CHAPTER 1

1. INTRODUCTION

Biosurfactants are diverse groups of surface active molecules/chemical compounds produced by microorganisms (Desai and Banat, 1997). These are amphiphilic compounds produced on living surfaces; mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension (ST) and interfacial tensions between individual molecules at the surface and interface, respectively (Karanth et al., 1999). Surfactants are the active ingredients found in soaps and detergents with the ability to concentrate at the air-water interface and are commonly used to separate oily materials from a particular media due to the fact that they are able to increase aqueous solubility of non-aqueous phase liquids (NAPLS) by reducing their surface/interfacial tension at air-water and water-oil interface (Yin et al., 2009). Though, surfactants are widely used in industrial, agricultural and pharmaceutical industries, most of the compounds synthesized are chemically and potentially toxic and cause environmental problems due to the recalcitrant and persistent nature of these substances (Makkar and Rockne, 2003). With current advances in biotechnology, attention has been paid to the alternative environmental process for the production of different types of biosurfactants from microorganisms (Lotfabad et al., 2009).

Primarily, biosurfactants attracted attention as hydrocarbon dissolution agents in the 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry. Most biosurfactants are either anionic or neutral and the hydrophobic moiety can be a carbohydrate, an amino acid, a phosphate group, or some other compounds. The hydrophobic moiety is mostly a long carbon chain fatty acid. These molecules reduce surface and interfacial tensions in both aqueous
solutions and hydrocarbon mixtures. This property of biosurfactant makes them as potential
candidates for enhancing oil recovery (Sarkar et al., 1989). Because of the surface active
property of biosurfactants, micro emulsions are created in which micelle formations occur
where hydrocarbons can solubilize in water or water in hydrocarbons (Banat 1995).
Biosurfactants enhance the emulsification of hydrocarbons, and also have the potential to
solubilize hydrocarbon contaminants and increase their availability for microbial degradation.

The use of chemicals for the treatment of hydrocarbon polluted sites may contaminate
the environment with their by-products, whereas, biological treatment may efficiently destroy
pollutants, while being biodegradable themselves. Hence, considerable attention has been
given in the past to the production of surface-active molecules of biological origin because of
its diversity, flexibility in operation, potential utilization in food-processing, pharmacology
and oil industry (Saharan et al., 2011; Gudina et al., 2011). Though the type and quantity
of the microbial surfactants produced depends primarily on the producer organism, factors like
carbon and nitrogen, temperature, trace elements, temperature and aeration also affect the
production of the organism.

Biosurfactants are derived from renewable resources and are low or nontoxic,
biodegradable, has low critical micelle concentration, high emulsification activity,
demonstrate excellent surface activity, possess high specificity, show effectiveness under
extreme conditions and can be reused through regeneration when compared to the synthetic
surfactants (Koglin et al., 2010; Xu et al., 2011a; Lima et al., 2011b), hence commercially
exploited. Recently, biosurfactants and biosurfactant producing microorganisms are used for
the production of nanoparticles. The area of the biosurfactant mediated process of
nanoparticle synthesis is emerging as a part of Green chemistry (Kiran et al., 2011).
Petroleum industry has traditionally been the major users of biosurfactants for enhanced oil removal recovery applications. In this application, surfactants increase the solubility of petroleum components (Falatko 1991). However, the only drawback of biosurfactants is their production costs when compared to the chemical surfactants (Gautam and Tyagi 2006; Pacwa-Plociniczak et al., 2011). Their future completely depends upon the economic balance between their production costs, functional benefits and the development of economical processes by the use of low cost raw materials (Cameotra and Makkar 1998; Desai and Banat 1997). Therefore, a lot of wastes are getting attention in response to reduce the production cost of biosurfactant (Makkar and Cameotra 2002; Otto et al., 1999; Rodrigues et al., 2006a, 2006b; Das and Mukherjee 2007; Joshi et al., 2008; Rivera et al., 2007; Moldes et al., 2007).

