ENHANCED OIL RECOVERY BY MICROBIAL FLOODING

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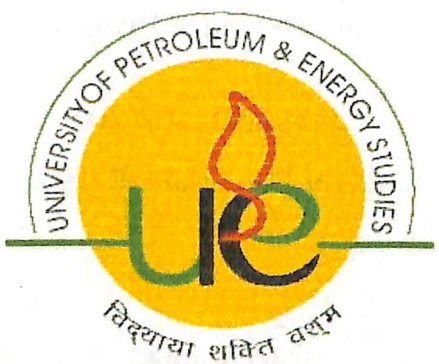

College of Engineering
University of Petroleum & Energy Studies
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ENHANCED OIL RECOVERY BY MICROBIAL FLOODING

A thesis submitted in partial fulfillment of the requirements for the Degree of Bachelor of Technology (Applied Petroleum Engineering)

By

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CERTIFICATE

This is to certify that the work contained in this thesis titled "ENHANCED OIL RECOVERY BY MICROBIAL FLOODING" has been carried out by Saurabh Arora & Saurabh Asthana under my/our supervision and has not been submitted elsewhere for a degree.

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ABSTRACT

The residual oil remaining after secondary water flooding is the target for further recovery by microbial methods. Microbial Enhanced Oil Recovery (MEOR) relies on microbes to ferment hydrocarbons and produce a by–product that is useful in the recovery of oil. MEOR functions by channeling oil through preferred pathways in the reservoir rock by closing/plugging off small channels and forcing the oil to migrate through the larger pore spaces. Nutrients such as sugars, phosphates, or nitrates frequently must be injected to stimulate the growth of the microbes and aid their performance. Microbial biomass or biopolymers might: plug high-permeability zones and lead to a redirection of the waterflood; produce surfactants, leading to increased mobilization of residual oil; increase gas pressure by producing CO_2 or methane; or reduce oil viscosity due to digestion of large molecules. Microbial growth can be either within the oil reservoir (in situ) or on the surface where the byproducts from microbes grown in vats are selectively removed from the nutrient media and then injected into the reservoir. MEOR has two distinct advantages: (1) microbes do not consume large amounts of energy, and (2) the use of microbes is not dependent on the price of crude oil, as compared to many of the other EOR processes. In some reservoirs, beneficial microbes are indigenous and only need nutrients to stimulate growth. Because microbial growth occurs at exponential rates, it should be possible to produce large amounts of useful products rapidly from inexpensive and/or renewable resources. Thus, MEOR has the potential to be more cost-effective than other EOR processes. Studies have shown that several microbially-produced bio-surfactants compare very favorably with chemically synthesized surfactants. The ability to produce effective surfactants at a low price may make it possible to recover substantial amounts of residual oil.

The report covers the basic science of microbes including isolation, identification and growth of the microbes, the basic process of microbial flooding along with an experimental work explaining the process of growth of microbes at surface conditions. The report also describes the microbial flooding procedure to be required at the suitable stage of flooding. A chapter also includes case histories of implementation of MEOR at different reservoir conditions on different fields. In the end conclusion and recommendations comprehend the report based on recent researches worldwide.

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CHAPTER – 1

INTRODUCTION

Oil & natural gas are the major but nonrenewable sources of energy. The accretion of the reserves is not proportionate to the steep depletion of the proved reserves. The probability of a major discovery is becoming more and more remote in the ease to find and already explored areas. This leaves the only option to go to logistically more hostile and technologically more fragile areas, such as deep sea, hilly and icy terrain, deeper depths etc. for future exploratory efforts. This option poses more economical and physical risks and technological constrains Exploration in these areas demands advanced technology and cost intensive inputs. This naturally leads to assume that reserve accretion is continuously being outpaced by the ever increasing demand for oil and natural gas; gas sector being comparatively better placed.

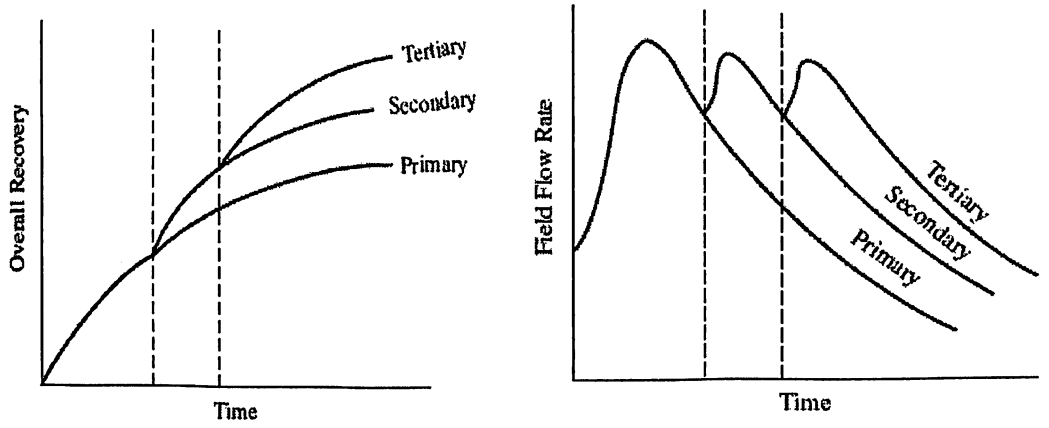


Figure 1.1: Overall recovery vs. Time Graph

The remaining oil, after primary and water flood operations, of about 70%, becomes the target for EOR techniques. The above figures explain that the tertiary recovery if combined with secondary can help gain residual oil and may result in increased oil recovery. As the reservoir pressure deplete, it requires to be supplemented with further energy to maintain the oil productivity and flow-rate of oil. During the primary recovery, pressure maintenance helps to maintain oil flow-rate and initial recovery without loss of reservoir pressure. As soon as reservoir pressure starts to deplete the oil mobility starts to decrease and permeability of the

pay zone starts to decrease due to plugging. Therefore secondary recovery helps to improve permeability along with recovering residual oil. The incremental recovery, depending upon the situation and the process may vary from 15% to as high as 40% of oil initially in place (OIIP).

1.1 CLASSIFICATION

The first and foremost development in EOR was water injection which has stood the test of time through more than one hundred years of oil industry. It is techno-economically a versatile method as water is abundantly available every where in the world, it provides favorable hydrostatic head, can be readily injected in the reservoirs at desired rate, it spreads well throughout the formation and finally cost in terms of dimes in the overall economics of oil production. However, in normal practice it is not considered as an EOR process. It of course forms a basis for comparison of some of the EOR methods and also for economical evaluation purposes. The EOR process may be classified into four typical categories based upon the mode of application of energy supplementation into the oil pool.

I. **Chemical EOR Processes**

1. Polymer Flooding
2. Surfactant Polymer Flooding
3. Alkaline Flooding
4. Alkali Surfactant Polymer Flooding

II. **Gas Injection EOR Processes**

A. Hydrocarbon Gas Injection Process

1. LPG Injection
2. Enriched Gas Injection
3. Lean Gas Injection.

B. Non- Hydrocarbon Gas Injection

1. Carbon dioxide Flooding
2. Nitrogen and Flue Gas Flooding

III. Thermal EOR Processes

1. Hot water flooding
2. Steam Injection
3. In-situ Combustion

IV. Emerging EOR Processes

1. Microbial EOR Process (MEOR)
2. Electric Current Induced EOR Process
3. Magnetic water flooding

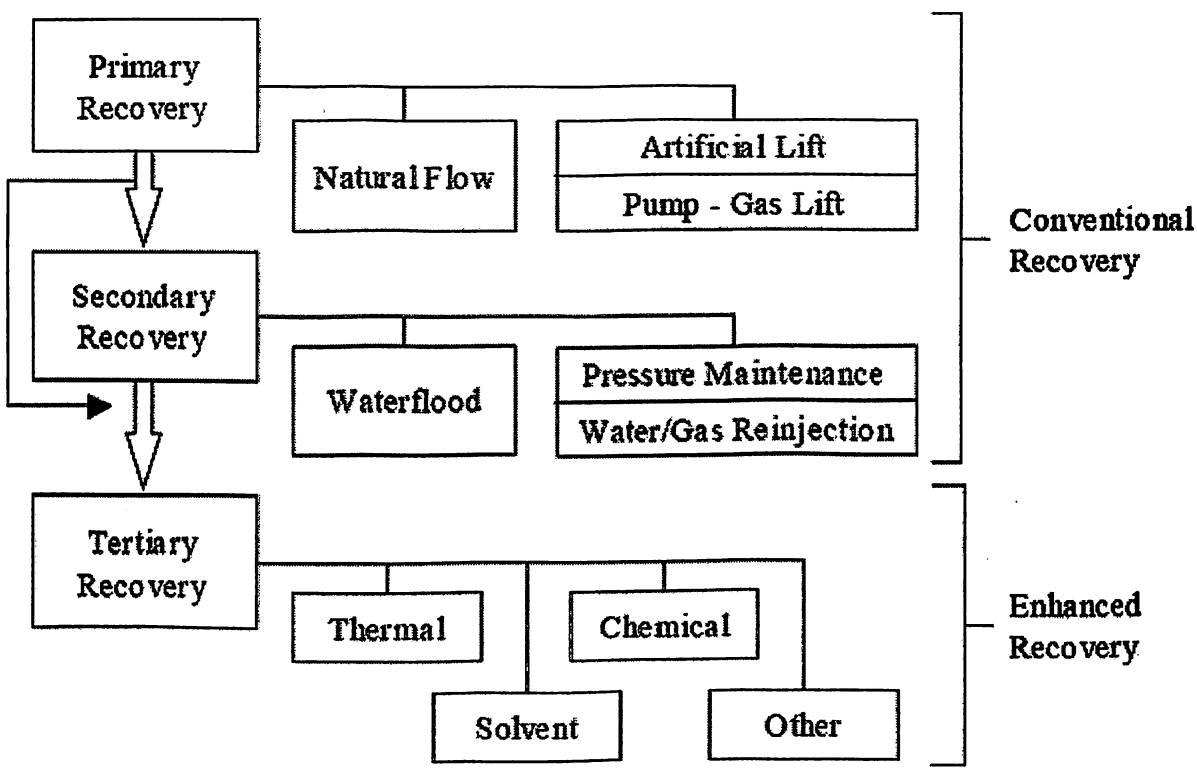


Figure 1.2: Oil recovery classifications (courtesy – oil and gas journal biennial surveys)

The EOR processes are fluid and rock dependent and are effective under specific conditions and hence are not universally applicable in diverse petroleum reservoir conditions. As such

each process is to be analyzed with respect to the specific reservoir fluid and rock environmental conditions. Only techno-economically viable processes qualify for commercial applications. It is essential to understand the mechanism of the process to identity a suitable candidate reservoir and evaluate its response to a particular and well scrutinized and evaluated process.

1.2 INTRODUCTION TO MEOR

Microbial enhancement of oil recovery has recently received good attention in the oil industry. In-situ MEOR processes involves the injection of microorganisms and suitable nutrients into the reservoir. The use of microorganism to enhance oil recovery (MEOR) has for many years been considered as possible by many investigators. Based on the fact that MEOR processes were recognized nearly 70 years ago, one would think that this technology is in wide use today. On the contrary, the general perception is quite the opposite. The negative perception on the use of bacteria for EOR has been attributed to inadequate methods for transfer of technology. MEOR processes are potentially cost effective and particularly well suited for today's economic climate. After review of hundreds of projects, it was concluded that the cost of MEOR process ranges from $ 0.25 to $ 0.50 per barrel of oil produced at the time MEOR begins, and does not go up as oil production increases. The total cost of incremental oil produced from MEOR is only about $2.00 per barrel. In addition, MEOR processes are considered environmentally friendly as verified by tests conducted by public health laboratories that reported that the mixed culture of bacteria is safe to handle and poses no threat to plants, animals, or human beings. Microorganisms used for MEOR processes rely on microscopic oil displacement for improving the efficiency of oil recovery. The most commonly species are those of Bacillus and Clostridium. These microorganisms have a greater potential for survival under petroleum reservoirs, harsh environmental conditions than other species because they produce in-situ spores, which are dormant, resistant forms of the cells that can survive under stressful environmental conditions. Clostridium species produce surfactants, gases, alcohols, and solvents, whereas some Bacillus species produce surfactants, acids, and some gases. In the MEOR process, it is vital that microorganisms can travel through the porous

media and mobilize oil. The processes that facilitate oil production are complex and usually involve multiple biochemical processes. MEOR systems represent high-risk processes to oil producers looking for efficient and predictable oil recovery. Microbial biomass or biopolymers might: plug high-permeability zones and lead to a redirection of the waterflood, produce surfactants, leading to increased mobilization of residual oil; increase gas pressure by producing CO_2 or methane; or reduce oil viscosity due to digestion of large molecules. The net effect of the entire MEOR process causes previously immobile, unrecoverable oil to become mobile so that it is now available to be swept into producing well bores, causing an incremental improvement in oil production.

The application of MEOR technology has promising prospects since it is supported by;

a) Simple technology and equipments which are easy to operate,
b) The process can be easily monitored,
c) It is environmental friendly and does not cause pollution,
d) It is low cost process,
e) The technology is capable of recovering the remaining oil in trapped in the reservoir rock, which quantity is quite considerable.

Although thermal and gas injection methods find the widest commercial applications, MEOR has two distinct advantages: 1) microbes do not consume large amounts of energy, and 2) the use of microbes is not dependent on the price of crude, as compared with other EOR processes. In some reservoirs, beneficial microbes are indigenous and only need nutrients to stimulate growth. Because microbial growth occurs at exponential rates, it should be possible to produce large amounts of useful products rapidly from inexpensive and/or renewable resources. Thus, MEOR has the potential to be more cost-effective than other EOR processes. Studies have shown that several microbially produced bio-surfactants compare favorably with chemically synthesized surfactants. The ability to produce effective surfactants at a low price may make it possible to recover substantial amounts of residual oil. Not every technique can be used in every oil reservoir.

CHAPTER – 02

SCIENCE OF MEOR

2.1 MEOR TECHNOLOGY

Microbial enhanced oil recovery (MEOR) is well-known as a developing and promising method to improve the oil recovery through microbial activities. Specifically selected suitable nutrients are introduced into the producing formation near the wellbore. When the well is shut-in, after nutrients injection, the microbes are encouraged to produce bio-metabolites and extend colonization outward into the producing formation. Bio-production of solvent, polymer, and surfactant is done in-situ when the well is put into production; the fluids flow toward the wellbore, and result in increasing oil recovery. The microorganisms used in MEOR can be applied to a single oil well or to an entire oil reservoir. They need certain conditions to survive, so nutrients and oxygen are often introduced into the well at the same time. MEOR also requires that water be present. Microorganisms grow between the oil and the well's rock surface to enhance oil recovery by the following methods:

Reduction of oil viscosity – Oil is a thick fluid that is quite viscous, meaning that it does not flow easily. Microorganisms help break down the molecular structure of crude oil, making it more fluid and easier to recover from the well.

Production of carbon dioxide gas – As a by-product of metabolism, microorganisms produce carbon dioxide gas. Over time, this gas accumulates and displaces the oil in the well, driving it up and out of the ground.

Production of biomass – When microorganisms metabolize the nutrients they need for survival, they produce organic biomass as a by-product. This biomass accumulates between the oil and the rock surface of the well, physically displacing the oil and making it easier to recover from the well.

Selective plugging – Some microorganisms secrete slimy substances called exo-polysaccharides to protect themselves from drying out or falling prey to other organisms. This substance helps bacteria plug the pores found in the rocks of the well so that oil may move past

rock surfaces more easily. Blocking rock pores to facilitate the movement of oil is known as selective plugging.

Production of biosurfactants – Microorganisms produce slippery substances called surfactants as they breakdown oil. Because they are naturally produced by biological microorganisms, they are referred to as biosurfactants. Biosurfactants act like slippery detergents, helping the oil move more freely away from rocks and crevices so that it may travel more easily out of the well.

Table 2.1: Microbial products and their contribution to EOR

MICROBIAL	EFFECT
ACIDS	Modification of reservoir rock Improvement of porosity & permeability Reaction with calcareous CO_2 production
BIOMASS	Selective or non-selective plugging Emulsification through adherence to hydrocarbons. Modification of solid surfaces example : Wetting Reduction of oil viscosity & pour point
GASES (CO_2, CH_4,H_2)	Reservoir Repressurization Oil swelling Viscosity reduction
SOLVENTS	Dissolving of oil
POLYMERS	Mobility control Selective or non-selective plugging
SURFACTANTS	Lowering of Inter-facial tension

2.2 MEOR MECHANISM

In MEOR, microbial growth can be either in situ or on the surface, where by-products from microbes grown in vats are selectively removed from the nutrient media, and injected into the reservoir. MEOR processes are similar to in-situ bioremediation processes. Injected nutrients, together with indigenous or added microbes, promote in-situ microbial growth and/or generation of products that mobilize additional oil and move it to producing wells through reservoir depressurization, interfacial tension/ oil viscosity reduction, and selective plugging of the most permeable zones. Alternatively, the oil-mobilizing microbial products may be produced by fermentation and injected into the reservoir. For in-situ MEOR processes, the microorganisms must not only produce the chemicals necessary for oil mobilization, but must also thrive in the reservoir environment. In a MEOR process, conditions for microbial metabolism are frequently supported by nutrient injection. In some processes, this involves injecting a fermentable carbohydrate into the reservoir. Some reservoirs also require inorganic nutrients as substrates for cellular growth or for serving as alternative electron acceptors in place of oxygen or carbohydrates

The mechanism of the enhanced oil recovery includes one or combination of the following processes:

- Gas produced by fermentation (produced from molasses) such as CO_2, H_2 and CH_4 aid in the repressurization of the reservoir and dissolution of gases like CO_2 in oil reduces the oil viscosity.
- Production of low molecular weight organic acids should help to dissolve/erode limestone rock thus leading to increase movement of bacteria through the reservoir as well as dislodgment of the oil the at was attached to such rocks.
- Production of large amount of lower molecular weight solvent (primary alcohols and acetone). Presence of these compounds in sufficient quantities should result in solubilization of crude oil that is attached or trapped in rock pores.

- Production of low molecular weight-non-ionic emulsifiers that would form oil in water emulsion and thus result in solubilization of the crude oil from the surface and rock pores.
- Degradation of heavy fraction in the oil leading to decrease in oil viscosity.
- Production of water soluble high molecular weight polymers for mobility control.
- Affinity of bacteria for solid surfaces will force the oil from the rock through wettability alterations.

