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

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## TABLE OF CONTENTS

LIST OF TABLES .....	5
ABSTRACT .....	6
EXECUTIVE SUMMARY .....	7
CHAPTER 1 Introduction .....	8
CHAPTER 2 Laboratory Procedures .....	
Introduction .....	
Background .....	9
Experimental .....	9
Results and Discussion .....	11
Conclusions .....	12
CHAPTER 3 Flooding Test Procedures, Flooding Apparatus .....	13
Introduction .....	13
Background .....	13
Experimental .....	13
Results and Discussion .....	14
Conclusions .....	15
CHAPTER 4 Flooding Regimes and Protocols .....	16
Introduction .....	16
Background .....	16
Experimental .....	17
Results and Discussion .....	17
Conclusions .....	18
CHAPTER 5 Oil Recovery Tests .....	
Introduction .....	19
CHAPTER 6 Data Correlation and Interpretation .....	20
Introduction .....	
CHAPTER 7 Field Evaluation of New Technology/Products .....	21
Introduction .....	21
CHAPTER 8 Reports & Technology Transfer .....	22
Introduction .....	22

**REFERENCES.....**

**23**

## LIST OF TABLES

Table 1. Strains selected for experiments. _____	10
Table 2. Media for nitrogen source experiment. _____	10
Table 3. Media for carbon source experiment. _____	10
Table 4. Viscosity and polymer description of isolates. _____	11
Table 5. Growth of strains with various nitrogen sources. _____	12
Table 6. Growth of strains with various carbon sources. _____	12
Table 7. Composition of synthetic brine. _____	14

## 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. Coreflood experiments have begun and initial comparative experiments demonstrating in situ polymer production have been conducted. In research that is planned for future work, comparative laboratory studies demonstrating in situ production of microbial products as oil recovery agents will be conducted in sand packs 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 used for 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 Task 4 was started. Construction of flooding apparatus is in progress and experimental design of flooding regimes has begun.

## 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 month four through month nine of the project. The ongoing tasks are in their early research stages. Chapter 2 describes the selection of suitable microbial strains, development of test procedures, and status of polymer production experiments. Design and development of physical models for studying fluid flow and diversion is detailed in Chapter 3, as well as coreflood results. Physical models were used in Chapter 4 to begin testing the concepts of controlled microbial ecology for creating a fluid diversion system. Several corefloods were completed. Chapter 5 gives a brief summary of the start-up procedures for oil recovery tests.

Construction of flooding apparatus is in progress and experimental design of flooding regimes has begun. Chapter 6 introduces the beginning of data correlation and interpretation. Chapter 7 gives a brief introduction of the field evaluation of new technology/products, which is not scheduled to begin until later in the project. Chapter 8 describes the work thus far on reports and technology transfer.

## 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. Polymer producing strains were isolated from produced water samples as described in the previous Semi-Annual report (5).

Four strains were selected for further study. Initial characterization of these strains is shown in Table 1.

Table 1. Strains selected for experiments.

Isolate	Gram Reaction	Description
Circle 1	ND	ND
AERA	+	Mixed culture, rods
41A	ND	ND
33	+	very short large rods

ND = not determined

The strains were grown on DNB (denitrifying bacteria) media for viscosity measurements on a Brookfield viscometer. Cells were harvested and suspended in a 25% glycerol solution for cryostorage.

An experiment was run using various nitrogen sources. The strains were grown in anaerobic Hungate tubes containing the media listed in Table 2, plus a nitrogen source, at pH 7.2. The cultures were incubated 7 days at 40°C.

Table 2. Media for nitrogen source experiment.

Component	Amount per liter
Na <sub>2</sub> HPO <sub>4</sub> •7H <sub>2</sub> O	1.5 g
K <sub>2</sub> HPO <sub>4</sub>	1.5 g
MgSO <sub>4</sub> •7H <sub>2</sub> O	0.1 g
Hutner's trace minerals	20 ml
Na Acetate	1.64 g
NaCl	7.5 g
Yeast extract	0.5 g

An experiment was run using various carbon sources. The strains were grown in anaerobic Hungate tubes containing the media listed in Table 3, plus a carbon source, at pH 7.2. The carbon sources were added at concentrations of 0.1%, 1%, and 10%. The cultures were incubated 8 days at 40°C.

