

TITLE:
**IMPROVEMENT OF CARBON DIOXIDE SWEEP EFFICIENCY BY UTILIZATION OF
MICROBIAL PERMEABILITY PROFILE MODIFICATION TO REDUCE THE
AMOUNT OF OIL BYPASSED DURING CARBON DIOXIDE FLOOD**

FIFTH SEMI-ANNUAL PROGRESS REPORT

REPORTING PERIOD START DATE:

1 October 2007

REPORTING PERIOD END DATE

31 March 2008

PRINCIPAL AUTHORS

Darrel Schmitz

Lewis R. Brown

F. Leo Lynch

Brenda L. Kirkland

Krystal Collins

William Funderburk

DATE OF REPORT:

March 31, 2008

DOE AWARD NUMBER:

DEFC2605NT15458 05090806

RECIPIENT:

Mississippi State University

Sponsored Program Administration

P.O. Box 6156

Mississippi State, MS 39762

Principal Investigator: Dr. Darrel Schmitz, P.G.

662-325-2904

FAX 662-325-9423

schmitz@ra.msstate.edu

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

TABLE OF CONTENTS

ABSTRACT.....	4
REPORT DETAILS	
Introduction and Objectives	6
Results and Discussion.....	7
Task 1.0	7
Task 2.0	18
Task 3.0	21
Task 4.0	31
Task 5.0	30
Task 6.0	31
SUMMARY	33
REFERENCES.....	34

ABSTRACT

The objective of this project is to significantly improve oil recovery at Eucutta Field, Wayne County, Mississippi and Little Creek Field, Lincoln and Pike counties, Mississippi by combining an established procedure, carbon dioxide flood, with a new technology, microbial permeability profile modification (MPPM), developed at Mississippi State University (MSU) under prior DOE contract. MPPM technology, which utilizes environmentally friendly nutrient solutions to simulate the growth of the indigenous microflora in the most permeable zones of the reservoir, is compatible with the use of carbon dioxide, which is diverted to less permeable, previously unswept zones of the reservoir, thus improving production.

The first task in carrying out this objective was to isolate and culture live, indigenous bacteria from the producing formation. Enrichment cultures were prepared from formation water samples from the Eucutta and Little Creek fields and tested for viable microorganisms. Specialized culture vessels were designed to accommodate the high temperatures in the Little Creek Field. The second task was to simulate combination of the MPPM procedure combined with carbon dioxide in laboratory core flood experiments, which required modification of the equipment to be used to conduct core flood experiments in the laboratory to accommodate experiments carried out at higher temperatures and carbon dioxide pressures.

The Eucutta field produces from the Eutaw Formation, which occurs as relatively thin, variably-lithified, well-laminated sandstone interbedded with heavily-bioturbated, clay-rich sandstone and shale. Petrographic analysis of the Cook-McCormick core from the Heidelberg field reveals that quartz overgrowths are more abundant in sandstones without oil than those with oil. Oyster shells are found in the core, and calcite cement associated with those shells can completely occlude porosity, however, calcite cement is never present in sandstones with oil even when shells are present. Siderite is a locally significant authigenic iron-rich phase in the core (found in 5 of 40 thin sections); in contrast, pyrite is found in small amounts in all thin sections. Glauconite is also found in most samples. Live core samples of Eutaw Formation from the Heidelberg Field treated with nutrients and held in an anerobic chamber resulted in growth of biofilm.

Quantitative analysis of SEM photomicrographs shows that MPPM permeability modification occurs ubiquitously within pore and throat spaces of 10-20 μm diameter.

INTRODUCTION AND OBJECTIVES

As much as two thirds of the oil discovered in the U.S. (350 BBO) is economically unrecoverable with current enhanced oil recovery (EOR) technology (Brown et al, 2002). After thermal procedures, CO₂ flood is the most used EOR technique in the United States, responsible for almost 200 MBPOD in 1998 (Jarrell et al., 2002), even so, the recovery efficiency using CO₂ in oil reservoirs is low. Based on the experience of the industrial partner in this project (Denbury Resources, Inc.), in the study area approximately 20% of oil in place is produced initially, another 20% can be produced by water flooding, and approximately another 20% can be produced with carbon dioxide flooding. This leaves at least 40% of the original oil still in place in the reservoir.

The microbial permeability profile modification (MPPM) procedure, wherein the pathway of the injection water is redirected to unswept areas of the reservoir, therefore increasing the sweep efficiency of both the water flood and the carbon dioxide flood, was previously developed at Mississippi State University under DOE support and successfully field tested at North Blowhorn Creek Oil Unit (NBCU) in Lamar County Alabama (Stephens et al., 2000). MPPM involved adding compounds containing nitrogen and phosphorus (essentially dilute fertilizer) to the injection water of a conventional waterflood operation. These nutrients stimulate growth of naturally occurring, *in situ* microbes. Growth of the microbes reduces the flow of fluids in the originally most permeable zones, thus diverting water flow from these areas to less permeable thereby increasing the waterflood sweep efficiency.

The objective of this study is to combine carbon dioxide flooding with the microbial permeability profile modification (MPPM) procedure (Stephens et al., 2000) by first testing the procedure in the laboratory and then applying that knowledge in the field. The ultimate goal of this project is to significantly improve oil recovery at Eucutta Field, Wayne County, Mississippi by combining MPPM with conventional carbon dioxide flooding. A detailed understanding of the geology of the unit will help to gain a better understanding of the MPPM technology combined with CO₂ flooding technology and its applicability to other oil fields.

RESULTS AND DISCUSSION

Task 1.0 - Determine the Growth Characteristics of the Indigenous Microflora in the Eutaw Formation in Terms of Nutrient Supplements Necessary for Activation, Growth, and Tolerance to Carbon Dioxide

Most, if not all, of the microorganisms in oil reservoirs are dormant because vital nitrogenous and phosphorous-containing nutrients are missing and the bacteria are usually in the form of ultramicrobacteria (UMB). Furthermore, most of these microbes will be attached to the surface of the strata rather than be free-floating. This is the reason that isolation of cultures from production fluids is difficult. Furthermore, some of the most important microbes (*i.e.*, those needed in the project) may not be present in the fluids. Previous studies of the microflora of oil reservoirs conducted under DOE sponsorship (DOE Contract No. AC22-90BC14665) demonstrated that many of the indigenous microflora can grow under aerobic conditions, but *in situ* conditions are anaerobic. Essentially all of the indigenous microbes can use oil as a source of carbon and energy, and most of them can also use other compounds in place of oil. In many cases growth on other carbon sources, such as molasses, is faster than growth on oil.

After discussions with the operators of the Eucutta Oil Field, it was learned that because of the mechanics of the CO₂ flooding operation there will be very little time after introduction of the microbial permeability profile modification MPPM phase of the project to obtain an assessment of its ability to enhance the CO₂ flooding operation. While our laboratory work on the Eucutta Oil Field will continue there is an opportunity to proceed with a field demonstration of the impact of the MPPM on CO₂ flooding in another oil field-namely the Little Creek Oil Field, situated in Mississippi. Unfortunately, the temperature of the producing formation is 115°C which according to the literature is near the highest temperature at which microbial growth has been shown to occur. Nevertheless, samples of production water and oil were obtained from this field and tests have been conducted to determine if there are any viable microbes present that are capable of growing at 90°C, which is the highest temperature at which most of our studies can be conducted. The experimental design for the test of the Little Creek Oil Field production water was as follows and is the prototype to be used for samples from the Eucutta Field.

Due to a laboratory accident all of the samples using Eucutta production water were lost and the experiment was set up again as follows.

The culture vessels were 70 ml serum vials closed with grey rubber stoppers. Because the conditions in the reservoir are anaerobic, all work in the laboratory was conducted under anaerobic conditions and most of the work was performed in a Coy Glove Bag with incubation at 66 °C.

The atmosphere overlying the cultures was analyzed using a Fisher Model 1200 Gas Partitioner. This instrument is a dual column dual detector instrument. Column 1

was a 20' x 1/8" aluminum column packed with 37.5C-200/500 on 80/100 mesh chromosorb PAW. Column 2 was a 6' x 3/16" aluminum column packed with 60/80 mesh molecular sieve, 13X. The column temperature was 70°C and the injector temperature was 65°C. The carrier gas was helium and employed at a flow rate of 35 ml per min. All analyses were performed using a 50µl samples. Identification of gases was achieved by comparison of the retention time of peaks on the chromatogram to the retention time of standard gases. Quantification was accomplished by comparison of the area under the curve for a given gas to a standard curve prepared with known quantities of a pure sample of that gas. Specifically for this project, carbon dioxide production from the substrate is the objective. Ideally, samples of the formation are desired to obtain cultures but because samples are not yet available, production water and oil are being employed.

