Oil & Natural Gas Technology

DOE Award No.: DE-FC26-06NT15458

Final Report

Improvement of Carbon Dioxide Sweep Efficiency by Utilization of Microbial Permeability Profile Modification to Reduce the Amount of Oil Bypassed During Carbon Dioxide Flood

Submitted by:
Darrel Schmitz, Lewis R. Brown, F. Leo Lynch (posthumous), Brenda L. Kirkland,
Krystal M. Collins, and William K. Funderburk

Department of Biological Sciences and Department of Geosciences
Mississippi State University
Mississippi State, MS 39762

Denbury Resources, Inc.
5320 Legacy Drive
Plano, TX 75024

Prepared for:
United States Department of Energy
National Energy Technology Laboratory

Office of Fossil Energy

DISCLAIMER

ABSTRACT

The objective of this project was to couple microbial permeability profile modification (MPPM), with carbon dioxide flooding to improve oil recovery from the Upper Cretaceous Little Creek Oil Field situated in Lincoln and Pike counties, MS. This study determined that MPPM technology, which improves production by utilizing environmentally friendly nutrient solutions to simulate the growth of the indigenous microflora in the most permeable zones of the reservoir thus diverting production to less permeable, previously unswept zones, increased oil production without interfering with the carbon dioxide flooding operation. Laboratory tests determined that no microorganisms were produced in formation waters, but were present in cores. Perhaps the single most significant contribution of this study is the demonstration that microorganisms are active at a formation temperature of 115°C (239°F) by using a specially designed culturing device. Laboratory tests were employed to simulate the MPPM process by demonstrating that microorganisms could be activated with the resulting production of oil in coreflood tests performed in the presence of carbon dioxide at 66°C (the highest temperature that could be employed in the coreflood facility). Geological assessment determined significant heterogeneity in the Eutaw Formation, and documented relatively thin, variably-lithified, well-laminated sandstone interbedded with heavily-bioturbated, clay-rich sandstone and shale. Live core samples of the Upper Cretaceous Eutaw Formation from the Heidelberg Field, MS were quantitatively assessed using SEM, and showed that during MPPM permeability modification occurs ubiquitously within pore and throat spaces of 10-20 μm diameter. Testing of the MPPM procedure in the Little Creek Field showed a significant increase in production occurred in two of the five production test wells; furthermore, the decline curve in each of the production wells became noticeably less steep. This project greatly extends the number of oil fields in which MPPM can be implemented.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

The objective of this project was to determine if microbial permeability profile modification (MPPM), which was developed under DOE sponsorship to enhance oil production from a rapidly declining oil field (North Blowhorn Creek Oil Field) undergoing water flooding, could be applied to an oil field undergoing carbon dioxide flooding. The Little Creek Oil Field situated in Lincoln and Pike counties, MS was selected for the demonstration.

Cores were obtained from the Upper Cretaceous portion of the Heidelberg Oil Field, situated in Jasper County, MS, and tested for the presence of microorganisms. Although the temperature of the stratum from which the cores were obtained was 115ºC, the presence of microorganisms that grew anaerobically at 66ºC on molasses was shown by an increase in deoxyribonucleic and (DNA).

In order to demonstrate the presence of microorganisms in the cores that could grow at 115ºC, a special culturing device was devised to maintain water in a liquid state at 115ºC. Using this culturing device with oil as the substrate and incubation at 115ºC for 50 days, the growth of microorganisms was shown by an increase in DNA in the samples. The presence of DNA in samples was shown using the two different DNA stains.

Because the oil field was using CO_2 flooding as the secondary recovery method, the question of the impact of CO_2 on microbial growth was examined. Tests were conducted under both aerobic and anaerobic conditions and it was found that high levels of CO_2 did reduce microbial growth 96.7% aerobically (from 9.1×10^5 to 3.0×10^4) but only 63.69%, anaerobically (from 14.0×10^5 to 3.2×10^5). Equipment used to conduct core flood experiments was modified to accommodate experiments carried out at high temperatures and high carbon dioxide pressures and demonstrated that microorganisms could be activated and resulted in the production of oil in coreflood tests performed at 66˚C.

Geological analyses were carried out on the Cook-McCormick core from the Heidelberg Field. The Upper Cretaceous Eutaw Formation occurs as relatively thin, variably-lithified, well-laminated sandstone inter-bedded with heavily bioturbated, clay-rich sandstone and shale. Petrographic analysis of the Cook-McCormick core 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 of those samples shows that MPPM permeability modification occurs ubiquitously within pore and throat spaces of 10-20 μm diameter.

Testing of the MPPM procedure in the Little Creek Field was carried out between December 2008 and October 2010. After approximately nine months, a significant increase in production occurred in two of the five production test wells; furthermore, the decline curve in each of the production wells became noticeably less steep. The test was terminated in October 2010, due to operational issues unrelated to nutrient injection. The field test of the MPPM procedure in association with carbon dioxide flood was successful and the MPPM procedure has the potential to increase production if implemented in other fields.

REPORT DETAILS

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, CO_2 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 CO_2 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.

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) because of this lack of vital nutrients. Furthermore, most of these microbes will be attached to the surface of grains and cements in the rocks rather than be free-floating. This is the reason that isolation of cultures from production fluids is difficult if at all possible. Furthermore, some of the most important microbes (*i.e.*, those needed in this 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.

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 water flood 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 water flood 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 an active oil field. The goal of this project was to significantly improve oil recovery by combining MPPM with conventional carbon dioxide flooding. The project was originally focused on Eucutta Field, Wayne County, Mississippi, but was shifted to Little Creek Field, Pike County, Mississippi. A detailed examination of the geology of the producing interval was carried out to help gain a better understanding of the MPPM technology combined with CO_2 flooding technology and increase its applicability to other oil fields.

EXPERIMENTAL METHODS

Tests of Water from the Eucutta Oil Field

After discussions with the operators of the Eucutta Oil Field, it was learned that because of the mechanics of the CO_2 flooding operation there would 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 CO_2 flooding operation and the laboratory experiments were transferred to the study of the Little Creek Oil Field.

Complications arose during the conduct of the tests but no evidence of microbial growth was evident in any of the samples and no further work was conducted with water from the Eucutta Oil Field.

Tests of Water from the Little Creek Oil Field

Unfortunately, the temperature of the producing formation in the Little Creek Oil Field 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.

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 which was the highest temperature at which the samples could be incubated in the hood.

For this experiment, 20 ml samples of production water from the Little Creek 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 KNO_3 solution (10g/100 ml H_2O) and 0.1 of K_2HPO_4 (5 g/100 ml H_2O). 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.

Cores from the Heidleberg Oil Field

Samples of cores were obtained from a well being drilled near Heidelberg, MS and kept under anerobic conditions. The Heidelberg Oil Field is located near the Eucutta Oil Field and both produce from similar reservoir sequences within the Upper Cretaceous Eutaw Formation. The temperature of the oil stratum from which oil is produced from the Heidelberg Oil Field is 115˚C. Conventional microbiological techniques could not be employed to demonstrate growth of microorganisms from the cores at that temperature. Therefore, tests for growth were carried out at 66˚C. Heidelberg core samples from 4,773 ft and 4,779 ft were tested for viable microorganisms using production water and oil from the Eucutta Oil Field supplemented with 0.05 mg KNO_3 and 0.025 mg K_2HPO_4. Incubation was carried out at 66°C under anaerobic conditions.

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 were initiated. The protocol of the experiment was 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 KNO_3 and 0.2 mg K_2HPO_4 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.

Flagella stains were prepared using 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 66ºC were vigorously vortexed and then passed through a 0.45µ membrane filter and the filtrate passed through a 0.22µ 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.

Deoxyribonucleic acid stains were prepared using propidium iodide by Dr. Dwayne Wise of the Biological Sciences Department at Mississippi State University. 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 KNO_3 and 0.048 g K_2HPO_4 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 above were repeated with CO_2 production evident.

Tests to demonstrate that microbial growth could occur at 115˚C required a special culturing device to maintain water in the liquid state. This device consisted of a 4 cm piece of threaded stainless steel pipe fitted on both ends with a steel screw cap. The pipe had an inside diameter of 1.1 cm and the device contained approximately 10 ml of sample.

Core Flood Experiments Using Cores from Heidelberg Oil Field

In preparation for laboratory core flood experiments the existing equipment was 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 were used in the next round of flow tests.

Changing from various injection waters to liquid CO_2, in the next set of flow tests presented a new challenge. In-cylinder conditions for CO_2 run near 21°C and maximum pressure approximately 700 psi. We injected the liquid CO_2 through stainless steel tubing at approximately 200 psi into cores heated to 72°C. When the CO_2 entered 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 showed a 36.8/1 volume ratio of liquid to vapor CO_2, 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 CO_2 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 were 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.

Field Experiments

Dr. Lewis Brown met with the Little Creek Field team from Denbury Resources, Inc. to discuss procedures for beginning the demonstration phase of the project in the Little Creek Field. Application of nitrates/phosphates to the reservoir to revive the dormant microflora was designed to allow slight growth, in order to redirect the injected CO_2 into unswept areas of the reservoir, thereby increasing the sweep efficiency of the CO_2 flooding operation. In order to carry out this field test, the Little Creek team submitted a request for a new line item for the Development Schedule for $400M. This provided funds for changing out the injection string, installing a short, connecting water line to the well and provided the start-up chemicals needed for this year-long project.

The team chose a well in a pattern with two other injectors and five producers, two of which were shut-in. This injection well had to have its current tubing and packer replaced in addition to changes to the well head and acquisition of a new tank for mixing.

Gas Analysis

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.

Detailed analysis of Eutaw Formation mineralogy and porosity using petrography and SEM and high-resolution CT imagery

The geological and biological team at MSU held multiple telephone and in person conferences 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 1). 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 1. MSU geologists were on site in the summer of 2006 while approximately 400 ft of core were 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 approximately 200 ft of slabbed reservoir core from Cook-McCormick #4 well in the Heidelberg Field near Laurel, Mississippi. Core photos, 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 was documented as unlithified rubble and drillers' reports indicated extremely poor recovery.

