FOSSIL ENERGY

NIPER-351
CONF-870858
(DE88001232)

PROCEEDINGS OF THE SYMPOSIUM ON APPLICATIONS OF MICROORGANISMS TO PETROLEUM TECHNOLOGY

Bartlesville, Oklahoma
August 12-13, 1987

Thomas E. Burchfield
Rebecca S. Bryant

September 1988

Performed Under Cooperative Agreement No. FC22-83FE60149

IIT Research Institute
National Institute for Petroleum and Energy Research
Bartlesville, Oklahoma

National Petroleum Technology Office
U. S. DEPARTMENT OF ENERGY
Tulsa, Oklahoma

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Printed in the United States of America. Available from:
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes
Paper copy: **A14**
Microfiche copy: **A01**

NIPER-351
CONF-870858
Distribution Category UC-122

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U.S. Department of Energy
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Sponsored by
IIT Research Institute
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EXECUTIVE SUMMARY

The interest in applications of microorganisms in petroleum technology results from the potential low cost and versatility of microbial formulations. With recent declines in oil prices, the petroleum industry has needed an oil recovery technology that can be applied in a cost-effective manner to increase production from declining oil fields. Microbial enhanced oil recovery (MEOR) methods may be able to increase our recoverable oil reserves when oil prices are such that other methods are not economically attractive. The purpose of this symposium was to review the state-of-the-art in development and application of microbial methods to petroleum technology. The focus of the conference was on application of the technology in the field, particularly by independent petroleum producers.

More than 120 interested persons attended the conference from 14 states including the major oil producing states of Texas, Oklahoma, Kansas, California, Illinois, Colorado, and New Mexico. The international interest in microbial EOR was evidenced by the attendees from four foreign countries including Australia, Japan, Canada, and the United Kingdom. Representatives from four domestic and two foreign major oil companies also attended. The symposium was cosponsored by the IIT Research Institute/National Institute for Petroleum and Energy Research (NIPER), the Independent Petroleum Association of America (IPAA), the Oklahoma Independent Petroleum Association (OIPA), the Kansas Independent Oil and Gas Association (KIOGA), the Illinois Oil and Gas Association (IOGA), and the U.S. Department of Energy.

Microorganisms can be thought of as minute factories, which produce certain chemical products when provided with an appropriate feedstock and operating conditions. Laboratory studies are required to select, isolate, and adapt the microbial species with the best properties. Microbial methods are attractive for oil recovery applications because bacteria are self-replicating and therefore inexpensive to produce using inexpensive, readily-available nutrient sources, such as molasses. Furthermore, microbes can be chosen which do not pose a threat to the environment, and in the unlikely event that deleterious effects are observed from a microbial treatment, the injection of nutrient can be stopped, or a biocide can be injected to mitigate the problem. Through careful design, microbes can produce a diverse spectrum of chemical products under a variety of conditions and are therefore potentially applicable in many different reservoir environments.

There are numerous applications of microorganisms in petroleum technology. The papers in this proceedings describe several applications for microbial systems that are currently being developed. Well-stimulation treatments have been used to increase well productivity through mitigating near-wellbore damage or increasing oil mobilization. Injection of polymer-producing microbes or bacteria that produce large quantities of biomass have been used to alter permeability contrasts in heterogeneous reservoirs and modify injection profiles. Microbial systems are also being developed to mitigate the effects of water coning at producing wells. Microbial-enhanced

waterflooding methods are also currently being developed and applied. Microbial systems can be devised to provide the improvements in displacement and sweep efficiency that are typically achieved through application of different chemical products.

The mechanisms of oil mobilization in reservoir rock by injection of bacteria are still not well understood. Microbes can produce chemical products such as surfactants, polymers, acids, gases, and solvents that can assist in oil mobilization by reducing interfacial forces and crude oil viscosity, and by altering rock wettability and permeability. However, in addition to the effects of the chemical products from the microbial metabolism of a nutrient, there are physical interactions between the microbes and both the rock and oil. In reservoir rock the greatest concentration of microbes is usually in the brine phase; however, the physical nature of many bacteria will result in interactions at the rock-brine and oil-brine interfaces. Chemical and physical interactions between the microbes at the rock-brine interface can lead to alterations in wettability, which can affect the fractional flow of oil and brine. At the oil-brine interface, relatively high local concentrations of chemical products can reduce the interfacial and viscous forces that hold oil droplets trapped in the porous rock matrix or lead to alteration in the properties of crude oil. Further understanding of the mechanisms of oil mobilization by microorganisms and of the interactions between bacteria and both reservoir rock and fluids is needed to fully determine how best to apply this advanced technology.

Microbial oil recovery methods are complex because the biological bacterial systems are complicated living cells. However, microbial systems are also versatile, being able to produce a wide spectrum of chemical products under a variety of conditions, and they offer the unique ability to be self-perpetuating when supplied with a suitable nutrient. This characteristic is unlike that of any other currently used oil recovery fluid. As advances are made in bioengineering and bioprocessing, the means to design microbial formulations to produce copious quantities of a specific chemical product at a relatively low cost may become a reality. Other applications of biotechnology in petroleum and chemical processing technologies include production of chemicals such as biosurfactants and biopolymers through industrial fermentation, upgrading of crude oil through bioprocessing, and removal of deleterious components of gas such as hydrogen sulfide through application of microbial technology.

It is extremely important to prevent any adverse effects on the environment when applying any oil recovery method. Most of the general public perceive bacteria as a cause of disease. While this is true for certain classes of organisms, it is not, in general, true of the microbes that are used in MEOR. The organisms that are used in MEOR field tests usually have been isolated from oil field environments and are part of the native microbial flora and are not pathogenic. While mutation is possible, it is highly unlikely that any of the types of bacteria that are currently being used in the field would mutate to form a dangerous pathogen. One possible adverse environmental effect of applying MEOR is stimulation of indigenous sulfate-reducing bacteria (SRB), which can produce toxic hydrogen sulfide and cause corrosion. Most of the chemical

products from microbial fermentation are no different from chemicals that are commonly injected into oil reservoirs. Field test results have indicated that in many cases SRB are not stimulated, and that methods can be devised to mitigate the problem if they are. MEOR methods are frequently applied in aging wells that may have problems with integrity of the casing. Care must be taken through laboratory testing and proper engineering design to ensure that contamination of potable water aquifers does not occur. The environmental concerns when applying MEOR are really no different than those associated with any EOR method. Laboratory compatibility testing and reservoir engineering design procedures should be used to avoid potential adverse environmental effects.

The proceedings consist of 19 papers submitted by authors from the United States and 7 foreign countries and encompass laboratory studies to understand the mechanisms of oil recovery by microorganisms, results of field applications of MEOR methods, and economics. The widespread interest in the technology and the scientific results presented at this symposium indicate that MEOR is an emerging technology that may significantly impact the petroleum industry.

General

The problems associated with development and application of various MEOR methods are discussed in the paper entitled "MEOR Systems: Problems of Design and Scale-up" by V. Moses (Queen Mary College, UK) and M. J. Brown (QMC Industrial Research, Ltd, UK). The authors present a comprehensive discussion of the different ways that microbial technology can be applied to improve oil recovery. The important parameters in designing MEOR systems for applications such as well stimulation, selective plugging, and polymer and surfactant flooding are discussed. The authors make the important point that MEOR is not one process but a set of different techniques that must be designed for specific applications.

The need for development of a cost-effective technology that can be applied by independent petroleum producers in stripper wells is discussed in the paper by J. W. King (Hardin-Simmons University), entitled "MEOR Update and Technical Assessment." The paper summarizes the statistics which indicate the need for development of cost-effective methods for increasing oil production. The paper also summarizes the technical constraints and advantages of MEOR methods and outlines priorities for future research that is needed to develop the technology.

Environmental

The potential environmental impact of injection of microbial cultures into oil reservoirs is an important concern, as it is with any EOR method. The paper entitled "Environmental Aspects of Microbial Enhanced Oil Recovery" by J. Douglas, R. S. Bryant, and K. Bertus (National Institute for Petroleum and Energy Research) discusses laboratory and field methods for determining the complex interactions

between the injected bacteria and the indigenous microbial population, which is present in all oil reservoirs. The paper emphasizes the importance of laboratory studies in preventing potential environmental problems and indicates that MEOR methods can be applied in an environmentally safe manner if they are properly designed.

The potential of sulfate-reducing bacteria (SRB) to cause biocorrosion in oilfield equipment is discussed in the paper by G. Horacek (Conoco), which is entitled "Biocorrosion in the Oilfield." The paper emphasizes potential problems that EOR and MEOR methods may encounter because of biofilms formed by SRB. An experimental procedure for studying the development of biofilms and corrosion is described.

The use of biotechnology to mitigate environmental problems that may be associated with EOR projects is considered in the paper entitled "Potential Roles for Bioprocessing in Enhanced Oil Recovery," by J. S. Watson and C. D. Scott (Oak Ridge National Laboratory). If the petroleum industry comes under increasingly stringent environmental regulations with regard to produced brines, microbial methods may be useful in treatment prior to disposal. The authors also discuss the need for further study on the merits of surface production of chemicals for injection and bioprocessing to upgrade crude oil.

Field

In the paper entitled "Review of Microbial Enhanced Oil Recovery Field Tests" D.O. Hitzman (INJECTECH, Inc.) updates his prior summary, which was published in 1982, of the results of MEOR field tests in the U.S. and foreign countries. His recent report indicates that there have been more than 200 MEOR field tests conducted in the U.S. of which 22 field tests have been performed since 1982. In the more recent field tests (i.e., since 1982), more extensive testing is being conducted in the laboratory prior to field testing and a smaller volume of microbial formulation is being injected. Alternate MEOR techniques such as fluid diversion and paraffin removal have been advanced to the stage of field testing. The reservoir data and oil recovery performance are summarized for selected field tests.

T. R. Jack and L. G. Stehmeier (Nova/Husky Research Corporation, Ltd., Canada) describe a novel application for microbes in their paper entitled "Selective Plugging in Watered Out Oil Reservoirs." A microbial method for selectively reducing the permeability of zones in a heavy oil reservoir in Canada is described. The laboratory procedures used to design the microbial system and the injection scheme for the microbial treatment are discussed. A polymer-producing organism, which produced polymer only in the presence of sucrose, was injected into the reservoir in a media containing glucose. Polymer production was subsequently activated by injection of a sugar beet molasses medium containing sucrose. In addition, anaerobic slime forming organisms either from the formation or the molasses were stimulated in the reservoir. Further field tests are being planned to test this patented method.

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water samples 5 weeks after its appearance. Microbial and carbohydrate assay methods to detect stimulated indigenous microbes and molasses, respectively, can be used to characterize flow patterns in oil reservoirs.

D. H. Oppenheimer and F. K. Heibert (Alpha Environmental) describe a novel microbial stimulation method in the paper entitled "Microbial Enhanced Oil Production Field Tests in Texas." A microbial formulation consisting of a mixed culture of hydrocarbon-degrading organisms, inorganic nutrients, and a biocatalyst was injected into sandstone and limestone formations in Texas. The microbial formulation is believed to decompose crude oil under reservoir conditions by the use of a proprietary biocatalyst and to improve oil recovery through production of surfactants, carbon dioxide and to cause a decrease in pH. Results of microbial stimulation treatments are described.

In the paper entitled "Bacterial Activities and their Inhibition in Oil Reservoirs During Waterflooding Processes" W. Xiuyuan (Institute of Microbiology, Academia Sincia, China) discusses a treatment method to reduce losses in injectivity when injection water components lead to undesired microbial growth near injection wells. Mineral and microbial components of injection waters in two oil fields in China led to significant losses in water injectivity and to production of H_2S. The treatment method consisted of backflushing injection wells to determine the extent of the microbial damage and to remove some of the debris for the near-wellbore region followed by injection of a surfactant, a biocide, and 3% hydrochloric acid. The treatment method was shown to be effective in 25 of the 27 wells that were treated.

Laboratory Studies

In the paper entitled "Some Considerations Concerning the Nutrient Support Injected into the Reservoirs Subjected to Microbiological Treatment" by I. Lazar, S. Dobrota and M. Stefanescu (Institute of Biological Sciences, Romania), laboratory studies comparing the effects of nutrient composition on gas and acid production and oil recovery efficiency for four reservoirs in Romania are presented. These studies show that addition of nitrate and phosphate additives to a molasses nutrient did not have a significant effect. The addition of small quantities of pantothenic acid significantly increased gas production. The addition of a scum from sugar processing plants, which contained 80% of bicarbonate salts, increased production of acids and gases. The role of bicarbonate in buffering the pH of the nutrient solution during microbial metabolism indicates that application of certain adapted acid and gas producing organisms may be more favorable in reservoirs that contain a significant amount of carbonate minerals.

H. H. Russell and M. M. Grula (Oklahoma State University) discuss the importance of rock mineralogy on microbial fermentation in the paper entitled "Nutritional Aspects of MEOR: Effect of Core Minerals on Production of Substances Potentially Enhancing

Oil Recovery by Clostridium." Systematic studies are reported that were conducted to determine the effects of carbonate content of rock on fermentation by *Clostridium.* The products of the fermentation process change from acids to solvents when the pH drops below approximately 4.5. Reservoir rocks with mineral constituents such as calcite, dolomite, or clays can provide buffering capacity and enhance the fermentation process.

The paper entitled "Microbial Selective Plugging and Enhanced Oil Recovery" by R. A. Raiders, R. M. Knapp, M. J. McInerney, and D. E. Menzie (University of Oklahoma), discusses laboratory studies on fluid diversion by injection of microorganisms. Parallel coreflooding studies were conducted both with and without crossflow. Flow diversion was found to be caused primarily by selective reductions in permeability and biogenic gas production. A computer model was developed to describe the crossflow and fluid diversion phenomena.

The paper entitled "MEOR Studies in a Radial Flow System – The Research Outlooks" by D. Momeni, J. R. Chen, and T. F. Yen (University of Southern California) discusses the advantages of using physically scaled models to study MEOR processes. The construction of a scaled laboratory model is described. The factors that affect the performance of MEOR systems are discussed and the need for data from experiments in scaled models for development of computer simulators is emphasized.

K. J. Chauhan (Oil and Natural Gas Commission, Institute for Reservoir Studies, India) reports on research being conducted to develop MEOR methods for selective plugging in the paper entitled "Prospects of MEOR in India." Application of MEOR methods in oil reservoirs in India requires microorganisms that can tolerate temperatures in excess of 80° C (180° F), which are not conducive to microbial growth. Laboratory studies are reported showing the effects of increasing temperature on growth of some adapted microbial cultures and on their ability to reduce permeability in cores. When compared at equivalent numbers of microbes injected, a greater reduction in core permeability was achieved by injection of a culture with lesser concentration.

T. E. Burchfield and H. B. Carroll, Jr (National Institute for Petroleum and Energy Research) present an economic analysis of MEOR stimulation and waterflooding methods in the paper entitled "Economics of Microbial Enhanced Oil Recovery Methods." The requirements for design and implementation of MEOR methods are discussed. The potential economic returns from microbial stimulation methods are discussed and the effects of oil price, number of wells treated, and cost of nutrient are examined. The economics of microbial-enhanced waterflooding are compared with polymer flooding, a widely practiced EOR method, through adaptation of a polymer flooding computer model.

Concluding Remarks

The papers presented in this symposium exemplify the development of MEOR technology. A variety of different applications for microorganisms are being developed in the laboratory and applied in the field. In comparison with previous symposia on MEOR, the research is more focused on development of laboratory methods for designing optimal systems and on field tests. Mechanistic studies are concentrating on understanding the interactions between the microbes, rock, oil , brine, nutrients, and microbial metabolic products under reservoir conditions. Results of field tests are encouraging and are providing important information that is needed to guide future research and development efforts. Clearly, the technology has advanced significantly since the prior symposium on MEOR in 1984. When compared with other conventional EOR methods, MEOR has advanced to field application at a considerably faster rate with a relatively small expenditure of funds for research. Further development of the technology through a coordinated effort of laboratory research and field testing will hopefully lead to the development of a cost-effective microbial enhanced oil recovery technology. Development of MEOR technology will benefit the petroleum industry through increased oil production from declining oil fields and the agricultural industry through increased sales of nutrient products such as molasses.

The financial support of the U.S. Department of Energy is gratefully acknowledged. The support of the sponsors of the symposium and the contributions from each of the authors are appreciated. The assistance of the members of the organizing and program committees including Dr. Fred Burtch, Jim Chism and Tom Wesson of the Bartlesville Project Office, U.S. Department of Energy; Linda McGrew and Bill Linville for publicity and arrangements; Virginia Foreman and Tina Long for registration, and the management of the National Institute for Petroleum and Energy Research for supporting the effort required to conduct the symposium are all gratefully recognized.

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MEOR SYSTEMS: PROBLEMS OF DESIGN AND SCALE-UP

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ABSTRACT

Much oilfield microbiology is potentially a branch of reservoir engineering, a series of possible, perhaps even plausible, techniques for operating practice. The procedures are not easy to transfer from the laboratory to the field partly because, in their attitudes to practical matters, microbiologists are typical of most experimental scientists. They want a precise description of a problem and to develop a solution by altering variables one at a time; field operations rarely offer them such opportunities. In realistic terms, the further into the reservoir from an injection well a procedure is designed to be operated, the greater the difficulty of modeling it in the laboratory and hence the more problematic the transfer of the technology to field use. For the near future, at any rate, near-well activities are likely to be the easiest to put into practice. They include: (1) well stimulation by the removal of metallic scales, tarry materials and, perhaps, waxy deposits both in the tubing and in the neighboring reservoir matrix; (2) the local diversion of water flow to improve the sweep pattern and control coning; and (3) the establishment of polymer and surfactant floods based on the downhole bacterial synthesis of the appropriate chemicals. Results have already been reported for field pilots presumably intended to test some of these techniques, but the data tend to be limited, and their rigorous evaluation is usually impossible. Perhaps this is because microbiologists and operators frequently fail to understand each other's priorities and ways of thought.

INTRODUCTION

Microbiological methods for the improvement of oil recovery from underground reservoirs are becoming recognized as offering important new opportunities to the operator in his quest for increased oil production. The term *Microbial Enhancement of Oil Recovery (MEOR)* is, however, frequently used in a way which conveys the sense of it being a single methodology. Thus, having noted the reported beneficial effects of microbial applications in a number of field pilots, some people seek an explanation of "how MEOR works." There is no single explanation to be had: MEOR represents a collection of possible technologies analogous to those of non-microbiological EOR. Just as the operation of different fields might call for the use of steam soak, pressure maintenance or polymer flooding, each of which has a quite distinct mechanism and addresses a specific objective, so the various forms of MEOR, too, need to be clearly defined and perhaps specifically adapted to a number of enhanced recovery objectives.

The initiatives for the development of MEOR systems have come in the past primarily from microbiologists interested in the problems of petroleum microbiology. It is largely they who have acquired some understanding of reservoir and operating problems in the industry and who have sought to use their knowledge of microbiology to offer new solutions to existing and recognized problems. Only rarely have reservoir engineers with direct experience in oil production taken much interest in microbiology; most engineers without microbiological training have usually been unwilling to engage themselves with methods they often regard as exotic and of marginal interest to their primary activities.

The microbiologists have clearly been heavily influenced by the nature of their individual relationships with the oil industry. It is very noticeable, but hardly surprising, that those based in the mature oil-producing areas of the United States (Texas, Oklahoma, Kansas, Colorado and the neighboring states), areas in which much of the production involves low-yield stripper wells operated by independents, have developed their concepts of MEOR largely to address technical problems in their own regions. Microbiologists in other countries have usually been equally concerned primarily with their own local problems: heavy oil in Alberta, the deep, hot reservoirs of the North Sea in the United Kingdom, and the declining industry in Romania are obvious cases in

point. In each of these countries contact between microbiologists on the one hand, and reservoir engineers and operators on the other, reflects those differences of emphasis: in the U.S. it has been mostly with independents, while in those other countries the interactions are most frequent with major (often multinational) corporations or national oil companies. It is hardly surprising that the different groups of petroleum microbiologists have different priorities for MEOR.

The value of microorganisms for the enhancement of oil recovery lies in their ability to generate a wide variety of chemical products: if these can be shown to be effective and less expensive to use than similar substances injected from the surface as inert chemicals, microbial methods may win acceptance by a wide range of operators. It is important to recognize precisely what their value might be, and in any particular reservoir procedure their intended mechanism should be clearly defined and a series of benchmarks described by which success or failure may be measured. Only in that way will it become possible to understand and improve MEOR methods so that they become progressively more effective and attractive to operators.

EOR AND MEOR

EOR methods, in general, depend upon the injection from the surface of water and/or prepared materials (surfactants, polymers, gases, steam and others) in order to mobilize residual oil. MEOR methods, in particular, suppose the generation of some of these chemicals by microorganisms (more specifically, by bacteria since other types of microbes do not show the requisite properties) in an underground location using inexpensive feedstocks in order significantly to reduce the cost of the operation. The cost saving is expected to result from the very low capital requirement (no factory installations as well as simple wellhead equipment) together with the use of cheap agricultural products as feedstocks. Various sugar preparations, including molasses and perhaps raw or even refined sugar, are those most commonly considered. Their prices fluctuate according to normal market forces but, in recent years at least, there has been a surplus of such sugar preparations in the world commodity markets and prices have been low. Whether they would remain so if they become widely used for oil recovery is a matter of opinion. In the modern world, microbial methods may have a further

significant advantage: their products are all biodegradable and will not accumulate in the environment. However, biodegradability might in some circumstances impair their usefulness in the reservoir unless they were to be protected from attack by indigenous organisms.

Bacteria share with other living organisms great powers of chemical catalysis. All living cells possess hundreds if not thousands of biocatalysts (enzymes) each of which is specific in its ability to catalyze either one chemical reaction or a single category of related reactions. The presence within each living cell of such a range of biocatalysts, coupled both with the existence in nature of enormous numbers of different species or *strains* of bacteria, each with its own complement of enzymes, and the recent elaboration of genetic methods for generating new combinations of enzymes within cells, opens up a very wide range of possibilities for configuring specific microbial systems for enhanced oil recovery. There are, of course, some limitations to the use of biocatalysts, and Table 1 summarizes their advantages and disadvantages. The objective of EOR systems which depend on the use of certain living bacteria emplaced in the reservoir matrix itself is to generate EOR chemicals *in situ* inexpensively in the oil-bearing strata. Such processes must employ organisms capable of survival under the often inhospitable conditions of the reservoir and the characteristics of the microbial population need to be designed carefully to assure long-term stability. Some of these considerations are noted in Table 2.

USING BACTERIA TO IMPROVE OIL RECOVERY

There are a number of ways in which bacteria emplaced downhole might contribute to improved oil recovery, and these will be outlined below. All living systems behave so as to maximize their numbers. In all environments, resources are in some way limiting, and living organisms always compete with one another for access to those resources. In some cases, of course, organisms of different types are obviously competing but often the competition is most acute between individuals of the same type since their demand for resources are identical. And because even small differences between ostensibly identical bacteria may confer marginal advantages in growth rates, inevitably those which grow the fastest capture the greatest share of the limited resources, and their numbers increasingly dominate the population.

Such factors must be incorporated into the system design. They are the more vital in an underground MEOR operation in which close and frequent monitoring of downhole events cannot be performed. For example, it might be possible to improve the recovery of heavy viscous oil by injecting bacteria into the reservoir in order to attack long chain compounds in the oil at sensitive sites to break them into smaller molecules and reduce the viscosity of the crude. No bacterium is likely to undertake such an attack, however, unless it is thereby able to acquire a competitive advantage, that of consuming much or all of an otherwise refractory molecule. Thus, heavy oil molecules might indeed be metabolized, but the products would probably be bacterial mass rather than less viscous oil.

THE DESIGN OF MEOR SYSTEMS

The choice of particular bacterial strains, or mixtures of strains, for deployment downhole, and the way the system is configured, must be done in such a way as to maximize the probability that the chosen bacteria will be the most competitive in the environment in which the system is to operate. Should foreign bacteria by chance gain access, or the injected population itself develop variant forms (both normal hazards of microbiological practice, particularly in outdoor environments difficult to control precisely), the system must be designed in such a way as to minimize their chance of survival. This is essential practice in all microbiological procedures: it becomes even more necessary when, as in an oil reservoir, it would be difficult if not impossible to remove the contaminants and start again.

In the light of our understanding of bacterial growth and metabolism it is possible to enumerate a series of factors which are likely to influence the behaviour and success of any downhole system. Not all are equally important nor do they all apply to every case. But they do constitute a set of questions to be addressed and to which acceptable and satisfactory answers must be obtained. Many of them are listed in Tables 3 to 8. They include nutrition, tolerance of the reservoir environment, penetration through the porous reservoir matrix, generation of products suitable for oil mobilization, probable stability of the injected population under reservoir conditions (including possible interactions with an indigenous flora), and certain questions of how the system is to be operated.

In situ MEOR systems are generally understood to have four potentially useful rôles in stimulating oil recovery. Each requires a specific design although in some cases it might prove possible to combine more than one method into a single operating system.

Well Stimulation (Clean-Out).

In time, many wells show a fall in the rate of fluid production which may be due to the progressive development of an obstructive "skin" in the reservoir matrix close to the wellbore. The obstruction may result from the impaction of mobile sand or clay particles, the deposition of insoluble metallic scales (perhaps from an incompatibility of injected and reservoir brines), the presence of tarry residues from the crude itself, and the accumulation of wax in the wellbore and tubing. Nonbiological techniques already exist for correcting some of these effects and restoring the rate of fluid flow in the well, but they may be too expensive for application to low-producing stripper wells. Downhole bacterial systems may be able to help out but in order for them to do so effectively it is necessary to know the cause of the problem and construct the bacterial system accordingly. Many of the single well field pilots reported either in the literature or anecdotally at conferences and elsewhere are probably of this type. Rarely, if ever, do the reports define the objective of the pilots other than in the most general terms of improving oil recovery. Thus, the reasons for the diminution of the flow rate appear often not to have been explored, and the bacterial inoculum and nutrient support seem rarely if ever to have been designed to meet the specific problems of the particular reservoir or well.

Most commonly, a consortium of bacteria known or believed to produce gas (usually carbon dioxide), surfactant, solvents, and organic acids is introduced into the well together with molasses or other sugar preparation, perhaps supplementary inorganic nutrients, and water. The well may be shut in for a period to allow the (anaerobic) fermentation downhole to complete; it is then reopened and production resumed through the same well. Often the rate of oil (and water) production is increased, in some cases apparently for several years from a single treatment. The reasoning behind such procedures (rarely explicit) appears to involve both a local rise in reservoir pressure from the fermentation gases displacing impacted particles and the other chemicals

acting collectively as solvents in helping to remove the other accumulations. It is usually clear that the total volumes of fluid injected in the course of these treatments would not extend more than a few meters at most from the injection well. Additional yields of oil for long periods following stimulation treatment could hardly originate solely from that volume of the reservoir contacted by the injected fluid: the treatment most probably facilitates the flow from a wider area of the reservoir by improving fluid access to the well.

A valuable set of techniques could be developed from this general approach by designing and testing *in situ* bacterial systems directed to resolving particular identified causes of poor well productivity. All of the limitations to fluid flow listed above are frequently susceptible to resolution by bacterial methods but, of course, the correct method must be applied in each case. For some wells, suffering from a multiplicity of defects, multipurpose bacterial consortia might be effective but in others single action systems will be more effective. The removal of wax accumulations would be difficult solely by bacterial action in the anaerobic environment and restricted volume of a well bottom, but a combination of bacterial and nonbiological methods can be envisaged to remove wax and thereafter control further deposition. Because of their limited cost, all of these techniques are of particular value to operators of low yield stripper wells.

Selective Plugging - the Control of Water Flow

Major operational problems arise in some reservoirs from the preferential flow of water between injector and producer wells through fractures, fissures, or high-permeability beds. When this happens, the injected water fails to act as a drive, and the production of oil is curtailed. Various nonbiological methods have been proposed for the plugging of such "thief zones" and these have included the use of cements and of time-setting polymers. The purpose of the latter is to avoid the injection of a viscous solution which will probably flow in channels different from those of the more mobile water. The injection of low viscosity monomer solutions which are allowed to polymerize *in situ* is intended to avoid the difficulty of viscous flow.

Microbiological systems can be designed to achieve a similar result. A number of protocols have been suggested the details of which depend on the nature of the zone to be plugged. Interwell fracture thief zones in some reservoirs can be filled with injected water within a day or two. A microbial thief zone plugging system might therefore consist of a dilute inoculum of a suitable bacterium capable of generating anaerobically an insoluble (or highly viscous) polymer, together with the appropriate inexpensive nutrients formulated both to give a viscosity close to that of water (so ensuring passage of the injected fluid through the water channels) and to allow the development of viscosity at reservoir temperatures in four or five days. The characteristic growth of bacteria by doubling of numbers in each successive generation means that almost all the viscosity will be acquired late in the fermentation. There would accordingly be sufficient time for the whole fracture system from injector to producer to be filled with the microbial inoculum before appreciable viscosity were to develop.

In other cases the problems are likely to be more difficult. In large offshore fields, where wells may be spaced as much as a kilometer apart, fluid might take months to fill a thief zone. Ways must be found (a) of ensuring that bacterial cells are able reliably to migrate for hundreds of meters through the reservoir without becoming attached to rock particles or impacted in the pore throats; and (b) of delaying the onset of viscosity for a protracted period in order to avoid alteration of the pattern of water flow by premature gelling, with a consequent failure to block the whole of the high permeability zone. There is a discussion elsewhere in this symposium of bacterial migration through porous matrices but not of methods for delaying polymer production under reservoir conditions for very long periods.

Yet another kind of difficulty arises with respect to the plugging of high permeability beds in the reservoir. In some offshore fields they extend the whole distance between wells, with a thickness of several meters and a width of perhaps tens or hundreds of meters. They might as a result require millions of barrels of viscous solution or precipitate for their plugging. Quite apart from the difficulties of emplacement, the cost of any feedstock for such an operation would be very high. An alternative option might be to use a microbial system to deposit a plug of an inorganic material, likely to be a good deal less expensive than a biopolymer in terms of feedstock cost.

Plugging with bacterial polymer will also be a valuable way of controlling coning. This phenomenon often arises during pumping because of the proximity of the downhole pump to the underlying aquifer; it is particularly troublesome if the viscosity of the oil is significantly greater than that of the brine. When the water cut becomes too high, production may have to be interrupted to allow the cone to subside, with obvious economic disadvantages. The introduction of polymer-producing bacteria via the production well into the water channels of a cone would enable a local seal to be generated between the aquifer and the oil-bearing horizon. Such a near-well operation is ideally suited to the downhole use of bacteria.

The interest in microbial plugging shown in recent years by a number of major oil companies, coupled with the work of several laboratories in developing such systems, suggests that they will be successfully field tested in the fairly short term. In relatively cool reservoirs with short interwell distances there are likely to be few microbiological difficulties and the systems will be fairly easy to design. Solutions to the larger and more difficult problems inevitably lie further in the future.

Polymer and Surfactant Floods

The third major opportunity for MEOR technologies lies in using bacteria emplaced in the reservoir to generate polymer and/or surfactant floods. The advantage of using microorganisms for this purpose lies in their simplicity of operation and low cost. Such a flood will use bacteria chosen for their ability to produce chemicals in sufficient quantities using low cost feedstocks and in the conditions of the reservoir *near the injector well.* The last point is important. Although some bacteria are known to be capable of growing at very high temperatures, a few as high as 100° C, most will not. Most organisms thrive best at modest temperatures around blood heat and the higher the temperature of an environment the smaller the range of organisms able to survive there.

In a polymer and/or surfactant flood the objective must be to confine microbial growth to the minimum necessary to provide an adequate rate of product formation and to maximize the conversion of feedstock to product. When a reservoir is seeded with bacteria through an injection well and feedstock then introduced through the same injector, the microbial population

will probably develop as an annulus around that well. The bacteria will have a high affinity for the nutrient (one of the reasons for choosing a particular feedstock) and will begin to use it as soon as it becomes available. The microbial inoculum will therefore grow as close to the injection well as the scouring effect of the injected water will allow. Once the nutrient contacts bacterial cells, its concentration in the waterflood will begin to fall as greater and greater numbers of cells remove the substrate molecules for their own metabolism. All the time the nutrient solution will be flowing outwards from the injector in more or less of a radial pattern, driven by the continuing waterflood. At some point in every direction, all the nutrient materials will have been removed from the waterflood and no bacterial growth will be possible along that radius: the limit of bacterial growth will have been reached and the band or annulus will be in place. Its dimensions will depend primarily on the *rate of injection of nutrients*; the higher the input concentration and the greater the rate of water injection the further the nutrient will travel from the injector well before it has all been depleted by the bacteria. It seems probable that such a configuration is unavoidable with this type of continuous feeding, but note how it differs from the limited injection of nutrient which would be required for selective plugging as we have described above. The objective of the method will thus be the most favorable conversion of injected feedstock to polymer and/or surfactant which will be flushed deeper into the reservoir by the waterflood, there to contact and mobilize additional oil.

The prime requirements for such bacterial floods are organisms able to convert inexpensive feedstock nutrients under reservoir conditions to effective products at sufficient concentration to improve the recovery of oil. An important requirement is the decoupling of product formation from growth; if this is not done the growth of bacterial cells in the annulus will eventually plug the formation.

One encouraging aspect of this system design is that mutation of the injected bacteria, or contamination with extraneous forms, will have little effect on the system unless such contamination becomes massive. This is because the flow patterns in the annulus will be predominantly radial with little lateral mixing in the porous matrix. Thus, a contaminant or mutant, even if it were to dominate a local point in the annulus, would exert its

effect only in a narrow sector extending from that point to the outer margin of the annulus and the great bulk of the operation would continue to be unaffected: this is illustrated in Figures 1 and 2.

Heavy Oil Recovery

In some reservoirs there are opportunities for using *in situ* microbial techniques to improve the recovery of heavy oil by selective plugging for profile improvement or by polymer and surfactant flooding; while the details might vary such methods would, in principle, be no different from their deployments with lighter crudes. Additional techniques are highly problematic: they all seek to reduce the viscosity of the crude either by modification of those components in the oil primarily responsible for the high viscosity or by generating surfactants to promote the formation of an oil/water emulsion.

In practical terms, both would be difficult. For reasons advanced above, it does seem unlikely, though not totally inconceivable, that bacteria might be found which would preferentially attack a sufficient number of the long-chain, high-molecular-weight molecules in heavy crudes to reduce viscosity. On the contrary, it is perhaps more probable that the *shorter* chain molecules would be the main targets of microbial attack; metabolizing them would serve only to exacerbate the problem.

The alternative is to generate surfactants to encourage the formation of an oil/water emulsion with a viscosity lower than that of the crude; separation of oil and water would be carried out on the surface. The problem here may be mainly one of access. We have earlier discussed the very limited ability of bacteria to use crude oil as a feedstock in a strictly anaerobic environment. Thus, the production of surfactant would be dependent on the injection of nutrients from the surface. Either this would operate as a surfactant flood (in the manner we have already described) or the bacteria and their nutrients would need to migrate into the deposits of crude to produce the surfactant at the oil/water interface. If that were possible, it might indeed generate the desired emulsion, but it is difficult to see how to achieve infiltration except, perhaps, quite close to the injection well. Thus, the most reasonable protocol might resemble a huff-and-puff steam soak: the injection of a measure of inoculated medium, followed by production

of emulsified heavy crude from the vicinity of the injector after an interval for the fermentation to complete. The following and subsequent cycles might seek to mobilize further tranches of the crude, each progressively farther from the injector. To the best of our knowledge, few studies along these lines have been undertaken.

SCALE-UP

Scaling up microbial methods from the laboratory to the field is no different in principle from doing so for any other type of EOR. A reservoir is a large structure compared with a laboratory model, and the environmental conditions prevailing in reservoirs are often difficult to simulate on the surface. This applies to the construction of radial models using genuine reservoir rock (normally available only as small diameter cores) and even more to the construction of apparatus rigs in which the high pressure of deep reservoirs can be simulated. It may be difficult also to reproduce in the laboratory the microflora already present in the reservoir. But without doing so in some way there will be only a restricted ability to predict how an MEOR system will behave in reality and over an extended period.

Independent operators are clearly limited in their ability to test new methods extensively by themselves although with the help of governmental agencies such testing may be possible. On the other hand, major oil companies with adequate technical resources are certain to require computer simulation of any MEOR protocol before they would consider its use in the field. Thus the design and planning of MEOR methods intended for use by majors must include provision for defining and acquiring the input parameters which will be needed for computer modeling.

TOWARDS ACCEPTANCE OF MEOR BY THE INDUSTRY

Microbiological methods will, of course, be accepted and brought into use by the industry only if they are effective, economically attractive, compatible with existing practice, and understood by the operators. Effectiveness depends on translating successful laboratory experiments into convincing field pilots. Economic benefits are difficult at present to evaluate because of very limited experience, confined almost entirely to watered-out fields. Input costs, at least for feedstocks, can be roughly

calculated, and an example is presented in Table 9. But there are for the moment very few data to relate input costs to output benefits. Most MEOR methods now being designed and developed seem compatible with many types of reservoir operation but it is essential to remember that no fields have yet been developed with microbial methods in view. Finally, the microbiologists need to keep in mind that their ideas are unfamiliar in the oil patch. Few operators or field managers will have much understanding of microbiology as a subject. Inevitably there will be reluctance to venture into methods unknown and not well understood even if the promise may seem to be high. There is a major task of education ahead.

It is clear that a number of opportunities exist now in the industry for the deployment of microbial methods of enhanced oil recovery which do already or soon will meet the criteria we have discussed. But readiness on the part of the industry actually to use them will be influenced by many factors in addition to technical success. In particular, perceptions of the future movement of oil prices, changes in tax regimes, and political uncertainties as well as individual company policy and experience are all major factors influencing decision making. MEOR will have its place in the scheme of things although just when, how, and where are much more difficult to predict.

TABLE 1. - The potential advantages and disadvantages for MEOR of biocatalysis

Advantages:	- specificity
	- absence of side reactions
	- great variety of specificities (and combinations of specificities) theoretically available
	- possibility of fine-tuning the properties of the system for specific problems
	- avoidance of capital investment for production facilities
	- simple wellhead facilities
Disadvantages:	- the range of specificities, though wide, is not infinite
	- biocatalysts have restricted abilities to activate variant substrates
	- it is therefore important to know as much as possible about the chemistry of the proposed system.

TABLE 2. - The use of living bacteria downhole

Advantages:	- reproduction; self-maintenance of production capability, potentially for long periods, with a minimum of external intervention
	- inexpensive nutritional feedstocks
	- minimal problems of producing adequate quantities of organisms for injection *once the starter inoculum is available;* bacterial populations are easy and cheap to grow but considerable effort may be necessary at the development stage in order to obtain the desired set of properties with the types of bacteria to be employed.
Risks:	- the injected bacteria may not survive the environmental conditions of the reservoir
	- foreign organisms may gain access to the reservoir and contaminate the injected bacterial system
	- mutations may take place within the injected population and change its properties in undesirable ways
	- in the case of a population consortium, the possibility exists of a change in the ratio between the different strains with undesirable and perhaps unpredictable effects
	- the injected bacteria may fail to generate the expected products
	- reservoir damage may occur by plugging at the injection face or elsewhere
	- undesirable interactions might take place with a bacterial population already resident in the reservoir; e.g., the injected feedstock may stimulate the growth of the wrong population or pre-existing bacteria may destroy the oil-mobilizing chemicals produced by the injected population.

TABLE 3. - Nutritional options

Use of injected organic nutrients (e.g. sugar-containing feedstocks of various types, and including molasses):

- inexpensive
- utilized by a wide variety of bacterial species: therefore an acceptable feedstock for the growth of a consortium of different species compounded to produce a "cocktail" of desirable products
- will support comparatively rapid rates of growth and product formation even in the absence of molecular oxygen
- can be utilized to generate a range of possible products
- no additional electron acceptor (oxidizing agent) required
- considerable experience exists in the use of such feedstocks
- but microbial *growth and metabolism* will be limited by the penetration into the reservoir of the injected nutrients which are likely to be confined to a near-well location. However, microbial *products* could be washed further into the reservoir by a waterflood and their oil-mobilizing actions thus not limited to the region near the injector well

Use of *in situ* crude oil:

- widely dispersed in the reservoir
- effectively cost-free
- but studies already complete show that with present methods growth on crude oil in the anaerobic environment of the reservoir is very slow and probably inadequate to establish a downhole MEOR system
- there is little experience of its use; no systematic study has been made with this feedstock of the generation of products for oil mobilization
- an electron acceptor (e.g. nitrate) would need to be injected from the surface and bacterial growth might be limited by its availability; however, the electron acceptor would not necessarily be confined to a near-well location.

TABLE 4. - Microbial tolerance of the reservoir environment

Environmental factors limiting microbial growth might include:

- temperature
- pressure
- mineralization of the aqueous phase
- possibly toxic substances in the crude oil

TABLE 5. - Penetration of bacteria through the reservoir matrix

Requirements include:

- minimal risk of plugging the face during injection
- minimal risk of plugging the matrix by biomass or bacterial products unless (as in water profile improvement) this is a specific objective
- avoidance of damage to the organisms resulting from excessive shear forces during injection
- attachment of the bacteria to the sand particles of the matrix at some distance from the injector where the oil-mobilizing products are to be produced; the need for such attachment and its location in relation to the injector and producer wells will depend on the design and objectives of the MEOR system

TABLE 6. - Bacterial products for oil mobilization

Those most likely to be of value include:

- surfactants to reduce interfacial tension
- polymers to increase waterflood viscosity or for selective plugging
- gases (especially carbon dioxide and methane)
- solvents (predominantly alcohols and ketones)
- acids (various organic molecules) for improving fluid flow, probably mainly in the near-well environment
- other products or reactions which might significantly reduce the viscosity of heavy oil deposits

TABLE 7. - Considerations affecting bacterial population composition and stability

In the complex relationships between living organisms a number of factors may influence behavior and the production of useful products. Among others are:

- growth dynamics of the injected population under the conditions of the MEOR operation
- likely stability of a consortium for the period of the MEOR operation
- probability and consequences of genetic mutation
- probability and consequences of contamination by extraneous species
- response of an indigenous bacterial population, if present, to
  (a) the introduction of nutrients into the reservoir
  (b) the oil-mobilizing products generated by the bacteria of the injected MEOR system

TABLE 8. - Operational considerations

- flux of injected water
- concentration of bacteria in the injected water
- requirement for and availability of microbiological skills and facilities at the wellhead
- effects of MEOR activities on the produced oil and water
- possible environmental impacts
- possible existence of regulations governing the use of bacteria downhole

TABLE 9. - Feedstock costs: estimates for a North Sea pilot

EOR using Xanthan

- requirement: 500-1,500 tons per year
- concentration: 500-2,000 ppm
- price per ton for xanthan: £3,100-4,700 ($5,000-7,500)
- volume of water to be injected per year: 1.57-18.8m bbl.

cost per bbl of injected water: at 500 ppm = 25-37p (39-60¢)

2,000 ppm = 99-149p (158-230¢)

Annual cost = £1.55-7.05m ($2.48-11.3m).

MEOR using Molasses

- price per ton: £62.50 ($100.00)
- concentration required: 2-5%

cost per bbl of injected water: at 2% = 20p (32¢)

5% = 50p (80¢)

Annual cost: 1.57m bbl at 5% molasses = £0.78m ($1.25m)

18.8 m bbl at 2% molasses = £3.74m ($5.98m)

Notional feedstock cost: molasses/xanthan = 0.50-0.53.

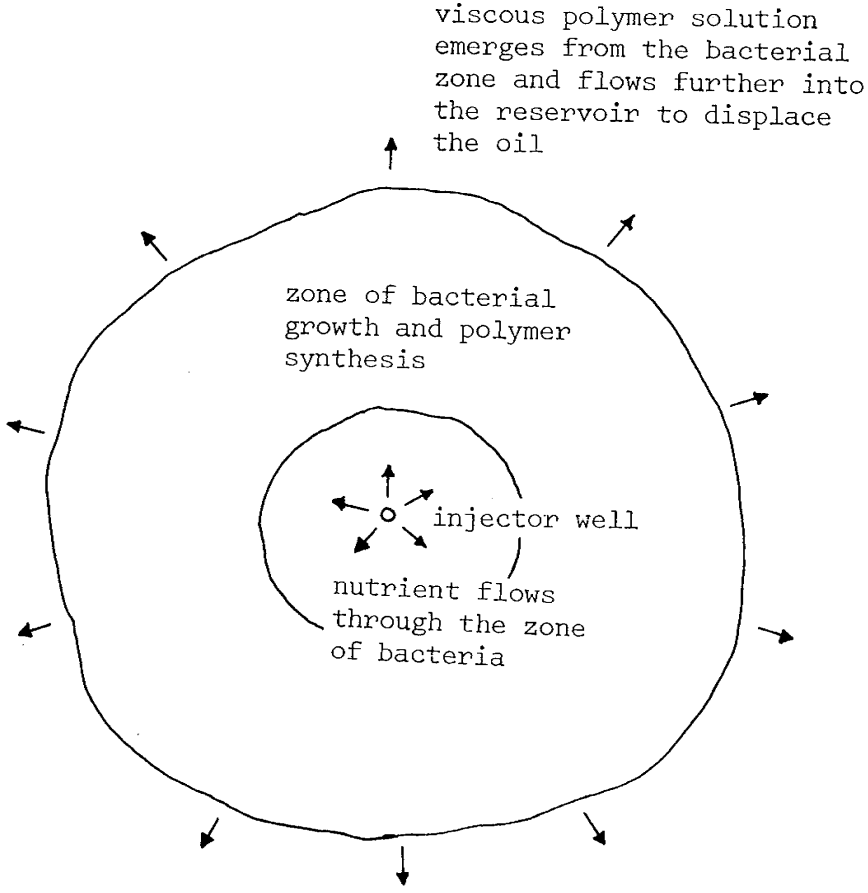

FIGURE 1. - Model system for the continuous synthesis downhole of bacterial polymer.

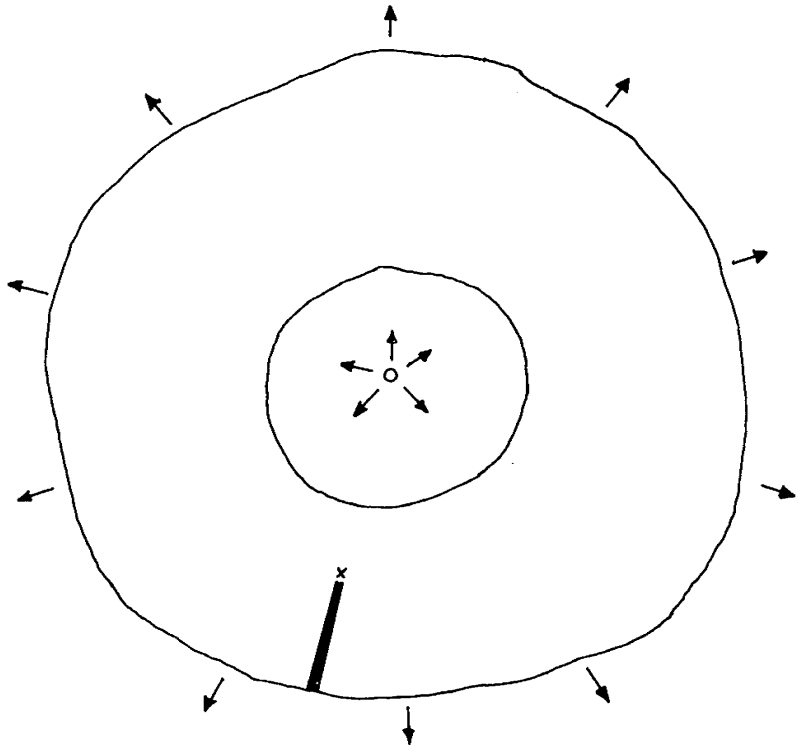

FIGURE 2. - The effects of mutation and contamination.

In the model shown in Figure 1 note that there is little or not lateral mixing in the zone of bacterial activity and that fluid flow is essentially radial away from the injection well. The effect of a mutant or a contaminant sited at position "X" will be minimal and confined to a narrow radial sector.

MEOR--A SOLUTION TO OUR CURRENT DOMESTIC OIL SUPPLY

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ABSTRACT

Until recently in this country only thermal recovery, chemical flooding and miscible flooding have been considered as economical enhanced oil recovery (EOR) techniques. Last year the DOE sponsored international workshop concluded that MEOR has merit as a cost-effective method for recovering residual oil. However, reliable assessments of the technical aspects of MEOR as compared to other EOR techniques are lacking. The conclusions of the MEOR workshop and related literature have been summarized to present a state-of-the-art of MEOR technology, its advantages, limitations and future.

Microbial Enhanced Oil Recovery (MEOR)

Is it a technology that can actually contribute to this nation's continually growing need for oil or is it simply a black art?

The current critical situation of our domestic oil industry is obvious to all of us. The bad news is all around us - in the oil business and the economy of those states directly and indirectly affected by the oil industry. The good news is that more than two-thirds of the original oil is still in place waiting to be recovered - just like penicillin was waiting to be developed as an antibiotic or the double helix was always there just waiting to be discovered so a whole era of biotechnology could begin.

A few statistics will reemphasize the significance that MEOR can have in revitalizing our oil industry. (Figure 1.) The world has a total of 697 billion proved reserves; 24.6 billion (3.52%) in the United States and 88.64% in non-communist countries; 57.62% in Middle East, and if we include Libya that amounts increases to 60.68%. In the United States where 28 billion barrels of oil were estimated as reserves in 1984, that figure is now 24.6 billion barrels available for conventional recovery; however, we still have the additional 327 billion barrels of oil targeted for EOR. Conventional technology is not enough. (Figure 2.)

In 1985 the United States consumed nearly 75% more oil than it produced; that trend will continue; our style of life demands it and has not prepared us for anything else. There are no alternatives. (Figure 3.)

In 1958 John Kenneth Galbraith in his book, The Affluent Society said, "We have solved the problems of production." He was expressing a common attitude of our society and asserting that production had been mastered. He was referring to manufacturing industries, but the oil industry was no different. This attitude was reflected in executives who turned production over to caretaker managers and before we knew it we were saddled with the problems of laborers and labor unions and we were being seriously challenged by countries like Japan who were encouraging dedicated and innovative workers and energetic scientists and engineers. The American standard of living has been accused of sleeping while our once unassailable lead in high technology is narrowing.

How do these facts apply to us here? Biotechnology is becoming stronger in the United States and we can take advantage of it. As biotechnology becomes one of the high technologies of today and energy continues to be one of our major marketplaces, we have the perfect niche for MEOR. Two years ago when a request was made about suggestions for the proposed MEOR program in 1986, only two experienced investigators responded - Dr. Bubela, for whom we will always feel a great loss, and Dr. Moses who wrote that "we must make discernable progress towards a technology to offer the industry, not simply a set of applied science projects." We have made some progress, but we still have alot to do.

Many times microbiology has been considered an art rather than a science and like that of any artist our first concern is finding a patron - and "patrons for our art" have been somewhat lacking for the last few years. But the market place is there; the technology is here, and getting from here to there is our present concern. Our objective cannot be simply to solicit funds to support additional academic exercises, but to develop processes for applying technologies for recovering oil. We must know that we have that technology and we must be able to convince industry that we can do it.

The United States is the largest consumer of oil today. (Figure 4) In 1984 34.1% of our oil supply was imported and early projections were that our imports would reach 46.3% by the year 2000. (Figures 5 and 6). However, earlier this year Energy Secretary John Herrington, warned that our imports could rise from 33% in 1986 to 50% by 1990. An oil import fee will not solve our problems; that solution would be like putting a band aid on cancer - creating more problems and covering their source. Other major oil producing countries have been reducing production for the last several years. Only Mexico and China have increased production significantly. (Figure 7). As technologists we can be improving and demonstrating advanced technologies for developing our targeted reserves and thereby increasing our own competetiveness.

Forty years ago the average daily rate of oil production in the United States was 5,196,000 bbl. (Burns, 1948). In 1949 Dr. Morris Muskat, Director of Physics Divisions for Gulf Research and Development Company made the following observations:

> A very conservative estimate as to the average quantity of oil remaining underground in the oil fields which have already been economically depleted and abandoned is that this residual oil is as great in volume as that produced. If the future exploitation of the proved oil reserves should be associated with this unit ratio for the residual oil left in the producing horizons, there will still remain some 125 billion barrels of oil distributed throughout the developed oil fields after they have been condemned and abandoned.
>
> Why? A quantitative answer to this question cannot be given....
>
> Will the ratio of unrecovered residual oil to that produced necessarily remain as high in the future as it has been in the past?...
>
> Must these residual billions of barrels of oil be considered as forever and irretrievably lost?...

As recently as 1985 Allen E. Murray, president of the Mobil Corporation, reported some significant observations that apparently went unnoticed:

> We're seeing patterns like those that led to the 1973-74 and 1979-80 energy crises.
>
> --revived domestic consumption that exceeds domestic production.
>
> --Approximately one-third of our oil supplies now comes from abroad, the same level as before the 1973-74 energy crisis.
>
> --With enough thoughtful attention to energy now, the crisis I'm predicting need never happen - at least not as severely as before. (Murray)

But a crisis did happen - not in the same form as before, but a crisis nevertheless. Crude oil prices fell below what anyone would have speculated and oil-producing states suffered, and are still suffering drastic consequences. Solutions are all around us - long term and short term solutions. One of those short term solutions is the application of EOR processes to the many stripper wells which have been the most severely

affected by the present crisis from which we are now emerging. Relief may be only temporary. Until we can free our selves from being held hostage to the prices demanded by OPEC, energy crises will be lingering around every corner. We must eliminate this crisis to crisis energy planning.

In an interview on NBC's "Today Show," March 18, 1987 Energy Secretary Herrington stressed that the United States is too dependent on foreign oil. Quoting him from Chemical and Engineering News, March 23, 1987 he said: "even with continued conservation and efficiency and substantial contributions from other energy resources like coal, nuclear, energy and renewables, our economic and energy security is inextricably tied to the fate and fortune of our domestic petroleum industry through this century."

Can MEOR make a difference? I think so.

Stripper wells operated by independent oil operators produce approximately forty-one percent of our domestic oil, and stripper well operators have been affected the most severely by this recent oil crisis. (Figure 8.) In Texas 62% of the total producing oil wells are stripper wells and the proportion nationwide is 71% percent. Stripper wells represent the largest target for MEOR. Making MEOR available as a cost-effective oil recovery process will decrease the number of stripper wells being forced to shut down, increase state and federal revenues from crude oil, and contribute to more jobs.

The preface to this meeting states that MEOR is an "emerging technology which holds promise as a cost-effective method for improving oil recovery." Getting that technology to the market place is the challenge presented to us here. MEOR has some constraints, it also has distinct advantages and we need to be aware of what we have and what we need.

TECHNICAL CONTRAINTS

- Unknown Risks -

1. No culture respository with defind mechanisms of action
2. Lack of:
   a. controlled field test data,
   b. standardized field monitoring techniques, and
   c. formal long range planning program.

TECHNICAL ADVANTAGES

1. MEOR topics that have been shown positive results:
   a. Bacteria do produce biogases that are highly soluble in oil and less soluble in water and, therefore, increase oil displacement.
   b. Bacteria can produce in-situ surfactants that reduce

interfacial tension and increase oil mobility.
   c. Polymers that give increased mobility to a water flood can be produced by bacteria.
2. MEOR is less expensive to install and operate than other EOR methods.
3. MEOR can be easily adapted to any field unit.
4. MEOR will not cause any reservoir deterioration or other adverse environmental effects.
5. Bacteria can be maintained as long as carbohydrate is available.
6. MEOR is more easily applied than other EOR techniques.
7. The chemical counterparts of MEOR, namely gases, surfactants and polymers have already been field tested and proven.

The conclusions from the 1986 workshop outlined research priorities in each MEOR category which are summarized below:

WELL EVALUATION

1. We need laboratory modeling studies to simulate reservoir conditions on a scale large enough to be predictable.
2. Field pilot tests, carefully documented and monitored, must be performed.

MICROBIAL SCREENING

1. Studies must be done which define the mechanisms of action.
2. Simple, rapid analytical procedures for screening and monitoring microbial activity must be developed.
3. We need a well-defined modeling system for demonstrating EOR via microbial activity.
4. We must develop a substrate catalog.
5. We need models for evaluating the compatibility of microorganisms with reservoir environments.
6. We must define the mechanisms and effects of microbial plugging.
7. We must plan cooperative research on recombinant DNA technology for MEOR.
8. We need a respository of defined microorganisms which have special applications to MEOR.
9. The life expectancy of desired MEOR activity and effects of "refeeding" must be determined.
10. Selected, controlled field tests in various types of reservoirs are necessary.
11. A microbiological reference manual must be prepared and include:
    a. micronutrient factors affecting microbial efficiency,
    b. standard methods,
    c. methodology for pathogenicity and toxicity testing,
    d. a glossary of terminology, and
    e. a compilation of microorganisms with referenced application to MEOR.

SCALING-UP FROM LAB TO FIELD

1. We must determine the kinetics of scaling-up.
2. Comparisons must be established between microbial systems and other EOR systems.

MEOR APPLICATIONS TO HEAVY OIL

1. We need more complete knowledge about the structure of heavy oil.
2. Anaeorobic and thermophilic bacteria must be screened.
3. We must screen and optimize and then synthesize biosurfactants that promote oil-in-water emulsions.
4. Microbes that degrade paraffins must be selected.

LABORATORY CORE STUDIES

1. Must represent the target reservoirs.
2. Reasonable and reproducible starting points must be identified.
3. Two determinants for MEOR core studies must be established, to include:
   a. constant injection pressure, and
   b. constant injection flow rate.
4. Accurate mass balance must be obtained.
5. Permeability changes must be determined.
6. Additional experiments should be conducted:
   a. to determine facial plugging or penetration effects,
   b. dual core experiments for determining different permeabilities, and
   c. kinetic studies to determine flow of nutrient through each zone.

ENVIRONMENTAL CONSTRAINTS

1. Risk analysis assessment:
   a. information survey should include:
      (1) ecosystem description,
      (2) effects of various biological and abiological components on ecosystems, and
      (3) information about groundwater research.
   b. environmental modeling predictions considering:
      (1) economics,
      (2) technical feasibility of monitoring and disposal of environmental wastes,
      (3) toxicity and pathogenicity testing, and
      (4) use of recombinant versus non-combinant organisms
         (a) legal and
         (b) regulatory.
2. Wastes disposal methods must be selected for
   a. on-site,
   b. off-site, or
   c. containment facilities.
3. Sampling methods must be established.
4. Sulfate-reducing populations must be determined.

INJECTION PROTOCOL

1. We must understand the mechanisms of formation penetration and distribution.
2. We need to determine injection sequencing and timing.
3. Pre and post injection monitoring techniques must be improved.
4. We need to design a broad range injection protocol from available information for general use.
5. Data acquisition must continue with studies for the development of more specific techniques.

The secret to our success, making MEOR available to those independent operators who represent more than 98.6% of our oil companies and produce 41% of our domestic crude oil supply, will require unitization from all of us - the scientists and oil men. As a team in which all of the members are working in a concerted effort toward a defined goal, we will share the rewards for the quality of our work.

Our group is still small enough and yet representative enough to be able to develop the foundation for a technology which will bring an effective relief to the oil industry.

The National Energy Policy Plan (1985) states:

> Given the right market signals, the right mix of Federal cooperation, and the right regulatory climate, the American Economy has the competitive vigor and the inherent strengths to take new technologies off the drawing boards and put them into commercial practice. Using these tools, the Nation can look to a future of energy stability, security, and strength.

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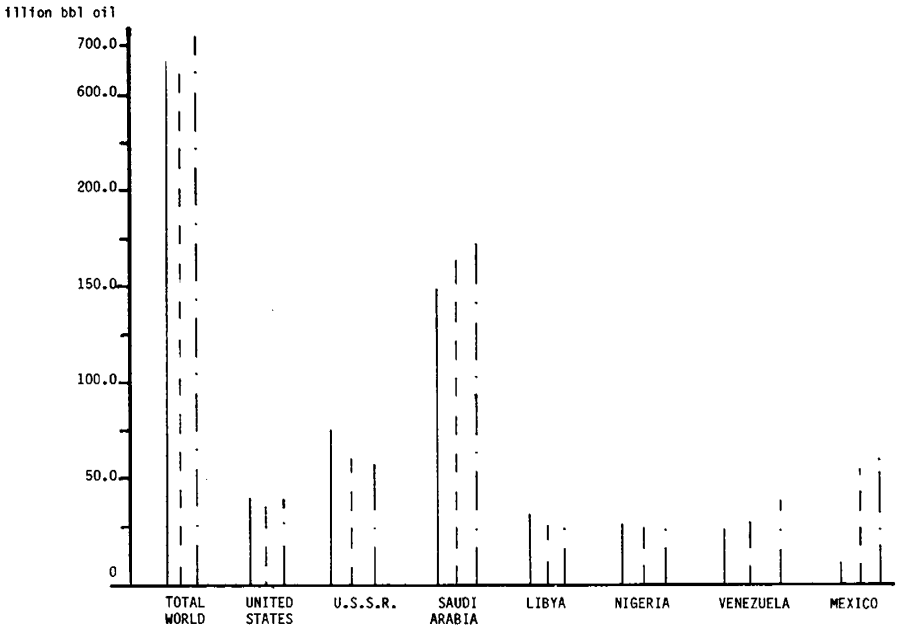
ACKNOWLEDGMENTS

Thanks to Debra Stevens who designed and made all the graphs from various publications.

RESERVE DATA



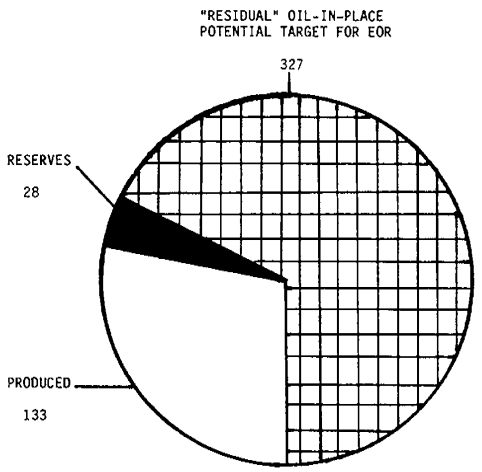
Information Source: IPE, 1986.

FIGURE 1

DOMESTIC RESOURCE AVAILABLE FOR EOR

(billions of barrels)

TOTAL ORIGINAL OIL-IN-PLACE - 488

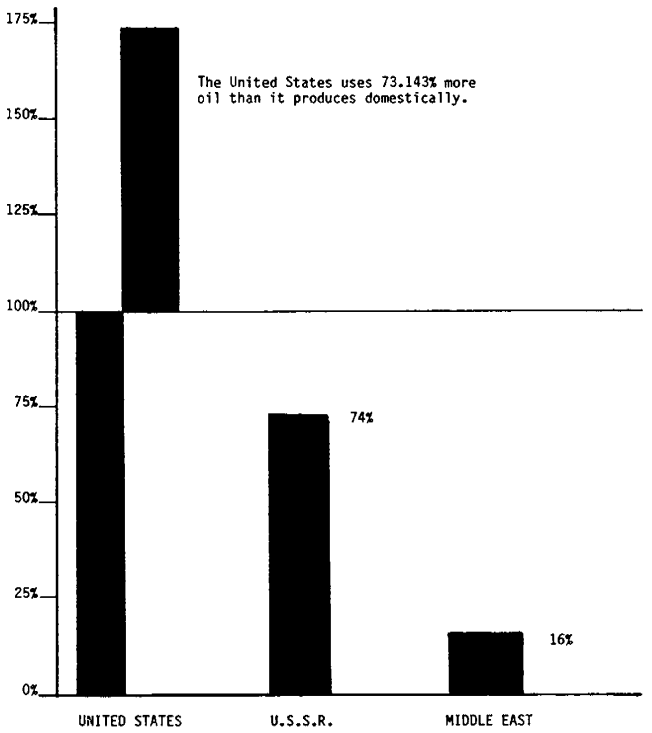


Sources: EIA, 1984; API/AGA/CPA, 1979.

Ref: GAO, June 1986.

FIGURE 2

COMPARISON OF OIL CONSUMED TO OIL PRODUCED

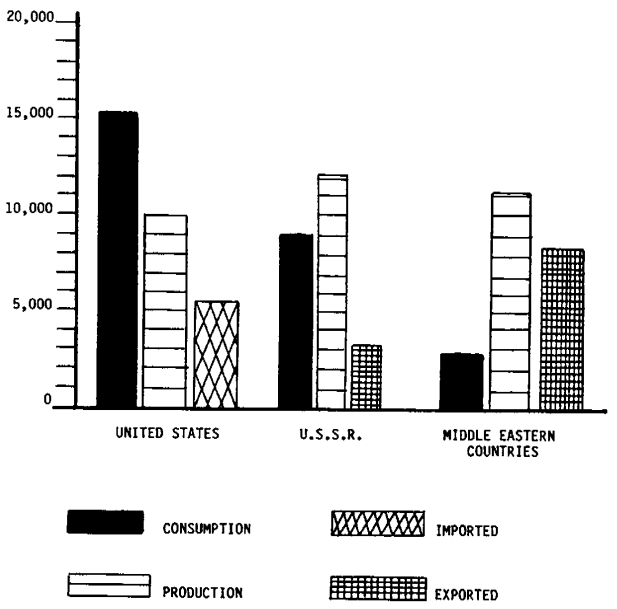


Information Source: IPE, 1986.

FIGURE 3

CONSUMPTION - PRODUCTION - IMPORT/EXPORT CHART

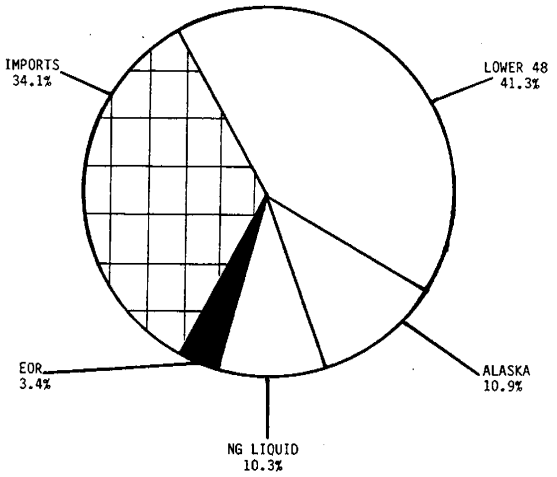
(1,000 bbl/day)



Information Source: IPE, 1986.

FIGURE 4

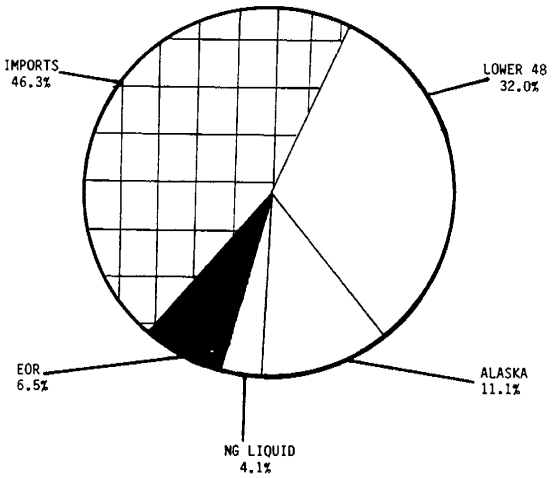
1984 UNITED STATES OIL SUPPLY BY SOURCE



Ref: GAO, 1986 (Sources: EIA, 1985; O&GJ, 1985; DOE, 1985.)

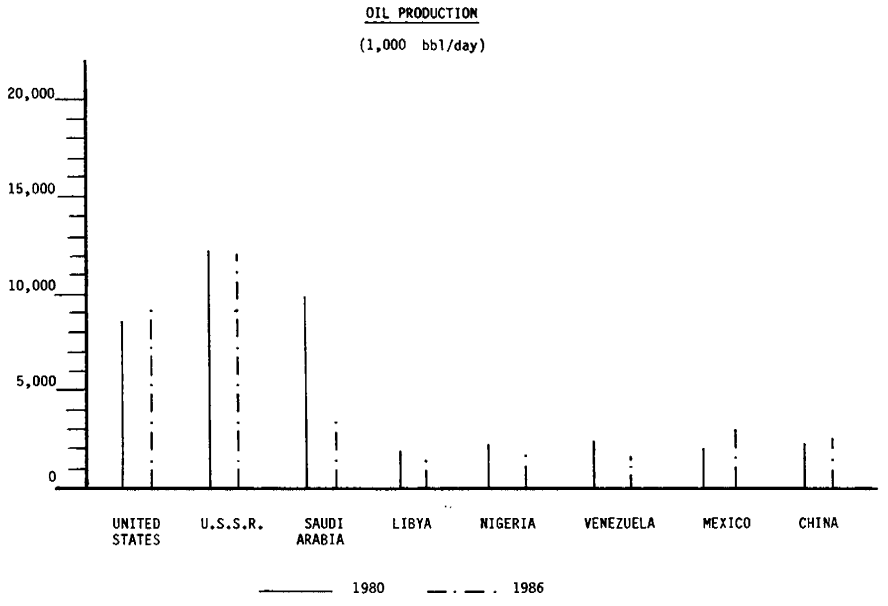
FIGURE 5

2000 UNITED STATES OIL SUPPLY BY SOURCE



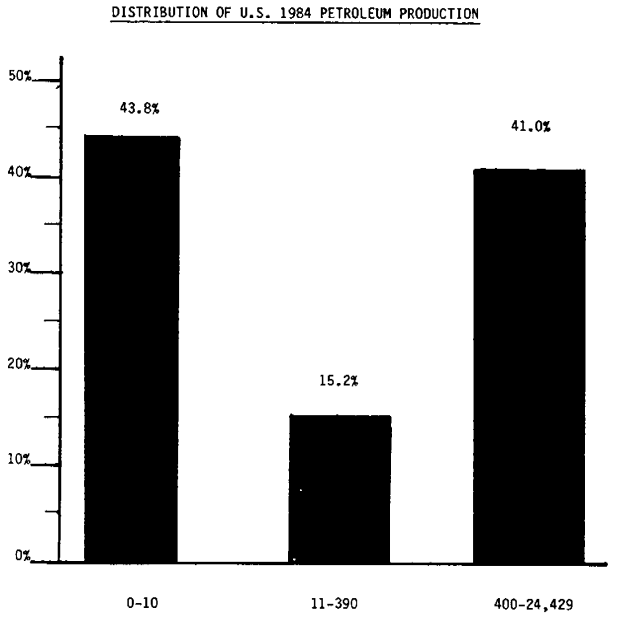
Ref: GAO, 1986 (Sources: EIA, 1985; O&GJ, 1985; DOE, 1985.)

FIGURE 6



Information Source: IPE, 1986.

FIGURE 7



Ref: GAO, 1986.

FIGURE 8

ENVIRONMENTAL ASPECTS OF MICROBIAL ENHANCED OIL RECOVERY

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ABSTRACT

With the increased interest and implementation of microbial enhanced oil recovery (MEOR), the environmental aspects of MEOR are of great importance. For the past 4 years, the National Institute for Petroleum and Energy Research (NIPER) has been studying the interactions between MEOR bacteria and indigenous reservoir microorganisms. Core experiments designed to simulate reservoir conditions were used to investigate microbial interactions in porous media. NIPER's environmental work has also included core studies to determine how the indigenous microflora are affected by the introduction of a nutrient, such as molasses. Berea sandstone cores have been colonized with strains of sulfate-reducing bacteria to determine the effects of MEOR systems on these bacteria. Results of the coreflooding experiments have shown that certain species of *Clostridium* and *Bacillus*, two microorganisms commonly used in the field and laboratory, can co-exist and recover oil in the presence of indigenous bacteria, even without refeeding for as long as 6 months. Some *Bacillus* and *Clostridium* species are able to survive and inhibit the sulfate-reducing bacteria present in the core. Core studies have also shown that nonsterile nutrients may interfere with the injected MEOR organisms. The laboratory environmental work has been correlated with field sample data. Recommendations for monitoring field projects will also be presented.

INTRODUCTION

The use of microorganisms to enhance oil recovery from partially depleted petroleum reservoirs is a technology with great potential. Microbial enhanced oil recovery (MEOR) is a method of tertiary chemical oil recovery, consisting of the injection of bacteria with nutrient for in situ production of such products as surfactants, acids and gases, polymers, and solvents. Thermal and chemical enhanced oil recovery accounts for more than 5% of the total U.S. oil production. Microbial enhanced oil recovery could be a major contributor to this overall EOR production within the next few years.

Currently, there are several groups performing microbial field projects. Many of these field tests have involved the use of shallow, abandoned, or stripper wells in which the oil production has decreased. Many of these stripper well projects often suffer from corrosion of the field equipment and/or subsurface casings. Leakage of microbial injectants from these wells could result in contamination of freshwater aquifers or other water supplies. If a microbial formulation is injected into one of these wells without sufficient laboratory and field assessment, serious environmental consequences could occur.

The two major environmental concerns associated with MEOR field applications are (1) possible contamination of surface and groundwater and (2) possible contamination of agricultural land during bacterial transport or injection. There are other environmental concerns involved with releasing microorganisms and/or nutrients into oil reservoirs. First, since microorganisms have a rapid mutation rate, the injected bacteria could mutate to a pathogenic form or produce potentially toxic chemical products. Secondly, the introduction of nutrients and nonindigenous bacteria may stimulate the growth of sulfate-reducing bacteria present in the reservoir. Sulfate-reducing bacteria, such as *Desulfovibrio* species, are strict anaerobes and are often isolated from reservoir waters. The stimulation of these bacteria could cause "souring" of the crude oil by the production of hydrogen sulfide, plugging of injection wells, and corrosion of the well. Also, injecting complex nutrients, such as molasses, may stimulate indigenous microflora to produce copious amounts of polymer slime which could plug the formation and the equipment lines.

At the present time some people may say, no guidelines or regulations have been established by the Environmental Protection Agency (EPA) that address the area of MEOR field technology. The EPA does have regulations for toxic waste disposal and salt water disposal injection wells. The Clean Water Act could also be used to control and monitor microbial solutions used for MEOR processes. Some states require a permit to carry out MEOR field projects. In Oklahoma no permit is required unless the process is implemented on a commercial scale. The regulations vary from state to state.

During the past few years, the Department of Energy has provided funds for laboratory and field investigations relating to MEOR. One concern of the DOE has been the area of environmental effects of MEOR. With support from the DOE, the National Institute for Petroleum and Energy Research (NIPER) has established laboratory facilities to study MEOR processes and evaluate the environmental concerns related to MEOR. The objective of the research is to design and develop methods for safe and successful field applications of MEOR techniques. The MEOR research at NIPER has involved a study of the interactions of two microbes, *Bacillus* and *Clostridium*, with indigenous reservoir bacteria. The survival and performance of these two organisms in the presence of indigenous bacteria have been studied in porous media. The effect of injecting a nutrient, such as molasses, has also been investigated in the laboratory. This work has included a study to determine whether sulfate-reducing bacteria can survive and grow in Berea sandstone cores. The interactions between sulfate-reducers and *Bacillus* and *Clostridium* species were also studied.

ACKNOWLEDGMENTS

The authors wish to thank James W. Chism and Dr. Fred W. Burtch of the Bartlesville Project Office, U.S. Department of Energy for their valuable suggestions in conducting this research, and Tina Long for her typing assistance. This work has been supported by the U.S. Department of Energy under Cooperative Agreement No. DE-FC22-83FE60149.

MATERIALS AND METHODS

To adequately evaluate the environmental impact of MEOR laboratory data are required. Microbial experiments in sandstone cores were used to study the interactions between injected bacteria and indigenous reservoir bacteria in porous media. The effects of nutrients on indigenous bacteria and sulfate-reducing bacteria were also examined using cores. The core studies were designed to simulate reservoir conditions in which MEOR processes would be applied.

Microbial Selection

Several samples of oilfield waters were obtained and examined for the predominant bacterial types. About 50% contained *Bacillus* species (Gram-positive, spore-forming rods) and 30% contained Gram-negative facultative anaerobes. These bacteria were isolated, purified, and designated as "indigenous bacteria." A *Clostridium* species and a *Bacillus* species were chosen to represent the MEOR process microorganisms. Both of these genera are presently being used in field tests. Four different *Bacillus* species were used: (1) Bac A, (2) Bac B, (3) Bac C., *B. licheniformis* (American Type Culture Collection, (ATCC) No. 27811), and (4) Bac. D, *B. licheniformis* (ATCC No. 39307). Both *B. licheniformis* strains produce surfactant and acids from the fermentation of sucrose. The *Clostridium* species used is a strict anaerobe which produces CO_2, acids, and solvents when fermenting sucrose. The sulfate-reducing bacterium selected was *Desulfovibrio desulfuricans* (ATCC No. 29577). It was used as the "type strain" for sulfate-reducing bacteria in the core studies. This organism is a strict anaerobe and was originally isolated from a tar-sand mixture in water-saturated clay.

The microbial consortia, NIPER BAC 1, is the bacterial system presently being used in the DOE-supported microbial waterflood project in the Delaware-Childers field. This consortia was used in core experiments with the *Desulfovibrio* type strain. It consists of two *Bacillus* species, one clostridial strain, and a facultative anaerobic Gram-negative rod. These organisms were selected on the basis of their ability to produce surfactants, solvents, acids and gas in previous core experiments. All of these microorganisms were tested for their compatibility with field fluids, reservoir conditions, and the molasses nutrient.

Preparation of Cores

Cylindrical and rectangular cores were cut from Berea sandstone obtained from Cleveland Quarries in Amherst, Ohio. The dimensions of the cylindrical cores were 25.4 X 3.7 cm and the dimensions of the rectangular cores were 30.5 X 4.7 X 4.7 cm. The cores were fired at 800° F for 24 hours to stabilize the clays present.[1] The round cores were either sealed in epoxy resin or encased in rubber sleeves and placed in stainless steel Hassler coreholders. The square cores were sealed in epoxy resin to confine the flow of injected fluids to a linear path. Inlet and outlet tubing with valves were attached to both ends of the encapsulated cores. The cores were then evacuated for 2 to 3 hours and flooded with sodium chloride brine or field brine. Darcy's law was used to calculate the absolute permeability of each of the cores. The

absolute permeabilities to brine ranged from 45 to 759 millidarcies. The pore volumes of the cores ranged from 53 to 138 cm^3. The cores were saturated with crude oil until no additional water was produced to simulate connate water saturation. The oil saturated cores were then flooded with brine before microbial injection until oil production ceased. This is done to simulate residual oil saturation after a waterflood. The average oil saturation after waterflood, designated by Sowf% in the tables, was 29.6%.