Table 3. Media for carbon source experiment.

Component	Amount per liter
Na <sub>2</sub> HPO <sub>4</sub> •7H <sub>2</sub> O	1.5 g
K <sub>2</sub> HPO <sub>4</sub>	1.5 g
NH <sub>4</sub> Cl	1.3 g
MgSO <sub>4</sub> •7H <sub>2</sub> O	0.1 g
Hutner's trace minerals	20 ml
NaNO <sub>3</sub>	1.7 g
Yeast extract	0.5 g
NaCl	7.5 g

## Results and Discussion

Viscosity and polymer production results are shown in Table 4. Culture 33 had the highest viscosity, hence the most polymer production. This culture will be used for flooding tests.

Table 4. Viscosity and polymer description of isolates.

Isolate	Product	Viscosity (cP)
Circle 1	Biomass	1.6
AERA	Flocculent	1.2
41A	Polymer	5.6
33	Polymer	14.7

Results for the nitrogen source experiment are shown in Table 5. The AERA culture exhibited the best growth with most of the nitrogen sources. This culture will be used for further tests.

Table 5. Growth of strains with various nitrogen sources.

Nitrogen source	33	Circle 1	41A	AERA	None
None	- p	- p	- p	+/- p	- p
NH <sub>4</sub> NO <sub>3</sub>	-	+	-	+ sp	- p
NaNO <sub>3</sub>	-	+	- p	+ sp	- p
NH <sub>4</sub> Cl	-	+	-	-	- p
NaNO <sub>3</sub> •NH <sub>4</sub> Cl	-	+	+/- p	+ sp	- p
NaNO <sub>2</sub>	-	-	+/- p	+ sp	- p
Urea	+ rp	+	+ rp	+ rp	- p
Alanine	-	+	+/- p	+	- p

+ = growth

+/- = may be growth or chemical precipitate

- = no growth

p = precipitate

rp - ropy-looking precipitate

sp = sticky precipitate

Results for the carbon source experiment are shown in Table 6. The addition of 10% sucrose increased growth of the cultures considerably.

Table 6. Growth of strains with various carbon sources.

Carbon source	33	Circle 1	41A	AERA	None
Acetate 10%					
Acetate 1%			-		
Acetate 0.1%			+/- p		
Lactate 10%			-		
Lactate 1%			+/- p	+	
Lactate 0.1%	-		+ p	+	
Glucose 10%	+ sp		-	+ sp	
Glucose 1%	+		+	+ sp	
Glucose 0.1%	-		+ rp	+	
Sucrose 10%	+++ rp		+++ sp	+++ sp	
Sucrose 1%	+ rp		+ sp		
Sucrose 0.1%			+/- sp		

+ = growth

+++ = very good growth

+/- = may be growth or chemical precipitate

- = no growth

p = precipitate

rp - ropy-looking precipitate

sp = sticky precipitate

All isolates and undefined consortia enriched from environmental samples are currently being maintained as active cultures. Additional strains that are isolated will be categorized as this project continues. More extensive testing will be done to determine which cultures produce more polymer with various nitrogen sources.

### Conclusions

- The AERA culture exhibited good growth with a variety of nitrogen sources. It will be used for further studies.
- Culture 33 had the highest viscosity, so it will also be used for further studies.

## 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 have 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 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.

Additional sandpack experiments will be conducted with other synthetic and natural brines.

#### Experimental

The cultures which were identified in the 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.

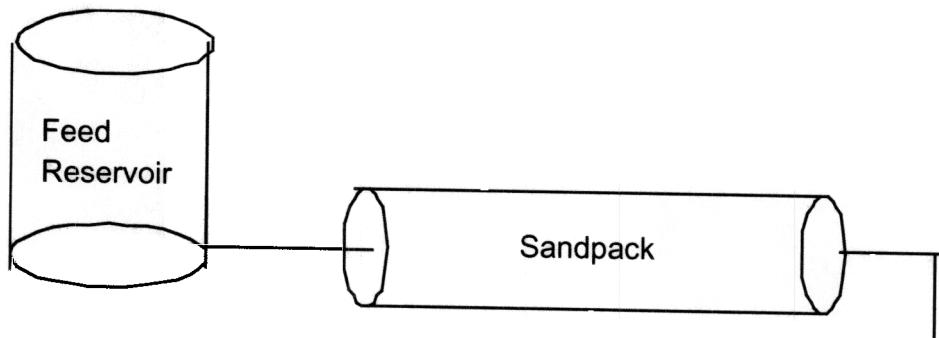


Figure Sandpack flooding schematic.