Detailed analysis of Eutaw Formation mineralogy and porosity using SEM and high-resolution CT imagery

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 (Figure 16). 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 (Fratesi et al, 2004). High-resolution CT imagery carried out at the Center for Advanced Vehicular Systems on the on the MSU campus.

RESULTS

Tests of Water from the Little Creek Oil Field

After 163 days, the sample of water from the Little Creek Oil Field with added nitrogen and phosphorus showed a slight amount of carbon dioxide and the atmosphere above the samples containing molasses had twice as much carbon dioxide. Therefore, it is possible that there might have been some microbial activity although the carbon dioxide could have been produced abiotically. In other tests after incubation for nine months, the only vials showing any CO_2 production were those vials containing molasses and there was no visual evidence of microbial cells in any of the vials. Therefore, the CO_2 must have been produced abiologically. This was not unexpected since most, if not all, of the microorganisms in the reservoir are attached to the rock and absent in the water from the well.

.

Core Samples from the Heidleberg Oil Field

Good CO_2 production was noted in all vials containing core material. Microscopic examination suggested microbial growth but, what was believed to be microbial growth was at the limits of detection with the light microscope and therefore of questionable value.. Also, the presence of core material makes observation of the samples difficult, since it can be confused with microbial cells. 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 in an attempt to increase the amount of microbial growth. Since the flagella stain coats the flagellum in order to make it visible, it was employed in an attempt to make the cells large enough to view. Vials containing core material from a depth of 4773 ft and incubated at 66ºC were vigorously vortexed and then passed through a 0.45µ membrane filter and the filtrate passed through a 0.22µ 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 stained 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 stains prepared using core material from a depth of 4,779 ft demonstrated the same visual images.

To be even more definitive that the sample contained living material samples were stained by Dr. Dewayne Wise with a DNA (deoxyribonucleic acid) stain, propidium iodide which intercalates between the bases of the DNA and fluoresces red in the presence of ultraviolet light. This is illustrated in Figure 2. This is further evidence that there are living microorganisms in the cores from the Heidleberg Oil Field.

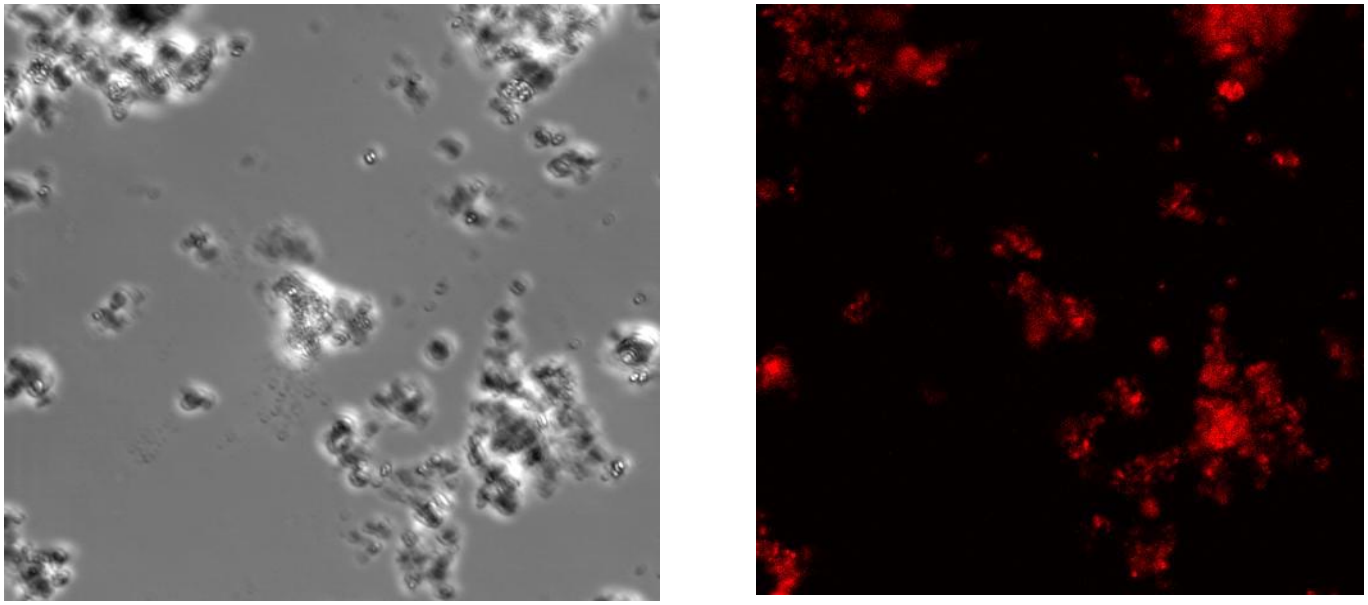


Figure 2. Photographs of Heidelberg core material in the presence of KNO_3, and K_2HPO_4, and oil after incubation for 50 days at 66°C stained with the DNA stain propidium iodide. Photograph on the left is a photograph using the confocal laser scanning microscope with white light. Photograph on the right is the same slide viewed with ultraviolet light. (Red spots indicate DNA.)

All of the enrichments were subcultured using a 10% inoculum and new enrichments were prepared using Heidleberg cores from 4,773 ft and 4,779 ft, Brookhaven production water and nitrogen and phosphorus nutrients. After incubation was at 66°C under anaerobic conditions all enrichments 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 KNO_3 and 0.048 g K_2HPO_4 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 were repeated and CO_2 production was shown to occur as shown in Figures 3 and 4.

Figure 3 shows CO_2 production for crushed core material from 4,773 ft and Figure 4 shows CO_2 production for core material from 4,779 ft. The evidence suggests that the CO_2 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 CO_2 production is not abiotic.

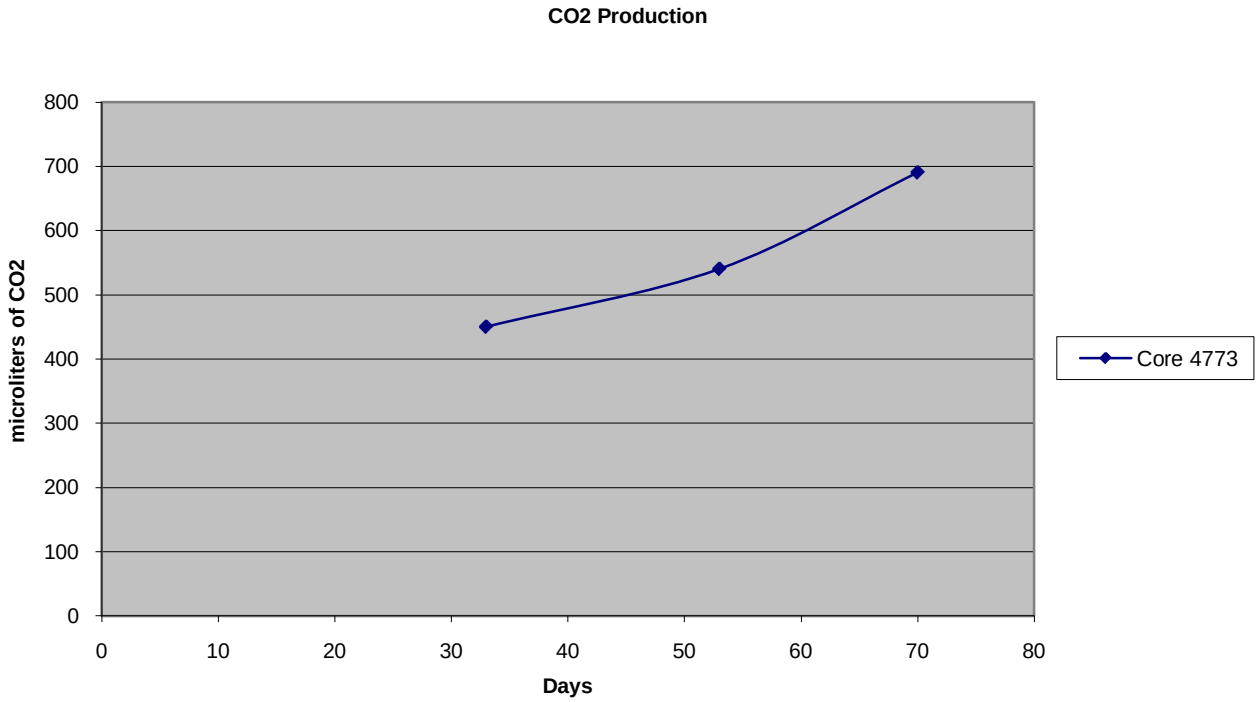


Figure 3. Carbon dioxide production by core material from the Heidelberg Oil Field from a depth of 4,773 feet.