For this experiment, 20 ml samples of production water from the Eucutta Oil Field and two ml of oil from the same field were placed into each of 16 vials. To four of the vials was added 0.1 ml of KNO₃ solution (10g/100 ml H₂O) and 0.1 of K₂HPO₄ (5 g/100 ml H₂O). To another four vials 0.1 ml of molasses was added. Four of the vials contained only the production water and oil. An additional set of four vials contained only 20 ml of distilled water and oil.

After 163 days, the samples containing nitrogen and phosphorus showed a slight amount of carbon dioxide and the atmosphere above the samples containing molasses had twice as much carbon dioxide. This suggests some microbial activity although the carbon dioxide could have been produced abiotically. Microscopic examination later will determine if the carbon dioxide was produced by microorganisms. After nine months,

the only vials showing any CO₂ production were those vials containing molasses and there was no visual evidence of microbial cells in any of the vials.

Samples of cores were obtained from a well being drilled near Heidelberg, MS. The Heidelberg oilfield is located near the Eucutta oilfield and both produce from similar reservoir sequences within the Eutaw Formation. Heidelberg core samples from 4,773 ft and 4,779 ft are being tested for viable microorganisms using production water and oil from the Eucutta Oil Field supplemented with 0.05 mg KNO₃ and 0.025 mg K₂HPO₄. Incubation is at 66°C under anaerobic conditions. After 88 days, the atmosphere above the cultures contained carbon dioxide, suggesting that microbes were growing in the cultures. The production of CO₂ did not continue and there has been no visual evidence of microbial growth.

Samples of production water and oil from the Heidelberg Oil Field were received on November 8, 2006, and tests to determine the presence of viable microorganisms have been initiated. The protocol of the experiment is as follows. Twenty ml of production water from the Heidelberg Oil Field was placed into each of six 70 ml Wheaton vials. One ml of a solution containing 0.4 mg KNO₃ and 0.2 mg K₂HPO₄ was added to each vial. A small quantity (< 1 g) of crushed core from a depth of 4,773 ft added to each of three vials and a similar amount of crushed core from a depth of 4,779 ft added to the other three vials. All work was conducted in the COY Glove Bag under anaerobic conditions and incubated at 66°C. Good CO₂ production was noted in all vials. Microscopic examination suggested microbial growth but, what is believed to be microbial growth is at the limits of detection with the light microscope. Also, the presence of core material confuses observation of the samples. Therefore, the following

procedure was performed to enhance detection of microbial growth in the samples. Specifically, because of the extremely small size of the potential microbial cells, larger portions of the core material were filtered out and attempts to increase the size of the microbial growth was accomplished by using the flagella stain. The flagella stain was prepared by making a 1% solution of Leifson's Flagella Stain (BBL) in distilled water. Vials containing core material from a depth of 4773 ft and incubated at 66 C were vigorously vortexed and then passed through a 0.45 μ membrane filter and the filtrate passed through a 0.22 μ membrane filter. The material on the filter was suspended in distilled water, placed on a glass slide, and air dried. The slide was then flooded with the flagella stain until the precipitate was formed (10 min) in the flagella stain solution. The stain material was then washed from the slide, allowed to air dry, and then viewed with the microscope using the oil immersion lens. Examination of the slide revealed stained material believed to be microbial cells. The cultures prepared using core material from a depth of 4,779 ft also demonstrated the same thing.

Other samples (not filtered) from the same vial as above were stained with propidium iodide, which intercalates between the bases of the DNA and fluoresces red. Dr. Dyane Wise stained and then examined the sample using a confocal laser-scanning microscope.

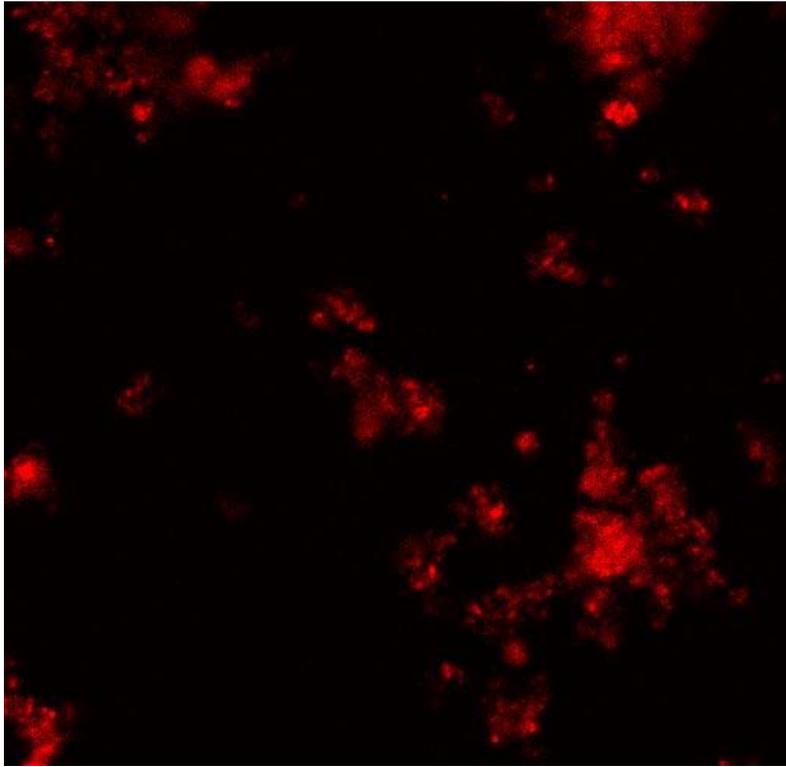


Figure 1. Sample of vial with core material from a depth of 4,773 ft. stained with propidium iodide.

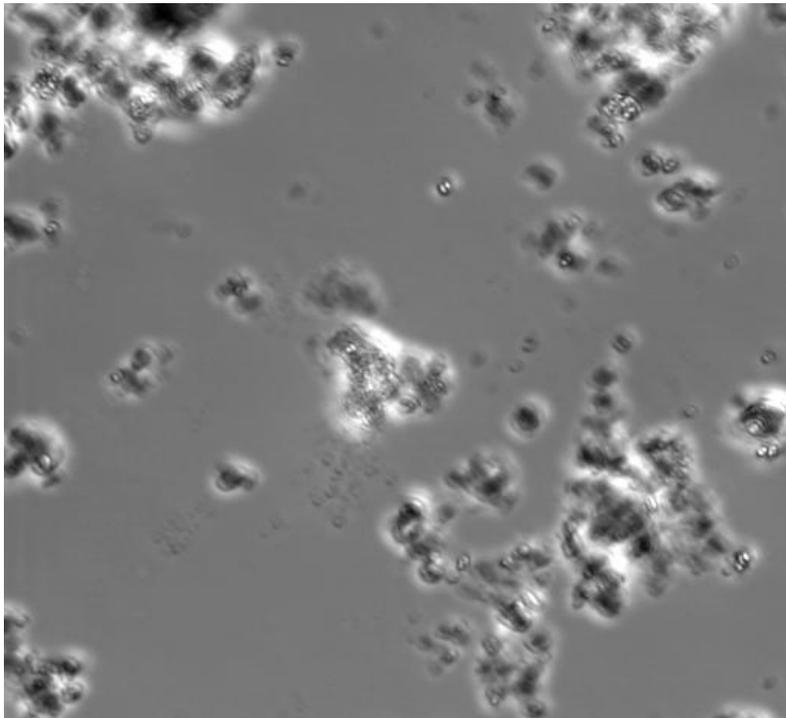


Figure 2. A duplicate image of Figure 1 above but viewed using differential interference contrast.

All of the enrichments were subcultured using a 10% inoculum and new enrichments were prepared using Heidelberg cores from 4,773 ft and 4,779 ft, Brookhaven production water and nitrogen and phosphorus nutrients. Incubation is at 66°C under anaerobic conditions.

All enrichments were streaked on an agar medium prepared as follows. Brookhaven production water (250 ml) was saturated with Brookhaven oil and 0.125 g of KNO₃ and 0.048 g K₂HPO₄ added. The pH was adjusted to 7.0 using 10% (w/v) KOH and 4.25 g of granulated agar added. The medium was sterilized in the autoclave at 121°C for 15 min and after cooling to 45°C, poured into 60 mm glass Petri plates in the anaerobic glove bag. After streaking with the enrichment cultures the plates were placed in sealed jars contained in 1-gal paint cans containing sand (to help maintain a constant temperature) and the cans placed in the 66°C incubator. [Note: the agar remains solid at this temperature.]

Work with cores from the Heidelberg field continued with incubation at 66°C under anaerobic conditions. The experiments described on page 9 of this report have been repeated with CO₂ production evident.

Figure 3 shows CO₂ production for crushed core material from 4,773 ft and Figure 4 shows CO₂ production for core material from 4,779 ft. The evidence suggests that the CO₂ production is the result of microbial action, particularly since we have been able to demonstrate that microbial cells are present. Nevertheless, other studies are in progress to insure that the CO₂ production is not abiotic.

