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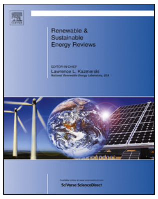

Recent developments in microbial enhanced oil recovery

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ABSTRACT

The world continues to rely heavily on petroleum as a primary energy source. However, a great fraction of the oil-in-place remains inaccessible to traditional recovery means. This review presents an update on the use of biotechnology to improve residual crude oil production from oil wells as a tertiary oil recovery method known as “microbial enhanced oil recovery” (MEOR). Our focus has been to critically discuss and analyze the recent research trends in this field, with special attention devoted to separately assessing both laboratory and field cases to better demonstrate the progress being made across different MEOR techniques. MEOR strategies reviewed here include the uses of selective plugging, biopolymers, wettability alterations, bioacids, biosolvents, and biosurfactants. Additionally, the emerging contributions of genetically-engineered microorganisms for MEOR purposes (GEMEOR) and enzyme-enhanced oil recovery (EEOR) are also analyzed. While further research must still be done to optimize MEOR methods for the oil industry, biotechnology-based methods hold much promise for oil recovery operations as well as for oil spill remediation.

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1. Introduction

1.1. The need for MEOR

Despite recent efforts to introduce renewable energy sources to the market, the world still relies heavily on crude oil and petroleum-based products. Until such "green" options can become truly viable replacements, current research undertakings would be prudent to help (1) extract hard-to-reach sources of crude, (2) lower the price of crude, and (3) lessen the impact crude oil has on the environment. Such endeavors would allow countries like the U.S. to assert more energy independence until a proper alternative energy infrastructure can be put into place.

It is estimated that over two-thirds of the crude in an oil reservoir remains untouched [1]. Primary oil recovery—the process through which simple drilling and pressure differences allows gushing oil to be captured—harvests only 5–10% of the original oil in place (OOIP) [1]. Enhanced recovery methods include the introduction of fluids to physically displace the OOIP to make it easier to recover. This secondary oil recovery is often done through the injection of water at the well-head in a process called waterflooding. It is estimated such secondary recovery methods will recoup about 10–40% of the OOIP [1–5]. Together, the primary and secondary recovery methods encompass the primary physical and mechanical means through which reservoir oil is recovered.

In recent years, a new set of oil recovery methods has been introduced. In a process known as enhanced oil recovery (EOR), chemicals such as surfactants, emulsifiers, polymers, acids, dispersants, and solvents have been used in conjunction with the aforementioned secondary recovery techniques in abandoned oil fields to improve crude oil yield, as well as for bioremediation efforts after oil spills [6]. However, these methods carry with them their own inherent risks—in addition to the economic costs, the chemical pathways through which these products are generated often use toxic chemicals, such as ethylene oxide in the production of nonionic surfactants [7,8]. Additionally, the products themselves may be damaging to the environment, especially when present with oil [9,10].

Such risks have directed attention towards finding environmentally-friendly but economically-feasible alternatives. First noted in 1926, much recent research has focused on the use of microbes and microbial products to enhance oil recovery [2]. Microbial enhanced oil recovery (MEOR) applies biotechnology to the problems of the petroleum industry, and products such as biosurfactants, biofilms, biopolymers, and biologically-produced acids and solvents have been shown to improve crude recovery. This method has advantages over traditional EOR techniques because these bioproducts can be generated from cheaper substrates and are largely biodegradable and nontoxic. Furthermore, these products have a further economic benefit because they are independent of the price of crude, unlike many of the other petroleum-based chemicals used in EOR [1].

An established library of laboratory data and an ever-growing volume of field trials indicate that tertiary oil recovery through MEOR can potentially serve as an important industrial tool. Recent developments in genetically-engineered microbial enhanced oil recovery (GEMEOR) and enzyme enhanced oil recovery (EEOR) constitute particular areas of interest to the industry.

In order to make this review different from the previous and contemporary ones, we have consciously highlighted various new aspects of MEOR research including genetically-engineered microorganisms for MEOR (GEMEOR) and enzyme-enhanced oil recovery (EEOR). To our knowledge, GEMEOR and EEOR for depleted oil reservoirs have not been critically reviewed so far. We have also included and discussed on the results of some recent field trials conducted both in India and abroad. We have also tried to highlight the need and implication of mathematical modeling in MEOR while imparting an engineering perspective to the review.

1.1.1. MEOR strategies: an overview

The use of biologically active organisms to enhance petroleum recovery is not an entirely new concept. In 1926, Beckman introduced the idea that microorganisms could be employed to free oil from porous media [3,11]. Afterwards, ZoBell reported a procedure in which sulfate-reducing bacteria was used enhance oil recovery in 1946 [3,12]. More modern MEOR research has predominantly focused on *ex situ* and *in situ* methods of delivering MEOR products into oil wells, as well as on the primary challenges of extraction, which include the immiscibility of the oil in water, the high viscosity of the crude, the size of crude oil components.

The *ex situ* method of delivery draws from the approach of chemically enhanced oil recovery (CEOR) in that it produces the desired bioproducts outside of the well and then injects them into the wellhead to enhance recovery. Such a method is appealing because it allows for more directed control by reservoir operators, as specific compositions, compounds, and products can be selected and injected. The *ex situ* method of MEOR uses microbes either grown or engineered in the laboratories to increase sweep efficiency. Microbial products of interest such as biosurfactants are often extracted from these laboratory microbes and mixed with the water before flooding, sometimes in combination with synthetic chemicals. In other approaches, isolated laboratory microbes themselves may be injected into the well, with the hope that they will produce their desired products within the reservoir.

Despite the apparent plausibility of the *ex situ* method, numerous concerns with it exist. For example, these bioproducts currently suffer from high production costs. While it has been

Nomenclature

ALD	alcohol dehydrogenase
AMEC	adapted mixed enrichment cultures
CEOR	chemically enhanced oil recovery
CMC	critical micelle concentration
CMR	cyclic microbial recovery
CO_2	carbon-di-oxide
DOE	Department of Energy
EEOR	enzyme EOR
EOR	enhanced oil recovery
FD	finite difference
GEMEOR	genetically engineered microorganisms for microbial OR
IFT	interfacial tension
IMPEC	implicit pressure, explicit concentration
IMPES	implicit pressure, explicit saturation
IRS	Institute of Reservoir Studies
K	absolute permeability
K_o	absolute permeability at initial condition
K_o	filtration coefficient
LSOR	linewise success over relaxation method
MEOR	microbial enhanced oil recovery
MFR	microbial flooding recovery
MSPR	microbial selective plugging recovery
NaCl	sodium chloride
$(NH_4)_2HPO_4$	ammonium hydrogen phosphate
OIL	Oil India Limited
ONGC	Oil and Natural Gas Corporation
OOIP	original oil in place
PDS	pressure development and implemented
PIMP	*Pseudomonas aeruginosa* (P–I) and its metabolic products
SL	stream line
SRB	sulfate reducing bacteria
TERI	The Energy and Resources Institute
TRINTOC	Trinidad and Tobago Oil Company Limited
UAE	United Arab Emirates
UTCHEM	University of Texas Chemical Composition Simulator

Latin symbols

Φ	porosity
Φ_o	porosity at initial condition

mentioned that the price would drastically decrease if more crude forms of the bioproducts were used, the cost of *ex situ* methods will nonetheless be a concern for the petroleum industry moving forward [13,14]. Furthermore, from a scientific standpoint, the method of directly injecting laboratory microbes presupposes that the laboratory strains will out-compete those strains already acclimated to the harsh well conditions and indigenous to the reservoirs. This presupposition is often not the case. The *ex situ* method of MEOR thus faces many hurdles it must overcome to establish itself as a widespread industry practice.

In contrast with the *ex situ* approach, the *in situ* approach stimulates the microbial populations indigenous to the wells to produce the desired bioproducts. While the *ex situ* methods have shown promise in controlled laboratory settings, the *in situ* approach has often been relied upon for field trials of MEOR tactics. Indigenous microbes of interest are often stimulated with cheap substrates to produce and release compounds such as biosurfactants, bioacids, and biosolvents. The stimulation of biofilm production to decrease sand permeability has also been employed in the field. While both *in situ* and *ex situ* approaches have potential, and while both tactics could be used in tandem, the available literature indicates that *in situ* operations are of higher industrial importance [1,15–17].

In addition to the pH, temperature, and pressure considerations in these wells, a number of other challenges in this field have sustained the research interest in MEOR. For example, one unique challenge is the need for microbes employed in MEOR to be anaerobic; in addition to the lack of oxygen in most of these underground wells, the injection of oxygen by drill operators can lead to metal corrosion and damage to the equipment. Furthermore, the injection of an electron acceptor such as oxygen can cause imbalances in the microbial environment and lead to desired *in situ* strains being out-competed by other indigenous microbes [3,18]. These factors taken together form the most basic parameters for most applications of MEOR. Employed bioproducts and microbes must be able to withstand and thrive in the oil well environment, and as each well may have a unique composition, different microbial consortiums and varied “cocktails” of bioproducts may need to be used [1,19–21].

The primary challenges regarding tertiary oil recovery have to do with the nature of the oil's interactions with everything around it. For example, the relatively high permeability of the rocks can lead to thief zones and soaked regions of sand that make oil inaccessible to recovery via traditional water-flooding means [1,22,23]. Water and oil are immiscible, and the different viscosities of the fluids combined with the interfacial tension forces makes recovery by water flooding more complex. The oil reservoirs worldwide represent very complicated biological systems for which laboratory simulations of microbial activities become very challenging. The microbial consortia that are introduced into an oil field would have to compete with the indigenous microflora [1]. An analysis of the data from the data base information collected from 322 projects employing the same MEOR methods resulted in the evaluation of technical effectiveness and process economics of this particular technology and provides a source of information useful for predicting treatment response in the given reservoir [24]. The application of MEOR in these trials has resulted in a substantial and sustained increase in production compared to control operations on the same reservoir. Obviously, there are differences in the extent of improvement in oil recovery, which is influenced by various factors including individual reservoir characteristics like lithology, nature of the sands, porosity, permeability, reservoir temperature, crude oil gravity and the drive types [1]. Being a unique process, MEOR jobs are successful under specific conditions and are mostly targeted for stripper wells (wells that produce less than 10 bbl/d (i.e.1056 l/d)). The DOE reservoir data base [25] and Institute of Reservoir Studies (IRS), Ahmedabad, India has standardized certain well selection criteria for MEOR jobs as in Table 1.

Strategies used to remedy the rock permeability problems have included the use of polymers, solvents, and biomass such as biofilms to selectively plug permeable rock regions [1,3,14,22,23,26,27]. Furthermore, the use of bio surfactants decreases the interfacial tension between oil and water and increases sweep efficiency [1,3,14,15,28–30]. Polymers which increase the viscosity of flood water and consequently increase the capillary number can also improve oil recovery [1,18,19,29,31]. The degradation of large alkyl chains by microbes can also improve sweep efficiency by lowering

Table 1
Standardized certain well selection criteria for MEOR jobs.

