Faculty of Resources Science and Technology

Production of Xylitol From Sago Lignocellulosic Waste

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Production of Xylitol From Sago Lignocellulosic Waste

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Destarched sago fibre which contained lignocellulosic material can be exploited for the production of value-added product such as xylitol. Microbial xylitol production has received much attention because of its flexibility in term of adaptation, ease of operation and eco-friendliness. The aim of this study is to produce xylitol from sago lignocellulosic waste by focusing on the pre-treatment technique and fermentation parameters. Pre-treatment parameters such as dilute sulphuric acid concentration (0% (v/v) - 6% (v/v), solid to liquid ratio of hydrolysis system (5:100 – 40:100), hydrolysis process reaction time (30 – 90 minutes) using high-pressure steam pre-treatment, mild hydrothermal pre-treatment, and microwave pre-treatment were studied. The hydrolysate obtained from the selected pre-treatment was characterised and selected for fermentation. In order to obtained the highest xylitol production, fermentation parameters such as inoculum age (9, 18, 27 hours), inoculum concentration (10 – 30%), and effects of yeast extract and peptone were studied. The result revealed that pre-treatment using high pressure steam produced the highest xylose concentration with acid concentration of 2% v/v, reaction time of 30 minutes and solid to liquid ratio 30:100. The acid hydrolysis which was integrated with high pressure steam pre-treatment exhibits highest xylose (11.56 g/L) and glucose (22.51 g/L) production in which the temperature was set at 121°C and solid to liquid ratio of 30:100. The leftover hydrolysate from pre-treatment of high-pressure steam were then used for xylitol fermentation using *C. tropicalis*. The inoculum age of 28 hour with inoculum level of 20% was chosen as it consumes glucose and xylose efficiently in the hydrolysate without any supplementation and produces highest xylitol concentration of 0.86 ± 0.11 g/L compared with the control experiments containing commercial xylose and glucose which produced xylitol concentration of 2.56 ± 0.11 g/L. The fermentation of destarched sago fibre hydrolysate with addition of yeast extract significantly increases the consumption of glucose and xylose and produces maximum xylitol production which was 11.75 $\pm$ 0.09 g/L which was higher than the control experiment which produced 7.07 $\pm$ 0.06 g/L after 60 h of batch fermentation process. This work indicates that the optimal pre-treatment of destarched sago fibre and selected parameter of fermentation of *C. tropicalis* can influences xylitol production. The studies suggests that destarched sago fibre can be potentially served as an alternative raw material for xylitol production.

Keywords: *Candida tropicalis*, destarched sago fibre, fermentation, pre-treatment, xylitol