Crude Oil

Crude oil samples were obtained from the Bartlesville sand, Delaware-Childers field in northeastern Oklahoma. The specific gravity of the oil is 31° API and the viscosity is 10 cp at 25° C. Samples of crude oil from the Mink lease, Delaware-Childers field were also used for core tests. This oil sample has a specific gravity of 31° API and its viscosity is about 7 cp at 25° C.

Chemicals and Media

All chemicals and bacteriological media were obtained from Sigma Chemical Company and American Scientific Products. The molasses used in these experiments was Mr. Blackstrap 87, a cane molasses used for cattlefeed. Its composition is as follows: crude protein, 5%; crude fat, 0.5%; and total sugars, 38%. It was filtered through cheesecloth to remove the suspended fibers. Another cane molasses (OKC molasses) being used in the microbial waterflood project was also used in some core experiments. It was obtained from a company in Oklahoma City and its composition is as follows: total ash, 8.1%; calcium, 0.8%; phosphorus, 0.08%; magnesium, 0.35%; potassium, 2.4%; sulfur, 0.8%; sodium, 0.2%; and the pH is 5.5. The amount of total solids is 74% of which 3% is total protein, and 48% is total sugar (sucrose). The concentration of OKC molasses used was 4% by weight in tap water. In some core studies, sterile sucrose (1.5% by weight in distilled water) was used as a nutrient. The growth medium for *Desulfovibrio desulfuricans* was medium number 1249 which is recommended by the American Type Culture Collection for growth of this organism. The contents are as follows: $MgSO_4$, 0.2%; Na Citrate, 0.5%; $CaSO_4$, 0.1%; NH_4Cl, 0.1%; K_2HPO_4, .05%; yeast extract, 0.1%; and Na lactate, 0.35%. The medium is adjusted to a pH of 7, autoclaved, and tubed anaerobically. A 5% solution of $(Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was prepared in distilled water, filtered through a 0.45-micron Millipore filter, and 0.1 milliliter was added to 5.0 milliliters of the number 1249 medium just prior to inoculation. Growth of the *Desulfovibrio desulfuricans* can be detected by H_2S gas production and blackening of the medium due to the reduction of sulfates to FeS.

Coreflooding Apparatus

Figure 1 illustrates the apparatus used for coreflooding. The fluid separators are piston devices used to inject microbial solutions and other fluids into the cores. They are made of Lexan and were constructed at NIPER. The fluid separators prevent microbes, crude oils, and brines from passing through the pumps, which extends the life of the pump.

Experimental Protocol

Figure 2 illustrates the injection protocol used for the MEOR-indigenous microbial experiments. The selected indigenous microorganisms were cultured in pairs in tubes of trypticase soy broth (TSB). After an incubation period of 48 hours at 37° C, the tubes were examined for the following: (1) relative numbers of *Bacillus* species and Gram-negative rods, (2) endospore formation by the *Bacillus* species, and (3) alterations in the relative ratio of the two species due to the addition of Delaware-Childers crude oil and Berea sandstone. The indigenous microbes were injected (0.2 pore volume (PV) in TSB with 0.3 PV of sterile sucrose) into Berea sandstone cores that had been waterflooded to residual oil saturation (S_{owf}%). After the cores were incubated 1 week at 30° C, they were reinjected with the MEOR bacteria (0.2 PV) and 0.3 PV of nutrient, usually nonsterile molasses.

Short-term Indigenous Microorganisms Studies

For the short-term indigenous microbial compatibility tests, two microbial species, isolated from formation water obtained from oil wells in Oklahoma were used. The indigenous bacteria selected were designated as FR (Gram-negative facultatively anaerobic rod) and H1 (Gram-positive *Bacillus* species) and were injected together into the core (0.2 PV) at an average concentration of 5.0×10^6 cells/ml. The cores were incubated at 37° C for 1 week. After incubation, 0.3 PV of the MEOR microorganism (either *Bacillus*, *Clostridium*, or both) was injected into the core at an average concentration of 2.0×10^7 cells/ml. These organisms were injected in a sterile sucrose nutrient. The H1 and MEOR *Bacillus* strains could always be differentiated microscopically. The cores were then incubated another week at 37° C and were waterflooded at 2 foot/day. During the flood, fractions of the core effluent were collected. Plate counts and microscopic observations of all effluents were recorded.

Long-term Indigenous Microbial Studies

The methodology of the long-term indigenous microbial-*Bacillus* or *Clostridium* compatibility experiments differed from the short-term experiments only in the length of incubation time. Some of these cores were incubated at 25° C for up to 6 months. In both short and long-term experiments, control cores containing no indigenous microorganisms were also studied.

Core Studies With Sulfate-Reducing Bacteria

Core experiments were also performed to test the effects of *Bacillus* and *Clostridium* species on a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*. The cores were prepared as described previously. The cores were first colonized with *Desulfovibrio* sp. in medium 1249 (0.2 PV) and allowed to incubate for 1 week anaerobically at 30° C. At the end of the incubation period, the core was waterflooded (0.5 PV) and the core effluent was analyzed for the presence of *Desulfovibrio* sp. The cores were then re-injected with the *Bacillus* or *Clostridium* species, or both, along with 0.3 PV of 1.5% sterile sucrose or 4% molasses. All cores were incubated at 25° C for 1 week. The

cores were then waterflooded to determine the residual oil recovery efficiency and to check the effluent to see whether all of the injected microorganisms survived. Aerobic and anaerobic plate counts were also made from the core effluents during the waterflood. These cores were shut-in for 3 to 6 months to evaluate the long-term interactions of *Desulfovibrio desulfuricans* with the other injected bacterial strains. The experimental protocol for the *Desulfovibrio* core experiments is given in figure 3.

Single-Well Survivability Test

In addition to laboratory data, field data are also required to adequately evaluate MEOR. A single-well microbial injection experiment was done in Delaware-Childers field, near Nowata, Oklahoma. A consortium of microorganisms (NIPER BAC 1) was injected with molasses into an off-pattern injection well. NIPER BAC 1 system was injected along with OKC molasses. After injection, the well was shut-in for twelve days. It was then backflushed and samples of the gas and well fluids were collected. A field laboratory was set up near the wells to observe the field samples for microorganisms and to inoculate bacteriological media while the samples were still fresh. All field samples were inoculated into sulfate-reducing bacterial media to assay for stimulation of sulfate-reducers due to the injection of molasses and microorganisms. The gas samples from the well were analyzed for the presence of hydrogen sulfide. Microbial counts of the samples were also performed.

RESULTS AND DISCUSSION

Recovery Efficiencies for *Clostridium* and *Bacillus*

Clostridium Results

Tables 1 and 3 present coreflood data for *Clostridium* and *Bacillus*, respectively. A number of core experiments were performed to determine the ability of injected microbial strains to recover residual crude oil. A few of the cores were chosen as representatives for all the core studies.

Table 1 gives the results for the *Clostridium* species studied. Cores 1, 3, 4, 5 were injected with pairs of indigenous microbes and later re-injected with *Clostridium* species. Cores 2, 6, 7, 8 served as controls and were injected with only *Clostridium* and a nutrient. The residual oil recovery efficiency (E_r) of these cores containing indigenous microorganisms and *Clostridium* ranged from 15 to 21.4%. The average E_r was 18.4% ±3%. This average was lower than a previously reported E_r average for *Clostridium* alone which was 45%.[2,3] The E_r of the control cores ranged from 5.0 to 46.2%. The best E_r of 46.2% occurred in core 7 which had been injected with *Clostridium* and sterile sucrose (1.5%). The next best recovery, 23%, was seen in core 6 which was injected with *Clostridium* and nonsterile molasses. The average E_r of the *Clostridium* control cores was 21.3% ±4%. Cores which had microorganisms and nonsterile molasses as nutrient showed oil recoveries of 16.7% (core 1), 21.4% (core 3), 15.0% (core 4), 20.5% (core 5), and 23% (core 6). The average recovery efficiency of these cores was 19.3% ±3%. All of the effluents from these cores contained a Gram-variable rod, a microorganism present in the molasses, which is also capable of enhancing crude oil recovery

from sandstone cores. It probably contributed to the overall recovery seen in these cores. The lower recovery efficiencies in cores 2 and 8 are due to the presence of the microbial contaminant in the molasses which overgrew the *Clostridium* species. Even sterilizing the molasses at 121° C, 15 psi, for 15 minutes did not destroy this organism. The results from the *Clostridium* core studies indicate that the presence of indigenous bacteria will decrease to some degree the ability of *Clostridium* species to recover residual oil from sandstone cores.

Bacillus Results

The results of the *Bacillus* coreflood experiments to determine oil recovery efficiency in the presence of indigenous bacteria are given in table 3. Cores 1,2, and 4 were injected with a pair of indigenous microorganisms, H1 and FR, and selected strains of *Bacillus*. Sterile 1.5% sucrose was used as a nutrient in all three cores to prevent interference from microbial contaminants in the nutrient. Cores 3, 5, 6, 7 and 8 served as controls for this study. The recovery efficiencies of cores 1, 2 and 4 ranged from 10.7 to 21.7%. The highest E_r was seen in core 1 which had been injected with Bac. C, H1 and FR. This strain of *Bacillus* gave a residual oil recovery efficiency of 21.7% in the presence of indigenous bacteria, H1 and FR. This was unexpected since Bac. C with sterile sucrose alone (core 6) was only able to recover 8.4% residual oil. The indigenous microbes must have contributed to this increase in oil recovery. Both indigenous bacteria produce acid and gas from sucrose which could enhance the movement of crude oil in the core.

The other two *Bacillus* strains tested with the same indigenous microorganisms gave recoveries of 10.7 and 11.5%. The average E_r for the *Bacillus* species and indigenous microbes was 14.6% ±6%. This average is slightly lower than the average oil recovery of 22% for *Bacillus* without indigenous microbes present.[4] The best oil recovery of 22.5% occurred in core 3 which was injected with Bac. D and sterile sucrose. This strain gave an E_r of 10.7% in the presence of indigenous bacteria (core 2). The Bac. D strain did not perform as well in the presence of indigenous bacteria as it did when injected by itself. The E_r of the *Bacillus* control cores ranged from 0 to 22.5% The low recovery of core 8 was attributed to the fact that Bac. A did not survive well in this core and was overgrown by a *Pseudomonas* species present in the molasses or other injected fluids. The average oil recovery efficiency of the control cores was 11.7% ±8%. From these coreflood studies, we concluded that certain *Bacillus* and *Clostridium* species are able to enhance the recovery of residual crude oil even in the presence of indigenous bacteria in Berea sandstone cores.

Short-term Core Results for *Bacillus* and *Clostridium*

Core experiments were used to evaluate the compatibility of *Clostridium* and indigenous microorganisms following an incubation period of 1 week at ambient temperature. All *Clostridium* cores were injected with a *Clostridium* inoculum having a concentration of about 1×10^6 cells/ml. The concentration of the indigenous microorganisms injected into cores 1, 3, 4 and 5 was 4.7×10^6 cells/ml. All of the core effluents had the injected *Clostridium* species present after 1 week. The only exception was core 1 in which very

few *Clostridium* cells were present in the effluent (table 2). Usually, other microbial strains were also present, depending on the injectant and the nutrient used. A molasses bacterium, designated C*, was also found in the effluents of cores 1 through 7 after 1 week incubation. Of the cores injected with indigenous microorganisms, cores 1, 3, 4, and 5 showed the indigenous *Bacillus* (H3) and H13 present in the effluents prior to the injection of the *Clostridium* species. After injection of the *Clostridium*, H3 *Bacillus* was not found in effluents from the injected cores. H13, a Gram-negative rod, was isolated from all the core effluents. The indigenous *Bacillus* species was either overgrown by faster-growing microbes, such as the C* organism in the molasses, or inhibited by some chemical produced by the *Clostridium* or another organism present in the core. This finding was surprising since *Bacillus* species are spore-formers which should make them more resistant to environmental stresses and enable them to survive better than other non-sporeforming bacteria under reservoir conditions. However, results of the 1-week experiments suggest that these indigenous microorganisms could be inhibited by *Clostridium* species or by other bacteria present in the nutrient.

Cores 1, 2 and 4 were designed to study the effects of indigenous bacteria on *Bacillus* species. These cores were injected with indigenous microbes, H1 and FR, at a concentration of 3.5×10^6 cells/ml in Trypticase Soy Broth. Table 4 gives a list of the injectants for each core. After 1 week the core effluents were checked for the presence of H1 and FR. Both bacteria were found in all three core effluents at a concentration of 10^5 cells/ml. The cores were then re-injected with a *Bacillus* species at a concentration of about 2×10^7 cells/ml. After injecting *Bacillus*, the cores were shut-in for 1 week. The results from the 1 week studies showed that all the injected MEOR *Bacillus* strains (Bac. A, B, C, D) survived in all the cores after 1 week. The Bac. D strain seemed to be the most stable and the most compatible with the indigenous bacteria, H1 and FR. Of the indigenous microorganisms, FR seemed to be more abundant in the core effluents and was found in all the cores injected with H1 and FR. Control cores 3, 5, 6, 7 and 8 also had Gram-positive coccoid rods present in their effluents after short-term incubation. The short-term results for *Bacillus* showed that the injected MEOR *Bacillus* strains survive after 1 week incubation in the presence of indigenous H1 and FR. Only in core 8 did the *Bacillus*, Bac. A, not grow well and was overgrown by a *Pseudomonas* species. The FR organism was usually the most predominant microorganism present in the short-term indigenous core experiments. This result indicates that some strains of *Bacillus* will be overgrown by faster-growing indigenous microorganisms in reservoirs.

Long-term *Clostridium* Results

Table 2 presents the long-term core results from the *Clostridium* experiments which were performed to determine the length of time that certain *Clostridium* spp. could survive in sandstone cores. Cores 3 through 8 were shut-in for periods varying from 12 to 27 weeks. The *Clostridium* was able to survive for up to 27 weeks in control core 8, which had no indigenous bacteria injected. In all control cores the *Clostridium* was found in the core effluent after 1, 20, 24, and 27 weeks incubation at ambient temperature. The *Clostridium* cells were not refed during the incubation period. Since the *Clostridium* species are spore-formers, they would be expected to survive for long periods under reservoir conditions.

One observation made from the cores injected with indigenous bacteria, H3 and H13, was that the *Clostridium* species did not survive after 1, 12, or 16 weeks. Only in core 4 which was injected with indigenous strains, H2 and H12, did the *Clostridium* survive after 12 weeks. All three cores (1, 3, 5) had C* present in their effluents. This organism was also present after the short-term incubation period. The C* organism even overgrew the indigenous microbes, H3 and H13. When autoclaved molasses was used as nutrient in cores 8 and 12, the C* was still not completely eliminated. The C* bacterium overgrew the *Clostridium* in cores 3 and 5 after short-term incubation and this same result was seen after 16 weeks. These data stress the importance of testing the compatibility of bacteria present in injected fluids, such as nutrients, brine, and oil, with the selected MEOR bacteria and core bacteria.

Again, as in the short-term experiments with *Clostridium*, the injected indigenous *Bacillus* species did not survive; however, after 12 weeks the Gram-negative rods (H12 and H13) were also eliminated. Also, the results indicate that indigenous microbes affect the survival of the MEOR *Clostridium* species. Core data show that the microorganisms present in the nutrients may suppress the indigenous microflora in the reservoir. Most of the *Clostridium* species can survive under reservoir conditions for more than 6 months if they are not overgrown by other microorganisms present.

Long-term *Bacillus* Results

The results from the long-term experiments indicate that most of the *Bacillus* species can survive in Berea sandstone cores for up to 27 weeks (table 4). All four *Bacillus* species tested were isolated from the core effluents during waterflood after long-term incubation at ambient temperature. The indigenous microbes, H1 and FR, were also found in effluents from cores 2 and 4. FR was also evident in core 1 effluent. Usually, other microorganisms were also present in the effluents, *Pseudomonas* being the most common. It may have been introduced into the core in the injected fluids or stimulated by the sucrose or molasses. The use of sterile nutrients eliminated the problems of competition by microbial contaminants present in nonsterile nutrients.

Two species of *Bacillus licheniformis* (Bac. C and D) were evaluated for long-term survival in sandstone cores. Bac. C was able to survive for up to 24 weeks when injected with either sterile sucrose or nonsterile molasses as nutrient (cores 6 and 7, table 4). The Bac. D strain survived after 12 weeks in control core 3 and in core 2, which had also been injected with H1 and FR. The presence of these indigenous organisms did not affect Bac. D's ability to survive, as seen in the microbiological analysis of the core effluent. In the short-term experiments, it was observed that the injected MEOR *Bacillus* could survive but was usually outnumbered by the indigenous Gram-negative bacteria. The same result was seen after long-term incubation. The *Bacillus* species were present but in much lower concentrations than the other indigenous bacteria or the *Pseudomonas* species. Of all the *Bacillus* strains tested, Bac. D exhibited the greatest ability to grow and survive over long time periods in sandstone cores and was found to be the most compatible with other indigenous bacteria. The microbial counts remained high even up to 27 weeks, aerobically 10^5 cells/ml and aerobically 10^3 cells/ml. This indicates that the core environment was not too harsh for the injected bacteria even over longer shut-in periods.

Desulfovibrio Core Flood Experiments

Cores D2, D5, D13, and D14 were designed to test the ability of a strain of *Desulfovibrio* to recover residual crude oil. Table 5 gives the results for these core experiments with sulfate-reducing bacteria. All cores were injected with *Desulfovibrio desulfuricans* in medium #1249. The recovery efficiency for *Desulfovibrio* in D2 was 10.2% and the bacterium was found in the core effluent during waterflooding. Core D5 was injected with the *Desulfovibrio* type strain and nonsterile cane molasses (4%). The residual oil recovery efficiency for this core was 5.6%. No *Desulfovibrio* was seen in the core effluent which indicated that the microorganisms from the nonsterile molasses or the bacteria present in the core, oil, or brine overgrew the *Desulfovibrio* species. Core D13 was injected with the type strain in 1.5% sterile sucrose. Core D14 was injected with *Desulfovibrio* and nonsterile cane molasses. The recovery efficiencies for D13 and D14 were 5.5% and 0.43%, respectively. The *Desulfovibrio* species was recovered from the D13 core effluent; however, no *Desulfovibrio* was seen in the effluent from D14. In all four cores, the *Desulfovibrio* was able to survive in the core after 1 week at 30° C anaerobically. Using sterile nutrients seemed to improve the growth and survival of the sulfate-reducing bacterium but did not greatly increase the residual oil recovery by the *Desulfovibrio* species. The average recovery efficiency for *Desulfovibrio desulfuricans* with Delaware-Childers crude oil and 0.5% NaCl brine was 5.4% in Berea sandstone cores. Figure 3A presents a graph of the residual oil saturation versus brine pore volume for core D13.

The results of core experiments performed to study the effects of *Desulfovibrio* on *Bacillus* and *Clostridium* species are presented in table 5. Results from the core studies conducted with both *Desulfovibrio* and *Bacillus* species indicate that the presence of sulfate-reducing bacteria does not greatly affect the ability of the *Bacillus* strains to recover residual crude oil. The recovery efficiency (E_r) for the *Bacillus* species ranged from 6.7 to 26.2%. The average E_r for the *Bacillus* species with *Desulfovibrio desulfuricans* present was 16%. This result is similar to an earlier reported recovery efficiency average of 22% for *Bacillus* species alone in Berea sandstone cores.[2,3] The highest residual crude oil recoveries with *Bacillus* species occurred when a sterile sucrose nutrient was used. The S_{ocf}% versus pore volume for core D10 is shown in figure 3B.

The coreflood data show that *Clostridium* species are more affected as far as their ability to recover oil by the presence of sulfate-reducing bacteria than are *Bacillus* species. The recovery efficiencies for *Clostridium* ranged from 11 to 31%. The average E_r for *Clostridium* with *Desulfovibrio* was 20% ±5%. This value is lower than the average E_r of 45% for *Clostridium* alone.[3] A representative graph of the residual oil saturation versus brine pore volume for the *Clostridium* species tested is given in figure 3C. Two cores, injected with the microbial system being used in the microbial waterflood test in Delaware-Childers field, were also done to test the effect of sulfate-reducing bacteria on this system. Cores D8 and D12 were first colonized with the type strain of *Desulfovibrio* and incubated anaerobically at 30° C for 1 week. The NIPER BAC 1, a consortia of four microorganisms, was then injected along with nonsterile OKC molasses (4%). The recovery efficiencies of these two cores were 6.7 and 14.6%. The recoveries were somewhat lower than the recoveries obtained for NIPER BAC 1 without *Desulfovibrio* present. The effluent from core D12 after waterflooding showed

no sign of *Desulfovibrio*. However, the sulfate-reducing bacteria were found in the effluent from Core D8. The results from these core studies indicate that the presence of sulfate-reducing bacteria can decrease the oil recovery efficiency of NIPER BAC 1. However, this microbial system is able to survive and still recover residual oil even with sulfate-reducing bacteria present. In some cases, NIPER BAC 1 may inhibit the growth of sulfate-reducing bacteria or reduce their numbers in the core.

The pressure data from these core studies indicate that the injected *Bacillus* and *Clostridium* species are able to metabolize the injected nutrient to produce CO_2 and H_2 gas. The pressure increases during incubation of the cores after injection of bacteria ranged from 17 to 40 psi. The production of gas by the microorganisms is one mechanism by which they may enhance oil recovery. The gas pressure of the cores following injection of *Desulfovibrio* remained low, usually less than 10 psi. This was as expected, since no nutrients other than the medium in which the *Desulfovibrio* was grown in, was injected into the cores. The pressure in core D5 rose to 21 psi and to 25 psi in core D14 after introduction of the sulfate-reducing bacteria and molasses. This microorganism can produce some CO_2 by fermenting lactate, but the molasses bacteria are also gas producers and probably contributed to the higher pressure seen in these cores. The production of gas by the *Bacillus* and *Clostridium* species, indicated by the pressure increases during incubation of the cores, suggests that these *Bacillus* and *Clostridium* species are able to grow and perform in the presence of sulfate-reducing bacteria.

Survival of Microbial Systems with *Desulfovibrio*

Results of the survivability and compatibility of the sulfate-reducing bacteria and injected MEOR bacteria in porous media are given in table 6. The average aerobic plate counts for the cores after production were 8.60×10^5 cells/ml and the average anaerobic plate counts were 1.00×10^5 cells/ml. The types of bacteria found in the core effluents during production are presented in table 6. The *Bacillus* species seem to outcompete and overgrow the *Desulfovibrio* species in the core tests. Cores D1, D6, and D10 indicated that the *Bacillus* species inhibited the growth of sulfate-reducing bacteria in the core. Only in D3 was sulfate-reducing bacteria found in the effluent and they were present at a very low concentration (less than 1×10^2 cells/ml). The *Bacillus* strains appeared healthy and viable after incubation in the core with the *Desulfovibrio* species.

The results from the tests with *Clostridium* indicate that it is not as inhibitory for *Desulfovibrio* as are the *Bacillus* species. The data from core D2 showed that both the *Desulfovibrio* and *Clostridium* were able to survive and grow together in the core. Core D7 showed that both the *Desulfovibrio* and *Clostridium* were overgrown by the *Bacillus* species which was a contaminant introduced in the injected fluids or present in the core itself. The results from core D9 indicated that the *Clostridium* species could inhibit the sulfate-reducing bacteria present in the core. The use of sterile nutrients enhanced the survival of *Clostridium* with *Desulfovibrio* and allowed the *Clostridium* to overgrow the *Desulfovibrio* as in the case of core D9. Core results from D8 and D12 showed that the *Desulfovibrio* species was inhibited by the presence of the microbial consortia, NIPER BAC 1, which is being used in the microbial waterflood project. The MEOR bacterial system grew well in the core and the

Desulfovibrio species did grow in core D8, but the concentration of sulfate-reducers was very low in comparison with the other microbial populations present. The control cores which were injected with only *Desulfovibrio desulfuricans* and a nutrient, indicated that the type strain could survive in Berea sandstone cores after short-term incubation. In a few cases, such as with core D5, the microorganisms present in the nutrients or in the core fluids overgrew and inhibited the sulfate-reducing bacteria. The use of sterile nutrients, such as sterile sucrose, seemed to give the *Desulfovibrio* a better chance of survival in the core experiments.

The inhibition of sulfate-reducing bacteria by injected microbial strains could be a major asset to microbial enhanced oil recovery techniques. Inhibition of sulfate-reducing bacteria by clostridia has been reported previously by Oklahoma State University.[5] It was thought that the presence of sulfate-reducing bacteria would interfere with the ability of *Bacillus* and *Clostridium* species to recover oil. Data from the core experiments indicate that these bacteria are affected very little by the presence of sulfate-reducers and can even inhibit their growth in sandstone cores. The extent to which this effect lasts is not yet known; however, the long-term core studies should give more information. After waterflooding to produce residual crude oil from the cores, the epoxy cores were shut-in and incubated for long time periods. Some of the cores have been incubated at room temperature for up to 6 months.

Long-Term Core Results for *Desulfovibrio*

Data from the long-term core experiments are given in table 7. A total of seven cores were re-opened after being shut-in for 3 to 6 months. Cores D1 through D3 were encased in Hassler coreholders and were not shut-in for long-term testing. The cores were waterflooded with brine (1 PV) and the bacteria from the effluents were identified. Most of the results indicate that the *Desulfovibrio desulfuricans* was unable to survive and grow in the cores after 3 to 6 months. In cores D4 and D5, the bacteria present in the nutrients or indigenous core bacteria were able to inhibit the sulfate-reducing bacteria after 5 to 6 months. The inhibition effect due to either the injected bacterial strains or nutrient limitations in cores D6, D9 and D10, seemed to have permanently inhibited the sulfate-reducing bacteria. It was encouraging to see that injected MEOR strains survived after long-term incubation with *Desulfovibrio* species in the cores. Cores D7 and D8 were the only cores whose effluents had sulfate-reducing bacteria present. The anaerobic plate counts of *Desulfovibrio* from these cores were 1.0×10^1 cells/ml. The bacteria were seen early in the waterflood core effluent, usually before 0.2 PV of brine, and were not detected in later core samples. After waterflooding for oil recovery, core D7 showed no *Desulfovibrio* sp. present. But, after 4 months of incubation the *Desulfovibrio* were seen in low concentrations in the core effluent. The clostridia species did not survive and were not seen in the effluent during production or after long-term incubation in the core. The clostridia were overgrown by the other microorganisms present in the core. The low microbial population in this core and elimination of clostridial species may have allowed for the growth of some *Desulfovibrio* sp.

The pH of the core effluents did not change significantly from the pH at the time of the initial waterflood. The anaerobic microbial counts from the

core effluents were much lower than have been previously reported for *Bacillus* and *Clostridium* species. This may indicate that environmental conditions in the core are not as optimal for growth of these bacteria when *Desulfovibrio* species are present. However, the microbial analysis of the core effluents indicate that *Bacillus*, *Clostridium* and other microbes are able to survive in the presence of *Desulfovibrio* after 3 to 4 months in sandstone cores. In some cases, they are even able to inhibit the growth of sulfate-reducing bacteria over long time periods.

Single-Well Survivability Test

The field test results from the single-well study substantiate the experimental core results obtained in the laboratory. All species of injected microorganisms were recovered and observed directly in effluents from the well backflush samples. Previous well samples had shown that sulfate-reducers were present in these wells. There was no evidence of stimulation of sulfate-reducing bacteria by the introduction of NIPER BAC 1 and molasses. In fact, the anaerobic plate counts and direct microscopic examination of backflush samples showed no sulfate-reducing bacteria present. Less than 5 ppm of hydrogen sulfide was detected in the samples from the two single-wells. The aerobic and anaerobic plate counts of the samples showed that the numbers of injected bacteria did not decrease from the initial inoculum by more than a factor of 10. The bottomhole temperature of the reservoir when injected was 52° F, and after 12 days, the temperature was 60° F. This increase in temperature was attributed to the heat produced due to microbial metabolism downhole.

CONCLUSIONS

1. Certain species of *Bacillus* and *Clostridium* can co-exist in the presence of indigenous bacteria, even as long as 6 months.

2. The injection of nutrients into petroleum reservoirs could stimulate the growth of indigenous microbes.

3. The compatibility of injected MEOR systems and nutrients must be tested with field microbes and fluids.

4. Some *Bacillus* and *Clostridium* species are able to inhibit the sulfate-reducing bacteria present in sandstone cores for up to 6 months.

5. *Bacillus* species are more likely to survive and overgrow the *Desulfovibrio* species in porous media than *Clostridium* species.

6. The ability of *Bacillus* species to recover residual crude oil is less affected by the presence of sulfate-reducing bacteria than is *Clostridium*.

7. In situ injection of some microorganisms may inhibit sulfate-reducing bacteria in petroleum reservoirs.

8. MEOR is an environmentally safe process if properly designed and monitored.

Recommendations for Environmental Monitoring of MEOR Field Projects

1. Perform preliminary laboratory experiments to test the compatibility of all injected fluids and reservoir fluids. Also, determine whether undesirable products or undesirable bacterial mutations occur over a period of months prior to injecting the bacteria.

2. Design a sampling and monitoring procedure which will detect the production of environmentally hazardous products or the stimulation of undesirable microorganisms such as the sulfate-reducing bacteria.

3. Be prepared with a contingency plan in case undesirable results or environmentally unsafe conditions result (i.e. have biocides or other chemicals at injection site).

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TABLE 1. - Recovery efficiencies from *Clostridium* core studies

Core[1]	Pore volume, cc	K, md[2]	S_{owf}[3]	S_{ocf}[4]	E_r[5]
1	58.0	315	37.07	30.86	16.7
2	58.1	330	32.42	30.88	5.0
3	58.0	374	38.55	30.29	21.4
4	57.7	287	34.66	29.46	15.0
5	57.6	331	37.33	29.51	20.5
6	57.7	339	36.90	28.40	23.0
7	56.7	344	34.00	18.30	46.2
8	59.3	331	32.46	28.92	10.9

[1]Cores 1, 3, 4, 5: indigenous microbes + *Clostridium*.
Cores 2, 6, 7, 8: *Clostridium* alone.
[2]K = Absolute permeability to brine in millidarcies.
[3]S_{owf} = Residual oil saturation after waterflood (%).
[4]S_{ocf} = Residual oil saturation after microbial treatment (%).
[5]E_r = Residual oil recovery efficiency (%).

$$\frac{S_{owf} - S_{ocf}}{S_{owf}} \times 100\%$$

TABLE 2. - Core experiments with *Clostridium*

Core	Injectant[1]	Incubation, wks[2]	Core Effluent[3]
1	H-3, H-13 *Clostridium* NS molasses[4]	1	No *Clostridium*, C*
2	*Clostridium* St. molasses	1	*Clostridium*, C*
3	H-3, H-13 *Clostridium* NS molasses	12	No *Clostridium*, C*
4	H-2, H-12 *Clostridium* NS molasses	12	*Clostridium*, C*
5	H-3, H-13 *Clostridium* NS molasses	16	No *Clostridium*, C*
6	*Clostridium* NS molasses	20	*Clostridium*, C*
7	*Clostridium* Sterile sucrose	24	*Clostridium* Gram-positive rods
8	*Clostridium* Sterile molasses	27	*Clostridium*, *Pseudomonas*

[1]Cores first injected with indigenous microbes H-3, H-13 or H-2, H-12.
[2]At ambient temperature, 25° C.
[3]Microscopic and bacteriological examination of effluent after incubation period.
[4]NS = Nonsterile.

TABLE 3. - Recovery efficiencies from *Bacillus* core studies

Core[1]	Pore volume, cc	K, md[2]	S_{owf}[3]	S_{ocf}[4]	E_r[5]
1	56.6	300	35.0	27.4	21.7
2	53.0	246	35.1	31.3	10.7
3	57.1	306	31.9	24.7	22.5
4	137.0	321	23.6	20.9	11.5
5	138.0	388	23.9	22.2	7.3
6	52.4	162	31.7	29.0	8.4
7	55.4	192	37.9	34.6	8.6
8	56.8	175	30.6	30.3	0.0

[1]Cores 1, 2, 4: indigenous microbes + *Bacillus* spp.
Cores 3, 5-8: *Bacillus*
[2]K = Absolute permeability to brine in millidarcies.
[3]S_{owf} = Residual oil saturation after waterflood (%).
[4]S_{ocf} = Residual oil saturation after microbial treatment (%).
[5]E_r = Residual oil recovery efficiency (%).

$$\frac{S_{owf} - S_{ocf}}{S_{owf}} \times 100\%$$

TABLE 4. - Core experiments with *Bacillus*

Core	Injectant[1]	Incubation, wks[2]	Core Effluent[3]
1	H-1, FR *Bac. C*[4] St. Sucrose	8	*Bac. C*, FR, *Pseudomonas*
2	H-1, FR, *Bac. D*[5] St. Sucrose	12	*Bac. D*, FR, H-1, *Pseudomonas*
3	*Bac. D* St. Sucrose	12	*Bac. D*, *Pseudomonas*, Gram-pos. rods
4	H-1, FR, *Bac. B* St. Sucrose	20	H-1, *Bac. B*, FR, *Pseudomonas*
5	*Bac. B*, St. Sucrose	20	*Bac. B*, Gram-pos. coccoid rods
6	*Bac. C*, St. Sucrose	24	*Bac. C*
7	*Bac. C* NS molasses	24	*Bac. C*, Gram-pos. coccoid rods
8	*Bac. A*, NS molasses	27	*Pseudomonas*, a few *Bac. A*

[1]First injected indigenous microbes H-1, FR.
[2]at 25° C.
[3]Microscopic and bacteriological examination of effluent after incubation.
[4]Bac C = B. licheniformis.
[5]Bac D = B. licheniformis.

TABLE 5. - *Desulfovibrio* core experiments

Core	K,[1] md	Injected fluids,[2]	Core information	Pressure,[3] psi	E_r[4]
D-1	477	0.5 PV D.d.[5] in SRB media 0.2 PV *Bacillus* 0.3 PV Barley malt	Round, fired Berea SS;[6] Hassler holder; DC[7] oil, 0.5% NaCl	0 34	9.5
D-2	633	0.5 PV D.d., then 0.5 PV *Clostridium* in sucrose	Same as D-1	15 40	10.2 31.4
D-3	563	0.5 PV D.d. then (0.5 PV) *Bacillus* in sucrose	Same as D-1	0 33	0 9.7
D-4	-	0.2 D.d.; 0.3 PV sucrose	Square, fired Berea SS;[8] Epoxy; 0.5% NaCl; no oil	23	-
D-5	730	0.2 PV D.d., 0.3 PV nonsterile molasses	Round, fired Berea SS	21	5.6
D-6	759	0.2 PV D.d., then 0.5 PV *Bacillus* in sucrose	Same as D-5	0 24	0 20.6
D-7	701	0.2 PV D.d., then 0.5 PV *Clostridium* in sucrose	Same as D-5	0 26	- 17.7
D-8	329	0.2 PV D.d., then 0.2 PV NIPER BAC 1 0.3 PV 4% NS molasses	Square, fired Berea SS;[9] Expoxy; field brine and oil	0 20	- 6.7
D-9	299	0.2 PV D.d., then 0.2 PV *Clostridium* 0.3 PV sucrose	Same as D-8	0 20	- 11.3
D-10	368	0.2 PV D.d., then 0.2 PV *Bacillus* 0.3 PV sucrose	Same as D-8	0 28	- 26.2
D-11	412	0.2 PV D.d., then 0.1 PV *Bacillus* 0.1 PV *Clostridium* 0.3 PV Sucrose	Same as D-8	0 17	- 8.4

TABLE 5. - *Desulfovibrio* core experiments (continued)

Core	K,[1] md	Injected fluids,[2]	Core Information	Pressure,[3] psi	E_r[4]
D-12	278	0.2 PV D.d.[5] 0.2 PV NIPER BAC 1, 0.3 PV molasses	Square, fired Berea; Epoxy; field brine	0 35	14.6
D-13	76.1	0.2 PV D.d., 0.3 PV Sucrose	Square, fired Berea; Epoxy; DC oil; 0.5% NaCl	10	5.5
D-14	362	0.2 PV D.d., 0.3 PV molasses	Same as D-13	25	0.4

[1]Absolute permeability to brine in millidarcies.

[2]0.5 PV = 0.5 pore volume. Cores were first injected with *Desulfovibrio desulfuricans*.

[3]Maximum pressure in psi during incubation.

[4]Recovery efficiency of residual oil remaining in core after waterflood $\left(\frac{S_{owf} - S_{ocf}}{S_{owf}}\right)$ X 100%.

[5]D.d. = *Desulfovibrio desulfuricans*.

[6]SS = Berea sandstone core.

[7]DC = Delaware-Childers crude oil.

[8]Core D4 plugged on one end; no oil injected into core or recovery efficiency calculated.

[9]Field = Brine and oil samples from the Mink lease, Delaware-Childers field.

TABLE 6. - Microbial analysis of core experiments

Core	Injectant[1]	First effluent[2]	Second effluent[3]
D1	0.5 PV D.d.[4] 0.2 PV *Bacillus* 0.3 PV Barley Malt	D.d.	No D.d. *Bacillus*
D2	0.5 PV D.d. 0.2 PV *Clostridium* 0.3 PV Sucrose	D.d.	D.d. *Clostridium*
D3	0.3 PV D.d. 0.2 PV *Bacillus* 0.3 PV Sucrose	D.d.	Few D.d. *Bacillus*
D4	0.2 PV D.d.	-	D.d. *Bacillus*
D5	0.2 D.d. 0.3 PV molasses	D.d.	No D.d. *Bacillus*
D6	0.2 PV D.d. 0.2 PV *Bacillus* 0.3 PV Sucrose	D.d.	No D.d. *Bacillus* Gram + rods
D7	0.2 PV D.d. 0.2 PV *Clostridium* 0.3 PV Sucrose	D.d.	No D.d. No *Clostridium* *Bacillus*
D8	0.2 PV D.d. 0.2 PV NIPER BAC 1 0.3 PV 4% molasses	D.d.	Few D.d. *Bacillus*, *Clostridium* M18, Gram + rods
D9	0.2 PV D.d. 0.2 PV *Clostridium* 0.3 PV sucrose	Few D.d.	No D.d. *Clostridium* Gram + coccoid rods
D10	0.2 PV D.d. 0.2 PV *Bacillus* 0.3 PV sucrose	No D.d.	No D.d. *Bacillus* Gram + coccoid rods
D11	0.2 PV D.d. 0.1 PV *Bacillus* 0.1 PV *Clostridium* 0.3 PV sucrose	D.d.	No D.d. *Bacillus* Few *Clostridium* Gram + coccoid rods
D12	0.2 PV D.d. 0.2 PV NIPER BAC 1 0.3 PV 4% molasses	D.d.	No D.d. *Bacillus* *Clostridium*

TABLE 6. - Microbial analysis of core experiments (continued)

Core	Injectant[1]	First effluent[2]	Second effluent[3]
D13	0.2 PV D.d.[4] 0.3 PV Sucrose	D.d.	Few D.d. Gram + coccoid rods Gram - rods
D14	0.2 PV D.d. 0.3 PV 4% molasses	D.d.	No D.d. *Bacillus* Gram + and Gram - rods

[1]0.5 PV = 0.5 pore volume. Cores were first injected with *Desulfovibrio desulfuricans*.

[2]Microscopic observation of first effluent, 1 week at 30° C; D.d. average anaerobic counts = 4.0×10^3 cells/ml.

[3]Microscopic observation of second effluent, 1 week after injection of *Bacillus* and/or *Clostridium* sp.

[4]D.d. = *Desulfovibrio desulfuricans*.

TABLE 7. - Long-term core experiment results

Core	Injectant[1]	Months	Microbial analysis	Anaerobic[2] plate count, cells/ml	Effluent[3] pH
D4	D.d. and sucrose	6	No D.d., *Bacillus* Gram + rods Gram - rods	4.50×10^3	6.45
D5	D.d. and molasses	5	No D.d., Gram + coccoid rods, Gram - rods	7.00×10^4	6.15
D6	D.d., *Bacillus*	4	No D.d. *Bacillus*, Gram + coccoid rods	6.30×10^4	6.60
D7	D.d. and *Clostridium*	4	Few D.d.[4] *Bacillus* *Pseudomonas* Gram + coccoid rods	1.00×10^2	6.40
D8	D.d. and NIPER BAC 1	3	Few D.d.[4] *Bacillus*, *Clostridium*	3.90×10^2	6.70
D9	D.d. and *Clostridium*	3	No D.d., Gram + coccoid rods, Gram - rods *Clostridium*	5.70×10^4	6.80
D10	D.d. and *Bacillus*	3	No D.d. *Bacillus* Gram + coccoid rods	2.80×10^3	6.85

[1]Injected 5.00×10^6 cells/ml of *Desulfovibrio desulfuricans* into each core.
Injected 3.00×10^6 cells/ml of *Bacillus* sp.
Injected 7.00×10^6 cells/ml of *Clostridium* sp.

[2]After waterflood to produce residual oil, the average aerobic plate count was 8.60×10^5 cells/ml and the average plate count anaerobically was 1.00×10^5 cells/ml.

[3]Average pH of the core effluent samples taken during the waterflood.

[4]D.d. (*Desulfovibrio desulfuricans*) microbial count = 1.00×10^1 cells/ml.

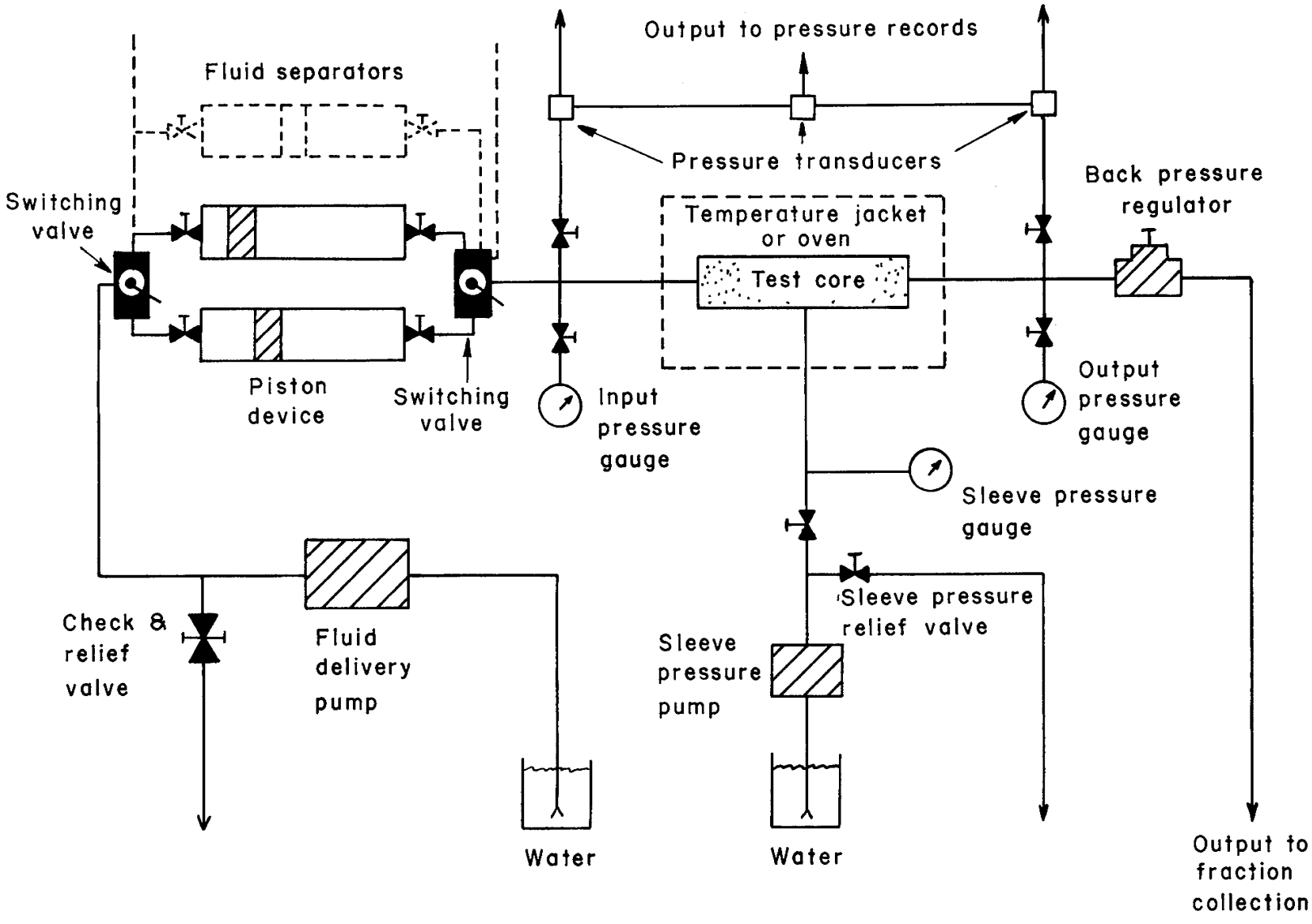

FIGURE 1. - Schematic diagram of experimental apparatus.

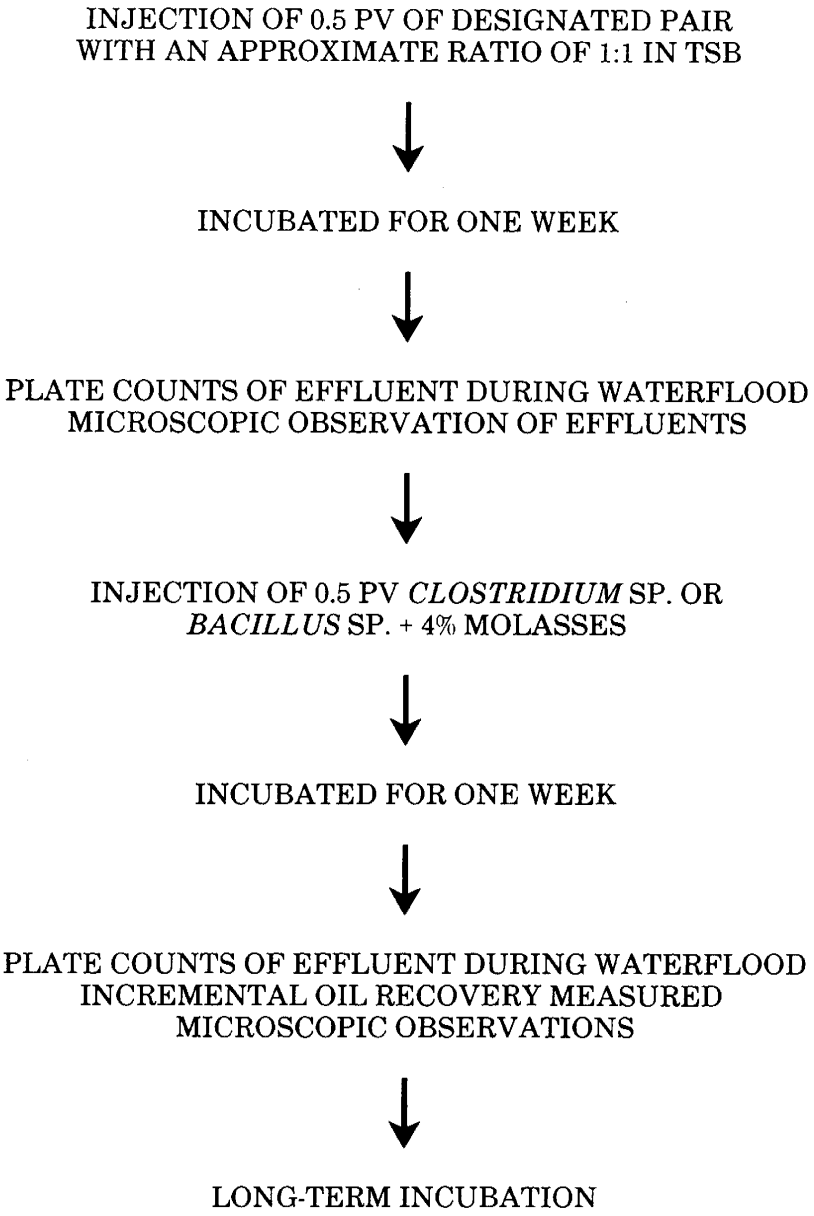

FIGURE 2. - Protocol for indigenous core experiments.

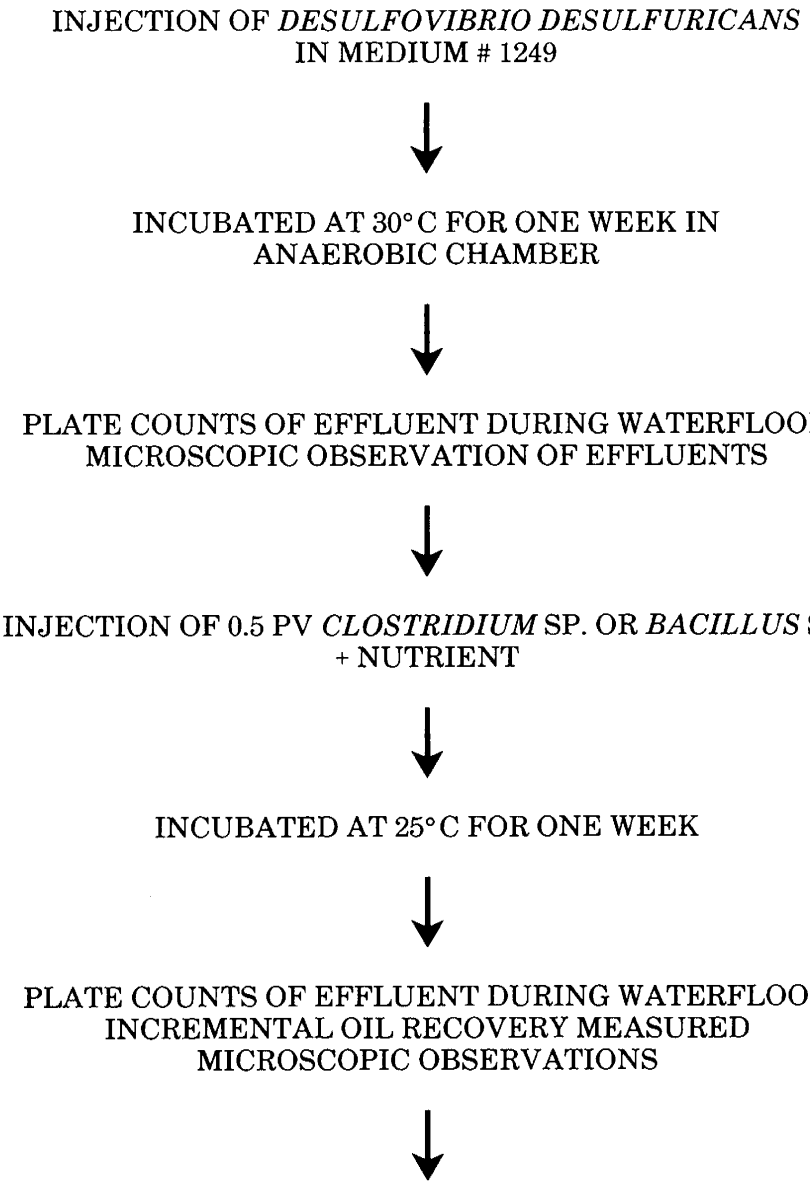

FIGURE 3. - Flow chart of protocol for *Desulfovibrio* core experiments.

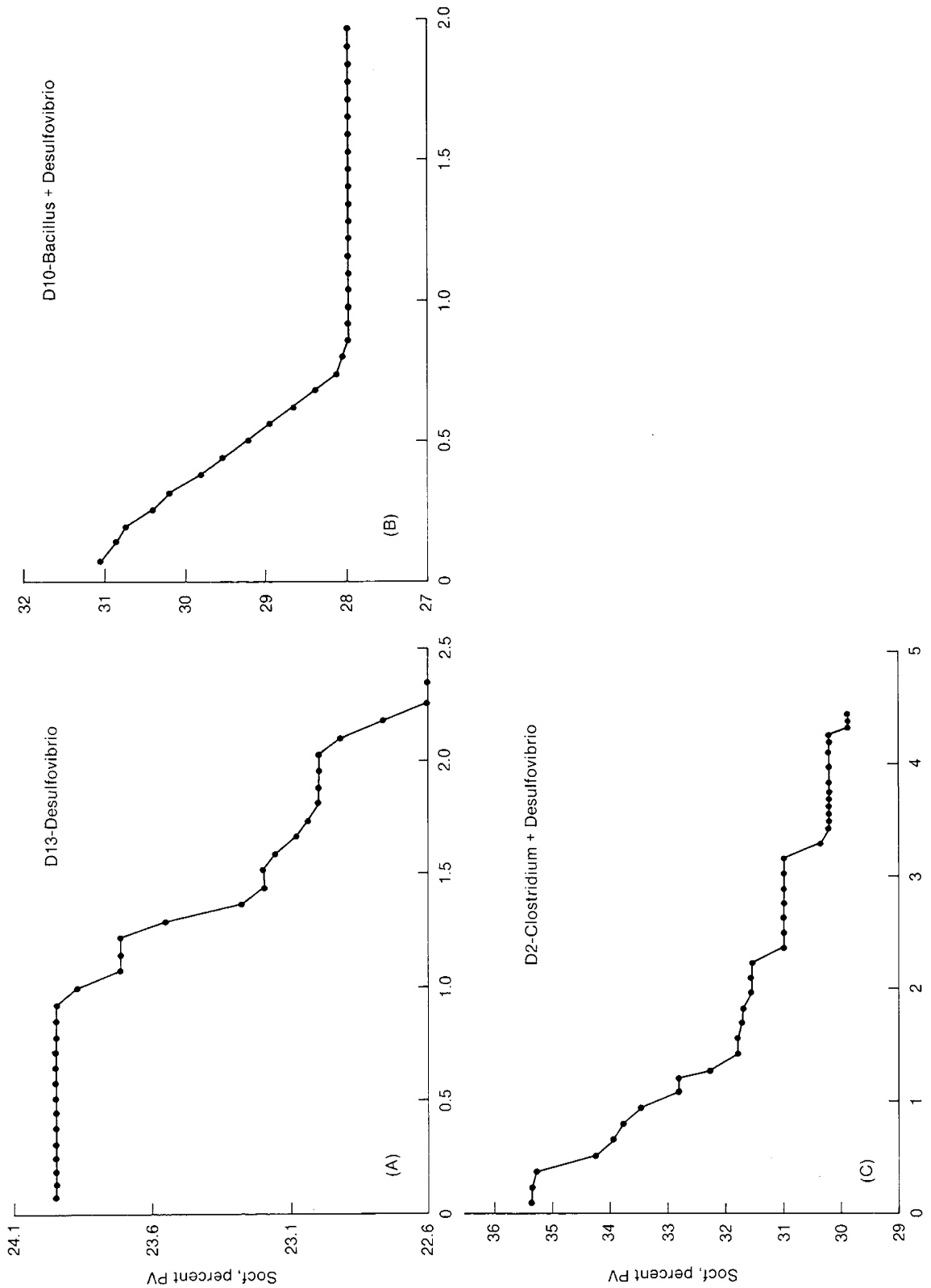

FIGURE 4. - S_{ocf}% vs. brine PV for *Desulfovibrio* core experiments.

BIOCORROSION IN THE OILFIELD

I. Experimental Methods Development: Scanning Electron Microscopy Techniques

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ABSTRACT

Biocorrosion is a problem routinely encountered, but not always recognized, in the oilfield. Examples of biocorrosion in the oilfield abound, but may be masked or overshadowed by other corrosion. Microbially enhanced oil production is likely to increase the occurence of biocorrosion. The sulfate-reducing bacteria (SRB), which are the most common bacterial type associated with biocorrosion, exist under virtually every environmental condition. SRB-caused biocorrosion not only results in material loss but in safety problems from the released hydrogen sulfide and the danger of catastrophic failures. SRB survive and persist in environmental niches, including hydrocarbon production and waterflooding systems, primarily by their ability to attach to surfaces. Bacterial exopolymers "cement" the bacteria to the substratum and to each other. The total consortium of attached bacteria (sessiles) and their exopolymers is called a biofilm. Classical microbiological methods do not assay for sessile bacteria or biofilms. In the work reported here, biofilming bacteria, their associated exopolymer, and the early time-course events of biofilm development on steel were visualized by use of the scanning electron microscope (SEM). Resulting scanning electron photomicrographs show that corrosion occurred directly under the bacterial growth. Prevention of bacterial growth with a biocide prevented corrosion. These SEM results represent progress in Conoco's attempt at correlating biofilm development with detrimental effects in the oilfield. Monitoring, and controlling, biofilming bacteria in oilfield systems would appear to be more worthwhile than classical microbiological field procedures which are most useful for planktonic (free-floating) bacteria.

INTRODUCTION

Recent work (1,2) has shown that attached (sessile) bacteria represent a significant proportion of the total bacterial population in many environments. Similarly, many activities commonly associated with planktonic (free-floating) bacterial populations are in fact more representative of the sessile bacterial populations in that environment. Sessile bacteria mixtures produce extracellular biopolymers that "cement" individual cells to each other and to surfaces. The resulting film of bacteria, exopolymer, and entrained inorganic matter is called a biofilm. Biofilms may reach 100 microns in thickness and are known to increase heat transfer resistence across, and restrict flow

through, tubing (3). However, the cummulative metabolic activity that causes the most problems is surface deterioration. Metal surface deterioration is termed biocorrosion.

Biocorrosion mechanisms operating within a biofilm often result in corrosion processes and products identical to those of classical non-biological corrosion mechanisms (4). Biofilm corrosion mechanisms can enhance classical corrosion processes. Extensive literature reports link one common sessile bacteria type, the sulfate reducing bacteria (SRB), to corrosion in many diverse environments (4,5,6). This bacteria type is ubiquitous in the oilfield and Microbially Enhanced Oil Recovery (MEOR) processes are sure to introduce significant SRB contamination into the production reservoir. Previous Enhanced Oil Recovery (EOR) attempts have resulted in unintentional microbial contamination of process equipment and reservoir (7). As a consequence, EOR, and undoubtedly MEOR, operations face drastically increased corrosion, particularly in oil production equipment.

The hidden life style of sessile bacteria may mask the root corrosion cause in many cases. This is unfortunate because treatment regimes for biocorrosion and classical corrosion are often significantly different. In order to prevent inappropriate treatment, analysis and monitoring methods for sessile bacteria and biocorrosion are required. Developing appropriate analysis and monitoring methodology is complicated by the fact that traditional microbiological assay methods are not readily applicable to measuring the activity of biofilming bacteria or even useful in enumerating sessile populations (1,2). There is no known correlation between planktonic bacterial populations, which are measurable by classical methods, and sessile bacterial populations (8). As the relative importance of sessile bacteria becomes apparent, particularly with respect to corrosion (4), new methods must be developed to quantitate sessile bacterial activity in petroleum operations. The work presented here is a preliminary attempt at developing methodology for the qualitative analysis of sessile bacteria and their effect on petroleum operations.

EXPERIMENTAL

Mild steel nails were cleaned according to the procedure outlined in Table 1. Cleaned nails were added to duplicate test systems containing a dilute bacterial growth medium. The bacterial growth medium used is shown in Table 2. Both test systems were sealed in rubber septumed serum vials and autoclaved at 121° C for 15 minutes. Following cooling, ten milligrams of filter sterilized cysteine-hydrochloride were added to each test system to assure reduced conditions. Both systems were inoculated with a mixed bacterial culture originally isolated from oilfield produced fluids from the Hobbs, New Mexico area. One nail was removed at random from one of the test systems and immediately prepared for scanning electron microscopy (SEM) (Table 3). One test system then received enough formaldehyde to yield a 1000 ppm (final) solution. Both systems were incubated at 30° C. Nails were aseptically removed from the two test systems after 1 week, 2 weeks', six weeks', and ten weeks' incubation. At each sampling, one nail was removed from each test system. The nail from the bacterial growth system was designated nail #1; the nail from the biocide treated, or control, system was designated nail #2. Both nails were immediately submitted to the fixation and dehydration procedure required for SEM. Both systems were resealed and 10 mg

cysteine-hydrochloride was added. Additional growth media was not used during the course of this experiment.

During photography, SEM personnel indexed the location of each photograph. It was estimated that subsequent SEM examination and photography (see below) could return to within one SEM field. After photography, the nails (mounted, indexed, and coated with gold and palladium) were cleaned by immersion in 0.1 N HCl for six minutes. This immersion took place in a sonic bath. Following spent acid removal by aspiration, the procedure was repeated. The nails were rinsed in deionized water and resubmitted to the SEM for photography.

RESULTS

Figures 1-8 are SEM photomicrographs of several steel specimens prepared during this experiment. For brevity, only the two weeks and ten week exposed nails are shown. The omitted one week and 6 weeks samples gave results that were consistent with the two and ten week specimens, respectively.

Figure 1 shows the surface appearance of the first experimental nail, i.e. the nail removed immediately after bacterial inoculation. Unlike succeeding nails, this nail was not cleaned with 0.1 N HCl (above) and serves as a baseline control of the nails' surface.

Figure 2 is a photomicrograph of the nails' surface after two week's incubation (nail #1,test). Biofilm is well developed. Biofilm removal (Figure 3) reveals that general surface corrosion has occurred. Figure 4 (nail #2, biocide treated system) shows that this corrosion had not occurred in the absence of bacteria (or bacterial products). Small surface irregularities noted were likely the result of acid cleaning procedures.

Figure 5 is a photomicrograph of a test nail after ten weeks' incubation. After biofilm removal, definite corrosion is now noted on the nail (Figures 6-7). The control nail (with biocide) shows increased surface irregularity but not the strong corrosion noted on the test nail (Figure 8). This surface irregularity may be evidence that general corrosion has occurred. Based on past photos, this corrosion is probably not due to acid cleaning but may represent some generalized corrosion mechanism not addressed in this experiment (i.e., non-biological corrosion). Biocorrosion mechanisms were not involved, as bacteria could not be isolated from the fluid or be seen by the SEM.

DISCUSSION

Traditional microbiological assay methods are not readily applicable to measuring biofilming bacterial activity, enumerating sessile populations, or even useful in detecting their prescence qualitatively (1,2). There is no known correlation between planktonic bacterial populations which are measured by classic methods and sessile bacterial populations (9). It is clear that new methods are required for the quantitation of sessile bacterial populations and their relative activity in petroleum operations. EOR pilot project results indicate that significant SRB populations can be expected to develop in production equipment (8). Microbially enhanced oil recovery (MEOR) programs will experience similar problems.

Work detailed here utilizes scanning electron microscopy (SEM) to investigate sessile bacteria effects on mild steel. Experimentation was performed in dilute bacterial growth medium under static conditions. Production systems are more accurately viewed as dynamic, flowing systems which bear fairly low organic (nutrient) loads. However, to demonstrate SEM's usefulness, it was important to produce a good biofilm in a short time period and on a specific surface usable without undue sample preparation. The system employed here met these criteria.

The described experimental methodology employed random selection of duplicate specimens from a host of similar ones. The only variables were whether: 1) bacteria were present, and 2) the biocide (formaldehyde) had any effect other than limiting biological growth. From this experiment, it is readily apparent that biofouling had a definite positive effect on mild steel corrosion. Lack of corrosion in the controls was due to the absence of bacteria or bacterial products (iron sulfide); formaldehyde has no corrosion inhibiting properties reported in the literature. The striking longitudinal corrosion (Figures 6-7) was a result of preferential corrosion of nail areas exhibiting greater stress caused by metal sizing operations during the manufacturing process.

The experimental procedure used here is applicable to field systems, but a longer exposure period will be required. A likely field test would be to employ modified corrosion coupons. The coupons' physical size must be reduced to dimensions more amenable to SEM procedures without undue sample preparation. For example, physically sizing a biofouled coupon will destroy the biofilm. A reduced coupon size will also allow multiple samples to be taken from the same flow regime so that multiple tests can be performed on the same "coupon". Ultimately we need to develop the appropriate methodology to decide whether corrosion inhibitors or biocides are more cost effective for corrosion

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8. Personal communication with J. W. Costerton, University of Calgary, B.C., Canada.

TABLE 1. - Nail cleaning procedure

1. Use common, nonplated nails of appropriate size.
2. Cover with 15 percent HCl for 5 minutes.
3. Rinse with deionized water.
4. Rinse twice in acetone and store under acetone.
5. To use, rinse twice with isopropyl alcohol and air dry.
6. Add to test system; do not touch with hands

TABLE 2. - Bacterial growth medium

Ingredient	Quantity
Deionized water	70 ml
KH_2PO_4	0.0200 g
Na_2HPO_4	0.0400 g
$MgCl_2.6H_2O$	0.0400 g
NH_4Cl	0.0250 g
$CaCl_2.2H_2O$	0.0020 g
$FeSO_4.7H_2O$	0.0001 g
Na_2SO_4	0.0500 g
Ascorbic acid	0.0120 g
Sodium lactate (60%)	0.6 ml
Deionized water	to 100 ml
Resazurin	0.0010 g

Adjust pH to 6.5–7.0

TABLE 3.- Sample fixation and dehydration for SEM

1. Fix specimen for one hour in 5 percent glutaraldehyde made in 0.067 M cacodylic buffer (pH 7.0).

2. Rinse twice in deionized water. Aspirate water away.

3. Dehydrate in the following solvent mixtures. Soak in each for 3 minutes. Aspirate fluid away between soakings.

   - 30 percent ethanol
   - 50 percent ethanol
   - 75 percent ethanol
   - 85 percent ethanol
   - 95 percent ethanol
   - 100 percent ethanol
   - 100 percent ethanol
   - 50:50 ethanol:Freon 113
   - 100 percent Freon 113
   - 100 percent Freon 113

4. Deliver specimen in Freon 113 to SEM laboratory.

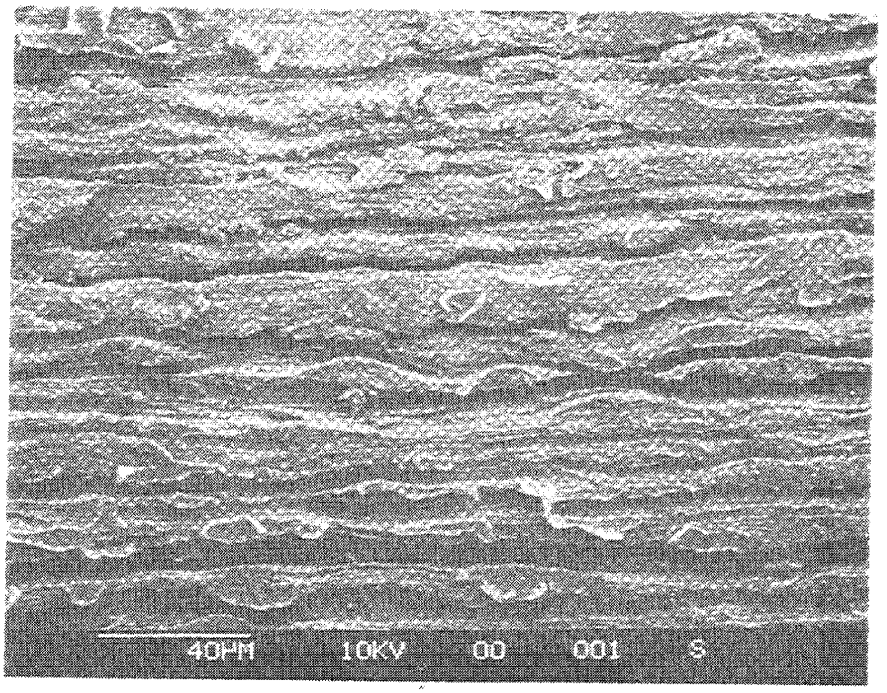

FIGURE 1. - Scanning electron photomicrograph of the steel specimen (nail) used as a time zero control (before biofouling).

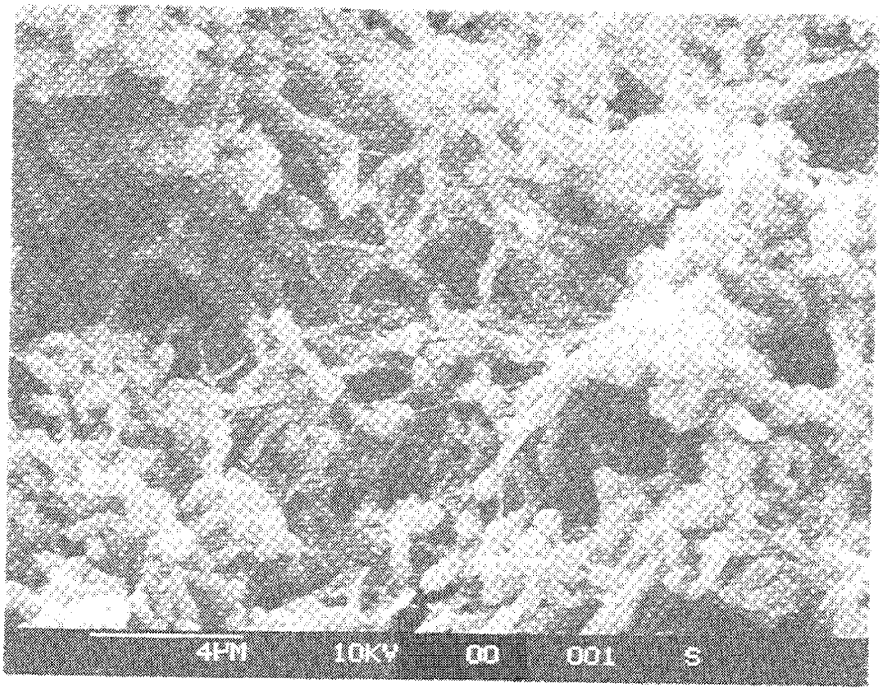

FIGURE 2. - Photomicrograph of a nail from the biofilm test system after two weeks bacterial growth.

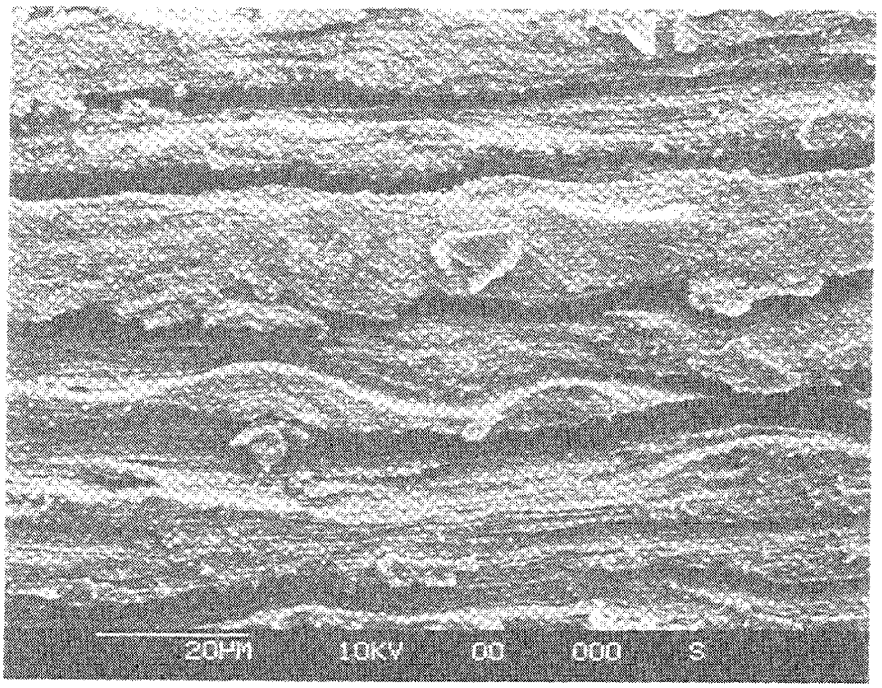

FIGURE 3. – Photomicrograph of a nail cleaned of biofilm after two weeks bacterial growth.

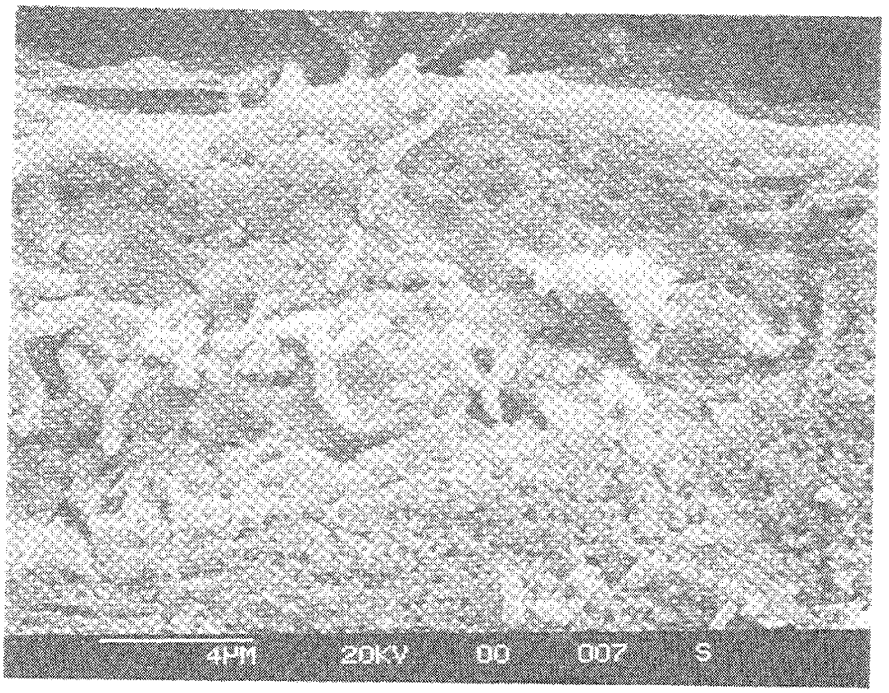

FIGURE 4. – Photomicrograph of a control nail after two weeks elapsed time.

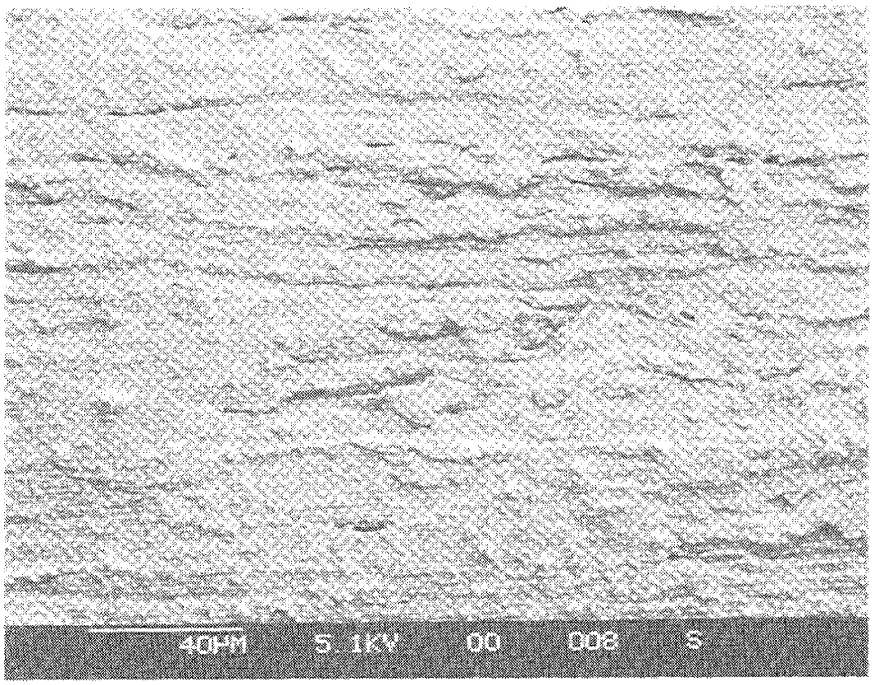

FIGURE 5. - Photomicrograph of a biofouled nail after 10 weeks growth.

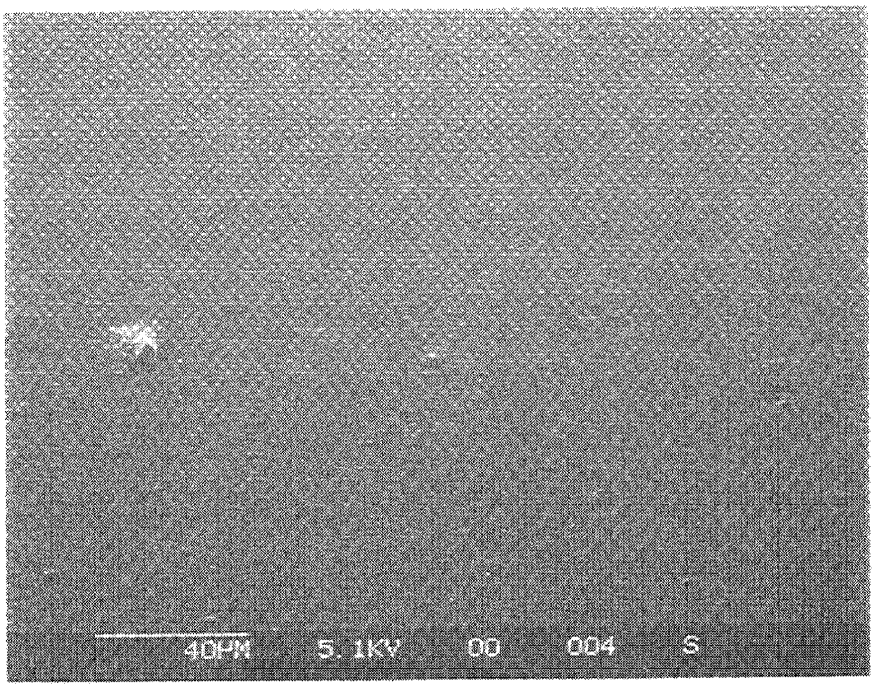

FIGURE 6. - Photomicrograph of a nail cleaned of ten weeks biofilm growth.

