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Biosurfactants and their Use in Upgrading Petroleum Vacuum Distillation Residue: A Review

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ABSTRACT: It has been known for years that microbial surface active agents have a wide range of applications not only in oil spill environment but also in many industries. Their properties including: (*i*) changing surface active phenomena, such as lowering of surface and interfacial tensions, (*ii*) wetting and penetrating actions, (*iii*) spreading, (*iv*) hydrophylicity and hydrophobicity actions, (*v*) microbial growth enhancement, (*vi*) metal sequestration and (*vii*) anti-microbial action attract the biotechnologist's attention to be substituted instead of synthetic ones. There are many advantages of biosurfacants in comparison with chemically synthesized counterparts like *biodegradability*, generally *low toxicity, biocompatibility and digestibility, availability of cheap raw materials, acceptable production economics, use in environmental control, specificity and Effectiveness* at extreme temperatures, pH and Salinity. Hydrophobic petroleum hydrocarbons require solubilization before degradation by microbial cells. Surfactants can increase the surface area of hydrophobic materials, such as oil spills in soil and water environment, thereby increasing their water solubility. Hence, the presence of surfactants would increase biodegradation of complex hydrocarbons like asphaltenes and resins. Increasing supply of heavy crude oils, bitumens, distillation vacuum residue in most of oil producing countries has increased the interest in transportation and conversion of the high-molecular weight fractions of these materials into refined fuels and petrochemicals and also the interest of conversion of heavy fraction of crude oil like vacuum distillation residue to more valuable components.

Key words: Vacuum Bottom Residue, Biosurfactant, Heavy crude oil, Microorganisms

INTRODUCTION

Recent BP's catastrophic oil spill has been a massive one. World experts believes this is the largest ever spill in the Gulf of Mexico, they have come to this conclusion after studying the oil flow for more than two months. That is why pressure is mounting on the oil giant to halt the gusher that has done immense damage to the environment, businesses and sea species in the region. It is estimated that in the last two and a half months more than 140 million gallon crude oil has leaked from a blown-out well in the Gulf, endangering species and plants deep in the sea. Crude oil spills results of covering soil or water surfaces, the oxygen supply to the bulk of the soil or water is cut off causing environmental disasters such as the death of oxygen-dependent organisms. The use of surfactants is among the most effective ways of removing hydrocarbons from the environment. Oil spills can be removed using different mixtures of surfactants.

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Originally, biosurfactants attracted attention as hydrocarbon dissolving agents in the late 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry (Deisi *et al.*, 1997; Banat *et al.*, 2000; Nasrollahzadeh, *et al*., 2007). The reason for their popularity as high value microbial products is primarily because of their specific action, low toxicity, higher biodegradability, effectiveness at extremes of temperature, pH, salinity and widespread applicability, and their unique structures which provide new properties that classical surfactants may lack (Cooper *et al.*,1984; Kosaric *et al.*,1992). Biosurfactants possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as chemical surfactants. Unlike chemical surfactants, which are mostly derived from petroleum feedstock, these mol-

ecules can be produced by microbial fermentation processes using cheaper agro-based substrates and waste materials (Muthusamy *et al.,* 2008). Hydrophobic pollutants present in petroleum hydrocarbons, and soil and water environment require solubilization before being degraded by microbial cells. Mineralization is governed by desorption of hydrocarbons from soil. Surfactants can increase the surface area of hydrophobic materials, such as oil spills in soil and water environment, thereby increasing their water solubility. Hence, the presence of surfactants may increase microbial degradation of pollutants. Use of biosurfactants for degradation of oil in soil and water environment has gained importance only recently (Karanth *et al.,* 2008). Bacteria degrade and use *n*-Alkanes and polycyclic hydrocarbons (PAHs) as carbon substrates in presence of synthetic surfactants more efficiently than without surfactants (Edwards *et al,* 1991; Tiehm, 1994); biosurfactants may likewise facilitate biodegradation of hydrocarbons (Zhang and Miller, 1992, 1995; Van Dyke *et al.*, 1993). Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (C_xH_y), microorganisms facilitate their diffusion into the cell by producing a variety of substances, the biosurfactants. Some bacteria and yeasts excrete ionic surfactants which emulsify the C_xH_y substrate in the growth medium. Some examples of this group of biosurfactants are rhamnolipids which are produced by different *Pseudomonas* sp.(Mazaheri Assadi *et al.,* 2004; Mazaheri Assadi and Tabatabaee, 2008), or the sophorolipids which are produced by several *Torulopsis* sp (Cooper *et al.,*1984). Some other microorganisms are capable of changing the structure of their cell wall, which they achieve by synthesizing lipopolysaccharides or nonionic surfactants in their cell wall. Examples of this group are: *Candida lipolytica* and *C. tropicalis* which produce cell wall-bound lipopolysaccharides when growing on *n*-alkanes; and *Rhodococcus erythropolis,* and many *Mycobacterium* sp. and *Arthrobacter* sp. which synthesize nonionic trehalose corynomycolates (Kretschmer *et al.,*1982*;* Rosenberg *et al.,*1979). There are lipopolysaccharides, such as Emulsan, synthesized by *Acinetobacter* sp. (Rosenberg *et al.,*1979; Chamanrokh *et al.,* 2010), and lipoproteins or lipopeptides, such as Surfactin and Subtilisin, produced by *Bacillus subtilis* (Arima *et al.,*1968; Haghighat *et al.,* 2008). Other effective BS are: (i) Mycolates and Corynomycolates which are produced by *Rhodococcus* sp., *Corynebacteria* sp., *Mycobacteria* sp., and *Nocardia* sp.; and (ii) ornithinlipids, which are produced by *Pseudomonas rubescens, Gluconobacter cerinus*, and *Thiobacillus ferroxidans*. Biosurfactant produced by various microorganisms together with their properties are listed in Table 1. (Das *et al.,*2008, Karanth *et al.,*2008).

The bioavailibity of many organic compounds such as petroleum hydrocarbons is limited by their water solubility (leathy and colwell, 1990; Atlas and Bartha,1992). Surfactants and emulsifiers facilitate degradation of hydrophobic materials by making them more bioavailable to microorganisms. Therefore, they may have application in oil spill remediation, as well as in the textile, pharmaceutical, cosmetic, and paper industries. All surfactants possess both hydrophilic and hydrophobic domains and thus can interact with both aqueous and nonpolar materials (Georgiou *et al*., 1992; Desai and Desai, 1993). They facilitate dispersion of hydrophobic materials into aqueous phases (Mazaheri Assadi *et al*.,2004).

Biosurfactants

Biosurfactants are microbially produced surface-active compounds have amphiphilic molecules. These amphiphilic molecules have both hydrophilic and hydrophobic regions causing them to aggregate at interfaces between fluids with different polarities such as water and hydrocarbons (Banat, 1995a; Fiechter, 1992; Georgiou, 1992; Kosaric, 1993; Karanth *et al*., 1999) hence, decreases interfacial surface tension (Fiechter, 1992; Georgiou *et al*., 1992;Rouse *et al*., 1994 ; Lin, 1996; Shafi and Khanna, 1995; Volkering *et al*.,1998; Karanth *et al*., 1999). It has been proved that these secondary metabolites enhance nutrient transport across membranes and affect in various host-microbe interactions. Usually provide biocidal and fungicidal protection to the producing organism (Banat, 1995a; Banat,1995b; Lin,1996). The ability of these specific biomolecule is to reduce interfacial surface tension, which has important role in petrolrum industry like in tertiary oil recovery and bioremediation consequences or upgrading the heavy crude oil (Rouse *et al*., 1994; Lin, 1996; Volkering *et al*., 1998). Many of the biosurfactant producing microorganisms are also hydrocarbon-degraders (Rouse *et al*., 1994; Willumsen and Karlson, 1997; Volkering *et al*., 1998). However in the past decades, many studies have showed the effects of microbially produced surfactants not only on bioremediation but also on enhanced oil recovery (Jenneman *et al*., 1984; Jack, 1988; Volkering *et al*., 1998, Tabatabaee *et al*., 2005). Most of these studies typically involved a single microbe or group of microbes isolated and identified in a laboratory and then applied to either *ex situ* soil core experiments or injected into existing oil reservoirs for observation. In addition, the majority of biosurfactant production, hydrocarbon recovery, heavy crude oil and vaccum residue upgradingwere conducted with known species such

