

## CONTENTS

Content	Page
Title Page	i
Approval Sheet	ii
Acknowledgments	iii
Abstract	v
List of Tables	xii
List of figures	xiii
Abbreviations and Symbols	xv
<b>Chapter 1. Introduction</b>	
1.1. Pesticides and Its Impact on the Environment	1
1.2. Carbaryl and Environment	2
1.3. Biodegradation of Pesticides and Its Importance	4
1.4. Improvement of Microorganisms for Higher Biodegra- dation of Pesticides	6
1.5. Literature Review: Biodegradation of Carbaryl and Carbamate Insecticides	7
1.6. Research Scope and Objectives	10
<b>Chapter 2. Materials and Methods</b>	
2.1. Apparatus	12
2.2. Chemicals	13
2.3. Methods	14
2.3.1. Preparation of Minimum Minerals (MM) Media	14
2.3.2. Bacterial Culture	15
2.3.3. Biodegradation of Carbaryl	15

2.3.3.1. Temperature	15
2.3.3.2. pH	15
2.3.3.3. Nutrients	16
2.3.3.4. Enrichment	16
2.3.3.5. Cross-feeding	16
2.3.3.6. Bacterial Mutation	17
2.4. Bacterial Identification Test	18
2.5. DNA Isolation and Preparation	18
2.6. HPLC Condition for Analysis of Carbaryl	19
2.7. Preparation of Mobile Phase	20
2.8. Preparation of Standard Curve	20
2.9. Confirmation Analysis	21
3.0. Recovery Analysis	21
Chapter 3. Results	
3.1. Optimization of Biodegradation of Carbaryl	22
3.1.1. pH	22
3.1.2. Temperature	26
3.1.3. Nutrients	28
3.2. Carbaryl Enriched Bacteria	31
3.3. Cross-feeding	31
3.4. Bacterial Mutation	35
3.5. Bacterial Identification	40
3.6. DNA Preparation and Isolation	43
3.7. Confirmation of Carbaryl	44
3.8. Recovery Analysis of Carbaryl	45

Chapter 4. Discussion	47
4.1. Optimization of Biodegradation	47
4.1.1. pH	48
4.1.2. Temperature	49
4.1.3. Nutrients	50
4.2. Bacterial Enrichment	51
4.3. Cross-feeding	52
4.4. Bacterial Mutation	53
4.5. Bacterial Identification	54
4.6. Isolation of DNA	55
Chapter 5. Conclusion	56
Chapter 6. Recommendations	57
References	64
Appendix	84
Curriculum Vitae	

## List of Tables

Table.	Page
Table 1. Carbaryl degradation with UV-60 seconds bacteria	38
Table 2. Results of different identification tests of selected bacterial strain of isolate-5	41
Table 3. Acid and gas production test with different sugars	42
Table 4. Data for confirmation analysis of carbaryl	44
Table 5. Data for recovery analysis of carbaryl	45
Table 6. Repetition of biodegradation study with U3 strain at pH 6.8 at 37 and 34 °C	53
Table A. 1. Effect of pH on bacterial degradation of carbaryl	77
Table A.2. Effect of temperature on bacteria in degradation of carbaryl in minimum minerals media	78
Table A.3. Bacterial degradation of carbaryl in nutrient broth	78
Table A.4. Bacterial degradation of carbaryl in minimum minerals media with yeast-extract	79
Table A.5. Bacterial degradation of carbaryl in MM with vit. B1, B6 and nicotinamide	79
Table A.6. Degradation of carbaryl in minimum minerals media with carbaryl enriched bacteria	80
Table A.7. Bacterial degradation of carbaryl in presence of carbofuran and carbosulfan	80
Table A. 8. Bacterial growth in MM with 1-naphthol and carbofuran	80
Table A.9. Effect of UV-radiation on the mortality of bacteria	81

## List of Figures

Figure	Page
Figure 1. Carbaryl degradation in MM medium at pH 6.0, 6.5, 6.8 and 7.0 at 34 °C	23
Figure 2. Carbaryl degradation in MM medium at pH 7.2, 7.5, 8.0 and 8.5 at 34 °C	25
Figure 3. Carbaryl degradation in MM medium at 30 °C, 34 °C, 37 °C and 41 °C at pH 6.8	27
Figure 4. Carbaryl degradation in nutrient broth at pH 6.8 at 34 °C	29
Figure 5. Carbaryl degradation in MM with yeast-extract at pH 6.8 at 34 °C	29
Figure 6. Carbaryl degradation in MM in presence of vit. at pH 6.8 at 34 °C	30
Figure 7. Carbaryl degradation in MM with enriched bacteria at pH 6.8 at 34 °C	32
Figure 8. Carbaryl degradation in MM with carbofuran at pH 6.8 at 34 °C	33
Figure 9. Carbaryl degradation in MM with carbosulfan at pH 6.8 at 34 °C	33
Figure 10. Degradation of 1-naphthol in MM Medium at pH 6.8 at 34 °C	34
Figure 11. Effect of UV-radiation on selected bacterial strain	36
Figure 12A. Bacterial strain with UV-10 seconds	37
Figure 12B. Bacterial strain with UV- 30 seconds	37

Figure 12C . Bacterial strain with UV-60 seconds	37
Figure 12D. Bacterial strain with UV-120 seconds	37
Figure 13. DNA band of the selected bacterial strain	43
Figure 14. The peak of carbaryl and its intermediate product 1- naphthol	46
Figure A.1. Standard curve of carbaryl	76

มหาวิทยาลัยเชียงใหม่  
Chiang Mai University

**Abbreviations and Symbols**

%	percent
oC	degree celsius
μl	microliter
cfu	colony forming unit
cm	centimeter
<i>et al</i>	and others
g	gram
HPLC	High Performance Liquid Chromatography
ml	milliliter
M	Molar
nm	nanometer
rpm	revolution per minute
UV	ultraviolet