CHAPTER 1

INTRODUCTION

This chapter deals with the general introduction of the bio-surfactants. The definition, classification, microorganisms involved in production, advantages as well as disadvantages over chemical surfactants and their applications in different industries are discussed.

1.1 Bio-surfactants: General Introduction

Bio-surfactants are the diverse group of surface active molecules/chemical compounds synthesized from various microorganisms. The bio-surfactants are produced as secondary metabolites when microbes, instead of using nutrients for their growth utilize them for the production of bio-surfactants or the actual cell itself from its surface chemistry [Zajic et. al., 1983]. These metabolites or cells themselves are responsible for the balanced functioning of the biological systems and are related to exchange of energies, matter and signals at dissimilar interfaces. An important example is the similarity of phospholipids to form bio-membranes, where membrane proteins in addition to glycolipids work as an important platform for molecular recognitions, signal transduction, trans-membrane transportation, cell adhesion etc. [Lang, 2002]. The uniqueness in the properties of phospholipids (bio-surfactant) is because of their amphipathic/amphiphilic nature [Kitamoto et. al., 2009]. The amphipathic/amphiphilic nature of bio-surfactants corresponds to the presence of both hydrophilic as well as hydrophobic moieties in their chemical structure that
reduce the surface and interfacial tensions between two immiscible liquids. The common hydrophilic groups present in the bio-surfactants are non-ionic, positively or negatively charged or amphoteric (a carbohydrate, an amino acid, a phosphate group, etc.) groups, while the common hydrophobic groups present are different types of fatty acids, saturated or unsaturated, branched or hydroxylated hydrocarbons [Cameotra and Makkar, 1998; Kitamoto et al., 2009]. These polar and non-polar moieties present in the bio-surfactants allow them to accumulate at inter-phases (a/w; o/w) between liquids of different polarities and form micelles thereby reducing surface tension, allowing them to mix easily due to reduced forces of repulsion and facilitating hydrocarbon uptake and emulsification.

A variety of micro-organisms produce bio-surfactants that are diverse in chemical composition. The nature and amount of the bio-surfactant produced solely depends upon the site from where the micro-organism is isolated and the various nutritional factors available for their growth. Many microorganisms have been isolated from contaminated soils, effluents and waste water sources for industrial utilization of the various types of agro-industrial waste products.

1.2 Microorganisms producing bio-surfactants

Many types of microorganisms (bacteria, fungi, actinomycetes) are known for bio-surfactant production with distinct molecular configurations [Biria et al., 2009; Cooper, 1986]. Depending upon their structural diversity the microbial surfactants are classified into various classes’ viz. glycolipids, lipopeptide and lipoprotein, fatty acids, phospholipids and neutral lipids, polymeric bio-surfactants and particulate bio-
surfactants [Anandraj and Thivakaran, 2010; Pansiripat et. al., 2010; Bhardwaj et. al., 2013b; Thavasi et. al., 2009; Khopade et. al., 2012; Desai and Banat, 1997]. The bio-
surfactants are produced during the growth of microorganisms on carbon sources
which are either water soluble or water insoluble substrates. The varieties of carbon
sources are utilized by microorganisms to get energy for their growth. In case of
insoluble carbon sources like a hydrocarbon, microorganisms facilitate their diffusion
into the cell by producing bio-surfactants which emulsify the hydrocarbon at the
junction of the cell wall of the microorganism and makes its uptake easy. Some of the
hydrocarbon substrates are emulsified in the growth media by ionic surfactants which
are produced by bacteria and yeast [Banat, 1995; Gautam and Tyagi, 2006]. The well
known bio-surfactants of this group are rhamnolipids and sophorolipids produced by
Pseudomonas sp. or Torulopsis bombicola. Other types of bio-surfactants are
lipopolysaccharides or non-ionic surfactants which upon secretion changes the cell
wall structure of the microorganism respectively.

