

AN ANALYTICAL STUDY OF MICROBIAL ENHANCED OIL RECOVERY

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CERTIFICATE

This is to certify that Mr. Ankur Rana and Mr. Vinay Rawat have completed their major project on "An Analytical Study of Microbial Enhanced Oil Recovery" under the guidance of Mr. Arvind Chittambakkam during their 7th and 8th semester.

Arvind Chadd for
Arvind

(Mr. Arvind Chittambakkam)

Project mentor

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Abstract

Throughout the life of an oil well, it goes through three distinct phases where various techniques are employed to achieve optimum production and maximize ultimate recovery.

These three phases of oil field development are:

Primary recovery: The term refers to the production of the hydrocarbons from the reservoir due to the pressure generated by the gas present in the oil, without the use of any process (such as fluid injection). The production in this phase is due the natural energy of the reservoir.

There are basically six driving mechanism that provides the natural energy for oil recovery:

- (1) rock and liquid expansion drive;
- (2) depletion drive;
- (3) gas cap drive;
- (4) water drive;
- (5) gravity drainage drive;
- (6) combination drive

Secondary recovery: The lack of sufficient natural drive led to practice of supplementing the natural energy of the reservoir introducing some kind of artificial drives, the most common method includes the injection of water or gas.

Tertiary recovery: Enhanced oil recovery (EOR) or the tertiary recovery is the last recovery phase. EOR is achieved by several methods such as gas injection, chemical injection, thermal recovery and MICROBIAL ENHANCED OIL RECOVERY (MEOR).

Microbial Enhanced Oil Recovery (MEOR) is a biological technology, manipulating function or structure, or both, of microbial environments existing in oil reservoirs. The aim of MEOR is to improve the recovery of oil entrapped in porous media and also increasing economic profits.

MEOR involves the use of microorganisms. Several ways, how microorganisms may contribute to EOR, are:

- 1) Microorganisms can produce bio surfactants and biopolymers on the surface
- 2) Microorganisms grow in reservoir rock pore throats to produce gases such as $\text{CO}_2, \text{H}_2, \text{NH}_3, \text{CH}_4$, surfactants, and other chemicals to recover trapped oil

- 3) Microorganisms can selectively plug high-permeability channels in reservoir rock, so that sweep efficiency increases
- 4) Bio cracking, where microbes metabolize carbon atoms from the interior of an alkane chain
- 5) Changes in interfacial and surfacial tensions can be induced.
- 6) Acid production can cause dissolution of rock, changes in water PH and stimulate in situ surfactant formation

Microorganism comprises of five major groups of organism: viruses, Fungi, algae, protozoa, and bacteria. Bacteria are the only microbe that have been used so far for the development of processes for enhance oil recovery, because of their several accountable properties: small size, exponential growth rate when supplied with essential nutrients, and production of metabolic compounds, such as gases, acids, low-molecular-weight solvents, surfactants and polymer.

There are many types of bacteria which are capable to exist in severe conditions such as high salinity, high temperature and high pressure which are too encountered in subsurface geological formations or even in the absence of oxygen.

Due to a wide range of physical and chemical properties of bacteria it may be beneficial to use the symbiosis of different bacteria's to enhance the oil recovery.

Most of the conventional oil recovery processes are able to retrieve only half of the oil available in the reservoir. The life of the reservoir can be extended by the utilization of this technology.

We are also taking the reference from different MEOR case studies of different field which is helpful for us to understand the MEOR related field problems and their remedial measures in the reservoir.

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1. Introduction

The technology used today for production of petroleum from subsurface reservoirs has not been advanced beyond the stage at which the ultimate production of oil is only one third to half of the original oil-in-place. Thus the potential target for enhanced oil recovery is greater than reserves that can be produced by conventional methods. A new approach to enhance of oil recovery is using microbes. Basic strategy behind this technique is injection of microbe with suitable nutrients which produces their metabolic products like gases, surfactants, acids etc or extracellular product of metabolism may be extracted from cultures grown at the surface and solutions of these materials may be injected into the Petroleum reservoir that can aid in releasing oil from the reservoir rock.

Microbial cultures are capable of synthesizing a large variety of biochemical products from crude oil constituents when provide with essential nutrients and proper environmental conditions. The range of metabolic products from microbial attack of petroleum is very broad, depending on environmental conditions (Pressure, temperature, salinity, pH, and the presence and absence of oxygen), supporting nutrient available for cells metabolism (nitrogen, phosphorus etc.) and the specific bacteria interacting with the petroleum.

In very general terms, metabolic products may be :- gases (methane, hydrogen, carbon dioxide, hydrogen sulfide), carboxylic acids (formic, acetic, valeric), solvents (alcohols, aldehydes, ketones), polymers (proteins, polysaccharides), surface active compounds (poly-anionic lipids) and many other compounds ranging from simple to very complex micromoleules.

With respect to petroleum, the study of microbes and their products is carried out to:

- 1) Develop products that can enhance secondary and tertiary recovery;
- 2) Cause the mobilization of heavy oils by viscosity and interfacial tension reduction;
- 3) Enable injection of cells into petroleum reservoirs, which will produce bioproducts in situ to enhance the recovery of oil; and
- 4) Study the petroleum reservoir microbial ecology.

1.1 MEOR Essentialities

The practical application of microbial cultures to subsurface oil reservoirs imposes several restrictions on the microbial culture. The microbes must be able to migrate, or to be transported, deep within the reservoir for any in situ applications to be of practical significance of oil recovery. Furthermore they must be able to multiply in the subsurface environment and, therefore, nutrients required for growth which are not available in the petroleum reservoir must be supplied in the injection water.

Microbial toxicity to heavy metals increases at elevated temperature; and, heavy metals are frequently present in reservoir brines, the increase of temperature in the subsurface ($25\text{ }^{\circ}\text{C} + 18\text{ }^{\circ}\text{C}/\text{km}$ of depth) might inhibit the effective in situ application of microbial cultures. Consequently, microbes intended for use in petroleum reservoirs should be tested with the reservoir fluids at surface conditions of temperature and pressure. High pressure reduces the growth rate of microbes at all temperatures. Microbial systems intended for subsurface applications, therefore, should be tested at simulated subsurface environment conditions, or selected microbial cultures should be isolated under these conditions.

1.2 Microbiological Enhanced Oil Processes

The use of microorganism and their metabolic products to stimulate oil production is now receiving renewed interest worldwide. This technique involves the injection of selected microorganism into reservoir and the subsequent stimulation and transportation of their in-situ growth products in order that their presence will aid in further reduction of residual oil left in the reservoir after secondary is exhausted. The MEOR is unlikely to replace conventional EOR methods, because MEOR itself has certain constraints. This unique processes seems superior in many respects, however, because self-duplicating units, namely the bacteria cells, are injected into the reservoir and by their in-situ multiplication they magnify their beneficial effects. Some of the mechanisms are proposed, by which these microbial agents could stimulate oil release are shown in Table-1. The CO_2 and /or CH_4 produced from fermentation and neutralization of acid products by the reservoir rock could lower the pH of water and repressurize the reservoir. The solvents, primarily alcohols such as methanol, ethanol, propanol, isobutanol, n-butanol, as well as formaldehyde and acetone could oil viscosity.

Table1. Microbial products and their contribution to EOR

Bioproduct	Effect
Acids	<ul style="list-style-type: none"> • Modification of reservoir rock • Improvement of porosity and permeability • Reaction with calcareous rock and CO₂ production
Biomass	<ul style="list-style-type: none"> • Selective and nonselective plugging • Emulsification through adherence to hydrocarbons • Modification of solid surfaces • Degradation and alteration of oil • Reduction of oil viscosity and oil pour point • Desulfurization of oil
Gases (CO ₂ , CH ₄ , H ₂)	<ul style="list-style-type: none"> • Reservoir repressurization • Oil swelling • Viscosity reduction • Increase of permeability due to solubilization of carbonate rocks by CO₂
Solvents	<ul style="list-style-type: none"> • Dissolving of oil
Surface-active agents	<ul style="list-style-type: none"> • Lowering of interfacial tension • Emulsification
Polymers	<ul style="list-style-type: none"> • Mobility control • Selective and non-selective plugging

1.3 Methods of using bacteria for recovery of residual oil

Fig1. Displacement of oil by metabolites of inoculated bacteria grown insitu

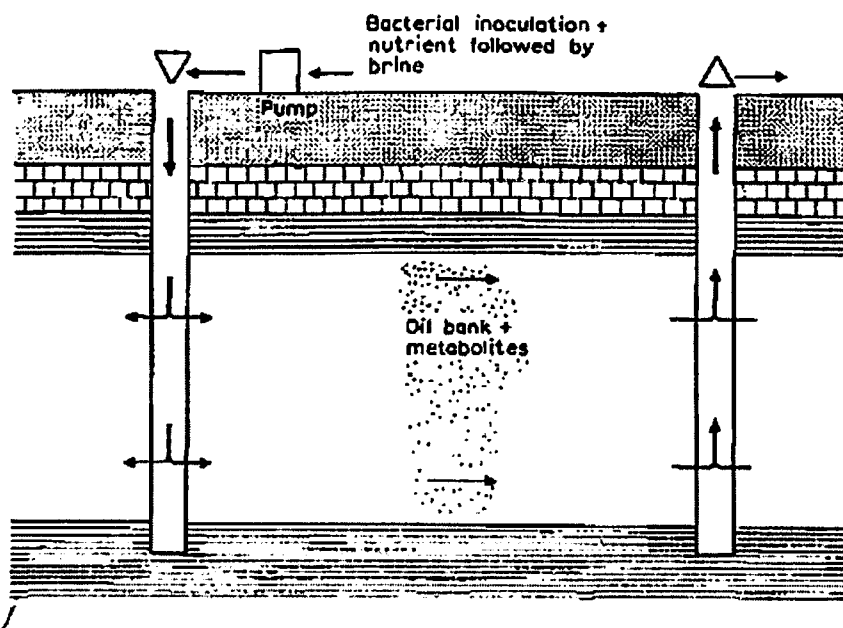


Fig2. Selective plugging of highly permeable zones by bacteria

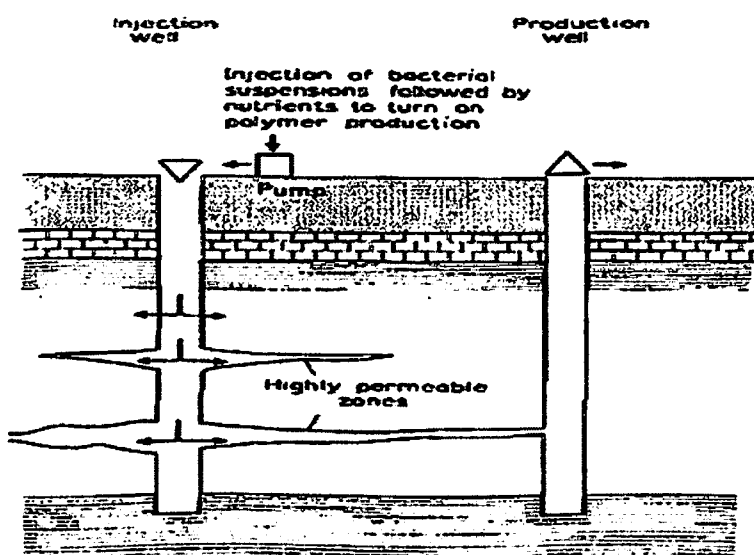


Fig3. Migration of cells and the synthesis of metabolic products around the well bore following inoculation and the closing the injection well.

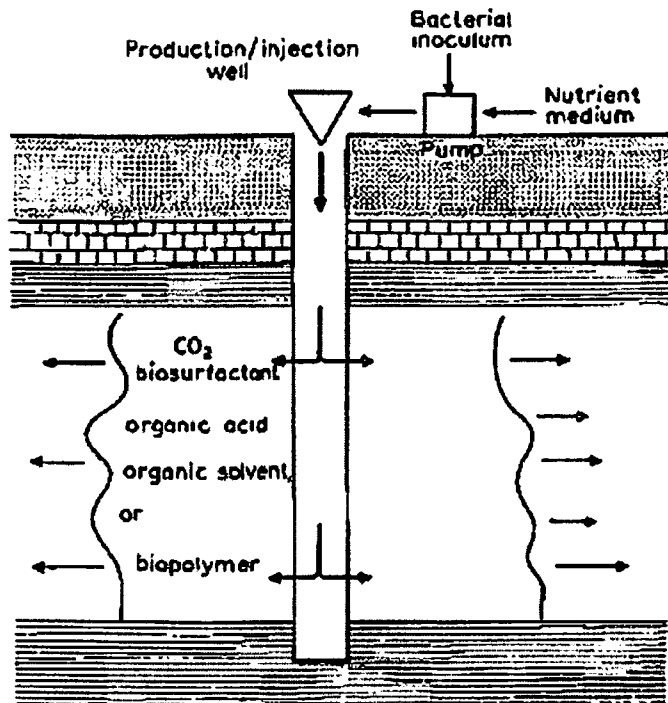
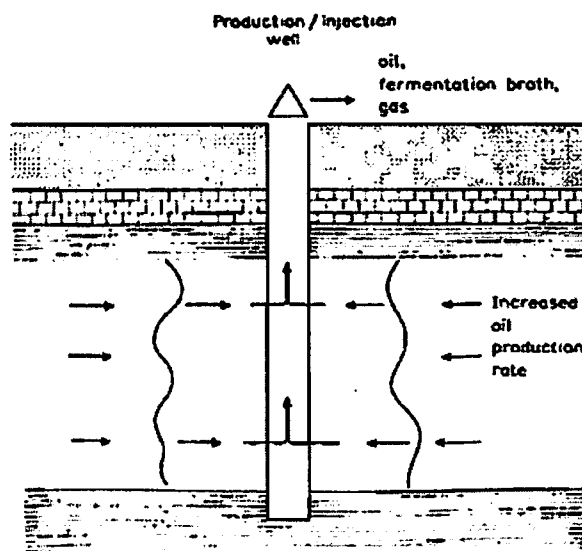


Fig4. Production of oil at the end of the incubation period, when the well is responded



1.4 Factors affecting the penetration of cells in porous matrix

- 1) Physical and chemical properties of the rocks, such as permeability and pore size distribution, porosity, wettability, surface charge, type of oil (e.g polar v/s non-polar), and total salinity and ionic composition of formation water;
- 2) Cell properties, such as shape, size, motility, type of cell growth (individually or in clumps or chains), surface charge, production of capsules slimes, chemical reaction products (acids or gases);
- 3) Mode of injection, such as rate of injection, salt content of injection water and density of cell suspension.

