COMBINED MICROBIAL SURFACTANT-POLYMER SYSTEM FOR IMPROVED OIL MOBILITY AND CONFORMANCE CONTROL

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Many domestic oil fields are facing abandonment even though they still contain two-thirds of their original oil. A significant number of these fields can yield additional oil using advanced oil recovery (AOR) technologies. To maintain domestic oil production at current levels, AOR technologies are needed that are affordable and can be implemented by independent oil producers of the future.

Microbial enhanced oil recovery (MEOR) technologies have become established as cost-effective solutions for declining oil production. MEOR technologies are affordable for independent producers operating stripper wells and can be used to extend the life of marginal fields. The demonstrated versatility of microorganisms can be used to design advanced microbial systems to treat multiple production problems in complex, heterogeneous reservoirs.

The proposed research presents the concept of a combined microbial surfactant-polymer system for advanced oil recovery. The surfactant-polymer system utilizes bacteria that are capable of both biosurfactant production and metabolically-controlled biopolymer production. This novel technology combines complementary mechanisms to extend the life of marginal fields and is applicable to a large number of domestic reservoirs. The research project described in this report is performed jointly by, Bio-Engineering Inc., a woman owned small business, Texas A&M University and Prairie View A&M University, a Historically Black College and University.

This report describes the results of our laboratory work to grow microbial cultures and the work done on recovery experiments on core rocks. We have selected two bacterial strains capable of producing both surfactant and polymers. We have conducted laboratory experiments to determine under what conditions surfactants and polymers can be produced from one single strain. We have conduct recovery experiments to determine the performance of these strains under different conditions. Our results do not show a significant influence of nutrient regime on alternate production of surfactants or polymers.

A no cost extension has been awarded for this project; therefore, the next report will contain all the significant findings of this research project.
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STATEMENT OF WORK (SOW)

Under this Statement of Work (SOW), Dr. Jorge Gabitto from the Chemical Engineering Department at Prairie View A&M University (PVAMU), Dr. Maria Barrufet from the Petroleum Engineering Department at Texas A&M University (TAMU) and Dr. Rebecca Bryant from Bio-Engineering International Inc. (BEI) will conduct research and training in the area of microbial improved oil recovery techniques.

A research project is proposed to develop and prove the concept of a combined surfactant-polymer microbial system for advanced oil recovery. The surfactant-polymer system will utilize bacteria that are capable of both biosurfactant production and metabolically-controlled biopolymer production. This novel technology combines complementary mechanisms to extend the life of marginal fields and is applicable to a large number of domestic reservoirs.

This research will involve training of graduate and undergraduate students in state of the art techniques. Technology transfer of the results generated by the project will be achieved through Dr. Bryant’s efforts, presentations in professional meetings and publications in refereed journals.

Dr. Gabitto will act as coordinator of the research team and he will be responsible for the laboratory experimental program. Dr. Barrufet will be Co-Principal Investigator, and she will be responsible for the recovery experimental program. Dr. Bryant will provide the microorganisms to be used in the project, advise the research team, and she will be responsible for transferring the project’s findings to small independent producers.
TECHNICAL DESCRIPTION

INTRODUCTION

Enhanced Oil Recovery

The most common EOR methods are the chemical and the physical methods\(^1\). Chemical methods of enhanced oil recovery are characterized by the addition of chemicals to water in order to generate fluid properties or interfacial conditions that are more favorable for oil displacement. Polymer flooding, using polyacrylamides or polysaccharides, is conceptually simple and inexpensive, and its commercial use is increasing despite the fact that it raises potential production by only small increments. Surfactant flooding is complex, requiring detailed laboratory testing to support field projects. It is also expensive and is only used in few large-scale projects.

Microbial Enhanced Oil Recovery (MEOR) is an EOR method that uses microorganisms and their metabolic products to improve oil production in the reservoir. The majority of the MEOR work leading to field trials has been completed in about the last 20 years. The technology has advanced from a laboratory-based evaluation of microbial processes, to field applications internationally. MEOR has been recognized as a potentially cost-effective method, particularly for stripper wells (well that produces less than 10 bbl/day)\(^2\).