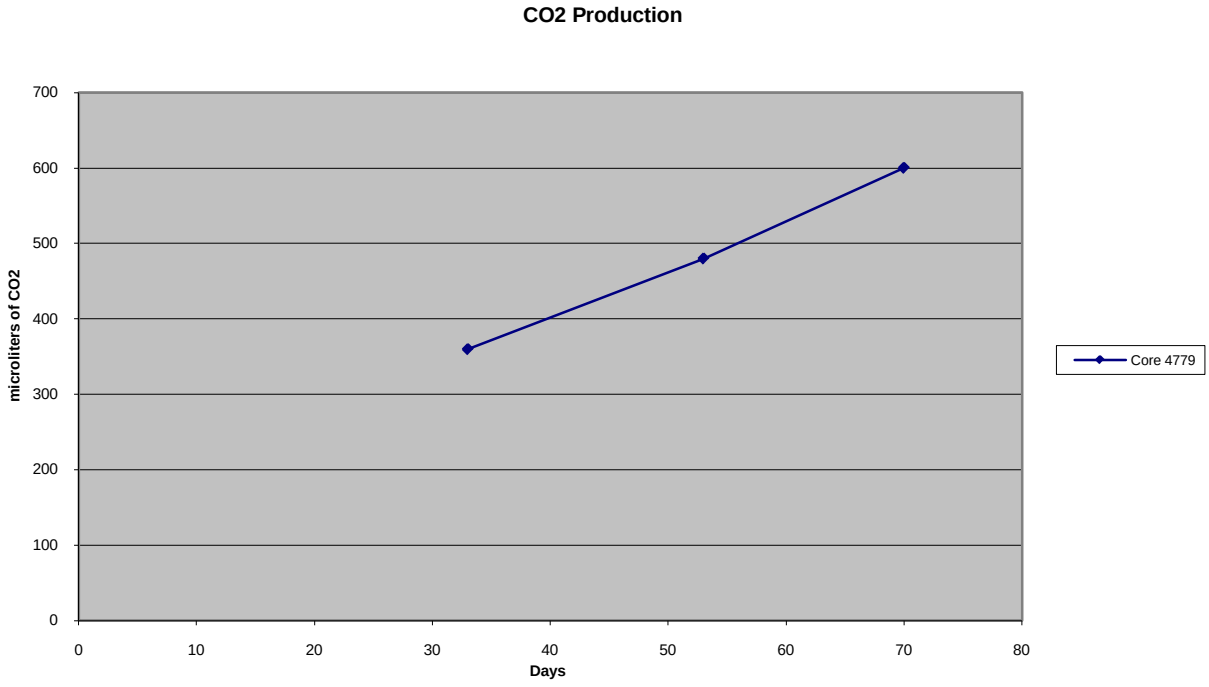


Figure 4. Carbon dioxide production by core material from the Heidelberg Oil Field from a depth of 4,779 feet.

Core Flood Experiments Using Cores From the Heidelberg Oil Field

Because it was proposed to conduct a field demonstration of the impact of MPPM on CO_2 flooding, it was desirable to determine if there were viable microorganisms in the producing formation that would grow 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. Therefore, a growth chamber that could be incubated at 115°C was designed and fabricated as shown in Figure 5.

Figure 5. 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 of 115°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 6 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 6). The photographs in Figure 7 are samples from the same core material stained using another DNA stain. Tests demonstrated that the positive tests for DNA resulted from growth in the samples at 115°C, not merely present in the material but had not grown.

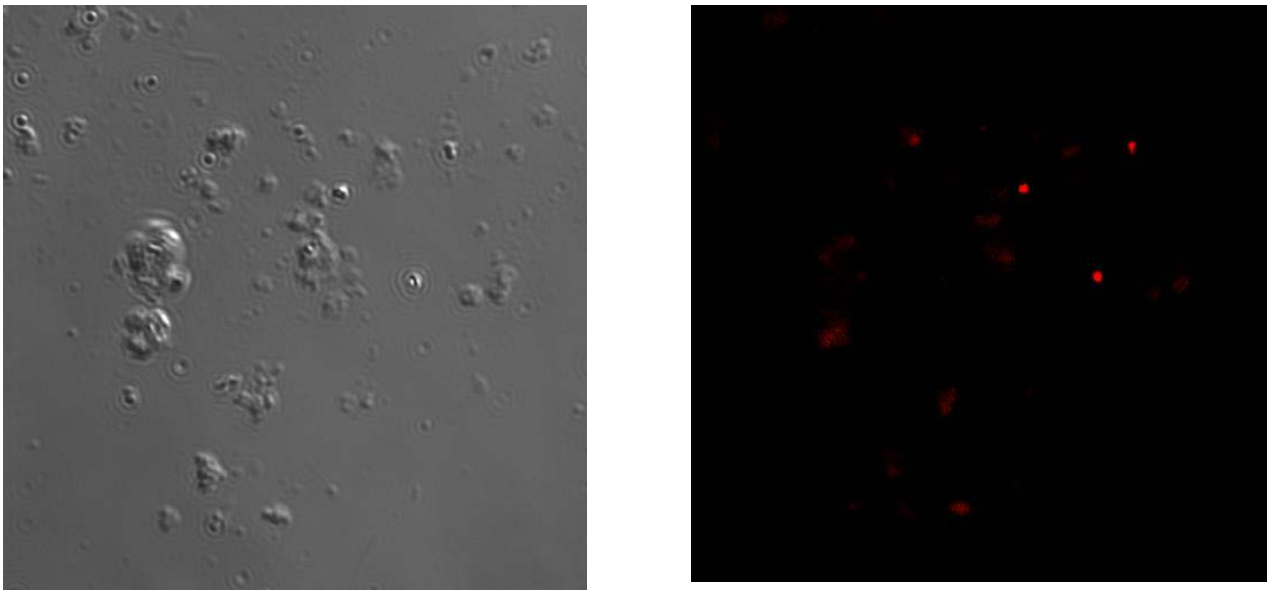


Figure 6. Photographs of core material incubated for 50 days at 115°C stained with propidium idodide. Photograph on the left is a photograph using the confocal laser scanning microscope with white light. Photograph on the right is the same slide viewed with ultra violet light. (Red spots indicate DNA.)

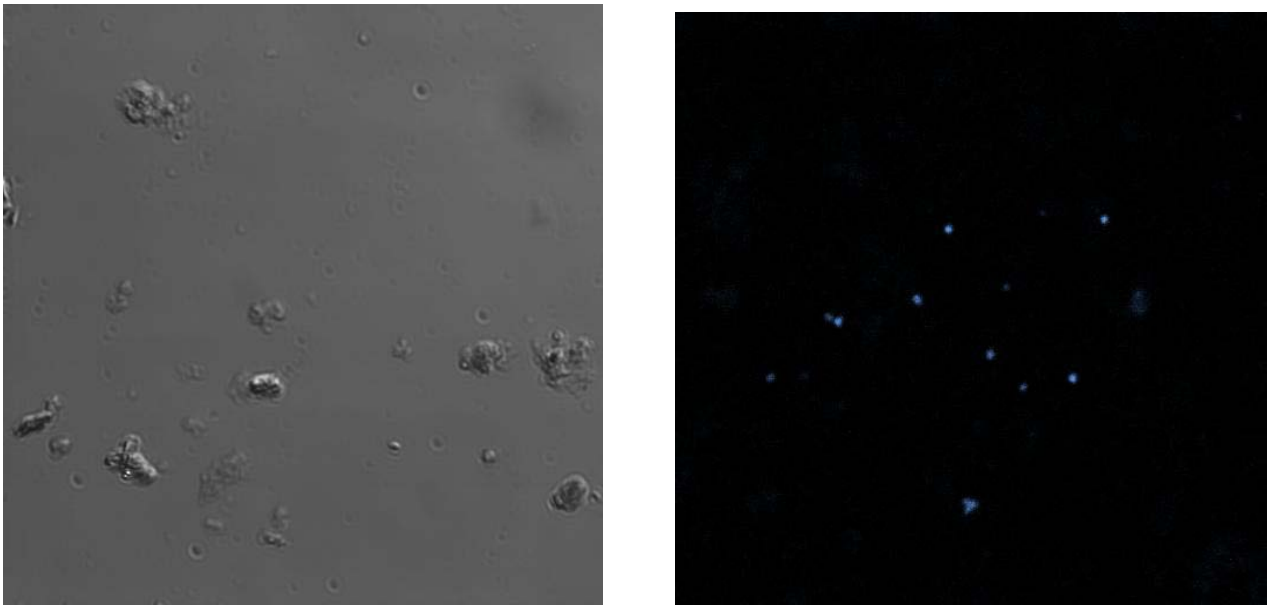


Figure 7. Photographs of core material incubated for 50 days at 115°C stained with DAPI (4′-6-diamidino-2-phenylindole). Photograph on the left is a photograph using the confocal laser scanning microscope with white light. Photograph on the right is the same slide viewed with ultraviolet light. (Blue spots indicate DNA.)

Effect of CO_2 On Microorganisms

Over the course of this study, questions arose about the growth of microorganisms in the presence of high CO_2 concentrations. Samples of water from the Little Creek Oil Field, received on Oct. 11, 2007, had a pH of 5.3 and when CO_2 was bubbled into the water the pH went down to 4.2.

An experiment was conducted to determine the impact of a high CO_2 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 CO_2 or, anaerobic conditions with the atmosphere being predominately CO_2. 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, CO_2 reduced the number of cells from 9.1 x 10^5 per ml to 0.3 x 10^5 per ml or 96.7% under aerobic conditions. Under anaerobic conditions, CO_2 only reduced the number of cells from 8.8 x 10^5 per ml to 3.2 x 10^5 per ml or 63.6%. Therefore, while a high concentration of CO_2 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 CO_2 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 (in air)	AEROBIC (with CO_2)	ANAEROBIC (in N_2)	ANAEROBIC (in N_2 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	2.5	0.3	5.0	2.0
AVG.	9.1	0.3	8.9	3.2

Note: The number of cells divided by 1 x $10^{5.}$

In preparation for laboratory core flood experiments the existing equipment was 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 was used in the next round of flow tests.

Changing from various injection waters to liquid CO_2, in the next set of flow tests presented a new challenge. In-cylinder conditions for CO_2 run near 21°C and maximum pressure is approximately 700 psi. We injected the liquid CO_2 through stainless steel tubing at approximately 200 psi into cores heated to 72°C. When the CO_2 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 showed a 36.8/1 volume ratio of liquid to vapor CO_2, segued within the injection circuit from pre-oven to oven temperatures, viz. 21 to 72°C.

Two additional safety valves were needed and had to be designed and installed. First, a diode valve at the tank will allow CO_2 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 was 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 and made the core flood equipment 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 each approximately 4 inches long and 1 inch in diameter. 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 CO_2. As may be seen in Figure 8 oil was produced from the test core while no oil was produced from the control core during the water flooding operation.

