Potential Uses of Microorganisms in Petroleum Recovery Technology

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Microorganisms were considered to be detrimental to the petroleum industry in the past. It is now known that they can also be beneficial in terms of oil recovery. There are three ways in which microorganisms may contribute to enhanced oil recovery (EOR): (a) microorganisms can produce biosurfactants and biopolymers on the surface; (b) microorganisms grow in reservoir rock pore throats to produce gases, surfactants, and other chemicals to recover trapped oil in reservoirs; and (c) microorganisms can selectively plug high-permeability channels in reservoir rock so that the sweep efficiency of the recovery process can be increased. In this paper progress in enhanced oil recovery through the use of microorganisms, whether *in situ* or on the surface, is reviewed, and field tests of the *in situ* process are reported.

INTRODUCTION

Enhanced oil recovery (EOR) processes rely upon the use of chemical or thermal energy to recover crude oil that is trapped in pores of reservoir rock after primary and secondary (waterflood) crude oil production has ceased. The residual crude oil in reservoirs makes up about 67% of the total petroleum reserves, indicating the relative inefficiency of primary and secondary production.

Chemicals used for EOR include surfactants to reduce the interfacial tension between oil and water, and oil and rock interfaces. Many microorganisms produce biosurfactants and perform this activity by fermentation of inexpensive raw materials such as molasses. Several biosurfactants are being evaluated for use in EOR.

Polymers are used in EOR as water-thickening agents, allowing injected water to sweep the reservoir rock more efficiently. A biopolymer, xanthan gum, is already in use in oilfield EOR processes, and other biopolymers are being screened for improved properties such as increased stability at higher temperatures.

The chemicals used for EOR must be compatible with the physical and chemical environments of oil reservoirs. In the use of microorganisms *in situ* for EOR, it is necessary to use microbial cultures that can survive and grow at the temperatures, pressures, and salinities present. Microorganisms produce several compounds that have potential for enhanced oil recovery, including carbon dioxide, acids, and alcohols. Carbon dioxide may increase reservoir pressure and decrease the viscosity and gravity of the crude oil, allowing it to move more freely to the producing wells.

A major problem in EOR processes is the variation of permeability in petroleum reservoirs. When water is injected to displace oil the water will preferentially flow through areas of highest permeability, and bypass much of the oil. When bacteria are injected they also will initially flow preferentially into high-permeability zones with the water, but then will grow and block those zones. They will not be so likely to go into lower-permeability zones because of their size. When high-permeability zones are blocked, sweep efficiency is improved, and thus oil recovery. Another factor affecting bacterial penetration is the negative charge on the cell surface. This charge may affect both mobility and penetration of the bacteria.

SURFACTANT PRODUCERS

Surfactants produced by microorganisms are usually lipids, and often they are glycolipids. Microorganisms, often coryneform bacteria, producing trehalose are described in the literature. Trehalose glycolipids are also produced by several strains of *Arthrobacter*, *Mycobacterium*, and *Nocardia* (1). The most commonly studied surfactants come from the cell wall of mycobacteria.

These are called cord factors, and are 6, 6 ' -dimycolates of trehalose.

Cooper (2) screened several organisms for their ability to release bitumen from tar sands. When the isolates that released bitumen were grown using kerosene as the carbon source, he showed that they produced large amounts of biosurfactant that release bitumen at ambient temperatures. The biosurfactant, after extraction from the medium, was added to the tar sands along with kerosene. The amount of released bitumen was compared with controls run with only kerosene added to tar sands. The biosurfactant enhanced the release of bitumen.

Generally the organisms that produce surfactants are aerobes. However, Cooper et al. (3) isolated a neutral lipid biosurfactant from the anaerobe *Clostridium pasteurianum*, and an anaerobic biosurfactant from a *Bacillus* sp. was recently reported (4).

Several combinations of surfactants and bacteria have been suggested for use in EOR drive fluids. Harvey (5) patented a process using dead bacterial and/or yeast cells to carry nonionic surfactants [poly(ethylene oxide) ethers or thioethers] as EOR slugs through the reservoir. Using such slugs in laboratory sand column reservoir models, he obtained 20 to 30% recovery of residual oil. Hitzman (6) and Wagner and Stratton (7) claim that commercial surfactant-treated dead bacterial cells can be used effectively in EOR drive fluids, without the need for injecting live bacterial cells into a reservoir for effective channeling of the injection water.

