

CONTENTS

ACKNOWLEDGEMENTS	i
ABSTRACTS	ii
CONTENTS	iii
LIST OF TABLES	ix
LIST OF FIGURES	x

Chapter One

1.0	Introduction	1-1
1.1	Characteristics of enzymes	1-4
1.2	Immobilization of enzymes	1-7
1.3	Objectives of the present study	1-8

Chapter Two

2.0	Literature review	2-1
2.1	Immobilization of enzymes	2-2
2.1.1	Historical development	2-2
2.1.2	Advantages and disadvantages of immobilized enzymes	2-5
2.2	General methods of immobilization	2-10
2.2.1	Immobilization of enzymes by adsorption	2-12
2.2.2	Immobilization by ionic binding	2-14
2.2.3	Binding of enzymes using metals	2-15

2.2.4	Immobilization by covalent binding	2-16
2.2.5	Immobilization by Crosslinking	2-18
2.2.6	Entrapment method of immobilization of enzymes	2-19
2.2.6.1	Gel entrapment	2-20
2.2.6.2	Fiber entrapment	2-20
2.2.6.3	Microencapsulation of enzymes	2-21
2.3	Carriers for enzyme immobilization	2-22
2.4	Properties of urease	2-27
2.5	Studies on immobilization of urease	2-30
2.6	Application of immobilized urease	2-38

Chapter Three

3.0	Materials and methods	3-1
3.1	Materials	3-1
3.2	Estimation of urease activity	3-1
3.2.1	Urease activity of a soluble enzyme	3-2
3.2.2	Urease activity of immobilized urease	3-2
3.3	Protein estimation	3-3
3.3.1	Protein estimation of the sample	3-4
3.4	Immobilization of urease	3-5
3.4.1	Immobilization of urease on nylon	3-5
3.4.1.1	Preparation and activation of nylon	3-5
3.4.1.2	Immobilization of urease on nylon	3-5

3.4.1.2.1	Effect of immobilization pH	3-6
3.4.1.2.2	Effect of enzyme amount	3-6
3.4.1.2.3	Effect of time on urease immobilization	3-6
3.4.1.3	Properties of soluble and immobilized urease	3-7
3.4.1.3.1	Effect of temperature	3-7
3.4.1.3.2	Determination of the thermal stability	3-7
3.4.1.3.3	Effect of buffer pH	3-8
3.4.1.3.4	Storage stability	3-8
3.4.1.3.5	Determination of optimum substrate concentration	3-8
3.4.1.3.6	Reusability	3-9
3.4.2	Adsorption of urease on vermiculite saturated with different cations	3-10
3.4.2.1	Preparation of cationic vermiculite	3-10
3.4.2.2	Adsorption of urease on vermiculite	3-10
3.4.2.2.1	Equilibrium time	3-11
3.4.2.2.2	Effect of temperature	3-11
3.4.2.2.3	Effect of pH	3-12
3.4.2.2.4	Effect of dose of cationic vermiculite	3-12
3.4.2.2.5	Effect of enzyme concentration	3-12
3.4.2.2.6	Storage stability	3-12

3.4.3	Immobilization of urease on vermiculite	3-13
3.4.3.1	Preparation of vermiculite	3-13
3.4.3.2	Immobilization of urease	3-13
3.4.3.2.1	Method 1: Vermiculite+EDTA+Urease	3-13
3.4.3.2.2	Method 2: Vermiculite+Glutaraldehyde+ EDTA+Urease	3-13
3.4.3.2.3	Effect of immobilization pH	3-14
3.4.3.2.4	Effect of urease concentration	3-14
3.4.3.2.5	Effect of time on urease immobilization	3-15
3.4.3.3	Properties of soluble and immobilized urease	3-15
3.4.3.3.1	Effect of temperature	3-15
3.4.3.3.2	Determination of the thermal stability	3-15
3.4.3.3.3	Effect of buffer pH	3-16
3.4.3.3.4	Storage stability	3-16
3.4.3.3.5	Determination of optimum substrate concentration	3-16
3.4.3.3.6	Reusability	3-16
3.4.4	Immobilization of urease on Amberlite MB-1	3-17
3.4.4.1	Immobilization of urease	3-17
3.4.4.1.1	Effect of immobilization pH	3-17
3.4.4.1.2	Effect of urease concentration	3-18
3.4.4.1.3	Effect of time on urease immobilization	3-18

3.4.4.2	Properties of soluble and immobilized urease	3-18
3.4.4.2.1	Effect of temperature	3-18
3.4.4.2.2	Determination of thermal stability	3-19
3.4.4.2.3	Effect of buffer pH	3-19
3.4.4.2.4	Storage stability	3-19
3.4.4.2.5	Determination of optimum substrate concentration	3-19
3.4.4.2.6	Reusability	3-20

Chapter Four

4.0	Results and discussion	4-1
4.1	Immobilization of urease on nylon-6	4-1
4.1.1	Studies on immobilization parameters	4-1
4.1.2	Properties of soluble and immobilized urease	4-6
4.2	Adsorption of urease on vermiculite saturated with cations	4-15
4.3	Immobilization of urease on vermiculite-glutaraldehyde	4-24
4.3.1	Studies on immobilization parameters	4-24
4.3.2	Properties of soluble and immobilized urease	4-29
4.4	Immobilization of urease on Amberlite MB-1	4-40
4.4.1	Studies on immobilization parameters	4-40
4.4.2	Properties of soluble and immobilized urease	4-43

Chapter Five	5-1
CONCLUSIONS	5-1
APPENDIX	A-1
REFERENCES	R-1