

Table of Contents

List of Figures	i
List of Tables	iv
Abbreviations	v
Chapter 1. Introduction	1
1.1 Crop residues as feedstock for chemicals and biochemicals	1
1.2 Microbial surfactants and their uses	2
1.3 Surfactin	5
1.4 Objective of this investigation	6
1.5 Introduction to contents of various chapters	7
Chapter 2. Literature Survey	9
2.1 Overview of agro based residues with potential for biosurfactant production	9
2.1.1 Potato	10
2.1.2 Molasses	11
2.1.3 Rice	13
2.2 Biosurfactants overview	14
2.2.1 Classification of biosurfactants	14
2.2.2 Biosurfactants in comparison with chemically synthesized surfactants	19
2.3 Microbial origin of biosurfactants	20
2.4 Metabolic pathways for the synthesis of biosurfactant	22
2.5 Parameters affecting the growth of microorganisms and subsequent biosurfactant production	23
2.5.1 Effect of nutrients provided	23
2.5.2 Effect of temperature	25
2.5.3 Effect of agitation and gas supply	26
2.5.4 Effect of alkalinity or acidity (pH)	27
2.6 Low cost agro based substrates for the production of biosurfactants	27
2.6.1 Starchy substrate	28
2.6.2 Molasses, a byproduct of sugar industry	28
2.6.3 Soapstock, a byproduct from oilseed processing industry	29
2.6.4 Olive oil industry effluent	29
2.6.5 Animal fat	30
2.6.6 Lactic whey and distillery wastes	30

2.6.7	Vegetable oil and oil wastes	30
2.7	Upstream processing techniques for biosurfactant production with emphasis on surfactin – a lipopeptide type of biosurfactant	31
2.8	Downstream processing techniques for recovery and purification of biosurfactant	33
2.8.1	Ultrafiltration	33
2.8.2	Foam Fractionation	35
2.8.3	Adsorption-desorption on polystyrene resins	35
2.8.4	Ion exchange chromatography	35
2.9	Applications of biosurfactants	36
2.10	Isolation and identification of biosurfactants with emphasis to surfactin	38
2.10.1	Thin layer chromatography (TLC)	39
2.10.2	Fourier transform infrared spectroscopy (FTIR)	39
2.10.3	Nuclear magnetic resonance spectroscopy (NMR)	39
2.10.4	High pressure liquid chromatography (HPLC)	40
2.10.5	Electrospray ion mass spectrometry (ESI-MS) and Secondary ion mass spectrometry (SIMS)	41
2.10.6	Surface activity of biosurfactants	41
2.11	Conclusions	42
Chapter 3. Materials and Methods		43
3.1	Materials	43
3.1.1	Agro based wastes as substrates	43
3.1.2	Microorganisms	44
3.1.3	Mineral salts	44
3.1.4	Miscellaneous chemicals used	45
3.2	Methods	45
3.2.1	Inoculum preparation and culture conditions	45
3.2.2	Substrate preparation and analysis	46
3.2.3	Experimental procedure	48
3.2.4	Biosurfactant characterization	50
Chapter 4. Results and Discussion		54
4.1	Background	54
4.2	Potato starch as substrate for biosurfactant production	55
4.2.1	Drop collapse test	56
4.2.2	Surface tension reduction with time	57

4.2.3	Cell growth and carbohydrate utilization by <i>B.subtilis</i> MTCC 2423	59
4.2.4	Growth of <i>Bacillus subtilis</i> using potato starch	59
4.2.5	Effect of nutrients	62
4.3	Biosurfactant production using Molasses and Rice bran as substrate	63
4.3.1	Molasses as substrate	63
4.3.2	Rice bran as substrate	65
4.4	Surfactant concentration in the fermentation media	67
4.5	Growth of <i>B.subtilis</i> and modelling of growth curves	68
4.6	Concentration of biosurfactant using foam fractionation and subsequent acid precipitation	72
4.7	Characterization of synthesized biosurfactant	76
4.7.1	Thin layer chromatography (TLC)	77
4.7.2	FTIR analysis	78
4.7.3	Nuclear magnetic resonance spectroscopy (NMR)	80
4.7.4	High pressure liquid chromatography (HPLC)	84
4.7.5	Electrospray ionization mass spectrometry (ESI-MS)	86
4.8	Dynamic light scattering studies of surfactin solutions	90
4.9	Scale up and production economics	93
4.9.1	Scale up	93
4.9.2	Production economics	96
4.10	Summary	96
Chapter 5. Summary and Conclusion		98
5.1	Low cost agro based substrates as potential sources for biosurfactant production	100
5.2	Growth of <i>B.subtilis</i> , biosurfactant production and downstream processing	101
5.3	Identification and characterization of the biosurfactant	104
5.4	Scope for future work	106
References		109
Appendices		
Appendix 1		
Appendix 2		
Appendix 3		