

Comparative Analysis of Alkaline Pretreatment Methods for Levulinic Acid Production from Oil Palm Biomass Waste

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Abstract

The effectiveness of alkaline pretreatment at elevated temperatures in the acid hydrolysis of palm mesocarp fiber (OPMF) was compared to ultrasonic-assisted alkaline pretreatment at room temperature. The study varied temperatures (60°C, 80°C, and 100°C) and NaOH concentrations (4% and 8% v/v) for the alkaline pretreatment at elevated temperatures. For the ultrasonic-assisted alkaline pretreatment, the effect of sonication power (40%, 60%, and 80%) and NaOH concentrations (4% and 8% v/v) were evaluated without heating to a higher temperature. After pretreatment, the samples were subjected into acid hydrolysis under constant condition. The highest percentage of levulinic acid (LA) was obtained from the sample pretreated with alkaline pretreatment with heating at 100°C with 8% NaOH, yielding 3.28% LA. In contrast, the sample from ultrasonic-assisted pretreatment at 80% sonication power using 8% NaOH resulted in a yield of 2.20% LA during acid hydrolysis reaction. The findings indicate that alkaline pretreatment at elevated temperatures is more effective in enhancing the acid hydrolysis reaction, producing higher LA yields than the ultrasonic-assisted alkaline pretreatment. Further study is recommended to explore the potential of combining ultrasonic-assisted alkaline pretreatment with high temperatures to enhance LA yields. It is important to highlight that ultrasonic-assisted pretreatment was able to improve LA production from OPMF compared to no pretreatment sample. Ultrasonic pretreatment has been demonstrated successful in achieving delignification proven by FTIR results, which is its main purpose. To achieve higher LA yields, further study is needed to explore additional reactions involved within the process.

Highlights

- The potential of alkaline pretreated oil palm biomass for producing LA
- Assessed alkaline pretreatment at elevated temperatures versus ultrasonic-assisted alkaline pretreatment.
- Different conditions during alkaline pretreatment were studied.
- Higher yields of sugars and LA with alkaline pretreatment at elevated temperatures.

Keyword

Oil Palm Mesocarp Fiber; Levulinic Acid; Alkaline Pretreatment; Ultrasonic-Assisted; Hydrolysis

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I. Introduction

Levulinic acid (LA) serves a vital role as a key precursor in catalytic transformations with diverse potential industrial application for producing various chemicals and fuels [1]. LA is one of the products formed from acid-catalyzed hydrolysis of lignocellulosic biomass [2]. LA is an organic acid containing a carboxylic acid group and a ketone group which make LA a potentially versatile building block for production of many chemicals and organic compounds [3]. Gundekari et al. (2024) [4] found that LA is synthesized through a two-step process using glucose generated from cellulose which was followed by degradation into 5-hydroxymethylfurfural (HMF) as the precursor. Next, HMF is subjected to hydrolysis in an aqueous environment, leading to the production of LA with formic acid generated as a secondary product.

Oil palm is a major agricultural crop in Malaysia, making the fourth largest contribution to the country's Gross domestic product (GDP) [5]. Oil palm biomass is an attractive renewable biomass for the production of many high value products. Since oil palm biomass is abundantly available in palm oil plantations, the biomass can be utilized to produce various valuable products such as catalysts, animal feeds, healthy food products and biofuels [6]. After the palm fruitlets are detached from the bunches, they undergo digestion and reconditioning in a digester to eliminate the mesocarp before being processed in a screw press for the extraction of oil [7]. Hence, oil palm mesocarp fibre (OPMF) is one of oil palm biomass. Approximately 15,700 tons of fiber can be produced from every 100,000 tons of oil palm fresh fruit bunches that are processed to extract oil [8]. In regions with significant production of palm oil, these waste materials are already posing challenges in terms of landfill management and OPMF is a significant byproduct that remains after the processing of fresh palm oil fruit [9]. The current utilization of these fibers however has created huge environmental pollution to the environment. Therefore, it is important to find a more profitable application of this valuable biomass. Synthesis of green chemicals such as LA and other derivatives from OPMF is the best opportunity for Malaysia to intensify the valorization of its biomass.

Pretreatment is the first and important step in conversion of lignocellulosic biomass including OPMF into high value added products. Pretreatment assists in breaking carbohydrates-lignin complexes and thereby releasing the lignin from biomass and enhancing the accessibility to chemicals or enzymes [10]. Cellulose is a biopolymer made up of individual units of D-glucose that are connected in straight chains without any branches, using β -1,4 glycosidic connections while hemicellulose is a complex polysaccharide made up of various types of sugar monomers, including pentose units like xylose and arabinose, and hexose units like mannose, glucose, and galactose. Meanwhile, lignin is a complex polymer with a three-dimensional structure and high molecular weight [11]. Chemical pretreatments include both alkaline pretreatment and acid pretreatment under different various processing parameters such as reaction time and reaction temperature [12]. Alkali pretreatment is the most widely used technology among the various chemical pretreatments in enhancing the biomass conversion. Among the frequently used reagents in alkaline pretreatment are ammonium hydroxides, potassium, sodium, calcium hydroxide and sodium (NaOH) but the latter is the most common because of its outstanding delignification capacity [13].

Although alkaline pretreatment has been effective in delignification process, it requires a large amount of alkali and is also a time-consuming method. An appropriate physical step is necessary to enhance the alkaline pretreatment of lignocellulose in order to shorten the pretreatment time and/or reduce the usage of bases [14]. Thermal or ultrasound technology can be used together with alkaline pretreatment to mitigate the processing issues. It has the potential to enhance the biomass conversion and increase the production of sugar through the deconstruction of biomass structure from cavitation forces [15]. However, to date, only a few reports are available on the ultrasound-assisted alkaline pretreatment of oil palm biomass. In this paper, the effects of alkaline pretreatment using thermal or ultrasound techniques were explored on the acid hydrolysis of OPMF. Only dilute NaOH solutions were evaluated in this study. Two alkaline pretreatments, with and without ultrasonication were compared. Although alkaline pretreatment has been extensively studied and demonstrated successful in delignification, there is currently no study that explicitly evaluates the use of alkaline pretreatment for the LA production from OPMF. This gap in the literature underscores the need for further exploration in this area to better understand and optimize the process for sugars, HMF and LA production from OPMF.

