REVIEW ARTICLE

MICRO-ORGANISMS IN ENHANCED OIL RECOVERY

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ABSTRACT

The extent of bacterial involvement in petroleum genesis has been a subject of debate even while evidence indicates that million years of heat and pressure changed the remains of microscopic plants and animals into oil and natural gas. Microbial-enhanced oil recovery (MEOR) involves processes where microorganisms and their products are used to recover oil from either wells or reservoirs. MEOR mechanism could be in the production of biosurfactant, acids, gas or biomass. The major advantage of MEOR is that it is economically-attractive for marginally-producing oil fields; less expensive than other enhanced oil recovery methods and environmentally-friendly. MEOR activities commenced as far back as 1926 in most developed countries and is ongoing till date. The alarming rate of decrease in the supply of petroleum and its products makes the potentially-inexpensive method of MEOR useful; hence developing a method such as this in Nigeria would not only stabilize future worldwide oil production but will also ensure adequate energy supply. However, despite its advantages, this technology is still barely recognized by the oil and gas industries in the Federal Republic of Nigeria probably due to lack of published data as well as little or no cooperation between microbiologists, reservoir engineers, geologists and the Nigerian Government.

Keywords: Microbial-Enhanced Oil Recovery (MEOR), Microorganisms, Oil and Natural Gas, Federal Republic of Nigeria

INTRODUCTION

Bacterial activity has undoubtedly been involved in petroleum genesis, but the extent to which bacteria have contributed to the formation of petroleum is debatable. The essential elements and processes (generation, migration, accumulation and preservation) that occur leading to the formation of petroleum, and accumulations whose provenance is a single pod of active source rock is known as a petroleum system. Typically, it consists of a source rock, a migration route, a reservoir rock, a seal rock and a trap (Gullapalli *et al.*, 2000).

Crude oil is commonly found in oil reservoirs and formed in the Earth's crust from the remains of living things; it is properly known as petroleum and is used as fossil fuel. Evidence according to Bryant *et. al.* (1992) indicates that millions of years of heat and pressure changed the remains of microscopic plants and animals into oil and natural gas. When buried with the accumulating sediment, they reach an adequate temperature (50°C-70°C), are transformed and changed into liquid hydrocarbons that migrate and become oil and gas reservoir. The formation of hydrocarbon in a sedimentary basin requires the following: organically-rich source rocks which must be heated enough and mature to penetrate hydrocarbons, migration pathways, reservoir, correct trapping mechanisms and correct timing of the elements.

To obtain the contents of the oil reservoir, it is usually necessary to drill into the Earth's crust, although surface oil seeps exist in some parts of the world, such as the La Brea Tar pits in California, numerous seeps in Trinidad and tar sand belt in the south-western Nigeria and other oil-rich Niger Delta regions such as the Ogoni-land in Nigeria. A virgin reservoir may be under sufficient pressure to push hydrocarbons to surface; as the fluids are produced, the pressure will often decline and production will falter. The reservoir may respond to the withdrawal of fluid in a way that tends to maintain the pressure (Gullapalli *et al.*, 2000).

PETROLEUM GENESIS AND HABITAT

MICRO-ORGANISMS IN OIL RECOVERY

Microbial-enhanced oil recovery (MEOR) processes has recently been called "microbial increased oil recovery" (MIOR) and involves processes where both micro-organism and microbially-derived products (such as surfactants, acids and polymers) are used to recover oil from individual wells or entire reservoirs. Injection of micro-organisms, nutrients or microbial by-products can take place one well at a time (through the cyclic injection method also called the bio huff and puff method) or it can take place in conjunction with a water flood (the field flood method) or using the wel-prep treatment which is adapted to either cyclic injection or field flooding (Zhang and Qin, 1983).

HISTORY OF MICROBIAL-ENHANCED OIL RECOVERY (MEOR)

In 1926, Beckman a scientist suggested for the first time that micro-organisms could be used to release oil from porous media. Between 1926 and 1940, very little research was done on this topic. In the 1940s, ZoBell and his research group started a series of systematic laboratory investigations that marked the beginning of a new era of research in petroleum microbiology with application for oil recovery (Zobell, 1947).

ZoBell explained the main mechanisms responsible for oil release from porous media involving processes such as dissolution of inorganic carbonates by bacterial metabolites, production of bacterial gases which decrease the viscosity of oil thereby promoting its flow, production of surface-active substances or wetting agents by some bacteria; as well as the high affinity of bacteria for solids, later attached to crowd off the oil films (Zobell, 1947).

ZoBell in 1947 also described and patented processes by which bacterial products such as gases, acids, solvents, surface-active agents and cell biomass released oil from sand pack columns in laboratory tests. ZoBell's experiments were later repeated (Updegraff and Wren, 1954; Davis and Updegraff, 1954), resulting in the Updegraff's patent that is based on the use of underground injected micro-organisms which can convert cheap substrates like molasses into agents of oil recovery such as gases, acids, solvents, and biosurfactants.

