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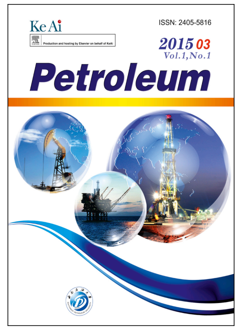

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Abstract

The energy resources mainly petroleum and petroleum hydrocarbons are major pollutants of the environment. The oil and oil products contamination may cause severe harm and hence, the attention has been remunerated in the development of alternative technologies for elimination of these contaminants. Biosurfactants were used in the remediation of oil pollution due to advantages such as biodegradability and low toxicity. The biosurfactants are produced from low cost substrates like agro-industrial wastes which reduce the cost of production. Biosurfactants and bioemulsifiers are amphiphilic compounds and are produced as extracellular or a part of the cell membrane by bacteria. The insight view, how hydrocarbons are degraded by microorganisms and thereby reduce the damage of ecosystem is highly essential to target the problem. Biofilms are the bacterial communities which protects the bacterial cells from various adverse conditions. The present review describes the biosurfactants and its synthesis from bacteria and also emphases on the role of surfactants in oil remediation.

Key words: Biosurfactant, Bioremediation, Microbial enhanced oil recovery (MEOR), Biofilm

1. Introduction

1.1 Hydrocarbon contamination sites

Rapid growth of industries leads to the environmental pollution and other environmental hazards. One of the prevalent ecological hazards is petroleum pollution, which show harmful effects on all aquatic living organism's particularly microbial population. The first step in this effect is hydrocarbon transportation to the surface of the microbial cell from oil phase to cell surface through the contact and then transportation across the cell membrane. Even though a great amount of work was done in this area, n-alkane transportation into the bacterial cell and assimilation mechanism of the hydrocarbons in the microbial cells were poorly understood [1]. It has already been reported that some bacterial populations exhibited resistance to oil transportation and also few bacterial population efficiently degrade oils/hydrocarbons. Two different types of interactions normally observed in the processes of oils/hydrocarbon biodegradation. Oil adhesion, pseudo-solubilization and degradation of hydrocarbons to form small droplets of oils are the sequential steps involved in one of the mechanisms. Microbial cells adhere to the drops of hydrocarbons whose size was less than the cells and the substrate uptake has taken place by active transport or by diffusion at the point of interference between cells and hydrocarbons [2]. Bioemulsifiers that reduce the surface tension are termed as biosurfactants. Biosurfactants may be located inside the cells (intracellular) or secreted outside the cells (extracellular) [3]. There are many reports available on bacterial biosurfactants, but the spectrum of activity depends on their chemical composition. A strain of *Pseudomonas aeruginosa* was reported to produce the rhamnolipid type biosurfactant which was mono as well as di-rhamnolipid [4]. It has been proved that the rhamnolipids and its producing microorganisms specifically degraded hexadecane, hence there is a clean correlation exists between the type of surfactant and the type of hydrocarbon/oil that gets degraded. It has been noted that several studies were done on phenanthrene degradation by various chemical surfactants. It was also indicated that the increased phenanthrene degradation when it was associated with bacterial isolate that produced a non-ionic surfactant (Itrich, et al., 2015). In another instance, oil degradation capacity of a chemical surfactant ‘FinasolOSR-5’ was multiplied when supplemented with a biosurfactant trehalose-5, 5’- dicorynomycolatesand reported to be the complete removal of aromatic hydrocarbons from the contaminated soil within a given period [5]. In another study, polycyclic aromatic hydrocarbons (PAHs) were significantly degraded by a group of bacteria that produced glycolipids and sophorose lipids. Surface active glycolipids when added to the hydrocarbon sites have increased the biodegradation of 2,4-DCPIP. In the presence of glycolipids, most of the PAH's are almost removed completely in less than a month in soil contaminated sites [6].

