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# **Innovative MIOR Process Utilizing Indigenous Reservoir Constituents**

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## ABSTRACT

This research program is directed at improving the knowledge of reservoir ecology and developing practical microbial solutions for improving oil production. The goal is to identify indigenous microbial populations which can produce beneficial metabolic products and develop a methodology to stimulate those select microbes with inorganic nutrient amendments to increase oil recovery. This microbial technology has the capability of producing multiple oil releasing agents. The potential of the system will be illustrated and demonstrated by the example of biopolymer production on oil recovery.

Research has begun on the program and experimental laboratory work is underway. Polymer-producing cultures have been isolated from produced water samples and initially characterized. Concurrently, a microcosm scale sand-packed column has been designed and developed for testing cultures of interest, including polymer-producing strains. In research that is planned to begin in future work, comparative laboratory studies demonstrating in situ production of microbial products as oil recovery agents will be conducted in sand pack and cores with synthetic and natural field waters at concentrations, flooding rates, and with cultures and conditions representative of oil reservoirs.

## EXECUTIVE SUMMARY

This project is an experimental laboratory study aimed to improve the understanding of reservoir ecology, and establishing methods of manipulating indigenous microorganisms to utilize naturally occurring water soluble organic acids to produce beneficial oil recovery agents. The objectives of this research program are to demonstrate in-situ production of oil recovery agents in reservoir waters by indigenous microbial populations, and to enhance and control the content and concentration of the bioproducts by the selective addition of low concentrations of inorganic salts as an alternate electron system.

The research program has been divided into a series of seven tasks that are designed to determine feasibility of developing a practical and cost effective in-situ microbial system for increasing the effectiveness of oil-recovery agents in oil reservoirs. Research in this program will focus on stimulating in situ polymer production to reduce permeability of porous zones and alter fluid flow patterns in heterogeneous formations. Experimental work on the project begins in Task 1 with selection of suitable microbial strains and development of test procedures for subsequent studies. Research in Task 2 will begin to develop physical models which can be used to quantify fluid diversion in different types of porous media. The objective of Task 3 is to demonstrate that nutrient amendments can be used to selectively stimulate polymer-producing microbes to modify matrix permeability and cause changes in flow patterns. Results from Tasks 1 through 3, will be applied in Task 4 into an increased oil recovery system. This task will be incorporated in conjunction with the preceding flooding tests. Task 4 tests may involve a significant portion of the test program and will involve demonstrating and optimizing the effectiveness of the oil recovery biosystem. Data from experimental work will be correlated and integrated for the effects of the biosystems on oil recovery in Task 5, and reported in a form which could be offered for technology transfer to the oil industry for commercial applications. As results are obtained from the laboratory investigations and are made available to field operations through technology transfer, work in Task 6 will be directed toward applying the new technology to field studies, situations, and operations. This approach allows rapid introduction and evaluation of any system and/or product which is developed by this program, and will provide directly comparable data to be collected. Technical reports will be prepared and offered to industry under Task 7 to complete the project.

The described research project was designed as a three-year experimental study. Work on the project commenced on October 1, 1999 and research projects were initiated as planned at that time. Active experimental projects are now in progress in Tasks 1, 2, and 3. Samples of produced water have been obtained from actively producing fields and enriched for polymer-producing microorganisms. Several promising strains of microbes have been isolated and are

currently being maintained for use in subsequent experimental work. Microcosm scale sand-  
packed columns were designed and tested for developing selected cultures by nutrient  
stimulation. Experimental design of flooding regimes is in progress to test the effects of nutrient  
stimulation on flow behavior in physical models. No problems have been encountered in the  
project to date. Experimental work on additional Tasks is scheduled to begin later in the project.

## CHAPTER 1

### Introduction

It is known that microorganisms can survive and multiply in and under reservoir conditions, and have the capability to significantly influence oil practices and production (credited to Beckman, 1926). Using such data, it has been proposed that microorganisms can also exert and have a positive effect on oil production (1, 2). Areas being actively studied include the production of biopolymers and biosurfactants by microorganisms, and the injection of these products for viscosity and surface tension modifications. In addition, microorganisms have been tested for their ability to grow in oil reservoirs and by their growth in-situ cause the increased mobilization of oil through various mechanisms and/or products such as CO<sub>2</sub> and other gases, surfactants, organic acids and solvents. Successful field tests employing Microbial Improved Oil Recovery (MIOR) technologies have been reported and more field tests are now in progress (3).