The first field test was carried out in the Lisbon field, Union County, AR, in 1954 (Yarbrough and Coty, 1983). From the USSR, Kuznetsov concluded that oil deposits contain bacteria capable of anaerobically destroying oil to form gaseous products CH_4, H_2, CO_2 and N_2). (Kuznetsov *et al*., 1963)

In the 1960s and 1970s, significant research activities took place in some European countries such as former Czechoslovakia, Hungary, and Poland (Dostalek and Spurny, 1958; Senyukov *et al*. 1970; Lazar, 1978). The field trials developed in these countries were based on the injection of mixed anaerobic and/or facultative anaerobic bacteria (*Clostridium, Bacillus, Pseudomonas, Arthrobacterium, Micrococcus, Peptococcus, Mycobacterium*, etc.) selected on their ability to generate high quantities of gases, acids, solvents, polymers, surfactants, and cell-biomass. Details about such activities are also in the diagrams of Lazar's review papers (1991 and 1998).

At the same time, a new technology (selective plugging recovery) was suggested based on the idea of improving oil recovery from water floods by producing polysaccharide slime *in situ* from an injected microbial system based on molasses. This technology has been recognized as an important additional mechanism of oil release from reservoir rocks. Very important efforts were put into producing biopolymers of xanthan or scleroglucan types as viscosifying agents for enhanced oil recovery {EOR} (Lazar, 1978; Lazar, 1991; Lazar, 1998).

Investigations carried out in the period 1970 - 2000 have established the basic nature and existence of indigenous microbiota in oil reservoirs, as well as reservoir characteristics essential to a successful MEOR application. All these investigations proved that cyclic microbial recovery (single well stimulation), microbial flooding recovery, and selective plugging recovery are feasible to applications, as well as the technology based on activation of stratal microbiota successfully developed in former

Soviet Union. MEOR research was actually boosted by the petroleum crisis of the 1970s and later became a scientifically-substantiated EOR method supported by research projects carried out all over the world in countries such as the U.S., Canada, Australia, China, Russia, Romania, Poland, Hungary, Czech Republic, Great Britain, Germany, Norway and Bulgaria (Bryant and Douglas, 1988).

Many international meetings have been organized periodically on the MEOR topics and proceedings on the advances in the knowledge and practice of MEOR have been published. It is also important to recognize and acknowledge the role of the U.S. Department of Energy (DOE) which sponsored MEOR basic research and field trials as well as periodically organizing international meetings. Several books on MEOR have also been published and by the end of the 1990s, MEOR was already a scientific and interdisciplinary method for the increase of oil recovery (Dietrich *et al.*, 1996).

Today, MEOR technologies are well suited for application, whenever the need for oil arises at a rate of 3 to 4%/year, while oil production constantly decreases. It is of interest to mention that the abandonment of stripper wells has increased to 175% since 1980. Taking this into account, the U.S. could have access to less than 25% of its remaining oil resources within 15–25 years.

However, in spite of the long history of MEOR activities, none of the proceedings has been used in the Federal Republic of Nigeria and MEOR technologies are still barely recognized by the oil and gas industries in Nigeria. This may be due to the lack of published data especially in widely-available journals, as well as to little cooperation between microbiologists, reservoir engineers, geologists, economists, owner operators and the Nigerian Government (Dietrich *et al.*, 2008).

MEOR RECOVERY MECHANISMS

There are many different mechanisms by which bacteria can increase oil recovery from a reservoir; usually more than one of these mechanisms will be in action for a given species/strain assuming it has access to nutrients, a carbon source and oxygen (for aerobic bacteria) and that the environmental conditions are favourable for its development. Understanding these mechanisms, how they help increase oil recovery and how the specific bacteria produce them is the key for developing efficient MIOR technology (Karim *et al.*, 2001).

We will discuss four MEOR mechanisms:

Gas Generation and Solvent Production

Gas and solvent-producing strains of the micro-organism *Clostridia* have been extensively studied by Bryant and Douglas and used for commercial solvent production during the last half century (Bryant and Douglas, 1988). This group of bacteria and some others produce hydrogen (H_2), carbon dioxide (CO_2), acetate, and butyrate during their initial growth phase (acidogenic phase) of the fermentation process. As growth continues through the second phase of fermentation, the stationary growth phase, there is seen to be a shift in the metabolism of the cells to solvent production (solventogenic phase) and solvents produced include acetone, butanol, ethanol, isopropanol, and other solvents in lesser amounts. These gaseous and liquid metabolites dissolve into the oil resulting in reduced viscosity. In addition to reducing the viscosity of oil, microbes increase the pressure in the reservoir by producing gases (H_2, CO_2, N_2, CH_4) in the pore spaces that would have been normally bypassed with conventional gas flooding operations. (Bryant and Douglas., 1988).