Sandpack flooding experiments to demonstrate production of polymer in-situ were begun using synthetic brine based on Velma rural water. Brine composition is shown in Table 7. Several floods were run to develop the procedure and apparatus. After the technique was developed, a successful flood was completed. Sandpack, PB-2, was 25.3 cm in length and 1.27 cm in diameter. It was packed with Mill Creek sand. The initial permeability was 10.4 darcies. The sandpack was inoculated with 1 PV of culture 33 grown in DNB broth, then flooded with additional DNB broth with no nitrogen source at 40° C.

Table 7. Composition of synthetic brine.

Component	g/L
NaCl	20
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.35
MgCl <sub>2</sub> •6H <sub>2</sub> O	0.25
Na <sub>2</sub> SO <sub>4</sub>	0.20
NaHCO <sub>3</sub>	0.075

### Results and Discussion

Treatment with DNB broth without nitrate stimulated polymer production which reduced the sandpack permeability from 10.4 darcies to 8.1 darcies, as shown in Figure 2.

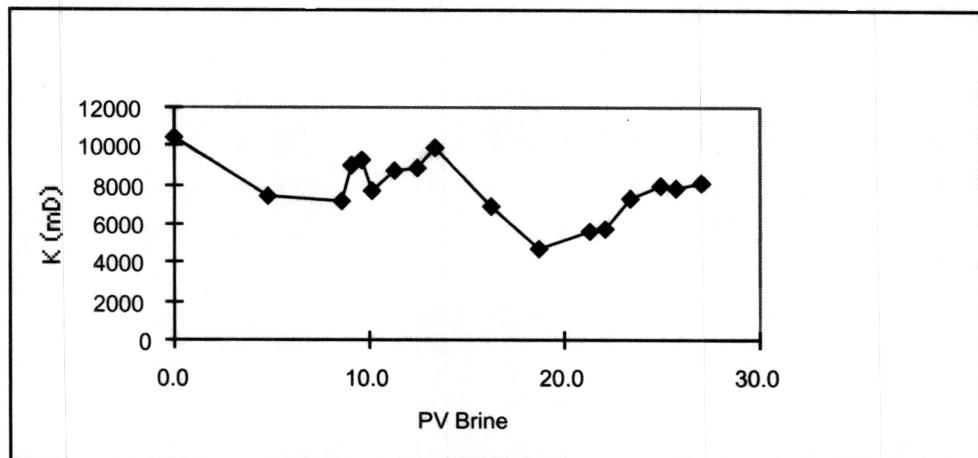


Figure 2. Sandpack PB-2 treated with DNB without nitrogen.

Planned activities include more flooding experiments with sandpacks and core material.

### Conclusions

- Development of selected cultures by nutrient stimulation is underway.
- A sandpack flooding apparatus was developed which works well for these experiments.

## 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.

#### Background

Preliminary data suggest that it is possible and that the technology has the potential to be able to manipulate and modify the production of biopolymer by changing the form of the inorganic salts (nitrate). This ability, which is in addition to effects caused by changes in the concentration of the salts, will have an important effect on the production of microbial products. This new and important development which complements the use of the VFA and indigenous microflora offers a uniqueness to the proposed system that previously has not been considered. Thus, in addition to studies which measure effects of nitrate/nitrite on polymer production, the systems would be studied in regard to the effects of adding various sodium, calcium, or ammonium forms of such salts. This increases the effectiveness of the process. For example, using the ammonium form of nitrate would have an added effect on the types and numbers of the microbial species which are established. The resultant polymer production can be increased. Previous work with different nitrate salts added for sulfide control have shown that biomass/polymer production will be influenced and it is usually observed that lower concentrations of nitrogen will increase biopolymer production. This ability to modify biopolymer composition/concentration by minor

modifications of the added nitrogen sources is being investigated. This directed and controlled addition of the nitrogen compounds can be expected to provide additional advantages for the selective development of a unique in-situ polymer generating system.