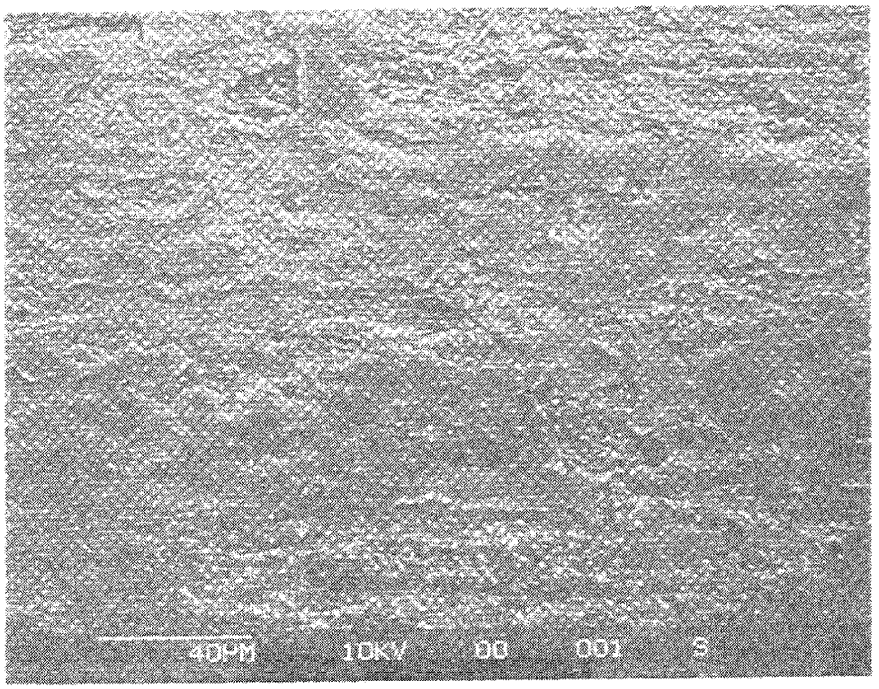

FIGURE 7. - Enlarged photomicrograph of the area shown in figure 6; nail cleaned of 10 weeks biofilm growth.

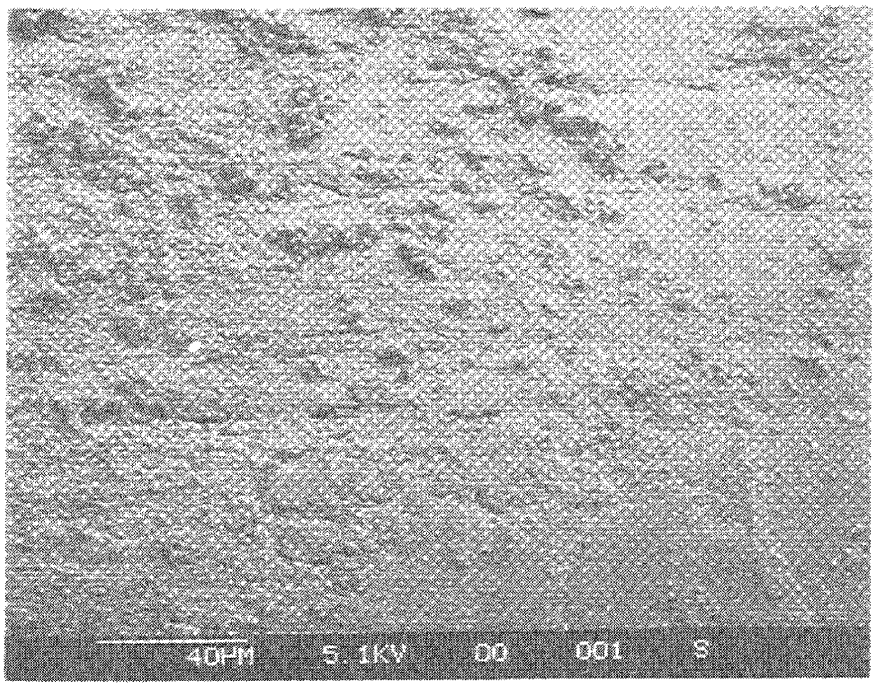

FIGURE 8. - Photomicrograph of a control nail after ten weeks exposure.

POTENTIAL ROLES FOR BIOPROCESSING IN ENHANCED OIL RECOVERY

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ABSTRACT

Biotechnology can, and is likely to, play an important role in many aspects of microbial-enhanced oil recovery (MEOR). Most current research is directed toward in-situ production of surfactants, polymers, and other materials that can enhance the recovery of oil by altering the water/oil interfacial properties or viscosities or the bulk flow patterns in the field. The mechanisms of MEOR are still not well understood, and better evaluations of the relative merits of in-situ and surface production of these chemicals that enhance oil recovery are needed. Field tests of MEOR must be planned and executed to answer specific questions that will increase our understanding and predictions of MEOR results.

Other potential uses of biotechnology to enhance oil recovery have been identified which should be explored. This paper provides a brief review of current MEOR studies and assesses the additional roles which biotechnology is likely to have in future oil recovery operations. For example, the use of microbial action to reduce environmental problems from release or reinjection of flood waters could become very important if current exemptions of oil recovery operations from environmental regulations are not extended.

INTRODUCTION

The role of biotechnology in increasing the production of oil from older and less productive fields is usually considered to involve the injection of selected microorganisms and nutrients into oil fields where the organisms can produce materials that enhance the effects of water flooding and increase oil production. This is called microbial-enhanced oil recovery (MEOR) and has been used successfully to increase oil production in several fields. However, that is only one approach, and there are several roles which biotechnology could play in increasing oil production.

This paper is derived from a longer report currently in preparation (Watson and Scott, 1987). The object of the study was to assess opportunities for bioprocessing in MEOR, rather than to recommend one area of MEOR study

*Research sponsored by the Office of Fossil Energy, U.S. Department of Energy, under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

over another. The present understanding of MEOR (and of some other methods for enhancing oil recovery) is limited, and it is early to recommend detailed choices in these areas.

REVIEW OF MEOR ACTIVITIES

The use of microbes to assist in oil recovery has been considered for several decades, and there have been numerous laboratory and field studies of organism injection into oil fields.* The most recent counting of field tests (Hitzman, 1987) lists more than 200 documented tests, although the documentation is often limited, either because few measurements were made or because the tests were proprietary. Many of these field tests were made in eastern Europe, and few details from the results were reported in the open literature. There is general agreement that, in many oil fields, proper additions of microbes and nutrients can increase the production rate significantly and thus extend the field's production period and total oil recovery. Quantitative predictions of these effects have been difficult, and some field tests have resulted in little or no confirmed productivity increase.

The need for a better understanding of microbial actions that increase oil production is evident, despite the numerous laboratory and field tests that have been made. Laboratory tests in this area have usually simulated field conditions with Berea sandstone filled with petroleum crude oil. A number of surfactants and polymers, several organisms and nutrients, and a variety of field and flow conditions have been tested. The principal measurement in these tests has been the rate of petroleum "wash" from the sandstone.

Potential mechanisms for microbiological enhancement of oil recovery have been hypothesized, and these are probably qualitatively correct (Smith and Collins, 1984; Bryant and Douglas, 1986). Microorganisms can produce biopolymers which will increase the viscosity of the water flood and result in more efficient washing of oil from the field. In an extreme case, such prodigious amounts of polymer, cell matter, and other biological materials can be produced that they actually block the flow in regions of the field (Knapp et al., 1985). Although plugging can be a serious problem when using microbes in oil fields, microbial plugging can also be helpful when used in reducing or eliminating flow in very porous regions where large fractions of the water flood would bypass the oil-bearing regions. Surfactants produced by the microbes can alter the interfacial wetting effects between the oil, water, and solids (rock or sand), and microbe-produced gases such as methane or carbon dioxide can help to repressurize the field. When large quantities of gas are produced, it may even be possible to alter the oil properties, as in carbon dioxide or hydrocarbon flooding. There are also several other materials produced by these microorganisms (such as alcohols, solvents, and acids) that can affect the oil, its interfacial properties, or the rock matrix. For instance, acids reacting with the limestone found in some formations could both increase the porosity of the field and produce carbon dioxide to pressurize the field.

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*A more detailed review of MEOR literature is given in the report currently in preparation (Watson and Scott, 1987); only a summarized review is presented here.

OPPORTUNITIES FOR BIOTECHNOLOGY

Obviously, oil recovery enhancement can be a very complex operation, and there are many ways for microbial reactions and biotechnology to make important contributions. These opportunities extend far beyond the MEOR approaches that have received the most study recently. A few such opportunities are listed in Table 1. Note that the materials which are believed to contribute to the favorable results achieved by MEOR can also be produced on the surface by microbial processes (Compere et al., 1983; Grune et al., 1979) and injected into the formation. This is usually called "chemical flooding," but it could be viewed as a variation of MEOR. In-situ and surface microbial production parameters need to be compared to determine their relative cost and effectiveness; this will be discussed later in this paper.

Microbial action has been an important factor in reducing environmental damage from the release of process waters used in the treatment of petroleum, coal, and gas (Healy et al., 1985). It could also become an important part of enhanced oil recovery (by MEOR or by other methods). There have been a few studies on the environmental impact of MEOR (e.g., Bryant and Chism, 1986), but little work has been done on the potential for using biotechnology to reduce the impacts of other types of enhanced oil recovery. Oil recovery is currently exempt from many restrictions of the Resource Conservation and Recovery Act (RCRA), but the exemptions are not permanent and could be totally or partially removed. If future environmental restrictions should require treatment of some oil flood waters prior to their release on the surface or injection into wells, bioprocessing could be the most effective treatment available. Microbes are also likely to be useful in reducing the effects of accidental oil releases to ground waters or to oceans (Atlas, 1981; Bryant, 1986).

There are possibilities that microbial actions could favorably alter the composition of petroleum. Although petroleum is relatively inert to microbial attack, especially to the action of anaerobic organisms, there have been a few favorable reports of anaerobic microbial reactions with petroleum. Under aerobic conditions, a number of organisms will attack petroleum and, it is believed, will eventually decompose it completely (Atlas, 1981; Bryant, 1986). This is thought to be the basis for the eventual dissipation of oil spills on the oceans. Since traces of hydrocarbons exist throughout the world, organisms capable of metabolizing hydrocarbons are probably equally widespread. When a spill occurs, those organisms can multiply to eventually eliminate the oil. Unfortunately, these organisms usually do not induce changes that improve the quality of the oil. The lighter and more valuable hydrocarbon fractions are usually attacked first, leaving the heavier, aromatic fractions that are more resistant to degradation. If microbial reactions could be found that would remove heteroatoms, such as sulfur, or selectively alter the heavier fractions of the oil while leaving the lighter material, a beneficial bioprocess might be developed.

RECOMMENDED AREAS FOR RESEARCH

As noted earlier, the physical and chemical processes which occur when enhanced oil recovery chemicals are added to a water flood are complex and

not well understood. These uncertainties, in addition to the lack of knowledge concerning microbe growth and metabolism, make the complexity of MEOR apparent.

Meaningful field tests will be difficult until the mechanism of MEOR and the field characteristics are better known. Our inability to predict the results of field tests (Bryant and Douglas, 1986; Hitzman, 1987) reflects our lack of understanding of either MEOR or field properties. We must also have a better understanding of the mechanisms and transport processes involved, before we can make quantitative estimates of MEOR performance or assess the reliability of field test results. Field tests must be carefully planned to obtain specific needed data. Models for MEOR performance should be developed so the tests can be planned to address the principal uncertainties in the models and to guide an evaluation of the results. Furthermore, the oil field used for such tests should be sufficiently characterized (and instrumented) to eliminate data uncertainties. Smaller test fields and shorter spacing between wells in the field could reduce the potential for faults or other important nonhomogeneous features of the field to go undetected. It would generally be desirable for the test field to be relatively shallow, to restrict drilling costs in case additional well drilling should be needed for characterization or test monitoring.

Another of the principal needs in MEOR research appears to be an improved assessment of the relative merits of in-situ and surface production of the materials which lead to enhanced oil recovery. Currently, it seems to be a common opinion that the lack of requirements for surface facilities makes MEOR (in-situ production) a less costly but effective way to increase oil production, but that is not documented.

A major portion of the cost for MEOR is for the nutrients to feed the injected organisms. One recent estimate lists the cost of nutrients as only 20 to 30% of the costs required to purchase the chemicals needed to enhance oil recovery separately and inject them into the field (Moses and Brown, 1987). This certainly is a potentially significant advantage, but care must be taken to ensure that the correct conclusions are reached from these figures. First, it is necessary to note that such estimates require the assumption of a value for the efficiency of in-situ material production, apparently assuming maximum production for the field temperature and chemical environment. However, it is possible for the in-situ efficiency to be much lower than that assumed, since several factors may affect the chemical production. For example, nutrients could be washed from the experimental portion of the field and/or could be consumed by other organisms.

Furthermore, it is possible to overestimate the cost of surface chemical production by using listed chemical prices. Errors can result in at least two ways. First, the quoted product price may include purification and/or concentration costs that would be unnecessary for chemicals used in enhanced oil recovery. A direct comparison of the price of purified and concentrated products made for the commercial market with costs involved in producing the dilute mixtures produced in-situ in the oil fields may be misleading. To minimize shipping costs, chemicals need to be concentrated if they are produced in remote plants, but no concentration would be necessary for "on-site" production.

Second, the production scale should be taken into account when estimating costs. There are usually economies of scale; so the costs of chemicals could decrease, if the demand justifies larger plants and equipment.

Despite the complexities involved in comparing the costs of surface and in-situ production of enhanced oil recovery chemicals, this is one of the major questions to be answered. Laboratory studies are needed to determine the relative effectiveness and costs of various in-situ and surface production processes.

Another area where current research on biotechnology for enhanced oil recovery appears to be especially limited is environmental control. A few studies have been done on the potential environmental impacts of MEOR, but little research has investigated the use of biotechnology to reduce or eliminate environmental impacts of MEOR or any enhanced oil recovery operations. The success of microbial processes in reducing the hydrocarbon content of waste waters from petroleum, gas, and coal processing facilities certainly indicates that they are likely to be useful in enhanced oil recovery operations as well. They are most likely to be helpful in treating waste waters that are to be released to surface waters and possibly in treating flood waters prior to reinjection into deep wells. They may also help in the clean-up of spills around enhanced oil recovery operations.

An important factor in assessing environmental control needs for enhanced oil recovery is the role of the RCRA. Oil recovery operations are currently exempt from RCRA, but that exemption is currently under review by the EPA. If oil recovery operations have to meet all of the RCRA regulations, several changes may be needed. Some flood waters may contain toxic components which would limit their injection into deep wells until those wells are certified for handling them. That could result in pretreatment of the flood waters prior to injection and/or greater recycle or reuse of flood waters (provided reuse is allowed). Biological treatment could be helpful in controlling the content of either reused or rejected flood waters. If the waters are not reused, the volume of waters pumped into disposal wells can be very large; so treatment by any means would be a difficult task requiring efficient bioreactors.

SUMMARY AND RECOMMENDATIONS

Until the merits of in-situ production are proven, research on surface production should continue. There may be significant differences between the oil recovery rates achieved using chemicals produced in surface facilities and those achieved with chemicals produced in situ, but those differences have not been conclusively demonstrated in either laboratory or field tests. For instance, if the organisms concentrate near the oil-water interface, the chemical nutrients could be more usefully introduced there than with the bulk water flood. But that is only conjecture, not a proven fact. So little is known about the mechanism of MEOR that it is difficult to make even basic comparisons between surface and in-situ processes.

Bioprocessing should also be considered for reducing the environmental impact of enhanced oil recovery operations. This could be an especially

important role for biotechnology if oil production facilities are forced to meet RCRA regulations.

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TABLE 1. - Potential uses of microbial processes in enhanced oil recovery

Activity	Surface production	In-situ production
Preparation of flooding chemicals		
Surfactants	X	X
Polymers	X	X
Gases	X	X
Miscellaneous (acids alcohols, etc.)	X	X
Selective plugging of porous region		X
Environmental control		
Waste water treatment	X	
Spill treatment	X	X
Modifications to the oil		
Alterations to molecular size	X	X
Removal of heteroatoms	X	

REVIEW OF MICROBIAL ENHANCED OIL RECOVERY FIELD TESTS

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ABSTRACT

A review of Microbial Enhanced Oil Recovery (MEOR) field tests was presented in 1982 at the International Conference on MEOR at Shangri-La, Afton, Oklahoma. In order to provide a current report of MEOR field tests, this review has been updated to include information which has been made available since its publication. A survey of these data will provide a basis for determining the status of developments in MEOR since 1982.

INTRODUCTION

The application of microbial techniques to improve oil recovery has been termed Microbial Enhanced Oil Recovery (MEOR). The MEOR technologies normally involve the introduction of viable microorganisms into the oil reservoir which by their growth, products, or actions, cause the release, mobilization, and recovery of oil that has remained trapped after secondary waterflood operations. The potential of the MEOR technology has been evaluated in the laboratory by studies which have demonstrated the growth of candidate cultures at simulated reservoir conditions. These experiments were expanded to include model core flooding tests which demonstrated that oil release by microbial action was successful. However, field testing remains the final criterion for evaluating the potential of MEOR technology to become a successful tertiary oil recovery method.

BACKGROUND

A review of the available literature which concerns field tests involving MEOR techniques was published in l982 (Petroleum Microbiology and the History of Its Role in Enhanced Oil Recovery. D.O. Hitzman, Inter. Conference on Microbial Enhancement of Oil Recovery. Shangri-La, Afton, Oklahoma May 6-21, 1982 p.162-218). This review has been expanded in this report to include information, both of new field tests and also an update of past tests, which has become available since the l982 publication. A survey of the combined data in these two reports will provide a basis for determining the status of MEOR field developments.

Where possible, the same format has been used in this report as in the l982 review to provide continuity to the data. However, it should be emphasized that the data, even though it was collected and correlated from various publications and personal communications is limited in some cases. Some of the information is incomplete because it either involves on-going field tests, or it is restricted for proprietary reasons.

DISCUSSION

Most of the previously reported MEOR field tests occurred in Eastern Europe. Currently, the activity has shifted to the United States which has experienced a renewed interest in the MEOR technologies. This increased emphasis in MEOR has resulted from the maturing of oil fields, economic considerations, and a better understanding of the broader applications that the MEOR technology may offer. In addition to past tests which stressed microbial well stimulation and waterflooding, it should be noted that field tests in the U.S now embrace microbial applications such as water diversion and paraffin removal. These alternate MEOR technologies appear to have developed to a state that field tests are now being considered or are in operation.

The microbial oil releasing mechanisms (Table 1) listed in the l982 report are very similar to those currently proposed.

TABLE 1.

Proposed Mechanisms Involved in MEOR

1. In situ generation of gases for pressure and solubilization.
2. Production of organic acids.
3. Production of surface active agents.
4. Conversion of hydrocarbons to lower molecular weight.
5. Consolidation of oil to droplets.
6. Production of polymers.

Previously reported tabulations showing trends in MEOR field tests have been updated to include the currently reported data. These comparisons can be seen in Tables 2 & 3.

TABLE 2.

MEOR: Number of Wells Treated

Country	Number of wells treated	
	1982 Report	1987 Update
Poland	17	-
Hungary	10	-
Czechoslovakia	6	-
USSR	2	-
Romania	9	-
Netherlands	2	-
Canada	1	-
USA	178	
200?		

TABLE 3

MEOR Tests: Well Depth

Well depth (meters)	Number of Wells Inoculated	
	1982 Report	1982 - 1987
50-250	7	3
251-500	1	2
501-750	16	3
751-1000	4	3
1001-1250	5	-
125l-1500	2	-
>1500	1	1

The extremes of reservoir parameters restricting microbial growth have not changed since the 1982 report. Most tests have been conducted in reservoirs that have conditions favorable for microbial growth.

There have been changes in the type of microorganisms that are used as inocula in the field (Table 4). In general, the inoculum now consists of mixed, pre-conditioned and selected cultures of anaerobes and facultative anaerobes. Spore forms of the cultures are used in many cases. Sulfate-reducing bacteria are not employed but may already be present in the formation.

TABLE 4.

Microbial Cultures Currently Employed in MEOR Field Tests

1. Anaerobes and facultative anaerobic cultures
   Clostridium
   Bacillus
   Pseudomonas
   Leuconostoc
2. Pre-conditioned proprietary mixed cultures

While the amount of inoculum injected depends on the test site and proprietary considerations, it appears that current injections favor a smaller inoculum than previously reported (Table 5).

TABLE 5.

MEOR: Concentrations of Inoculum and Nutrient Additions Employed in Field Tests

	Number of wells treated	
Inoculum Size (liters)	1982 report	1982-1987
10-50	4	3
51-100	6	2
101-200	1	1
201-300	-	3
301-400	2	1
401-500	9	-
501-600	2	-
601-700	-	-
701-800	1	-
>800	4	2
Molasses/Nutrients (kg.)		
10-100	4	3
101-500	4	4
501-1000	-	2
1501-2000	10	-
2001-10,000	1	2
>10,000	4	-

The practice now is to test the inoculum more extensively in the laboratory at simulated reservoir conditions to be sure that the cultures will grow in the reservoir. This decreases the need for a large inoculum because the injected quantity has a greater chance of being established rapidly. The amount of nutrient added (molasses usually is the main carbon source) is dependent on the MEOR technique employed. In addition, nutrients may be added in stages or continously rather than all at one time. However, the amount and type of nutrient added is again dependent on the culture, technique, and proprietary applications.

In general, the MEOR field tests reported since I982 conform to previous field tests. However, there are noticeable improvements in field techniques for applying the inoculum and nutrients. The cultures used are better selected and their growth conditions are more defined. It is becoming evident that the field tests are being designed with a greater consideration being given to field parameters and reservoir engineering data. However, it also remains evident that much better data collection before, during, and after the test will be necessary before a true evaluation of the success of the MEOR can be made.

FIELD TEST REPORTS

The following data on field tests reported since 1982 together with the revised data complementing the earlier test data are presented in the format of the 1982 report.

Romanian Field Test Update

Years of Test: 1975-1983
Location: Romania
Geological data: Reservoirs are sand or sands with a high content of marls (diorite sand) and clays.
Reservoir characterstics: Nine wells were inoculated in 7 oil reservoirs with the following range of characterstics.

TABLE 1.

PHYSICAL-CHEMICAL CHARACTERISTICS OF THE RESERVOIRS SUBJECTED TO MICROBIOLOGICAL TREATMENT

(Oil field reservoirs: BAICOI, VATA, SUTA SEACA, BRAGADIRU, BECIU, TINTA, MORENI)

LIMITS OF	RANGE	
Depth (m)	336	1559
Temperature, °C.	27	55
Permeability, mD	100	1500
NaCl (g/l)	5	180
Oil viscosity (cP)	6	53
Oil Density (kgf/dm^3)	0.850	0.908
Oil saturation (%)	71	81
Co-produced water (%)	15	96
Possible reaction wells	2	6

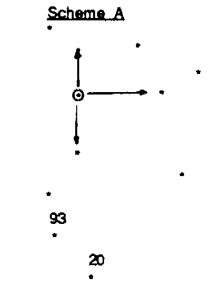
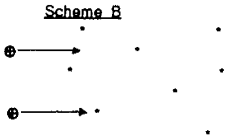
The Beciu reservoir was selected as the control since it had a low permeability (100 md). All the reservoirs were characterized by low oil and high co-produced water. The inoculum was specifically adapted for each target reservoir by growth of the culture in the formation water and reservoir oil at the conditions of the reservoir and in the presence of 2-4% molasses. The mixed inocula released from l9-49% of the oil from laboratory test collectors and utilized from 2-51% of the oil under aerobic conditions. The number of bacteria ranged from $4 \times 10^8 - 9 \times 10^9$/ml inoculum.

The main genera identified in the inoculum were: *Pseudomonas, Escherichia, Arthrobacter, Mycobacterium, Peptococcus, Bacillus, Clostridium*, with sporadic occurrence of *Aerobacter, Flavobacterium, Brevibacterium, Cellulomonas, Micrococcus, Sarcina, Spirillum, Desulfovibrio*, and some yeasts. The bacteria predominating in the inoculum belonged to genera: *Pseudomonas, Clostridium, Mycobacterium, Bacillus, Peptococcus*, and the family *Enterobacteriaceae*..

The wells were injected with 300 liters of inoculum, 2 tons of molasses and 110-120m^3 of formation water.

The homogenization and injection of the nutrient and inoculum was carried out by tank truck. Two schemes (Figure 2) were employed for the injection program.

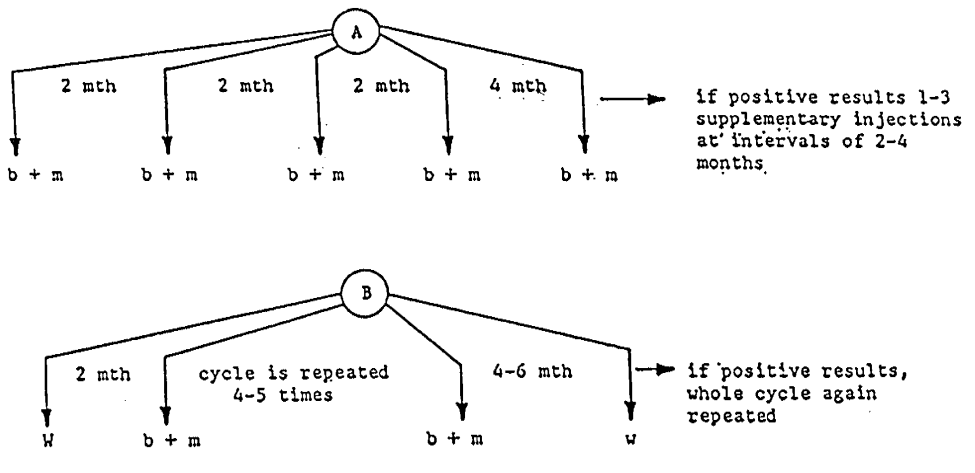
FIGURE 2.
PROTOCOLS FOR THE
INJECTION OF THE WELLS WITH BACTERIAL INOCULUM AND
NUTRIENT SUPPORT OF MOLASSES

WELL INJECTION TECHNOLOGY	NO. & INTERVALS BETWEEN INJECTIONS
Scheme A 	Initially 4 injections with bacterial inoculum and nutrient support at intervals of 2 months, followed by a 5th injection after a further interval of 4 months. Afterwards, 1 - 3 supplementary injections of 4-6 months, were made only when positive results were obtained.
Scheme B 	Injection well kept closed for 1-2 months after_bacteria + molasses injection. Water injection for 1-2 months, and this cycle was repeated 5 times. If positive results were obtained, this whole cycle was repeated.

⊙ = Well for injection of bacterial inoculum + molasses
⊕ = Wells for alternative injections of water and bacterial inoculum + molasses
* = Reaction wells

The Baicoi, Vata, and Suta Seaca reservoirs were treated with supplementary injections by the following protocol. (Figure 3).

FIGURE 3.



Legend
mth = month
w = water injection
b+m = bacterial inoculum and molasses injection

The injection wells, both from Baicoi and Vata, as well as for the other five reservoirs, were put into operation about 6 months from the last injection with bacteria and molasses. Although these wells were out of operation for about one year, no increased oil production was recorded when they were started. It is reported that plugging may have occurred due to the repeated bacteria and molasses injection without also flooding with a large quantity of fresh water. Chemical treatment to restore the formation permeability of the injection wells could have been helpful.

After finishing the cycle of bacteria and molasses injections, it was established that some geological and technical deficiences made the Beciu and Moreni reservoirs inadequate for microbiological treatment. For this reason, it was considered that positive results were obtained in two of the five (not from seven) reservoirs subjected to the microbial treatment.

Response:

Microbiological and physicochemical analysis of the fluids from the wells were usually made at 3 month intervals on the seven reservoirs from 1976-83.

It was found that the injected aerobic, facultative anaerobic or anaerobic heterotrophic bacteria, and the natural reservoir bacteria increased in number and the injected bacteria were transported toward the reaction wells. The counts decreased after 2 years to the initial value or less. The natural reservoir bacteria decreased less after 2 yrs. than the injected bacteria. It was concluded the bacteria involved in acid and gas production were dependent on the molasses and the injected amount of molasses was not able to support growth longer than 2-3 years.

The three reservoirs which responded to the microbial treatment were the Baicoi, Vata, and sporadically the Suta. As a result of bacterial activity in the reservoir, the physicochemical changes were measured every 3 months for 27 wells. The average values (Table 2) for the two responding reservoirs (Baicoi and Vata) show that the oil density and oil viscosity increased and the Engler distillation indicated the light fractions increased in the oil. However, the lack of correlation between these factors could not be explained.

TABLE 2

THE AVERAGE VALUES OF SOME PHYSICO-CHEMICAL CHARACTERISTICS OF RESERVOIRS WHICH RESPOND POSITIVELY TO MICROBIOLOGICAL TREATMENT

(BAICOI AND VATA RESERVOIRS)

PERIOD	OIL DENSITY AT 30°C (Kgf/dm^3)	OIL VISCOSITY AT 30° C (c ST)	ENGLER DISTILLATION INITIAL	ENGLER DISTILLATION FINAL (70%)
		BAICOI		
Before treatment	0.870	6.61	65	109
After treatment (1977-1981)	0.875	6.68	98	125
		VATA		
Before treatment	0.924	99.39	298	362
After treatment (1978-1983)	0.925	103.15	305	365
Tendency	Increasing 0.12%	Increasing slightly	Increasing 0.60%	Increasing 0.13%

THE AVERAGE OF SOME MODIFIED PHYSICO-CHEMICAL CHARACTERISTICS OF THE CO-PRODUCED WATER FROM REACTION WELLS

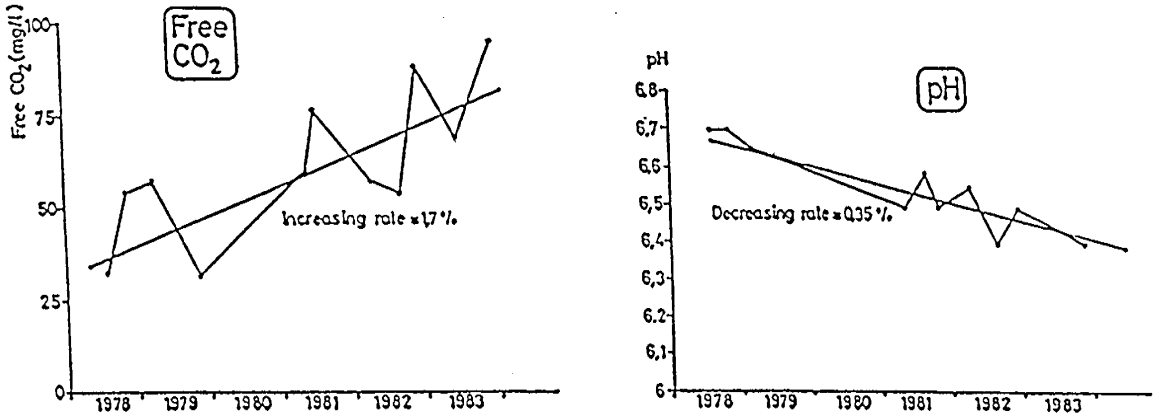
PERIOD	NaCl (g/l)	SO_4 (mg/l)	HCO_3 (mg/l)	Fe_2O_3+ Al_2O_3 (mg/l)	Total M* (g/l)	Iodine (mg/l)	Free CO_2 (mg/l)	pH
				BAICOI RESERVOIR				
Before treatment	65.2	3.29	1450	75.0	64.5	5.40	127	6.8
After treatment (1977-1981)	66.5	2.58	1400	66.0	66.5	6.66	331	6.4
				VATA RESERVOIR				
Before treatment	74.3	4.46	940.0	190.0	74.6	19.9	49	6.62
After treatment (1978-1983)	77.0	4.13	936.3	180.7	78.2	21.7	62	6.45
Tendency	Inc. 0.10%	Dec. 0.01%	Dec. 0.82%	Dec. 1.70%	Inc. 0.08%	Inc. 0.10%	Inc. 1.70%	Dec. 0.35%

* - Mineralization Inc. - Increasing Dec. - Decreasing

The average changes observed during the test period in the co-produced water are shown in the Baicoi and Vata reservoirs. The most notable changes were observed in the free CO_2 and pH values. (Figure 4).

FIGURE 4.

CO_2 AND pH CHANGES OBSERVED IN THE RESERVOIR WATERS



Oil Production Analysis

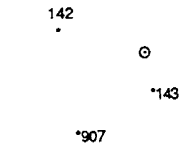
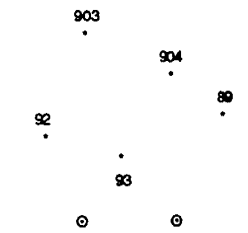
Oil production was measured in 24 wells in the 7 injected reservoirs before and after the treatment. Positive and significant results were reported for only the Baicoi and Vata reservoirs (Table 3). The oil production was not significantly changed in the other reservoirs (Table 4).

The effect of the microbial treatment occurred in the Baicoi reservoir 4-6 months after bacterial injection while the Vata reservoir responded in 7-8 months due to the greater distances between wells.

In some wells, decreases in oil production occurred which were greater than expected. The decrease in production was unexplained and the influence of the microbes was unknown. The situation was viewed as being very complex. This complexity can be seen in Baicoi well I42, at Vata well 1868, Tintea well 85, and Bragadiru well 263. At these locations, bacteria could not spread; probably due to some geological characteristics of the reservoirs. Data reported and estimates using oil decline curves for wells in the Baicoi and Vata reservoirs are shown. (Figures 5-11).

TABLE 3

SIGNIFICANT OIL PRODUCTION OF SOME RESERVOIRS
SUBMITTED TO MICROBIOLOGICAL TREATMENT 1977 - 1983

	Well #	Oil production (Tons/Day) Before treatment	Oil production (Tons/Day) After treatment	Period Increased oil production reported (Tons)
BAICOI RESERVOIR				
	142	1.0 (0.8)	0.8 (0.7)	1977-1983
	143	1.0 (1.0)	1.9 (1.6)	+ 2633
	907	0.6 (0.4)	1.0 (0.6)	
VATA RESERVOIR				
	1016	1.6 (0.5)	2.0 (0.5)	
	1019	1.2 (1.5)	1.7 (1.6)	
	1393	2.5 (1.4)	2.8 (1.6)	1979-1983
	1863	0.2 (0.7)	0.5 (0.8)	+ 3201
	1864	3.0 (1.2)	3.8 (1.4)	
	1869	8.8 (7.1)	10.2 (8.0)	
	1868	3.5 (1.9)	1.9 (1.5)	
SUTA SECA RESERVOIR				
	89	1.0	0.8	
	92	1.0	1.9	1978-1981
	93	0.5	0.5	+ 288
	903	1.0	0.6	
	904	0.5	0.6	

Values in ( ) for the period 1981-1983 =Injection well *=Possible reaction wells

TABLE 4

NON-SIGNIFICANT OIL PRODUCTION OF SOME RESERVOIRS SUBMITTED TO MICROBILOGICAL TREATMENT 1977 - 1982

	Well #	Oil production (Tons/Day) Before treatment	After treatment	Period Oil production reported (Tons)
TINTEA RESERVOIR				
85 ⊙ 553 555	85	1.0	0.7	1977-1980
	553	0.5	0.6	-
	555	1.4	1.6	
MORENI RESERVOIR				
367 363 366 ⊙	366	0.3	0.4	1979-1982
	363	0.8	0.6	-
	367	1.0	1.1	
BRAGADIRU RESERVOIR				
⊙ 263 481 482	263	5.0	3.9	1977-1980
	481	1.0	1.1	-
	482	0.8	0.8	

⊙ = Injection well * = Possible reaction well

Conclusions

Estimated oil decline curves and analyses of each reservoir and well established that the increased oil production dropped to or below the pre-treatment level if no more bacteria and molasses were injected. Two of the 7 reservoirs were incompatable with the bacteria treatment and should not have been tested. A significant amount of increased oil production was observed from 2 of the other 5 reservoirs injected and increased production was maintained for periods of 7-8 months to 5 years. Increased oil production was considered to be dependent on maintaining the nutrient addition. The injected bacteria spread to the production wells and were found for 2 years. The increases of free CO_2, lower pH, and changes in the mineralization of the produced water indicated microbial action in the reservoir. The reservoirs that had an oil viscosity below l0 cP responded best to the microbial treatment.

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CANADA TEST I

Bioplug Well Test

Nova/Husky Operations Ltd.

Year of Test: 1985-86

Location: Lloydminster field, Canada

Geological Data: The Lloydminster field produces from the lower Sparky formation at 650 meters with a 5.4m pay thickness. It is an unconsolidated sand of 1500mD with a 30% porosity and 3957KPa initial pressure. The oil is heavy with a viscosity at 2l°C of 4927mm^2/s. The API gravity is 15° with density at 15°C of 973.8 kg/m^3. Thegas quanitity is very low containing C_1 (0.9436), C_2 (0.0061), C_3 (0.0001), C_4 (0.0007), and C_{5+} (0.0010) with no H_2S or H_2 . The field water is a 6+% brine (89mS conductivity) and 20% saturation. The injection water or the field is fresh (drinking quality).

Microbial Treatment: The microbial treatment was designed to improve the water-oil ratio (WOR) on primary production through the selective plugging of water zones. The proposed microbial mechanism to achive the results was by production of insoluble polymer. A well was injected on June 5, 1985 with 2000 liters of a *Leuconostoc mesenteroides* culture, which was a "special" facultative strain that tolerates oxygen. After inoculation, the well was treated with a single injection of 11.6m^3 of sugar beet molasses/sucrose in 120m^3 of water. Due to field problems (rain and poor roads), the dilute nutrient was left in the well for 5 days at end of the injection sequence and the subsequent bacterial growth sealed the formation face. The well was reperforated without difficulty. The well was shut in for 10 weeks.

Response: The bacterial counts were reported as -- aerobes 10^2 -10^3/ml and anaerobic counts were 10^4/ml (50% slime formers). The microbial products monitered showed fatty acids (lactic, acetic and pyruvic), and alcohols (ethonol and propanol). No sugar was evident. There was no evidence of sulfate reducing bacteria. The pH of the fluid decreased from 6.3± 0.l to 6.0+ 0.1 with surface tension decreasing from 66 to 59 dynes/cm. There was no change in pressure or gas or oil composition.

The fermentation products detected in the bailed water showed the well bore population was different from the inoculum or was a mixed population related to populations deeper in formation. No permanent formation damage was evident and good fluid flows were obtained at high pump rates after reperforation. However, it was recommended that well bore damage be prevented by either moving the nutrients deeper into formation or by limiting growth in the well bore by other means.

References: Personal communication.

FIGURE 5

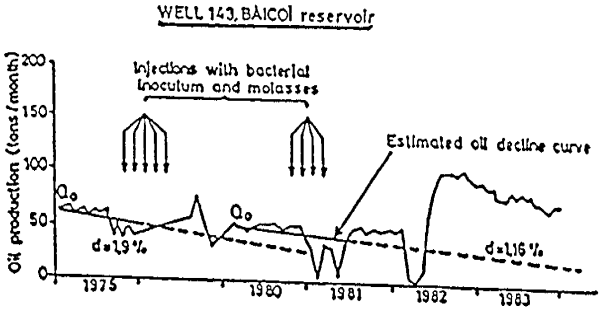


FIGURE 6

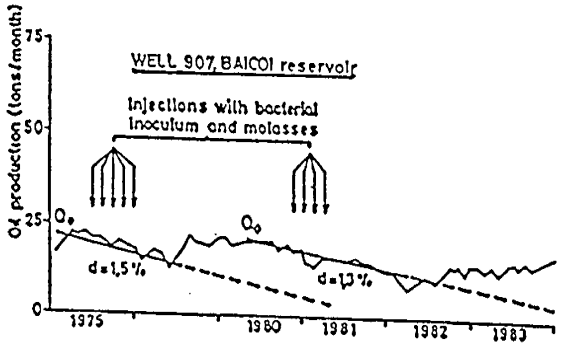


FIGURE 7

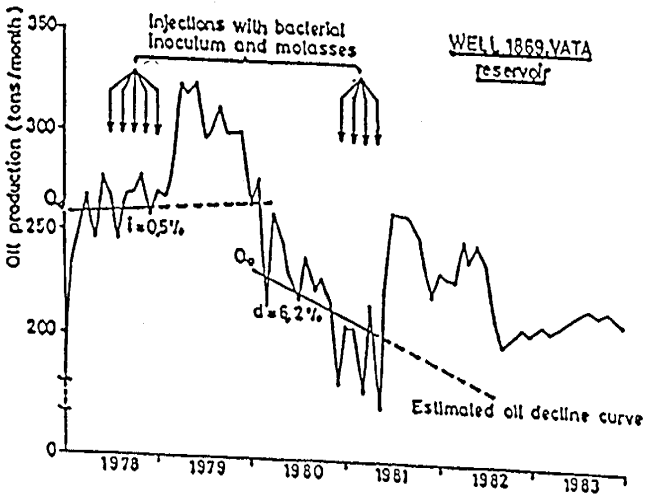


FIGURE 8

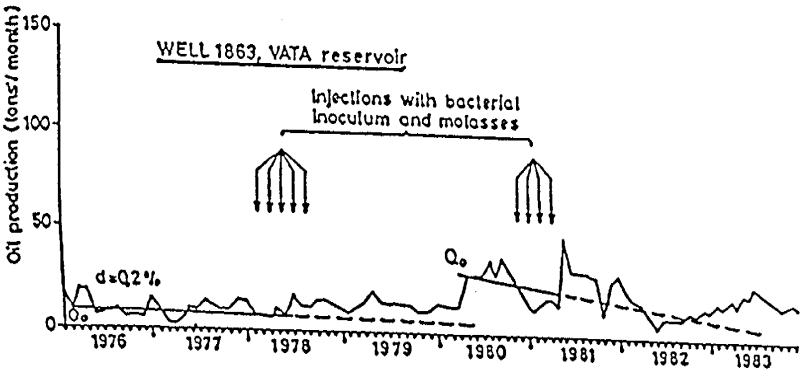


FIGURE 9

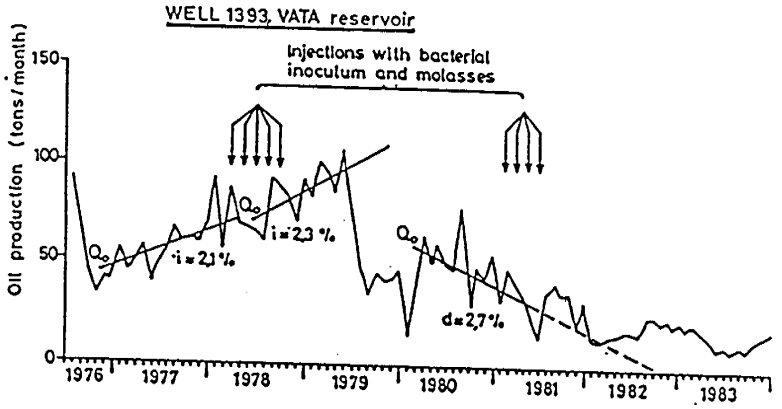


FIGURE 10

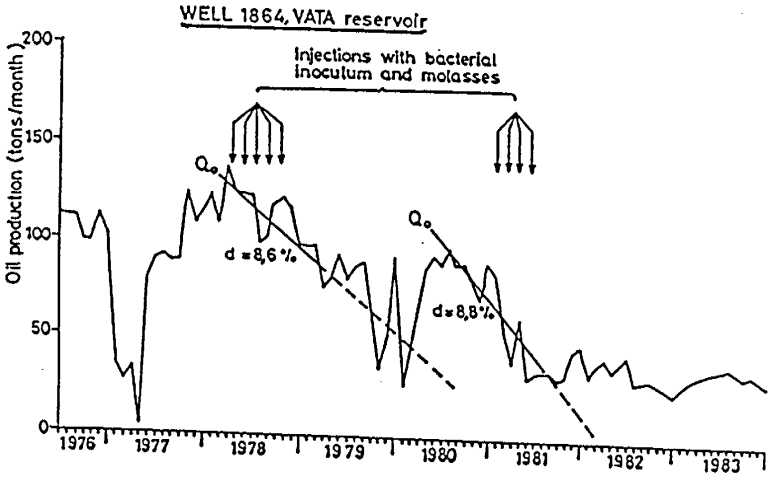
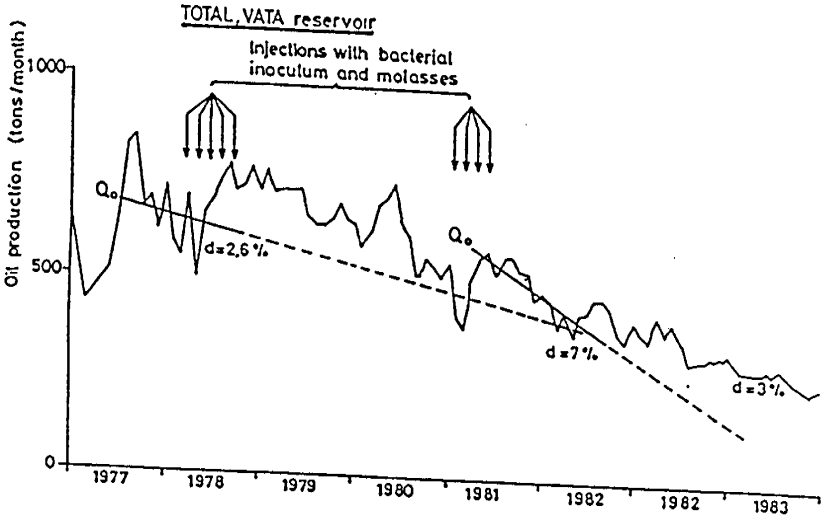


FIGURE 11



U.S.A. 1

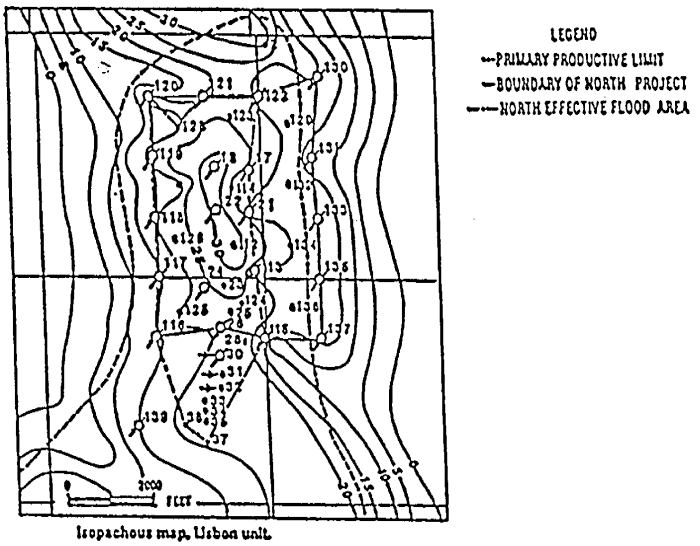
Mobil Field Test

Year of Test: 1954

Location: Lisbon Unit, Union Co., Arkansas.

Geological Data: Production from Nacatoch sand; a loosely consolidated sand with a carbonate content ranging from 1-16% over the field and 8% in the test area. Structure of Lisbon field believed to be a terrace on a continuation of the El Dorado anticline. Subsea depth to top of Nacatoch averages 1920 ft. Net pay thickness 0-30 ft. (averaging 11.3 ft.). Test area located between 20-25 foot contours on isopach map (Figure 1). Bottom hole temperature was 90-l05 degrees F.

FIGURE 1



Isopachous map, Lisbon unit.

Data on adjacent well cores from wells 112 through 137 are:

Residual oil saturation, rotary cores percent pore space	4.5
Residual oil saturation, cable tool cores,percent pore space	8.5
Interstitial water saturation, rotarycores, percent pore sp	77.3
Interstitial water saturation, cable toolcores, percent pore spa	72.3
Porosity, percent	30.5
Permeability to air, mD	408
Permeability range, mD	1-5770

The test was conducted on a 2 spot pattern with injection well 30 being 400 ft. from producing well 31 and 665 ft. from producing well 32.

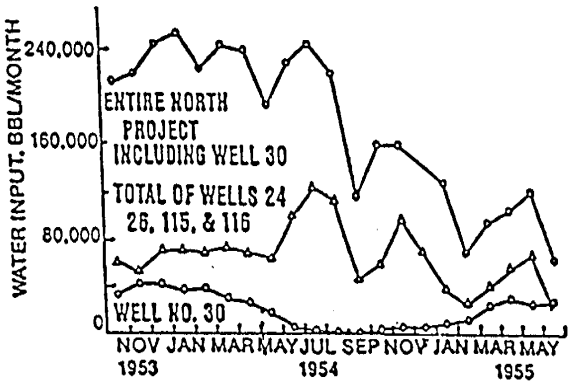
Prior Production History

The Lisbon field was discovered in l925 and covers approximately 4400 acres. Waterflood operations started in 1949 and the area surrounding the test wells was almost completely flooded out prior to this test. The reservoir properties at the beginning of the waterflood were:

Estimated oil formation volume factor,reservoir bbl/stock tank bbl	1.00
Formation oil viscosity at 100°F, cp	4.48
Formation oil gravity at 60°F, °API	36
Fresh injection water viscosity at 100°F, cp	0.59
Formation water salinity, ppm NaCl	42,000

Water movement was in a southward direction and the rate of flow between wells 30 and 3l was determined by field flood rather then by injection in well 30. Plots of total north section water injection, injection into the low wells immediately north of well 30, and into well 30 itself are shown in Figure 2.

FIGURE 2



Lisbon unit water injection.

Microbial Well Treatment

The field test was begun in May, l954 with the injection of fresh water (less than 200 ppm sodium chloride) into Well 30. Previously, a mixture of produced and fresh waters (20,000-25,000 ppm sodium chloride) had been used for flooding. Two months later, beet molasses was added to the injection water first in a 60 bbl slug and then in amounts necessary to maintain a two percent concentration. This mixture was injected continuously for 5 1/2 months. At first, the injection mixture was allowed to flow into the well under gravity at a rate of approximately l00 bbl/day.

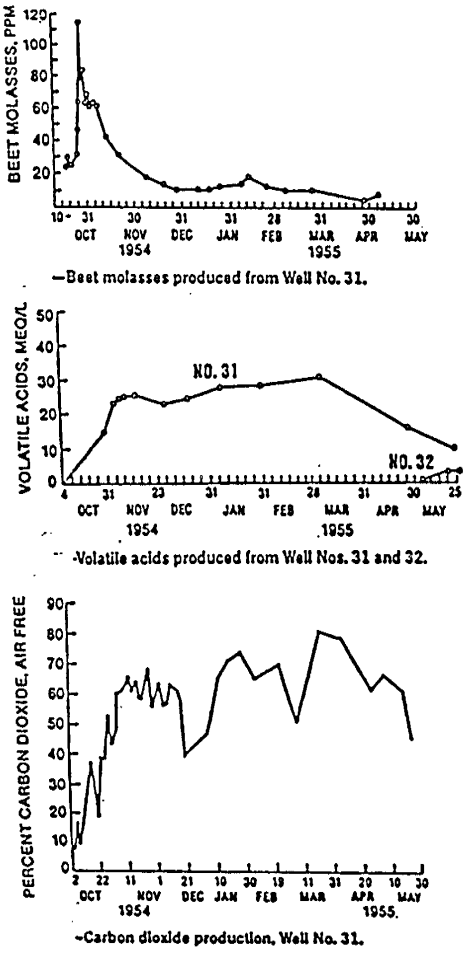
Later, because of a reduction in injectivity due to either gas or bacterial cells, it became necessary to install an injection pump in order to maintain rates of 100 to 500 bbl/day.

Bacterial inoculations were begun simultaneously with the addition of molasses. Approximately 4000 gal. of heavy *Clostridium acetobutylicum* suspensions were flooded into the test well in 18 separate batches of 220 gallons each during the four month period (July-November).

Response - Microbiological

Fresh water breakthrough occurred in Well 31 in 70 days when average injection rate was 160 bbl/day in Well 30. Fermentation products and molasses appeared in 80-90 days after the initial inoculation when the average injection rate was 70 bbl/day. The production of sugar, acids, and CO_2 are shown in Figure 3.

FIGURE 3



—Beet molasses produced from Well No. 31.

—Volatile acids produced from Well Nos. 31 and 32.

—Carbon dioxide production, Well No. 31.

After 286 days, fermentation products were recovered from the producing well 665 feet from Well 30 and 265 feet from Well 3I. No producing wells more than I000 feet from Well 30 showed any evidence of bacterial fermentation after 11 months. The principal fermentation products recovered were 7 short-chain fatty acids and CO_2. The observed and calculated amount of products produced during the test were compared to the expected products. (Table 1).

TABLE 1.

Products	Products of fermentation Comparsion lbs of products produced	Calculated % of sugar injected	Expected % of sugar yield
7 shot chain fatty acids (formic through caproic acids)	77,000	59%	
formic, acetic, butyric			35 - 40%
Alcohols (ethyl & butnal)	small	-	
acetone	small	-	
CO_2	25,000 (200,000 cu ft)	I9%	47%
H_2		-	2%
CH_4	I8-6I% of gas produced	?	0
Cell biomass	?	?	-

The CO_2 content of the gas produced varied between 39 - 82% (air free) from Well 3I while wells not effected had ranges of 0.3-8.8%. The remainder of the gas from Well 3I was methane with little or no hydrogen detected as had been expected.

It was believed that the products observed were produced by a mixed culture fermentation. Although many types of bacteria were isolated erratically and in small numbers from the produced water, cells of *Clostridium acetobutylicum* were never isolated. Laboratory tests had shown that the fermentation products observed could be produced under simulated field conditions in sand packs. Analysis of the produced gas indicated that 20% of the carbon was derived from a modern carbon source (molasses). Evidently, some of the CO_2 and H_2 was converted to methane. It was also

shown that 20% of the produced CO_2 was ancient carbon; presumably resulting from acid attack on reservoir carbonate. No changes in the oil were observed.

Production Rate

The production data for Well 31 is shown from the time it was opened on Oct. I, I953 until its abandonment on May 27, I955. (Figure 4).

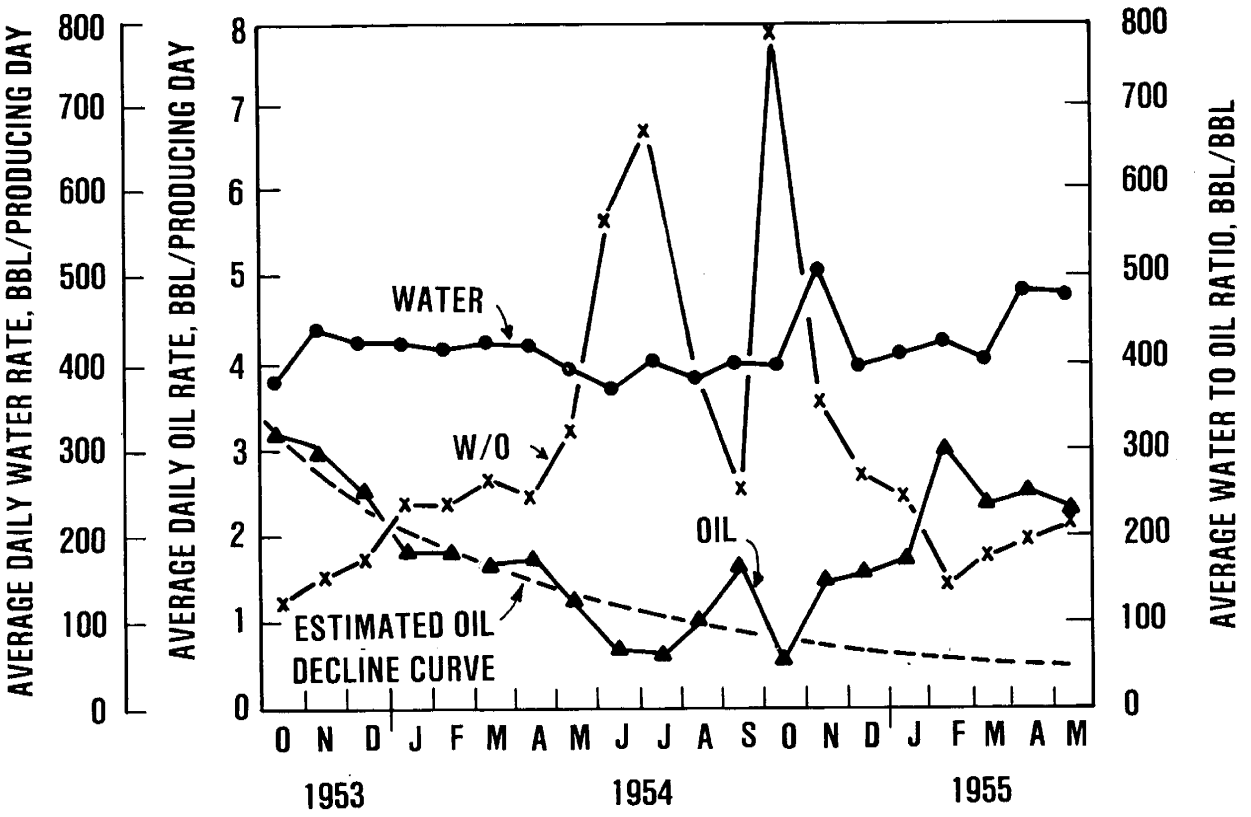


FIGURE 4. - Average daily production per producing day, Lisbon Well No. 31.

A statistically significant increase in oil production occurred after October 1954. (Figure 5).

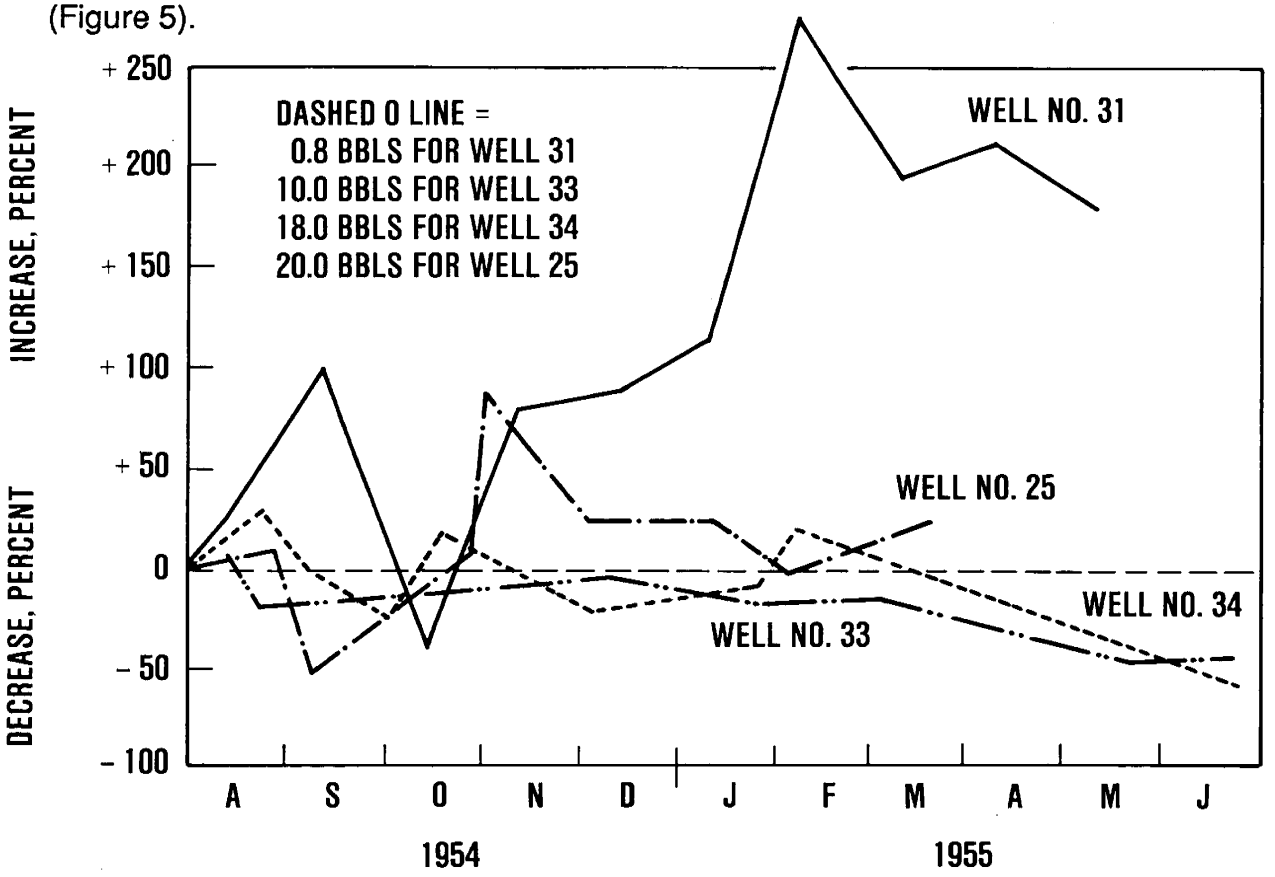


FIGURE 5. - Percent change in oil production from August 1, 1954.

Oil production from Well 31 had decreased to small amounts as a result of extensive previous waterflooding. However, oil production increased from 0.6 bbl/day (Nov. 1954) to 2.1 bbl/day in May 1955. The actual rate was 3.5 times the normal production rate (250% increase). A comparison of nearby producing wells indicated that the percentage increase in Well 31 differ significantly from the 3 adjacent wells. The test was stopped after 8+ months due to the low cost of oil.

Remarks

The Mobil test successfully demonstrated an underground fermentation. The mechanism for the increased oil production was unknown. It was assumed that H_2 was produced, but it was not detected. Free H_2 could have resisted fluid displacement and acted as an effective blocking agent, which would force water into new flow patterns. The CO_2 that was produced by fermentation and from the neutralization of fermentation acids by reservoir carbonates may have exerted beneficial effects in the reservoir. It

was unlikely CO_2 contributed to a free gas phase. The high yields of acids may have reacted with the carbonates and, combined with the production of CO_2 at the wetted surfaces, caused appreciable effects on flood patterns and total recovery.

Reference:

Yarbrough, H.F. and Coty, V.F. (1983). Microbially Enhanced OilRecovery from the Upper Cretaceous Nacatoch Formation, Union County, Arkansas. International Conference on Microbial Enhancement of Oil Recovery, May 16-2l, 1982. Shangri-La, Afton, Okla. Conf. 8205140. pps. 149-153.

U.S.A. V

Oklahoma State University Field Tests

Year of Test: 1983
Location: Okmulgee, Okla.
Geologial Data: Sandstone reservoir, gas drive
Depth: 1750 ft.
Prior production history: Well first opened in 1981 and produced 10 bbl/day. By 1983 production was 1 bbl/day.

Microbial Well Treatment:

First Test

Clostridium cells were grown in a 50 gal. fermentor and allowed to sporulate (viable spore concentration was 10^{15} units/ml). Spores and 80 bbl of 8.6% molasses (not beet) were injected and the well was shut in for 30 days.

Response: Produced water showed presence of ethanol, acetone, n-butanol, and CO_2.

Production response - After opening the well, the 7th bbl. produced appeared to be pure oil with an emulsifier. Oil increased in viscosity indicating slight oxidation and loss of dissolved CO_2.

Second test

The same well was inoculated in Sept. with a spore suspension prepared as previously. The inoculum consisted of 2 strains (HR-69E and HR-51B) chosen for their ability to produce large amounts of CO_2 and solvents (n-butanol) and emulsifier (strain HR-69E). The injection medium was 160 short bbl of 4% beet molasses in a 1-4 dilution of Dutcher salt water (salinity = 3% Na Cl) with fresh water. The medium also contained 0.09% dry milk solids. Forty liters of spore suspension (2.6×10^{15} spores/ml) was injected followed by 40 liters of molasses - milk medium. The remainder of the medium (160 bbl) was introduced into the casing without pressure at a rate of 3 short bbl/min. until the casing was full. The rate of injection was slowed to 1.5 bbl/min. until 160 bbl was injected. The system was closed for 32 days (opened Oct. 25, 1983).

Microbial response - The gas pressure and gas composition was measured weekly for 4 weeks (Table 1).

TABLE I.

Reservoir Gas Pressure and Composition
During Field Test Near Okmulgee, Oklahoma
September - October 1983

Total gas pressure		Gas composition post-inoculation		
Weeks post-inoc	psi	gas	2 weeks percent	3 weeks percent
1	55	O_2	10.3	
2	100	N_2	49.0	
3	15	CH_4	36.8	21.5
4	80	CO_2	4.0	4.9
		H_2S		0
		H_2		0

Data on the observed solvent production is shown in Table 2.

TABLE 2.

Observed Solvent Production

Solvents	Solvents present in in Molasses from Well	% Solvent* Content of Recovered Inoculum Time After Opening	
	Percent	2 weeks	3 weeks
ethanol	60-90	80	40.7
2-propanol	2-6		
1-propanol	3-4	4.7	1.7
2 butanol	0.5-2		1.2
1 butanol	trace	15.3	5.4
acetone	trace		
methylethylketone	trace		
methanol	trace		
isobutanol	-		6.9
tertiary butanol	-		0.6
isoamyl alcohol	-		5.8
tertiary amyl alcohol	-		4.7
n-amyl alcohol	-		32.8

*Percent of each substance of the total solvent content of the sample.

The pH before the test was 6.0 while the pH of the molasses - oil mix recovered was 4.0. The only acids identified (Table 3) were either acetic or isobutyric, neither of which were present prior to inoculation.

TABLE 3.

Organic Acid Production from Well

Sample Number	Percent of total acids	
	Acetic	Isobutyric
1	61.6	38.4
2	17.8	82.3
3	21.6	78.4
4	20.6	79.4
5	17.3	82.7

These acids were not present in the laboratory tests so they were assumed to result from changes in the metabolic pathways in the reservoir environment. The microbial count 2 weeks after inoculation were $1x10^7$ viable cells/ml.

Oil Production:

The well was shut in for 32 days and then the gas released for 2 hours during pumping off the well. The oil production before the test was I bbl/day. Following the shut in period, the well: produced 2 bbl/day for more than a 30 day period. Two months following inoculation (Dec. I), the well produced little molasses and then only after being pumped 2 hours or more. The test was stopped because the lease was sold.

Remarks:

The test was considered a success but the mechanism was unknown. The microbial activity continued for 6+ weeks with the production of solvents, CO_2 , and acids being considered as playing a role in the oil recovery. All the CO_2 observed was considered biogenic and was attributed to the metabolic activity of microorganisms but the role of the *Clostridium* was unknown.

Reference:

Grula, E.A., Russell, H.H., and Grula, M.M. (1985). Field Trials in Central Oklahoma Using *Clostridium* Strains for Microbially Enhanced Oil Recovery. Microbes and Oil Recovery (Vol. 1). International Bioresources Journal, pps. 144-150.

Micro - Bac Gulf Coast Inc.
Paraffin Removal Treatment

Year of test: 1985-86.
Location : Little River Oil and Gas Co. Milam County Rice Cemetary #l and 2 - Mineiuz-Rockdale field
Geological data: Production is from the Navajo "A" formation - fine silty sandy shale - highly lamanated from sandy shale to shaley sand.

Depth - #l Rice casing to 1022 ft. - l l/l6" perforations 922-935 ft.
#2 Rice casing to 1007 ft. - 1 1/16 perforations 908-919 ft.
and 923-928 ft.

Bottom hole temperature ± 90° F.

Prior Production History: The #1 Rice was drilled in June 1983 and the #2 Rice drilled in Jan. 1984 with production being 14 and 17 BOPD. After first year decline, the production was ± 4.8 BOPD. Oil production at time of microbial treatment was ±1 to 1.5 BOPD. Water production was less than 5%. Wellhead pressure was unknown, but 0 psig at start of treatment. The wells experienced severe paraffin and formation skin problems which were treated by pulling and steam cleaning the wells.

Microbial treatment: The wells were treated by the Micro-Bac process which consisted of injecting a proprietary Para-Bac formulation. The formulation is a liquid culture of mixed marine source microorganisms in a saline solution of nutrients for use in crude oil to control the deposition of paraffin. Each well was treated with a total of 99 gallons introduced at intervals.

Response: The production from the wells increased from about 1.5 BOPD before the treatment (11-20-85) to 4 BOPD (l-10-86). (Figure 1). As important as the increase in production was the observation that no paraffin was visible after the treatment. Prior to this treatment, the wells required servicing every month as production declined from 1.5 to 0.5 BOPD. The test was considered a successful demonstration of paraffin treatment by microorganisms.

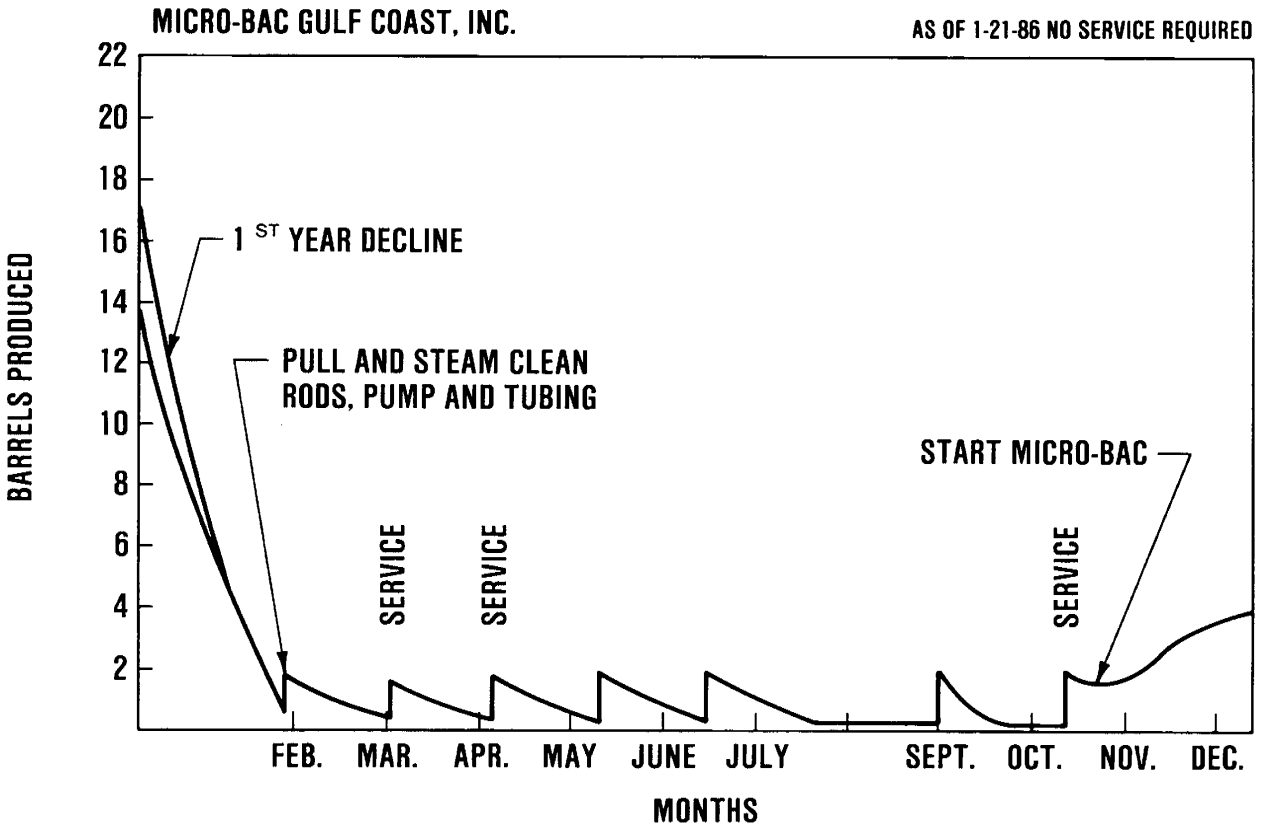


FIGURE 1

References: Personal communication.

U.S.A. VII

Covington Leases

Year of Test: 1985

Location: Jack County, Texas

Geological Data: Wells were cased and completed without packers in the Gunsight formation and are 1-2 years old.

		Well Data	
Well	Depth ft.	Perforated zone	Pay zone
Covington 18	442	6.5	18
Covington 22	405	5.5	16
Covington 24	481	9.0	12

Two wells on the Ellison lease (Ellison 2 and 4) were used as control wells. The characteristics of the lease fluids were: (Table 1).

TABLE 1.
Composition of Waters and Produced Oil Analysis

	Injection	Produced
specific gravity	0.990	0.992
pH	8.3	8.2
Resistivity (ohms/m^2/m)	0.03	0.02
temperature (°F)	75	75
HCO_3	529	783
Cl	1200	2200
SO_4	219	0
Ca	109	100
Mg	265	133
Fe	10	15
Na	475	1360
TDS	2820	4610
API gravity at 60°F		36
Paraffin wax		1.46%
Asphaltene		0.74%

Microbial Well Treatment: The 3 wells were injected with *Clostridium* spore suspensions (SEG-3) and a nutrient mixture composed of: (Table 2).

TABLE 2.

Inocula and Nutrient Addition

Well	Vol(l)	Spores/ml	Date prepared	Date injected
Covington 18	210	$3.1x10^{13}$	2/28-3/14	3/9/85
Covington 22	210	$2.3x10^{12}$	2/22-2/26	3/7/85
Covington 24	157	$1x10^{17}$	3/4-3/7	3/9/85

Nutrient Mixture

Injection water	100 bbl
Sucrose (table sugar)	1000 lbs
Beet molasses	25 gal
Powdered milk solids	25 lbs
30% NH_4NO_3 fertilizer	33 lbs

The injection protocol for the wells was: (Table 3).

TABLE 3.

	Amount Injected (gal)		
	Cov. 18	Cov.22	Cov.24
injection water spacer	889	602	280
bacterial spores	55.5	55.5	41.5
nutrient mixture	3850	4195	3654
water displacement	1196	2005	0

The 3 test wells and the 2 control wells (Ellison 2 and 4) were then shut in for approximately 3 months.

Microbial Response:

The microbial counts made before the test (Table 4) were:

TABLE 4.

Pretreatment Bacterial Counts

Well	Aerobic	Anaerobic
Covington 18	$5.9x10^4$	$4.5x10^4$
Covington 22	$1.3x10^2$	$2x10^2$
Covington 24	$3x10^4$	$5.3x10^4$
Nutrient mixture	$1.9x10^5$	$1x10^6$

These pre-treatment counts could be compared to counts made after the treatment at the Covington 24 well (Table 5).

TABLE 5.

Bacterial Counts on Covington 24 After Treatment

Times (hrs)	Post opening pumping Bacteria/mlx10^6	Description
1	0.2	SEG-3
	0.5	Gram + facultative yellow
	2.0	Gram - facultative cream
2	0.01	SEG-3
	0.04	Gram + facultative white
	0.01	Gram - facultative cream
4	0.5	SEG-3
	0.03	Gram + facultative white
6	2.0	SEG-3
	0.02	Clostridium - lavender
	0.8	Gram + facultative white

On June 25, 1985 bacterial counts on the Covington 24 were $3.23x10^7$/ml while the Covington 22 had $1.06x10^8$/ml.

The production of solvents was measured on the Covington 24 at two intervals (Table 5).

TABLE 5.

Solvent production on Covington 24

Solvent	After 6 hr production ($x10^{-4}$M)	after 18 days production ($x10^{-3}$M)
ethanol	-	3.3
isopropanol	-	2.6
n-butanol	2.5	2.0
isobutanol	-	1.0
Acetone	0.37	-

No detectable solvents were observed in the Covington 18 at 60 days.

Production response: Graphs of the Covington and Ellison tank battery data are compared in Figure 1. They concluded the Covington 24 showed a slight increase in oil production, while the Covington 22 production increased and then leveled off. The Ellison 2 and 4 control wells produced negligible amounts of oil.

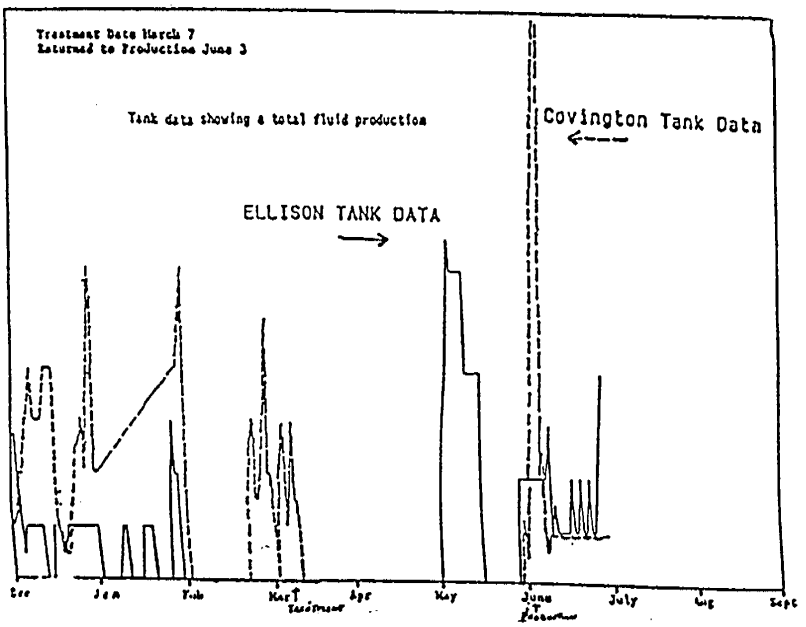


FIGURE 1.

References: Personal communication.

Single Well Stimulation Test
Microbial Systems Corp.

Year of Test: 1984

Location: Steel Lease - Washington Co., Okla.

Geological Data: The Steel #7C-239 well on the Steel lease operated by D & J Enterprises produces from the Peru sandstone formation at 700 ft. There are 75 producing acres of which 10 were in the test area. (10 acre spacing). The produced oil viscosity was 34° API with negligible (below 5 psi) shut in gas. The salinity of the field water was 11%.

