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Review

Exploiting Microbes in the Petroleum Field: Analyzing the Credibility of Microbial Enhanced Oil Recovery (MEOR)

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Abstract: Crude oil is a major energy source that is exploited globally to achieve economic growth. To meet the growing demands for oil, in an environment of stringent environmental regulations and economic and technical pressure, industries have been required to develop novel oil salvaging techniques. The remaining ~70% of the world's conventional oil (one-third of the available total petroleum) is trapped in depleted and marginal reservoirs, and could thus be potentially recovered and used. The only means of extracting this oil is via microbial enhanced oil recovery (MEOR). This tertiary oil recovery method employs indigenous microorganisms and their metabolic products to enhance oil mobilization. Although a significant amount of research has been undertaken on MEOR, the absence of convincing evidence has contributed to the petroleum industry's low interest, as evidenced by the issuance of 400+ patents on MEOR that have not been accepted by this sector. The majority of the world's MEOR field trials are briefly described in this review. However, the presented research fails to provide valid verification that the microbial system has the potential to address the identified constraints. Rather than promising certainty, MEOR will persist as an unverified concept unless further research and investigations are carried out.

Keywords: microbial enhanced oil recovery (MEOR); crude oil; petroleum biotechnology; microbial metabolic by-products; biosurfactants; microbial metabolic pathways; species properties; reservoir dynamics; field trials; numerical simulation and modelling

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1. Introduction

The global demand for energy is expected to escalate in the coming years due to steady increases in the population, urbanization, industrialization, and economic development. The financial situation of most nations is based on crude oil and its products [1]. For example, India is a large consumer of energy, and in 2019 its consumption was around 806.1 million tonnes of oil equivalent (of which crude oil was 254.39 million metric tonnes), accounting for 5.8% of the world's primary energy consumption. As of 31 March 2020, India had 603.36 million metric tonnes (mmt) of crude oil reserves, and its production contributed about 32.17 mmt [2]. To meet the energy demands and overcome the constraints on its resources, oil operators (or companies) will be required to exploit considerably more complex reservoirs. However, for this purpose, highly advanced technologies will be

required. Therefore, oil reserves, whether they are undeveloped, mature, or yet to be discovered, require Enhanced Oil Recovery (EOR) techniques for the extraction of the trapped oil. Conventional primary and secondary techniques cannot successfully extract the remaining oil from capillary rock pores, particularly those with a diameter <10 nm. The reduced pressure in oil reservoirs after a certain period of oil production, the high viscosity of crude oil, and the reduced flow rates make the recovery of oil difficult [3–8].

The primary objective of all petroleum refineries is to extract the residual oil that remains following the use of conventional techniques, which amounts to approximately 5 trillion barrels, i.e., 70% of the total oil reserves [9]. A large number of companies that cannot afford to recover residual oil are currently pursuing profitable and efficient equipment for salvaging conventional oil. About 35% to 55% of the residual crude oil in reservoirs is left behind after conventional recoveries; hence, EOR or tertiary methods are used to enhance the crude oil recovery production [10,11].

These applications are converted into projects only if they best suit the economic constraints of the current oil market, thereby extending the life span of the wells. Moreover, they are eco-friendly and an effective alternative to other EOR methods. The microbial enhanced oil recovery (MEOR) method combines microbial characteristics with petroleum engineering oil recovery methods to form a new bioengineering technology. This approach eventually maximizes the recovered oil reserves, extends the life of fields, and increases the well's recovery factor [5,6]. MEOR promotes microbial activity, allowing the microbes to release their metabolic products, such as biosurfactants, biopolymers, biogases, biomass, bioacids, and biosolvents [12]. Each of these metabolic products contributes to the microbial system differently and individually improves the recovery of oil. The ability of these microbes to intensify the oil's mobility by minimizing its viscosity is considered beneficial. This property is highly exploited by providing the microbes with adequate nutrients to produce a sufficient quantity of metabolic by-products and by extending the well's life [13,14].

Microorganisms may produce useful, biodegradable, and less toxic products with the help of low-cost substrates or raw materials. The establishment of MEOR as a viable alternative to chemically enhanced oil recovery (CEOR), which is mostly considered to be cost-intensive technology that is not eco-sustainable. In MEOR, particular microbial strains are used to synthesize compounds analogous to those used in common extravagant CEOR processes, to escalate the recovery of oil from depleted and marginal reservoirs. Microbial technologies are becoming ratified universally as lucrative and eco-sustainable methods for improving oil production [6,15,16].

This review article provides a holistic overview of the oil recovery stages, focusing mainly on the microbial technique due to the versatile benefits of its secreted metabolic by-products, particularly biosurfactants. Various studies assessing biosurfactants' MEOR potential and economics are summarized. The authors also elaborate on basic MEOR fundamentals, in addition to its promising applications and the challenges faced. Furthermore, the paper deals with the history and current scenario of MEOR, in addition to its dynamics, and provides a brief outline of the mechanism of hydrocarbon-degrading microbes. Through the examination of case studies throughout the world involving laboratory and field trials, we assess past performances and the potential of MEOR. The recent trends and ventures related to MEOR are addressed briefly. This paper also presents potential future opportunities for the development and utilization of MEOR, in addition to highlighting the importance of an integrated approach towards numerical modelling and simulation.

2. Phases of Oil Recovery

Oil recovery is usually separated into a trio of phases, namely, the primary, secondary, and tertiary stages. Conventional primary and secondary methods of oil extraction quickly exhaust between a fraction of a quarter and half of a well's oil reserve. This high level of extraction is addressed with the aid of a tertiary technique, Enhanced Oil Recovery, or EOR (Figure 1) [17].

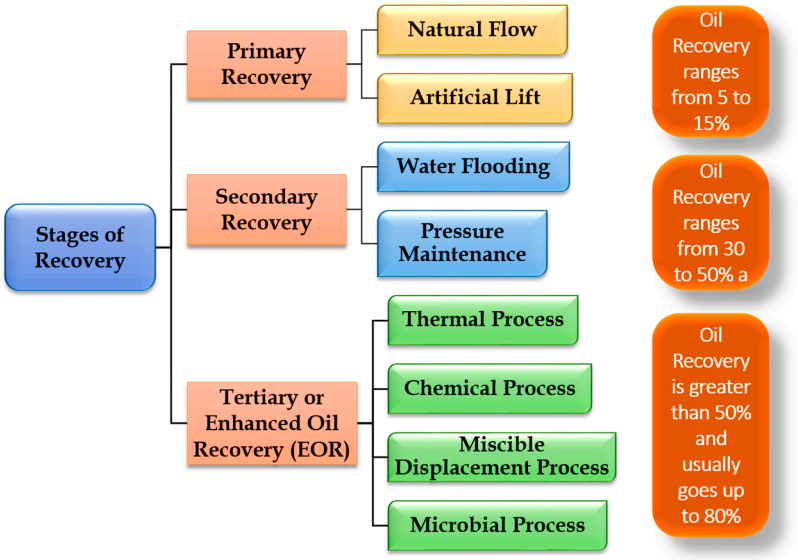

Figure 1. Schematics on the classification of the oil recovery phases.

2.1. Primary Recovery Stage

This process of extracting oil occurs either naturally or via artificial lift devices such as pump jacks. The oil in the reserves occurs in the small pores, thin crevices, and gaps within the reservoir rocks below the surface of the Earth. In this stage, the pressure of the reservoir plays a vital role because it causes the proper flow of oil towards the surface. This pressure depends on the internal energy and properties of the hydrocarbon fluids [9]. This natural force includes the driving energy of water from a large aquifer (a natural underground water flow), i.e., aquifer drive, dissolved gas drive, gas cap drive, and expansion by rock and fluid. Because this technique solely targets the oil and its susceptibility or accessibility, it has limited extraction potential [6,18].

In the dissolved gas drive, volatile fluids are released to form tiny effervescences of gas in the reservoir. These bubbles are trapped in the pore and when they expand, cause oil displacement. Subsequently, when the pressure drops, the gas bubbles merge to form a continuous rapid gas flow towards the upper portion of the reservoir, leading to the formation of a gas cap. The more the pressure decreases, the greater the displacement of the oil, i.e., gas cap drive [6,18]. Artificial lifting is preferred when the natural drive energy of the reservoir is weak and fails to thrust the oil to the surface. In this case, the pump jacks are submersed and used to lift the oil, whereas the gas lift technique injects compressed gas into the well to restore the same pressure [19].

2.2. Secondary Recovery Stage

This stage involves the injection of water or gas into the reservoir to restore the pressure or to maintain the movement of oil in the reservoir. This is undertaken when the pressure decreases due to the gradual production of oil in the reservoir, and the low pressure is no longer able to push the oil to the surface. At this stage, a critical point is reached when external energy is needed for the recovery of oil. Gas injection is mainly used with a gas cap to displace the oil in reservoirs. The pores of the rocks are prevented from clogging via the use of dissolved gases in the water used for flooding. At this point, the viscosity of the oil is minimized, consistent with thermodynamics. In this situation, a maximum quantity of oil is displaced under a specifically maintained gradient of pressure. At subsequent stages, when the quantity of oil relative to the water increases, it is no longer economical to continue the process, and the oil reserve should be abandoned [18,20,21]. Around 50% to 70% of the world's unrecoverable crude oil remains in reserves that have been exhausted by conventional methods [22–24]. Hence, in this paper, we focus on tertiary methods where the trapped oil is extracted for use from the existing and abandoned reserves.

2.3. Tertiary Recovery Stage (Enhanced Oil Recovery)

The only objective of this stage is to maximize the utilization of the crude oil that cannot be recovered during the previous oil recovery stages. This includes both residual oil and the remaining oil. The former refers to the immobile crude oil residue in the reservoir pores, whereas the latter refers to un-swept crude oil (which is stuck due to the formation of heterogeneity). This step is used in fields that are characterized by poor permeability, heavy oil, or irregular faultlines; it either alters the reservoir fluid properties or the rock's characteristics, to make the reservoir more conducive to extraction. The economics of this method must be valid. Therefore, to determine which type of EOR will suit the reservoir, each field must be precisely evaluated by screening, reservoir characterization, scoping, and reservoir modelling, in addition to simulation. This stage aids in improving the crude oil's mobility, the properties of the oil-water interface, and the microscopic and macroscopic sweep efficiency. Further extensive literature and laboratory research is required on the fluid, the reservoir rocks, and the individual processes of this stage [6,9,25].

