Application of Biotechnology in Oil and Gas Industries

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Abstract

Biotechnology has wide application in medical, agriculture, bioremediation, non-renewable energy production, and food production. Nowadays, environment friendly biotechnological approaches are also popular in petroleum industry. Conventional approaches are high in cost, involve complex implementation process and environmental hazard, while biotechnological approaches are environment friendly. In this chapter, we discuss about biotechnological approaches used by oil and gas industries for resolving production related problems and also for enhancement of production. In oil fields microbial souring, corrosion, and sulfur content in crude oil are major problems faced during or after the production. Another is, production of trapped oil through various enhanced oil recovery processes, in which microbial enhanced oil recovery process has shown great potential. At last, oil sands and shale gas production and its related problems are also been discussed.

6.1 Introduction of Oil Production System

Modernization and technological evolution have developed this era at an exponential phase. With the advent of modern tools and equipment's, the focus on tapping renewable energy potential is the center of every scientific development. However, the non-renewable fossil fuels are still a major requirement of the present world. This global energy demand is presently met by the conventional and non-conventional oil reserves such as onshore, offshore oilfields and oil sands. Currently, the discovery of

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new oil reservoirs along with the development of improved crude oil recovery methods has led to optimal production of crude oil in a sustainable way.

Typically, the recovery of crude oil from these oil reserves proceeds as three different processes: primary, secondary, and tertiary oil recovery. During the primary recovery process, the existence of natural geological pressure is sufficient to produce oil from beneath the earth surface. This pressure leads to the recovery of about 10–20% of original oil in place. However, with time this natural geological pressure gradually decreases and therefore results in reducing percentage of oil been recovered. The secondary oil recovery process is then implemented at this point to re-pressurize the reservoir bottom-hole by water injection. Injection of water in the injection well sweeps the oil along the flow path leading to recovery of crude oil from the production well. About 40–50% of the residual oil in place is recovered during this process (Gieg et al. 2011).

Secondary flooding process is also limited for a period of time. With continuous injection of water, channels are formed along the flow path, termed as *viscous fingering*, which further lowers the efficiency of the recovery process. Moreover, much of the oil still remains in the reservoir, as the water to oil ratio increases in the production fluid and thus leads to unprofitable production of oil. Recovery of the rest percentage of crude oil involves implementation of different methods to recover residual oil based on reservoir nature. These recovery processes are termed as tertiary oil production or enhanced oil recovery (EOR) processes and are typically adopted after secondary flooding. The EOR process is of different types, such as chemical, thermal, miscible gas, and microbial based process. The chemical EOR process (CEOR) involves injection of solvents, surfactant, and mostly polymers such as xanthan gum and partially hydrolyzed polyacrylamide to improve the sweep efficiency of the drive fluid. Thermal recovery process such as SAGD (steam assisted gravity drainage) involves the injection of steam to lower oil viscosity and especially applied during heavy oil recovery. Microbial enhanced oil recovery (MEOR) is further implemented as a tertiary process which is recognized as a greener and sustainable way of oil recovery process. In MEOR, microbial activity is utilized for producing crude oil. Microbes are known to use oil organics for production of biosurfactant, bioemulsifier, biopolymers, various acids and gases. These microbial products aids in the oil recovery process by improving the efficiency of the drive fluid (Gbadamosi et al. 2019).

Additionally, two different types of oil fields are present throughout the world, i.e., onshore and offshore oil fields. Onshore oil fields are those fields discovered on the land, while offshore oil fields are found in the sea. Both these oil fields constitute similar oil production systems that are composed of two types of facilities. The downhole facilities include injection and production well components, while above ground facilities comprise injection pipelines, production pipelines, and processing terminal facilities. Water during the secondary oil recovery process passes from the processing terminal to the injection well, and then is injected downhole which leads to its recovery from the production well. The produced water then passes through the three-phase separator and finally to the processing terminal where oil is separated. This whole secondary process, therefore, requires large amounts of injection water.

This large quantity is generally drawn from nearby water aquifers (e.g., sea, river, and lake or ponds) and sewage treatment plants. Besides, the onshore oil fields adopt a sustainable process, produced water re-injection (PWRI), wherein the produced water after oil separation is reused for re-injection in oil field. The offshore oil fields, on the other hand, have access to large sums of sea water, and, therefore, do not require PWRI strategies (Prajapat et al. 2019).

6.2 Microbiology of Oil and Gas Industry

Microbes are ubiquitous in nature and are present in a variety of extreme environments. However, earlier beliefs added oil and gas industries as an exception, till the nineteenth century, when the microbiology of oil reservoirs was introduced (Bastin et al. 1926). It was found that the oil reservoir heterogeneous nature itself provides different extreme environments such as high pressure, temperature, salinity, and depth barriers for the growth of organisms. There are varied microbial communities thriving in different oil reservoir zones. Some of these communities are sulfate reducing bacteria (SRB), fermentative bacteria, nitrate reducing bacteria (NRB), iron reducing bacteria (IRB), iron oxidizing bacteria (IOB), and methanogens. These bacterial groups are known to perform their metabolism based on different hydrocarbon sources. Hydrocarbons or oil organics present in the reservoir act as energy sources for these microorganisms with inorganic molecules as an electron acceptor.

Among the different microbial communities, SRB are known to be the dominant species present in the oil fields (Voordouw et al. 1996). Many studies have also stated, they are indigenous to the oil reservoir (Stetter et al. 1987), however, controversies exist whether SRB are indigenous to oil fields or are been introduced with injection water. Though, the existence of similarities between the oil field and hydrothermal SRB population shows the mobility/transportation of SRB communities between two geological distinct environments (Stetter et al. 1987; Voordouw et al. 1996). SRB communities are further known to belong to both eubacterial and archeal lineages. Besides SRB population two different types of NRB are present in the oil reservoir, i.e., heterotrophic nitrate reducing bacteria (hNRB) and sulfide oxidizing nitrate reducing bacteria (so-NRB). The hNRB are known to perform dissimilatory heterotrophic nitrate reduction (into N_2 or ammonia) with the oxidation of oil organics, while so-NRB reduce nitrate into N_2 or ammonia with the oxidation of sulfide into elemental sulfur or sulfate. The NRB abundance is privileged when the nitrate is injected in the reservoir in order to control microbial souring.