More recently it has been shown that the presence of inorganic nutrients can control reservoir ecology, and adding inorganic nutrients as alternate electron acceptors can stimulate distinct groups of bacteria (4). Several discoveries resulting from this understanding of reservoir ecology are of key importance for the present research project:

- Low concentrations of selected nitrogen salts stimulate populations of indigenous denitrifying microbes,
- Such denitrifying populations are heterotrophs known to produce copious amounts of biopolymers (and biosurfactants) at reservoir conditions,
- Beneficial polymer-producing populations can be established and maintained within the reservoir by supplying low-cost nitrogen salts.

This line of investigation has been expanded in the present research program to develop an understanding of a methodology to use low-cost inorganic nutrient amendments that stimulate indigenous microflora to utilize natural reservoir constituents to produce beneficial products. The three-year research project began in October, 1999. This report describes the first three months of the project. The ongoing tasks are in their early research stages. Chapter 2 describes the selection of suitable microbial strains and development of test procedures. Design and development of physical models for studying fluid flow and diversion is detailed in Chapter 3. Physical models were used in Chapter 4 to begin testing the concepts of controlled microbial ecology for creating a fluid diversion system. Additional tasks are scheduled to begin later this year.

## **CHAPTER 2**

### **Laboratory Procedures**

#### **Introduction**

Oil reservoirs contain diverse microbial populations, including species introduced during drilling and production activities, and species native to the reservoir environment. Except in cases of extreme biological constraint (i.e., temperature, salt, etc.), oil reservoirs establish indigenous microbial communities which adapt to the prevailing reservoir conditions. These complex microbial communities demonstrate they contain the metabolic capabilities to produce known oil recovery agents such as biosurfactants and biopolymers. The indigenous communities are in dynamic equilibrium with their environment, and must be restructured in a directed way to favor production of beneficial products. The presence of inorganic nutrients can control reservoir ecology, and adding inorganic nutrients as alternate electron acceptors can stimulate distinct groups of bacteria. However, to understand how these groups contribute to the process, we must first assess their individual contributions.

This research program will focus on developing an understanding of a methodology to use low-cost inorganic nutrient amendments that stimulate indigenous microflora to utilize natural reservoir constituents to produce beneficial products. In order to assess effects that the distinct physiological groups have on oil mobilization, it is necessary to develop procedures to measure the multiplicity of effects. Experimental work on the project began with selection of suitable microbial strains and development of test procedures for subsequent studies.

#### **Background**

Previous investigations of oilfield waters have endowed us with an extensive culture collection of oilfield microflora. Numerous cultures have been isolated from a wide range of field waters and facilities, including primary production wells and waterflooded fields, ranging from fresh waters to highly saline formation waters, and at various reservoir temperatures. The cultures have been isolated on varied media, and in particular the standard API acetate-lactate SRB medium used widely by the oil industry. The collection has been supplemented with isolates from several other environmental sources including activated sewage sludge, polluted marine waters and sediments, naturally attenuated remediation sites, and historically contaminated production sites. Selected cultures from the collection were used as a primary source of inocula for enrichments.

## Experimental

The objective of the culture studies is to select high polymer producing cultures from natural microbial consortia and to determine and develop conditions which encourage maximum polymer production. Produced water samples were collected from production fields in Washington County Oklahoma, Coleman County Texas, Ector County Texas, and Natrona County Wyoming.

Water samples were added directly to liquid selective culture media and incubated anaerobically at 40°C. Selective media enriched environmental samples for denitrifying bacteria (DNB) and general anaerobic bacteria (GAB). Enrichment media formulations are listed in Tables 1 and 2.

Table 1. Composition of denitrifying bacterial (DNB) medium.

	g/liter
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	1.5
$\text{KH}_2\text{PO}_4$	1.5
$\text{NH}_4\text{Cl}$	0.3
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1
Trace minerals	2.0
Na Acetate	1.64
$\text{NaNO}_3$	1.7
$\text{NaCl}$	7.5
YE	0.5

Table 2. Composition of general anaerobic bacterial (GAB) medium.

	g/liter
Sea Salts	22.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	0.45
$\text{KNO}_3$	0.6
Yeast Extract	3.0
HEPES	0.5
EDTA Disodium Salt	0.75
Glucose	10.0
Cysteine HCl	0.5
Agar	17.5

Mixed cultures enriched in the liquid medium were transferred to solid medium and incubated at 40°C in an oxygen-free atmosphere containing 5% CO<sub>2</sub>, 10% H<sub>2</sub>, and 85% N<sub>2</sub>. Isolates were picked by colony appearance from streak plates of the enrichment cultures. Cultures were maintained on solid media at 25°C in an anaerobic environment. Isolates were partially characterized using general physiological and cell morphological characteristics.

