LACTIC ACID PRODUCTION FROM SOLID PINEAPPLE WASTE USING *Rhizopus oryzae* NRRL 395

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A thesis submitted in fulfilment of the
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Special dedicated:

To my dearly husband, Muhamad Ashhep Samsori
To my lovely son, Zayn Maleeq
To my Abah, Aziman Kastor
To my Ibu, Noriyah Embok Entang
To my siblings:
Siti Nurhazwani
Siti Nurshahira
Muhammad Nur Zaki
Nur Elysya Amirah

"For indeed, with the hardship, there is relief.
Indeed, with the hardship, there is relief."
Al- Insyirah, 94: 5-6

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'In the name of Allah, the Most Beneficent, the Most Merciful'

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ABSTRACT

Solid pineapple waste (SPW) is one of the most abundant agricultural wastes found in tropic region. It was reported about 40-50% of the wastes generated from pineapple canning industry are from solid wastes. Their disposal poses a serious environmental pollution problem. This study looks into the potential of utilizing a mixture of solid pineapple waste including residual pulp, peels, cores, stems and leaves for the production of lactic acid by *Rhizopus oryzae* NRRL 395 in solid state fermentation (SSF). Characterization of SPW using Fourier transform infrared (FT-IR) and Scanning electron microscope (SEM) indicate that the structural and chemical composition of autoclaved SPW was suitable for use as SSF substrate. Screening studies through 2-level factorial (2LF) design revealed that *R. oryzae* NRRL 395 was best suited for lactic acid metabolism under the conditions of SSF system. The optimum SSF condition in shake flasks conducted based on central composite design was obtained at 67.53%, 3 days of incubation, at temperature of 32.2°C, pH of 5.6, and inoculum size of 1×10^7 spores/g, with 1.21 fold increment of lactic acid yield compared to that produced in one-factor-at-a-time experiment. This study has successfully designed a novel modified Memmert-tray bioreactor to analysed the effects of lactic acid production in larger scale (1kg) SSF of SPW. The highest concentration of lactic acid in the bioreactor was obtained at condition variables of 70 ± 2% of humidity chamber with 2 Liters per minute (LPM) aeration rate, incubation temperature of 30°C, pH 6 and 70% of initial moisture content of SPW bed, where the SSF was run for 3 days.The lactic acid yield ($Y_{p/s}$), maximum and overall lactic acid productivity of *R. oryzae* NRRL 395 in modified Memmert-tray bioreactor were 1.03, 1.05, 1.14 fold higher than those under optimum condition performed in shake flask system. As a conclusion, a significant lactic acid production from SPW by *Rhizopus oryzae* NRRL 395 has proved that it could be contributed towards the sustainability of agricultural industry by creating wealth from waste and promoting economic biotechnology for future development.

ABSTRAK

Sisa pepejal nanas merupakan salah satu daripada bahan buangan pertanian yang paling banyak boleh didapati di kawasan tropika. Kira-kira 40-50% sisa buangan yang dihasilkan daripada industri pengetinan nanas adalah dari sisa pepejal nanas. Pembuangan sisa nanas juga menyebabkan masalah pencemaran alam sekitar yang serius. Tujuan kajian ini dijalankan ialah untuk melihat keboleh-upayaan penggunaan sisa pepejal nanas termasuk pulpa, kulit, teras tengah, batang dan daun, bagi menghasilkan asid laktik oleh *Rhizopus oryzae* NRRL 395 melalui penapaian keadaan pepejal. Pencirian kandungan sisa pepejal nenas menggunakan alat Penjelmaan Fourier Inframerah dan Mikroskop Elektron Imbasan telah membuktikan bahawa struktur dan komposisi pepejal nenas yang telah diautoklaf sesuai untuk digunakan sebagai substrat penapaian keadaan pepejal. Saringan menggunakan Rekabentuk 2-Aras-Faktoran menunjukkan bahawa *R. oryzae* NRRL 395 berkebolehan melakukan metabolisme asid laktik di dalam keadaan SSF. Keadaan optimum SSF bagi penapaian di dalam kelalang yang diperolehi melalui Rekabentuk Pusat Komposit adalah pada 67.53% kandungan lembapan, pengeraman selama 3 hari, 32.2°C, pH 5.6, dan 1×10^7 spora/g, dimana hasil asid laktik telah meningkat 1.21 kali lebih tinggi berbanding kawalan yang menggunakan kaedah satu-faktor-pada-satu-masa. Kajian ini berjaya merangka sebuah bioreaktor Memmert-dulang yang telah diubahsuai bagi menganalisis kesan penghasilan asid laktik dalam kapasiti yang lebih besar (1kg). Jumlah tertinggi asid laktik yang diperoleh dalam bioreaktor ialah pada pembolehubah kelembapan kebuk pada 70±2%, kadar pengudaraan sebanyak 2 liter seminit (LPM), suhu 30°C, pH 6 dan kelembapan awal sampel nenas pada 70%, masa pengeraman selama 3 hari. Hasil asid laktik ($Y_{p/s}$), produktiviti maksimum dan produktiviti keseluruhan penghasilan asid laktik oleh *R. oryzae* NRRL 395 dalam bioreaktor Memmert-dulang yang diubahsuai adalah 1.03, 1.05, 1.14 kali lebih tinggi daripada keadaan optimum di dalam kelalang. Kesimpulannya, penghasilan asid laktik daripada SPW oleh *Rhizopus oryzae* NRRL 395 membuktikan bahawa ianya dapat menyumbang ke arah kemapanan industri pertanian dengan mewujudkan kekayaan dari sisa terbuang dan mempromosikan ekonomi bioteknologi bagi pembangunan masa hadapan.