The iron reducing bacteria (IRB) and oxidizing bacteria (IOB) are yet other dominant bacterial groups present in various oil fields setup. Iron reducing bacteria uses ferric iron as a terminal electron acceptor, while iron oxidizing bacteria are found to oxidize the ferrous ions. Additionally, IOB are known to oxidize the Fe under different set of conditions, e.g., acidic, neutral pH, phototrophically, anaerobically, autotrophically, and heterotrophically (Ionescu et al. 2015).

The methanogens are strict anaerobic archaea that produce methane during anaerobic respiration. Methanogens are found in deeper regions of oil reservoirs, utilizing the acetate, one carbon compounds, and H_2 and CO_2 (Prajapat et al. 2019). Generally, methanogens are present with syntrophs (an obligate proton producing bacteria).

6.2.1 Sulfate Reducing Bacteria

Sulfate reducing bacteria (SRB) are morphologically diverse and anaerobic organisms obtaining energy by dissimilatory reduction of sulfate to sulfide (Bernardez and De Andrade Lima 2015). The SRB and sulfate reducing archaea (SRA) are together known as sulfate reducing prokaryotes (SRP). During the sulfate reduction process, these SRP communities oxidize the organic compounds (oil components in the oil reservoir) and reduce sulfate into hydrogen sulfide (H_2S). SRP are also found to have the ability to utilize other sulfur compounds such as sulfite, dithionite, thiosulfate, trithionate, elemental sulfur, and polysulfides.

The sulfate reduction processes are of two types, assimilatory sulfate reduction and dissimilatory sulfate reduction. During assimilatory sulfate reduction, the final product of sulfate reduction is used for the biosynthesis of sulfur containing biomolecules, while dissimilatory sulfate reduction produces H_2S which is released in the surrounding environments. Dissimilatory sulfate reduction is a three step process: In the initial step, sulfate is reduced into adenosine-5′-phosphosulfate (APS) with the help of the ATP sulfurylase enzyme. Then, APS is converted into sulfite and adenosine monophosphate (AMP) with the help of APS reductase enzyme. During the last step, produced sulfite is further converted into H_2S with the help of dissimilatory bisulfite reductase (Dsr) enzyme (Prajapat et al. 2019).

SRP communities are known to be phylogenetically distributed variably along the oil fields. Such as, the presence of SRB depends upon the oil field origin, oil composition, downhole temperature, pH of oil–water mixture, depth of the reservoir, stage of oil production, and salinity of produced water. The variance of SRP communities also differs worldwide in different oil fields. The general SRB communities present in varies oil field setup are *Desulfotomaculum, Desulfosporosinus, Thermodesulfobacterium, Thermodesulfovibrio, Thermodesulfobium, Thermotoga, Thermodesulfohabdus, Desulfacinium, Desulfobulbus, Desulfocapsa, Desulfofustis, Desulforhapalus, Desulfobotulus, Desulfotignum, Desulfobacter, Desulfobulbus*, and *Desulfovibrio* (Prajapat et al. 2019). While SRA present in various oil fields are *Archaeoglobus fulgidus, Archaeoglobus profundus, Archaeoglobus veneficus,* and *Caldivirga maquilingensis* (Prajapat et al. 2019).

Moreover, beside there divergence the SRP population are widely distributed among the oil field facilities. These microbes thrive in-between the oil and water phases. They mostly inhabit in the injection, production, and crude oil processing facilities. As these SRP population grow in different environments, they showed varied types of physiology. The SRP present in the oilfields are mesophilic, thermophilic, halotolerant, and halophilic in nature. They are the causes for corrosion of pumping facilities and storage tanks, lower quality of oil products, e.g., sulfur content and a number of limiting factors.

6.2.2 Iron Reducing and Oxidizing Bacteria

Iron reducing bacteria (IRB) reduce the ferric iron (Fe^{3+}) into ferrous (Fe^{2+}) iron with the oxidation of organic substrates. This reduction of ferric can be coupled with the fermentation process. Besides, the ferric iron acts as a terminal electron acceptor, and with other terminal electrons can be used by the IRB (e.g., sulfate). Moreover, as iron is insoluble in nature; therefore, during the iron reduction process, membrane bound ferric reductase enzyme is present in the IRB (Lovley 1993). Additionally, the assimilation of iron after reduction is not possible in IRB. Also, the phylogeny of IRB is closely related to SRB phylogeny as some IRB have the capabilities to reduce sulfur. However, in the presence of H_2, IRB outcompete SRB and methanogens (Fredrickson and Gorby 1996). Examples of IRB are *Geobacter, Shewanella, Desulfuromonas,* and *Pelobacter*.

Iron oxidizing bacteria (IOB), on the other hand, oxidize the ferrous iron (Fe^{2+}) into ferric iron. These types of bacteria are generally microaerophilic in nature. They are also found to be autotrophic, heterotrophic, and mixotrophic in nature (Bridge and Johnson 1998). Examples of some IOB species are *Sulfobacillus thermosulfidooxidans, Sulfobacillus acidophilus, Acidimicrobium, Gallionella ferruginea, Leptothrix, Sideroxydans, Mariprofundus ferrooxydans,* and *Ferritrophicum radicicola*. One hypothesis also concludes that IOB indirectly involve microbial influenced corrosion by deoxygenation and lead to SRB growth. Thus, these communities are actively involved in corrosion (Emerson et al. 2010).

