UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION ON ENZYMATIC SACCHARIFICATION OF PRETREATED SAGO HAMPAS FOR FERMENTABLE SUGAR PRODUCTION

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OPTIMIZATION ON ENZYMATIC SACCHARIFICATION OF PRETREATED SAGO HAMPAS FOR FERMENTABLE SUGAR PRODUCTION

By

ROSFARIZA MD REJAB

Thesis submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, as a fulfillment of the requirement for the degree of Bachelor of Science (Honours) Biotechnology

June 2015

FAKULTI BIOTEKNOLOGI DAN SAINS BIOMOLEKUL

UNIVERSITI PUTRA MALAYSIA

DATE :

LETTER OF PERMISSION

It is thereby to state that I, ROSFARIZA MD REJAB (Matric No: 162632) have done a final year project entitled "**Optimization on Enzymatic Saccharification of Pretreated Sago Hampas for Fermentable Sugar Production**" under supervision of Dr. Mohamad Faizal Ibrahim from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

I hereby give permission to my supervisor to write and prepare manuscript from the results of this research to be published in any form, if I do not do so in six (6) months from the date above, in condition that my name is also added as one of the article's authors. The arrangement of the name depends on the supervisor himself.

Yours sincerely,

(ROSFARIZA MD REJAB)

FAKULTI BIOTEKNOLOGI DAN SAINS BIOMOLEKUL

UNIVERSITI PUTRA MALAYSIA

APPROVAL SHEET

This thesis entitled "**Optimization on Enzymatic Saccharification of Pretreated Sago Hampas for Fermentable Sugar Production**" is submitted by ROSFARIZA MD REJAB (Matric No: 162632) in fulfillment of the requirement for the Degree of Bachelor of Science (Honours) Biotechnology in Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Approved by,

Date:

(Dr. Mohamad Faizal Ibrahim)
Project Supervisor
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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfillment of the requirement for the Degree of Bachelor of Science (Honours) Biotechnology

OPTIMIZATION ON ENZYMATIC SACCHARIFICATION OF PRETREATED SAGO HAMPAS FOR FERMENTABLE SUGAR PRODUCTION

By :

ROSFARIZA MD REJAB

June 2015

Supervisor : Dr. Mohamad Faizal Ibrahim

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Nowadays, lignocellulosic feedstocks such as corn stover, oil palm biomass, rice straw, sugarcane bagasse and also sago biomass are used for the production of many valuable bioproducts. Sago biomass is a starch and lignocellulosic-based biomass produce from the processing of sago palm. There are three majors sago biomass produced which are sago bark (24 t), sago hampas (50 to 110 t) and sago waste water (300 to 1000 m^3) generated daily in the sago processing mills. The waste generated usually discharged to the river without any treatment and this has contributed to high Biological Oxygen Demand (BOD), Chemical Oxygen demand (COD) and suspended solid in the river causing a serious environmental pollution.

Sago hampas contained 58% starch, 23% cellulose, 9.2% hemicellulose and also 4% of lignin. In this study, sago hampas were pretreated using the steaming process (autoclave) to remove the starch before proceeded with the enzymatic saccharification to produce fermentable sugar. The saccharification was conducted using a commercial cellulase enzyme (Acremonium Cellulase) and the optimization study was conducted using a two-level factorial design with four parameters which are substrate concentration (1-10%), enzyme concentration (10-50 FPU/g), agitation speed (50-250 rpm) and incubation period (24-96 h). The optimization of enzymatic saccharification of cellulose and hemicellulose of pretreated sago hampas by a cellulase enzyme (10 FPU/g) had produced the highest fermentable sugar (5.94 g/L) at parameter : 10% of substrate concentration, 10 FPU/g of enzyme concentration at 250 rpm of agitation speed in 24 h of incubation period. Through the optimization, the production of fermentable sugar was increased up to 35.2% as the sugar produced before the optimization was about 3.85 g/L.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Fakulti Bioteknologi dan Sains Biomolekul sebagai memenuhi sebahagian daripada keperluan untuk Bacelor Sains (Kepujian) Bioteknologi

PENGOPTIMUMAN SAKARIFIKASI ENZIM DARI HAMPAS SAGU TERAWAT UNTUK PENGHASILAN GULA FERMENTASI

Oleh:

