PRODUCTION OF SORBITOL FROM *MERANTI* WOOD SAWDUST USING SOLID STATE FERMENTATION (SSF) PROCESS

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ABSTRACT

The main objective of this research is to produce a high concentrations of sorbitol using solid state fermentation (SSF) of *meranti* wood dust by bacteria *Lactobacillus plantarum* (BAA 793; NCIMB 8826). Before the fermentation process, *meranti* wood dust has been treated through physical and chemical processes for the recovery of cellulose, followed by enzymatic hydrolysis process to produce glucose. The resulting glucose is then used as the carbon sources in fermentation to produce sorbitol. Parameters studied in sorbitol production using solid-state fermentation is fermentation time (2 hours to 14 hours), moisture content (40% to 90%) and temperature (25^{o}C to 45^{o}C). Method one factor at a time (OFAT) conducted on all parameters to determine an appropriate range before the response surface methodology (RSM) is implemented. From this study, the results showed that the optimum condition for the production of sorbitol is at 10 hours, with 50% moisture content and temperature of 35^{o}C where the production of sorbitol is 29.0625g/L. Results of this experiment showed that SSF produce high sorbitol than SMF

ABSTRAK

Objektif utama kajian ini adalah untuk menghasilkan kepekatan sorbitol yang tinggi menggunakan proses penapaian keadaan pepejal (SSF) daripada habuk kayu *meranti* dengan bacteria *Lactobacillus plantarum* (BAA 793; NCIMB 8826). Sebelum proses penapaian, habuk kayu meranti telah dirawat melalui proses fizikal serta kimia untuk pemulihan selulosa dan diikuti dengan proses enzim hidrolisis untuk menghasilkan glukosa. Glukosa yang terhasil kemudiannya dijadikan sumber karbon dalam penapaian untuk menghasilkan sorbitol. Parameter yang dikaji dalam penghasilan sorbitol menggunakan proses penapaian keadaan pepejal adalah masa penapaian (2 hingga14 jam), kandungan lembapan (40% hingga 90%) dan suhu (25^{o}C hingga 45^{o}C). Kaedah satu faktor pada satu masa (OFAT) dijalankan terhadap semua parameter bagi menentukan julat yang sesuai sebelum kaedah tindak balas permukaan (RSM) dilaksanakan. Daripada kajian ini, keputusan menunjukkan bahawa keadaan optimum untuk penghasilan sorbitol adalah pada 10 jam, dengan 50% kandungan lembapan dan suhu 35^{o}C dimana penghasilan sorbitol adalah sebanyak 29.0625 g/L. Hasil ekperimen ini menunjukkan bahawa SSF dapat menghasilkan sorbitol yang tinggi berbanding SMF.

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LIST OF SYMBOLS

SSF	-	Solid State Fermentamin
SmF	-	Submerged Fermentationb
OFAT	-	One Factor At a Time
RSM	-	Response Surface Methodology
CCD	-	Central Composite Design
G	-	Gram
g/L	-	Gram per Litre
Ml	-	Mililiter
Mm	-	Milimeter
Mg	-	Miligram
Nm	-	Nanometer
µl	-	Microliter
mol/L	-	Mol per Liter
FRIM	-	Forest Research Institute of Malaysia
w/dw	-	Weight per Dry Weight
w/v	-	Weight per Volume
LAB	-	Lactic Acid Bacteria
GRAS	-	Generally Recognised As Safe
ATCC	-	America Type Culture Collection

NaOH	-	Sodium Hydroxide
PAA	-	Peracetic Acid
CH_3COOOH	-	Peracetic Acid
H_2O_2	-	Hydrogen Peroxide
H_2SO_4	-	Sulfuric Acid
N_2	-	Nitrogen Gas
O_2	-	Oxygen Gas
OD	-	Optical Density
DNS	-	Dinitrosalicylic
MC	-	Moisture Content
RH	-	Relative Humidity
a_w	-	Water Activity
HPLC	-	High Performance Liquid Chromatography
FTIR	-	Fourier Transform Infrared Spectrocopy
XRD	-	X-ray Diffraction

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND STUDY

Malaysia generates an abundance of agricultural wastes such as sawdust, sugar cane baggage, rice husk, rice straw, rubber wood dust, palm kernel cake and many other waste materials. The volume of the agricultural wastes' produced is approximately 5 million tons per year. To avoid this environmental problem, the management of agricultural wastes in this country must be given priority to ascertain the reduction of environment pollution (Pang *et al*., 2006). The production of new chemicals and biochemical's today must be produced by utilizing minimum energy requirements and zero environmental pollution in order to achieve the environmental friendly status. Production of waste materials is actually an undeniable part of human society. Nowadays many products are produced from these wastes materials and one such product is sorbitol *from meranti* (*Philippine mahogany*) wood sawdust. *Meranti* tree sawdust is an inexpensive raw material and is currently being investigated as an absorbent (Anees *et al.*, 2009).