Table 1. Biosurfactants with their microbial sources

Biosurfactants	Microbial origin	
	Bacteria	Fungi
Surfactin	*Bacillus subtilis* (Arima *et al*. 1968) *Bacillus licheniformis* F2.2(Thaniyavarn *et al.* 2003) *Bacillus subtilis* ATCC 21332(Nitschke and Pastore, 2003) *Bacillus subtilis* LB5a(Nitschke and Pastore, 2006) *Bacillus subtilis* MTCC 1427 and MTCC 2423 (Makkar and Cameotra, 1999)	
Surfactant BL86	*Bacillus licheniformis* 86 (Horowitz and Currie, 1990)	
Arthrofactin	*Arthrobacter* sp. MIS38 (Morikawa *et al*. 1993)	
Viscosin	*Pseudomonas fluorescens* (Neu and Poralla, 1990)	
Plipastatin	*Bacillus licheniformis* F2.2 (Thaniyavarn *et al.* 2003)	
Massetolides	*Pseudomonas fluorescens* SS101 (Tran *et al.* 2007)	
Iturin	*B. amyloliquefaciens* B94 (Yu *et al*. 2002) - *Bacillus subtilis* RB14 (Rahman *et al*. 2006)	
Lichenysin A	*Bacillus licheniformis* BAS50 (Yakimov *et al*. 1995)	
Lichenysin B, C	*Bacillus* sp. (Yakimov *et al*. 1995, Yakimov *et al*. -1998, Yakimov *et al.* 1999)	
Bamylomycin	*B. amyloliquefaciens* (Lee *et al*. 2007)	
Halobacillin Marine	*Bacillus sp*. (Trischmann *et al.* 1994)	
Isohalobacillin	*Bacillus sp*. A1238 (Hasumi *et al.* 1995)	
Bioemulsifier	*Bacillus stearothermophilus* VR-8 *Candida lipolytica* (Gurjar *et al.* 1995) IA 1055 (Vance-Harrop *et al.* 2003)	
Flavolipid	*Flavobacterium* sp. MTN11 -(Bodour *et al.* 2004)	
Mannosylerthritol		*Candida antarctica* lipid (MEL) (Kitamoto *et al*. 1990a) *Candida* sp. KSM-1529 (Kobayashi *et al.* 1987) *Pseudozyma antarctica* JCM 10317T (Morita *et al.* 2007)
Rhamnolipids R1 and R2	*Pseudomonas aeruginosa* (Guerra-Santos *et al*. 1986)	
Rhamnolipid	*P. aeruginosa* EM1 (Wu *et al*. 2008) *Pseudomonas aeruginosa* GS3 (Patel and Desai 1997) *Pseudomonas aeruginosa* BS2 (Dubey and Juwarkar 2001) *P. putida* 300-B mutant (obtained from *Pseudomonas putida* 33 wild strain by gamma ray mutagenesis) (Robert *et al.* 1989) *Pseudomonas aerogiosa MM1011* (Mazaheri Assadi M., et al.2004)	
Rhamnolipid RL1	*Pseudomonas* sp. 47T2 NCIB 400044 and RL2 (Mercade *et al*. 1993)	
Rhamnolipids (RLLBI)	*Pseudomonas aeruginosa* strain LBI (Benincasa *et al*. 2002)	
Emulsan	*Acinetobacter calcoaceticus* ATCC 31012 -(RAG-1) (Shabtai 1990) *Acinetobacter venetianus* RAG-1 (Panilaitis *et al.*2006)	
Liposan -		*C. lipolytica* (Cirigliano and Carman 1985)
Biodispersan	*A. calcoaceticus* A2 (Shabtai 1990) -	
Lactonic sophorose lipid		T. *bombicola* KSM-36 (Ito *et al.* 1980)
Fructose-lipids	*Arthrobacter* sp., *Corynebacterium* sp., - *Nocardia* sp., *Mycobacterium* sp. (Itoh and Suzuki, 1974)	
Sophorolipids		*Candida bombicola* (Deshpande and Daniels 1995)
Bioemulsan	*Gordonia* sp. BS29 (Franzetti *et al*. 2008)	
Circulocin	*Bacillus circulans*, J2154 (He *et al*. 2001) AP-6 *Pseudomonas fluorescens* 378 (Persson *et al*. 1988)	

as *Pseudomonas aeruginosa.*, *Pseudomonas fluorescens.*, *Bacillus licheniformis* strain JF-2, *Bacillus subtilis*, or Acinetobacter calcoaceticus and many unknown ones either reservoir indigenous ones or from other hydrocarbon recourses and hydrocarbon contaminated sites(Adkins *et al.*, 1992; Banat, 1995a; Banat, 1995b; Lin, 1998, McInerney *et al.*,300 Proceedings of the 2000 Conference on Hazardous Waste Research 1990, Jenning and Tanner.,2000; Tahzibi *et al.*,2004; Tabatabaee *et al.*,2005).

Classification and chemical nature of biosurfactants

Biosurfactants are specific molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. (Fig. 1 to 4). Usually structurally elucidated surfactants were obtained by a procedure of precise purification processes. The high molecular weight biosurfactants are generally polyanionic heteropolysaccharides containing both polysaccharides and proteins which are more effective at stabilizing oil-in-water ((Rosenberg and Ron 1999; Chamanrokh *et al.*, 2008).

Mechanisms proposed for the enhancement of aqueous solubility of hydrophobic substances by surfactants include solubilization in the hydrophobic core of multimolecular surfactant structures formed at above-aggregation concentrations, such as micelles (Edwards *et al.*,1991; Volkering *et al.*,1995, Jordan *et al.*,1999, Schippers *et al.*,2000) and liposomes (Miller and Bartha, 1989); decreased surface tension of the solvent ; and interaction with hydrophobic tails of surfactant monomers(Barkay *et al.*,1999).

The low molecular weight biosurfactants which lower surface and interfacial tensions are often glycolipids such as trehalose lipids, sophorolipids and rhamnolipids, or lipopeptides, such as surfactin, gramicidin S and polymyxin (Rosenberg and Ron 1999,Tahzibi *et al.*, 2004); and ones with low (micrograms per milliliter) critical micelle concentrations (CMC) can increase the apparent solubility of hydrocarbons by incorporating them into the hydrophobic cavity of micelles (Miller and Zhang ,1997).

Three main roles for biosurfactants are supposed to be: (i) increasing the surface area of hydrophobic water-insoluble growth substrates; (ii) increasing the bioavailability of hydrophobic substrates by increasing their apparent solubility or desorbing them from surfaces; (iii) regulating the attachment and detachment of microorganisms to and from surfaces (Rosenberg and Ron, 1999).The yield of microbial surfactants varies with the environmental requirement i.e including their nutrition requirements. Intact microbial cells that have high cell surface hydrophobicity are themselves surfactants. In some cases, surfactants themselves play a natural role in growth of microbial cells on water-insoluble substrates like C_xH_y, sulphur, etc. Exocellular surfactants are involved in cell adhesion, emulsification, dispersion, flocculation, cell aggregation, and desorption phenomena. Biosurfactants generally classified into six major groups: Glycolipids,Fatty acids, Phospholipids, Surface active antibiotics, Polymeric microbial surfactants and particulate surfactants. (Karanth *et al.*,2008; Gautam and Tyagi, 2006, Shakerifard *et al.*, 2009).

Glycolipids

The most common types of biosurfactant are glycolipids which constituent mono-, di-, tri- and tetrasaccharides include glucose, mannose, galactose, glucuronic acid, rhamnose, and galactose sulphate. The composition of fatty acid has a similar structure to that of the phospholipids of the same microorganism. The glycolipids usually are classified as:

Trehalose lipids: The production of trehalose lipids seen in many members of the genus *Mycobacterium.* The typical structure is due to the presence of trehalose esters on the cell surface (Asselineau *et al.*,1978) from different species of *Mycobacteria* (Asselineau *et al.*,1978), *Corynebacteria*, *Nocardia*, and *Brevibacteria* differ in size and structure of the mycolic acid esters.

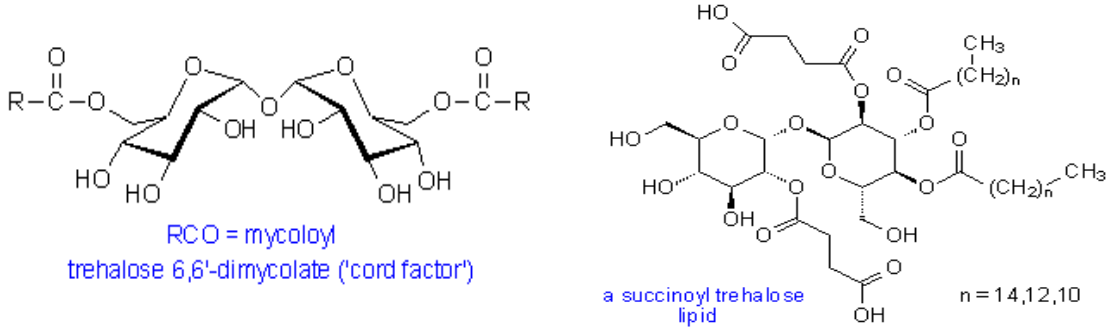

Fig.1. Different chemical Structure of Trehalose lipids (Marqués *et al.*, 2009)

Sophorolipids: *Torulopsis bombicola* are major specis of yeast which are capable of producing glycolipids (Rosenberg *et al.*,1979). There are several yeasts such as:

T. petrophilum and *T. apicola* consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage.Usually sophorolipids occur as a mixture of macrolactones and free acid form. The lactone form of the sophorolipid is most important molecules, known for many applications. According to Muthusamy *et al.*(2008), these biosurfactants are a mixture of at least 6-9 different hydrophobic sophorolipids

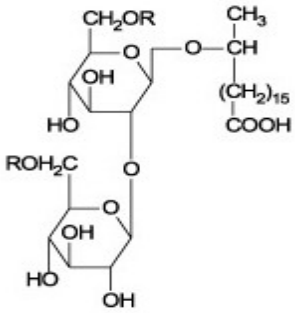

Fig. 2. chemical Structure Sophorolipid (Van Bogaert, *et al.*, 2007)

Rhamnolipids: Genus *Pseudomonas* are one of the most important producers of large quantities of a glycolipid, consisting of two molecules of rhamnose and two molecules of b-hydroxydecanoic acid (Jarvisand Johnson1949; Edward and Hayashi1965; Reiling etal., 1986,). In glycosidic linkage, the OH group of one of the acids is involved with the reducing end of the rhamnose disaccharide, the OH group of the second acids is involved in ester formation. Since one of the carboxylic acid is free, the rhamnolipids are anions above pH 4.0((Karanth *etal.*, 2008, Hisatsuka *atal*1971). Formation of rhamnolipids by *Pseudomonas* species was greatly increased by nitrogen limitations (Mazaheri Assadi *etal.*, 2004) . The pure rhamnolipid lowered the interfacial tension against *n*-hexadecane in water to about 1 mN/m and had a critical micellar concentration (cmc) of 10 to 30 mg/L depending on the pH and salt conditions (Karanth *et al.*, 2008).

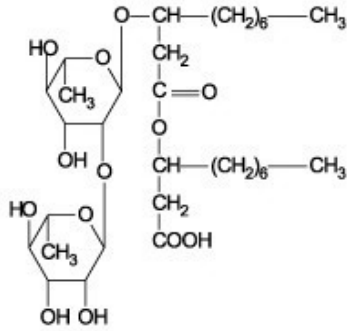

Fig. 3. chemical Structure of Rhamnolipid (Tazhibi *et al.*, 2005)

Mannosylerythritol and Cellobiose Lipids

The yeast *Candida (Pseudozyma) antarctica* secretes an extracellular *mannosylerythritol lipid* (4-*O*-(2',6'-di-*O*-acyl-β-D-mannopyranosyl)-D-erythritol), with biosurfactant properties, when grown on a vegetable oil substrate. When grown on glucose, the same lipid accumulates intra-cellularly as an energy store until it amounts to 10% or more of the dry weight of the cell.

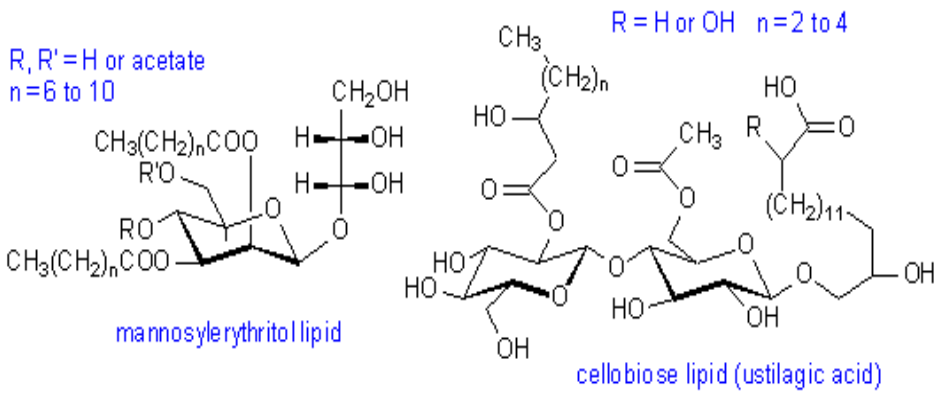

Fig. 4. Chemical structure of Mannosylerythritol and Cellobiose Lipids (Arutchelv, *et al.*, 2008)

One or two of the hydroxyls on the mannose residue are acetylated, and there are two esterified fatty acids, which are both are odd- and even-numbered from C_8 to C_{12} in chain-length (longer in related species). While this organism gives the greatest yields of these lipids, they were first found in the fungus *Ustilago maydis* and termed 'ustilipids'. In this instance, the 2-hydroxyl group of the mannose residue is esterified with a C_2 to C_8 fatty acid, while the 3-hydroxyl group is esterified by a C_{12} to C_{20} fatty acid. Several other species of the genus *Pseudozyma* are now known to produce similar lipids in which the nature, number and positions of the acyl groups vary. As with other biosurfactants, these compounds are believed to facilitate dissolution of organic hydrophobic compounds so that they can be consumed by the organism. Mannosylerythritol lipids have been shown to have a number of profound biological effects in animals, but especially to induce the differentiation of certain cancer cells.

Ustilago maydis also contains distinctive cellobiose lipids (or 'ustilagic acid'), consisting of the disaccharide cellobiose linked *O*-glycosidically to the ω-hydroxyl group of the unusual long-chain fatty acid 15,16-dihydroxyhexadecanoic acid or 2,15,16-trihydroxyhexadecanoic acid. Others of the hydroxyl groups are esterified either to acetate or a medium-chain 3-hydroxy fatty acid. A further unusual cellobiose lipid is produced by the fungal biocontrol agent, *Pseudozyma flocculosa*, and has been show to be 2-(2',4'-diacetoxy-5'-carboxy-pentanoyl)octadecyl cellobioside (flocculosin), the compound responsible for the antifungal activities of the organism

Fatty acids

Using alkanes as substrates, the fatty acids produced by microbial oxidations, have received highest attention as biosurfactants. Straight-chain acids, microorganisms produce mixed fatty acids containing OH groups and alkyl branches(Figs.5 &6). Some of these mixed acids, are corynomucolic acids, which are considered as surfactants (Kretschmer *et al.,*1982; Cooper *et al.,* 1984; Karanth *et al.*,2008).

Phospholipids

Phospholipids are major components of microbial membranes. When certain C_xH_y-degrading bacteria or yeast are grown on alkane substrates, the level of phospholipids increases greatly. Phospholipids from hexadecane-grown *Acinetobacter* sp. have potent surfactant properties. Phospholipids produced by *Thiobacillus thiooxidans* have been reported to be responsible for wetting elemental sulphur, which is necessary for growth (Kappeli *et al* ., 1979; Karanth *et al.*,2008).

Surface active antibiotics

Gramicidin S: Many bacteria produce a cyclosymmetric decapeptide antibiotic, gramicidin S. Spore preparations of *Brevibacterium brevis* contain large amounts of gramicidin S bound strongly to the outer surface of the spores. Mutants lacking gramicidin S germinate rapidly and do not have a lipophilic surface. The antibacterial activity of gramicidin S is due to its high surface activity (Karanth *et al.*,2008).

Polymixins: A group of antibiotics produced by *Brevibacterium polymyxa* and related to bacilli species. A decapeptide known as Polymixin B contain amino acids 3 through 10 form a cyclic octapeptide. Polymixins are able to solubilize certain membrane enzymes(Karanth *et al.,*2008).

Surfactin (subtilysin): One of the most active biosurfactants produced by *B. subtilis* is a cyclic lipopeptide surfactin (Arima *etal*1968). The yield of surfactin produced by *B. subtilis* can be improved to around 0.8 g/l by continuously removing the surfactant by foam fractionation and addition of either iron or manganese salts to the growth medium (Karanth *et al.*,2008).

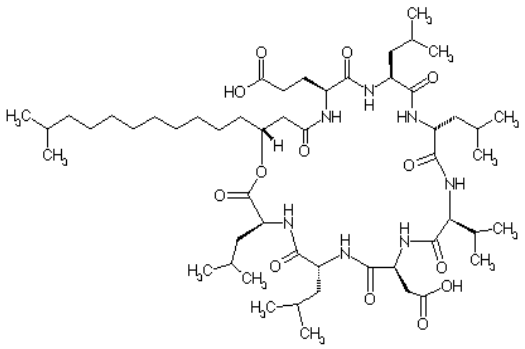

Fig. 5.Chemical structure of Surfactin (López, *et al.*, 2009)

Antibiotic TA: *Myxococcus xanthus* produces antibiotic TA which inhibits peptidoglycan synthesis by interfering with polymerization of the lipid disaccharide pentapeptide. Antibiotic TA has interesting chemotherapeutic applications (Karanth *et al.*,2008).

Polymeric microbial surfactants

Most of these are polymeric heterosaccharide containing proteins.

