Comparative Study of Xylose Extraction from Lemongrass Leaves via Autoclave Assisted Acid and Alkali Hydrolysis

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Lemongrass (*Cymbopogon* species) is an aromatic plant that is cultivated mainly as a source of essential oils. It is a perennial species grown extensively in tropical and subtropical regions around the world. Lemongrass leaves (LL) are a waste product generated by the agricultural industry that is rich in lignocellulosic material such as lignin, cellulose and hemicellulose. Xylose is a sucrase inhibitor which is very useful as a functional sugar complement to inhibit blood glucose levels from rising. Autoclave-assisted acid and alkaline hydrolysis methods were used to extract xylose from lemongrass leaves. The yields obtained were used to determine the most efficient hydrolysis method for xylose extraction. The acid and alkaline hydrolysis of lemongrass leaves were performed using a range of hydrolysis times (10 - 150 min), initial acid/alkali concentrations (0.6 % - 5 %), solid-liquid ratios (1:30 - 8:30) and temperatures (105 - 140 °C). The maximum xylose yield via acid hydrolysis was 69.57 g/L, under the optimum conditions of 90 min hydrolysis time, 0.8 % sulphuric acid, a solid-liquid ratio of 1:5 and an autoclave temperature of 125 °C. However, with alkaline hydrolysis the maximum yield achieved was only 4.119 g/L, under optimum conditions which were 120 min hydrolysis time, 2% sodium hydroxide, a solid-liquid ratio of 2:15 and autoclave temperature of 125 °C. Thus, acid hydrolysis was much more efficient in the production of xylose compared to alkaline hydrolysis.

Keywords: Xylose; hydrolysis; autoclave assisted; lemongrass leaves

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Lignocellulosic materials such as agricultural, hardwood and softwood residues, are potentially viable sources of sugars for many useful applications such as biofuels, bioethanol, biocomposites and biosorbents. Its main constituents are cellulose, hemicellulose and lignin. Lemongrass (*Cymbopogon citratus*) is an abundant source of agricultural waste in Malaysia. Globally, approximately 30,000,000 tons per annum of lignocellulosic lemongrass residues are generated mainly from industrial extraction processes [1]. In addition, lemongrass is one of the most extensively-grown perennial plants in tropical and subtropical regions. It is mainly cultivated for the essential oils found in the stems of this aromatic plant [2]. In the leaves and stems of lemongrass, an essential oil called citral (3,7-dimethyl-2,6-octadienal) can be readily obtained by hydro-distillation. The essential oils in lemongrass have useful chemical components that are utilized for their anti-fungal, anti-microbial, anti-inflammatory, antioxidant, cancer chemo-preventive, cardioprotective, anti-depressive, anti-spasmodic, diuretic, sedative and analgesic properties [3]. In addition, *Cymbopogon citratus* is used worldwide as an ingredient in herbal teas. According to Anal (2014) [4], research has been conducted on the concentrations of twelve trace elements (including magnesium, calcium, copper, manganese, nickel, zinc, chromium and iron) in *Cymbopogon citratus* which showed that this species possesses beneficial health properties.

Lemongrass contains cellulose-rich fibres that can serve as a potential raw material source for various applications including pulp and paper [5]. However, there have been no current studies on the use of lemongrass as a source of xylose. Xylose is used in blood xylose level measurement (DNA). It is classified as a monosaccharide of the aldopentose type because it contains 5 carbon atoms. Pentose sugars make up the majority of the hemicellulose backbone in the cell walls of plants. Xylose can be extracted from lignocellulosic waste using very common methods such as acid hydrolysis. Xylose has many applications in food, health and also in the cosmetics industry where it is used in disinfectants or liquid antibacterial soaps [6]. It can be included in the daily diet because it is a partly glycosidic compound like flavonoids or polymeric sugars [6]. Unlike glucose, xylose cannot be broken down in humans. Thus, it does not increase blood sugar levels. In short, xylose is a healthy alternative to white sugar [7].

