Extraction of Xylose from Rice Straw and Lemongrass Leaf via Microwave Assisted

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Abstract. In recent years, agricultural wastes cause serious pollution to the environment. Agricultural residues can be used to produce value-added products in order to control environmental pollution, therefore these wastes are a promising feedstock as it has many advantages such as wide range of source, low cost and renewable. Rice straw and lemongrass leaves which are the renewable sources for the production of many useful products such as xylose. The aim of this study is to compare the content of xylose produced from rice straw and lemongrass leaves, in order to obtain highest yield of xylose and to optimize the acid hydrolysis time, concentration of sulphuric acid, liquid-solid ratio and the power of the microwave towards the maximum extraction of xylose. Microwave assisted acidic hydrolysis method was used to extract xylose from rice straw and lemongrass leaves. The acid hydrolysis of both rice straw and lemongrass was performed at different time (1-5 minutes), acid concentration (1%-5%), liquid solid ratio (1:30, 2:30, 3:30, 4:30 and 5:30) and power (160W, 320W, 480W, 640W, 800W). The results obtained indicate that the yield of xylose from rice straw (2.98g/L) via the microwave assisted is slightly higher than lemongrass (2.91 g/L) with optimum conditions of 3 minutes of hydrolysis time, 2% of sulphuric acid concentration, 1:30 of liquid solid ratio and 320W of microwave power. Meanwhile, the optimum conditions for lemongrass leaves are 4 minutes of hydrolysis time, 4% of sulphuric acid concentration, 2:30 of liquid solid ratio and 480W of microwave power. Thus, rice straw has slightly higher capability in production of xylose compared to lemongrass.

1. Introduction

Agricultural wastes can be obtained from the growth of plant and crop production. Agricultural wastes can play an important role in meeting the growing energy demands of the society in a sustainable manner. Generally, wastes like wheat straw, rice straw, sugarcane bagasse consists of a large amount of lignocellulose substance compared to fruit and vegetable wastes which have less content of lignocellulose [1]. Lignocellulosic material consists of cellulose, hemicelluloses and lignin cellulose are responsible for strength and the resistance to the degradation of linear polymer of D-glucose. Hemicellulose is a group of polysaccharides where it depends on the plant type and plant tissue. Lignin is held together by different linkages of phenolic polymer of phenylpropane units [2].

The agricultural wastes used in this research were rice straw (RS) and lemongrass leaves (LG). Lemongrass which is scientifically known as Cymbopogon citratus is one of the abundant agricultural wastes in Malaysia. Citral is the main constituent of lemongrass which associated with lemon flavour
essential of synthetic vitamin A. Moreover, lemongrass also has high content of lignocellulose where it contains 28.5-47% (w/v) of hemicelluloses, 7-11% (w/v) of lignin and 29.9-35% (w/v) of cellulose [4].

Rice straw is a fibrous by-product from rice production industry which is considered as an important feed resource. Rice straw contains 32-47% of cellulose, 5-24% of lignin and 19-27% of hemicellulose. Cellulose is the most abundant component that present in the rice straw. Rice straw is low in digestibility and protein but the content of carbohydrate is high. Glucose is the highest percentage of carbohydrate in rice straw, followed by xylose, arabinose, mannose and galactose. The conversion of rice straw to fermentable sugar is hindered due to the presence of lignin and hemicellulose. Therefore, pretreatment is required to break lignin and hemicellulose prior to enzymatic hydrolysis of polysaccharides [5].

Malaysia supplies about 65% of rice which is approximately 660 million tons of rice while another 35% will be imported from Thailand, Vietnam and India. At the same time, during harvesting and milling process, it also produces a large amount of rice residues which will be considered as agricultural wastes. Thus, these residues will be burnt through open burning that leads to significant air pollution [6]. Carbon dioxide (CO$_2$) is produced from the burning of rice straws which is the major pollutants in the atmosphere. Although it is a cost-effective method for straw disposal, but the burnt rice straw will lose its benefits. About 80% of nitrogen, 25% of phosphorus, 20-21% of potassium and 40-60% of sulphur will be loss due to burning of the wastes [7]. Thus, the rice straw waste can be used in the production of xylose instead of being an agricultural waste and it can be processed as renewable source for complementing from waste to wealth ideology.

