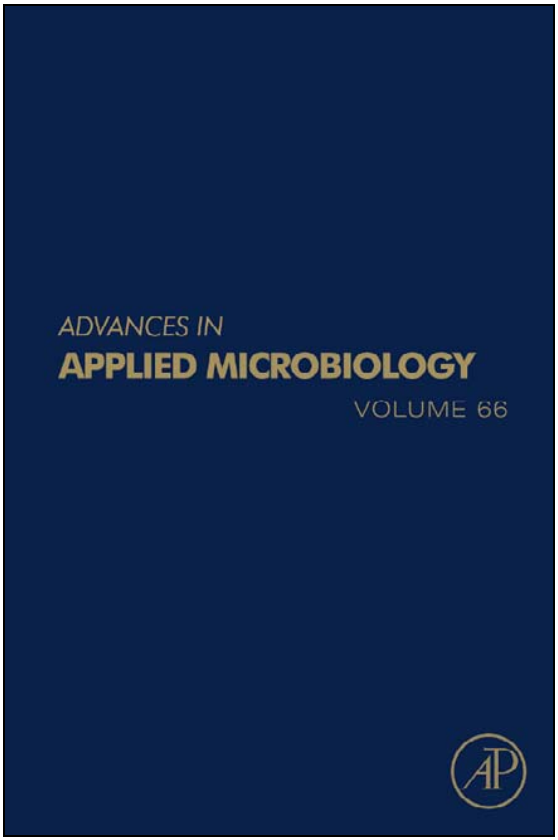

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CHAPTER 6

Microbial Processes in Oil Fields: Culprits, Problems, and Opportunities

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Contents

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Abstract Our understanding of the phylogenetic diversity, metabolic capabilities, ecological roles, and community dynamics of oil reservoir microbial communities is far from complete. The lack of appreciation of the microbiology of oil reservoirs can lead to detrimental consequences such as souring or plugging. In contrast, knowledge of the microbiology of oil reservoirs can be used to enhance productivity and recovery efficiency. It is clear that (1) nitrate and/or nitrite addition controls H_2S production, (2) oxygen injection stimulates hydrocarbon metabolism and helps mobilize crude oil, (3) injection of fermentative bacteria and carbohydrates generates large amounts of acids, gases, and solvents that increases oil recovery particularly in carbonate formations, and (4) nutrient injection stimulates microbial growth preferentially in high permeability zones and improves volumetric sweep efficiency and oil recovery. Biosurfactants significantly lower the interfacial tension between oil and water and large amounts of biosurfactant can be made *in situ*. However, it is still uncertain whether *in situ* biosurfactant production can be induced on the scale needed for economic oil recovery. Commercial microbial paraffin control technologies slow the rate of decline in oil production and extend the operational life of marginal oil fields. Microbial technologies are often applied in marginal fields where the risk of implementation is low. However, more quantitative assessments of the efficacy of microbial oil recovery will be needed before microbial oil recovery gains widespread acceptance.

Key Words: Petroleum microbiology, Biosurfactants, Sulfate reducers, Souring, Plugging, Oil recovery.

I. INTRODUCTION

World population is projected to increase by nearly 45% over the next 4 decades and by the middle of the century there will be more than 9 billion people (U.S. Census Bureau, International Data Base, August 2006;

http://www.census.gov/ipc/www/idb/worldpopinfo.html). The per capita energy consumption is a good predictor of the standard of living, which means that the demand for energy will continue to increase with the world's population and its desire to improve living standards (Hall *et al.*, 2003). The economic prosperity and security of nations will depend on how societies manage their energy resources and needs. An important question is how will we meet the future demand for more energy. Historically, the combustion of fossil fuels–oil, coal, and natural gas–has supplied more than 85% of world energy needs (Energy Information Agency, 2006). The reliance on fossil fuel energy has increased CO_2 emissions and fostered global climate change. For these reasons, it is advantageous to diversify our energy sources with the inclusion of more carbon-free or carbon-neutral fuels. However, even the most optimistic projections suggest that renewable energy sources will comprise less than 10% of the world's requirements through 2030 (Energy Information Agency, 2006). The most critical energy need is in the transportation sector. The use of nonpetroleum sources such as ethanol and of unconventional oil sources (shale oil, gas-to-liquids, and coal-to-liquids) will increase substantially, but each will only account for <10% of the demand by 2030 (Energy Information Agency, 2007). Thus, crude oil will likely continue as the dominant source of transportation fuels in the near future.

Current technologies recover only about one-third to one-half of the oil contained in the reservoirs. Globally, about 1 trillion barrels (0.16 Tm^3) of oil have been recovered, but about 2–4 trillion barrels (0.3–0.6 Tm^3) remain in oil reservoirs and are the target of enhanced oil recovery (EOR) technologies (Hall *et al.*, 2003). In the United States, more than 300 billion barrels (47.6 Gm^3) of oil remain unrecoverable from U.S. reservoirs, after conventional technologies reach their economic limit (Lundquist *et al.*, 2001). A critical feature of U.S. oil production is the importance of marginal wells whose production is <1.6 m^3 of oil or <1600 m^3 of natural gas per day. Currently, 27% of the oil (about the same amount imported from Saudi Arabia) and 8% of the natural gas produced in the U.S. onshore (excluding Alaska) is produced from marginal wells. These wells are at risk of being prematurely abandoned and it is estimated that about 17.5 million m^3 of oil was lost because of the plugging and abandonment of marginal wells between 1994 and 2003. New technologies to recover entrapped oil and to slow the decline in oil production in marginal wells are needed to increase oil reserves.

The amount of oil recovered by EOR technologies is not large, about 0.3 million m^3 per day (Anonymous, 2006), even though a number of economic incentives have been used to stimulate the development and application of EOR processes. Chemical-flooding technologies such as micellar or alkaline-surfactant-polymer flooding displace tertiary oil efficiently, but they have been marginally economic because of high

chemical costs. Chemical losses because of adsorption, phase partitioning, trapping, and bypassing when mobility control is not maintained can be severe (Green and Willhite, 1998; Strand *et al.*, 2003; Weihong *et al.*, 2003). The only way to compensate for these losses is by increasing the volume of the surfactant solutions (Green and Willhite, 1998). Further, the implementation of these processes is complicated by reservoir heterogeneity and the need for large capital investment. All of these factors make chemical flooding a high-risk venture.

Microbially EOR (MEOR) processes have several unique characteristics that may provide an economic advantage. Microbial processes do not consume large amounts of energy as do thermal processes, nor do they depend on the price of crude oil as many chemical processes do. Because microbial growth occurs at exponential rates, it should be possible to produce large amounts of useful products rapidly from inexpensive and renewable resources. The main question is whether microbial processes do, in fact, generate useful products or activities in amounts and at rates needed for significant oil recovery (Bryant and Lockhart, 2002).

In this chapter, we will review what is known about the microbiology of oil fields from cultivation-dependent and cultivation-independent approaches. The technical feasibility of MEOR processes will be assessed by analyzing laboratory and field data to determine (1) if microbial products or activities mobilize entrapped oil from laboratory model systems, (2) if it is possible to produce the needed products or to stimulate the appropriate activity in the reservoir, and (3) if oil production coincides with the *in situ* product formation or activity stimulation. The recent book on petroleum microbiology provides an excellent resource (Ollivier and Magot, 2005). A number of compilations of the results of microbial field trials are available that provide detailed information on the characteristics of the reservoirs and the microorganisms and the nutrients used (Bass and Lappin-Scott, 1997; Hitzman, 1983, 1988; Lazar, 1991). Metabolism of hydrocarbons has been recently reviewed (Van Hamme *et al.*, 2003) and will only be covered here in its relationship to EOR. Other reviews and summaries of MEOR are available (Finnerty and Singer, 1983; Islam and Gianetto, 1993; Jack, 1988; McInerney *et al.*, 2005a). Methodologies for MEOR have been reviewed (McInerney and Sublette, 1997; McInerney *et al.*, 2007). Two other reviews provide an excellent summary of the early history of MEOR (Davis and Updegraff, 1954; Updegraff, 1990).

II. FACTORS GOVERNING OIL RECOVERY

An understanding of the multiphase flow properties of reservoir rock and the mechanisms that entrap oil is important for the success of any EOR project, including those involving microorganisms. When a well is drilled

into an oil reservoir, oil and water are pushed to the surface by the natural pressure within the reservoir. As this pressure dissipates, pumps are placed on the well to assist in bringing the fluids to the surface. This stage of oil production is called primary production (Planckaert, 2005). Eventually, additional energy must be added to the reservoir to continue to recover oil. Often surface water, seawater, or brine from a subterranean formation is injected into the formation to push the oil to recovery (production) wells. This stage of oil production is called secondary oil production. After extensive water flooding, a large amount of oil still remains entrapped in the reservoir (called residual oil) and is the target of EOR (or the tertiary stage) processes.

The capillary pressure within the vicinity of the well governs the rates of oil and water production (Craig, 1980; Donaldson, 1985). The relative fluid saturations of water and oil in this region are functions of the capillary pressure between these two fluids, which is determined by the pore entrance size distribution of the rock. If the pore entrance size distribution decreases, the capillary pressure shifts and causes oil production to cease at an oil saturation, which otherwise would normally allow oil production. The accumulation of small particles, scale, paraffins, and asphaltenes precipitates, and the compaction of the sand plug drainage channels for oil and cause changes in capillary pressure. Mobile oil may be available only a short distance from the well, if the appropriate drainage patterns can be reestablished. Technologies that remove particulates, scales, paraffin, and asphaltene deposits can restore the drainage pattern or alter fluid saturations making oil mobile and increasing the rate of oil production from the well (Donaldson, 1985).

The goal of EOR, MEOR, when microorganisms are involved, is to increase the ultimate amount of oil recovered from a reservoir and not just to increase the productivity of individual wells. The efficiency of oil recovery is defined by the following equation (Craig, 1980; Green and Willhite, 1998):

$$E_r = E_d \times E_v \tag{6.1}$$

where E_r is the recovery efficiency expressed as a fraction of the original oil-in-place, E_d is the microscopic oil displacement efficiency expressed as the fraction of the total volume of oil displaced from a unit segment of rock, and E_v is the volumetric sweep efficiency expressed as the fraction of the total reservoir that is contacted by the recovery fluid. The microscopic displacement efficiency is a measure of the amount of oil that remains in small pores or dead-end pores after a recovery process. The viscosity and the capillary forces that hold the oil in place are expressed as a ratio called the capillary number (N_{ca}) (Tabor, 1969):

$$N_{ca} = (\mu_w v_w)/(s_{ow}) \tag{6.2}$$

where μ_w is the viscosity, v_w is the volumetric fluid flux, and s_{ow} is the oil–water interfacial tension (IFT). Chemical methods such as surfactant, micellar–polymer, or caustic or polymer flooding increase the capillary number by reducing interfacial tension or increasing water viscosity, respectively. Thermal methods reduce oil viscosity. Significant oil recovery requires a 100- to 1000-fold increase in capillary number (Reed and Healy, 1977). Some biosurfactants generate the very low interfacial tensions needed for significant oil recovery (Lin *et al.*, 1994; McInerney *et al.*, 1990; Nguyen *et al.*, 2008; Youssef, *et al.*, 2007a).

The volumetric sweep efficiency often dominates the recovery process when large variations between the viscosity of the recovery fluid and the oil or between the permeability of different zones of the formation exist (Craig, 1980). Large differences between the oil and aqueous phase viscosities will result in irregular movement of these fluids with water moving more rapidly than oil and reaching the production well first. The relative mobility of the two phases is expressed in the mobility ratio (Craig, 1980):

$$M = (k_w \mu_o)/(k_o \mu_w) \tag{6.3}$$

where M is the mobility ratio, k_w is the relative permeability of water in the waterflooded zone, k_o is the relative permeability of oil in the oil saturated zone, μ_o is the viscosity of the oil, and μ_w is the viscosity of water. Mobility ratios less than 1 are favorable and result in a uniform displacement of oil. Mobility ratios much greater than 1 are unfavorable and result in water channeling through the oil. The addition of polymers, such as xanthan gum, increases the viscosity of the water phase, resulting in favorable mobility ratios.

Poor sweep efficiency also occurs in reservoirs that have large permeability variations (Craig, 1980; Hutchinson, 1959). Because most oil reservoirs are composed of heterogeneous layers of rock, permeability variation is often an important factor controlling the sweep efficiency and the ultimate recovery of oil. Water preferentially flows through high permeability layers and little or no flow occurs in low permeability layers. Waterfloods will push the oil out of high permeability layers but the oil in low permeability layers remains unrecovered. Bacteria will preferentially plug high permeability layers, which will lead to more uniform water movement through the reservoir and improved oil displacement (Crawford, 1962, 1983; Raiders *et al.*, 1986a).

III. MICROBIAL ECOLOGY OF OIL RESERVOIRS

Multiple groups of microorganisms with diverse physiological and metabolic abilities and phylogenetic affiliations have routinely been recovered from oil reservoirs. The ability of microorganisms to sustain an underground deep biosphere, which is independent of above ground primary productivity (Chapelle *et al.*, 2002; Krumholz *et al.*, 1997; Lin *et al.*, 2007), coupled to the proved abilities of anaerobic microorganisms to utilize multiple oil components (Heider *et al.*, 1998) attest to the presence of indigenous microbial communities in oil reservoirs, and currently, it is a well-established scientific fact that oil reservoirs harbor and sustain diverse bacterial and archaeal communities. This section will summarize past efforts to characterize the microbial communities in oil reservoirs by culture-dependent and culture-independent approaches and highlight the effect of prevalent geochemical *in situ* conditions on the microbial communities' compositions.

A. Origins of microorganisms recovered from oil reservoirs

Determining whether a microorganism is autochthonous (indigenous) or allochthonous (foreign or transient) to an oil reservoir is essential before any conclusions can be made, regarding its role in the ecosystem. Contamination of oil-reservoir materials obtained during sampling is a thorny issue that constantly concerns petroleum microbiologists due to the number of possible sources of contamination upon sampling. Another important point is the effect of water-flooding procedure on the native-microbial community (Vance and Thrasher, 2005). Reinjecting produced water after being exposed to surface conditions will result in the reinoculation of the reservoir with surface microorganisms. Water, brine, or seawater injection, besides introducing exogenous microorganisms, could alter the geochemistry of the formation temporally or permanently, such as with the introduction of sulfate or oxygen, that could result in changes to the indigenous-microbial community structure. Further, some microorganisms could possess exceptional survival abilities and could be detected by various culture-dependent and culture-independent efforts long after being introduced to the formation.

The issue of contamination during sampling oil reservoirs has previously been debated in great detail (Magot *et al.*, 2000). Although more elaborate and expensive sampling procedures have been used to sample the deep terrestrial subsurface (Griffin *et al.*, 1997; Krumholz *et al.*, 1997; Lin *et al.*, 2007), they have often not been used while sampling oil reservoirs due to cost. Therefore, personal judgment becomes a critical factor in

determining the origin (indigenous or nonindigenous) of isolates and 16S rRNA gene sequences encountered in oil reservoirs. Magot (2005) suggests two main criteria to determine the indigenous nature of microbial strains obtained from reservoir fluids: (1) comparing the isolate's growth optima to the *in situ* conditions in the oil reservoir, and (2) comparing the global distribution of the strain's phylotype in oil reservoir samples worldwide. While useful, both approaches could be judged as harsh or exclusive. The optimum temperature of a microorganism is not necessarily an accurate reflection of the *in situ* temperature and could also be governed by other physiological and ecological considerations. Thermophilic isolates with much lower temperature optima than their environment (Vetriani *et al.*, 2004), and thermotolerant isolates with a low temperature optima have been reported from high-temperature ecosystems (Takai *et al.*, 2004). Similarly, some halophilic and halotolerant microorganisms recovered from salt crystals have a relatively low salt tolerance (Mormile *et al.*, 2003; Vreeland *et al.*, 2002, 2007). A more reasonable approach may be considering range (minimum and maximum growth limits) or the ability to survive for prolonged periods of time at the *in situ* reservoir condition. However, care should be taken while assessing the growth limits of slow-growing isolates, as false negatives may result because of the extended incubation time. The global presence of specific microbial lineages in geographically isolated oil reservoirs is indeed a good indication of their indigenous nature. However, this criterion could theoretically exclude novel groups that are indigenous to a specific oil reservoir where specialized niche exists.

B. Microorganisms isolated from oil reservoirs

In general, oil reservoirs have low redox potentials and hence harbor mainly anaerobic and facultative microorganisms. Electron donors in oil reservoirs include hydrogen, volatile fatty acids (VFAs) such as acetate, propionate, and benzoate (Fisher, 1987), petroleum hydrocarbons (aromatic hydrocarbons of various ring numbers and aliphatic hydrocarbons of various chain lengths), and inorganic electron donors (e.g., sulfide). Sulfate and carbonate minerals are important electron acceptors in many oil reservoirs. Some oil field isolates use iron (III) as an electron acceptor, but it is unclear how prevalent iron (III) is in oil reservoirs. Nitrate and oxygen are limiting in most oil reservoirs unless added with injected fluids.

In addition to redox potential and the availability of electron donors and acceptors, temperature and salinity appear to be the most important environmental factors that shape oil reservoir microbial communities. Below, we will summarize the various types of microorganisms that have been isolated from oil reservoirs and highlight the effect of prevalent environmental conditions on the phylogenetic diversity of isolated

species. It is important to note that it is difficult to determine whether a microorganism is indigenous or not to an oil reservoir and the reader should consult the original manuscript if this decision is critical.

1. Methanogens

Methanogens metabolize hydrogen and CO_2, acetate, methylamines, and dimethylsulfides with the concurrent production of methane. Currently, methanogens are distributed among five orders (Methanomicrobiales, Methanobacteriales, Methanosarcinales, Methanococcales, and Methanopyrales) within the subkingdom Euryarchaeota, domain Archaea (Euzeby, 2008). Oil reservoir methanogenic isolates capable of metabolizing all the above-mentioned substrates that have been described, and collectively belong to four out of the five recognized methanogenic orders. No oil reservoir Methanopyrales have been described so far.

a. Hydrogenotrophic methanogens Mesophilic, hydrogenotrophic methanogens isolated from low-salinity oil reservoir include members of the genus *Methanobacterium* within the order Methanobacteriales (Belyaev *et al.*, 1986; Davydova-Charakhch'yan *et al.*, 1992a), and the genus *Methanoplanus* within the order Methanomicrobiales (Ollivier *et al.*, 1997). At higher temperatures, hydrogenotrophic methanogens include members of the genera *Methanobacterium*, *Methanothermobacter* (Davydova-Charakhch'yan *et al.*, 1992a; Jeanthon *et al.*, 2005; Ng *et al.*, 1989; Orphan *et al.*, 2000), *Methanoculleus* (order Methanomicrobiales (Orphan *et al.*, 2000)), and *Methanococcus* and *Methanothermococcus* (order Methanococcales (Nilsen and Torsvik, 1996a; Orphan *et al.*, 2000)). Halotolerant hydrogenotrophic methanogens (e.g., *Methanocalculus halotolerans*) has also been recovered from oil reservoirs with elevated salinities (Ollivier *et al.*, 1998).

b. Methylotrophic methanogens Methylotrophic methanogens have also been isolated from oil fields. Most of these isolates are mesophiles, such as *Methanosarcina siciliae* (Ni and Boone, 1991), *Methanosarcina mazei* (which can also utilize acetate (Obraztsova *et al.*, 1987)), in addition to the non-thermophilic halophile *Methanhalophiluus euhalobius* (Obrazstova *et al.*, 1988). However, a recent thermophilic methylotrophic isolate (*Methermicoccus shengliensis*) that belongs to the order Methanosarcinales and represents a novel family (Methermicoccaceae) within this order has been reported (Cheng *et al.*, 2007).

c. Aceticlastic methanogens Acetate-utilizing methanogens belong to the order Methanosarcinales. A single Methanosarcinales-affiliated isolate (*Methanosarcina mazei*) has been reported from oil fields (Obraztsova *et al.*, 1987). However, active mesophilic (Belyaev and Ivanov, 1983; Grabowski *et al.*, 2005b,c) and thermophilic (Bonch-Osmolovskaya *et al.*,

2003; Orphan *et al.*, 2000, 2003) aceticlastic enrichments derived from oil reservoir materials have frequently been reported. The scarcity of aceticlastic methanogenic isolates from oil reservoirs is probably a reflection of the difficulty of isolating this group of microorganisms rather than a reflection of their rarity in oil fields.

2. Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) were the first microorganisms recovered from oil fields (Bastin *et al.*, 1926). Sulfate-reducing capability is currently identified in four different bacterial phyla (Proteobacteria, Firmicutes, Nitrospira, and *Thermodesulfobacterium*), as well as in the phyla Euryarchaeota and Crenarchaeota within the Archaea. Sulfate-reducing oil-reservoir isolates belonging to three out of the four bacterial phyla, as well as the Euryarchaeota within the archaeal domain have been reported.

a. Proteobacteria Sulfate-reducing Proteobacteria belong to the class δ-Proteobacteria. Within the δ-Proteobacteria, eight orders are currently described, five of which are predominantly composed of sulfate-reducing microorganisms (Euzeby, 2008). Two of these six orders (Desulfarculales and Desulfurellales) currently contain very few recognized species, and no isolates belonging to these orders have been encountered in oil reservoirs. SRB isolated from oil reservoirs are members of the orders Desulfovibrionales, Desulfobacterales, and Syntrophobacterales.

i. Desulfovibrionales Desulfovibrionales isolates recovered from the oil field are predominantly mesophiles. The majority are members of the genera *Desulfovibrio* and *Desulfomicrobium* (Birkeland, 2005; Leu *et al.*, 1999; Magot *et al.*, 1992, 2004; Miranda-Tello *et al.*, 2003; Nga *et al.*, 1996; Rozanova *et al.*, 1988; Tardy-Jacquenod *et al.*, 1996). Members of both genera use H_2, lactate, and pyruvate as electron donors. The apparent ubiquity of these two genera in oil reservoirs suggests their pivotal role in hydrogen metabolism in sulfidogenic oil fields, although this might be a reflection of the relative ease of their isolation. In addition, to the previously mentioned genera, a novel isolate that represents a new Desulfovibrionales genus (*Desulfovermiculus halophilus* gen. nov., sp. nov.) has recently been recovered from a Russian oil-field (Beliakova *et al.*, 2006) and is capable of completely degrading several organic compounds (malate, fumarate, succinate, propionate, butyrate, crotonate, ethanol, alanine, formate, etc.), in addition to using hydrogen, lactate, and pyruvate. More interestingly, this novel microorganism can grow at NaCl concentrations up to 23%.

ii. Desulfobacterales Collectively, members of the order Desulfobacterales are capable of degrading H_2, organic acids, ethanol, as well as various small molecular weight petroleum hydrocarbons. Most Desulfobacterales genera are known for their ability to completely oxidize substrates to CO_2, while others (e.g., members of the genus *Desulfobulbus*) are incomplete oxidizers, metabolizing substrates only to the point of acetate. Oil field isolates belonging to the order Desulfobacterales include members of the genera *Desulfobacter* (Lien and Beeder, 1997), *Dsulfobulbus* (Lien *et al.*, 1998b), *Desulfotignum* (Ommedal and Torsvik, 2007), and *Desulfobacterium* (Galushko and Rozanova, 1991). Most oil field Desulfobacterales isolates are VFA degraders. However, *Desulfobacterium cetonicum* has been shown to metabolize *m*- and *p*-cresol (Muller *et al.*, 1999, 2001). In addition, *Desulfotignum toluenicum* (Ommedal and Torsvik, 2007) and strains oXyS1 and mXyS1 (Harms *et al.*, 1999) were isolated from oil reservoir model columns and oil separators, respectively, and are capable of metabolizing toluene, *o*- and *m*-xylene anaerobically.

iii. Syntrophobacterales Oil-well sulfate-reducing Syntrophobacterales described so far are members of the exclusively thermophilic genera *Desulfacanium* (Rees *et al.*, 1995; Rozanova *et al.*, 2001b) and *Thermosulforhabdus* (Beeder *et al.*, 1995). However, it is important to note that the order Syntrophobacterales encompasses, in addition to thermophilic sulfate-reducers mentioned above, mesophilic sulfate-reducers as well and microorganisms that are capable of syntrophic degradation of organic compounds. Hydrocarbon-degrading capabilities have also been reported by mesophilic sulfate-reducing isolates belonging to this order (Cravo-Laureau *et al.*, 2004; Davidova *et al.*, 2006), none of which however, originated from oil-reservoirs.

b. Firmicutes Within the gram-positive Firmicutes, multiple genera with sulfate-reduction abilities have been reported, all of which belong to the order Clostridiales, for example, *Desulfotomaculum*, *Desulfurispora*, *Desulfovirgula*, *Desulfosporosinus*, and *Thermodesulfobium* (Euzeby, 2008). The genus *Desulfotomaculum* is the most ubiquitous amongst gram-positive SRBs, and the only Firmicutes that has been encountered in oil fields so far. Mesophilic oil reservoir *Desulfotomaculum* isolates have been reported (Tardy-Jacquenod *et al.*, 1998), but the majority of *Desulfotomaculum* isolates from oil reservoir are thermophiles (Nazina *et al.*, 1988; Nilsen *et al.*, 1996b; Rosnes *et al.*, 1991). Hydrocarbon-degrading capabilities have been reported within the members of this genus (Londry *et al.*, 1999; Tasaki *et al.*, 1991) but not in any of the oil-reservoir isolates so far.

c. Thermodesulfobacteria *Thermodesulfobacteria* represents a distinct bacterial phylum with only two genera (*Thermodesulfobacterium* and *Thermodesulfatator*) both of which are thermophilic sulfate-reducers. Oil reservoir isolates belonging to the genus *Thermodesulfobacter* have been obtained from thermophilic terrestrial and marine oil reservoirs (Christensen *et al.*, 1992; L'Haridon *et al.*, 1995).

d. Archaea Only Euryarchaeota sulfate-reducing microorganisms, but no sulfate-reducing Crenarchaeota (members of the genus *Caldivirga*), have been isolated from oil fields so far. Sulfate-reducing Archaea recovered from oil reservoirs are members of the genus *Archaeoglobus* and have been recovered mainly in various North Sea oil wells (Beeder *et al.*, 1994; Stetter *et al.*, 1993). The indigenous nature of this hyperthermophilic sulfate-reducer has been a matter of intense debate (see below).

3. Fermentative microorganisms

A number of fermentative microorganisms have been isolated from high-temperature and low-temperature oil reservoirs. It is important to note that many microorganisms in this group possess dual fermentative and respiratory metabolic abilities (e.g., sulfur and thiosulfate reduction) and could theoretically utilize both strategies for their *in situ* growth and survival.

A large fraction of thermophilic fermentative microorganisms recovered from oil fields are either members of the phylum Thermotogae or members of the order Thermoanaerobacteriales within the class Clostridia, phylum Firmicutes. Thermotogae is a phylum exclusive for thermophilic, anaerobic fermenters, although recent phylogenetic and metagenomic evidence for the presence of low-temperature ''Mesotoga'' have recently been reported (Nesbo *et al.*, 2006). Thermotogae isolates have consistently been shown to be members of high-temperature oil reservoirs, suggesting an indigenous nature of these microorganisms to oil reservoirs (Davey *et al.*, 1993; L'Haridon *et al.*, 2001, 2002; Lien *et al.*, 1998a; Miranda-Tello *et al.*, 2004, 2007; Takahata *et al.*, 2001). Members of the Thermotogae can grow on complex substrates, as well as sugars, with acetate and hydrogen being the final end products. Oil field isolates have been identified in four out of the six currently recognized genera in this phylum (*Thermotoga*, *Thermosipho*, *Geotoga*, and *Petrotoga*) with members of the last two genera being exclusively recovered from oil reservoirs. Sulfur and/or thiosulfate reduction is widely distributed among members of this phylum.

Members of the order Thermoanaerobacteriales within the Firmicutes are also commonly encountered in oil fields, and include isolates belonging to the genera *Thermoanaerobacter* (Cayol *et al.*, 1995; L'Haridon *et al.*, 1995), *Thermoanaerobacterium* (Grassia *et al.*, 1996), *Caldanaerobacter* (Fardeau *et al.*,

2004; Grassia *et al.*, 1996), and *Mahella* (Bonilla Salinas *et al.*, 2004), all of which are thermophilic sugar fermenters. In addition to Thermoanaerobacteriales isolates, other thermophilic, Firmicutes-affiliated oil reservoir microorganisms have been identified, including the organic acid fermenter *Anaerobaculum thermoterrenum* (Rees *et al.*, 1997) and the amino acid degrader *Thermovirga lienii* (Dahle and Birkeland, 2006), both of which are members of the order Clostridiales.

Few studies have focused on isolating fermentative microorganisms from oil reservoirs with elevated salinities. Fermentative halophilic oil reservoir isolates exclusively belong to the genus *Haloanaerobium* (Order Haloanaerobiales) such as *H. acetethylicum*, *H. salsuginis*, *H. congolese*, and *H. kushneri* (Bhupathiraju *et al.*, 1994, 1999; Ravot *et al.*, 1997). Most *Haloanaerobium* spp. are saccharolytic and proteolytic, and produce H_2, acetate, and CO_2 as end products of fermentation.

In spite of the general metabolic and phylogenetic diversity of mesophilic fermentative microorganisms, few isolates that belong to this metabolic group have been recovered from oil fields. In general, studies on the isolation of mesophilic fermentative microorganisms from oil fields are extremely sparse and are far from adequate to identify any global distribution patterns or link members of any of the identified phylogenetic groups to a specific ecological role in oil reservoirs. Fermentative, mesophilic Firmicutes-affiliated isolates include *Fusibacter paucivorans*, a new genus belonging to the order Clostridiales that utilizes a limited number of carbohydrates and was isolated from an offshore oil field in Congo (Ravot *et al.*, 1999), *Dethiosulfovibrio peptidovorans* a proteolytic microorganism that grows on peptones and individual amino acids but not sugars (Magot *et al.*, 1997b), and ''*Acetobacterium romashkovi*,'' an acetogenic microorganism that is also capable of fermenting sugars and amino acids (Davydova-Charakhch'yan *et al.*, 1992b). In addition to Firmicutes, a novel species within the genus *Spirochaeta* has been identified from oil reservoirs (Magot *et al.*, 1997a). A novel genus (*Petrimonas*) within the phylum Bacteroidetes has recently been isolated from oil reservoirs as well (Grabowski *et al.*, 2005c), making it the first member of this phylum to be isolated from oil reservoirs. A recent study has enriched for a wide range of fermentative mesophilic microorganisms from a low-temperature oil reservoir in Canada, and 16S rRNA gene-based analysis of bacterial enrichments identified various members of putatively fermentative members of the Clostridiales, genus *Bacteroides*, and genus *Spirochetes* (Grabowski *et al.*, 2005b).

4. Other microbial isolates

a. Hyperthermophiles Although the presence and recovery of hyperthermophiles from oil fields has previously been demonstrated, the indigenous nature of these microorganisms remains in question

(L'Haridon *et al.*, 1995; Stetter *et al.*, 1993). Geological studies correlating *in situ* biodegradation of oil components to temperatures in oil reservoirs (Head *et al.*, 2003) and those correlating VFA levels to temperatures (Fisher, 1987) suggest an upper limit of 80–90 °C for *in situ* biological activities in oil reservoirs, which is far from being the upper growth limit within the microbial world (Kashefi and Lovley, 2003). In addition, it has been observed that these hyperthermophilic microorganisms recovered in high-temperature reservoirs could remain viable for long periods of time at seawater temperatures, increasing the possibility that they are immigrants to the ecosystem introduced during well manipulations. Hyperthermophiles recovered from oil reservoirs include members of the archaeal genera *Archaeoglobus*, *Pyrococcus*, and *Thermococcus* (Grassia *et al.*, 1996; L'Haridon *et al.*, 1995; Stetter *et al.*, 1993; Takahata *et al.*, 2000).

b. Syntrophic microorganisms Syntrophic microorganisms are responsible for the degradation of a wide range of organic compounds in association with hydrogen- and acetate-utilizing methanogens. A previous report demonstrated that glycerol fermentation by two oil reservoir-derived species of the genus *Halanaerobium* is greatly facilitated when grown in a coculture with the hydrogen-scavenging sulfate-reducer *Desulfohalobium retbaense* (Cayol *et al.*, 2002). To our knowledge, no pure isolate capable of degrading compounds that obligatory-require syntrophic interactions under methanogenic conditions have been retrieved from oil reservoirs. The notoriously fastidious nature of syntrophic microorganisms could partly be responsible for the lack of syntrophic oil well isolates. Thermodynamic considerations argue for the involvement of syntrophic microorganisms in the anaerobic degradation of most aromatic and aliphatic components of oil under methanogenic conditions. It follows that documenting the degradation of oil components *in situ* or in methanogenic laboratory enrichments derived from oil reservoirs could be regarded as an indirect evidence for the presence of syntrophic microorganisms in oil fields. Recent work demonstrating active methanogenic hydrocarbon metabolism in oil field reservoirs clearly attests to the presence of a native syntrophic population within the oil fields (Atiken *et al.*, 2004; Jones *et al.*, 2008). Indeed, methanogenic alkane-degrading enrichments derived from North Sea oil reservoirs have been reported (Jones *et al.*, 2008) and 16S rRNA gene clone libraries derived from these enrichments indicated the presence of a clone affiliated with the genus *Syntrophus*, all members of which are capable of syntrophic metabolism. Finally, members of the genus *Syntrophus* were also identified in enrichments degrading heptadecane under methanogenic conditions that was derived from Pelikan lake oil field in western Canada (Grabowski *et al.*, 2005a).

c. Autotrophs Respiratory microorganisms capable of utilizing hydrogen as a sole electron donor coupled to sulfate (Birkeland, 2005; Leu *et al.*, 1999; Magot *et al.*, 1992, 2004; Miranda-Tello *et al.*, 2003; Nga *et al.*, 1996; Rozanova *et al.*, 1988; Tardy-Jacquenod *et al.*, 1996) or nitrate and/or iron (III) (Greene *et al.*, 1997; Myhr and Torsvik, 2000; Nazina *et al.*, 1995b; Slobodkin *et al.*, 1999) as electron acceptors have frequently been isolated from oil fields. Acetogenic microorganisms utilizing H_2 and CO_2 to produce acetate have also been reported. ''*A. romashkovii*,'' a member of the order Lactobacillales within the Firmicutes, has been isolated from an oil field in Siberia (Davydova-Charakhch'yan *et al.*, 1992b). Also, an acetogenic enrichment containing a microorganism is highly similar to a known acetogen (*Acetobacterium carbinolicum*) (99% 16S rRNA gene sequence similarity) has been reported (Grabowski *et al.*, 2005b). Finally, chemolithoautotrophic sulfide-oxidizing, nitrate-reducing bacteria (NRB) have been isolated from oil production fluids in a Canadian oil field (Gevertz *et al.*, 2000).

d. Nitrate, iron, and manganese reducers Various nitrate-reducing microorganisms with autotrophic (Myhr and Torsvik, 2000), heterotrophic (Nazina *et al.*, 1995b), and chemolithotrophic (Gevertz *et al.*, 2000) abilities have been isolated. Many of the NRB, including some that were recovered from oil fields, are facultative and metabolically versatile, raising the question of their contribution to nitrate reduction in oil fields (Huu *et al.*, 1999; Nazina *et al.*, 1995b). Two thermophilic respiratory genera that appear to be especially abundant in oil reservoirs are *Geobacillus* and *Deferribacter*. The genus *Geobacillus* belongs to the order Bacillales within the Firmicutes, and oil reservoir-derived *Geobacillus* isolates are thermophilic microaerophiles that degrade alkanes only under aerobic conditions and some could reduce nitrate anaerobically (Nazina *et al.*, 2001). The genus *Deferribacter* contains three species, all of which are thermophiles and are capable of anaerobic respiration using multiple electron acceptors. *Deferribacter thermophilus* was isolated from Beatrice oil field in the North Sea and is capable of coupling oxidation of hydrogen, organic acids, and complex substrates to manganese and iron reduction (Greene *et al.*, 1997).

C. Culture-independent analysis of microbial communities in oil reservoirs

Isolation efforts have identified numerous bacterial and archaeal species that are capable of mediating various metabolic processes occurring in oil fields. Nevertheless, culture-dependent characterization of microbial communities is governed by several factors that limit its utility to describe the ecosystem's community completely. Isolation of a single

microorganism mediating a specific metabolic process could hardly be a representative of the entire community mediating this process *in situ* because microorganisms that are easiest to obtain in pure cultures are not necessarily the most numerically abundant and/or metabolically active *in situ*. The media routinely used for isolation of environmental microorganisms (including those in oil reservoirs) are often carbon and nitrogen-rich compared to the prevailing environmental condition. Culturing from samples collected at a specific time and production stage of oil reservoir does not capture the changes in microbial community that occur throughtout the entire exploration and exploitations stages. In addition, the relatively low number of isolates obtained from oil fields, especially from low-temperature oil reservoirs, severely limits our ability to deduce distribution patterns or unifying ecological themes regarding oil-reservoir community. For example, while the recent isolation of a spirochete (Magot *et al.*, 1997a) and a member of the phylum Bacteroidetes (Grabowski *et al.*, 2005a) establishes the presence of members of these phyla in oil reservoirs, these isolations hardly capture the intra-phylum diversity, abundance, and distribution of members of these phyla in various oil fields. Finally, the inability of microbiologists to isolate a large fraction of microorganisms present in nature is a well-established phenomenon (Rappe and Giovannoni, 2003; Zengler, 2006; Zengler *et al.*, 2002).

In comparison, culture-independent 16S rRNA gene-based surveys are extremely valuable in providing an overall view of the community composition in a specific ecosystem, regardless of the metabolic abilities of the community members. These studies also provide preliminary information on the relative abundance of different groups within the ecosystem and could be used for monitoring temporal and spatial changes in an ecosystem. Thousands of culture-independent 16S rRNA gene-sequencing surveys have already been reported in almost all accessible ecosystems on Earth (Keller and Zengler, 2004; Pace, 1997). The collective conclusion from these studies clearly enforces the notion that the scope of microbial diversity is much broader than implied by culture-dependent studies (Janssen, 2006; Rappe and Giovannoni, 2003). The discovery of novel phyla and subphyla as the most numerically abundant members of several habitats drastically changed our view of the community compositions of multiple globally relevant ecosystems, including soil (Janssen, 2006) and pelagic marine environments (DeLong, 2005; Rappe and Giovannoni, 2003).

Several culture-independent sequencing surveys have been conducted in high and low temperatures, and in marine and terrestrial oil reservoirs. Compared to the majority of previously studied ecosystems, the information (number of studies and number of sequences analyzed per study) currently available is very sparse. Surprisingly, 16S rRNA gene-based analysis of oil reservoir communities have not lead to any significant, paradigm-shifting discoveries and have not drastically altered our

view of the oil reservoir communities. To our knowledge, this is one of the few environments in which culture-independent analysis did not dramatically alter our view of its microbial community composition. Culture-independent studies have rather been confirmatory of the results previously obtained by culture-dependent surveys, in spite of the inherent limitations outlined above. The lack of significant discoveries does not necessarily mean that novel, yet-uncultured microbial groups are not present in oil reservoirs, because the absence of novel phyla could also be attributed to (1) the small number of studies conducted so far, (2) the relatively small number of sequences that have been analyzed in these studies, and (3) the fact that most studies, so far, have been conducted in thermophilic oil reservoirs where extreme conditions could limit the overall microbial diversity within the ecosystem (Lozupone and Knight, 2007).

In addition, a review of currently available culture-independent sequencing surveys show that these studies, similar to isolation-based efforts, are effected by the issues of contamination and reinoculation during water flooding described above, which confers uncertainty regarding the indigenous nature of identified oil-reservoir populations. Therefore, while many of the culture-independent studies generated fairly large clone libraries (Li *et al.*, 2006, 2007a), the detection of perceived contaminants and their exclusion from further analysis severely diminished the number of ''relevant'' sequences recovered. This issue deprives many of these studies of one of the most important strengths of culture-independent surveys, for example, the ability to identify large number of microorganisms in a single experiment.

Culture-independent surveys of high-temperature oil reservoirs have been conducted in oil fields in continental and offshore California (Orphan *et al.*, 2000, 2003), an offshore oil field in Qinghuang unit, China (Li *et al.*, 2006), Huabei oil field in continental China (Li *et al.*, 2006, 2007a), and in Troll oil formation in the North sea (Dahle *et al.*, 2008). Collectively, the results highlight the problem of sample contamination that plagues the work. While thermophilic enrichment and isolation studies could partially alleviate this problem by setting enrichments targeting thermophiles, the indiscriminatory nature of culture-independent surveys does not selectively detect indigenous oil field thermophiles. As a result, a large number of sequences that belong to marine and terrestrial mesophilic lineages (e.g., *Pseudomonas*, *Marinobacter*, *Sinorhizobium*) often represent a majority of the clones in a specific library and microorganisms perceived as native, for example, *Thermotogales*, while readily enriched from the same samples and often represent the majority of the population based on microscopic observation, are often the minority (Li *et al.*, 2006), or entirely absent (Orphan *et al.*, 2000) from these clone libraries.

The main characteristics of these libraries (site, reservoir conditions, target group of microorganisms, library size, and salient findings) are

TABLE 6.1 Culture independent 16S rRNA gene sequencing surveys conducted in oil reservoirs

Study site	Reservoir conditions	Primers target	Clones /OTUs	Lineages detected	Novel lineages previously unidentified by culturing studies, general comments	Reference
Troll oil formation, North sea	70 °C, low-salinity, not water flooded	Bacteria, Archaea	88/29 (Bacteria), 22/3 (Archaea)	Firmicutes, γ, δ-Proteobacteria, *Thermotogales*, *Spirochetes*, *Bacteroidetes*, *Methanococcus*, *Methanolobus*, *Thermococcus*	Detection of moderately thermophilic members of the *Bacteroidetes* (genus *Anaerophaga*) that have not been previously isolated from oil reservoirs. Large fraction of clones belongs to nonthermophilic lineages, and appears to be contaminants	Dahle *et al.* (2008)
Multiple oil fields, California	Multiple high-temperature, low salinity reservoirs at different stages of flooding	Unviersal, Archaea	118/41 (Bacteria), 168/11 (Archaea). Archaeal sequences identified in both clone libraries	α, β, γ, δ-Proteobacteria, *Bacteroidetes*, Firmicutes, *Methanomicrobiales*, *Methanosarcinales*, *Thermococcales*	Majority of Proteobacteria, *Bacteroidetes*-affiliated clones belong to nonthermophilic lineages and appear to be contaminants. No *Thermotogales* clones detected in spite of being identified in a parallel enrichment effort	Orphan *et al.* (2000)

Hubei oil field, China	75 °C, low salinity (1.6%), water flooded	Bacteria	337/74	α, β, γ, ε-Proteobacteria Firmicutes, *Actinobacteria*, *Thermotogales*, Nitrospira	Majority of γ and β-Proteobacteria and *Actinomycetes*, as well as some Firmicutes clones belong to nonthermophilic lineages and appear to be contaminants. Detection of clones affiliated with sulfate-reducing genus *Thermodesulfovibrio* (Phylum Nitrospira) that has not been previously isolated from oil reservoirs	Li *et al.* (2006)
Hubei oil field, China	75 °C, low salininty (1.6%), water flooded	Archaea	237/28	*Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*	First identification of *Methanocorpusculum* clones (order *Methanomicrobiales*) in high-temperature oil reservoirs, origin still uncertain.	Li *et al.* (2007a)

(*continued*)

TABLE 6.1 (*continued*)

Study site	Reservoir conditions	Primers target	Clones /OTUs	Lineages detected	Novel lineages previously unidentified by culturing studies, general comments	Reference
Qinghuang offshore oil field, China	65 °C, low salinity, water flooded	Bacteria, Archaea	338/60 (Bacteria) 220/28 (Archaea)	Firmicutes, Nitrospira, *Thermotogae*, α, β, γ, ε-Proteobacteria, *Methanobacteriales*, *Methanococcales*, *Crenarchaeota*	Detection of clones affiliated with sulfate-reducing genus *Thermodesulfovibrio* (Phylum Nitrospira) that has not been previously isolated from oil reservoirs. Most α, β, γ-Proteobacteria and *Crenarchaeota* clones belong to nonthermophilic lineages and appear to be contaminants	Li *et al.* (2007b)
Pelikan lake oil field	Low-temperature (18–20 °C), low salinity	Bacteria, *Archaea*	151/1, 192/12	ε-Proteobacteria, *Methanomicrobiales*, *Methanosarcinales*	Detection of *Methanocorpusculum*, and *Methanosaeta* clones both of which have not been previously isolated from oil reservoirs. Only one OTU identified in the entire bacterial clone library	Grabowski *et al.* (2005b)

Western Canadian oil fields	Low-temperature (25 °C), low salinity	Bacteria	36/ND*	δ-Proteobacteria, *Deferribacteres*, Firmicutes	Most clones appear to be indigenous, minimum amount of contaminants clones. Detection of *Synergistes*-like microorganisms (Phylum *Deferribacteres)* that have not been previously isolated from oil reservoirs	Voordouw *et al.* (1996)

* ND; not determined.

listed in Table 6.1. Clones that we subjectively judge as indigenous that were identified in these clone libraries mainly belong to lineages previously identified as inhabitants of high-temperature oil reservoirs by using culture-based approaches (e.g., *Thermotogales*, *Thermoanaerobacteriales*, *Thermococcus*, *Methanomicrobiales*, *Methanosarcinales*, and δ-Proteobacteria). Novel microorganisms/lineages identified in these studies that could potentially be indigenous, but have not previously been cultured, include thermophilc *Bacteroidetes* (thermophilic members of the genus *Anaerophaga*) (Dahle *et al.*, 2008), clones affiliated with members of the genus *Methanocorpusculum* (order Methanomicrobiales) (Li *et al.*, 2007a) and clones affiliated with members of the sulfate-reducing genus *Thermodesulfovibrio* (Phylum Nitrospira) (Li *et al.*, 2006, 2007a).

