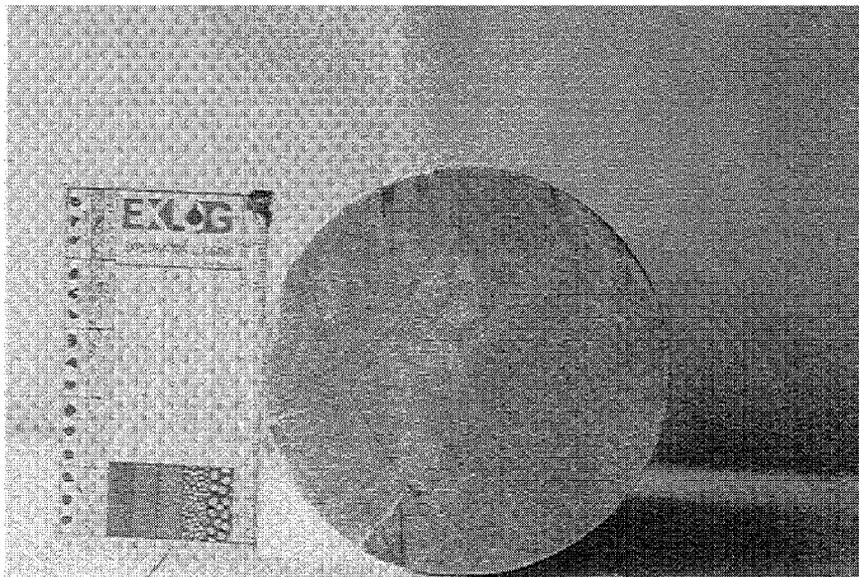


Plate 1c

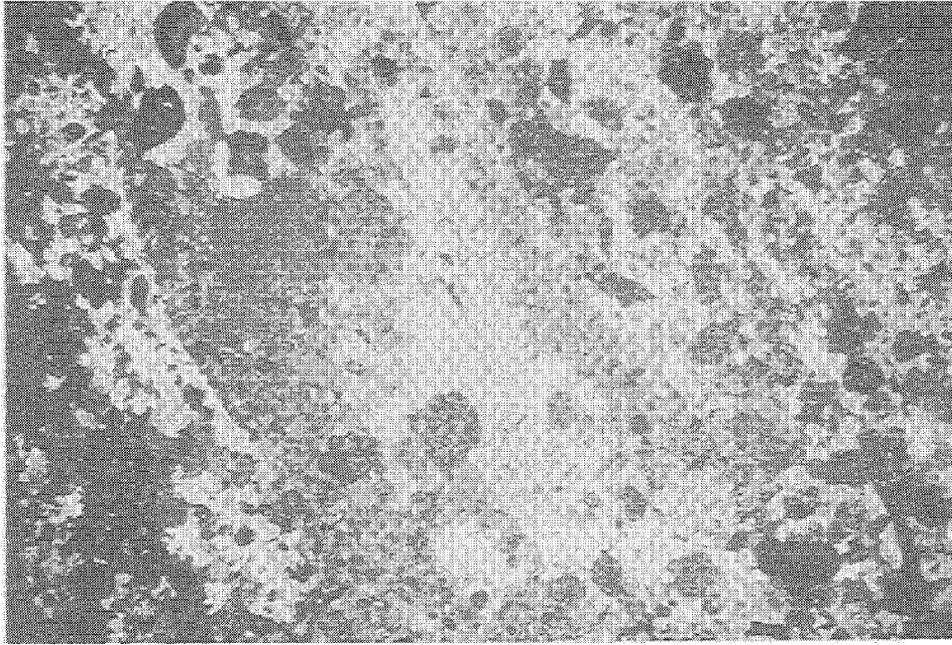


Plate 2a

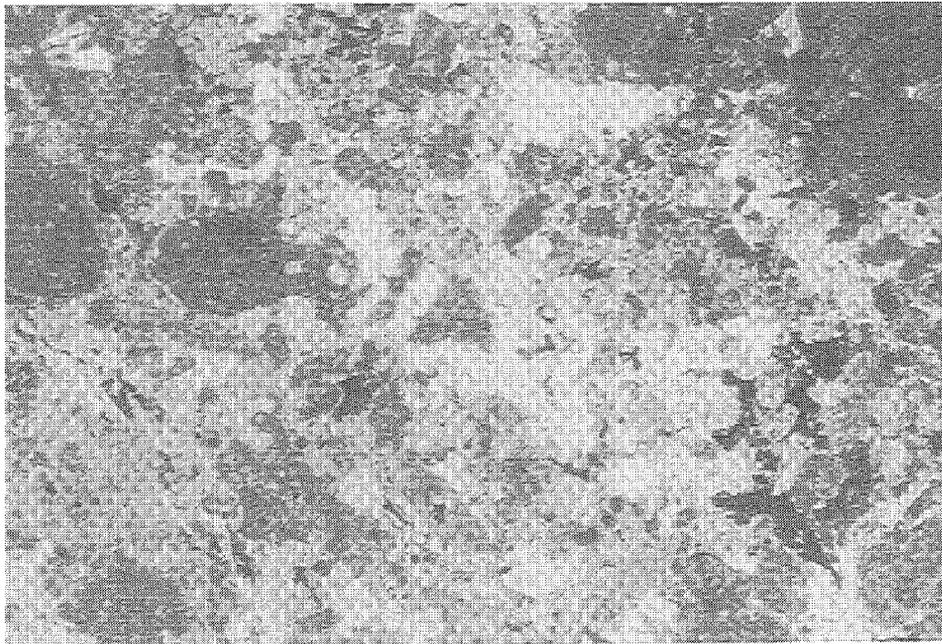


Plate 2b

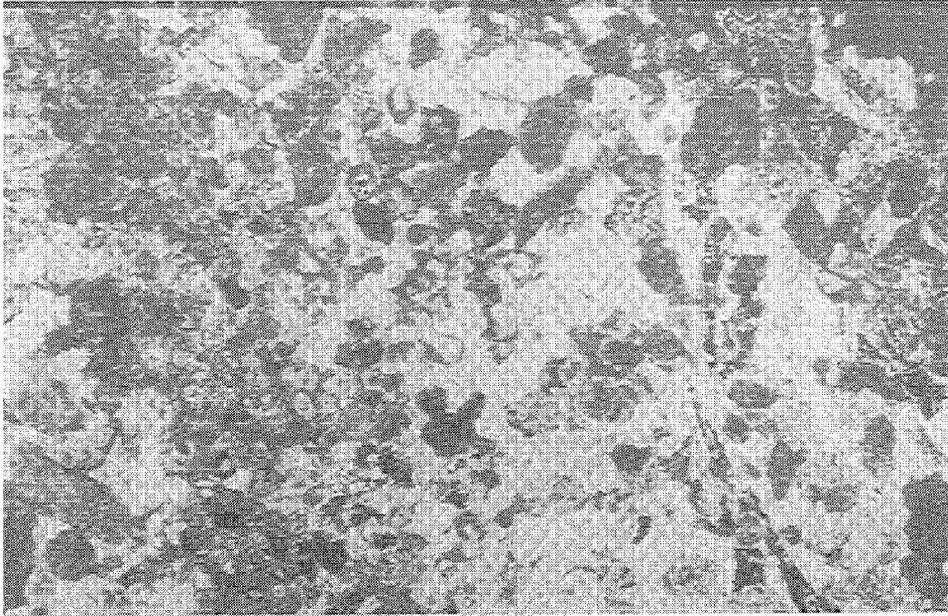


Plate 2c

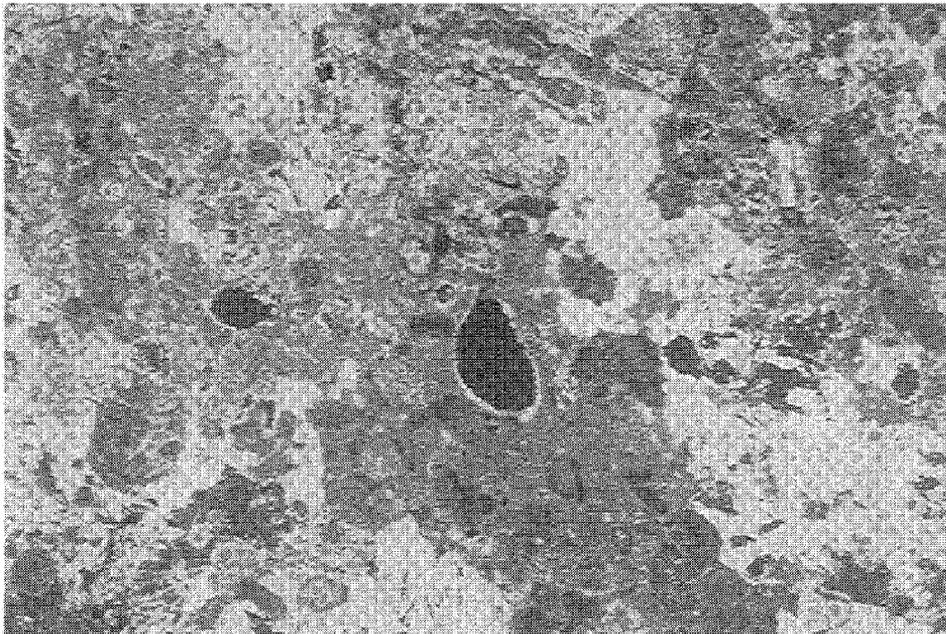


Plate 2d

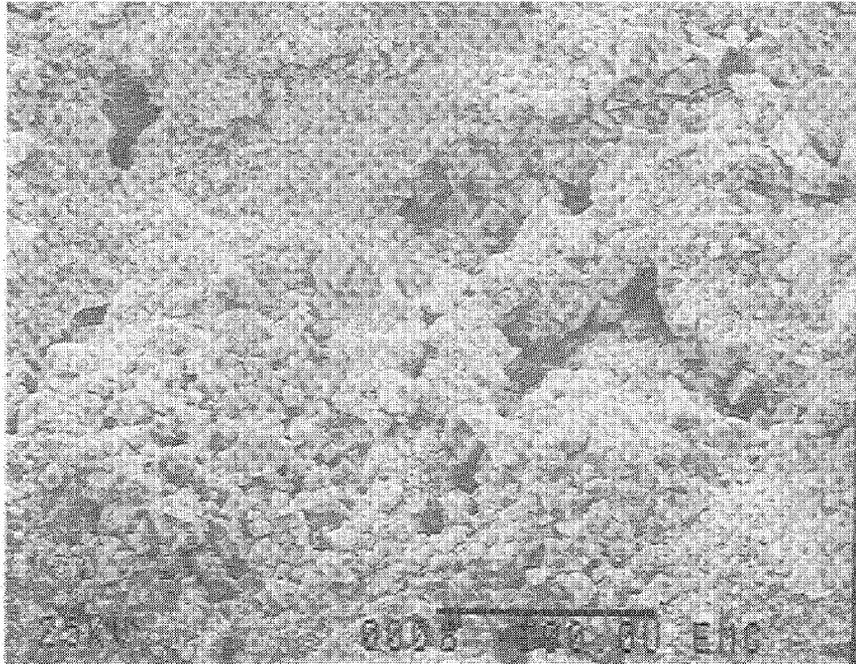


Plate 3a

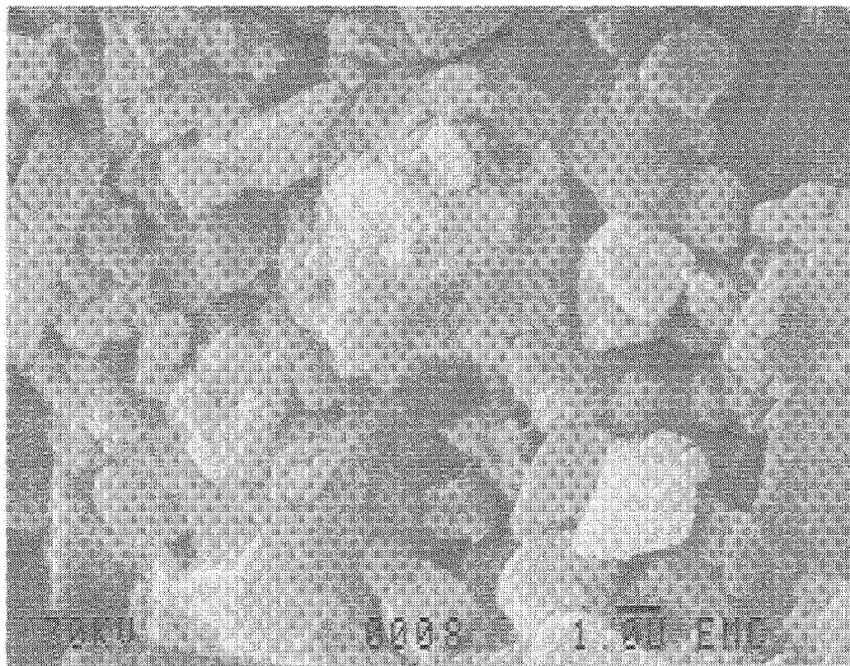


Plate 3b



Plate 3c

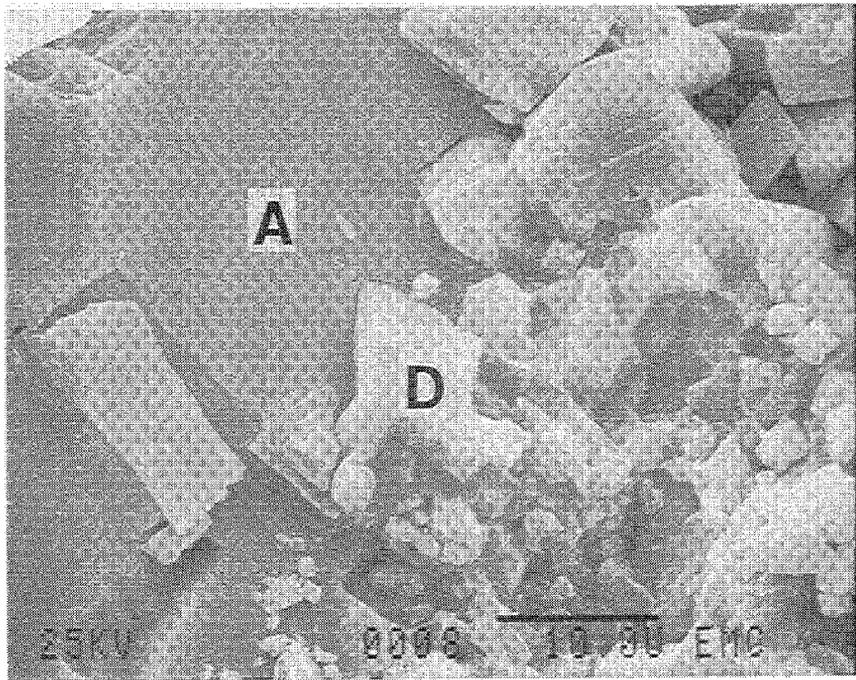


Plate 3d

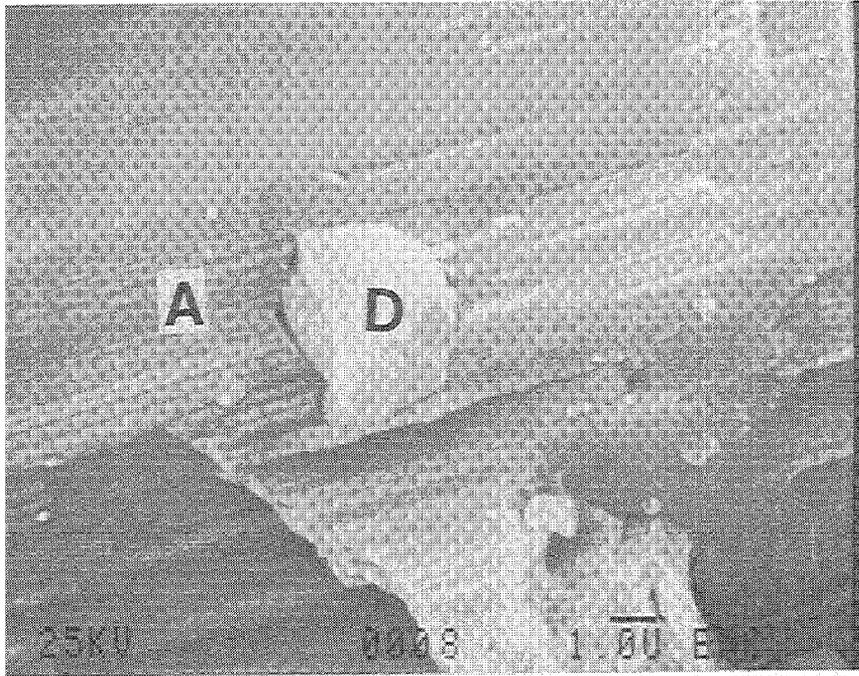


Plate 3e

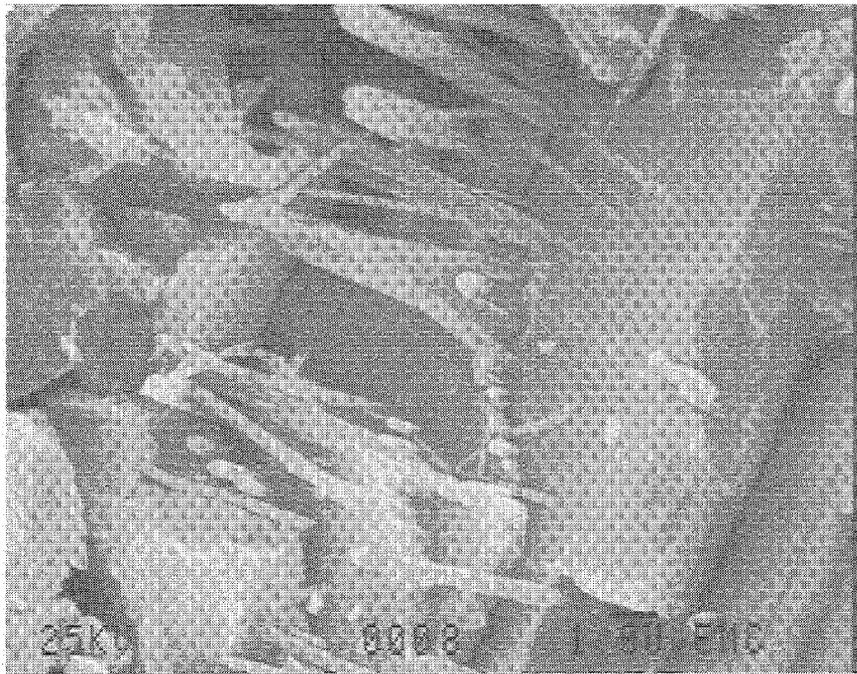


Plate 3f

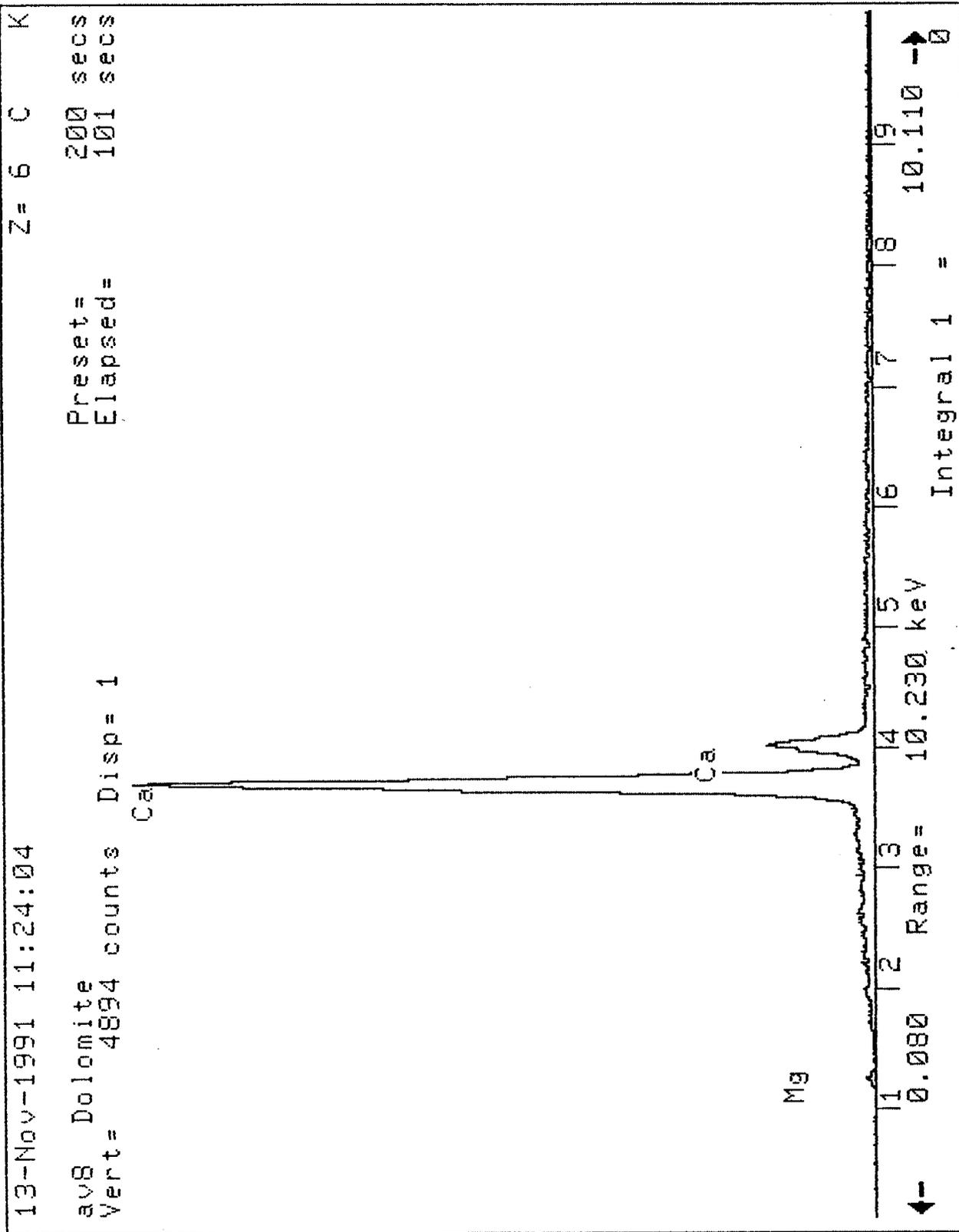


Plate 4a. Dolomite Ca,Mg(CO₃)₂

13-Nov-1991 11:25:51

Z= 6 C K

av8 Dolomite

Preset= 200 secs

Vert= 500 counts

Elapsed= 101 secs

Disp= 1

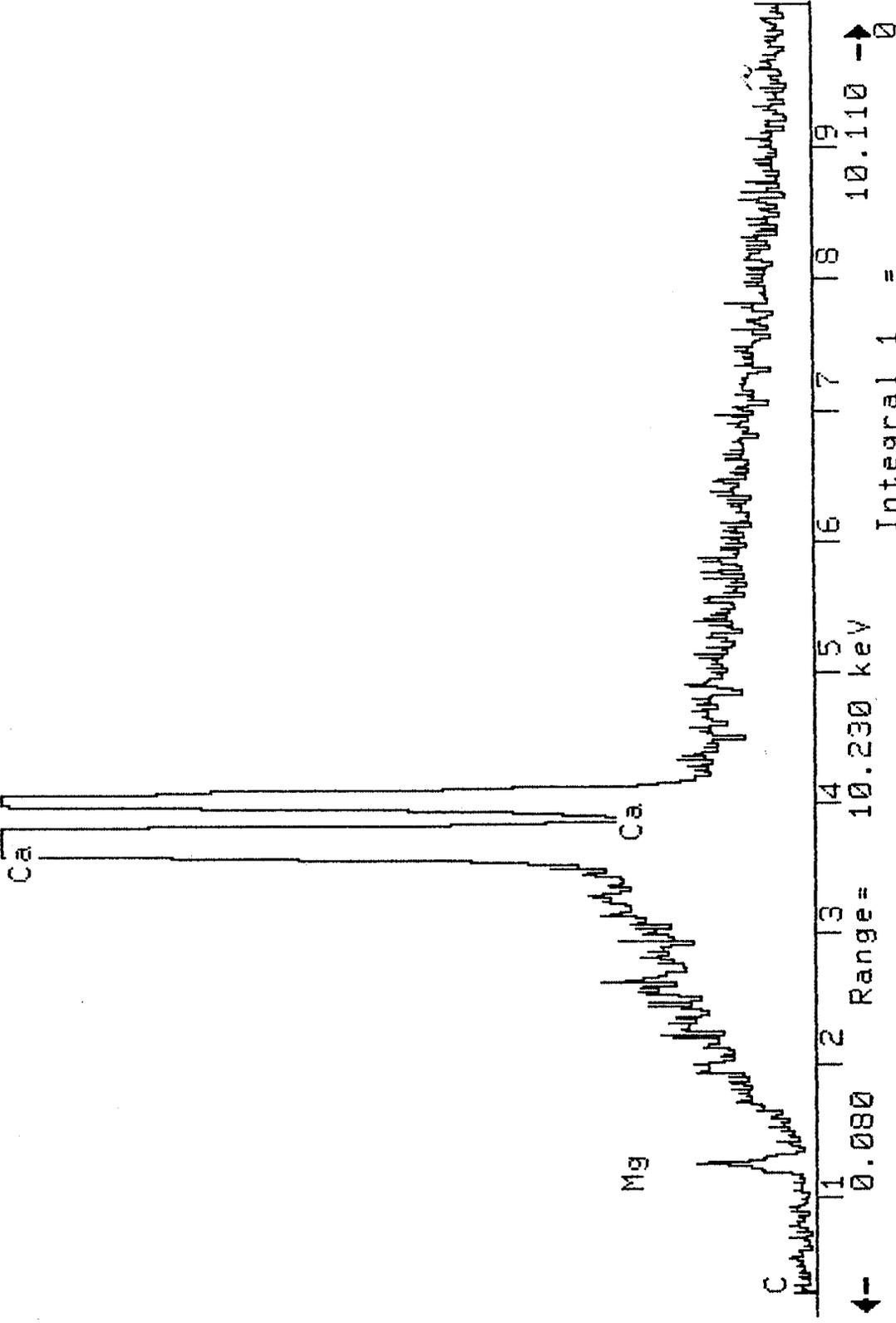


Plate 4b. Dolomite Ca,Mg(CO₃)₂

30-Oct-1991 17:25:30

AV8 Dolomite

Vert= 1391 counts

Disp= 1

Preset=

100 secs

Elapsed=

100 secs

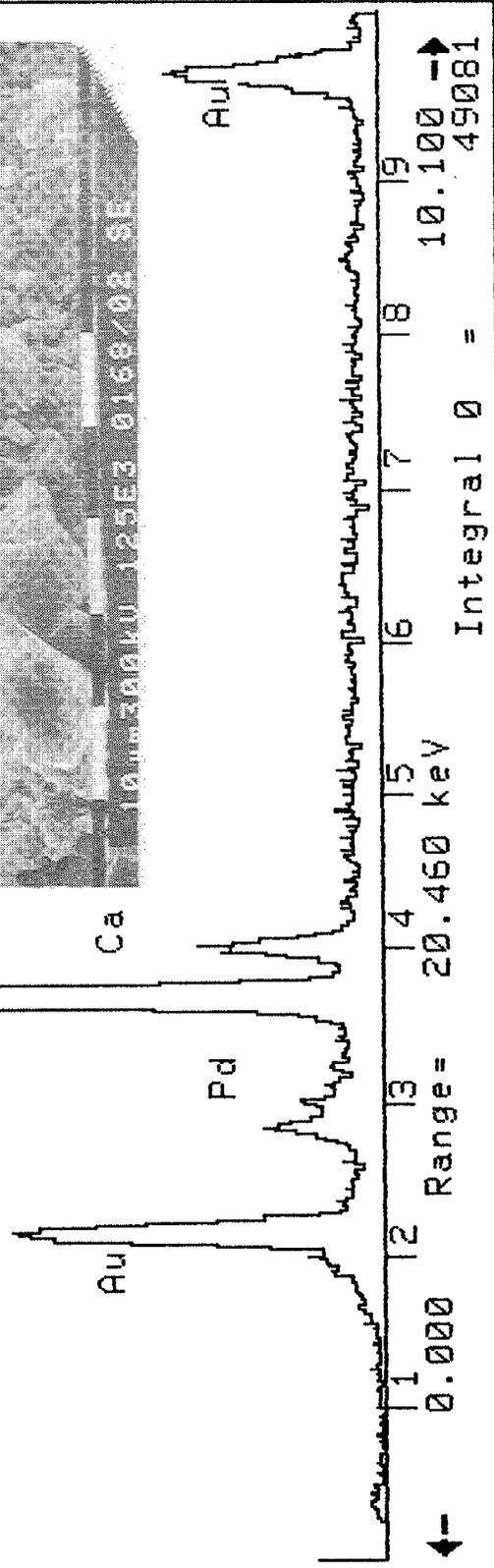
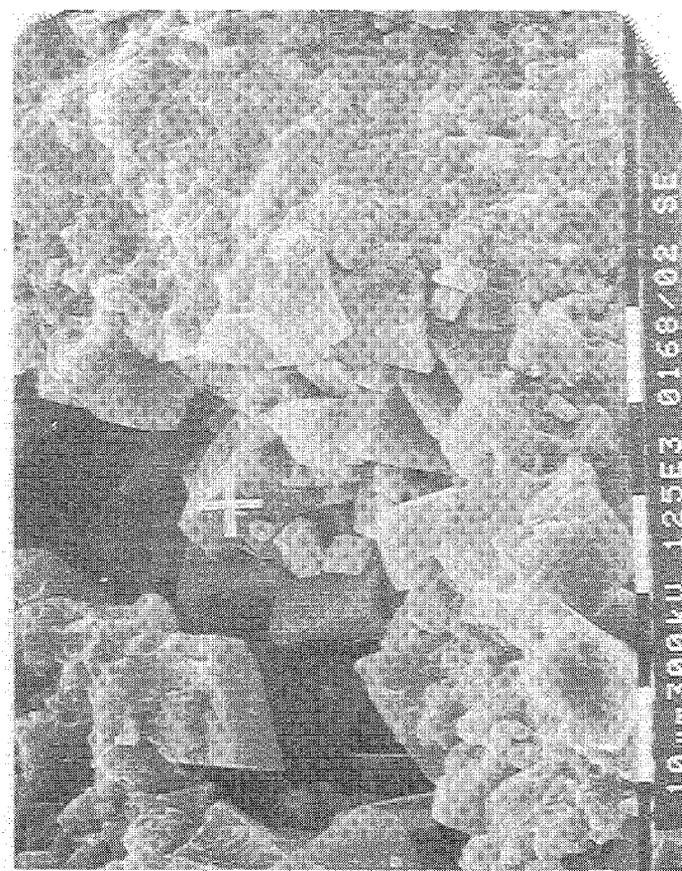


Plate 4c. Vug-lining dolomite crystals Ca,Mg(CO₃)₂

30-Oct-1991 17:55:10

AV8 Anhydrite
Vert= 3457 counts
Quantex>

Disp= 1

Preset= 100 secs
Elapsed= 100 secs

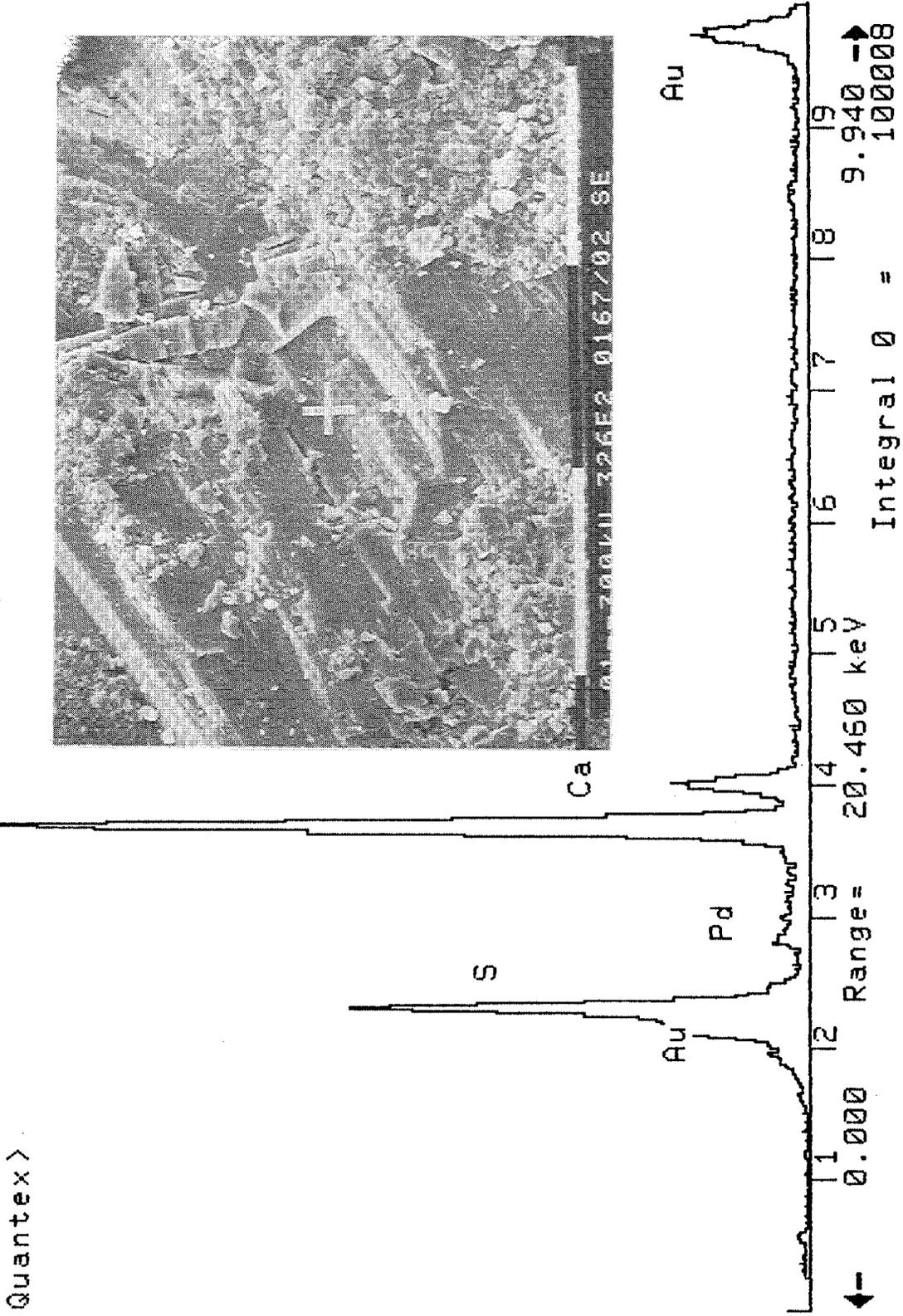
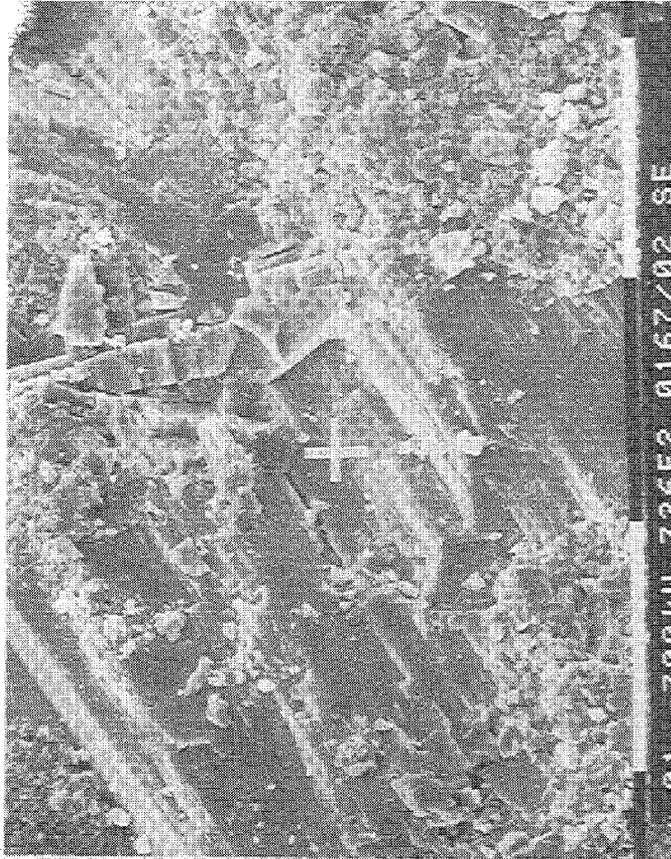