The concept of using microorganisms and their activities in an enhanced oil recovery procedure is not new. The first suggestion for using MEOR was made as early as in 1926, when Beckman reported the action of bacteria on mineral oil\(^3\). As a result of his work, he proposed that bacterial enzymes could be used in oil recovery. However, little work was done until ZoBell started a series of systematic laboratory investigations in the 1940’s in connection with the American Petroleum Institute\(^4\)-\(^6\). The results and ideas presented in ZoBell’s articles marked the beginning of a new era of research in petroleum microbiology. Much more experimental work will have to be done by trained microbiologists in cooperation with petroleum engineers before any definite recommendations can be made.”

A number of field trials were initiated during the 1980’s and 1990’s. The conventional method used was to inject an adapted mixture of bacteria into the reservoir together with a cheap carbon source such as molasses. This process is limited to small onshore installation due to the amount of molasses needed. In later years considerable work has been done in analyzing the potential for doing MEOR on larger offshore fields\(^7\).

In this study we will concentrate on microbial enhanced waterflooding. MEOR is applied to existing waterfloods to improve their performance and enhance oil production by treating the entire reservoir. MEOR materials are added, either continuously or periodically,
to the water holding tanks at the primary injection stations. Normal waterflooding operations are not interrupted when the MEOR process is initiated in a field.

In conventional MEOR method a special culture of microorganisms is injected into the reservoir. This bacterial culture is selected for each individual reservoir and it could be injected as a pure culture, a mixed culture or an adapted culture. During the process, a complete growth medium containing all major nutrients necessary for microbial growth including carbon, nitrogen, and phosphorus, is injected into the reservoir to feed the injected microorganisms. The microorganisms replicate and produce chemicals for oil mobilization based entirely on the cost of nutrients. Bryant\textsuperscript{8} presents the results of two microbial enhanced waterflood fields performed by NIPER (National Institute for Petroleum and Energy Research) at the Mink Unit site, Delaware, Oklahoma. After this pilot project demonstrated a 13\% increase in oil production, an expanded field pilot program at the Phoenix field site in the same area was initiated. The Phoenix field showed a 19.6\% improvement in oil production, and using the nutrient cost only, $2.33/incremental bbl of oil was calculated. The cost for the Mink unit site was calculated to be $3.23/incremental bbl of oil. Unlike commercial surfactant processes, which are linked to the cost of the chemicals and energy involved in their manufacture, microbial surfactant can be produced using inexpensive feedstock\textsuperscript{8}. The microorganisms can be regulated by the amount of nutrient present. In other words, if they are not fed, they will disappear.

In the use of microorganisms \textit{in-situ} for MEOR, it is necessary to use microbial cultures that can survive in the reservoir environment, but also produce the chemicals in sufficient quantities that are necessary for oil mobilization. Before the bacteria are injected into the reservoir they must have very good growth under facultative anaerobic conditions (e.g., can survive under both aerobic and anaerobic conditions) and a high metabolic activity of molasses with important production of oil displacement agents\textsuperscript{9}.

Laboratory research has demonstrated that microbial products can change the chemical and physical properties of oil, and selectively plug high permeability zones to improve sweep efficiency\textsuperscript{10-12}. The transport of microorganisms in the reservoir rock has also been studied in the laboratory and the results indicate that certain strains of microbes can be transported through the reservoir rock under proper conditions. Associated with these laboratory investigations mathematical simulations have been introduced to help understand the mechanisms involved in the MEOR process\textsuperscript{9,13}.

