

**TABLE OF CONTENTS**

---

<b>Contents</b>	<b>Page No.</b>
List of figures	i-vi
List of tables	vii-ix
List of abbreviations and symbols	x-xii
Preface	xiii-xiv
<b>Chapter 1</b>	
<b>Introduction and Objectives</b>	<b>1-4</b>
1.1 Introduction	1
1.2 Objectives	4
<b>Chapter 2</b>	
<b>Literature Review</b>	<b>5-32</b>
2.1 L-asparagine amidohydrolase (EC 3.5.1.1)	5
2.2 L-asparaginase reaction mechanism against cancer cells	6
2.3 L-asparaginase relevance in food industry	9
2.4 Immunological reactions associated with L-asparaginase enzyme	11
2.5 Properties of a good L-asparaginase for diverse applications	12
2.6 Sources of L-asparaginase enzyme	13

2.6.1	Bacterial sources of L-asparaginase	13
2.6.2	Fungal sources of L-asparaginase	14
2.6.3	Other sources of L-asparaginase	15
2.7	Microbial production of recombinant L-asparaginase from diverse sources	15
2.8	Submerged (SmF) and Solid state fermentation (SSF) processes	18
2.9	Cost-effective production of L-asparaginase enzyme	20
2.10	Optimization approaches for enhancement of L-asparaginase production	20
2.10.1	Screening of process parameters using the Plackett Burman screening design	20
2.10.2	Optimization using Response surface methodology approach (RSM)	21
2.10.3	Optimization using Artificial neural networks (ANN)	23
2.11	Asparaginase Quantitative enzyme assays	25
2.11.1	Aspartyl $\beta$ -hydroxamate (AHA) method	25
2.11.2	Nesslerization method	26
2.11.3	Indoosine method	27
2.12	Purification procedures for L-asparaginase enzyme	28
2.13	L-asparaginase role in the virulence and survival of pathogenic microorganisms	31

### Chapter 3

<b>L-asparaginase production using <i>Aspergillus niger</i></b>	<b>33-59</b>
---	--------------

3.1	Introduction	33
3.2	Materials and methods	35
3.2.1	Substrates and Microorganism	35
3.2.2	Screening for amidohydrolases activities using Plate Assay	35
3.2.3	Preparation of Substrates	36
3.2.4	Elemental composition analysis	36
3.2.5	Fermentation and extraction	36
3.2.6	Assay for the enzymatic activity of L-asparaginase	38
3.2.7	Process parameters optimization using RSM and ANN approach	38
3.2.8	Authentication of the experimentally designed model	42
3.3	Results and Discussion	42
3.3.1	Qualitative screening for asparaginase and glutaminase activities	42
3.3.2	L- asparaginase production parameters	43
3.3.3	Elemental composition analysis of the agro-substrates	45
3.3.4	Process modeling using statistical and machine learning models	45
3.3.5	Effects of Process parameters on L-asparaginase production in SSF	54
3.3.6	Validation of experimentally designed models	55
3.4	Conclusion	58

**Chapter 4**

<b>L-asparaginase production using <i>Bacillus indicus</i> bacteria</b>		<b>60-75</b>
4.1	Introduction	60
4.2	Materials and Methods	62
4.2.1	Microorganism growth parameters and screening for amidohydrolase activity	62
4.2.2	Assay for the enzymatic activity of L-asparaginase	62
4.2.3	Screening of medium components using Plackett Burman design (PBD)	63
4.2.4	Process modeling using central composite design of experiments (CCD)	64
4.2.5	Bioreactor operation for model validation and scale-up	65
4.3	Results and discussion	66
4.3.1	Plate assay based screening for enzyme activity	66
4.3.2	Screening of significant medium constituents affecting L-asparaginase production	67
4.3.3	Production and optimization using central composite design of experiments	68
4.3.4	Scale-up and validation of the experimental model	74
4.4	Conclusion	75

**Chapter 5**

<b>L-asparaginase purification and characterization from <i>Bacillus indicus</i></b>		<b>76-90</b>
5.1	Introduction	76

5.2	Materials and Methods	77
5.2.1	Enzyme purification steps	77
5.2.2	Ammonium sulfate precipitation	77
5.2.3	Ion exchange chromatography (IEC)	77
5.2.4	Size exclusion chromatography (SEC)	78
5.2.5	Assessment of molecular weight and purity	78
5.2.6	Evaluation of kinetic parameters and substrate specificity	78
5.2.7	Influence of temperature and pH on activity and pH stability of the purified L-asparaginase	79
5.2.8	Determination of the secondary structure of purified L-asparaginase	79
5.3	Results and Discussion	80
5.3.1	Purification of L-asparaginase enzyme	80
5.3.2	Ammonium sulfate precipitation	80
5.3.3	Anion exchange chromatography	80
5.3.4	Size-exclusion chromatography purification	82
5.3.5	Determination of molecular weight and purity	83
5.3.6	Evaluation of kinetic parameters and substrate specificity	84
5.3.7	pH and temperature effects on the purified L-asparaginase	87
5.3.8	Secondary structure evaluation	89
5.4	Conclusion	89

**Chapter 6**

<b>Evaluation of the therapeutic potential of purified L-asparaginase from <i>Bacillus indicus</i></b>	<b>91-105</b>
6.1 Introduction	91
6.2 Materials and Methods	92
6.2.1 L-asparaginase enzyme preparation	92
6.2.2 Chemicals and equipments utilized	92
6.2.3 Antitumor potential of the purified L-asparaginase - MTT cytotoxicity assays	93
6.2.4 Assessment of live/dead cells by acridine orange/propidium iodide (AO/PI) dual staining	94
6.2.5 Assessment of apoptosis using Annexin V/Propidium iodide assay	94
6.3 Results and Discussion	96
6.3.1 Cytotoxic evaluation of the anti-leukemic potential purified L-asparaginase	96
6.3.2 Live / dead cells assay using the AO / PI dual staining	98
6.3.3 Quantitative confirmation of apoptotic process using flow cytometry	101
6.4 Conclusion	104

**Chapter 7**

<b>Summary and Conclusions</b>	<b>106-109</b>
--------------------------------	----------------

<b>References</b>	<b>110-143</b>
-------------------	----------------

<b>List of Publications</b>	<b>144</b>
-----------------------------	------------