

Review

# Development of Microbial Consortium and Its Influencing Factors for Enhanced Oil Recovery after Polymer Flooding: A Review

Hui Xiao, Zulhelmi Amir \*  and Mohd Usman Mohd Junaidi 

Department of Chemical Engineering, Faculty of Engineering, Universiti Malaya, Kuala Lumpur 50603, Malaysia; s2178714@siswa.um.edu.my (H.X.); usmanj@um.edu.my (M.U.M.J.)

\* Correspondence: zulhelmi.amir@um.edu.my

**Abstract:** After polymer flooding, substantial oil and residual polymers remain in reservoirs, leading to plugging and reduced recovery. MEOR (Microbial Enhanced Oil Recovery) aims to release trapped oil by utilizing microorganisms and their byproducts. The microorganisms can use residual HPAM (hydrolyzed polyacrylamide) as an energy source for polymer degradation, addressing reservoir plugging issues and improving oil recovery. However, microorganisms are sensitive to environmental conditions. This paper presents a detailed update of MEOR, including microbial products, mechanisms, and merits and demerits. The effect of the displacement fluid and conditions on microorganisms is thoroughly demonstrated to elucidate their influencing mechanism. Among these factors, HPAM and crosslinkers, which have significant biological toxicity, affect microorganisms and the efficiency of MEOR. Limited research exists on the effect of chemicals on microorganisms' properties, metabolism, and oil displacement mechanisms. The development of microbial consortium, their metabolic interaction, and oil displacement microprocesses are also discussed. In addition, prior studies lack insights into microorganisms' interaction and mechanisms using chemicals. Finally, field trials exist to examine the microbial consortium's efficiency and introduce new technologies. This review mainly explores the influencing factors on microorganisms, and confirms the credibility of MEOR after polymer flooding, providing a scientific basis for improving the theory of MEOR.

**Keywords:** MEOR; microbial consortium; mechanism; effect; HPAM and crosslinkers; oil displacement



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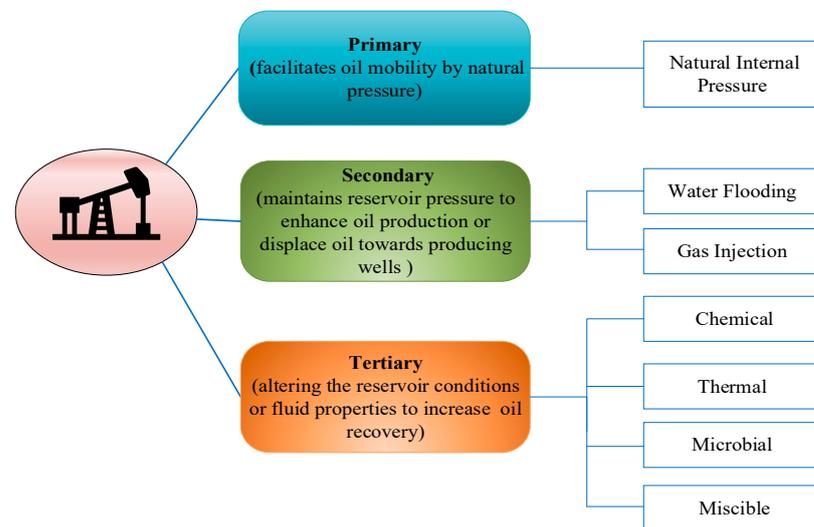
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## 1. Introduction

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.

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].



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

Nowadays, the majority of oil fields have reached the stage of high-water cuts, leading to challenges in exploitation, high costs, low recovery efficiency, and significant pressure [9]. Conventional techniques often come with high costs and complex applications, resulting in a considerable volume of unrecoverable oil once the conventional technologies have reached their economic limit [10]. Hence, there is a need to explore alternative recovery methods. In recent years, microbial enhanced oil recovery (MEOR) has emerged as a prominent research topic in energy due to its environmentally friendly nature and low operating costs [11]. This essential tertiary oil recovery technique utilizes microorganisms and their metabolites [12]. The effectiveness of MEOR hinges on the capability of microbial communities and their metabolites to improve the fluidity of oil [13]. MEOR progress has been extensively reviewed from both theoretical and practical perspectives [14], and it has been applied in various scenarios such as sandstone, carbonate, light oil, heavy oil, marginal reservoirs, post-polymer flooding reservoirs, and low/medium/high-permeability reservoirs [15]. A particular approach involves conducting exploratory investigations on microorganisms to enhance residual oil recovery from reservoirs following polymer flooding, potentially improving oil recovery [7].

Following polymer flooding, a significant amount of remaining oil, approximately 50%, is retained in the reservoir, along with residual polymer that has adsorbed onto the rock formations [5]. Microorganisms alter polymer molecules by breaking the amide group into carboxylic acid through hydrolysis [16]. Nuclear magnetic resonance tests have demonstrated a decrease in the content of the polymer amide group from 74.6% to 60.8%, accompanied by a noticeable increase in the carboxylic group [14]. In a study by Shi et al. [17], five strains of facultative anaerobes were screened from the Daqing oil field in MEOR after polymer flooding. These strains were found to grow and reproduce using oil and polymer as carbon sources. The results revealed that the screened bacteria could produce organic acids and active substances, degrade polymers and crude oil, and enhance oil recovery by 5%. Patel et al. [18] found that the microorganisms involved in MEOR utilize residual HPAM as a nitrogen resource to grow, reproduce, metabolize crude oil, and produce surfactants in micro-pores, which can degrade polymers providing a solution to solve the reservoir plugging problem and improve the oil recovery. Furthermore, all additives used in MEOR are biodegradable and environmentally friendly [19]. Neverthe-

less, the widely used HPAM and its crosslinkers in polymer flooding exhibit substantial biological toxicity and adverse impacts on the characteristics of subsequent oil-displacing microorganisms and oil recovery processes [20,21].

The previous research focuses on improving the oil recovery after polymer flooding, while there are few recent investigations on the effect of the chemicals on oil-displacing bacteria. This review aims to provide insights into and critically explore the influence mechanism of HPAM and crosslinkers on microorganisms and the performance of microorganisms in MEOR, which can provide a scientific basis for improving the theory of microbial oil recovery.

## 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 [14]. According to Quraishi et al. [22], 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 [14]. 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 [23].

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.

### 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 [24]. 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 [25,26]. 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 [27].

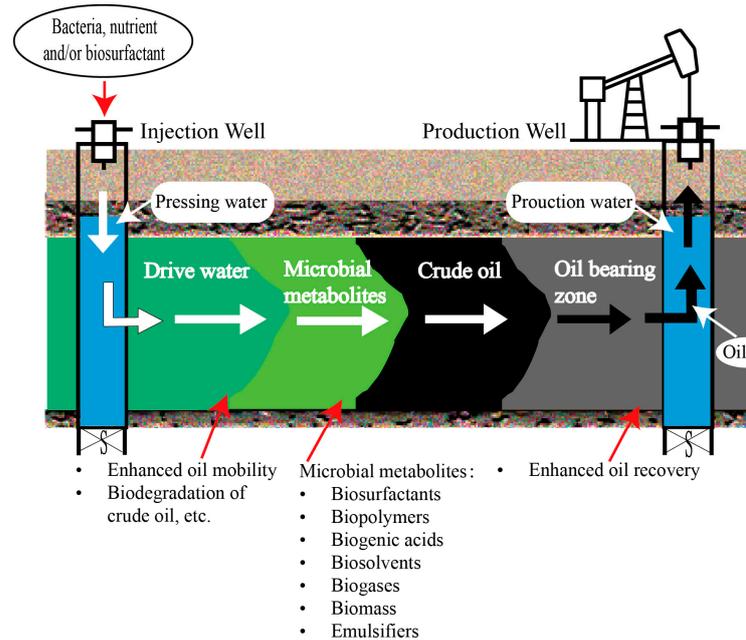


Figure 2. Diagram of MEOR.

The microbial activities in oil reservoirs significantly impact oil’s chemical composition and physical–chemical properties [28]. 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 [29,30]. A large number of studies have highlighted the significance of microbial community dynamics within petroleum oilfield ecosystems [27]. The spatial distribution of microbial communities within long cores plays a role in the mechanism of oil displacement by microorganisms [31]. 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 [12]. 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<sub>2</sub>, CO<sub>2</sub>, small molecule acids, and alcohols through anaerobic fermentation [32]. Within the anoxic conditions of the reservoir’s deep environment, methanogenic microbes increase crude oil fluidity by generating CH<sub>4</sub> [33]. Figure 3 depicts the distribution of microorganisms in MEOR.

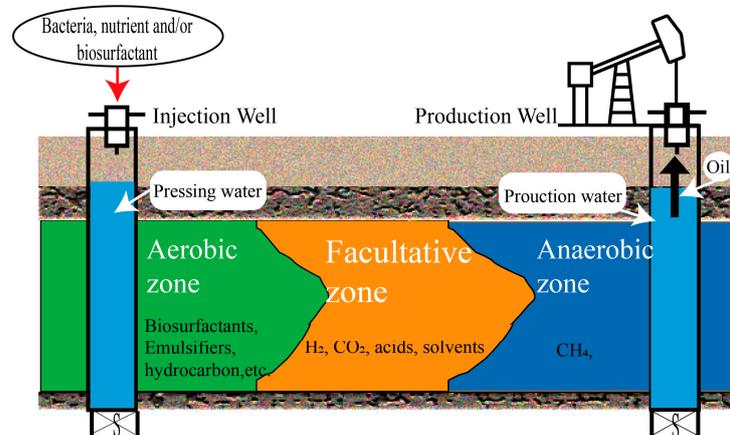


Figure 3. The spatial distribution of microorganisms in MEOR.

Oil reservoirs are known for adverse environments characterized by high temperature, high pressure, high salinity, and stringent anoxic conditions [25,34]. 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 [35]. A biofilm matrix acts as a protective coating, providing resistance against external stressors in oil fields [36]. 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 [37]. 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 [35], forming gel-like networks that confer mechanical stability to biofilms in oil environments [38]. EPS contain diverse functional groups, such as carboxyl, phosphoric, sulfhydryl, phenolic, and hydroxyl groups, contributing to cell aggregation and attachment to various surfaces [37]. 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 [39]. Furthermore, EPS play a significant role in oil displacement mechanisms, influencing oil through processes such as emulsification, degradation, dispersion, aggregation, and sedimentation [40]. 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. [41] 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 [42].

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 [42]. 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 lipopro-

teins, and polymeric surfactants [43]. 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 [44,45]. 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 [46].

As stated by Niu et al. [47], 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  $10^{-4}$  [48].

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 [49]. 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.

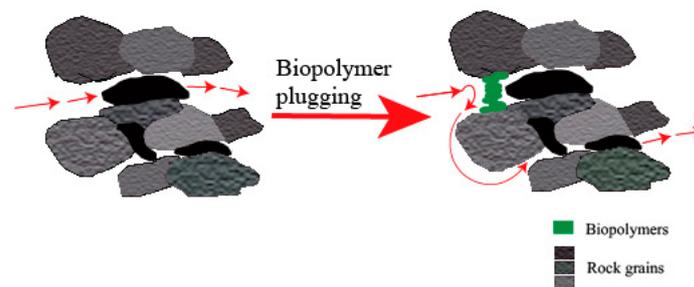
Biosurfactants offer several advantages for EOR applications, including their biodegradability, outstanding surface and interfacial activities, low toxicity and capital cost, enough raw materials for production, and substantial productivity. These attributes make biosurfactants a cost-effective and efficient 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 [50]. 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 [45,51].