Plate 4d. Anhydrite crystal, CaSO₄

13-Nov-1991 10:51:48

Vert= 386 counts Disp= 1
Preset= 200 secs
Elapsed= 200 secs

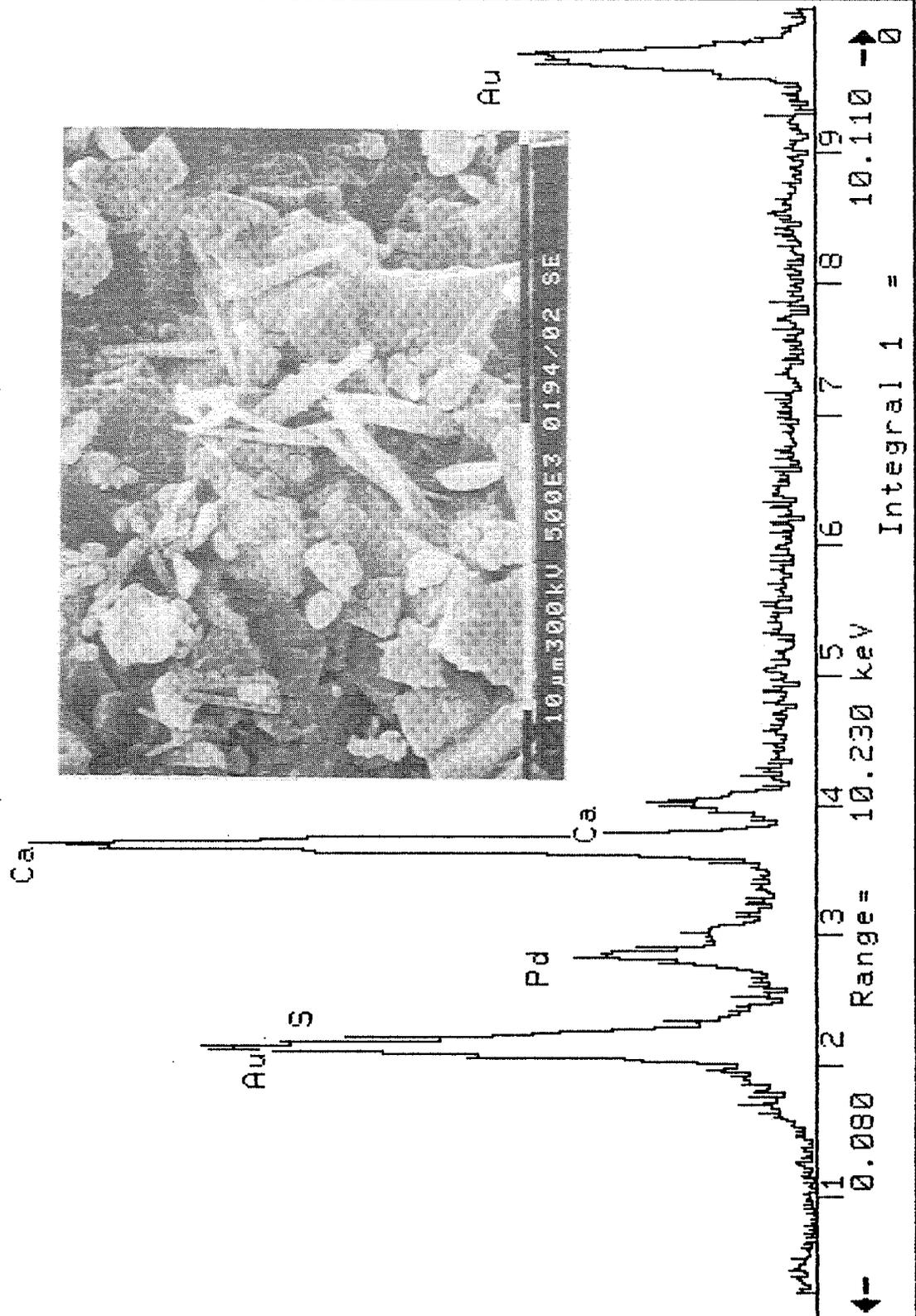


Plate 4e. Needle like crystals of anhydrite, CaSO_4

FN: AV8.NI ID: DOLOSTONE SCINTAG/USA
 DATE: 08/19/91 TIME: 10:14 PT: 0.225 STEP: 0.0300 WL: 1.54060

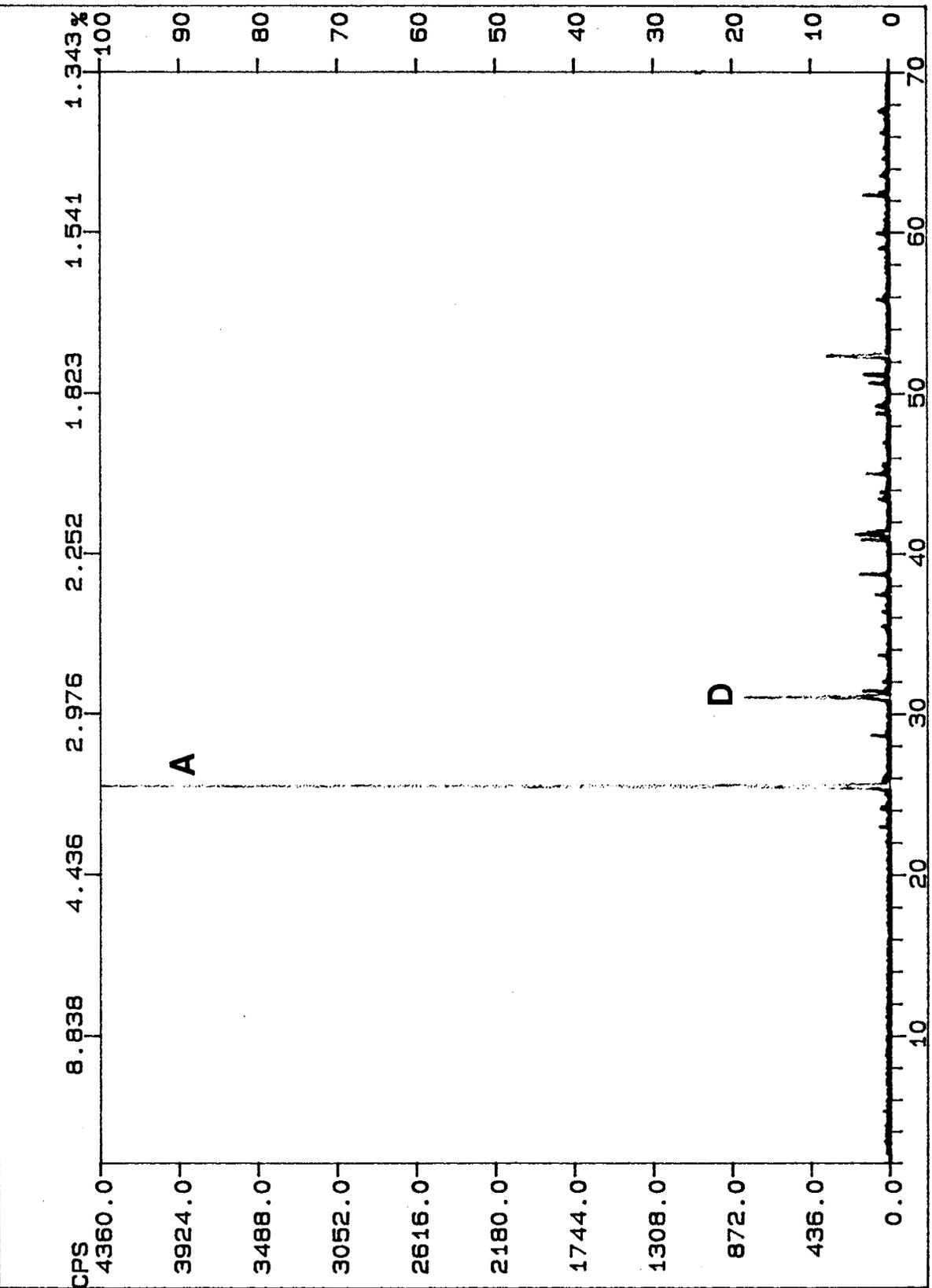


Plate 5a. D: Dolomite A: Anhydrite

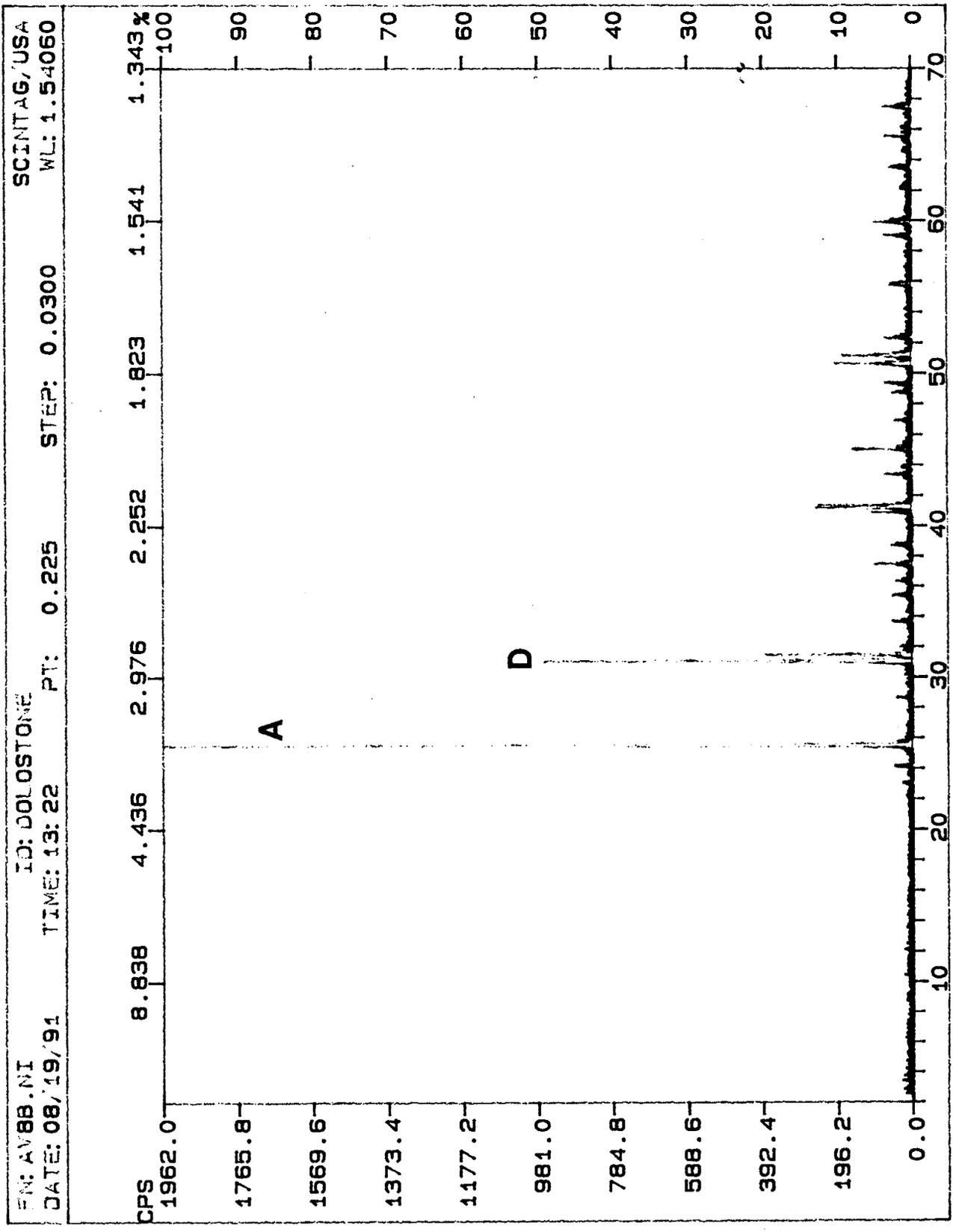


Plate 5b. D: Dolomite A: Anhydrite

RUC AVBTS.NI ID: AV8 THIN SECTION SCINTAG/USA
 DATE: 08/19/91 TIME: 14:35 PT: 0.225 STEP: 0.0300 WL: 1.54060

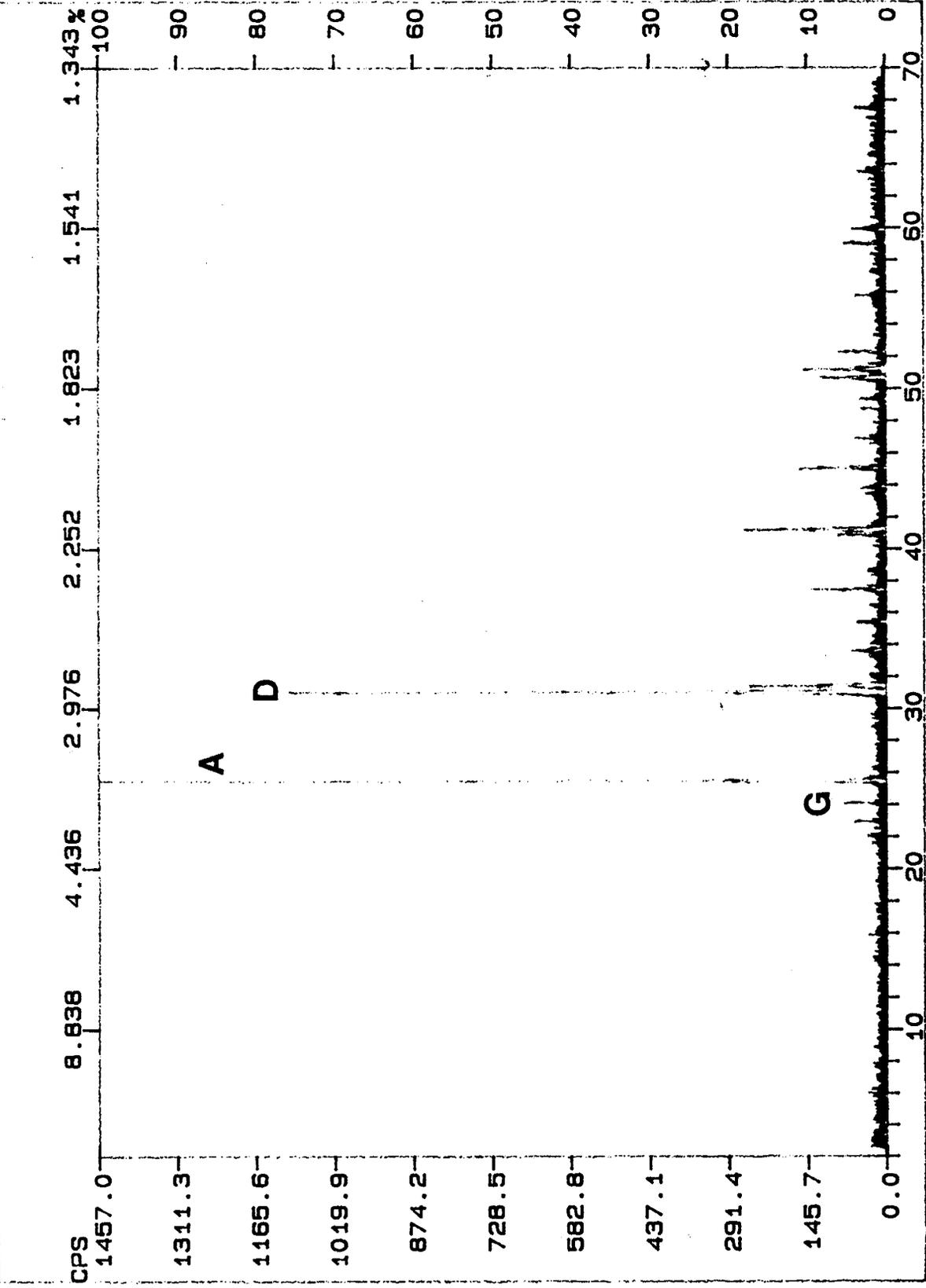


Plate 5c. D: dolomite A: Anhydrite G: Gypsum?

Core No. 5

Oil Company: Chevron

Well Name: 679

Field Name: Emsu

State: New Mexico

County: Lea

Formation Name: Grayburg

Formation Age: Upper Permian; Guadalupian

Formation Type: Dolomite

Core Depth: 4300 - 4350 ft.

Core Sample Length: 15 cm.

Core Diameter: 10 cm.

Rock Description

A brown to light brown dolomitic wackestone/fossiliferous dolostone. Hydrocarbons are present but do not alter the color of the rock. Allochems within the rock are matrix supported and range from 2.5 mm to less than 0.5 mm in size. Allochems are composed of fragmented bioclasts of echinoderms, mollusks, bryozoans and peloids. All allochems have been completely dolomitized. The rock is composed of fine dolomite and locally of coarser dolomite crystals. The rock contains stylolite structures, but is void of any primary depositional structures.

Detailed Petrography

Grain Size: Allochems range from 2.5 mm to less than 0.5 mm in size. Very fine dolomite consists of rhombohedral crystals generally less than 5 μm .

Roundness, Angularity and Detrital Grain Shape: Allochems are well rounded. Peloids are both spherical and elliptical in shape and display very little deformation. Bioclasts are rounded to subrounded.

Fabric (Orientation and Packing): Random, no preferred orientation. Allochems are matrix supported.

Porosity: Percentages measured by point counting thin sections.

$$\text{Porosity} = 2.4 \pm 1.2 \%$$

Vugs are common in the dolostone. Vugs generally were less than 0.5 mm in diameter. The porosity is probably greater due to intercrystalline porosity between dolomite crystals. Pore sizes between dolomite crystals generally do not exceed a maximum of 10 μm .

Mineral Composition: Percentages measured by point counting thin sections. The rock consists of less than 99.5 % dolomite. The rock contains minor amounts of quartz grains and authigenic clay (less than 0.5 %).

Detrital Grains

Allochems: Percentages were measured by point counting thin sections

(Dolomitized allochems).

- Crinoidal 4 ± 1.4 %
- Peloidal and unidentified grains 7 ± 1.8 %
- Minor amounts of bryozoan and mollusk skeletal fragments.

All allochems have been completely dolomitized. Allochems contain voids formed during replacement.

Minor amounts of sub-rounded to rounded quartz grains were identified.

Post-Depositional Minerals

Dolomite micrite makes up 89 ± 2.3 % of the rock. Dolomite often occurs as microscopic rhombohedral crystals.

Authigenic clay, (probably a variety of smectite) with a crinkley interwoven morphology occurs as pore-lining layers or pore-filling clusters.

Diagenesis

List of diagenetic events (the events are not necessarily in the correct chronological order):

1. Dolomitization of micrite matrix.
2. Dolomitization of allochems.
3. Formation of vugs.
4. Growth of authigenic clays.
5. Compaction and stylolite formation.

See the chart.

RELATIVE SEQUENCE OF DIAGENETIC EVENTS FOR CORE NO. 5

Increase in Burial

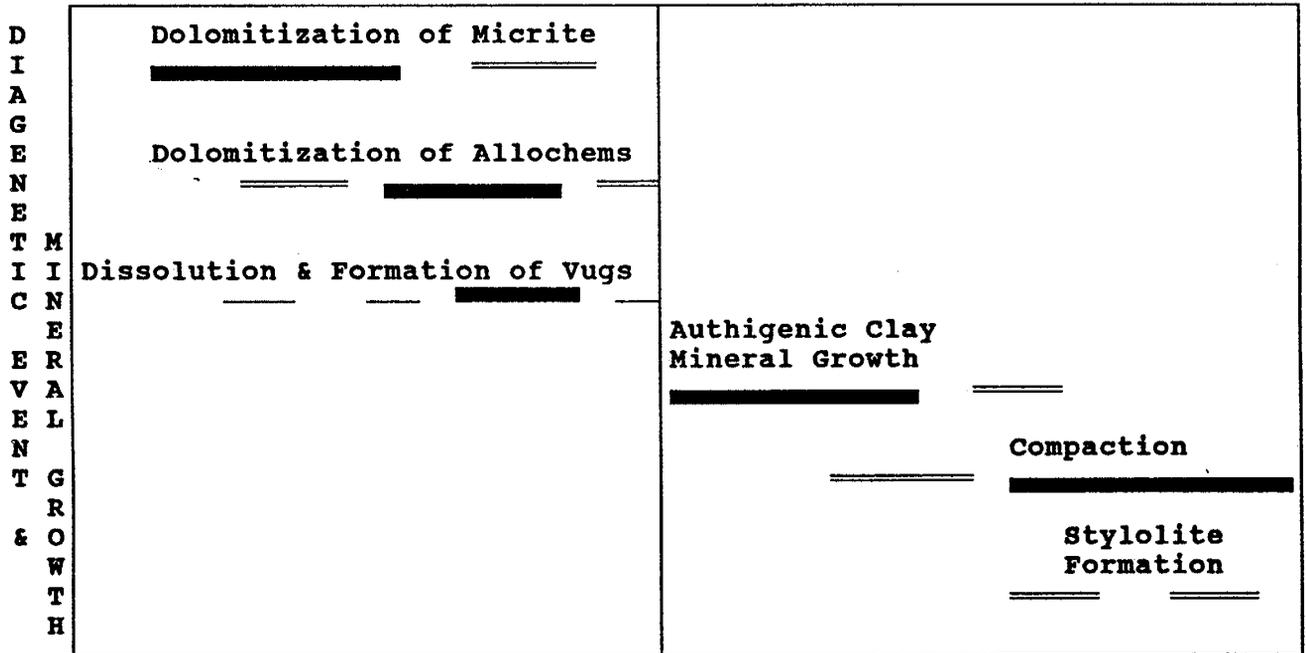
>>>

Shallow Burial: Eogenetic Zone
(Authigenetic minerals formed due to hypersaline fluids)

Deep Burial: Mesogenetic Zone
(Authigenetic minerals formed due to circulating meteoric fluids)

Middle Permian

Recent



Key:  Relative timing of diagenetic event is certain.
 Relative timing of diagenetic event is uncertain.
 ——— Dissolution, timing uncertain.
 Dissolution

Note: Sequence of diagenetic events are identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No. 5 was cored from the Permian, Grayburg Formation of the EMSU Field located in the extreme southeast of New Mexico. The Grayburg Formation has been described in detail by Bebout et al. (1987). Bebout characterized the Grayburg Formation for Crane County, west Texas and divided the formation into three units using sand or silt beds picked out by logged gamma ray markers (see description for Core No. 4). The EMSU field is a structural trap formed due to the eastward loss of porosity along the western flank of the Central Basin Platform (Ward et al., 1986). The facies grades into a crinoidal packstone and grainstone to the east, further onto the Central Basin Platform (Bebout et al., 1987).

The 15 cm length of studied core was a crinoid/ wackestone dolostone probably originated from the central part of the formation. The studied core generally was found to be of a homogeneous lithology.

Depositional Environment

During the Permian Period the area of southeast New Mexico and west Texas was a broad shallow basin trending north-northwest to south-southeast on a continental shelf known as the Central Basin platform. The Central Basin was bordered by deeper basins. To the east by the Midland Basin and to the west by the Delaware Basin. Core No. 5 was deposited in a shallow water shelf environment on the western edge of the Central Basin Platform. Subtidal bars probably were well developed subparallel to the shelf edge (Bebout et al., 1987). Dolomitization probably took place in the shallow subsurface. This occurred when hypersaline waters flowed downward from arid supratidal sabkha settings originating through the evaporation of seawater.

Description of Plates

Plate 1: The core sample studied.

- 1a. Side views of the studied lengths of core.
- 1b. An end view of the core.

Plate 2: Photomicrographs of thin sections.

- 2a. Plane polarized light view of fine micritic dolomite. Allochems within this photomicrograph are mainly peloids. In the center of the photomicrograph is a pale brown skeletal fragment.
- 2b. Cross polarized light view of spherical peloid grains within a fine dolomite matrix.

Plate 3: Scanning electron micrographs of Core No. 5

- 3a. Bar is $10\mu\text{m}$, x 400.
Large dolomite crystals, representing a dolomitized allochems, within a matrix of small (less than $10\mu\text{m}$ in diameter) rhombohedral dolomite crystals.
- 3b. Bar is $10\mu\text{m}$, x 860.
Large dolomite crystal, representing a dolomitized allochem. The allochem crystal contains voids which may have been inherited from the original grain or a result of replacement.
- 3c. Bar is $10\mu\text{m}$, x 2000.
Rhombohedral dolomite crystals which make up the matrix of the rock.
- 3d. Bar is $1.0\mu\text{m}$, x 10000.
Pore-filling authigenic clay (AC) with a crinkly interwoven morphology occurring in a cluster and attached to surrounding dolomite crystals.
- 3e. Bar is $1.0\mu\text{m}$, x 11000.
Pore-filling authigenic clay (AC). The clay forms plates within the center of pores and have thin extensions attaching it to surrounding dolomite (D) crystals.

Plate 4. Energy dispersive spectra analysis

- 4a. Rhombohedral crystals of dolomite $\text{Ca,Mg}(\text{CO}_3)_2$
- 4b. Large crystallized allochem of dolomite $\text{Ca,Mg}(\text{CO}_3)_2$
- 4c. Fe, Ca, Mg clay. Probably a variety of smectite.
- 4d. Fe, Ca, Mg, K clay. Probably a variety of smectite.

Plate 5. X-Ray diffraction analysis

- 5a. D: Dolomite
- 5b. D: Dolomite

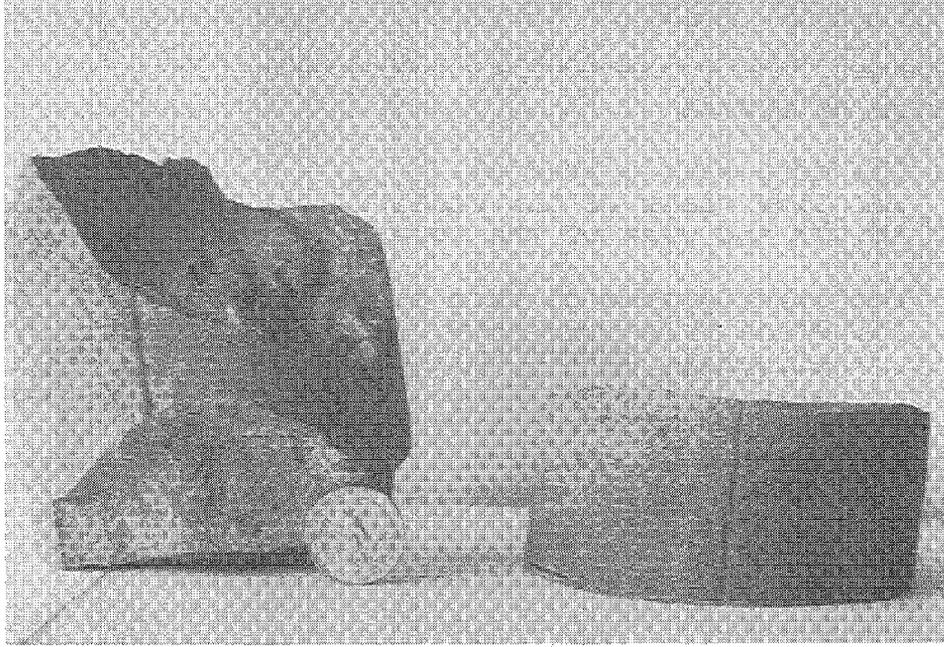


Plate 1a

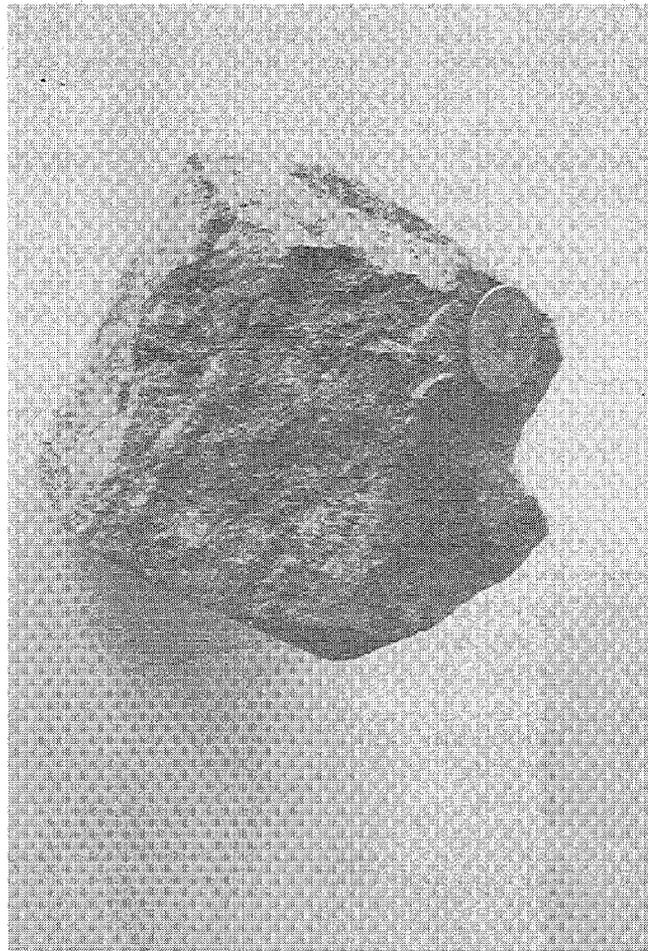


Plate 1b

Plate 2a

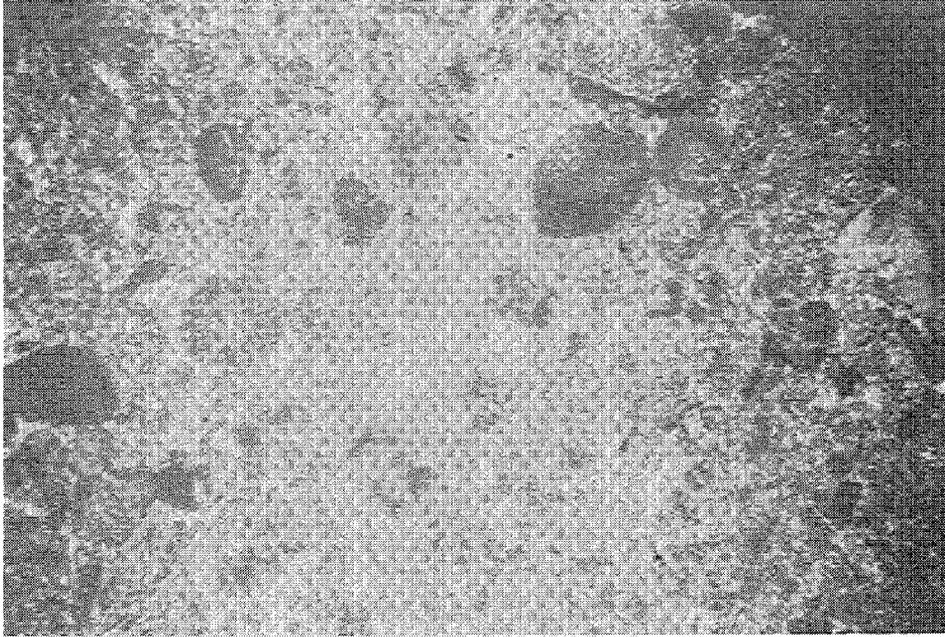


Plate 2b



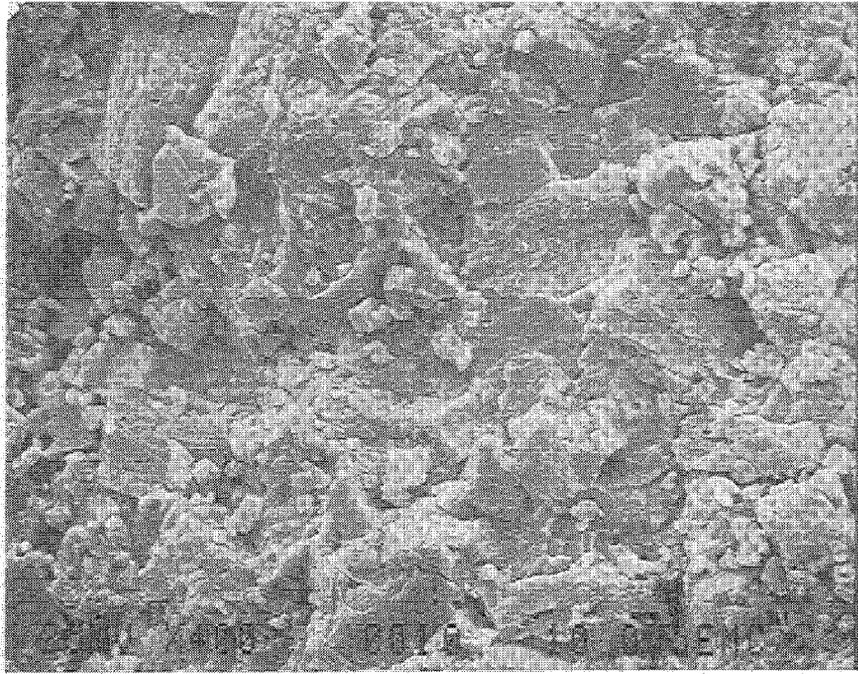


Plate 3a

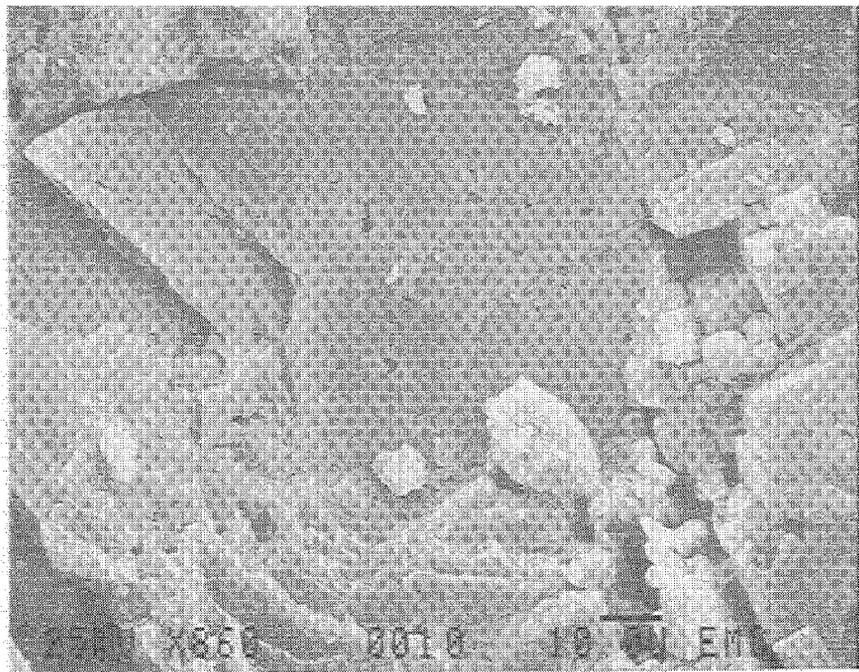


Plate 3b

Plate 3c

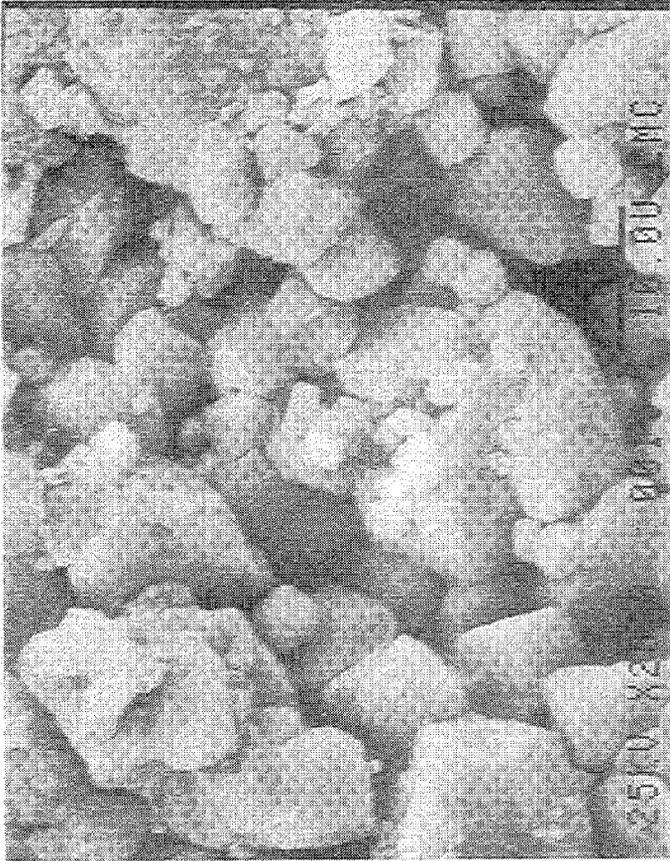


Plate 3e

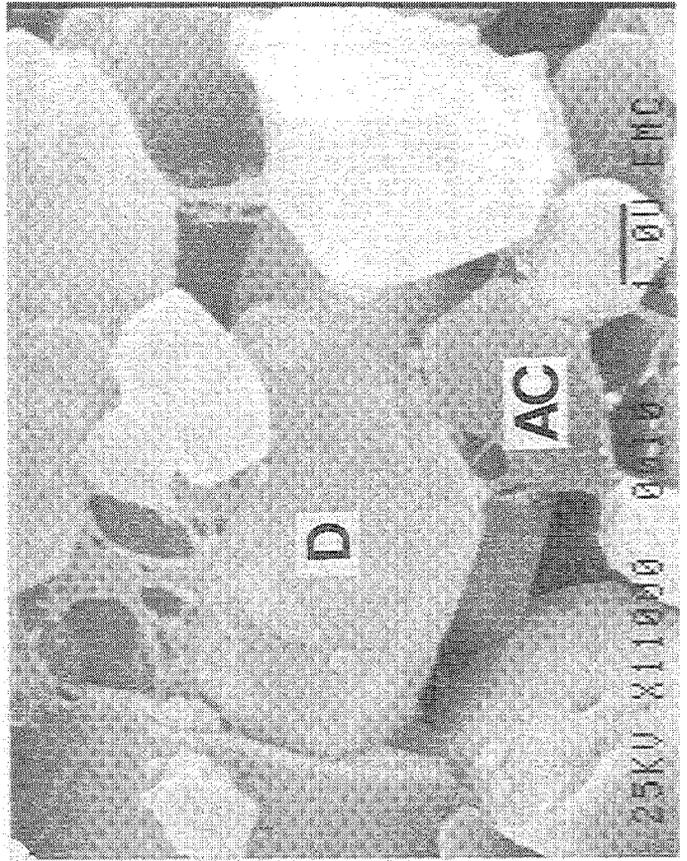
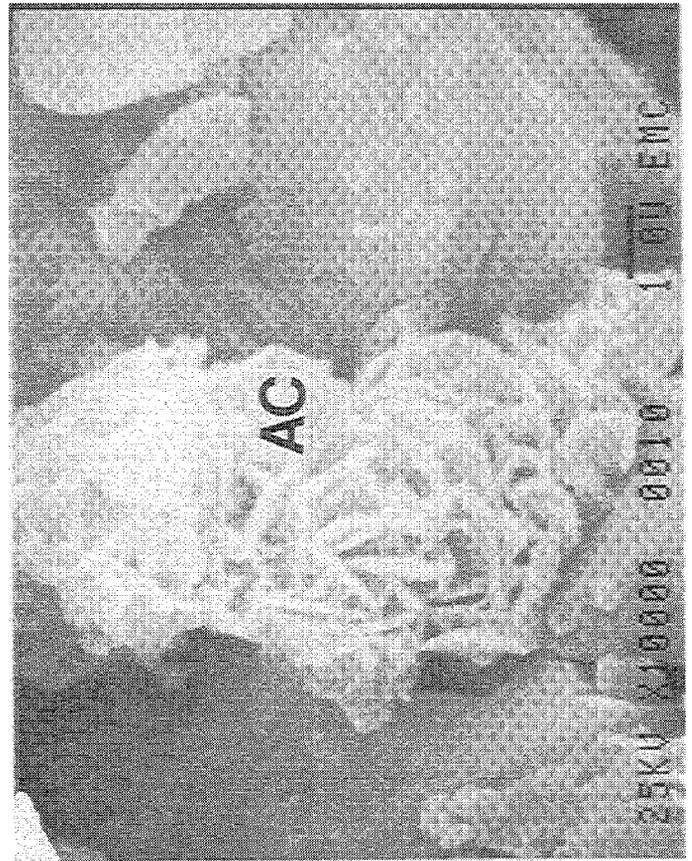


Plate 3d



13-Nov-1991 12:40:49

av10 Dolomite
Vert= 2451 counts
Quantex >

Disp= 1

Preset= 200 secs
Elapsed= 102 secs

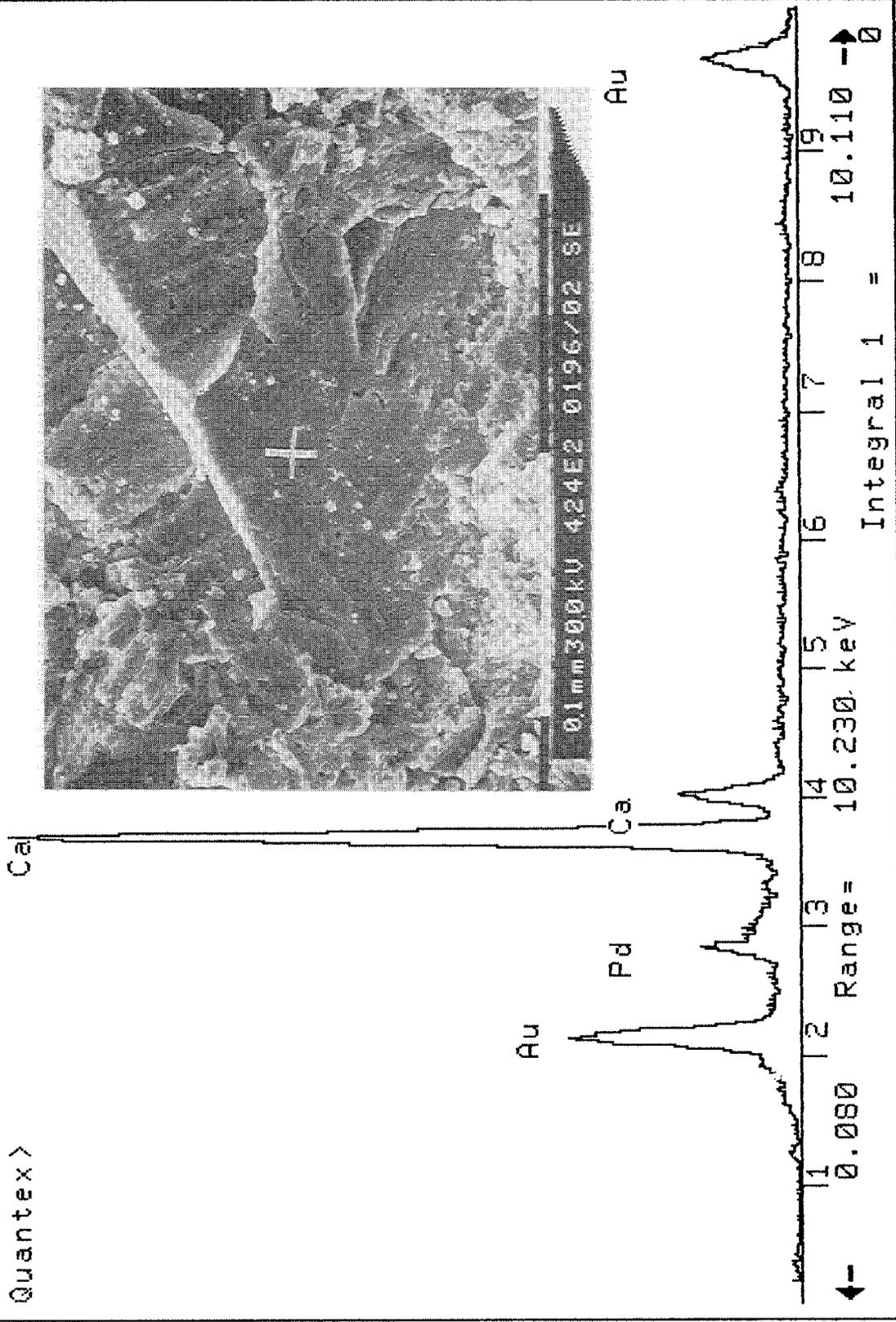


Plate 4a. Rhombohedral crystals of dolomite $\text{Ca,Mg}(\text{CO}_3)_2$.

13-Nov-1991 12:30:39

av10

Vert= 1000 counts Disp= 1

Preset=

200 secs

Elapsed=

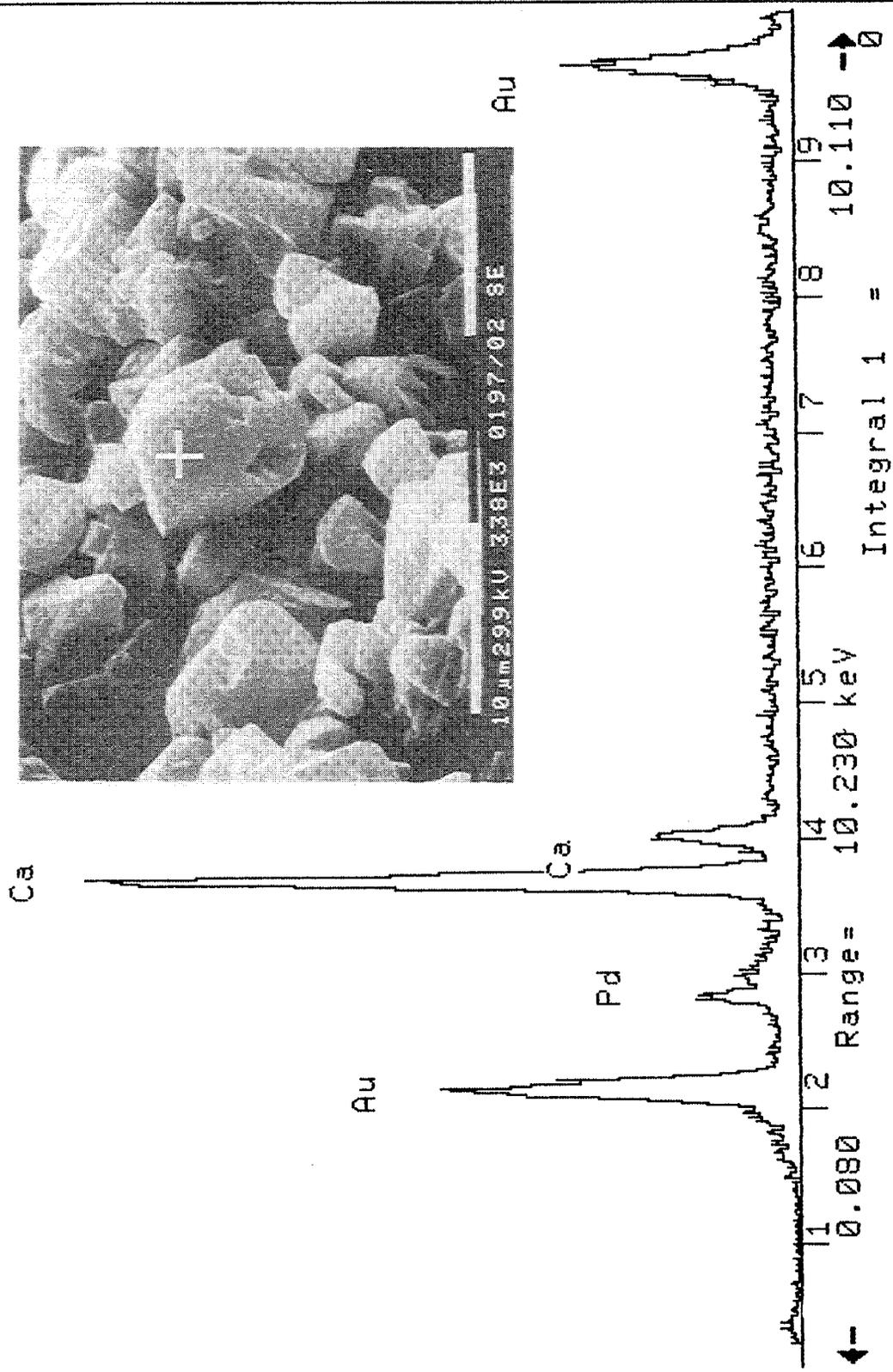
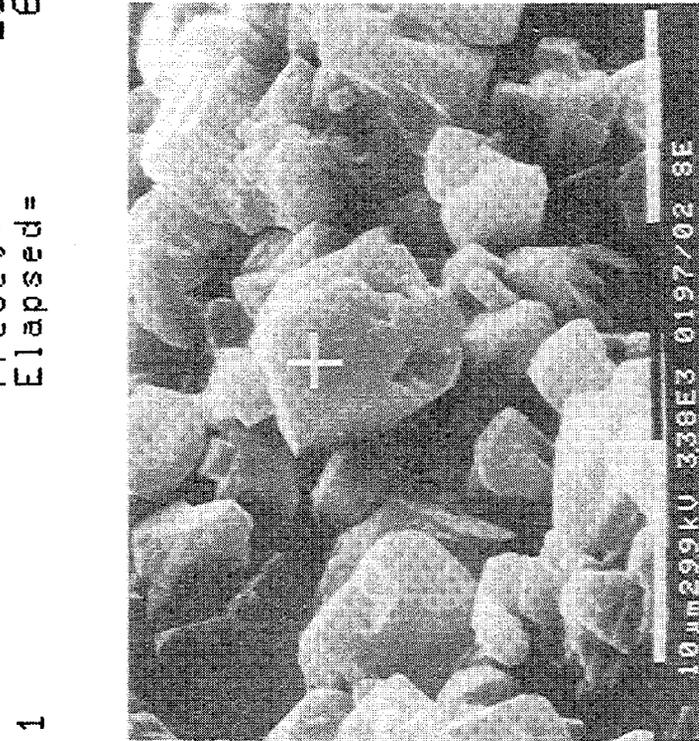


Plate 4b. Large crystallized allochem of dolomite $\text{Ca,Mg}(\text{CO}_3)_2$.

