

Roles and Applications of Bacteria in Petroleum Industry: A Review

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Abstract

Roles and applications of bacteria in petroleum industries spanned across the discovery of petroleum hydrocarbon, bacterial enhanced oil recovery (BEOR), solubilisation, emulsification and bioremediation of petroleum. Numerous species of bacteria have evolved which can oxidize petroleum and its products for their sole source of carbon and energy, similarly bacteria have played a crucial role in the process of petroleum formation by catalyzing many biological and reactions in the marine sediments rich in organic matter. Bacterial enhanced oil recovery technology was recognized as the most efficient and cost-effective technique that enhanced oil recovery from mature reservoir or abandoned wells. In recent years, chemically-synthesized surface-active agents were used to enhanced oil recovery (EOR), however, bacteria produced biological surface-active compounds (biosurfactants) which can reduce surface tension and interfacial tension in petroleum mixture. In this review we provided current and recent literature available concerning the roles of bacteria in the petroleum industry.

Keywords: Bacteria, Biosurfactant, Bioemulsifiers, Petroleum, Biodegradation.

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INTRODUCTION

The roles of bacteria in the petroleum industry are as enormous as it touches many aspects of the oil and gas industry, particularly the downstream processing including, petroleum formation and discovery, recovery of petroleum hydrocarbon from abandoned or depleted oil well, biodegradation of petroleum hydrocarbon pollutants, bioconversion of petroleum hydrocarbon (Coronelli, 1996; Gudina *et al.*, 2013; Mohsin *et al.*, 2018). Nowadays, bacteria can be manipulated and applied in the oil and gas industries due to its metabolic capabilities (Rashedi *et al.*, 2012). Bacterial biodegradation of hydrocarbon is one of the primary mechanisms for the elimination of petroleum and other hydrocarbon pollutants from the environments (Calvo *et al.*, 2008; Abdel-shafy and Mansour, 2018). Biosurfactants and emulsifiers are by-products of bacterial metabolism of petroleum hydrocarbon which are now used for bacterial enhanced recovery of residual oil, managing oil spill pollution, transporting petrol in pipelines, mobilization of heavy crude oil, cleaning of sludge from oil storage facility (Banat, 1995; Rashedi *et al.*, 2012). Petroleum and its products have now become one of the major sources of environmental pollution worldwide (Perfumo *et al.*, 2010). Nowadays, the hydrocarbon pollution caused by the activities of petroleum industries is a serious issue that need a scientific approach, thus, bacteria can be exploited in a variety of ways in the petroleum industry due to its nutritional and metabolic diversity. Numerous types of bacteria have evolved which can oxidize petroleum and its products for their sole source of carbon and energy (Mohsin *et al.*, 2018). It's generally believed that all kinds and classes of petroleum hydrocarbon are susceptible to bacterial degradation under certain conditions (Zobell, 1947b). Furthermore, *in situ* stimulation of biosurfactant or bioemulsifiers-producing bacteria reduces viscosity in the reservoir and subsequently increases oil flow and production (Calvo *et al.*, 2008; Rashedi *et al.*, 2012). The first documented studies on the bacterial oxidation of hydrocarbons of various classes began in the 70s of the 20 century (Kolotilova, 2011; Coronelli, 1996). Bacteria capable of interaction and oxidation with hydrocarbons were previously reviewed by Rozanov (1967), Rozanov (1971), Foster (1962), Foster (1966) and Klug & Markvetz (1971), Abdel-shafy and Mansour, (2018). According to a recent analysis, the most active destructors of petroleum hydrocarbons are found among bacteria of the following genera: *Pseudomonans*, *Arthobacter*, *Rhodococcus*, *Acinetobacter*, *Flavobacterium*, *Corynebacterium*, *Xanthomonans*, *Alcaligenens*, *Norcardia*, *Brevibacterium*, *Mycobacterium*, *Beijerinckia*, *Bacillus*, *Enterobacteria*, *Klebsiella*, *Micrococcus*, *Sphaerotilus* (Mohsin *et al.*, 2018; Coronelli, 1996; Rozanov, 1967; Rozanov, 1971; Abdel-shafy and Mansour, (2018)). The objective of this review is to establish and develop an overview of the microbiological aspect of the oil and gas industry with exclusive roles and applications of bacteria.