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the site from where the micro-organism is isolated and the various nutritional factors
available for their growth (Table-1.1). Many microorganisms have been isolated from
contaminated soils, effluents and waste water sources for industrial utilization of the
various types of agro-industrial waste products. Thus, these have an ability to grow on
substrates considered potentially noxious for other non-producing microorganisms.
Table 1.1: Potential bio-surfactants producing micro-organisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Sources of isolation</th>
<th>By-products/ Carbon Sources</th>
<th>Bio-surfactant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Oil spilled soil</td>
<td>Glucose/ Molasses/ Cheese whey</td>
<td>Rhamnolipid</td>
<td>Anandaraj and Thivakaran, 2010</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Used edible oil</td>
<td>Used edible oil/ Rice-water/ Diesel/ Petrol/ Whey</td>
<td>Rhamnolipid</td>
<td>Soniyamby et. al., 2011</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Crude oil contaminated localities</td>
<td>Glucose/ Rapeseed oil supplemented with crude oil</td>
<td>Iturin</td>
<td>Bayoumi et. al., 2010</td>
</tr>
<tr>
<td><em>Bordetella hinizi-DAFI</em></td>
<td>Crude oil contaminated localities</td>
<td>Sucrose/ Molasses supplemented with crude oil</td>
<td>Trehalose-2,3,4,2’-tetraester</td>
<td>Bayoumi et. al., 2010</td>
</tr>
<tr>
<td><em>Trichosporon asahii</em></td>
<td>Petroleum-contaminated soil</td>
<td>Diesel oil</td>
<td>Sophorolipids</td>
<td>Chandran and Das, 2010</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa* LBI</td>
<td>Petroleum contaminated soil</td>
<td>Soap-stock</td>
<td>Rhamnolipids</td>
<td>Benincasa et. al., 2002</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Petroleum contaminated soil</td>
<td>Glycerol</td>
<td>Lipopeptide</td>
<td>Anyanwu et. al., 2011</td>
</tr>
<tr>
<td><em>Candida</em> sp. SY-16</td>
<td>Oil-containing soil sample</td>
<td>Soybean oil and glucose (Glyco-lipid)</td>
<td>Mannosylerythritol</td>
<td>Kim et. al., 1999</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa* SP4</td>
<td>Petroleum contaminated soil</td>
<td>Palm oil</td>
<td>Rhamnolipid</td>
<td>Sarachat et. al., 2010</td>
</tr>
<tr>
<td><em>Rhodococcus</em> sp.</td>
<td>Oil-contaminated soil</td>
<td>Sucrose/ Kerosene/ n-heptane/ n-octane/ n-hexadecane/ n-paraffin/gas oil</td>
<td>Extracellular lipids and glycolipid</td>
<td>Shavandi et. al., 2011</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Oil contaminated soil</td>
<td>Vegetable oil/ Kerosene/Petrol/Diesel</td>
<td>Surfactin</td>
<td>Priya and Usharani, 2009</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa*</td>
<td>Oil contaminated soil</td>
<td>Vegetable oil/ Kerosene/Petrol/Diesel</td>
<td>Rhamnolipid</td>
<td>Priya and Usharani, 2009</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa* J4</td>
<td>Waste water of petrochemical factory</td>
<td>Glucose/ Diesel, Kerosene/Glycerol/Olive Oil/ Sunflower oil/ Grape seed oil</td>
<td>Rhamnolipid</td>
<td>Wei et. al., 2005</td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa* EM1</td>
<td>Oil contaminated site</td>
<td>Glucose/ Glycerol/ Sucrose/ Hexane/Olive oil/ Oleic acid/ soybean oil</td>
<td>Rhamnolipid</td>
<td>Wu et. al., 2008</td>
</tr>
</tbody>
</table>
1.3 Classification and Chemical structure

Unlike the chemically synthesized surfactants that are generally categorized on the basis of the type of polar group present, bio-surfactants are in general classified chiefly by their chemical composition dictated by different molecules forming the hydrophilic and hydrophobic moieties and microbial origin. Another classification of bio-surfactants is on the basis of their molecular masses; they can be classified into low molecular mass molecules (lipopeptides, glycolipids and phospholipids) that efficiently lower surface and interfacial tension, and high molecular- mass polymers (polymeric and particulate surfactants), that are more efficient as emulsion-stabilizing agents [Rosenberg and Ron, 1999]. The majority of bio-surfactants are either anionic or neutral and their hydrophobic moiety is based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic moiety can be a carbohydrate, phosphate, amino acid, or cyclic peptide [Nitschke and Coast, 2007].

1.3.1 Glycolipids

Glycolipids are the major class of bio-surfactants which possess many subclasses. Basically the glycolipids are the mono or disaccharide, acylated in combination with the long chain fatty acids or hydroxyl fatty acids. The connection is by means of either an ether or ester group. Among the glycolipids, the best known are rhamnolipids [Pantazaki et. al., 2011; Pornsunthorntawee et. al., 2008; Loftabad et. al., 2010; Gunther et. al., 2005; Sotivoro et. al., 2009; Syldatk et. al., 1985], sophorolipids
trehalolipids [Cameotra and Makkar, 1998], while the less known are mannosylerythritol lipids [Kitamoto et. al., 2001; Kim et. al., 1999; Kim et al., 2002; Konishi et. al., 2007] and monoacylglycerol [Thanomsub et. al., 2004].

