Microbial Enhanced Oil Recovery

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Microbial enhanced oil recovery (MEOR) involves the utilization of microbes and their by-products, such as biosurfactants, biopolymers, biogenic acids, solvents, biogases, biomass, and emulsifiers, to stimulate the production of oil by mobilizing residual reserves.

Keywords: MEOR ; microbial consortium ; mechanism ; effect

1. Introduction

Crude oil is the primary energy source within the intricate capillary network in porous reservoir media. Enhanced oil recovery aims to economically extract the maximum amount of original oil in place (OOIP). It can be categorized into three stages based on the development process: primary recovery (natural energy extraction), secondary recovery (injecting water or gas to maintain reservoir pressure), and tertiary recovery (known as enhanced oil recovery or EOR) [1]. Tertiary recovery methods include chemical flooding (including polymer flooding, surfactant flooding, and alkaline flooding [2]), thermal flooding, microbial enhanced oil recovery (MEOR), and miscible flooding, which are designed to increase the amount of oil recovered by altering the reservoir conditions or fluid properties [3][4]. **Figure 1** illustrates the different stages of oil recovery and their respective operating mechanisms.

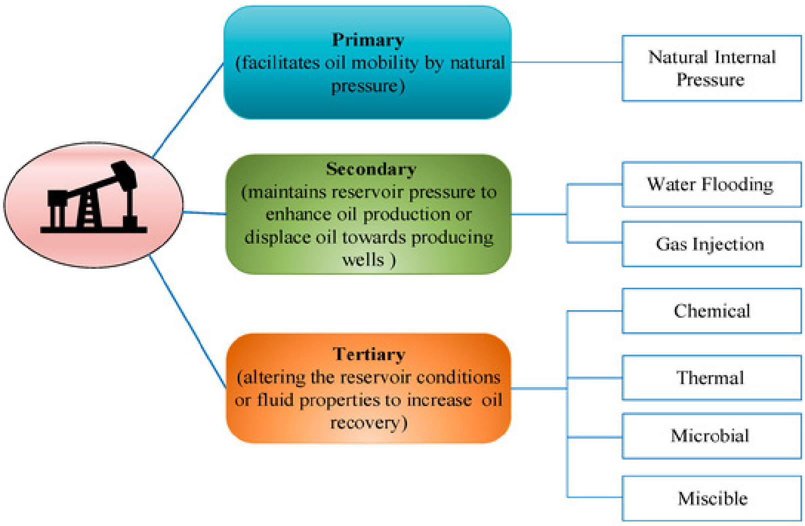

Figure 1. The distinct stages of oil recovery and their corresponding operational mechanisms.

In generally, primary production allows for recovery of approximately 10% of the OOIP, and secondary recovery techniques can improve the overall oil recovery by 12% [5]. Tertiary recovery techniques come into play when primary and secondary recovery methods have become ineffective and significant amounts of oil remain trapped in the reservoir. As one of the methods of tertiary oil recovery, polymer flooding technology, particularly using partially hydrolyzed polyacrylamide (HPAM), has emerged as a crucial tertiary oil recovery method and has gained widespread application [6]. This technique involves the application of HPAM to block large channels, increase the viscosity of the displacing phase, and effectively enhance the oil recovery rate by 8–15% [7]. However, polymer flooding encounters challenges related to severe plugging issues and a reduction in oil recovery. This is a consequence of the polymer solution's potential reactivity with metal ions in the original formation, resulting in the formation of compounds that are difficult to degrade [8].

2. Microbial Enhanced Oil Recovery

Microbial enhanced oil recovery (MEOR) involves the utilization of microbes and their by-products, such as biosurfactants, biopolymers, biogenic acids, solvents, biogases, biomass, and emulsifiers, to stimulate the production of oil by mobilizing residual reserves [9]. According to Quraishi et al. [10], microbial flooding can be categorized into exogenous and indigenous. The category is based on the source of the strains. Exogenous microbial flooding involves the injection of microbes that have been screened under conditions similar to, but not within, the reservoir. These microbes are introduced underground to enhance oil production through their propagation and metabolites. Indigenous microbial flooding involves the utilization of remaining oil as a carbon source by microbes, making use of the active substances present in the formations. During water injection, air, inorganic salts, a phosphorus source, and a nitrogen source are introduced to facilitate the proliferation of these native microorganisms [9]. The key aspect of exogenous microbial flooding lies in developing effective production strains, and challenges include ensuring the compatibility of microorganisms, performance degradation, and high costs [3]. Indigenous microbial flooding shows excellent adaptability but lacks a subsequent procedure for developing production strains. In the process of MEOR implementation, microorganisms are commonly cultured ex situ and subsequently introduced into the reservoir by injection. As the injected water is transported, these microorganisms build up in the caprock pores, where oil is present at the interface between oil and water. After the injection of MEOR bacteria, in situ production persists, resulting in continuous alterations in the oil and reservoir properties, which facilitates the mobilization of tightly trapped oil to the surface [11].

The MEOR diagram in **Figure 2** illustrates introducing a water mixture, including bacteria and/or biosurfactants, accompanied by a nutrient medium, into the reservoir as part of the MEOR process. Inside the reservoir, the bacteria facilitate the biodegradation of heavy crude oil into light components through biosurfactants. This degradation process aids in improving the movement of oil toward the production well. Furthermore, the injected bacteria experience metabolic activities that result in the production of metabolites. These metabolites contribute to multiple mechanisms, including reducing oil viscosity, lowering interfacial tension (IFT), promoting emulsification, and re-pressurizing the reservoir. These mechanisms collectively enhance the recovery of residual oil.