Although each of the above metabolic end products and mechanism should function individually to bring about release of oil in a reservoir, the combined activities are not understood. It is necessary that all of the above desired metabolic end products be produced from cheap and plentiful sources of carbon and energy such as molasses with some form of ammonia salt present.

2.2.1 Bioproduct Formation.

Microbes undergo metabolism process in their lives, producing metabolites in the form of enzymes which are useful for the microbes themselves, and bioproducts which are useful for their environment. Enzymes are used as catalyst, for degradation of nutrient and food materials. Bioproducts such as surfactant, bioacid, biogas, biopolymer, biosolvent, etc. are useful for the MEOR process. The type of bioproducts depends on the type and composition of the nutrients consumed by the microbes from their environment. Nutrients of food materials required by the microbes consist basically of seven types, namely water, energy source, carbon source, electron acceptor, essential minerals, nitrogen source, and growth factor.

2.2.2 Effect of Bioproduct on Oil Recovery.

Out of the positive effects of microbial activities in oil field environment is their ability to result in enhancing oil recovery. This is due to the fact that the microbes, in their activities and metabolism, produce bioproducts in the form of chemicals. Biosurfactant can reduce the interfacial tension between oil and the formation water, while biopolymer controls the mobility of water use in waterflooding. Biofilm and biomass may plug the pores of reservoir rocks and

then change the direction and pattern of fluid flow in the rock. Biogas production increases reservoir pressure and assists in forcing oil out of the rocks. Bioacid, on the other hand, assists in dissolving rock particles and open pore mouths, thus increasing rock porosity and permeability and allowing more fluid to flow.

The following flow diagram explains the conventional MEOR field implementation. The isolated bacteria obtained from soil, formation water, crude oil is fed into the biotechnological unit. On the other hand molasses is sent to the biotechnological unit which mixes with the bacteria and helps them to grow. This combined mixture is injected into the injection well with water. Water here acts as a nutrient as well as a medium for the growth of the microbes in the reservoir. Bacterial colonization helps to recover residual oil with the generation of metabolites, thereby improving oil mobility. The generated products pressurize the oil to exit though the production wells.

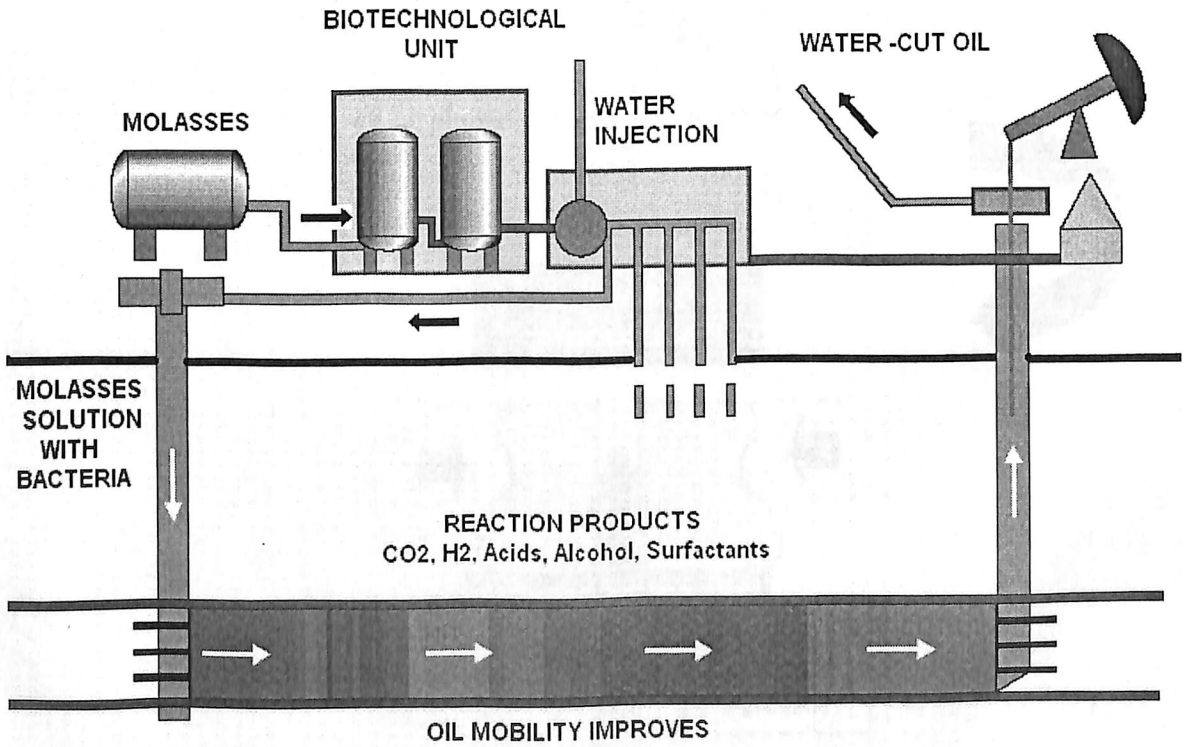


Figure 2.1: MEOR Flow diagram

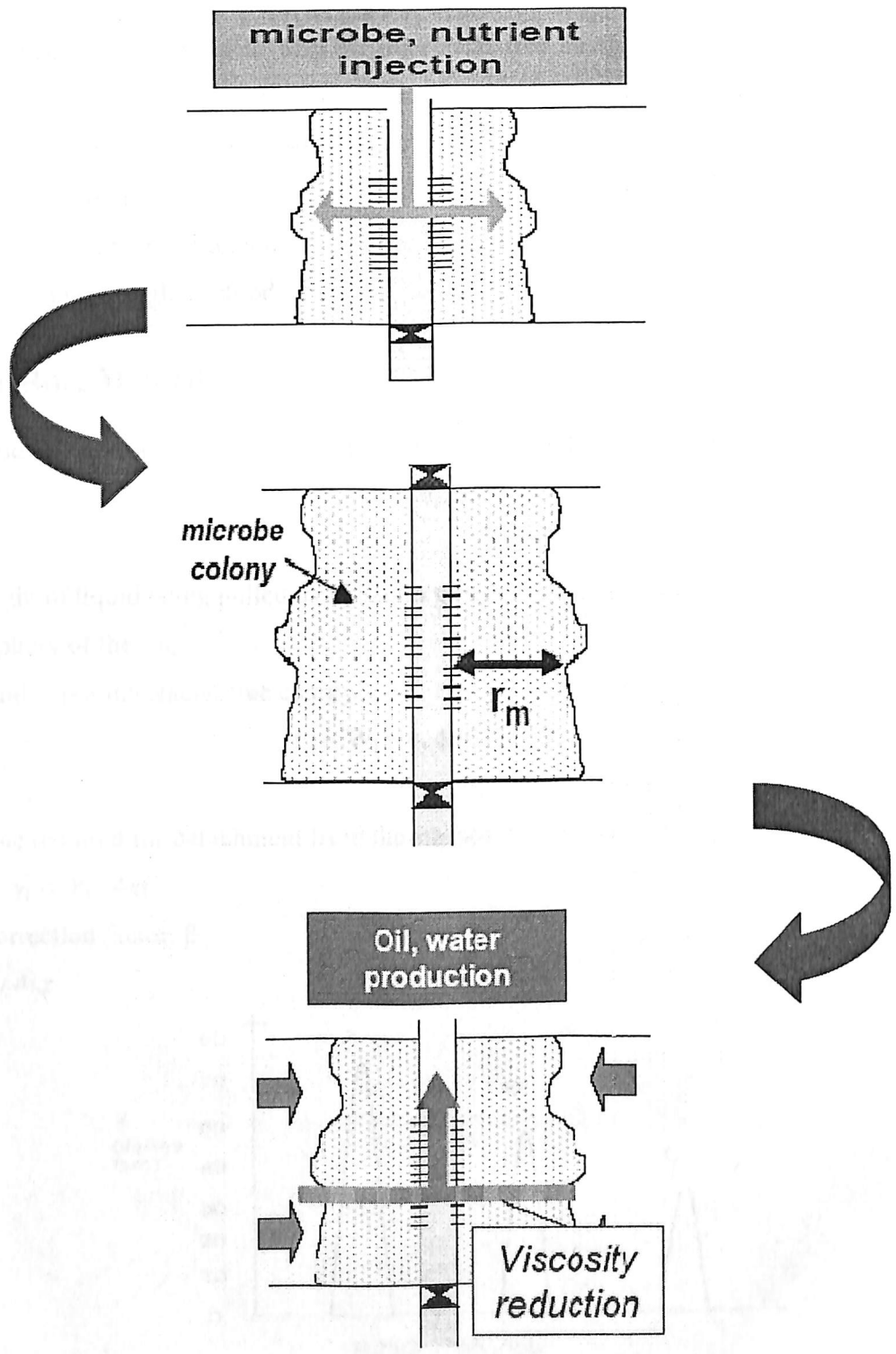


Figure 2.2: MEOR Mechanism

2.3 CALCULATION OF PARMETERS:

Interfacial phenomena relating to MEOR: Interfacial free energy (γ) is used to describe the boundary or interface between physical phases. There are three methods to calculate interfacial energies based on experimental calculations.

1. The Ring Method
2. The Spinning Drop Method
3. The Contact Angle Method

2.3.1 The Ring Method:

This method is used for measuring interfacial energies more then 1.0mN/m and is given by-

$$W_l = \gamma_l 4\pi r$$

Where,

W_l = weight of liquid being pulled using strain gauge

$4\pi r$ = periphery of the ring

γ_l = liquid vapor interfacial free energy

Also, $$F_l = W_l = \gamma_l 4\pi r$$

Where,

F_l = force required for detachment from the surface

Therefore $\gamma_l = F_l / 4\pi r$

Using a correction factor, β

$\gamma_{lv} = F \beta / 4\pi r$

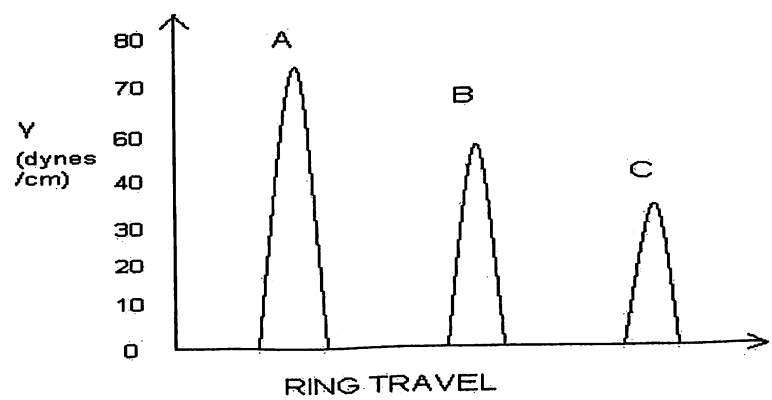


Figure 2.3: Interfacial energy vs. Ring travel

Typical experiment using a bacteria culture of corynebacterium fascians in a mineral salts medium with 2% sucrose and 1% yeast extract gives surface and interfacial readings as shown above. Curve A is for water while curve B is the surface tension of a whole broth of C fascians. The curve C is the interfacial tension between a whole broth of C fascians and hexadecane. The vertical axis measures the strain upon the ring supporting wire, while the horizontal axis is a measurement of the vertical distance the ring travels before it breaks away from the surface. The maximum force (the peak of each strain vs. travel curve) is that which satisfies the equations and is the surface or interfacial tension (γ_{lv}).

2.3.2 The Spinning Drop Method

The contact angle method allows one to study interfacial phenomena of a solid- liquid- vapour system. Once surface tension of liquid used is known, the method enables the calculation of both the solid- vapor interfacial free energy and the solid- liquid interfacial free energy. A drop of liquid placed on a uniform flat surface, will spread over the surface, or the drop will form an angle with the solid surface and not spread.

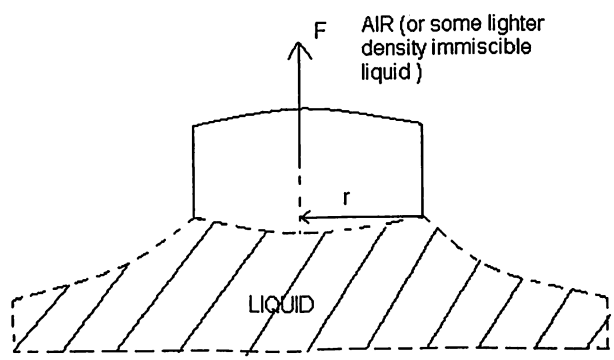


Figure 2.4: The interfacial tension or energy relationship between a drop, surface of smooth solid, and a vapor

γ_{lv} = liquid- vapor surface tension,

γ_{sl} = solid – liquid interfacial tension,

γ_{lv} = solid surface interfacial energy,

p = equilibrium pressure

CHAPTER 03

SELECTION OF MICROBES

3.1 ISOLATION OF BACTERIA

3.1.1 Microbial Selection

The microbes which have potential for use in MEOR are generally bacteria, as this type of microbes can easily adapt to their environment, compound to other types such as fungi and others. These bacteria must be of the non pathogenic type. The morphology of bacteria can be inspected by means of microscope as their size is quite large, being 0.5-50 mm in diameter. Bacteria appear in various forms, and these can be classified into three types, namely round, rod, and spiral forms. The success in application of MEOR depends on that of the selection of microbes which are capable of producing metabolites in an effective quantity to assist oil displacement.

3.1.2 Identification of Bacteria.

To obtain isolates of bacteria from an oil field, samples must be taken from oil field environment. Samples that represent oil field environment can be those of formation water, crude oil, or soil collected near the wellhead. From the isolate taken from such sample, a pure culture can be prepared and bacteria presence can be determined. Each pure isolate is then identified to determine the type of bacteria.

3.1.3 Isolation of Bacteria.

Prior to isolation the samples are prepared for the purpose. Crude oil and soil samples are first extracted with water. Formation water samples, on the other hand, being already a suspension of microbes need no prior extraction Isolation of microbes is then conducted from the suspension. Inoculation is made and each one is incubated at temperature of 30°C and 55°C for a period of 24- 48 hours, until colonies are observed.

3.1.4 Identification and Determination.

A number of bacterial isolates have been obtained from the above activity. To differentiate the isolates from one another, identification and determination procedures are applied to each isolate. The genus/species of each bacterial isolate is determined. This is done by observing the morphology and testing the activity of the bacteria by various treatments. Isolate identification and determination are based on the macroscopic and microscopic observation as well as biochemical testing of the isolate. The identification and determination activities allow the worker to differentiate one isolate from another.

The isolates can be of the following genera -***Staphylococcus***, ***Bacillus***, ***Corynebacterium***, ***Flavobacterium***, ***Enterobacter***, ***Alcaligenes***, ***Actinobacillus***, ***Neisseria***, ***Pseudomonas***, ***Hafniae***, ***Chromobacterium***, ***Micrococcus***, and ***Streptococcus.***

Each isolate is then given coded identification number as shown in an Example from Indonesian Field following Tables 3.1-3.3.

Table 3.1 Cepu oil field microbes isolated at 30oC

No.	Isolate Code	Genus/Species Code
1.	GAN-1	Bacteria KKL-1
2.	GAL-1	Bacteria KKL-2
3.	GAK-1	Bacteria KKL-3
4.	GAK-2	Bacteria KKL-4
5.	GMK-1	Bacteria KKL-5
6.	GMN-1	Bacteria KKL-6
7.	GMN-2	Bacteria KKL-7
8.	GMN-3	Bacteria KKL-8

Table 3.2 Cirebon oil field microbes isolated at 30 oC		
No.	Isolate Code	Genus/Species Code
1.	GAJ-1	Bacteria KKL-9
2.	GAC-1	Bacteria KKL-10
3.	GAC-2	Bacteria KKL-11
4.	GAC-3	Bacteria KKL-12
5.	GAT-1	Bacteria KKL-13
6.	GMJ-1	Bacteria KKL-14
7.	GMT-1	Bacteria KKL-15
8.	GMC-1	Bacteria KKL-16
9.	GTJ-1	Bacteria KKL-17

Table 3.3 Rantau oil field microbes isolated at 30 oC		
No.	Isolate Code	Genus/Species Code
1.	GAR-1	Bacteria KKL-18
2.	GAR-2	Bacteria KKL-19
3.	GAR-3	Bacteria KKL-20
4.	GAP-1	Bacteria KKL-21
5.	GAS-1	Bacteria KKL-22
6.	GAS-2	Bacteria KKL-23
7.	GAS-3	Bacteria KKL-24
8.	GAS-4	Bacteria KKL-25
9.	GMR-1	Bacteria KKL-26
10.	GMS-1	Bacteria KKL-27
11.	GMS-2	Bacteria KKL-28
12.	GMP-1	Bacteria KKL-29
13.	GTP-1	Bacteria KKL-30

3.1.5 Desirable Properties of Microbes

1. Small size – to permit most ready penetration through rock strata.
2. Resistance to high pressure since reservoir is deep.
3. Maximum tolerance to high temperature prevailing in large number of economically important reservoirs.
4. Ability to withstand brines and sea water, since these are often present in water flooding.
5. Ability to thrive and live on simple mineral salts already present in the water flooding.
6. Capacity to use foodstuffs an-aerobically since molecular oxygen cannot be provided in sufficient amount downhole.
7. Absence of any unacceptable property which might lead to plugging of formation.

3.1.6 Types of Microbes

The term microorganism encompasses five major groups of organisms: viruses, fungi, algae, protozoa, and bacteria. These are organisms that exist as individual cells or undifferentiated aggregates of cells (cells that are not differentiated into tissues that have distinct functions). The size of microbial cells is so small that a microscope is required for observation. The viruses are the smallest form of recognized microbial life. They are not cells because they differ in many respects from the multifunctional cells: the viruses are much simpler in structure containing only protein and nucleic acid surrounded by a lipid or protein membrane and they do not reproduce by growth followed by division as cells do. Viruses must use other living cells in order to reproduce.

A second division of microorganisms is the eucaryotic microbes which include the fungi, algae, and protozoa. Eucaryotic microbes are distinguished from viruses and bacteria by virtue of possession of a true nucleus, which is enclosed by a membrane that contains the genetic material of the cell (the deoxyribonucleic acid (DNA)), organized into structures known as chromosomes. Eucaryotic microorganisms also have specialized organelles in their cells, such as Golgi bodies that conduct specialized functions of transport of materials within the cell and secretion of materials to the exterior (Gaudy and Gaudy, 1980).