Few 16S-based analysis of the microbial community in low-temperature reservoirs has been reported. Compared to surveys of thermophilic oil reservoirs, judging the indigenous nature of encountered microorganisms is more problematic because there is no litmus test (i.e., affiliation with thermophilic lineages) available to attest to the indigenous nature of the community. Grabowski *et al.* (2005b) investigated the bacterial diversity in a low-temperature, nonwater flooded oil reservoir (Pelikan lake oil field) in western Canada and reported a bacterial community with extremely low diversity; only one phylotype related to the genus *Arcobacter* (ε-Proteobacteria) was encountered in the bacterial clone library. Voodrouw *et al.* (1996) presented an analysis of 36 16S rRNA gene clones from a low-temperature oil reservoir in western Canada. The study remains one of the few culture-independent surveys of oil reservoirs in which contaminants' clones do not appear to represent a significant fraction of the total number of clones in the library and hence plausible ecological functions could be assigned to the majority of the sequenced clones. Several potential metabolic groups of microorganisms were identified: fermentative and/or acetogenic microorganisms, sulfide-oxidizers, and sulfate-reducers (Voordouw *et al.*, 1996).

Besides 16S rRNA gene-based analysis, a few hybridization-based studies on the oil reservoir microbial communities have been reported. Voordouw *et al.* used a reverse sample genome probing approach, in which labeled environmental DNA is hybridized to genomes of target microorganisms, to detect and quantify sulfate-reducing microorganisms in multiple oil reservoir-derived samples and enrichments (Voordouw *et al.*, 1991, 1992, 1993). A more recent study used an oligonucleotide microchip that targets key genera of thermophilic *Bacteria* and *Archaea* (17 probes with varying degrees of specificity) to identify communities in a high-temperature reservoir in Western Siberia (Bonch-Osmolovskaya *et al.*, 2003). The study identified several microorganisms (members of the phylum Aquificales, genus *Thermus* within the phylum Deinococcus-Thermus, and members of the genus *Desulfurococcus* within the Crenarchaeota, Domain *Archaea*) that

have escaped a parallel cultivation effort and have not been previously detected in oil reservoirs members. This significant contribution with a relatively limited number of probes highlights the potential of hybridization-based community investigations using newly available broad range microchips that are capable of reliably detecting thousands of microbial species with a high degree of sensitivity simultaneously.

IV. DELETERIOUS MICROBIAL ACTIVITIES: HYDROGEN SULFIDE PRODUCTION (OR SOURING)

The onset of hydrogen sulfide production (or souring) often occurs when reservoirs are flooded with brine or seawater that contains high levels of sulfate (McInerney *et al.*, 1991; Sunde and Torsvik, 2005). The presence of sulfate and nitrogen and phosphorous sources in the injection water, the reduction in reservoir temperature due to the injection of cooler displacement fluids, and the electron donors present in the reservoir (organic acids and hydrocarbons) create conditions favorable for the growth of SRB and the production of hydrogen sulfide (Sunde and Torsvik, 2005; Vance and Thrasher, 2005). The increase of H_2S (known as souring) is associated with corrosion of pipelines, platform structures, and other equipment; increases refining costs of oil and gas; plugs reservoirs by the accumulation of sulfides minerals; and increases health risks due to the toxicity of H_2S (Chen *et al.*, 1994; Davidova *et al.*, 2001; Eckford and Fedorak, 2002; Myhr *et al.*, 2002; Nemati *et al.*, 2001a,b).

A. Current souring control approaches

The detrimental consequences of souring have caused the oil industry to invest heavily in strategies for souring control. Biocides such as bronopol, formaldehyde, glutaraldehyde, benzalkonium chloride, cocodiamine, and tetrakishydroxymethyl phosphonium sulfate (THPS) are commonly applied to injection waters and production facilities to reduce H_2S concentrations below the threshold levels defined by NACE international standards (Vance and Thrasher, 2005). The problem with biocides is the need for high concentrations and frequent treatments to achieve the desired results, especially when dealing with biofilms (Burger, 1998; Kjellerup *et al.*, 2005; Vance and Thrasher, 2005). Some biocides may also pose a health risk to operators. If the reservoir was sour prior to water flooding, H_2S was probably not of recent biological origin and liquid- or solid-phase H_2S scavengers (e.g., triazines, sodium hydroxide, aldehydes, metal oxides, and iron and zinc oxide-based biocides) could be used. Another approach to control souring is to remove sulfate or significantly lower sulfate concentrations below 50 mg/l from the injection

waters. Nanofiltration technology has been applied to water injection facilities to achieve desulfation (Rizk *et al.*, 1998; Vance and Thrasher, 2005). Manipulation of the injection water's salinity could potentially inhibit H_2S production, if NaCl concentrations above 12% can be achieved (Vance and Thrasher, 2005).

B. Microbial control of souring

Recently, the stimulation of NRB by addition of nitrate, nitrite, or nitrate/molybdate mixtures with or without the addition of NRB has been used to control souring (Hubert *et al.*, 2003; Sunde and Torsvik, 2005). The idea of using nitrate to abate hydrogen sulfide production is not new and has been used to control odors in sewage (Carpenter, 1932; Heukelelekian, 1943). There are several mechanisms by which nitrate addition can control souring: (1) competition for electron donors between NRB and SRB, (2) sulfide oxidation by NRB, (3) increase in redox potential and subsequent inhibition of SRB, and (4) the inhibition of SRB by nitrite, or other nitrogen oxides (e.g., nitrous oxide) (Hubert *et al.*, 2003, 2004; Jenneman *et al.*, 1986; Montgomery *et al.*, 1990; Nemati *et al.*, 2001b; Reinsel *et al.*, 1996; Sunde and Torsvik, 2005). Two physiological types of NRBs are involved in the control of SRB activity; heterotrophic, NRB (hNRB) and sulfide-oxidizing, NRB (SO-NRB) (Eckford and Fedorak, 2002; Sunde and Torsvik, 2005). hNRB compete with SRB for common electron donors. The reduction of nitrate or nitrite is energetically more favorable than sulfate reduction. Thus, hNRB have higher molar growth yields and faster growth rates than SRB so that hNRB effectively outcompete SRB for common electron donors (Sunde and Torsvik, 2005). If hNRB are present, the prediction is that they will prevent the growth of SRB (Kjellerup *et al.*, 2005). On the other hand, SO-NRB oxidize H_2S to sulfur or sulfate with nitrate or nitrite as the electron acceptor, but do not inhibit the growth of SRB (Kjellerup et al., 2005; Montgomery *et al.*, 1990). Some microorganisms such as *Sulfurospirilum* spp. are capable of both hNRB, and SO-NRB types of metabolism (Hubert and Voordouw, 2007).

Most of the laboratory experiments indicate that sulfide oxidation by SO-NRB is an important mechanism for sulfide inhibition (Montgomery *et al.*, 1990; Myhr *et al.*, 2002; Nemati *et al.*, 2001a). In presence of pure cultures of SO-NRB and nitrate, sulfide concentrations decreased (McInerney *et al.*, 1992, 1996; Montgomery *et al.*, 1990; Nemati *et al.*, 2001a). The addition of nitrate or small amounts of nitrite to an up-flow packed-bed bioreactor inoculated with produced water from an oil field inhibited sulfide accumulation and resulted in an increase in SO-NRB populations but the SRB population was not affected (Hubert *et al.*, 2003). Similarly, the addition of nitrate inhibited sulfide production and increased the NRB populations (Davidova *et al.*, 2001). Because the SRB

population was not affected after treatment, it is likely that SO-NRB were responsible for the observed decrease in sulfide concentration.

One study involving a coculture of *Sulfurospirilum* sp. and *Desulfovibrio* sp. implicates the accumulation of nitrite as an important mechanism for inhibiting SRB activity (Haveman *et al.*, 2004). In an up-flow packed-bed bioreactor inoculated with produced water from an oil field and amended with nitrate, *Sulfurospirilum* spp. were dominant. The authors argued that the metabolic versatility of *Sulfurospirilum* spp. (the ability to perform heterotrophic or sulfide-oxidizing nitrate reduction) coupled with the ability to produce inhibitory concentrations of nitrite were the main reasons that SRB activity was inhibited. Similarly, nitrite accumulation concomitent with the inhibition of sulfide was observed following nitrate addition to porous columns inoculated with brine from North Slope of Alaska reservoirs (Reinsel *et al.*, 1996).

Competitive exclusion of SRB by hNRB is also possible. An increase in the population of hNRB was observed when the concentration of NO_2^- was increased in an up-flow packed-bed bioreactor inoculated with produced water from an oil field (Hubert *et al.*, 2003). Eckford and Fedorak (Eckford and Fedorak, 2002) found both hNRB and SO-NRB populations increased in batch experiments when sulfide-containing produced water was amended with nitrate. Finally, some studies show that nitrite and molybdate act synergistically to inhibit sulfide production by pure cultures of SRB or by sulfate-reducing enrichments from produced waters (Kjellerup *et al.*, 2005; Nemati *et al.*, 2001b; Reinsel *et al.*, 1996).

Several field trials show that nitrate injection is effective in inhibiting or reducing reservoir souring (Table 6.2). The injection of about 58 kg per day of ammonium nitrate for 45 days into a hypersaline oil reservoir reduced sulfide levels by 40–60% in produced fluids from three production wells (McInerney *et al.*, 1991). A reduction of sulfide in produced fluids of two wells was detected 20 days after nitrate injection began and sulfide levels dropped to 25% of their pretreatment values 55 days after nitrate injection (Telang *et al.*, 1997). Reverse sample genome probing indicated that a sulfide-oxiding, nitrate-using bacterium became a dominant member of the microbial community implicating that sulfide oxidation to sulfate coupled to nitrate reduction was an important mechanism for the reduction in sulfide levels in production wells. The addition of 3 m^3 of calcium nitrate (0.3 mM nitrate) per day in the injection waters reduced the number of sulfate reducers by 50–90%, increased the numbers of nitrate-using bacteria, and decreased corrosion rates in biofilms that formed on metal coupons placed inside of the Gullfaks North Sea injection system (Sunde *et al.*, 2004; Thorstenson *et al.*, 2002). Hydrogen sulfide concentrations of eleven Gullfaks production wells decreased about 12–18 months after continuous nitrate injection began (Sunde and Torsvik, 2005). The decrease in sulfide levels coincided with time

TABLE 6.2 Use of nitrate to control hydrogen sulfide production in reservoirs

Method	Results	References
Continuous NO_3^- injection till breakthrough in production wells	Decline in H_2S after breakthrough of treated water	Jenneman *et al.* (1999), Larsen *et al.* (2004), Sunde *et al.* (2004), Thorstenson *et al.* (2002)
Continuous injection of NO_3^- and PO_4^{2-} for 50 days	Reduction in sulfide levels; population of a sulfide-oxidizing, nitrate reducer increased	Telang *et al.* (1997)
Injection of NO_3^- and NO_2^-	Reduction in dissolved sulfide levels in production equipment and produced water	Hitzman *et al.* (2004)
Continuous injection of NH_4NO_3	40–60% decrease in sulfide levels decreased in 3 wells 45 days after injection began	McInerney *et al.* (1991)

predicted based on the breakthrough of the nitrate-treated water. Fluids collected by backflowing nitrate-treated injection wells had barely detectable levels of sulfide (<1 mg/l) while those of biocide-treated wells had 39 mg/l of sulfide (Sunde and Torsvik, 2005). A reduction in sulfide concentration in production wells after continuous injection of 100–150 mg/l of nitrate was observed in the Halfdan North Sea field (Larsen *et al.*, 2004). The continuous injection of ammonium nitrate and sodium phosphate decreased sulfide concentrations and suppressed sulfate reducers in injection and production wells (Jenneman *et al.*, 1999). A mixture of nitrate, nitrite, and inorganic nutrients decreased sulfide levels in production facilities and hydrogen sulfide levels from a gas well (Hitzman and Sperl, 1994; Hitzman *et al.*, 2004). The use of nitrate to control souring shows that it is possible to manipulate the reservoir ecology in a predictable manner. In addition, the injection of nitrate can have a second benefit by slowing the natural decline in oil production (Brown, 2007; Brown *et al.*, 2002; Hitzman *et al.*, 2004).

While nitrate is effective in controlling souring, the effect of adding nitrate on corrosion is not as clear. The addition of nitrate to mixed cultures with SO-NRB (Nemati *et al.*, 2001c) or to up-flow packed-bed

bioreactor studies (Hubert *et al.*, 2005) increased corrosion. The rate of corrosion was high during the time when sulfide was oxidized (Rempel *et al.*, 2006). The addition of nitrite, on the other hand, seems to have a less pronounced effect on corrosion when used in small concentrations and added gradually (Hubert *et al.*, 2005; Rempel *et al.*, 2006). The addition of a single high dose of nitrite completely eliminated microbial activity and hence corrosion (Hubert *et al.*, 2005). The inhibitory effect of high nitrite concentrations may be due to the production of nitric oxide (Kielemoes *et al.*, 2000).

V. MICROBIAL ACTIVITIES AND PRODUCTS USEFUL FOR OIL RECOVERY

Microorganisms produce a number of products (acids, solvents, gases, biosurfactants, biopolymers, and emulsifiers) and have activities (hydrocarbon metabolism and plugging) that are potentially useful for oil recovery (Table 6.3) (ZoBell, 1947a–c). Although the microbial mechanisms for oil recovery will be discussed independently, it is likely that microbial processes act synergistically. For example, significant mobilization of entrapped oil from sand-packed columns and sandstone cores required multiple products, an alcohol, a biosurfactant, and a polymer (Maudgalya *et al.*, 2004). The relative importance of each will depend on factors that limit oil production within a given reservoir, the strains of the microorganism involved, and the protocols used for injection of nutrients and inocula.

Microbial technologies are often grouped into three main categories, paraffin removal, microbial well stimulations, and microbially enhanced waterfloods (Knapp *et al.*, 1990). Hydrocarbon degradation is the mechanism for microbial paraffin removal (Table 6.3). Commercial companies use proprietary inocula and nutrients to degrade paraffins and other hydrocarbons that may have accumulated on production equipment, within the well, or within the reservoir (Fig. 6.1A and Table 6.4). The microbial treatment may be localized to the well-bore region or occur several meters to ten or more meters in the reservoir. For the sake of this discussion, we will call other microbial technologies that treat individual wells and do not involve *in situ* hydrocarbon metabolism well stimulations technologies. The objective of well stimulation technologies is to stimulate the production of large amounts of acids, gases, solvents, biosurfactants, and/or emulsifiers in the well and the near well region of the reservoir to improve oil production rates. The volumes of nutrients and cells injected are large enough to treat several to tens of meters of the resevoir. In addition to removing scale, paraffins, asphaltenes, and other debris, well stimulations may change wettability and flow patterns to

TABLE 6.3 Microbial products and activities useful for MEOR, their mechanism of action, the production problem they target, and the most suitable type of reservoir for their application

Microbial product/ activity	Microorganisms	Production problem	Mechanism of action	Type of formation/reservoir
Hydrocarbon metabolism	Aerobic hydrocarbon degraders	Paraffin deposition; poor microscopic displacement efficiency	Remove paraffin deposits; metabolites mobilize oil	Wells with paraffin deposition; mature waterflooded reservoirs
Gases (CO_2, CH_4)	Fermentative bacteria; Methanogens	Heavy oil	Reduce oil viscosity	Heavy oil-bearing formations (API <15)
Acids	Fermentative bacteria	Low porosity, poor drainage, formation damage	Dissolve carbonaceous minerals or deposits	Carbonate or carbonaceous reservoirs
Solvents	Fermentative bacteria	Heavy oil	Reduce oil viscosity	Heavy oil-bearing formations (API <15)
		Poor microscopic displacement efficiency	Alter wettability	Strongly oil-wet, waterflooded reservoirs

Emulsifiers	*Acinetobacter* sp., *Candida*, *Pseudomonas* sp., *Bacillus* sp.	Paraffin and oil sludge deposition, poor microscopic displacement efficiency	Emulsify oil to form o/w emulsions (or less commonly w/o emulsions)	Waxy oil (>C22 alkanes); paraffinic oil and asphaltene-bearing formations
Biosufactants	*Bacillus* sp., Pseudomonads, *Rhodococcus* sp.	Poor microscopic displacement efficiency	Lower interfacial tension	Sandstone or carbonate reservoirs with moderate temperatures (<50 °C) and relatively light oil (API >25)
Biomass/ polymer production	Many kinds	Poor volumetric sweep efficiency	Plug water channels and reduce permeability in water-swept regions by biofilm formation	Stratified reservoirs with variations in permeabilities

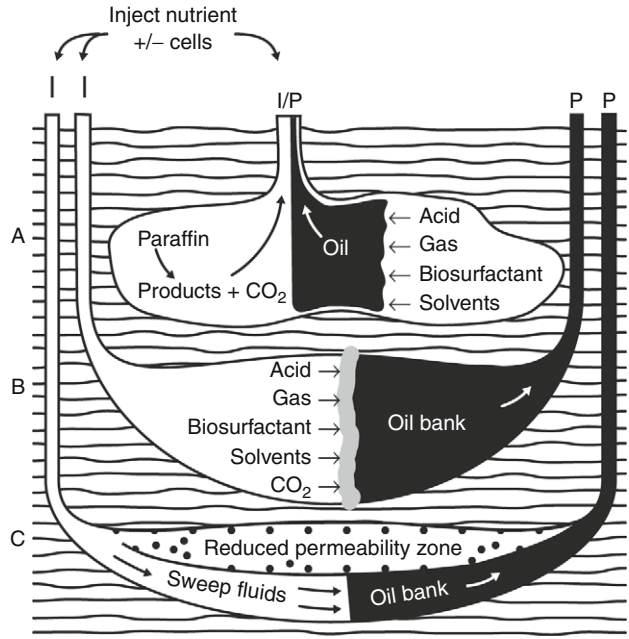

FIGURE 6.1 Microbial approaches for oil recovery. Panels A–C refer to different processes described in the text. (A) Microbial paraffin removal (left side) and microbial well stimulation (right side). Paraffin removal could be applied to either injection (I) or production (P) wells; well stimulations are done in production wells; most often, paraffin removal and microbial well stimulations involved the injection of nutrients and cells. (B) Microbially-enhanced water flooding where the stimulation of microbial metabolism creates useful products to mobilize oil; an inoculum maybe used. (C) Microbial selective plugging blocks high permeability zones (upper region) and redirects the recovery fluid into bypassed regions (lower) of the reservoir; nutrients and inoculum (if used) enter the high permeability zone (upper panel) and *in situ* microbial growth reduces permeability in this region.

allow more oil to flow to the well (Fig. 6.1A, Table 6.3). Microbially enhanced water flooding differs from the above in that nutrients with or without inocula are injected into one well in order to stimulate microbial activity in a large portion of the reservoir and the oil is recovered in wells different from that used for the injection (Fig. 6.1B and C, Table 6.3). The goal of microbially enhanced water flooding is to increase the ultimate oil recovery factor of the reservoir. This is done by improving the microscopic displacement efficiency through a reduction in the capillary forces that entrapped oil or by improving the volumetric sweep efficiency of the recovery fluid by blocking water channels and high permeability zones to push bypassed oil to production wells (Fig. 6.1B and C, Table 6.3).

Although well clean up and well stimulation technologies are not technically EOR processes, many times these processes extend the economic life of a field, either by reducing operating costs or increasing daily revenue (Brown *et al.*, 2002; Portwood, 1995b). By doing so, more oil is recovered from the reservoir than could have been recovered by conventional technology. Common usage in the microbial oil recovery discipline rarely distinguishes between microbial processes that improve oil production rate from those that improve the ultimate oil recovery factor and all microbial processes have been labeled MEOR. A recent analysis of numerous field trials concluded that MEOR is successful (Maudgalya *et al.*, 2007). Of the 403 MEOR projects that were analyzed, the overwhelming majority (96%) was considered to be successful by the investigators.

A. Paraffin control

The removal of paraffin and other deposits from the well and production equipment reduces operating costs and can improve the flow of oil into the well by altering drainage patterns and/or fluid saturations near the well. Expensive physical and chemical treatments are frequently needed to keep wells operative (Barker *et al.*, 2003; Becker, 2001; Etoumi, 2007; Ford *et al.*, 2000; Lazar *et al.*, 1999; Misra *et al.*, 1995). Chemical methods include the use of solvents, surfactants, dispersants, and wax crystal inhibitors. Thermal methods include the treatment of wells with hot fluids, usually hydrocarbons or water, to remove deposits. Formation damage may occur if the oil used as the solvent has paraffin content (Etoumi, 2007). Physical removal of paraffins by scraping is also used (Etoumi, 2007).

Stimulation of *in situ* hydrocarbon metabolism is the most common microbial approach to treat paraffin deposition problems (Fig. 6.1). Many hydrocarbon-degrading microorganisms have been isolated and there is a vast literature on the ability of microorganisms to degrade hydrocarbons aerobically (Van Hamme *et al.*, 2003) and anaerobically (Heider *et al.*, 1998; Spormann and Widdel, 2001). Usually, the procedure involves the injection of hydrocarbon degraders along with nutrients. Fluid production from the well is stopped (shut in) for several days to several weeks to allow *in situ* microbial growth and metabolism. The microbial treatments are repeated on regular schedule (every several weeks or monthly). Unfortunately, many of the published reports about microbial paraffin removal use proprietary mixtures of hydrocarbon-degrading bacteria and nutrients or ''biocatalysts,'' making it difficult to provide a scientific assessment of the technology.

The stated mechanism for paraffin removal involves the conversion of long-chain hydrocarbons to short-chain hydrocarbons, resulting in oils

with lower viscosities and improved mobilities (Brown *et al.*, 2005; Lazar *et al.*, 1999; Maure *et al.*, 2005; Nelson and Schneider, 1993; Smith and Trebbau, 1998). How long-chain alkanes are converted to short-chain alkanes is unclear. There are no microorganisms known to catalyze such a reaction. A number of studies report changes in the composition of the oil and its physical properties as a result of microbial activity. The proportion of low carbon number alkanes to high carbon number alkanes increases and viscosity decreases after microbial treatment (Brown, 1992; Deng *et al.*, 1999; He *et al.*, 2003; Nelson and Schneider, 1993; Partidas *et al.*, 1998; Smith and Trebbau, 1998; Trebbau *et al.*, 1999; Wankui *et al.*, 2006). Additional evidence for microbial metabolism such as the loss of electron acceptors and the production of metabolites are not provided. While the mechanism of action of commercial hydrocarbon-degrading microbial formulations is at least debatable, there is geochemical evidence that suggests that low molecular weight alkanes may be derived from fatty acids in certain oil reservoirs (Hinrichs *et al.*, 2006). There is also a number of reports of microbial paraffin degradation.

