



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 ± 0.09 g/L which was higher than the control experiment which produced 7.07 ± 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 mengandung 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 sago 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-perawatan 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 inokulum (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 C. tropicalis. 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 sago tanpa kanji, penapaian, pra-perawatan, xylitol

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
<i>C. tropicalis</i>	<i>Candida tropicalis</i>
CBY	Commercial Bakers' Yeast
CG	Commercial Glucose
cm	Centimetre
DNS	Dinitrosalicylic Acid
DSF	Destarched sago fibre
DSFH	Destarched sago fibre hydrolysate
g/L	Gram per litre
H ₂ SO 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 hydrolyse 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 hydrolyse 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 there were about 85 % reduction of dental plaque 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 plaque. This makes xylitol has the properties to prevent cavity by formation of the carriers which is less fermentable when compared with other polyols. Therefore,

xylitol is often being used in toothpaste formulation as it can help to decrease the bacterial plaques mainly caused by *Streptococcus mutans* (Ur- Rehman et al., 2015; Mohamad et al., 2015; Runnel et al., 2013). Furthermore, xylitol was frequently combined with preventive substances such as fluoride in the formulation of dental products since it has a synergistic impact in the prevention of caries (Mohamad et al., 2015).

Not only that, xylitol can be commonly found in sugar-free chewing gum because it is very convenient. It has organoleptic properties, good solubility, and controllable crystallization. These properties are beneficial in the coating and give dental benefits (Zachari., 2012; Roberto et al., 2003). Also, xylitol is widely used in the confectionery range as a sweetener. For example, in candies, jellies, chocolate, ice-cream, yogurt, and gelatins (Zacharies et al., 2012; Roberto et al., 2003). In 1983, the World Health Organization (WHO) and Agriculture Organization of the United Nations (FAO) carried out an inclusive evaluation of the toxicological safety data for xylitol through the Joint Committee of Experts on Food Additives (JECFA). According to the findings, xylitol has a very low toxicity and has no negative health consequences. It is classed as a GRAS type additive, or better known as Generally Recognized Safe, it is allowed as a food additive in Europe, Japan, and Canada.

Xylitol is also listed in the Food and Drug Administration's Inactive Ingredient Guide for oral solution, chewing gum, and the list of non-parenteral medications licenced in the United Kingdom (Zacharies et al., 2012; Musatto & Roberto., 2003). Another benefit of xylitol is that it has a prebiotic effect. Xylitol can decrease the faecal pH, thus increasing the amount of gram-positive flora in the faeces. Nevertheless, overconsumption of xylitol will produce gastrointestinal adverse effects due to its low digestion and absorption in the small intestine whereby only 25 % to 50 % of ingested xylitol is absorbed (Zacharies et al., 2012).

2.1.1 Xylitol Industry and Global Market

Daniso (DuPont), Futaste Pharmaceutical, Yucheng Luijan, and Hangzhou Shouxing are the main xylitol manufacturers (Zihare & Blumberga, 2020). The resources used lignocellulosic waste as feedstock and production of xylitol strongly affected the price of the xylitol (Zihare & Blumberga, 2020). It is estimated 6 EUR/kg for wood-derived xylitol (Rafiqul et al., 2012) and it is approximately only 2.5 EUR/kg for corn-derived xylitol (Muizniece et al., 2017).

As shown in Figure 2.1, xylitol demand has steadily increased by 6% annually, mainly in the food markets, as a result of multiple advantages (Dasgupta et al., 2017). The rising demand has been driven by two main factors: health concerns, which have speed up the growth of the alternative sweetener business, and increased awareness of naturally produced low-calorie sugar-free chemicals (Dasgupta et al., 2017). Xylitol consumption is highly recommended for people with diabetes or in need of low-calorie diets. Many foods and pharmaceutical products have incorporated xylitol into their formulations (Mohamad et al., 2015). In addition, it is also used in cosmetic applications due to its emollient and humectant properties. It can be topical use as xylitol has bacteriostatic and bactericidal properties. Not only that, it is also being used as the coating for tablets in order to enhance the stability of the product. Furthermore, sugar-free chewing gums, candies, mints, diabetes-friendly foods, and oral-care items all include xylitol which subsequently, there is increasing demand of xylitol usage in these products (Arcaño et al., 2020).

Based on Figure 2.1, the global xylitol market was predicted to be worth of US\$725.9 million, with 190.9 thousand metric tonnes produced. Based on Figure 2.1, the forecast for 2022 is 266.5 thousand metric tons, with an amount above US\$1 Billion (<https://www.grandviewresearch.com/industry-analysis/xylitol-market>).

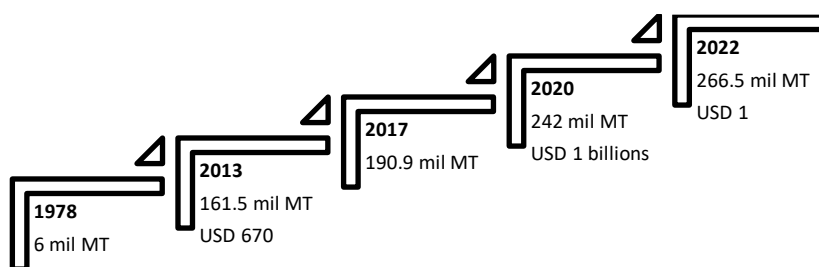


Figure 2.1: Global xylitol market, MT:metric tons.

2.1.2 Industrial Production of Xylitol

At an industrial level, the production of xylitol from chemical hydrogenation of xylose involves four fundamental stages. The first stages involve acid hydrolysis to obtain the xylose; Next stage is purification of the hydrolysate; the third stage is the catalytic hydrogenation of xylose; the fourth stage the purification of the xylitol. The third and fourth stages, which are hydrogenation and purification, involves a high energy consumption because the usage of pressure for the hydrogenation process of xylose required high temperature, and pressure subsequently lead to the high price of the final product (Zhang et al., 2019; Rafiqul & Mimi Sakinah, 2012).

The significant energy used during the separation and purification processes contributes to the high pricing of the final product which is xylitol. The high sensitivity of the catalyst is required to raise production costs, and its products can be up to ten times more expensive than sucrose to sorbitol conversion (Peng et al., 2012). The separation of the by-product resulting from the reduction reaction is necessary for absolute purity of xylitol, and there is a need to create effective and selective purification procedures (Arcaño et al., 2020).

Based on Figure 2.2, the lignocellulosic material, which is rich in xylan, is decomposed into monomeric sugar, which dissolves in the reaction medium. The hydrolysate is then obtained, which is a soluble liquid fraction including mainly consisted of xylose, glucose,

and related sugar such as arabinose. The proportions of sugar depending on the reaction conditions and the type of biomass (Gírio et al., 2012; Arcaño et al., 2020). The lignin precipitates, together with the cellulose, contained most of the compounds of the solid fraction. Hemicellulose is easily hydrolysable due to its amorphous and branching nature.

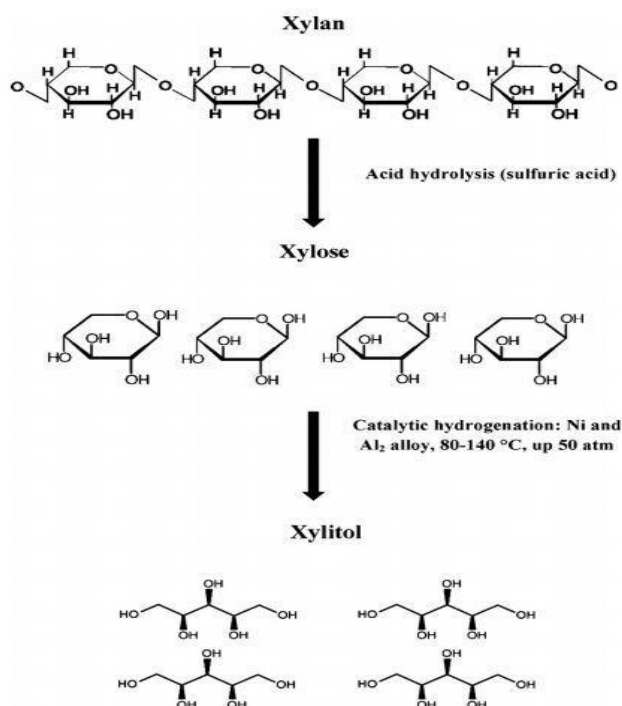


Figure 2.2: The stages of process using chemical way to obtain xylitol (Arcaño et al., 2020).

It is feasible to recover up to 95% of the sugar content (Gírio et al., 2012). Due to its high degree of polymerization and minimal accessibility to surface area, cellulose does not breakdown using this approach (Sun et al., 2014). Before pure xylitol is obtained, the xylose undergoes xylose hydrogenation. This method necessitates the use of a catalyst, as well as a high temperature ranging from 79.85 °C to 139.85 °C and hydrogen pressures of up to 725.189 psi. Other sugars present in this aqueous solution except xylose will all be hydrogenated to their respective polyols (Vallejos et al., 2016). Then, the hydrogenated solutions will undergo several stages which are purification, concentration, and

crystallization of xylitol. After that, the hydrolysate undergoes xylose purification. This step is required to decrease the undesirable by-products such as phenolics (Vallejos et al., 2016). The separation of the solid fraction is created from the hydrolysate in crystallization. The crystallization process can occur by precipitation, evaporation, or cooling and this depending on the solubility and temperature (Girio et al., 2012).

By comparison, the traditional method of xylose extraction is conducted using diluted sulphuric acid and high temperature (Girio et al., 2012). The disadvantage of this method is that it simultaneously appears to cause unwanted side reactions. The unwanted product releases are grouped into three. First, by products released from hemicellulose structure, such as acetic acid; second, sugar degradation products, such as furfural and hydroxymethylfurfural (HMF); and third, lignin degradation products, such as aromatic and polyaromatic compounds (Fehér et al., 2017). The pre-treatment parameters, include temperature, pressure, reaction time, acid concentration, and solid to liquid ratio, should be chosen to maximize the high production concentration and reduce unwanted products' yield (Pensri et al., 2016; Rafiqul & Sakinah, 2013).

The temperature and pressure needed is high and employs high-cost equipment to achieve conditions needed for the process (Clauser et al., 2018; Koutinas et al., 2014; Franceschin et al., 2011; Nigam & Singh, 1995). Not only that, but several steps of purification also needed to make the process of obtaining xylitol expensive (Clauser et al., 2018; Nigam & Singh, 1995). Therefore, a new method to produce xylitol is studied to be as effective as many traditional techniques (Ping et al., 2013). Currently, the main international supplier of xylitol from China, known as Danisco or name as industry of Dupont Company, produces xylose using catalysis from hardwood sources (Franceschin et al., 2011).

2.1.3 Biotechnological Production of Xylitol

Xylitol is produced from waste lignocellulosic waste by using biotechnological processes such as hydrolysis of lignocellulosic biomass which produces sugars. The primary type of sugar production obtained from hydrolysis are glucose and xylose, together with other types of sugar such as galactose, mannose, and arabinose (Arcaño et al., 2018; Gírio et al., 2012). These sugars have broad applications in both the pharmaceutical and food industry (Sasaki et al., 2012). Xylose, the second most common sugar found in lignocellulosic waste, plays a significant economic role as it is often being employed in the biotechnological process to convert the xylose into xylitol (De Albuquerque et al., 2014). Therefore, methods to produce xylose from lignocellulosic waste have been extensively developing and optimised to convert the xylose into xylitol, a higher added product (De Albuquerque et al., 2014).

Figure 2.3 shows a simplified process for the bioproduction of lignocellulosic materials to produce xylitol. The usage of lignocellulosic waste materials has been abundantly studied for the potential use of its raw materials in xylitol production. Based on the previous studies by Rao et al. (2016), biomass waste such as corn fibre and sugar bagasse can be used as raw materials to produce fermentable sugars. It is also known that these lignocellulosic materials needed to undergo several pre-treatments to enhance sugar production.

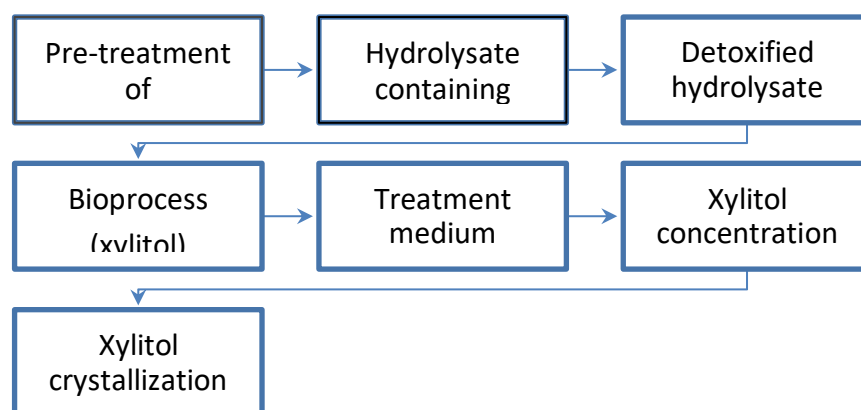


Figure 2.3: The overall bioproduction of xylitol using lignocellulosic materials (D Albuquerque et al., 2014).

A study conducted by Eryasar & Karasu. (2016), found that biomass waste such as wheat bran, potato peels could be used as raw materials for microbial xylitol synthesis by yeasts. The experiment conducted using acid hydrolysis found the highest xylose yield for 10 g/100 mL solid/liquid ratio yielded the highest xylose from the chestnut shell, which was 8.33 g/L, followed by the potato peel, which is 4.67 g/L and 0.89 g/L and 2.34 g/L for wheat bran and barley brain respectively. Then, hydrolysate is further fermented by the *C. tropicalis* into xylitol, which is a valuable product. 83.28 g/L and 54.07 g/L xylitol production from 100 g/L xylose (Eryasar & Karasu., 2016). To date, there was no study that has been conducted using destarched sago fibre as the lignocellulosic waste to produce xylitol.

However, the acid hydrolysis of the lignocellulosic waste generates many compounds that can inhibit the fermentation process. These inhibitors developed mainly are hydroxymethylfurfural, furfuralaliphatic acids, phenolic compounds, and vanillin (Sousa et al., 2015; Vajzovic et al., 2012; Zhang et al., 2012). These unwanted by-products from hydrolysates can decrease the consumption of the carbon source and inhibit the fermentation process from happening (Vajzovic et al., 2012). Due to these inhibitors, several purification methods have been reported to be effective in reducing the inhibitors (Kamal et al., 2011).

Furthermore, other factors that affect the production of the xylitol also has been studied such as usage of the enzyme and the pH of the fermentation media (Umai et al., 2022). In this study, the fermentation parameter such as inoculum age, the inoculum concentration and the effects of the xylitol production has been studied.

2.2 Physical Pre-Treatment of Lignocellulosic Waste

Physical pre-treatment methods such as chipping, grinding, freezing, and radiation were examples of pre-treatment used to pre-treat the lignocellulosic waste. This method helps to maximise the surface area of biomass materials. Usually, these physical pre-treatments were often combined with other pre-treatments methods. Sirohi et al. (2020) reported that acid hydrolysis using corn cob with different particle size with 0.2 and 0.6 mm yields 9.0 g/ml, and 4.2 mg/ml of xylose, respectively. The physical structure of the feed, such as surface area, plays an essential role in the yield of xylose (Sirohi et al., 2020).

2.2.1 High Pressure Steam Pre-treatment in An Autoclave

Pre-treatment using high pressure steam in an autoclave is commonly employed as pre-treatment of several lignocellulosic waste materials. This pre-treatment involved high temperature and steam to solubilize the hemicellulose fraction and transformation of lignin and subsequently helps the lignocellulosic waste to be accessible to either acid or cellulolytic enzymes (Debiagi et al., 2020; Sun et al., 2014). Debiagi et al. (2020) reported the pre-treatment of oat hulk and wheat bran using high pressure steam with 1% of sulphuric acid can effectively releases most sugars and produces 2.7 g/L and 24 g/L of xylose respectively. The cellulose-hemicellulose-lignin complex can be broken down by high-pressure steam pre-treatment carried out in an autoclave (Debiagi et al., 2020).

2.2.2 Mild Thermal Pre-treatment

Mild-thermal pre-treatment has been reported to be the most viable procedure in term of energy efficiency, operational expenses, economic and environmental sustainability (Crasta et al., 2021). Dilute acid with hydrothermal pre-treatment conducted at relatively low temperatures can be employed for optimal utilization of the xylose released from the hemicellulose (Pedersen et al., 2011). Pre-treatment of the wheat straw which utilised maximum 140°C of temperature with pH 1 produces 197 g/kg of xylose. It is concluded that pre-treatment of wheat straw with influence of pH is the significant factor for the yields of glucose and xylose (Pedersen et al., 2011). However, this pre-treatment has the disadvantage which required large additional loads of lime or solvent, as well as extremely extended treatment periods ranging from 60 minutes to several hours (Pedersen et al., 2011).

2.2.3 Microwave Pre-treatment

The structure of lignocellulosic compound can be altered by using microwave irradiation. Pre-treatment using a microwave helps to degrade the hemicellulose part and lignin. It disrupts the silicified waxy surface, and this helps to enhance the enzymatic sensitivity of reducing sugar (Kumari & Singh, 2018). It is also reported by Hu & Wen., (2008) that pre-treatment of switchgrass using microwave help to increase about 58% of sugar yield. Also, pre-treatment using microwave irradiation showed a 56% increment of glucose yield from rape straw (Lu et al., 2011). Previous research used 6 seconds of microwave pre-treatment after 6 minutes hydrolysis pre-treatment produces 88% of glucose and 76.5% of xylose yield using sago pith waste (Thangavelu et al., 2019).

Hu and Wen. (2008), reported a 58% increase in sugar yield using a microwave to pre-treat switchgrass compared to conventional heating. The main benefits of using microwave irradiation aids in cellulosic disintegration. The main benefits of this procedure are high

selectivity and uniformity, short reaction time, and low energy needed compared to traditional heating.

2.3 Physicochemical Pre-treatment of Lignocellulosic Waste

Physicochemical approaches are pre-treatment methods that use two combined approaches to alter physical characteristics and intermolecular interactions of the lignocellulosic waste (Zhao et al., 2012).

Pressure and temperature are employed in conjunction with a chemical process to break down lignocellulosic waste utilising methods such as high-pressure steam, mild thermal pre-treatment and microwave pre-treatment (Kumari & Singh, 2018; Lu et al., 2011).

The most common chemical pre-treatment is by using an acid hydrolysis process. This pre-treatment solubilised the hemicellulose fraction in the lignocellulosic waste and it employed high acid concentration with low temperature or low acid concentration with high temperature (Kumari & Singh 2018). The pre-treatment condition can be manipulated based on the raw materials to be pre-treated and the process requirement. The acid concentration can be from 0.5 % and the solids loading and reaction time vary from 100 min and 15% respectively (Manaf et al., 2018). However, the major drawback of this pre-treatment is that it produces various inhibitors such as furfural and 5-hydroxymethyl furfural (Manaf et al., 2018; Hong et al., 2016).

Based on Table 2.1 shows the comparison of different types of raw materials as substrate which undergo acid pre-treatment to produce xylose hydrolysate which subsequently use as fermentation media to produce xylitol. When corncob was used as the raw material, the highest xylitol obtained was 45.4 g/L with initial xylose concentration of 65.9 g/L followed by 81.4 g/L of initial xylose concentration which produces 38 g/L of xylitol (Cheng et al.,

2009; Ping et al., 2013). Whereas 22.66 g/L of xylose concentration of oil palm empty fruit bunches produces 10.3 g/L of xylitol (Manjarres-pinzón et al., 2017). The lowest xylitol obtained were 5.17 g/L using exhausted olive pomace with initial xylose concentration of 32.59 g/L (López - Linares et al., 2020). This shows different high initial concentration of xylose does not contribute to high production of xylitol.

Table 2.1: Dilute sulphuric acid pre-treatment of selected lignocellulosic waste to produce xylitol.

Raw materials	Pre-treatment process	Xylose production in the hydrolysate (g/L)	Micro organism	Concentration of xylitol produce (g/L)	Yield (g/g)	Productivity (g/L/h)
Corn cob (Cheng et al., 2009)	Acid hydrolysis of 40 g corncobs at a solid loading of 10% (w/w) was mixed with dilute sulfuric acid (1% (w/w) and pre-treated in an autoclave at 120 °C with residence time of 1 hour.	65.9	<i>C. tropicalis</i> W103	45.4	0.71	0.71
Corn cob (Ping et al., 2013)	Dilute-acid hydrolysis of corncobs at a solid loading of 15% (w/w) and dilute sulfuric acid (1%, w/w) and processed in an autoclave at 125 °C for 1 hour.	81.4	<i>C. tropicalis</i>	38	0.7	0.46
Corn Stover (Hong et al., 2016)	Pre-treatment of corn stover with 2.5% (w/v) sulphuric acid for 1.5 h, with a solid slurry concentration of 90 g/L at 120 °C	11.2	<i>C. tropicalis</i>	10.9	0.97	0.03

Table 2.1 Continued

Oil palm empty fruit bunches (Manjarrés-pinzón et al., 2017)	Dilute-acid hydrolysis of biomass was performed in 125 ml Erlenmeyer flasks at 121 °C for 30 minutes with a 12.5% substrate load and a 2% (w/v) aqueous H ₂ SO ₄ solution.	32.59	<i>C. guilliermondii</i>	10.3	0.43	0.11
Exhausted olive pomace (López - Linares et al., 2020)	Pre-treated with 2% (w/v) H ₂ SO ₄ at 170 °C and 20% biomass loading	22.66	<i>C. boidinii</i>	5.17	0.36	0.07

2.4 Inhibitor in Acid Pre-treatment

However, there are some major drawbacks of acid pre-treatment as this pre-treatment causes lignocellulosic waste to produce inhibitory by-products under acidic conditions. As shown in Figure 2.4, by product from acidic pre-treatment can be groups based on their origin, chemical functionality and properties of the fermenting microorganism (Jönsson & Martín, 2016). The existing pentose and uronic acid are dehydrated to generate 2-furaldehyde which then further becomes furfural, whereas the hexoses are dehydrated to form 5-hydroxymethyl-2-furaldehyde, hereafter referred to as HMF, when the hemicelluloses are hydrolysed. HMF is then converted to levulinic and formic acids under harsh pre-treatment conditions, such as a long reaction time, high temperature, and acid concentration (Jönsson & Martín, 2016). Furfural, like HMF, is unstable in the dehydrative medium and can be susceptible to additional degradation to formic acid and condensation processes, resulting in resin production (Danon et al., 2013). Furfural potentially can harm microbial cell walls and membranes, impede growth, fermentation rate and decrease

enzyme activity (Liu et al., 2008; Dragone et al., 2007; Modig et al., 2002). The degradation products such as furan derivatives, acetic acid and phenolic compounds inhibit fermentation by different mechanism and a combination of the action of several compounds other than sugars present in the hydrolysate may be one of the reasons for the inhibition (Behera et al., 2014

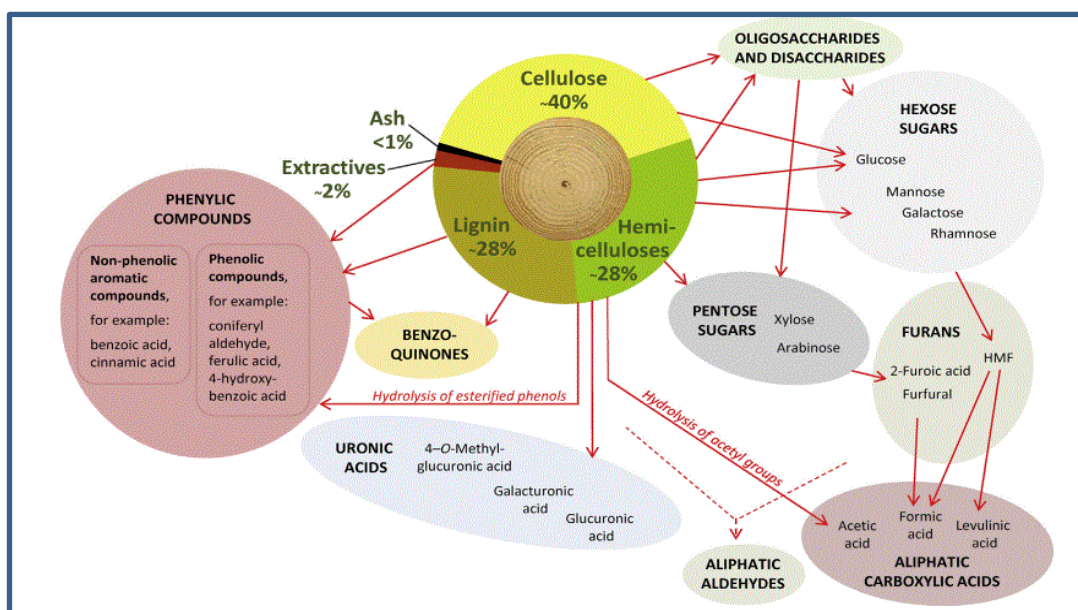


Figure 2.4: Inhibitor produced from pre-treatment of lignocellulosic materials using acid pre-treatment (Danon et al., 2013).