After considering all of these factors, it is essential to treat whole reservoir as a bioreactor and study displacement efficiency and the applicability of releasing oil by bacteria under reservoir conditions. The wettability alteration and effective oil permeability modification when bacteria are injected in to the reservoir have to be investigated. In any event, the addition of growth-promoting nutrients is required to obtain maximum bacterial activity of the type desired. Production of gas, acids and surfactants throughout the oil bearing formation is, theoretically, the principal objective. Ideally, without regard to development within the formation of extraneous microbes, cells or spores of the specific microbe to be employed may be introduced along with the nutrients, or before or after the nutrient is injected in to the formation. It is only through multidisciplinary integration that MEOR can become a technically proven and feasible technology.

2. The Subsurface Environment: Physical and Environmental constraints in microbial activity

In considering the use of viable microbial cultures within the petroleum reservoirs for enhancement of oil recovery through metabolic activities of microbes, one is immediately

confronted with an unusual environment existing in the subsurface. Not only is this environment completely foreign to life systems adapted to surface conditions, but it changes in a generally predictable manner as the depth from the surface increases. The porosity and permeability of the sedimentary formations decreases with depth of burial. The pressure and temperature increases with depth.

Although certain types of microorganism may grow prolifically at surface conditions and produce metabolic products that would obviously be beneficial to EOR, they may not be able to survive in a deep subsurface environment. If they do grow at subsurface conditions, they may not produce the same metabolic products because of the adverse influences of the high salt content and increased temperature of subsurface water. In addition, microbes are known to undergo a change of morphology at high pressure which may cause a change of metabolism.

There are four broad divisions of microorganism have emerged base on environmental tolerance:

- 1) **Aerobes**, capable of life process only in the presence of oxygen;
- 2) **Anaerobes**, which exist in the absence of oxygen and derive their energy from degradation of oxygenated molecules;
- 3) **Facultative**, which are able to exist aerobically and anaerobically

Microbes can be classified according to their optimum temperature range as **psychrophiles** (< 25 °C), **mesophiles** (25-45 °C), and **thermophiles** (45-60 °C). The relatively recent discovery of microbes that can survive in water at temperatures above 100°C has considerably extended the range of conditions under which life can be expected to exist. Microbes that thrive under such extreme conditions are generally referred to as '**extremophiles**'. Within these broad groupings, there are microbes that metabolically produce gases (methane, hydrogen, nitrogen, carbon dioxide), polymer (polysaccharides, proteins), surface active compounds that are generally poly-anionic lipids, and many more compounds from simple alcohols to very complex macromolecules. The type and yield of metabolic products can be controlled to a large extent by modifications of environmental conditions and nutrients. Additionally, the microbes exhibit amazing properties for adaptation to new circumstances, both environmentally and nutritionally, through mutation.

There are several factors affecting the influence of microbial activities and they can be broadly classified as:

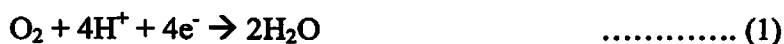
- Physical- temperature, pressure, pore size/geometry
- Chemical- pH, E_h , electrolyte composition
- Biological

In the following subsections, these constraints, and their interactions, are discussed

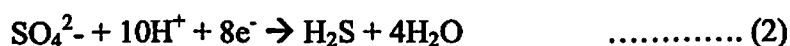
- 1) **Pore size**: - Perhaps the most obvious constraint that applies to deep subsurface microbes is the size of the pores. No metabolic activity was detected in core samples with pore throats narrower than $0.2\mu\text{ m}$, although in some cases it was after extended incubation. The observation of much higher levels of metabolic activity in more permeable samples led to conclude that sustained bacterial activity require interconnected pores of diameter at least $0.2\mu\text{ m}$.
- 2) **Acidity**: - The acidity or (alkalinity) of the surrounding aqueous medium, measured by the pH. Of biochemical parameters which affect the growth of metabolism of microorganisms, pH appears to be the least extreme in oil reservoirs. The rates of the enzymic processes that occur in respiration are strongly dependent on the pH. There generally exists an optimal pH, lying between 2 and 9.5, for the rates of such processes. The pH not only affect the growth and metabolism directly but also in an indirect manner by affecting the solubility of toxic materials. Most notable of these effects are the one that affect the solubilization of heavy metals. Heavy metals can be very toxic to organisms if they are found at levels highly in excess of what is needed for nutrition, generally in range of 10^{-3} - 10^{-4} M. heavy metals such as copper, ferric ions, zinc etc; can exist in concentrations greater than this range; however organisms are affected in different ways and certainly organisms are known that can tolerate very high concentrations of most any heavy metal. At lower pH the solubility of heavy metals and, therefore, their toxicity increases. Some organic acids become protonated and may enter cells with toxic effect. At high pH, some essential co-substrates or inorganic components required for growth may precipitate as carbonates, phosphates or hydroxides and may become a limiting parameter in controlling bacterial growth. The distribution of electric charges on bacterial cells depends on the pH of the microenvironment; therefore, bacterial distribution due to

their hydrophobic-hydrophilic properties at the oil-water interfaces may become affected by pH.

- 3) **Oxidation potential:** - Cellular respiration consists of enzymically mediated electron transfers from an electron donor (reducing agent, in chemical parlance) to a terminal electron acceptor (oxidizing agent). Apart from a few rare cases where only one mole of electrons is transferred from each mole of reductant, this electron transfer almost always involves a number of intermediate electron transfer steps, which can be quite numerous if the original electron sources are complex molecules such as sugars. The thermodynamic driving force for these electron transfer processes is expressed quantitatively in terms of the oxidation potential, E_h (measured in volt), which is the Gibbs energy change divided by the number of moles of electrons transferred. According to the Nernst equation [$E=E_0-RT \{\ln k\}/nf$], this quantity depends logarithmically on the concentrations (strictly speaking, the activities; k) of not only the oxidized and reduced forms of the electron acceptor, but also of hydrogen ions and other species that might be involved. Thus, for aerobic respiration, the terminal electron acceptor is oxygen, which is reduced to water according to the overall equation



A particularly important electron acceptor in hydrocarbon reservoirs that are not supplied by surface water is sulfate:



Some organism can use ferric ions:



The oxidation potentials corresponding to the first two of these reactions depend on pH, while that of the third does not.

- 4) **Pressure:** The effects of pressure on microorganisms are closely associated with those of temperature, since elevated pressures in natural environments are always associated with

temperature variations. Specifically, the pressure in the ocean increases by about 10 MPa for every km of depth, while the temperature of the ocean is about 3 °C below about 100 m. On land, the pressure increases by about 3 MPa per km depth, but the temperature increases by about 25 °C per km. Thus, in terms of the earlier terminology introduced to describe the temperature tolerance of bacteria, a marine bacterium that thrives on the seafloor at a depth of 3 km would be a psychrophile, while its terrestrial counterpart at the same depth underground would be a thermophile. Growth rates of normal bacteria decrease to zero as hydrostatic pressure approaches about 40 MPa. The term 'barophilic' is used to describe bacteria whose growth rate is enhanced at elevated pressure. (The prefix 'baro-' is sometimes replaced by 'piezo-'.) It is also customary to refer to bacteria for which the diminution of growth rate commences at pressures above 40 MPa as 'piezotolerant'. A third class of bacteria, which cannot be grown under ambient conditions, are referred to as 'obligatory piezophiles'. Pressure tolerance is dependent on biophysical conditions present. The ability to grow at high pressure can be shown to depend on the energy source present, inorganic salts present, E_h , pH, and temperature. However, it is difficult in most cases to determine exactly which of these parameters is affecting growth, as they are all affected by pressure. Of particular interest with regards to oil reservoir is that salts such as NaCl, as well as divalent cations such as Mg^{2+} and Ca^{2+} , which are commonly found in oil reservoirs, can confer a greater barotolerance to some marine organisms.

- 5) **Lithology:** The solid phase of reservoir is composed of various rocks and minerals. Oil is predominantly found trapped in sedimentary rocks. Sandstones and carbonates (limestones and dolomite) compose the major classes of sedimentary rocks in which oil is found. Silicates and carbonates pose little restriction on microbial activity, the adsorptive capacity of clays and some other minerals contained within the porous rock can interfere with biological processes. Under proper condition of pH and ionic strength, clays and rock possess charges on their surface that act to adsorb bacteria and inhibit their migration through the porous medium. Montmorillonite clays exhibit the greatest ion exchange capacity, whereas kaolinites are least adsorptive. Illites have intermediate exchange capacity. Clay can also adsorb water resulting in their swelling, thereby restricting

microbial transport through the rock matrix. This swelling is reduced by presence of salts (NaCl, KCl, CaCl₂) found in most reservoir brine.

- 6) **Heavy metals:** Heavy metals are present in reservoir waters at ppm concentration. Despite the fact that quite frequently metals at such concentrations are not toxic to microorganisms, their presence may be detrimental to biological activity when other environmental stresses, like temperature or limitation in available molecular oxygen, are present. The responses to heavy metals by microorganisms may take a number of forms. Specific requirement for a supplementary substrate may arise and there may be a change in the mean generation time or in morphology of the organism. In some cases, detoxification processes involving metals operated by the cells are simple, like the reduction of sulfate to sulfide by the sulfate-reducing organisms and subsequent precipitation of the toxic metal.

3. Geobiology and Microbiologically Enhanced Oil Recovery

MEOR utilizes microorganism and their products to improve the recovery of crude from reservoir rocks. The microorganisms produce metabolites which are introduced into the reservoir to decrease interfacial tension in the oil-water-rock system, to improve mobility ratio of the reservoir fluids, or to induce preferential plugging of pore spaces to facilitate a better directional distribution of sweeping fluids. The selection of appropriate organism and their activities are independent of the environmental conditions in the reservoir under consideration.

The whole concept of microbiologically enhanced oil recovery can be divided into three parts:

1. Geology and mineralogy
2. Fluids
3. Biology

Any significant beneficial changes due to biological activity introduced into the geological and mineralogical component are limited, with the exception of some minor diagenetic changes in the mineral component.

Fluids, the second component of the system, can be subject to manipulation. Changes in the relative viscosities of the oil-water system, changes in the interfacial tension between water and oil, the composition of the aqueous phase and, to some extent the composition of the oil phase can be biologically altered.

The third component, biology, is the manipulator, through which the beneficial changes in the fluids may be achieved.

3.1 In-Situ Microbial Enhanced Oil Recovery

Three major biotechnological approaches may be contemplated:-

- Significant decrease in the interfacial tension of the water-oil system.
- Improvement in the mobility ratio.
- Selective plugging.

Before a specific organism can be selected for microbiologically enhanced oil recovery, the environment and the conditions under which it has to operate have to be known. This knowledge is necessary for the following reasons: Firstly, the biological activity of the organisms and their production of the required metabolite will be influenced by environmental conditions like temperature, pressure, availability of a suitable substrate, electrolyte concentration, and crude oil composition. Secondly, the effectiveness of the metabolite itself will be influenced by some if not all of the parameters just mentioned.

Interfacial tension

The reasons leading to chemically or biologically enhanced oil recovery using surfactants are obvious from the following example:

In water-wetted reservoirs, a substantial amount of the residual oil is located in the form of individual droplets and ganglions. Considering a droplet of oil residing in a pore throat 0.4 mm

long with end curvatures of $R_1=9 \times 10^{-3}$ mm and $R_2= 4 \times 10^{-2}$ mm, respectively, the water-oil interfacial tension, σ , being 30 mN m^{-1} , the pressure difference Δp can be determined using Laplace's equation:

$$\Delta p = 2\sigma \left(\frac{1}{R_1} - \frac{1}{R_2} \right)$$

The differential Δp required to move this oil droplet through its limiting pore would be 1-3 MPa. The practical limits achievable in the field are usually in the range of $20\text{-}30 \times 10^{-3}$ MPa. This example indicates clearly the necessity for an ultra-low interfacial tension of less than 10^{-2} mNm^{-1} to be achieved by the introduction of a surfactant, to obtain a significant enhancement in recovery.

Regardless of the surfactant formulation, to achieve a displacement of a residual oil the following criteria have to be satisfied:

- (1) The surfactant employed has to be capable of mobilizing residual oil.
- (2) The ability to displace oil must be maintained as the surfactant progresses from the injection point or the location of its biological production to the production well.
- (3) An optimal mobility relationship between the crude oil and the aqueous phase must be satisfied.

Since the amount of surfactant produced by an organism is finite in its concentration, consideration must be given to mechanisms rendering it ineffective. The major ones are:

- (1) Retention of the surfactant by the porous matrix.
- (2) Mixing and dilution of the surfactant with and by the reservoir fluids.
- (3) Partitioning of the surfactants between the oil and the aqueous phase.
- (4) Biological degradation.

To sustain a favorable mobility ratio, the viscosity of the surfactant solution should be in the vicinity of 8 cP to avoid viscous instabilities that could augment mixing of the surfactant with the reservoir fluids thus decreasing the miscibility. There is probably no single surfactant which would work satisfactorily in all reservoirs. This is simply because the operational situation varies from reservoir to reservoir, depending on the geological, mineralogical, and chemical

characteristics of the reservoir, its physical parameters, and the physicochemical composition of the reservoir fluids.

Surfactants

Surfactants can be divided into the following groups: anionic, cationic, amphoteric, and non-ionic. Biological activity can produce surfactants belonging individually to all of these groups:

- (1) Anionic: containing carboxylic acids.
- (2) Cationic: including amines and heterocycles.
- (3) Amphoteric: represented by amino acids and peptides.
- (4) Non-ionic: like esters.

The surfactants have different abilities to reduce interfacial tension and characteristics of crude oils depending on the composition (paraffinic, naphthenic, aromatic or mixed), the colloidal chemistry of the crude oil and, thus, on the presence of asphaltenes and resins. The efficiency of the surfactants depends on the environmental conditions in the reservoir like diffusion rates from the bacterial side to the oil-water interfaces. The diffusion coefficient of the surfactant depends on the viscosity of the reservoir fluids. The efficiency of the anionic and amphoteric surfactants is affected by the Ca^{2+} and Mg^{2+} cations in the reservoir waters.

The efficiency of the surfactant is also affected by the lithology of the reservoir rock. Different rocks adsorb surfactant to a different degree. The ability of clays to adsorb surfactants decreases in the following order: cationic, non-ionic, anionic compounds. Silicates show slight adsorption of non-ionic surfactants, but adsorb strongly cationic surfactants. Therefore, cationic surfactants should not be applied to silicate-rich reservoirs. For enhanced oil recovery, a surface adsorption of a surfactant of $0.5 \times 10^{-4} \text{ mg cm}^{-2}$ on quartz is generally considered as an acceptable level. A surfactant to be acceptable to MEOR should demonstrate as many as possible of the following characteristics:

- (1) Concentration enrichment at the oil-water interface and, thus, biological production of a surfactant at the oil-water interface. This would be a great advantage.
- (2) Ability to permanently lower the interfacial tension below $10^{12} \text{ mN m}^{-1}$
- (3) Partial solubility in oil.
- (4) Capability of stabilizing oil-water emulsions.
- (5) Solubility or at least dispersibility in highly saline reservoir waters.