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LIST OF ABBREVIATIONS

°C	-	Degree celcius
µ	-	Micro
$(NH_4)_2SO_4$	-	Ammonium sulphate
2LFD	-	Two-level factorial design
ADF	-	Acid Detergent Fibre
ANOVA	-	Analysis of variance
ATP	-	Adenosine triphosphate
CCD	-	Central composite design
CCRD	-	Central Composite Rotatable Design
CO_2	-	Calcium hydroxide
DHAP	-	Dihydroxy acetone phosphate
DNS	-	Dinitrosalicyclic acid
EM	-	Effective microorganism
EMP	-	Embden-Meyerhof-Parnas
FDA	-	Food and Drug Administration
$Fe_2(SO_4)_3$	-	Iron(III) sulfate
FT-IR	-	Fourier Transform Infrared Spectroscopy
g	-	Gram
GRAS	-	Generally recognized as safe
H	-	Hour
HCl	-	Hydrochloric acid
HPLC	-	High performance liquid chromatography
H_2SO_4	-	Sulphuric acid
kg	-	Kilogram
KH_2PO_4	-	Potassium dihydrogen phosphate
L	-	Liter
LAB	-	Lactic acid bacteria

LDH	-	Lactate dehydrogenase
LPM	-	Liter per minute
Mg/mL	-	Milligram per mililitre
$MgSO_4$	-	Magnesium sulfate
min	-	Minute
mL	-	Milliliter
NaOH	-	Sodium hydroxide
mm	-	Millimeter
NADH	-	Nicotinamide adenine dinucleotide hydrogen
NDF	-	Neutral Detergent Fibre
nm	-	Namometer
OAA	-	Oxaloacetate
OFAT	-	One Factor At a Time
PDA	-	Potato Dextrose Agar
PDC	-	Pyruvate decarboxylase
PDH	-	pyruvate dehydrogen complex
RID	-	Refractive index detector
rpm	-	Rotation per minute
RSM	-	Response surface methodology
SEM	-	Scanning Electron Microscopy
SmF	-	Submerged fermentation
SPW	-	Solid pineapple waste
SSF	-	Solid state fermentation
TCA	-	Tricarboxylic acid cycle
TPP	-	Thiamine pyrophosphate
USDA	-	United States Department of Agriculture
XDH	-	Xylitol dehydrogenase
XK	-	Xylulose kinase
XR	-	Xylose reductase
$ZnSO_4$	-	Zinc sulfate

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Pineapple (*Ananas comosus*) is a tropical plant that is commonly cultivated on peat soil and is believed to have originated from the Eastern region South America (Ahmed *et al*., 2004; Botella and Smith, 2008). Malaysia is one of the world's major producers of pineapple industry which includes Thailand, Philippines, Indonesia, Hawaii, Kenya, Brazil, Taiwan, Australia, India and South Africa (Rohrbach *et al*., 2002). Pineapple was introduced to Tanah Melayu in the early 16th century by the Portuguese and was commercially planted in Johor and Selangor in 1921 as cash crop (Siti Roha *et al.*, 2013).

