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APPLICATION OF PROTEINS IN ENHANCED OIL RECOVERY-A REVIEW

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Abstract

Enhanced oil recovery acquires sustained attention due to the continued consumption of fossil fuels. The traditional EOR techniques involve chemical, thermal and miscible flooding. On the other hand, biotechnology, including proteins, enzymes, and other microbial products applied only on a lab scale, not on the industrial scale, so great interest should be paid for these agents in the upcoming days. Proteins and enzymes may act to alter wettability and influence the capillary forces in the oil/brine system so can improve the oil recovery factor. This article summarizes the applicability of proteins and their enzyme-constituting unit as a flooding agent in the field of enhanced oil recovery (EOR).

Keywords: *Proteins; Enhanced oil recovery, Enzymes; Sandpack flooding.*

1. Introduction

Enhanced oil recovery (EOR) defined as any procedure comprises the inoculation of external agents into the reservoir to recover some of the remaining oil after conventional treatment processes [1]. Improved oil recovery through different technologies involving: thermal methods (in-situ combustion, steam injection), chemical methods (polymers, surfactants, solvents, alkali), microbial, and miscible gaseous injection increased progressively [2-5] owing to depletion of oil reservoirs, and increased global energy demand, in addition to poor recovered oil amount by primary and secondary methods [6-12]. On the microscopic scale, the capillary force subjects a certain oil amount estimated to be 1/3 of the reserve to be left behind after water flooding [13]. Oil production occurs through three distinct phases [1, 14-16] as follow; **1) primary recovery** which recovers 12-15% OOIP through utilizing reservoir energy to relocate the oil [7]; **2) secondary recovery** which recover 10-15% OOIP via injection of water and gas to enhance crude oil mobility and fluidability [7]; **3) tertiary recovery** which involves the injection of the external agent into the reservoir to retrieve some of the trapped oil. One of the recent EOR techniques is the microbial-enhanced oil recovery (MEOR) that firstly presented in 1926 by Beckman, and further preliminary studies on MEOR generated until 1940. After that, Zobell introduced and elucidated MEOR technologies and patented a MEOR technique [17]. In 1954, the first MEOR field trial generated through the Lisbon field, Union County, Arkansas, USA, followed by several field-trials as reported by Geetha *et al.* [5]. MEOR is one of the promising tertiary recovery techniques in the future owing to its simple implementation, environmentally friendly, large potential and better applicability [5, 18, 19]. There are numerous microorganisms in the reservoir bio-flora, which regarded as probable creators of lipase-esterase and urease enzymes. Investigation of the microbial municipal in petroleum reservoirs is vital for MEOR as the competence of these approaches rest on how original microbes respond to the oil well ecological aspects like temperatures, pressure, and pH [20]. MEOR technology utilizes the microbial activities of microorganisms (either indigenous or injected in the reservoir) and their metabolites to emulsify the oil, reduce oil/brine interfacial tension, in addition to oil solubility and hydrocarbons disintegration to form minor oils droplets [21] and hence mobilize entrapped oil [18, 22]. The microbial metabolites may be biosurfactants, biopolymers, enzymes/or protein, solvents, acids, biogases (carbon dioxide, methane, and hydrogen) [23-26]. Microbial biopolymers applied in EOR for controlling mobility ratio and improving the sweeping efficacy of water flood by discriminating plowing of high permeable districts [27-29]. Proteins and/or enzymes-EOR (EEOR) characterized by ecological benefits as a green chemistry agent in the industrial processes. Proteins are complex macromolecules possess high molecular weight and high interfacial activity as they hang at the interfaces to generate high viscoelastic coatings with high surface activity [30-31], consequently employed as emulsifying agents in food, pharmaceutical or chemical industries, and enhanced oil recovery (EOR) in oil reservoirs [32]. Moreover, the protein retains about ~80-95% of their functionality even under harsh reservoir conditions [33]. Protein functionality defined as the rate of catalyzing of a chemical reaction, uncoupling of biochemical processes, lowering of interfacial tension, or lowering of critical micelle concentration [33]. Feng *et al.* [34]] reported that a recovery factor of 5-10% could be achieved at reservoir temperature 50 to 80 °C on lab scale using modified enzyme solution at 10% salinity. In the protein architectural arrangement, hydrophilic moieties face the aqueous phase while the hydrophobic moieties face the oil phase. Protein amphipathic molecules made up of hydrophobic and hydrophilic moieties that form a micelle [31]. Protein foam employed as a substitute foaming candidate for EOR processing owing to their higher steadiness [31]. Enzymes are naturally occurring biological moieties created by biological cells to serve as a catalytic agent for many biochemical responses and consist of amino acid units that wrinkle and form three-dimensional architecture [35] as shown in Figure 1.