The microorganisms play various roles in the reservoir. The most important ones considered in this project are\textsuperscript{14}: production of surfactants, selective plugging of the reservoir, and alcohol production. Microorganisms can produce surfactants that can decrease surface and oil water interfacial tension to as low as $5\times10^{-3}$ mN/m\textsuperscript{17}. Interfacial
tension between oil and water is normally about $10^1$ mN/m$^{15}$. In general, a biosurfactant is easily dissolved in connate water or injection water and acts favorable on the interface between oil and water. A surface tension reduction decreases the pressure required to release oil trapped in the rock pores by capillary forces, which displaces oil from the pores into the mobile liquid phase. A microbial biosurfactant usually act in the same way as synthetic surfactants and have the same characteristics$^{15}$.

Another application for microorganisms in a waterflood is fluid diversion. Since many types of microorganisms produce polymers, biomass and slimes, it has been suggested that some microorganisms could be used in-situ to preferentially plug high permeability zones in the reservoir, and thus improve sweep efficiency $^{16-17}$.

Microorganisms can also produce organic solvents like alcohol. Solvents decrease oil viscosity, making it thinner and increasing flow$^{14}$.

**EXPERIMENTAL PART**

**Growth Experiments**

**Introduction**

The main goal of the research described in this proposal is to develop a combined microbial surfactant-polymer system using bacteria that produce both biosurfactant and biopolymer. This concept is based on our experience with a bacterium, *Bacillus licheniformis*, that is known to produce both products in sufficient quantities to make the process feasible. Strains of *B. licheniformis* have been previously reported both for surfactant production (Bryant et al.$^{18}$, Lin et al.$^{19}$) and for polymer production; (Ramsay et al.$^{20}$, Bae et al.$^{21}$) there are no reports in the literature of the two products being investigated together for a combined process. A developed strain of *B. licheniformis*, NIPER 1A, is used. NIPER 1A transports well through oil reservoirs and produces copious amounts of biosurfactants. However, NIPER 1A produces only minimal amounts of biopolymer.

Biosurfactants cause a decrease in the interfacial tension between oil and water phases, which is the primary mechanism by which surfactant-producing bacteria mobilize oil. NIPER 1A strains that produce large amounts of biosurfactant are being tested for their effects on interfacial tension between aqueous and oil phases. Interfacial tension are measured at various stages of culture growth and over a reasonable range of nutrient levels.
using appropriate laboratory tensiometer methods such as spinning drop or the du Nuoy ring method.

Polymer-producing bacteria polymerize carbohydrates present in growth substrate into extra-cellular polysaccharide biopolymers. The production of soluble biopolymers causes viscosity increases in the microbial growth medium. Polymer production can be stimulated by nutrient manipulations and/or other external factors. Optimum polymer production is determined by chemical assays and by measuring viscosity increases with a tubular viscometer.

An important part of this research is to validate the concept that a microbial surfactant-polymer system can be designed to advance current technology for improving oil recovery from porous media in the laboratory. We do this in a series of experiments designed to show advantages of concerted microbial polymer system blockage of watered out high permeability zones and microbial surfactant-improved mobilization of oil from oil-bearing zones. The two mechanisms for oil recovery that are developed in this research program, increased oil mobility and profile modification, are tested and optimized individually in coreflood and/or sandpack experiments. The processes can then be combined in more complex models to test the performance of the combined system under simulated reservoir conditions.

The majority of pure culture studies have used glucose as the substrate. When grown in a batch culture, the change in microbial population with time follows the classical growth curve (Fuyimoto, 1963\textsuperscript{22}). This curve has three quite distinct phases, namely lag, exponential and stationary. The lag phase of growth represents the acclimation period of an organism to its new environment. After the onset of cell division the organism moves out of the lag period and continues dividing exponentially until such time as the medium is no longer able to support growth. This exponential phase of growth is the most important part of the growth curve as it represents the maximum rate of substrate removal, and in wastewater treatment the aim is to remove the substrate as rapidly as possible (Gaudy and Gaudy\textsuperscript{23}). After a given period of time the microorganism population reaches the stationary phase of growth and it is reasonable to assume that this is a direct result of substrate depletion. In this case glucose and/or other sugars are said to be the growth limiting substrate. The decline in population appearing in the last portion of the growth curve represents the death period. In this period population decline outnumbers population growth and the cells still alive remain dormant.
Bacterial Strains

The work done in the first two years of this project showed that NIPER 1A, a laboratory modified strain of *Bacillus licheniformis* that produces copious surfactant, and NIPER 11, a laboratory modified polymer producing *Leuconostic mesenteroides* are the microorganisms relevant to this study. Therefore, the experimental work is related primarily to them.