Acinetobacter calcoaceticus RAG-1 (ATCC 31012) emulsan: A bacterium, RAG-1, was isolated during an investigation of a factor that limited the degradation of crude oil in sea water. This bacterium efficiently emulsified C_xH_y in water. This bacterium, *Acinetobacter calcoaceticus*, was later successfully used to clear a

cargo compartment of an oil tanker during its ballast voyage. The cleaning phenomenon was due to the production of an extracellular, high molecular weight emulsifying factor, emulsan(Karanth *et al.*,2008, Amiryan *at al.*,2004).

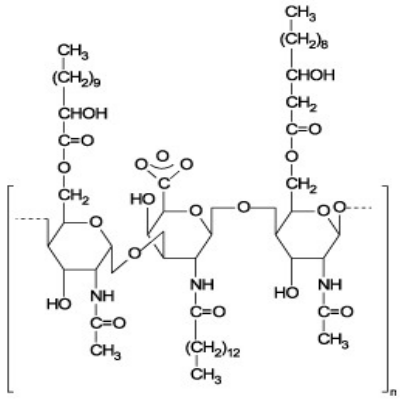

Fig. 6.Chemical structure of Emulsan (Amiryan, *et al.*, 2004)

Emulsan has potential applications in the petroleum industry, including formation of heavy oil-water emulsions for viscosity reduction during pipeline transport and production of fuel oil-water emulsions for direct combustion with dewatering(7; M. E. Hayes, K. R. Hrebenar, P. L. Murphy, L. E. Futch, Jr., and J. F. Deal, U.S. patent 4,618,348, October 1986). The affinity of emulsan for the oil-water interface suggests that it might affect microbial degradation of emulsified oils. This has implications both for the stability of the oil emulsions during storage and transport and for their biodegradability should the emulsions accidentally be spilled in the environment(Foght *et al.*,1989; Amiryan *at al.*,2004).

The polysaccharide protein complex of Acinetobacter calcoaceticus BD413: A mutant of *A. calcoaceticus* BD4, excreted large amounts of polysaccharide together with proteins. The emulsifying activity required the presence of both polysaccharide and proteins.

Other Acinetobacter emulsifiers: Extracellular emulsifier production is widespread in the genus Acinetobacter. In one survey, 8 to 16 strains of *A. calcaoceticus* produced high amounts of emulsifier following growth on ethanol medium. This extracellular fraction was extremely active in breaking (de-emulsifying) kerosene/ water emulsion stabilized by a mixture of Tween 60 and Span 60.

Polysaccharide-lipid complexes from yeast: The partially purified emulsifier, liposan, was reported to contain about 95% carbohydrate and 5% protein. A C_xH_y-degrading yeast, *Endomycopsis lipolytica* YM, produced an unstable alkane-solubilizing factor. *Torulopsis petrophilum* produced different types of surfactants depending on the growth medium (Copper and Paddock1984). On water-insoluble substrates, the yeast produced glycolipids which were incapable of stabilizing emulsions. When glucose was the substrate, the yeast produced a potent emulsifier.

Emulsifying protein (PA) from Pseudomonas aeruginosa: The bacterium *P. aeruginosa* has been observed to excrete a protein emulsifier. This protein PA is produced from long-chain *n*-alkanes, 1-hexadecane, and acetyl alcohol substrates; but not from glucose, glycerol or palmitic acid. The protein has a MW of 14,000 Da and is rich in serine and threonine (Hisatsuka *et al*.,1971, Kappeli *et al.*,1979).

Surfactants from Pseudomonas PG-1: *Pseudomonas* PG-1 is an extremely efficient hydrocarbon-solubilizing bacterium. It utilizes a wide range of C_xH_y including gaseous volatile and liquid alkanes, alkenes, pristane, and alkyl benzenes.

Bioflocculant and emulcyan from the filamentous Cyanobacterium phormidium J-1: The change in cell surface hydrophobicity of *Cyanobacterium phormidium* was correlated with the production of an emulsifying agent, emulcyan. The partially purified emulcyan has a MW greater than 10,000 Da and contains carbohydrate, protein and fatty acid esters. Addition of emulcyan to adherent hydrophobic cells resulted in their becomeing hydrophilic and detach from hexadecane droplets or phenyl sepharose beads (Karanth *et al.*,2008).

Alasan, the bioemulsifier complex of A. radioresistens KA53: Alasan is made of a polysaccharide (apo-alasan) containing covalently bound alanine and proteins. The proteins of alasan plays an essential role in both the structure and surface activity of the complex, because in contrast with alasan, apo-alasan had no emulsifying activity and did not show the large temperature-induced hydrodynamic shape changes while alasan emulsifying activity increased greatly after exposure to high temperature under neutral or alkaline conditions(Navon-Venezia *et al*.,1995). Bioemulsifier alasan can increases the solubility of some PAHs, that this activity is likely due to a reversible binding of these compounds, and that it enhances the biodegradation of PAHs. As the mechanism of solubilization by high-molecular-weight polymers may be fundamentally different than that of small micelle-forming biosurfactants, research on the nature of this process might lead to the development of new approaches and tools for environmental management and industrial applications (Toren *et al*.,2001). The hydrophobic regions in alasan are the most plausible explanation for the mechanism of solubilizing compounds with limited aqueous solubility. Recently (Chamanrokh et al., 2010) isolated two autochthonous strains which are

capable of producing an extracellular, emulsifying agent when grown in Mineral Salt Medium containing Soy oil, ethanol or local crude oil. Analysis of purified emulsion was performed to prove the molecular structure by ^{13}CarbonNuclear Magnetic Resonance (^{13}CNMR), Proton1Nuclear Magnetic (^{1}HNMR) Resonance and Fourier Transform Infrared Radiation (FTIR) methods. These investigations showed that the molecular weight of emulsion produced by species isolated from Iranian crude oil reservoirs are comparable with *Acinetobacter calcoaceticus PTCC 1318*.

Particulate surfactants

Extracellular vesicles from Acinetobacter sp. H01-N: *Acinetobacter* sp. when grown on hexadecane, accumulated extracellular vesicles of 20 to 50 mm diameter with a buoyant density of 1.158 g/cm^3. These vesicles appear to play a role in the uptake of alkanes by *Acinetobacter* sp. HO1-N.

Microbial cells with high cell surface hydrophobicities: Most hydrocarbon-degrading microorganisms, many nonhydrocarbon degraders, some species of *Cyanobacteria*, and some pathogens have a strong affinity for hydrocarbon-water and air-water interfaces. In such cases, the microbial cell itself is a surfactant (Karanth *et al.*,2008).

Biosurfactants in oil industry

Biorefinery currently use biosurfactant of different forms and therefore face the increasing environmental awareness and tightening of regulations in this regard (Fig. 7). Microorganisms have long been known to be able to produce a variety of surface active compounds that display properties and activities comparable to those of synthetic surfactants. (Daisi and Banat, 1979; Singh *eal.*, 2007). Biological surface active molecules can potentially replace chemical analogue compounds, even offering additional advantages, all through the chain of petroleum processing including; Extraction, Transportation, Upgrading and refining and Petrochemical manufacturing (Van Dyke *etal.*,1991, Tabatabaee *et al.*, 2005; Tabatabaee *et al.*, 2006; Haghighat *et al.*, 2008, Planckaert, 2005). Application and activity attributed to use of biosurfactant in oil industry is presented in fig.8.

Heavy fractions of crude oil and Distillation Vacuum Residue

Increasing supply of heavy crude oils, bitumens, distillation vacuum residue in most of oil producing countries has increased the interest in transportation and conversion of the high-molecular weight fractions of these materials into refined fuels and petrochemicals.

In refineries crude oil is first preheated in a heat exchanger network en then heated up to 350°C in a gas fired heater. Hot crude oil is then separated in an atmospheric distillation column (CDU) into different fractions (naphtha, kerosene, gasoline). Heavy fuel oil related streams produced by atmospheric distillation comprise fractions of crude oil separated by heating (650-700 degrees °F) at atmospheric pressure. They include atmospheric distillates (heavy gas oils) and the heavier residual materials (The Petroleum HPV Testing Group, 2004).The bottom stream of the column is further separated in a vacuum distillation column into other fractions. vacuum residual refinery streams com-