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 [52]. 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 [53], 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. [52] 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 [54]. 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 [55].

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 [18]. Generally, low molecular weight organic acids like formic acid, acetic acid, and propionic acid are commonly employed in MEOR [19]. 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 [14].

### 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 [18]. Furthermore, solvents contribute to the reduction in oil viscosity and the decrease in IFT between water and oil [55].

#### (2) Biogases

Certain microorganisms capable of in situ gas production by fermenting carbohydrates or hydrocarbons like  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{H}_2$  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 [55]. 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 [10,56].

### (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 [18].

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.

## 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 [64]. 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 [19]. A thorough comprehension of the mechanisms of MEOR lays the groundwork for evaluating its practicability and prospective benefits.

Table 1. Microbial products.

Microbial Products	Representative Microorganisms	Effect	Limitation	Type of Formation/Reservoir	Reference
<ul style="list-style-type: none"> <li>Biosurfactant (Alasan, Surfactin, Rhamnolipid, Emulsan)</li> </ul>	<ul style="list-style-type: none"> <li><i>Acinetobacter</i></li> <li><i>Arthrobacter paraffineus</i> sp.</li> <li><i>Calcoaceticus</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Emulsify crude oil into an oil–water mixture.</li> <li>Decrease IFT.</li> <li>Modify rock wettability.</li> </ul>	<ul style="list-style-type: none"> <li>Low displacement efficiency at the microscopic level.</li> </ul>	<ul style="list-style-type: none"> <li>Reservoirs composed of sandstone or carbonate formations with temperatures below 50 °C and containing lighter oils with an API gravity greater than 25.</li> </ul>	[57]
<ul style="list-style-type: none"> <li>Biopolymers (Xanthan gum, Pullulan, Levan)</li> </ul>	<ul style="list-style-type: none"> <li><i>Bacillus polymyxa</i> sp.</li> <li><i>Pseudomonas</i> sp.</li> <li><i>Brevibacterium viscogenes</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Raise the viscosity of the displacing fluid while diminishing the mobility ratio between water and oil.</li> <li>Optimize the sweep regions and effectiveness with selective plugging.</li> </ul>	<ul style="list-style-type: none"> <li>Low efficiency in sweeping fluids across the volume.</li> </ul>	<ul style="list-style-type: none"> <li>Stratified reservoirs exhibiting distinct permeable zones.</li> </ul>	[58]
<ul style="list-style-type: none"> <li>Bioacids (Acetic acid, formic acid, propionic acid)</li> </ul>	<ul style="list-style-type: none"> <li><i>Clostridium</i> sp.</li> <li><i>Enterobacter aerogenes</i> sp.</li> <li><i>Methanobacterium</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Improve the rock’s dissolution in the pore throats to increase porosity and permeability.</li> </ul>	<ul style="list-style-type: none"> <li>Low porosity.</li> <li>Inefficient fluid drainage.</li> <li>Reservoir impairment.</li> </ul>	<ul style="list-style-type: none"> <li>Carbonate or carbonaceous reservoirs</li> </ul>	[59]
<ul style="list-style-type: none"> <li>Biogases (CO<sub>2</sub>, H<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>)</li> </ul>	<ul style="list-style-type: none"> <li><i>Clostridium</i> sp.</li> <li><i>Enterobacter aerogenes</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Enhance the mobility of crude oil by reducing its viscosity.</li> <li>Re-establish reservoir pressure</li> <li>Gas injection for partial or miscible displacement.</li> </ul>	<ul style="list-style-type: none"> <li>Equipment corrosion.</li> <li>Reservoir souring.</li> </ul>	<ul style="list-style-type: none"> <li>Formations containing heavy crude oil with an API gravity of less than 25.</li> </ul>	[22]
<ul style="list-style-type: none"> <li>Biosolvents (Alcohols, ketones, acetone, butanol)</li> </ul>	<ul style="list-style-type: none"> <li><i>Zymomonas mobilis</i> sp.</li> <li><i>Clostridium acetobutylicum</i> sp.</li> <li><i>Klebsiella</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Decrease the viscosity of oil through its dissolution in crude oil.</li> <li>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.</li> <li>Optimize the porosity and permeability by dissolving heavy oil in the pore throats.</li> </ul>	<ul style="list-style-type: none"> <li>Low displacement efficiency at the microscopic level.</li> </ul>	<ul style="list-style-type: none"> <li>Formations containing heavy crude oil with an API gravity of less than 25.</li> <li>Reservoirs with high oil wettability have undergone water flooding.</li> </ul>	[60]

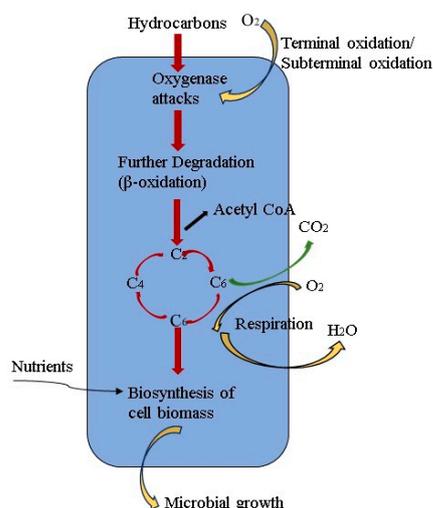
Table 1. Cont.

Microbial Products	Representative Microorganisms	Effect	Limitation	Type of Formation/Reservoir	Reference
<ul style="list-style-type: none"> <li>Biomass (Microbial cells, biofilms, EPS)</li> </ul>	<ul style="list-style-type: none"> <li><i>Licheniformis</i></li> <li><i>Bacillus</i></li> <li><i>Xanthomonas</i></li> <li><i>Leuconostocmesenteroides</i></li> <li><i>Campestris</i></li> </ul>	<ul style="list-style-type: none"> <li>Change rock wettability.</li> <li>Employ selective plugging to optimize sweep effectiveness.</li> <li>Gas-based biopolymers.</li> <li>Specific decomposition of heavy oil.</li> </ul>	<ul style="list-style-type: none"> <li>Low displacement efficiency at the microscopic level.</li> </ul>	<ul style="list-style-type: none"> <li>Stratified reservoirs exhibiting distinct permeable zones.</li> </ul>	[61]
<ul style="list-style-type: none"> <li>Emulsifiers</li> </ul>	<ul style="list-style-type: none"> <li><i>Candida</i></li> <li><i>Acinetobacter</i> sp.</li> <li><i>Bacillus</i> sp.</li> <li><i>Pseudomonas</i> sp.</li> </ul>	<ul style="list-style-type: none"> <li>Emulsification of oil with water.</li> </ul>	<ul style="list-style-type: none"> <li>Sludge buildup.</li> <li>Low displacement efficiency at the microscopic level.</li> </ul>	<ul style="list-style-type: none"> <li>Formation containing high-molecular-weight waxy oil (&gt;C<sub>22</sub> alkanes), paraffinic oil, and asphaltene.</li> </ul>	[62,63]

### 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 [14]. From one perspective, through this process, bacteria can obtain carbon for energy, growth, and reproduction by degrading hydrocarbons in crude oil [65]. 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 [66]. Biosurfactants are commonly utilized to degrade the heavy fractions of crude oil.

Biodegradation pathways can be classified into aerobic and anaerobic biodegradation [19]. 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 [14]. 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 [41]. Cell biomass biosynthesis occurs in the central precursor metabolites such as acetyl-CoA, succinate, and pyruvate [67], as depicted in Figure 5.



**Figure 5.** The fundamental concept underlying aerobic hydrocarbon degradation by microorganisms [68].

Anaerobic degradation has demonstrated the capability of biodegrading various hydrocarbons, including benzene, toluene, naphthalene, asphaltene, phenanthrene, alkane, branched alkane, and hydrocarbon mixtures [69]. Nonetheless, the application of anaerobic degradation in MEOR is restricted, possibly because of the choice of unsuitable oil-degrading microorganisms or suboptimal conditions [70]. 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 [69].

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

Microorganisms	Range of Degradation	Reference
<i>Rhodococcus erythropolis</i>	C <sub>10</sub> –C <sub>30</sub>	[71]
<i>Pseudomonas fluorescens</i>	C <sub>12</sub> –C <sub>32</sub>	[72]
<i>Thermophilic Bacillus</i>	C <sub>15</sub> –C <sub>36</sub>	[73]
<i>Gordonia amici</i>	C <sub>18</sub> –C <sub>36</sub>	[74]
<i>Chelatococcus daeguensis</i>	C <sub>23</sub> –C <sub>37</sub>	[75]
<i>Pseudomonas aeruginosa</i>	C <sub>36</sub> –C <sub>40</sub>	[76]

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. [77], 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 [19]. Nevertheless, a complete understanding of the biochemical processes involved in anaerobic hydrocarbon mechanisms is still being explored and investigated.

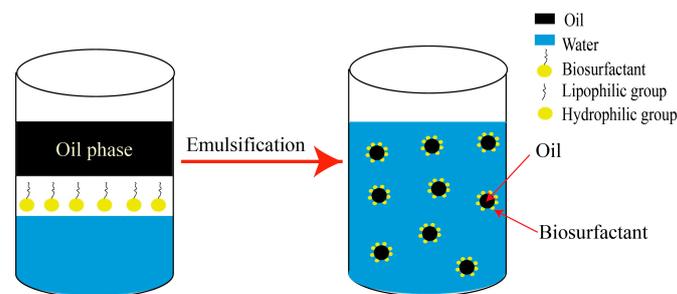
In summary, biodegradation plays a crucial role in MEOR, as research indicates that microorganisms and their products can degrade carbon atoms by utilizing them as a carbon source for metabolic processes. As a consequence, the viscosity of the oil is reduced, and the flow properties are improved; hence, 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 [78]. 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) [79]. Emulsification can change the reservoir’s wettability, shifting it from oil-wet to water-wet, thus making trapped oil easier to recover [14].

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 [80]. 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

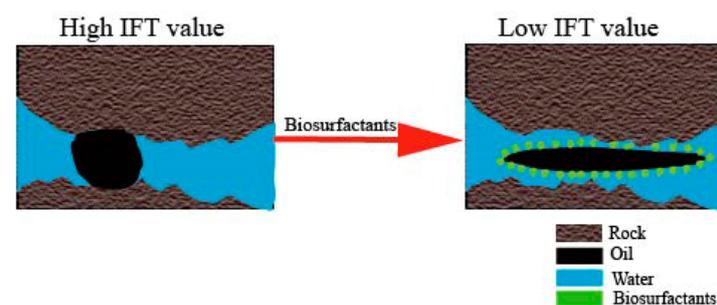
movement until the biosurfactant is either diluted or completely adsorbed on the caprocks. Emulsification of crude oil leads to the generation of various droplet sizes, exhibiting tensile, deformed, and seepage flow characteristics, which alter the permeability of the reservoir, and increase the contact area between the droplets and the reservoir medium, thereby facilitating the flow and recovery of oil [14]. 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.

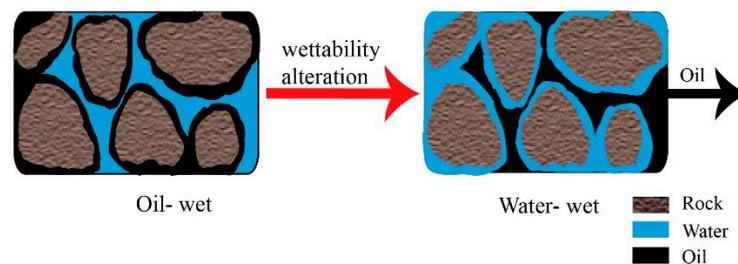
### 2.3.3. Interfacial Tension Reduction

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 [81]. Secondly, the elasticity of the oil/water interface is changed, enabling the two phases to mix more easily [82]. 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 [83]. The formation of foams using organic materials also can lead to a reduction in IFT at the oil/water interface [23]. A visual representation of reducing IFT can be seen in Figure 7.