Microorganisms require suitable nutrients as well as a favorable environment for growth. First, the *culture medium* must contain those nutrients essential for the growth of a given microorganism. Second, this medium must provide suitable surroundings for growth, the proper pH, osmotic pressure, oxygen, temperature, etc. Many different substances will serve satisfactorily as a culture medium. We have worked so far with liquid cultures.

The microorganisms listed above were grown under different conditions in different media. A broth, Brewer Thyoglycolate by Difco, was selected as the most suitable medium to grow and maintain the aforementioned organisms. This broth contains meat extract and sucrose as principal nutrients. It also contains phosphorus and nitrogen compounds. Good growth was achieved working under anaerobic or oxygen limiting conditions. The growth under aerobic conditions was significantly less and in some strains almost negligible. The original microorganisms were provided in this culture; therefore, it was thought that it was convenient to keep the original cultures in this broth, placed in an oven at constant temperatures of 33 °C. The cultures used in laboratory and recovery experiments were prepared by taking a 10 ml sample from the Brewer Thyoglycolate broth medium and placing it in 250 conical flasks filled with a 1% peptone in deionized water solution. Later sugars were added to study the influence of different sugar sources. Sucrose, glucose and mannose were added. Typically, 10% sugar solutions in peptone water were prepared. These solutions will be referred as, sucrose -peptone broth, glucose -peptone broth, etc.

Growth conditions for NIPER 11A have been published in literature 24. NIPER 11A was observed to be limited to a salinity range of 0 to 4 wt% NaCl, with an upper temperature limit of 35° C. This microbial system can tolerate a pH range from 4.5 to 8.0. Other microbial strains were observed to tolerate a salinity up to 14 wt% NaCl, when grow in Tryptic soy broth, and up to 5 wt% when grown in sucrose peptone broth. Its upper temperature limit was 45° C, with a pH tolerance from 6.0 to 10.0.

Measuring Techniques

A brief description of the laboratory techniques used in this project is provided below.
Colony Counting Procedures

In this work, the Standard Plate count technique (SPC\textsuperscript{25}) was used to enumerate the microbial population. It is a direct quantitative measurement of the viable aerobic and facultative anaerobic bacteria in a water environment, capable of growth on the selected plating medium. An aliquot of the water sample is pipetted into a sterile petri dish where a tempered agar medium has been added. The plate is rotated to evenly distribute the bacteria. Each colony that develops on or in the agar medium originates theoretically from one single bacteria cell.

Although no one set of plate count conditions can enumerate all organisms present but the number and types of bacteria that developed are influenced by the time and temperature of incubation, the level of oxygen, the presence of specific nutrients in the growth medium, the pH of the medium and competition among cells for nutrients. The Standard Plate Count Method provides the uniform technique required for comparative testing water quality in most situations. When the method applies to research work, however, the consistency of the parameter and media become more important.

The preparation process was, dissolve 1 g of peptone water (crystals) into 1 liter of distilled water, dispense 9 ml to each test tube. Autoclave at 121-124 °C for 15 minutes and allow to cool to room temperature. Dissolve 23 grams of Nutrient Agar into 1 liter of water and boil completely. Autoclave at 121-124 °C for 15 minutes, and place agar in a tempering water bath, maintained at a temperature of 44-46 °C. Do not hold agar at this temperature longer than three hours because it may form precipitates which confuse the counting of colonies.