II. Materials and Methods

2.1. Raw Materials

Oil palm mesocarp fiber was obtained from the Sungai Tinggi, Kuala Kubu Baru, Selangor. The fiber was grounded by using a grinder into approximately 1000 μ m particle size. Afterwards, the grounded fiber was screened and sieved through 20 and 80 mesh siever to obtain particles less than 0.5mm. The dry weight of OPMF was measured after oven-drying at 105^o C to remove moisture.

The alkaline used for the pretreatments was sodium hydroxide (NaOH) while acid hydrolysis used sulfuric acid (H₂SO₄) solution as catalyst. Both catalysts were purchased from Friendemann Schmidt and Fisher Scientific (Malaysia) respectively. HPLC standards were kindly provided by different companies for example Friendemann Schmidt, Sigma Aldrich, Rankem and Acros Organic and all the standards were in HPLC grade.

2.2. Alkaline Pretreatment at Elevated Temperatures

A pretreatment of oil palm mesocarp fiber with dilute sodium hydroxide was carried out in 500 mL beaker. 15g of OPMF was added into NaOH solution. Sodium hydroxide solution of 4% and 8% (v/v) were prepared and used for the pretreatment of oil palm mesocarp fiber. The alkaline pretreatment experiments for each concentration were performed at different temperatures which were 60 °C, 80 °C and 100 °C to investigate the effects of alkaline concentration and temperature on acid hydrolysis reaction. The pretreated solids were separated from the liquid phase by filtration and a small amount of liquid phase was taken to be analyzed by HPLC. The

pretreated solids were later used in acid hydrolysis which will be explained in the next section. A summary of the experimental work for alkaline pretreatments is shown in Table 1 below.

Table 1. Initial set-up conditions during alkaline pretreatments.

Sample	Alkali concentration (v/v)	Temperature (°C)
7		60
8	4%	80
9		100
10		60
11	8%	80
12		100

2.3. Ultrasonic-assisted Alkaline Pretreatments

300 VT Ultrasonic Homogenizer with 230 volts was used to provide the ultrasonication to the ultrasonic-assisted alkaline pretreatments. This model delivers up to 300 watts of ultrasonic disruption with analog controls for superior sample processing. This model also contains Titanium Tips for processing sample volumes ranging from 5 ml to 2000 ml.

Ultrasonic-assisted alkaline pretreatments were conducted using 15 g of OPMF sample and 500 mL of sodium hydroxide aqueous solution. The probe was immersed partially into the glass beaker. The power and time was switched on to the required power to start the pretreatment. The ultrasonic pretreatment was performed at room temperature and atmospheric pressure. Since the effect of pulsar was found to be negligible as stated by Yunus et al., (2010) [16], the pulsar of ultrasonic was kept constant at 90%. The parameter for ultrasonic-assisted alkaline pretreatments were concentration of NaOH and ultrasonication power as shown in Table 2. The liquor from the pretreatments was diluted and neutralized with calcium carbonate (CaCO₃) before being analyzed using high pressure liquid chromatography (HPLC).

Table 2. Ultrasonic-assisted pretreatments.

Pretreatments	Catalyst used	Power (%)
		40
	4% NaOH (v/v)	60
Ultrasonic-assisted alkali pretreatment		80
	8% NaOH (v/v)	40
		60
		80

2.4. Acid Hydrolysis

The acid hydrolysis reaction was carried out in a 1-liter pilot batch reactor. The reactor was loaded with 15g of biomass and 500mL of dilute sulfuric acid. The concentration of sulfuric acid used in this reaction was 2% (v/v) sulfuric acid at the temperature of 140 ° C for 3 hours. After hydrolysis and separation, the solid OPMF was kept for further characterization such as scanning electron microscopy and Fourier Transform Infrared Spectroscopy while the liquid phase from hydrolysis was analyzed by high performance liquid chromatography (HPLC) for the compositions of main reaction products.

2.5. Analytical Methods

Fourier transform infrared (FTIR) spectrometer (model Perkin-Elmer Spectrum GX) was employed for investigating the effects of pretreatment on the chemical structure and crystallinity of oil palm mesocarp fiber. The spectra were collected at a range of 400 cm⁻¹ to 4000 cm⁻¹. A high performance liquid chromatography (HPLC) equipped with Refractive Index Detector (RID) was used to measure the concentration of sugars, HMF, furfural and several acids such as formic acid and levulinic acid. The column used was a Rezex RFQ Fast Acid column from Phenomenex, (California, USA), a cation guard cartridge (Bio-Rad Laboratories). 0.01 N sulfuric acid at a flow rate of 1.0 mL/min was used as the mobile phase. Before the samples were analyzed, the samples were neutralized and diluted with CaCO₃ to pH 5 to 6.

III. Result and Discussion

3.1 Alkaline Pretreatment at Elevated Temperatures

Sodium hydroxide (NaOH) was selected as the alkaline catalyst in this pretreatment study due to its outstanding delignification capability [17]. The effects of temperatures and sonication power were investigated at two different NaOH concentrations which were 4% (v/v) and 8% (v/v) NaOH. Subsequently the pretreated

samples were subjected to acid hydrolysis to evaluate the production of sugars and other derivatives particularly LA.

