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Production, Characterisation and Applications of Biosurfactants-Review

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Abstract: Biosurfactants are surface active compounds released by microorganisms. They are biodegradable non-toxic and ecofreindly materials. In this review we have updated the information about different microbial surfactants. The biosurfactant production depends on the fermentation conditions, environmental factors and nutrient availability. The extraction of the biosurfactants from the cell-free supernatant using the solvent extraction procedure and the qualitative and quantitative analysis has been discussed with appropriate equipment details. The application of the biosurfactant includes biomedical, cosmetic and bioremediation. Rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* DS10-129 showed significant applications in the bioremediation of hydrocarbons in gasoline-spilled soil and petroleum oily sludge. Rhamnolipid biosurfactant enhanced the bioremediation process by releasing the weathered oil from the soil matrices and enhanced the bioavailability of hydrocarbons for microbial degradation. It is having potential applications in the remediation of hydrocarbon contaminated sites.

Key words: Rhamnolipid, fermentation, emulsification, bioremediation, qualitative analysis, quantitative analysis

INTRODUCTION

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi (Chen *et al*., 2007; Mata-Sandoval *et al*., 1999, 2000) from various substances including sugars, oils and wastes. However, carbohydrates and vegetable oils are among the most widely used substrates for research on biosurfactant production by *Pseudomonas aeruginosa* strains (Rahman *et al*., 2002a, b, 2003; Raza *et al*., 2007). The amphiphiles that form micelles can be potentially used for surface chemical works, are termed as SURFace ACTive AgeNTS or SURFACTANTS. Soaps and detergents can be described as having similar characteristics as surfactants. All surfactants have two ends namely, a hydrocarbon part which is less soluble in water (hydrophobic end). The hydrophobic part of the molecule is a long-chain of fatty acids, hydroxy fatty aids, hydroxyl fatty acids or α-alkyl-β-hydroxy fatty acids. The water soluble end (hydrophilic) can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. Additionally, the hydrophobic moiety is usually a C8 to C22 alkyl chain or alkylaryl that may be linear or branched (Van Ginkel, 1989).

The unique properties of biosurfactants allow their use and possible replacement of chemically synthesised surfactants in a number of industrial operations (Kosaric, 1992). Biosurfactants reduce surface tension, Critical Micelle Concentration (CMC) and interfacial tension in both aqueous solutions and hydrocarbon mixtures (Rahman *et al*., 2002c, d; Banat, 1995).

GENERAL CLASSIFICATION OF BIOSURFACTANTS

Surfactants can be classified according to the nature of the charge on individual polar moiety. Anionic surfactants are negatively charged usually due to a sulphonate or sulphur group. Non-ionic surfactants lack ionic constituent and the majority of all non-ionics are polymerisation products of 1, 2-epoxyethane. Cationic surfactants are characterised by a quaternary ammonium group which is positively charged. Lastly, amphoteric surfactants have both positively and negatively charged moieties in the same molecule (Van Ginkel, 1989). Biosurfactants can also be grouped into two categories namely; low-molecular-mass molecules with lower surface and interfacial tensions and high-molecular-mass polymers, which bind tightly to surfaces (Rosenberg and Ron, 1999). Examples of low-molecular-mass molecules are rhamnolipids (Lang and Wullbrandt, 1999; Cohen and Exerowa, 2007), sophorolipids (Davila *et al*., 1997) whilst food emulsifiers (Sheperd *et al*., 1995) and biodispersan (Rosenberg, 1993) are some of the examples of high-molecular-mass polymers.

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Table 1: Type and microbial origin of biosurfactants (Mulligan, 2005)