- (6) Low adsorption coefficient relative to the reservoir rock.

3.2 Microbiology of Reservoirs

The origin of microbial populations in reservoirs is probably due to the introduction of the organisms from the surface by reservoir waters. Such a source of the reservoir population is quite feasible despite the long periods of time required for the organisms to travel from their place of origin. The surface water usually contains some components capable of supporting bacterial life. A great variety of organisms are usually found in reservoir including the genera *Bacillus*, *Pseudomonas*, *Micrococcus*, *Mycobacterium*, *Clostridium* and some *Enterobacteriaceae*. Microorganisms were present at all points of collection with a maximum concentration of sulfate reducers at the oil-water contact area. This location coincided with maximum H₂S production, thus suggesting that the organisms used some components of the crude oil for their activity. Biological activity of sulfate reducers and the subsequent production of H₂S results in the souring of oil with a consequent corrosion of plant equipment. Sulfate reducers are found in environments which may differ considerably in temperature. Thermophilic organisms and mesophiles are quite common among sulfate reducers. Salt tolerance is quite a common characteristic of sulfate reducers. The end product of the sulfate reduction is sulfide ion which has an equilibrium Eh (at 15°C and pH 7) in the region of -320 mV. In such a medium, free oxygen can exist only at very low concentration and only anaerobic respiratory processes should take place. Sulfate reducers are not true autotrophs as they require organic material for their growth.

Inhibition of sulfate reducers is required. Air is the cheapest inhibitor of sulfate-reducing organisms, but its practical application is limited. The introduction of sufficient oxygen into a reservoir may induce biological and abiological formation of ferric hydroxide and thus potentially plug the reservoir rock. Furthermore, the sulfate reducers are not killed by oxygen just inhibited; therefore, reintroduction of anaerobic conditions or formation of anaerobic micro-environments would result in renewed production of H₂S. It is evident that a variety of organisms are able to grow under reservoir conditions. This observation is of great significance to MEOR, because any introduced microorganism will have to compete with the endogenous bacterial population. As it may be necessary, to introduce an additional substrate to the reservoir in order

to facilitate the required biological activity, a possible "bloom" of the endogenous population may create problems.

When considering the MEOR techniques, one may pose the following question: Why the biological activity is apparently low in reservoirs where endogenous bacterial populations have been detected? The reasons may be numerous. Conditions in oil reservoirs are usually anaerobic or at their best just microaerobic. So far, no conclusive evidence has been offered that microorganisms can use hydrocarbons as a substrate under such conditions. Readily available carbon, therefore, may be one of the limiting factors. This aspect is discussed later. Furthermore, phosphorus is usually present at concentrations below 0.05 ppm, thus possibly becoming a limiting factor. Practically no data are available from oilfields on the form and concentration of nitrogen in reservoir waters. Its low concentration may be the reason for the low-density endogenous bacterial population. It is envisaged that both elements may need to be supplied into the reservoir before a satisfactory MEOR activity will be achieved.

3.3 Physicochemical aspects of microbial ecology as related to microbial enhanced oil recovery

Knowledge of the behavior of microorganisms within microhabitats is essential for the definition of their activity in different ecosystems. One of the microhabitats which is important to microorganisms is an interface. An interface may be defined in physicochemical terms as the boundary between two phases in a heterogeneous system. In the reservoir, interfaces exist between oil and water, fluids and gases, fluids and solids, and solids and gases. The gases may be gaseous hydrocarbons or CO₂. The fluids are crude oil and water. Solid surfaces are provided by rock or the bacterial cell.

Interfaces

Microbial activity near surfaces is affected by micro environmental changes like pH variation, resulting from the attraction and the repulsion of H⁺ ion, concentration of substrates and inhibitors, and the availability of gases. Microorganisms with hydrophobic surfaces have tendency to accumulate at the water-oil interfaces. Should such organisms produce a surfactant; their location would be of significant advantage to MEOR as it would be produced at the site

where it is needed. Despite their average size being larger than generally accepted for colloidal particles, bacteria in free suspension behave as colloids in an aqueous colloidal suspension. Bacterial cells possessing both hydrophobic and hydrophilic surfaces may behave similar to surfactant molecules in an aqueous suspension. At low concentration, they are singularly distributed through the medium. When the concentration of the cells reaches a critical value, they cluster and form rosettes with their hydrophobic parts closely attached inside the rosette, while the hydrophilic parts are facing the aqueous environment. Under such a condition, microorganisms behave similar to surfactants molecules. Such a situation is called Critical Micelles Concentration, a parameter important in enhanced oil recovery.

During the MEOR processes, it is essential that microorganisms remain as "colloidal" suspension to obtain the maximum penetration of the cells into reservoir spaces and not to decrease the permeability of the rock. Suspension of microorganisms is facilitated by repulsive forces due to like electrostatic charges on their surfaces. Low electrolyte concentration in the reservoir waters, therefore, is more favorable to MEOR, as high concentration could in some cases facilitate the formation of bacterial aggregates and diminish the penetrating capacity of the bacterial population. Microorganisms reach interfaces by Brownian movement, mobility, buoyancy, currents in the interstitial fluids, or movement of the surfaces themselves. Microorganisms prefer surfaces of high interfacial tension. When interface between two immiscible liquids (e.g., water and oil) moves, it sweeps bacterial masses ahead into the aqueous phase. With films of low interfacial tension, the phase boundary may be stretched into a peninsular projection, which may break off and leave droplets of water containing bacteria in the organic phase. All or some of the above processes may affect the MEOR by influencing the distribution of microorganisms.

Substrate

One of the parameters to be considered in the assessment of a given reservoir for MEOR is the possibility that the substrate introduced to support the bacterial growth may be adsorbed onto the walls of the pores. Such adsorption will depend on the mineralogy, chemistry and ionic state of the rock surface, and the molecular structure and concentration of the organic matter.

Many natural habitats have low nutritional status. Solid surfaces capable of absorbing organic matter are potential sites of nutrient concentration and, therefore, sites of intensified microbial activities. A paucity in substrate availability through the aqueous phase and its concentration on pore surfaces could result in localized formation of biomass and may, thus, cause a decrease in

the reservoir rock permeability. If the substrate is adsorbed, its availability to the organisms will depend on the substrate location relative to the organisms, on the presence or absence of extracellular enzymes, the tendency of the enzymes to be adsorbed, and the configuration of the enzyme and the substrate in the adsorbed state. Adsorption of bacteria and chemical compounds on clays is affected by cations present in the reservoir waters. Sodium saturation of clays increases their adsorption capacity, whereas calcium decreases it. The adsorption is further affected by pH, and the ionic strength of the electrolyte present. Thus it is evident that plugging and obstruction of interstitial spaces can be affected by a large number of parameters associated with bacterial population in a reservoir.

4. Microbial Plugging in Enhanced Oil Recovery

Although bacteria have long been recognized as agents of formation damage by those involved in the production of oil, the importance of bacterial plugging in water flooding operations is generally underrated. This is due partly to the fact that early laboratory investigations concentrated on the plugging effects of suspensions of dead cells and failed to systematically evaluate the more damaging effects resulting from the metabolism of the viable microorganisms found in the field.

In this section, an exploration of the microbial plugging problems associated with conventional water flooding operations and evaluate the mechanisms responsible are explained. Then, the characteristics required of bacterial MEOR cultures for their successful injection and examine the microbial problems anticipated during other EOR operations such as polymer flooding is also mentioned here.

4.1 Bacteria in water flooding injection water

In the secondary production of oil, water is injected under pressure into the producing formation via designated injection wells in such a way that oil underground is displaced through the formation to production wells. The quality of water used in this operation varies from field to field and may include fresh water from wells or surface sources, produced water or, in some locations, sea water. In some instances, poor water quality results in plugging of the injection well at the formation face. This loss of permeability in the wellbore region results in a loss of "injectivity" (a decline in the rate of injection), which can seriously jeopardize the overall production of oil. This problem is not confined to water flooding but is of general importance in all types of enhanced oil production (polymer flooding, alkaline flooding, micellar or microemulsion flooding, etc.), which rely on an injected "pusher" phase to displace the oil from the reservoir.

Different types of microbes found in injection water are: - Molds, Yeasts, Algae, Diatoms, Slime-forming bacteria, Sulfate-reducing bacteria, Iron bacteria, Aerobes, Anaerobes.

They all are potentially able to cause plugging. The sulfate-reducing bacteria are a group of obligate anaerobes, which reduce sulfate to sulfide in a manner analogous to that in which aerobes reduce oxygen to water during aerobic metabolism. The production of copious quantities of sulfide results in a health hazard from H₂S release, problems of corrosion, and potential plugging through the precipitation of iron sulfide minerals. The iron bacteria can potentially cause plugging through the precipitation of iron oxides often in association with microbial slime. The slime-forming bacteria found have been implicated in the plugging of sand packs and soils.

4.2 Mechanism of Microbial Plugging

Particulate plugging

Bacterial cells alone can cause permeability reduction by plugging and filter cake formation. The importance of particulate plugging, however, depends on the ratio of the average throat size of the pores in the formation to the size of the bacterial cells or their aggregates (chains, clusters, etc.) as shown in figure 5. Thus, in very tight formations even singly dispersed bacterial cells

could cause an injectivity problem, whereas in more permeable formations, only larger aggregates of cells would be of concern.

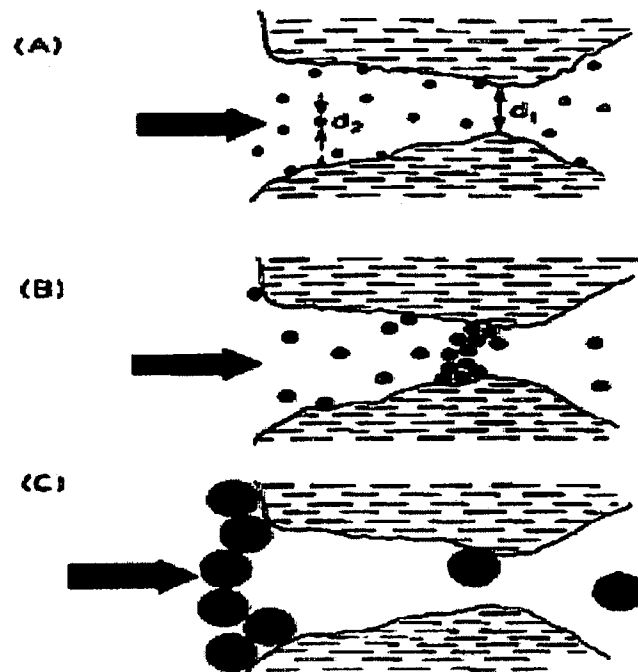


Fig 5. Particulate plugging categorized into three distinct phases

Plugging due to biofilm formation

Most bacteria have natural tendency to grow and attached to the rock surfaces rather than free floating in liquid surface. In petroleum reservoir bacteria may attach to the rock, start to grow and then produce exopolymers – sugars – that help them attach to each other and rock surfaces. Such growth is termed as biofilm and offers the advantages of protection from biocides while encouraging the bacteria to interact he best use nutrients and other resources. Bacteria that are introduces to reservoir through water flooding will flow over preexisting biofilm; some bacteria will attach themselves to these biofilms and grow. From time to time, some bacteria detach from the biofilm and move with the liquid flow or by their own motility and colonize other areas deeper in the reservoir.

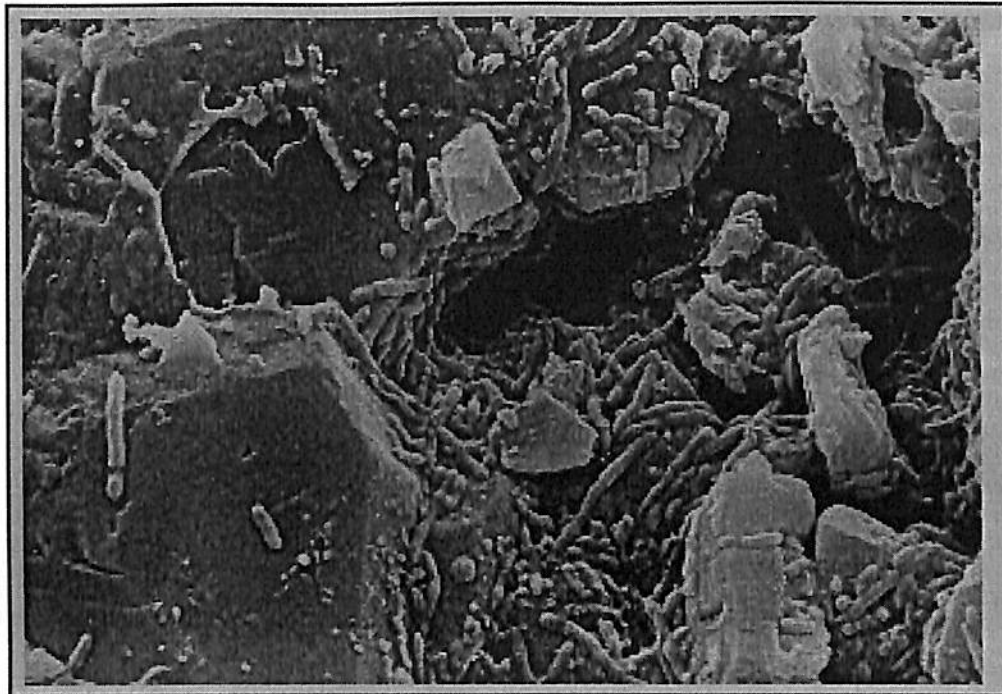


Fig 6. Electron micrograph of biofilm inside rock. The blocking of pores by bacteria can clearly be seen.