3.2 Effect of Temperature

In this alkaline pretreatment, the effect of temperature (60 °C, 80 °C and 100 °C) on the production of sugars, precursors and acids was evaluated. Previous reports found that 60 to 100 °C was a suitable temperature range for pretreatment [12]. To evaluate the effect of temperature, the NaOH concentration was kept constant at 4% (v/v). The yields of the products after pretreatment are shown in Figure 1 while Figure 2 indicates the yield of products from acid hydrolysis of the alkaline-pretreated samples.

In general, the pretreatment temperature has no immediate effect on the pretreated samples based on the result presented in Figure 1. The consistent production of glucose and xylose at temperatures of 60 °C and 80 °C demonstrated that these temperatures are efficient for biomass decomposition without substantial degradation. However, after pretreatment at 100 °C, the yields of glucose, xylose and other sugars decreased and the concurrent increase in HMF and furfural slightly possibly due to the degradation of sugars. Cellulose and hemicellulose in the biomass undertakes hydrolysis to produce glucose, which in turn forms HMF or furfural by dehydration [18]. The degradation is evidenced in the marked increase in HMF and furfural contents after pretreatment at 100 °C. Glucose and xylose degrade into HMF and furfural upon exposure to excessive heat or chemical treatment [17].

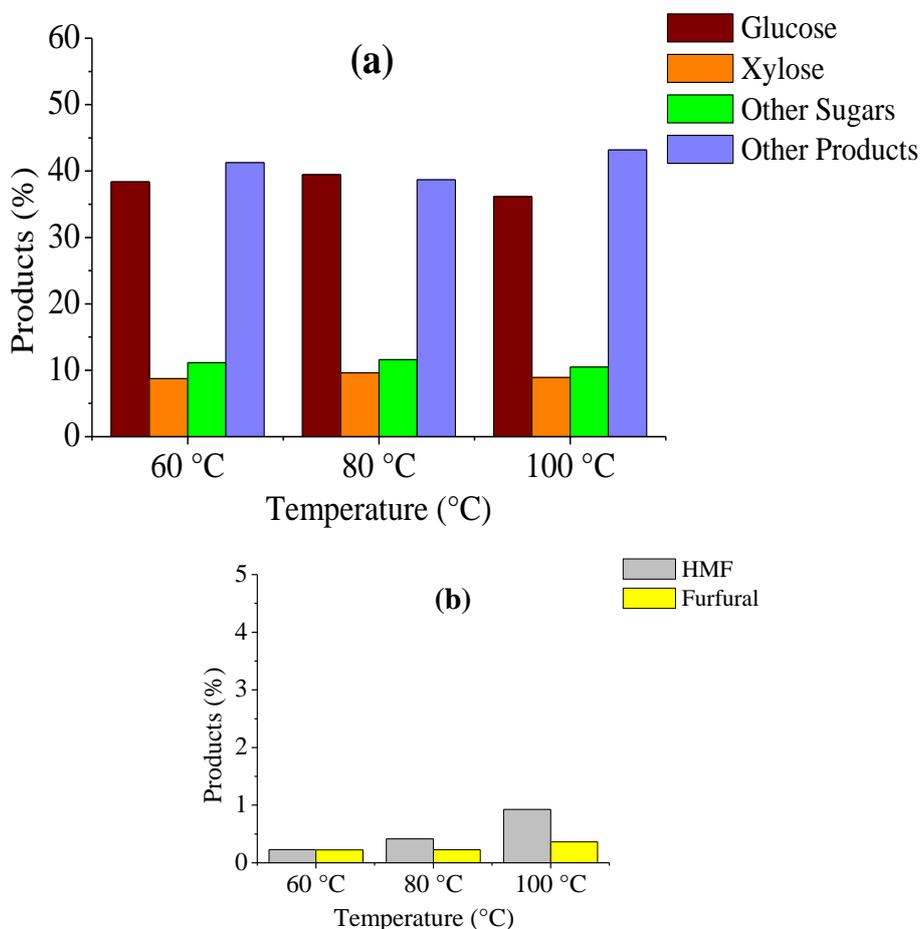


Figure 1. Effect of temperature on alkaline pretreatment at 4% (v/v) NaOH.

Figure 2 shows that the pretreatment temperature has a positive effect on glucose yield but not on xylose after the pretreated samples were subjected into an acid hydrolysis reaction. The increasing glucose content after hydrolysis with pretreatment temperature suggested that not all cellulose have been hydrolyzed to monosugars during pretreatment. Before hydrolysis, the sample pretreated at 100 °C produced only 0.92% of furfural but after hydrolysis, the hydrolyzed sample contained about 24.06% of furfural. When samples are exposed to high temperature alkaline pretreatment, xylose formed from the acid hydrolysis degraded to furfural as evidenced in

the increase in furfural content (Figure 2). Moreover, Solé-bundó et al. (2017) [12] stated that the use of high temperatures especially higher than 100 ° C may lead to the production of inhibitory intermediates and refractory organic compounds caused from intramolecular reactions. These results also suggest that conducting acid hydrolysis at 140 ° C using 2% H₂SO₄ for 3 hours is not sufficient to convert all the monosugars to intermediate chemicals even with the assistance of high temperature alkaline pretreatment. However, the result contradicts with a finding by Xu et al. (2011) [19] which demonstrated that the application of alkaline pretreatment, particularly with NaOH resulted in much higher sugar yields even at lower temperatures. The efficiency of this process is associated with a notable preservation of carbohydrates in the solid residue, which facilitates further hydrolysis. In contrast, in high-temperature acid pretreatments, carbohydrates dissolve in the solution and interact with inhibiting degradation products.