6.2.3 Nitrate Reducing Bacteria

Nitrate reducing bacteria (NRB) use nitrate as a terminal electron acceptor and contribute in the nitrogen cycle. Generally, NRB are heterotrophic in nature and can be facultative anaerobes in nature, sometimes. Also, few of the NRB species can grow autotrophically with the oxidation of sulfur and iron containing compounds, and use carbon dioxide or bicarbonate as the carbon source (Matějů et al. 1992). These different types of their metabolism switch according to the surrounding environmental conditions. NRB respire nitrate in the limiting oxygen condition through the membrane bound nitrate reductase enzyme. Fewer NRB species can also oxidize the sulfide into sulfate or sulfur elements, and are known as sulfide oxidizing nitrate reducing bacteria (so-NRB). In the oil fields, NRB activity is induced when the nitrate is injected as a strategy for controlling microbial souring. During injection of nitrate, NRB outcompete the sulfate reducing bacteria for similar energy source (oil components). Besides, the so-NRB activity also increases during this process and decreases sulfide concentration in the production fluids by sulfide oxidation. Some of the NRBs are also capable of incomplete reduction of nitrate under stress conditions such as temperature, high nitrate concentration in environment which leads to conversion of nitrate to nitrite only. The nitrite so produced is a strong inhibitor of the Dsr enzyme of SRB and therefore limits the growth of SRB population. Besides, nitrate reduction occurs at a high redox potential which also limits SRB growth with increasing redox potential.

6.2.4 Methanogens

Methanogens are strictly anaerobic archaea which produce methane as a by-product during metabolism. These organisms can be present in any part of the sediments when the sulfate is depleted. Methanogens typically use methanol, methylamines, methyl sulfate, formate, H_2 + CO_2 or CO and acetate as a carbon and energy source (Schlegel and Müller 2011). They are of two types: acetoclastic methanogen and hydrogenotrophic methanogen. Acetoclastic methanogens use acetate, while hydrogenotrophs use hydrogen and CO_2. These communities are present in swamps, digestive systems of animals, marine sediments, hot springs, hydrothermal vents, and oil fields.

Methanogens belong to the phylum Euryarchaeota in the archaea domain. They are further classified into seven orders: Methanococcales, Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanopyrales, Methanocellales, and Methanomassiliicoccales (Enzmann et al. 2018). In Methanosarcinales order acetoclastic methanogens are present, while in order Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanopyrales hydrogenotrophic methanogens are present. Methylotrophic methanogens use methylated compounds like methanol, methylamines, or methylated thiols. These are present in the order Methanomassiliicoccales, Methanobacteriales, and Methanosarcinales.

6.2.5 Acid Producing and Fermenting Bacteria

Acid producing bacteria (APB) produce the acids (e.g., sulfuric acids) during their metabolic activities. This produced acid causes corrosion in the oil field installations. These are fermentative bacteria known to gain energy from the substrate level phosphorylation. These bacterial population use a wide range of organic compounds like sugars, peptides, amino acids, and organic acids. Many reports have also shown APB to use inorganic sulfur compound, ferric ions, and nitrate as a terminal electron acceptor. Besides, fermentative bacteria can grow at wide ranges of salinity and temperature conditions. Mesophilic, saccharolytic fermentative *Halanaerobium* uses disaccharide and monosaccharide in fermentative pathways (Ollivier and Magot 2005). Fermentative bacteria in oil fields are present in separators and filters where extensive involves in biodegradation of oil. Thermophilic fermentative bacteria are also present in oil fields and belong to the order Thermotogales (Prajapat et al. 2019).

6.3 Oil Field Souring

Biogenesis of hydrogen sulfide (H_2S) by SRB (known as a souring) has a deleterious effect on the human which could lead to death after inhalation. This anthropogenic H_2S generation is not only dominated in the oil industry but also found in paper and pulp industry, rayon textile production, chemical manufacture, and waste disposal. Annual cost estimated $90 billion associated with the microbially produced H_2S in reservoir and fluids (Gieg et al. 2011). While, injection of water during the secondary recovery is found to be mostly responsible for souring in oil fields. The probable reason for such an effect could be the sulfate content in the injection water used by oil industries. Water sources used for secondary recovery are seawater, municipal waste water, and the saline aquifer that contain moderate to high sulfate concentration (5–30 mM). In offshore oilfields the seawater are injected which contain high sulfate concentration of 25–30 mM (Voordouw and Grigoryan 2009). Sulfate containing water injected in an oil reservoir is conducive for SRB activity in the reservoir matrix and leads to conversion of sulfate to sulfide. While, injection water also act as a source of both inoculum and electron acceptor. Therefore, both indigenous and adventitious populations thrive in reservoir rock and are responsible for reservoir souring. Also, during the secondary oil recovery process, water-flooding decreases the temperature of the reservoir and also dilutes the harmful petroleum fluids. These further creates a zone at the near injection wellbore region (NIWR), making it a suitable condition for boosting microbial growth.

Additionally, in the reservoir, more labile electron donors are present in the form of simple organic acids (acetate, propionate, butyrate, etc.) at high concentrations as much as 1500 mgL^{-1} (Vance and Thrasher 2005). Besides utilizing these electron donors, SRB are also capable to utilize diverse aliphatic and aromatic hydrocarbon (Anderson and Lovley 2000; Annweiler et al. 2000; Abu Laban et al. 2009). Therefore reservoir conditions provide a limitless carbon and energy supply to activate microbial population. The composition or quality of injection water is a key factor to the extent of SRB activity in situ. Produced water re-injection (PWRI) strategy also provides the soluble oil organics like volatile fatty acids (VFA). VFAs include acetate, butyrate, and propionate and other organic, for example, lactate. VFAs are completely oxidized by SRB in CO_2 and incompletely oxidized into acetate that is excreted into the environment (Widdel and Rabus 2001).