Put 9 ml of 0.1% peptone water in 10 different sterile test tubes, label them as 10\textsuperscript{-1}, 10\textsuperscript{-2}, ....,10\textsuperscript{-10}, make a 1:10 dilution using 1 ml of solution A with a 1 ml pipette, mix each tube carefully by vortexing. Obtain 11 sterile petri plates, label them with the appropriate dilution i.e. 1, 10\textsuperscript{-1}, ...10\textsuperscript{-10}. Begin with highest dilution, and working backward, pipet 1.0 ml of aliquot from every diluted sample into their appropriate petri dishes including the straight sample. Pour approximately 10 ml of nutrient agar (held at 45 °C) into each petri dish, swirl gently to disperse the sample evenly, put on all the cover of the petri dishes, allow to cool down for 10 minutes. After the agar plates have hardened on a level surface, invert the plates and immediately incubate at 35 °C for 24-48hrs. Also pipet 1 ml of peptone water into a petri dish, add agar mix and incubate with the others. This is the control plate, it will check the sterility of pipets, agar, dilution peptone water and petri dishes.

After the required incubation period, examine plates and select those with 30-300 colonies. Count these plates immediately by placing the plate to be counted on a colony
counter, a standard plastic petri dish has a surface area of 57 cm$^2$; count the colonies in 13 squares usually highlighted on the surface of the colony counter a representative distribution of colonies. Select 7 consecutive horizontal squares and 6 consecutive vertical squares for counting, some colonies will contact with grid lines, count the colonies individually, even if they are in contact with each other.

Record the sum of the colonies in these 13 cm$^2$, and multiply by 4.32 to estimate the colonies per plate. Then multiply by dilution to determine the count/ml, i.e., the number of bacteria/ml of your original sample, record as S.P. Count /ml (Standard Plate Count per ml).

If there are less than 30 colonies on all plates, record the actual number of colonies on the lowest dilution plated and report the count as, Estimated Standard Plate Count per milliliter e.g. ESPC = 100 cfu/ml. If 1ml volumes of original sample produce counts less than 30, actual counts are reported.

**Surface Tension Measurements**

Measurements of the surface tension of the different cultures were made using a Fisher model 21 surface tensiometer. All measurements were made at a temperature range of 20 - 23°C. The method is based on the force needed to lift a platinum-iridium ring from the bulk of a sample fluid through the surface.

**Viscosity Measurements**

Viscosity was measured using an Ube-Lode type viscometer. The apparatus allows viscosity measurements in the range 1-10 cp at 25 °C. Only 2 ml of sample are needed for the measurements. The apparatus is based on the Hagen-Pousielle equation that predicts a linear relationship between a fluid viscosity and the time required for this fluid to transverse the distance between two fixed marks on a cylindrical calibrated glass tube.

**Recovery Tests**

One of the purpose of the microbial polymer systems for improving oil recovery is to block fluid flow through watered out thief zones in the reservoir and divert fluid flow into less hydrologically accessible regions of the reservoir. Polymer producing microbes (NIPER 11) were used for its ability to produce abundant amount of polymer as verified in our laboratory tests. Polymer-producing bacteria identified in this study were tested for their ability to reduce the permeability of porous media. The ability of the polymer-producing bacteria to block fluid flow and cause fluid diversion was also tested using coreflood experiments. The other main purpose was to mobilize oil by decreasing surface tension at the oil-water interface. Surfactant producing microbes (NIPER 1A) were tested in
coreflood experiments for their ability to mobilize oil. These experiments were conducted at the Petroleum Engineering Department at Texas A&M University. Dr. Bryant facilitated oil samples and the original microbial samples.

The experimental set-up is shown in Figure 1. Blocks of Berea sandstone were obtained and cut in cylindrical shape, 25 cm in length and 2.5 cm in diameter. The cores were encased in rubber sleeves and placed inside Hassler coreholders. Coreflow experiments were conducted to determine permeability reduction due to \textit{in-situ} biopolymer production. Berea cores were injected with the sucrose-peptone growing medium. Later, microbes were injected and left incubating for six days and permeability was determined.