This mechanism of oil release functions by reduction of viscosity and interfacial tension has been demonstrated by the conventional EOR technique of miscible displacement which involves the injection of solvents like alcohols, refined hydrocarbons, condensed hydrocarbons, liquefied petroleum gases or CO_2. The injected solvents/gases dissolve in the reservoir oil and reduces the capillary forces contributing to oil retention (Yen, 1990).

In the laboratory, research has been conducted with heavy crude oil to determine efficacy of injecting surfactant, gas, and solvent-producing bacteria in porous media for improving oil recovery. Corefloods with Sho-Vel-Tum crude oil (26.4°API, 41.3 cp at 30°C) and Wilmington crude oil (17°API) showed an increase in crude oil recovery efficiency with microbial systems.

Corefloods were conducted with the combination of a *Bacillus* sp. known to produce biosurfactant and a *Clostridium* sp. which produces solvents and CO_2. The recovery efficiencies were 44.4% for the Wilmington and 35.5% for the Sho-Vel-Tum crude oil (Bryant and Douglas, 1988).

Davidson and Russell in 1987 conducted a pilot test in the Royal Loco Unit, Stephens County, Oklahoma using *Clostridium* sp. for neutral solvent and gas production. Crude oil from the Loco Field is 21°API and has a high viscosity. Spores of *Clostridium* sp. were injected into an injection well on a five spot pattern followed by 308 bbls of a sucrose medium. All wells were shut in for one week after nutrient injection. Acetone, butanol, and a small suite of low-molecular-weight acids were detected in production wells after production resumed. Oil production increased after the microbial stimulation. Increased oil production was attributed primarily to viscosity reduction and profile modification by biomass and foam (Davidson and Russell, 1987).

Biosurfactant and Acid Production

Specific microorganisms produce biosurfactant that reduces oil/water interfacial tension and cause formation of stable oil-and-water emulsions. Also these surfactants act by increasing the relative permeability of the reservoir to oil by changing the wettability of the reservoir core to a more water-wet condition (Stepp *et al.*, 1992). Previous research shows that a *Bacillus* sp., and a *Clostridium* sp. are capable of altering wettability of cores to a more water-wet condition (Chase *et al.*, 1990).

Alcohols also act as co-surfactants and work synergistically with surfactants to lower the surface and interfacial tension and mobilize the trapped oil. Microorganisms also produce acids, primarily low-molecular-weight fatty acids, which cause rock dissolution and decrease interfacial tension between the oil and reservoir brine. *In situ* acid production by microorganisms can be used to treat various production problems, such as formation damage, low oil relative permeability, trapped oil due to capillary forces and paraffin and scaling problems. Biosurfactant-producing microorganisms have been shown in laboratory studies to also be effective on heavy crude oil (Yen, 1990).

Singer and his research team (1983) were able to isolate surfactant-producing microorganisms from soils containing heavy crude oils and asphaltenes by enriching for growth on crude oil. When incubated with Venezuelan Monagas crude oil (>25,000 cp), some consortia produced stable emulsions which reduced viscosity by up to 98% compared to abiotic emulsions (Singer *et al.*, 1983).

Zhang and Qin (1983) found out that the fermentation fluids produced during aerobic fermentation of crude oil were able to form stable oil-and-water microemulsions with a viscous crude oil. The viscosity of the stable emulsion was 12–46 PaS as compared to 23-44 PaS for the crude oil. They also evaluated the use of a rhamnolipid biosurfactant for recovery of both light and heavy crude oils. The rhamnolipid biosurfactant, produced by a *Psuedomonas aeruginosa* performed the same function as petroleum sulfonates for recovery of both light and heavy crudes. A recovery efficiency of 83% was achieved using this polymer. Recovery efficiency was defined as the ratio of the cumulative dry weight of oil recovered by bacteria to the dry weight of the initial residual oil (Zhang and Qin, 1983).

Research has also shown that solvent- and gas-producing bacteria combined with a biosurfactant-producing strain were effective for improving oil production for both medium-weight and heavy crude oils. Specially adapted strains of *Bacillus licheniformis* and *Clostridium* sp. added to a waterflood were able to improve oil production by approximately 20% when they were injected into the Chelsea-Alluwe field (34°API, 8.1 cp at 25°C) in Rogers County, Oklahoma. The cost of the microbial treatment in the Chelsea-Alluwe field was $2.33 per incremental barrel of oil recovered above the baseline waterflood production level. One of the key advantages to the efficiency of microbial surfactant systems is that microbial cells move to the hydrocarbon/water interface and produce compounds at that interface to mobilize trapped residual oil. Microbial technologies such as these are cost-effective at current oil prices (Bryant *et al.*, 1993).