The large quantities of H_2S generated by SRB cause a variety of problems (Larsen 2002), such as contamination of crude oil, metal corrosion, and the precipitation of metal sulfides. The souring in production facilities and the reservoir leads to additional costs associated with the prevention of operators exposure to toxic H_2S, reduced oil–water separator performance, management of iron sulfide solids, and accumulation of iron sulfide deposits that are responsible for the enhancement of equipment corrosion and fouling of equipment corrosion (Vance and Thrasher 2005). Sulfide production has been broadly classified to have three negative impacts; firstly, the toxicity of sulfide as a concern to the worker and public health. Secondly, sulfide production decreases the oil quality, and, therefore, sulfur content must be lowered by processing facilities. Lastly, sulfide is corrosive in nature, which causes leaks or ruptures in pipelines; increases the potential of environment damage in a remote location and negatively impacts the facility integrity. Also, the sulfide production can arise abiotically by thermo-geochemical processes, but water injection induces the souring significantly by increased SRB activity. The distinguishing character between this biological and non-biological sulfide production can be identified by isotope signature analysis of the ratio of ^{34}S to ^{32}S (Frazer and Bolling 1991).

6.3.1 Souring Control by Traditional Methods

Prevention of souring can be done by choosing suitable make-up water sources without sulfate, but this is generally not possible as oil companies have to depend on the nearby water source. Additionally, as a prevention measure the sulfate contained in the injection water can be physically removed by nanomembrane filtration or by reverse osmosis (Robinson et al. 2010). This protective control technology has earlier been adopted by some of the North Sea oilfield platforms (McElhiney and Davis 2002; Odgen et al. 2008). However, the drawback of this technology is the high cost of installation and maintenance of the filtration system. Therefore, commonly used methods are the application of a chemical to terminate sulfate reduction or removal of sulfide from injection water. Chemical sulfide scavenger involves triazines, sodium hydroxide, aldehyde, metal oxides, and nitrite (Vance and Thrasher 2005). These chemical treatment removes sulfide from the downstream operation, but are unable to shortfalls of inhibiting sulfide production at the NIWR. The biocide injection process is regularly followed in the oil industry. Regular treatment of injection and produced water inhibits a broad spectrum of the attack on the particular microbial community. However, repeated dosing of biocide develops the biocide resistance community and therefore alternate injection strategies are proposed (Telang et al. 1998).

Depending on their function these industrial biocides are broadly divided into two categories: oxidizing and non-oxidizing biocide. Oxidizing biocides are ozone and chlorine; these biocides are reactive with microbes, organic matter, solids, and pipeline material. Non-oxidizing biocides are tetrakis hydroxymethyl phosphonium sulfate (THPS), glutaraldehyde (Glut), acrolein, bronopol, and quaternary ammonium salts (Fig. 6.1).

Glut is the second most used biocide in oilfields after the THPS. The use of Glut has found to develop resistance in microbes; therefore, alternation of dosage of biocide has been used to prevent such occurrence (Gieg et al. 2011). Two reactive terminal aldehyde group of Glut cross link amino and sulfhydryl group of proteins and nucleic acids. Quaternary ammonium compounds such as benzalkonium chloride (BAC) work as surfactant, reacting with the cell membrane and rupturing it. THPS is a biodegradable and less toxic compound (Downward et al. 1997) and also dissolves ferrous sulfide precipitation. The mechanism of THPS action is still unknown. Bronopol is an alcoholic compound and inactivates the sulfhydryl group containing protein via the free radical of electron deficient bromine atom (Legin

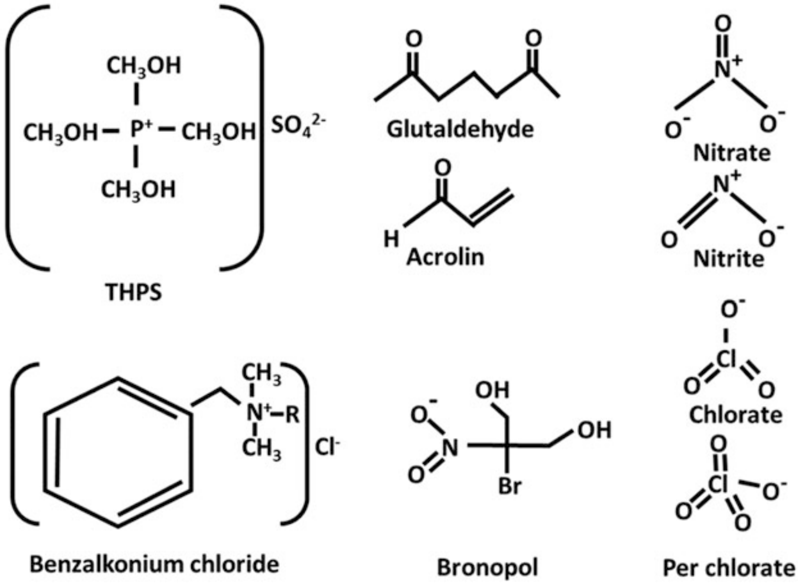

Fig. 6.1 Structure of different types of biocides used in the oil industry

1996). Bronopol is soluble in water but not in a hydrocarbon solvent. This is also tested in laboratory and field study of the oil industry (Tischler et al. 2010). Acrolein is a three carbon unsaturated aldehyde and high chemical reactivity. It has biocidal activity with the activity of scavenging sulfide and also dissolves iron sulfide precipitation. All the biocides have been well studied and different injection schemes have been studied by various researchers to predict the efficiency of various biocides in controlling sulfide production (Penkala et al. 2006).

