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Comparison of Acid Hydrolysis and Combination System Method for Production of Xylose from Meranti Wood Sawdust (MWS)

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ABSTRACT

Background: Meranti wood sawdust (MWS) is a liglocellulosic materials that contains of three main components of polymer which are cellulose, hemicellulose and lignin. It is consist of > 29% of xylan that made up from pentose sugar xylose. **Objective:** To compare the production of xylose from Meranti wood sawdust (MWS) by using chemical method and combination system method (physical and biological process). **Results:** Chemical process showsmuch faster reaction timein order higher xylose productionthan combination system method, but the production of byproduct (furfural) is main disadvantagesin chemical process. The maximum yield obtained were 14.13 g/l and 13.60 g/g using 40 minutes acid hydrolysis and 1455 minutes reaction period in combination system method respectively. **Conclusion:** The results obtained from the present study clearly indicate that the potential utilization of MWSby using both methodfor the production of xylose which can serve as functional food ingredients such as xylitol.

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INTRODUCTION

Meranti wood is a most common and popular species of hardwood in Malaysia. Most of sawmill has processed this species of wood since it's a lot of growth in peninsular and east of Malaysia. The residue generated after the processing timber logs is known as Meranti wood sawdust (MWS) which is a fully renewable and organic resources residues. It contains of three main components of polymer which are cellulose, hemicellulose and lignin. It is estimated that MWS biomass consist of >29% of xylan which is a sugar polymer that made up from pentose sugar xylose (Islam and Mimi Sakinah., 2011).

Bioconversion and hydrolysis of the lignocellulosic material can be producing a valuable product (eg: fuels, chemicals or food ingredients) since it is renewable, widespread and cheap sources of raw material in the nature. It is also can become an environment friendly option to reducing generated of waste. However, there are still limited uses of hardwood species residue in Malaysia. Usually, it is used as animal feed, fuel manufacturing plants or treated as a waste and dump it into the river. Because of that, most of the industries especially timber industries are increasing their interest to reuse and recycle back agro industrial residue to innovate the waste into wealth (Zainab *et al*., 2014; Garrote *et al*., 2002).

There are many different approaches have been used for the production of xylose from lignocellulosic biomass such as chemical, physical, biological and combination system method (Sabiha Hanim *et al*., 2011). Dilute acid hydrolysis is a popular studied and widely used method compared to other hydrolysis method. This method is effective and inexpensive with a single stage hydrolysis. It is conducted by diluted acid (less than 5% acid concentration) under the controlled conditions at temperature (120-230 oC) and pressure 10 atm. In the under control treatment conditions, acid act as a catalyst in acid hydrolysis of lignocellulosic biomass that mainly produce xylose from hemicellulose while cellulose and lignin component remain unaltered. This method can be applied either as a pre-treatment steps proceeding with enzymatic hydrolysis or as a main method of hydrolysis. Hemicellulose is easily to degrade during acid hydrolysis due to its amorphous, brunched structure compared to cellulose which needs severe treatment conditions due to its crystalline nature (Parajo *et al*., 2004).

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The utilization of lignocellulosic waste into xylose can be involves with both physical and biological methods. Pretreatment of lignocellulosic with saturated steam (steam explosion) or hot water (autohydrolysis) has been widely studies to weaken lignocellulosic structure that mainly focused on lignin and cellulose fraction. It is known as prehydrolysis process before proceeding enzymatic hydrolysis process (Xiao *et al*., 2013). It is conducted under mild operational conditions (non-isothermal condition) at temperature of 120-260oC. This method will separated the feedstock into two fractions which are an aqueous extract composed mainly of hemicellulose derivatives along with some water soluble lignin and a solid fraction composed of cellulose and extractable lignin. Basically, the liquors that obtained from autohydrolysis assays is contain a mixture of sugar oligomers (mainly xylooligomers), monosaccharides (mainly xylose), sugar decomposition products (furfural or hydroxyfurfural) and acetic acid that come out from acetyl groups. Hence, enzymatic hydrolysis is desirable to conduct for the further steps to recover xylose due to it does not produce undesirable by-products and high amount of monosaccharide (Akpinar *et al*., 2010). The study focuses on two difference hydrolysis methods (acid hydrolysis and combination system methods) for xylose production. Then, comparison of the methods will be made.

Methodology:

Raw material:

The MWS that collected from Seng Peng Sawmills Sdn Bhd was sun dried and screened to remove oversized particles. Then, it was sieve using sieve shaker to obtain the particle size less than 1200µm. The sample was analyse using mastersizer to make sure the samples was in range of size required. After that, it was dried in the oven at temperature 105°C for 24 hours before stored in a polypropylene plastic bag at room temperature for further use (Rafiqul and Mimi Sakinah 2012).