## Results and Discussion

Enrichment cultures from all production fields sampled in this period yielded consortia which grew anaerobically at 40°C. Undefined consortia from liquid cultures were streak plated on solid culture media and probable polymer-producing strains were selected by colony appearance. Promising polymer-producing strains produced copious volumes of biomass when grown on solid medium and had slimy or mucoid colony appearance.

The most promising polymer-producing strains from each location are listed in Table 3. Utilization of alternate carbon sources for growth was examined in isolates by cross testing for growth on both DNB medium and GAB medium. Isolates from three of the locations were able to utilize both acetate (DNB medium) and glucose (GAB medium) as growth supporting carbon sources. Those isolates also grew aerobically at 40°C and 55°C when tested. The polymer-producing strain isolated from the Ginnings Lease did not exhibit growth on GAB medium or aerobically on GAB or DNB medium in these early tests. More complete characterization of these polymer-producing strains will be performed as this project progresses.

Table 3. Polymer-producing isolates enriched from produced water samples.

Source	Growth					
	DNB	GAB	Anaerobic	Aerobic	40°C	55°C
Marco Lease, Washington <u>County, Oklahoma</u>	+	+	+	+	+	+
Ginnings Lease, <u>Coleman County, Texas</u>	+	—	+	—	+	/
NPU Lease, <u>Ector County, Texas</u>	+	+	+	+	+	+
NPR-3 (Teapot Dome), <u>Natrona County, Wyoming</u>	+	+	+	+	+	+

+ Growth confirmed

— Growth not confirmed

/ Not tested

All isolates and undefined consortia enriched from environmental samples are currently being maintained as active cultures. Additional strains have been isolated from these sources will be categorized as this project continues. More extensive characterization will be needed to adequately assess the performance of these cultures.

## **Conclusions**

- Produced water samples from multiple sources have yielded polymer-producing cultures.
- Initial characterization of cultures indicates that reservoir populations contain desirable properties of interest.
- Continued research is necessary to further develop cultures and procedures.

## **CHAPTER 3**

### **Flooding Test Procedures, Flooding Apparatus**

#### **Introduction**

The research project focuses on stimulating in situ polymer production to reduce permeability of porous zones and alter fluid flow patterns in heterogeneous formations. Experimental designs and protocols for examining cultures in porous media and conducting flooding experiments in sand-packed columns and Berea cores were needed. Because of the versatility for examining different porous media, and multiple cores in series or parallel, the sand-packed column system was chosen for preliminary testing. Sand-packed column systems has been previously developed and used extensively for studying sequential effects of nitrate-based stimulation systems. However, the need to screen a large number of cultures required modifications of the traditional sand packed columns.

Research in the flooding test procedures and flooding apparatuses began with the development of a microcosm-scale the physical model that can be used to examine enrichment cultures and isolates for growth characteristics and polymer production. The microcosm model was tested in a preliminary screening of isolates and mixed cultures.

#### **Background**

A large number of waterflood operations use seawater as the injection and drive fluid. A seawater-based medium was chosen for the initial column growth studies as the representative flood water. This seawater base medium was fortified with sodium acetate at levels which have been measured and reported in many major oil reservoirs such as the Alaska North Slope and North Sea. The combination of the choice and the selection of cultures, together with the known composition of the base growth fluids which were easily amended to realistic fluid water compositions allowed the preliminary test protocol to be established and controlled.

#### **Experimental**

Representatives from the stock culture collection and recent isolates were enriched and used as composite inocula for growth studies. The primary base medium, Medium A, was a derivative of seawater salts amended with microbial nutrients (Table 4). The use of Medium A allowed for the rapid preliminary screening of numerous cultures and isolates. All growth studies were run at room temperature (~23°C), 40°C, and 55°C, under anaerobic conditions in 10-ml serum vials

sealed with butyl rubber septa. Control tests were run with select cultures under aerobic conditions.

Table 4. Compositions of primary screening and growth media.

