CHAPTER 1

INTRODUCTION

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1.1 Surfactant

The term *surfactant* is a blend of ***surf**ace **act**ive **agent***. Surfactants are usually organic compounds that are amphiphilic, they contain both hydrophobic groups (their "tails") and hydrophilic groups (their "heads"). Therefore, they are soluble in both organic solvents and water. The term surfactant was coined by Antara products in 1950 (Rahman and Randhawa 2015).

Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Surfactants can assemble in the bulk solution into aggregates, examples of such aggregates are vesicles and micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge) (Shaw 1994; Rosen and Kunjappu 2012).

Global surfactant demand increases day by day due to its large application in various sectors such as enhanced oil recovery, soil and aquifer remediation, foods, pharmaceuticals, cosmetics, herbicides, pesticides, leather industries, house hold, paint industries etc. Global surfactants market demand was 15.93 million tons in 2014 and is expected to reach 24.19 million tons by 2022, growing at a compound annual growth rate (CAGR) of 5.4% from 2015 to 2022 (http://www.marketwired.com/press-release/surfactants-market-to-be-worth-4620-billion-by-2022-grand-view-research-inc-2074859.htm).

Almost all synthetic surfactants currently in use are chemically derived from petroleum-based. These compounds are usually toxic to environment, and they can release toxic chemicals when they decompose, they are toxic to human skin and also there by-products can be environmental hazardous, due to its persistent (non-biodegradable) nature they can negatively affect the soil fertility and also contaminate the water bodies (Md 2012). Apart from these disadvantages still there use increases day by day globally from many industries to household's uses. That's where biosurfactants come in, biosurfactants are surface-active compounds produced by living cells. Their nature and surface tension-reducing abilities depend on the type and strain microorganism (bacteria, yeast, fungi) used and the available nutrient substrate for cell growth (Singh, Jain et al. 2017).

1.1.1. Classification

A surfactant can be classified by the presence of formally charged groups in its head. A non-ionic surfactant has no charge groups in its head. The head of an ionic surfactant carries a net charge. If the charge is negative, the surfactant is more specifically called anionic; if the charge is positive, it is called cationic. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic (Myers 2002).

Anionic (based on sulfate, sulfonate or carboxylate anions

Cationic (based on quaternary ammonium cations)

Zwitterionic (amphoteric)

Nonionic

Biosurfactants are mainly classified according to their chemical composition, molecular weight, physico-chemical properties, chemical structure and their microbial origin. The main classes of biosurfactants are glycolipids, phospholipids, polymeric biosurfactants and lipopeptides (surfactin) (Pacwa-Płociniczak, Płaza et al. 2011).

1.1.2 Surfactant Uses:-

Surfactants having various potential applications as shown in table 1.1 shows its application over different areas and figure 1.1 shows its contribution in different sectors in percentage form (surfactant market).

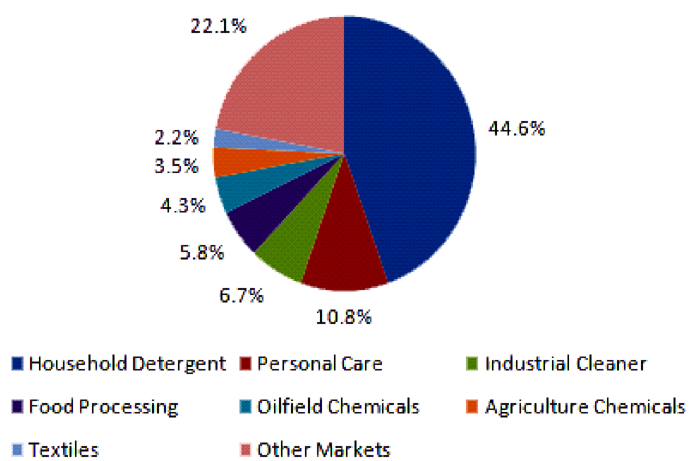

Figure 1.1 Surfactant contributions in different sector

(https://www.grandviewresearch.com/industry-analysis/biosurfactants-industry)

Table 1.1 Potential applications of surfactants (Banat, Makkar et al. 2000)

