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Potential applications of bioprocess technology in petroleum industry

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Abstract Petroleum refining is traditionally based on the use of physicochemical processes such as distillation and chemical catalysis that operate under high temperatures and pressures conditions, which are energy intensive and costly. Biotechnology has become an important tool for providing new approaches in petroleum industry during oil production, refining and processing as well as managing environmentally safe pollutant remediation and disposal practices. Earlier biotechnology applications in the petroleum industry were limited to microbial enhanced oil recovery, applications of bioremediation to contaminated marine shorelines, soils and sludges. The potential role of bioprocess technology in this industry has now expanded further into the areas of biorefining and upgrading of fuels, production of fine chemicals, control of souring during production and air VOC biofiltration. In this paper we provide an overview of the major applications of bioprocesses and technology development in the petroleum industry both in upstream and downstream areas and highlight future challenges and opportunities.

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Introduction

Biotechnology, the use of living organisms to produce or modify desirable products for specific applications, has become an important tool for providing new approaches in agriculture, food, pharmaceutical and environment industries (Gavrilescu and Chisti 2005; Singh 2010). Biological catalysts operate in a wide range of ambient temperature and pressure and are endowed with a high selectivity, resulting in reduced energy costs, low emissions and minimal generation of undesirable by-products (Le Borgne and Quintero 2003; Singh et al. 2006; Sen 2008). Because of these unique characteristics, biotechnology has become an attractive alternative or complementary to the development of new petroleum refining processes as well as managing environmentally safe remediation and disposal practices during the last two decades (Fig. 1).

Crude oil is a complex mixture of organic compounds including some organometallic constituents complexing vanadium and nickel (Salanitro 2001; Van Hamme et al. 2003; Scullion 2006). Petroleum refining is traditionally based on the use of physicochemical processes. However, application of microorganisms

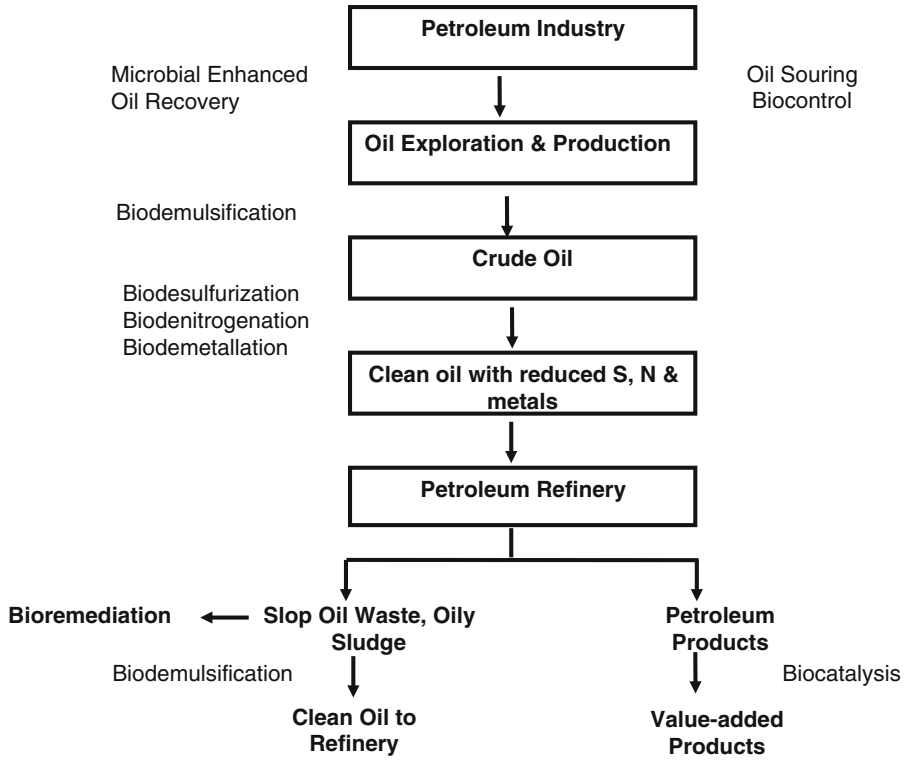

Fig. 1 Biorefining and microbial enhanced oil recovery approaches in petroleum industry

and their enzymes in petroleum biorefining has also been extensively studied at the laboratory and pilot scale. Examples are biodesulfurization, biodenitrogenation, biodemetallation, biodemulsification and microbial enhanced oil recovery (MEOR), biological control of reservoir souring, and transformation of heavy crude to light crude (Singh et al. 2006; Sen 2008; Huang et al. 2010). It appears that in the distant future, the use of biotechnology could also be extended to other areas of petroleum refining, such as hydrocarbon cracking, isomerization, polymerization or alkylation by biological catalysts (Alvarez and Cervantes 2011). Although there are practical challenges in commercialization of these bioprocesses, significant progress have been made in this area (Kilbane 2006; Ward et al. 2009; Voordouw 2011). All the operations in the petroleum industries like exploration and production of oil, transportation, refining, refined product handling and oily waste management activities are potential sources of water, soil and air pollution (Caliman et al. 2011). Crude oil spills that occur during transportation, and refinery wastes, pose serious threats to the environment. Of various technologies available for cleaning of hydrocarbon contaminations, biological remediation methods using microbes and plants are considered more environmentally friendly and less expensive (Glick 2010).

In this paper, we have provided an overview of the recent advances in understanding and development of bioprocess technology related to petroleum production, refining and remediation, and also highlighted constraints associated with field applications and approaches to overcome those challenges.