In this stage, oil can be recovered using several thermal methods, such as steam flooding [26–30] and in situ combustion or fire flooding [12,28]; chemical methods using alkaline displacement, surfactants, and polymers [28,31–35]; miscible displacement methods using CO_2, N_2, flue gases, kerosene, gasoline, benzene, etc. [28,36]; and via the microbial method. A study by Alvarado and Manrique [37] showed that these methods are recurrently used in sandstone reservoirs rather than in other lithologies such as turbiditic and carbonate formations. Figure 2 depicts the number of EOR projects applied to the respective lithologies and highlights that gas injection has not been heavily applied to the respective lithologies compared to chemical and thermal methods. However, implementing these techniques (with the exception of MEOR) requires massive energy consumption for the generation of steam, the use of costly chemicals, a huge quantity of fresh water, and expensive equipment for their groundworks, which increases the oil price and the associated ecological risks [38]. Modern technologies, such as seismic [39] or sonic stimulations [40], and electromagnetic methods [41], are also currently being implemented. These methods are discussed in detail elsewhere, and this article focuses only on the microbial method.

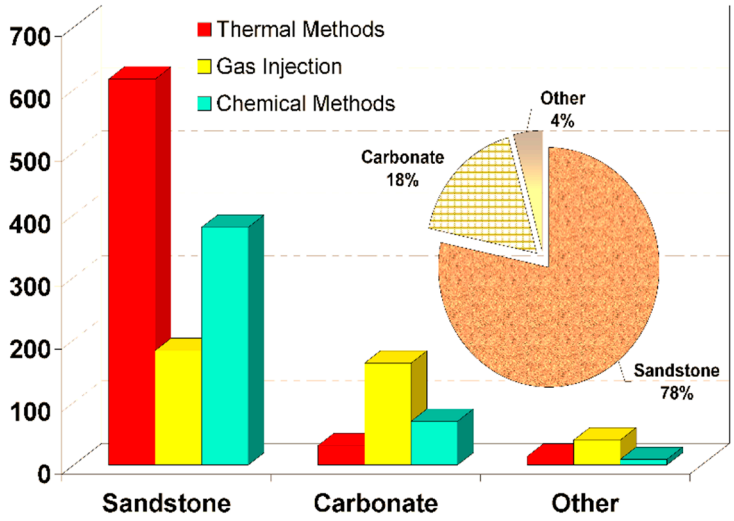

Figure 2. Lithology-based implementation of tertiary EOR methods. Adapted from [37].

2.3.1. Microbial Method (MEOR)

MEOR is a vital tool for organizations searching for a novel means to increase their returns on older investments. Unfortunately, not all indigenous microbes can ensure the recovery of oil. The application of microbial knowledge to oil reserves through reservoir engineering designs is often considered to be uncertain due to the lack of understanding of the mechanism of microbial activity. Although a large amount of theoretical data is available on MEOR, the lack of standardized field results, the shortage of post-trial investigation, the

lack of understanding of the mechanism (ecophysiology), and the shortage of structured research that presents promising evidence of this process contributes to the current low acceptance of MEOR [42]. Due to its remarkably low operating costs, MEOR has the potential to be implemented [9]. Extremophiles such as halophilic (*Salicolamarasensis* sp. and *Halobacterium* sp.), piezophilic (*Shewanella* sp., *Thermococcus barophilus*, and *Photobacterium* sp.), alkaliphilic (*Natronococcus occultus*), and thermophilic (*Pyrolobus fumarii* and *Thermus aquaticus*) bacteria and polyextremophiles (*Halonatronum saccharophilum* and *Natranaerobius thermophilus*) are the only microbes that are sufficiently small in size, can survive harsh environments (pH, temperature, salinity, pressure), and are capable of producing vital metabolic products [43–46]. For better adaptation to the surrounding oil environment, these hydrocarbon-consuming, non-virulent, and naturally occurring microbes are preferred and have attracted the attention of the researchers of MEOR [47–49].

A synopsis of the MEOR process is depicted in Figure 3. As shown, after the subsequent application of conventional techniques, oil remains in the reservoir substratum. Several microbes that come into contact with the trapped oil release metabolic products to alter the Interfacial Tension (IFT) and rock/sand grain wettability conditions that contribute to emulsion formation and mobility enhancement. The highly permeable zones allow water to flow to the thief areas via a preferential path. These areas are then plugged by microbes (selective plugging) that disturb the channel flow and drive the water to areas with low permeability. This water flows laterally to increase the sweeping efficiency of the crude oil (reducing the residual oil saturation), and help to manage excess water production. The microbial consortium clusters can disappear and displace during water flooding, causing a dynamic stimulation of new flow channels [50–52].

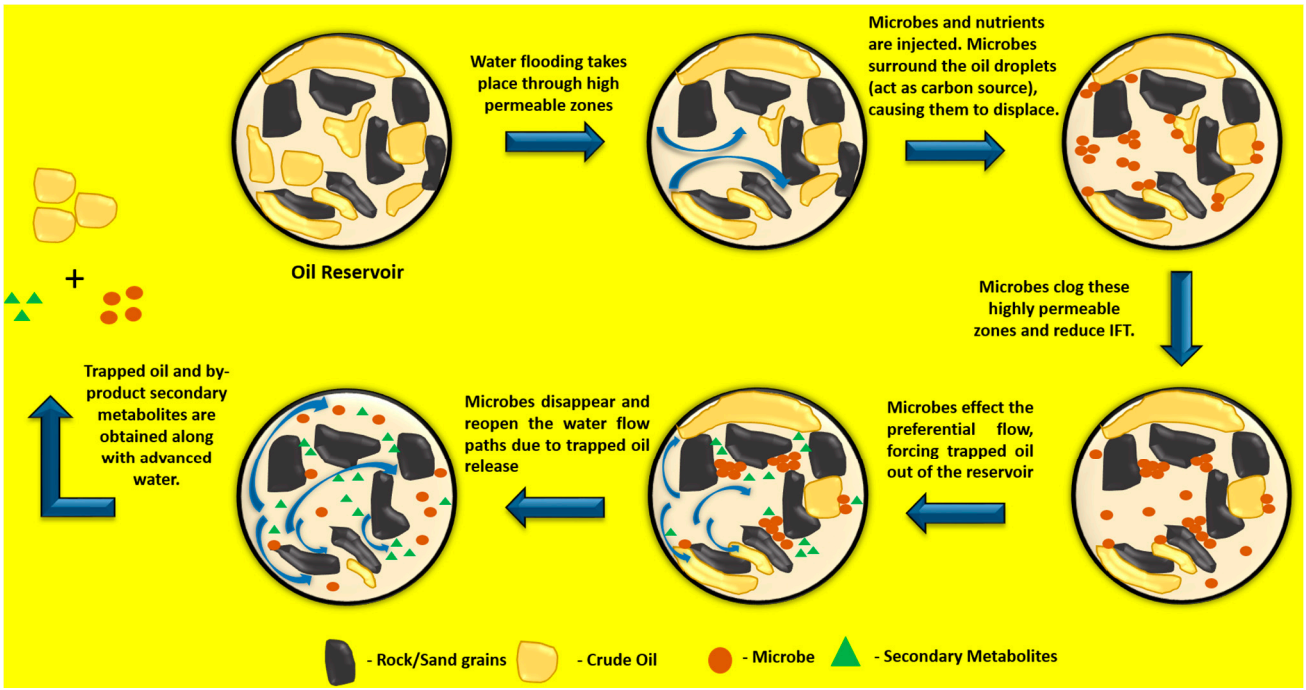

Figure 3. Diagrammatic illustration of a synopsis of the MEOR process.

The strategy of this stage is to characterize microbial communities and to understand their cultivation methods. This overall biological MEOR method, therefore, depends on parameters such as depth, the viscosity of oil, permeability, pH, temperature, pressure, water reduction, the specific gravity of crude oil, brine salinity, porosity, residual oil saturation, wax content, and the microbial species involved [22,28,53].

The two main justifications for the use of the MEOR technique are oil advancement and upgrading [10]. In the former technique, the oil films are displaced through the porous permeable capillaries, creating oil-water IFT where microbial activities alter the oil

fluidity by viscosity reduction, increasing the sweep efficiency, and restoring the reservoir pressure [54,55]. In the latter technique, the heavy oil present in the reservoir is degraded to lighter oil due to microbial activity, helping in the removal of heavy metals and sulphur [56]. In the 21st century, the oil and gas industry considers MEOR to be among the promising future research areas, and as a technology that is capable of exploiting 377 billion barrels of hidden oil that could potentially be recovered [10,57].

MEOR has been a popular research subject in recent years and has long been expected to offer an economic approach to refining retrieval [58]. Field studies have shown that an ideal projected oil production phase-down curve can be reversed by MEOR because microbial growth and the produced metabolites can have varying results on different characteristics of both the reservoir rock and the crude oil [4]. MEOR from unconventional petroleum deposits, such as oil sands, tar sands, and bituminous sands, are in the ongoing research phase due to the originality and specific ecological problems of these sources, such as water scarcity and high hydrocarbon concentrations [59].

Microbial by-products have an effect that is consistent with the consequence of the previously used chemicals, or those considered for use in an EOR, except for biomass. Therefore, in principle, MEOR is not an innovative novel oil recovery process, indicating that MEOR only differs from EOR in terms of the addition of chemicals into the reservoirs. To be significantly more efficient than EOR, MEOR is usually performed on-site to reduce logistical costs [12,60].

2.3.2. Microbial Metabolic Products Involved

A variety of microbes produces six chief metabolites (biosurfactants, biopolymers, biogas, biomass, bioacids, and biosolvents) that perform a dynamic role in the oil recovery process. These mechanisms include the reduction of oil viscosity, wettability modification, microbial plugging, reducing IFT, long hydrocarbon chain breakdown, and release of acids [14,17], as shown in Table 1. Azarhava et al. [61] reported that when *Bacillus licheniformis* LMG 7559 was injected into the oil-saturated heterogeneous porous medium, it secreted extracellular Poly gamma—(glutamic acid), which enabled oil recovery of up to 31.45% using biopolymer flooding. Another study by Ashish and Debnath [62] stated that, when used in a sand-packed method, *Candida tropicalis* MTCC230 secretes a biosurfactant, namely, lipopeptide surfactin, leading to oil recovery of 39.80%. Many of these case examples can be observed in the review paper by Saravanan et al. [28].