Serial no.	Parameter	IRS, Ahmedabad	US DOE
1.	Type of formation	Sandstone	Sandstone
2.	Depth, ft (m)	<8000 (2400)	$<10{,}000$ (3048)
3.	Temperature, °C	<90	<71
4.	Pressure, kg/cm^2	<300	–
5.	Reservoir rock permeability, md	>50	>100
6.	^{0}API gravity of crude oil	>20	18–40
7.	Viscosity of oil under reservoir condition, cp	<20	
8.	Water cut, %	30–90	
9.	pH	6–9	
10.	Residual oil saturation, %	>25	>25
11.	Salinity of NaCl, %	<10	<10

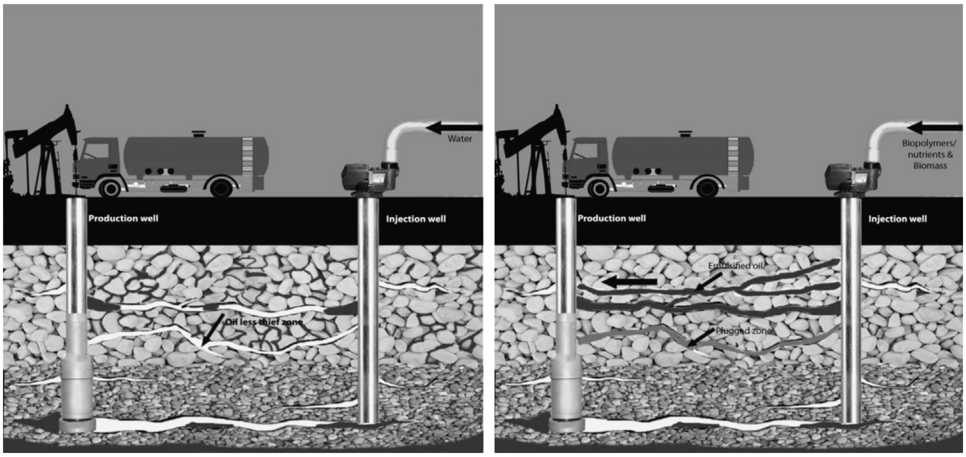

Fig. 1. MEOR strategy.

the viscosity of crude, and pressure increases due to biogenic production of gases can help oil flow out of wells [14–16,32,33]. However, despite these available methods for MEOR, caution must be exercised when introducing new compounds or changing the microenvironment of the oil reservoir. The accidental promotion of microbes such as sulfate-reducing bacteria (SRBs) harmful to recovery efforts can lead to equipment damage, increased costs, and safety hazards [1,3,16,34–37].

MEOR processes are evaluated on the basis of different applications. Microbial well stimulation or cyclic microbial recovery (CMR) is being applied on a commercial basis throughout the world. In CMR a solution of microorganism and nutrients is introduced in an oil reservoir during injection. The injection well is then shut for incubation period allowing the microorganisms to produce carbon-dioxide (CO_2) gas and surfactants that help to mobilize oil (Fig. 1). The well is then opened and oil and products resulting from the treatment are produced. This CMR process may be repeated. The major applications have been in the heavier oil reservoirs dealing with problems associated with paraffin and asphaltene deposits. The major areas of application include the United States, Venezuela, China, Indonesia and to some extent in India. Microbial Enhanced Water flooding or microbial flooding recovery (MFR) requires the transport of nutrients over a long distance within the reservoir, is still in the development phase. Oil and gas recovery by MFR method utilizes the effort of the microbial solution in a reservoir. The reservoir is usually conditioned by water pre-flush, after which a solution of microorganisms and nutrients is introduced. As the solution is pushed through the reservoir by the drive water, it forms gases and surfactants that help to mobilize the oil. The resulting oil and product solution is then pumped out through the production well.

2. MEOR strategies

2.1. *Selective plugging*

One of the most crucial problems related to oil recovery is the high porosity of the media (rocks, sand, earth, *etc.*). The oil often saturates the media and collects into inaccessible regions called "thief zones" [1,22,23]. It is this permeability of the media that tends to make extraction of crude so difficult. While some methods (such as the use of biosurfactants and EEOR) aim to release the entrapped oil, other methods attempt to selectively plug the media to keep oil from getting into the pores to begin with. This technique is often carried out through the use of biomass and biopolymers.

When indigenous bacteria grow in oil reservoirs, they take up space and their surface molecules often keep them attached to substrates near where they feed. Consequently, microbes tend to grow in the porous media themselves, creating a biofilm that help prevent more oil from being drawn into the porous zones [38]. Furthermore, these microbes tend to form colonies and cluster together as groups of biomass. Such clustering has been found to have an evolutionary advantage [39]. Some MEOR research has focused on exploiting this biomass plugging effect to lessen the number of possible paths the oil can flow through. Such a method typically requires the stimulation of indigenous microbes or the injection of selected microbes. It consequently keeps more oil together in more accessible regions in the reservoir, improving sweep efficiency. However, opportune biomass growth is not the only way in which microbes can decrease media permeability; the release of biopolymers could also influence and aid selective plugging procedures.

Bacteria growing in oil reservoirs often produce surface molecules in the form of biopolymers. Many of these polymers are exopolysaccharides which function to enhance cell adhesion and protect the bacterial cells from desiccation and predation [40]. Others, such as xanthum gum, are often used in MEOR as thickening agents for water flooding [1,26,41]. These bioproducts derived from naturally plugging microbes may be produced *ex situ* and injected directly into the well with the flood water. Other methods, in both *ex situ* and *in situ* forms, rely on stimulating injected or natural microbes to produce the desired biopolymers to enhance flood recovery.

2.1.1. *Lab scale studies*

Bacterial species and their ability to selectively plug porous media have been extensively studied in the lab. Of particular note are studies which incorporate trials directly involving the selective plugging of biomass and those that use biopolymers to thicken floodwater.

2.1.1.1. With biomass. The targeted growth of particular microbial species for biomass that can selectively plug porous media has been a considerable research topic in MEOR. Biomass can accumulate in highly permeable pore zones, channeling floodwater towards available oil [42]. Furthermore, such biomass could have surface properties favorable enough to enhance wettability by plugging in rock pores [38]. The stimulation and use of viable strains for selective plugging has been a topic of MEOR research. It was proposed that four criteria needed to be met for successful biomass plugging [43]. These criteria included that the cells must be transported throughout the rock media (1), nutrients must be supplied for proper growth (2), the strains must grow and/or produce bioproducts adequate for selective plugging (3), and that the growth of the microbes must not be so rapid that it clogs the well bore (4). A recent laboratory study found that a strain known as *Bacillus licheniformis BNP*29 fit all of the criteria when injected into low-permeability cores and could also produce bioproducts suitable for MEOR purposes [26]. This strain allows for better selective plugging than sulfur-reducing bacteria, which non-selectively plug porous media in addition to releasing products harmful to MEOR operations. It is thus important to selectively stimulate selective plugging microbes rather than stimulating all of the microbes that are indigenous to a well. Future laboratory and pilot scale research on selective plugging should incorporate a focus on selective stimulation to make biomass selective plugging a viable MEOR tool for the industry.

2.1.1.2. With biopolymers. A variety of organisms are known to produce polymers that can aid in enhanced oil recovery techniques. Bacteria such as *Xanthomonous*, *Aureobasidium*, and *Bacillus* in particular have been singled out for their production [1]. Important biopolymers include xanthan gum and curdlan.

Xanthan gum is one of the most versatile products available, with wide applicability in the food, cosmetic, chemical, and oil industries [44]. Because of its high temperature and salt tolerance, xanthan gum is a superior polymer capable of injection into drilling operations [44]. Because of this, much research has been done to find high producing mutant strains and strains that can subsist on cheap substrates [44–46]. Xanthan gum alters the viscosity of floodwater and allows it to reach and recover oil at levels far above simple water flooding. Most often directly injected into flood water, it can also be generated by stimulated bacterial strains in the reservoir.

While not as effective as xanthan gum, curdlan has also been mentioned as a biopolymer capable of enhancing MEOR efforts [1]. Curdlan modifies the permeability of the rock. One laboratory study described mixing a particular version of the biopolymer with acid-producing bacteria and injecting the mixture into Berea Sandstone cores. The mixture decreased the permeability from 850 to 2.99 mD and from 904 to 4.86 mD, respectively, giving residual resistance factors of 334 and 186 [47].

Typical research involving biopolymers has directly mixed the products with flood water as opposed to stimulating injected or indigenous microbes to produce the biopolymers [48]. In addition to biopolymers such as xanthan gum and curdlan, other microbial products have also been known to selectively plug porous regions. Emulsions and other bacterial products can become viscous enough to aid in plugging efforts and can allow better channeling for crude oil recovery [14]. Different products can thus provide multiple advantages for MEOR efforts.

2.1.2. *Field studies*

This MEOR strategy has predominantly been done by stimulating indigenous bacteria that are capable of selective plugging and forming bioproducts that accomplish similar goals [1,49]. It has been found in laboratory experiments that the use of cornsteep liquor and $(NH_4)_2HPO_4$ can adequately stimulate microbial growth that induce the desired plugging effects [50]. In Canada, a new concept based on selective plugging uses ultra-micro bacteria formed by selective starvation [51]. Another new concept in selective plugging is based on the idea of using bio-mineralization to form calcite cements capable of sand consolidation and fracture closure in carbonate formations. A study in Canada showed that selective plugging strategies remain the most promising [52–54]. Here problems center on the difficulties of displacing viscous treacle-like material with the more mobile water phase in a heterogeneous matrix with zones of high permeability. In these Canadian fields the temperature (21–33 °C) are well below the upper limit temperature tolerance level of *Leuconostoc mesenteroides* 40 °C. In deeper fields, use of *Leuconostoc* might still be feasible as in contrast to *in situ* production of MEOR oil mobilizing agents, the blocking effect of biomass and polymers could be effectively much closer to the injector well, within the region cooled by water flooding. A 2010 application of selective plugging research to the Block Zhan-3 oil field upheld the results of this study by finding that indigenous microbial flooding can achieve better water reduction and an increase in oil production [50]. A field study in Brazil also found that, with the right blend of nutrients and electron acceptors, the stimulation of indigenous bacterial species can lead to improved vertical sweep efficiency due to the plugging of high permeability zones due to the *in situ* production of biopolymers and biomass [55]. The primary focus of research for this strategy is to figure out optimal nutrient blends that stimulate desired populations. It should be noted that many alternative options exist in addition to the corn steep liquor method. The employment of selective plugging and most uses of generated bioproducts in oil wells are primarily done through the *in situ* stimulation of microbes by the addition of proper nutrients and additives. Early research had established that MEOR could not be applied to all petroleum reservoirs. It has been studied that the effect of permeability and pore size distribution on the penetration of bacteria through petroleum reservoir rock, and concluded that MEOR was only applicable to petroleum reservoirs where the average permeability exceeds 100 md [56]. Field trials have been performed with different degrees of success [16,25,43,49,57–66].