Autoclaved-assisted acid hydrolysis is considered an effective method as it can cleave strong chemical bonds under high temperatures to release monosaccharides, oligosaccharides and lignin. Sulphuric acid (H_2SO_4) is most commonly utilized in acid hydrolysis [8]. Hydrochloric acid (HCl) is not recommended as it is too corrosive for most machinery [9]. In alkaline hydrolysis, the addition of alkaline solution hinders the binding of lignin to hemicellulose which enhances the digestibility of hemicellulose and boosts enzyme access to cellulose. This promotes the internal surface and solubilizes the binding wax between lignin, hemicellulose and cellulose [10]. Alkaline hydrolysis has very good solubilization properties with the substrate, but when agricultural waste is mixed with alkali the reading rate of 40 % (v/w) is inaccurate [11]. The present work aims to compare the performance of autoclave-assisted acid and alkaline hydrolysis methods in extracting xylose from lemon-grass leaves (LL). The hydrolysis para-meters analysed included hydrolysis time, concentration of sulphuric acid/sodium hydroxide, weight of lemon- grass leaves and autoclave temperature.

MATERIALS AND METHODS

Collection and Preparation of Lemongrass Leaves

A sample of lemongrass leaves (LL) was collected from a hatchery located at the Faculty of Engineering and Life Science, Universiti Selangor, Malaysia. The LL sample was washed with distilled water and dried under sunlight for 24 h. Subsequently, the LL were crushed with a blender (Model Butterfly B-592) to an average size of 2 mm. The LL sample was kept in a container at room temperature till further use [12].

Compositional Properties of Lemongrass Leaves

Moisture content, ash content and pH content were determined according to Mukherjee *et al.* (2018) [13] and Mabai *et al.* (2018) [14]. Lignin, cellulose and hemicellulose were determined using the methods of Lewandowski *et al*. (2018) [15].

Autoclave-Assisted Acid and Alkaline Hydrolysis

For treatment purposes, a 1 g sample of LL was soaked in 10 mL of 0.1 M NaOH for 15 min, then washed with distilled water and filtered prior to acid hydrolysis. Subsequently, 1 g of LL was added to a 250 mL conical flask with 30 mL of 1 % (v/v) H_2SO_4 which was placed in an autoclave (Model: Hirayama) set at 105 °C for 10 min for acid hydrolysis [16]. These steps were repeated using an alkali (NaOH) for comparison purposes. Next, for both H_2SO_4 and NaOH hydrolysis methods, various parameters were optimized using one factor at a time (OFAT) as shown in Table 1[17]. The laboratory work for both hydrolysis procedures was carried out in triplicate.

Preparation of Dinitrosalicyclic Acid (DNS) Reagent

Dinitrosalicyclic acid (DNS) was prepared by dissolving 5 g of 3,5-dinitrosalicylic acid, 201.5 g of potassium sodium tartrate tetrahydrate and 8 g of sodium hydroxide in 500 mL of distilled water. The beaker containing this solution was wrapped with aluminium foil. Then, the mixture was boiled on a hotplate in the dark until the solution became clear. A magnetic stirrer was used to thoroughly stir the mixture, which was initially a thick yellow suspension. After a clear yellow solution was obtained, the solution was allowed to cool and then poured into a volumetric flask. The DNS reagent was kept in the chiller and used within 2 weeks [19].

Determination of Xylose Content

After cooling, the hydrolysate was separated from the LL using Whatman filter paper No. 1. The clear filtrate was used to determine the xylose yield [20]. About 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added to the filtrate. The mixture was then incubated at 100 °C in a water bath for 5 min. The reducing sugar (xylose) content of the sample was determined at 540 nm using D-xylose as a standard. Distilled water was used as the control for the xylose assay during the optical density measurement [21].

RESULTS AND DISCUSSION

Structural and Morphological Characterization

The characteristics of the lemongrass leaves used in this study are given in Table 2. The composition of cellulose, hemicellulose and lignin in this sample were in line with the values reported in a study by Norazlina et al. (2020)[2].