Recently a Swiss biotechnology company announced plans to field test three biosurfactants in the United States. They utilize continuous culture methods (fermentation apparatus) for the production of these compounds (8).

POLYMER PRODUCERS

Hussain et al. (9) suggested the use of bacterially produced polysaccharides (dextrans) as floodwater thickening agents. Lindblom et al. (10) documented the use of *Xanthomonas* sp. to ferment glucose and produce a thermally stable heteropolysaccharide that can be used in waterflooding systems. Using a model reservoir in the laboratory, they injected an aqueous mixture of the polysaccharide (xanthan gum) and 50×10^3 ppm sodium chloride. By using data from these experiments they showed that the properties of the xanthan gum were not altered by the sodium chloride. Precipitation and plugging of the model reservoir also did not occur. Processes based on the uses of xanthan gum in EOR are: (a) use of biopolysaccharides to thicken injection waters; (b) use with other chemicals, which are sacrificial adsorption agents to prevent adsorption of the polysaccharide on to rock surfaces; (c) use with multivalent cations to bind the polysaccharide molecules together (crosslinking) for preferential partial plugging of the reservoir to prevent water channeling; and (d) use with gel particles, hydrolyzed polyacrylamides, and ferric chloride solutions to assist in the water-thickening process.

Since Lindblom's patent, several laboratories have investigated the production of xanthan gum. It is now used commercially as a mobility control agent for EOR. Its properties of viscosity, shear resistance, and temperature and salt tolerance make it almost an ideal polymer for use in EOR. It is considered superior to polyacrylamides for the above reasons, but it is more expensive (11) and more susceptible to bacterial degradation (12).

Another biopolymer, scleroglucan, is produced by species of the fungus *Sclerotium.* These polymers were examined for possible use in EOR (13). The scleroglucans are neutral polysaccharides with a cellulose-type backbone (14). Scleroglucan and xanthan gum possess similar rheological properties. *Sclerotium* sp. can grow on a variety of carbon and energy sources. It is postulated that the fungus can grow on waste plant material such as hydrolyzed lignin and other industrial wastes, and produce the polymer. These nutrients would be less expensive than hydrocarbons or most other commercially available ones (13).

IN SITU MECHANISMS

Under favorable conditions and in a liquid culture, bacteria can ferment carbohydrates to produce gases such as CO_2 and H_2. Such gases produced *in situ* can contribute to re-pressurization of a pres-

sure-depleted petroleum reservoir. These gases also may dissolve in the crude oil and reduce its viscosity. Additional benefits of this bacteria-induced fermentation process are the production of acids, such as acetic and propionic acids, which can dissolve carbonate rock and increase its permeability and porosity, and the production of solvents, such as acetone, ethanol, 1-butanol, and butanone.

Grula et al. (15) used a microbial screening program to isolate an anaerobic *Clostridium* species that produced gases, acids, alcohols, and surfactants. They isolated several such strains, but tolerance of the bacteria to high salt concentrations (>7%) remains a problem. Success of *in situ* microbial EOR (MEOR) operations depends upon finding microorganisms that can survive and produce the desired metabolic products in reservoirs containing hydrocarbons and saline water. Other researchers continue to investigate *Clostridium* species, *Bacillus* species, and *Enterobacter* for better adaptation to reservoir conditions.

LABORATORY AND FIELD MEOR PROJECTS

C.E. Zobell, in 1946, patented a process for the secondary recovery of petroleum, using anaerobic, hydrocarbon-utilizing, sulfate-reducing bacteria such as *Desulfovibrio* species *in situ* (16). The mechanism of oil recovery with this organism was thought to be similar to those with other microorganisms such as *Clostridium* sp., i.e., through the production of organic acids, gases, and surfactants. He reported that an aqueous mixture of nutrients, *Clostridium,* and *Desulfovibrio* resulted in hydrogenase production by the bacterial cells. These microorganisms were considered to use this enzyme, hydrogenase, as a catalyst for producing acetic acid, butyric acid, and ammonium hydroxide from CO_2, water, and nitrates in the reservoir. Some of these products are known to enhance the release of oil from reservoir rock. Other patents were granted to Updegraff and Wren (17), who proposed injecting *Desulfovibrio* and molasses downhole for the production of organic acids and gases. In later work, other fermentation-type bacteria were used to produce substantial amounts of organic acids and carbon dioxide to enhance oil recovery in laboratory experiments (18). A drawback to this early work was the fact that *Desulfovibrio* species produce hydrogen sulfide and thus should not be considered for MEOR processes.