The mechanisms by which microbial systems work are fundamentally sound and can be adapted for

heavier oil. The same microbial system that proved successful in the Chelsea-Alluwe field was used for single well stimulations in the Taylor-Ina field in Medina County, Texas, which produces a viscous heavy oil. The microbial system caused the API gravity to increase from 11–16°, causing the oil viscosity to decrease from 1,000 cp to 711 cp (both viscosity measurements at 50°C) and stimulated increased oil production (Rowe, 1998).

These results were seen to be very promising, considering that the microbial system was optimized for medium weight oil and viscosity reduction was not the primary goal with this microbial system. Microbial systems based on the same concept can be designed specifically for heavy oil by increasing solvent and gas production to further lower the viscosity and increase the API gravity of heavy oil (Rowe, 1998).

Biomass and Biopolymer Production

Another method of improving oil recovery is by modifying the fluid flow through the reservoir. This is achieved by shifting fluid flow from the high permeability zones in a reservoir to the moderate or low permeability zones; the sweep efficiency is increased resulting in improved oil recovery. MEOR processes traditionally associated with fluid flow changes include *in situ* biomass and biopolymer production. Successful engineering of an *in situ* microbial plugging system must take into consideration the ability to transport both the microorganism and the required growth nutrients through the reservoir, as well as the ability of the microorganism to selectively block the higher permeability zones of the reservoir as a result of microbial growth and metabolism (Bryant *et al.,* 1992).

Biomass Production also referred to as "bacterial plugging", is a fluid flow modification process that is achieved by an increase in microbial cell mass within the reservoir. Production in biomass can be achieved by stimulating either indigenous microbial populations or injected microorganisms with growth nutrients. The injected nutrient and microbes preferentially go into the high permeability zones of the reservoir and as cell growth occurs, the biomass selectively plugs these zones to a greater extent than the moderate or low permeability zones. This results in an increase in sweep efficiency by forcing the injected water to pass through previously by-passed zones of the reservoir (Bryant *et al.*, 1992).

MEOR/MIOR methods, thus, hold the promise of a superior recovery and for recovery of unrecoverable residual reservoir oil. Biopolymer production can be used in addition to bacteria plugging by cell growth; certain bacteria have been observed to produce water-insoluble biopolymers under growth conditions. Selective plugging of the high permeability zones by the biopolymers results in high oil recovery efficiency by increasing the sweep efficiency. Production of these biopolymers in high permeability channels reduces water channeling by blocking these zones. Similarly, microbial polymer systems can be applied to producing formations experiencing water coning problems. Direct injection of the bacteria into the production well, followed by a nutrient slug, will allow the bacteria to enter the water channels. The accumulation of insoluble biopolymer in the pore throats of the formation results in pore closure and effectively plugging the water-bearing zone. Studies have found wettability changes both towards more water-wet and towards more oil-wet as a consequence of bacterial biomass applications, depending on properties of the rock, the fluids and the metabolites (Jack and Diblasio, 1985).

Changes in the Flow Pattern of Oil

Changes in the flow pattern can be caused by bacterial plugging of the pore space and sometimes by biopolymers. This effect has been studied thoroughly and it is acknowledged as a very important MIOR mechanism (Gullapalli *et al.*, 2000; Yusuf *et al.*, 1999; Stepp *et al.*, 1996; Udegbunam *et al.*, 1991). Some of the experiments that serve as a proof that this mechanism had taken place include increase with time of the pressure drop along a core after bacterial inoculation, visualization of plugged fractures with a scanning electron microscope (Zerki and El-Mehaideb, 2002), permeability profiles along cores (Lee *et al.*, 1998), and the movements of bacteria and metabolites by liquid samplings along the core during flooding.

At the beginning of the bacterial flooding, the bacteria present in the water phase will flow

through the largest pore channels with greatest ease. This is also where nutrients will be most abundant. Therefore, the bacterial growth rate will be directly proportional to the effective rate of water flowing through a given pore channel. Bacterial adhesion and growth will lead to a reduction of the effective flow area of the pore channel, which will eventually make the water deviate through other previously un-swept (or poorly-swept) flow paths (Zobell, 1952a).
Consequently the sweep efficiency will be improved, even in cases where there has been water fingering, making possible the displacement of bypassed oil. Field tracer tests support this theory. Bacterial slime can also contribute to this mechanism (Nagase *et al.*, 2002).