Bacteria produce biosurfactants in the form of biofilm which interacts with an interface and alters the surface properties such as wettability and other properties. A marine bacterium isolated from sea water polluted with oil, *Pseudomonas aeruginosa*, has shown the ability to break hexadecane, octadecane, heptadecane as well as nonadecane after 28 days of incubation. The degradation ability of this bacterium has been proved due to the production of a biosurfactant. It was also proved that *Pseudomonas aeruginosa* has effectively degraded a range of hydrocarbons like 2-methylnaphthalene, tetradecane and pristine [7]. In another experiment, the hydrocarbon contaminated soil was inoculated with *Acinetobacter haemolyticus* and *Pseudomonas* ML2 (biosurfactant producing strains) and the degradation of hydrocarbons were studied, after the completion of the 2 months period of incubation, a tremendous reduction of hydrocarbons (39-71%) and (11-71%), was achieved by *Acinetobacter haemolyticus* and *Pseudomonas* ML2 respectively. These results suggested that cell free biosurfactant produced by bacteria had the remarkable hydrocarbon degradation ability. The rhamnolipid content of *Pseudomonas aeruginosa* was extensively characterized for its hydrocarbon degradation ability. The growth of economy of any country increases along with the demand for oil which should be met by all the new discoveries and technologies. The major pollutants from the oil production companies lead to the deposition of oil sludge which gets strongly bound to the effluents during conditioning and treatment by the treatment process. As the sludge deposition increases, the hydrocarbons penetrate through the top layer of soil and then slowly diffuses into subsoil which causes high risk of contamination to the ground water. Hence, the oil sludge needs to be treated to prevent the environmental toxicity. Even if the sludge is burned, it would cause undesirable air pollution [8]. There are two major factors in the formation of the oil sludge. The first factor is residual inorganic substances which has scales, sand and dust while the second major factor is the precipitation of paraffin wax, as the paraffin wax was in less soluble form. Oxidation of organic heavy material present in the crude oil leads to various climatic changes. These changes cause changes in material balance of various components resin, polymeric compounds and asphaltenes of the oil sludge. There are many technologies being used for the cleaning up of the contaminated sites include thermal evaporation, excavation and soil vapour extraction. Bioremediation is the most important method which has been accepted treatment by using indigenous microbial flora. Certain biosurfactant producing bacteria can metabolize several classes of hydrocarbons. Technologies have already been developed and used in middle east and Canada for bioremediation of hydrocarbon contaminated soil by using biosurfactant producing bacteria Most of the hydrocarbon contaminated soil sites in middle east and Canada were added with biosurfactant producing microorganisms for the bioremediation since glycolipid rich biosurfactants act as the nutrients to the soil. Microorganisms oxidize the organic hydrocarbon compounds by dissolving or emulsifying them while the major limiting factor of the biodegradation of the oil is its solubility rate, biosurfactants increase the rate of biodegradation of the organic compounds by increasing their solubility by emulsification. Most of the crude oil-degrading bacteria release extracellular biosurfactants to facilitate microbial oil uptake and facilitate degradation by emulsifying the hydrocarbon. Biosurfactants can increase the pseudo-solubility due to their specificity and degradability. Biosurfactants were in different complex nature namely rhamnolipids, trehalolipids, sophorolipids, peptide-lipid complexes and carbohydrate-peptide-lipid complexes. Biosurfactants play a role in bioremediation by increasing the surface area of substrates. Biosurfactant producing microorganisms create their own micro-environment and promotes emulsification by the release of certain compounds through various mechanisms such as quorum sensing. Compounds exhibit hydrophobicity show poor water solubility and prolonged environmental persistence. Alasan, a known bioemulsifier has increased the solubility of polyaromatic compounds (PAHs) by many folds. *Alcanivorax borkumensis* and *A. calcoaceticus* RAG-1 were well known standard bioemulsifiers. Surface active biomolecules could replace chemical analogues offer various advantages in various ecological aspects. The activity and application attributed to the use of biosurfactant in oil industry has been presented by many researchers [9]. Biological processing was being considered as a suitable constituent due to its less severity and more selectivity to specific reactions [10]. The low water solubility nature of hydrocarbon compounds limited the capability of microorganisms to emulsify. The microorganisms that degrade the hydrocarbons normally produce a variety of extracellular biosurfactants and were observed when mixed with chemical surfactants, increased the efficiency of the hydrocarbon removal from solid or soil surfaces, but the inhibition and enhancement of the hydrocarbon degradation was observed [11]. Many biosurfactants with low molecular weight such as lipopeptides and glycolipids are lot effective in decreasing the surface tension. Biosurfactants emulsify the compounds, increase the water solubility and make the compounds more accessible for the microorganisms. In the past few years, a lot of research being focused on the study of biosurfactants for their spreading, emulsifying, wetting and foaming properties, but recently biosurfactants have been extensively studied for their applications in oil and food industries. Soil and groundwater contamination by organic hydrocarbons, which are the reasons for majority of environmental problems worldwide, affects the health of living organisms and the quality of the environment they are surviving in [12]. The constant sources are organic hydrocarbons, such as hydrocarbons of petroleum products, solvents and poly aromatic hydrocarbons; these sources are usually persistent in the list of soil contaminants [13]. Industrial activities fall under the category of contamination sources as the emissions of various levels of hydrocarbons. Transportation and refining of petroleum are considered as major contributors to environmental contamination. However, organic hydrocarbons could be released accidentally or deliberately [14]. The physical nature of the contaminant is determined based on whether it is in a solid or a liquid state. Organic contaminants of liquids have low solubility and remain in different phase and are called non-aqueous phase liquids (NAPLs). Differences exist between liquids that are lighter than water and those that are heavier than water. This condition implies that liquids are heavier than water. Lighter liquids will float in water and spread on the water bodies. One of the examples of light non-aqueous phase liquid (LNAPL) is diesel that contains a homogenous mixture of complex compounds that are aromatic in nature. Normally branched cyclic alkenes are extracted from distillation by the fraction of the gasoline during the petroleum separation process [15]. A frequently reported hydrocarbon pollutant is diesel oil, which when leaked from pipelines or storage tanks cause accidental spills. Diesel oil is the common pollutant of groundwater, which is a result of underground storage tank and pipeline leakage [16]. The negative influence on water and soil properties caused due to the contamination of diesel oil, resistance to various types of degradation, toxicity to the living biota, and intrinsic chemical stability [17]. Different technologies such as flushing, bioremediation, chemical treatment as well as incineration are used for the site remediation that contains diesel oil contaminated soil. One of the best approaches is bioremediation among different technologies for cleanup of soil and ground water, which is contaminated [18]. Franzetti et al. (2010) had reported that the most economical tool is bioremediation that could be used for contamination management of the polluted sites. Chen et al. (2011) has categorized bioremediation as eco friendly and effective technology for the sites, which are contaminated mainly with hydrocarbons. It involves in increasing the pace of the process, which is naturally biodegradable [19]. Bioremediation majorly includes the application of phosphorus and nitrogen fertilizer, pH management of the effluent and addition of the bacteria to the contaminated sites, but the availability of hydrocarbons to microorganisms is the important limiting factor [20]. Diesel is hydrophobic and has less water solubility, hence less available to microorganism [21]. The compounds with high hydrophobicity as well as low solubility have the ability to adhere strongly to particles of soil and slowly released into the water phase that could cause time to be the factor for the increase in bioremediation [22].