Microbial Well Treatment: The well was treated on Oct. 17, 1984 with a proprietary, mixed anaerobic microbial culture followed by molasses. The well was shut in for 3 months after which the well was pumped.

Response: The wellhead pressure increased from 5 to 36 psi at the end of the shut-in period. The measured production increase observed was:

Production	Before test	After test
Avg. Prod. (BOPD) per well	0.5	2
Avg. Prod. (BWPD) per well	13	40

The oil increased 230% of the baseline value for 7 months (including 3 month shut-in time) with no observed changes in its composition. The water increased 300% of the baseline value then gradually decreased. There was no indication of sulfate reducing bacteria activity.

References: Personal communication.

U.S.A. IX

Single Well Stimulation Test
Fairleigh Dickinson Laboratories Inc.
Conners Project

Year of Test: 1986
Location: Gailjo field, Texas
Geological Data: The formation treated was the Flippin sand, which is a limey sand, in the Gailjo field. The average well depth was 2,550 ft. and the oil viscosity was 40° API with 21% gas.

Microbial Treatment: One well was treated on Dec. 18, 1986 with anaerobic and facultative cultures for the purpose of microbial polymer production. The nutrient addition was 75 gallons of a 5% molasses and liquid fertilizer (32-0-0) mixture.

Response: At the end of 2 months the microbial numbers were reported as $1x10^5$ to $1x10^8$/ml.
The test ended due to operator leaving.

Reference: Personal communication.

U.S.A. X

Single Well Stimulation Test
Fairleigh Dickinson Laboratories Inc.
Vernor Project

Year of Test: 1987
Location: Edwards Co., Texas
Geological data: The Wildcat field in Edwards County, Texas covers 3000 producing acres and is a sandstone reservoir at 350 ft. with a 20° API gravity oil and negligible quantity of gas. The salinity of the water is >800 ppm.

Microbial Treatment: The proposed mechanisms to release the oil was by microbial gas and solvent production. Three wells were injected on Feb. 13, 1987 with anaerobic and facultative anaerobic cultures and with 75 bbl of 10% molasses. The wells were shut in for 3 weeks.

Response: Average production before the test was 0.25-0.5 BOPD. A slight increase in oil production occurred after the test. Changes were noted in the pH and pressure and water production increased. The test was stopped due to lack of funds.

Reference: Personal communication.

U.S.A. XI

Berry Energy and Petroleum Bio-Resources
Westfork Field Test

Year of Test: 1983-84
Location: Washington Co., Colorado
Geological Data: The test was in the J sand of the Westfork field at a depth of 5200 ft..

Microbial Treatment: The reservoir was preconditioned for good distribution of the microorganisms and nutrients and to avoid plugging. The injected inoculum consisted of a surfactant-cosurfactant gas- producing culture together with a polymer-polysaccharide gas-producing culture. The culture was grown as a 6-10,000 gal. inoculum and was transported with 200,000 gal of injection medium to the field where it was diluted with recycle water. The injection period lasted 3 weeks. No further nutrient was added.

Response: The field injection was completed in August 1983 and by December the production increased from 15 BOPD to 40 BOPD. Initially, the production increases were attributed to field practices and changes in the flood pattern rather than to microbial growth. However, production increased rapidly following injection: then decreased rapidly. The production decrease could have resulted from the lack of additional nutrients.

Reference: Personal communication.

U.S.A. XII

Alpha Environmental

Year of Test: 1986-87
Location: Longwood Field - Shreveport, Lousiana
Geological Data: The test were run in the La Vernia field which is a cretaceous sandstone and in the Longwood field which is Goodland limestone.

Microbial Treatment: The cultures used for injection were proprietary and were injected with a proprietary catalytic mixture which released oxygen for aerobic growth. The objectives of these projects were to test the use of mixed, natural populations of hydrophilic oil-degrading microorganisms selected to enhance oil release and to control paraffin during production. The Longwood field has 13 producing wells; of which 3 wells were injected and shut-in for approximately 1 week, then returned to production. The La Vernia well was injected and shut-in and then produced.

Response: In the La Vernia field, the pretest production rate was 4.6 BOPD and the post-production rate was 4.2 BPOD. In one well, the viscosity was changed from 8.6 to 19.4 cp. There was a reduction in BS & W from 7.6 before the test to 2.2% after the microbial treatment.

Reference: DOE / BC-86-/4 Progress Review 48, Sep. 30, 1986.

U.S.A. XIII

NIPER, Microbial Systems, Inc. and INJECTECH, INC.
Mink Unit Waterflood

Year of Test: 1986-87
Location: Nowata Co., Okla.
Geological Data: The 1720 acre Deleware Childers field is a Bartlesville sandstone reservoir with an average depth of 600 ft. The wells were completed open hole, rag packer, and cemented. The test unit consists of 60 acres with 5 acre spacing and has a total of 21 injection wells and 15 producing wells which are flowing and produce a total of 6.5 BOPD. The average

permeability is 63 MD, the average porosity is 20%, and the estimated oil saturation is 30%. In the test pattern, there are 10 producing wells and 4 injection wells with an average injection volume of 40 BWPD at 535 psi.

A map of the Mink Unit test area shows the position of the injection and producing wells and distances between wells in the test area. (Figure 1).

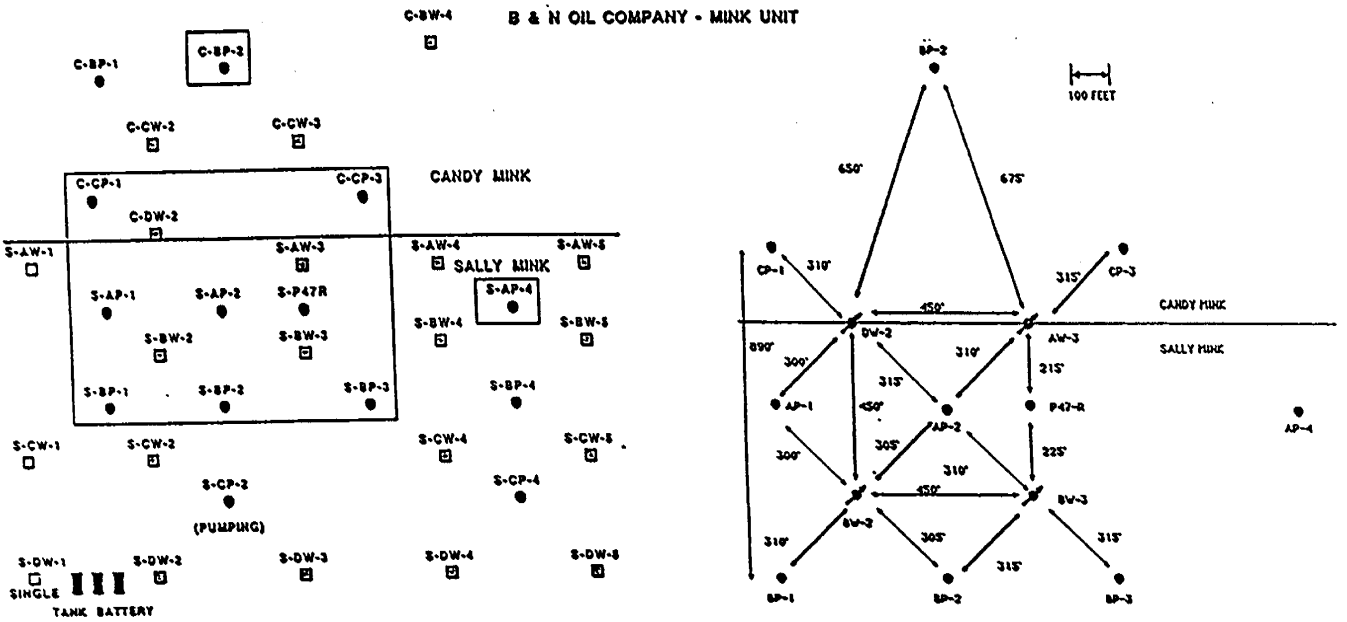


FIGURE 1

Baseline Information: In order to determine the effects of the microbial treatment on the waterflood, the test area was monitored for a period of 5 months (Oct. 28, 1986 through March 3, 1987), to establish a baseline. A comprehensive field sampling program was initiated in Nov. 1986 and was continued to the end of the test. All data were tabulated and graphed, including: a). total oil production (Mink Lease); b). total water production (Mink Lease); c). injection well pressures and fluid rates from each well; d). production well fluid rates and the water oil ratio from each well; e). microbial counts from the producing wells including aerobic counts, anaerobic counts, and SRB counts; and f). physicochemical analyses of the produced fluids and gases. In addition, a series of pretreatment tests were made in the field which included a tracer study to show flow patterns, and a microbial survivability field study to demonstrate that the inoculatated cultures would grow at reservoir conditions.

Oil Production: The Mink unit was selected as the test site because the oil decline curve was well established for the period 1976-86 (Figure 2) and the oil production from the Mink unit was extremely constant during 1985-86 (Figure 3).

FIGURE 2

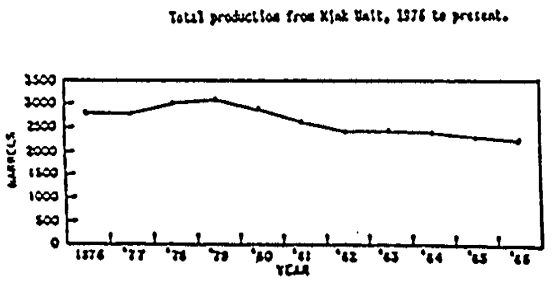
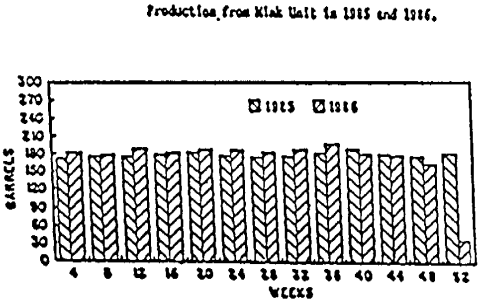


FIGURE 3



The production from Mink unit was 2,300 bbl in 1985 and 2,366 bbl in 1986. The 8 week production average (363 bbl) in 1986 compared to the 8 week production average (345 bbl) in 1987 showed a difference of only 18 bbl. The injection pressure from Dec. 1986 through March 1987 ranged from 525 psi to 555 psi and was very stable.

Microbial Counts: The microbial counts made weekly or bi-weekly over a 20 week period covering 10 producing wells and the injection waters are shown in Table 2.

TABLE 2 .

Average Microbial Counts during Baseline Period

	cfu/ml
Aerobic plate counts	
Injection water	3000-5000
Produced water	0-100
Tank battery water	300-500
Anaerobic plate counts	
Injection water	100-300
Produced water	0-10
Tank battery water	5-50

cfu/ml = colony-forming units per milliliter water.

Sulfate reducing bacteria (SRB) were consistently present in the tank battery water and intermittently present in the plant injection water. There were sporadic occurrences of SRB in the produced waters.

Tracer Studies - Fluorescein Injection: To determine flow patterns of fluids in the Mink Unit a fluorescein tracer test was initiated. Forty-six grams of fluorescein was injected into wells BW2 and BW3 on Jan. 13, 1987. Sampling of each producing well was initiated on a twice-weekly basis. On March 5, 1987, wells DW2 and AW3 were injected with 250 gms of fluorescein and sampled daily for the first 5 days. The data showed every producing well received some fluorescein with wells P47R and AP2 receiving the highest amount.

Microbial Survivability Tests: To determine survivability of cultures in the field, two off-pattern wells (DW-1 and CW2) were each inoculated with 100 liters of culture. Well DW3 was used as a control and no microorganisms were injected. The inoculum were grown in 4% molasses and injected on Feb. 4, 1987. The 4 injection wells were shut-in for 12 days. Backflush samples taken at 10 to 15 minute intervals showed that the cultures survived and multiplied even though the pH decreased to 5. The injection pressures and rates after the microbial treatment were normal and indicated no plugging of either well. No sulfate reducing baceria were detected. The control well remained unchanged. The test showed the inoculum was successfully established in the reservoir.

Psysicochemical Data: Weekly samples from each producing well were analysed for crude oil viscosity, TDS, and pH. Trace mineral ion analyses were performed. Based on the data from Oct. 28, 1986 through March 3, 1987 it was determined the TDS of the produced and injection water remained stable (within 0.01%). The pH remained between 6.4 and 7.0 and the oil viscosity varied no more than 2 centipoises. There were no abnormal concentration of trace minerals.

Microbial Treatment: Extensive laboratory and core flooding tests were conducted to develope cultures which were compatable with the flood waters and which showed the greatest potential for recovering oil. The inoculum was selected to produce acids, gas, and surfactants and consisted of a mixed culture of a *Clostridium*, 2 cultures of *Bacillus*, and a gram-negative facultative organism.

The mixed cultures were grown in 20 liter carboys and 100 liters of this inoculum (containing $1x10^7$ cells/ml) was injected into each of the four injection wells. Two wells (BW2 and DW2) were inoculated on March 19 and 2 wells (BW3 and AW3) were inoculated on March 23. Twenty-five gallons of molasses were injected with the inoculum and all four wells were shut-in until April 3. The wells were backflowed and viable microbial cells were observed. All injection wells were then fed twice weekly with 5 gallons of molasses on a routine basis. The molasses feed rate to each of the 4 injection wells was increased to 2 gallons per day starting in the week of July 14 and is continuing.

Response: Although the test is still in its early stages, preliminary results show that oil production is increasing in the test area (Figure 4).

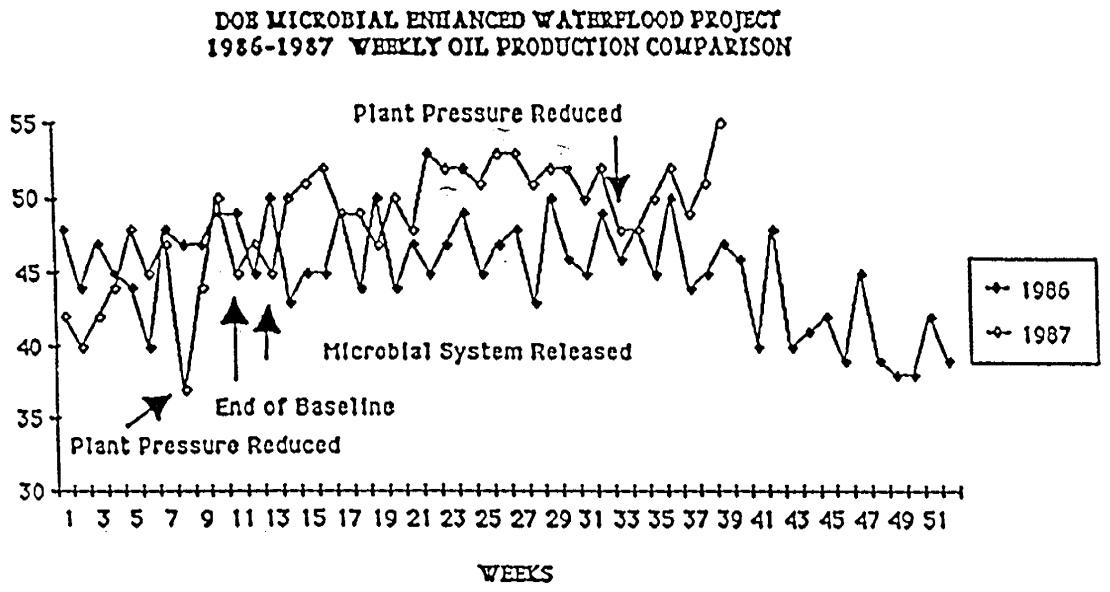


FIGURE 4

The duration and the amount of increased production directly attributable to the microbial treatment will be determined as the project continues to operate.

Reference: Personal communication.

SELECTIVE PLUGGING IN WATERED OUT OIL WELLS

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ABSTRACT

In the early 1980's, NOVA/Husky Research Corporation Ltd. developed a microbial system to selectively plug water bearing zones in heavy oil fields in Western Canada. Water incursion results in uneconomical water cuts and is the usual fate of heavy oil wells on primary production. One field attempt has been made to return a watered out oil well to economic production using this microbial system. A second field test is currently planned for this summer.

The lab development program will be briefly reviewed along with some observations on the first field test. Current restraints and future prospects for this technology will be discussed.

INTRODUCTION

The concept of microbially enhanced oil recovery is not new. In the late 1940's, work done in Zobell's lab led to the suggestion that bacteria could be used to assist in the recovery of oil in-situ (1). In the following thirty years, many field applications were carried out but the organisms used and mechanisms involved were more or less obscure (2,3). In the late 1970's, high oil prices and declining reserves rekindled interest in enhanced oil recovery techniques. As part of this renaissance, a number of research projects were begun more or less simultaneously in several countries.

This paper describes the research project carried out at NOVA/Husky Research Corporation Ltd. from its inception in 1980 to its first field test in 1985. Since much of this work has been previously described in detail, the intention here is to highlight findings which may be of general use to future projects.

TARGET RESERVOIRS

Heavy oil reservoirs around Lloydminster on the Alberta-Saskatchewan border were chosen as the target for our research program. Reservoirs in this region hold sufficient crude oil to warrant a basic research program. Corporate holdings and experience are extensive and current production techniques are limited to a total recovery of less than 8% of the oil in place. Thermal recovery methods and sophisticated EOR schemes are largely thwarted by the thin pay zones (less than 7 meters) typical of the region. There is

a need for low cost ways to increase production from thin pay zones under ambient conditions.

These reservoirs are located in clean unconsolidated sand formations having an average permeability in excess of 1 Darcy. In-situ temperatures are about 22°C. Pressures are modest with an average reservoir depth in the order of 600 meters. The only apparent challenge to microbial growth is the high salinity of the connate brine (6.5-7.0% salinity).

BACTERIA IN PLACE

That these reservoirs were suitable for microbial growth was clearly evident from microbial analyses of core samples taken from a reservoir near Wainwright, Alberta. Core sections were taken on site and handled as aseptically as possible. Growth tests carried out on samples of the pristine material from the center of the core indicated the presence of viable aerobic and anaerobic bacteria. Since these occurred in approximately equal numbers, it seems probable that most of the organisms were actually facultative anaerobes able to grow with or without oxygen. Populations of up to 45,000 per gram of sand were noted in the high permeability sections of the core (4). Obviously, bacterial growth in these reservoirs occurs naturally. The excellent correlation noted between bacterial numbers and permeability observed further suggests that these organisms and their essential nutrients enter the formation along higher permeability streaks.

NUTRIENTS

Oil

One of the first questions addressed in our program was whether the oil itself could support microbial growth under anaerobic conditions found in the reservoir.

A research project carried out under contract at B.C. Research in Vancouver screened numerous wild mixed populations for an ability to produce gas on various kinds of crude oil in an anaerobic chamber (4,5). Extensive studies indicated methane, carbon dioxide and hydrogen sulfide gases could be produced in measurable amounts over several months incubation; however, the rate of gas production was found to depend on the concentration of trace oxygen present indicating that oxygen may be needed to initiate oil degradation (4). Only certain oils were susceptible. Light paraffinic crudes favoured gas production. Heavy oil did not.

One potentially useful observation was that the inclusion of even small amounts of nitrate in the medium prevented both methane and hydrogen sulfide production. Metabolic carbon dioxide production was sustained suggesting that only those organisms responsible for methane and H_2S were being inhibited (5). Explanations for this inhibition have been developed elsewhere (6) but the observation offers a way to control noxious H_2S formation in a field application.

Based on the above studies, the use of heavy oil itself as a nutrient to support MEOR activity seemed a dubious concept. A complete MEOR system was therefore defined as the bacteria plus their essential nutrients and it was recognized that microbial activity underground would be limited in both extent and location by the nutrient package.

Sugar Beet Molasses

Sugar beet molasses was selected as a feasible growth medium. Certainly, sugar rich molasses is a well known fermentable substrate used by a wide range of anaerobic organisms. Sugar beet molasses was specifically chosen because of its ready availability near the target oilfields and its low stable price in Western Canada.

MECHANISMS

At this point we began to screen in earnest for anaerobic bacteria able to grow under the in-situ conditions of our heavy oil fields near Lloydminster using sugar beet molasses as the basis for the nutrient package. Selection of microbial isolates was based on their ability to produce agents which might facilitate oil recovery.

Potential mechanisms of heavy oil release can either operate on the oil itself or modify the reservoir.

Alteration of Heavy Oil

Heavy oil is difficult to recover because of its high intrinsic viscosity. Two potential ways in which fermentation products could theoretically reduce viscosity would be through emulsification or through dissolution of CO_2 under pressure.

Emulsification

Emulsifying agents for heavy oil have been reported by Finnerty et al. (7). These agents are produced during aerobic growth of bacteria on crude oil and can generate oil-in-water emulsions under low shear conditions. Emulsion viscosities approach that of water, the continuous phase. Unfortunately, our screening program for anaerobes growing on sugar beet molasses under reservoir conditions failed to turn up any surfactants or emulsifiers of note.

Viscosity Reduction

The reduction of heavy oil viscosity through CO_2 dissolution is a well known phenomenon (8). Under 1000 psi pressure, CO_2 dissolution can swell heavy crude by 10% and cut its viscosity by an order of magnitude (8). A number of aggressive gas producing isolates were found in our screening program. The best of these could operate at full reservoir salinity (6.5-7.0%) and were identified as *Enterobacter sp.* Unfortunately, these isolates failed to produce significant gas pressures when grown under pressures likely to be encountered even in our shallow reservoirs (9,10). Any screening program specifically seeking gas producers for use in situ should be done at reservoir pressure.

Alteration of Reservoir

Well Bore Cleaning

Past MEOR treatments on single wells have stimulated production in both a short and a long term manner. A likely mechanism for long term stimulation appears to be a well bore cleaning action based on fermentation gases, solvents, surfactants and acids (2). While this process may be of considerable interest to small companies or stripper well owners, alternate technologies are well established. Little need was perceived for our further development of this approach.

Limestone Dissolution

Organic acids produced by fermentation have been demonstrated to be extremely effective agents of calcite and dolomite dissolution (11). This could be of considerable use in increasing the porosity and permeability of limestone reservoirs especially in the critical vicinity of the well bore. The target reservoirs in our case were not limestone and this avenue was not pursued.

Flow Diversion

Selective plugging offers the opportunity to divert or control fluid flow in the reservoir. Use of bacterial agents to plug off certain zones or to improve permeability profiles underground has a number of potential applications in oil recovery schemes. In heavy oil production, the common fate of wells is to "water out" long before the oil in place is exhausted. In primary production total recoveries of 4% are common. Control of water production through bacterial action in situ was thus identified as a useful target for a MEOR system in the heavy oil area.

BACTERIA FOR SELECTIVE PLUGGING

Work by Shaw et al. (12) established that the production of biopolymers was a key to formation plugging. Screening for isolates able to make polymer from sugar beet molasses under reservoir conditions was very successful. The organism finally selected for field application was a *Leuconostoc* species known to be non-pathogenic and capable of converting dilute molasses into a gel through the production of insoluble dextrans (13). This polymer is produced only from sucrose; other sugars lead to growth without polymer formation (13).

LAB STUDIES

Plugging of Single Test Cores

Early lab tests were carried out in fused glass bead cores (9) having 6.5 Darcies permeability. These small cores were used principally to determine factors liable to lead

to facial plugging of the formation around the well bore in field applications. This can be an important effect since rapid facial plugging can pinch off injectivity preventing the successful placement of the MEOR system into the reservoir.

Active gas bubble formation, chains or clumps of cells exceeding one-fifth the pore throat size and particulates (usually carmelized sugar) in molasses based media were all found to cause plugging and a loss of injectivity which might compromise a field injection (14). Surprisingly, the production of even a soluble polymer by microbial suspensions led to serious facial plugging during injection (15). While subsequent water flushes readily removed this type of plugging, injection of the organisms was limited by the effect. It was decided that organisms intended for selective plugging in the field could not form polymer during their injection. This meant that polymer production would have to be turned on once the bacteria reached the site for plug formation underground. These criteria were met by choosing a microbe which produced polymer only in the presence of a trigger compound (16). In the case of *Leuconostoc*, polymer is only made from sucrose through the action of an enzyme called dextran sucrase (13). The bacteria are injected into the reservoir growing in a medium based on glucose. There they are overrun by a sugar beet molasses medium rich in sucrose to instigate polymer formation in situ. To test the efficacy of plugging by the insoluble dextrans produced, a series of experiments were performed using test cores made from coproduced sand from a Lloydminster oilfield. In a typical test, one pore volume of bacterial suspension was followed by one pore volume of sugar beet molasses medium. After incubation, the initial permeability of 6 D was reduced by more than 92% and polymer was found throughout the length of the core (17).

Parallel Cores

Simultaneous injection into two parallel cores of differing permeability clearly demonstrated the selective plugging effect. Most bacteria and nutrients entered the more permeable core leading ultimately to more polymer formation and a greater permeability reduction in that core. In effect the fraction of flow being carried by each core initially was reversed. This process could lead to more uniform permeabilities in inhomogeneous reservoirs and improved sweep efficiencies in water flooding or more sophisticated EOR technologies.

DISPERSION OF A MEOR SYSTEM IN THE RESERVOIR

As noted above, a complete MEOR system consists of bacteria and their essential nutrients. In order to introduce the MEOR system deep in the reservoir, bacteria and nutrients must be able to pass through the formation in such a way that they arrive in the target site together. It is therefore necessary to know the rates of travel for both bacteria and nutrient underground. In our case, long sand packed cores (2m) were used to measure the rates of travel and retention of the system components.

Nutrients

A sucrose enriched sugar beet molasses medium partitioned only slightly on passage through a core of coproduced sand from the field. Protein was slightly delayed relative to the water front but retention was not serious. All effluent fractions were able to support microbial growth and polymer formation when inoculated. Apparently in Lloydminster reservoirs, nutrient loss or partitioning is not a critical problem for injections of modest depth (4).

Bacteria

Similar studies with bacteria were not so encouraging. Early work done with washed and sorted sand had revealed no special problems with respect to retention of the cells; however, studies done with whole coproduced sand including fines showed that serious problems do exist.

Our first hypothesis was that the clay fraction present as fines in the test cores was responsible for cell retention. This proved to be largely true. When cores were pretreated with proprietary sacrificial agent developed by B.C. Research (18), the rate of arrival of cells through the core and the extent of passage were greatly enhanced (Figure 1).

In the core used in Figure 1, the fines fraction (0.3% by weight) was made up of quartz (16%), feldspar (5), kaolinite (47%), illite (26%) and chlorite (6%). After some twenty pore volumes, about 100% of the cells entering the column were passing through in the pretreated cores. Nevertheless, there was still significant cell retention. Multiple mechanisms appear to be operating. The spikes seen in Figure 1 correspond to momentary shutdowns of the injection pump in the experiment. Apparently, loss of injection pressure and flow allows cells caught in the core to relax and resume travel when pumping is resumed. This suggests that a pulsed injection protocol in the field may be more effective at moving cells through the formation than sustained pumping. It also implies that significant numbers of cells will be left behind in the vicinity of the well bore during an injection and that this region may have to be treated specially to prevent premature plugging and loss of injectivity during the MEOR treatment. Results to date do not encourage the idea that cells will travel easily through tight formations.

In summary, the major constraint on placing bacterial systems in Lloydminster reservoirs appears to be the dispersion of bacteria through the unconsolidated sand matrix. Bacteria travel more slowly than injected nutrients and are retained especially on clay surfaces. Pretreatment of clays with a sacrificial agent which adsorbs on the clay surface can extend the depth and extent of bacterial injection.

FIELD APPLICATION

Targets for the microbial selective plugging system include bottom waters, channels, casing problems, thief zones and inhomogeneities in formation permeability. In the Lloydminster region, bottom water, that is water lying below the pay zone in direct contact with the oil, represents a major production limitation. Any attempt to produce

oil on a sustained basis is thwarted by water being drawn up from below to the production well. This problem, called "coning", presents a challenging target for a microbial plugging system in view of the volume of the plug likely to be required. A more tractable target was selected for the first field test of a microbial selective plugging system.

A single well which had had a long production history but which had ultimately watered out was chosen as the target site. Geology and well logging had indicated that water influx was limited to about half a meter at the bottom of the well. It was suggested that this water had been drawn up formation from a nearby oil/water contact. A single injection of the microbial plugging system was attempted to try to plug off the high water permeability zone which had developed. The well was then returned to production to see if an economic water/oil ratio resulted.

Injection Scheme

Figure 2 illustrates the surface facilities used for injecting the microbial system. The bacteria were injected first in the volume shown followed by a slug of water (8 m^3) and then a sucrose enriched sugar beet molasses medium. The intention was to overrun the bacteria with nutrients. No sacrificial agent was employed in this test. Unfortunately, the well was shut in with residual molasses medium still in the well bore due to an access problem caused by heavy rainfall. The injection sequence was then completed by squeezing a slug of oil into the well to encourage oil flow.

On reopening the well, it was found that it was completely plugged. A sample of water was bailed from the well for analysis and the well was reperforated. This succeeded in restoring good fluid flow indicating that the plugging had been due to surface growth on the formation face presumably promoted by the nutrients left standing in the well bore.

Water Analyses

Produced water was characterized by chemical and biological analyses and compared to baseline data collected prior to the microbial treatment. Results are presented in Figures 3 to 6.

Biological Analyses

Prior to treatment, no slime formers were present in the coproduced brine although up to 10^4 anaerobes/mL and 10^3 aerobes could be cultured on general growth agar per mL of produced water. After the test, up to 10^4 anaerobes were still present but up to 75% of these were polymer producers. Aerobic counts remained the same. Not all of the polymer producing organisms found in the produced water were the injected organism, *Leuconostoc*. Apparently, the molasses medium had favoured growth of anaerobic slime formers in situ but some of these were either indigenous or came from the molasses itself. After 124 days production, anaerobes persisted at 10^4 cells/ml, 66% of which were slime formers.

Chemical Analyses

Chemical analyses on the produced water after the bacterial treatment were carried out to assess whether the injected freshwater slug came back immediately and the extent and kind of fermentation that had proceeded underground.

Plots of the conductivity of the produced water over time (Figure 3) show that the injected freshwater did not return quickly and quantitatively to the well. Only slight freshening of the produced water occurred early in the production period after treatment. Large amounts of brine continued to be produced almost undiluted by the injected water.

A slight decline in pH (6.4 ± 0.1 to 6.0 ± 0.1) and surface tension (66.5 ± 0.6 to 59.6 ± 2.4 dynes/cm) were evident in the first eleven days of production consistent with a slow release of fermentation products. Sugar content of the water was negligible.

To ascertain the kind and extent of fermentation which occurred in situ, the produced water was analyzed for volatile and non-volatile fatty acids as well as alcohols. Elution patterns revealed the presence of at least two microbial populations.

Bailed water taken from the plugged well prior to reperforation contained a mixture of fatty acids atypical of organisms such as *Leuconostoc* (Figure 4). The concentration of these acids dropped off rapidly in the first days of production, consistent with their occurrence in the immediate vicinity of the well bore. It seems probable that these are fermentation products formed by the mixed community of organisms which grew on the residual nutrients in the well bore and which caused the facial plugging initially observed on reopening the well.

In contrast, fermentation products typical of *Leuconostoc* continued to be produced and achieved their maximum concentrations over a more extended period. These include acetic acid (Figure 4), lactic acid (Figure 5), and ethanol (Figure 6). This is consistent with the growth of heterolactate fermenting organisms deeper in the formation. Products continued to be eluted up to 96 days after reopening the well. This would be the expected result had a plug been formed which allowed only modest water passage. Elution of the coincident fermentation products would be expected to occur slowly. After 96 days, some 91% of the lactic acid and some 69% of the acetic acid anticipated for the amount of nutrient injected had been produced back to the surface.

While these observations are consistent with growth and polymer production by *Leuconostoc* or similar bacteria, there was also evidence of concommitant or secondary fermentation. The absence of significant sugar and production of unfamiliar products such as pyruvate (Figure 5) indicate the participation by other kinds of bacteria. Gas analysis for the casing gas in the well showed a very modest increase in CO_2 but no H_2S. There was no evidence for sulfate-reducing bacteria either before or after the MEOR treatment. Excess barium in the connate brine kept soluble sulfate levels below 20 ppm. Since no special precaution was taken to inhibit these noxious organisms, the lack of soluble sulfate may have prevented their appearance.

Summary

Field application indicated that injection of bacteria was a feasible procedure requiring no special equipment or modification of the well. While only a small (possibly insignificant) change was observed in the oil/water ratio, fermentation products in the produced water indicated that the sort of fermentation expected had occurred at some distance from the well bore. Secondary growth by other organisms was also evident but there was no evidence of potential problems with sulfate-reducing organisms. A further field test in a better defined reservoir situation is planned.

CONCLUSIONS

The research program at NOVA/Husky Research proceeded from conception to field in a logical fashion. This included a number of fundamental investigations which need not be repeated by future researchers. The technology developed is patented but has yet to demonstrate economic success. A further field test is planned.

In some senses, the microbial system developed represents "second generation" technology and has opened the way for more sophisticated approaches to the selective plugging problem in heavy oil fields. It is our hope that efforts underway in a number of laboratories will generate new and better microbial plugging systems. The economic opportunity for the application of these systems especially in conventional oil fields is tangible.

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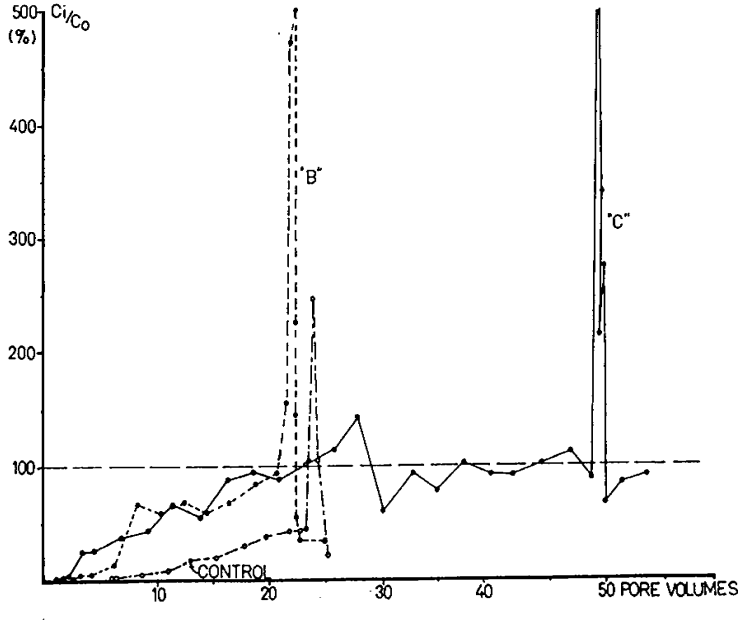

FIGURE 1. - Percent passage of cells through a sand pack as a function of volume injected with (B and C) and without (control) pretreatment with a sacrificial agent. Spikes in the plot are due to interruption of pumping.

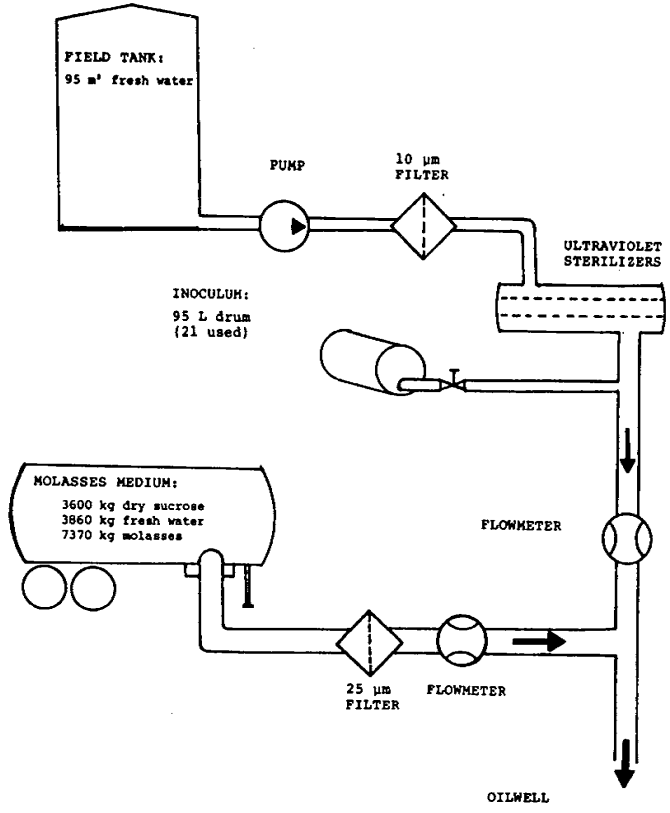

FIGURE 2. - Schematic diagram of field injection system.

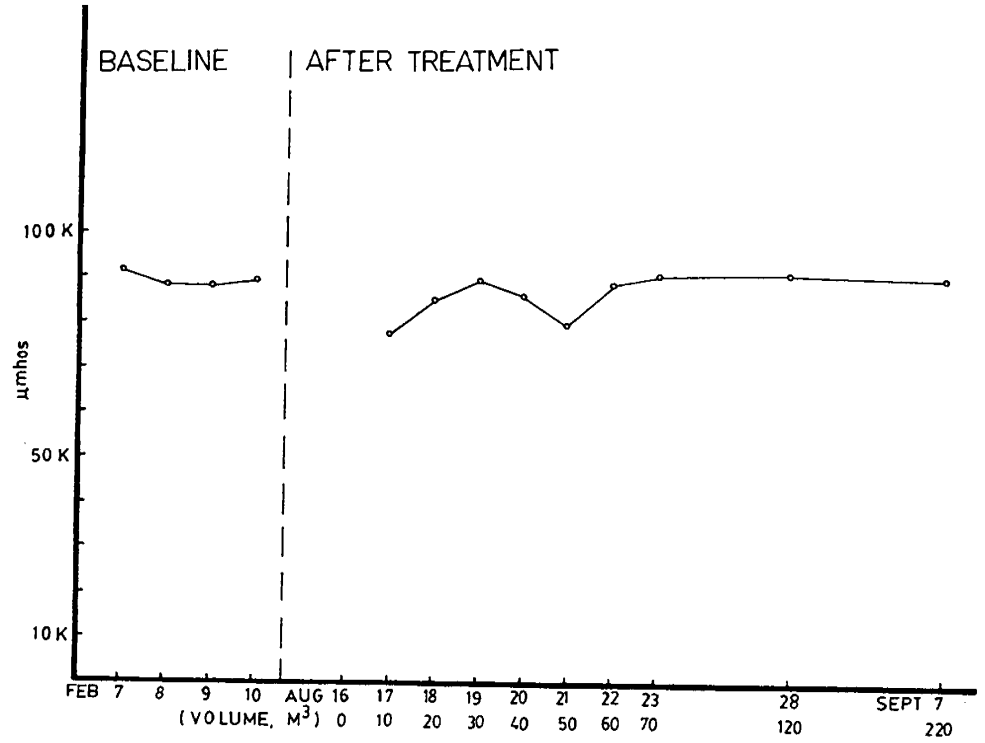

FIGURE 3. - Conductivity of produced water before and after injection of the bacterial system.

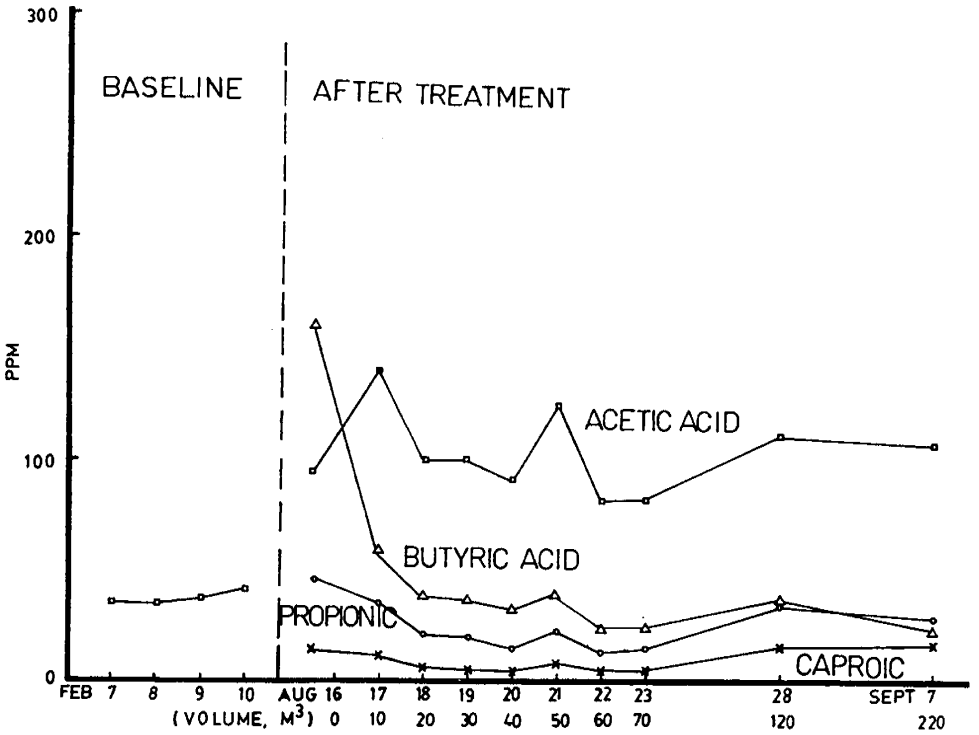

FIGURE 4. - Volatile fatty acids in produced water before and after injection of the bacterial system. August 15th data are for bailed water. Molasses medium injected contained 290 ppm acetic acid.

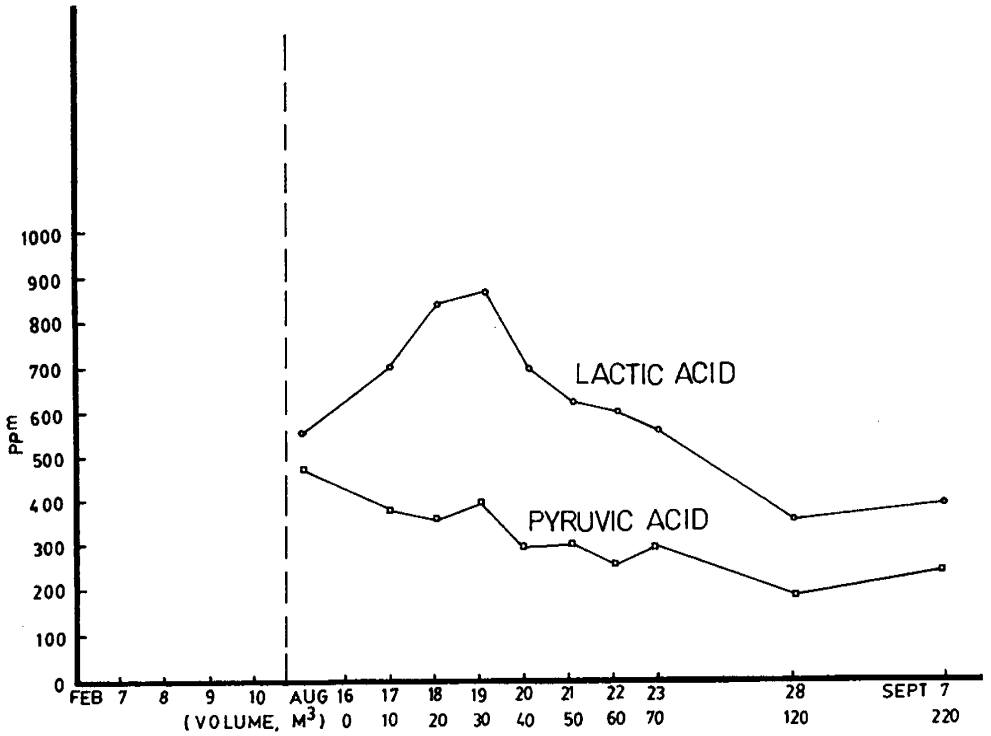

FIGURE 5. - Fatty acids in produced water before and after injection of the bacterial system. August 15th data are for bailed water. Molasses medium injected contained 987 ppm of lactic acid and 0 ppm of pyruvic acid.

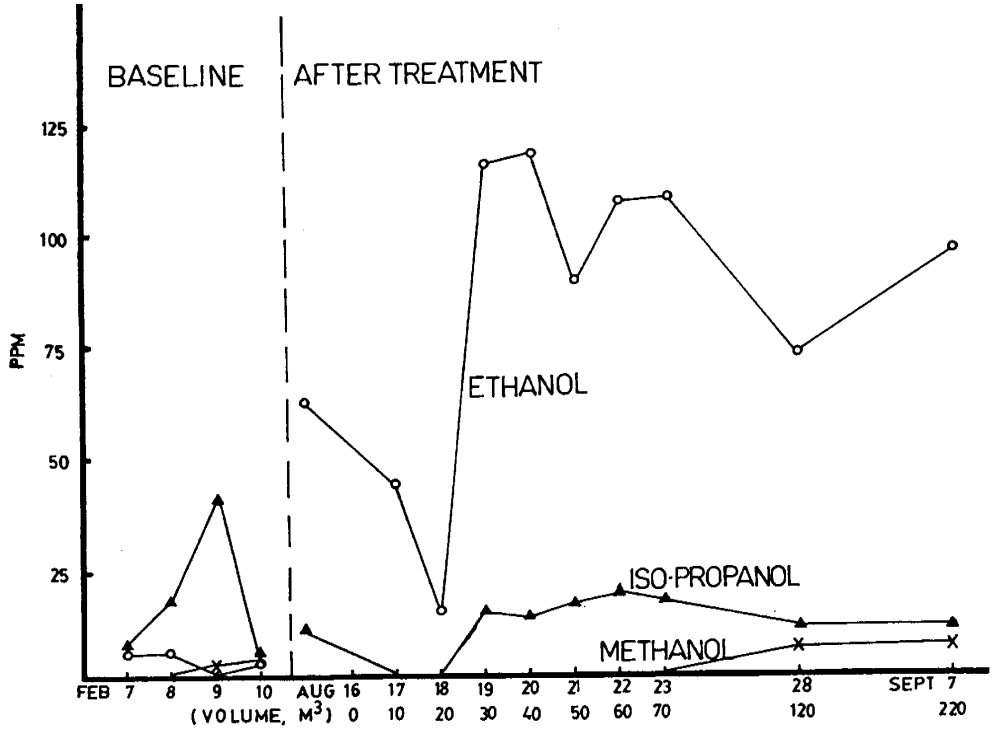

FIGURE 6. - Alcohols in produced water before and after injection of the bacterial system. August 15th data are for bailed water.

AN MEOR PILOT TEST IN THE LOCO FIELD

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ABSTRACT

A field test to evaluate the neutral solvent/microbial enhanced oil recovery process, which is an *in situ* fermentation utilizing a specially adapted bacterium of the genus *Clostridium*, as well as the effect of such a fermentation on oil productivity is described. The test was conducted on the Royal Loco Unit, Stephens County, Oklahoma, using an irregularly spaced inverted five-spot. A 2,000 pound/308 barrel water/sucrose/mineral mixture was injected in a Loco sand lease (Permo-Pennsylvanian Age) at low rates as nutrient for a 40 gallon inoculum. After a shut in period and subsequent production, fermentation products appeared in surrounding wells. This fermentation is discussed and compared with earlier work.

Oil production increased after the initial shut in. This increased production will be discussed in terms of mechanisms involved and the possible economic significance of the process.

INTRODUCTION

At present, most oil reservoirs contain large amounts of oil that cannot be produced by conventional primary and secondary (waterflooding) methods. This residual oil is estimated to be some 327 billions of barrels (1,2). This residual oil is primarily the result of poor areal and vertical sweep efficiency by current secondary and tertiary recovery methods.

One enhanced oil recovery (EOR) method, microbial enhanced oil recovery (MEOR) may be economically feasible to recover a portion of this large remaining oil resource at present low prices. Of the various bacteria thought useful for MEOR processes, bacteria of the genus Clostridium, specifically the "butylic" group are the most appealing for the following reasons:

1. Production of inert spores, which within reason can be handled without respect to temperature, oxygen availability and pH. This is not true with vegetative cells. The spores do not contain a charge and as such will not react with the fluids or rock matrix. Additionally, because of the spores small size, they are fairly easily injected into the oil reservoir and are resistant to pressures and shear during the injection process.

2. Production of a number of metabolic end-products useful in EOR processes, allowing the development of a neutral solvent type in situ fermentation. The products of the neutral solvent fermentation; carbon dioxide (CO_2), normal butyl alcohol (NBA), isopropyl alcohol (IPA), acetone, etc., are known to be useful in EOR (3,4,5).

3. Production of a water-external emulsifier compound (6) which is able to keep minute droplets of oil in the aqueous phase, and has some ability to lower surface tensions.

4. Vegetative growth within the confines of a reservoir, especially the more permeable; (the portion of the reservoir that has been most subjected to waterflooding) will change the conformance of injected flood waters. Improvements in oil production and water oil ratio (WOR) from profile modification and improved mobility control can be very important (7).

5. Clostridia, being anaerobic in nature, are able to cope with the lack of oxygen in static oil reservoirs.

BACTERIAL SELECTION

A number of Clostridia are capable of fermenting various carbon sources to carbon dioxide and neutral solvents (8). Yarbrough and Coty (9) had some success using a Clostridium acetobutylicum in a field test of MEOR. However, the Clostridium acetobutylicum was injected as vegetative cells (10) and may not have actually fermented additional injected substrate as the expected metabolic products were not found in either the produced water or oil phase.

Laboratory experiments were conducted on specially adapted Clostridia to determine if the bacteria was viable in the oil and brine phase of the Loco reservoir. Several adaptions were performed that resulted in the following prediction as to the final concentration of various end-products from the injected nutrient:

Carbon Dioxide-	1200#	(10,548 cu. ft.)
Hydrogen-	40#	
N-Butanol-	300#	
Acetone-	20#	
Ethanol-	60#	
Acetic Acid-	20#	
Butyric Acid-	140#	

The produced water contained approximately 38,000 PPM Total Dissolved Solids (TDS), or approximately 3.5% sodium chloride. Laboratory metabolic production was consistent with this level of sodium chloride.

RESERVOIR SELECTION

The Loco Field represents an ideal target for the neutral solvent/microbial enhanced oil recovery process (NS/MEOR). The field was first discovered in 1913, with early production by solution gas drive. During the early 1960's, Conoco unitized the bulk of the field, some 2,300 acres, and began secondary recovery operations. The oil is a heavy, API 21 degrees, high viscosity crude with a very adverse mobility ratio resulting in approximately 14% of the initial oil in place (101P) being produced by primary and secondary methods. Residual oil is estimated to be approximately 1,500 BBL per acre foot. The field is estimated to contain 73.4 million barrels of 101P in the deeper Loco sands (12).

Figure 1 is a plat of the project area.

INJECTION PROTOCOL

Upon introduction of any fluid into an oil reservoir, extreme care must be taken when injecting fluids that contain solids. Figure 2 shows the approximate permeability/pore throat diameter relationship in sandstone formations (13). A pore throat (PT) may be bridged by particles as small as 1/7th the PT diameter (14). As a rule of thumb, pore throat bridging (plugging) will occur with particles 1/7th to 1/3rd the pore throat diameter. For this reason, spores (less than 1 micron) are the ideal choice for inoculation. It is also the reason that molasses (which contains filterable material larger than 5 microns) was not chosen as the substrate for the pilot flood.

For the Loco pilot, 20 barrels of potable water were injected as a preflush. This was followed by staging the 40 gallons of spores in 20 barrels of potable water and injecting at 1/2 barrel per minute. A nutrient system, as developed and tested for optimum butanol production above the breakpoint (pH 4.5) in the adaption procedure, was then injected at 1/2 BPM (barrel per minute). Total injected nutrient was 308 bbls at approximately 350 psi.

Injection rates were kept low to insure that the preflush, spores and nutrient would be distributed and remain in the high permeability, relatively lower residual oil zone. The lease was shut in seven days after the injection.

RESULTS AND DISCUSSION

After a one week shut in, all wells were returned to production. Produced water was reinjected through the indicated injection well. At weekly intervals, the surrounding wells were sampled and the aqueous phase analyzed by gas chromatography for metabolic end-products. Sample data appears in Table 1. Of interest is the butanol production during the week of October 31st. The 7,208 ppm yield is higher than the theoretical yield (substrate to product) achieved industrially (15) and in the above laboratory tests. We believe that this concentration is the result of NBA forming a bank and subsequently being displaced to the producing well. Eight weeks post-injection, no other metabolic products were detected from the sampled wells.

The oil phase was examined by the crude oil purchaser for gravity. To the extent that any metabolic products were dissolved in the oil phase, they did not result in a change (higher) API gravity designation.

Throughout the sampling, no hydrogen sulfide was detected. Additionally, the sample analysis indicated that the fermentation was accomplished by the injected bacterium rather than any indigenous or contaminating bacteria. Results of published (16) and unpublished field tests involving Clostridia similar to the one used in the Loco test indicate that this, while unexpected to the uninitiated due to the lack of aseptic conditions during injection and the ecosystem utilized for the fermentation, is the rule rather than the exception.

Figure 3 shows daily oil production (solid line) with the above metabolic products overlayed as the dashed line. The ten day period (Days 16-26) of low production was the result of equipment problems and low temperature causing the oil to remain in the flow lines and free water knockout. On day 27, this oil was produced; however, it has been omitted from the graph so as

not to distort daily production quantities.

The bimodal peaks in oil production are thought to be, first, associated with carbon dioxide production, and second, with the arrival of the metabolic products (principally NBA).

Figure 4 shows the lease plot with the estimated microbially affected area. Based on the per well metabolic production, directional permeability trending in a N-S direction is indicated. The very high metabolic levels in Well 5 suggest a highly saturated zone or water channel from the injector to that produced.

Although there are many factors affecting oil recovery in MEOR, we believe that the viscosity reduction from in situ carbon dioxide production and profile modification by biomass and perhaps foam, were the two most important factors affecting the oil production in this test.

The higher viscosity alcohol (NBA 2.9 CP) may have contributed some mobility control and with the carbon dioxide improved oil viscosity, would have achieved a more favorable mobility ratio. It is not clear whether at the pore level, miscibility is occurring and if not, whether it is important, as immiscible CO_2 huff and puff operations have proved effective in producing waterflood residual oil (17). Additionally, the produced CO_2 would be effective as carbonation during the subsequent waterflood/production operations (18, 19).

The bacterium used in the Loco test exhibits some surfactant attributes that are also useful in EOR. The surfactant/CO_2 reaction creates foam and an oil-in-water emulsion. Foams are very effective methods of altering permeability profiles (20, 21). These foams and cellular mass alter the injection profile and the areal sweep of the subsequently injected flood waters. We believe this did occur later in the test as injection pressures rose to approximately 500 psi and visual inspection of the fluid samples indicated an improving water-oil ratio.

From the foregoing, it is apparent to use that much larger treatments would be required to mobilize and produce significant quantifies of oil. This is substantiated by Bryant (22) wherein 30% nutrient pore volume injection was required to produce an average, greater than 30% residual oil. Larger treatments necessarily require substantial amounts of substrate in the NS/MEOR process. The relative availability of solids free corn syrup (glucose) was evaluated as the carbon source. Laboratory tests indicated metabolic yields not substantially different than with sucrose. Utilizing a delivered cost of $0.10/lbs in 200,000 lb quantities, the following is an order

of magnitude estimate of the economics for a 200,00 lb NS/MEOR inoculation in the Royal Loco Unit lease:

Treatment size:
25% Pore Volume per one surface acre,
20 lb substrate per barrel of injected water
10,000 barrels of water
200,000 Substrate (d-glucose)

Expected Produced Products:	
Crude oil (30% of 50% residual oil)	4,350 BBL
Butanol (20% yield on substrate)	40,000 lbs
Acetone (2% yield on substrate)	4,000 lbs
Ethanol (2% yield on substrate)	4,000 lbs
Carbon Dioxide (55% yield on substrate)	110,000 lbs

Expected Revenues (80% Net Revenue Interest)	
Crude Oil (4350 bbl @ $15.00/bbl)	$65,250
Metabolic Byproducts:	
Butanol* (40,000 @ $0.30/lb)	$12,000
Acetone* (4,000 @ $0.20/lb)	800
Ethanol* (4,000 @ $0.20/lb)	800
Carbon Dioxide* (110,000 @ $0.03/lb)	3,300
Total Metabolic Byproduct	$16,900

*These values represent replacement costs and, as such, may not justify actual recovery.

Total Expected Revenues	$65,250
Expected Expenses	
Substrate 200,000 lb @ $0.10/lb	$20,000
Microorganisms, additional nutrients and injection costs ($0.25/injected bbl)	7,500
Production operations 2 years @ $600/year	1,200
Total Expected Expenses (one acre)	$28,700
Expected Pretax Cash on Cash Return	$41,550
Expected Pretax Cost to Produce a Working Interest BBL	$ 6.60

A problem may occur if produced water with high concentrations of NBA is to be reinjected in an area of microbial activity. Concentrations greater than 2% (W/W) of NBA are toxic to the adapted strain of Clostridia used in the Loco test. In this event, a NBA separation would have to be effected, probably by solvent extraction, which costs may exceed the value of the recovered NBA. Such costs could reasonably add at least $1.50 per barrel to the incremental cost of oil produced. However, most operating waterfloods will have other injection locations that, in early time operations, could be used to dispose of high concentrations of NBA as an alcohol flood or other chemical flooding procedures such as polymer or miscellare-polymer operation.

CONCLUSIONS

1. The NS/MEOR process is a promising enhanced oil recovery method, particularly for heavier oils. The Loco test suggests waterflood residual oil recoveries by CO_2 mechanisms which result in improved vertical and areal sweep efficiencies.

2. Laboratory and field results indicate that a microorganism specific in situ fermentation is possible by a strain of Clostridia especially adapted for the Loco reservoir.

3. Laboratory tests indicate that refeeding the microorganisms would result in additional incremental oil recovery.

4. Laboratory and field results indicate that much more work will be required to adequately describe the mechanism of MEOR, particularly the phase relationships between oil, brine, CC_2, and the metabolic products.

5. Economics of the NS/MEOR process appear favorable on a large scale basis because of the substantial availability of glucose from corn syrup for the microbiological carbon source.

6. Large scale NS/MEOR processes could cause a neutral solvent (NBA) bank to form allowing profitable separation of the valuable constituents and/or provide feedstocks for other EOR operations.

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Table 1. Metabolic end-products detected at the producing wells [PPM].

Well Number	4	5	6	7	8
End-Product					
10/26/86					
Acetone	0	0	163	0	0
Butanol	0	0	157	0	0
Acetic Acid	0	0	0	0	0
Butyric Acid	0	101	0	0	0
10/31/86					
Acetone	0	0	0	0	0
Butanol	0	7208	707	396	0
Acetic Acid	0	19	0	0	0
Butyric Acid	0	188	158	126	0
11/07/86					
Acetone	0	0	0	0	0
Butanol	112	71	59	41	19
Acetic Acid	0	19	0	0	0
Butyric Acid	0	0	0	0	0
11/18/86					
Acetone	0	0	0	0	0
Butanol	33	0	0	0	0
Acetic Acid	0	0	21	11	0
Butyric Acid	0	0	0	41	47

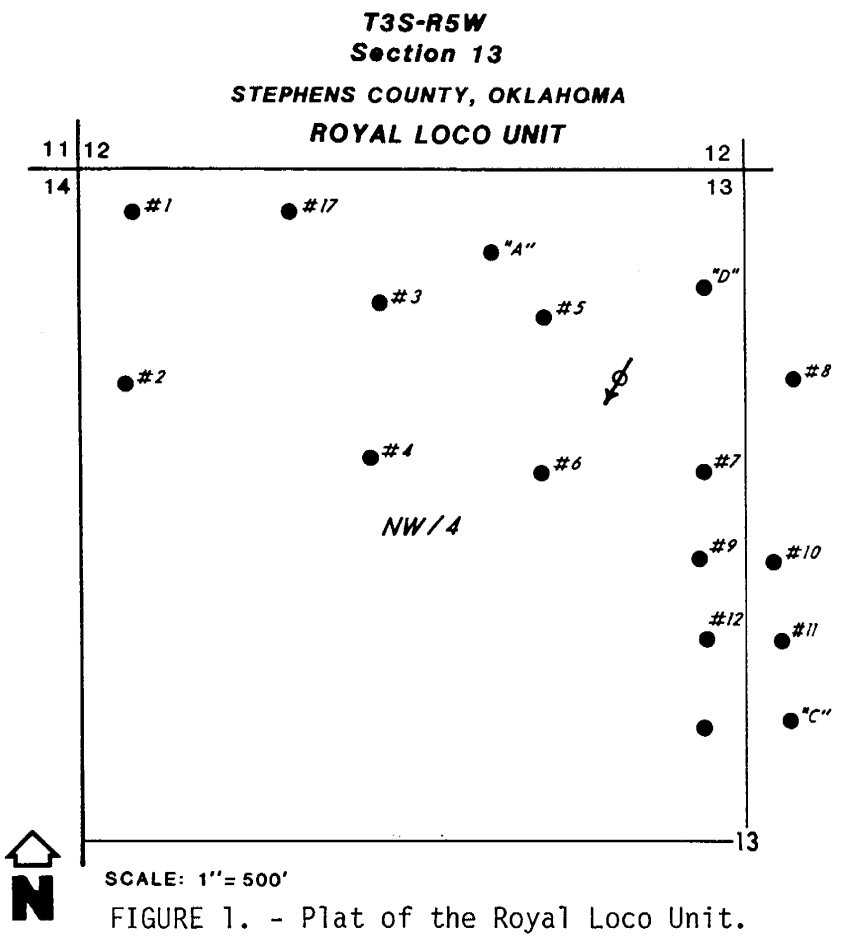

FIGURE 1. - Plat of the Royal Loco Unit.

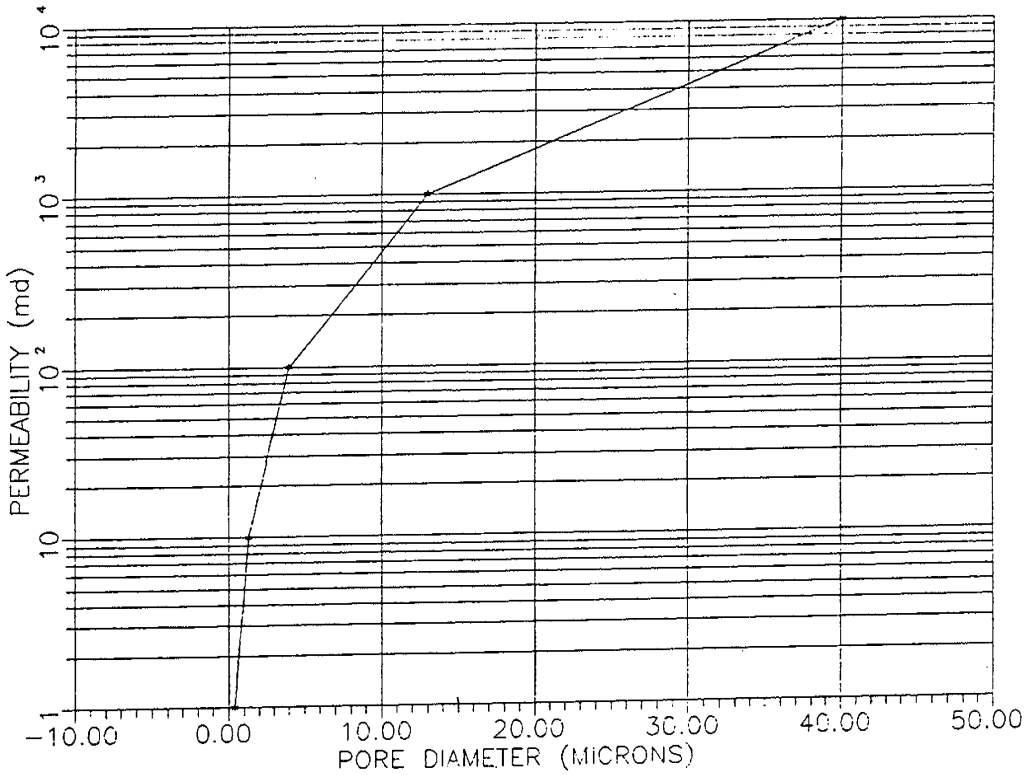

FIGURE 2.

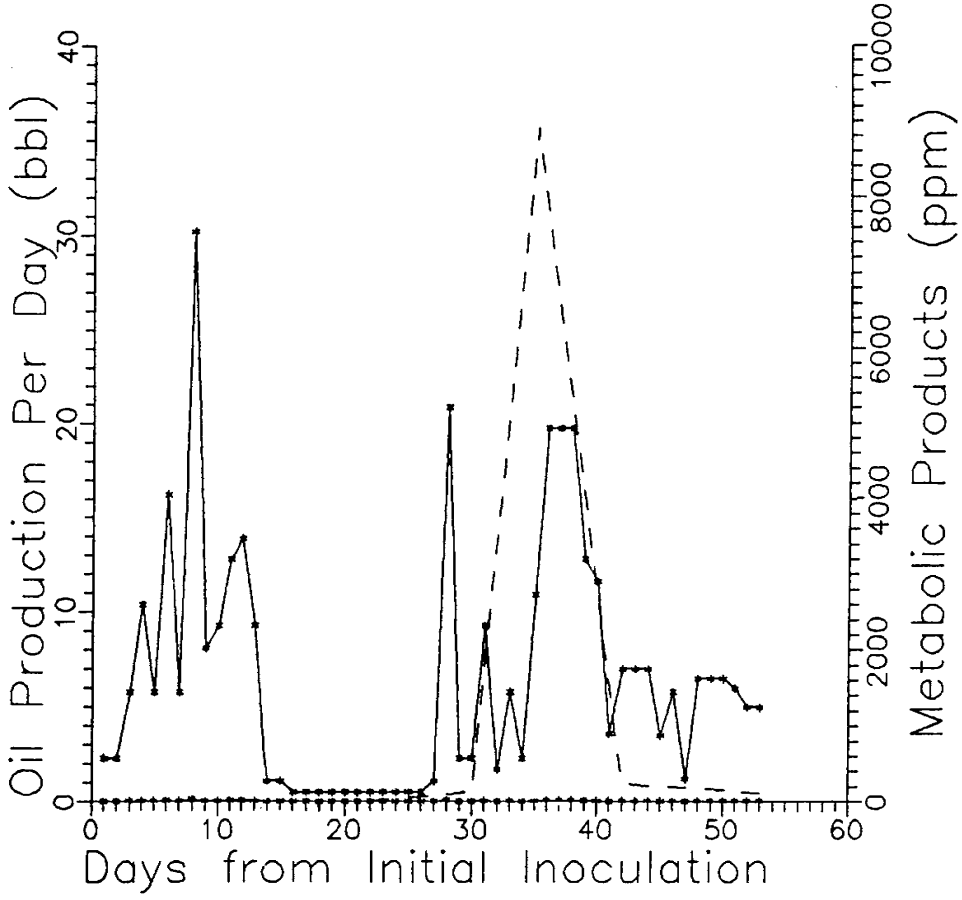

FIGURE 3. - Daily oil production after microbial treatment.

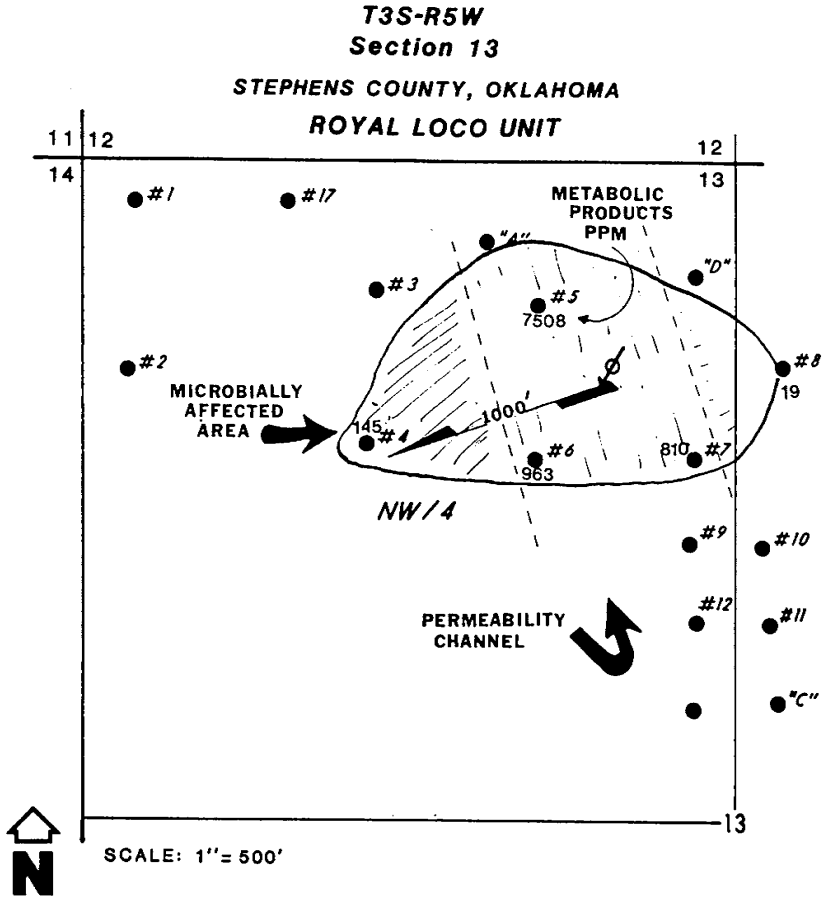

FIGURE 4. - Plat of Royal Loco Unit showing area affected by treatment.

MICROBIAL-ENHANCED WATERFLOOD FIELD PROJECT

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ABSTRACT

A microbial enhanced waterflood field project sponsored by the U.S. Department of Energy (DOE), Microbial Systems Corporation (MSC), and INJECTECH, Inc. and conducted in cooperation with the National Institute for Petroleum and Energy Research (NIPER) was initiated in October, 1986. MSC's project responsibility was to conduct a comprehensive field operation to include: site selection; data base compilation; baseline monitoring program; chemical tracer application; microbe injectivity and survivability field tests; microbial system field injection; post-injection monitoring and nutrition program; and field data analysis.

An extensive site survey resulted in selection of the "Mink Unit" of the B & N Oil Company waterflood project at Childers, Oklahoma. A satisfactory data base was constructed from historical records, reports, maps, operator information and the U.S. Bureau of Mines abstracts for the Delaware-Childers oilfield. A baseline program was initiated in October, 1986, and continued to March, 1987. This program included periodic well sampling, testing, rates, pressures, and fluid ratios. All samples were transported to participating laboratories for further testing and analyses. During the baseline period, a chemical tracer study was conducted to help determine flow patterns of the project site. Off-pattern microbial system injectivity and survivability tests were also performed during the baseline to indicate the degree of expected success for the project site. Based on these optimistic results, the microbial system was introduced to the project site formation in mid-March, 1987, and allowed a two-week "soak" period before resuming normal water injection.

The project group is continuing a post-injection field monitoring, sampling and nutrition program, with emphasis given to data analysis.

INTRODUCTION

The first activity undertaken by the project group was to characterize the project and establish the goals we intended to accomplish. The microbial formulation was designed to target the "trapped" residual crude oil that remains in the swept reservoir areas of a mature and active waterflood property. The principal project goals were (1) to demonstrate and document the technical and commercial feasibility of this type of MEOR process; and (2) to define the critical parameters that must be incorporated in planning of an MEOR field project.

PROJECT PLAN

A formal project plan was developed to provide a sequential blueprint of all the activities that would take place during the course of the project, and

the approximate time frame for each. The following steps are provided in chronological order, and noted if they were conducted concurrently.

Candidate Screening and Site Selection

A set of criteria was established for the project to guide selection of the final site. Area operators were interviewed and over 200 individual properties were analyzed for their suitability according to the criteria. Site selection criteria included:

- Operator Cooperation
- Site Accessibility
- Mature, Active Waterflood
- Low Brine Salinity Values
- Established Oil Production Decline Curve
- Established Oil Saturation
- Reasonably Uniform Injectivity Values
- Favorable Well Spacing and Pattern
- Availability of Historic and Current Project and Reservoir Data
- Ability to Isolate Project Site
- Ability to Easily Measure Incremental Oil Increase
- Compatibility with Microbial Inventory
- Permeability above 50 md

The candidate list of 200 was narrowed by the process of elimination, and the final site was selected based on its reasonable fulfillment of all project criteria. The project site is located in the Delaware-Childers Field near Childers, Oklahoma. The Mink Unit is a 60 acre site situated on the northeast extension of a large waterflood comprising approximately 1700 acres (fig. 1). These wells are all open hole completions (fig. 2). The actual field test is being conducted on approximately 20 acres of the Mink Unit and involves 4 of the 21 Mink injection wells, and 10 of the Mink producing wells. The target producing formation zone is the Bartlesville Sand at 650 feet in depth, which has been waterflooded for nearly 35 years.

Reservoir and Field Data Base

The current operator was very helpful in providing information, some of which dated back to the initiation of the waterflood by Sinclair Oil Company. These data included surface maps, drilling reports, core analysis, logs, geologic and reservoir reports, and injection and production records. Also utilized were historic U.S. Bureau of Mines abstracts for this area. All data were organized and compiled for the permanent project record.

Preparation of Field Site

Field personnel installed several valves, meters and miscellaneous equipment to prepare for sampling and testing of the field site.

Baseline Program

A four month field sampling program was initiated to establish a baseline for the parameters considered necessary for the project monitoring. Additionally, chemical tracer studies were initiated in December to determine

the flow patterns of the injected fluids in the Mink Unit. Fluorescein was found to be compatible with the formation fluids as well as the microbial cultures, and it was chosen as the tracer for the test. Samples of each producing well were transported to NIPER and the fluorescein concentration was determined using a spectrophotometric method. Every producing well received some fluorescein, and indicated that communication occurred between all wells. The final analysis of the tracer study is still in progress.

Other parameters that were monitored during the baseline period were:

- Microbial Testing and Analysis
- Crude Oil Analysis
- Crude Oil Production
- Brine Analysis
- Brine Production
- Gas Analysis
- Injection Pressures and Rates
- Production Well Water-Oil Ratios

The baseline values and results for these parameters were presented by Bryant et al.[1]

Microbial System Optimization

During the baseline period, various porous media techniques such as linear coreflooding and micromodel testing were used to optimize the microbial formulation. Various microbial consortia were tested to demonstrate the most effective formulation for improving oil mobilization.[2] Results from these studies will be presented in another symposium paper.

Single Well Injectivity and Survivability Test

The project group elected to perform a trial injection on an off-pattern injection well to determine if there were any injectivity or microbial survivability problems. The microbial system was injected and monitored in this well several weeks prior to inoculation of the test site. Using an uninoculated well for comparison, no increase in injection pressure was observed. The microorganisms survived and metabolized very well during the shut-in period.

Scale-Up and Injection of the Test Site

Microbial solution volumes were prepared in the laboratory for the four injection wells involved in the experiment, and transported to the field with the molasses. Injection of the system was completed with no difficulty and the injection wells were "shut in" to allow the microbial system to become established in the formation. Water injection was resumed after this two week soak period when it was determined that copious amounts of viable microorganisms and metabolites (surfactant) were present in the near wellbore region.

Monitoring and Field System Maintenance

Sampling, testing and monitoring of scheduled parameters is performed periodically, and the results analyzed and compared with the project baseline information. A constant program of nutritional maintenance is in place to ensure growth and continued viability of the microbial system.

CONCLUSIONS

The microbial system has been established in the four Mink injection wells for a total of six months. As might be expected, it is too early in the project to make conclusions. However, based on the data accumulated, several observations have been made:

1. Molasses is being metabolized before reaching the producing wells in detectable amounts.

2. The introduced microbial consortium is still viable and producing metabolites in the near wellbore region.

3. No change in injection volumes or pressures has occurred.

4. There is evidence of lowered water-oil interfacial tension and water surface tension at some producer wells.

5. There has been some improvement of water-oil ratio at all producing wells (fig. 3).

6. A statistically significant increase in oil production has occurred (fig. 4).

This MEOR field experiment will continue to be monitored and maintained until the end of the project. All results will be compiled, documented, analyzed and compared to the baseline data to determine the relative success of this project.

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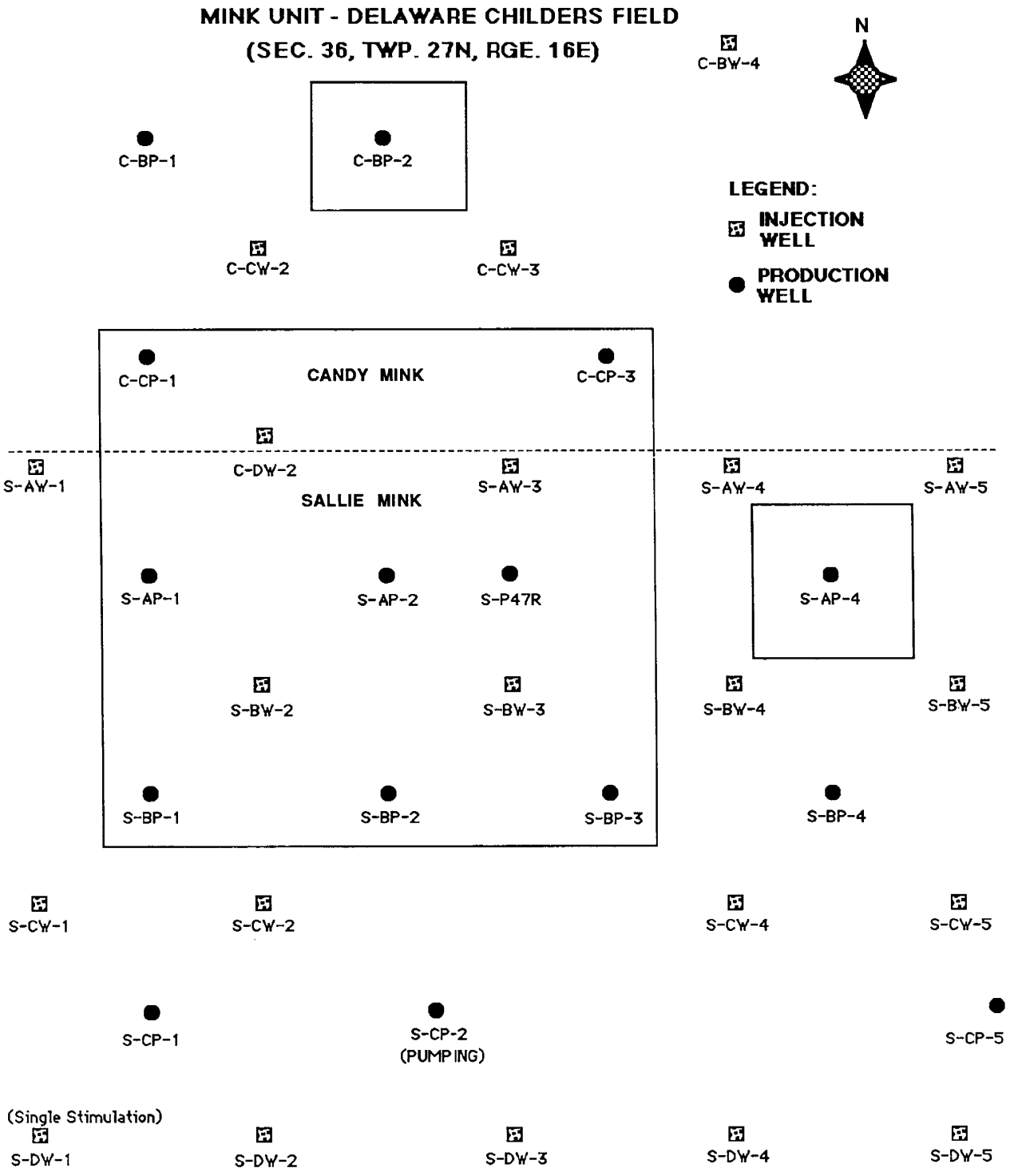

FIGURE 1. - Map of Mink Unit.