### 1.3.1.1 Rhamnolipids

The glycolipids, in which one or two molecules of rhamnose are connected to one or two molecules of β-hydroxydecanoic acid, are the most studied ones. They are generally referred as mono-rhamnolipids having one rhamnose ring and di-rhamnolipids having two rhamnose rings (Figure 1.1) [Desai and Banat, 1997]. The -OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the -OH group of the second acid is occupied in ester formation [Karanth et. al., 1999]. The production of rhamnose, which contains glycolipid was first studied in *Pseudomonas aeruginosa* by Jarvis and Johnson, [1949]. The principal rhamnolipids produced by *P. aeruginosa* are L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate and L-rhamnosyl-β-hydroxydecanoyl-β-hydrtocydecanoate [Edwar, and Hayashi, 1965].

![Monorhamnolipid](image1.png)  ![Dirhamnolipid](image2.png)

**Figure 1.1:** Rhamnolipids
1.3.1.2 Sophorolipids

Sophorolipids bio-surfactants are known to produce by various fungi *viz.* *Candida bombicola* [Casas et. al., 1997; Cavalero and Cooper, 2003; Casas and Garcia-Ochoa, 1999a; Deshpande and Daniels, 1995; Felse et. al., 2007; Williams, 2009; Zhou et. al., 1992], *Candida lipolytica* [Sarubbo et. al., 2007; Rufino et. al., 2007], *Candida ishiwadai* [Thanomsub et. al., 2004], *Candida batistae* [Konishi et. al., 2008], *Aspergillus ustus* [Kiran et. al., 2009], *Ustilago maydis* [Aljendro et. al., 2011], *Trichosporon ashi* [Chandran and Das, 2010], *Torulopsis bombicola*, [Cooper and Paddock, 1984] and *T. petrophilum* [Cooper and Paddock, 1983]. The structure consists of a dimeric carbohydrate sphere attached to a long-chain hydroxyl fatty acid by a glycosidic linkage. Generally, sophorolipids are found as a mixture of free acid form and macrolactones. It is seen that the lactone form of the sophorolipid is essential, for various applications [Hu and Ju, 2001]. These bio-surfactants are a combination of at least six to nine varied hydrophobic sophorolipids. The structures of the acidic and lactonic form of sophorolipids are given in Figure 1.2.

1.3.1.3 Trehalolipids

Various structural types of microbial trehalolipid bio-surfactants have been reported. Disaccharide trehalose linked at C-6 and C-6 to mycolic acid is related to most species of *Mycobacterium, Corynebacterium* and *Nocardia*. Mycolic acids are the long chain, α-branched and β-hydroxy fatty acids. Trehalolipids from diverse organisms differ in the size and structure of mycolic acid, the number of carbon atoms present and the extent of unsaturation [Asselineau and Asselineau, 1978]. Trehalose lipids obtained from
*Rhodococcus erythropolis* and *Arthrobacter sp.*, reduced the surface tension and interfacial tension in the culture broth to a good extent [Kretschmer et. al., 1982]. The structure of the trehalolipids is given below, where \( m+n = 23 \) (Figure 1.3) [Makkar et. al., 2011].

**Figure 1.2:** Sophorolipids

**Figure 1.3:** Trehalolipids
1.3.2 Lipopeptide and Lipoprotein

There are several reports on the production of lipopeptide bio-surfactants mainly by *Bacillus* sp. [Makkar and Cameotra 1999; Ullrich et. al., 1991]. A great number of cyclic lipopeptides, including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) are produced. These contain a lipid linked to a polypeptide chain. Lipopeptides usually appear as mixtures of closely related compounds which show slight variations in their amino acid composition and/or lipid portion which is mostly a hydroxy fatty acid. A family of cyclic lipopeptides consists of 8 to 17 amino acids and a lipid portion which is composed of 8 to 9 methylene groups and a mixture of linear and branched tails [Desai and Banat, 1997]. These include surfactin produced by *B. subtilis* [Sen and Swaminathan, 2004] and viscosin from *P. fluorescence* [Kitamoto et. al., 2002].

1.3.2.1 Surfactin

The cyclic lipopeptide surfactin, synthesized by *Bacillus subtilis*, is among the most effective bio-surfactants known so far (Figure 1.4). It is made up of a seven amino-acid ring structure joined to a fatty-acid chain by means of lactone linkage. Surfactin reduces the surface tension of water from 72 to 27.9 mN/m, even at very low concentrations [Arima et. al., 1968; Gautam and Tyagi, 2006].
1.3.2.2 Lichenysin

*Bacillus licheniformis* synthesizes several bio-surfactants that act synergistically and exhibit great temperature, salt, and pH stability. They are also similar in their structural and physico-chemical properties to surfactin. The surfactants that are produced by *B. Licheniformis* are able to reduce the surface tension of water up to 27 mN/m and the interfacial tension between water and n-hexadecane to 0.36 mN/m [McInerney et. al., 1990].