### Experimental

Sandpack flooding experiments to demonstrate production of polymer in-situ were begun using synthetic brine based on Velma rural water. Brine composition is shown in Table 7. Several successful floods were completed. Sandpack, PB-3 was 25.8 cm in length and 1.27 cm in diameter. It was packed with Mill Creek sand. The initial permeability was 12.6 darcies. The sandpack was inoculated with 1 PV of culture 33 grown in DNB broth, then flooded with additional DNB broth at 40° C.

Another flooding experiment was done with the addition of sucrose. Sandpack PB-1 was 26.8 cm in length and 1.27 cm in diameter, also packed with Mill Creek sand. The initial permeability was 11.1 darcies. It was inoculated with 1 PV of culture 33 grown in DNB broth, then flooded with additional DNB broth containing 10% sucrose to determine if sucrose would stimulate polymer production more than the addition of nitrate alone. This flood was also tested at 40° C.

### Results and Discussion

Treatment with DNB broth stimulated polymer production in PB-3 which reduced the sandpack permeability from 12.6 darcies to 0.6 darcies, as shown in Figure 3. This demonstrates that the addition of nitrate stimulated polymer production, as compared with the flood in which no nitrate was added. Treatment with DNB broth containing 10% sucrose also stimulated polymer production in PB-1, reducing the permeability from 11.1 darcies to 5.5 darcies, as shown in Figure 4. The treatment with nitrate gave better results than the flood without nitrate and the flood with sucrose added to the nitrate.

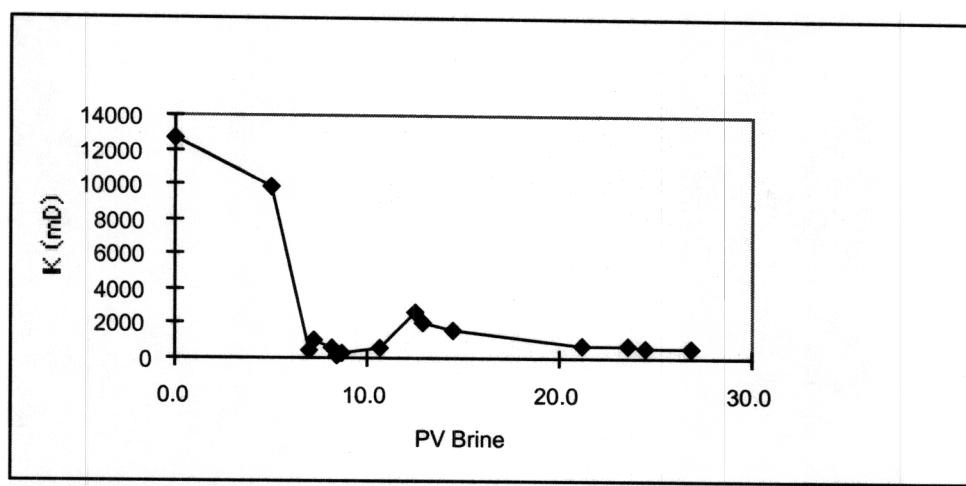


Figure 3. Sandpack PB-3 treated with DNB broth.