Biorefining and upgrading petroleum oil

Crude oil contains about 0.05–5.0 % sulfur, 0.5–2.1 % nitrogen and heavy metals such as Ni and V associated with the asphaltene fraction. High temperature- and pressure-requiring expensive hydrotreatment processes are generally used to remove sulfur and nitrogen compounds from petroleum. Current refining processes are able to reduce the sulfur content of diesel fuels to less than 50 ppm. However, lower sulfur standards are being enforced in some jurisdictions and

future values for diesel fuel are expected to be <10 ppm in the US and Europe (Kilbane 2006). Availability of advanced molecular techniques has facilitated exploration and engineering of improved biocatalysts (both microbes and enzymes) suitable for the oil biorefining and recovery processes (Brown 2010; Gieg et al. 2011; Ward et al. 2012). Since conventional technologies cannot cost effectively achieve the target, a combination of biodesulfurization (BDS) and hydrodesulfurization (HDS) technology has the potential to achieve the future goals. The focus of this section is the biorefining processes to improve oil quality that have gained significant interest in the last two decades (Le Borgne and Quintero 2003; Kilbane 2006).

Biodesulfurization

The removal of sulfur from crude oil requires costly and extreme conditions using processes such as HDS because most of the sulfur is contained in condensed thiophenes in crude oil. Major microbial species known for BDS activity belong to genera *Agrobacterium*, *Gordona*, *Klebsiella*, *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Paenibacillus* and *Xanthomonas* that are capable of selective desulfurization of organic sulfur (Table 1). The sequence of catabolism of dibenzothiophene (DBT) by the most studied bacteria *Rhodococcus* is mediated by two monooxygenases and a desulfinase, with the result of successive production of dibenzothiophene-5-oxide (DBTO), dibenzene-5,5-dioxide ($DBTO_2$), 2-(2-hydroxybiphenyl)-benzenesulfinate (HPBS) and 2-hydroxybiphenyl (HBP) with associated release of inorganic sulfur (Gupta et al. 2005; Nandi 2010). Desulfurization (dsz) genes have been manipulated by gene shuffling and directed evolution to broaden substrate specificity including DBT and benzothiophene (BT) and improved biocatalysts have been engineered (Kilbane 2006). Application of extracellular peroxidases has also been proposed as attractive biocatalysts for desulfurization for petroleum processes (Ayala et al. 2007). Peroxidase treatment combined with a distillation step reduced the sulfur content of diesel fuel from 1.6 to 0.27 % in a reaction system of an aqueous medium with a low percentage of water-miscible organic solvent.

Biodesulfurization in two phase aqueous-alkane solvent systems exhibited increased sulfur removal rates as compared with aqueous systems (Caro et al. 2008). The extent of biodesulfurization has been shown to vary from 20 to 90 % with the nature of the oil feedstock. The key techno-economic challenge to the viability of biodesulfurization processes is to establish cost effective means of implementing the two phase bioreactor system and de-emulsification steps as well as the product recovery step (Soleimani et al. 2007; Yang et al. 2007).

Since the feedstock and the biocatalysts are required to be suspended in the aqueous phase to maintain continuous contact with each other in a bioreactor, different types of loop bioreactors have been proposed to avoid mechanical stirring and shear damage of microbial cells (Yazdian et al. 2010). Use of multiple stage air-lift reactors can reduce mixing costs, overcome poor reaction kinetics, and achieve greater oxygen utilization, continuous growth and regeneration of the biocatalyst in the same system rather than in a separate reactor (Nandi 2010; Irani et al. 2011). Furthermore, use of nanotechnology (enzymes/microbes encapsulated in nanomaterial) may overcome this challenge as the ability of encapsulated enzymes/microbes to withstand mechanical stress is well documented (Singh 2010).

Biodenitrogenation

Nitrogenous compounds in crude oil consist of pyrroles, indoles and carbazole. Carbazole is not only a toxic and mutagenic compound, but also a potent inhibitor of hydrodesulfurization process, can denature petroleum cracking catalysts and has deleterious environmental impacts by contributing to the formation of air polluting NO_x.

Some species of *Alcaligenes*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Comamonas*, *Mycobacterium*, *Pseudomonas*, *Serratia* and *Xanthomonas* can utilize indole, pyridine, quinoline and carbazole compounds (Table 1). Pyrrole and indole are easily degradable, but carbazole is relatively resistant to microbial attack (Bai et al. 2010). Biofilm-immobilized *Burkholderia* sp. IMP5GC in a packed reactor has demonstrated good activity for the semi-continuous degradation of carbazole present in a mixture of gas oil and light cycle oil (Castorena et al. 2008). The genes responsible for carbazole degradation by *Pseudomonas* sp. CA10 have been identified and cloned to generate recombinant strains that were able to transform a wide range of

Table 1 Applications of biotechnology in petroleum industry

Biotechnology	Biocatalyst	Application
Biodesulfurization	Species of *Agrobacterium*, *Arthrobacter*, *Corynebacterium*, *Gordona*, *Nocardia*, *Rhodococcus*	Biotransformation of organic sulfur compounds, selective removal of sulfur in crude oil or refined petroleum products
Biodenitrogenation	Species of *Comamonas*, *Nocardioides*, *Pseudomonas*, *Rhodococcus*	Biotransformation of organic nitrogen compounds in crude oil, nitrogen removal
Biodemetallation	Species of *Bacillus megaterium*, *Caldariomyces fumago*, *Escherichia coli*	Enzymatic removal of Ni and V from crude oil using chloroperoxidase, cytochrome C reductase and heme oxygenase
Biocatalysis	Microbial enzymes; cytochrome p450-dependent-monooxygenases; dioxygenases, lipoxygenases; peroxidases	Bioconversion/biotransformation of petroleum hydrocarbons to produce fine chemicals
Biotransformation/ biodegradation	Species of *Thiobacillus*, *Achromobacter*, *Pseudomonas*, *Sulfolobus*	Biotransformation of heavy crude into light crude
Biodemulsification	Species of *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Nocardia*, *Pseudomonas aeruginosa*, *Rhodococcus*	De-emulsification of oil emulsions, oil solubilization, viscosity reduction, wetting
Microbial enhanced oil recovery (MEOR)	Bacteria, biosurfactants, biopolymers, solvents, organic acids, gases	Tertiary oil recovery technique employing microbes or their metabolic products to mobilize residual oil to enhance crude oil recover from the wells
Biosouring biocontrol	Nitrate addition to induce nitrate-reducing bacteria (NRB)	Nitrate induces growth of heterotrophic NRB and sulfide-oxidizing NRB, thus inhibiting sulfidogenesis to shift the sulfate-reducing bacteria (SRB) community away from sulfide production
Bioremediation	Bioreactor, engineered biopile, landfarming, bioventing, biosparging, monitored natural attenuation	In situ or ex situ method for biodegradation of contaminants enhanced by bioaugmentation and biostimulation
Phytoremediation	Plants and bacteria	Decontamination by plants and plant growth promoting rhizobacterial interactions through extraction, degradation or stabilization
Biofiltration	Biofilters containing VOC (e.g. benzene, toluene, ethylbenzene, xylenes etc.) degrading microbes	Mobile VOC-contaminated gas stream together with air passes through a stationary porous medium phase which supports microbial growth in the form of a biofilm