2.2. Wettability alteration

The immiscibility of crude oil and water leads to a myriad of challenges for oil recovery. One of these challenges is the low water wettability of the porous media. Much of the world's oil is found in fractured carbonate rock reservoirs [67], with matrix blocks that range from mixed to oil-wet. The difficulty of water to become adsorbed onto the surfaces of these matrix blocks contributes to lower sweep efficiencies, as the oil in these entrapped regions can avoid the draw of water flooding techniques [1]. Barriers formed from microbial colonies growing on the fractured reservoir rock can further keep such oil intact during recovery operations [1,3,68]. By increasing the water wettability of the reservoir rock under certain conditions, studies have shown that oil recovery improves [1,3]. Methods through which wettability has been increased to enhance oil recovery include the introduction of small concentrations of surfactants and the stimulation of bacteria that adhere to the reservoir rock directly and increase the ability of water to mix with oil (Fig. 2). Such techniques improve sweep efficiency and hence, lead to better yields for oil recovery.

2.2.1. Lab scale studies

The wettability of reservoir rock is commonly modified through MEOR by the formation of a biofilm and through the application of biosurfactants. They provide advantages such as improved resistance to antimicrobial agents over suspended cells and by attaching to rock reservoir surfaces they have the ability to change the surface properties of the media [38]. Through such changes, wettability often increases. One particular study of note, titled *Investigating wettability alteration during MEOR process, a micro/macro scale analysis*, simulated the effects of aging onto glass surfaces to witness the effects upon wettability due to an *Enterobacter cloacae* strain in the lab. The study found that although many microbial products including biosurfactants could enhance wettability, biofilm formation was the most important factor [38]. Furthermore, it was found that increasing surface roughness and aging allowed for better enhancement of water wettability [38]. MEOR field trial procedures which allow for incubation and which can exploit, or create additional, surface roughness could allow for better industrial crude oil recovery. Amongst microbial products used for wettability alterations, biosurfactants have been noted as holding extreme promise. A study researching the effects of ion charges and polarity on surfactants found that the inclusion of such compounds in surfactant blends enhanced the ability of these surfactants to alter wettability [67]. Moreover, the same study found that, in the laboratory setting, the potent biosurfactant surfactin performed well in comparison to synthetic alternatives [67]. Thus, the production of surface-altering products by bacteria in oil reservoirs can enhance oil recovery by helping to improve the water-wetness of reservoir rock. Whether the production of such products—or of the more effective biofilms—is performed *in situ* or *ex situ*, the directed modification of rock wettability serves as a viable way to use microbes to enhance oil recovery.

2.3. Bioacids/solvents/gases

The problem posed by the high permeability of carbonate reservoir rock to oil can also be solved by other means. Microbial versions of traditional chemical methods can be employed to enhance oil recovery through a variety of means. Microbes can produce important acids, solvents, and gases to aid in MEOR. The production of extracellular acids by microbes aids oil recovery by dissolving parts of the carbonate rock [1,3]. While other measures like plugging or wettability alteration aim to use the properties of the reservoir rock to enhance recovery, the use of bioacids is predicated on removing the rocks themselves to make it easier for flooding to access hidden oil. Acetic and propionic acids in particular are notably used in this regard [1]. Similarly, solvents such as acetone and ethanol also dissolve the carbonate rock [27]. Thus, the bioproduction of acids and solvents aids in hydrocarbon recovery by changing the porosity and permeability of the media entrapping the oil. Traditional enhanced oil recovery methods include attempts at re-pressurization [69]. By introducing more pressure into the well, well operators can force oil out of the reservoir in a manner reminiscent of primary recovery means. To produce such a phenomenon *in situ*, microbes that can generate gases are stimulated. By fermenting carbohydrates, bacteria can produce gases such as methane, carbon dioxide, and hydrogen, which re-pressurize the reservoir and enhances oil recovery [1]. This same fermentation can lead to the formation of organic acids and solvents used to dissolve the carbonate rock. In addition to increasing reservoir pressure, the gases can dissolve into the crude and reduce its viscosity, increasing the sweep efficiency [1,3].

2.3.1. Lab scale studies

Biosolvents, biogases, and bioacids have all been studied at the laboratory level for potential MEOR applications. Biosolvents produced by bacteria including *Zymomonas mobilis*, *Clostridium*

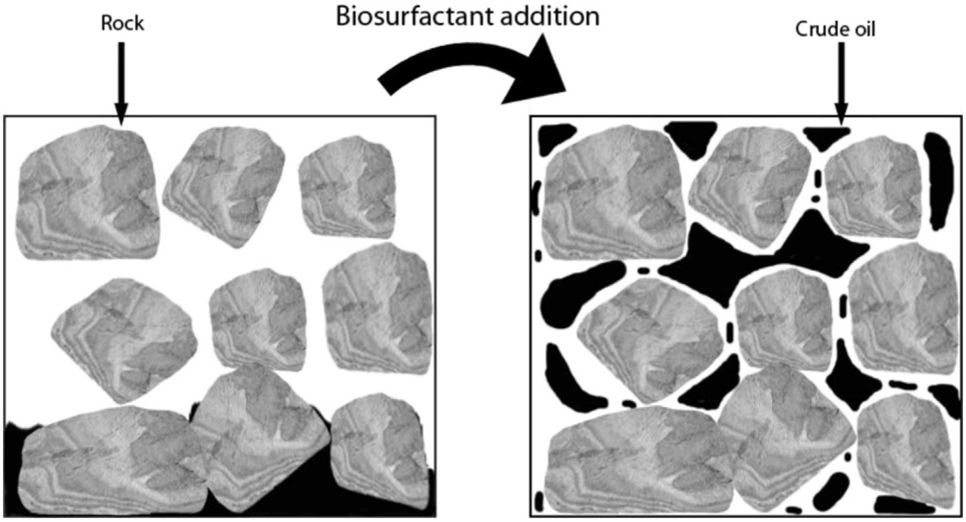

Fig. 2. MEOR by modifying wettability of rocks.

acetobutylicum, and *Clostridium pasteurianum* and the stimulation of indigenous or injected microbes make the most sense to use this product [18]. Widespread generation of solvents such as ethanol by microbes is still an emerging technology [70], and it therefore appears that biosolvents are unlikely to be produced in large enough quantities to directly inject into reservoirs. However, the use of advantageous bacteria that are resistant to injected chemical solvents could be a potential avenue for further MEOR investigations [71]. Bioacids allow for the dissolution of carbonate rock. This makes it easier for flood recovery to access the oil hidden in thief zones. Bacteria such as *Clostridium* have been known to release acetate and butyrate [72], which lead to bioacids. *Bacillus* strains, which have often been injected for their biosurfactant production, can also introduce bioacids into oil wells [72]. However, few studies have been published attempting to use biosolvents, biogases, or bioacids in *ex situ* production and direct injection into oil wells. Field studies incorporating biosolvents, biogases, and bioacids are currently lacking. In comparison to other methods such as plugging and biosurfactants, these three bioproducts appear to be on the periphery of effective MEOR techniques. While indigenous or injected microbes which can produce these bioproducts can be stimulated to enhance MEOR, these effects should not be the ones focused on enhancing oil recovery through biotechnological means. Other methods remain more effective, controllable, and efficient.

2.4. *Degradation, clean-up of build up*

Many of the microbes living in oil reservoirs consume hydrocarbons. In order to do this, they must break down the long alkyl chains present in heavy crude oil. By degrading these chains, bacteria increase the amount of valuable, light crude [1,15]. This lighter crude is less viscous and therefore, easier to recover after flooding. The ability of a microbe to degrade crude is amongst the most fascinating and practical uses of MEOR for the petroleum industry. In addition to stimulating *in situ* or injecting *ex situ* strains of hydrocarbon-degrading bacteria to obtain lighter crude, oil-degrading bacteria have shown tremendous promise in other aspects of interest to the petroleum industry. For example, many oil extraction processes lead to hazardous waste products. Certain waste, such as oily sludge, can be degraded by bacteria in an environmentally friendly and economical manner [73]. Furthermore, these microbes can aid in the upkeep and maintenance of industry equipment: microbes have been used to clean tanks and storage containers, and are often injected into wellheads specifically to keep them clean and keep them from being plugged [14,26,73]. Thus, microbes which can degrade long alkyl chains provide numerous opportunities for the industry and serve as amongst the best examples of successful MEOR applications.

2.4.1. *Lab scale studies*

2.4.1.1. Environmental remediation. Oily sludge left over from drilling operations poses a substantial risk to the environment: it can contaminate soil, air, and groundwater [73]. One fruitful application of MEOR has been to remediate contaminated sites by recovering the contaminating oil [73]. While other conventional tactics may generate their own toxic by-products, an application of suitable microbes is significantly more environmentally-friendly [73]. The use of a rhamnolipid-biosurfactant for bioremediation was chronicled as being one successful method [73]. Another study, which compared the performances of many biosurfactants to remediate crude-contaminated oil found that the most successful biosurfactants were those with low CMCs, high miscibility with oil and soil, and low molecular weights [74].

2.4.1.2. Clean up of paraffin deposits. Other uses of microbes to enhance drilling operations include the maintenance of equipment. One problem encountered by the oil industry is the formation of paraffin in storage tanks, wellheads, and pipes. While some methods incorporate the use of harsh chemicals, employing microbes that can readily degrade these alkyl chains is a viable alternative. For example, one study successfully employed special microbial consortiums designed to degrade paraffin deposits [75].

2.4.1.3. Alkyl chain degradation and MEOR. In addition to degrading paraffin deposits to upkeep equipment, microbial consortiums can also be used to degrade alkyl chains to enhance oil recovery. Oil-degrading bacteria can get closer to porous media and enhance the wettability of the rock [32]. Furthermore, the degradation of long alkyl chains leads to a reduction in viscosity that can enhance flood recovery. It has been shown in the lab that certain strains of *Bacillus subtilis* can do exactly that at 40 °C [15]. Furthermore, bacterial strains that can both degrade long alkyl chains and also produce biosurfactants hold tremendous promise for MEOR and are ideal for future studies [15].

2.4.2. *Field studies*

The use of oil-degrading bacteria in MEOR field trials has been well-documented. One reason for this is because the removal of wax depositions from high pour-point oil reservoirs is a crucial step that is furthered significantly by stimulating *in situ* oil-degrading bacteria [5,14,76,77]. Other applications of *in situ* MEOR involve the use of degrading microbes to improve flood recovery. One such recent application of oil-degrading bacteria to the field was the stimulation of aerobic bacteria that could degrade heavy oil fractions to increase recovery by injecting dissolved oxygen in water in the Kongdiong oilfield of the Chinese Daqang oilfield [78]. Furthermore, consortiums of oil-degrading bacteria can be used to enhance the mobility of oil in transport pipes [79].