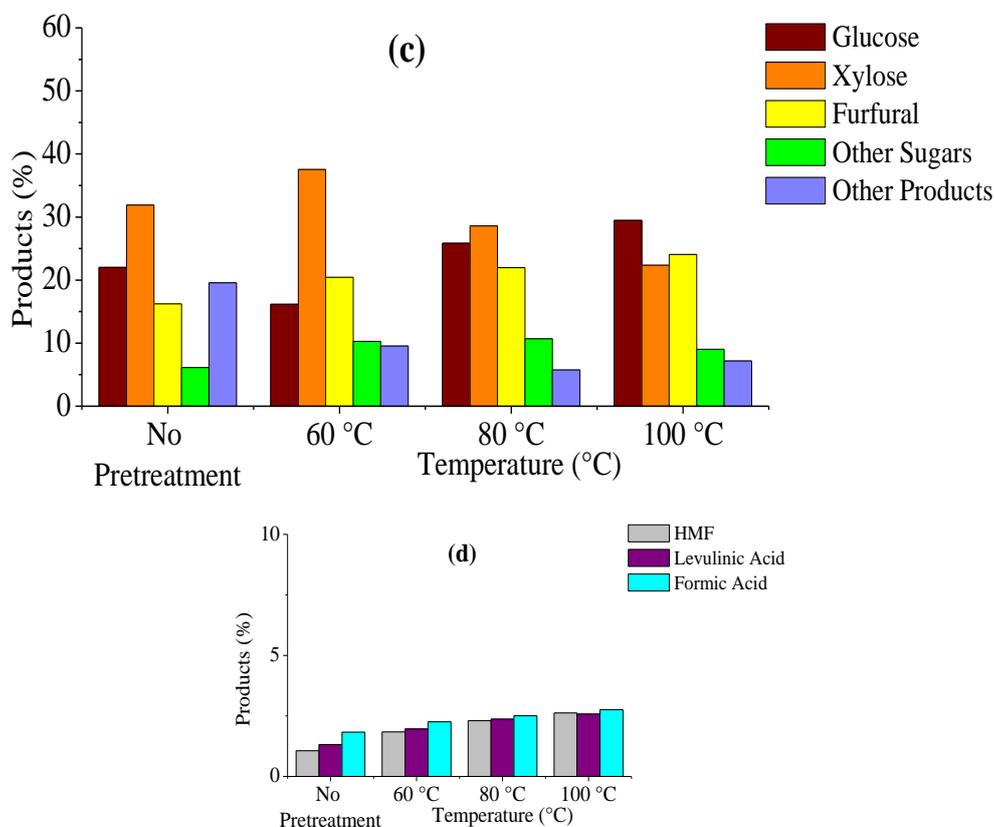


Figure 2. Effect of temperature of alkaline pretreatment on hydrolysis of pretreated OPMF at 140 ° C using 2% H₂SO₄ for 3 hour

However, there is a positive outcome to high temperature alkaline pretreatment, the content of LA increased gradually with temperature. The highest content of LA after hydrolysis was 2.57% from the sample pretreated at 100 ° C. The content of HMF in the hydrolyzed product was still high, indicating that the HMF has not been fully rehydrated to LA. It is postulated that the LA may have degraded into acetic acid more than the rehydration of HMF into LA during acid hydrolysis. This is because a large content of humic acids and black soluble materials are evidenced as unwanted materials from reaction in HMF [20]. It is also suggested that higher temperature hydrolysis is necessary to enhance the conversion of pretreated samples to LA.

3.3 Effect of Alkaline Concentration

To evaluate the effect of alkaline concentration, the results from previous experiments conducted at 4% NaOH were compared with results obtained using 8% NaOH. The effects of temperatures in both experiments (4% and 8% NaOH) were also evaluated. The results after acid hydrolysis are presented in Table 3. Samples from pretreatment using 8% alkaline produced higher content of glucose (32.25%) compared to 4% alkaline (29.46% glucose) at 100 ° C. As expected, the production of xylose after hydrolysis dropped with pretreatment temperature. This is mainly due to degradation at higher alkaline concentration. As a result, low content of xylose was collected after hydrolysis from the sample pretreated at 100 ° C (16.38% xylose). Furfural, which is one of the degradation products of xylose, was the highest in this sample (27.87% furfural).

Similar to furfural, the content of HMF after hydrolysis increased slightly with temperature of alkaline pretreatment. The highest content of HMF collected was 2.73% from the sample pretreated at 100 ° C using 8% alkaline solution. LA and formic acid are the products from HMF degradation. Based on Table 3, the highest yield of LA and formic acid after hydrolysis came from the same sample which was pretreated at 100 ° C using higher concentration of NaOH which was 8% NaOH. The finding was similar to Xihui et al. (2018) [21]. The highest value for LA was 3.28% while formic acid was 3.47%. Jiang *et al.* (2016) [22] suggested that high alkaline concentration favored the removal of lignin so that the conversion of biomass into products such as levulinic acid and HMF will be more effective. They found that 5 g/L of NaOH can remove 15.8 % lignin while 1 g/L of NaOH only can remove 14.5% lignin. However, simply raising the alkali concentration does not necessarily lead to improved outcomes but the synergistic effect is essential [23]. It is expected that, if the reaction time for acid hydrolysis is longer, more LA can be produced from HMF.

Table 3. Analysis of hydrolyzed samples pretreated with different alkaline concentrations.

Products (%)	Pretreatment conditions					
	4% (v/v) alkaline			8% (v/v) alkaline		
	60 ° C	80 ° C	100 ° C	60 ° C	80 ° C	100 ° C
Glucose	16.16	25.82	29.46	23.49	25.04	32.25
Xylose	37.53	28.60	22.35	25.97	21.25	16.38
Furfural	20.43	21.97	24.06	24.34	26.40	27.87
HMF	1.84	2.30	2.62	2.12	2.28	2.73
Levulinic Acid	1.96	2.37	2.57	2.41	2.81	3.28
Formic Acid	2.26	2.50	2.76	3.11	3.14	3.47

3.4 Ultrasonic-assisted Alkaline Pretreatment

To evaluate the effects of ultrasonication on alkaline pretreatment, both sonication power and alkaline concentrations at 4% (v/v) alkaline solution and 8% (v/v) alkaline solution were considered. The products (liquor) after pretreatment and after acid hydrolysis were analyzed.