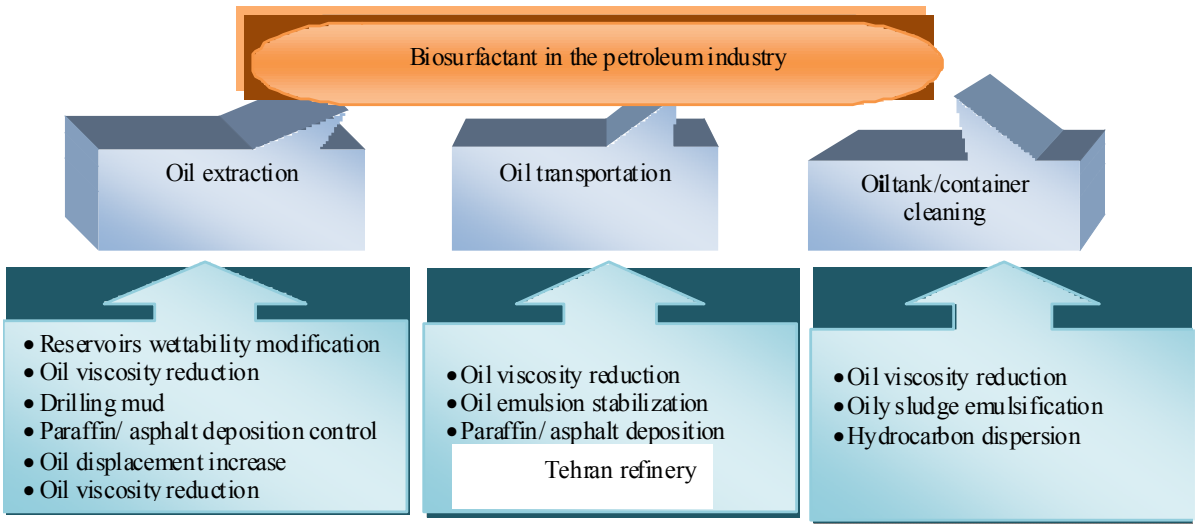

Fig. 7. Biosurfactant application in petroleum industry

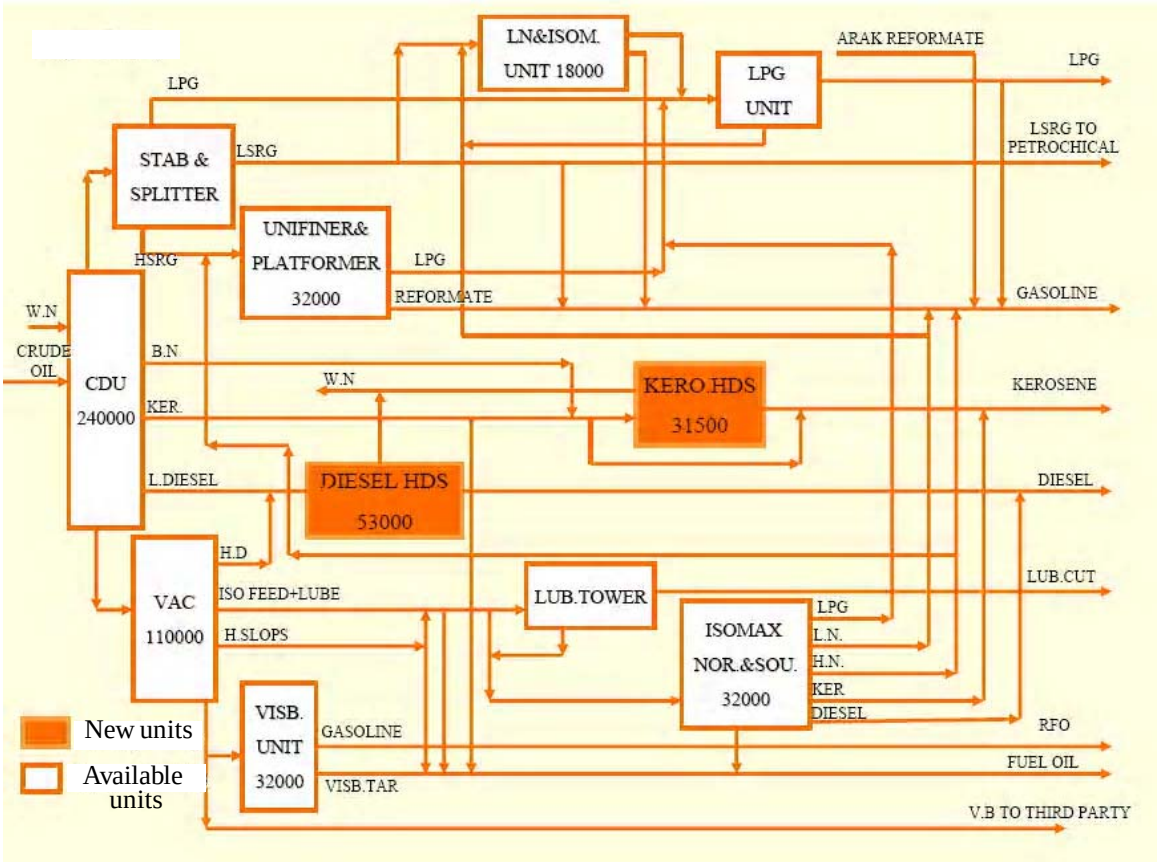

Fig. 8. Tehran Oil refinery distillation column scheme (2008)

prise a heterogeneous group of poorly defined, viscous, high boiling hydrocarbon streams that usually contain suspensions of resin/asphaltene complexes in the form of colloidally dispersed particles and the central part of the asphaltene micelle consists of high molecular-weight compounds surrounded and peptized by neutral resins of aromatic hydrocarbons(kim *et al.*,1996). These streams often have high levels of heterocyclic aromatic and naphthenic compounds. Varying percentages of sulfur, nitrogen, oxygen, and other elements are present as heterocyclic inclusions, primarily in the aromatics fraction (The Petroleum HPV Testing Group, 2004).

Crude oils with high viscosity require addition of a solvent in order to allow pipelining over a significant distance. Since the cost of suitable solvents, such as naphtha or natural gas condensate, has led to study of new methods to reduce the viscosity of heavy crudes and vacuum residue. In refineries once heavy crudes and bitumens enters, requires conversion of the vacuum residue components, including waxes and asphaltenes, into distillable oils and hence is considered as upgrading (Kirwooda *et al.*, 2004).The whole processes have typically been practiced with either thermal conversion (cracking or coking). Thermal conversion, due to expensive equipment and supporting infrastructure for supply of hydrogen and treatment of hydrogen sulfide in cracked off-gases, is a costly process. Biological processing considered being suitable constituents thanks to its less severity and more selectivity to specific reactions. (Kirwooda *et al.*, 2004). The characteristics of the molecules in the vacuum residue fraction of crude oils and the prospects for using biological processes to upgrade them is discussed in this part of our review.

Vacuum distillation upgrading goals

Reducing the molecular weight of residue fractions to low molecular weight materials, increasing the hydrogen to carbon (H/C) ratio by hydrogenation, and removal of heteroatoms, specially sulfur and nitrogen are the matters of attention in processing the heavy crudes. According to Kirwooda *et al.*, 2004, there are five key areas of heavy oil upgrading where biological treatment could have an impact. Viscosity reduction, composition improvement, deposition control, de-emulsification, and naphthenic acids removal are considered as their five key components of vacuum distillation upgrading processed (Kirwooda *et al.*, 2004).

Oxidation of aliphatic and aromatic carbon groups, oxidation of naphthenic acids, and oxidation and desulfurization of aromatic and aliphatic sulfur groups are studied interactions between microbes and the high molecular weight components of crude oils. Hydrogenation and dehydrogenation reactions have been demonstrated only on lower-molecular weight components. All of these reactions are of potential interest for upgrading heavy crude oils and bitumens, but a major barrier is the transport of reactants to the active site of reaction, particularly for intracellular enzymes in bacteria. Although membranes may give significant barriers for bioprocessing of heavy hydrocarbons, the interactions of cell membranes with oil/water interfaces may be of interest in deemulsifying oil and in dispersing asphaltenic material to prevent deposition (Kirwooda *et al.,* 2004).

Vacuum distillation residue contents

The asphaltene content of petroleum is an important aspect of fluid processability (Fig. 9). Therefore SARA method is conveniently used to separate the crude oil into four major fractions: saturates (including waxes), aromatics, resins and asphaltenes (SARA), based on their solubility and polarity as shown in Fig. 10 (Harald Auflem, 2002).

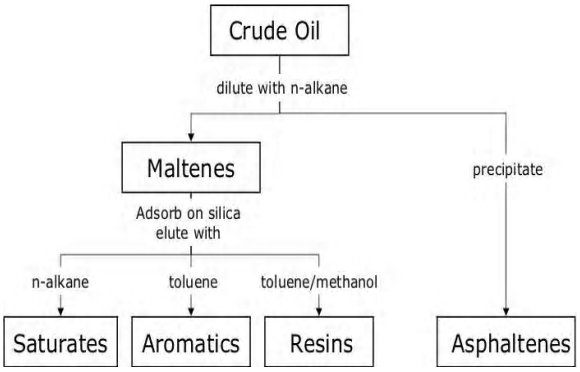

Fig. 9. Typical scheme for separating crude oil into saturate, aromatic, resin and asphaltene (SARA) components (Harald Auflem, 2002)

Asphaltene and resin fraction of VR:

Asphaltenes with a heavy polar structure, are insoluble in low normal alkanes (*n*C5 *n*C8) and soluble in such solvents as benzene and toluene and so on (Fig.10). Generally crudes have a dynamic stable system of asphaltenes, resins and petroleum alkanes, similar to a colloidal system, in which the petroleum alkanes act as solvents, the asphaltenes as micelle and the resins as stabilizers (Spight ,1996;Spight and Long,1996, Storm, 1995). Resins in crude oil consist mainly of naphthenic aromatic hydrocarbons, generally aromatic ring systems with alicyclic chains. The resins are to a degree interfacially active in crude oils and they are effective as a dispersant of tensions of asphaltenes (Schorling 1999) leading to formation of micelles with different polarities, which can further aggregate to form supermicelles and molecular solutions(Fig.11). This process is summarized in Fig. 9 (Premutzic And lin,1999; Schorling *et al.*,1999). Any changes in dynamic stable system of crude oil like changes of temperature, pressure and/or compositions in crude oils may couse asphaltene precipitation. (Spight ,1996;Spight and Long,1996; Leontaritis,1996; Zewen and ansung, 2000, Harald Auflem *at al.*,2002).