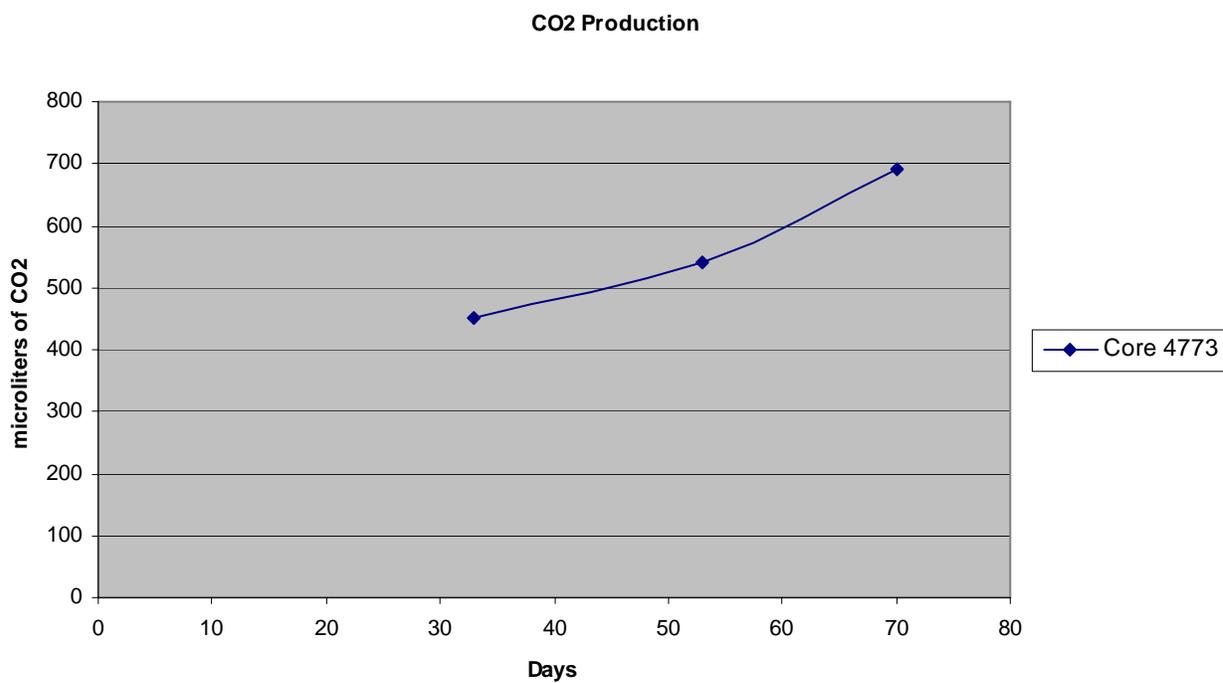


Figure 3. Carbon dioxide production by core material from the Heidelberg oil field at a depth of 4,773 feet.

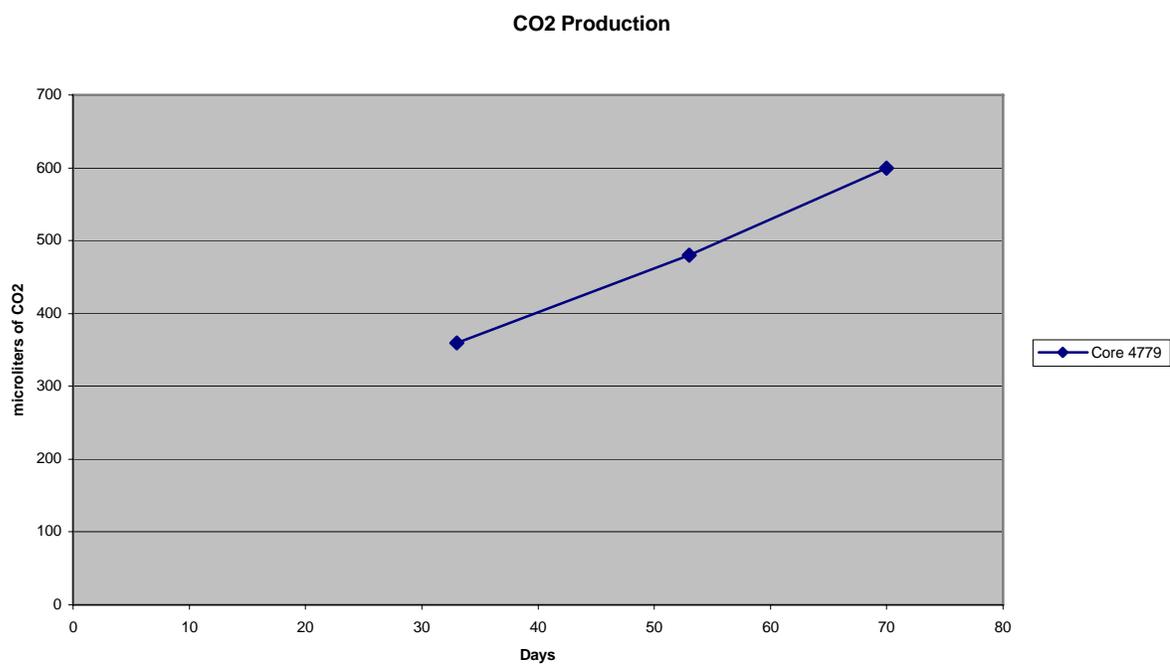


Figure 4. Carbon dioxide production by core material from the Heidelberg oil field at a depth of 4,779 feet.

Because it was proposed to conduct a field demonstration of the impact of MPPM on CO₂ flooding, it is desirable to determine if there are viable microorganisms in the producing formation at 115°C. As stated earlier, most of our studies cannot be conducted at this temperature since water will only be liquid at this temperature under pressure. A growth chamber that can be incubated at 115°C in an oven has been fabricated and tests have shown that the device should yield valid results (Fig. 6). Tests for microbes that will grow at 115°C have been initiated.

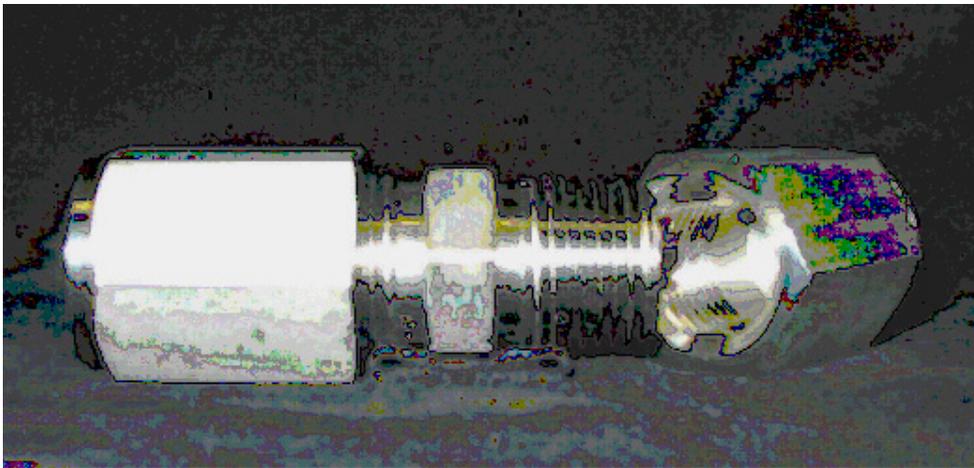


Figure 6. Device designed to act as a growth chamber that can be incubated in an oven at a temperature of 115°C.

The first attempt to utilize the newly developed devices for incubation at temperatures greater than 100°C caused the contents to leak out when the temperature was raised above 100°C, but corrections were made in handling the devices to prevent leakage. Samples of core material from the Brookhaven field were placed in some of the devices along with production water and oil and incubated for 50 days at 115°C. After incubation the contents were removed and slides of the contents prepared for staining. Figure 7 is a photograph of the contents of one of the devices after staining with

propidium iodide. It should be noted that the material stained red indicates the presence of DNA (see figure 7). The photographs in Figure 8 are samples from the same core material stained using another DNA stain. Tests are underway to determine whether the positive tests for DNA resulted from growth in the samples at 115°C or whether they were present in the material but had not grown.