6.3.2 Souring Control by Nitrate Treatment

From the past many years, nitrate injection is used as a “green” technology to control microbial souring in sewage treatment facilities. Though, the first oil field trials of nitrate injection occurred in the 1990s (Jenneman et al. 1999). Successful application was later reported for both offshore and onshore oil operations (Dinning et al. 2005; Sturman et al. 1999; Thorstenson et al. 2002; Larsen et al. 2004; Kuijvenhoven et al. 2007; Bødtker et al. 2008; Voordouw and Grigoryan 2009). Few of the benefits of using nitrate are, it is cheaper and does not harm the workers and environment. Also, nitrate effectively penetrates the SRB biofilms (Gardner and Stewart 2002) and controls biosouring by different mechanisms. High redox potential of nitrate than sulfate, favors bio-competitive exclusive growth of heterotrophic NRB (hNRB) that outcompetes SRB for the same electron donors (VFA or oil organics) (Hubert et al. 2005; Sunde and Torsvik 2005). Alternatively, nitrate allows the growth of sulfide oxidizing nitrate reducing bacteria (so-NRB) which oxidize sulfide into sulfate coupled with nitrate reduction. Hence, the sulfate reduction into sulfide by SRB and re-oxidation into sulfate by so-NRB are complementary metabolic cycles.

The reduction of nitrate by hNRB and so-NRB forms metabolic intermediate, i.e., nitrite which further inhibits the SRB growth. Since the structure of nitrite acts as a sulfide analog, it therefore easily binds with dissimilatory sulfite reductase (Dsr) enzyme, which is responsible for sulfate reduction in SRB. This interaction further inhibits the Dsr enzyme thereby limiting the sulfide production. Also, excess accumulation of nitrite can chemically oxidize the sulfide into sulfur, polysulfide, and sulfate (Sanders and Sturman 2005; Kaster et al. 2007; Lin et al. 2009). In such reaction, nitrite is also reduced in N_2 and NH_4^+. Besides, an increase in redox potential by conversion of nitrite to NO and N_2O also inhibits the SRB growth (from −400 mV to +100 mV) (Nemati et al. 2001; Hubert et al. 2009). Moreover, limiting nitrate concentrations during nitrite reduction continuously leads to end products N_2 and NH_4^+ formation (Hubert and Judd 2010). NRBs are known to catalyze the reduction of nitrate via two main pathways in the presence of carbon and energy sources. Conversion of ammonium by dissimilatory nitrate reduction or de-nitrification of nitrogen results in the reduction of nitrate. In the oil reservoir, both these processes of nitrate reduction have been observed (Gevertz et al. 2000; Hubert and Voordouw 2007).

The use of nitrate for controlling souring has different advantages. In general, the biocide treatment has its efficacy against all the resident microbial population present in the reservoir. However, nitrate is known to target SRB specifically and thus can have an added advantage over biocides. Though nitrate can also have some negative effects such as accumulation of biomass leads to pipeline bio-fouling and downstream corrosion due to the change in oxidizing potential (Martin 2008). Dissimilatory nitrate reduction present in the facultative organism also utilizes nitrate as an alternative to aerobic or fermentative growth. Some of the SRB are known to upregulate the nitrite reductase (nrf) gene which detoxifies nitrite to ammonium whereby down-regulating Dsr gene (Haveman et al. 2004). This strategy is not much common among SRB, though nrf transiently inhibits the sulfate reduction by nitrite. Some SRB, for example, *Desulfovibrio gracilis* has the ability to reduce nitrate in the presence of high sulfate concentration.

In comparison to nitrate, nitrite acts as a good inhibitor because it directly inhibits the Dsr enzyme. Some successful field trials have also demonstrated the nitrite application as a biocide for the inhibition of microbial souring, e.g., New Mexico field (Sturman et al. 1999). However, nitrate is easier to handle and is less toxic than nitrite. Additional researches have also proposed the synergistic effect of biocide with nitrate injection. Nitrite and other biocide synergy have also been tested with *Desulfovibrio* sp. SRB consortium. Few reports have mentioned, injection of biocide with a nitrite combination or mixture of different biocides can inhibit the SRB community successfully (Greene et al. 2006).

6.3.3 Souring Control by Novel Biotechnological Approaches

By Injection of Bacteriophage for Killing the SRB Bacteriophage is a virus that infects the bacteria specifically. In order to control souring, these viruses can act as a good approach for bacteriocidal activity. Summer et al. (2017) patented method for prevention and remediation of reservoir souring and corrosion by treatment of virulent bacteriophage (Summer et al. 2017). Their approach was to control souring by specifically targeting the problematic bacteria by virulent lysogenic bacteriophages. These problematic microbes are sulfate reducing bacteria and acid producing bacteria that cause souring and corrosion in oil fields.

By Injection of (per)Chlorate Perchlorate and chlorate (combinedly known as a (per)chlorate) are new metabolic inhibitors that can be applied in the oil fields for souring control. (per)chlorate oxyanion is present in nature and anthropogenically synthesized due to its wide uses (Liebensteiner et al. 2014). Perchlorate (ClO_4^-) is a soluble anion that has a central chlorine atom and is surrounded by four oxygen atoms. To control souring (per)chlorate has the following effects: (1) Bio-competitive exclusion (2) Oxidation of sulfide, (3) Inhibition of sulfate reduction pathway (Okpala and Voordouw 2018).

6.4 Microbial Corrosion Control

Besides souring, corrosion is one of the major problems faced by the oil industries. Corrosion can be CO_2 mediated corrosion, oxygen corrosion, galvanic corrosion, crevice corrosion, erosion corrosion, and microbiological induced corrosion (MIC) (Popoola et al. 2013). Among all the different types of corrosion, microbiologically influenced corrosion occurs mostly in the oil fields. Microbiologically influenced corrosion occurs due to microbial activities. Microbial activities produce CO_2, H_2S, and organic acids and corrode the pipelines. The general principle for the working of corrosion inhibitors to inhibit corrosion is the formation of protective barriers to the metal/metal oxide through chemisorption, physisorption, complexation, or precipitation. Corrosion inhibitors either neutralize the action of corrosive substances or form protective films/stabilizing the pre-existing protective films. Therefore, they prevent the access of oxygen to the cathode, prevent the hydrogen diffusion from the cathode, or inhibit the dissolution of the metal. Inhibitory efficiency of corrosion inhibitors depends on the pH, temperature, duration, metal composition of pipeline, and the structural properties of inhibitor molecules (Lukovits et al. 2001). Corrosion inhibitors also slowdown the corrosion reaction. In the biological context, some corrosion inhibitors prevent the adhesion of microbes to metal surfaces (Videla and Herrera 2009).