According to the official portal of the Malaysian Pineapple Industry Board (MPIB), Johor is the biggest contributor to the Malaysian pineapple industry because of its peat soil nature that makes it suitable for pineapple plantation (Siti Roha *et al.*, 2013). Furthermore, in 2015, almost 329,954 metric tonnes of pineapples were produced in Johor, which covers almost 73% of the total pineapple producers (452,020 metric tonnes) in Malaysia (MPIB, 2016). The export of pineapples-based-products in Malaysia has recorded a positive growth. In 2015, the total value of pineapples exports (including fresh, canned, juices and ornamental) are approximately RM161,169,968, at which was increased by 41% compared to theprevious year (RM14,115,983). The product that appeared with the highest export value is canned pineapples, which is about 50.15% of the total pineapple exports (RM 80,834,669). Demands on the export of fresh pineapples in Malaysia is expected to increase at 5% per annum due to the higher demand on MD2 type of pineapple (hybrid pineapple also known as super sweet pineapple) in the international market (Mensah and Brummer, 2015; Wan Rizzal *et al.*, 2014).

Pineapple canning industry is one of the contributors of solid waste accumulation in Malaysia (Jusoh *et al.*, 2014). It was reported about 40-50% of the wastes generated from pineapple canning industry are from solid wastes, where it was comprises of residual pulp, peels, core, stems and leaves (Buckle, 1989; Abdullah and Mat, 2008). Sometimes, the pineapple wastes are used by dairy farmers to feed cattle (Sruamsiri, 2007). In some places, the pineapple's peat is reutilized by burning process (Ahmed *et al.*, 2004). However, it needs extra work in controlling the burning peat and in the worst condition, it generates lots of ash that may cause haze to occur. According to Ahmed and his co-workers (1999), 1.31 mg per hectare (ha^{-1}) of ashes has been released after burning 4.34 Mg per hectare (ha^{-1}) of pineapple leaves per cropping season (Ahmed *et al.*, 2004). At present, dumping pineapple waste into the environment involves a considerable cost due to the handling process and transportation (Dacera and Babel, 2008). The solid pineapple wastes are usually left accumulated or disposed on the soil as waste, causing environmental pollution. Therefore, research on the utilization of solid pineapple waste into a value added product is one of the best solutions in managing pineapple waste, especially in Johor.

Pineapple waste is classified as a lignocellulosic compound at which it consists of cellulose, hemicelluloses and lignin (Zhang *et al*., 2007). Usually, pineapple waste contains high amount of sugars and nutrients that make it economically feasible for the conversion of value added products such as organic acids, bromelain, ethanol, phenolic antioxidant, etc. (Kareem *et al*., 2010; Larrauri *et al*., 1997; Nigam, 1999, Dacera *et al*., 2009). Besides that, pineapple waste also contains organic substances that could be used for biogas production, for example methane gas (Rani and Nand, 2004). Furthermore, pineapple waste has been reported to contain fibers where in some Southeast Asian countries, the pineapple leaves has been used to make coarse textiles and threads (Tran, 2006). In Malaysia, pineapple waste has been used as Bio-Organic fertilizer by using effective microorganism (EM) technology (Zakaria, 2006). Recently, research on renewable materials conversion into value added product in maintaining a sustainable environment is an attractive idea especially in applying the lignocellulosic-based material since it contains a possible component that can be used as a substrate in the fermentation process (Prados *et al*., 2010). Based on the compositional analysis of the SPW component by previous researchers, it is logical that it can be used as sole carbon source of organic acid production, specifically L(+) type of lactic acid (Rani *et al*., 2004; Bardiya *et al*., 1996; Ban-Koffi *et al*., 1990).

Recent worldwide demand for L(+) lactic acid has been recently estimated around 130 000 to 150 000 (metric) tonnes per year (John *et al*., 2007). The prices of lactic acid depends on the grade of the lactic acid itself, where food-grade of lactic acid is around 1.38 US$/kg (50% purity) and 1.54 US$/kg (88% purity), whereas technical-grade of lactic acid is around 1.59 US$/kg (88% purity) (Wee *et al*., 2006). Lactic acid is a type of organic acid that serves as feed in certain industries, such as food, cosmetic, medical, etc. (Wee *et al*., 2006). Lactic acid can exist in several forms, either D (−) or L (+) lactic acid, or as a racemic mixture of both, depending on the type of microorganism used in the fermentation process. L(+) lactic acid is a well-known monomer being used in the production of biodegradable plastic, called poly lactic acid (PLA) (Garlotta, 2002).