Paraffin and naphthenic of VR

The petroleum crudes typically consists of paraffin hydrocarbons (C18 - C36) known as paraffin wax and naphthenic hydrocarbons (C30 - C60) which are straight chain saturated hydrocarbons (Hoa *et al.*, 2008). Hydrocarbon components of wax can exist in various states of matter (gas, liquid o r solid) depending on their temperature and pressure. When the wax freezes it forms crystals. The crystals formed of paraffin wax are known as macrocrystalline wax. Those formed from naphthenes are known as microcrystalline wax (Himran *et al.*, 1994; Mansoori *et al.*, 2003).

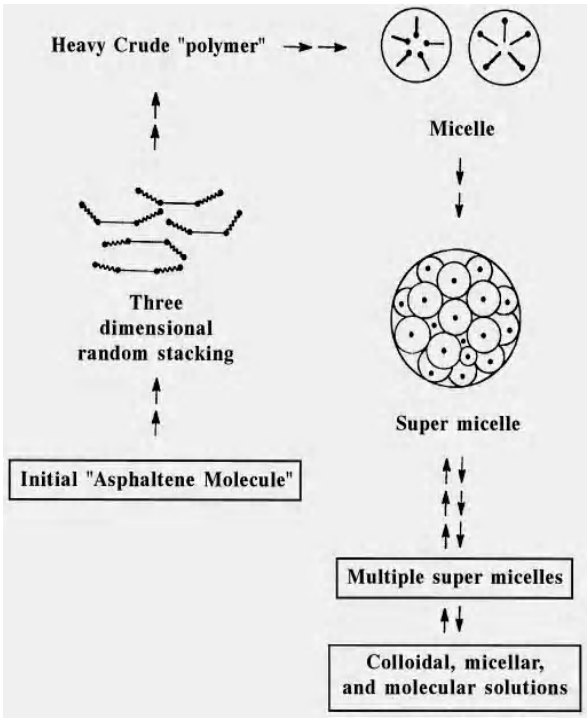

Fig. 10. Formation of molecular solutions. Dark circles represent heteroatoms and active sites (Harald Auflem *at al.*,2002)

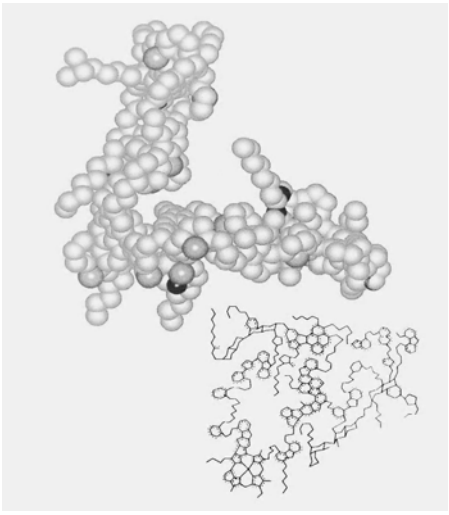

Fig. 11. Space filling model of an asphaltene molecule (Strausz and Yen, 1994). Color code: blue, nitrogen; red, oxygen; yellow, sulfur; black, carbon; small white, hydrogen; large white, metal

Problems generated by vacuum distillates

Problems associated to asphaltenes are classified in five general groups: (Leon *et al.*,1999;Nalwaya.,1999) extraction, transport, processing, crude economical profit and leaking.Asphaltenes with a large capability of blocking the porous spaces of the deposit reduce the permeability and a remarkable diminishment of the crude's exit flux would occur.(Calemma *et al*,1998.,Nalwaya *et al.*,1999;Wu *et al.*,2000).

As asphaltenes precipitates broadly particularly in metal pipelines in presence of ferric ions combined with acidic conditions will form a solid known as "asphaltenic mud" which deposits in conducts, blocking them and obstructing the free flow of crude.(Artok *et al.*,1999;Kaminski *at al.*,2000) When this kind of mud develops, solvents, such as toluene and xylene are applied in order to dissolve them. This process increases production costs and generates residues of a high toxicity degree (Kaminski *et al.*,2000).

During oil refining asphaltenic mud cause problems by deactivating catalysts for desulfurization (Calemma *et al*,1998;Wu *et al*.,2000)which causes a general limitation in the maximal conversion of less-sulfured petroleum(Mitra-kirtley *et al.*,1993;*Rogel*,1997 and shirokoff *et al.*,1997). And finally asphaltenic crude oils (18-22% asphaltenes) called "Heavy" have a low quality product and also difficulties in its extraction and refining and thus less economical profits (Pineda-frores and Mesta-Howard, 2001).

Environmental petroleum leakages are the most evident way by which asphaltenes and microorganisms get in touch (Cernigla *et al.*,1973; Calemma *et al.*,1998). One of the greatest problems of these compounds in the environment is their resistance to biodegradation by microbial metabolic activity. (Atlas,1981; Guiliano,2000) Due to this fact, metabolic routes involved in this process are the less known ones in these days, although, there is some evidence suggesting that some microorganisms have the potential capability of transforming asphaltenes, and in the best case, eliminating them (Pineda-frores and Mesta-Howard, 2001).

On the other hand, microcrystalline waxes harbor more branched and cyclic hydrocarbons therefore can relatively infrequently solidify and deposited at room temperature. Presence of paraffin in crude oil cause an increase in freezing point and viscosity and consequently a decrease in fluidity of oil which will result in low recovery and pipeline blockades in oil production and transportation (fig. 12).

Microbes can control paraffin in three principle ways: i) direct biodegradation (Xue *et al*.,2003; Salgodo-Brito *et al*.,2007; Sood and Lal.,2008), ii)microbial products like fatty acids and biosurfactants which prevent crys-

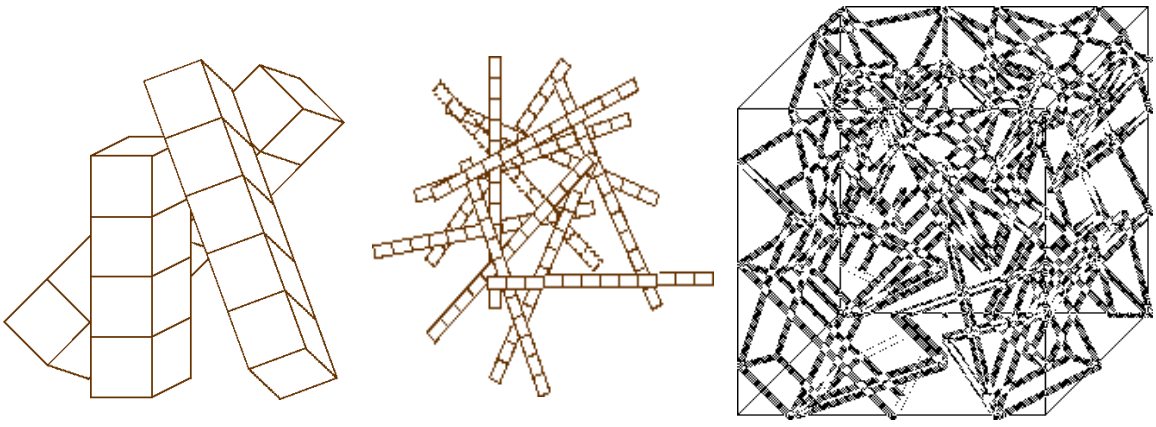

Fig. 12. Macrocrystalline , Microcrystalline, and Crystal Deposit Network of Wax (Mansoori *et al*.,2003)

tallization and cause solubilization (Gieg *et al*.,2006, Hasanuzzaman *et al*., 2007,). iii) attaching to the paraffin surface in the form of biofilm to prevent crystallization and deposition(Hoa *et al*.,2008).