6.5 Microbial Enhanced Oil Recovery (mEOR)

Microbial enhanced oil recovery is the biotechnological approach for the tertiary oil recovery process. This is applied by oil industries after the secondary oil recovery process or some times during secondary oil recovery. mEOR process involves use of microbes for enhancing residual oil production from the reservoir. In mEOR, microbes enhance oil recovery by producing gas (CO_2, CH_4, H_2, and N_2), low molecular weight acids, solvent, biosurfactant, polymers, and biomass production (Kaster et al. 2012). These microbial products contribute to the enhanced oil recovery process by different mechanisms. During the mEOR process, either microbial product (mEOR chemicals) may be injected in well or nutrients are added in the injection water for in situ microbial growth. Adding microbes or microbial products in an oil well is also known as bioaugmentation, while adding growth supplement for increasing in situ microbial growth is termed as biostimulation.

During the mEOR process, biosurfactants mobilize the oil by reducing the interfacial tension (IFT) between oil and water phases. Another mechanism is microbial growth on reservoir rocks and biofilm formation changes its rocks wettability. Further microbial growth in reservoir rocks leads to bioclogging of the water flow path. This bioclogging alters the water flow path and ultimately increases a reservoir sweep efficiency. Other than these mechanisms, microbes generate gases during metabolism (for example, methane and carbon dioxide) and these gases increase pore pressure. These gases also dissolve in the oil phase, therefore reduces the viscosity and swelling of the oil. Another mechanism is the biodegradation of the hydrocarbon. Microorganisms utilize the crude oil components as a carbon source and alter oil viscosity (Armstrong and Wildenschild 2012).

6.5.1 Conventional Methods of EOR

Conventional EOR methods are: thermal (steam injection, in situ combustion), chemical (surfactant, polymer, alkali and solvents injection), and miscible gas (CO_2) injection.

Thermal EOR (TEOR): This is a commercially available technique of EOR. In this process, steam is generated from water using natural gas. This steam is injected in the reservoir to heat heavy oil, for reducing its viscosity and also impact on thermal expansion and crude oil vaporization. This process allows the oil to flow easily. Other than using natural gas, alternatively solar energy is also used for steam generation. This TEOR process is unsuitable where thin pay zone and increased depth are present (Gbadamosi et al. 2019).

Chemical EOR (CEOR): CEOR enhances oil production by altering oil–water/oil–rock interactions in the reservoir. Chemical is injected in water slug that lowers the IFT between fluids (water–oil), alter the wettability of rock to increase oil permeability, and increment in the viscosity of injection fluid improves the mobility.

Traditional CEOR methods are polymer, surfactant, and alkaline flooding. In polymer flooding, polymer increases the viscosity of injected fluids and further increases mobility. In surfactant flooding, surfactant reduces the IFT of injected fluid, thus mobilization of residual oil improves oil production. In alkaline flooding, the pH of injected fluids is increased by the addition of alkaline agents (sodium carbonate, sodium silicate, sodium hydroxide, and potassium hydroxide) (Kumar et al. 1989). Other than these, a binary mix of alkaline-surfactant (AS), surfactant/polymer (SP), alkaline/polymer (AP), and alkaline/surfactant/polymer (ASP) methods are also applied in tertiary chemical enhanced oil recovery. Combined CEOR methods have improved efficiency for more oil production.

Other than traditional CEOR nanofluids flooding in the field has also been evaluated by few researchers (Gbadamosi et al. 2019). Injected nanofluid contributes to wettability alteration, IFT reduction, and improved viscosity of the injected fluid. Nanofluid flooding can be combined with polymer flooding. Polymeric nanofluids flooding improved rheological properties, increases stability at high temperature and salinity conditions.

Miscible CO_2 gas injection is yet another EOR method in the tertiary oil recovery process. CO_2 has unique displacing properties; it is due to low minimum miscibility pressure with various types of crude oil. During CO_2 injection, it forms a single-phase fluid with hydrocarbon and disrupts the capillary effect to retaining oil in place. CO_2 injection also promotes oil swelling, reduces fluid viscosity, and increases oil mobility.

6.5.2 Novel Approaches for mEOR Using Toluene and Nitrate

During mEOR process, molasses and nitrate are also injected in the reservoir to stimulate the growth of residents and introduced bacteria. Other than these, toluene addition (light oil component) can be a good option, where toluene is present in limiting concentration. Toluene is the preferable energy source of nitrate reducing bacteria. Molasses is used in mEOR due to its high solubility in water and insolubility in oil, while toluene is oil soluble and low molecular weight mEOR substrate. Hence, toluene binding to oil is prevented from been lost during water injection. Gassara et al. (2015) investigated the effect of toluene and nitrate injection on mEOR in laboratory scale (Gassara et al. 2015). They found that additional oil can be produced from heavy oil containing bioreactors. In these bioreactors, they stimulated the hNRB activity by the injection of aqueous nitrate and toluene. The mechanism behind this lab trial is the biosurfactant and biopolymer production by microbes and the stimulation of microbial activity by sequential addition of toluene and nitrate.

6.5.3 Polymers for Enhanced Oil Recovery

Polymer injected during polymer flooding can be categorized into two categories: synthetic polymers and biopolymers. Synthetic polymers are polyacrylamide or its derivatives (hydrolyzed polyacrylamide) and other copolymers of acrylamide. There are a number of biopolymers tested for EOR efficiency such as xanthan gum, scleroglucan, welan gum, schizophyllan, mushroom polysaccharide, cellulose, and lignin. Biopolymers have added advantage over the synthetic polymer like biopolymer has more rigidity, increased viscosity at higher temperature, superior tolerance to salts/temperature, outstanding thickening capability and stability in harsh condition of reservoirs. Along with these mentioned benefits biopolymer also has limitations like well plugging due to cell debris, bacterial sensitivity, and poor filterability. Other than these, nano-celluloses based nanofluids have better capability to involve in oil displacement (Pu et al. 2018). During the selection of polymer/ biopolymer different rheological properties to be tested at each field condition.

