Faculty of Resource Science and Technology

RECOVERY OF XYLOSE FROM SAGO HAMPAS VIA DILUTE SULPHURIC ACID PRETREATMENT : EFFECTS OF HYDROLYSIS REACTION TIME AND TEMPERATURE

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Bachelor of Science with Honours
(Resources Biotechnology Programme)
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This project is submitted in partial fulfillment of the requirement for degree of
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ABSTRACT

Metroxylan sagu (Sago) produced sago hampas as a by-product after starch extraction and are abundantly found as an industrial waste. On dry basis, sago hampas extraction consist lignocellulosic materials of 58% of starch, 23% of cellulose, 9.3% of hemicellulose which eventually contains glucose and xylose and 4% of lignin, approximately. This study focused on how 2.5% (v/v) of dilute sulphuric acid (H_2SO_4) pre-treatment with studied parameters of hydrolysis time and temperature affected the xylose concentration produced from sago hampas. Xylose which can be further fermented to produce xylitol which can be used in many commercial applications in different sectors of food and dental related products. Liquefaction and saccharification enzymes, termamyl 0.5µL/g and dextrozyme 0.6µL/g are added to help in enzyme hydrolysis process of sago hampas. In this experiment, the result of xylose production showed an increase as the parameters increase but to certain point the xylose production decrease. The best xylose production are produce at 60 minutes of hydrolysis time with 9.14% of xylose recovery and at 120°C of temperature with 9.14% of xylose recovery. From the present of study, it can be concluded that hydrolysis time and temperature plays great role in inducing the xylose production from sago hampas.

Key words: dilute sulphuric acid pre-treatment, sago hampas, enzyme hydrolysis, xylose

ABSTRAK

Metroxylan sagu (Sagu) menghasilkan hampas sagu sebagai produk sampingan selepas proses pengeluaran kanji dan banyak didapati sebagai bahan buangan industry. Hampas sagu mengandungi bahan lignoselulosa yang terdiri daripada 58% kanji, 23% selulosa, 9.2% hemiselulosa yang mana mengandungi glukosa dan xilosa, dan 4% lignin secara amnya. Kajian ini memfokuskan tentang bagaimana proses prarawatan 2.5% (w/v) asid sulfuric cair dengan objektif kajian iaitu masa hidrolisis dan suhu memberi kesan kepada pehgasilan xilosa daripada hampas sagu. Xilosa boleh digunakan dalam proses penapaian untuk menghasilkan xilatol yang boleh digunakan dalam pelbagai aplikasi komersial dalam pelbagai sektor makanan dan produk berkaitan pergigian. Enzim pencairan dan sakarafikasi, termamyl 0.5µL/g dan dextrozyme 0.6µL/g digunakan untuk membantu dalam proses enzim hidrolisis untuk hampas sagu. Kajian ini menunjukkan peningkatan kepada penghasilan xilosa apabila parameter yang dikaji meningkat tetapi selepas itu menurun apabila mencapai sesuatu tahap. Kadar xilaso yang dihasilkan terbaik adalah pada minit masa hidrolisis 60 minit dengan catatan 9.14% penmbahbaikan xilosa dan pada suhu 120 °C. Melalui kajian ini, konklusinya, masa hidrolisis dan suhu memainkan peranan dalam penghasilan xilosa daripada hampas sagu.

***Kata kunci**: prarawatan asid sulfurik cair, hampas sagu, hidrolisis enzim, xilosa*

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List of Abbreviations

SH	Sago Hampas
SHH	Sago Hampas Hydrolytes
HPLC	High Performance Liquid Chromatography
DSA	Dilute Sulphuric Acid
g/L	gram per liter
min	minutes
(v/v)	(volume/volume)
(w/v)	(weight/volume)

CHAPTER 1

Introduction

Early in the last century, sugars was the bane of human existence, and pioneers in the area of health and longevity almost universally agreed the necessity of removing it from the diet. To overcome this problem, the industry has gained enormous demand on making low-cost xylitol from renewable stock and to be used as one of the alternative sweeteners. Nowadays, sugar free food are very much popular as people now are aware of the effect of high calorie sugar towards one's health such as obesity. A study show a prepondance of attempting to lose and maintain weight was 28.8% and 35.1% among men and 43.6% and 34.4% among women, respectively (Serdula et al., 1999). Xylitol is a natural sweetener that commonly found in fruits, berries, vegetables, plants and trees. During normal metabolism the human body even produces a few grams of xylitol each day.