1.1 Biosurfactant Producers

Many microorganisms have been reported to produce several classes of biosurfactants such as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants (Cooper and Zajic 1980; Cooper 1986; Kosaric 1993). These compounds are produced during the growth of microbes on water soluble and insoluble substrates (Sheppard and Mulligan 1987; Desai et al., 1988; Ron and Rosenberg 2001). Microorganisms utilize a variety of organic compounds as a source of carbon and energy for their growth. When the carbon source is an insoluble substrate like hydrocarbons (CnHn), microorganisms facilitate their diffusion into the cell by producing a variety of biosurfactants. Some bacteria and yeasts excrete ionic surfactants which emulsify the CnHn substrates in the growth medium. Some examples of this group of biosurfactants are rhamnolipids which are produced by different Pseudomonas sp. (Burger et al., 1963; Guerra-Santos et al., 1984; Guerra-Santos et al., 1986), or the sophorolipids which are produced by several Torulopsis sp. (Cooper and Paddock 1983). Some other microorganisms are capable of changing the structure of their
cell wall, which they achieve by synthesizing lipopolysaccharides or nonionic surfactants in their cell wall. Examples of this group are: *Candida lipolytica* and *Candida tropicalis* which produce cell wall-bound lipopolysaccharides when growing on n-alkanes (Osumi et al., 1975), *Rhodococcus erythropolis*, many *Mycobacterium* sp. and *Arthrobacter* sp. which synthesize nonionic trehalose corynomycolates (Kretschmer et al., 1982; Ristau and Wagner 1983). These also include lipopolysaccharides, such as emulsan, synthesized by *Acinetobacter* sp. (Rubinowitz et al., 1982) and lipoproteins or lipopeptides, such as surfactin and subtilisin, produced by *Bacillus subtilis* (Cooper et al., 1981). Other effective biosurfactants are mycolates and corynomycolates which are produced by *Rhodococcus* sp., *Corynebacteria* sp., *Mycobacteria* sp. and *Nocardia* sp. (MacDonald et al., 1981; Kretshmer et al., 1982) and ornithinlipids which are produced by *Pseudomonas rubescens*, *Glucobacter cerinus*, and *Thiobacillus ferroxidans* (Knoche and Shively 1972; Tahara et al., 1976).

Till now, the most commonly isolated and the best studied group of biosurfactants are mainly glycolipids and phospholipids in nature. Rhamnolipids are glycolipid compounds produced mainly by *Pseudomonas* sp. which could reduce water surface tension and emulsify oil (Babu et al., 1996; Patel and Desai 1997; Deziel et al., 1999; Rahman et al., 2002). These compounds are environmental friendly since they are biodegradable and have potential industrial and environmental applications.

### 1.2 Characteristics of Biosurfactants

The biosurfactants offers the following benefits over their chemically synthesized counterparts:
(a) Biodegradability: Owing to low toxicity and simple chemical structure, the compounds do not persist in the environment and are degraded easily preventing the problem of being accumulated.

(b) Biocompatibility and digestibility: Biological origin imparts them the inherent characteristic of compatibility, which allows their unabated usage in cosmetics, pharmaceuticals and as functional food additives.

(c) Availability of raw materials: Biosurfactants can be produced from relatively cheap raw materials available in abundance. The carbon source ranging from hydrocarbons, carbohydrates and lipids may be used separately or in combination with each other for microbial production.

(d) Acceptable production economics: Depending on the intended use, biosurfactants can be produced even from industrial wastes and byproducts, which remains a promising area for bulk production (use in petroleum recovery).

(e) Environmental control: Processes for stabilization of industrial emulsions, control of oil-spills, biodegradation and detoxification of industrial effluents and the bioremediation of contaminated soil can be favored with use of biosurfactants.