The pretreatment is an important step to convert biomass into fermentable sugars. Pretreatment also helps to free the cellulose by breaking hemicellulose and lignin through enzymatic hydrolysis. A pretreatment process should be eco-friendly, cost-effective and simple to be used. An effective pretreatment should prevent the formation of by-products which might affect the enzymatic hydrolysis [8]. Thus, the pretreatment used in this research is microwave assisted acidic hydrolysis pretreatment. Microwave pretreatment method is used in various studies because it has a high heating efficiency which helps to degrade lignin and hemicellulose. This method is also able to operate easily and reduce the energy requirements [9]. Acidic hydrolysis via microwave assisted pretreatment is considered effective where it helps to split strong chemical bonds under high temperatures to release monosaccharides, oligosaccharides and lignin. Sulphuric acid (H$_2$SO$_4$) is used most commonly in acidic hydrolysis pretreatments. Hydrochloric acid (HCl) is not recommended to be used as it is too corrosive [10].

Xylose is a reducing sugar which is extracted from plants. It is the major components of hemicellulose which contains five carbon atoms and an aldehyde functional group. The chemical formula of xylose is C$_5$H$_{10}$O$_5$ [11]. Xylose has wide applications in food and medical industries. Xylose can be used as a substrate to produce chemical compounds through chemical or biotechnological process. Xylose from palm oil biomass, corncobs and rice straw are great alternative to white sugar. It has no negative side effects of the sugar produced. Xylose is secreted from the kidneys and not be metabolized by humans. Moreover, xylose can be reduced to produce xylitol by catalytic hydrogenation. Xylitol is a sugar substitute which is used extensively in food, odontological and pharmaceutical industry [12].

The aims of this study are to compare the maximum content of xylose between RT and LG leaves via microwave assisted pretreatment and to optimized the acid hydrolysis time, the concentration of sulphuric acid, liquid-solid ratio and power of the microwave to maximize the content of xylose.

### 1.1 Xylose

Xylose is a sugar which belongs to monosaccharide group. It is classified as a monosaccharide of aldopentose type because it contains five carbon atoms and includes an aldehyde functional group. Xylose can be found in the cell walls of many plants. Xylose is a pentose sugar that can makes up majority of the hemicellulose backbone in the cell walls of plants. This sugar can be used to convert into several valuable products using biotechnological methods. The production of xylose from agricultural wastes give many benefits to mankind [13]. Xylose has many applications in food industry, including
the dietary carbohydrates. Xylose can appear as part of glycosidic composition along with flavonoids, catechins or in polymeric sugars like starch. Xylose as a pentose sugar also contributes to the flavour and colour of the processed food. It is safe to be used in food because xylose is antibacterial, antifungal and contains natural healing agents [14]. Unlike glucose, xylose cannot be broken down in humans. Thus, it does not increase the level of blood sugar. People with diabetes or people who need to control their weight can consume xylose instead of glucose. Shortly, it can be said that xylose is an alternative to white sugar [15].

1.2 Lemongrass
Lemongrass is a plant that contains essential oil with fine lemon season. It plays an important role in the production of biofuel. Lemongrass can be a good source for biofuel because of it high-value essential oil content in it. Since the biomass would be a by-product of essential oil production, the cost for production of biomass for biofuel will be cheap [16]. Besides that, lemongrass is also used as herbal to treat minor health problems such as constipation, heat burn and bloating. Tea which is made from lemongrass is very good to improve healthy digestive tract. Citral that is found in lemongrass has anti-cancer properties. It helps to slow down the growth of breast cancer cells by fighting free radicles. Studies have shown that lemongrass does not give any toxic effects to human. Low dose of lemongrass extract can be harmful for species such as Salmonella typhi and Staphylococcus aureus [17]. Lemongrass contains phytochemicals such as flavonoids and phenolic compounds which consist of quercetin, kaempferol, luteolin and apigenin. Compounds such as alcohols, terpenes, ketones and aldehyde are also identified in the lemongrass. Hasim et al. (2015) [18] highlighted that lemongrass is also considered to have an anti-bacterial, anti-diarrheal, anti-fungal, anti-amoe bic and anti-inflammatory properties. Other properties such as anti-malarial, antioxidants, anti-mycobacterial, hypoglycemia and anti-mutagenicity were also indicated in some studies.

1.3 Rice Straw
Rice straw can be used to make some quality papers. Raw rice straw can be converted into pulp for the production of handmade papers. Rice straw is also excellent in fiber strength and thickness. The bottom layer of the paper will be function as bloating paper when the paper is coated with heavy paint. The bottom layer takes up extra moisture from top layer. The paper made from rice straw is stable towards ink response during printmaking [24]. Other than that, rice straw can be also used in the construction materials. The material produced from rice straw contains good acoustic which can control the noise and sound problems in indoor environment.