Nutrient	Amount (per liter)	
	Medium A	Medium G
Instant Sea Salts	35 g	35 g
Sodium acetate	1000 mg	2000 mg
Sodium nitrate	100 mg	200 mg
Sodium phosphate (dibasic anhydrous)	25 mg	25 mg

As the screening program progressed, Medium A was modified and supplemented to establish and isolate active cultures. Media B through G were composed of Medium A fortified with rich supplements including ammonium nitrate, yeast extract, glucose, or defined media including NIH thioglycollate, DNB medium, or API acetate-lactate SRB medium. However, because of the requirement for media composition consistent with nutrient levels in reservoir brines, the addition of rich nutrient supplements was restricted and a reformulated sea salts base medium, Medium G, was tested (Table 4).

At the same time that the media compositions were modified, the column systems were employed and refined to demonstrate the growth, activity, and potential of the candidate cultures. Numerous variations of the slim tubes, batch reactors, and sandpacks were tested for versatility, applicability, and ease of assembly while maintaining conditions which represented field environments. Fluid-filled sandpack columns were employed and lead to the development of a modified microcosm scale sand-packed column which had the attributes of both a sand pack while maintaining the advantages of a small-scale bottle test procedure.

The preferred system which met the criterion and offered practical and controlled test conditions centered on the use of a sand-packed slim tube constructed from 6 mm ID glass tubing (Figure 1). A 7.5 cm section of the tube was packed with washed Mill Creek sand confined with glass wool plugs to yield a sand-packed column with a bulk volume of 2 cm<sup>3</sup> and a void mixing volume of approximately 2.66 cm<sup>3</sup> on each end. The sand-packed slim tubes were saturated with growth medium, sealed on each end with butyl rubber septa to maintain anaerobic conditions, and mounted vertically. Inocula and treatments were injected into this system by a syringe through the lower septum.