Table 1. Various microbial metabolic products and their uses [28,55,63].

Metabolic Bioproducts	Preferred Reservoir Type	Microbes	Role in Oil Recovery
Cell biomass	Stratified reservoir swith different permeable zones	*Bacillus licheniformis*, *Leuconostoc mesenteroides*, *Xanthomonas campestris*	Selective and as non-selective plugging, viscosity relaxation, wettability alteration, oil degradation, and emulsification.
Biosurfactants	Mature water flooded reservoirs, sandstone or carbonate reservoirs with less than 50 °C (moderate temperatures)	*Acinetobacter calcoacetiens*, *Arthrobacter paraffeninues*, *Bacillus* sp., *Clostridium* sp., *Pseudomonas* sp.	Emulsification, IFT reduction, viscosity relaxation, and wettability alteration.
Biopolymers	Stratified reservoirs with different permeable zones	*Bacillus polymyxa*, *Brevibacterium viscogenes*, *Leuconostoc mesenteroides* *Xanthomonas campestris*	Injectivity profile and viscosity modification, mobility checking, and decrease in permeability in water-swept regions.

Table 1. *Cont.*

Metabolic Bioproducts	Preferred Reservoir Type	Microbes	Role in Oil Recovery
Biosolvents	Highly oil-wet, water flooded reservoirs	*Clostridium* sp., *Enterobacter aerogens*, *Klebsiella* sp., *Zymomonas mobilis*	Increase in permeability and oil viscosity relaxation with long-chain hydrocarbon removal from pore throats.
Biogases	Heavy oil reservoirs	*Clostridium* sp., *Enterobacter aerogens*, *Methanobacterium* sp.	Oil swelling, IFT, and viscosity relaxation increase pressure and permeability.
Bioacids	Carbonate or Carbonaceous reservoirs	*Clostridium* sp., *Enterobacter* sp., *Mixed acidogens*	Permeability increases, emulsification, CO_2 production, and dissolves minerals present in the reservoirs.

Biomass

Microbes multiply to form clusters of colonies, which act beneficially due to their plugging effect. This biomass comprises 27% of microbial biofilms and their extracellular products, water channels, and void space (constituting from 73% to 98% in total). A bacterial cell multiplies every 20–30 min and is reported to proliferate as quickly as 20 times under aerobic conditions. This mechanism encompasses high permeability zonal selective plugging to ensure the growth of microbial cells in the reservoir. This limits the undesirable water flow through the pore throats, ultimately helping the penetrating water to move through the small pores under pressure, and thus recover the trapped oil from the wells. Furthermore, it reduces the viscosity and pour point of the crude oil, and also contributes to oil desulfurization and emulsification, thus making MEOR strategies more favourable. Some studies have attempted to starve the microbes to minimize their size and enhance their penetration depth into the reservoir, and subsequently provide them with nutrients to form biofilms of biomass [23,64].

Biosurfactants

Chemical surfactants have been used for a long period. However, due to the low toxicity and eco-sustainability of biosurfactants, the research on these biomolecules has increased significantly. Biosurfactants are amphipathic compounds [65,66] that ease IFT and surface tension [67], in addition to enabling O/W or W/O emulsion stabilization, thus increasing the mobility of insoluble organic compounds [68,69]. They further alter the wettability of the well-bore rocks to displace more oil films from the rock pores. Because biosurfactants reduce the interfacial activity and improve the oil recovery proficiency compared to other by-products, they have gained prominence in MEOR processes [24]. The recent growth of environmental concerns has led to the development of cost-effective, biodegradable, and low toxicity biosurfactants for use in tolerable conditions, such as temperature, salinity, and pH (especially during tertiary oil recovery). Due to the superiority of these properties compared to those of chemically synthesized surfactants, biosurfactants are a high-value product in applications such as cleaning oil tankers, conveying heavy crude oil through pipelines, use as an anti-corrosive agent, extracting bitumen from tar sand, use as a demulsifying agent, and recovering oil from reservoir sludge in the oil enhancement industry. These applications of biosurfactants, in addition to their use in MEOR, are being recognized and are expected to expand within the petroleum industry [68,70–72]. Many firms have commercialized products containing biosurfactants, and have further developed strategies to improve the economics of biosurfactant production [73,74].

Microbial contamination should be seriously considered to prevent oil pollution from oil spills, oil tanker accidents during transport, and anthropogenic activities [75]. The categories of biosurfactants produced by various microbes are lipopeptides, phospho-

lipids, glycolipids, and polymeric fatty acids. The most common are glycolipids (comprising sophorolipids, rhamnolipids, trehalolipids, etc.), whereas lipopeptides (surfactin, syringomycin, viscosin, polymyxin, etc.), phospholipids, and polymeric fatty acids (emulsan, liposan, alasan, etc.) are complex structures [28,76,77]. At present, the most favourable biosurfactants are glycolipids, sophorolipids secreted by *Candida* yeasts, and rhamnolipids produced by *Pseudomonas*. A study was conducted by Renard et al. [78] in which his team analyzed cloud water and found 42% of biosurfactants secreted microbes (from 480 strains). Of these, the most prominent were *Pseudomonas syringae* PDD-32b-74 and *Xanthomonas campestris* PDD-32b-52, which secreted linear lipopeptides called syringafactin; and Pseudomonas sp. PDD-14b-2, which secreted viscosin, cyclic lipopeptides, and massetolide E. Despite the industrial interest in these compounds, numerous problems persist [7,79,80].

Critical Micelle Concentration (CMC) determines the efficiency of a biosurfactant. The biosurfactant activity depends on its concentration until CMC is reached because, above this limit, bilayers, micelles, and vesicles are formed. This formation aids in the reduction of IFT, and further increases the solubility and the bioavailability of hydrophobic organic compounds, resulting in a linear function for oil recovery [81]. For more detailed properties of various species' strains, see Table 2.

The quality and quantity of the produced biosurfactants depend on the nutrient medium supplied to the microbial consortia and the carbon substrate [113]. Their activity is also affected by the concentration of NaCl and pH (optimum range is between 4 and 10). The sodium ions form sodium acetate with the oil molecules, thus making the oil wetter, which is detrimental to the recovery process. In contrast to these ions, magnesium ions alter the surface of calcite by making the oil less sticky, enhancing the process [57,114]. The total biomass produced is directly proportional to the O/W emulsion formed; the more the quantity increases, the better the quality. Therefore, a generic microbial technique cannot be applied to all oil reservoirs [115].

Biopolymers

Several microbial strains secrete polysaccharides in self-defence during short-term desiccation, predation, and adhesion to surfaces. This mechanism involves selective plugging of the high permeability zone to modify the permeability of the oil reservoir to the floodwater, to effectively reach the oil-rich pores. The viscosity of the penetrating water is further increased using a mobility control agent to improve the oil sweep efficiency and the mobility stoichiometry by equalizing the permeability across the reservoir. The global capital market for biopolymers is anticipated to increase 17% and reach USD 10 billion by 2021 [116–118].

The most prominent bacterial commercial biopolymer is Xanthan gum [119], produced by *Xanthomonas* sp. and sold at USD 12 kg^{-1}. It is thermally stable, has sheer resistance, and is salt tolerant, making it a potential candidate for EOR [120]. Other biopolymers include scleroglucan (USD 50 kg^{-1}) produced by the fungus *Sclerotium* sp., and levan produced by the bacterial *Bacillus* species. The former exhibits high potential for commercialization, is water-soluble, has viscosifying ability, reduces IFT, and is stable under a variety of abiotic stresses [117,121,122], whereas the latter exhibits strong adhesivity, high solubility in oil and water, low intrinsic viscosity, and non-gelling, biocompatibility, and film-forming properties. As a result, these are promising candidates for EOR [123,124].

Table 2. Properties of biosurfactants produced by various microorganisms of several strains. Abbreviations—BS: Biosurfactant, ST: Surface tension, CMC: Critical Micelle Concentration, AOR: Additional oil recovery, E_{24}: Emulsification Index (mostly against kerosene, diesel, or sunflower oil), Temp: Temperature, P: Pressure, API G: American Petroleum Institute Gravity, OV: Oil Viscosity, Por: Porosity, PV: Pore volume, Sub: Substrate, FR: Flow Rate, d: diesel, k: kerosene and HCCL: Hemi-cellulosic Corncob Liquor.