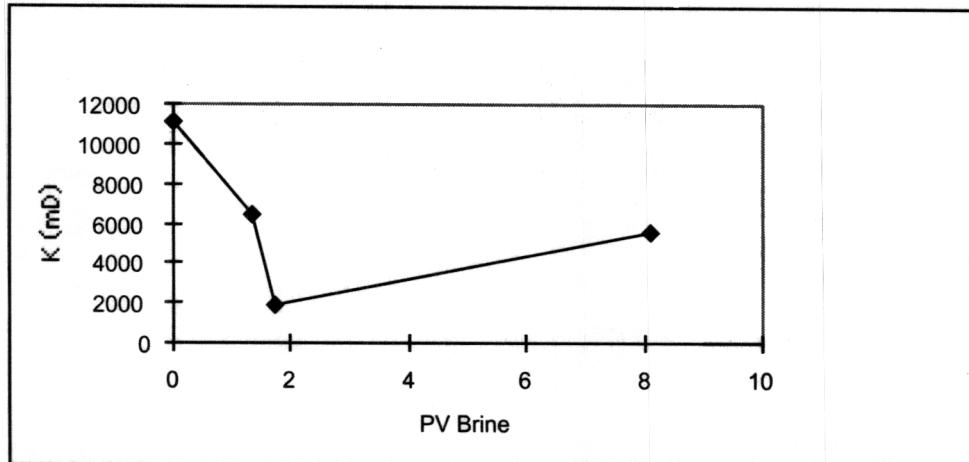


Figure 4. Sandpack PB-1 treated with DNB broth and sucrose.

Additional floods will be completed to determine which nutrients produce the largest decrease in permeability. Floods will also be conducted with field brine and at various temperatures.

### Conclusions

- A flooding apparatus and protocol was developed which worked successfully.
- Treatment with nitrate gave better results than the flood without nitrate and the flood with sucrose added to the nitrate.

## CHAPTER 5

### Oil Recovery Tests

#### Introduction

Protocols and regimes developed in the other Tasks will be used to identify the conditions and parameters which would demonstrate the effective increase of polymer production by the biosystem operations. Such tests will also identify the limitations of the biosystem and lead to the development of alternate treating and flooding schemes to overcome such limitations. This exploratory study approach will allow many variables to be determined rapidly and provide the information needed to conduct more effective tests which measure oil increase. This approach is based on the known observation and data which demonstrated that biopolymer production causes viscosity increase which will have an impact on profile modification, flow patterns, etc. As the system is optimized and treatment procedures and techniques are developed, the flooding tests will include similar systems which contain oil.

It is proposed to introduce oil into the flooding systems as rapidly as possible to develop data on increased oil recovery. This task is being incorporated in conjunction and simultaneously with the preceding flooding tests. The same analytical procedures and testing program will be followed and will be expanded to include all factors and test measurements that concern the oil phase, content, and characteristics. These oil flooding tests could involve a significant portion of the test program and will involve demonstrating and optimizing the effectiveness of the oil recovery biosystem.

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.

## **CHAPTER 6**

### **Data Correlation and Interpretation**

#### **Introduction**

The program will develop extensive data on the microbiology, geochemistry, polymer production, profile modification, etc. of flooding systems. These data will be correlated and integrated for the effects of the biosystems on oil recovery. All results will be examined and correlated for identifying conditions and treatments which maximize the polymer production. The interactions of the results from the various test conditions and parameters will be integrated to present a composite evaluation of the biosystem actions on oil recovery. These results will be reported in a form which could be offered for technology transfer to the oil industry for commercial applications.

## **CHAPTER 7**

### **Field Evaluation of New Technology/Products**

#### **Introduction**

As results are obtained from the laboratory investigations and are made available to field operations through technology transfer, it is anticipated that the findings would be offered and applied to field studies, situations, and operations. As the laboratory results are incorporated into pilot field projects, these field operations would be closely followed and monitored. The identifications of such fields and participation of the operators would provide additional feedback data from such projects. These pilot field evaluations would be conducted in conjunction with ongoing projects whenever possible. By utilizing such ongoing projects, the requirements for collection of base line data, flood responses, field operations, etc. would be minimized and a pilot study could rapidly be implemented with operator assistance. This approach would allow rapid introduction and evaluation of the any system/product which was developed by this program and would provide directly comparable data to be collected. This method of field testing offers a low cost and easily approved and operated system to introduce the technology/products which will be developed in this research program.

## **CHAPTER 8**

### **Reports and Technology Transfer**

#### **Introduction**

The data will be presented in the form of tables, graphs and reports. Such data will be offered in a form most suitable for technology transfer to industry. Reports will be issued semiannually, and as a final comprehensive report. Reports will be issued and offered to industry.

The first Semi-Annual report was delivered on schedule. A presentation on the project was made at the Oil Technology Program Contractor Review Meeting in Denver in June by Scott Bailey.

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