3.4.1 Effect of Sonication Power

The effect of ultrasound power was evaluated based on the production of sugars, acids and precursors as shown in Figure 3. Sonication power has no marked effect on glucose and xylose contents but affects the content of HMF and furfural slightly. Not much xylose was observed after pretreatment compared to glucose. The highest content of xylose was only 10.08% as compared to glucose at 32.80%. It is suggested that hemicellulose may have been converted into other products than xylose. Xylose may also have degraded into furfural as indicated by the small increase in furfural content.

After hydrolysis as shown in Figure 4, the glucose content increased sharply as observed earlier in high temperature alkaline pretreatment and the xylose content decreased. Most of the xylose may have degraded to furfural as the furfural content increased drastically after hydrolysis. Degradation of HMF to LA via HMF dehydration was expected to take place during acid hydrolysis [24]. As a result, the LA content increased significantly at higher sonication power as depicted in Figure 4 (b). Ultrasounds induce acoustic cavitation, leading to the formation of concentrated pressure and heat [25]. However, the increasing of sonication power was still unable to generate LA from the ultrasonic-pretreated sample. It was expected that the heat produced during treatment was not sufficient enough for the conversion.

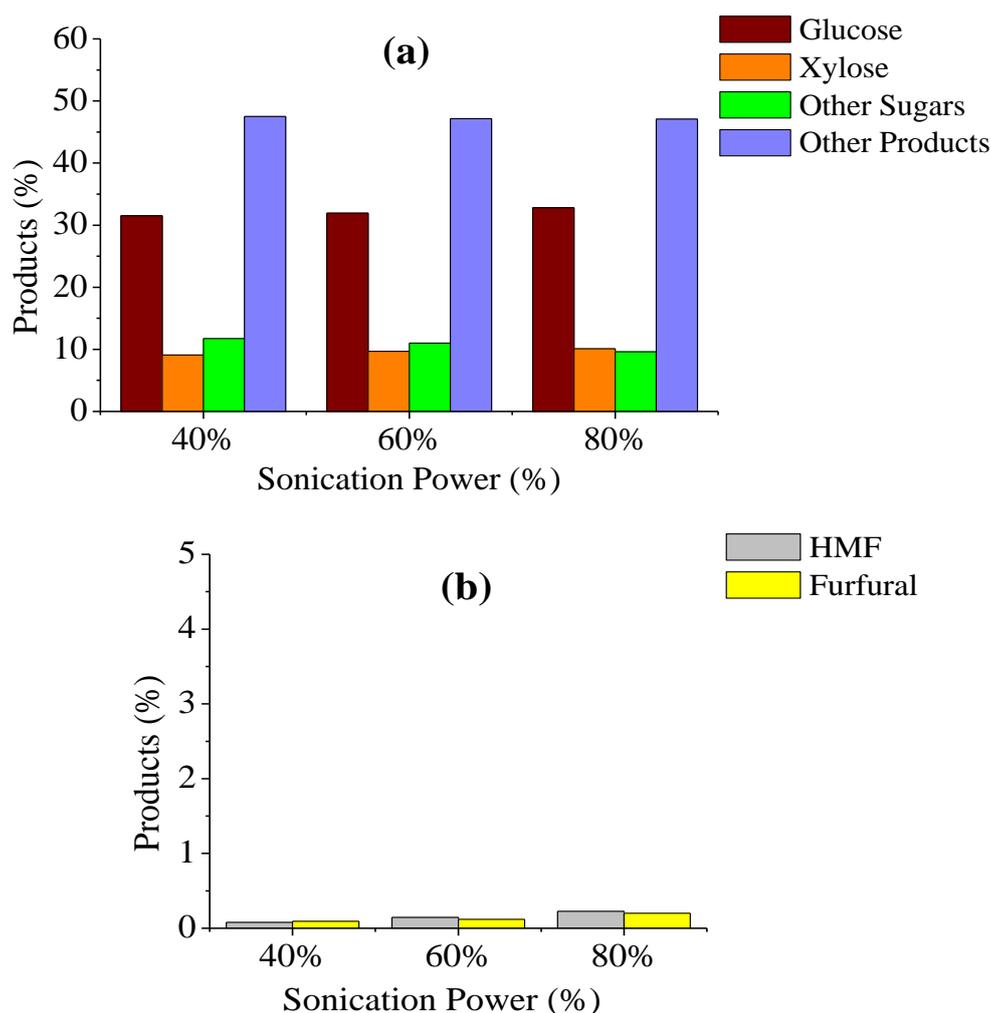


Figure 3. Effect of ultrasound power on ultrasonic-assisted alkaline pretreatment

The highest content of LA (1.91%) was produced at the highest sonication power 80%. The sonication power output corresponds to the ultrasonic wave amplitude, so sono-chemical and mechano-acoustic effects will be increased by the increasing of sonication power which increased the severity and occurrence of cavitation [26]. The presence of glucose in the sample proved that the HMF can still be produced during the process even though the degradation of HMF to LA already took place. The increasing production of LA while the sugar production is decreasing is similar to the report by Amarasekara and Wiredu (2012) [27]. The yield of formic acid is higher than levulinic acid, since formic acid may also be produced from furfural. This is evidenced in the yield of furfural shown in Figure 4 which is lower relative to xylose as compared to the result shown in Figure 2. This may infer that under ultrasonication, the degradation of furfural occurs at a higher rate than under high temperature conditions. Chen *et al.* (2017) [28] also found that the LA (0.3 g/L) content was lower than formic acid (1.1 g/L).

