

ACKNOWLEDGEMENT

The greatest gratitude to God for His grace and guidance provided to the author to complete this thesis, entitled “IMMOBILIZATION OF *Providencia stuartii* USING PAPAYA TRUNK WOOD AND ITS APPLICATION ON N-ACETYLGLUCOSAMINE PRODUCTION”, promptly, starting from the making of the proposal, throughout the conduct of research, and until the writing of the report.

The author acknowledges that the completion of this thesis would not have been possible without the guidance, support, as well as prayers from many people. Therefore, the author would like to express a huge gratitude towards these people:

1. Mr. Eric Jobiliong, Ph.D. as the Dean of Faculty of Science and Technology.
2. Mrs. Dela Rosa, S.Si., M.M., M.Sc.Apt. as the Vice Dean of Faculty of Science and Technology.
3. Mr. Laurence, M.T. as the Director of Administration and Student Affair of Faculty of Science and Technology.
4. Ir. W. Donald R. Pokatong, M.Sc., Ph.D. as the Head of Food Technology Study Program, who has given the permission for the author to work on this thesis.
5. Ms. Yuniwaty Halim, M.Sc., as the thesis supervisor, who has given the time, guidance, as well as enlightenment, starting from the making of the proposal up to the completion of this report.
6. Dr. Ir. Hardoko, MS. and Ms. Ratna Handayani, MP, as the lecturers of Food Technology Study Program, who have given counsel, insight and guidance in regarding the research on glucosamine.
7. Dr. Adolf J. N. Parhusip, as the head of microbiology lab, who has given the permission for the author to conduct the research in the laboratory.
8. Mr. Adi, Mr. Yosafat Rudju, Mr. Denny, Mr. Darius, Mr. Regy, Mr. Deni and Mr. Adzie as the laborants, who has helped the author in the laboratory.

9. Dearest family, including father (Hasan Nurtan), mother (Megawaty), little sisters (Stephanie Esperansa and Vivi Julietta), as well as little brother (Rizky Akbar Tantradjaja) for the endless support and prayers to the author.
10. Devianita, Cindy, Elita Feby Susanto and other members of “Glucosamine Family”, who have worked side by side with the author, as well as helped, supported and entertained the author throughout the research and completion of this report.
11. Friends from Class C of Food Technology Study Program, Universitas Pelita Harapan, especially, “Loloers” and “The Boys”.
12. Friends from high school, for the enlightenment, support, as well as prayers.
13. Other friends and relatives who have not been mentioned by the author yet have also supported the author up to the completion of this report.

The author realizes that this report is still far from perfect and requires further improvement. Therefore, the author would gladly receive any suggestions and critics that are useful to improve this report. Moreover, the author also hope that this report can be informative and beneficial for the readers. Thank you.

Tangerang, January 24th, 2020

(Steven Fausta Tantradjaja)