Coreflow experiments were also carried out to determine residual resistance factors ($F_{rr}$). The aforementioned Berea cores were saturated with 0.5\% brine solution and injected with 1 PV sucrose-peptone medium inoculated with NIPER 11. The cores were shut in for six days then flooded with brine, and flow rates and pressures were measured. The residual resistance factors ($F_{rr}$) were then calculated using the following equation,

$$F_{rr} = \frac{Q_w/\Delta P}_{\text{brine}} / \frac{Q_w/\Delta P}_{\text{AfterMicrobialInjection}}$$  \hspace{1cm} (1).

Here $Q_w$ is the flow rate, and $\Delta P$ is the pressure difference between fluid input and output.

Recovery experiments were carried out using NIPER 1A and NIPER 11 cultures. After been placed in Hassler coreholders the Berea sandstone cores were evacuated and flushed with a brine 2\% solution. Crude oil was injected into the cores until no additional water was produced, about 24 hours. Then, brine was injected until no more crude oil was produced. The core thus simulated a waterflooded ROS condition designated by $S_{orwf}$.

Oil samples were obtained from the Delaware-Childress field in northeastern Oklahoma and from the Wilmington field in California. Delaware-Childress oil has a gravity of 31 °API (0.87 g/cm3), and Wilmington 17 °API (0.97 g/cm3). The first qualifies as a light/medium crude while the second was used as representative of heavy oils.

The following experimental procedure was adopted. The Berea sandstone cores that have been waterflooded to the residual saturation $S_{orwf}$ are prepared for microbial injection. The pore volume of these cores varied from 55 to 60 ml. The cores were injected with 0.2 PV of a bacterial cells solution (at a concentration of about $10^6$ cells/ml) in 1 \% peptone solution and 0.3 PV of 10\% sugar solution. Glucose, mannose and sucrose
solutions were used as sugars solutions. The cores were allowed to incubate at 33 °C for 1 week. The cores are flooded with brine at a rate of 0.3 to 0.6 m/day, and fractions of the core effluents were collected. The amount of oil in each fraction was determined, and the residual oil in the core, $S_{orf}$, was calculated. The oil recovery efficiency ($E_r$, %) was calculated using the following equation,

$$E_r = 100 \left( \frac{S_{orf} - S_{orf}}{S_{orf}} \right)$$

(2).

Effluent fluids were separated and the water phase was separated for further analysis. Surface tension, viscosity and colony count measurements were conducted on the separated water phase. The same procedure was followed for the NIPER 1A and the NIPER 11 cultures.

**Results**

Surface tension and viscosity for NIPER 1A and NIPER 11 samples were recorded for more than 11 consecutive months. Periodic plate counts, SPC/ml, were also measured in order to determine microbial numbers. Figure 2 shows results for NIPER 1A samples grown in Tryptic soy and Thyoglycolate broth media. Glucose was used as hydrocarbon source for microbial growth. The growth curve depicts all the classical kinetic periods. At first, the number of microorganisms did not show appreciable growth (lag period). After this lag period the microbial population growths exponentially until reaches equilibrium and after approximately nine months started to decline in numbers. The same behavior was appreciated for all our experiments in different growth media. Similar results were obtained for the NIPER 11A samples. The lag period was computed adding the time the original microbial cultures were grown in ACORN Biotechnical26 and the time we incubated them in our laboratories.