Overview of enhanced oil recovery (EOR)

Nowadays, the majority of world's energy come from crude oil, a large proportion of this valuable and non-renewable resource is left behind in the ground after the application of conventional oil extraction methods. Moreover, there is a need to produce more crude oil to meet the world rising energy demand, in view of this, enhanced oil recovery (EOR) processes were established and developed (Rashedi *et al.*, 2012). Previous studies have recognized three (3) stages of petroleum production (Gudina *et al.*, 2013), these include: primary petroleum production, when the natural pressure of the reservoir is high enough and causes the oil to flow up to the surface, the second stage is secondary petroleum production when the natural pressure of the reservoir declined during primary production a critical point is reached when it is necessary to provide external energy for the reservoir to achieve desired oil recovery (Singer and Finnerty, 1984). The third stage is tertiary petroleum production, after some years of exploration, the injected fluid (gas or water) flows

preferentially along with high permeable layers that cause these fluids to by-pass oil saturated areas in the reservoir, thus, causes a large quantity of water or gas to rise with the oil, subsequently the ratio of oil to water will be decreased. However, the properties of the reservoir fluids and characteristics of the rock can be modified to increase recovery efficiencies more than the one obtained by primary and secondary stages (Banat *et al.*, 2000; Cameotra & Makkar, 2004; Rashedi *et al.*, 2013). Bubela (1987) have previously reviewed factors affecting tertiary production of petroleum which can cause poor oil recovery from producing wells, they includes, low permeability of some reservoirs, the high viscosity of the oil (poor mobility) and high interfacial tension between the water and oil (high capillary force). In recent years, there are only three (3) methods by which properties of the reservoir fluids and characteristics of the reservoir rock can be modified, and the choice of method depending on the nature and specification of the oil well (Banat, 1995; Wagner, 1991; Xu *et al.*, 2009; Rashedi *et al.*, 2012): they include thermal process which is specifically for the recovery of heavy or viscous oil and has been applied for the recovery of many oil reservoir, the chemical method which involves the used of chemical and miscible displacement method which involves the used of injected solvent or gas, however, these three aforementioned processes are not environment-friendly due to their negative environmental impact (Lazar *et al.*, 2007; Scheibenbogen *et al.*, 1994). Nowadays, the alternative process of enhanced oil recovery with no negative environmental impact is the microbial process, which was first described by Beckman in 1926. To date several studies were conducted on microbial enhanced oil recovery (Zobell, 1947b, Zobell, 1947a; Zhang and Miller, 1992; Banat, 1993, Banat, 1995; Lazar *et al.*, 2007; Rashedi *et al.*, 2012; Safdel *et al.*, 2017; He *et al.*, 2018; Haicheng *et al.*, 2019). Microbial enhanced oil recovery is also known as tertiary petroleum production, its an environment-friendly and low operating cost treatment technology that increase oil production by propagation and metabolites of bacteria (Haicheng *et al.*, 2019). Currently, microbial enhanced oil recovery has become the most widely used method of dealing with poor oil recovery in existing producing or abandoned wells. Microbial enhanced oil recovery stands as the most rapidly developed method of tertiary petroleum production that uses microorganisms, particularly bacteria or their metabolites to enhanced recovery of residual oil (Banat 1995; Xu *et al.*, 2009; Rashedi *et al.*, 2012).

Roles of bacteria in petroleum industry

Our study has identified five (5) major ways by which bacteria played tremendous roles in the petroleum industries, these include: bacterial enhance oil recovery (BEOR), bacteria in petroleum discovery and formation, bacterial-biosurfactant production and its application, role of bioemulsifiers and role of bacteria in bioremediation of hydrocarbon pollutants.

Bacterial enhanced oil recovery (BEOR)

Bacteria were now considered as the best microorganisms to be used in petroleum industries for the recovery of residual oil, because bacterial enhanced oil recovery does not consume a large amount of energy as in the case of the thermal process of enhancing oil recovery, it's also cost effective and environmentally friendly. The technique involves the use of bacteria and their by-products (biomass, biopolymers, gases, acids, solvents, enzymes and biosurfactants) for oil immobilization in the reservoir (Sen, 2008; Brown, 2010). Recently, Seema (2013) conducted a research on bacterial enhanced oil recovery using potent biosurfactant- produced by *Pseudomonans sp* . According to a survey of previous bacterial enhanced oil recovery in China, there are more than 4600 bacterially-enhanced oil recovery wells and bacterial wax removal of more than 3000 oil wells, which accounted for 65% (Haicheng *et al.*, 2019). Bacterial enhanced oil recovery can be achieved through inoculation of bacteria in the reservoir , with the view that bacteria and their by-products cause some