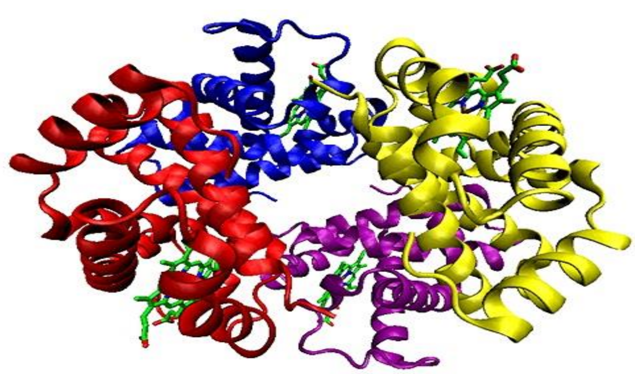

Figure 1. 3D structure of the enzyme

Enzymes serve to lower the reaction activation energy and accelerate the reaction rate. Enzymatic hydrolysis is a bioconversion process in which the enzyme can convert cellulose to glucose. Moreover, the enzyme acts on lipoprotein and hydrolyze it to simple lipid and protein moieties, thus render oil accessible for extraction [36]. Enzymes applied on an industrial scale, including detergents, textile, and food industry, as well as the oil industry [3]. Enzymes exhibit exceptional superficial action, owing to the presence of hydrophobic and hydrophilic moieties [37].

Enzymes are familiar candidates in petroleum engineering applications, including pre-treatment of biopolymers, gel infringement, sulfur removal, and acid production [38]. Summary of enzymatic field applications and types of enzymes commonly used in enhanced oil recovery are reported elsewhere [37]. Recently, enhanced oil recovery by biological agents such as enzymes and biopolymers acquire incremental focus since they are environmentally friendly and surface-active agents. They serve to 1) alter rock wettability towards more water-wet state by formation of protein film on the rock surface [39], this protein film consists of hydrophilic amino and carboxylic groups ($-NH_2$ & $-COOH$) groups which bind through hydrogen bonding to water molecules and render a water wet surface; 2) reduce either of oil viscosity or interfacial tension through emulsification [40], since they consist of hydrophilic and lipophilic moieties in the same structure, so exhibit amphipathic structure which can be micellized at the interfaces and reduce surface tension; 3) and remove high molecular weight paraffin's through breaking of ester and double bond [41], as it contains terminal amino group ($-NH_2$) which can initiate the double bond through aza Michael addition reaction and form highly reactive propagating radical accessible for further addition reaction. Generally, the enzyme consists of two amino acids linked by peptide bond and linked to the hydrophobic side chain as illustrated in Figure 2.