1.3.3 Fatty acids, Phospholipids and Neutral lipids

Fatty acid and phospholipids produced during growth on n-alkanes by several bacteria and yeast, has received considerable attention as surfactants [Desai and Desai, 1993]. These bio-surfactants are able to produce optically clear micro-emulsions of alkanes in water [Singh et. al., 2007]. The hydrophilic lipophilic balance (HLB) of fatty acids is found clearly related to the length of the hydrocarbon chain. Example of microorganisms that produced these types of bio-surfactants are sulfur-reducing bacteria, *Thiobacillus thiooxidans* and *Corynebacterium lepus* [Hauser and Karnovsky, 1958; Desai and Desai, 1993; Desai and Banat, 1997]. Extracellular free fatty acids produced by microorganisms grown on alkanes also showed surfactant activity. They include saturated fatty acids in the range of C-12 to C-14 and the complex fatty acids containing hydroxyl groups and alkyl branches. Phosphatidylethanolamine biosurfactant produced by *Rhodococcus erythropolis* caused a lowering of interfacial tension against hexadecane to less than 1 mN/m and a CMC of 30 mg/L. The structure
of phospholipid (Phosphatidylethanolamine) is given in Figure 1.5 [Lang and Philp, 1998; Kretschmer et. al., 1982].

Figure 1.5: Phosphatidylethanolamine

1.3.4 Polymeric bio-surfactants

Many bacteria and yeast synthesize large amounts of polymeric bio-surfactants when grown on water immiscible carbon sources [Cirigliano and Carman, 1984; Cirigliano and Carman, 1985]. The bio-surfactants having higher molecular weights generally exhibit useful properties like high viscosity, tensile strength and resistance to shear. It is therefore polymeric bio-surfactants possess the various useful industrial applications. Fatty acids are covalently linked to the polysaccharide through α-ester linkages [Bodour et. al., 2004; Desai and Banat, 1997]. The most common polymeric bio-surfactant known is emulsan. The emulsan has been characterized as a polyanionic amphipatic heteropolysaccharide [Rosenberg et. al., 1979; Desai and Desai, 1993]. Emulsan is known to be very potent emulsifier known till date; it can stabilize an emulsion in very low concentration of 0.001 to 0.01% and resists inversion even at a water-oil ratio of 1:4 [Desai and Banat, 1997; Gautam and Tyagi, 2006; Zosim et. al., 1982;]. The structure of Emulsan is given in Figure 1.6 [Gautam and Tyagi, 2006].
1.3.5 Particulate bio-surfactants

This type of bio-surfactant includes vesicles and fimbriae produced by *Acinetobacter* spp. When *Acinetobacter* sp. was grown on hexadecane the accumulation of an extracellular-vesicular component was observed and characterized as phospholipid-rich, lipopolysaccharide particle [Kappeli and Finnerty, 1979]. The purified vesicles are composed of protein, phospholipid and lipopolysaccharide. The extracellular membrane vesicles partition hydrocarbons form a microemulsion, which plays an important role in the alkane uptake by microbial cells [Desai and Banat, 1997]. Generally, bio-surfactant molecules consisted of both hydrophilic and hydrophobic moieties, which enable them to accumulate at the interfaces and mediated between phases of different polarity such as oil-in-water or water-in-oil interfaces [Hauser and
Karnovsky, 1958]. The polar water soluble part of a bio-surfactant may be as simple as a carboxylate or hydroxyl group or a complex mixture of phosphate, amino acids or peptides, anions or cations, or mono-, di- or polysaccharides. Whereas the lipophilic portions are the hydrocarbon tail that usually made of long chain, saturated, unsaturated, hydroxyl or α-alkyl, β-hydroxy fatty acids and may contain cyclic structures. This fatty acid is linked to the hydrophilic group by a glycosidic, ester or an amide bond [Banat, 1995; Kitamoto et. al., 2002; Desai and Desai, 1993].