beneficial effects including; extending the life of depleted or abandoned oil reservoir. Investigation showed that 30% of oil present in reservoir can be recovered using this technique (Singer and Finnerty, 1984). Bacterial specific abilities for enhancing oil recovery were recently reviewed (Lazar, 1991, 1996, 1997, 1998), anaerobic and facultative anaerobic bacteria can be considered as potential microorganism for microbial enhanced oil recovery of residual oil due to their ability to produce large quantities of acids, gases, solvents, polymers, surfactants and cell biomass (Lazar *et al.*, 2007). Similarly, the roles and application of bacteria in petroleum industries were reviewed by Behesht *et al.*, (2008). The rationale behind the use of bacteria in microbial enhanced oil recovery was due to the possession of several features that are of practical importance.

Mechanisms of bacterial enhanced oil recovery

The mechanisms of bacterial enhanced oil recovery were previously reviewed by Jenneman *et al.* (1984), Bryant *et al.* (1989), Chrisholm *et al.* (1990), Sarker *et al.* (1994), Desouky *et al.* (1996), Delshad *et al.* (2002), Feng *et al.* (2002), Gray *et al.* (2008) and Nielsen *et al.* (2010). There are some important factors which must be taken into consideration before the commencement of bacterial enhance oil recovery for a given oil well, these include temperature, pressure, PH, porosity, salinity, geological make-up of the oil reservoir, availability of nutrients and presence of indigenous bacterial flora (Nielsen *et al.*, 2010). Our study identified four major mechanisms of operations by which bacteria can enhance recovery of residual oil from depleted or abandoned oil wells these includes; reduction of oil-water interfacial tension by using bacterial-biosurfactants (Banat *et al.*, 2010), selective plugging of porous media by using bacteria and their metabolites (Cameotra and Makkar, 2004), oil viscosity reduction by using gases or solvent which are produced by anaerobic bacteria such as clostridia (Youssef *et al.*, 2007; Youssef *et al.*, 2013) and Acid production by bacteria which dissolved rock to improve porous media permeability (Bryant and Douglas, 1988; Bodoloi and Konwar 2008). Similarly, according to Haifcheng *et al.*, (2019), mechanisms of bacterial enhanced oil recovery in China can be categorised in to four (4): bacterial flooding recovery, cycle bacterial recovery, bacterial selective plugging recovery and others, however, according to a worldwide implemented field trials survey, bacterial flooding recovery was rank first in the world and it was described as the best among all other bacterial enhanced recoveries (Tanner *et al.*, 1991; Desai and Banat 1997; Banat *et al.*, 2000; Cameotra & Makkar, 2004).

Advantages of bacterial enhanced oil recovery

Bacterial enhanced oil recovery offers a greater advantage over the other enhanced oil recovery, the properties of being safe and environment-friendly where it does not cause or imposed any negative impact on the environment after the operations. The advantages of bacterial enhanced oil recovery were reviewed by Lazar *et al.* (2007) and Rashedi *et al.* (2012). Bacterial enhanced oil recovery causes the formation of stable oil-water emulsions and mobilization of residual oil. Moreover, they are economically cheap, easy to apply and handle in the fields (Rashedi *et al.*, 2012), it also requires little input of energy which is in contrast to other methods of enhanced recovery such as a thermal process that requires a high input of energy. Another important advantage is that its cost is not dependent on the global crude oil price, since it involves use of bacteria or their by-products. Products of bacterial enhanced oil recovery are all biodegradable and will not be accumulated in the environment after the process (Lazar *et al.*, 2007; Sen 2008; Xu and Lu, 2011).

Bacteria in petroleum discovery and formation

The origin of petroleum hydrocarbon has been described extensively (Brooks, 1936; Skeleton & Skelton, 1942). According to geologist, crude oil was formed from the remains of plants