At present time, natural lactic acid production by using biological synthesis has received much attention compared to chemical synthesis because it could reduce the production cost and prevent serious environmental problems (Wee *et al.*, 2006). Lactic acid production from lignocellulosic material through the fermentation process is the best alternative to chemically synthetic method since production through chemical technique might generate toxic by-products and is quite expensive due to the chemical components, and solvents needed in the manufacturing process (Gavrilescu and Chisti, 2005). Fermentative production of lactic acid from lignocellulosic biomass can be conducted either through solid state fermentation (SSF) or submerged fermentation (SmF) (Barrios-Gonzalez, 2012). SSF involves the fermentation of solid or non-soluble material in absence or near absence of free water, where the condition promotes the growth of fermentative organisms (Pandey, 2003). In contrast to that, SmF requires excess amount of water, where the fermentative substrate is suspended in a high volume of water (Moo-Young *et al.*, 1983). SSF has been applied since ancient times (2600 B.C.); but it has been ignored in 18th century due to the emergence of submerged fermentation (SmF) for penicillin commercialization (Pandey, 1992). At present times, it can be seen that there is a growing interest in modern SSF systems since more industries employs the SSF system due to the higher production of desired product in large scale volume within low production time compared to the SmF method (Barrios-Gonzalez, 2012; Acuna-Arguelles *et al*., 1995; Diaz-Godinez *et al*., 2001; Elinbaum *et al.*, 2002). In addition, SSF promotes low cost spending on energy investment since it requires less amount of water during fermentation and most of SSF process does not involve mechanical mixing technique (Chen, 2013). Plus, SSF easily handles separation of products from the solid substrate and at the same time has fewer requirements in the downstream process (Chen, 2013).

Selection of suitable microorganism is another important feature in the production of lactic acid (Pandey, 2003). Lactic acid bacteria (LAB) are classified as a group of microbes that are usually used in producing lactic acid. Previously, *Lactobacillus* was the best strain used in the lactic acid industry (Wahidin, 2008). However, over the past few years, filamentous fungi have played an important role in producing L(+) lactic acid (Miura *et al*., 2004a; Soccol *et al.*, 1994). The fungi belonging to the *Rhizopus* genome has received much attention for its amylolytic activity, pure lactic acid production and low nutrient requirement (Tay and Yang, 2002; Maas *et al*., 2006; Zhang *et al*., 2007). *Rhizopus oryzae* is one of the common fungi that have been used in the lactic acid industry (Zhang *et al*., 2007). The non-fastidious nature of *Rhizopus oryzae* provides a low nutritional requirement advantage compared to using lactic acid bacteria. Moreover, *Rhizopus oryzae* is preferable because it does not require the purification process as it has the capability of directly produce pure L(+)-lactic acid (Skory, 2004). Ruengruglikit and Hang (2003) successfully produced 299.4±6.8 g of L(+) lactic acid per kg dry matter of corncobs with the addition of commercial apple juice using *Rhizopus oryzae* NRRL 395 through the SmF process. Phrueksawan *et al.* (2012) has enhanced the production of lactic acid from 206.20 to 463.18 mg/g of cassava pulp by using *Rhizopus oryzae* NRRL 395 through direct SSF with the help of commercial cellulase and glucoamylase. Since *Rhizopus oryzae* is available in several types of strains, Saito *et al.* (2012) found that *Rhizopus oryzae* NBRC 5378 was the best among 56 strains of *R. oryzae* for the production of lactic acid, where they effectively obtained 0.23 g/g of lactic acid from a wheat straw. Therefore, the selection of *Rhizopus oryzae* NRRL 395 as fermenting agent in employing direct SSF technique to produce the L(+) lactic acid is the best idea and should be investigated.

In ancient times, large scale handling SSF was a great concern due to its difficulties in controlling the operational conditions (Jou and Lo, 2011). In response to that, improvement on upscalling conditions can be achieved by understanding the overall process through optimizing the operating conditions including temperature, moisture content of the sample, humidity and aeration rate within the chamber (Vaseghi *et al*., 2013). Various types of bioreactors have been designed in a large scale of SSF including tray, packed bed, stirred bed, rotating drum and fluidized bed bioreactors (Couto and Sanroman, 2005; Ali and Zulkali, 2011; Mitchell *et al.*, 2006). Among these types of bioreactors, the simplest type is the tray bioreactor (Couto and Sanroman, 2005). Its non-complicated nature (easy to decontamination), low cost (affordable to be constructed) and probability of the whole chamber considered as a single bioreactor makes the tray bioreactor one of the promising bioreactors to be applied in the upscale stage (Ruiz *et al*., 2012; Vaseghi *et al*., 2013; Mitchell *et al.*, 2006). Besides that, an improved novel type of tray bioreactor has been developed as a new approach in dealing the SSF problems. Mohseni *et al.* (2012) have designed a noble tray bioreactor in producing high concentration of lipase (142.732 U/gds) from rice bran using *A. niger* NCIM 584. They designed an effective tray bioreactor by incorporating side by side fans (individual tray was in the middle) in order to improve the air circulation within the chamber. Hence, in this study, a novel modified-Memmert tray bioreactor was developed in order to improve the SSF performance since no report has been found on using this type of bioreactor. In addition, comparison on individual tray production was conducted in order to evaluate the possible errors in different trays.