13-Nov-1991 13:08:50

av10 Clay

Vert= 1179 counts Disp= 1

Preset= 200 secs
Elapsed= 200 secs

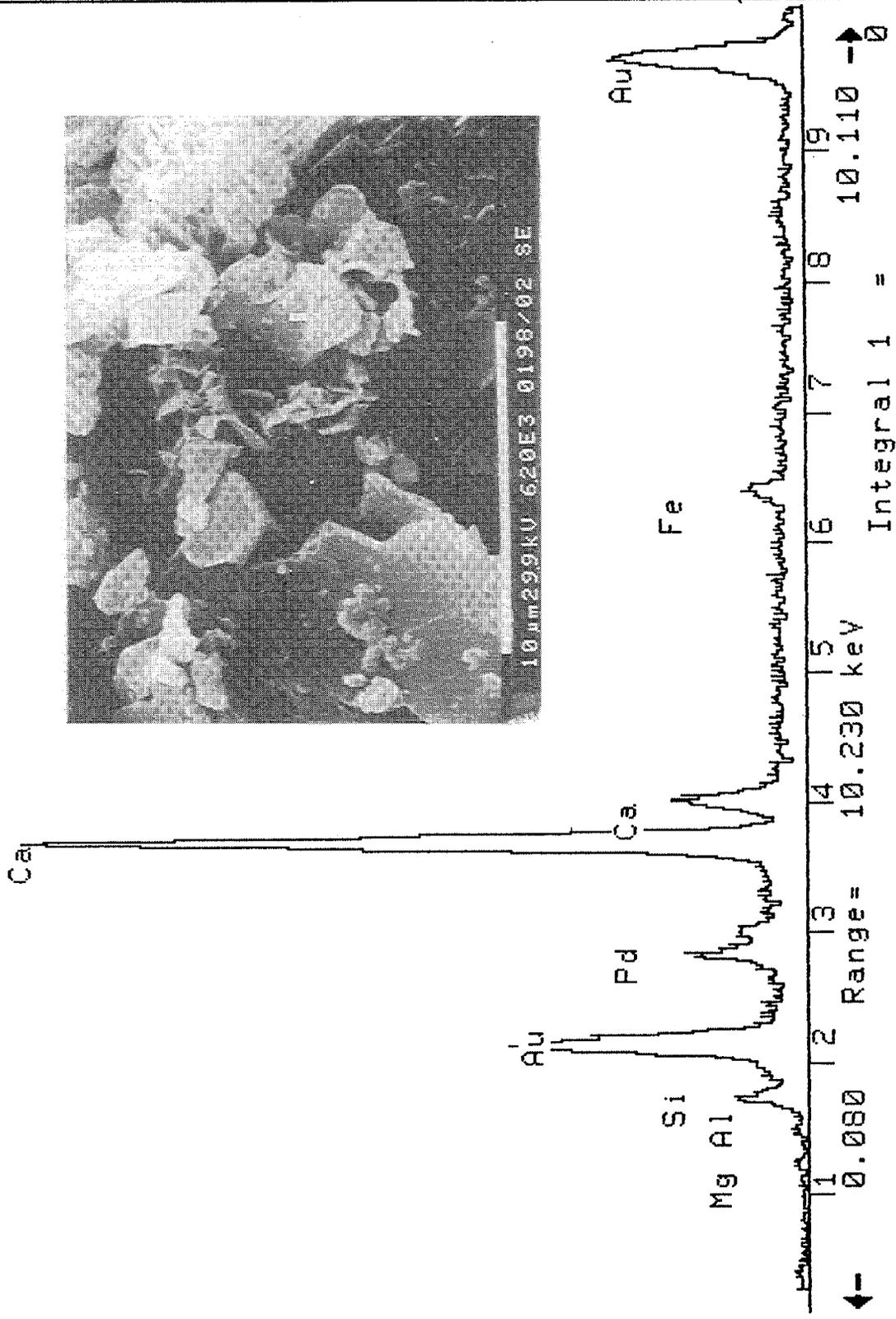


Plate 4c. Fe, Ca, Mg, clay. Probably a variety of smectite.

13-Nov-1991 13:41:11

AV10-clay

Vert =

500 counts

Disp 1

Preset =
Elapsed =

200 secs
200 secs

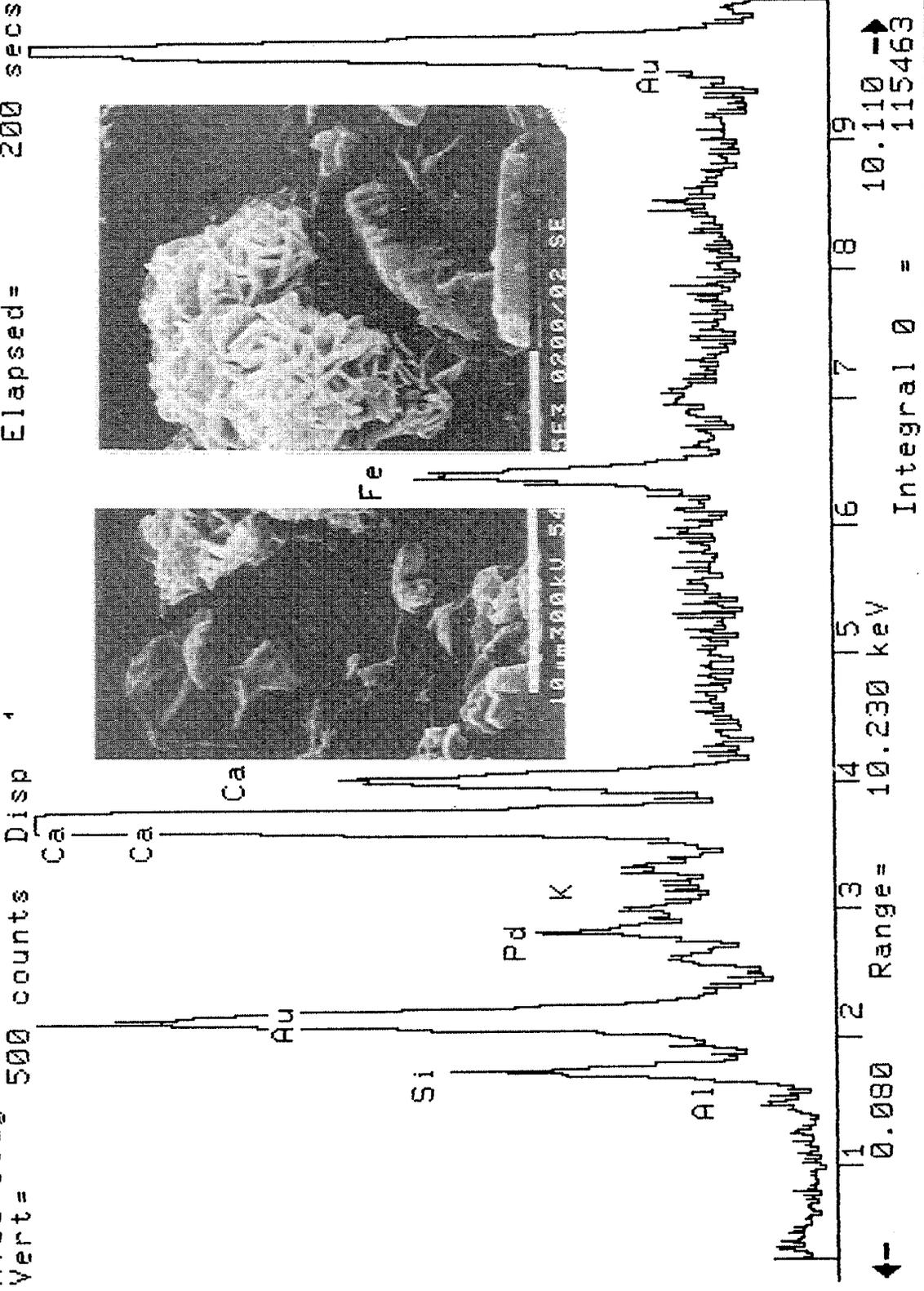
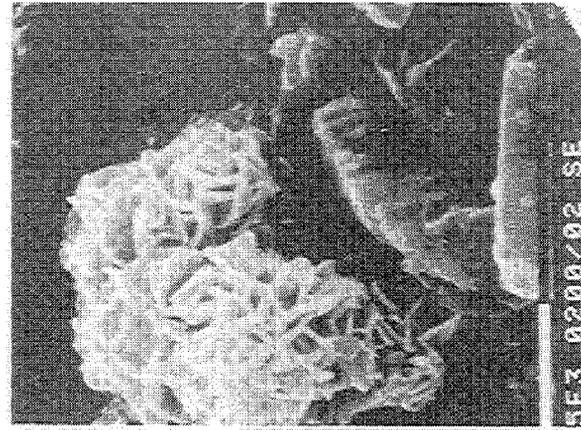


Plate 4d. Fe, Ca, Mg, K, clay. Probably a variety of smectite.

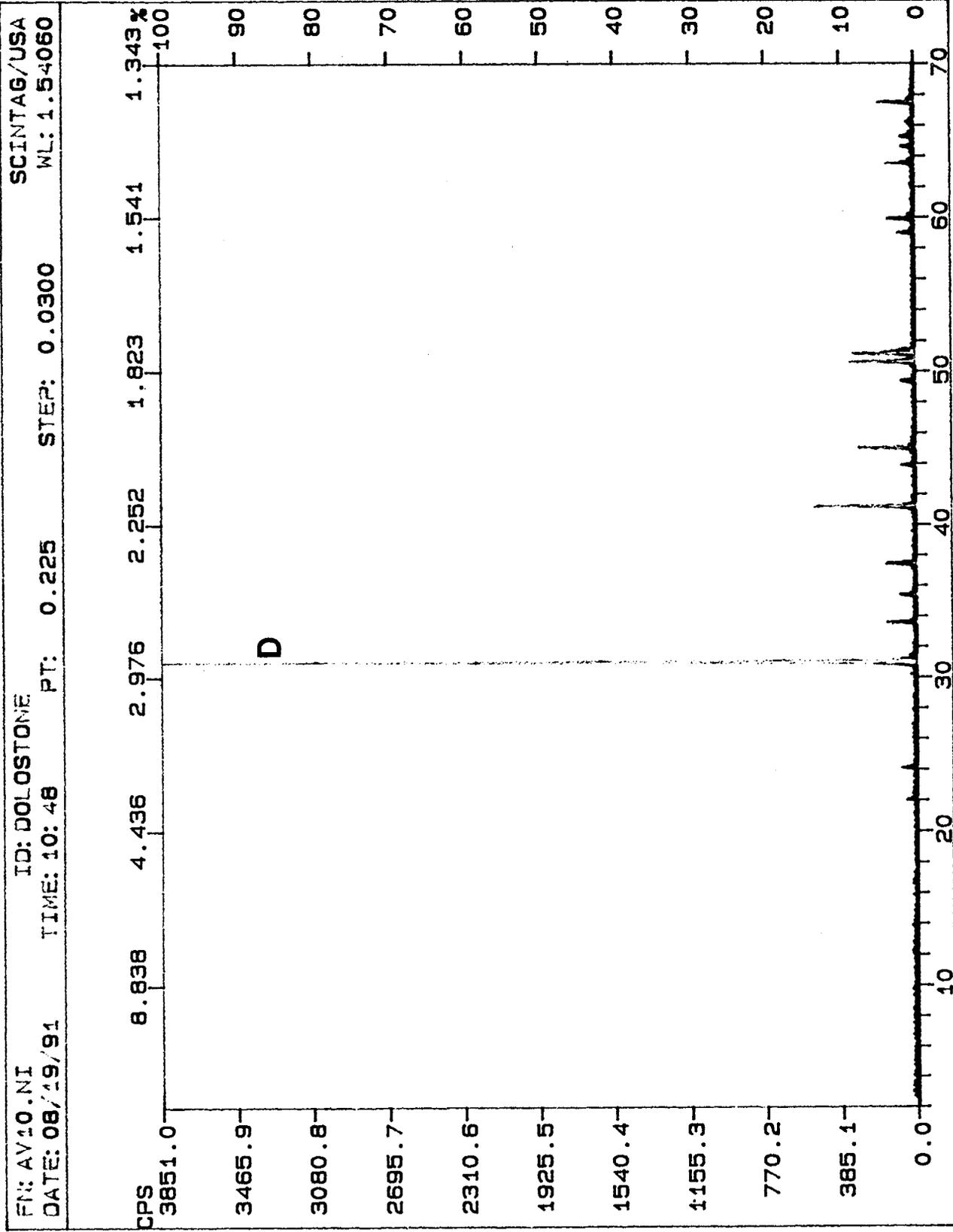


Plate 5a. D: Dolomite

FN: AV408.NI ID: DOLOSTONE SCINTAG/USA
 DATE: 08/19/91 TIME: 13:35 PT: 0.225 STEP: 0.0300 WL: 1.54060

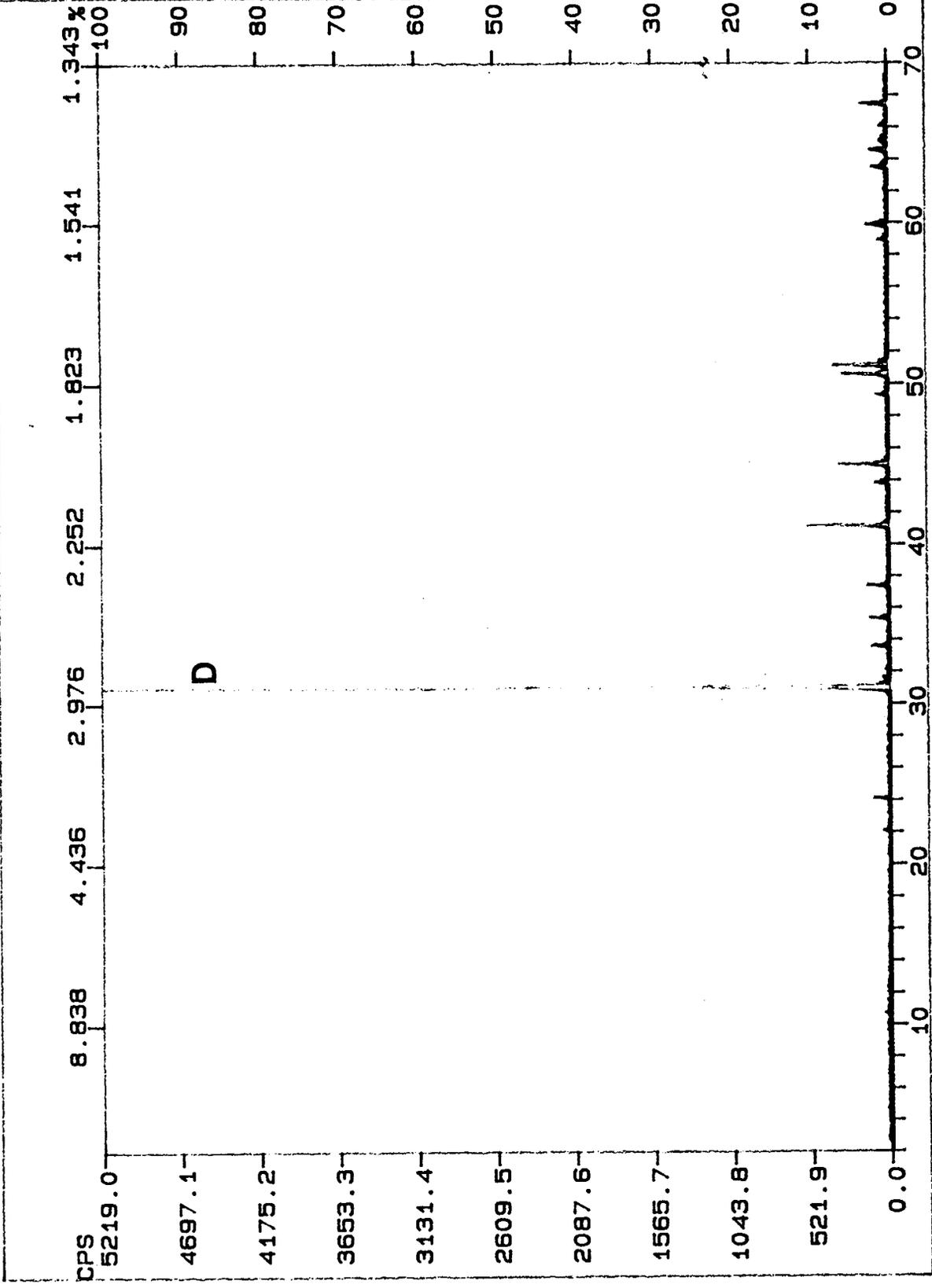


Plate 5b. D: Dolomite

Core NO. 6

Oil Company: Chevron
Well Name: JT McElroy 1156
Field Name: McElroy
State: Texas
County: Crane
Formation Name: Grayburg
Formation Age: Upper Permian; Guadalupian
Formation Type: Dolomite
Core Depth: 2705 - 2706 ft.

Core Length: 21 cm.
Core Diameter: 10 cm.

Rock Description

The rock is dark yellow/brown to light brown due to its hydrocarbon content. It is a dolostone consisting of crystalline dolomite, anhydrite and minor amounts of detrital grains. White crystalline anhydrite occurs as a cement and as large nodules, locally 2 cm in diameter. Detrital grains are rounded to sub-rounded, well sorted, coarse-grained sand, eolian quartz grains, and localized heavy mineral grains. Sedimentary structures identified include wispy parallel laminations, locally disrupted by burrows.

Detailed Petrography

Grain Size: Crystalline dolomite and anhydrite matrix.
Detrital grain average diameter is 0.1 mm.

Roundness, Angularity and Detrital Grain Shape: Detrital grains are rounded to sub-rounded and spherical in shape. Quartz grains occasionally are angular in appearance due to overgrowths.

Fabric (Orientation and Packing): Random, no preferred orientation.
Detrital grains are matrix supported.

Porosity: Percentages measured by point counting thin sections,
Porosity = $15 \pm 1.7 \%$
Most porosity was found to exist within a three dimensional network of dolomite crystals. Pore sizes do not exceed a maximum of 20 μm .

Mineral Composition: Percentages measured by point counting thin sections,
Dolomite $48 \pm 2.5 \%$
Anhydrite $32 \pm 2.3 \%$
Quartz
(Detrital grains including heavy minerals) $19 \pm 1.7\%$
Opagues $1 \pm 0 \%$

The rock also contains minor amounts of authigenic clay; kaolinite and a K, Fe, Ca, Mg clay mineral group. See Plate 4, the energy dispersive spectra analysis, and Plate 5, x-ray diffraction analysis.

Detrital Grains

Quartz: Grains have pitted surfaces. Pits are 1-2 μm in diameter and characteristic of eolian wind blown sediment (Tucker, 1988). Detrital quartz grains also have overgrowths of quartz.

Heavy Minerals: Zircon, tourmaline.

Post-Depositional Minerals

Dolomite often occurs as microscopic rhombohedral crystals generally less than 10 μm in size.

Anhydrite forms large tabular crystals with well developed flat crystal surfaces. Anhydrite crystals have a poikilotopic texture that encapsulates dolomite crystals and detrital grains.

Authigenic kaolinite clay occurs as pore-filling, stacked pseudo hexagonal plates. Hexagonal plates range from 3 to 20 μm in diameter. The K, Fe, Ca, Mg clay mineral group occurred as a pore-lining authigenic clay (generally less than 5 μm thick). It has a crinkly interwoven morphology. This clay was found to occur in localized spots.

Diagenesis

List of diagenetic events (these events are not necessarily in the correct chronological order).

1. Early dolomitization of calcium carbonate (Bebout et al., 1987).
2. Possible replacement of gypsum nodules by anhydrite.
3. Growth of poikilotopic, anhydrite, pore-filling cement and nodule enlargement.
4. Formation of quartz overgrowths.
5. Growth of pore-filling authigenic kaolinite.
6. Localized growth of pore-lining K, Fe, Ca, Mg clay mineral.
7. Late-stage dolomite dissolution forming serrated rhomb-shaped crystals.
8. Anhydrite and quartz dissolution.

See Chart.

RELATIVE SEQUENCE OF DIAGENETIC EVENTS FOR CORE NO. 6

Increase in Burial

>>>

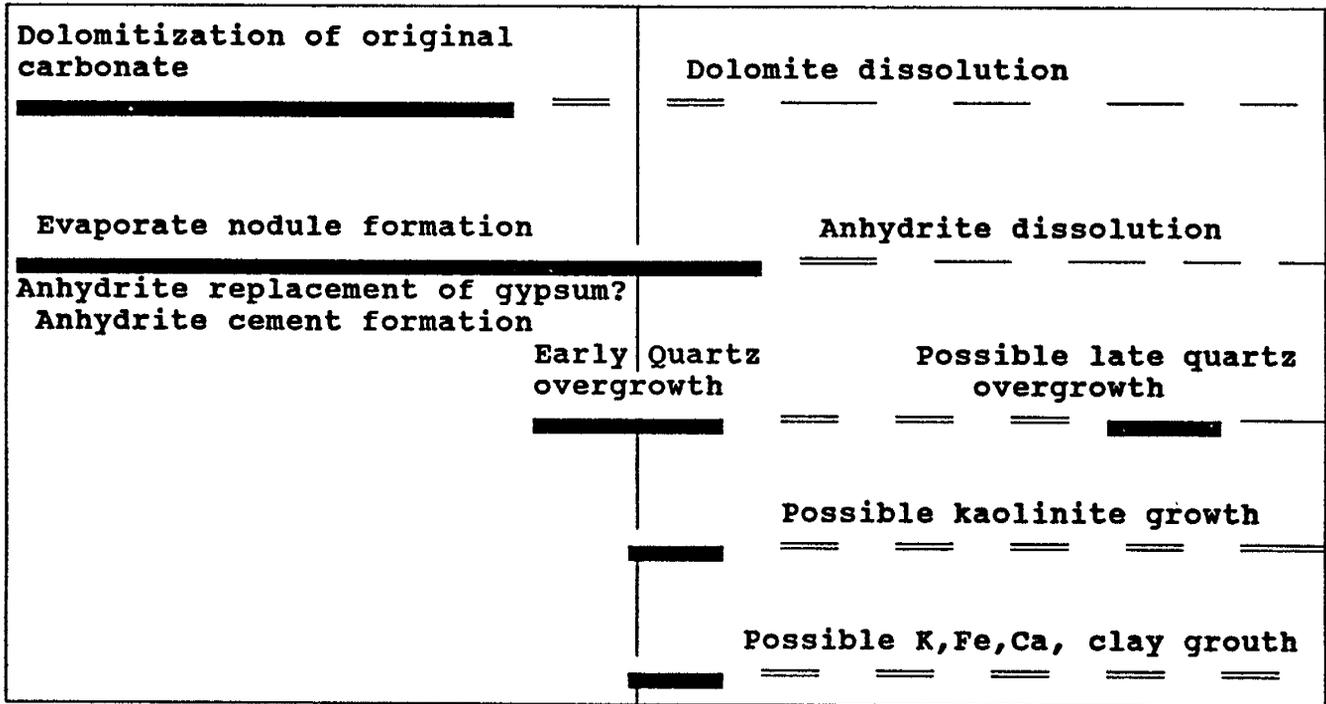
Shallow Burial: Eogenetic Zone
(Authigenetic minerals formed due to hypersaline fluids)

Deep Burial: Mesogenetic Zone
(Authigenetic minerals formed due to circulating meteoric fluids)

Middle Permian

Recent

D
I
A
G
E
N
E
T
I
C
O
W
T
H
M
I
N
E
R
A
L



Key: ■ Relative timing of diagenetic event is certain.
 — Dissolution
 == Relative timing of diagenetic event is uncertain
 - - - Dissolution, timing uncertain

Note: Sequence of diagenetic events are identified using textures displayed by S.E.M. and the petrographic microscope. Due to the limited extent of sampling this chart may not be characteristic for the whole formation.

Regional Geology and Rock Homogeneity

Core No. 6 was obtained from the Permian, Grayburg Formation of the McElroy Field, west Texas. The Grayburg Formation has been described in detail by Bebout et al. (1987). Bebout characterized the Grayburg Formation for Crane County, west Texas and divided the formation into three units using sand or silt beds picked out by logged gamma ray markers (see description for Core No. 4). The McElroy field is a stratigraphic trap formed by the disappearance of porosity by the infilling of evaporites updip to the west.

The 21 cm length of studied core is a silty dolostone from the top of the formation. The studied core was found to be generally of a homogeneous lithology. It is important to note that the silty dolostone lithology studied was not the predominant lithology within the reservoir.

Depositional Environment

During the Permian Period, the west Texas area was a broad shallow basin trending north-northwest to south-southeast on a continental shelf known as the Central Basin platform. The Central Basin was bordered by deeper basins; to the east by the Midland Basin and to the west by the Delaware Basin. Within the interior of the Central Basin platform, low energy carbonates and evaporates were deposited. Dolomitization probably took place in the shallow subsurface. This occurred when hypersaline waters flowed downward from arid supratidal sabkha settings originating through the evaporation of seawater. Siliclastic sediments were intermittently deposited within the carbonate shelf. They probably were eolian in origin being wind blown onto intertidal sabkha flats.

Description of Plates

Plate 1: The core sample studied.

- 1a and 1b show end views of the core.
- 1c shows the complete length of the core.

Plate 2: Photomicrographs of thin sections.

- 2a. Plane polarized light view of white, translucent, low relief anhydrite and quartz grains. Patchy brown areas are dolomite.
- 2b. Cross polarized light view of large tabular anhydrite crystals showing 2nd to low 3rd order birefringence. Anhydrite crystals poikilotopically enclose detrital quartz grains.

Plate 3. Scanning electron micrographs of Core No. 6

- 3a. Bar is $10\mu\text{m}$, x 1200.
Rhombohedral dolomite crystals (D) and kaolinite (K) stacks. A void (intercrystalline pore space) is seen in the center of the photomicrograph.
- 3b. Bar is $10\mu\text{m}$, x 1800.
Tabular crystals of pore-filling anhydrite.
- 3c. Bar is $10\mu\text{m}$, x 2000.
Initiation of a quartz overgrowth (QO) on the surface of a detrital quartz grain (QG). The surface of the quartz grain is pitted while the quartz overgrowth has well developed crystal faces.
- 3d. Bar is $10\mu\text{m}$, x 1000.
Quartz overgrowths with rhombohedral shaped voids (RV) within the overgrowths, indicating removal or dissolution of dolomite rhombs. Authigenic kaolinite (K) shows delicate "books" of hexagonal plates. There are also areas of scattered dolomite (D).

Plate 4. Energy dispersive spectra analysis

- 4a. Rhombohedral crystals of dolomite $\text{Ca,Mg}(\text{CO}_3)_2$
- 4b. Anhydrite crystal, CaSO_4 .
- 4c. Detrital quartz, SiO_2 grain with quartz overgrowths.
- 4d. Kaolinite, $\text{Al}_4\text{O}_{10}(\text{OH})_8$ clay.
- 4e. Fe, Ca, Mg, K clay. Probably a variety of smectite.

Plate 5. X-Ray diffraction analysis

- 5a. D: Dolomite, A: Anhydrite, G: Gypsum, Q: Quartz
- 5b. D: Dolomite, A: Anhydrite, G: Gypsum, Q: Quartz,
K: Kaolinite
- 5c. D: Dolomite, Q: Quartz, K: Kaolinite

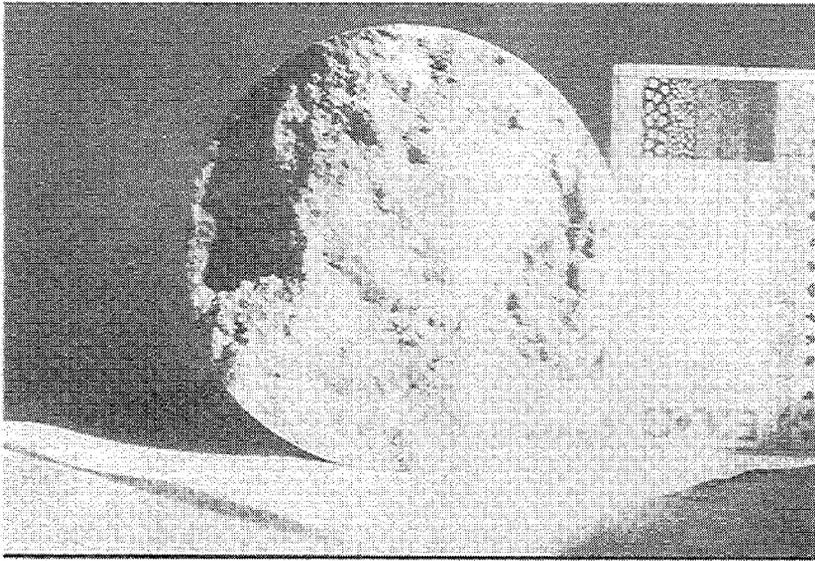


Plate 1a

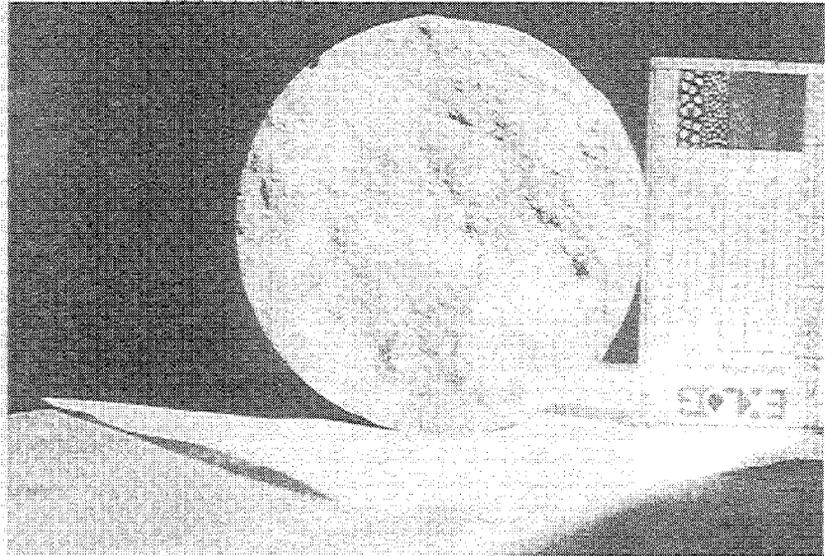


Plate 1b

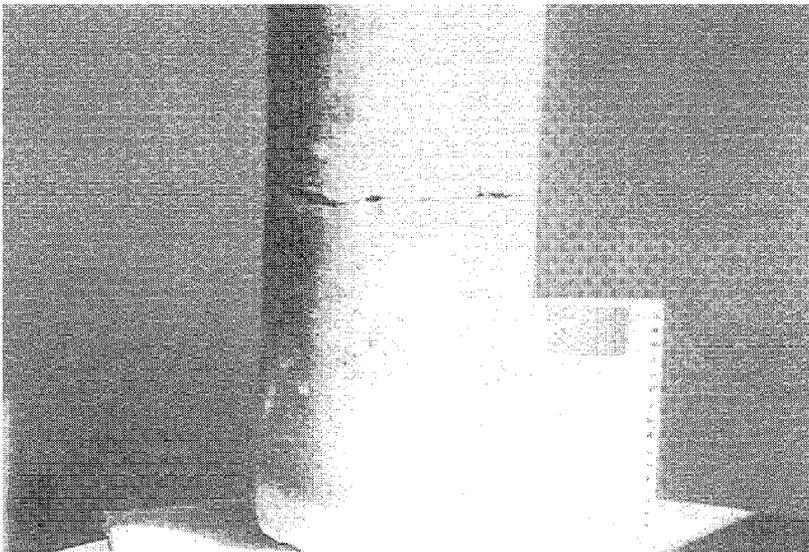


Plate 1c

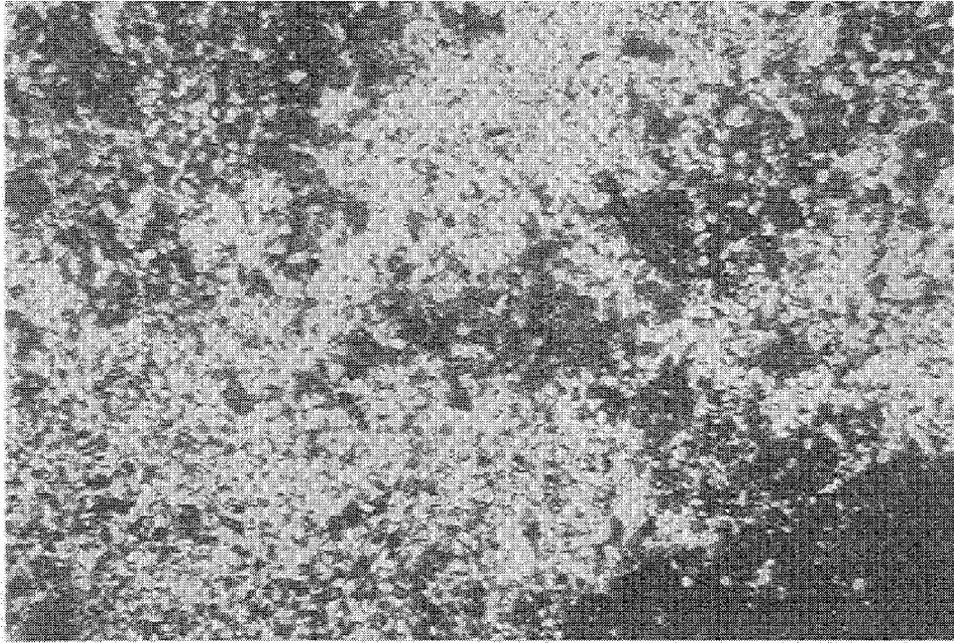


Plate 2a

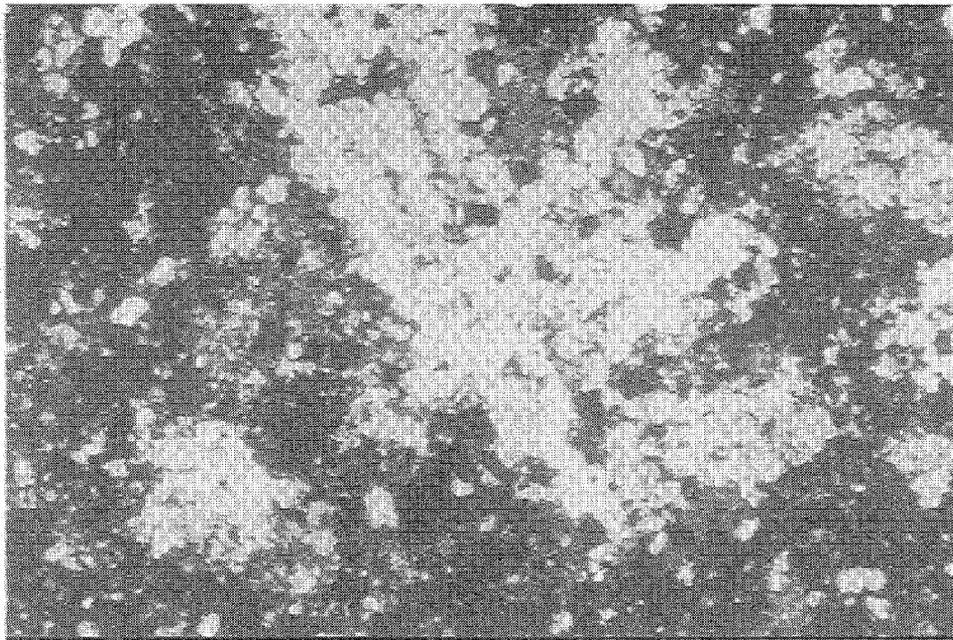


Plate 2b

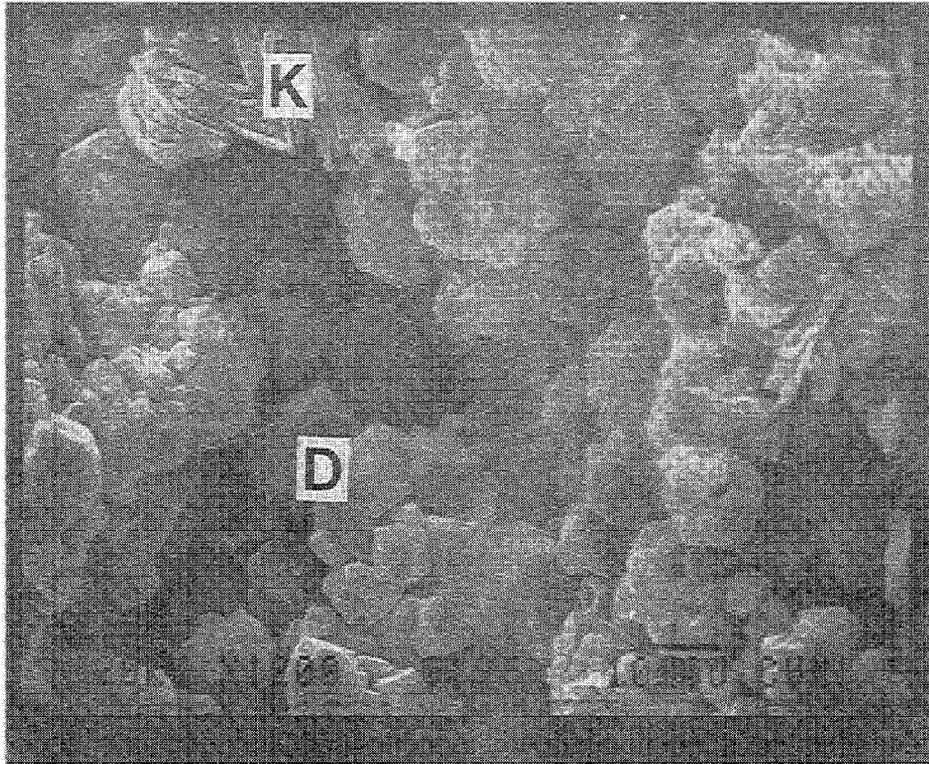


Plate 3a

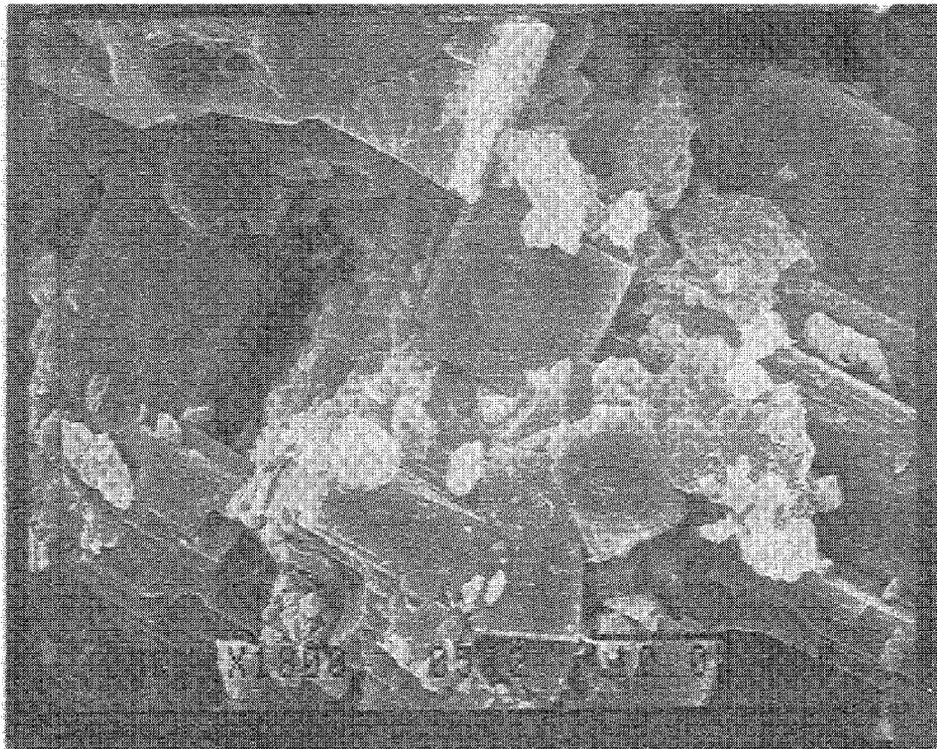


Plate 3b

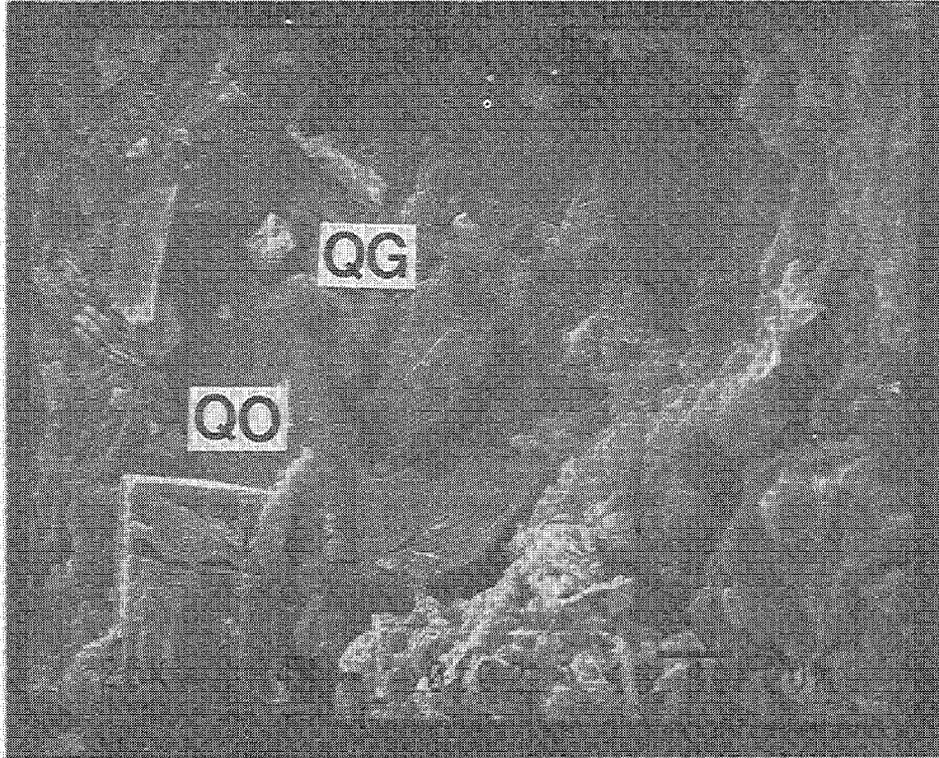


Plate 3c

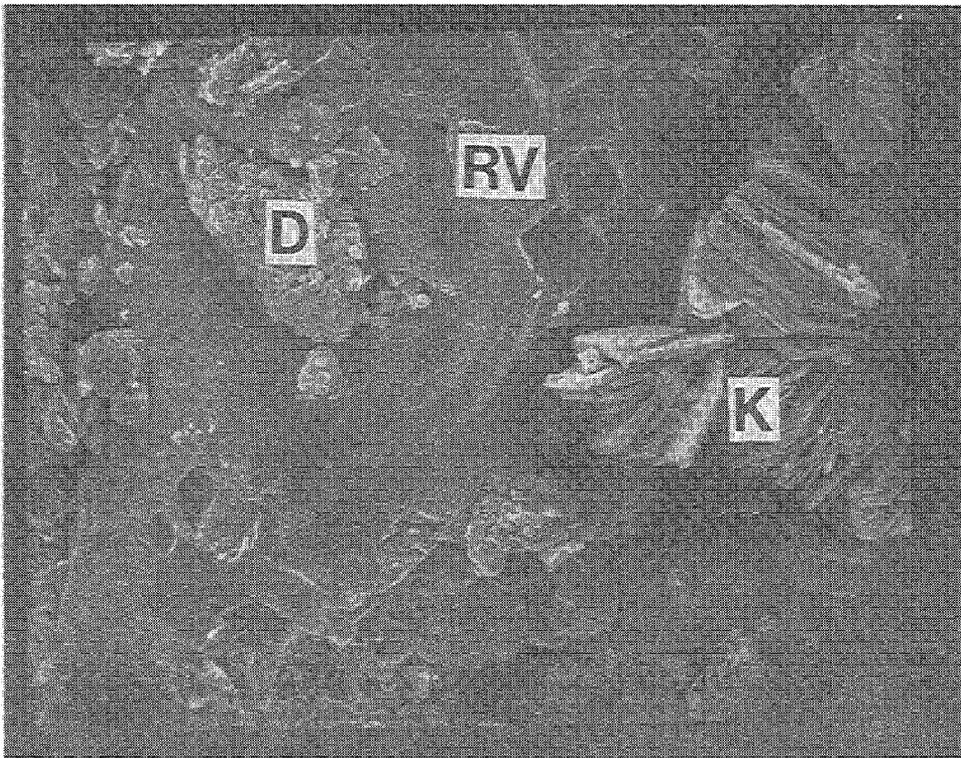


Plate 3d

12-Apr-1992 14:19:04
 Execution time = 3 seconds
 DOLOMITE
 Vert# 1921 counts Disp= 1
 Preset = 200 secs
 Elapsed = 159 secs

Core No. 6

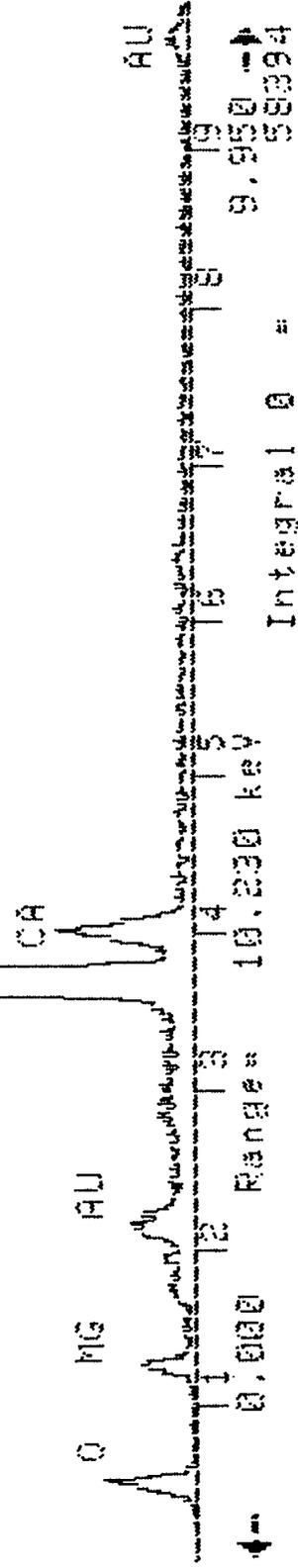
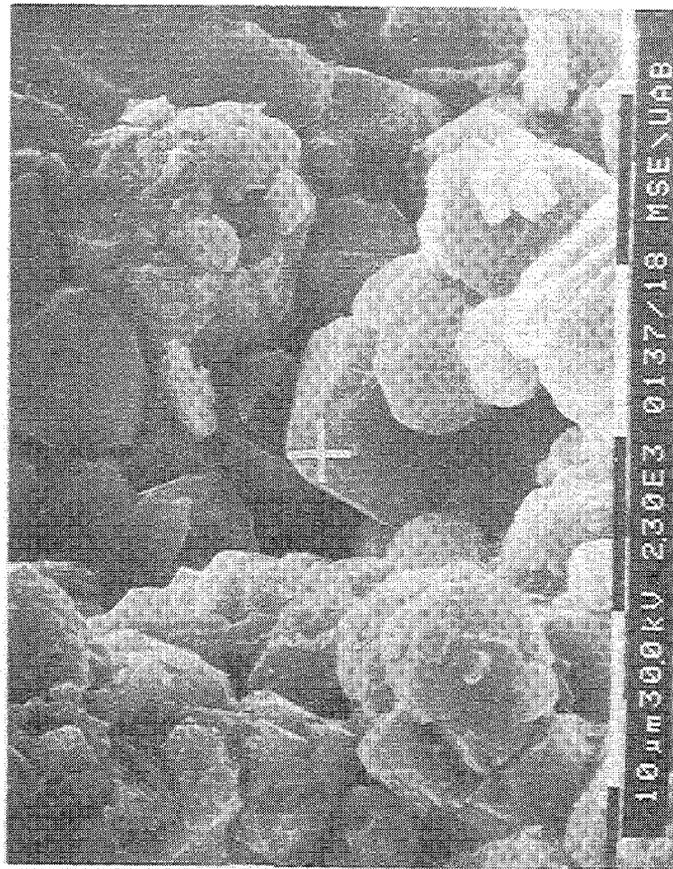


Plate 4a. Rhombohedral crystals of dolomite $\text{Ca}_2\text{Mg}(\text{CO}_3)_2$.

