

## TABLE OF CONTENTS

	Pages
<b>COVER</b> .....	<b>ii</b>
<b>STATEMENT OF THESIS APPROVAL</b> .....	<b>iii</b>
<b>DECLARATION</b> .....	<b>iv</b>
<b>ACKNOWLEDGEMENT</b> .....	<b>v</b>
<b>LIST OF TABLES</b> .....	<b>ix</b>
<b>LIST OF FIGURES</b> .....	<b>x</b>
<b>LIST OF APPENDIXES</b> .....	<b>xii</b>
<b>ABSTRACT</b> .....	<b>xiii</b>
<b>I. INTRODUCTION</b>	
1.1. Background.....	1
1.2. Problem Statements.....	3
1.3. Objectives.....	4
1.4. Benefits.....	4
<b>II. LITERATURE REVIEW</b>	
2.1 Proteases.....	6
2.2 Classification of proteases.....	7
2.2.1 Aspartic Proteases.....	9
2.2.2 Serine Proteases.....	11
2.2.3 Cysteine Proteases.....	14
2.2.4 Metallo-Proteases.....	16
2.3 Sources of proteases.....	18
2.4 Microbial Proteases.....	18
2.5 Application of proteases.....	19
2.6 Factors effect on enzyme activity.....	20
2.6.1 Enzyme concentration.....	21
2.6.2 Substrate concentration.....	21
2.6.3 Temperature.....	21

2.6.4	pH.....	21
2.6.5	Activators.....	22
2.6.6	Inhibitors.....	22
2.6.6.1	Serine protease inhibitor (PMSF).....	22
2.6.6.2	Cysteine protease inhibitor (Iodoacetamide).....	22
2.6.6.3	Metalloprotease inhibitor (EDTA).....	23
2.7	Thermostable enzyme.....	23
2.8	Thermophilic Microorganism.....	24
2.9	Thermophilic proteases.....	25
2.10	<i>Brevibacillus</i> .....	26
2.11	Protein Purification techniques.....	26
2.11.1	Separation based on solubility.....	27
2.11.2	Separation based on size and density.....	28
2.11.3	Chromatographic technique.....	30
2.11.3.1	Gel filtration chromatography or size- exclusion chromatography.....	30
2.11.3.2	Ion exchange chromatography.....	31
2.12	Hypothesis.....	32
<b>III. RESEARCH METHODS</b>		
3.1.	Materials and Equipment.....	34
3.1.1.	Materials.....	34
3.1.2.	Equipment.....	34
3.2.	Scope, place, and time of study.....	34
3.3.	Research Implementations.....	35
3.3.1.	Preliminary protease production from <i>Brevibacillus</i> sp. using Solid Media.....	35
3.3.2.	Optimizing time and temperature for protease production using Liquid Media.....	35
3.3.3.	Protease Assay.....	36
3.3.4.	Protein content.....	36

3.3.5. Partial purification of protease by ammonium sulfate precipitation.....	36
3.3.6. SDS PAGE Assay.....	37
3.3.7. Effect of pH and temperature and its stability.....	38
3.3.7.1. Effect of pH.....	38
3.3.7.2. Effect of temperature and its stability.....	38
3.3.8. Effect of inhibitors on protease activity.....	38
3.3.9. Enzyme kinetic assay.....	39
3.4. Data Analysis.....	39
<b>IV. RESULTS AND DISCUSSION</b>	
4.1. Preliminary protease production from <i>Brevibacillus</i> sp. using Solid Media.....	40
4.2. Optimum time and temperature for protease production using Liquid Media.....	42
4.3. Partial purification of protease by ammonium sulfate precipitation.....	44
4.4. Molecular weight of protease.....	46
4.5. Characterization of protease.....	47
4.5.1. Effect of pH on protease activity.....	47
4.5.2. Effect of temperature and stability of protease activity.....	48
4.5.3. Effect of inhibitors on protease activity.....	51
4.5.4. Kinetic Assay.....	52
<b>V. CONCLUSIONS AND SUGGESTIONS</b>	
5.1. Conclusions.....	54
5.2. Suggestions.....	54
<b>REFERENCES.....</b>	<b>55</b>
<b>APPENDIXES.....</b>	<b>65</b>