Etoumi (2007) found that *Pseudomonas* and *Actinomyces* spp. emulsified crude oil and hexadecane. Gas chromatographic (GC) analysis showed a decrease in the proportion of alkanes with carbon numbers greater than 22 and an increase in the proportion of alkanes between C13 and C21. Wax appearance temperature and crude oil viscosity decreased. Two *Bacillus* spp. and one *Pseudomonas* sp. isolated from fluids produced from the Liaohe field in China grew with wax as the sole carbon source, indicating their ability to degrade paraffins (He *et al.*, 2003). Bacteria isolated from hydrocarbon-polluted site degraded up to 84% of crude oil, 88% of semisolid or solid paraffin added to the cultures under aerobic conditions, and 47% of semisolid or solid paraffin under facultative anaerobic conditions (Lazar *et al.*, 1999). In laboratory flow experiments, the most active consortium did not significantly alter the total paraffinic content of crude oil but a decrease in viscosity was observed. Biosurfactants and biosolvents were detected during the test and may have contributed to the reduction in viscosity. A decrease in the apparent molecular weight of crude oil and its cloud point temperature indicated that microbial degration of heavy paraffinic hydrocarbons is possible (Sadeghazad and Ghaemi, 2003). Chemical and physical analyses showed that *P. aeruginosa* degraded normal alkanes (C16–C25) and *B. licheniformis* degraded cyclo and isoalkanes (C20–C30). Kotlar *et al.* isolated a strain identified as *Acinetobacter* sp. strain 6A2 from enrichments containing paraffin with melting temperature 52–54 °C (Kotlar *et al.*, 2007). The strain degraded alkanes with carbon numbers of C10–C40 and harbored enzymes involved in the degradation of high carbon number components.

Alterations of the physical properties of crude oil such as the viscosity, pour point (the lowest temperature where oil flows when cooled), and cloud point (the temperature where paraffins begin to precipitate from a liquid state) may indicate the production of emulsifiers or biosurfactants (Barkay *et al.*, 1999; Etoumi, 2007; Lazar *et al.*, 1999; Rosenberg *et al.*, 1983; Trebbau de Acevedo and McInerney, 1996). Another possible mechanism of action is the partial oxidation of hydrocarbons to alcohols, aldehydes, or fatty acids, which could act as solvents or surfactants (Pelger, 1991). There is a large body of evidence that shows that hydrocarbons are incompletely metabolized with the production of alcohols, fatty acids, etc. (Abbott and Gledhill, 1971; Connan, 1984). The injection of oxygen or a chemical that can be converted to oxygen (hydrogen peroxide) is needed to stimulate *in situ* aerobic metabolism. It is not possible to know whether the commercial approaches provide the needed electron acceptor. Anaerobic metabolism is a possibility (Spormann and Widdel, 2001), but this is usually a very slow process and it is difficult to envision significant anaerobic hydrocarbon metabolism occurring in the 1- to 2-day shut in period. Another concern regarding commercial microbial paraffin-degrading formulations is that laboratory studies do not support the ability of the inocula to degrade hydrocarbons or to recover oil. Gieg *et al.* (2004) found some emulsification, but no evidence of hydrocarbon metabolism when several crude oils were incubated under a variety of conditions with a proprietary mixture of hydrocarbon-degrading bacteria according to manufacturer's guidelines. *In situ* growth of commercial formulations of bacteria and nutrients did not mobilize oil entrapped in sandstone (Lazar *et al.*, 1999; Rouse *et al.*, 1992).

Microbial paraffin removal has survived in the market place for many years and published information supports the effectiveness of the approach (Table 6.4). A large number of wells from many different reservoirs all over the world have been treated. The conclusions of these studies are that the use of the proprietary inocula reduces the frequency of physical and chemical paraffin control treatments (Brown, 1992; He *et al.*, 2000, 2003; Nelson and Schneider, 1993; Santamaria and George, 1991; Streeb and Brown, 1992), reduces other operating costs (pump current) (He *et al.*, 2003; Streeb and Brown, 1992), and increases oil production (Table 6.4). In many cases, the natural decline in oil production was slowed or stopped for periods of months to years (Table 6.4). In quantitative terms, the results can be impressive, for example, daily oil production rate improvements of 47–210 m^3/d (Abd Karim *et al.*, 2001) or increases in oil production of 1700% (Nelson and Schneider, 1993) (Table 6.4).

Two users of commercial microbial paraffin treatment products indicate that oil production did not change, but the microbial treatments were cheaper to use than chemical or physical remediation approaches (Ferguson *et al.*, 1996; Santamaria and George, 1991) (Table 6.4). The

TABLE 6.4 Field results of microbial treatments to control paraffin deposition

Method	Results	References
Use of proprietary inocula and nutrients	50 wells treated; 78% had an increase in oil production above 4 m^3/d; overall average increase was 10 m^3/d; better performance if well produced from one formation and if layer was <7 m thick; shift in alkane ratio and reduction in oil viscosity noted	Partidas *et al.* (1998), Smith and Trebbau, (1998), Trebbau *et al.* (1999)
	Less frequent chemical wells treatments; oil production increased by 0.2–0.6 m^3/d	Hitzman (1988), Lazar *et al.* (1993)
	Two fields treated; oil production increased from 0.2 to 0.6 m^3/d for about 1 year; less frequent servicing of wells needed	Bailey *et al.* (2001)
	6 fields treated; 20–1700% increase in reserves over 3 years; saved $348 per well in operating costs; reductions in cloud and pour point and viscosity noted	Nelson and Schneider (1993)
	3 fields treated; 160–600% increase in oil production for 7–9 months; less frequent hot oil treatments and reduced chemical costs	Pelger (1991)
	72 wells treated; reduced frequency of hot oil and chemical treatments; arrested the natural decline in oil production; oil viscosity decreased	Brown (1992)

TABLE 6.4 (*continued*)

Method	Results	References
	Numerous wells treated; reduced frequency of hot oil and chemical treatments; reduced fuel consumption; arrested the natural decline in oil production; 4760 m^3 incremental oil production in 1 year	Streeb and Brown (1992)
	Treated 27 wells; 0–48% increase in oil production; 2950 m^3 of incremental oil in 3–6 months. Treated another 20 wells; 18% increase in oil production for 15–30 days; wax content altered	Deng *et al.* (1999)
	Treated 2 wells in two different fields; 36% and 46% increase in oil production; 3080 and 2200 m^3 incremental oil	Maure *et al.* (2005)
	9 fields treated; oil production increased from 22% to 320% for 14–44 months; incremental oil recovery ranged from 340 to 4110 m^3	Portwood (1995a,b), Portwood and Hiebert (1992)
	3 of 4 wells had no response, one well had an 18% increase in production; less expensive than hot oil treatments	Ferguson *et al.* (1996)
	5 wells treated: no change in oil production, reduced frequency of hot oil treatments; savings of $8000 per month	Santamaria and George (1991)
	Unnamed supplier of microbial paraffin treatment; 4 wells treated; overall, 16% increase in production (8 months); 65 m^3 incremental oil; microbial treatment ($1031 per well) had lower cost than chemical treatment ($3414 per well)	Giangiacomo (1997)

(*continued*)

TABLE 6.4 (*continued*)

Method	Results	References
Hydrocarbon-degrading *Bacillus* strains	43 of 60 wells showed positive response; 10,630 m^3 incremental oil; oil viscosity and oil composition altered	Wankui *et al.* (2006)
	Stopped natural decline and increased oil production in 3 of 4 wells; 650 m^3 incremental oil; chemical treatments reduced from every 15 days to every 4 months; pump current reduced	He *et al.* (2003)
Anaerobic hydrocarbon degrader with kerosene	Five different fields treated; increases in oil production of 1–2 m^3/d noted for all fields	Nelson and Launt (1991)
Unspecified treatments	Inoculate and shut in 1–5 weeks; oil production in 3 wells increased by 47% on average for 5 months (47–210 m^3/d)	Abd Karim *et al.* (2001)
	Undescribed microbe reapplied every 28 days; 26 wells treated; 9 wells had less maintenance and increased oil production; inconclusive results or no change in other wells	Wilson *et al.* (1993)

commercial formulations were tested in four production wells at the Department of Energy's Rocky Mountain oil storage facility (Giangiacomo, 1997). A slight increase in oil production and low costs of the microbial treatments were noted.

Several independent groups have developed their own hydrocarbon-degrading inocula (Table 6.4). Scientists in China used three hydrocarbon-degrading *Bacillus* spp. to treat a number of paraffin-laden wells in the Liaohe oilfield (He *et al.*, 2003; Wankui *et al.*, 2006). Forty-three of the sixty treated wells showed a positive response (Wankui *et al.*, 2006). The need for frequent thermal and chemical treatments dropped markedly and oil production increased substantially. Another study showed that the

TABLE 6.5 Results of microbial processes not involving hydrocarbon metabolism applied to individual wells to improve performance

Method	Results	References
Early tests		
Injection of aerobic and anaerobic bacteria with peat biomass, silt extracts and aerated water	Oil production increased by 28–48 m^3/day	Hitzman (1983), Senyukov *et al.* (1970)
Injection of a mixed culture with 4% molasses and a 6-month shut in period	Oil production increased 3.5 m^3/month for 3 months	Hitzman (1983), Senyukov *et al.* (1970)
Stimulation of fermentative metabolism: acid, gas, and solvent production		
Injection of a mixed culture of anaerobic microorganisms and molasses	Oil production increased 230% for 7 months	Lazar (1991)
Injection of an adapted mixed microbial culture with 2–4 % molasses	Oil production increased 300% from 0.1 to 0.35 m^3/day and 500% from 0.2 to 1.1 m^3/day	Lazar *et al.* (1993)
Injection of *Bacillus* sp. and *Clostridium* sp. with 4% molasses	350% increase in oil production if done correctly; most effective in carbonate wells with 15–39 API gravity crude oil and less than 10% salt	Hitzman (1983)
Injection of clostridial strains and 20 ton of molasses into carbonate formation	480% increase in oil production from 0.6 to 2.9 m^3/day	Wagner (1991)
Injection of clostridial spores and 9% molasses with a 30-day shut in period	Oil production increased from 0.16 to 0.32 m^3/day in one well	Grula *et al.* (1985)

(*continued*)

TABLE 6.5 (*continued*)

Method	Results	References
Injection of clostridial spores with sucrose, molasses, and NH_4NO_3 and a 3-month shut in period	Slight increase in oil production for 12 weeks	Hitzman (1983), Lazar (1991)
Injection of a mixed culture with 3.2% sugar and a 40-day shut in period	2086 m^3 of incremental oil; microbial numbers increased and metabolites detected	Wang *et al.* (1993)
Injection of a mixed culture with 4–5% molasses and a 15–21-day shut in period	42 of 44 wells tested had positive response with 33–733% increase in daily oil production; 261 m^3 of incremental oil per well; microbial numbers increased; CO_2 gas increased; microbial metabolites detected	Wang *et al.* (1995)
Injection of facultative anaerobic and anaerobic polymer producers and molasses	Cell numbers increased in the produced water	Lazar (1991)
Injection of molasses with 7-day shut in period	Oil production increased 216% (10 m^3/day)	Yusuf and Kadarwati (1999)
Stimulation of fermentative metabolism and biosurfactant production		
Injection of surfactant, alcohol, and polymer producers with sugar, molasses, yeast extract, $PO_4^{=}$, and NO_3^{-} and a 3-week shut in period	Oil production increased from 2.4 to 6.3 m^3/ day	Zaijic (1987)
Injection of a mixed culture of acid, gas, solvent and biosurfactant	Oil production increased by 79%	Bryant *et al.* (1988), Lazar (1991)

TABLE 6.5 (*continued*)

Method	Results	References
producers with 4% molasses		
Injection of *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *Bacillus licheniformis* with unspecified nutrients and a 40–64-day shut in period	Oil production increased by 2.3 and 3.4 m^3/day for 8 and 18 months, respectively, in two treated wells; 1140 m^3 of incremental oil	Zhang and Zhang (1993)
Injection of two *Bacillus* strains and one pseudomonad with waste fluids from a fermentation industry and a 7-day shut in period	Oil production increased 60% (1.9 m^3/day) in one well; no change in other well	He *et al.* (2000)
Injection of biosurfactant-producing *Bacillus* sp. and clostridia with unspecified nutrients and a 7-dayshut in period	Oil production increased in 2 wells (percent increase, incremental oil (m^3), duration in days): 50%, 56 m^3, 70 days and 40%, 137 m^3, 137 days; no change in 3 other wells	Buciak *et al.* (1994)
Injection of *B. licheniformis* and *Bacillus subtilis* subsp. *subtilis spizizenii* with glucose-nitrate-trace metals and a 4-day shut in period	Oil production increased 30–100% in two wells for 100 days; 38 m^3 of incremental oil; increase in microbial numbers, microbial metabolites including the lipopeptide biosurfactants detected	Simpson *et al.* (2007)
Stimulation of fermentative metabolism, polymer production, or growth to plug flow channels		
Injection of polymer-producing *Enterobacter cloacae* and	7 of 12 wells had increased oil production; 1730 m^3 of	Maezumi *et al.* (1998)

(*continued*)

TABLE 6.5 (*continued*)

Method	Results	References
biosurfactant-producing *Bacillus licheniformis* with 10–20% molasses and an 11–21-day shut in period	incremental oil recovered	
Injection of *Enterobacter cloacae* followed by 1% molasses in 2 wells or 5% molasses in 2 wells and *E. cloacae* and 5% molasses coinjected into 2 wells, each followed by a 10-day shut in period	4 wells showed increased oil production (1.7 m^3/day) for about 1 year; no change in two wells	Nagase *et al.* (2001)
Injection of *Enterobacter cloacae* or a polymer-producing *Bacillus* sp. and 5 or 10% molasses with and without $PO_4^=$ and a 14–21-day shut in period	7 of 14 wells that received molasses and an inoculum had increased oil production and decreased water production; pH decreased, water viscosity increased, and microbial metabolites detected; only 1 of 4 wells that received only molasses had an increase in oil production	Ohno *et al.* (1999)
Injection of unspecified inorganic nutrients	Oil production in one well increased by 30% (0.5 m^3/day)	Sheehy (1990)
Injector clean-out: 6 tons of molasses and adapted mixed culture of microbes	Injection pressure decreased, oil viscosity decreased, and cell numbers increased 1–4 logs	Lazar *et al.* (1991)
Microbial fracturing of carbonate formations: treat with unspecified	Increased oil production by 20% for about 30	Moses *et al.* (1993)

TABLE 6.5 (*continued*)

Method	Results	References
nutrients and microbes; shut in 7 days	days then returned to pretreatment levels	
Fracture damage repair: treat with microbes that degrade guar gum-based gels	Oil production restored in two wells that had lost production due to previous fracturing treatment	Bailey *et al.* (2001)
Use of unspecified microbial strains and nutrients		
Unspecified	24 wells treated with a 42% average increase in oil production; 75% showed an increase in pressure	Hitzman (1983)
Unspecified strains and nutrients	Increased oil production in 20 wells for about 15–30 days	Deng *et al.* (1999)
Unspecified strains and nutrients with a 40-day shut in period	Oil production of well increased from 4 to 6.4 m^3/day for 8 months; 556 m^3 of incremental oil	Hitzman (1988)

injection of hydrocarbon degraders and kerosene into production wells from five different formations increased oil production (Nelson and Launt, 1991). The success of independently-derived hydrocarbon-degrading inocula and independently tested commercial formulations provides support that the stimulation of *in situ* hydrocarbon metabolism is an effective approach to improve operations and oil production. However, other investigators found that these formulations were ineffective or provided inconclusive results (Ferguson *et al.*, 1996; Wilson *et al.*, 1993). None of the studies mentioned so far address the issue of whether an inoculum is required or if indigenous microorganisms caused the changes observed in the field.

TABLE 6.6 Field results of microbial processes to enhance oil production in oil reservoirs

Process	Method	Results	References
Multiple products (acids, gases, solvents)	Injection of *Clostrdium acetobutylicum* and molasses into poorly consolidated sand with carbonate minerals	Oil production increased 250% and water production decreased for 7 months; 22,000 m^3 of CO_2 and 11, 340 kg of fermentation acids accounting for 78% of the sugar carbon were produced	Yarbrough and Coty (1983)
	Injection of *Clostrdium tyrobutyricum* and molasses into carbonate reservoir	Water production decreased by ~20% and oil production increased by 50–65%; 2550–4900 m^3 of incremental oil recovered after 3 years; calcium and bicarbonate increased indicating dissolution of the rock; 350,000 m^3 of CO_2; 6 g/l organic acid concentration; isotopically light methane detected	Nazina *et al.* (1999b), Wagner *et al.* (1995)
	Injection of an adapted mixed culture of facultative and anaerobic microorganisms and 2–4% molasses followed by 2–4% molasses until molasses detected in production wells	Increased oil production by 400–600% (1–2 m^3/day/well) in two calcareous sandstone formations for 3 years; cell numbers increased and microbial metabolites detected in production wells. For carbonate or calcareous sandstone formations	Lazar (1992, 1993), Lazar *et al.* (1991, 1993)
	Injection of an adapted mixture of facultative and anaerobic microorganisms with molasses, four times at 4-month intervals	Around 15–50% increase in oil production rate in 2 of 8 reservoirs treated; large pH decease and microbial metabolites detected. Effective in carbonaceous reservoirs	Lazar (1987), Lazar and Constantinescu (1985), Lazar *et al.* (1988)

Injection of anaerobes derived from the field with molasses, NH_4Cl, polyphosphate and soda into carbonate reservoir; shut in until pressure increases at production wells	Oil production increased from 300% to 600% for nine months; 7000 m^3 incremental oil recovered; water production decreased 30–40%; 2 MPa increase in wellhead pressure; microbial metabolites and increase in cell numbers detected in production wells	Wagner (1991)
Indigenous microorganisms stimulated by two injections of local food manufacturing waste (sugars, proteins, amino acids) with N, P and K; shut in for 40 days	Oil production rate increased for 1.5 years; one well doubled oil production; 360 m^3 of incremental oil recovered; microbial metabolites detected; aerobic hydrocarbon degraders and heterotrophs increased; decrease of the C12 to C22 alkane fraction in oil.	Murygina, *et al.* (1995)
Injection of molasses with mixed microbial population containing sulfate reducers and pseudomonads	Significant increase in cell numbers in wells 500 m distant from injection wells; oil viscosity decreased; oil production increased 5–35% in some wells	Dostalek and Spurny (1957a,b), Hitzman (1983)
Injection of mixed microbial population (facultative and strict anaerobes, sulfate reducers, and pseudomonads), molasses, sucrose, NO_3^-, and $PO_4^=$	Oil production increased from 10% to 120% for several weeks to 18 months in some production wells; large decreases in oil viscosity and pH observed; CO_2 detected	Dienes and Jaranyi, (1973), Hitzman (1983), Jaranyi (1968)

(*continued*)

TABLE 6.6 (*continued*)

Process	Method	Results	References
	Injection of mixed microbial population from soils or sewage with molasses; shut in wells for 4–6 months	Oil production increased by 28–340% in some wells for 2–8 years	Hitzman (1983), Karaskiewicz (1975)
Multiple products with biosurfactant production	Injection of a biosurfactant-producing *Bacillus* sp., a *Clostridium* sp. and molasses; shut in for 2 weeks; periodic injection of molasses for 2 years	Oil production increased 13% and water to oil ratio decreased 35%; 88 m^3 of incremental oil recovered at \$20/m^3; surface tension of produced water decreased	Bryant *et al.* (1990)
	Injection of a biosurfactant-producing *Bacillus* sp. a *Clostridium* sp. and molasses; continuous molasses injection for 1 year	Oil production increased 19% for 3 years; 400 m^3 of incremental oil recovered at \$15/m^3	
Stimulation of indigenous hydrocarbon metabolism	Injection of aerated mineral salts solution for 3–6 months	Oil production rate increased 18–45% in 3 of 4 reservoirs; 5660, 12,440, 83,400, and 105,800 m^3 of incremental oil produced	Ibatullin (1995), Matz *et al.* (1992)
	Cyclic injection of aerated water with N and P sources	Oil production rate increased 10–46%; 2670–48,160 m^3 of incremental oil produced at \$6/m^3; metabolites, δ-C^{13} of bicarbonate increased and δ-C^{13} of CH_4 decreased; increased methanogenesis; acetate detected in production wells. Multiple formations treated	Belyaev *et al.* (1998, 2004)

	Cyclic injection of aerated water with N and P sources, but treated immediately before wells scheduled for shut in	376,000 m^3 of incremental oil produced from 118 wells (59 injection wells treated) over 11-year period	Belyaev *et al.* (2004)
	Cyclic injection of aerated water with N and P sources with addition of crude oil	4830, 35,100, and 67,000 m^3 of incremental oil produced from 3 different formations in 4, 12, and 7 years, respectively	Belyaev *et al.* (2004)
	Inject air–water mixture with mineral salts 2–3 days every month for 3 years	16200 m^3 of incremental oil produced in 3.5 years from Dagang oil field; inter-well permeability profile modified; methanogens, sulfate reducers and aerobes increase 1–2 logs; metabolites detected and surface and interfacial tension decreased	Nazina *et al.* (2007b)
Stimulation of hydrocarbon metabolism using an inoculum	Cyclic injection of aerated water with N and P sources with halophilic hydrocarbon degraders	2760, 6180, and 44380 m^3 of incremental oil recovered from 3 formations over 4, 3, and 8 years, respectively	Belyaev *et al.* (2004)
	Two cycles of injections of *Bacillus cereus* and *Brevibacillus brevis* hydrocarbon-degrading strains	Oil production in Daqing oil field increased 165% for about 200 days; 6700 m^3 of incremental oil from 10 producers; oil viscosity decreased, alkane profile changed, microbial counts increased 2 logs	Wankui *et al.* (2006)

(*continued*)

TABLE 6.6 (*continued*)

Process	Method	Results	References
	Periodic injection of a proprietary mixture of microorganisms, inorganic nutrients (N, P, and trace metals), and a ''biocatalyst''	Multiple reports: 78% of the projects either increased oil production or arrested natural decline in oil production; oil production increased by an average of 36%; 1740–3086 m^3 of incremental oil; oil viscosity decreased; cost of \$1.6–12.50 per m^3 of incremental oil	Dietrich *et al.* (1996), Maure *et al.* (1999, 2001, 2005) Portwood (1995a, b), Portwood and Hiebert (1992), Strappa *et al.* (2004), Yu and Schneider (1998)
Permeability profile modification	Indigenous microorganisms stimulated by weekly injections of molasses, NO_3^-, and $PO_4^=$	Increased oil production in 13 of 19 production wells in the treated area; oil from 9 wells had altered oil composition; extended economic lifetime of field by 5–11 years; 63,600 m^3 of incremental oil recovered at a cost of \$8.30/m^3	Brown (2007), Brown *et al.* (2002)
	Indigenous denitrifiers stimulated by injection of nitrate, nitrite, and other unspecified nutrients	Slowed natural decline of oil production in two fields; 1315 and 1225 m^3 of incremental oil recovered in 7–10 months; sulfide levels in brine decreased	Hitzman *et al.* (2004)
	Indigenous microorganisms stimulated with 3 batch injections of molasses and NH_4NO_3, each followed by cessation of injection fluid for 2–4 weeks	Major flow channel blocked; inter-well permeability variation reduced; oil production reinitiated (0.16 m^3/day); microbial metabolites detected	Knapp *et al.* (1992)

Indigenous microorganisms stimulated with maltodextrins and ethyl acid phosphate followed by cessation of injection fluid for 2 weeks	Fluid injection into upper portion of reservoir, which initially took 28% of the injected fluid, stopped; microbial metabolites detected	Jenneman *et al.* (1996)
Injection of *Bacillus* spores, sucrose, yeast extract, polyphosphate, and NO_3^- followed by cessation of injection fluid for 3 weeks	One injection zone blocked, another zone reduced by 50%, and 7 new injection zones detected; biofilm stable for 8 months	Lee Gullapalli *et al.* (2000)
Injection of polymer-producing *Enterobacter* strain for one week followed by 10% molasses for 2 months	Flow diversion occurred based on tracer analysis; water production decreased and oil production increased in seven of eight wells in the patterns; PCR analysis detected injected strains in produced fluids	Nagase *et al.* (2002)

B. Biogenic acid, solvent, and gas production

End products of anaerobic sugar fermentation include gases (CO_2 and H_2), acids (acetate, propionate, butyrate, valerate, and lactate), and solvents (ethanol, propanol, butanol, acetone, and 2, 3 butanediol) (Nakano *et al.*, 1997). H_2 is produced in large quantities in anaerobic ecosystems, but H_2 is quickly used by many different kinds of microorganisms. Acid, gas, and solvent production are used to improve oil production from individual wells (Fig. 6.1A, Table 6.5) or to mobilize entrapped oil during waterfloods (Fig. 6.1B, Table 6.6). In both approaches, readily fermentable carbohydrates with or without an inoculum are injected into the formation. If sufficient CO_2 and CH_4 are made, these gases will result in swelling of crude oil and reduce its viscosity (Bryant and Burchfield, 1989). *In situ* gas production may also lead to repressurization of oil reservoirs and hence improve oil recovery especially in mature reservoirs, but very large volumes would have to be made (Bryant and Lockhart, 2002). Organic acid production can lead to the dissolution of carbonates in source rocks, increasing porosity and permeability, and enhancing oil migration (Adkins *et al.*, 1992a; Udegbunam *et al.*, 1991). Given that large amounts of acids can be made by *in situ* microbial fermentation, it is possible that microbial processes could replace conventional acid treatments (Coleman *et al.*, 1992). Solvents alter the rock wettability at the oil–rock interface, releasing the oil from the porous matrix. Solvents could also dissolve in oil and lower its viscosity (McInerney *et al.*, 2005a).