ROSFARIZA BINTI MD REJAB

Jun 2015

Penyelia : Dr. Mohamad Faizal Ibrahim

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Pada masa kini, bahan mentah lignoselulosa seperti batang jagung, biomas kelapa sawit, jerami padi, hampas tebu dan biomas sagu digunakan untuk pengeluaran banyak bioproduk berharga. Biomas sagu adalah kanji dan lignoselulosa berasaskan biomas hasil dari pemprosesan pokok sagu. terdapat tiga jenis biomas utama sagu dihasilkan iaitu kulit kayu sagu (24 t), hampas sagu (50 hingga 11 t) dan air sisa sagu (300 hingga 1000 m^3) yang dihasilkan setiap hari di kilang-kilang pemprosesan sagu. Sisa yang dihasilkan biasanya dilepaskan ke sungai tanpa sebarang rawatan dan ini telah menyumbang kepada permintaan oksigen biologi (BOD), permintaan oksigen kimia (COD) dan pepejal terampai yang tinggi di dalam sungai menyebabkan pencemaran alam sekitar yang serius.

Hampas sagu mengandungi kanji 58%, 23% selulosa, hemiselulosa 9.2% dan 4% lignin. Di dalam pembelajaran ini, hampas sagu dirawat dengan menggunakan proses pengukusan (autoklaf) untuk mengeluarkan kanji sebelum diteruskan dengan pengoptimuman melalui sakarifikasi enzim menjadi gula fermentasi. Sakarifikasi ini dijalankan dengan menggunakan enzim selulase komersial (Acremonium Sellulase) dan kajian pengoptimuman telah dijalankan dengan menggunakan reka bentuk dua tingkat faktorial dengan empat parameter iaitu kepekatan substrat (1-10%), kepekatan enzim (10-50 FPU/g), kelajuan pergolakan (50-250 rpm) dan tempoh pengeraman (24-

96 h). Pengoptimuman sakarifikasi enzim daripada selulosa dan hemiselulosa daripada hampas sagu terawat oleh enzim selulase (10 FPU/g) telah menghasilkan gula beragi tertinggi (5.94 g/L) pada parameter : 10% daripada kepekatan substrat, 10 FPU/g kepekatan enzim pada 250 rpm kelajuan pergolakan dalam 24 jam tempoh pengeraman. Melalui pengoptimuman, penghasilan gula fermentasi telah meningkat kepada 35.2% di mana gula yang terhasil sebelum pengoptimuman adalah 3.85 g/L.

ACKNOWLEDGEMENTS

Alhamdulillah, praise to Allah S.W.T, by the blessing of Him and His willing, I was able to complete the final year project and this thesis entitled 'Optimization on enzymatic saccharification of pretreated sago hampas for fermentable sugar production' for my bachelor degree. In completing this project, I would like to take this opportunity to express my deep appreciation to my project supervisor, Dr. Mohamad Faizal Ibrahim for his willing to guide, critic, monitoring and also gives a constant encouragement to me throughout of this project and writing this thesis. Besides, I would like to express my special thanks to my co-supervisor, Ms. Hazwani Husin for her valuable information, support and guide which helped me a lot in completing this project through the semester.

I also would like to deliver my appreciation to Dr. Dayang Salwani Awang Adeni (UNIMAS) who helped me a lot especially during collecting sago hampas in Sarawak. I am grateful for her cooperation in guiding, giving information and advising me regarding the project. Not to forgotten, many thanks to the Environmental Biotechnology (EB) Research Group members, especially Dr. Ezyana Kamal Bahrin, Mr. Azwan Jenol, Ms. Atheera Aiza Md Razali and Ms. Ruqayyah Masran for their willingness to help me a lot in understanding my project, improving my laboratory skills and laboratory management practices. Last but not least, I would like to thank to my parent and to all my colleagues for their unconditional support from the beginning until the end of the project.

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LIST OF ABBREVIATIONS

ANN	Artificial neural network
ANOVA	Analysis of variance
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DNS	Dinitrosalicyclic acid
FPU/g	Filter paper unit per gram
g/L	Gram per litre
h	Hour
Kg	Kilogram
M	Molar
m^3	Cubic metre
mg/L	Milligram per litre
min	Minute
MJ/Kg	Megajoule per kilogram
mL	Mililitre
MSG	Monosodium glutamate
OPEFB	Oil Palm Empty Fruit Bunch
rpm	Revolutions per minute
RSM	Response surface methodology
spp	several species
SPR	Sago pith residue
t	Tonnes
TSS	Total suspended solid
w/v	Weight per Volume
w/w	Weight per Weight

