



**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERTAAN DISERTAI MUTAKHIR
SATU (1) NASKAH**

Nama Penyelia: Prof Madya Dr Husnul Azan Bin Tajarudin

Bahagian: Bioproses Teknologi

Saya telah menyemak semua pembedahan/pindaan yang dilaksanakan oleh
Encik/Puan/Cik Nur Shuhada binti Baharuddin

mengemui disertainya sebagaimana yang dipersetujui oleh Panel Pemeriksa di Viva
Vocanya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembedahan/pindaan
yang dilaksanakan oleh calon.

Sekian, terima kasih.

Prof Madya Dr Husnul Azan Bin Tajarudin
Bahagian Bioproses Teknologi,
Pusat Pengajian Teknologi Industri,
Universiti Sains Malaysia,
11800, Pulau Pinang

(Tandatangan dan cop)

18/07/2020

Tarikh



LACTIC ACID PRODUCTION FROM BROKEN RICE VIA FERMENTATION PROCESS

By

Nur Shuhada binti Baharuddin

**A dissertation submitted in partial fulfillment of the requirement for the degree of
Bachelor of Technology (B. Tech) in the field of Bioprocess Technology**

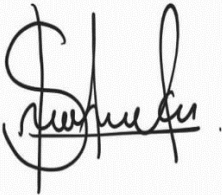
School of Industrial Technology

Universiti Sains Malaysia, USM

JUNE 2020

DECLARATION BY AUTHOR

This dissertation is composed of my original work and contain no material previously published or written by another person except where due reference had been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution



NUR SHUHADA BINTI BAHARUDDIN

JUNE 2020

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to the God, the Almighty, for his showers of blessings throughout my research work to complete the research successfully.

I would like to express my deep and sincere gratitude to my supervisor, Prof Madya Dr Husnul Azan Tajarudin for his endless guidance and support throughout this project carried out. He shared so many ideas and advises to me when I faced problems in this project. I really thank him for his patience, commitment and tolerance of helping me when I faced difficulties and get confused during the halfway of my research.

I also would like to express my gratitude to the laboratory assistants Encik Azmaizan and Puan Najmah for their cooperation and support throughout this research work.

I am extending my thanks to postgraduate student, Mr. Charles for his guidance and advises to me throughout my final year project periods as well as sharing a lot of his knowledge with me.

I would like to say thanks to my friends and all post graduate students for their constant encouragement.

I would like to take this opportunity to express my gratitude to my parents for their support and encouragement to me throughout this period.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly because this project would be impossible for me to complete if there is no support from many individuals.

Nur Shuhada Baharuddin

June 2020

TABLE OF CONTENT

| | Page |
|---|-------------|
| ACKNOWLEDGEMENT | iii |
| TABLE OF CONTENT | iv |
| LIST OF TABLES | vi |
| LIST OF FIGURES | vii |
| LIST OF ABBREVIATIONS | viii |
| LIST OF SYMBOLS | ix |
| ABSTRAK | x |
| ABSTRACT | xi |
| CHAPTER 1: INTRODUCTION | 1 |
| 1.1 Research Background | 2 |
| 1.2 Problem Statement | 2 |
| 1.3 Objectives | 2 |
| CHAPTER 2: LITERATURE REVIEW | 3 |
| 2.1 Broken Rice | 3 |
| 2.2 Rice Milling process | 3 |
| 2.3 Fermentation method for lactic acid production | 5 |
| 2.4 Mode of fermentation | 5 |
| 2.4.1 Batch fermentation | 5 |
| 2.4.2 Fed – batch fermentation | 5 |
| 2.4.3 Continuous fermentation | 6 |
| 2.5 Physical and chemical properties of lactic Acid | 6 |
| 2.6 Selection of substrates for lactic acid production | 6 |
| 2.7 Selection of microorganism for lactic acid production | 7 |
| 2.8 Kinetics and Modelling | 7 |
| 2.9 Applications of lactic acid | 8 |
| CHAPTER 3: MATERIALS AND METHODS | 9 |
| 3.1 Overall Flowchart | 9 |
| 3.2 Raw material preparation | 9 |
| 3.2.1 Preparation of broken rice as a substrate in the fermentation process | 9 |
| 3.3 Cultivation of the strain culture | 10 |
| 3.4 Preparation of inoculum | 10 |