TABLE OF CONTENTS

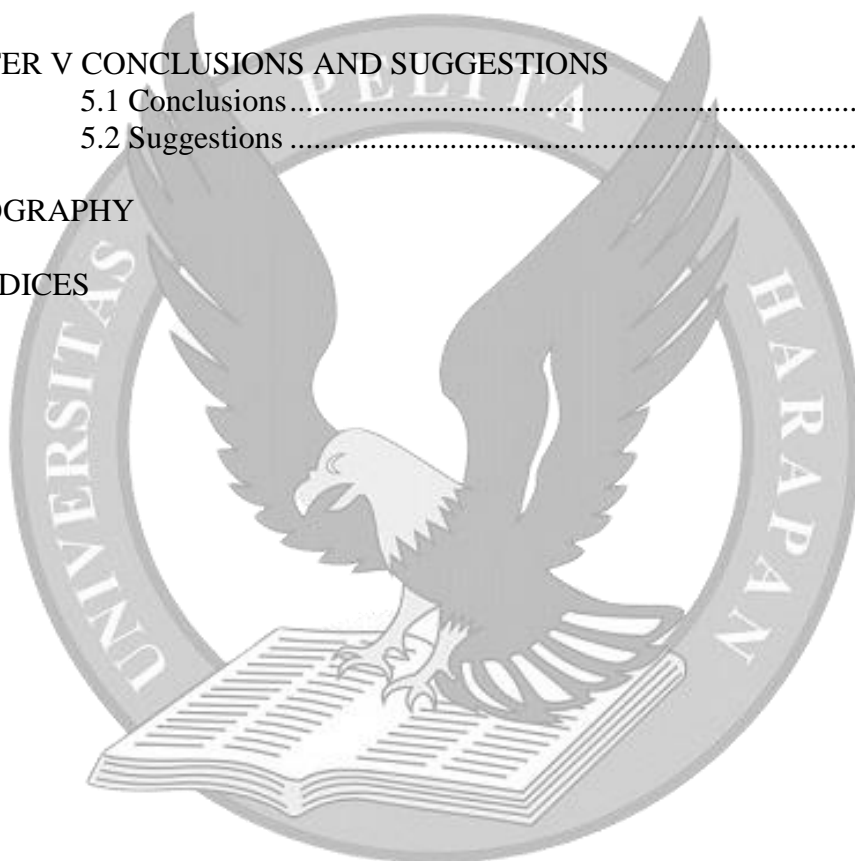
	page
COVER.....	
FINAL ASSIGNMENT STATEMENT AND UPLOAD AGREEMENT.....	
APPROVAL BY THESIS SUPERVISOR.....	
APPROVAL BY THESIS EXAMINATION COMMITTEE.....	
ABSTRACT.....	v
ABSTRAK.....	vi
ACKNOWLEDGEMENT.....	vii
TABLE OF CONTENTS.....	ix
LIST OF TABLES.....	xii
LIST OF FIGURES.....	xiii
LIST OF APPENDICES.....	xiv
 CHAPTER I INTRODUCTION	
1.1 Background	1
1.2 Research Problem.....	4
1.3 Objectives	4
1.3.1 General Objective	4
1.3.2 Specific Objectives	5
 CHAPTER II LITERATURE REVIEW	
2.1 <i>Penaeus vannamei</i>	6
2.2 Glucosamine.....	8
2.3 Fermentation.....	9
2.4 Chitinolytic Microorganisms.....	10
2.5 <i>Providencia stuartii</i>	11
2.6 Cell Immobilization.....	11
2.7 Structural Fibrous Network (SFN) of Papaya Trunk Wood...13	
 CHAPTER III RESEARCH METHODOLOGY	
3.1 Materials and Equipment.....	16
3.2 Research Method	17
3.2.1 Preliminary Steps.....	18
3.2.1.1 Shrimp Shells Powder Production.....	18
3.2.1.2 Stock Culture and Starter Culture Preparation	18
3.2.1.2.1 Preparation of Medium.....	19
3.2.1.2.2 Preparation of Stock Culture	19

3.2.1.2.3 Preparation of Starter Culture.....	20
3.2.1.3 Morphological Characterization of Bacteria	20
3.2.1.4 Bacterial Cell Counting	21
3.2.2 Main Research	22
3.2.2.1 First Stage Research	22
3.2.2.1.1 Preparation and Inoculation of Growth Medium	22
3.2.2.1.2 Immobilization of <i>Providencia stuartii</i>	23
3.2.2.1.3 Immobilized Cells Counting.....	24
3.2.2.1.4 Preparation of Fermentation Media.....	25
3.2.2.1.5 Determination of Optimum SFN of Papaya Trunk Wood Size and Ratio (w/v) between SFN of Papaya Trunk Wood and Growth Medium	25
3.2.2.2 Second Stage Research.....	26
3.3 Experimental Design	28
3.3.1 First Stage Research	28
3.3.2 Second Stage Research	30
3.4 Methods of Analysis.....	31
3.4.1 Analysis of Shrimp Shells Powder	31
3.4.1.1 Moisture Content Analysis (AOAC, 2005)	31
3.4.1.2 Ash Content Analysis (AOAC, 2005)	32
3.4.1.3 Protein Content Analysis (Nielsen, 2010)	32
3.4.1.4 Shrimp Shells Powder Yield Analysis (Demir <i>et al.</i> , 2016).....	33
3.4.2 N-Acetylglucosamine Analysis (Halim <i>et al.</i> , 2018).....	34
3.4.2.1 Preparation of N-Acetylglucosamine Standard Curve ..	34
3.4.2.2 Quantification of N-Acetylglucosamine.....	34
3.4.2.3 Scanning Electron Microscopy (SEM) Analysis.....	35