4.3 Demonstrations of mechanisms of microbial plugging

Fig7. A). Low-magnification scanning electron microscope image of the thick filter cake on top of the model core. B) High-magnification image of the filter cake showing it to be a collection of chains of cells

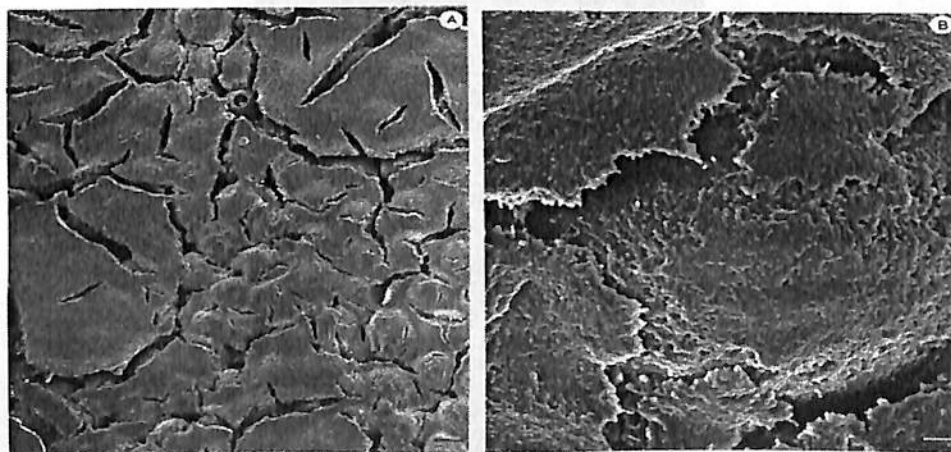


Fig 8. High-magnification scanning electron microscope image of bacterial cells inside the model glass bead core below the filter cake. No exopolymer (slime) production is evident

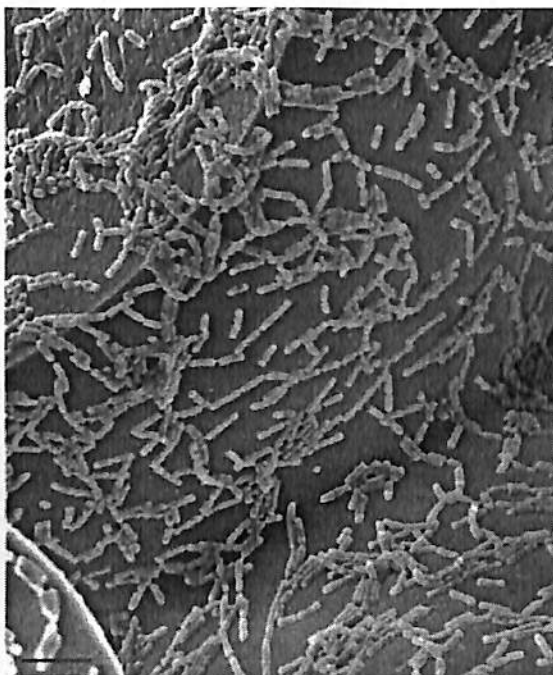


Fig 9. High-magnification scanning electron microscope image of the mixed population of bacteria plugging the model core

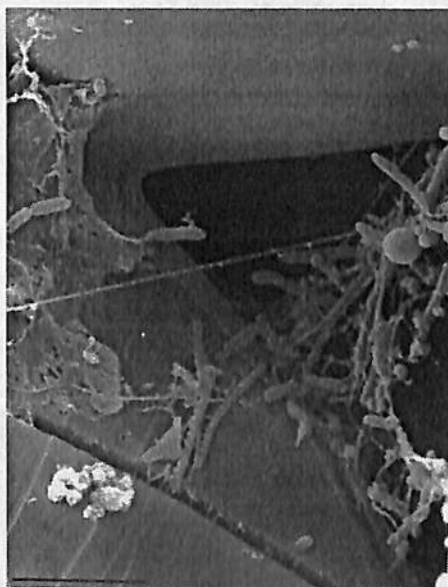


Fig 10. Low-magnification scanning electron microscope image showing extensive polysaccharide (slime) production by the live cells of *Pseudomonas*

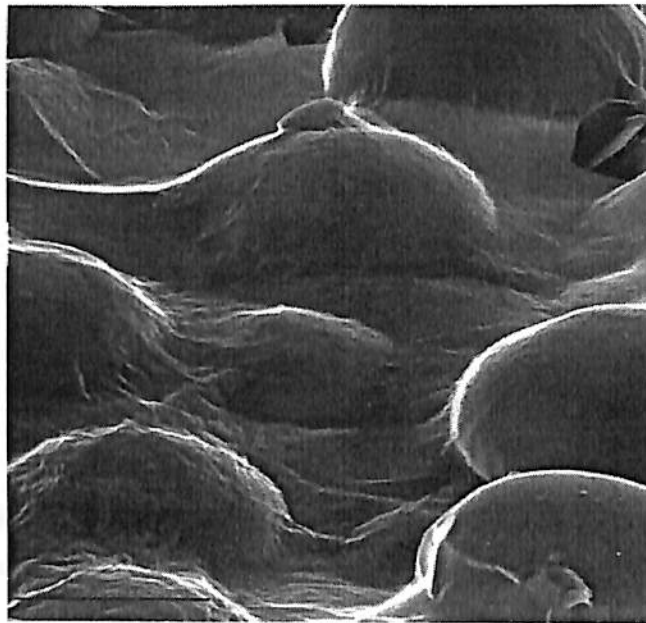
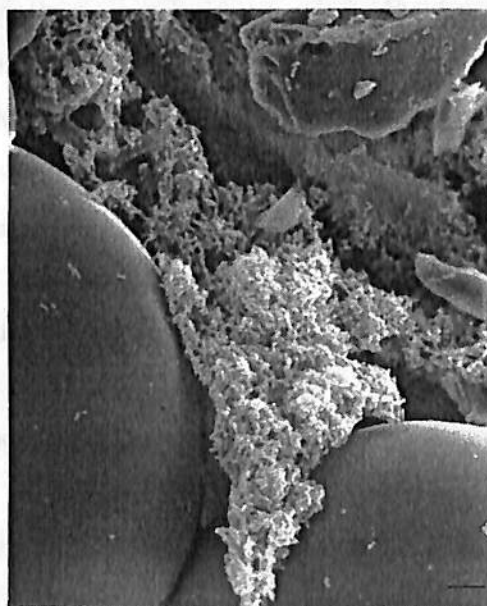


Fig 11. High-magnification scanning electron microscope image of the clumps of dead cells of *Pseudomonas* which cause particulate plugging



4.4 Implications

Waterflooding operations

Except where special precautions such as biocide treatment are taken, it is apparent that live bacteria will enter the formation face via the injection well. This is true even for apparently clear waters taken from freshwater wells. It is also apparent that bacteria can plug even very permeable structures having a large average pore throat size. Two mechanisms of plugging can be distinguished: (1) particulate plugging by the microbial cells themselves, and (2) viable bacterial plugging through biofilm formation. By either mechanism, plugging is concentrated in the formation face immediately adjacent to the wellbore. In particulate plugging, this is due to the retention of aggregates or clumps of cells and possibly other debris, to which bacteria are attached, in the near-wellbore zone. In the case of biofilms, the continual introduction of oxygen and fresh nutrients in the injection waters favors those bacteria which attach and proliferate in this zone. The relative importance of these two mechanisms is a function of pore throat size distribution, with tighter formations being more sensitive to particulate plugging. Biocide treatment of the injection waters inhibits the establishment of biofilms in a new injection well, but may not be effective in older wells where biofilm development is already extensive

MEOR

Based on the above-discussed examples, the following criteria are suggested for viable bacterial cultures being developed as MEOR agents. Successful injection requires the following:

- (1) Bacteria must be of a size appropriate to the underground formation; preferably, as small as possible and singly dispersed. The size depends on the pore throat size distribution in the formation face.
- (2) Bacteria must not actively produce extracellular polysaccharides or other exopolymers even if these are soluble.
- (3) Bacteria should not adhere to the rock surface and form biofilms through glycocalyx production.
- (4) The culture must not be capable of generating gas bubbles in the formation face during injection, because these will occupy pore space and in effect reduce the permeability of the formation to the aqueous phase.

Polymer flooding operations

The initial problem to be faced in a polymer flooding operation is the proliferation of polymer-degrading bacteria in surface handling facilities and the transfer of these potentially troublesome organisms into the injection well. Commercial EOR polymers contain combinations of biocides to prevent bacterial growth, but it must be clearly established that bacterial proliferation does not occur following dilution of the concentrated material with water of doubtful quality. Most polymer flood EOR operations make use of water injection wells that have been used extensively in secondary waterflood operations. Thus, one must assume that the boreholes and proximal formations of these wells are already heavily fouled by bacteria that have developed very extensive biofilms. Because these pre-existing populations may adsorb the polymer into their biofilms, and many act as a bacterial reservoir to "seed" the initial moving polymer "front" with bacteria, a fastidious clean-up of proposed polymer injection wells would seem to be indicated.

Xanthan gum is indeed degraded to form low-viscosity fragments, within the formation, but that an overall "push" on the oil-bearing stratum has sometimes been achieved. These unconfirmed and unpublished data raise the very interesting possibility that refractory nutrients and bacteria may be carried into the "fingering" zones, responsible for water breakthrough in secondary recovery with subsequent plugging of these water channels through bacterial growth and biofilm development. To initiate EOR using degradable biopolymers without firm evidence of the long-term control of bacterial activity is naive in the extreme, because the basic precepts of microbial ecology dictate that a complex series of population changes will be "set in train" by the introduction of a potential nutrient into the formation. Given the high cost of polymer flooding, careful laboratory study using the polymer, the formation water, and the formation matrix would seem to be required prior to field application.

5. Case Studies

5.1 A Three – Dimensional Numerical Simulator for Microbial Enhance Oil Recovery

5.1.1 Mathematical formulation

The mathematical formulation is written to describe multiphase flow through porous media. The flow equation for oil, water and gas as follows:

Water:

$$\left[\frac{kk_{rw}}{\mu_w B_w} \Phi_w \right] + q_w = \frac{\partial}{\partial t} \left[\phi \frac{S_w}{B_w} \right] . \quad (1)$$

Oil:

$$\left[\frac{kk_{ro}}{\mu_o B_o} \Phi_o \right] + q_o = \frac{\partial}{\partial t} \left[\phi \frac{S_o}{B_o} \right] . \quad (2)$$

Gas:

$$\begin{aligned} & \left[\frac{kk_{rg}}{\mu_g B_g} \Phi_g + \frac{R_{sw}kk_{rw}}{\mu_w B_w} \Phi_w + \frac{R_{so}kk_{ro}}{\mu_w B_w} \Phi_o \right] \\ & + q_g + q_w R_{sw} + q_o R_{so} \\ & = \frac{\partial}{\partial t} \left[\phi \frac{R_{sw}S_w}{\mu_w B_w} + \phi \frac{R_{so}S_o}{\mu_o B_o} + \phi \frac{S_g}{B_g} \right] . \quad (3) \end{aligned}$$

The bacterial transportation can be described by the following equation:

$$\left[\frac{C_{wb}kk_{rw}}{\mu_w B_w} \Phi_w \right] + q_w C_{wb} = \frac{\partial(\phi S_w \rho_w C_{wb} + \sigma)}{\partial t} . \quad (4)$$

The bacterial capture kinetics is given by following equation:

$$\frac{\partial \sigma_{np}}{\partial t} = -\alpha(u_{np} - u_c)\sigma_{np} + \beta C, \quad (5)$$

$$\frac{\partial \sigma_p}{\partial t} = -(\delta + \rho\sigma_p)u_p C, \quad (6)$$

and

$$\phi_i \sigma = \phi_i f \sigma_p + (1 - f)\sigma_{np}, \quad (7)$$

Where volumetric flux densities in the pluggable and no pluggable pathways are related through respective permeabilities:

$$\frac{u_p}{u} = \frac{k_p(\sigma_p)}{k_p(\sigma_p) + k_{np}(\sigma_{np})}, \quad (8)$$

Where 'u' denotes volumetric flux density in any given direction. Permeability damage is expressed through the following empirical relationship:

$$k_p \approx k_{pi} e^{-a\sigma_p^4}, \quad (9)$$

and

$$k_{np} \approx \frac{k_{npi}}{1 + \varepsilon\sigma_{np}}. \quad (10)$$

In the nutrient transport, there are several considerations. Very often, carbon and mineral sources are required to support the growth and metabolic activities of bacteria. It is assumed that blocking of porous channel takes place by exponential growth of bacteria. This is given by:

$$C_t = C_o e^{\mu t}, \quad (11)$$

Where C_t is the concentration at time t and C_o is the initial concentration.

Reservoir simulation runs were conducted in order to simulate following cases:

1. Bacterial growth and plugging through biomass.
2. IFT- reduction surfactant generation.

3. Oil viscosity – reducing surfactant generation.
4. CO₂ generation.

5.1.2 Result of Simulator run

Bacterial Plugging

The first series of numerical simulations run was conducted using single phase fluid. Both injection and production rates were assumed to be 50 m³ for all these cases. This was done to investigate the extent of bacterial plugging in a three-dimensional case. The use of single phase enabled one to compare overall permeability variation for different cases. Bacterial plugging may occur by two means:

1. Shear multiplication of the number of bacteria.
2. The generation of polymer in situ.

In the present work, the first case is modeled. Results of reservoir modeling are compared with that predicted on a linear core. These results are extrapolated on the basis of pore volumes of fluid injected. Figure 12 shows that comparison. All these runs are conducted assuming 20% pore volume of bacteria injected, followed by nutrient injection and then by chase water. First, note the difference between the results of one – dimensional and three – dimensional modeling. All the parameters except dimensionality were kept constant for these two cases.

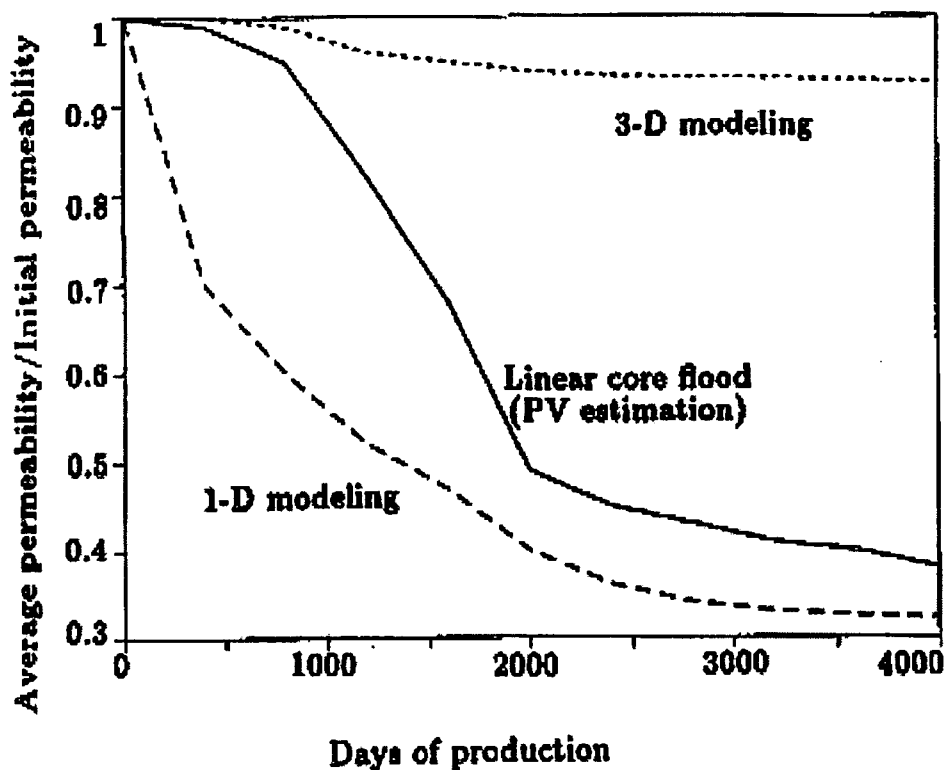


Fig.12 Comparison of permeability reduction due to bacteria injection

In one dimensional analysis, the bacterial plugging occurs quickly in the first block and reduces the permeability to such an extent that further fluid injection leads to continuing bacterial build-up in first few blocks. This reduces overall permeability drastically.