Type of biosurfactant	Microorganism
Trehalose lipids	*Arthrobacter paraffineus*
	Corynebacterium sp.
	Mycobacterium sp.
	Rhodococcus erythropolis, *Norcardia* sp.
Rhamnolipids	*Pseudomonas aeruginosa*
	Pseudomonas sp., *Serratia rubidea*
Sophorolipids	*Candida apicola*, *Candida bombicola*
	Candida lipolytica
	Candida bogoriensis
Glycolipids	*Alcanivorax borkumensis*
	Arthrobacter sp., *Corynebacterium* sp.
	R. erythropolis, *Serratia marcescens*
	Tsukamurella sp.
Cellobiose lipids	*Ustilago maydis*
Polyol lipids	*Rhodotorula glutinus*
	Rhodotorula graminus
Diglycosyl diglycerides	*Lactobacillus fermentii*
Lipopolysaccharides	*Acinetocbacter calcoaceticus* (RAG1)
	Pseudomonas sp., *Candida lipolytica*
Arthrofactin	*Arthrobacter* sp.,
Lichenysin A, Lichenysin B	*Bacillus licheniformis*
Surfactin	*Bacillus subtilis*, *Bacillus pumilus*
Viscosin	*Pseudomonas fluorescens*
Ornithine, lysine peptides	*Thiobacillus thiooxidans*
	Streptomyces sioyaensis
	Gluconobacter cerinus
Phospholipids	*Acinetocbacter* sp.
Sulfonylipids	*T. thiooxidans*
	Corynebacterium alkanolyticum
Fatty acids	*Capnocytophaga* sp.
(Corynomycolic acids,	*Penicillium spiculisporum*
spiculisporic acids, etc.)	*Corynebacterium lepus*
	Arthrobacter paraffineus
	Talaramyces trachyspermus
	Norcadia erythropolis
Alasan	*Acinetobacter radioresistens*
Streptofactin	*Streptomyces tendae*
Particulate surfactant (PM)	*Pseudomonas marginalis*
Biosur PM	*Pseudomonas maltophila*

Various micro-organisms are known to produce specific kind of biosurfactants. This depends on mainly the molecular composition of the type of biosurfactant produced. For instance, *Pseudomonas aeruginosa* DS10-129 was used to produce rhamnolipid (Rahman *et al*., 2002a, b, 2003), sophorose lipid by *Torulopsis bombicola* and *Bacillus subtilis* ATCC 2132 which was used by Davis *et al*. (2001) to produce surfactin. Kosaric (1992) classified biosurfactants based on their structure namely; hydroxylated and cross-linked fatty acids, polysaccharide-lipid complexes, glycolipids, lipoproteins-lipopeptides, phospholipids and complete cell surfaces. On the other hand, Biermann *et al*. (1987) group biosurfactants as glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids, polymeric and particulate compounds (Table 1). Lastly, Healy *et al*. (1996) group biosurfactants into four main categories namely, glycolipids, phospholipids, lipoproteins/ lipopepetides and polymeric.

TYPES OF BIOSURFACTANTS

There are many types of biosurfactants each produced by a specific micro-organism. The following are some of the various types of biosurfactants.

Glycolipids: Most known biosurfactants are glycolipids. They consist of mono-, di-, tri- and tetrasaccharides which include glucose, mannose, galactose, glucuronic acid, rhamnose and galactose sulphate. The fatty acid component usually has a composition similar to that of phospholipids of the same micro-organism (Veenanadig *et al*., 2000; Chen *et al*., 2007). Also, they are made up of carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids (Desai and Banat, 1997). Among the glycolipids, the best known are the rhamnolipids, trehalolipids and sophorolipids (Desai and Banat, 1997; Karanth *et al*., 1999) and the best-studied glycolipid bioemulsifiers, rhamnolipds, trehalolipids and sophorolipids are disaccharides that are acylated with long-chain fatty acids or hydroxyl fatty acids (Rosenberg and Ron, 1999).