1.2 Objectives

The objectives of this study are:

1. To analyze and characterize the composition of solid pineapple waste (SPW).
2. To screen the factors that affect lactic acid production from SPW by *Rhizopus oryzae* NRRL 395 using two-level factorial design (2LFD) in flask.
3. To optimize lactic acid production using central composite design (CCD) in flask.
4. To investigate the effects of lactic acid production in modified-Memmert tray bioreactor.

1.3 Scope of Research

This study focuses on investigating the capability of *Rhizopus oryzae* NRRL 395 in producing L(+) lactic acid from solid pineapple waste (SPW) through SSF. The SPW component was chemically and physically figured prior to the fermentation process. The image of raw SPW was examined through scanning electron microscope (SEM) and fourier transform infrared spectroscopy (FT-IR). The capability of direct utilization of untreated SPW was tested where the lignocellulosic content of SPW was compared before and after sterilization using autoclave. Preliminary analysis on working parameters that possibly affect the lactic acid optimization study by *R. oryzae* NRRL 395 were conducted including incubation time, particle size, initial pH and calcium carbonate concentrations.

The ability of *R. oryzae* NRRL 395 in solid-fermenting the SPW to lactic acid in flask scale was statistically studied using Design Expert® Software (Version

6.0.4). At first, the variables that possibly affect lactic acid production were screened through 2-Level Factorial Design (2LFD). Next, a standard Response Surface Methodology (RSM) known as Central Composite Design was used to optimize the lactic acid production condition. Factors that were involved in the optimization study were moisture content (45 - 85%), incubation time (0 - 7 days), temperature (25 - 45°C), pH (4 - 8) and inoculum size (1×10^5 - 1×10^9 spores/g). Besides that, the effect of variables towards by-products formation (fumaric acid and ethanol) and sugar utilization (polyoses and reducing sugar) after optimization was also investigated. Moreover, the efficiency of lignocellulosic degradation of SPW by *R. oryzae* NRRL 395 before and after optimization was also compared.

After completing the optimization study on process parameters of lactic acid production in flask scale, the potential of large scale analysis by a novel modified-Memmert tray bioreactor (1kg) was also investigated. The working parameters involved in the bioreactor analysis were incubation time (0 - 6 days), humidity of the chamber (50±2 - 90±2%), aeration rate (control 1 and control 2, 1 - 5 LPM), initial moisture content (60 - 85%), incubation temperature (25 - 50°C) and initial pH of solid substrate (4.5 - 7.5). Besides that, an attempt to detect the SPW structural transformation after SSF of lactic acid in the bioreactor was also investigated using SEM and FT-IR. At the final stage, the lactic acid performance in flask and bioreactor scale in terms of lignocellulosic degradation and sugar utilization were also evaluated.

1.4 Significance of Research

As Johor is the biggest contributor of pineapple plantation in Malaysia, there is a high possibility that it might give rise to environmentally sensitive disposal issues in the future. Thus, this encouraged us to utilize the raw material of solid pineapple waste and convert the possible residual components into a value added product for example L(+) lactic acid. Below are several addressed issues that make this research is significant:

i. Increased production of pineapple by 10% (from 412,665 to 452,020 metric tonnes) from 2014 to 2015 (MPIB, 2016) directly caused the accumulation of pineapple waste which may trigger environmental concerns among certain communities including local societies, agricultural-based industries and government authorities. Disposal of SPW through burning may not only lead to air pollution (haze), but also causes long-term effects that lead to serious diseases especially heart and lung diseases (Ahmed *et al*., 2004). Instead of serving it as animal feeding to farmers and disposed to the environment, it is beneficial to utilize the SPW as a substrate to produce valuable product and it also advantageous towards sustainable technology.