1.4 Bio-surfactants vs surfactants

The bio-surfactants possess several advantages over their counterparts’ i.e. synthetic surfactants including high biodegradability, low toxicity, and low irritancy etc. ‘Commercialization’ owing to their large scale and cheap production cost is the only disadvantage of bio-surfactants till date. However, the most important advantage they bear over chemical surfactants is their environmentally friendly nature which makes them ecologically acceptable. Some of the well known advantages in details are as given below:

1.4.1 Biodegradability

Biodegradability is a major concern in today’s life concerning to the environmental pollution. The bio-surfactants are able to be broken down by microorganisms or other simple organisms into more basic components and this shows their biodegradable nature. So, these are well suitable for remediation processes where the pollution needed to be controlled [Mohan et. al., 2006; Mulligan, 2005]. The increasing environmental concern among consumers and the regulatory bodies of many
governments imposing stringent rules have compelled industries to search for more environmentally friendly products such as bio-surfactants for applications in food, cosmetics, and pharmaceutical industries [Cameotra and Makkar, 1998].

1.4.2 Lower toxicity

The bio-surfactants are considered low or non-toxic to the environment, so do not cause any harm to the surroundings. On the other side, the synthetic surfactants are toxic and cause serious threats to the environment. Even though little data is available in the literature concerning the toxicity of microbial surfactants, they are usually considered non-toxic products and therefore, suitable for pharmaceutical, cosmetic and food uses. It was reported that a synthetic anionic surfactant (Corexit) displayed an LC50 (concentration lethal to 50% of test species) against *Photobacterium phosphoreum* 10 times lower than rhamnolipids,signifying the high toxicity of the chemically derived surfactant [Poremba et. al., 1991]. When compared to the toxicity of six bio-surfactants, four synthetic surfactants and two commercial dispersants, it was found that most bio-surfactants degraded faster, except for a synthetic sucrose-stearate that showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids (rhamnolipids, trehalose lipids, and sophorose lipids. A bio-surfactant from *P. aeruginosa* was compared with a synthetic surfactant (Marlon A-350) commonly used in the industry in terms of toxicity and mutagenic properties. Higher toxicity and mutagenic effect were indicated by both assays of the chemical-derived surfactant whereas bio-surfactant was considered slightly to non-toxic and non-mutagenic [Flasz et. al., 1998].
1.4.3 Ionic strength, pH and temperature tolerance

The bio-surfactants show their activity in a wide range of NaCl concentrations, pH and temperature. The Antarctic psychrophilic strain *Arthrobacter protophormiae* produced a bio-surfactant that was thermostable (30-100°C) and pH (2-12) stable [Pruthi and Cameotra, 1997]. Industrial processes recurrently involve exposure to extreme temperature, pressure, pH and ionic strength; hence there is a continuous need to separate new microbe derived products capable to function under these conditions [Cameotra and Makkar, 1998].

1.4.4 Biocompatibility and digestibility

The term ‘biocompatibility’ means their well tolerance by living organisms. Bio-surfactants when interact with the living organisms do not change their bioactivity. Therefore, are beginning to acquire a status as potential performance-effective molecules in various fields [Banat et al., 2000]. This shows their applications in various industries such as cosmetics, pharmaceuticals, food etc.

1.4.5 Availability of raw material

One of the most important advantages of bio-surfactants is their production by using the raw material from various industries like agricultural, oil, food, etc. There are so many reports on the production of bio-surfactants by using wastes of various industries.
1.4.6 Specificity

Bio-surfactants are the complex organic molecules with specific functional groups and hence have specific actions. This is of very interest in detoxification of specific pollutants, de-emulsification of industrial emulsion, cosmetic products, pharmaceutical and food applications.

1.5 Applications

Bio-surfactants possess numerous applications ranging from environmental, bioremediation, food and biomedical, cosmetic and pharmaceutical industries. These all properties show their potential of usage at industrial level for a greener environment. Some of the applications are given below in Table 1.2:

Table 1.2: Applications of bio-surfactants

<table>
<thead>
<tr>
<th>Industry</th>
<th>Applications</th>
<th>Role of surfactants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>Emulsification and de-emulsification, Functional ingredients</td>
<td>Emulsifier, solubilizer, demulsifier, suspension, wetting, foaming, defoaming, thickener, lubricating agent</td>
</tr>
<tr>
<td>Cosmetic</td>
<td>Health and beauty products</td>
<td>Emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agent, mediators of enzyme action</td>
</tr>
<tr>
<td>Environmental</td>
<td>Bioremediation, Soil remediation and flushing</td>
<td>Emulsification of hydrocarbons; lowering of interfacial tension, metal sequestration</td>
</tr>
<tr>
<td>Petroleum</td>
<td>Enhanced oil recovery</td>
<td>Improving oil drainage into well bore; stimulating release of oil entrapped by capillaries; wetting of soil surfaces; De-emulsification of oil emulsions; oil solubilization</td>
</tr>
<tr>
<td>Agriculture</td>
<td>De-emulsification Biocontrol</td>
<td>Facilitation of bio-control mechanisms of microbes such as parasitism, antibiosis</td>
</tr>
<tr>
<td>Biological</td>
<td>Pharmaceuticals</td>
<td>Antimicrobial, antifungal, antivirus agents, adhesive agents</td>
</tr>
</tbody>
</table>
1.5.1 Anti-adhesive properties