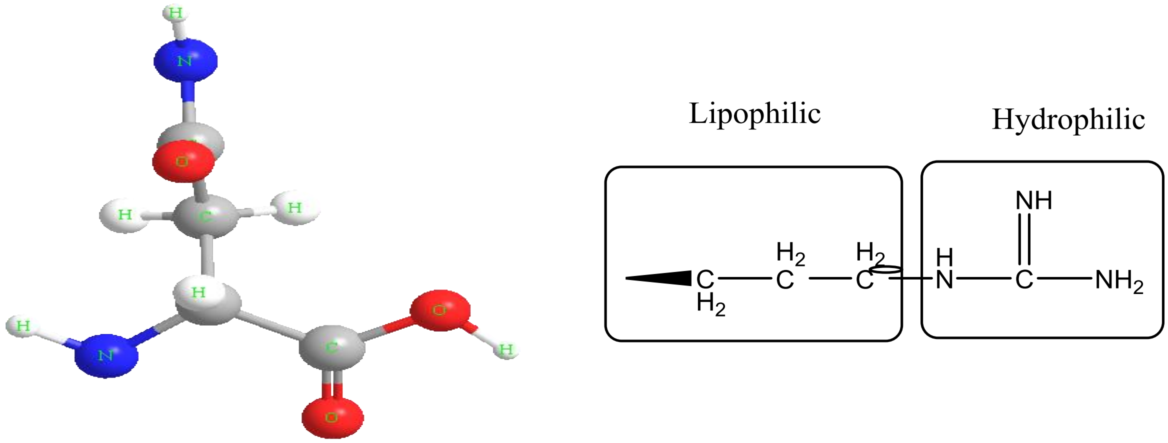

Figure 2. Chemical and amphiphilic structure of the enzyme

Enzymes like esterases, lipases, carbohydrases, proteases, and oxidoreductases may be applied as wettability modifying agents in carboniferous petroleum reservoirs [37]. Figure 3 summarizes the different roles of enzymes in EOR. Generally, the advantages of microbially enhanced oil recovery (MEOR) conclude two main aspects

1. Low cost, as microbial outcrops generated from cheap, regenerated resources [42]. Moreover, it does not consume large energy amounts like thermal processes, nor depend on the oil price as chemical processes [26, 29].
2. The injected agents have lower toxicity, highly biodegradable and compatible with a wide range of temperature and pH [28].

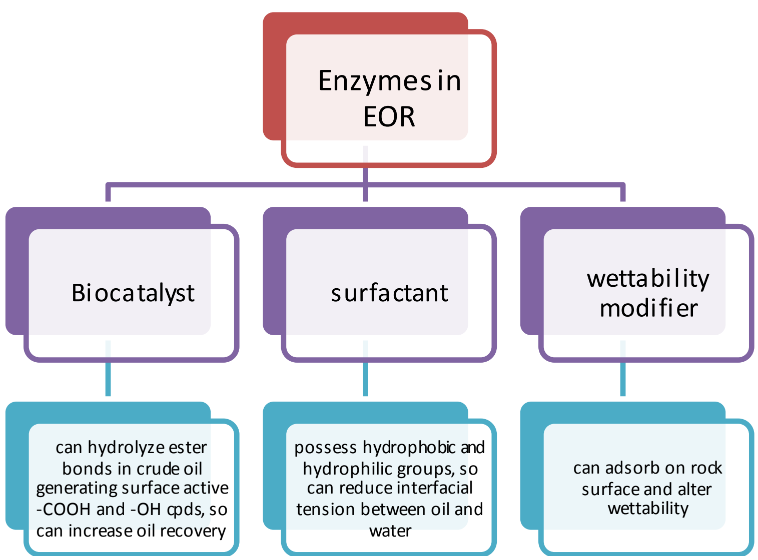

Figure 3. Schematic illustration of enzymes role in EOR

2. Structure and function of enzymes

Enzymes are spherical proteins consist of amino acids that contain $-NH_2$ and -COOH groups [3]. Reactions carried out on the enzyme active site. Enzyme activity affected by temperature, chemical environment, the substrate, and activators concentration. Enzymes catalyze chemical reactions through binding of the substrate to the enzyme active sites. The substrate/enzyme linkage results in the disturbance of electronic charge around the substrate and formation of the products that separated from the enzyme surface to stimulate the enzyme for another trip. The active site has a sole geometric figure that is corresponding to the geometric figure of a substrate moiety. Consequently, enzymes react with a rare number of similar compounds. There are two concepts that pronounce the binding of enzymes and substrates. The first concept is the lock and key theory, and the second concept is the induced fitting model, which is a modification of the lock and key model that first hypothesized in 1894 by Emil Fischer [43]. In this model, the enzyme act as a lock and the substrate act as a key as shown in Figure 4. Only the correctly sized key (substrate) fits into the keyhole (active site) of the lock (enzyme).

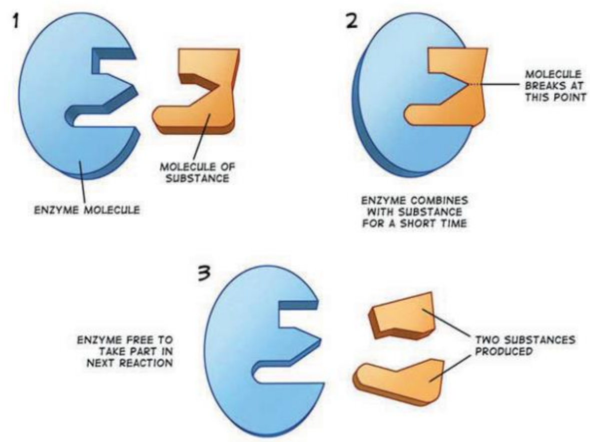

Figure 4. General mechanism of enzymes (Lock and key theory) [3]

