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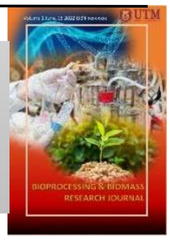

Research Article

SCREENING OF LOW-PRESSURE STEAM HEATING PRETREATMENT PARAMETERS FOR ENHANCED DELIGNIFICATION OF PINEAPPLE WASTES

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ABSTRACT

Pineapple waste is classified as lignocellulosic biomass that can potentially be used in the production of biofuel (bioethanol) to replace the current resources of fossil fuels. Carbohydrate compositions of pineapple waste (cellulose and hemicellulose) are excellent properties to be exploited as fermentable sugars that can be converted into bioethanol through the fermentation process. In producing fermentable sugars from pineapple wastes, a pretreatment process is required to breakdown the complex crystalline structure of lignocellulose. However, this process is challenging due to the recalcitrant structure of lignocellulosic material. Extensive researches have been done to improve the efficiency of the pretreatment method for maximum removal of lignin from the lignocellulosic biomass. In this study, low-pressure steam heating pretreatment was introduced to examine the delignification effectiveness of pineapple wastes. In this study, screening of three parameters using low-pressure steam heating pretreatment (biomass loading, pressure, and residence time) were carried out using one-factor-at-a-time (OFAT) analysis. From the results obtained, the best condition for low-pressure steam heating pretreatment was found to be at biomass loading of 5 % w/v, 80 kPa pressure, and 30 minutes residence time. A total of 46.89 % lignin had successfully been removed from pineapple wastes by using this pretreatment condition. The proposed pretreatment in this present study was proven to be a practical approach for biomass delignification.

INTRODUCTION

Biomass is referred to as organic material originated from plants and animals. Biomass waste can be used for energy production and as alternative renewable energy to replace current resources of fossil fuel. Biomass waste can be converted into fuels such as biofuel or biogas through combustion or gasification. Most of the biomass wastes in Malaysia are generated from the agriculture sector (Chuah et al., 2006). Malaysia has high availability and a wide variety of biomass wastes from forestry, agriculture, and industry, this creates an opportunity for Malaysia to convert lignocellulose into significant value-added products.

Pineapple (*Ananas comosus*) is among the most significant tropical fruits in Malaysia's processing industry. Although the pineapple canning industry in Malaysia is comparatively small, however, it played a vital role in improving the economic conditions of Malaysia. The pineapple canning industry produces a large number of

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unutilized pineapple waste materials, which include stems, crowns, cores, peels, residual pulps, and leaves. This pineapple wastes contain lignocellulosic materials, which included celluloses, hemicelluloses, and lignin. The complex crystalline structure of lignin had restricted the conversion of lignocellulose into fermentable sugars. Thus, the pretreatment process is a prerequisite before the enzymatic hydrolysis to disrupt lignin and thus boost enzymatic hydrolysis's efficiency by enhancing the enzyme accessibility onto the carbohydrate sources (Nordin et al., 2020). As mentioned by Nauman Aftab et al. (2019), numerous types of pretreatments have been introduced for the process of delignification, including physical (milling, grinding, and fragmentation), physiochemical (liquid hot water (reference), low-pressure steam heating), ammonia fiber explosion and steam explosion), chemical (acidic and alkaline) and biological (enzymatic and whole-cell pretreatment). However, most of these pretreatment processes showed slow cellulose digestion, low sugar yields and require extreme reaction conditions (high temperature and/or high pressure), which demand the use of expensive equipment. These pretreatments thus have high processing cost, have limited effectiveness, and may generate side products that can inhibit subsequent fermentation.

In this study, we proposed low-pressure steam heating pretreatment to enhance the delignification process of pineapple wastes. Screening of three low-pressure steam heating pretreatment parameters (biomass loading, pressure, and residence time) were carried out using one-factor-at-a-time (OFAT) analysis in this study to achieve high delignification. The effect of the pretreatment condition on the percentage of lignin removal was screened and explored.

MATERIALS AND METHOD

Materials

The pineapple wastes were obtained from Lee Pineapple Co Pte Ltd. Toluene (99.9%, HmbG, Johchem Scientific & Instruments Sdn Bhd), Ethanol (95%, QReC, QReC Chemical Co LTD), Sulfuric acid (98%, Sigma-Aldrich, Sigma-Aldrich Corporation) and distilled water were purchased as specified.

Raw materials preparation

The pineapple wastes were first dried for 5-7 days at 70˚C using an oven until constant weight. The pineapple wastes were ground into a fine particle size using a grinder machine and passed into a sieve pan with a specific pore size of 500 microns to obtain the maximum 500 microns particle size of samples. The dried-ground pineapple wastes were then kept and stored in a sealed plastic container prior the pretreatment processes described below.