CHAPTER IV RESULTS AND DISCUSSIONS

4.1 Shrimp Identification and Shrimp Shells Powder Characteristics	36
4.2 <i>Providencia stuartii</i> Culture Morphology	38
4.3 First Stage Research	39

4.3.1 Effect of Papaya Trunk Wood Size and SFN of Papaya Trunk Wood : Growth Medium Ratio (w/v) on Percentage of Immobilized Cells	39
4.3.2 Effect of SFN of Papaya Trunk Wood Size and SFN of Papaya Trunk Wood : Growth Medium Ratio (w/v) on N- Acetylglucosamine Production	42
4.4 Second Stage Research.....	45
4.4.1 Immobilized <i>Providencia stuartii</i> Cells Count.....	45
4.4.2 Effect of Fermentation Cycles on N-acetylglucosamine Production	46
CHAPTER V CONCLUSIONS AND SUGGESTIONS	
5.1 Conclusions	49
5.2 Suggestions	49
BIBLIOGRAPHY	
APPENDICES	



LIST OF TABLES

	page
Table 3.1 Statistical design for first stage research.....	28
Table 3.2 Statistical design for second stage research	30
Table 4.1 Shrimp shells powder composition	36
Table 4.2 Percentage of immobilized <i>Providencia stuartii</i> cells	41



LIST OF FIGURES

	page
Figure 2.1 <i>Peneus vannamei</i>	7
Figure 2.2 Transformation of chitin into NAG and GlcN	9
Figure 2.3 Papaya trunk wood	14
Figure 3.1 Flowchart of shrimp shells powder production	18
Figure 3.2 Flowchart of stock culture preparation	19
Figure 3.3 Flowchart of starter culture preparation	20
Figure 3.4 Gram staining procedure	21
Figure 3.5 Bacterial cell counting procedure	22
Figure 3.6 Flowchart of lignin removal process	23
Figure 3.7 Flowchart of <i>Providencia stuartii</i> immobilization	24
Figure 3.8 Flowchart of optimum SFN of papaya trunk wood size and ratio (w/w) between SFN of papaya trunk wood and growth medium for the fermentation of <i>Penaeus monodon</i> shell	26
Figure 3.9 Flowchart of determination of optimum fermentation cycles	27
Figure 4.1 (a) <i>Providencia stuartii</i> by Chamberland (2013) (b) Result of microscopic observation of <i>Providencia stuartii</i> (1000x magnification)	38
Figure 4.2 SEM result (a: 150x magnification; b: 400x magnification)	40
Figure 4.3 SEM result (a: 1000x magnification; b: 3500x magnification)	40
Figure 4.4 Effect of SFN of papaya trunk wood size on NAG production	43
Figure 4.5 Effect of SFN of papaya trunk wood : growth medium (w/v) on NAG production	44
Figure 4.6 Effect of fermentation cycles on NAG production	47

LIST OF APPENDICES

	page
Appendix A.	
Identification Result of Shrimp	A-1
Appendix B.	
Documentation of Shrimp Shell Powder Manufacturing Process	B-1
Appendix C.	
Data and Yield Calculation	C-1
Appendix D.	
Data and Moisture Content Results	D-1
Appendix E.	
Data and Ash Content Results	E-1
Appendix F.	
Data and Protein Content Results	F-1
Appendix G.	
Documentation of Shrimp Shells Powder Fermentation Process	G-1
Appendix H.	
Standard Curve of N-acetylglucosamine	H-1
Appendix I.	
Initial Bacterial Cells Count	I-1
Bacterial Cell Count in Growth Medium	I-2
Immobilized Cells Count	I-5
Absorbance and N-acetylglucosamine Concentration	I-6
N-Acetylglucosamine Concentration and Yield	I-8
Statistical Analysis of First Stage Research	I-9
Post Hoc Test Results of First Stage Research	I-10
Appendix J.	
Initial Bacterial Cells Count	J-1

Bacterial Cell Count in Growth Medium	J-2
Immobilized Cells Count.....	J-3
Absorbance and N-acetylglucosamine Concentration.....	J-4
Statistical Analysis of Second Stage Research.....	J-6
Post Hoc Test Results of Second Stage Research	J-7