Lignocellulosic from agricultural waste mainly used as source of raw material for production of fermentable sugar (Fet et al., 2018). It is abundantly available for sustainable sources and usually exploited to produce valuable products such as xylose for production of xylitol (Manjarres-pinzón et al., 2017). Lignocellulosic biomass is majorly consisted of cellulose, hemi-cellulose, and lignin.

2.4.1 Cellulose

Cellulose is a polymer of β -D-glucopyranose moieties linked via β -(1,4) glycosidic bonds with well documented polymorphs. Cellulose is insoluble in water and this polymer is water insoluble unless the pH is exceptionally low or high (Baruah et al., 2018). Cellulose is a flexible resource for derivatized materials such as fibres, films, composites, as well as fuels and chemicals, due to its beneficial features such as biocompatibility, stereoregularity, hydrophilicity, and reactive hydroxyl groups (Baruah et al., 2018).

2.4.2 Hemicellulose

The hemicellulose fraction contained a sugar polymer known as xylan. This xylan is made up of the pentose sugar and commonly used as substrate for chemical and biological processes to produce a variety of chemicals (Manjarres-pinzón et al., 2017). As shown in Figure 2.5, the heterogeneous component of hemicellulose consists of pentoses, hexoses and some acids (Manjarres-pinzón et al., 2017; Sindhu et al., 2016).

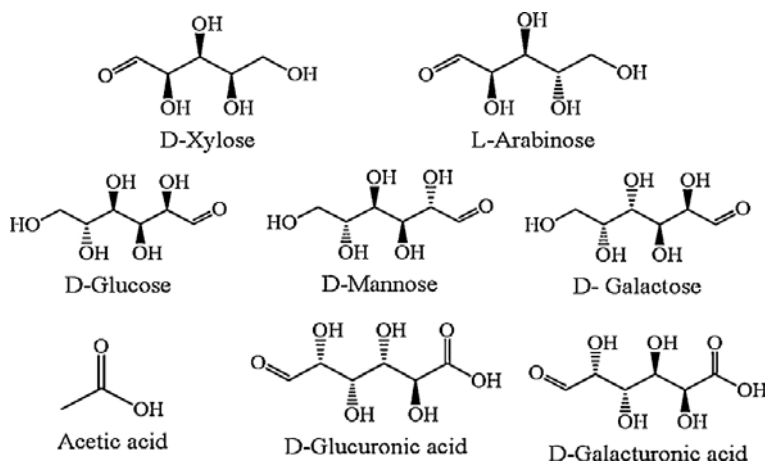


Figure 2.5: Hemicellulose components (Sindhu et al., 2016).

Xylose is the major building block of hemicellulose can be further undergone microbial fermentation or enzyme technology to produce xylitol (Mohamad et al., 2015). Due to the amorphous structure of the xylose in the hemicelluloses, the xylose is much more

susceptible to hydrolysis by mild acid pre-treatment compared to cellulose which needs severe treatment due to its crystalline nature (Manjarres-pinzón et al., 2017).

2.4.3 Lignin

Lignin is formed by a large molecular structure containing cross-linked phenolic polymers (Kumari & Singh, 2018). Types of lignocellulosic biomass influences the components of the lignin which are associated with each other (Baruah et al., 2018). The composition of cellulose, hemicellulose and lignin are different in biochemical and thermo-chemical conversion processes. The composition of lignin can cause a hindrance in cellulosic bioethanol production compared to cellulose and hemicellulose (Baruah et al., 2018).

2.5 Sago Industries

The sago industries in Sarawak have been produced annually about 44,000 t of starch mainly Peninsular Malaysia, Japan, Singapore, and other countries (Awg-Adeni et al., 2013). Over 90% of the commercially farmed sago palm is located in Sarawak which was estimated to be 15–25 tons/ha. This makes the sago palm a significant commercial species and resource for this region. When compared to other types of plants like corn (5.5 tons/ha), rice (6 tons/ha), potato (2.5 tons/ha), and wheat (5 tons/ha), the sago palm has the largest starch content (25 tons/ha) (Awg-Adeni et al., 2013).

2.5.1 Sago Waste

The mechanical process involved in production of this sago is ineffective and commonly unable to remove residual starch found in the fibrous portion of the trunks and this waste which are mixed together with wastewater usually are washed off into nearby streams or deposited in the factory's compound (Awg-Adeni et al., 2013). Sago bark, sago

hampas and sago waste water are waste that are generated by the sago palm industry (Awg-Adeni et al., 2013). Hence, the damaging impacts of these agro-wastes have become a major source of worry and have sparked interest among academics working to find a sustainable solution.

2.5.2 Utilisation of Sago Waste

Sago waste such as sago bark is utilised as a source for firewood. Apart from these, bark is used as flooring material in the mill, while locals used it to construct ceilings, fences and wall materials (Mohammad et al., 2020). Furthermore, sago starch waste contains 58% starch, 23% cellulose, 9.2% hemicellulose, and 4% lignin based on dry basis (Awg-Adeni et al., 2013).

Recent studies shown sago fibre has been utilised for production of fermentable sugars (Alias et al., 2021; Mohammad et al., 2020). Hence, the study utilizing the leftover sago hampas known as destarched fibre that has been used for bioethanol utilisation and emphasising on utilising leftover sago fibre and convert it into fermentable pentose sugar and maximise the use of hydrolysed sago fibre in the fermentation process.

2.6 Utilisation of Yeast in The Industry

Yeast plays has wide usage in the industry process to produce enzymes, polysaccharides, vitamin, polyhydric alcohols, pigments, lipids, and glycolipids (Adrio & Demain, 2003). The products produced are potentially valuable in biotechnology thus some of the products are being produced commercially. The secondary metabolites produced by yeast are essential to health due to its nutrition value and have major economic affluence (Adrio & Demain, 2003).

2.6.1 Roles of Yeast in Xylitol Production

Studies on xylitol production using fungi are limited but some researchers have reported positive results using the yeast *C. tropicalis*. Misra et al. (2013), reported xylitol production from 18 strains of yeast and concluded *C. tropicalis* shown higher production performance. Using 50 g /L initial xylose concentration and 72 hours at 30 °C, pH 5, and 200 rpm, a strain of *C. tropicalis* produced 12.11 g /L of xylitol. Also, Yablochkova et al. (2003) reported that found that *C. tropicalis* Y-456 had the highest specific activity of XR: 6.57 mol min⁻¹ mg protein⁻¹. Microorganisms with NADPH-dependent and high XR activity are potential producers of xylitol from D-xylose (Yablochkova et al., 2003).

Table 2.2 summarises the studies for the production of xylitol using yeasts *C. tropicalis* using lignocellulosic waste as the substrate. From Table 2.2, the results depending on the types of microbial and fermentation parameter conditions such as pH and aeration. Controlling the fermentation parameter is important to maximise the xylitol production process. In addition, Zikou et al. (2013), reported that maximum xylitol production which is 31 g/L achieved by *Thamnidium elegans* strain. The fermentation takes place with the presence of mixture sugar which is xylose and glucose. Furthermore, Zhang et al. (2012) also reported that yeast *Candida athensensis* SB18 in flasks with agitation and bioreactors performed in both batch and fed-batch systems containing 200 g/L xylose. This resulted in productivity of 0.98 g/L/h and yield of xylitol 0.81 g/g/L were obtained.

Table 2.2: Xylitol production by using yeasts *Candida* species from industrial residue.

Micro organism	Raw materials	Initial xylose concentration (g/L)	Maximum xylitol production (g/L)	Fermentation condition	Productivity (g/L/h)	References
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Table 2.2 Continued

<i>C. tropicalis</i> As2.1776	Corncob	160	96.5	pH 5.5, 30 °C, 120 Hours	1.01	Li et al. (2012)
<i>C. tropicalis</i> CCTCC M201246 2	Corncob	81.4	38.8	pH 6, 35 °C, 14 Hours	0.46	Ping et al. (2013)
<i>C. tropicalis</i> HDY 02	Corncob	60	58	pH 7, 35 °C, 78 Hours	0.71	Hong et al. (2016)
<i>C. tropicalis</i> 31949	sugarcane rice straw	100	62.98	30 °C, 104 Hours	-	Xu et al. (2019)

2.6.2 Metabolism of Xylose in Microorganism

Initial studies of xylose metabolism in yeast cell were demonstrated using strain *Pichia stipitis*, *Pichia heedii*, *Candida shehatae* and *Candida intermedia* (Ghindea et al., 2010). The studies concluded there are two transport systems involved. The first transport system involved facilitated diffusion with low affinity. Examples of genes involved in *P. stipites* is SUT1 or known as sugar transporter 1 and *GXF1* expressed genes that code the glucose and xylose transporter protein for *C. intermedia*. Second transport system symporter with high affinity. The genes involved are GXS1 or known as glucose and xylose symporter assist in the symport monosaccharides-protons transport in fungi and yeast (Ghindea et al., 2010).

Xylitol is the intermediate metabolites produced by the yeast during the break down of d-xylose (Ghindea et al., 2010). Yeast metabolism involves NADPH – dependent enzyme known as xylose reductase and xylitol dehydrogenase (XDH) which needs NADP+. Metabolism of xyloses in yeast can be briefly described as in Figure 2.6. The first step

involved the assistance of the special membranes that carry the xylose into the cell. After that, enzyme xylose isomerase will convert xylose into xylulose and subsequently broken down in the pentose phosphate pathway. In another pathway, xylose reductase will convert xylose into xylitol which can be further converted by xylitol dehydrogenase into xylulose.

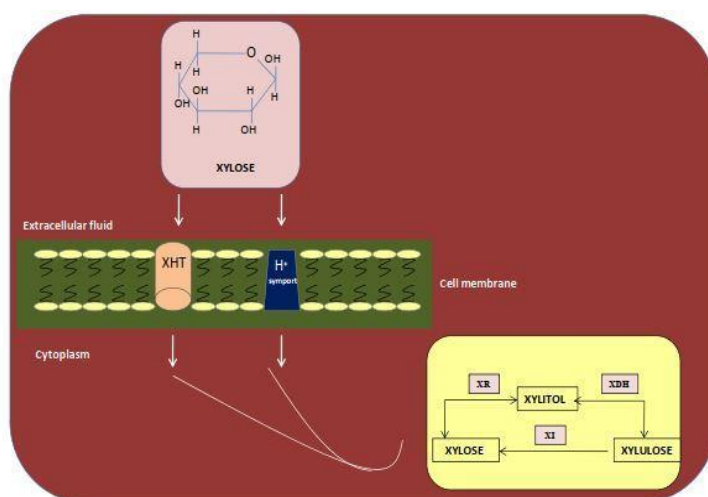


Figure 2.6: The transport of xylose within the yeast cell. Notes: XHT- hexose transporters; XR-xylose-reductase; XDH-xylitol-dehydrogenase;XI – xylose-isomerase) (Ghindea et al., 2010).

Since there are two types of enzymes, thus the d-xylitol is broken into in two steps. NADPH will reduce D- xylose into d-xylitol. NADP⁺ will oxidise this d-xylitol into d-xylulose. Both reactions are consider as limiting for fermentation of d-xylose fermentation (Zhang et al., 2012; Franceschin et al., 2011). Some microbial species have cofactors which NADPH - dependent of xylose reductase and NAD⁺ dependent of xylose dehydrogenase. Both of these enzymes are produced with the presence of xylose (Sullivan et al., 2008).

On the contrary with the yeast, the metabolic pathway of other microorganisms is through the express's isomerase gene. As shown in Figure 2.7, isomerase gene in the cell of the microorganism will break down the xylose (Dumon et al., 2012). In some

microorganism unlike yeast, the xylose can be directly metabolised into xylulose with the present of xylose isomerase (Liu et al., 2019). It is difficult to express the isomerase gene in the yeast and this pathway only has been discovered recently. In 2003, Kuyper et al. (2003), expressed the yeast with gene xylose isomerase from fungi *Piromyces sp.* The discovery of this studies showed that the biotechnological ways for fermentation of xylitol from the xylose can be achieved however the there is a need to study the best strain in order to achieve optimum fermentation in the industry (Dumon et al., 2012). Figure 2.7 shows the comparison between type 1 and type 2 xylose metabolism in the microorganism.

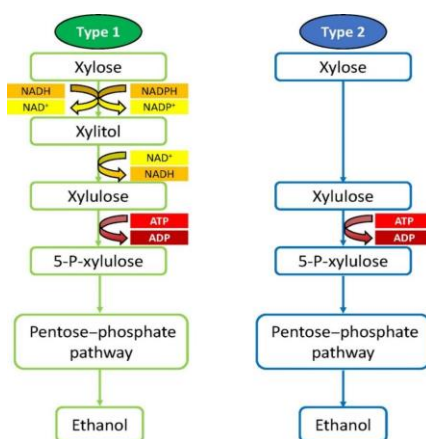


Figure 2.7: Comparison of Xylose metabolism in yeast (Type 1) and lower bacteria (Type 2) (Liu et al., 2019).

2.7 Fermentation Mode

Similarly, with fermentation producing bioethanol, xylitol production also produced with three types of mode which were batch, fed batch and continuous mode.

2.7.1 Batch fermentation

In the batch fermentation, there is no addition or removal of the medium and the substrate is provided at early of the process (Ramesh et al., 2013). This bioreactor is known as the simplest system that easy control process. Batch fermentation known as closed system with high sugar concentration introduced in the beginning of the fermentation which

often resulted in high product concentration. The advantage of this batch system is complete sterilization and easy to manage as it also needs no labour skill and the feedstocks can be easily managed. In addition, it is easy and flexible to various product specifications (Manjarres et al., 2017). In contrast, batch fermentation required high labour cost and sometimes the productivity is low as high sugar concentration introduced in the early fermentation could potentially lead to osmotic pressure for the yeast. This subsequently result in inhibition of the fermentation due to the cell growth (Hernández-Pérez et al., 2016).

2.7.2 Factors Affecting Fermentation Xylitol Production

Xylitol production from the fermentation can be optimised by several factors such as pH, temperature, nutrients and inoculum age. In order to maximise the xylitol production, several conditions should be studied and be chosen based on the types of microorganism. There have been several studies of the fermentation parameter for fermentation of the xylose in order to maximise the xylitol production.

2.7.3 Temperature

The temperature commonly employed in fermentation process conducted using yeast is from 30 °C to 37 °C as this range of temperatures has been proven to be the best for xylitol production by the yeast (De Albuquerque et al., 2014). A significant decrease of the productivity by the yeast can be observed if the temperature employed is beyond 37°C.

However, there is an exception for some microorganism where high temperature is required. For example, *K. marxianus* is a thermotolerant microorganism which stands out in fermentation when compared to another microorganism (Dasgupta et al., 2017). It required high temperature up to 45°C to produce ethanol and xylitol production from the hemicellulose hydrolysate. High temperature helps the activation of the enzyme of the

microorganism to produce xylitol and ethanol (Rodrussamee et al., 2011). In addition, it is revealed the strain can grow at high temperature because cell growth and sugar consumption were observed at all temperatures studied.

For *C. tropicalis* yeast, the most suitable temperature that has been studied is 30 °C to 37 °C for xylitol production. A significant decrease in xylitol production can be observed if the temperature higher than 45 °C was employed in the fermentation. High temperature can cause denaturation of the enzyme xylose reductase (Rao et al., 2016). Li et al. (2012) reported that *C. tropicalis* As2.1776 produced 96.5 g/L of xylitol production from corn cob hydrolysate at temperature of 30 °C after 120 hours of fermentation. Also, studies conducted by Ping et al. (2013) revealed that *C. tropicalis* CCTCC M2012462 produced 38.8 g/L of xylitol from corn hydrolysate at temperature of 35 °C after 8 hours of fermentation.

2.7.4 Carbon source

Carbon sources comes from lignocellulosic materials is often used as the raw materials for production of value-added products such as ethanol, biobutanol and xylitol. This is because lignocellulosic waste can be easily obtained as it can be found widely in Malaysia.

The hemicellulose fraction obtained from the lignocellulosic waste can be sources of xylose sugar which subsequently fermented to xylitol (De Albuquerque et al., 2014). Research has proven experimentally yeast *C. tropicalis* utilised glucose as substrate for growth and this enhance the xylitol production in the hydrolysate containing mixture of glucose and xylose (De Albuquerque et al., 2014). Low concentration of glucose helps to promote growth and production efficiency. Usually, glucose is being consumed first for cell growth whereas xylose is consumed afterward. However, too much concentration of

glucose can cause allosteric competition and causes the repression of the yeast to utilised other sugars (Kawaguchi et al., 2006).

In addition, high concentration of xylose was required to produce maximum values of xylitol. Research by Cheng et al. (2009), reported that *C. tropicalis* W103 fermented in only xylose medium as the carbon sources can produced 200 g/L of xylitol. Lorliam et al. (2017), found that *C. tropicalis* A26 addition of 4 g/L of peptone can enhance the xylitol production whereby 42.52 g/L of xylitol was produced from 80 g/L of xylose. Research by Lorliam et al. (2017) indicated the initial xylose concentration of 80 g/L can produced maximum xylitol concentration of 52 g/L and increased xylose concentration of 150 g/L and 156 g/L could produce xylitol yield 0.47 g/g and 0.77 g/g respectively using *C. boidinii* and *D. hansenii* (Lorliam et al.,2017).

2.7.5 Nitrogen Sources

There are different types of nitrogen sources such as peptone and yeast extract that have been researched in fermentation parameter in order to maximise the production of the fermentation product. Studies has found that synthetic medium supplemented with various types of nitrogen sources such as amino acid and urea could affect the production of the xylitol (Zhang et al., 2012). Example of the nitrogen sources that were used in the studies were amino acid, urea, ammonium nitrate and ammonium sulphate and this study proves that urea produces highest xylitol yield which is 0.75 g/g (Zhang et al., 2012). Other studies conducted using yeast extract and peptone as additional nutrients to the fermentation medium using *C. tropicalis* OMV5 produces maximum xylitol yield was obtained by using 1.32 % peptone and 0.48 % yeast extract (Sreenivas et al., 2008)

Sreenivas et al. (2008), using Taguchi approach to investigate the effect of nitrogen sources using yeast extract and peptone for xylitol production using mutant *C. tropicalis*

OMV5 and the result showed that maximum xylitol yield was obtained by using 1.32% peptone and 0.48% yeast extract. By comparing yeast extract and peptone, it was reported peptone as only one organic nitrogen source that had a significant effect on xylitol production of *C. tropicalis* (Lorliam et al., 2017). Lorliam et al. (2017), discovered that 4 g/L of peptone helps to enhance and produces highest xylitol production which was 42.52 g/L using yeast *Candida*.

2.7.6 Inoculum Age

Inoculum ages can affect the product formation in the fermentation as inoculum ages can of the yeast does affect the viability and the metabolic activity of the yeast. Kadam et al. (2006), investigated the influences of inoculum age for the production of lactic acid. The study showed inoculum age of 24 hours and 48 hours of affecting the lactic acid production and 24 hours of incubation age is effective for the lactic acid bacteria. There were limited studies regarding the inoculum age of yeast for fermentation. According to Kadam et al., (2006), different inoculum age does influence the yield of fermentation products. Two crucial parameters affecting the fermentation which were inoculum age and inoculum concentration were comprehensively studied. Thus, this study utilised different inoculum age 9, 18, 28 hours and different inoculum concentration of 10%, 20% and 30% (v/v) in order to investigate the effect of inoculum age and inoculum concentration on the concentration of xylitol and ethanol. Xu et al. (2019), discovered that xylitol production increases at maximum xylitol with concentration of 36.62 g/L with 26h of inoculum age and the concentration decreases with increasing inoculum age. The present studies concluded that there is an effect with the concentration of the fermentation product when different inoculum age was employed during the fermentation.

2.7.7 Inoculum Concentration

Juanssilfero et al. (2018), found that increasing inoculum size helps to decrease the fermentation time significantly and the inoculum size does influence the fermentation. Increasing fermentation size during the initial fermentation can increase the sugar consumption rate which subsequently increase the rate of the fermentation (Juanssilfero et al., 2018). Not only that, increase inoculum size during the initial fermentation could lead to increase efficiency of the fermentation as the increase inoculum could dilute the inhibitors concentration in the medium (Juanssilfero et al., 2018). This is supported by Wang et al. (2015), which found that fermentation of xylitol production using undetoxified corn straw hydrolysate can be repressed by increasing the inoculum size to 1.6 g/L (Wang et al., 2015). Xu et al. (2019) studied fermentation of *C. tropicalis* in sugar bagasse found that 10% of inoculum quantity helps to maximise the xylitol production while higher than this can cause decrease in xylitol production as a lot of xyloses has been used a lot of xyloses has been used for the growth of bacterial cells.

2.7.8 Aerobic Condition

In the breakdown of xylose by yeasts, oxygen plays a significant role. It has been demonstrated that in completely anaerobic conditions, the metabolic process that creates xylitol from xylose comes to a complete halt (F et al., 2014). The oxygen level needed for xylose metabolism is varies among yeast species. For example, *C. tropicalis* shows a maximum of productivity in semi-anaerobic conditions, reaches its peak productivity under semi-anaerobic conditions with compared to *Debrayomyces hansenii* strains (F et al., 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sago Fibre Collections

Sago fibre samples used as raw materials in this research were obtained from Herdsen Sago Mill Sdn. Bhd near Pusa. The samples were packed into porous plastic bags prior to delivery to UNIMAS. The leftover sago fibre from previous hydrolysis for glucose production by Mohammad et al. (2020), was used in this study. The leftover sago fibre samples known as “destarched” fibre due to starch removal were used as raw materials in this research. Generally, this project contains two main sections of experimental design involving pre-treatment of the destarched sago fibre using dilute sulphuric acid pre-treatment and fermentation of destarched sago fibre hydrolysate for the production of xylitol. Figure 3.1 shows the overall research flow chart of the study conducted in this project.

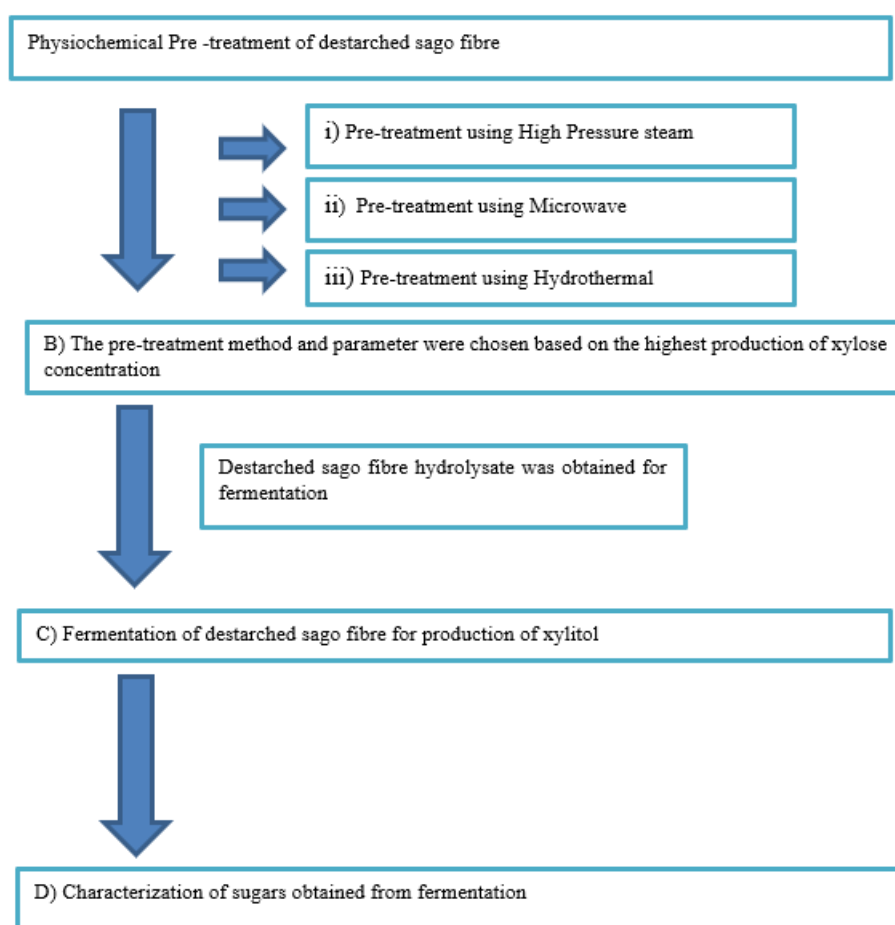


Figure 3.1: The overall pre-treatment and fermentation of the destarched sago fibre for xylitol production (Hong et al.,2016).