**Figure 7.** Graph of IFT reduction.

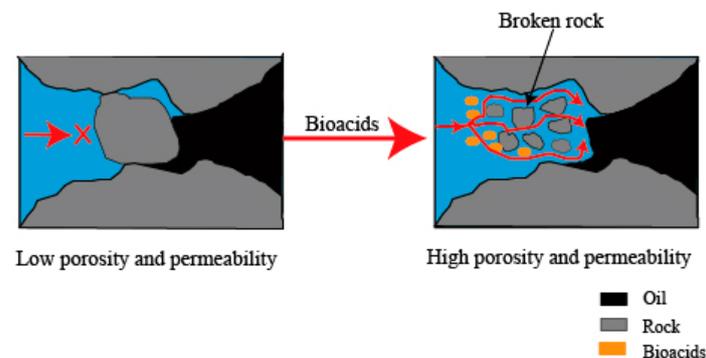
IFT reduction and wettability alteration are closely related mechanisms that occur concurrently and contribute significantly to EOR [84]. The reduction in IFT increases the capillary number by 3–4 orders of magnitude, resulting in improved oil flow. Simultaneously, wettability alteration promotes oil production by supporting the mobilization and displacement of oils attached to the rock surface or trapped in the pores [85,86]. The wettability condition of the rocks significantly influences the oil recovery [84]. 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 [84]. 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 [14]. The mechanism is believed to predominantly occur in carbonate reservoirs [87]. 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 [88]. 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 [87]. The modification of wettability accelerates oil production by improving the permeability of injected water within the reservoir matrix and boosting the capillary pressure [89]. In porous media, fluids' location, distribution, and flow are governed by the wettability of rock/fluid systems [90]. 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 [91]. 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 [92]. Compared to thermal flooding and gas flooding, microbial flooding exhibits notable advantages, primarily its eco-friendly attributes and cost-effectiveness for augmenting oil production [50]. 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 [93]. 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.

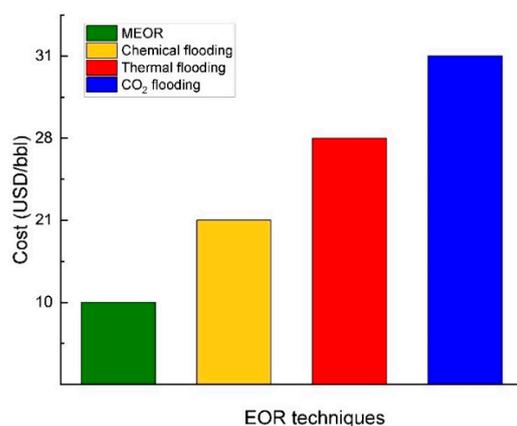


Figure 10. Estimations of cost for various EOR techniques [14].

There are also some disadvantages of MEOR. For example, the presence of SRB in MEOR is recognized for its detrimental effects [94], leading to corrosion, plugging, and reservoir souring issues in oil production, transportation, and storage [26]. Moreover, the hydrogen sulfide (H<sub>2</sub>S) generated by SRB acts as an inhibitor, impeding the growth and metabolism of numerous bacteria, including those responsible for producing rhamnolipids [95]. Table 3 displays the current advantages and disadvantages of MEOR technology.

Table 3. Benefits and drawbacks of MEOR technology.

Benefits	Drawbacks
<ul style="list-style-type: none"> <li>• Cost-effectiveness and simplicity of facility setup</li> </ul>	<ul style="list-style-type: none"> <li>• Equipment corrosion</li> </ul>
<ul style="list-style-type: none"> <li>• Affordable injection cost of materials</li> <li>• Minimal energy consumption associated with microbial metabolic activities</li> <li>• Minimal environmental pollution</li> <li>• Progressive enhancement of microbial metabolic activities over time</li> <li>• Achieving improved effects through the concurrent activation of multiple mechanisms</li> <li>• Significantly effective in sandstone and carbonate reservoirs</li> </ul>	<ul style="list-style-type: none"> <li>• Microorganisms' constrained ability to withstand reservoir conditions</li> <li>• The toxicity of microorganisms induced by the heavy metal ions</li> <li>• Challenges in constructing a comprehensive model that covers every aspect of MEOR</li> <li>• Restricted applications on offshore platforms due to the high demand for sugar in anaerobic bacteria activities</li> </ul>
<ul style="list-style-type: none"> <li>• Ability to apply to both light and heavy crude oils</li> </ul>	

### 3. The Effect of a Reservoir Fluid on Microorganisms

#### 3.1. Crude Oil

Crude oil is commonly classified into four primary categories: saturated hydrocarbons, aromatic hydrocarbons, asphaltenes, and resins, which is a mixture composed of hydrocarbons and various heteroelements such as oxygen, sulfur, nitrogen, and trace metals [96]. The properties of oil can exert a notable influence on microorganisms and subsequently affect the oil recovery process [97]. The oil's viscosity plays a crucial role in determining the concentration of metabolites needed for the dissolution, emulsification, or dispersion of oil [42]. Heavy oil's high viscosity and low flow properties pose challenges in oil extraction from the reservoir [98]. Among light components, alkanes with long carbon numbers may produce varying degrees of microbial activity [99].

In addition, heavy components in crude oil, such as bitumen, adversely affect microbial activity [100]. Moreover, volatile light components are toxic to microorganisms. Compounds such as sulfides, aromatics, and phenols are toxic and inhibit microbial growth and metabolism. Because microorganisms need a certain amount of water to carry out metabolic activities, the moisture content in crude oil also affects the activities of microorganisms [101].

#### 3.2. Injection Water

Water injections are essential for MEOR. The quantity of water injected relies on the well maturity, reservoir pressure, or water displacement in the oil reservoir. Through water injection, exogenous microorganisms, new electron donors, and acceptors are introduced into the oil reservoir [102]. A multitude of researchers has explored microbial variabilities, metabolic activities, and the composition of microorganisms within oil reservoirs subjected to water flooding.

In certain reservoirs, chemicals or nutrients are added to the injection waters to control the native microbial structure [103]. For example, nitrite and nitrate injections are implemented to mitigate reservoir souring resulting from microbial hydrogen sulfide production. Injecting oxygen can improve aerobic hydrocarbon metabolism and facilitate oil mobilization within the well. This is accomplished by lowering the IFT between the oil and water phases by the activity of biosurfactant-producing microorganisms or changes in the oil matrix [104]. Alternatively, injecting fermentative bacteria and carbohydrates produces acids, gases, and solvents, leading to an augmentation in oil production [105].

Water injections induce a lowering in the oilfield temperature, creating a temperature gradient. Moreover, the quantity of injection impacts the chemical composition of the production water, leading to decreased levels of sulfate, nitrate, potassium, nitrite, and magnesium [106]. Production water, a byproduct of oil extraction, is transported alongside the oil phase and brought to the surface. It constitutes a combination of formation and injection water, potentially carrying particles and soluble compounds from the oil [107]. Two comparative studies have indicated that formation water exhibits higher levels of fatty acids and ammonium than production water [106]. Produced fluids with an injection water content below 10% demonstrated no notable effect on microbial community and metabolic potential.

Conversely, it was shown that fluids containing a higher proportion of injected water could affect the microbial structure and metabolic potential due to variations in the water component. A strong association was observed between the relative abundance of the genus family *Deferribacteres*, *Flexistipes*, the percentage of injection seawater, and the levels of nitrite, nitrate, magnesium, sulfate, and potassium. Some wells with the highest water injection cut exhibited a higher abundance of *Epsilonproteobacteria* and *Gammaproteobacteria* isolates [106]. Numerous research studies have documented a correlation between the chemical component of oil reservoirs and the presence of operational taxonomic units (OTUs) within them. In Algerian oil fields, notable associations were observed between the relative abundance of bacterial OTUs or phyla and the concentrations of  $K^+$  and  $Cl^-$  ions in the production waters [103].

Nonetheless, it remains uncertain whether this relevance is causative. It is plausible that the disparities in microbial community composition may arise due to factors such as variations in water content and reservoir structure, and the ion composition may merely indicate geological dissimilarities [103]. Distinct microbial structure differences between injection and production waters were evident in the offshore Halfdan and Algerian oilfields [108]. In the Algerian oilfields, the injection water exhibited higher cell richness, and bacteria predominantly populated it. In contrast, the production water had approximately one-tenth of the cell count and was mainly dominated by *Archaea* [106]. This disparity suggests a negligible relationship between oil degradation processes in the methanogenesis-dominated reservoir and the microbial structure in the production water.

### 3.3. The Residual Chemicals

#### 3.3.1. Hydrolyzed Polyacrylamide (HPAM)

The concentration and composition of residues can have varying effects on microorganisms, potentially impacting negatively on the production efficiency of the field. Chemicals dissolved in formation water affect the morphology, structure, and growth metabolism of microorganisms that passively influence oil recovery. HPAM and crosslinkers are the common chemical components after polymer flooding, and this review focuses on them.

PAM (polyacrylamide) is derived from the acrylamide monomer and is commonly employed in its hydrolyzed form, known as HPAM [109]. HPAM is synthesized by either copolymerizing sodium acrylate with acrylamide or partially hydrolyzing polyacrylamide and polyacrylic acid [110], which is a water-soluble, stable, anionic, and linear polymeric compound comprising high molecular weight chains composed of acrylamide ( $C_3H_5NO$ ) monomers [111]. While HPAM is not considered toxic, it is important to note that acrylamide, the starting building block used in its synthesis, is classified as a substance of very high concern [112]. The main concern revolves around the possible presence of residual acrylamide monomer in HPAM polymeric material and the associated risks linked to its degradation [113]. This degradation process can result in the conversion of acrylamide ( $CH_2=CHCONH_2$ , depicted in Figure 11). Acrylamide is a toxic chemical to many microorganisms and is included in the candidate list for authorization due to its potential health and environmental risks [114].

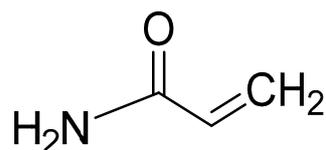


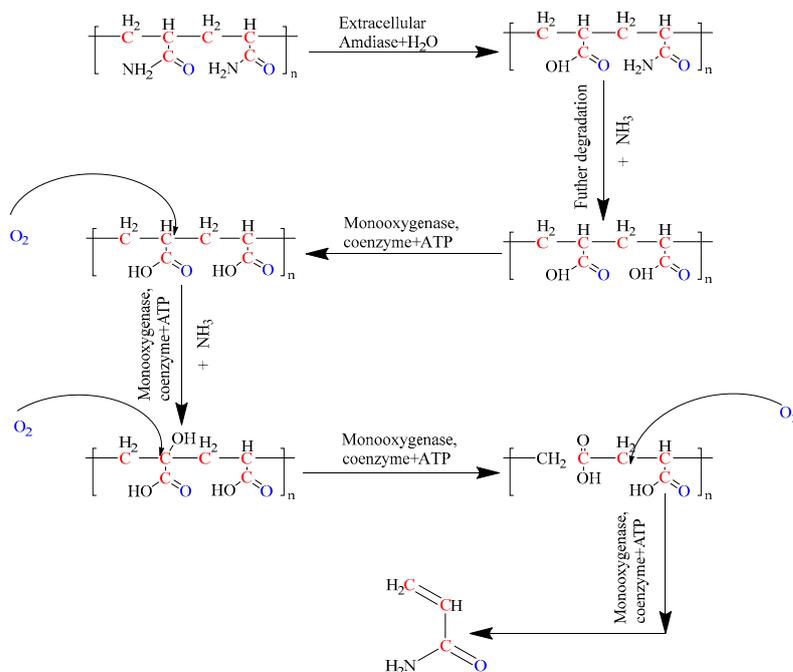
Figure 11. The chemical structure of acrylamide.

