

REVIEW

BIOSURFACTANTS AND ITS PROSPECTIVE APPLICATION IN THE PETROLEUM INDUSTRY

MUHAMMAD SAIFULLAH OSMAN¹, ZAHARAH IBRAHIM¹, AZURAIEN JAPPER-JAAFAR²
AND SHAFINAZ SHAHIR^{1*}

¹Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor,

²Centre for Advanced and Professional Education (CAPE), Universiti Teknologi PETRONAS, 50470 Kuala Lumpur

*Corresponding author email: shafinazshahir@utm.my

Abstract: Increasing number of reports on the oil degrading performances from microbes shows a good prospective for successful microbial remediation in treating environmental pollution caused during the production and accidental spillage of petroleum hydrocarbon. Biosurfactant-producing and hydrocarbon-utilizing microbes increase the bioavailability of complex hydrocarbons by synthesizing biosurfactant thus enhancing the bioremediation process. Bioremediation has been proven as a method that is effective in treating oil contaminated sites as well as cost effective and gives complete degradation of organic contaminants. Biosurfactant is well-known for its unique properties such as not exhibiting toxicity effect, biodegradable, efficient at low concentration and having high stability towards extreme environment. Their advantages over synthetic surfactants make them a better alternative for application in many fields. Most emphasis to date has been on the optimization and application of biosurfactant in petroleum-related activities. Optimization involving physical and nutrient factors has been attempted as a primary step to improve biosurfactant production. This mini-review article aims to emphasize recent findings on microbial biosurfactant and its potential applications and limitations particularly in the petroleum industry.

Keywords: Bioremediation, amphiphilic, hydrocarbon, crude oil

Introduction

Surfactants are widely recognized as amphiphilic compounds consisting of hydrophobic and hydrophilic moieties that are able to reduce surface and interfacial tension between fluid phases with different polarities such as oil-water or air-water interfaces (Hoskova *et al.*, 2013; Souza *et al.*, 2014; Moya *et al.*, 2015; Varjani *et al.*, 2016). Surfactants have the ability to accumulate at the air-water interface making them key ingredients in soaps and detergents. Surfactants are also able to increase aqueous solubility of Non-Aqueous Phase Liquids (NAPLS) by decreasing their surface or interfacial tension between two different phases (Yin *et al.*, 2009). These surface active compounds are also commonly used for the petroleum industry, agriculture, foods, cosmetics and pharmaceutical application. Petroleum industries prefer to use petrochemical-based synthetic surfactants as mobilizing agents to enhance crude oil extraction and other processes related to it (Hazra *et al.*, 2012; Silva *et al.*, 2014). However, these synthetically synthesized surfactants are creating environmental problems as their residues end up in soil, water or sediment potentially exerting toxic effects on various living organisms (Ivanković & Hrenović,

2010; Rebello *et al.*, 2013). Therefore, demands for sustainable alternatives have influenced the progressive research for more environmentally friendly compounds.

The potential of using biosurfactant as an alternative solution in oil treatment and oil recovery plant has been widely studied among researchers and industrial people (De Almeida *et al.*, 2016). The use of organic sources are more beneficial as compared to chemical based treatment. Biosurfactants are produced by a wide variety of microorganisms (Gudiña *et al.*, 2015; Hu *et al.*, 2015; Elshafie *et al.*, 2015; Ferhat *et al.*, 2017; Oluwaseun *et al.*, 2017). Biosurfactants exhibit hydrophobic and hydrophilic regions which assist the uptake mechanism of hydrocarbon into the bacterial cells. By having this trait, biosurfactant molecules can accumulate at interfaces of two liquids, able to form micelles, thus reducing the surface tension and increasing the solubility of compounds in water (Kuiper *et al.*, 2004).

Crude petroleum oil is the most essential energy resource and raw medium for various industries that has brought global economic growth to another stage for the past century (Okoliegbe & Agarry, 2012; Silva *et al.*, 2014). Crude oil is refined to produce various petroleum products such

as gasoline, jet fuels, lubricants, asphalt, ethane, propane and others. According to the U.S. Energy Information Administration (EIA), global consumption of petroleum and other liquid fuels increased by 1.4 million barrels per day (b/d) in 2017, showing an average of 98.4 million b/p for the year. It has been estimated that the consumption growth will increase to an average of 1.7 million b/d in 2018, led by the countries outside the Organization for Economic Cooperation and Development (OECD) such as India and China. The main concerns, besides increasing demand for petroleum-based product are leaks and accidental spills which occur during the exploration, extraction, transportation, processing and storage of petroleum and its derivatives products. Poorly treated hydrocarbon waste released into the environment either accidental or through human activities can lead to water and soil pollution as well as threatening the ecosystem and biota (Reddy *et al.*, 2011; Wang *et al.*, 2015). Biosurfactants may help to repair this environmental pollution while offering less harmful alternative compared to synthetic surfactants (De Almeida *et al.*, 2016).