3.2 Preparation of Sago Fibre for analyses

The destarched sago fibre collected from starch removal from previous hydrolysis by Mohammad et al. (2020), was air dried and ground to pass a 1 mm screen prior to compositional analysis. The destarched fibre was then analysed for then analysed for moisture content prior for acid pre-treatment (AOAC, 2012).

3.3 Pre-treatment of Destarched Sago Fibre

Three parameters affecting sugar extraction from destarched sago fibre which were the acid concentration, reaction time and solid/liquid ratio were investigated in this study. The destarched sago fibre were treated with dilute sulphuric acid ranging from 0 % to 9 % (w/v). The solid/ liquid ratio (g/ml) which ranges from 5 g/100 ml to 40 g/100 ml was also investigated. After the hydrolysis process, the solids were separated from the aqueous solution by using muslin cloth. The hydrolysate was used as fermentation media for *C. tropicalis*. The pH of the hydrolysate was adjusted to pH 6 by using sodium hydroxide pellet (Ping et al., 2013). The resulting precipitation was removed by using centrifugation at (10,000 g, 10 minutes).

3.3.1 Dilute Sulphuric Acid Hydrolysis Using High Pressure Steam

The high-pressure steam autoclave pre-treatment was performed in a vertical autoclave (Shimadzu, Japan) at a pressure of 1.5 atm and a temperature of 121°C. As shown in Figure 3.2, different sago fibre concentration (5 %,10 %, 20 %, 30 %), dilute sulphuric acid concentration (2 % (v/v), 4 % (v/v), 6 % (v/v) and reaction time (30 minutes, 60 minutes and 90 minutes) were employed in the hydrolysis process. Reactions were carried out at 121°C in an autoclave. After hydrolysis, the resulting solid material was separated by filtration using a muslin cloth and the filtrate (hemicellulose hydrolysate) was analysed for solubilised sugars which are glucose and xylose (Manjarres-Pinzon et al., 2017).

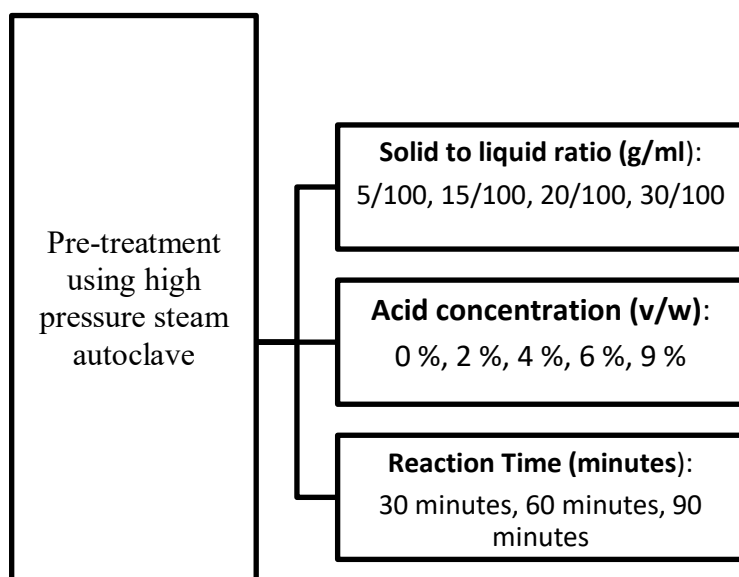


Figure 3.2: The schematic diagram of dilute H₂SO₄ pre-treatment using high pressure steam.

3.3.2 Dilute Sulphuric Acid Hydrolysis Using Microwave

The acidic pre-treatment of destarched sago fibre was carried out in a microwave at a frequency of 2450 MHz. As shown in Figure 3.3 the study was conducted by using a 500 mL beaker filled with a specific amount of substrate loading of 5 %, in 100 mL of dilute sulphuric acid concentration with different concentrations which were 2 %, 4 % and 6 % of v/v. The pre-treatment took 8 minutes of reaction time and was irradiated at 510 W.

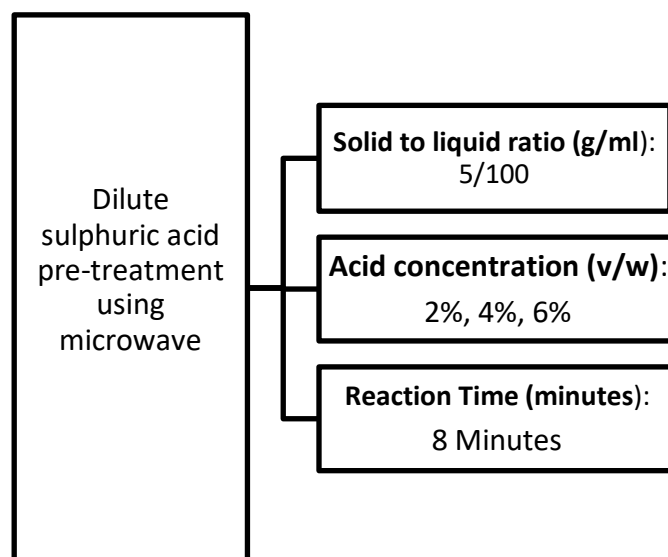


Figure 3.3: The schematic diagram of dilute H_2SO_4 pre-treatment using microwave.

3.3.3 Dilute Sulphuric Acid Hydrolysis in Hydrothermal Condition

Different sago fibre concentration (5%, 10%, 20%, 30%), sulfuric acid concentration (2 % (v/v), 4% (v/v), 6% (v/v) and reaction time (30 minutes, 60 minutes and 90 minutes) were employed in the hydrolysis process. As shown in Figure 3.4, reactions were carried out at 100 °C in a water bath. After hydrolysis, the resulting solid material was separated by filtration using a muslin cloth and the filtrate (hemicellulose hydrolysate) was analysed for solubilised sugars which are glucose and xylose (Manjarres-Pinzón et al., 2017).

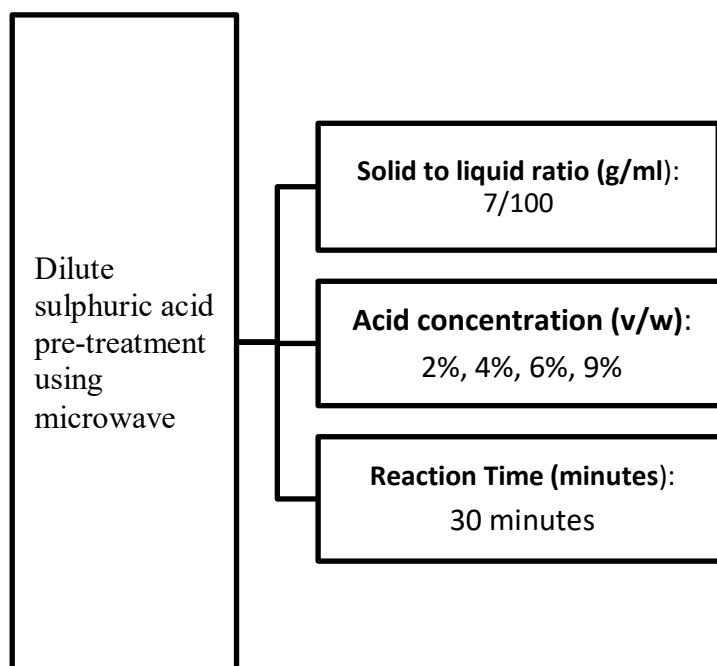


Figure 3.4: The schematic diagram of dilute H_2SO_4 pre-treatment using microwave.

3.4 Preparation of Fermentation Media

All media were prepared in at least in duplicate and performed aseptically in a laminar air flow cabinet (ERLA Series). PDA agar was prepared using 39 g Bacto TM PDA powder, dissolved in 1000 mL distilled water and sterilised at 121 °C for 15 minutes, left to cool to about 50-60 °C and then poured into sterile Petri dishes. Yeast malt broth medium was prepared by dissolving 1.05 g of Sigma-Aldrich TM YMB powder (Peptic digest of animal tissue, 5.00 g/L, yeast extract, 3.00 g/L, malt extract, 3.00 g/L, dextrose, 10g/L) in 50 mL shake flask of distilled water and sterilised at 121 °C for 15 minutes. In order to study the effects of nitrogen sources on production of xylitol and ethanol in destarched sago fibre (DSF) hydrolysate, 5 g/L of yeast extract and 5 g/L of peptone were added into 100 ml of fermentation media respectively.

3.5 Activation of *C. tropicalis* and Preparation of Inoculum

The *C. tropicalis* from glycerol stock was revived in yeast malt broth on a shaking incubator at 30 °C and 200 rpm for 24 h, before being transferred into the fermentation medium (Hong et al., 2016). After 24 hours, the yeast was subculture onto potato dextrose agar to obtain single colonies and to test for viability (Bellissimi & Ingledew, 2005). Figure 3.5. shows the colonies of the yeast on PDA agar after 24 hours. The microorganism was subculture for every two weeks on PDA agar. A single colony from the colonies formed from the agar were selected for preparation of inoculum. One loopful single colonies of *C. tropicalis* was inoculated in 150 ml of Erlenmeyer flask of preculture media containing 20 g/L of glucose, 20 g/L xylose and 5 g/L of yeast extract and cultivated at 30°C on the Orbital Incubator Gyromax TM706 Shaker (Hotech Instruments Corp) at 100 rpm.



Figure 3.5: The yeast colonies on the PDA agar plate.

3.6 Determination of Inoculum Age and Inoculum Ratio for Fermentation

Different inoculum ratio 10, 20 and 30% (v/v) were taken from the pre - culture flask and then was centrifuged. The pellet washed twice with distilled water and then used as inoculum for fermentation. Then, the experiment was repeated by using 20% (v/v) with

different inoculum age of 9,18, 28 hours. All the experiments were carried out at least in duplicate.

3.7 *C. tropicalis* fermentation

The fermentation media were supplemented with yeast extract and peptone at the same concentration as nitrogen sources. 5 g/L of yeast extract and peptone were used in the fermentation. DSFH without any supplementation and Commercial sugar (xylose and glucose) with nitrogen source were used as control experiments. Figure 3.6. shows the fermentation media for *C. tropicalis* fermentation.

After that, two nitrogen sources were selected and the experiments were repeated with addition the chosen nitrogen sources. Again, DSFH without any supplementation and Commercial sugar (xylose and glucose) with nitrogen and respective salt were used as control experiment.

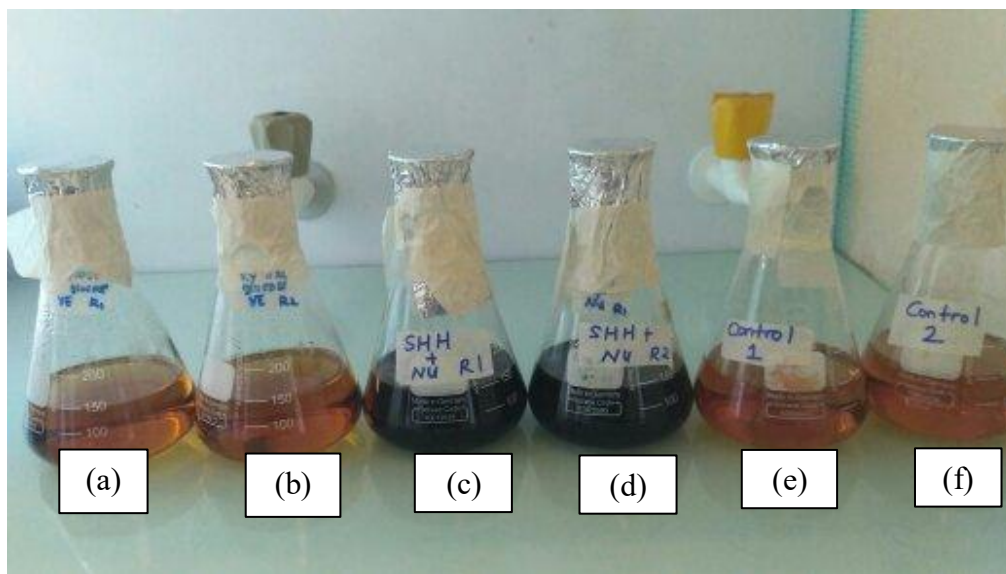


Figure 3.6: Fermentation media for production of xylitol; (a) DSFH + Yeast extract (R1), (b) DSFH + Yeast extract (R2), (c) DSFH + Peptone (R1), (d) DSFH + Peptone (R2), (e) standard medium (R1), (f) standard medium (R2).

For all experiments, samples were obtained every 24 hours and then promptly centrifuged at 10,000 rpm for about 5 minutes. The supernatant was then used for the determination of ethanol and xylitol concentration and glucose and xylose consumption by High Performance Liquid Chromatography system (Shimadzu Corporation, Japan). The data obtained from series of fermentation process was compiled and analysed for xylitol and ethanol yield ($Y_{p/s}$), xylitol and ethanol volumetric productivity (Q_P) and xylitol and ethanol fermentation efficiency (E_f). The outcomes were analysed based on these three different criteria to observe which combination would produce the highest xylitol as well as bioethanol yield, efficiency and productivity. Table 3.1 shows the media composition of the fermentation media in this study.

Table 3.1: Media compositions for fermentation for production xylitol and ethanol.

Trials	Standard medium	Experiment
1.	50 g/L glucose + 40 g/L xylose (Without supplementation)	50 g/L glucose, 40 g/L xylose of destarched sago hydrolysate (without supplementation)
2.	50 g/L glucose + 40 g/L xylose + 5 g/L peptone	50 g/L glucose, 40 g/L xylose destarched sago fibre hydrolysate + 5 g/L peptone
3.	50 g/L glucose and 40 g/L xylose + 5 g/L yeast extract	50 g/L glucose, 40 g/L xylose destarched sago fibre hydrolysate + 5 g/L yeast extract

3.8 Analyses

3.8.1 NDF and ADF of Sago Fibre Analysis

The preliminary analysis was conducted to identify the cellulose and hemicellulose content in the destarched sago fibre. Neutral detergent fibre analysis and acid detergent fiber analysis were conducted by method of Van Soest & Robertson (1979).

3.8.2 Neutral Detergent Fibre (NDF) Analysis

One gram of dried and ground ed sago fibre (W_0) was transferred into 250mL conical flask and added with 100 mL of neutral detergent fibre solution (Appendix B). The sample was heated and boiled for 1 hour. The sample was cooled and filtered through a sintered funnel which was accommodated with 0.2 whatman filter paper and connected to a vacuum pump for suck dry. The trapped residue on filter paper was washed three times with hot distilled water and followed by another three-time washed with acetone. An empty crucible was weight (W_1). The washed residue was removed from filter paper and

transferred into a weighted crucible and dried in the oven at 105 °C for 3 hours. Then, the crucible with dried residue was placed in a desiccator and weighted (W_2). The percentage of NDF was calculated by using a formula as below:

$$ADF (\%) = \frac{(W_2 - W_1)}{W_0} \quad \text{Equation 3.2.1}$$

Where;

W_0 = Weight of sample (dried and ground sago waste)

W_1 = Weight of empty crucible

W_2 = Weight of crucible contain dried residue (after dried in oven)

3.8.3 Acid Detergent Fibre (ADF) Analysis

The NDF sample (W_0) was placed into a 250mL conical flask and added with 100mL of acid detergent fibre solution (Appendix A). The sample was heated and boiled for 1 hour. The sample was cooled and filtered through a sintered funnel which was accommodated with 0.2 whatman filter paper and connected to a vacuum pump for suck dry. The trapped residue on filter paper was washed three times with hot distilled water and followed by another three-time washed with acetone. An empty crucible was weight (W_1). The washed residue was removed from filter paper and transferred into a weighted crucible and dried in the oven at 105 °C for 3 hours. Then, the crucible with dried residue was placed in a desiccator and weighted (W_2). Cellulose was determined as the loss in weight from acid detergent residue. The percentage of NDF was calculated by using a formula as below:

$$ADF (\%) = \frac{(W_2 - W_1)}{W_0} \quad \text{Equation 3.2.4}$$

Where;

W_0 = Weight of sample (dried and ground destarched sago fibre)

W_1 = Weight of empty crucible

W_2 = Weight of crucible contain dried residue (after dried in oven)

3.8.4 Determination of Hemicellulose in The Destarched Sago Fibre Hydrolysate

The present percentages of hemicellulose and cellulose calculated from the NDF and ADF values were as the following:

$$\text{Hemicellulose content (\%)} = \% \text{ NDF} - \% \text{ ADF} \quad \text{Equation 3.2.5}$$

3.8.5 Glucose, Xylose, Ethanol and Xylitol Determination

Glucose and xylose content in both destarched sago fibre hydrolysate (DSH) and fermentation broth were analysed via High Performance Liquid Chromatography (HPLC) by using a Shimadzu Chromatographic System (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT and Shimadzu RID-10A. Prior to the analysis, the samples were centrifuged at 10,000 x g for about five minutes, filtered using 0.2 µm puradisc filter membrane and kept at 4 °C before the sample were injected into an Aminex Fermentation Monitoring Column (Aminex HPX-87H column, 150 x 7.8 mm, BioRad Laboratories, Inc) kept at 60 °C. The mobile phase used was 5 mM H₂SO₄ with a flow rate set at 0.8 mL/min. Standard samples were then prepared using different known concentrations in % w/v of all components of interest, such as glucose, xylose, acetic acid, lactic acid and ethanol. The kinetic parameters for all fermentation profiles were calculated and evaluated based on the following equations (Unrean et al., 2018).

$$Y_{p/s} = \frac{p}{s} \quad \text{Equation 3.8.1}$$

$Y_{p/s}$ = ethanol or xylitol yield

P = actual ethanol/xylitol produced (g)

S= glucose utilised

$$Q_p = \frac{P}{t}$$

Equation 3.8.2

Q_p = volumetric ethanol or xylitol productivity

P = Actual ethanol/xylitol concentration (g/L)

T = fermentation time (h) giving the highest ethanol/xylitol concentration

$$E_f = \frac{Y_{ps} \times 100}{0.511} \quad \text{Equation 3.8.4}$$

E_f = ethanol fermentation efficiency

$Y_{p/s}$ = ethanol yields

0.917 = maximum theoretical ethanol yield of glucose consumption

$$E_F = \frac{Y_{ps} \times 100}{0.917}$$

Equation 3.8.5

E_f = xylitol fermentation efficiency

$Y_{p/s}$ = ethanol yields

0.917 = maximum theoretical xylitol yield of xylose consumption

3.8.6 Statistical Analysis

All the data obtained were calculated for the mean and standard deviation. One-way ANOVA ($\alpha = 0.05$) was used to analyse the data and value less than 0.05 was considered as significant. Significant data was analysed with F-distribution to compare means between treatments. Significant difference between treatments was obtained if p value is less than 0.05. LEAD Technologies, MINITAB Version 17.1.0 was used to perform the statistical analyses.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview

The feasibilities of the xylose production from the destarched sago fibre hydrolysates (DSFH) depends on the operating conditions including residence time which were 30 minutes, 60 minutes, and 90 minutes and dilute sulphuric acid concentration ranges from 0% v/v, 2% v/v, 4% v/v, 6% v/v and 9% v/v and solid to liquid ratio of 5:100, 10:100, 20:100, 30:100 and 40:100. Different pre-treatment approaches give different results (Rafiqul et al., 2014). In this experiment, three approaches were used which were autoclave high pressure steam, hydrothermal and microwave assisted with dilute H₂SO₄ as pre-treatment of the destarched sago fibre. The best approaches pre-treatment method which produces the highest xylose production was chosen and the xylose was further fermented as carbon source by *C. tropicalis* for production of xylitol.

4.2 Characterisation of Destarched Sago Fibre

The composition of hemicellulose and cellulose content of the destarched sago fibre were analysed in order to quantify the raw material for the production of xylose and glucose. This means, the efficiency of the pre-treatment used can be optimised through the rate of conversion from the DSH to glucose and xylose. Table 4.1 summarises the comparison of the composition of raw sago fibre by different studies. However, previously there has no studies about destarched sago fibre. In this study, experiment conducted using NDF and ADF methods showed the composition of cellulose and hemicellulose were 20.72 ± 0.49 % and 10.12 ± 0.24 %. By comparison, the hemicellulose and cellulose content was almost similar with other raw sago fibre found shown in Table 4.1.

Table 4.1: Comparison of the composition of raw sago hampas with a different collection of sago hampas.

Sago fibre collection	Composition(%)		References
	Cellulose	Hemicellulose	
Pusa Sarawak	26.0	14.5	Jeno et al., 2014
Pusa Sarawak	23.5	8.2	Vincent et al., 2019
Pusa Sarawak	21.0	13.4	Jeno et al., 2015

4.3 Physicochemical Pre-Treatment of Destarched Sago Fibre

Physicochemical pre-treatment of destarched sago fibre (DSF) was conducted using three pre-treatment approaches which were high pressure steam, microwave and hydrothermal pre-treatment by employing different concentration of dilute sulphuric pre-treatment which ranges from 0% v/v, 2% v/v, 4% v/v, 6% v/v and 9% v/v of dilute H_2SO_4 . In this experiment, the solid to liquid ratio of DSF was fixed at 7 g/L and the reaction time was fixed at 20 minutes. The purpose of this study is to compare the best physicochemical that yields the highest glucose and xylose concentration. Figure 4.1 shows the glucose and xylose concentration obtained using these three approaches.

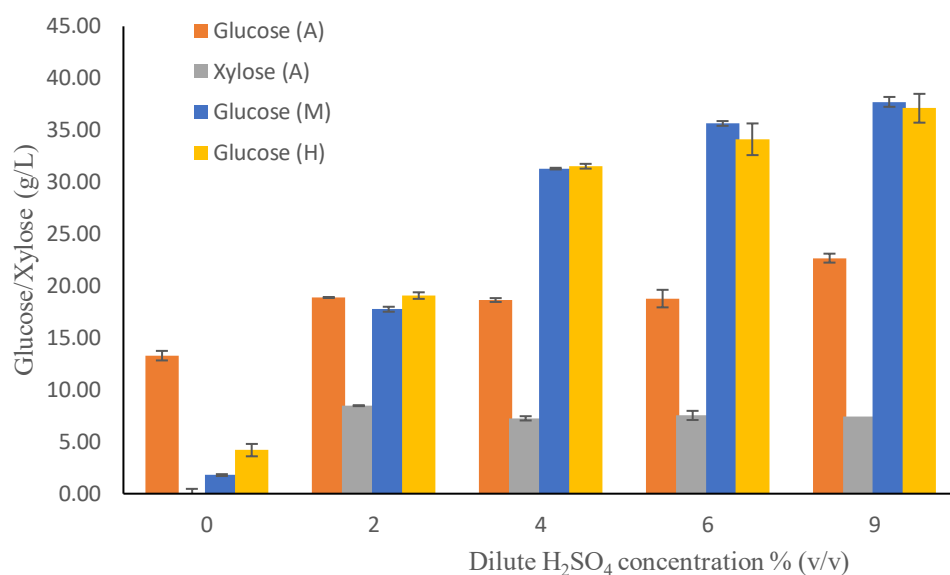


Figure 4.1: Profile of glucose and xylose obtained using different dilute H₂SO₄ concentration, Glucose (A) is glucose obtained from high pressure steam autoclave, Xylose (A) is xylose obtained from high pressure autoclave, Glucose (M) is glucose obtained from microwave and Glucose (H) is glucose obtained from hydrothermal pre-treatment.

4.3.1 Pre-treatment using High Pressure Steam

Pre-treatment of dilute H₂SO₄ using high pressure steam in an autoclave involved disruption of the physical and chemical reaction to disrupt the structure of the lignocellulosic materials (Kurian et al., 2013). This study was conducted to determine the best dilute H₂SO₄ concentration for pre-treatment of the destarched sago fibre (DSF) for xylose production.

Based on the results in Figure 4.1, pre-treatment of dilute H₂SO₄ with 2 % (v/v) of concentration produces highest xylose concentration 6.98 ± 0.03 g/L followed by 9% (v/v) acid concentration 6.95 ± 0.06 g/L then 6% (v/v) acid concentration with of 6.54 ± 0.21 g/L, 4 % (v/v) acid concentration with 6.26 ± 0.04 g/L and lastly 0% with no xylose detected.

