Microbial Biosurfactants – Review

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Abstract

Biosurfactants are surface active compounds released by microorganisms. They are biodegradable non-toxic and eco-friendly 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. The type of microbial biosurfactants include trehalose lipids, rhamnolipids, sophorolipids, glycolipids, cellobiose lipids, polyol lipids, diglycosyl diglycerides, lipoloysaccharides, arthrofactin, lichensyn A and B, surfactin, viscosin, phospholipids, sulphonyl lipids and fatty acids. 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. Biosurfactants from marine microorganisms also offer great potential in bioremediation of oil contaminated oceanic environments.

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

1 Introduction

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi (Mata-Sandoval et al., 1999, 2000; Chen et al., 2007) 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., 2002 a,b, 2003; Raza et al., 2007). The amphiphiles that form micelles can be potentially used for surface chemical works which are termed SURFace ACTive AgeNTS or SURFACTANTS. Soaps and detergents can be described as having similar characteristics as surfactants. All surfactants have two ends namely, hydrophobic end and hydrophilic end. The hydrophobic part of the molecule is a long-chain of fatty acids, hydroxy fatty aids, hydroxyl fatty acids or á-alkyl-â-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 synthesized surfactants in a great number of industrial operations (Kosaric, 1992). Chemically synthesized biosurfactants are mostly derived from oil and are widely used in cosmetics. Biosurfactants reduce surface tension, critical micelle concentration (CMC) and interfacial tension in both aqueous solutions and hydrocarbon mixtures (Banat, 1995; Rahman et al., 2002c, d).

2 General Classification

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 contain no ionic constituent and the majority

of all non-ionics are polymerisation products of 1, 2-epoxyethane. Cationic surfactants are characterized 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 efficiently 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; Cohan 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.

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, rhamnolipid from *Pseudomonas aeruginosa* DS10-129 (Rahman et al., 2002a,b, 2003), sophorose lipid from *Torulopsis bombicola* and *Bacillus subtilis* ATCC 2132 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, lipoprteins/lipopepetides and polymeric.

2.1 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), whilst 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).

2.1.a 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 (RhC10C10), RL2 (RhC10), RL3 (Rh2C10C10) and RL4 (Rh2C10) (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. 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 mNm^{-1}); high average emulsifying activity of (10.4 - 15.5 Uml^{-1} filtrate), low critical micelle concentration (CMC) (5 - 65mgL^{-1}) and high affinity for hydrophobic organic molecules (Van Dyke et al., 1993).

2.1.b Sophorolipids

(SLs) are group of biosurfactants produced by Torulopsis sp. Sophorolipids 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 deodorant (Mager et al., 1987).

2.1.c Trehalolipids

This is another group of glycolipids. The serpentine growth 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. Trehalose lipids from Rhodococcus erythropolis and Arthrobacter sp. were found to lower the surface and interfacial tensions in culture broth from 25 to 40 mNm^{-1} and 1 to 5 mNm^{-1} respectively (Li et al., 1984).

2.2 Lipoproteins and Lipopeptides

Lipopepetides also called surfactins, have been produced by Bacillus sp. contain 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 to 27 mNm^{-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 being used to detect surfactin production in bacteria through haemolysis on blood agar.

2.3 Fatty Acids

Fatty acids produced from alkanes as a result of microbial oxidations have been considered as surfactants (Rehm 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).

2.4 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 mNm^{-1} and CMC of 30 mgL^{-1} (Kretschmer, 1982).

2.5 Polymeric Compounds

Emulsan, liposan, mannoprotein and polysaccharide-protein complexes are known to be the best-studied polymeric biosurfactants (Desai and Banat, 1997). Rosenberg et al., (1979) extracted a potent polyanionic amphipathic heteropoly saccharide bioemulsifier called emulsan from

Acinetobacter calcoaceticus RAG-1. It is a very effective emulsifying agent for hydrocarbons in water even at a concentration as low as 0.001% to 0.01%. It is also 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) synthesized liposan, an extracellular water-soluble emulsifier using *Candida lipolytica*. It 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) reported production of large amounts of mannoprotein from *Saccharomyces cerevisiae* with excellent emulsifying activity toward several oils, alkanes and organic solvents. When purified, the emulsifier contains 44% mannose and 17% protein. The mannoprotein exhibited other polymeric biosurfactants such as biodispersan, alasan, food emulsifiers, protein complexes and insectide emulsifiers.

3 Biosurfactant Production

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).

3.1 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 nutritional factors.

3.1.a 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). In using bacteria for Microbially Enhanced Oil Recovery (MEOR) in situ, bacteria 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 ranges found in many oil reservoirs (Karanth et al., 1999). Desai and Banat (1997) also affirm the fact that environmental factors such as pH, temperature, agitation and oxygen availability and growth conditions 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).

3.1.b Nutritional factors

A number of carbon substrates have been used for 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 was also reported that 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 acid delivered poor yields (12 - 36 mg g^{-1} substrate) (Mata-Sandoval et al., 2000). Robert et al. (1989) reported production of rhamnolipid from *Pseudomonas aeruginosa* using

a variety of carbon sources such as C11 and C12 alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol.