1.2 Surfactants and biosurfactants

Surfactants are used for bioremediation of the hydrocarbons and make them available for the microorganisms to degrade. Hence, the transfer of the hydrocarbons to the aqueous phase in bulk is an important process for its bioavailabity [23]. Among various methods, surfactants can be seen as the promising method for bioavailability related problems. The use of surfactants could increase the hydrocarbons mobility as well as bioavailability, which promotes the rate of biodegradation [24]. Mulligan (2001) stated that industry of petroleum has been using surfactants majorly, as they can increase the solubility of petrol and its byproducts. The diverse groups of surfactants are divided on structural basis depending on the type of microorganisms that produced them [25]. Biosurfactants produced by microorganisms are the biological active surface molecules with vast applications due to their specific versatile properties, minute toxicity and biological acceptability [26]. They are used as additives for the production of organic chemicals, petro-derivatives ,and petrochemicals. Bioremediation of waste water effluents can be done effectively by using biosurfactant producing microorganisms due to their specificity of utilizing the organic waste and hydrocarbon waste as raw materials. Biosurfactants have higher surface activity with high tolerance to various environmental factors and can withstand from mean to extreme conditions such as acidity or basicity of an aqueous solution, temperature, salt concentration, ionic strength, biodegradable nature, demulsifying-emulsifying ability, anti-inflammatory potential and anti-microbial activity. Microorganisms living in extreme environments such as extremophiles have gained much attention for the last few decades, as they possess different properties by producing certain useful compounds. Surfactants derived from chemicals expose severe environmental problems, hence it demands for screening of biodegradable surfactants from the extreme marine environments for biosorption of polyaromatic cyclic compounds [27]. Biosurfactants are amphiphilic compounds consists 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 [28]. One of the key features of biosurfactant is the hydrophilic - lipophilic balance, which causes the hydrophobic as well as hydrophilic portions to be determined in substances that are surface active. Because of the amphiphilic structure, biosurfactants not only have the ability to increase the hydrophobic substance surface area, but also have the ability to change the property of cell surface of the microorganisms. Surfactants behave as an excellent foaming agents, emulsifiers and dispersing agents attributed to their surface activity [29]. Biosurfactants show selectivity of the substrate to degrade and functionally active at extreme conditions of high temperatures, high salt concentrations as well as pH that can be attributed by the products and generated waste from industries. Different properties of surfactants are dispersion, emulsification or de-emulsification, wetting, foaming as well as coating that makes them effective in bioremediation and physiochemical technologies of metal and organic contaminants [13]. Biosurfactants form different complexes with metals and perform surface removal of heavy metals that may cause the increase of ion concentration of metals and the bioavailability in the soils with heavy-metal pollution [30]. Surfactants have the property of increasing hydrophobic particle surface area like pesticides applied in the soil and water, which in turn increases the solubility [31]. Increase in the microbial production of surfactants and its wide use for the degradation of insecticides and pesticides has gained attention in the past few years [17]. The biosurfactants which are produced by various microorganisms are identified and characterized by Lin, (1996), Desai, (1987) and Parkinson, (1985) [32,33,34]. Hence there are various types of biosurfactants based on the properties such as characterization, antimicrobial activity, production, efficiency of hydrocarbon removal from environment and its ability of reducing the surface tension [35]. Wide range of compounds used by microorganisms as the energy rich source and carbon source for their growth. But, if carbon is insoluble hydrocarbon, microorganisms diffuse various substances as biosurfactants, where as some of the yeast and bacteria diffuse biosurfactants that can emulsify hydrocarbons in the medium [36]. Some examples for this type are different species of *Pseudomonas* producing rhamnolipids and sophorolipids, which is produced by different species of *Torulopsis*. Most of the microorganisms could change the cell wall structure, which was caused by the production of lipopolysaccharides in the cell wall [37]. *Candida lipolytica*produce lipopolysaccharide, which are cell wall-bound when the medium contains n-alkanes. *Rhodococcus erythropolis* along with different *Mycobacterium species* and *Arthrobacter* species produce non-ionic trehalose corynomycolates. *Acinetobacter* species produce emulsan as well as lipoproteins like subtilisin, are produced by *Bacillus subtilis*. *Rhodococcus sp.* synthesises Mycolates, Corynomycolates synthesized by *Pseudomonas rubescens, Thiobacillus ferroxidans* and *Gluconobacter cerinus* synthesizes ornithinlipids.