Acid Hydrolysis:

10 g of MWS was mixed with 3.24% w/w concentration of Sulfuric Acid in 100 ml Erlenmeyer flasks (Lavarack *et al*., 2002). The flasks were loaded with samples to a liquid solid ratio of 8 g/g. The slurries were allowed to stir using magnetic stirrer for 10 minutes at room temperature until completely mixed. Then, the hydrolysis process was carried out in an autoclave (Hiclave HVE-50, Hirayama, Japan) at constant temperature of 121oC for 20 minutes. After completing the acid hydrolysis process, the flasks were cooled at room temperature for desired length of time. Then, the content of the flasks were filtered using a filter paper (Whatman No. 1). The hemicellulosic hydrolysate was obtained at the filtrate (pH 1.25) and the cakes were contained cellulose and lignin (Musatto *et al*., 2005). The filtrate solution that obtains in acidic solution was neutralized with calcium Hydroxide (CaO). The CaO was added a little bit and stirred the solution by using magnetic stirrer for 15 minutes. After that, the stirred solution was checked the pH reading until it reach an average pH 6.5. The neutral sugar solution will be separated by filtration to obtain the clear solution.

Combination system method:

10 g of oven dried MWS was placed in 250 ml screwable capped Erlenmeyer flask. Distilled water was added to obtain slurries with liquid solid ratio (LSR) of 8 g/g. The slurries were mixed and stirred with magnetic stirrer for 10 minutes at room temperature to equilibrate the slurries. Autohydrolysis process was conducted in vacuum oven at 140 °C for 10 minutes. The thermocouple was used to monitor the temperature during the process. After the time interval elapsed, the flask was collected and cooled down at room temperature. Then, the slurries were filtered through vacuum filter to separate solid and liquid phases. The filtrate was known as hemicellulosic hydrolysate solution and the solid residue.

20ml of hemicellulosic hyrolyste from autohydrolysis treatment was dissolved in 100 ml 0.05M sodium acetate buffer, pH 5.0 in 250ml erlenmeyer flask. The hydrolysis of substrate was started by added 1 ml 4.1 U/ml commercial xylanase into the flask. The sample was allowed the incubation process at the incubator shaker. The process was conducted at 37 oC, 150 RPM for 24 hours (Akpinar *et al*., 2009). After the time interval elapsed, the sample was taken and it was heated in the water bath at 100oC for 5 minutes to inactivate the enzyme. Then, the sample was taken and cooled at room temperature for a certain time. The sample was filtered by using a filter paper (Whatman No 1) to separate the solid and liquid fraction named as xylose solution.

Sugar analysis:

High performance liquid chromatography (HPLC) analysis was used to estimate the concentration of xylan, xylose, glucose, arabinose, furfural, hydroxymethylfurfural and acetic acid. This analysis was carried out using an Agilent 1200 chromatograph (Agilent, USA) equipped with a refrective index detector (RID) and an ultraviolet diode array detector (UV-DAD). The concentration of the xylan, xylose, glucose, arabinose and acetic acid was detected by Refractive Index (RI) detector and a Rezex RHM Monosaccharide H+ column (300 mm x 7.8 mm; Phenomenex USA) in combination with guard column (50 mm x 7.8 mm; Phenomenex, USA) operate at 80oC using ultrapure water as mobile phase, 0.6 ml/min flow rate and 20µL as an injection volume (Rafiqul and Mimi Sakinah., 2012).

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RESULTS AND DISCUSSIONS

Composition of MWS:

A detailed amount of the MWS composition is necessary to determine for assessing the reactivity of constituents that present in the biomass. The main chemical composition of MWS used in this study was compared with the composition of MWS from In house method by forest research institute of Malaysia, FRIM laboratory (shown in Table 2.1). Based on the experiment conducted, the MWS contains cellulose, hemicellulose and lignin as the major biopolymers with content value are 41.06, 33.20 and 25.22% respectively. From the results, it is shows that the hemicellulose was a second highest component in the MWS. According to Saba 2003, Hardwood hemicellulose contains mostly xylan which is a heterogeneous polymer of pentoses (xylose and arabinose). Xylan was a sugar polymer made of xylose was being accounted in this study about 29.20%. Hence, the high amount of xylan makes the biomass adequate for xylose production.

Table 2.1: Main constituents of meranti wood sawdust (oven dry basis).

Constituents	*Content (%, w/w)*	*In house method by forest research institute of Malaysia, FRIM laboratory*
Cellulose (alpha)	41.06	48.1
Hemicellulose	33.20	23.6
Xylan	29.20	20.77
Lignin (acid insoluble)	25.22	33.9
Extractive	3.08	na
Ash	0.43	0.4
Pentosan	na	12.4

na : not available

Table 2.2: Comparison of acid hydrolysis and combination system method of MWS for production of xylose.