Although eucaryotic microbes are probably responsible for some microbial plugging problems of injection wells, they are not important to enhanced oil recovery processes at this time. The third division of microorganisms that can be distinguished by its physical characteristics is the procaryotes. The procaryotes are in general about ten times smaller than the eucaryotes and the structural features within the cells are not distinguishable with an optical microscope. The intracellular features of some procaryotes can be observed by staining, but an electron microscope is required for detailed structural observation. The only food utilized by procaryotes (or bacteria) comprises soluble molecules that can be assimilated through the cell wall. On the other hand, the eucaryotic protozoa contain a flexible outer membrane that can surround particles of food to form a vacuole where digestion of the food takes place.

Bacteria have two distinctive structural features that are: a rigid cell wall that determines the shape of the organism, which may be either cylindrical or spherical, and those that are mobile possess flagella which are responsible for their movement. Differences in the cell walls of bacteria furnish the basis for classification into two broad groups: Gram-positive and Gram-negative bacteria. The cell wall of Gram-positive bacteria consists of multiple layers of peptidoglycan, cross-linked through amino acid bridges, and teichoic acids bonded to the peptidoglycans. The three-dimensional network of molecules provides a strong (rigid) structure. The wall of the Gram-negative bacteria also contains peptidoglycan; however, the wall is very thin and surrounded by a lipid layer of lipoprotein and lipo-polysaccharide, sometimes referred to as the outer membrane.

The Gram stain test is conducted by applying crystal violet, which is adsorbed by the bacteria. Next, a solution of iodine is added, which forms an iodine-dye complex. Then the mixture is treated with ethanol which removes the iodine-dye complex from the Gram-negative cells where the complex is not chemically bound. Finally, a red stain is applied, which colors the Gram-negative bacteria (that could not retain the dye complex) red. The two types of bacteria are then easily distinguished under the microscope: Gram-positive bacteria appear purple and the Gram-negatives are pink in color. Retention of the iodine-dye complex is

related to the structure of the cell wall; it is retained by the Gram-positive bacteria that contain the thick, multilayered, cross-linked cell walls described above (Nester et al., 1978; Gaudy and Gaudy, 1980). The bacterial cytoplasm is a concentrated solution of organic and inorganic molecules which are prevented from leaving the cell by the cell membrane; however, water and other small molecules can move freely through the semi-permeable membrane. This concentration gradient between the molecules inside the cell and the surroundings produces an osmotic pressure within the cell. If the cell did not have a rigid wall that can withstand the osmotic pressure, it would expand and burst. The osmotic pressure may be as much as 2.5 MPa (25 atm). If the concentration of low-molecular-weight compounds in the surroundings is high, the osmotic pressure in the cell decreases. Indeed, bacteria that occur in the Dead Sea water (containing approximately 30% salt) do not have a rigid cell wall and will burst if the salt concentration is decreased (Nester et al., 1978).

There are many species of bacteria having a variety of sizes and shapes. Some have flagella that are used for movement within an aquatic environment. Bacteria that are not mobile are transported only by motion of the fluid. Some bacteria are cylindrical (or rod-shaped), whereas others are spherical. Some exist as individual cells, whereas others grow in aggregates or chains of cells. They range in size from 0.2 to about 5 μm and are able to penetrate consolidated rocks that typically have pores as large as 100 μm. Bacteria are the only microbes that have thus far been proposed for development of processes for enhancement of oil recovery, because they possess several important 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 polymers. Various types of bacteria also tolerate harsh environments similar to those encountered in subsurface geological formations, such as high salinity, high pressure, and high temperature. Also, many bacteria are anaerobic (grow in the absence of oxygen). The ranges of physical and chemical properties of bacteria are so wide that in many cases it may be prudent to select a culture of mixed bacteria to live in a symbionic relationship in a petroleum reservoir to improve the recovery of oil. Thus, microorganisms have a large capacity for chemical synthesis. They produce a wide range of products, generally from

relatively simple nutrient compound, and propagate vigorously under favorable conditions. Gases produce a metabolic byproduct from inorganic anions which include N_2 from NO_3- reducing bacteria and H_2S from SO_4- reducing bacteria. Production of H_2S from SO_4- could dissolve sulphate containing minerals to increase the permeability of the reservoir or remove inorganic matrices. A class of anaerobes capable of fermenting or metabolizing injected water soluble carbohydrate in-situ to generate gases. Members of the genera of Aerobacter, Escherichia are organisms. A class of spore forming bacteria namely desulfovibrivo and clostridium as patented by Hitzman in US is used to erase the problem of bio-mass build-up in the well-bore by inoculation of a reservoir by smaller and more robust bacterial spores. It may also reduce the formation permeability with consequent fluid flow loss (injectivity) for the reservoir having water-oil contact interface bacteriaciae and pseudomona deceae may be an alternate to production zone. These micro-organisms are injected into the water bearing zone below the oil production zone. The organism utilizes hydrocarbon and producing gases CO_2 or CH_4 in-situ. They migrate to the water oil interface and proliferate with concomitant gas formation which enhances production from the overlying oil bearing zone. The use of strong oxidizing agents and extreme pH solutions like potassium permagnate (2-20%), Hydrogen or alkali metal peroxides followed by an aqueous acid injection (3.5-5% HCL) to remove organic material. Corrosion control can be done by the use of hypohallites (hypochlorite and hypobromide) as oxidizing agents but includes water dispersed soluble silicated or hydroxides (pH-13) as corrosion inhibitors.

3.1.7 Microbial Growth & Metabolism

Populations of microbes are found everywhere in nature, even found in areas that will not support any other form of life. The actual species growing in a particular environment comprise those that have been able to successfully adapt to the prevailing environmental and nutritional conditions and the extremes of variations of those conditions. They also have been the most successful in competition with other microbes that may have entered the particular ecosystem. There may be several species living in an area, apparently as a homogeneous population. On closer inspection by division of part of the material

supporting the growth (soil, decaying matter, etc.) into squares of approximately 100 μm^2, however, it is found that only one species occupies this microzone where it has been able to competitively exclude all other microbes. The combined effect of all of the species in a given zone develops a symbiotic relationship that results in recycling of essential chemical compounds and elements required for maintenance of life. The population will usually remain stable as long as the environmental and nutrient conditions do not undergo drastic changes. Small changes of the environmental conditions can result in rapid changes of the relative populations of the species living in a particular zone. Utilization of nutrients in the environment to maintain metabolism and growth depends on the enzyme inventory of a given species of bacteria. Enzymes are protein molecules endowed with the specific characteristics of organic catalysts. They lower the chemical activation energy of metabolic materials (substrates) in the surroundings, allowing them to undergo various organic reactions at low temperatures which are compatible to the living organism. The enzymes are true catalysts in that they remain unchanged by the reaction which causes a rearrangement of substrate molecules or decompositions into smaller units. The enzymes increase the rates of reaction as well as facilitating low-temperature reactions. In the absence of the enzyme, the same reaction will only take place at an elevated temperature, which may be too high for living organisms to tolerate. The relationship among the rate of reaction, temperature, and the activation energy is expressed by the Arrhenius equation (Levenspiel, 1972):

$$Log\ (K) = \log\ (A) - E/(2.3RT) \qquad (3\text{-}1)$$

Where,

K = reaction rate constant;

A = constant, mol/l;

E = activation energy, cal;

R = gas constant (1.987 cal/deg-mol); and

T = temperature, ° K.

Above Equation (3-1) is used to determine the activation energy by conducting the reaction at various temperatures and measuring the rate of product formation. A plot of the rates of reaction made versus the reciprocal of the absolute temperature. The activation energy is obtained from the slope of the line and the constant, A, from the vertical intercept. Differentiation, as to whether one or another type of bacteria will survive in a given environment containing food resources, depends on the types of enzymes associated with a given species. This is due to the fact that the enzymes have a high degree of specificity with regard to the substrates with which it will interact as a catalyst. Thus, one type of bacteria can assimilate paraffin hydrocarbons, whereas another cannot do so. In the majority of cases, the enzymes remain within the cell and the substrates must penetrate the cell wall, with the aid of a protein which is made specifically for transportation of substrates through the cell wall. This special protein is called permease. In some cases, enzymes are present on the outside of the cell wall, or may even be released as free molecules into the solution surrounding the microbe. Mostly it is the enzyme inventory of the given species, and the permease, which control the type of substrate that can be utilized by the microbes and the rate of transport into the cell. Excretion of molecules from the cell also appears to be a controlled type of process (Nester et al., 1978).

3.1.8 Catalytic function of microbes:

The catalytic function of enzymes depends on the presence of special groups and the spatial configuration of other groups on the protein. Any change of these special functional groups results in a decrease, or complete termination, of catalytic activity with fatal consequences to the microorganism. A change of pH, above or below the optimum for the microbes, will change the functional groups enough to slow down their activity. With a more severe change of pH (+ or -, 2-4 pH units) the functional groups may be destroyed (denatured).

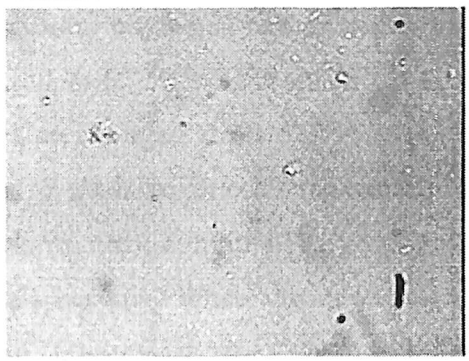


Figure 3.1 *Microphotograph showing inlet bacteria concentration*

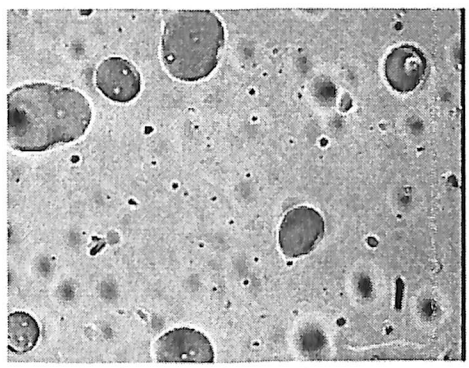


Figure 3.2 *Microphotograph showing bacteria concentration after flooding and presence of micro-emulsion (x1000)*

3.1.9 Effect of the temperature and pressure on microbes:

The temperature of the environment also has a profound influence on the microbe. As indicated by equation (3-1), with increasing temperature the rate of reaction also increases; but, an optimum temperature level exists for any given species. When the temperature is raised above the optimal level, the microbial rate of metabolism decreases and finally stops as the proteins making up the enzymes are denatured. Most of the enzymes cannot withstand temperatures greater than 70°C; however, a few enzymes which are possessed by thermophilic microbes remain active at temperatures up to 100°C.

Bubela (1983) found that an increase of pressure increased the optimum metabolic temperature and rate of growth of rod-shaped bacteria (6-8 μm long, 3-4 μm in diameter). At atmospheric pressure (101 kPa) the optimum growth temperature was 50 °C with a

mean generation time of 17 hours, but when the pressure was increased to 20 MPa the maximum growth rate occurred at 65 °C with a mean generation time of 12 hours. The morphology of the microbe also changed from rod-shaped to coccoidal (about 5 μm in diameter). Cores from offshore wells in Brazil's Namorado oilfield at a depth of more than 3000 m (about 30 MPa pressure), contained large populations of *Desulfovibrio* and clusters of coccoidal (spherical) bacteria. When the coccoidal bacteria were grown at one atmosphere pressure in the laboratory, they reverted to rod-shaped morphology, indicating either *Clostridium* or *Bacillus* (Petzel and Williams, 1986). Similar results were discussed by Marquis (1983). Moses and Springham (1982) also reported that bacteria have been found to be catalytically active at 179 MPa and that other bacterias were found to raise their optimum growth temperature from 65 °C to 85 °C, when the pressure of the growth medium was increased from 101 kPa to 60 MPa. Thus it is evident that the denaturization of some enzymes is inhibited by an increase of pressure.

3.1.10 Other Environmental Factors

Another environmental factor that is important to microbial enhancement of oil recovery is the salt concentration (NaCL and CaCL) of the surroundings. A difference of concentration between the surroundings and the cytoplasm within the cell produces a large osmotic pressure difference which can result in dehydration of the cell followed by growth inhibition or death of the cell. This is the basis of the high salt or sugar concentrations used for preservation of foods. Some bacteria, can tolerate a low concentration of salt within the cell walls, which diminishes the osmotic pressure. Others, called halophiles, may actually require high salt concentrations for growth. Grula et al. (1983) readily grew *Clostridium* in salt concentrations up to 75,000 ppm and 45 °C, which are representative of conditions that are encountered in petroleum reservoirs that are generally less than 1000 m deep

.

Three divisions of microbes are based on their ability to utilize oxygen. Aerobic bacteria contain enzymes that can decompose peroxides, which are formed as part of the metabolic processes involving oxygen, but the strict aerobes cannot grow in an

oxygen-free environment. The facultative bacteria contain a group of enzymes that allow growth in both aerobic and anaerobic environments. The strict (obligate) anaerobes cannot utilize oxygen because they do not contain the appropriate complement of enzymes that are necessary for growth in an aerobic environment. Many biological reactions in microbes will occur only if adenosine triphosphate (ATP) is present for interaction with the enzymes, because even though the activation energy of the reaction is lowered by the enzymes, ATP is required to furnish additional energy. This leads to another method of classification of microbes on the basis of their ability to use carbon dioxide as a source of carbon for synthesis. Autotrophic bacteria can use carbon dioxide as their source of carbon and they can make ATP from oxidation of inorganic compounds, deriving their energy from the sun (photosynthesis) or from metabolism of inorganic compounds. The other class of microbes, known as heterotrophic microbes, must have preformed organic compounds as both their source of carbon and for energy (Nester et al., 1978; Gaudy and Gaudy, 1980; Moses and Springham, 1982). In assessing microbial nutrient requirements and their metabolic products from the standpoint of microbial enhancement of oil recovery, it is more appropriate to classify the microbes according to their ability to utilize oxygen. Numerous microbial cultures (pure and mixed) are capable of synthesizing a variety of biochemical products using petroleum fractions as the substrates. The range of metabolic products from microbial consumption of petroleum is very broad, depending on environmental conditions (pressure, temperature, salinity, pH, and the presence or absence of oxygen), supporting nutrients available for cell metabolism (nitrogen, phosphorus, minerals, etc.), and the specific bacterial cell interaction with petroleum.

Obligate Aerobes

They are organisms that must have oxygen for their metabolism and growth, but they can exist dormant in the absence of oxygen. The bacteria decompose sugars to carbon dioxide, water and, often, acids: $C_5H_{12}0_2 + 80_2 = 6C0_2 + 6H_20 +$ energy. When the microbes are furnished hydrocarbons and an ample supply of oxygen, a wide variety of products will result which depend on the type of microbe, environmental conditions, and the specific

type of hydrocarbon substrates. The products may be carbon dioxide, low-molecular-weight acids, phenols or biopolymers (proteins, polyanionic lipids, glycolipids, or polysaccharides). The compounds are waste products from the microorganism and some may even be toxic if accumulated in the surrounding fluid.

Obligate Anaerobes

They cannot utilize free oxygen; small quantities of oxygen (10 ppm) are even toxic to some anaerobes. Spores produced by some anaerobes, however, can remain dormant for long periods in an aerobic environment, germinating when they enter anaerobic environments. The anaerobes use low-molecular-weight organic compounds, such as sugars, as a source of carbon and energy. In the process of metabolism, the microbes release various products. Sugars undergo anaerobic fermentation yielding acids, alcohols, ketones, aldehydes, carbon dioxide, and hydrogen. Some species of the anaerobic genus *Clostridium* have been found to produce all of these compounds (Grula et al., 1983). These anaerobes also may reduce sulfur occurring as inorganic sulfates, or as part of the molecular structure of organic compounds, to hydrogen sulfide. Petroleum reservoirs have been known to become sour (produce large quantities of H_2S with the hydrocarbons) when infected by *Desulfovibrio* bacteria from injected water used in secondary recovery (Crawford, 1983). Anaerobes also can produce chemicals, some of which are surface-active agents that lower the oil-water interfacial tension and promote emulsification of oil. The anaerobic bacteria also may produce biopolymers (primarily polysaccharides) that can be used as mobility control agents.

Facultative bacteria can change their metabolism for growth either in anoxidizing or reducing environment. Their products of metabolism are quite similar to those described above. They may produce hydrogen sulfide from organic sulfides and inorganic sulfates, and reduce low-molecular-weight compounds (sugars, aldehydes, etc.) to methane, hydrogen and carbon dioxide. In the proper environment, they produce biosurfactants and biopolymers. The three classes of bacteria noted above are generally mesophilic (existing

within a temperature range of 20-50°C), but there are thermophilic and caldoactive bacteria that live quite well in the temperature range from 40 ° to 100 °C. These have not yet been studied in reference to MEOR, but they reduce sugars and other carbohydrate compounds to methane and carbon dioxide, while reduction of sulfur compounds yields hydrogen sulfide. They are used in the secondary (anaerobic) treatment of sewage. There has been some concern regarding the injection of microorganisms into petroleum reservoirs in the past because of possible pathogenicity (disease-causing) of the microorganisms. Pathogenic microorganisms have never been used for any MEOR field test in the United States. There have been reports of raw sewage injection in Hungary in 1969 and 1970 (Hitzman, 1983). Raw sewage may contain pathogenic or potential pathogenic bacteria and viruses; however, microbial researchers in the United States who have injected petroleum reservoirs with bacteria are not using bacteria that can cause disease.

The literature on bacteria genetics indicates that microorganisms do not easily mutate into pathogenic strains. Specific microorganisms can cause human and animal diseases, and these microbes are generally fastidious, i.e., they require specific growth factors and specialized environments in order to survive. The environment is not favorable for pathogenic microorganisms in a petroleum reservoir; however, any microorganism to be used in field experiments should be tested for pathogenicity before injection.

3.2 RESERVOIR CHARACTERISTICS

Many reservoir characteristics must be determined before applying MEOR. This technology requires consideration of the physicochemical properties of the reservoir in terms of salinity, pH, temperature, pressure and nutrient availability. Rock factors are also important. Natural fractures may alter how microbes can effectively be introduced to the reservoir. The presence of clays may preferentially adsorb biopolymers and biosurfactants, rendering them useless. Carbonates may quickly utilize acids and produce larger quantities of beneficial gasses, such as carbon dioxide.