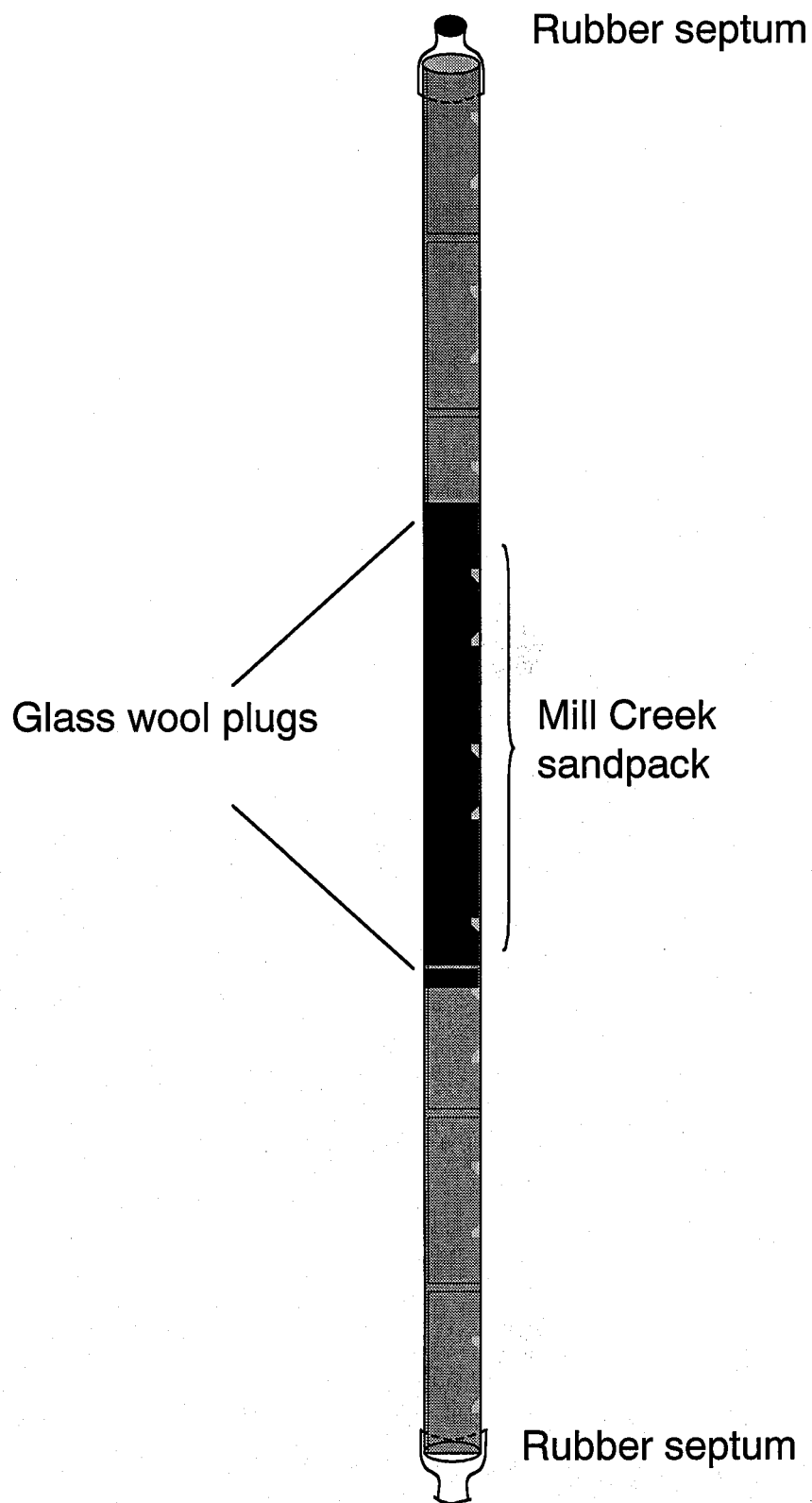


Figure 1. Sand-packed slim tube (6 mm ID).✓

This test system offered the advantages of visual observations of both the bottom (influent) and top (effluent) separated by the porous medium. The presence of the sand-packed zone reduced exchange of cells throughout the column and required that cells penetrate the sand while nutrients were readily exchanged. The columns were maintained anaerobically and incubated at room temperature, 40°C, and 55°C. Samples were withdrawn periodically from influent and effluent mixing zones by syringe and new medium was added as required. The sand-packed slim tube system fulfilled all the requirements for rapidly and effectively identifying and growing cultures which showed potential of being candidates for further testing.

## Results and Discussion

This research program was initiated after a review of the currently available literature, and previous studies of the microflora and biochemical constituents of oil field waters. The review identified areas that could be targeted to offer the greatest potential for developing and improved oil recovery system and technology. Techniques for the identification of the capabilities of isolated cultures are currently being developed and tested in the preliminary screening program. The modified sand-packed slim tube system has thus far proven to be versatile and easily handled apparatus for employing screening methodologies. Cultures currently being grown in bottle systems can be transferred to the sandpack system for additional observation and control.

The initial test period successfully established a collection of cultures which are able to grow and show activity at conditions which are representative of many reservoir waters. Growth conditions employed consisted of supplying a minimal carbon source (acetate) combined with the use of nitrate as an electron acceptor in a seawater base medium. This restricted growth medium limited the number of cultures able to survive and grow (Table 5). Results show that several cultures produced good growth and polymer production (i.e., flocculant appearance) within a period as short as 48 hours. The identified inocula included cultures able to grow anaerobically at room temperature, 40°C, and 55°C. Results also identified the presence of sulfate reducing bacteria (SRB) which are indicative of the anaerobic conditions that exists within the conditioned columns. Grey to black coloration of the white sand indicated the presence of sulfide generation. In several cases the top of the slim tube developed more growth than the bottom, suggesting that a sulfide-utilizing population was being encouraged. This was expected with several cultures which had been isolated from sulfide-containing waters and had been maintained in this condition.

Table 5. Growth and appearance of selected mixed and composited cultures in sand pack columns.

Culture #	Medium	Inocula	Room Temp			40 ° C			55 ° C		
			Top	Sand	Bottom	Top	Sand	Bottom	Top	Sand	Bottom
1	B	Mixed	+	-	+/-	+/-	grey	+/-	+/-	-	+/-
2	C	Mixed	+	-	+/-	+/-	grey	+/-	+/-	-	+/-
3	C	20 cultures	+	grey	+	+/-	black	+	+/-	black	+/-
4	C	Mixed	+/-	grey	+	+	black	+	+/-	black	+
5	F	Composite	+	grey	+/-	++	black	+	+/-	grey	+
6	G	Composite	+	grey	+/-	++	black	+	+/-	grey	++
7	H	Composite	+	grey	+/-	++	black	+	+/-	grey	+
8	G	Composite	+	grey	+/-	+/-	-	+ floc	+/-	-	++ floc
9	G	T1	+	-	+	+/-	-	+	+/-	-	+ floc
10	G	T2	+	-	++	+	-	+ floc	+/-	-	+
11	G	D1	+/-	-	+/-	+	grey	++	+/-	-	+/-
12	G	D2	+/-	grey	+	+	grey	++	+/-	-	+/-
13	G	D3	+/-	-	+	+/-	grey	+	+/-	-	+
14	G	D4	+	-	+	+/-	-	+/-	+/-	-	++ floc
15	G	Polybac	+/-	-	+/-	+/-	grey	+/-	+/-	-	+ floc
16	G5	D5	+/-	-	+	++	-	+	+/-	-	+ floc
17	DNB	D5	+	-	++	+++ floc	grey	+++	+++ floc	-	+++ floc
18	NIH	D5	n/a	n/a	n/a	++	grey	++	+++	-	++
19	G5	D6	+/-	grey	+	+/-	-	+	+	-	+
23	G5	D7	+/-	-	+	+/-	grey	++	+/-	-	+
24	G5	D8	+/-	-	+	+	-	+	+/-	-	+ floc
25	G5	D9	+/-	-	+/-	+/-	-	+	+/-	-	+
26	G5	D10	+/-	-	+/-	+/-	-	+	+/-	-	+
27	G5	D11	+/-	-	+	+/-	-	+	+/-	-	+
28	G5	456	+/-	-	+	+/-	-	++ floc	+/-	-	+ floc