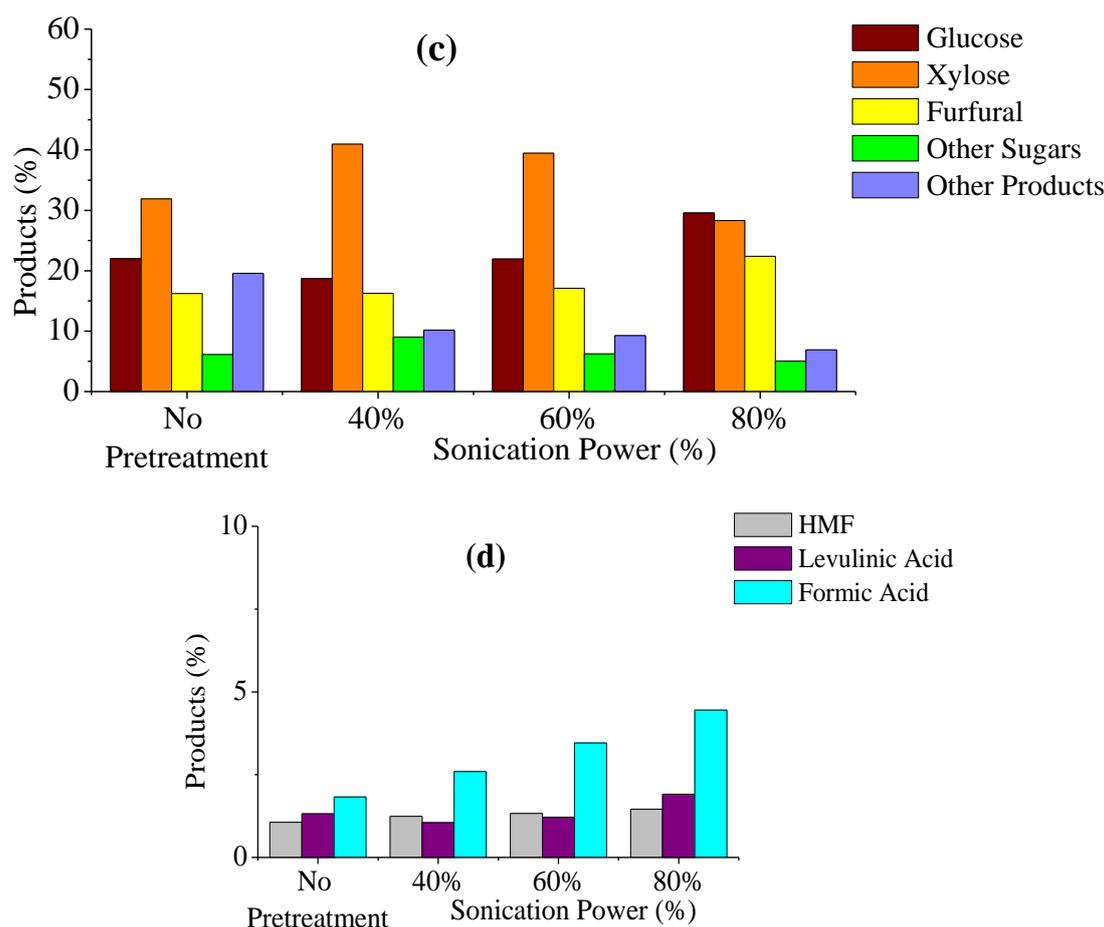


Figure 4. Effect of ultrasound power on ultrasonic-assisted alkaline pretreatment after hydrolysis of pretreated OPMF at 140 °C using 2% H₂SO₄ for 3 hours.

3.5 Comparison between Alkaline and Ultrasonic-assisted Alkaline Pretreatments

Based on the two alkaline pretreatments with and without ultrasonication, the yields of the reaction products were analyzed. LA can only be produced after hydrolysis and not after any pretreatments suggests that the condition during pretreatment was not sufficient to convert HMF into LA. The highest content of LA after acid hydrolysis was obtained from sample pretreated by alkaline pretreatment at 3.28% using 8% NaOH while ultrasonic-assisted alkaline pretreatment managed to obtain only around 2.28% LA. No heat was applied during alkaline pretreatment when ultrasonication was applied. This shows that ultrasonication is a less efficient technique than using heat for alkaline pretreatment. However, it is important to highlight that ultrasonic-assisted pretreatment was able to improve LA production compared to without pretreatment. Ultrasonic pretreatment has been demonstrated successful in achieving delignification proven by FTIR results, which is its main purpose. To achieve higher LA yields, further study is needed to explore additional reactions involved within the process. Further study is recommended to explore the potential of combining ultrasonic-assisted alkaline pretreatment with high temperatures to enhance LA yields.

Furthermore, the application of different solvents enhanced the conversion. Ong et al. (2021) [29] demonstrated an excellent yield when combined ultrasonic-assisted alkaline pretreatment with deep eutectic solvent (DES) pretreatment. DES is created by combining a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) in precise ratio. The HBD component in the DES has the ability to form interactions with the hydroxyl groups present in hemicellulose and cellulose. On the other hand, the HBA component may form interactions with the aliphatic and phenolic hydroxyl groups found in lignin [25].

The production of LA is related to the content of HMF, evidently the HMF content for both pretreatments was also not too far different. Yuan *et al.* (2018) [30] and Saha and Cotta (2006) [31] were unable to detect HMF after hydrolysis even though the samples were pretreated by alkaline pretreatment. Lima *et al.* (2013) [32] used

4% NaOH in their research which is similar to this study but the content of HMF could not be detected. These reports suggest that alkaline pretreatment cannot be used to assist in HMF production from acid hydrolysis of lignocellulosic biomass. However, our study proved that the alkaline pretreatment is indeed able to assist in the conversion of the biomass into HMF and later to LA. A large amount of glucose and xylose still present in the reaction products indicate that the acid hydrolysis at 140 °C using 2% H₂SO₄ for 3 hours is not adequate to convert all these sugars to furans and later to acids. [33] reported that the glucose yields for untreated poplar and woods pine were 13.1% and 8.4%, respectively. Nevertheless, subjecting the poplar and pine to pretreatment at a temperature of 93 °C with NaOH resulted in a significant increase in the yield of glucose reaching 69.3% and 46.5%, respectively.