Hitzman (19) patented a process for the injection of bacterial spores along with nutrients into a reservoir. He asserted that the spores would germinate in the reservoir and enhance removal of oil from reservoir rock. The hypothesis was tested in the laboratory by using an oil-saturated sandpacked column. An aqueous solution containing spores of *Clostridium roseum* and molasses was passed through the column and an improved release of oil (about 30%) was obtained.

Later patents by Hitzman concern the use of microorganisms that feed on injected polymers and the byproducts of CO_2 floods, to produce chemicals for EOR, i.e. gases, acids, solvents, and surfactants (20). In polymer floods the injected organisms feed on polymer that was adsorbed on the reservoir rock. In CO_2 floods, the microbes feed on soluble compounds of carbon, nitrogen, and sulfur left behind the CO_2-crude oil slug. The process has mobilized residual oil in sandpack tests but no core or field tests are reported.

Knapp et al. (21) reported the isolation of 22 microorganisms that produce biopolymers and emulsifiers. One strain was isolated that grew in 10% salt concentrations, over a pH range of 4.6 to 9.0, at temperatures up to 50 ˚C, and in the presence of crude oil. They demonstrated in the laboratory that glucose, ammonium sulfate, and potassium phosphate were easily transported through sandstone cores. Viable bacterial cells in aqueous solutions of 2% NaCl and 0.01% $CaC1_2$ injected into these cores were not recovered in the effluent. The addition of glucose to cores previously inoculated with bacteria resulted in a significant decrease in permeability, indicating that bacteria were multiplying and plugging the pores. Bacteria indigenous to all of the cores treated were *Pseudomonas* sp., *Bacillus* sp., and Actinomycetes. A major problem in these experiments was in determining how much of the plugging was caused by

injected bacteria and how much by indigenous bacteria. The problem persisted even when cores were steam-cleaned and autoclaved. "Sterilization" of cores with chlorine dioxide diminished the problem, but the bacterial populations returned at 48 hr incubation.

Jack et al. (22) considered that emulsification of viscous crude oil *in situ* is not feasible because of the problems in transporting the bacteria through the reservoir rock.

Yarbrough and Coty (23) recently reported on a field test they performed in 1954 in Arkansas. A 2% solution of beet molasses in fresh water was injected during a 6-month period, along with 18 200-gal containers of broth containing *Clostridium acetobutylicum.* Freshwater breakthrough occurred at the production well 70 days after injection started. Fermentation products (short-chain fatty acids, CO_2, and traces of ethanol, 1-butanol, and acetone) and sugars appeared 80 to 90 days after the injection started. No increase in hydrogen content was noted. Production of oil increased from 0.6 bbl/day to 2.1 bbl/day. The experiment was considered successful, but no further field tests were conducted.

Bond (24) injected 5,000 gal of 290 agar gel agent medium containing sand and *Desulfovibrio hydrocarbonoclasticus* (no longer a valid species) into a sandstone reservoir at a depth of 3,000 ft. Prior to the injection, the well produced 15 bbl/day. After the injection, the well was shut in for 3 months to allow the bacteria to act. When the well was placed back on production, it produced 25 bbl/day.

Johnson (25) injected 150 stripper wells in the U.S. that produced, on an average, 2 bbl/day, with no wellhead pressure. The reservoir porosities were 10 to 30%, depths 200 to 1,000 ft, with an average reservoir temperature of 38 ˚C. The work was done during the years 1977-81. The inoculum was 1 to 10 gal of a mixed culture of *Bacillus* and *Clostridium* spp. (non-hydrocarbon-utilizing) with crude molasses and mineral salts. In the typical situation, the bacterial inoculum required 10 to 14 days to adequately multiply within the treated area of the reservoir. The results varied, but in suitable reservoirs, 20 to 30% additional oil-in-place was recovered. These suitable conditions include oil of 15 to 30˚ API gravity, formation water of less than 10×10^4 ppm salt content, preferably a carbonate reservoir, and a temperature of approximately 38 ˚C.