1.3 Classification of biosurfactants

Biosurfactant classification is mainly based on the origin of the microbes and their chemical composition. Biosurfactants are not classified like the artificial chemical surfactants, which are categorized, based on the polarity of the functional group [38]. Biosurfactants are divided into two types based on the molecular weight, low molecular weight compounds, which lower the interfacial surface tension, polymers of high molecular weight that are most of the efficient stabilizing agents. Glycolipids, lipopeptides and phospholipids constitute the majority of low mass biosurfactants, while particulate and polymeric surfactants come under the large mass biosurfactants [37]. Mostly are anionic biosurfactants and some are neutral, while hydrophobic moiety is based on the derivatives of fatty acid long chains and have the hydrophilic moiety that could be an amino acid, phosphate group, carbohydrate part and a cyclic peptide [39]. Glycolipids have a long-chain of aliphatic acids. They form a connection of either ester group or ether group. Some of the glycolipids are sophrolipds, rhamnolipids and trehalolipids [40]. Rhamnolipids are the glycolipids in which any of the rhamnose sugar moieties linked to the myrmicacin, which is a derivative of *β*-hydroxycarboxylic *acid* hydroxyl group at the reducing end of rhamnose disaccharide, or present as one of the hydroxyl group is occupied by ester formation [41]. Trehalolipids are present in most of the species such as *Corynebacterium sp.,Mycobacterium sp.*, and *Nocardia sp.* Trehalose is a disaccharide sugar,which is linked at 6^{th} position of the carbon backbone to long chain fatty acids of mycolic acid. The structure and size of the mycolic acid vary from organism to organism by different number in the presence of atoms of carbons and its unsaturation rate. Trehalose lipids obtained from *Arthrobacter sp.* and *Rhodococcuserythropolis*decreased the interfacial as well as surface tension in the growth medium [42]. *Torulopsis bombicola* synthesizes three types of glycolipids. *T. Petrophilum*as well as *T. apicola* contains a carbohydrate sophorose that is dimeric through the glycosidic linkage attached to the hydroxyl fatty acid. Generally sophorolipids are heterogenous mixture of macrolactones and a free acidic group. Lactones, ester groups of hydroxycarboxylic acids extracted from sophorolipid molecules are required for various biomedical applications as polymers [43]. Cell walls of wide range of microorganisms have cyclic lipopeptides, which triggers the responses of immune system that include decapeptide-lipopeptide antibiotics. Lipopeptides and lipoproteins contain lipid as the functional group linked to the polypeptide chain. *Bacillus subtilis* synthesizes the cyclic lipopeptide surfactant, which is the most effective biosurfactant. Surfactin is made of seven-ring structure of amino-acid, which is joined to fatty acid chain with the help of a lactone linkage. Surfactin was reported that it has reduced the surface tension below 28 mN/m [44]. Several of the biosurfactants synthesized by *Bacillus licheniformis*have exhibited great stability towards salt, temperature as well as pH.It have similar structure as well as physio-chemical properties that of surfactin. Surfactant of *Bacillus licheniformis*is capable of lowering the surface tension of various liquids [45]. Yeast and bacteria when grown on n-alkane medium synthesize a large number of phospholipid and fatty acid molecules. *Acinetobacter* species produces rich vesicles of phosphatidylethanolamine form microemulsions. *Rhodococcus erythropolis* produce phosphatidylethanolamine when grown on n-alkane decreases the surface tension of water and hexadecane [46]. Liposan and Alasan are some of the most popular polysaccharide–protein complexes. Heteropolysaccharide biosurfactants show extracellular polyanionic activities that are synthesized by most of the *Acinetobacter species.* Emulsan is used to emulsify hydrocarbons present in water, which is considered to be one of the effective emulsifying agent even if the concentration is lesser than 0.01%. Extracellular polymeric emulsifier, liposan is a water-soluble emulsifier synthesized by *C. lipolytica,* which consists more than 80% of carbohydrate and less than 20% of protein part [47].

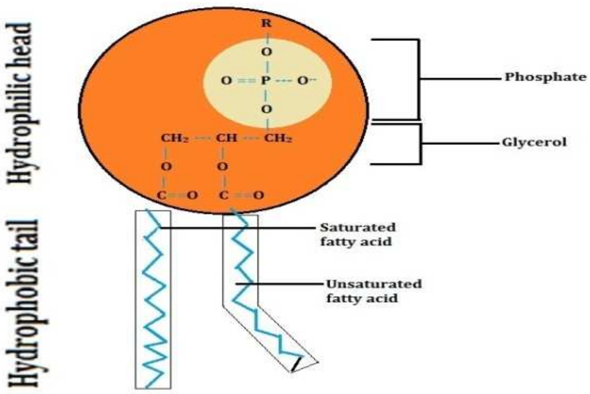

Fig.1 Structure of phospholipids

1.4 Properties of biosurfactants

1.4.1 Surface and interface activity

An effective surfactant or a biosurfactant is the one that lowers the surface tension of water. *Bacillus Subtilis* produces surfactin that lowers surface tension of liquids most effectively even at adverse extreme conditions. *Pseudomonas aeruginosa* produces biosurfactant of rhamnolipid nature that decreases the water surface tension effective than many other surfactants [48]. Sophorolipids produced by *T. bombicola* reduces the surface tension. Biosurfactants are effective as well as efficient, their CMC is from 10 to 40 times lower than chemical surfactants, because of the reason very less amount of biosurfactant is required to reduce the surface tension [49].