According to Research and Markets (2018), the global biosurfactant market was estimated at USD 4.20 billion in 2017 with a projected growth of USD 5.52 billion by 2022. The expanding market growth is triggered by the increasing awareness among consumers around the world who support green products. Sophorolipid-type biosurfactant reportedly accounted for the largest share of the global biosurfactant market in 2016 due to strong demand from the home care industry such as detergents. Biosurfactants are widely used in the home care industry products especially detergents to replace chemical surfactants which bring about environmental issues. Biosurfactants are known to have better properties as compared to conventional surfactants in terms of oil removal, stains, and environmental friendliness. Furthermore, global awareness toward environmental pollution increases the demand for low-cost and eco-friendly biosurfactants in various end-use industries.

This mini-review paper aims to discuss the properties and classification of biosurfactants, mechanism of biosurfactant production, factors influencing biosurfactant production, potential application of biosurfactant in petroleum industry and mechanisms of surfactant in enhanced oil recovery. Some comparative oil recovery data are summarized to show the potential application of biosurfactant in real environment. This mini-review

also highlights some limitations of using biosurfactant for enhanced oil recovery.

Properties of Biosurfactant

Biosurfactants are commonly defined as surface-active compounds synthesized by living cells, usually by microorganisms (Ferhat *et al.*, 2017). Interestingly, biosurfactants possess characteristics similar to synthetic surfactants with additional advantages such as highly biodegradable, low toxicity, cost effective and ability to maintain activity under extreme conditions of temperatures, salinity and pH (Singh *et al.*, 2007; Kapadia & Yagnik, 2013; Santos *et al.*, 2013; Silva *et al.*, 2014). These characteristics make biosurfactants relevant to be applied in various industries such as oil industry (Silva *et al.*, 2014). Cost effectiveness and economical production process are two important factors that have successfully promoted applications of biosurfactant in the markets (Banat *et al.*, 2010). Such applications require lower purity of biosurfactants, thus total production cost is reduced up to 60% by eliminating purification downstream processing steps (Sarubbo *et al.*, 2015). Almost half of the total biosurfactant production cost comes from substrate composition which constrains its market growth. Interestingly, cheaper substrates such as renewable agricultural resources and waste products can be used to produce biosurfactants which significantly reduce the production cost (Nitschke & Pastore, 2006; Rodrigues *et al.*, 2006; Helmy *et al.*, 2011; Rufino *et al.*, 2014). Waste cooking oil, frying rice bran oil, molasses and corn steep liquors have been found to produce high yield of biosurfactants (Al-Bahry *et al.*, 2012; Henkel *et al.*, 2012; Venkatesh & Vedaraman, 2012; Lan *et al.*, 2015; Gudiña *et al.*, 2015).

Biosurfactants have different functional properties depending on the molecular structure of hydrophilic and hydrophobic moieties. Surface activity involving surface tension and interfacial tension reduction are the principal properties of biosurfactant. Biosurfactant reduce the strong intermolecular bonds between liquid molecules by accumulating at the surface or interface of fluids (Beraet *al.*, 2017). For example, in water-oil interface, biosurfactant molecules tend to form a new layer around the hydrocarbon droplets as the hydrophobic tail diffuse into the hydrocarbon particles as shown in Figure 1 below.

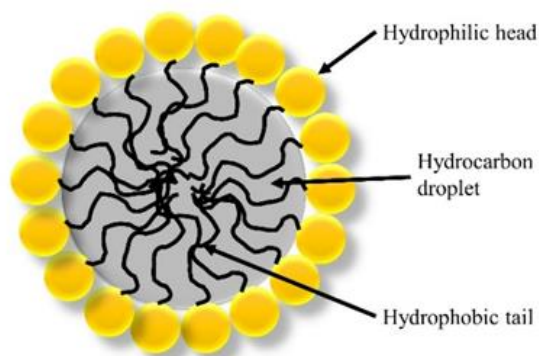


Figure 1: Micelle formation of biosurfactant

Emulsification is the process of two immiscible liquids mixed together forming a colloid. De-emulsification is the process of breaking down emulsions by altering the stable surface between two non-homogenous liquids in an emulsion. The ability of a liquid to maintain contact with a solid surface is known as wetting. Biosurfactants can alter wet ability of a liquid by reducing the attractive forces between the same molecules and increasing the repulsive forces between different molecules (Saxena *et al.*, 2017; Pal *et al.*, 2018). In addition, biosurfactant improves solubilisation of insoluble compound by forming micellar structure at certain concentration (Satpute *et al.*, 2010). The insoluble compounds are surrounded by micellar structure and improve the mobilization through the solution.

Table 1 below summarizes the biosurfactants production by different species of bacteria and their prospective in petroleum industry.

Classificaton of Biosurfactants

In general, biosurfactants are classified according to their chemical structure, function, and microbial origin. Five major groups of biosurfactants are lipopeptides, glycolipids, phospholipids, neutral lipids and fatty acids (Silva *et al.*, 2014; Santos *et al.*, 2016). The most common biosurfactants used in petroleum industry are lipopeptides (surfactin and lichenysin) and glycolipids (rhamnolipids, sophorolipids and trehalolipids) (Liu *et al.*, 2015; Santos *et al.*, 2016). Biosurfactants can be grouped into two main categories according to their molecular weight. Low molecular weight biosurfactants consist of glycolipids and lipopetides while high molecular weight biosurfactants consist of polyanionic heteropolysaccharides.

The hydrophobic part of biosurfactants consists of hydrocarbon chain of saturated, unsaturated, hydroxylated, or branched fatty acid.