In three dimensional modeling, the problem of local plugging is overcome and overall permeability reduction is much lower than what is obtained by one dimensional modeling. Also, three dimension modeling correctly tracks the path of nutrients, which do not necessarily follow that of bacteria.

The effect of in situ polymer generation is modeled by simply using higher growth rate of bacteria. Figure 13 shows the effects of $\mu_m = 6.5/\text{hr}$ on permeability loss.

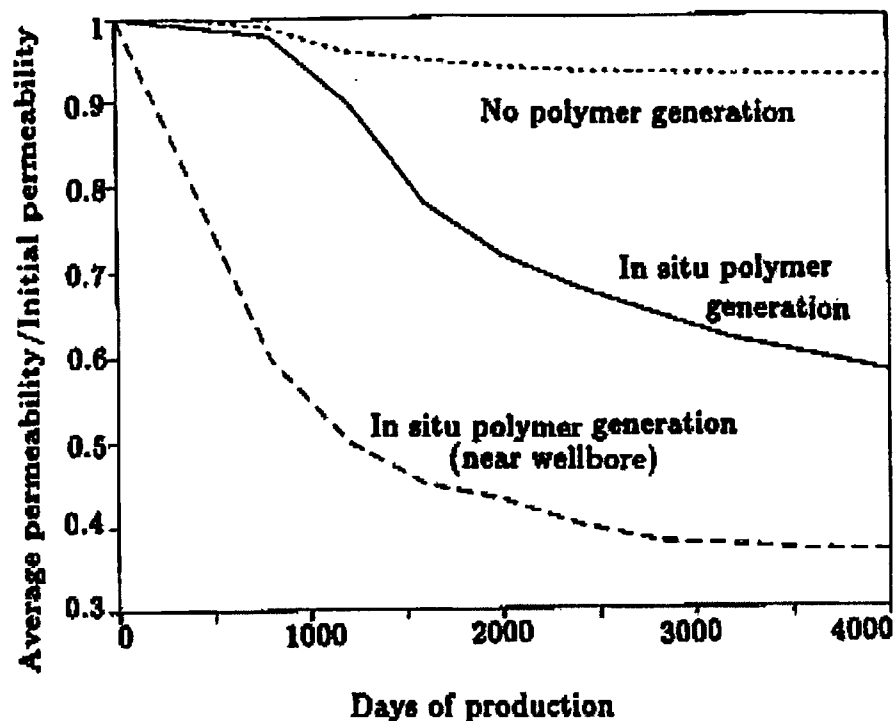


Fig.13 Comparison of permeability reduction due to bacteria injection and polymer generation

As can be seen from this figure, substantial permeability loss is observed for this case. This effect is more intense for the near well bore permeability reduction. A low near well bore permeability may lead to injectivity problem in a field situation.

The effect slug size of bacteria solution on permeability reduction for the case of polymer generation is shown in Figure 14. A 5% slug does not appear to give any appreciable permeability reduction. In a three dimensional case, 5% bacteria solution leads to a relatively small surface area to be in contact with the nutrient solution, which follows the bacteria. Consequently, nutrients find only a limited amount of bacteria in order to trigger bacterial growth and consequent plugging.

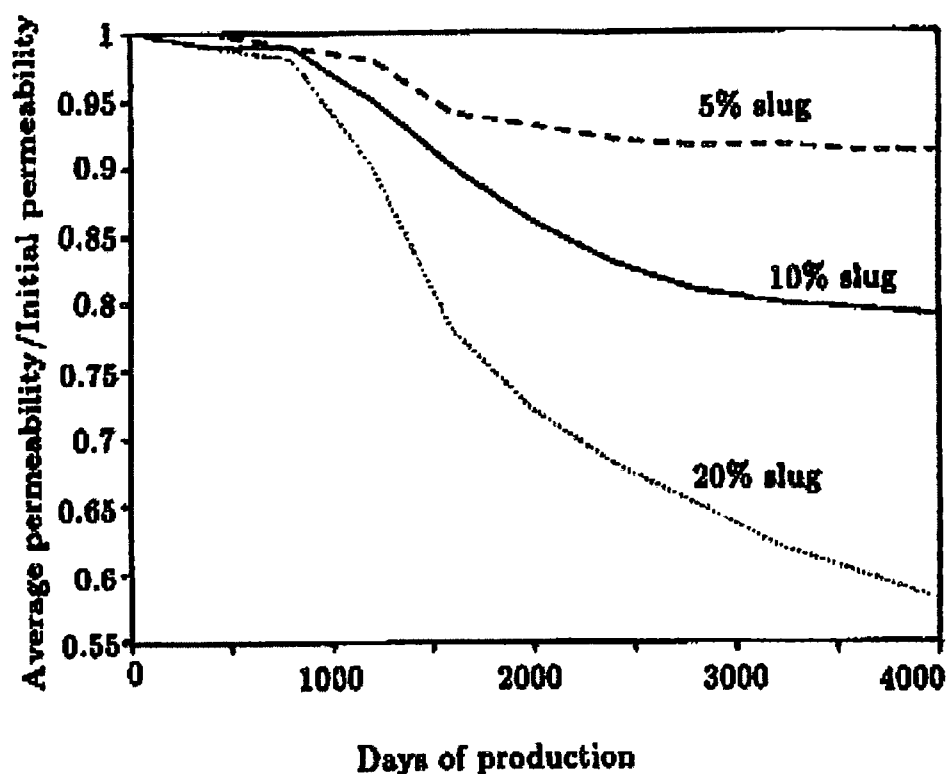


Fig.14 Effect of slug size on permeability reduction due to polymer generation

IFT Reduction with Bacteria

In order to model bacteria-generated surfactant flood, it is assumed that interfacial tension (IFT) as a function bacteria concentration. Figure 15 shows the IFT vs bacteria concentration curve. Also, the relative permeability curves were related to IFT values in the following manner:

$$k_{ro} = k_{ro}(S_o) + [S_o - k_{ro}] \frac{\sigma_{max} - \sigma(C_b)}{\sigma_{max}} \quad (12)$$

$$k_{rw} = k_{rw}(S_w) + [S_w - k_{rw}] \frac{\sigma_{max} - \sigma(C_b)}{\sigma_{max}} \quad (13)$$

In this formulation, it is assumed that the relative permeabilities to water and oil are straight line (extending from 0 to 1) when the IFT, s , is zero. As IFT approaches 0, these relative permeability curves approach straight line forms.

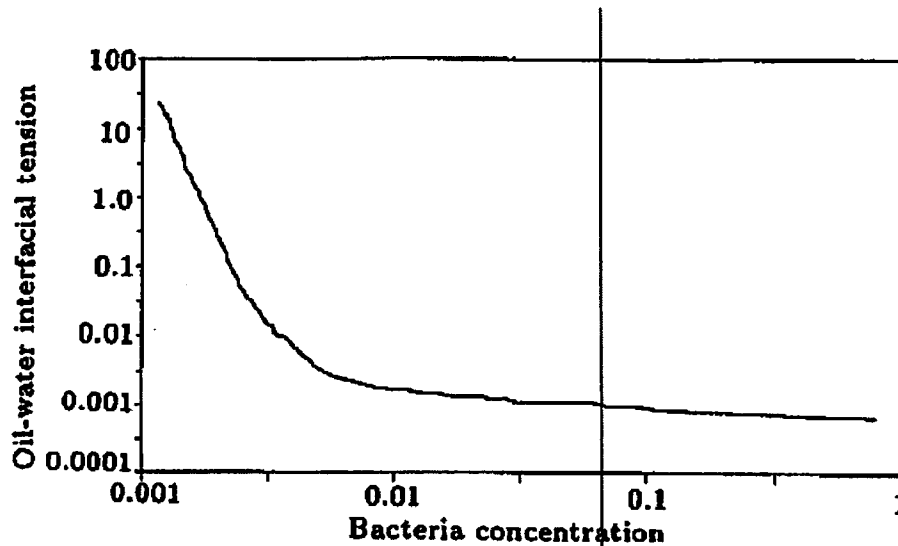


Fig.15 Correlation of bacteria concentration vs. oil-water interface tension.

Also, following capillary pressure curves were used in order to incorporate dependence of the capillary pressure on $s(C_b)$:

$$p_c[\sigma(C_b)] = p_1 p_c(\sigma_{max}, S_w) \cdot [\sigma(C_b) / \sigma_{max}] , \quad (14)$$

Where, p_c becomes 0 if s is 0.

Initial reservoir conditions and saturations are given in Table 2. In order to compare results, a base case of water flooding was carried out. Similar to the field practice, surfactant – generating bacteria were injected following water flooding when the oil cut was less than 5%.

Table2. Reservoir model parameters

Grid block in x-direction	20
Grid block in y-direction	20
Grid block in z-direction	10

Porosity	30%
Permeability, x-direction	$5\mu\text{m}^2$
Permeability, y-direction	$5\mu\text{m}^2$
Permeability, z-direction	$1\mu\text{m}^2$
Oil viscosity	10 mPa.s
Water viscosity	1 mPa.s
Oil/Water IFT	30 dynes/cm
S_{wi}	40%

Such a low oil cut was obtained after 2400 days, at which time bacteria injection was initiated. In order to minimize the effect of bacterial plugging, the deposition was assumed to be 0.002/cm, a value 5 times smaller than that of plugging bacteria. The injection rate was kept constant at 50 m³/ day for all the cases. The production well was operated at a total production constraint of 50 m³/ day. For this case, a 20% pore volume of bacteria solution was injected. This was followed by nutrient injection. Unlike the plugging case, nutrient injection was carried out throughout the final phase of water injection. This ensured bacterial growth and surfactant generation in an uninterrupted fashion. Results of water flood and bacteria injection are compared in Figure 16.

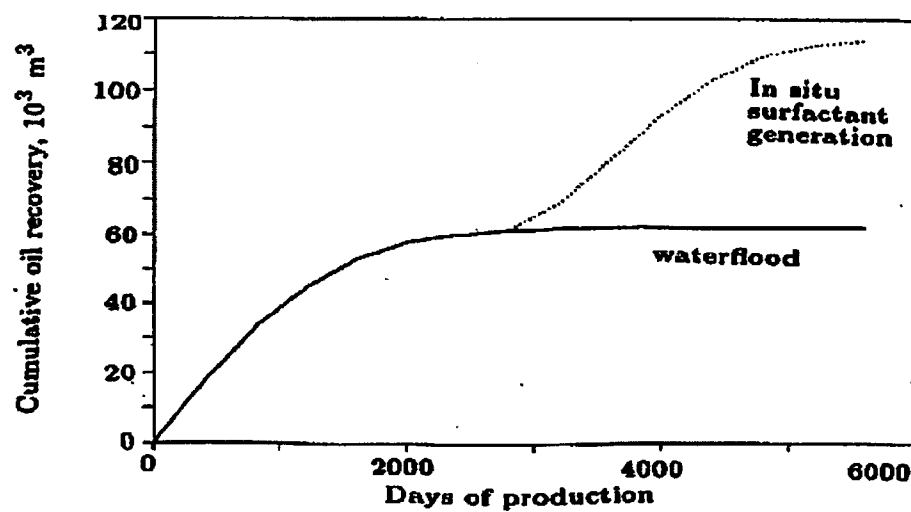


Fig.16 Comparison of oil recovery between bacteria- generated surfactant flood and water flood.

Note that there is no improvement with bacteria for over 3000 days of injection. This delay is expected, since bacteria concentration has to increase substantially away from the injection well to mobilize oil near the production well. However, as the oil mobilization takes place, oil cut increases rapidly. This leads to an incremental oil recovery of some 50.10 m^3 . This is an improvement of 80% over a conventional water flood. Whereas, the exact amount of incremental oil recovered will depend largely on the nature of IFT vs. bacteria concentration, this clearly shows that surfactant-producing bacteria can be used as an improved water flooding technique.

A final run of surfactant – generating bacteria was conducted in huff and puff mode. Only one well was used for this particular run. Bacteria were injected at a rate of $50 \text{ m}^3/\text{day}$ for 10 days, followed by nutrient injection at $50 \text{ m}^3/\text{day}$ for 10 days. Following this the well was closed for 20 days prior to production. Similar to the previous case, post water flood conditions were used as the initial conditions for the bacteria injection. The first cycle gave an average oil cut of 60% for 20 days for a total of 600 m^3 of oil. Several cycles of operation were conducted. As the process progressed, the oil cut decrease gradually, after 5 cycles, a total of $14,500 \text{ m}^3$ of oil was produced. This result was shown in Figure 17.

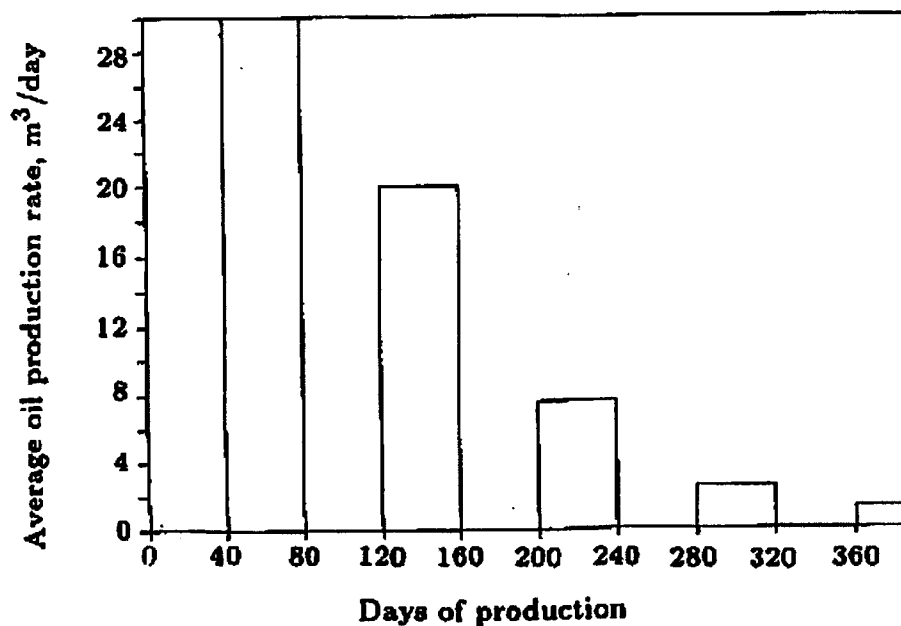


Fig.17 Oil flow rate of huff and puff with bacteria – generated surfactant.