12-Apr-1992 13:59:09

Execution time = 3 seconds

ANHYDRITE

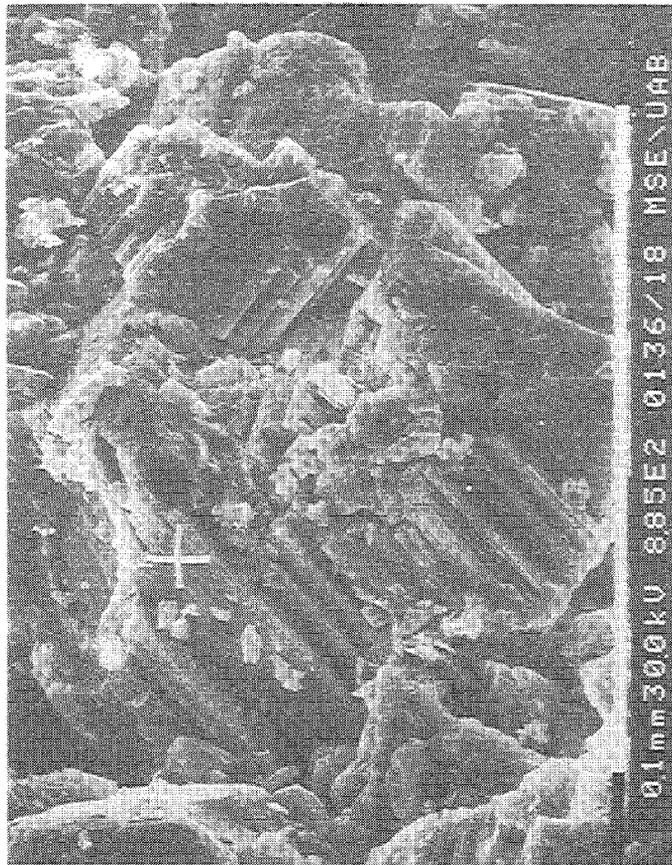
Vert# 281 counts Disp= 1

Presets =
Elapsed =

200 secs
200 secs

Core No. 6

CA



S

FD

CA

AU

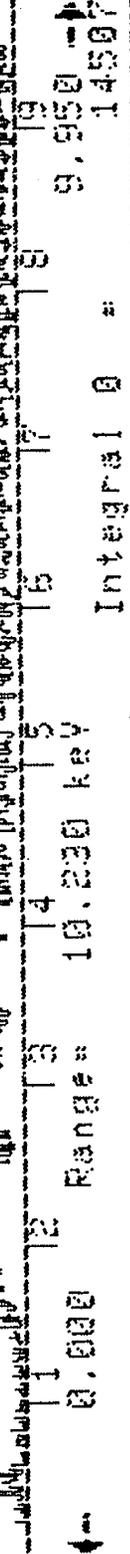


Plate 4b. Anhydrite crystal, CaSO₄.

12-APR-1992 13:35:22
 Execution time = 4 seconds
 QUARTZ
 Vert = 556 counts Disp = 1
 Preset =
 Elapsed = 2:00 : 2:00

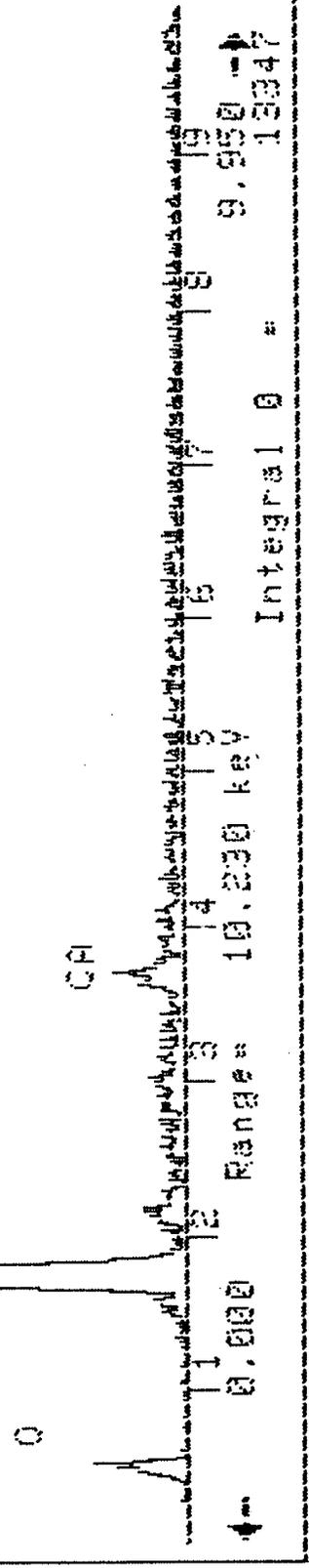
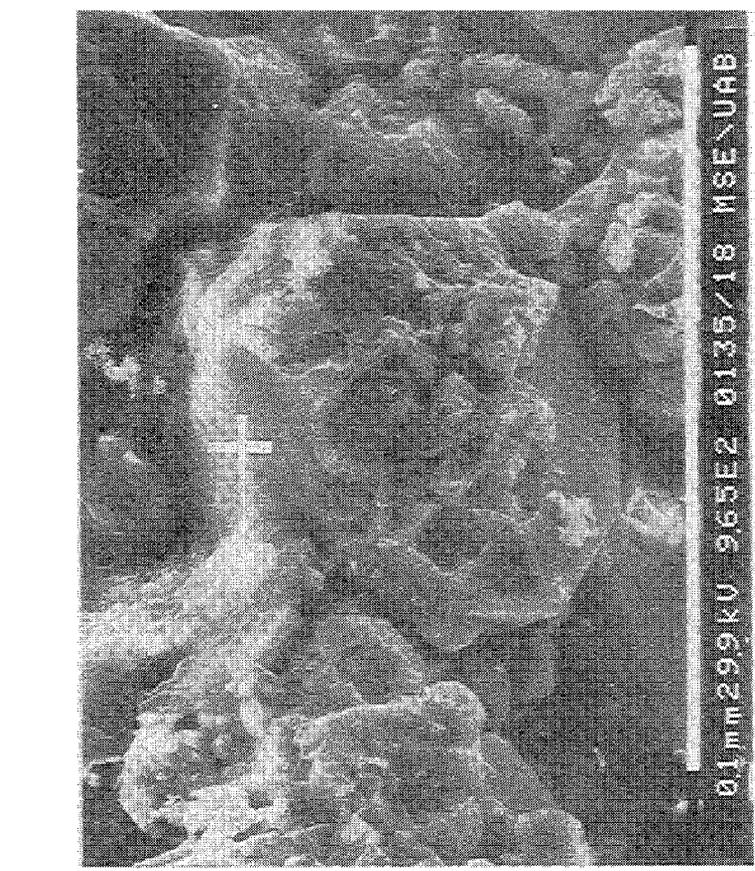


Plate 4c. Detrital quartz, SiO₂ grain with quartz overgrowths.

12-APR-1992 13:12:55
 Execution time = 18 seconds
 KAOLINITE
 Vert = 100 counts Disp = 1

Preset =
 Elapsed = 200 secs
 100 secs

Core No. 6

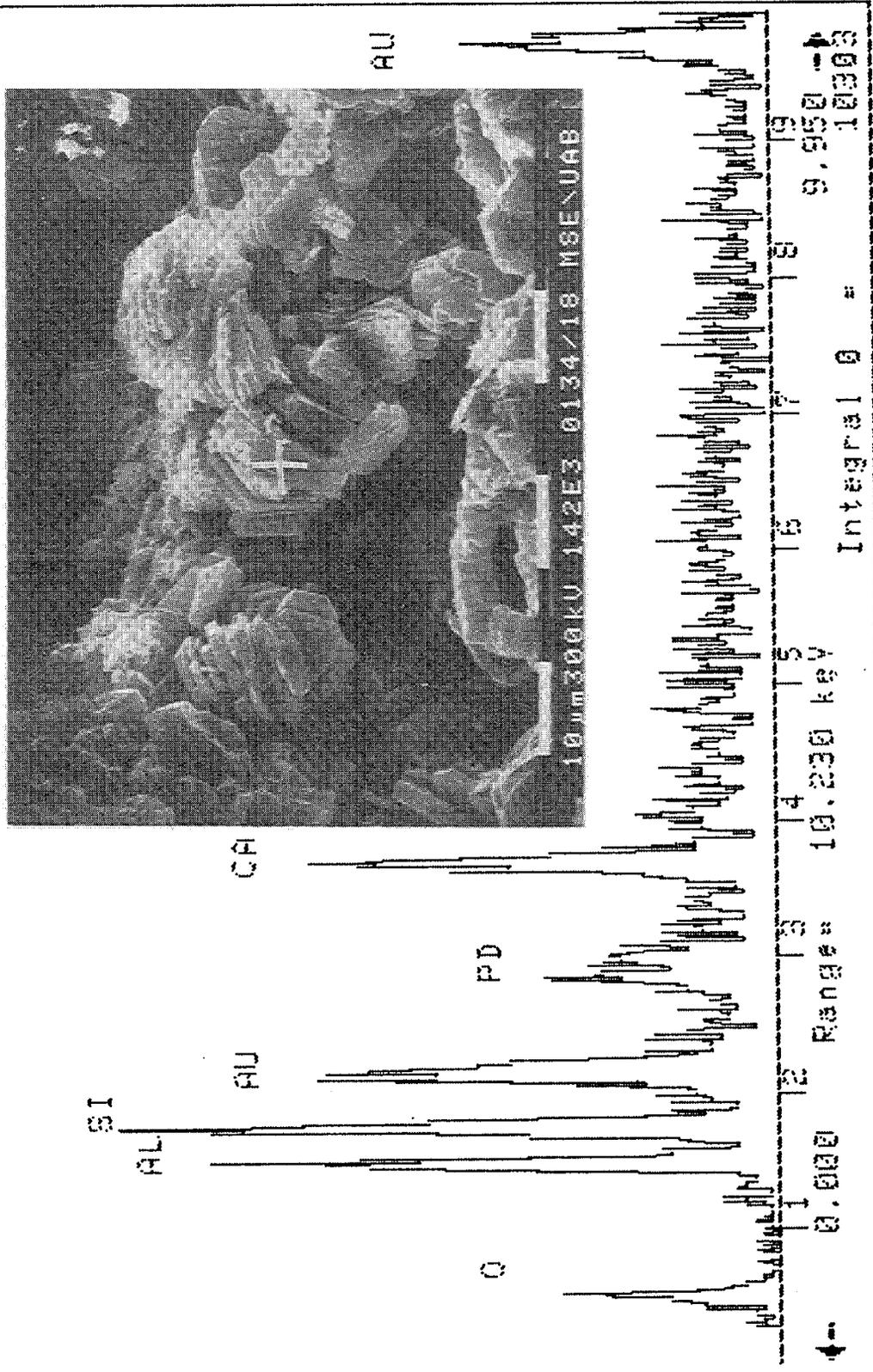
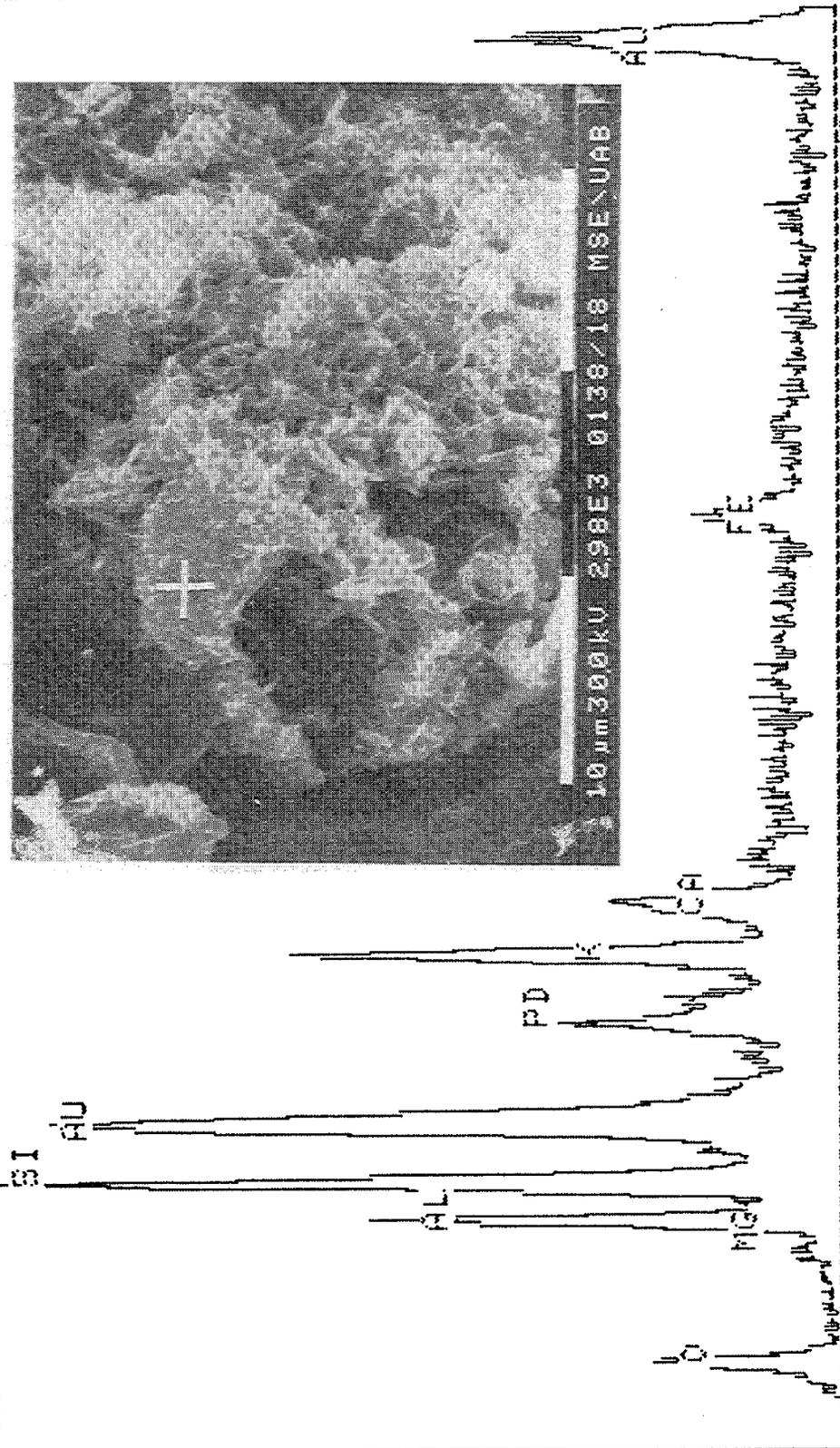


Plate 4d. Kaolinite, $Al_4Si_4O_{10}(OH)_8$ clay.

12-Apr-1992 14:41:52 Z# 95 Am
 Execution time = 37 seconds Preset = 200 secs
 SMECTITE? Elapsed = 110 secs
 Vert# 550 counts Disp# 1

Core No. 6



0.000 Range = 10.230 keV Integral 0 = 9.950
 11 12 13 14 15 16 17 18 19 60007

Plate 4e. Fe, Ca, Mg, K clay. Probably a variety of smectite.

FN: AV9.NI ID: SANDY DOLOSTONE SCINTAG/USA
 DATE: 08/19/91 TIME: 10:29 PT: 0.225 STEP: 0.0300 WL: 1.54060

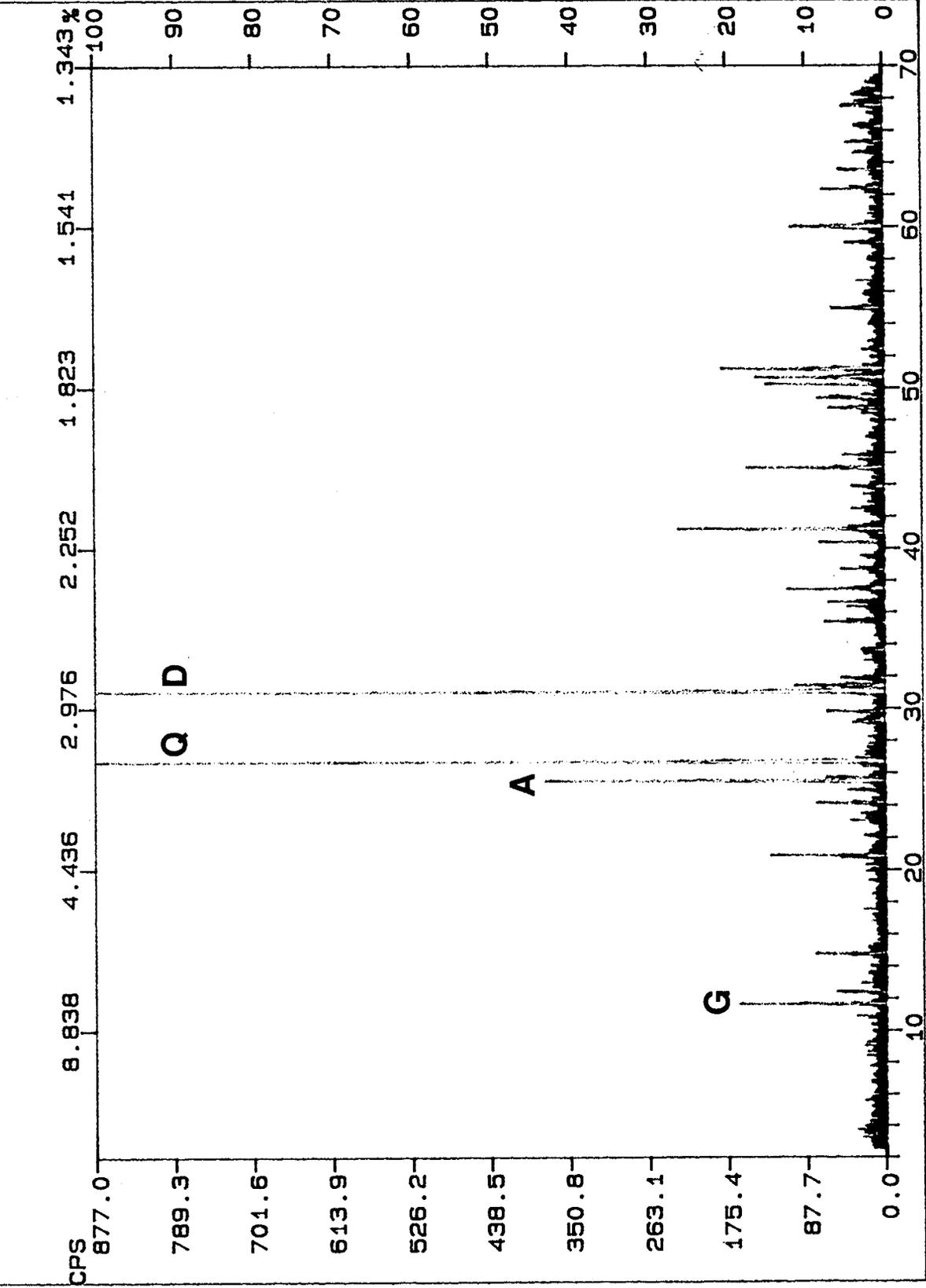
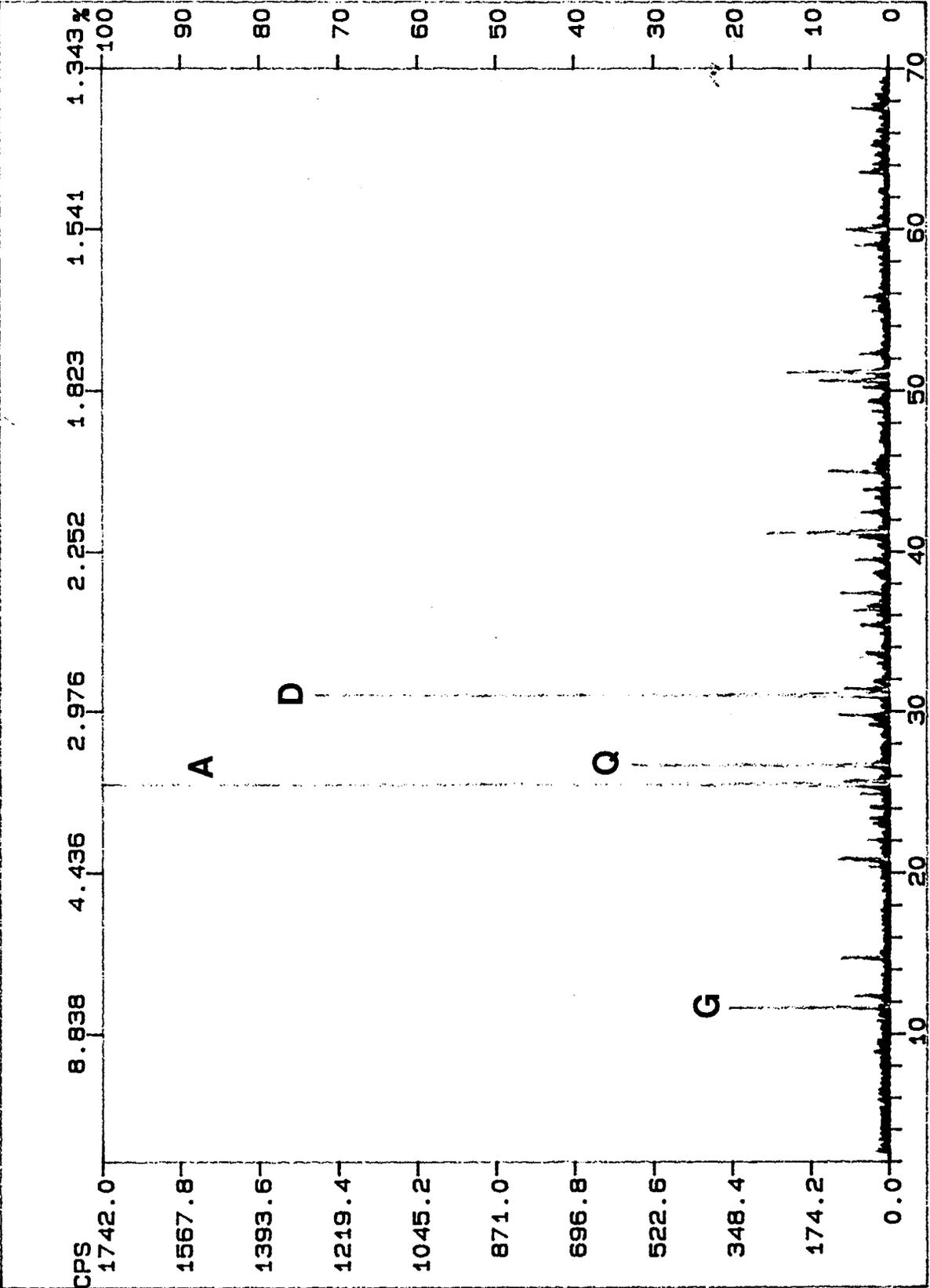


Plate 5a. D: Dolomite, A: Anhydrite, G: Gypsum, Q: Quartz

FN: AV98.NI ID: SANDY DOLOSTONE SCINTAG/USA
 DATE: 08/19/91 TIME: 13:47 PT: 0.225 STEP: 0.0300 WL: 1.54060



FN: AV9TS.NI ID: AV9 THIN SECTION SCINTAG/USA
 DATE: 08/19/91 TIME: 14:50 PT: 0.225 STEP: 0.0300 WL: 1.54060

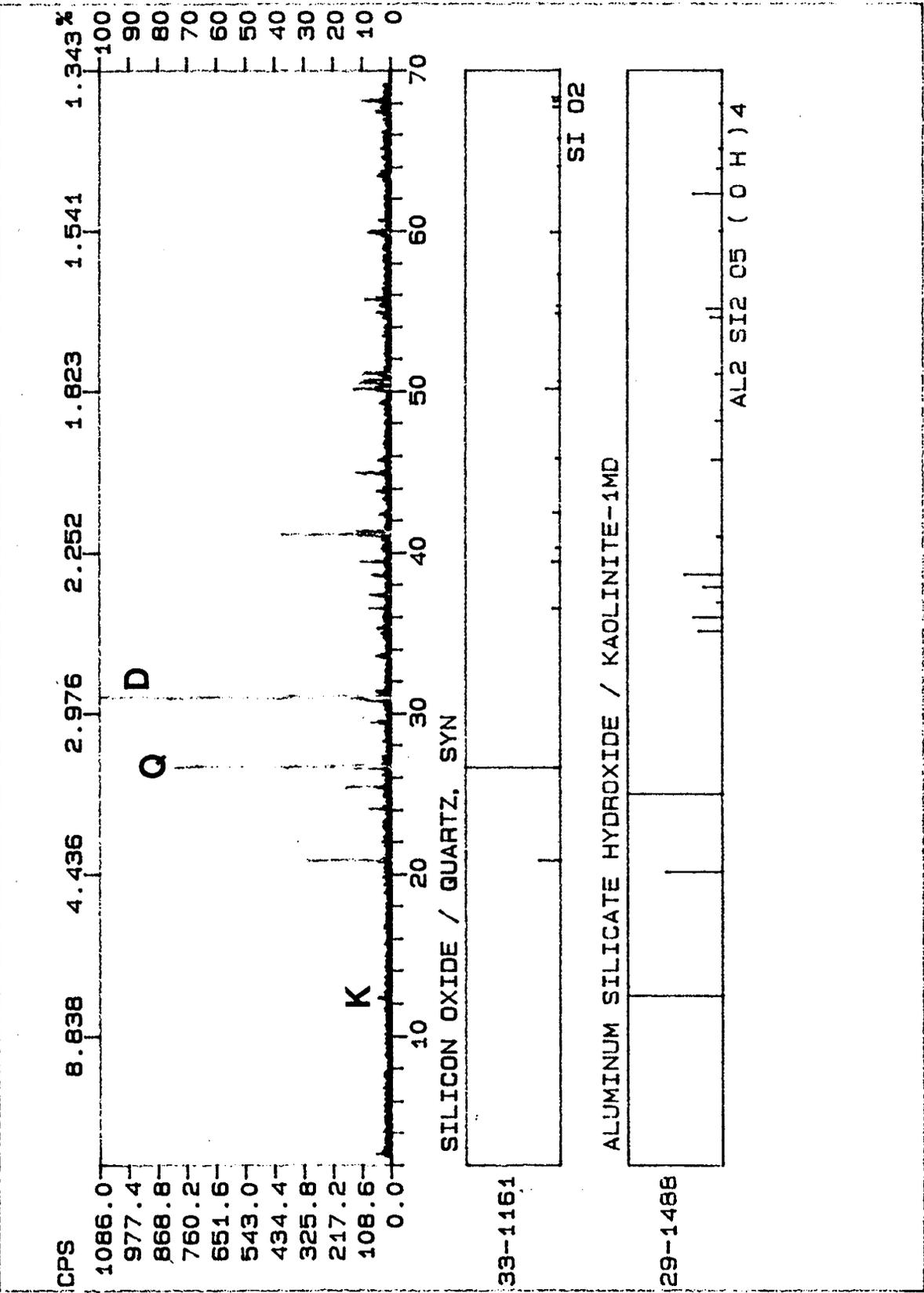


Plate 5c. D: Dolomite, Q: Quartz, K: Kaolinite

Data Sheet: Core No. 7

Oil Company: Texaco
Well Name: Texaco State of Wyoming # 2
Field Name: Cellers Ranch
State: Wyoming
County: Johnson
Formation Name: Tensleep
Formation Age: Pennsylvanian
Formation Type: Sandstone
Core Depth: 6568 - 6695 ft.

Core Sample Length: 9 cm.
Core Sample Diameter: 10 cm.

Rock Description

The rock is light gray and dark to light brown due to its hydrocarbon content. It is a clean, mature, well sorted quartz arenite (sandstone) consisting predominantly of fine grained (0.25 - 0.125mm), subrounded to subangular, translucent quartz grains. There are small (generally less than 7 mm in diameter) patches of a white crystalline mineral that are composed of dolomite. The rock appears massive.

Detailed Petrography

Grain Size: Detrital grains range from 0.5 to 0.1 mm in diameter. They average 0.2 mm in diameter.

Roundness, Angularity and Detrital Grain Shape: Detrital grains are subrounded to subangular and spherical to sub-prismoidal in shape.

Fabric (Orientation and Packing): Random, no preferred orientation. High degree of close packing due to compaction. Contacts between grains are long contacts, locally concave-convex, angular or sutured.

Porosity: Percentages measured by point counting thin sections - Porosity = 16 ± 3.2 %
Porosity was found to exist within a three dimensional interparticle/ intercrystalline network. Pore sizes did not exceed 100 μ m. Porosity was reduced due to compaction, quartz overgrowth and carbonate cementation.

Mineral Composition: Percentages measured by point counting thin sections;

Quartz, 87.0 ± 4.2 %
Dolomite (ferroan), 12.0 ± 3.0 %
Feldspar, 0.7 ± 0.2 %
Heavy Minerals, 0.3 ± 0.1 %

Description of Detrital Grains

Quartz: Well sorted, rounded to subangular monocrystalline grains, with uniform extinction. Grain contacts usually are sutured. Surface features are lost due to the pressure solution between grains. Grains have an irregular furrowed surface. Grains

frequently possess quartz overgrowths recognized by euhedral crystal surfaces on grains, ghosts of original grains, and the formation of angular pore spaces.

Orthoclase feldspar: Identified by staining thin sections, cloudy-brown color due to corrosion (alteration to clay) and twinning. Feldspar grains display dissolution.

Heavy minerals: Most commonly tourmaline.

Post-Depositional Minerals

Quartz overgrowths were extensively well developed forming euhedral surfaces on all grains. Overgrowths are pore-filling. Ferroan dolomite cement occurs as euhedral rhombohedral crystals ranging from a maximum size of 30 μm and occasionally forming interpenetrating crystals. The carbonate cement is pore-filling. It has a patchy and random distribution.

Diagenesis

List of diagenetic events (the events are not necessarily in the correct chronological order):

1. Compaction causing pressure solution between detrital quartz grains.
2. Feldspar dissolution.
3. Formation of quartz overgrowths.
4. Carbonate pore-filling growth.

Regional Geology and Rock Homogeneity

Core No.7 was cored from the Tensleep Sandstone Formation of the Cellers Ranch Field in central northeast Wyoming. The Tensleep Formation is a clean quartz arenite varying from 20 to 60 ft. in thickness and is interbedded with dolomite and anhydrite units. The Tensleep Sandstone Formation was thrust to the southwest and folded during the Laramide orogeny producing the structural trap which forms the Cellers Range Field (Stone, 1986). The reservoir is capped by impermeable Permian salts which lie unconformably above. There is also a loss of porosity within the Tensleep Sandstone off-structure within the Cellers Range Field. The sandstone lithology sampled is probably a good representation for most of the reservoir rock.

Depositional Environment

During the Pennsylvanian period central northeast Wyoming was covered by an intracratonic shallow basin trending south-southeast called the Powder River Basin. The basin was bordered by uplifts, the Front Range Uplift to the south, the Canadian Shield to the north and possibly the Transcontinental Arch to the east which acted as sediment source areas. The core sample AV3 was deposited in a shallow nearly featureless bottom and shoreface sea which allowed for the deposition of fine quartz arenites with a uniform lithology. The Tensleep Sandstone Formation is diachronous and are interfingered with dolostones and evaporites. Associated with these sandstones are fringing carbonates which formed on a carbonate shelf to the southwest of the basin.

Description of Plates

Plate 1. The core sample studied.

- 1a and 1b show end views of the core.
- 1c, shows the complete length of the core.

Plate 2. Photomicrographs of thin sections.

- 2a. Plane polarized light view of sub-angular to sub-rounded well sorted, quartz detrital grains. The thin section has been impregnated with a light pink colored epoxy. The epoxy has infilled pore spaces.
- 2b. Cross polarized light view of sub-angular to sub-rounded, low birefringence quartz grains. Mottled brown patches are of dolomite. Dark black areas are pore spaces.

Plate 3. Scanning electron micrographs of core No. 7

- 3a and 3b, bars are 100 μm x 150.
Detrital quartz grains with sutured contacts. Detrital grains have an irregular furrowed surface. Flat surfaces on the detrital quartz grains are quartz overgrowths.
- 3c. Bar is 100 μm x 400.
Detrital quartz grains displaying quartz overgrowths. The central grain has been corroded and probably is a detrital feldspar grain (F).
- 3d. Bar is 10 μm x 860.
Pore-filling authigenic dolomite (D) between detrital quartz grains (Q). The dolomite occurs as interpenetrating rhombohedral crystals.

Plate 4. Energy dispersive spectra analysis

- 4ai. Detrital quartz, SiO_2 grain.
- 4aaii. Quartz overgrowth, SiO_2 .
- 4aaiii. An iron(Fe) rich pore-filling dolomite $\text{Ca,Mg}(\text{CO}_3)_2$.
- 4b. Dissolved, K rich, orthoclase feldspar, KAlSi_3O_8 .
- 4c. Dissolved, K rich, orthoclase feldspar, KAlSi_3O_8 .