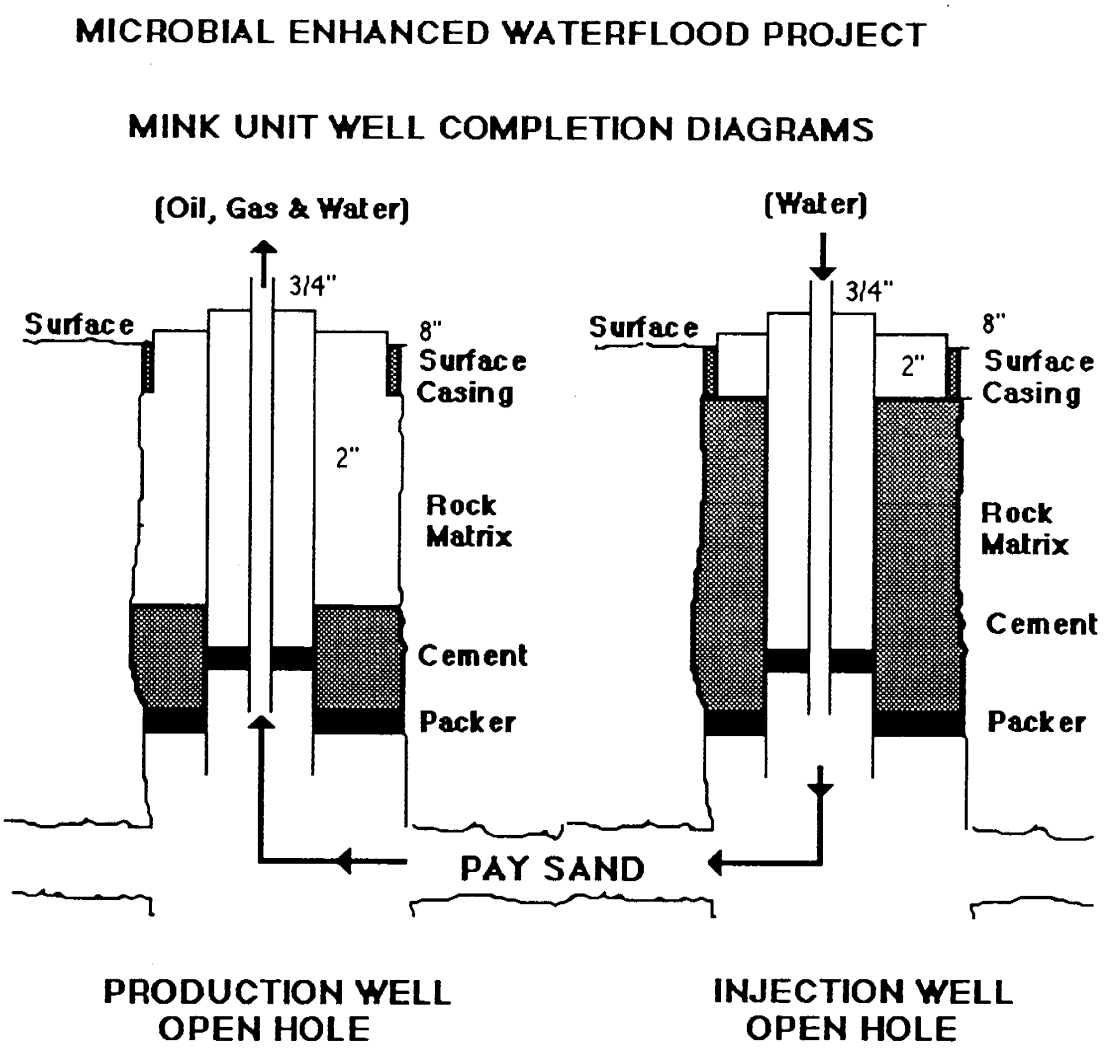

FIGURE 2. - Diagram of Mink Unit wells.

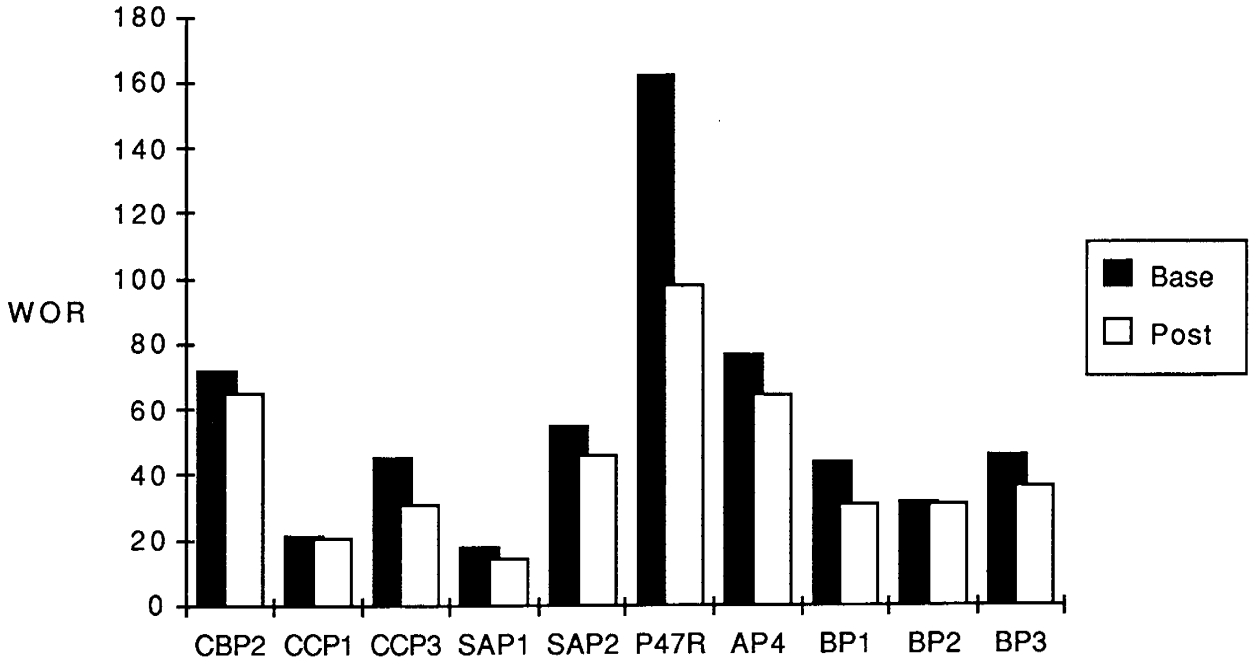

FIGURE 3. - WOR graph.

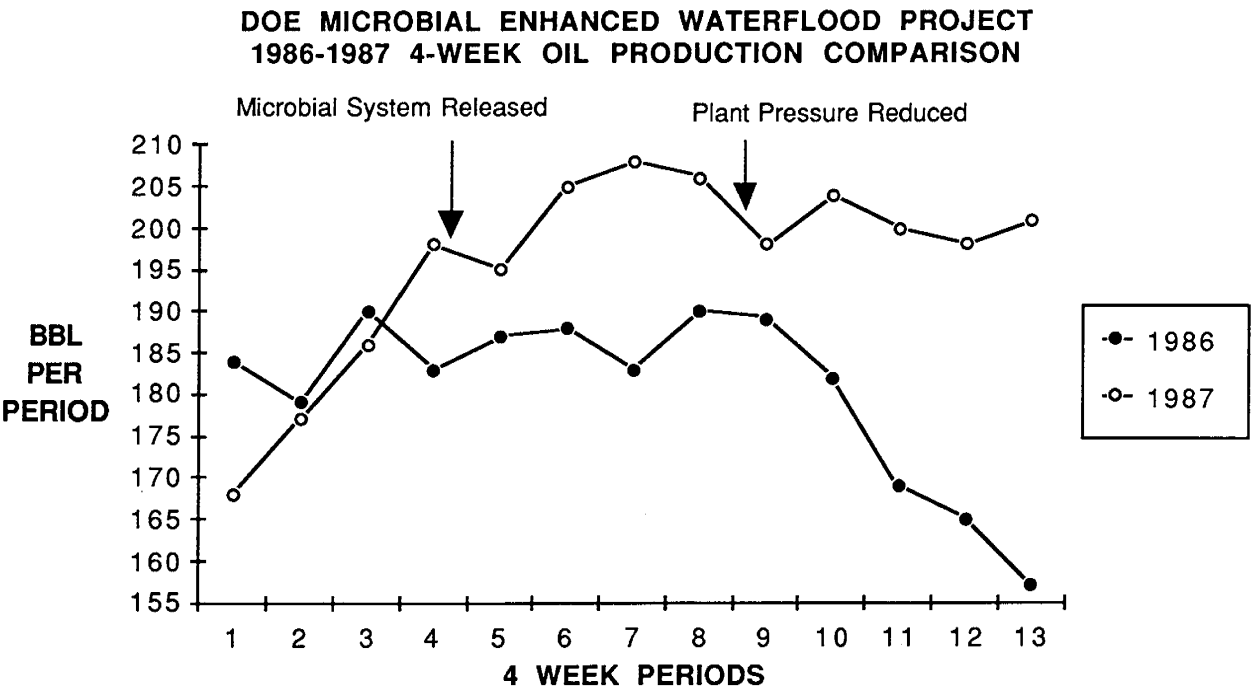

FIGURE 4. - Oil production - 4-wk chart.

LABORATORY OPTIMIZATION OF MICROBIAL FIELD PROJECTS

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ABSTRACT

A microbial-enhanced waterflood field project sponsored by the U.S. Department of Energy (DOE), Microbial Systems Corp. (MSC), and INJECTECH, Inc. and conducted in cooperation with the National Institute for Petroleum and Energy Research (NIPER) was initiated in October 1986.

The site is operated by B & N Oil Co., Nowata, OK, and consists of four adjacent inverted five-spot patterns drilled on 5-acre spacing in the Mink Unit of Delaware-Childers field. A baseline monitoring phase before microbial injection was conducted from October 1986 to March 1987. Field samples were collected weekly and analyzed for microbial populations, total dissolved solids (TDS), trace mineral concentrations, pH, and oil viscosities.

Several different microbial formulations were tested in Berea sandstone cores with Mink Unit water and oil to determine oil recovery efficiency. A field core produced 28% more residual Mink crude oil than the waterflood process. Glass-etched micromodels were run concurrently with this coreflood to determine if the microbial formulation could mobilize oil in simulated porous media. It was visually observed a large amount of the residual oil was mobilized after the microbial treatment. The oil recovery mechanism for this system could be partially due to alteration of wettability caused by surfactant and alcohol production.

Optimization of the microbial system for this project has identified several key parameters that should be considered when planning MEOR field projects. Laboratory compatibility studies and field efforts must be coordinated to fully evaluate the results.

INTRODUCTION

The design of a microbial enhanced oil recovery (MEOR) field project is just as important as its implementation. There are two major goals for the design of a microbial formulation to be injected into a petroleum reservoir. First and foremost, the microbial formulation must be able to grow prolifically under the environmental conditions of the reservoir. Temperature, salinity, and trace mineral concentrations must all be considered for optimal growth of the microorganisms. The second major criterion is that the microorganisms not only grow but also produce the desired chemical products for increasing oil recovery. Although fundamental research has been conducted in characterizing microorganisms, their products, and the mechanisms

of oil recovery, questions regarding which microbial product is the most important for improving oil recovery remain unanswered.

A key concept for in situ MEOR processes that must be considered is that injection of a living system is not comparable to the injection of a pure chemical. A living microbial system duplicates itself and produces chemicals in the rock pore matrix. The concentrations and compositions of microbially produced chemicals can be totally different from chemicals produced above ground. It becomes important, therefore, to establish certain criteria for microbial oil mobilization under the conditions of the targeted reservoir.

Generally, compatibility testing of microbial formulations with reservoir fluids is conducted in test tubes, and as long as all fluids are screened, this is usually adequate. Most microbial systems used in field tests have been microorganisms that were originally isolated from petroleum reservoirs and thus could be adapted to the temperatures, pressures, and salinities encountered in a petroleum reservoir. Factors to consider include compatibility of microbes with produced waters, injection water (in the case of a microbial waterflood), nutrients, crude oil, and reservoir rock. After completion of compatibility testing, the first criterion of laboratory optimization has been satisfied.

The task of optimization of the microbial formulation is performed using porous media testing. If Berea sandstone cores are used, efforts must be made to match the permeability of the target reservoir. Reservoir cores should be used, if at all possible, to duplicate the mineralogy and permeability of the reservoir formation. Microbial coreflooding will yield useful results concerning oil recovery efficiency and compatibility of the microbial formulation with reservoir rocks and fluids, particularly if reservoir core is used. It should be pointed out, however, that the oil recovery obtained in the laboratory cores will usually be higher than field results because of the difference in sweep efficiency of a coreflood and a full-scale reservoir.

This paper describes the specific experiments designed and conducted for the microbial-enhanced waterflooding field project in Delaware-Childers field. The project is located in the Mink Unit of Delaware-Childers field located about 20 miles from Nowata, OK. The Mink Unit produces from the Bartlesville sand formation and is owned and operated by B & N Oil Co. B & N owns 1,720 acres in Delaware-Childers field, with more than 200 injectors and producers, and has been waterflooding the field since 1953. Oil production for the field is about 160 bbl/day. Overall, the Mink Unit contains 21 injectors and 15 producers, with all producers flowing except one (S-CP-2). The pilot area of the Mink Unit is shown in figure 1. It contains four injectors, eight on-site producers, and two off-site producers. The Mink Site has four adjacent inverted five-spots, but is not a perfectly geometric well pattern because some of the wells have been plugged. An off-pattern well, S-P47R, is only 215 ft away from the injection wells and serves as an excellent monitoring well for the experiment. The average injection pressure is 535 psi, and the average injection volume is 40 bbl/day/well. The oil production averages 6.4 bbl/day for the Mink Unit, which is 0.4 bbl/day for each producer. Core analyses obtained from historical records indicate that the current residual oil saturation in the Mink Unit is 30%, the average permeability is 63 md, and the porosity is 20%. A baseline monitoring program was initiated that included weekly sampling for 18 weeks and ended March 17,

1987. A comprehensive field sampling program included monitoring (1) total oil production (Mink lease); (2) total water production (Mink lease); (3) injection well pressures and fluid rates from each well; and (4) production well fluid rates and water-oil ratio of each well.

ACKNOWLEDGMENTS

The authors wish to thank James M. Chism and Dr. Fred W. Burtch of the Bartlesville Project Office, U.S. Department of Energy for their valuable suggestions in conducting this research, and Tina Long for her assistance in typing this manuscript. This work has been supported by the U.S. Department of Energy under Cooperative Agreement No. DE-FC22-83FE60149.

MATERIALS AND METHODS

Core preparation

Blocks of Berea sandstone were obtained from Cleveland Quarries (Amherst, Ohio) and cut into cylindrical cores 10 inches in length and 1½ inches in diameter. The cores were fired at 800° F for 24 hours to stabilize the clays.[1] Cores were either encapsulated in epoxy resin with inlet and outlet valves at the ends or encased in rubber sleeves and placed inside stainless steel Hassler coreholders. The cores were then evacuated and flushed with brine (0.5% NaCl or Mink Unit formation water). Darcy's Law was applied to determine absolute permeability of each core. Crude oil from Delaware-Childers field in Nowata County, OK, was injected into the cores until no additional brine was produced (about 24 hr; pressure drop was less than 1 psi), then brine was flushed through the core until no more crude oil was produced. The cores thus simulated a waterflooded residual oil-saturated condition designated by S_{owf} (% pore volume).

Coreflood Apparatus

The experimental equipment used for microbial coreflooding experiments has been described previously.[2] Cores were encapsulated in epoxy resin or encased in Hassler coreholders. Fluid piston devices were used to inject the microbial solutions and other fluids into the cores and prevent corrosive fluids from contacting the pumps. The frontal advance rate for the cores was 1 ft/day.

Micromodels

The glass micromodels used in this study have been described previously by Bryant and Douglas[2] and Chatzis.[3] The micromodels were saturated with produced water from the Mink Unit and oil flooded with Mink Unit crude oil, and finally waterflooded with Mink Unit injection water to residual oil saturation before microbial injection.

Chemicals and Media

All chemicals and bacteriological media were obtained from Sigma Chemical Co. and Curtin Matheson Scientific, Inc., respectively. Two types of molasses were used for compatibility studies and microbial coreflooding experiments: one, Mr. Blackstrap 87, has been described previously[2]; and the other was supplied by Pacific Molasses Co., Oklahoma City. Both types are cane molasses; Pacific molasses contains at least 46% total sugars, while Mr. Blackstrap 87 contains 38% total sugars.

Microorganisms

NIPER 1 is a strain of *Bacillus licheniformis*, ATCC #39307, that produces surfactants from metabolism of molasses. NIPER 2 is a strain of *Bacillus* that produces surfactant, acids, and some carbon dioxide. NIPER 3 is a species of *Clostridium* that produces carbon dioxide, ethanol, butyric acid, and surfactant when fermenting sucrose. NIPER 4 is a Gram-negative, facultatively anaerobic rod that produces carbon dioxide and acids when fermenting sucrose. NIPER 12 is another Gram-negative facultatively anaerobic rod with products similar to NIPER 4.

Methodology

Coreflooding experiments with these microorganisms have been described previously by Bryant and Douglas.[2] Berea sandstone cores that had been waterflooded to residual oil saturation (S_{owf} percent) were prepared for injection with microorganisms. The cores were injected with 0.2 PV of a solution of bacterial cells (at a concentration of approximately 1 X 10^6 cells/ml) in trypticase soy broth and then 0.3 PV of 4% molasses solution. The cores were allowed to incubate at 37° C for 1 week. A pressure gauge was used on the core to determine the increase in pressure while the core was incubating.

RESULTS AND DISCUSSION

Laboratory studies were conducted on samples obtained weekly from the Mink Unit during Oct. 28, 1986, - March 17, 1987. Only the laboratory data will be given in this paper. Samples were analyzed to determine crude oil viscosity, total dissolved solids (TDS), pH, and trace minerals. Table 1 gives the results of the baseline measurements from each producing well sampled. The concentration of TDS (percent) from each well has remained relatively stable (within ±0.03%) during the baseline sampling period. The TDS values range from 0.30 to 1.12% for the Mink Unit producing wells. The average pH of water from each producing well has remained between 6.5 and 6.7. Trace mineral and ion analyses indicated no abnormal concentrations of sodium, calcium, magnesium, strontium, barium, potassium, iron, copper, zinc, nickel, chloride, sulfate, bicarbonate, carbonate, hydroxide, or phosphate. No unusually high concentration of any trace mineral that might interfere with the growth and metabolism of the microorganisms was noted.

Oil viscosities of samples taken from each producing well during the baseline period showed that although they vary from week to week by as much as 2 centipoises, most oil viscosity average ranges were relatively stable (±0.75 to 2.0 cp, table 2). The post-microbial injection averages, though not really statistically different, showed some interesting trends. S-P47R well, the well closest to the injectors, showed a significant increase in oil viscosity. This increase may represent oil from previously unswept regions, although there are not enough data to support this hypothesis at present.

Test tube compatibility studies were performed with several different microbial formulations from INJECTECH, Inc. and NIPER. Results from these tests indicated that the nutrient molasses to be used for the field experiment supported good growth and metabolite production from all microorganisms. Also, the reservoir brine and crude oil appeared to have no adverse affects on their growth and metabolite production. Berea sandstone corefloods were then used to determine oil recovery efficiencies (E_r) for various microbial formulations. Representative data from these corefloods are given in table 3. More complete information may be obtained from Bryant and Douglas, 1987.[2] NIPER 2 and 3 had the highest E_r of all microbes tested, 53 and 60%, respectively. NIPER 1 was selected because of its ability to perform consistently in Berea sandstone and survive better than other microbial species. NIPER 4 was selected because of its prolific gas production and survival in Berea sandstone.

The microbial formulation selected for the waterflood injection was NIPER Bac 1; a bacterial mixture comprised of NIPER 1, 2, 3, and 4 (table 4). After the microbial formulation was selected based upon laboratory and single well injection results, a field core from Delaware-Childers field (Costen lease) was obtained and tested with NIPER Bac 1. This field core had not been preserved but represents reservoir lithology more accurately than Berea sandstone. Table 5 and figure 1 summarize the results from this microbial coreflood. Based upon these data, it was found that NIPER Bac 1 recovered 28.3 percent of the residual Mink crude oil remaining in the core after waterflooding. The pH of the core effluent fractions was lower during the oil recovery period, and higher at the beginning and end of the waterflood, which indicates that the microbes are producing acids that may aid in oil recovery improvement. NIPER Bac 1 produces ethanol, butyric acid, and other short chain fatty acids.

Two single-well injection tests were performed with NIPER Bac 1 and another formulation both of which were optimized for this field. These tests provided valuable information regarding the survivability of the microbial formulations and the injectivity of the microbes and nutrient. Backflush water samples were analyzed to determine if injection of nutrients stimulated the indigenous population of sulfate-reducing microorganisms. Microbes and nutrients were injected into the wells which were then shut in for 12 days, and backflushed. During backflushing, samples were collected every 10 to 15 minutes until microorganisms and molasses were detected. Several interesting observations were noted during this test. Samples from the injection wells showed a dramatic change in color and turbidity after about 1½ hours of backflushing. The samples also contained gas and foamed. The injection rates and pressures after the microbial injection and shut in activity were normal, indicating no plugging in either well. A control well was monitored to determine whether backflush pressures were normal, and the pressures observed

were equivalent to those of the two test wells. All microorganisms that were injected were also detected after 12 days of incubation in the reservoir. The microbial counts were high, around 10^4 and 10^5 cells/ml, which indicated that the microorganisms were growing and metabolizing after 12 days under reservoir conditions. The pH of the backflush samples showed a dramatic decrease from 8.0 to 5.0 when the microorganisms were found in the sample which indicates that some acid is being produced by the microbes. No sulfate-reducing bacteria were found in the backflush samples, nor was any hydrogen sulfide detected.

Micromodel studies were carried out simultaneously with this coreflood to determine if the microbial formulation could mobilize oil in the simulated porous media. A glass micromodel was saturated with brine from the Mink tank battery and flooded with crude oil from the Mink lease. The micromodel was then flooded with Mink plant injection water until no more oil movement was obtained (residual oil saturation). NIPER Bac 1 (0.1 PV) in molasses was injected and the micromodel shut in at room temperature for 3 days. The micromodel was then waterflooded and the progress of the waterflood was monitored using a video-enhanced microscopy apparatus. It was observed that some gas bubbles were produced which tended to swell the oil in the pores. During the waterflood, a large amount of the oil was subsequently mobilized.

SUMMARY OF RESULTS

1. Baseline data obtained from weekly sampling of producing wells from the Mink Unit have been very stable. This stability allows us to determine any changes that might be occurring.

2. Single well injection tests showed that the microbial formulation (NIPER Bac 1) survived and metabolized during a 12 day shut-in period. The injection pressures were unchanged, indicating that no plugging occurred.

3. Laboratory porous media studies showed that NIPER Bac 1 significantly increased oil production over that of a waterflood. Results were corroborated with a Delaware-Childers field core and glass micromodel experiments.

RECOMMENDATION

Careful design and testing of microbial formulations in the laboratory using reservoir fluids and preferably reservoir cores should be done prior to any MEOR field project. There are some microorganisms that grow very well under laboratory conditions, but could not mobilize residual oil in porous media studies.

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TABLE 1. - TDS, pH, and trace mineral analyses from Mink Unit field water samples

Well	TDS, %		pH	
	Baseline Average	Post[1] Average	Baseline Average	Post[1] Average
CBP2	0.66 ± 0.02	0.66 ± 0.05	6.63 ± 0.28	6.57 ± 0.17
CCP1	1.08 ± 0.05	1.00 ± 0.07	6.61 ± 0.30	6.59 ± 0.24
CCP3	1.12 ± 0.01	1.08 ± 0.07	6.54 ± 0.18	6.60 ± 0.21
SAP1	0.66 ± 0.03	0.67 ± 0.06	6.63 ± 0.16	6.62 ± 0.24
SAP2	1.06 ± 0.10	1.08 ± 0.06	6.54 ± 0.27	6.53 ± 0.27
SP47R	0.28 ± 0.03	0.28 ± 0.02	6.53 ± 0.11	6.58 ± 0.17
SBP1	0.48 ± 0.03	0.49 ± 0.04	6.66 ± 0.18	6.65 ± 0.23
SBP2	0.55 ± 0.11	0.50 ± 0.04	6.56 ± 0.22	6.66 ± 0.29
SBP3	0.48 ± 0.02	0.48 ± 0.03	6.65 ± 0.30	6.59 ± 0.18
SAP4	0.73 ± 0.03	0.68 ± 0.02	6.55 ± 0.14	6.61 ± 0.21
PLANT	0.03 ± 0.00	0.03 ± 0.01	6.65 ± 0.34	6.68 ± 0.18
T.B.	0.50 ± 0.03	0.52 ± 0.06	7.40 ± 0.15	7.58 ± 0.25

Cation mg/l	Plant mg/l	Tank Battery mg/l	Well SAP2 mg/l
Sodium	12	1,183	3,176
Calcium	34	156	330
Magnesium	53	289	217
Strontium	0.4	30	20
Barium	0.2	134	144
Potassium	4.5	8.7	14
Iron	0.7	5	2
Copper	<0.1	<0.5	<0.5
Zinc	<0.1	<0.5	<0.5
Nickel	<0.1	<0.1	<0.5

Anion mg/l	Plant mg/l	Tank Battery mg/l	Well SAP2 mg/l
Chloride	17	2,037	5,294
Sulfate	15	12	12
Bicarbonate	135	1,450	1,800
Carbonate	0	0	0
Hydroxide	0	0	0
Phosphate	<0.5	<2	<2

[1]Last sampling date for the average was Aug. 26, 1987.

TABLE 2. - Viscosity of crude oil samples from the Mink Unit wells

Well	Viscosity, cp	
	Baseline, Avg.	Post[1], Avg.
CBP2	6.79 ± 0.78	7.03 ± 0.76
CCP1	5.90 ± 0.81	6.11 ± 0.76
CCP3	6.71 ± 1.0	6.08 ± 0.79
SAP1	5.73 ± 0.35	5.79 ± 0.54
SAP2	7.42 ± 1.0	6.71 ± 0.99
SP47R	7.50 ± 0.75	8.87 ± 0.68
SBP1	6.43 ± 1.1	7.87 ± 1.4
SBP2	6.23 ± 0.75	6.95 ± 0.85
SBP3	6.92 ± 1.2	6.57 ± 0.73
SAP4	8.11 ± 2.0	7.05 ± 1.2

[1]Last sampling date was Oct. 14, 1987.

TABLE 3. - Microbial coreflood experiments with Delaware-Childers crude oil

Core	Microorganism	k	S_{oi}	S_{owf}	S_{ocf}	E_r	Total oil recovery, bbl
			Non-sporeforming genera				
M44	NIPER 12	304	65.4	29.7	19.3	35.0	70.5
M61	NIPER 12	293	63.1	27.5	19.1	30.4	69.7
M40	NIPER 4	439	63.8	29.3	20.9	28.8	67.1
M43	NIPER 4	164	65.4	27.2	20.5	26.6	68.7
			Sporeforming genera				
M68	NIPER 2	180	73.9	25.0	11.7	53.0	84.1
M94	NIPER 1	332	77.4	34.8	27.6	20.7	64.3
M69	NIPER 3	134	82.3	22.8	8.7	60.4	89.1
M77	NIPER 3	344	71.1	34.0	18.3	46.2	74.2

k = Absolute permeability to brine in millidarcies.
S_{oi} = Initial oil saturation prior to waterflood (%).
S_{owf} = Residual oil saturation after waterflood (%).
S_{ocf} = Residual oil saturation after microbial treatment (%).
E_r = Residual recovery efficiency (%). $\frac{S_{oi} - S_{ocf}}{S_{oi}} \times 100\%$.

Total oil recovery = $\frac{S_{oi} - S_{ocf}}{S_{oi}} \times 100\%$.

TABLE 4. - NIPER Bac 1 characterization: four different microbial species designated as NIPER 1, NIPER 2, NIPER 3, and NIPER 4

NIPER 1 - *Bacillus licheniformis* ATCC #39307, surfactant and acid producer
NIPER 2 - *Bacillus* species, gas, acid, and surfactant producer
NIPER 3 - *Clostridium* species, gas, surfactant, alcohol, and acid producer
NIPER 4 - Gram-negative facultatively anaerobic rod, gas, and acid producer

TABLE 5. - Microbial field core data and preparation protocol - Core No. MSC23

Rock composition:	field core	Porosity, %:	19.35	Viscosity, cp:	7
Dry weight, g:	2461.5	Encapsulation:	epoxy	Water vol., ml:	32.7
Wet weight, g:	2509.8	Brine conc., %:	0.55	Oil vol., ml:	17.5
X-sec area, cm^2:	11.045	Flow rate, ml/s:	0.058	Residual oil, ml:	15.2
Length, cm:	22.6	Pressure, psi:	33.5	S_{oi}, % PV:	67.70
Core vol., ml:	249.6	Permeability, D:	0.052	S_{owf}, % PV:	31.47
Pore vol., ml:	48.3	Oil type used:	Mink lease		

Tube Vol, ml		Oil/	S_{ocf},	Recovery,	Brine,	
Water	Oil	Water	% PV	%	PV	pH
8.50		0.000	31.47		0.17	7.70
8.90		0.000	31.47		0.36	7.75
9.20		0.000	31.47		0.55	7.00
9.40		0.000	31.47		0.74	6.35
9.20	0.50	0.054	30.44	3.28	0.94	6.20
9.20	0.20	0.022	30.02	4.60	1.14	6.25
9.30		0.000	30.02	4.60	1.33	6.15
9.35	0.05	0.005	29.92	4.93	1.53	6.55
9.40		0.000	29.92	4.93	1.72	6.65
9.40		0.000	29.92	4.93	1.92	6.70
9.30		0.000	29.09	4.93	2.11	6.85
9.30	0.40	0.043	29.09	7.56	2.31	6.50
9.30		0.000	29.09	7.56	2.50	6.60
9.60		0.000	29.09	7.56	2.70	6.85
9.50		0.000	28.26	7.56	2.90	6.75
9.35	0.40	0.043	27.64	10.19	3.10	6.50
9.50	0.30	0.032	27.64	12.17	3.30	6.55
9.50		0.000	27.64	12.17	3.50	6.55
9.40		0.000	26.81	12.17	3.70	6.65
9.30	0.40	0.043	26.81	14.80	3.90	6.65
9.40		0.000	26.81	14.80	4.09	6.70
9.30		0.000	25.77	14.80	4.28	6.80
9.20	0.50	0.054	25.16	18.09	4.48	6.55
9.50	0.30	0.032	24.43	20.06	4.69	6.55
9.50	0.35	0.037	24.22	22.36	4.89	6.45
9.85	0.10	0.010	23.60	23.02	5.10	6.50
9.90	0.30	0.030	23.60	25.00	5.31	6.85
10.00	0.10	0.010	23.40	25.65	5.52	6.55
9.50	0.40	0.042	22.57	28.28	5.72	6.55
7.90		0.000	22.57	28.28	5.89	6.55
7.90		0.000	22.57	28.28	6.05	7.10
8.00		0.000	22.57	28.28	6.21	7.10
14.00		0.000	22.57	28.28	6.50	7.35
11.50		0.000	22.57	28.28	6.74	7.80
11.50		0.000	22.57	28.28	6.98	7.80
13.00		0.000	22.57	28.28	7.25	7.75
13.00		0.000	22.57	28.28	7.52	7.75
10.10		0.000	22.57	28.28	7.73	7.60

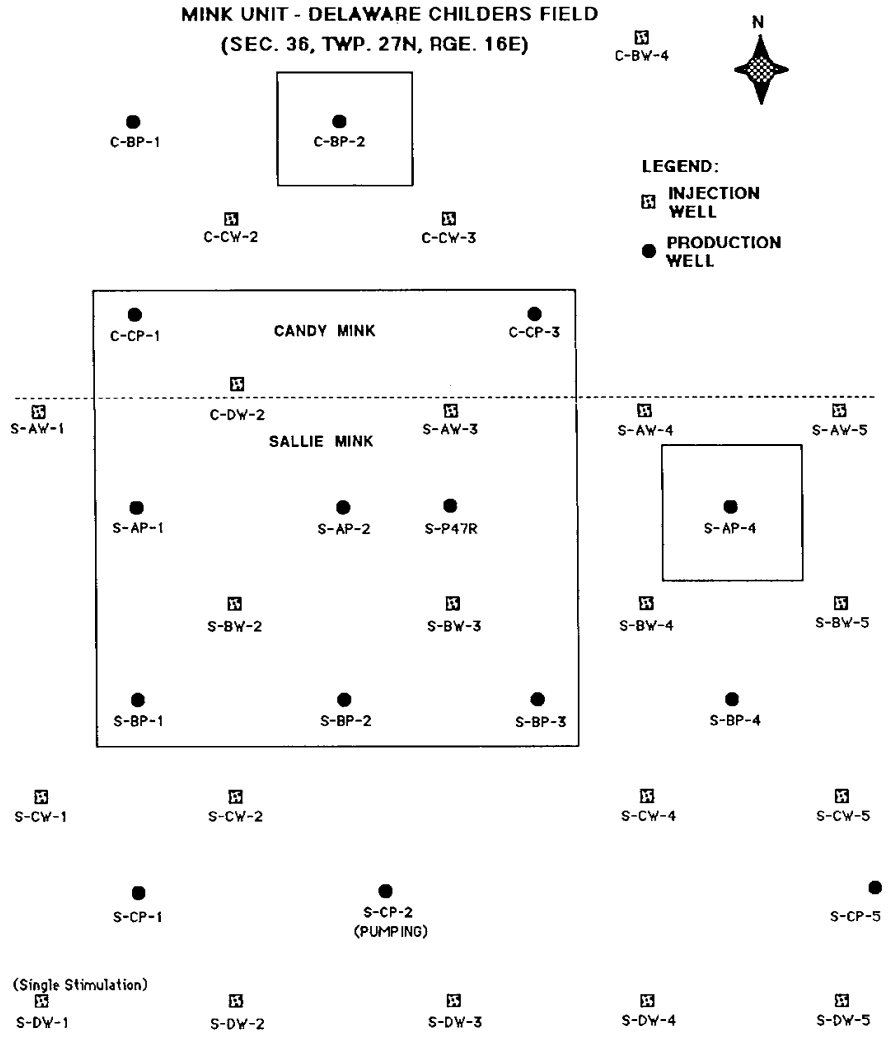

FIGURE 1. - Map of Mink Unit/DOE Project Site.

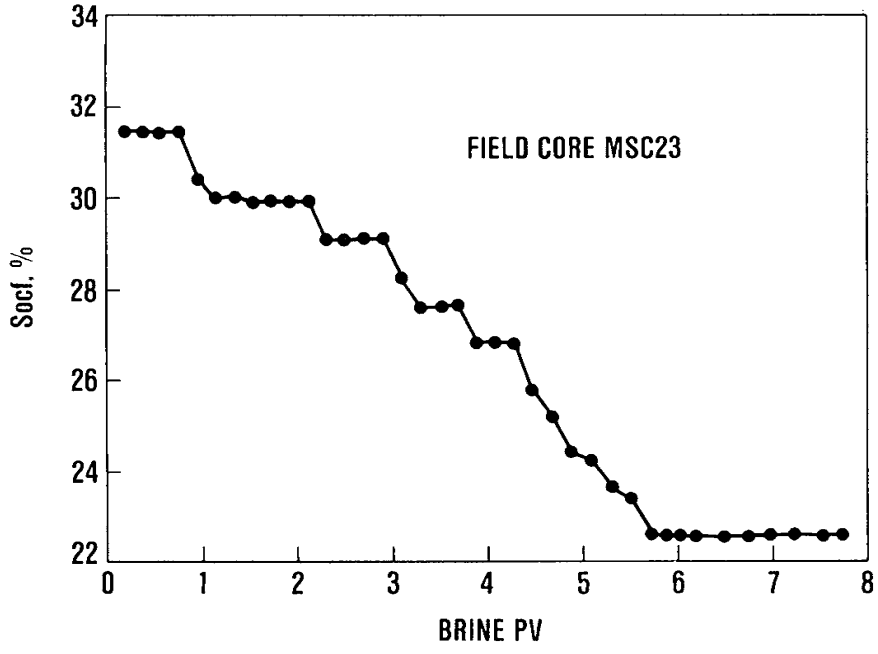

FIGURE 2. - Residual oil saturation versus brine injected.

MICROBIAL FIELD SAMPLING TECHNIQUES FOR MEOR PROCESSES

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(918)535-2281

ABSTRACT

Field studies of MEOR processes require routine monitoring to determine effects microorganisms exert in the release of oil from petroleum-bearing formations. Baseline monitoring of oil production, flow rates, oil-water ratios, temperature, pH, viscosity, ionic strength, etc. allow the investigator to observe real changes that occur as a result of microbial activities after selected microbes are injected. Simple techniques to determine microbial counts can be utilized to determine viability and transport of injected microbes. The effect of injected energy sources, such as molasses, on indigenous microbes inhabiting a reservoir can also be detected. We have participated in a MEOR-waterflood project sponsored by the DOE in Nowata County, Oklahoma. Data accumulated from this ongoing project demonstrates the feasibility of field testing of MEOR.

INTRODUCTION

Field investigations of MEOR processes, by their size and nature, limit the investigator's experimental freedom. The starting conditions of a multitude of variables are generally not known, which necessitates frequent sampling over a long period of time. A battery of tests must be incorporated into any experimental scheme to cover both known and

unknown variables. However, simple methods can often reveal much information if proper planning for routine monitoring programs are implemented.

Microbial formulations for a given MEOR project must be designed in the laboratory under conditions which approximate the reservoir environment. The candidate formulation should be well characterized if one wishes to distinguish injected microbes from the microbes indigenous to the reservoir. Of course, the investigator should have knowledge of the indigenous microbial population as well.

Methods to estimate the total number of living microbes in environmental samples have relied upon many methods. The "total viable plate count" is the method most widely utilized. The term "viable" is used to describe those cells in the sample being analyzed that are capable of multiplying and forming colonies on solid media. Microorganisms vary greatly in their nutritional and environmental requirements. No single medium or set of growth conditions will permit the growth of all the microorganisms present in a given ecosphere. Consequently, any medium suitable for the growth of a specific organism is selective for that organism. As inefficient as the plate count method may be however, it can and does yield valuable information as long as the investigator remembers its inherent limitations.

We used plate count methods and a "total carbohydrate" assay to monitor the progress of injected fluids in a DOE-sponsored MEOR field test in Nowata County, Oklahoma. Microbial counts were established for 10 producing wells, the injection water, and the tank battery water before and after injection of the microbial formulation and molasses feed. Sulfate-reducing bacteria (SRB) were also monitored following API recommended procedures. This field test is presently underway and data are still being accumulated.

FIELD STUDIES

Sampling

Any competent field study of MEOR requires frequent sampling. The field study in Nowata County encompasses 10 producing wells, 4 water injection wells, and the

associated tank battery. Weekly samples were collected from each well for 18 weeks prior to the injection of the microbial formulation. Catch samples from a flowing stream were collected in sterile 4 oz. flint glass bottles. Each bottle was filled completely and tightly capped. The samples were taken to the laboratory and processed immediately.

It was recognized early that, as expected, the field under study was anaerobic. Although the samples were taken aerobically, samples from the producing wells were capable of reducing resazurin to the colorless state within one hour. Redox potentials were frequently measured at less than -100 mV at pH 7. Additionally, the majority of the iron content of the produced water was found to be in the ferrous state. Measurements performed with a Clark dissolved oxygen electrode were useless, because the mineral content of the produced water interfered with the probes proper function. The true redox potential within the oil reservoir was not accurately determined. However, the data obtained from air-exposed samples indicate that the E^o of the reservoir must be less than -100 mV. Sample collection techniques for water samples that exclude exposure to air were conceived, but they could not be implemented within the time and budgetary constraints of the study. Consequently, the determination of the microbial population that were strict anaerobes, particularly the methanogens, was not possible. Also not possible was the estimation of the sessile microbial population residing within the reservoir that cannot be determined by water sampling techniques.

The microbial formulation that was injected into the reservoir consisted of three facultatives and one anaerobe; each microbe possessing the capability of fermentative growth on a carbohydrate-based feedstock. Short-term exposure to air was tolerated by each microbe. Therefore, the sampling techniques and the subsequent plating techniques utilized in the laboratory were adequate for the detection and quantification of the injected microbes from the oil field waters.

Plate Counts

Water samples from the field were immediately processed within a Forma 1024 anaerobic chamber. Pour plates of appropriate dilutions using Bacto Plate Count Agar (Difco) were prepared. Plates incubated anaerobically within the chamber

incorporated sodium thioglycollate (0.1% w/v) as a reducing agent and resazurin (0.0001% w/v) as an Eh indicator. Plates for aerobic plate counts were removed from the anaerobic chamber and were incubated at room temperature. No reducing agent or redox indicator was added to the aerobic plates.

Composite aerobic and anaerobic plate counts from the ten producing wells are illustrated in Fig. 1 and Fig. 2. As a rule, the anaerobic plate counts yielded less sporadic results than the aerobic plate counts. An increase in the anaerobic and aerobic counts over baseline values occurred shortly after the injection of the microbial formulation and molasses. However, at this time, no formulation microorganisms have been identified from the production wells. The microbial count increases are believed to result from molasses penetration into the reservoir which stimulated the indigenous microbial population. Backflush samples from the water injection wells have yielded formulation microorganisms in high concentrations. Evidence to date indicates that the injected microbial formulation can survive within the reservoir and produce microbial products.

Molasses Detection

Molasses was detected in the production well waters using an anthrone carbohydrate assay. One week after injection of the microbial formulation and molasses, molasses was detected in 7 producing wells and the tank battery (Fig. 3). Interestingly, the injection wells had been shut-in during this time period. The injection wells were turned on two weeks after injection and molasses was subsequently fed twice a week to each of the 4 injection wells. No molasses was detected in any of the produced water samples after 5 subsequent weeks of its initial appearance. At week 7 after injection, molasses sporadically appeared in a few production wells for about 4 weeks and then disappeared. The appearance of molasses early in the test indicated the presence of low volume stringers within the reservoir. The subsequent disappearance of detectable molasses indicated the establishment of a microbial population that consumed all of the fed molasses. The transport rate of molasses through the formation appeared to be much greater than that of the injected microbial formulation. This could be the result of adsorption of the microbial cells to the rock surface. The presence of molasses and/or stimulated indigenous microbial populations occurred

primarily in the north wells of the pattern. This directional flow pattern from the injection wells supplied additional evidence for the presence of low volume stringers within the formation.

Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) were monitored using lactate-based API sulfate broth. No significant change has occurred in the SRB population after injection of the microbial formulation and molasses. Additionally, no "black water" has appeared before or after injection. The low SRB activity is probably more a reflection of the low sulfate content of the reservoir than any other factor.

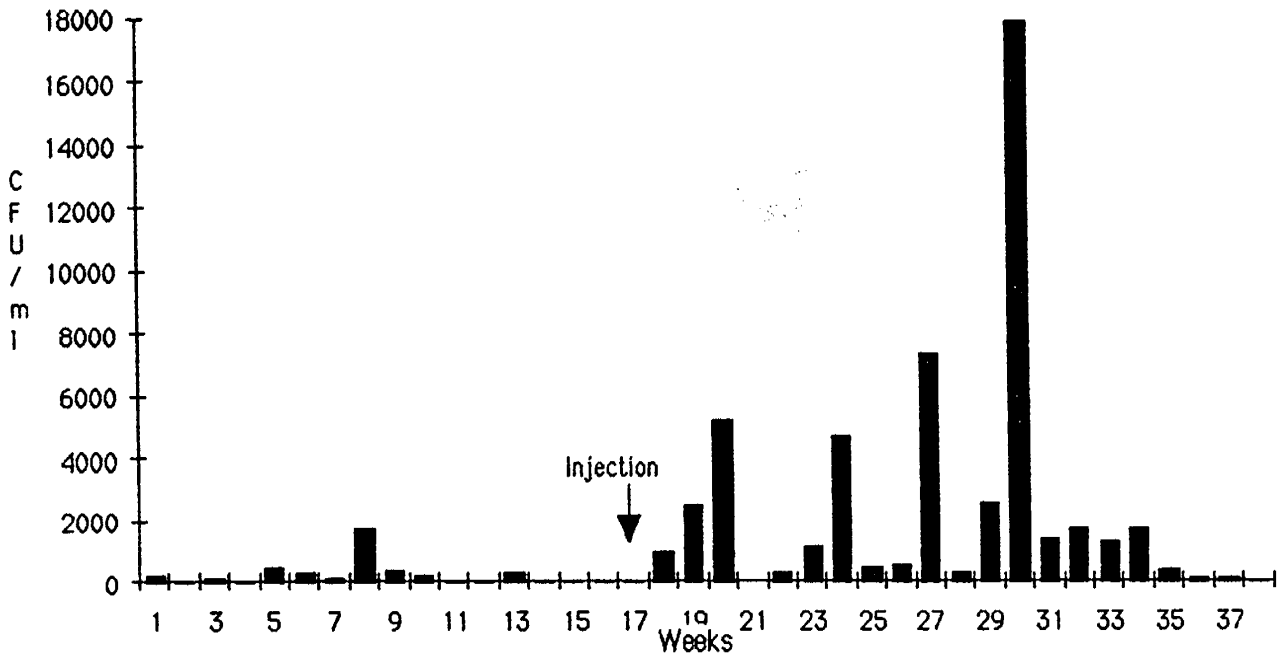

FIGURE 1. - Composite Aerobic microbial count.

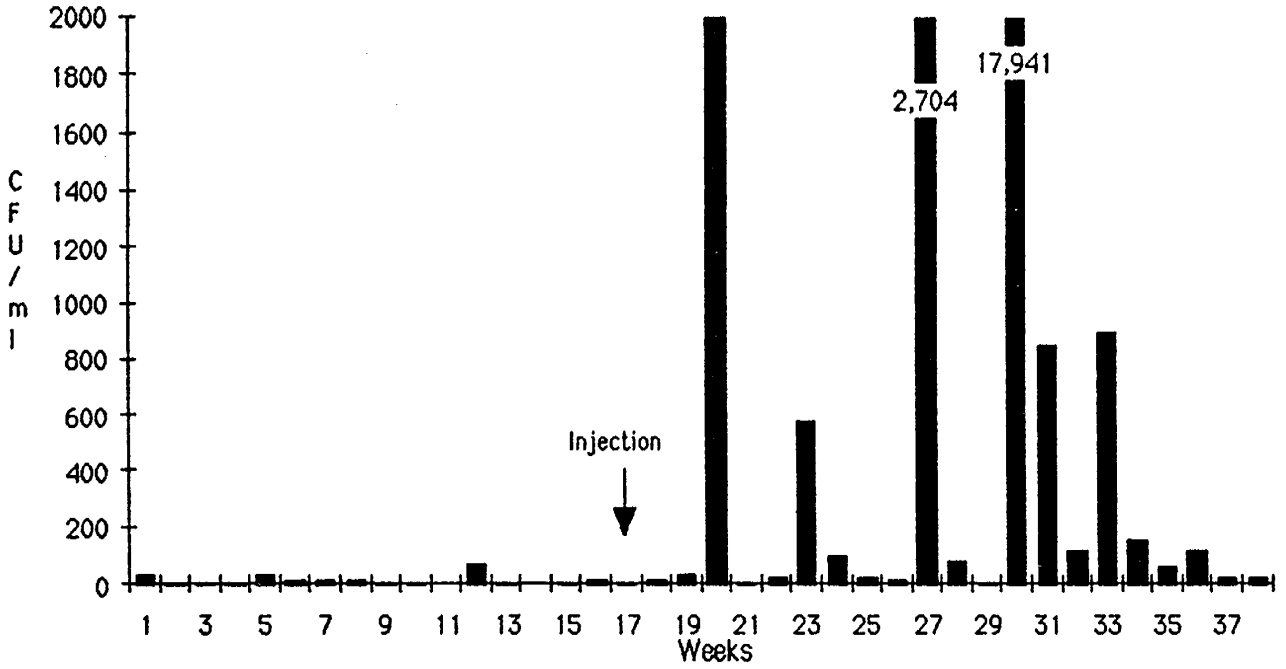

FIGURE 2. - Composite Anaerobic microbial count.

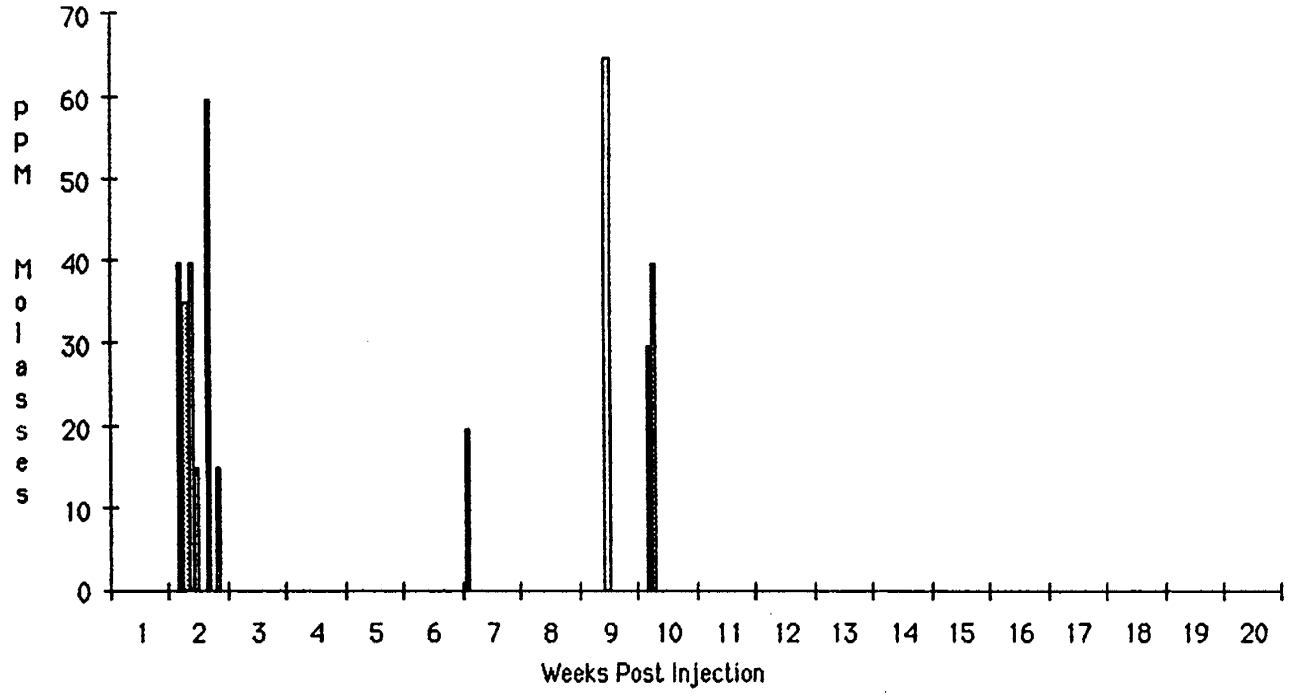

FIGURE 3. - Detected molasses in production wells

MICROBIAL ENHANCED OIL PRODUCTION FIELD TESTS IN TEXAS

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ABSTRACT

Alpha Environmental Corporation uses a mixed community of hydrocarbon degrading microorganisms together with inorganic nutrients and a biocatalyst as the basis of a microbial enhanced oil recovery system. We have developed very active mixed cultures of bacteria by screening and isolating microorganisms from areas of known historical oil contamination. Alpha's mixed community of microorganisms decompose crude oil and produce detergents and CO_2, decrease the pH, and mechanically and chemically release oil from pore spaces. We have tested these organisms in two different oil fields. Results show a highly promising increase in oil production, a qualitative decrease in paraffin, and a reduction of Basic Sediment and Water (BS&W) in storage tanks. The action of the bacteria is enhanced by the use of a biocatalyst that provides catalytic oxygen in the formation and allows activity of the microorganisms in highly saline conditions, i.e., to twenty percent salinity. Data on the method of treatment and the oil production yield of the fields tested is presented.

INTRODUCTION

The principal role of microorganisms in nature is to form and destroy organic matter. Without this latter oxidative cycle, essential nutrients would be irreversibly bound in protoplasm. Photosynthesis and chemosynthesis result in the transformation of carbon dioxide to organic matter. From 50 ppm to 1 percent of living matter is hydrocarbon. Thus hydrocarbon oxidizing microorganisms evolved with early life. It is only in ancient sedimentary environments that hydrocarbons accumulate. In oxidizing environments, hydrocarbons have been continually decomposed since life began. Products of hydrocarbon degradation are co-oxidation and metabolic products, organic acids, carbon dioxide and microbial cells. Early microbiologists suggested that the different types of crude oil found in different sedimentary environments were the result of different types of microbial activities.

As microorganisms are associated with the genesis of petroleum it is only natural that one explore those microbial activities that may be responsible for the movement, concentration, and recovery of commercial amounts of hydrocarbons . It becomes obvious from a survey of the available literature that microbial production

of carbon dioxide, formation of detergents as fatty acids, production of gases, changes in hydrogen ion concentration, and the physical action of microbial cells during growth on hydrocarbons displace other hydrocarbons in sedimentary systems (Hitzman, 1983).

Alpha Environmental, A Texas Corporation, has completed a year of a field orientated, cooperative research program with the U.S. Department of Energy (Grant DE-FG 19-86BC 14014) to demonstate a process using microorganisms and a catalyst to economically recover residual oil in reservoirs. The target of our process is that portion of original oil unrecoverable by conventional techniques. The objective of the research program was to develop an economically viable (treatment cost between two thousand and four thousand dollars for an eight-well field) method of MEOR using natural hydrocarbon-oxidizing microorganisms to release crude oils in shallow, low-production oil fields. The results of the year-long field test indicate that we have met the cost restriction and have observed a substantial increase in crude oil production, a qualitative drop in viscosity and reduction of paraffin and BS&W.

The application of bacteria to oil reservoirs as a tool for secondary enhanced oil recovery is not a new concept or process (ZoBell, 1946, Shennan and Vance, 1987). Oil originates from organic matter generated, deposited, and preserved under sedimentary conditions (Hunt, 1979). This organic matter is converted to crude oil by several biological, chemical, and physical processes which include pressure and temperature, microorganisms, natural catalysts and geologic time (Beerstecher, 1954). It is also believed that microorganisms play a part in the migration of oil through sediments (Davis, 1967,p74). Oppenheimer (1980) describes the role of microorganisms in the ecological cycles of oil in the environment. In reviewing the vast amount of international literature on hydrocarbon microbiology, it is realistic to assume that biogeochemical processes may also be employed to release oil from reservoirs.

Professor Claude ZoBell was awarded U.S. Patent #2,413,278 in 1946 for a process to use bacteria to remove oil from sediments. He described the salt tolerant bacterium Desulfovibrio that used indigenous organic material in sediments as an energy source to reduce inorganic sulfates to acids and to produce detergents. During this period of history, oil operators found that Desulfovibrio was responsible for pipe and equipment corrosion. The patent of ZoBell was field tested but never accepted because of the corrosion impact on pipes and oilfield storage tanks.

In 1955, La Riviera demonstrated the release of hydrocarbons from laboratory sediment cores by Desulfovibrio, using lactate as an energy source. He suggested detergent production as the release mechanism. In 1956, ZoBell applied for a Patent for a process to reduce paraffin by microbial action, but the process was never implemented due to the poor economics of the time (U.S. Patent # 2,742,398, 1956).

Petroleum microbiology has an experimental history extending back 100 years. However, the field has only in the last 30 years become a major scientific

subject relating to oil pollution and secondary recovery (API Oil Spill Annual Conferences 1969 to present, and King and Stevens, I987). Bacteria, which utilize organic substances as an energy source, produce acids which in turn produce carbon dioxide from carbonates. Detergents and biomass are two by-products of bacterial metabolism and co-oxidation. All of the above can act at the oil-sediment interface and have been shown to release oil from sedimentary surfaces in laboratory experiments (Yen, A State of the Art Review on MEOR, no date). The oil-release mechanism involves acid-base equilibria, detergents, physical displacement and other surfactant phenomena. The practical restrictions to applying the process have been the cost of treatment, brine inhibition of microorganisms, lack of oxygen, and high subsurface temperatures. Pressure does not appear to be a problem as bacteria can tolerate high pressures and also can produce gas pressures up to 9000 psi (Oppenheimer, 1951).

At present, there is no published economical commercial application of MEOR (King and Stevens, I987). The primary deterent to the process is the high cost of the bacteria and field application, and the inability of microorganisms to survive and be active at the high salt concentrations in oil field brines. Temperature is a limiting factor for MEOR, but as many oil fields are within the temperature range of microorganisms this latter becomes a deterant only for deep, hot formations. Mechanical plugging of reservoir-rock pores and oil field pipes by bacteria cells and introduced nutrients is a second inhibitor.

As a student of Professor ZoBell, and a petroleum microbiologist, Oppenheimer has followed the scientific reports on MEOR since ZoBell's Patent in 1946. The salt problem and oxygen have remained the primary technical obstacles.

Alpha has acquired a unique biocatalyst that opens a pathway for an economic microbial process whereby microorganisms can be cultivated in very high salt concentrations and adapted to live and be active in brines up to 20 percent salt. This biocatalyst has an ability to stimulate microbial growth and increase the normal biomass of microbial fermenters in general by three orders of magnitude. Efficient laboratory production of a highly concentrated hydrocarbon degrading, salt-tolerant microbial community, combined with a method of preserving the bacteria in a dry powder form has permitted the development of an inexpensive and effective field application material.

Test Microorganisms

The microorganisms used in the field and laboratory tests were isolated as the result of 20 years of sampling throughout the world from many sites, ranging from the tropics to the polar regions, where hydrocarbons have been historically spilled in the environment. Composite soil and water samples were cultivated in inorganic nutrient-enriched sea water and fresh water, with crude oil as the only organic energy source. The crude oils used as substrate ranged from light South Texas crude to heavy Kuwait and Bunker C fuel oil. The enrichment media were made up from either filtered tap water or filtered sea water to which was added inorganic nitrogen and phosphate at 1 and .01 percent and adjusted to a pH of 7.6.

The continuous cultivation of these worldwide enrichment cultures on a wide variety of crude oils has produced a mixed culture of microorganisms, primarily bacteria, that actively decompose many types of crude oil. During the progess of several research projects at the Florida State University and the University of Texas, the rate of degradation of crude oil and the molecular fingerprint of changes in the crude during microbial activity was measured (Miget et. al. 1969, Kator, 1972, Oppenheimer 1971). The enriched cultures repeatedly utilized both aliphatic and aromatic molecules of a wide range of molecular weight in the various crude oil tested. No molecular hydrocarbon sparing action was noted.

Alpha Environmental's mixed cultures have been maintained in simple fermenters in the laboratory for several years and continue to be very active oil degraders, utilizing on average one ml of oil per liter of sea water medium in less than 24 hours. Under optimal conditions the oil is utilized in 12 hours. The oil is converted to fatty acids, metabolic by-products and microbial cells. The cells are harvested and concentrated in a dry powder by a proprietary technique. In the enriched sea water the cells grow consistantly to concentrations up to 10^{12} cells per ml. They are harvested from the mass culture and mixed into a dry biodegradable powder with a viability of 10^{12} cells per gram. This dry material has a shelf life of approximately one year. The bacteria consist of motile and non-motile bacteria, including rods, cocci and spirilli. When introduced to an oil-water emulsion they orient to the oil-water interface where they are biologically active as oil degraders. Sea water was selected as the culture medium because it effectively elliminates possible contamination by human pathogens.

For the field demonstrations Alpha Environmental selected enrichment cultures of microorganisms that convert the hydrocarbons to detergents with an acid reaction. The microorganisms were selected to displace hydrocarbons in the oil reservoir by three mechanisms: (1) to mobilize oil droplets by detergent action, (2) to physically displace oil by growing between the oil and the sediment, and (3) to produce an acid condition in which carbonates dissolve producing carbon dioxide which helps displace oil. It is possible that the microorganisms produce gases other than CO_2 as well.

The microorganisms actively grow up to temperatures of 60 degrees C and at salinities from fresh water to 20 percent salts, and in molecular oxygen concentrations near zero. This wide range of growth conditions, which increases the biological scope of the microorganisms, is due to the effect of a biocatalyst developed by ALPHA. The catalyst is used both to cultivate the microorganisms in the laboratory and is added to the injected treatment mixture and brine in the field applications. The catalyst is a natural liquid product that is not toxic and contains no visible turbidity or hydrocarbons.

FIELD TESTS

During the past year Alpha Environmental has studied and treated four producing oil leases in two different types of oil fields. Maps of these leases and

the spatial relationships between injection and producing wells are shown in Figures I and 2. Three leases in the LaVernia Field, a sandstone reservoir located South East of San Antonio, Texas were studied , two of which were treated with the Alpha MEOR system. A second location, the Boynton Heirs lease, a limestone reservoir in East Texas near Schreveport, LA. was also treated with MEOR techniques.

Field application of Alpha's MEOR system was preceeded by a geological survey to characterize the producing reservoir and to locate optimal wells for injection. A summary of geological data on the two demonstration reservoirs is presented in Tables 1 and 2. Historical data on well completion, well service, chemical treatments, and oil production were collected and analyzed to build a baseline field characterization for comparison with post-treatment results.

Prior to injection samples of oil and brine were tested for microorganism compatibility, toxicity, and for the occurence of indigenous hydrocarbon-oxidizing microorganisms. After injection the treated well and surrounding producing wells were sampled for hydrocarbon microorganisms at frequent intervals. The population and activity of hydrocarbon-oxidizing microorganisms were monitored at each well in order to determine if, and at what rate, they moved through the formation from the injection well. Numbers of microorganisms were determined by standard dilution methods using sea water medium enriched with nitrogen and phosphate according to Oppenheimer et.al., 1979.

The first field test was initiated in well #7 of the Vorphal Lease near LaVernia, Texas (Figure 1). Well bottom temperatures were less than 120 degrees F. Brines ranged up to 11 percent salts. A solution consisting of microorganisms (45.35 Kg dry weight, 10^9 cells/gm), 200 liters of catalyst, 2000 liters of formation water, and inorganic nutrients was injected into a well by gravity feed. Immediately before injection, the well was pumped for 24 hours to create a vacuum and to remove fluids from the casing. During the injection all other producing wells were pumped on their established schedule.

Well #7 was treated for five days, shut-in for five days and then put back on production. Microbial populations were monitored in the surrounding wells. Little to no increase in microbial population and activity was observed in the wells that were down dip from the treatment well (Vorphal#4,11,12,13, and 14, fig. 1). Significant increases in microbial population were observed in the wells to the North East of the treatment well(Vorphal# 1,2,1A, 9, and Penshorn Lease wells). The wells to the North East lie updip from the treated well and along the trend of the underlying Luling Fault Zone.

Oil production in the wells to the North East increased within three weeks of the treatment. Figure 3 shows oil production from the Penshorn lease before and after the treatment of the Vorphal well #7. It appears that the treatment of the Vorphal #7 well stimulated oil production in the nearby Penshorn Lease. The reservoir in the LaVernia Field dips down to the south south east. Microbial activity and increased oil production appeared up-dip from the treatment well in the producing formation.

Four wells on the Wiseman Lease (wells 16-19, on the extreme left of fig. 1) are located directly updip from well #20, the deepest completion and furthest down dip well on the lease. Well # 20 was treated in November, 1986 and wells 19-16 observed for signs of microbial activity and changes in oil production. Production history for the Wiseman lease before and after injection is reported in Figure 4. Substantial increases in microbial population were observed in a sequential pattern from well #19 to well # 16. We believe this confirms our observation that the microbes migrate up-dip in this reservoir.

The Boynton Heirs Lease was studied and treated with essentially the same procedure in July, 1986. Two wells were selected as treatment sites on a lease of twelve producing wells. Post-treatment production history superimposed over a decline curve calculated from pre-treatment production data is illustrated in figure 5.

The production data of each field was statistically treated by calculating five day running averages of daily production. The data presented in figures 3-5 are reported as five day running averages in units of barrels-per-day. Actual daily production values varied erratically due to variations in tank gauging time and tank gauging technique. Five day running averages smooth the production data, helping illuminate trends from the background variability of production volumes caused by operational inconsistencies.

The produced water from individual wells was tested before and after injection at various time intervals to determine the concentration of hydrocarbon oxidizing microorganisms. The results for representative wells presented in Figure 6, show a pattern of increased microorganisms through time for several wells. The increased microbial population and activity count measured at production wells near the treatment well is a typical response to the treatment.

In addition to the production results, the oil field operator observed several other changes in his field that may be attributable to the activity of hydrocarbon degrading microorganisms. Procedures to measure these effects were not yet developed for the first two field tests. Paraffin buildup at the wellhead was reduced as was equipment maintenance resulting from paraffin problems. The API gravity of the produced oil, as determined by the company collecting the crude oil, increased. Experimental procedures are being developed to accurately measure these parameters in subsequent field operations.

DISCUSSION

As the natural, in-situ microbial population in the subsurface formation may be assumed to be static, the rapid increase in numbers of microorganisms in producing wells may be explained by the movement of the injected organisms through the field. Figure 6 illustrates the low pre-injection microbial population and the time when microorganisms appeared in the adjacent wells. Comparing the distance between the observation wells and injection well with the time of first arrival of the microorganisms at the observation wells suggests microbial migration

rates of several hundred feet per day. This transport rate is tremendously higher than commonly accepted for particle transport through porus media. Alpha reports this data as a field observation. We are currently designing laboratory experiments in cooperation with the Department of Petroleum Engineering at the Univeristy of Texas at Austin to study bacterial transport phenomena.

Figures 7 and 8 illustrate a hypothetical action of the microorganisms in an oil reservoir. Figure 7 represents a microscopic view of the relationship between formation particles, porosity, water, and oil. Figure 8 illustrates how we believe the microorganisms locate between the rock and the oil. The microbial production of acids, detergents, and biomass would then tend to mobilize the oil, shifting it to the pore water phase. The oil droplet, displaced from the rock surface, would then be free to move with formation water.

The figures 3, 4, and 5 show oil production after injection. There is a clear indication of an increase over the decline curve as determined by historical production and extrapolation. The field operation procedures were not changed after treatment (except that the injection wells were shut in for 5 days after injection) so the records of production should be representative of the effects of the microbial treatment.

CONCLUSIONS

A simple, efficient, and economical method of producing microorganisms and catalyst for oil field treatment has been developed.

Alpha Environmental's MEOR system has been tested on four stripper-well leases. Oil production after treatment increased above an extrapolated decline curve calculated from historical production data. The oil production increase occurred after approximately two weeks and continued for several months.

Observations by the field operator indicated a reduction of paraffin and BS&W from normal levels in the treated leases. Run tickets provided by the oil buyer indicated an increase in API gravity after treatment.

We believe that the injected microorganisms moved rapidly up-dip through the producing zone from the injection well to the surrounding wells. It appears that consideration of the attitude (dip) of the producing formation is important to the location of injection wells and to the successful application of MEOR.

ACKNOWLEDGMENTS

Partial funding for this study was provided by U.S. D.O.E. grant #DE-FG 19-86BC 14014 to ALPHA ENVIRONMENTAL. LaDonna Hays performed many of the microbial activity analyses and prepared the graphs in Figure 6.

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TABLE 1. Background data on the LaVernia Field leases.

FIELD NAME: LaVernia **FORMATION:** Navarro (Cret.)

LOCATION: LaVernia, Texas (S.E of San Antonio)

PERMEABILITY (MD): 37-43 **POROSITY:** 16-25%

RESERVOIR DEPTH: 1500 ft. **PAY THICKNESS:** 1-10 ft broken sands

LITHOLOGY: Fine grained, shaley, limey sand with swelling clays.

TRAP: regional monocline dipping SE, with subtle local stratigraphic and porosity pinchouts.

OIL: API gravity 30-33.

WELL SERVICE: 22,000 LBS Sand frac with 28 BBLS lease oil. Pumps rarely serviced for sanding. Small parrafin problem.

 # WELLS: 15 on three leases **SPACING:** 10 acre, wells drilled along strike,no standard production pattern

PREVIOUS EOR TREATMENTS: None

TABLE 2. Background data on Boynton Heirs Lease, Longwood Field.

FIELD NAME: Longwood **FORMATION:** Goodland Lime

LOCATION: Harrison County, Texas, (east of Shreveport, LA.)

PERMEABILITY (MD): 25 **POROSITY:** 25%

RESERVOIR DEPTH: 2,350 ft. **PAY THICKNESS:** 30 ft.

LITHOLOGY: Chalky limestone

TRAP: subtle structural high.

OIL: API gravity 39-40.

WELL SERVICE: Acid treated with 5000 gals. HCl., Wells chemically treated bi-weekly (prior to Alpha treatment) for paraffin and gypsum buildup.

 # WELLS: 13 on one lease **SPACING:** 20 acre

PREVIOUS EOR TREATMENTS: none

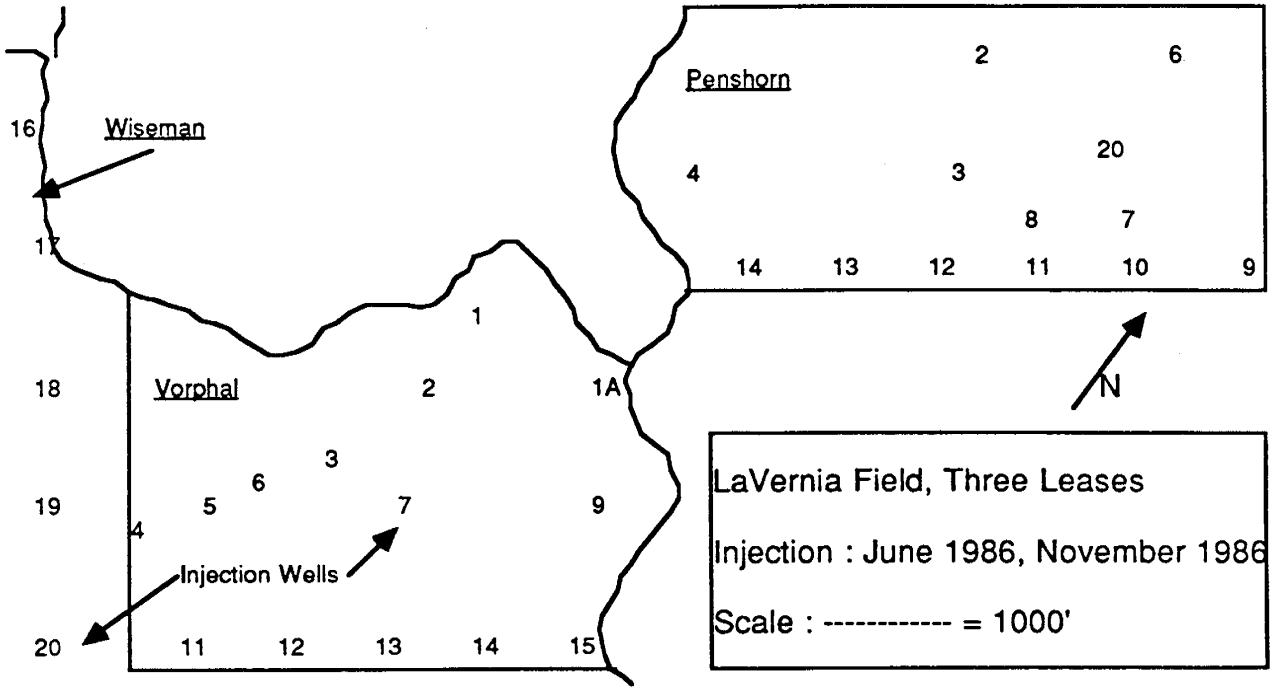


FIGURE 1. - Well location map of three leases in the LaVernia Field, Texas.

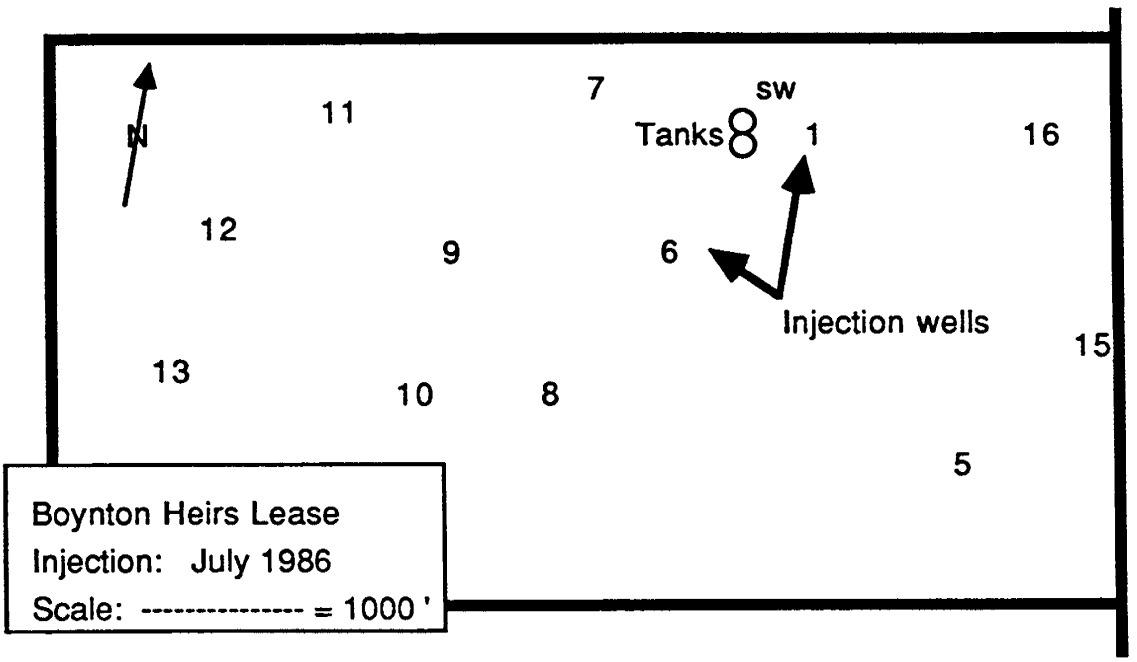


FIGURE 2. - Well location map of the Boynton Heirs lease near Shreveport, LA.

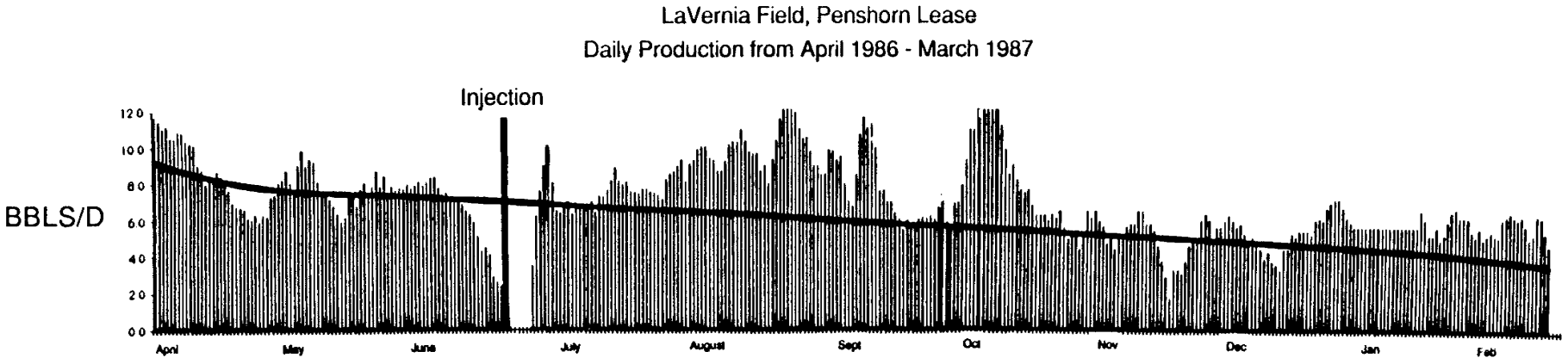


FIGURE 3. - Production history of the Penshorn Lease, LaVernia Field, Texas. Decline curve calculated from historical pre-injection production data and extrapolated from date of treatment injection. Production is reported as five day running averages in units of BBLS/D.