Rhamnolipids: Bacteria of the genus *Pseudomonas* are known to produce glycolipid surfactant containing rhamnose and 3-hydroxy fatty acids (Lang and Wullbrandt, 1999; Rahman *et al*., 2002b). Rhamnolipids produced by *Pseudomonas aeruginosa* have been widely studied and reported as a mixture of homologous species RL1 ($RhC_{10}C_{10}$), RL2 (RhC_{10}), RL3 ($Rh_2C_{10}C_{10}$) and RL4 (Rh_2C_{10}) (Syldatk and Wagner, 1987; Lang and Wagner, 1987; Rahman *et al*., 2002b). Using virgin olive oil (Healy *et al*., 1996), a rhamnolipid was produced by *Pseudomonas fluorescens* NCIMB 11712 that is a methyl pentose monosaccharide. Disaccharide rhamnolipids are formed by condensing two moles of rhamnose sugar and an acetal group links the hydrophobic group. However, the lipid part of the molecule contains ester and carboxyl groups. Rhamnolipids produced by *Pseudomonas aeruginosa* strains are among the most effective surfactants when applied for the removal of hydrophobic compounds from contaminated soils (Rahman *et al*., 2006). They posses low average minimum surface tension of (30-32 mN m^{-1}); high average emulsifying activity of (10.4-15.5 U mL^{-1} filtrate), low critical micelle concentration (CMC) (5-65 mg L^{-1}) and high affinity for hydrophobic organic molecules (Van Dyke *et al*., 1993).

Sophorolipids: They are group of biosurfactants produced by *Torulopsis* sp. Sophorolipids (SLs) consist of a dimeric sugar (sophorose) and a hydroxyl fatty acid,

linked by a β-glycosidic bond (Asmer *et al*., 1988). According to Hu and Ju, (2001) there are two types of SLs namely, the acidic (non-lactonic) SLs and the lactonic SLs. The hydroxyl fatty acid moiety of the acidic SLs has a free carboxylic acid functional group whilst that of the lactonic SLs forms a macrocyclic lactone ring with the 4″-hydroxyl group of the sophorose by intramolecular esterificaion. Until recently, lactonic SLs have been reported to have attracted more commercial and scientific attention than their acidic counterparts. They have measurable biocide activity (Lang *et al*., 1989), whilst the acetylated lactonic SLs have been applied in cosmetics as antidandruff, bacteriostatic agents and deodorants (Mager *et al*., 1987).

Trehalolipids: Another group of glycolipids are the trehalolipids, the serpentine group seen in many members of the genus *Mycobacterium* is due to the presence of trehalose esters on the cell surface (Asselineau and Asselineau, 1978). Disaccharide trehalose linked at C-6 and C-6′ to mycolic acid is associated with most species of *Mycobacterium*, *Norcardia* and *Corynebacterium*. Mycolic acids are long-chain, α-branched-β-hydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms and the degree of unsaturation (Desai and Banat, 1997). Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. were found to lower the surface and interfacial tensions in culture broth from 25-40 and 1-5 mN m^{-1}, respectively (Li *et al*., 1984).

Lipoproteins and Lipopeptides: Lipopepetides called surfactin are produced by *Bacillus* sp. containing seven amino acids bonded to a carboxyl and hydroxyl groups of a 14-carbon acid. Surfactin just as any other biosurfractant reduces surface tension from 72-27 mN m^{-1} with concentrations as low as 0.005%, making surfactin one of the most powerful biosurfactants (Kakinuma *et al*., 1969). The cyclic lipopeptide surfactin produced by *Bacillus subtilis* ATCC 21332 is an example of one of the most powerful biosurfactants. Another important characteristic of surfactin is its ability to lyse mammalian erythrocytes and to form spheroplasts (Bernheimer and Avigad, 1970). This property is been used to detect surfactin production through haemolysis on blood agar.

Fatty acids: Fatty acids produced from alkanes as a result of microbial oxidations have been considered as surfactants (Rehn and Reiff, 1981). In addition to the straight-chain acids, micro-organisms produce complex fatty acids containing OH groups and alkyl branches. Examples of such complex acids include Corynomucolic acids that are also surfactants (Kretschner *et al*., 1982). The hydrophilic or lipophilic balance of fatty acids is clearly related to the length of the hydrocarbon chain. For lowering surface and interfacial tensions, the most active saturated fatty acids are in the range of C12-C14 (Rosenberg and Ron, 1999).

Phospholipids: Phospholipids are known to form major components of microbial membranes. When certain hydrocarbon-degrading bacteria or yeast are grown on alkane substrates, the level of phospholid increases greatly. For instance, using hexadecane-grown *Acinetobacter* sp. HO1-N, phospholipids (mainly phosphatidylethanolamine) rich vesicles were produced (Kaeppeli and Finnerty, 1979). Phospholipids have been quantitatively produced from *Thiobacillus thiooxidans* that are responsible for wetting elemental sulphur necessary for growth (Beeba and Umbriet, 1971). Phosphatidylethanolamine produced by *Rhodococcus erythropolis* grown on n-alkane resulted in the lowering of interfacial tension between water and hexadecane to less than 1 mN m^{-1} and CMC of 30 mg L^{-1} (Kretschner *et al*., 1982).