1. Microorganisms

The most common microorganisms used for acid, gas, and/or solvent production for MEOR processes include members of the genera *Bacillus* and *Clostridium* (Bryant, 1988; Bryant and Douglas, 1988; Chang, 1987; Donaldson and Clark, 1982; Tanner *et al.*, 1993; Udegbunam *et al.*, 1991; Wagner, 1985; Wagner *et al.*, 1995). Spore production by these species is an advantage because spores survive harsh conditions and penetrate deep into petroleum reservoirs. *Clostridium* spp. produce gases (CO_2 and H_2), alcohols (ethanol and butanol), solvents (acetone), and acids (acetate and butyrate). *Bacillus* spp. produce acids (acetate, formate, lactate, etc.), gas (CO_2), alcohols (ethanol and 2,3-butanediol), and biosurfactants. Lactic acid bacteria (LAB) have also been used in oil recovery operations (Coleman *et al.*, 1992). Homofermentative LAB produce only lactate from sugars, while heterofermentative LAB produce ethanol and CO_2 beside lactate. Methane production by methanogens could potentially aid in oil release (Belyaev *et al.*, 2004).

2. Laboratory flow studies

A number of studies show that *in situ* production of acid, solvent, and gas increases oil recovery from laboratory models (Almeida *et al.*, 2004; Behlulgil and Mehmetoglu, 2002; Bryant, 1988; Bryant and Burchfield, 1989; Bryant and Douglas, 1988; Chang, 1987; Desouky *et al.*, 1996; Jinfeng *et al.*, 2005; Marsh *et al.*, 1995; Rauf *et al.*, 2003; Wagner, 1985; Wagner *et al.*, 1995). Most of these studies employed allochthonous microorganisms and molasses or some type of readily-fermentable carbohydrate.

Gas production has often been mentioned as an important mechanism for oil recovery (Jack *et al.*, 1983). The authors pointed out that what works effectively in the laboratory might not be as effective in the field due to low gas transfer. Isolates that showed promise for field applications include *Enterobacter* sp. with production of 1.6 moles of gas per mole of sucrose utilized (Jack *et al.*, 1983) and *Clostridium* strains that metabolize at 5–7.5% salt concentrations (Grula *et al.*, 1983). *Vibrio* sp. and *Bacillus polymyxa* were found to be the most proficient gas-producing strains under conditions that simulated actual oil reservoirs conditions (Almeida *et al.*, 2004). *In situ* growth of a consortium containing *B. polymyxa* in sand-packed column recovered 18% of the residual oil (which could not be recovered by extensive water flooding). The consortium containing *Vibrio* sp. recovered 16% of the residual oil. *In situ* growth of *Streptococcus* sp., *Staphylococcus* sp., or *Bacillus* sp. with molasses or glucose in sand-packed columns produced more gas (CO_2, H_2), and higher pressures than that observed by the stimulation of indigenous microorganisms with the same substrates (Desouky *et al.*, 1996). Large recoveries of residual oil (>50%) occurred when an inoculum was used. Although the cultures were selected based on gas production, the large decrease in interfacial tension to $\leq$1 mN/m suggests that the main mechanism for oil recovery may have been biosurfactant production rather than gas production.

Clostridium acetobutylicum and related species have been used in a number of studies due to the production of copious amounts of gases, acids, and solvents produced from carbohydrate substrates (Wagner, 1985; Wagner *et al.*, 1995). Large pressure increases and oil viscosity decreases were observed (Behlulgil and Mehmetoglu, 2002). Flow experiments with crushed limestone showed that *in situ* growth and metabolism recovered 49% of the residual oil. The core pressure increased, the pH decreased by 3 units, and the weight of crushed limestone decreased after microbial growth occurred. Residual oil was recovered when cores were treated with cell-free culture fluids (containing acids and alcohols) probably due to changes in wettability and oil viscosity. Larger oil recoveries (up to 30%) were observed due to the *in situ* growth and metabolism of *Clostridium tyrobutyricum* (Wagner, 1985; Wagner *et al.*, 1995) or *C. acetobutylicum* in sandstone cores (Marsh *et al.*, 1995). In the latter

study, the cores were incubated at an initial pore pressure of about 7000 kPa to mimic actual reservoir conditions where free gas phases are often not present. Large amounts of acetate, butyrate, butanol, ethanol, and CO_2 were made and their production coincided with oil recovery. The injection of cell-free culture fluids that contained the acids and solvents did not recover residual oil, consistent with *in situ* CO_2 production being the main mechanism for oil recovery. However, large reductions in permeability occurred, making it likely that multiple mechanisms were involved in oil recovery. *In situ* growth of a *Clostridium* sp. (acid and gas producer) or a *Bacillus* sp. (biosurfactant and gas producer) in a medium containing sodium pyrophosphate mobilized entrapped oil from sand-packed columns and sandstone or limestone cores (Chang, 1987).

Acid production by microorganisms may be an important mechanism for oil recovery from carbonates. The *in situ* growth of a halophilic, acid-producing bacterium in columns packed with crushed limestone lead to the dissolution of the carbonate minerals as evidenced by the presence of Ca^{2+} in the core effluents and significant amounts of carbonate particulates in the dissected cores (Adkins *et al.*, 1992b). Significant amounts of residual oil were recovered. Dissolution of the carbonate matrix was confirmed by studying the effect of *in situ* microbial growth and metabolism on the pore entrance size distribution of carbonate and sandstone cores (Udegbunam *et al.*, 1991). *Clostridium acetobutylicum* and a polymer-producing *Bacillus* strain were unable to penetrate the carbonate core. However, an unidentified halophilic, acid-producing anaerobe was able to grow through the carbonate core and electrical conductivity, permeability, porosity, and capillary pressure measurements showed pore enlargement and porosity increase due to acid dissolution of carbonate mineral. Acid production by *Lactobacillus* sp. and *Pediococcus* sp. dissolved $CaCO_3$ and iron scales in media containing goethite and magnetite (Coleman *et al.*, 1992).

3. Well stimulations

The first report of the use of acid, solvent, and gas production for oil recovery was the patent granted to Updegraff (Davis and Updegraff, 1954; Updegraff, 1956) for the use of *Clostridium* sp. and molasses for oil recovery. Later, Russian scientists tested whether gas production would decrease oil viscosity and improve oil recovery (Kuznetsov, 1962; Kuznetsov *et al.*, 1963; Senyukov *et al.*, 1970; Updegraff, 1990) (Table 6.5). They injected 54 m^3 of a mixed bacterial culture in 4% molasses into a well in the Sernovodsk oil field, which was then shut in for 6 months. A large increase in well pressure (1. 5 atm) indicated that *in situ* metabolism occurred. However, oil viscosity increased rather than decreased and only a slight increase in oil production was observed

(3.5 m^3 for 3 months) (Table 6.5). Another early approach used a geobioreagent (190 m^3) consisting of a mixture of aerobic and anaerobic bacteria and a nutrient solution containing peat and silt biomass (Hitzman, 1983; Senyukov *et al.*, 1970). This was followed by the injection of fresh water (650 m^3) (Table 6.5). Analyses of the produced fluid from the well showed that microbial populations in the produced fluids had changed and the pH increased from 5–5.6 to 6.5–8.3 and the gas/oil ratio increased from 17 to 70 m^3/ton. All of these studies showed that it was possible to stimulate *in situ* microbial growth and metabolism. The geobioreagent treatment increased oil production from 28 to 48 m^3 per day (Table 6.5).

Improvements to well treatment technologies continued for several decades and lead to increases in the size of the inoculum and nutrients and the use of mixed cultures adapted to the nutrients and the environmental conditions of the reservoir (Grula *et al.*, 1985; Hitzman, 1983; Lazar, 1991; Petzet and Williams, 1986; Wang *et al.*, 1993, 1995) (Table 6.5). These improvements decreased shut-in periods. *In situ* acid, gas, and solvent formation appears to be most effective in carbonate wells with an oil gravity of 875–965 kg/m^3, salinity less than 100 g/l, and a temperature around 35–40 °C (Hitzman, 1983). The data indicate that the injection of *Clostridium* and *Bacillus* spp. with molasses can increase oil recovery reproducibly, if done correctly (Hitzman, 1983). Seventy-five percent of the wells (24 wells total) treated in one study showed an increase in well-head pressure and an increase in oil production for 3–6 months (Hitzman, 1983). Another study states that 64 of 80 wells that were treated showed an increase in well-head pressure indicating that *in situ* microbial growth and metabolism occurred and more than 40 of these wells showed some increase in oil production (Petzet and Williams, 1986). Forty-two of forty-four wells had an increase in oil production of 33–733% (261 m^3 of incremental oil per well) (Wang *et al.*, 1993, 1995). The use of an adapted, mixed culture of microorganisms or clostridial strains with molasses increased oil production by 300–500% in low productivity wells (Lazar, 1987, 1991, 1992, 1993, 1998; Lazar and Constantinescu, 1985; Lazar *et al.*, 1988, 1991 1993; Wang *et al.*, 1993, 1995). One study indicates that an inoculum may not be needed as oil production increased by 216% with the addition of molasses only (Yusuf and Kadarwati, 1999), but details of this test are limited and control wells that received an inoculum were not used.

In situ microbial production of acids, gases, and solvents can also remove scale and debris in injection wells and increase injectivity (Table 6.5). Microorganisms have also been used to degrade guar gum and other injected chemicals to correct the damage caused by nonmicrobial treatments (Table 6.5).

4. Fermentatively-enhanced water flooding

Analogous to well stimulation approaches, fermentative bacteria and carbohydrate-based nutrient (usually molasses) are injected deep into the reservoir and fluid injection is stopped to allow for *in situ* growth and metabolism (Fig. 6.1B). The large number of cells in the inoculum along with the large amount of readily degradable carbohydrate provides a competitive advantage for the inoculum and selects for its growth and metabolism (Table 6.6).

One of the earliest and best-documented tests of MEOR was done by Mobil Oil Company in 1954 in the Upper Cretaceous Nacatoch Formation in Union County, Arkansas (Yarbrough and Coty, 1983) (Table 6.6). Laboratory studies showed that *C. acetobutylicum* produced 8–30 volumes of gas (CO_2 and H_2) per volume of 2% molasses medium and large amounts of acids (formic, acetic, and butyric) and solvents (acetone and butanol) in oil-saturated sand packed columns or cores (Updegraff, 1990). Based on the encouraging laboratory results, a field test of the technology was conducted. The Nacatoch sand formation is a loosely consolidated sand of high permeability and porosity about 700 m deep with bottom hole temperatures of 35–39 °C and a salinity of about 4.2%. The temperature and salinity were ideal for the growth of the *C. acetobutylicum*, but the high permeability and porosity and low residual oil saturation (4.5–8.5%) made the field undesirable in terms of oil release studies (Yarbrough and Coty, 1983). Two percent beet molasses was injected into a well at a rate of 16 m^3 per day for 5.5 months. A heavy inoculum of *C. acetobutylicum* was injected in 18 separate batches of 0.8 m^3 each (15.1 m^3 total) over a 4-month period. Breakthrough of the injection fluids in the production well (well 31), 88 m from the injection well, occurred after 70 days; fermentation products and sucrose were detected between 80 and 90 days after injection began. Fermentation products were detected 286 days after injection in another well that was 220 m from the injection well. Short chain organic acids in the produced fluids accounted for about 59% of the sugar injected. About 22,000 m^3 of CO_2 gas was produced from the well, which accounted for 19% of the sugar added. Some of the CO_2 was probably made by neutralization of fermentation acids by the carbonate minerals. Oil production in well 31 increased about the same time that microbial products were detected from about 0.1 m^3 per day prior to treatment to about 0.34 m^3 per day after treatment (250% increase) and lasted for at least 7 months. The formation of a free gas phase by CO_2 production was not likely given the reservoir pressure, but fermentation acids and CO_2 production at the surface of the sands may have lead to oil release.

Although the Nacatoch sand trial clearly showed that it was possible to generate large amounts of microbial metabolites throughout an oil

reservoir and more oil was recovered, the results did not generate much excitement in the U. S for MEOR. Oil prices were low and the amount of oil recovered from the Nacatoch sand trial was low because of the low residual oil recovery and high porosity. A series of tests were conducted in Eastern Europe throughout the 1960s where the amounts and type of nutrients and inocula were optimized (Table 6.6). In general, residual oil recovery in many of these trials increased by 10–340% for 2–8 years (Dienes and Jaranyi, 1973; Dostalek and Spurny, 1957a,b; Hitzman, 1983; Jaranyi, 1968; Karaskiewicz, 1975; Lazar, 1987, 1991). One approach involved the use of sulfate reducers and pseudomonads and improved oil production slightly (Table 6.6). Concern over the detrimental effects of sulfate reducers limited the application of this approach elsewhere. These studies did provide evidence that it is possible to propagate the growth of microorganisms throughout the reservoir as the numbers of sulfate reducers in production wells 500 m from the injection well increased significantly (Dostalek and Spurny, 1957a,b; Hitzman, 1983).

Improvements to the technology included the use of mixture cultures adapted to the nutrients and reservoir salinities and temperatures and much larger volumes of nutrients (Table 6.6). One test involved the use of a mixed culture of anaerobes obtained from the reservoir, molasses, NH_4^+, and pyrophosphate (Wagner *et al.*, 1995). Oil production increased by 300–600% for 7 months (7000 m^3 incremental oil recovered) and large amounts of CO_2 and organic acids were detected. The injection of a mixed culture of anaerobes adapted to molasses and reservoir conditions and large amounts of molasses increased oil production by 400–600% (1–2 m^3/d/well) in two calcareous sandstone formations for 3 years (Lazar, 1987, 1991, 1992, 1993, 1998; Lazar and Constantinescu, 1985; Lazar *et al.*, 1988, 1991, 1993). A large decrease in pH (1–2 units) and microbial metabolites were detected in production wells. Lazar concludes that this approach works best in carbonate or carbonaceous reservoirs. Based on the amount of molasses used, the amount of incremental oil recovered and the price of sucrose, we estimate that the cost was about \$19 per m^3 of incremental oil recovered.

Two studies used large amounts of molasses and *Clostridium tyrobutyricum* to treat carbonate reservoirs (Nazina *et al.*, 1999b; Wagner *et al.*, 1995). Both studies report large incremental oil recoveries (2550 and 4900 m^3 in 3 years). Each study provides very strong evidence that links microbial activity to oil recovery. Large amounts of CO_2 were detected in the gas phase (3,50,000 m^3) (Wagner *et al.*, 1995). The concentration of calcium and bicarbonate in production fluids increased consistently with a partial dissolution of the rock matrix. Organic acids (up to 6 g/l) were also detected in produced fluids. Highest increases in oil production were detected in wells where the greatest isotopic fractionation of methane was

detected, that is, where the strongest evidence for biological activity was present (Nazina *et al.*, 1999b; Wagner *et al.*, 1995).

The feasibility of *in situ* carbon dioxide production for oil recovery has been questioned because it is doubtful that sufficient amounts of CO_2 can be made to create free gas phase and that the amount of biomass needed to generate large quantities of CO_2 would lead to serious plugging problems (Bryant and Lockhart, 2002; Sarkar *et al.*, 1989). Nonetheless, the approach appears to be effective in carbonate and carbonaceous sandstone formations (Hitzman, 1983, 1988; Lazar and Constantinescu, 1985; Tanner *et al.*, 1993; Wagner *et al.*, 1995). In these types of formations, the production of organic acids could dissolve carbonate minerals and alter the pore structure (Udegbunam *et al.*, 1991, 1993), which would release oil from surfaces of the rock (Yarbrough and Coty, 1983). Consistent with this mechanism, many field trials report a large reduction in the pH (1–2 units) of produced fluids, large concentrations of organic acids in produced fluids, and increased calcium concentrations in produced fluids (Lazar, 1987; Lazar *et al.*, 1988; Yarbrough and Coty, 1983). The production of solvents (butanol, acetone, and isopropanol) may also be beneficial because solvents are known to decrease oil viscosity and alter rock wettability, both of which improve oil mobility. One study reported that large amounts of butanol were generated after the injection of fermentative anaerobes and carbohydrate-based nutrients (Davidson and Russell, 1988). However, no improvement in oil production was observed suggesting that solvent production alone may not be a viable approach for MEOR.

C. Biosurfactant production

Biosurfactants are surface-active agents produced by a wide variety of microorganisms. Biosurfactants are low molecular weight amphiphilic compounds that form micelles. Due to the presence of hydrophilic and lipophilic moieties in their structure, biosurfactants are able to partition at the oil–air or the oil–water interfaces and to lower surface or interfacial tension, respectively. This property makes them good candidates for MEOR (Youssef *et al.*, 2004). Towards the end of the secondary stage of oil recovery, the high capillary pressure traps crude oil in small pores within the rock matrix. To recover this entrapped or residual oil, a large decrease in interfacial tension between the oil and aqueous phases is needed (see Section III) (McInerney *et al.*, 2005a). Biosurfactants are ideal agents because they partition at the oil–rock interface and promote the mobilization of oil from the rock by the displacing fluid.

The most common biosurfactants used in MEOR are lipopeptides produced by *Bacillus* and some *Pseudomonas* spp., glycolipids (rhamnolipids) produced by *Pseudomonas* sp., and trehalose lipids produced by

Rhodococcus sp. (Banat, 1995a,b; Bodour and Miller-Maier, 2002; Youssef *et al.*, 2004) (Fig. 6.2). Lipopeptides and rhamnolipid biosurfactants lower interfacial tension between the hydrocarbon (crude oil or pure hydrocarbons) and aqueous phases to values of 0.1 mN/m or lower (Lin *et al.*, 1994; Maier and Soberon-Chavez, 2000; McInerney *et al.*, 1990; Nguyen *et al.*,

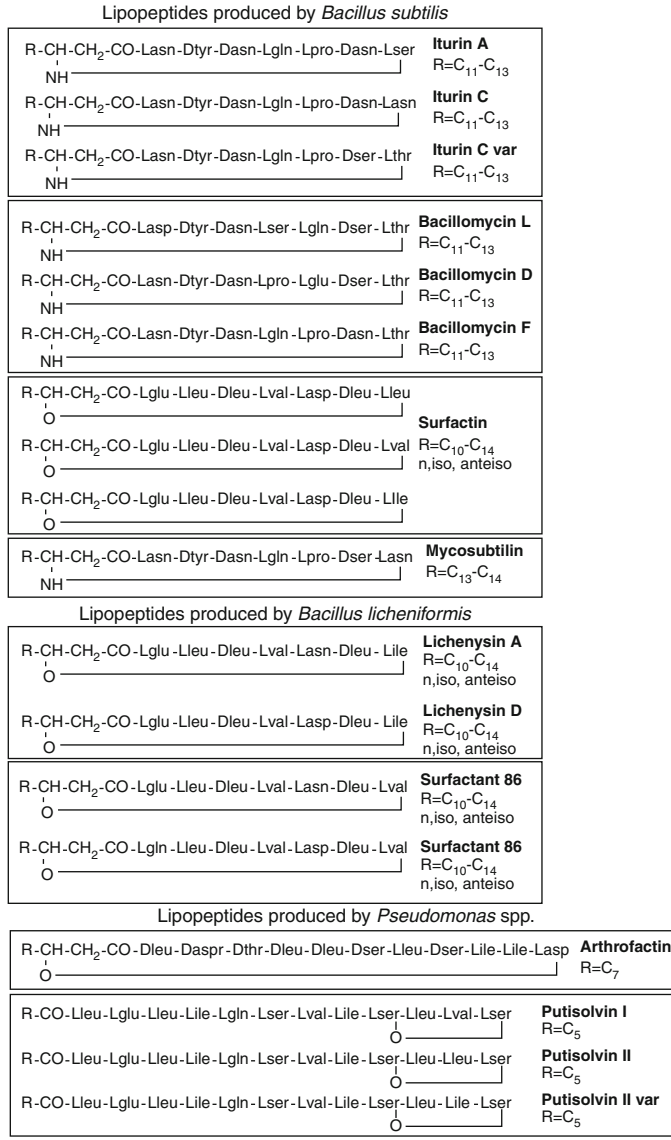

FIGURE 6.2 (Continued)

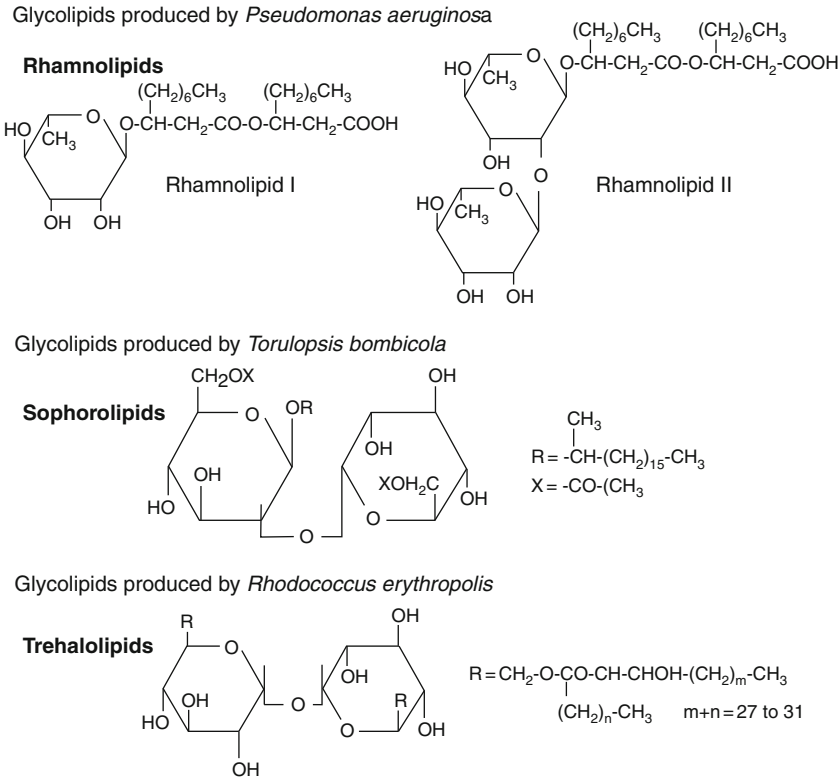

FIGURE 6.2 Structures of microbial biosurfactants. (A) Lipopeptide biosurfactants made by *Bacillus* and *Pseudomonas* sp. . . (B) Glycolipids including rhamnolipids, sophorolipids, and trehalolipids.