aromatic compounds including carbazole, *N*-methylcarbazole, *N*-ethylcarbazole, dibenzofuran, dibenzothiophene, dibenzo-*p*-dioxin, fluorene, naphthalene, phenanthrene, anthracene, and fluoranthene (Fetzner 1998). *Pseudomonas* sp. degrades carbazole to anthranilate and 2-hydroxypenta-2,4-dienoate through angular dioxygenation, meta-cleavage, and hydrolysis (Zhao et al. 2011). The enzyme carbazole 1,9-dioxygenase, which participates in the angular dioxygenation and cleaves one of the two carbon–nitrogen bonds, is composed of terminal oxygenase (CarAa), ferredoxin (CarAc), and ferredoxin reductase (CarAd). A potential pathway for the selective removal of nitrogen from carbazole could be created using metabolic engineering to combine the CarA enzyme from carbazole degraders such as *Sphingomonas* sp. GTIN11 with a suitable deaminase (Kilbane et al. 2002).

From a practical perspective denitrogenation and desulfurization processes need to be integrated. An effective biodenitrogenation and biodesulfurization process requires removal of sulfur and nitrogen through specific enzymatic attack of the C–N and C–S bonds, respectively, but without C–C bond attack, thereby preserving the fuel value of the residual products. *Gordonia* sp. strain F.5.25.8 was the first reported strain that has the ability to simultaneously metabolize DBT and carbazole (Santos et al. 2005), however, its efficacy under industrial setting yet to be tested.

Biodemetallation

Asphaltenes are high molecular weight compounds containing aromatic and aliphatic constituents, heteroatoms and heavy metals. The complex molecular structure contains sulfur (0.3–10.3 %), oxygen (0.3–4.8 %), nitrogen (0.6–3.3 %), and metal elements, such as Fe, Ni, and V at a trace level. Although microorganisms have been shown to associate with bitumen and asphaltene, only some fractions are susceptible to enzymatic or microbial attack. However, some hemeproteins such as chloroperoxidase, cytochrome c peroxidase, cytochrome c reductase and lignin peroxidase from *Caldariomyces fumag*, *Bacillus megaterium*, *Catharanthus roseus*, and *Escherichia coli* can perform biocatalytic modifications of the asphaltene fraction for removal of Ni and V from petroporphyrins and asphaltenes (Mogollon et al. 1998; Garcia-Arellano et al. 2004). It has been suggested that the biocatalyst interacts with heavy oil at the heteroatom and organometallic sites redistributing and fragmenting the heavy polar fractions into lighter fractions, thus facilitation removal of Ni and V. The enzymatic treatment with chloroperoxidase has been demonstrated to remove up to 93 % of Ni from nickel octaethylporphine, 53 % of V from vanadyl octaethylporphine and 20 % of the total Ni and V from the asphaltene fraction of heavy oil (Fedorak et al. 1993).

Metal-containing fossil fuels that can be treated with enzymes include crude petroleum oil, distillate fractions, coal-derived liquid shale, bitumens, gilsonite, tars and synthetic fuels derived from them. While cytochrome c reductase and chloroperoxidase enzymes have potential applications in metal removal from petroleum, further investigations on the biochemical mechanisms and bioprocessing aspects are required for development of a commercially feasible biodemetallation process.

Biocatalysis for novel compounds

The unique regio- and stereo-specificity properties of enzymes and their capacities to catalyze reactions in non-aqueous media can be exploited in systems for biotransformation of petroleum compounds into novel high-value chemicals. Enantiospecific conversions of petrochemical substrates and their derivatives can be achieved by stereoselective biocatalytic hydroxylation reactions using cytochrome p450-dependent monooxygenases, dioxygenases, lipoxygenases and peroxidases (Kikuchi et al. 1999). A range of diol precursors for chemical synthesis can be produced by naphthalene dioxygenase (NDO) which also catalyzes a variety of other reactions including monohydroxylation, desaturation, *O*- and *N*-dealkylation and sulfoxidation (Resnick et al. 1996). These approaches may be extended to create powerful biocatalysts with applications for specific transformations for upgrading of petroleum fractions through applications of molecular/protein engineering methods in expanding substrate specificity, enhancing enzyme stability in non-aqueous media and manipulating reaction rates (Cherry 2000).

Novel biocatalysts have been obtained from metagenomic libraries that has increased our capabilities in adapting the biocatalysts to specific reactions (Singh 2010) and process improvements by rational and random mutagenesis further broadens the scope for application of biotechnology in the fine chemical industry. Cytochrome $P450_{cam}$ monooxygenase from *P. putida* has been successfully evolved to function more efficiently in the hydroxylation of naphthalene and dioxygenases with improved thermostability and substrate specificity (Furukawa 2000). Similarly improved cytochrome P450 mutants have been described with enhanced activity towards benzene, styrene, 1-hexane and propane, direct conversion of ethane to ethanol, hydroxylation of octanoic acid, *n*-octane, ionone, naphthalene and anthracene, oxidation of terminal alkanes to either (R-) or (S-) epoxides, and increased total activities by using air as oxidant in whole cell bioconversions of propane to propanol under mild conditions (Kumar 2010).