Polymeric biosurfactants: Emulsan, liposan, mannoprotein and polysaccharide-protein complexes are known to be the best-studied polymeric biosurfactants (Desai and Banat, 1997). Using *Acinetobacter calcoaceticus* RAG-1, Rosenberg *et al*. (1979) extracted a potent polyanionic amphipathic heteropolysaccharide bioemulsifier called emulsan. It is a very effective emulsifying agent for hydrocarbons in water even at a concentration as low as 0.001-0.01%. Additionally, it is noted as one of the most powerful emulsion stabilizers known with the ability to resist inversion even at a water-to-oil ratio of 1:4 (Zosim *et al*., 1982). Ciriglian and Carman (1984) synthesised liposan, an extracellular water-soluble emulsifier using *Candida lipolytica*. It is composed of 83% carbohydrate and 17% protein with the carbohydrate portion being a heteropolysaccharide consisting of glucose, galactose, galactosamine and galactoronic acid. Cameron *et al*. (1988) demonstrated the production of large amounts of mannoprotein by *Saccharomyces cerevisiae*. When purified, the emulsifier contains 44% mannose and 17% protein. The mannoprotein exhibited excellent emulsifying activity toward several oils, alkanes and organic solvents. Other polymeric biosurfactants such as biodispersan, alasan, food emulsifiers, protein complexes and insectides emulsifiers have also been reported.

BIOSURFACTANT PRODUCTION

Biosurfactants are usually produced extracellularly or as part of cell membrane by yeast, bacteria or filamentous fungi (Mata-Sandoval *et al*., 1999). Different kinds of bacteria have been employed by many researchers in producing biosurfactant using culture media. Most of such bacteria used are isolated from contaminated sites usually containing petroleum hydrocarbon by products and/or industrial wastes (Rahman *et al*., 2006; Benincasa 2007).

Factors affecting biosurfactant production: A number of factors affect the production of biosurfactants. These factors include environmental factors as well as source of carbon substrate among others.

Environmental factors: Biosurfactant production like any other chemical reaction is affected by a number of factors that either increase its productivity or inhibit it. Accordingly, environmental factors such as pH, salinity and temperature affect biosurfactant production (Rahman *et al*., 2002b; Ilori *et al*., 2005; Raza *et al*., 2007). During *In situ* applications, bacteria for Microbially Enhanced Oil Recovery (MEOR) must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity and low oxygen level. Additionally, it was found out that biosurfactant produced from *Pseudomonas strains* MEOR 171 and MEOR 172 were not affected by temperature, pH and Ca, Mg concentration in the ranges found in many oil reservoirs (Karanth *et al*., 1999). Desai and Banat (1997) also affirm the fact that environmental factors and growth conditions such as pH, temperature, agitation and oxygen availability also affect biosurfactant production through their effects on cellular growth or activity. Salt concentrations also affect biosurfactant production depending on its effect on cellular activity. Some biosurfactants however, were not affected by salt concentrations up to 10% (w/v), although slight reductions in the CMCs were detected (Abu-Ruwaida *et al*., 1991).