HPAM can usually be crosslinked to form a gel using a crosslinker to improve the polymer's performance, creating a highly controllable polymer network in the well. The categorization of cross-linked polymers as metallic or organic cross-linked systems is determined by the type of cross-linker employed in the process [115]. Inorganic cross-linked polymer gels that are commonly used involve the utilization of trivalent cations such as aluminum ( $Al^{3+}$ ) and chromium ( $Cr^{3+}$ ) as cross-linkers [116]. Metallic crosslinkers, including  $Cr^{3+}$  and  $Al^{3+}$ , have been identified for their toxicity and potential detrimental effects on microorganisms [117]. These microorganisms play a direct role as active or passive promoters of microbial metabolism [118].

Furthermore, microorganisms associated with changes in metal speciation can result in variations in metal mobility, resulting in both favorable and unfavorable consequences [119]. Polyethyleneimine (PEI) and a combination of phenol and formaldehyde are widely employed as organic cross-linkers for polymer gel formation [120]. Although the PEI crosslinker is considered non-toxic and environmentally friendly, its high cost restricts its widespread application [121]. Phenol and formaldehyde are cost-effective, but

the primary concern is their toxicity, with formaldehyde mainly known for its carcinogenic properties. Research has identified alternative substitutes, such as hydroquinone (HQ) and hexamethylenetetramine (HMTA), which exhibit lower toxicity compared to phenol and formaldehyde [120].

Following polymer flooding, reservoirs contain diverse toxic components, including residual chemicals, crosslinking agents, and heavy metals [20]. Regarding residual metals, microorganisms have the capacity to induce significant alterations in metal composition, amplifying or mitigating the toxicity of metals. Moreover, microorganisms can influence mineral precipitation, formation, dissolution, and deterioration due to the diverse properties exhibited on microbial-charged surfaces and metabolic activities [122]. In the context of the residual presence of HPAM/PAM in the reservoir, an observed phenomenon is the potential blockage of reservoir pores. Based on the former research, the biodegradation of PAM/HPAM takes place while microorganisms apply the carbon backbone of the polymer as a carbon source or the amide group of the polymer as a nitrogen source [123]. Observations indicate that microorganisms use acrylamide, PAM, and its derivatives as their exclusive carbon or nitrogen sources. In MEOR, certain microorganisms like *Bacillus* can utilize HPAM as a nitrogen or carbon source, enabling their growth, reproduction, and metabolization of crude oil within micro pores. This microbial activity produces surfactants, which aid polymer degradation, enhance crude oil flow, and modify reservoir properties [12]. Under aerobic and anaerobic conditions, certain bacterial species within the genera *Enterobacter* sp., *Azomonas* sp., and *Clostridium* sp. have exhibited the ability to degrade PAM/HPAM, achieving degradation rates ranging from 16% to 91% [124]. Microbial degradation of the polymer results in a decrease in molecular weight, viscosity, and the conversion of amide nitrogen into ammonia, so bacterial degradation of PAM/HPAM typically leads to modifications in the structure of the polymer. The transformation of amide groups (NH<sub>2</sub>) into carboxylic acid (COOH) in PAM or HPAM has been consistently demonstrated through the application of analytical methods such as FT-IR, LC/MS, and GC/MS [125]. A proposed degradation pathway of PAM and HPAM, leading to the formation of lower molecular weight products like acrylamide, is depicted in Figure 12.



**Figure 12.** The pathway for the biodegradation of PAM and HPAM [124].

However, microorganisms are affected by the reservoir environment, especially HPAM, and crosslinkers have enormous biological toxicity and adverse effects on the properties of

microorganisms and the oil displacement effect [124,126]. On the one hand, the residual agents after polymer flooding affect the growth and structure of microorganisms. According to Daqing Oilfield and Shengli Oilfield researchers, the number of microorganisms in water following polymer flooding is two orders of magnitude lower than that after water flooding [127]. Compared with water-flooded oil wells, the prevailing bacteria and archaea exhibited dissimilarity within alkali-surfactant-polyacrylamide (ASP)-flooded oil wells [128]. In both polymer-flooded and ASP-flooded production wells, *Arcobacter* and *Pseudomonas* were prevalent, while *Halomonas* was exclusively observed in the latter [21,128]. The outcomes imply that additional studies are necessary to delve into the potential effects of polymer flooding on the indigenous microbial structure in oil reservoirs.

On the other hand, after polymer flooding, there was a substantial reduction in microbial community diversity, accompanied by an increase in the prevalence of strains capable of degrading polymers. Huang et al. [129] studied microbial community structure and diversity of reservoirs after polymer flooding in the Daqing oil field at the earlier stage of microbial profile modification, which showed that the microbial community structure had undergone significant changes for the impact of exploitation by the polymer flooding. Ren et al. [21] found that the structure composition and abundance of dominant bacteria of endogenous bacteria were significantly changed after polymer flooding.

HPAM and crosslinkers in low concentrations can cause obvious negative impacts on microorganisms. Still, the primary focus of the research is centered on enhancing oil recovery following polymer flooding. At the same time, there is a lack of research on the physiological and biochemical properties, metabolic cycle, and mechanism of the influence of chemicals on the processes of MEOR.

### 3.3.2. Polymer Nanospheres

In the realm of petroleum field development, nanotechnology has emerged as a promising avenue for EOR applications, thanks to the benefits of nanoparticles (NPs), including their outstanding properties [130]. NPs can be categorized into inorganic NPs and organic NPs [4]. Inorganic NPs encompass metal oxide, ceramic, metal, and semiconducting NPs, whereas organic NPs include polymeric NPs and biomolecules [131]. Polymer-based NPs are colloidal entities composed of non-toxic, small, stable, water-soluble, and biodegradable polymers. Polymer NPs come in two varieties: nanospheres and nanocapsules [132].

Due to the nano size, selective water absorption, high water-absorbing capacity, tolerance to brine, low viscosity in aqueous solutions, effective dispersion in water, and other properties, nanospheres can readily penetrate interior layers to achieve comprehensive profile control, water shutoff, and flooding, with the ultimate goal of enhancing sweep efficiency [130,133]. Polymer nanospheres (PNPs) exhibit a positive impact on EOR. They have been utilized in low permeability oilfields within China and have demonstrated strong applicability in the Changqing Oilfield [133,134]. For example, polymer nanospheres and microspheres have been implemented in water mobility control, both on a pilot scale and across full fields, yielding remarkable outcomes in reducing water production, enhancing sweep efficiency, and auguring oil recovery [135]. Nonetheless, a comprehensive exploration of the mechanisms underlying the effectiveness of PNPs in enhanced oil recovery has not been conducted.

While PNPs offer numerous advantages, including cost-effectiveness, low toxicity, and exceptional durability, they also have the potential to inhibit microbial activity. Li et al. achieved the successful synthesis of antimicrobial materials through the conjugation of PNPs with Salphen Ce (IV) complexes (HSpCe) [136]. The research revealed that HSpCe@PNPs exhibited exceptional antibacterial efficacy, surpassing that of HSpCe alone. This enhancement in antibacterial activity is attributed to the synergistic antibacterial effects between HSpCe and PNPs.

PNPs are employed in EOR as an emerging technology, aiding in surmounting significant constraints associated with chemical EOR and enhancing its effectiveness. PNPs offer numerous exceptional advantages but exhibit inhibitory effects on microbial activity. More-

over, research efforts primarily focus on improving oil recovery, with a limited exploration of the mechanisms through which chemicals influence microorganisms in MEOR.

#### 4. The Effect of Reservoir Conditions on Microorganisms

The effectiveness of MEOR is heavily influenced by the reservoir environment, including temperature, pressure, salinity, pH, permeability, porosity, crude oil viscosity, etc. [11,29]. Physicochemical conditions within oil reservoirs can profoundly affect microbial survival, growth, and metabolites [34]. Consequently, these physicochemical conditions directly impact the microbial concentration and, in turn, influence the MEOR [137].

##### 4.1. Temperature

In the process of MEOR, temperature stands out as the foremost factor that determines the microbial structure and imposes constraints on microbial growth, metabolism, and activity [106,138]. Due to the temperature-dependent metabolic rate of bacteria, the efficiency of transport processes can be hindered at low temperatures, while excessively high temperatures can adversely affect enzymes and proteins, disrupting vital cellular activities [139]. Therefore, the microbial concentration within the reservoir, biosurfactant production, and ultimately the residual oil saturation of the reservoir varies as a function of temperature.

Petroleum oil reservoirs typically exhibit a natural temperature range from 10 to 124 °C [140]. Based on the findings of Maudgalya et al. [141], the majority of MEOR field trials have temperature limitations below 100 °C. To be specific, aerobic microorganisms are predominantly present in formations with temperatures ranging from 70 °C to 90 °C, whereas anaerobic microorganisms and facultative anaerobic microorganisms typically thrive in oil reservoirs at relatively low temperatures, usually from 30 °C to 60 °C [142]. According to Sivasankari and Govindarajan [143], microbes utilized in enhanced oil recovery processes must withstand the average temperature conditions prevalent in the reservoir.

Firstly, temperature directly influences the growth of microorganisms, which have an optimal temperature range for growth, and exceeding this range can significantly impact their growth and activity [103]. Laboratory experiments indicated that for a given microorganism, an increase in temperature from 37 °C to 73 °C resulted in a substantial decrease in bacterial concentration, and when the temperature surpassed 80 °C, the growth rate experienced a significant reduction and became extremely slow [14]. Specific temperature conditions dictate the suitability of microorganisms to inhabit the reservoir [103].

In addition, there was a notable alteration in the structure of the microbial community as the formation temperature increased [144]. Li et al. [142] employed a culture-independent approach involving CARD-FISH, qPCR, and 16S rDNA to examine the abundance, community structure, and diversity of microbes across a significant thermal gradient (50–90 °C) in the Tengchong Geothermal Field. Their findings revealed that temperature strongly influenced microbial community structure, cell growth, and activity, and the number of microorganisms exhibited a negative correlation with temperature. Moreover, at higher temperatures within the oil reservoir, there was a reduction in the diversity of the dominant bacterial species. Lin et al. [144] compared two oil reservoirs, R-A and R-G, which exhibited similar salinity and permeability but differed in temperature, with R-A being at a milder temperature of 55 °C and R-G at a higher temperature of 71 °C. The results indicated that the R-A reservoir contained a greater variety of bacterial species and displayed more intricate predominant metabolic pathways than R-G. Previous research revealed reservoirs with moderately hot temperatures around 55 °C observed the highest microbial diversity. Microorganisms inhabiting oil reservoirs exhibit varying temperature preferences, as indicated in Table 4.