CHAPTER 1

INTRODUCTION

A carbohydrate, which also known as saccharides are one of the most important biomolecules in the world. The main function of these sugar biomolecules is to provide a basic backbone towards the energy storage, act as a fuel in the cellular functions and also render the metabolic processes stable. Besides, the carbohydrate also plays an important role in controlling the interaction of cells themselves (Flitsch *et al*., 2003). Carbohydrate can be divided into three classes, there are consist of monosaccharides, disaccharides and polysaccharides. Monosaccharides refers to a single and simple sugar such as glucose, fructose and galactose that generally formed a basic unit for the carbohydrate, whereas disaccharides are referring to two unit monomers of a monosaccharide which combined and joined together with the removal of the water molecules. The combination of these two monosaccharides formed a sugar called maltose, lactose and sucrose. The complexity classes of sugar are called polysaccharides (Horton, 2008).

Polysaccharides consist of a long and complex chain of monosaccharides which bound together by a linkage called glycosidic bond. Basically, polysaccharides structure may be in a form of linear or highly branched. Starch and glycogen are the examples of linear polysaccharides, while cellulose and chitin are in the branched formed. In plants, there are a lot of polysaccharide insides which has a function in a plant metabolic process and it also provides a lot of advantages. Nowadays, polysaccharides that are broken down into sugars and undergo a several process such as fermented or chemically altered tends to produce a valuable product such as in biofuel and chemical industries. In the production of fuel and chemical, the biological conversion of cellulosic biomass is frequently used. According to Wayman *et al*. (1999), the conversion of cellulosic biomass in the meantime has become economically competitive as it gives an important strategy, environmental and economic advantages in industries. Thus, the biological conversion of these structures may give an advantage by producing a valuable product.

In recent years, there is an increasing towards the uses of agriculture residue for the production of a valuable product. It has been a lot of sustainable feedstocks that has been obtained from a variety of resources in order to extract it into a fermentable sugar through a bioconversion. A feedstock which contained a lignocellulosic material such as corn stover, oil palm empty fruit bunch (OPEFB), rice straw, sugarcane bagasse and also sago biomass (Linggang *et al*., 2012; Awg-Adeni *et al*., 2012; Jenol *et al*., 2014) has widely been used for the production of the fermentable sugar. Nowadays, an abundant presence of sago biomass in the state of Sarawak (Linggang *et al*., 2012) giving advantages to the residue to serve as an alternative substrate for the production of fermentable sugar. Sago biomass is a starch and lignocellulosic-based biomass produce from the processing of sago palm for the production of sago starch in industries. Currently, Malaysia is the world's biggest exporter of sago starch. Malaysia's exporting of sago starch annually is about 44 000 t, mainly in peninsular Malaysia, Japan, Singapore and other countries (Awg-Adeni *et al*., 2012).

Sago is a starch extracted in the spongy center or pith of various tropical palms stems. Sago which also known as *Metroxylon* sagu can be found in tropical lowland forest and freshwater swamps across Southeast Asia and New Guinea. The plant has the ability to thrive in the harsh swampy peat environment. Generally in the production of sago, several types of waste have been generated as a byproduct such as sago bark, sago hampas and sago wastewater (Awg-Adeni *et al*., 2012). Currently, the waste produced had been dumped into nearby river which then caused an increasing of the river pollution (Linggang *et al*., 2013). Thus, it has contributed to an environmental pollution. A study by Ozawa *et al*. (1996), waste such as sago hampas had contains 58% starch, 23% cellulose, 9.2% hemicellulose and 3.9% lignin. According to Awg-Adeni *et al*. (2012), sago hampas consists of a starchy lignocellulosic by-product which is generated from the pith after the starch extraction.

There are two types of the saccharification process that commonly being used in converting the biomass into a fermentable sugar. The process can be done either by using the enzyme (enzymatic saccharification) or by using an acid (acid saccharification) as a catalyst. An enzymatic saccharification is a process involves specific enzymes that facilitate the cleavage of bond in molecules in the presence of water (Broda *et al*., 1996). There are three major enzymes usually involves which are exoglucanase, endoglucanase and also β-glucosidase. During the hydrolysis process, a physical barrier of the plant (sago) cell wall is distrupted, thus, resulting the degrading of the biomass structure (Wyman, 1999). Nowadays, the production of fermentable sugar is started to be important as it is able to be further used for the production of biofuel, bio-based, bioenergy and chemical products in industries.

Therefore, the objective of this study is to optimize the conditions of enzymatic saccharification of a pretreated sago hampas as a substrate by using two-level factorial design in order to produce a fermentable sugar.

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