Pengeluaran Xylitol Dari Sisa Lignoselulosa Sagu

ABSTRAK

Serat sago yang mengandungi bahan lignocellulosa boleh digunakan untuk penghasilan produk bernilai tambah seperti xylitol. Penghasilan xylitol melalui mikrobial telah menerima banyak perhatian kerana fleksibiliti dalam hal penyesuaian, kemudahan pengendalian dan mesra alam. Tujuan kajian ini adalah untuk menghasilkan xylitol daripada sisa lignoselulosa sagu dengan memberi tumpuan kepada teknik pra-rawatan dan parameter penapaian. Parameter pra-perawatan seperti konsentrasi asid sulfur cair (0% (v/v) - 6% (V/v), perbandingan nisbah pepejal kepada cecair sistem hidrolisis (5:100 - 40:100), masa reaksi proses hidrolisa (30 - 90 minit) menggunakan pra-perawatan wap tekanan tinggi, pra-prawatan hidrotermal ringan, dan pra-perawatan gelombang mikro telah dikaji. Hidrolisat yang diperoleh daripada parameter pra-perawatan yang terpilih akan dijadikan sebagai fermentasi media. Untuk mendapatkan pengeluaran xylitol yang tertinggi, parameter fermentasi seperti umur inokulum (9, 18, 27 jam), kepekatan inoculum (10 – 30%), dan kesan ekstrak yis dan pepton telah dikaji. Hasil kajian mendedahkan bahawa pra-perawatan menggunakan wap tekanan tinggi menghasilkan kepekatan xylos tertinggi dengan konsentrasi asid sulfurik cair 2% v/v, masa tindak balas 30 minit dan perbandingan nisbah pepejal kepada cecair 30:100. Hidrolisis asid yang disepadukan dengan pra-perawatan wap tekanan tinggi menunjukkan pengeluaran xylos tertinggi (11.56 g / l) dan glukosa (22.51 g / L) di mana suhu ditetapkan pada 121 °C dan nisbah pepejal kepada cecair 30:100. Hidrolisat sisa daripada pra-perawatan wap tekanan tinggi kemudian digunakan untuk fermentasi penghasilan xylitol menggunakan <u>C. tropicalis</u>. *Umur inokulum 28 jam dengan kepekatan inokulum 20% telah dipilih kerana glukosa dan xylose dihabiskan secara berkesan dalam hidrolisat tanpa tambahan dan menghasilkan kepekatan* *xylitol tertinggi 0.86 ± 0.11 g/L berbanding dengan percubaan kawalan yang mengandungi xylos dan glukosa komersial yang menghasilkan konsentrasi xylitol 2.56 ± 0,11 g / L. Fermentasi hidrolisat serat sago yang dengan penambahan ekstrak yis secara signifikan meningkatkan pengambilan glukosa dan xylos dan menghasilkan pengeluaran maksimum xylitol yang adalah 11.75 ± 0.09 g / L yang lebih tinggi daripada percubaan kawalan yang menghasilkan 7.07± 0.06 g / l selepas 60 jam proses fermentasi tertutup. Kajian ini menunjukkan bahawa parameter pra-perawatan sisa sago tanpa kanji yang optimal dan parameter fermentasi* C. tropicalis *yang dipilih boleh mempengaruhi pengeluaran xylitol. Kajian ini menunjukkan bahawa serat sago tanpa kanji yang boleh diguna pakai sebagai bahan mentah alternatif untuk pengeluaran xylitol.*

Kata kunci: *Candida tropicalis, hampas sagu tanpa kanji, penapaian, pra-perawatan, xylitol*

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
C. tropicalis	Candida tropicalis
CBY	Commercial Bakers' Yeast
CG	Commercial Glucose
cm	Centimetre
DNS	Dinitrosalicyclic Acid
DSF	Destarched sago fibre
DSFH	Destarched sago fibre hydrolysate
g/L	Gram per litre
H_2SO_4	Sulphuric acid
PDA	Potato Dextrose Agar
PE	Peptone
Q_p	Product Volumetric Productivity
vvm	volume of liquid per minute
v/v	Volume per volume
YE	Yeast extract
Yp/s	Product yield based on substrate

CHAPTER 1

INTRODUCTION

1.1 Study Background

Xylitol is a sugar alcohol made from the sugar D - xylose. It is commonly utilised as a sweetener instead of aspartame, saccharin, and cyclamate, among others (Rafiqul et al., 2014). Xylitol usually used as substitute sugar as it is low in calories and can be digested by the body cell without insulin (Rafiqul et al., 2014).

Due to its anti-cariogenic properties and insulin-independent metabolism which is a type of metabolism that occurs without the use of insulin, it is being used as an ingredient in toothpaste and a substitute for sucrose (Ur-Rehman et al., 2015). In addition, the reports from 2004 and 2010 from the United States Department of energy stated that xylitol is the most demanded sugar as it has wide application in pharmaceutical and food industries (Ur-Rehman et al., 2015).

Current commercial xylitol manufacturing procedures, on the other hand, necessitate high temperatures (80 – 140 °C) and high pressures (up to 50 atm), as well as the toxic nickel catalyst, making the chemical process both costly and ecologically unfriendly. Therefore, biological approaches using microorganisms have been studied extensively. Microbial production using the fermentation process is attractive since it is more environmentally friendly and does not need high temperatures or the presence of catalyse (Arcaño et al., 2020).

Using agricultural waste, the production of xylitol produces an alternative to xylitol production via biotechnology rather than using a chemical route that requires extensive energy. Biomass resources from agricultural waste are found abundantly in Malaysia. It is reported by Agamuthu et al. (2009), that the waste accounted for more than 70 million tons annually. This waste continuously increases each year due to rapid development happening in Malaysia. Agricultural biomass such as wheat straw, rice straw, corn cob, and corn fibres are an example of lignocellulosic waste that contains a significant number of lignocellulosic fractions such as cellulose hemicellulose lignin. These lignocellulosic wastes are usually pre-treated to yield many valuable sugar products such as glucose and xylitol.