Rice straw that are left in the field can lead to environmental issues. It may release methane gas which can cause effect to the greenhouse [20]. Open burning can cause emission of gas such as carbon dioxide (CO2), methane (CH4), carbon monoxide (CO) and nitrous oxide (N2O) which leads to global warming. It can also easily invade the lungs which causes respiratory disease especially people with asthma [21].

Thus, it is necessary to utilize the rice straw and lemongrass leaves in order to convert them into economic values by extracting the xylose that has many benefits in different industries.

2. Methodology

2.1 Characteristics of Rice Straw and Lemongrass
Moisture content, ash content, pH content were determined according to [27] (Mukherjee et al., 2018) and [28] (Mabai et al., 2018) while lignin, cellulose and hemicellulose were determined by referring to [29] (Norazlina, 2016).
2.2 Acid Hydrolysis Via Microwave Assisted Pretreatment

The rice straw and lemongrass leaves were pretreated using microwave. Microwave pretreatment was carried out using a microwave oven (Model Sharp) with an operating frequency of 2450MHz. The investigated parameters were selected after pretesting based on a study by Kuittinen et al., (2015) [3]. Thus, four different parameters were investigated as simplified in Table 2.1:

| Parameter                  | Range used               |
|----------------------------|--------------------------|
| Acid Hydrolysis time       | 1,2,3,4,5 min            |
| Concentrations of H$_2$SO$_4$ | 1,2,3,4,5 % w/v          |
| Liquid-solid ratio         | 1:30, 2:30, 3:30, 4:30, 5:30 |
| Microwave power            | 160, 320, 480, 640, 800W  |

Table 2.1. Different hydrolysis parameters investigation via microwave assisted

About 1g of powdered rice straw was soaked in 10ml with 0.1M of NaOH and left for 15 minutes. Then, distilled water was used to wash and extract the solid substrate from NaOH. The substrate was separated from the solution through filtration technique using Whatman filter paper No 1. The filtered substrate is placed in a beaker filled with 30ml of 2% H$_2$SO$_4$ and the mixture was stirred. The beaker was placed on the rotating ceramic plate inside the microwave oven. Firstly, the mixture was exposed to five different pretreatment time which which were 1, 2, 3, 4 and 5 min. The process was done in three replicates for each sample [25]. The same steps were repeated using different concentration of sulphuric acid, different liquid-solid ratio and different pretreatment power.

At every interval of 2 min, the microwave was stopped, and the beaker was taken out to be stirred to ensure the uniform heating process [9]. After the microwave pretreatment, the solution was left to cool down to room temperature. The mixture was filtered using Whatman filter paper no. 1 and the filtrate obtained was used as crude xylose [33]. The same procedures were carried out for the lemongrass leaves sample.

2.3 Preparation of DNS Reagent

DNS is prepared by dissolving 5g of 3,5-dinitrosalicylic acid, followed by 201.5g of potassium sodium tartrate tetrahydrate and 8g of sodium hydroxide into 500ml of distilled water. The beaker containing this solution was wrapped with aluminium foil. Then, the solution was let to boil till the solution become clear in a dark place. A magnetic stirrer was used to stir the solution thoroughly. After a clear yellow solution is obtained, the solution was let to cool and was poured in a volumetric flask. The flask is kept in the chiller [26].

2.4 Determination of Xylose Content

Solutions obtained from procedure 2.3 were used in the xylose assay. The cold solutions were separated from the substrate using Whatman filter paper. The clear filtrate obtained, was used as crude xylose preparation [33]. About 2ml of DNS reagent was added to the solution. The mixture was then incubated at 100°C in water bath for 5 minutes. DNS reagent was used to measure the reducing sugar content at 540nm wavelength, with D-xylose as the standard and distilled water as the control by using spectrophotometer [27].