Xanthan is a bacterial polysaccharide and has rigid polysaccharide chains, thus less sensitive to mechanical shear, elevated salinity, and divalent ion concentration. Demerits of using xanthan gum are its high susceptibility to biodegradation and high risk of plugging in rock pores (Pu et al. 2018; Gbadamosi et al. 2019). Other biopolymers such as scleroglucan, produced by fermentation of a plant pathogen fungus (genus Sclerotium) are also been proposed as EOR agent. This is a good substitution of HPAM due to its good solubility and eco-friendly nature. This biopolymer is durable to various pH and high mineralization. Scleroglucan has an excellent viscosifying ability due to its high molecular weight and rigid structure. Some demerits of scleroglucan are its cost, ease of biodegradation, and poor filterability.

Hydroxymethycellulose (HEC) is a derivative of amorphous cellulose and non-ionic in nature. HEC has high molecular mass, rigid backbone structure, cheap in cost, non-toxic, and eco-friendly features suitable for uses as an ideal biopolymer. HEC is a shear resistant, salinity durable, and temperature tolerant biopolymer. HEC has some drawbacks like oxidation, biodegradation, and enzymatic degradation.

6.6 Biotechnological Upgradation of Produced Oil

Biotechnological upgradation of produced oil is industrial interest. This is due to the increase in concentration of sulfur in produced oil. Increased sulfur content in oil is a major environment concern. Biodesulfurization is a biological method for the removal of sulfur content in produced oil. Biodesulfurization was initially developed for the removal of inorganic (pyrite) and organic sulfur from coal by microorganisms. In petroleum, organic sulfur removal in transportation fuels is also a major concern. In crude oil, benzothiophene (BT) and dibenzothiophene (DBTs) are two main organosulfur compounds that are recalcitrance to removal by refinery process (e.g., hydrodesulfurization). DBT is the model compound for biodesulfurization research. Many microbes were investigated for their ability to utilize DBT as a sole sulfur source. Anaerobic desulfurization is less investigated, thus lack of anaerobic biochemistry and genetics of the desulfurization process are a major hurdle for commercial desulfurization process development. In the biodesulfurization process, there is low energy requirement, less generation of unwanted products, and low emission of sulfur containing compounds compared to hydrodesulfurization. The biodesulfurization process is carried out at low pressure and mild temperature. In biodesulfurization, DBT and other sulfur compounds are transformed in harmless compounds by microbes. For increasing the efficiency of biodesulfurization process, genetic engineering technology has also been applied (Ma 2010).

Rhodococcus erythropolis has a high ability to remove sulfur from crude oil. This species is aerobic, chemoorganotrophic, gram negative, non-motile, and non-endospore forming. It also has a variety of catabolic enzymes, more than one plasmid. Thus, all these properties give greater biotransformation capability across a wide range of compounds. It utilizes the DBT as a source of sulfur, rather than a source of carbon and uses 4S pathway for DBT desulfurization. Other than *Rhodococcus, Agrobacterium, Mycobacterium, Gordonia, Nocardia, Sphingomonas, Stenotrophomonas, Sphingobacterium, Klebsiella, Pseudomonas, Arthrobacter*, and *Bacillus* species have shown capabilities for desulfurization of crude oil and oil products (Alkhalili et al. 2017).

Other than the desulfurization of produced crude oil, heavy oil to light oil conversion is also a bio-upgradation process for crude oil. This is now been developed as a new focus area by oil industries and petroleum microbiologist. Heavy crude (bitumen) is majorly produced by unconventional oil reservoir (e.g., Venezuela oil sands, Athabasca oil sands). This heavy oil is very viscous in nature and contains a high concentration of asphaltene, resins, nitrogen, and sulfur containing hetero-aromatics and several metals (nickel and vanadium). This heavy oil has a high production cost, difficult in transportation, and requires a conventional refining process (Leon and Kumar 2005). For transportation of heavy oil, solvent addition is needed for easier flowing in pipelines. The cost of solvent and vast heavy oil production require another method for decreasing its viscosity.

The upgradation of heavy oil is generally done by thermal cracking or by catalytic hydro conversion. But both processes have some common concern like been energy and cost intensive, less selective and environment reactive. Biological conversion could be an alternative process for the upgradation of heavy crude oil. Biological process is less severe and highly selective for specific reactions. This biological upgradation can be done by the microbial and enzymatic transformation process.

6.7 Intervention of Biotechnology in Unconventional Oil and Gas Production (Oil Sands and Shale Gas Fields)

Oil sands are heavy oil deposits that have a composition of sand, clay, and petroleum. These unconventional oil reserves are present in the Orinoco Oil Belt (Venezuela), Athabasca (Alberta, Canada), Olenik (Siberia, Russia), and Maya (Mexico) heavy oil sands. These reserves contain oil with high levels of bitumen. For the recovery of oil from such oil sands, very few biotechnological approaches are proposed. Therefore, biotechnological intervention can be applied on tailing ponds management. Oil sands tailing ponds are engineered dam and dyke, contain the waste by-product such as water, sand, slit, clay, and residual bitumen produced during oil sand extraction and upgrading. Thus a proper management of tailing ponds is the main concern of oil sands operators.

Tailing ponds act as a good habitat for microbial growth and raise a major environmental concern. SRB and methanogens are easily grown in tailing ponds due to the availability of sulfate and anaerobic environment. Currently, oil sands mining companies are operating under a zero effluent discharge policy to the environment. In Canada alone, 1 billion m^3 tailings are stored in tailing ponds and cover approx. 220 km^2 area (Foght et al. 2017). For reclamation of land, some proposals related to placing tailing semi solids in the basin and capping with fresh water (end pit-lake) ecosystem or wetlands have been proposed. Other options available are placing de-watered tailing and covering with sand, soil, and vegetation for the generation of the boreal forest.