At 0% (v/v) concentration of dilute H₂SO₄, there is no xylose detected. This justify at 121°C, dilute acid is needed to solubilise the hemicellulose fraction in the DSF to produces sugars. If lignocellulosic waste is pre-treated with water as the solvent, higher temperature is needed for autohydrolysis to occur in order to solubilise the hemicellulose fraction (Cuevas et al.,2015).

From Figure 4.1, 2% v/v dilute H₂SO₄ is efficient to solubilise the hemicellulose for the DSH. This finding is almost similar with Canilha et al. (2011), which discovered that the 2.5 % of dilute H₂SO₄ is the best concentration in maximizing extensive hemicellulose removal. Increasing the acid concentration to 9% did not cause significant in the xylose concentration. Based on statistical analysis, $p < 0.05$ and showed that 2% v/v and 9% v/v were effective to produce the highest xylose concentration. This finding concluded the best dilute H₂SO₄ to obtain high xylose concentration was 2% v/v and 9% v/v produced highest xylose recovery with xylose concentration of 6.98 ± 0.03 g/L and 6.95 ± 0.06 g/L respectively.

In term of glucose concentration obtained, previous Figure 4.1 shows that increasing dilute H₂SO₄ concentration helps to increase the glucose production however the xylose production shows no significance differences. In term of glucose concentration, the highest concentration was observed using 9% v/v dilute H₂SO₄ with glucose concentration of 22.68 ± 0.43 g/L followed by 6% v/v dilute H₂SO₄ with glucose concentration of 18.79 ± 0.84 g/L, 4% v/v with 18.65 ± 0.18 g/L, 2% v/v with glucose concentration of 18.89 ± 0.01 g/L and lastly 0 % with glucose concentration of 13.29 ± 0.46 g/L. Based on statistical analysis by comparing the glucose concentration obtained, $p < 0.05$ which showed it is statistically significant. Different concentrations of H₂SO₄ can influence the glucose production. The

results showed that the most suitable dilute H_2SO_4 concentration to produce the highest glucose is 9% v/v.

Based on the results obtained in Figure 4.1, it can be concluded that dilute H_2SO_4 pre-treatment yields higher glucose concentration compared to xylose concentration. This is because the acid hydrolysis releases most glucose from the hemicellulose fraction (Debiagi et al., 2020). Also, the cellulose fraction was also solubilised by the dilute acid pre-treatment which contributed to the higher glucose concentration (Lenihan et al., 2010). This study concluded dilute H_2SO_4 with concentration of 9% v/v produced the highest xylose and glucose concentration. By comparison, 2% and 9% v/v of dilute H_2SO_4 produced similar concentration of xylose, therefore, 2% v/v of dilute H_2SO_4 was chosen for further study.

4.3.2 Pre-treatment Using Microwave

In this experiment, the pre-treatment of destarched sago fibre using microwave with different concentration of the acid were tested. The reaction time conducted was 10 minutes with substrate to liquid ratio of 7:100.

Based on result from Figure 4.1, it is shown that the glucose concentration achieved higher yields compared to glucose concentration obtained when using autoclave pre-treatment. The highest glucose concentration obtained when 9 % v/v and 6% v/v of dilute H_2SO_4 were used which were 37.73 ± 0.48 g/L and 35.65 ± 0.23 g/L respectively. The lowest glucose concentration was obtained using 0% v/v of dilute H_2SO_4 with glucose concentration of 1.79 ± 0.09 respectively. Based on statistical analysis by comparing the glucose concentration obtained, $p < 0.05$ which prove that different concentration of dilute H_2SO_4 does have influence on the glucose production and 9% v/v of dilute H_2SO_4 can be used to achieved highest glucose concentration using microwave pre-treatment.

However, in contrast with glucose, there is no xylose detected even though different concentration of acid was used. The result justifies that short reaction time conducted which was 10 minutes were not efficient enough to solubilise the hemicelluloses fraction.

In this experiment, the reaction time for the microwave assisted dilute H_2SO_4 pre-treatment is 10 minutes with low voltage which is 550 W, it is strongly believed that no degradation of hemicelluloses occurred and longer reaction time is needed to yield xylose from DSH. This is supported by Sunarti et al. (2017), which suggested that higher the acid concentration and the longer hydrolysis time is the higher the total sugar and reducing sugar amount of the hydrolysate will be. According to Ji et al. (2017), hydrolysis of lignocellulosic waste for xylose production such as wheat straw requires long reaction time like 90 minutes and 0.5% (v/v) of H_2SO_4 .

The finding from this study concluded that dilute H_2SO_4 pre-treatment using microwave assisted to produce high concentration of glucose. The highest glucose concentration achieved was 37.73 ± 0.48 g/L using 9% (v/v) which was obtained during the hydrolysis.

4.3.3 Pre-treatment Using Hydrothermal Pre-treatment

In hydrothermal pre-treatment, the pre-treatment was conducted with the same reaction time as the autoclave pre-treatment and microwave which was 30 minutes reaction time and same solid to liquid ratio which is 7:100 but at different temperature which was 100 °C.

Based on the result in Figure 4.1, the glucose concentration obtained from hydrothermal pre-treatment were 4.20 ± 0.60 g/L, 19.08 ± 0.32 g/L, 26.53 ± 0.23 g/L, 34.13 ± 2.52 g/L and 37.11 ± 1.39 g/L respectively when using 0, 2, 4, 6, and 9% (v/v) dilute H_2SO_4 concentration. Based on statistical analysis by comparing the glucose concentration obtained, the $p < 0.05$ which showed the result is significant. The highest glucose

concentration achieved was when 9% v/v of dilute H_2SO_4 concentration was used in the hydrolysis. The higher the concentration of dilute H_2SO_4 , the higher the glucose concentration.

Similar with the microwave pre-treatment, this hydrothermal pre-treatment also was not able to yield any xylose from the DSF. The result justifies that hydrothermal pre-treatment using 100 °C was not high enough to solubilise the hemicellulose fraction to produce xylose. The glucose recovery may come from the cellulose-glucose conversion in the DSF. The result suggested that the hydrolysis of lignocellulosic at low temperature requires the help of enzyme. This is supported by the finding of Sathitsuksanoh et al. (2010), which discovered the hydrolysis of bamboo can be conducted at 50 °C with 60 minutes reaction time with the presence of enzyme which yield 86 % and 82.6 % of glucose and xylose respectively.

Another possible reason why xylose was not detected is probably due to the conversion of xylose into another by product. This is due to the fact that prolong reaction time with dilute acid hydrolysis could cause a conversion of xylan through cyclodehydration process into furfural, which is an inhibitory product to fermentation (Mamman et al., 2008).

From the results, it is clearly showed microwave and hydrothermal assisted pre-treatment were not able to recover xylose sugar from the hemicellulose of the DSH. One factor contributes to this result could be due to the crystalline structure of the lignocellulosic. The recalcitrance property of the sago fibre decreases the solubilities of the hemicellulose part to break down into sugars such as xylose and other oligosaccharides (Pang et al., 2018). From this study, it is concluded that high pressure steam is considered the best option to yield xylose sugars as this pre-treatment produces highest xylose

concentration using 2% v/v of dilute H₂SO₄. Further parameter such as reaction time and solid to liquid ratio were further investigated using high pressure steam pre-treatment.

4.4 Effects of Different Reaction Time of Hydrolysis on Glucose and Xylose Concentration

Based on the previous result discussed earlier, pre-treatment of DSF in high pressure steam autoclave produced highest xylose recovery compared with that microwave and hydrothermal. Therefore, the following stage of this study focuses on the effects of different reaction time using dilute H₂SO₄ pre-treatment assisted with high pressure steam in an autoclave on xylose and glucose recovery.

To investigate the effect of reaction time, 7% (w/v) of DSF were treated with three different reaction time which are 30 minutes, 60 minutes and 90 minutes. Based on the previous result, the concentration of the acid was fixed with 2% (v/v) as this concentration produces highest xylose sugar and the temperature was maintained at 121 °C by using autoclave throughout the experiment.

In autoclave pre-treatment, as shown in Figure 4.2, 30 minutes of reaction time produced the highest xylose concentration with 8.42 ± 1.23 g/L, followed by 60 minutes which produces 7.64 ± 0.08 g/L and lastly 90 minutes which produced 6.32 ± 0.58 g/L of xylose concentration. The highest glucose concentration obtained was at 60 minutes followed by 30 minutes and 90 minutes reaction time with glucose concentration of 20.62 ± 0.15 g/L, 16.27 ± 1.22 g/L and 19.67 ± 1.08 g/L respectively. Based on the result from Figure 4.2, the glucose concentration obtained was opposite with the xylose concentration. Higher glucose recovery was observed in the 60 minutes of reaction time. This justifies that cellulose fraction was solubilised to produce glucose and most glucose come from the hemicellulose fraction during the pre-treatment which give higher glucose recovery

compared to xylose. In addition, in longer reaction time, xylose can decompose into furfural (Batista et al., 2019).

In autoclave pre-treatment, glucose and xylose concentration showed decreases concentration with increases reaction time. It is possible that this might cause by the decomposition of the sugar due to the prolong time of heating. The sugar might dehydrate into furan compounds (Yemiş & Mazza., 2011). Also, the result showed the presence of high acetic acid with concentration of 17.67 g/L and 21.98 g/L at 60 minutes and 90 minutes of reaction. High acetic acid concentration in the liquid indicates the sugars are being degraded after their release from the lignocellulosic matrix (Batista et al., 2019). Acetic acid is a product of the auto-hydrolysis of the acetyl ($-\text{COCH}_3$) groups present in the hemicellulose molecules. These compounds are detrimental to the fermentation due to their inhibitory behaviour besides causing the pH decrease of the medium (Batista et al., 2019).



Figure 4.2: Effects of different reaction time on xylose and glucose concentration during high pressure steam autoclave pre-treatment. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

Based on the results, the best reaction time for pre-treatment to produce xylose concentration is by using autoclave with 30 minutes reaction time as this reaction time

produced the highest xylose concentration for xylitol fermentation. Intermeconomical value, shorter reaction time with high sugars recovery is preferred in the industry.

4.5 Effects of The Solid Liquid Ratio on Glucose and Xylose Concentration

In order to investigate the influences of solid: liquid ratio on xylose recovery, four different solid: liquid ratios (5:100, 15:100, 20:100 and 30:100) were studied. Based on previous result, the best acid concentration and reaction time were chosen and were kept constant which is 2% (v/v) of dilute H₂SO₄ and the reaction time is 30 minutes.

In autoclave pre-treatment, based on the result in Figure 4.3, the glucose and xylose concentration increased starting from substrate load 5:100 until 20:100. Based on Figure 4.3, when the solid to liquid ratio was increased from 5% to 20%, the glucose concentration increased from of 18.89 ± 0.01 g/L to 83.61 ± 0.05 g/L respectively.

However, starting from 30:100 of solid to liquid ratio, the glucose concentration obtained from the pre-treatment decreased. The glucose concentration obtained using solid to liquid ratio for 30% and 40% were 78.63 ± 2.22 g/L and 61.11 ± 0.99 g/L respectively. Xylose concentration shows not significant different which were 24.75 ± 0.77 g/L and 28.19 ± 0.78 g/L respectively. This might due to the solvent which is insufficient to fully soak the surface area of the substrate for acid hydrolysis to place. This is supported by Kristensen et al. (2009), which stated that high substrate concentrations can result in substrate inhibition, which significantly lowers the hydrolysis rate. Additionally, the DSH have the ability to absorb the water like a sponge. This caused the sample to expand and reduce the amount of dilute H₂SO₄ solution to react with the DSF. This will reduce the accessibility of the acid attach to the DSH. Thus, not all surface area of the DSF can be covered by the acid for effective hydrolysis to occur.

Based on the ANOVA analysis by comparing the different solid to liquid ratio, the p-value is <0.5 , solid to liquid ratio does influence the production of glucose and xylose and the best solid to liquid ratio for pre-treatment is 305% which produced highest glucose and xylose concentration which were 83.61 ± 0.05 g/L and 28.19 ± 0.19 g/L respectively.. This concentration was chosen for fermentation of xylitol.

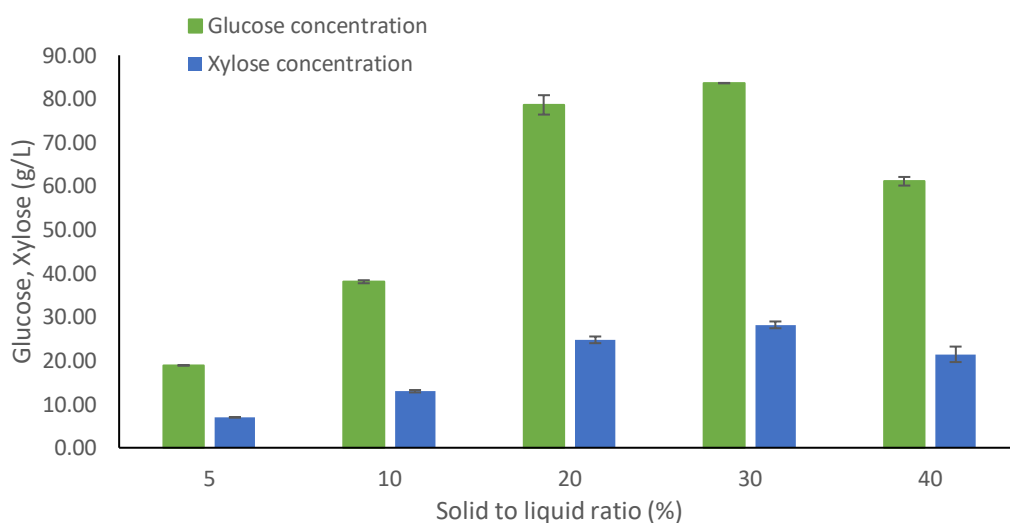


Figure 4.3: Effects of different substrate to liquid ratio (%) on glucose and xylose concentration (g/L) using high pressure steam autoclave pre-treatment. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

4.6 The Dry Cell Weights of The Yeast in The Inoculum Media

Based on Figure 4.4, the dry cell weight of the *C. tropicalis* was analysed before conducting xylitol fermentation. In this study, the growth curves of the *C. tropicalis* during the transition period were characterised by the rapid changes in slope. The lag phase of the yeast is short with significant change in dry cell weight at from 0 hour to 12 hours of incubation. The dry weight of the yeast increased from $0.200 \text{ g/L} \pm 0.001$ at 0 hour to $2.24 \text{ g/L} \pm 0.001$ at 12 hours. During the lag phase, the cell is adjusted to the new environment. It is the transition to the exponential phase after the initial population has doubled (Yates & Smotzer, 2007).

Vrabl et al. (2019), reported that the slopes in the transition periods were either continuously decreasing until a plateau was reached (second transition period) or increasing until the log phase was reached (first transition period). Therefore, precise determination of these two periods is important to determine the log phase. Based on Figure 4.4, the exponential phase of the yeast falls at 6 to 18 hours with rapid increasing weight of the yeast. The weight of the yeast increases rapidly at 6 hours with dry cell of 1.12 ± 0.005 g/L to $2.840 \text{ g/L} \pm 0.001$ g/L. During this 6 to 18 hours, incubation of the yeast leads to increase biomass of the yeast.

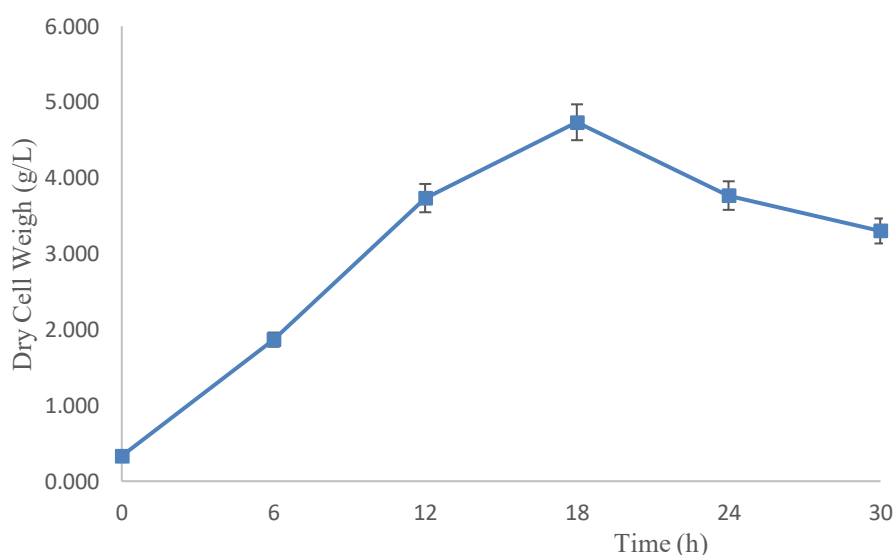


Figure 4.4: The dry weight of the *C. tropicalis* in the inoculum media consisted of yeast extract and commercial glucose and xylose. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

However, after 18 hours, the dry cell weight of the yeast began to decrease. At 18 to 24 hours, there is a decrease in the dry cell weight of the yeast. The dry cell weight of the yeast decreases from 2.840 ± 0.011 g/L to 2.260 ± 0.005 g/L. Based on this study, it is concluded that the yeast entered a short lag phase in the first 6 hours, then entered an exponential phase

at 12 to 18 hours then continued with stationary phase and death phase after 18 hours. In this experiment, the yeast did not show an obvious death phase but the dry cell weight of the yeast decreases after 18 hours which indicated the yeast entering the death phase. Therefore, based on this growth profile curve, the exponential curve which occurred between 6 hours and 18 hours and also 28 hours were taken to study the effect of inoculum age on the fermentation of xylitol.

4.7 Effects of Inoculum Age on Xylitol Fermentation

In this study, three different inoculum ages of *C. tropicalis* were selected in attempt to study the effects of inoculum age for xylitol production. From the previous growth curve obtained from Figure 4.2, three inocula with ages of 9 hours, 18 hours and 28 hours were chosen for fermentation in destarched fibre hydrolysate to produce xylitol. 15% of inoculum level were used and batch fermentation with working volume of 200 ml was conducted at 30 °C with pH 6 and 100 rpm. No external nutrient sources were supplemented for the fermentation. This could be used as a method to determine the optimal inoculum age for *C. tropicalis* for xylitol production using destarched sago fibre hydrolysate.

Based on Table 4.2, the initial glucose concentration in destarched sago fibre hydrolysate (DSFH) for inoculum age of 9, 18 and 28 hours were 22.64 ± 0.35 g/L, 22.51 ± 1.17 g/L and 22.17 ± 0.83 g/L respectively. After 54 hours of fermentation, the remaining glucose concentration for respective inoculum age for 9, 18 and 28 hours were 10.15 ± 0.81 g/L, 9.35 ± 0.50 g/L and 3.14 ± 0.70 g/L. By comparing the glucose consumption in Table 4.2, the consumption of glucose by the yeast in DSFH for inoculum age of 9 hours and 18 hours showed no significance different which were 58.46% and whereas in 28 hours showed significance different with glucose consumption of 85.83%.

Table 4.2: The kinetic parameter of ethanol with different inoculum age of *C. tropicalis*.

Kinetic Parameter	9hours		18hours		28hours	
	DSFH	Control	DSFH	Control	DSFH	Control
Initial glucose concentration (g/L)	22.64±0.35	22.14±0.14	22.51±1.17	22.26±0.03	22.17±0.83	22.18±0.05
Residual glucose (g/L)	10.15±0.81	0.60±0.05	9.35±0.50	0.28±0.06	3.14±0.70	0.23±0.11
glucose consumption efficiency (%)	55.16	97.28	58.46	98.70	85.83	98.96
Maximum Ethanol produced (g/L)	4.60±0.10	7.10±0.40	4.53±0.03	7.24±0.56	5.60±0.10	7.25±0.57
Y _{p/s} (g/g)	0.36±0.004	0.33±0.021	0.35±0.067	0.33±0.027	0.29±0.007	0.33±0.028
Q _P (g/Lh ⁻¹),	0.09±0.001	0.13±0.007	0.08±0.0005	0.13±0.01	0.10±0.007	0.13±0.01
Fermentation efficiency(%)	71.29±0.78	64.55±4.22	68.53±9.19	64.47±5.23	0.58±0.01	64.64±5.49

Notes: Parameters studied: Y_{p/s} ethanol yield (g/g), Q_P ethanol volumetric productivity (g/Lh⁻¹), Ef fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical ethanol yield is 0.511 g/g glucose.

Based on the results in Figure 4.5 and 4.6, in DSFH, it showed the yeast consumed only glucose in the first 9 hours of fermentation as the xylose concentration remained unchanged. In all experiments, the glucose is not fully consumed by the yeast even after 54 hours of fermentation. After 54 hours of fermentation, the glucose consumption in 9,18 and 28 hours of inoculum age were 65%, 59.41 % and 85.83% respectively. The possible reason the glucose is not fully consumed could cause by the absent of nitrogen sources to support the cell growth. This is supported by previous studies which reported that nitrogen sources such as urea, amino acid and peptone helps to promote the cell growth and subsequently increase the xylitol production (Zhang et al., 2012; Rao et al., 2016). The absence of these

nitrogen sources causes prolonged time of glucose consumption for cell growth. Therefore, yeast could enhance the cell growth and consume the sugar present in the DSFH efficiently with the presence of nitrogen sources.

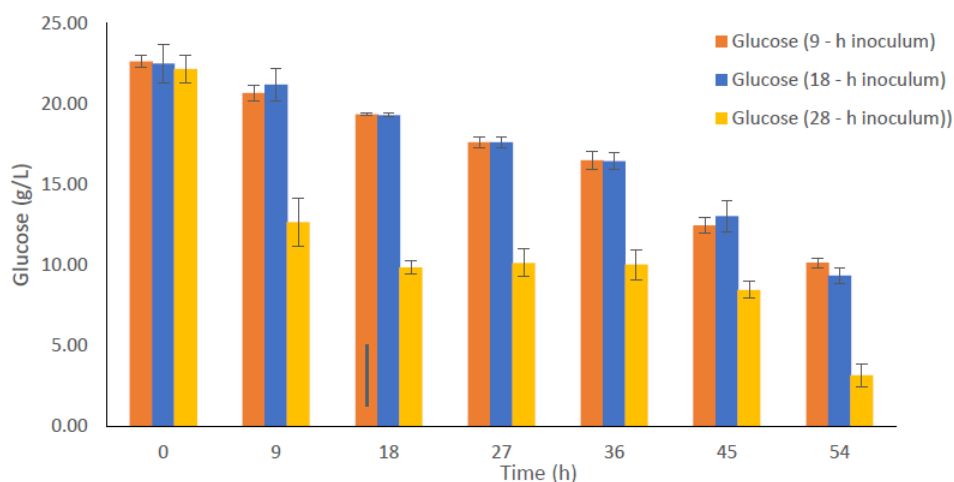


Figure 4.5: The glucose consumption in destarched sago fibre hydrolysate (DSFH) by different inoculum age of 9,18 and 28 h. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

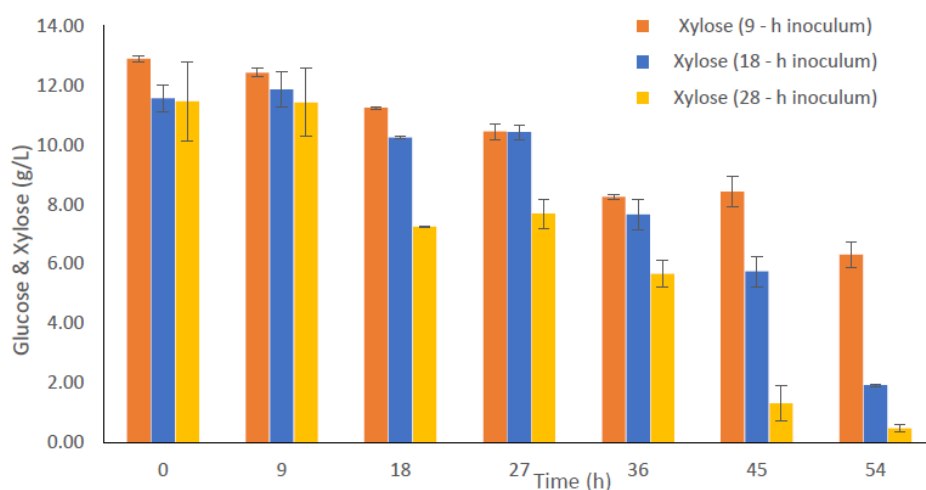


Figure 4.6: The xylose consumption in destarched sago fibre hydrolysate (DSFH) by different inoculum age of 9,18 and 28 h. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

From Table 4.3, the initial xylose concentration for respective experiments were 12.90 ± 0.09 g/L, 11.56 ± 0.45 g/L and 11.46 ± 1.35 g/L. Based on Figure 4.6, the xylose consumption increases and after 54 hours of fermentation, the residual xylose is 0.81 ± 0.07 g/L, 1.92 ± 0.04 g/L and 0.47 ± 0.12 g/L respectively. Based on the Table 4.3, the highest xylitol production for 9 hours and 18 hours inoculum age in DSFH was the same which is at 45 hours with the xylitol concentration of 0.57 ± 0.05 g/L and 0.70 ± 0.14 g/L respectively. The xylose consumptions at 45 hours for inoculum age of 9 hours and 18 hours is 51% and 50.3% respectively. However, after 45 hours of fermentation, the concentration of the xylitol decreases to 0.41 ± 0.16 g/L and 0.29 ± 0.03 g/L respectively. Based reported literature, the decreasing concentration of xylitol due to the presence of enzyme xylitol dehydrogenase (XDH) in *C. tropicalis* prevents the xylitol accumulation by oxidizing it to xylulose (Zhang et al., 2021).