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

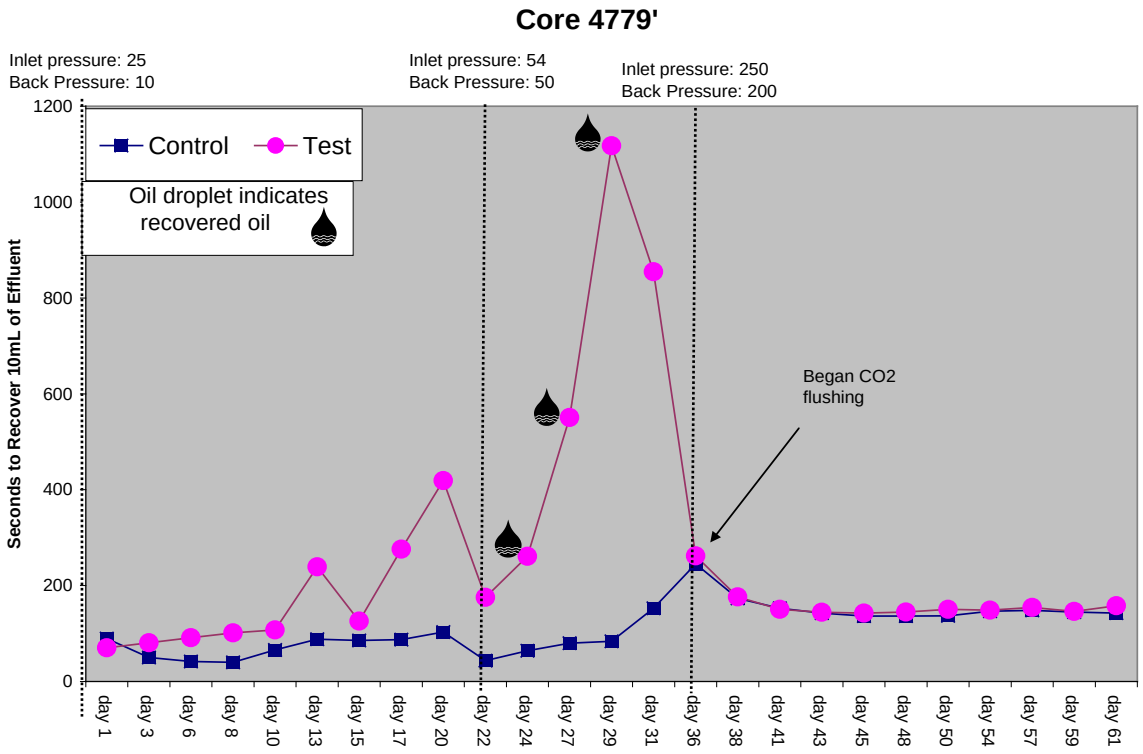
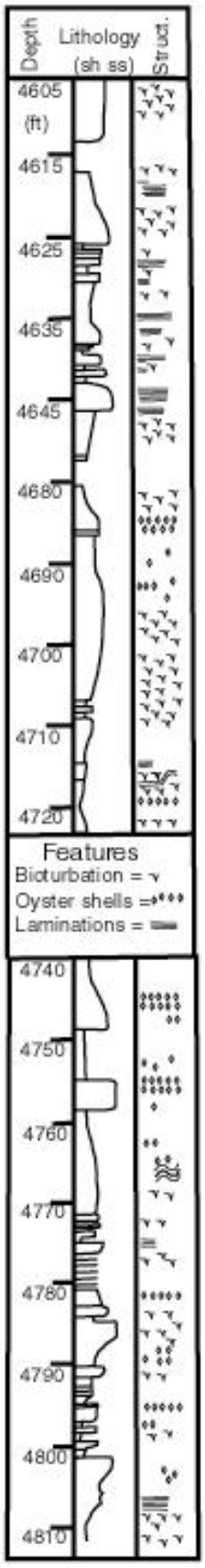


Figure 8. Effluent from cores from the Heidelberg Field (4,779 feet) being treated in the core-flood facility.

Detailed analysis of Eutaw Formation mineralogy and porosity using petrography and SEM and high-resolution CT imagery

In the Upper Cretaceous of the Heidelberg Field the Eutaw Formation occurs as upward coarsening paralic sandstone and siltstone. 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 9). The framework composition of the entire section is subarkosic, and glauconite, chert, and muscovite are abundant. Limited core flood tests were performed on plugs of the Cook-McCormick core to determine the effects of CO_2 on the different iron-rich minerals (pyrite, siderite, glauconite), but were not definitive.



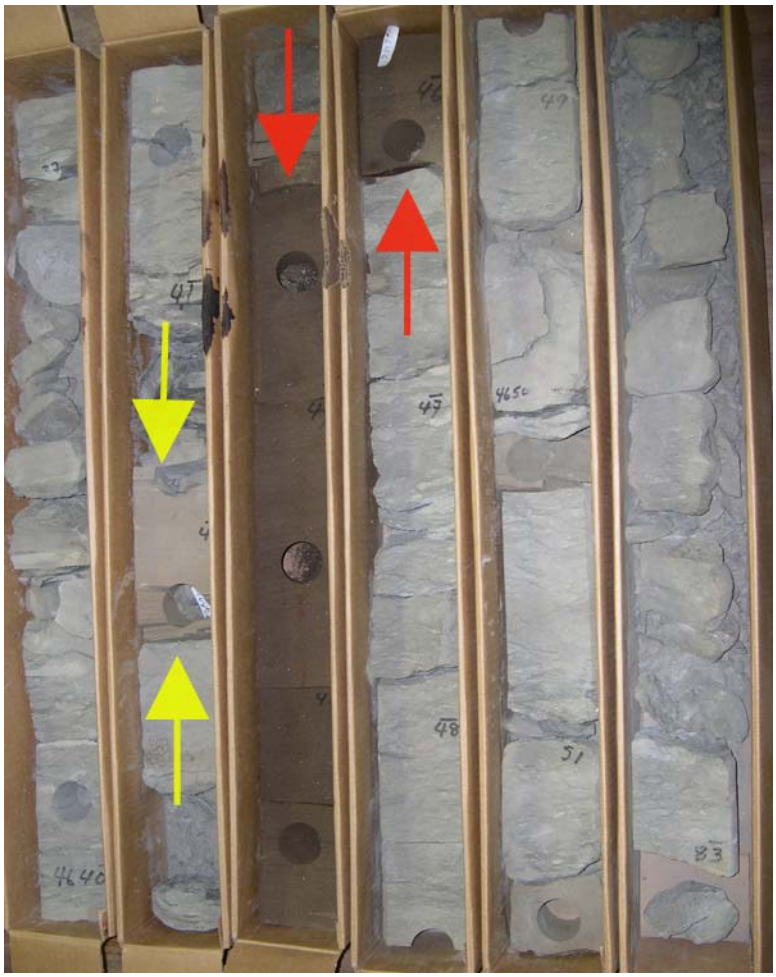
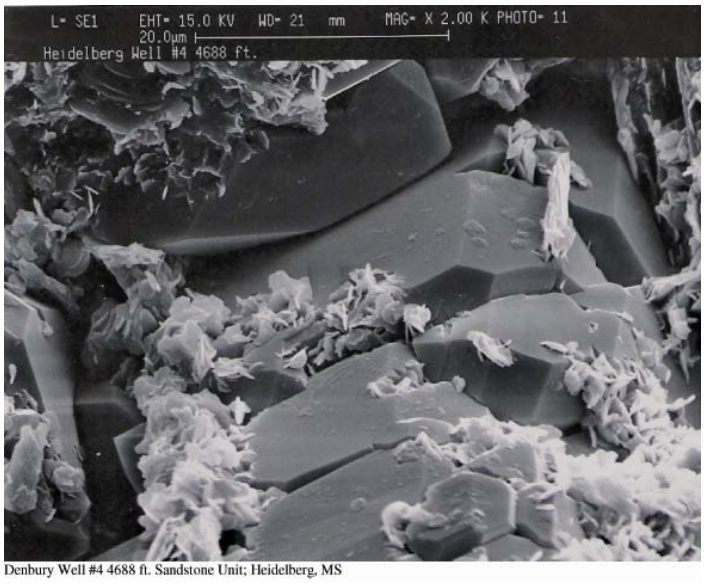


Figure 9. The Eutaw Formation occurs as relatively thin, variably-lithified, well-laminated sandstone interbedded with heavily-bioturbated, clay-rich sandstone and shale. Left-Log of Cook-McCormick # 4 core from Heidelberg Field. Below-Core from approximately 4,627ft (left top) to 4,651 ft (right bottom). The red arrows delineate oil-filled, well laminated sandstone. The yellow arrows delineate a second, well laminated sandstone with no oil show.

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 10). These relations imply that the migration of oil into the sandstones stopped porosity-occluding diagenesis.



Denbury Well #4 4688 ft. Sandstone Unit; Heidelberg, MS

Figure 10. 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 (width approximately 4 in).

Alteration of glauconite (Figure 11) in the burial diagenetic environment is relatively minor and siderite is locally abundant (Figure 12 and 13). 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 13).

A

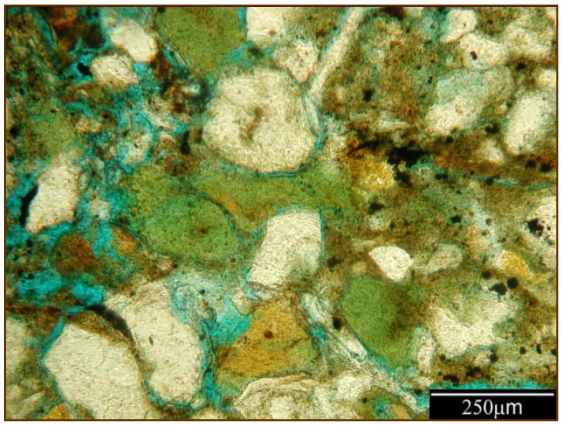


Figure 11 A. Glauconite as psuedomatrix in thin section from 4,606 ft, Cook McCormick well, Heidelberg Field.