Carbon substrates for biosurfactant production: A number of carbon substrates have been used in many researches during biosurfactant production. Indeed the type, Quality and quantity of biosurfactant production are affected and influenced by the nature of the carbon substrate (Singer, 1985; Raza *et al*., 2007). Diesel and crude oil were identified to be good sources of carbon for biosurfactant production by organisms (Ilori *et al*., 2005). Other water soluble compounds such as glucose, sucrose and glycerol have also been reported to be a source of carbon substrate for biosurfactant production (Desai and Banat, 1997; Rahman *et al*., 2002a). In the treatment of wastewater (Pagilla *et al*., 2002) used soluble acetate and sparingly soluble hexadecane as carbon substrate for *Gordonia amarae* growth and biosurfactant production in large scale batch reactors. It has become evident that the importance of carbon substrates does have a major role to play on the biosurfactant production. It was noted that carbon sources such as nutrient concentrations, pH and age of the culture affects the yield of rhamnolipid production. On a positive note, hydrophobic substrates like corn oil, lard (rich in unsaturated and saturated fat) and long chain alcohols maximized biosurfactant production (100-165 mg g^{-1} substrate). Contrarily, hydrophilic substrates like glucose and succinate delivered poor yields (12-36 mg g^{-1} substrate) (Mata-Sandoval *et al*., 2000). Lastly, Robert *et al*. (1989) attests to the fact that *Pseudomonas aeruginosa* can be produced from a variety of carbon sources such as C11 and C12 alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol.

Estimation of biosurfactant activity: This involves measuring the changes in surface and interfacial tensions, stabilization/destabilization of emulsions and hydrophilic-lipophilic balance (HLB). Using a tensiometer, the surface tension at air/water and oil/water interfaces can be easily determined. The surface tension of distilled water is noted to be 72 mN m^{-1} and an addition of biosurfactant lowers it to as low as 28 mN m^{-1} (Rahman *et al*., 2006). Thus adding a biosurfactant to water reduces its surface tension to a critical level above which amphiphilic molecules readily form supramolecular structures like micelles, bilayers and vesicles known as Critical Micelle Concentration (CMC). CMC is therefore defined as the ability of a biosurfactant within an aqueous phase and is commonly used to measure the efficiency of a biosurfactant (Desai and Banat, 1997).

Analytical methods: A number of analytical methods have been employed by many researchers in their analyses and in some cases characterisation of biosurfactants. In the Table 2, the type of biosurfactant, bacteria, solvent, supporting references and type of analytical method used are shown.

Table 2: Analytical methods used for the qualitative and quantitative analysis of biosurfactant

Biosurfactant and bacteria	Analytical method	Chemicals/Solvents required	Reference
Rhamnolipids			
Pseudomonas aeruginosa	TLC	$CHCl_3/CH_3OH/CH_3COOH$	Arino *et al.* (1996)
	HPLC	$CHCl_3/CH_3OH$	Rahman *et al.* (2002b)
	HPLC	CH_3CN	Chayabutra *et al.* (2001)
		2-Propanol-NH_4OH-H_2O	Chayabutra *et al.* (2001)
	Western blot		Olvera *et al.* (1999)
	TLC	Carbenicillin, Tetracycline	Olvera *et al.* (1999)
	HPLC	CH_3CN-H_2O	Schenk *et al.* (1995)
	HPLC	Tetrahydrofuran-H_2O	Sekelsky and Shreve (1999)
	HPLC	CH_3CN/Phosphate buffer pH 6	Wild *et al.* (1997)
	TLC	CH_3OH/H_2O	Rahman *et al.* (1999)
	FTIR		Wu and Ju (1998)
	TLC	Solv. A: $CHCl_3/CH_3OH/CH_3COOH$	Wu and Ju (1998)
		Solv. B: 2-Propanol-NH_4OH-H_2O	Wu and Ju (1998)
P. aeruginosa LBI	HPLC	CH_3CN/H_2O	Benincasa *et al.* (2002)
	TLC	$CHCl_3/CH_3OH/H_2O$	Benincasa *et al.* (2002)
P. aeruginosa 57RP	HPLC-MS	CH_3CN/H_2O	Deziel *et al.* (2000)
	TLC	$CHCl_3/CH_3OH/CH_3COOH$	Deziel *et al.* (2000)
P. aeruginosa UG2	HPLC	CH_3CN-H_3PO_4	Mata-Sandoval *et al.* (2000)
	ESI	N_2	Mata-Sandoval *et al.* (2000)
	HPLC-UV	CH_3CN-H_3PO_4	Mata-Sandoval *et al.* (1999)
P. aeruginosa 47T2	HPLC	CH_3CN/CH_3COOH	Haba *et al.* (2000)
	TLC	$CHCl_3/CH_3OH/CH_3COOH$	Haba *et al.* (2000)
P. fluorescens	TLC	CH_3CN/H_2O	Caldini *et al.* (1995)
Lpopeptide			
Bacillus licheniformis	FTIR		Thaniyavarn *et al.* (2003)
	HPLC-MS	CH_3CN/TFA	Thaniyavarn *et al.* (2003)
Sophorolipid			
Candida bombicola	HPLC with ELSD		Davila *et al.* (1997)
Torulopsis sp.	HPLC-UV	CH_3CN/H_2O	Hu and Ju (2001)
	FTIR		Hu and Ju (2001)
Phospholipid			
Acinetobacter sp.	GC-MS	$CHCl_3/CH_3OH$ (Extraction Method)	Koma *et al.* (2001)
Trehalose lipid			
Rhodococcus sp. P32C1	HPLC	CH_3CN	Maghsoudi *et al.* (2001)
Surfactin			
Bacillus subtilis ATCC 21332	HPLC	CH_3CN/TFA	Davis *et al.* (2001)