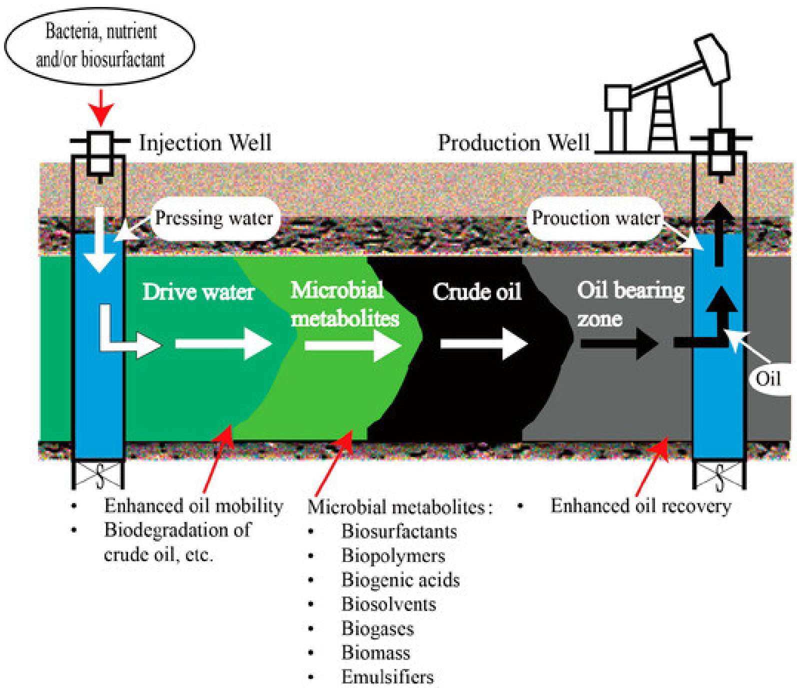

Figure 2. Diagram of MEOR.

2.1. Microorganism

Oil reservoirs can be viewed as extensive geo-bioreactors primarily populated by sulfate-reducing bacteria (SRB), methanogens, syntrophic bacteria, and fermentative bacteria [12]. Accordingly, the dominant microbial processes observed in oil field ecosystems affected by petroleum hydrocarbons include sulfate reduction, fermentation, acetogenesis, methanogenesis, nitrate reduction, as well as iron and manganese reduction [13][14]. In addition, oil reservoirs contain a significant number of organic substances, including various inorganic ions such as sulfate and nitrate, as well as organic compounds like alkanes, alkenes, cycloalkanes, and aromatic hydrocarbons [15].

The microbial activities in oil reservoirs significantly impact oil's chemical composition and physical–chemical properties [16]. This influence can be either positive, such as reducing viscosity in heavy oil to enhance its exploitation, or negative, leading to corrosion of drilling equipment or reservoir souring [17][18]. A large number of studies have highlighted the significance of microbial community dynamics within petroleum oilfield ecosystems [15]. The spatial distribution of microbial communities within long cores plays a role in the mechanism of oil displacement by microorganisms [19]. Generally, in the vicinity of the injection water, aerobic oil-displacement functional bacteria can decrease oil–water IFT by producing biosurfactants and emulsifying crude oil [20]. Additionally, aerobic hydrocarbon-loving microorganisms are abundant in the aerobic zone, which enhances crude oil's physical properties and residual oil fluidity through aerobic hydrocarbon metabolism. Within the middle section of the reservoir, facultative and anaerobic microorganisms coexist and produce H_2, CO_2, small molecule acids, and alcohols through anaerobic fermentation [21]. Within the anoxic conditions of the reservoir's deep environment, methanogenic microbes increase crude oil fluidity by generating CH_4 [22]. **Figure 3** depicts the distribution of microorganisms in MEOR.

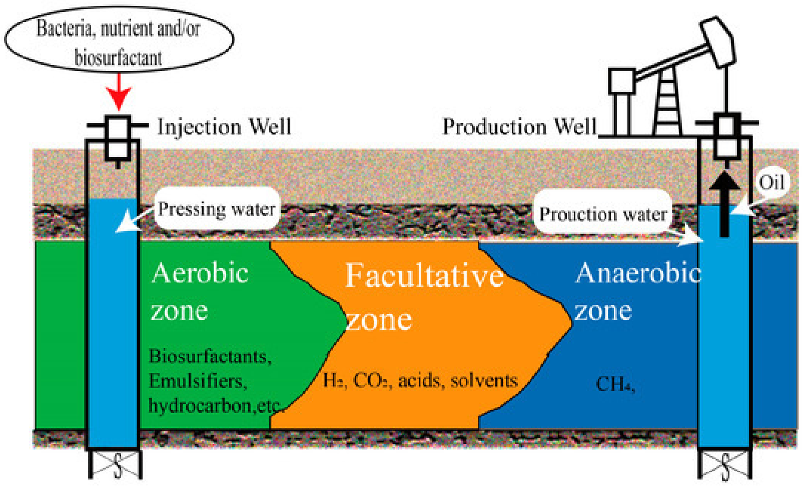

Figure 3. The spatial distribution of microorganisms in MEOR.

Oil reservoirs are known for adverse environments characterized by high temperature, high pressure, high salinity, and stringent anoxic conditions [13][23]. These environmental factors usually limit the range of microorganisms suitable for in situ processes. One adaptation of microorganisms to such harsh conditions is their ability to form biofilms. Biofilms are generally described as clusters of microorganisms surrounded by a self-produced matrix of extracellular polymeric substances (EPS), which adhere to each other or surfaces [24]. A biofilm matrix acts as a protective coating, providing resistance against external stressors in oil fields [25]. In addition, biofilms create a conducive environment for mutualistic microorganisms, promote habitat diversity through confined gradients, enable nutrient sorption for resource capture, aid nutrient transport via channel systems, facilitate the exchange of signaling molecules, and offer increased tolerance to toxic compounds through horizontal gene transfer, acting as a barrier [26]. Biofilm formation emerges as the predominant mode of microbial existence in oil reservoirs due to the advantageous protection and adaptation it provides in toxic and extreme environments.