2008; Wang *et al.*, 2007). These low IFT values are sufficient to significantly lower the capillary number and mobilize significant amounts of oil (see Section III). The critical micelle concentrations of biosurfactants are orders of magnitude lower than synthetic surfactants, indicating that biosurfactants are effective at much lower concentrations (Georgiou *et al.*, 1992; McInerney *et al.*, 2005b; Youssef *et al.*, 2004).

Several laboratory studies investigated the improvement of biosurfactant yield and/or activity via cultivation conditions-related or genetic manipulation methods (for detailed review see Abu-Ruwaida *et al.*, 1991; Bordoloi and Konwar, 2007; Das and Mukherjee, 2005; Davis *et al.*, 1999; Joshi *et al.*, 2008a; Makkar and Cameotra, 1998; Mukherjee *et al.*, 2006; Schaller *et al.*, 2004).

TABLE 6.7 The effect of biosurfactant addition on interfacial tension and residual oil recovery in model porous systems

Microorganism	Biosurfactant	Type of experiment	Effect on IFT, wettability, and/or residual oil recovery	References
Aerobic mesophilic hydrocarbon-degrading bacteria	Unidentified	Core flood	IFT lowered; Wettability alteration	Kowalewski *et al.* (2006)
Isolates from Egyptian and Saudi oil fields	Unidentified	Berea sandstone core and sand-packed columns	IFT lowered; Wettability alteration; Increased oil recovery	Sayyouh (2002)
Thermophilic bacterial mixtures obtained from UAE water tanks	Unidentified	Core flood under reservoir conditions	IFT of 0.07 mN/m against four crude oils; Average residual oil recovery of 15–20%	Abdulrazag *et al.* (1999)
Five microorganisms from Persian reservoirs	Unidentified	Glass micromodels and carbonate rock with or without fracture	IFT reduction; Wettability alteration	Nourani *et al.* (2007)
Indigenous microorganisms from Persian reservoirs (45 °C)	Unidentified, Lipopeptides	Core flood	Residual oil recovery of 14.3%	Abhati *et al.* (2003)

(*continued*)

TABLE 6.7 (*continued*)

Microorganism	Biosurfactant	Type of experiment	Effect on IFT, wettability, and/or residual oil recovery	References
Bacillus subtilis and *Pseudomonas* strain	Unidentified	Crushed limestone-packed column	IFT of 0.052 mN/m, Injection pressure decreased 5–40%, Residual oil recovery of 5–10%	Li *et al.* (2002)
Facultative anaerobes from Daqing oil field	Unidentified	Anaerobic core flood	IFT lowered; pH decreased; oil viscosity decreased; light alkane proportion increased; residual oil recovery of 10%	Peihui *et al.* (2001)
Anaerobic enrichments from high-temperature oil reservoir	Unidentified	Sand-packed column at reservoir conditions	Residual oil recovery of 22%	Banwari *et al.* (2005)
Biosurfactant-producing microorganisms from Indonesian oil fields	Unidentified	Native and model core floods	Residual oil recovery of 10–60%	Sugihardjo and Pratomo (1999)
Bacillus mojavensis strain JF-2	Lipopeptide	Sand-packed columns	Residual oil recovery increased	McInerney *et al.* (1985b)

		Sand-packed columns and Berea sandstone cores	Residual oil recovery proportional to biosurfactant concentration; residual oil recovery of 83% with 920 mg/l biosurfactant + 10mM butanediol + 1% PHPA	Maudgalya *et al.* (2004, 2005), McInerney *et al.* (2005b),
		Crushed limestone columns	Residual oil recovery of 27%; calcium carbonate minerals dissolved	Adkins *et al.* (1992a,b)
Bacillus subtilis	Lipopeptide	Sand-packed columns flooded with sodium pyrophosphate	Residual oil recovery of 35%	Chang (1987)
Bacillus subtilis strain MTCC1427	Lipopeptide	Sand-packed column with kerosene	Residual kerosene recovery of 56% with 100 ml of 1 mg/ml crude biosurfactant	Makkar and Cameotra (1998)
	Lipopeptide	Sand-packed column with crude oil	Residual oil recovery of 34–39%	Makkar and Cameotra (1997)
Bacillus subtilis strains DM03, DM04 (thermophilic)	Lipopeptide	Sand-packed column	Residual oil recovery of 56–60%	Das and Mukherjee (2007)
Bacillus subtilis 20B, *B. licheniformis* K51, *B. subtilis* R1, *Bacillus* strain HS3	Lipopeptide	Sand-packed columns	Residual oil recovery of 25–33%	Joshi *et al.* (2008a,b)

(*continued*)

TABLE 6.7 (*continued*)

Microorganism	Biosurfactant	Type of experiment	Effect on IFT, wettability, and/or residual oil recovery	References
B. subtilis	Surfactin	Adsorption to carbonates	Wettability alteration; surfactin adsorbed	Johnson *et al.* (2007)
Acinetobacter calcoaceticus	Unidentified	Sand-packed column at 73 °C	IFT lowered; residual oil recovery of 36.4%	Sheehy (1992)
Engineered strains of *Pseudomonas aeruginosa* and *Escherichia coli*	Rhamnolipids	Sand-packed column	Residual oil recovery of 50% with 4 pore volumes of 250 mg/l rhamnolipid solution	Wang *et al.* (2007)
Pseudomonas strain	Glycolipid and phospholipids	Sand-packed column	Residual oil recovery of 52%	Okpokwasili and Ibiene (2006)
Pseudomonas strains	Glycolipid	Sand-packed column	Residual oil recovery of 64%	Das and Mukherjee (2005)
Pseudomonas aeruginosa strains	Glycolipid	Sand-packed column	Residual oil recovery of 50–60%	Bordoloi and Konwar (2007)
Rhodococcus strain	Glycolipid	Sand-packed column	Residual oil recovery of 86% with 5 pore volumes of broth	Abu-Ruwaida *et al.* (1991)

1. *Ex situ* biosurfactant flooding

Even though the low interfacial tensions and critical micelle concentrations exhibited by various biosurfactants strongly argue for their effectiveness in oil recovery, there was considerable skepticism about their use in EOR (Bryant and Lockhart, 2002; McInerney *et al.*, 2002). The lipopeptide-producing *Bacillus* strain JF-2 (Javaheri *et al.*, 1985) has been used in core flood experiments and in field trials (Bryant and Douglas, 1988; Bryant *et al.*, 1990), but oil recoveries were low and inconsistent. It is important to note that strain JF-2 has been reclassified as a strain of *Bacillus mojavensis* (Folmsbee *et al.*, 2006). The JF-2 lipopeptide was partially purified and tested in flow experiments with sand-packed columns and Berea sandstone cores (Knapp *et al.*, 2002; Maudgalya *et al.*, 2004, 2005; McInerney *et al.*, 2005b) (Table 6.7). Even very high concentrations of the JF-2 biosurfactant alone (12.3 g/l) were not effective in oil mobilization. The formation and subsequent disappearance of an oil bank in sand-packed columns during flooding suggested that mobility control was needed. Pre- and post-flushing of the column with a viscous solutions of 1% partially hydrolyzed polyacrylamide (PHPA) and the addtion of a cosurfactant (10 mM of 2,3 butanediol) to the biosurfactant solution resulted in very large oil recoveries (up to 83%) that were proportional to the biosurfactant concentration. 2,3 Butanediol was chosen as the cosurfactant because it is an end product of glucose fermentation by *Bacillus* sp. (Nakano *et al.*, 1997), including JF-2 (Folmsbee *et al.*, 2006). The lipopeptide is effective at very low concentrations so long as 2,3 butanediol and a PHPA were present (Maudgalya *et al.*, 2005). Culture fluids with as little as 16 mg/l recovered 22% of the residual oil recovery and concentrations above 40 mg/l recovered >40% of the residual oil from sandstone cores. Residual oil recovered was proportional to biosurfactant concentration in what appears to be a curvilinear relationship, which provides a relationship to predict oil recovery; on average, 2.2 ml of crude oil can be recovered per mg of biosurfactant used (Youssef *et al.*, 2007b).

Others have also shown that low concentrations of the biosurfactants can recover residual oil from model porous systems at elevated temperatures and salinities (Table 6.7). Very low concentrations (1 pore volume of 1 mg/l solution) of lipopeptides purified from *B. subtilis* MTCC 1427 cultures recovered 56% of the residual kerosene from sand-packed columns (Makkar and Cameotra, 1998). The effective concentration of the lipopeptide is much lower than that of rhamnolipid biosurfactants (Wang *et al.*, 2007). The lipopeptide lowered the surface tension to 28 mN/m and was stable at pH ranges of 3–11 and temperatures up to 100 °C. The lipopeptide produced from molasses-grown cells lower surface tension to 29 mN/m and recovered 34–39% of residual oil from sand-packed columns (Makkar and Cameotra, 1997). Two thermophilic

Bacillus subtilis strains DM-03 and DM-04 produced a lipopeptide biosurfactant when grown with cheap nutrients (potato peel) that lowered surface tension to 32–34 mN/m and recovered 56–60% of residual oil from sand-packed columns (Das and Mukherjee, 2007). Joshi *et al.* (2008a,b) used *Bacillus subtilis* strain 20B, *B. licheniformis* K51, *B. subtilis* R1, and *B.* strain HS3 to produce lipopeptide biosurfactants from different carbon sources including molasses and whey. The biosurfactant-containing culture broth lowered the surface tension to 29.5 mN/m and recovered 25–33% of residual oil from sand-packed columns. Lipopeptides are active over a wide range of environmental conditions often present in oil reservoirs, temperatures up to 100 °C, pH from 6 to 10, and salt concentration up to 8% (Cameotra and Makkar, 1998; Jenneman *et al.*, 1983; Joshi *et al.*, 2008b; Makkar and Cameotra, 1997, 1998; McInerney *et al.*, 1990). Surfactin produced by *B. subtilis* was more effective than sodium lauryl sulfate (an anionic chemical surfactant) in changing the rock wettability from oil–wet to water–wet system (Johnson *et al.*, 2007). The use of environmental scanning electron microscope showed that only small pores in flooded areas duing MEOR have altered wettability (Kowalewski *et al.*, 2005).

Glycolipids, in particular, rhamnolipids produced by *Pseudomonas aeruginosa*, are also effective in lowering IFT and recovering residual oil (Table 6.7). *Escherichia coli* and a *Pseudomonas* strain were genetically engineered to produce rhamnolipids from cheap renewable substrates (Wang *et al.*, 2007). The engineered *Pseudomonas* strain produced two kinds of rhamnolipids (with one or two rhamnose sugars) while the *E. coli* strain produced only rhamnolipids with one rhamnose. Crude preparations of biosurfactants from both strains reduced IFT at different pH and NaCl concentrations and recovered about 50% of the residual oil entrapped in sand-packed columns. A *Pseudomonas* strain that produced a mixture of glycolipid and phospholipids biosurfactants recovered 52% of the residual oil from sand-packed columns (Okpokwasili and Ibiene, 2006). A *Rhodococcus* strain isolated from oily soil produced a glycolipid biosurfactant that reduced surface tension to <30 mN/m. The biosurfactant was stable at high temperatures (120 °C), at high salt concentrations (up to 10%), and over a wide range of pH (2–12) (Abu-Ruwaida *et al.*, 1991). In sand-packed columns, 5 pore volumes of the biosurfactant-containing broth recovered 86% of residual oil. In a similar study, two *Pseudomonas* strains produced biosurfactants stable at pH 3–12 and temperatures up to 100 °C and recovered 64% of residual oil from sand-packed columns (Das and Mukherjee, 2005). The injection of cells of four biosurfactant-producing *Pseudomonas aeruginosa* strains into sand-packed columns resulted in clogging (Bordoloi and Konwar, 2007). Cell-free biosurfactant-containing culture fluids did not clog and recovered 50–60% of the residual oil. The authors suggested the use of *ex situ* produced biosurfactant to treat high-

temperature reservoirs as most biosurfactant producers are mesophilic and would not be able to grow in thermophilic reservoirs.

A number of studies with glycolipids were directed towards bioremediation. Rhamnolipids removed 79% of the crude oil from oil-contaminated soil (Urum *et al.*, 2003). Removal efficiencies of 80–95% were observed with biosurfactant-containing broths from *Rhodococcus* strain ST-5, a thermophilic *Bacillus* AB-2, and a proprietary strain Pet1006 compared to 58% with the synthetic surfactant, petroleum sulfonate (Banat, 1995a). Very low concentrations of biosurfactant produced by *Bacillus* strain C-14 released oil from contaminated sand. The biosurfactant produced by *Rhodococcus ruber* removed 80% crude oil from contaminated soil despite the adsorption of the biosurfactant to clay present in the soil (Kuyukina *et al.*, 2005).

The addition of rhamnolipids to synthetic surfactant used for alkaline surfactant polymer (ASP) flooding reduced the amount of the synthetic surfactant required for the efficiency of oil recovery (Daoshan *et al.*, 2004) and adding biosurfactant-containing culture fluids decreased the amount of synthetic surfactant and alkali required to generate low IFT against crude oil (Feng *et al.*, 2007).

2. Efficacy of *in situ* biosurfactant production

Biosurfactant producers isolated from a number of oil reservoirs are effective in mobilizing residual oil from a variety of laboratory test systems (Table 6.7). *In situ* growth of *B. mojavensis* strain JF-2 recovered residual oil from sand-packed columns (McInerney *et al.*, 1985b) and columns packed with crushed unconsolidated viola limestone (Adkins *et al.*, 1992b) (Table 6.7). In the latter study, oil recovery increased by 27% and increase in dissolved calcium suggested that some of the rock matrix had been dissolved. A number of other studies report effectiveness of lipopeptide-producing *Bacillus* strains for oil recovery (Table 6.7). The growth of *B. subtilis* in a sand-packed columns recovered 35% of the residual oil compared to 21% when only nutrients were added (Chang, 1987). *In situ* growth of biosurfactant-producing bacteria also recovered residual oil from sandstone cores (Thomas *et al.*, 1993; Yakimov *et al.*, 1997) (Table 6.7). In general, oil recoveries were low (<20%) and multiple pore volumes were needed. One study reported a residual oil recovery of 39% (Zekri *et al.*, 1999). Isolates from Saudi Arabian and Egyptian oilfields reduced IFT and altered rock wettability (Sayyouh, 2002). A thermophilic bacterial mixture reduced IFT to 0.07 mN/m at temperatures up to 100 °C and salinities up to 10% and recovered 15–20% of the residual oil from cores (Abdulrazag *et al.*, 1999). Five microorganisms isolated from a Persian reservoir reduced IFT, changed wettability, and recovered oil from glass micromodel systems and carbonated cores (Abhati *et al.*, 2003; Nourani *et al.*, 2007). Hydrocabon degraders from the Daqing oil

field in China decreased the IFT between crude oil and water to ultra low values (0.052 mN/m) and recovered 5–10% of the residual oil from columns packed with crushed limestone (Li *et al.*, 2002; Peihui *et al.*, 2001). Anaerobic enrichments of biosurfactant producers from fluids produced from a reservoir with temperature between 70 and 90 °C recovered about 22% of the residual oil recovery from sand-packed columns (Banwari *et al.*, 2005). Alternating cycles of nutrient starvation may be an approach to stimulate *in situ* biosurfactant production (Sheehy, 1992). This approach increased residual oil recovery from sand-packed columns by 36.4%.

Combining multiple microbial mechanisms by the use of a consortium of microbes with different properties is clearly an effective strategy for oil recovery (Bryant, 1988; Bryant and Burchfield, 1989; Bryant and Douglas, 1988). The combination of a biosurfactant producer (*B. licheniformis*), an acid, gas, and solvent-producer (*Clostridium* sp.) and a facultatively anaerobic, gram-negative rod increased oil recovery by 60% from etched glass micromodels and 28% from Berea sandstone. Residual oil recoveries of 39% and 10–60% from sand-packed columns were observed with two *Bacillus* strains grown with molasses (Rauf *et al.*, 2003). A consortium of three strains, an *Arthrobacter* and a *Pseudomonas* spp. both known to degrade oil and a biosurfactant-producing *Bacillus* sp. (Jinfeng *et al.*, 2005), when grown at 73 °C in a medium with molasses and crude oil, produced a biosurfactant that lowered the IFT between the oil and aqueous phases and decreased oil viscosity and cloud point. However, residual oil recovery was low, only 5%. Stimulation of *in situ* hydrocarbon metabolism by injection of oxygenated water into model core systems lowered the interfacial tension between oil and aqueous phases and changed the matrix from strongly water-wet to less water-wet which led to residual oil recovery (Kowalewski *et al.*, 2006).

3. Field trials

While stimulation of acid, gas, and solvent production appears to be very effective in recovering residual oil in carbonate reservoirs, fermentative metabolism alone may not be as effective in sandstone formations. One would predict that biosurfactant production would be needed based on laboratory studies (Table 6.7). Surprisingly, very little field information is available on the efficacy of biosurfactant use. Several studies have used biosurfactant-producing bacteria with those that make acid, gas, and solvent and report oil production increases of 30–100% for up to 18 months (Table 6.5).

Recently, it has been conclusively shown that large amounts of a lipopeptide biosurfactant can be made *in situ* and that that inoculation of the wells with lipopeptide-producing strains is required (Simpson *et al.*, 2007; Youssef *et al.*, 2007b). Although the test was conducted as a

proof of principle for *in situ* biosurfactant production, incremental oil was recovered (Simpson *et al.*, 2007; Youssef *et al.*, 2007b).

Two tests made use of a biosurfactant producer during water flooding (Bryant and Burchfield, 1991; Bryant *et al.*, 1990, 1993) (Table 6.6). In both tests, a mixed culture of bacteria was used and the biosurfactant producer was *Bacillus* strain JF-2 (Javaheri *et al.*, 1985). The surface tension of produced fluids from production wells decreased 6 weeks after injection suggesting that a biosurfactant was made. However, the identity of the surface-active agent in the produced fluids was not determined. When molasses was periodically added to four injection wells, oil production from the field increased by 13% after the microbial treatment and improvements in the water to oil ratio were noted in the treated area (Bryant and Burchfield, 1991; Bryant *et al.*, 1990). In a different field where continuous injection of molasses was used, oil production of the field increased by about 19% for 3 years (Bryant *et al.*, 1993). Total incremental oil recoveries in each case were low, 88 and 400 m^3 over a 2-year span, which means that the daily oil productivities (m^3 of oil per day) were low and probably not that much different from pretreatment values. The low incremental oil recoveries and small increases in daily productivity probably made the economics of the process unattractive.

D. Emulsifiers

Bioemulsifiers are high molecular weight amphiphilic compounds that are produced by a wide variety of microorganisms (Banat *et al.*, 2000; Bognolo, 1999; Dastgheib *et al.*, 2008; McInerney *et al.*, 2005a). Bioemulsifiers form stable emulsions with hydrocarbons (usually oil-in-water and less commonly water-in-oil) (Dastgheib *et al.*, 2008). Compared to biosurfactants, bioemulsifiers may not lower surface or interfacial tension (Dastgheib *et al.*, 2008). Members of the genus *Acinetobacter* produce the most commonly used bioemulsifier called emulsan (Rosenberg and Ron, 1999). Emulsan is an anionic heteropolysaccharide and protein complex. Other bioemulsifiers are heteropolysaccharides such as those produced by *Halomonas eurihalina* and *Pseudomonas tralucida*, protein complexes such as those produced by *Methanobacterium thermoautotrophicus*, protein–polysaccharide–lipid complex such as those produced by *Bacillus stearothermophilus*, carbohydrate–protein complex as liposan produced by *Candida lipolytica*, mannan protein as that produced by *Saccharomyces cerevisiea*, and others (Rosenberg and Ron, 1999).

Emulsan emulsifies hydrocarbon mixtures, but not pure hydrocarbons (Rosenberg *et al.*, 1979a,b). Emulsan did not adsorb to sand or limestone saturated with oil, emulsified a wide range of hydrocarbons and crude oils, and recovered 90% of the crude oil present in oil-contamined sand and 98% of crude oil saturating crushed limestone ($CaCO_3$) in shake

flask experiments (Gutnick *et al.*, 1986). A bioemulsifer obtained from a *Bacillus licheniformis* strain emulsified different hydrocarbons in shake flask experiments, but did not recover residual oil from sand-packed columns (Dastgheib *et al.*, 2008). However, *in situ* growth of the organism resulted in residual oil recoveries of 22%. The mechanism of oil recovery may have been profile modification rather than emulsification. Another possible application for bioemulsifier in oil recovery involves its use in preventing paraffin deposition (McInerney *et al.*, 2005a; Wentzel *et al.*, 2007). While bioemulsifiers alone may not be effective for oil recovery, their use in oil storage tanks cleanup has been well documented (Banat, 1995a; Banat *et al.*, 2000). A proprietary bioemulsifying strain removed hydrocarbons from the sludge floating on top of an oil storage tank (Banat, 1995a; Banat *et al.*, 2000) and emulsan reduced the viscosity of Venezuelan heavy oil and increased its mobility in the pipeline (Bognolo, 1999).