Production of sorbitol is important as an industrial chemical. It can be produced by the fermentation of glucose using a number bacteria such as *Lactobacillus sp., Zymomonas mobilis, E. Coli* as well as several others microorganisms. However, only a few microorganisms have been suggested as a potential sorbitol producer. Sorbitol or also known as glucitol, is a six-carbon sugar polyol and it has a variety of applications in the pharmaceutical industry, in the food industry as a low-calorie sweetener, humectants, texturing agent and also as a softener (Reinout *et al*., 2010). Moreover, a polyol like sorbitol is generally used extensively in its liquid form as in in oral care products and it is

also expensive in its crystalline form. The world-wide production of sorbitol is estimated to be approximately 500,000 tons annually and the market for the product is continuously increasing (Ladero *et al*., 2007).

The fermentation process to produce sorbitol can be divided in two types, namely solid state fermentation (SSF) which is still under intensive research and submerged fermentation (SmF), which is well established. Most of the industries, especially in Malaysia, rely on submerged fermentation where the bacteria or microorganisms are grown in liquid media and yet some industries also use the solid state fermentation process. Some authors such as Manpreet, (2005) have mentioned that SSF has a good option compared to the SmF process because less chances of contamination due to low water activity, better product recovery, low waste water output and others benefits. Besides that, the process using SSF has been increasing nowadays because it is an important process and has applications in bio-pesticides, production of enzymes and aroma compounds, biopharmaceutical and the production of organic acids. The development of the SSF process was achieved sometime around 1950 to 1960 when steroid transformation was reported using fungal culture followed by mycotoxin production using the SSF process (Manpreet *et al*., 2005).

The present study on sorbitol fermentation is focused primarily on the effect of parameters (fermentation time, moisture content and temperature) on the solid state fermentation (SSF) process using the *Lactobacillus plantarum* (BAA793;NCIMB 8826) strain on *meranti* wood sawdust as substrate.

1.2 PROBLEM STATEMENT

Malaysia is a large country that produces many types of waste materials and one such waste material is wood sawdust. Malaysian sawmills were produced 3.4 million m^3 annually of wood wastes. This waste production can result in a significant environmental problem if not disposed of in proper manner. The agro-industry likes sawdust as it has great potential as a substrate for sorbitol fermentation because it contains cellulose that can be

converted to glucose and then utilized by *Lactobacillus sp.* Such utilization would further increase profitability for the sorbitol industry and consequently solving an environmental problem.

Sorbitol can be produced by the fermentation of glucose by using either the solid state fermentation (SSF) process or the submerged fermentation (SmF) process. Solid state fermentation has gained renewed attention in the recent years. To produce a high yield of sorbitol, the solid state fermentation is used. SSF has the potential for the economical production of sorbitol. SSF also has many advantages over submerged fermentation including economy of space needed for fermentation, superior yield, less energy demand, low capital and recurring expenditure. The submerged fermentation (SmF) process on the other hand, has many drawbacks including lower production quantity compared to the solid state fermentation (SSF). In addition, submerged fermentation requires processed ingredients that are expensive and the media concentration is very much lower as compared to the water content. Submerged fermentation also uses a large amount of water in the process and therefore it becomes a major cause of contamination besides making the downstream process difficult and very expensive. In the SmF process, the level of liquid waste produced is very high and it will cause difficulties in dumping later on (Manpreet *et al.*, 2005).

1.3 RESEARCH OBJECTIVE

The main objective of this project is to produce a high yield of sorbitol by solid sate fermentation (SSF) using the *meranti* wood sawdust. To achieve this objective, the following steps has been carried out:

- To identify the effect of fermentation time, moisture content and temperature on the sorbitol concentration during solid state fermentation (SSF).

- To determine the optimum condition of solid state fermentation (SSF) which can produce high yield of sorbitol.

- To compare the conversion yield of sawdust and yield of sorbitol between solid state fermentation (SSF) and submerged fermentation (SmF), thereafter to compare the yield of sorbitol for commercial glucose between glucose pretreated from *meranti* wood sawdust using solid state fermentation (SSF).

1.4 SCOPE OF THE STUDY

There are mainly four scopes in this research:

- The characterization of *meranti* wood sawdust (raw material), cellulose (after pretreated *meranti* wood sawdust) and glucose (after enzymatic hydrolysis) was done before the reaction and separation was carried out. The *meranti* wood sawdust was characterized based on the Forest Research Institute of Malaysia (FRIM) laboratory by using in house (FRIM) methods and functional groups (FTIR), while the cellulose and glucose characterized by its functional group (FTIR) and quantitative analysis (HPLC).

- The glucose produced after the enzymatic hydrolysis process was used in this study in order to investigate the parameters (fermentation time, moisture content and temperature) controlling the sorbitol production for the (OFAT) study. The amount of sorbitol was analyzed using the HPLC method.

- Prior to its application and in order to determine the optimum condition for sorbitol production (fermentation time, moisture content and temperature), the response surface methodology (RSM) was performed. This phase is very important in order to determine the optimum yield of sorbitol production.

- Commercial glucose was used in this study to produce sorbitol in order to compare the concentration of sorbitol and/between glucose from pretreated *meranti* wood sawdust using the optimum condition of parameters (fermentation time, moisture content and temperature).