SOME MAJOR ADVANTAGES OF MEOR TECHNOLOGIES

1. The injected bacteria and nutrient are inexpensive and easy to obtain, produce and handle in the field.
2. The technology is economically-attractive for marginally producing oil fields; a suitable alternative before the abandonment of marginal wells.
3. According to a statistical evaluation (1995 in U.S.), 81% of all MEOR projects demonstrated a positive incremental increase in oil production and no decrease in oil production as a result of MEOR processes.
4. The implementation of the process needs only minor modifications of the existing field facilities. It is less expensive to install and more easily applied than another EOR method.
5. The costs of the injected fluids are not dependent on oil prices.
6. MEOR processes are particularly suited for carbonate oil reservoirs where some EOR technologies cannot be applied with good efficiency.
7. The effects of bacterial activity within the reservoir are magnified by their growth whole, while in EOR technologies the effects of the additives tend to decrease with time and distance.
8. MEOR products are all biodegradable and will not be accumulated in the environment, so the technology is environmentally-friendly.

Microbial-enhanced oil recovery processes can be supplemented with:

1) Production of various bi-products in large scale fermenters and injection of these products into individual wells or entire fields.
2) Continuous injections of selected bacterial cultures into single wells or entire fields.
3) Continuous injections of selected bacterial cultures and nutrients into single wells or entire fields.
4) One time inoculation with selected bacterial cultures followed by injection of nutrients.
5) *In situ* stimulation of indigenous micro-organisms beneficial to oil recovery by injection of biocatalytic agents and micro nutrients.

ENVIRONMENTAL CONSTRAINTS TO THE GROWTH/ACTIVITY OF BACTERIA IN MEOR

Physical factors such as temperature, pressure and pore size/geometry; chemical factors including pH and electrolyte composition and biological factors constrain microbial activity in hydrocarbon reservoirs (Bryant *et al.*, 1992).

It is observed that the same factors control the existence and behavior of bacteria in other subterranean environments which are of relevance in other practical contexts notably the management and remediation of groundwater resources. These factors provide a set of criteria by which the suitability of organisms for use in MEOR can be assayed and compared (Bryant *et al.*, 1992).

Pore Size

The most obvious constraint that applies to deep subsurface microbes is the size of the pores. The existence of bacteria in deep subsurface rocks has been disputed in the past, but since the advent of modern tracer techniques and improved sampling protocols, it is now generally accepted. In some studies, the lower limit of mean pore sizes has been shown to be smaller than the size of known bacteria. For example, through phosphor-lipid fatty acid assays, Frederickson and his team in 1996 assessed shale and sandstone cores from a site in northwestern New Mexico for microbial activity.

They found no metabolic activity was detected in core samples with pore throats narrower than 0.2μm, although in some cases it was after extended incubation. The observation of much higher levels of metabolic activity in more permeable samples led these authors to conclude that sustained bacterial activity require interconnected pores of diameter at least 0.2μm (Frederickson and Phelps, 1996).

Depletion of Nutrients

The most limiting factor of bacterial activity in organic sedimentary material is a lack of free oxygen. The oxygen demand of the sediments is apparently great enough to deplete free oxygen at an early stage in sedimentation. Lack of free oxygen results in the accumulation of sedimentary organic matter which otherwise would be oxidized (or mineralized) ultimately to carbon dioxide, minerals and water. Bacterial decomposition under anaerobic conditions proceeds at a relatively slow rate; the hydrogen from the decomposable organic compounds being transferred through the bacterial enzyme system to hydrogen acceptors such as oxidized organic compounds or to forms of oxidized sulfur (Emery and Rittenberg, 1952).

Thus, general anaerobic bacterial activity ultimately leads to an accumulation of more reduced organic material and hydrogen sulfide. The activities of the sulfate-reducing bacteria (*Desulfovibrio* spp.) have received a great deal of attention whereas other anaerobic bacteria which may be active in marine sediments have received little. *Desulfovibrio* because of its peculiar metabolism, primarily reduces oxidized forms of sulfur rather than organic matter. If sulfate becomes limiting in the environments, activity of *Desulfovibrio* spp. ostensibly ceases. Connate waters associated with petroleum reservoirs are notably low in sulfate although there are many exceptions (Zobell, 1952b).

Nitrogen in available form must be present in order for bacterial activity to proceed. As the bacteria incorporate nitrogen into their cells, it is largely converted into protein. Upon death of the cell and its subsequent decomposition the protein nitrogen is converted into ammonia and is therefore susceptible to dissipation. In this way, the sediments could become depleted of available nitrogen, and the consequence would be a decrease in bacterial activity. Actually, very little is known about the bacterial activity that ensues in recent marine sediments, and practically nothing is known of such activity in source beds productive of petroleum as we know it and the various stages of petroleum formation have yet to be clearly defined (Zobell, 1952b).