and animals in a marine environment at a pressure of 5,000 psi and relatively low temperature (Cox, 1946). Bacteria have contributed to the process of the formation of petroleum hydrocarbon, because its believed that the organic remains of plants and animals are susceptible to bacterial modification. Zobell (1952) have recently reviewed on the part played by bacteria in petroleum formation and similarly reported that marine sediments bacteria usually converted organic compounds composition of crude oil. It has been shown by several laboratory analyses that marine sediments rich in organic matter contain large numbers of living indigenous bacteria (Zobell and Anderson 1936; Zobell 1938; Rittenberg, 1940). Multiplication and metabolism of bacteria in sediments occurs under conducive environmental conditions of temperature, PH, lack of toxics, it has been shown that bacteria grow in oil-well and caused a lot of changes (Ginter,1934;Zobell,1950). Similarly, Zobell (1952) has reported that various anaerobic bacteria can convert marine humus into a substance that can be converted to petroleum through decarboxylation of organic compounds, deamination reaction and transmethyllation. Methane, a constituent of natural gas and crude oil is produced by anaerobic fermentation of organic matter by bacteria, similarly methane can also be produced by the reduction of carbon dioxide or by molecular hydrogen as source energy (Barker 1936; Zobell 1947a; Zobell 1952). It has also been reported that many bacterial species produced carotene, flavorhod in and other hydrocarbon, however, there is no enough evidence for the bacterial formation of propane, butane and ethylene (Rawn *et al.*, 1939). Furthermore, Updegraff (1948) reported bacterial degradation of proteins which lead to the formation of phenol, cresol and other aromatic compounds found in petroleum. Similarly, anaerobic degradation of fats by bacteria under natural condition resulted in the formation of liquid hydrocarbon (Zobell, 1952). Anaerobic bacteria played another important role in marine sediments which include catalyzing the oxidation of hydrogen. The conversion of paraffinic hydrocarbon higher than C₁₀ into naphthenic hydrocarbon and preferential attract of sulfur compounds has been reported by sulfate reducing bacteria, (Maliyantz, 1935).Mechanisms of formation and recovery of hydrocarbon from oil-bearing materials were described by Zobell (1952),which shows that growth and metabolic activities of bacteria in marine sediments may contribute to the liberation, formation and recovery of oil from oil bearing materials (Zobell 1947b; Zobell 1950), these mechanisms include: bacterial decomposition of the organic matrix of plants bodies bearing oil, bacterial dissolution of carbonate or sulfate rocks, which may result in the release of adherence oil and increase oil channels, bacterial production of carbon dioxide which resulted in the decrease in the viscosity of the oil, thus favors the flow of oil more readily and the entrapped oil may be expelled, bacterial growth on a solid surface and subsequent production of detergents and fatty acids which bring about water emulsion of oil that caused inherent oil to be removed from surface of sedimentary surface.

Bacterial-biosurfactants

Biosurfactants mean biological surface active compounds produced by bacteria. Biosurfactants have been described as high value products of bacterial-hydrocarbon metabolism which posses certain characteristic such as low toxicity, ease of application, high, biodegradability, tolerance of temperature and salinity, these characteristics were lacking in chemically-synthesized surface active compound (Banat *et al.*, 2000; Cameotra & Makkar, 2004).The role of bacteria in biosurfactants production and potential application in petroleum microbiology were previously reviewed by Banat (1995). Laboratory analysis of *Bacillus subtilis* strains has shown that recovery of residual oil can greatly be enhanced using sand-pack columns couple with oil degrading ability of *Bacillus subtilis* (Gudina *et al.*,2013). Biosurfactants have crucial roles to play in petroleum industries including, critical micelle concentration reduction, surface tension reduction, interfacial tension reduction between petroleum mixture and water. The mechanism of actions of biosurfactants involves the

formation of micro-emulsions followed by micelle accumulation, which can cause hydrocarbon to solubilized in water or water in hydrocarbons (Banat,1995).The diversity, structure and properties of biosurfactants has been extensively reviewed by Cooper (1986), Rosenberg (1986), fietchter (1992a), Georgiou *et al.* (1992), Kosaric (1993) and Banat (1995). The structure of biosurfactants have been identified as a key factor that contribute to its roles and advantages in petroleum industries, biosurfactants consist of hydrophilic moiety with amino acids, anions or cations and carbohydrates. Also, it contains hydrophobic portion which is made up of saturated, unsaturated or hydroxylated fatty acids (Georgiou *et al.*, 1992).According to several recent fields and laboratory experiments, biosurfactants were produced spontaneously as a result of hydrocarbon uptake by petroleum degrading microorganisms, particularly bacteria (Yarbrough & Coty, 1983; Hitzman, 1988; fry *et al.*, 1993, Cooper and Goldenberg,1987; Chang 1987).

Similarly, some water-soluble compound such as glucose, glycerol and sucrose has been described as potential substrate for biosurfactants production (Hommel and Huse 1993; Paseri *et al.*, 1992; Palejwala & Desai, 1989).