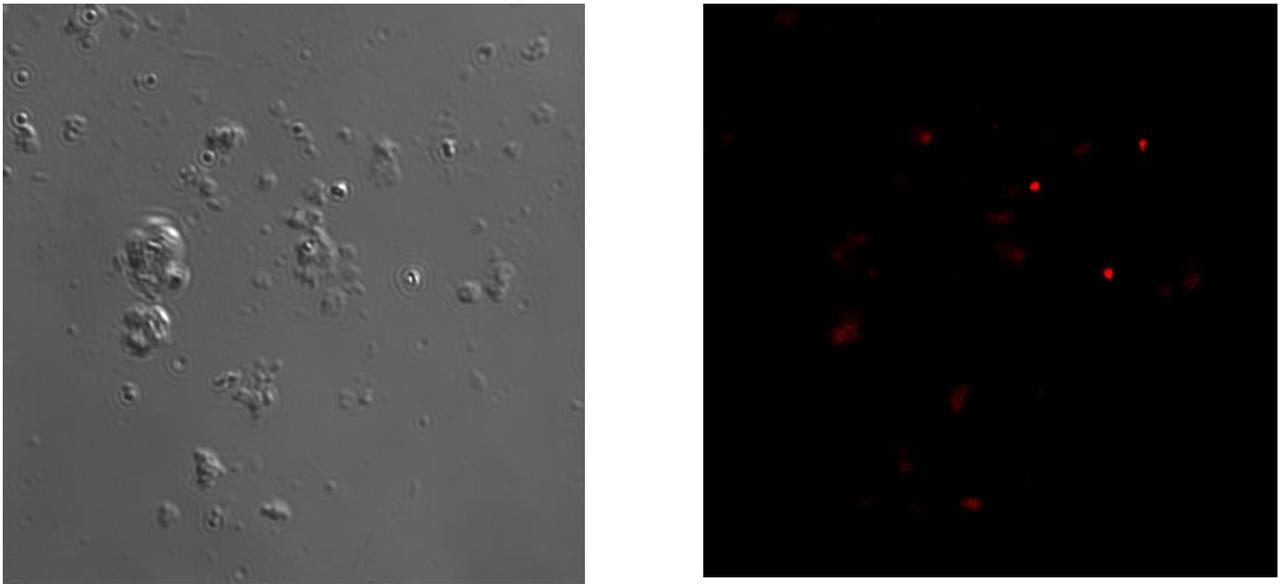


Figure 7. Photographs of core material incubated for 50 days at 115°C stained with propidium iodide. Photograph on the left is a photograph using the confocal laser scanning microscope with white light. Photograph on the right is the same slide viewed with ultraviolet light. (Red spots indicate DNA.)

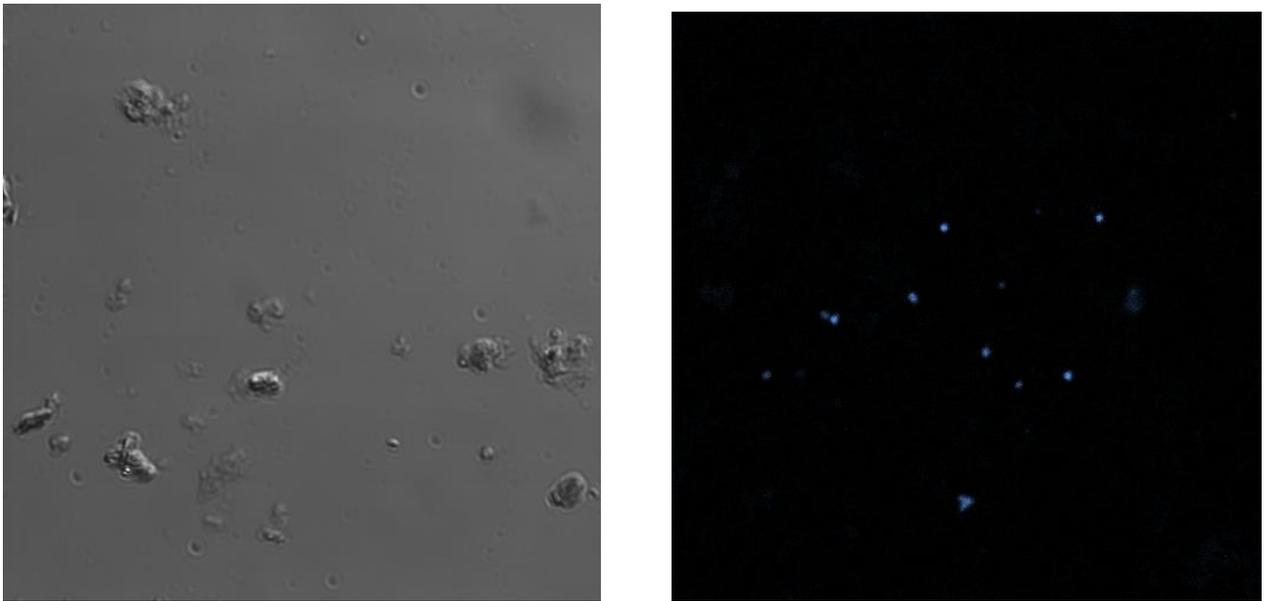


Figure 8. Photographs of core material incubated for 50 days at 115°C stained with DAPI (4'-6-diamidino-2-phenylindole). Photograph on the left is a photograph using the confocal laser scanning microscope with white light. Photograph on the right is the same slide viewed with ultraviolet light. (Blue spots indicate DNA.)

Over the course of this study, questions arose about the growth of microorganisms in the presence of high CO₂ concentrations. Samples of water from the Little Creek Oil Field, received on Oct. 11, 2007, had a pH of 5.3 and when CO₂ was bubbled into the water the pH went down to 4.2.

An experiment was conducted to determine the impact of a high CO₂ concentration on the microbial population in the Heidelberg cores. The cores had been incubated with oil, potassium nitrate, disodium hydrogen phosphate and injection water under anaerobic conditions at room temperature for months. Samples of the aqueous suspensions were mixed with Brookhaven water, placed in 70 ml Wheaton Vials, and incubated under aerobic conditions, anaerobic conditions, aerobic conditions with the atmosphere being predominately CO₂ or, anaerobic conditions with the atmosphere being

predominately CO₂. After incubation, plate counts using Tryptic Soy Agar and the spread plate technique were conducted with incubation under aerobic conditions for the two aerobic systems and under anaerobic conditions for the two anaerobic systems. Duplicate plate counts were done using triplicate plates at each dilution and the experiment was repeated a second time.

As may be seen in Table I, CO₂ reduced the number of cells from 9.1 x 10⁵ per ml to 0.3 x 10⁵ per ml or 96.7% under aerobic conditions. Under anaerobic conditions, CO₂ only reduced the number of cells from 8.8 x 10⁵ per ml to 3.2 x 10⁵ per ml or 63.6%. Therefore, while a high concentration of CO₂ does have a negative effect on microbial numbers, substantial numbers of microbes are not killed or prevented from growing. Furthermore, the impact of a high CO₂ concentration is less under anaerobic conditions (as is found in the subterranean oil stratum).

Table I. The Effect of Increased Carbon Dioxide on Microorganisms Under Both Aerobic and Anaerobic Conditions.

TEST	AEROBIC	AEROBIC WITH CO ₂	ANAEROBIC	ANAEROBIC WITH CO ₂
1	18.0*	0.3	14.0	4.6
1	13.0	0.3	13.0	4.3
2	2.7	0.3	3.6	1.8
2	<u>2.5</u>	<u>0.3</u>	<u>5.0</u>	<u>2.0</u>
AVG.	9.1	0.3	8.9	3.2

* Number of cells divided by 1 x 10⁵.

Task 2. - Testing the effectiveness of the designed demonstration procedure in laboratory core flood experiment.

In preparation for laboratory core flood experiments the existing equipment is being modified to accommodate for problems encountered with experiments carried out

at higher pressures and temperatures than the equipment was originally designed to handle. In previous flow tests under conditions of 300 psi annulus and at 90° C, confinement pressure (annulus) was lost after approximately 20 days due to core sleeve deformation. The sleeves were too long and the excess length had no physical support. In order to accommodate for this, 3/8 in Teflon support bushings were fashioned for each end, but the combined temperature and pressures caused the Teflon bushings to deform after about 30 days with a loss of annulus pressures again. Next a lathe was used to fashion stainless steel bushings, which will be used in the next round of flow tests.

Changing from various injection waters to liquid CO₂, in the next set of flow tests presents a new challenge. In-cylinder conditions for CO₂ run near 21°C and maximum pressure is approximately 700 psi. We expect to inject the liquid CO₂ through stainless steel tubing at approximately 200 psi into cores heated to 72°C. When the CO₂ enters the oven it changes phase and expands. Using the stainless steel tubing, we have created coiled expansion zones for the injection circuit of roughly 40 ft in length for each foot of the circuit outside the oven. Calculations show a 36.8/1 volume ratio of liquid to vapor CO₂, segued within the injection circuit from pre-oven to oven temperatures, viz. 22 to 80°C.