No.	Function	Application Field
1.	Emulsifiers and dispersants	Cosmetics, paints, additives for rolling oil
2.	Solubilizers and microemulsions	Toiletries, pharmaceuticals
3.	Wetting and penetrating agents	Pharmaceuticals, textile industry, paints
4.	Detergents	Household, agriculture products
5.	Foaming agents	Toiletries, cosmetics, ore floatation
6.	Thickening agents	Paints
7.	Metal sequestering agents	Mining
8.	Vesicle forming materials	Cosmetics, drug delivery systems
9.	Microbial growth enhancers	Sewage sludge treatments for oily wastes, fermentation
10.	Viscosity reducing agents	Pipeline transportation
11.	Dispersants	Coal-oil mixture, coal-water slurry
12.	Resource recovery agents	Tertiary recovery of oil

1.2 Biosurfacatants

Microbial surfactants/Biosurfactants are surface-active metabolites produced by microorganisms when grown on water miscible or oily substrates: they either remain adherent to Microbial cell surfaces or are secreted in the culture broth. They possess the characteristic property of reducing the surface and interfacial tensions using the same mechanisms as chemical surfactants.

Microbial surfactants constitute a diverse group of surface-active molecules and are known to occur in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, and polymeric and particulate structures. The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and, hence, greater environmental compatibility, better foaming properties (useful in mineral processing), and stable activity at extremes of pH, salinity and temperature (Mukherjee, Das et al. 2006).

Biosurfactant activities can be determined by measuring the changes in surface and interfacial tensions, stabilization or destabilization of emulsions, and hydrophilic-lipophilic balance (HLB). Surface tension at the air/water and oil/water interfaces can easily be measured with a tensiometer. When a surfactant is added to air/water or oil/water systems at increasing concentrations, a reduction of surface tension is observed up to a critical level, above which amphiphilic molecules associate readily to form supramolecular structures like micelles, bilayers, and vesicle. This value is known as the critical micelle concentration (CMC). CMC is defined by the solubility of a surfactant within an aqueous phase and is commonly used to measure the efficiency of a surfactant. Microbial culture broth or biosurfactants are diluted severalfold, surface tension is measured for each dilution, and the CMC is calculated from this value (Desai and Banat 1997).

Originally, biosurfactants attracted attention as hydrocarbon dissolution agents in the late 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry (Banat, Makkar et al. 2000; Cao, Liao et al. 2009).

Biosurfactants have unique properties that chemical synthesized surfactants do not have, such as high surface activity, environmental friendliness, lower toxicity, biodegradability, ecological acceptability, good anti-microbial activity and do not lose physico-chemical properties at different temperatures, pH and salinity levels (Banat, Makkar et al. 2000; Mukherjee, Das et al. 2006).

They are widely used in microbial-enhanced oil recovery (MEOR), agriculture, food, cosmetics, and pharmaceuticals industries. Today crude oil and petroleum products are the major source of hydrocarbon pollutants for soil and marine environments. Due to the insoluble nature of these pollutants in water, their removal from the environment is very difficult. Biodegradation of hydrocarbons by microorganisms is one of the promising ways to remove them from the soil and marine environments (Inakollu, Hung et al. 2004; Moldes, Paradelo et al. 2011; Ławniczak, Marecik et al. 2013).

In number of studies it was found that the genetically modified microorganisms will survive less in environment condition like when they were introduce into the natural condition of soil, because expression of inserted gene require extra energy that could reduce their environmental fitness (Viebahn, Smit et al. 2009). Survival chances could become more lesser of genetically modified microorganisms when they were introduce under stress environmental condition such as crude oil contaminated soil. To overcome this problem one of the best approach is to acclimatize the microbial strain under stress environmental (Hydrocarbon) condition for the production of desirable product (Biosurfactant).

1.2.1. Market value of Biosurfactant

The global market for biosurfactants is expected to reach USD 2,308.8 million by 2020. Escalating consumer preference towards the use of bio-based products, particularly in Europe and North America is expected to increase biosurfactant penetration. The global biosurfactant market is expected to reach 462 kilo tons by 2020, growing at a CAGR of 4.3% from 2014 to 2020 (https://www.grandviewresearch.com/industry-analysis/biosurfactants-industry). Europe was the largest regional market for biosurfactants, with consumption of 178.9 kilo tons in 2013. Figure 1.2 shows the market size biosurfactant in various areas in 2015 and expected in 2023. North America was the second largest consumer of biosurfactants in 2013 with a share of over one quarter.