The hydrophilic part consists of:

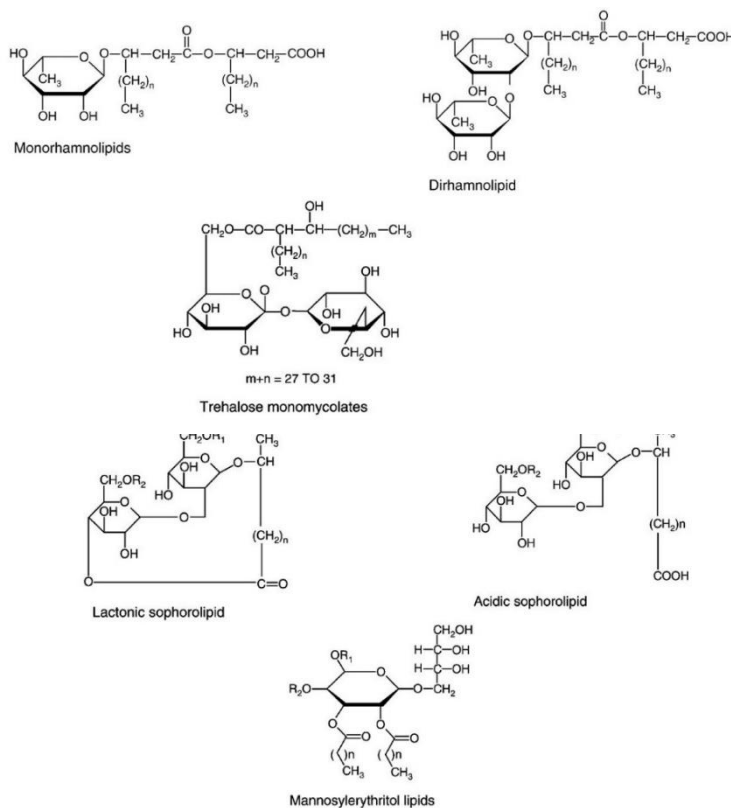
- carboxylate group of fatty acids or amino acids or the phosphate-containing portions of phospholipids,
- mono-,di-, and polysaccharides of glycolipids, and the polar side chains and peptide backbone of lipopeptide

Table 1: Current findings on some biosurfactants and their potential applications in the petroleum industry

Type of biosurfactant	Microorganism	Potential application	References
Rhamnolipid (Mono- and Di-)	<i>Pseudomonas aeruginosa</i> PG1	Bioremediation of crude oil contamination sites	Patowary <i>et al.</i> (2017)
Rhamnolipid(Di-rhamnolipid)	<i>Pseudomonas aeruginosa</i> C1501	Biomedical, Food industries, Agriculture, Bioremediation	Oluwaseun <i>et al.</i> (2017)
Trehalolipid	<i>Rhodococcus</i> sp. strain PML026	Bioremediation	White <i>et al.</i> (2013)
Sophorolipid	<i>Candida bombicola</i> ATCC 22214	Enhanced oil recovery	Elshafie <i>et al.</i> (2015)
Phospholipid	<i>Klebsiellapneumoniae</i> IVN51	Biodegradation of petroleum hydrocarbons	Nwaguma <i>et a.</i> (2016)
Surfactin	<i>Bacillus subtilis</i> K1	Enhanced oil recovery	Pathak and Keharia, (2014)
Lipopeptide	<i>Bacillus subtilis</i> A1	Bioremediation of crude oil contaminated environments	Parthipan <i>et al.</i> (2017)
Lichenysin	<i>Bacillus licheniformis</i> W16	Enhanced oil recovery	Joshi <i>et al.</i> (2016)
Emulsan	<i>Acinetobactercalcoaceticus</i> PTCC 1318	Emulsification of crude oil	Amani and Kariminezhad, (2016)

Rhamnolipids, sophorolipids, trehalose lipids and mannosylerythritol lipids are the four main classes of

glycolipids (Banat *et al.*, 2010). Figure 2 depicts the chemical structures of some common glycolipids.

Figure 2: Chemical structures of some common biosurfactants (Smyth *et al.*, 2010)

Pseudomonas aeruginosa strains are well-known as rhamnolipids producer (Patowary *et al.*, 2017; Oluwaseun *et al.*, 2017). The hydrophilic part of rhamnolipids consists of either one or two rhamnose molecules while the hydrophobic part consists of long chain aliphatic acids or hydroxyl aliphatic acids (Oluwaseun *et al.*, 2017). Trehalose lipids consist of a trehalose linked to α -branched β -hydroxy fatty acids by an ester bond (Lang & Philp, 1998; White *et al.*, 2013). Sophorolipids can be divided into two main classes, lactonic and acidic, according to the bonding position between sophorose and the fatty acid chain. Sophorolipids are produced mainly by *Candida* species (Elshafie *et al.*, 2015). Mannosylerythritol lipids, also known as MELs, are produced mainly by *Pseudozyma* yeasts species (Fukuoka *et al.*, 2007). Isolation, purification and characterization of biosurfactant-producing bacteria are very important aspects in microbial biosurfactant study as the information can be used for further optimization analysis and production of biosurfactant (Makkar *et al.*, 2011).