Table 4.3: The kinetic parameter of different inoculum age on the production of xylitol.

Kinetic Parameter	9hours		18hours		28hours	
	DSFH	Control	DSFH	Control	DSFH	Control
Initial xylose concentration (g/L)	12.90 ± 0.01	12.90 ± 0.09	11.56 ± 0.45	12.96 ± 0.05	11.46 ± 1.35	13.05 ± 0.05
Xylose residual (g/L)	8.43 ± 0.01	0.47 ± 0.16	5.74 ± 0.52	0.49 ± 0.07	0.47 ± 0.12	0.48 ± 0.09
Xylose consumption Efficiency (%)	34.65	96.36	50.34	96.21	95.90	96.32
Maximum Xylitol produced (g/L)	0.57 ± 0.05	2.41 ± 0.16	0.70 ± 0.14	2.56 ± 0.11	0.86 ± 0.11	2.56 ± 0.11

Table 4.3 Continued

$Y_{p/s}$ (g/g)	0.13±	0.19±	0.13±	0.204±	0.08±	0.203±
	0.02	0.01	0.05	0.007	0.008	0.008
Q_p (g/Lh)	0.011±	0.07±	0.012±	0.049±	0.021±	0.05±
	0.001	0.002	0.003	0.001	0.001	0.003
Fermentation	14.04±	21.11±	13.94±	22.35±	8.48±	22.15±
efficiency(%)	2.42	1.32	4.97	0.71	0.09	0.85

Notes: Parameters studied: $Y_{p/s}$ xylitol yield (g/g), Q_p xylitol volumetric productivity (g/Lhr), Ef fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical xylitol yield is 0.917 g/g xylose

On the other hand, the possible reason low concentration of xylitol due to the viability of the yeast cell. The DSFH was not supplemented with any nitrogen sources to promote the growth of the yeast cell. The addition of nitrogen sources could increase the production of xylose to xylitol (Zhang et al., 2012). The result of the consumption of sugars by different inoculum age is shown in Figure 4.6.

Based on Figure 4.7 and 4.8, it is demonstrated the inoculum age of 28 hours produced the highest xylitol at 54 hours of fermentation in DSFH with xylitol concentration of 0.86 ± 0.11 g/L with xylose consumption of 96%. This result showed prolonged fermentation can help to increase the final xylitol concentration.

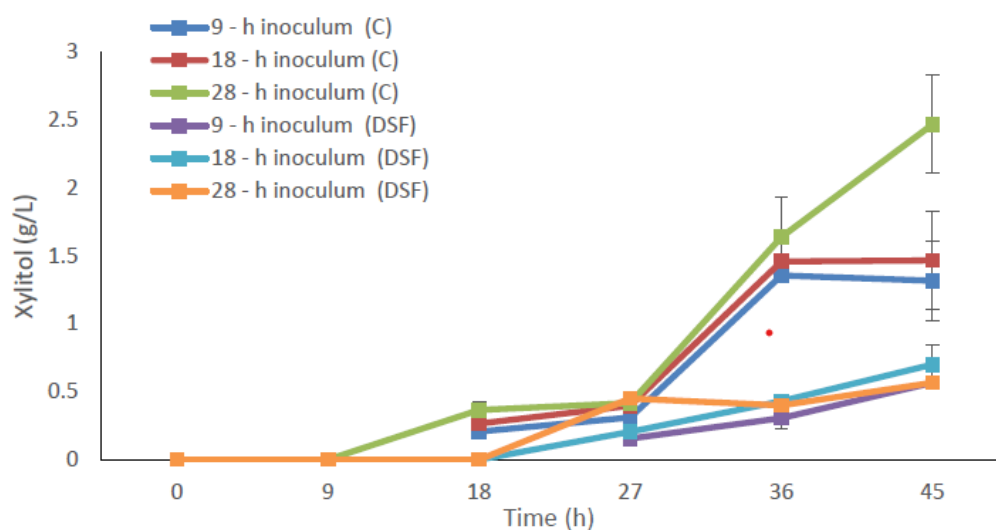


Figure 4.7: The production of xylitol from destarched sago fibre hydrolysate and control experiment (commercial glucose and xylose) using different inoculum age of 9, 18 and 28h. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

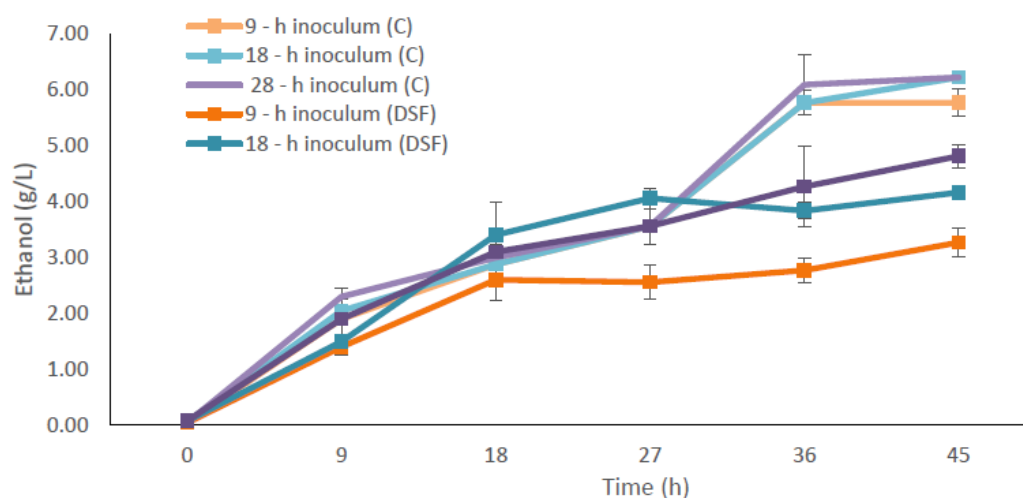


Figure 4.8: The production of ethanol from destarched sago fibre hydrolysate and control experiment (commercial glucose and xylose) using different inoculum age of 9, 18 and 28h. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

Along with xylitol, batch fermentations of this DSFH by the *C. tropicalis* also produced ethanol as by-products. Based on the sugar consumption results from Figure 4.6, the decreasing concentration of the glucose indicated *C. tropicalis* consumed the carbon sugar

for the production of ethanol. This is proven with the production of ethanol with decreasing concentration of glucose. In the early stationary phase, an increased number of active viable cells used carbon producing the ethanol as the product of fermentation; however, as the inoculum age increased, the number of viable cells decreased as a result of nutrient depletion (Kadam et al., 2006).

By comparison of xylitol and ethanol production in DSF hydrolysate, the xylitol was only produced at 27 hours of fermentation with concentration of 0.16 g/L, 0.21 g/L 0.45g/L in respective 9 hours while ethanol was produced even at 0 hours of fermentation. The reason for this is because the glucose was first consumed by yeast as the xylose concentration remained constant before 28 hours. The yeast ferments the glucose into ethanol and then starts to ferment xylose into xylitol. Similarly, with the result of the study, the finding from Leathers (2003)., also discovered the yeast produces ethanol until the glucose is consumed and then they start to ferment xylose and arabinose. The highest ethanol production for inoculum age 9, 18 and 28 hours are at 54 hours which are 4.60 ± 0.10 g/L, 4.53 ± 0.03 g/L and 5.60 ± 0.10 g/L with glucose consumption of 55.16%, 58.45% and 85.83% respectively. It demonstrates that prolonged fermentation could possibly increase the ethanol concentration. Contrary with experiment conducted by Mohammad et al. (2020), 50 g/L glucose from sago fibre media is capable of generating maximum ethanol concentration at 20.33 ± 0.15 g/L, with highest glucose consumption efficiency (97.78 %) during 24 hours of fermentation by using baker's yeast. In this study, fermentation using yeast inoculum age of 28 hours showed higher glucose consumption compared with yeast inoculum age 9 and 18 hours.

Based on Figure 4.9, the result of the control experiment using commercial glucose and xylose showed consistent increases of xylitol and ethanol. The initial glucose concentration

is 22.14 ± 0.14 g/L, 22.26 ± 0.03 g/L and 22.18 ± 0.05 g/L with xylose concentration of 12.90 ± 0.09 g/L, 12.96 ± 0.05 g/L and 13.05 ± 0.05 g/L respectively. The yeast produced highest ethanol at 54 hours of fermentation with concentration of 7.10 ± 0.40 g/L, 7.24 ± 0.56 and 7.25 ± 0.57 g/L with consumption 97 %, 99 % and 99 % of glucose respectively. Unlike in destarched fibre hydrolysis, the glucose is almost consumed completely by the yeast in all control experiments.

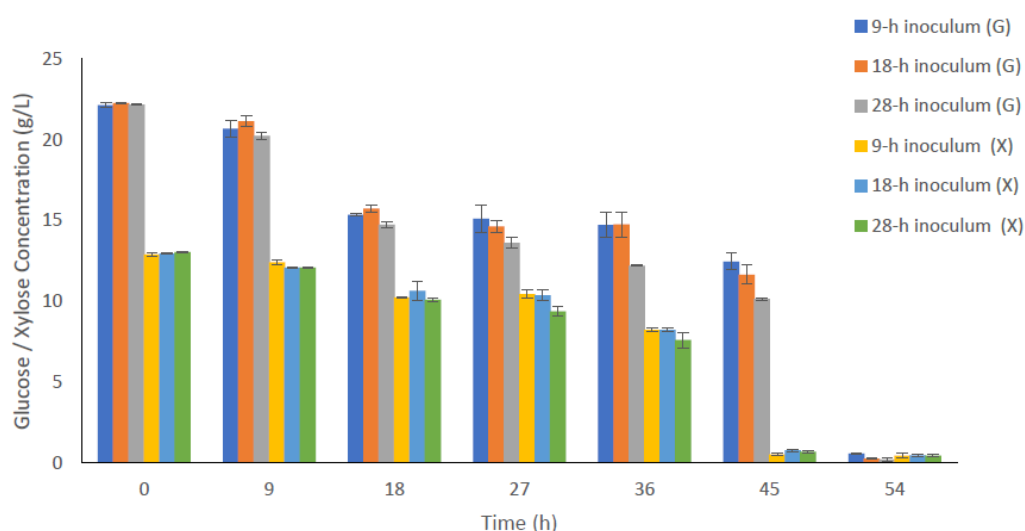


Figure 4.9: Consumption of glucose and xylose during fermentation using 9 h , 18 h and 28 h of inoculum age in control experiment using commercial glucose and xylose. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments. Notes: G is glucose and X is xylose.

By comparison, the xylitol produced in control experiment was at 18 hours unlike in destarched fibre hydrolysate which xylitol only produced at 27 hours. The xylitol produced in the control experiment was also higher compared in the hydrolysate. The highest xylitol fermentation in the control experiment was at 54 hours. The xylitol produced was 2.41 ± 0.16 g/L, 2.56 ± 0.11 g/L and 2.56 ± 0.11 g/L respectively with xylose consumption of 96 % respectively.

The result as shown in Figure 4.9 demonstrated the yeast consume sugar better in control experiments compared in hydrolysate. The reason for this is because the earlier pre-treatment of destarched fibre using dilute H_2SO_4 hydrolysis causes the existing pentose and uronic acid in the lignocellulosic to undergo dehydration with formation of 2-furaldehyde, hereafter referred to as furfural, while the hexoses are dehydrated to 5-hydroxymethyl-2-furaldehyde, hereafter referred to as HMF. Furfural has been described to damage microbial cell wall and membrane, inhibit growth, reduce enzymatic activities and the rate of fermentation (Moysés et al., 2016).

The result of this study suggested that inoculum age of yeast 28 hours can be chosen for fermentation as it produces higher xylitol with maximum xylitol production of compared to inoculum ages of 9 hours and 18 hours after 54 hours of fermentation with xylitol concentration of 0.86 ± 0.11 g/L with xylose consumption of 96%. Also, the yeast age 28 hour of inoculum consume almost 85.83% which is highest compared to inoculum age 9 hour and 18 hours.

Table 4.5 summarises the kinetic parameters of xylitol productions using destarched sago fibre hydrolysate. The result has proven the inoculum age of the yeast does influence the xylitol production and addition of nitrogen sources in the destarched fibre hydrolysate is needed to increase the cell growth for better sugar consumption and xylitol production. Therefore, the inoculum age of 28 hours was chosen for further fermentation experiment.

Table 4.4: The kinetic parameter of xylitol productions using destarched sago fibre hydrolysate.

Kinetic Parameter	9 hours		18 hours		28 hours	
	DSFH	Control	DSFH	Control	DSFH	Control
Initial xylose concentration (g/L)	12.90± 0.09	12.90± 0.09	11.56± 0.45	12.96± 0.05	11.46± 1.35	13.05± 0.05
Xylose residual (g/L)	8.43± 0.01	0.47± 0.16	5.74± 0.52	0.49± 0.07	0.47± 0.12	0.48± 0.09
Xylose consumption	34.65	96.36	50.34	96.21	95.90	96.32
Efficiency(%)						
Maximum Xylitol produced (g/L)	0.57± 0.05	2.41±0.16	0.70±0.14	2.56±0.11	0.86± 0.11	2.56± 0.11
$Y_{p/s}$	0.13± 0.02	0.19± 0.01	0.13± 0.05	0.204± 0.007	0.08± 0.008	0.203± 0.008
$Q_p(g/Lh^{-1})$	0.011± 0.001	0.07± 0.002	0.012± 0.003	0.049± 0.001	0.021± 0.001	0.05± 0.003
Fermentation efficiency(%)	14.04± 2.42	21.11± 1.32	13.94± 4.97	22.35± 0.71	8.48± 0.09	22.15± 0.85

Notes: Parameters studied: $Y_{p/s}$ ethanol yield (g/g), Q_p xylitol volumetric productivity (g/Lh⁻¹), Ef fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical xylitol yield is 0.917 g/g xylose.

4.7.1 Growth Profile of Fermentations Using Different Inoculum Age

The dry cell weight of the yeast was taken in order to investigate the growth profile of the different age of the yeast in the DSFH media and control media. Based on Figure 4.6.6, the yeast with inoculum age of 9 hours, 18 hours and 28 hours enters the lag phase in the first 9 hours of fermentation. At 18 hours of fermentation, the yeast with inoculum age

of 9 hours shows highest dry cell weight of 11.13 ± 10.50 g/L compared to inoculum age of 18 hours and 28 hours which were 8.56 ± 2.81 g/L and 3.19 ± 2.69 g/L respectively. However, in terms of xylitol production the inoculum age of 9 hours produces the low xylitol production compared with inoculum age 28 hours.

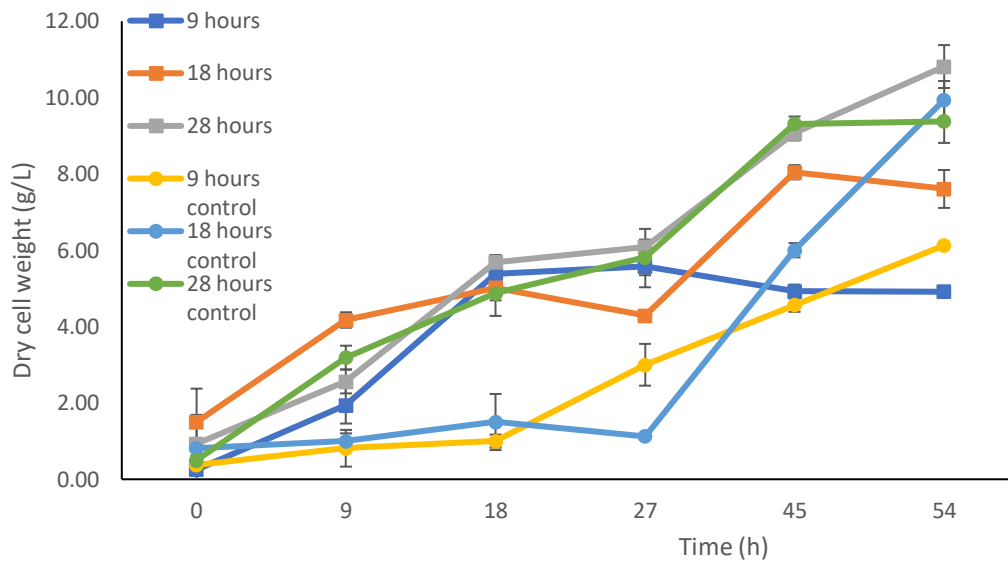


Figure 4.10: The dry cell weight of the *C. tropicalis* in fermentation media of destarched sago fibre hydrolysate (DSH) and control media by using different inoculum age of 9, 18 and 28h. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

Fermentation using the inoculum age of 9 hours reached the maximum weight at 27 hours of fermentation with weight of 10.06 ± 1.94 g/L while inoculum age of 18 hours and 28 hours achieved maximum weight at 54 hours with dry cell weight of 14.38 ± 8.13 g/L and 16.94 ± 5.56 respectively. In control experiment, fermentation using inoculum age of 9, 18 and 28 hours showed consistent increase in weight and reached maximum dry cell weight at 54 hours of fermentation with dry cell weight of 6.12 ± 4.88 g/L, 9.94 ± 9.56 g/L and 9.37 ± 0.12 g/L. The result showed that the yeast grows better in DSH compared with commercial media consisting glucose and xylose. This is because the DSH itself has nutrients and minerals to support the growth of the yeast (Mohammad et al., 2020).

From this study, it is concluded that the dry cell weight of the yeast with inoculum age of 9 achieved the fastest maximum weight compared with inoculum age of 18 hours and 28 hours. Inoculum age of 9 hours with short lag phase, will be chosen for the fermentation of DSH for production of xylitol.

4.8 Effects of Inoculum Concentration on the Fermentation

In order to investigate the effect of the inoculum concentration towards carbon sources consumption and production of xylitol and ethanol, inoculum concentration with 10 %, 20% and 30% were conducted in this study. Based on the previous result, the inoculum age of 9 hours was chosen for fermentation and the experiments were conducted at 30 °C with pH 6, agitation rate 100 rpm. No external nutrient sources were supplemented for the fermentation and the pH was not maintained throughout the fermentation.

Based on the result on Figure 4.11, the initial glucose concentration for fermentation using inoculum concentration of 10%, 20% and 30% were 22.64 ± 0.64 g/L, 22.17 ± 0.83 g/L and 22.64 ± 0.36 g/L meanwhile xylose concentration were 12.90 ± 0.09 g/L, 11.46 ± 1.35 g/L and 12.90 ± 0.09 g/L respectively.

In the first 9 to 18 hours, the glucose was consumed first by the yeast. At 18 hours, the glucose consumption of all fermentation showed significant difference whereas the xylose concentration only showed slight decreases. Almost 61.8% of glucose was consumed by the yeast with a starting inoculum concentration of 30% whereas for the glucose consumption were 55% and 3.5% for fermentation using inoculum size of 20% and 10% respectively.

Unlike glucose, the xylose consumption does not show an obvious decreased at 18 hours. The cells in fermentation using inoculum of 20% consumes the highest xylose

followed by 30% and 10% with xylose consumption of 26%, 56% and 3%. The sugar consumption continues to increase with increasing fermentation time. This result illustrated the higher inoculum density, the higher the consumption of carbon source by the yeast cells.

After 54 hours of fermentation, fermentation using 30% inoculum concentration consumes most glucose followed by fermentation using 20% and 10% inoculum concentration with glucose consumption of 98%, 86% and 22%. Nearly all glucose was consumed in 30% of inoculum quantity. The 10% starting inoculum quantity showed the lowest sugar consumption compared to 20 % and 30%.

On the other hand, the xylose consumption at 54 hours fermentation for fermentation using 30%, 20% and 10% inoculum concentration were 41%, 96% and 18% correspondingly. The 20% starting inoculum quantity consumed the highest xylose followed by fermentation using 30% and 10%. Fermentation using Inoculum concentration of 30% was not able to consume the xylose efficiently even though it can almost consume the glucose completely after 54 hours. This result suggested the high number of active yeast cells might compete to consume glucose for the cell growth. On the contrary, fermentation using 10% starting inoculum quantity was not able to consume both glucose and xylose after 54 hours of fermentation. The possible reason fermentation using 10% starting inoculum concentration does not consume the sugar efficiently is due to the presence of inhibitors in the fermentation media (Juanssilfero et al., 2018). Low level of inoculum level will be affected by the inhibitor which causes the consumption of glucose to be affected (Juanssilfero et al., 2018). Prior acidic pre-treatment produces degradation compounds which can inhibit the fermentation process. The degradation products such as furan derivatives (furfural and HMF), acetic acid and phenolic compounds inhibit fermentation by different mechanism and a combination of the action of several compounds other than

sugars present in the hydrolysate may be one of the reasons for the inhibition (Behera et al.,2014).

Furthermore, the inoculum concentration of 10% might not be sufficient to overcome the inhibitors in the fermentation media. Juanssilfero et al. (2018), highlighted the significant effect that inoculum size employed on the fermentation and found that high inoculum sizes could dilute the rate-limiting substrate concentration in the medium. In other words, the limiting substrate in the medium can be hindered by increasing the inoculum size. This is supported with the finding by Wang et al. (2015), the inhibition for fermentation of xylitol production using undetoxified corn straw hydrolysate can be overcome by increasing the inoculum size to 1.6 g/L.

Based on Figure 4.11, in a control experiment, the initial glucose and xylose concentration was shown according to the sugar concentration in destarched fibre hydrolysate. The glucose concentration for fermentation using 10 %, 20 % and 30 % starting inoculum level were 22.21 ± 0.08 g/L, 22.18 ± 0.05 g/L and 22.02 ± 0.01 g/L and xylose concentration were 12.05 ± 0.08 g/L , 13.05 ± 0.05 g/L and 13.05 ± 0.05 g/L respectively. By comparing the lignocellulosic hydrolysate with the control experiment, the control experiment showed higher glucose and xylose consumptions. After 54 hours of fermentation, the 20% and 30% starting inoculum level consumed almost all glucose with 99% of glucose consumption whereas 10% starting inoculum level only consumed 34% of glucose. The result proved that lower inoculum level increases the fermentation time. Unlike glucose, the xylose does not fully consume at 54 hours. Only 3% of xylose was consumed by 10% starting inoculum level and 50% in 30% starting inoculum level. 20% starting inoculum level consumed the highest xylose with xylose consumption of 96%. Contrasting with the result in destarched fibre hydrolysate, the xylose consumption was

very low compared to xylose consumption in the control experiment. This illustrated the presence of inhibitors in the hydrolysate could slow down the fermentation process.