The sago palm plantation area in the Mukah division contributes one of the agricultural wastes in Malaysia, mainly in Sarawak. Sarawak, notably in the Mukah division, has the most sago palm plantation areas. Sago fibre, bark, and wastewater are all waste products of the sago business. Starch and lignocellulosic substances, such as lignin, cellulose, and hemicellulose, make up the majority of this waste (Awg-Adeni et al., 2010).Pre-treatment of lignocellulosic waste can produce several value-added products. It is one of the cost-effective downstream processes (Sahoo et al., 2018). Several pre-treatment approaches have been made to hydrolysed the lignocellulosic fraction. This fraction can further produce fermented sugar, which was glucose, mannose, xylose, xylitol, arabinose, acetic acid, glycerol, methanol, methane, butanol, furfural, hydroxymethylfurfural, 5-hydroxymethyl furfural, succinic acid, and many other products (Ghaffar et al.,2017). To date, the most efficient way to hydrolysed the hemicellulose-rich materials is by using acid hydrolysis. Acid hydrolysis can hydrolyse the hemicellulose fraction, leaving the cellulose residue and the lignin fraction unaltered (Fehér et al., 2017).

1.2 Problem Statement

The chemical hydrogenation of sugars like D–xylose in the industry to produce xylitol requires nickel catalyst, high temperature, and pressure conditions. The biotechnology approach of manufacturing xylitol by microorganisms has attracted a lot of attention because the chemical route needs a lot of money and energy (Arcaño et al., 2020; Rafiqul et al., 2014).

The usage of lignocellulosic waste materials has been abundantly studied for the potential use of its raw materials in xylitol production.

Several studies have been done by using different types of raw materials to produce xylose hydrolysate which subsequently used as fermentation media to produce xylitol. Pre-treatment of corncob by using 1% (w/w) of dilute sulphuric acid was able to produce 65.9 g/L of xylose concentration and other studies found pre-treatment by using the same acid concentration was able to produce higher xylose concentration which was 81.4 g/L of xylose (Cheng et al., 2009; Ping et al., 2013). The xylitol produced were 45.4 g/L and 38 g/L respectively (Cheng et al., 2009; Ping et al., 2013). Pre-treatment of oil palm empty fruit bunches by using 2% (w/v) of dilute sulphuric acid was able to produce 22.66 g/L of xylose concentration and produced 10.3 g/L of xylitol concentration through fermentation (Manjarres-pinzón et al., 2017). Also, a studies using 2% (w/v) exhausted olive pomace was able to produce xylose concentration of 32.59 g/L and produced 5.17 g/L of xylitol though fermentation (López – Linares et al., 2020). Different concentrations of dilute sulphuric acid and different solid to liquid ratio were employed in these studies and it was found that these pre-treatment parameters produced different xylose concentration by using different raw materials. This showed that pre-treatment parameters such as concentration of dilute sulphuric acid and solid to liquid ratio can influence the production of the xylose sugar. However, other pre-treatment parameters such as different ranges of concentrations of dilute sulphuric acid, hydrolysis time, solid to liquid ratio has not been evaluated by using sago lignocellulosic waste. Also, fermentation parameters to produce xylitol such as inoculum age, inoculum concentration and effects of nitrogen sources were not studied in the previous research. Therefore, this study aimed to determine the suitable parameters (dilute sulphuric acid concentration, solid to liquid ratio of hydrolysis system, hydrolysis process reaction time) on xylose recovery from sago waste lignocellulosic material using high-pressure steam pre-treatment, mild hydrothermal pre-treatment, and microwave pre-treatment.