Biofilms are of great attention in many industries such as medical and food because bacteria colonize medical devices and food processing surfaces by changing their properties [Zeraik and Nitschke, 2010]. One of the very important applications of bio-surfactants is their role as anti-adhesive agents to pathogens, making them useful for treating many diseases and as therapeutic and probiotic agents. Bio-surfactants not only prevent adhesion of bacteria to the surfaces, but also induce detachment of already adherent cells. Involvement of bio-surfactants in microbial adhesion and desorption has been widely described, and constitutes an effective strategy to reduce microbial adhesion and combat colonization by pathogenic microorganisms, not only in the biomedical field, but also in other areas, such as the food industry. When the bio-surfactant produced by Bacillus spp. was subjected to anti-adhesion assay, the pathogenic bacteria Escherichia coli and Staphylococcus aureus biofilm formations was decreased to 97% and 90% respectively [Rivardo et. al., 2009]. The bio-surfactant produced by various species of the genus Lactobacillus (L. paracasei, L. plantarum, L. delbrueckii, L. acidophilus, L. casei, L. fermentum, L. rhamnosus L. spp., L. reuteri, L. brevis) was screened for their anti-adhesive properties. The highest anti-adhesive activity was recorded for L. fermentum bio-surfactant against Candida albicans (84.69%). On the other hand, the lowest activity was recorded for L. delbrueckii bio-surfactant against Proteus vulgaris (9.66%) [Gomaa, 2013]. The lipopeptide bio-surfactant produced by a marine bacterium in concentration 0.1 g/L was found to have anti-adhesive properties against A. faecalis, K. aerogenes, and S. typhimurium in percentage 19, 15, and 25%. However, at higher concentrations of the lipopeptide bio-
surfactant almost 100% anti-adhesion was observed [Das et al., 2009]. Bio-surfactant produced by *Candida lipolytica* showed anti-adhesive activity against most of the microorganisms tested from the minimum concentration used (0.75 mg/L). The anti-adhesive property was proportional to the concentration of the bio-surfactant. Since, against *Lactobacillus* sp. anti-adhesive values around 81% were observed in the minor concentration tested (0.75 mg/L). The major anti-adhesive specificity was observed against *L. casei* with values of 91 and 99% with the minimum concentration used. Lower inhibitions were observed for *S. epidermidis* and *E. coli*, with values of 27% and 21%, respectively, at the maximum bio-surfactant concentration. For the other microorganisms, the anti-adhesive activity was above 45% [Rufino et al., 2011]. When *Trichosporon montevideense* was subjected to anti-adhesive property, it was found to release a polymeric bio-surfactant which may significantly reduce the bio-film formation ability of *C. albicans* cells on polystyrene surfaces [Monteiro et al., 2011]. The lipopeptide bio-surfactant produced by *Bacillus cereus* was found to inhibit the biofilm formation by the pathogenic microorganisms, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The highest biofilm inhibition was observed against *S. epidermidis* with the inhibition percentage of up to 57%. The biofilm inhibition was found to be concentration dependent and highest inhibition was observed at a concentration of 15 mg/mL in case of both the microorganisms [Sriram et al., 2011].

### 1.5.2 Food and oil industry

Bio-surfactants are able to stabilize various types of emulsions, so are valuable to the food industry in food additives acting as thickening, emulsifying, dispersing or stabilizing agents [Campos et al., 2013]. The bio-surfactants from the *Candida*
*lipolytica* and *Saccharomyces cerevisiae* are good choices for the food and oil industries [Sarubbo et. al., 2007]. As an example, a nutrition rich bio-surfactant from *Saccharomyces cerevisiae* is used as a single cell protein [Cameron et. al., 1988]. The liposan (bioemulsifier) from *Candida lipolytica* was able to stabilize the emulsion of vegetative oils and water. It was also able to stabilize the cottonseed oil, corn oil, soybean oil and peanut oil emulsion [Cirigliano and Carman, 1985].