Chemically enhanced oil recovery (CEOR) has for long been applied in petroleum industries to enhanced oil recovery from oil reservoir and also to control oil spill pollution, however these chemically-synthesized surfactants are hazardous and toxic to the environment, also, they are costly and not biodegradable (Bordoloi & Konwar,2008; Suthar *et al.*, 2008),these properties make chemical surfactants to be an inefficient alternative to the petroleum industry. In comparison, biosurfactants do not suffer the disadvantage of chemical surfactants, but, they are biodegradable, cost effective, easy to handle and low toxicity. These characteristics make it to be an efficient alternative to the petroleum industries and have replaced the chemical surfactants (Morita *et al.*, 2007; Fox & Bala, 2000;Abalos *et al.*,2001;Sarker *et al.*, 1989).

Application of biosurfactants to the petroleum industry

The application of biosurfactants to the petroleum industries was demonstrated by several researchers (Banat 1991; Banat, 1993; Banat *et al.*, 1995; Banat *et al.*, 2000; Cameotra and Makkar, 2004; Boidoloi & Konwar, 2008; Calvo *et al.*, 2008; Banat *et al.*, 2010; Simpson *et al.*, 2011; Gudina *et al.*, 2013; Seema, 2013; Haicheng *et al.*, 2019). Similarly, it was reported that substantial amounts of oil can be recovered using bacterial-biosurfactants at a certain concentration which can mobilize residual oil (Boidoloi and Konwar, 2008). Biosurfactants possess the ability to reducing the interfacial tension between the hydrocarbon and aqueous phase, thus played significant roles to the petroleum industries including; bacterial enhanced oil recovery, mobilizing heavy crude oil, transporting petroleum in pipelines, managing oil spills, oil pollution control, cleaning oil sludge from oil storage facilities and bioremediation of hydrocarbon concentrated soil or water (Simpson *et al.*, 2011).

Biosurfactants-producing bacteria

Various biosurfactants which are produced by bacteria has been reviewed recently (Banat, 1995;Georgiou *et al.*, 1992; Passeri *et al.*, 1992; Hommel & Huse 1993).The distribution of biosourfactants producing bacteria has been studied by Perfumo *et al.*(2010). Biosurfactants producing-bacteria are ubiquitous, inhabiting the sea, fresh water, ground water and lands as well as the extreme environment (oil reservoirs).Biosurfactants producing-bacteria can also thrive at wide range of PH, temperature salinity (Perfumo *et al.*,2010). Similarly, biosurfactants-producing bacteria have been reported for their physiological roles within the environment e.g antimicrobial activity, biofilm formation and process of motility and colonization of surfaces, in addition to the mobilization of residual oil and bioremediation of

hydrocarbon, this concept lead to the establishment of interaction and relationship between the oil degrading bacterial communities and their environment (Perfumo *et al.*, 2010). Several fields and laboratory experiments showed that bacteria are the most dominant among all biosurfactant -producing microorganism (Shulga *et al.*, 1993;Abu-Ruwaida *et al.*, 1991b; Banat et al 1991).List of different species of bacteria and their various biosurfactants is shown in table 1.

Investigations on biosurfactants-producing bacteria revealed the following bacterial genera as the most common producers of biosurfactant: *Pseudomonas*, *Bacillus*, *Sphingomonans* and *Actinobacter* in soil and sediments, while *Pseudoalteromonans*, *Halomonans alcanivorax* and *Acinetobacteria* in the marine ecosystem (Rahman *et al.*, 2002; Bodour *et al.*, 2003).Perfumo *et al.*(2010) have reviewed the production and role of biosurfactants, similarly, biosurfactant production has been reported by different species of bacteria: Rhamnolipid biosurfactant from *Pseudomonans aeruginosa*, surface active lipids from *Rhodococcus*, Glycolipids from *Alcanivorax* in the marine environment and Lipopeptide biosurfactant from *Fluorescent pseudomonans* and *Bacilli*. Similarly, *Bacillus subtilis* strain has been used to produced surfactin-biosurfactants using a liquid medium containing hydrocarbon under aerobic and anaerobic conditions (Perfumo *et al.*, 2010).