Typical results corresponding to our laboratory experiments are shown in Table 1. We can see that NIPER 1A cultures produced a significant reduction in the medium surface tension, more than three orders of magnitude. This behavior was observed for all the NIPER 1A cultures grown independently of the sugar used. In general glucose and mannose cultures produced similar results while the values measured using sucrose cultures were lower. The same trends were observed for standard plate counts (SPC) per ml and viscosity measurements. These results verified that NIPER 1A is a good surfactant producer and that there is a direct relationship between population size and surfactant production. The bigger the population the bigger the amount of biosurfactant produced and, therefore, the lower the
surface tension is. NIPER 11 results showed a much smaller reduction in surface tension even when the population of these microbes was about the same size of NIPER 1A populations. This result reflects the fact that NIPER 11 is a poor surfactant producer and no significant change has resulted from the use of different sugars as nutrients. NIPER 11 microbes produced significant increase in the viscosity of the cultures (more than 100%) while the population growth of NIPER 1A microbes only produced a small increase in viscosity. These results were also confirmed by visual observation of a big amount of deposited solids in the bottom of the NIPER 11 culture tubes. Our results confirmed than NIPER 11 is a good polymer producer, but it is not a good surfactant producer.

There were not significant differences between the results measured using glucose and mannose while the property values measured for sucrose grown cultures were generally lower. Glucose and mannose were better nutrients than sucrose producing faster microbial growth than the sucrose solutions. Most of the cultures grown during this project exhibited the same behavior independently of the type of microbe.

The difference in measured properties appears to be produced by the difference in population growth and not by an extra production of surfactant and/or polymer.

Figures 3 and 4 show the experimental results obtained for residual resistance factors for NIPER 11 and 1A cultures. The best results were obtained using a constant backpressure set using the regulator shown in Figure 1. NIPER 11 produced a significant increase in the residual resistance factors values for the entire range of flow values studied. The high residual resistance factor values are explained by a high microbial growth rate inside the core pores. These results were supported by permeability measurements before and after microbial injection. The residual resistance factor values decreased as the volumetric flow rate increased. This behavior is attributed to looser microbial attachment to the solid surface as flow rate increases. No significant differences in the experimental results were observed changing the nutrient sugar.

Figure 4 shows equivalent results for NIPER 1A cultures. Residual resistance factor values measured for NIPER 1A cultures were significantly lower than the ones measured for NIPER 11. It seems that NIPER 1A microbes only change the porosity of the cores due to cellular growth and not by high production of biopolymers as NIPER 11 cultures do. This conclusion is supported by the smaller viscosity measured in NIPER 1A cultures compare with the values measured for NIPER 11 solutions (Table 2).
Table 2 shows typical results of our recovery experiments using the two types of oils and cores of different absolute permeability. NIPER 1A populations produced a significant decrease in the solutions surface tensions while NIPER 11 cultures only slightly changed the surface tension of the produced solutions. NIPER 11 cultures increased the viscosity of the solutions while NIPER 1A cultures did it only slightly. Similarly to the laboratory measurements no significant differences in population size values were observed between the two kinds of microbes using the same sugars as nutrients. Glucose and mannose performed better as nutrient than sucrose, but acceptable values were measured for the sucrose solutions. NIPER 1A solutions showed better recovery efficiency \( (E_r) \) than the NIPER 11 ones.

Better recovery was measured using medium permeability cores than low permeability cores. A systematic study of core permeability influence on recovery was not carried out at this time.

Oil recovery results are shown in Figures 5 and 6. The results in Figure 5 were obtained using NIPER 1A samples while the results shown in Figure 6 were measured using NIPER 11 samples. Both microbial samples show increase recovery as the injected brine volume increases compared to the residual oil saturation after waterflooding. Residual recovery efficiency \( (E_r) \) in the NIPER 1A cultures was above 53\% (Table 2). NIPER 11 cultures showed lower, but still respectable recovery values. Recovery values for sucrose nutrient solutions were only slightly smaller than the glucose and mannose values in the NIPER 1A experiments and practically the same in the NIPER 11 experiments. The recovery curves are very similar, but the main mechanism of oil recovery in the case of NIPER 1A is surface tension reduction while in the case of NIPER 11 is improved areal sweep efficiency. NIPER 1A samples are well-known producers of surfactant while NIPER 11 cultures produce abundant amounts of glucose biopolymers.