Two additional safety valves were needed and had to be designed and installed. First, a diode valve at the tank will allow CO₂ to flow in the direction of injection, but restrict back flow, due to gas expansion, at 19,000 psi. Second, a pop-off valve rated at about 100 psi above the annulus pressure will be inserted into the circuit parallel to the expansion zone as a safety precaution. Valves already available in the lab were used to make these modifications to the existing equipment.

The core flood equipment has been repaired and is operable. The first tests were conducted at 65°C (the temperature of the oil-producing stratum at the Heidelberg field is 66°C) on cores from a depth of 4,779 feet. All tests consist of a test core and a control core. The control core had simulated injection water pushed through the core while the test core was flooded with simulated injection water plus potassium nitrate followed by simulated injection water plus disodium hydrogen phosphate. On day 36 both cores were flooded with liquid CO₂. As may be seen in Figure 9 oil was produced from the test core while no oil was produced from the control core during the water flooding operation.

The experiment is being repeated with the exception that a dilute solution of ethanol is also being used to treat the test core.

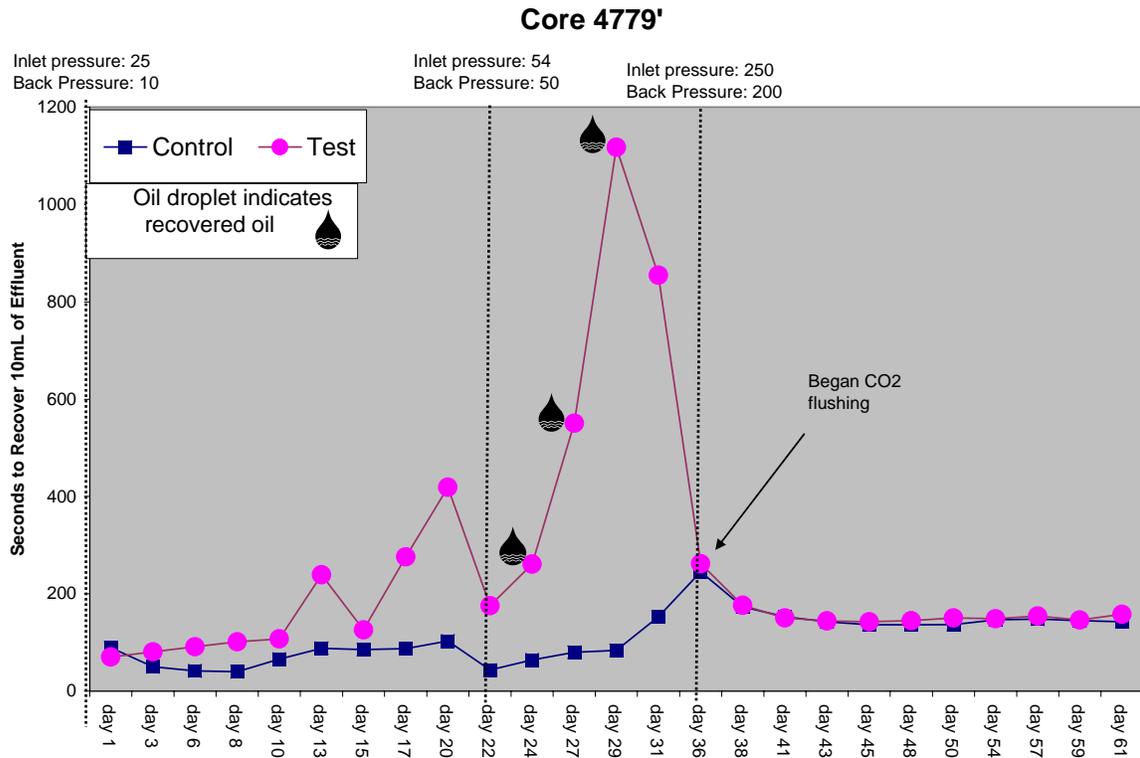


Figure 9. Effluent from cores from the Heidelberg field (4,779 feet) being treated in the core flood facility.

Task 3.0 – Detailed analysis of Eutaw Formation mineralogy and porosity using standard, SEM, and confocal microscopy, X-ray diffraction, and high-resolution CT imagery.

The geological and biological team at MSU have held multiple telephone conferences and met in person three times with Denbury reservoir engineers and geologists. With the assistance of a team of Denbury Resources personnel, live core from an active drilling site near Laurel, Mississippi was obtained by the MSU geological and biological team. This locality, the Heidelberg Field, was chosen because of its proximity and geologic similarity to the Eucutta Field (Figure 10). Samples were taken within minutes after the core reached the surface and were returned as quickly as possible to anaerobic conditions for storage. Part of these cores were used for laboratory core flood experiments and part were used to document growth of *in situ* bacteria to be imaged in SEM and with high-resolution CT imagery as described below.

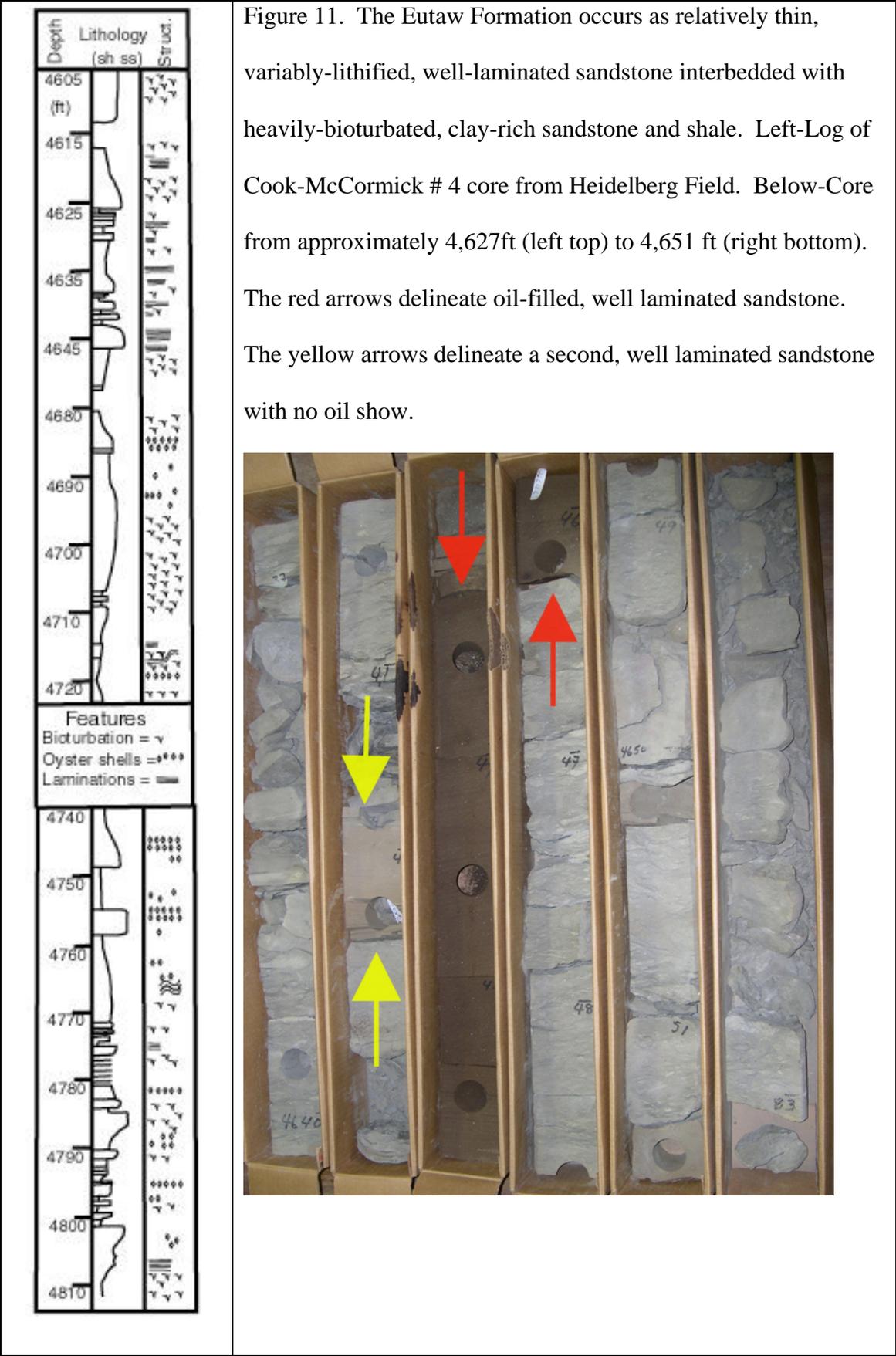
Figure 10. MSU geologists were on site in the summer of 2006 while approximately 400 ft of core was taken from this Eutaw Formation well near Laurel, MS. Fourteen samples of live core (approximately 8 in by 4 in) were acquired and placed in anaerobic storage at the drill site. Sample locations were based on electric log interpretation, drillers' reports, and visual inspection of the ends of the core.