Biosurfactant Synthesis and Stability in Culture Medium.

The basic structure of biosurfactant is composed of hydrophobic and hydrophilic moieties. There are a number of variations in amphiphilic structures yielded by different microorganisms. Generally, two different synthetic pathways are involved to produce two different moiety of biosurfactant. The hydrophobic moieties are synthesized by common lipid metabolism pathway while hydrophilic moieties exhibit a wide range of biosynthetic pathways due to various structural complexity (Santos *et al.*, 2016).

There are four possible principles for biosynthesis of amphiphilic molecules:

1. Both moieties are synthesized *de novo* via independent pathways
2. The hydrophilic moiety is synthesized *de novo* and the substrate induces the formation of hydrophobic moiety.
3. The hydrophobic moiety is synthesized *de novo* while the substrate induces the formation of hydrophilic moiety.
4. Both moieties are synthesized depending on the substrate available in the surrounding environment.

Rhamnolipid is a well-studied glycolipid biosurfactant produced mainly by *Pseudomonas* sp. The structural and regulatory genes responsible for rhamnolipid synthesis have been isolated and characterized (Irfan-Maqsood & Seddiq, 2014).

The *rhl* quorum sensing system in *Pseudomonas aeruginosa* regulates the production of rhamnolipid. Genes responsible in rhamnolipid biosynthesis are plasmid-encoded such as *rhl* A, B, R and I which transcribed in 5'-*rhl*ABRI-3' direction in heterologous host. Different types of rhamnosyltransferase catalyze two sequential glycosyl transfer reaction in the pathway. The *rhl*AB gene produces enzyme rhamnosyltransferase which catalyzes rhamnolipid 1 synthesis. Rhamnolipid synthesis and regulation in *Pseudomonas aeruginosa* have been well elaborated by Chong & Li, (2017).

Previous studies have demonstrated that biosurfactants exhibit good stability in culture medium with a wide range of temperature and pH (Pathak & Keharia, 2014; Ferhat *et al.*, 2017; Almatawah, 2017; Sharma *et al.*, 2018). Rhamnolipid-type biosurfactant produced by *Pseudomonas aeruginosa* PBS showed consistent surface tension reduction at an average of 25 mN/m when grown in glucose minimum salt medium with pH range 4 – 10 and temperature up to 100°C (Sharma *et al.*, 2018). Ferhat *et al.*, (2017) successfully extracted a thermostable biosurfactant from *Ochrobactum intermedium* that reduces surface tension with an average of 31 mN/m at temperature range from 20°C to 100°C. The biosurfactant was also able to maintain its stability in medium with a wide pH range (pH 4 to 11). Stability of biosurfactant in a wide range of pH and temperature are very crucial to ensure optimum result is achieved in the real application for oil recovery.

Factors Influencing Biosurfactant Production

Diverse microorganisms produce biosurfactants with different chemical structures and surface activity (Youssef *et al.*, 2009; Santos *et al.*, 2016). Among the factors that influence biosurfactant production are types of microorganisms, carbon sources, nitrogen sources, pH, temperature, salinity, carbon to nitrogen ratio, aeration and agitation (Luna *et al.*, 2013; Rahman & Gakpe, 2008; Santos *et al.*, 2002; Mukherjee *et al.*, 2006; Banat *et al.*, 2010). Figure 3 summarizes the factors that influence biosurfactant production.

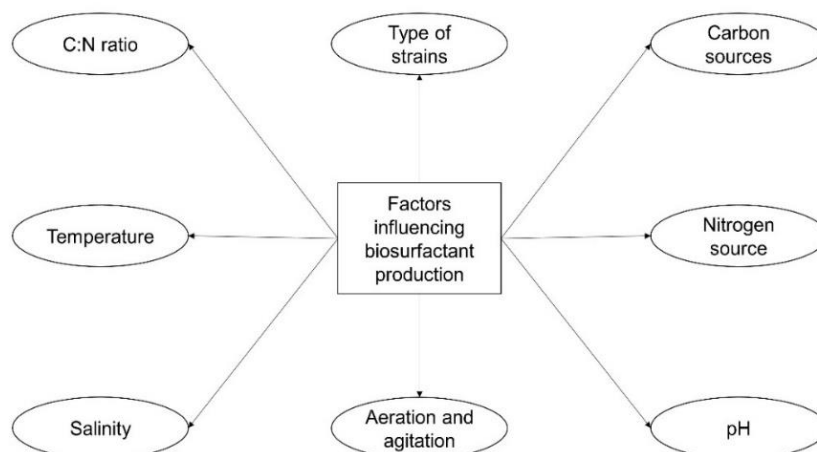


Figure 3: Factors influencing biosurfactant production

The availability of carbon source affects the quality and quantity of biosurfactant production. A recent study by Parthipan *et al.* (2017) reported that *Bacillus subtilis* A1 was able to degrade hydrocarbon by synthesizing biosurfactant in the presence of crude oil as carbon source. They used eight different carbon sources namely crude oil, coconut oil, diesel oil, sucrose, starch, glycerol, mannitol and maltose to optimize the biosurfactant production. Sucrose was identified as the best carbon source and the type of biosurfactant produced by the bacteria was lipopeptide which exhibited high emulsification activity of up to 78%. Another interesting report by Sharma *et al.* (2018) showed that of the twelve carbon sources tested for biosurfactant production with *Pseudomonas aeruginosa* PBS, sodium citrate resulted in maximum surface tension reduction. Most of the carbon sources in the study led to surface tension reduction of up to 30-34 mN/m with respect to 71.80±0.96 mN/m (uninoculated media) except for fructose, sorbitol, maltose and lactose.