E. Exopolymer production and selective plugging

Microbial polymers have been used as mobility control agents to reduce viscous fingering of waterfloods and EOR processes for many years (Craig, 1974; Dabbous, 1977; Dabbous and Elkins, 1976; Harrah *et al.*, 1997; Malachosky and Herd, 1986; Trushenski *et al.*, 1974). The use of biopolymer for mobility control will not be discussed here. Instead, we will focus on the role of biopolymers and/or microbial biomass as plugging agents to improve volumetric sweep efficiency. Gelled polymer systems are widely used in petroleum reservoirs to reduce flow in high permeability zones and thereby redirect the displacement fluid into previously by-passed portions of the reservoir (Abdul and Farouq Ali, 2003; Ali and Barrufet, 1994; Kantzas *et al.*, 1995; Vossoughi, 2000). Curdlan is a β-1,3-D-glucan polymer that is soluble at alkaline pH and forms an insoluble gel as the pH decreases (Bailey *et al.*, 2000; Buller and Vossoughi, 1990; Harrah *et al.*, 1997). The gelation of the polymer can be induced by acid production by alkaliphilic bacteria. This approach was tested in sandstone cores and was shown to reduce permeability by two to four orders of magnitude and to divert flow from a high permeability core to a low permeability core that were connected in parallel (Bailey *et al.*, 2000). A number of biopolymers (xanthan gum, poly-β-hydroxybutyrate (PHB), guar gum, polyglutamic acid, and chitosan) were tested in a laboratory-pressurized flow system (Khachatoorian *et al.*, 2003). All of the polymers reduced the permeability of the sand-pack with PHB being the most effective.

Another mechanism to rectify permeability variation and to improve volumetric sweep efficiency is to stimulate the *in situ* growth of microorganisms in high permeability zones (Brown, 1984; Crawford, 1962, 1983; Jenneman *et al.*, 1984; McInerney *et al.*, 1985b) (Fig. 6.1C). Selectivity

of a microbial plugging process is controlled by water movement. Most of the fluid injected into a reservoir flows through regions of high permeability (Crawford, 1962, 1983). Because of this, most of the nutrients injected into the formation will enter the high permeability regions and little will enter the low permeability regions. Thus, microbial growth will preferentially occur in the high permeability regions because these regions receive most of the nutrients. The growth of microorganisms in high permeability zones of the reservoir or dominant flow channels will reduce the movement of water in these regions and divert the water into regions of the reservoir with higher oil saturations (Brown, 1984; McInerney *et al.*, 1985a). The microbial selective plugging is a generic one that can be applied to almost any reservoir because all that is required is to stimulate the growth of indigenous microorganisms by nutrient injection. It does not depend on the production of a specific chemical or the growth of a specific bacterium.

1. Laboratory studies on plugging and oil recovery

The growth of microorganisms in high permeability zones of the reservoir or dominant flow channels will reduce the movement of water in these regions and divert the water into regions of the reservoir with higher oil saturations (Brown, 1984; McInerney *et al.*, 1985a). A number of studies have shown that the *in situ* growth of bacteria in sandstone cores or other reservoir model systems results in significant reductions in permeability (Bae *et al.*, 1996; Cusack *et al.*, 1990; Jack and DiBlasio, 1985; Jack and Steheier, 1988; MacLeod *et al.*, 1988; Raiders *et al.*, 1986b, 1989). The injection of sucrose-nitrate medium stimulated the growth of indigenous microorganisms and resulted in large permeability reductions (>90% of the initial permeability) of Berea sandstone cores (Jenneman *et al.*, 1984; McInerney *et al.*, 1985a; Raiders *et al.*, 1985, 1986b, 1989). Large amounts of gas were produced and it is likely that a free gas phase formed and blocked water movement in some of these experiments. Later experiments with cores inoculated at reservoir pressure where a free gas phase would not form confirmed that *in situ* biomass production results in large permeability reductions (Knapp *et al.*, 1991). Two cores of differing permeability connected in parallel and two slabs of sandstone with different permeabilities layered on top of each other to allow crossflow between the slabs severed as model systems to test the selectivity of the microbial plugging process (Raiders *et al.*, 1986b, 1989). The injection of nutrients followed by an incubation period stimulated *in situ* microbial growth and permeabitiy reductions preferentially occurred in the high permeability core or slab. After *in situ* growth, most of the fluid was diverted to the low permeability core or slab. *In situ* growth of *Klebsiella pneumoniae* preferentially occurred in high permeability regions of a model porous system (Cusack *et al.*, 1990, 1992). The use of less readily

degradable carbohydrates (maltodexrins vs. glucose) and sodium trimetaphosphate, which adsorbs less to rock surfaces than phosphate allowed more uniform microbial growth and permeability reductions throughout the core (Davey *et al.*, 1998).

Residual oil recoveries ranging 8–35% were observed with sandstone cores in the absence of biosurfactant production (Raiders *et al.*, 1986b, 1989). The mechanism for oil recovery was postulated to be microscopic sweep efficiency where microbial growth blocks large pores and diverts fluid flow into smaller pores. In this mechanism, *in situ* growth of microorganisms would occur preferentially in large pores because these pores receive most of the nutrients and thus support most of the biomass production. Pore size distribution analysis of sandstone cores (Torbati *et al.*, 1986) and fused-glass columns (Stewart and Fogler, 2002) showed that microbial growth preferentially plugged the large pores or flow channels. The distribution of microbial activity in a shallow aquifer is strongly correlated with regions of high porosity where nutrient levels would be high (Musslewhite *et al.*, 2007).

Inocula have also been used for microbial selective plugging. Spores of a halotolerant mesophilic biopolymer-producing penetrated deeper into cores and, upon germination, permeability reduction and biofilm formation were observed uniformly throughout the core rather than only at the core inlet when vegetative cells were used (Bae *et al.*, 1996). Starved ultramicrocells of *K. pneumoniae* transported uniformly throughout the core and, when resuscitated, uniform reductions in permeability and biofilm formation throughout the porous model systems were observed rather than at the inlet end when vegetative cells were used (Lappin-Scott *et al.*, 1988; MacLeod *et al.*, 1988). Attachment and transport of *Lactobacillus casei* in a vertical, two-dimensional, packed-bed flow system depended on the concentration of injected cells concentration and the flow rate (Yang *et al.*, 2005).

Significant reduction in permeability in high permeability sand-packed and fused glass columns requires biopolymer production in addition to cell biomass (Geesey *et al.*, 1987; Jack and Steheier, 1988; Jenneman *et al.*, 2000; Lappan and Fogler, 1992; Robertson, 1998; Shaw *et al.*, 1985). Bacteria capable of secreting extracellular polymers and forming biofilms, for example, *Cytophaga*, *Arcobacter*, and *Rhizobium* were able to plug fractured limestone cores (Ross *et al.*, 2001). *Leuconostoc mesenteroides* produces the polysaccharide, dextran, when grown with sucrose (Lappan and Fogler, 1994). Batch experiments showed a correlation between the concentration of sucrose and other growth conditions and the amount of dextran produced (Lappan and Fogler, 1994, 1996; Wolf and Fogler, 2001). Visualization of the plugging process by use of glass bead-micromodel systems showed that plugging occurred in three phases: (1) an induction phase characterized by initiation of dextran

production, (2) a plugging phase characterized by biofilm and dextran production in large flow channels, and (3) plug propagation phase characterized by sequential development and breakthrough of plugs (Stewart and Fogler, 2001). *In situ* growth and polymer prodution of *L. mesenteroides* selectively plugged the high permeability core of a parallel core system (Lappan and Fogler, 1996). Injection of *L. mesenteroides* into fractured etched-glass micromodels and subsequent dextran production led to the plugging of matrix–fracture interface (Soudmand-asli *et al.*, 2007). Extracellular polymer production by four *B. licheniformis* strains was observed when sandstone cores were analyzed by scanning electron microscopy (Yakimov *et al.*, 1997). Biogenic acid production and microscopic selective plugging resulted in 9–22% residual oil recoveries. The permeability of sand-packd columns and sandstone cores was reduced by 65–95% reduction by the *in situ* growth and polymer production of two strains of *B. licheniformis* (Silver *et al.*, 1989). The degree of permeability reduction depended on the amount of polymer produced. The authors suggested the use of spores pretreated with lysozyme for deeper penetration and showed the importance of adding sodium triphosphate, citric acid, and aluminum for polymer formation.

2. Field trials

Surprisingly, the use of polymer-producing bacteria is effective in stimulating oil recovery from individual wells. The injection of the polymer producer, *Enterobacter cloacea*, and the biosurfactant-producer, *B. licheniformis*, with 10–20% molasses increased oil production in 7 out of 12 treated wells with the total incremental oil recovery of 1730 m^3 (Maezumi *et al.*, 1998) (Table 6.5). The effect of an inoculum (*E. cloacae*) and molasses concentration was studied (Nagase *et al.*, 2001; Ohno *et al.*, 1999). Seven of the 14 wells that received an inoculum and molasses had an increase in oil production and a reduction in the amount of water produced while only 1 of 4 wells treated with molasses had an increase in oil production (Table 6.5). Molasses concentrations greater than 5% were more effective than when 1% molasses was used. The produced fluids from the wells after treatments had a decreased pH and an increase in CO_2 concentration and H_2O viscosity compared to the fluids prior to treatment. Fermentations acids (acetic, propionic, and butyric) and 2,3-butanediol were detected. The presence of 2,3-butanediol and the biopolymer, known products of the metabolism of *E. cloacae*, indicated that the inoculum was active in the reservoir. The injection of inorganic nutrients to stimulate *in situ* microbial growth increased oil production by 30% for 6 months (Sheehy, 1990, 1991). Economic analysis indicated that additional oil was recovered at less than two Australian dollars per barrel (12.5 Australian dollars per m^3). The suggested mechanism of action is the blockage of water channels. Microbial plugging in the vicinity of the well

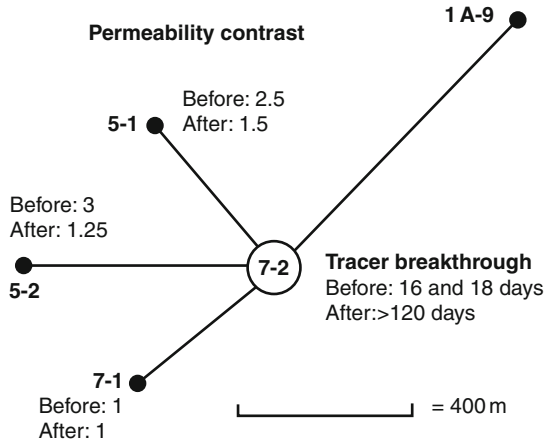

FIGURE 6.3 Efficacy of a microbial selective plugging process. Well 7-2 is an injection well that received molasses and nitrate; wells 1A-9, 5-1, 5-2, and 7-1 are production wells. Permeability contrast was calculated by dividing the inter-well permeabilities between wells 7-2 and 5-1 and 7-2 and 5-2 (before treatment permeabilities of 0.15 and 0.18 μm^2, respectively) by the interwell permeability between wells 7-2 and 7-1 (before treatment permeability of 0.06 μm^2). The time for tracers to travel between well 7-2 and well 1A-9 is given in days (Knapp *et al.*, 1992).

probably alters the relative permeability of the rock to oil and water and improves the migration of oil to the well (Donaldson, 1985).

The *in situ* growth of microorganisms in a hypersaline sandstone formation blocked a major channel and reduced interwell permeability variation in the reservoir (Fig. 6.3) (Knapp *et al.*, 1992). Large amounts of molasses and nitrate were injected until sucrose was detected in production well fluids. The further injection of brine was stopped for about 1 month to allow time for the microorganisms to grow. Evidence for *in situ* microbial activity included an increase in alkalinity and an increase in microbial numbers in the produced fluids (Knapp *et al.*, 1992). Prior to nutrient injection, the inter-well permeabilities between the injection well 7-2 and production wells 5-1 and 5-2 were 2.5 and 3 times that between well 7-2 and well 7-1 (Fig. 6.3). After nutrient treatment, the inter-well permeability between 7-2 and 5-1 and 5-2 decreased resulting in more uniform permeability throughout this region of the reservoir (Fig. 6.3). In addition, trace studies showed that a major water channel between the injection well 7-2 and the production well 1A-9 was blocked. The recovery of residual oil occurred. The reservoir brine contained large concentrations of divalent cations (Mg^{2+} and Ca^{2+}) and the interactions of these ions with the CO_2 made microbially probably resulted in the formation of calcium/magnesium carbonate minerals that could have blocked flow channels and reduced permeability. Such a mechanism has been

proposed to explain the reduction in groundwater flow of a drinking water aquifer (Chapelle and Bradley, 1997) and biogenic sealing of carbonate reservoirs (Ashirov and Sazonova, 1962).

The injection of a spore preparation of a *B. licheniformis* strain with molasses and nitrate blocked one highly transmissive zone and drastically reduced fluid intake by another zone (Gullapalli *et al.*, 2000) (Table 6.6). Well logs showed that fluid entered the formation by seven new zones after microbial treatment and that the biofilm was stable for 8 months. The stimulation of indigenous microorganisms with the injection of maltodextrins and ethyl acid phosphate followed by cessation of injection fluid for 2 weeks reduced fluid injection into one transmissive zone, but the biofilm was not stable for long periods of time (Jenneman *et al.*, 1996).

The injection of a polymer-producing *Enterobacter* CJF-002 for 1 week followed by the injection of 10% molasses for 2 months altered the interwell flow pattern of a reservoir as indicated by changes in tracer breakthrough times (Nagase *et al.*, 2002) (Table 6.6). The change in flow patterns lead to a large increase in oil production (38%) for 6 months and the amount of incremental oil recovered was large, 2144 m^3. Produced fluids contained large amounts of polymer and microbial metabolites. The numbers of CJF-002, estimated by most probable number analysis and restriction fragment length polymorphism, showed that CJF-002 propagated throughout the reservoir.

Several studies have used LAB as inocula to improve sweep efficiency of water floods (Jack and Steheier, 1988; Jenneman *et al.*, 1995; von Heiningen *et al.*, 1958; Yulbarisov, 1990). In one case, a reduction in the water to oil ratio of the produced fluids and an increase in water viscosity occurred (von Heiningen *et al.*, 1958). In the other studies, the permeability reductions were not large enough for significant flow diversion or oil recovery, probably because the permeabilies were very large (>1 darcy, 1 μm^2) (Jack and DiBlasio, 1985; Jack and Steheier, 1988).

Several studies show that stimulating indigenous denitrifiers will result in improved oil recovery (Brown *et al.*, 2002; Hitzman *et al.*, 2004) (Table 6.6); the mechanism for one of these studies is believed to be selective plugging (Vadie *et al.*, 1996). The injection of nitrate, phosphate, and molasses into portions of an oil field undergoing water flooding to stimulate *in situ* growth and metabolism and block water channels slowed the natural decline in oil production (Brown, 2007; Brown *et al.*, 2002). Thirteen of the nineteen production wells in the treated area had an increase in oil production while only two production wells from the control pattern that did not receive nutrients had an increase in oil production. Most of the production wells in the control patterns exhibited their normal decline in oil production or were shut-in due to low oil production during the test. The production of oil with a different composition in the treated wells compared to the control wells suggested that oil

previously by-passed by the waterflood was recovered. The economic lifetime of the field was extended by 5–11 years and resulted in the recovery of 63,600 m^3 of incremental oil at a cost of \$8.30/m^3 (Brown, 2007; Brown *et al.*, 2002). The stimulation of indigenous denitrifiers by the addition of nitrate, nitrite, and unspecified inorganic nutrients slowed the natural decline in oil production in two fields and recovered 1315 and 1225 m^3 of incremental oil in 7–10 months (Hitzman *et al.*, 2004).

F. *In situ* hydrocarbon metabolism

The *in situ* stimulation of hydrocarbon metabolism by injection of oxygen and inorganic nutrients was also an early approach to recover additional oil (Andreevskii, 1959, 1961). This idea has been studied intensively in Russia and very strong evidence links microbial activity with oil recovery (Belyaev *et al.*, 1982, 1998, 2004; Ivanov and Belyaev, 1983; Ivanov *et al.*, 1993; Nazina *et al.*, 1995a, 1999a, 2000a,b; Rozanova and Nazina, 1980; Rozanova *et al.*, 2001a; Yulbarisov, 1976, 1981, 1990; Yulbarisov and Zhdanova, 1984). In this approach, the stimulation of aerobic hydrocarbon metabolism in the vicinity of the injection well results in the production of acetate, other organic acids, and alcohols. These metabolites are converted to methane by methanogenic consortia deeper in the reservoir. Methane production would swell the oil and make it more mobile. Several push–pull tests were conducted to test this mechanism (Belyaev *et al.*, 1982; Ivanov and Belyaev, 1983). In a push–pull test, fluid is injected into a well for a period of time and then produced back from the same well. High concentrations of aerobic hydrocarbon degraders and organic acids were detected in fluids close to the injection well. Fluids recovered further away from the injection well had much lower numbers of aerobic hydrocarbon degraders and high rates of methanogenesis. The carbon in the carbonate pool was substantially heavier (average $\delta\ ^{13}C$ of −10.1) than that from untreated areas (average $\delta\ ^{13}C$ of −24.9) and the carbon of methane was substantially lighter (average $\delta\ ^{13}C$ of −64.1) than that from untreated areas (average $\delta\ ^{13}C$ of −55.7) (Belyaev *et al.*, 1998; Ivanov and Belyaev, 1983; Nazina *et al.*, 2007a). These data are consistent with the recent biological origin of some of the methane.

Laboratories studies showed that aerobic hydrocarbon degraders obtained from the produced fluids from the reservoir produced a mixture of organic acids and alcohols when incubated in minimal medium with crude oil and limiting amounts of oxygen and that methanogenic consortia converted these products of aerobic hydrocarbon metabolism to methane (Belyaev *et al.*, 1982; Groudeva *et al.*, 1993; Nazina *et al.*, 1985, 1995a; Rozanova and Nazina, 1980). Laboratory core studies showed that the continued injection of oxygenated brine and inorganic nutrients decreased the residual oil saturation in sandstone cores (Kulik *et al.*,

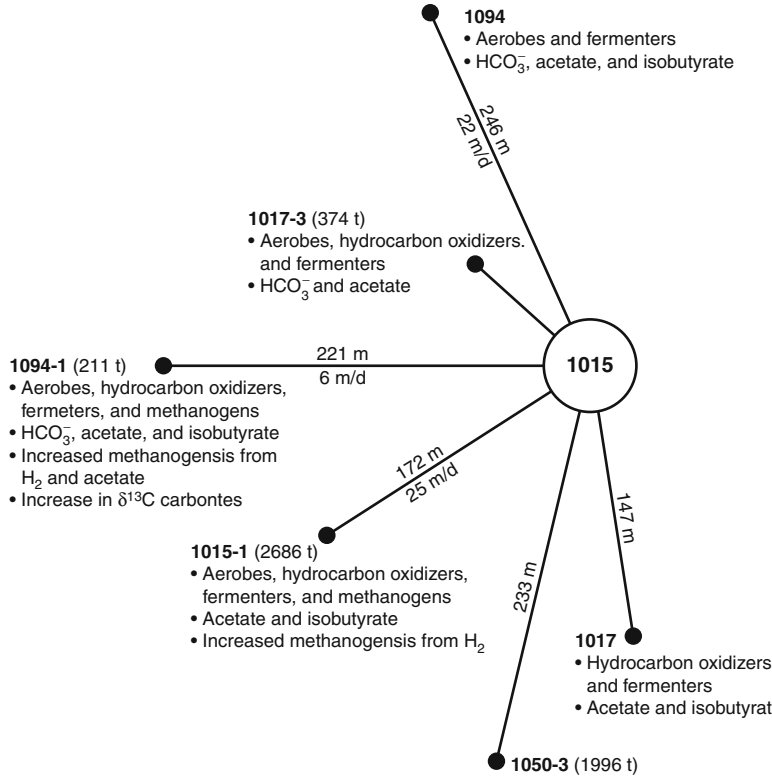

FIGURE 6.4 Efficacy of an *in situ* hydrocarbon metabolism process. Well 1015 is an injection well; wells 1094-1, 1017-3, 1094, 1015-1, 1050-3, and 1017 are production wells. Aerated water with nitrogen and phosphorous sources was injected two to three times a month from March to September over a 3-year period (15 treatments total) into well 1015. Inter-well distances and rates of fluid flow are given above and below the lines connecting the wells, respectively. The amount of additional oil recovered (metric tons) is given in parentheses next to the well number. Evidence for microbial activity is given under the well number. Only those parameters that had a large change from pretreatment values are given. Data are from Nazina *et al.* (2007a).

1985; Nazina *et al.*, 1985, 1995a; Sunde *et al.*, 1992). When sufficient oxygen was supplied, aerobic hydrocarbon-degrading cultures formed metabolites that decreased oil/aqueous phase interfacial tension by four orders of magnitude (Kowalewski *et al.*, 2005, 2006).

A number of field tests of this technology have been conducted and are summarized in Table 6.6. The results of one field trial are given in Fig. 6.4. Analysis of the microbial populations showed that aerobic hydrocarbon degraders were present near the injection well and their concentration increased with the injection of aerated water. The numbers of anaerobes

and methanogens increased in production well fluids. Rates of methanogenesis increased and high concentrations of acetate and bicarbonate were detected in production well fluids (Belyaev *et al.*, 1998; Nazina *et al.*, 2007a) (Fig. 6.4). Isotopically heavy carbon in carbonates and isotopically light carbon in methane was detected in the production well fluids consistent with the idea that some of the methane was of recent biological origin. Oil production increased by 10–45% in many different formations (Ibatullin, 1995; Ivanov *et al.*, 1993; Matz *et al.*, 1992; Murygina *et al.*, 1995; Nazina *et al.*, 2007b). The amounts of incremental oil recovered were very large (Table 6.6). The process is very economic with treatment costs near \$6 per m^3 of incremental oil recovered (Belyaev *et al.*, 2004).