Xylitol has application and potential for food and pharmaceutical industries. Globally, xylitol is known for the ability to avoid dental carries in young children and mothers as it decreases mutans streptococci levels in plaque and saliva (Ly et al. 2008) and can be helpful to the diabetic patients (Natah, et al., 1997) because it is low calorie sugar and can reduce blood glucose, triglyceride, and cholesterol level with 40% less energy. More examples on the type of low calorie sweeteners would be aspartame, tagatose and sucralose (Chattopadhyay et al., 2014). The preparation cost of xylitol is high which affect these applications that are supposed to be beneficial towards human being. The production of xylitol will include the hydrolysis which then will breaks the cellulose and the hemicellulose polymers to fermentable sugars, mainly xylose which then will be proceeded by the fermentation process that will converts the sugars to xylitol (Kamal et al., 2011).

Sago palm are commercially grown in Sarawak especially in the Mukah where most of saho hampas are produced from the sago starch processing industries. During the processing of sago starch, three major component of by-product were generated which are the sago trunk, sago hampas which is also known as fibrous pitch residue and wastewater where they are all can be readily available for exploitation as there are abundantly found (Awg-Adeni et. al., 2013). It was estimated that there are about 7 tons of sago hampas produced from daily single starch processing mill being washed off into the nearby stream (Awg- Adeni et al., 2013).

This will surely cause a serious issues towards the environment as it has high chemical demand (COD) and biological demand (BOD). Apart from that, sago hampas residue can cause pollution to our clean water supply.

The most commonly organisms used to extract starches are corn, wheat, rice, potatoes and legumes while sago is the only example of commercial starch that can be derived from other part of the tree. Sago palms are the species of the genus Metroxylon belonging to the Palmae family. On dry basis, sago hampas extraction consist lignocellulosic materials of 58% of starch, 23% of cellulose, 9.3% of hemicellulose which eventually contains xylose and 4% of lignin, approximately (Awg-adeni et al., 2013). Other hemicellulosic sugars also consist of arabinose, galactise, mannose and glucose besides xylose (Walther, Hensirisak, & Agblevor, 2001). Lignin is a complex 3- 2 dimensional polyaromatic matrix that forms a seal around cellulose micro fibrils and exhibits limited covalent associated with hemicelluloses which limiting the effectiveness of direct hydrolysis of the sago hampas as enzymes cannot directly produce a reaction with the substrate. So this is where the importance of pretreatment is needed as it can alter the structure of the hemicellulosic biomass to make it more accessible by the enzyme to produce required fermentable sugar.

In recent years, people have gained more awareness on the importance of more efficient utilization of agro-industrial by-products into a value added products and in this case of study, it is on how to extract xylose which then will eventually converted to xylitol. Unfortunately, the production cost is high and it is harmful to the environment thus proper pretreatment method need to be apply to improve the efficiency of the whole process. Dilute sulphuric acid pretreatment will be used as it is one of the key to the utilization of hemicellulose from sago lignocellulosic waste (Butto et al., 2017). Dilute acid pretreatment has the advantages of solubilizing the hemicellulose and convert the solubilized hemicellulose to fermentable sugars which will eventually reduce the use of hemicellulose enzymes and can result in higher xylose yields (Butto et al., 2017). Current commercial requires very high temperature in the range of 80 - 140°C to initiate xylose production and high-pressure condition up to 50 atm (Hong et al., 2016).