3. Application of enzymes in the petroleum industry through MEOR

Enzymes may be considered as a promised EOR candidates since they are produced microbial, ecologically friendly, surface-active materials, even at tremendously low doses [41]. They serve to improve the oil recovery factor through the following aspects; 1) Enzymes can initiate alteration of wettability and capillary forces at the liquid/solid interface [41] through catalytic cleavage of ester bonds in the crude oil and produce acidic moieties which in turn contribute to increase the repulsive electrostatic forces and alter the communication at the interfaces [3]; 2) Enzymes able to change the fluids interactions by promoting the breakdown of the oil components [44]. For example, hydrolase enzymes catalyze bond breaking between molecules into (I) smaller molecules with enhanced water solubility and reduced interfacial action (ii) more polar molecules, including acid and alcohol. These compositional alterations may affect wettability and interfacial tension; 3) Enzymes can adsorb onto interfaces. The portentous nature of the enzymes permits the changes in the interaction energy between crude oil, brine, and rock; 4) Decreasing the interfacial tension (IFT) and the oil viscosity [41]. The application of enzymes in the petroleum industry had been reported recently. In March 2004, enzymes were implemented on Shengli oil field, China. Enhanced production was continued for six months with 10,961 bbl. of additional oil. Similar behavior was detected in Baise oil field, China [34]. Most of the published scientific reports have used enzymes in the form of commercial mixtures. In such mixtures, enzymes are usually present in combination with stabilizers and surfactants as stated in Apollo GreenZyme™ Material Safety Data Sheet [45]. Although lipases have been reported as effective EOR enzymes, some authors stated about protein and enzyme application in the field of the oil industry as follow;

Kohler *et al.* [46] relate enzymes to enhance xanthan gums Injectivity. They reported that enzymes could destroy the bacterial cells and improve the flow behavior of xanthan gum solutions. Harris and McKay,1998 [38] reported some implementation of enzymes in the oil industry. The applications comprised biopolymers treatment; gel breakage through drilling in order to disturb filter cake construction, water shut-off and sand consolidation [3]. Beverung et al., 1999 [47] accomplished IFT detection between heptane and spherical proteins solutions and stated a reduction in IFT by addition of enzyme to the solution. They clarified it by transition arrangement in the spherical protein particle that allows noteworthy proteins unfolding at the oil/water boundary. Nemati and Voordouw, [48] have utilized an enzyme to adapt porous media permeation. They exhibited that enzymes promote the creation of $CaCO_3$ is an effective substitute for plummeting porous media permeation. The consequences of their work designate that growth in enzyme doses enhances the amount of $CaCO_3$ precipitation and directed to an important reduction in permeability. Feng *et al.*, [45] described the use of interfacial active enzymes from hydrolases class to improve oil production on laboratory and pilot scale as reported elsewhere [3]. They accomplished experiments to observe the modified enzyme compatibility with the oil type, salinity, and temperature. After that, core displacement runs were generated. They established that at optimum circumstances, recovery increased by 16.9% on average with decreased water production and increased oil recovery. Samuel *et al.* [49] reported the usage of the enzyme to eliminate formation impairment that convinced by the drilling fluids. Armstrong *et al.*, [50] use enzymes as breakers for highly viscous fluids such as guar gum and solid proppants in hydraulic fracturing, where the enzyme reduces the fluid's viscosity by disintegrating the polymer chain and permits the proppant fluid to settle down into the crack and facilitate fluid flow ability back to the wells. Nasiri *et al.* [51]] reported that lipase and esterase are the most effective enzymes for rock wettability alteration on carbonate rock. Rajaram *et al.* [36] reported that enzymes enhance oil lipopolysaccharides, lipoproteins hydrolysis, rendering ease extraction and consequently improve oil recovery. Khusainova *et al.* [41] reported enhanced oil recovery by Genzyme injection using Berea sandstone cores. Five pore volumes (PV) of enzyme solutions were injected along with the core samples after water flooding. Moreover, they studied the mechanism of various enzymes, including esterase/lipases, carbohydrases, proteases and oxidoreductases, along with two commercial blends on wettability alteration through contact angle measurements and adhesion behavior tests. They concluded that the applied enzymes have the capability to separate oil from the surface, at extreme lower enzyme concentration. Esterase/lipases greatly alter the wettability and reduce oil adhesion at concentrations of 0.1%. Yela *et al.*, [7] recorded a recovery factor of 12%OOIP by using bio-surfactants generated from transmembrane proteins. Samin *et al.*, [31] stated that protein foaming could be applied for EOR processing. Aurepatipan *et al.*, [20] stated the probability of conducting an in-situ EOR methodology by means of a combination of enzyme enhanced oil recovery (EEOR) and microbial enhanced oil recovery (MEOR) approaches, in the presence of lipase-esterase producing *Bacillus licheniformis* isolate.