Denbury Resources, Inc. provided the MSU team over 200 ft of slabbed reservoir core from Cook-McCormick #4 well in the Heidelberg field near Laurel, Mississippi. Core photos and logs and petrographic images from that highly-lithified, well-recovered material are shown below. In comparison, material available from the Eutaw Formation at the Eucutta oilfield is unlithified rubble and drillers' reports indicate extremely poor recovery.

In the Heidelberg Field the Eutaw Formation occurs as upward coarsening paralic sandstones and siltstones. Sandstone layers at the bottom of the studied core are only inches thick and very-fine grained; these relatively clay-poor rocks are surrounded by heavily bioturbated siltstones and shales. In the upper portion of the 150 ft core the relative amount of usually fine- to medium-grained laminated sandstone increases and the units can be as thick as 5 to 6 ft, again separated by siltstones and shales (Figure 11). The

framework composition of the entire section is subarkosic, and glauconite, chert, and muscovite are abundant. Future core flood tests will be performed on plugs of the Cook-McCormick core to determine the effects of CO₂ on the different iron-rich minerals (pyrite, siderite, glauconite).



In the upper portion of the core, oil occurs almost exclusively in sandstones thicker than 3 or 4 ft. In the sandstone-poor lower section of the core oil occurs in sandstones just a few inches thick. The first significant burial diagenetic minerals present in all the sandstones are quartz overgrowths, which are more abundant in sandstones without oil than those with oil. Oyster shells are found in many of the sandstones, and calcite cement associated with those shells can completely occlude porosity, however, calcite cement is never present in sandstones with oil even when shells are present (Figure 12). These relations imply that the migration of oil into the sandstones stopped porosity-occluding diagenesis.

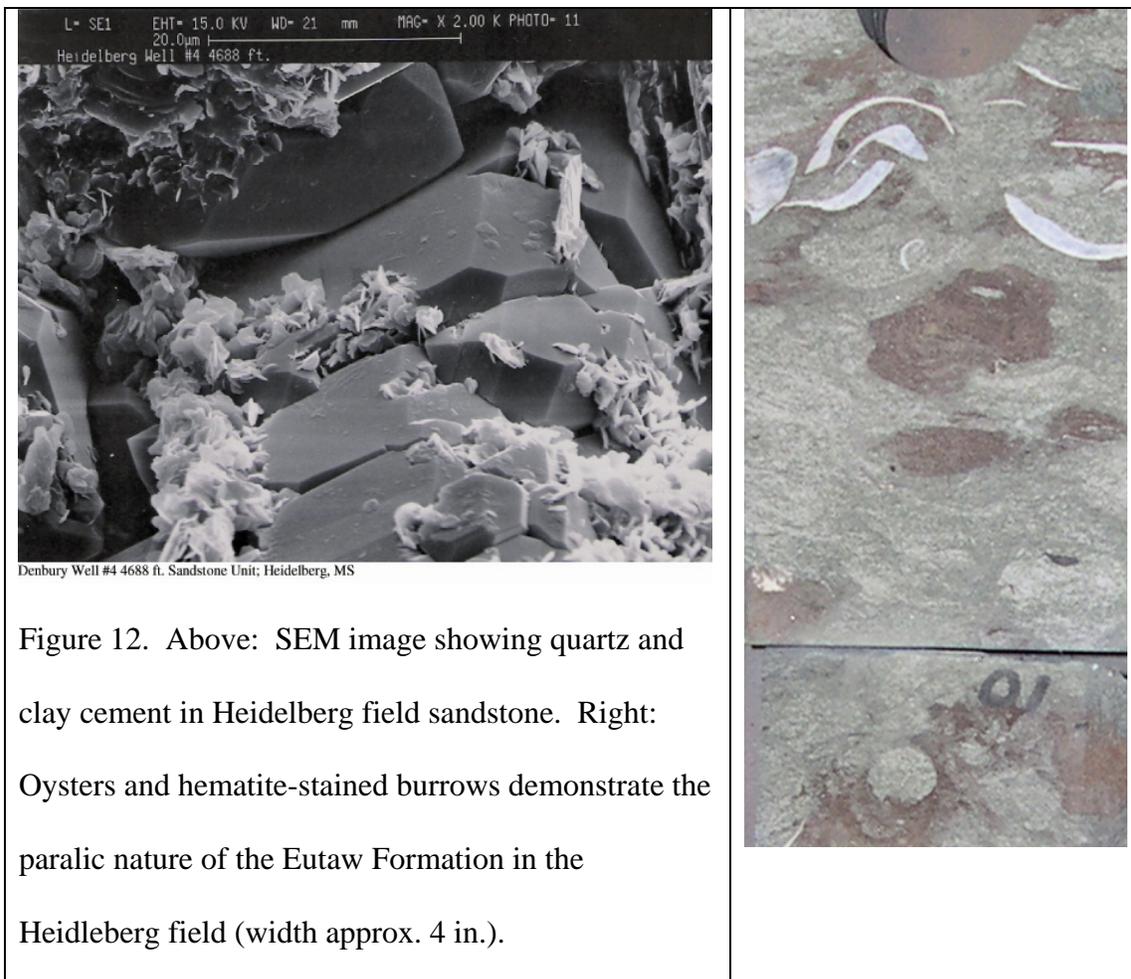


Figure 12. Above: SEM image showing quartz and clay cement in Heidelberg field sandstone. Right: Oysters and hematite-stained burrows demonstrate the paralic nature of the Eutaw Formation in the Heidelberg field (width approx. 4 in.).

Alteration of glauconite (Figure 13) in the burial diagenetic environment is relatively minor and siderite is locally abundant (Figure 12 and 14). This is in contrast to alteration of Eutaw Formation outcrops in the northern part of the state in which glauconite is extensively altered and iron oxides and hydroxides are the most common cements (Figure 15).

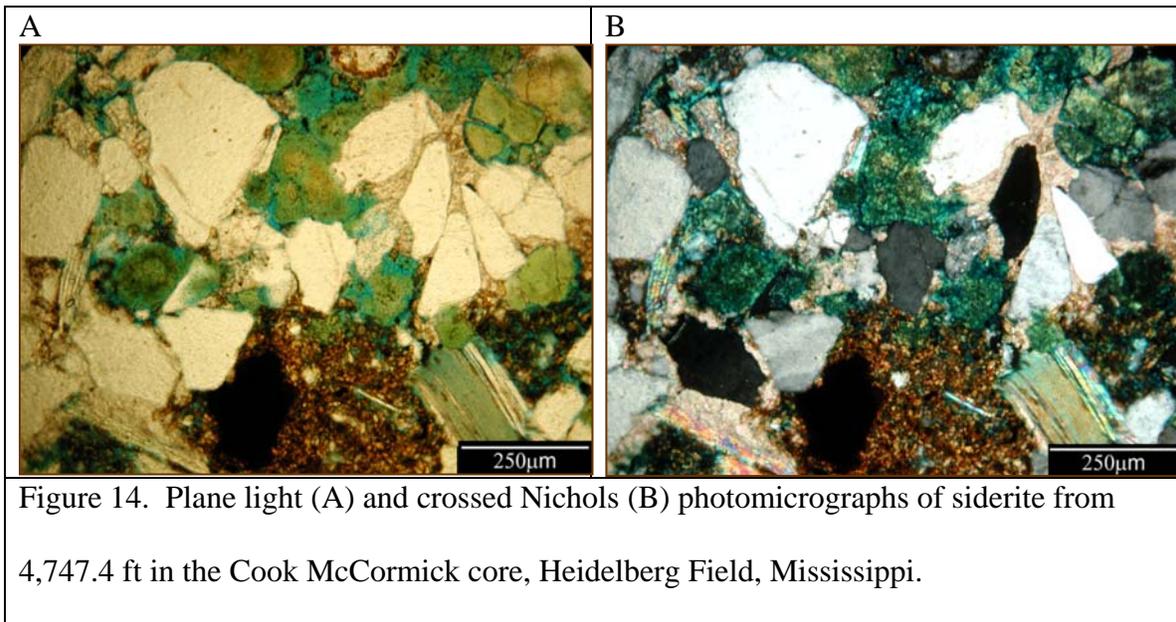
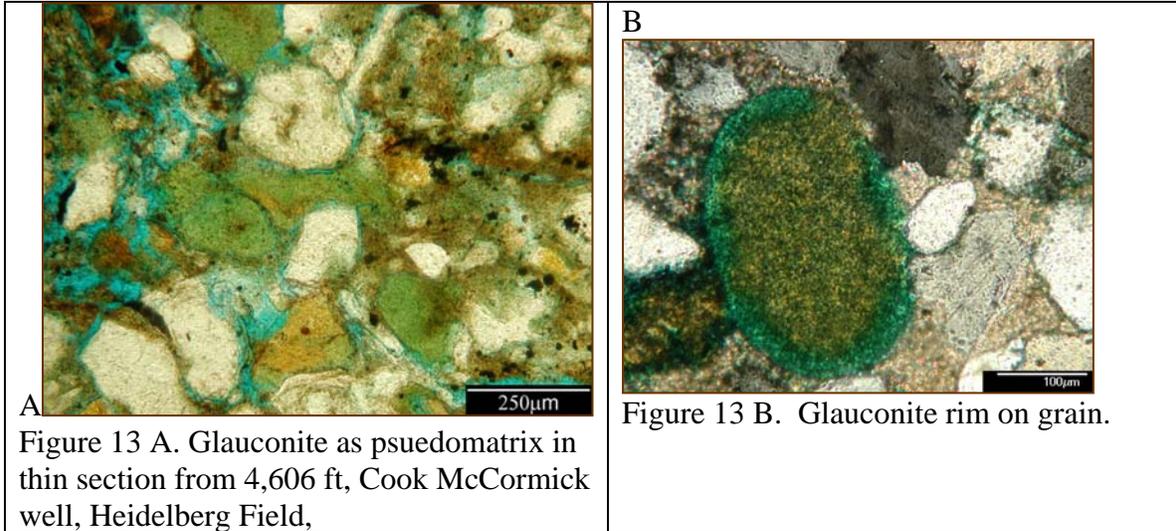
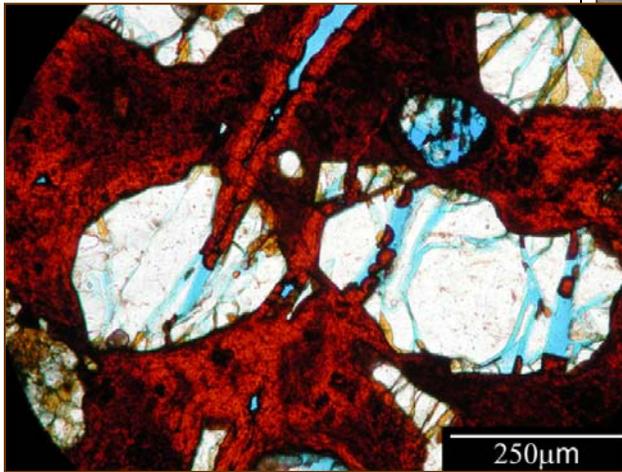