TLC = Thin Layer chromatography; HPLC = High Performance Liquid Chromatography; FTIR = Fourier transform infrared spectroscopy; GC/MS = gas Chromatography with Mass Spectroscopy

APPLICATIONS OF BIOSURFACTANT

A number of applications of biosurfactants have been researched into and published. Its usefulness to man in most aspects of human life can not be over emphasised. The enormous market demand for surfactants is currently met numerous synthetic, mainly petroleum-based chemical surfactants. These compounds are usually toxic to the environment and as well as been non-biodegradable. Furthermore, they may bio-accumulate and their production, processes and by-products can be environmentally hazardous. It has become necessary that tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest in biosurfactants as possible alternates to chemical surfactants (Banat *et al.*, 2000; Benincasa, 2007). Biosurfactants are beginning to acquire a status as potential performance-effective molecules in various fields. Presently, biosurfactants are mainly used in studies on enhanced oil recovery and hydrocarbon bioremediation (Rahman *et al.*, 2004, 2006). The worldwide production of surfactants amounted to 17 million metric tonnes (t) in 2000 (including soaps) with expected future growth rates of 3-4% $year^{-1}$ globally and 1.5-2.0% in the EU (Whalley, 1995). Industrial applications of surfactants are classified according to how they are applied. These are surfactants used in detergents and cleaners (54%); as auxiliaries for textiles, leather and paper (13%); in chemical processes (10%); in cosmetics and pharmaceuticals (10%); in the food industry (3%); in agriculture (2%) and in others (8%).

Biosurfactants and bioremediation: Oil spillage during offshore production (drilling) and its transport from one location to another is seriously affecting aquatic life. An explicit example is the massive oil spillage as well as release during the Gulf War from 1991 to 1992. It was estimated that some 11 million barrels of oil was released into the Arabian Gulf from January to May 1991, polluting

more than 800 miles of Kuwait and Saudi Arabian coastline. The cost of clean-up has been estimated at more than $700 million. The oil released in to the Gulf produced devastating consequences on the marine wildlife of the area, including endangering hawksbill and green turtles, thousands of cormorants (a type of marine bird) as well as 400-500 tons of fishes died in the Gulf as a result of exposure to oil or polluted water. Additionally, (Shaw, 1992; Burns *et al*., 1993; Burger, 1993) it was identified that several oil pollution accidents at high seas and on beaches have resulted in enormous ecological and social catastrophes. Rahman *et al*. (2003, 2004, 2006) examined the bioremediation of n-alkanes in petroleum sludge containing an oil and grease content of 87.4%. Remarkably, 10% of the sludge constituting C8-C11 alkanes were degraded 100%; whilst C12-C21, 83-98%; C22-C31 between 80-85% and finally C32-C40, 57-73% after 56 days with addition of a bacterial consortium, nutrients and rhamnolipids. In another experiment, (Hayes *et al*., 1986) demonstrated that when Boscan Venezuelan heavy crude oil was treated with emulsan, oil viscosity was reduced from 200,000 to 100 Cp. Hence, it became visible to pump heavy oil 26,000 miles in a commercial pipeline after this treatment although conventional chemical surfactant treatment failed. Biosurfactants are also used in bioremediation of sites contaminated with toxic heavy metals like uranium, cadmium and lead (Miller, 1995; Mulligan and Wang, 2006). Shafeeq *et al*. (1989) showed that hexadecane, octadecane and nanodecane incubated for a 28 day period under laboratory conditions with the *Pseudomonas aeruginosa* isolate S8 obtained from oil-polluted sea water degraded the hydrocarbons by 47, 58, 73 and 60%, respectively. The application of rhamnolipid produced by *Pseudomonas aeruginosa* DS10-129 along with poultry litter and coir pith enhanced *ex situ* bioremediation of a gasoline-contaminated soil (Rahman *et al*., 2002a). Benzene, toluene, ethylbenzene, xylene and trimethylbenzene were degraded according to Kosaric (2001) by adding microbial consortium to soil contaminated with gasoline and enriched with nutrients and oxygen.