EPS, accounting for approximately 90% of biofilm composition, are generated through the cellular lysis and hydrolysis of high molecular weight macromolecules by microorganisms [24], forming gel-like networks that confer mechanical stability to biofilms in oil environments [27]. EPS contain diverse functional groups, such as carboxyl, phosphoric, sulfhydryl, phenolic, and hydroxyl groups, contributing to cell aggregation and attachment to various surfaces [26]. They primarily consist of polysaccharides, proteins, glycoproteins, glycolipids, and nucleic acids, which can accumulate multiple substances (such as nutrients or ionic) that function as substrates in extracellular chemical reactions. Bacteria produce EPS as a survival strategy in harsh reservoir conditions characterized by extreme temperature, salinity, toxic agents, and limited nutrient availability [28]. Furthermore, EPS play a significant role in oil displacement mechanisms, influencing oil through processes such as emulsification, degradation, dispersion, aggregation, and sedimentation [29]. Previous research has predominantly focused on investigating the temporal changes in bacterial communities and genera associated with hydrocarbon degradation. As an example, Bacosa et al. [30] effectively cultured nine bacterial strains from the surface waters of the northern Gulf of Mexico that exhibited the dual ability to produce EPS and degrade hydrocarbons. EPS also act as a bio-emulsifier, facilitating efficient oil mobilization by releasing oil from rocks and creating stable oil/water emulsions [31].

To summarize, the oil reservoirs are considered harsh conditions. Microorganisms secrete EPS to protect the bacteria from adverse conditions and play an important role in MEOR. Since microorganisms are the base of MEOR, isolating and screening suitable microorganisms are vital steps in establishing an effective MEOR process. The primary selection criterion for microorganisms is their capability to degrade hydrocarbons, survive in the reservoir, and generate the desired metabolic products. Given the variations in conditions among different reservoirs, customization of the MEOR process according to specific reservoir conditions is crucial for its success.

2.2. Microbial Products

The effectiveness of the MEOR process relies on metabolite production by either exogenous or indigenous microorganisms [31]. These metabolites play a crucial role in altering the rock properties of the reservoir, including permeability, porosity, wettability, and oil viscosity. This alteration facilitates oil recovery by reducing viscosity, emulsifying the oil, pressuring the reservoir, and dissolving residual oil [3]. The bioproducts can generally be categorized into seven main groups: biosurfactants, biopolymers, biogenic acids, biosolvents, biogas, biomass, and emulsifiers.

2.2.1. Biosurfactants

Biosurfactants, a group of surface-active molecules that are synthesized by microorganisms via fermentation processes, can be categorized into five primary groups, including glycolipids, phospholipids and fatty acids, particulate surfactants, lipopeptides and lipoproteins, and polymeric surfactants [32]. These molecules exhibit great potential for improving oil recovery on account of their ability to lower IFT and surface tension, alter wettability, and create water/oil or oil/water emulsions, thereby facilitating the mobilization of oil to the surface [33][34]. The abilities mentioned above result from biosurfactants forming biofilms that interact with reservoir rocks and water/oil formations, bringing about modifications in their characteristics. *Pseudomonas aeruginosa* is an illustrative example of a biosurfactant-producing microorganism that exhibited the capability to degrade alkanes, including hexadecane and octadecane, within a 28-day incubation period [35].

As stated by Niu et al. [36], biosurfactants with lower molecular weights can reduce surface tension and IFT, whereas those with higher molecular weights display emulsifying activities, forming stable emulsions at the interface due to the close arrangement of surfactant molecules. The effectiveness of reducing IFT relies on the number of biosurfactants required to attain the desired reduction and the adsorption of biosurfactants onto caprocks. To achieve significant oil production, it is necessary to reduce the IFT to a range of approximately 10^{-2} to 10^{-3} mN/m, which corresponds to a capillary number of 10^{-3} to 10^{-4} [37].

Numerous studies have reported significant oil recovery achieved through the use of biosurfactants. To produce biosurfactants of high quality with minimal losses, careful consideration of various conditions, including temperature, salinity, pH, oxygen, nutrient composition, and physical and chemical parameters, is necessary. Additionally, it is essential to investigate the characteristics of compounds such as carbon sources and nitrogen sources, as well as the C:N ratio and the selection of bacterial strains, to ensure the production of biosurfactants that are effective [38]. Prior studies have shown that in situ production of surfactants is typically restricted by the requirement of most surfactant-producing microorganisms for oxygen to support their growth. In the past few years, numerous experimental studies have shown that specific microorganisms have the ability to generate surfactants under anaerobic conditions. This ability is exceedingly advantageous for extracting trapped oil residues situated within oxygen-depleted reservoirs' deep pores. It facilitates surfactant production and subsequently improves oil recovery without needing oxygen during metabolic processes.

Biosurfactants offer several advantages for EOR applications, including their biodegradability, outstanding surface and interfacial activities, low toxicity and capital cost, enough raw materials for production, and substantial productivity. These attributes make biosurfactants a cost-effective and efficient approach for achieving enhanced oil recovery.

2.2.2. Biopolymers

Injected microorganisms and their metabolites encounter significant limitations in migrating within low- and high permeability zones; they face hindrances in contacting residual oil caused by preferential fluid flow [39]. To resolve this problem, biopolymers as plugging agents are employed to selectively plug high permeability zones and redirect injected water towards low permeability zones to enhance the recovery of residual oil [34][40].

Figure 4 visually illustrates the mechanism of selective plugging. The left-hand side diagram initially portrays water flowing effortlessly through wide pore channels, neglecting the narrow and low permeable zones resulting in poor sweep efficiency. In contrast, the right-hand side diagram illustrates the effective plugging of the wide pore channel via biopolymers. When the biopolymers adhere and grow, they deviate the water flow towards the less permeable zones, facilitating oil displacement and sweep efficiency [41]. The biopolymer plugging process can be accomplished through either the injection of bacteria or the in-situ production of bacteria in the reservoir. As mentioned by Sen [42], microorganisms enter the reservoir via high permeability zones and establish themselves in specific laminae. The presence of the growth of biopolymers in the pore throat leads to the plugging of the pore space, causing a reduction in the permeability rate. As a result, plugging pore throats with biopolymer growth helps in restoring a balanced permeability across the reservoir, thereby allowing water-flooding operations to resume with conventional sweep efficiency.