Microbe	BS Type	ST (mN/m)	CMC (mg/L)	AOR (%)	E_{24} (%)	IFT (mN/m)	Temp (°C)	P (atm)	API G (°)	OV (cP)	Por	PV	Sub	FR (ml/min)	BS Yield	Reference
(a) Various Pseudomonas species																
Pseudomonas putida CB-100	Rhamnolipid	47.5 ± 1.32	430	-	20	-	37 ± 2.0	-	-	-	-	-	Phenanthrene	0.8	27	[82]
Pseudomonas nitroreducens	Rhamnolipid	37	28	-	-	-	30	-	-	-	-	-	Glucose	-	5.46	[83]
Pseudomonas otitidis P4	Glycolipid	33.4	40	-	68.7	-	40	-	-	-	-	-	2% Sodium acetate	100	2.75 ± 0.07	[84]
Pseudomonas DYNA270	Rhamnolipid	22	20	-	-	0.005	120	1.22	-	-	-	-	4% Mannitol	-	5.32	[85]
Pseudomonas sp. TMB2	Rhamnolipid	33.4	120	16.7	78.6	0.8	30	40.7	34.2	12.43	22, 21.3 19.7	21.5, 20.81, 19.25	2% Glucose	0.8	2.8	[86]
Pseudomonas balearica Z8	Rhamnolipid	41	90	-	44	-	40	-	-	-	-	-	Oily sludge waste	1	-	[87]
(b) Various Pseudomonas aeruginosa strains																
Pseudomonas aeruginosa OBP1	Rhamnolipid	31.1	45	-	82	1.5	25 ± 1	-	-	-	-	-	2% n-hexadecane	0.4	4.57	[88]
Pseudomonas aeruginosa WJ-1	Rhamnolipid	24.5	14	23.02	95	-	37	-	-	-	36.77	-	5% Waste Sun flower oil	-	50.2	[89]
Pseudomonas aeruginosa MM1011	Rhamnolipid	26	120	-	80	2	25	0.05	19	-	23	12.6	Sun flower oil	0.14	0.7	[90]
Pseudomonas aeruginosa JBK1	Rhamnolipid	33.7	540	10.8	62	4.7 (d) 3.4 (k)	90	-	-	-	-	-	3% Raw Bio-glycerol	0.5	3.9	[91]
Pseudomonas aeruginosa TMN	Rhamnolipid	34	18.75	-	46	-	25 to 37	-	-	-	-	-	40 g/L Glucose or Glycerol	-	0.30.25	[92]
Pseudomonas aeruginosa KVD-HR42	Rhamnolipid	30.14	83	-	-	100	37	-	-	-	-	-	23.85 g/L Karanja oil	-	5.90 ± 2.1	[93]

Table 2. *Cont.*

Microbe	BS Type	ST (mN/m)	CMC (mg/L)	AOR (%)	E_{24} (%)	IFT (mN/m)	Temp (°C)	P (atm)	API G (°)	OV (cP)	Por	PV	Sub	FR (ml/min)	BS Yield	Reference
Pseudomonas aeruginosa ATCC-10145	Rhamnolipid	31.9	80	-	97.4	-	25 to 30	-	-	-	-	-	Soy molasses	0.5	11.70	[94]
Pseudomonas aeruginosa	Rhamnolipid	-	55.87	-	58.43 ± 0.3	1.17 ± 0.01	55	-	-	-	-	-	Kitchen waste oil	0.5	2.47 ± 0.03	[95]
Pseudomonas aeruginosa SNP0614	Lipopeptide	25.4	45	-	90	-	37	-	-	-	-	-	Crude oil	-	-	[96]
Pseudomonas aeruginosa PBS	Rhamnolipid	23.76	-	56.18 ± 1.59	50 to 60	-	100	-	-	-	-	29.83 ±0.3	2.17% Sodium citrate	0.4	2.65	[97]
Pseudomonas aeruginosa DQ3	Rhamnolipid	33.8	-	5.22	58	-	42	-	-	10	15.26	93	-	0.2	-	[98]
Pseudomonas aeruginosa	Rhamnolipid	-	9.25 ± 0.27	35.26	69	127	30	-	21.9	-	20.49 ± 0.69	-	125 g/L Glycerol	1	0.877	[7]
Pseudomonas aeruginosa HAK01	Rhamnolipid	28.1	120	43	60	2.52	40 to 121	1	19.5	1.8	-	0.4	20 g/L Sun flower oil	-	2.07	[99]
Pseudomonas aeruginosa YM4	Di-Rhamnolipid	28	50, 60	-	61, 57	<1	25	-	-	-	-	-	Glycerol Soybean Oil	-	79.7 ± 4.083.5 ± 4.6	[80]
Pseudomonas aeruginosa KT1115	Rhamnolipid (Mono as well as di)	28	167, 8	-	41.4, 52.1	<1	20 to 80	-	-	-	-	-	Rapeseed oil	-	44.39	[100]
Pseudomonas aeruginosa SG	Rhamnolipid	28.1	60	-	76.1	2.09	4 to 100	-	-	118.9	-	-	Soybean oil	0.6	10.32	[101]
(c) Other bacterial strains																
Bacillus mojavensis JF-2	Lipopeptide	-	10 to 40	14	-	1.0	37	-	32	6.0	16.7	29	DNA with medium E	0.515	-	[102]
Fusarium sp. BS-8	Glycolipid	32	-	46	71	-	30	-	-	-	-	400	9% Sucrose	36	5.25	[103]
Bacillus salmalaya 139SI	Cyclic lipopeptide	27	5%	-	65 ± 1.1	-	36	-	-	-	-	10 to 11	1% Sun flower oil	-	1.9	[104]
Candida tropicalis MTCC230	Lipopeptide, Surfactin	32	32.5	39.80	62	-	30 to 90	-	-	-	-	31	0.5% Glucose + 1.5% Petrol	1	-	[62]

Table 2. *Cont.*

Microbe	BS Type	ST (mN/m)	CMC (mg/L)	AOR (%)	E_{24} (%)	IFT (mN/m)	Temp (°C)	P (atm)	API G (°)	OV (cP)	Por	PV	Sub	FR (ml/min)	BS Yield	Reference
Bacillus subtilis ANSKLAB03	Surfactin	38	0.01	-	82	-	40	-	-	-	-	-	2% Sucrose	-	0.324	[105]
Bacillus atrophaeus L193	Fengycin, Surfactin, Bacillomycin Iturin	33	9.38	-	51.53 ± 1.39	-	28	-	-	-	-	-	1% Colloidal chitin	-	2.04	[94]
Streptomyces sp. DPUA1559	Glycoproteic Surfactant	25.34	10,000	-	41, 95	-	4 to 80	-	-	-	-	-	1% Residual frying soybean oil, Residual motor oil	-	1.74	[106]
Serratia marcescens UCP 1549	Polymeric compounds	25.92	1.5%	-	-	-	28	-	-	-	-	-	0.2% Lactose 5% Corn oil	-	-	[107]
Bacillus subtilis ICF-PC	Surfactin	27	100	-	65.74	-	30 to 45	-	-	-	-	-	HCCL + Glucose	-	3.95	[108]
Bacillus licheniformis DS1	Lipopeptide	-	157.5	5.4	65.19	12.0	40	1	-	-	12.84	14.84	2% Crude oil	0.2	-	[109]
Rhodococcus Erythropolis HX-2	NK	28.89	100	-	90	-	20 to 100	-	-	-	-	-	2% Petroleum	-	5.0	[110]
Saccharomyces cerevisiae URM 6670	Glycolipid	26.64 ± 0.06	-	-	-	9.12 ± 0.04	40 to 400	-	-	-	-	-	1% Waste Soybean oil	50	5.84 ± 0.17	[111]
Candida bombicola URM 3718	Sophorolipid	30.79 ± 0.04	0.5	-	66.77 ± 0.15	0.73 ± 0.05	28 to 50	1	-	-	-	-	-	-	2 ± 1.02	[112]

The impressive stability of schizophyllan, which is secreted by the fungus *Schizophyllum commune*, under high temperature and high salinity, renders its potential in enhancing the recovery of crude oil. Cellulose (USD 4 kg^{-1}), curdlan (*Agrobacterium* sp.), pullulan (*Aureobasidium pullulans, Pullularia pullulans*), guar gum (USD 2 kg^{-1}), polyglutamic acid (*Bacillus licheniformis*, *Bacillus subtilis*), dextran (*Leuconostoc mesenteroides*), poly-βhydroxybutyrate (*Azotobacter vinelandii, Bacillus* spp., *Alcaligenes eutrophus*), and chitin or chitosan (USD 220 kg^{-1}) are other examples of biopolymers [64,117,125]. Bi et al. [16], showed that the polymer-secreting bacteria *Enterobacter cloacae* FY-07, in addition to the surfactant-secreting bacteria *Pseudomonas aeruginosa* WJ-1, successfully reduced surface tension and increased crude oil recovery. Another successful case was reported in the Fuyu oilfield, China, in which a biopolymer was used as a plugging agent. This MEOR field trial used the strain, *Enterobacter* sp. CJF-002 secreted insoluble jelly-like biopolymers, aiding in recovering twice the oil by minimizing the channelling effect and regulating the water flow across the reservoir [126].

Biogases

During the fermentation of carbohydrates, the bacterial strains release CO_2, N_2, H_2, H_2S, and CH_4, which are then also rapidly consumed for other microbial activity. In addition to methanogens, which uptake H_2 for reducing CO_2 to CH_4, sulphate-reducing bacteria reduce sulphate to sulphide, and nitrate-reducing bacteria reduce nitrate to nitrite, and then to N_2. These gases help to repress the oil reservoir by altering IFT and the pH of the formation water and lower the heavy oil viscosity by dissolving the gases in crude oil. These gases build up in the pressure-depleted areas of the reservoir, aiding the mobility of the oil, and acting as an ideal alternative to conventional gas flooding. Thus, gas-producing microbes such as *Clostridium sp.*, *Pseudomonas sp.*, and *Methanogens* are the preferred products. Methanogens produce about three-fifths CH_4 and two-fifths CO_2, from which the methane separates the oil and gas phases, whereas the carbon dioxide separates the water phases. Furthermore, they are responsible for changing the pour point of oil and for plugging in the form of inorganic mineral ($CaCO_3$) precipitates [15,20,52,64,127,128].

Enterobacter sp., *Vibrio* sp., *Staphylococcus* sp., *Clostridium* strains, *Bacillus polymyxa*, and *Streptococcus* sp. are the few microbes that have been shown to release biogases and can be used for MEOR. Because methane is a cheap gas, methanogenesis is a facile method to salvage the trapped crude oil. When the gas is sufficiently swollen in the reservoir, the secretion of biogases may stop, aiding in the production of more valuable substances. Therefore, to hinder methanogenesis, inhibitors (methyl fluoride and 2-bromoethanesulfonic acid) can be used. It should be noted that harvesting energy in the gaseous form from heavy oils and tar sand, rather than as hydrocarbons, is economically feasible and a sustainable approach [64].

Bioacids and Biosolvents

Lactic, acetic, and butyric acids are the most common bacterial acids used to improve absolute permeability and porosity by dissolving minerals in carbonate rock. They usually occur in carbonate reservoirs or under specific nutrients during sandstone formation. The capillary forces contributing to oil retention continue to be reduced [129,130]. For example, during the anaerobic process, *Clostridium* sp. produces 0.0034 moles of acid (acetic and butyric) per kg of molasses [131,132].