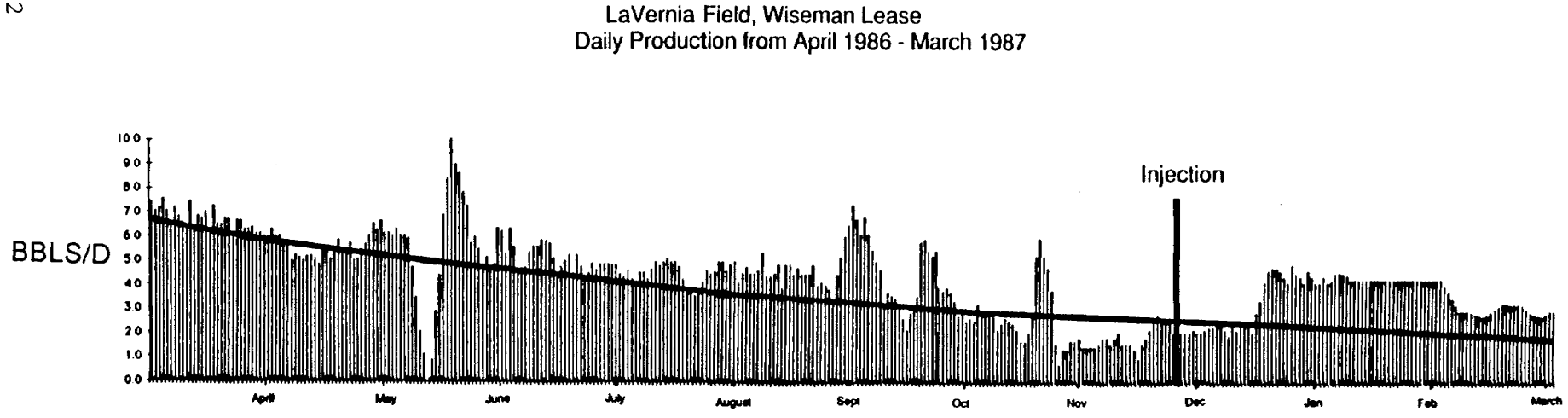


FIGURE 4. - Production history of the Wiseman Lease, LaVernia Field, Texas. Decline curve and units as in Figure 3. Flat production in January and February, 1987 represents average production for those months (lease production was not gauged daily).

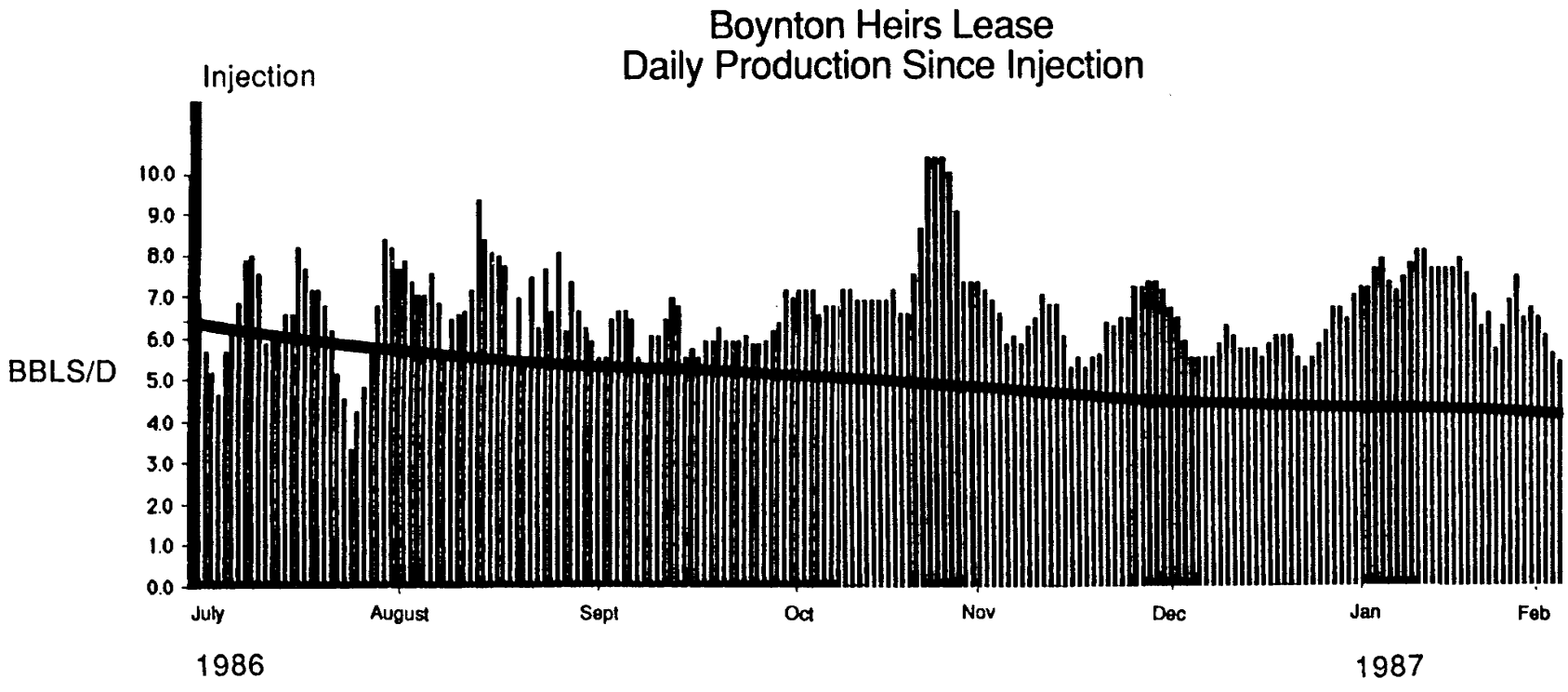
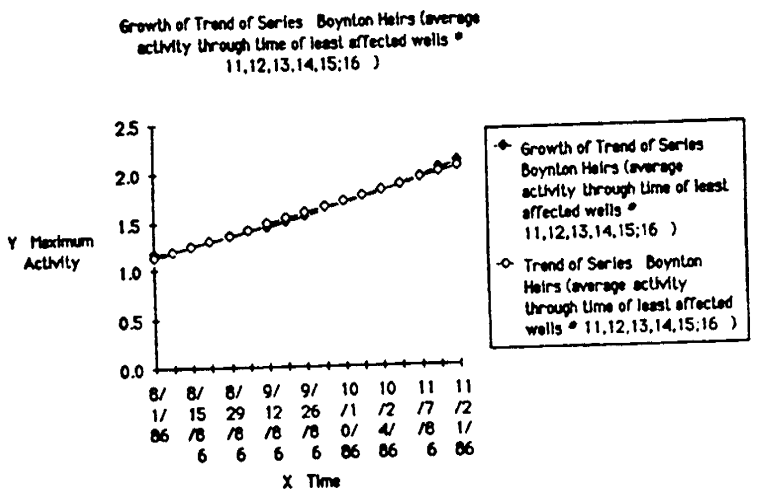
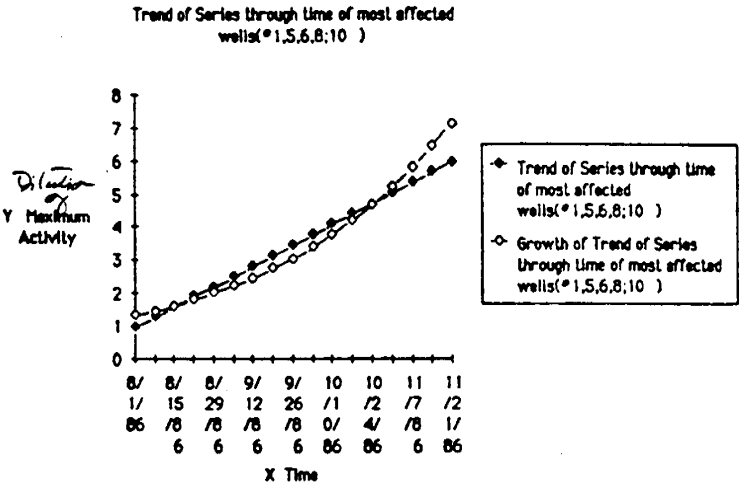
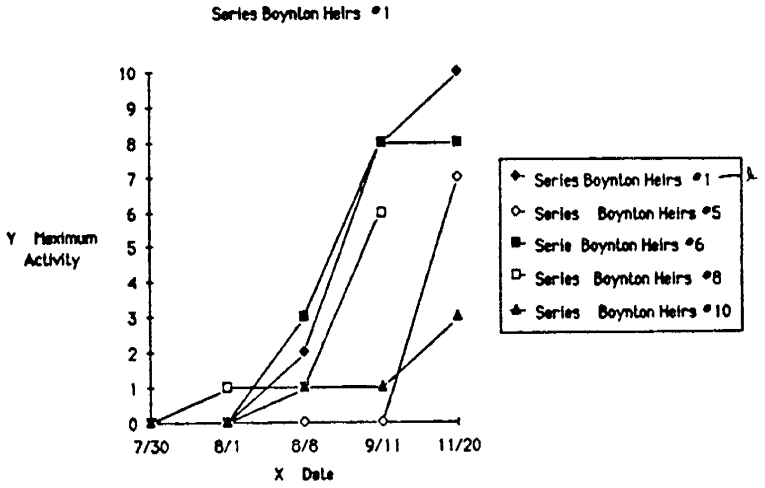
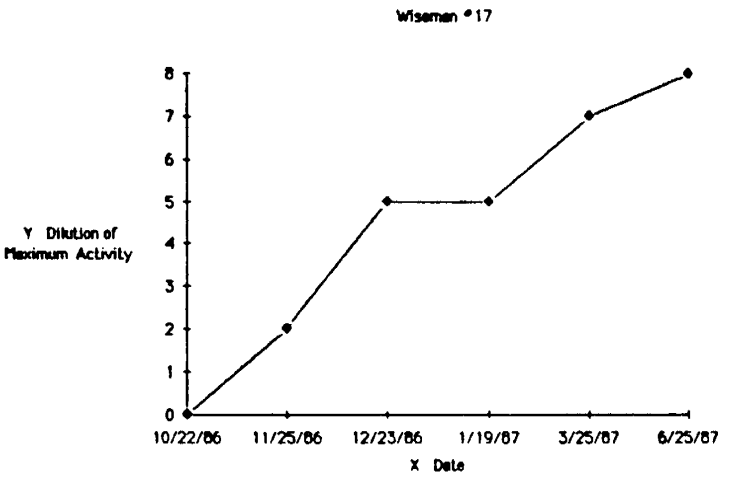


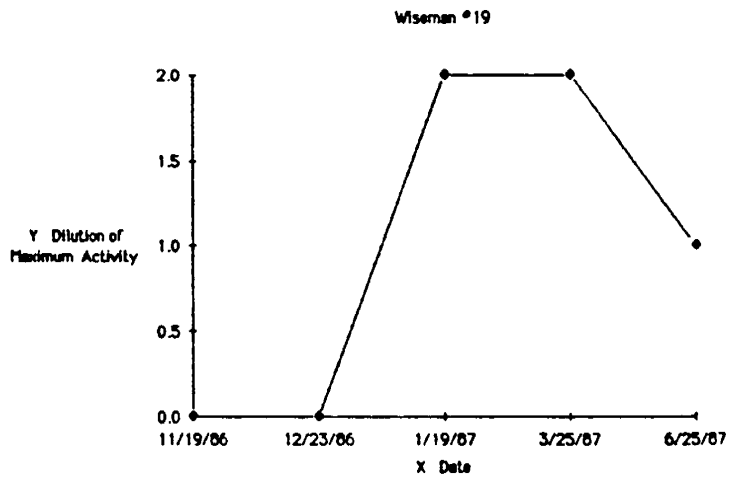
FIGURE 5. - Production history of Boynton Heirs Lease near Shreveport, LA. Decline curve and units as in Figure 3.











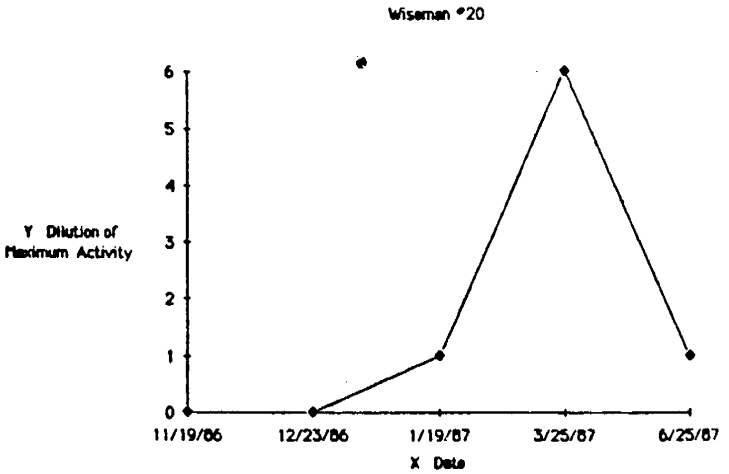


FIGURE 6. - Six graphs representing typical changes in the concentration of hydrocarbon oxidizing bacteria in the producing wells following injection. Numbers of bacteria are given in log units. Dates of sampling are reported on the abscissa. This type of information was used to estimate the rate and direction of microorganism movement through the tested formations.

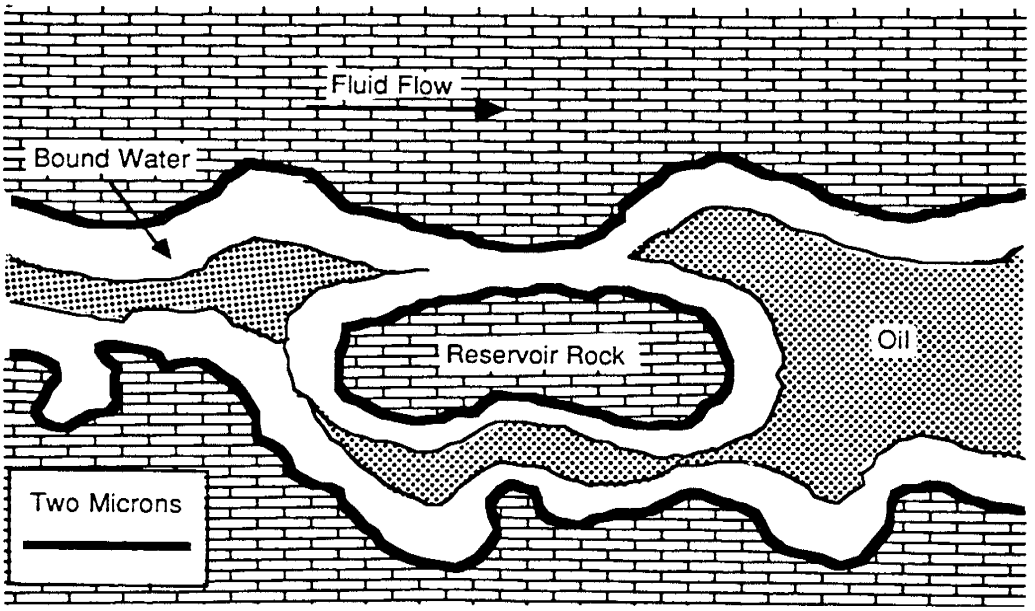


FIGURE 7. - Hypothetical view of how oil and water fill porosity in reservoir rock.

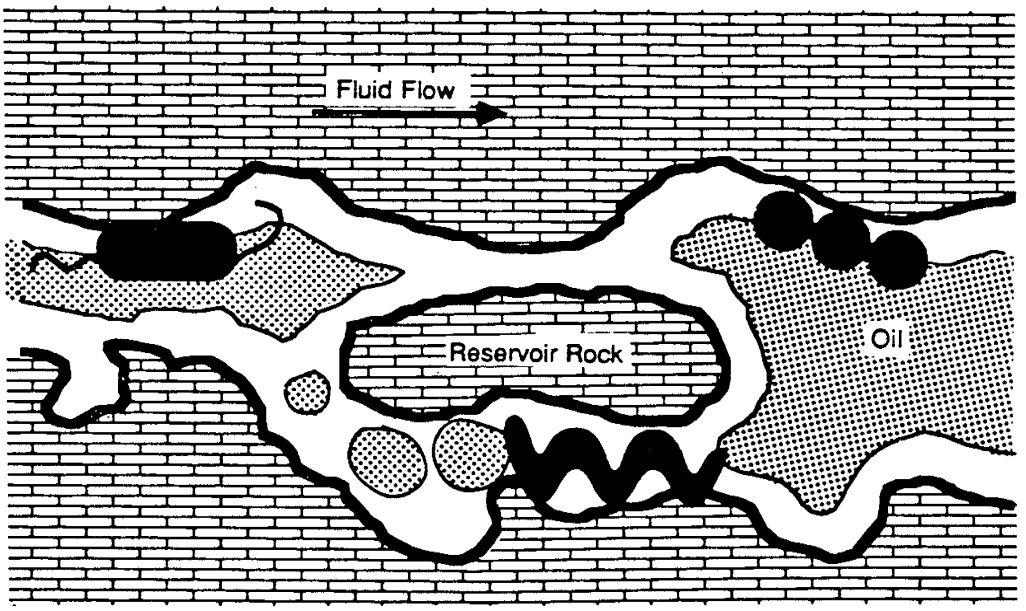


FIGURE 8. - Hydrocarbon-degrading microorganisms transported by formation brines attach to reservoir rock surfaces at the oil/water interface. As the microbes metabolize some of the hydrocarbon, they produce acids, gases, and new cells which displace the oil droplets. The previously immobile oil is now mobile and available to be swept to production wells in the oil/brine emulsion.

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BACTERIAL ACTIVITIES AND THEIR INHIBITION IN OIL RESERVOIRS DURING WATERFLOODING PROCESSES

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ABSTRACT

Microbiological investigations were carried out at two oil fields, one of which was the Laojunmiao oil field (in the Gansu Province) waterflooded earlier. The maximum counts of sulphate reducers were hundred thousands to ten millions/ml. The H_2S contents reached maxima of 91 to 136 mg/l. The active development zones were located at 6.8 to 9.4 m around the bottoms of injection wells. The other field, the Shengli (in the Shangdong Province), were waterflooded later. The maximum counts of sulphate reducers were thousands to hundred thousands/ml. The H_2S contents were 0.62 to 10.12 mg/l. The active development zones were located at 2.4 to 2.7 m around the well bottoms.

A method for sterilizing bacteria in the active development zones and for increasing the water-intake capacity of injection wells has been elaborated. Its applicability was certified by a series of field experiments. After treatments the water injection rates were increased, and the periods of stability water injection were longer.

INTRODUCTION

Studies on the distribution of bacteria and their geochemical activity in oil-bearing strata were mostly related to the petroleum deposits developed by waterflooding.[1-2]

It has been indicated that the prolonged flooding changed the ecological environment of petroleum strata, created suitable development conditions for the microbiota composed of hydrocarbon oxidizers and sulphate-reducing bacteria etc., and thereby improved the distribution of bacteria in oil strata and enhanced their biogeochemical intensity, mainly including the petroleum ozidation process, sulphate reduction process, and methanogenesis.[3-7]

The water-intake capacity of the injection well is usually decreased with the increase of injection duration. It is due to the physical, chemical, and biochemical changes caused by the extraneous water injected into reservoirs. In biochemical changes resulting in bacterial biomass and metabolites (the slimes produced by slime-forming bacteria), the H_2S produced from sulphate by sulphate reducers might result in the plugging of oil strata and the corrosion of iron, which are severe to oil recovery.[7-8]

The oil horizon L of Laojunmiao oil field was early developed by waterflooding with spring water. In 1958, hydrogen sulphide was already found in the backflushed water from injection wells. Up to now the phenomenon has been widespread at the southeastern part. The water-intake capacity of the injection well has decreased sharply.

The third area of Shengli oil field was lately developed by waterflooding with river water. After 1 year, H_2S was found in the backflushed water from injection wells. The water-intake capacity of several injection wells also decreased.

Microbiological and hydrochemical changes in water samples from water sources and flooded oil horizons at two oil fields were examined. This report describes the development intensity and distribution of bacteria and the effect of a combination of sterilizing and deplugging in the flooded horizons.

MATERIALS AND METHODS

Samples

Water samples were directly collected from sampling valves of water lines into sterilized flasks. The backflushed water samples were taken from well heads.

Bacterial Counting

The quantitative analyses of physiological groups were carried out by the dilution method of counting with five tubes at each dilution with 8 hours after sampling.

Nutrient broth was used for saprophytes.[9] Hydrocarbon oxidizers were determined in Bushnell-Hass medium with liquid paraffin as the C-source and sulphate-reducing bacteria in Starkey medium.[10] The samples were cultured at 30° to 32° C for 15 or 45 days, depending on the physiological groups.

Chemical Analyses

Oxygen dissolved in water was determined by the colorimetric method with indigo carmine. Sulphate was determined by titration with FDTA, and H_2S by photoelectric colorimetry with methylene blue.[11]

RESULTS

I. Changes of Bacterial Counts

Laojunmiao Oil Field[12]

The water source for waterflooding is spring water. Its mineralization is about 300 mg/l, and the $SO_4^{=}$-content is about 96 mg/l. The bacterial counts were low and increased in water samples from water lines and well holes (table 1).

Bacteriological analyses and H_2S determinations of water samples from 21 injection wells were performed at Laojunmiao oil field. The following two wells are examples to show their general regularities.

1. L_3-horizon of the injection well 714

In August 1958, the well was used for water injection. The beginning water injection rate was 183 m^3/day under 104 atm at the well head. After 1960, the well was used for separate zone water injection into L_1, L_2 and L_3. It water-intake capacity was coming down month by month. The well was repeatedly treated by fracturing or acidizing, but the effective period was short. At the beginning of 1965, the water injection rate into L_3 after acidizing was 32 m^3/day under 80 atm at the wellhead. In April the well was stopped because water could not be injected into it.

Microbial activities in backflushed water of well 714 (Fig. 1) indicted that the counts of sulphate reducers increased gradually to maximum by ten millions/ml by 120 m^3 of water, then began to reduce. After reaching the maximum, the hydrocarbon oxidizers and saprophytes were decreasing. The changes of H_2S concentration were parallel with those of sulphate reducers and increased from initially 20 to 120 mg/l.

2. L_3-horizon of the well L261

In May 1965, the well was used for water injection. Its water injection rate was initially 50 m^3/day, but in September had reduced to 8 m^3/day and finally to zero.

Microbial activities in backflushed water (Fig. 2) showed that the numbers of sulphate reducers increased from the initial ten to millions/ml by 193 m^3 of backflushed water. The concentration of H_2S increased from 60 to 100 mg/l. Saprophytes and hydrocarbon oxidizers reached ten thousands and humdreds/ml, respectively.

Shengli Oil Field[13]

Treated river water is the water source for waterflooding. It contains dissolved oxygen, 5 to 10 ug/l; $SO_4^=$, 69.0 to 110.0 mg/l; and low concentration of hydrogen sulphide. Its mineralization is about 334.4 to 467.0 mg/l. The counts of different physiological groups of microorganisms fluctuate between tens and thousands/ml (table 2).

Microbiological and hydrochemical analyses of water samples from six injection wells were carried out at Shengli oil field. The following two wells are examples to show their general changes.

1. The injection well 3-6-20

The water samples were neutral or nearly equal to alkaline. The temperature at the wellhead was between 26.5° and 41° C. The oxygen dissolved in water was not detected after the 23 m^3 of backflushed water. The $SO_4^=$ concentration decreased gradually from 119.1 mg/l to zero. The H_2S concentration increased to maximum 10.12 mg/l after 771 m^3 of backflushed water, while the mineralization kept on increasing.

Microbiological and hydrochemical analyses (Fig. 3) indicated that the maximum counts of bacteria were as follows: sulphate-reducers, thousands/ml in 62 m^3, saprophytes, ten millions/ml in 447 m^3; and hydrocarbon oxidizers, millions/ml in 401 m^3.

2. Injection well 3-2-9

Water samples were of pH 7.8-8.3. The temperature at the wellhead was between 34° and 42° C. $SO_4^=$ decreased from 69.2 mg/l to zero at the backflushed water 80 m^3. The H_2S concentration reached a maximum of 0.45 to 0.62 mg/l in 70 m^3 of backflushed water. Its mineralization increased from 344 to from 454 to 506 mg/l and remained constant.

Microbiological and hydrochemical analyses (Fig. 4) showed that the maximum counts of different physiological groups were basically the same, ten thousands/ml.

II. The Active Development Zones of Bacteria

The above-mentioned data indicated that both the bacterial counts and H_2S changed from low to high to low with the increase of backflushed water. To calculate the radius (r) of active zones, the data obtained above were put into the following formula:[14]

$$r = \sqrt{\frac{V}{\pi h m \eta}}$$

V = the backflushed water (m^3) containing the maximum content of bacteria or of H_2S, h = the thickness (m) of flooded horizons, m = porosity (%) of the flooded horizons, and η = flushing coefficient of the water.

Some results obtained at the two oil fields are presented in table 3. Ranges of the active development zones varied with the injection wells. At the Laojunmiao oil field, it ranged from 6.8 to 9.4 m. At the Shengli oil field, it was located at 2.4 to 2.7 m around the well bottoms.

III. Combination of Sterilization and Deplug

Treatment processes

1. Stratum water was backflushed and collected for microbiological and hydrochemical analyses. The active development zones of bacteria carried a part of the precipitates produced by bacteria and by mixing the injected water with the stratum water out of the horizons and thus reduced a part of the plug.

2. Surfactant (sodium aminobenzenesulphonate) was injected to remove the oil residues.

3. Bactericides (formaldehyde) and hydrochloric acid (3.0%) were injected. The injected amounts were calculated on the basis of the active

development zone. Hydrochloric acid is able to dissolve the precipitates (ferrosulphide, etc.), thereby reducing a part of the plug, and enhancing the bactericidal effect.

Treatment Effects

At the beginning of the field experiments, injecton well L261 was selected for treatment because the strata around the well bottom were already plugged and unable to repair by acidizing. Before the treatment, the water-intake capacity was 0 under 90 atm at the wellhead. After the treatment, the controlled injection rate was 50 m^3/day under 0 atm. After 2 months of steady water injection, the production wells around it were flooded by the high injection rate. Although the well was closed, the effect of the treatment process was confirmed.

Twenty-seven of the injection wells were treated, of which only one or two wells showed insignificant effects. The field experiments (table 4) indicated that the treatment effects were correlated with the active development degrees of bacteria around the well bottoms and with the effective permeabilities of injected horizons. In general, evident effects were obtained at the horizons with higher bacterial counts and permeabilities, otherwise the effects were not evident.

The evaluation of a treatment process not only depends on the comparison of the situations before and after treatments, but also on the effective periods. It is shown in table 4 that the pressure was decreased and the injection rate was increased after treatments. Wells 714 and 777 are examples that show the effective periods were as follows.

After the onset of injection, well 714 was treated three times by acidizing and twice by backflushing and fracturing, but the effective periods were usually short or insignificant. After these treatments, the effect was obvious. After 368 days of controlled water injection, the water injection rate did not obviously decrease.

After beginning water injection, well 777 was treated by jet fracturing etc., but without success. The well is near wells L261 and 756 at the northeastern part of Laojunmiao oil field in the active development area. Thus, the well was treated by the injection of bactericide and hydrochloric acid without backflushing. The treatment gave good results. After 274 days of water injection, the water injection rate did not decrease.

At Shengli oil field the horizons 1^{4-1}-2^{3-1} of injection well 3-2-9 were treated by sterilizing after backflushing, and compared with its acidized horizons 2^{5-1}-2^{6-1}. Data in table 5 indicate that the effects of sterilizing were better than those of acidizing. The period of stable water injection was longer. The same result was obtained at well 3-6-4.

DISCUSSION

It is well known that an adequate conclusion in geomicrobiological investigations can be made only by the combination of the presence of

microorganisms with their necessary ecological conditions.[1] Microorganisms present in flooded oil reservoirs are in general carried by the water injected into them and are so adapted to their environment.[2]

At Laojunmiao oil field, the reservoir water belongs to the type of calcium chloride. Its mineralization is about 18,550 to 98,333 ppm; salt content, 16,353 to 66,570 ppm; $SO_4^=$ 0.01 to 4.96 in equivalent percentage. The temperature of horizon L is between 20° and 36° C. The pH of the backflushed water is 6.0 to 8.0. Saprophytes, denitrifying bacteria, anaerobic hydrocarbon-utilizing bacteria, and sulphate reducers have been found in oil-bearing cores.[16] The injected fresh water carried bacteria and $SO_4^=$ into the reservoir and diluted the stratum water, thereby resulting in enhancement of bacterial activities,[17] and formed an active development microbiota around the well bottom, especially enhancing the sulphate-reduction process. The sulphate reducers in stratum water were as high as hundred thousands to ten millions/ml. H_2S reached a maximum concentration of 91.3 to 136.4 mg/l. The active development zones of bacteria were located at 6.8 to 9.4 m around the bottom of injection wells.

The backflushed fluid (water-cut 68%) from production well M24 (located 300 m away from injection well 714 and in communication with each other) was found to contain sulphate reducers 1.7 x 10^3 ml, and H_2S, 8.4 mg/l (table 6). This indicated that the distribution of bacteria was enlarged.

At Shengli oil field, the stratum water contains no $SO_4^=$. Its temperature as high as 70° to 80° C, is obviously a significant limiting factor to bacteria developing in the reservoir, but the temperature at the well bottoms is 52° to 66° C. During sampling, the temperature of backflushed water was measured from 26.5° to 42° C; therefore, the temperature around the well bottom during continuous water injection was not very high. It depends upon both the injection period and the injection rate.

To establish the range of growth temperature, the mixed cultures obtained from the experiments were inoculated and cultered at 30°, 45°, 55°, and 65° C. Results indicated that the growth percentages of sulphate reducers at these temperatures were 100, 97.4, 25.3, and 10.5, respectively. Saprophytes and hydrocarbon oxidizers could not develop over 45° C, so it seems that the bacterial groups carried with the injected water are able to grow at and below 45° C around the well bottom during water injection. The ability of sulphate reducers to adapt to the higher temperature was enhancing, and the biota adapted to the higher temperature of the stratum has not been established. The active development zones of bacteria were located at 2.4 to 2.7 m around the well bottoms. After the treated river water containing dissolved oxygen, $SO_4^=$, and microorganisms was injected into Shengli field, the chemical components were obviously changed, principally including the increase of mineralization, H_2 and HCL_3^-, and decrease of dissolved O_2 and $SO_4^=$. The water was changed from sodium sulphate into that of sodium carbonate. In a limited area around the well bottom having temperature below 45° C, a food chain was formed, in which the hydrocarbon ozidizers utilized the oil hydrocarbon and the dissolved O_2 to produce acid acetic acid used as C-source for $SO_4^=$-reducers, and created an anaerobic conditions for them.[5-6] They participated in petroleum oxidation processes and reduced a certain amount of sulphate in

the injected water into H_2S. But both the development intensity of bacteria and the radius of the active development zones around the well bottom were smaller than those of Laojunmiao field.

A method for sterilizing bacteria in the active development zones and for increasing the water-intake capacity of injection wells has been elaborated. Its applicability was certified by a series of field exeriments. This results in the ability to control bacterial plugging and corrosion by the breakdown of microbiota formation, which might be of great importance to enhanced oil recovery processes.

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TABLE 1. - Bacterial counts in the water source and water lines of Laojunmiao oil field

Sampling points	Bacterial counts/ml		
	Saprophytes	Hydrocarbon oxidizers	Sulphate reducers
Spring water	7.0×10^2	3.5×10^1	0
Pump house	1.3×10^2	1.3×10^1	0.7×10^0
G7 pump house	1.7×10^1	1.4×10^1	0.8×10^0
Water distributor No.3	3.5×10^2	3.0×10^2	1.7×10^0
" " No.4	8.0×10^1	4.5×10^1	2.0×10^0
Well hole L261	9.5×10^2	5.0×10^3	1.4×10^1
" " 714	2.5×10^4	2.0×10^2	8.0×10^3
" " 761	2.5×10^5	8.0×10^2	1.3×10^3
" " 756	9.0×10^3	3.5×10^3	6.0×10^4

TABLE 2. - Characterization of the water source for waterflooding at Shengli oil field

Sampling points	O_2 (μg/l)	$SO_4^{=}$ (mg/l)	H_2S (mg/l)	HCO_3^{-} (mg/l)	Mineralization (mg/l)	Bacterial counts/ml		
						Saprophytes	Hydrocarbon oxidizers	$SO_4^{=}$-reducers
Water-treating plant		75.0	0.01	107.4	333.4			7.0×10^1
Water distributor	5-10	72.0	0.02	112.3	343.0			7.7×10^2
3-2-9 well head		69.2	0.05	112.3	344.0	3.5×10^3	3.6×10^2	
Water-treating plant		119.1	0.01	136.7	463.0	8.0×10^1	5.0×10^1	1.3×10^1
Water distributor	5	119.1	0.01	136.7	467.0	5.0×10^2	2.5×10^1	2.5×10^1
3-6-20 well head		119.1	0.01	136.7	466.0	8.0×10^2	2.5×10^1	2.5×10^1

TABLE 3. - The active development zones of bacteria around the injection well bottoms

Well	Horizon	Thickness of horizon (m)	Maximum counts of $SO_4^{=}$-reducers/ml	Maximum H_2S (mg/l)	Backflushed water at the max. content (m^3)	Active development zones of bacteria (m)
Laojunmiao oil field						
714	L_3	3.4	2.5×10^7	120.0	120.0	7.9
"	$L_{1,2}$	10.2	2.5×10^6	136.4	358.9	7.5
L261	L_3	5.1	2.5×10^6	100.0	193.0	8.0
756	$L_{1,2}$	9.0	5.0×10^3	91.3	269.1	6.8
761	L_2	1.6	3.5×10^5		45.7	6.8
23	L_3	3.2	2.5×10^4		177.0	9.4
Shengli oil field						
3-6-20	mix.		2.5×10^3	10.12	62.0	2.7
3-2-9	$1^{4-1}_{-2}3^{-1}$		1.6×10^5	0.62	22.0	2.4

TABLE 4. - Relationship of the treatment effects with some of the factors

Wells	Horizon	Effective permeability(md)	Max.counts of bacteria/ml			Active development zone(m)	Treatment effects			
							Before		After	
			$SO_4^=$-reducers	Hydrocarbon oxidizers	Saprophytes		Pressure (atm.)	Injection rate(m^3/d.)	Pressure (atm.)	Injection rate(m^3/d.)
L261	L_3	1190	$2.5X10^6$	$2.0X10^2$	$4.5X10^1$	8.0	90	0	0	50
714	$L_{1,2}$	656	$2.5X10^6$	$3.5X10^3$	$5.0X10^5$	7.5	100	0	25	70
761	L_2	714	$3.5X10^5$	$3.5X10^4$	$2.5X10^6$	6.8	180	0	100	24
756	$L_{1,2}$	350	$5.0X10^3$	$9.5X10^3$	$3.5X10^4$	6.8	98	16	85	22
L23	L_3		$2.5X10^4$	$5.0X10^3$	$2.5X10^4$	9.4	85	0	80	66
777	$L_{1,2}$	456					93	13	70	20

TABLE 5. - Comparison of the sterilizing with the acidizing of the well 3-2-9 at Shengli oil field

Horizon	Treatment	Before treatment		After treatment
		Pressure (atm.)	Injection rate($_{m^3/day}$)	
$1^{4-1}_{-2}3-1$	Backflushing Sterilizing	54	0	Stable injection $40m^3$/day for more than one year
$2^{5-1}_{-2}6-1$	Backflushing Acidizing	54	0	Stable injection 30 m^3/day for about 10 months

TABLE 6. - Bacterial counts and H_2S content in the backflushed water of production well M^{24}

Sulphate reducers	Hydrocarbon oxidizers	Saprophytes	H_2S
1.7×10^3/ml	1.1×10^1/ml	3.0×10^2/ml	8.4 mg/l

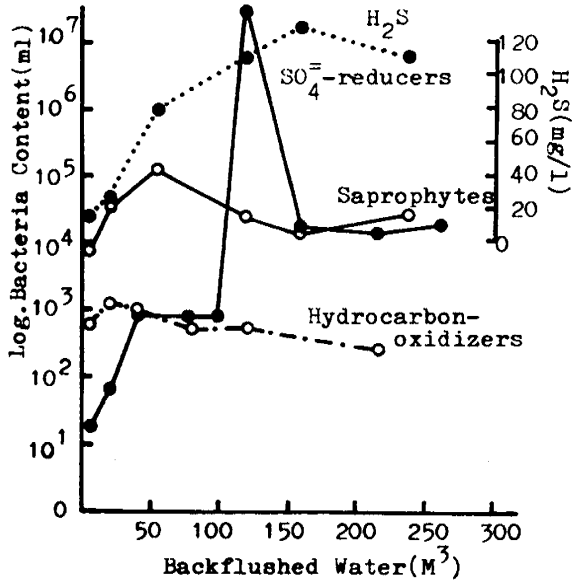

FIGURE 1. - Microbiological changes in backflushed water of the injection well 714

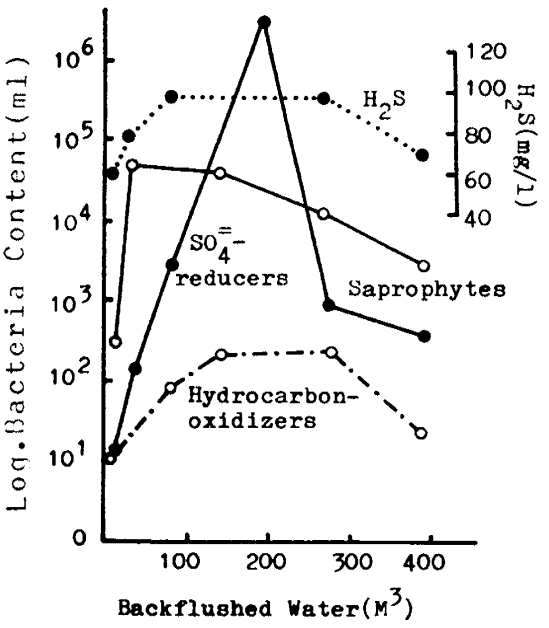

FIGURE 2. - Microbiological changes in backflushed water of the injection well L261

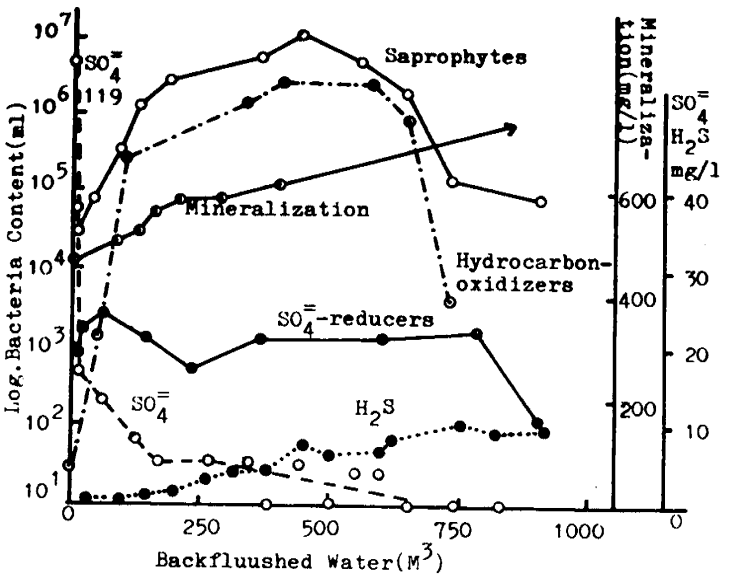

FIGURE 3. - Microbiological and hydrochemical changes in backflushed water of the injection well 3-6-20

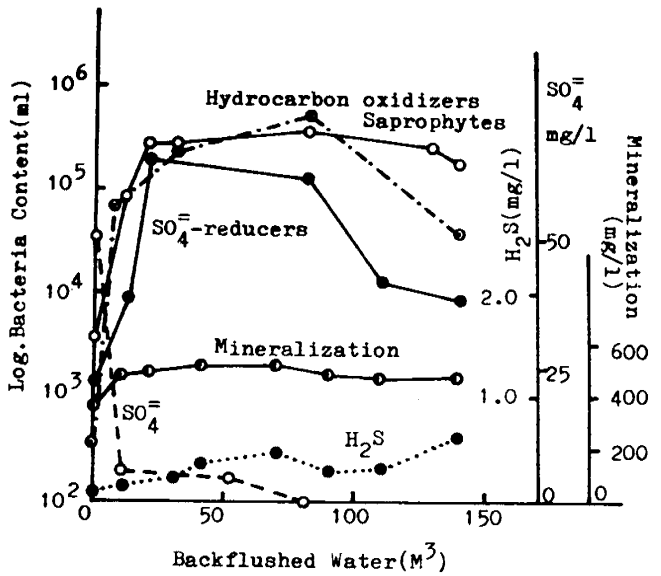

FIGURE 4. - Microbiological and hydrochemical changes in backflushed water of the injection well 3-2-9

SOME CONSIDERATIONS CONCERNING NUTRIENT SUPPORT INJECTED INTO RESERVOIRS SUBJECTED TO MICROBIOLOGICAL TREATMENT

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ABSTRACT

In the period 1987-1988, four reservoirs in Romania will be subjected to microbiological treatment using two technologies of well injection. To establish the suitable nutrient support to be injected into these reservoirs together with a specially selected bacterial inoculum, several nutrient supports based on molasses were tested. It was found that the addition of several N, C, K, or P sources to molasses does not seem necessary because the molasses used and the formation water of the reservoirs permit a good growth of bacteria.

INTRODUCTION

During the past 30 years, the United States, Czechoslovakia, Hungary, Poland, Soviet Union, and Romania have reported field trials for microbial enhanced oil recovery. These reports (Jaranyi et al., 1967; Karaskiewicz, 1974; Hitzman, 1983; Lazar et al., 1983 and 1985; and Zajic, 1987) mention that along with bacterial inoculum into reservoirs a nutrient support of molasses was also injected. Most of the reports, except those from Poland and Romania, underline that the nutrient support injected into reservoirs contained molasses as well as other substances as supplementary sources of carbon, nitrogen, and phosphorous or other nutrients for stimulation of bacterial activity.

In our previous papers (Lazar 1983, 1985), it was mentioned that the molasses and the formation water from the reservoirs submitted to microbiological treatment proved to be suitable and adequate for bacterial growth and activity in the formation. Based on these results as well as those of Zajic (1987), we carried out a new series of experiments to establish the most suitable nutrient support for the field trials mentioned above and in progress in Romania. In this paper, the results of these experiments are presented.

MATERIALS AND METHODS

Nutrient Supports (NS) Tested

NS_1 = formation water + molasses 3% + crude 2%. This nutrient was used in field trials, mainly in Poland and Romania (Karaskiewicz, 1974; Lazar et al., 1983 and 1985).

NS_{1a} = NS_1 + 10-15% fermented scum from sugar processing plants.

NS_2 = formation water + molasses 3% + sugar 0.5% + NH_4NO_3 0.2% + K_2HPO_4 0.2%.

This nutrient support was used in field trials, mainly in Hungary (Jaranyi et al., 1967, 1968).

NS_{2a} = NS_2 + 10-15% fermented scum from sugar processing plants.

NS_3 = formation water + molasses 3% + sucrose 0.5% + NH_4NO_3 0.2% + K_2HPO_4 0.2% + yeast extract 0.05%.

This nutrient support is recommended for field trials by Zajic (1987).

NS_{3a} = NS_3 + 10-15% fermented scum from sugar processing plants.

NS_4 = NS_{1a} + pantothenic acid 10 µg/l (recommended by Jaranyi (1975).

Bacterial Inoculum Used

Several "adapted" bacterial populations obtained for each of the four reservoirs submitted to microbiological treatment were used. The procedure to obtain these "adapted" bacterial populations was previously described (Lazar, 1983; Lazar et al., 1985, 1987).

The Formation Water and Crude Oil Used

The formation water and crude oil used for these studies were taken from four reservoirs located near Bucharest (see table 1). These reservoirs have temperatures between 36° and 51° C, salinity from 3 to 40 g/l NaCl, permeability from 150 to 400 or even 1,000 md, crude oil viscosity from 9 to 50 cp, a content in carbonates from 15 to 20 to 70 to 80%, and coproduced water (formation water) up to 70 to 90%.

Crude Oil Released From Porous Media

The capacity of "adapted" bacterial populations supplied with NS_1, NS_2, NS_3, and NS_4 as nutrient supports to release crude oil was tested on sand columns saturated with crude oil, then washed with water until no more drops of crude oil eluted from the column, then the bacterial inoculum and nutrient support were injected, followed by 7 days of incubation at the temperature of each reservoir, then washed again with water until no more drops of crude oil were produced. The crude oil released by bacterial action was calculated. Details are given in two recent patents (Lazar et al., 1987).

The experiments were carried out in incubators set at the temperature of each of the four reservoirs, which were the sources for the crude oil, formation water and "adapted" bacterial populations, namely at 36°, 43° to 45°, and 51° C for a period of 5 to 7 days.

RESULTS AND DISCUSSION

Gases and Acid Production

Table 1 presents the results concerning gases and acid production. These results show the following: In the four reservoirs treated, gases and acid production were not influenced by the addition to nutrient support of molasses of some supplementary sources of carbon, nitrogen, potassium, or phosphorus. Thus, for the nutrient support recommended, for instance, by Jaranyi (NS_2) and by Zajic (NS_3), gases and acid production was not higher than that from the nutrient support we used, (NS_1).

The formation water of those four reservoirs subjected to microbiological treatment, as well as the molasses we used, seems to contain all of the nutrients that the "adapated" bacterial populations needed.

It was noticed that no improvement in gases and acid production took place when a correction of the pH of nutrient support was made using 10% NaOH, but when scum from sugar processing plants, very rich in bicrabonates (80%) was added for pH correction, an important increase of gas and acid production was observed. It seems that the scum from sugar processing plants played an important role in buffering the nutrient support, so that the pH never dropped below 5.5 to 6.0, while in the cases of variation N_1, NS_2, or NS_3 when pH dropped below 6.0 or 5.5, gas production decreased drastically. For this reason, we think that reservoirs with a content in carbonates higher than 20% are more suitable for treatments with high gas and acid-producing bacterial inoculum.

The addition to nutrient supports of molasses of substances like panthotenic acid seems to be helpful as the variation with such substances (NS_4) gave a higher quantity of gas than all of the other variations tested.

Crude Oil Release

In table 2, results concerning the crude oil released by "adapted" bacterial populations supplied with several nutrient supports of molasses are presented. These results showed insignificant differences between variations based on using NS_1 or other ones as NS_{1b}, NS_2 or NS_3, which have additional substances. So these results seem to prove that the addition to nutrient support to molasses of several N, P, K or C sources does not seem to be necessary because the molasses and formation water permit good growth of the "adapted" bacterial populations.

CONCLUSIONS

1. The bacterial populations adapted to the conditions of four reservoirs located in areas close to Bucharest were tested for their gas and acid production on several nutrient supports on molasses bases. The same "adapted" bacterial populations were tested for crude oil release from sand columns injected with same nutrient supports.

2. It was found that the nutrient support containing 2 to 4% molasses, 1 to 2% crude oil, and formation water from the reservoirs for which bacterial populations were adapted, gave similar or even better results than nutrient support with supplementary additions of K, P, N, and C sources. These results are based on gas and acid production as well as crude oil release from sand columns.

3. The addition of very small quantities (10 μg/1) of pantothenic acid to nutrient support containing 2 to 4% molasses, 1 to 2% crude oil, and formation water, seems to be useful because gas production had a quite significant increase.

4. The content of carbonates proved to play an important role in buffering the nutrient support when high gas and acid-producing bacteria are used. This led to the recommendation that reservoirs with a carbonate content of more than 20% must be submitted to microbiological treatment.

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TABLE 1. - Gas and acid production by the "adapted" bacterial population supplied with several nutrient supports of molasses

The nutrient support (NS) of molasses	Gas production (in ml) in the first 5 days, from 15 ml of nutrient support				Limits of the pH	
	Reservoirs				Initial	After 5 days
	1	2	3	4		
NS_1	49	76	105	68	6.8 - 7.3	4.5 - 5.0
NS_2	40	49	60	63	7.5 - 8.0	5.0 - 5.3
NS_3	47	80	57	70	7.0 - 8.0	5.0 - 5.4
NS_{1a}	90	118	110	95	7.5 - 8.0	5.8 - 6.7
NS_{2a}	84	90	85	75	8.0 - 8.2	5.9 - 7.3
NS_{3a}	80	94	90	90	7.4 - 8.3	5.2 - 6.8
NS_4	115	124	137	120	7.4 - 7.9	5.6 - 5.9

Legend: 1 = Balaceanca (43° C) 2 = Bragadiru (36° C)
3 = Caldararu (45° C) 4 = Gradinari (51° C)

TABLE 2. - The crude oil release by the "adapted" bacterial populations supplied with several nutrient supports of molasses

Reservoirs from which "adapted" bacterial populations were obtained	% of crude oil released using various nutrient supports			
	NS_1	NS_{1a}	NS_2	NS_3
Balaceanca	12.5	14.4	14.6	13.0
Bragadiru	12.3	15.1	12.8	11.2
Caldararu	13.6	14.5	11.8	11.3
Gradinari	16.3	15.3	15.0	14.5

NUTRITIONAL ASPECTS OF MEOR

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ABSTRACT

We have focused on the chemical environments in which bacteria potentially useful in MEOR will be growing. Strains of Clostridium recently isolated from natural environments (in some cases, oil reservoirs) have been used to determine the effects of various cores on gas, solvent, and acid production.

The strains used were fermentative clostridia of the butyric-butanol type; these organisms are among the highest bacterial gas producers known. Cores were provided by grinding to the particle size of medium sand; known amounts of ground core were added to a certain volume of liquid medium. In general, limestone cores (e.g., Austin Chalk) provide better buffering capacity than mainly sandstone cores. One result of this is much more abundant gas production upon a refeed (addition of more energy source after exhaustion of initial supply), and much less butanol production during the first fermentation. This may be because of the pH in limestone cores does not fall down to the break point. Data on production of acids, solvents, and gases in the presence of various limestone and sandstone cores, initially and upon refeeding will be presented. Different cores result in widely varying production of all end products. These differences appear to be related to several factors: buffering capacity of the core; chemical composition of the core; and the ability of the strain to grow in the core environment.

INTRODUCTION

The growth *in situ* of bacteria requires the addition to the formation a nutrient medium consisting of those growth factors necessary for the propagation of numbers as well as the production (in sufficient quantities) of those end products that would facilitate the movement of oil from a reservoir.

The composition of this medium as well as the concentrations of the components is of direct interest to anyone involved in MEOR. It would be in the best interest of the science and the economics of MEOR, to pump only those medium components downwell that will be readily utilized; in concentrations such that there is complete utilization at the end of the fermentation. There is no real advantage in placing an unusable or highly concentrated nutrient source in a reservoir only to pump it back at a later date.

In this light we have studied the interactions of cores and the clostridial fermentation we utilize as an EOR tool. To understand our interest in the effect of various cores on the fermentation of clostridia, we must first understand the butylic fermentation, to the extent of what is presently known about the butylic fermentation.

The butylic or acetone-butanol fermentation is a complex set of enzymatic reactions that are regulated by a series of interactions which to this date are not fully understood. The fermentation begins by the Embden-Meyerhof-Parnas pathway, whereby glucose is broken down to two pyruvate molecules. From there pyruvate is activated; this involves four distinct steps, including release of CO_2, oxidation of the two carbon moiety, reduction of an iron-sulfur protein (which releases H_2), and the ultimate formation of acetyl phosphate. This is the key intermediate for every clostridial end product (ethanol which is not a sole characteristic of clostridial fermentations is formed by the reduction of acetyl CoA by two molecules of NADH). A diagram is shown in Figure 1.

The clostridial fermentation is divided into two distinct phases: the acid-forming, early, or acidogenic fermentation and the solvent-forming, late, or solventogenic fermentation. In its simplest form, we have acetyl phosphate converted to acetic acid and butyric acid early in the fermentation. Formation of a 4-carbon chain necessitates the condensation of two molecules of acetyl CoA to acetoactyl CoA. In the later stage of the fermentation, the solvents acetone and n-butanol are formed. The conversion or "break point" usually occurs when the pH has dropped to around 4.5, and is associated with the beginning of sporulation. A key factor in the switch is the formation of the enzyme acetoacetate decarboxylase, which forms acetone and CO_2. Numerous articles and researchers attribute this switch in fermentation to a number of complex interactions and

concentrations of various medium constituents. The culture condition thought to be most responsible for the conversion of acids to solvents is the lowering of the pH of the medium below 4.5, the so-called "break-point" for solvent formation. The scheme usually put forth for the formation of acetone and butanol does not indicate that they are formed from their corresponding acids, yet ethanol and n-butanol are formed, at least in part, by reduction of the corresponding acids, as shown by the use of ^{14}C labelled compounds. This conversion no doubt is a direct result of the culture being unable to grow at a pH much below 4.5, but is not the sole mechanism for the product of solvents by the butylic group of clostridia (2).

For this reason, we were interested in the buffering capacity that a core might be able to provide. While we wish the pH to eventually reach the break point, we also need for the medium to be buffered sufficiently to cause a 80-100% conversion of substrate (sugar) to end products before the medium becomes too acidic and growth ceases. While most limestone or carbonate cores conceivably should contain the buffering capacity necessary, we wondered whether sandstone cores which contain smaller amounts of calcite and dolomite do likewise, or if the clays present contribute buffering capacity by acting as ion exchangers.

While the pH is most important for conversion and subsequently solvent production, other ions and their availability, notably $PO_4^{\equiv}$, Mg++, Fe++ and Mn++ (3,4,5,6,7 contribute to the overall fermentation and solvent ratio. This becomes a second question; while the cores no doubt contain an adequate supply of said (8,9), can they be released as the result of metabolic action for bacterial use, or if said are not available in the water to be used to make up the nutrient support, would they need to be added? It would not be in our best interest to argue that connate or produced waters do not contain sufficient quantities of most ions required for a complete and viable clostridial fermentation, although phosphate concentrations in reservoirs are considered low (10). Still, it should be of interest to know that these ions can be supplied by the reservoir rock.

While our strains require little or nothing more than a sugar and nitrogen source for growth, various amino acids are needed for germination. Since clays such as Kaolinite, which has an affinity for the binding of aspartic and glutamic acids (11), do indeed bind amino acids, we need to determine if this binding is strong enough to affect the levels of free amino acids incorporated into the medium for spore germination.

The final question that needs to be addressed is what the effects are of various cores on the fermentation products. It should be determined how the ratio of acid soluble constituents to non-acid soluble constituents in a core affect the ratios of end-products.

To this end we designed a series of experiments to answer each of these questions, growing one of our isolates under conditions which it would encounter in the reservoir. For all intents and purposes, the buffering capacity and trace elements would have to come from the cores themselves.

MATERIALS AND METHODS

Culture

The strain chosen for the core tests was strain SEG-3, a saccharolytic Clostridium able to grow in a medium which contains any one of a number of sugar and sugar alcohols. Amino acids from the aspartic or glutamic families are required for germination of viable spores. Major metabolic end-products are ethanol, acetone, butanol, acetic and butyric acids as well as carbon dioxide and hydrogen. Minor metabolic products are isopropanol, isobutyric acid and acetoin.

Medium

The medium for thé core tests was 1% sucrose, 0.1% yeast extract and 0.1% ammonium nitrate. At various times, the yeast extract or ammonium nitrate was omitted in an effort to determine amino acids needed for germination, effect of yeast extract on growth or effect of various other ammonium compounds on growth and production of metabolic end-products. Omission of the yeast extract did not effect the total gas production, but did affect growth rates and hence the rate of gas production.

For refeeds, (at the time nutrients became depleted, or gas production ceased) various additions were made to bring the final concentration to:

1. 1% sucrose
2. 1% sucrose, 0.1% yeast extract
3. 1% sucrose, 0.1% yeast extract, 0.1% ammonium nitrate
4. 1% sucrose, 0.1% ammonium nitrate

All additions were aseptic, using components sterilized by autoclaving.

Cores

Cores were gifts from various oil interests. They were analyzed (mineral content) by X-ray diffraction.

Before use, cores were ground to a fine powder with a mortar and pestle. Once ground, the powder was thoroughly mixed to get a homogenous mixture of all core contituents, and to ensure that all components of the core were evenly distributed.

Cores were usually added to Hungate tubes at 0.5 gm/tube. Experiments were also done with enough core material to simulate a 15% porosity within a Hungate tube, using the formula $\pi r^2 \times H \times 0.85$, where r is the radius of the tube, H the height of the medium in the tube, and 0.85 a factor that varies according to the porosity one desires to simulate (1-0.15=0.85). Gas production and growth appeared to mirror conditions under which less core material was used, but the actual gathering of data was difficult and these experiments were abandoned.

Refeed Procedure

Normally, refeeding was done 288 hr post-inoculation, at which time cell proliferation and gas production had stopped. A 3" needle (alcohol flamed) was used to aseptically remove from the culture 2 ml of spent medium. At 288 hours post-inoculation, a large majority of the cells have lost motility and have fallen to the bottom of the tube. The top 2 ml which were removed contained very few cells as compared to the remaining 8 ml. This procedure assured a low washout rate of viable cells. Next, 2 ml of fresh medium, which upon a 1/5 dilution would give the proper fresh concentration of nutrients were added with a sterile syringe.

End-Product Analysis

Gas production (mm^3) was determined by the distance the plunger on a syringe was pushed as it penetrated the septum of a Hungate tube using the formula $\pi r^2 \times h$ in mm.

Acids and Solvents- All analysis were done with a Sigma 2 series Perkin-Elmer gas chromatograph equipped with a flame ionization detector and a Perkin-Elmer M-2 calculating integrater. Samples were prepared by adding to 1 ml of spent culture medium (cell free) 0.1 ml of 50% phosphoric acid.

For analysis 1 µl of the prepared sample was injected. Internal standards of propanol or valeric acid were used, if questions arose as to the validity of peak areas.

Standard curves were prepared using appropriate solvents and acids at appropriate concentrations. A plot of peak area versus concentration of each end-product was used to determine unknown concentrations. Co-chromatography was used to confirm initial end-product analysis.

RESULTS

First, determinations of the total gas that culture SEG-3 could be expected to produce in the core base medium, with all trace elements and buffering capacity supplied extraneously, were made. In order to do this, the core base medium was supplemented with Erwinia trace minerals and 20 mg/100 ml of magnesium sulfate and 10 mM combined

phosphate buffer. Tubes were inoculated, after which 10 mM phosphate buffer was added back to the tubes every 48 hours. Results (n=16) are shown in figure 2. From this figure, we see that total gas production in a non-phosphate limited system is approximately 37,400 cubic millimeters.

We next must address what effect, if any, the level of phosphate (added back) has on the total gas production. The total phosphate (mg/100mg) of yeast extract is 3.6, giving us a final concentration per tube of 0.36 mg from yeast extract. Total phosphate per 100 mg of the Austin chalk is 0.2 mg. Then, if we combine the experiment described above, with one where the Austin chalk supplied not only the buffering capacity but phosphate above that supplied by the yeast extract, we can get an idea of the effect of phosphate on gas production. Figure 2 gives the total gas production by SEG-3 under the two conditions of buffering capacity and phosphate concentration, where the sucrose concentration in each tube is varied. In the case of tubes without core as before, 10 mM phosphate buffer is added back every 48 hours to rebuffer the system. We see that the two curves overlap completely from 0.25% to 2.5% sucrose and that there is a linear relationship between sucrose concentration (all other components of the core base medium at base levels) and gas production. It is not clear what the upper limit would be for gas production with increasing sucrose concentration. The large discrepancy between the Austin chalk and 10 mM phosphate at 3.5% sucrose has not been explained. If we look at the fermentation pathway carefully and calculate the ATP requirements, it becomes evident that little phosphate is needed in the case of the butylic clostridia for ATP. The acetone-butanol pathway yields a net of 3 ATP synthesized for every molecule of glucose fermented; more than the two from the Embden-Meyerhof pathway.

Core Experiments

Looking at Table 1, the X-ray analysis of each core, we see that the calcite concentration goes from high in the Kansas City Lime to medium in the Austin Chalk and low in the Redden Sand. The other cores contain little or no detectable calcite. Small amounts of dolomite are present in the Kansas City Lime and Austin Chalk. Clays abound in the Dutcher, Bryden and Douglas sands, with some clay present in the Austin Chalk.

Total gas production as a function of time of Culture SEG-3, grown in the presence of each core (0.5 gm/tube) is shown in figure 3. Here we see that the gas production values from each core are similar during approximately the first 24 hr. At 48 hr the two limestone cores, and the Redden Sand have allowed significantly more gas production than the other three cores. Yet, initially, sandstone cores are almost as adept at allowing gas production as the limestone, chalk or carbonate cores.

We can, therefore, safely assume that sandstone cores can provide sufficient buffering capacity for a complete fermentation; this leads to the conclusion that clays must provide some buffering capacity. With certain clays, that is not surprising. In situ, where that ratio of medium to reservoir rock will be lower than we employ in the laboratory test, the fermentation can go to completion, and it is not necessary to add any buffering agent to the medium.

Experiments using spore preparations as inoculum show that there is a longer lag period between inoculation and gas production than if exponentially growing cells were used. The extent of this differential varies with the core. In all cases, this extended lag is the result of the time required for germination of spores. Similar curves are obtained in any medium when exponential cells and spore preparation are compared as to length of lag. This leads us to the conclusion that the clays do not bind the amino acids required for germination avidly enough to prevent germination. Of all the cores, the Redden Sand (low clay content) appears to have the longest delay in gas production when spore inoculations are compared to exponential cells. Since the Redden Sand contains no detectable clay, the longer lag could result from the unavailability or deficiency of almost any molecule necessary for spore germination. Comparing total gas production figures of the Redden Sand with any other core (log or spore inoculations) show that after the lag in time for gas production to occur in the case of a spore inoculum, total production is better than or equal to all other cores.

Table 2 shows the concentrations of products (mM) from each core after the initial fermentation. If we rank each core after the initial fermentation, from the lowest concentration of butanol to the highest, we have the Austin chalk, Kansas City lime, Redden sand, Bryden sand, Douglas sand and Dutcher sand in increasing order of butanol production. This is approximately the order one would place the cores as to ability to supply buffering capacity because of their carbonate composition. The final concentration of ethanol also appears to be somewhat correlated with the buffering capacity. Levels of acetone and acetic acid are for the most part comparable.

It is interesting to note, in comparing the data in Tables 2 and 3, that there is a close relationship between the final pH and the production of n-butanol in the initial fermentation. In cores where the final pH was 5.0 or higher, (Redden Sand, Kansas City Lime, and Austin Chalk) the yield of n-butanol was very low or zero. With a pH of 4.74 (Bryden Sand), the yield was intermediate (14.68 mM), and in the case of the cores with the lowest pH values, the yield of n-butanol was over 20 mM, a figure probably close to the theoretical maximum. This is strong evidence that the break point is reached at a pH very close to 5.0, and that substantial butanol production does not occur until the pH

falls below 5.0. The butyric acid figures do not lend themselves to a simple interpretation involving a direct conversion of butyric acid to n-butanol.

Major differences occur only upon the refeed of the cores, when we remove 2 ml of spent medium and add back 2 ml of fresh medium, then let the fermentation proceed again to completion or as far as the system (core) will allow it to proceed. The "refeedability" of a core can be determined in this way, by the number of times the system can be refed, with a vigorous fermentation resulting from the addition of fresh nutrients.

Figure 4 details the refeed gas production of the three cores that contain calcite: the Austin Chalk, Kansas City Lime and Redden Sand. We see very little difference between the three on the first or second refeed. On the 3rd refeed, however, the Redden sand begins to degenerate and can no longer supply the necessary components for a complete fermentation. Gas production falls off drastically (data not shown). No limit has yet been found for the Austin Chalk or Kansas City Lime, if a structured refeed schedule is kept. In fact, tubes of the Austin Chalk have been kept going for over a year, with refeeds every two weeks.

Figure 5 shows the refeeds of the remaining three sandstone cores. We see that the first refeed does give some gas production in the Bryden sand, but not the Douglas or Dutcher. We can conclude that the refeed limit of the Bryden sand is one and the Dutcher and Douglas sand cannot with efficiency be refed.

Table 4 gives the acid and solvent ratio of each core after the first refeed. We see that butanol is being produced in the carbonate cores, even though the pH break point has not been reached. (This could happen if the break point were not an "all or nothing" phenomenon; i.e, the changeover occurs gradually over a range of pH, say 5.1 to 4.6.) Also it is very likely that factors other than pH are involved in the break point. It is documented that with Cl. Acetobutylicum, the occurrence of the break point is associated with sporulation. From studies of the genetics of spore formates in Bacillus, it is known that certain genes whose expression results in sporulation are "turned on" only when specific metabolites fall below a critical level. These gene products initiate a series of events resulting in spore formation. Likewise, in Clostridium, there may be certain genes that are not expressed during exponential growth, but when they are, the break point ensues. One such gene could code for acetoacetyl decarboxylase.

Table 5 shows the final pH of the spent medium, after the first refeed of various cores. We see that the final pH of the carbonate cores is high enough to allow growth to occur, while that of the sandstone cores is too low to permit much growth.

CONCLUDING STATEMENT

Cores can provide sufficient buffering capacity for a complete or near-complete fermentation to occur. This buffering capacity can even be extended in the case of certain cores to a first, second or even further refeed. As would be expected, the carbonate cores or cores with some carbonate are much better on refeed that cores that do not contain carbonate. A buffer addition to the injection medium, upon the first fermentation, is not needed. Should it prove economically advantageous to refeed, buffers or acid neutralizers would have to be added after the second refeed.

Cores also provide the trace elements necessary for a complete fermentation, which can satisfy the need, even if the yeast extract (which contains trace nutrients) is left out of the medium. We can therefore safely assume that should the produced water or make-up water not contain an element necessary for a complete fermentation such as iron, the core will provide same, no doubt as a result of organic acids causing the release of not only iron, but other divalent cations required for a complete clostridial fermentation. Again, we are not concerned with the concentrations of most trace elements since produced waters generally contain a plethoria of same.

Evidently, the clays in cores do not bind amino acids avidly enough to cause any problem as to their availability for germination. There is a slight difference in time required for germination between the carbonate and sandstone cores, but the core with the longest delay in germination (based on turbidity and delay in measurable gas production) is the Redden sand, which contains very little clay.

Differences occur in the fermentation pattern from various cores. As one would expect, the yield of butanol in the initial fermentation is much higher in cores with a low buffering capacity; the concentration of ethanol also appears to parallel the buffering capacity, although total quantities of ethanol are much lower. Other end-products are comparable from core to core. On refeed, the carbonate or highly buffered cores begin to produce butanol, in response to some signal other than pH.

The time has come to extend experiments such as these to whole cores. We need to determine the differences between growth in situ and the effects of flooding with the end-products, at concentrations comparable to those produced in the cores themselves. We should also determine how cost-effective refeeding a reservoir would be. One major question remains: if we get a recovery of 18-21% during the initial fermentation, can we expect a similar recovery on refeed?

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TABLE 1. - Core analysis by x-ray differation

CONSTITUENT	KANSAS CITY LIME	DUTCHER SAND	BRYDEN SAND	AUSTIN CHALK	DOUGLAS SAND	REDDEN SAND
QUARTZ	5-10	30-40	40-50	5-10	40-50	50-100
FELDSPAR	-0-	2-5	15-20	2-5	15-20	-0-
CALCITE	80-100	-0-	-0-	50-65	-0-	10-20
DOLOMITE	5-10	-0-	-0-	-0-	-0-	0.5-2
KAOLINITE	-0-	5-10	5-10	2-5	5-10	2-5
ILLITE	-0-	5-10	-0-	2-5	5-10	-0-
MIXED LAYER	-0-	20-30	5-10	-0-	5-10	0.5-2
CHLORITE	-0-	5-10	5-10	0.5-2	5-10	-0-
PYRITE	-0-	0.5-2	0.5-2	-0-	0.5-2	-0-
ANHYDRITE	-0-	-0-	-0-	-0-	-0-	0-1
SIDERITE	-0-	2-5	0.5-2	-0-	0.5-2	-0-
SMECTITE	-0-	-0-	-0-	10-50	-0-	-0-
MICA	-0-	-0-	5-10	-0-	-0-	-0-

TABLE 2. - Initial solvent and acid production (mM) by culture SEG-3 in the core medium where various powdered cores provide trace element and buffering capacity. All incubations 288 hours at 30°C

CORE	ETHANOL	ACETONE	N-BUTANOL	ACETIC	BUTYRIC
KANSAS CITY LIME	-0-	6.60	0.58	0.09	20.00
AUSTIN CHALK	0.63	6.44	-0-	0.10	17.64
DUTCHER SAND	3.32	6.31	25.55	0.11	18.20
REDDEN SAND	-0-	7.08	1.09	0.09	21.35
DOUGLAS SAND	0.64	4.27	21.73	0.01	11.33
BRYDEN SAND	1.92	8.10	14.68	0.05	15.05

TABLE 3. - Final pH of spent medium in the presence of various cores after initial fermentation

CORE	pH FINAL	YIELD OF N-BUTANOL mM
DOUGLAS SAND	4.34	21.73
DUTCHER SAND	4.63	25.55
BRYDEN SAND	4.74	14.68
REDDEN SAND	5.01	1.09
KANSAS CITY LIME	5.58	0.58
AUSTIN CHALK	5.71	-0-

TABLE 4. - Comparison of initial solvent and acid data with refeed solvent and acid data. Powdered cores supply trace element and buffering capacity. All analyses done 288 hr post-inoculation or refeed

CORE	FERMENTATION	ETHANOL	ACETONE	N-BUTANOL	ACETIC	BUTYRIC
DOUGLAS	INITIAL	0.64	4.27	21.73	0.01	11.33
DOUGLAS	1ST REFEED	1.50	7.61	21.88	0.03	17.21
BRYDEN	INITIAL	1.92	8.10	14.68	0.05	15.05
BRYDEN	1ST REFEED	1.74	7.11	26.94	0.13	10.10
DUTCHER	INITIAL	3.32	6.31	25.55	0.11	18.20
DUTCHER	1ST REFEED	1.70	6.44	20.52	0.29	10.17
REDDEN	INITIAL	-0-	7.08	1.09	0.09	21.35
REDDEN	1ST REFEED	1.19	13.50	0.90	0.57	13.48
AUSTIN CHALK	INITIAL	0.63	6.44	-0-	0.10	17.64
AUSTIN CHALK	1ST REFEED	-0-	7.87	4.19	0.20	14.03
K.C. LIME	INITIAL	-0-	6.60	0.58	0.09	20.20
K.C. LIME	1ST FEED	1.50	5.12	2.16	0.17	10.12

TABLE 5. - Final pH of spent medium in the presence of various cores after first refeed

Core	pH Final
Douglas Sand	3.95
Dutcher Sand	4.30
Bryden Sand	4.55
Redden Sand	4.74
Austin Chalk	5.35
Kansas City Lime	5.50

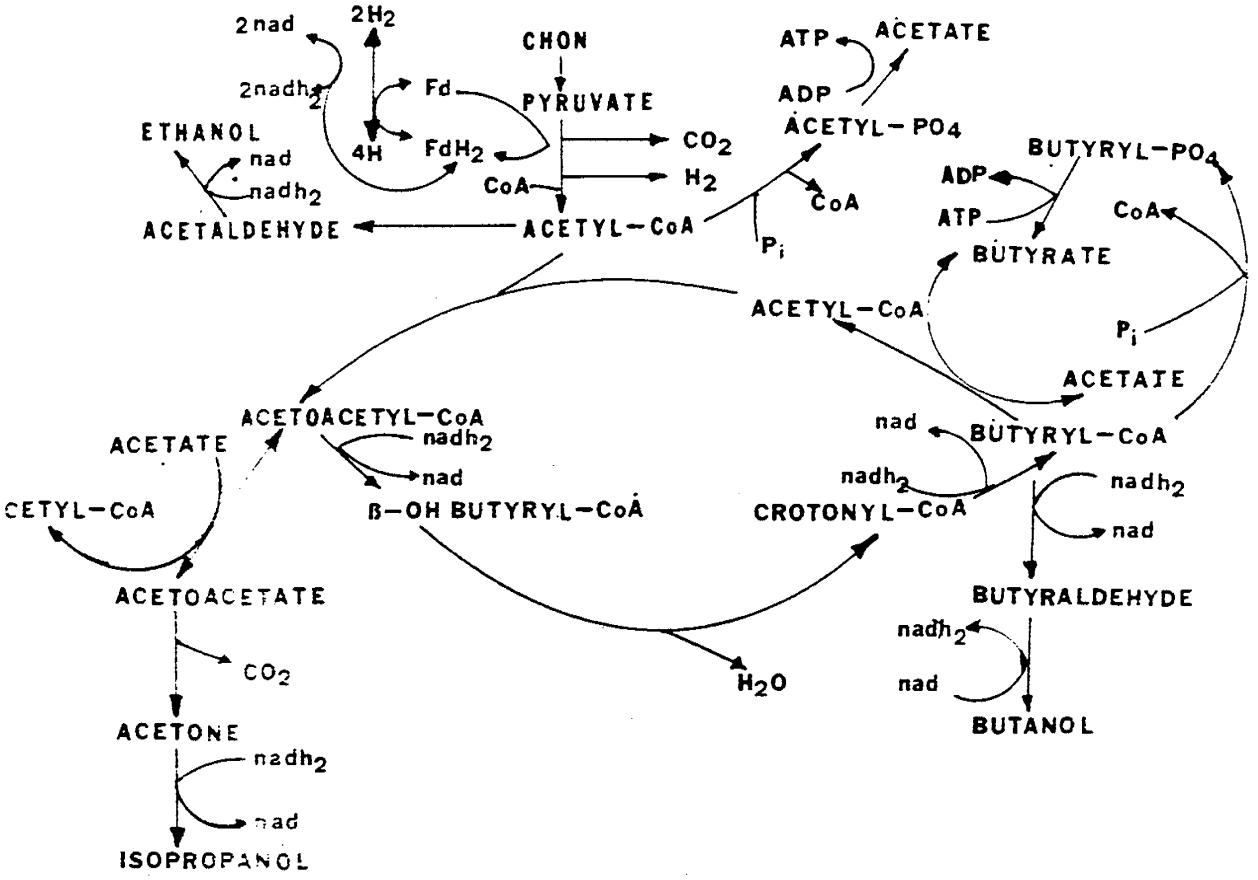

FIGURE 1. - Pathways in the butylic (acetone-butanol) fermentation.

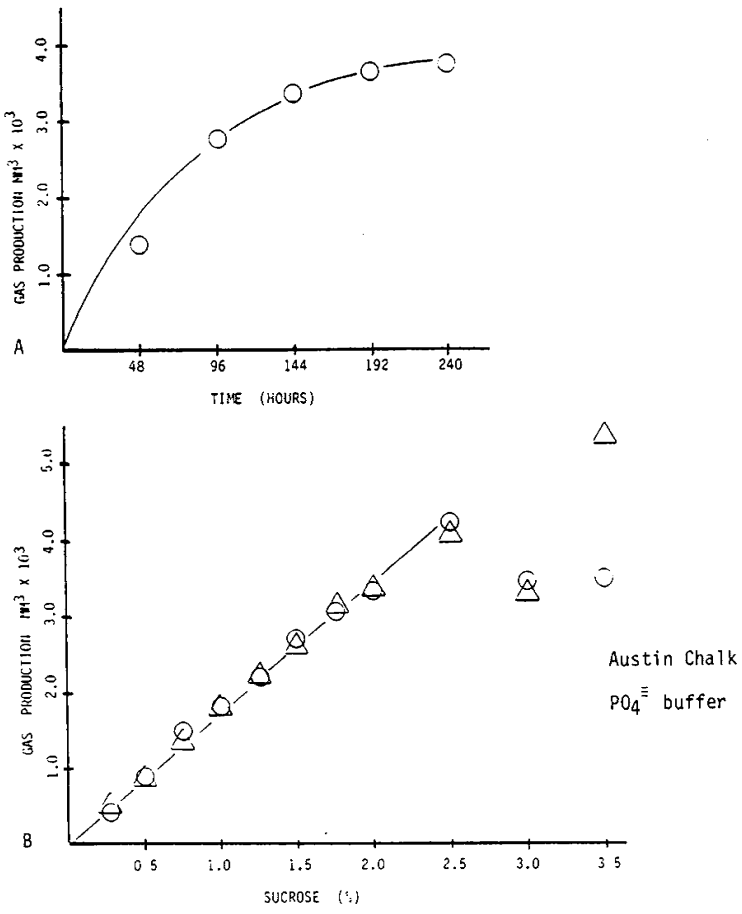

FIGURE 2A. - Total gas production by SEG-3 in core basal medium, with buffer added every 48 hr.

2B. - $PO_4^{\equiv}$ buffer (10mM) added every 48 hr, or Austin chalk, 0.1g/tube. No added buffer.

