Chapter 12
Biotechnology in Enhanced Petroleum Oil Recovery

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12.1 Introduction

Enhanced oil recovery (EOR) methods are conventionally applied in the petroleum industry to recover residual oil from wells and oilfield emulsions. In petroleum oil well, residual high viscous oil is often located in areas inaccessible to fluids used for flooding, or the oil is adhered to sand or carbonate particles in the reservoir making it difficult to recover further and usually more than two-third of the oil in the reservoir is left unrecovered after primary and secondary extraction (Sen 2008; Brown 2010). Conventional EOR methods make use of chemicals (solvents, polymers, surfactants), injected gases (CO_2, N_2, flue gas), and thermal methods (steam flood, hot water) to extract remaining oil.

Oilfield emulsions, both oil-in-water and water-in-oil, are formed at various stages of exploration, production, oil recovery, and processing and represent a major problem for the petroleum industry. North American producers estimate that as much as 2 % of their oil production ends up as an emulsion during production and pipeline transport, which translates into millions of dollars in lost revenue and potential environmental damage (Becker 1997). Traditional physical and chemical de-emulsification methods to recover oil include centrifugation, heat treatment, electrical treatment, and chemicals containing soap, fatty acids, and long-chain alcohols. However, physical and chemical de-emulsification processes

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are capital intensive, and emulsions often generated at the wellhead have to be transported to central processing facilities.

During last two decades, interest in using microorganisms and their product has continuously increased due to their biodegradability, no reliance on the cost of crude oil, and representation of a cost-effective and environmentally friendly alternative (Gao and Zekri 2011). Both microbial-enhanced oil recovery (MEOR) and biodemulsification methods apply microorganisms or their metabolic products (solvents, polymers, organic acids, and surfactants) to mobilize residual oil left over in the wells or de-emulsify petroleum oil emulsions (Zhou et al. 2008; Khire 2010). A brief overview of oil recovery methods is presented in this chapter.

12.2 Microbial-Enhanced Oil Recovery

MEOR methods have been actively pursued both in laboratory and field conditions with many successful attempts. Although more than 70 % of the low temperature oilfield wells treated by bacteria achieved increases in oil production rate, considerable uncertainty remains regarding process performance due to reservoir heterogeneity.

12.2.1 Mechanisms of MEOR

The residual oil is held in porous rocks by capillary pressure that is equal oil–water interfacial tension (IFT). MEOR methods improve the mobility of oil through decreasing oil viscosity, dissolution of carbonates in the reservoir, physically displacing oil, and plugging of highly permeable areas in the reservoir to increase the sweep efficiency of water flooding (Lazar et al. 2007; Elshahed 2010). MEOR mechanisms include microbial plugging, biofilm growth production of bioproducts (biosurfactants, biopolymers and solvents) and, gases (CO_2 and CH_4) (Youssef et al. 2009; Kaster et al. 2012). While biosurfactants reduce IFT to make residual oil flow, certain bacteria produces biopolymers that can plug the high-permeability zones with large pores, thus forcing injected water to sweep the oil in low permeability zones. Biofilms growing on the surface of the porous rock may lead to a change of surface properties and a decrease in permeability. Bacterial gases and solvents can dissolve in crude oil and reduce its viscosity while increasing reservoir pressure also leads to improved mobility and oil recovery (Voordouw 2011). Certain bacterial species can degrade the paraffin deposits near the wellbore region to improve permeability and oil production. Only bacteria are considered promising candidates for MEOR due to their higher tolerance of extreme reservoir properties in terms of high salinity, pH, temperature, pressure, and nutrient availability (Ward et al. 2009). Potentially useful MEOR isolates including extremely thermophilic anaerobes have been isolated and cultured in the laboratory (Brown 2010).