In Sarawak, the sago palm plantation land in the Mukah division contributes one type of agricultural waste. This state, most notably the Mukah division, contains the largest area of sago palm plantation. Sago fibre, bark and wastewater are all waste products of the sago business. Starch and lignocellulosic substances, such as lignin, cellulose, and hemicellulose, comprise most of this waste. However, the literature shows that limited studies have attempted to use sago fibre to produce xylose which subsequently converted into xylitol by using fermentation. It was reported sago fibre has been utilised by focusing on the production of fermentable sugars such as glucose to produce ethanol (Alias et al., 2021; Mohammad et al., 2020). Hence, the idea was using the leftover sago hampas known as destarched hampas that has been used for bioethanol utilization and emphasising on utilising leftover destarched sago and characterise sugars obtained in the hydrolysate of destarched sago fibre hydrolysate material upon completing the selected pre-treatment process. After that, this study aimed to determine the feasibility of producing xylitol from xylose using destarched sago hydrolysate through a batch fermentation process using *C. tropicalis* with selected fermentation parameters (inoculum age, inoculum concentration , and effects of yeast extract and peptone). The usage of agricultural waste is a promising renewable alternative to produce xylitol due to the abundant resources and low-price sago biomass as an alternative feedstock for xylitol production.

1.3 Rationale of the study

The significance of the study is that the leftover destarched sago waste lignocellulosic compound can be used in production of a value-added product which is xylitol which will consequently help to fulfil the increasing demand for xylitol. This project emphasises the pre-treatment of the destarched sago waste subsequently the production of xylitol from the fermentation of the destarched sago hydrolysate.

Several dilute sulphuric acid pre-treatments, such as the type of physicochemical pre-treatment, the concentration of dilute sulphuric acid, reaction time, and solid to liquid load concentration, were studied. In addition, the effect of inoculum concentration on fermentation, as well as the effect of yeast extract and peptone sources during fermentation, needed to be investigated in order to select less severe conditions that maximise xylose yield while minimising by-product formation from the dilute sulphuric acid pre-treatment. The fermentation parameter was also studied to produce the highest xylitol production, such as inoculum age, concentration, yeast extract, and peptone effects on xylitol production.

1.4 Objective

This research aims to study the feasibility of converting destarched sago fibre to xylitol by *C. tropicalis.* The specific objectives this study was:

i) To determine the suitable parameters (dilute sulphuric acid concentration, solid to liquid ratio of hydrolysis system, hydrolysis process reaction time) on xylose recovery from sago waste lignocellulosic material using high-pressure steam pre-treatment, mild hydrothermal pre-treatment, and microwave pre-treatment.

ii) To characterise sugars obtained in the hydrolysate of destarched sago fibre hydrolysate material upon completing the selected pre-treatment process.

To study the feasibility of producing xylitol from xylose using destarched sago hydrolysate through a batch fermentation process using *C. tropicalis* with selected fermentation parameters (inoculum age, inoculum concentration , and effects of yeast extract and peptone).

CHAPTER 2

LITERATURE REVIEW

2.1 Xylitol

Xylitol is a natural sugar alcohol made up of five carbon sugars that can be found in fruits and vegetables (Arcaño et al., 2020). Xylitol is similar to saccharose in terms of sweetness. In contrast with saccharose, xylitol has a third fewer calories. Diabetic people utilise xylitol as a sugar substitute because of its sweetness (Ghosh & Sudha, 2012). Another advantage of xylitol is that its two main absorption mechanisms in the liver and gut flora are insulin-independent. Because of the permeability of liver cells and the large number of enzymes present, xylitol can be swiftly digested and converted into energy by liver cells (Mohamad et al., 2015). All D-glucose produced by xylitol metabolism is stored as glycogen in the liver before being progressively released and the intestine absorbs nutrients at a slower rate than the liver. As a result, the blood sugar content will not rise suddenly which usually caused by sucrose and glucose. Due to this reason, xylitol is the greatest option for diabetics looking for a sweetener alternative. Not only that, xylitol is widely used as a sweetener for restricted diet as it has satiety effects, a low glycaemic response and a better nutritional profile when consumed (Kresnowati et al., 2015; Mohamad et al., 2015; Zhang et al., 2015).

Since the 1970s, the use of xylitol in oral health has been researched and recognised and it was revealed that were about 85 % reduction of dental plague in adults when substituting sucrose for xylitol (Runnel et al., 2013). The cariogenic bacteria are unable to use xylitol as an energy source or a substrate to make toxic compounds like acid which can cause the dental plague. This makes xylitol has the properties to prevent cavity by formation of the carriers which is less fermentable when compared with other polyols. Therefore,