Biotransformation of heavy crudes

The progressive depletion of high-quality light crudes has led to investigations on biochemical conversion of heavy crudes into lighter crudes utilizing extremophile bacterial species of *Thiobacillus*, *Achromobacter*, *Pseudomonas* and *Sulfolobus* (Premuzic and Lin 1999). The strategy was to adapt these bacteria to resist high temperatures, pressures, and salt and hydrocarbon concentrations, followed by introducing them into the crudes in aqueous solution and incubated at temperatures of 50–65 °C. The treated crudes became lighter and contained about 24–40 % less sulfur, nitrogen, oxygen and heavy metals. Although

the exact mechanism was not known, the microorganisms might have specifically directed their action to the heteroatoms and organometallic structures involving oxidation and degradation of higher hydrocarbons, shortening of paraffin chains, depolymerization of asphaltenes, increasing the solvent fraction and allowing the liberation of smaller molecules. The increase in the concentration of saturated chains from C8 to C26 indicated the degradation of high molecular weight hydrocarbons, probably alkanes. More research is needed to elucidate the metabolic pathways responsible for these transformations at molecular level and to further improve the activity of these strains (Le Borgne and Quintero 2003). Biocatalytic cracking, also known as biocracking, is probably one of the most interesting future biotechnology target for heavy oil upgrading (Ayala et al. 2007). The possibility of asphaltene cracking by a biocatalytic process is a potential alternative to the energy intensive and inefficient physicochemical processes.

Biodemulsification

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

Since biological processes can be carried out at non-extreme temperature and pressure conditions, an effective microbial de-emulsification process could be used directly to treat emulsions at the wellhead, thus saving on transport and high capital equipment costs.

Several microorganisms are known to possess de-emulsification properties (Table 1), e.g. *Nocardia amarae*, *Corynebacterium petrophilum*, *Rhodococcus auranticus*, *Bacillus subtilis*, *Micrococcus* sp., *Torulopsis bombicola*, *Acinetobacter calcoaceticus*, species of *Alteromonas*, *Rhodococcus*, *Aeromonas*, *Brevibacillus*, *Dietzia*, *Ochrobactrum*, *Pusillimonas*, *Sphingopyxis*, *Achromobacter*, and mixed bacterial cultures (Kosaric 1996; Das 2001; Nadarajah et al. 2002a; Huang et al. 2009; Huang et al. 2010).

Microbes exploit hydrophobic cell surfaces and some biologically produced chemicals such as acetoin, polysaccharides, glycolipids, glycoproteins, phospholipids and rhamnolipids, to displace the emulsifiers that are present at the oil–water interface (Das 2001; Singh et al. 2007). The mixed bacterial culture used in biodemulsification studies of Nadarajah et al. (2002a, b) had two cell populations distributed at different phases of the inoculum, an upper non-pelletable hydrocarbon-associated cell population (floating cells) and a lower pelletable population (pelleted cells). Floating cells consistently provided higher de-emulsification activity than the pelleted cells. Due to their hydrophobic nature floating cells might influence the de-emulsification activity by interacting at the oil–water interface. The dual hydrophobic/hydrophilic nature of biosurfactants decreases the interfacial tension resulting in removal of thin liquid film from the surface of dispersed droplets to cause coalescence of droplets and phase separation (Wen et al. 2010; Liu et al. 2011).

Cell surface hydrophobicity (CSH) of bacteria plays a significant role in nonspecific adsorption to all kinds of biological or non-biological surfaces and interfaces and the degradation of hydrophobic organic materials in addition to bacterial migration and adsorption at the oil–water interface. Bacterial surfaces contain proteins and peptides, polysaccharides, lipids and lipopolysaccharides. Chang et al. (2009) have reported that the CSH of *Rhodococcus* sp. was enhanced via accumulation of fatty acids on the cell surface to adhere to the oil phase. The above observations recently led Huang et al. (2012) to further investigate the CSH of the demulsifying bacterial strain of *Alcaligenes* sp. S-XJ-1 grown on different vegetable oils as carbon sources to obtain various surface properties. Better performance was achieved with demulsifying bacteria S-XJ-1 possessing a relatively high (CSH and total unsaturated degree for the cell-wall bound fatty acids mainly composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). C18:1 and C18:2 had a positive effect on the formation of CSH, while C18:0 and C18:3 had the opposite effect.

A range of oil emulsions obtained from different oil companies have been tested using mixed bacterial culture with proven demulsifying ability (Nadarajah et al. 2002b). The mixed culture grown on crude and motor oil could cause separation of oil from water and solids in various oilfield emulsions within 24–96 h. The initial demulsification rate varied significantly among the various emulsions tested possibly due to the variation in the composition and viscosity of the emulsions and the nature of the emulsifier. Generally, emulsions with higher water content were easier to break than the ones with lower water content. Due to variability in the properties of oilfield crude oil emulsions, inconsistencies have been experienced in performance of the different demulsification processes. Further research on biodemulsification processes with field emulsions needs to be aimed at development of more reliable and universally effective systems. Genetic manipulations on biodemulsifying organisms have not been attempted so far.

Microbially enhanced oil recovery

Enhanced oil recovery (EOR) is used to recover residual oil from the wells. Residual oil is often located in areas inaccessible to fluids used for flooding, or the oil is adhered to sand or carbonate particles in the reservoir making it difficult to recover further and usually more than two-thirds of the oil in the reservoir is left unrecovered after primary and secondary extraction (Brown 2010). Sometimes high oil viscosity may also result in incomplete recovery. Conventional EOR methods make use of chemicals (solvents, polymers, surfactants), injected gases (CO_2, N_2, flue gas), and thermal methods (steam flood, hot water) to extract remaining oil (Sen 2008).

Microbially enhanced oil recovery (MEOR) is considered as a tertiary oil recovery technique that relies on microorganisms or their metabolic products to mobilize residual oil (Khire 2010). Both conventional and MEOR methods are applied to improve the mobility of oil through decreasing oil viscosity, dissolution of carbonates in the reservoir, physically displacing oil, and plugging of highly permeable areas in the reservoir to increase the sweep efficiency of water flooding. MEOR methods are considered more economical and environmentally friendly compared to conventional EOR (Lazar et al. 2007; Zhou et al. 2008).