4. Surfactants and bio-surfactants in EOR

Chemical surfactants are amphiphilic surface-active moieties, hanged to both oil/water interfaces as they consist of a hydrophilic head and hydrophobic tail [5, 52] as exposed in Figure 5.

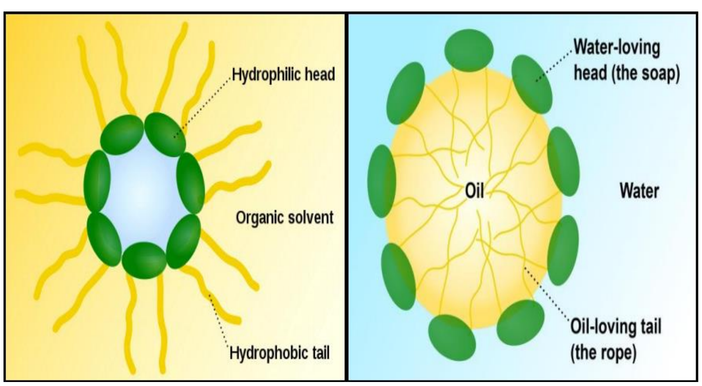

Figure 5. Surfactant skeletal structure (http://conf.sej.org/pollution-environmental-health/, 2011)

Surfactants are utilized for hydrocarbons bioremediation and microbial lysis [21]. They are classified based on ionic charge, polarizability, and molecular size. Surfactants widely implemented in petroleum field as EOR agent [5, 53] in order to;

A. Decrease interfacial tension between the entrapped oil and inoculated aqueous phase to ultra-low values reaches 0.001mNm^{-1} thus increase the capillary number (N_c) and increase recovery factor [54].
B. Altering rock wettability to water-wet, so increase the brine imbibition amounts.
C. Modify polymeric systems criteria for various applications [55].
D. Surfactants utilized in oil recovery for particle diffusion, emulsion steadiness, foam creation, reservoir wetness, and several other applications [56].
E. The promising surfactant characterized by low slug adsorption and longitudinal steadiness at reservoir environment as well as suitable compatibility towards the reservoir hardness, including divalent cations (Ca^{2+} and Mg^{2+} ions).