Only bacteria are considered promising candidates for MEOR. Molds, yeasts, algae and protozoa are not suitable due to their size or inability to grow under the conditions present in reservoirs. Many petroleum reservoirs have high NaCl concentrations and require the use of bacteria that can tolerate these conditions. Bacteria-producing biosurfactants and polymers can grow at NaCl concentrations of up to 8% and selectively plug sandstone to create a biowall to recover additional oil. It is unlikely that a single MEOR method can be applied to all types of reservoirs. One MEOR approach, thermophilic isolates, successively limits the carbon sources and increases the temperature, pressure, and salinity of the media to select microbial strains capable of growing on crude oil at 70 to 90°C and 2,000 to 2,500 psia, and a salinity range of 1.3 to 2.5%. Extremely thermophilic anaerobes that grow at 80 to 110°C have been isolated and cultured. MEOR-participating microorganisms produce a variety of fermentation products, e.g., carbon dioxide, methane, hydrogen, bio-surfactants and polysaccharides from crude oil, pure hydrocarbons, and a variety of non-hydrocarbon substrates. Xanthum gum, a microbial biopolymer, is frequently used in MEOR field testing. Xanthum may be used in well drilling to lubricate drill strings, to help remove rock cuttings from the borehole, and in MEOR, to compensate for decreased pressure in depleted oil wells, thereby aiding production. Desirable properties of polymers for MEOR include shear stability, high solution viscosity, and compatibility with reservoir brine, stable viscosity over a wide pH range, temperature, pressure and resistance to biodegradation in the reservoir environment. Organic acids produced through fermentation readily dissolve carbonates and can greatly enhance permeability in limestone reservoirs, and attempts have been made to promote their anaerobic production. Organic solvents and dissolved CO_2 can decrease oil viscosity. Fermentation gases can repressurize wells, leading to displacement and production of light or conventional crude oil through a revitalized gas-driven mechanism. Residual oil in reservoirs can be recovered when highly permeable, watered-out regions of oil reservoirs are plugged with bacterial cells and biopolymers. Bacteria and nutrients are injected into the reservoir, and the system is shut in to allow the biomass to plug the more permeable region as it grows. Water is then injected (water flooding) to force oil, trapped in less permeable regions of the reservoir, out into the recovery well. The residual oil remaining after water-flooding is a potential target for selective reservoir plugging of porous rocks with in-situ bacterial growth on injected nutrients.

Bacteria may exert a much greater plugging effect when they multiply within the reservoir rock rather than when they are injected and accumulated at the surface. This process is technologically relatively simple and is a dynamically stable system. As water enters the reservoir rock, biomass grows vigorously, diverting flow to the next permeable layer, which then promotes more growth. The process can be limited by restricting nutrients at any time. In a field MEOR study in the Southeast Vassar Vertz Sand Unit, a salt-containing reservoir in Oklahoma, nutrient injection stimulated growth of microbial populations, including several aerobic and anaerobic heterotrophic bacteria, sulfate-reducing bacteria and methanogenic halophiles. Nutrient-stimulated microbial growth produced a 33% drop in the effective permeability in an injection well at North Burbank Unit in Oklahoma, plugging off high-permeability layers and diverting injection fluid to zones of lower permeability and higher oil saturation.

In contrast to the poor experience with exogenous organisms for bioremediation (bioaugmentation), injection of selected microbial species into oilfield pilots in Japan and China resulted in improved oil recoveries of 15 to 23%. MEOR technology has advanced from lab-based studies in the early 1980s, to field applications in the 1990s. Field MEOR projects have been conducted in the US, Australia, China, Rumania, Peru and Russia. These projects report beneficial results in most cases. Reported EOR resulting from MEOR processes vary from no impact, to 13%, 19%, 36%, 50 - 65% and even 204%. In addition to increased oil production, some projects report decreased water production, increased GOR and improved injectivity. While the potential use of MEOR has been of great interest, field use has been limited. However, Petronas has been at the forefront of using MEOR in their Bokor field to increase oil recovery.

More than 400 MEOR field tests have been conducted in the US alone, mostly as single-well stimulation treatments on low-productivity wells, so that reliable data are sparse. Reservoir heterogeneity significantly affects oil recovery efficiency. MEOR technology may be particularly attractive to small, independent oil producers, operators of the approximate 470,000 "stripper wells" in the US. A single-well stimulation treatment might increase the

production rate to 2.8 bopd from 1.4 bopd and sustain the increased rate for 2 to 6 months without additional treatments.

3.2.1 Remaining Oil Saturation. Large quantity of remaining oil will assist the microbes to enter and distribute in the pores of the reservoir rock. The process will assist oil recovery. The lowest level of remaining oil that can be recovered by MEOR application is 25%.

3.2.2 Distance Between Wells. If the flow into the wellbore is radial in nature; beyond the outer limit no fluid can enter the radial system. The quantity of gas produced by the microbes assists in maintaining the pressure in the system. Too close distance between wells can cause interference and affect production. The distance between wells should not be more than 40 acres.

3.2.3 Well Base Temperature. For MEOR application, the well temperature should be such that it allows the microbes to grow and proliferate, and quickly produce metabolites. Certain thermophilic microbes can grow well at a temperature of 55- 85°C. If MEOR injection is planned to wells with higher temperature, then laboratory tests should be first conducted at that temperature.

3.2.4 Depth of Reservoir. The deeper a reservoir, the higher its temperature. Very high pressure would not only modify the morphology of the microbes, but also influence their metabolism mechanism, which may result in inhibiting or stopping their growth. Therefore the depth of the reservoir should be limited to around 8000 ft.

3.2.5 Salinity of Formation Water. Besides inhibiting microbial growth, too high salinity would exert pressure on the electrically charged double layer between the surface of the rock and the bacteria, and this increases their adhesion, and thus hampers the transportation of the bacteria in the oil reservoir. The composition and concentration of the salts are important in

determining the type of microbes which are compatible with the condition. Generally the NaCl concentration that can be tolerated is no more than 10%.

3.2.6 Trace Minerals. Trace minerals such as As, Hg, Ni and Se are used in the MEOR technology. But their concentrations should not be more than 10-15 ppm. Higher trace mineral contents would limit microbial growth, thus affecting the ultimate recovery.

3.2.7 Permeability. Microbe cells should flow through the reservoir layers which still contain oil and produce metabolites there. The contact between the metabolites and oil would eventually result in more oil flowing in the reservoir pores towards the wellbore. Low permeability would limit microbial penetration. Good oil flow and microbial penetration would occur, if layer permeability is no less then 50 md.

3.2.8 Oil Gravity. Crude oils with higher gravity than 15 °API have hydrocarbon chains that can be used by the microbes as their carbon source. At this situation the microbes will become active and produce bioproducts that will be useful for enhancing oil recovery.

Table 3.4 Reservoir parameters suitable for MEOR

Sl. No.	Parameter	Desirable Limit
1.	Oil gravity API	15
2.	Depth Meters	2940
3.	Reservoir Temperature °C	66
4.	Reservoir PressureKg/cm^2	300
5.	ROS, percent	25
6.	Permeability, Md	100
7.	Porosity percent	10
8.	Lithology	Sand stone preferred.
9.	Gas cap	Non to minor
10.	Salinity	100000
11.	Natural Water Drive	Non to minor
12.	Fractures	Non to minor

CHAPTER 4

SUSTAINABLE DEVELOPMENT AND MEOR

As MEOR reduces or eliminates the need to use harsh chemicals during oil drilling, it is an environmentally compatible method of carrying out tertiary oil recovery. MEOR will become increasingly economically feasible as genetic engineering develops more effective microbial bacteria that may subsist on inexpensive and abundant nutrients. Methods for developing and growing MEOR bacteria are improving, thereby lowering production costs and making it a more attractive alternative to traditional chemical methods of tertiary oil recovery.

Microbes offer a number of possible solutions to help improve production or increase the ultimate amount of oil recovered. These microbial processes are usually called MEOR (microbially enhanced oil recovery) or MIOR (microbially improved oil recovery). Most of the time, microbes are often seen as the bad actors in oil fields, causing plugging, corrosion, souring, etc. However, microbes can also be helpful, if one understands how to use them. A number of field-tested microbial processes indicate that additional oil is recovered economically (less that three dollars per barrel of additional oil).

Table 4.1: MICROBIAL INSITU ACTIVITIES - BIOGAS

Microbial Products	Primary Effect	Oilfield Parameters
CO2 (80%), H2 (20%) upto 380ml per gram of molasses,	Increases the pressure upto 20 bar in model experience	Leads to a higher energy potential in the oil field
CH4 as final product from organic acids, alcohol's and hydrogen	Increases the oil volume factor and therefore decreases the oil viscosity changes the pressure	To give a better oil mobilization

Table 4.2 *MICROBIAL INSITU ACTIVITIES – ORGANIC ACIDS*

Microbial Products	Primary Effect	Oilfield Parameters
Acetic-, proponoic- acid	Decrease the pH upto 4.8	Increase the permeability of rock
	Dissolve the rock carbonate rock by about 0-2 tons of rock per ton of molasses	Partially including of fresh not yet drained rock sessions to the fissure system building up new ways of flow

Table 4.3: *MICROBIAL INSITU ACTIVITIES – ACETONE AND ALCOHOLS*

Microbial Products	Primary Effect	Oilfield Parameters
Methanol, ethanol, propanol, butanol	Decreasing the interfacial tension for example against heptane up to 12 – 46, 5 mNm-1	Forces the imbibition of injected, fermented molasses media into the pore canals and fissures to give more oil from rock
Biolipids	Alternated the rock wettability	Break up oil/water barriers and emulsions lead to a better fluid rate

CHAPTER 5

DIVERSE APPLICATIONS OF BIOTECHNOLOGY

5.1 Historical Applications of Microbial Culture Products

5.1.1 Paraffin Control:

Microbial culture products (MCPs) were first used in 1986 in the Austin Chalk formation in Texas to control paraffin deposition. The theory behind these products was that microorganisms can be isolated and combined in novel mixtures which will produce biochemicals that will mimic the action of classic oil field chemicals such as pour point depressants, crystal modifiers and wax dispersants.

The advantage of using such biological products is the fact that the microorganisms will

1) Produce these biochemicals continuously and
2) Attach to surfaces where paraffin deposition is occurring and act directly at the site of deposition.

The first successful application of these products began a pattern of expansion that continued throughout the 80s and 90s. Paraffin deposition results in a variety of problems for oil field operators, ranging from plugging of tubulars to occult formation deposition that reduces formation permeability. A continual increase in the number of products available to the industry allowed the expansion of the microbial technology for paraffin control into a variety of different oil types and formations. Conventional technologies to control paraffin deposition are thermal and chemical treatments. Both of these technologies have limitations that restrict their long-term effectiveness. In particular, hot oil or water treatments may lead to increased formation damage by forcing deposited high molecular weight paraffin into the formation where they can contribute to pore throat plugging and lead to production loss. Development of MCPs represents a successful alternative technology to remove paraffin deposits without

causing lasting formation damage. Long term use of MCPs showed no damage to the oil field production system and their use increased throughout the mid continent region in the early 1990s. An example of the type of changes produced in paraffinic oils that were associated with control of wax deposition is shown in Figures 5.1 and 5.2.

In the first Figure, the viscosity of the oil has been reduced by microbial treatment. This reduction in viscosity is probably due to the production of small solvent molecules by the microbial population. These solvent molecules include alcohols, ketones and aldehydes and are functionally similar to oil field chemicals used as wax dispersants and pour point depressants. This "thinning" action can also be seen in Figure 5.2 where the metabolic capacity of the microorganisms to degrade high molecular weight paraffin molecules results in a change in the hydrocarbon profile of the oil as detected by gas chromatography. With a reduction in the average molecular weight in the hydrocarbon component, increases in API gravity and reductions in viscosity are frequently seen. This reduction in viscosity may lead to increased relative permeability and increased oil production.

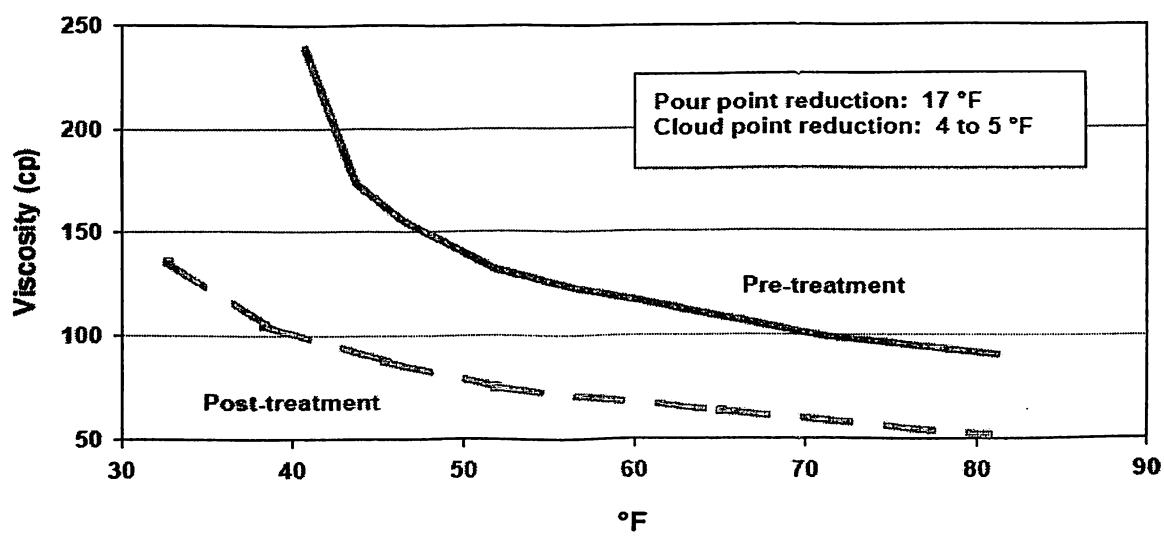


Figure 5.1: Effect of treatment with Microbial Culture Products on crude oil viscosity.

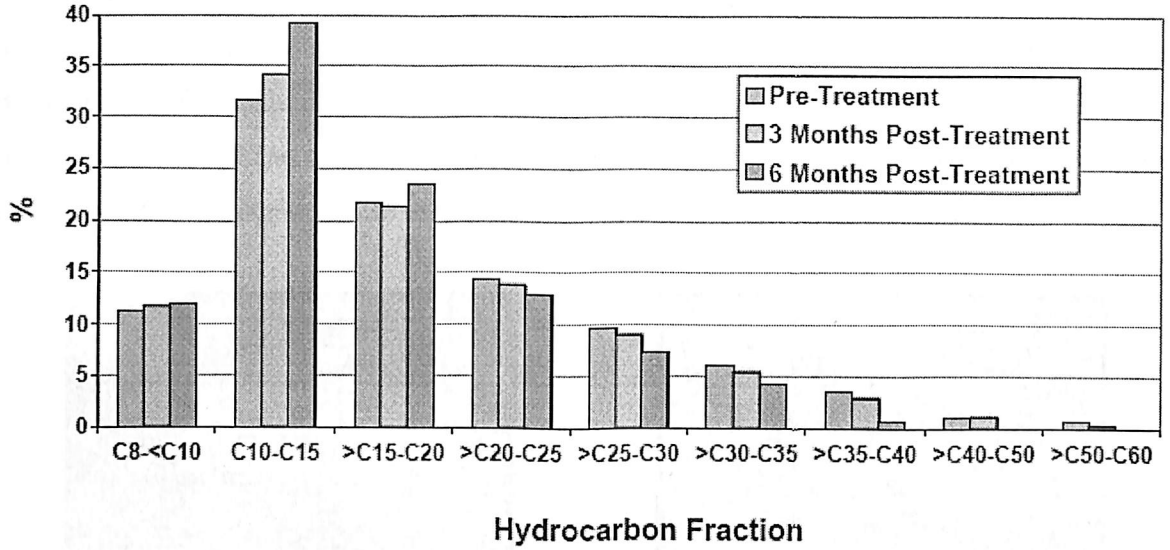
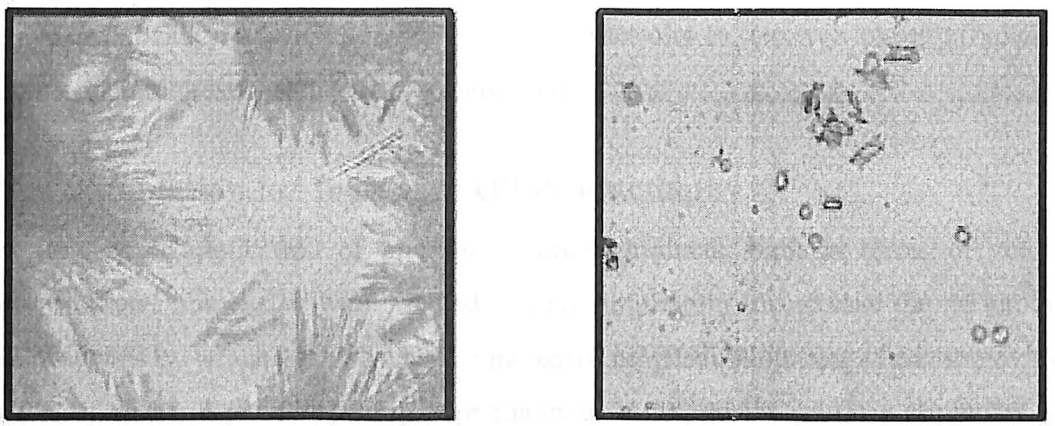


Figure 5.2: Effect of treatment with Microbial Culture Products on hydrocarbon distribution in wellhead samples of crude oil.

5.1.2 Scale and Corrosion Control:

Observations that some of the biochemicals produced by microorganisms had properties similar to scale and corrosion control chemicals lead to the development of new MCP product lines to address these oil field problems. The deposition of mineral scales in oil wells is a well-understood phenomenon. It is often related to the commingling of waters of different chemical types that produce a blend of ions that exceeds the solubility limit for compounds such as calcium carbonate, calcium sulfate or barium sulfate (to name the most commonly encountered oil field scales). Scale deposition can also be related to temperature and pressure changes occurring in the production string as the fluid column is brought to the surface. Conventional chemical technologies utilize compounds that control scale through either chelation or dispersant mechanisms. Microbial biochemicals such as organic acids are naturally occurring chelating agents that bind cations and thus restrict their capacity to form mineral deposits. Other microbial compounds may act as filming agents, coating surfaces and preventing nucleation sites for scale growth from forming. The ability of MCPs to prevent scale growth *in vitro* is shown in Figures 5.3.These photomicrographs show how the growth of typical oilfield scale crystals in brine can be controlled by treatment with MCP's. The mechanisms by which MCPs control scaling are chelation, crystal modification, and dispersion of scale nuclei.