Mechanisms involved in MEOR include production of bioproducts (biosurfactants and biopolymers), gases (CO_2 and CH_4) biofilm growth and microbial plugging (Youssef et al. 2009; Gao and Zekri 2011; Kaster et al. 2012). The residual oil is held in porous rocks by capillary pressure, which is proportional to the interfacial tension (IFT) between oil and water. Biosurfactants reduce the oil–water IFT to enhance the potential for flow of residual oil. Certain bacteria can generate biopolymers that plug the high-permeability zones with large pores, thus forcing injected water to sweep the oil in low permeability zones. Bacterial biofilms can grow on the surface of the porous rock, which may lead to removal of paraffin deposits, change of surface properties and a decrease in permeability to enhance oil recovery. Carbon dioxide and methane produced by bacteria can dissolve in crude oil to reduce oil viscosity and increase the reservoir pressure to improve the mobility of oil.

MEOR technology requires consideration of reservoir properties in terms of salinity, pH, temperature, pressure and nutrient availability (Sen 2008; Khire 2010). Many petroleum reservoirs have high NaCl concentrations and high temperature, and require use of microbes which can tolerate these conditions. Since molds, yeasts, algae and protozoa are not suitable due to their size or inability to grow under the conditions present in reservoirs only bacteria are considered promising candidates for MEOR. Potentially useful MEOR thermophilic isolates have been described and extremely thermophilic anaerobes growing at 80–110 °C have been isolated and cultured in the laboratory (Van Hamme et al. 2003).

Field application of MEOR has been tested in various oilfields in USA, China, Malaysia, and Argentina with some success (Gao and Zekri 2011). More than 400 MEOR field tests with some success have been conducted in the United States alone, mostly as single-well stimulation treatment on low productivity wells. The reservoir heterogeneity significantly affects oil recovery efficiency. Microbial flooding process is generally used where bacteria and nutrients are injected into a reservoir, and a normal water flooding operation is then resumed. While being transported inside the reservoir, bacteria produce bioproducts or plug the high permeability zones that improve the recovery of oil. However, most of the successful MEOR treatments were conducted for formations with a low temperature (below 55 °C),

low water salinity (less than 100,000 ppm), high water cut (above 75 %), and low production rate. In these field studies around 15,000–70,000 bbl of additional oil recovered using MEOR techniques was reported (Gao and Zekri 2011). Among the proposed MEOR mechanisms in field studies, mainly selective plugging and biosurfactant production are believed to be the main contributors to better recovery. Although no clear relationship between the success of MEOR projects and reservoir permeability could be established, reduction in oil viscosity, IFT and paraffin content were observed in the above field studies. Although there has been only limited number of studies on using MEOR for oil extraction from oil sands, MEOR strategies involving biosurfactants-producing bacteria has potential for use in oil recovery from oil sands, especially processes focusing on decreasing the viscosity of oil and reducing the interfacial tension between oil and water interfaces (Harner et al. 2011).

MEOR methods have been actively pursued both in laboratory and field conditions. More than 70 % of the low temperature oilfield wells treated by bacteria achieved increases in oil production rate. Despite numerous MEOR tests, considerable uncertainty remains regarding process performance due to reservoir heterogeneity. Insuring success requires an ability to manipulate environmental conditions to promote growth and/or product formation by the participating microorganisms. Opportunities for MEOR in enhanced gas production might be limited as no field case reported a significant increase in gas production.

Biological souring and biocontrol

Both non-biological and biological mechanisms are responsible for souring in oil reservoirs or oilfield systems. While non-biological mechanisms include the thermal decomposition of sulfur-containing hydrocarbons, thermochemical sulfate reduction and pyrite dissolution, biological souring occurs when sulfate-reducing bacteria (SRB) enzymatically reduce sulfate, thiosulfate, or elemental sulfur to sulfide to gain energy for growth (Liamleam and Annachhatre 2007; Agrawal et al. 2010). Souring negatively impacts the petroleum industry in several ways including higher oil production costs, lower value petroleum products, enhanced corrosion, reservoir plugging by iron sulfide precipitation, odor complaints, and by lowering the air quality leading to worker health and safety issues (Tang et al. 2009; Williamson 2011).

Biological souring is commonly the consequence of secondary oil recovery, during water-flooding operations by injecting water (or seawater) down hole to re-pressurize the reservoir after natural pressure is lost during primary recovery and to sweep the oil towards production wells (Barton and Fauque 2009). However, some biological souring may occur during primary oil production process from the reservoir. SRB populations in reservoir ecosystems use available sulfate resulting in increased sulfide concentrations in the associated water, oil, and gas and eventually in sour oil and natural gas. Souring can readily occur in low salinity (<6 %) low-temperature (<45 °C) and high temperature (45–80 °C) reservoirs. However, SRB activity can be naturally constrained by high salinities (>10 %) and temperatures (>80 °C) in reservoirs (Wilhelms et al. 2001; Voordouw et al. 2011).

Biological souring requires the simultaneous presence of viable bacteria, sulfate, carbon and energy sources, nutrients, and suitable temperature. Many of these components can be present in the water used for secondary recovery, thus the water source can play a key role in souring. Readily metabolized carbon sources volatile fatty acids (acetate, butyrate and propionate) and labile hydrocarbons such as alkanes and monoaromatics (toluene) are frequently present in the injection water used for water flooding, thus providing carbon sources for SRBs (Grigoryan and Voordouw 2008). Diverse thermophilic SRB genera have been detected in reservoir fluids such as species of *Desulfovibrio*, *Desulfobotulus*, *Desulfohalobium*, *Desulfonema*, *Desulfotalea*, *Desulfomonas*, *Desulfonatronum*, *Desulforhopalus*, *Thermodesulfovibrio* and *Desulfomicrobium* by culture-dependent or 16S rRNA gene sequencing (Grigoryan et al. 2008; Bødtker et al. 2009; Wei et al. 2010).

