Chapter 1
Introduction

India is an agricultural economy; more than fifty percent of Indian population depends on agriculture for their primary source of income. Agriculture accounts for 14% of our nations GDP. India has made a significant progress in agriculture in the last five decades and produced a record of 257.4 million tons of food grains in 2011-12. There has been an increase of 6.45 times in agricultural exports in the last ten years and currently it accounts for 13.08% of India’s total exports.

With ever increasing production of food grains and other agricultural produce there is also a corresponding increase in crop residue, it is estimated that more than 500 million tonnes of crop residues are generated in India every year. Crop residues encompass all agricultural wastes such as straw, stem, stalk, leaves, husk, shell, peel, lint, seed/stones, pulp, stubble, etc. which come from cereals (rice, wheat, maize or corn, sorghum, barley, millet), cotton, groundnut, jute, legumes, coffee, cacao, olive, tea, fruits, molasses, bagasse, oilseed cakes, maize milling by-products and brewer’s wastes etc. These residues are used as a source of domestic and industrial fuel, cattle feed, for thatching of homes, also as a source of industrial products such as ethanol, solvents etc.

The use of agricultural products such as wheat, rice, corn etc. for the production of chemicals or bio-fuels is well established, but with the rise in human population and ensuing food shortages around the globe, the use of crop biomass for production of industrial chemicals raises an ethical issue worldwide. However, the use of agricultural residues as substrates for fermentation products is a different issue altogether because, use of residues for production of chemicals and biochemicals is not only an economically viable proposition because of the abundance of agricultural residues as raw materials, but also, because it helps abet pollution arising out of combustion of such residues. The major task is then to develop appropriate process technologies to convert such crop residues into commercially viable products.

1.1 Crop residues as feedstock for chemicals and biochemicals

Traditionally crop residues have numerous competing uses such as animal feed, fodder, fuel, roof thatching, packaging and composting. The residues of cereal crops are mainly used as cattle feed. Rice straw and husk are used as domestic fuel or in boilers for parboiling rice. The surplus residues i.e., total residues generated minus residues used for various purposes, are typically burnt on farm. Agro-industrial residues can be used in many ways because they are abundant and cheap, their use provides us environmental and economic benefits. In USA and Canada, the straws of wheat, barley, oats and rye, and the husks of rice have been utilised in mixture with wood fibres in the production of pulp, particle boards and fibreboards (Hesch, 1978; Loken et al.,1991; Knowles, 1992). In Asia, husks of rice have been used to produce cement-boards (Govindarao, 1980). China and Japan also have made attempts to utilize Indian cane fibers in combination with wood fibers and foamy plastics to produce various kinds of wood boards, (Wang and Joe, 1983) production of charcoal and briquettes (TDRI, 1983; Hulscher et al., 1992) etc.

Although there are several technologies in India for managing crop residues, agro residues even now are either poorly valorised or left to decay on the land. (Sarath et al., 2008; Zhang, 2008). Constraints that limit the large-scale adoption of these technologies are the high cost and labour requirement for its collection and transportation.

Production of chemicals from agro-industrial residues and derivatives has been in focus for quite some time. Production of ethanol from renewable biomass has received considerable attention in recent years because ethanol as a gasoline fuel additive as well as transportation fuel helps to alleviate global warming and environmental pollution. Agro-residues also have the potential to produce biogas under anaerobic fermentation conditions. Currently, there is considerable activity on the use of agro-residues for production of biodiesel. In general the agro-residues are rich sources of proteins and polysaccharides that can be converted to value added products by the fermentation route. Almost all end products obtained from agro residues are fermentation products. Hence, appropriate biotechnology serves as the pathway to valorisation of the agro residues. Table 1.1 highlights some of the major chemicals that have been synthesized using agro residues or by products obtained from agro based chemical industries such as molasses and lignin.

1.2 Microbial surfactants and their uses

One important class of chemicals with considerable commercial interest are the biosurfactants or microbial surfactants. Biosurfactants are surface-active biomolecules produced by microorganisms, they are abundant in nature. Lung surfactants and bile are absolutely necessary for human life to survive. According to Nitschke and Pastore (2006) majority of microbial surfactants are complex biomolecules, comprising different structures that include lipopeptides, glycolipids, polysaccharide protein complex, fatty acids and phospholipids.