12.2.2 Field Applications of MEOR

MEOR has been tested in various oilfields around the world particularly in the USA, China, Malaysia, and Argentina with some success (Gao and Zekri 2011; Ward et al. 2012). Most of the successful MEOR treatments were conducted for formations with a low temperature (below 55 °C), low water salinity (less than 100,000 ppm), high water cut (above 75 %), and low production rate. Additional oil recovery with the MEOR in these field studies were reported in the range of 15,000–70,000 bbl. Although the reservoir heterogeneity significantly affects oil recovery efficiency, single-well stimulation treatment with MEOR may increase the rate of production from 0.2 to 0.4 ton of oil per day for 2–6 months without additional treatments. Microbial flooding processes are mostly used, where bacteria and nutrients were injected and carried deep into the reservoir with the normal water flooding operation. Selective plugging and biosurfactant production were believed to be the main contributor to the better oil recovery in the successful field studies. Reductions in IFT, crude oil viscosity, and paraffin content were also observed in some studies.

Although MEOR has potential for use in oil recovery from oil sands, there has been only limited number of studies on oil extraction from oil sands using MEOR (Harner et al. 2011). The oil sands deposits in Western Canada Sedimentary Basin (WCSB) cover an area of 1,400,000 km^2 in the western part of North America with the depth varying from 0 to 500 m and containing 6–18 % bitumen. The oil sands deposits are a mixture of a sand, clay, water, and bitumen and comprise of at least 85 % of the total immobile bitumen in place in the world (Huang et al. 2008). Common oil sand extraction methods decrease the viscosity of the bitumen through steam, chemical solvents, or hot air injection followed by hot water extraction and agitation process to facilitate separation of oil from the sand and water. Microbial communities, including sulfate-, nitrate-, and iron-reducing fermentative bacteria and methanogens, interact in the oil sands environment, metabolize crude oil compounds, and produce biosurfactants, solvents, gases, and acids to displace oil from mineral surfaces and reduce oil viscosity (Youssef et al. 2009; Harner et al. 2011).

12.3 Microbial De-emulsification of Petroleum Oil Emulsions

Oilfield emulsions are formed at various stages of petroleum production, processing, and transportation. While traditional physical de-emulsification methods are capital intensive, disposal of the chemical de-emulsifier in the aqueous phase and removal of the de-emulsifier from the oil phase create further complications in the chemical processes. Further, chemical treatment methods require a series of tests and tedious exercise to find a suitable emulsion-breaking chemical for every emulsion type. Often, emulsions are transported to centralized facilities for oil recovery operations. On the other hand, microbial processes can be carried out at non-extreme conditions and an effective biodemulsification process may be used directly to treat emulsions on site at the wellhead, thus saving on transport and high capital equipment costs (Van Hamme et al. 2003).

A number of microbial species are known to possess de-emulsification properties (Table 12.1). Microbes exploit hydrophobic cell surfaces and the dual hydrophobic/hydrophilic nature of biosurfactants to displace the emulsifiers that are present at the oil–water interface in the biodemulsification process (Kosaric 1996; Singh et al. 2006; Huang et al. 2010). Some microbes produce certain compounds with de-emulsification properties, e.g., acetoin, polysaccharides, glycolipids, glycoproteins, phospholipids, and rhamnolipids (Das 2001; Singh et al. 2007). In a study to understand process mechanisms of demulsification using demulsifier from *Alcaligenes* sp. S-XJ-1, it was observed that the process appeared to begin with the adsorption of the biodemulsifiers onto the water–oil interface due to their amphiphilic nature (Wen et al. 2010; Liu et al. 2011). The reaction of the biodemulsifiers with the emulsifiers then results in the removal of thin liquid film from the surface of dispersed droplets to cause coalescence of droplets and phase separation.

Cell surface hydrophobicity (CSH) of bacteria plays a significant role in nonspecific adsorption to all kinds of biological or nonbiological surfaces and interfaces and bacterial migration and adsorption at the oil–water interface. The CSH of *Rhodococcus* sp. and *Alcaligenes* sp. is generally enhanced via accumulation of fatty acids on the cell surface to adhere to the oil phase (Chang et al. 2009; Huang et al. 2012). Demulsifying bacteria possessing a relatively high CSH and total unsaturated degree for the cell wall-bound fatty acids perform better demulsification activity. Generally Oleic acid (C18:1) and linoleic acid (C18:2) had a positive effect on the formation of CSH, while stearic acid (C18:0) and linolenic acid (C18:3) had the opposite effect.