2.5. *Surface tension alteration: biosurfactants*

Biosurfactants are arguably amongst the most exciting of the MEOR tools available. Biodegradable, temperature tolerant, and pH-hardy, these amphipathic molecules are viable alternatives to the conventional surfactants used by the petroleum industry. Biosurfactants lower surface and interfacial tensions and consequently improve flood recovery [1,15,29]. While there are some concerns with the cost of biosurfactants, using effective crude biosurfactant products instead of the expensive pure forms derived for medical purposes is one viable way to make this method economically competitive with current surfactants [13,14,80]. Biosurfactants can be applied to many fields in addition to MEOR. Current uses include use as a biopesticide, eco-friendly detergent, antimicrobial agents in the health industry, emulsifiers in the food industry, bioremediation, and cleaning oil storage tanks [80]. Because of their general nontoxicity to humans, biodegradability, and the wide applicability of biosurfactant properties, these products have a promising future in the developing green economy. Biosurfactants are secreted by microbes in oil reservoirs primarily to alter surface tension, adsorb onto immiscible interfaces, emulsify crude oil that is to be consumed, and increase the mobility of bacterial cells [1,3,73]. In fact, it has been noted that biosurfactants can aid in the mass motility of bacterial colonies across different local environmental regions [81]. As with many biological phenomena, we can exploit these natural processes to suit human goals including MEOR, medicine, and environmental remediation [82]. Biosurfactants have an advantage over traditional chemical surfactants because lower concentrations of biosurfactants can be used to achieve similar results [1,15].

The threshold concentration for a given biosurfactant to reach its ability to turn its emulsifying ring structures into spherical micelles is known as the critical micelle concentration (CMC). This is the primary mechanism used to improve MEOR yields using biosurfactants.

2.5.1. Different types of biosurfactants

There are various types of biosurfactants, each with its own particular set of properties. Major classification groups of biosurfactants include glycolipids, fatty acid biosurfactants, lipopeptides, emulsifying protein, and particulate biosurfactants [80]. From these, lipopeptides and a type of glycolipid known as rhamnolipids have particular importance in MEOR trials because they can lower the hydrocarbon-aqueous IFT to below 0.1 mN/m [83]. Consequently, they are the most used biosurfactants for MEOR purposes [83].

2.5.1.1. Rhamnolipids. Rhamnolipids are derived from the bacterium *Pseudomonas aeruginosa*, and have a rhamnose head and fatty acid tail. These biosurfactants are composed of either one or two molecules of the sugar rhamnose, and they are amongst the best studied of all biosurfactants. They have natural bacterial uses ranging from biofilm production to helping cell mobility through a process known as swarm motility [84]. Furthermore, in addition to aiding in MEOR flooding operations, rhamnolipids have been noted as fine degraders of crude oil for remediation purposes [80]. Rhamnolipids produced by certain strains of bacteria have been noted as killing off competing strains of bacteria [85]. This function as an antimicrobial agent has been exploited in the health industry. Rhamnolipids come in many shapes, including a conical dirhamnolipid [80].

2.5.1.2. Lipopeptides. Lipopeptides include surfactin, a widely known powerful surfactant with the ability to drop the surface tension of water from 72 to 27.9 mN/m at a CMC of 0.017 g/L [86]. Lipopeptides are derived from bacteria, most notably from strains of *B. subtilis* and *Pseudomonas*, and are often used as antibiotics. Some lipopeptides are cyclical in shape [80].

2.5.2. Use of biosurfactant in MEOR application

The main purpose of biosurfactant-based MEOR methods is to lessen the interfacial tension between crude oil and water to increase the yield of flood recovery. In order to do this, viable biosurfactants must first be screened, developed, and economically scaled up. Optimal biosurfactants will possess strong interfacial activity, a low CMC, broad tolerance to temperature and pH, good solubility, and high emulsion capacities [87]. Suitable products can be either directly injected *ex situ* or produced indirectly either by stimulated indigenous microbes or injected *ex situ* microbes. Sometimes, additional products such as various metal ions are also introduced with the biosurfactant to induce better properties due to polar interactions between the ions and the biosurfactant [88]. With lab trials that have reported up to 95% oil recovery from sand-packed columns, biosurfactant-mediated MEOR is a promising technology [89].

2.5.2.1. Lab scale studies. The hardiness and effectiveness of biosurfactants have been well-noted in laboratory trials. For example, a 2011 study made the case for isolating suitable microbial species and exploiting their abilities to create biosurfactants [15]. This study isolated various *Bacillus* strains that produced biosurfactants that lowered the surface tension of water from 72 to 30 mN/m at temperatures as high as 40 °C for the bacteria and 121 °C when the biosurfactants were used alone [15]. Such results indicate that biosurfactants have high stand-alone potential and may be directly mixed with floodwater, after considering salinity and temperature limitations of the water. Furthermore, other studies have demonstrated that biosurfactants often outperform their synthetic counterparts [29]. That being said, it is important to evaluate the individual properties of each unique biosurfactant; sometimes, while some biosurfactants may have more favorable growth times, others have better surface activity [29] and it is imperative that MEOR ventures are performed after a heavy consideration of such differences. However, it is important to note that the reduction of IFT is not the exclusive factor in improving MEOR yields, and further research must still be done [14]. As noted in previous sections, biosurfactants do more than simply reduce interfacial and surface tensions. They can also enhance oil recovery through altering wettability, aiding in the degrading of long alkyl chains, and cleaning up contaminated soil [1,15,29,73]. This demonstrates that many MEOR strategies are interlinked because of the multifaceted nature of the properties of bioproducts. Because many factors can blend with one another, the cascade effect caused by introducing a bioproduct such as a biosurfactant must be thought of, researched, and tested when MEOR is applied to the field.

2.5.2.2. Field studies. The two most common methods of employing biosurfactants in the field are direct injection and the *in situ* stimulation of indigenous or injected microbes. The direct injection of biosurfactants into oil reservoirs is amongst the most widely used tactics to increase sweep efficiency. Biosurfactants may be injected alone or in a cocktail of sorts with other forms. One recent study used a strain of *P. aeruginosa* in conjunction with its metabolites as a biosurfactant. This mixture, called PIMP, was found to increase oil recovery and prolong the life of oil wells in Daqang through the mechanisms of IFT reduction and the lessening of oil viscosity [28]. Furthermore, the relatively low concentration of biosurfactant needed to induce a favorable effect allows it to be a viable additive to flood recovery operations. That being said, it has been noted that amongst the biggest barriers to the direct injection of biosurfactants are cost and scale. The expenses to maintain the bioreactors, facilities, and purification efforts compared to the low yield of production make the direct injection of most biosurfactants currently economically unfeasible [79]. It has been argued, however, that stimulating the indigenous populations of biosurfactant-producing bacteria would be amongst the most cost-effective MEOR strategies [79]. It is important to find ways to selectively stimulate only the desired populations, however. Recent research has shown that desired populations can be exclusively stimulated *in situ* by nutrients while inhibiting SRBs and other species harmful to MEOR efforts [16]. A variety of field trials have found ways to induce the *in situ* production of biosurfactants—whether by stimulating existing populations or injecting exogenous strains. For example, a 2006 study incorporated the *in situ* metabolism of a bacterial population to produce biosurfactant in a limestone reservoir, demonstrating that this method was both cost-effective and technically-feasible [90]. Another study from 2011 injected exogenous *B. subtilis* strains that then produced enough biosurfactant after 60 days to allow for an additional 52.5 cubic meters of oil recovery [17]. This study also demonstrated that the stimulation of *in situ* biosurfactant production can be economically viable. Further research upon either of these strategies could bolster the credibility of biosurfactant use in oil reservoirs and lead to a mainstream adoption of MEOR technologies. Going forward, future research on biosurfactants should focus on ways to make these products more cost-effective. More research should be done to evaluate how best to stimulate indigenous strains, as it has been argued that stimulating these strains is better than adding nonnative species that could be outcompeted [79]. Additional field trials on the other benefits of biosurfactants, such as their abilities to clean up oily sludge and enhance oil mobility in transport can also help this technology gain more credibility in the industry [91].

Economic analyses are needed to compare direct biosurfactant injection with *in situ* production. But by exploring other properties of biosurfactants, finding cheaper means of production, and testing for better implementation in the field, it is possible for biosurfactants to eventually be adopted for widespread use.

3. GEMEOR

Conventional *in situ* and *ex situ* methods make use of existing native microbes with a finite number of possible trait combinations. For much of MEOR's history, the bacteria studied each possessed their own unique traits but each also possessed their own unique limitations. For example, a bacterial strain that produces large amounts of a vital bio surfactant could not survive at temperatures whereas others, more thermophilic bacteria could thrive. Due to such constraints posed by conventional MEOR techniques, a new trend in petroleum biotechnology research has emerged. Genetically-engineered microbial enhanced oil recovery (GEMEOR) uses genetic engineering methods such as recombineering, protoplast fusion, and mutagenesis to enhance oil recovery by performing tasks such as combining favorable traits from various organisms and creating more efficient strains [82,92,93]. This technique aims to expand the scope of MEOR by introducing aspects of metabolic engineering as well as, allowing for the production and employment of more efficient biochemical products and cells in oil wells. Moreover, engineering bacterial strains helps to obtain microbial products with desired properties. By adjusting growth conditions and applying such genetic approaches, the yields of bioproducts is most likely to be enhanced which can make it economically feasible. While still predominantly tested in lab and pilot scale levels, this approach has recently emerged successful in field trials and represents a potential cost-effective and highly efficient application of MEOR in the industry.

3.1. Lab scale studies

The advances in genetic engineering tools and techniques offers the advantage of manipulating, engineering and producing microorganisms that can withstand extreme environmental conditions and at the same time grow on cheap substrates and produce metabolites in significant amount. A considerable amount of research has focused on the search for indigenous microorganisms suited for MEOR applications. However, they suffer from disadvantages which make it difficult to employ at the commercial scale. Such strains have considerably low metabolic activities and produce metabolic products which are usually dilute. Such problems can be addressed with the aid of genetic engineering approaches. The main purpose is to obtain microorganisms with inherent ability of surmounting and surviving under harsh environmental conditions such as high temperature, pH, pressure and salinity. The genetic engineering based approaches allow the DNA sequence of organism to get inserted into a host by protoplast fusion or by incorporation of recombinant plasmid DNA into the competent cells [94]. Protoplast fusion offers the advantage of developing and improving hybrid strains. Little literature exists on the genetic manipulation of microorganisms as an application for MEOR. The genetic manipulation can be done by two ways. First, the structural, chemical and functional characteristics of the protein help in creating site-specific mutation of the target enzyme [95]. Second, the processes of random mutagenesis and high-throughput screening have been employed to generate mutants with desired characteristics which don't entail information about structural and functional properties of the proteins [96,97]. A transformant constructed by employing an *E. cloacae* and a thermophilic *Geobacillus* strain showed enhanced exopolysaccharide in the range of 8.83 g/l in molasses medium at elevated temperatures of 54 °C. The RAPD analysis was done followed by core flooding experiments which demonstrated the potential of such approach in MEOR technology [82]. The similar experiment involving generation of fusant strain ZR3 using above two strains was also demonstrated by protoplast fusion which resulted in production of 7.5 g/L of exopolysaccharide in pH range of 7–9 and thus holds a promise in future applications of MEOR [98]. In another study, engineered strains named STP-1 and STP-5 was produced using *Enterobacter sakazakii*, capable of highly water insoluble polysaccharide production, and *B. subtilis I,* using protoplast fusion. STP-1 showed higher ability of enhanced oil recovery as compared to STP-5 upon sand-pack flooding tests [33]. A study involving use of biosurfactant-producing strains of *E. cloacae* and *Bacillus stearothermophilus SUCPM*#14 on carbonated cores was done to measure interfacial tension (IFT). *E. cloacae* and *B. stearothermophilus SUCPM*#14 showed significant reduction of IFT from 30 to 2.7 mN/m and 30 to 15 mN/m in 24 hours [99]. The aim of microbial biopolymer is to ameliorate the efficiency of selective plugging.