These results indicate that even though huff and puff has much quicker response time than does the drive process, total incremental oil recovery is considerably smaller for this case.

Viscosity - Reducing Bacteria

An oil viscosity of 50 mPa.s was used for this study. This viscosity can be decreased considerably in the presence of bacteria. However, no conclusive experiment has been conducted to find out how oil viscosity correlates with bacterial concentrations. By analogy to solvent flood, a μ_o vs. bacteria concentration curve, as in Figure 18, is proposed for present study. An improved prediction may be done by providing experimental curves for μ_o vs. bacterial concentration.

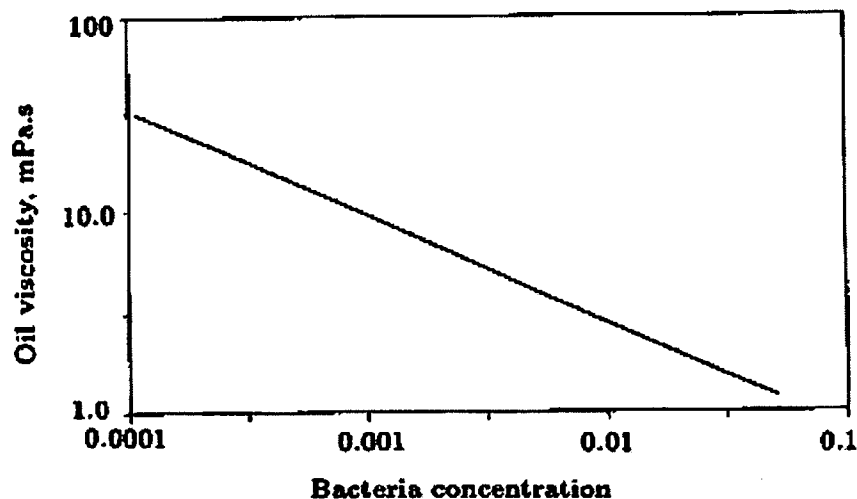


Fig.18 Correlation of oil viscosity vs. bacteria concentration

Figure 19 compares recovery results of viscosity – reducing bacteria with that of water flooding. The main difference between the two curves is the delay in water breakthrough for the case of bacteria injection.

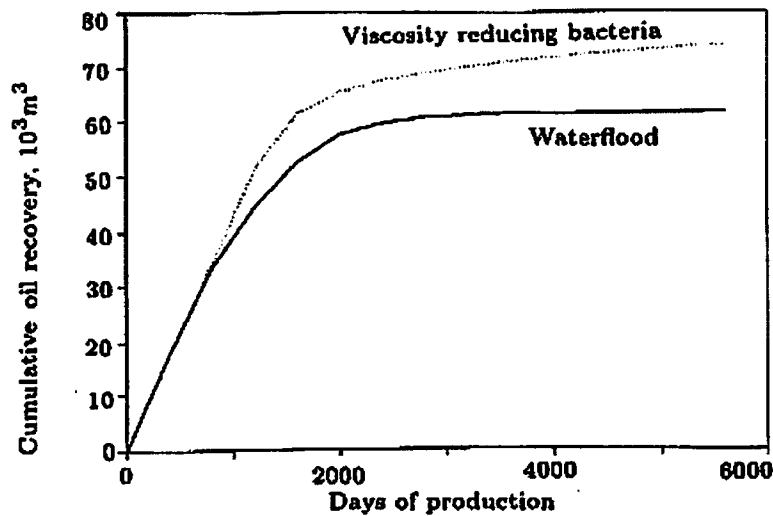


Fig.19 Comparison of oil recovery between viscosity reducing bacteria and water flooding

Following water breakthrough, oil recovery declines in a way similar to the water flood case. However, during this process, close to $15,000 \text{ m}^3$ of additional oil is recovered. This improvement is much less than that shown during bacteria- generated surfactant flood, even though this is an indication that decreasing IFT is a better way to recover residual oil. Two factors of uncertainty are involved here. The first factor is the nature of IFT reduction or oil viscosity reduction is not known. The second factor is the presence of moderately viscous oil limits the benefits of oil viscosity reduction. It would probably be more appropriate to compare results with very high – viscosity oil for which a drastic decrease in oil viscosity would lead to great improvement over a water flood.

CO₂ - Generating Bacteria

The final run was conducted to simulate performance of CO₂- generating bacteria. Once again correlation between bacteria and CO₂ volume was assumed. Figure 20 such a correlation.

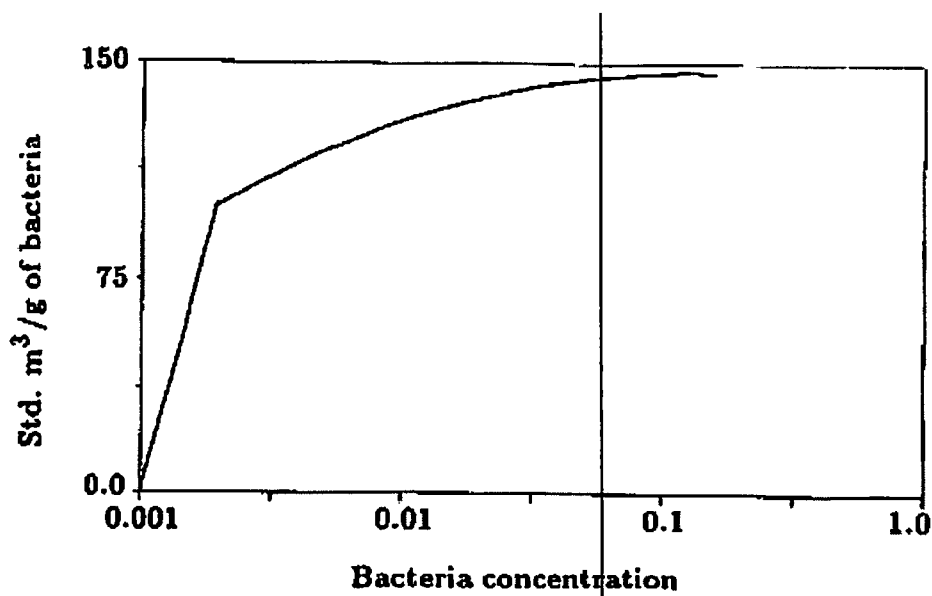


Fig.20 Correlation of CO₂ generation vs. bacterial concentration

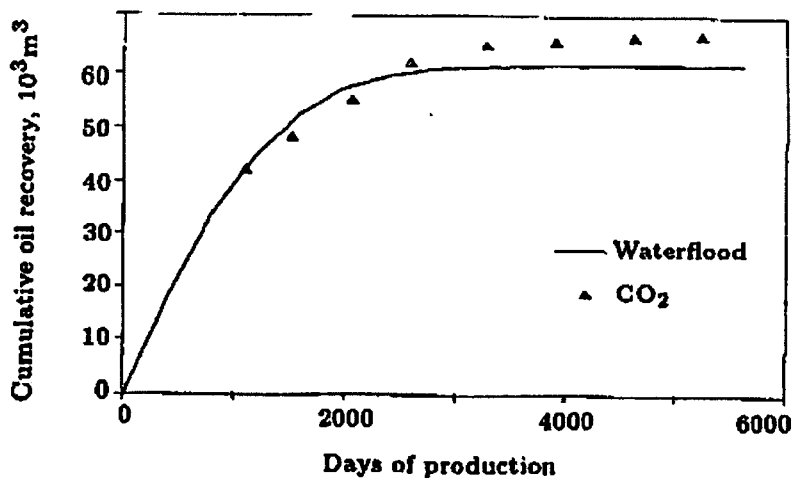


Fig.21 comparison between bacterial generated CO₂ injection and water flood

The nature of this curve will determine the oil recovery performance. CO₂ generation was simulated by a source term in each block where the bacteria concentration is higher than 0.0001. In order to eliminate the numerical problem the appearance of gas phase, a continuous gas phase was assumed to be existent in the reservoir (at 15%). A R_s value of 50 m³/ m³ was used throughout. This value is reasonable for heavy oil reservoir at 300 psi as in the present case. Figure 21 compares results of CO₂ generation with water flooding. Bacteria injection was carried

out 20% pore volume of the slug and was followed by continuous nutrient injection. This led to increasing bacteria population and the generation of large amount of CO₂. However, as Figure 20 shows, results of CO₂ generation were not very encouraging. It appears that while CO₂ is beneficial for pressurizing the reservoir, it is detrimental to oil flow, much of which is restricted by presence of a third phase. In any case, the bacteria - generated CO₂ appears to recover as much oil as an immiscible CO₂ and water would.

5.2 Effect of Temperature and Salinity on MEOR

5.2.1 Introduction

The present work aimed at investigating the effect of temperature and salinity on oil recovery by employing two types of bacteria: *Bacillus subtilis* (PTCC 1365), *Bacillus licheniformis* (PTCC 1595). Oil recovery experiments were performed in the sand pack column and sucrose was used as nutrient. The column was mounted horizontally under 15 bars during shut-in period (3 days). The temperature and salinity range from 40°C to 70°C and 0 to 10000 ppm NaCl respectively. Results show that for both of the bacteria which were used in this investigation at different salinities the microbial recovery efficiency decreased with increasing the temperature. The maximum recovery for *B. subtilis* was 27% of water flood residual oil saturation at 40°C and 0% of salinity while for *B. licheniformis* was occurred (33.9%) at 40°C and 5% NaCl. In the present paper, the effect of temperature and salinity on oil recovery by bacteria was investigated in sand pack column. The final goal of our study is to identify the best condition of each bacterium for MEOR processes.

5.2.2 Material

MICROORGANISMS:

Bacillus subtilis (PTCC 1365) and *Bacillus licheniformis* (PTCC 1595) were preferred to use in this investigation.

OIL:

A non-detergent motor oil (°API = 21) was used in the experiments

5.2.3 Experimental procedure

The column was filled and packed with the sterile quartz sand and flushed with CO₂ and five pore volume of brine (pH ~ 7) were passed through the column then column was flooded with oil until residual water saturation was reached. The column then flooded with the same brine until no more oil was observed in the effluent. The flow rate was 10 ml/min. 0.2 PV of bacterial solution (5×10^8 cells/ml) was injected to the column followed by 0.2 PV of nutrient (2% sucrose, 0.01% (NH₄)₂HPO₄). The column was mounted horizontally at constant temperature for 3 days (shut-in period) under nitrogen pressure (15 bars). After shut-in period the column was flooded with the same brine and at the same flow rate until oil production was complete. Oil viscosity was measured before and after shut-in period.

5.2.4 Results

Effect of Temperature:

Effect of temperature for each bacterium observed at different temperatures is illustrated in Fig. 22, 23.

B. subtilis (especially at low concentration of salinity)

It is shown that recovery at different salinities decreased with increasing the temperature from 40°C to 70°C for all of the bacteria.

B. licheniformis (especially at high concentration of salinity)

It shows a great potential at 40°C for MEOR processes and their abilities to releasing the oil at 55°C are relatively acceptable.

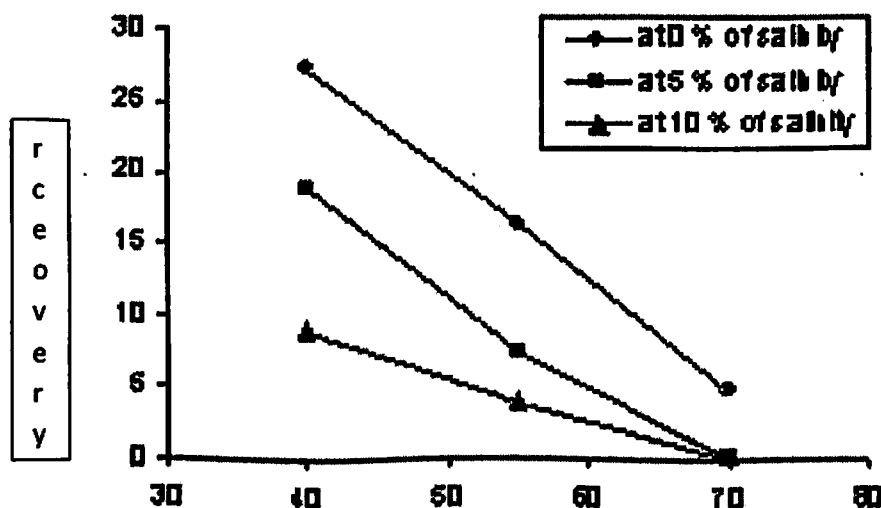


Fig.22 Effect of Temperature on recovery for Bacillus Subtilis

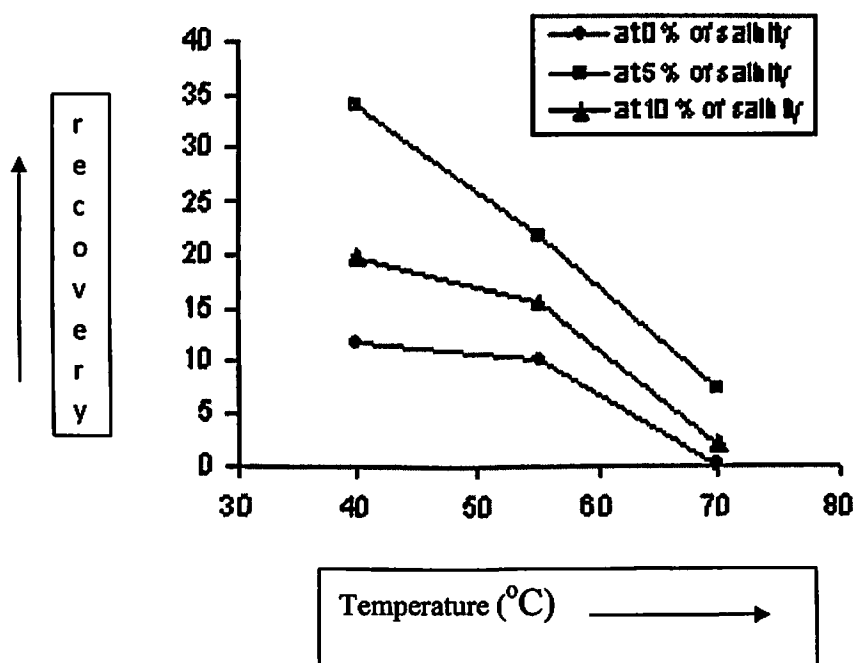


Fig.23 Effect of Temperature on recovery for Bacillus Licheniformis

Effect of Salinity:

The effects of salinity on at different temperatures for both of the bacteria were given in Fig. 24, 25. The figures illustrate that the microbial recovery for *B. subtilis* decreases with increasing the salinity from 0 to 10 % NaCl. But the maximum microbial recovery for *B. licheniformis* was achieved at 5% of salinity.