3.2 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 mNm^{-1} and an addition of biosurfactant lowers it to as low as 28 mNm^{-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).

A number of analytical methods have been employed by many researchers in their analyses and in some cases characterization of biosurfactants. Table 2 shows the type of biosurfactant, bacteria, solvent, supporting references and type of analytical method used.

4 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 emphasized. The enormous market demand for surfactants is currently met by 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. Stringent 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 to 4% per year globally and 1.5 to 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%).

4.1 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 to 500 tons of fishes died in the Gulf as a result of exposure to oil or polluted water. Additionally, several oil pollution accidents at high seas and on beaches have resulted in enormous ecological and social catastrophes (Shaw, 1992; Burns et al., 1993; Burger, 1993). Rahman et al. (2003, 2004, 2006) examined the bioremediation of n-alkanes in petroleum sludge containing 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 using a bacterial consortium, nutrients and rhamnolipids. In another experiment, it was demonstrated that when Boscan Venezuelan heavy crude oil was treated with emulsan, oil viscosity was

reduced from 200,000 to 100 Cp (Hayes et al., 1986). 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 *Pseudomonas aeruginosa* isolate S8 obtained from oil-polluted sea water degraded the hydrocarbons hexadecane, octadecane and nanodecane by 47%, 58%, 73% and 60% respectively when incubated for a 28-day period under laboratory conditions along The treatment of poultry litter and coir pith with rhamnolipid produced by Pseudomonas aeruginosa DS10-129 enhanced ex situ bioremediation of a gasoline-contaminated soil (Rahman et al., 2002a). Benzene, toluene, ethylbenzene, xylene and trimethylbenzene were degraded by adding microorganism mixture to soil contaminated with gasoline and enriched with nutrients and oxygen (Kosaric, 2001).

4.1.b Other Applications

Biosurfactants have also been used in food industries usually as food additives (emulsifiers). For example, 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 come a long way in improving the flavour, taste and quality of products with minimal health hazards. Biosurfactants have also application in agriculture industry. 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.* Purified mono- and di-rhamnolipids with concentrations ranging from 5 to 30 mgL^{-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).

4.2 Reduction of 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 wonder the 1997 UNFCCC Kyoto Protocol was adopted to curtail the emission of greenhouse gases (Kyoto Protocol, 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 880kt in 1998 to approximately 1,100kt 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 CO_2 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).

5 Biosurfactants from Marine Microorganisms

Marine microorganisms are relatively unexplored group of organisms put under production and recent studies revealed that the culturable diversity of marine microorganisms is only 1% suggesting great scope for metagenomics. Among the various bioactive substances being searched, both from cultured and uncultured marine microorganisms, biosurfactants

captures great attention as they are ideally suited for the bioremediation of oil contaminated sites in the sea, which is a regular feature in the high seas these days. The marine microorganisms produce 4 major classes of biosurfactants such as glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric biosurfactants and particulate biosurfactants (Desai and Banat, 1997). Important biosurfactants from marine microorganisms is given in Table 4.

In order to screen biosurfactant producing microorganisms, various assays such as production of rhamnolipid by calorimetric method (Yuste et al., 2000), ability to form clear halos in methylene blue/ cetyl trimethyl ammonium bromide (CTAB) or N-acetyl pyridinium chloride - methylene blue agar (Lin et al., 1998), haemolysis of RBC (Carrillo et al., 1996; Feibig et al., 1997) are in practice. Morikawa et al. (1992) described a method where colony surrounded by an emulsified halo on L-agar plate coated with oil was considered as biosurfactant producer. A drop collapsing test was also suggested as a rapid and sensitive method for bacterial colonies producing surfactants (Haba et al., 2000).

For the measurement of biosurfactant activity use of tensiometer is suggested (Dungar and Schink, 1995) which measures the surface tension at the air/ water or oil/water interface. Other assay methods suggested include checking the ability of biosurfactant to generate a suspension of hydrocarbon such as n-hexadecane (Kim et al., 2000) or kerosene (Haba et al., 2000) or a mixture of n-hexadecane and 2 methyl naphthalene (Navon - Venicia et al., 1995) or dodecane (Burd and Ward, 1996) in an aqueous assay system.

Marine biosurfactants are considered highly useful in combating the pollution of the oceanic environment resulting from oil spills and frequent ship operations. The biodegradation of a given carbohydrate depends on its dispersion site and the degradation is maximized when the water insoluble substances in dissolved, solubilized or emulsified (Mattai et al., 1986). While synthetic detergents add to the environmental problems of the aquatic environment, the biodegradable biosurfactants from marine microorganisms increases bioavailablity of poorly soluble polycyclic aromatic hydrocarbons such as phenanthrene (Gilewics et al., 1997; Olivera et al., 2003) and resins (Venkateswaran et al., 1995). Control of marine algal (Cochlodinium) blooms by spraying a mixture of biosurfactant sophorolipid and yellow clay has been reported (Lee et al., 2008). The removal efficiency of biosurfactant-yellow clay mixture was more than 90% after 30 minutes treatment.