3.2. Field studies

GEMEOR has not been extensively applied to the field. Hence, a review of field studies is omitted here.

4. EEOR

The use of enzymes to enhance oil recovery is another novel concept under much recent research consideration. Enzymes are proteins used to catalyze various biochemical reactions, and when applied to enzyme enhanced oil recovery (EEOR), they are often used in consortium with other enzymes or surfactants. It is believed that these surfactants aid in EEOR by enhancing active site mechanisms for the enzymes [100]. These enzyme mixtures alter the oil-rock-water interface dynamic through accompanying changes in wettability and capillary action, rendering oil easier to recover [100]. Harris and Mckay first suggested the application of enzymes to the oil and gas industry for uses including the desulphurization of hydrocarbons and pretreating biopolymers to make them easier to handle [101]. Later studies such as those by Nemati and Voordouw suggest that enzymes can be used to modify reservoir rock permeability by aiding in plugging using the products generated in catalyzed reactions [102]. Enzymes may also aid MEOR plugging methods by breaking down insoluble bacterial cells to improve the injection efficiency of biopolymers such as xanthan gum [103]. Furthermore, many hydrolases have been shown to break down crude oil components, making recovery much easier [100]. However, it is the adsorption ability of enzyme proteins and the accompanying increase in water-wettability that most aids EEOR [82]. Enzyme enhanced oil recovery is amongst the newest *ex situ* research trends. Despite its high cost, it shows potential promise for the industry. A number of companies have introduced products, such as Greenzyme, that have undergone both laboratory and limited field trials. The addition of EEOR to existing microbial oil recovery techniques could eventually lead to more successful brine injection solutions for use in industry.

4.1. Lab scale studies

Recent laboratory research on EEOR has found that enzyme-assisted oil recovery techniques can improve the yield of water flooding [100]. One comprehensive study found that Greenzyme and the NZ enzymes from the Novozymes group both gave “incremental increase[s] in oil recovery of 1–11% OOIP” when added to a brine solution, and that no major difference was detected between the two types of enzymes [100]. These experiments were performed using sandstone and carbonate rocks and also found that the enzyme-brine solution could mobilize oil from areas not swept by the water flooding [100]. This study found that wettability alterations and a reduction of interfacial tension were the primary mechanisms. Future lab scale work on EEOR should continue to focus on improving OOIP recovery, perhaps focusing on the effects of mixing various additives such as biosurfactants and enzymes. Furthermore, finding or engineering microbial strains that could produce viable enzymes *in situ* could improve EEOR yield in a cost-effective manner that could later be applied to the field.

4.2. Field studies

Despite the relative youth of this technique, enzymes have been increasingly applied to the field. Much of the limited field study trials on record have taken place in Asia, and have focused on both directly increasing oil yield and on using enzymes to clean blockages and separate miscellaneous well components from the crude. Field trials applying enzymes to directly enhance oil recovery have mirrored the results found in the laboratory. One study, which applied the commercial product Greenzyme to 2 mature oil wells in Myanmar, found that the enzyme enhanced water flooding by increasing the water-wetness of the reservoir rock, provided that the oil was above a certain fluidity point [104]. The same study also found that Greenzyme was able to function in many extreme pH, temperature, and salinity conditions, though it proved to be unstable if left in the well for many days [104]. Another study using different types of enzymes including alcohol dehydrogenase (ADH) and xylanase found that oil recovery can increase by 3% primarily through wettability alterations that make the reservoir rock more water wet and by emulsification [105]. These enzymes can be used in homogenous reservoirs with medium to low permeability and with high resin and asphaltene content [105].

Enzymes have also been used in the field for separating well components from crude and for operation treatments. In another Shengli field study, researchers found that the use of an agent known as SUN enhanced the maintenance and upkeep for a wide variety of operations and oilfield treatments; the agent, composed of proteinases and bacteria, was able to aid blockage removal in heavy crude oil wells, remove and inhibit the formation of wax, and even enhance water injectivity [106]. Field applications using such a mixed microbial-enzyme approach seem to hold promise for the oil industry. Enzyme-enhanced oil recovery is a new tool that could potentially aid the industry by increasing productivity of both oil wells and existing machinery. However, despite the commercial attempts to go mainstream with this technology, this technique is still a developing body of work and many things must be considered in order to advance forward. In addition to cost concerns, the productivity of injected enzymes must be improved for EEOR to viably compete with other MEOR approaches. While it has been suggested that increasing the enzyme's well incubation times could do just that, considerable research must still be done at all levels to develop worthwhile EEOR strategies [105].

5. Status of MEOR

5.1. Global scenario

Since the mid 1950s numerous MEOR field trials have been conducted in the US, Eastern Europe, the USSR and the Netherlands [107,108]. The oil crisis of 1970 triggered a great interest in active MEOR research in many countries. Table 2 gives the list of field trials carried out in various countries. The first field test was carried out in the Lisbon field, Union County, Arkansas, USA [18,25,107–113]. A 2% solution of the beet molasses was injected along with 4000 gallons of bacterial suspension *i.e.*, *C. acetobutylicum*, and molasses injection was continued for six months. Breakthrough of fermentation products was obtained 80–90 days after inoculation. Samples of the produced water contained source bacteria but no *C. acetobutylicum*; samples of produced oil contained no bacteria or fermentation products and were not different from the produced oil from parts of the reservoir not affected by the fermentation. Literature also reports the experiments with molasses, volatile acids and carbon-dioxide (CO_2) production [114]. They found a highly increased production by injecting bacterium of the clostridium. The actual rate of oil production from a well was 3.5 times the normal predicted value, an increase in oil production of approximately 250%. The mechanism responsible for this great increase in oil recovery but probably involve four mechanisms *i.e.*, gas production, acid production resulting in solution of carbonate rocks in the reservoir with release of CO_2, surfactant production and reduction of oil viscosity by solution of produced gas in the oil. In addition permeability stratification rectification may have been achieved by selective plugging of thief zones. In Soviet Union (now Russia) efforts were started in the 1960s towards this direction. Experiments were also carried out a field test in which 54 m^3 of a mixed bacterial culture growing in a 4% molasses solution was injected into a well in the Sernovodek oil field [115]. The well was shut in for six months. The well head pressure increased to 1.5 atm. When the well was reopened, oil production increased slightly, and then fell [116,117]. Wagner's experience was then used for some MEOR applications in Tataryia oil fields in Russia [52]. Simulated by Soviet effort, research group in four Eastern European countries, namely Czechoslovakia, Poland, Romania and Hungary also carried out field tests. In 1955 La Riviere correlated oil release with reduced surface tension in laboratory experiments using rapidly growing cultures of sulfate reducing bacteria. Although this work has been criticized [118], similar observation were reported subsequently from Czechoslovakia. Dostalek's group in Czechoslovakia [119] reported the isolation of a soil clostridium which would grow on petroleum, or on carbohydrate or yeast extract, with the production of large quantities of gas. Laboratory trials indicated that gas production was the critical factor in releasing oil. In a series of field trials in 1954, cultures of *Desulfovibrio* and *Pseudomonas* were injected together with molasses. Bacterial counts in the formation water were increased in every case. In three of the seven trials increased production of oil was reported. In one individual wells showed increase of 12–36% and the whole formation showed an increase of 7% over the six month trial. Trials in which either inoculums or molasses were omitted gave no increase in oil production. In the successful trials, permeabilities were in excess of 3 Darcy. In Romania, experiments were carried out many field tests from 1971 to 1982, which were reported successful results of MEOR field trials both in single-well stimulation and microbial flooding recovery technologies at several Romanian oil fields, where adapted mixed enrichment cultures (AMEC) and molasses were injected into reservoirs after an improved protocol of injection [52]. He injected 20% molasses medium inoculated with bacteria isolated from formation waters. He injected in one well and oil and water were produced from a nearby well like water flooding. After 6–8 months, there was evidence of gas, acid, biopolymer or surfactant production. Two reservoirs out of 7 showed increased oil recovery. The reasons for failure in 4 wells were identified as follows:

- low permeability rock;
- lack of strata continuity from the injection well to the production well;
- movement of unconsolidated sand in the reservoir;
- undesirable high temperature (52–56 °C) and high salt concentration in the connate water (170–190 g NaCl/L).

Field trials by injecting genus clostridium were also performed in other countries and encouraging results have been reported. In Hungary after injecting a mixed culture growing in a medium containing molasses, potassium nitrate, sodium

Table 2
World experience in MEOR field trials.

Country	Acronyms of MEOR technology	Microbial systems	Nutrients	Incremental of oil production	References
USA	CMR, MFR, MSPR	Pure or mixed cultures of Bacillus, Clostridium, Pseudomonas, Gram-negative rods	Molasses 2–4%, Molasses and ammonium nitrate	+	[18,25,107–114]
		Mixed cultures of hydrocarbon degrading bacteria	Free corn sirup C mineral salts		
		Mixed cultures of marine source bacteria	Maltodextrine and organic phosphate esters (OPE)		
		Spore suspension of Clostridium	Salt solution		
		Indigenous stratal microflora	Sucrose 10% C, Peptone 1% C, NaCl 0.5–30%		
		Slime-forming bacteria	Brine supplemented with nitrogen and phosphorous sources and nitrate, biodegradable paraffinic fractions + mineral salts		
		Ultra microbacteria	Naturally contain inorganic and organic materials C, N, P sources		
Russia	MFR, MSPR	Pure cultures of *Clostridium tyrobutiricum*	Molasses 2–6% with nitrogen and phosphorous salt addition	+	[115–117,121,171,172]
		Bacteria mixed cultures	Water injection with nitrogen and phosphorous salt and air addition		
		Indigenous microflora of water injection and water formation	Waste waters with addition of biostimulators and chemical additives		
		Activated sludge bacteria	Industrial wastes with salts addition		
		Naturally occurring microbiota of industrial (food) wastes	Dry milk 0.04%		
Former Czechoslovakia	CMR, MFR	Hydrocarbon oxidizing bacteria (predominant Pseudomonas sp.)	Molasses	+	[118,119]
		Sulfate-reducing bacteria			
Poland	MFR	Mixed bacteria cultures (Arthrobacter, Clostridium, Mycobacterium, Pseudomonas, Peptococcus)	Molasses 2%	+	[173]
Romania	CMF, MFR	Adapted mixed enrichment cultures (predominant: Clostridium, Bacillus, Pseudomonas, and other gram-negative rods)	In first field trial 20% Molasses was introduced and after wards 2–4% molasses was introduced		[52,75]
Hungary	CMF, MFR	Mixed culture growing in a medium containing	Molasses, potassium nitrate, sodium phosphate and sucrose	+	[120,174]
Canada	MSPR	Pure culture of *Leuconostoc mesenteroides*	Dry sucrose + sugar beet molasses dissolved in water	−	[51] [54,175–177]
Former East Germany	MFR	Mixed cultures of thermophilic: Bacillus and Clostridium	Molasses 2–4% with addition of nitrogen and phosphorous sources	+	[121]
		Indigenous brine microflora			
Australia	MFR	Ultra microbacteria generated from indigenous reservoir	Nutrient manipulation		[122,178]
China	CMR, MFR, MSPR	Mixed enriched bacterial cultures of Bacillus, Pseudomonas, Eurobacterium, Fusobacterium, Bacteroides	Molasses 4–6% Molasses 5% C Residue sugar 4% C Crude oil 5%	+	[179]
		Slime-forming bacteria: *Xanthomonas campestris*, *Brevibacterium viscogenes*, *Corynebacterium gumiform*, Microbial products as biopolymers, biosurfactants	Xanthan 3% in waterflooding		
The Netherlands	MSPR	Slime-forming bacteria (*Betacoccus dextranicus*)	Sucrose–molasses 10%	+	[108,123]
Saudi Arabia	CMR, MFR, MSPR	Adequate bacterial inoculum according to requirements of each technology	Adequate nutrients for each technology	−	[124,125]
Argentina	CMR			+	[126–128]
Venezuela	CMR	Isolated bacteria from water associated with biodegraded oil wells	Yeast and glucose	±	
Trinidad-Tobago	CMR	Fac. anaerobic bacteria high producers of gases	Molasses 2–4%	−	[129]
Norway (Norne, offshore)	MWPC	Nitrate-reducing bacteria naturally occurring in North Sea water	Nitrate and 1% carbohydrates addition to injected sea water	−	[130,131]
Malaysia (Bokor, offshore)	CMR			±	[180]