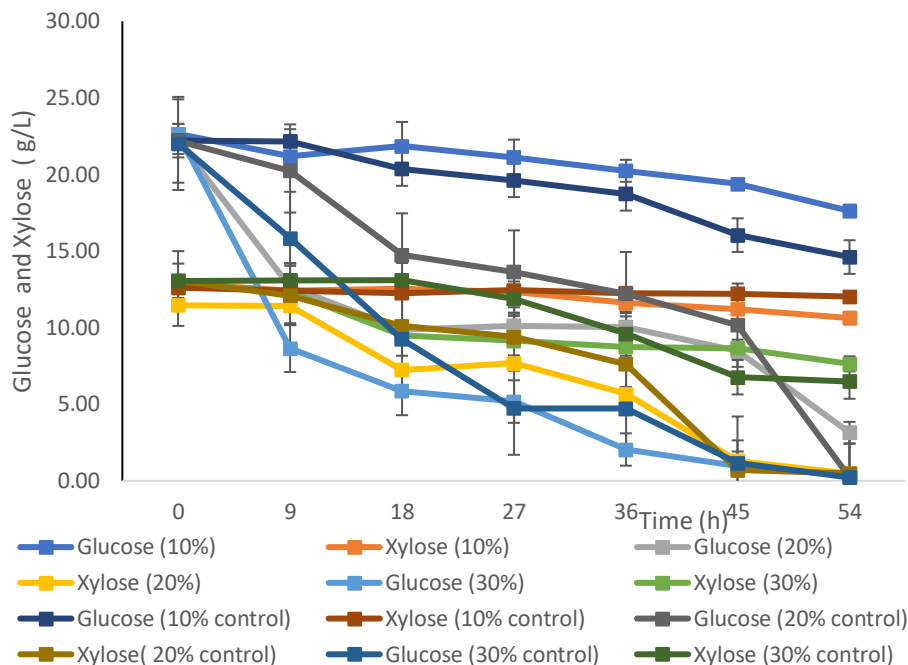


Figure 4.11: The glucose and xylose consumption in DSFH and commercial xylose with glucose (control) using different inoculum quantity 10%, 20% and 30%. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

Based on Figure 4.12, in terms of ethanol and xylitol production in destarched sago fibre hydrolysate fermentation using 10% starting inoculum concentration showed the slowest ethanol production. At 18 hours, only 0.10 ± 0.01 g/L of ethanol was produced. Unlike fermentation using 20% and 30% starting inoculum quantity, the ethanol was produced in small concentrations even at 0 hours with concentration of 0.08 ± 0.01 g/L and 0.12 ± 0.10 g/L respectively. The reason that can be deduced from this result is the higher concentration of starting inoculum ratio, the higher the consumption of carbon source in the fermentation. Subsequently, the faster the glucose metabolized into ethanol. The highest ethanol production was at 54 hours for fermentation using 10%, 20% and 30% inoculum were 2.38 ± 1.18 g/L 5.60 ± 0.10 g/L 4.27 ± 0.59 g/L, respectively.

As shown in Figure 4.12, in destarched sago fibre hydrolysate, xylitol was produced at 36 hours of fermentation for 10% inoculum ratio, 27 hours for 20% inoculum quantity and 18 hours for 30% inoculum quantity. The delay in the xylitol production could be caused by delay consumption of the xylose because xylose utilisation only took place only when glucose had been completely metabolised (Tamburini et al., 2010). By comparison in Figure 4.12, in the control experiment, *C. tropicalis* started to consumed the xylose at 18 hours and produced xylitol. The xylitol was produced at 18 hours when fermentation using 20% and 30 % with xylitol concentration of 0.37g/L and 0.17 g/L respectively.

Based on Figure 4.12, at 54 hours fermentation, fermentation using 20% inoculum showed that the highest xylitol production with 0.86 ± 0.11 g/L followed by fermentation using 30% and 10% inoculum with concentration of 0.44 ± 0.01 g/L and 0.14 ± 0.01 g/L. Fermentation using 5% of starting inoculum quantity (data not shown) not produce any xylitol even after 54 hours of fermentation. The result demonstrated fermentation using 20% inoculum is effective for xylitol fermentation. The reason 30% of starting inoculum quantity showed lower xylitol concentration might be due to the viability of the yeast cell. The high concentration of yeast cells may not be viable after long hours of fermentation due to the competition of the glucose for cell growth. This is proven with the dry cell weight of the yeast and glucose consumption. The dry cell weight of the yeast achieved consistent growth at 36, 45 and 54 hours with dry cell weight of 15.13 ± 7.27 g/L, 15.47 ± 3.87 and 15.83 ± 0.83 g/L respectively. Also, the glucose was almost fully consumed at 45 hours of fermentation with leftover glucose concentration of 0.97 ± 0.26 g/L.

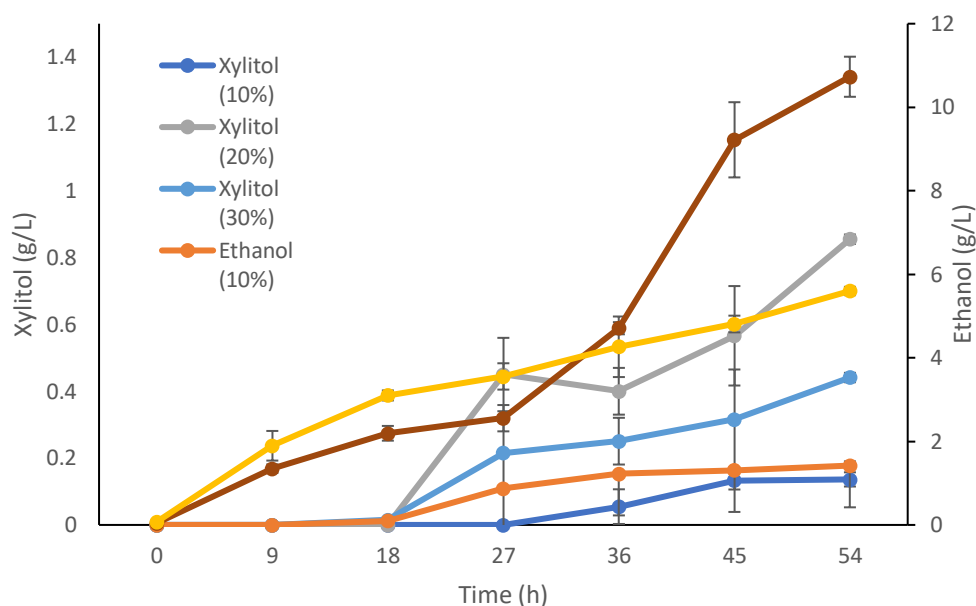


Figure 4.12: Ethanol and xylitol production in fermentation in destarched sago fibre hydrolysate using 10%, 20% and 30% of inoculum quantity.

As shown in Figure 4.13 in control experiment, both fermentation using 20% and 30% inoculum quantity produced xylitol at 54 hours with concentration of 3.10 ± 0.12 g/L and 0.02 ± 0.01 g/L. The result illustrated higher concentration of inoculum ratio may not be effective to produce xylitol because the yeast cell compete for glucose for cell growth as there was no nutrients added into the media. Accordingly, increasing the initial inoculum quantity will lead to an increase in the active cell fraction whereas a higher number of active cell fractions will increase the probability that one or more cells in the population is able to grow. This is also justified with the dry cell of the different inoculum concentration for 20 % and 30% at 54 hours of fermentation with dry cell weight of 5 ± 0.07 g/L and 13.47 ± 2.47 g/L respectively. Also, this explained the reason the fermentation using 10% starting inoculum ratio produced little xylitol at 27 hours with concentration of 0.02 ± 0.01 g/L.

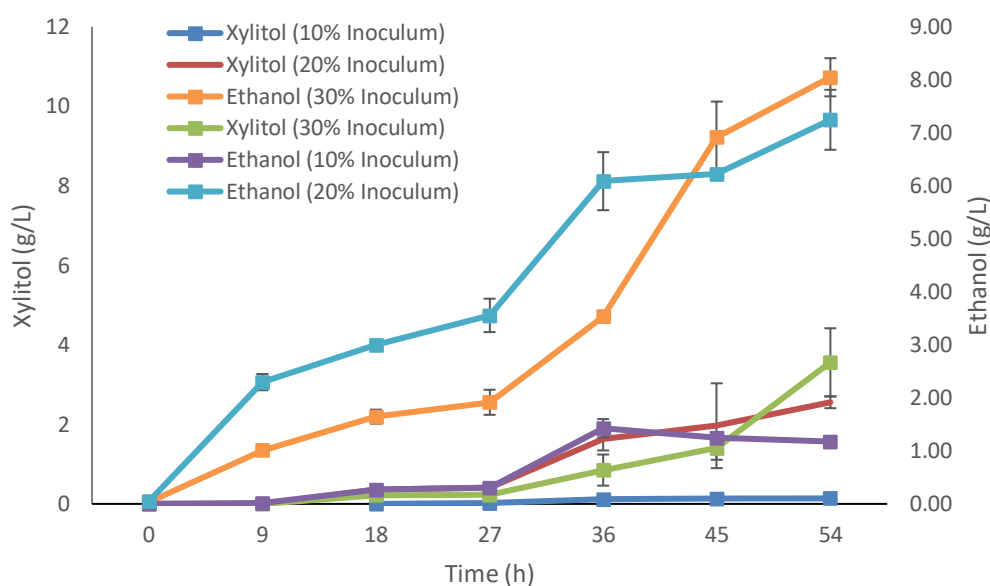


Figure 4.13: Ethanol and xylitol production in fermentation using control media consisting of commercial glucose and xylose with the inoculum density of 10%, 20% and 30% of inoculum quantity.

From industrial point of view, higher specific carbon source consumption rate reflects the potential economic benefit of lower capital and operating costs from reduced fermenter capacity. Based on the discussion and Table 4.6, the highest xylose consumption and highest fermentation efficiency achieved by fermentation using 20% inoculum was 72.60 % and 22.15 ± 0.85 %.

It is concluded that 20% inoculum was chosen for the fermentation due to effective glucose and xylose consumption as well as high xylitol production as early at 27 hours compared with other inoculum concentration investigated in this study.

Table 4.5: The kinetic parameter of xylitol production by using different inoculum age.

Kinetic Parameter	10%		20%		30%	
	DSFH	Control	DSFH	Control	DSFH	Control
Initial xylose concentration(g/L)	12.90± 0.09	12.47± 0.16	11.46± 1.35	13.05± 0.05	12.90± 0.09	13.05± 0.05
Residual xylose	10.62± 0.41	10.62± 0.41	3.14± 0.70	0.48± 0.09	9.15± 0.04	6.48± 0.09
Xylose consumption Efficiency(%)	17.67	14.83	72.60	96.32	29.07	50.34
Maximum Xylitol produced	0.14± 0.01	0.14± 0.02	0.86± 0.11	2.56± 0.11	7.63± 0.49	2.67± 0.45
$Y_{p/s}$ (g/g)	0.062± 0.015	0.011± 0.001	0.077± 0.0009	0.309± 0.008	0.079± 0.0003	0.57± 0.07
Q_P (g/Lh)	0.003± 0.0003	0.003± 0.0003	0.102± 0.001	0.047± 0.002	0.009± 0.003	0.049± 0.008
Fermentation efficiency(%)	6.78± 1.65	1.19± 0.12	57.61± 1.43	22.15± 0.85	6.35± 2.48	44.31± 7.76

Notes: Parameters studied: $Y_{p/s}$ xylitol yield (g/g), Q_P ethanol volumetric productivity (g/L_{hr}), E_f fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical xylitol yield is 0.917 g/g xylose.

4.9 Effects of Selected Nitrogen Sources on *C. tropicalis* Fermentation

The aim of this study is to investigate whether different nitrogen sources in the hydrolysate could increase the yeast ability to consume the carbon sources and produces

high ethanol and xylitol. DSF hydrolysate without any supplements and commercial sugars (xylose and glucose) was used as control in all experiments.

4.9.1 Growth Profile of *C. tropicalis*

Based on the *C. tropicalis* growth profile from Figure 4.14, the yeast achieved different maximum growth it was observed that in destarched sago fibre hydrolysate with addition of yeast extract have the highest yeast growth rate at 72 hours with dry cell weight of 6.20 ± 0.05 g/L followed by that attained in fermentation using destarched sago fibre hydrolysate with addition of yeast extract (6.14 ± 0.002 g/L) achieved at 84 hours. In fermentation using destarched sago fibre without any supplementation, the yeast achieved maximum dry cell weight at 5.61 ± 0.000 g/L.

In contract, the yeast achieved earlier maximum growth weight in control experiments. The *C. tropicalis* showed maximum growth weight in fermentation using commercial medium containing peptone and yeast extract at 36 hours (21.65 ± 0.20 g/L and 28.98 ± 0.93 g/L) respectively. This is consistent with the finding by Zhang et al. (2019), which showed that the yeast extract was the best nutrient supplier for *C. tropicalis* cell growth.

As shown in Figure 4.14, the culture containing solely commercial glucose and xylose showed lower yeast growth compared to destarched sago fibre hydrolysate without any supplementation of nitrogen sources. The cell showed the highest cell growth at 84 hours with dry cell weight of 5.06 ± 0.004 g/L growth whereas in culture using destarched sago fibre, the yeast achieved the highest cell growth at 48 hours with dry dell weight of 5.55 ± 0.001 g/L. This is because the culture does not contain nitrogen sources to support the yeast growth.

In this study, it is concluded that yeast extract alone could enhance the growth of the *C. tropicalis* yeast in fermentation using destarched sago fibre hydrolysate. Furthermore, studies by Mohammad et al. (2020) discovered sago fibre hydrolysate contained essentials such as magnesium and phosphorus to support the yeast growth. According to Silva et al. (1998), metal ions, such as K^+ , Cu^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Zn^{2+} and Co^{2+} , are needed in yeasts to activate the enzymes involved in biosynthetic reactions. The result obtained contradicts with the finding of Zhang et al. (2019) which discovered different nitrogen sources with equal content under the same condition does not enhance the xylitol productions by the yeast.

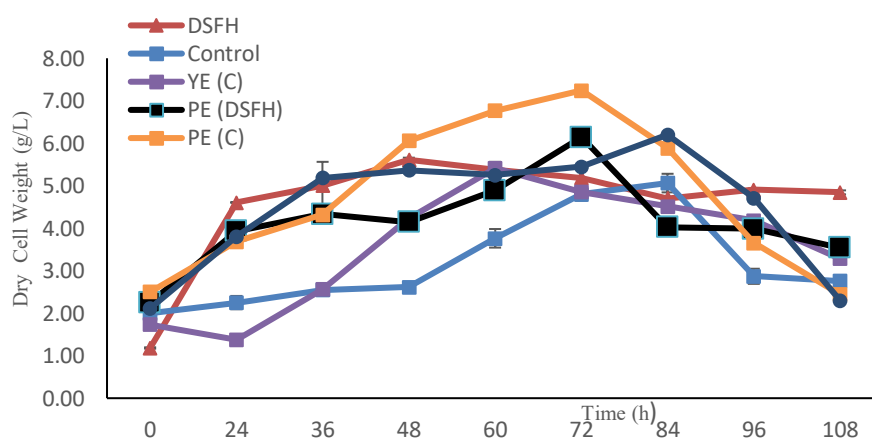


Figure 4.14: Cell growth profile of *C. tropicalis* in commercial xylose and glucose (C), in commercial xylose and glucose supplemented with yeast extract (C+YE), in destarched sago fibre hydrolysate supplemented with yeast extract (DSFH+YE), and in destarched sago fibre hydrolysate supplemented with peptone (DSFH+PE) and commercial xylose and glucose supplemented with peptone (C+PE).

4.9.2 Carbon Source Consumption

Based on the Figure 4.15, the glucose consumption by *C. tropicalis* in fermentation using destarched fibre hydrolysate with nitrogen sources reduces significantly after 24 hours were 66.56 %, 61.96 % and 5.19% respectively. The residual glucose concentration after 24 hours was 19.19 ± 10.83 g/L with in fermentation added with peptone and 16.73

± 0.55 g/L in fermentation added with yeast extract respectively. However, the remaining glucose in cultures without any addition of nitrogen sources was still high even after 24 hours of fermentation which was 48.99 ± 0.09 g/L. In control experiment, the cultures using commercial sugar media consisting glucose, xylose and added peptone and yeast extract showed a complete glucose consumption after 24 hours which was earlier than the control experiment without nitrogen sources. In comparison, *C.tropicalis* only consumed glucose completely after 36 hours in media without addition of nitrogen sources. The result concluded that the addition of nitrogen sources significantly helps the yeast to consume glucose significantly.

In contrast with glucose consumption, the xylose consumption remained unchanged in the first 24 hours both in destarched fibre hydrolysate with and without nitrogen sources. The same result was also illustrated in the control experiments. The yeast only consumed xylose after the glucose has been consumed by the yeast. The result demonstrated that the composition of the glucose in the fermentation media does influence the consumption of the xylose by the yeast.

The hydrolysate with added yeast extract consumed highest xylose after 24 hours with consumption of 7.62 % followed by hydrolysate with added peptone which was 1.14 % and lowest is by the hydrolysate without any nitrogen sources which was 0.21 %. The consumption of xylose increases significantly at 48 hours for the hydrolysate with the nitrogen source. The possible reason could be the depletion of the glucose in the media.

At 48 hours of fermentation, hydrolysate with added yeast extract, consumption of the glucose more efficiently which was 80.23% followed by 64.34 by hydrolysate with added peptone and lastly without any addition of nitrogen sources which was 5.46%. This result

illustrated might be due to the decrease of the glucose in the fermentation. The yeast consumes glucose rather than xylose. Same result was illustrated in the control experiment.

In control experiment, the glucose was depleted faster than xylose. After 24 hours of fermentation, the culture supplemented with peptone and yeast extract consumed the glucose completely. The yeast consumes glucose faster with the addition of nitrogen sources. The highest glucose consumption was shown in culture supplemented with yeast extract at 108 h where the consumption of glucose was 92.23 %. This was followed by culture supplemented with peptone with glucose consumption of 76.33 %. The lowest performances were shown in non-supplemented cultures hydrolysate without any supplementation which was 28.70%. The result is similarly with the finding by Arrizon & Gschaedler., (2007), using *Saccharomyces cerevisiae* which was able to consumed 6.55 g/L of sugar with the addition of ammonium sulfate in the tequila fermentation. The research has proved the sugar consumption was faster with addition of nitrogen sources.

In the control media without nitrogen sources, the consumption of glucose by yeast was only completed at 36 hours. The result proves nitrogen sources in the fermentation media helps to enhance the yeast glucose consumption subsequently helps to improve xylose consumption in the fermentation media. Similar result was found by Mohamad et al. (2013), whereby the yeast *C. tropicalis* consumed glucose rather than xylose and faster consumption of xylose in the control media showed earlier accumulation of xylitol.

After 108 hours of fermentation, the hydrolysate with added yeast extract achieved the highest xylose with consumption of 96% followed by hydrolysate with added peptone which was 29.93% and hydrolysate without nitrogen sources which was 21.8 %.

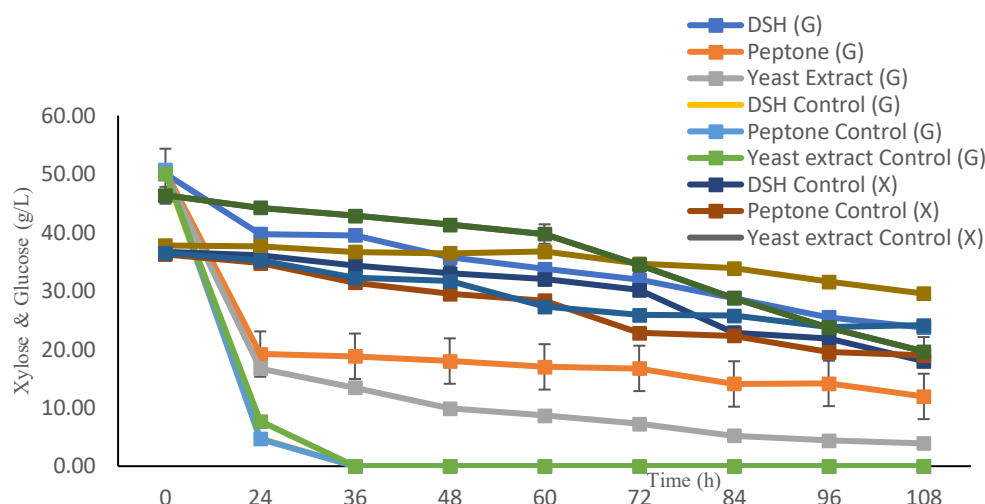


Figure 4.15: The residual glucose and xylose during fermentation using destarched sago fibre hydrolysate and in control experiments supplemented with various nitrogen sources. Notes: G indicates glucose and X indicated xylose. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

4.9.3 Ethanol and Xylitol Production

Based on Figure 4.16 and Figure 4.17, the fermentation profiles indicate ethanol and xylitol production in destarched sago fibre hydrolysate by *C. tropicalis* during 0 hour until 108 hours. The hydrolysate with the addition of 5 g/L of peptone showed the highest ethanol production followed with hydrolysate without any added nitrogen and lastly hydrolysate with the addition of 5 g/L of yeast extract.

The maximum ethanol produced by fermentation with added peptone was 13.74 ± 0.47 g/L at 108 hours followed by fermentation without nitrogen sources which was 10.84 ± 0.05 and lastly was hydrolysate with added yeast extract which was 8.42 ± 0.96 g/L. At 108 hours, the maximum ethanol production by the control experiment produced lower ethanol concentration 6.11 ± 0.10 g/L compared with the experiment 13.74 ± 0.47 g/L.

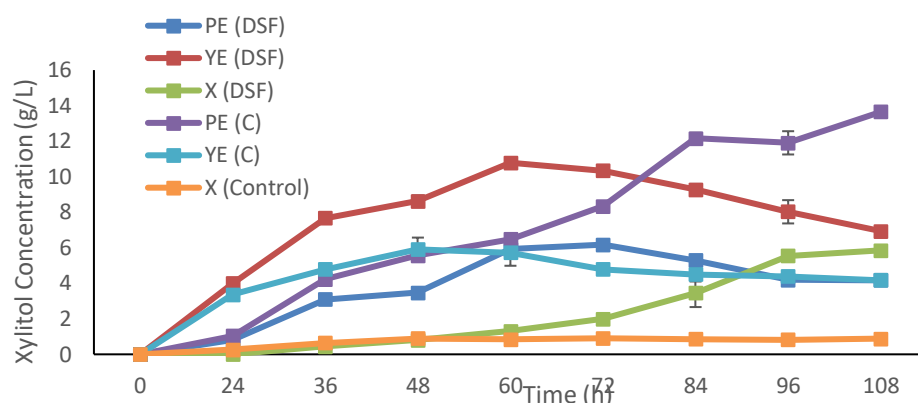


Figure 4.16: Xylitol production by *C. tropicalis* in fermentation using destarched sago fibre hydrolysate and in commercial xylose and glucose. Notes: YE(C), control media supplemented with yeast extract, PE(C), control media supplemented with peptone, YE(DSFH), in destarched sago fibre hydrolysate supplemented with yeast extract and PE(DSFH), in destarched sago fibre hydrolysate supplemented with peptone, X(DSFH) in destarc sago fibre without any nutrient addition and X(C) without addition of nutrients in control media. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

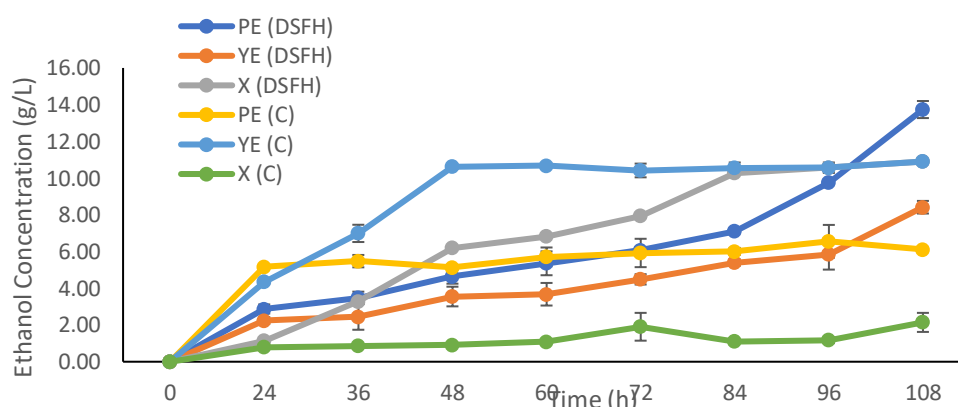


Figure 4.17: Ethanol production by *C. tropicalis* in destarched sago fibre hydrolysate and in commercial xylose and glucose. Notes: YE(C), control media supplemented with yeast extract, PE(C), control media supplemented with peptone, YE(DSFH), in destarched sago fibre hydrolysate supplemented with yeast extract and PE(DSFH), in destarched sago fibre hydrolysate supplemented with peptone, X(DSFH) in destarched sago fibre without any nutrient addition and X(C) without addition of nutrients in control media. Error bars indicate the mean $\pm$ standard deviation of duplicate experiments.

The results supported the finding by Li et al. (2017), which revealed yeast extract is an ideal nitrogen source with for ethanol production with fermentation efficiency 84.5%, by *S. cerevisiae* in very high gravity (VHG) fermentation of corn starch (340 g/L). Contrary

with the carbon consumption, the hydrolysate with added peptone showed higher ethanol concentration compared with addition of yeast extract. The possible reason for this might be because the yeast extract has been used by the yeast for cell growth instead of production of ethanol. The resulting biomass and by-product could repress the formation of ethanol (Li et al., 2017). In this study, peptone helps to enhance ethanol production in *C. tropicalis*.