Plate 5. X-Ray diffraction analysis

- 5a. Q: Quartz, D: Dolomite
- 5b. Q: Quartz, D: Dolomite
- 5c. Q: Quartz, D: Dolomite

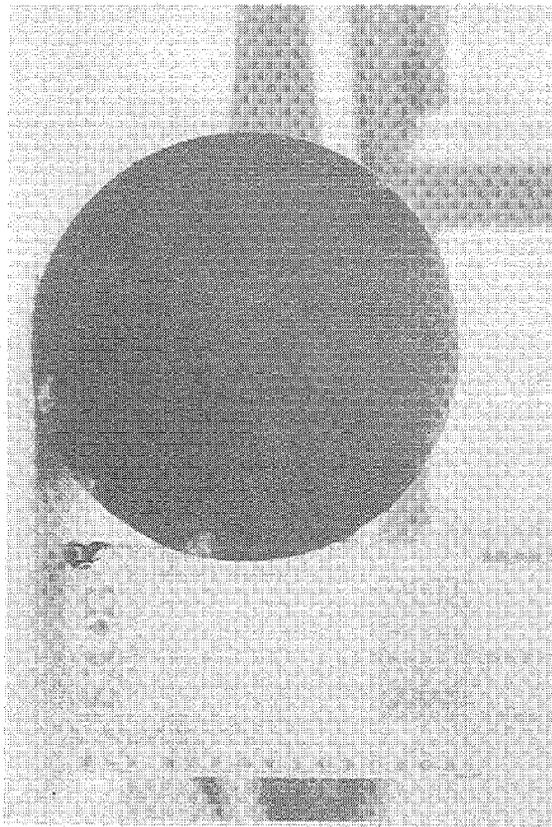


Plate 1b

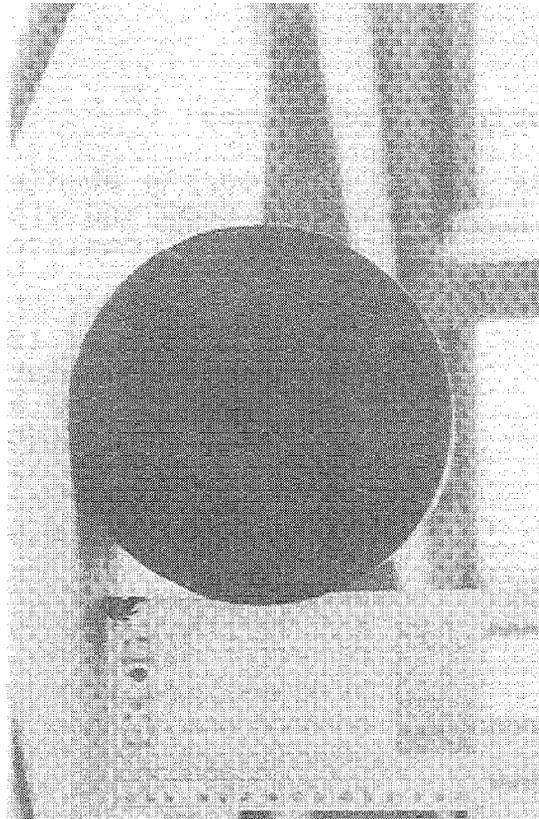


Plate 1a

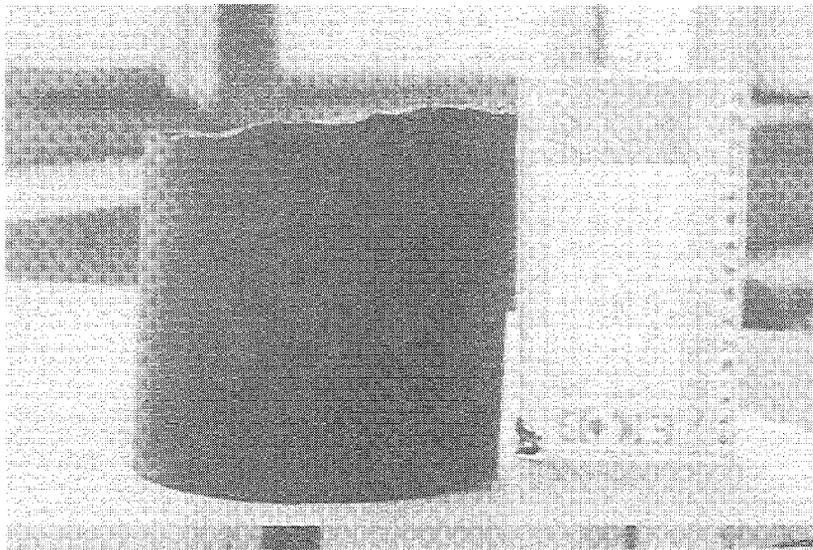


Plate 1c

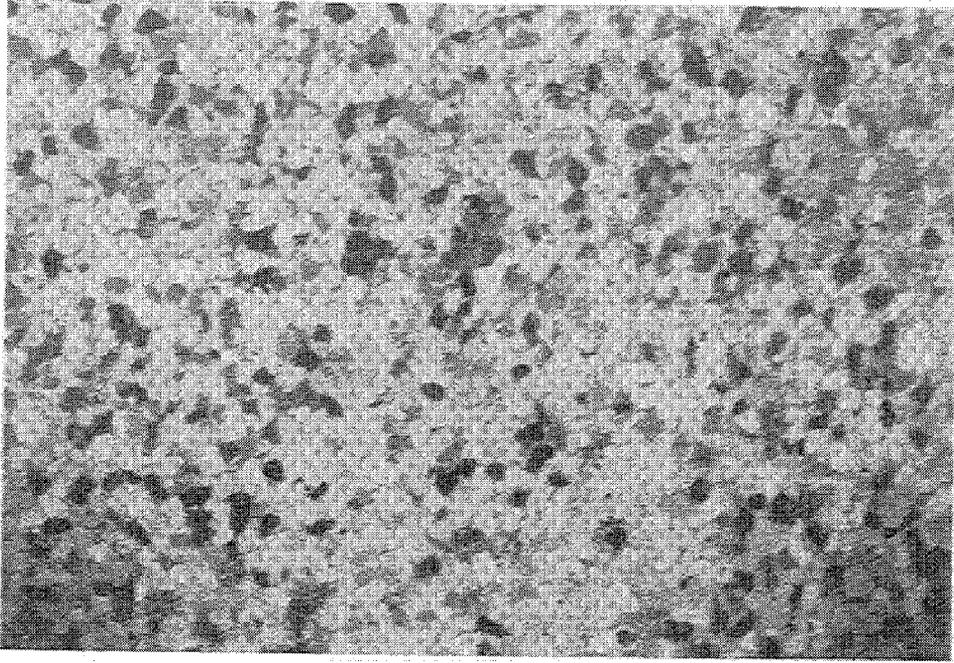


Plate 2a

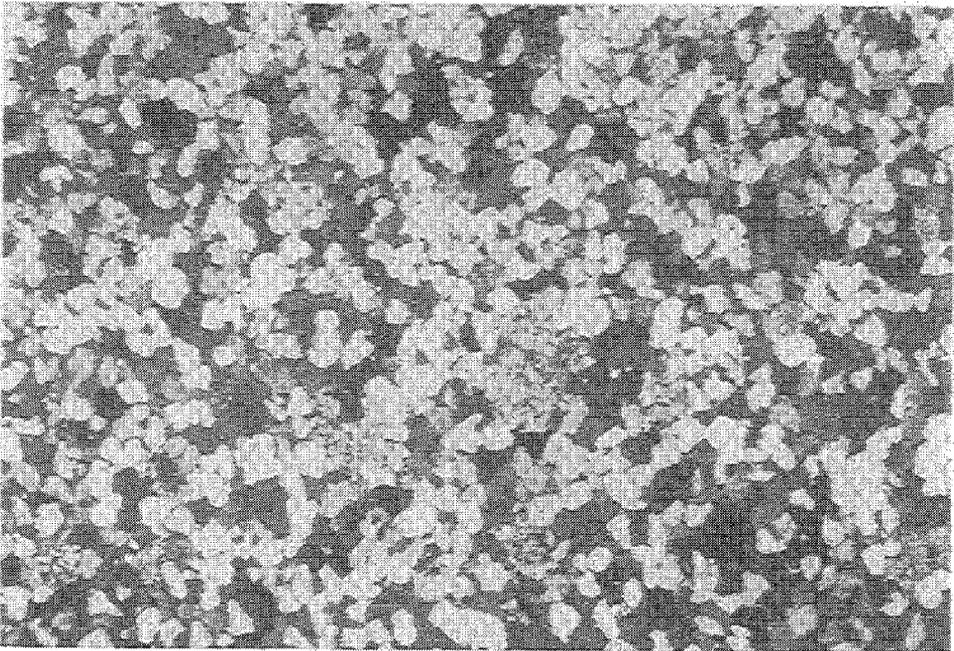


Plate 2b

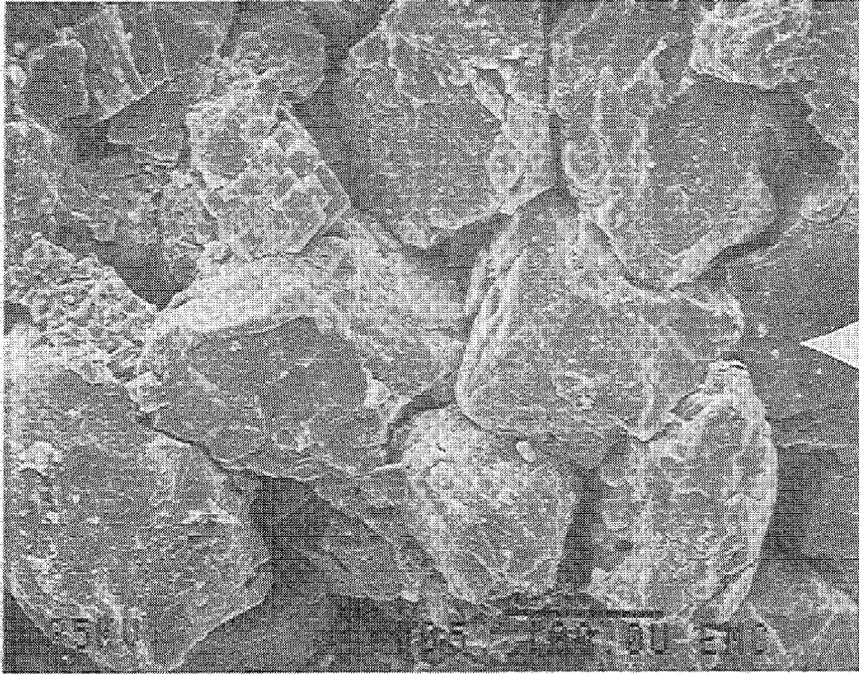


Plate 3a

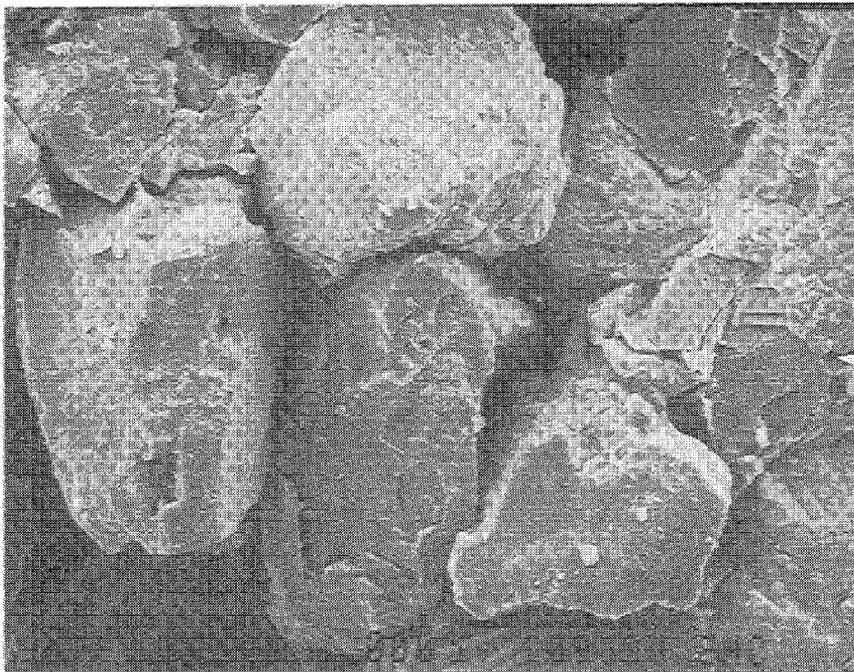


Plate 3b

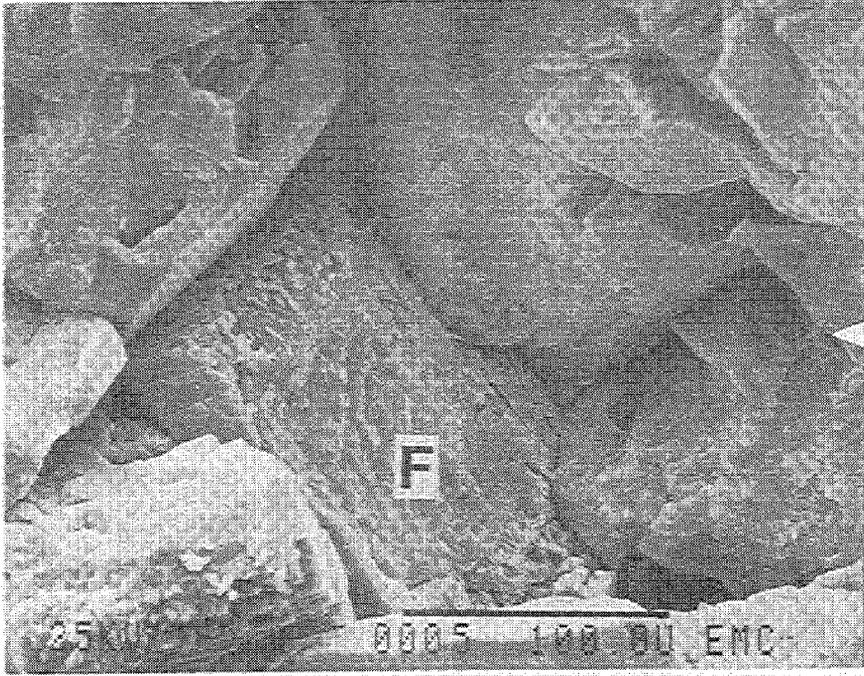


Plate 3c

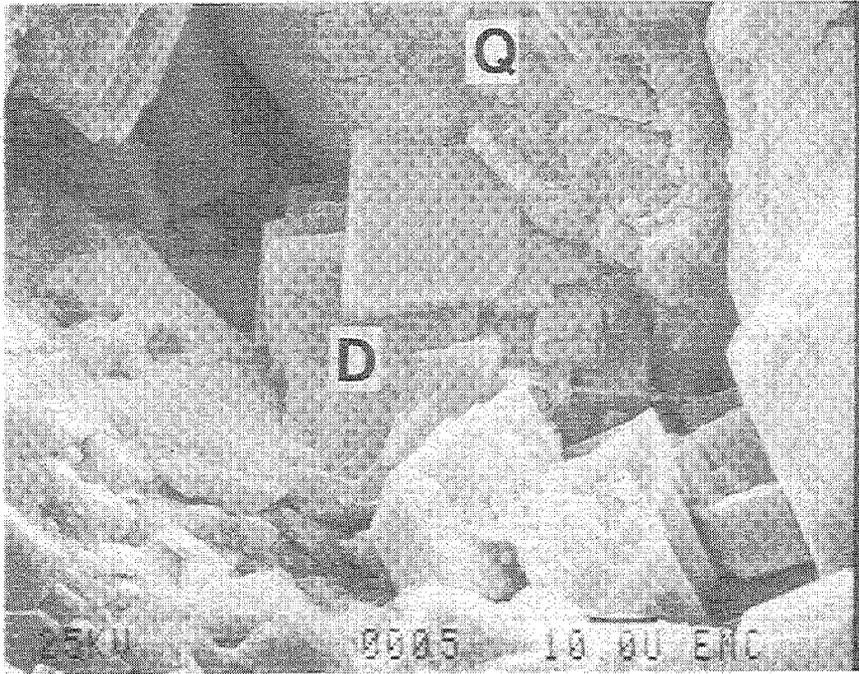
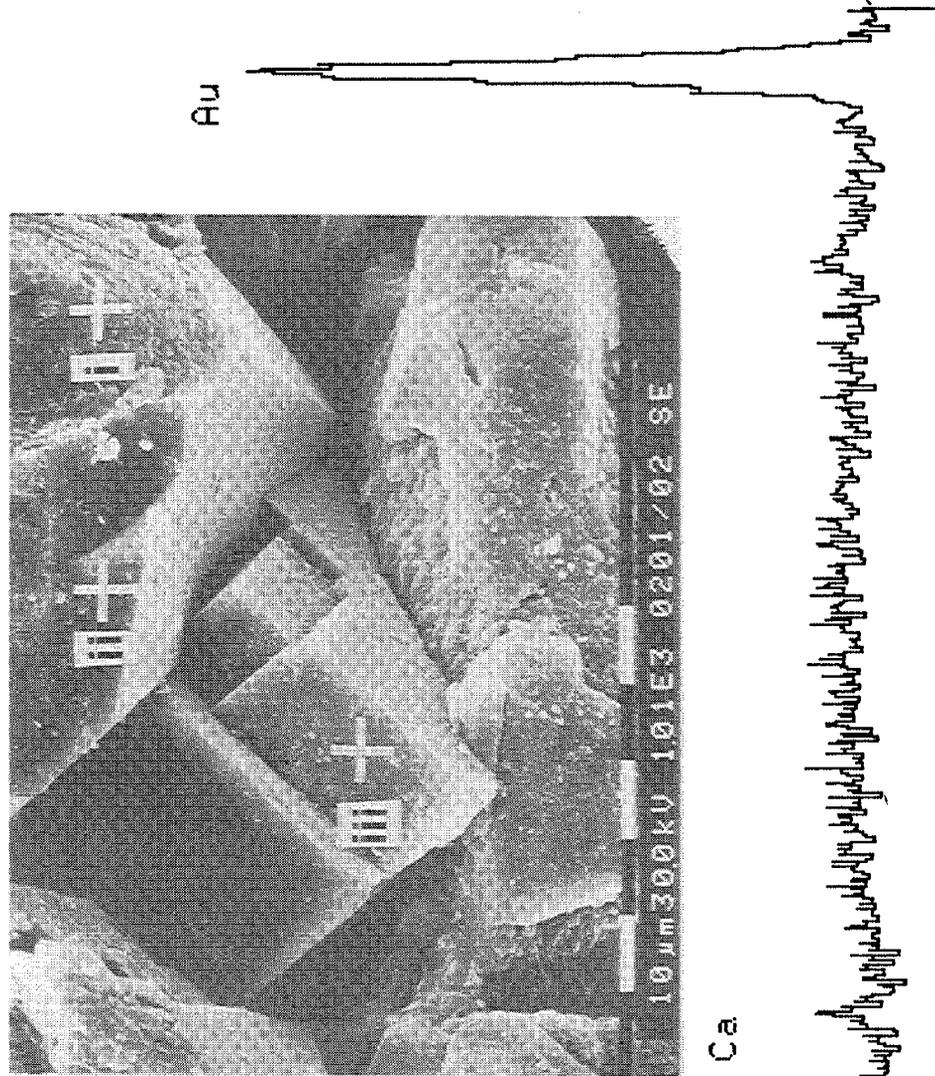


Plate 3d

13-Nov-1991 14:44:41

Quartz
Vert = 563 counts
Core No. 7
Preset = 200 secs
Elapsed = 113 secs

Disp = 1



← 0.080 Range = 10.230 keV
Integral 0 = 58968
11 12 13 14 15 16 17 18 19
10.110 →

Plate 4a1. Detrital quartz, SiO₂ grain.

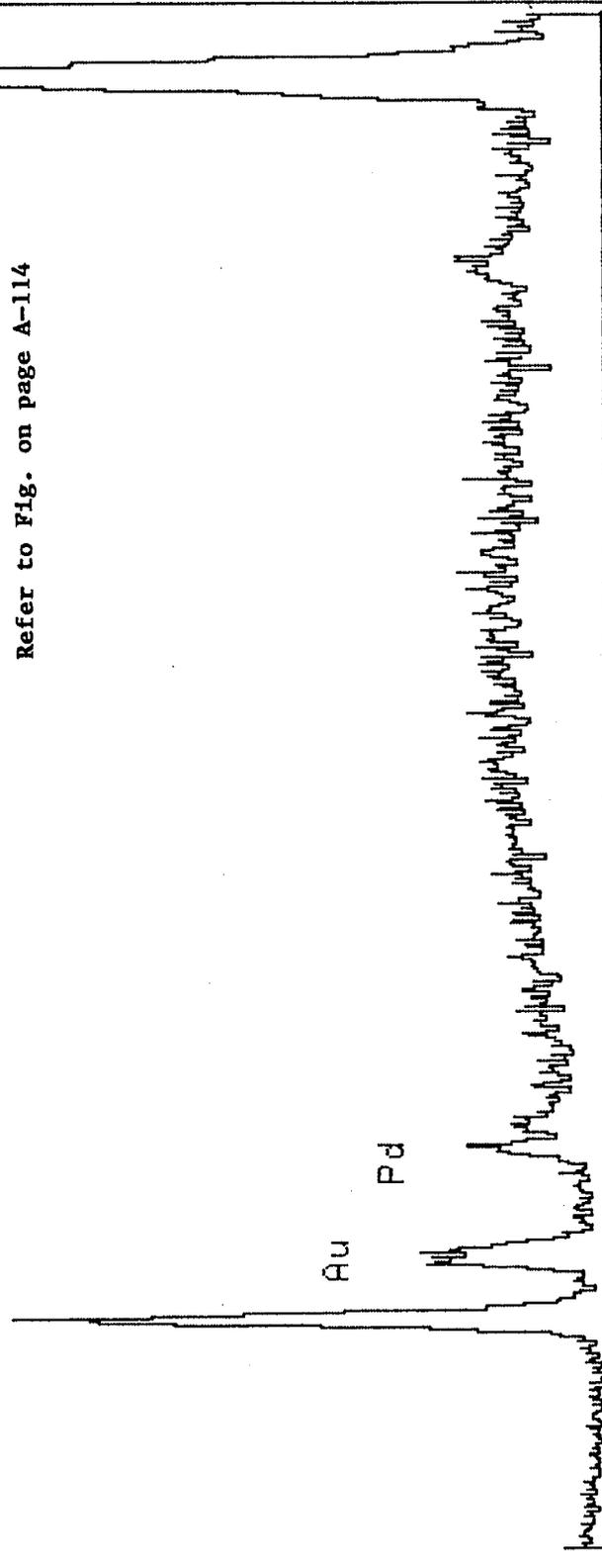
13-Nov-1991 14:53:16

-Quartz overgrow
Vert = 534 counts Disp = 1
Core No. 7

Preset = 200 secs
Elapsed = 100 secs

Au

Si



Refer to Fig. on page A-114

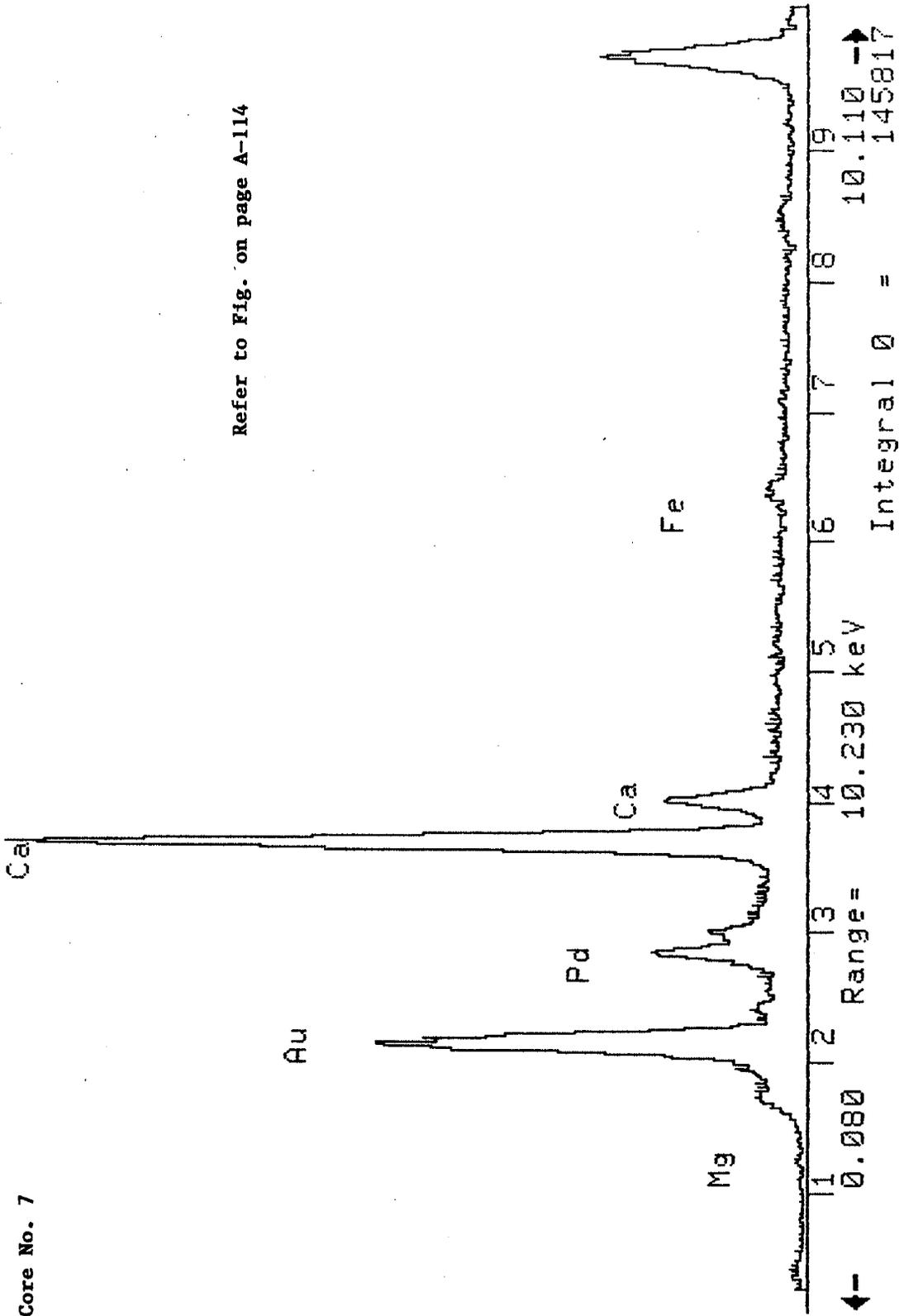
← 0.080 Range = 10.230 keV 10.110 →
Integral 0 = 55438

Plate 4a11 . Quartz overgrowth, SiO₂.

13-Nov-1991 14:49:17

-Dolomite
Vert= 2236 counts
Core No. 7
Preset= 200 secs
Elapsed= 102 secs

Disp= 1



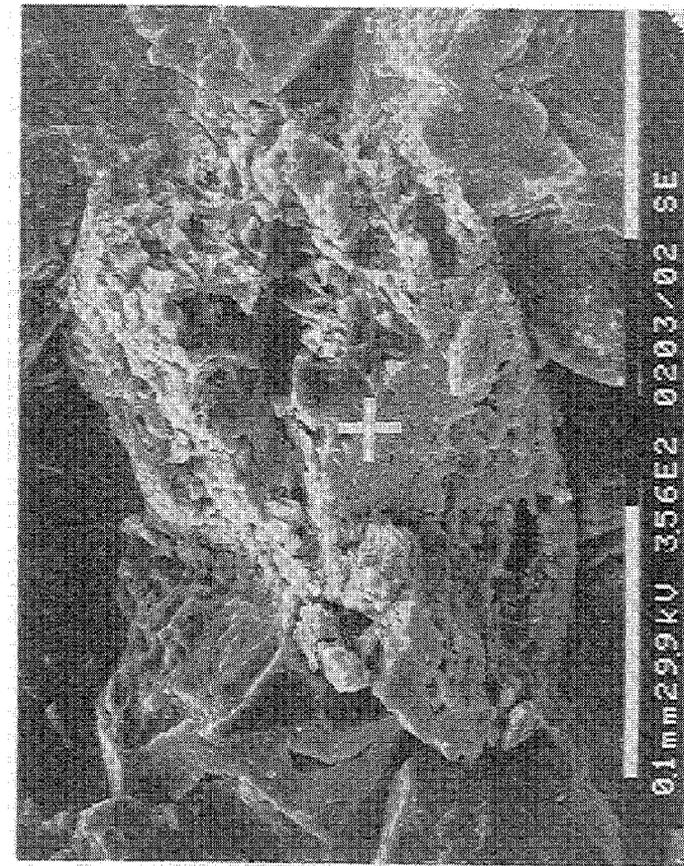
Refer to Fig. on page A-114

Plate 4a111 - An iron (Fe) rich pore-filling dolomite Ca,Mg(CO₃)₂.

13-Nov-1991 15:33:22

-Orthoclase Feldspar
vert= 856 counts Disp= 1
Quantex > K

Preset= 200 secs
Elapsed= 60 secs



Si Au

Core No. 7

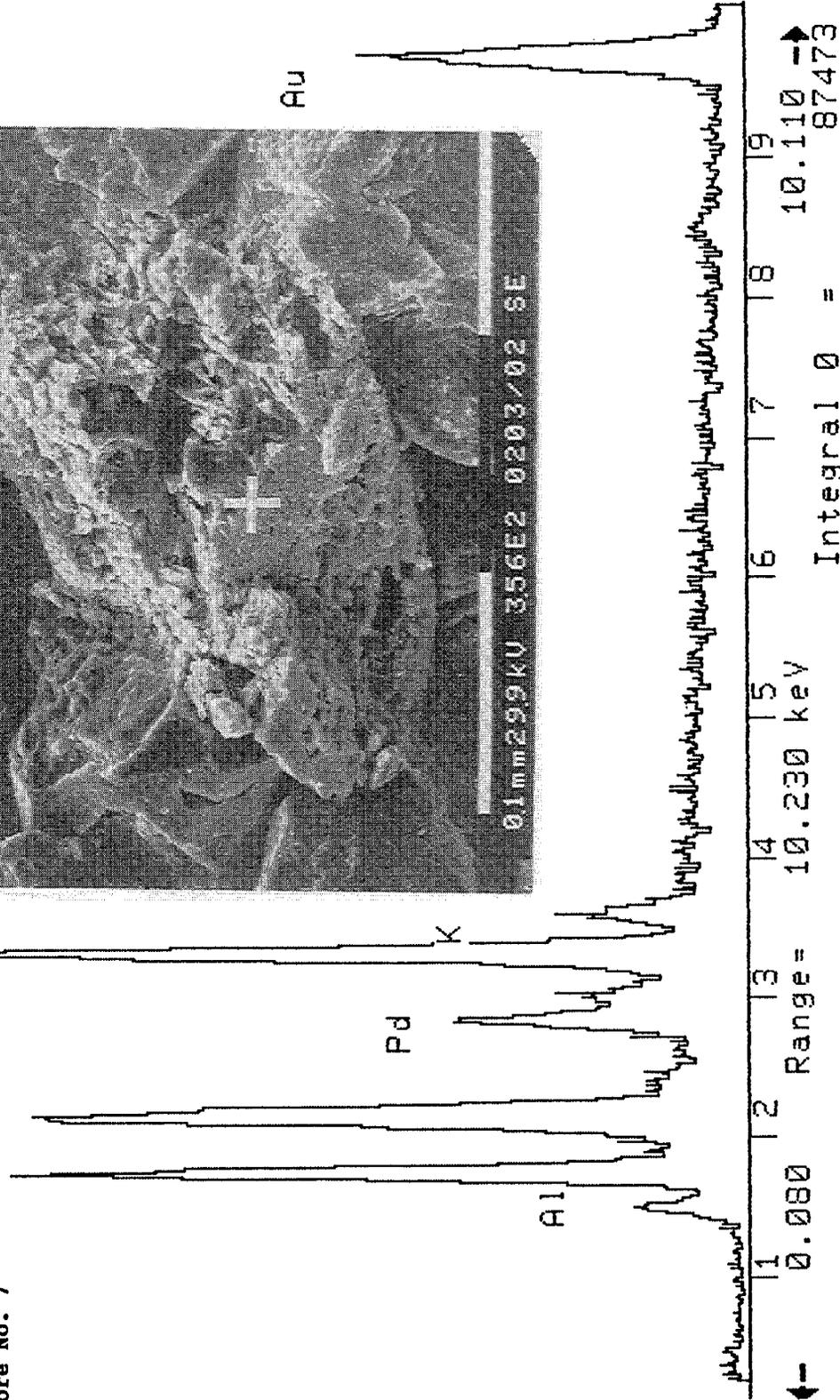


Plate 4b. Dissolved, K rich, orthoclase feldspar, KAlSi₃O₈.

13-Nov-1991 15:06:08

-Orthoclase Feldspar
Vert= 1090 counts Disp= 1

Preset=
Elapsed=

200 secs
99 secs

Core No. 7

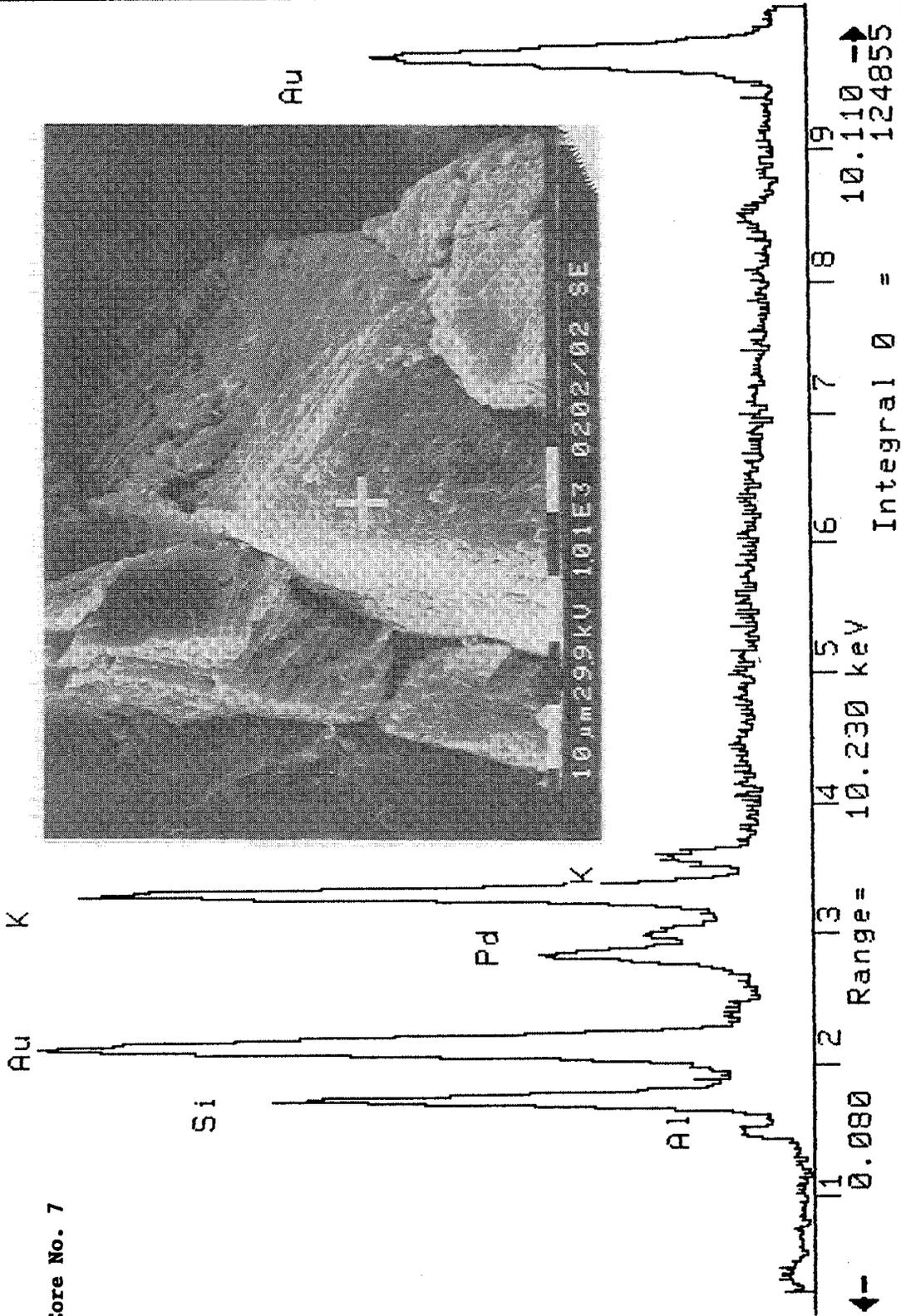


Plate 4 c. Dissolved, K rich, orthoclase, $KAlSi_3O_8$.

FN: AV5.NI
DATE: 08/19/91

ID: SANDSTONE
TIME: 10:00
PT: 0.225
STEP: 0.0300

SCINTAG/USA
WL: 1.54060

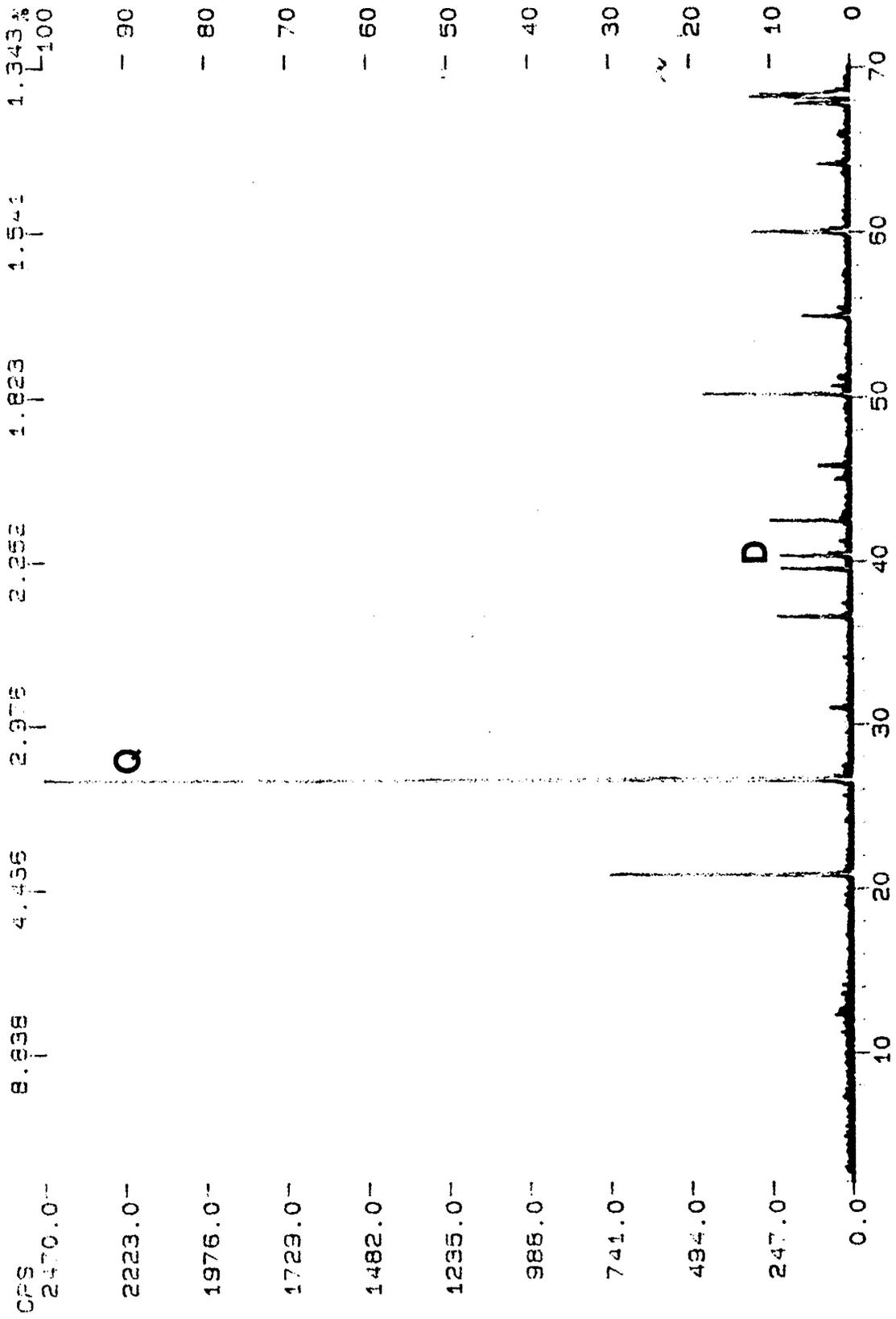


Plate 5a. Q: Quartz, D: Dolomite

FN: AV58.NI ID: SANDSTONE SCINTAG/USA
 DATE: 08/19/91 TIME: 13:08 PT: 0.225 STEP: 0.0300 ML: 1.54060

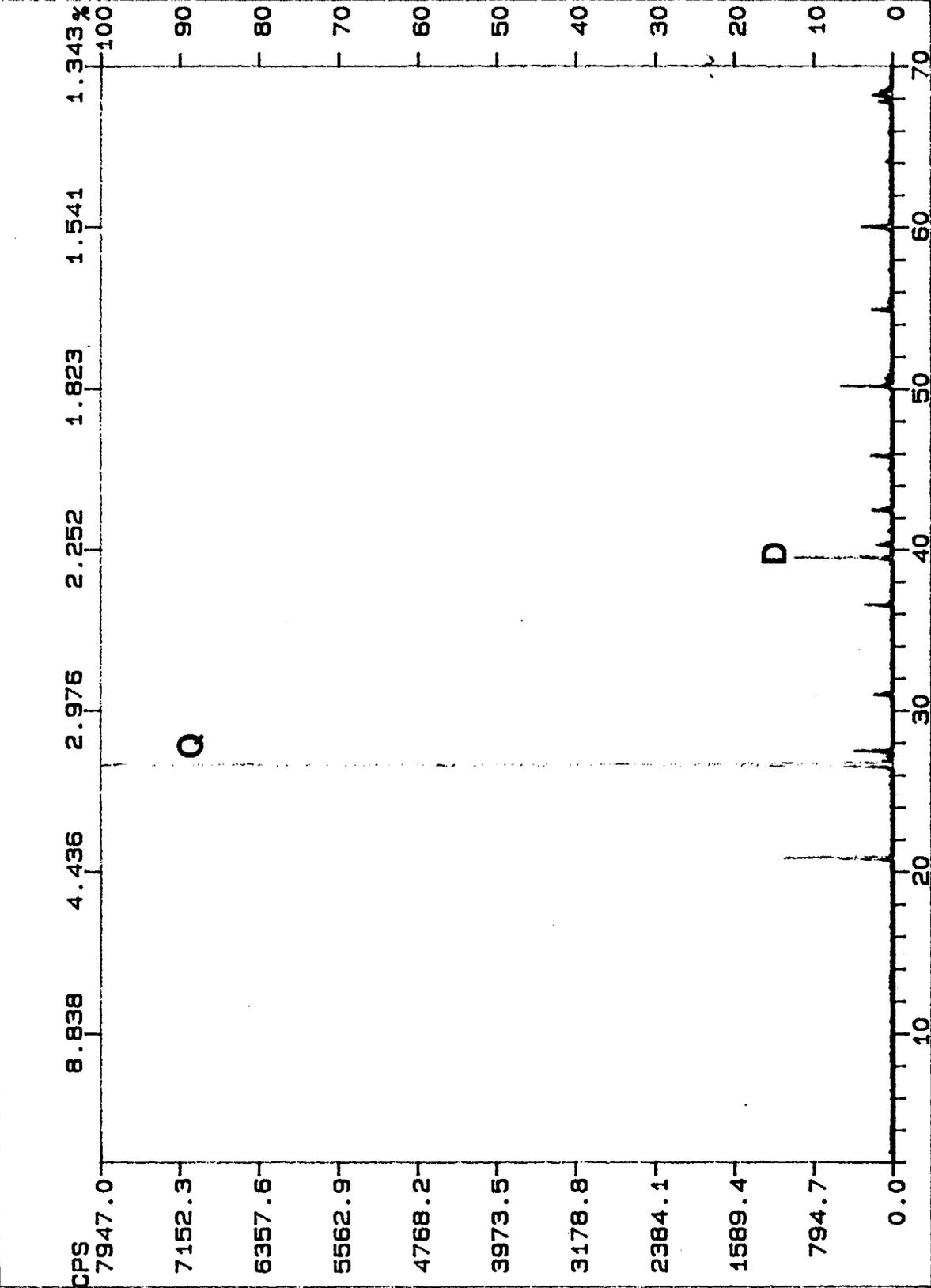


Plate 5b. Q: Quartz, D: Dolomite

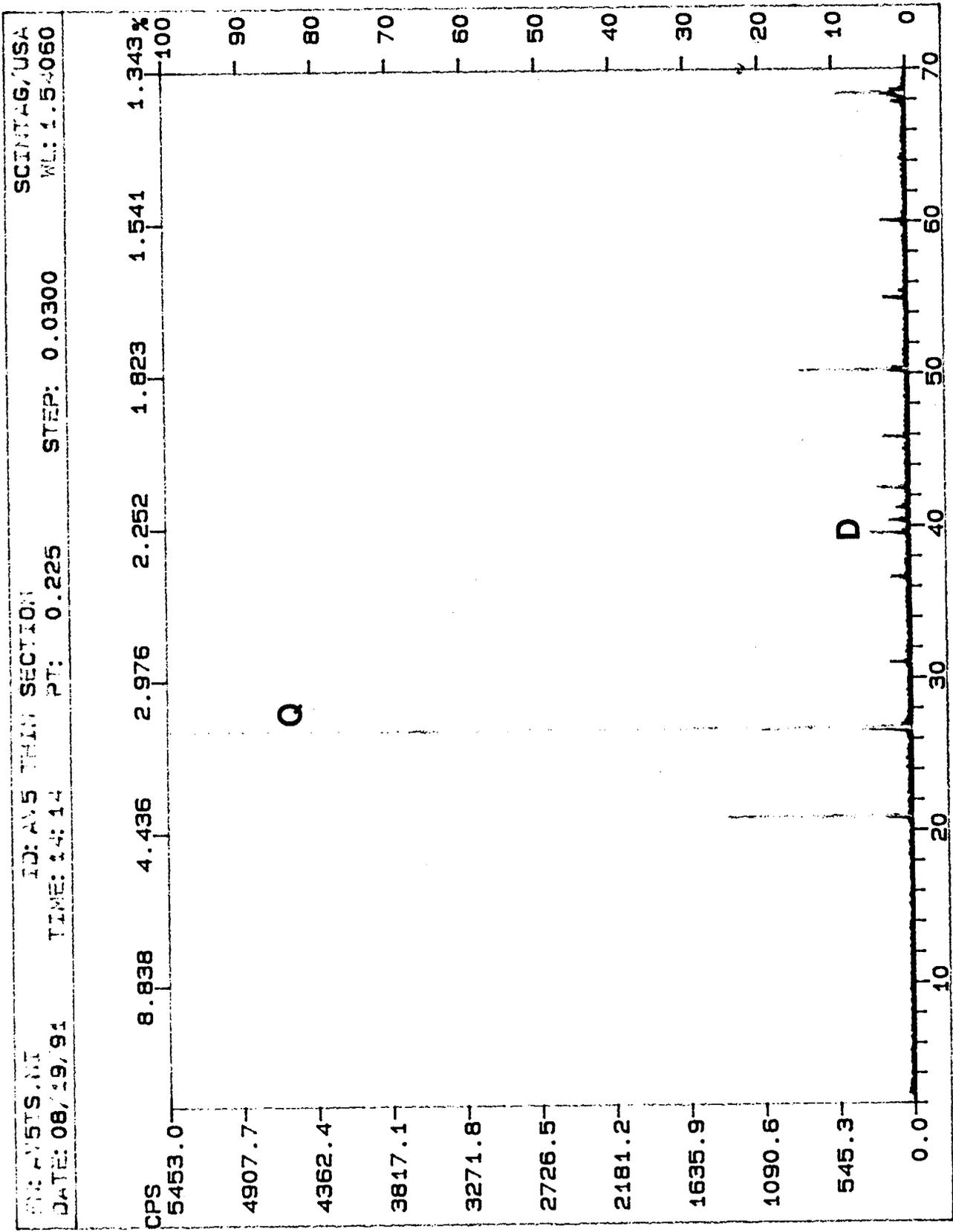


Plate 5c. Q: Quartz, D: Dolomite

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CULTURE NUMBER

7 DIGIT CODE

(Core)(O₂, no O₂)(Original Medium)(Isolation Medium)(Rep.)(Dil.*)(Tube No.*)