+ = yes; − = not yet reported; ± = some reported and some not reported.
CMR – cyclic microbial recovery (Huff and Puff, Single Well Stimulation).
MFR – microbial flooding recovery.
MSPR – microbial selective plugging recovery.

phosphate and sucrose, an increase in oil production was observed over an 8 months period from a reservoir with permeability of 600–700 md, but in another field with a permeability of only 10–70 md, no effects were observed [120]. In Germany, an experiment was carried out for successful enhancement of oil production from a carbonate reservoir where Clostridia species, such as inoculum and molasses as the main nutrient support, have been used [121]. In Australia, a new concept for enhanced oil production has been developed [122]. This concept consists of using ultra microbacteria generated from indigenous reservoir microbiota through nutrient manipulation. The outer cell layers of such ultra microbacteria have surface-active properties. Such a microbial system was successfully demonstrated in increasing oil production in the Alton oil field in Queensland, Australia. The literature shows that China is leading in the area [3]. From China came very documented results concerning the production and application in China oil fields of biopolymers produced by *L. mesenteroides* and *P. aeruginosa* strains, as well as by *Brevibacterium viscogenes*, *Corynebacterium gumiform*, and *Xanthomonas campestris*—the last three species using hydrocarbons for biopolymer production. In 1958, researchers in the Netherlands conducted a selective plugging experiment using *Betacoccus dextranicus* and reported significant increases in oil production as well as an improved water/oil ratio. Two Dutch trials reported are of interest because of the explicit intention to enhance oil production by selective plugging [123]. Few details are provided but *B. dextranicus*, *Bacillus polymyxa* and *Clostridium gelatinosum* were used in the other, with molasses was the nutrient. An increase in oil production was reported in the first case and an increase in oil–water ratio in the second case. A study [124] by Sayyouh on the applicability of MEOR for recovering more oil under the Arab reservoir conditions where data was obtained from more the 300 formations from seven Arab countries (Saudi Arabia, Egypt, Kuwait, Qatar, UAE, Iraq and Syria). He anticipated that MEOR technology may recover up to 30% of the residual oil under the Arab reservoir conditions [124]. Some initiatives were taken in the Sultanate of Oman at Sultan Qaboos University to experimentally investigate the potential of MEOR in Omani oil fields [125]. Argentina MEOR operations include huff-and-puff projects in Piedras Coloradas field [126]. Receiving the bacteria treatments were two wells in Barrancas formation and four wells in Blanco formation. The Barrancas formation has a 120-md permeability and 170 °F. temperature, while the Blanco formation has a 5–10 md permeability and 180 °F. temperature. The operator injected bacteria solution in the 6 producers, followed by a 72-h shut-in. The treatment increased oil production rates decreased water cut, and decreased crude viscosity. Another Argentina MEOR was a bacteria flood of the Papagayos reservoir in Vizcacheras field [127]. The top of reservoir is at 1850 m. The reservoir has a 1-darcy permeability, 1400-psia pressure, and 198°F. temperature. Before MEOR, the Papagayos reservoir was water flooded. The production had high 96% water cut. Nine producing wells had a positive response from the bacteria solution injected in an injection well. In Venezuala bacteria were Isolated from water associated with biodegraded oil wells located on the eastern coast of the Maraciabo lake. Wells were incubated at 32–50 °C in mineral media supplemented with yeast and glucose extract for growth and multiplication of bacteria and oil recovery [128]. The Trinidad and Tobago Oil Company Limited (TRINTOC) possesses approximately 1300 active oil wells, of which 75% produce less than fifteen barrels per day. The decline in natural production was 15–18% per annum over the last five years. Efforts are underway to examine ways to enhance the oil recovery from existing reservoirs. Since Trinidad and Tobago produces sugar, it was anticipated that MEOR using sugar by-products is a technique by which stripper oil wells may economically produce incremental oil [129].The majority of the field trials were done in sandstone reservoirs and very few in fractured reservoirs and carbonates [130]. The only known offshore field trials were in Norne, Norway [130,131]. From 1970s to late 1990s, MEOR research boosted by the petroleum crisis and later became a scientific substantiated EOR method. The research of MEOR has been done worldwide, and most of oil producing countries has applied this technology into oil fields for pilot tests. Recently this technology has been widely used in oilfields of China, such as Daqing, Shengli, Jilin, Dagang, Liaohe, Henan, Changqing, Xinjiang, and Qinghai.

5.2. Indian scenario

Application of MEOR processes in Indian oil fields have been reported in TERI, India (2001). In India, the Oil and Natural Gas Corporation (ONGC) Limited, in collaboration with The Energy and Resources Institute (TERI, New Delhi) and the Institute of Reservoir Studies (IRS), Ahmedabad, conducted some field trials by employing a Huff and Puff process and using an indigenously developed MEOR technology based on a consortium of anaerobic extremophiles isolated from the candidate reservoirs. A three-fold increase in oil production and a significant reduction in water cut were achieved by applying this technology in 9 wells out of 12 wells treated in 4 oil fields, mostly in the state of Gujarat [132]. Recently, a US patent has been granted on the same process and microbial consortium, which was developed in TERI in collaboration with IRS and was evaluated in field trials by ONGC [19]. As far as India is concerned the application of MEOR technology is very new. Table 3 gives the MEOR field trials carried out in various places of India. The ONGCL and OIL which is facing declining crude oil production, has taken steps to increase oil outputs at its existing wells. The focus is on its wells in Gujarat and Assam. ONGCL introduced the MEOR process conceived with technical experts of IRS, Ahmadabad and The Energy and Resources Institute (TERI) during the last ten years has given encouraging results in the fields of Cambay and Assam basins. This technology has already been patented by ONGCL for its operations in India. The MEOR process is basically CMR – cyclic microbial recovery (Huff and Puff, Single Well Stimulation) technique applied in oil wells producing with high water cut and also in wells producing waxy crude for MSPR – microbial selective plugging recovery. The physic-chemical changes induced to saturate hydrocarbons by injecting bacteria into oil reservoirs along with suitable nutrients medium reduces the pour point and viscosity of oil in reservoir condition.

The indigenously developed IRS consortiums IRSM-1 and IRSM-2 are thermophillic and halophillic (3% salinity) anaerobic bacteria consortium with small cocci and short rods (1.5–2.0 µm), with a pH tolerance of 6–8.5 for low temperature upto 65 °C microbial system application in the oil fields. Produces useful metabolites like fatty acids, surfactants and gases and it is non-pathogenic. The two consortiums are active up to 65 °C for MEOR, which are field trialed for MEOR through huff and puff in Badarpur (3 wells), Kosamba (1 well) and Padra (1 well) of the Mehsana asset belonging to Cambay basin. The MEOR jobs were quite successful in all these wells, especially in Badarpur and Kosamba where the incremental oil production was more than 1200 m^3 and 1100 m^3 in two cycles respectively. Average lives of the jobs were around ten months. The successful response of MEOR in the above fields encouraged IRS to develop a third consortium namely S-2 Multi-bacterial Consortium which is Halophilic, barophilic, thermophilic, anaerobic bacteria consortium (*Clostridium* type *Thermoanaero bacterium* sp. and *Thermococcus sp.*) with small cocci, short rods (0.1–1.3 µm), with a pH tolerance of 4–9 for high temperature up to 90 °C microbial system application in the oil fields. The nutrient media is the 3% glucose and produces useful metabolites like volatile fatty acids and carbon dioxide and it is non-pathogenic. The consortium is active up to 90 °C for MEOR, which are field trialed for MEOR through huff and puff in 109 wells of 9 different fields of ONGC (Kalol, Viraj, Jhalora, Sanand, Wadu, Sobhasan, Jotana, North Kadi and Lakwa) and 8 wells of Naharkatia field of OIL, Duliajan. Total oil recovery was around 61,000 m^3 and the gain was round 550 m^3 per well per job with a success ratio of 70%. Average life cycle takes 6–8 months. MEOR by stimulation of

Table 3
India's experience in MEOR field trials.