1.4.2 Temperature, pH and ionic strength tolerance

Functions and parameters such as temperature and pH of most of the biosurfactants are not altered by the environmental conditions. Research studies suggesting that lichenysin, which is produced by *B. licheniformis* was less affected by pH (4.5–9.0), temperature (up to 50°C) and by NaCl as well as Ca concentrations. At high temperatures beyond autoclavable temperature (121°C) and at low temperatures below minus 15°C, lipopeptides produced by *Bacillus subtilis* found to be stable when stored for 180 days. At NaCl concentrations, greater than 15% and pH range between 4 and 12 ,the activity was found to be stable [25].

1.4.3 Biodegradability

Biosurfactants are regarded as non-toxic agents, as they are one of the best options to use in cosmetic, food and pharmaceutical fields. One of the recent studies suggest that the polyanionic surfactant named emulsan has shown LC_{50} against *Photobacterium phosphoreum*, which is much lesser than *Pseudomonas* rhamnolipids. Commercially, if we compare ten of the biosurfactants based on the toxicity, seven of them were synthetic surfactants, while others are dispersants, most of the biosurfactants are easily degradable in nature [50]. Biosurfactants produced by *Pseudomonas* species are widely in use in industries because of its wide applications and environmental toxic friendly nature compared with artificial surfactants. Many of the laboratory tests were available to assess the toxicity levels of biosurfactant and chemical surfactant. Studies indicated the range of mutagenic and toxicity effects of biosurfactant when compared to that of chemical surfactant were less [17]. Formation and breaking of emulsion could be produced within a month, emulsion may be stabilized or destabilized by the biosurfactants. Emulsifiers are generally a class of biosurfactants with high molecular weight compared with low mass biosurfactants. *T. bombicola* produces sophorolipid surfactant that can lower the surface tension and surface area. Stable emulsions were formed by the use of polymeric biosurfactants and have the additional advantage that they consists of oil coat droplets to form oil/water emulsions for cosmetics and food that are stable. Liposan produced by *C. lipolytica*can emulsify edible oils but does not reduce surface tension effectively. Biosurfactants contain hydrophilic group which may be a sugar, or a protein, where as hydrophobic group usually contains fatty acids or fatty alcohols. Biosurfactants perform several functions as they increase the surface area, thus increase the bioavailability of water-insoluble complexes and finally bound to heavy metals for removal [51]. Biosurfactants have been shown to possess antioxidant, antimicrobial and anti-inflammatory activities [52]. Different complexes involve in versatile biological functions and the common characteristic was to reduce the surface tension of liquids(Table 2.1). Bioactive surfactant molecules were potent to perform several functions include inhibition, fibrin clot formation, antimycoplasmic, antitumorigenic and insecticidal activities. Microorganisms that produce surfactants were used for nanoparticle synthesis, tend to give different applications in the field of biology. Polyphilic polymers contain deoxy sugars and hydrophobic constituents. Bioemulsan is the best ever studied polymer produced by *Acinetobacter*. Microbes use many of the pathways including de-novo pathway. Most of the amphipathic polysaccharides were produced by *Acinetobacter* species. Rhamnolipids which are carbohydrate-lipid derivatives has been produced by *Pseudomonas* sp., and showed good emulsification ability, peptide linked bioemulsifiers produced by *Methyl bacterium* sp., and *Methanobacteriumsp.*, *A.calcoaceticus*has carbohydrate-protein derivative. Lipid-protein derivatives produced by *Bacillus velezensis*and *Streptococcus gordonii*. Lipid-fatty acid derivatives produced by *Myroides*species. Surface active agents show the surface property are made up of biological molecules such as carbohydrates, lipids and proteins in various combinations and compositions. Microorganisms that produce bioemulsifiers have typical physiological behavior which was poorly understood by researchers as they perform definite functional roles in the microbes.