As with microbial paraffin removal, there are many reports on the effectiveness of proprietary inocula and nutrients to increase oil recovery (Table 6.6). The use of commercial formulations slowed or arrested the natural decline in oil production and large incremental recoveries of oil have been reported (Bailey *et al.*, 2001; Brown *et al.*, 2005; Dietrich *et al.*, 1996; Maure *et al.*, 1999, 2001, 2005; Portwood, 1995b; Strappa *et al.*, 2004; Yu and Schneider, 1998). Supporting evidence for the involvement of microorganisms is limited to changes in the physical properties of the oil, reductions in oil viscosity, cloud point, or pour point, and an alteration in the alkane composition of the oil (Table 6.6) (Dietrich *et al.*, 1996; Strappa *et al.*, 2004; Wankui *et al.*, 2006). The cost of the treatment was usually less than \$20 per m^3 of incremental oil recovered. A computer simulation approach showed that the reductions in oil viscosity and relative permeability could explain the observed oil recoveries by the commercial formulations (Dietrich *et al.*, 1996). Independently, Wankui *et al.* (2006) used two hydrocarbon-degrading strains, *Bacillus cereus* and *Brevibacillus brevis*, to treat part of the Daqing oil field. Oil production increased by 165% for about 200 days and about 6700 m^3 of incremental oil was produced from 10 production wells (Table 6.6). A decrease in oil viscosity, alteration in the alkane profile, and an increase in microbial cell concentration by two orders of magnitude support the role of microorganisms in oil recovery. Halophilic hydrocarbon-degrading inocula have also been used to treat formation with high salinities (Belyaev *et al.*, 2004). Coinjection of these inocula with aerated water containing nitrogen and phosphorous nutrients resulted in large incremental oil recoveries (Table 6.6).

VI. IMPLEMENTATION OF MEOR

As shown in Table 6.3, there are a number of mechanisms that could be used to increase oil production (Bryant, 1991; McInerney *et al.*, 2005a). Surveys of the environmental conditions indicated that about 27% of the

reservoirs in the U. S. could be candidates for MEOR (Bryant, 1991; Clark *et al.*, 1981). The initial screen criteria to determine the suitability of a reservoir for application of MEOR (Bryant, 1991; Clark *et al.*, 1981; Sayyouh *et al.*, 1993) include: temperatures less than 80 °C; rock permeability >75 mDarcies (0.075 μm^2); salinities <10%; depth <2500 m; crude oil viscosity >15 °C API; As, Hg, Ni, and Se <10–15 ppm; and residual oil saturation >25%. The problem is to match the MEOR process with the production problem. This is often done heuristically based on sound petroleum engineering analysis of historical production data. Once the the problem is defined, then the appropriate microbial approach can be selected and a treatment strategy developed (Bryant, 1991).

A. Treatment strategies

Regardless of the MEOR process, three general strategies exist for the implementation of MEOR: (1) injection of nutrients to stimulate indigenous microorganisms, (2) in absence of the suitable indigenous population, the injection of exogenous microorganisms(s) and nutrients, or (3) in cases where the reservoir conditions are too harsh to allow growth of exogenously added microorganisms and the absence of suitable indigenous microorganisms, the injection of *ex situ* produced products (Banat *et al.*, 2000).

1. Injection of nutrients to stimulate indigenous microorganisms

This strategy requires the presence of indigenous microorganisms that perform the desired function (e.g., plugging, gas, solvent, acid, or biosurfactant production). To choose this approach, one must first determine if the appropriate microorganism or activity is present and then decide on how to stimulate the microbe or activity. Often, this decision is based on the analysis of produced fluids. However, core material should be considered if available. A number of procedures are available for sampling core material that can minimize the problems associated with contamination (Kieft *et al.*, 2007). Both molecular and microbiological techniques can be used to verify the presence of the appropriate microorganism or activity. Molecular techniques require the removal of oil from the samples and concentration of the cells usually by filtration to provide sufficient biomass for DNA extraction (Youssef *et al.*, 2007b). Once the DNA is available, the presence of specific genes can be detected by PCR or quantitative PCR (qPCR). For some approaches such as biosurfactant production, identification of suitable target genes such as *srfA* for surfactin, *licA* for lichenysin, *rhlR* for rhamnolipid production is straightforward. For others such as acid, solvent, and gas production, the choice of the appropriate gene target is less clear. The analysis of the 16S rRNA content using universal bacterial primers could be valuable in identifying

useful members of the community in cases where activity is linked to phylogeny (e.g., methanogenesis).

Once the appropriate indigenous microorganisms are identified, further tests are needed to confirm the production of the desired metabolite or activity. This requires a suitable medium to support growth and metabolism of the organism in question. The use of undefined media to grow indigenous microorganisms might not be the best approach as high concentrations of nutrients can be inhibitory to indigenous microorganisms. Also, the presence of multiple carbon compounds in these media may make it hard to control the process *in situ*. Systematic amendment of C, N, and P sources and other nutrients (trace metals, vitamins, etc.) to produced fluids incubated under conditions that stimulate reservoir conditions as closely as possible is an effective approach to detect the appropriate microbe of activity (Harvey and Harms, 2001). This approach also defines the nutrients that need to be injected into the reservoir. Subjecting indigenous microorganisms to nutrient-limiting conditions may increase the production of surface-active metabolites (Sheehy, 1992). Periodic cycles of nutrient-excess and nutrient-limitation are suggested because microorganisms become hydrophobic and cell wall components act as surface-active agents when starvation is induced.

2. Injection of exogenous microorganisms and nutrients

If the appropriate microorganisms or activity is absent, then inoculation of the reservoir with exogenous microorganism is needed. The use of exogenous microorganisms may also be an effective way to establish the appropriate activity quickly in the reservoir. Long incubations times may be needed before indigenous microorganisms grow sufficiently to produce the amounts required. The problem here is that long incubations time mean economic loss as the operator is not receiving revenue and significantly add to the cost of the treatment. Foremost, the exogenous microorganism must be able to grow under the environmental conditions present in the reservoir and in presence of competing indigenous population (Bryant, 1991). Some exogenous microorganisms may be adapted to reservoir conditions by stepwise challenging the microorganism to differing temperature and salt regimes (Banwari *et al.*, 2005). One advantage of the use of exogenous microorganisms is that a nutrient package can be designed specifically to stimuate their growth and metabolism in the reservoir (Youssef *et al.*, 2007b).

A critical factor is the transport abilities of the exogenous microorganism. Ideally, the injected microorganisms should have minimal adsorption to reservoir rock material. Some studies provide conflicting recommendations on the use of starved versus nonstarved cells (Camper *et al.*, 1993; Cunningham *et al.*, 2007). However, starved cells are smaller and smaller cells have less retention and higher transport efficiency than larger cells

(Fontes *et al.*, 1991). A number of laboratory studies show that starved cells penetrate porous material more effectively than vegetative cells (Lappin-Scott *et al.*, 1988; MacLeod *et al.*, 1988). Spores are also desirable in this respect (Bae *et al.*, 1996; Jang *et al.*, 1983; Gullapalli *et al.*, 2000; McInerney *et al.*, 2005b). Alternatively, addition of nutrients may allow microorganisms to grow throughout the reservoir (Jenneman *et al.*, 1985; Sharma and McInerney, 1994; Sharma *et al.*, 1993).

3. Injection of *ex situ*-produced metabolites (Biosurfactant or polymer)

If indigenous microorganisms are not suitable for the desired outcome and conditions in the reservoir are too harsh for survival of exogenous microorganisms, the last resort is to add *ex situ*-produced products. Lipopeptides and rhamnolipids are stable at high temperatures (100 °C), a wide range of pH (2–12), and at high salt concentrations (up to 10%) (Abu-Ruwaida *et al.*, 1991; Bordoloi and Konwar, 2007; Das and Mukherjee, 2005; Joshi *et al.*, 2008a,b; Makkar and Cameotra, 1998). Some biopolymers show some deterioration at reservoir conditions, but not as extensively as synthetic polymer (Buller and Vossoughi, 1990).

Loss of the injected chemical is a major concern and a problem that plagues chemical EOR approaches (Green and Willhite, 1998; Strand *et al.*, 2003; Weihong *et al.*, 2003). Adsorption of surfactin and rhamnolipids to rock was higher than synthetic surfactants (Daoshan *et al.*, 2004; Johnson *et al.*, 2007). Studies on biopolymer loss due to adsorption have been conducted (Huh *et al.*, 1990). Synergistic effects of biosurfactants and chemical surfactants have been reported (Daoshan *et al.*, 2004; Feng *et al.*, 2007; Singh *et al.*, 2007), which would decrease the amount of chemical surfactant required and account for loss due to adsorption of the biosurfactant. Biosurfactants and polymers can be produced from cheap renewable sources in amounts sufficient for injection without extensive purification (Maneerat, 2005; Mukherjee *et al.*, 2006). There is also the possibility of designing recombinant strains that over-produce the biosurfactant or produce biosurfactant with specific structure or quality (Mukherjee *et al.*, 2006; Wang *et al.*, 2007).

B. Nutrients selection

The choice of nutrients largely depends on the desired outcome and the organism(s) involved. When exogenous microorganisms are used, the extensive knowledge base generated by studying the organism can be used to develop nutrient packages to maximize growth and/or product formation. For other MEOR approaches, there are a few guidelines that can be used. Selective plugging requires biomass production in the reservoir. Biomass formation can be maximized by supplying nitrate as the

electron acceptor and its presence will also help control detrimental activities such as souring (Section V). Many LAB such a *Leuconostoc* sp. produced biopolymers (dextran) only when sucrose is added (Jenneman *et al.*, 2000; Lappan and Fogler, 1994). Nutrient manipulation showed that *B. licheniformis* could produce a biosurfactant or a biopolymer (Gabitto and Barrufet, 2005). Acid, solvent, and gas formation occurs by anaerobic fermentation of carbohydrates so the injection of large amounts of a readily degraded carbohydrate will create the conditions needed. Subjecting indigenous microorganisms to cycles of nutrient-excess and nutrient-limiting conditions may be a mechanism to promote the production of bioemulsifiers or other surface-active metabolites (Sheehy, 1992).

Biosurfactant production, on the other hand, requires fine balance between carbon and nitrogen. Davis *et al.* (1999) showed that surfactin production by *Bacillus subtilis* was enhanced when nitrate was limiting ($Y_{p/x}$ of 0.075 g surfactin per gram of biomass compared to 0.012 g surfactin per gram of biomass when nitrate vs. ammonium was limiting in the growth medium, respectively). Nitrogen limitation causes overproduction of the biosurfactant made by *Candida tropicalis*, and C/N ratio of >11 maximized rhamnolipid production by *Pseudomonas* sp. (Cameotra and Makkar, 1998; Gautam and Tyagi, 2006). Extensive laboratory study is worth the effort as the systematic testing of different media components to maximize lipopeptide production from two *Bacillus* sp. under anaerobic conditions (McInerney *et al.*, 2005b) gave success in the field (Youssef *et al.*, 2007b). One important aspect to consider is the partial loss of nutrients due to adsorption to rock material. Concentration of the nutrient or a different form (organophosphates instead of inorganic phosphates) (Jenneman and Clark, 1994a,b) can minimize this problem.

C. Monitoring the success of MEOR field trials

The ultimate goal of MEOR is to increase in the amount of oil recovered or the rate of oil production, or to alleviate a production problem. It is important to monitor the microbiology to ensure that the process worked. In cases where both microorganisms and nutrients are injected, the process can be monitored by assessing the strain survivability, usually through a cell counting approaches, or by quantifying the loss in substrates injected and the appearance of metabolites (Youssef *et al.*, 2007b). When nutrients are injected to stimulate indigenous microorganisms, the process can be monitored by the increase in the number of indigenous population following treatment, the loss of substrate, and appearance of specific products of metabolism (Nazina *et al.*, 2007a,b).

TABLE 6.8 Methods to manipulate lipopeptide biosurfactant structure and its activity

Manipulation method	Effect on structure	Effect on surface activity	Reference
Growth in Landy's medium with valine as nitrogen source	Replacement of leucine in position 7 with the less hydrophobic valine, [Val7]-surfactin variant	Decreases the hydrophobicity and increases the CMC of surfactin	Peypoux and Michel (1992)
Growth in Landy's medium with leucine or isoleucine as nitrogen sources	Replacement of the valine in position 4 with more hydrophobic leucine or isoleucine makes the molecule more hydrophobic, [Leu4]- or [Ile4]-surfactin variant	Improves surfactant activity	Bonmatin *et al.* (1995)
Growth in Landy's medium with isoleucine as nitrogen source	[Ile4, 7]-surfactin variant; [Ile2, 4, 7]-surfactin variant	Increased hydrophobicity, CMC decreased from 220 to 90 μM, increased affinity for calcium ions	Grangemard *et al.* (1997)
Genetic manipulation by module swapping between fungal and bacterial genes	[Val7]-, [Phe7], [Orn7], and [Cys7]-surfactin variants	Hemolytic activity lowered; surface activity not tested	Stachelhaus *et al.* (1995)

(*continued*)

TABLE 6.8 (*continued*)

Manipulation method	Effect on structure	Effect on surface activity	Reference
Addition of precursors of branched chain fatty acids (valine, leucine, isoleucine) to the growth medium of *Bacillus licheniformis*	Lichenysin with variable fatty acid tail composition	The increased proportion of branched chain fatty acid of lichenysin a lowered the surface activity. Normal chain C14 fatty acid is important for surface activity	Yakimov *et al.* (1996)
Addition of precursors of branched chain fatty acids (valine, leucine, isoleucine) to the growth medium of *Bacillus subtilis* subsp. *subtilis*	Surfactin with variable fatty acid tail composition	Oil diplacement activity against crude oil increased with the increase in the ratio of iso to normal even numbered fatty acid in surfactin	Youssef *et al.* (2005)

VII. CURRENT AND FUTURE DIRECTIONS

A. Biosurfactant formulations

Significant improvements in microscopic displacement efficiency will require the use of biosurfactants that generate very low interfacial tensions against the crude oil present in the reservoir. Biosurfactants vary in their hydrophobicity/hydrophilicity such that a hydrophilic biosurfactant partitions in the aqueous phase and a hydrophobic biosurfactant partitions in the oil phase rather than at the interface between the two phases. The success of biosurfactant-mediated oil recovery depends on formulating biosurfactant mixtures with the appropriate hydrophobicity/hydrophilicity so that they partition at the interface. This can be done by measuring the activity of the biosurfactants against hydrocarbons with different equivalent alkane carbon number (Nguyen *et al.*, 2008;

TABLE 6.9 Formulating effective mixtures of biosurfactants and synthetic surfactants to minimize interfacial tension

Mixture components	Effect on IFT	References
Lipopeptides with different fatty acid tail composition	IFT against a hydrophilic hydrocarbon (toluene) was lowered when the mixture has less than 70% of the FA as C14 and C15, and the ratio of C16 to C18 FA is ≥ 8	Youssef *et al.* (2007a)
Lipopeptides and rhamnolipids	IFT against toluene lowered when the hydophilicity of the mixture was increased. Rhamnolipids are more hydrophilic than lipopeptide and hydrophilic lipopeptides were required	Youssef *et al.* (2007a)
Lipopeptides with hydrophobic synthetic surfactant	IFT lowered against more hydrophobic hydrocarbons (e.g., hexane and decane) when the hydrophobicity of the mixture increased	Youssef *et al.* (2007a)
Rhamnolipid with hydrophobic synthetic surfactants	IFT against more hydrophobic hydrocarbons (e.g., hexadecane) was lowered as the hydrophobicity of the mixture increased	Nguyen *et al.* (2008)

Youssef *et al.*, 2007a). The biosurfactant structure and the properties of the displacing fluid can then be manipulated to maximize IFT reduction. Table 6.8 shows the different methods that could be used to change the structure of lipopeptide biosurfactants. Most of these methods are simple manipulations of the growth medium to obtain a more hydrophobic or more hydrophilic lipopepetide. If altering the structure does not yield a biosurfactant with the appropriate properties, for example, very low IFT against the crude oil in question, mixtures of two or more biosurfactants or synthetic surfactants can be used to obtain very low IFT against crude oil. Table 6.9 shows the effect of different biosurfactant/synthetic surfactant mixtures on IFT (Youssef *et al.*, 2007a). The salt concentration and the pH of the displacement fluid can be manipulated to enhance interfacial activity (Nguyen *et al.*, 2008).

B. Understanding the microbial ecology of oil reservoirs

Our understanding of the phylogenetic diversity, metabolic capabilities, ecological roles, and community dynamics of oil reservoir microbial communities is far from complete, and several fundamental ecological questions remain partly or completely unanswered. There are three general areas of research in which we believe significant advances could be achieved by using traditional and molecular ecological approaches. It is important to note that at the heart of all current and future opportunities is the issue of sampling.

1. Detailed understanding and comparative analysis of microbial diversity in oil reservoirs

As outlined in Section IV, none of the published studies has satisfactorily documented a complete or near complete census of the bacterial or archaeal communities in a reservoir and the relatively small number of clones sequenced per study prevents statistical estimation of species richness, evenness, or diversity patterns. Detailed high throughput investigations of the bacterial and archaeal communities (e.g., studies generating >1000 clones) in multiple oil reservoirs are needed to obtain the needed information. Such effort will also result in the identification of less abundant members of the community, but ones that could potentially be useful for MEOR. In addition, coupling high throughput investigations with thorough documentation of the geochemical conditions in oil reservoirs will allow for detailed statistical analysis correlating presence and/or abundance of specific groups of microorganisms to specific environmental conditions, and provide a means to manipulate the appropriate microbial activity.

The utilization of group specific primers to provide an in-depth view of the microbial community is a commonly used approach but rarely applied to oil reservoirs. Lineage specific 16S rRNA gene-based primers are either currently available, or could readily be designed (e.g., using Greengenes database or ARB software package, (DeSantis *et al.*, 2006; Ludwig *et al.*, 2004)). Also, primers targeting genes other than the 16S rRNA could be used to study the diversity within specific metabolic groups, for example, *dsr* gene for the sulfate reducers (Wagner *et al.*, 1998), *mcrA* gene for methanogens (Luton *et al.*, 2002), *soxB* genes for sulfide oxidizers (Petri *et al.*, 2001). The higher level of resolution obtained by using group specific primers usually results in identifying microorganisms that escaped detection by bacterial and archaeal 16S rRNA gene clone libraries.

A novel, powerful tool for microbial community analysis is the high-density oligonucleotide 16S rRNA gene-based microarray (phylochip) (DeSantis *et al.*, 2007). This microchip was developed by scientists in

Lawrence Berkley National Laboratory and uses the constantly updated global 16S rRNA gene database Greeengenes (DeSantis *et al.*, 2006; Wilson *et al.*, 2002) to design high-density microarrays that could theoretically detect all phylogenetic lineages available in Greengenes. The most recent phylochip, to be introduced in 2008 will contain ~30,000 probes derived from 16S rRNA genes of pure cultures as well as uncultured sequences currently available (Todd DeSantis, personal communication). The phylochip achieves higher sensitivity than small-sized clone libraries (e.g., less than 1000 clones) as recent validation studies have clearly demonstrated (Brodie *et al.*, 2006, 2007).

2. Monitoring changes in oil reservoir community composition in response to manipulations

Another promising area of research is to identify changes in community structure that occur during various stages of production or in response to specific manipulations, for example, water flooding, injection of nutrients, and/or exogenous microorganisms. Various molecular typing approaches, for example, differential or temperature gradient gel electrophoresis (DGGE or TGGE), internal transcribed spacer (ITS) amplification, and restriction fragment length polymorphism (RFLP) that have routinely been used in microbial ecology, but rarely in oil reservoirs, could be used to monitor changes in microbial community composition. In addition, comparing microbial communities using the previously described phylochip could be a powerful tool for monitoring changes in communities. More specific source tracking goals, for example, monitoring the survivability of an injected exogenous microorganism, or monitoring genes expression *in situ* could be achieved with quantitative or real time PCR.

3. Metagenomic analysis of oil-reservoir communities

Metagenomics (direct random sequencing of environmental DNA fragments from the environment) represents another tool that could potentially be extremely powerful in oil-reservoir community studies. Metagenomic analysis of a specific oil reservoir, or comparative genomics between reservoirs with different geochemical properties could provide important insights into the reservoir's ecology. For example, metagenomics could identify and compare relative abundance and diversity of genes involved in specific processes in an ecosystem (Venter *et al.*, 2004), document the occurrence or the potential of occurrence of processes not yet encountered in the ecosystem (Hallam *et al.*, 2004), identify novel functions in microorganisms not previously known to mediate these processes (Beja *et al.*, 2000), or provide diagnostic genes associated with an ecosystem in general, or with specific processes occurring within this system in particular (Tringe *et al.*, 2005).

VIII. CONCLUSIONS

Oil reservoirs are home to phylogenetically and metabolically diverse microbial communities. Our understanding of the phylogenetic diversity, metabolic capabilities, ecological roles, and community dynamics of oil reservoir microbial communities is far from complete. The lack of a complete consensus of the number of species or phylotypes prevents the statistical estimation of species richness, evenness, or diversity patterns needed to understand how the community changes over time and responds to different exploitation practices (water flooding, etc.). Even simple questions such as whether an organism is autochthonous or allochthonous are difficult to answer without a more complete description of the microbial ecology of the reservoir. The lack of appreciation of the microbiology of oil reservoirs often leads to detrimental consequences such as souring or plugging. However, an understanding of the microbiology can be used to enhance operations. It is clear that biotechnology can also be used to mobilize entrapped oil in reservoirs. Laboratory and field studies clearly show that (1) nitrate and/or nitrite addition control H_2S production, (2) oxygen injection stimulates hydrocarbon metabolizers that feed methanogenic communities to make methane and help mobilize crude oil, (3) injection of fermentative bacteria and carbohydrates generates large amounts of acids, gases, and solvents that increase oil recovery, particularly in carbonate formations, and oil production rates of individual wells, and (4) nutrient injection stimulates microbial growth preferentially in high permeability zones and improves volumetric sweep efficiency and oil recovery. Other work shows that biosurfactants significanly lower the interfacial tension between oil and water and that large amounts of biosurfactant can be made *in situ*. However, there have not been enough field trials of biosurfactant-mediated oil recovery to determine the efficacy of the process. There are still many questions that need to be resolved particularly whether an inoculum is needed or not. Metagemonic and high-throughput community analysis should be able to provide an answer to this and other questions concerning the metabolic capabilities of the microbial community.

Many of the commerical microbial technologies have been shown to slow the rate of decline in oil production and extend the operational life of marginal oil fields. With marginal oil fields, the goal is to keep the well producing rather than maximizing the ultimate amount of oil recovered from the reservoir. The risk for implementing MEOR is low in marginal fields as these fields are near the end of their economic lives. The data needed to assess performance, for example, oil production rates and operating costs, are relatively easy to obtain. With larger, more productive oil fields, increasing the ultimate recovery factor is the goal, but this

requires more extensive analysis of the reservoir and mathematical models to predict the outcomes of treatments. Many companies simply do not have the microbiogical expertise to obtain the information needed to make these assessments. Microbial oil recovery processes will only gain more widespread acceptance and application when quantitative measures of performance can be reliably obtained. Given the future demand for energy and the likely dependence on petroleum resources to meet this demand, petroleum engineering and microbiology disciplines must come together to develop the needed technologies.

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