ii. As mentioned before, the pineapple canning industry is one of the contributors of lignocellulosic waste accretion. SPW was reported to possess useful remaining components especially celluloses, sugars and nutrients (Abdullah and Mat, 2008; Siti Roha *et al.*, 2013). SPW could serve as a promising substrate in the production of a valuable fermented product, named lactic acid. Nowadays, lactic acid has been used as a precursor to generate a biodegradable plastic called poly-lactic acid (PLA).

iii. Production of lactic acid from lignocellulosic agrowaste is an effective alternative because of their cost effectiveness, renewable, and availability worldwide (Zhang, 2008). Moreover, production of lactic acid in the biological pathway provides a significant economic potential by reducing the usage of chemicals, and perhaps reduce the cost for the downstream process especially in using the purification method. In fact, direct utilization of lignocellulosic waste as sole carbon with no expensive pretreatment involved, was able to cut 40 - 60% of the total production cost (Howard *et al*., 2003).

iv. Fermentation of solid pineapple waste could be accomplished through solid state fermentation (SSF). SSF process is more complex than submerged fermentation (SmF) due to its heterogeneity condition (Rokem, 2010; Kapilan, 2015). However, SSF offer various advantages over SmF, mostly on a laboratory scale including higher fermentation product, generating a high degree of product stability, lower possibility on catabolic repression and less requirement on sterility due to low water activity needed in SSF (Holker *et al*., 2004; Singhania *et al*., 2009). Plus, lactic acid production using *Rhizopus oryzae* NRRL 395 is the best alternative to microbial fermentation as fungi were listed as the most suitable microorganism for SSF process due to the penetration capability of fungi to absorb nutrients from solid substrate and resembling its natural habitat (Pandey, 2003; Ruengruglikit and Hang, 2003; Gowthaman *et al.*, 2001; Phrueksawan *et al.*, 2012).

v. Instead of producing the specific L(+) lactic acid, *Rhizopus oryzae* is capable secreting heterogeneous products include ethanol and fumaric acid (Abe *et al*., 2007). *R. oryzae* NRRL 395 tends to secrete by-products at poor fermentation conditions (Soccol *et al.*, 1994). Hence, optimization of the physical conditions to produce the maximum concentration of lactic acid using statistical analysis through response surface methodology (RSM) is one of the best solutions in reducing the production of unwanted products. RSM promotes a simple, fast and cost-effective system compared to conventional methods due to simultaneous analysis of several working factors at a specific time (Tarley *et al.,* 2009). Moreover, RSM would generate a mathematical model that can be used to study the relevance and statistical significance of the selected factors.

vi. Difficulties in handling process parameters in large scale has been a crucial concern in order to achieve the optimum working condition over the last few decades. The moisture, aeration rate and temperature of the bioreactor play important roles towards the lactic acid fermentation performance. Optimization of the process parameters in the bioreactor would improve the lactic acid yield compared to the flask scale analysis. Therefore, a novel modified-Memmert tray bioreactor that contains all controlling unit has been designed, perhaps to improve the lactic acid production. Besides that, it would significantly impact the SSF research field, particularly in terms of upscale understanding, knowledge and its future potential.

1.5 Thesis Organization

This thesis is organized into seven chapters. **Chapter 2** covers relevant literatures and information regarding on the potential of lignocellulosic of SPW to be used as a raw material for production of lactic acid. This chapter provides an overview of lignocellulosic degradation via solid state fermentation (SSF) approach and the role played by the fungus named *Rhizopus oryzae*. The effect of bioprocess parameters in the SSF of lactic acid production was also reviewed. Literatures related to application of various types of bioreactors in SSF industries are briefly summarized.

Chapter 3 describes the general experimental procedures performed in this research. All common methods and procedures are placed in this chapter and be referred to in specific chapters, respectively.

The results and discussions are divided into three main chapters. **Chapter 4** describes the characterization of raw and autoclaved SPW composition chemically and physically prior to the SSF process. **Chapter 5** presents the process of preliminary, screening and optimization of lactic acid production from the SPW using *Rhizopus oryzae* NRRL 395 via SSF. The effect of important bioprocess factors (moisture content, incubation time, temperature, pH and inoculum size) towards the lactic acid production was also studied. In **Chapter 6**, an upscale analysis was made in the novel modified-Memmert tray bioreactor and comparison was also has been done between the lactic acid productions in flask system with the bioreactor performance.

The conclusions from this research are given in **Chapter 7**. This chapter also states specific achievement and some recommendations for future works.

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