Table 1.1: Major chemicals synthesized using agro residues

Agro residues/by products	Application	Reference
Biomass(corncobs, corn stalks, sugarcane waste, wheat or rice straw, starchy materials	Ethanol production, Vitamins, amino acids, antibiotics	Lin and Tanaka, (2006); 1
Wheat bran	Ferulic acid production	Faulds et al. (1997)
Cassava bagasse	Organic acids, flavour, aroma compounds	Pandey et al. (2000b)
Sugarcane bagasse	Production of protein enriched cattle feed and enzymes	Pandey et al. (2000a)
Molasses	Extracellular polysaccharides	Israilides et al. (1994)
Cotton stalk, rice straw, bagasse, banana plant waste	Extraction of lignocellulosic fibres	Habibi et al. (2008)
Wheat bran, rice bran, rice straw,	Production of endoxylanase, natural waxes	Poorna and Prema, (2006)
Sugarbeet pulp, potato pulp, Brewery grain	Methane production	Kang and Wetland, (1993)
Soyabean oil and canola oil waste	Glycerol	1
Waste of biodiesel producing refinery	Succinic acid	1

Microbial surfactants have gained attention in recent years due to their unique properties like higher biodegradability, lower toxicity, and effectiveness at extremes of temperature, pH and salinity (Mukherjee et al., 2006). Bio-surfactants are used for diverse applications such as synthesis of antibiotics to enhanced oil recovery. They draw their applications from their capability to drastically reduce surface tension at very low concentrations. Bio-surfactants are projected to replace synthetic surfactants in several industrial processes, such as lubrication, wetting, softening, fixing dyes, making emulsions, stabilizing dispersions, preventing foaming, as well as in food, biomedical and pharmaceutical industry, and bioremediation of organic- or inorganic-contaminated sites.

Glycolipids and lipopeptides are the most important bio-surfactants (BS) for commercial purpose. The major classes of bio-surfactants and their applications are listed in Table 1.2. In general biosurfactants are microorganism specific high molecular weight compounds. In spite of their importance, not much appears to have been done to develop technologies to synthesize them in quantities that make their application more wide spread. Table 1.3 lists some of the commercial scale products incorporating bio-surfactants that are in use.

Table 1.2: Major classes of biosurfactants and their applications

			Microorganism	**Applications**	**Reference**
Biosurfactants	**Glycolipids**	Rhamnolipids	*P. aeruginosa and P. putida*	Bioremediation	Kumar et al. (2006)
			P. chlororaphis	Biocontrol agent	Reis et al. (2013)
			Bacillus subtilis	Antifungal agent	Reis et al. (2013)
			Renibacterium salmoninarum	Bioremediation	Reis et al. (2013)
		Sophorolipids	*Candida bombicola and C. apicola*	Emulsifier, MEOR, alkane dissimilation	Banat et al. (2010)
		Trehalose lipids	*Rhodococcus spp.*	Bioremediation	Wei et al. (2005)
			Tsukamurella sp., Arthrobacter sp.	Antimicrobial agent	Reis et al. (2013)
		Mannosylerythritol lipids	*Candida antartica, Candida sp.* SY16	Neuroreceptor antagonist, antimicrobial agent	Wakamatsu et al. (2003); Kim et al. (2006)
			Kurtzmanomyces sp	Biomedical application	Zhao et al. (1999); Zhao et al. (2000)
	Lipopeptides	Surfactin	*Bacillus subtilis*	Antimicrobial agent, biomedical application, cosmetic items, Antiviral, anti-tumor, anti-mycoplasma activity, thromobolytic activity, MEOR	Seydlova and Svobodova, (2008)
		Lichenysin	*B. licheniformis*	Hemolytic and chelating agent	Singh et el. (2008)

Usually as of now most bio-surfactants are synthesized only on laboratory scale and are available in very small quantities at unaffordable costs. One gram of surfactin sells for almost Rs 12,00,000/-. Therefore, there exists a very promising case for developing technology for synthesizing biosurfactant from agro- residues and agro based substrates to make them available commercially in tangible amounts and high purity for the benefit of man and society.

Table 1.3: Commercial scale products incorporating biosurfactants (Reis et al., 2013)

Origin	Biosurfactant (Supplier)	Application
Bacterial Rhamnolipid	BioFuture, BioFuture Ltd. Ireland	Bioremediation of contaminated soil with hydrocarbon
	EC-601, Ecochem Ltd, Canada	Dispersive agent of water insoluble hydrocarbons
	JBR products Jeneil Biosurfactant Co., LTC, USA	Used in different industries, such food and agro-industrial markets
Bacterial consortium	EC-2100W, Ecochem Ltd, Canada	Degrades hydrocarbon based compounds in waste water treatment plants, lagoons, storage tanks, sumps and other aqueous environments
	EC-1800, Ecochem Ltd, Canada	Cleans up oil spills in soil, sand and gravel
	Petrosolv Enzyme Technologies Inc,USA	Oil removal; oil recovery and processing
Bacillus subtilis	Surfactin, Sigma-Aldrich Co. LLC, USA	Antifungal, antibacterial and antitumor activities

1.3 Surfactin

Surfactin – a biosurfactant, is perhaps the most potent surfactant known to man, is a cyclic lipopeptide, exhibits high surface activity and has an antibiotic potential. Surfactin as membrane active agents is a promising alternative to synthetic medicine and antimicrobial compounds, and could be used as effective therapeutics, especially at time when drug resistance among causal organisms for many life-threatening diseases increases. Surfactin also exhibits anti-viral activity against several viruses including semliki forest virus, herpes simplex virus (HSV-1a HSV-2), vesicular stomatitis virus, simian immunodeficiency virus, feline calicivirus and murine encephalomyocarditis virus. Surfactin has been explored for anti-mycoplasma effect, anti-tumor activity, and thrombolytic activity, and has found to be effective hence, suggested for possible use in the treatment of above mentioned conditions. Further investigations on human cells and natural biota are required to be carried out for surfactin so that it could be utilized in biomedical and other health-related areas.