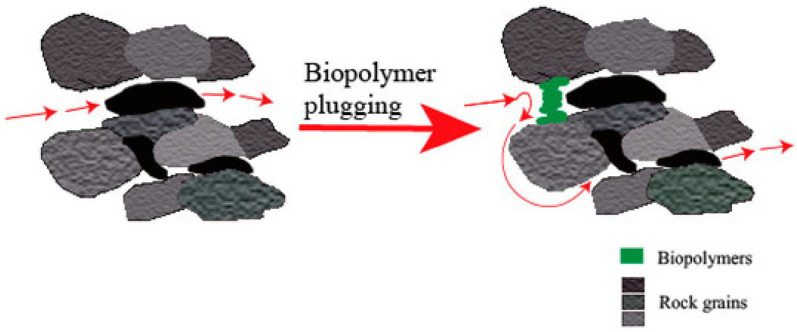

Figure 4. Illustration of the selective plugging mechanism.

Moreover, biopolymers can be employed as displacement agents to augment the viscous pressure gradient in water, thus improving the efficiency of oil sweeping. Halim et al. [41] extensively evaluated the selective plugging method using a sand pack column and the thermotolerant, anaerobic, biofilm-producing microorganism called *Bacillus licheniformis*. The study demonstrated that the biopolymer produced by the *Bacillus licheniformis* substantially augmented the biofilm thickness, resulting in the diversion of flow towards low permeability areas and subsequent oil displacement. More and more evidence indicates that microbially produced polysaccharides, including xanthan gum, have been utilized for many years and have consistently demonstrated successful outcomes in terms of achieving higher oil recovery [43]. Xanthan gum is generated through the fermentation of carbohydrates and is subsequently introduced into the water used for well flooding and wide pore channels. Microorganisms effectively plug the high permeability zones as they grow. The remarkable physical properties of xanthan gum, including shear resistance, salinity tolerance, thermal stability, and viscosity, make it an efficient option for EOR [44].

In conclusion, the application of biopolymer flooding enhances recovery effectiveness by reducing the mobility ratio between the injected water and entrapped crude oil, triggering a substantial improvement in MEOR, particularly in heterogeneous reservoirs.

2.2.3. Biogenic Acids

The production of bioacids contributes to the dissolution of carbonate rocks in the reservoir, leading to increased porosity and permeability and assisting oil displacement into the remaining reservoir [45]. Generally, low molecular weight organic acids like formic acid, acetic acid, and propionic acid are commonly employed in MEOR [46]. It has been reported that organic acids serve as the active metabolites responsible for reducing oil viscosity, thereby elevating their mobility. Laboratory experiments conducted in Daqing Oilfield demonstrated a 36% reduction in viscosity through the utilization of microbial acids [9].

2.2.4. Others

(1) Biosolvents

One significant benefit of the fermentation process is its capability of producing solvents and bioacids, which play a vital role in dissolving carbonate rocks to reveal concealed oil in the reservoir. Common examples of solvents include acetone, ethanol, and butanol. Consequently, these simultaneous processes contribute to a higher oil recovery yield [45]. Furthermore, solvents contribute to the reduction in oil viscosity and the decrease in IFT between water and oil [44].

(2) Biogases

Certain microorganisms capable of in situ gas production by fermenting carbohydrates or hydrocarbons like CH_4, CO_2, and H_2 are employed to achieve oil mobilization through re-pressurization. The reservoir containing an accumulation of gases leads to a buildup of pressure and subsequent swelling of the oil, thus promoting its mobilization to the surface [44]. Moreover, these gases can contribute to the reduction in oil viscosity by dissolving into the oil, increasing sweep efficiency.

(3) Biomass

Biomass, including microbial cells, biofilms, extracellular products, extracellular structures, water channels, and void space, plays a role in the selective plugging effect. Additionally, it contributes to the reduction in crude oil viscosity, as well as oil desulfurization and emulsification, thereby favoring the effectiveness of MEOR. Some studies have explored the approach of starving microorganisms to minimize their size and increase their penetration depth into the reservoir, followed by nutrient supply to promote the formation of biomass biofilms [47][48].

(4) Emulsifiers

Emulsifiers produced by a diverse range of microorganisms lead to the achievement of oil emulsification. This process results in the formation of stable emulsions, typically oil in water, where hydrocarbons are effectively dispersed and incorporated [45].

In summary, producing various highly advantageous metabolites by microorganisms ultimately leads to an increase in the overall oil recovery in MEOR. The widespread distribution of microbial bioproducts can significantly influence the rock characteristics of reservoirs. **Table 1** comprehensively compiles the bioproducts generated by various microorganisms, including their major effects and the most suitable reservoir candidates for MEOR processes.

Table 1. Microbial products.

Microbial Products	Representative Microorganisms	Effect	Limitation	Type of Formation/Reservoir	Reference
• Biosurfactant (Alasan, Surfactin, Rhamnolipid, Emulsan)	• *Acinetobacter Arthrobacter paraffineus* sp. • *Calcoaceticus* sp.	• Emulsify crude oil into an oil–water mixture. • Decrease IFT. • Modify rock wettability.	• Low displacement efficiency at the microscopic level.	• Reservoirs composed of sandstone or carbonate formations with temperatures below 50 °C and containing lighter oils with an API gravity greater than 25.	[49]
• Biopolymers (Xanthan gum, Pullulan, Levan)	• *Bacillus polymyxa* sp. • *Pseudomonas* sp. • *Brevibacterium viscogenes* sp.	• Raise the viscosity of the displacing fluid while diminishing the mobility ratio between water and oil. • Optimize the sweep regions and effectiveness with selective plugging.	• Low efficiency in sweeping fluids across the volume.	• Stratified reservoirs exhibiting distinct permeable zones.	[50]
• Bioacids (Acetic acid, formic acid, propionic acid)	• *Clostridium* sp. • *Enterobacter aerogenes* sp. • *Methanobacterium* sp.	• Improve the rock's dissolution in the pore throats to increase porosity and permeability.	• Low porosity. • Inefficient fluid drainage. • Reservoir impairment.	• Carbonate or carbonaceous reservoirs	[51]

