

## LIST OF CONTENTS

	Pages
<b>TITLE .....</b>	<b>I</b>
<b>THESIS APPROVAL .....</b>	<b>II</b>
<b>DECLARATION .....</b>	<b>III</b>
<b>ACKNOWLEDGMENT .....</b>	<b>IV</b>
<b>LIST OF CONTENTS .....</b>	<b>VI</b>
<b>LIST OF TABLES .....</b>	<b>VIII</b>
<b>LIST OF FIGURES .....</b>	<b>IX</b>
<b>ABSTRACT .....</b>	<b>X</b>
<b>INTISARI .....</b>	<b>XI</b>
 <b>I. INTRODUCTION .....</b>	 <b>1</b>
1.1 Background .....	1
1.2 Problems .....	3
1.3 Objectives .....	3
1.4 Expected Outcomes .....	3
 <b>II. LITERATURE REVIEW AND BASIC THEORY .....</b>	 <b>4</b>
2.1 Literature Review .....	4
2.1.1 Naphthalene .....	4
2.1.1.1 Effect and Exposure of Naphthalene .....	6
2.1.1.2 Naphthalene Degradation .....	10
2.1.2 Bioremediation .....	17
2.1.3 <i>Pseudomonas</i> sp. Involved in Biodegradation .....	18
2.1.4 <i>Pseudomonads</i> .....	20
2.1.4.1 <i>Pseudomonas citronellolis</i> .....	21
2.1.4.2 <i>Pseudomonas aeruginosa</i> .....	22

2.1.4.3 <i>Pseudomonas putida</i> .....	24
2.2 Basic Theory .....	26
2.3 Hypothesis .....	29
<b>III. METHODOLOGY .....</b>	<b>30</b>
3.1 Location and Periods of the Research .....	30
3.2 Materials and Equipment .....	30
3.2.1 Materials .....	30
3.2.2 Equipment. ....	31
3.3 Methods .....	31
3.3.1 Purification and Cultivation of Bacteria .....	31
3.3.2 Experiments of Naphthalene Degrading Bacteria Capability by strains <i>Pseudomonas citronelolis</i> (SBT4), <i>Pseudomonas aeruginosa</i> DSM50071(M3), and <i>Pseudomonas putida</i> . ....	32
3.3.2.1 Culture of Bacteria on Agar Bushnell Haas Minimal Medium with Naphthalene as a sole Carbon Source .....	32
3.3.2.2 Culture of Bacteria in Liquid Bushnell Haas Minimal Medium with Naphthalene as a sole Carbon Source .....	32
3.3.3 Naphthalene Metabolism Products Extraction for Gas Chromatography Analysis .....	33
3.3.4 Gas Chromatography Analysis (GC) .....	34
3.3.5 Naphthalene Metabolism Products Extraction for Gas Chromatography– mass spectrometry Analysis .....	35
3.3.5 Gas Chromatography–Mass Spectrometry Analysis (GC-MS) .....	36
<b>IV. RESULT AND DISCUSSION .....</b>	<b>38</b>
4.1 Naphthalene Metabolism by <i>Pseudomonas</i> spp. ....	38



4.2 Gas Chromatography Analysis of Naphthalene Degradation by Pseudomonas	
spp. ....	43
4.3 Naphthalene Degradation Assay by Gas Chromatography-Mass Spectrometry	
Analysis .....	47
 <b>V. CONCLUSION</b> .....	 <b>48</b>
<b>REFERENCES</b> .....	<b>49</b>
<b>APPENDICES</b> .....	<b>59</b>

## LIST OF TABLES

	Pages
Table II.1. Naphthalene characteristics .....	5
Table III.2. Bacteria that used in research.....	30
Table IV.3. Mass spectrometry analysis of the remarkable metabolites detected in the degradation of naphthalene by <i>Pseudomonas citronellolis</i> (SBT4), <i>Pseudomonas aeruginosa</i> DSM 50071 (M3) and <i>Pseudomonas putida</i> . R.times: Retention times. ....	48

## LIST OF FIGURES

	Pages
Figure II.1: Chemical structure of naphthalene.....	4
Figure II.2: Naphthalene 1,2-dioxygenase multicomponent enzyme. ....	12
Figure II.3: Proposed catabolic pathways of naphthalene by <i>Pseudomonads</i> . ....	13
Figure II.4: Proposed pathway for the degradation of naphthalene by strains <i>Pseudomonas</i> sp. CZ2 and CZ5. I, Naphthalene dioxygenase; II, catechol 1, 2-dioxygenase; III, catechol 2, 3-dioxygenase. CZ2, <i>Pseudomonas</i> sp. CZ2; CZ5, <i>Pseudomonas</i> sp. CZ5. ....	14
Figure II.5: Proposed scheme of precursor-product relationship for naphthalene degradation by thermophilic isolate <i>Geobacillus</i> sp. G27. Brackets indicate metabolites, proposed elsewhere. ....	15
Figure II.6: Proposed catabolic of anaerobic naphthalene degradation pathways by a sulfate-reducing enrichment culture. ....	16
Figure IV.7: The growth <i>Pseudomonas</i> spp. on agar Bushnell Haas minimal medium with 1,28gr/L of naphthalene for 7 days; (a: SBT4), (b: M3), (b: <i>P.putida</i> ): (Control: area without bacteria streaking ) ....	39
Figure IV.8: Growing of bacteria in Bushnell Haas minimal medium containing naphthalene (1,28gr/L), incubated in shaker 150 rpm for 21 days. ....	40
Figure IV.9: Growth averages of three strains of bacteria <i>Pseudomonas</i> spp. in Bushnell Haas minimal medium containing naphthalene (1,28gr/L) for 21 days from three time of replications. ....	41
Figure IV.10: Gas Chromatogram, analysis results of samples Internal Standard(a), Naphthalene+ Internal standard(b), M3(c), <i>P. Putida</i> (d), SBT4(e), 1: Internal Standard (Phenanthrene), 2: Naphthalene. ....	44
Figure IV.11: Remaining of Naphthalene after inoculated with three strains of <i>Pseudomonas</i> spp. bacteria from gas-chromatography analysis result. ....	46