Microbial souring is controlled by applying mechanical and/or chemical treatments (Voordouw 2011). Early prevention of souring can be achieved by using injection water that is naturally low in sulfate concentration, VFAs, and biomass. Alternatively sulfate, thiosulfate and sulfite are removed from injection water at a significant cost using reverse osmosis or membrane filtration (Robinson et al. 2010). Broad-spectrum biocides such as glutaraldehyde, tetrakis (hydroxymethyl) phosphoniumsulfate (THPS), benzalkonium chloride,

formaldehyde, sodium hypochlorite, and cocodiamines are often used to inhibit SRB communities (Kaur et al. 2009). However, biocides are generally expensive and require repeated application to be effective and their continued use can lead to the development of biocide-resistant microbial populations.

Seawater containing nitrate has been effective in preventing souring in fields with temperatures above 60 °C (Sunde and Torsvik 2005). The use of nitrate may have many benefits over biocide application. Nitrate induces growth of heterotrophic nitrate-reducing (NRB) and sulfide-oxidizing NRB, thus inhibiting sulfidogenesis to shift the SRB community away from sulfide production. The NRBs compete for the same oil organics as the SRB and production of nitrite by NRB can strongly inhibit the growth of SRB (Bødtker et al. 2009). Compared to a non-treated field, produced waters from a nitrate-treated field had heterotrophic NRB of the genus *Deferribacter* and lower SRB activity at 60 °C (Gittel et al. 2009). In Enermark field (Alberta, Canada) with low resident temperature (30 °C), injection of low sulfate concentration water and nitrate to control souring, decreased sulfide production by 70 % within the first 5–7 weeks (Voordouw et al. 2008). Pulse injection of 760 mM nitrate for 1 h/week in an injection well from the Enermark field caused complete removal of sulfide from a neighboring production well. Injected nitrate was reduced to nitrite and then to N_2 (95 %) or ammonium (5 %) inducing anammox activity ($NH_4^+ + NO2 \rightarrow N_2 + 2H_2O$) and utilizing ammonium to increase NRB biomass (Cornish-Shartau et al. 2010).

Although capital costs for chemical injection are relatively low, injected water volumes and chemical requirements increase over time. More work and better understanding is needed to define long-term dosage requirements of nitrate/nitrite addition to make a cost effective treatment process. Better nitrate injection strategies, possibly in combination with selective biocides, and reservoir simulation tools need to be developed to predict the success of various treatment options and better control of microbial souring.

Biodegradation and bioremediation

The majority of molecules in crude oil and refined products are biodegradable and oil-degrading microorganisms are ubiquitous (Van Hamme et al. 2003). Biodegradation of complex hydrocarbons usually requires the cooperation of more than a single species because the individual microorganism can metabolize only a limited range of hydrocarbon substrates (Ward et al. 2003). Mixed populations with overall broad metabolic capabilities are generally required to increase the rate and extent of petroleum degradation. Some members of the microbial community secrete important degradative enzymes, growth factors, whereas others may produce biosurfactants leading to the enhanced solubilization of hydrophobic hydrocarbons for their better utilization (Van Hamme et al. 2003; Juhasz et al. 2005; Singh et al. 2007).

Soil and sludge bioremediation

The fate of hydrocarbons in soil is governed by various factors related to the soil environment as well as properties and chemical characteristics of the compound (Husain 2008). Polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, chrysene, phenanthrene, benzo(a)pyrene and others, and BTEX compounds (benzene, toluene, ethyl benzene and xylene isomers) have been listed as priority pollutants in several countries including in the USA. Bioavailability and biodegradation of PAHs and BTEX compounds is affected by the distribution and partitioning of the contaminants in soil particles and ageing of the contaminated soil (Singh et al. 2009).

In the natural environment, hydrocarbons are biodegraded by a diverse group of microbes including bacteria, yeast and filamentous fungi, algae, cyanobacteria and some protozoan organisms and that are widely distributed in nature (Van Hamme et al. 2003). Among the most common and efficient hydrocarbon degrading bacteria in both soil and marine environments are species of *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Achromobacter*, *Bacillus*, *Flavobacterium*, *Nocardia*, and *Pseudomonas;* among the yeasts are *Aureobasidium*, *Candida*, *Rhodotorula and Sporobolomgers* (Singh and Ward 2009).

Among saturated hydrocarbons, *n*-alkanes are generally considered to be readily degradable components of crude oil. Based on the chain length, *n*-alkanes are divided into three groups: lower alkanes (C_8–C_{16}), medium alkanes (C_{17}–C_{28}), and higher alkanes, (>C_{28}). Most of the microorganisms have been reported to show satisfactory growth and faster degradation of *n*-alkanes up to C_{16} carbon chain length mainly due to increased solubility and reduced

surface tension. The resins and asphaltene are generally considered to be recalcitrant to biodegradation and the metabolic pathways for the degradation of these fractions are less understood (Van Hamme et al. 2003). However, Tavassoli et al. (2012) have recently reported isolation and kinetics of asphaltene biodegradation by bacterial strains of *Pseudomonas* and *Bacillus* capable of degrading up to 48 % of asphaltene in single or mixed culture.

Types of bioremediation

For bioremediation of petroleum hydrocarbon-contaminated sludge and soil, landfarming composting and biopiling processes have been traditionally used. In landfarming, contaminated soil or sludge placed on a piece of dedicated land, augmented with nutrients, and periodically tilled and irrigated to stimulate the natural microbial population that degrades the contaminants over a long period of time, usually 3–24 months (Ward and Singh 2004). Long treatment times in landfarming methods are generally due to the lack of control of parameters affecting microbial activity such as moisture, oxygen, temperature, pH and mixing. Composting involves mixing of contaminated soil with organic materials such as straw, wood chips, and sewage sludge to improve C:N ratio, and placing the mixture in piles or windrows to support growth of hydrocarbon degrading microorganisms. In an engineered biopile technology system aeration is provided through a network of air distribution pipes and a leachate collection system is used for further treatment.