Other biosurfactant applications: Biosurfactants have also been applied in food industries usually as food additives (emulsifiers). For instance, lectin and its derivatives, fatty acid esters containing glycerol, sorbitan or ethylene glycol and ethoxylated derivatives of monoglycerides including recently synthesized oligopeptide (Bloomberg, 1991). These emulsifiers have a long way to improving the flavour, taste and quality of products with minimal health hazards. The agriculture industry has also benefited from the production of biosurfactants. Stanghellini and Miller (1997) demonstrated that rhamnolipids are highly effective against three representative genera of zoosporic plant pathogens; *Pythium aphanidermatum*, *Phytophthora capsici* and *Plasmopara lactucea-radicis*. Hence, purified mono-and di-rhamnolipids with concentrations ranging from 5-30 mg L^{-1} caused cessation of motility and lysis of the entire zoospore population in less than 1min. Bioemulsifiers are potentially used in various formulations of herbicides and pesticides (Rosenberg and Ron, 1999). An example is the use of bioemulsifiers (glycolipopeptides) produced by strains of *Bacillus* for emulsifying immiscible organophosphorus pesticides (Patel and Gopinathan, 1986). Biosurfactant applications in cosmetic and pharmaceutical industries have also been reported (Cameotra and Makkar, 2004).

BIOSURFACTANT AND CO_2 EMISSIONS

Greenhouse effect is a naturally occurring process that aids in heating the earth's surface and atmospheric gases such as CO_2, water vapour and methane that are able to change the energy balance of the planet by absorbing long wave radiation (infra red) emitted from the earth's surface. Studies have shown that biosurfactants a have a role to play in the reduction, if not total elimination of CO_2 emission into the atmosphere. No

Table 3: Potential to substitute petrochemical by oleochemical surfactants in the EU by 2010 (Patel, 2004)

Surfactants	EU production 1998 (kt)	High RRM scenario 2010 (kt)	Change (%)
Anionic			
LAS-Pc	409	409	0
SAS-Pc	69	69	0
AS-Pc	43	16	-63
AS-Oleochemical	64	91	42
AE_3S-Pc	74	37	-50
AE_3S-Oleochemical	172	209	21
Other anionics-Pc	47	28	-42
Other anionics-Oleochemical	32	51	63
Non-ionic			
AE-Pc	255	128	-50
AE-Oleochemical	383	510	33
Other-ethoxylates-Pc*	26	26	0
Other-ethoxylates-Oleochemical†	233	233	0
Total	1,807	1,807	-
Oleochemical surfactants	884(49%)	1,095(61%)	+24
Petrochemical surfactants	923(51%)	712(39%)	-23

This table covers only the most important surfactants while cationic, amphoteric and some of the nonionic surfactants are excluded, RRM = Renewable raw materials; AE = Alcohol ethoxylate; AES = Alcohol ether sulphate; AS = Alcohol sulphate; LAS = Linear alkylbenzene sulphate; Pc = Petrochemical feedstock; PKO = Palm kernel oil; CNO = Coconut oil; PO = Palm oil; SAS = Secondary alkane sulphonate, * Containing 7 ethylene oxide (EO) units on average, † Average of 7 EO units based on PKO and CNO and 11 EO units based on PO