Core: No. of core

O₂, no O₂: O₂ = 1, no O₂ = 2

Original Medium:

TSA	= 1
Oil Agar	= 2
Peptone iron Agar	= 3
CH ₄ H ₂ - CO ₂	= 4
CH ₄ formate	= 5
NO ₃ broth	= 6
NO ₃ Red-HC util	= 7
SO ₄ Red-HC util	= 8
PCA	= 9

Isolation Medium: Same as original medium

Rep: from same original medium

Dil: Dilution, 1/2, 1/20, or 1/200

Tube No.: 1, 2, or 3

*Only used for cultures isolated from liquid media

ABBREVIATIONS

G(-)	: Gram Negative
G(+)	: Gram Positive
GV	: Gram Variable
AF	: Acid Fast
NAF	: Non-Acid Fast
CO	: Cocci
B	: Bacilli
Cap	: Capsule
NCap	: Non-Capsule
S	: Spore Former
NS	: Non-Spore Former
SG	: Surface Growth
MAF	: Microaerophile
FA	: Facultative Anaerobe
OA	: Obligate Anaerobe
M	: Motile
NM	: Non-Motile
PLEO	: Pleomorphic
FIL	: Filamentous
CSH	: Cell Surface Hydrophobicity

BACTERIAL FATTY ACIDS

1. undecanoic
2. 2-Hydroxydecanoic
3. dodecanoic
4. tridecanoic
5. 2-hydroxydodecanoic
6. 3-hydroxydodecanoic
7. tetradecanoic
8. 13-methyltetradecanoic
9. 12-methyltetradecanoic
10. pentadecanoic
11. 2-hydroxytetradecanoic
12. 3-hydroxytetradecanoic
13. 14-methylpentadecanoic
14. cis-9-hexadecenoic
15. hexadecanoic
16. 15-methylhexadecanoic
17. cis-9,10-methylenehexadecanoic
18. heptadecanoic
19. 2-hydroxyhexadecanoic
20. cis-9, 12-octadecadienoic
21. 9-octadecenoic
22. trans-9-octadecenoic &
cis-11-octadecenoic
23. octadecanoic
24. cis-9,10 methyleneoctadecanoic
25. nonadecanoic
26. eicosanoic

CULTURE

Core # 2 Referred to as Culture 1 in Task 1-4
 Culture # 32112
 Orig. Medium TSA

Cellular:

Size	2
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Rhizoid
Elevation	flat
Margin	fil.
Motility	No
Oxygen	OA

Solvents: Medium Acetate + NH₄

Acetylald.	
Ethanol	.15
Acetate	
Methanol	3.3
Acetone	.064
Butanol	
Benzene	
Toluene	
E. Acetate	4.6
Xylene	

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	3	4.5	1.59		.9657
	NH4	++	13.58	3.4	94.10		
	Urea	+	10.97	6.3	9.30		1.0620
Oil	NO3	+	3.13	6.9	4.89	-12.5	
	NH4	+	24.3	6.2	1.59	12.7	.8564
	Urea	+	2.3	7.9	10.40	41.6	
Hexadecane	NO3	+	5	6.7	0		
	NH4	+	.52	6.6	4.89		
	Urea	+	1.02	8.3	3.24		
Acetate	NO3	+	.5	8.3	5.99		
	NH4	+	25.5	6.9	1.59		
	Urea	+	16.2	8.9	0		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	.347
2		15	
3		16	
4	2.164	17	1.581
5		18	
6		19	
7		20	
8	.794	21	
9		22	.339
10		23	
11	.383	24	
12		25	1.678
13		26	

CULTURE

Core # 3
 Culture # 22113
 Orig. Medium TSA

Referred to as Culture 2 in Task 1-4

Cellular:

Size	1.5
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	entire

Motility	No
----------	----

Oxygen	FA
--------	----

Solvents:	NH ₄ + Acetate	NH ₄ + Molasses	NO ₃ + Oil
Acetylald.	.013		26.4
Ethanol	.44	.013	.003
Acetate		.029	
Methanol			
Acetone	.0027		
Butanol			
Benzene	2.6		
Toluene	.013		
E. Acetate	3.3		
Xylene			

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	70.5	4.6	2.69		
	NH4	+	18.41	3.6	165.69		.9961
	Urea	+	11.5	6.8	11.72		
Oil	NO3	+	3.1	5.5	36.83	20.4	.8581
	NH4	+	24.3	6.0	1.59	58.2	.8513
	Urea	+	1.02	8.2	21.41	38.2	
Hexadecane	NO3	+	3.3	6.6	9.30		
	NH4	+	.8	6.6	2.14		
	Urea	+	.5	8.4	1.59		
Acetate	NO3	+	3.13	8.4	1.59		
	NH4	+	27.2	6.7	13.70		
	Urea	+	1.31	8.5	1.59		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1	.170	14	
2		15	
3	.753	16	
4		17	
5	1.323	18	
6	2.477	19	
7		20	
8	1.601	21	
9	3.212	22	
10		23	
11	6.385	24	
12	1.668	25	
13	2.341	26	

CULTURE

Core # 2
 Culture # 32112
 Orig. Medium TSA

Referred to as Culture 3 in Task 1-4

Cellular:

Size	.5
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Convex
Margin	Fil.
Motility	No
Oxygen	MAF

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	5.00	6.3			
	NH4	+	4.18	4.8			
	Urea	+	1.80	7.0			
Oil	NO3	+	2.10	7.0	2.69	50.0	
	NH4	+	2.00	7.0	3.24	62.0	
	Urea	+	.50	7.0	3.24	34.7	
Hexadecane	NO3	+	.80	7.0			
	NH4	+	1.50	7.0			
	Urea	+	.80	7.0			
Acetate	NO3	+	1.30	7.0			
	NH4	+	.80	7.0			
	Urea	+	.50	7.0			

Bacterial Acid Methyl Esters

Media Oil + NO₃

Media Oil + NH₄

1		14	
2		15	
3	.657	16	
4		17	20.147
5	3.009	18	
6	2.691	19	5.111
7		20	
8	2.558	21	
9	11.129	22	
10		23	2.714
11		24	2.224
12	1.940	25	
13	.504	26	9.804

1		14	
2		15	
3	1.642	16	
4		17	9.230
5	5.076	18	
6	4.725	19	3.440
7		20	
8	.761	21	3.424
9	5.535	22	
10		23	3.890
11	14.864	24	3.126
12		25	-
13		26	3.083

CULTURE

Core # 2
 Culture # 32113
 Orig. Medium TSA

Referred to as Culture 4 in Task 1-4

Cellular:

Size	1-1.5
Shape	Pleo
Gram RXN	(v)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Raised
Margin	Undulate
Motility	Yes
Oxygen	MAF

Solvents:	Oil + NO ₃	Oil + Urea
Acetylald.	138	194.7
Ethanol	.004	.016
Acetate		
Methanol		
Acetone	631	
Butanol	352.7	507
Benzene		
Toluene	42.4	72.6
E. Acetate		.309
Xylene	3.04	

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	-					
	Urea	+	3.40	6.6	24.72		
Oil	NO3	+	1.57	7.2	5.99	25.7	
	NH4	+	1.04	7.0	3.79	25.7	.8867
	Urea	+	.50	8.0	99.61	29.0	
Hexadecane	NO3	+	2.09	6.6	0		
	NH4	+	.52	6.5	0		
	Urea	+	.80	8.3	24.72		
Acetate	NO3	+	15.50	8.3	2.69		
	NH4	+	2.09	6.8	1.59		
	Urea	+	1.3	8.8	0		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3	1.279	16	
4		17	17.996
5	4.537	18	
6		19	4.089
7		20	
8		21	
9		22	
10		23	3.208
11		24	3.148
12		25	
13		26	6.146

Media Oil + Urea

1	2.383	14	
2		15	
3		16	10.012
4		17	
5	2.582	18	2.545
6		19	
7	1.856	20	
8	6.002	21	
9		22	1.693
10	3.393	23	
11		24	2.420
12		25	
13		26	

Media Oil + NH₄

1		14	
2		15	
3	2.110	16	
4		17	
5	2.800	18	
6		19	2.813
7		20	
8		21	
9	2.178	22	
10		23	5.212
11	28.168	24	4.480
12	1.840	25	
13		26	

CULTURE

Core # 3
 Culture # 22193
 Orig. Medium TSA

Referred to as Culture 5 in Task 1-4

Cellular:

Size	2
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Flat
Margin	Curled
Motility	No
Oxygen	SG

Solvents: Medium Oil + NO₃

Acetylald.	13.7
Ethanol	.051
Acetate	
Methanol	
Acetone	
Butanol	
Benzene	
Toluene	
E. Acetate	
Xylene	.577

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	-					
	Urea	-					
Oil	NO3	+	2.09	6.2	8.19	30.7	.8919
	NH4	+	13.50	6.2	8.20	12.7	.8355
	Urea	+	2.30	8.3	3.79	38.2	
Hexadecane	NO3	-					
	NH4	+	3.90	6.3	12.60		
	Urea	-					
Acetate	NO3	+	5.0	8.6	1.59		
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NO₃

Media Oil + Urea

Media Oil + NH₄

1	.916	14	
2		15	
3		16	0.235
4		17	12.916
5	2.316	18	
6	5.701	19	3.632
7		20	
8	1.097	21	
9	8.000	22	
10		23	4.566
11	10.370	24	3.788
12	2.290	25	
13	2.383	26	3.895

1	2.020	14	
2		15	
3	1.623	16	22.040
4		17	
5	4.389	18	4.769
6		19	
7	2.208	20	
8		21	
9		22	2.834
10	5.382	23	
11		24	6.755
12		25	
13	1.204	26	

1		14	0.340
2		15	
3		16	
4	1.217	17	2.133
5		18	
6		19	
7		20	
8	0.799	21	
9		22	0.254
10		23	
11	0.357	24	0.247
12		25	0.997
13		26	

CULTURE

Core # 3
 Culture # 21293
 Orig. Medium Oil

Referred to as Culture 6 in Task 1-4

Oil
+
NO₃

Cellular:

Size	1
Shape	B
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	flat
Margin	lobate
Motility	No
Oxygen	MAF

Solvents: Medium Oil + NO₃

Acetylald.	15.5
Ethanol	.052
Acetate	
Methanol	
Acetone	
Butanol	
Benzene	
Toluene	
E. Acetate	
Xylene	.656

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	-					
	Urea	-					
Oil	NO3	+	1.31	6.3	8.20	12.7	.8647
	NH4	+	12.52	6.1	2.69	49.8	.8529
	Urea	-					
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NH₄

1	.650	14	
2		15	
3		16	
4		17	
5	2.495	18	7.467
6	3.264	19	2.753
7		20	
8	1.360	21	
9	4.511	22	
10		23	2.892
11	3.799	24	2.529
12		25	
13		26	3.071

CULTURE

Core # 2
 Culture # 32293
 Orig. Medium Oil

Referred to as Culture 7 in Task 1-4

Cellular:

Size	1
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Fl.
Elevation	flat
Margin	lobate
Motility	No
Oxygen	SG

Solvents: Medium Acetate + NH₄

Acetylald.	.003
Ethanol	.32
Acetate	2.80
Methanol	
Acetone	.43
Butanol	
Benzene	
Toluene	.019
E. Acetate	6.50
Xylene	2.60

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	13	4.5	2.69		
	NH4	-					
	Urea	-					
Oil	NO3	+	2.09	6.5	9.30	36.8	.8251
	NH4	+	24.29	5.4		32.2	
	Urea	+	2.00	8.0	40.14	32.2	
Hexadecane	NO3	+	2.00	6.6	15.90		
	NH4	+	2.00	6.3	3.79		
	Urea	+	1.00	8.0	1.59		
Acetate	NO3	+	11.30	8.7	1.59		
	NH4	+	15.00	6.8	0		
	Urea	-					

Bacterial Acid Methyl Esters

Media Mol + NO₃

Media Oil + NH₄

Media Oil + Urea

Media Oil + NO₃

1		14	
2		15	
3	1.437	16	1.829
4		17	6.757
5	1.351	18	
6	4.340	19	2.856
7		20	
8	1.899	21	.631
9	6.000	22	
10		23	3.264
11	7.581	24	2.820
12	2.454	25	
13	1.258	26	1.955

1		14	
2		15	
3		16	
4		17	6.327
5	1.343	18	
6	2.501	19	3.656
7		20	
8	1.818	21	2.305
9	3.155	22	
10		23	3.530
11	5.871	24	3.279
12	.467	25	
13	2.314	26	3.860

1		14	
2		15	
3	.763	16	.865
4		17	7.043
5	2.220	18	
6	4.909	19	3.452
7	.433	20	
8		21	.622
9	4.006	22	.579
10		23	4.707
11	10.089	24	4.016
12	1.304	25	
13	1.808	26	1.553

1		14	
2		15	
3	.666	16	
4		17	13.160
5	3.567	18	
6	5.498	19	3.403
7		20	
8	2.149	21	1.095
9	8.060	22	
10		23	4.394
11		24	3.892
12		25	
13	.930	26	5.307

CULTURE

Core # 2
 Culture # 32293
 Orig. Medium Oil

Referred to as Culture 8 in Task 1-4

Cellular:

Size	1.5
Shape	Pleo
Gram RXN	(v)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Fil.
Elevation	Convex
Margin	Undulate
Motility	Yes
Oxygen	FA

Solvents:	NH ₄ + Molasses	NO ₃ + Oil	Urea + Oil
Acetylald.			45.8
Ethanol	.0028	.018	.01
Acetate			
Methanol			
Acetone			
Butanol		140.4	116.2
Benzene			
Toluene			16.1
E. Acetate			
Xylene		.044	.011

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	6.5	5.0	7.10		
	NH4	+	21.19	3.6	59.96		.9769
	Urea	+	2.87	6.3	31.32		
Oil	NO3	+	1.83	6.2	7.10	39.6	
	NH4	+	24.04	6.3	4.89	36.8	
	Urea	+	2.30	7.3	11.50	38.2	
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3		16	
4		17	
5	4.012	18	
6	5.560	19	3.670
7		20	
8	1.956	21	1.434
9	6.090	22	.364
10		23	4.211
11	12.809	24	3.511
12		25	
13	1.692	26	2.589

Media Oil + Urea

1		14	
2		15	
3	1.660	16	
4		17	15.193
5	1.832	18	
6	4.973	19	3.206
7	2.611	20	
8		21	
9	10.618	22	
10		23	4.225
11	7.135	24	3.625
12	2.881	25	
13		26	8.073

Media Oil + NH₄

1		14	
2		15	
3		16	.131
4		17	
5	2.344	18	9.910
6		19	3.355
7		20	
8	2.181	21	.396
9	8.531	22	
10		23	4.074
11	5.232	24	
12		25	
13	1.341	26	

CULTURE

Core # 3
 Culture # 22293
 Orig. Medium Oil

Referred to as Culture 9 in Task 1-4

Cellular:

Size	1
Shape	Co
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	undulate
Motility	Yes
Oxygen	FA

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	55	4.5	3.79		
	NH4	-					
	Urea	+	17.3	6.4	25.82		
Oil	NO3	+	3.13	5.6	8.20	-4.96	.8882
	NH4	+	17.76	5.4	5.99	32.2	
	Urea	+	.52	8.0	53.35	35.3	
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NH₄

Media Oil + NO₃

1	.503	14	
2		15	
3	1.432	16	
4		17	
5	1.722	18	8.834
6		19	3.128
7		20	
8	1.186	21	1.369
9	1.823	22	
10		23	3.282
11	2.939	24	2.836
12	1.399	25	
13		26	2.947

1		14	
2		15	
3	.743	16	1.757
4		17	13.426
5	4.757	18	
6	4.932	19	2.040
7	.309	20	
8		21	
9	9.399	22	
10		23	3.290
11	17.845	24	2.903
12		25	-
13	1.446	26	8.706

CULTURE

Core # 3 Referred to as Culture 10 in Task 1-4
 Culture # 22442 1/200 D2
 Orig. Medium CH_4, H_2-CO_2

Cellular:

Size	.5
Shape	Co
Gram FXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Entire
Motility	No
Oxygen	O _A

Solvents:	NO ₃ + Oil	Urea + Oil
Acetylald.	88.3	21.2
Ethanol	.022	
Acetate		
Methanol		
Acetone	2206.1	287.2
Butanol	799.2	
Benzene		
Toluene		
E. Acetate		
Xylene	.311	1.16

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3						
	NH4						
	Urea						
Oil	NO3	+	1.31	6.2	28.02	35.3	.8763
	NH4	+	10	7.0	40.12	6.45	
	Urea	+	1.78	8.1	31.32	8.0	
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	1.707
2		15	
3	1.195	16	
4		17	16.943
5	4.261	18	
6	4.419	19	3.458
7	1.706	20	
8		21	
9	8.089	22	
10		23	3.619
11	17.266	24	2.748
12		25	
13		26	6.490

Media Oil + Urea

1	2.069	14	
2		15	
3		16	
4		17	10.049
5	1.657	18	
6	1.919	19	.737
7		20	
8	1.082	21	
9	5.432	22	
10		23	1.472
11	4.644	24	1.300
12	1.208	25	
13		26	3.713

CULTURE

Core # 4 Referred to as Culture 11 in Task 1-4
 Culture # 42442 1/200 D3
 Orig. Medium CH₄, H₂-CO₂

Cellular:

Size	1
Shape	Co
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Lobate
Motility	No
Oxygen	SG

Solvents:

	NO ₃ + Acetate	NH ₄ + Hexa	NO ₃ + Hexa	Urea + Molasses	Urea + Hexa
Acetylald.					
Ethanol	.37	.081	.090	.0035	.10
Acetate					
Methanol	5.50				
Acetone			.059	.0440	.06
Butanol					
Benzene	4.10				
Toluene					
E. Acetate	2.90				
Xylene					

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	+	.8	4.8	1.12		
	Urea	+	5.75	6.3	28.02		
Oil	NO3	+	1.04	6.3	4.89	36.8	.8554
	NH4	+	.50	6.2	4.89	42.3	.8829
	Urea	+	.50	8.7	126.70	12.7	.8519
Hexadecane	NO3	+	.78	6.6	0		
	NH4	+	1.83	6.5	0		
	Urea	+	1.04	8.0	1.59		
Acetate	NO3	+	16.46	7.1	3.79		
	NH4	+	6.01	7.0	3.79		
	Urea	+	.50	8.9	0		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3	.626	16	.393
4		17	6.373
5	4.182	18	
6	6.083	19	3.615
7		20	
8	.706	21	1.089
9	5.902	22	
10		23	4.759
11	14.019	24	4.367
12		25	
13	2.072	26	2.076

Media Oil + Urea

1		14	
2		15	
3		16	6.318
4		17	
5	4.002	18	2.389
6		19	
7	.524	20	.753
8	3.553	21	
9		22	2.473
10	8.820	23	
11		24	1.543
12	.865	25	
13		26	

Media Oil + NH₄

1		14	
2		15	
3	.626	16	1.57
4		17	1.193
5	2.141	18	2.692
6	2.805	19	2.474
7		20	
8	1.978	21	2.086
9	2.445	22	.940
10		23	2.838
11	2.415	24	2.197
12	1.714	25	
13	2.423	26	3.506

CULTURE

Core #

4

Referred to as Culture 12 in Task 1-4

Culture #

42492 1/2 D1

Orig. Medium

CH₄, H₂-CO₂

Cellular:

Size	1
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used

PCA

Form	Irregular
Elevation	Flat
Margin	Lobate
Motility	No
Oxygen	FA

Solvents: Medium Oil + Urea

Acetylald.	117.1
Ethanol	.006
Acetate	
Methanol	
Acetone	23793
Butanol	468.2
Benzene	
Toluene	10.7
E. Acetate	
Xylene	.231

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	70.50	4.5	2.69		
	NH4	-					
	Urea	+	18.50	6.6	14.81		.9794
Oil	NO3	+	3.10	8.5	1.59	12.7	.90704
	NH4	+	14.00	6.2	2.69	38.2	
	Urea	+	2.30	8.2	177	52.0	.85337
Hexadecane	NO3	+	3.50	7.0	0		
	NH4	+	3.90	6.4	0		
	Urea	+	.80	8.3	0		
Acetate	NO3	+	2.30	8.8	17.01		
	NH4	+	17.00	6.8	28.02		
	Urea	+	2.35	8.5	8.20		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3		16	.941
4		17	3.712
5	2.389	18	4.588
6	4.393	19	3.975
7		20	
8	1.255	21	1.127
9	3.772	22	.457
10		23	3.650
11	10.455	24	3.096
12	1.128	25	
13	.609	26	

Media Hex. + Ures

1		14	
2		15	
3	.777	16	1.021
4		17	4.779
5	.736	18	
6	1.960	19	1.792
7		20	
8	.848	21	.690
9	3.082	22	
10		23	1.858
11	6.638	24	1.641
12	.937	25	
13	1.110	26	1.694

Media Oil + NH₄

1		14	16.776
2		15	
3	.554	16	
4		17	11.792
5	3.363	18	
6		19	4.175
7	.713	20	
8		21	
9	5.983	22	
10		23	3.191
11	13.903	24	2.517
12		25	
13	1.295	26	4.092

Media Oil + Urea

1		14	1.234
2		15	2.935
3	.479	16	3.136
4	1.277	17	12.130
5	2.446	18	
6	3.940	19	5.211
7		20	
8	2.098	21	2.362
9	4.313	22	1.234
10		23	3.214
11	9.670	24	2.772
12	2.158	25	
13	1.724	26	1.116

CULTURE

Core # 4 Referred to as Culture 13 in Task 1-4
 Culture # 42592 1/200 D3
 Orig. Medium CH₄ formate

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Undulate
Motility	Yes
Oxygen	MAF

Solvents:

	NO ₃ + Oil	Urea + Oil
Acetylald.	29.0	37.7
Ethanol	.034	.009
Acetate		
Methanol		
Acetone		735.6
Butanol		237.6
Benzene		
Toluene		36.5
E. Acetate		
Xylene	.024	.018

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	45	4.4	5.99		.9649
	NH4	+	23	3.2	36.83		.
	Urea	+	17	6.3	22.51		
Oil	NO3	+	1.31	6.2	3.79	32.2	.8786
	NH4	+	3.40	6.1	1.59	14.7	.8418
	Urea	+	.50	8.7	3.79	55.2	.86425
Hexadecane	NO3	+	3.00	7.0	0		
	NH4	-	1.00	7.0	20.31		
	Urea	+	.80	8.2	0		
Acetate	NO3	+	9.93	7.0	3.79		
	NH4	+	2.61	6.9	0		
	Urea	+	4.80	8.7	0		

Bacterial Acid Methyl Esters

Media Oil + NO₃

Media Oil + NH₄

1		14	
2		15	
3		16	
4		17	21.805
5	5.635	18	
6		19	3.927
7	1.291	20	
8		21	
9	10.468	22	
10		23	2.759
11	4.276	24	2.575
12		25	
13	1.829	26	11.658

1		14	
2		15	
3	.830	16	
4		17	4.150
5	1.114	18	
6	3.847	19	3.139
7		20	
8	.667	21	
9	6.536	22	
10		23	3.375
11	3.191	24	2.845
12	1.643	25	
13		26	3.329

CULTURE

Core # 4 Referred to as Culture 14 in Task 1-4
 Culture # 42552 1/2D1
 Orig. Medium CH₄ formate

Cellular:

Size	.75
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	entire
Motility	No
Oxygen	MAF

Solvents:

	Oil + NO ₃	Oil + Urea
Acetylald.		
Ethanol		.016
Acetate		
Methanol		
Acetone	128	135.60
Butanol		
Benzene		
Toluene		
E. Acetate	.016	
Xylene	.454	.435

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	55.02	4.4	17.01		
	NH4	+	43.90	3.2	7.10		
	Urea	+	21.40	6.5	22.51		
Oil	NO3	+	1.83	6.0	2.69	36.80	.8505
	NH4	+	24.30	5.9	3.79	38.20	
	Urea	+	2.30	8.1	2.14	30.70	.86344
Hexadecane	NO3	+	4.50	6.5	0		
	NH4	+	3.00	6.6	0		
	Urea	+	.80	8.2	1.59		
Acetate	NO3	+	11.50	8.2	3.79		
	NH4	+	6.00	6.8	1.59		
	Urea	+	11.50	8.8	0		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3		16	
4	.464	17	12.685
5	1.439	18	
6	2.317	19	3.966
7		20	
8	2.587	21	2.910
9	6.350	22	
10		23	3.565
11	7.641	24	3.562
12		25	
13		26	7.616

Media Oil + Urea

1	.933	14	
2		15	
3	4.303	16	12.946
4		17	
5	3.792	18	1.680
6		19	2.287
7	1.479	20	
8		21	
9		22	2.230
10	11.750	23	
11	2.996	24	6.287
12	1.459	25	
13		26	

Media Oil + NH₄

1		14	
2		15	
3	2.517	16	8.632
4		17	
5		18	6.021
6	.661	19	
7		20	2.504
8	7.111	21	
9		22	
10	3.381	23	5.000
11	1.350	24	4.290
12		25	
13		26	5.763

CULTURE

Core # 5 Referred to as Culture 15 in Task 1-4
Culture # 62592 1/2D3
Orig. Medium CH₄ formate

Cellular:

Size	1
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	No
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Undulate
Motility	No
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	+	4.96	4.8			
	Urea	-					
Oil	NO3	+	1.31	7		50.3	
	NH4	-					
	Urea	-					
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

CULTURE

Core # 3 Referred to as Culture 16 in Task 1-4
 Culture # 22593 1/2ooD3
 Orig. Medium CH₄ formate

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Puntiform
Elevation	Convex
Margin	fil.
Motility	No
Oxygen	MAF

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	39.5	5.7	46.74		
	NH4	+	11	3.3	88.59		
	Urea						
Oil	NO3	+	3.1	6.0	20.31	38.2	
	NH4						
	Urea						
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

CULTURE

Core # 3 Referred to as Culture 17 in Task 1-4
 Culture # 22592 1/200D1
 Orig. Medium CH₄, formate

Cellular:

Size	1.5
Shape	B
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Convex
Margin	entire
Motility	Yes
Oxygen	FA

Solvents: Medium Oil + NO₃

Acetylald.	18.5
Ethanol	
Acetate	
Methanol	
Acetone	
Butanol	
Benzene	
Toluene	
E. Acetate	
Xylene	.892

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3						
	NH4						
	Urea						
Oil	NO3	+	2.5	6.2	3.79	22.2	.8863
	NH4						
	Urea						
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

CULTURE

Core # 4
 Culture # 42662 ³/₂D1
 Orig. Medium NO₃ Broth

Referred to as Culture 18 in Task 1-4

Cellular:

Size	1
Shape	Co
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	entire
Motility	No
Oxygen	SG

Solvents:	Acetate + NH ₄	Oil + Urea
Acetylald.		0.006
Ethanol	.18	
Acetate		
Methanol		
Acetone	.0097	123.1
Butanol	8.6	
Benzene		
Toluene	.39	
E. Acetate	2.5	
Xylene	2.9	0.447

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	0.78	4.4			
	NH4	+	42.31	3.3			0.9793
	Urea	-					
Oil	NO3	+	1.83	6.2	75.38	12.7	0.8948
	NH4	+	0.5	6.0	26.92	36.8	0.8400
	Urea	+	0.5	7.9	33.53	29.0	0.8451
Hexadecane	NO3	+	1.57	6.7	8.20		
	NH4	+	1.83	6.3	40.12		
	Urea	+	1.83	8.2	28.02		
Acetate	NO3	+	0.5	8.8	5.99		
	NH4	+	1.3	6.7	17.01		
	Urea	+	0.5	8.1	2.69		

Bacterial Acid Methyl Esters

Media Oil + NH₄

1		14	0.229
2		15	
3		16	0.445
4		17	9.694
5	4.447	18	
6	5.002	19	3.045
7	1.905	20	
8		21	
9	6.203	22	0.665
10		23	4.308
11	15.079	24	3.680
12	1.468	25	
13		26	3.321

Media Oil + Urea

1		14	0.200
2	0.541	15	
3	1.500	16	
4	1.295	17	9.984
5	2.281	18	
6	1.710	19	3.681
7		20	
8	2.012	21	1.137
9	7.478	22	
10		23	4.953
11	14.574	24	4.149
12		25	
13		26	4.766

Media Oil + NO₃

1	0.550	14	
2		15	2.883
3	0.450	16	1.106
4		17	
5	1.403	18	6.857
6		19	3.281
7		20	
8	2.023	21	2.328
9	4.031	22	
10		23	3.298
11	7.820	24	3.100
12	1.839	25	
13	2.429	26	2.438

CULTURE

Core # 4 Referred to as Culture 19 in Task 1-4
 Culture # 42663 1/20 D3
 Orig. Medium NO₃ Broth

Cellular:		Colonial: Medium used PCA	
Size	1.5	Form	Circular
Shape	Co	Elevation	Convex
Gram RXN	(+)	Margin	entire
Acid Fast	No		
Spore	Yes	Motility	No
Capsule	Yes		
Storage	No	Oxygen	MAF

Solvents:	Urea + Acetate	NH ₄ + Acetate	NH ₄ + Hex.	NO ₃ + Oil
Acetylald.	2.5			24.8
Ethanol	.36		.096	.015
Acetate		3.9	3.100	
Methanol	2.5	13.5		
Acetone	.079	.046	.055	428.2
Butanol				
Benzene				
Toluene		.013		
E. Acetate		5.100		
Xylene	3.0			.003

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	15.50	5.2	10.40		
	NH4	+	19.07	3.8	39.03		
	Urea	+	21.40	7.0	9.30		
Oil	NO3	+	1.50	6.1	36.83	29.0	.8604
	NH4	+	24.30	5.8	1.59	29.0	
	Urea	+	1.50	8.0	2.14	33.8	
Hexadecane	NO3	+	5.00	6.6	5.99		
	NH4	+	3.96	6.1	8.20		
	Urea	+	1.00	8.0	20.31		
Acetate	NO3	+	14.00	7.0	4.89		
	NH4	+	27.20	6.8	5.99		
	Urea	+	16.02	8.7	15.91		

Bacterial Acid Methyl Esters

Media Oil + NH₄

1	1.043	14	
2		15	
3		16	
4		17	
5	4.928	18	
6	5.073	19	4.250
7	1.846	20	
8		21	
9	9.176	22	
10		23	3.740
11	4.619	24	3.219
12	2.105	25	
13		26	5.552

Media Oil + Urea

1		14	
2		15	
3	1.007	16	0.796
4		17	
5	4.268	18	
6	6.729	19	2.188
7	4.873	20	3.287
8	2.491	21	
9	5.770	22	
10		23	3.947
11	3.993	24	3.267
12	5.911	25	
13	3.837	26	3.054

Media Oil + NO₃

1	1.073	14	
2		15	
3	0.916	16	
4		17	
5	1.928	18	11.068
6	3.247	19	2.858
7	1.608	20	
8	1.264	21	
9	2.378	22	
10		23	2.439
11	9.314	24	2.106
12	1.502	25	
13	0.848	26	2.910

CULTURE

Core # 5 Referred to as Culture 20 in Task I-4
 Culture # 62662 1/200D3
 Orig. Medium NO₃ Broth

Cellular:

Size	1
Shape	B
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Flat
Margin	Entire
Motility	Yes
Oxygen	MAF

Solvents:	Acetate	Hexadecane	Oil
	+ NH ₄	+ NH ₄	+ NO ₃
Acetylald.			
Ethanol	.33	.034	
Acetate			
Methanol			
Acetone			
Butanol			
Benzene			
Toluene			
E. Acetate	3.4		
Xylene	2.9		.469

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	70.52	4.1	23.62		
	NH4	+	18.02	3.6	23.62		
	Urea	+	3.92	4.5	64.36		
Oil	NO3	+	.5	5.5	35.73	36.8	.9771
	NH4	+	1.3	5.8	20.31	60.2	.8826
	Urea	-					
Hexadecane	NO3	+	.78	6.6	12.60		
	NH4	+	3.4	6.2	6.55		
	Urea	+	.8	8.2	13.70		
Acetate	NO3	+	.78	7.1	13.70		
	NH4	+	5.2	6.7	15.91		
	Urea	+	.5	8.6	17.01		

Bacterial Acid Methyl Esters

Media Mol. + NH₄

1		14	
2		15	
3	.817	16	2.750
4		17	13.062
5	2.023	18	
6	3.300	19	3.019
7		20	
8	1.816	21	.626
9	6.187	22	
10		23	2.638
11	11.042	24	2.124
12	2.394	25	
13		26	3.128

CULTURE

Core # 4 Referred to as Culture 21 in Task 1-4
 Culture # 42332 300D2
 Orig. Medium peptone iron

Cellular:

Size	.75
Shape	B
Gram RXN	(v)
Acid Fast	No
Spore	Yes
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Rhizoid
Elevation	Umbonate
Margin	Lobate
Motility	Yes
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	1.31	4.4	75.38		.9624
	NH4	+	14.63	3.1	138.15	29.0	
	Urea	+	1.57	6.3	64.36		
Oil	NO3	+	.52	5.9	83.08	36.8	.8972
	NH4	+	.78	5.9	14.81	38.2	
	Urea	+	.78	7.9	80.33	55.2	.87081
Hexadecane	NO3	+	1.57	7.0	30.22		
	NH4	+	.52	6.5	8.22		
	Urea	+	1.3	8.2	17.00		
Acetate	NO3	+	1.5	7.1	52.25		
	NH4	+	3.40	6.8	41.24		
	Urea	+	.50	8.7	17.01		

Bacterial Acid Methyl Esters

Media Oil + NO₃

1		14	
2		15	
3	.3096	16	
4		17	3.2056
5	1.6387	18	
6	2.2367	19	1.1845
7		20	
8	.5605	21	.4357
9	3.0786	22	
10		23	1.5479
11	5.7537	24	1.5788
12	1.0871	25	
13	1.8910	26	1.2991

CULTURE

Core # 2 Referred to as Culture 22 in Task 1-4
 Culture # 32772 1/20 D3
 Orig. Medium NO₂RED-HC UTIL.

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Convex
Margin	entire
Motility	No
Oxygen	MAF

Solvents:	Oil + NO ₃	Oil + Urea
Acetylald.	27.5	29.7
Ethanol	.001	.006
Acetate	110.5	
Methanol		
Acetone		190.3
Butanol		
Benzene		
Toluene		10.4
E. Acetate		
Xylene		

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	+	16.19	3.2	154.67		
	Urea	+	1.83	6.4	75.38		
Oil	NO3	+	1.31	7.2	8.20	12.7	.907
	NH4	+	.52	6.0	1.59	38.2	.870
	Urea	+	.52	7.9	83.09	32.2	.872
Hexadecane	NO3	+	1.83	6.5	0		
	NH4	+	.5	6.3	0		
	Urea	+	1	8.1	28.02		
Acetate	NO3	+	.52	7.1	2.69		
	NH4	+	2.61	6.7	0		
	Urea	+	.5	8.7	0		

Bacterial Acid Methyl Esters

Media Oil + Urea

1		14	
2		15	.527
3	.429	16	.552
4	65.095	17	
5	.846	18	5.216
6	.694	19	.840
7	.991	20	
8		21	.418
9	4.513	22	
10		23	1.245
11	2.606	24	1.015
12	1.199	25	
13	.756	26	3.879

Media Oil + NH₄

1		14	
2		15	
3		16	
4		17	12.730
5	3.573	18	
6	5.099	19	2.424
7		20	
8	1.605	21	1.034
9	6.520	22	
10		23	3.600
11	18.424	24	2.850
12	2.057	25	
13	1.419	26	4.630

CULTURE

Core # 1 Referred to as Culture 23 in Task 1-4
 Culture # 12692 $\frac{1}{2}$ DI
 Orig. Medium NO₃ Broth

Cellular:

Size	.5
Shape	B
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Lobate
Motility	No
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	8	6.1	4.89		
	NH4						
	Urea						
Oil	NO3	+	2.31	6.1	2.69	33.8	
	NH4						
	Urea						
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

CULTURE

Core # 5 Referred to as Culture 24 in Task 1-4
Culture # 62492 $\frac{1}{2}$ D2
Orig. Medium CH₄, H₂, CO₂

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Entire
Motility	No
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	51.72	6.3	111.72		
	NH4	+	43.9	3.8	1.12		
	Urea	+	2.09	6.4	28.02		
Oil	NO3	+	.52	6.2	28.02	55.2	
	NH4	+	.78	5.8	13.70	30.7	
	Urea	+	.52	8.2	4.34	30.7	
Hexadecane	NO3	+	.52	7.0	28.02		
	NH4	+	1.04	6.4	1.59		
	Urea	+	3.40	8.1	2.69		
Acetate	NO3	+	2.09	7.9	4.89		
	NH4	+	4.70	6.7	2.69		
	Urea	+	3.40	7.2	0		

CULTURE

Core # 4 Referred to as Culture 25 in Task 1-4
 Culture # 42662 3D2
 Orig. Medium NO₃Broth

Cellular:

Size	1.5
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	raised
Margin	entire
Motility	Yes
Oxygen	MAF

Solvents: Medium NH₄ + Acetate

Acetylald.	.055
Ethanol	.39
Acetate	-
Methanol	-
Acetone	.064
Butanol	-
Benzene	-
Toluene	-
E. Acetate	4.8
Xylene	-

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	4.70	4.3	31.32		
	NH4	+	38.14	3.4	19.21		
	Urea	+	1.78	6.4	9.3		.9876
Oil	NO3	+	3.13	6.0	90.80	-12.5	.86095
	NH4	+	1.62	5.9	40.14	33.8	
	Urea	+	.52	7.5	53.35	12.7	
Hexadecane	NO3	+	2.09	6.7	50.29		
	NH4	+	1.83	6.9	0		
	Urea	+	.8	8.1	13.70		
Acetate	NO3	+	3.13	7.8	1.59		
	NH4	+	4.44	6.6	4.34		
	Urea	+	.78	8.4	1.59		

Bacterial Acid Methyl Esters

Media Mol. + NH₄

1		14	1.018
2		15	
3	.528	16	.802
4		17	4.383
5	1.428	18	
6	2.592	19	3.736
7		20	
8	1.799	21	.964
9	3.366	22	
10		23	3.168
11	5.376	24	3.121
12	1.479	25	
13	2.466	26	4.096

Media Oil + NH₄

1		14	
2		15	
3		16	
4		17	11.985
5	4.908	18	
6	5.373	19	4.563
7	2.147	20	
8		21	
9	7.655	22	
10		23	4.472
11	2.542	24	3.898
12		25	-
13		26	7.762

CULTURE

Referred to as Culture 26 in Task 1-4

Core # 2
 Culture # 32983
 Orig. Medium PCA

Cellular:

Size	1
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Puntiform
Elevation	Convex
Margin	entire
Motility	Yes
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	22	5.5	9.30		
	NH4	+	43.9	3.5	66.57		
	Urea	-					
Oil	NO3	+	3.1	6.4	44.54	55.24	
	NH4	+	11.5	6.9	52.25	49.8	
	Urea	-					
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	+	12	6.8	4.89		
	NH4	-					
	Urea	-					

CULTURE

Core # 3 Referred to as Culture 27 in Task 1-4
 Culture # 22893 1/2 D1
 Orig. Medium SO₄ Red-HC Util.

Cellular:

Size	1
Shape	B
Gram RXN	(+)
Acid Fast	NO
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	convex
Margin	entire
Motility	No
Oxygen	SG

Solvents: Medium Oil + NO₃

Acetylald.	28.2
Ethanol	.012
Acetate	
Methanol	
Acetone	256.8
Butanol	
Benzene	
Toluene	4.6
E. Acetate	
Xylene	1.57

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3						
	NH4						
	Urea						
Oil	NO3	+	3.1	6.1	89.59	.24	
	NH4	+	24.3	5.8	40.14	33.80	
	Urea						
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

Bacterial Acid Methyl Esters

Media Oil + NH₄

1		14
2		15
3	1.136	16
4		17
5	2.232	18
6	6.309	19 4.647
7		20
8	1.066	21
9	4.245	22
10		23 5.023
11	20.221	24 3.935
12	1.258	25
13	1.551	26 3.610

CULTURE

Core # 2 Referred to as Culture 28 in Task 1-4
 Culture # 22582 1/200 D1
 Orig. Medium CH₄ formate

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Pulvinate
Margin	Undulate
Motility	Yes
Oxygen	FA

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	4.18	6.3			
	NH4	+	6.01	4.5			
	Urea						
Oil	NO3	+	.3	6.8	14.81	27.4	
	NH4	+	.5	7.0	26.92	49.8	
	Urea	+	1.3	7.4	20.31	12.7	
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

CULTURE

Core # 1 Referred to as Culture 29 in Task 1-4
 Culture # 12792 $\frac{1}{200}$ D2
 Orig. Medium NO₃ Red-HC utilizer

Cellular:

Size	2
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Flat
Margin	entire
Motility	No
Oxygen	MAF

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	1.83	6	2.14		
	NH4						
	Urea						
Oil	NO3	+	1.83	7.1	53.35	12.7	
	NH4	+	1.31	5.9	40.12	4.6	
	Urea						
Hexadecane	NO3						
	NH4						
	Urea						
Acetate	NO3						
	NH4						
	Urea						

CULTURE

Core # 1
 Culture # 12692 1/2D2
 Orig. Medium NO₃ Broth

Referred to as Culture 30 in Task 1-4

Cellular:

Size	1.5
Shape	Pleo
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Convex
Margin	Entire
Motility	No
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	13	5.8	1.59		
	NH4	+	8	4.2	1.12		
	Urea	-					
Oil	NO3	-					
	NH4	-					
	Urea	-					
Hexadecane	NO3	+	2.5	6.5	10.40		
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

CULTURE

Core # 2
 Culture # 32992
 Orig. Medium PCA

Referred to as Culture 31 in Task 1-4

Cellular:

Size	2.5
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	irregular
Elevation	raised
Margin	lobate
Motility	yes
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	-					
	NH4	+	19	4.7	4.89	2.04	
	Urea	-					
Oil	NO3	+	3.1	6.8	1.59	30.7	.8331
	NH4	-					
	Urea	-					
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NH₄

Media Oil + NO₃

1		14	
2		15	
3	1.013	16	
4		17	
5	2.525	18	
6	3.855	19	3.562
7		20	
8	2.571	21	
9	7.532	22	
10		23	2.954
11	7.501	24	2.476
12		25	
13	2.280	26	4.547

1		14	
2		15	
3	1.094	16	
4		17	14.687
5	4.972	18	
6		19	3.761
7		20	
8		21	
9	4.590	22	
10		23	4.341
11	15.937	24	3.692
12	3.409	25	-
13		26	3.036

CULTURE

Core # 5 Referred to as Culture 32 in Task 1-4
 Culture # 62892 1/200 D2
 Orig. Medium SO₄ Red-HC Util.