(f) Specificity: The presence of specific functional groups imparts specificity in the action by the biosurfactant molecules. This property can be of paramount importance in detoxification of specific pollutants, de-emulsification of industrial emulsions, development of specific cosmetic, specialized pharmaceutical and food applications.

1.3 Critical Micelle Concentration

Critical micelle concentration is the concentration of an amphiphilic compound in solution at which micelle is initiated. The CMC is an important parameter during the evaluation of biosurfactant activity. The surface-active properties of biosurfactant mainly depend on its
ability to lower surface and interfacial tensions, CMC value and formation of stable emulsion. The ability to reduce the surface tension depends on the specific concentration of surface-active compound, that is, the CMC, which is defined as the minimum concentration of biosurfactant required to give maximum surface tension reduction of water and initiate micelle formation. Efficient surfactants have very low CMC values, that is, less biosurfactant is required to decrease surface tension (Sarubbo et al., 2006; George and Jayachandran 2009). Biosurfactants are most effective and efficient at their CMC which can be 10–40 times lower than that of chemical surfactants (Desai and Banat, 1997). This is one of the key features which makes biosurfactants more efficient than their counterparts.

1.4 Classification of Biosurfactants

The chemically synthesized surfactants are generally classified on the basis of the polar group present but the biosurfactants are categorized mainly by their chemical composition and microbial origin. Generally, their structure includes a hydrophilic moiety, consisting of aminoacids or peptides; mono-, di-, or polysaccharides; and a hydrophobic moiety comprising saturated or unsaturated fatty acids. Accordingly, Rosenberg et al. (1999) suggested that biosurfactants can be divided into low-molecular weight molecules, which lower surface and interfacial tension efficiently, and high-molecular weight molecules, which are more effective as an emulsion stabilizing agents. The major low-molecular weight molecules are glycolipids, lipopeptides and phospholipids, whereas high-molecular weight molecules include polyanionic heteropolysaccharides containing polysaccharides and proteins, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers. The most important groups of biosurfactants and some of their classes are described below.
1.4.1 Glycolipids

Glycolipids are the most common type of biosurfactant. They are conjugates of carbohydrates and fatty acids. The constituents mono, di, tri and oligosaccharides include glucose, mannose, galactose, glucoronic acid, rhamnose and galactose sulphate. The linkage is by means of either an ether or ester group. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids.

1.4.1.1 Rhamnolipids

Rhamnolipids are the group of biosurfactants that are studied extensively. These are glycolipids in which one or two molecules of rhamnose are connected to one or two molecules of β-hydroxydecanoic acid. These are produced by many species of *Pseudomonas* and have tremendous antimicrobial activity against several common microbes. Production of rhamnose containing glycolipids was first described in *Pseudomonas aeruginosa* by Jarvis and Johnson (1949). Because of their excellent surface activity, the physico-chemical properties of rhamnolipids have received considerable interest (Abalos *et al.*, 2001; Chen 2004; Cohen *et al.*, 2004; Cohen and Exerowa 2007; Hansen *et al.*, 2008; Abdel-Mawgoud *et al.*, 2009; Pornsunthorntawee *et al.*, 2009).

![Chemical structure of rhamnolipid](image)

1.4.1.2 Trehalolipids

It is another type of glycolipids containing trehalose hydrophobic moieties. Various structural type of microbial trehalolipid biosurfactants 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 long chain, α-branched-β-hydroxy fatty
acids. Trehalopids from different organism differ in size and structure of mycolic acid, the number of carbon atoms and the degree of unsaturation (Asselineau and Asselineau 1978). These trehalose lipids are mainly produced by *Rhodococci* and present interesting physico-chemical and biological properties (Lang *et al.*, 1998). A number of possible applications have been proposed for these compounds. In addition, succinoyl trehalose lipids have been found to induce differentiation of leukemia cell lines (Sudo *et al.*, 2000) and to inhibit protein kinase activity (Isoda *et al.*, 1997).