Nitrogen is a crucial element in biosurfactant production because protein and enzyme syntheses rely on it. Microorganisms require nitrogen as raw material to build protoplasm and other cellular structures. Various sources of nitrogen have been used in previous reports for the production of biosurfactants such as ammonium nitrate, ammonium sulphate, ammonium chloride, ammonium phosphate, peptone, potassium nitrate, urea, sodium nitrate, yeast extract, beef extract, soybean meal and corn meal (Parthipan *et al.*, 2017; Hu *et al.*, 2015; Sharma *et al.*, 2018). Yeast extract was found to give a high production of biosurfactant by *Bacillus subtilis* A1 exhibited by the highest emulsification activity (68%) (Parthipan *et al.*, 2017). Another study by Hu *et al.* (2015) reported that marine *Vibrio* sp. strain 3B-2 produced the highest yield of biosurfactant with yeast extract as nitrogen source. The respective strain also preferred to utilize

organic nitrogen sources rather than inorganic nitrogen sources.

Physical factors that affect the biosurfactant production are pH, temperature, salinity, aeration and agitation level. Most biosurfactant productions are reported to be optimized under pH 7.0 (Parthipan *et al.*, 2017; Sharma *et al.*, 2018). When the pH changes, there is substantial effect on the biosurfactant production. But, it is important to highlight that the biosurfactant itself is very stable in wide ranges of pH as reported by Sharma *et al.* (2018). Sharma *et al.* (2018) showed that the surface tension of the cell free supernatant containing biosurfactant retained its activity over pH 4-10. In addition, the mesophilic bacterium *Bacillus subtilis* A1 was found to optimally produce biosurfactant at 40°C with an emulsification activity of 76% (Parthipan *et al.* (2017). Biosurfactant production by marine *Vibrio* sp. strain 3B-2 was found to gradually increase from 25°C to 28°C with maximum production at 28°C (Hu *et al.*, 2015). Further increase in the temperature above 28°C caused increased energy consumption and decreased bacterial growth resulting in reduced biosurfactant production.

Another factor that has a corresponding effect on the biosurfactant production is salinity or the concentration of salt in growth medium. Higher salinity in the medium indicates high osmotic pressure (Wood, 2015). In general, isotonic solution is a favorable environment for bacterial growth. Hu *et al.* (2015) reported that in the absence of sodium chloride, marine *Vibrio* sp. strain 3B-2 showed almost no growth compared to other medium with salt. They used the analytical hierarchy process (AHP) method to determine the optimal salt concentration for biosurfactant production. Based on the results, the optimal concentration for biosurfactant production by marine *Vibrio* sp. strain 3B-2 was 20 g/L. Aeration and agitation are other physical factors that influence the biosurfactant production by controlling the oxygen

availability in the growth medium. A previous study by Adamczak and Bednarski (2000) had found that the optimum biosurfactant production (45.5 g/L) was achieved when the air flow rate was 1 vvm with 50% dissolved oxygen concentration.

Factors Influencing Biosurfactant Production

Hydrocarbons are hydrophobic substrates and the role of biosurfactant is to assist its bioavailability to the cells. Surfaces of bacterial cells are mostly hydrophilic in nature, thus allowing them to interact with water-soluble compounds effectively and keeping the membrane-bound enzyme system operating at a normal rate. Biosurfactant can be applied during extraction, transportation, cleaning of crude oil storage tank and treatment of crude oil waste. The main mechanisms of biosurfactant augmented oil recovery are reduction of surface and interfacial tension, wettability alterations, emulsification, de-emulsification, adsorption, solubilization and viscosity reduction (Satpute *et al.*, 2010; Silva *et al.*, 2014; Beraet *et al.*, 2014; Babu *et al.*, 2015; Pal *et al.*, 2017; Saxena *et al.*, 2017; Pal *et al.*, 2018).

Extraction of Crude Oil

Generally, the oil production strategies begin with primary depletion followed by secondary oil recovery. Tertiary recovery process may be included in certain cases. In the first step, oil is extracted under natural pressure and usually recovers about 10-20% of crude oil. As the pressure declines, the crude oil yield also reduces. Secondary oil recovery involving either water and/or gas injection can improve the oil recovery up to 40-50% (Bachmann *et al.*, 2014). Almost half of the crude oil remains trapped in the pores of the rock formation in the reservoir. In this situation, tertiary step or also known as enhanced oil recovery methods are conducted. Conventional tertiary recovery process involves chemical and/or thermal methods. Chemicals commonly used are hydrocarbon solvents, synthetic surfactants, gas or combination of them to reduce interfacial tension between oil and water (Bachmann *et al.*, 2014). Thermal methods involve steam, hot water or combustible gas to increase the temperature in the reservoir increasing their mobility. However, these methods are not economical and potentially hazardous to the environment, thus demanding better alternatives (Perfumo *et al.*, 2010).