Cellular:

Size	1.5
Shape	B
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Undulate
Motility	Yes
Oxygen	SG

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	.52	4.5	20.31		
	NH4	+	6.79	3.4	111.72		
	Urea	-					
Oil	NO3	+	3.1	6.2	91.90	12.7	
	NH4	-					
	Urea	-					
Hexadecane	NO3	-					
	NH4	-					
	Urea	-					
Acetate	NO3	-					
	NH4	-					
	Urea	-					

Bacterial Acid Methyl Esters

Media Oil + NH₄

1	1.023	14	
2		15	
3		16	
4		17	13.651
5	3.090	18	
6		19	3.228
7	1.215	20	
8		21	1.083
9	9.877	22	
10		23	.112
11	7.136	24	.116
12	2.172	25	
13		26	.279

Media Oil + Urea

1	.436	14	.131
2		15	
3	3.695	16	
4		17	
5	4.751	18	
6	4.940	19	2.362
7	1.836	20	3.925
8		21	1.072
9	5.921	22	
10		23	4.391
11	3.100	24	3.893
12	1.871	25	
13	3.219	26	3.963

CULTURE

Core # 2
 Culture # 32993
 Orig. Medium PCA

Referred to as Culture 33 in Task 1-4

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Fil
Elevation	Flat
Margin	Fil
Motility	Yes
Oxygen	MAF

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	55.6	4.6	13.70		
	NH4	-					
	Urea	+	11.5	7.0	13.70		
Oil	NO3	+	2.5	6.8	31.32	30.7	.8595
	NH4	+	15.3	6.0	2.69	27.4	
	Urea	+	.8	8.2	165.69	35.3	
Hexadecane	NO3	+	.5	7.0	44.54		
	NH4	+	.8	7.0	40.12		
	Urea	+	1.2	7.0	28.02		
Acetate	NO3	+	10.5	7.0	3.79		
	NH4	+	27.2	6.8	0		
	Urea	+	24.2	6.5	17.01		

Bacterial Acid Methyl Esters

Media Oil + Urea

Media Oil + NO₃

1	.276	14	
2		15	
3		16	5.666
4		17	
5	2.527	18	4.638
6		19	
7	1.798	20	2.103
8	2.958	21	1.183
9		22	3.087
10	4.705	23	
11	2.447	24	2.526
12	1.997	25	
13		26	

1		14	
2		15	
3	.836	16	
4		17	7.182
5	5.226	18	
6	6.139	19	6.113
7		20	
8	1.251	21	1.891
9	3.367	22	
10		23	4.945
11	8.254	24	3.984
12	1.548	25	
13	1.659	26	2.435

CULTURE

Core # 4 Referred to as Culture 34 in Task 1-4
 Culture # 42693 1/2D1
 Orig. Medium NO₃ Broth

Cellular:

Size	.5
Shape	CO
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Flat
Margin	Entire
Motility	No
Oxygen	MAF

Solvents:	Acetate + NH ₄	Hexa + Urea	Hexa + NO ₃	Molasses + NH ₄	Oil + Urea
Acetylald.					
Ethanol	.34	.091	.084	.014	.016
Acetate	9.3				151.2
Methanol					
Acetone	.004	.046	.055	.037	
Butanol		7.1			
Benzene					
Toluene	.074				
E. Acetate					
Xylene					

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	55.5	4.5	4.89		
	NH4	+	43.9	3.4	3.79		
	Urea	+	18	6.5	15.9		
Oil	NO3	+	2.1	6.0	30.22	29.0	
	NH4	+	22	6.0	1.59	53.6	
	Urea	+	1.57	7.4	2.14	55.2	
Hexadecane	NO3	+	5	6.8	8.20		
	NH4	+	3.9	6.4	4.34		
	Urea	+	1.04	7.5	0		
Acetate	NO3	+	16.4	8.8	23.62		
	NH4	+	12.02	6.6	31.32		
	Urea	+	11.5	8.5	13.70		

Bacterial Acid Methyl Esters

Media Oil + NH₄

1	14	
2	15	
3	16	
4	17	9.415
5	18	5.489
6	19	6.405
7	20	
8	21	2.234
9	22	8.037
10	23	6.109
11	24	2.891
12	25	5.233
13	26	3.397

Media Oil + Urea

1	.568	14	
2	.828	15	2.153
3	4.906	16	
4	3.873	17	7.690
5	3.868	18	
6	1.700	19	4.331
7		20	
8	.466	21	
9	3.772	22	
10		23	3.787
11		24	3.196
12	1.269	25	2.047
13	1.973	26	2.724

Media Oil + NO₃

1		14	
2		15	
3	5.810	16	5.539
4		17	2.316
5	2.904	18	
6	4.272	19	2.970
7		20	5.067
8	.504	21	
9	3.893	22	
10		23	4.770
11	2.080	24	4.809
12	3.512	25	
13	2.145	26	4.608

CULTURE

Core #

4

Referred to as Culture 35 in Task 1-4

Culture #

42692 1/2 D1

Orig. Medium

NO₃ Broth

Cellular:

Size	1.5
Shape	B
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Puntiform
Elevation	Convex
Margin	Lobate
Motility	Yes
Oxygen	OA

Solvents:

	Urea + Acetate	Urea + Oil
Acetylald.	.026	17.5
Ethanol	.375	
Acetate		
Methanol	5.3	9.2
Acetone	.038	239.5
Butanol		
Benzene		
Toluene	.01	
E. Acetate	3.5	
Xylene		

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	69	4.5	2.69		
	NH4	+	40.3	3.1	1.12		
	Urea	+	19	5.9	18.11		
Oil	NO3	+	2.5	6.0	1.59	18.5	.8828
	NH4	+	13	5.9	1.59	30.7	
	Urea	+	2.35	7.9	12.6	36.8	
Hexadecane	NO3	+	5	6.8	3.79		
	NH4	+	2.5	6.1	0		
	Urea	+	.8	7.9	2.69		
Acetate	NO3	+	11.5	8.2	2.14		
	NH4	+	6	6.8	3.79		
	Urea	+	16.2	8.1	14.81		

Bacterial Acid Methyl Esters

Media Hex. + NH₄

1		14	
2		15	
3	.249	16	.137
4		17	1.065
5	.803	18	
6	1.714	19	1.013
7		20	
8	.355	21	.415
9	.822	22	
10		23	1.546
11	3.226	24	1.214
12	.357	25	
13	.516	26	.155

Media Oil + Urea

1		14	
2		15	3.717
3	.896	16	15.845
4		17	
5	3.634	18	2.820
6		19	
7	1.900	20	
8	7.597	21	
9		22	
10	2.056	23	
11	2.600	24	5.921
12	1.550	25	
13		26	

Media Oil + NO₃

1		14	
2		15	
3	5.907	16	
4		17	7.702
5	3.795	18	
6	4.608	19	3.010
7		20	
8	1.407	21	
9	4.053	22	
10		23	4.495
11	3.283	24	4.186
12	2.386	25	
13	1.556	26	3.710

Media Oil + NH₄

1		14	
2		15	
3	.982	16	
4		17	
5	5.022	18	4.761
6		19	
7		20	2.957
8	3.254	21	4.009
9		22	1.495
10	15.047	23	5.253
11	1.163	24	4.153
12	1.417	25	
13		26	1.650

CULTURE

Core # 2
 Culture # 32992
 Orig. Medium PCA

Referred to as Culture 36 in Task 1-4

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Flat
Margin	undulate
Motility	Yes
Oxygen	FA

Solvents: Medium Oil + NO₃

Acetylald.	40.5
Ethanol	.02
Acetate	
Methanol	
Acetone	
Butanol	
Benzene	
Toluene	13.9
E. Acetate	
Xylene	.025

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	70.5	4.7	37.93		
	NH4	+	43.9	3.6	12.60		
	Urea	+	21.4	5.9	28.02		
Oil	NO3	+	3.1	6.5	14.81	30.7	.8729
	NH4	+	22.3	6.0	4.89	49.8	
	Urea	+	2.3	8.2	5.99	20.4	
Hexadecane	NO3	+	5.0	6.7	9.3		
	NH4	+	3.9	6.4	1.59		
	Urea	+	.8	8.1	4.89		
Acetate	NO3	+	11.5	7.2	1.59		
	NH4	+	27.2	6.7	0		
	Urea	+	5.2	8.7	0		

CULTURE

Core # 2
 Culture # 32993
 Orig. Medium PCA

Referred to as Culture 37 in Task 1-4

Cellular:

Size	.5
Shape	Co
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	rhizoid
Elevation	Pulvinate
Margin	entire
Motility	Yes
Oxygen	MAF

Physiology:

		Growth	Gas	Acid	Emulsifier	CSH	Polymers
Molasses	NO3	+	11.04	4.7	34.63		
	NH4	+	21.16	3.7	12.6		
	Urea	+	7.05	6.4	48.95		
Oil	NO3	+	2.35	7.1	51.15	29.0	.8563
	NH4	+	24.03	6.1	61.06	50.3	.8809
	Urea	+	1.04	7.7	9.30	36.8	
Hexadecane	NO3	+	2.87	6.4	5.99		
	NH4	+	1.31	6.8	1.59		
	Urea	+	1	8.0	1.59		
Acetate	NO3	+	1.83	8.2	25.82		
	NH4	+	3.92	6.6	2.14		
	Urea	+	.52	8.3	0		

Bacterial Acid Methyl Esters

Media Oil + NH₄

1		14	
2		15	
3		16	
4		17	12.280
5	3.435	18	
6	5.212	19	
7		20	
8	2.239	21	
9	9.381	22	
10		23	4.096
11	15.230	24	3.345
12	1.755	25	
13	1.852	26	3.725

Media Oil + Urea

1		14	
2		15	
3		16	7.397
4		17	1.350
5	6.966	18	3.579
6	.227	19	
7		20	1.192
8	1.873	21	
9		22	4.405
10	11.071	23	
11	2.791	24	
12	2.230	25	
13		26	

Media Oil + NO₃

1		14	1.679
2		15	2.871
3		16	1.248
4		17	3.440
5	1.026	18	
6	2.546	19	3.476
7		20	
8	1.461	21	1.420
9	3.020	22	
10		23	3.141
11	2.330	24	2.940
12	1.	25	
13	2.361	26	3.345

Media Mol + NH₄

1		14	
2		15	
3		16	21.913
4		17	
5	3.241	18	3.790
6		19	
7		20	1.704
8	11.413	21	
9		22	
10	23.067	23	2.778
11		24	2.489
12		25	
13		26	8.023

CULTURE

Core # 3
Culture # 22112
Orig. Medium TSA

Cellular:

Size, μm	.75
Shape	Bacilli
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Filamentous
Elevation	Convex
Margin	Filamentous

Motility	Yes
----------	-----

Oxygen	OA
--------	----

CULTURE

Core # 3
Culture # 22222
Orig. Medium Oil

Cellular:

Size, μm	↓.5
Shape	Pleo
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	OA
--------	----

CULTURE

Core # 2
Culture # 21293
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Filamentous

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22292
Orig. Medium Oil

Cellular:

Size, μm	1.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Filamentous

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 5
Culture # 62593 1/20 D1
Orig. Medium CH₄ formate

Cellular:

Size, μm	.75
Shape	Bacilli
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Erose

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22111
Orig. Medium Oil Agar

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Curled

Motility	Yes
----------	-----

Oxygen	OA
--------	----

CULTURE

Core # 3
Culture # 21294
Orig. Medium Oil

Cellular:

Size, μm	1.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Rhizoid
Elevation	Flat
Margin	Filamentous

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32112
Orig. Medium TSA

Cellular:

Size, μm	2
Shape	Bacilli
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Filamentous
Elevation	Umbonate
Margin	Curled

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 2
Culture # 32123
Orig. Medium TSA

Cellular:

Size, μm	1.5
Shape	Bacilli
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Rhizoid
Elevation	Raised
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32122
Orig. Medium TSA

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Pulvinate
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32193
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Bacilli
Gram RXN	(-)
Acid Fast	No
Spore	Yes
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Entire

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22113
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Entire

Motility	Yes
----------	-----

Oxygen	OA
--------	----

CULTURE

Core # 2
Culture # 32223
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Flat
Margin	Curled

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22123
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Pulvinate
Margin	Entire

Motility	Yes
----------	-----

Oxygen	SG
--------	----

CULTURE

Core # 2
Culture # 32223
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Flat
Margin	Filamentous

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22223
Orig. Medium Oil

Cellular:

Size, μm	.75
Shape	Bacilli
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Raised
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32223
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Flat
Margin	Lobate

Motility	No
----------	----

Oxygen	OA
--------	----

CULTURE

Core # 2
Culture # 32293
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Bacilli
Gram RXN	(v)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Convex
Margin	Entire

Motility	Yes
----------	-----

Oxygen	FA
--------	----

CULTURE

Core # 3
Culture # 22224
Orig. Medium Oil

Cellular:

Size, μm	1.5
Shape	Bacilli
Gram RXN	(-)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	OA
--------	----

CULTURE

Core # 2
Culture # 32223
Orig. Medium Oil

Cellular:

Size, μm	$\downarrow .5$
Shape	Cocci
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Convex
Margin	Undulate

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 2
Culture # 32222
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Undulate

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22223
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Flat
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22223
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Pulvinate
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22114
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Filamentous
Elevation	Raised
Margin	Filamentous

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 2
Culture # 33112
Orig. Medium TSA

Cellular:

Size, μm	1.5
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Filamentous
Elevation	Flat
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	SG
--------	----

CULTURE

Core # 5
Culture # 61593 1/20 D1
Orig. Medium CH₄ formate

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Pulvinate
Margin	Entire

Motility	Yes
----------	-----

Oxygen	FA
--------	----

CULTURE

Core # 2
Culture # 32222
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Convex
Margin	Filamentous

Motility	Yes
----------	-----

Oxygen	FA
--------	----

CULTURE

Core # 2
Culture # 32222
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	FA
--------	----

CULTURE

Core # 3
Culture # 22113
Orig. Medium TSA

Cellular:

Size, μm	1.5
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Rhizoid
Elevation	Umbonate
Margin	Filamentous

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 3
Culture # 22292
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Bacilli
Gram RXN	(-)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Flat
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22222
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(v)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Spindle
Elevation	Convex
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 21294
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Undulate

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 21294
Orig. Medium Oil

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Spindle
Elevation	Flat
Margin	Entire

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32993
Orig. Medium PCA

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Umbonate
Margin	Erose

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 1
Culture # 12692 1/2 D1
Orig. Medium NO₃ Broth

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	No
Storage	Yes

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 21293
Orig. Medium Oil

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	FA
--------	----

CULTURE

Core # 3
Culture # 22224
Orig. Medium Oil

Cellular:

Size, μm	.75
Shape	Bacilli
Gram RXN	(+)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Flat
Margin	Entire

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 2
Culture # 32113
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(v)
Acid Fast	No
Spore	Yes
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Filamentous
Elevation	Convex
Margin	Filamentous

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 2
Culture # 32113
Orig. Medium TSA

Cellular:

Size, μm	.5
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Irregular
Elevation	Flat
Margin	Curled

Motility	No
----------	----

Oxygen	FA
--------	----

CULTURE

Core # 2
Culture # 31992
Orig. Medium PCA

Cellular:

Size, μm	1.0
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Filamentous
Elevation	Convex
Margin	Undulate

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 3
Culture # 22293
Orig. Medium Oil

Cellular:

Size, μm	.75
Shape	Pleo
Gram RXN	(+)
Acid Fast	Yes
Spore	No
Capsule	No
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Flat
Margin	Erose

Motility	Yes
----------	-----

Oxygen	SG
--------	----

CULTURE

Core # 4
Culture # 42229
Orig. Medium Oil

Cellular:

Size, μm	1.0
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Flat
Margin	Entire

Motility	No
----------	----

Oxygen	SG
--------	----

CULTURE

Core # 3
Culture # 22192
Orig. Medium Oil

Cellular:

Size, μm	2
Shape	Bacilli
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Filamentous
Elevation	Umbonate
Margin	Filamentous

Motility	No
----------	----

Oxygen	FA
--------	----

CULTURE

Core # 5
Culture # 62443 1/20 D1
Orig. Medium CH₄ H₂-CO₂

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Filamentous
Elevation	Umbonate
Margin	Filamentous

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 5
Culture # 61593 1/200 D1
Orig. Medium CH₄ formate

Cellular:

Size, μm	.75
Shape	Cocci
Gram RXN	(+)
Acid Fast	No
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Undulate

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 5
Culture # 62583 1/2 D1
Orig. Medium CH₄ formate

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(-)
Acid Fast	Yes
Spore	Yes
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Punctiform
Elevation	Flat
Margin	Entire

Motility	Yes
----------	-----

Oxygen	MAF
--------	-----

CULTURE

Core # 3
Culture # 22223
Orig. Medium Oil

Cellular:

Size, μm	↓ .5
Shape	Bacilli
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	No

Colonial: Medium used PCA

Form	Punctiform
Elevation	Convex
Margin	Entire

Motility	No
----------	----

Oxygen	MAF
--------	-----

CULTURE

Core # 5
Culture # 61593 1/20 D2
Orig. Medium CH₄ formate

Cellular:

Size, μm	↓.5
Shape	Cocci
Gram RXN	(-)
Acid Fast	No
Spore	No
Capsule	No
Storage	No

Colonial: Medium used PCA

Form	Circular
Elevation	Convex
Margin	Entire

Motility	No
----------	----

Oxygen	FA
--------	----

CULTURE

Core # 4
Culture # 42552 1/2 D1
Orig. Medium CH₄ formate

Cellular:

Size, μm	1
Shape	Cocci
Gram RXN	(-)
Acid Fast	Yes
Spore	No
Capsule	Yes
Storage	Yes

Colonial: Medium used PCA

Form	Irregular
Elevation	Umbonate
Margin	Undulate

Motility	Yes
----------	-----

Oxygen	SG
--------	----

TABLE 2-5

The Ability of Oil-grown Mixed Cultures to Produce Gases.

Culture No	Nitrogen Source		
	NO ₃ ions	NH ₄ ions	Urea
7+ 9	2.35	1.04	0.52
7+37	1.83	7.31	1.80
9+25	0.32	2.87	1.50
9+37	1.31	0.78	0.00
21+37	2.50	0.78	0.52
21+33	1.57	0.52	1.52
33+37	2.09	1.31	1.31

1. CO₂ is reported as μ l gas/30 ml cell culture.
2. All figures are reported after deduction from controls.

TABLE 2-6

The Ability of Oil-grown Mixed Cultures to Produce Acids.

Culture No	Nitrogen Source		
	NO ₃ ions	NH ₄ ions	Urea
7+ 9	6.85	6.10	8.30
7+37	7.20	6.03	7.90
9+25	6.15	5.35	7.16
9+37	7.40	5.88	7.80
21+37	7.37	6.05	7.65
21+33	6.25	6.05	7.77
33+37	7.05	6.00	8.05

1. The initial pH for all system was 7.
2. The final pH for controls were: NO₃ ions, 7.03; NH₄ ions, 6.98; and Urea, 7.37.

TABLE 2-7

The Ability of Oil-grown Mixed Cultures to Produce Emulsifiers.

Culture No	Nitrogen Source		
	NO ₃ ions	NH ₄ ions	Urea
7+ 9	7.10	1.6	46.74
7+37	53.35	28.22	75.38
9+25	14.81	25.82	31.32
9+37	25.82	28.02	91.20
21+37	12.60	1.60	1.60
21+33	91.50	50.49	177.00
33+37	3.18	17.00	41.24

Emulsifiers concentration is reported as μg of emulsifier produced/ml of cell suspensions.

TABLE 2-8

Cell Surface Hydrophobicity Measurements of the Mixed Bacterial Culture Grown on Oil.

Culture No	Nitrogen Source		
	NO ₃ ions	NH ₄ ions	Urea
7+ 9	-20.0	36.8	-6.2
7+37	45.3	58.0	59.0
9+25	13.8	36.1	36.1
9+37	32.2	29.5	33.8
21+37	12.7	32.0	32.2
21+33	42.5	-4.6	29.6
33+37	67.0	55.5	50.3

Data are reported as percent decreased in turbidity of cell suspension after mixing with 1000 μ l of n-Hexadecane.

TABLE 3-1

The Effect of Formation Materials on Microbial Gas Production.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	2.09	0.78	5.91	1.70	1.78	0.57
7	6.79	2.48	17.82	4.70	6.03	1.31
9	1.78	0.58	3.31	1.78	4.91	1.31
18	3.13	0.78	3.40	3.31	5.50	2.26
19	14.93	8.75	19.53	17.00	3.30	1.83
20	6.79	5.20	7.59	5.50	2.87	3.13
21	14.70	8.73	13.31	2.87	2.09	2.22
25	3.04	3.92	2.91	14.70	2.09	2.55
27	11.35	5.55	14.70	22.59	5.50	4.55
33	3.40	0.78	6.79	10.19	5.78	2.09
37	3.92	3.92	8.88	10.19	5.49	1.83

1. CO₂ is reported as μ l of gas/30 ml of cell culture.
2. All figures are reported after deduction from controls.

TABLE 3-2

The Effect of Formation Materials on Microbial Acid Production.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	7.00	6.90	7.44	6.25	6.83	6.99
7	6.75	6.83	7.10	6.40	7.00	6.83
9	7.05	7.15	6.34	6.00	7.35	7.02
18	6.60	6.52	6.76	5.80	7.75	7.00
19	6.90	7.05	6.60	6.13	6.93	7.31
20	6.65	6.93	6.35	5.91	7.15	7.15
21	7.33	6.97	7.66	6.25	7.15	7.01
25	6.90	7.15	6.62	6.22	6.95	7.28
27	7.05	6.87	6.95	6.00	7.09	6.97
33	7.30	7.10	7.30	6.36	6.88	7.00
37	6.91	6.91	6.51	5.85	7.10	7.23
Control	7.37	7.00	7.15	6.40	6.90	6.90

The initial pH for all systems was adjusted 7.0.

TABLE 3-3

The Effect of Formation Materials on Microbial Emulsifiers Production.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	3.18	2.10	7.10	31.32	5.18	3.50
7	7.10	7.50	11.69	14.81	12.60	9.03
9	4.53	7.50	17.00	7.10	3.88	3.80
18	42.81	11.32	24.50	91.20	50.49	28.02
19	3.50	5.18	7.61	1.60	17.00	2.15
20	5.18	1.60	9.45	1.60	50.49	11.22
21	5.50	3.18	17.60	5.18	14.81	3.80
25	31.32	17.85	25.82	110.63	75.00	52.15
27	90.26	15.38	28.52	31.32	46.74	28.22
33	11.22	1.60	15.50	1.65	28.22	7.10
37	9.03	5.00	21.80	75.38	31.32	7.10

Emulsifier concentration is reported as μg of emulsifier produced/ml of cell suspension.

TABLE 3-4

pH of the Spent Fermentation Broth..

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6+ 7+ 9	6.73	6.50	6.97	5.50	6.76	6.72
18+19+20	7.00	6.85	7.25	6.40	7.15	6.70
21+25+27	6.50	6.50	6.22	5.63	6.85	6.95
6+33+37	6.85	7.00	7.48	6.35	7.22	7.19
7+25+18	6.85	6.70	6.65	6.35	7.10	7.05
9+20+21	6.85	6.65	6.22	5.98	6.77	6.90
18+21+33	6.50	6.50	6.37	6.45	6.83	6.85

The initial pH for all systems was 7.

TABLE 3-5

The Effect of Formation Materials on Cell Surface Hydrophobicity.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	20.00	52.50	14.70	29.70	39.60	38.20
7	38.20	63.60	76.00	52.80	52.00	49.60
9	-4.00	33.80	38.50	29.70	32.20	19.00
18	-4.60	12.70	30.70	26.00	-2.20	12.70
19	32.20	32.20	36.80	32.50	50.30	62.90
20	18.50	22.50	33.50	49.20	53.60	55.50
21	12.70	24.00	14.70	12.70	27.40	12.70
25	14.50	27.40	22.50	30.90	38.20	46.60
27	6.40	-4.00	0.00	-2.00	0.00	0.00
33	36.80	38.10	25.60	27.40	27.40	22.50
37	26.45	52.50	36.40	38.20	32.00	32.00

Data are reported as percent decrease in turbidity of the cell suspension after mixing with 1000 μ l of n-hexadecane.

TABLE 3-6

Microbial By-products and Cell Surface Hydrophobicity of Oil-grown Cultures in the Absence of Formation Materials.

	<u>NO₃ ions</u>		<u>NH₄ ions</u>	
	Low	High	Low	High
Gas	4.3	25.3	0.3	16.5
pH	7.1	8.2	5.5	6.9
Emulsifier	1.6	81.0	1.6	75.0
HC Adherence	-14.5	62.2	0.0	70.6

1. CO₂ is reported as μ l of gas produced/30 ml of cell culture.
2. Emulsifier concentration is reported as μ g of emulsifier produced/ml of cell suspension.
3. The initial pH for all system was 7.
4. Data are reported as percent decrease in turbidity of the cell suspension after mixing with 1000 μ l of n-hexadecane.

TABLE 3-7

pH of the Packed Column Effluent.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	6.91	6.65	6.86	5.99	7.50	7.55
7	5.73	6.85	6.35	5.65	7.62	7.07
9	7.00	6.93	7.55	6.00	6.99	6.99
18	5.50	5.80	6.22	5.52	6.75	6.70
19	6.34	6.55	6.43	5.87	6.80	6.70
20	6.55	5.99	7.02	6.00	7.08	6.83
21	7.08	7.00	7.99	6.19	7.00	7.10
25	6.33	6.88	6.90	6.19	7.73	7.55
27	6.75	6.95	6.90	5.75	7.30	6.96
33	6.91	7.00	7.35	6.19	7.25	7.94
37	6.98	7.00	6.71	6.10	7.55	7.50
Control	6.88	6.90	6.97	6.52	7.35	7.22

The initial pH for all systems was 7. .

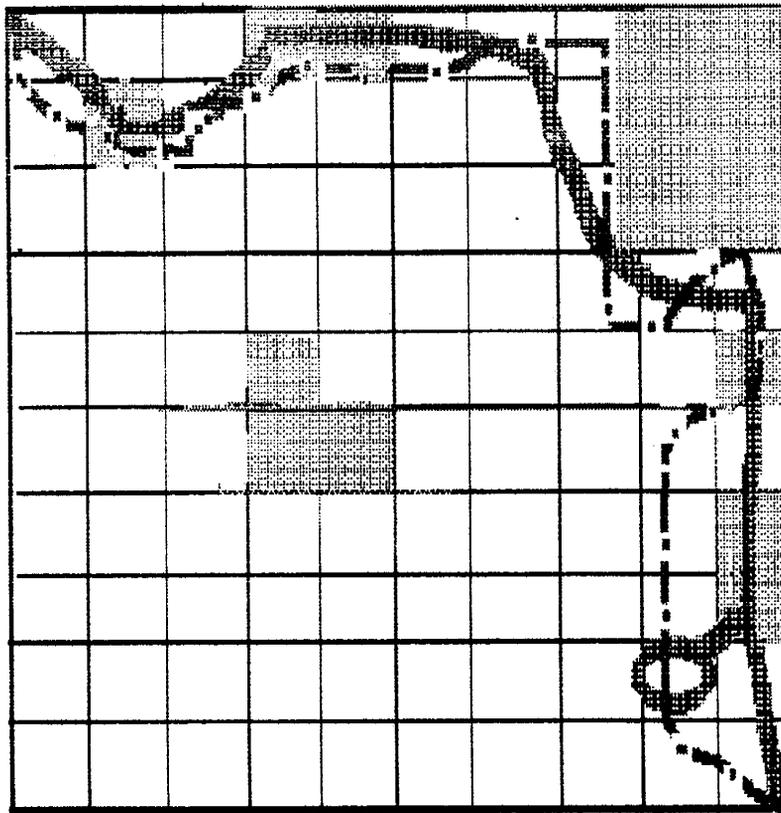
TABLE 3-8

The Concentration of the Emulsifier in the Packed Column Effluent.

Culture No	Limestone		Sandstone		Clay Shale	
	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions	NO ₃ ions	NH ₄ ions
6	0.00	0.00	9.03	14.81	12.60	3.50
7	7.10	9.03	11.55	11.55	3.88	1.60
9	7.61	1.60	9.03	12.60	5.18	1.60
18	40.12	83.09	165.69	31.32	35.69	60.00
19	11.22	9.03	28.82	3.50	11.69	11.69
20	1.60	9.03	7.61	3.88	3.88	5.18
21	7.20	9.38	50.49	42.69	17.00	3.88
25	38.83	17.00	9.38	14.22	11.69	1.60
27	35.88	50.49	77.25	28.02	28.02	60.00
33	1.60	0.00	0.00	0.00	0.00	0.00
37	40.12	28.22	53.55	24.50	35.69	17.00

Emulsifiers concentration is reported as μg of emulsifier produced/ml of cell suspension.

INLET



OUTLET

SANDPACK No 1

Effluent after First Cl-36 injection: Released small amount of fines. More fines were released after two weeks.

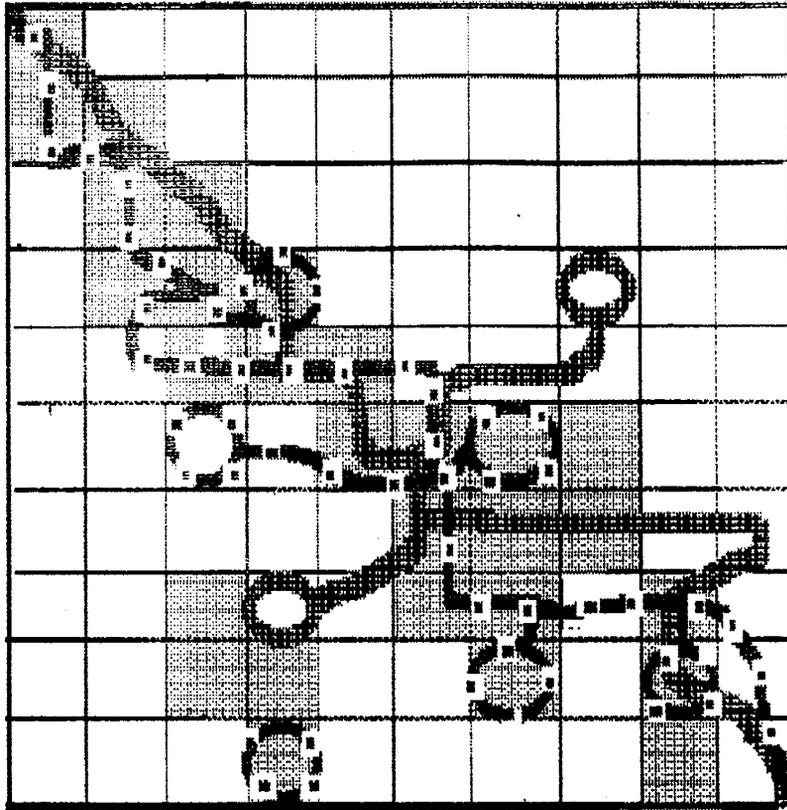
Effluent after Last Cl-36 injection: Released fines. pH was 6.7.

Cl-36,1 

Cl-36,2 

P-32 

INLET



OUTLET

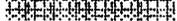
SANDPACK No 2

Effluent after First Cl-36 injection: Clear

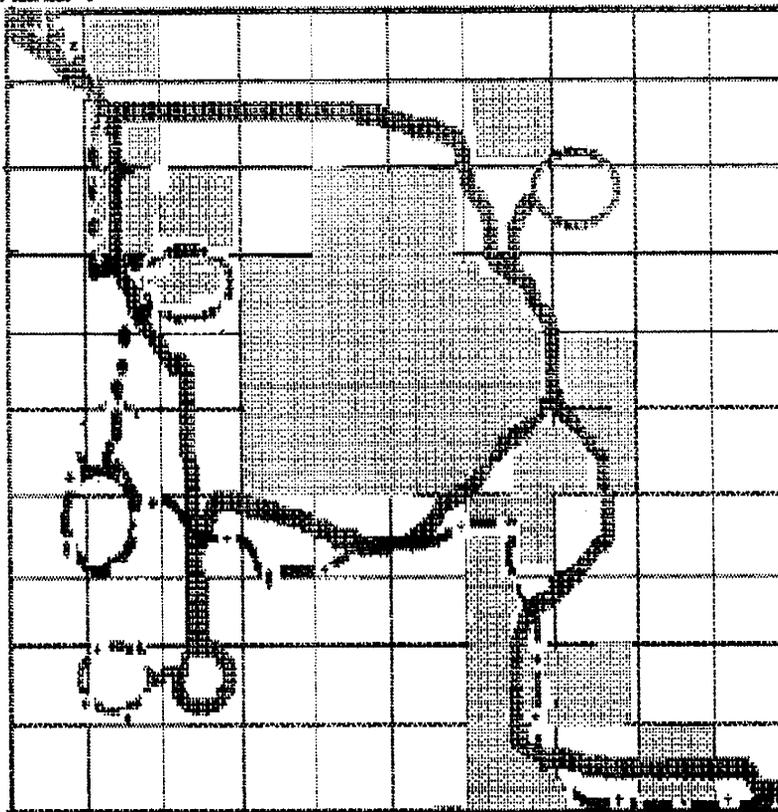
Effluent after Last Cl-36 injection: pH was 7.6.

Cl-36,1 

Cl-36,2 

P-32 

INLET



OUTLET

SANDPACK No 3

Effluent after First Cl-36 injection: Clear

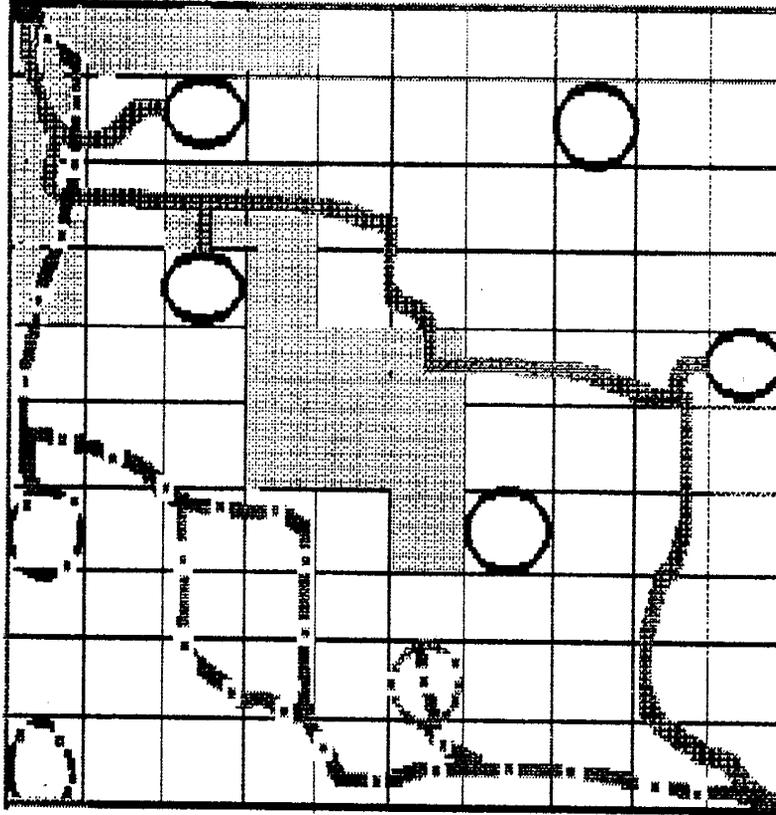
Effluent after Last Cl-36 injection: pH was 7.1.

Cl-36,1

Cl-36,2

P-32

INLET



OUTLET

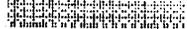
SANDPACK No 5

Effluent after First C1-36 injection: Contained fines. After two weeks, HC sheen was evident, no more fines were released.

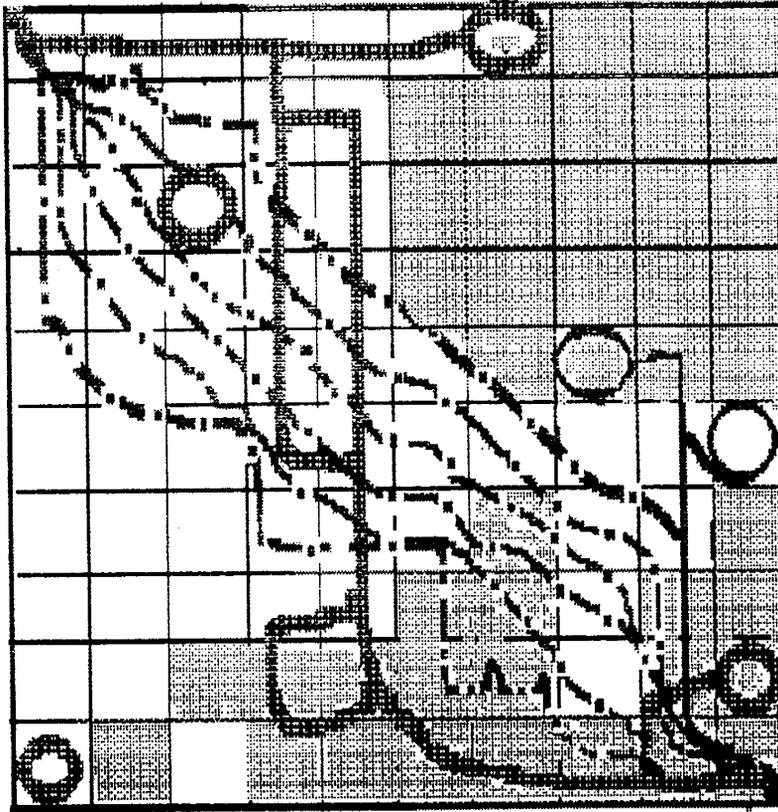
Effluent after Last C1-36 injection: pH was 7.

C1-36,1 

C1-36,2 

P-32 

INLET



OUTLET

SANDPACK No 6

Effluent after First Cl-36 injection: Clear

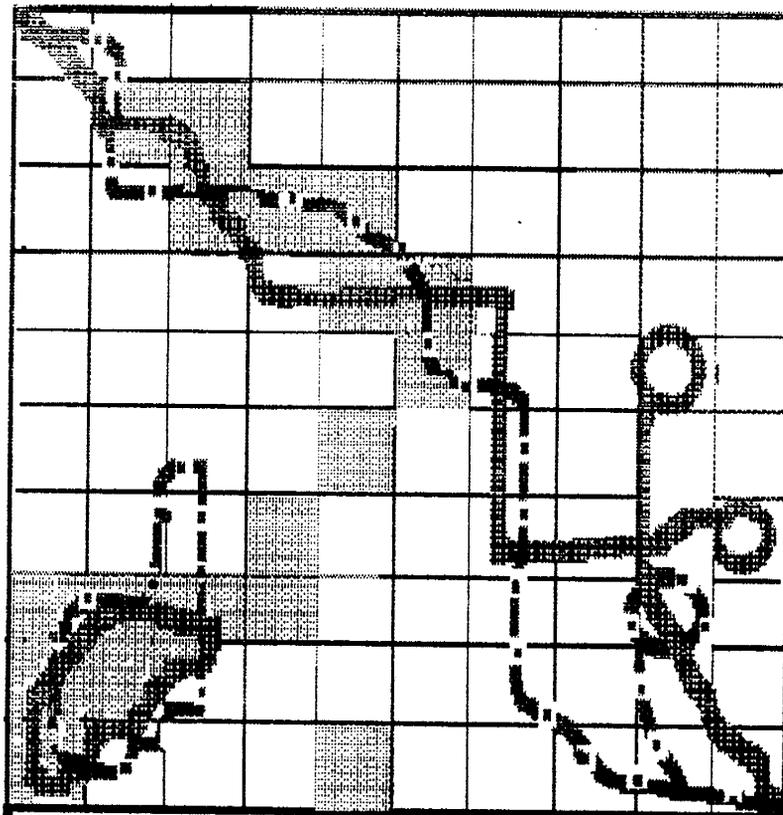
Effluent after Last Cl-36 injection: pH was 7.3.

Cl-36,1

Cl-36,2

P-32

INLET



OUTLET

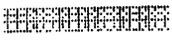
SANDPACK No 8

Effluent after First CL-36 injection: Clear

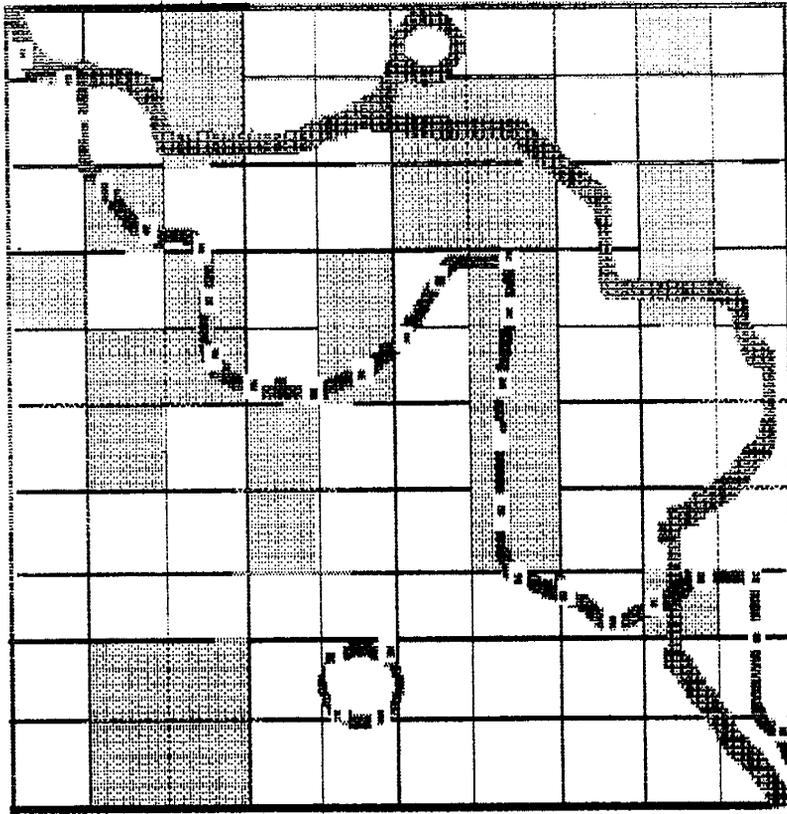
Effluent after Last CL-36 injection: pH was 7.