Indian company	Acronyms of MEOR technology	Microbial systems	Nutrients	Incremental of oil production	References
(A) ONGCL					
Mehsana Asset *Sobhasan* *Jotana* *N. Kadi* **Ahmedabad Asset** *Kalol* *Viraj* *Jhalora* *Sanand* *Wadu* **Assam Asset** *Lakwa Rudrasagar* *Geleki*	CMR	Multi-bacterial Consortium: Clostridium type Thermo anaero bacterium sp. and Thermococcus sp.	Molasses 3%	+	Personal communication and visit to ONGCL libraries, Woodward, 2006, TERI, India (2001) [181]
(B) OIL, Duliajan					
Assam Asset *Nahorkatiya Moran* *Chabua*	CMR, MSPR			+	Personal communication and visit to OIL library, Woodward, 2006, TERI, India (2001) [181]

+ =yes; − =not yet reported; ± =some reported and some not reported.
CMR – cyclic microbial recovery (Huff and Puff, Single Well Stimulation).
MFR – microbial flooding recovery.
MSPR – microbial selective plugging recovery.

native microbes through injection of optimized nutrients eliminates use of extraneous microbes is also being applied. The nutrient media is carbon source mainly glucose, inorganic salts are mainly nitrogen, phosphorus, trace elements and vitamins. Field trials were done successfully in 2 wells of Kalol field with an increase of over 2–4 in oil production rate. More than 3000 m^3 incremental oil was produced. Before the job the oil production was 4 m^3/d with a water cut of 82% and after the job the oil production was 20 m^3/d with a water cut of 70%. IRS further developed two more consortiums NJS7-91 and NJS4-96 prepared from the formation waters of Nandej and Sobhasan wells of Ahmedabad and Mehsana oil fields. These are Anaerobic, Hyperthermophilic and grow at 91 and 96 °C and halophilic which grow in 7% and 4% salinity. The primary nutrient media which is a carbon source is cane sugar and the nitrogen source is Urea/Ammonium sulfate while the secondary nutrient source is trace elements and vitamins. The optimum incubation period is 14 days. This application is applied in two wells of South Kadi oil field. Initial oil production was observed in post MEOR job. Presence of motile microbes observed even after four months, short lived effect was observed. These consortiums needs more field trials to establish the process. Biosurfactant producing microbial system is consortia HS4-2 with *B. licheniformis,* the source of which is the hot water spring, with a salinity tolerance of 5% and product stability up to 95 °C. There was a reduction in interfacial tension between the aqueous and oleic phase from 35 dynes/cm to 0.064 dynes/cm. The additional oil recovery was 19% of the original oil in place (OOIP). Field trials were conducted in one well of Kalol and one well of Limbodra. Water cut decreased from 92% to 85% in case of Kalol well and in case of Limbodra well, water cut showed decreasing trend but with fluctuations. In both the cases marginal increase in oil production was observed for 4 months. The Microbial System for Wax Deposition control in well tubulars is the PDS-10. PDS-10 is the *Geobacillus kaustophillus* which is gram positive and rod shaped derived from the oil contaminated soil of Sobhasan field. It is thermophilic, microaerophilic consortium, thrives up to 90 °C with optimum activity at 55 °C. The nutrient source for the bacteria is glucose and wax. Paraffin degradation efficiency is less than 82% at 55 °C and best grows in the pH range of 6–8. It is non-pathogenic and the optimum incubation period is 7 days. FIB-19 Consortium improves flow efficiency in surface flow lines. The source of this consortium is oil contaminated soil of Limbodra field. The bacterium is mesophilic and microaerophilic. The nutrient medium is minimal salt medium and the carbon source is glucose and glycerol. Minerals and vitamins are also added. At 37 °C, 67% degradation of paraffin takes place. The incubation period is 5 days and it is non-pathogenic.

6. Modeling and simulation in MEOR

Comprehensive research and development studies and annual reports on the development of microbial strains with improved transport and biosurfactant activity for enhanced oil recovery (EOR) and on the biosurfactant-mediated oil recovery in model porous systems with its computer-aided simulations have tremendously contributed to the design and development of effective microbial enhanced oil recovery (MEOR) strategies and at the same time have enormously enriched the scientific literature on MEOR [1]. Developing mathematical models for MEOR is very challenging since physical, chemical and biological factors need to be considered. Structured mathematical models are required to describe the MEOR processes in a better way. In order to develop a proper field strategy, formulation of an efficient reservoir simulator capable of predicting bacterial growth and transport through porous network and *in situ* production and action of the metabolites called MEOR agents is of paramount importance [1]. Published MEOR models, as given in Table 4, are composed of transport properties, conservation laws, local equilibrium, breakdown of filtration theory and physical straining [1,133–137]. Such models are so far simplistic and they were developed based on:

(A) Fundamental conservation laws, cellular growth, retention kinetics of biomass, and biomass in oil and aqueous phases. The main aim was to predict porosity retention as a function of distance and time.

Table 4
Showing the different models.

S. no.	Model	Description	References
1.	General models for MEOR	Based on the general description of isothermal, multiphase, multicomponent fluid flow in porous media from the basic conservation laws	[152,153]
2.	Numerical	To simulate a microbial plugging process	[141,154]
3.	Mathematical model	To simulate bacterial growth that leads to plugging, reduction of oil viscosity and interfacial tension, and production of gas was developed	
4.	Mathematical model	To describe adsorption, growth and decay of microorganisms, consumption of nutrients, and other physical processes.	[143]
5.	Laboratory model	To study the penetration rates in the laboratory	[114]
6.	The model used a modified Monod equation	To describe bacterial growth, when two nutrients (substrates) were present. Permeability modification is assumed to be due to both pore-surface retention and pore-throat plugging by bacterial cells	[138]
7.	One-dimensional isothermal model	To study the displacement of oil by water containing bacteria and substrate for their feeding. The bacterial products are both bacteria and metabolites	[157]
8.	One dimensional, two phase compositional numerical model	To depict the transport of bacteria in a MEOR process where oil recovery was by bio-surfactant based interfacial tension reduction and selective plugging of higher permeability regions by biomass generated by microbial growth	[158]
9.	Models considering mechanisms	To describe the mechanisms of transport and biological processes	[148,160]
10.	Model considering mechanisms	To demonstrate various mechanisms such as surfactant production and adsorption, salinity effects, adsorption of microorganisms, reduction of interfacial tension, and wettability changes.	[147]
11.	Finite element model	A modified model for residual oil saturation under several assumptions.	[28]
12.	The latest reactive transport model	To incorporate convection, bacterial growth, substrate consumption and surfactant production	[157]

(B) Filtration model to express bacterial transport as a function of pore size; and relate permeability with the rate of microbial penetration by applying Darcy's law.

Researchers use either two or three phases presenting either an oil–water or oil–water–gas system. Modeling of MEOR includes several approaches which may be both one-dimensional models [138–140] and models extendable to two and three dimensions [133,141–147]. Only models show how the gas phase influences the flooding system [141]. MEOR is one of the built-in features in the simulator. MEOR can be coupled with other chemical features such as the effects from gas, surfactant and polymer. Simulation results for MEOR cases agree well with core flooding experiments [145]. In the MEOR literature, the oil phase generally consists of oil only. The water phase includes the remaining components being bacteria, substrates and metabolites. The two flowing phases and their components are considered immiscible. Bacteria attach to the pore walls, where they form biofilm. The mathematical description of the bacterial attachment and detachment processes in connection with biofilm formation has overall two approaches. One approach utilizes equilibrium partitioning of bacteria assuming that equilibrium is fast compared to convection. This gives a relation between flowing and adsorbed bacteria. The adsorption is often described by the Langmuir isotherm [139,140,145,147]. The other approach applies rate expressions for the attachment and detachment processes. This implies an extra mass balance for the attached bacteria, where rate processes describe that bacteria grow, enter and leave the biofilm [138,141,143]. The attachment and detachment rate expressions exist in many versions, but they are generally modified derivations from the colloid filtration theory [148]. The porosity is reduced due to formation of biofilm influencing the absolute permeability. Generally, the permeability is modified according to the Carman–Kozeny equation or modifications thereof. The Carman–Kozeny equation is

$$\frac{K}{K_o}=\frac{\phi}{\phi_0} \tag{1}$$

where K is absolute permeability, ϕ is porosity. The index 0 indicates initial value [138,145].

Some models introduce a limit for how much the water phase pore space can be occupied by biofilm. In the UTCHEM simulator, the biofilm comprises 90% of water phase volume at the maximum. Nutrients and metabolites adsorb to the pore walls. Their adsorption is also modeled using the Langmuir isotherm [141,147]. In MEOR models, it is usually assumed that nutrients do not adsorb [139,141,143]. However, surfactants were allowed to adsorb in their model developed by others [147]. Several methods are used to model relative permeability changes as a function of interfacial tension, where a correlation between surfactant concentration and interfacial tension was used. Generally, a reduction of interfacial tension decreases residual oil saturation affecting relative permeability curve endpoints, but it also straightens the relative permeability curves approaching full miscibility [149,150]. Some methods use interpolation of different parameters and others apply capillary number and residual oil dependencies or use a capillary number dependent interpolation function [150]. This work investigates three methods: (1) capillary number and normalized residual oil saturation correlation; (2) Coats' interpolation between relative permeability curves; and (3) interpolation of parameters of Corey type relative permeability curves [149,151].

6.1. Modeling

The models for MEOR are based on the general description of isothermal, multiphase, multicomponent fluid flow in porous media from the basic conservation laws [152,153]. A numerical model was developed to simulate a microbial plugging process. The model investigated the growth and retention of microbes as a stationary phase leading to reduction of permeability of the porous media. The model assumed that development of the stationary phase was due to biomass retention and convective transport was the dominant method of microbial transport [154]. Mathematical model describes the process of MEOR, where bacterial growth leads to plugging, reduction of oil viscosity and interfacial tension, and production of gas was developed [141]. Interfacial tension was directly correlated with bacterial concentration avoiding actual surfactant production in the model. The mathematical formulation describes microbial movement in a multidimensional system where microbe and nutrient transport equations were coupled to phase flow equations. A drawback of this formulation was that it neglected physical dispersion as a transport mechanism. A mathematical model was developed describing adsorption, growth and decay of microorganisms, consumption of nutrients, and other physical processes [143]. Porosity and permeability were changed due to deposition of microorganisms. It was also demonstrated that the oil recovery increased by microbial plugging.

Quantitative studies in the laboratory of penetration rates have been made [114], which found negative semi logarithmic relationships between spore numbers and distance moved, using a series of sample points along a length of sandpacks [155], using Pseudomonas putida, Clostridium sp. and *B. subtilis*, found that, in general, cells were detectable in the fluids emerging from their rock cores after one pore volume of fluid cell concentrations remained more or less constant at a fraction of the input concentration. Eventually cell concentrations rose to equal the input level. They interpreted their data in terms of deep bed filtration model [156], calculation the filtration coefficient (K_0) for cells and spores as

$$K_0 = \frac{\ln\left[\frac{C_i}{C_L}\right]}{L} \qquad (2)$$

where C_i=initial cell concentration; C_L=emerging cell concentration and L=length of core

They studied the effects of ion and chelating agents and concluded that at low cell concentrations, filtration was mainly due to cell adsorption on to rock surfaces. Spores have low filtration coefficient than cells and the presence of residual oil lowered the filtration coefficient, suggesting that cells might penetrate water flooded reservoirs more readily than most experiments with rock cores might suggest. Later, a one dimensional multi-component model was developed to simulate biomass growth, metabolic product formation and nutrient consumption in a MEOR process [138]. The model used a modified Monod equation to describe bacterial growth when two nutrients (substrates) were present. Permeability modification is assumed to be due to both pore-surface retention and pore-throat plugging by bacterial cells [138]. The one-dimensional isothermal model comprises displacement of oil by water containing bacteria and substrate for their feeding. The bacterial products are both bacteria and metabolites [157]. A one dimensional, two phase compositional numerical model was presented for bacteria transport in a MEOR process where oil recovery was by bio-surfactant based interfacial tension reduction and selective plugging of higher permeability regions by biomass generated by microbial growth [158]. The continuing development of a three-dimensional, three-phase, multiple-component numerical model was included to describe microbial transport phenomena in porous media [159]. Laboratory data were used to develop correlations and mathematical models for specific phenomena; linear core flooding data were used to test the simulator in an iterative process. The simulator development and laboratory testing aspects of this project were coordinated so that the results could be used to design other laboratory experiments to clarify and quantify certain physical and chemical effects. An accurate reservoir simulator for MEOR methods can best be developed through an integrated program of acquisition of laboratory and field data with the feedback loop being the reservoir simulation model.