Filming activity of biosurfactants also prevents attachment of scale crystals to surfaces. Such filming agents may also act as passivating agents for controlling corrosion of metal surfaces. By coating surfaces, the interaction between corrosive compounds such as carbonic acids and sulfides is mitigated and corrosive processes reduced. Another effect associated with microbial culture product use is a decrease in the number and activity of sulfate reducing bacteria.



Untreated Treated with MCPs

Figure 5.3: Effect of microbial culture products on formation of calcium sulfate scale in brine. Note large scale crystals in untreated solution.

5.1.3 Waterflood Treatments

MCPs have found successful applications in waterfloods as well. In waterflood operations where injection of the drive fluid is restricted by scale and/or organic deposits, MCPs have been used to open up injector wells and improve injectivity. Deposits of mineral scale can form at any location in an injection system from surface equipment to downhole in the formation, and preformed scale from the surface may be carried downhole by the fluid flow. Skin and formation damage can also occur in injection wells by organic deposits such as paraffin or asphaltene precipitates, or stable emulsions formed from residual oil and grease carried over during re-injection of produced water. Buildup of scale and/or organic deposits in the near wellbore region blocks pore channels, which decreases permeability and can severely restrict fluid flow into the formation. These occlusions are a leading cause of reduced waterflood efficiency. MCP scale and corrosion control products can be used to inhibit and remove these occlusions from injection wells. Treatment of injection systems with MCPs has been shown to

increase volumes of water injected as well as reduce injection pressures and energy costs. In the reservoir, MCPs produce several enhanced oil recovery compounds that decrease capillary forces and increase oil mobility. Microbial metabolites such as surfactants, solvents, low-molecular-weight organic acids, and gases are well-known oil mobilizing agents. These products work by the same mechanism as traditional EOR chemicals to reduce interfacial tension, decrease oil viscosity, and improve the microscopic sweep efficiency of the waterflood. Application of MCPs in waterflooded reservoirs to improve sweep efficiency and increase recoverable reserves has been widely used.

5.2 Well Stimulation for Increased Oil Production:

By far the largest application of microbial culture products, both in terms of volume of products used and number of wells treated is well stimulation to increase the oil production rate. Mechanisms by which MCPs stimulate increased oil production range from removing skin and/or formation damage and opening pore channels, to improving oil flow properties. As oil flows through the reservoir toward the wellbore, high-molecular-weight fractions such as waxes, paraffins, and asphaltenes precipitate out of the oil, forming deposits on the rock matrix and occlusions in the pore channels. This deposition can become much more severe if the producing temperature is near or below the cloud point, or if decreased pressure near the wellbore allows dissolved gases to bubble out of the oil. Buildup of organic deposits can decrease permeability, change relative permeability, and restrict or block fluid flow through pore channels. In addition to organic deposition, skin and formation damage can also be caused by precipitation of mineral scales, or by formation of stable emulsion in the near-wellbore region. Stimulation of wells with MCPs involves injection of specific microorganisms into the damaged zone, where they can colonize the region and produce bioproducts *in situ*. The production of the aforementioned solvent molecules can directly solubilize hydrocarbon deposits present in pore throats, increasing the effective porosity and permeability of the formation. Biosurfactants can also solubilize such deposits, in addition to mobilizing scale particles and other occlusions from the fluid channels. When the well is reopened after stimulation, damage from the near-wellbore region is mobilized in the fluid flow and removed from the well.

A significant advantage of MCPs when compared to traditional technologies is that colonies remain in the formation for some period of time after production is resumed, and continue to metabolize specific compounds and produce bioproducts, which affect oil properties. Strain-specific metabolic activity targets long-chain paraffins and shortens the paraffin chain, causing a distinct shift in the hydrocarbon distribution. Direct metabolic action of the microorganisms on the oil can reduce oil viscosity and increase relative permeability. Biosurfactants can also produce changes in wettability, increasing relative permeability. A variety of positive mechanisms are thus available in using MCPs to increase oil flow from the formation and increase production. The typical protocol for such treatments to increase production involves a bullhead injection of product-water mixture into the formation at matrix rates. Injections are generally planned for delivery 3 to 6 feet into the formation. This maximizes the likelihood of removing hydrocarbon deposition, emulsion blockages or other types of near wellbore formation damage. Stimulation of production wells with MCPs commonly results in a 20–50% increase in oil production rates. Production curves illustrating the types of increases obtained are given in Figure 5.4 and 5.5. The wells shown in these figures were producing medium gravity (~ 36 ° API) paraffinic oil from a sandstone formation. Wells exhibited signs of skin and formation damage, and production rates were declining due to paraffin deposition in the near-wellbore region. Stimulation injections were designed to treat a 5-6 ft. zone around the wellbore for paraffin damage and increased oil production. Oil production increased by an average of 39% (field average) after stimulation with MCPs and production rates remained elevated for more than one year.

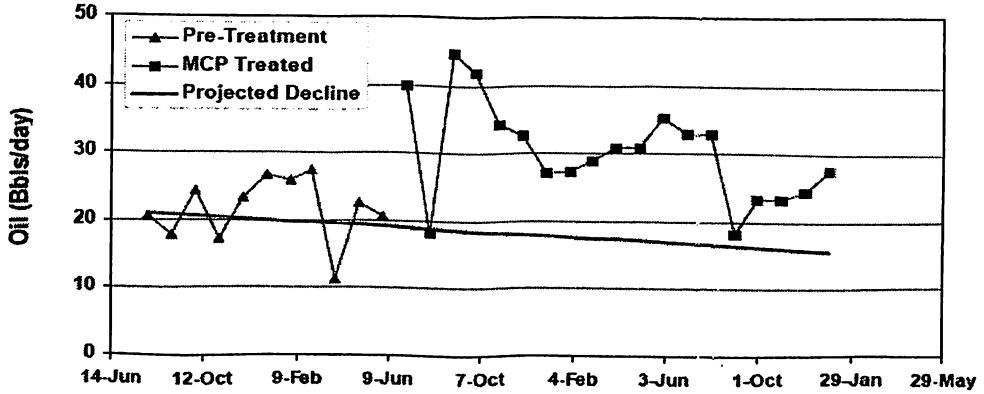


Figure 5.4: Oil production history for well group #1.

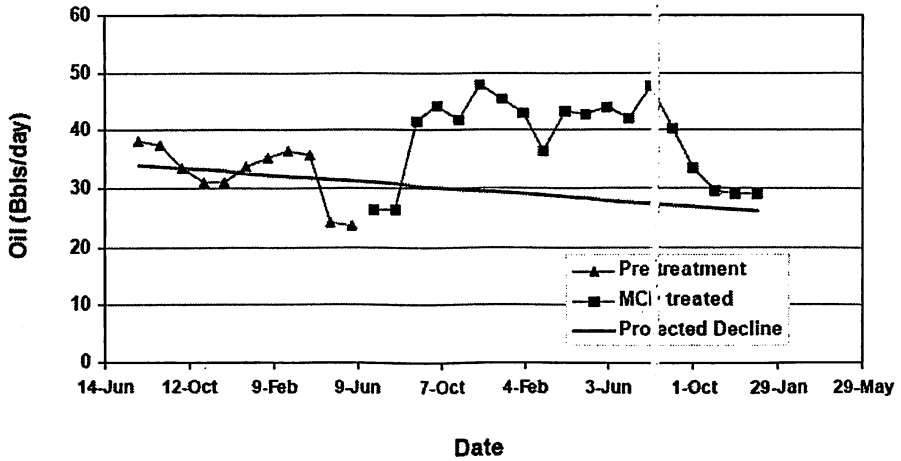


Figure 5.5: Oil production history for well group #2.

5.2.1 Frac Damage Repair

Another area where MCPs have been used successfully is to remediate polymer damage caused during well fracturing. Hydraulic fracturing is a widely used stimulation method to increase production from oil and gas wells. It is routinely used in new completions to increase drainage rates and maximize field development. Also, favorable economics in today's markets have caused many producers to fracture stimulate developed or mature fields in order to get the most out of existing wells and maintain production rates. However, the full potential of fracture stimulation is often not realized because gelling agents used in the frac fluids cause formation damage. Polymer solutions or gels are normally used in frac fluids to increase viscosity. A common problem that frequently plagues the use of frac polymers and gels is incomplete or inadequate breakage of gel. Residual gelling agents cause damage to the formation by decreasing permeability and blocking flow from producing zones. This can lead to lower than expected production increases from fracturing jobs or in extreme cases, complete loss of production. Microbial culture products have been developed which will degrade specifically with minerals or other chemicals, and they are not consumed by the reaction. Instead, MCPs act as true catalysts to break down the polymer. They catalyze specific metabolic reactions such as breaking the polymer backbone and/or removing or modifying functional side groups. This specific metabolic activity decreases the molecular weight of the polymer and reduces

fluid viscosity. An example of polymer degradation and subsequent viscosity reduction in a frac fluid is shown in Figure 5.6.

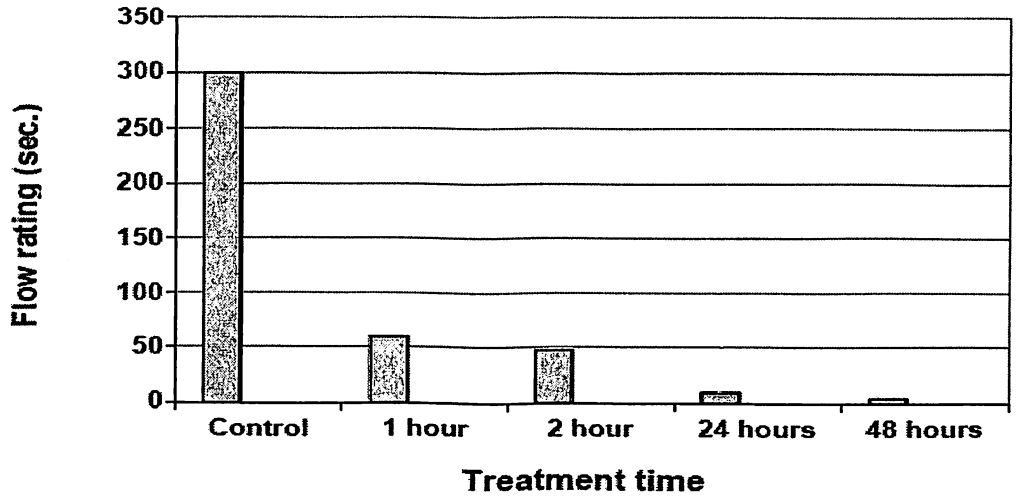


Figure 5.6: Viscosity reduction of frac fluid (guar cross linked with borate) with Microbial Culture Products

The frac fluid is a guar-based gel cross linked with borate. After the MCPs initially attack the gel and significantly reduce viscosity, they continue to degrade the polymer long after a reactive chemical breaker would have been expended. This biocatalytic activity is inherent of MCPs and insures that their activity will continue until the polymer is degraded. Continued degradation and removal of both broken and unbroken gel material through the long-term activity on MCPs allows improved formation flow, and leads to increased production in the well. Examples of two wells which sustained severe damage after fracture stimulation with guar-based fluids are shown in Figures 5.7 and 5.8.

The well in Figure 5.7 was producing approximately 150 Mcf/d of gas from a carbonate formation before fracture stimulation with a guar-based fluid. Swabbing was performed after fracturing, but the well would not flow back the frac fluid and gas production was completely blocked. Treatment with MCPs designed to degrade the guar polymer resulted in flow back of frac fluid and commenced gas production. The well continued to release fluid with residual frac polymer for several months after production resumed.

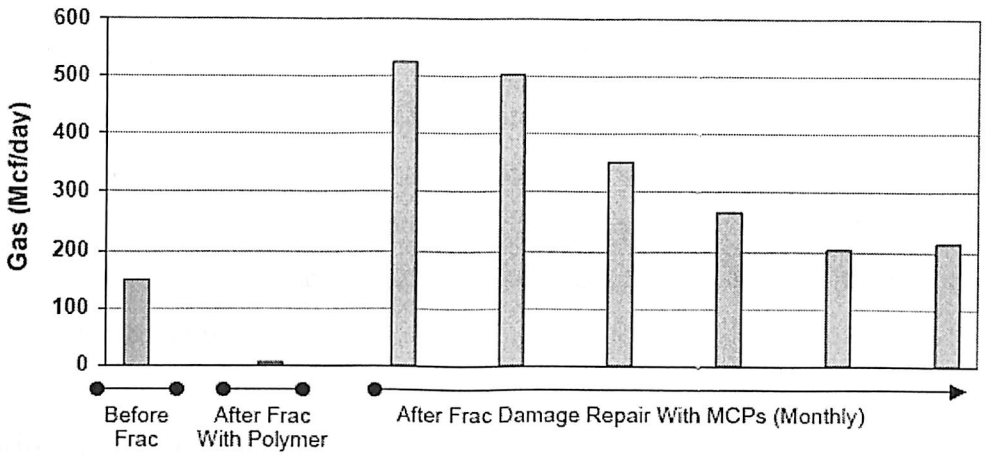


Figure 5.7: Production history for gas well treated with Microbial Culture Products to repair frac polymer damage.

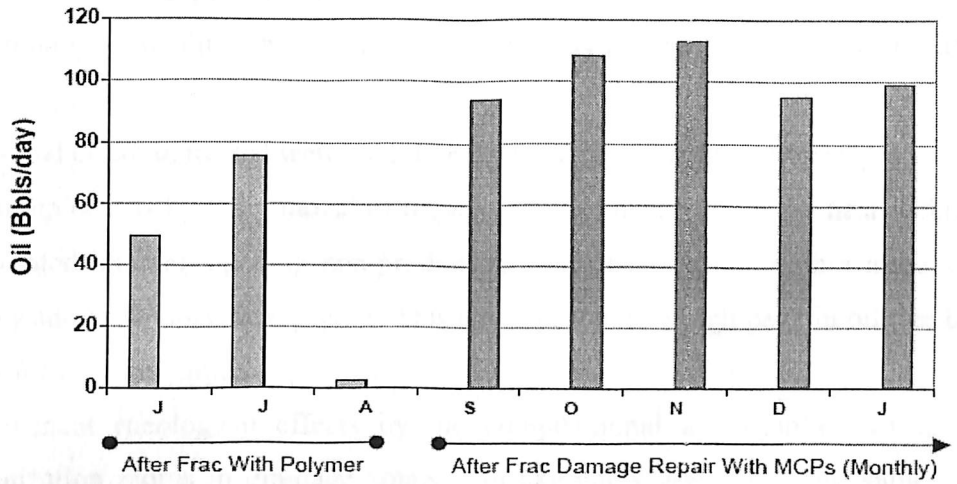


Figure 5.8: Production history for oil well treated with Microbial Culture Products to repair frac polymer damage.

An oil well shown in Figure 5.8 flowed for approximately two months after fracture stimulation before residual polymer in the produced fluids reached a plug point and the well plugged off completely. MCPs were injected into the well to degrade the residual polymer and reopen the flow channels to fluid flow. When flow was resumed the oil production rate quickly exceeded the maximum rate the well had produced at before treatment with MCPs, indicating that even before reaching the point of plugging off, production was severely restricted by residual polymer damage. After treating the residual polymer damage, oil production stabilized at the elevated rate.

5.2.2 MEOR in Waterflooding Schemes: Conceptual frame

Microbial Enhanced Oil Recovery (MEOR) technology is based on the systematic inoculation of producing and/or injecting wells with hydrocarbon-degrading anaerobic facultative microorganisms and complementary nutrients. The primary goal of this method is to extensively colonize the poral medium of the oil-bearing formation. Two main procedures are used to colonize a target reservoir:

1) Treating producer wells

By pumping downhole periodic microbial batches in order to reach the near wellbore poral space via tubing or annulus. This methodology is usually referred to as simply MEOR.

The microbial effect in treated wells could be detected in two consecutive stages:

A) Clean up effects by the removal of organic damage occurring in the near wellbore of the perforated interval, opening non-productive zones bearing oils with a more segregated, heavy and pseudoplastic behavior. This effect produces a high peak in oil rate but usually only for a limited time.

B) Permanent rheological effects by the compositional alteration occurring at deeper colonization radius in drainage zones with extremely low shear rate values (low fluid velocity). This effect is the most important MEOR objective to pursue in treating producers, as this improvement is sustainable for a long time if appropriate microbial inoculation schedule is continued.

2) Treating injector wells

By introducing the microbial treatment using the injected water as carrier to introduce colonizing microorganisms into active stream tubes of flooded space of the target layers. This

is frequently termed as MEOR2, because it is usually used in combination with the simultaneous treatments in injectors and producers described above.

The advantages of treating injectors are potentially larger size of colonized zones, longer residence time of microorganisms in target layers and the role of multiple enhanced recovery mechanisms:

1) Rheology alteration of moveable oil in active stream tubes and its direct impact on fractional flow and oil/water mobility ratio,
2) Depletion in residual oil saturation (S_{or}) by in-situ production of biosurfactant, and
3) Conformance efficiency improvements created by the colonizing biomass in active stream tubes that diverts fluids to unflooded zones.

5.2.3 Possible Mechanisms of Oil Gains

Three possible mechanisms to explain the changes of the well performances have been made:

1. **Skin reduction and Improvement in PI** – Based on the pressure buildup tests, it is proven that the microbes can clean up the damaged formation. For this case there could be a solubilization of heavy components that plugged the near wellbore formation.
2. **Demulsification & Destabilization of emulsion** – It was physically observed that emulsion problem has been reduced. Though this is not the ultimate objective of the technology application, it helps to improve the oil production. It can be explained by coalescence of oil droplet which bacteria cells act as wetting bridges between oil droplets in the continuous phase.
3. **Watercut reduction** – This could be explained by the possible change in fluid relative permeability due to wettability change and reduce in interfacial tension between oil and water.
4. A combination of microorganisms is necessary to achieve a successful adjustment of the bacterial community to specific substrates (oils), enhancement mechanisms and reservoir conditions. Microbial products are also conditioned to have an adequate balance in C/N and C/P ratios and type of complementary nutrients, buffers, trace elements (K+, Na+, Mg++,

Ca++, Fe++/+++, Zn++, Co++) and bio-catalyzers, since formation water usually lacks sufficient nitrogen and phosphorous.