B

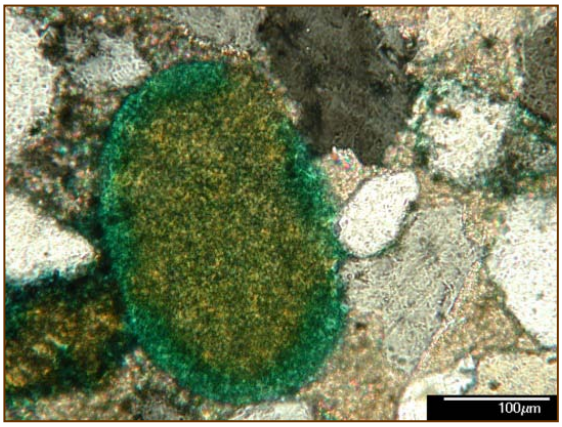
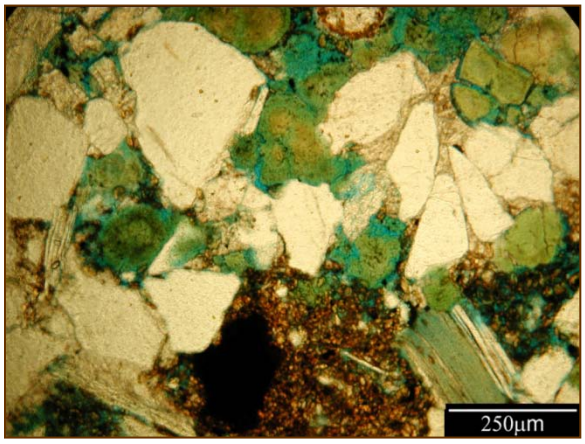


Figure 11 B. Glauconite rim on grain.

A



B

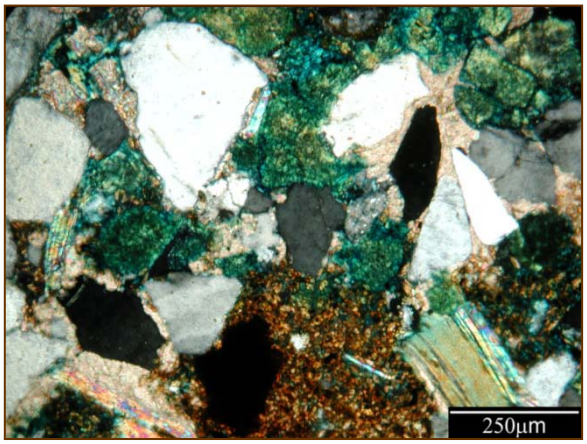


Figure 12. Plane light (A) and crossed Nichols (B) photomicrographs of siderite from 4,747.4 ft in the Cook McCormick core, Heidelberg Field, Mississippi.

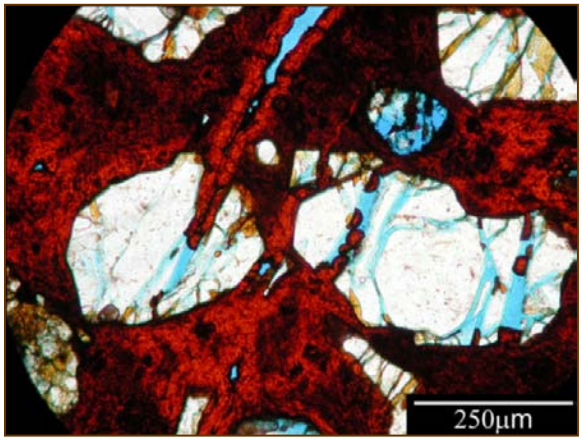


Figure 13. Comparison to Eutaw Formation outcrops in northeastern Mississippi reveals that in outcrop glauconite is altered to iron oxide as shown in thin section below.

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 future studies are recommended.

Detailed analysis of Eutaw Formation mineralogy and porosity using SEM and high-resolution CT imagery

Samples of core stored in anerobic conditions were successfully cultured (Figure 14) and were imaged with SEM to determine the distribution of microbes and mucilage, particularly in association with pore throats (Figure 15). High-resolution CT imagery was used to create 3-D images of the core without (Figure 16 and 17) and with (Figure 18) microbial growth. We were able to obtain very high-resolution images with sub-micron 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 (Figures 16, 17, and 18).

Figure 14. Live core samples were cultivated within an anerobic 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.

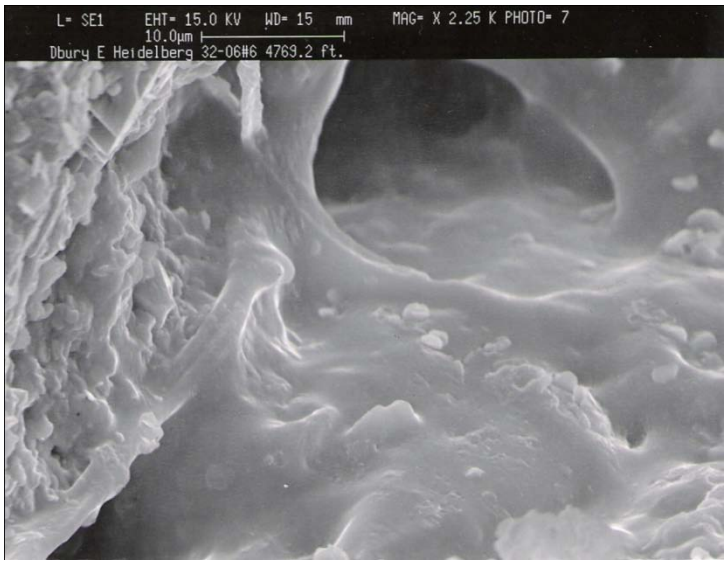


Figure 15. This SEM photomicrograph illustrates a piece of live core treated with nutrients in the anaerobic chamber illustrated above. Mucilage lines most of this pore.

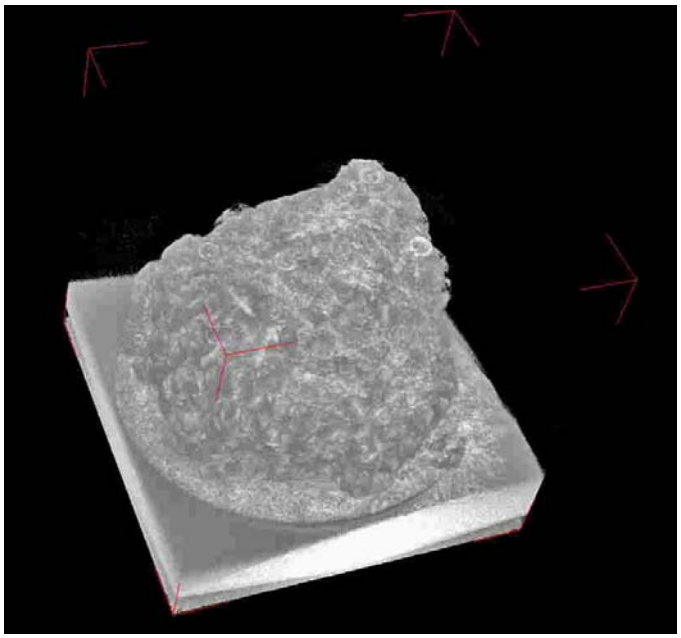


Figure 16. Three-dimensional image of fragment of sandstone sample. Sample is approximately 5 mm in diameter.