Microbial enhanced oil recovery (MEOR) can be applied during the tertiary recovery process which involves biosurfactant-producing microorganisms or their metabolic products used in the reservoir as depicted in Figure 4.

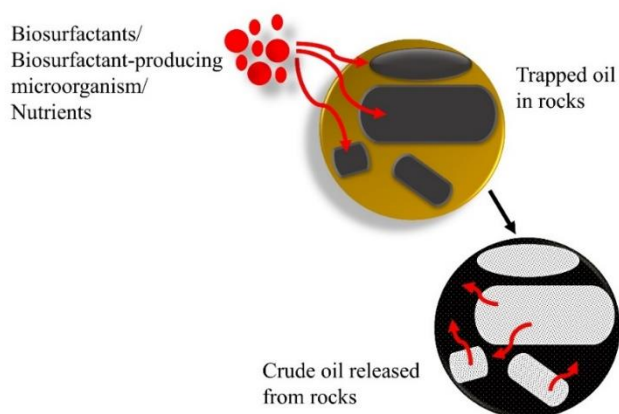


Figure 4: Proposed method to recover trapped oil in rocks

The principle of using biosurfactant to improve oil recovery is by improving crude oil mobilization throughout the process (Perfumo *et al.*, 2010). Biosurfactant exhibits adsorption property which can enhance oil recovery by replacing the oil attached to the rocks. This method is cost effective compared to synthetic surfactant due to the low-cost substrates required by the respective microorganisms to produce sufficient biosurfactants (Sarafzadeh *et al.*, 2014; Silva *et al.*, 2014). The process of crude oil recovery occurs, mediated by the activity of microorganisms and/or their metabolites, such as biosurfactants, cell biomass, biopolymers, acids, gases and others, applied ex-situ or in-situ (Youssef *et al.*, 2009). There are three ways proposed in MEOR (Banat *et al.*, 2000; Al Bahry *et al.*, 2013; Bachmann *et al.*, 2014):

1. Biosurfactants are produced ex-situ using bioreactors in industrial conditions and injected directly into the reservoir. Biosurfactants must maintain activity under environmental conditions. In this strategy, the production of biosurfactant is totally dependent on the medium composition with optimum environment. However, this strategy is expensive because of the bioreactor operation, biosurfactant purification and introduction into the reservoir.
2. Biosurfactant-producing microbes are cultured in the laboratory followed by injection into the reservoir. Specific active biosurfactant producing microbes are introduced at the cell-oil interface inside the reservoir to enable *in-situ* microbial growth. Usually, low-cost substrates are provided to enhance the growth and biosurfactant production.
3. Selected nutrients are injected into the reservoir to stimulate and enhance the growth of indigenous biosurfactant-producing microorganisms. Under nutrient-rich environment, the growth of indigenous biosurfactant-producing bacteria are stimulated and subsequently enhance the biosurfactant production

Mechanisms of Surfactants in Enhanced Oil Recovery

The mechanisms of synthesized surfactants in enhanced oil recovery have been well studied recently and the results can be used for comparison with biosurfactants produced by microorganisms since both have common amphiphilic molecules.

Pal *et al.*, (2018), reported that synthesized cationic gemini surfactants (GS) show—great physicochemical properties such as increment of critical micelle value (CMC) with temperature rise and stability maintained at high temperature after 30 days of continuous heating at 343 K. In the real application situation, surfactant solution is subjected to non-ambient environment for a period of time which requires thermally stable surfactant. The reduction of interfacial tension enhances the oil recovery by forming more capillary number which improves oil mobilization. Moreover, GS is able to self-aggregate at a low concentration which makes the displacement of the remaining trapped oil more

effective. Interfacial tension also decreases when the salinity increases due to synergistic effect until it reaches optimal salinity value. Furthermore, GS also exhibited wettability alteration when maximum zeta potential values perceived at CMC value, explaining the minimum disjoining pressure and maximum oil-wetting feature.

Wettability alteration is an important characteristic for successful application of surfactant. A recent report by Saxena *et al.*, (2017) found that surfactant synthesized from palm oil known as alpha sulfonated ethyl ester (α -SEE) exhibited thermal stability, ultralow interfacial tension and good wetting properties. Increase in salinity enhanced the wetting result of surfactant (Bera *et al.*, 2014; Babu *et al.*, 2015; Pal *et al.*, 2017). The additional length of carbon chain in surfactant can significantly reduce the interfacial tension between crude oil and surfactant by altering the adsorption and micellization behavior of the respective surfactant (Kumar & Mandal, 2018). More than 30% additional oil recovery was obtained in flooding experiment due to synergistic effect between alkali, surfactants and polymer. Micellization and adsorption properties are important mechanisms of surfactants in enhanced oil recovery application. A detailed study by Pillai *et al.*, (2017) showed that synthesized imidazolium-based ionic liquids exhibited good thermal stability, able to reduce surface tension, possess high adsorption efficiency and great micellization mechanism.