Table1. Various biosurfactants produced by bacteria

<i>Bacteria</i>	<i>Biosurfactants</i>	<i>References</i>
<i>A rthrobacter</i> RAG- 1	hetropolysaccharides	Rosenberg (1986)
<i>A rthrobacter</i> MIS 38	lipopeptide	Ron & Rosenberg (2002)
<i>Arthrobacter</i> sp.	rehalose and sucrose	Hitzman (1983)
<i>Bacillus licheniformis</i> JF-2	lipopeptides	Rosenberg (1986)
<i>Bacillus licheniformis</i> 86	lipopeptides	Banat (1993)
<i>Bacillus subtilis</i>	surfactin	Eliseev <i>et al.</i> (1991)
<i>Bacillus pumilus</i> A1	surfactin	Banat (1993)
<i>Bacillus</i> sp. AB-2	rhamnolipids	Rashedi <i>et al.</i> (2012)
<i>Bacillus</i> sp. C- 14	hydrocarbon-lipid- protein.	Banat (1993) Eliseevetal. (1991)
<i>Pseudomonas aeruginosa</i>	rhamnolipid	Cameotra & Makkar (2004)
<i>Pseudomonas fluorescens</i>	lipopeptide	Van dyke (1993a)
<i>Rhodococcus erythropolis</i>	trehalosedicorynomycolate.	Shulga <i>et al.</i> (1993)
<i>Rhodococcus</i> sp. ST-5	glycolipid	Abu-Ruwaida <i>et al.</i> (1991b)
<i>Rhodococcus</i> sp. H13-A	glycolipid	Singer & Finnerty (1984)
<i>Rhodococcus</i> sp. 33	polysaccharide	Banat (1995)

Application of biosurfactants in hydrocarbon pollution control

Environmental pollution due to petroleum and its products have increased to a critical level and caused a serious catastrophe in the environment (Burger 1993;Bums *et al.*, 1993).The capability of biosurfactants in the emulsification of hydrocarbon-water mixture has been reported by several types researches and reviews (Harvey *et al.*, 1990;Francy *et al.*, 1991;Sutton 1992; Drouin and Cooper, 1992; Zhang and Miller, 1992). We summarized and tabulated previous findings for the roles of biosurfactants in hydrocarbon pollution control, and it was presented in table 2..Furthermore, biosurfactants have been described as a potential tools for oil spill pollution control because of their hydrocarbon degradation abilities (Banat, 1995).

Table 2. Previous findings for the roles of biosurfactants in hydrocarbon pollution control.

<i>Experiment</i>	<i>Findings</i>	<i>Reference</i>
Biosurfactant produced by <i>P.aeruginosa isolate S8</i> were mixed with hydrocarbon Contaminated Soil.	Showed its ability to degrade hexadecane, heptadecane, octadecane and nona-decane in sea water by up to 47, 58, 73and 60% respectively.	Shafeeq <i>et al.</i> , 1989
Biosurfactant produced by <i>P. aeruginosa UG2</i> were mixed with petroleum contaminated soil.	showed degrading of all hydrocarbons except 2-methylnaphthalene.	Jain <i>et al.</i> , 1992
Biosurfactants produced by <i>Acinetobacterhaemolyticus</i> were mixed with hydrocarbon contaminated soil.	showed 39-71% reduction in hydrocarbon.	Vrdoljak <i>et al.</i> , 1992
Biosurfactant produced by <i>Pseudomonans ML2</i> (108 cell/mol) were mixed with hydrocarbon contaminated soil.	showed11-72% reduction in hydrocarbon.	Vrdojak <i>et al.</i> , 1992
Biosurfactant produced by <i>RhodococcusST-5</i> were mixed with hydrocarbon contaminated sand in a culture media.	showed significant ability to remove residual oil from sand packed columns by 95%	Aburuwaida <i>et al.</i> , 1991a
Biosurfactant produced by <i>Thermophilic bacillus AB-2</i> were mixed with sand packed, which was saturated with hydrocarbon.	showed significant ability to release 95% residual oil from sand packed column.	Banat 1993
Biosurfactant produced by <i>Bacillus C-14</i> were mixed with Hydrocarbon contaminated sand..	showed significant reduction of oil at concentration of 0.04mg/ml.	Eliseev <i>et al.</i> , 1991;Banat 1993
Biosurfactant produced by <i>Acinetobacter calcoeticus RAG -1</i> were mixed with hydrocarbon contaminated soil in centrifuge tubes containing culture broth.	demonstrated best solubilization of hydrocarbon at 41.9%	Vandyke <i>et al.</i> ,1993b
Biosurfactant produced by <i>Pseudomonansaeruginosa UG2</i> were mixed with hydrocarbon containing culture broth in a centrifuge tubes.	demonstrated best solubilization of hydrocarbon at 48%.	Vandyke <i>et al.</i> ,1993a
Rhamnolipid-biosurfactant were mixed with sandy-loamy soil containing hydrocarbon mixture.	rhamnolipid at a Conc. of 5g/L were found to increase recovery of hydrocarbon by 25-75% in silt-loamy soil and 40-80%. in sandy-loamy soil.	Vandyke <i>et al.</i> ,1993b
0.08% mixture of rhamnolipid biosurfactant were mixed with sandy-loamy soil, aliphatic, aromatic and hydrocarbons.	showed reduction of aliphatic and aromatic hydrocarbon by 36%. and 40% respectively.	Scheibenbogen <i>et al.</i> ,1994