### 1.4.1.3 Sophorolipids

These glycolipids are mainly synthesized by yeast such as *Torulopsis bombicola* (Cooper and Paddock 1984; Hommel *et al.*, 1987), *Torulopsis petrophilum* and *Torulopsis apicola*. They consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Generally, sophorolipids occur as a mixture of macrolactones and free acid form. It has been shown that the lactone form of the sophorolipid is necessary, or at least preferable, for many applications (Hu and Ju 2001). It is found to have better surface tension lowering property whereas acid form has better foam and solubility properties (Nuneza *et al.*, 2003).

### 1.4.2 Lipopeptides and Lipoproteins

Lipopeptides biosurfactants are cyclic compounds which are mostly isolated from *Bacillus* and *Pseudomonas* type bacteria. Lipopeptides mainly consists of hydrophilic peptides which are generally 7 to 10 aminoacids long, linked to a hydrophobic fatty acid structure. *Bacillus* cyclic lipopeptides consists of three major groups known as surfactin, iturin and fengycin families. Among those, surfactin is the most commonly studied and are said to have superior surface activity (Kakinuma *et al.*, 1969).
1.4.3 Fatty acids, Phospholipids and Neutral lipids

Several bacteria and yeast synthesize large quantities of fatty acids and phospholipid surfactants during their growth on n-alkanes (Cirigliano and Carman, 1985). The hydrophilic and lipophilic balance (HLB) is directly proportional to the length of the hydrocarbon chain in their structures (Kretschmer et al., 1982). In Acinetobacter sp., phosphatidylethanolamine rich vesicles are synthesized (Kappeli and Finnerty 1979), which form optically clear microemulsions of alkanes in water. Phosphatidylethanolamine produced by Rhodococcus erythropolis grown on n-alkane causes a lowering of interfacial tension between water and hexadecane to less than 1mN/m and a critical micelle concentration (CMC) of 30 mg/l (Muthusamy et al., 2008).

1.4.4 Polymeric Biosurfactants

Polymeric biosurfactant are high molecular weight biosurfactants. The best studied polymeric biosurfactants are emulsan, liposan, alas an, mannoprotein and other polysaccharide protein complexes. Emulsan is an effective emulsifying agent for hydrocarbons in water (Zosim et al., 1982) even at a concentration as low as 0.001 to 0.01%. Liposan is an extracellular water soluble emulsifier synthesized by Candida lipolytica and is composed of 83% carbohydrate and 17% protein (Cirigliano and Carman 1984).

1.4.5 Particulate Biosurfactants

Particulate biosurfactants are of two types, extracellular vesicles and whole microbial cell. Extracellular membrane vesicles partition hydrocarbons to form microemulsions, which play an important role in hydrocarbon uptake by microbial cells. Sometimes the whole bacterial cell itself can work as surfactant.
1.5 Natural Role of Biosurfactants

Although biosurfactants are produced by a wide number of microbes and are clearly significant in several aspects of growth, it is difficult to generalize or specify on their role in microbial physiology. Mostly, they are known for only one common role, that is, they enable the growth of microorganisms on hydrocarbons. Owing to their diverse chemical structures and properties, various groups of biosurfactants may have different roles in the growth of the microorganisms which produces it, and probably provide advantages in a particular ecological niche. Recently, Ron and Rosenberg (2001) and Van Hamme et al. (2006) reviewed the physiological role associated with biosurfactants. They observed that some biosurfactants are essential for the motility of the microbes, such as, gliding and swimming. Bioemulsifiers also play an important role in regulating the attachment-detachment process of microorganisms.