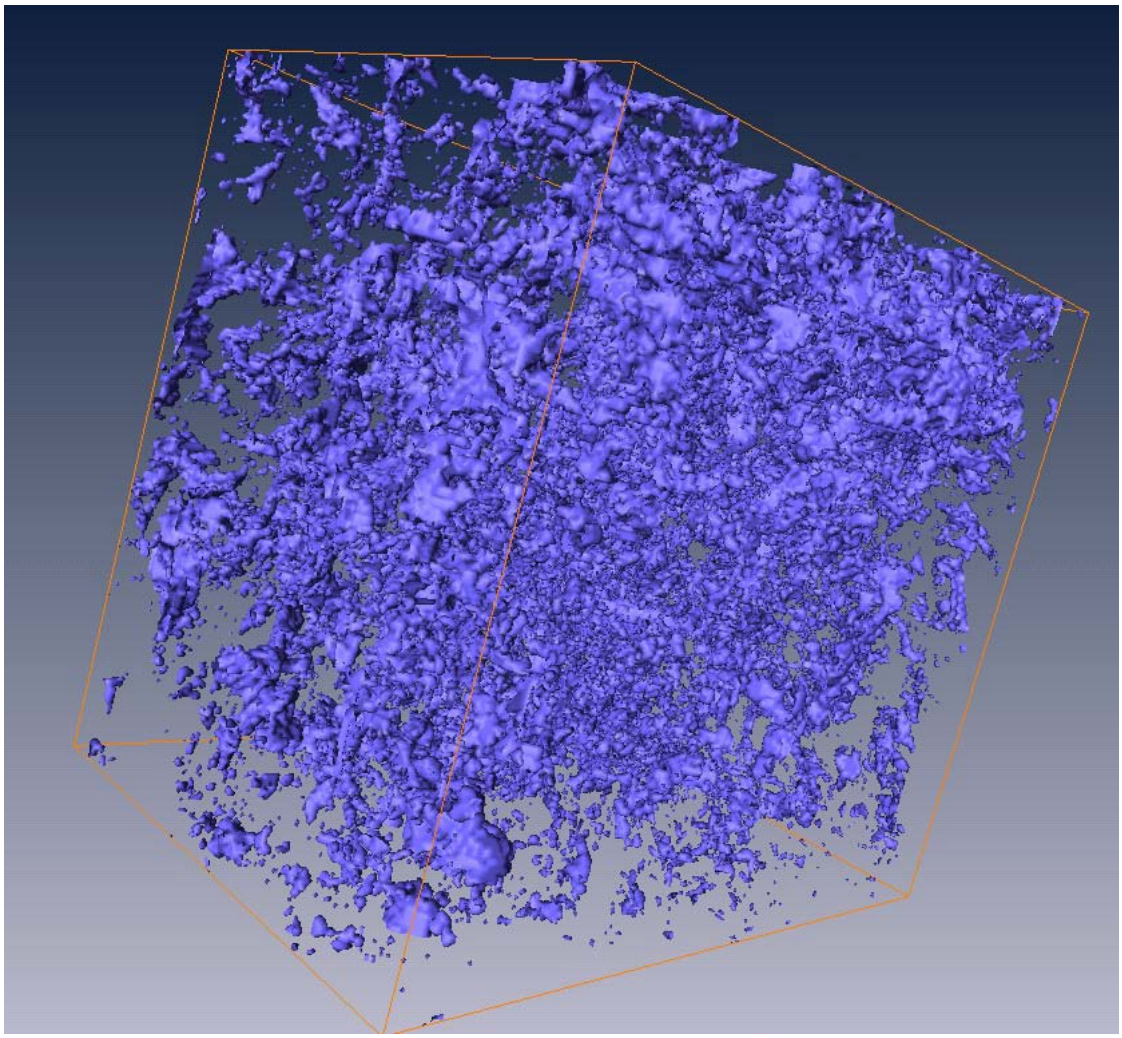


Figure 17. Three-dimensional image of the pore and throat network of a dry sample of Stanley Sandstone. Constructed in conjunction with the MSU High Performance Computing Collaboratory (HPC^2). Cube imaged is approximately 4 x 4 x 4 mm.

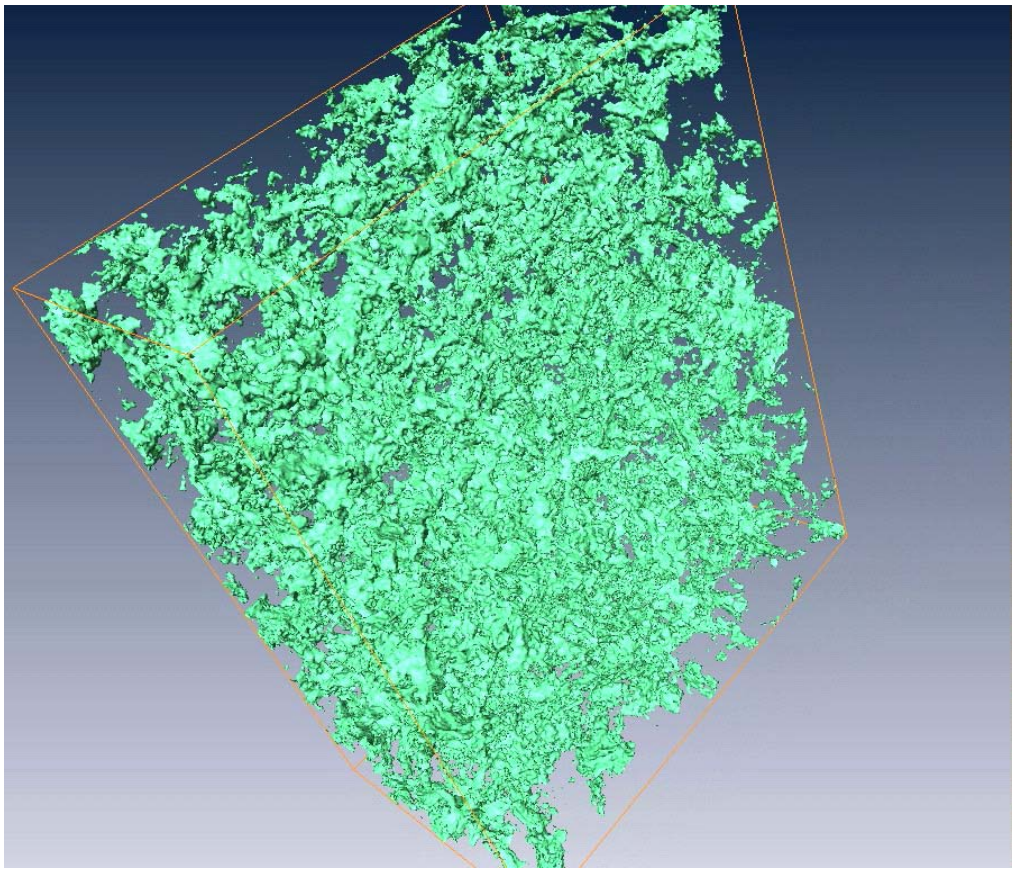


Figure 18. Three-dimensional image of the pore and throat network of a sample of Stanley Sandstone immersed in injection brine, K_2HPO_4 (0.01%), KNO_3 (0.01%), with cultured bacteria. Imaged section is approximately 4 mm in longest axis.

Extensive data was collected using scanning electron microscopy and analyzed quantitatively to determine the nature and distribution of biofilm in core. Particle edges on the sandstone pore surfaces appear as grayscale intensity maxima on SEM micrographs and are countable as number of spikes (N) over chord-length (d) (Figure 19). Analysis of grayscale intensity maximums in SEM images of pores in dry core samples from the Eutaw Formation yield a fractal distribution. The fractal dimension of the surface serves as a measure of the roughness of that surface over several orders of magnitude (1-200 μm) (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 (i.e. quantitatively smoother), and the 10µm – 20µm data conspicuously fall out of the distribution (Figure 21). Concerning the conspicuous 10-20 µm data, as the mucilage spreads itself between particles and across a pore throat, its meniscus feature has no edges, i.e., no intensity spikes, and appears smooth. Meniscus features which span a full gap thus reveal themselves in the distribution. Meniscus features stretched >20µm 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.

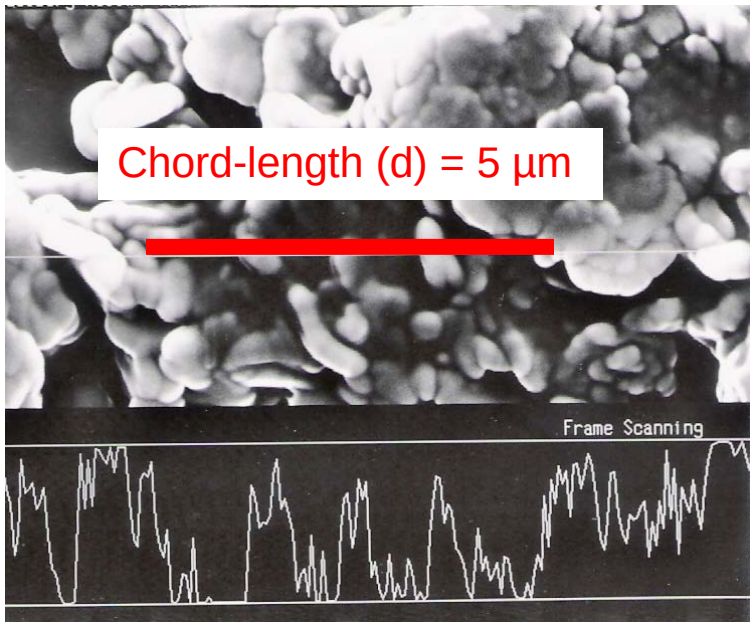


Figure 19. The number of intensity spikes (N) is counted for a given cord length (d) shown here by red line. These data are plotted on a graph like the one shown below.

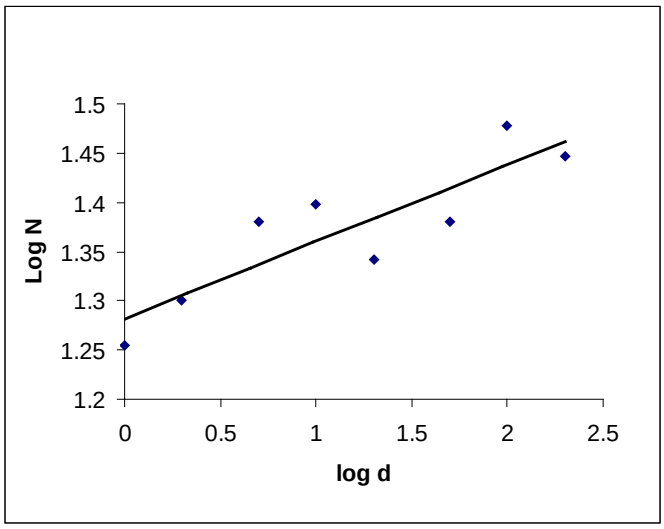


Figure 20. The photomicrograph in Figure 19 was used to generate just one of the data points on the graph in Figure 20. Where this type of log-log data regress nicely to give a line, the distribution is called a power-law, and this slope is a measure of the roughness of the pore surface.

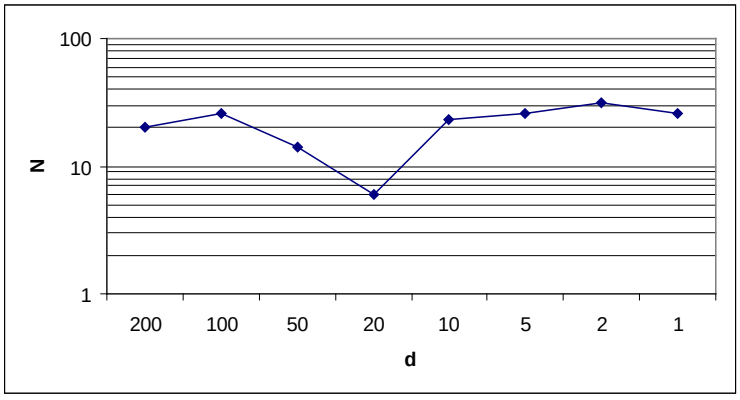


Figure 21. MPPM sample yields an altered edge-feature distribution.