### 1.5.3 Biomedical Industry

The bio-surfactants are extensively useful in the biomedical industry. They possess significant anti-biological activities [Singh and Cameotra, 2004]. The bio-surfactant from the *Aspergillus ustus* MSF3 has significant antimicrobial activity against the *Candida albicans* and gram-negative bacterium [Kiran et. al., 2009]. The modified bio-surfactant of *Tsukamurella sp.* showed novel biological activities [Langer et. al., 2006]. Glycolipids from *Ustilago maydis* FBD 12 showed significant antimicrobial activities against *Salmonella enteric Var. Typhimurium* and *Staphylococcus aureus* [Alejandro et. al., 2011].

One of the most exciting findings that have been reported for bio-surfactants is their ability to control a variety of mammalian cell functions and therefore their potential to act as antitumor agents interfering with some cancer progression processes [Gudina et. al., 2013]. The cytotoxic effects of the sophorolipid produced by *Wickerhamiella domercqiae* on cancer cells of H7402, A549, HL60 and K562 were investigated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. The results showed a dose-dependent inhibition ratio on cell viability, according
to the drug concentration ≤62.5 µg/ml [Jing et. al., 2006]. The anti-proliferative activity of monoolein produced by *Exophiala Dermatitis* was checked against 4 different varieties of cancer cell lines. The antiproliferative activity was specific to the cancer cell types, those being HeLa and U937, and did not affect normal cell growth even when used at high concentrations. The most prominent anti-proliferative effect was found to be against the cervical cancer (HeLa) and leukemia (U937) cell lines in a dose-dependent manner mode of cell death triggered by monoolein might be the process of apoptosis, which is recognized as a novel approach for anti-cancer drugs [Paramaporn et. al., 2010]. The anti-tumor activity of a surfactin produced by *Bacillus subtilis* and a glycoprotein (BioEG) produced by *Lactobacillus paracasei subsp. paracasei* was evaluated. Both bio-surfactants were tested against two breast cancer cell lines, T47D and MDA-MB-231, and a non-tumor fibroblast cell line (MC-3 T3-E1), specifically regarding the cell viability and proliferation. Surfactin was found to decrease the viability of both breast cancer cell lines without affecting normal fibroblasts. Moreover, BioEG induced the cell cycle arrest at G1 for both breast cancer cell lines. The bio-surfactant BioEG was shown to be more active than surfactin against the studied breast cancer cells. The results gathered in this work were very promising regarding the bio-surfactants potential for breast cancer treatment and encourage further work with the BioEG glycoprotein [Duarte et. al., 2014].

### 1.5.4 Cosmetic industry

These are the cosmetic products which uses surfactants: insect repellents, antacids, bath products, acne pads, antidandruff products, contact lens solution, hair colours and care products, deodorants, nail care, body massage accessories, lipsticks,
lipmakers, eye shades, mascaras, soap, tooth pastes and polishes, denture cleansers, adhesives, antiperspirants, lubricated condoms, baby products, foot care, mousses, antiseptics, shampoos, conditioners, shampoos, conditioners, shave and depilatory products, moisturizers, health and beauty products [Kosaric, 1992]. The sophorose lipids use in cosmetics may be valuable as compared to the use of the synthetic surfactant, for the same cosmetic applications due to their skin friendly properties. Sophorolipids from the mutant strain *Candida bombicola* ATCC 22214 have great uses in the cosmetic industries due to their anti-radical properties, stimulation of fibroblast metabolism and hygroscopic properties to support healthy skin physiology [Williams, 2009].

1.5.5 Bioremediation

In bioremediation processes, cost effective, contaminant specific treatment is done to reduce the concentration of individual or mixed environmental contaminants. The process of bioremediation can be done by utilizing plants, microbes or microbial products. Bio-surfactants, in contrast to chemical surfactants, have lower possible toxicity and shorter persevere in the environment [Begley et. al., 1996]. The ability of a surfactant to increase the biodegradation of slightly soluble organic compounds depends on the amount to which it increases the bioavailability of the compound [Bertrand et. al., 1994]. There will be a double benefit if the industrial wastewaters which generally contains organic pollutants can be utilized as substrates for bio-surfactant production. This idea can reduce the cost of wastewater treatment with even a potential of creating net income through the sale of the bio-surfactant [Kosaric, 1992].
1.5.6 Microbial enhanced oil recovery and cleaning of oil tanks