Petrogen, Inc. did some preliminary field testing with 24 wells during 1977-82. Wells varied in depth from 300 to 4,600 ft. Some 75% of the wells showed a pressure increase of 10 to 200 psi. The type of microbial or nutrient injectant was not defined. Four wells doubled production for 6 months, and 12 increased production by 50% for 3 months. The production increase was indicated to average 42%; however, the final results remain to be reported (26).

The first MEOR project in the Rocky Mountains was initiated in 1983 (27). An independent oil operator purchased three field service operations from Petroleum Bio-Resources Company. These were (a) a reservoir field conditioning system to avoid plugging; (b) use of a microorganism that produces gas and surfactant; and (c) use of a microorganism that produces a polysaccharide for mobility control. The most recent information regarding this field project indicated that production immediately doubled, which was attributed to well stimulation, whereas the remainder of the wells in the field increased from 26 to 60 bbl/day, which was considered due to mobilization of oil by microorganisms and waterflooding (28).

Microbial flooding is being tested for oil recovery in an oil field near Nowata, Oklahoma, by NIPER, the U.S. Department of Energy, and two industrial paticipants (29). A microbially enhanced waterflood is being implemented in a field that has been waterflooded for more than 35 years and in a lease where the average oil production per well is currently 0.4 bbl/day. This microbial waterflood test is a carefully monitored field experiment, and should provide much needed documentation and information for future MEOR testing. Laboratory compatibility testing and coreflooding studies were done to optimize microbial formulations for this test (30, 31, 32).

Bryant and Douglas (30) have demonstrated the oil recovery efficiency of several different bacterial strains in Berea

sandstone cores. Treatment with microorganisms could recover an average of 32% more residual light crude oil than waterflood recovery. There were some spore-forming bacteria that brought about a recovery of 60 and 50% more crude oil. Heavy oil (API 14 and 17) Berea sandstone core experiments showed that selected microbial strains could recover up to 72% of the heavy oil left after waterflooding.

EUROPEAN FIELD TESTS

The United States has not been as active in field tests of microorganisms for EOR as some of the eastern European countries.

Kuznetsov (3) reported that in 1950 bacteria were present in certain oil-gas-bearing strata in the Saratov and Guguruslan areas of the USSR in such numbers that large quantities of CO_2 were generated (depth was approximately 3,300 ft). Apparently methane was also formed. In subsequent work, Kuznetsov et al. injected mixed aerobic and anaerobic bacteria together with acid-hydrolyzed substances from peat and soils, shut in the well for 6 months, and then put the well in production (26). The rate of oil production rose from 275 to 300 bbl/day; however, 4 months later it had fallen to 270 bbl/day. No other work has been reported concerning these field tests.

Von Heningen et al. (34) reported on two field tests performed in the Netherlands. The year and location of the tests are unknown. In one test they used *Betacoccus dextranicus* in a sucrose-molasses medium of 10% total sugar content and obtained a 30% increase in cumulative oil recovery. The second test used a mixed culture of slime-forming bacteria in 50% molasses. The oil-to-water production ratio changed to 1:20 compared to 1:50 before treatment.

Field tests in Czechoslovakia in the mid 1950s by Dostalek and Spurney (34) utilized injected sulfate-reducing *(Desulfovibrio)* and hydrocarbon-utilizing *(Pseudomonas)* bacteria with nutrients (generally molasses). The daily average oil production increased by nearly 7% during the 6-month experiment period. No further work has been reported since 1958.

In Hungary, Jaranyi et al. (35) used a mixture of anaerobic thermophilic bacteria that fermented molasses in an MEOR field test to recover naphthenic crude. They also used raw sewage as an inoculum (100 L, along with 20 to 40,000 kg molasses) in later trials (1969-70). The deepest reservoir was 8,200 ft, where the pressure was 228 atm and the temperature was 97 ˚C. In most reservoirs tested (7 out of 10), the introduced microbial populations produced a positive effect on cumulative oil recovery.