Microbial Products	Representative Microorganisms	Effect	Limitation	Type of Formation/Reservoir	Reference
• Biogases (CO_2, H_2, CH_4, N_2)	• *Clostridium* sp. • *Enterobacter aerogenes* sp.	• Enhance the mobility of crude oil by reducing its viscosity. • Re-establish reservoir pressure • Gas injection for partial or miscible displacement.	• Equipment corrosion. • Reservoir souring.	• Formations containing heavy crude oil with an API gravity of less than 25.	[10]
• Biosolvents (Alcohols, ketones, acetone, butanol)	• *Zymomonas mobilis* sp. • *Clostridium acetobutylicum* sp. • *Klebsiella* sp.	• Decrease the viscosity of oil through its dissolution in crude oil. • Alcohols and ketones can act as co-surfactants in the formation of micelles, leading to a decrease in critical micelle concentration (CMC) and IFT, thereby facilitating emulsification. • Optimize the porosity and permeability by dissolving heavy oil in the pore throats.	• Low displacement efficiency at the microscopic level.	• Formations containing heavy crude oil with an API gravity of less than 25. • Reservoirs with high oil wettability have undergone water flooding.	[52]

Microbial Products	Representative Microorganisms	Effect	Limitation	Type of Formation/Reservoir	Reference
		• Change rock wettability.			
Biomass (Microbial cells, biofilms, EPS)	• *Licheniformis* • *Bacillus* • *Xanthomonas* • *Leuconostoc mesenteroides* • *Campestris*	• Employ selective plugging to optimize sweep effectiveness. • Gas-based biopolymers. • Specific decomposition of heavy oil.	• Low displacement efficiency at the microscopic level.	• Stratified reservoirs exhibiting distinct permeable zones.	[53]
• Emulsifiers	• *Candida* • *Acinetobacter* sp. • *Bacillus* sp. • *Pseudomonas* sp.	• Emulsification of oil with water.	• Sludge buildup. • Low displacement efficiency at the microscopic level.	• Formation containing high-molecular-weight waxy oil (>C_{22} alkanes), paraffinic oil, and asphaltene.	[54][55]

2.3. Mechanisms of Microbial Enhanced Oil Recovery

MEOR is founded on two fundamental principles. The first principle involves modifying the interfacial properties of oil–water minerals to enhance oil movement through porous media, thereby improving displacement efficiency (IFT reduction or permeability increase), driving force (reservoir pressure), fluidity (miscible flooding, viscosity reduction), and sweep efficiency (selective plugging, mobility control). The second principle focuses on microbial activity for the degradation and removal of sulfur and heavy metals from heavy oils [56]. Due to the diverse range of strains and metabolites involved, the mechanism of MEOR is intricate and comprehensive, encompassing processes such as biodegradation, emulsification, IFT reduction, and alteration of reservoir rock properties [46]. A thorough comprehension of the mechanisms of MEOR lays the groundwork for evaluating its practicability and prospective benefits.

2.3.1. Biodegradation

Biodegradation is of paramount importance in MEOR. The main aspect of this mechanism involves the breakdown of crude oil’s long-chain hydrocarbons into shorter hydrocarbons, which results in an increase of up to 30% in the light components of crude oil [9]. From one perspective, through this process, bacteria can obtain carbon for energy, growth, and reproduction by degrading hydrocarbons in crude oil [57]. From another perspective, converting heavy components into lighter ones brings about fundamental alterations in crude oil properties, leading to a reduction in viscosity and an enhancement in fluidity. Consequently, this contributes to improved oil recovery rates. This biodegradation mechanism entails direct cell contact and the involvement of biosurfactants, which decrease IFT and facilitate the emulsification of hydrocarbons [58]. Biosurfactants are commonly utilized to degrade the heavy fractions of crude oil.

Biodegradation pathways can be classified into aerobic and anaerobic biodegradation [46]. Typically, aerobic biodegradation occurs near the injection wells, while anaerobic biodegradation takes place in deep reservoirs. Aerobic biodegradation involves dissolving oxygen and injecting it into the reservoir alongside water rich in nutrients in MEOR, which has been an established and extensively employed technique for many years. Field trials have been conducted in China’s Daqing Oilfield, where dissolved oxygen was injected to stimulate aerobic bacteria for degrading heavy components, thereby enhancing oil recovery [9]. During aerobic biodegradation, *Pseudomonas putida* with *alkB* genes on the OCT plasmid is capable of degrading aliphatic alkanes. This degradation process involves the enzyme alkane hydroxylase, which consists of rubredoxin reductase, oxygenase, and membrane-bound rubredoxin. These cofactors facilitate the transport of electrons from nicotinamide adenine dinucleotide phosphate to hydrocarbon substrates. The degradation of alkanols occurs through alcohol dehydrogenase, which converts them into alkanals. Subsequently, alkanals are transformed into fatty acids by aldehyde dehydrogenase and further converted to acetyl CoA by its synthetase [30]. Cell biomass biosynthesis occurs in the central precursor metabolites such as acetyl-CoA, succinate, and pyruvate [59], as depicted in **Figure 5**.