Table 1. Experimental setup for autoclave-assisted acid and alkaline hydrolysis

Parameters	***Range***
Hydrolysis time	10 – 150 min
Concentration of H_2SO_4 or NaOH	0.6 - 5% (v/v)
Weight of lemongrass leaves	1 – 8 g
Hydrolysis temperature	105 – 140 °C

Table 2. Composition of lemongrass leaves

pH	*Moisture content (%)*	*Ash content (%)*	*Cellulose (%)*	*Hemicellulose (%)*	*Lignin (%)*
5.38	27.41%	24.05%	29.91%	15.89%	3.4%

Effects of Hydrolysis Time

Hydrolysis time is a significant parameter in the hydrolysis process. Many researchers include this parameter in their optimization studies, e.g. Kasangana et al. (2022), Zhang et al. (2020) and Temis et al. (2017). While investigating hydrolysis time, other parameters such as temperature (105 °C), acid/ alkali concentration (2%) and LL weight (1 g) were fixed. Figure 1 indicates the correlation between the concentration of xylose and hydrolysis time for both H_2SO_4 and NaOH between 10 to 150 minutes. For acid hydrolysis, the concentration of xylose increased up to 90 min, and then decreased. The xylose content was at a maximum of 37.67 g/L at 90 min. With alkaline hydrolysis, the xylose yield increased with time from 10 to 60 min, decreased at 90 min, and then slightly increased at 120 min, followed by another decrease when the hydrolysis time was extended to 150 min. The highest yield of xylose was 2.364 g/L at 120 min. This shows that the xylose yield was 59.3% higher with acid hydrolysis compared to alkaline hydrolysis. These results were in agreement with Islam and Ali (2018) [22], who had studied different hydrolysis extraction methods with jute, and found that between sulphuric acid (H_2SO_4), distilled water and sodium hydroxide (NaOH), sulphuric acid (H_2SO_4) showed the highest xylose yield of 19.5 % (w/w). Ethaib et al. (2015) [29] noted that an extended hydrolysis time may lead to a decline of biomass digestibility and cause the decomposition of released sugars. For example, dissolved xylose can be decomposed into furfural, thus reducing the concentration of xylose. Kasangana et al. (2022) stated that xylose content increased with acid concentration and then decreased after reaching its maximum value.

H_2SO_4 and NaOH Concentration Effects

The optimum hydrolysis times using acid and alkali were determined at 90 and 120 min respectively, thus these values were used in the following experiments. The hydrolysis temperature and solid-liquid ratio were set at 105 °C and 1:30 respectively. For acid hydrolysis, the concentrations of H_2SO_4 used in this study were 0.6 % to 5 %. Figure 2 illustrates the xylose concentration versus H_2SO_4 and NaOH concentrations. Initially, the concentration of xylose increased. The maximum yield of xylose was 51.25 g/L with an acid concentration of 0.8 %. For the alkaline hydrolysis, the maximum yield of xylose was 2.364 g/L with an NaOH concentration of 2 %. The difference in the xylose yield between acid and alkali hydrolysis was a very significant 117.6%. According to Bekele et al. (2017) [23], acid hydrolysis enhanced the process without affecting the quality of the product. This is related to the 'lock and key' principle which represents the enzyme and substrate during hydrolysis. Acid hydrolysis reduced process time and energy consumption. In addition, extraction of lignocellulosic biomass with acid hydrolysis resulted in the liberation of mainly hemicellulose fractions, whereas alkaline hydrolysis combined with microwave heating efficiently removed both hemicellulose and lignin from lignocellulose [24]. Thus, acid hydrolysis was more efficient than alkaline hydrolysis at extracting xylose.