3.6. FTIR Analysis

FTIR spectra of untreated, alkaline pretreatment and ultrasonic-assisted alkaline pretreatment samples after being hydrolyzed are shown in Figure 5 below. The stretching vibration of the C=O and C-O bonds of the acetyl ester units available in hemicellulose are indicated by the peaks of 1730 and 1245 cm⁻¹ and the reducing intensity of those peaks is purportedly due to the partial removal of hemicellulose that happened during alkaline pretreatment. Basically, the band intensity will be increased due to migration or release and relocation of lignin on the surface of OPMF samples but FTIR spectra above shows that the intensity of this band for treated samples is lower than untreated sample. This is because some amount of lignin might be released during pretreatment into hydrolysate and this hydrolysate was separated and filtered before solid biomass being hydrolyzed in acid hydrolysis. As a result, a lower amount of lignin is identified in the FTIR spectra of OPMF samples after acid hydrolysis as shown in Figure 5.

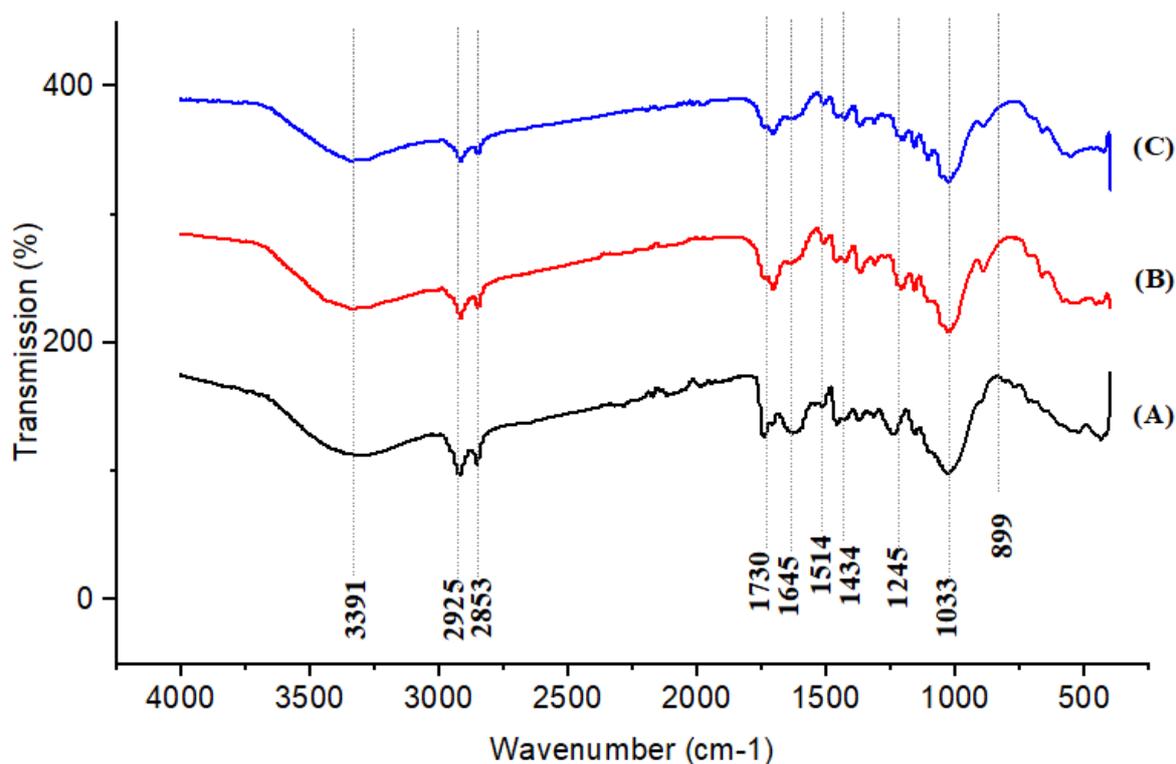


Figure 5: FTIR spectra of (A) raw biomass and samples after acid hydrolysis which were pretreated by (B) 80% power of ultrasonic-assisted alkaline pretreatment by 8% NaOH and (C) 100 °C of alkaline pretreatment at by 8% NaOH.

Impregnation of samples in 8% NaOH during the pretreatments has improved the delignification process as evidenced in the result for FTIR analysis of all samples after acid hydrolysis showing the decreasing of band intensity. A similar study by Nakashima et al. (2016) [26] compared the FTIR results for samples pretreated by ultrasound only with the sample pretreated by combining ultrasound and alkaline pretreatment. Their results showed that the combination ultrasound and alkaline pretreatment had a synergistic effect on the removal of lignin

while pretreatment using only ultrasound hardly influenced lignin removal. On the other hand, peaks at 2925 and 2853 cm^{-1} indicate the stretching vibration of C-H group which is related to hemicellulose and cellulose. The intensity of the bands at 2920 cm^{-1} which indicates C-H stretching vibration decreased in all the treated samples. This indicates that the alkaline pretreatment disrupted the hydrogen bonds inside and between cellulose molecules [21]. The presence of lignin and hemicellulose can be determined by the absorption band observed at 1730 cm^{-1} [33]. Both pretreatment samples experienced a decrease in the absorption of lignin and hemicellulose. It was evident that all samples at a wavenumber of 1033 cm^{-1} exhibited a prominent peak, albeit with a relatively low intensity for pretreated samples. This observation can be attributed to the alteration in the fiber structure induced by the treatment, similar to the finding by Vieira et al. (2024) [11]. From the FTIR spectra above, it may suggest that alkaline pretreatment also affected the structural damage of cellulose-hemicellulose-lignin link by cleavage of ester bonds between carbohydrate and lignin complex as well as the removal of hemicellulose in OPMF biomass.