**Table 4.** Bacteria and archaea linked to temperature ranges within reservoirs.

Temperature Optimum	Phylum/Class	Order/Genus	References
• Ubiquitous	<ul style="list-style-type: none"> <li>• Proteobacteria/Epsilonproteobacteria</li> <li>• Proteobacteria/Gammaproteobacteria</li> <li>• Euryarchaeota</li> <li>• Acinetobacter</li> <li>• Proteobacteria/Alphaproteobacteria</li> </ul>	<ul style="list-style-type: none"> <li>• Campylobacterales/Sulfurospirillum/Arcobacter</li> <li>• Pseudomonadales/Pseudomonas</li> <li>• Rhizobiales//Sphingomonas/Sphingomonadales/Rhizobium</li> </ul>	[145]
• Only above 50 °C	<ul style="list-style-type: none"> <li>• Atribacteria</li> <li>• Acidobacteria</li> <li>• Crenarchaeota/Thermoprotei</li> <li>• Euryarchaeota/Halobacteria</li> <li>• Thaumarchaeota/Nitrososphaeria</li> <li>• Euryarchaeota/Thermoplasmata</li> <li>• Euryarchaeota/Archaeoglobi</li> </ul>	<ul style="list-style-type: none"> <li>• Atribacter</li> <li>• Acidobacterium/Terriglobus</li> <li>• Fervidicoccales/Sulfolobales/Thermoproteales</li> <li>• Halobacteriales/Haloferacales</li> <li>• Nitrososphaerales/Nitrospira</li> <li>• Thermoplasmatales</li> <li>• Archaeoglobales</li> </ul>	[105,146]
• Mostly above 50 °C	<ul style="list-style-type: none"> <li>• Firmicutes/Clostridia</li> <li>• Firmicutes/Bacilli</li> <li>• Euryarchaeota/Thermococci</li> <li>• Euryarchaeota/Halobacteria</li> <li>• Euryarchaeota/Thermoplasmata</li> <li>• Proteobacteria/Hydrogenophilalia</li> <li>• Proteobacteria/Alphaproteobacteria</li> <li>• Firmicutes/Clostridia</li> <li>• Thermotogae</li> <li>• Bacteroidia</li> <li>• Deinococcus-Thermus/Deinococci</li> <li>• Nitrospirae/Nitrospira</li> <li>• Actinobacteria/Thermoleophilia</li> </ul>	<ul style="list-style-type: none"> <li>• Clostridiales/Thermosyntropha</li> <li>• Bacillales/Bacillus/Ancrobacillus</li> <li>• Thermococcales</li> <li>• Halobacteriales/Halogeometricum</li> <li>• Thermoplasmatales/Thermogymnomonas</li> <li>• Hydrogenophilales/Tepidiphilus</li> <li>• Rhodospirillales/Tistrella</li> <li>• Thermoanaerobacterales/Thermoanaerobacter</li> <li>• Kosmotoua</li> <li>• Thermals/Thermus</li> <li>• Nitrospirales/Thermodesulfovibrio</li> </ul>	[106,145,147]
• Mostly below 50 °C	<ul style="list-style-type: none"> <li>• Planctomycetes</li> <li>• Spirochaetes</li> <li>• Synergistetes</li> <li>• Chloroflexi</li> <li>• Proteobacteria</li> <li>• Euryarchaeota</li> <li>• Crenarchaeota</li> </ul>	<ul style="list-style-type: none"> <li>• Planctomyces/Gemmata/Isosphaera/Pirellula/Rhodopirellula/Brocadia</li> <li>• Treponema/Borrelia/Leptospira</li> <li>• Aminobacterium/Synergistes/Thermovirga/Jonquetella</li> <li>• Anaerolinea/Dehalococcoides/Chloroflexus/Herpetosiphon/Caldilinea</li> <li>• Marinobacterium/Paracoccus/Donghicola</li> <li>• Methanosarcinales/Methanomicrobiales/Methanocellales/Methanocorpusculum/Methanolinea</li> <li>• Desulfurococcales</li> </ul>	[105,145,146]

Furthermore, a correlation has been observed between the occurrence of functional genes of microorganisms and reservoir temperature. Cai et al. [148] examined four production wells and detected a progressive increase in the relative abundance of genes associated with the degradation of aromatic compounds, carbon cycling, and the metabolism of other organic compounds as the temperature and depth of the oil-containing stratum decreased. Correspondingly, there was a reduction in genes related to stress response, antibiotic resistance, and sulfur metabolism with diminishing temperature. For instance, optimal oil degradation rates are observed at lower temperatures, while microbial activity within reservoirs is largely absent beyond 80 °C. Therefore, general predictions can provide valuable indications for determining the in situ temperature of a reservoir. However, it is still impossible to accurately determine microbial community composition solely based on temperature.

The global demand for hydrocarbons and subsequent exploration efforts have recently shifted towards deep and ultra-deep environments, where reservoir temperatures frequently exceed 100 °C. The studies conducted by Li et al. [149] and Junzhang et al. [150] highlighted the impact of high-temperature oil reservoirs on microbial activities during oil recovery. They emphasized the necessity of identifying and isolating high thermophilic microorganisms suitable for MEOR in deep and ultra-deep reservoirs. The Jiangnan Oil-field in central China documented the cultivation of thermophilic bacteria, specifically *Geobacillus kaustophilus*, capable of thriving at 100 °C, and a pilot test for paraffin removal in a well with a temperature of 117 °C demonstrated effective paraffin and plug removal [14]. But the temperature criteria for MEOR reservoirs in China is still set at 80 °C. Thermophilic bacterial genera such as *Bacillus*, *Thermus*, *Thermoanaerobacter*, *Thermococcus*, and *Thermotoga* have been successfully isolated from high-temperature reservoirs in regions like China, California, and the North Sea. These thermophilic bacteria represent excellent candidates for MEOR applications [144].

#### 4.2. Salinity

The salinity in reservoirs varies widely, ranging from 100 g/L to 300 g/L depending on the corresponding depth. This significant variation in salt concentration profoundly impacts the MEOR process, as microorganisms exhibit varying degrees of tolerance to different salinity levels [151]. Using microbial flooding technology is not feasible in reservoirs with high salinity in water formation [14]. An apt illustration of this phenomenon is the reduction in the metabolic generation rate of biosurfactants, gases, alcohols, and acids under high salinity conditions [152]. The high mineralization in the reservoir also tends to form precipitates when interacting with other substances, leading to blockages [92].

First and foremost, salinity directly influences microbial growth [92]. According to the report, the quantity of isolated microorganisms diminishes when the salinity of the reservoir surpasses 10% [11]. She et al. [14] isolated *Pseudomonas aeruginosa* and *Bacillus subtilis* from the production fluid, and these microorganisms demonstrated successful growth within a salinity range of  $1 \times 10^5$ – $2 \times 10^5$  ppm. But once the salinity exceeded  $2 \times 10^5$  ppm, the growth rate of these microorganisms decreased.

Secondly, salinity plays a pivotal role in determining the composition of microbial communities in oil reservoirs by influencing growth and restricting bacterial activity [25, 153]. The effectiveness of MEOR is contingent upon the microbial community within the reservoirs. Usually, higher levels of formation water salinity tend to suppress bacterial diversity. Reservoirs with lower salinity levels exhibit a significantly higher microbial diversity compared to reservoirs with higher salinity levels, leading to an increased oil recovery [144]. For example, Xiao et al. [154] studied the community diversities of two oil reservoirs in Changqing, China, exhibiting substantial salinity differences. The findings suggested that reservoirs exhibiting relatively lower salinity levels showcased increased diversity, and the efficiencies of MEOR were quantified at 8.12% (lower salinity) and 6.56% (higher salinity), correspondingly.

Additionally, salt concentrations in oil reservoirs impact the methanogenesis of microbes. The process of hydrogenotrophic methanogenesis from carbon dioxide with hydrogen was observed to occur only up to an in situ salt content of 9% [155]. Except for the above adverse effects, salinity exerts positive effects on microorganisms, reflected in the reduced CMC leading to a decrease in the required amount of ex-situ biosurfactants for surface tension reduction [156]. Table 5 displays the bacteria and archaea associated with the optimal salinity conditions in petroleum reservoirs.

**Table 5.** Bacteria and Archaea correlated with salinity conditions in oil reservoirs.

Phylum/Class	Order/Genus	Preferred Salinity	Reference
• Firmicutes/Clostridia	• Clostridiales/Deulfotomaculum	• higher salinity	[106]
• Proteobacteria/Deltaproteobacteria	• Desulfovibrionales/Desulfovermiculus halophilus	• higher salinity	[157]
• Firmicutes/Clostridia	• Halanaerobiales/Haloanaerabium	• higher salinity	[158]
• Euryarchaeota/Methanococci	• Methanococcales/Metanothermmeococcus	• higher salinity	[145]
• Thermotogae	• Petrotogales/Petrotoga	• higher salinity	[106]
• Euryarchaeota/Methanobacteria	• Methanobacteriales/Methanobacterium	• lower salinity	[103]
• Euryarchaeota/Methanomicrobia	• Methanomicrobia/Methanoplanus	• lower salinity	[159]

Salinity was identified as an essential screening parameter of MEOR. The salinity of the formation water directly impacts the osmotic pressure within microbial cells, thereby influencing microbial proliferation, overall abundance, and community composition. Utilizing microbial oil recovery methods is impractical in forming water with high salinity. The salinity in the reservoir varies with depth, exerting a significant influence on the MEOR process due to the varying salinity tolerance of microorganisms. Each microbe possesses its optimal salinity range, crucial in MEOR effectiveness.

#### 4.3. pH

After polymer flooding in oil reservoirs, the variation in pH can be related to several scenarios:

Firstly, the choice of polymer for flooding can affect the pH of the injected fluid [160]. Some polymers, especially synthetic ones, may have inherent acidic or alkaline properties, leading to pH variations in the injected solution. For example, PAM/HPAM can influence the pH of the injection fluid.

Secondly, polymers injected into the reservoir can interact with reservoir rocks and fluids. These interactions can alter the pH of both the injected and reservoir fluids. On the one hand, polymers may adsorb onto rock surfaces, releasing acidic or alkaline components into the solution, affecting pH. On the other hand, chemical reactions between the injected polymer solution and reservoir fluids can result in changes in pH [161]. For instance, interactions with minerals or brines within the reservoir can lead to pH shifts due to precipitation or dissolution reactions.

Furthermore, the initial pH of the reservoir fluids and rock properties can also play a role [162]. Depending on the reservoir's geological formation and water chemistry, the pH of the injected polymer solution may need to be adjusted to optimize the recovery process.

Additionally, the reservoir's rock mineralogy and porosity variations can impact the pH response to polymer flooding [163,164]. Different regions of the reservoir may exhibit varying pH shifts based on their geological characteristics.

After polymer flooding, pH variations mainly relate to the choice of polymers, their interactions with reservoir components, chemical reactions, initial reservoir conditions, and reservoir heterogeneity. Therefore, understanding these pH effects is crucial for optimizing its impact on enhanced oil recovery.

Generally, the pH levels within oil reservoirs fall between 3 and 7 [29]. pH plays a significant role in determining the presence and distribution of microorganisms [102]. Microorganisms typically exhibit the ability to thrive across a spectrum of pH levels spanning approximately 3 to 4 units. They are classified as acidophiles ( $\text{pH} < 5$ ) when their optimal growth occurs in acidic environments, neutrophiles ( $5 < \text{pH} < 9$ ) in neutral conditions, and alkaliphiles ( $\text{pH} > 9$ ) in alkaline environments.