Acidity/Alkalinity (pH)

The acidity or alkalinity of the surrounding aqueous medium, measured by the pH, is significant in several respects. Firstly on the cellular scale, pH controls the extent of ionization of the protein molecules that are embedded in the cell walls. As a result, cellular surfaces are generally charged and surrounded by diffuse double layers, the thickness of which is controlled by the overall electrolyte concentration and interaction of these ionic space-charge regions with those that also surround small particles of mineral phases can strongly affect the motion of the cells through a natural porous medium. The effect of pH on the surface charge of a protein depends on the relative numbers of acidic and basic groups in the side chains. Protein molecules are often characterized by pH called the iso-electric point, at which the positive and negative charges resulting from ionization of side chains are balanced. Secondly, some of the embedded cell wall proteins play a crucial role in the uptake of nutrients, elimination of waste products and maintenance of correct electrolyte concentrations (Portwood, 1995).

On a molecular scale, their ability to perform these functions also depends on their extent of ionization. The rates of the enzymatic processes that occur in respiration is strongly dependent on the pH. There generally exists an optimal pH, lying between 2 and 9.5 for the rates of such processes. The mineral phases in a porous medium (particularly carbonates), and the proteins themselves can exert a buffering effect, which can mitigate the lowering of the pH by the acids generated by primary metabolism (Portwood, 1995).

Oxidation Potential

Cellular respiration is a process that enzymatically mediates electron transfers from an electron donor (reducing agent in chemical parlance) to a

terminal electron acceptor (oxidizing agent). Apart from a few rare cases where only one electron is transferred from each mole of reductant, this electron transfer almost always involves a number of intermediate electron transfer steps, which can be quite numerous if the original electron sources are complex molecules such as sugars. The thermodynamic driving force for these electron transfer processes is expressed quantitatively in terms of the oxidation potential, (*bE* measured in volt) which is the Gibbs energy change divided by the number of moles of electrons transferred (Lee, 2000).

According to the Nernst equation, this quantity depends logarithmically on the concentrations (strictly speaking the activities) of not only the oxidized and reduced forms of the electron acceptor, but also of hydrogen ions and other species that might be involved (Lee, 2000).Thus, conclusively speaking for aerobic respiration, the terminal electron acceptor is oxygen, which is reduced to water. A particularly important electron acceptor in hydrocarbon reservoirs that are not supplied by surface water is sulphate, some organisms can use ferric ions (Ginter, 1934).

Thermodynamic Considerations

It has been demonstrated in the laboratory that anaerobic bacteria convert fatty acids into methane although the production of significant amounts of higher paraffin homologues has not been accomplished. There are small amounts of reduced organic matter in the Desulfovbrio bacterial cells in the form of lipids and hydrocarbons. Occurrence of reduced compounds is possible under anaerobic conditions (Thayer, 1931).

Furthermore, two mechanisms have been developed for bacterially-formed methane dependent upon the bacteria involved. One mechanism involves a reduction of carbon dioxide, the other a reduction of the methyl group of methanol or acetic acid. Thus, it is conceivable that still other, longer alkyl radicals can be reduced to corresponding paraffinic hydrocarbons by anaerobic bacteria. While most attempts to demonstrate this have failed, recently it found that other gaseous hydrocarbons including ethane, in the order of a few parts per million is involved in methane fermentations (Bernard *et al.*, 1994).

As organic matter becomes more reduced in the sediments, presumably because of hydrogen transfer resulting from anaerobic oxidations, it becomes progressively more difficult to oxidize because it is less susceptible to activation from a thermodynamic standpoint. The anaerobic conversion of compounds such as tyrosine to yield phenol or cresol, the alleged production of even benzene and the already mentioned methane formation from fatty acids indicates a bacteriological means of carrying organic matter to a state as reduced petroleum but these observations are not indicative of anaerobic bacterial activity in general or of such activity in sedimentary rock (Hammer, 1934).

Oligotrophy and Heterotrophy

To explain the existence of active microbial communities in environments such as deep granitic and basaltic aquifers, where nutrient levels are expected to be extremely low, it was suggested by Stevens and McKinley (1995) that such organisms can be sustained by hydrogen generated by reduction of minerals in groundwater. Although many species of hydrogen-consuming lithotrophic bacteria have been described and it is well known that appreciable hydrogen fugacities can be 'buffered' by some naturally-occurring mineral assemblages (Stevens and McKinley, 1995).

The suggestion that microbial communities could be sustained geochemically in this way has however, been disputed by Anderson and Chapelle (1998). These authors argued that basalt does not produce hydrogen under slightly-alkaline conditions and that the production of hydrogen under slightly-acidic conditions cannot be sustained over geological time scales. These discussion points out that the most important and difficult issue to be established is the long-term independence of such communities from the products of photosynthesis; at present this is best regarded as an open question. Considerable attention has been devoted to the study of heterotrophic microbes in sandstones and shales and the possibility that these organisms are sustained by organic material co-deposited with the sediments (Anderson and Chapelle, 1998).