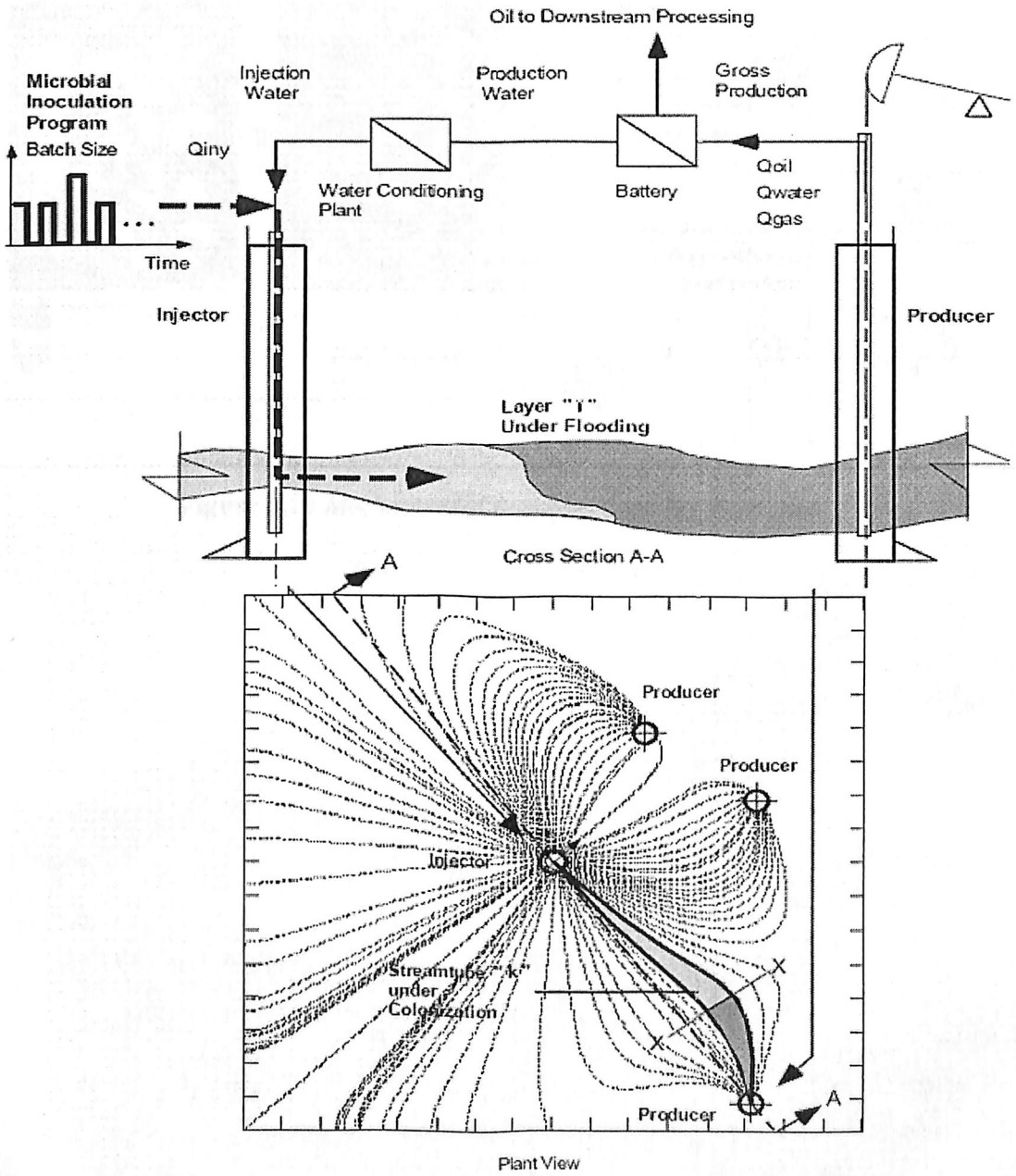


Figure 5.9: Streamtube colonization in Target Sand by Inoculating Injection water, conceptual frame

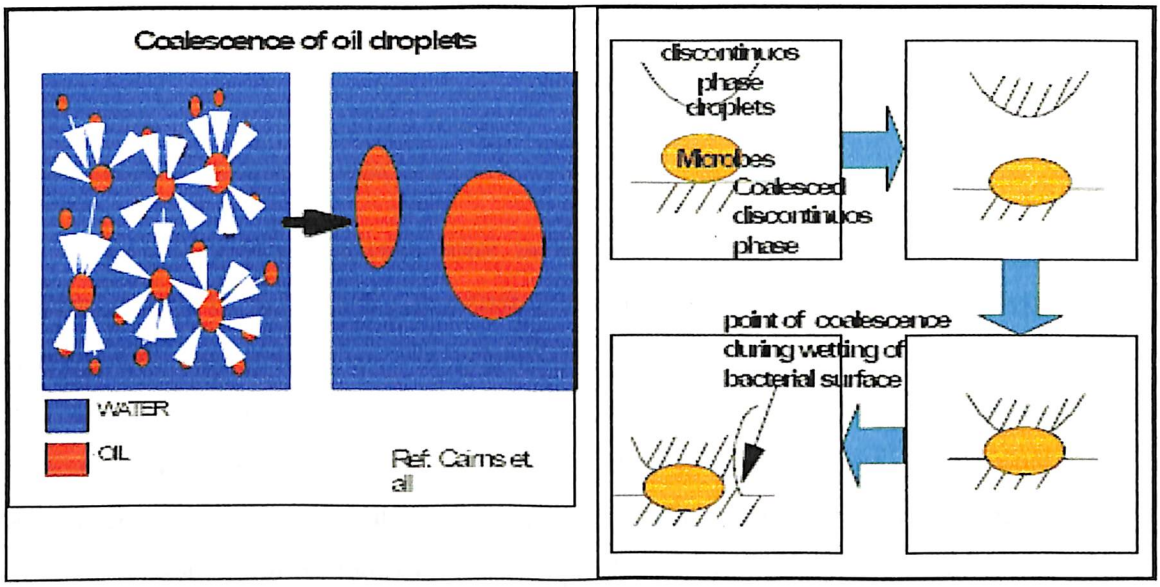


Figure 5.10 *Mechanism of Emulsion Stability Reduction.*

CHAPTER 6

CASE HISTORIES

6.1 Research Studies in the Arabic Area:

More than ten strains of bacteria were isolated from Saudi and Egyptian crude oils and formation waters. Experimental investigation was carried out to identify the bacterial isolates, determine the compositions of the appropriate nutrients, and conduct surface phenomena measurements. Based on the results obtained, it was found that presence of bacteria affected the solution phase volume, interfacial tension between oil and water, rock wettability, and relative permeability characteristics. These effects depend upon the bacterial type, nutrient type and concentration, salinity, temperature, composition of the crude oil. A series of microbial displacement laboratory tests were carried out in homogeneous sand packs and Berea sandstone cores using different types of bacterial solutions of glucose, sucrose and molasses based nutrients. The effects of nutrient type and its concentration, bacterial type, salinity, API, and permeability on oil recovery were investigated. Results show that the greatest oil recovery was obtained from activation of the indigenous bacteria by 1% molasses concentration. Some strains of bacteria were found to produce biogas, biosurfactant and biopolymers which increased oil recovery. The changes in sand-pack permeability or API gravity have no effect on oil recovery.

6.1.1 Role of Microorganisms on Interfacial Forces, Phase Variation and Rock Wettability:

Studies were performed to investigate the effect of biochemical's from microorganisms, originally present in the crude oils, on the interfacial forces, phase variation of oleic/aqueous systems and rock wettability. In some of these studies it was found that interfacial and surface tension was markedly affected by nutrient type and concentration (see Fig. 6.1).

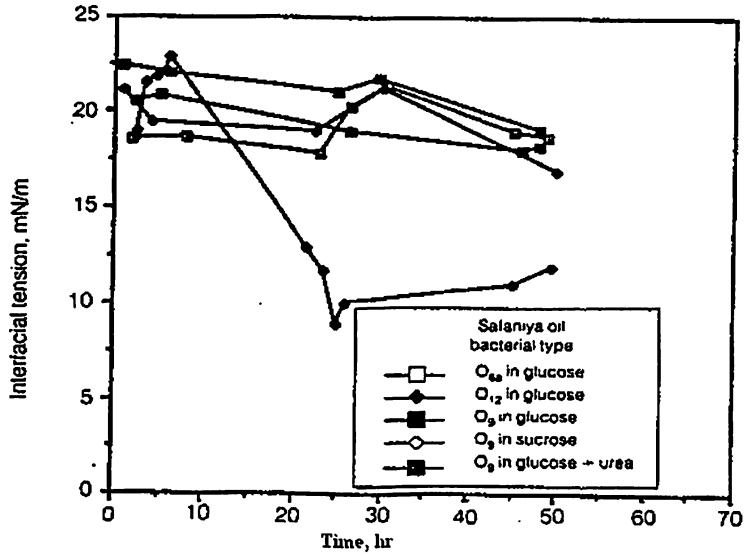


Figure 6.1: Variation of IFT w.r.t. Time for different bacterial types

This effect depends on the temperature at which the tests were carried out. In another study, two Egyptian crude's were used, one of them contained bacteria of *Clostridium* type and the other contained *Bacillus* type. The investigators found that, for each crude oil, the phase variation and interfacial tension was affected not only by the bacterial nutrient type and concentration but also by salinity, temperature and time of contact between the crude oil and the nutrient used. The effect of temperature, as an example, on the phase variation is shown in Figs. 6.2.

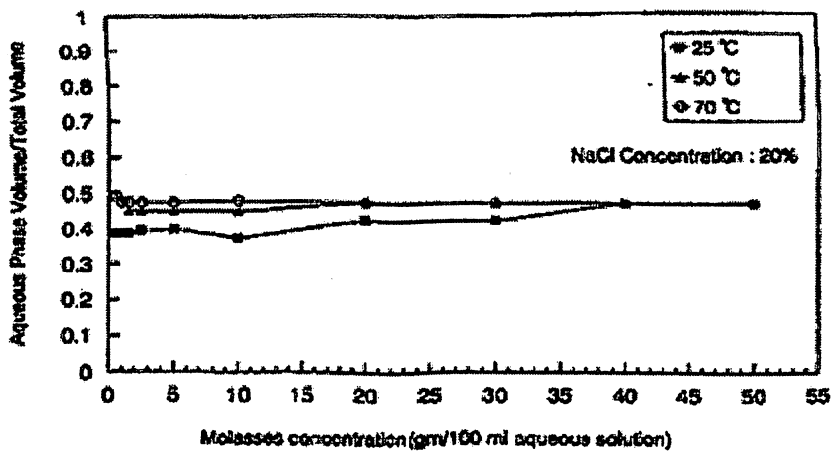


Figure 6.2: Effect of Temperature on phase volume for molasses solution and crude oil using 20% NaCL

This figure indicates that this effect depends on the type of crude oil used. A study of the applicability of MEOR for recovering more oil under the Arab oil field conditions was presented by Sayyouh. Based on the analysis of data obtained from more than 300 formations in seven Arab counties, (Saudi Arabia, Egypt, Kuwait, Qatar, UAE, Iraq and Syria); the possibility of the application of MEOR to the Arabian area was investigated.

6.2 Measurement of parameters of AH, UZ and UAD crude oil:

Fig. 6.4 shows the effect of temperature on the IFT of UAD crude. Clearly at low temperature, the microbes have insignificant activity, which results in no side products and no alteration of IFT. At the elevated temperature 60°C, an abrupt decrease in the interfacial tension of the system coupled with gas bubbles evolving from the solution was observed. This indicates the increased activity of the microorganisms. A bacteria free solution and a bacteria containing solution. Results are shown in Fig. 6.4. These results clearly demonstrate that bacteria metabolism is essential in reduction of IFT at relatively high temperature of around 60°C. A 70 % reduction in IFT of the AH crude for the microbial rich solution was observed. Results of the measurement of IFT employing the UZ crude indicate the reduction of IFT starts gradually at a slightly lower temperature than 60°C as shown in Fig. 6.5. The microbial solution did not significantly affect the IFT of the St Crude oil. The presence of sulfur in the oil seems to reduce or eliminate the microbial metabolism, thus preventing the bacteria from producing a surfactant.

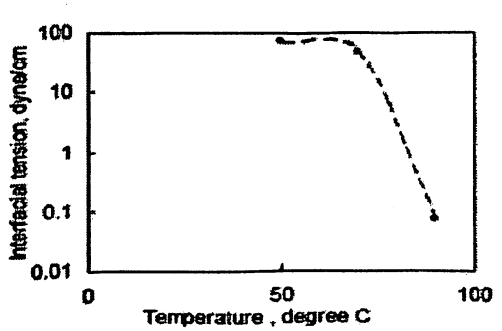


Figure 6.3 : Effect of temperature on interfacial tension, UAD crude oil and bacteria solution

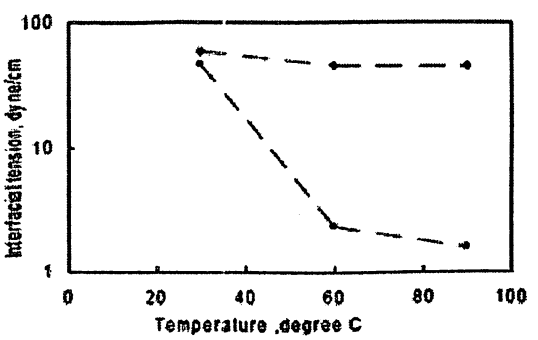


Figure 6.4: Effect of temperature on the interfacial tension, AH crude oil and Bacteria solution

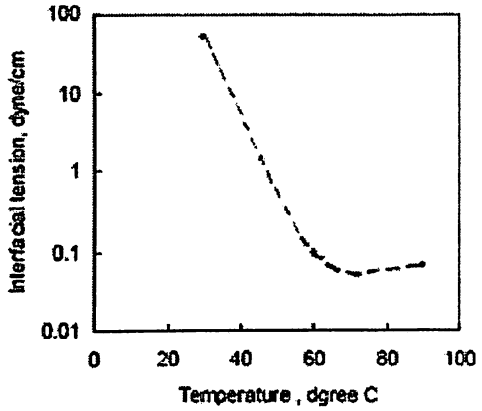


Figure 6.5 Effect of temperature on interfacial tension, UZ crude oil and bacterial solution

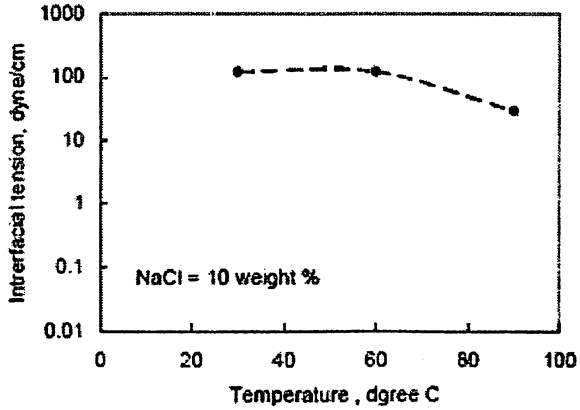


Figure 6.6 Effect of temperature on interfacial tension, St crude oil and bacterial solution

Different limestone cores were used to assess the viability of microorganisms in a tertiary flooding process. Figure 6.7 (for core which has a pre-microbial flooding absolute permeability of 19 md) is a typical plot for tertiary floods conducted. Microbial flooding started after water flooding at 45% residual oil saturation. As a result of microbial action, the residual oil saturation dropped to around 11%.

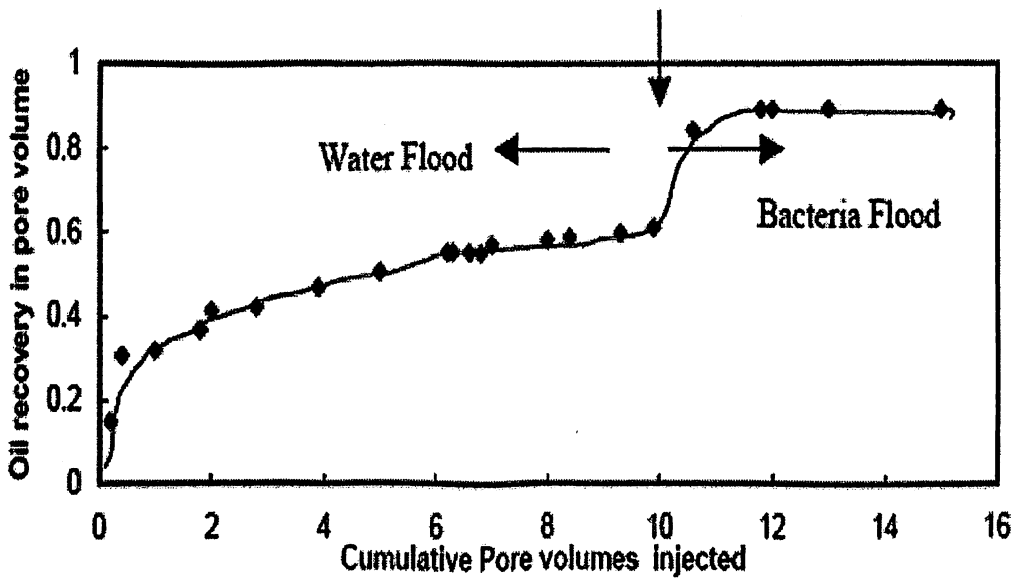


Figure 6.7: Oil recovery vs. pore volume injected for Core#BF3, K=19md, Flow rate=1.2 ml/min

Another mechanism responsible for the improvement of oil recovery is the selective plugging. Selective plugging of high permeability zones by these microorganisms can improve sweep efficiency. Two types of microorganism plugging have been identified: plugging by viable cells and plugging by nonviable microbes. Non-viable cells (dead bacteria), were observed for the rod bacteria type used in this work which could not tolerate reservoir conditions, act as particulate agents, since they don't produce slime. Viable microorganisms have the ability to adhere to the rock surface and produce excellurlal which cover the cell and the rock surface forming a polymer film. This phenomenon was observed and is shown in Figure 6.8. It is an electronic image of the outlet bacteria solution dried on the surface of glass. The bacteria are forming sessile (attached) which occupy the pore space. The pores surface characteristics are changed by the presence of sessile bacteria. This selective plugging will result in microscopic change in the limestone rock. Jenneman *et al.* reported that bacteria are capable of producing enough bio-mass inside a rock core to produce significant permeability reduction (60- 80%)

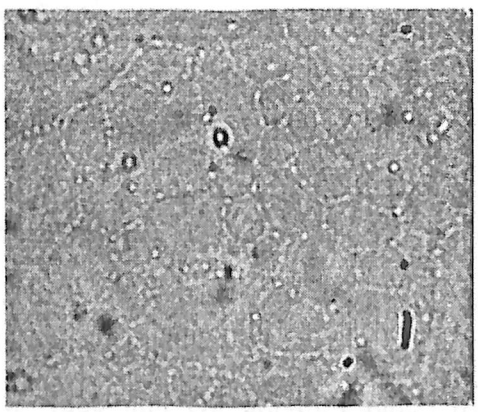


Figure 6.8: The Sessile Bacteria plugging the pore space(x1000)

Conclusions

Based on the study, the following conclusions can be stated:

1. Increases in oil recovery by tertiary injection of thermophilic bacteria obtained from UAE environment in limestone cores was observed.
2. The mechanism for enhanced recovery seems to be through production of bio-surfactant, bio-gas, and biomass by the bacteria.