-: No growth

+/-: Slight growth (turbidity)

+, ++, +++: Growth (turbidity)

grey, black: Coloration of sand

floc: Particulate or stringy growth

The cultures which were identified in this preliminary screening program are now being further developed by altering medium composition to stimulate growth and polymer production. Additional screening tests are being conducted to continue identifying new cultures that merit further development.

## **Conclusions**

- A sand-packed slim tube system was developed and tested for the growth and identification of selected cultures.
- Preliminary isolations have identified cultures which have potential for polymer production at reservoir conditions.
- Development of selected cultures by nutrient stimulation has been initiated.

## **CHAPTER 4**

### **Flooding Regimes and Protocols**

#### **Introduction**

Biogeochemical properties of an oil reservoir are dominant factors which govern the composition and action of reservoir microflora. It can be expected that the indigenous microbial community will be in a dynamic equilibrium at reservoir conditions. Alterations of the reservoir environment will cause a selective shift in both the numbers of microbes present, and the metabolic activities of the community. Thus, an understanding of the interactions of the geochemical factors and the biological response, will influence the uses and capabilities of such natural indigenous microbial populations in oil recovery systems.

Nutrient amendments in reservoir waters have been shown to modify reservoir ecology and stimulate distinct groups of microorganisms. Of key importance are the nitrate-reducing, polymer producing bacteria. Through controlled addition of nitrogen compounds into reservoir waters, reservoir ecology can be manipulated and restructured to stimulate denitrifying bacteria. The onset of polymer production by this distinct group of microbes can be induced by certain chemical and physical conditions, and is often triggered by availability of specific nitrogen compounds. There is evidence to indicate that the introduction of selected and low levels of nitrogen containing salts, such as nitrate, combined with an appreciation of the natural reservoir ecology, can lead to the ability to produce biopolymers in-situ in areas deep within the formation where such viscosity increases would have the greatest and most effective impact.

The research objective is to demonstrate that amendments of certain nitrogen containing salts to reservoir waters can be used to selectively stimulate polymer-producing microbes to modify matrix permeability and cause changes in flow patterns. To establish an understanding of conditions that stimulate polymer production, comparative tests will be conducted with a variety of nitrogen containing salts. Effects of these directed nutrient manipulations on fluid flow properties will be measured in artificial and natural brine floods of the physical models.

Construction of flooding apparatus is in progress and experimental design of flooding regimes has begun. Work is progressing as planned, however, we have not completed work on any defined elements at this early stage.

## REFERENCES

1. ZoBell, C. E. 1946. Bacteriological Process for Treatment of Fluid-Bearing Earth Formations. U.S. Patent No. 2,413,278.
2. Hitzman, D. O. 1962. Microbiological secondary recovery of petroleum. U.S. Patent No. 3,032,472
3. Hitzman, D. O. 1983. Petroleum Microbiology and the History of Its Role in Enhanced Oil Recovery. Proc. of the 1982 International Conference on Microbial Enhancement of Oil Recovery, Afton, OK, May 16 – 21, 1982.
4. Sperl, G. T., P. L. Sperl, and D. O. Hitzman. 1993. Use of Natural Microflora, Electron Acceptors and Energy Sources for Enhanced Oil Recovery, In: E. T. Premuzic and A. Woodhead (ed.), Microbial Enhancement of Oil Recovery - Recent Advances, Elsevier Science Publishers, New York, NY.