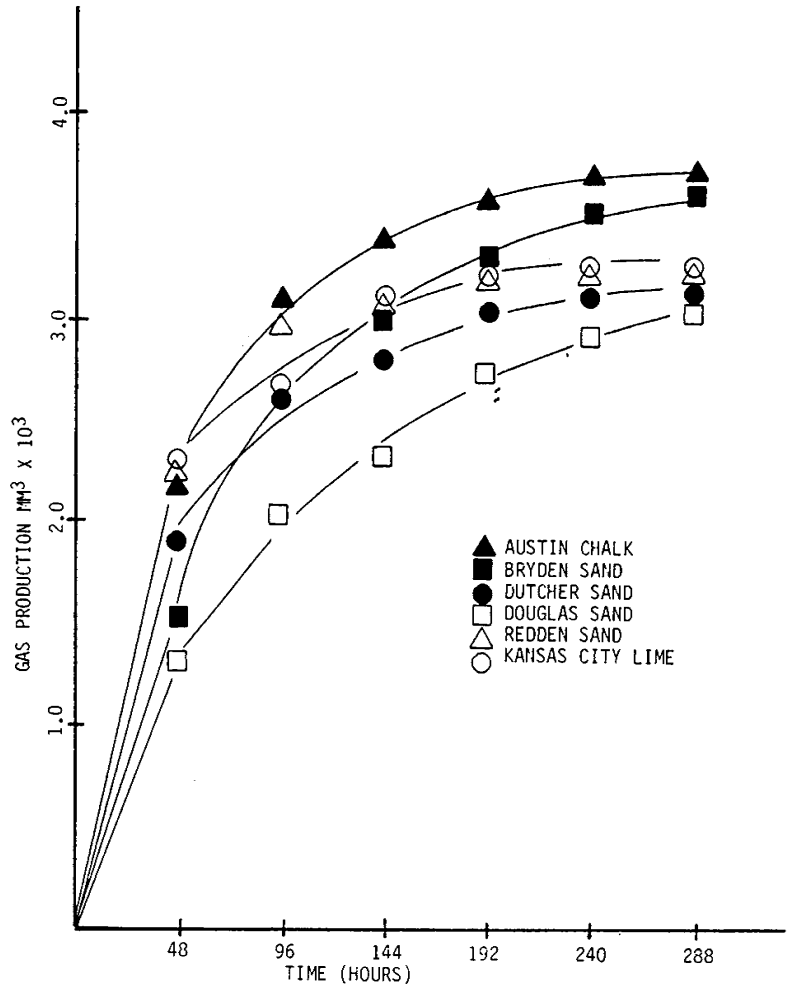

FIGURE 3. - Total gas production in the presence of various cores as a function of time.

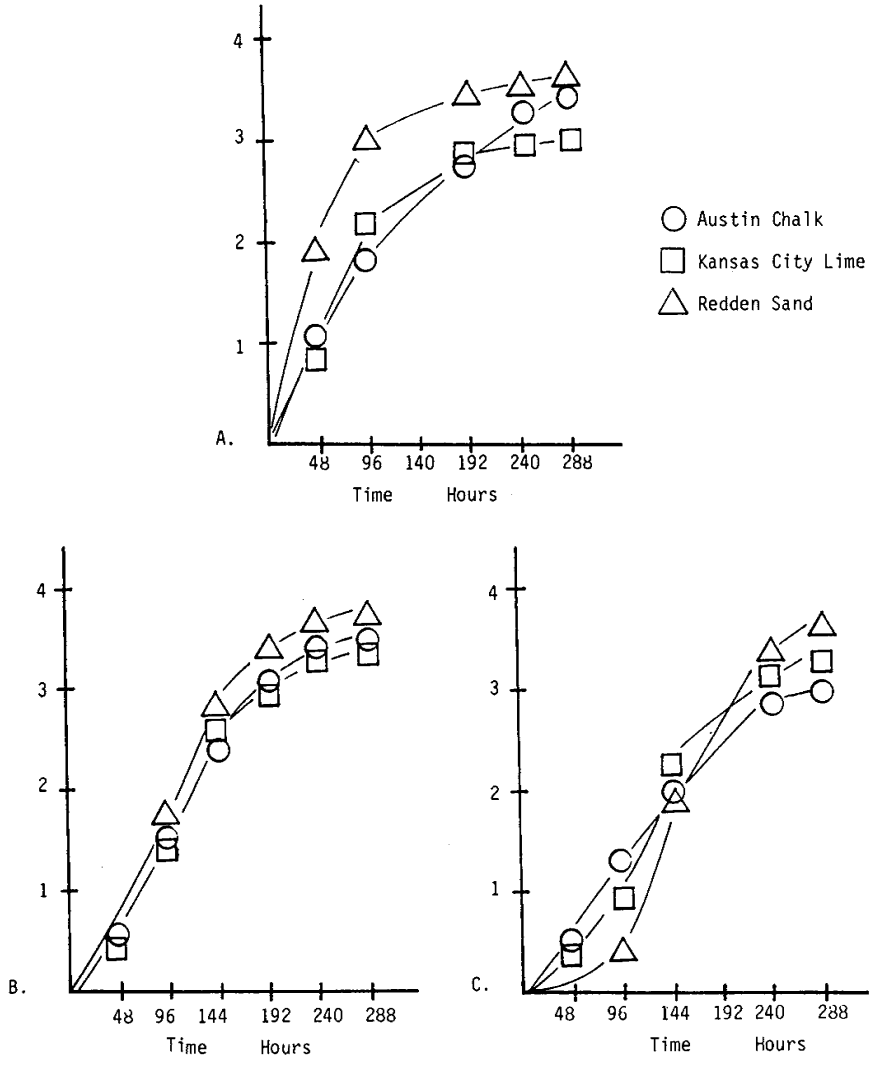

FIGURE 4. - Total gas production in various cores. (A.) Initial fermentation. (B.) First refeed. (C.) Second refeed. Vertical axis in all cases represents gas production $mm^3 \times 10^{-4}$.

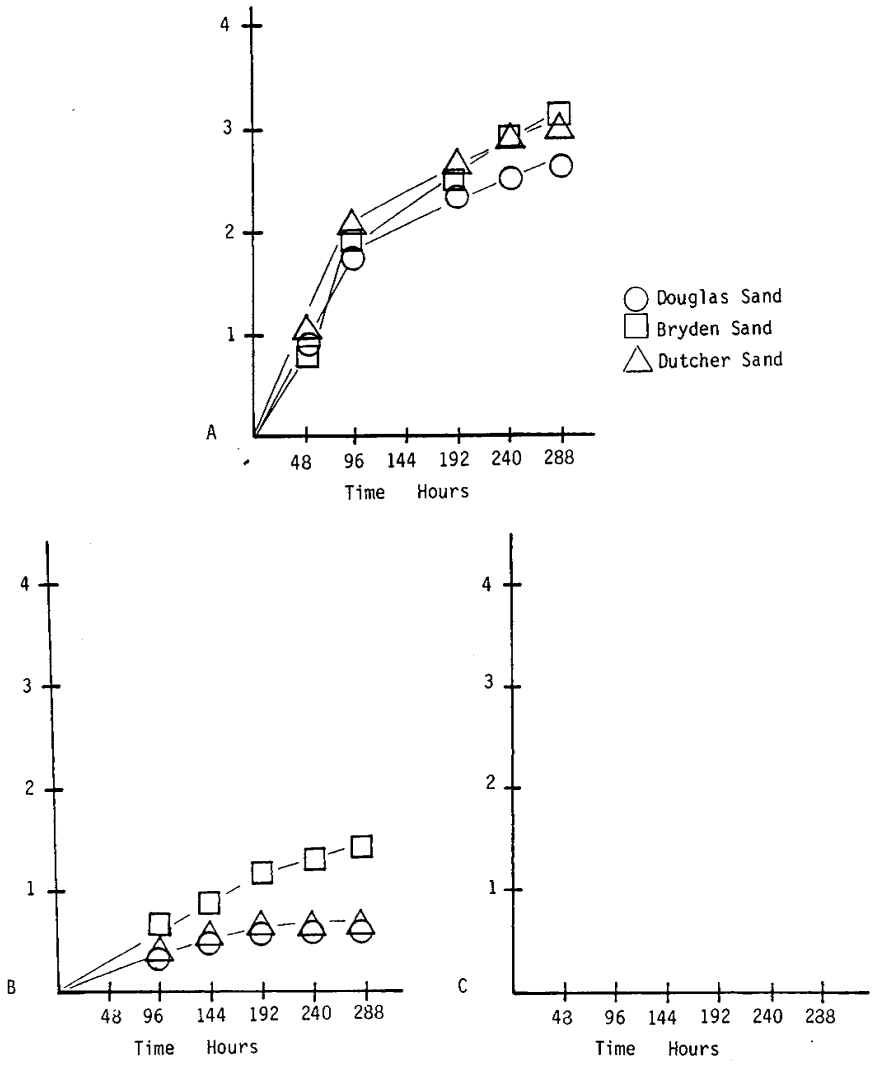

FIGURE 5. - Total gas production in sandstone cores. (A.) Initial fermentation. (B.) First refeed. (C.) Second refeed. Vertical axis in all cases represents gas production $mm^3 \times 10^{-4}$.

MICROBIAL SELECTIVE PLUGGING AND ENHANCED OIL RECOVERY

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ABSTRACT

A method for oil recovery enhancement by stimulating growth of indigenous microbial populations in Berea sandstone cores by nutrient injection was investigated. Two systems were used in this study: one using sets of two parallel cores of differing permeabilities without crossflow, and another using sets of two cores of differing permeabilities with crossflow.

In the parallel core experiments, microbial activity was responsible for recovering 10% to 38% of the original oil in place. Volumetric and microscopic sweep efficiencies were improved by the selective plugging process. Biogenic gas production accompanied the oil production, and much of this gas was entrained within the produced oil. Similar results were obtained in crossflow experiments. Biomass (as phospholipid) was detected throughout the cores. Biomass was evenly distributed through the high permeability cores, but biomass concentration decreased with increasing length in low permeability cores. The energy source did not limit growth, since sucrose was detected in the effluent. Biopolymer was produced by the cells, and may have acted as a supplementary carbon source and a oil recovery mechanism. Most of the carbohydrate found in the effluent after growth occurred in the core was polymeric. The primary limiting condition for bacterial growth, and oil recovery, is likely the sizes of the pores within the rock.

When the crossflow system was used to study the process, similar results were obtained. Flow was diverted to the lower permeability layer, apparent permeability was reduced preferentially in the high permeability layer, and oil was released by the process. Gas production occurred preferentially in the high permeability layer once permeability reduction occurred.

INTRODUCTION

Permeability variation and crossflow limit oil recovery from petroleum reservoirs.[1-7] When water is injected into a reservoir, flow will be preferentially conducted into the high permeability layer, and flow into lower permeability layers will be diverted into high permeability layers whenever possible. Increased permeability variation decreases volumetric sweep efficiency of enhanced oil recovery processes, and crossflow complicates this problem by allowing flow between contrasting layers.[8-12]

Several methods to selectively plug petroleum reservoirs have been proposed, including polymers, gels, clays, paraffins, and resins.[12-16] These have not been widely used in the field since they do not selectively plug larger pores, do not penetrate deep into the reservoir, migrate when injection pressure is decreased, and are not easily matched to the reservoir in question.

Bacterial selective plugging has been proposed to correct microscopic and volumetric sweep efficiencies within geologic formations.[17-18] This selective plugging could correct stratification problems within a reservoir by penetrating the high permeability layers of a reservoir. Since bacteria multiply their mass at exponential rates, plugging could be enhanced under growth conditions. This increase in cell mass could divert flow to less permeable zones, and increase oil recovery in those zones.

Much laboratory work has been directed to the study of microbial selective plugging. Jenneman et.al[19-20] have shown that nutrients necessary for growth and metabolism can be transported through sandstone cores. Jang et.al.[21-22] and Jenneman et.al.[19-20] have shown that bacteria can penetrate through sandstone cores. Jenneman et.al.[19] showed that microbial penetration is reduced in cores below 100 mD due to increasing probability of cells encountering pores too small to penetrate. Torbati et.al[23] and Jack and DiBlasio[24] suggested that live cells preferentially plug larger pore throats. Hart et.al[25] observed that dead cells do not plug large pore throats.

Shaw et.al[26] found that bacteria produce an exopolysaccharide film, which contributes to pore plugging by suspending clay particles, cells, and other suspended solids. This polymer was not removed by fluid passing through the porous media or by bacteriocidal agents. Cusack et.al[27] reported that a bleach and acid treatment will remove microbial facial plugging in cores and waterflood injection wells. Raiders et.al[28] have reported that semi-continuous nutrient injection under anaerobic conditions will limit facial plugging in cores.

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Jack and DiBlasio[24] and Jang et.al[22] have reported that injecting pure cultures of a specific bacterial strain will increase oil recovery beyond residual oil saturation. Jang et.al[22] and Jenneman et.al[19] noted that some of the incremental oil recovery may be due to indigenous or contaminating organisms. Freeman[29] showed that growth of indigenous bacteria is responsible for increased oil recovery from linear sandstone cores, and suggested that sweep efficiency improvements observed by Torbati et.al[23] and biogenic gas production are important mechanisms in recovering residual oil.

Mathematical and physical model systems have been developed to study crossflow.[5,6,12,30-32] While wellbore plugging was not effective, a plug penetrating deeply into the formation should alter flow patterns enough to recover additional oil.[12,33] Mathematical models assuming no flow between stratified layers were not found adequate to simulate experimental crossflow situations. Mathematical and physical models agreed when the mathematical models accounted for crossflow.[2,12,30,33] This shows the importance of crossflow to the flow of fluids in actual oil reservoirs.

Most physical models simulating stratified reservoirs have been made from Ottawa sand or glass beads.[7,34-39] These are much more permeable than consolidated oil reservoirs. We have constructed a crossflow model using two slabs of Berea sandstone with differing permeabilities. We also developed a corresponding mathematical model to examine the effects of bacterial growth and metabolism on fluid flow patterns under simulated reservoir conditions.

EXPERIMENTAL PROCEDURE

Blocks of Berea sandstone (Cleveland Quarries, Amherst, OH) were cut into 6 inch long, 2 inch diameter cores for parallel core experiments or 12 inch x 12 inch x 2 inch slabs for crossflow experiments. Cores were steam cleaned for 2 weeks,[40] dried for 24 hours at 125 C, and stored in a vacuum desiccator until use. Two inch outcrops were removed from two sides of the crossflow slabs to determine initial core properties. (Figure 1) These slabs were cored in the three principal directions to determine initial conditions in each direction.[41] All cores were coated with Conap Easypoxy. Plexiglas endplates were epoxied to the parallel cores, and pressure taps were drilled into the crossflow cores (Figure 2). The crossflow system was assembled with a sheet of Whatman chromatography paper between layers. All cores and crossflow systems were then wrapped with fiberglass cloth and coated with fiberglass resin. An additional layer of epoxy was applied to the cores.

Each sandstone system was flushed with CO_2 for 15 minutes and vacuum saturated with 5% NaCl + 0.1 M $CaCl_2$ brine. The calcium was added to stabilize clays in the rock.[42] All injected fluids were filtered through 0.22 um filters and saturated with N_2 to limit the amount of dissolved O_2 in the brines. After saturation, 100 pore volumes (PV) of brine were flowed through each parallel core (10 PV through the crossflow systems) at constant flow rate, (0.02 ml/sec for parallel cores, 0.05 ml/sec for crossflow) using a Gilson HP4 Miniplus peristaltic pump. Fifteen PV of 5% NaCl brine were then injected, to reduce calcium concentrations in the cores, since high calcium concentrations may interfere with microbial growth. Initial core properties were then measured.

Pressure across parallel cores was measured by Validyne DP-15 pressure transducer, converted by a Validyne CD-380 signal demodulator, and routed to a calibrated linear chart recorder. Pressures for injection, and each pressure tap, for the crossflow experiments were measured using PermaCal pressure gauges. Flow was measured as a volumetric rate.

Parallel cores were connected at the injection source at this time.[43] Crossflow injection pumps were calibrated to inject fluids into the core at the same potential.[44] All parallel cores were saturated with n-hexadecane, as was one crossflow system. All oil saturated systems were waterflooded to residual oil saturation.

A series of treatments was then performed on each system. Treatments consisted of injection of one pore volume of nutrients[23] into the high permeability zone or until pressure response was observed, incubation for 24 hours, and waterflood until stable pressure was reached. Gas was collected in an inverted graduated cylinder filled with 5% NaCl brine at pH 3. The treatment process was repeated until stable permeability reduction factor (PRF = % of remaining initial permeability) was reached or the pressure limits of the system were reached.

The pH of effluent samples collected within the first pore volume of each waterflood was measured using a Fisher Accumet Model 630 pH meter. The oil-effluent interfacial tension was measured using a Fisher Autotensiomat Dunouy ring tensiometer.

Gas analysis was performed with a Packard 427 gas chromatograph with a Porapak Q column and a thermoconductivity detector.[45] Volatile fatty acid concentrations were determined with a Varian 3400 gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard fused silica column.[46] Carbohydrate and nitrate concentrations of effluent samples were determined colorimetrically.[47] The amount of polymeric carbohydrate was determined after dialyzing samples in dialysis tubing with a 500

dalton exclusion size. Biomass concentrations along the length of cores were determined by extraction and quantitation of the phospholipids as described by White et.al.[48]

CROSSFLOW SIMULATION MODEL

A mathematical model was developed to simulate the core system. The computer program solved Poisson's equation in three dimensions for the potential in each grid block using second order correct, centered finite difference approximations. The system of linear algebraic equations were solved using the SOR method.[49-53] The validity of the model was tested by simulating several three dimensional experiments in steady-state, non-growth systems. The model was utilized to predict if permeability reduction alone could explain changes in flow patterns within the crossflow systems.

The simulation model agreed with previously published data, suggesting that selective plugging is effective in changing crossflow patterns within plugged regions. Wellbore plugging alone will not correct flow profile if layers are in capillary contact.[12,33,39]

RESULTS

Parallel Core Experiments

Physical parameters of the cores are shown in Table 1. Oil recovery and permeability reduction data are shown in Table 2. For the first three experiments, nutrients were injected into the cores until pressure response was observed. In the fourth experiment, only one PV of nutrients were injected into the high permeability core after the initial treatment, which was continued until pressure response was observed. Experiment 3 was not waterflooded completely to determine if mobile oil saturation affected the microbial plugging process. The first 20 ml produced from each core during each waterflood were analyzed. Samples from the first three experiments were analyzed for pH changes and interfacial tension changes. Samples from experiment four were analyzed for material balance determinations.

In all experiments, the level of permeability reduction was similar, 6-8% for high permeability cores, 14-18% for low permeability cores. Permeability reduction occurred more quickly in high permeability cores than in low permeability cores. (Figure 3) Oil recovery and gas production did not occur in significant quantities until PRF dropped below 50%. Most of the oil recovered by the treatment process in high permeability cores

was produced within 3 or 4 treatments after PRF had fallen below 50%. Gas bubbles were entrained in the oil as it was produced from the cores.

Correcting for the initial waterflood front from the third experiment, the oil recovery from that low permeability core correlates with similar cores. Between 10 and 39% of the oil left behind in the cores after waterflood was recovered by the process. Generally more oil was recovered from the high permeability cores than from low permeability cores.

Figure 4 shows the volumetric sweep efficiency improvements in the core system as each experiment progressed. The best sweep efficiency improvements were observed in the third dual core experiment, which had the greatest difference in permeability contrast between cores. The smallest improvement in sweep efficiency occurred in experiment 2, where the permeability contrast was the least.

Material balance calculations for carbon were performed for each treatment in the fourth experiment. The carbon balance data are shown in Table 4. These results are summarized in Figure 5. Initially, some nutrients were found in the initial effluent sample, L0, taken from the first 20 ml of the first nutrient injection. This suggests that the pore structure of core L4 was very heterogeneous, and that most of the flow was through a few large pores. The initial peak in both curves showed that little metabolism of the nutrients occurred at the start of the experiment. Some of the nutrients injected into the cores during the first treatments may have been adsorbed onto the clays. After this initial adjustment period, the microorganisms utilized the carbon source and produced acids, gases, and polymers. At that time, significant oil was released from the cores, and the PRF values decreased below 50%.

Figure 5 shows the cell concentration found in cores H4 and L4 after the treatment process. While the cell concentration in core H4 was fairly constant from inlet (1) to outlet (4), the cell concentration in core L4 decreased by two orders of magnitude form inlet to outlet. Growth was more concentrated at the inlet sections of core L4.

The effluent from the eleventh waterflood of core H4 was analyzed to determine the numerically dominant bacteria. Viable cell counts using plate count agar (Difco, Inc.) incubated under aerobic and anaerobic conditions were $5.7 * 10^7$ cells/ml effluent and $2.4 * 10^7$ cells/ml effluent, respectively. The dominant types of bacteria found were Klebsiella pneumoniae and a facultative rod-shaped cell similar to Photomicrobium. Both of these organisms were facultative, salt tolerant, polymer producing

bacteria. These indigenous organisms grew under the experimental conditions of this study.

A decrease in pH of the effluent from 7.0 to 6.0 occurred and a decrease in the oil-effluent interfacial tension of 20% was found throughout the study. Some CO_2 was observed in the gas samples (0.1 - 1% of the gas phase). The remainder of the gas is presumed to be N_2, since other common gaseous products (H_2, NO, N_2O, and CH_4) were not detected in any gas samples. Nitrite was detected in early effluent samples of core L4 (less than 0.2 mM). Nitrate was not detected in any effluent samples. Since the concentration of nitrate in the influent was 17mM, this data suggests that denitrification or dissimilatory nitrate reduction was the dominant metabolism in the cores.

Crossflow Experiments

Flow diversion and selective plugging by indigenous bacteria in crossflow core systems were first examined in a brine saturated system. The porosity of the high and low permeability layers was 20% and 17%, respectively. Average permeabilities for the high permeability layer were 423 mD in the x direction, 415 mD in the y direction, and 342 mD in the z direction. For the low permeability core, average permeabilities were 107 mD in the x direction, 105 mD in the y direction, and 54 mD in the z direction.

Five treatments were conducted in this experiment. In the first two treatments, very little change was observed in the potential distributions from the baseline data (Table 4). This was due to the small number of bacteria residing in the core, or found in the effluent, at the beginning of the treatment process. During later treatments, large numbers of bacteria were found in the effluent. As the bacterial populations became large enough to modify pore structure, the potential distribution was modified.

Figure 6 shows the percentage of flow into and out of the high permeability layer from this experiment. After microbial plugging occurred, the amount of flow conducted by the low permeability layer increased, to up to 60% of the total flow from the system. Relative injectivity into the low permeability layer increased by as much as threefold.

Wellbore plugging could not have caused the uniform plugging and flow diversion found in the crossflow system, according to our simulation data (not shown). Since the plug, and sustained increases in potential, occurred throughout the system, and not just in the injection and production ports, we conclude that flow diversion occurred throughout the entire high permeability layer.

The mathematical simulator predicted the actual waterflood data and data from treatments before the microbial plugging began (data not shown). After the increase in potential, the simulator no longer uniquely solved for the permeability, injectivity, productivity, or the amount of crossflow. None of the solutions adequately explained the amount of crossflow from Figure 6 that occurred in the brine saturated system. All solutions showed diversion into the low permeability layer, but the attempted solutions showed an order of magnitude less flow diversion than the experimental data.

After five treatments, the pressure on the system exceeded the operating limits of the equipment. One PV (atmospheric) of biogenic gas was collected from the system. No significant changes in crossflow patterns were observed after the gas was drained from the system. Potential decreased slightly during this waterflood.

When an oleic phase was introduced into the system, similar results were obtained, except that the magnitude of the observed changes was reduced (Table 5). This was expected since the volume of active pore space available for bacteria is reduced by introducing oil into the system.[28] The porosities of this system were 17% and 14% for the high and low permeability cores, respectively. Average permeabilities for the high permeability layer were 225 mD for the x direction, 235 mD in the y direction, and 80 mD in the z direction. For the low permeability layer, permeabilities were 95 mD in the x direction, 88 mD in the y direction, and 27 mD in the z direction. Initial oil saturations were 52% and 44% for high and low permeability layers, respectively. Residual oil saturations were 34% and 25%. Relative permeabilites to brine were 40% and 10%.

Significant flow diversion was observed, and the potential distribution was changed as bacterial growth occurred. Relative injectivity into the low permeability core increased by 50%. In the five treatments, 10% of the residual oil in the high permeability layer was recovered through the high permeability port, and 2% of the residual oil in the low permeability layer was recovered through the corresponding port.

The simulator was able to duplicate the initialization data and the results for treatments before the plug was established. Simply reducing permeability in the high permeability layer did not explain the observed crossflow in the later treatments. Since the endpoint of the relative permeability (to oil) curve is moved each time oil is recovered from the core, relative permeability changes not accounted for in the simulator may affect interlayer flow.

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DISCUSSION

The decrease in permeability observed in these experiments is believed to be the result of the in situ growth and metabolism of microorganisms, rather than abiotic factors, because of the following observations. The time lag between the initiation of nutrient injection and the pressure increase in the cores, and the exponential pressure increase observed throughout each experiment are indicative of microbial growth. Since clay swelling was controlled by $CaCl_2$ injection, and NaCl concentration was not changed during experiments, clay swelling would not cause the pressure increase.[42,54] The fluid velocity was kept below the critical fines migration velocity, so fines migration would not be responsible.[55]

Cells and biologically-produced substance were observed in the effluent from each core, and the amount of these substances increased as the PRF decreased. After 4 to 6 treatments, gas, acid, and polymer production began from each core. The production of N_2 and CO_2 suggests that the denitrification pathway was selected for by the choice of nutrients. Since gas was not initially produced from cores, and the quantity of gas produced increased as PRF decreased, the gas produced from the cores resulted from microbial action.

While all experiments exhibited some volumetric sweep efficiency improvement, the best improvement was shown in the third parallel core experiment, where the permeability contrast was greatest. The sweep efficiency improvement decreased with decreased contrast. Mobile oil saturation in a core seems to decrease microbial activity in that core. PRF declined more slowly and erratically in core L3 than in comparable cores. Once the mobile oil was produced from the core, PRF decline and oil and gas production correlated with other low permeability cores.

While PRF data for crossflow experiments was not directly available, the plugging process is comparable to the parallel cores through other experiments. The plugging process occurs more slowly in oil saturated cores than in brine-only cores without crossflow.[28] When crossflow models were tested, the plugging process was again slower for oil-saturated systems than for brine-only systems. The relative injectivity increases observed in the crossflow experiments were far more dramatic than those observed in parallel cores. We attribute the increased injectivity changes on the influence of interlayer flow.

Microscopic sweep efficiency may have also contributed to the oil recovery process. Very little oil was recovered from any of the swept cores until a substantial reduction in PRF occurred. Larger pores are preferentially plugged during in situ microbial growth

in porous media.[23,24] The lack of oil recovery until the PRF declined suggests that plugging the larger pores diverted flow into smaller pores containing higher oil saturations, which had been previously unswept.

Biogenic gas production was an important factor in the oil recovery process. At the beginning of each experiment, little oil and no gas were produced from each core. During the first 4 to 6 treatments in which gas was produced, most of the incremental oil was produced. Since in the crossflow experiments, only two treatments were run after the beginning of gas production, little of the oil recovery activity was observed before apparatus pressure limits were reached. Much of the gas produced at this time was entrained in the oil. The gas acts to increase pressure, increase mobility, and change flow patterns by introducing another mobile phase. As oil production decreased, gas was produced as a free phase, not entrained in the oil.

The largest increases in oil recovery correlated with the largest increases in the amount of gas produced. These increases occurred after most of the PRF decline and the initiation of acid and polymer production. The relatively small pH and interfacial tension decreases suggest that fermentation acids or surface-active agents were not of major importance in the recovery process,[56] although they may act with the gas, with the selective plugging, or separately to increase oil production.

The large number of PV of nutrients injected into the cores through most of this study was a result of experimental design. Since nutrients were injected into cores until pressure increases were observed, large amounts of nutrients were injected into cores at the beginning of each experiment. Since initial cell populations are low and the residence time of nutrients is short compared to the time initially required to metabolize the nutrients, much of the nutrients initially injected into the cores were not metabolized. Thus, in later experiments, the nutrient injections were restricted to 1 PV into the high permeability core or layer, and no significant changes in the process were observed. As the experiments progressed and the cell density increased from in situ growth, the amounts of nutrients needed to observe a pressure increase was much less than initially required.

Throughout the fourth parallel core experiment, not all of the sucrose injected was metabolized in the core, and significant quantities of exopolymer were found in the effluent. This polymer may serve as a carbon source for bacteria downstream from the point of sucrose utilization. When the cell density in the core was increased by injecting bacteria, PRF declined more rapidly and less nutrients were required.[28] Also, treatments were

continued until PRF stabilized or no more oil was produced from the cores. Thus, a greater number of treatments were used in this study than would be necessary in a field trial, with far more nutrients per treatment than would be needed.

Significant reductions in permeability were observed in low permeability cores in dual core experiments, even during the first several treatments. This was particularly true when the permeability contrast was small, and little sweep efficiency correction occurred. Much of this plugging was likely due to facial plugging. The amount of nutrients injected into low permeability cores during early stages of experiments often were less than 1 PV. This would force most of the growth near the entry. Also, the penetration rate of bacteria through Berea sandstone of permeability < 100 mD is slow and dependent upon the length of the core.[41] Since the average pore-throat size of these cores is less than the size of the average bacterial cell (1-5 um), the probability that a pore will end or be constricted increases with increasing length. This suggests that bacteria will not deeply penetrate low permeability regions. This is verified by decreased lipid concentration observed in the outlet end of low permeability cores, relative to that of high permeability cores. This effect is enhanced in the crossflow experiments, since facial plugging in the low permeability layers was minimal relative to the growth in the high permeability layers. The plugging in the low permeability layers was never enough to divert significant flow back into the high permeability layers.

The observation of such high numbers of bacteria in the effluent and the isolation of cells compatible with the applied experimental conditions suggests that injecting cells into cores is not necessary to increase oil recovery, and the process could the applied on a larger scale without cell injection.

CONCLUSIONS

1. Growth of <u>in situ</u> bacteria selectively plugged high permeability cores in parallel flow systems with oil present.

2. Oil recovery was increased due to increased microscopic and volumetric sweep efficiency, with or without capillary contact between layers.

3. Flow diversion by microorganisms is due to many factors, including selective permeability reduction and biogenic gas production.

4. Relative injectivity into low permeability layers increases during the plugging process, with or without oil present, and with or without capillary contact.

5. Simple permeability reduction modelling is not adequate to account for the observed incremental oil recovery.

6. Oil recovery was increased due to gas, acid, and biomass produced by in situ bacteria.

7. Growth of in situ bacteria was possible with decreased amounts of nutrients injected into cores per treatment.

ACKNOWLEDGEMENTS

This work was supported under U.S. Department of Energy contract DE-AS19-80BC 10300. We thank Mike Rozmin for performing the chemical analyses, Fish Greenfield for bacterial count data, John Grow for determining initialization data for crossflow experiments, Dave Freeman for developing the parallel core system, and Tom Maher for developing the crossflow apparatus.

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TABLE 1. - Physical parameters of cores before treatment

	Core Number[a]							
	H1	L1	H2	L2	H3	L3	H4	L4
Length of core (cm)	14.2	14.2	13.5	13.5	14.2	14.2	11.4	11.4
Porosity (%)	21	15	20	17	19	17	23	20
Pore Volume (cc)	65	48	59	51	59	55	51	44
Initial Brine Permability (mD)	866	103	292	109	1210	112	460	110
Initial Oil Saturation (%)	62	52	45	57	54	50	68	55
Effective Oil Permeability (mD)	250	55	179	37	435	65	120	52
Effective Brine Permeability (mD)	90	5.7	67	7.5	79	5.8	67	11.3
Residual Oil Saturation (%)	41	41	30	39	39	50	41	34

[a]H and L represent the high and low permeability cores, respectiveiy, from each experiment, followed by the experiment number in which they were used.

TABLE 2. - Oil recoveries and permeability reductions obtained in core experiments

	Core Number							
	H1	L1	H2	L2	H3	L3	H4	L4
Number of Treatments	13	13	12	12	17	17	13	13
Waterflood Brine Injected (PV)	2.9	.3	7.4	.5	.3	0	.5	.1
Total Brine Injected (PV)	12.8	3.3	35.4	4.3	23.1	10.1	25.1	5.3
Average Brine Injected per Treatment (PV)	1	.3	3	.4	1.4	.6	1.9	.4
Total Nutrients Injected (PV)	13	2.7	26.3	2.5	28.5	6.6	18.4	10
Average Nutrient Injected per Treatment (PV)	1	.2	2	.2	1.7	.4	1.4	.8
Waterflood Oil Recovery (% OOIP)	33.7	21.1	34	31.7	27.9	0	39.4	36
Total Oil Recovery (% OOIP)	48.4	35.9	56.3	42	55.1	38.9	56.5	54
Average Oil Recovery per Treatment (% OOIP)	1.1	1.1	1.8	.9	1.6	2.3	1.3	2
Final PRF (%)	8.3	14	6.6	15	7	18	7.7	14.2
Total Gas Produced (cc)	82	15	55	13	78	25	82	26

TABLE 3. - Carbon balance for effluent samples from core H4 and L4

SAMPLE[a]	ACETIC ACID (mM)	BUTYRIC ACID (mM)	CO_2[b] (mM)	SUCROSE (mM)[c]	POLYMER (mM)[c]	TOTAL (mM)	CARBON RECOVERY[d] (%)
L0	1.7	0[e]	0.9	106.8	0.0	109.4	31.2
L1	2.7	0	1.4	429.3[f]		433.4	123.7
L2	3.1	0	1.6	372.7[f]		377.4	107.7
L3	6.9	0	3.5	123.3[f]		133.7	38.1
L4	13.1	0	6.6	25.1	7.2	52.0	14.8
L5	16.3	0	8.2	21.5	52.5	98.5	28.1
L6	14.3	0	7.1	0.0	77.8	99.2	28.3
L7	13.3	0	6.7	52.6	103.7	176.3	50.3
L8	13.5	0	6.8	48.2	24.8	93.3	26.6
L9	14.3	0	7.1	167.8	14.2	203.4	58.0
L10	13.9	0	7.0	34.0	80.1	135.0	38.5
L11	12.7	0	6.4	53.7	19.3	92.1	26.3
L12	15.9	0	8.0	46.1	23.9	93.2	26.8
L13	18.7	0	9.4	90.0	39.7	157.8	45.0
H0	0.0	0	0.0	0.0	0.0	0.0	0.0
H1	2.3	0	1.2	252.2	22.5	278.2	79.4
H2	2.6	0	1.3	19.1	23.7	46.7	13.3
H3	7.3	0	3.6	333.8	16.5	361.2	103.1
H4	20.4	0	10.7	340.7[f]		371.8	106.1
H5	27.5	1.1	15.9	122.7	21.3	188.5	53.8
H6	30.2	1.2	17.5	86.9	46.4	182.2	52.0
H7	25.4	1.0	14.7	47.3	24.5	112.9	35.0
H8	38.2	1.5	22.1	0.0	97.3	159.1	45.5
H9	31.8	1.6	19.1	130.9	17.0	200.4	57.2
H10	27.6	1.2	16.1	53.0	115.0	212.9	60.8
H11	31.2	1.5	18.7	0.0	148.2	199.6	57.0
H12	38.0	1.8	22.7	73.4	102.1	238.4	68.0
H13	36.2	1.6	21.4	103.3	92.2	254.7	73.0

[a]H and L represent the core H4 and L4, respectively, followed by the treatment number in which the sample was collected. Samples from treatment 0 were collected during the first 20 ml of the first nutrient injection. All other samples were collected during the first 20 ml of waterfloods.

[b]CO_2 was determined by calculating carbon balance by known metabolic pathways (32). one mM CO_2 would be made from each mM acetic acid, and two mM CO_2 would be made from each mM butyric acid.

[c]Sucrose and polymer concentrations are given in mM hexose equivalent.

[d]Sucrose was injected into the cores at 350.4 mM during nutrient injection.

[e]Butyrate was detected in measurable quantities only in samples H5 through H13.

[f]This represents the sum of the polymer and sucrose concentrations.

TABLE 4. - Potential distributions in atmospheres at discrete points in the brine saturated crossflow system

	TREATMENT					
LOCATION[a]	0	1	2	3	4	5
1	1.095	1.109	1.143	1.211	1.449	2.374
3	1.054	1.054	1.061	1.095	1.299	2.143
5	1.027	1.027	1.048	1.088	1.286	2.143
7	1.034	1.027	1.034	1.082	1.279	2.129
9	1.024	1.02	1.02	1.082	1.279	2.143
2	1.095	1.102	1.143	1.224	1.449	2.401
4	1.054	1.061	1.075	1.122	1.327	2.129
6	1.408	1.034	1.048	1.088	1.313	2.075
8	1.054	1.048	1.054	1.088	1.299	2.102
10	1.054	1.054	1.068	1.102	1.286	2.061

[a]Odd numbers represent the high permeability layer, which was placed on top of the system. The potential at points 11 and 12 is given as atmospheric.

TABLE 5. - Potential distributions in atmospheres at discrete points in the oil-brine saturated crossflow system

	TREATMENT					
LOCATION[a]	0	1	2	3	4	5
1	1.102	1.095	1.109	1.177	1.367	1.728
3	1.082	1.061	1.082	1.163	1.265	1.422
5	1.068	1.054	1.068	1.150	1.259	1.408
7	1.054	1.054	1.054	1.136	1.245	1.395
9	1.041	1.020	1.048	1.109	1.231	1.388
2	1.102	1.095	1.116	1.177	1.395	1.952
4	1.082	1.068	1.082	1.163	1.259	1.414
6	1.082	1.054	1.075	1.136	1.244	1.401
8	1.054	1.034	1.054	1.122	1.224	1.381
10	1.082	1.054	1.068	1.129	1.204	1.361

[a]Odd numbers represent the high permeability layer, which was placed on top of the system. The potential at points 11 and 12 is given as atmospheric.

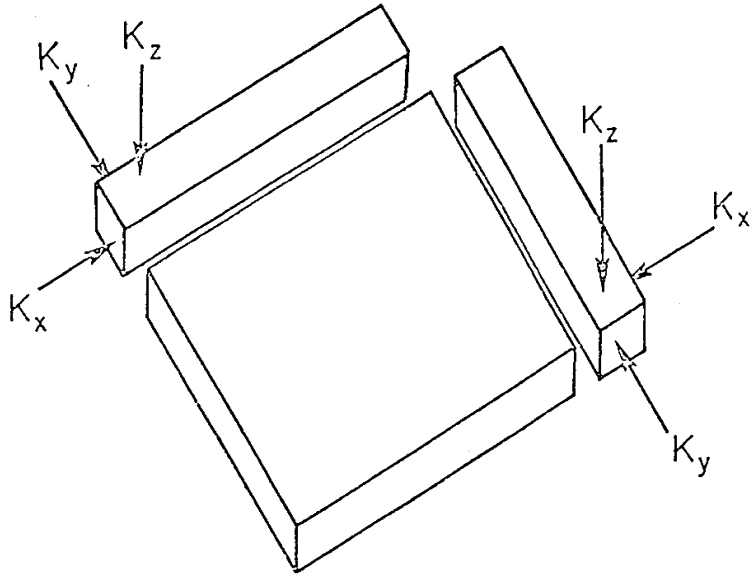

FIGURE 1. - Outcrops from crossflow slabs for directional permeability measurements.

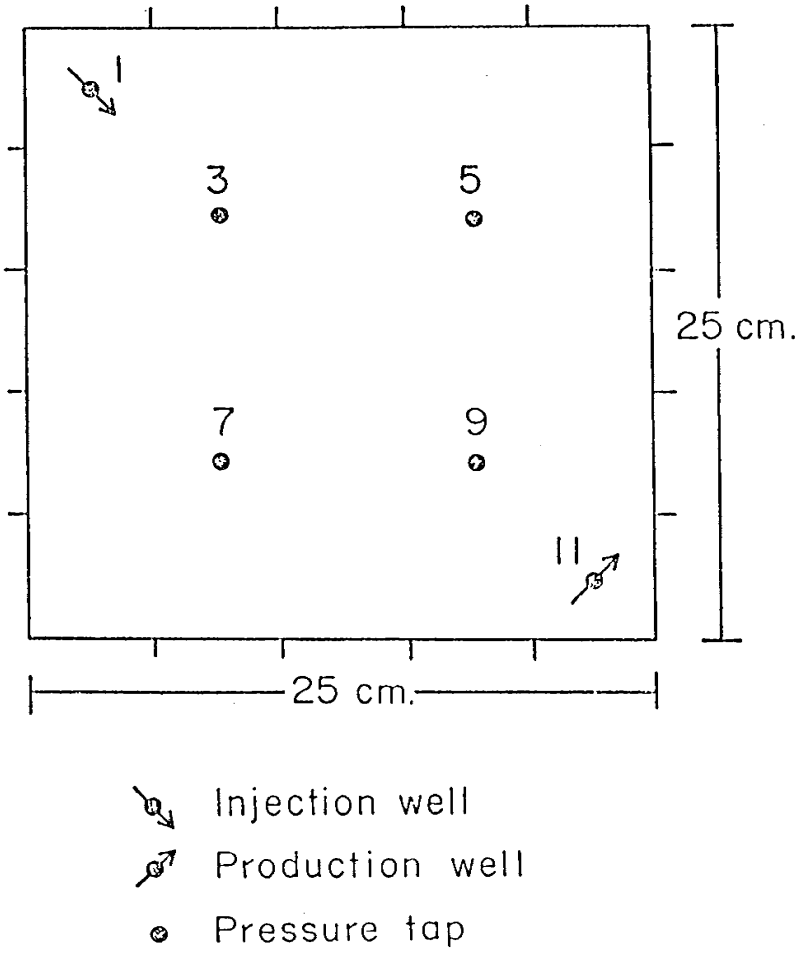

FIGURE 2. - Locations of pressure taps on crossflow systems.

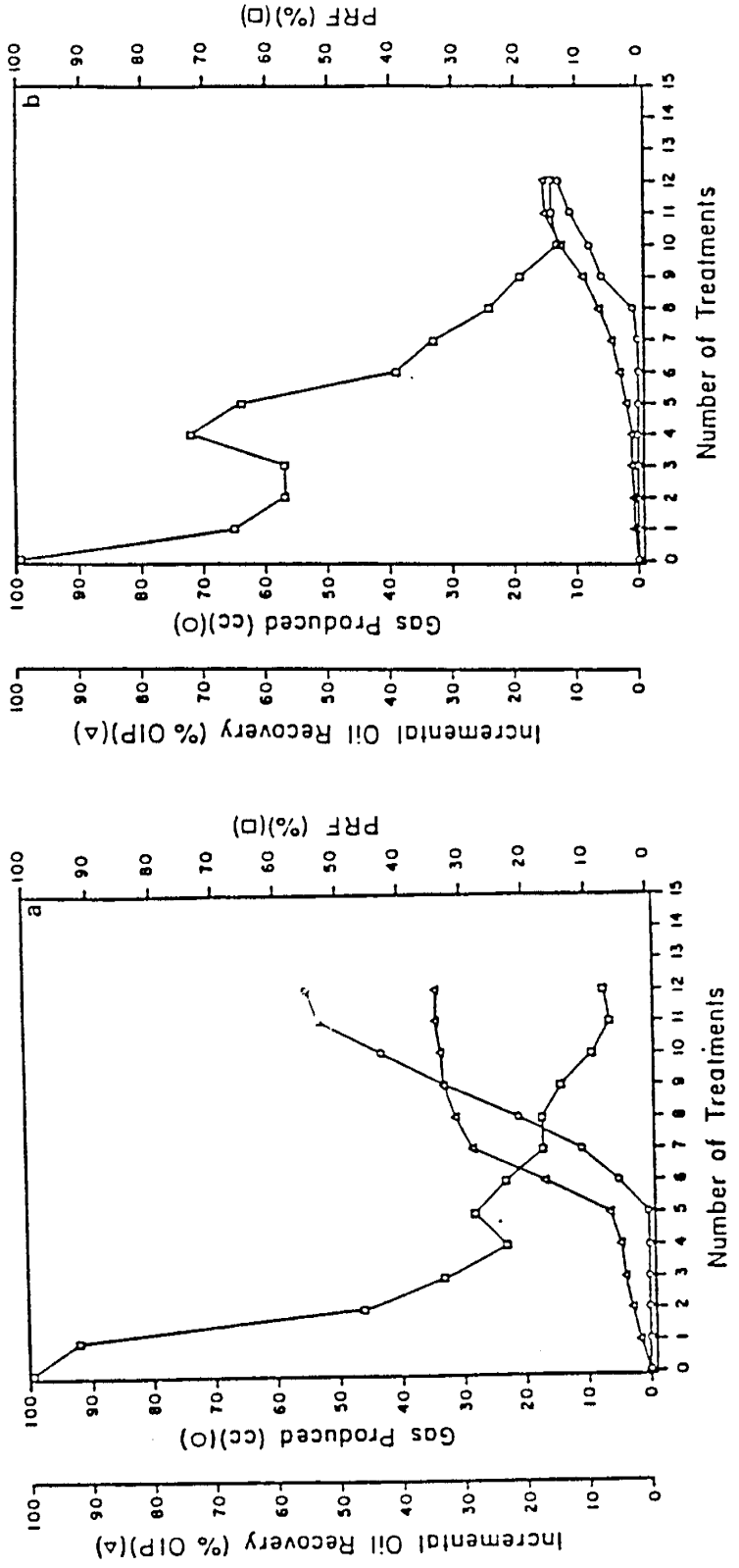

FIGURE 3. - Effect of nutrient treatments on the PRF, cumulative oil recovery, and gas production for core H2 (a) and core L2 (b).

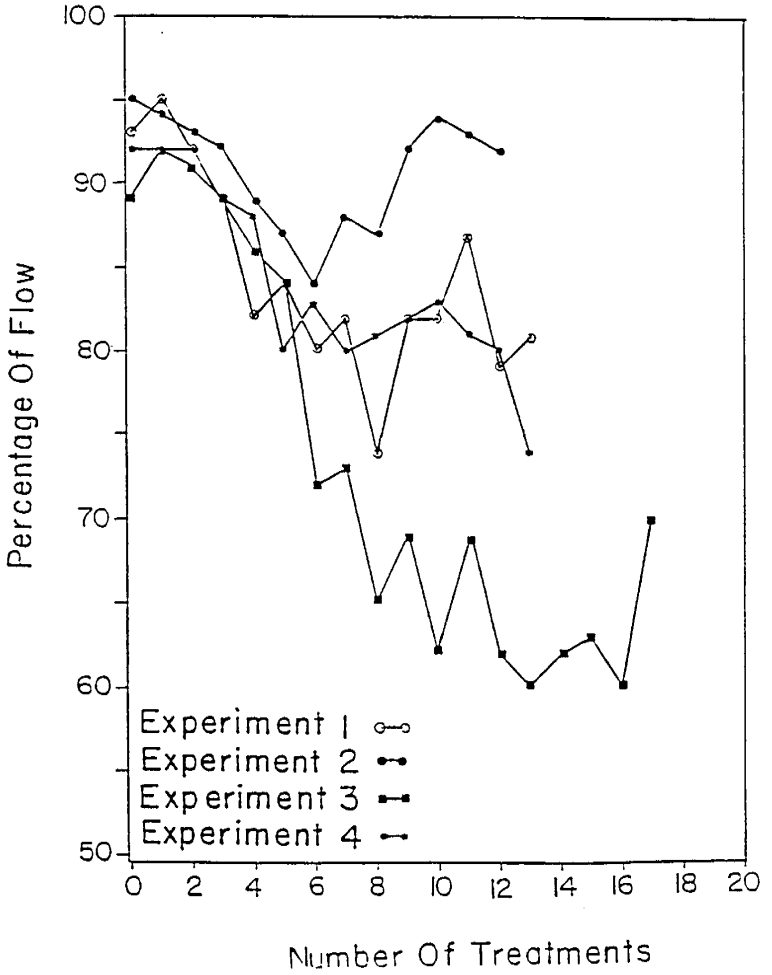

FIGURE 4. - Comparison of the percentage of total flow through the high permeability core per treatment in all experiments.

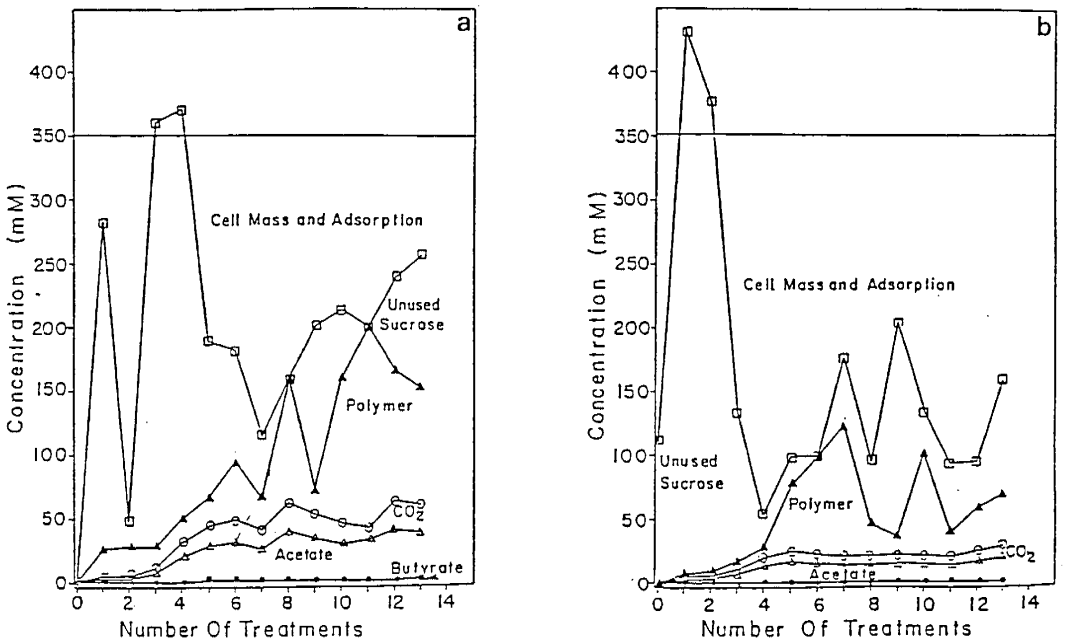

FIGURE 5. - Carbon balance for effluent samples from core H4 (a) and L4 (b). Linear interpolation was used to fill in missing points for polymer concentration.

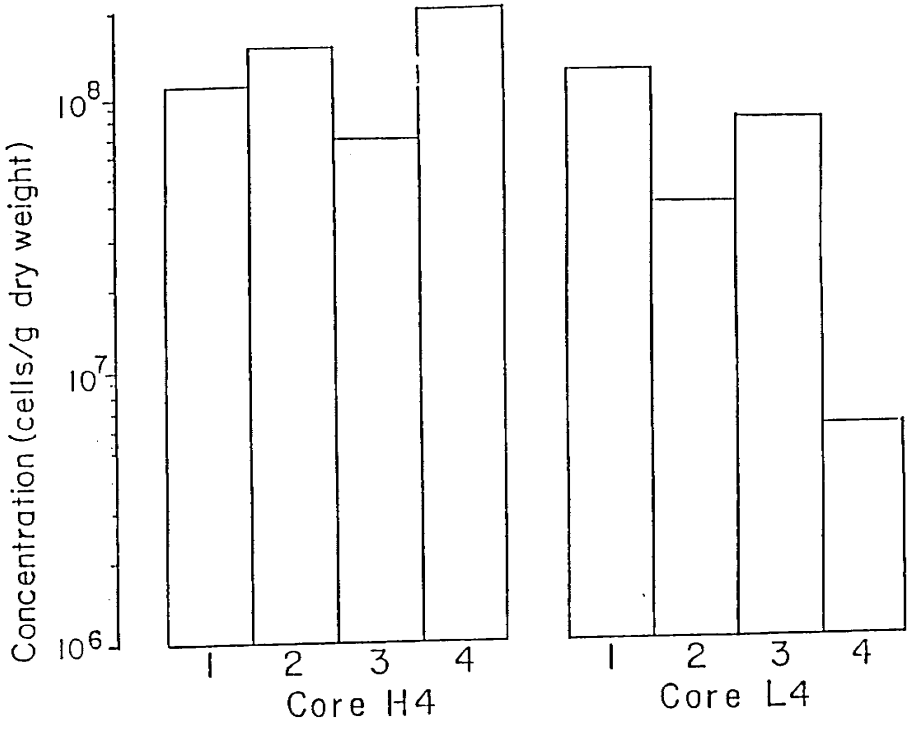

FIGURE 6. - Cell concentrations in cores H4 and L4 after treatment process.

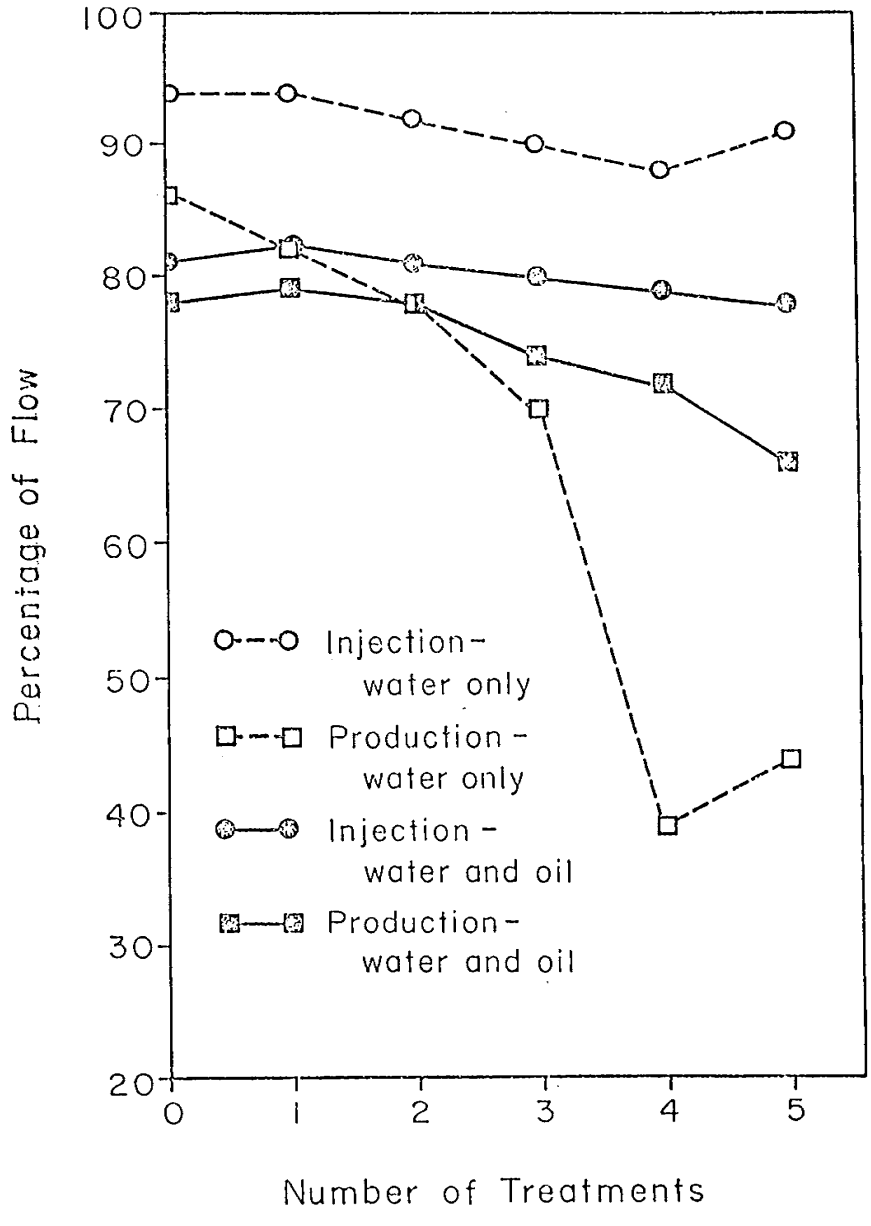

FIGURE 7. - Percentage of flow through high permeability layer into and out of crossflow systems.

MEOR STUDIES IN A RADIAL FLOW SYSTEM-- THE RESEARCH OUTLOOK

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ABSTRACT

This work is aimed at developing a physical model to be used as a strong and reliable predictive tool in large scale application for the-state-of-the art technology of MEOR. This physical model is designed in such a way that could simulate both the radial flow of fluids toward the wellbore and bacteria transport through porous media simultaneously and could also perfectly scale relevant resrevoir operational parameters over wide operating ranges.

The commonly used core flooding experiments performed on real or synthetic core samples usually assume that flow of fluids is longitudinally linear. But in the real cases, the wellbore intersects natural fractures or layers of different permeabilities where the porous media are more sophisticated and diversified in their structures and the reservoir fluids flow radially in cylinderical coordinates toward the point of lower pressure at the wellbore. The near-wellbore region of a radial flow system, a region of rapid convergence of streamlines through an ever decreasing cross-sectional area of the porous medium, is critical to reservoir fluids recovery performance.

It is therefore has been an important objective for us to design and costruct a workable physical model which could adequately categorize the followings: a) geometry of the well pattern and reservoir spacing configuration, b) simultaneous flow of fluids and bacteria transport inside the reservoir, c) initial and boundary conditions, d) reservoir pressure and temperature, and e) reservoir heterogeneities effects. Furthermore, the model would be proficient to produce pertinent data on saturation distribution and relative permeabilities of wetting and nonwetting phases, upon the transport of bacteria through the porous media, for a finite-difference simulator to corroborate the experimental data obtained from the laboratory studies.

INTRODUCTION

The purpose of this paper is to set the stage for development of a reliable physical model to be employed in performance prediction of MEOR processes. To make MEOR a technically proven technology, much work remains to be done. It is no doubt that most of the laboratory scale research efforts have demonstrated that some bacterial strains are fairly effective in releasing oil from oil-bearing porous media. Through the aid of the in-situ metabolic products (such as solvent, bio-gas, bio-surfactant, biopolymer, etc.) produced from the bacterial activities when they are emplaced into an oil reservoir, the oil recovery efficiency could be increased substantially. However, to scale up MEOR processes directly from bench scale to field production would be more difficult than other non-biological recovery processes. Not only many known intervening factors come into play, but also a variety of unknown or inherent difficulty to define parameters exist. The development of a predictive physical model discussed in this paper, would prompt the researchers to look more closly to the problems one may face in the field experiments. These problems may not be biological in nature. But without carefully and scientifically characterizing these factors, large scale tests are likely to yield useless information.

Extensive research conducted by Yen et al (1-13) in the past on the transport of bacteria through porous media for MEOR processes were resulted in understanding the fundamentals of bacteria-rock interaction as well as identifying all the important parameters that govern the cell penetration. Table 1 summarizes the major accomplishments of these studies having had the ultimate goal of providing paths to successful field applications.

In order to narrow the gap between laboratory work and large scale application, an approach to design a physical model which could simulate both the radial flow of fluids toward the wellbore and bacterial transport through porous media is a necessity (14-15). The commonly used core flooding experiments performed on real or synthetic cores usually assume that fluid flow is longitudinally linear. But in real cases, the wellbore intersects natural fractures or layers of different permeability where the porous media are more sophisticated and diversified in their structure and the reservoir fluids flow radially in cylinderical coordinates toward the point of lower pressure at the wellbore. The near-wellbore region of a radial flow system, a region of rapid convergence of streamlines through an ever decreasing cross-sectional area of the porous medium is critical to reservoir fluids recovery performance. Pressure gradients and flow velocities become extremely high in that region and all flow effects (including those responsible for the accumulation of wellbore damage) are magnified and accelerated close to the wellbore.

It is therefore the major intent of this paper to describe the development aspects of a physical model to be used as a strong and reliable predictive tool in large scale application for the-state-of-the art technology of MEOR. This model will simulate the actual conditions in a reservoir prototype and will perfectly scale relevant reservoir operational parameters over wide operating ranges. The model itself should be designed and constructed in such a way which could adequately categorize the followings: a) geometry of the well pattern and reservoir spacing configuration; b) stipulated flow of fluids inside the reservoir; c) boundary and initial conditions; d) reservoir pressure and temperature; and e) reservoir heterogeneities effects. Moreover, the model should be proficient to produce pertinent data on saturation distribution and relative permeability of wetting and nonwetting phases for a finite-difference simulator to corroborate the experimental data obtained from the laboratory studies.

ACKNOWLEDGMENTS

We gratefully appreciate the principal support of NSF grant CBTE-8614019 for this project.

THE TECHNOLOGY PROSPECTS

The Physical Model Layout

The reservoir model is designed in a cylindrical shape geometrically similar to a reservoir with a circular boundary. Drainage radius is determined by a pre-examined scaling factor from prototype to the model. Figure 1 shows a simplified view of the model. A small diameter tube (Figure 2) is sealed to the center of upper and lower planes of the vessell. The tube, which serves as a central well, is perforated uniformly across its perimeter to ensure radial fluid flow, and is wrapped in a slotted liner to control the sand migration to the tube. Four side wells are constructed around the perimeter of the model for sampling and collection of effluent fluids without interfering the radial flow pattern inside the reservoir. The objective is to physically model a uniform five-spot flooding pattern which is the best for simulating a radial flow system. The porous media could be either packed materials or consolidated sand as required for the test. The flow lines are connected to the center as well as side wells for both injecting and monitoring purposes. Pressure gauges are mounted on the lines close to the wells to record the pressure variations during the experiments.

In terms of the radial flow model, it is considered that the well is surrounded and confined by a flow barrier, at its outer boundary, which prevents the flow of fluids into the radial cell. The cell pressure will decline in such a

way that rate pressure variation remains constant for all locations in the drainage area at all times. Experimental analysis of this semi-steady state, before and after the bacteria is emplaced, will determine the efficiency of bacteria strains for raising and maintaining the pressure inside the reservoir. The optimized condition would then prevail when the reservoir pressure remains constant due to sufficient gas production from bacterial activity. This will then simulate the steady state condition of a radial flow system.

It is important to perform a careful analysis of these two states before and after the bacteria is emplaced for the pressure enhancement purposes. Sensitivity or duration of these two states to flow rates and to various injection modes of nutrients and bacteria should also be investigated. Subsequently, an optimum mode of injection and perforation sizes will be derived. Results of these studies would have extensive applications for inflow well performance in MEOR applications.

Formation Damage and Clay Stabilization

Flow of a suspension through a porous medium would result in the deposition of suspended particles onto the pore walls or in the entrainment of deposited particles. The known mechanisms involved in particle retention and entrainment are: (a) surface deposition due to surface interaction, (b) straining or size exclusion due to particles being larger than pore throats and (c) sedimentation or gravity settling in pores due to difference in densities of particles and fluids. Any of these factors migh dominate the retention process depending on the operating conditions. Besides the clogging and blockage due to hydrocarbon (wax) and asphaltene, some procedures used for facilitating the transport of bacteria might knock loose the clay components present in the formation. This is highly undersirable since large permeability reductions would result from clay migration and plugging.

Since the designed physical model has the capability of simulating the wellbore conditions, it could be subjected to investigation of the bacteria influence for removal of formation damage caused by asphaltene deposition around the wellbore.

Reservoir Conditions Effects

The conditions present in many subsurface reservoirs hinder the activity of most microorganisms. The question of whether bacteria life is possible under adverse conditions of temperature and pressure, has been open to debate until this day. Most bacteria will tolerate pressures up to 20mpa and above this value an increasing proportion become inhibited, even when other conditions are optimal (16, 17, 18, 19). Even if bacteria could grow at reservoir

temperatures, the combination of different adverse conditions, especially temperature and pressure (19), and of heavy metals (20) are unknown. The effect of pressure and temperature in combination with salinity, heavy metals are needed to be investigated. No information is available to assess the suitability of using extreme thermophilic bacteria for transport studies. It is therefore a necessity to study the effects of pressure and temperature on bacterial behavior such as growth and mobile activity and to provide criteria for appropriate ranges of temperature and pressure in which the bacteria activity are favorable.

It is also necessary to check bacteria strains carefully for genetic stability once the high pressure is applied to obtain high-yielding mutants (e.g. surfactant producers). It is predicted that a mutant, unless it arises very early, could affect only a comparatively small sector of the reservoir due to very little assessment of heterogenous reservoirs.

It is no doubt that effect of reservoir heterogeneities on bacterial transport through porous media is a critical step in the physical modeling studies. Variations in rock and fluid porperties from one location to another may result from deposition, folding and faulting, post-depositional changes in reservoir lithology, and changes in fluid type or properties. Reservoir heterogeneities may be small scale as in carbonates reservoirs where the rock has two constituents, matrix and fractures, vugs, or solution cavities. They also may be large-scale, such as physical barrier, faults, fluid-fluid contacts, thickness changes, lithology changes, several layers with different properties in each layer, etc.

A related characteristic with heterogeneities is permeability anisotropy. An anisotropic reservoir has permeability that varies with flow direction. Anistropy can occur in both homogenous and heterogenous reservoirs. As a result, anistropy does not necessarily imply heterogeneity in the sense defined before. Most reservoir rocks have a lower vertical permeability than horizontal permeability, and so they are anisotropic in that regard.

With the type of the model being developed for this research work, it is possible to create synthetic heterogeneity in the area around the wellbore and the different points in the model. From study on the pressure behavior at the wellbore, a unique and specific test could be derived for field application in identifying type of reservoir heterogeneities and their effects on the pressure behavior analysis.

Two or three layers of different mesh size sands of different particles (e.g. shale, sands, glass bits) could be packed in the model to simulate the layered reservoir systems in which each layer has its own permeability-thickness product and the bacterial transport in each of the variety of layer constrained in the model.

Application of a Lysogenic System

Experiments involving substrate specific bacterial species and wildtype species are needed to be carried out to determine the feasibility of a lytic system for either applied or indigenous bacteria in a radial flow system.

The bacterial population which develops in the reservoir may not be the one injected due to interaction of bacteria with the residing fluids and the rock materials. The influence of indigenous bacteria on the injected bacteria species should be carefully examined. The numbers of indigenous bacteria could be reduced locally by a biocide injected prior to inoculation. The effects of competition could also be minimized by using a large inoculum of organisms with a high growth rate. It is herefore necessary to study the phage-bacteria-rock interaction to clarify the important variables that will be encountered in real reservoir conditions.

The data gathered from these studies would aid in developing a computer model for the prediction of phage, bacteria and rock interactions. The development of a lysogenic system will ultimately lead to an efficient method of in-situ microbial population control.

Effect of Chemical Additives and Ionic Strength

An important problem in the process of injecting bacterial strains into a reservoir is the retention of the bacteria on rock surfaces, preventing their penetration into the formation. In certain cases where the pore throat sizes are comparable in size to the bacterial species, a filter cake of bacteria would quickly form and prevent any further injection. In cases where the pores are larger than the bacteria, the dominant mechanism by which the bacteria are retained is absorption. The electrostatic interactions between bacteria and rock are dominant factors in their retention. In addition to these, properties of aqueous solutions, including pH and ionic strength would alter the extent of deposition.

Our previous experiments on the transport of bacterial species through sand packs have shown that their transport could significantly be enhanced by the presence of polyelectrolytes. We also proved that for two common species of bacteria, Bacillus subtilis and Pseudomonas fluorescenes, surface charge modification could change the extent of bacterial adhension drastically. Additives such as polyphosphate or pyrophosphate definitely will alter the charge nature of the porous media. Experiments of similar approaches for the radial flow model have also the equal merit.

Development of Scaling Factors and a Mathematical Simulator

The bacteria ability to penetrate and its rate of movement through porous media is affected by both the reservoir and bacteria cell characteristics. In addition to study the effects of important reservoir characteristics such as permeability, pore structure, the chemical nature of the rock, nutrient and salt content of the connate water and temperature and pressure on bacteria transport, a detailed investigation on bacteria motility, i.e. an important characteristics of microbial cell, under simulated hydrodynamic conditions is a necessity.

It is the purpose of this reserach work to present a theoretical background for the performance of laboratory tests that are more representative of field behavior. To correctly simulate the field behavior when bacteria injected into the oil reservoir, laboratory tests under conditions selected in accordance with definite scalling laws would be conducted.

The formation of conditions required for bacterial transport in porous media, where the multiphase flow phenomena subjected to gravity and capillary forces requires a detailed survey on parameters which have similar values in the model and prototype. This would aid in derivation of the dimensionless groups, to which the variable that govern both the bacterial transport and flow of fluids througout the reservoir rock. These dimensionless groups consist of independent variables such as dimensionless distance and time, dependent variables such as recovery and pressure which could be measured during the experiment, and independent constant groups such as the ratio of length to height of the reservoir and ratio of viscosities of the reservoir fluids whose values are known a priori. If the values of these groups are the same for the model and for the reservoir, the model is then properly scaled, which means that for equal values of the independent variable groups in prototype and the model, the values of dependent groups are equal too. It is then appropriate to directly interpret the results of such a dimensionally scaled model in terms of field performance.

Moreover, to satisfactorily model and predict the the transport of bacteria in an oil reservoir for a field project based on the laboratory study, development of a computer simulator is a matter of necessity. For this, a generalized compositional simulator which incorporates material balance in aqueous and solid phases, and the amounts as well as their associated effects of surfactant, gas, biopolymer produced needs to be developed. The experimental results from the physical model will first be served to test the validity of the simulator. The simulator is to be developed in such a way that it is flexible enough to account for various mechanisms such as motility, chemotaxis, and diffusion simultaneously. For example, if

selective plugging is one of the dominant mechanisms for oil recovery in addition to biosurfactant generation, this should be accounted for by making the permeability a function of position and time, and estimating the flow rate of surfactant and bacteria at a particular location based on the permeability calculated in the previous iteration. In this manner, by taking permeability as a dependent variable the simulator can account for its continuous variation.

CONCLUSION

The design and application of the physical model in progressive MEOR studies as they were discussed in this paper strongly suggests the necessity for physical simulation of relevant reservoir operational parameters over wide operating ranges. As important as the actual flow conditions prevail in real reservoirs, they have been ignored in the past on small scale laboratory experiments of MEOR processes. The radial flow model, however, like any other models for different applications, is a true representation of reality. It is needed because currently researchers cannot effectively deal with all of variables which affect the MEOR field applications. This could also reveal the interchanging effects of some of the important variables. By careful experimenting and data analysis, the model would serve to decrease the difference between the projected and actual results from a MEOR field experiment. A lot of time and money could thus be saved and technology be advanced.

Data obtained from this radial flow model would be used to derive analytical correlations between the actual flow conditions of the reservoir and performance of bacterial transport process. It would enhance our ability for better understanding of the effects of hydrological dynamic and structural reservior conditions on the bacterial transport in general and/or in MEOR applications. The detailed physical as well as mathematical modeling analysis of the process, would generate a realistic, quantitative as well as qualitative picture of bacterial transport in porous media which would contribute significantly to some advancing areas of interest of biotechnology. One could benefit from outcome of this research work in development of bioengineering methods, to their fullest potential, in other scientifc applications such as soil decontamination or aquifer purification.

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TABLE 1. - Summary of major accomplishments

1. Transport of Bacillus subtilis, Pseudomonas putida, and Clostridium acetobutylicum through Berea sandstone and Cleveland sandstone cores was investigated.

2. Influence of influent concentration, aggregation modes of bacteria, and chemical additives on the process dynamics were assessed.

3. Addition of nonionic surfactants and some anionic polyelectrolytes could improve the transport of bacteria.

4. Bacteria in their spore form penetrated sandstone cores more readily. The spores and bacteria were also more easily transported through oil saturated cores as opposed to cores with no oil in them.

5. It was shown that surface charges of the rock and bacteria were crucial to the bacterial transport. A detailed electrochemical description of the rock and bacterial surfaces has been developed.

6. Transport of bacteria due to their motility was investigated.

7. Chemical modification of bacterial cells was shown to increase surface charge of the bacteria appreciably and resulted in substantial improvements of penetration rates.

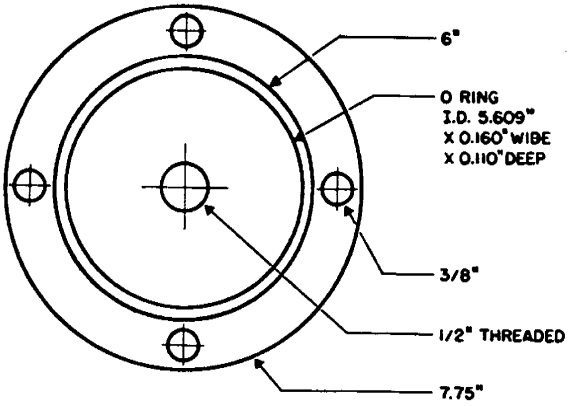
8. Pyrophosphate and lignosulfonate proved to be excellent chemicals for increasing the net negative charge of rock surfaces and hence facilitate transport of bacteria.

9. Bacterial phages were used to demonstrate their ability to selectively lyse bacterial cells and control subsurface bacterial populations.

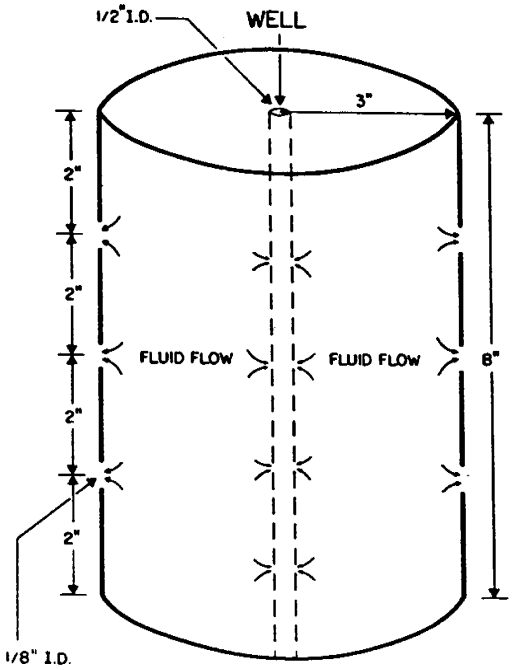
10. Production of metabolites was shown to improve oil recovery substantially. A variety of injection modes showed substantial improvement in oil recovery efficiency.

11. Theoretical deposition rate of bacteria onto rock was analyzed incorporating bacterial motility, surface interactions, and hydrodynamic regime.

12. Streaming potential equipments and measurements during core flooding have been developed.

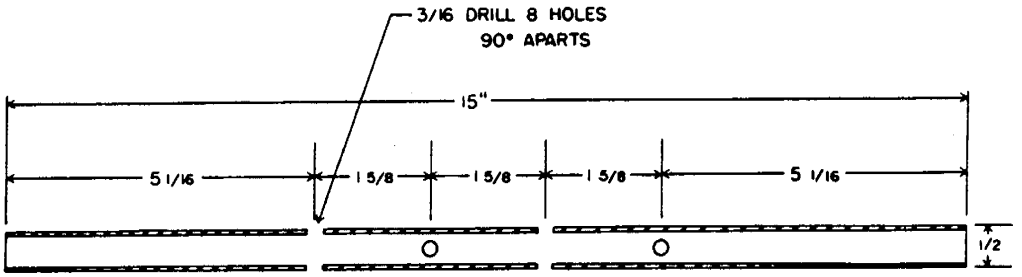


END PLATES OF THE WELL MODEL



FRONT VIEW OF THE WELL MODEL

FIGURE 1. - Dimensional description of the physical model.



HORIZONTAL VIEW OF PRODUCTION TUBING

FIGURE 2. - Detailed description of the production tubing.

PROSPECTS OF MEOR IN INDIA

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ABSTRACT

Microbial enhanced oil recovery (MEOR) methods have attained a great significance in view of the high cost of some of the conventional EOR processes. The application of MEOR in Indian reservoirs, where the temperature is over 80° C, requires bacteria that can grow in extreme environmental conditions. This paper describes the studies on transport of bacteria through porous media with a view to plugging the high-permeability streaks for improving the sweep efficiency of waterfloods. This study revealed that although the bacteria could plug the high-permeability cores effectively, they could not be grown at elevated temperatures that exist in the reservoirs.

INTRODUCTION

Our untiring efforts in developing suitable methods for the recovery of about 70% of the oil that remains underground after primary and secondary recovery have resulted in the development of valuable tertiary recovery methods broadly classified as thermal and chemical flood processes. A unique tertiary recovery process, conceived as early as 1926 but attracted systematic attention only after the unprecedented oil price hike of the last decade, envisages introduction of living microbes and their metabolic products into reservoirs. The injected microbes are expected to grow in reservoirs and produce various products which bring about changes in oil properties and increase recovery by one or more chemical flood mechanisms.

Conceptually, microbes can be used in two ways for EOR applications. The first concept is based on injection of microbes into depleted oil reservoirs to increase ultimate oil recovery by in situ microbial processes such as breakdown of heavy hydrocarbons and paraffins, generation of gases, and production of surfactants, organic acids, alcohols and microbial cells themselves. In the second concept some of these metabolic products; for example, surfactants and biopolymers, are produced at the surface in a commercial bioreactor and subsequently injected into reservoirs to create the desired effect.

The technology for surface production of some of the EOR biochemicals is well developed. The current research in this aspect is focused on reduction of the product cost to make them cost effective. On the contrary, the in situ MEOR process appears to be of potential commercial value as it does not require much material input for field applications. Here, the major expenditure is for development of suitable bacterial cultures.

GROWTH OF BACTERIA IN RESERVOIR

The success of an in situ MEOR process depends upon the growth of the injected microbes in the reservoir and producton of the desired products at the required rate. Most of the field tests and laboratory investigations conducted thus far have been in shallow reservoirs where the environment, particularly temperature, is not unfavorable for microbial growth. The biologically acceptable limit for high temperature is 80° C, above which most of the naturally occurring bacteria cease to multiply. The optimum growth temperature for most of the thermophilic bacteria,, as shown in figure 1, is 55° to 65° C.[1] Hence, temperature is a limiting factor for the growth of microbes. Keeping this in mind, reservoirs having temperatures below 66° C are considered suitable for the MEOR process.[2] Temperatures in Indian reservoirs range from 62° to 120° C and in most cases are above 70° C.[2] (See table 1.)

Thus, high reservoir temperatures are major hinderances to MEOR applications in India where suitable microbes have to grow at temperatures above 80° C. This limits the scope of selection of microbes to the caldoactive bacteria only. These bacteria are reported to grow at about 80° to 85° C. It may, however, be emphasized that although some bacteria are capable of growing at very high temperatures, their growth rate generally is too slow to create the desired magnitude required in reservoirs.

Laboratory studies on three mesophiles--*Bacillus* Spp. *Aerobacter aerogenus* (*Enterobacter aerogenes*) and *Pseudomonas fluorescens*--isolated from oil field waters for their growth at high temperatures, showed that the maximum growth temperature could be raised from 55° to 70° C by a gradual adaptation method for the *Bacillus* Spp. while the *Pseudomonas* and the *Aerobacter* could not be grown above 55° C even after prolonged adaptation.[3] (See table 2.) When the *Bacillus* was grown at 70° C, the growth rate was very slow, and the cell size, morphology, and cellular arrangements were altered significantly. Efforts for further increasing the growth temperature and growth rate of these bacteria and also for isolation of other more suitable bacteria are in progress.