The governing equations for microbial and nutrient transport into a three dimensional, three phase black oil model. It simulated microbial activities from the net flux of microbes by convection and dispersion, microbial growth and decay, chemotaxis, nutrient consumption and deposition of microbes on rock grain surfaces. An IMPES simulator solved for pressure and phase saturations and a direct sparse matrix solver was used to obtain solutions for component transport equations.

In the MEOR literature, there are different approaches for implementation of the mechanisms. There exists literature from other research areas, where these mechanisms are also important. As an example, bioremediation deals with bacteria in the underground working with models that describe the transport and biological processes [148,160]. A MEOR model was developed, where several mechanisms are taken into account; surfactant production and adsorption, salinity effects, adsorption of microorganisms, reduction of interfacial tension, and wettability changes [147]. Polymer is additionally injected in order to reduce permeability and increase viscosity. A fully coupled biological and hydrological finite element model has also been developed that introduces a modification to the residual of saturation under several assumptions [28].

The latest reactive transport model incorporates convection, bacterial growth, substrate consumption and surfactant production [157]. It is a two-phase flow comprising five components; oil, water, bacteria, substrate, and surfactant. The water phase may consist of water, bacteria, substrate and surfactant. In the context of MEOR, a novel approach is the partition of surfactant between both phases. The oil phase consists primarily of oil, but contains also surfactant. The fractional flow function and the relative permeability are calculated by Corey type expressions. The reactions are substrate consumption, bacteria multiplication and surfactant production. The bacterial growth rate is the Monod expression for one limiting substrate, so the reaction rate depends on the bacteria and substrate concentrations. Surfactant reduces IFT, modifying the relative permeability. The relative permeability depend on the water phase concentration, so when surfactant is moved into the oil phase, there will be a smaller effect from the surfactant on the flow. Therefore, transfer part of the surfactant to oil phase is equivalent to its “disappearance”, so that the total effect from surfactant is reduced. The bacteria partition in between the phases is according to the Langmuir expression dependent on the bacteria concentration in the water phase. The adsorbed bacteria constitute the biofilm phase. The surface available for adsorption is scaled by the water saturation, as bacteria only adsorb from the water phase. No transport limitations were assumed in the biofilm, causing the bacteria in the water and biofilm phases to have the same growth rate. The formation of biofilm leads to porosity reduction, which is coupled to the modification of permeability. The modification of absolute permeability that could take place is not investigated as the model is one-dimensional. An effect contributing to the fluid diversion mechanisms is microscopic fluid diversion, where the relative permeability for water only, is modified. This happens due to the fact that the biofilm is formed only at the water-occupied zones or pores where bacteria live. Bacteria only influence the water and biofilm phases, while the oil phase remains the same.

6.2. *Simulation*

A one-dimensional, two-phase, compositional numerical simulator was developed to model the transport and growth of bacteria and oil recovery in MEOR process [158]. The basic equation governing the transport of oil, water, bacteria, nutrient and metabolites in porous media were component mass conservation equations. Permeability reduction was modeled using the effective medium theory. The oil recovery model is based on mechanisms such as interfacial tension reduction by bio surfactant and selective plugging by biomass. In this model, an implicit-pressure, explicit-concentration (IMPEC) algorithm was used to solve pressure and mass conservation equations.

A mathematical formulation was used to describe microbial transport in multidimensional porous media [141]. In this formulation, multiphase flow equations were coupled with microbe and nutrient transport equations. Physical dispersion terms were neglected in the component transport equations. Since metabolic products were not included in this model, correlations which relate biomass to metabolites and their activities were defined. Numerical simulation runs were conducted to investigate bacterial plugging, interfacial tension reduction and carbon dioxide effects *etc.* The results showed that surfactant-producing bacteria appeared to be promising.

The equations was incorporated for microbial and nutrient transport into a three-dimensional, three-phase black oil model [143]. Microbial activities simulated included net flux of microbes by convection and dispersion, microbial growth, decay and chemotaxis, nutrients consumption and decomposition of microbes on rock grain surfaces. The alteration of rock wettability during microbial treatment was considered as the mechanism for oil recovery. Based on experimental results, empirical correlation between cell concentrations and rock wettability and between the rock wettability and residual phase saturations were established. In this simulator the IMPES procedure, was employed to solve pressure and saturations while a direct sparse matrix method was used to obtain solutions for component transport equations.

The mathematical model of microbial influence on the reservoir was made on the basis that the injection of molasses water solution with Clostridium bacteria into the mixed type of rock was used. And the results of calculations were compared with experimental data [161]. The conventional simulators are often based on finite difference methods, where many variations exist. An example is UTCHEM developed by University of Texas, Austin. The latter also has a wide utilization range for both water-flooding and chemical EOR methods. UTCHEM has the possibility for applying both MEOR and bioremediation [145] but more extensive studies could be presented using UTCHEM. Multiphase pressures and saturations for the MEOR model were calculated with a black oil model that employed the IMPES formulation and LSOR solver [162,163]. Three phases; oil (o), water (w) and gas (g) were considered. Mass transfer between the water and oil phases and water or oil vaporization into the gas phase was neglected. The basic mass balance and continuity equations are also known [164].

A three-dimensional multi-component transport model in a two-phase oil–water system was developed. The model includes separated terms to account for the dispersion, convection, injection, growth and death of microbes, and accumulation. For the first time, effects of both wettability alteration of reservoir rock from oil wet to water wet and reduction in interfacial tension (IFT) simultaneously on relative permeability and capillary pressure curves were included in a MEOR simulation model. Transport equations were considered for the bacteria, nutrients, and metabolite (bio-surfactant) in the matrix, reduced interfacial tension on phase trapping, surfactant and polymer adsorption, and effect of polymer viscosity on mobility of the aqueous phase. The model was used to simulate effects of physico-chemical parameters, namely flooding time schedules, washing water flow rate, substrate concentration, permeability, polymer and salinity concentration on Original Oil In Place (OOIP) in a hypothetical reservoir [147].

A simulator was developed in order to analyze the mechanisms of MEOR using polymer-producing microorganism [146]. The numerical model in this simulator consists of 2-phases (oil and water) and 5-components (oil, water, microorganism, nutrient and polymer). This model includes almost all processes of MEOR such as growth and death of microorganism, nutrient consumption, polymer production, water viscosity increment, improvement of the flow profile in a reservoir and enhancement of oil recovery. The validity of this simulator was shown by a comparison of both results of the numerical simulation and a flooding experiment.

The certain correlation is stated to be used in many commercial simulators for modeling the effect of miscibility on relative permeability, even though it is not based on any theory, but developed to describe the changes in the relative permeability curves by IFT reductions [149,150]. A change was suggested in model of two-phase gas and oil relative permeability curves due to reductions of IFT [149].

Today's streamline (SL) methods have been developed from the stream tube approach. The flow domain is divided into stream tubes and the geometry of the tubes is taken into account[165]. Their geometry delivers a side of disadvantages, when simulating in multiple dimensions. The SL application has gone through several steps of development since the stream tubes were used. The details of development in the streamline approach can be found [166]; an excellent review has been proposed on the application of SL simulators [165,167]. An advantage of the SL simulation over the finite difference (FD) approach is that the computation time is often smaller and has a smaller impact of numerical dispersion [168]. On the other hand, the finite difference simulators better handle physical phenomena that transport fluid across the streamlines [166]. The SL simulator has been extended to include the MEOR two-phase model, enabling the study of MEOR in two and three dimensions. The SL simulator is found to produce similar results with the corresponding FD simulator. The general characteristics found for MEOR in one-dimensional simulations are also demonstrated both in two and three dimensions: It is accumulation of water together with mobilization of residual oil producing a traveling oil bank, and the creation of two displacement fronts.

The reservoir is divided into grid blocks in a conventional manner, where each grid block has a porosity, permeability and initial composition assigned. Several solution methods have been used for solving the system of equations. A fully implicit method can be applied, but it produces a substantial amount of numerical dispersion [169]. Therefore, the solution procedure for both simulators is based on the standard IMPEC framework (implicit pressure explicit composition) [170].

7. Suggestions and future perspectives

MEOR is a process in which microorganisms are used to recover the oil remaining in the reservoir. MEOR appears to be least expensive. Till date a report of many experiments and field trials were found successful all over the world and is reported in this work. The efficiency of MEOR process varies between fields and between reservoirs, depending on the viscosity of crude oil, the characteristics of the reservoir rock, the specific microbial communities present in the reservoir fluid and the technology and economics of production of microbial system for EOR. It was also observed that some MEOR methods have progressed from laboratory-scale trials to field studies in actual oil fields because they complied with certain strategies the chief amongst them are the selective plugging, production of biosurfactants, biopolymers, bioacids and biosolvents. In case of the advanced MEOR methods like the GEMEOR, and EEOR much work still remains to translate MEOR into accepted oil industry practice.

At present MEOR might be able to contribute to the increased oil production if they can be shown to be economically feasible, because an industry will use a technology only if it sees advantages in doing so. Such methods need not replace physical–chemical methods of EOR but rather could complement them by being used in situations where physical–chemical methods are not applicable.

Future research is aimed to focus not only on the production of cost-efficient microbial products but also on optimizing yields and strategies by using mathematical models and analyses. Since each oil well differ from location to location, therefore, MEOR strategies face the inherent challenge of having to study each well individually before choosing an appropriate method; no one-size-fits-all, "blanket", solution exists for MEOR operations. Despite this, many MEOR methods hold tremendous promise for EOR and most of the bio products (biosurfactants and biopolymer in particular) which are produced during MEOR process can have uses that transcend oil recovery operations valuable. MEOR can allow us to obtain more oil in eco-friendly ways and tilt the global energy balance towards lower prices and more domestic production.

8. Conclusion

Many factors are at play regarding microbial enhanced oil recovery. Amongst them are considerations concerning the unique nature of each oil well, the wettability of reservoir rock, the varying viscosities of crude oil, and the specific microbial communities present in each recovery operation. While many different possible MEOR strategies exist, not all of them are viable for industry practice due to concerns with cost, time, and production. Such concerns can help answer why few methods have progressed from laboratory-scale trials to field studies in actual oil fields. While some select strategies have shown the ability to be used on a mass scale through both lab and field trials—chief amongst them strategies employing plugging, biosurfactants, GEMEOR, and EEOR—much work still remains to translate MEOR into accepted oil industry practice. Future research should focus not only on more cost-efficient production of viable microbial products but also on optimizing yields and strategies by using mathematical models and analyses not covered in this review. Because each particular oil well is different, MEOR strategies face the inherent challenge of having to study each well individually before choosing an appropriate method; no one-size-fits-all "blanket" solution exists for MEOR operations. Despite this, many MEOR methods do in fact hold tremendous promise for oil recovery and many of the most heralded bio products (biosurfactants in particular) can have uses that transcend oil recovery operations, which can make their production valuable to a myriad of industries. While the application of biotechnology to enhanced oil recovery cannot solve our global need for renewable energy sources, microbial enhanced oil recovery can allow us to obtain more oil in eco-friendly ways and tilt the global energy balance towards lower prices and more domestic production.

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