In a review, Krumholz (2000) considers

formations containing alternating layers of sandstone and shale and discusses experimental evidence that organic matter and fermentation products present in the shales can diffuse across sandstone shale boundaries and support microbial communities in the sandstone adjacent to the sandstone-shale interfaces. Similar phenomena have been identified (McMahon and Chapelle, 1991) in clay-sand sequences and by Ulrich *et al.* (1998) in lignite/clay deposits.

Water and Electrolytes

The concentrations of electrolytes and other dissolved species required for proper cellular function is maintained by enzyme-mediated exchange of solutes or solvent with the surrounding medium. Dissolution of electrolytes reduces the thermodynamic activity of water, (*aw),* and this effect is measured by the ratio of the fugacity of water above the solution to that of pure water. At temperatures far below the critical point of water, the fugacity of water is approximately equal to the vapor pressure for example, *aw* in sea water is about 0.98 while in inland salt lakes it can be as low as 0.75. Since the water activity corresponding to appreciable electrolyte concentrations differs only slightly from 1, an alternative measure is provided by the osmotic pressure of the solution which is defined as the hydrostatic pressure that must be applied to the fundamental aspects of microbial-enhanced oil recovery (Todar, 2008).

Differences in ionic strength across membranes provide a powerful driving force for diffusion of water into cells (when the surrounding medium is a more dilute electrolyte) or out of cells (when it is more concentrated). While most bacteria are incapable of surviving in media with *aw* below 0.95, minimum water activities for *Pseudomonas* species (which are of interest as candidates for MEOR) are considerably lower (0.91). Extreme halophiles, such as *Halococcus*, can survive when *aw* =0.75 (Todar, 2008).

Aerobic degradation of benzene, toluene and xylene by halotolerant *Marinobacter* species in soil contaminated with oilfield brines was demonstrated by Nicholson and Fathepure (2004), suggesting potential usefulness in environmental remediation. Anaerobic bacteria from hypersaline environments are of particular interest to MEOR, considering the high salinity of connate water often found in oil-bearing formations. A review of such organisms by Ollivier *et al.* (1994) devoted considerable attention to sulfate-reducing organisms that feed on polymeric substrates such as starch, cellulose, and chitin, and the work of McMeekin *et al.* (1993) on anaerobic microorganisms isolated from concentrated salt lakes in Antarctica suggests applications to hydrocarbon degradation. In addition to MEOR and environmental remediation, the study of halotolerant bacteria is also relevant to food preservation (Vilhelmsson *et al.*, 1997). In addition to the specific chemical and biochemical effects that are often associated with high electrolyte concentrations, non-specific effects can be expected.

Solubility of the vast majority of non-electrolytes decreases with increasing ionic strength; this phenomenon which is known as the 'salting out' effect, is particularly pronounced for non-polar solutes (which tend to have low solubilities in pure water). Important examples are oxygen (the concentration of which controls the thermodynamic driving force for aerobic metabolism) and carbon dioxide ionization of which controls the pH of many natural waters. In this way, high electrolyte concentration could affect both pH and *hE*. In this connection, it is also worth mentioning that the position of the autoprotolysis equilibrium also depends weakly on ionic strength primarily through the activity of water (Vilhelmsson *et al.*, 1997).

Temperature

The increase in random molecular motion resulting from an increase in temperature generally exerts negative effects on enzyme function since the active-site configurations required for catalysis are disrupted. At still higher temperatures, the hydrogen-bonded three-dimensional arrangement of the amino-acid chains also becomes disordered subsequently resulting in irreversible denaturation. This molecular picture of the effects of temperature on enzyme function is generally accepted but it is also to be observed that the temperatures at which these phenomena occur vary widely between organisms. In general, microbes can be classified according to their optimum temperature range as psychrophiles

(<25°C), mesophiles (25-45°C), and thermophiles (45-60°C). The relatively recent discovery of microbes that can survive in water at temperatures above 100°C has considerably extended the range of conditions under which life can be expected to exist; microbes that thrive under such extreme conditions are generally referred to as 'extremophiles' (Todar, 2008).

In 1946, Cox proposed a "geological fence" secured to "posts", namely, organic matter, marine environment, temperature, pressure and time, within which the herd of facts pertaining to petroleum formation should be brought. Observations relative to bacterial activity should logically be considered in the light of known temperature and pressure ranges existent in sedimentary rock. Definite ranges of temperature and pressure exist beyond which bacteria are no longer physically stable or biochemically active. Cox (1946) points out that petroleum is probably formed in sedimentary sections not exceeding 5,000 ft in thicknes. The minimum temperature expected would be about 65^{0}C and the maximum would be slightly higher than 100^{0}C. Certain bacteria can metabolize at temperatures of 55 to 75^{0}C, and some spore-forming bacteria can resist temperature up to 100^{0}C (Cox, 1946).