By comparison, the ethanol was produced after 24 hours of fermentation which was earlier than the production of xylitol. The result is the same with the consumption of xylitol by the yeast as the xylitol was consumed later than the glucose. The glucose was first consumed by the yeast for fermentation to produce ethanol. The xylitol produced after 24 hours of fermentation in hydrolysate with added peptone and yeast extract except for hydrolysate without added nitrogen sources which was at 48 hours.

In this study, the maximum concentration of xylitol produced by the yeast in sago fibre hydrolysate varies at different hours. The yeast fermented in hydrolysate with added peptone produced maximum concentration 6.18 ± 0.04 g/L at 72 hours followed by hydrolysate with added yeast extract at 60 hours 10.79 ± 0.74 g/L and lastly in hydrolysate without any nitrogen sources at 108 hours. In this study, it was proven different nitrogen sources do affect the rate of fermentation as well as the products of the fermentation. Table 4.6 summarises the kinetic parameter of xylitol fermentation using various media. The maximum xylitol produced is 11.75 ± 0.09 g/L by DSH supplemented with yeast extract.

Table 4.6: The kinetic parameter of xylitol in fermentation using destarched sago fibre hydrolysate supplemented with peptone, yeast extract, without any supplementation and in control experiments.

Kinetic parameter	Peptone	Control	Yeast extract	Control	DSH	Control
Initial xylose Concentration (g/L)	36.49±0.265	36.24 ± 0.11	44.87 ± 1.45	36.44 ± 0.13	37.77 ± 0.355	36.69 ± 0.21
Residual xylose (g/L)	24.12±0.005	18.98 ± 0.88	1.13 <i>i</i> ± 0.0106	18.89 ± 0.91	29.54 ± 0.34	17.98 <i>i</i> ± 0.12
Xylose consumption efficiency (%)	33.91	47.63	42.35	48.16	21.79	50.99
Maximum xylitol produced (g/L)	6.18 ± 0.04	13.64 ± 0.07	11.78 <i>i</i> ± 0.09	7.07 ± 0.06	5.853 ± 0.034	0.875 ± 0.007
Y _{p/s} (g/g)	0.59 ± 0.05	0.79 ± 0.0392	0.379 ± 0.0213	0.22 ± 0.0031	0.716 ± 0.0557	0.05 ± 0.00
Q _p (g/L/h)	0.09 ± 0.000625	0.126 ± 0.000616	0.164 ± 0.001319	0.0031 ± 0.00155	0.0277 ± 0.000295	0.0081 ± 0.0000463
Fermentation efficiency(%)	0.639897 ± 0.057166	0.864 ± 0.04272	0.413 ± 0.023258	0.432 ± 0.0061	0.703 ± 0.047545	0.05 ± 0.00

Notes: Parameters studied: Y_{p/s} xylitol yield (g/g), Q_p ethanol volumetric productivity (g/Lhr), E_f fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical xylitol yield is 0.917 g/g xylose.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Upon the completion of this project, it was discovered that the pre-treatment of destarched sago fibre hydrolysate (DSFH) with dilute H_2SO_4 using high pressure steam autoclave yielded highest recovered high concentration of xylose and glucose in this study. The glucose and xylose recovered from this pre-treatment were 32.40% and 9.34%. Microwave and mild hydrothermal pre-treatment were unable to produce xylose using the acidic pre-treatment however it was found to be alternative methods for conventional heating to produce glucose as this pre-treatment method produced higher glucose concentration with recovery of 35.62% and 32.50% compared with the high-pressure steam pre-treatment.

In microwave and hydrothermal pre-treatment, the highest glucose concentration achieved was using 9% v/v of dilute H_2SO_4 which produces 37.73 ± 0.48 g/L and 37.11 ± 1.39 g/L respectively. By comparison using the (% v/v of dilute H_2SO_4 , high pressure autoclave produced glucose with concentration of 22.68 ± 0.43 g/L which is lower compared to other two physicochemical pre-treatments. These studies found the highest xylose concentration (8.42 ± 1.23 g/L) produced using high pressure autoclave was 30 minutes and 60 minutes of reaction time produced the highest glucose concentration (20.62 ± 0.15 g/L). The best solid to liquid ratio for pre-treatment is 30%. which produced high glucose (83.61 ± 0.05 g/L) and xylose concentration (28.19 ± 0.78 g/L).

In this study, it was shown that parameters such as inoculum age, inoculum concentration and addition of nitrogen sources does influence the productions of sugars obtained from the fermentation. Fermentation using 27 hours of inoculum age has the highest glucose consumption of 85.83% and xylose consumption of 96%. Fermentation using 27-hour inoculum age produced the highest ethanol (5.60 ± 0.10 g/L) and the highest xylitol (0.86 ± 0.11 g/L) at 54 hours of fermentation in DSFH. Fermentation using inoculum 20% produced the highest xylitol at 54 hours with concentration of 0.86 ± 0.11 g/L with glucose consumption 99% and xylose consumption, 96% compared with others selected inoculum investigated in these studies. the inoculum level does influence the fermentation of the DSFH. Interestingly, the inhibition in the DSFH can be overcome by increasing the inoculum level of the fermentation. The growth of *C. tropicalis* was studied in the inoculum media consisting 5 g/L yeast extract, 20 g/L commercial glucose and xylose. The study discovered that in a control media, the lag phase of the yeast started from 0 to 6 hours. Then the exponential phase of the yeast occurred at 12 to 42 hours and after 48 hours the yeast started declining in weight. This study proves that different inoculum age from taken from these phases influenced the glucose and xylose consumption which subsequently affect the production of the xylitol and ethanol.

In addition, the study discovered the fermentation of the DSFH with supplementation of yeast extract and peptone has dramatically increased the consumption of glucose and xylose in the DSFH as well as the production of xylitol and ethanol in the DSFH. *C. tropicalis* showed maximum growth weight in commercial medium containing peptone and yeast extract at 36 hours (21.65 ± 0.20 g/L and 28.98 ± 0.93 g/L) respectively. Fermentation media added with yeast extract produced highest xylitol 10.79 ± 0.74 g/L at 60 hours whereas the maximum ethanol produced by fermentation with added peptone was $13.74 \pm$

0.47 g/L at 108 hours. This proves different nitrogen sources do affect the rate of fermentation as well as the products of the fermentation.

In the future research, it is recommended on the utilisation of enzymes such as xylitol dehydrogenase to better break down the xylose sugar in the DSFH. The usage of enzymes could enhance the production of the xylitol. It is also recommended for the destarched fibre to be treated with higher temperature using hydrothermal pre-treatment as higher temperature of hydrothermal pre-treatment can help to degrade the hemicellulose and subsequently recover higher xylose sugar for xylitol production. In addition, it is recommended for the destarched sago fibre to undergo detoxification. This process could decrease the inhibition in the hydrolysate for fermentation of the xylitol. In addition, in the future work, the engineered yeast *C. tropicalis* with efficient xylose utilisation with accumulated more pentose phosphate pathway intermediates can be used for xylitol fermentation. Also, the yeast *C. tropicalis* showed better growth in DSFH compared to commercial xylose and glucose. This proves that DSFH can be used as growth media for yeast *C. tropicalis* in the future. For future studies, it is recommended to study other parameters affecting the fermentation such as the pH of the fermentation media, the rate of agitation during the fermentation and the temperature of the fermentation. It is also recommended to conduct multifactorial design experiments to select the best parameters used in the future studies.

Finally, destarched sago fibre can be an alternative for production of ethanol and glycerol in the future. Thus, sago wastes can be used for production of value-added products and managed properly at the sago mills instead of being discharged into nearby rivers or deposited at another place.

REFERENCES

- Adrio, J. L., & Demain, A. L. (2003). Fungal biotechnology. *International Microbiology*, 6(3), 191-199.
- Agamuthu, P. (2009, November). Challenges and opportunities in agro-waste management: An Asian perspective. In *Inaugural Meeting of First Regional 3R forum in Asia*, 11, 12
- Alias, N. H., Abd-Aziz, S., Phang, L. Y., & Ibrahim, M. F. (2021). Enzymatic Saccharification with Sequential-Substrate Feeding and Sequential-Enzymes Loading to Enhance Fermentable Sugar Production from Sago Hampas. *Processes* 2021, 9, 535. *Biotechnology for Sustainability and Social Well Being*, 9, 225.
- AOAC. (2012). Official Methods of Analyses of Association of Analytical Chemists. *AOAC International*, 954.01.
- Arcaño, Y. D., García, O. D. V., Mandelli, D., Carvalho, W. A., & Pontes, L. A. M. (2020). Xylitol: A review on the progress and challenges of its production by chemical route. *Catalysis Today*, 344, 2-14.
- Arrizon, J., & Gschaedler, A. (2007). Effects of the addition of different nitrogen sources in the tequila fermentation process at high sugar concentration. *Journal of Applied Microbiology*, 102(4), 1123-1131.
- Awg-Adeni, D. S., Bujang, K. B., Hassan, M. A., & Abd-Aziz, S. (2013). Recovery of glucose from residual starch of sago hampas for bioethanol production. *BioMed Research International*, 2013.

- Barathikannan, K., & Agastian, P. (2016). Xylitol: Production, optimization and industrial application. *International Journal of Current Microbiology and Applied Sciences*, 5(9), 324-339.
- Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C., & Kalita, E. (2018). Recent trends in the pretreatment of lignocellulosic biomass for value-added products. *Frontiers in Energy Research*, 6, 141.
- Batista, G., Souza, R. B., Pratto, B., dos Santos-Rocha, M. S., & Cruz, A. J. (2019). Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. *Bioresource Technology*, 275, 321-327.
- Behera, S., Arora, R., Nandhagopal, N., & Kumar, S. (2014). Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, 36, 91-106.
- Bellissimi, E., & Ingledew, W. M. (2005). Metabolic acclimatization: preparing active dry yeast for fuel ethanol production. *Process Biochemistry*, 40(6), 2205-2213.
- Canilha, L., Santos, V. T., Rocha, G. J., Almeida e Silva, J. B., Giulietti, M., Silva, S. S., ... & Carvalho, W. (2011). A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid. *Journal of Industrial Microbiology and Biotechnology*, 38(9), 1467-1475.
- Cheng, K. K., Zhang, J. A., Ling, H. Z., Ping, W. X., Huang, W., Ge, J. P., & Xu, J. M. (2009). Optimization of pH and acetic acid concentration for bioconversion of hemicellulose from corncobs to xylitol by *Candida tropicalis*. *Biochemical Engineering Journal*, 43(2), 203-207.

- Clauser, N. M., Gutiérrez, S., Area, M. C., Felissia, F. E., & Vallejos, M. E. (2018). Alternatives of small-scale biorefineries for the integrated production of xylitol from sugarcane bagasse. *Journal of Renewable Materials*, 6(2), 139-151.
- Crasta, I., Sivakumar, S., Banuvalli, B., Murugesan, S., & Mudliar, S. (2021). Mild-thermal pretreatment of agro-residues enhances biomethanation potential: a comparative study of napier grass and rice straw. *Clean Technologies and Environmental Policy*, 1-9.
- Cuevas, M., Garcia, J. F., Hodaifa, G., & Sanchez, S. (2015). Oligosaccharides and sugars production from olive stones by autohydrolysis and enzymatic hydrolysis. *Industrial Crops and Products*, 70, 100-106.
- Damião Xavier, F., Santos Bezerra, G., Florentino Melo Santos, S., Sousa Conrado Oliveira, L., Luiz Honorato Silva, F., Joice Oliveira Silva, A., & Maria Conceição, M. (2018). Evaluation of the simultaneous production of xylitol and ethanol from sisal fiber. *Biomolecules*, 8(1), 2.
- Danon, B., Van der Aa, L., & De Jong, W. (2013). Furfural degradation in a dilute acidic and saline solution in the presence of glucose. *Carbohydrate Research*, 375, 145-152.
- Dasgupta, D., Bandhu, S., Adhikari, D. K., & Ghosh, D. (2017). Challenges and prospects of xylitol production with whole cell bio-catalysis: A review. *Microbiological Research*, 197, 9-21.
- De Albuquerque, T. L., da Silva Jr, I. J., de Macedo, G. R., & Rocha, M. V. P. (2014). Biotechnological production of xylitol from lignocellulosic wastes: A review. *Process Biochemistry*, 49(11), 1779-1789.

- Debiagi, F., Madeira, T. B., Nixdorf, S. L., & Mali, S. (2020). Pretreatment efficiency using autoclave high-pressure steam and ultrasonication in sugar production from liquid hydrolysates and access to the residual solid fractions of wheat bran and oat hulls. *Applied Biochemistry and Biotechnology*, 190(1), 166-181.
- Dragone, G., Mussatto, S. I., & Almeida e Silva, J. B. (2007). High gravity brewing by continuous process using immobilised yeast: effect of wort original gravity on fermentation performance. *Journal of the Institute of Brewing*, 113(4), 391-398.
- Dumon, C., Song, L., Bozonnet, S., Fauré, R., & O'Donohue, M. J. (2012). Progress and future prospects for pentose-specific biocatalysts in biorefining. *Process Biochemistry*, 47(3), 346-357.
- Eryasar, K., & Karasu-Yalcin, S. (2016). Evaluation of some lignocellulosic by products of food industry for microbial xylitol production by *Candida tropicalis*. *3 Biotech*, 6(2), 1-7.
- Fehér, A., Fehér, C., Rozbach, M., & Barta, Z. (2017). Combined approaches to xylose production from corn stover by dilute acid hydrolysis. *Chemical and Biochemical Engineering Quarterly*, 31(1), 77-87.
- Franceschin, G., Sudiro, M., Ingram, T., Smirnova, I., Brunner, G., & Bertucco, A. (2011). Conversion of rye straw into fuel and xylitol: A technical and economical assessment based on experimental data. *Chemical Engineering Research and Design*, 89(6), 631-640.
- Ghaffar, A., Yameen, M., Aslam, N., Jalal, F., Noreen, R., Munir, B., ... & Latif, F. (2017). Acidic and enzymatic saccharification of waste agricultural biomass for biotechnological production of xylitol. *Chemistry Central Journal*, 11(1), 1-6.

- Ghindea, R., Csutak, O., Stoica, I., Tanase, A. M., & Vassu, T. (2010). Production of xylitol by yeasts. *Romanian Biotechnological Letters*, 15(3), 5217-5222.
- Ghosh, S., & Sudha, M. L. (2012). A review on polyols: new frontiers for health-based bakery products. *International Journal of Food Sciences and Nutrition*, 63(3), 372-379.
- Gírio, F. M., Carneiro, F., Duarte, L. C., & Bogel-Lukasik, R. (2012). Deconstruction of the hemicellulose fraction from lignocellulosic materials into simple sugars. *D-xylitol*, 3-37.
- Granström, T. B., Izumori, K., & Leisola, M. (2007). A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. *Applied Microbiology and Biotechnology*, 74(2), 273-276.
- Hernández-Pérez, A. F., Costa, I. A. L., Silva, D. D. V., Dussán, K. J., Villela, T. R., Canettieri, E. V., ... & Felipe, M. G. A. (2016). Biochemical conversion of sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates for xylitol production. *Bioresource Technology*, 200, 1085-1088.
- Hong, E., Kim, J., Rhie, S., Ha, S. J., Kim, J., & Ryu, Y. (2016). Optimization of dilute sulfuric acid pretreatment of corn stover for enhanced xylose recovery and xylitol production. *Biotechnology and Bioengineering*, 21(5), 612-619.
- Hu, Z., & Wen, Z. (2008). Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochemical Engineering Journal*, 38(3), 369-378.
- Jenol, M. A., Ibrahim, M. F., Kamal Bahrin, E., Kim, S. W., & Abd-Aziz, S. (2019). Direct bioelectricity generation from sago hampas by *clostridium beijerinckii* sr1 using microbial fuel cell. *Molecules*, 24(13), 2397.

- Ji, X., Ma, H., Tian, Z., Lyu, G., Fang, G., Chen, J., & Saeed, H. A. (2017). Production of xylose from diluted sulfuric acid hydrolysis of wheat straw. *BioResources*, 12(4), 7084-7095.
- Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, 199, 103-112.
- Juanssilfero, A. B., Kahar, P., Amza, R. L., Miyamoto, N., Otsuka, H., Matsumoto, H., ... & Kondo, A. (2018). Effect of inoculum size on single-cell oil production from glucose and xylose using oleaginous yeast *Lipomyces starkeyi*. *Journal of Bioscience and Bioengineering*, 125(6), 695-702.
- Kadam, S. R., Patil, S. S., Bastawde, K. B., Khire, J. M., & Gokhale, D. V. (2006). Strain improvement of *Lactobacillus delbrueckii* NCIM 2365 for lactic acid production. *Process Biochemistry*, 41(1), 120-126.
- Kamal, S. M. M., Mohamad, N. L., Abdullah, A. G. L., & Abdullah, N. (2011). Detoxification of sago trunk hydrolysate using activated charcoal for xylitol production. *Procedia Food Science*, 1, 908-913.
- Kawaguchi, H., Vertès, A. A., Okino, S., Inui, M., & Yukawa, H. (2006). Engineering of a xylose metabolic pathway in *Corynebacterium glutamicum*. *Applied and Environmental Microbiology*, 72(5), 3418-3428.
- Kim, S. (2019). Xylitol production from byproducts generated during sequential acid-/alkali-pretreatment of empty palm fruit bunch fiber by an adapted *Candida tropicalis*. *Frontiers in Energy Research*, 72.

- Kresnowati, M. T. A. P., Mardawati, E., & Setiadi, T. (2015). Production of xylitol from oil palm empty fruits bunch: A case study on bio refinery concept. *Modern Applied Science*, 9(7), 206-213.
- Kristensen, J. B., Felby, C., & Jørgensen, H. (2009). Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnology for Biofuels*, 2(1), 1-10.
- Kumari, D., & Singh, R. (2018). Pretreatment of lignocellulosic wastes for biofuel production: a critical review. *Renewable and Sustainable Energy Reviews*, 90, 877-891.
- Kurian, J. K., Nair, G. R., Hussain, A., & Raghavan, G. V. (2013). Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 25, 205-219.
- Kuyper, M., Harhangi, H. R., Stave, A. K., Winkler, A. A., Jetten, M. S., de Laat, W. T., ... & Pronk, J. T. (2003). High-level functional expression of a fungal xylose isomerase: the key to efficient ethanolic fermentation of xylose by *Saccharomyces cerevisiae*?. *FEMS Yeast Research*, 4(1), 69-78.
- Lenihan, P., Orozco, A., O'Neill, E., Ahmad, M. N. M., Rooney, D. W., & Walker, G. M. (2010). Dilute acid hydrolysis of lignocellulosic biomass. *Chemical Engineering Journal*, 156(2), 395-403.
- Li, M., Meng, X., Diao, E., & Du, F. (2012). Xylitol production by *Candida tropicalis* from corn cob hemicellulose hydrolysate in a two-stage fed-batch fermentation process. *Journal of Chemical Technology & Biotechnology*, 87(3), 387-392.

- Li, Y. J., Wang, M. M., Chen, Y. W., Wang, M., Fan, L. H., & Tan, T. W. (2017). Engineered yeast with a CO₂-fixation pathway to improve the bio-ethanol production from xylose-mixed sugars. *Scientific Reports*, 7(1), 1-9.
- Liu, C. G., Li, K., Wen, Y., Geng, B. Y., Liu, Q., & Lin, Y. H. (2019). Bioethanol: New opportunities for an ancient product. In *Advances in Bioenergy*, 4, 1-34.
- Liu, R., Li, J., & Shen, F. (2008). Refining bioethanol from stalk juice of sweet sorghum by immobilized yeast fermentation. *Renewable Energy*, 33(5), 1130-1135.
- López-Linares, J. C., Romero, I., Cara, C., Castro, E., & Mussatto, S. I. (2018). Xylitol production by *Debaryomyces hansenii* and *Candida guilliermondii* from rapeseed straw hemicellulosic hydrolysate. *Bioresource Technology*, 247, 736-743.
- López-Linares, J. C., Ruiz, E., Romero, I., Castro, E., & Manzanares, P. (2020). Xylitol production from exhausted olive pomace by *Candida boidinii*. *Applied Sciences*, 10(19), 6966.
- Lorliam, W., Akaracharanya, A., Krajangsang, S., Tolieng, V., & Tanasupawat, S. (2017). Optimization of xylitol production by *Candida tropicalis* A26. *Chiang Mai Journal of Science*, 44(1), 50-58.
- Lu, X., Xi, B., Zhang, Y., & Angelidaki, I. (2011). Microwave pretreatment of rape straw for bioethanol production: focus on energy efficiency. *Bioresource Technology*, 102(17), 7937-7940.
- Mamman, A. S., Lee, J. M., Kim, Y. C., Hwang, I. T., Park, N. J., Hwang, Y. K., ... & Hwang, J. S. (2008). Furfural: Hemicellulose/xylo-derived biochemical. *Biofuels, Bioproducts and Biorefining: Innovation For a Sustainable Economy*, 2(5), 438-454.

- Manaf, S. F. A., Jahim, J. M., Harun, S., & Luthfi, A. A. I. (2018). Fractionation of oil palm fronds (OPF) hemicellulose using dilute nitric acid for fermentative production of xylitol. *Industrial Crops and Products*, 115, 6-15.
- Manjarres-Pinzón, K., Arias-Zabala, M., Correa-Londoño, G., & Rodriguez-Sandoval, E. (2017). Xylose recovery from dilute-acid hydrolysis of oil palm (*Elaeis guineensis*) empty fruit bunches for xylitol production. *African Journal of Biotechnology*, 16(41), 1997-2008.
- Misra, S., Raghuwanshi, S., & Saxena, R. K. (2013). Evaluation of corncob hemicellulosic hydrolysate for xylitol production by adapted strain of *Candida tropicalis*. *Carbohydrate Polymers*, 92(2), 1596-1601.
- Modig, T., Liden, G., & Taherzadeh, M. J. (2002). Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. *Biochemical Journal*, 363(3), 769-776.
- Mohamad, N. L., Mustapa Kamal, S. M., & Mokhtar, M. N. (2015). Xylitol biological production: a review of recent studies. *Food Reviews International*, 31(1), 74-89.
- Mohammad, S., Awg-Adeni, D. S., Bujang, K. B., Vincent, M., & Baidurah, S. (2020). Potentials of sago fibre hydrolysate (SFH) as a sole fermentation media for bioethanol production. In *IOP Conference Series: Materials Science and Engineering* (Vol. 716, No. 1, p. 012001). IOP Publishing.
- Moysés, D. N., Reis, V. C. B., Almeida, J. R. M. D., Moraes, L. M. P. D., & Torres, F. A. G. (2016). Xylose fermentation by *Saccharomyces cerevisiae*: challenges and prospects. *International Journal of Molecular Sciences*, 17(3), 207.

- Muizniece, I., Gravelsins, A., Brauners, I., Blumberga, A., & Blumberga, D. (2017). Innovative bioproducts from forest biomass. Method of analysis. *Energy Procedia*, 113, 434-441.
- Mussatto, S. I. (2012). Application of xylitol in food formulations and benefits for health. In *d-Xylitol* (pp. 309-323). Springer, Berlin, Heidelberg.
- Mussatto, S. I., & Roberto, I. C. (2003). Xylitol production from high xylose concentration: evaluation of the fermentation in bioreactor under different stirring rates. *Journal of Applied Microbiology*, 95(2), 331-337.
- Pang, S. C., Voon, L. K., & Chin, S. F. (2018). Conversion of sago (*Metroxylon sagu*) pith waste to fermentable sugars via a facile depolymerization process. *Applied Biochemistry and Biotechnology*, 184(4), 1142-1154.
- Pedersen, M., Johansen, K. S., & Meyer, A. S. (2011). Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw. *Biotechnology For Biofuels*, 4(1), 1-10.
- Peng, B., Shen, Y., Li, X., Chen, X., Hou, J., & Bao, X. (2012). Improvement of xylose fermentation in respiratory-deficient xylose-fermenting *Saccharomyces cerevisiae*. *Metabolic Engineering*, 14(1), 9-18.
- Pensri, B., Aggarangsi, P., Chaiyaso, T., & Chandet, N. (2016). Potential of fermentable sugar production from Napier cv. Pakchong 1 grass residue as a substrate to produce bioethanol. *Energy Procedia*, 89, 428-436.