The main objectives of this study is to produce xylose via dilute sulphuric acid pretreatment using sago hampas as a substrate. The specific objectives of this research were

1. To determine the xylose production at various hydrolysis reaction time.

2. To analyze the xylose production at various hydrolysis temperature.

Chapter 2

Literature review

2.1 *Metroxylon Sago* Palm

Sago palm is also known by its scientific name of *Metroxylon sago* are in the Palmae family. Sago palm has been acknowlegde as a starch crop of the 21st century for its ability to thrive in most soil condition without the need of pesticide and herbicide (Singhal et al., 2008). Numerous efforts have been done to produce and develop cultivation on sago palm and some of the commercial value production of sago palm can be found around Singapore, in West Malaysia, and in the Riau archipelago, Indonesia (Flach, 1997). In Malaysia, sago starch has been commercially used in the production of glucose (Abd-Aziz, 2002). The major contained in the pith of sago palm is mainly starch, which the components need to be separated from the cellulosic materials by undergoing certain processing such as enzyme and acid hydrolysis in a way to extract the best sago starch by quality and quantity (Awg-Adeni, et al., 2013). Sago palm are known for its advantages of being a crop that is economically acceptable, relatively sustainable, environmentally friendly, uniquely versatile and it also promotes socially stable agroforestry systems (Flach, 1997).

2.2 Sago Hampas

Sago hampas is a by-product after the starch extraction from sago (*Metroxylan sagu*). In Sarawak, especially in Sibu and Mukah Division, about 50 - 110 t of sago hampas are produced daily as an industrial waste from a starch processing mill and these waste are washed off into the nearby river stream that will lead to serious environmental issues due to the rich substrate content (Awg-Adeni et al., 2013). This situation will cause various environmental pollution such as chemical oxygen demand (COD), and biological oxygen demand (BOD) as SH waste water contained high starch and lignocellulosis materials. This might as well cause residual to be embedded to the bottom of the river and reduce the overall river water quality, as population grows, the size of towns and cities also grows. On dry basis, sago hampas extraction consist lignocellulosic materials of 58% of starch, 23%

of cellulose, 9.3% of hemicellulose which eventually consist xylose and 4% of lignin, approximately (Awg-Adeni et al., 2013).

2.3 Xylitol in Industry

Early in the last century, sugars was the bane of human existence, and pioneers in the area of health and longevity almost universally agreed the necessity of removing it from the diet. The interest in xylitol has increased due to many commercial applications in different sectors such as food, dental related products and pharmaceuticals but the chemical production cost of producing xylitol by itself are considered high (Ravella et al., 2012) Therefore, much efforts has been dedicated to the development of a cost-effective and environmentally-friendly biotechnological method process in evaluating a cheaper lignocelulosic substrates.

Xylitol which can be derived from D-xylose, is a five carbon sugar that have lower calorie but has the same high sweetening power, requires high temperature approximately, 80 - 140 °C and high pressure condition up to 50 atm (Hong et al., 2016). These parameters needed to produce xylitol makes the production cost to kick in and affect the applications. The process of obtaining xylose will be needing the hydrolysis of cellulose and hemicellulose polymers into fermentable sugars and will be needing a fermentation process to convert xylose into pure xylitol (Kamal et al., 2011). The use of sago hampas substrate in this study will provide an abundant raw materials that can actually help in reducing the cost of producing the valuable bio-product, xylitol.

2.4 Dilute Sulphuric Acid Pretreatment of Lignocellulosic Compound

In order to increase xylitol production rate during the submerged fermentation process, substrate load, pH, reaction time, and temperature will be optimized. The maximum specific growth rate and the yield coefficient of biomass on initial xylose concentration of 30 g/L occurred at pH 5.5 but the best productive performances in xylitol will be at the pH of 2.5 (Tamburini et al., 2015) which is too much acidic content and will definitely affect the environment. In order to able to extract the maximum amount of xylose from sago hampas, the sago hampas hydrolytes particles will be treated with 0.5~4.5% (v/v) dilute sulphuric acid solutions with the reaction time of 0.5~1.5 hours (Hong et al., 2016). The slurry of SHH are to be filtered to separate the solid contains of cellulose and lignin from the liquid portion where it primarily contained hemicellulose (Kamal et al., 2011). DSA pretreatment is an effective commercialize method that can be used to improve the conversion of lignocellulose to xylose which normally performed at approximately 180 - 200 °C (Hong et al., 2016).