Roles of bioemulsifiers

Bioemulsifiers are also by-products of bacterial metabolism of hydrocarbon, however, they are high molecular mass biopolymers of polysaccharide, proteins, lipopolysaccharides, lipoproteins, or even a mixture of these compounds. Bioemulsifiers are complex biosurfactants which are amphiphatic in nature. Furthermore, bioemulsifiers can bind tightly with hydrocarbons and oil to form a barrier that prevents drop coalescence; thus show the ability to stabilize emulsions (Ron and Rosenberg, 2002), this is the reason why they are called bio-emulsifiers. Like biosurfactants, bioemulsifiers are also produced by different species of microorganisms, particularly bacteria. Many different types of bioemulsifiers have been reported so far, but the first bioemulsifier reported was *Acinetobacter* RAG-1 emulsan (Rosenberg and Ron 1995). Another bioemulsifier called Alasan, produced by *Acinetobacter radioresistens* KA53, its formed by anionic alanine-containing polysaccharides and proteins (Navon-venezia *et al.*, 1995). Furthermore, Perfumo *et al.* (2010) have extensively reviewed the roles of bioemulsifiers in accessing hydrophobic substrates. One of the most important role of bioemulsifiers was reported to be the bioremediation of hydrocarbon, and the first two bacteria reported for bioemulsifiers-production and application in the bioremediation process are *Pseudomonans aeruginosa* and *Corynebacterium* (Macdonald *et al.*, 1981). Similarly, bioemulsifier has been described as bio-stimulating agent in bioremediation process by several analysis meanwhile, the application of bioemulsifier in soil bioremediation has been recently reviewed (Calvo *et al.*, 2008). Several investigation in the laboratory and also in the field have described the biodegradation of hydrocarbon using bioemulsifier (Banat 1995; Barkaye *et al.*, 1999, Kosaric, 1993; Choi *et al.*, 1996).

Bacteria in bioremediation of petroleum hydrocarbons

Interest in the bacterial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments, which may be land or water. (Mangino, 1975). Bioremediation and biotransformation by bacteria have now become a recipe for boosting the economy through the degradation of pollutants and transformation of huge range of compounds into useful chemicals. Biodegradation of hydrocarbons is a cost effective technique that involves the use of microorganisms to clean and detoxify soil or water contaminated with hydrocarbon. Biodegradation of petroleum hydrocarbons can be achieved using different microorganisms, however, bacteria were reported as the most active in the biodegradation of PAHS (Polycyclic aromatic hydrocarbon) as the only carbon source (Abdel-shafy *et al.*, 1988). Similarly, several laboratory analyses have shown that bacteria are capable of converting petroleum hydrocarbon in to the required carbon and energy (Chaudhary, 2016). Abdel-shafy and Mansour (2018) have recently reviewed on the microbial degradation of hydrocarbons in the environment, this study has identified bacteria as the most potential microorganism for biodegradation. Human activities in industrialized countries have been the causative agents of environmental pollution due to hydrocarbon contaminants. Petroleum is a very intricate mixture containing several hydrocarbons, which includes, the combination of cyclic, linear, and branched alkenes, aromatic hydrocarbons and asphaltenes as well as resins. The polycyclic aromatic hydrocarbon (PAH) compounds are mostly stable, toxic and carcinogenic (Abdel-shafy and Mansour 2016).