On the other hand, MEOR is recently generated through biosurfactants. Biosurfactants application in microbially improved oil recovery depends on their steadiness in at severe reservoir environment [57]. They are amphiphilic complexes entails of hydrophilic polar moiety as oligo or monosaccharide and proteins as well as polysaccharides or peptides, and the hydrophobic moiety has unsaturated, saturated fatty alcohols or hydroxylated fatty acids [21]. Owing to their amphipathic nature, biosurfactants can increase the surface area of hydrophobic substance, change the property of the microorganisms cell surface [21], reduce the interfacial tension between brine and petroleum, and hence reduce the capillary forces that entrap the oil in rock pores [29,58,59]. Biosurfactants are heterogeneous group of surface active molecules [58] produced by microorganisms and possess unique benefits over traditional surfactants including; lower critical micelle concentration (CMC), can be synthesized from renewable origins, high biodegradation rates, active under hard environmental conditions, worthy foaming features, high discrimination in severe conditions, minute toxicity, acceptable production economics and biological acceptability [21, 60-62]. Biosurfactants are extensively utilized in different applications, as reported by Karlapudi *et al.*, [21] including cosmetics, industries of chemicals, food, pharmaceutics, cultivation, domestics and microbially enhanced oil recovery (MEOR) [63]. traditional biosurfactants for MEOR fit to the rhamnolipids group, which are glycolipid-type, produced by *Pseudomonas* species [64]; they are manufactured as a combination of complexes comprising one or two rhamnose assemblies associated to one or two 3-hydroxy fatty acids of different chain intervals. The blends of these groups produce a large number of rhamnolipid congeners [65]. Numerous biosurfactants, particularly lipopeptides created by *Bacillus* strains, diminishes the IFT between oil and the aqueous interfaces, so mobilize residual hydrocarbons [18, 29, 66-68]. Stimulations of biomass growth and biosurfactant production can be achieved through the addition of nitrate, which could be used for respiration by nitrate-reducing microorganisms [69]. Such stimulation results in the disintegration of heavy oil portions in-situ, decreases the capillary forces that entrap the oil into the reservoir, and declines oil viscosity, therefore endorsing its movement and increase oil recovery [70]. Owing to biosurfactant chemical nature, they are classified as glycolipids, lipoproteins, lipopeptides, fatty acids, phospholipids, natural lipids, polymeric microbial surfactants and particulate biosurfactants [5, 23, 71-73]. Among them, lipopeptides are the most common and well-organized biosurfactant with high surface activity, emulsion creating capability and stabilizing, wetness, anti-adhesive and antimicrobial activity. Surfactin, a cyclic lipopeptide biosurfactant produced by different strains of *Bacillus subtilis*. It is a multipurpose bioactive particle applied in inhibition of fibrin clot creation, enhanced oil recovery to mobilize the entrapped oil [23], bioremediation applications [74]. These surfactin properties reveal its probable commercial uses [75]. Biosurfactant injection in enhanced oil recovery involves [7,22,76];

1) Ex-situ injection: Biosurfactant and other microbial products, including biosurfactants, biopolymers, biogases, biomass, and biosolvent were produced outside by aerobic fermentation and then injected into oil reservoirs as a slug trailed by water displacement or dissolved directly in the injected water [18].
2) In-situ injection: Biosurfactant-producing bacteria and their nutrients were injected into oil reservoirs, followed by a shut-in phase where the biosurfactant was in-situ created in the oil reservoirs to improve oil recovery. nutrients comprise syrup, corn steep liquor,

glucose, sucrose, nitrate salts [5]. It can be considered more advantageous for MEOR applications [77]. However, the oxygen-depleted circumstances in oil reservoirs can adversely affect biosurfactant production [77]. Consequently, anaerobic microorganisms can yield in-situ biosurfactants [18,78].

Comparing to the ex-situ application, production of in-situ biosurfactant is more beneficial for MEOR use, such as low cost and simple employment [22]. On the other hand, oil reservoirs microbial flora will be more adaptive and more competitive for nutrients to stably grow and metabolize. Since oil reservoirs suffer from oxygen-deficiency [79]. Therefore, anaerobic biosurfactants producing bacteria are promised candidates for in-situ MEOR processes [18].

4.1. Characteristics of biosurfactants

Biosurfactants are a better alternative to conventional surfactants in EOR owing to the following aspects;

1. Biosurfactants have advanced surface action with extraordinary adapting to numerous environmental conditions, and their properties remain stable under extreme physicochemical circumstances [21].
2. Ecological friendly, minor poisonousness, biodegradability, environmental adequacy, and do not lose physicochemical criteria at severe temperatures, salinity, and pH [75].
3. Biosurfactants able to emulsify and decrease the crude oil viscosity; thus it is reasonable for enhanced oil recovery processing [75,80].
4. Biosurfactants create diverse complexes with metals and remove heavy metals that cause different biological disorders [81].
5. Biosurfactants accumulate at the oil/water interface and reduce interfacial tension, so implemented in tertiary oil recovery [63].

Several reports stated a successfully implemented uses related to petroleum activities, including (MEOR, tank scrubbing, increasing the oil flow-assurance), ecological bioremediation, pharmacological and cosmetic applications, food, and beverages, as antimicrobial mediators, cleaning and domestic applications, as stated elsewhere [5]. A list of synthetic biosurfactants application and approaches used to enhance the biosurfactant production finances, as well as patents granted on the biosurfactants application and microbes related to MEOR, are reported elsewhere [5]. The successfully implemented MEOR projects are summarized in these reports stated by Geetha *et al.* and Youssef *et al.* [5,67,68,82]. These manuscripts illustrate the applicability and cost saving related to the incremental oil recovery per barrel. The biosurfactant application relies on oil-spreading effectiveness, interfacial tension activity [5,59]. Numerous low-priced substitutes are stated for biosurfactant production, including molasses, starch, and dairy waste, waste of vegetable oil industry, as well as agriculture and lignocellulosic wastes [5,83]. Biosurfactants enhance oil recovery through wettability alteration, decreasing surface and interfacial tensions at oil/water interfaces [5]. Consequently, in the subsequent section, we will give a brief note on porous media wettability.