The components of the autochthonous microflora of marine environment such as Acinetobacter spp T4 start acting on the oil pollutant initially resulting in the production of metabolite that could support the growth of *Pseudomonas putida* (Nakamura et al., 1996; Delille, 2000). The dissolved organic compounds through activity of marine bacteria are also found to have important consequences on the physico chemical characteristics of water (Goutx et al., 1990). They have also recorded that 1mg of bacterial protein releases 16-289µg of carbohydrates and 8 to 188 µg of lipids in the extracellular lipid medium. Maneerat and Phetrong (2007) identified 8 strains of biosurfactant producing marine bacteria such as *Vibrio parahaemolyticus, Bacillus subtilis, Micrococcus luteus, Myroides sp., Acinetobacter anitratus* and *B. pumilus* by 16s rRNA analysis from oil spilled seawater along Thailand coast and studied the characteristics of selected biosurfactants. Batista et al. (2006) isolated good number of Gram positive bacteria from petroleum contaminated sites that are capable of producing biosurfactants based on drop collapse test. It was also observed that glucose was a better carbon source than fructose, sucrose or kerosene for screening surfactant and/or emulsifier-producing isolates. Seafloor gas hydrate formation resulting from surfactant production by *Bacillus subtilis* and other species under prevailed anaerobic conditions around seafloor gas hydrates was studied

(Zhang et al., 2007). It is found that this bioproduct has propensity to be dispersed in the porous media by natural gas vents.

6 Advantages and disadvantages of biosurfactants

Scientific 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).

6.1 Merits

- 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 species* 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 for particular interest for 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.

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

6.2 Demerits

- 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 which 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 may require multiple consecutive steps.
- Over producing strains of bacteria are rare and those found generally display a very 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 in 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).

Table 1. Type and microbial origin of biosurfactants (Biermann et al., 1987 & Mulligan, 2005)

Type of biosurfactant	**Microorganism**
Trehalose lipids	*Arthronbacter paraffineus*
	Corynebacterium sp.
	Mycobacterium sp.
	Rhodococus 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., Corynebacterium 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	*Capnoytophaga sp.*
(Corynomycolic acids, spiculisporic acids, etc)	*Penicillium spiculisporum*
	Corynebacterium lepus
	Arthrobacter paraffineus
	Talaramyces trachyspermus
	Norcadia erythropolis
Alasan	*Acinetobacter radioresistens*
Streptofactin	*Streptomyces tendae*
Particulate surfactant (PM)	*Pseudomonas marginalis*
Biosur PM	*Pseudomonas maltophilia*

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

Biosurfactant & 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: $CHCl_3/CH_3OH/CH_3$ COOH	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)
Lipopeptide			
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 & Ju, (2001)
	FTIR		Hu & 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

Table 3. Potential to substitute petrochemical by oleochemical surfactants in the EU by 2010.

Surfactants	EU Production	High RRM Scenario	Change %
	1998 (kt)	2010 (kt)	
ANIONICS			
LAS-Pc	409	409	0
SAS-Pc	69	69	0
AS-Pc	43	16	-63
AS-Pc	64	91	42
AS-Oleochemical	74	37	-50
AE3S-Oleochemical	172	209	21
Other anionics-Pc	47	28	-42
Other anionics-Oleochemical	32	51	63
NONIONICS			
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

Note: 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.

Table 4. Marine microorganisms and some types of biosurfactants (Maneerat et al., 2005)

Biosurfactant	Organisms	Reference (s)
Glycolipids		
Glucose lipids	*Alcanyvorax borkumensis*	Abraham et al. (1998)
	Alcaligenes sp.	Poremba et al. (1991)
Trehalose lipids	*Arthrobacter sp.*	Schulz et al. (1991)
Lipoproteins		
Ornithine lipids	*Myroides sp. SM1*	Maneerat et al. (2005)
Phospholipids and Fatty acids		
Bile acids	*Myroides sp. SM1*	Maneerat et al. (2005)
Polymeric biosurfactants		
Lipid-carbohydrate-	*Yarrowia lipolytica*	Zinjarde and Pant (2002)
protein	*Pseudomonas nautica*	Hussein et al. (1997)
Particulate biosurfactants		
Whole cells	*Variety of bacteria*	Denger and Schink (1995)
		Gilewicz et al. (1997)
		Zinjarde and Pant (2002)

7 Conclusions

This review, provided information about the biosurfactant production by microorganisms. In this review we have provided an overview about the availability of various analytical equipments to detect and quantify the biosurfactant. Few organisms in the indigenous microbial flora are producing biosurfactants in the natural environment to adapt to various adverse conditions. The requirement of the purity of the biosurfactants depends on the application, for example if we use the surfactant for environmental remediation, the final product should be free from the 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. The scale up of biosurfactants for industrial production is still challenging. Since the composition of the final products is affected by the nutrient, microorganism, micronutrient and environmental factors, it is obvious to find a right surfactant for industrial scale up. Further understanding of the microbial physiology and genetics of these microorganisms is needed to make them to work in the industrial sector.

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