Cl-36,1 

Cl-36,2 

P-32 

INLET



OUTLET

SANDPACK No 10

Effluent after First Cl-36 injection: Clear

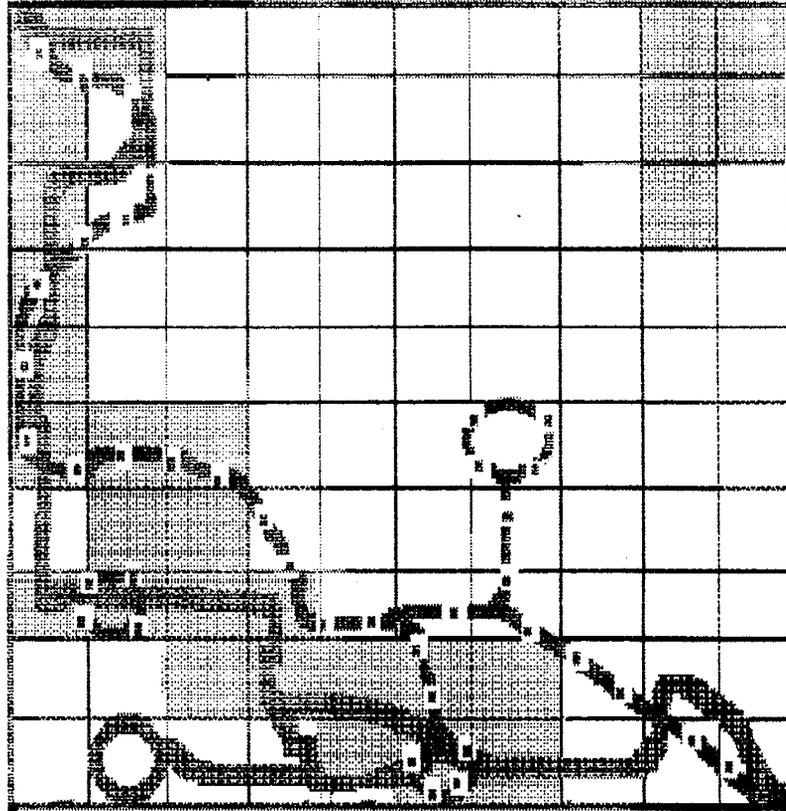
Effluent after Last Cl-36 injection: pH was 7.

Cl-36,1

Cl-36,2

P-32

LET



OUTLET

SANDPACK No 11

Effluent after First Cl-36 injection: White color effluent, released some fines.

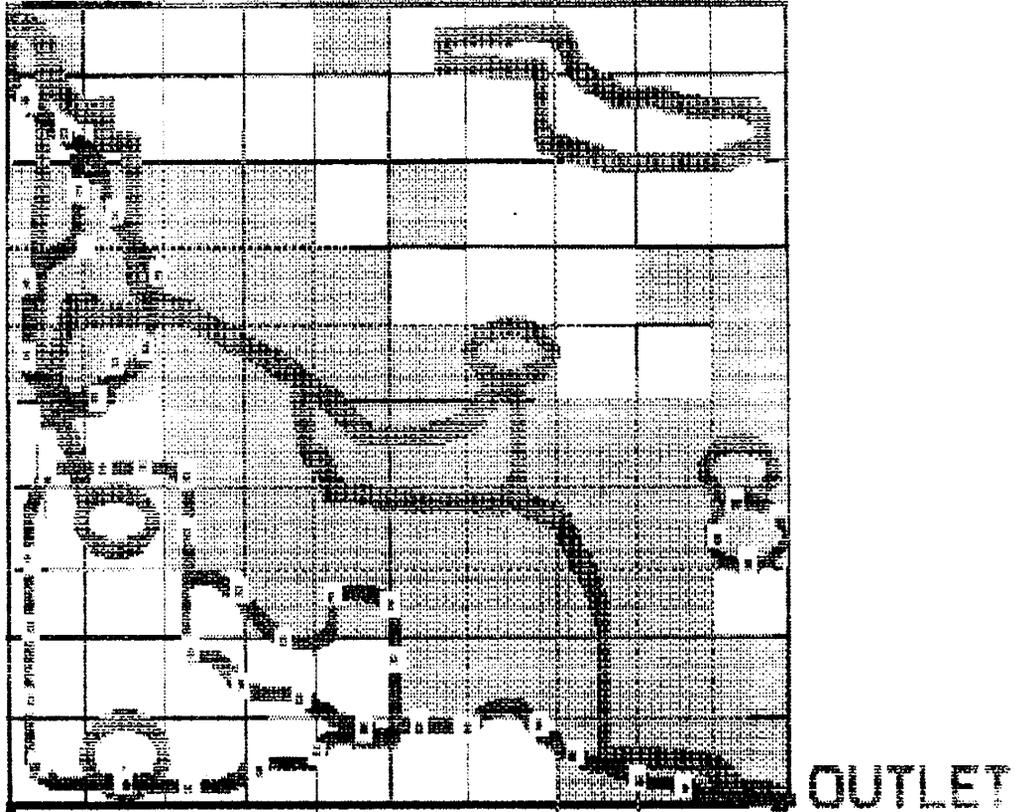
Effluent after Last Cl-36 injection: pH was 7.3.

Cl-36,1

Cl-36,2

P-32

LET



SANDPACK No 12

Effluent after First C1-36 injection: Released fines.

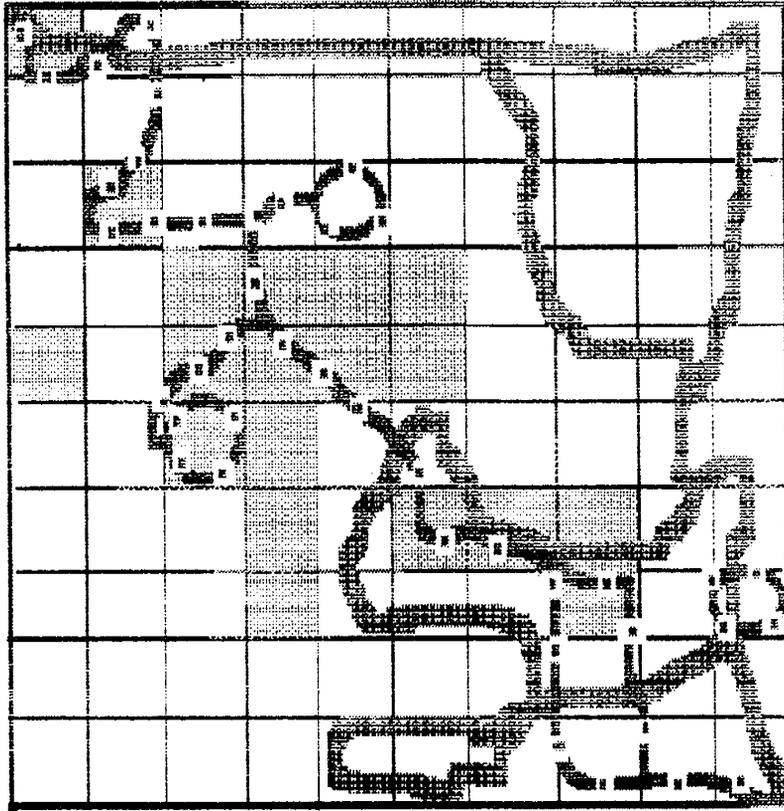
Effluent after Last C1-36 injection: pH was 6.5

C1-36,1 

C1-36,2 

P-32 

INLET



OUTLET

SANDPACK No 13

Effluent after First Cl-36 injection: Released large amount of HC and fines.

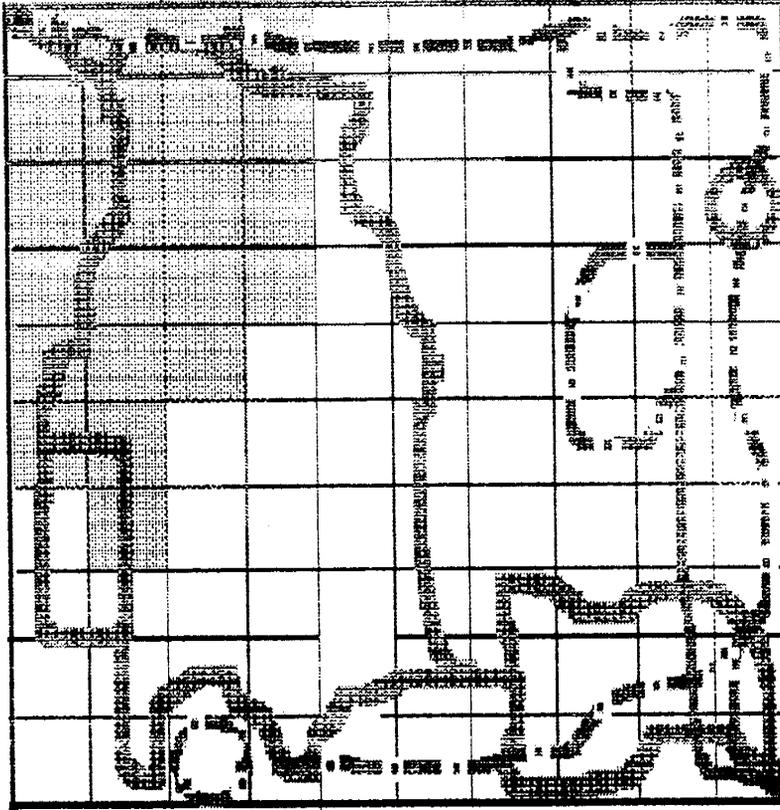
Effluent after Last Cl-36 injection: pH was 7.0.

Cl-36,1

Cl-36,2

P-32

INLET



OUTLET

SANDPACK No 14

Effluent after First Cl-36 injection: Clear

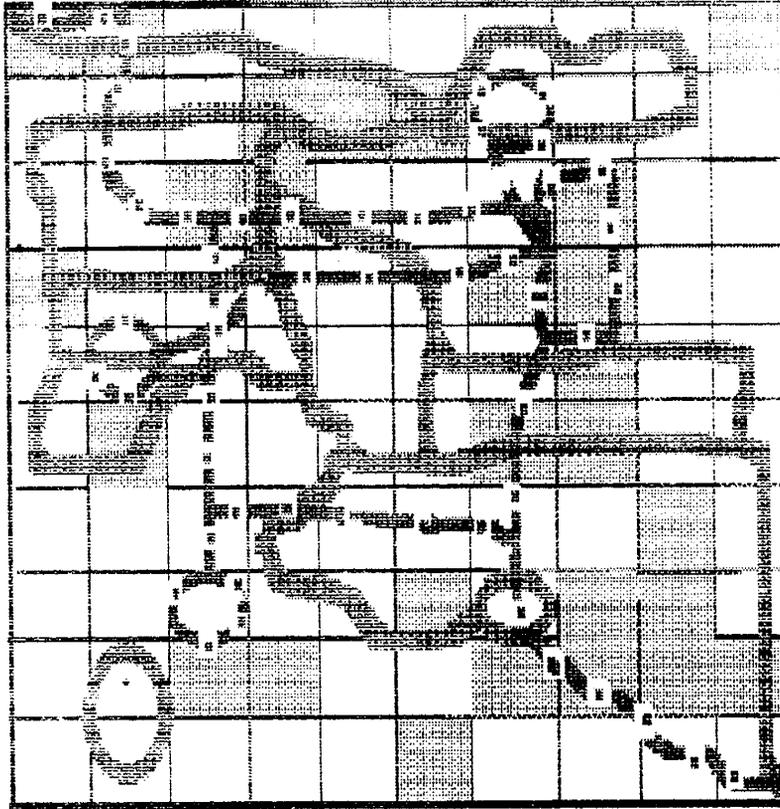
Effluent after Last Cl-36 injection: pH was 7.0.

Cl-36,1

Cl-36,2

P-32

PLAN



OUTLET

SANDPACK No 15

Effluent after First Cl-36 injection: Turbid.

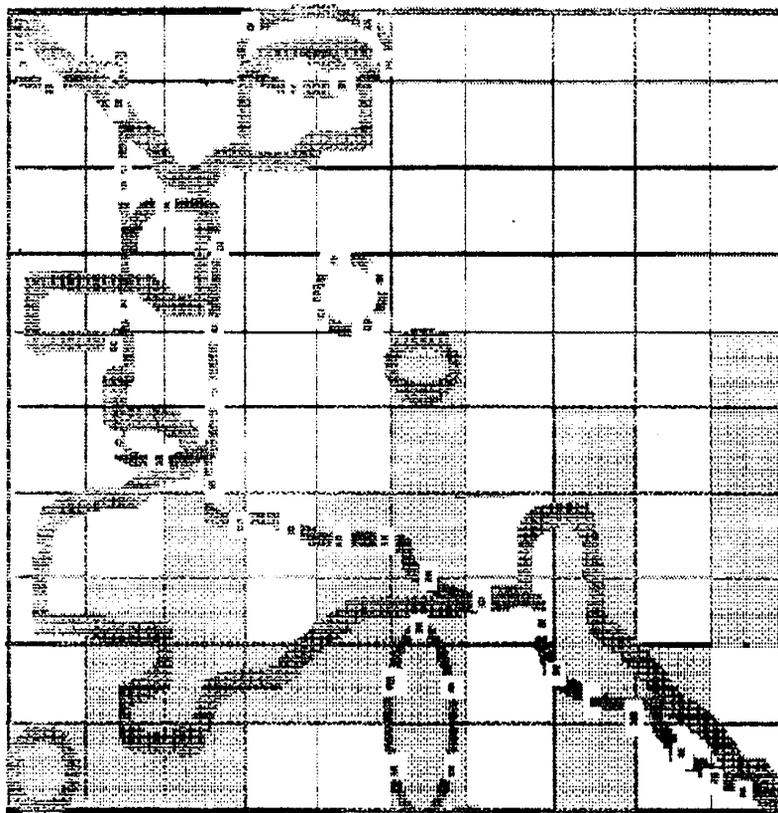
Effluent after Last Cl-36 injection: pH was 6.9.

Cl-36, 1 

Cl-36, 2 

P-32 

200



200

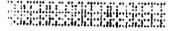
SANDPACK No 16

Effluent after First Cl-36 injection: White color effluent.

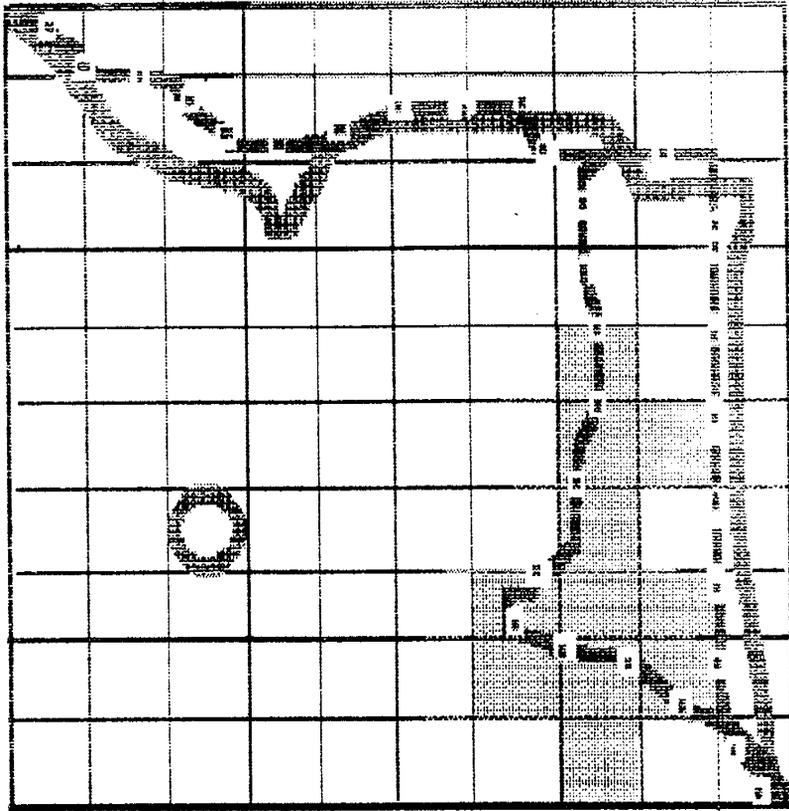
Effluent after Last Cl-36 injection: pH was 6.4.

Cl-36,1 

Cl-36,2 

P-32 

11/18/81
11/18/81
11/18/81
11/18/81
11/18/81



11/18/81
11/18/81
11/18/81
11/18/81
11/18/81

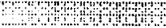
SANDPACK No 17

Effluent after First Cl-36 injection: Clear.

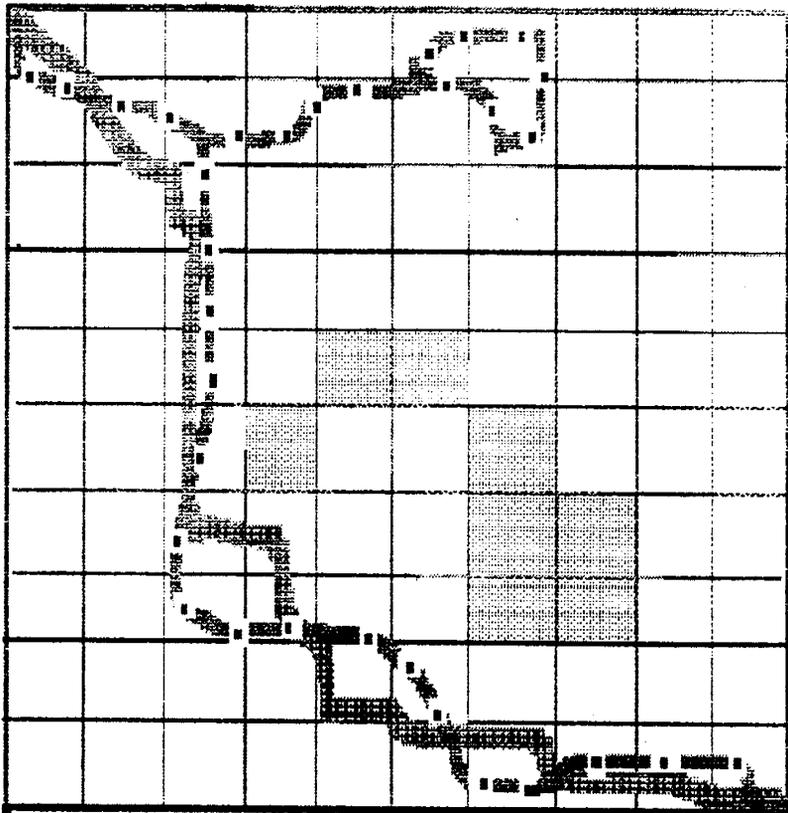
Effluent after Last Cl-36 injection: pH was 7.2.

Cl-36,1 

Cl-36,2 

P-32 

INLET



OUTLET

SANDPACK No 18

Effluent after First Cl-36 injection: Clear

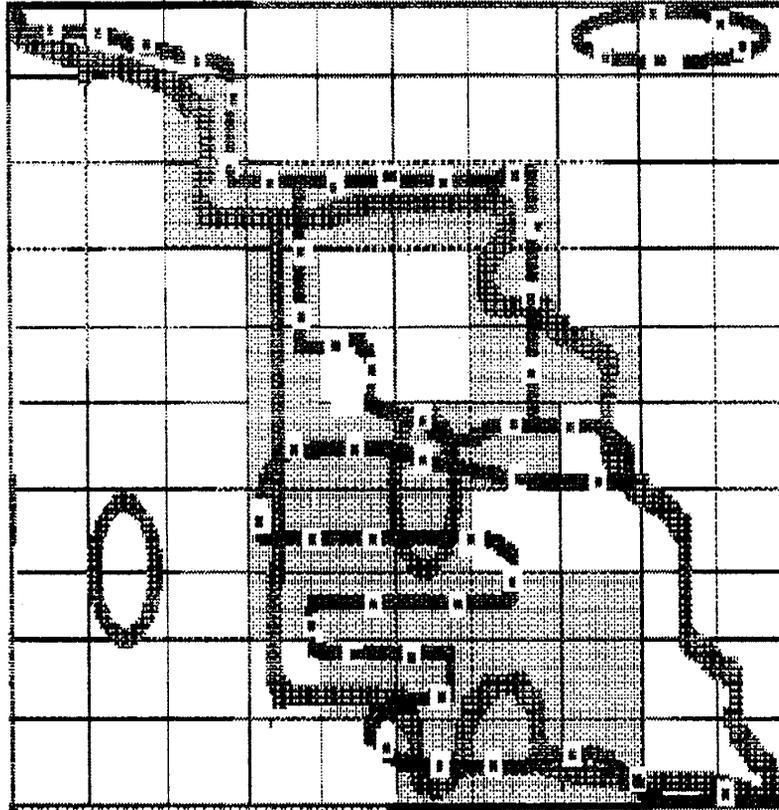
Effluent after Last Cl-36 injection: pH was 7.2.

Cl-36,1

Cl-36,2

P-32

INLET



OUTLET

SANDPACK No 21

Effluent after First Cl-36 injection: Released small amount of fines.

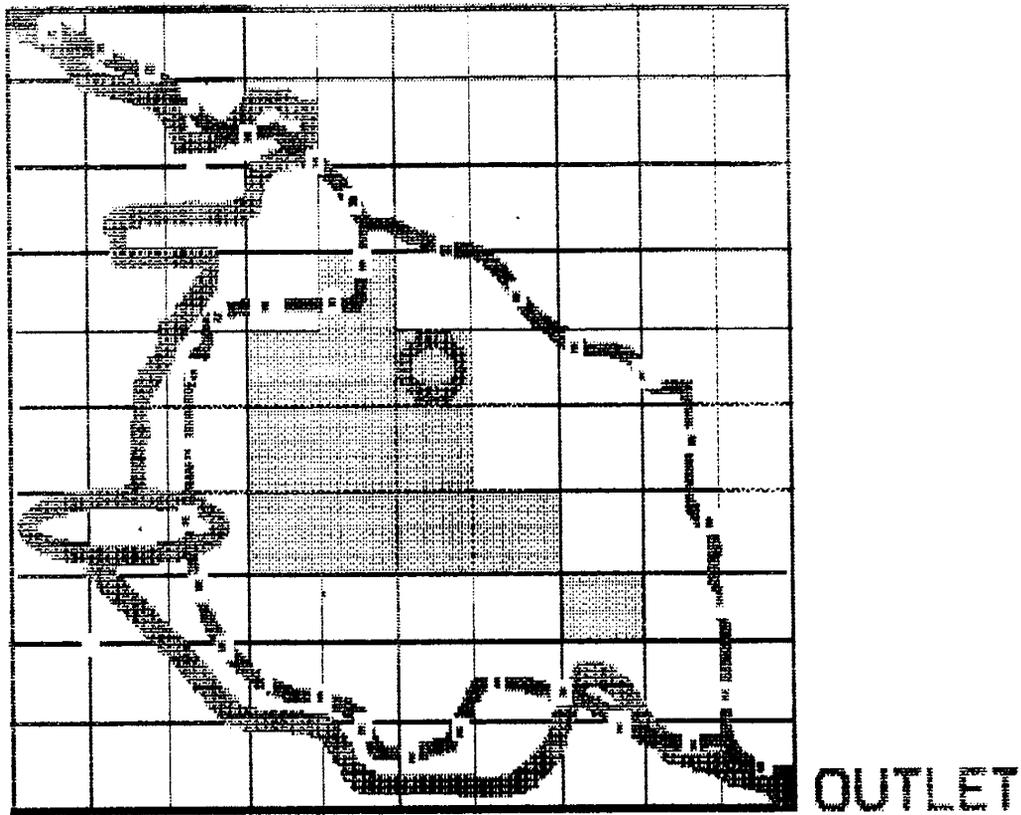
Effluent after Last Cl-36 injection: pH was 7.3.

Cl-36,1

Cl-36,2

P-32

NET



SANDPACK No 22

Effluent after First Cl-36 injection: HC sheen was evident after the first week.

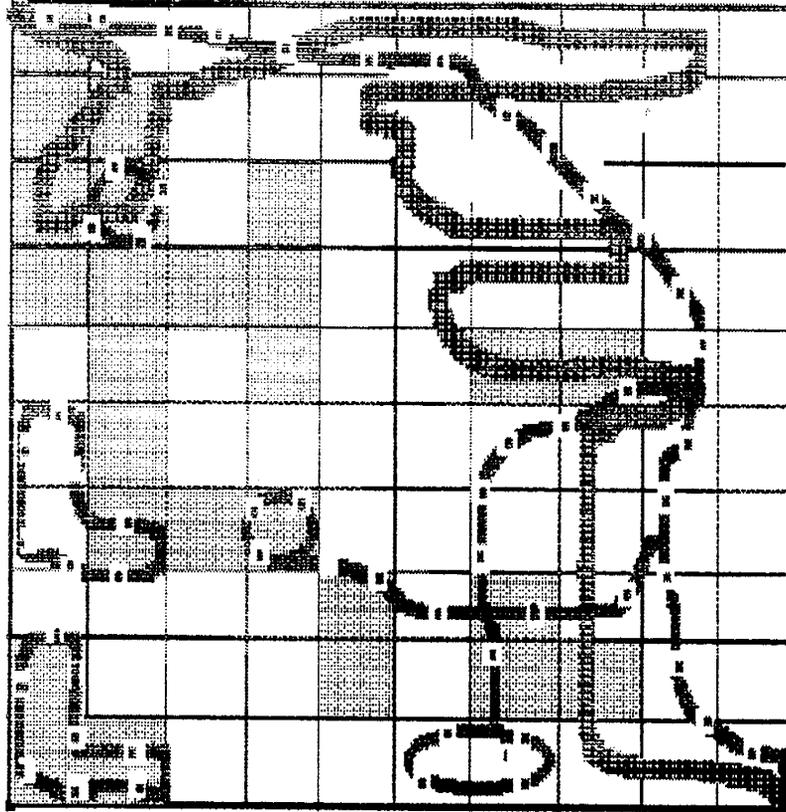
Effluent after Last Cl-36 injection: HC sheen slightly evident. pH was 6.8.

Cl-36,1

Cl-36,2

P-32

PLAN



SANDPACK No 23

Effluent after First Cl-36 injection: Released large amount of fines. HC sheen was evident.

Effluent after Last Cl-36 injection: pH was 7.1.

Cl-36,1 

Cl-36,2 

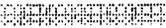
P-32 

TABLE 4-1

The Ratio of the Area under the Curve for the Low P-32 Activity from the GC Chromatograms.

Sandpack No	C-7	C-8	C-9	C-10	C-11	C-12	C-14	C-16	C-18	C-20	C-24	C-28
1	0.8	3.6	**	0.5	3.3	0.8	0.4	0.3	0.4	1.1	0.7	1.9
2	1.2	0.5	0.5	1.3	1.5	3.4	2.3	20.6	1.1	2.1	1.5	3.8
3	2.3	1.0	4.8	0.95	0.81	1.1	1.4	0.46	2.0	1.4	0.35	23.4
4	3.1	1.1	*	1.9	2.5	2.1	2.0	1.2	3.5	2.6	1.0	1.5
5	0.2	1.1	3.3	1.9	3.3	1.4	2.6	5.4	1.3	2.5	9.4	35.8
6	0.7	1.8	***	***	***	0.7	0.3	0.6	1.0	0.3	1.0	5.7
7	1.3	1.6	***	***	***	**	6.0	1.7	1.7	0.8	0.1	0.2
8	8.3	0.5	3.4	0.9	1.1	0.7	0.9	5.7	0.5	0.9	6.5	1.9
9	1.1	0.6	1.6	0.5	0.5	1.1	0.4	0.4	0.6	2.7	6.6	8.1
10	1.2	1.0	*	1.0	1.6	0.7	1.0	1.3	2.2	1.1	0.2	0.1
11	0.7	0.6	***	***	***	**	0.8	0.7	0.8	0.96	1.5	9.3
12	1.2	1.9	1.8	0.6	0.2	0.4	0.3	1.4	0.2	0.6	0.8	0.97
13	1.2	**	***	***	***	2.5	4.7	9.7	5.5	8.4	16.3	5.2
14	2.3	15.7	9.7	6.4	3.4	6.1	5.7	1.1	8.0	1.2	1.6	1.7
15	2.6	.98	***	***	**	**	2.5	1.9	2.5	4.1	11.6	0.2
16	0.7	*	***	***	***	0.98	1.1	1.1	11.4	5.4	1.8	1.3
17	1.1	6.0	**	**	15.4	1.9	1.2	0.3	2.0	0.7	1.3	3.5
18	0.9	**	***	1.1	0.3	0.6	1.1	0.6	1.5	0.5	1.1	2.4
19	0.6	*	***	***	***	0.8	1.1	0.8	0.3	0.4	0.3	0.2
20	1.3	**	***	***	***	0.3	0.4	0.3	0.4	1.2	0.8	18.9
21	0.9	2.2	2.1	0.4	0.5	0.3	0.6	2.8	0.3	0.7	0.7	1.5
22	0.8	***	***	***	***	**	6.8	6.7	3.3	1.9	1.7	7.1
23	1.2	1.2	***	***	***	1.2	1.2	1.1	0.6	3.4	0.6	0.3
24	1.1	1.8	0.5	1.1	1.1	0.5	1.2	0.6	0.8	1.2	0.5	0.5
25	1.8	0.5	0.5	1.9	2.2	0.3	1.5	1.1	1.1	0.96	1.0	1.3

C-7 through C-28 refers to the length of the carbon chain in each aliphatic compounds; * area for the low P-32 activity was zero; ** area for the high P-32 activity was zero; *** area for the high P-32 activity and the low P-32 activity was zero.

TABLE 4-2

The Culture No and Growth Condition of the Oil-grown Microbial Cultures used for Sandpacks Preparations.

Sandpack No	Culture No	Culture employed in Sandpack Grown on:	
		Nitrogen Source	Formation Material
1*	18	NO ₃ ions	Sandstone
2*	18	NH ₄ ions	Clay shale
3	37	NH ₄ ions	Limestone
4	7	NH ₄ ions	Sandstone
5	37	NO ₃ ions	Limestone
6	9	NO ₃ ions	Clay shale
7	9	NO ₃ ions	Sandstone
8	21	NH ₄ ions	Sandstone
9	9	NO ₃ ions	Limestone
10	21+ 37	NH ₄ ions NH ₄ ions	Clay shale Clay shale
11	21	NO ₃ ions	Clay shale
12	37	NO ₃ ions	Clay shale
13	18	NH ₄ ions	Clay shale
14	21	NO ₃ ions	Limestone
15	18	NO ₃ ions	Limestone
16	21	NO ₃ ions	Sandstone
17	6	NH ₄ ions	Limestone
18	37+ 7	NH ₄ ions NH ₄ ions	Sandstone Clay shale
19	18+ 37	NO ₃ ions NO ₃ ions	Limestone Limestone
20	18+ 9	NO ₃ ions NO ₃ ions	Sandstone Sandstone
21	18	NO ₃ ions	Sandstone
22	21+ 18	NH ₄ ions NH ₄ ions	Limestone Sandstone
23	18	NH ₄ ions	Limestone
24	18	NO ₃ ions	Clay shale
25	7	NH ₄ ions	Limestone

*Sandpack was used as control.

Treatment Schedule and Observations for Coreflooding Experiment Using Cores from Reservoir No 3.

Day	Column No	Additions	Rate, ml/h	Observation
0	1	-	8.60	pH, 6.90 pH, 7.12
	2	N	0.30	
3	1	-	8.00	MC*, 8E4 cfu/ml MC, 26E4 cfu/ml
	2	P	0.50	
6	1	-	8.50	
	2	N	1.50	
10	1	-	8.00	MC, 12E4 cfu/ml Effluent was turbid, milkish color, HC odor. MC, 9E6 cfu/ml
	2	P	0.50	
13	1	-	8.00	
	2	N	0.50	
16	1	-	8.50	pH, 7.20 Flow restarted under syringe Pressure. Turbid and oily effluent. pH, 6.80
	2	P	0.00	
19	1	-	14.00	
	2	N	2.50	
28	1	-	12.50	MC, 14E4 cfu/ml MC, 54E6 cfu/ml
	2	N,P,G	2.50	
31	1	-	15.00	HC sheen is evident, small oil drops.
	2	N,P,G	1.60	
34	1	-	17.50	
	2	N,P,G	0.50	
37	1	-	17.50	MC, T1CE4 MC, 39E6 cfu/ml
	2	N,P,G	0.50	
40	1	-	16.00	Released one large oil drop, some fines.
	2	N,P,G	0.00	
43	1	-	16.50	Flow restarted by syringe pressure. Effluent was very turbid, released HC.
	2	N,P,G	0.00	
46	1	-	19.00	MC, 4E4 cfu/ml; pH, 6.90 MC, 10E6 cfu/ml; pH, 6.50
	2	N,P,G	3.00	
51	1	-	28.00	HC odor
	2	N,P,G	2.50	
55	1	-	1.67	
	2	-	0.00	

- pH of simulated production water was adjusted to 7 prior to waterflooding.
- P=PO₄, 0.5% (W/V) Na₂HPO₄ in simulated production water (5 ml).
- N=NO₃, 0.1% (W/V) NaNO₃ in simulated production water (5 ml).
- E=Ethanol 15 μM in simulated production water (5 ml).
- Conditions:
 - 1- Starting Day 0 cores were flooded for 48 hr. After flooding and nutrients injection, cores were incubated for 24 hr at 31 C.
 - 2- Starting Day 42, cores were incubated at 25 C.
 - 3- Core Column No 1 was used as control.
- *MC, Microbial count.
 - Microbial content was measured from the effluent (first 5 ml) which was collected aseptically into sterile twist top 6 oz prescription bottles.
 - Plate counts were carried on TSA.

Treatment Schedule and Observations for Coreflooding Experiment Using Cores from Reservoir No 4.

Day	Column No	Additions	Rate, ml/h	Observation
0	1	-	47.00	pH 7.5
	2	N	5.20	pH 7.2
3	1	-	48.00	MC*, TFTCE6
	2	P	5.50	MC, 37E6 cfu/ml
6	1	-	46.00	Released some oil drops.
	2	N	6.00	Released high amount of fines.
10	1	-	45.00	MC, 5E4 cfu/ml
	2	P	7.00	MC, 61E6 cfu/ml
13	1	-	46.00	
	2	N	7.00	Released fines, HC sheen, odor.
16	1	-	17.00	pH 6.9
	2	P	0.00	Core plugged. Flow restarted under syringe pressure. Effluent contained black oil drops, HC sheen evident, large amount of fines, tar balls. pH, 5.9
19	1	-	2.00	
	2	N	20.00	
28	1	-	21.00	MC, 17E4 cfu/ml
	2	N,P	2.30	MC, 50E6 cfu/ml
31	1	-	36.00	
	2	N,P	2.50	Released large amounts of fines, HC sheen was evident.
34	1	-	36.00	
	2	N,P	1.20	
38	1	-	36.00	
	2	N,P	1.20	
41	1	-	31.00	
	2	N,P	1.50	
44	1	-	28.00	
	2	N,P	2.50	Gas produced, released HC, fines.
47	1	-	32.00	
	2	N,P	0.00	Core plugged, Flow restarted with syringe pressure. Released fines, HC sheen was evident.
52	1	-	30.00	MC, 16E6 cfu/ml
	2	N,P	8.50	Released fines, produced gas. MC, 46E6 cfu/ml
56	1	-	1.79	
	2	N,P	10.42	Released fines, turbid, produced gas.
59	1	-	1.59	pH, 7.30
	2	N,P	10.00	pH, 6.50
62	1	-	2.50	
	2	N,P	13.00	
69	1	-	1.00	MC, 2E6 cfu/ml
	2	N,P	8.50	MC, 150E6 cfu/ml
72	1	-	0.80	
	2	N,P	10.00	
76	1	-	1.50	
	2	N,P	11.00	
79	1	-	1.00	Some turbidity.
	2	N,P	11.00	Released small amount of fines.
82	1	-	0.00	
	2	N,P	11.00	

(cont.) Core No 4.

Day	Column No	Additions	Rate, ml/h	Observation
85	1	-	0.00	Milkish color effluent, layer of HC, large amount of fines.
	2	N,P	10.42	
88	1	-	1.50	MC, 19E6 cfu/ml MC, 15E6 cfu/ml
	2	N,P,E	11.71	
91	1	-	1.00	pH, 7.50 pH, 6.50
	2	N,P,E	90.00	
94	1	-	2.00	
	2	N,P,E	132.50	
97	1	-	1.50	pH, 7.20 Released high amount of fines. pH, 6.00
	2	N,P,E	2600.00	
100	1	-	0.00	
	2	N,P	4400.00	
103	1	-	0.00	Released large amount of fines.
	2	N,P	4635.00	

- pH of simulated production water was adjusted to 7 prior to waterflooding.
- P=PO₄, 0.05% (W/V) Na₂HPO₄ in simulated production water (5 ml), from Day 31 concentration changed to 0.5% (W/V).
- N=NO₃, 0.01% (W/V) NaNO₃ in simulation production water (5 ml), from Day 31 concentration changed to 0.1% (W/V).
- G=Glucose 0.5% (W/V) in simulation production water (5 ml).
- H₂O₂ 0.2M in simulated production water (5 ml).
- E=Ethanol 15 μm in simulated production water (5 ml).
- Conditions:
 - 1- From Day 0 to 15 cores were flooded for 48 hr using simulated production water under hydrostatic pressure (38 inches of water).
 - 2- Starting Day 15 cores were flooded for 24 hr. After flooding and nutrients injection, cores were incubated for 24 hr at 31 C.
 - 3- Starting Day 42, cores were incubated at 25 C.
 - 4- Core Column No 1 was used as control.
- *MC, Microbial count.
- Microbial content was measured from the effluent (first 5 ml) which was collected aseptically into sterile twist top 6 oz prescription bottles.
- Plate counts were carried on TSA.

Treatment Schedule and Observations for Coreflooding Experiment Using Cores from Reservoir No 5.

Day	Column No	Additions	Rate, ml/h	Observation
0	1	-	4.55	pH, 6.80
	2	N,P	4.08	pH, 6.70
3	1	-	6.00	
	2	N,P	5.50	
6	1	-	5.50	
	2	N,P	8.00	
10	1	-	4.00	MC* , 3E4 cfu/ml; pH, 7.0
	2	N,P,E	4.00	MC , 8E4 cfu/ml; pH, 6.4
13	1	-	1.88	
	2	N,P,E	0.50	
16	1	-	1.55	
	2	N,P,E	2.30	
20	1	-	0.80	pH, 7.20
	2	N,P	4.50	pH, 6.50
23	1	-	0.80	
	2	N,P	5.00	HC sheen is evident.
26	1	-	0.50	
	2	N,P	6.00	
29	1	-	0.00	
	2	N,P	7.67	
32	1	-	0.00	
	2	N,P,E	0.188	
35	1	-	1.20	MC, 5E4 cfu/ml
	2	N,P,E	84.00	MC, 17E6 cfu/ml; Released small amount of fines.
38	1	-	1.50	
	2	N,P,E	96.00	HC sheen is evident.
44	1	-	2.50	MC, 10E4 cfu/ml; pH, 6.90
	2	N,P	43.00	MC, 24E6 cfu/ml; pH, 6.80
47	1	-	0.00	
	2	N,P	94.00	

- pH of simulated production water was adjusted to 7 prior to waterflooding
- P=PO₄, 0.05% (W/V) Na₂HPO₄ in simulated production water (5 ml), from Day 31 concentration changed to 0.5% (W/V).
- N=NO₃, 0.01% (W/V) NaNO₃ in simulation production water (5 ml), from Day 31 concentration changed to 0.1% (W/V).
- G=Glucose .5% (W/V) in simulation production water (5 ml).
- Conditions:
 - 1- From Day 0 to 15 cores were flooded for 48 hr using simulated production water under hydrostatic pressure (38 inches of water).
 - 2- Starting Day 15 cores were flooded for 24 hr. After flooding and nutrient injection, cores were incubated for 24 hr at 31 C.
 - 3- Starting Day 42, cores were incubated at 25 C.
 - 4- Core Column No 1 was used as control.
- *MC, Microbial count.
 - Microbial content was measured from the effluent (first 5 ml) which was collected aseptically into sterile twist top 6 oz prescription bottles.
 - Plate counts were carried on TSA.

Treatment Schedule and Observations for Coreflooding Experiment Using Cores from Reservoir No 6.

Day	Column No	Additions	Rate, ml/h	Observation
0	1	-	7.20	pH, 7.80 pH, 7.00
	2	N,P	6.25	
3	1	-	7.00	
	2	N,P	9.50	
6	1	-	7.50	
	2	N,P	13.00	
10	1	-	7.50	
	2	N,P	15.17	
13	1	-	5.20	MC*, 15E4 cfu/ml MC, 19E6 cfu/ml
	2	N,P	3.83	
16	1	-	5.50	Effluent is turbid gas produced, HC odor was evident.
	2	N,P	6.00	
21	1	-	5.00	pH, 7.30 pH, 6.10
	2	N,P	5.00	
24	1	-	5.00	
	2	N,P	8.50	
27	1	-	4.50	
	2	N,P	9.50	
30	1	-	4.17	Released high amount of HC.
	2	N,P	9.75	
33	1	-	1.30	MC, 2E6 cfu/ml MC, 22E6 cfu/ml
	2	N,P,E	4.04	
36	1	-	7.00	Effluent was clear.
	2	N,P,E	243.00	
39	1	-	6.00	
	2	N,P,E	132.00	
45	1	-	4.60	MC, 5E6 cfu/ml. pH, 7.00 MC, 65E6 cfu/ml. pH, 6.50
	2	N,P	289.00	
48	1	-	9.40	
	2	N,P	486.00	

- pH of simulated production water was adjusted to 7 prior to waterflooding.
- P=PO₄, 0.5%(W/V) Na₂HPO₄ in simulated production water (5 ml).
- N=NO₃, 0.1%(W/V) NaNO₃ in simulation production water (5 ml).
- E=Ethanol 15 μM in simulated production water (5 ml).
- Conditions:
 - 1- Starting Day 0 cores were flooded for 48 hr after flooding and nutrients injection, cores were incubated for 24 hr at 31 C.
 - 2- Starting Day 42, cores were incubated at 25 C.
 - 3- Core Column No 1 was used as control.
- * MC, Microbial count.
 - Microbial content was measured from the effluent (first 5 ml) which was collected aseptically into sterile twist top 6 oz prescription bottles.
 - Plate counts were carried on TSA.

Treatment Schedule and Observations for Coreflooding Experiment Using Cores from Reservoir No 7.

Day	Column No	Additions	Rate, ml/h	Observations
0	1	-	11.50	pH, 7.30 pH, 6.90
	2	N,P	11.50	
3	1	-	3.25	Released fines, high amount of oil in effluent.
	2	N,P	6.26	
6	1	-	3.33	MC*, 34E4 cfu/ml MC, 53E6 cfu/ml
	2	N,P	34.80	
9	1	-	10.50	Effluent is very clear.
	2	N,P,E	238.00	
12	1	-	5.80	
	2	N,P,E	3400.00	
15	1	-	1.00	MC, 25E4 cfu/ml; pH, 6.90 Released large amount of oil. MC, 73E6 cfu/ml; pH, 4.50
	2	N,P,E	2960.00	
18	1	-	0	
	2	N,P	2800.00	
22	1	-	0	pH, 6.50 pH, 5.00
	2	N,P	3360.00	

- pH of simulated production water was adjusted to 7 prior to waterflooding.
- P=PO₄, 0.5% (W/V) Na₂HPO₄ in simulated production water (5 ml).
- N=NO₃, 0.1% (W/V) NaNO₃ in simulation production water (5 ml).
- E=Ethanol 15 µM in simulated production water (5 ml).
- Conditions:
 - 1- Starting Day 0 cores were flooded for 48 hr. After flooding and nutrients injection, cores were incubated for 24 hr at 31 C.
 - 2- Starting Day 42, cores were incubated at 25 C.
 - 3- Core Column No 1 was used as control.
- *MC, Microbial count.
 - Microbial content was measured from the effluent (first 5 ml) which was collected aseptically into sterile twist top 6 oz prescription bottles.
 - Plate counts were carried on TSA.

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