5. Wettability of the porous media

Wettability well-defined as the favored affinity of the substrate towards the aqueous or oil phases or defined as the affinity of the wetting fluid to extend on or spread over a solid substrate in the presence of other non-wetting fluid [41]. Reservoir wettability greatly affects enhanced oil recovery procedures [84]. Wettability acquire incremental interest for the last 60 years owing to its effects on capillary pressure, which is the driving force for the spontaneous imbibition process[85], relative permeability, electrical properties, water cut production, water flood behavior, initial water saturation (S_{wi}), residual oil saturation (S_{or}), mineralogy of the rock, surfactant/polymer adsorption, formation conditions such as pH and salinity, oil and water composition, reservoir temperature and simulated tertiary recovery [86,87]. Historically, all petroleum sandstone and carbonate reservoirs proposed to be water-wetness. This philosophy relies on the fact that all porous rocks are fashioned during sediment deposition through an aqueous phase; moreover, sedimentary rocks are mainly water-wet. However, a handful of studies had suggested that sandstone reservoirs vary from water-wet to oil-wet [88]. This can be attributed to;

A. In high saline environments, clay particles lining the pores of sandstone reservoirs are extremely hydrophobic, resulting in oil wetness properties in sandstone reservoirs [89].
B. Under hard reservoir conditions, the chemical structure of sandstone altered and develops molecular interaction with the oil and hence become oil wet. This wettability alteration during oil migration into the reservoir [90] can be explained on the basis of, when oil first invades the rock pores which is covered by a thick water film, the water films rupture as a critical capillary pressure increased, resulting in direct contact of the crude oil with the pore wall. Surface-active agents in the crude oil accumulate on the rock surface, converting it to more oil-wet.
C. Although sandstone is anticipated to be water wet [41], Treiber *et al.* [91] stated that sandstone rocks do not possess a uniform wettability condition and have mixed wettability [92] due to adsorption of organics from petroleum crudes, thus creating various wettability degrees.

There is a consensus in the petroleum industry that favorably recovered oil through water-wet cores is higher than oil-wet ones [93]. This can be attributed to oil displacement increasing owing to imbibition and other interactions occurring in the reservoir [94].

5.1. Wettability evaluation

Different quantitative and qualitative methods had been proposed for wettability measurement. The most common and universal quantitative method on laboratory and industrial scales for characterizing wettability of crude oil/brine/rock systems (COBR-system) is contact angle measurement [41]. On the other hand, one of the widely used qualitative methods is two-phase separation [95].

5.1.1. Quantitative assessment

Wettability is a surface phenomenon regulating the position, movement, and circulation of the reservoir fluids and defined as the contact angle that arises at the intersection between two immiscible fluid phases and the rock surface. One of the applied techniques in the petroleum industry is the static sessile drop technique [96]. As shown in Figure 6a-d, the contact angle represented by the angle between the tangent to the droplet at the three-phase line and the solid surface, where contact angle (θ) measured through the aqueous phase. Wettability can be quantified by contact angle value [86] such that;

A) If the contact angle is between 0 and 60 to 75^0, the surface is preferentially water-wet [i.e., total hydrophilicity (zero hydrophobicity)].
B) If the contact angle is between 180 and 105 to 120^0, the surface is preferentially oil wet [i.e., total hydrophobicity (zero hydrophilicity)].
C) If the contact angle is between 60 to $75^0$and 105 to 120^0, neither fluid preferentially wets the solid (i.e., intermediate wettability).
D) Fractional and mixed wettability [41] where some pores and throats are water wet while others are oil wet [95].