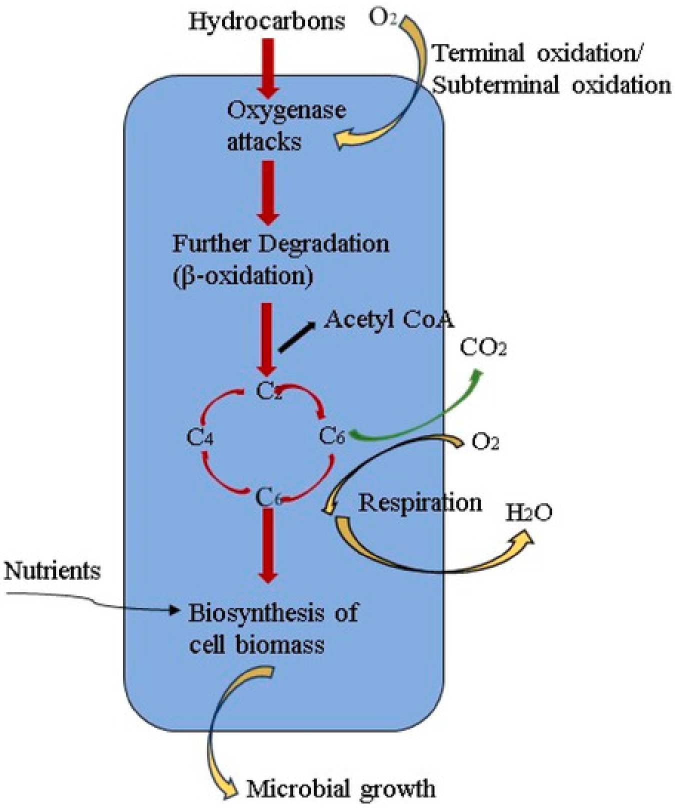

Figure 5. The fundamental concept underlying aerobic hydrocarbon degradation by microorganisms [60].

Anaerobic degradation has demonstrated the capability of biodegrading various hydrocarbons, including benzene, toluene, naphthalene, asphaltene, phenanthrene, alkane, branched alkane, and hydrocarbon mixtures [61]. Nonetheless, the application of anaerobic degradation in MEOR is restricted, possibly because of the choice of unsuitable oil-degrading microorganisms or suboptimal conditions [62]. Of all the options, the anaerobic degradation of long-chain alkanes holds the greatest promise for achieving enhanced oil recovery. **Table 2** displays the commonly encountered long-chain degrading microorganisms and the scope of hydrocarbons they can degrade. Furthermore, oil-degrading microorganisms that have the ability to degrade asphaltenes are frequently regarded as highly effective, while hydrocarbon-degrading microorganisms generating biosurfactants are considered optimal for MEOR [61].

Table 2. Microorganisms that exclusively use long-chain n-alkanes as their carbon source.

Microorganisms	Range of Degradation	Reference
Rhodococcus erythropolis	C_{10}–C_{30}	[63]
Pseudomonas fluorescens	C_{12}–C_{32}	[64]
Thermophilic Bacillus	C_{15}–C_{36}	[65]
Gordonia amici	C_{18}–C_{36}	[66]
Chelatococcus daeguensis	C_{23}–C_{37}	[67]
Pseudomonas aeruginosa	C_{36}–C_{40}	[68]

Since reservoirs are generally anaerobic environments, anaerobic degradation predominantly governs the biodegradation of hydrocarbons in deep reservoirs. The thorough examination of this process has led to a clear understanding of the biochemical mechanisms involved in the anaerobic biodegradation of organisms. An illustration of this is the work by Purwasena et al. [69], who effectively isolated a thermophilic anaerobic bacterium strain belonging to the *Petrotoga* sp. from a Japanese oil reservoir. Through core flooding methods, their findings demonstrated that the bacterium could degrade long-chain n-alkanes, reduce oil viscosity, and enhance oil recovery under 80 °C. Additionally, researchers have effectively identified the key metabolites and associated genes involved in this specific biodegradation mechanism [46]. Nevertheless, a complete understanding of the biochemical processes involved in anaerobic hydrocarbon mechanisms is still being explored and investigated.

In summary, biodegradation plays a crucial role in MEOR, as research indicates that microorganisms and their products can degrade carbon atoms by utilizing them as a carbon source for metabolic processes. As a consequence, the viscosity of the oil is reduced, and the flow properties are improved; hence, promoting oil displacement towards production wells for recovery. While aerobic and anaerobic biodegradation pathways exist, anaerobic degradation predominantly controls the process. The duration of the biodegradation process varies based on factors such as hydrocarbon composition, bacterial population, reservoir environment, and the amount of oxygen available or introduced into the wells.

2.3.2. Emulsification

Emulsions have an impact on increasing the oil–water interface, leading to enhance the accessibility of oil [70]. Different types of emulsions are classified based on particle size, including transparent microemulsion (nm), translucent colloid emulsion (below 1 μm), milky white emulsion (1–2 μm), fine dispersion (1 mm), and coarse emulsion (100 mm) [71]. Emulsification can change the reservoir's wettability, shifting it from oil-wet to water-wet, thus making trapped oil easier to recover [9].

Figure 6 provides a general visual representation of the emulsification process. On the left-hand side of the figure, water containing biosurfactant comes into contact with oil. Biosurfactants composed of both hydrophobic and hydrophilic structures, through their hydrophobic interaction properties, facilitate the formation of steady oil emulsions in water, as depicted on the right-hand side of **Figure 6**. These emulsions allow for the coexistence of both liquids by creating amphiphilic films at the oil–water interface [72]. To ensure a consistent dispersion of oil in water, biosurfactants are incorporated to provide stabilization. The hydrophobic interaction properties of biosurfactants enable them to attach to the interface between oil and water, improving the dispersion and facilitating movement until the biosurfactant is either diluted or completely adsorbed on the caprocks. Emulsification of crude oil leads to the generation of various droplet sizes, exhibiting tensile, deformed, and seepage flow characteristics, which alter the permeability of the reservoir, and increase the contact area between the droplets and the reservoir medium, thereby facilitating the flow and recovery of oil [9]. Additionally, the structural alteration of bioacids contributes to changes in wettability, converting water-in-oil emulsion into oil-in-water emulsion, followed by a subsequent reduction in oil viscosity.