3.0 Results and Discussion

The characteristics of the rice straw and lemongrass leaves used in this study are shown in Table 3.1. The result show that the rice straw sample has greater cellulose, hemicellulose and lignin content compared to the lemongrass leaves.
Table 3.1. Chemical composition of treated rice straw and lemongrass

| Agricultural Wastes | pH    | Moisture content (%) | Ash content (%) | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---------------------|-------|-----------------------|-----------------|---------------|-------------------|-----------|
| Rice Straw          | 6.97  | 11.7                  | 75.0            | 44.9          | 17.8              | 6.2       |
| Lemongrass Leaves   | 5.35  | 8.4                   | 80.2            | 29.7          | 16.4              | 3.1       |

3.1 Optimum Conditions of Rice Straw and Lemongrass using Microwave Assisted Acidic Pretreatment

3.1.1 Acid Hydrolysis Time

Figure 3.1 shows the concentration of xylose versus microwave acidic pretreatment time for both rice straw and lemongrass sample. The time intervals used in this research were from 1 to 5 minutes. For the rice straw sample, the concentration of xylose increases as the time increases until it reached the maximum xylose content (2.86 g/L) at the third minute. Then, the xylose concentration started to decrease at minute 4 and minute 5. This is due to the further degradation of xylose under certain pretreatment conditions.

The same trend was observed for the lemongrass sample, with a further slight increase of xylose concentration at 4 minutes, followed by a reduction when the pretreatment time is increased to 5 minutes. The highest concentration of xylose is 1.54 g/L at fourth minute. During the optimization process, the time interval varies whereas the power is set at 160W and 2% of acid concentration was used. Increasing the hold time increases the yield of reducing sugars from the available carbohydrate [28] and therefore, the maximum sugar removal for rice straw was achieved with a pretreatment time of 3 min producing up to 2.86g/L of xylose. It is clearly demonstrated that the time taken for the production of xylose from rice straw is lesser than lemongrass. In a short period of time, rice straw was able to produce higher yield of xylose compare to the concentration of xylose produced by lemongrass at minute 4 which is 1.54g/L.

According to Yan et.al. (2018) [35], the highest xylose yield (93.2%) was obtained during the pretreatment using oxalic acid at 120 °C for 10 min, implying that almost all of the xylan in the bagasse was released and depolymerized into xylose sugar, under the given pretreatment conditions. In this study, it proves that the optimum time taken by both the samples to produce xylose is relatively less than the study conducted by Yan et al. (2018) [35].

![Figure 3.1. Concentration of xylose versus pretreatment time for rice straw and lemongrass](image-url)

3.1.2 Concentration of H₂SO₄

Figure 3.2 shows the concentration of xylose versus the concentration of sulphuric acid used for microwave acidic pretreatment of rice straw and lemongrass. For rice straw, the concentration of sulphuric acid used in this research were from 1% to 5%. Initially, the concentration of xylose increases.
The maximum content of xylose is reached at 2% of acid concentration. Then, the xylose concentration started to decrease from 3% till 5% of sulphuric acid concentration. The highest concentration of xylose is 2.86 g/L at 2% concentration of acid. During the optimization, time is set for 3 minutes.

For lemongrass, the xylose yield increases with concentration of H₂SO₄ ranging from 1% to 4%. However, with further increase in concentration of H₂SO₄, there was a decrease in xylose yield which suggested that the optimal range of H₂SO₄ concentration was 4%. The highest concentration of xylose is 1.67 g/L. During the optimization, time is set for 4 minutes. The microwave power used is 160W whereas the liquid-solid ratio was maintained at 1:30 for both the samples.

Second parameter, concentration of sulphuric acid, also clearly show that rice straw needs less concentration of acid in xylose production. H₂SO₄ is a powerful agent for acid hydrolysis which can be used to break the glycosidic bond to produce sugar monomers. According to Bekele et.al. (2017) [20], acid hydrolysis helps to enhance the process yield as it does not affect the quality of the product. This is related with the 'lock and key' principle which represents the enzyme and substrate during the process. Acid hydrolysis reduces the time of process and reduces the energy consumption.

H₂SO₄ produces a higher sugar release than NaOH. In the case of rice straw pretreatment, when the acid concentration is increased from 2% to 5%, the total sugar production declined, due to the stronger acid condition facilitating further degradation of xylose. But it was vice versa in the lemongrass pretreatment condition. As 4% of H₂SO₄ leads to optimum reducing sugar release, it can be predicted that maybe a severe condition can have better reducing sugar release for lemongrass [11].

Figure 3.2. Concentration of xylose versus concentration of H₂SO₄

3.1.3 Liquid-Solid Ratio.
Figure 3.3 shows the concentration of xylose versus liquid solid ratio used in microwave acidic pretreatment for rice straw sample. About 30ml of 2% sulphuric acid was used in the hydrolysis process [30]. The liquid solid ratio used in this research is 1:30, 2:30, 3:30, 4:30 and 5:30. The mass of sample was changed but the volume of sulphuric acid was kept constant. For rice straw, the maximum content of xylose (2.86 g/L) is reached when 1g of sample was used. Then, the xylose concentration started to decrease till 3g of samples used. There is a sudden increase when 4g of sample was used and then eventually decrease. The highest concentration of xylose is 2.86 g/L. During the optimization process, the time was set for 3 minutes and the concentration of acid is fixed at 2%.