In addition, bioemulsifiers are involved in cell–cell interactions such as bacterial pathogenesis, maintenance and maturation, and in quorum sensing and biofilm formation. For example, rhamnolipids are essential to maintain the architecture of the biofilms and are considered as one of the virulence factors in the Pseudomonas sp (Arutchelvi et al. 2008; Ron and Rosenberg 2001). Rhamnolipids, surfactin, and mannosylerythritol lipid possess antimicrobial and antibiotic properties thus conferring a competitive advantage to the organism during colonization and cell–cell competition. In addition, there are other roles such as, cellular differentiation, substrate accession and resistance to toxic compounds which are attributed to microbial surfactants. However, their most widespread role is believed to be the interaction between microorganisms and insoluble substrates such as hydrocarbons. Some biosurfactants/bioemulsifiers enhance the growth of microbes on hydrophobic water insoluble substrates by increasing their bioavailability, presumably by increasing their surface
area, desorbing them from surfaces and increasing their apparent solubility (Neu, 1996; Van Hamme et al., 2006).

1.6 Environmental Factors Affecting Biosurfactant Production

The composition and emulsifying activity of the biosurfactant not only depends on the producer strain but also on the culture conditions. Thus the nature of carbon and nitrogen source as well as the C:N ratio, nutritional limitations, chemical and physical parameters such as temperature, pH, aeration, etc., influence not only the amount of biosurfactant synthesized but also the type of polymer produced (Salihu et al., 2009). Environmental factors and growth conditions such as temperature, pH, agitation and O₂ availability affect the production of biosurfactant through their effects on cellular growth or activity.

1.6.1 Carbon Source

Carbon source plays an important role in growth as well as in the production of biosurfactant by various microbes and it varies from species to species. The quality and quantity of biosurfactant are affected directly by the nature of the carbon substrate (Rahman and Gakpe, 2008). Diesel, crude oil, corn oil, glucose, sucrose and glycerol have been reported to be a good source of carbon substrate for biosurfactant production (Desai and Banat, 1997).

1.6.2 Nitrogen Source

This is the second most important supplement for the production of biosurfactant as it is essential for the growth of microbes, as protein and enzyme synthesis depends on it. Different nitrogen compounds have been used for the production of biosurfactants, such as urea, yeast extract, peptone, ammonium sulphate, ammonium nitrate, sodium nitrate, meat extract and malt extracts. Though yeast extract is the most commonly used nitrogen source for biosurfactant production, its usage with respect to concentration is organism and culture medium dependent.
1.6.3 pH

pH plays an important role in the biosurfactant production. Maintaining an optimal pH is an essential factor for the growth of microorganisms and it varies between species to species.

1.6.4 Temperature

Various microbial processes are temperature dependent and gets affected even by a little change. To obtain large quantities of biosurfactants, it is always necessary to optimize the bioprocess at the optimal temperature. Most biosurfactant productions are reported to be performed in a temperature range of 25–30°C (Desai and Banat 1997).

1.6.5 Incubation Time

Incubation time also have a significant effect on the production of biosurfactants. It seems to be an important factor for the biomass production and maximum growth. Different microbes are able to produce biosurfactant at different time intervals.

1.6.6 Aeration and Agitation

Aeration and agitation are important factors which influence the production of biosurfactant as both facilitates the oxygen transfer from the gas phase to the aqueous phase. It may also be linked to the physiological function of microbial emulsifier. It has been suggested that the production of bioemulsifiers can enhance the solubilization of water insoluble substrates and consequently facilitate nutrient transport to microorganisms.

1.6.7 Salt Concentration

Salt concentration of a particular medium also has a corresponding effect on the biosurfactant production as the cellular activities of microbial cell are dependent on the salt concentration. Nevertheless, contrary observations were also noticed for some biosurfactant products which
were not affected by concentrations up to 10% (weight/volume) although slight reductions in the CMC were detected.