Although the demulsification capability of most of the cultures is not affected by lyophilization or freezing and thawing, it can be completely destroyed by autoclaving or alkaline methanolysis or significantly reduced by washing of cells with any lipid solubilizing solvent such as *n*-pentane, *n*-hexane, kerosene or chloroform–methanol. Nutrient (carbon and nitrogen) sources in the growth media and cultural conditions significantly impact the biodemulsifying properties of the bacteria (Kosaric 1996; Das 2001; Huang et al. 2009; Singh et al. 2012). The cultures grown on petroleum fractions produce better biodemulsifiers compared to the cultures grown on carbohydrate sources (Nadarajah et al. 2002a, b). The biodemulsification activity also depends upon growth media, cell density, and age of the culture (Mohebali et al. 2012).

Mixed bacterial cultures with proven demulsifying ability have been tested on a range of oil emulsions obtained from different oil companies (Nadarajah et al. 2002a, b). The mixed culture grown on petroleum oil products caused separation of oil from water and solids in various oilfield emulsions within 24–96 h. The initial demulsification rate varied significantly among the various emulsions tested possibly due to the variation in the composition and viscosity of

Table 12.1 Applications of biotechnology in petroleum oil recovery

Biotechnology	Biocatalyst	Application
Microbial enhanced oil recovery (MEOR)	Biomass/bacteria (*Bacillus subtilis*, *Arthrobacter protoformiae*, *Serratia marcescens*); biosurfactants like rhamnolipid, trehaloselipids, surfactin, emulsan, viscosin (*Acinetobacter calcoaceticus*, *Arthrobacter paraffineus*, *Bacillus licheniformis*, *Corynebacterium fascians*, *Pseudomonas rubescens*); biopolymers like curdlan, dextran, pullulan, levan, xanthan (*Bacillus polymyxa*); solvents like acetone, butanol, propanediol (*Clostridium acetobutylicum*, *C. pasteurianum*, *Brevibacterium viscogenes*, *Xanthomonas campestris*); acids like propionic and butyric (*Clostridium* spp., *Enterobacter aerogenes*); Gases like CO_2, CH_4, H_2 (*Clostridium acetobutylicum*, *Clostridium tetanomorphum*, *Enterobacter aerogenes*, *Methanobacterium* sp.)	Tertiary oil recovery employing microbes or their products to mobilize residual oil to enhance crude oil recovery; biosurfactants and chemicals produced by microbes help in oil dissolution, viscosity reduction; selective biomass plugging, increased permeability; increased pressure and oil swelling
Biodemulsification	*Acinetobacter calcoaceticus*; *Bacillus subtilis*; *Corynebacterium petrophilum*; *Nocardia amarae*; *Ochrobactrum anthropi*; *Pseudomonas aeruginosa*; *Rhodococcus globerulus*	Oil recovery through de-emulsification of oil emulsions, oil solubilization, viscosity reduction, and wetting

the emulsions and the nature of emulsifier. Generally, emulsions with higher water content were found easier to break compared to the ones with lower water content. Due to variability in the properties of oilfield crude oil emulsions, inconsistencies have been experienced in performance of the different biodemulsification processes. Further research on biodemulsification processes with field emulsions needs to be aimed at development of more reliable and universally effective systems.

12.4 Conclusion

With current crude oil prices around \$85–100/barrel, there is strong economic incentive to recover residual oil from petroleum oil reservoirs. MEOR methods have been actively pursued both in laboratory and field conditions with many successful attempts. However, considerable uncertainty remains regarding process performance due to reservoir heterogeneity. Oily wastes such as oil emulsions, slop oils, and oily sludges, which are produced at many stages of exploration, production, refining, and recovery contain significant amounts of oil associated with them. Biological methods for de-emulsification of oil emulsions and slop oils have been developed and evaluated at the laboratory scale and biodemulsifiers have shown potential in providing alternative to physicochemical oil recovery while operating at significantly lower cost than conventional processes such as centrifugation. Microbial de-emulsifiers represent potential alternatives to the chemicals, but most of the studies have been done on mechanism of biodemulsification in the laboratory conditions only. Field scale studies will further explore the potential of commercial biodemulsification application in the petroleum industry. Genetic manipulations on biodemulsifying organisms have not been attempted so far and would further help developing efficient biodemulsifiers.

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