Table 2: Comparative oil recovery data using biosurfactants

Methodology	Results	References
Core-flooding studies. Biosurfactant produced by <i>Bacillus subtilis</i> B20 was used in this study.	Pore volume: 24.2 cm ³ , initial oil saturation after oil flooding: 67%. No more oil was produced after 10 pore volumes of formation water were injected, where the residual oil saturation was 38%. The oil recovery was observed to be increased at 9.7%, after 4 pore volumes of biosurfactant were injected into the core sample.	Al-Bahri <i>et al.</i> , (2013)
Core-flooding studies. Biosurfactant produced by <i>Bacillus subtilis</i> B30 was used in this study	Pore volume: 18-24 cm ³ , initial oil saturation after oil flooding: 55-67%. No more oil was produced after 5-8 pore volumes of formation water were injected, where the residual oil saturation was 25-38%. The oil recovery was observed to be increased at 17-26% after 4-5 pore volumes of biosurfactant were injected into the core sample.	Al-Wahaibi <i>et al.</i> , (2014)
Core-flooding studies. Sophorolipid-type biosurfactant produced by <i>Candida bombicola</i> ATCC 22214 was used in this study	Pore volume: 18 cm ³ , initial oil saturation after oil flooding: 55-60%. No more oil was produced after 5-8 pore volumes of formation water were injected, where the residual oil saturation was 20%. The oil recovery was observed to be increased at 27.27% after 4-5 pore volumes of biosurfactant were injected into the core sample.	El-Shafie <i>et al.</i> , (2015)
Core-flooding studies. Lipopeptide-type biosurfactant produced by <i>Bacillus licheniformis</i> W16 was used in this study	Pore volume: 17-19 cm ³ , initial oil saturation after oil flooding: 73-77%. No more oil was produced after 7 pore volumes of formation water were injected, where the residual oil saturation was about 15-20%. The oil recovery was observed to be increased at 24-26% after 4-5 pore volumes of biosurfactant injected into the core sample.	Joshi <i>et al.</i> , (2016)
Sand-pack column test. Rhamnolipid-type biosurfactant produced by <i>Pseudomonas aeruginosa</i> PBS was used in this test.	Pore volume: approximately 30cm ³ , initial oil saturation was 60.90±0.84%. Secondary oil residues after water flooding were 13.76±1.41%, residual oil saturation was 86.24±1.41% and additional oil recovery was 56.18±1.59% when biosurfactant was applied to the column.	Sharma <i>et al.</i> , (2018)

Core-flooding studies were conducted to investigate the ability of the bacteria in enhanced oil recovery. The experimental condition can be controlled to mimic the real reservoir condition. Table 4 above summarized some recent core-flooding studies which have shown that oil recovery improved when biosurfactant was injected into the column

Transportation of Crude Oil

Crude oil is mostly transported via pipelines over long distances to shipping ports or refineries. Problems arise particularly for heavy or extra-heavy crude oil which increases the transportation cost because of slower flowing rate as this type of crude oil is more viscous because it contains high paraffin and asphaltenes. Asphaltenes may form precipitates or asphaltene mud on metal surface under acidic condition and in the existence of ferric ions, creating obstruction to the flow rate of crude oil (De Almeida *et al.*, 2016). Crude oil becomes thicker with high presence of paraffin as cyclic hydrocarbons may solidify and accumulate at room temperature leading to blockage problem during shipping of crude oil (Assadi & Tabatabaee, 2010). High viscosity of crude oil also can lead to sludge build up on the inner walls of the pipeline which later will reduce the pressure inside and create blocking problems (Perfumo *et al.*, 2010). Conventional treatment has been done by heating or diluting crude oil with solvents, such as xylene and toluene to minimize viscosity and dissolving any semisolid compounds formed in the crude oil. However, this method is not cost effective and leaves solvent that contains harmful waste (Assadi & Tabatabaee, 2010; Mulligan *et al.*, 2014).

The production of a stable oil-in-water emulsion has been recently developed using biosurfactants specifically bioemulsifiers to enhance oil mobility. Bioemulsifiers are high-molecular weight biosurfactant with different properties that are very effective in stabilizing oil-in-water emulsion, but lack the ability in reducing interfacial tension. Bioemulsifiers contain a high number of reactive groups which make them bind strongly to oil droplets and create a barrier that obstructs drop coalescence (Perfumo *et al.*, 2010). *Acinetobacter* strains are well-known producers of bioemulsifier such as emulsan, alasan and biodispersan (Mulligan *et al.*, 2014). Bioemulsifiers have been widely studied and promising applications have been shown during crude oil transportation in pipelines by reducing viscosity of crude oil (Assadi & Tabatabaee, 2010; Perfumo *et al.*, 2010; Mulligan *et al.*, 2014;). An interesting investigation by Amani & Kariminezhad (2016) used an emulsan type biosurfactant synthesized by *Acinetobacter calcoaceticus* PTCC1318 to remove crude oil from a stainless steel tubing and the result signified efficacious tube washing at room temperature.

Cleaning of Crude Oil Storage Tanks

Extracted crude oil is kept in oil tanks in refineries or transported by oil trucks and barges over a longer period. Those tanks need consistent cleaning or maintenance which often leads to environmental pollution due to significant amount of harmful waste generated (Perfumo *et al.*, 2010; Matsui *et al.*, 2012; Mulligan *et al.*, 2014). Conventional method such as pumping cannot remove the oil sludge fraction formed at the walls and bottom of the tanks because it is highly sticky and sometimes may be in semisolid forms. Even though this cleaning process can be done manually using steam or hot water and solvents, it involves harmful chemicals and it is also time consuming and requires a large workforce. This conventional method is also expensive and generates huge volumes of waste product (Perfumo *et al.*, 2010; Matsui *et al.*, 2012).