Similar residual oil saturation curves were obtained when heavy oil samples were used (Chaffee). The residual recovery efficiency was higher in this case for NIPER 1A samples, 72.2\%, than in the experiments done using NIPER 11, 47.1\%. A possible explanation could be the higher permeability of the core used in the experiments with NIPER 11 cultures. The NIPER 11 microbes could only partially shut-off the high permeability pores, therefore, a significant amount of by-pass led to lower recovery efficiency. These results are similar to the ones reported by Bryant and Douglas\textsuperscript{24}. 

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Conclusions

NIPER 1A, surfactant producer, and NIPER 11, polymer producer, showed a lifetime cycle of more than 9 months. This cycle reproduces the classical periods for bacterial growth. Bacterial cultures with high number of surfactant producing microorganisms produced lower surface tension than the low number cultures. Monosaccharides solutions are slightly better nutrients than sucrose solutions. Sucrose solutions still performed well enough, and are in general more economical than monosaccharides solutions. Similar number of polymer-producing microorganisms produced similar values of viscosity in the different growth media.

The improved recovery efficiency for different nutrients seems related to population numbers and not to different recovery mechanisms. The residual recovery curves were similar for NIPER 1A and NIPER 11 samples using both light and heavy oils. Medium permeability cores produced more recovery than low permeability cores.

The improved recovery efficiency for different nutrients seems related to population numbers and not to different recovery mechanisms. High surfactant production is the main recovery mechanism during the NIPER 1A recovery experiments while improved sweep efficiency is the main recovery mechanism during the NIPER 11 experiments. These conclusions are supported by low surface tension values during the NIPER 1A experiments and high viscosity during the NIPER 11 experiments.

Our experiments do not support metabolically change in recovery mechanisms at this time. Probably mixed microbial cultures hold more promise than single microbial cultures.

FUTURE WORK

Laboratory experiments will be continued using different carbon sources in order to determine if the microorganisms respond differently to different nutrients. Microbial samples grown for 2 months will be transfer to flasks with different carbon sources. Weekly samples will be withdrawn and colony counting, surface tension and viscosity measurements will be taken. Some experiments using mixed NIPER 1A and NIPER 11 cultures will also be conducted. Some of these cultures will also be used in residual recovery experiments.
Experiments to determine residual resistance factors and oil recovery will be performed. Flow rates and pressure measurements will be used to determine permeability and residual resistance factors. The fluids will be collected and the final oil saturation will be determined from mass balance.

All experimental results will be collected, conclusions will be drawn and a final report will be written.

**PRESENTATIONS**

A paper summarizing the work done on this project has been accepted for presentation at the American Institute of Chemical Engineers Annual Meeting to be held at Austin, TX, November 11-15, 2004.

**STUDENTS**

Ms. Sharmila Enayet has been working on this project as part of his Master thesis. Ms. Sherronda Martin has conducted undergraduate research on this project.

**REFERENCES**


## TABLES AND FIGURES

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<th>Microbe</th>
<th>Time</th>
<th>Nutrient</th>
<th>SPC/ml (c.f.u.)</th>
<th>Viscosity (cp)</th>
<th>Surface Tension (dynes/cm)</th>
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Table 1. Summary of typical laboratory experiments.

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<th>Oil</th>
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<th>SPC/ml</th>
<th>Viscosity (cp)</th>
<th>Surface Tension (dynes/cm)</th>
<th>( S_{\text{orw}} ) (%PV)</th>
<th>( S_{\text{orcf}} ) (%PV)</th>
<th>( E_r ) (%)</th>
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Table 2. Summary of typical coreflood experiments.
Figure 1. Experimental set-up for oil recovery experiments.

Figure 2. Lifetime cycle for NIPER 1A microorganisms.
Figure 3. Residual resistance factor as a function of volumetric flow for NIPER 11 cultures.

Figure 4. Residual resistance factor as a function of volumetric flow for NIPER 1A cultures.
Figure 5. Residual oil saturation versus injected PV for NIPER 1A cultures.

Figure 6. Residual oil saturation versus injected PV for NIPER 11 cultures.