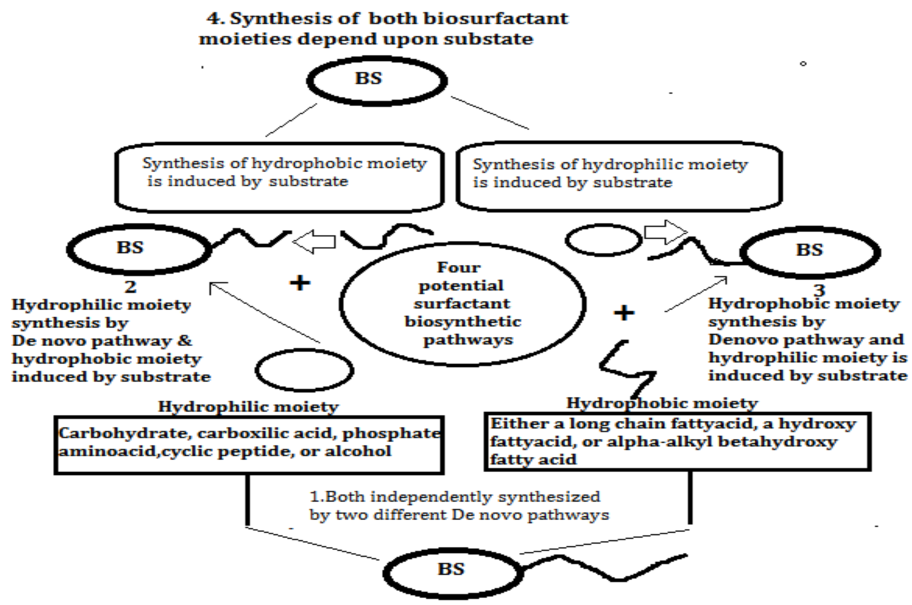

Fig.2 Biosynthetic pathway of biosurfactant in bacteria

1.4.4 Biofilm formation

Biosurfactants made use of wettability property by creating a suitable environment for the attachment of bacterial adhesion. Biofilms are the microbial communities that produces extracellular matrix. Bioemulsifiers were exopolymeric substances that help the bacteria in the biofilm formation, these substances help the cells in survival and protect themselves from adverse extreme conditions, predators and especially from the loss of water from the cell. Bacterial adhesion occurs in mobile and stagnant phases. Biofilm formation is a complex process of surface attached community transition from numerous free-floating cells. Based on the planktonic cells engaged, the biofilms can be of different types. Biofilms formed by single species are highly regulated by signal circuits relating the same species of organisms. Multiple species that generate specific signals are responsible for the formation of surface attached community of various bacterial planktonic cell species. The biofilm development factors such as surface area, smoothness, flow velocity, nutrients influence the biofilm by providing suitable environment for bacterial growth and attachment [53]. The formation of biofilm is a complicated process involves the following crucial steps:

The organic molecules adhere to the material submerged in water and neutralize the surface charge repelling the bacteria. Planktonic bacteria temporarily attach by electrostatic and physical forces. The permanent attachment can be created by producing extracellular polymeric substances (EPS). The EPS cements the cell to the substratum material forming an ion exchange system entrapping nutrients. The adequate nutrients can lead to doubling of the organisms by reproduction. The slimy nature of the biofilm was due to the presence of maximum percentage of EPS and water. The metabolites produced by the primary colonizers were utilized by the secondary colonizers and grow on them to settle, thereby forming a biofilm. Bacteria secreting extra cellular polysaccharides (EPS) by the regulation of respective genes via Quorum Sensing (QS) systems in forming biofilms have beena fabulous asset to microbes [54].QS systems assist the microorganisms in the quorum to survive against antimicrobial compounds, and also to avail nutrients in a nutrient limited condition. The well studied multiple species type of biofilms are the dental plaques/ biofilms. The oral bacteria interact competitively and cooperatively to exhibit the most sophisticated communication of metabolites released by them. As a consequence, biofilms that contaminate medical devices, manufacturing surfaces and fluid systems were extremely difficult to eliminate. Several mechanisms of biofilm resistance have been described and those are believed to work in synergy to bring about reduced susceptibility in biofilms [55]. The existence of cells in a biofilm allows a community response, whichwould be greater when compared to that from a single cell (planktonic). In addition, the three dimensional structures provide protection of the persisted cells, which, when these are disrupted, might result in the cells becoming susceptible to the antimicrobial agents. More recently high magnetic field and ultrasound have been reported to be useful in eradicating biofilms [56]. The use of chemical biocides (disinfectants, sanitizers and detergents) was also common in the control of biofilms [55]. These are divided into two main groups: oxidizing and non-oxidising agents. The commonly used oxidizing agents include chlorine, ozone, iodine and hydrogen peroxide. These agents can act by depolymerising the EPS matrix, thereby disrupting the biofilm integrity. Non-oxidising agents include quaternary ammonium compounds (QACs), formaldehyde, anionic and non-ionic surface-active agents were widely used [57].