Pre-coated vinyl urethral catheters with surfactin were shown to reduce biofilm formation by several *Salmonella* species and other infectious bacteria (Mireles et al., 2001). Surfactin has been shown to be more efficient than chemical surfactants (sodium dodecyl sulphate or dodecylamine) in improving floatability of metal laden sorbents under similar conditions (Zouboulis et al., 2003). Surfactin was also shown to contribute to reduce colonization of pathogenic bacteria, such as *L. monocytogenes*, *Enterobacter sakazakii* and*Salmonella enteritidis*, when applied to solid surfaces prior to infection (Nitschke et al., 2009; Korenblum et al., 2012).

Surfactin has been found to have remarkable properties to prevent fibrin clot formation and has been found to be potent clotting inhibitor and works by inhibiting the conversion of fibrin clot monomer in to fibrin polymer (Arima et al., 1968). It can therefore be used in future to release the blood clot which can be life saving for the people. This aspect is still being investigated and not utilized professionally. However, it is just a matter of time, before full potential of surfactin is fully exploited and used in medical science (Seydlova and Svobodova, 2008).

Bacillus subtilis, a non-pathogenic species of bacteria is utilized in the production of surfactin which makes the process safe in comparison with rhamnolipid - biosurfactant that is synthesized by *Pseudomonas* species of bacteria which is classified among pathogenic species and requires utmost care during its handling and production.

In spite of its importance and wide range of applications, the potential of surfactin has not been realized commercially because of its very limited availability and high cost.

1.4 Objective of this investigation

It is envisaged that the use of surfactin commercially in medicinal and other applications would see explosive growth if it is made available at reasonable cost. The key to achieve this objective would be to use low cost agro substrates /agro wastes and appropriate downstream processing techniques for production, recovery and concentration of surfactin. This investigation is a humble effort undertaken to address the following:

- To study the viability of the production of surfactin by fermentation of low cost and abundantly available agro based substrates such as, effluent from potato wafer industry, molasses and rice bran, obtained during the polishing of rice using *Bacillus subtilis*.
- To study the carbohydrate consumption pattern and growth behaviour of *Bacillus subtilis* in the varied fermentation media. Develop growth curves and model the data so as to use this information during scale-up of the fermentation system.
- Use foam fractionation as the downstream processing technique to concentrate and purify the biosurfactant.
- Characterize the surfactant using TLC, FTIR, NMR for chemical group identification, HPLC for identification, ESIMS, to determine fatty acid content, the peptide sequence and for molecular weight determination.
- To estimate the size of micelle formed by surfactin in solution using dynamic light scattering (DLS) technique.
- To develop a process for the production and recovery of the biosurfactant and work out the overall economics of the project for the production of biosurfactant at an industrial scale.

1.5 Introduction to the contents of various Chapters

The work is presented in five Chapters. Chapter 1 introduces the overall scope of the study and lists its objectives.

Chapter 2: Literature survey, presents an overview and statistical data of the various agricultural produce available in India and in the world. It lists the different types of biosurfactants and the various low cost substrates that can be utilized for production of biosurfactants, microorganisms utilized by different investigators for biosurfactant production, different fermentation techniques, purification and recovery techniques for production and extraction of biosurfactants.

Chapter 3: Materials and Methods, provides information about the chemicals and other materials utilized in this work. The basic fermentation technique involving growth of BS and the method for production of biosurfactant utilizing various low cost agro wastes are discussed. The chapter also describes the foam fractionation technique as a method of concentration, various analytical techniques and the biosurfactant characterization techniques utilized in this study.

Chapter 4: Results and Discussion, reports the results obtained for various fermentation experiments carried out for the production of biosurfactant in terms of yield, surface tension reduction, colony forming units for enumeration of bacterial growth. The production of biosurfactant with different types of substrates is evaluated. Product characterization by techniques such as ESI-MS, HPLC, FTIR, TLC, and NMR is documented. Discussion and inferences drawn from the results obtained are presented. The scale up and cost estimation for the production at commercial scale is attempted.

The Chapter 5: Summary and Conclusions, summarizes the results obtained and draws significant conclusions pertaining to production, recovery, application of surfactin and the role of the agro based residue in its production.