

Contents

Title Page	i
Certificate of Approval	ii
Certificate	iii
Acknowledgements	iv
Declaration	vi
List of Symbols and Abbreviations	vii-ix
List of tables	x
List of figures	xi-xiii
Abstract	xiv
Contents	xv-xviii
Chapter 1 Introduction and review of literature	1
1.1. Microbial lipopeptides: Global scenario	3
1.2. Strategies for feasible commercial lipopeptide production	6
1.2.1. Lipopeptide production using inexpensive raw materials	6
1.2.2. Improvement in lipopeptide bio-manufacturing technology	7
1.2.2.1. Process optimization	7
1.2.2.2. Artificial neural network (ANN) and particle swarm optimization (PSO)	8
1.2.3. Modes of operation	9
1.2.4. Inexpensive and efficient downstream processing	11
1.2.4.1. Recovery and separation of lipopeptides	11
1.2.4.2. Preparative purification of lipopeptides	13
1.3. Lipopeptides as green stabilizers for microbubbles	14
1.3.1. Microbubbles	14
1.3.2. Role of surface active molecules in microbubble synthesis	16
Scope of the present investigation	18
Objectives	19
Chapter 2 Process optimization for enhanced lipopeptide production by marine <i>Bacillus megaterium</i> using food waste	21
2.1. Introduction	23
2.2. Materials and methods	25
2.2.1. Microorganism, medium and growth conditions	25
2.2.2. Isolation of lipopeptide	26
2.2.3. Analytical methods	26
2.2.4. Optimization of lipopeptide production in batch fermentor	27

2.2.4.1. Statistical experimental design	27
2.2.4.2. Artificial Neural Network Modeling	29
2.2.4.3. Particle Swarm Optimization	30
2.3. Results and Discussion	31
2.3.1. Optimization of lipopeptide production in batch fermentor	31
2.3.1.1. ANN-PSO Optimization	32
2.3.1.2. Validation studies	36
2.4. Conclusion	39
Chapter 3 Development of a bioprocess in fed-batch mode for improved lipopeptide productivity	41
3.1. Introduction	44
3.2. Materials and methods	45
3.2.1. Microorganism, medium and growth conditions	45
3.2.2. Analytical methods	45
3.2.3. Fed-batch mode of operation	45
3.2.3.1. DO-stat fed-batch culture	45
3.2.3.2. Repeated fed-batch culture	47
3.3. Results and discussion	48
3.3.1. DO-stat fed-batch fermentation	48
3.3.2. Repeated fed-batch fermentation	50
3.4. Conclusion	55
Chapter 4 Separation and purification of lipopeptide families by macroporous resin column chromatography	57
4.1. Introduction	60
4.2. Materials and Methods	61
4.2.1. Adsorbent resins	61
4.2.2. Preparation of lipopeptide solution	62
4.2.3. Analytical Methods	62
4.2.3.1. HPLC analysis	62
4.2.3.1. MALDI-ToF analysis	63
4.2.4. Static Adsorption and Desorption Studies	63
4.2.4.1. Selection of Adsorbent for Lipopeptide Recovery	63
4.2.4.2. Factors influencing adsorption	64
4.2.4.3. Adsorption Isotherms	64
4.2.5. Dynamic adsorption and desorption studies	65
4.2.6. Purity measurement	66
4.3. Results and Discussion	66
4.3.1. Static adsorption and desorption tests	66
4.3.1.1. Factors Affecting Static Adsorption	67
4.3.1.2. Adsorption isotherms	69
4.3.2. Dynamic breakthrough studies	69

	4.3.2.1. Effect of column H/D ratio	70
	4.3.2.2. Effect of feed flow rate	72
	4.3.3. Separation and purification of three lipopeptide families by MAR column chromatography	72
	4.3.3.1. Step gradient elution	72
	4.3.3.2. Influence of mobile phase pH	74
	4.3.3.3. Stepwise pH-solvent dual-gradient elution	75
	4.3.3.4. Concurrent recovery and purification of lipopeptides from cell free broth	80
	4.4. Conclusion	80
Chapter 5	Development of a semi-preparative HPLC strategy for simultaneous separation of homologues of lipopeptides	81
	5.1. Introduction	83
	5.2. Materials and Methods	85
	5.2.1. Sample preparation	85
	5.2.2. RP-HPLC	85
	5.2.2.1. Analytical method development	85
	5.2.2.2. Validation	86
	5.2.2.3. Scale-up of the purification process in Semi-preparative HPLC	86
	5.2.3. MALDI-ToF analysis	87
	5.3. Results and discussion	88
	5.3.1. Process development for separation of lipopeptide homologues in analytical HPLC	88
	5.3.2. Validation	90
	5.3.3. Scale-up to Semi-preparative Scale	92
	5.3.4. MALDI-ToF analysis	93
	5.3.5. Comparison of degrees of lipopeptide purity achieved by different downstream processing methods	97
	5.4. Conclusion	98
Chapter 6	Synthesis and characterization of lipopeptide stabilized microbubbles	101
	6.1. Introduction	104
	6.2. Materials and methods	105
	6.2.1. Materials	105
	6.2.2. Sample preparation	106
	6.2.3. Surface Tension Measurements	106
	6.2.4. Dynamic Light Scattering	106
	6.2.5. Circular Dichroism	106
	6.2.6. Preparation of microbubbles	106
	6.2.7. Measurement of bubble size distribution and stability	107
	3. Results and discussion	107

3.1. Conformation and assembly of surfactin molecules	107
3.1.1. Influence of pH	107
3.1.2. Role of counter-ion	109
3.2. Surfactin stabilized microbubbles	110
3.2.1. Influence of pH on microbubble properties	110
3.2.2. Role of counter-ion on microbubble properties	112
3.2.3. Dependency of microbubble properties on conformation and assembly of surfactin molecules	114
6.4. Conclusion	120
Chapter 7 Summary and conclusions	121
7.1 Summary	123
7.2 Conclusion	125
7.3 Scope of further investigation	126
References	127
Appendix	139
Publications	
Curriculum vitae	