1.7 Advantages of Biosurfactants

When compared to the synthetic surfactants, biosurfactants have many advantages owing to their unique functional properties, high biodegradability, low toxicity, low irritancy and compatibility with human skin (Banat et al., 2000; Cameotra and Makkar 2004). Therefore, they are superior to the synthetic ones. The most significant advantage of a microbial surfactant over chemical surfactant is their ecological acceptance (Desai and Banat 1997; Karsa et al., 1999; Banat 2000). Most important feature of the biosurfactant is their environmental acceptability, as they are readily biodegradable and have low toxicity than synthetic surfactants. These unique properties of biosurfactants allow their use and possible replacement of chemically synthesized surfactants in a great number of industrial operations. Moreover, they are ecologically safe and can be applied in bioremediation and waste-water treatment. Some of the potential applications of biosurfactants in pollution and environmental control are microbially-enhanced oil recovery (MEOR), hydrocarbon degradation in soil, removal of heavy-metal from contaminated soil and hydrocarbons from aquatic environment (Singh et al., 2007).

1.8 Application of Biosurfactants

With their high surface activity and environmental compatibility, biosurfactants are widely used in environmental, biomedical and therapeutic, food, agricultural and also in cosmetic industries. They are briefly described as follows:

1.8.1 Anti-adhesive Agents

A biofilm is a group of bacteria that have colonized as a surface. They are potential sources of contamination of food and sterile things. Biosurfactants have been reported to inhibit the
adhesion of pathogenic organisms to solid surfaces or to infection sites, hence, prior adhesion of biosurfactants to solid surfaces of implant materials is essential. Thus controlling the adherence of microorganisms to contact surfaces is an essential step in providing safe and quality products to consumers. Therefore, the involvement of biosurfactants in microbial adhesion and detachment from surfaces has become very important in food and medical fields.

1.8.2 Anti-cancer Activity
There are many reports describing the anticancerous activity of biosurfactants. The biological activities of seven microbial extracellular glycolipids, includingmannosylerythritol lipids (MEL)-A, mannosylerythritol lipids-B, polyol lipid, rhamnolipid, sophorose lipid, succinoyltrehalose lipid (STL)-1 and succinoyltrehalose lipid-3 have been investigated (Isoda et al., 1999). Recently, it has been demonstrated that these interesting microbial products can control a wide variety of mammalian cell functions. They are considered to participate in various intercellular molecular recognitions such as signal transduction, cell differentiation, cell immune response, etc. (Osada, 1998).

1.8.3 Immunomodulatory Action
Biosurfactants show potential immunomodulatory actions. Bacterial lipopeptides constitute potent non-toxic, non-pyrogenic immunological adjuvants when mixed with conventional antigens. Park and Kim (2009) reported the role of surfactin on the inhibition of the immunostimulatory function of macrophages through blocking the NK-κB, MAPK and Akt pathway. This provided a new insight into the immunopharmacological role of surfactin in autoimmune disease and transplantation.
1.8.4 Antimicrobial Activity

Many biosurfactants have strong antiviral, antibacterial and antifungal activity. The antimicrobial activity of several biosurfactants has been reported in the literature for many different applications.

1.8.5 In the Food Industry

Biosurfactants have been used for various food processing applications. They are used as emulsifiers for the processing of raw materials. They are also used in bakery and meat products. Improvement in dough stability, texture, volume and conservation of bakery products are obtained by the addition of rhamnolipids (Van Haesendonck and Vanzeveren 2004). Their study also suggested the use of rhamnolipids to improve the properties of butter cream and frozen confectionery products.

1.8.6 In Agricultural Industry

Maintaining soil health and protecting crops from various diseases are two prime issues in agriculture. Biosurfactants have been used to deal with these issues. There are many studies demonstrating the use of biosurfactants as bio-control agents. Biosurfactants are used as mobilizing agents to enhance the solubility of bio-hazardous chemical compounds such as PAH. These are also used for the hydrophilization of heavy soils to obtain good wettability and to achieve even distribution of fertilizer in soil.