Factors affecting oil degradation

Microbial degradation of crude oil or oil waste depends on a variety of factors, including the physical conditions and the nature, concentration, and ratios of various structural classes of hydrocarbons present, the bioavailability of the substrate, and the properties of the biological system involved (Winter *et al*,1993; Ko and Lebeault,1999, Sugiura *et al.*,1997; VanHamme And Ward,1999; Yuste *et al.*,2000). A generalized sequence of petroleum components in order of decreasing biodegradability is represented as follows (Huesemann,1995): *n*-alkanes > branched-chain alkanes > branched alkenes>low-molecular-weight *n*-alkyl aromatics >monoaromatics> cyclic alkanes > polynuclear aromatics > asphaltenes.

Many new predictive models developed to estimate the extent of petroleum hydrocarbon biodegradation (Huesemann,1995) and diffusion-controlled bioavailability of crude oil components (Urazizii *et al*,1998). For example, properly chosen chemical surfactants may enhance biodegradation (Cameotra *et al*,1998;Bruheim and Eimhjellen,1998;Kroutil and Faber,2000;Rouse *et al*.,1994; Van Hamme and Ward,1999). The efficiency of processes for degradation of hydrocarbons will also depend on the nature of the hydrocarbon-contaminated material, the environmental conditions, and the characteristics of the microbial population that is present (Van Hamme *et al*,2003).

Biosurfactants are among the most important factors in oil degradation from two different points of view, the increase in bioavlibility of hydrocarbon molecules and as an aid of Molecular weight reduction in the vacuum residue components of heavy oils.

Effect of biosurfactants on bioavailability of oil fractions

The low water solubilities of most of the petroleum hydrocarbon compounds have the limit the capability of microbes, which generally exist in aqueous phases, to access and degrade these substrates. Hydrocarbon-degrading microbes produce a variety of biosurfactants as part of their cell surface or as molecules released extracellularly (Sar and Rosenberg,1983; Rosebnerg *et al*.,1988; Fiecher, 1992; Navon-Venezia *et al*,1995(a,b); Burd and Ward,1996(a&b); Rosenberg and Ron1997; Burd and ward,1997; Sim and Ward,1997; Barathi *et al.*,2001; Maker and Cameorta; 2002). Biosurfactants in addition to chemical surfactants enhance removal of petroleum hydrocarbons from soil or solid surfaces(fig.13). However, both enhancement and inhibition of biodegradation of hydrocarbons have been observed (Tumeo *et al.*,1994; Bai *et al*,1997; Laurie and Lioyd – Jones,2000). To examine the biological degradation of hydrocarbons their production was suppressed by mean of inhibitors or mutagens. This process resulted in decrease in their biodegradability (Banat ,1998; Prince, 1998).

The low-molecular-weight biosurfactants (glycolipids, lipopeptides) are more effective in lowering the interfacial and surface tensions, whereas the high-molecular-weight biosurfactants (amphipathic polysaccharides, proteins, lipopolysaccharides, and lipoproteins) are effective stabilizers of oil-in-water emulsions (Banat,1995; Lin,1996; Desai and Banat,1997; Cameotra and Makker,1998; Rosenberg and Ron,1999; Makker and Cameotra, 2002).

By observing the effects of fractionated preparations, many studies have declared the roles of biosurfactants in biodegradation (Foght *et al.*,1989; Jain *et al*,1992; Falatko and Novak,1992; Rouse *et al*.,1994; Zhang *et al*,1994; Zhang and Miller,1995; Churchil *et al.*, 1995; Ermolenko *et al.*,1997; Herman *et al.*,1997, Kanga *et al*,1997; Noordman *et al*, 1998; Rosenberg and Ron,1997&1999; Banat *et al.*,2000). However, the successful application of biosurfactants in bioremediation of petroleum pollutants will require precise targeting to the physical and chemical nature of the pollutant-affecting areas.

Chemical surfactants in some extent can emulsify or pseudosolubilize water-soluble compounds and make them accessible for microorgansims. Chemical surfactants have some properties that influences their efficacy like their charge (nonionic, anionic or cationic), hydrophiliclipophilic balance (a measure of surfactant lipophilicity), and critical micellar concentration (the concentration at which surface tension reaches a minimum and surfactant monomers aggregate into micelles). Surfactants with hydrophilic-lipophilic balance values from 3 to 6 and 8 to 15 generally promote formation of water-in-oil and oil-in-water emulsions, respectively. Biodegradation of certain poorly soluble petroleum hydrocarbons may be inhibited by surfactants as a result of (i) toxicity by high concentration of surfactant or soluble hydrocarbon; (ii) preferential metabolism of the surfactant itself; (iii) interference with the membrane uptake process; or (iv) reduced bioavailability of miceller hydrocarbons (Efroymson and Alexander 1991, Mulligan *et al*.,2001; Rouse *et al.*, 1994; Van Hamme *et al.*,2003).

It has been known by Edward etal., since the year 1991, that , the effect of a surfactant through three

mechanisms can increase availability of organic compounds: dispersion of nonaqueous-phase liquid (NAPL) organics, leading to an increase in contact area caused by a reduction in the interfacial tension between the aqueous phase and the nonaqueous phase; increased apparent solubility of the pollutant, caused by the presence of micelles that contain high concentrations of HOCs (Edwards *et al*., 1991); and facilitated transport of the pollutant from the solid phase, which can be caused by lowering of the surface tension of the soil particle pore water, interaction of the surfactant with solid interfaces, and interaction of the pollutant with single surfactant molecules. The first mechanism is involved only when there is nonaqueous-phase liquid organics . Because both of the latter two mechanisms can cause an increase in the rate of mass transfer to the aqueous phase, the relative contributions of these two mechanisms to the enhancement of bioavailability of the substrate are confounded by Schippers *et al*. (Schippers, 2000), they gave three suppositions for the promotion of the biodegradation of PAHs by surfactants. In their comments, the first proposed pathway by bacteria were able to take up the hydrocarbons from the micellar core (Miller and Bartha,1989). In the second pathway, biosurfactants increased the mass transfer of hydrophobic organic compounds to the aqueous phase to make them accessible for microbes. In the third approached, the direct contact between cells and NALP facilitates by making changes in hydrophobicity by mean of surfactants (Randhir *et al*.,2003).

In another proposed mechanism, surfactants help microbes to be adsorbed to soil particles occupied by Hydrocarbon compounds, thus decreasing the diffusion path length between the site of adsorption and site of bio-uptake by the microbe (Tang *et al.,*1998; Poeton *et al,*1999; Randhir *et al.,*2003).

Effect of biosurfactants on Molecular weight of oil VR fractions

Molecular weight reduction in the residue fraction of heavy oils by a biological agent has been reported (Miller *et al*.,1989; Widdel and Rabus, 2001). There are few bacterial strains reported that act on paraffines and functionalize them. British Petroleum coined the concept of biological dewaxing in 1970 with some value added as a by product (Hamer and Al-Awadhi, 2000). Microbes can help in deposition control by producing metabolites (from carbon sources other than the oil) that improve the solubility of either waxes or asphaltenes, biotransform waxes and asphaltenes to more soluble products (through molecular weight reduction or functionalization), and biodegrade to remove the problematic compounds either from the oil or from existing deposits (lazer *et al,*1999). Rocha *et al*. Disclosed a method for preparing biosurfactants for use in making emulsions of high viscosity hydrocarbons such as high viscosity crude oil wherein the biosurfactant is a metabolite of Pseudomonas aeruginosa (USB-CS1). The resulting biosurfactant can be used to produce emulsion having a viscosity below about 500 centipoise and, more preferably, below about 100 centipoise at ambient temperatures.The production of biosurfactants in situ by microbial organisms grown in the presence of crude oil has also been reported in literature (Iqbal *et al*.,1995; Abalos *et al*,2004;

Mazaheri *et al.,* 2004; Amirian *et al.,* 2004; Chamanrokh *et al.,* 2010). Mostly the microbial produced biosurfactants assisted in the dispersal of crude oil in aquatic environment, thus facilitating the bioremediation of oil spills and chronic petroleum pol-

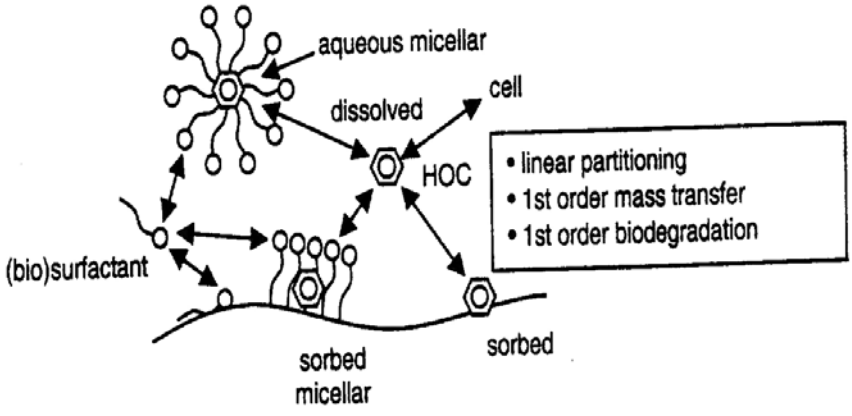

Fig. 13. The bioavailability model for syrfactant-enhanced biodegradation (Jordan *et al.,*1999)

lution. Of special authochthonous microorganisms or/ and genetically modified microorganisms used for bioremediation purposes, however, are not generally compatible with petroleum extraction and refining processes because they also attack and catabolize (destroy) combustible hydrocarbons(Leon and Kumar,2005; Chamanrokh *et al.,* 2008).