Landfarming and biopiling are cost-effective and proven methods of treating readily biodegradable petroleum contaminants in the soil where the treatment system is designed in such a way that the transfer of pollutants to other environmental media is minimized or prevented (Salanitro 2001). However, it is difficult to reduce hydrocarbon concentration by more than 80–85 % in contaminated soil, particularly persistent hydrocarbons like high molecular weight polycyclic aromatic hydrocarbons, which may not be adequate to meet regulatory standards in some countries. There is also evidence that significant amount of hydrocarbons (15–60 %) including semi-volatile 4- and 5-ring PAHs can be volatilized instead of biodegraded (Salanitro 2001; Hejazi and Husain 2004).

Bioreactors designed for accelerated hydrocarbon degradation provides greater control of operational parameters compared to biopile or landfarm processes (Van Hamme et al. 2003). Continuous aeration and mixing facilitates desorption of hydrocarbons from soil to the liquid phase and provides sufficient oxygen for microbes to enhance the biodegradation rate. In contained bioreactors, optimized operational parameters such as pH, temperature, oxygen, moisture, mixing, and bioavailability of nutrients promote desirable microbial growth and hydrocarbon degrading activity (Ward et al. 2003).

Approaches to enhance efficacy of microbial bioremediation

Bioremediation of oily sludge-contaminated soil in the presence of a bacterial consortium, inorganic nutrients, compost and a bulking agents, like wheat bran, coconut, charcoal, cellulose, straw, soybean hulls, saw dust, wood ash, oat, poultry litter and surfactants showed degradation in the range of 60–80 % of total oil (Vasudevan and Rajaram 2001; Rahman et al. 2002). A variety of food-grade organic substrates such as vegetable oil, sucrose esters of fatty acids and whey were found to support bioremediation processes (Pannu et al. 2003; Borden and Rodriguez 2006; Yap et al. 2010; Jonsson and Östberg 2011; Taccari et al. 2012). The combined use of mature compost and of a selected microbial consortium is a useful strategy for improving total petroleum hydrocarbon removal, achieving a high degradation (96 %) at the end of the bioremediation process (Taccari et al. 2012).

Treatment of contaminated soil may also involve biostimulation (addition of nutrients and oxygen to stimulate the indigenous microbial population) and bioaugmentation (inoculation of enriched mixed bacterial consortia) or a combination of both processes (Xu and Lub 2010; Singh et al. 2011). Understanding how remediation methods influence the diversity of the soil microbial community and providing appropriately designed nutrients based on the nature of the contaminant, soil structure and chemistry, better insight into the behavior and function of microbial populations and maximum benefits of bioremediation technology can be achieved (Singh and Ward 2009; Ros et al. 2010; Tyagi et al. 2011).

Weathering, sorption, evaporation or volatilization, leaching and photo-oxidation processes may cause the

removal of certain hydrocarbon compounds during bioremediation resulting in overestimation of the extent of biodegradation (Salanitro 2001; Mphekgo et al. 2004). Hydrocarbon biodegradation can occur over a wide pH and temperature range. The optimum pH for petroleum bioremediation in soil ranges from 6.0 to 8.0. The biodegradation rate generally increases from the psychrophilic to mesophilic temperatures. The optimum temperature for biodegradation has been reported in the range of 25–40 °C (Van Hamme et al. 2003). Temperature influences hydrocarbon biodegradation by affecting the physical state and chemical composition of oil as well as the metabolic activities and composition of the microbial community. Oil sludge biodegradation was found optimal at C:N and C:P ratios of 60:1 and 800:1, respectively. To maintain metabolic activities of microbial cells, the oxygen supply rate must match the overall oxygen consumption rate (Huesemann 1995). Appropriate aeration is provided by a network of air spargers in biopiles and optimum mixing and aeration in bioslurry reactors (Ward et al. 2003). Reduced bioavailability of hydrocarbons can limit biodegradation, particularly in aged soils that have been contaminated for many years and during final stages of a soil bioremediation treatment processes (Singh et al. 2009). Mass transfer of hydrocarbons into microbial cells is a significant determinant of biodegradation rates and extents. Hydrocarbon bioavailability and subsequent degradation can be improved by addition of chemical surfactants and biosurfactants. Biosurfactants are generally more environmentally friendly alternative to synthetic surfactants because of their lower toxicity and higher biodegradability (Mukherjee et al. 2007; Franzetti et al. 2010). Full-scale application of biosurfactant products in bioremediation may be economically prohibitive due to the high cost for their production. Therefore, the production of biosurfactants in situ by the identification and the selective stimulation of autochthonous biosurfactant-producing bacteria would be a better strategy rather than separate bioproduction and augmentation (Singh et al. 2006).

Successful soil bioremediation depends on numerous environmental, nutritional and operational factors. Since it is unlikely that all contaminants would be removed from a contaminated soil even under optimal conditions, the effectiveness of a biological process depends on the success in identifying the rate-limiting factors and optimizing them in order to achieve maximum treatment benefits. Inadequately designed systems are likely to fail to achieve the required local regulatory treatment criteria. A number of potential hydrocarbon-degrading strains have been isolated and characterized using advanced molecular techniques in the last two decades and further increase in our understanding of the ecology of hydrocarbon-degrading microbial communities, nature of contaminants, soil chemistry and engineering design of the appropriate treatment system will help in developing practical soil bioremediation strategies.

Phytoremediation of petroleum contaminations

Phytoremediation methods involve some specific plants and their rhizospheric microorganisms by either providing favorable conditions for contaminant degradation by plant root colonizing microbes, or accessing contaminants through the plant roots (Krämer 2005; Macek et al. 2008). Decaying biomass and plant root exudates provide nutrients and stimulate co-metabolic transformations of organic contaminants. Soil phytoremediation methods include phytostabilization—stabilization of inorganic pollutants in the soil to make them less bioavailable; phytovolatilization—volatilization of the contaminant to the atmosphere in its original or modified form after its uptake by the plant; phytostimulation—stimulation of growth and activity of rhizospheric bacteria to enhance contaminant degradation by the root exudates from the plant; phytotransformation—transformation of contaminants into less toxic forms within the plant; and phytoextraction—extraction and concentration of soil contaminants in their above-ground tissues for further drying, incinerating and disposing to a landfill.