Figure 15. Comparison to Eutaw Formation outcrops in northeastern Mississippi reveals that in outcrop glauconite is altered to iron oxide as shown in thin section below.



The near ubiquitous nature of the glauconite, a poorly crystalline iron-rich clay, in the reservoir, may be problematic. Iron-rich minerals, particularly chlorite, react adversely when exposed to acid; they dissolve and re-precipitate as an amorphous Fe-Si gel, thus ruining the reservoir. This phenomenon has not been identified with glauconite or siderite, another iron-rich phase found in the Heidelberg core; however, the reaction between these minerals and carbon dioxide flood has never been investigated. These reactions may affect the efficiency of the carbon dioxide MPPM procedure and will be investigated by the laboratory simulations.

In order to find the best combination for imaging the distribution of living biofilm within the core samples we tested multiple sample preparation techniques. Samples (below) were kept in anaerobic conditions, immersed in solutions of different

combinations of injection brine pH 7, sodium phosphate, potassium nitrate, molasses and cultured bacteria. After 16 days, samples were fixed in 2.5% gluteraldehyde in 0.1M phosphate buffer, pH 7.2. They were then post fixed in 2% osmium tetroxide, dehydrated through a graded acetone series, followed by HMDS and air-drying overnight. The dried specimens were then mounted on aluminum stubs, coated with Au/Pd of 30nm thickness, and viewed on a JEOL JSM 6500F at 5kV.



Figure 16. Live core samples were cultivated within an anaerobic chamber in the MSU Department of Biology. These samples were used to image the distribution of microbes and biofilm in SEM and with high-resolution CT imagery.

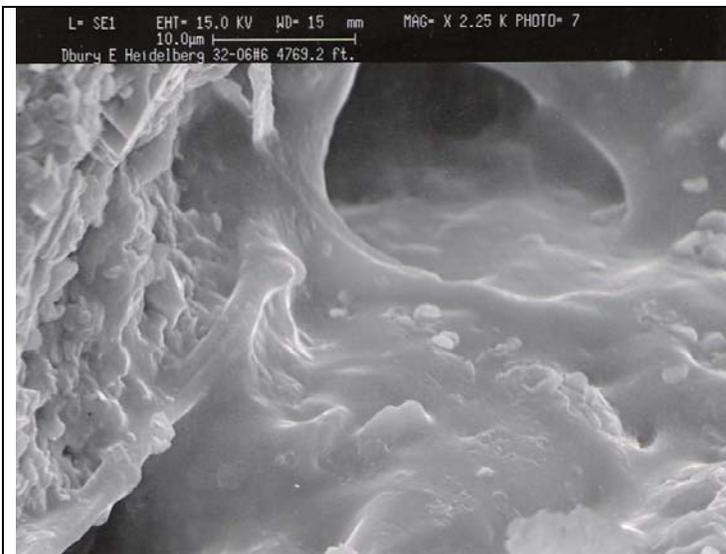


Figure 17. This SEM photomicrograph illustrates a piece of live core treated with nutrients in the anaerobic chamber illustrated above. Mucilage lines most of this pore.

High-resolution CT imagery carried out at the Center for advanced Vehicular Systems on the on the MSU campus resulted in 3-D images of core. We were able to obtain very high resolution images with sub-micro scale resolution, by using a very small (mm-scale sample). We also learned that the grains and porosity had adequate density contrast to produce a viable data set. This work was carried out in preparation for work to be carried out at the Center for Nanophase Materials Sciences at the Oak Ridge National Laboratory to use neutron scattering to create very high-resolution images of pore networks and microbial growth. We are currently in correspondence with experts at Oak Ridge regarding the best possibilities for creating these images.

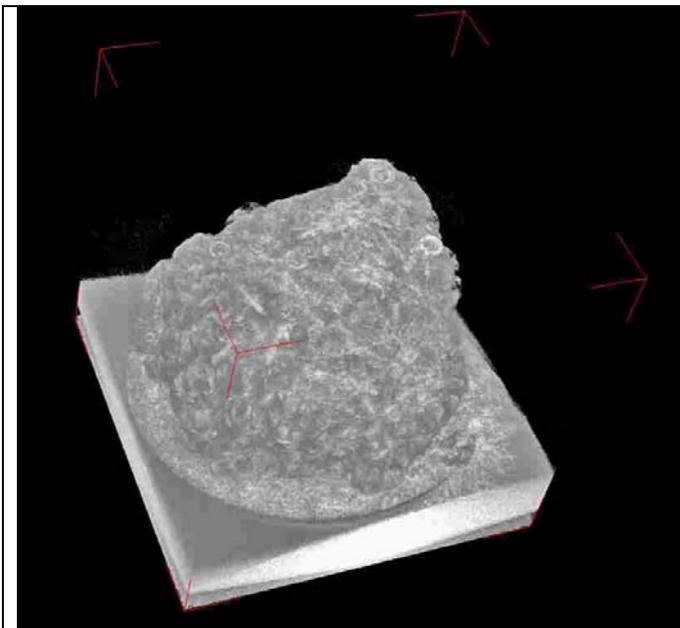


Figure 18. Three dimensional image of fragment of sandstone sample. Sample is approximately 5 mm in diameter.

Extensive data has been collected using scanning electron microscopy and analyzed quantitatively to determine the nature and distribution of biofilm in core. Particle edges appear as grayscale intensity maxima on SEM micrographs and are countable as number of spikes (N) over chord-length (d). Analysis of grayscale intensity

maximums in SEM images of pores in dry core samples from the Eutaw Formation yields a fractal distribution. (Each micrograph is one data point on the graph shown in Figure 20.) Identical assessment of grayscale intensity maximums over biofilm coated core samples and meniscus features within these samples yields a non-fractal distribution of data points, and the 10 μm – 20 μm data conspicuously fall out of the distribution. As the mucilage spreads itself between particles and across throat pores, its meniscus feature has no edges, i.e., no intensity spikes, and appears smooth. Meniscus features stretched >20 μm tend to pinch out. Based on both qualitative and quantitative analysis of photomicrographs, bacterial mucilage does not completely fill porosity, but does clog pore throat spaces of 10 to 20 μm in diameter, thus significantly impacting porosity.