Undesirable water in oil (W/O) emulsions occurs throughout oil production, transportation, and processing, and represents a major problem in heavy crude oil. Crude oil emulsions are complex and the emulsifying agents may be amphiphilic molecules from the oil, especially the resin fraction, including naphthenic acids, asphaltenes, fine solids, including clays, scale, wax crystals or by microorganisms. De-emulsification in the oil industry is challenging due to the variety of possible emulsion properties, and treatments are currently tailored to each site and adapted over time. Various microbes including Nocardia amarare, Pseudomonas sp., Corynebacterium petrophilum, Rhodococcus auranticus, Bacillus subtilis, and Micrococcus sp. are known to exhibit demulsification activity. Some biologically produced agents like glycolipids, polysaccharide, glycolipids, glycoproteins, phospholipids and rhamnolipids destabilize petroleum emulsions.

Since 1982, it have been proven that , the bacterial cell surface is responsible for major demulsifying activity of some microorganisms (Cairns *et al.*,1982, Cooper 1982). It is more than three decades of research on biosurfactant but till today, the applicability of biotechnology to asphaltene- or solids- stabilized emulsions have not been studied throughly. There is a work done by (Leon and Kumar,2005) proved that, biologically produced molecules may be effective in removing or dispersing asphaltenes or wax crystals, particularly in combination with suitable cell-surface properties to aid in dispersion of the heavy crude oil or in aiding flocculation. Further more, not much work implemented in this regards.

Biosurfactant Genetic engineering supporting the concept of "biorefining"

Genetic engineering consists in modifying in a determinate way the genetic material of microorganisms of industrial interest so that they acquire new or enhanced capabilities.

For this, novel DNA sequences are created by artificially joining together strands of DNA from different organisms through the use of recombinant DNA technology. The design of recombinant microorganisms for petroleum biorefining includes the construction of microorganisms:

- able to transform the different types of compounds present in petroleum,
- possessing higher activities compatible with the design of efficient and economically viable processes,
- capable to secrete biosurfactants to increase the bioavailability of hydrocarbons to be transformed,
- stable under process conditions (e.g. solvent-resistant microorganisms)(Borgne and Quintero, 2003).

One of the main purposes of microbial genetic engineering in oil industry is to increase the biosurfactant secretion to promote the bioavailability of hydrocarbons particularly the heavy fractions, to be transformed; or to be used in bioremediation of hydrocarbon contaminated soils or MEOR.

Among all the biosurfactants reported till date, the molecular biosynthetic regulation of rhamnolipid, a glycolipid type biosurfactant produced by *Pseudomonas aeruginosa* and a lipopeptide biosurfactant called surfactin produced by *Bacillus subtilis* were the first to be famous. Other biosurfactants whose molecular genetics have been introduced in later years included arthrofactin from *Pseudomonas* species, iturin and lichenysin from *Bacillus* species, mannosylerythritol lipids (MEL) from *Candida* and emulsan from *Acinetobacter* species (Mazaheri *et al*.,2004).

Quorum sensing, a cell density dependent gene regulation process allowing bacterial cells to express certain specific genes on attaining high cell density, regulates the production of some biosurfactants**.** It had been reported that low-molecular-mass signal molecules (such as the furanosyl borate diester AI-2) are involved in biosurfactant production from different bacteria (Daniels *et al*., 2004). However, whether quorum sensing is the environmental cue to biosurfactant production in general is not known(Das *et al.*,2008).

The yield of all biotechnological products relies upon the producer's genetic that cause the type and amount of the metabolite production. Furthermore, to economize further the production process and to obtain products with better commercially important properties, recombinant and mutant hyperproducers seems necessary (Mukherjee *et al*.,2006). Various methods and agents have been reported in literature to produce biosurfactant hyperproducers. A summery some examples of these mutants are given in Table 2.

CONCLUSION

Oil spill has been a massive catastrophic effect on the environment. World experts believes Crude oil spill have done immense damage to the biodiversity, environment and businesses. The use of surfactants is among the most effective ways of removing hydrocarbons from the environment. Oil spills can be removed using different mixtures of surfactants. These bioactive

Table 2. Mutant and recombinant strains of microorganisms with enhanced biosurfactant yields and with improved product characteristics after Mukherjee 2006

Mutant and/or recombinant strain	Characteristic feature	Increased yield and/or improved production properties
Pseudomonas aeruginosa 59C7	Transposon Tn5-GM induced mutant of *Pseudomonas aeruginosa* PG201(Koch., *et al*. 1991)	2 times more production
Pseudomonas aeruginosa PTCC 1637	Random mutagenesis with *N*-methyl-*N*?nitro-*N*-nitrosoguanidine (Tahzibi *et al.,* 2004)	10 times more production
Bacillus licheniformis KGL11	Random mutagenesis with *N*-methyl-*N*?nitro-*N*-nitrosoguanidine (Lin *et al.,* 1998)	12 times more production
B. subtilis ATCC 55033	Random mutagenesis with *N*-methyl-*N*?nitro-*N*-nitrosoguanidine (Carrera *et al.*, US Patent no. 5,264,363 & 5,227,294)	Approximately 4–6 times (2–4 g /l crude surfactin)
Pseudomonas aeruginosa EBN-8	Gamma ray induced mutant of *Pseudomonas aeruginosa* S8 (Iqbal, *et al.*, 1995)	2–3 times more production
Bacillus subtilis Suf-1	Ultraviolet mutant of *Bacillus subtilis* ATCC 21332 (Mulligan, C.N. *et al.,* 1989)	3–4 times more production
Acinetobacter calcoaceticus RAG-1 mutants	Mutant selection on basis of resistance to cationic detergent CTAB (Shabtai, Y. and Gutnick,1986)	2–3 times more production
Recombinant *Bacillus subtilis* MI 113	Incorporation of a plasmid containing *lpa-14* gene (Ohno, *et al*.,1995)	8 times more surfactin production
B. subtilis SD901	Random mutagenesis with N-methyl-N?-nitro-N-nitrosoguanidine (Yoneda, T. *et al.*, US patent no 7,011,969)	4–25 times more surfactin production (8–50 g/l)
Recombinant *Bacillus subtilis* strain ATCC 21332	Contains recombinantly modified peptide synthetase (Symmank *et al*.,2002)	Production of lipohexapeptide with reduced toxicity
Recombinant *Bacillus subtilis*	Produced by whole enzyme module swapping (Yakimov *et al*., 2000)	Production of lichenysin
Recombinant *Pseudomonas aeruginosa* strains	Insertion of *E. coli lacZY* genes into the chromosomes of *Pseudomonas aeruginosa* strains PAO-1 and PG-201 (Koch *et al*.,1988)	Use of lactose- and whey-based cheap substrates
Recombinant *Pseudomonas putida* KT2442 and *P. fluorescens*	Expression of cloned *rhl*AB genes in heterologous hosts (Ochsner *et al*.,1995)	Production of *P. aeruginosa* rhamnolipids in nonpathogenic stains
Recombinant *Gordonia amarae*	Stable maintenance and expression of *Vitreoscilla* hemoglobin gene (*vgb)* (Dogan *et al*.,2006)	4 times more production of trehalose lipid biosurfactant

material applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry The development of new surfactants has long been acknowledged to need continuous technology improvement and adoption for maximum economic benefit.

A part from environment, in refineries crude oil is first preheated in a heat exchanger network. Hot crude oil is then separated in an atmospheric distillation column (CDU) into different fractions (naphtha, kerosene, gasoline). Heavy fuel oil related streams produced by atmospheric distillation comprise fractions of crude oil separated by heating at atmospheric pressure. The vacuum residual refinery streams comprise a heterogeneous group of poorly defined, viscous, high boiling hydrocarbon streams that usually contain suspensions of resin/asphalting complexes in the form of colloidal dispersed particles. These streams often have high levels of heterocyclic aromatic and naphthenic compounds using special biosurfactant produced by specific strain of genetically engineered. Usually novel DNA sequences are created by artificially joining together strands of DNA from different organisms through the use of recombinant DNA technology. This review has focused on the identification of emerging and developing biosurfactant technologies that can, when fully developed, either be applied directly to not only cleaning the environment but also upgrade Vacuum bottom residue and very heavy crudes, or are integral to new approaches to upgrading. This approach can have some important and economically attractive side benefits in the main vacuum bottom residue upgrading process selection.

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