| | |
|---|----|
| 3.5 Submerged Batch Fermentation | 11 |
| 3.5.1 Fermentation Process | 11 |
| 3.6 Analysis on the effect of different process parameters to the growth of <i>Lactobacillus acidophilus</i> | 11 |
| 3.6.1 Analysis of the cell growth rate | 11 |
| 3.6.2 Determination of cell biomass | 12 |
| 3.6.3 Determination of carbohydrate content | 12 |
| 3.6.4 Determination of lactic acid concentration by using Spectrophotometric Method | 13 |
| 3.7 Analysis of kinetic parameter for the fermentation process by using 2.5 L batch bioreactor | 13 |
| 3.8 Mathematical model | 13 |
| 3.9 Kinetic models | 14 |
| 3.9.1 Microbial Growth kinetic | 14 |
| 3.9.2 Lactic acid production kinetic | 15 |
| 3.9.3 Substrate utilization kinetic | 15 |
| 3.10 Data analysis and modelling | 16 |
| 3.10.1 Regression and percentage error | 16 |
| 3.11 Feasibility study for production of lactic acid via fermented broken rice | 16 |
| CHAPTER 4: RESULTS AND DISCUSSION | 17 |
| CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH | 31 |
| 5.1 Conclusions | 31 |
| 5.2 Recommendations for future research | 32 |
| References | 33 |
| Appendix | 35 |

LIST OF TABLES

- Table 4.1 The optimized result for strain *Lactobacillus acidophilus* with 50 g/l of broken rice
- Table 4.2 The optimized result for strain *Lactobacillus acidophilus* with 100 g/l of broken rice
- Table 4.3 The optimized result for strain *Lactobacillus acidophilus* by using 50 g/l of broken rice and 100 rpm agitation speed in 2.5 L STR bioreactor.
- Table 4.4 The optimized result for strain *Lactobacillus acidophilus* by using 50 g/l of broken rice and 150 rpm agitation speed in 2.5 L STR bioreactor.
- Table 4.5 The optimized result for strain *Lactobacillus acidophilus* by using 50 g/l of broken rice and 200 rpm agitation speed in 2.5 L STR bioreactor.
- Table 4.6 Determination of optimum parameter values for the lactic acid production using broken rice as a main substrate
- Table 4.7 Kinetic parameter estimation for lactic acid production with broken rice as substrate using batch bioreactor system
- Table 4.8 Estimated experimental and predicted kinetic model parameter values for lactic acid production by *Lactobacillus acidophilus*
- Table 4.9 Lactic acid market price
- Table 4.10 Lab scale operational cost for lactic acid production (58.5 liter).

LIST OF FIGURES

- Figure 2.1 Diagram of the rice production process with inputs and outputs
- Figure 3.1 Summary of methodology used in this study
- Figure 4.1 The growth curve of *Lactobacillus acidophilus*
- Figure 4.2 Model and experimental (biomass concentration, product formation, substrate utilisation) data for lactic acid production using *Lactobacillus acidophilus* with broken rice as a substrate under optimum conditions in batch fermentation
- Figure 4.3 Comparison of experimental and predicted biomass concentration by using *Lactobacillus acidophilus*
- Figure 4.4 Comparison of experimental and predicted substrate utilization by using *Lactobacillus acidophilus*
- Figure 4.5 Comparison of experimental and predicted lactic acid production by using *Lactobacillus acidophilus*
- Figure 4.6 Lactic acid Market Size and Forecast

LIST OF ABBREVIATIONS

| | |
|----------------|---------------------------------|
| CAGR | Compound annual growth rate |
| USD | United States Dollar |
| etc | et cetera |
| STR | Stirred tank bioreactor |
| LAB | Lactic acid bacteria |
| R ² | Regression value |
| EPA | Environmental Protection Agency |
| SmF | Submerged Fermentation and |
| SSF | Solid-State Fermentation |
| MRS | de Mann Rogosa Sharpe |
| ATP | Adenosine triphosphate |
| CFUs | A colony-forming unit |
| rpm | Revolutions per minute |
| OD | Optical density |

LIST OF SYMBOLS

| | |
|--------------|--|
| μ | Specific growth rate (h^{-1}) |
| μ_{\max} | Maximum specific growth rate |
| X | Biomass concentration (g/l) |
| X_0 | Initial biomass concentration (g/l) |
| X_m | Maximum biomass concentration at stationary phase (g/l) |
| $Y_{x/s}$ | Biomass yield coefficient (g biomass g^{-1} glucose) |
| $Y_{p/s}$ | Product yield coefficient (g lactic acid g^{-1} glucose) |
| m_s | Maintenance coefficient (g glucose g^{-1} biomass h^{-1}) |
| P | Lactic acid concentration (g/l) |
| t | Time (h) |
| S | Substrate concentration (g/l) |
| ΔS | Amount of substrate consumed |
| α | Growth-associated product formation coefficient (g lactic acid g^{-1} biomass) |
| β | Non-growth-associated product formation coefficient (g lactic acid g^{-1} biomass h^{-1}) |