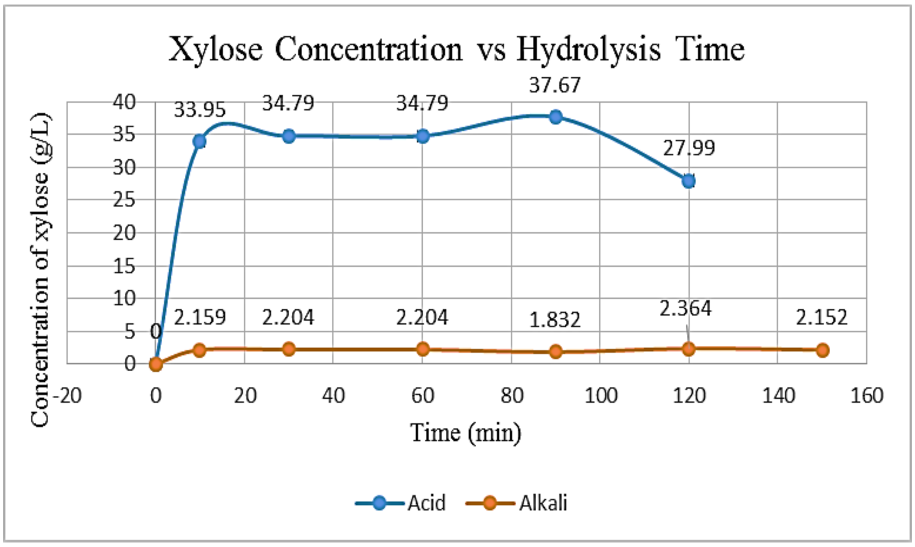

Figure 1 Concentration of xylose versus hydrolysis time for acid and alkaline hydrolysis

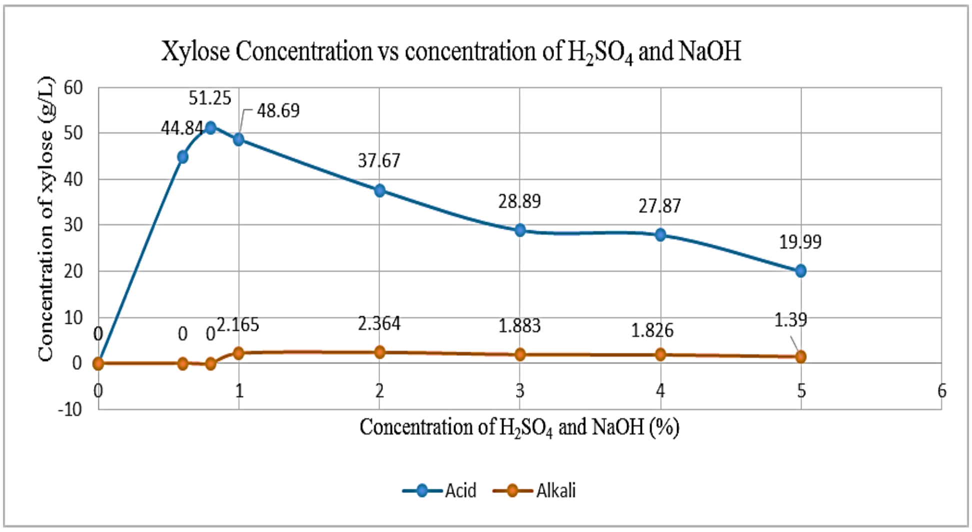

Figure 2 Xylose yield vs concentration of H_2SO_4 and NaOH

Effect of Weight of Lemongrass Leaves

As shown in Figure 3, the optimum LL weight for acid hydrolysis was 6 g in 30 mL of 0.8 % (v/v %) H_2SO_4 with a maximum xylose yield of 64.96 g/L. However, with alkaline hydrolysis the optimum LL weight was 4 g but the xylose yield was also lower, at 3.863 g/L. Therefore, acid hydrolysis was a better method of xylose extraction.

Deshavath et al. (2017) [25], investigated the extraction of xylose in sorghum stalks. The optimized parameters were 121 °C, 2 % sulphuric acid and 120 min hydrolysis time, which gave a xylose yield of 0.48 g/L. In a study by Choudhary et al. (2013) [26], sorghum bicolour straw was investigated to determine its maximum xylose content. The optimized parameters used were 2 % sulphuric acid at 121 °C for 10 minutes. The xylose yield was 0.205 g/L. The xylose content obtained in this study was higher compared to previous studies.

In order to investigate the effects of sample weight, 30 mL of 0.8 % sulphuric acid and 90 min of hydrolysis time were set as constant values while the liquid-solid ratio (g:ml) was varied, at 1:30, 2:30, 3:30, 4:30, 5:30, 6:30, 7:30 and 8:30. For acid hydrolysis, the maximum yield of xylose was reached when 6 g of LL was used. The xylose yield then started to decrease up to a sample weight of 8 g. The highest yield of xylose was 64.96 g/L.

