

UNIVERSITI TEKNOLOGI MARA

**PRODUCTION OF LACTIC ACID
FROM CULTIVATION OF
RHIZOPUS ORYZAE NRRL 395 AND
CHARACTERIZATION OF ITS
POLYMER DERIVATIVE**

WAN NASRIN AFIFA BINTI WAN DAUD

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Chemical Engineering)

Faculty of Chemical Engineering

September 2019

ABSTRACT

In this work, the project is to discover an alternative way to substitute non-biodegradable plastic with biodegradable plastic from poly(L)-lactic acid (PLLA) polyester, using direct polycondensation (DPC) process. The first objective is to determine the growth and morphology of *Rhizopus oryzae* NRRL 395 and its ability to produce L-lactic acid (LLA) from microbial lactic acid fermentation (MLAF) of cassava starch. The second objective is to study the effect of spores concentration, substrate concentration, temperature and time on LLA concentration, yield and productivity in MLAF. The third objective is to characterize the PLLA obtained from DPC process by using Fourier Transform Infrared (FTIR), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) analysis. From these research findings, the *R.oryzae* NRRL 395 fungi has the ability to grow on potato dextrose agar (PDA) at 37°C after 72 hour of incubation period. The *R.oryzae* NRRL 395 fungi also have successfully produce LLA in the MLAF from partially hydrolyzed cassava starch substrate, which was identified from the HPLC and FTIR analysis. From HPLC analysis of the compound of interest in the sample taken from the fermentation broth, LLA was well separated at $t_R = 4.58$ min and produced a sharp pointed peak at UV wavelength of 210 nm, at 1.0 mL/min flow rate by using 250 mm C-18 column with mobile phase of 0.001 M orthophosphoric acid, H_3PO_4 (pH 2.5). This indicates the presence of LLA compound in the samples as compared to the LLA standard. From the study of the effect of substrate concentration, the highest LLA concentration, 21.83 g/L was obtained at 96 h from 120 g/L cassava substrate concentration, followed by 100 g/L which produced 18.38 g/L of LLA after 72 h. The highest cassava starch concentration of 140 g/L used had resulted in producing the lowest LLA concentration, which was only 6.19 g/L after 120 h. In terms of the effect of temperature on LLA concentration, the highest LLA concentration was 24.17 g/L at 34°C obtained after 96 h of fermentation time. Nevertheless, it was found that the highest concentration for both $T = 37$ and 40°C were 22.19 g/L and 24.01 g/L, respectively at 72 h, faster than the highest concentration obtained at $T = 34^\circ C$ (24.17 g/L). Hence, the temperature of 37°C effectively produced 24.01 g/L of LLA in 72 h. In overall, the best operating condition for MLAF is at 37°C after 72 hour of fermentation with 120 g/L cassava substrate concentration. The PLLA was synthesized by DPC method, tested in the presence of stannous octoate (SnO) catalyst and without catalyst. The chemical compound of the synthesized PLLA was characterized by FTIR analysis and compared with the commercial PLA. From the analysis in TGA and DSC, the PLLA thermal properties were compared to the commercial PLA. In conclusion, the chemical compound and thermal properties showed that the PLLA should be improved and further enhanced.

ACKNOWLEDGEMENT

In the name of Allah, The Most Gracious and The Most Merciful, all praises to Allah, The One and Only, for giving me this chance to complete a long journey of success. Through His guidance and help, I am able to finish my research after all the hard work, tons of tears, patience and obstacles that I had been experienced. I am heartedly feeling blessed from Allah s.w.t for everything that He gave and put me through during my master journey, Alhamdulillah tsumma Alhamdulillah.

The highest appreciation and gratitude goes to my main supervisor, Mr Abdul Aziz bin Ishak for all his continuous kindness, advices, support and guidance along the completion of this study. Also, not to forget my co-supervisors, Madame Syafiza Abd Hashib and Madame Miradatul Najwa Muhd Rodi for their advices and supports, while guiding and assisting me during laboratory works and writing progress.