Chapter 3

Materials and methodology

3.1 Materials

3.1.1 Sago Hampas

The sago hampas is obtained at Pusa, Sarawak in October 2017, separated hampas is then packed into porous bag, promptly processed at Biochemistry Laboratory, Universiti Malaysia Sarawak. The climate area in this area is tropical with a significant rainfall with the average 3900 mm per year. The average annual temperature is 28°C.

3.1.2 Dilute Sulphuric Acid

Dilute sulphuric acid used in this case study is 2.5% (v/v). Acidic condition promotes solubilization of hemicellulose leaving the lignin mostly insoluble.

3.1.3 Enzymes

The saccharification enzyme used in this study is dextrozyme (0.6µl/g). Next, (0.5µl/g) termamyl is used in this study to liquefy the starch in the process of liquefaction.

3.1.4 Incubator

The incubator is used for the destarch of sago hampas. The temperature is maintained at 60 °C and 100 rpm.

3.1.5 Drying oven

The drying oven is used to dry the raw sago hampas and sago hampas hydrolysate before proceeding to the destarch process.

3.1.6 Centrifuge

The centrifuge machine is used to separate the solid residue from the liquid residue of sago hampas hydrolysate at 100 rpm for 5 minutes.

3.2 Methodology

3.2.1 Sample Collection and Raw Material Preparation

The sago hampas is collected from Pusa, Sarawak in October 2017. The climate area in this area is tropical with a significant rainfall with the average 3900 mm per year. The average annual temperature is 28 °C. The sago hampas are left to dry for a few days to ensure the mass of the SH will not be affected by the moisture content. The sago hampas is then grinded and sieved. The samples were filtered by using a filter to make sure the samples were small enough to enhance the reaction of the samples and the enzyme in the enzymatic hydrolysis.

Figure 1 : The sago hampas were grinded after the process of drying.

Figure 2 : Before and after grinding of sago hampas

Figure 3 : The samples were filtered by using a filter

3.2.2 Destarch of Sago Hampas

70 g of samples were boiled in 1 litre distilled water for 30 minutes at 100 °C. Liquefaction enzyme is then added into the mixture after the boling process of 30 minutes. In this case of study, termamyl 0.5 µl/g is added into the mixture once the temperature has cooled down to 90 °C because that will be the optimum temperature required for termamyl to function efficiently. This process required constantly stirring for 2 hours to make sure it is mix well and to maximize the liquefaction of starch from sago hampas. The mixture is then left to cool down until 60 °C and dextrozyme 0.6 µl/g is then added in the mixture. Then, they were left in the incubator shaker overnight (24 hours) at 100 rpm, 60 °C. This process is called sacharification that will turn starch in sago hampas into glucose. This method were obtained by the study conducted by Awg – Adeni, 2013.

Figure 4 : The destarched process through Enzymatic Hydrolysis of Sago Hampas

3.2.3 Filtration

The mixture that has been through enzymatic hydrolysis were filtered to separate the solid and liquid matter by using marcellin cloth. In this case study, the solid matter of the mixture is used as it contains hemicellulose, cellulose and lignin. The liquid matter will be used to produce bioethanol or lactic acid for other purpose of study and this will gives benefits to the environment as there will be no waste produce in this study as the waste will be fully utilized. The solid part of the mixture were then dried in the oven at 70°C for 24 hours. The samples were then grinded into smaller particles for the acid hydrolysis process. They were kept in a sealed container for acid hydrolysis. The samples has undergoes the destarch process where the starch from the sago hampas has been removed.