Quantitative assessment of wettability carried out by contact angle measurement through a static sessile drop technique. The contact angle determined by optically monitoring the angle of the liquid/solid or the liquid/liquid/solid interface. The measurement technique based on projected or photographed images in which contact angle measured in the water phase can be calculated from the mathematical analysis of image dimensions through measuring the angle formed between the tangents to both the rock surface and the oil droplet. The precision obtained when evaluating the contact angle through this method was within ±1° [97-98]. The accuracy of contact angle measurement depends on surface contamination, and requires carefulness to obtain precise values and carried out as follow;

A. The wettability tests were done on a spherical, evacuated core plate, then saturated with synthetic brine for a day.

B. After removing from the brine solution, the core plate was aged with crude oil for 24 hours at a raised temperature to compensate for long geological periods, so the core plate become oil-wet. After that, the core plate was water flooded with a brine solution.
C. The core plate was hanged vertically in the flooding/brine solution at simulated reservoir temperature. An oil drop was hanged on the bottom of the core plate with the help of a needle bent into the shape of a "J" so the drop could "hang" upward. The contact angle was observed for a period of two days by imaging the drop attached to the plate.

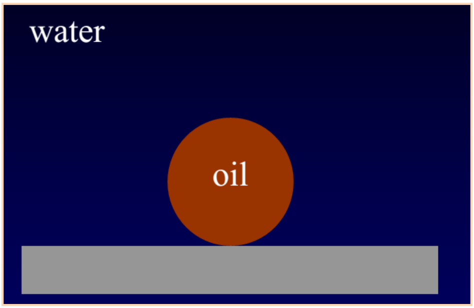

Figure 6a. Totally Water-wet (oil is non-wetting) θ=0°

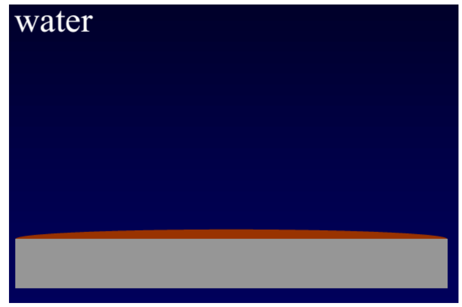

Figure 6b. Totally Oil-wet (oil is spreading) θ=180°

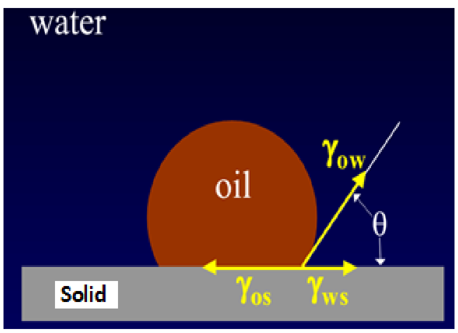

Figure 6c. Partial wetting; 0° < θ < 90°

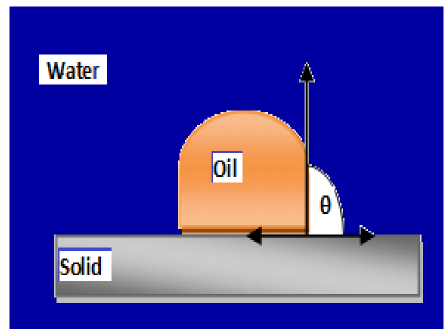

Figure 6d. Neutral wetting; θ = 90°

Figure 6. The contact angle (θ) as measured through the water phase

5.1.2. Qualitative assessment

Qualitative assessment of wettability carried out through Two-phase separation test, where the test carried out as follow;

1. Add 0.2 gm of crushed sandstone material in a 50 mL vial.
2. Add the flooded solutions to the crushed material, then add 20 mL of paraffin oil.
3. Prepare a blank sample by adding 20 cc brine solution to 20 cc of paraffin oil.
4. Shake the three vials, then left to settle.
5. The quantity of sandstone material dipped in each phase displays a qualitative sign of wettability (i.e., If all crushed material is oil-wet, it will settle in the oil phase, if it is water-wet, it will sink into the aqueous phase [89].

6. Conclusion

Retrieving trapped oil after primary and secondary techniques through microbially enhanced oil recovery should acquire increased spotlight in the upcoming days, especially some studies reported a promised recovery factor by applying this technique. Moreover, these technologies are economically and environmentally sustainable. In the nearest future, MEOR will acquire incremental application in the field of the oil industry. Since the first field-scale in 1954, numerous literature have been reported concerning the mechanisms of enhancing oil recovery. Proteins, enzymes, and biosurfactants are the most applied nutrients in the field of microbial-EOR

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