The pioneer in using biosurfactants for cleaning oil storage sludge is Gutnick and Rosenberg (1981). Among the advantages of using this alternative method is the fact that the recovered crude oil still has superb properties and could be retailed when mixed with fresh crude (Banat *et al.*, 1991). A previous investigation by Diab and El Din (2013) had assessed the effect of biosurfactant produced by *Pseudomonas aeruginosa* SH29 in cleaning oil-contaminated vessels and they reported successful oil elimination from the vessel's surface under appropriate conditions. Another related study by Rocha e Silva *et al.* (2013) reported similar result by using biosurfactant from *Pseudomonas cepacia* CCT6659 to clean beaker's wall covered with hydrocarbon.

Treatment of Crude Oil Waste

Crude oil waste or sludge is one of the main concerns in petroleum industry because it is produced in a significant amount through oil exploration, storage, transportation and refining. Oil sludge is composed of various petroleum hydrocarbons mixed with solid particles, water and heavy metals (Hu *et al.*, 2013). These impurities must be removed from the crude oil before the oil can be further refined. Conventional treatment often leads to formation of emulsion phase of variable composition and thickness at the interface of the oil and water. When the emulsion phase becomes stable and thickens, it is considered as a waste and will be removed to storage tanks. Extensive studies regarding biosurfactants in laboratory, pilot and field scale to treat this oily sludge have shown higher oil recoveries (Pornsunthorntawe *et al.*, 2008; Hu *et al.*, 2013). A study by Lima *et al.* (2011) showed that biosurfactant was able to reduce viscosity and enhance the creation of oil-water emulsions making the sludge easy to be pumped out, thus improving oil recovery and reducing the sludge volume by up to 95%. An

interesting investigation on the use of rhamnolipid produced by *Pseudomonas aeruginosa* F-2 to recover oil sludge reported oil recovery of up to 91.5% (Yan *et al.*, 2012). Even though many reports have shown successful application of biosurfactant, there are several cases of unsuccessful activities by biosurfactant (Franzetti *et al.*, 2011; Lawniczak *et al.*, 2013).

Limitation of using Biosurfactants for Enhanced Oil Recovery

The limitation of using biosurfactants can be associated with three main strategies during biosurfactant-mediated oil recovery. The first strategy involves the injection of purified biosurfactants produced in the laboratory into the reservoirs. Although many biosurfactants manifest great surface activities, the commercialization of this approach remains difficult and costly because chemically synthesized media were used for growing the microorganisms (Smyth *et al.*, 2010; Banat *et al.*, 2014). Most biosurfactants also have not achieved a sufficient economical level because of their low production (Smyth *et al.*, 2010).

The second strategy is to inject selective exogenous biosurfactant-producing microorganisms and specific nutrients into the reservoir. This requires a large concentration of microorganisms to induce their activity in the reservoir. Even though the respective microorganisms were already grown under industrial conditions that mimic the real environment, adaptation to the condition in the reservoir and competition with indigenous microorganisms need to be considered (Li & McInerney, 2016) as these factors may affect growth and biosurfactant production. In addition, the respective microorganisms should have good motility and minimal adsorption to reservoir rock material so that it can move freely in the reservoir (Li & McInerney, 2016).

The last strategy involves injection of specific nutrients to encourage the indigenous biosurfactant-producing microorganisms inside the reservoir. Detailed analysis is required to determine the potential indigenous microorganisms and their activities before proper action can be planned and executed. Many assessments are required to confirm the biosurfactant production and after that a suitable nutrient combination need to be formulated. A more detailed analysis is also required to provide sufficient information on the toxicity of biosurfactants toward

the environment since it has yet to be well studied and documented (de Cássia F.S. Silva *et al.*, 2014, Franzetti *et al.*, 2006).

Despite the limitations, the extraordinary properties of biosurfactant as surface active products make them potentially promising for a vast number of applications in different industries particularly in oil recovery.

Conclusion

Biosurfactants exhibit notable functional properties such as ability to reduce surface and interfacial tension, wettability alteration, emulsification, de-emulsification, adsorption, viscosity reduction and solubilization. The production of biosurfactants seems to be promising and is a better alternative to replace chemical surfactants currently used in the petroleum industry. The significant positive impact offered by biosurfactant is that it is environmental friendly which evidently can overcome the environmental issues compared to synthetic surfactant. At the moment, authors and the team are studying the biosurfactant produced by *Enterobacter xiangfangensis* strain SSP3b16 isolated from rag layer crude oil emulsion. There is not much information regarding rag layer as a source of bacteria isolation that has potential in producing biosurfactant. As the rag layer is one of the common issues faced by the petroleum industry, biosurfactant shows promising potential to be used as an alternative treatment solution to replace synthetic surfactants in the future. However, detailed research need to be conducted in the lab-scale before real environment application can be attempted so as to ensure its maximum effectiveness.

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