wonder the 1997 UNFCCC Kyoto Protocol was adopted to curtail the emission of greenhouse gases (United Nations Framework Convention on Climate Change, 1997). Assuming that the total surfactant production remains constant until 2010 in EU, it was estimated that the amount of oleochemical surfactants could be increased from about 880 kt in 1998 to approximately 1,100 kt in 2010, an increase of 24%. This substitution reduces the life- cycle CO_2 emissions from surfactants by 8%. The theoretical maximum potential for total substitution is 37% (Table 3). Since the surfactant market is expected to grow, the avoided emissions are expected to exceed 8% of the current life-cycle CO_2 emissions from surfactants. Furthermore, in 1998, an estimated 1.5 million tons of CO_2 emissions were avoided by the production of oleochemical surfactants (Patel, 2004).

MERITS OF BIOSURFACTANTS

Researches have shown that biosurfactants exhibit many advantages over chemically synthesized surfactants. The following are some of the advantages of biosurfactants (Kosaric, 1992; Mulligan and Wang, 2006).

- **Biodegradability:** Biosurfactants are easily degraded by bacteria and other microscopic organisms; hence they do not pose much threat to the environment.
- **Generally low toxicity:** For instance glycolipids from *Rhodococcus* sp. 413A were 50% less toxic than Tween 80 in naphthalene solubilization tests (Kanga *et al*., 1997).
- **Biocompatibility and digestibility:** This ensures their application in cosmetic, pharmaceuticals and as functional food additives.
- **Availability of raw material:** Biosurfactants can be produced from cheap raw materials that are available in large quantities.
- **Acceptable production economics:** Depending on its application, biosurfactants can also be produced from industrial wastes and by-products and this is of particular interest for their bulk production.
- **Use in environmental control:** Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and bioremediation of contaminated soil.
- **Specificity:** Biosurfactants being complex organic molecules with specific functional groups are often specific in their action. This would be of particular interest in detoxification of specific pollutants, de-emulsification of industrial emulsions, specific cosmetic, pharmaceutical and food applications.

DEMERITS OF BIOSURFACTANTS

Despite the numerous advantages that biosurfactants have been known to exhibit, it is also known to have the following associate demerits (Kosaric, 1992).

- Large scale production of biosurfactants may be expensive. However this problem could be overcome by coupling the process to utilization of waste substrates, combating at the same time their polluting effects that balance the overall costs.
- There is difficulty in obtaining pure substances (biosurfactants), which is of particular importance in pharmaceutical, food and cosmetic applications. This is because downstream processing of diluted broths involved that may require multiple consecutive steps.
- Over producing strains of bacteria are rare and those found generally display a low productivity. In addition, complex media need to be applied to the sample.
- The regulation of biosurfactant synthesis is hardly understood, seemingly it represent secondary metabolite regulation. Thus considering a batch culture, secondary metabolite production begins when the culture is stressed due to the depletion of a nutrient. This phenomenon is closely correlated with the transition phase- slow growth rate of culture and with the morphological changes that this phase implies. Among others O_2-limitation has been described as an essential parameter to govern biosurfactant production.
- An improvement of the production yield is hampered by the strong foam formation. Consequently, diluted media have to be applied and only immobilised systems provide an increased productivity of about 3 gl^{-1} h^{-1} (Fiechter, 1992).

CONCLUSIONS

This review provides information about the biosurfactant production by microorganisms. The scale-up of biosurfactants for industrial production is still challenging. Since the composition of the final products is affected by the nutrient, micronutrient and environmental factors, it is obvious to find a right surfactant for industrial scale-up. In this review we have provided an overview about the availability of various analytical equipments to detect and quantify the biosurfactant. The requirement of the purity of the biosurfactants depends on the application, for example the surfactants used for environmental remediation

should be free from microbial loading but the quality of the product could be compromised. But for pharmaceutical and cosmetic applications the biosurfactants should meet the requirement of various regulatory standards. Few organisms in the indigenous microbial flora are producing biosurfactants in the natural environment to adapt to various adverse conditions. We are just trying to exploit the process for the benefit of mankind. Still we need further understanding of the microbial physiology and genetics of these microorganisms to harness them for efficient industrial applications.

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