For alkaline hydrolysis, the maximum yield of xylose (3.863 g/L) was reached when 4 g of sample was used. The xylose yield decreased when a 5 g sample was used. During the optimization process, the time was set at 120 min and the acid concentration was fixed at 2 %. This shows that a higher yield of xylose was produced with acid hydrolysis.

In the present study, the optimized parameters for acid hydrolysis were 0.8 % sulphuric acid, 90 min and 105 °C with 6 g of sample, which produced a xylose yield of 64.96 g/L. Meanwhile, for alkaline hydrolysis, the optimized parameters were 120 min, 105 °C, 2 % alkali and 4 g lemongrass leaves which produced 3.863 g/L xylose.

The yield of xylose obtained with acid hydrolysis was higher compared to alkaline hydrolysis because of the biomass composition. The temperature, time and acid/alkali concentration could be adjusted by controlling the sample weight. If a longer time was needed, a higher sample weight could be used. However, the dosage should be reduced if a higher yield of xylose is required [13]. This in agreement with a study done by Devi, Dhaka and Singh (2016) [28], who reported that alkaline hydrolysis was a slow process that required neutralization, and the added alkali also needs to be recovered. Dilute NaOH treatment of the lignocellulosic biomass causes swelling, leading to an increase in the internal surface area, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrate and disruption of the lignin structure.

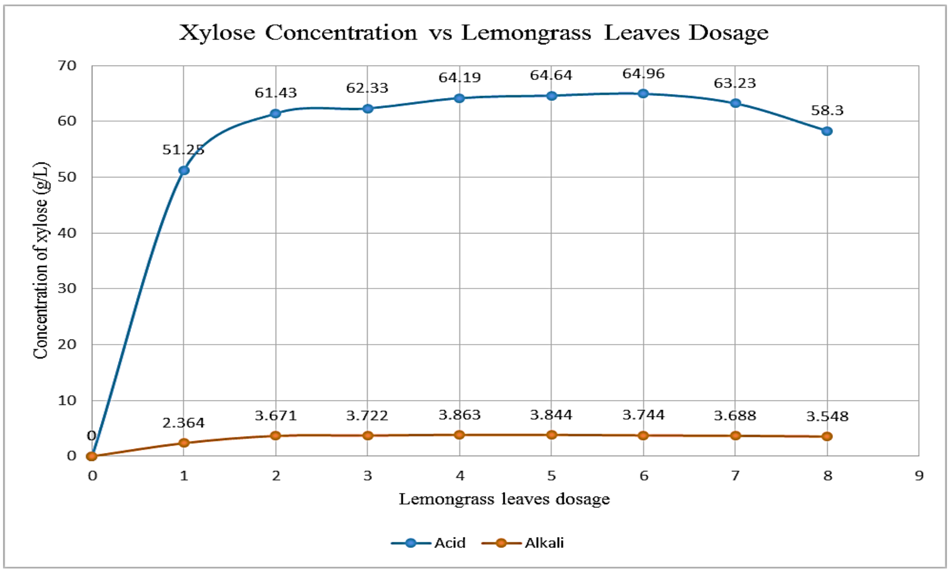

Figure 3 Concentration of xylose versus lemongrass leaves dosage/weight with acid and alkaline hydrolysis

Effect of Hydrolysis Temperature

Figure 4 reveals the effect of hydrolysis temperature on the yield of xylose with acid hydrolysis. The temperatures used in this study were 105 °C, 110 °C, 115 °C, 120 °C, 125 °C and 130 °C. The concentration of xylose increased with temperature from 105 °C to 120 °C. The maximum xylose content of 69.57 g/L was reached at 125 °C. Then, the xylose concentration decreased slightly as the temperature increased. In comparison, the maximum xylose content obtained by alkaline hydrolysis was only 4.119 g/L at 125 °C. These results agree with Kuittinen et al. (2016) who stated that the optimum temperature was 170 °C; this indicates that a higher temperature, and thus more energy, was needed when microwave heating was used for extracting xylose. According to a study by Pang et al. (2012) [27], different hydrolysis methods produced the highest yields of xylose at different extraction temperatures. Xylose is a major component of hemicellulose. Therefore, xylose-rich wastes can be recovered in good yield by acid hydrolysis. Lemongrass, which contains 15.89 % hemicellulose, produced 69.57 g/L of xylose via acid hydrolysis. This is a significant amount compared to the 4.119 g/L of xylose obtained via alkaline hydrolysis. It is also easier to produce xylose from lemongrass as it requires less time and energy [29].