PENGHASILAN LAKTIK ASID DARIPADA BERAS HANCUR MENGGUNAKAN PROSES FERMENTASI

ABSTRAK

Antara cabaran yang perlu industri pengeluaran makanan hadapi adalah penghasilan sisa yang banyak dan proses pengurusannya menjadi kekangan yang besar kerana memerlukan ruang yang luas dan jika tidak dikendalikan dengan baik pasti akan menjejaskan alam sekitar dan kesihatan manusia. Sisa pepejal utama yang paling banyak dihasilkan daripada industri padi adalah beras hancur. Beras hancur merujuk kepada serpihan butiran padi yang diperolehi daripada pengilangan dan diasingkan selepas fasa penggilapan. Ia mempunyai komposisi kimia yang sama dengan beras putih dan karbohidrat merupakan komponen terpenting. Tujuan penyelidikan ini adalah untuk mengkaji keadaan dan potensi optimum penggunaan beras hancur yang ditapai untuk penghasilan asid laktik menggunakan kultur bakteria yang berlainan. Parameter proses yang berbeza seperti pH medium, suhu, pengadukan dan kepekatan substrat digunakan untuk mengkaji keadaan optimum. Kemudian, pertumbuhan kinetik dan pemodelan pengeluaran asid laktik daripada beras hancur dalam sistem kelompok dikaji. Daripada proses saringan kepekatan asid laktik yang tertinggi diperolehi adalah 5.24 gL^{-1} daripada bakteria jenis *Lactobacillus acidophilus* pada pH 7, suhu 35°C , 150 rpm dan 50 gL^{-1} substrat. Model tidak berstruktur diusulkan menggunakan persamaan logistik untuk pertumbuhan, persamaan Luedeking-Piret untuk penghasilan asid laktik dan model Leudeking-Piret yang telah dimodifikasi untuk penggunaan substrat. Anggaran parameter untuk penapaian secara kelompok adalah $\mu_{\max} = 0.2582 \text{ h}^{-1}$, $X_0 = 2.81 \text{ g/l}$, $X_m = 8.60 \text{ g/l}$, $Y_{x/s} = 0.200 \text{ g/g}$, $Y_{p/s} = 0.74 \text{ g/g}$ dan pekali penyelenggaraan (m_s) = $0.017 \text{ g glucose g}^{-1} \text{ biomass h}^{-1}$. Daripada persamaan Leudeking - Piret, nilai α , β diperolehi adalah 0.179 dan 0.014. Nilai R^2 regresi biojisim, pengeluaran asid laktik dan penggunaan substrat adalah 0.8452, 0.9245, dan 0.9669. Penentuan nilai regresi telah menunjukkan bahawa model yang digunakan dapat menafsirkan kinetik dalam penapaian kelompok. Hasil kajian kebolehlaksanaan, menunjukkan beras hancur berpotensi tinggi untuk menjadi sumber lestari yang baru dalam penghasilan asid laktik.

LACTIC ACID PRODUCTION FROM BROKEN RICE VIA FERMENTATION PROCESS

ABSTRACT

One of the consequences of industrial food production activities is the generation of high volumes of waste, whose disposal can be problematic, since it occupies large spaces and when poorly managed can pose environmental and health risks for the population. The rice industry is an important activity and generates large quantities of waste. The main solid waste generated in the rice production cycle is broken rice. Broken rice refers to the fragments of rice grain obtained by milling and separated after the polishing phase. It has the same chemical composition as white rice and its most important components are carbohydrates. The aim of this research is to investigate the optimum condition and potential of utilizing fermented broken rice to the production of lactic acid using different strain of bacteria culture. Different process parameters such as pH of the medium, temperature, agitation speed and substrate concentration were used to study the optimum condition. Then, the growth kinetics and modelling of lactic acid production from broken rice were studied in a batch system. From the screening process, the highest lactic acid concentration of 5.24 g/L was obtained from *Lactobacillus acidophilus* strain at pH 7, Temperature 35°C, 150 rpm agitation speed and the substrate used was 50 g/L. Unstructured models were proposed using the logistic equation for growth, the Luedeking-Piret equation for lactic acid production and modified Leudeking-Piret model for substrate consumption. The parameters estimation for the experiment data for batch fermentation $\mu_{\max} = 0.2582 \text{ h}^{-1}$, $X_0 = 2.281 \text{ g/L}$, $X_m = 8.60 \text{ g/L}$, $Y_{x/s} = 0.200 \text{ g/g}$, $Y_{p/s} = 0.74 \text{ g/g}$ and maintenance coefficient (m_s) = $0.017 \text{ g glucose g}^{-1} \text{ biomass h}^{-1}$. From the Leudeking – Piret equations the α , β value obtained were 0.179 and 0.014 respectively. Excellent significant R^2 values of 0.8452, 0.9245, and 0.9669 were observed for biomass, lactic acid production and substrate consumption, respectively. The values indicated that the model used was able to interpret the fermentation kinetic in the batch fermentation. The feasibility study shown broken rice having high potential to be a new sustainable source for the lactic acid production.