1.5 Oil remediation and microbial enhanced oil recovery (meor)

Oil spills cause devastating effect on aquatic life on marine environment. Chemically synthesized surfactants had been reported for their toxicity on aquatic organisms, so were, treated them unsuitable for remediation. One of the inherent alternatives for this purpose wasto find the biomolecules which had surface activity as well as the emulsifying activity along with the low Critical Micelle Concentration (CMC) characteristics. The biosurfactants emulsify the hydrocarbons in water to form various mixtures and make them water soluble. Lichenysins, rhamnolipids and surfactin are the few surfactants which are found to be successful in the remediation of the oil contamination. Kim et al, (1997) isolated a bacterium from a crude oil sample which produced a biosurfactant that had good emulsifying properties on crude oil and paraffin. Literature suggested that biosurfactants produced from marine bacterium were capable enough to destroy the oil slicks which float on the surface of water in order to promote the dispersion of oil in water by forming a stable emulsion thereby enhancing the rate of biodegradation. Due to these factors, biosurfactants had shown potential in its applications of cleaning up the oil spills on shorelines and in the sea. The ubiquitous presence of the marine bacteria which degrade hydrocarbons have been recognized as hydrocarbonoclastic bacteria. These bacteria degrade the hydrocarbons present in the polluted sites of marine environments. Different studies revealed that the mixture of the biosurfactants stimulated the degradation of hydrocarbons in the marine environment. Hydrocarbonoclasticity bacterial consortium has a wide range of degradation capabilities on both aliphatic as well as aromatic fractions of crude oil. In general, biosurfactants produced by oil degrading bacteria can enhance the assimilation of the hydrocarbons as well as the nutrients available in the environment. Some groups of microorganisms synthesize emulsifying agents that could help in hydrocarbon degradation, hence emulsifiers have been used for cleaning up the oil [58]. Biosurfactants can be largely produced in the industrial scale by fermentation process; Lichenysins were produced from *B.licheniformis* JF-2 which was isolated from the well water, Lichenysin even at lower concentrations (10-60 mg/l) was able to reduce the surface tension between the interfacial surfaces into ultra lesser values (10^{-2}mN/m). The range of temperature (≤140°C), pH (6 -10), and salinity (up to 10% w/v NaCl) variation had no effect on its activity. Biosurfactant adsorbs the oil by altering the wettability capacity of the porous media. The emulsion produced by *Acinetobacter venetianus* ATCC 31012 at 0.1 mg/ml removes 89% of crude oil which had been reabsorbed to the samples of limestone and 98% of removal was achieved are used at 0.5 mg/ml concentration [59]. Majority of the studies had focused on the possibility of introducing the bacteria which produce biosurfactants in to the infected sites, so that they can utilize the nutrients present in the oil well for their growth, but it was more suitable for the strategy of microbially enhanced oil recovery where the bacteria would metabolically active even at extreme conditions in the petroleum reservoirs. Many bacterial species that produce biosurfactants had been described for the microbially enhanced oil recovery in-situ applications that belong to *Bacillus*sps. because of their thermal and halotolerance ability. A typical *Bacillus* strain was grown and produced lichenysin by both anaerobic and aerobic processes at relatively high temperatures ranging from 40-60°C [60]. Different processes can be approached to exploit the biosurfactant producing strains in oil recovery applications. A biosurfactant composed of rhamnolipid had the CMC of 70 mg/l, was stable even at 90°C and had shown good emulsifying activity at the low pH of 2.0, but it was slightly affected by the calcium ions and salinity. Cloning of the biosynthetic genes had been attempted to overcome the limitation possibilities of the microorganism. Biosurfactant synthesis under strict anaerobic conditions was desirable and essential characteristic for aerobic microbes in a microbially enhanced oil recovery procedure. *Anaerophaga thermohalophila* (DSM 12881T), a well known anaerobic bacterium which was able to grow at high temperatures like 50°C and high salinity such as 7.5%, produced the low molecular weight peptide which was a surface active compound. There were several factors that affect microbial degradation of crude oil such as nature, ratio of the structural classes of the hydrocarbons and bioavailability of the substrate. One of the vital features of microbial genetic engineering in oil industry was to increase the biosurfactant secretion and to provide the bioavailability of hydrocarbons, specially, the heavy fractions to be converted, or for use in bioremediation of hydrocarbon infected soils. Poly Aromatic Hydrocarbons (PAHs) always impose harm to aquatic creatures and human fitness, in addition, their removal capacity might have constrained with the aid of using low mass transfer phases at Poly Aromatic Hydrocarbons-contaminated soils. A lot of research was being focused to investigate novel molecules that improve the bioavailability on increasing solubility of hydrocarbon contaminating compounds. Bioremediation of PHAs was considered to be the most promising and environmentally useful cleanup approach as it involves the microbial transformation of pollutants to useful metabolites. In 2002,Zhuang et al, isolated and characterized a bacterium which degrades naphthalene contaminated site present in marine sediments. Microbial enhanced oil recovery has been extensively used for the recovery of oil. Some bacteria mobilize the sediments of oil trapped in the reservoirs and rocks for their metabolism to produce various metabolites [61]. Recently, it had been shown that the interfacial tension reduction and alteration in wettability were two important mechanisms of microbial enhanced oil recovery. Sarafzadeh et al, (2013) reported that biosurfactants played an important role on adsorption of oil from the rocks. It had been shown the effect of biosurfactant producing bacteria on laboratory sand packed columns to demonstrate the effectiveness of microbially enhanced oil recovery and been reported that surfactin from *Bacillus coagulans*30 could form emulsions with crude oil, which in turn increased the recovery of oil from 17 to 31% (Chaprao, et al., 2015), meanwhile, surfactin formed an emulsion which was stable at different pH, temperature and salinity ranges. Dinger et al. (2002) justified that the surfactin produced by *B. subtilis* was active even at high pH, temperature and salt concentration ranges (pH 3–10, temperature 21–70°C and NaCl0%–10%). Many studies had revealed that surfactants showed potential uses in microbially enhanced oil recovery. *Bacillus* species produced lipopeptides at a range between 85 and 95 mg/l in the reservoirs of oil. During the last decade, around ten of the microbially enhanced oil recovery methods had been implemented in USA, Malaysia, China and Argentina. Maudgalya reviewed about 26 different types of biosurfactants in field trialed of microbially enhanced oil recovery and found out 20 of the biosurfactants were capable of oil recovery (Chaprao, et al., 2015) and most promising results of the microbially enhanced oil recovery were seen in Shengli oil field of China till the date. Microbially enhanced oil research is the promising field of research and was known to show the high potential in increasing the oil production and extending the life of the oil field economically [62].