	Combination system method	**Acid Hydrolysis method**
Hydrolytic reagents	Distilled water (H_2O) (autohydrolysis), Xylanase (enzymatic hydrolysis)	3.24% w/w H_2SO_4
Time	15 min (autohydrolysis) + 24 hr (enzymatic hydrolysis) = 24 hr 15 min = 1455 min	40 min
Temperature	140°C (Autohydrolysis), 37°C (Enzymatic process)	121°C
Neutralization	-	Necessary (CaO)
Production of monosaccharide (mainly xylose)	13.60 g/l	14.13 g/l
Production of furfural	-	2.64

Comparison acid hydrolysis and combination system method on xylose production:

Table 2.2 was illustrated the comparison of acid hydrolysis and combination system method of MWS for production of xylose. Based on the results, both methods were showed the ability and tendency to produce xylose. Based on the experiment, it was found that in order to produce xylose, at least 1455 minutes hydrolysis period with enzyme was necessary. Acid hydrolysis is more faster (40 minutes) to convert the main constituent into xylose. Xylose production with respect to MWS was 14.13 g/l in acid hydrolysis while the yield was 13.60 g/g after 1455 minutes reaction period in combination system method.

A combination system method was conducted by combining physical process (Autohydrolysis) and biological process (enzymatic hydrolysis). Figure 2.1 (a) shows the process flow for xylose production using combination system method. In physical process, it was an alternative technology for the solubilisation of hemicellulose since no chemicals added other than water is used (Gerrote *et al*., 2002). However, the purity of liquor autohydrolysis that produce is low since there are consist of by product where liquor of autohydrolysis is partially

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fermentable by microorganism. Thus, a posthydrolysis step (enzymatic hydrolysis) was needed to produce more purify product. Xylanase from *Trichodema varied* was uses as a hydrolytic reagent to synthesis xylose from hemicellulosic hydrolysate (Jaskari *et al*., 1998).

Dilute hydrolytic reagent (3.24% w/w H2SO4) was used in the acid hydrolysis process. Figure 2.1 (b) shows the process flow for xylose production using acid hydrolysis process. The hydrolytic reagent used was toxic, corrosive and hazardous. Thus, a special stainless steel reactor that resistant to corrosion was needed to handle this hydrolysis process. Furthermore, the production of xylose through chemical process becomes highly expensive due to the difficulties to neutralize and purify the product. Other than that, the purity of xylose through the chemical process is low since there was producing a by-product substance which is furfural (2.64 g/l). This method also was facing a lot of problems at the end of the process especially to dispose waste itself. The waste that generated after acid treatment is hazardous and may cause environmental problems especially when it is directly throw without any treatment. The proper procedure is needed and highly cost should be taken to dispose the waste accordingly (Rafiqul and Mimi Sakinah., 2012).

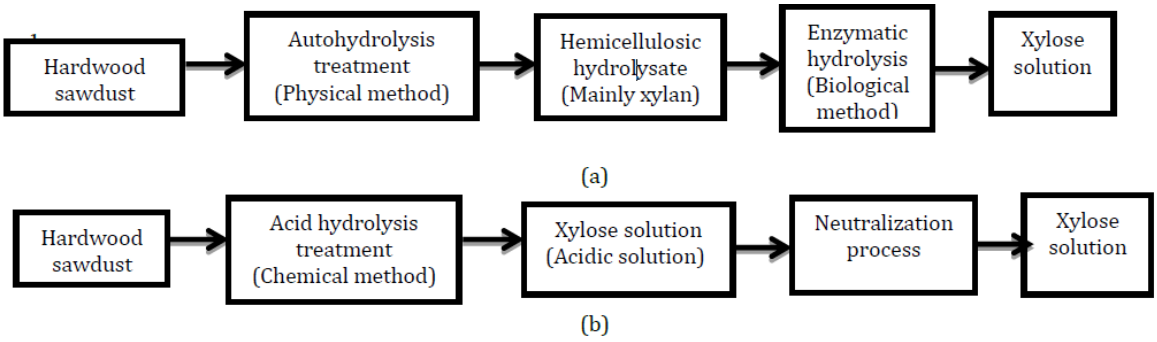

Fig. 2.1: Process flow for xylose production. a) Combination system method (Physical and biological methods). b) Acid hydrolysis method (Chemical method).

Conclusion:

MWS contains more than 29% xylan which promising source of xylose. It can be converted to xylose by either acid hydrolysis or combination system method. Acid hydrolysis was much faster reaction time than combination system method, but the production of byproduct (furfural) is main disadvantages. The maximum yield obtained were 14.13 g/l and 13.60 g/g using 40 minutes acid hydrolysis and 1455 minutes reaction period in combination system method respectively. The results obtained from the present study clearly indicate that the potential utilization of MWS, a residue of timber industry for the production of xylose which can serve as functional food ingredients such as xylitol.

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