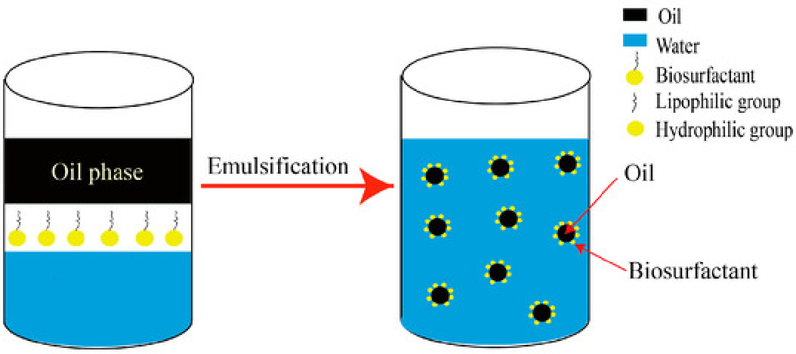

Figure 6. Schematic diagram illustrating the emulsification process.

2.3.3. Interfacial Tension Reduction

Reducing the IFT is an alternative mechanism that significantly enhances oil recovery. One of the three components contributing to the reduction in IFT is the work of adhesion, which represents the energy required to separate an oil film from an oil-wet rock surface [73]. Secondly, the elasticity of the oil/water interface is changed, enabling the two phases to mix more easily [74]. Thirdly, biosurfactants, characterized by their amphiphilic nature, have the ability to accumulate at the interface between immiscible fluids like oil and water or water and oil. This accumulation leads to a reduction in IFT, ultimately strengthening capillary forces and making oil easier to flow [75]. The formation of foams using organic materials also can lead to a reduction in IFT at the oil/water interface [11]. A visual representation of reducing IFT can be seen in **Figure 7**.

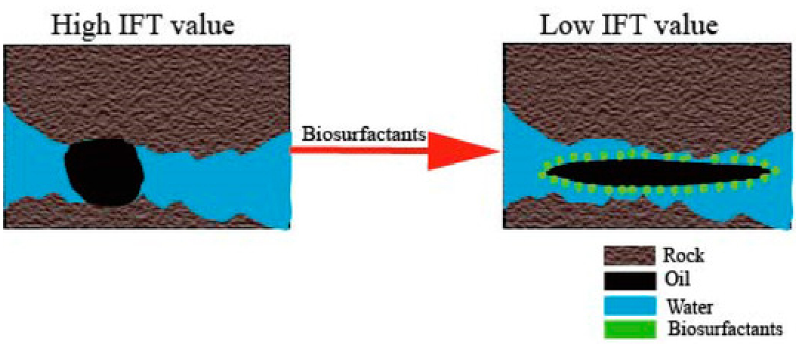

Figure 7. Graph of IFT reduction.

IFT reduction and wettability alteration are closely related mechanisms that occur concurrently and contribute significantly to EOR [76]. The reduction in IFT increases the capillary number by 3–4 orders of magnitude, resulting in improved oil flow. Simultaneously, wettability alteration promotes oil production by supporting the mobilization and displacement of oils attached to the rock surface or trapped in the pores [77][78]. The wettability condition of the rocks significantly influences the oil recovery [76]. In the case of oil-wet rocks, IFT reduction helps decrease the negative capillary force that hinders the entry of injection fluids. For intermediate-wet rocks, IFT reduction facilitates oil desorption and promotes mixing between oil and water. In the case of water-wet rocks, IFT reduction reduces positive capillary force and weakens interface elasticity, allowing trapped oil droplets to flow more easily [76]. **Figure 8** depicts the process of alteration in wettability.

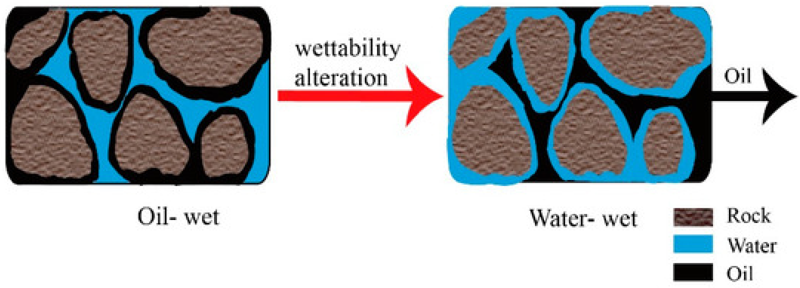

Figure 8. Illustration of alteration in wettability.

2.3.4. Altering Reservoir Rock Properties

By altering the properties of reservoir rocks, it is possible to increase their permeability and porosity. Bioacids have the potential to significantly decrease the permeability of reservoir rocks, reducing it from 284 mD to 24 mD while also diminishing the viscosity by approximately tenfold [9]. The mechanism is believed to predominantly occur in carbonate reservoirs [79]. The porosity and permeability modification can be seen in **Figure 9**.

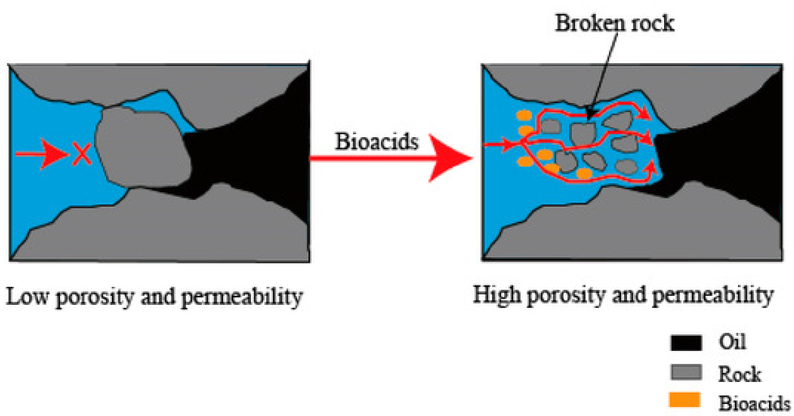

Figure 9. Diagram of the porosity and permeability modification.