I would also like to express my sincere gratitude to my friends and colleagues, Nik Salwani, Arbanah, Liyana, Normah, Fahana, Rosliza, Ain, Azlina, Siti Nuraida, Zuraida, Khairunnisa, Izni, Amira and many more who always been there whenever I need help and a shoulder to lean on. I am also grateful for continuous help and assistance from laboratory staffs, which are Encik Mohd Ridhuan Salleh from Biology Laboratory, Encik Mohd Yazid Yusof from Supercritical Laboratory, Puan Azizan Din from Science Laboratory and others who helps me whether directly or indirectly. I am also thankful to all staffs charged for High Performance Liquid Chromatography (HPLC) analysis in Faculty of Applied Science, Puan Julia Bt. Kassim and Faculty of Pharmacy UITM Puncak Alam, Encik Mohamad Ezalee Eisa. Without the help of them, I cannot finish my tedious laboratory works and analysis which take years to be completed.

Last but not least, I would like to address my deepest appreciation and gratitude to my beloved parent, Wan Daud bin Wan Mohd Amin and [REDACTED] and also to my sisters for all the love, kindness, patience and support during my master journey, emotionally, spiritually and financially. Alhamdulillah I am grateful to Allah s.w.t and millions of appreciation and thankfulness to all.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF PLATES	xv
LIST OF SYMBOLS	xvi
LIST OF ABBREVIATIONS	xvii
LIST OF NOMENCLATURES	xviii
CHAPTER ONE: INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Research Objectives	4
1.4 Scopes of Study	4
1.5 Significance of Study	5
CHAPTER TWO: LITERATURE REVIEW	
2.1 Introduction	6
2.2 Cassava in Malaysia	6
2.2.1 Cassava Starch Production	10
2.2.2 Cassava Starch Extraction	13
2.3 Lactic Acid	14
2.3.1 Lactic Acid Production	17
2.4 Microbial Lactic Acid Fermentation (MLAF)	18
2.4.1 Raw Materials	18
2.4.2 Fermentative Microorganisms	23

2.4.2.1	<i>Rhizopus Species</i>	24
2.4.3	Characterization of <i>Rhizopus Species</i>	27
2.4.3.1	<i>Morphology of Rhizopus Species</i>	27
2.4.3.2	<i>Growth Temperature</i>	31
2.4.4	Fermentation Conditions	32
2.5	High Performance Liquid Chromatography	36
2.6	Fourier Transform Infrared (FTIR) Spectroscopy Analysis	39
2.7	Polylactic Acid (PLA)	40
2.7.1	Polylactic Acid Properties	40
2.7.2	Application and Market of PLA	41
2.8	PLA Production	43
2.8.1	Direct Polycondensation (DPC) of PLA	44
2.9	PLA from Renewable Resources	45
2.9.1	Poly(L)-lactic Acid Production from MLAF	47
2.10	PLA Characterization	51
2.10.1	FTIR Spectroscopy Analysis	51
2.10.2	Thermal Gravimetric (TGA) Analysis	53
2.10.3	Differential Scanning Calorimetry (DSC) Analysis	53
2.11	Literature Review Highlight	54
CHAPTER THREE: METHODOLOGY		
3.1	Raw Material Preparation of Cassava	57
3.1.1	Peeling/Chopping	57
3.1.2	Cassava Starch Extraction	58
3.1.3	Starch Deposition/Settling (Decantation)	58
3.1.4	Drying/Storage	58
3.2	Characterization of Cassava Starch	58
3.2.1	Amylose Percentage	58
3.2.2	Amylose Percentage Standard Curve	59
3.2.3	Melting Temperature	59
3.3	Growth of <i>Rhizopus oryzae</i> NRRL 395	59
3.3.1	Potato Dextrose Agar (PDA)	60
3.3.2	<i>Rhizopus oryzae</i> Culture	60
3.4	Characterization of <i>Rhizopus oryzae</i> NRRL 395	60