- Ping, Y., Ling, H. Z., Song, G., & Ge, J. P. (2013). Xylitol production from non-detoxified corncob hemicellulose acid hydrolysate by *Candida tropicalis*. *Biochemical Engineering Journal*, 75, 86-91.
- Povelainen, M. (2008). Pentitol phosphate dehydrogenases: Discovery, characterization and use in D-arabitol and xylitol production by metabolically engineered *Bacillus subtilis*.
- Rafiqul, I. S. M., & Mimi Sakinah, A. M. (2012). A perspective: Bioproduction of xylitol by enzyme technology and future prospects. *International Food Research Journal*, 19(2), 405–408.
- Rao, L. V., Goli, J. K., Gentela, J., & Koti, S. (2016). Bioconversion of lignocellulosic biomass to xylitol: an overview. *Bioresource Technology*, 213, 299-310.
- Roberto, C., Mussatto, S. I., & Rodrigues, R. C. L. B. (2003). Dilute-acid hydrolysis for optimization of xylose recovery from rice straw in a semi-pilot reactor. *Industrial Crops and Products*, 17 (3), 171–176.
- Rodrussamee, N., Lertwattanasakul, N., Hirata, K., Limtong, S., Kosaka, T., & Yamada, M. (2011). Growth and ethanol fermentation ability on hexose and pentose sugars and glucose effect under various conditions in thermotolerant yeast *Kluyveromyces marxianus*. *Applied Microbiology and Biotechnology*, 90(4), 1573-1586.
- Runnel, R., Mäkinen, K. K., Honkala, S., Olak, J., Mäkinen, P. L., Nömmela, R., ... & Saag, M. (2013). Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables. *Journal of Dentistry*, 41(12), 1236-1244.

- Sahoo, D., Ummalyma, S. B., Okram, A. K., Pandey, A., Sankar, M., & Sukumaran, R. K. (2018). Effect of dilute acid pretreatment of wild rice grass (*Zizania latifolia*) from Loktak Lake for enzymatic hydrolysis. *Bioresource Technology*, 253, 252-255.
- Sasaki, M., Inui, M., & Yukawa, H. (2012). Microorganisms for xylitol production: focus on strain improvement. In *D-Xylitol*, 109-131.
- Sathitsuksanoh, N., Zhu, Z., Ho, T. J., Bai, M. der, & Zhang, Y. H. P. (2010). Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings. *Bioresource Technology*, 101(13), 4926–4929.
- Sindhu, R., Binod, P., & Pandey, A. (2016). Bioresource Technology Biological pretreatment of lignocellulosic biomass – An overview. *Bioresource Technology*, 199, 76–82.
- Sirohi, R., Pandey, J. P., Singh, A., Sindhu, R., Lohani, U. C., Goel, R., & Kumar, A. (2020). Acid hydrolysis of damaged wheat grains: Modeling the formation of reducing sugars by a neural network approach. *Industrial Crops and Products*, 149, 112351.
- Sousa, A. F., Vilela, C., Fonseca, A. C., Matos, M., Freire, C. S., Gruter, G. J. M., ... & Silvestre, A. J. (2015). Biobased polyesters and other polymers from 2, 5-furandicarboxylic acid: a tribute to furan excellency. *Polymer Chemistry*, 6(33), 5961-5983.
- Sullivan, R. P., Nair, N. U., & Zhao, H. (2008). Engineering a Fungal L-Arabinose Pathway Towards the Utilization of Pentose Sugars for Production of Xylitol and Ethanol. In *2008 AIChE Annual Meeting*, AIChE 100.

- Sun, S. L., Wen, J. L., Ma, M. G., & Sun, R. C. (2014). Enhanced enzymatic digestibility of bamboo by a combined system of multiple steam explosion and alkaline treatments. *Applied Energy*, 136, 519-526.
- Sunarti, T. C., Yanti, S. D., & Ruriani, E. (2017, May). Two-steps microwave-assisted treatment on acid hydrolysis of sago pith for bioethanol production. In *IOP Conference Series: Earth and Environmental Science* 65(1), 012052.
- Taylor, M. J., Alabdrabalameer, H. A., & Skoulou, V. (2019). Choosing physical, physicochemical and chemical methods of pre-treating lignocellulosic wastes to repurpose into solid fuels. *Sustainability*, 11(13), 3604.
- Umai, D., Kayalvizhi, R., Kumar, V., & Jacob, S. (2022). Xylitol: bioproduction and applications-a review. *Frontiers in Sustainability*, 3, 826190.
- Unrean, P., & Ketsub, N. (2018). Integrated lignocellulosic bioprocess for co-production of ethanol and xylitol from sugarcane bagasse. *Industrial Crops and Products*, 123, 238-246.
- Ur-Rehman, S., Mushtaq, Z., Zahoor, T., Jamil, A., & Murtaza, M. A. (2015). Xylitol: a review on bioproduction, application, health benefits, and related safety issues. *Critical Reviews in Food Science and Nutrition*, 55(11), 1514-1528.
- Vajzovic, A., Bura, R., Kohlmeier, K., & Doty, S. L. (2012). Novel endophytic yeast *Rhodotorula mucilaginosa* strain PTD3 II: production of xylitol and ethanol in the presence of inhibitors. *Journal of Industrial Microbiology and Biotechnology*, 39(10), 1453-1463.
- Vallejos, M. E., Chade, M., Mereles, E. B., Bengoechea, D. I., Brizuela, J. G., Felissia, F. E., & Area, M. C. (2016). Strategies of detoxification and fermentation for

- biotechnological production of xylitol from sugarcane bagasse. *Industrial Crops and Products*, 91, 161-169.
- Van Soest, P., & Robertson, J. (1979). Systems of analysis for evaluating fibrous feeds. In *Standardization of analytical methodology for feeds: proceedings....* IDRC, Ottawa, ON, CA.
- Vincent, M. A. (2015). Simultaneous co-saccharification and fermentation of sago hampas for bioethanol production. *Agricultural Engineering International: CIGR Journal*, 17(2).
- Vrabl, P., Schinagl, C. W., Artmann, D. J., Heiss, B., & Burgstaller, W. (2019). Fungal growth in batch culture—what we could benefit if we start looking closer. *Frontiers in Microbiology*, 10, 2391.
- Wang, W., Ling, H., & Zhao, H. (2015). Steam explosion pretreatment of corn straw on xylose recovery and xylitol production using hydrolysate without detoxification. *Process Biochemistry*, 50(10), 1623-1628.
- Xu, L., Liu, L., Li, S., Zheng, W., Cui, Y., Liu, R., & Sun, W. (2019). Xylitol production by *Candida tropicalis* 31949 from sugarcane bagasse hydrolysate. *Sugar Tech*, 21(2), 341-347.
- Xu, Y., Chi, P., Bilal, M., & Cheng, H. (2019). Biosynthetic strategies to produce xylitol: an economical venture. *Applied Microbiology and Biotechnology*, 103(13), 5143-5160.
- Xylitol Market Size, Share & Trends Analysis Report by Application (Chewing Gum, Oral Care, Confectionery), By Form (Powder, Liquid), By Region (Europe, Asia Pacific, North America, MEA), And Segment Forecasts, 2020 – 2028 (Sep,2021). Grand

viewresearch.com. Retrieved from <https://www.grandviewresearch.com/industry-analysis/xylitol-market>.

Yablochkova, E. N., Bolotnikova, O. I., Mikhailova, N. P., Nemova, N. N., & Ginak, A. I. (2003). The activity of xylose reductase and xylitol dehydrogenase in yeasts. *Microbiology*, 72(4), 414-417.

Yates, G. T., & Smotzer, T. (2007). On the lag phase and initial decline of microbial growth curves. *Journal of Theoretical Biology*, 244(3), 511-517.

Yemiş, O., & Mazza, G. (2011). Acid-catalyzed conversion of xylose, xylan and straw into furfural by microwave-assisted reaction. *Bioresource Technology*, 102(15), 7371-7378.

Zhang, C., Qin, J., Dai, Y., Mu, W., & Zhang, T. (2019). Atmospheric and room temperature plasma (ARTP) mutagenesis enables xylitol over-production with yeast *Candida tropicalis*. *Journal of Biotechnology*, 296, 7-13.

Zhang, J., Geng, A., Yao, C., Lu, Y., & Li, Q. (2012). Xylitol production from D-xylose and horticultural waste hemicellulosic hydrolysate by a new isolate of *Candida athensensis* SB18. *Bioresource Technology*, 105, 134-141.

Zhang, J., Zhang, B., Wang, D., Gao, X., & Hong, J. (2015). Improving xylitol production at elevated temperature with engineered *Kluyveromyces marxianus* through over-expressing transporters. *Bioresource Technology*, 175, 642-645.

Zhao, X., Zhang, L., & Liu, D. (2012). Biomass recalcitrance. Part II: Fundamentals of different pre-treatments to increase the enzymatic digestibility of lignocellulose. *Biofuels, Bioproducts and Biorefining*, 6(5), 561-579.

Zihare, L., & Blumberga, D. (2020). Bioeconomy Investments: Market Considerations.

Rigas Tehniskas Universitates Zinatniskie Raksti, 24(2), 79-91.

Zikou, E., Chatzifragkou, A., Koutinas, A. A., & Papanikolaou, S. (2013). Evaluating glucose and xylose as cosubstrates for lipid accumulation and γ -linolenic acid biosynthesis of *Thamnidium elegans*. *Journal of Applied Microbiology*, 114(4), 1020.

APPENDICES

Appendix A: Media/Reagents/Solution

Neutral detergent solution (NDF)

Reagents

1. In a 4 L beaker combine 600 g sodium lauryl sulfate, 200 mL ethylene glycol monoethyl ether, and 2 L deionised water. Mix this solution thoroughly and pour it into a 20 L carboy.
2. Combine 372.2 g disodium ethylenediaminetetra-acetate (EDTA) dihydrate crystals and 136.2 g of sodium borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) with 3 L of deionised water in 4 L beaker.
3. Heat and stir this solution until the crystals are dissolved, then add it to the 20 L carboy. Add 91.2 g of anhydrous sodium phosphate dibasic (Na_2HPO_4) to 1 L of deionised water, heat and stir to dissolve, then add it to the 20 L carboy. Dilute this mixture to 20 L with deionised water and mix thoroughly. pH should be between 6.9 - 7.1.

Acid detergent solutions (ADF)

1. Weigh 400 g Hexadecyltrimethyl-ammonium bromide in a 2 L beaker under the hood.
2. Add deionised water to this beaker and stir until mixture can be poured.
3. Transfer this solution to a 4 L beaker. Bring the volume up to about 3 L with water and stir thoroughly with a long stirring rod. Very slowly add 556 mL conc. H_2SO_4

to the solution and continue to stir vigorously with the stirring rod. At this point there may still be some undissolved material, so place the beaker on a heater/stirrer and stir with heat until the material is dissolved.

4. Transfer this solution to a 20 L carboy and dilute to 20 L with deionised H₂O.

Potato Dextrose Agar (PDA)

39.0 g Bacto™ PDA powder, dissolved in 1000 mL distilled water.

Yeast Malt Broth (YMB) Medium

1.05 g of Sigma-Aldrich YMB powder, dissolved in 50 mL distilled water.

Appendix C1: The glucose and xylose concentration obtained using different dilute sulphuric acid concentration using high pressure autoclave.

Acid concentration % (v/v)	Glucose Concentration (g/L)	Xylose Concentration (g/L)
0	13.29 ^C ± 0.46	0.00
2	18.89 ^B ± 0.01	6.98 ^A ± 0.03
4	18.65 ^B ± 0.18	6.26 ^A ± 0.04
6	18.79 ^B ± 0.84	6.54 ^{AB} ± 0.21
9	22.68 ^A ± 0.43	6.95 ^A ± 0.06

Appendix C2: The glucose and xylose concentration obtained using different dilute sulphuric acid concentration using microwave.

Acid concentration % (v/v)	Glucose Concentration (g/L)	Xylose Concentration (g/L)
0	1.79 ^E ± 0.09	
2	17.76 ^D ± 0.24	
4	31.30 ^C ± 0.08	
6	35.65 ^B ± 0.23	
9	37.73 ^A ± 0.48	

Appendix C3: The glucose and xylose concentration obtained using different dilute sulphuric acid concentration using hydrothermal.

Acid concentration % (v/v)	Glucose Concentration (g/L)	Xylose Concentration (g/L)
0	4.20 ^C ± 0.60	Not detected.
2	19.08 ^B ± 0.32	
4	26.53 ^A ± 0.23	
6	34.13 ^A ± 2.52	
9	37.11 ^A ± 1.39	

Appendix C4: Effects of reaction time on the glucose and xylose recovery using high pressure steam autoclave.

Reaction time (minutes)	Glucose recovery (%)	Xylose recovery (%)
30	16.27 ^A ± 1.22	8.42 ^A ± 1.23
60	20.62 ^A ± 0.15	7.64 ^A ± 0.08
90	19.67 ^A ± 1.08	6.32 ^A ± 0.58

Appendix C5: Effects of reaction time on the glucose and xylose recovery using high pressure autoclave pre-treatment

Reaction time (minutes)	Glucose recovery (%)	Xylose recovery (%)
30	16.27 ^A ± 1.22	8.42 ^A ± 1.23
60	20.62 ^A ± 0.15	7.64 ^A ± 0.08
90	19.67 ^A ± 1.08	6.32 ^A ± 0.58

Appendix C6: Effects of different substrate to liquid ratio on xylose yield using autoclave

Solid: liquid ratio	Glucose concentration (%)	Xylose concentration (%)
5 :100	18.89 ^c ±0.01	6.98 ^c ±0.03
15:100	38.07 ^B ±0.35	12.97 ^B ±0.28
20:100	78.63 ^A ±2.22	24.75 ^A ±0.77
30:100	83.61 ^A ±0.05	28.19 ^A ±0.78

Appendix C7: The effects of inoculum age on productions of xylitol and ethanol at 9, 18 and 28 hours

Hours	9 Hours		18 hours		28 hours	
	Xylitol (g/L)	Ethanol (g/L)	Xylitol (g/L)	Ethanol (g/L)	Xylitol (g/L)	Ethanol (g/L)
0	Not detected	0.04 ± 0.03	Not detected	0.07±0.02	Not detected	0.08±0.01
9		1.4 ± 0.14		1.49±0.05		1.90±0.36
18		2.60 ± 0.38		3.40±0.58		3.10±0.12
27	0.16 ± 0.04	2.56±0.31	0.21±0.09	4.06±0.19	0.45±0.03	3.56±0.32
36	0.31 ± 0.08	2.77 ± 0.23	0.43±0.01	3.84±0.15	0.40± .02	4.27±0.72
45	0.57 ± 0.05	3.27 ± 0.26	0.70±0.14	4.16±0.05	0.57±0.05	4.81±0.20
54	0.41 ± 0.16	4.60 ± 0.10	0.29±0.03	4.53±0.03	0.86±0.11	5.60±0.10

Appendix C8: The effects of inoculum level on consumption of glucose and xylose in destarched fibre hydrolysate fermentation (DHH)

Hours	10 %		20%		30%	
	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose (g/L)
0	22.64±0.64	12.90±0.09	22.17±0.83	11.46±1.35	22.64±0.36	12.90±0.09
9	21.18±1.00	12.42±0.14	12.68±1.50	11.42±1.14	10.14±1.54	12.34±0.22
18	21.85±1.57	12.53±0.25	9.85±0.43	7.24±0.03	8.64±1.57	9.49±0.28
27	21.12±1.15	12.34±0.33	10.12±0.85	7.68±0.50	5.64±1.39	9.15±0.04
36	20.23±0.72	11.60±0.57	10.03±0.92	5.67±0.45	2.04±1.05	8.75±0.58
45	19.37±0.15	11.21±0.11	8.46±0.50	1.31±0.60	0.97±0.26	8.67±0.54
54	17.62±0.40	10.62±0.41	3.14±0.70	0.47±0.12	0.36±0.12	7.63±0.49

Appendix C9: The dry cell weight of the different inoculum age in destarched sago hampas hydrolysate (DHH) and control experiment

Hours	9 Hours		18 hours		28 hours	
	DSH	Control	DSH	Control	DSH	Control
0	0.25±0.12	0.38±0.13	1.50±0.88	0.81±0.31	2.06±1.19	0.50±0.25
9	2.87±2.00	0.81±0.06	7.94±3.56	1.00±0.12	6.06±3.19	3.19±2.19
18	11.13±10.50	1.00±0.75	8.56±2.81	1.50±1.00	3.19±2.69	4.87±0.88
27	10.06±1.94	3.00±2.38	8.63±5.63	1.13±0.75	12.56±6.94	5.81±0.06
45	9.50±3.50	4.56±4.06	11.13±5.25	6.00±5.25	16.56±7.69	9.31±5.69
54	9.69±7.44	6.12±4.88	14.38±8.13	9.94±9.56	16.94±5.56	9.37±0.12

Appendix C10: The effects of inoculum ratio on consumption of sugars in control experiment.

Hours	10 %		20%		30%	
	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose (g/L)
0	22.21±0.08	12.47±0.16	22.18±0.05	13.05±0.05	22.02±0.01	13.05±0.05
9	22.17±0.01	12.42±0.19	20.23±0.23	12.10±0.02	15.83±0.60	13.10±0.02
18	20.35±0.07	12.24±0.05	14.74±0.21	10.10±0.08	9.24±0.71	13.10±0.08
27	19.62±0.36	12.45±0.37	13.62±0.35	9.39±0.28	4.73±0.54	11.89±0.22
36	18.73±0.22	12.25±0.12	12.21±0.05	7.61±0.49	4.71±0.55	9.61±0.50
45	16.04±0.92	12.03±0.83	10.16±0.08	0.70±0.07	1.16±0.08	6.76±0.49
54	14.60±0.05	12.05±0.08	0.23±0.11	0.48±0.09	0.21±0.09	6.48±0.09

Appendix C11: The effects of inoculum age on productions of xylitol and ethanol at 9, 18 and 28 hours

Hours	9 Hours		18 hours		28 hours	
	Xylitol (g/L)	Ethanol (g/L)	Xylitol (g/L)	Ethanol (g/L)	Xylitol (g/L)	Ethanol (g/L)
0	Not detected	0.04 ± 0.03	Not detected	0.07±0.02	Not detected	0.08±0.01
9		1.4 ± 0.14		1.49±0.05		1.90±0.36
18		2.60 ± 0.38		3.40±0.58		3.10±0.12
27	0.16 ± 0.04	2.56±0.31	0.21±0.09	4.06±0.19	0.45±0.03	3.56±0.32

36	0.31 ± 0.08	2.77 ± 0.23	0.43±0.0 1	3.84±0.1 5	0.40± .02	4.27±0.7 2
45	0.57 ± 0.05	3.27 ± 0.26	0.70±0.1 4	4.16±0.0 5	0.57±0.0 5	4.81±0.2 0
54	0.41 ± 0.16	4.60 ± 0.10	0.29±0.0 3	4.53±0.0 3	0.86±0.1 1	5.60±0.1 0

Appendix C12: The effects of inoculum age of 9, 18 and 28 hours on consumption of sugars in control experiment.

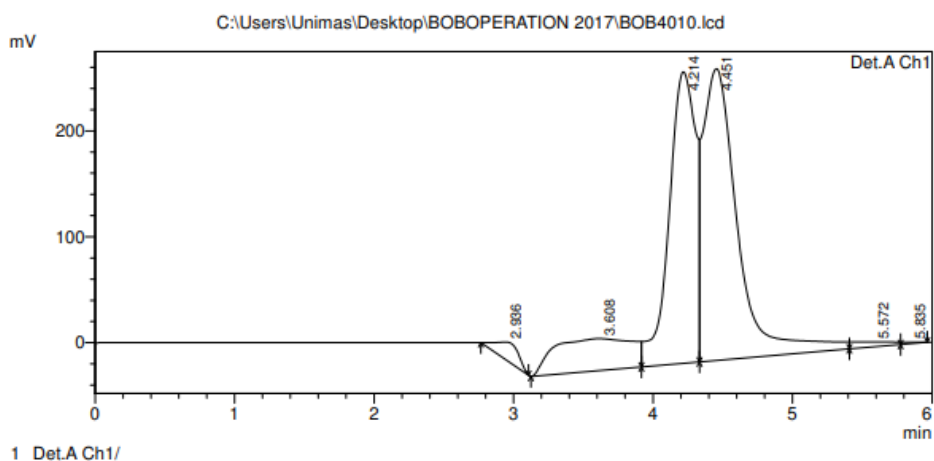
Hour s	9 hours		18 hours		28 hours	
	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)	Xylose
0	22.14±0.1 4	12.90±0.0 9	22.26±0.0 3	12.96±0.0 5	22.18±0.0 5	13.05±0.0 5
9	20.68±0.5 0	12.42±0.1 4	21.13±0.3 2	12.09±0.0 3	20.23±0.2 3	12.10±0.0 2
18	15.35±0.0 7	10.24±0.0 3	15.74±0.2 1	10.65±0.5 7	14.74±0.2 1	10.10±0.0 8
27	15.12±0.8 5	10.45±0.2 6	14.62±0.3 5	10.39±0.3 2	13.62±0.3 5	9.39±0.28
36	14.73±0.7 8	8.25±0.08	14.75±0.7 6	8.23±0.10	12.21±0.0 5	7.61±0.49
45	12.46±0.5 0	0.53±0.09	11.66±0.5 8	0.80±0.08	10.16±0.0 8	0.70±0.07
54	0.60±0.05	0.47±0.16	0.28±0.06	0.49±0.07	0.23±0.11	0.48±0.09

HPLC Chromatogram of Sugar Component in Destarched Sago Fibre Hydrolysate (DSFH)

==== Shimadzu LCsolution Analysis Report ====

C:\Users\Unimas\Desktop\BOBOPERATION 2017\BOB4010.lcd
 Acquired by : Admin
 Sample Name : 0H R1
 Sample ID :
 Vial # :
 Injection Volume : 20 uL
 Data File Name : BOB4010.lcd
 Method File Name : Adilla-method.lcm
 Batch File Name :
 Report File Name : Default.lcr
 Data Acquired : 4/22/2019 11:04:31 AM
 Data Processed : 4/22/2019 11:10:33 AM

<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.936	199811	15913	2.014	2.638
2	3.608	1190903	30207	12.002	5.008
3	4.214	3692711	275264	37.214	45.637
4	4.451	4727371	275365	47.641	45.653
5	5.572	98578	4799	0.993	0.796
6	5.835	13551	1615	0.137	0.268
Total		9922925	603164	100.000	100.000

Retention time

- a) 3.606: Oligosaccharide
- b) 4.214: Glucose
- c) 4.451: Xylose
- d) 5.5722: arabinose

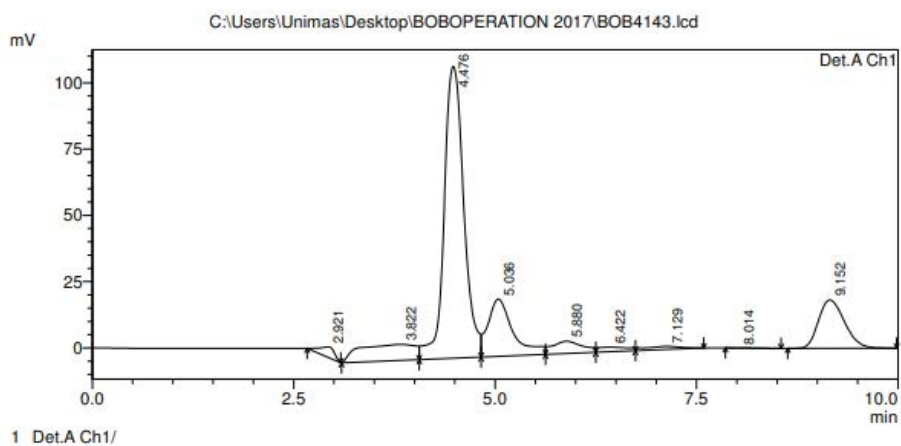
e) 5.835: Glycerol

HPLC Chromatogram of Sugar Component in Destarched Sago Fibre Hydrolysate (DSFH)

==== Shimadzu LcSolution Analysis Report ====

C:\Users\Unimas\Desktop\BOBOPERATION 2017\BOB4143.lcd
Acquired by : Admin
Sample Name : 30H R1
Sample ID :
Vial # :
Injection Volume : 20 µL
Data File Name : BOB4143.lcd
Method File Name : Adilla-method.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 5/8/2019 2:58:20 PM
Data Processed : 5/8/2019 3:08:23 PM

<Chromatogram>



PeakTable

Detector A Ch1					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.921	49850	3864	1.513	2.309
2	3.822	297907	6041	9.041	3.609
3	4.476	1867851	110065	56.688	65.752
4	5.036	464542	21638	14.098	12.926
5	5.880	121904	4633	3.700	2.768
6	6.422	43848	1721	1.331	1.028
7	7.129	37885	1157	1.150	0.691
8	8.014	1314	70	0.040	0.042
9	9.152	409875	18204	12.439	10.875
Total		3294975	167393	100.000	100.000

Retention time:

- a) 5.036: Xylitol
- b) 6.422: Acetic acid
- c) 8.014: Hydroxy Methyl Furfural
- d) 9.152: Ethanol