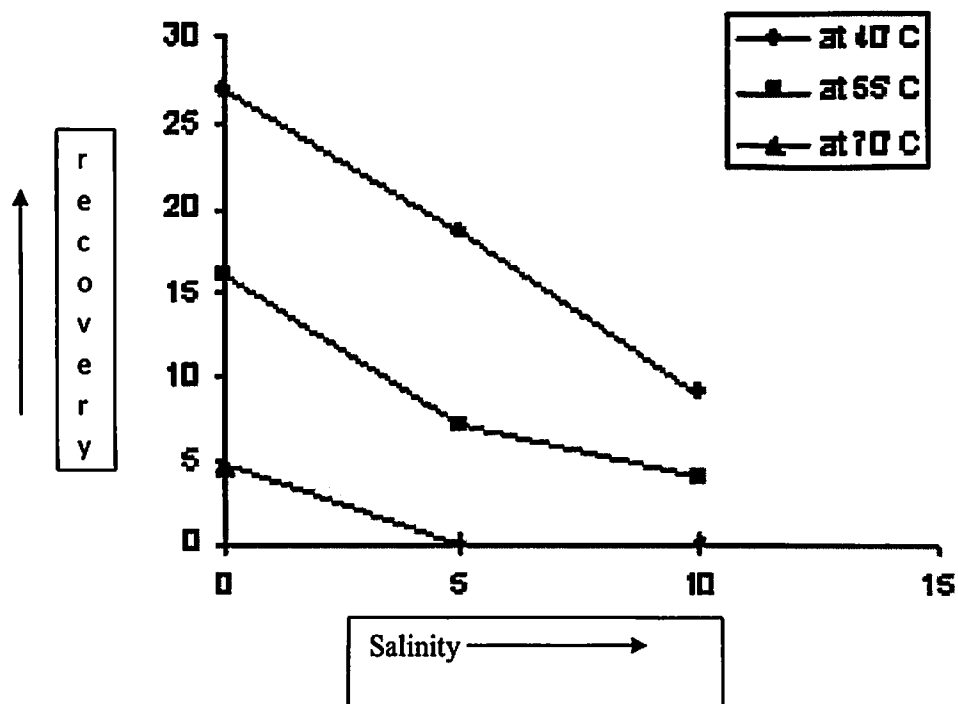


Fig.24 Effect of Salinity on recovery for Bacillus Subtilis

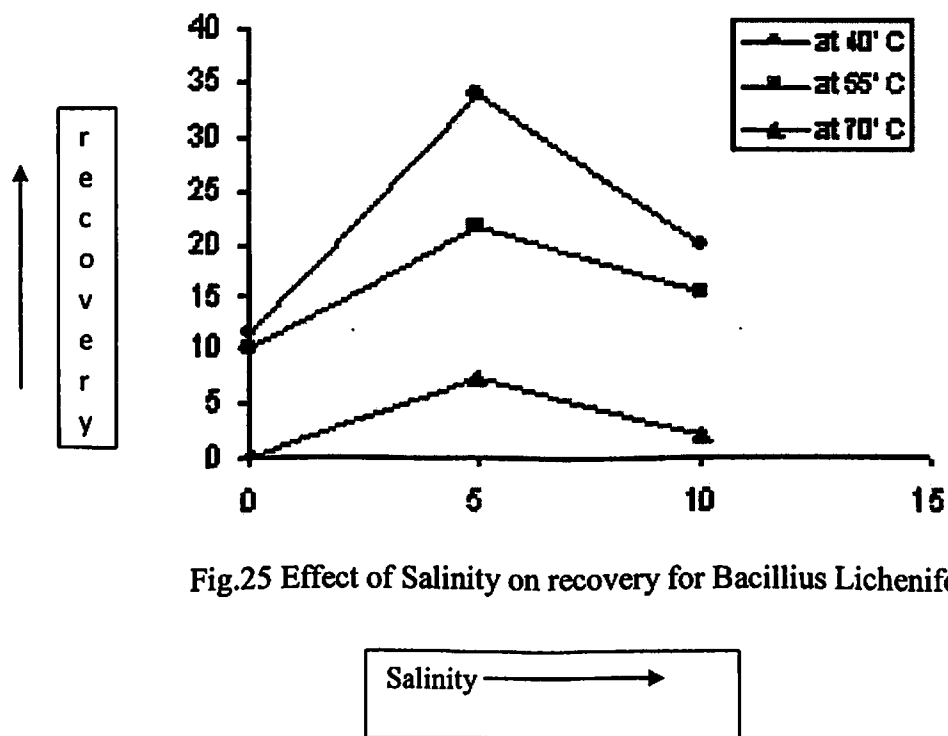


Fig.25 Effect of Salinity on recovery for Bacillus Licheniformis

5.2.5 Conclusion:

Several conclusions can be drawn from the results:

- ❖ The microbial recovery efficiency for *Bacillus subtilis* decreased with increasing temperature and salinity from 40°C to 70°C and from 0% to 10% NaCl respectively. Hence this bacterium can be more practical in reservoirs with low concentration of salt.
- ❖ The microbial recovery efficiency for *Bacillus licheniformis* decreased with increasing temperature from 40°C to 70°C while its maximum takes place at 5% NaCl, making it suitable for microbial enhanced oil recovery in relatively high salty reservoirs.

Experimental data is shown in Table 3.

Table 3: Experimental data

Ex No	Bacteria	Temp (°C)	Salinity (w/v, NaCl)	?	K _{abs} (D)	S _{ooip} (%) PV	S _{orwf} (%) PV	Water flood oil recovery (% PV)	S _{ormf} (%) PV	E _r (%) PV	μ _r %
1	B. Subtilis	40	0	33.0	33.7	80.1	56.4	29.6	41.1	27.0	26.6
2	B. Subtilis	40	5	33.7	31.1	78.0	56.9	27.0	46.3	18.5	15.9
3	B. Subtilis	40	10	36.6	37.2	79.3	57.8	27.1	52.5	9.0	8.0
4	B. Subtilis	55	0	34.5	44.6	71.1	46.9	34.0	39.3	16.1	19.3
5	B. Subtilis	55	5	34.5	35.1	69.3	43.5	37.1	40.3	7.2	-
6	B. Subtilis	55	10	35.8	39.7	69.7	44.0	36.8	42.2	3.9	-
7	B. Subtilis	70	0	36.6	41.9	66.1	35.5	46.3	33.8	4.7	-
8	B. Subtilis	70	5	35.8	43.8	61.9	33.7	44.7	33.7	0	-
9	B. Subtilis	70	10	35.0	34.0	63.9	35.1	45.1	35.1	0	-

10	B.Licheni.	40	0	37.0	31.5	83.2	58.0	30.2	51.2	11.6	11.8
11	B.Licheni.	40	5	36.6	39.1	79.9	59.0	26.1	40.0	33.9	31.8
12	B.Licheni.	40	10	35.8	43.0	78.5	57.3	27.0	45.9	19.8	22.5
13	B.Licheni.	55	0	36.6	32.1	68.4	43.9	35.8	39.5	10.0	4.3
14	B.Licheni.	55	5	34.5	37.6	70.1	45.4	35.2	35.5	21.7	18.9
15	B.Licheni.	55	10	34.0	42.0	70.8	44.2	37.5	37.3	15.5	9.6
16	B.Licheni.	70	0	35.8	41.7	63.8	37.0	41.9	37.0	0	-
17	B.Licheni.	70	5	37.0	39.7	61.6	34.0	44.8	31.5	7.2	-
18	B.Licheni.	70	10	34.5	33.1	64.5	36.5	43.3	35.7	2	-

Where;

S_{ooip} = original oil in place

S_{orwf} = Residual oil saturation after water flooding

S_{ormf} = Residual oil saturation after microbial flooding

μ_{bm} = Oil recovery before shut in period

μ_{am} = Oil recovery after shut in period

E_r = Microbial recovery efficiency;

$$E_r = \frac{S_{ormf} - S_{orwf}}{S_{orwf}} \times 100$$

μ_r = Viscosity reduction;

$$\mu_r = \frac{\mu_{Em} - \mu_{Em}}{\mu_{Em}} \times 100$$

5.3 Microbial EOR Laboratory Studies and Application Results in Daqing Oilfield

5.3.1 Introduction

Microbe inoculation of Daqing crude samples in the laboratory reduced oil viscosity, indicating microbe effectiveness. Field application of the same strains of microbes resulted in a thousand-fold increase in both concentration and total number of microbes in the water produced from the reservoir. The data proves existence of active, thriving microbe colonies in the reservoir in a manner not previously reported, compatibility between the microbes and Daqing oil in the reservoir, and propagation in the reservoir. The microbe detection method is reviewed along with microbe growing details in four wells. Overall field results in twenty-five wells are described; proving microbial EOR (MEOR) is suitable for use in Daqing Oilfield.

Mechanisms by which MEOR Microbes can improve oil recovery as reported in the case study are:-

1. Generating gases that increase reservoir pressure and reduce oil viscosity,
2. Generating acids that dissolve rock and improve absolute permeability,
3. Reducing permeability in channels thereby improving displacement conformance (With injected water microbes move into highly permeable channels and pores. Permeability is reduced with gas, metabolic products and aggregation of microbe body mass),
4. Altering wettability,
5. Producing bio-surfactants by metabolizing hydrocarbons that decrease interfacial tension, and
6. Reducing oil viscosity by degrading long-chain saturated hydrocarbons.

For Daqing reservoirs and the microbes used in this project, items 5 and 6 are considered the most significant mechanisms for residual oil reduction and production increase. The commercially available microbe products used in this

project are mixtures of several strains of facultative anaerobic microbes for improving oil properties and recovery. The microbes are 1 to 4 microns in length, and 0.1 to 0.3 microns in diameter. They are motile and can migrate into reservoir pores. Metabolic products are organic acids, ethanol and surfactants as well as decomposition of long-chain hydrocarbons into solvents. The products are mixed cultures of microbes that are able to metabolize hydrocarbons and produce beneficial byproducts. The MEOR process is that microbes convert the reservoir into a bioreactor. Unmovable residual oil and water in the reservoir provide the media for microbe propagation and migration. No external nutrients are supplied.

5.3.2 Laboratory Screening Test

Oil and water samples from 30 wells were tested to determine the reduction in viscosity caused by the microbes. Viscosity was reduced by more than twenty percent on all but four wells (wells 15-24, 15-233, 16-232 and 17-24 of Production (Division 6). Table 4 lists the 25 wells on which inoculation tests were done, which tested effective and were chosen for the pilot study. The microbes significantly reduced the average viscosity for all of these wells:

- 13 wells of Production Division 2 were reduced by 63.1%,
- 8 wells of Production Division 3 were reduced by 39.5%, and
- 4 wells of Production Division 6 were reduced by 43.7%.

The laboratory studies indicate that the microbes are effective at reducing oil viscosity which enhances the oil mobility by increasing short chain hydrocarbons through degrading long-chain normal alkanes.

Table 4: Viscosity Lab Testing and Production Performance Before and After MEOR Treating

Well No.	Visc. Red. in lab %	Before Treatment			After treatment									Cumulative Toas of Oil	
		Base			After 1st. Treatment			After 2nd. Treatment			After 3rd. Treatment				
		Fluid t/d	Oil t/d	Water cut %	Fluid t/d	Oil t/d	Water cut %	Fluid t/d	Oil t/d	Water cut %	Fluid t/d	Oil t/d	Water cut %		
S3-1-B22	51.8	5	1	79.2	29	7	77	29	7	77	33	7	79.6	1400	
S3-3-24	61	26	5	76	49	11	77.5	34	9	77.5	41	10	75.5	676	
S4-20-424	82.5	31	6	79	32	7	76	23	4	82	23	5	76.5	300	
S3-50-625	76	8	4	54	7	3	68	10	4	68	12	6	55	144	
S4-10-423	44.9	42	17	59	68	29	62	68	21	62	68	21	62	127	
S3-2-124	85	38	12	68.6	39	8	78.4	42	15	62	46	12	73.5	111	
S4-D1-118	80.6	7	4	87	10	5	56	10	6	50	12	5	58.4	64	
S3-1-B20	42.0	5	3	31	4	2	77.9	3	2	50	3	2	47	1	
S3-1-B21	38.9	4	1	77.9	6	1	89	4	1	78.5	4	1	75.4	0	
S3-51-622	80.2	17	7	58	23	10	60.1	18	7	67	22	8	64.6	-36	
S4-20-423	36.5	44	6	86	42	8	81	42	8	80	44	7	84.5	-46	
S3-50-623	83.2	15	6	58	12	6	54	9	3	64	8	3	65	-196	
S4-21-523	57.4	23	3	87	27	1	97	27	1	97	25	2	90	-196	
S 4-J1-120	.	17	9	16	20	17	13							641	542
S 3-2-125	.	37	11	68.8	45	14	69								200
S 3-J5-319	.	12	7	38	11	7	36								28
S 3-J3-119	.	25	9	64.2	29	10	65								-12
N5-9-C63	33.6	4	2	50	7	4	42.8	10	6	42	16	11	34		
N5-9-C64	38.9	31	3	90.32	21	1	95	25	2	90	33	2	94.3		
N5-8-C62	31	1	0	100	2	1	50	1	1	30	2	2	24		
N5-9-C66	50.5	8	1	87.5	10	1	90	11	1	90	10	1	92.8		
N5-9-C65	35.3	7	5	28.6	9	7	22	13	8	42	4	3	24		
N5-9-C68	31	18	2	88.9	11	1	90	11	1	90	23	2	92.6		
N5-10-C64	42.3	27	3	88.9	33	3	93.3	26	2	91.6	33	2	92.8		
N4-1-C64	53.1	42	5	88.1	36	4	89	28	3	91	40	4	91		
N 4-2-C73	21.3	15	3	80.0	10	2	82							0	
La 78	24.5	4	2	41	4	2	38							0	
N 5-9-C67	22.9	11	6	48.0	11	5	50							-111	
N 5-9-C69	21.1	12	4	69.2	11	3	73							-100	
14-231	45.7	14	1	89.7	14	4	74.5	12	3	73.4	15	4	75	254	
16-24	30.1	34	2	92.7	30	4	85.6	60	6	89.8	40	4	90.7	248	
14-251	46.5	23	2	90.7	17	2	85.7	19	2	94.4	25	2	92.4	-42	
15-232	52.4	23	2	92.8	49	5	89	24	3	88	18	2	91.4	-51	
Total		539	153		728	195		559	126		600	128		3305	

5.3.3 Microbe Concentrations in Produced Water

For effective MEOR field treating, the microbes are squeezed down hole into the near well-bore vicinity of the reservoir to facilitate better migration, colonization and propagation outward into the producing formation. The MEOR squeezes were performed down the casing-tubing annulus periodically with enough shut-in time after each treatment for microbes to become established and begin growing before resuming rod pumping. The treatments were approximately one month apart, with three days of shut-in time after the first treatment and one day after subsequent treatments. Table 5 presents living microbes concentrations before and after MEOR for five treatments on 4 different wells in Production Division 6 of Daqing Oil Field. Table 5 illustrates that living microbe concentrations increased a thousand-fold. Maximum microbe concentrations in the produced water at the wellhead were 10^3 to 10^4 cfu/ml above pre-MEOR levels, averaging more than 1000 for the four wells. The increase indicates favorable reservoir conditions for microbe growth and propagation. This excellent result which is indicative of a thriving microbe colony in the reservoir provides confirmation that sufficient microbes are active to reduce oil viscosity, resulting in higher oil flow rates and enhancing oil recovery. Figure 26 and related tables show that:

1. The concentration of living microbes increased 1000 times in produced water.
2. The total number of living microbes increased over 1,000 times. The concentration in the microbe-laden treating water was 104 to 105 cfu/ml, but concentrations reached 107 in produced water. The number of microbes squeezed into the formation was calculated by multiplying the treatment volume by the microbe concentration. The number of produced living microbes was calculated similarly, based on the 24 tons of produced fluid per hour at an average water cut of 90% over 46 days. The produced microbes totaled 1035 times more than the number squeezed into the reservoir.