The growth rates of microorganisms show variations depending on the pH levels, often represented by bell-shaped or triangular curves. These curves illustrate the limits of pH at which cell structural integrity is maintained and the impact of pH on cellular metabolism. In addition to influencing the metabolic activity of microorganisms, pH also impacts the performance of biosurfactants. A lower pH can result in the aggregation and sedimentation of biosurfactants, a frequently observed phenomenon in experiments aimed at the crude purification of surfactants produced by microorganisms [11].

In addition, pH can influence both the thermodynamics and kinetics of microbial respiration, ultimately shaping the composition and functionality of microbial communities [165]. A distinct relationship between pH and the compositions of microbial communities was identified at the site level for two separate pits located within the La Brea Tar Pits in Los Angeles [166]. Furthermore, variations in pH showed a positive correlation with the divergence of bacterial communities [102]. pH also impacts microorganisms' surface charge and enzymatic activity [11].

Finally, some studies found a correlation between pH value and salinity among microorganisms in oil reservoirs. An investigation conducted in twenty-two geographically distinct Chinese oil reservoirs revealed that *Alphaproteobacteria*, *Actinobacteria*, and *Deltaproteobacteria* were the dominant bacterial groups in reservoirs with neutral to alkaline conditions, characterized by pH values ranging from 7.0 to 8.2. *Pseudomonas* correlated with a decline in the pH value of formation brine within the range of 5.5 to 7.6. *Betaproteobacteria*, *Gammaproteobacteria*, and *Epsilonproteobacteria* thrive in highly acidic environments and are identified in reservoirs with pH values ranging from 5.5 to 6.5 [145]. SRBs were discovered to exhibit resistance to a diverse range of salinity levels and a broad spectrum of pH values, spanning from 4 to 9.5 [146]. Table 6 presents data on the bacteria and archaea found in petroleum reservoirs that thrive under optimal pH conditions.

**Table 6.** Bacteria and Archaea linked to pH conditions [103].

Phylum/Class	Order/Genus	Preferred pH
• Euryarchaeota/Methanomicrobia	• Methanobacteriales/Methanocorpusulum/ Methanolobus/Methanolinea/ Methanocalculus/Methanosaeta/Methanoculleus	• alkaline pH
• Actinobacteria		• alkaline pH 7.0–8.0
• Euryarchaeota/Methanococci	• Methanococcales/Methanococcs	• alkaline pH
• Proteobacteria/Alphaproteobacteria	• Rhodobacterales/paracoccus	• alkaline pH 7.0–8.2
• Proteobacteria/Archaeoglobi	• Archaeoglobales/Archaeoglobus	• alkaline pH
• Proteobacteria/Deltaproteobacteria	• Desulfuromonadales/Desulfuromonas	• alkaline pH
• Proteobacteria/Betaproteobacteria		• acidic pH 5.5–6.5
• Proteobacteria/Epsilonproteobacteria		• acidic pH 5.5–6.5
• Proteobacteria/Gammaproteobacteria	• Pseudomonadales/Pseudomonas	• acidic pH 5.5–7.6
• Euryarchaeota/Methanobacteria	• Methanobacteriales/Methanothermobacter	• acidic pH

#### 4.4. Oxygen

Numerous microorganisms display high sensitivity to variations in oxygen concentrations [167]. Microorganisms can be classified into obligate aerobic/anaerobic bacteria, aerotolerant anaerobes, and facultative anaerobes/aerobes based on oxygen needs. Obligate aerobic bacteria rely on oxygen for their growth as their energy production and respiration mechanisms depend on the transfer of electrons to oxygen, which serves as the final electron acceptor in the electron transport reaction for these bacteria [168]. Obligate anaerobic bacteria can only survive and thrive in oxygen-free environments as their metabolic processes for energy generation are not reliant on oxygen consumption. Oxygen can be detrimental to vital enzymes in obligate anaerobic bacteria.

Nevertheless, specific bacteria are categorized as microaerophilic or aerotolerant anaerobes as they demonstrate improved growth in environments with low oxygen concentrations [152]. Oxygen frequently triggers minor metabolic processes within these bacteria, augmenting major energy production pathways. Facultative anaerobes possess the ability to adjust their metabolic processes based on the availability of oxygen. In the presence of oxygen, they employ the more efficient process of respiration, while in its absence, they resort to the less efficient process of fermentation [169]. As a result, facultative aerobes, which can adapt to aerobic and anaerobic conditions, are frequently encountered and can be effectively utilized to enhance oil recovery in oxygen-depleted reservoirs [152].

Compared to most anaerobes, aerobes possess a highly efficient metabolism and can reproduce rapidly [10]. The findings from previous studies indicate that aerobic bacterial growth and metabolic activities were hindered in the presence of anaerobic conditions [170]. Cai et al. [171] studied the impact of oxygen inoculation on oil biodegradation and the biodiversity of microorganisms in the Dagang oil field. They assessed and analyzed biodegradation through in vitro enrichment, denaturing gradient gel electrophoresis, real-time PCR, and GC-MS techniques. The results indicated that aerobic conditions could promote petroleum biodegradation and induce alterations in the microbial community structure, potentially benefiting MEOR applications. Many research studies have been conducted on injecting oxygen and nutrients to stimulate aerobic bacteria in situ for de-

grading heavy oil [10]. The application of this method is extensively practiced in both China and Russia [172]. In addition, as the depth and temperature of an oil environment increase, it tends to transition towards anaerobic conditions. Since oxygen concentrations in water are typically low at elevated temperatures, following the principles of Henry's law, main microorganisms exhibit anaerobic characteristics when subjected to high-temperature conditions.

In summary, petroleum oil reservoirs can be viewed as extensive geo-bioreactors that harbor diverse microorganisms. Each microorganism has specific oxygen requirements and can only achieve optimal growth under suitable conditions. Oxygen significantly impacts microbial growth, metabolism, and oil recovery.

#### 4.5. Pressure

Pressure is also acknowledged as a critical parameter in MEOR. When examining its impact, it is observed that high hydrostatic pressures can have conflicting implications for bacterial growth [173]. Fluctuating pressure levels can induce alterations in the solubility of reservoir gas, leading to changes in crude oil viscosity and possibly influencing oil displacement efficiency. However, in comparison to other factors, pressure has a minimal impact on the growth and metabolism of oil-producing microorganisms. Simulating high-pressure conditions in laboratory settings is exceptionally challenging, and there is a scarcity in the literature of studies assessing the tolerance of microorganisms to severe pressure.

#### 4.6. Minerals

The composition of rock layers affects the activity of microorganisms. The reservoir is mainly composed of sandstone and carbonate rock, so it has a large specific surface area, and microorganisms are readily adsorbed on the rock surface, directly affecting microorganisms' activities. In addition, the injected nutrient solution can be concentrated, thus affecting the growth and reproduction of microorganisms. The clay minerals in the reservoir have a high cation exchange capacity. They are easy to hydrate, expand, and disperse under the action of formation water, resulting in a negative charge on the surface of the particles and a positive control on the end face, which significantly impacts the activity and migration of microorganisms. Therefore, in reservoirs, the influence of rock composition, chemical properties, and formation water should be fully considered to optimize the activity environment of microorganisms.

Moreover, porosity and permeability have an essential impact on the growth and migration of microorganisms, which substantially influence the efficiency of MEOR [174]. More precisely, the porosity determines the microbial penetration and the degree of growth in the pores and crevices [42]. The increase in pore size reduces permeability and adversely affects the fluid transport process [22]. When the core permeability is higher than  $100 \times 10^{-3} \mu\text{m}^2$  and lower than  $1500 \times 10^{-3} \mu\text{m}^2$ , the reservoir conditions have adsorption and retention effects on oil-producing bacteria [14]. There is a direct relationship between capillary pressure, porosity, permeability, and microbial growth rate [175]. When the capillary pressure increases, it decreases pore diameter and permeability [176]. As a result, the volume of microorganisms within the pores diminishes, causing a slowdown in their growth and metabolic rate [177].

In conclusion, the growth and activity of microorganisms in reservoirs are influenced by the physical and chemical conditions of the environment, including (i) the reservoir fluid: oil properties, residual chemicals, and injection water and (ii) the reservoir conditions: temperature, pH, oxygen, salinity, pressure, porosity, permeability, etc. Additionally, the availability of essential nutrients is crucial for supporting the growth and metabolic processes of the microorganisms. The specific type of microorganism introduced into the reservoir can greatly affect reservoir properties and oil recovery, and its optimal growth is determined by the ecological conditions present in the reservoir. Oil displacement efficiency increases as the reservoir conditions approach the optimum for microbial growth.

## 5. Microbial Consortium on Oil Displacement

### 5.1. Microbial Consortium

Various microorganisms possess the ability to degrade distinct components of crude oil. While a diverse range of microorganisms can degrade the simpler compounds found in crude oil, complex compounds such as polycyclic aromatic hydrocarbons (PAHs), asphaltenes, and resins are limited to fewer microorganisms. Mixed microbial populations are frequently employed as mixtures or formulations, along with their metabolic products, to optimize oil recovery and extend the productive lifespan of oil wells [178]. The metabolic by-products of diverse bacteria in MEOR processes possess distinct functionalities to enhance oil recovery [179]. For instance, bacteria belonging to the genera *Clostridium*, *Zymomonas*, and *Klebsiella* produce solvents like propane-2-diol acetone and butanol.

Additionally, *Enterobacter* and *Clostridium* are accountable for generating methane and hydrogen gas. These fermentation gases can contribute to re-pressurize wells, facilitating the displacement of light crude oil within the well and aiding its recovery [180]. *Clostridium* is considered one of the most suitable microorganisms for MEOR due to its highly resilient endospores, which allow them to survive in unfavorable conditions [179]. *Bacillus* is also beneficial in MEOR processes because it can produce biosurfactants that have favorable properties contributing to the effectiveness of the MEOR [181]. Thus, the concept of employing a microbial consortium was initiated. A study by Xia et al. [182] developed a microbial consortium consisting of *Serratia proteamaculans*, *Rhodococcus erythropolis*, *Rhizobium* sp., and *Alcaligenes* sp. The results showed that the microbial consortium achieved the consumption of 85.26% of the crude oil within 15 days, demonstrating a significantly higher efficiency compared to individual strains and which provided evidence to support the viability of the concept.

One of the common methods to develop the microbial consortium is cultivating a variety of microorganisms with different features to take advantage of their diverse functions and inject them into the reservoir. According to I. Lazar et al. [183], the patented Multi-strain Mixed Microbial Application consortium, which includes thermophilic, barophilic, acidophilic, and anaerobic strains isolated from oil reservoir water, has the ability to produce biosurfactants, fatty acids, alcohols, CH<sub>4</sub>, and CO<sub>2</sub> at temperatures up to 90 °C, mirroring in situ conditions. The introduction of the microbial consortium into reservoirs, along with the addition of targeted nutrients, resulted in a three-fold increase in oil recovery.

Some field trials involve directly indigenous microorganisms to develop microbial consortia. Rathi et al. [184] developed the methanogenic consortium named TERIL146, consisting of indigenous microorganisms from a high-temperature oil reservoir, resulting in significant gas production (12 mmol/L) and other metabolites. The TERIL146 exhibited an incremental oil recovery of 8.3% in a sand pack assay. These findings demonstrated the successful recovery of both oil and gas by the developed indigenous methanogenic consortium, highlighting its potential application in thermophilic depleted oil wells in the Indian subcontinent.

Numerous field trials have been conducted to screen and isolate microorganisms with specific metabolites, thus enhancing oil recovery rates. In a study by Okoro et al. [185], two petroleum thermotolerant consortia were successfully isolated and characterized from reservoir sandstones. This validation confirmed that the injection of selected microorganisms to enhance oil recovery and promote the production of specific metabolites was an effective approach.