Microbial-assisted phytoremediation technology has gained attention during the last decade with much better understanding of the contribution of microorganisms in phytoremediation and the efficacy of these approaches in removal of petroleum products and PAHs (Doty 2008; Al-Awadhi et al. 2009; Gerhardt et al. 2009; Glick 2010). Phytoremediation over a 2 year period decreased the total PHC concentration by 30 %, which was double that of non-vegetated soils at a highly contaminated site (Siciliano et al. 2003). A field study conducted on a site contaminated by a crude oil spill showed a 42–50 % decrease in total petroleum hydrocarbon concentration after 21 months using ryegrass, St. Augustine grass (Nedunuri et al.

2000). Gerhardt et al. (2009) have reported many advantages of phytoremediation including the cost effectiveness of the technology. The estimated cost of conventional ex-situ remediation methods such as excavation, soil washing, incineration or in situ stabilization are generally much more expensive ($200–1,500/ton) than in situ rhizoremediation ($10–50/ton), due to minimal maintenance cost associated with phytoremediation technologies.

Two main constraints to phytoremediation are (1) lack of technology to simultaneous remove organic and inorganic pollutants (2) buildup of organic pollutants inside the plant tissues, which slows down the growth of plants and the rate of remediation (Singh 2011). To overcome these challenges, further improvement in technology has been made. For example, degrading microbes were introduced inside the plant tissues (Singh 2011; Abhilash et al. 2012). This technology utilizes capability of plant to remove inorganic pollutants with soil/rhizospheric/endophytic microbes and to completely degrade organic pollutants. To increase economic viability, phytoremediation technology may also be applied for multi-purpose products. For example, use of bioenergy plants (such as poplar, willow) in remediation can provide biomass for energy generation and biochar for improved soil health and carbon sequestration (Abhilash et al. 2012).

Deep sea and shoreline oil bioremediation

While many oil spills occur at sea each year, the high profile explosion at the Deepwater Horizon drilling rig in April 2010, spilling almost five million barrels of crude oil into the waters of the Gulf of Mexico, was viewed by many as one of the world's largest environmental disasters. Nevertheless, that disaster also revealed the astonishing power of oil degrading microbes to participate in the clean-up of this huge oil spill as well as the effectiveness of the bioremediation strategy which was employed (Ward et al. 2012). About 7,600 m^3 of the biodegradable surfactant-containing dispersants, Corexit 9500 and 9527 from Nalco, promoted emulsification of the oil. As a result, in just 3 weeks after the damaged oil well was eventually capped, the US government concluded 50 % of the oil was already gone. Although microbial biodegradation appeared to be the dominant process, volatilization of lighter hydrocarbon fractions cannot be ruled out. Twenty-four bacterial strains from 14 genera were isolated from the hydrocarbon-contaminated Pensacola Beach in Florida (Kostka et al. 2011). Strains were predominantly from the *Gammaproteobacteria* and *Alphaproteobacteria*, and included some well-established oil-degrading genera such as *Alcanivorax*, *Marinobacter*, *Pseudomonas and Acinetobacter* and from the family *Rhodobacteraceae*.

Twenty years after the Alaskan shoreline was contaminated as a result of the running aground of the Exxon Valdez tanker, some residual oil still remained on the local beaches. While bioaugmentation of petroleum-degrading microbial inoculum were mostly ineffective in the oil bioremediation process, biostimulation through addition of oleophilic fertilizers facilitated colonization of the oil by biodegrading microbes, and effected substantial bioremediation of the contaminated coastline in a 2 year period (Atlas and Hazen 2011).

Air biofiltration processes

Petroleum contamination is not confined to solid and aqueous media, but also occurs in the atmosphere. Because a significant fraction of crude oils as well as a more substantial proportion of refined petroleum products, including petrochemicals, is made up of volatile organic carbons (VOCs), air streams become contaminated not only due to oil spills but also during processes for production and refining of petroleum. Benzene, toluene, ethylbenzene and xylenes isomers are some of the most dominant VOCs in petroleum. While emissions of these toxic contaminants are frequently controlled by physical separation and/or destruction methods, microbes which can biodegrade these components are widely distributed in the environment.

In a biofilter, the VOC-contaminated gas stream together with air or oxygen represents the mobile phase, which passes through a stationary porous medium phase which typically supports microbial growth in the form of a biofilm (Singh and Ward 2005; Mudliar et al. 2010). The contaminants and oxygen move from the gas stream to the biofilm populated with the VOC-degrading microbes. Biofilter media may consist of a variety of natural constituents including wood bark or chips, peat, compost and/or synthetic media including ceramics, plastics or other biofilm support components. Biofilters are designed with humidified gas streams to insure there is sufficient water for microbial growth and activity to

biodegrade the pollutant and decontaminate the gas stream (Yang et al. 2010).

Conclusions and future perspective

In recent years, we have seen some significant advances in biotechnology including biomolecular, metabolic and protein engineering developments, which will undoubtedly result in creation of powerful biocatalysts for applications in enhanced oil recovery from petroleum reservoirs, biodemulsification of oilfield emulsions and slop oils, bioremediation of contaminated sites, biorefining and upgrading of crude oil and petroleum fractions, and specific biotransformation of pure hydrocarbon compounds into fine chemicals. These advances will help us address the heterogeneity issues of many sites that will require specifically-tailored strategies and custom-designed technologies. All emerging technologies including bioremediation not only need to be environmental friendly but are also required to be economically viable. Emerging metagenomics, transgenic and nanotechnological approaches may revolutionize this area of science but their application is limited by the high cost of monitoring, maintenance and strict regulatory requirements. The multi-purpose remediation technology approach can revolutionize the industry as it will bring economic, environmental and social benefits to all stakeholders; however further improvement is required to make the remediation cost effective, less labour intensive and more efficient (Abhilash et al. 2012). In order to harness the real potential of environmental microbes in petroleum industries, the use of a multi-disciplinary approach combining conventional microbiology, genomics, and nanotechnology, along with soil sciences, chemistry and engineering design is needed. This can be achieved by employing a concerted and sustained effort from all stakeholders (researchers, industries and regulatory agencies) which in turn, may generate new biotechnological products in the near future.

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