Table 4. Crystallinity indices for raw untreated OPMF and alkaline pre-treated OPMF after hydrolysis calculated from FTIR spectra.

Samples	Crystallinity indices		
	Lateral order index ^a	Total crystallinity index ^b	Hydrogen bond intensity ^c
Raw OPMF	1.57	0.56	1.96
Alkaline pre-treated OPMF (with ultrasonic, 80% power, 8% (v/v) NaOH)	0.94	0.35	2.69
Alkaline pre-treated OPMF (100 °C temperature, 8% (v/v) NaOH)	0.73	0.51	2.55

^a The band intensity ratio of wavelength 1437 cm^{-1} and 899 cm^{-1}

^b The band intensity ratio of wavelength 1378 cm^{-1} and 2900 cm^{-1}

^c The band intensity ratio of wavelength 3308 cm^{-1} and 1330 cm^{-1}

The crystallinity of untreated and treated samples after being hydrolyzed was evaluated using total crystallinity index (TCI), lateral order index (LOI) and hydrogen bond intensity (HBI). TCI was calculated based on the band intensity ratio of wavelength 1437 cm^{-1} and 899 cm^{-1} , LOI based on the band intensity ratio of wavelength 1378 cm^{-1} and 2900 cm^{-1} while HBI based on the band intensity ratio of wavelength 3308 cm^{-1} and 1330 cm^{-1} . Three parameters above presented in Table 4, are strictly connected to the status of the intermolecular regularity and degree of crystallinity.

The LOI quantifies both quantitative and qualitative changes in cellulose crystallinity in the cellulose structure [34]. The LOI value for raw OPMF (Oil Palm Mesocarp Fiber) was 1.57, showing a cellulose structure that is relatively ordered. Following an alkaline pre-treatment with ultrasonic assistance at 80% power and 8% NaOH, the LOI value decreased to 0.94. This drop suggests that the pre-treatment process caused a disruption of the ordered structure and resulting in a more disordered form. Similarly, subjecting the material to alkaline pre-treatment at a temperature of 100°C with a solution containing 8% NaOH resulted in a LOI value of 0.73, indicating additional disruption of the structure of cellulose. These data are consistent with the conclusions from Oluwasina et al. (2024) [35] who observed a correlation between the LOI and the total level of organization in cellulose. The TCI correlates with the degree of crystallinity in cellulose [36]. The raw OPMF exhibited a TCI of 0.56, reflecting its crystalline nature. After alkaline pre-treatment with ultrasonic assistance, the TCI dropped to 0.35, suggesting that the pre-treatment converted some of the crystalline structure into an amorphous form. Alkaline pre-treatment at 100°C with 8% NaOH resulted in a TCI of 0.51, indicating partial retention of the crystalline structure. The lower TCI values for pre-treated samples compared to the untreated sample suggest that both ultrasonic and thermal alkaline pre-treatments degrade crystalline regions assuming the higher swelling power of alkaline solutions. However, the difference is not significant, consistent with the result from Fadavi et al. (2022) [37]. The HBI relates to the degree of intermolecular regularity, crystallinity, and the amount of bound water within the cellulose structure. Basically, an increase of crystallinity decreased the value of HBI [38]. Raw OPMF had an HBI of 1.96. Alkaline pre-treatment with ultrasonic assistance increased the HBI to 2.69, indicating enhanced intermolecular hydrogen bonding. Similarly, alkaline pre-treatment at 100°C resulted in an HBI of 2.55. The significant increase in HBI values for pre-treated samples suggests that the pre-treatment process did not disrupt the functional groups forming intermolecular forces and hydrogen bonds within the lignocellulosic structure.

The application of heat at 100°C in the presence of 2% acid led to a TCI of 0.04, the lowest among all samples, demonstrating substantial crystalline degradation. This likely occurred because all the amorphous parts had been converted into sugars, leaving behind a structure with very low crystallinity. Conversely, when the acid concentration was increased to 6% during thermal treatment, the TCI rose to 0.14, suggesting some remaining crystalline areas. This higher TCI value indicates that only crystalline areas remained after the amorphous components had been converted. In summary, alkaline pre-treatment significantly alters the crystallinity and order of OPMF. The reduction in LOI and TCI values for pre-treated samples compared to raw OPMF indicates the conversion of crystalline cellulose to amorphous forms. The increase in HBI values suggests that pre-treatment enhances intermolecular hydrogen bonding, despite the disruption of the ordered cellulose structure. These findings highlight the effectiveness of alkaline pre-treatment in modifying the structural properties of OPMF, with potential implications for its subsequent processing and utilization.

IV. Conclusion

The highest amount of LA was produced from the sample pretreated with alkaline pretreatment by heating at 100 °C using 8% NaOH (3.28%). Using ultrasonication, the sample pretreated at 80 % sonication power using 8% NaOH only yielded (2.20%) LA during acid hydrolysis reaction. High temperature of alkaline pretreatment produced higher amounts of LA than the ultrasonic-assisted alkaline pretreatment. The study demonstrated that alkaline pretreatment at elevated temperatures significantly enhances the acid hydrolysis of OPMF compared to ultrasonic-assisted alkaline pretreatment at room temperature. This indicates that heat application during alkaline pretreatment is more effective than ultrasonic-assisted methods for improving the efficiency of acid hydrolysis. The findings suggest that optimizing temperature and alkali concentration is crucial for maximizing LA production from lignocellulosic biomass.

Declaration of Competing Interest

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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CRediT authorship contribution statement

Z.N. Akhlisah: Writing – original draft, Conceptualization, Investigation, Methodology, **R. Yunus:** Writing – review & editing, Conceptualization, Methodology **W.A.K. Wan Azlina:** Writing – review & editing, Conceptualization, Methodology **Z.Z. Abidin:** Validation, Resources, Supervision **Y.H. Taufiq-Yap:** Validation, Resources, Supervision **U. Rashid:** Validation, Resources, Supervision

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