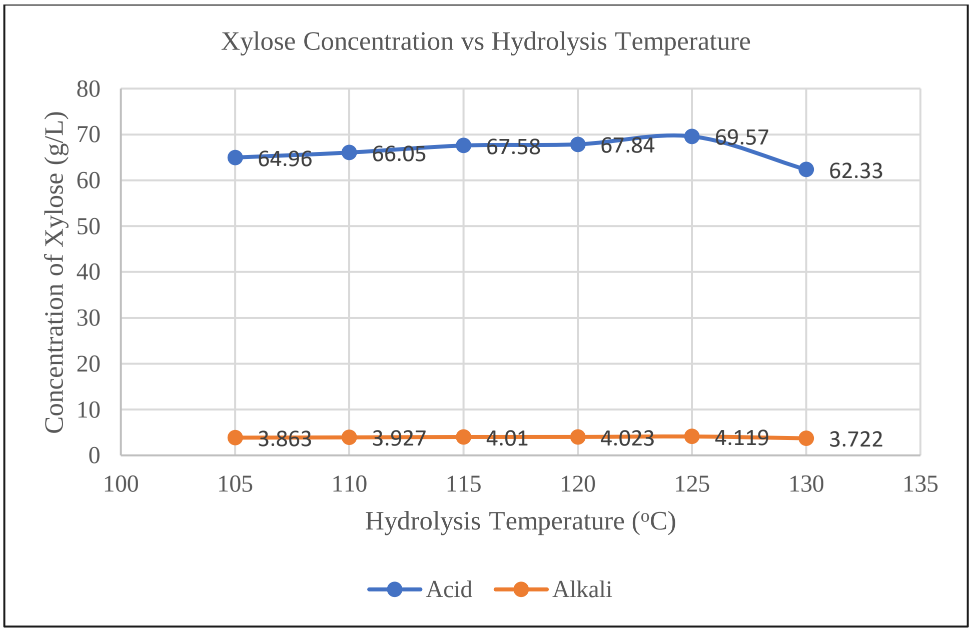

Figure 4 Concentration of xylose versus hydrolysis temperature for acid and alkaline hydrolysis

Table 3. Optimal conditions for xylose production by acid and alkaline hydrolysis

Hydrolysis	***Time (min)***	***Concentration (%)***	***Lemongrass leaves weight (g)***	***Temperature of Autoclave (°C)***	***Maximum Concentration of Xylose (g/L)***
Acid	90	0.8	6	125	69.57
Alkaline	120	2	4	125	4.119

As summarized in Table 3, the maximum xylose yield of 69.57 g/L was successfully obtained via autoclave-assisted acid hydrolysis.

CONCLUSION

The findings show that the LL sample contained holocellulose (45.80 %), α-cellulose (29.91 %), hemicellulose (15.89 %) and lignin (3.40 %). Its pH was 5.38, moisture content was 27.41 % and ash content was 24.05 %. The optimum acid (H_2SO_4) hydrolysis parameters were 90 min, 0.8 % (v/v) of H_2SO_4, 6 g of lemongrass leaves and 125 °C. The maximum xylose yield of 69.57 g/L was achieved with diluted H_2SO_4 hydrolysis (1.0 % v/v), and this value was significantly greater compared to the 4.119 g/L obtained using diluted NaOH (1.0 % v/v). Therefore, the extraction of xylose by autoclave-assisted acid hydrolysis was significantly more efficient than alkaline hydrolysis at extracting reducing sugars from lemongrass leaves.

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The authors declare that they have no conflict of interest.

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