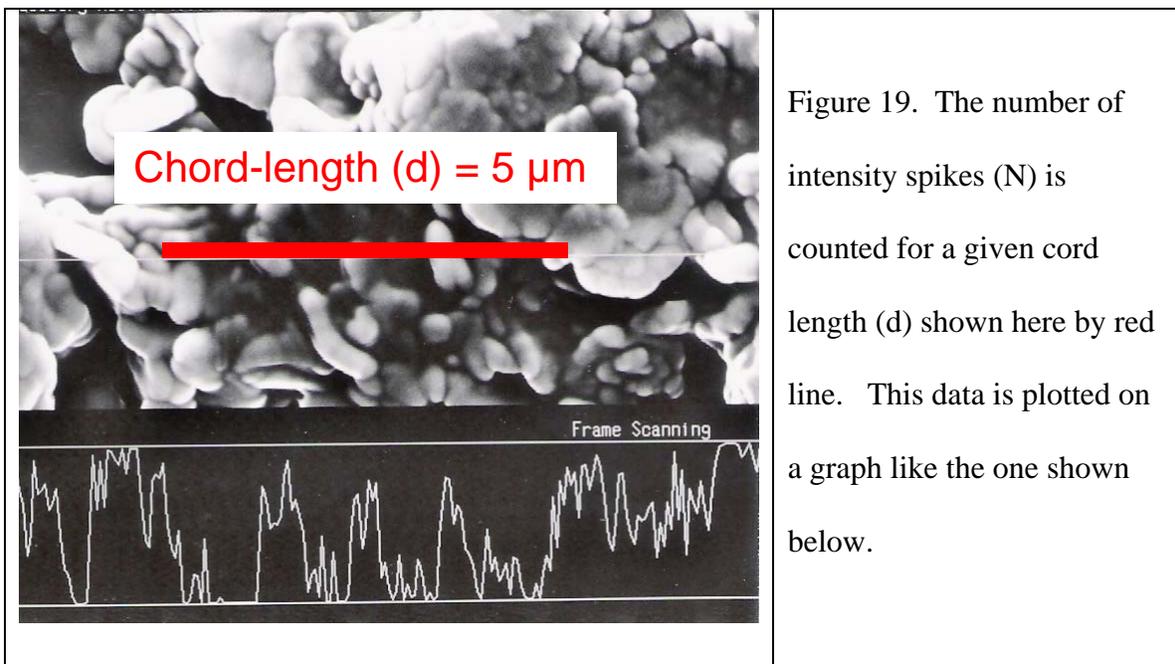


Figure 19. The number of intensity spikes (N) is counted for a given cord length (d) shown here by red line. This data is plotted on a graph like the one shown below.

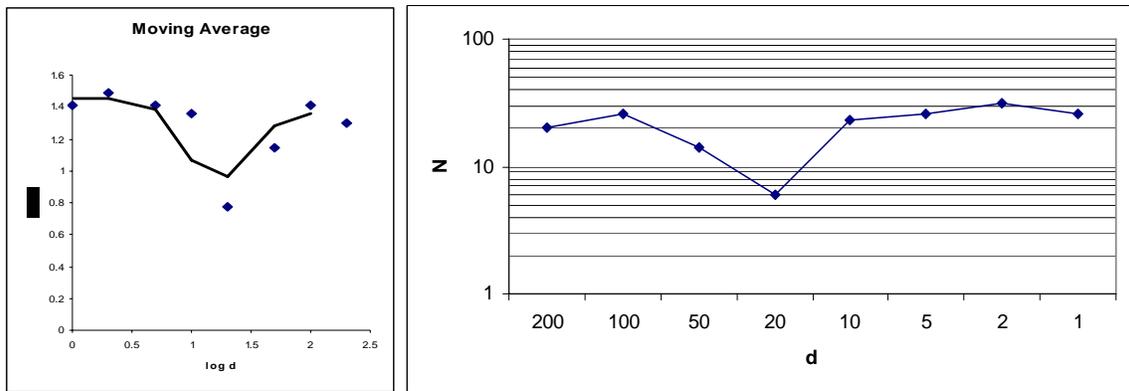
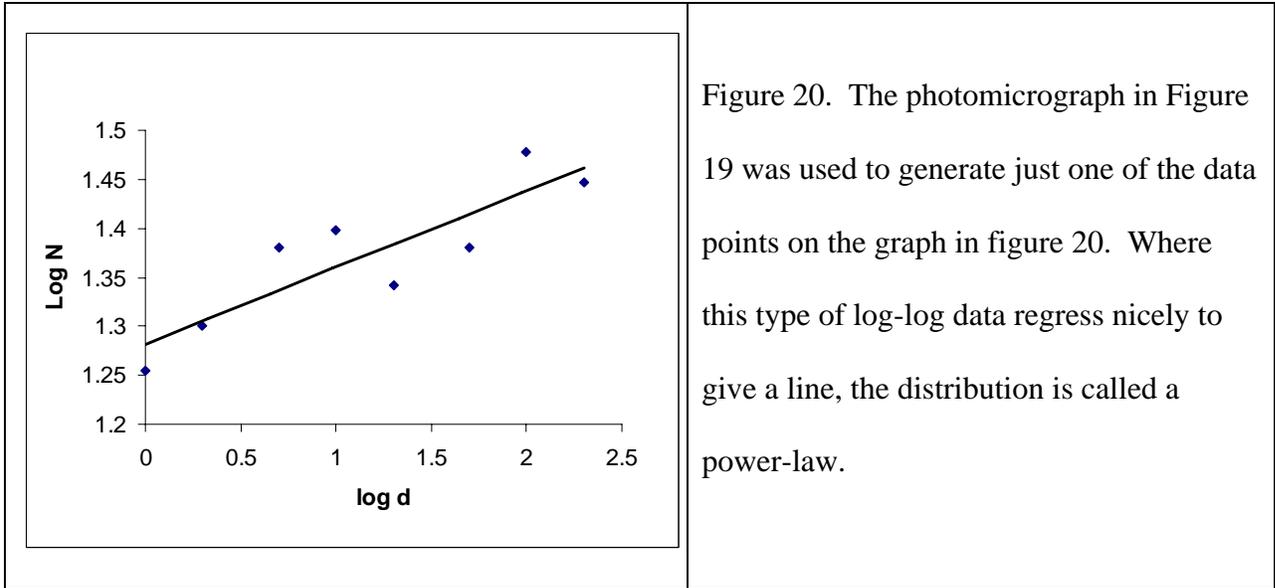


Figure 21. MPPM sample yields an altered edge-feature distribution.

Task 4.0 - Formulation of the protocols for the Demonstration Phase of the project (Decision Point).

Work on tasks 1-3 is nearing completion.

Task 5.0 - Addition of MPPM Protocols to CO₂ sweep in the Eucutta Field.

Meetings are scheduled to take place in January 2008 between the MSU researchers and staff of the Little Creek Field Office to discuss procedures for beginning

the demonstration phase of the project in the Little Creek Field. Depending on the results of this experiment and progress with production in the Eucutta Field, a second demonstration experiment in the Eucutta Field may begin at a later date.

Task 6.0 - Final Report Preparation

Recording of methodologies, data, and collection of references for the final report is ongoing.

SUMMARY

The enrichment cultures suggest that microorganisms are present in the samples. Cores obtained from a newly drilled well in the Heidelberg Oil Field and laboratory tests suggest that they contain microorganisms that grow at 66°C. A device has been fabricated that will allow us to determine if viable microorganisms exist in samples from formations at temperatures greater than 100°C and tests suggest that it will be satisfactory in testing for microorganisms growing above 100°C.

The Eutaw Formation occurs as relatively thin, variably-lithified, well-laminated sandstone interbedded with heavily-bioturbated, clay-rich sandstone and shale. Petrographic analysis reveals that quartz overgrowths are more abundant in sandstones without oil than those with oil. Oyster shells are found in many of the sandstones, and calcite cement associated with those shells can completely occlude porosity, however, calcite cement is never present in sandstones with oil. Live core samples of Eutaw Formation from the Heidelberg Field treated with nutrients and held in an anaerobic chamber resulted in growth of biofilm. This technique can be used to prepare samples for SEM and CT imagery. Quantitative analysis of SEM photomicrographs shows that MPPM permeability modification occurs ubiquitously within pore and throat spaces of 10-20 μm diameter.

REFERENCES

Brown, L.R., Azadpour, A., and A.A. Vadie, 1992, A study of the interactions between microorganisms, microbial by-products, and oil-bearing formations: Final report on DOE Contract No. AC22-90BC14665.

Jarrell, P. M., Fox, C. E., Stein, S. L., and S. L. Webb, 2002, Practical aspects of CO₂ flooding: Society of Petroleum Engineers Monograph Volume 22, 220 p.

Stephens, J. O., Brown, L. R., and A. A. Vadie, 2000, The utilization of microflora indigenous to and present in oil-bearing formations to selectively plug the more porous zones thereby increasing oil recovery during waterflooding: Final Report on DOE Contract No. DE-FC22-94BC-14962.