For lemongrass, the maximum content of xylose at 1.88 g/L is reached when 2g of rice straw sample was used. Then, the xylose concentration decreases during the usage of 3g sample. There is a sudden increase when 4g was used before it eventually decrease. During the optimization process, the time is set for 4 minutes and the concentration of acid was fixed at 4%. The mass of sample varies whereas other parameters are kept constant. This shows that high yield of xylose is produced by rice straw compare to lemongrass.
3.1.4 Microwave Pretreatment Power.

Concentration of xylose versus microwave assisted pretreatment power for rice straw and lemongrass samples are shown in Figure 3.4. The power used in this research is 160W, 320W, 480W, 640W and 800W. The concentration of xylose increases when the sample was kept at 160W. The maximum xylose content was reached at 320W. Then, the xylose concentration decreases till the end. The highest concentration of xylose is 2.98 g/L.

For lemongrass, the concentration of xylose increases gradually until the microwave power reached 480W. At this microwave power, xylose content was the highest at 2.91 g/L. Then, the xylose concentration decreases till the end. The highest concentration of xylose is 2.91 g/L. During this optimization process, about 2g of lemongrass was hydrolyzed using 4% H$_2$SO$_4$ for 4 minutes. It can be said that rice straw requires less power to produce high content of xylose.

Microwave irradiation power will give a high impact on the structure of plant cells. As the temperature rises, the degree of polymerization of cellulose declines and long cellulose chains collapse to shorter groups of molecules. It releases xylose that degrade to hydroxymethyl furfural. Thus, it is important to find a proper microwave power to avoid the degradation of some useful components [36]. According to a study by Pang et.al. (2012) [29], the highest yield of xylose was obtained at 540W microwave power using corn stover. In this present study, the highest yield of xylose for rice straw was produced at 320W whereas for lemongrass, the optimum microwave power is at 480W.

Figure 3.4. Concentration of xylose versus pretreatment power for rice straw

In this study, xylose produced from rice straw is slightly higher than lemongrass using microwave acidic pretreatment. It can be concluded that rice straw has higher capability to produce xylose. Rice
straw contains more hemicellulose compared to lemongrass as shown in Table 4.2. The hemicelluloses fraction of agricultural wastes contains xylose as the major sugar component. These xylose-rich wastes can be recovered in high yield through acid hydrolysis. Rice straw which contains 25.4% of hemicellulose is able to produce 2.98g/L of xylose whereas lemongrass which contains 22.6% of hemicellulose able to produce 2.91g/L. It is also easier to produce xylose from rice straw as it requires less time and energy [38].

Table 3.2. Optimum conditions for xylose production from rice straw and lemongrass

| Agricultural Wastes | Time (minutes) | Concentration of H$_2$SO$_4$ (%) | Liquid to Solid Ratio | Power of Microwave (W) | Maximum Concentration of Xylose (g/L) |
|---------------------|---------------|----------------------------------|-----------------------|------------------------|-------------------------------------|
| Rice Straw          | 3             | 2                                | 1:30                  | 320                    | 2.98                                |
| Lemongrass Leaves   | 4             | 4                                | 2:30                  | 480                    | 2.91                                |

4. Conclusion

The main aim of this study was to compare the content of xylose produced from rice straw and lemongrass and to optimize the important parameters of microwave pretreatment which are pretreatment time, concentration of acid, liquid-solid ratio and pretreatment power to maximize the content of xylose using one factor at a time. Pretreatments were able to change the structure of lignocellulosic biomass. Microwave acidic pretreatment helps to increase the saccharification of rice straw and lemongrass by removing lignin and hemicelluloses and increase its accessibility to xylose. The amount of sugar (xylose) released during microwave pretreatment and the xylose production in this study proved that microwave pretreatment has a significant performance on biomass pretreatment process.

It was found that the optimum conditions for rice straw were 2% H$_2$SO$_4$ at 320W of microwave for 3 minutes able to provide a maximum xylose yield of 2.98g/L while optimum conditions for lemongrass were 4% H$_2$SO$_4$ at 480W of microwave for 4 minutes with maximum of xylose at 2.91g/L. The results show that in comparison, the rice straw produces slightly more xylose than the lemongrass.

5. References

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