Karaskiewicz conducted 18 field trials in Poland between 1961 and 1969 (36). Microbial cultures were obtained from soil and water samples taken in the vicinity of the oil fields and from sugar factory waters. The mixed culture includes the genera *Arthrobacter, Clostridium, Mycobactetium, Peptococcus,* and *Pseudomonas* grown in 10-L bottles with formation water plus 4% molasses, and incubated at 32 ˚C. The wells ranged in depth from 1,650 to 5,000 ft. The rate of additional oil recovery ranged from 20 to 200% of the original production rate. A second injection of nutrients was considered a major factor in the increased oil recovery.

Lazar (37) recently published an extensive review of MEOR work done in Romania during the last decade. Three areas of research are emphasized: (a) examination of the bacterial population in the formation water of the reservoir; (b) adaptation of these microorganisms in the laboratory for oil release; and (c) field testing of such adapted cultures. Seven wells were treated with microbial formulations and two conclusions were drawn: the bacterial population caused an increase of oil flow up to 200% for 1 to 5 years in 2 out of 7 reservoirs (the other five were unaffected), and much information about the ecology of the reservoir is needed before initiating any MEOR activity.

Overall, MEOR field trials in eastern Europe have been promising. Field testing in Romania is still in progress, and is supported in part by the Romanian government. There are obvious difficulties in obtaining key information regarding European MEOR field tests; a large amount of information may have been accumulated, but at present it is unavailable to the general public.

SELECTIVE PLUGGING BY BACTERIA

In the early 1940s, it was observed that bacteria could clog or plug a reservoir. Early studies relating to bacterial plugging of a reservoir were mainly concerned with the prevention of this process.

Kalish et al. (38) studied the effects of bacterial plugging in sandstone cores and determined that the slime-forming and larger bacteria have a much greater effect on permeability reduction than the smaller and non-slime-forming ones. Jang et al. (39) and Jenneman et al. (40) examined the effect of rock permeability on bacterial transport. Both concluded that nutrients such as dissolved sucrose, inorganic salts, and yeast extract move easily through porous media. Jang et al. demonstrated that bacterial spores, because of their size and cell surface properties, travel faster and with less adsorption to rock through sandstone cores than bacterial cells. Jenneman et al. reported that nutrients and bacterial cells are selectively transported to zones of high permeability.

It is clear that microbial movement should receive more attention. Other microbial technological processes used in the environment may help to further transport knowledge and research. Virtually no studies have been done to examine microbial migration under actual reservoir conditions of pressure, temperature, and field brine and oil. These studies are vital to our understanding of microbial transport for MEOR processes.

CONCLUSIONS

Because of the decreasing supply of petroleum and the increasing production costs, a potentially inexpensive method such as MEOR may prove useful and economical. However, MEOR has only recently undergone an overall examination of the available technology. This review indicates that many reservoir characteristics must be determined before applying MEOR. Some of these characteristics are porosity, permeability, salinity, temperature, and pressure. It is unlikely that a single MEOR method can be applied to all types of reservoirs.

Areas of MEOR research that merit more investigation are listed here.

1. Data on the performance of biosurfactants compared with that of synthetic surfactants under reservoir conditions should be obtained.
2. Techniques for the bio-emulsification of oil within the reservoir formation need development.
3. Modifications to increase the salt and heat tolerance of biopolymers in the reservoir are needed. A new biopolymer showing promise for EOR is scleroglucan. Research should be increased to discover other microbially produced polymers for EOR. One disadvantage of biopolymers that further research may mitigate is their extreme biodegradability.
4. Parameters relating to transport, growth, and metabolite production by microorganisms in petroleum reservoirs need vigorous research. Microbial transport studies must be performed under reservoir conditions.
5. Potential environmental effects of introduction of microorganisms into reservoirs are important and it appears that they have been overlooked in zealous efforts to try to find "super microorganisms" for enhanced oil recovery. Research on microbial transport and activity in the reservoir should help alleviate environmental concern. This research is also necessary for adequate design of MEOR field projects.

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