Other microbes such as *Lactobacillus* sp., *Clostridium*, *Dsulfovibrio*, *Pediococcus* sp., mixed *Acidogens*, and *Bacillus* secrete bioacids including carboxylic acids of multiple molecular weights, butyric acid, formic acid, propionic acid, and low molecular weight fatty acids. Moreover, biogases such as carbon dioxide and hydrogen sulphide dissociate into the water to yield bioacids. Various studies further propose that aerobic hydrocarbon degraders secrete a blend of biosolvents and bioacids, which can then be converted to CH_4 via methanogenic microbial consortia [20].

Although it may be considered that solvents cannot be produced by microbes, they sometimes secrete solvents such as ethanol, acetone, and butanol. The microbes partially oxidize the hydrocarbons to biosolvents including alcohols, aldehydes, and fatty acids. They help relax oil viscosity and IFT, further acting as a surfactant. Moreover, they can moderately dissolve the carbonate rocks, enhancing the reservoir permeability and the porosity. They can also dissolve asphaltene and other heavy components present in the oil or from the pore throats of the reservoir, further reducing viscosity and aiding in the migration of oil.

The most common MEOR candidates are usually volatile fatty acids, lower water-soluble alcohols, and ketones (butanone and acetone). For instance, *Bacillus* spp. secretes ethanol and 2,3-butanediol; *Clostridium* spp. secretes ethanol, butanol, and acetone; and *Lactobacillus* sp. releases carbon dioxide, in addition to biosolvents. Other microbes include *Klebsiella*, *Arthrobacter*, and *Zymomonas mobilis*. Rather than producing biosolvents in large volumes in a laboratory, indigenous microbes with the capability to secrete bio-solvents should be used as a green alternative [20,64,129,133].

3. MEOR Dynamics

The global use of MEOR depends on various physical parameters. Moreover, its use is growing due to the gradual decrease in the development of novel fields and diminishing mature reserves. The success of MEOR is determined by multiple variables that affect the microbes, including:

1. Chemical factors such as electrolyte composition, redox potential, and nutrient composition;
2. Physical factors such as pore geometry, salinity, temperature, porosity, pore size, pressure, dissolved solids, lithology, and permeability;
3. Biological factors such as the type of microbe and extracellular product cytotoxicity.

Large-scale laboratory tests are essential to allow field applications to choose the appropriate micro-organisms and to understand the need for growth and production. Improved understanding of the MEOR method and its mechanisms from an engineering perspective is vital to improve the processes' productivity, in addition to the methodical estimation of the principal dynamics affecting this method, such as the characteristics of the reservoir and the microbial syndicates. Finally, several toxicity tests of the microbes to be handled in the area should be undertaken to ensure that they are safe to use and pose no risks for people or the environment. In addition to the dynamics or factors mentioned below, other dynamics affecting MEOR that should be studied carefully are the additional oil saturation of the remaining oil, the evaluation and the composition of the fluid, core laboratory tests, and the economic aspects of the procedure [4,12,14,53,64,73,134].

3.1. Selecting the Reservoir

Following the identification of the problem, a decision can be easily made about the species or microbial processes that provide the suitable resolution. For instance, for mobilizing the trapped oil from the porous media, or when facing paraffin problems or scaling, biosurfactant secreting microbes should be utilized. In the mature field, the residual oil is recovered through in situ MEOR following primary production and secondary recovery techniques. The parameters for the selection of an appropriate reservoir are –

- *Structural Analysis:* The regions of permeability and the area penetrated by oil can only be determined by this analysis for the plugging of specific pore throats to enhance the efficiency of sweeping. The depth of the oil well, the spatial distribution of oil films, and water saturation of water are included. This also comes with a high risk of drilling uncertainties. If the oil reservoir is not properly analyzed, the microbes will begin to destroy the method.
- *Geological Complexity:* This plays a crucial role in the injections of microbes due to the function they perform, and therefore needs to be carefully studied. The alteration in any of the different geological elements of salinity, porosity, wettability, or permeability leads to dysfunctional microbes or their absence.

- *Well Pattern to be Drilled:* This parameter is used when economic factors are to be considered. The pattern, i.e., a horizontal, directional, or extended range drill, determines the injectors and producers to be used.
- *Permeability Analysis:* This is critical for choosing the appropriate bacterial strain and the reservoir composition that are suited to the microbe's survival and feeding pattern. This factor should be reduced to be beneficial, as microbial consortium clogging will occur if the MEOR is not designed or implemented properly. In this case, the microbial metabolites themselves reduce the permeability by favouring plugging of thief zones as observed in Figure 3.
- *Temperature*: This is one of the key drivers for both the bacterial species variation and the oil reservoir's hydrocarbon development. Only extremophiles can survive extreme conditions, making them critical for the reservoir. Among these, *Pseudomonas* (predominantly), *Arcobacter*, *Marinobacter*, *Halomonas*, and *Caldicoprobacter* are found in the oil pits at high temperatures.

3.2. Selecting the Potential Microbes

Microbes should be capable of fulfilling the vital criteria of adapting to high temperature, pressure, salinity, and other reservoir parameters, and producing the desired by-products under aerobic conditions, thus ultimately degrading hydrocarbons (called hydrocarbon-o-clastic). The preferred microbes are usually thermophilic bacteria such as *Thermoanaerobacter* and *Thermotoga*; hyperthermophilic archaea such as *Thermococcus* and *Thermofilum*; methanogenic archaea (both hydrogenotrophic and acetotrophic) such as *Methanobacterium*, *Methanoregula*, *Methanosarcina*, and *Methanoculleus*; and mesophilic bacteria such as *Pseudomonas*, *Geobacter*, and *Desulfuromonas*. In addition to having high penetration permeability, these microbes are also dormant and resistant to endospores, thus helping them cope with stressful conditions [15,67,135]. They produce metabolites that help in the development of stable O/W emulsions, mobilize residual oil, and avert injection fluids via wet areas of the reservoir by clogging regions of high permeability. These factors act as a natural step towards reducing residual oil permeation [136].

3.3. Selecting the Right Nutrients

A variety of nutrients are injected into the wells, ranging from fermentable carbohydrates to inorganic electron acceptors that enable the micro-organisms to propagate using oil as the major carbon source. The by-products to be produced by microbes require a food source (in addition to trace minerals and suitable pH) for their survival, and therefore the nutrients act as a vital component. An ample quantity of suitable nutrients is required for microbes to colonize. When nutrients fail to perform this job, the microbes reduce their metabolic activity until the conditions become suitable to revitalize them. Nutrient deficiency causes severe damage to the catabolic activity of microbes in hydrocarbon-rich environments [1,16].

3.4. Pilot Testing

This stage determines the fate of MEOR and involves conducting the projects in phases with laboratory tests and analysis to identify the vital concerns. The combination of microbe–nutrient is checked for its compatibility, ability to grow in porous media, and competitiveness. Overall, the entire reservoir undergoes screening to optimize MEOR under strict assessment. It should be noted that many MEOR patents are solely centred on laboratory studies.

4. Microbial Metabolic Pathways Involved

The metabolic pathways associated with hydrocarbon-degrading microorganisms are either aerobic, i.e., they use O_2 as an electron acceptor, or anaerobic, i.e., they use alternative electron acceptors such as nitrate or sulphate. All metabolic pathways are mediated by specific enzymes, viz. monooxygenase, dioxygenase, peroxidase, reductase, hydroxylase,

and dehydrogenase. Under aerobic conditions, the fastest biodegradation of organic hydrocarbons occurs and is, therefore, thought to be better than anaerobic degradation because it requires less free energy expenditure, in addition to a lower energy yield per reaction. Initially, it pursues the oxidative attack route and triggers the incorporation of active oxygen to be catalysed by peroxidase and oxygenase. Subsequently, the hydrocarbons are converted into intermediate metabolites (as in the Citric Acid Cycle).

Pseudomonas putida comprising Pp alkB genes on OCT plasmid degrades aliphatic alkanes using the enzyme alkane hydroxylase consisting of membrane-bound oxygenase, rubredoxin, and rubredoxin reductase (these cofactors help transfer electrons from Nicotinamide Adenine Dinucleotide Phosphate (NADPH) to hydrocarbon substrates); and yields alkanols, which are degraded by alcohol dehydrogenase into alkanals, which are finally transformed into fatty acids by aldehyde dehydrogenase and then to acetyl CoA by its synthetase, as observed in Figure 4. Similar to this oxygenase's di-nuclear iron cluster, at the centre are methane monooxygenase and ribonucleotide reductase; for example, n-alkane by *Dietzia sp.* and *Marinobacter falvimaris*; octane by *Arthrobacter sp.*; decane by *Bacillus sp*. strain DHT; hexadecane by *Haloarculavallismortis*; and pristane by *Marinobacter aquaeolei*. Not all bacterial species can synthesize the same enzyme. The bacteria secrete specific enzymes, some of which can metabolize specific alkanes, whereas the others cleave aromatic hydrocarbons [137–139]. Enzymes associated with alkane degradation include monooxygenase, cyclohexanol-dehydrogenase, alcohol dehydrogenase, cyclohexanone 1, 2 monooxygenase, and methane monooxygenase [140].

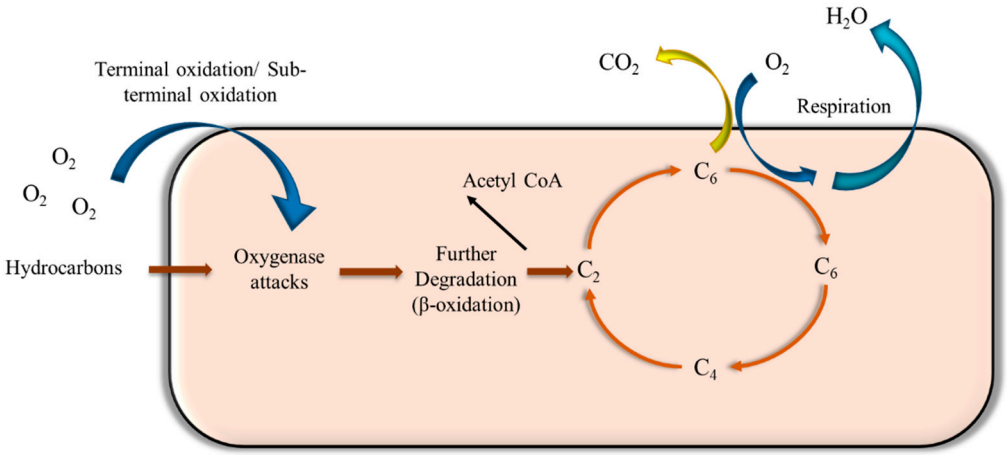

Figure 4. The principle behind aerobic hydrocarbon degradation by microbes [139,141,142].