Chemical and mechanical techniques being employed to eliminate petroleum contaminants in soil or water have now become cost ineffective, apart from their side effects on the environments, in contrast, bioremediation is the most appropriate techniques which can completely remove pollutants without causing any harm to the environment, they are efficient and also inexpensive (Das and Chandran 2011). The selection of bacteria for petroleum bioremediation depends on types of the hydrocarbon presents in the

environments, similarly, the ratio of the biodegradation of petroleum hydrocarbon is directly proportional to the degradation capabilities of the bacteria and equally the chemical nature of the pollutants (Chickere *et al.*, 2011). Bioremediation of hydrocarbon soil contaminated depends on the availability of water for bacterial growth and metabolism, decrease in water content, resulting in a decrease in microbial activity, thus decrease in bioemulsifiers production (Ayotamuno *et al.*, 2006).

Bioremediation of petroleum hydrocarbon suffer some challenges and difficulties due to environmental factors such as dissolved oxygen, temperature difference and dispersion of the petroleum, however, the most important among these factors is the temperature, because elimination of hydrocarbon has been reported with high value under optimum temperature, thus increase in temperature can lead to increase in the biodegradation of petroleum hydrocarbon. Another predominant factors which determined the value of the petroleum hydrocarbon biodegradation in both aquatic and terrestrial ecosystem is the presence of nutrients particularly phosphorus and nitrogen (Olajire and Essien, 2014). Bioremediation can be achieved using two main approaches these include :bio-stimulation and bio-augmentation.

Bio-stimulations

Bio-stimulations involve the addition of specific nutrients to waste or pollutants with the expectation that naturally indigenous bacteria were present in adequate numbers and type to metabolize and break down the waste effectively. This approach suffers disadvantage in that the indigenous bacteria may not be suitable and appropriate for the degradation of the hydrocarbon contaminants (Olajire and Essien, 2014; Foster, 1962).

Bio-augmentation

This approach which is the alternative to bio-stimulation, it's programmed biodegradation, which involves the addition of specific desired bacteria to a tangible petroleum hydrocarbon contaminants, it's achieved by designing and monitoring of an ideal environment for the bacteria. Calvo *et al.*, (2008) have reviewed the application of bioemulsifiers in the soil bioremediation process. Similarly, Abdel-shafy and mansour (2016) have reported the bacterial degradation PAHS (Polycyclic aromatic hydrocarbons), including naphthalene, phenanthrene, acenaphthene and anthracene. Furthermore, the degradation of naphthalene by *Pseudomonans specie S3 and F3* under optimum temperature and PH of 37c⁰ and 7 respectively were reported (Amenu, 2014).

Some of the bacterial species involved in biodegradation of petroleum hydrocarbon are *Bacillus*, *Pseudomonans*, *Acinetobacter*, *Calcoaceticus*, *Micrococcus*, *Nocardia*, *Erythropolis*, *Antarctica*, *Ochrobactrum*, *Serratia*, *Marcescens*, *Acenebacter*, *Alcaligenes*, *Odorans*, and *arthrobacter* (Bamforth and singleton, 2005).

Other bacteria reported for the biodegradation of the mono aromatic hydrocarbons include species of *Pseudomonans*, *Brevibacillus*, *Bacillus*, *Corynebacterium*, *Vibrio*, *Ochromobactrum* and *Achromobacter*. Similarly some bacterial families including *Vibrionaceae*, *Pseudomonanceae*, *Moraxella* and *Enterobacterianceae* has been described as a good candidate for degradation of petroleum hydrocarbon (Bamforth and Singleton 2005; Abdel-shafy and mansour, 2018). Furthermore, laboratory investigation of bacterial bioremediation of petroleum hydrocarbon revealed some bacterial genera including *Mycobacterium* and *Pseudomonans* which can be able to degrade and transform polycyclic aromatic hydrocarbon under the presence of dissolved oxygen (Mrozik *et al.*, 2003). Meanwhile, anthracene has been reported to be degraded by *Norcardia*, *Sphingomonans*, *Beijerinkia*, *Rhodococcus* and *dihydrodiolparacoccus* (Teng *et al.*, 2010). Cottin and Merlin (2007) have also reported some bacteria which produced

biosurfactants that can enhance biodegradation of PAHS (Polycyclic aromatic hydrocarbons) by making it to dissolve in the aquatic environment, these bacteria include: *Torulopsis bombicola*, *Pseudomonans aeruginosa* and *Bacillus subtilis*(Cottin and Marlin, 2007; Teng *et al.*, 2010).

CONCLUSION

The roles and applications of bacteria have been reviewed, in general, bacteria were described as the most important microorganisms that significantly contributes to the maintenance and development of oil and gas industries. Biosurfactants and bioemulsifiers production by different bacterial species was described as a potential reason why bacteria became a good candidates for microbial enhanced oil recovery.

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