Biosurfactants can undergo adsorption onto the pores of the reservoir rocks, leading to modifications in the wetting characteristics of the porous media. Interfacial forces dictate fluid flow at the microscopic level, making pore-scale wettability a crucial factor in enhancing oil recovery [80]. This phenomenon occurs due to the influence of the amphiprotic groups in biosurfactants. Biosurfactants primarily alter the reservoir rock's wettability or aid in the displacement of cells and biofilms within pore structures by adhering to them [79]. The modification of wettability accelerates oil production by improving the permeability of injected water within the reservoir matrix and boosting the capillary pressure [81]. In porous media, fluids' location, distribution, and flow are governed by the wettability of rock/fluid systems [82]. In non-fractured reservoirs, the transition from highly wet conditions to neutral wettability enhances oil extraction efficiency. In fractured reservoirs, changing the wettability to water-wet leads to favorable capillary forces, enabling the displacement of trapped oil through water infiltration into the rock formations in the opposite direction [83]. Nevertheless, the precise mechanisms underlying the alteration of reservoir wettability during MEOR processes remain insufficiently understood and necessitate additional research and exploration.

In practical terms, a mechanism must ultimately accomplish two objectives: (1) a substantial enhancement in oil recovery and (2) a financially viable yield ratio that ensures the incremental yield of oil surpasses the input material for MEOR. When these criteria are fulfilled, a mechanism can be regarded as promising and deserving of further investigation. The feasibility and robustness of each mechanism in the field are crucial considerations. Considering the reservoir conditions, it is essential to access fundamental reservoir data such as porosity, permeability, pressure, temperature, pH, viscosity, wettability, etc. These data are critical to determining which mechanisms are most suitable and effective for the specific reservoir conditions.

2.4. Advantages and Disadvantages of MEOR

Given the prevailing low oil prices, MEOR holds significant potential, particularly for marginal or uneconomical reservoirs. Microbial flooding has emerged as a viable substitute for other EOR techniques, showing a remarkably high success rate [84]. Compared to thermal flooding and gas flooding, microbial flooding exhibits notable advantages, primarily its eco-friendly attributes and cost-effectiveness for augmenting oil production [39]. Compared to other EOR technologies, microbial flooding possesses distinct features. Firstly, microbial products employed in the process are typically biodegradable and harmless. Secondly, implementing microbial processes is relatively straightforward in the field, as it necessitates minimal adjustments to existing facilities [85]. Thirdly, unlike thermal processes that demand significant energy consumption, MEOR is not energy-intensive, making it a cost-effective EOR method [3]. **Figure 10** illustrates various cost estimates for different EOR techniques.

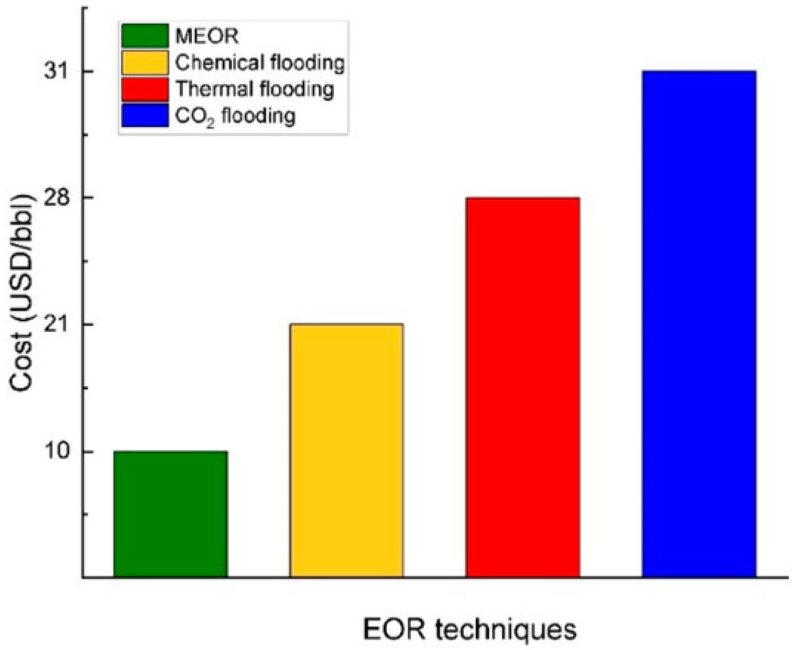

Figure 10. Estimations of cost for various EOR techniques [9].

There are also some disadvantages of MEOR. For example, the presence of SRB in MEOR is recognized for its detrimental effects [86], leading to corrosion, plugging, and reservoir souring issues in oil production, transportation, and storage [14]. Moreover, the hydrogen sulfide (H_2S) generated by SRB acts as an inhibitor, impeding the growth and metabolism of numerous bacteria, including those responsible for producing rhamnolipids [87]. **Table 3** displays the current advantages and disadvantages of MEOR technology.

Table 3. Benefits and drawbacks of MEOR technology.

Benefits	Drawbacks
• Cost-effectiveness and simplicity of facility setup	• Equipment corrosion

Benefits	Drawbacks
• Affordable injection cost of materials • Minimal energy consumption associated with microbial metabolic activities • Minimal environmental pollution • Progressive enhancement of microbial metabolic activities over time • Achieving improved effects through the concurrent activation of multiple mechanisms • Significantly effective in sandstone and carbonate reservoirs • Ability to apply to both light and heavy crude oils	• Microorganisms' constrained ability to withstand reservoir conditions • The toxicity of microorganisms induced by the heavy metal ions • Challenges in constructing a comprehensive model that covers every aspect of MEOR • Restricted applications on offshore platforms due to the high demand for sugar in anaerobic bacteria activities

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