Table 5: Number of Living Microbes in Produced Water (cfu/ml)

Colony forming units per milliliter	Daqing Oilfield, Production Division 6			
	Well 14-231	Well 14-251	Well 15-232	Well 16-24
Before MEOR Treating	4.3x10 ³		1.7x10 ³	2.7x10 ³
Days After Well Returned to Production				
After First MEOR Treatment				
1	1.4x10 ⁴	6.0x10 ⁴	0.7x10 ⁶	3.0x10 ⁴
2	Shut-in	2.0x10 ⁵	1.4x10 ⁶	2.0x10 ⁵
3	Shut-in	4.9x10 ⁵	1.4x10 ⁶	4.9x10 ⁵
5	Shut-in	Shut-in	1.7x10 ⁷	1.1x10 ⁶
8	5.0x10 ⁶	Shut-in	0.9x10 ⁷	1.2x10 ⁶
13	1.2x10 ⁶	1.7x10 ⁴	0.4x10 ⁵	1.1x10 ⁵
16	1.1x10 ⁴		1.7x10 ³	2.0x10 ³
20	1.1x10 ³	1.0x10 ³	1.7x10 ³	1.0x10 ³
23	0.9x10 ³		1.0x10 ³	4.9x10 ⁴
After Second MEOR Treatment				
1	0.9x10 ³	1.2x10 ⁴	1.1x10 ³	1.4x10 ³
2	1.1x10 ⁵	1.0x10 ⁵	0.8x10 ³	1.2x10 ⁴
3	1.2x10 ⁶	4.5x10 ⁶	1.7x10 ⁷	1.1x10 ⁵
5	2.0x10 ⁵	Shut-in	1.0x10 ⁵	1.2x10 ⁴
8		Shut-in		1.2x10 ⁴
12	1.0x10 ⁴	2.2x10 ⁵	0.7x10 ³	1.1x10 ⁵
15	0.8x10 ⁷	2.0x10 ⁷	1.0x10 ⁷	1.0x10 ⁶
After Third MEOR Treatment				
1	Flushing		0.2x10 ³	0.3x10 ³
2	Flushing		0.3x10 ³	0.4x10 ³
3	0.3x10 ⁶		0.4x10 ³	0.3x10 ³
7	1.1x10 ⁶		3.5x10 ³	1.6x10 ⁶
10	2.0x10 ⁵		1.1x10 ⁴	1.3x10 ⁶
14	0.7x10 ⁵		3.1x10 ³	1.5x10 ⁶
16	4.3x10 ³		2.5x10 ⁴	Shut-in

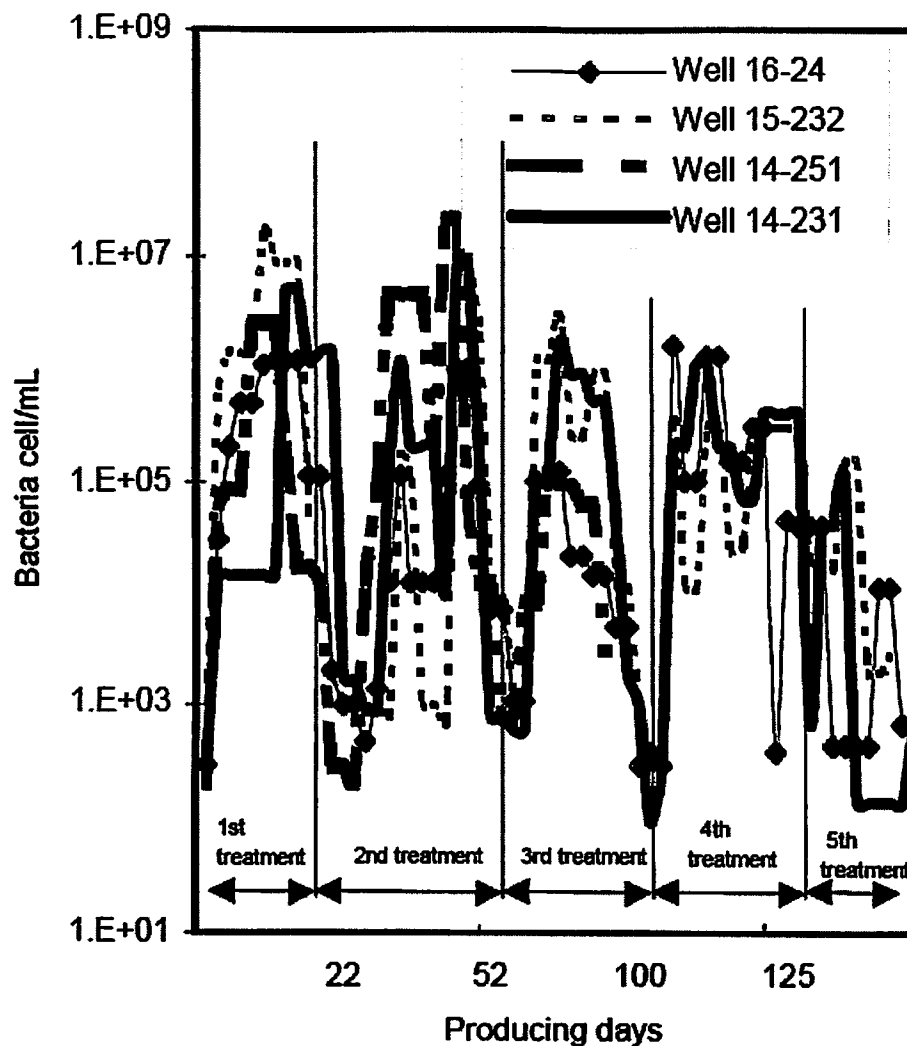


Fig.26 Bacterial Concentration of Produced Water of MEOR Test Well

The presence of a growing microbe colony as proven by this data and analysis indicates Daqing Oilfield chemical and physical reservoir environmental conditions are favorable for these microbes to live and propagate.

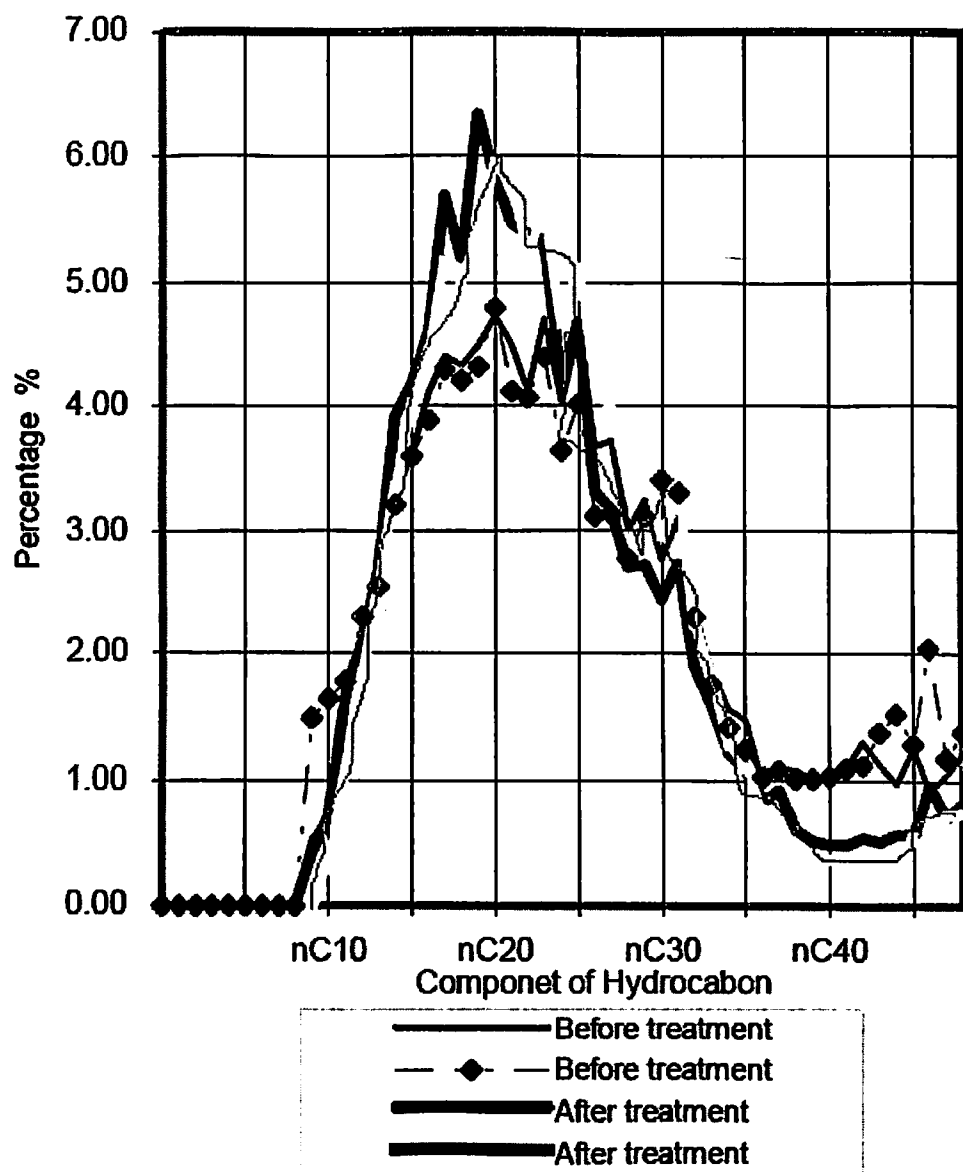


Fig.27 Chromatogram of Saturated Hydrocarbon of Well 14-231

Laboratory tests were done to measure microbial induced changes in the produced oil after the start of MEOR. Samples from treated wells showed wellhead oil viscosity was reduced by 25% as compared to before MEOR treatment. Well 14-231 and 16-24 show reduced viscosity of 23.2% and 25.4% respectively. Well 14-231 was also tested by comparing chromatography of normal alkane hydrocarbons in the wellhead oil samples before and after the treatment. Figure 27 shows that long-chain alkanes (C36 to C48) were reduced by 49.3%, while short chain alkane

(C14 to C24) increased by 21.5%. The microbe bioreactor altered the composition of the crude as it was being produced.

5.3.4 MEOR Squeeze Treatment Effectiveness

Individual well response to MEOR treatments is varied. For some wells oil production clearly increased. After the first or second period of microbe injection, produced fluid increased, water cut decreased and oil production increased for these wells: S 3-1-B22, S 3-3-24, S 4-20-424, S 4-D1-118, S 3-1-B20, N 5-8-C62, N 5-9-C63, N 5-9-C65, 14-231, 16-24 and 14-251. Total oil production increased 1796 tons for these eleven wells.

From Table 4 it is apparent that while some wells increased in oil production, others decreased. On many of these wells, factors other than microbes affected production, such as offset water injection changes and oil well production mechanical system problems. Any MEOR increase was obscured. Total oil production decreased 1300 tons for the 12 wells. Wells with oil production decreases fall into three categories:

- 1) Changes in offset water injection and mechanical problems.
 - a) S 4-10-423 of Production Division 2 was fractured and rod-pump parameters were adjusted. The offset injection rate dropped to $15 \text{ m}^3/\text{d}$ from $95 \text{ m}^3/\text{d}$. This well had a high production rate before MEOR. After MEOR, the MEOR production increase was obscured by the decreasing trend due to decreased offset water injection. The well decreased 159 tons.
 - b) N 5-10-C64 and well N 4-1-C64 in Production Division 3 decreased 267 tons due to decreases in offset water injection.
 - c) S 3-50-623 in Production Division 2 and 15-232 in Production Division 6 had leaking rod pumps for a time after MEOR treating. They decreased 316 tons of oil.
- 2) High water cut wells. Some of the reservoirs have been under water flood for a long period, and a great deal of oil has been produced over the past thirty years. The remaining oil saturation on these wells is very low, masking microbes, effectiveness.
- 3) Low reservoir productivity combined with high water cut, such as in S 3-1-C21, S 3-1-C20, and N 5-9-C66.

From the above analysis 938 tons, or most of the 1300 tons of the decreased oil production for 10 wells resulted from reservoir (decreased offset water injection) and mechanical (rod pump) problems.

5.3.5 Conclusions

- 1) MEOR has been effective on 17 of 25 oil wells in Daqing Oil Field and over three thousand tons of cumulative incremental oil has been achieved.
- 2) Living microbe concentrations in the produced water reached approximately the same level as in the microbe products. The concentration is one thousand times that in the diluted microbial products injected. Reservoir environmental conditions in Daqing Oilfield are favorable for growth of these strains of microbes.
- 3) These strains of microbes have obvious effects on produced oil composition, degrading long-chain normal alkanes and reducing viscosity.

NOMENCLATURE

a	dimensionless constant
B	formation volume factor
C	concentration
f	fraction of pore spaces containing pluggable pathways
k	absolute permeability
k_{ri}	relative permeability of phase i
p_i	pressure of phase i
q_i	injection rate of phase i
R_{si}	solution gas ration of phase i
S_i	saturation of phase i
t	time
u	volume flux density
u_c	critical volume flux density
a	constant, $1/cm$
β	constant, $1/s$
d	constant, $1/cm$
e	dimensionless constant
μ_i	viscosity of phase i
μ_m	maximum bacterial growth rate
$?$	constant, $1/cm$

- ρ_i density of phase i
- s volume of fine deposits per unit initial pore volume
- ϕ porosity

Subscripts

- b bacterial
- c critical condition
- i initial condition
- n nutrient
- o oil phase
- np non plugging path ways
- p plugging path ways
- t temporal
- w aqueous phase

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