Potential biotechnological approaches employed for the remediation of tailing ponds are the aerobic treatment of froth treatment tailings. This leads to biodegradation of hydrocarbons prior to deposition in tailing ponds. This aerobic treatment also decreases the toxicity of ponds and also decreases methane emission. Another method employed is the in situ aerobic biological treatment of toxic organics (e.g., naphthenic acid). In this, partial oxidation of naphthenic acid is performed by ozonation, followed by the biodegradation process. Other approaches involves use of biofilm based bioreactors and algal based bio-treatment. Besides, microbial sequestration of produced gases (e.g., CH_4, CO_2) by methane oxidation is known to occur by native tailing ponds methanotrophs. Currently, none of the approaches is applied in pilot scale or in situ. While some of the approaches are in conceptual stages such as improving bitumen recovery by pretreatment using mEOR methods (uses of biosurfactant), uses of encapsulated microbes to remediate tailing ponds water, electricity generation by the construction of microbial fuel cells (Foght et al. 2017).

Shale gas, on the other hand, is a natural gas trapped within shale formations/rocks. Shale rocks are clastic sedimentary rocks and formed from the mud, clay, and organic matter. Shale rocks porosity is very low (10–100 nanodarcies) and natural gas are trapped in the tiny pores. Initially, shale gas production is economical, but was not feasible due to the unviability of technology. At present, shale gas production is economically viable with the advancement of technology and improved methods. Shale gas is produced through horizontal drilling and hydraulic fracturing process. Major shale gas producing countries are China, USA, Canada, and Argentina. Shale gas production has some issues like leakage of extraction chemicals, high water requirement, and leakage of greenhouse gases during extraction and pollution due to the processing of natural gas. During the hydraulic fracturing, water based fluid is injected in well at high pressure to create cracks in shale rocks. This water based fluids contain water, diluted acids, biocides, breakers, corrosion inhibitors, friction reducers, gels, oxygen scavengers, proppant, scale inhibitors, and surfactant (Arthur and Layne 2008). Hydraulic fracturing fluid is susceptible to microbial growth. These microbes can be injected through fluids during drilling, drilling mud, and drilling water or indigenous to the shale gas field. To reduce microbial growth biocides are added in hydraulic fracturing fluids.

Among various microbes, acid producing and sulfate reducing bacteria induce corrosion in pipeline, while SRB create problem of souring. Growth of microbial biomass leads to well clogging due to biofilm formation, and subsequently inhibits the gas production. For better control of microbial growth, prior investigation of microbe types and concentration, carbon sources, nitrogen sources, electron acceptors, and growth limiting factors is required. The next generation sequencing tool is frequently used for knowing such microbial community composition in hydraulic fracturing fluids.

6.8 Conclusion

In oil field setup diverse microbial communities are present. In these microbial communities mainly, sulfate reducing bacteria, nitrate reducing bacteria, fermentative bacteria, iron reducing bacteria, iron oxidizing bacteria, and methanogens are dominated. Factors such as downhole reservoir temperature and pressure are extreme for the oil field microbial communities. In oil field system, oil organics are used as a carbon source, while inorganic compounds (such as sulfate, nitrate, and iron) are used as electron acceptor. In an oil reservoir, during oil biodegradation firstly higher oil components are converted into lower oil components by fermentative bacteria. These lower components are further used by sulfate or nitrate reducing bacteria. At the last stage, methanogens use one or two carbon compounds and converted into methane. Thus, due to microbial growth in oil field system, field operators face various problems such as microbial souring and biocorrosion, etc. The SRB is responsible for microbial souring in oil reservoir. To inhibit microbial souring various measures are taken by oil industries. The biotechnological approaches have great potential to control souring. Application of metabolic inhibitors and bacteriophage are the latest novel techniques to control souring with least environment impact and specifically target to problematic microbes only. Other than souring, different biotechnological approaches are applied in controlling microbial induced corrosion, EOR process, biodesulfurization, tailing ponds, and shale gas production. In mEOR, different microbial and plant origin biopolymers are applied to enhance oil production. Nanofluid with polymer is also applied as a novel strategy for the EOR process. For biodesulfurization, microbes and immobilized microbial cells are applied. For improving efficiency of desulfurization, recombinant DNA technology is becoming a good option. For reclamation of tailing pond affected land, aerobic treatment, biodegradation of oil components, and microbial sequestration are used. For upgradation of heavy oil, microbes can be used which utilize heavy oil components and convert it into lighter oil components. In shale gas fields, microbial souring, biocorrosion, and degradation of fracturing fluids are major problems. These problems are addressed by biocide injection in fracturing fluids. But prior to uses of biocide, microbial community composition should be known for the designing of appropriate control strategy.

6.9 Future Trends

Biotechnological intervention in oil industries is developed from the last three decades. Innovative biotechnology may offer a new way to reduce emission pathways in unconventional oil resources. New policies are aimed to maximize recovery from conventional and unconventional oil reserves. Production of fossil fuels from unconventional reserves is more expensive than conventional reserves. Therefore, there is a clear scope of less energy driven microbial process for recovering energy from these resources. Miscible flooding approaches can be applicable for heavy oil reserves (Shah et al. 2010). In situ catalytic upgrading of oil through Ni-, Co-, Mo-, and Pd-based catalyst is yet another purposed technique. Microbial deposited Pd nanoparticle has benefited over the physically prepared nanoparticles with respective to its application in in situ catalytic upgradation and oil recovery (Hart et al. 2016; Head and Gray 2016). Other than these possible intervention, enhanced energy recovery from residual oil, where secondary and tertiary recovery processes were no longer economical, is proposed. In this, methanogenic consortia convert alkanes to methane. This approach reduces the CO_2 emission (compared to oil and gas).

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