Field Demonstration

The field test began in northwestern portion of the Little Creek Field in December 2008. The field test originally consisted of one injection well and five production wells. Two additional production wells were added in May of 2010 (Figure 22 and Figure 23). The plan was to continue to inject CO_2 daily except on Tuesdays and Thursdays. The Little Creek Field team at the field site designed a special system to supply water to the injector well so that nutrient solutions could be added. On Tuesdays the aqueous nitrate solution was injected and on Thursdays the aqueous phosphate solution was injected. Additionally, oil/gas samples were collected along with a quantity of produced water and sent to Dr. Brown to be archived for future analyses.

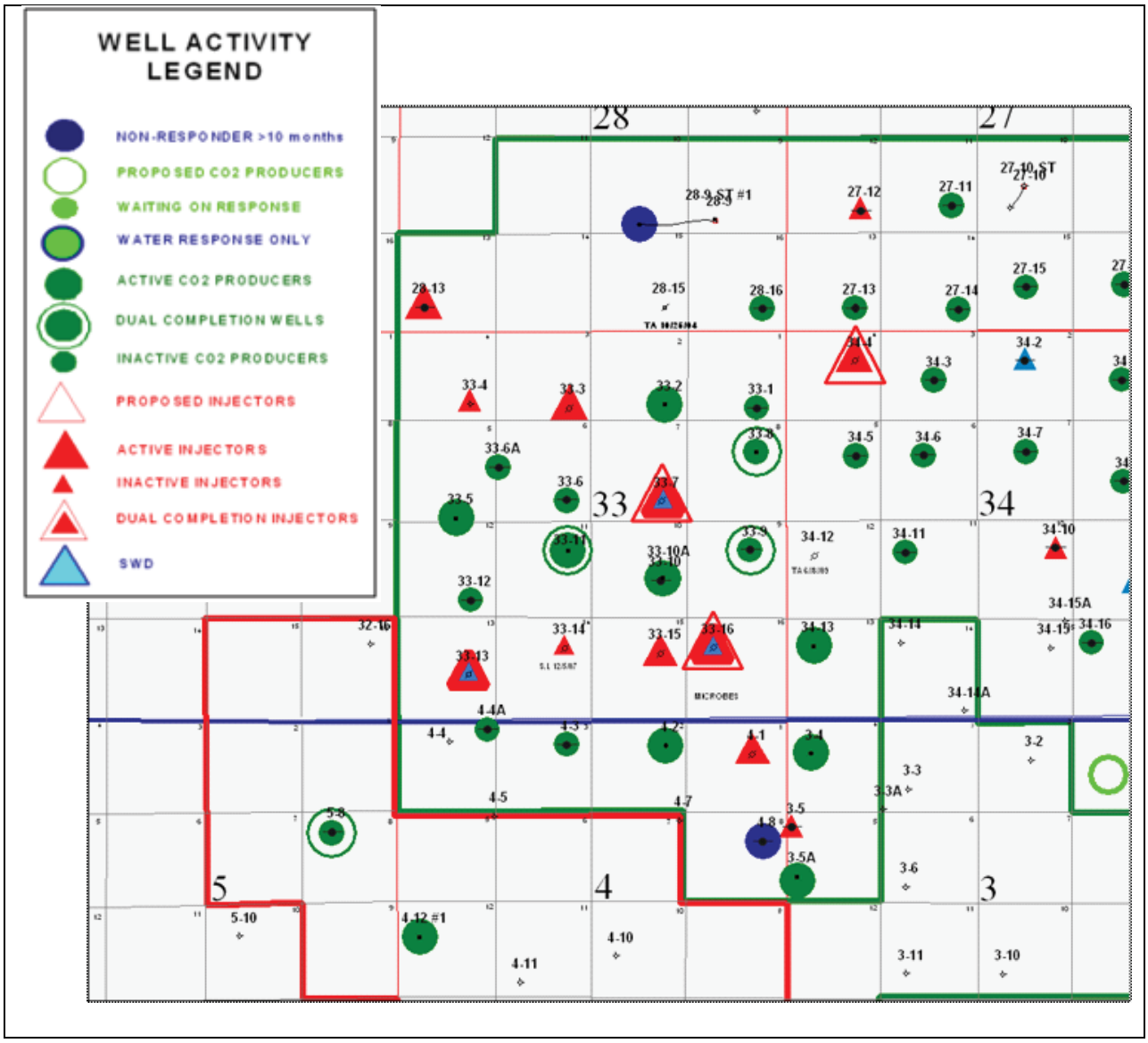


Figure 22. Portion of a map of Little Creek Field showing just the area used for the field demonstration.

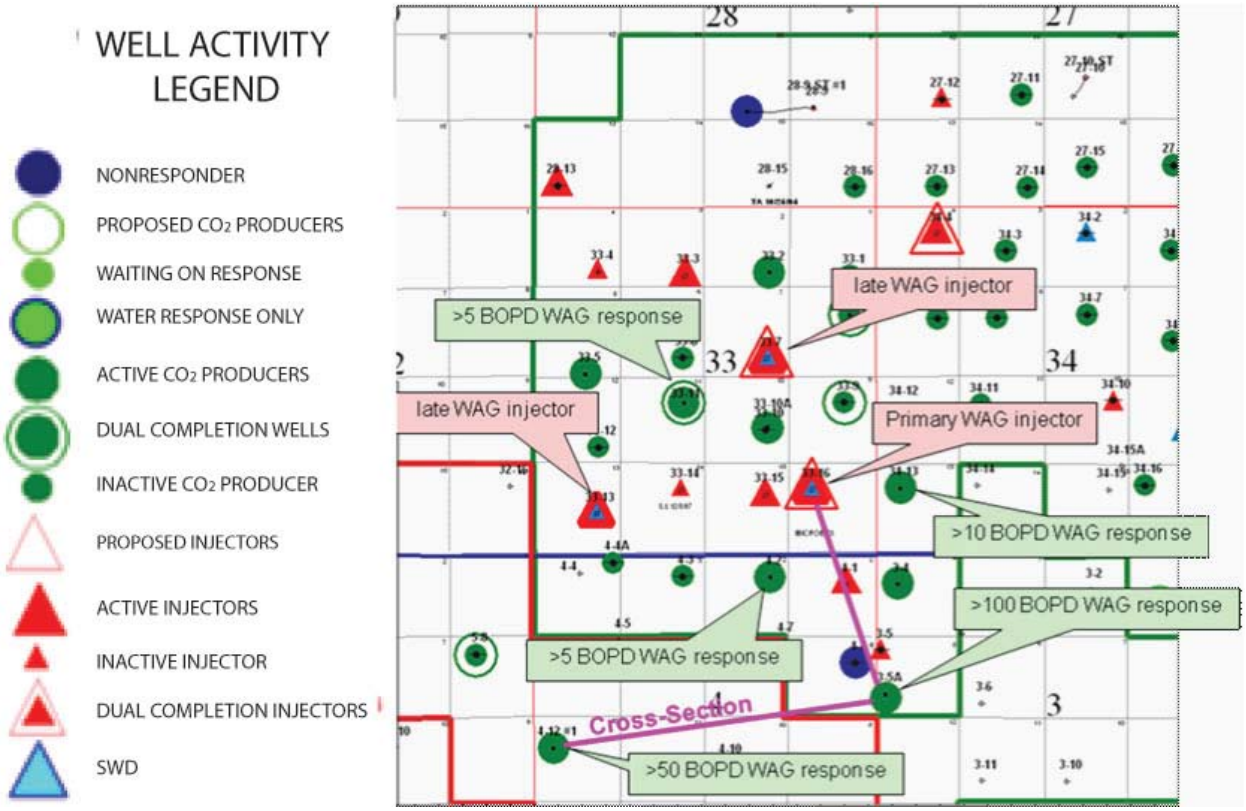


Figure 23. Northwestern portion of Little Creek Field showing WAG test area; injection wells are labeled in pink and wells with WAG response are labeled in blue.

Production data from the field are shown in Figure 24. The nutrient injection process continued from December 2008 until it was temporarily discontinued in March 2009 for a well work over. Initial production response was seen immediately after injection began in December 2008. Shortly after the nutrient injection process resumed in July of 2009, production again began to increase. Over the remaining ten months of the test, production from two of the test wells increased significantly, in one case as much as 80%, and in each of these two wells the decline curve became noticeably less steep.

