

## CONTENTS

	<u>Page</u>
<b>ACKNOWLEDGEMENT</b>	.. i
<b>ABSTRACT</b>	.. iii
<b>Chapter 1 INTRODUCTION</b>	.. 1
1.1 PREFACE	.. 1
1.2 REVIEW OF LITERATURE	.. 4
1.2.1 Glutaminase--Occurrence and distribution	.. 4
1.2.2 Synthesis, Isolation and Purification of glutaminase	.. 10
1.2.3 Solid State Fermentation (SSF)	.. 26
1.2.4 Glutaminase and treatment of cancer	.. 27
1.2.5 Industrial use of glutaminase	.. 33
1.3 Objectives of the Present Study	.. 37
<b>Chapter 2 MATERIALS AND METHODS</b>	.. 39
2.1 SUBSTRATE	.. 39
2.2 ISOLATION OF GLUTAMINASE PRODUCING BACTERIA FROM MARINE ENVIRONMENT	.. 40
2.2.1 Samples	.. 40
2.2.2 Collection of samples	.. 40
2.2.3 Preparation of media	.. 40
2.2.4 Plating procedures	.. 41

		<u>Page</u>
2.2.5	Isolation and maintenance of cultures ..	42
2.2.6	Identification of bacteria ..	42
2.3	SELECTION OF POTENTIAL L-GLUTAMINASE PRODUCING BACTERIA FOR FURTHER STUDIES	43
2.3.1	Media ..	44
2.3.2	Preparation of inoculum and inoculation procedures ..	44
2.3.3	Measurement of growth ..	45
2.3.4	Enzyme production ..	45
2.3.5	Determination of enzyme protein ..	46
2.3.6	Selection of strains ..	47
2.4	GROWTH STUDIES ..	47
2.4.1	Optimization of growth conditions for maximal enzyme production by bacteria ..	47
2.4.1.1	Media ..	47
2.4.1.2	Preparation of inoculum and inoculation procedures ..	48
2.4.1.3	Measurement of growth ..	48
2.4.1.4	Enzyme production ..	48
2.4.1.5	pH ..	49
2.4.1.6	Temperature ..	49
2.4.1.7	Substrate concentration ..	49
2.4.1.8	NaCl concentration ..	50
2.4.1.9	Carbon sources ..	50
2.4.1.10	Nitrogen sources ..	51

			<u>Page</u>
2.4.1.11	Glucose concentration	..	51
2.4.1.12	Inoculum concentration	..	51
2.4.1.13	Incubation time	..	52
2.4.2	Growth curve	..	52
2.5	COMPARISON OF INTRACELLULAR AND EXTRA-CELLULAR GLUTAMINASE PRODUCTION BY BACTERIA		53
2.6	ENZYME STUDIES	..	54
2.6.1	Isolation of enzymes	..	54
2.6.1.1	Media	..	54
2.6.1.2	Preparation of inoculum	..	55
2.6.1.3	Enzyme production in the medium	..	55
2.6.2	Enzyme assays	..	56
2.6.2.1	Buffers	..	56
2.6.2.2	Determination of enzyme activity	..	56
2.6.2.3	Determination of enzyme protein	..	56
2.6.3	Purification of enzymes	..	56
2.6.3.1	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fractionation	..	57
2.6.3.2	Dialysis	..	57
2.6.3.3	Chromatography upon seralite SRA-400	..	57
2.6.3.4	Chromatography upon seralite SRA-120	..	59
2.6.4	Characterization of glutaminase	..	60
2.6.4.1	Effect of pH on activity and stability of the enzyme	..	60
2.6.4.2	Effect of temperature on activity and stability of enzyme	..	61

		<u>Page</u>
2.6.4.3	Effect of substrate concentration on activity of the enzyme ..	62
2.6.4.4	Effect of incubation time on activity of the enzyme ..	63
2.6.4.5	Effect of NaCl concentration on activity of the enzyme ..	63
2.6.4.6	Determination of substrate specificity..	64
2.6.4.7	Effect of heavy metals on the activity of enzyme ..	64
2.6.4.8	Effect of other substances on the activity of the enzyme ..	65
2.7	FERMENTATIVE PRODUCTION OF GLUTAMINASE BY SOLID STATE FERMENTATION (SSF) ..	65
2.7.1	Preparation of solid substrate ..	65
2.7.2	Inoculation and incubation ..	66
2.7.3	Extraction and recovery of enzyme ..	67
2.7.3.1	Optimization of extraction parameters ..	67
2.7.3.1.1	Drying temperature ..	67
2.7.3.1.2	Extraction medium ..	68
2.7.3.1.3	pH of extraction media ..	68
2.7.3.1.4	Different buffer systems ..	69
2.7.3.1.5	Ratio of bran to buffer ..	69
2.7.3.1.6	Effect of contact time ..	69
2.7.3.1.7	Effect of contact temperature ..	70
2.7.4	Effect of operational parameters on enzyme production by SSF ..	70
2.7.4.1	Measurement of enzyme production ..	70
2.7.4.2	Effect of particle size of wheat bran ..	71

	<u>Page</u>
2.7.4.3	Effect of moisture content of WB medium 71
2.7.4.4	Effect of pH .. 72
2.7.4.5	Effect of temperature .. 72
2.7.4.6	Effect of substrate concentration .. 72
2.7.4.7	Effect of NaCl concentration .. 73
2.7.4.8	Effect of carbon sources .. 73
2.7.4.9	Effect of nitrogen sources .. 73
2.7.4.10	Effect of inoculum concentration .. 74
2.7.4.11	Effect of incubation time .. 74
<b>Chapter 3</b>	<b>RESULTS</b> .. 75
3.1	ENUMERATION AND ISOLATION OF L-GLUTAMINASE PRODUCING MICROORGANISMS .. 75
3.2	SELECTION OF POTENTIAL L-GLUTAMINASE PRODUCING BACTERIA . .. 76
3.3	IDENTIFICATION OF SELECTED STRAINS .. 77
3.4	GROWTH STUDIES .. 77-91
3.5	PRODUCTION OF EXTRA AND INTRACELLULAR GLUTAMINASE .. 91-93
3.6	PURIFICATION AND RECOVERY OF GLUTAMINASE FROM <u>PSEUDOMONAS</u> sp. AND <u>VIBRIO</u> sp. .. 93-105
3.7	PRODUCTION OF GLUTAMINASE BY SSF .. 105-119
3.8	COMPARATIVE ACCOUNT OF ENZYME PRODUCTION BY BACTERIA IN SSF AND SmF .. 119-121
	<b>FIGURES AND TABLES</b> .. 122-183
<b>Chapter 4</b>	<b>DISCUSSION</b> .. 184-212
<b>Chapter 5</b>	<b>SUMMARY</b> .. 213-220
<b>REFERENCES</b>	.. .. 221-241