Alkenes lead to secondary alcohols, followed by ketones, then esters, and eventually fatty acids by monooxygenase. Bacteria often cleave cyclic rings by forming cis-dihydrodiols with the aid of oxygenase and dioxygenase, e.g., phenol by *Halomonas* sp.; benzoate by *Haloferax* sp. D1227; gentisate by *Rhodococcus* sp.; cinnamic acid and ferulic acid by *Halomonasorganivorans*; benzene by *Halococcus* sp.; toluene by *Arhodomonas* sp.; and xylene by *Marinobacter hydrocarbonoclasticus* [137,143]. Biodegradation of any petroleum hydrocarbon depends solely upon the microbial consortia used. Enzymes involved in naphthalene degradation are cis-2,3-dihydrobiphenyl-2,3-diol dehydrogenase, naphthalene 1,2-dioxygenase ferredoxin reductase, and salicylaldehyde dehydrogenase, whereas those related to other petroleum hydrocarbon degradation are toluene dioxygenase and ethylbenzene dioxygenase [140,144].

Cytochrome P450 hydroxylases are frequently used for the aerobic bacterial activation of medium chain length alkanes. In addition, P450-monooxygenase is associated with the activation of aromatic hydrocarbons in mammals and filamentous fungi, suggesting the mechanism is CoA thioesterification. Hybrid pathways would be truly beneficial for microbial degradation of petroleum hydrocarbon, permitting flexibility and swift adaptation to inconsistent O_2 availability, because both oxic and anoxic conditions require CoA thioester

substrates [145]. Multiple reactions, specifically oxidation, reduction, dehydrogenation, and hydroxylation, are common in both anaerobic and aerobic metabolic pathways. Under anaerobic conditions, aromatic compounds are initially oxidized to organic acids or phenol and then distorted into long-chain unstable fatty acids, which are eventually metabolized to CO_2 and CH_4 [142]. Carboxylation and the addition of fumarate are the initially preferred biochemical mechanisms for the activation of alkanes [146,147]. The most common activation mechanism of microbial flora is the addition of fumarate to hydrocarbon aromatics via the enzyme benzyl-succinate synthase [141]. The mechanisms are summarized as a flow chart for enhanced understanding, see Figure 5.

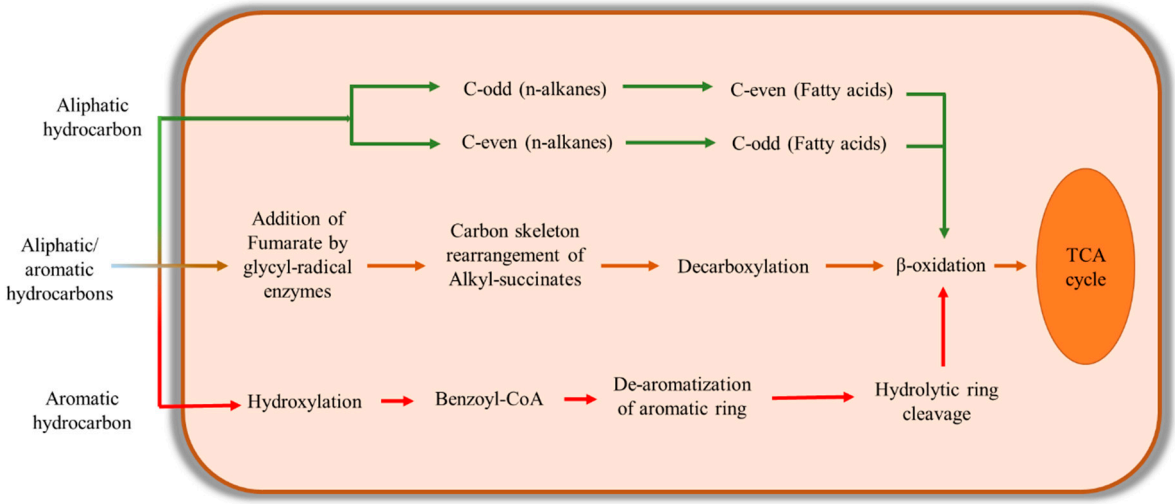

Figure 5. The principle behind anaerobic hydrocarbon degradation by microbes. The arrows represent different degradation reactions [139,141,142].

Evidence exists that links the growth of *D. alkenivorans* strain AK-01 (sulphate-reducing microbe) to anaerobic oxidation of n-hexadecane and n-pentadecane. An analysis of its metabolites revealed that C-odd and C-even cellular fatty acids were moulded from C-even and C-odd n-alkanes, respectively. This similarity was also seen in strain Pnd3 and *Desulfatibacillum aliphaticivorans* CV2803T. Furthermore, the latter strain and strain AK-01 secrete 2- to 8- branched ethyl branched fatty acids, in addition to 1-methylalkyl (succinates) having a chain length that is correlated with those of the parent substrates when catalysed by glycyl radical enzymes (n-alkanes: alkyl succinate synthase and cycloalkanes: succinate synthetase), thus signifying that n-alkanes are activated anaerobically by fumarate addition at the sub-terminal C-atom. Then, the metabolite undergoes a rearrangement of its carbon skeleton, which produces intermediates on undergoing β-oxidation, and is eventually transformed into acetyl CoA (benzoyl-CoA in the case of aromatic hydrocarbon), which is oxidized to CO_2. This whole fumarate addition reaction is exergonic, signifying that it does not need exogenous energy to function. The cyclic alkanes are completely activated through fumarate addition for additional degradation under anoxic conditions [145–147].

The next mechanism of activation is carboxylation, which, in previous research, was achieved by the growth pattern of the sulfidogenic strain Hxd3. Based on the study, Hxd3 growth in the presence of $H^{13}CO_3^-$ with varying chain lengths showed the formation of fatty acids that were one C-atom shorter than the parent alkane. It is also known that the carboxyl group consists of 13 C-atoms, which means that n-alkane is metabolized into fatty acid intermediates by carboxylation, with the consequent removal of two sub-terminal C-atoms from the alkane chain and the simultaneous inclusion of carbon at the third position. Subject to the selective environment, anaerobic degradation may be advanced through alternative activation mechanisms. As in the case of *Pseudomonas chloritidismutans* AW-1T, this can produce O_2 via chlorate respiration [145–147].

The critical anaerobic pathways associated with microbial oil reservoir consortia are the sulphate-reducing pathway (SRP), nitrate-reducing pathway (NRP), and methanogenic pathway [8,12]. SRP involves SRB such as *Desulfovibrionales*, *Desulfuromonadales*, and *Desulfobacterales* [128], which consume sulphate and sulphite, together with hydrocarbons and fatty acids in the form of terminal electron acceptors (TEA), to reduce them to H_2S gas, and thus leading to crude oil saturation and corrosion in the reservoir [52,75].

When sulphate is present, SRPs outperform methanogens due to their high affinity for substrates, and they avert electron flow from methanogenesis to sulfidogenesis. When sulphate acts as a restraint, some SRPs survive by cooperating with syntrophic bacteria and methanogens [128]. For example, archaeal members of the genus *Archaeoglobus* and the order *Thermococcales* usually thrive in hydrothermal and thermophilic environments [64,146]. To rectify this souring, nitrate, which is a powerful metabolic inhibitor, is injected. Nitrate-reducing bacteria (NRB) then reduce it to nitrite at a temperature range >45 °C and <65 °C, and to dinitrogen in temperatures up to 45 °C [148,149].

Past research has shown that aliphatic and aromatic hydrocarbons can only be biodegraded under anoxic conditions in which nitrate, sulphate, and metal ions function because TEA further helps to convert hydrocarbons into CH_4 and CO_2 in a process called methanogenic crude oil biodegradation. This process needs at least two dissimilar microbes (syntrophic bacteria and methanogenic archaea) due to thermodynamic constrictions [128]. Compared to other forms of anaerobic respiration, this is the least energetically demanding process [146]. The methanogenic pathway converts substrates such as H_2, CO_2, and CH_3COO^- into CH_4 [128]. The methanogens eradicate the inhibitory effect of the end metabolic biological products, thereby smoothing metabolic pathways and increasing oil displacement [150]. Via this pathway, the microbes degrade the crude oil to heavy oil deposits that settle at the bottom of the reservoir. To obtain clear reservoir zones, incessant injection of sulphate-containing, nitrate treated water is added. This restricts NRB to the wellbore and SRB to deeper regions [148].

5. Concept of Mathematical Modelling in MEOR

Various oil recovery techniques can be evaluated based on their variables (physical, chemical, and biological) and their practical field relationship through mathematical modelling. This need for improved and detailed models for MEOR is a highly time-consuming task [151,152]. Numerical calculations help enhance the yield of oil and minimize the cost of the technique. However, this is not straightforward, because the calculations involve microbial activities that are subject to strict geo-environmental restrictions [55,153].

The variables in the model are τ_{res}, the bacterial residue time in a cylindrical reaction zone [153] of radius r_m, depth h, and porosity ϕ. They are related as:

$$\tau_{res} = \frac{\left[\pi\, r_m.^2 h\, \phi\, (1 - S_{or})\right]}{Q} \quad (1)$$

where S_{or} is the residual oil saturation and Q is the volumetric flow rate to yield the required concentration c_{req} by time τ_{res} [128].

S_{or} is determined via the capillary number N_{cap}, which can be demarcated as the ratio of viscous force to capillary force:

$$N_{cap} = \frac{\text{viscous force}}{\text{capillary force}} = \frac{\upsilon\, \mu}{\sigma\, \cos\theta} \quad (2)$$

where υ and μ refer to the velocities of the displacing fluid, σ is the IFT between the forces, and θ is the contact angle. Because the MEOR mechanism is intricate, mostly due to a variety of microbial strains and their by-products, as shown in Table 1, S_{or} is inversely proportional to N_{cap} [55,154].