Pressure

The effects of pressure on microorganisms and their products of metabolism are closely associated with those of temperature, since elevated pressures in natural environments are always associated with temperature variations. Specifically, the pressure in the ocean increases by about 10 MPa for every 1 km of depth, while the temperature of the ocean is about 3^{0}C below about 100 m. On land, the pressure increases by about 3 MPa per km depth, but the temperature increases by about 25^{0}C per km. Thus, a marine bacterium that thrives on the seafloor at a depth of 3 km would be a psychrophile, while its terrestrial counterpart at the same depth underground would be a thermophile. An obvious exception to this generalization would be the bacteria in the vicinity of hydrothermal vents on the seafloor (the so-called 'black smokers'), some of which can withstand temperatures as hot as 121^{0}C (Miroshnichenko and Osmolovskaya, 2006).

Maximum pressure due to an overburden of 5,000 ft would be about 5,000 lb/sq in; hydrostatic head would be 2,000 psi. Furthermore, certain bacteria which do not even form spores can apparently withstand a mechanical pressure of 75,000 psi. However, definite changes in bacterial activity can be observed under the influence of 3,000 psi. ZoBell and Johnson (1949) give data to show that certain bacteria including spore formers are killed at pressures of 7,500 and 9,000 psi in 48 hours. The term "barophilic" has been coined by ZoBell and Johnson (1949) to describe certain bacterial strains (some of marine origin) that grow at a pressure of 9,000 psi. Careful scrutiny of their data reveals that no marked differences exist in the pressure tolerances of some terrestrial bacteria as compared with the marine bacteria. The interesting feature of their experiments was the concomitant increase in pressure tolerance with temperature over the ranges of 1 - 600 atmospheres and 20 - 40^{0}C (Miroshnichenko and Osmolovskaya, 2006).

Indirect and direct effects of pressure on cellular function can be identified. An example of an indirect effect is the augmentation of gas solubility with increasing pressure; this could affect the oxidation potentials if the gases concerned are electron donors or acceptors (such as hydrogen or oxygen, respectively). The study of direct pressure effects originated on bacterial growth under deep ocean conditions and on bacteria isolated from marine sediments. Growth rates of normal bacteria decrease to zero as hydrostatic pressure approaches about 40 MPa. ZoBell and Morita (1957) used the term 'barophilic' to describe bacteria whose growth rate is enhanced at elevated pressure. (The prefix 'baro-' is sometimes replaced by 'piezo-'). It is also customary to refer to bacteria for which the diminution of growth rate commences at pressures above 40 MPa as 'piezotolerant'. A third class of bacteria, which cannot be grown under ambient conditions, are referred to as 'obligatory piezophiles' (ZoBell and Morita, 1957).

DNA analysis reveals the extremophiles to be among the most ancient life forms known, this fact has given rise to intriguing speculations that life on earth could have originated in these extreme environments. The idea that life originated in the

depths of the oceans about 3.8 billion years ago has also been explored in detail (Dostalek and Spurny, 1958).

CONCLUSION

Conclusively, heavy crude oil and tar sands represent the largest potentially recoverable petroleum energy resources known. Worldwide there is an estimated 3 trillion bbls of heavy oil and bitumen resources, and an additional 2.5 trillion bbl of potentially recoverable oil in oil shale and tar sands. Development of efficient production technologies to recover this energy resource is necessary to stabilize future worldwide oil production and ensure an adequate energy supply to take us into the 21st Century.

The alarming rate of decrease in the supply of petroleum and its products and the subsequent increasing production costs makes the potentially inexpensive method such as MEOR useful and economical.

However, MEOR has only recently undergone an overall examination of the available technology and the above review indicates that many reservoir characteristics must be determined before applying it. Some of these characteristics are porosity, permeability, salinity, temperature, and pressure.

AREAS OF MEOR THAT SHOULD MERIT MORE RESEARCH

1. Comparison between the data gotten on the performance of biosurfactants and that of synthetic surfactants under reservoir conditions.
2. Modifications to increase the salt and heat tolerance of biopolymers in the reservoir are needed.
3. Techniques for the bio-emulsification of oil within the reservoir formation need development and intense investigation.
4 A new biopolymer showing promise for MEOR is scleroglucan. Further research should be encouraged to discover other microbially-produced polymers for MEOR.
5. Environmental effects of introducing microorganisms into reservoirs should also be researched into as it appears they have been overlooked in zealous efforts to try to find "super microorganisms" for enhanced oil recovery.
6. Logistics relating to transportation, growth, and metabolite production by microorganisms in petroleum reservoirs need more exposure and details about microbial transport studies specific to each reservoir is advisable under reservoir conditions.

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