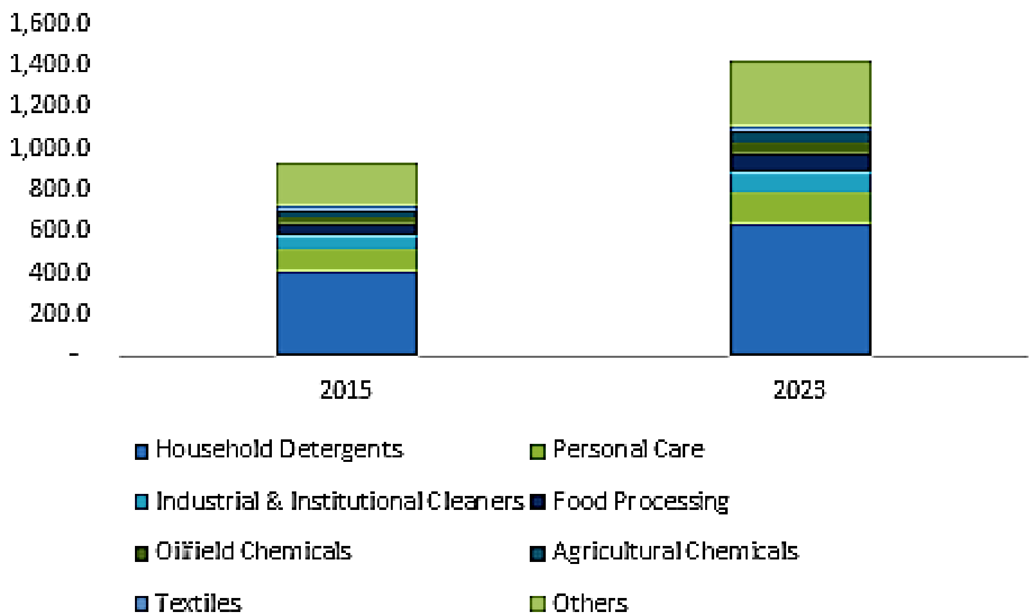

Figure 1.2. Europe Biosurfactants Market size, by application, 2015 & 2023 (USD Million) (https://www.fractovia.org/news/industry-research-report/biosurfactants-market)

Asia pacific was a relatively small market in 2013 but is expected to gain considerable share over the forecast period on account of growing application industry in the region. Countries such as India and China have extensive presence of textile, agricultural and personal care industry which is expected to augment the demand for biosurfactants over the next six years (https://www.gminsights.com/pressrelease/biosurfactants-market-size).

The applications of biosurfactants however, are still at the developmental stage of industrial level. The development of biosurfactant application in industries is focused mainly on high biosurfactant production yield and the production of highly active biosurfactants with specific properties for specific applications. Biosurfactants plays an important application in petroleum-related industries which such as enhanced oil recovery, cleaning oil spills, oil-contaminated tanker cleanup, viscosity control, oil emulsification and removal of crude oil from sludges (Desai and Banat 1997). These industries are known to be the potential target for the application of these compounds.

Considering wide potential applications of biosurfactant it is important to enhance its production on commercial level and also wide variety of diverse microorganism can able to produce biosurfactant with different chemical structure and surface properties. It is more advantageous when biosurfactant production through the microorganisms when they are grown on water-immiscible substrate by interact with the phase boundary between two phases in a heterogeneous system. Biosurfactants enhance the emulsification of hydrocarbons to solubilize hydrocarbon contaminants and increase their availability for microbes and as a result this phenomenon allows microbial growth on water-immiscible carbon source.

1.3. Thesis Objectives

Specific objectives for the thesis are as follows.

I- Acclimatization of microbial strain at high hydrocarbon condition and its Optimization using one-factor-at-a-time strategy for biosurfactant production.

Acclimatization of *C. tropicalic* MTCC230 under high hydrocarbon (Kerosene, Petrol, Mustered oil) as carbon source along with glucose for the production of biosurfactant. After acclimatization of *C. tropicalic* MTCC230 on hydrocarbon as carbon source, different nitrogen source (NH_4Cl, $NaNO_3$, NH_4NO_3), effect of microelements for the production of biosurfactant was studied and determine the emulsification index from different hydrocarbon source.

II- Optimization of media composition and other factors for biosurfactant production using biostatistical analysis (Response Surface Methodology).

To optimize the production of biosurfactant by using biostatistically based experimental design, Response Surface Methodology technique is not only less time consuming but also shows the interactive effect of different parameters. To the best of our knowledge, there have been no reports on the application of statistical methods for the optimization of biosurfactant production when *Candida tropicalis* MTCC230 is grown on two carbon sources of which one is water immiscible and another one is water-miscible. With the help of this study we can enhance the production of biosurfactant by achieving the acclimatized strain of *Candida tropicalis* MTCC230 in high hydrocarbon concentration media.