First-order kinetics includes a description of the reaction's complexity. A balanced equation can be applied for the formation of metabolic products but its rate of production

can only be found via experimentation. The multidimensional velocity flow of oil-water multiphase fluid in porous media, and adsorption and diffusion of by-products, microbes, and nutrients, are included in the modelling [155]. The model is divided into three portions: the law of conservation, transport properties, and biofilm clogging. The law of conservation lies at the heart of computational hydrology and the imitation of oil wells. The wettability alteration, permeability and porosity alteration, IFT relaxation between oil and water in porous media, and microbial clogging mechanism increase production of incremental oil and its sweep efficiency [134]. Three-dimensional modelling, including these parameters and additional assumptions, has helped in simulating a model for a hypothetical reservoir with the provided experimental data [21,53,134,150,153,155,156].

The mathematical model can be simulated and modified as per commercial feasibility and field requirements [157]. Different numerical calculations are based on various compositions and displacement mechanisms, and values of diffusion, focus point, adsorption, residual oil saturation, and fluid viscosity. For instance, Wang used a 1-D two-phase, five-component mathematical model to mimic the biopolymer flooding process in porous media [158]. Sugai and his colleagues [159] developed a numerical simulator to estimate souring induced by SRBs due to the injection of brine water into an oil reservoir. By comparison, Sivasankar and Govindarajan [160] used a mathematical model to mimic reservoir temperature and nutrient impact on biosurfactant production; and Hong and colleagues [152] constructed a model based on a coupled non-isothermal multiphase model and biokinetics to assess the potential of cold water MEOR in regions of high temperature.

6. A Vast Chronicle of MEOR History and Case Studies

Recent advances in petroleum microbiology have motivated refineries to be more transparent in assessing and enhancing microbial production. As per the microbe's compliance with the reservoir, they are used for injecting and in situ oil recovery [161]. Usually, bacterial strains are preferred due to their capacity to release trapped oil from mineral rocks, in addition to several other superior characteristics [162]. This mixture of anaerobic bacteria is recruited to effectively and efficiently produce metabolic bioproducts [11]. An oil reservoir may undergo either a single well treatment or a full field treatment [13,128].

MEOR methods originate from laboratory-based studies. A timeline depicting the long chronicle of MEOR history can be seen in Figure 6 [10,13,15,20,60,64,129,161,163].

Oman is a country known for its leading technologies in EOR and has sought to extend the limits of the existing technology to establish new methods [25,164]. To date, several field tests and laboratory tests have been carried out globally [165], and several successful applications and their biological systems were identified by Nikolova and Gutierrez [12]. Five commercially important projects that showed significant incremental oil production were located in the USA (two projects), Argentina (one project), and the Republic of China (two projects). These case studies were then analyzed based on their production rate or yield efficiency, incremental recovery, reduced water consumption, treatment design, reservoir frequency, and effect of microbes on oil viscosity [4]. Table 3 shows various project characteristics and compares them based on their screening criteria parameters (oil viscosity, permeability, temperature, porosity, salinity, and water cut) and oil production (treatment, shut-in period, well spacing, oil production rate, etc.). The data show that recent reservoir studies indicate that ranges in temperature of 80–120 °C, the salinity of >10,000 to 350,000 ppm, and permeability of 10 to 50 mD have expanded the horizon of MEOR parameters [166]. The main reason for oil being left behind (OIP) is the lack of funds. Theoretically, displaceable oil cannot be retrieved even if there is a route and ample energy, due to the laws relating to fluid displacement in porous media [167]; however, Liu and his team [168] showed a 19.58% increase in the oil recovery rate using biosurfactants produced by *Bacillus licheniformis* L20.

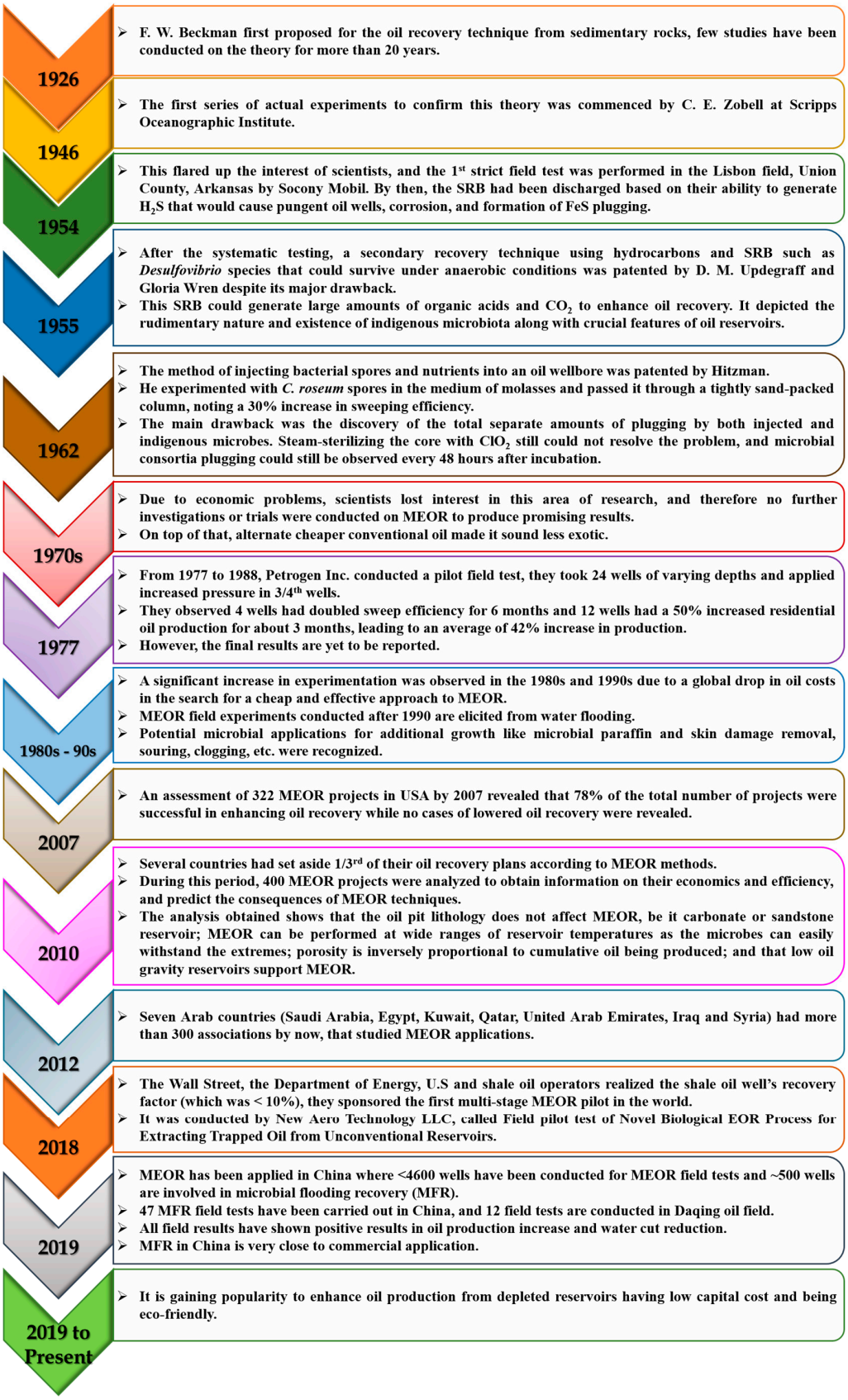

Figure 6. Timeline depicting the long chronicle of MEOR history.

Table 3. Comparison of different project characteristics based on their screening parameters and oil production ability. Abbreviations—OP: Oil Production, BL: Baseline, IP: Incremental Production, OIP: Oil in place, WF: Water flooding, ROS: Residual Oil Saturation [4,128,169].

Parameters	San Andres	Queen Sand	Tupungato-Refugio	Huabei	Xinjiang
Location and discovery	Hockley County, Texas, USA (in 1945)	Ector County, Texas, USA (in 1984)	Tupungato County, Medoza, Argentina (in 1930)	No. 3 plant, Renqiu, China (in 1987)	No. 1 plant, Xinjiang Uygur Autonomous Region, China (in 1991)
Comprises	30 producer and 15 injection wells	18 producer and 18 injector wells	Three producer wells (Victor Oscuro formation)	Seven producer wells	10 producer wells
Lithology	Fractured dolomite	Porous grey sandstone and siltstone interbedded with anhydrite and salt	Fractured sandstone	Sandstone	Sandstone
Depth (ft.)	4745	4450	5700	6900	4900
Porosity (fraction)	0.079	0.182	0.18	0.232	0.30
Permeability [Range] (mD)	1.7 [0.10–10.0]	13.0 [0.6–300]	300 [150–1500]	240 [20–640]	70 [0.2–440]
Temperature (°F)	115	110	160	180	110
Oil Density (API)	29	30	28	28	29.6
Viscosity (cp)	4.5	11	9	14	50
Pressure (psi)	1000	-	50	732	-
Water cut (%)	91	74	63.5 to 62	60	64 to 54
Drive Mechanism	Solution Gas (After 1967 water flooding was initiated)	Solution Gas replaced by water flooding in 1984	Gas drive, water flooding and water drive combination	Scattered water flooding	Dispersed water flooding
Treatment	19 months of microbe treating	24 months of Microbe treating	14 months of microbe treating	12 months of microbe treating (each well thrice)	6 months of microbe treating (each well thrice)
Shut-in period	3 days then overnight shut-in for 3 months	3 days; later, batch treating took 6 to 12 h	48 h on two wells and 24 h on the next	Not shut	Not shut
Spacing	25 acres	30 acres	42 acres	-	-
Avg. OP per well	14 bpd	42 bpd	90 bpd	-	-
% Decline (%/year)	Initially 6.5, after MEOR, it flattened to 0.6	Initially 39, after MEOR, it flattened for a few months then resumed at 31	Initially 7.1, after MEOR, it inclined to 7.3	Inclined and flattened at 150 bpd	Sustained a rate of about 300 bpd
OP Rate	Increased by 10% (40 bpd)	Increased by 47%	Increased by 29% (60 bpd)	Increased by 552%	Increased by 36% (80 bpd)
BL Production	OP of 440 bpd is 10% over BL	OP of 1000 bpd is 43% over BL	OP of 270 bpd is 29% over BL	OP of 150 bpd is 552% above BL	OP of 300 bpd is 36% over BL
IP	17,000 bbl, i.e., 7% over the BL	240,000 bbl, i.e., 34% above BL	19,000 bbl, i.e., 19% above BL	41,000 bbl, i.e., 216% over BL	14,000 bbl, i.e., 43% over BL
OIP after WF and MEOR (bbl/ac-ft.)	205 and 199	691 and 660	509 and 442	Due to short duration of the microbe treatment, no samples were available for the field treated oil.	Due to the short duration of the microbe treatment, no samples were available for the field treated oil.
ROS under WF	Drops from 35% to 34.1%	Falls from 51.4% to 49.1%	Drops from 38.3% to 33.3%		
Improvement with MEOR (%)	2.5	4.5	13		

As observed in Table 3, the Xinjiang project has the highest viscosity that creates surplus space for microbial colonization; the lowest temperature, which favours microbial growth; and the highest porosity and sandstone lithology. From this information, it can be

argued that the Xinjiang Project is the leading example in the consideration of oil production. All of these projects had a high water-cut percentage, which, following the application of MEOR, either decreased or remained constant [4,37]. Table 4 portrays multiple projects in powerful nations such as India, the U.S., and China [3,13,58,166].