Several developments of the microbial consortium have focused on combining biodegradation with its products. Munna Bhattacharya et al. [186] researched the simultaneous output of emulsifiers and the biodegradation of crude oil using a mixed culture consisting of *Ochrobactrum pseudintermedium* and *Bacillus cereus*. Under optimized culture conditions, the microbial consortium degraded up to 70.54% of total petroleum hydrocarbons within 72 h of incubation, with crude oil as the sole carbon source (4% v/v). Additionally, the microbial consortium produced two types of EPS with exceptional emulsification activity, reduced IFT in the crude oil–water system, and improved oil recovery by 46.85%.

Integrating multiple techniques utilizing microbial consortia with diverse properties is an effective strategy for increasing oil recovery. Developing a suitable microbial consortium with good performance is crucial for successfully implementing MEOR.

### 5.2. Microbial Activities and Interrelations within Oil Reservoirs

Microbial populations in nature do not function independently. Specific interactions occur between spatially separated species, and they utilize soluble or volatile metabolites to communicate information. Additionally, other interactions involve close species, either forming a biofilm on the same particle or being physically associated with each other. In the environment, microbial species can form different relationships, including mutualism, competition, commensalism, and parasitism [187,188].

When utilizing a microbial consortium to degrade various components of crude oil, individual members of the microorganisms can form mutually beneficial symbiotic relationships [189]. In the process of petroleum hydrocarbon degradation, certain bacteria have demonstrated the capability of degrading metabolites that accumulate from other members, which helps mitigate the inhibitive effects of metabolites on the degradation capacity, ultimately leading to an improvement in degradation [190]. For example, the consortium consisting of *Rhodococcus* sp. and *Mycobacterium* sp. demonstrated considerable efficiency in degrading phenanthrene. This was attributed to the fact that the *Rhodococcus* sp. degraded phenanthrene, producing 1-hydroxy-2-naphthoic acid, which acted as an inhibitor for its phenanthrene-degrading activity. However, the *Mycobacterium* sp. was capable of degrading the 1-hydroxy-2-naphthoic acid, relieving the repression of phenanthrene degradation caused by the *Rhodococcus* sp. [190].

Moreover, the investigators observed that the metabolites generated by the microorganisms within the microbial consortium also facilitated the degradation of other members. As an example, the addition of biosurfactants produced by the yeast *Pseudozyma* sp. to *Pseudomonas putida* significantly improved its ability to degrade crude oil, with a substantial increase of approximately 46% in the degradation of alkanes (C<sub>10</sub>–C<sub>24</sub>) [191]. This phenomenon can be attributed to the modulation of cell surface proteins and alteration of the cell membrane hydrophobicity by biosurfactants, which facilitate the adhesion of microbial cells to hydrocarbons, thereby enhancing microbial crude oil degradation [192]. In MEOR, interactions among microorganisms within populations can exhibit either synergistic or detrimental effects based on the metabolic capabilities of each microorganism [193]. As an illustration, the interactions between microalgae and bacteria can encompass a broad spectrum of relationships, ranging from mutualism and commensalism to competition and parasitism [194]. Increasing evidence indicates that microbial interactions within consortia are highly species-specific and remarkably sophisticated.

Given the variety of interactions observed among microorganisms, including competition, predation, commensalism, and mutualism, numerous researchers have investigated the correlation between two microorganisms employing the Lotka–Volterra (L-V) model. Lotka [195] and Volterra [196] introduced a well-known growth model that established a novel foundation for the mathematical ecology of populations. In a study by Guerra [197], the relationship between the absolute growth rates of *Lactococcus lactis* was elucidated using the L-V model, specifically, the two predators-one prey model. In addition, Fujikawa et al. [198] applied the L-V model to illustrate microbial growth in a mixed culture containing *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* in the study. They revealed that the competition coefficients in the model remained stable. In the research by Zheng et al. [199], the growth of *Rhodotorula* sp., *R. phaseoli*, and *A. ferrooxidans* within a leaching condition was modeled utilizing the L-V model. The findings showed that the individual growth curves of microorganisms followed a logistic pattern. Additionally, the correlation between *A. ferrooxidans* and *R. phaseoli* was identified as mutualism, while the correlation between *A. ferrooxidans* and *Rhodotorula* sp. was commensalism. To investigate the contribution of microorganisms in actual reservoirs to enhanced oil recovery, Wang et al. [200] developed a mathematical model for MEOR that incorporated a competition mechanism between

two bacterial species to study the impact of competitive growth and product interactions between bacteria on the efficiency of enhanced oil recovery.

Comprehensive studies conducted on the interactive growth models between single microorganisms and populations during oil recovery are comparatively scarce, while no research on the collaborative microorganisms using chemicals in oil recovery has been conducted to date. The investigations focus on oil displacement compound bacteria to establish a few bacteria with synergistic effects while there are limited studies on the relationship between microbial consortium using chemicals. Thus, based on previous works, future research is required to investigate the growth of microbial consortium using chemical agents at a specific stage during the MEOR process and verify the correlations and interactions between the microorganisms by the models. This can offer theoretical backing for the cooperative oil displacement by multiple microorganisms.

### 5.3. Microscopic Oil Displacing Process of Microbial Consortium

During the MEOR process, the saturation profile in a porous medium filled with oil and brine changes. However, the pore-scale physics and active mechanisms of MEOR are not entirely understood. Consequently, the flow visualization technique is employed to understand better the pore-scale behavior of MEOR mechanisms and the intricate processes involved.

The micromodel, which involves the etching of flow patterns on glass, is a valuable tool for examining various multiphase flow phenomena at the pore scale in porous media. A comprehensive literature review shows numerous researchers have extensively employed micromodels to study and visualize MEOR processes. Hongyan et al. [201] employed the dimensional microscopic visualization technique using a glass model to investigate the oil displacement mechanism and the impact of emulsified bacteria on the residual oil in a simulated reservoir environment, which revealed that the emulsified bacteria and their products exhibited a notable capability to emulsify crude oil effectively. As a result, the oil recovery was enhanced by 19%, providing valuable new insights and a theoretical foundation for applying microbial oil displacement techniques. Liu et al. [202] carried out a *Bacillus subtilis*-based MEOR process using a microfluidic device made of polymethyl methacrylate to mimic the MEOR process in reservoirs, which demonstrated that the ex situ MEOR process with *B. subtilis* achieved a recovery rate of 38.56%, while the in situ MEOR process achieved a recovery rate of 40.27%. This research provided a novel tool for comprehending the MEOR process, offering advantages in visibility and precise fluid control during the MEOR process.

While micromodels have proven to be effective in visualizing bacteria during the MEOR process, most prior investigations were performed using artificial pore-structure chips [203]. There is a consensus that artificial pore structure does not accurately represent the conditions found in natural reservoirs. Thus, Gaol et al. [204] utilized heterogeneous micromodels constructed from natural rock captured through  $\mu$ CT imaging to assess the MEOR process within porous media under conditions of increased pressure, temperature, and anaerobic and sterile environments, which revealed that microbial growth, along with the resulting end-products, play a crucial role in enhancing the efficiency of oil displacement during MEOR, offering novel perspectives into the pore-scale mechanisms and various factors influencing the performance of MEOR. Prior to field application, the findings may be utilized to support MEOR research on a wider scale.

Many studies describe the microscopic process of oil displacement using the flow visualization technique but rarely report the mechanism of microbial consortium on the oil displacement process using chemicals from a microscopic perspective.

## 6. Application of Microbial Consortium on Oil Recovery

Based on the applications and mechanisms of MEOR, its processes can be categorized as MFR (microbial flooding recovery), CMR (cycle microbial recovery), MSPR (microbial selective plugging recovery), MWR (microbial wax removal), and other methods [14]. The

microbial consortium is frequently employed as a mixture or formulation to improve recovery in MEOR. Over the past decades, a considerable quantity of field trials involving microbial consortia have been conducted in various countries to assess the feasibility of MEOR. These field experiments are conducted considering reservoir permeability, temperature, salinity, pH, the specific recovery method employed, the type of reservoir, etc. Numerous field trials for MEOR have been conducted worldwide, yielding diverse outcomes. Based on global statistics from field tests, over 90% of MEOR trials have demonstrated beneficial effects [11].

Among the pioneering nations to initiate field trials for MEOR, America operated a field trial in the Lisbon oil field in 1954, which involved the activation of *Clostridium acetobutylicum* through the injection of molasses, and the mechanisms employed in this trial included biosurfactants, gases, and acids [205]. Oklahoma carried out a field experiment in the Bebee field; an in situ application of biosurfactants using the *Bacillus* strain was tested, which remarkably demonstrated a biosurfactant concentration that was one-ninth of the minimum required concentration, still enhancing oil recovery [206]. Numerous implementations and field tests of CMR and MFR have been conducted in Romania, resulting in a remarkable average increase in oil production of 100% for CMR and 200% for MFR [11]. Furthermore, with the support of America, the Vizacheres oil field in Argentina underwent a 19-month injection process involving facultative anaerobic bacteria and nutrients, leading to a total increase of 20% in production, amounting to an aggregate production of 27,984 m<sup>3</sup> of crude oil [11]. In India, Ahmedabad carried out field trials utilizing the huff and puff technique that focused on meticulously isolating anaerobic microbial populations from the reservoir. The field trials, comprising 12 wells across 4 fields, demonstrated a remarkable threefold increase in crude oil production and a substantial decrease in water cut, showcasing the approach's efficacy [53]. In Poland, Karaskiewicz conducted a series of 18 field trials from 1961 to 1969 utilizing a microbial consortium comprising *Mycobacterium*, *Arthrobacter*, *Peptococcus*, *Pseudomonas*, and *Clostridium* [183].

Indeed, China stands among the leading nations in the field of MEOR because of its practical implementation techniques in recent times. MEOR has been extensively explored through applications and field tests in various oilfields in China, including Shengli, Daqing, Changqing, Qinghai, Xinjiang, Liaohe, and Jilin. Among these applications, the predominant focus has been on microbial wax removal. Following incomplete data, 11 fields comprising 1739 wells have undergone testing for MEOR in China [19]. Specifically, regarding the application of CMR in China, a combined sum of 678 wells distributed across 12 fields containing Shengli, Daqing, Zhongyuan, and Jilin oilfields have been implemented. Notably, the Shengli oil field conducted microbial huff and puff operations in 1640 wells, resulting in a remarkable production increase of  $2.19 \times 10^5$  tons of crude oil, while the Daqing oil field conducted polymer flooding in 518 wells across 10 blocks, with a production increase of  $6.4 \times 10^4$  tons. The quantity of MFR is relatively less compared to that of CMR. Table 7 displays MEOR field experiments conducted in various countries using microbial consortia. Owing to the absence of precise data in many field trial documents, certain depictions may be lacking in detail.

Table 7. Implementation of MEOR in oil fields.