1.8.7 In Cosmetics

In the cosmetic industry, due to its emulsification, foaming, water binding capacity, spreading and wetting properties, effects on viscosity and on product consistency, biosurfactants have been used to replace chemically synthesized ones. These surfactants are used as emulsifiers, foaming agents, wetting agents, solubilizers, cleansers, antimicrobial agents, mediators of enzyme action, insect repellents, deodorants, nail care, antacids, bath products, foot care,
acne pads, conditioners, anti-dandruff products, contact lens solutions, baby products, mascara, lipsticks, toothpaste, dentine cleansers, etc. (Gharaei-Fathabad, 2011).

### 1.8.8 In Bioremediation of Soil

Poly aromatic hydrocarbons (PAH) are the most hazardous contaminants present in the soil. Growing interests in biosurfactant applications for treating soils contaminated with hydrocarbon have been developed recently (Bartha 1986; Van Dyke et al., 1993b; Banat 1995). The primary method for removing hydrocarbon pollutants from the soil is by the degradation of hydrocarbon by microbes/microbial products present in the contaminated soil. Partially purified biosurfactants can be used either in bioreactors or *in situ* to emulsify and increase the solubility of hydrophobic contaminants. Moreover, surfactant producing microorganisms or growth limiting factors may also be added to the soil to enhance the growth of added or indigenous microorganisms capable of producing biosurfactants (Lang and Wagner 1993).

### 1.8.9 In Toxic Metal Remediation

Toxic metal contamination is a great threat to the ecosystem. Remediation of soil that is contaminated with heavy metal is possible by using biosurfactants. Biosurfactants help in the remediation of toxic metals from soil by making complexes with the metals. They create a non-ionic form with the metals by ionic bonds which is very stronger than the bonds formed by the soil and metals. By lowering the interfacial tensions, the metals are adsorbed in the soil.

### 1.8.10 Marine Bioremediation

Oil spill is a major pollution in marine environment. PAHs are the major constituent of oil. The most common role of biosurfactants is to enhance the dispersal of contaminants in the aqueous phase and increase the bioavailability of the hydrophobic substrate to
microorganisms, which is followed by the subsequent removal of such pollutants through biodegradation (Aparna et al., 2011, Olkowska et al., 2012).

1.8.11 In Microbial Oil Recovery
An area of considerable interest for biosurfactant application is in the field of microbial enhanced oil recovery (MEOR). Enhanced oil recovery methods were devised to recover oil remaining in reservoirs after primary and secondary recovery processes. It is an important tertiary recovery technology, which utilizes microorganisms and/or their metabolites for residual oil recovery (Banat 1995a). In MEOR, microbes in reservoirs are stimulated to produce polymers and surfactants which aid MEOR by lowering interfacial tension at the oil-rock interface. This reduces the capillary forces preventing oil from moving through rock pores and increases chances of recovery.

1.8.12 In Petroleum Industry
Petroleum industry generates large amounts of solid and semisolid wastes known as oily sludge. Removal of this oily sludge from storage tanks may be carried out by the application of biosurfactants which reduce viscosity and may develop emulsification which facilitates sludge removal thus making sludge pumping easier and causes crude oil recovery. Biosurfactants play a major role in petroleum extraction, transportation, upgrading and refining and petrochemical manufacturing.

1.9 Outline of the Study
Environmental pollution caused by the petroleum hydrocarbons represents a great risk to ecosystems. Biodegradation is an effective way to overcome this problem (Pirollo et al., 2008; Zhang et al., 2005). Petroleum is one of the most important energy resources and a raw material of the chemical industry. The world depends on oil and the use of oil as fuel has contributed to intensive economic development. Although petrochemical plants and oil
refineries are beneficial to society, they generate a large amount of solid and semisolid wastes known as oily sludge. A considerable amount of oily sludge can be generated from the petroleum industry during its crude oil exploration, production, transportation, storage and refining processes (Xu et al., 2011; Mrayyam and Battikhi, 2005). The composition of oily sludge varies due to the large diversity in the quality of crude oils, differences in the processes used for oil-water separation, leakages during industrial processes, and also mixing with the existing oily sludge. Usually, the oily sludge contains water, sand, oils, grease, organic compounds, chemical elements, and metals (Lima et al., 2011a). In particular, the sludge generated during the petroleum refining process has received much attention in recent years. It contains a high concentration of petroleum hydrocarbons (PHCs) and other recalcitrant components.