Table 4. Some vital screening criteria parameters of recent projects in powerful nations—India, U.S. and China.

Parameters	Institute of Reservoir Studies	U.S. Department of Energy	China National Petroleum Company
Location	Ahmedabad, India	Washington, DC, U.S.	Beijing, China
Lithology	Sandstone	Sandstone	Sandstone
Depth (ft.)	8000	10,000	-
Porosity (fraction)	-	-	0.17 to 0.25
Permeability (mD)	Less than 50	Less than 100	More than 150
Temperature (°F)	194	160	86 to 140
Oil Density (API)	20	18 to 40	-
Brine Salinity (g/L)	More than 10	More than 10	More than 100
pH	6 to 9	-	-
Viscosity (cp)	20	-	30 to 150
Residual Oil Saturation (%)	Less than 25	Less than 25	-
Pressure (psi)	Less than 4267	-	-
Water cut (%)	30 to 90	-	60 to 85

Oil production has collapsed in many reserve regions around the world as a result of oil field development; the best example is the North Sea Field [25,31]. Another important factor is the challenge to identify novel oil wells as a substitute for previously exploited, abandoned oil wells. Of the fossil fuels, 80% to 90% of the world's current energy generation is based on oil and gas [59]. In recent years, the majority of the oil remaining in reservoirs, particularly in peripheral and developed reserves, has been due to the lack of a low-cost operating method [170]. Research is underway in this field, with the results from both novel and longstanding approaches being carefully assessed [171]. Contemporarily, the oil sector has reached its peak; improving marginal and abandoned reserves will not only activate these wells for production but will also help operators to maximize their profit [59]. There are numerous benefits of employing MEOR compared to EOR [11,15,55,96,129,157,171]. Despite the positive results of field testing, MEOR has not received the attention of the petroleum refineries due to technical bottlenecks. In addition, a negative perception exists regarding the use of microbes, although the tests carried out by laboratories have proven these to be safe [60,156]. Numerous other factors are currently contributing to the lack of attention being paid to this approach [11,12,22,36,42,51,55,64,74,154,163]. Its limitation and benefits are listed in Figure 7.

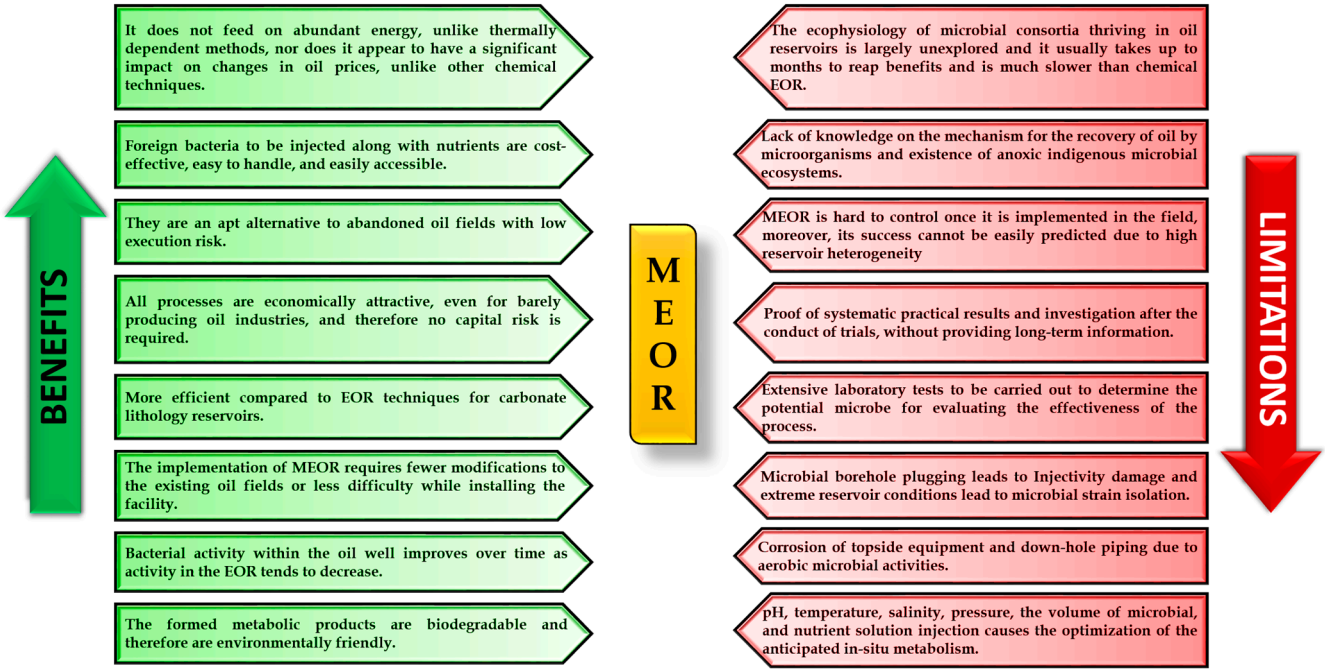

Figure 7. List of benefits and limitations of employing MEOR.

7. Trends and Ventures in MEOR

Genetically engineered microbial enhanced oil recovery (GEMEOR) and enzyme enhanced oil recovery (EEOR) are emerging areas of industrial interest. The former uses genetic engineering techniques, including recombination technology, protoplast fusion, and mutagenesis, to extract desirable features from multiple organisms and create more efficient strains, thereby enhancing oil recovery and making the process economically feasible. This approach has not yet been extensively applied to the fields, and it has been predominantly investigated in laboratories and pilot-scale testing. Enzymes with EOR potential are Greenzyme, the Zonase group, and the Novozyme group. The latter uses enzymes in consortium with other enzymes or surfactants to enhance oil recovery by altering IFT, wettability, mobility, and capillary action, leading to easy oil recoverability. The development of novel metagenomics approaches can also be observed as a growing trend [3,20,64].

Another practical strategy for the development of MEOR is switching its application direction from microbial processes to bioproduct utilization. Metabolic products, especially biosurfactants and biopolymers, have been extensively developed. Researchers have successfully produced these bio-based formulas for Selective Plugging Recovery (SPR), Flooding Recovery (MFR), Cycle Microbial Recovery (CMR), and Wax Removal (MWR). These technologies are all based on the contributions of microbes and their metabolic products [13,58].

Globally, several public and private sector ventures are currently underway concerning MEOR. Public sector ventures include Durham University (Durham, England, United Kingdom); Sultan Qaboos University (Muscat, Oman); Saudi Arabian Oil Company (Dhahran, Saudi Arabia); CSIRO—Commonwealth Scientific and Industrial Research Organisation (Canberra, Australia); Oil and Natural Gas Corporation Limited (ONGC) and The Energy and Resources Institute (TERI)–ONGC TERI Biotech Limited (New Delhi, India); United States Department of Energy; Equinor ASA (Stavanger, Norway); RIPI—Research Institute of Petroleum Industry (Tehran, Iran); DuPont Sustainable Solutions (Virginia Beach, VA, United States); Royal Dutch Shell (The Hague, The Netherlands); Pemex (Mexico City, Mexico); BP p.l.c. (London, England, United Kingdom); and Norwegian University of Science and Technology (Trondheim, Norway). Private sector examples include ONGC

IRS—Institute of Reservoir Studies (Gujarat, India); Glori Energy Inc. (Houston, TX, United States); ZFA Technologies Co. Ltd. (Jiangsu, China); Titan Oil Recovery, Inc. (Beverly Hills, CA, United States); Yara International ASA (Oslo, Norway); Oppenheimer Biotechnology, Inc. (Austin, TX, United States); Salym Petroleum Development (Moscow, Russia); Delft Inversion (Delft, The Netherlands); and Rawwater Engineering Company Ltd. (Warrington, England, United Kingdom). These are only a few of the well-known organizations or institutions that are currently working on or with MEOR [42].

8. Conclusions

- MEOR is undoubtedly a visionary approach to the field of oil recovery, although concerns have previously been raised about the Industrial Proposal Standards required to implement field microbial processes.
- A number of challenges and complications must be addressed before MEOR can be used. Although progress has been made, past approaches should also be scrutinized.
- It is argued that MEOR is cost-effective, eco-friendly, and most suitable for mature oil wells with a high water cut and that it is a feasible alternative to conventional methods.
- New biological trends such as GEMEOR and EEOR may be the innovative strategies required to produce the desired breakthroughs. These advances may tilt the global energy balance towards cheaper prices and encourage domestic production.
- An aspiring advancement program of studies would thus be required to verify the feasibility of MEOR. Integrated research and cross-disciplinary collaboration between the fields of petroleum engineering, economics, geology, bioengineering, and microbiology are highly recommended for better results.

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