III- Characterization and physicochemical properties of biosurfactant produced from an adaptive strain for microbial enhanced oil recovery (MEOR).

Biosurfactant produced from an acclimatized strain of *Candida tropicalis* MTCC230 was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Near IR spectroscopy, Reversed-phase high-performance liquid chromatography (RP-HPLC) and Mass Spectroscopy (MS) analysis. The physicochemical properties of the biosurfactant including Critical Micelle Concentration (CMC), oil spreading activity and emulsification ability were also studied. Surface tension variation study was done at the different temperature, pH and salinity for determining the effectiveness of biosurfactant under extreme environmental condition. Furthermore, a soil column saturated with crude oil was washed with biosurfactant by batch type soil washing this revels its potential use in MEOR. Waste mobile oil contaminated soil was washed with biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC230. This study is very much helpful for evaluating the properties, effectiveness and characterizing the biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC230 and its potential commercial application in MEOR.

IV- *InSilico* study: effect of surfactin against amyloid β peptide was studied by using computational approaches.

Insilico approach was applied to know the interaction site for single molecule of surfactin (Biosurfactant) with amyloid beta 42 and amyloid fibril. To reveal the common bindings of surfactin to both amyloid fibril and amyloid beta molecular docking was performed and molecular dynamics simulation studies were performed to validate the docking studies of amyloid beta and further used to analyse binding mechanism and effect of binding. This study constitutes a new frontier with a template for in vitro and in vivo experimentation in reference to new. In future this could potentially allow neuroscientists to adopt this *insilico* approach for the development of novel surfactin based therapeutic interventions in the neuroprotection and neurotherapy of Alzheimer‘s disease.

V- Radiological: Pre-clinical Comparative study of microbial derived surfactants with survanta for treatment of Respiratory Distress Syndrome (RDS).

The objective of present study was to do comparative study of microbial produced surfactant with FDA approved drug survanta by using Near IR and Far IR spectroscopy this is useful for preclinical test for RDS treatment. Lim et al. identified two spectral diagnostic windows for deep tissue imaging in Near IR region of the spectrum (700–900 nm) and for Far IR spectrum 1,200– 1,600-nm, these two IR spectrum regions used as “diagnostic window” of tissue imaging, where the absorption coefficients of body fluids are at their minimum. Near IR and Far IR spectrums of three surfactants (survanta, surfactin produced from *B. subtilis* MTCC2423 and lipopeptide (Biosurfactant) produced from an acclimatized strain produced from *C. tropicalis* MTCC230) was analyzed. Results showed that there is a specific set of frequencies for all three surfactants and these set of frequencies can be used to detect the presence of surfactant. Survanta (FDA approved drug used for RDS treatment) was used as a standard for the comparative radiological study of surfactin produced from *B. subtilis* MTCC2423 and lipopeptide (Biosurfactant) produced from an acclimatized strain produced from *C. tropicalis* MTCC230 that was characterized as surfactin. This study reveals that microbial surfactant may be considered for treatment of RDS in an infant.

1.4. Organization of thesis:

The whole work presented in the thesis is organized into following chapters based on committed objectives. The introduction section is brief detail about Surfactant, Biosurfactant, applications and their market value. The second chapter is reviews of literature consist of biosurfactant: How it is produced, role of hydrocarbon in biosurfactant production and other factors, applications, recovery processes, characterization. Further, each chapter consist problem-related review of literature *i.e.*, introduction then materials & methods, results & discussion and conclusion.

The thesis will be organized in following chapters.

Chapter 1: Introduction and objectives

Chapter 2: Review of literature

Chapter 3: Acclimatization of strain in high hydrocarbon condition and Optimization of media composition and other factors using one-factor-at-a-time strategy and biostatistical analysis for biosurfactant production

Chapter 4: Characterization and physicochemical properties of biosurfactant produced from an adaptive strain for microbial enhanced oil recovery (MEOR)

Chapter 5: *InSilico* study: effect of surfactin against amyloid β peptide was studied by using computational approaches

Chapter 6: Radiological: Pre-clinical Comparative study of microbial derived surfactants with survanta for treatment of Respiratory Distress Syndrome (RDS)

Chapter 7: Summary of work

Chapter 8: References