The term ‘microbial enhanced oil recovery’ or ‘MEOR’ means the utmost recovery of oil from the various reservoirs to its maximum extent with the help of bio-surfactant produced by microorganisms [Sen, 2008; Zhao et. al., 2015]. The Sophorolipids from *Candida lipolytica* and *Candida bombicola* are very promising in the cleaning of oil tanks, decontamination of polluted areas, microbial enhanced oil recovery, industrial cleaning, low-end consumer products and household applications [Rufino et. al., 2007; Felse et. al., 2007]. The bio-surfactants from *Torulopsis bombicola* and *Aspergillus ustus* MSF3 were used for the release of bitumen from the contaminated soil and for the degradation of hydrocarbons [Cooper and Paddock, 1984; Kiran et. al., 2009]. Mannosylerythritol lipids from *Candida antarctica* have potential applications in the removal and biodegradation of hydrocarbons in oil-contaminated soil and were also used to rinse oil and grease from the contaminated soil [Kitamtoto et. al., 2001]. Bio-surfactants isolated from *Candida antarctica* was also found to be the best choices in microbial enhanced oil recovery [Adamczak and Bednarski, 2000]. The bio-surfactant produced by *Lactobacillus delbrueckii* when grown on peanut oil was used in the bioremediation processes and helped in biodegradation of crude oil in laboratory scale microcosm experiments [Thavasi et. al., 2011]. *Rhodococcus sp.* isolated from the Iranian oil contaminated soil was able to recover 65% of the trapped oil in a sand pack column, which suggests its applications in the enhanced oil recovery [Shavandi et. al., 2011]. The bio-surfactant produced from *Serratia marcescens* NSK1 was able to remove 60% of the engine oil and 51% of kerosene in a soil column study, which suggest its various applications in microbial enhanced oil recovery [Anyanwu et. al.,
The bio-surfactant from the strain *Pseudomonas aeruginosa* EM1 isolated from the waste water of petrochemical industry was stated to be a good one to be used in the biodegradation processes [Wei et. al., 2005].

### 1.5.7 Bio-surfactants for agricultural use

To increase the rate of agricultural productivity to meet ever growing food demands of the human population and environmental pollution control has become a matter of great concern for all the nations. The agricultural residues left after field burns, produces various toxic compounds in the atmosphere and pollutes the environment; so, as an attractive alternative to the traditional field burning of this kind of residue, the utilization of agricultural wastes (barley bran, trimming vine shoots, corn cobs, and *Eucalyptus globulus* chips) for simultaneous bio-surfactant and lactic acid production is of great interest [Moldes et. al., 2007]. The degree of association of organic and inorganic pollutants is regulated by the complex physico-chemical interactions at boundaries of cell surfaces [Christofi and Ivshina, 2002]. In April 2009, a theme study entitled “Sustainable Agriculture and Food Security in Asia and the Pacific” was conducted by the United Nation’s Economic and Social Commission for Asia and the Pacific (ESCAP) which states the significance of the revitalization of native soil systems for improved crop yield was emphasized. Such rejuvenating processes can be carried out in an eco-friendly manner using various biological improvements. In this context, the bio-surfactants possess several functional properties and can be used effectively in agricultural industries [Sachdev and Cameotra, 2013].
1.5.8 Bio-surfactants use in mining

Bio-surfactants may also have some applications in mining and manufacturing processes. Enhanced metal extraction from the mining ores and partial solubilization of lignite coal are also known. Bio-surfactants can be used for the diffusion of inorganic minerals in mining and manufacturing processes [Singh et. al., 2007]. An anionic polysaccharide called biodispersan, produced by *Acinetobacter calcoaceticus*, prevented flocculation and dispersed a 10% limestone in water mixture. Biodispersan role out two functions: dispersant and surfactant. It catalyzes the fracture of limestone into smaller particles. Kao Chemical Corporation (Japan) used *Pseudomonas*, *Corynebacterium*, *Nocardia*, *Arthrobacter*, *Bacillus* and *Alcaligenes* to produce bio-surfactants for the stabilization of coal slurries to abet the transportation of coal. The removal of anionic contaminant arsenic from the mining tailings by rhamnolipids was observed [Mulligan, 2009].

1.6 Aims and Objectives

The above discussions have encouraged a lot to work on bio-surfactants emphasizing work on exploring the use of cheaper agro-industrial by-products of the oleo-chemical industry. The following objectives were outlined to achieve the aim:

1. Isolation of bio-surfactant producing micro-organisms from by-products of rice bran oil industry waste. Isolation and characterization of the microorganism(s) isolated.

2. Screening of bacterial strains for bio-surfactant production
3. Production of bio-surfactants [in presence of either isolated microorganisms (step 1) or screened bacterial strains (step 2)] using rice bran oil industry by-products as carbon source.

4. Purification and characterization of the bio-surfactant(s) produced using chromatographic and spectroscopic techniques.

5. Study of applications based on structure of the bio-surfactant produced.