3. Production of the bio-surfactant reduced the IFT for the AH, UZ, and UAD crude. The presence of sulfur in the St crude oil hindered the interfacial activity of the bacteria.
4. A successful bacteria EOR flood was obtained even in a tight limestone core whose permeability was as low as 1.7 md.
5. Minimum oil saturation (at or above 25%) is required for the success of MEOR process.

6.3 Feasibility Analysis of MEOR in Bokor Field, Sarawak

Bokor field was selected as the first field in Malaysia for Microbial Enhanced Oil Recovery (MEOR) technology application which utilizes micro-organisms to facilitate, increase or extend oil production from reservoir through the production of biochemical such as biosurfactant, solvents, gases and weak acids. The field was selected due to its high viscosity crude (4 to 10 cp) and low oil specific gravity of 20^0 API which resulted in low recovery factor in major reservoirs ranges from 19% to 25% of its original oil in place. This technology also seems to be attractive on the field as it was initially thought to be potential for reducing the viscosity of the oil and thus improve oil recovery. In addition, reservoir properties for major reservoirs in Bokor field conform to the basic screening criteria of the MEOR application.

6.3.1 Description of Bokor Field

Bokor field is located in the Baram Delta Area about 40 kilometers offshore Lutong (Miri) at a water depth of approximately 220 feet below msl. The porosities range between 15 and 32% and permabilities from 50 to 4000 md. Oil gravities range from 19^O to 22^O API in the shallower reservoirs (1500 - 3000 Ft.) to 37^O API in the deep reservoirs (6300 Ft.). The reservoirs in the Bokor field can be divided into two main groups i.e. the Main Reservoirs (A - F) and the Deep Reservoirs (H - L). The study is focused on A reservoir which is the shallowest reservoir for the field. The application of MEOR seems to be attractive for Bokor field mainly due to: -

- Low recovery factor in major reservoirs (19 % - 27%)
- Viscous oil (2 cp - 10 cp) produces from shallow reservoir
- Bokor data conform to the basic screening criteria of MEOR application (Table 6.1)

- In line with Petronas Initiative in promoting IOR/EOR technology application in Malaysia

Table 6.1: Screening Criteria

	Screening Criteria
Oil Gravity (°API)	10 to 50
Temperature (°F)	<270
Water Salinity (ppm Cl-)	<100,000
Parafin wax (%)	>=3
pH	5 to 8
Previous biodegration	Little or none
Avg. Permeability (md)	>=20 md
Porosity (%)	>=10
Oil viscosity (cp)	5 to 50
H_2S (ppm)	<10,000
Pressure Gradient (psi/ft)	>0.10
Water cut (%)	10 to 50

Feasibility Study:

The feasibility studies of MEOR technology application in Bokor field was carried out and completed in April 2000. During the feasibility studies, the focus was on the Main Reservoirs with priority on the shallowest reservoirs i.e. A reservoir. Out of five reservoirs, A reservoir was identified to be potential candidates for MEOR application [17,18]. Conclusions from the Lab studies were: -

- Analysis on the **crude properties** of this reservoir indicate low wax, low sulphur, low asphaltenes and low pour point.
- **Biodegradation study** indicates complete removal of normal/branched alkanes and partial removal of aromatics due to in-reservoir alteration.
- **Core study**: The product (MEOR) used is non-damaging to Bokor sandstone formation.
- **Rheology study**: There is a slight viscosity reduction in sample inoculated with microbes.
- **Emulsion breakout test**: Samples collected for this study are found to have emulsion problem. These emulsions are very stable at room condition The result indicate that the

product is capable of breaking-up the emulsion within 24 hours of inoculation for most samples.

- **Geochemical Test**: After inoculation, there is an increase in the solubility of high molecular weight component as a result of biosurfactant activity.

Pilot Project:

The pilot test was recommended to extend the laboratory the feasibility study to the field study. In July 2000, the pilot project was successfully carried out on three strings namely, B-1, B-2 and B-3. A dedicated team was formed for the pilot project implementation. The specific objectives of the pilot project were:-

- To demonstrate the feasibility of microbial application in the Bokor field.
- To assess the impact on oil production (incremental oil gain and sustainability) during 5 - 6 months monitoring period.
- To justify the potential of future full field scale implementation.

Performance Monitoring/Analysis Strategy for Pilot Project:

Performance monitoring/analysis strategy was developed to effectively measure or assess the performance of the project. The following parameters were being monitored/tested: -

Wellhead Sample/Lab Monitoring/testing

- Compositional analysis
- Dynamic viscosity
- Emulsion Stability (@ reservoir temp.)
- API gravity
- Asphaltene, wax, Sulphur, pour point
- Formation water analysis & Bacteria (SRB) analysis
- Emulsion stability (on-site @ room temp.)

Production Performance Monitoring/Testing

- Production well test (oil, water cut, FGOR, gaslift injection rates)
- Tubing and Casing head pressures.
- Sand production.
- BHP surveys (permeability, skin, and productivity index).

Wellhead Sampling

Wellhead samples were collected at specified frequency through out the project monitoring stage. To enable a reliable comparison of the MEOR treatment performances and effectiveness, pre MEOR samples were also included in samples collection as baseline information. Two types of samples were taken i) the crude oil and ii) the formation water samples. The samples were taken manually from the wellhead sampling port of each string. The crude samples were labeled and the formation water samples were preserved according to the test requirement. The preserved samples were sent to lab for detailed analyses. To minimize the operation cost, the project was carried out as part of the other planned acidizing campaign. Upon completion of injection, the wells were shut-in.

Pilot Project Results

This section discusses the production performance and wellhead sample results over the five months period after the MEOR treatment.

1) Production Performance Results

Production Baseline - Pre-MEOR production trends for the three wells were generated to forecast the well performance without MEOR treatment. This is required in order to assess the well production impact after the treatment. The trends were generated by performing a decline/incline curve analysis over the available historical production data (10–15 years) for oil, water cut or GOR. In order to minimize the effect of gaslift variation on production, the injection rate was closely monitored and controlled to its pre MEOR rates (0.2 - 0.3 MMscf/d).

B-1 Post MEOR Results - The well test results for the first two weeks indicate that the gross production has increased from 600 b/d (baseline) to 1500 b/d. Historical data confirms the

gross production for the string could only reach maximum 600 - 800 b/d even though after long shut in period. This indicates that the sudden high gross production could be due to the MEOR treatment. However, two weeks after the treatment, the gross production has dropped and maintained at its baseline. Formation GOR and wellhead pressures were also monitored and no change observed for these performances. Based on the above production test data and daily water cut performance, the monthly average net oil production data was estimated. Over the past 5 months (post MEOR), the average oil production increased from 152 b/d to 334 b/d. The increase in oil production was mainly due to the drop in water cut performance from 75% (pre MEOR) to average of 45% (water cut performance post MEOR fluctuated between 30% to 82%). The average oil gain of B-1 for 5 months is 182 b/d (equivalent to 120% oil incremental).

B-2 Post MEOR Results – Initially, the gross production for post MEOR was slightly higher. The gross production declines and approaches the baseline. Formation GOR and wellhead pressures were also monitored and no change observed for these performances. The water cut (Figure 6.9) declines from 30% to 20% before increases to the baseline. The average oil gain for B-2 for 5 months is 41 b/d (equivalent to 15% oil incremental).

B-3 Post MEOR Results - The gross production for post MEOR was higher than its baseline. Figure 6.10 shows the net oil and water cut performance. For 5 months post MEOR, no change observed in water cut but due to higher gross production average oil gain of 41 b/d (equivalent to 36% oil incremental) was realized.

Overall Production Performance – As shown in Figure 6.11, the average total oil gains from the three strings are 274 b/d (equivalent to 47 % oil incremental). This is beyond the expectation of the project which was estimated about 20% oil gains. The higher oil incremental is mainly contributed from the high water cut well, B-1.

Downhole Pressure Buildup Survey Results – Table 6.2 shows the downhole pressures buildup survey for the strings. No permeability change observed. However, there was a slight improvement in skin and PI for the low permeability wells, B-2 and B-3. This could be one of the causes that contributed the higher gross oil production as mentioned above.

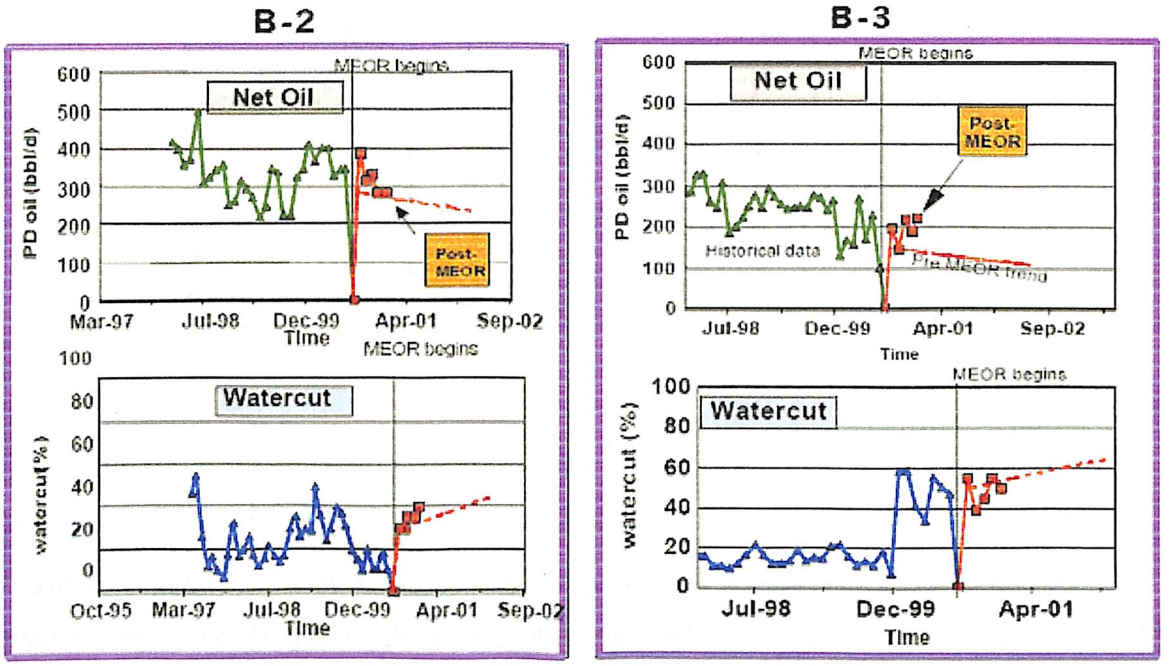
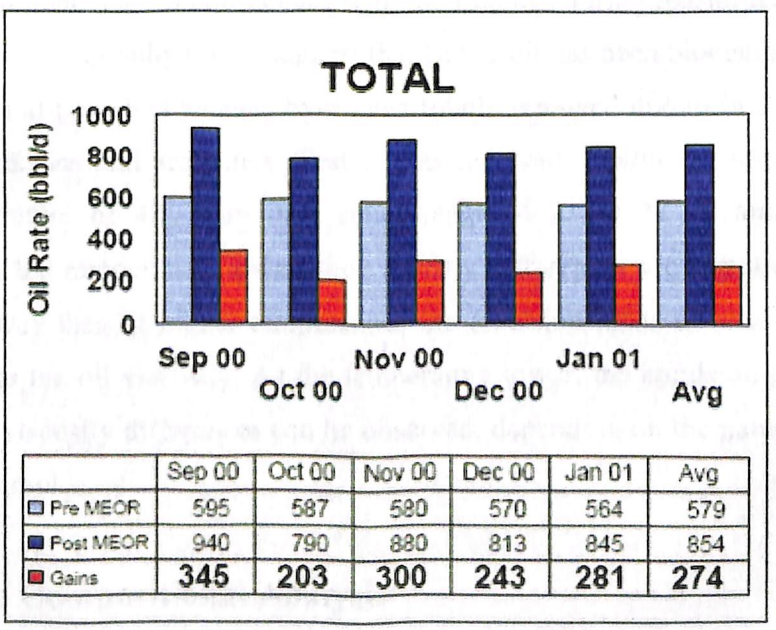


Figure 6.9 & 6.10: B-2, B-3 Net oil/water cut Post MEOR Performance respectively



	Sep 00	Oct 00	Nov 00	Dec 00	Jan 01	Avg
Pre MEOR	595	587	580	570	564	579
Post MEOR	940	790	880	813	845	854
Gains	**345**	**203**	**300**	**243**	**281**	**274**

Figure 6.11: Total Monthly Average Net Oil Production Performance

Table 6.3: Pre and Post Results

	Pre Treatment			Post Treatment		
Well	Permeability (mD)	Skin	Productivity Index (stb/d/psi)	Permeability (mD)	Skin	Productivity Index (stb/d/psi)
B-1	1926	25	4	1901	27	4.6
B-2	557	29	1.7	525	23	1.9
B-3	664	33	1.8	663	11	2.5

2) Wellhead Sample Analyses Results:

Dynamic Viscosity

Dynamic viscosity tests were conducted for crude oil viscosity and wellhead samples to measure the viscosity profile (viscosity vs. temperature) of the samples before and after microbes injection (after 6 hours, 3 days, 2 weeks and 1 months of microbes treatment). Analyses on crude oil viscosity for B-1 (Figure 6.12), B-2 and B-3 shows insignificant changes after MEOR treatment. This is consistence with the findings during feasibility studies whereby whole oil gas chromatography traces suggest that Bokor oil has been biodegraded to the extend that the normal and branched alkanes have been totally removed due to in reservoir alteration leaving cyclic alkanes and aromatics. Test on "as received' wellhead sample for B-1 at the reservoir temperature of 48°C are in a small range of 21 to 22 cP and 18 to 22.5 cP, respectively. As the temperature lower, the viscosity differences are significant. This can be explained in a way that, at higher temperature, the emulsion tends to break up resulted in a viscosity close to the oil viscosity. As the temperature lower, the emulsion starts to form and therefore larger viscosity differences can be observed, depending on the nature and severity of the emulsified samples.

Hydrocarbon Compositional Analysis

The objective of the analysis is to detect any changes in the properties of the crude oils. The bulk properties ot the oils that were used to monitor the effect of MEOR process on the Bokor

oils are API gravity, bulk composition, pour point temperatures, and wax, sulphur and asphaltene contents. The API gravities show that the Bokor oils are considered to be heavy with values ranging from 17 to 20 °API. The low API gravities of the oils as supported by whole oil gas chromatography, is due to the complete absence of normal and branched alkanes removed during in-reservoir biodegradation by indigenous microbes and/or water washing taking place in the reservoir after accumulation. The API gravity of the samples show that there is not much variation in the API values in samples collected after 6 hours, 3 days, 2 weeks and 2 months compared to the pre-treatment samples. This indicates that there is hardly any change in the bulk properties of the oils with soaking time. The results, as expected, are consistent with those reported in the feasibility studies. This finding is supported by other bulk property data such as wax (1.0 to 2.8 wt %), asphaltene (0.03 to 0.13 wt %) and sulphur (0.12 to 0.15 wt %) contents which do not show any significant change in the values with soaking time. The very little or no variation in the bulk property values may be explained by the fact that the Bokor oils accumulated in these shallow A reservoirs have been biodegraded. Bulk composition of the oils shows that the content of the aromatics (45 to 60 %) is relatively very high in all the samples at the expense of the saturates (34 to 49 %). This classifies the Bokor oils as napthenic-aromatic oils. The shift in the property of the oils from paraffinic to napthenicaromatic resulted from the removal of paraffin's during in reservoir biodegradation. The high aromatic fraction in the oils relative to the saturates could be one of the reasons for the high tendency of the Bokor oils to form emulsion. This is because the aromatic compounds are more polar and therefore relatively more soluble in water compared to the less polar saturated hydrocarbon compounds. Whole oil gas chromatographic (GC) traces of all the Bokor oils indeed indicate that the normal and branched alkanes have been totally removed due to in-reservoir alteration or biodegradation. In order to see more clearly the changes in the geochemical properties of the oils with soaking time (pretreatment, 6 hours, 3 days, 2 weeks and 2 months), histograms were plotted to reflect the distribution of the hydrocarbons contained in the oil. Results show that there is a slight difference between oils treated with demulsifier and those without. The analysis of oil treated with demulsifier show that there is an increase in the lower molecular weight components at the expense of the higher molecular components with soaking time (Figures 6.13). This shows that there is a breakdown of higher

molecular weight compounds to lower ones, which could result in a slight increase in the quality of the oils. This slight increase in oil quality may not be detected or noted in the bulk property data as the bulk property analyses are less sensitive to changes as compared to whole oil GC or GCMS which are very sensitive.