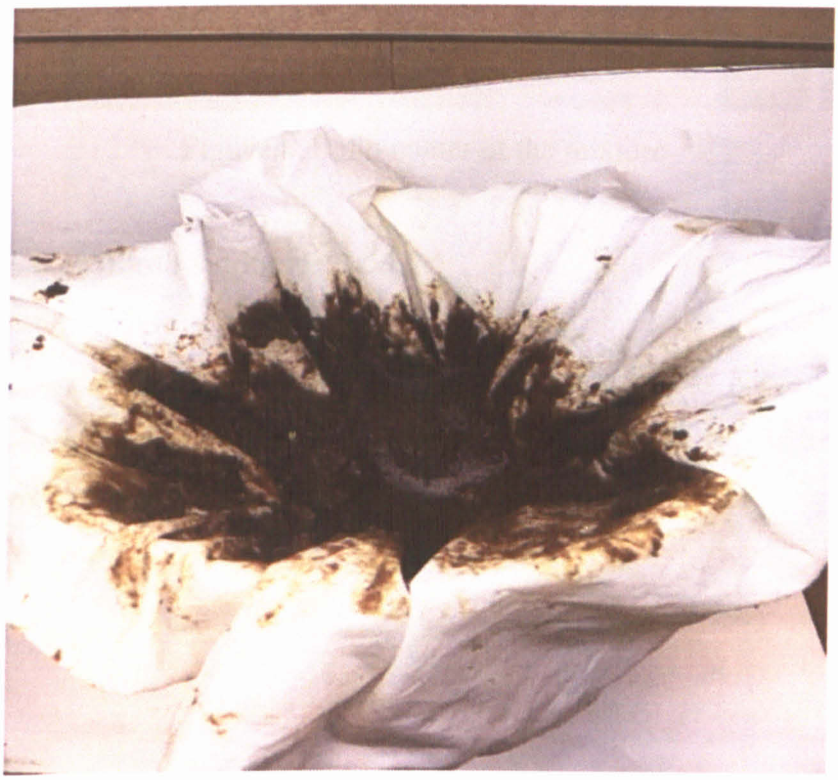

Figure 5 : The mixture was filtered by using Muslin cloth

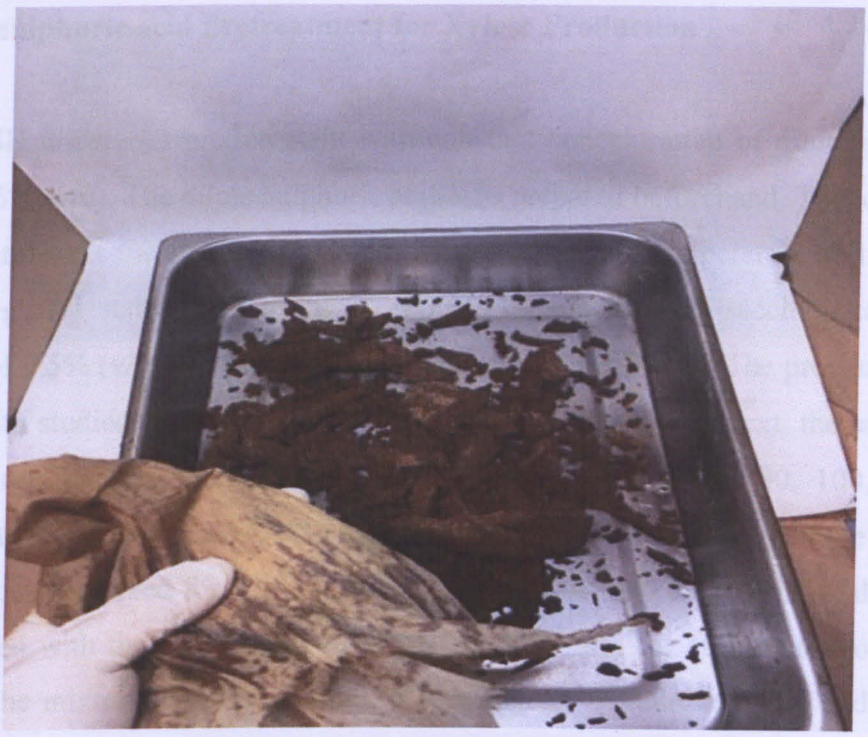

Figure 6: Solid matter of the mixture

3.2.4 Preparation of Dilute Sulphuric Acid

Dilute sulphuric acid of 2.5% (v/v) is the constant variable that is needed to perform the pretreatment of SH. 25 ml of concentrated sulphuric acid (H_2SO_4) was added into 1 litre of distilled water to produce 2.5% (v/v) of dilute sulphuric acid.