BACTERIAL TRANSPORT THROUGH POROUS MEDIA

The successful application of microbes in EOR also depends on the bacteria being transported into the reservoir. Permeability, porosity, and pore size distribution of reservoirs play important roles in the transport of bacteria through porous media. The pore size distribution curves of some Indian reservoirs are shown in figure 2. These indicate that a large fraction of the pores of formation rocks are of the same size as that of common bacteria or are smaller, from 0.5 to 2.0 m. The curves also indicate that generally higher permeability cores have more large pores than low-permeability cores. Therefore, to facilitate easy transport of the bacteria, high-permeability (above 100 md) reservoirs are considered suitable for MEOR processes.

Laboratory studies on the transport of *Pseudomonas fluorescens* through cores of different permeabilities by injecting a bacterial suspension containing approximately 1 x 10^9 viable cells/ml and determining their counts in the outlet fraction indicate that the bacteria can penetrate through the

cores.[3] (See table 3.) The penetration in high-permeability cores is faster and more pronounced than that of low-permeability cores. This supports the belief that in high-permeability cores the percent of pores of larger size is more when compared to low-permeability cores. It may be noted, however, that during the transport study the permeabilities of the cores were significantly reduced. The percent of permeability reduction was more for high-permeability cores than for low-permeability cores (Fig. 3). Thus, although the bacteria are capable of penetrating through the cores, the possibility of reservoir plugging cannot be ruled out.[4]

The capability of microbes to plug reservoirs can be beneficially used for plugging high-permeability streaks in heterogeneous formations to increase the sweep efficiency of waterfloods.[5] The studies on plugging of Berea cores of similar permeability by bacteria of different dimensions and morphology (i.e. *Pseudomonas fluorescens*, *Micrococcus* Spp. and *Bacillus* Spp.) revealed (Fig. 4) that the *Bacillus* Spp. could cause more plugging than the other two.[4] This may be due to the larger size of the bacillus and also to the large amount of slime produced by it.

Interestingly, it was observed that bacteria when injected in a low concentraiton could effect more permeability decline that the same number when injected at higher concentration. Figure 5 shows that the same number of bacteria injected, the reduction in permeability is much greater per unit number of bacteria when injected in lower concentration that in higher concentration.

The results indicate that the *Bacillus* Spp. has better plugging ability and can grow up to 70° C. Thus, it is considered to be a suitable organism for selective plugging purposes. Another advantage is its ability to form endospores in which form they can survive the operational environmental stresses and can be easily transported through porous media because of their smaller size. Subsequent injection of nutrients enable these bacterial spores to germinate and grow in vegetable form resulting in plugging of the zone.

An experiment was carried out to determine whether the bacteria can cause permanent plugging of the cores. *Bacillus* Spp. were grown in cores of known permeabilities. The bacterial growth resulted in reduction of core permeability from 200 to 57 md. Water was then flushed continuously through the core. It was observed that (Fig. 6) regaining the lost permeability was not significant until 45 pore volumes (PV) of the water was flushed.[4] About 69% of the original permeability was regained after flushing with over 90 PV of the water. There was no further significant improvement in the core permeability after flushing water beyond this value. This indicates that although the bacteria cannot plug the formation completely and permanently, they are still capable of causing effective plugging of high-permeability zones.

The use of aerobic bacteria for in situ MEOR processes requires oxygen and suitable nutrients which cannot be easily provided in quantities required in most reservoirs for the vigorous activity of bacteria.[6] To eliminate the need of oxygen and nutrients we have studied the isolation of suitable bacteria, particularly thermophilic anaerobic clostridia capable of thriving on a small fraction of hydrocarbons. This will make the process operationally much easier and less expensive.

SUMMARY

We are relatively new in the field of MEOR research. Considering the specialized requirements of Indian reservoirs for high-temperature-growing bacteria, our task becomes more difficult. However, with the advancement in molecular biology and biotechnology it can be achieved in the near future.

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TABLE 1. - Comparison of the desirable reservoir parameters and Indian reservoirs for MEOR

Sl. No.	Parameters	Optimum limit	Range of Indian parameters
1	Reservoir depth, meters	2,940	606-3,148
2	Gravity, °API	15	15-47
3	Reservoir temperature, °C	66	62-115
4	Reservoir pressure, Kg/cm^2	300	40-310
5	Permeability, md	100	10-10,000
6	Salinity, %	10	0.15-3.0

TABLE 2. - Growth of bacteria isolated from oil field waters after gradual adaptation at elevated temperatures

Sl. No.	Temperature, °C	Results after 48 hours		
		Pseudoma fluorescens	*Acrobacter acrogenus*[1]	*Bacillus* Spp.
1	30 (optimum -growth temp.)	+ + + +	+ + + +	+ + + +
2	40	+ + +	+ + +	+ + + +
3	45			
4	50	+	+	+ +
5	55	+	+	+ +
6	60	-	-	+
7	65	-	-	+
8	70	-	-	±
9	75	-	-	-
10	80	-	-	-
11	90	-	-	-

[1] *Enterobacter aerogenes*

+ + + + - denotes very heavy growth
+ + + - denotes heavy growth
+ + - denotes moderate growth
+ - denotes poor growth
± - denotes doubtful growth
- - denotes no growth

TABLE 3. - Penetration of *Pseudomonas fluorescens* through cores of various permeabilities

Sl. No.	K_{wi} of the core, md	K_w of the core, md	Injection bacteria/ml	Outlet bacteria	
				Initial count (after 2 PV)	Final count (after 10 PV)
1	27.86	13.2	1×10^9	1-10	1×10^4
2	247.0	44	1×10^9	1×10^2	1×10^5
3	455.0	65	1×10^{12}	1×10^3	1×10^6

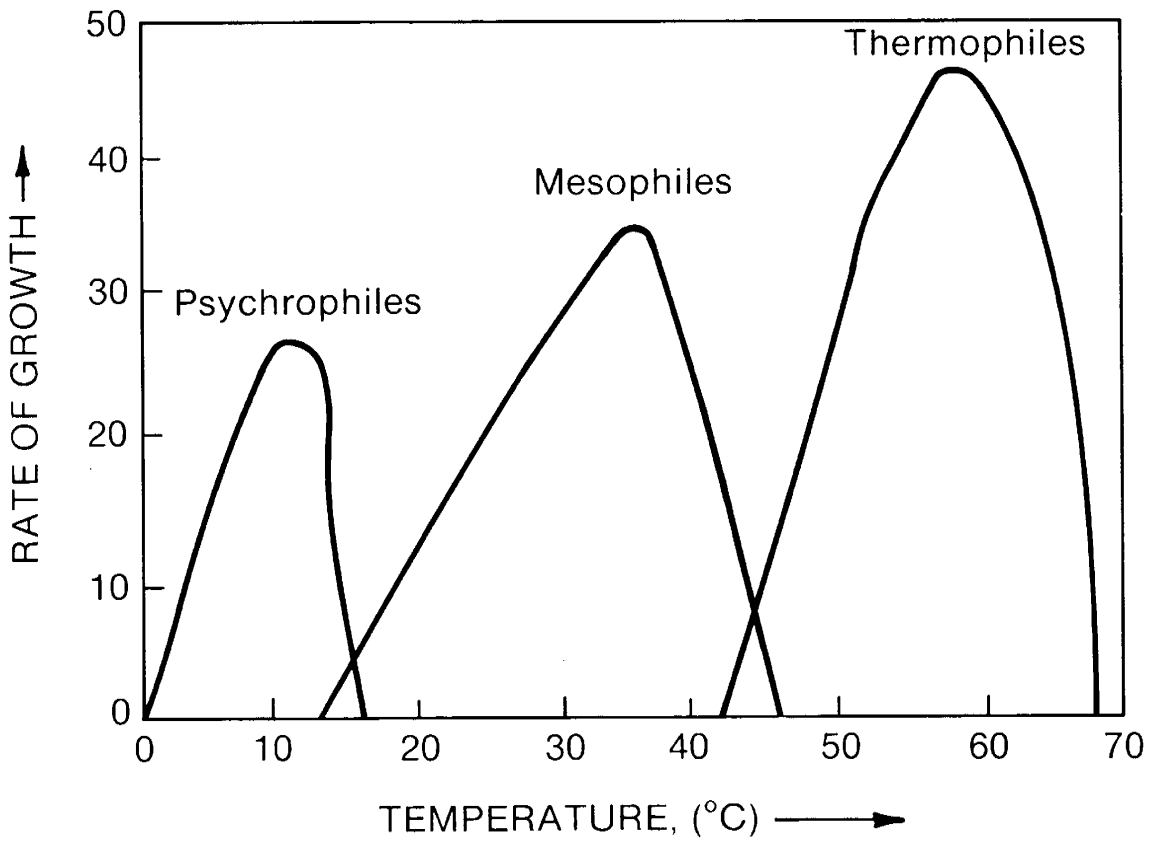

FIGURE 1. - Typical growth responses to temperatures for psychrophiles, mesophiles & thermophiles

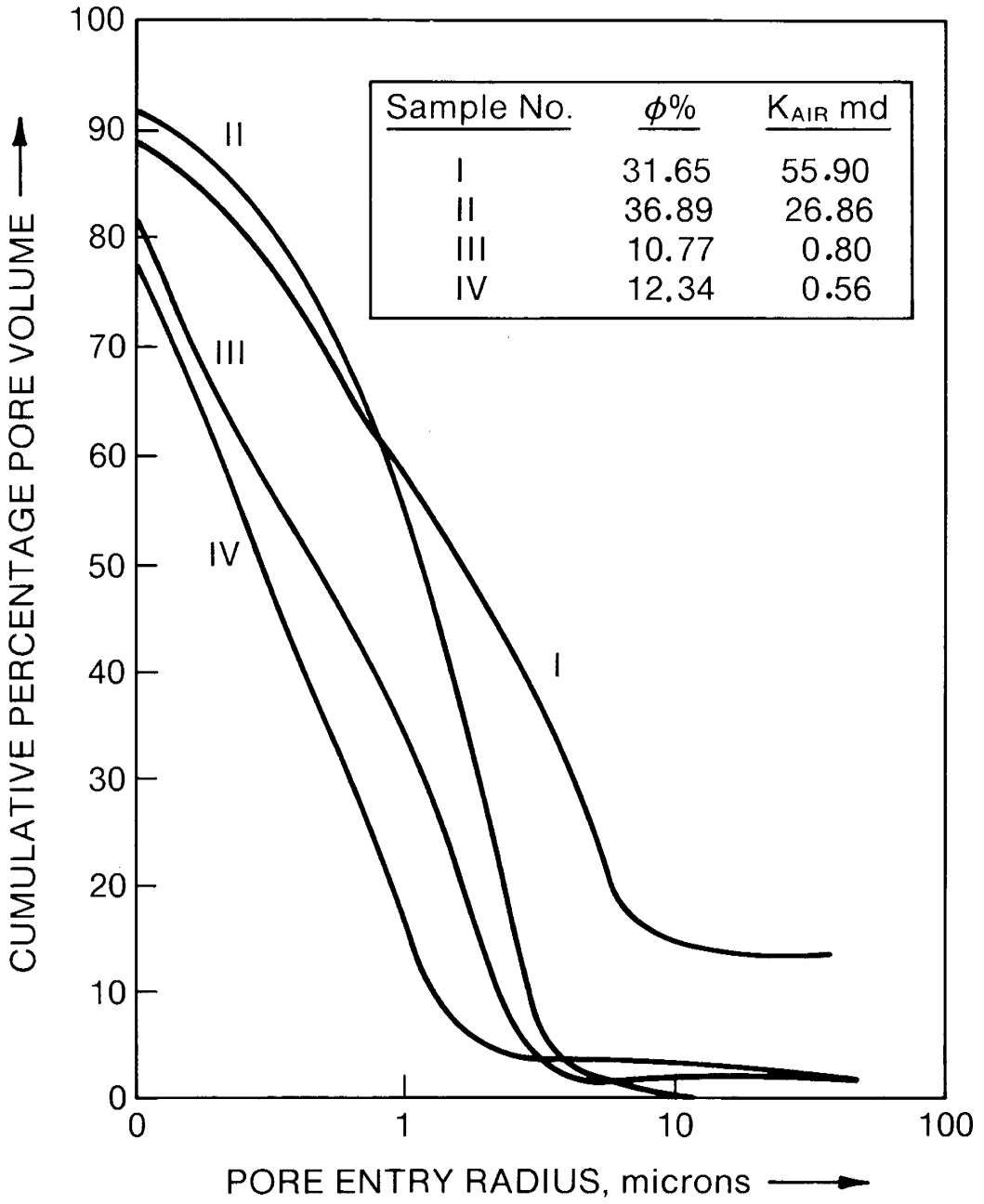

FIGURE 2. - Pore size distribution of different permeability cores

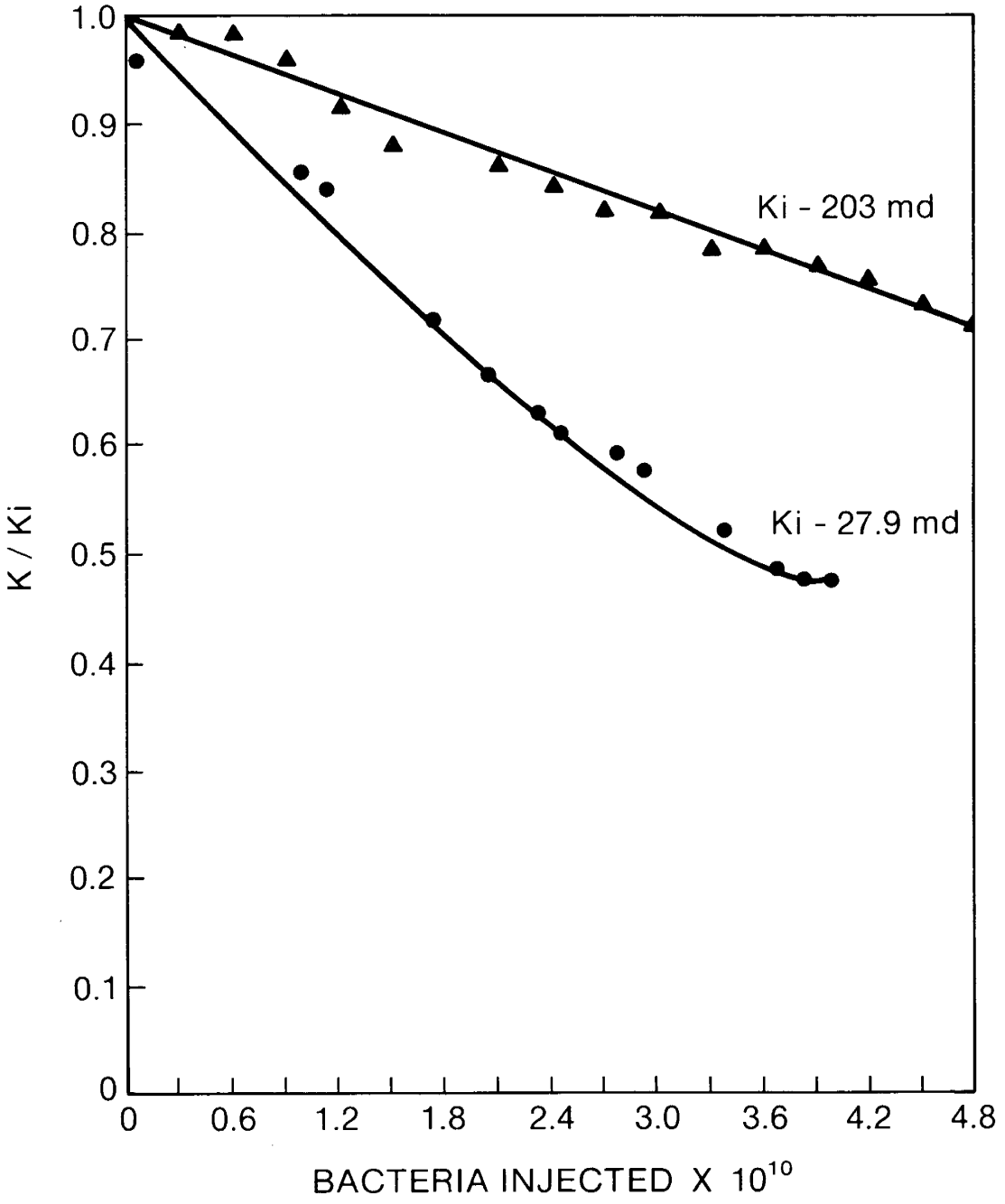

FIGURE 3. - Effect of initial core permeability on reduction of permeability caused by pseudomonas fluorescence

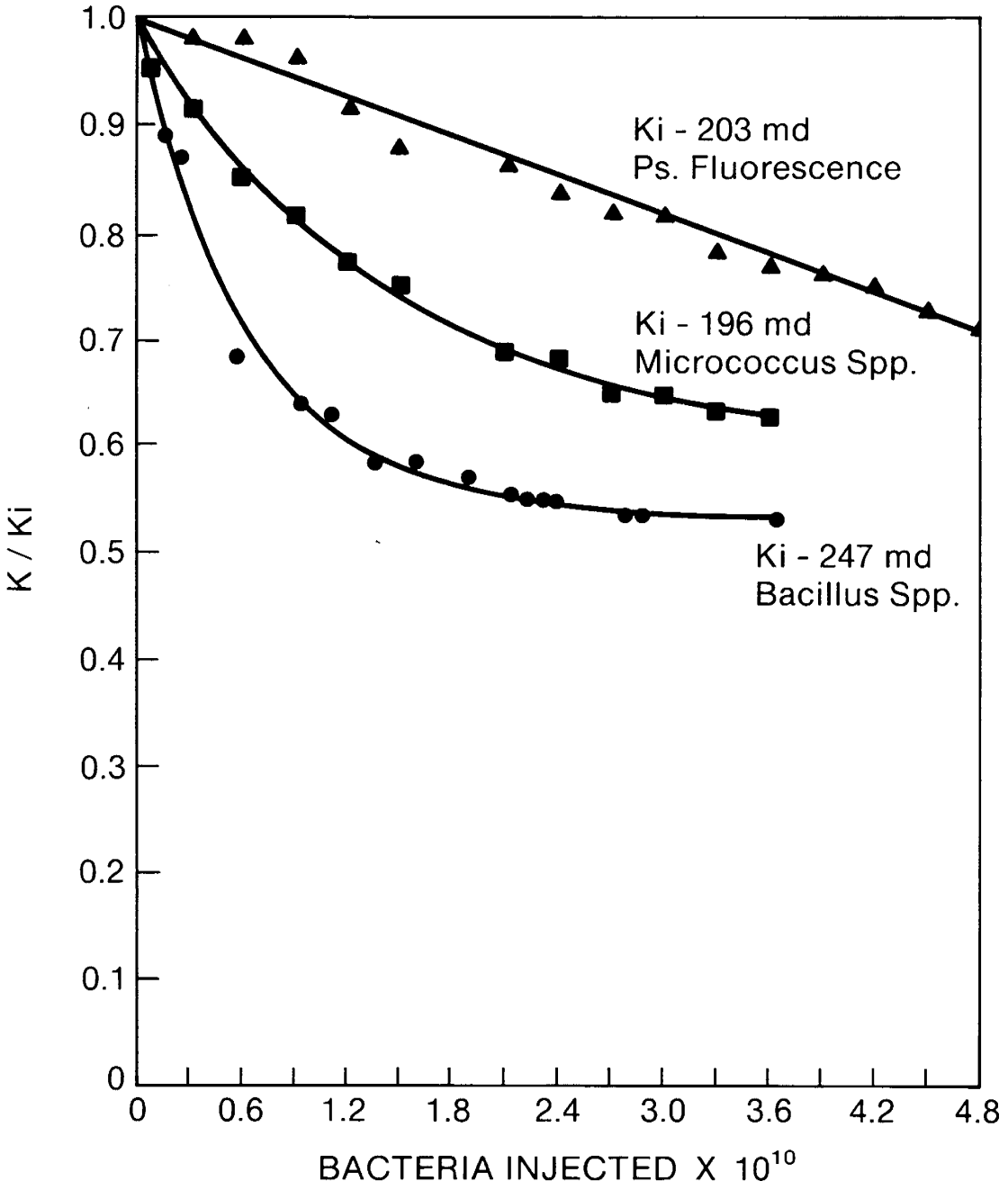

FIGURE 4. - Effect of bacterial species on permeability reduction of high permeability cores

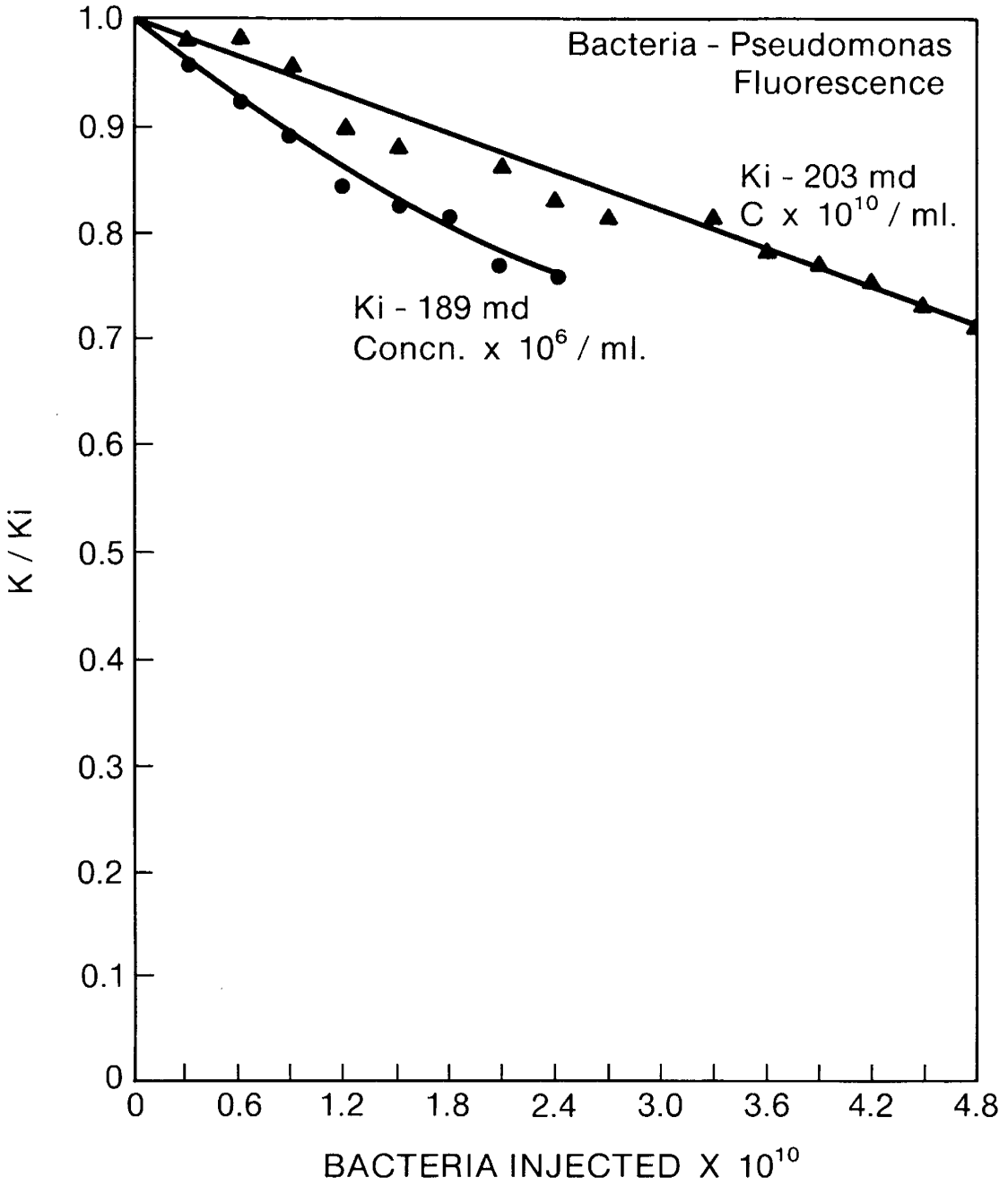

FIGURE 5. - Effect of bacterial concentration on permeability reduction

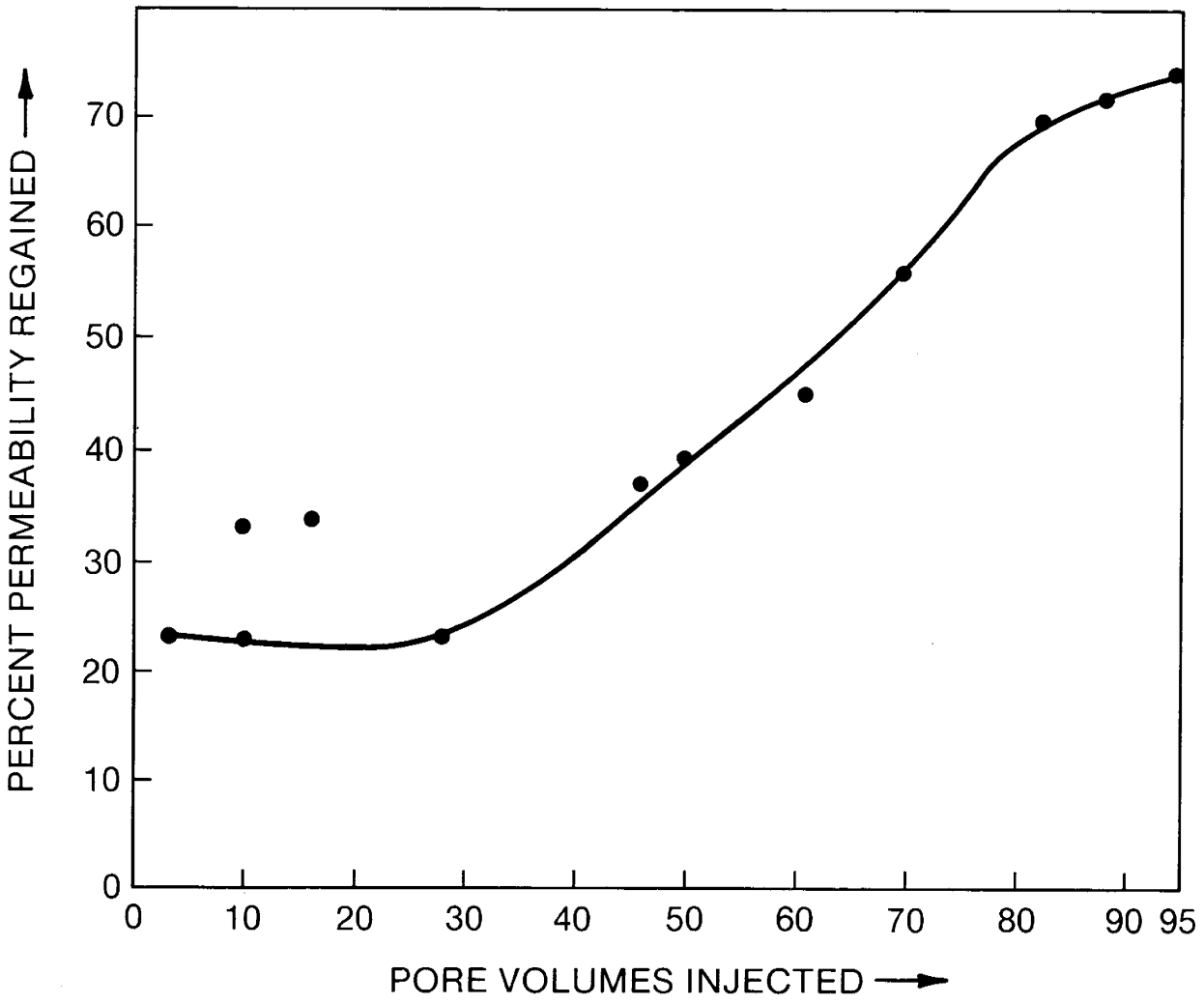

FIGURE 6. - Regain of permeability of a bacterially plugged core by flushing

ECONOMICS OF MEOR PROCESSES

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ABSTRACT

Cyclic microbial stimulation treatments and microbial-enhanced waterflooding methods are currently being applied in field pilot projects. There are few data available to assess the potential economic returns from applying these advanced EOR methods. The design and implementation of MEOR requires the same reservoir engineering considerations as does any EOR method. The parameters that need to be considered in designing and implementing an MEOR process are discussed. Economic analyses are presented for application of cyclic microbial stimulation treatments and microbial-enhanced waterflooding. The economic analysis indicates that application of MEOR methods may be economically attractive.

INTRODUCTION

Microbial methods for improving oil recovery are potentially feasible because the cost of the injectant is relatively low in comparison with other enhanced oil recovery methods. Microorganisms are self-perpetuating as long as they are supplied with a suitable nutrient and favorable conditions for sustained growth. Microbial enhanced oil recovery (MEOR) methods are potentially useful because the growth of the organisms can be sustained under reservoir conditions by injection of a relatively inexpensive and readily available nutrient such as molasses.

Microbial EOR methods are most commonly applied by single-well cyclic stimulation treatments in stripper wells (see Figure 1). In single-well stimulations, a marginally producing well is treated with a microbial formulation and nutrient. After a brief shut-in period, which ranges from a few days to a few weeks, the treated well is put back on production. Significant increases in production rate, which are sustained for several months, have been reported from single-well stimulation treatments. MEOR stimulation treatments are potentially cost-effective because the costs are low and the production response occurs quickly. However, the ultimate potential oil recovery from application of these treatments is somewhat limited because only a relatively small volume of the reservoir is affected by the small amount of microbes that is injected. In cases where the microbial treatment reduces near-wellbore damage, which has resulted from deposition of particulate or waxy precipitates, the method can be very effective.

Another MEOR method that is potentially effective, but has not been widely practiced, is microbial-enhanced waterflooding (see Figure 2). A microbial formulation is injected into water injection wells in an ongoing waterflood and followed by periodic injections of nutrient. Microbial-enhanced waterflooding requires the same careful reservoir engineering design as would any other EOR method. Reservoir engineering studies are required to characterize the reservoir to ensure balanced injection rates for maximizing sweep efficiency and to establish that a sufficient amount of oil remains to make the project economically feasible.

The costs associated with applying microbial methods include the expenses for laboratory tests that are required to design a microbial formulation to inject, procurement and transportation of the nutrient, and minor modifications to surface injection facilities. Periodic monitoring of the produced fluids is prudent to ensure that the process is operating properly and that no adverse environmental effects have occurred. In comparison with other commonly applied improved oil recovery methods such as polymer and steamflooding, MEOR methods are relatively easy to implement.

As with any EOR method, successful application of MEOR requires careful reservoir evaluation and engineering. It is important to design the microbial method for the specific reservoir properties. In reservoirs under waterflooding that have experienced early water breakthrough and high water-oil ratios, injection of a microbial formulation that reduces permeability by producing polymers or slimes (Jack and Diblasio, 1985; Raiders, et al., 1985; and Knapp, McInerney, and Menzie, 1987) can improve oil recovery by increasing sweep efficiency. In microbial waterflooding methods, it is also important to maintain adequate displacement and sweep efficiencies on a scale comparable to the interwell spacing in the field. Single-well stimulation treatments do not always require extensive reservoir evaluation.

It is difficult to accurately establish the cost-effectiveness of MEOR in field applications. It has been estimated (Hitzman, 1987) that more than 200 microbial field tests have been conducted in the United States, but the detailed results of these tests have seldom been reported in the literature. In many cases, the tests were not monitored closely enough to accurately establish the incremental recovery caused by the microbial injection. Also seldom have control wells been included in the testing program to determine the degree to which the oil production response was affected by changes in field operating procedures. Most of the field tests that have been conducted were single-well stimulation tests (Bryant, 1986). A number of MEOR field tests have recently been initiated by the U.S. Department of Energy and industrial companies (Hitzman, 1988; Donaldson and Clark, 1982). The results of these field pilot tests should provide important information that is needed to determine the cost-effectiveness of this novel technology. This paper provides an assessment of the economics of applying MEOR methods.

DESIGN AND FIELD APPLICATION OF MEOR METHODS

Reservoir Evaluation and Characterization

The success of any MEOR field application depends on the ability of the injected microbial formulation to mobilize and recover the residual oil that is trapped in the pores of the rock matrix. As with any improved recovery method, reservoir evaluation is required to determine if the candidate reservoir is suitable for application of the technology. Microorganisms produce acids, surfactants, solvents, and gases that can assist oil recovery. However, a microbial method should be designed to recover oil by a specific means. For profile modification applications, a microbe should be selected that produces polymers or slimes that can effectively reduce the permeability of the rock and increase sweep efficiency. In single-well stimulation treatments, microbial formulations that produce surfactants and solvents, which can alter wettability, emulsify waxy deposits, and disperse fine particulate matter in the near wellbore region, are desirable. In microbial waterflooding applications, the microbial formulation must be able to transport over long distances in reservoir rock and produce chemicals that can mobilize residual oil through reduction of interfacial forces or alteration in rock wettability. Acid-producing organisms can be useful in increasing permeability in reservoirs with carbonaceous cementing material. At least some limited understanding of the properties of the reservoir are necessary for designing an MEOR process.

The approximate conditions under which MEOR methods can be applied (Bryant, 1987) are summarized in Table 1. These screening criteria can be used to identify candidate reservoirs for application of MEOR. To be successful, an EOR method must be designed to efficiently and economically mobilize a significant amount of residual oil in the reservoir. Without adequate reservoir characterization, the injected fluids can quickly break through at producing wells or migrate out of the intended pilot area.

After a potential candidate reservoir has been identified, a reservoir engineering evaluation should be conducted to determine its characteristics. The evaluation may include log and core analyses, determination of fluid properties (i.e., salinity, oil viscosity, and oil composition), fluid saturations, analysis of oil production history, and analysis of the geologic depositional environment of the reservoir. For large-scale microbial flooding projects, where sweep efficiency and conformance of the injected microbial slug are important, more sophisticated techniques such as chemical or radioactive tracer tests and well testing methods may be used. The application of sophisticated reservoir characterization techniques is expensive and would usually be warranted only in large-scale projects in reservoirs that are known to be heterogeneous.

In many cases where single-well stimulation methods are to be applied, little additional information is required for a preliminary engineering evaluation. This information is usually available from lease operators. In relatively homogeneous sands with good permeability and a well-established production history, MEOR methods can probably be applied with minimal reservoir characterization.

The low cost of the microbial and nutrient injectants has led some operators to conduct MEOR field projects in marginal producing wells with very little reservoir analysis or laboratory design based on the assumption that they have little to lose and something to gain. The results from these field tests have been mixed, but some successes have been reported. However, there have also been some failures, most of which have probably not been reported. The potential gains in productivity that can be achieved through at least limited reservoir engineering may well outweigh the relatively minor cost of the analysis.

Design of a Microbial Formulation

A microbial formulation must be selected for the specific conditions of the target reservoir. Laboratory tests are necessary to design and optimize a microbial formulation (Bryant, Burchfield, Douglas, and Bertus, 1988). Microorganisms can mobilize crude oil by increasing sweep or displacement efficiencies. The injected microorganisms must multiply under reservoir conditions and produce sufficient quantities of the proper chemical products to efficiently mobilize the oil that is trapped in the pores of the reservoir rock. Laboratory studies are conducted to select a microbial formulation that is compatible with the brine, rock, and crude oil under reservoir conditions. It is important to conduct laboratory screening tests under conditions that are as close to those in the reservoir as possible. The ability of the injected organisms to grow in the presence of the indigenous organisms, which are present throughout the reservoir, should be tested. Laboratory tests should usually be conducted under anaerobic (oxygen-free) conditions. Most oil reservoirs are essentially anaerobic. Laboratory tests conducted under aerobic conditions may yield results that are not representative of behavior in the reservoir.

Laboratory tests can range from limited compatibility testing to more extensive studies to optimize the oil recovery efficiency of the microbial formulation. Limited compatibility testing is relatively inexpensive. More extensive optimization studies such as long-term compatibility testing in reservoir cores, coreflooding tests, capillary pressure tests to determine the extent of wettability alteration, interfacial tension measurements, and micromodel studies are more expensive and time-consuming but may also increase the ultimate recovery from applying the MEOR method and reduce the risk of formation damage.

Without at least minimal laboratory testing, injection of microbial formulations and nutrients can cause losses in well productivity. When nutrient is injected, the indigenous microorganisms can be stimulated and either overgrow the injected microbes or produce undesirable products that can cause souring of the crude oil or decreases in permeability. Laboratory coreflooding studies are recommended, especially in reservoirs having absolute permeabilities less than 100 md, to ensure that the injected organisms will not reduce injectivity. The size of individual microbial cells is typically a few microns, while the pore throats in reservoir rocks range from 1 to 10 microns for permeabilities from 10 to 500 md. Reductions in permeability can result from the accumulation of cell mass or debris in the reservoir. The cost of limited testing

is usually minimal when compared with the potential loss in income from formation damage. Single-well injectivity tests can also be conducted in which the microbial formulation and nutrient are injected into a well and the well is shut in and then produced. Microbial analysis of the produced fluids, which can be conducted in the field, can establish if growth of the organisms is being sustained in the reservoir. Monitoring of changes in injection pressure can also indicate if injection of the microbes or nutrient is causing formation damage or losses in injectivity.

Selection of Nutrient

Selection of the nutrient to be injected is an important consideration. The nutrient should be inexpensive and provide the necessary carbon source and, in some cases, trace minerals that will stimulate and sustain the growth of the injected microorganisms. Molasses is typically used as the nutrient for microbial field projects, primarily because it is readily available in bulk quantities and is relatively inexpensive. Corn syrup can also be a suitable nutrient for injection. The sugar content and cost for typical nutrients are given in Table 2. The cost of molasses and corn syrup is approximately the same when compared on the basis of active sugar content. The trace minerals in some molasses samples that are manufactured for cattle feed can be toxic to certain organisms. In oil field applications, molasses is diluted with field brine and injected at concentrations ranging from 2 to 10%. While molasses has been the most widely used nutrient in MEOR field applications, it is not necessarily the optimal nutrient for stimulating microbial growth. More research is needed to determine the optimal nutrient composition for MEOR applications.

Some commercially available molasses samples contain enough coarse fibrous material to cause formation damage when injected into low-permeability reservoirs. Laboratory coreflooding tests can be conducted to determine if a particular molasses sample will reduce injectivity in injection wells or cause formation damage. Ultimately the selection of the nutrient will depend upon availability, cost, and compatibility with the injected organisms. For microbial flooding applications, maintaining quality control on the sugar and fiber content of the molasses is important.

Modifications to Field Facilities

For single-well stimulation treatments, the modifications to field facilities are usually minimal (Carroll and Linville, 1986). The procedures for injection of the microbial formulation and nutrient are similar to those used for well workovers to remove formation damage. For producing wells that are being pumped, the microbes and molasses are injected under gravity or with a gas-powered pump and flushed into the formation by injection of brine. A relatively small volume (i.e., 0.5 to 5 bbl) of microbial formulation, which is contained in drums, is injected. The molasses is usually transported to the field in a water tank truck and is diluted in the field with formation brine or injection water to a concentration of 2 to 10% and then injected into the formation. Brine is usually

injected to flush the molasses and microbes out into the formation. After the brief shut-in period of a few days to a few weeks, the well is returned to production.

In waterflooding projects, injection of microbes and nutrients into a relatively small project area can be accomplished through use of a simple bypass header. For larger-scale projects, installation of a centralized tank and injection lines for the nutrient is more cost-effective.

Monitoring of Field Performance

Monitoring of produced fluid and gas samples can provide useful information. The injected microbes can be used as a tracer to determine breakthrough times in producing wells if they can be differentiated from the indigenous microbes. Gas chromatography (GC) and liquid chromatography (LC) analyses of produced brine samples can be used to determine if chemical products from the microbial fermentation are propagating through the reservoir. Microbial analysis can be conducted to determine if the microbes are remaining viable in the formation. In reservoirs where sulfate-reducing bacteria (SRB), which can produce dangerous hydrogen sulfide, are present, microbial analysis can determine if the SRB are being stimulated by the injection of nutrient. Changes in oil and gas composition can also be an indicator of the performance of the microbial treatment. Alteration in bottoms, sediments, and water (BS&W) and oil viscosity are frequently measured in single-well stimulation tests to determine if the injected microbes have survived in the formation and are affecting the composition of the crude oil. Periodic monitoring of produced fluids can be conducted for a relatively low cost.

Environmental Considerations

Although regulations governing the application of MEOR methods have not been specifically enacted, the protection of potable groundwater supplies is covered by environmental laws. Laboratory and field tests using microbes, which have been isolated from oil field environments, have identified no significant environmental hazards associated with applying this technology in a prudent manner (Douglas, Bryant and Bertus, 1988). Any operator injecting microorganisms into an oil reservoir should take every precaution to ensure that the injectant does not migrate into or contaminate any potable water aquifers. Periodic monitoring of drinking water wells in the vicinity of the project can help to establish that the injected organisms have not contaminated any sources of drinking water. Organisms that are usually injected into oil reservoirs are not pathogenic and are used at relatively low concentrations. If deleterious effects are observed, the injected organisms can usually be killed by injection of a biocide or by discontinuing the injection of nutrient. Many of the organisms used in oil field applications are strictly anaerobic and will not survive in an oxygen-containing atmosphere; therefore, surface spills of the organisms would not lead to long-term contamination. No evidence has been published to suggest that application of MEOR methods is any more hazardous than any other EOR methods which are routinely applied in oil fields.

ECONOMICS OF APPLYING MEOR METHODS

Very little reliable data are available on the performance and costs of applying MEOR methods. Most of the projects that have been conducted were single-well stimulation treatments. In single-well stimulation treatments, the important results are how much the oil production rate increased and how long the increase was sustained. To our knowledge, there have been no reports on any field test that present detailed data regarding the costs and economic returns for a specific MEOR field test. Several well-documented MEOR field tests currently being performed should provide quantitative information that is needed to determine the economic feasibility of this technology. The goal of this paper is to provide preliminary estimates of the costs associated with applying MEOR technology as a guideline for further development of these methods.

MEOR Well Stimulation Methods

The least-expensive and most widely practiced MEOR method has been single-well stimulation treatments in marginal stripper wells. These treatments have been conducted by a wide variety of entrepreneurs, organizations, and companies in many areas of the country. The following example is given to illustrate the costs of applying microbial single-well stimulation treatments. The costs assumed in this example are for MEOR methods that have already been developed. Application of undeveloped methods will require higher laboratory and development costs.

Assume that four stripper wells, which are each currently producing 1 bbl/d of crude oil, are stimulated by injection of a microbial formulation. Following injection of the microbial formulation, each well is shut in for 1 week and then returned to production. Each well receives 15 gallons of nutrient, which costs $100/ton, each month for 6 months for a total of 2 tons. The oil production for each well increases from 1 bbl/d for the first 2 months to 3 bbl/d for the next 6 months and then returns to 1 bbl/d for the remaining 4 months of the year. The cumulative oil production from the four-well pilot project when microbial stimulation is applied is compared with the baseline production in Figure 3. Over the 1-year term of the project, the MEOR treatment results in the production of an additional 1,410 bbl of oil above that obtained without any stimulation.

The costs of stimulating the four wells are assumed to be $2,000 for minimal laboratory testing to design the microbial and nutrient injectants, $600 for modifications to surface facilities, and $200 for the nutrient. In addition, approximately $300 in income is lost from the four wells during the 1-week shut-in period. Operating expenses are assumed to be $5.50/bbl of crude oil produced. The potential returns from hypothetical MEOR stimulation treatments under a variety of conditions are summarized in Table 3. The net income after expenses is illustrated for a base case with an oil price of $16.50 in Figure 4. The project breaks even sometime in the 4th month and results in a net increase in income of $12,700, which is 80% greater than would have been achieved without the MEOR treatment. The project has a return on investment greater than 355%.

The sensitivity of the hypothetical project to changes in crude oil price is illustrated in Figure 5. The return on investment exceeds 50% for oil prices greater than $10.50/bbl. A break-even point is typically achieved within 3 to 5 months. Because the cost of the nutrient is relatively small in relation to the other costs, the financial returns from the project are not very sensitive to this parameter. Changes in the cost of nutrient ranging from $100 to $400 per ton do not significantly affect the profitability of the project at a crude oil price of $16.50/bbl. The effect of the number of wells that are treated is illustrated in Figure 6. It is not possible to recoup the initial cost of implementing the MEOR stimulation unless two or more wells are treated. Since the incremental cost of treating more wells is essentially that of the nutrient and minor modifications to well heads, the economics become more attractive when a greater number of wells are stimulated.

This simplified analysis of MEOR stimulation treatments is only a hypothetical example which is based on the limited field data that are available. Efforts have been made to make the parameters that are used in the analysis realistic. However, there is little quantitative information available upon which to base these estimates. More data on well-controlled field tests are needed to establish the potential profitability of MEOR stimulation treatments. These methods are most likely to be successful when used to treat near wellbore formation damage problems. The potential recovery of crude oil from MEOR stimulation treatments by improvements in oil displacement efficiency is relatively low because the total volume of the reservoir that is affected by the treatment is usually small. The analysis suggests that MEOR stimulation treatments can be profitable, even at relatively low oil prices.

Microbial-Enhanced Waterflooding

Microbial-enhanced waterflooding has a significant potential for recovering a substantial amount of crude oil from U.S. oil fields that have been under waterflood for many years. The costs associated with designing a microbial-enhanced waterflood will be greater than that for MEOR stimulation treatments; however, the additional cost probably will be even a smaller fraction of the ultimate income.

More extensive laboratory studies should be conducted for a microbial-enhanced waterflood than for a well stimulation treatment to ensure that the microbes will be able to survive over long periods of time (i.e., years) that will be required to achieve production response. The ability of the microbes to transport through the reservoir rock is also more critical in a waterflooding operation than in stimulation treatments. It is important that the injected nutrient be able to transport to the microbial colony in the reservoir and that the chemical products, which are primarily responsible for the increase in oil recovery, be able to travel through the reservoir. Costs for laboratory testing for a microbial-enhanced waterflood would typically be 3 to 5 times that for a MEOR stimulation treatment. In addition, a more extensive reservoir engineering study would be required for the project. Simple chemical tracer tests may be needed, and reservoir data should be analyzed to determine the best way to implement the process.

The costs associated with engineering a microbial-enhanced waterflood would be similar to that for designing a polymer flood. The costs for implementing various EOR methods have been summarized in a recent paper (Carroll and Linville, 1986).

The following hypothetical example of a microbial-enhanced waterflooding project is presented to illustrate the potential income. The MEOR method is compared with polymer flooding, an EOR method that is widely applied by both major oil companies and independent operators. A model reservoir, which is typical of many mid-continent reservoirs under waterflooding, was selected. The reservoir parameters are given in Table 4. A 20-acre pattern with an original oil in place (OOIP) of 490 MSTB of oil was studied. At the beginning of the hypothetical project, 53% of the OOIP (260 MSTB) was assumed to remain in the reservoir (residual oil saturation = 37%). For economic calculations, a federal income tax rate of 34% and severance tax and royalty rates of 7% and 12.5%, respectively, were assumed.

A detailed reservoir simulator which can accurately predict the performance of a microbial-enhanced waterflooding process based on the physical phenomena that are involved has not yet been developed. A polymer flooding predictive model (U.S. DOE, 1986) was used to simulate the performance of a microbial-enhanced waterflood. The model is a three-dimensional, five-layer, two-phase (water and oil) model that computes water front breakthrough and oil recovery using fractional flow theory and models areal and vertical sweep efficiencies using a streamtube approach.

The effect of the improvement in oil displacement efficiency by injection of the microbial formulation was incorporated into the model calculations by assuming that the residual oil saturation was reduced from 30% (waterflooding) to 25% (MEOR). This effect was incorporated into the model calculations by changing the input value of the end point residual oil saturation to water from 30 to 25% in the relative permeability calcuations. While this is clearly not a mechanistically correct way to model oil displacement by microorganisms, it should provide a means of simulating increased displacement efficiency. Based on laboratory coreflooding results (Bryant and Douglas, 1987) this is a conservative estimate of the oil recovery efficiency of typical microbial formulations. The nutrient costs were assumed to be $100/ton, and 250 tons of nutrient was injected into the 20-acre test pattern each year for 2 years. The crude oil price was assumed to be $16.50/bbl. The operating costs and taxes were assumed to be the same for the polymer and the microbial floods. The polymer cost was taken as $1.39/lb.

The performance of the microbial-enhanced waterflood is compared with that of continued waterflooding and polymer flooding in Figure 7. The injection of 710 Mbbl of water over 19.6 years would produce 25 MSTB of oil, or 9.5% of the OOIP. The initial oil saturation is reduced from 37 to 33%, and 28 bbl of water is injected for each STB of crude oil that is recovered.

Injection of 33.5 tons of polymer (0.3 pore volume) at a concentration of 900 ppm with a mobility ratio of 0.24 (polymer buffer viscosity = 10.9 cp) over 19.6 years results in an incremental oil production of 19 MSTB (7% of the oil in place at the start of the flood). Approximately 3.5 lb of polymer is injected for each incremental barrel of crude oil that is recovered. The economic life of the polymer flood is 14 years with a payout based on an after-tax, mean discounted cash flow (DCF) of 12 years. A 10% discount rate was used in all calculations. The mean DCF net profit per barrel of oil is $0.79, and the mean DCF rate of return is 11.8%.

Innoculation of each injection well with a microbial formulation and injection of 500 tons (0.1 pore volume) of molasses in a 4% nutrient solution (viscosity =3.4 cp) over a 2-year period results in an incremental oil recovery of 21 MSTB (8% of the oil in place at the start of the flood) after 19.6 years of water injection. The project payout was 7 years, with an economic life of 8 years. The mean DCF net profit per barrel of oil is $1.57 and the mean DCF rate of return is 14.9%. At an oil price of $20/bbl the DCF net profit per barrel of oil for the microbial-enhanced waterflood is $3.22 (DCF ROR = 19.3%), whereas that for the polymer flood is $2.62 (DCF ROR = 15.5%)

The effect of improvement in sweep efficiency is compared with the additional oil production from increased displacement efficiency caused by the injection of the microbial formulation and molasses in Figure 8. The oil production from the microbial-enhanced waterflood is compared with that for a molasses flood in which the only effect is the improved sweep efficiency (i.e., residual oil saturation = 30% as for waterflooding case) caused by the increased viscosity (3.4 cp) of the injected 0.1 PV slug of molasses and microbes. The difference between the molasses and microbial flooding curves in Figure 8 is due to the simulated effects of the increased displacement efficiency of the injected microbial formulation. At 8 years, the alteration in the residual oil saturation accounts for 62% of the incremental recovery. At longer times, the proportion of the incremental oil recovery due to the increased displacement efficiency becomes greater.

The performances of the polymer and microbial floods are compared at the economic life of the microbial flood, 8 years, in Table 5. While the ultimate cumulative recovery of the microbial flood is only slightly greater than that for the polymer flood over the long term (i.e., 20 years), the microbial flood recovers oil in a shorter period of time. The microbial flood produces 11,180 more barrels of oil than the polymer flood and has a positive, after-tax discounted cash flow, whereas that for the polymer flood is negative. The cost of the injected nutrient per incremental barrel of oil, $2.75, is economically attractive.

If a microbial-enhanced waterflood were implemented in a 20-acre area with an equivalent of four injection and four production wells, the oil production on a per well basis could be compared with that from the MEOR stimulation treatment which was discussed in the previous section. At the economic life of the microbial-enhanced waterflood (8 years), 18,000 bbl of incremental oil would be produced from the 4

producing wells, which is an average of 560 bbl/yr for each well. In comparison, the 4 wells that were subjected to MEOR stimulation treatments would produce a total of 1,410 bbl of oil in one year, which would average 350 bbl/yr for each well.

The assumptions considered for these calculations are merely hypothetical and should be used only for comparative purposes. More reliable predictions of the potential profitability of microbial-enhanced waterflooding projects will require more data from field tests and the development of an accurate MEOR process simulator. The example illustrates that microbial-enhanced waterflooding does appear to offer significant potential for producing substantial quantities of oil with attractive economic returns when compared with commonly practiced EOR methods such as polymer flooding.

CONCLUSIONS

Successful application of MEOR methods requires the same reservoir engineering design considerations as would any other enhanced oil recovery process. MEOR methods are potentially financially attractive primarily because the cost of the injectant (microbes and nutrient) is relatively low in comparison with the costs of other EOR fluids. Based on a preliminary economic analysis, application of cyclic microbial stimulation methods appears to be financially attractive, but has a limited potential for ultimate oil production. Model calculations indicate that microbial-enhanced waterflooding can also be economically attractive when compared with polymer flooding. Further field data and better process simulators are needed to accurately assess the oil recovery potential from application of MEOR.

ACKNOWLEDGMENTS

This work has been supported by the U.S. Department of Energy under Cooperative Agreement No. DE-FC22-83FE60149. The authors thank Rex Thomas of NIPER for assistance in conducting the computer simulations. The graphic design work of Joe Lindley is gratefully acknowledged.

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U. S. Department of Energy: "Polymer Predictive Model," DOE/BC-86/10/SP (Dec. 1986).

TABLE 1. - Screening criteria for application of MEOR methods

• Permeability, md	> 100
• Temperature, °F	< 150
• Depth, ft	< 6,000
• Salinity, % TDS	< 10
• Rock type	Sandstone preferred
• Residual Oil Saturation, %	>30

TABLE 2. - Comparison of various nutrients

Nutrient	Sugar content, %	Cost, $/ton	Cost per active ton, $/ton
Beet molasses	45-50	50 - 200	100 - 450
Cane molasses	45-50	50 - 200	100 - 450
Corn syrup	85-90	100 - 300	100 - 350

TABLE 3. - Sensitivity study for cyclic MEOR stimulation treatments

Oil price $ /bbl	Number of wells treated	Time to payout, months	Increase in net income, $	Increase in net income, %	Return on investment, %	Cost of nutrient, $/ton
8.50	4	6	1,400	33	-49	100
9.50	4	5	2,848	49	-2	100
10.50	4	5	4,260	59	52	100
12.50	4	4	7,080	70	153	100
14.50	4	4	9,910	76	254	100
16.50	4	4	12,700	80	355	100
18.50	4	3	15,600	83	456	100
20.50	4	3	18,400	85	556	100
16.50	4	4	12,700	80	355	100
16.50	4	4	12,500	79	318	200
16.50	4	4	12,100	77	257	400
16.50	1	7	1,200	31	-53	100
16.50	2	4	5,100	64	88	100
16.50	3	4	8,900	75	224	100
16.50	4	4	12,700	80	355	100
16.50	5	4	16,600	84	481	100

Operating expenses are assumed to be $5.50/bbl

TABLE 4. - Model reservoir and fluid properties

- Pattern area, acres 20
- Depth, ft 1490
- Temperature, °F 79
- Average permeability, md 250
- Average porosity, % 20
- Net pay thickness, ft 23
- Original oil in place, MSTB 490
- Oil in place at start of flood, MSTB ..... 260 (53% of OOIP)
- Oil gravity, ° API 34.4
- Oil viscosity, cp 10.1
- Residual oil saturation at start of flood, % 37
- Ultimate waterflood residual oil saturation, % 30

TABLE 5. - Comparison of polymer and microbial floods at 8 years[1]

	Polymer Flood	Microbial Flood
Incremental oil recovered, Mbbl	6.9	18.0
Incremental oil recovery as a percentage of oil in place at the start of the project, %	2.62	6.86
After-tax discounted cash flow, $,000	-30	10
Chemical[2] injected, lb per incremental bbl of oil	9.72	55
Cost of Chemical[2] per incremental bbl of oil, $	13.50	2.75

[1]Economic life of the microbial flood at an oil price of $16.50/bbl.

[2]Polymer ($1.39/lb) or molasses ($0.05/lb), respectively.

CYCLIC MICROBIAL RECOVERY

A solution of microorganisms and nutrients is introduced into an oil reservoir during injection. The injection well is then shut in for an incubation period allowing the microorganisms to produce carbon dioxide gas and surfactants that help to mobilize the oil. The well is then opened and oil and products resulting from the treatment are produced. This process may be repeated.

Schematic portrays one well during the 3 phases of this process. Flow pattern is stylized for clarity.

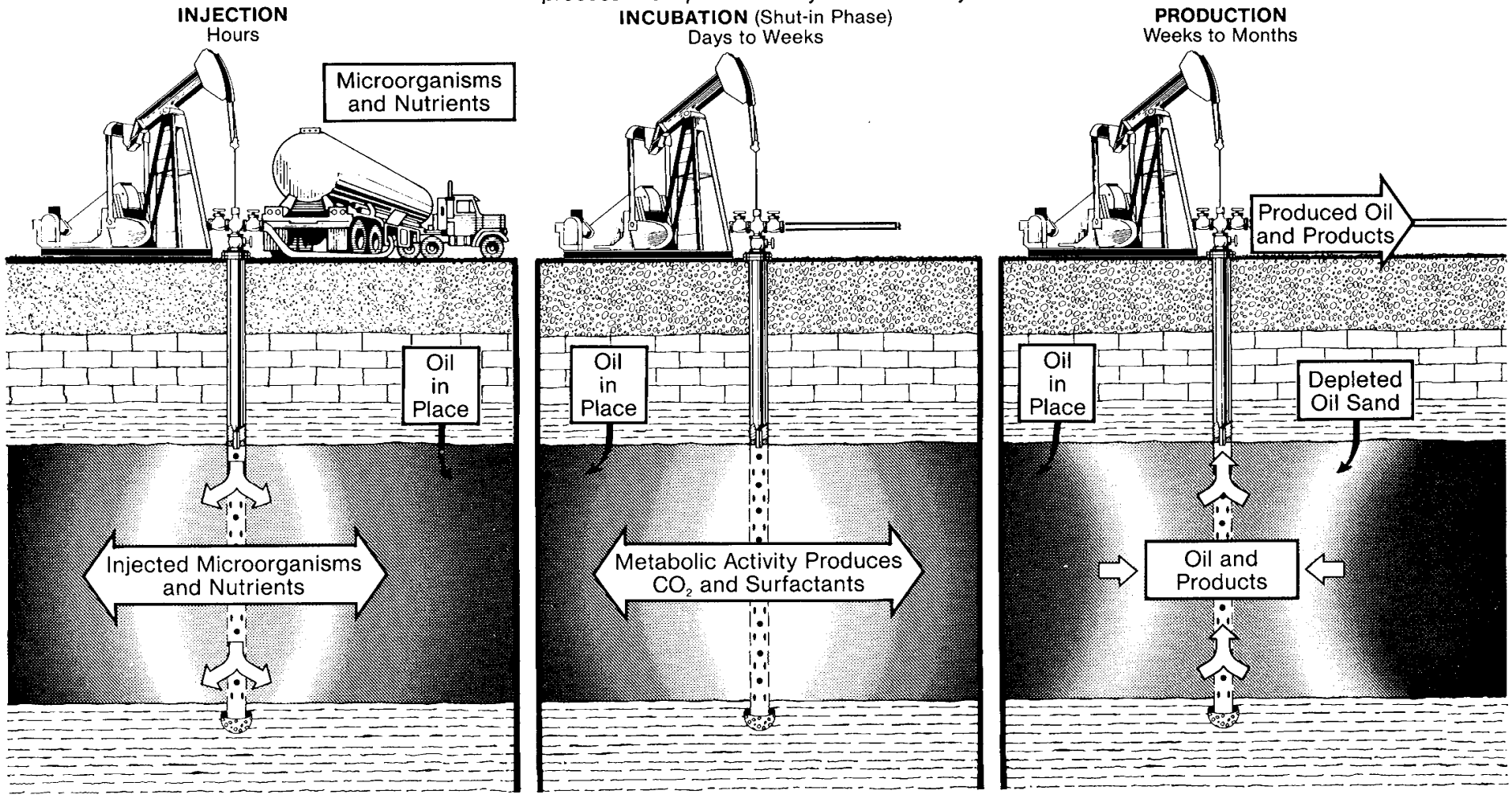

Figure 1. Cyclic Microbial Stimulation Process

MICROBIAL FLOODING

Recovery by this method utilizes the effect of microbial solutions on a reservoir. The reservoir is usually conditioned by a water preflush, then a solution of microorganisms and nutrients is injected. As this solution is pushed through the reservoir by drive water, it forms gases and surfactants that help to mobilize the oil. The resulting oil and product solution is then pumped out through production wells.

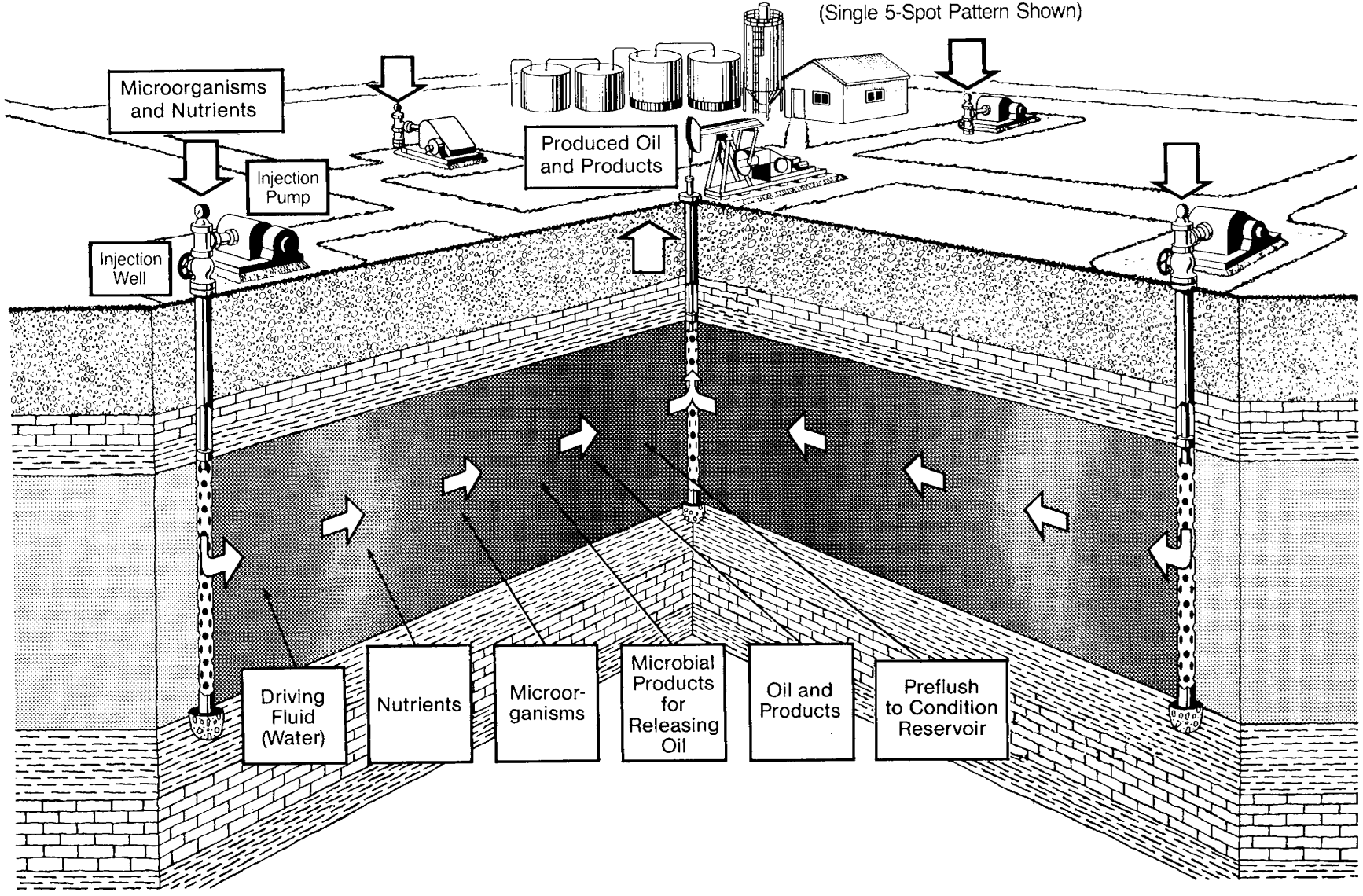

Figure 2. Microbial-enhanced Waterflooding Process

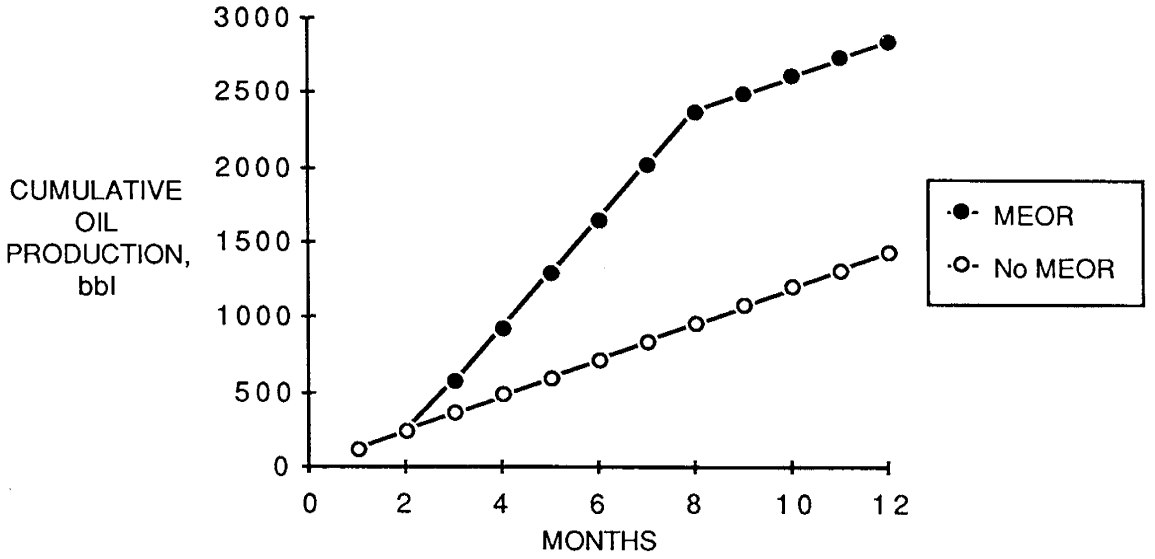

Figure 3. Increased oil production by MEOR well stimulation treatment of four wells.

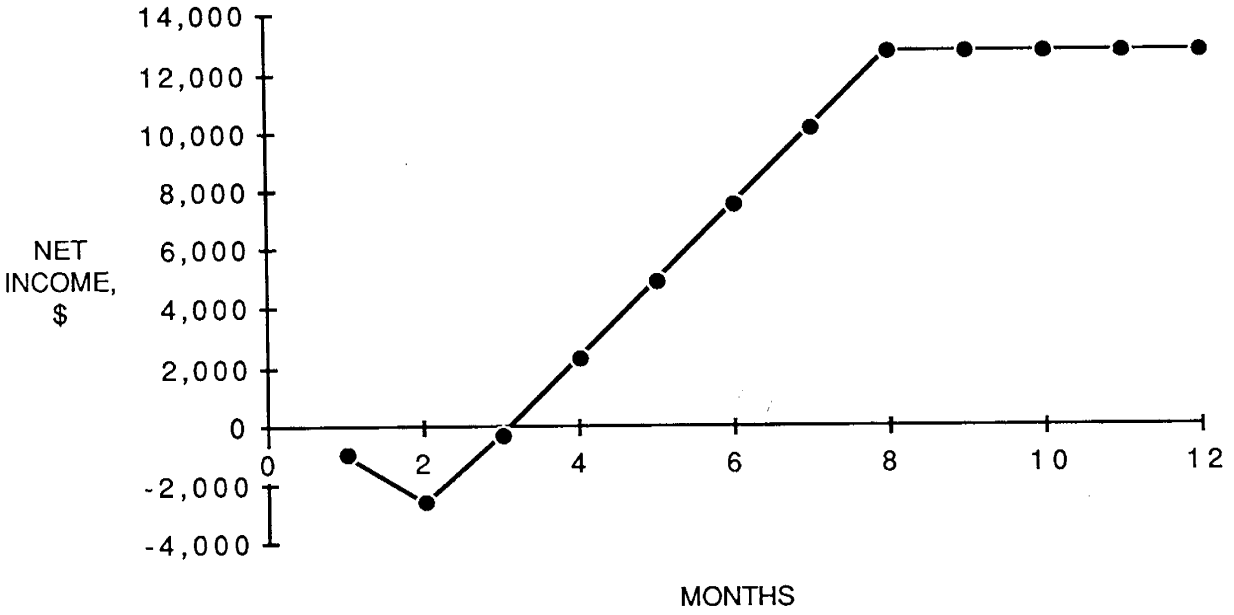

Figure 4. Net income after expenses for MEOR well stimulation treatment of four wells.

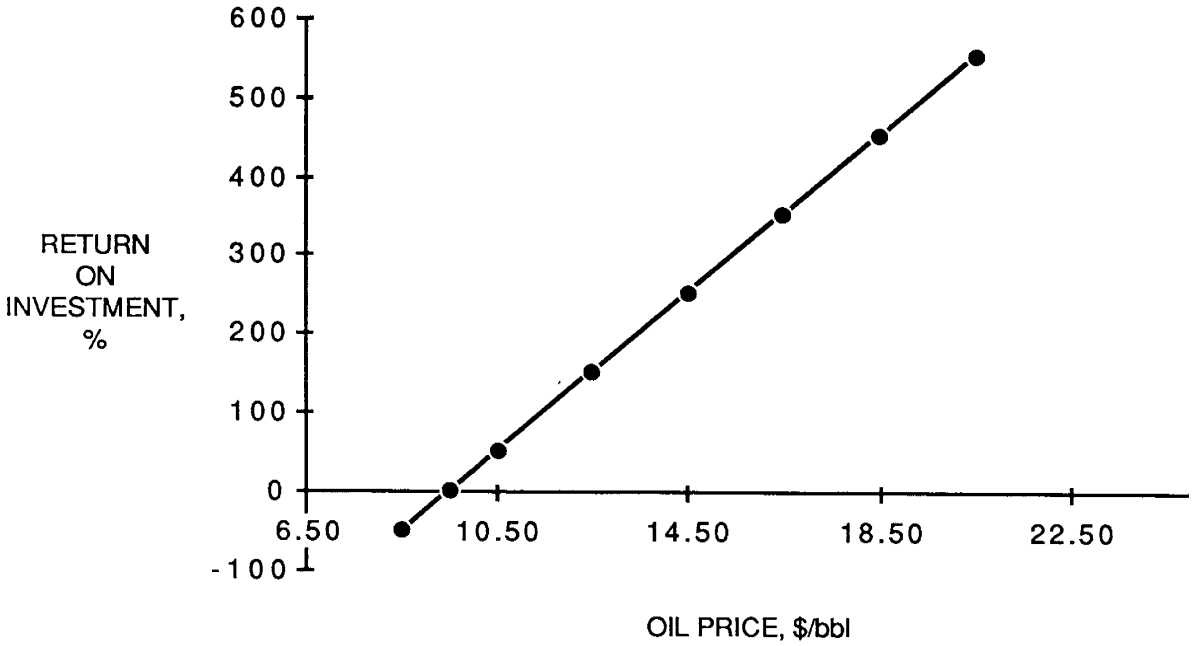

Figure 5. Effect of oil price on return on investment for MEOR well stimulation treatment of four wells.

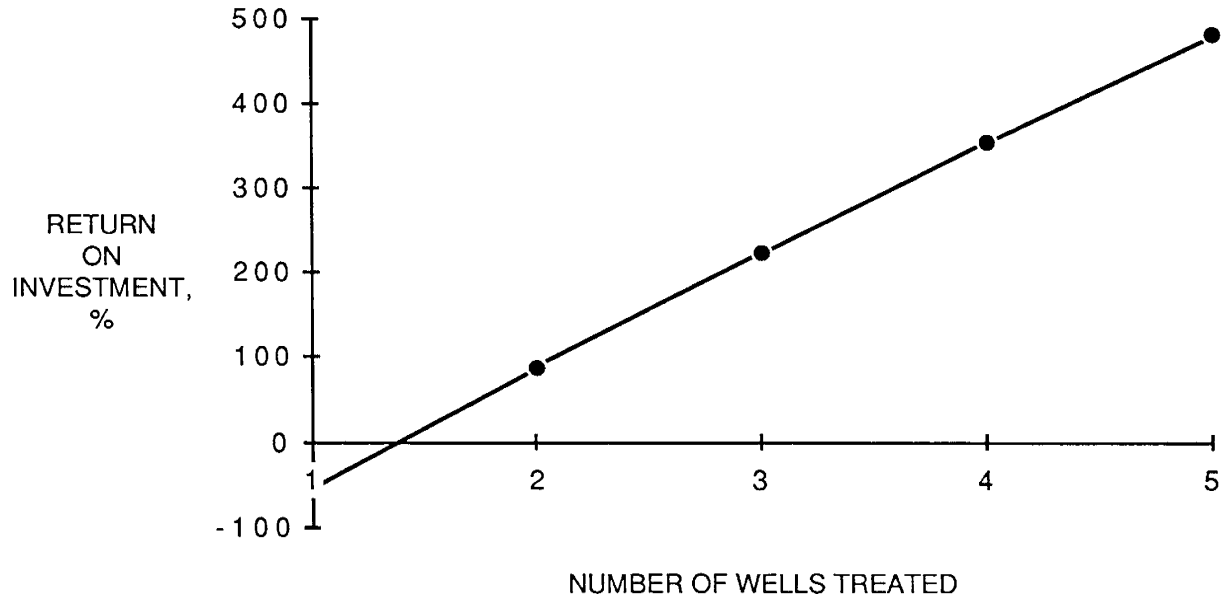

Figure 6. Effect of the number of wells treated by MEOR well stimulation on return on investment.

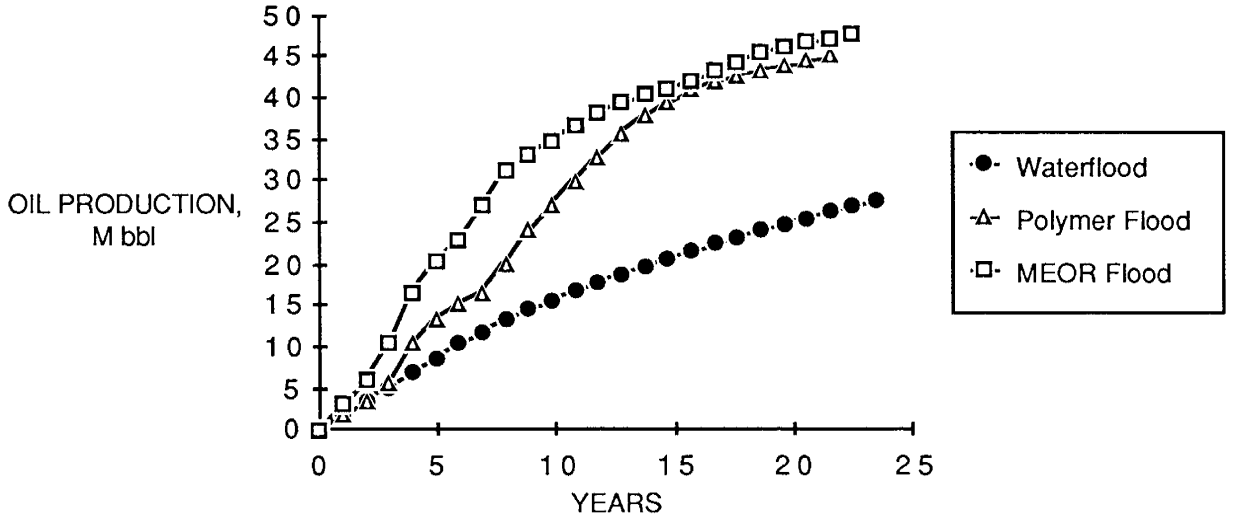

Figure 7. Comparison of oil production by waterflooding, polymer flooding, and MEOR flooding in a model mid-continent reservoir.

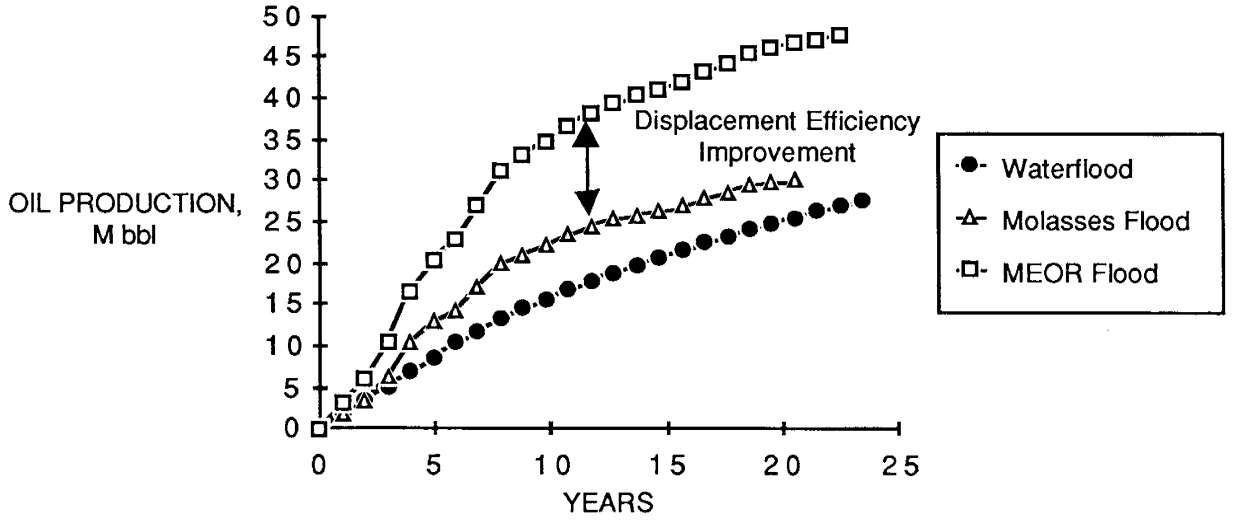

Figure 8. Effect of improvement in sweep and displacement efficiencies.

★ U.S. GPO: 1988 561-025/80019