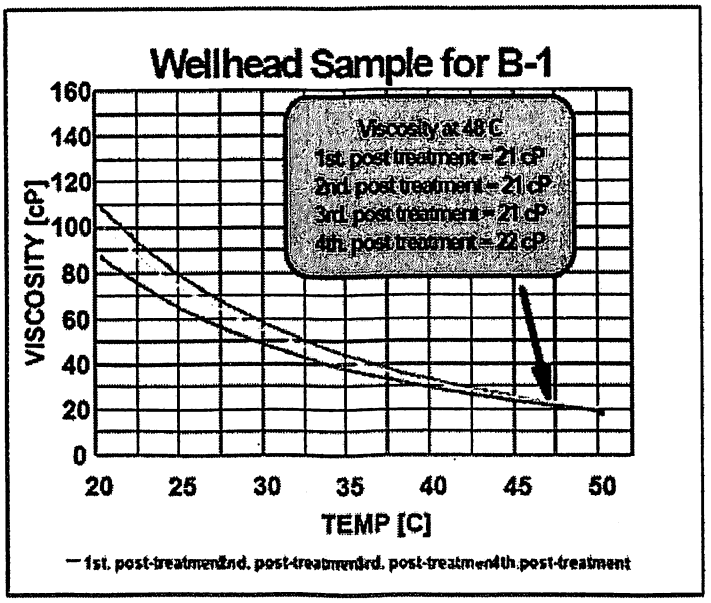


Figure 6.12: Post MEOR "Wellhead Sample" viscosity analysis

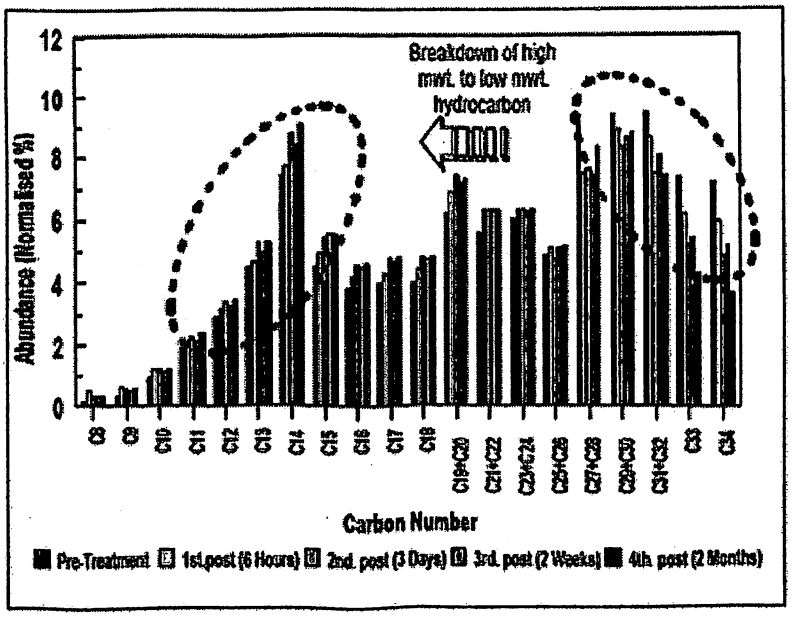


Figure 6.13: Hydrocarbon Compositional Analysis Results

In the case of oils not treated with demulsifier, the hydrocarbon distribution show that there is an increase in the higher molecular weight components relative to the lower ones in samples of up to two weeks soaking time. One possible explanation for this is the increase in the solubility of the higher molecular weight components as a result of biosurfactant activity of the microbes in dissolving the insoluble hydrocarbons found in higher abundance in biodegraded oils. These results, however, are just the opposite of the samples treated with demulsifier. The actual reason for this observation is still unknown but needs further laboratory investigation to understand the behavior of emulsions and the chemistry of demulsifier. However, for sample with two months soaking time, there seems to be a decrease in the high molecular weight components compared to the two weeks sample. This suggests possible breakdown of high molecular weight components as seen in samples treated with demulsifier.

Emulsion stability

It is anticipated that the treatment will reduce the stability of the crude oil emulsion. The stability of the emulsion is very important to be studied because it will provide information on net oil content (water cut), time to reach stable emulsion, percentage of stable emulsion, effect of temperature and effectiveness of MEOR treatment. Based on the analysis done for all samples on site, it is observed that the degree of emulsion stability has reduced significantly. Prior to the treatment, the emulsion separation tested using gravity settling without demulsifier method, showed a very low rate. After the treatment, the emulsion becomes less stable. For example, the rate of separation based on the 1, 4 and 24 hr settling, all the three wells showed a similar trend or curves. Immediately after the open up of the well, the rate of emulsion separation increase to more than 90%. However these effect only observed temporarily, after 2 weeks opened up, the rate decreased and continuously reverted to it initial stages. Under controlled condition in laboratory, the samples were allowed to settle for 7 days at both room and reservoir temperature (48°C). The volume of emulsion, oil and water was monitored. The observation (Figure 6.14) for all samples at reservoir temperature (48°C) indicates emulsified crude breakout completely into oil and water less than a week of laboratory time. This will slightly help the fluid to flow faster because the viscosity of the oil and water will be lower

compare to viscosity of emulsion. The trend of emulsion vs. time obtained for all three wells clearly described the effectiveness of the microbial products in reducing emulsion stability. The effectiveness of the microbes in breaking the emulsion decreases as the production time increases when most of oil near the wellbore is being produced.

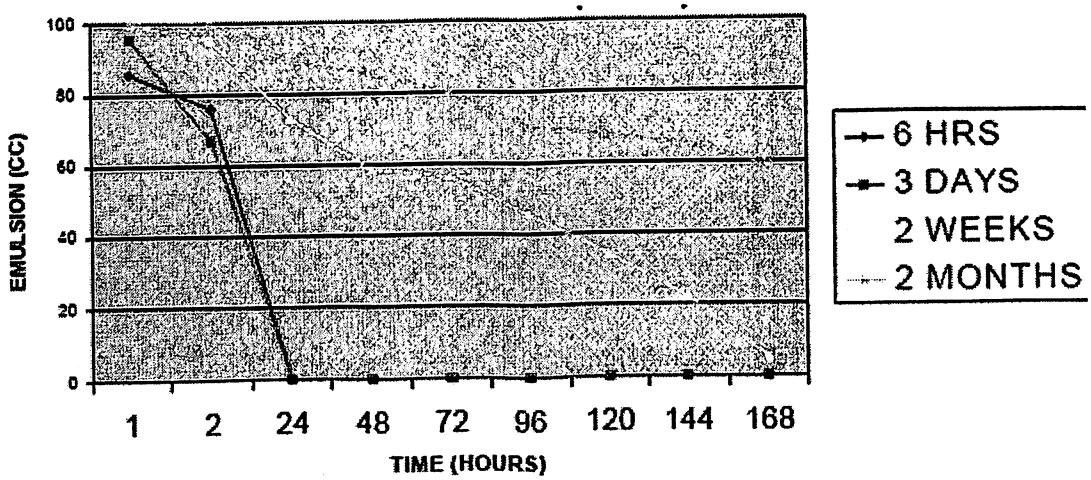


Figure 6.14: Emulsion Stability Analysis B-1

Conclusions

The results can be summarized as follows:-

Production Performance Results

1. Production increase observed is either due to high gross production (improvement in PI), reduction in emulsion stability or reduction in watercut.
2. The total average net oil gain after 5 months post MEOR is 270 bbl/d (47% oil incremental).

Wellhead Sample Results

1. At reservoir temperature, no significant viscosity change observed for both wellhead and crude oil samples. This is due to the fact that Bokor crude has been biodegraded.
2. Viscosity change observed for both wellhead and crude oil samples at surface temp.
3. Hydrocarbon compositional analysis indicates that there is an increase in the solubilisation of heavy components and breakdown of high molecular weight into low molecular weight components.
4. Emulsion stability reduces, thus improves the production/lifting performance of the well.

CHAPTER – 07

EXPERIMENTAL WORK

REQUIREMENTS

1. Soil sample
2. pH meter and pH paper
3. Autoclave
4. Glassswares
   a) Conical flask vol.500 ml
   b) Pipettes vol.10 ml and 1 ml
   c) Test tubes, vol. 15ml
   d) Measuring cylinder (25ml, 100ml,and 1000ml)

   (Glasswares were autoclaved 121°C for 15 minutes before use)
5. Magnetic Stirrer
6. Agar Medium
7. Nystatin
8. Distilled water
9. Culture tubes
10. Petriplates
11. Incubator
12. Weighing balance

Soil Sample

A total of 20 soil samples were collected from different places of dehradun. The soil samples were collected aseptically digging about 7cm below the soil surface. The samples were passed through a 0.71-mm mesh sieve after they were air dried at room temperature for 1 day and were stored at room temperature in plastic bags until they were used.

Soil pH measurement

One gram of soil sample was suspended in 1 ml of distilled water and mixed well. After 30 seconds of standing, the supernatant was used to determine the pH. If there is no supernatant found then another 1 ml of water is added to the soil sample. pH is determined by the use of pH meter. The electrode of the pH meter was dipped in the supernatant and pH was measured for the physiological significance of Pseudomonas in the soil sample. According to the alkalinity and salinity of the soil the soil samples were treated with saline water. To maintain the physiological condition or to provide the natural environment to the Pseudomonas.

Media preparation

Nutrient Agar

Peptone -5 gm

Nacl- 5 gm

Yeast Extract -1.5 gm

Agar – 15 gm

pH – 7.4

Water – 1 liter

Procedure for medium preparation

Proper amount of all constituents of the nutrient agar medium was weighed for preparation of medium. Then boil 1 litre of double distilled water with 5 ml of glycerol. Put the beaker with water on the magnetic stirrer for mixing of the media components. After that switch on the magnetic stirrer and add the constituents slowly one by one. When the media constitutes are mixed properly the pH should be adjusted to 7.0-7.2. Pour the media into three 500 ml flask in equal amount. Plug the flasks thoroughly and place them for autoclave. (121°C for 15min)

Sterilization of media

Water level of the autoclave was checked. The water level neither in the lower level nor in the upper level it should be just below the upper level. After checking the water level the media with flask and other glasswares were put in the basket of autoclave. Then switch on the autoclave and adjust it at 121°C for 15 minutes. After the autoclaving the glass was exhausted from the autoclave properly before opening the autoclave. Then cool down the media up to 45°C.for pouring.

Media dispensing

All the petriplates and test tubes properly washed and dried in the oven. Then rap them in newspaper and place them on autoclave. After autoclaving take the petriplates and media taken to the laminar air flow for pouring. Before pouring clean the laminar air flow thoroughly and put the UV for 10 minutes to avoid contamination for previous experiment. After 10 minutes switch off the UV and light Bunsen burner. Wash your hands with 70 % ethyl alcohol to avoid contamination of other microorganisms from hand. Pour the petriplates in the flask in front of the Bunsen burner. Then leave the petriplates for cooling and polymerization of the media.50 mg per / ml Nystatin to be added to the cooled medium before pouring.

Serial dilution technique

First of all autoclave 5 test tubes containing 85 % of saline water and each test tube contain 9 ml of water for dilution of each sample to make dilution. Dilution made in the sequence of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Then add one gram of soil to the test tube, which becomes 10^{-1} dilution. Shake the test tube vigorously and leave it for 10 minutes for settle down of soil. Then make 1 ml of supernatant water from the first test tube and put it into the second test tube. Shake the second test tube. It becomes 10^{-2} dilution. Then take 1 ml of water sample from the second test tube and mix it in the third test tube and shake it. It becomes 10^{-3} dilution and repeat the process for 10^{-4} and 10^{-5} dilution.

Spread plate technique

First of all wash your hands with 70 % ethyl alcohol to avoid contamination of other microorganisms from hand. Fit the 1 ml pipette in the sucker, and suck 1 ml of water from the dilution 10^{-2} and put it in the petriplate. Take the spreader and wash it also with 70 % ethyl alcohol and burn it fro a while and then spread the sample in the petriplate and repeat the process fro 10^{-3}, 10^{-4}, 10^{-5} dilution. Cover the petriplates by paraffin film to avoid the contamination of other aerial microorganisms. Incubate plates at 30-37°C.

Isolation procedure

Selective isolation procedure for the genus Pseudomonas

A small amount of soil suspended in 1 ml of sterilized water. 100 ml of aliquots of suspension was spread on dry agar plates. The plates were incubated at 27°C. Spores or mycelia of isolated colonies were picked up on a needle and streaked on various media for color determination and for microscopic and macroscopic examination.

Growth Curve

For the growth curve, pseudomonas was grown in the nutrient broth medium. After every hour the optical density of the samples were taken using spectro photometer in 600 nm wave length. The readings were taken 10-12 times in the gap of 1 hour. Then the graph and the chart were plotted.

The growth curve of the pseudomonas covers four phases:

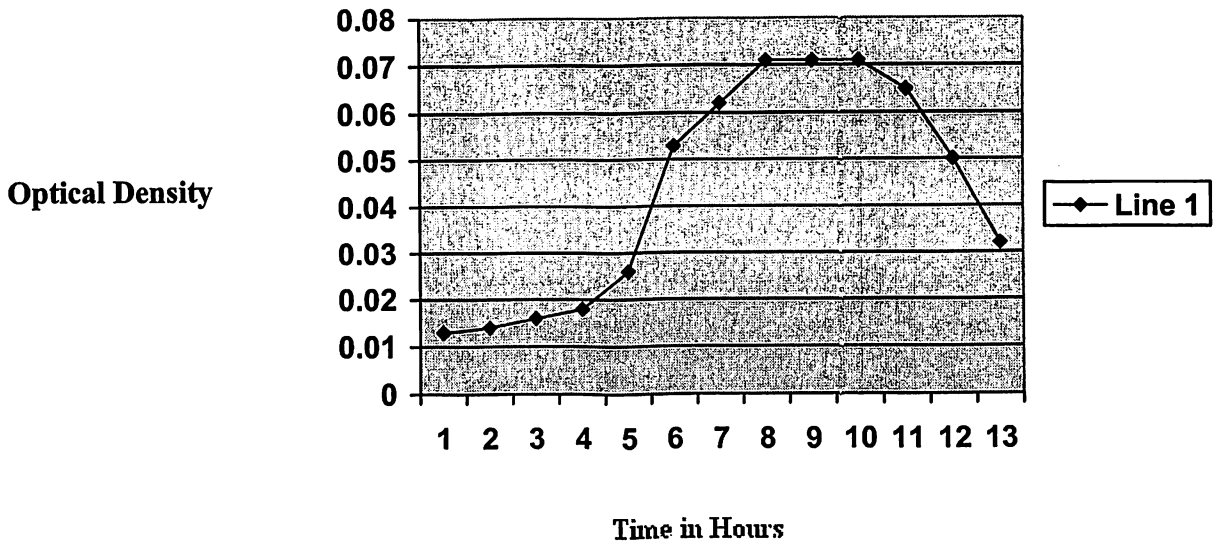
1. Lag Phase: After the introduction of pseudomonas in the growth medium, usually no intermediate increase in the growth number is observed. This phase of the growth cycle is known as the lag phase. Although the cell division does not take place in this phase so there is no increase in the net mass. In this phase the cell synthesizes the protein and enzymes required for the cell division.

2. Exponential Phase: During the exponential phase pseudomonas are growing and dividing at the maximal rate. Their rate of growth is constant during the exponential phase. The population is most uniform in this phase.

3. Stationary Phase: the stationary phase usually is attained by pseudomonas at a population level of around 10^9 cells per ml. Of course the population sizes can not increase in this phase due to the depletion of nutrients.

4. Death Phase: death phase s attained when the depletion of the nutrients occurred in the medium. Due to the death of the organisms the population structure also decreases in the medium.

GROWTH CURVE



Time in hours	Optical Density
1st hr	.013
2nd hr	.014
3rd hr	.016
4th hr	.018
5th hr	.026
6th hr	.053
7th hr	.064
8th hr	.071
9th hr	.071
10th hr	.071
11th hr	.065
12th hr	.050
13th hr	.032

CONCLUSION AND RECOMMENDATIONS

In MEOR technology, two types of microbes, the exogenous and the indigenous, have been studied and applied for flooding so far. For exogenous microbial flooding, displacing bacteria was derived from reservoirs. Field tests indicated a significant incremental oil recovery. The selected species usually came from reservoir or polluted soil related to oil spill. Cultured to be facultative in lab, the bacteria were fermented and injected into the reservoir. They had limited adaptability to the pay zones. Furthermore, the bacterial effect was restrained by staying time of fermentation fluid in anaerobic environment with a certain temperature/pressure window and long fluid cycle time from injectors to producers. As a result, flooding response was measured to be in stages, which reflected bacterial deterioration. For indigenous microbial flooding, the microbes were added in injection water, injected into the reservoir. They were later activated to be effective through periodic replenishing of necessary nutrient in the reservoir. It gave the same oil incremental production as that achieved in exogenous flooding. The indigenous microbes were also capable of anaerobic fermentation and could distinctly change reservoir ecological setting and produce polysaccharides, which not only expanded the swept volume and enhanced oil recovery rate but also improved the level of developed reserves. In addition, channeling of fermenting fluid would likely take place along pathways where the strong washout by injection water had appeared. It was necessary to plug these channeling zones before performing MEOR. Therefore, shutting off operation was incorporated in field tests.

Field Analysis:

1. Based on the experiences of microbial flooding and field tests for several years under various reservoir conditions, specifically those with average permeability exceeding 100 md and remaining with 40% oil saturation of pore volume can release oil to a residual level of 32% of pore volume.
2. The suitable conditions for flooding were better understood from actual production performance:

- During the MEOR process, no damage to the reservoir was found.
- Tests results show that the indigenous microbial flooding is better applied in low-temperature reservoir. A high temperature reservoir is not ideal for selecting the excitation agents and potential factors are limited. The latest research involves the use of bacteria surviving the metabolism at lower temperature thereby reducing the temperature.
- Deepened development will direct exogenous bacteria to lead oil displacement in one aspect. A series of these will compensate for each other. Selective combination of these bacteria can be used in reservoirs at higher temperature, which makes a unique effect.
- The oil recovery rate is found to be enhanced further with microbial flooding after polymer flooding.

Air assisting microbial flooding--In aerobic condition, facultative bacteria accelerate metabolic rate. Microbes can consume oxygen in the air injected into reservoir and hence some components of oil will be oxidized with a few products. No additional nutrients are required. Oxygen is not a challenge because of aerobic bacteria and oil oxidization at low temperature. As air application is affected by gravity, wells at structural high could be candidates.

Human interference with the existing biological environment in reservoir--Through analysis and separation of indigenous bacteria, identify the ones that produce polysaccharides. The function of bacteria will be limited in anaerobic condition of reservoirs. No bacterial culture and fermentation are needed, and the advantage is outstanding.

Microbial Analysis:

1. The class of microbe includes obligate or facultative thermophilic anaerobes utilizing readily available and inexpensive carbohydrate substrate.
2. The bacteria must produce active agents such as CO2, organic acids, surfactants and biopolymers.

Another important suggestion is the development of a continuous type of bioreactor which develops death rate curve equal to growth rate curve i.e. inlet stream of microbial flooding equal to outlet stream of microbial flooding.

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