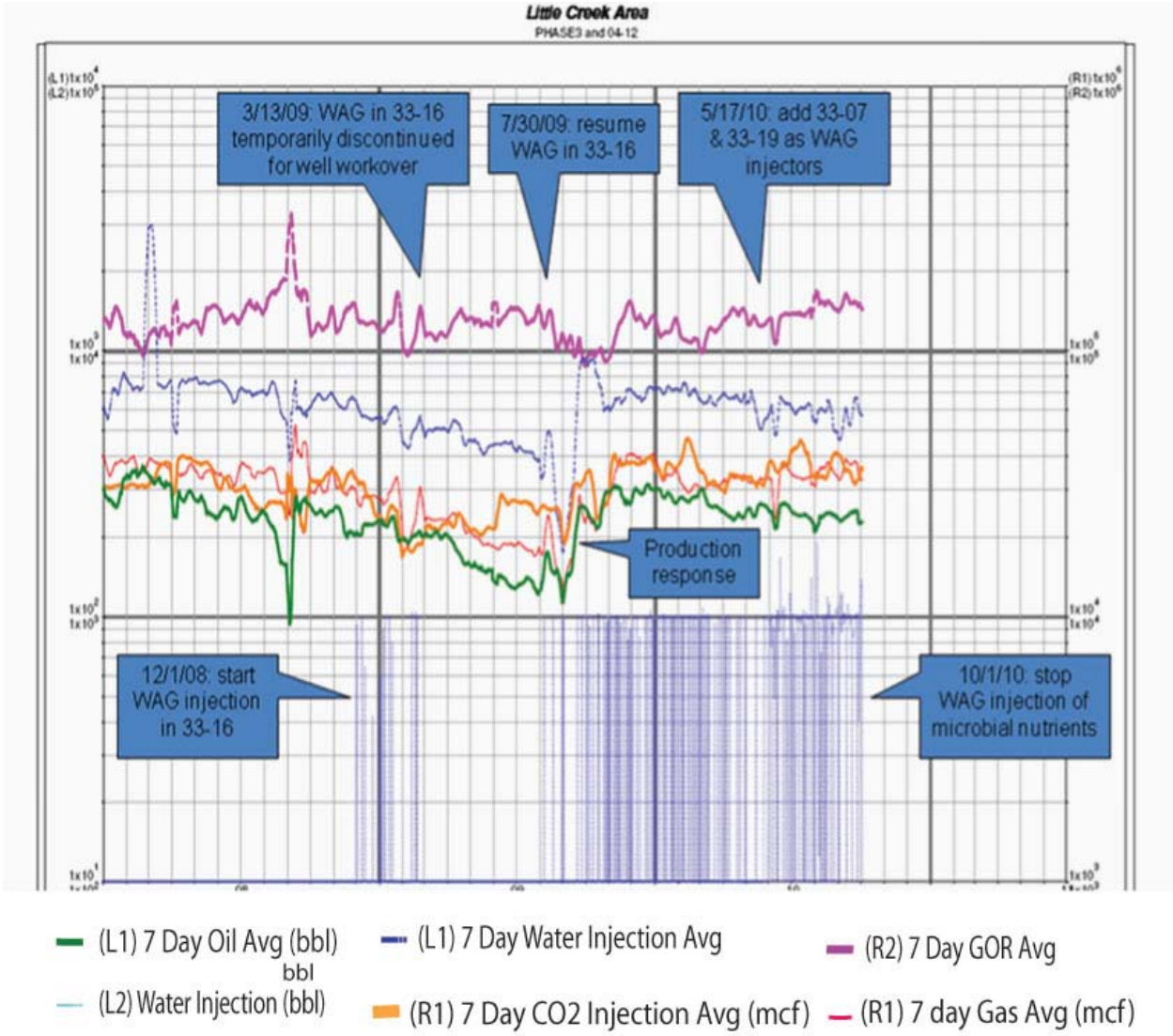


Figure 24. Production data from all wells in the Little Creek Oil Field test area. Blue labels document significant events in the test process.

The field test was terminated in October 2010 due to operational challenges. The test was considered successful based on both the increased production of oil, which was particularly significant in two of the production wells (>50 and >100 BOPD increases), and the reduction in the steepness of the decline curve for other production wells, which can have a significant economic impact over the long term (Figures 23 and 24).

CONCLUSIONS

The results of this investigation clearly demonstrated that MPPM was able to increase oil production from several wells in the area of nutrient injection into the formation. This was accomplished without interfering with the carbon dioxide flooding operation and without evidence of blockage in the petroleum-bearing formation. These results parallel and compliment results demonstrated in the North Blowhorn Creek Oil Unit (NBCU) in Lamar County, Alabama using water flooding as the secondary method of oil recovery. The current project indicates that MPPM is a viable option for increasing oil production from oil fields undergoing carbon dioxide flooding as the secondary method of recovery.

Perhaps the most significant contribution from this work is the demonstration that microorganisms can be cultured at 115°C (239°F) in core samples obtained from a newly drilled well in the Heidelberg Oil Field. Heretofore, it was uncertain as to whether viable microorganisms would be present at a temperature above the boiling point of water 100°C (212°F), much less grow at that temperature, but this project apparently proved that microbes can and will grow at temperatures above the boiling point of water, provided of course, that they are maintained under pressure. Growth of microorganisms was demonstrated at 115˚C by an increase in deoxyribonucleic acid (DNA) using two different stains. (DNA is only found in living cells.) Core flood simulation equipment was modified to simulate the MPPM process by activating microorganisms in live cores from the Heidelberg Field using dilute nutrient solutions and carbon dioxide under pressure. These core flood experiments were carried out at 66˚C (the highest temperature that could be employed in the coreflood facility). Tests were also conducted under both aerobic and anaerobic conditions to determine the effect of high levels of carbon

dioxide on microbial growth and showed a reduction in microbial growth of only 96.7% aerobically (from 9.1×10^5 to 3.0×10^4) and only 63.69%, anaerobically (from 14.0×10^5 to 3.2×10^5). All of these experiments demonstrated that viable microorganisms were present and would proliferate in the presence of carbon dioxide and at high temperatures.

The Upper Cretaceous 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 was never found to be 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 was used to prepare samples for SEM and CT imagery. Quantitative analysis of SEM photomicrographs showed that MPPM permeability modification occurs ubiquitously within pore and throat spaces of 10-20 μm diameter.

The field test of the MPPM procedure in association with carbon dioxide flood was carried out in the Little Creek Field between December 2008 and October 2010. After approximately nine months, a significant increase in production occurred in two of the five production test wells; furthermore, the decline curve in each of the production wells became noticeably less steep. The test had to be terminated in October 2010, due to operational challenges. Nutrient injection did not cause production problems. This field test of the MPPM procedure was considered successful and shows great potential for application in other oil and gas fields in other regions of the United States or globally.

The results of this study are particularly and directly applicable in the state of Mississippi, which hosts significant coal reserves and many partially depleted oil fields. Tremendous potential exists to extract more petroleum from these oil fields using tertiary and quaternary recovery practices such as MPPM combined with carbon dioxide sweep. Implementation of these combined procedures can help the state of Mississippi improve economic conditions and help the United States reduce dependence on imported oil.

GRAPHICAL MATERIALS LIST

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.

Fratesi, S.E., Lynch F.L., Kirkland, B.L., and Brown, L.R., 2004, Effects of SEM preparation techniques on the appearance of bacteria and biofilms in the Carter Sandstone, Journal of Sedimentary Research, v. 74, p. 114-123.

Jarrell, P. M., Fox, C. E., Stein, S. L., and S. L. Webb, 2002, Practical aspects of CO_2 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 water flooding: Final Report on DOE Contract No. DE-FC22-94BC-14962.

BIBLIOGRAPHY

Collins, K.M., Corley, M.E., Lynch, F.L., Schmitz, D.W., Russell, E.E., Kirkland, B.L., 2007, The role of oil migration in the diagenesis of Upper Cretaceous Eutaw Formation sandstone from the subsurface of central Mississippi, Geological Society of America Abstracts with Programs, v. 39, no. 6, p. 633.

Collins, K.M., 2008, Petrography of the Cook-McCormick Core, Eutaw Formation, Heidelberg Field Mississippi and Relationship to Microbial Permeability Profile Modification, Master's Thesis, Mississippi State University, 128 p.

Funderburk, W.K., 2009, Mathematical analysis of the effect of biofilm upon porosity and permeability in oil-bearing sandstone of the Eutaw Formation in Jasper County, Mississippi, Master's Thesis Mississippi State University, 83 p.

Funderburk, W.K., Kirkland B.L., and. Brown, L.R., 2009, Quantifying the fractal topology of micron-scale biofilm surfaces using SEM, Proceedings of the Southeastern Microscopy Society, v. 29, p. 15.

Funderburk, W.K., Kirkland, B.L., Lynch, F. L., Schmitz, D.W., and Brown, L.R., 2007, Fractal analysis of the effect of bacterial mucilage on porosity and permeability in sandstone, Geological Society of America Abstracts with Programs, v. 39, no. 6, p. 633.

Funderburk, W.K., Brown L.R., Kirkland B.L., and Lynch, F.L., 2007, SEM and microbial permeability profile modification of oil-bearing sandstones, Proceedings of the Southeastern Microscopy Society, v. 27, p. 20.

Kirkland, B.L., Lynch, F.L., Brown, L.R., and Schmitz, D., 2006, Improvement of carbon dioxide sweep efficiency using *in situ* microbes to modify reservoir permeability, Geological Society of America, Abstracts with Programs, v. 38, no. 7, p. 139.

LIST OF ACRONYMS AND ABBREVIATIONS

MPPM - Microbial Permeability Profile Modification

NBCU - North Blowhorn Creek Unit

ACKNOWLEDGEMENTS

Magan Green provided assistance with many aspects of this project. John Ed Parker was instrumental in carrying out the core flood experiments. Dr. Dwayne Wise provided confocal microscopy images of samples grown in culture. The staff of the Electron Microscopy Center, Amanda Lawrence, William Monroe, and Richard Kuklinski provided technical advice and assistance with microscopic imaging and sample processing. Mahnas Jean Mohammadi-Aragh (formerly of HPC^2) provided guidance needed to make the 3D sandstone porosity (density-isosurface) images, and Dr. Haitham El Kadiri (CAVS) provided guidance with the high-resolution CT X-ray techniques. Finally, at every level the men and women of Denbury Resources, Inc. have been extremely helpful in facilitating all aspects of this project from beginning to end. Their exceptional support, professionalism, and positive attitude made this project possible.

National Energy Technology Laboratory

626 Cochrans Mill Road
P.O. Box 10940
Pittsburgh, PA 15236-0940

3610 Collins Ferry Road
P.O. Box 880
Morgantown, WV 26507-0880

13131 Dairy Ashford, Suite 225
Sugarland, TX 77478

1450 Queen Avenue SW
Albany, OR 97321-2198

2175 University Ave. South
Suite 201
Fairbanks, AK 99709

Visit the NETL website at:
www.netl.doe.gov

Customer Service:
1-800-553-7681