1.6 Antibiotic degradation

There was a huge concern on the usage of antibiotics to treat various human ailments, because antibiotics may cause various adverse effects on human health. Recently, it had been indicated that antibiotics used to treat fish and shrimp might deposit in the bottom of the pond and damaged the herbal habitat found for shrimp, fish, hen and human race. Ponds that were used to culture shrimps could contaminate the water as it consists of number of materials which include nutrients like Phosphorous, Potassium, metabolic wastes, antibiotics, different drugs defending shrimp and suspended soil debris due to erosion. Focus on the fish farms had proven that most of the antibiotics were delivered into the feed were not passed by the fish but they entered into the environment causing damage to the ecosystem in the tropical mangroves. The only safest way for the elimination of these antibiotics was by the natural biodegradation, many of the antibiotics were absorbed in the nature so as many of the microorganisms that are found in nature make food out of these antibiotics and they can have a number of antibiotic resistance genes in common. In addition to this, a few soil bacteria can also live by the use of antibiotics serving as energy source of carbon. In order to remove the pollutants involved in chemical pollution and toxicity in the environment, biotransformation approach was always a great kind of ecofriendly process [63].

1.7 Purification and identification

Production and purification of biosurfactants were based mainly on their charge, solubility and selection of solvents. Biosurfactants that were secreted into the supernatant are extracted from centrifugation of the culture. Purification of individual components include acid hydrolysis, solvent extraction, filtration, chromatography and lyophilization methods [64]. Based on the species that produce biosurfactants of glyco and lipo conjugates, the selection of the solvent should be used for the precipitation of active fraction. Solvent mixtures like chloroform/methanol (2/1), acetone and ethyl acetate are used [65]. Generally most of the biosurfactants were less soluble in water due to their complex structure. Culture supernatant was applied to the column and different fractions were eluted with suitable solvents based on adsorption was one of the advanced separation techniques. Purification of the biosurfactants involved chromatography (Reiling, et al., 1986) separated by anion exchangers, preparative TLC using silica gel column, membrane filtration with a cut-off range of 10 kDa, foam fractionation through a column in a bioreactor, TLC offered simple and economic feasibility for the purification of biosurfactants. Mass spectrometry is one of the best proteomic analytical techniques that ionizes chemical groups based on their mass to charge ratio. Biosurfactant structural analysis can be done by tandem quadrupole mass spectrometry (TQMS), electro spray ionization (ESI). Identification of the target ions can be expertise by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS)/MS analysis using MASCOT search, the database that quantifies proteins using peptide mass spectrometry data. FTIR spectroscopy use the Infra red light for the irradiation of molecules that gives the characteristic frequencies of every molecule for the identification of chemical compound. Infra red spectra gives information about functional groups in given molecules [65]. Structural confirmation should be done using Nuclear Magnetic Resonance (NMR) spectroscopic analysis, as it was based on transitions in atoms and chemical shifts in their frequency of absorption. It allows more accurate structure and purity analysis than IR spectroscopy. Bacteria produce a number of biological active compounds that are aggregates of different molecules with different properties. Lipopeptides showed antibiotic property and were resistant to peptidases and proteases. Biosurfactants exhibited many pharmacological activities: antibacterial, antifungal antiviral anti-mycoplasma properties and biocontrol of plant pathogens [66].

Table 1 Economic importance of the selected biosurfactant producing strains

Biosurfactant	**Microorganisms**	**Economic importance**	**References**
Cellobiose lipids	*Ustilagomaydis*	Antifungal compounds	67
Rhamno lipids	*Pseudomonas aeruginosa*	Bioremediation	68
Trehalose lipids	*Rhodococcuserythropolis*	Dissolution of hydrocarbons	69
Sophoro lipids	*Candida bombicola*	Antimicrobial activity	70
Surafactin	*Bacillus subtilis*	Antimicrobial property	71
Lichenysin	*Bacillus licheniformis*	Microbially enhanced oil recovery	72

Emulsan Glycolipopeptide	*Acinetobacter calcoaceticus*	Microbially enhanced oil recovery	73
Microbactan Glycolipopeptide	*Microbacterium*	Emulsifier	74

Table.2 Surface tension values from the selected biosurfactant producing strains

Biosurfactant	**Organism**	**Surface Tension (mN m^{-1})**	**Reference**
Rhamnolipids	*P. aeruginosa*	29	68
Trehalolipids	*Rhodococcussp.*	36	75
Sophorolipids	*T. bombicola*	33	76
Peptide-lipid	*B. licheniformis*	27	77
Serrawettin	*S. marcescens*	33	68
Viscosin	*P. fluorescens*	26.5	78
Surfactin	*B. subtilis*	27-32	79
Emulsan	*A. calcoaceticus*	32	73
Mannan-lipid-protein	*C. tropicalis*	30	80
Liposan	*C. lipolytica*	29	81
Carbohydrate-protein-lipid	*Microbacterium*sp.	27	74

2. Conclusion

This review provided information on the application of biosurfactants as a promising alternative in the petroleum industry and the bioremediation of oil spills. Till date, the biosurfactants are not yet competitive with chemical surfactants in view of economic and hence, the extensive investigation on large scale production of biosurfactant from low cost substrates were needed to reduce the cost of production and allow the studies of large-scale production of these natural compounds from the novel bacterial stains.

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4 Conflicts of Interest

The authors declare no conflict of interest.

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