Country	Technologies	Cases	Microbial Consortium	Effect	References
USA	CMR	Single well stimulation	Mixed cultures of anaerobic bacteria	Oil production showed a substantial 230% rise over 7 months.	[207]
			<i>Clostridium</i> sp. and <i>Bacillus</i>	a 20 to 30% increase in the recovery of OOIP on average	[208]
	MFR	Single well stimulation waterflooding in Tulsa	<i>Bacillus</i> sp., <i>B. licheniformis</i> , <i>Clostridium</i> z., and a Gram-negative rod	Oil extraction saw a 13% increase and a 30% decrease in the water/oil ratio over ten months.	[209]
	CMR			Oil production witnessed an improvement of up to 79%.	[209]
	CMR	Scale-up of microorganisms in a single well stimulation in Oklahoma	Facultative and anaerobic bacteria	Oil production experienced significant growth.	[11]
MFR	Field test in Texas	Hydrocarbon-degrading bacteria	Increased oil recovery is achieved through surfactant usage, CO <sub>2</sub> production, pH control, and paraffin mitigation.	[210]	
China	CMR, MSPR, MFR, MWR	Xinjiang, Liuzhongqu, Jilin, Huabei, Changqing, and Jing'an oil fields	<i>Bacillus</i> , <i>Pseudomonas</i> , <i>Bacteroides</i> , <i>Fusobacterium</i> , and <i>Eurobacterium</i>	Oil production demonstrated a range of increases from 2001 to 122,800 tons.	[211–213]
		Daqing, Shengli oilfield	<i>Bacillus cereus</i> , <i>Brevibacillusbrevis</i> , Hydrocarbon-degrading strains	Oil production showed a substantial boost of 165%.	
	MFR	Shengli oilfield	Indigenous microbial community	Oil increment was about 2.1 × 10 <sup>5</sup> tons for 7 blocks	[214]
	MFR	Pilot tests in Baolige Oilfield	Six facultative anaerobes	Increase 2.1 × 10 <sup>5</sup> tons of crude oil over 43 months for 169 oil wells	[50]

Table 7. Cont.

Country	Technologies	Cases	Microbial Consortium	Effect	References
	MFR	Vostochno-Anzirscoe reservoir and Cheremukhovskoe heavy oil reservoir	Hydrocarbon-oxidizing bacteria, and aerobic bacteria <i>Rhodococcus</i> and <i>Gordonia amicalis</i>	Oil production experienced a significant rise of 1250 t.	[13]
	MSPR	Bashkiria reservoir	Aerobic and anaerobic activated sludge bacteria	Each 600-producing treated well contributed to an incremental oil recovery ranging from 1000 to 2000 tons per year.	[215]
Russia	MFR	Three pilot tests in the Romashkino oilfield		Oil recovery increased by 32.9% by generating surfactants, organic acids, carbonic acid, methane, and polysaccharides.	[216]
	MSPR		Aerobic bacteria anaerobic bacteria	The daily oil production escalated from 180 t to a range of 200–300 t.	[217]
		Oil field			
	MFR			Oil production experienced a rise of 8% in 4 months.	[218]
Romania	CMR	Oil fields	<i>Bacillus</i> , <i>Clostridium</i> , and Gram-negative rods	Oil production experienced a notable increase of 100–200% over a maximum of 5 months.	[183]
			Adapted mixed enrichment cultures predominated.	Oil production experienced an improvement of up to 200% in two wells over 1–4 years.	
India	MSPR, CMR	Oil fields	<i>Clostridium</i> , <i>Thermonaero bacterium</i> sp., and <i>Thermococcus</i> sp.	Improvement in oil production.	[18]
Former East German	ASMR, MFR	A carbonate reservoir	<i>Clostridium</i> and <i>Bacillus</i>	Improved oil production and reduced the water/oil ratio from 88% to 34%.	[219]
Former Czechoslovakia	MFR, ASMR, CMR	Pilot test	SRB, Oxidizing bacteria, Hydrocarbon, and <i>Pseudomonas SD</i> .	Microbial growth Oil viscosity decrease	[220]

Table 7. Cont.

• Country	• Technologies	• Cases	• Microbial Consortium	• Effect	References
• Poland	• MFR	• 18 field trials in Carpathian	• <i>Mycobacterium</i> , <i>Arthrobacter</i> , <i>Pseudomonas</i> , <i>Peptococcus</i> , and <i>Clostridium</i>	• The additional oil recovery rate varied from 20% to 200% of the original production rate over eight years.	[221]
• Hungary	• MFR	• Demjen field	• Bacteria from sewage-sludge cultures and anaerobic thermophilic bacteria	• An improvement in oil production by 12% to 60% was observed for a few weeks to 18 months.	[19]

Globally, experimental and modeling studies have been widely carried out at constant conditions to understand the effect of various microbial parameters on MEOR. In lab-scale research, most MEOR tests have shown promising results, and some field trials have demonstrated substantial oil production and greater economic effectiveness compared to chemical EOR. Despite these achievements, the predictability of MEOR's efficacy at the field scale has remained uncertain, raising doubts about the true potential of MEOR. The application of MEOR in the field is primarily constrained by the restricted understanding of microbial survival and metabolism in the oil reservoir. In particular, the residual chemicals left after polymer flooding have detrimental effects on microorganisms; however, the current research on the influencing mechanism of residual agents on microorganisms in MEOR is even less.

## 7. New Technologies in MEOR

Recently, innovative metagenomic methodologies have opened up new avenues for the in-depth characterization of microbial communities in oil reservoirs, allowing for a comprehensive understanding of their phylogenetic and functional profiles. These cutting-edge approaches have provided unprecedented resolution and insight into the microbial diversity and activities within oil reservoirs.

Employing metagenomic methods to explore the microbiology of oil reservoirs is anticipated to reveal previously unknown microbial strains, presenting novel discoveries in the field of science. Additionally, these approaches hold the potential to unlock opportunities for harnessing the untapped potential of these microbial strains for MEOR applications. The combination of metagenomic approaches and biotechnological methods holds tremendous promise for MEOR, and has the potential to significantly enhance MEOR processes and outcomes [18]. It is widely acknowledged that research aimed at improving MEOR necessitates the identification and isolation of new microbial strains, specifically those capable of thriving in anaerobic conditions and exhibiting rapid biomass production or the production of surface-active biopolymers [222].

The introduction of advanced DNA sequencing technology in the early 2010s enabled the precise identification and comprehensive characterization of the predominant microorganisms inhabiting oil reservoirs, thereby facilitating a deeper understanding of their metabolic pathways and functions. As a result, the MEOR technology transitioned towards directly injecting carefully selected microorganisms, typically cultivated under controlled laboratory conditions, to enable the in situ production of valuable metabolites. The underlying motivation for this approach was to substantially decrease expenses associated with procuring stimulating substrates and the need for modifying or installing additional equipment for treated water injection [178]. Due to its distinctive advantages, the utilization of sequencing technology built on 16S rDNA has gained significant traction

in research related to MEOR technology [189]. Zhang et al. [223] analyzed microbial community diversity in reservoirs after polymer flooding in the Daqing oil field using amplified ribosomal DNA restriction analysis. Numerous investigations have been carried out to explore microbial consortia in oil field environments [224]. These studies have employed diverse methodologies, including culture-based approaches, culture-independent methods based on the 16S rRNA gene, and metagenomic techniques [225,226]. A previous study utilizing the PhyloChip technique based on the 16S rRNA gene investigated samples from the Alaska North Slope oil field to identify microorganisms that have the potential to play a role in methane and hydrogen sulfide production, as well as hydrocarbon degradation [227]. Despite identifying various organisms from lineages that have not been cultivated, a comprehensive understanding of these organisms' complete diversity and functional potential remains elusive.

In previous metagenomic studies of microbial community composition in different systems, the genomic DNA of coexisting organisms was extracted and sequenced [228]. Ping Hu et al. [229] utilized a genome-resolved metagenomic method to analyze petroleum-reservoir-produced water samples obtained from six production wells under conditions of various depths, temperatures, and H<sub>2</sub>S concentrations. The findings demonstrated that the genome-resolved approach offered enhanced taxonomic resolution and enabled more detailed predictions of metabolic processes. An et al. [224] analyzed diverse hydrocarbon-containing samples from a shallow oil reservoir located in Alberta, Canada, at a depth of 850 m. Interestingly, several samples exhibited a remarkable presence of genes associated with aerobic hydrocarbon metabolism, while other samples demonstrated a higher representation of the genes related to anaerobic hydrocarbon metabolism and methanogenesis. In a recent study, fosmid libraries were sequenced to investigate hydrocarbon degradation pathways in an enrichment culture [230].

Genomic analysis can identify microbial strains that possess specific genes responsible for encoding stress resistant proteins and metabolite production, making them potentially well-suited for MEOR applications. These strains are anticipated to exhibit resilience to elevated temperatures, pressures, salinity, and anaerobic conditions in the oil reservoir. Furthermore, they are expected to possess genes encoding proteins that contribute to environmental resilience within the host organism or produce metabolites facilitating the mobilization of residual oil, thereby enhancing oil recovery [178]. Genes that encode proteins possessing characteristics such as tolerance to thermal, osmotic, and oxidative stress and the production of desired metabolites can serve as biomarkers. Strain 101C5, as identified by Kapse et al. [42], contains genes encoding crucial enzymes involved in synthesizing bio-emulsifiers with mannoprotein properties that indicate strain 101C5 can emulsify and solubilize crude oil. Utilizing these biomarkers makes it feasible to quickly identify potential strains in MEOR, thus promoting the development of an effective MEOR process that can be successfully implemented in high-temperature depleted reservoirs.

Microbial proliferation and the effectiveness of microbial displacement technology are significantly influenced by the intricate conditions present in the reservoir environment. Hence, prior to implementing microbial flooding, it is imperative to establish comprehensive reservoir screening criteria, systematically analyze the reservoir characteristics, and assess the microbial diversity in each well. This process will facilitate the development of an appropriate mathematical model for MEOR. Simultaneously, genetic engineering technology can be utilized to construct microbial strains that exhibit exceptional tolerance to the challenging conditions present in reservoir environments. Furthermore, the utilization of genetic engineering technology and the construction of a microbial consortium may represent a groundbreaking advancement for microbial oil recovery in the future. However, these approaches can be costly in terms of implementation. The former technique allows for the development of oil-recovery microorganisms with exceptional performance, while the latter facilitates the creation of a highly effective microbial consortium [189]. The integration of both approaches has the potential to augment the practicality and effectiveness of MEOR technology.

Currently, several metagenomic investigations focusing on oil reservoirs have provided insights into the phylogenetic profiles of indigenous microbial structures existing in these reservoirs. Nonetheless, there is a significant lack of knowledge regarding the genomic characteristics desired for MEOR. As a result, limited information is available for genetically assessing the suitability of bacteria or archaea for MEOR applications.

## 8. Conclusions

- (1) MEOR is a critical approach for enhancing oil recovery after polymer flooding. However, the presence of residual polymers in the formation has significant biological toxicity and negatively affects microorganisms' properties and oil displacement efficiency.
- (2) To improve MEOR, the key lies in screening, cultivating, and constructing a microbial consortium that can utilize HPAM and crude oil as energy sources while being compatible with indigenous bacteria, leading to the formation of dominant bacteria in the reservoir.
- (3) The research focuses mainly on improving oil recovery after polymer flooding, with limited reports on the impact of chemical agents on microorganisms' physicochemical properties, metabolic cycles, and mechanisms of oil flooding.
- (4) The relationships among microbial populations during the oil displacement process are highly complex, involving mutualism, benefits, competition, and potential harm. Existing studies mainly focus on creating a few bacteria with synergistic effects, but there are limited investigations into the interrelationships between microorganisms using chemicals during the MEOR process.
- (5) Many reports have been published on the microprocess of oil flooding. The parameters of MEOR can be optimized based on various reservoir conditions. Building on previous research, micro-visualization model flooding experiments can further elucidate the microscopic oil displacement process. However, the mechanism of microbial consortium on the oil displacement process using chemicals is rarely reported from a microscopic perspective.

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