Most of the oily sludge generated during oil production is flushed outdoors without any treatment causing serious threats as it contains toxic materials such as polycyclic aromatic hydrocarbons (PAHs), many of which are carcinogenic or mutagenic. As being recognized as a hazardous waste in many countries, the improper disposal or insufficient treatment of oily sludge can pose serious threats to the environment and human health (Xu et al., 2009; Mrayyan and Battikhi, 2005; Liu et al., 2009; Mater et al., 2006; da Rocha et al., 2010). Disposal of the oil containing sludge into pits and lagoons is hazardous for the environment on one hand as it can pollute ground water and on the other hand it is the wastage of energy. It would be appreciable if this sludge could be pre-treated to lessen its oil content before it is disposed. Once the viscosity and the binding affinity of the different components of sludge are reduced it becomes easier to remove the sludge from storage tanks.

The effective remediation of oily sludge has become a worldwide problem due to its hazardous nature and increasing production quantity around the world. During the past years,
a variety of the oily sludge treatment methods have been developed such as land farming, solvent extraction, ultrasonic treatment, photocatalysis, chemical treatment and biodegradation (da Rocha et al., 2010; Roldan-Carrillo et al., 2012; Zubaidy and Abouelnasr, 2010; Li et al., 1995; Yan et al., 2012). Conventional physico-chemical methods can rapidly remove the majority of spilled oil, but in most cases, removal simply transfers contaminants from one environment to another and can even produce toxic byproducts. Moreover, crude oil cannot be completely cleaned up with physico-chemical methods.

As a result, there is a need for an alternative method for the treatment of oily sludge. Thus, more attention is being given to biological alternatives (Malik and Ahmed, 2012; Lin et al., 2014). One of the most promising ways of pre-treatment of sludge to reduce its viscosity is through the use of microbe and/or microbial products because of their environmental acceptability. A variety of microbes and their products (biosurfactants) are reported as potential candidates for hydrocarbon recovery. Indeed, biosurfactants have applications in different industrial processes as well as they have possible novel uses in the future and are expected to become as multifunctional materials of twenty-first century (Marchant and Banat, 2012). Currently, the major market or need for biosurfactants is in the petroleum industry, in which these compounds can be used in the cleaning up of oil spills, removal of oil residue from storage tanks, microbial enhanced oil recovery and the bioremediation of soil and water (Sobrinho et al., 2013).

Though different types of microbes have been reported to be used in hydrocarbon recovery, Pseudomonads are the best known bacteria capable of utilizing hydrocarbons as the carbon and energy sources and produce biosurfactants which enhance the uptake of such immiscible hydrophobic compounds (Al-Tahhan et al., 2000; Beal and Betts 2000; Rahman et al., 2002; Cameotra and Singh 2008; Pornsunthorntawee et al., 2008b). Among
Pseudomonads, *Pseudomonas* is one of the most often reported genera for its availability to produce biosurfactant molecules (Koch *et al*., 1991; Santos *et al*., 2002).

Though many studies have been conducted to investigate microbial enhanced oil recovery, not much work has been reported in the recovery of hydrocarbons from refinery sludge. Hence, the present study was to evaluate an alternative process for the recovery of hydrocarbons from oily sludge by using microbial product to reduce viscosity. Microbes were isolated from the water and sediment sample collected from Ennore creek, biosurfactant producing bacteria were cultured, isolated and purified. The purified biosurfactant was then made to react with the oily sludge collected from two areas and the recovery of hydrocarbons was noted. Also, the anti-cancer activity and immunomodulant property of the rhamnolipids have been studied.