Determination of lignin (Klason Lignin)

Prior to klason method, the biomass was treated to make it extractive free (free of protein, waxes and resins) in accordance with method from Tappi (2004). Acid insoluble lignin content (Klason Lignin) was determined by a method described in (Carrier et al. 2011). The extractives free sample was prepared and dried at 45 °C in a vacuum oven overnight. A 200 mg of ground vacuum-dried sample was weighed into a 100 mL centrifuge tube. To the sample in a 100 mL centrifuge tube, 1 mL of 72 % (w/w) Sulfuric acid was added for each 200 mg of sample. The mixture was stirred and dispersed thoroughly using a glass rod twice, and then the tubes were incubated in a water bath at 30 °C for 60 min. An amount of 56 mL of distilled water was added into the tubes. This resulted in a 4 % solution for the secondary hydrolysis. The sample was then autoclaved at 121 °C, 15 psi, for 60 min. The sample was removed from the autoclave, and the lignin was filtered off with glass fiber filters (filters were rinsed into crucibles, dried, and tarred) in crucibles using suction, keeping the solution hot. The residue was thoroughly washed with hot water and dried at 105 °C overnight. The dried sample was moved into a desiccator, left for 1 hour, and weighed. The Klason lignin content was calculated on a weight basis (Sluiter et al.,2010).

Screening of low-pressure steam heating pretreatment condition

The dried pineapple waste samples were weighed into Schott bottles for the screening of different parameters which are biomass loadings, pressures, and residence times. The entire low-pressure steam heating pretreatment was carried out using a pressure cooker. The bottles that contain different biomass loading were immersed inside a pressure cooker. Then, the pressure and residence time were set. All of the screening processes were carried out one factor at a time (OFAT) in a triplicate. The range of biomass loading (1.25, 2.5, 3.75, 5.0, 6.25 % w/v); pressure (40, 50, 60, 70, 80 kPa); residence time (15, 30, 45, 60, 75 minutes) were screened. The first parameter screened was the biomass loading (% w/v) at fixed 80 kPa and 30 minutes followed by pressure (kPa) and residence time (minutes). The best-chosen value of the initial parameter was used for subsequent parameter screening. After every screening of each parameter, filtered the treated pineapple wastes until the filtrates became clearer before drying process using oven at 45°C for 24 hours.

Composition analysis of pineapple wastes

The effects of three parameters (biomass loading, pressure, and residence time) on the lignin content removal from pineapple wastes by low-pressure steam heating pretreatment were studied in this study. Biomass extraction and the Klason lignin method were repeated to determine the remaining lignin content in pineapple wastes after treatment (Equation 1 and 2 are in weight basis where W=weight (g)). The percentage of lignin removal was calculated for each run of the experiment. The best condition for low-pressure steam heating pretreatment was determined according to Rowell and Rowell (1996).

$$\%\,Extractives = \left[\frac{W_{oven-dried\ thimble+extract} - W_{oven-dried\ thimble+original\ sample}}{W_{original\ sample}}\right] \times 100\%$$

Equation 1

$$\%Klason\ lignin = \left[\frac{W_{dried\ insoluble\ lignin+filter\ paper} - W_{filter\ paper}}{W_{extractives-free\ original\ sample}}\right] \times 100\%$$

Equation 2

RESULTS AND DISCUSSION

Composition analysis of untreated pineapple wastes

The lignin content of raw pineapple wastes was firstly determined to establish a basis for comparison and as the controlled data in this study. For comparison, the compositions from published data were also included. **Table 1** summarizes the lignin content for the untreated pineapple wastes from different parts based on the previous studies done by other researchers. Mixed pineapple wastes were used in this study. The lignin content of the mixed pineapple wastes obtained in this study was 17.7 %w/v, which was much higher than other specific pineapple waste segments.

Table 1 Lignin content of pineapple wastes

Pineapple waste segment	Lignin content (%w/v)	Reference
Whole	4.7	(Casabar et al., 2019)
Skin	1.5	
Crown	4.5	
Pulp	2.3	
Leaves	22	Mansora et al., 2019)
Stem	20	
Root	7.0	
Leaves	4.2	(Daud et al., 2014)
Mixed pineapple wastes	17.7	This study

Effect of low-pressure steam heating pretreatment on the delignification of pineapple wastes

Lignocellulosic biomass is made up of a complex matrix of celluloses, hemicelluloses, and lignin. Chemical, physical, and physicochemical pretreatment are the crucial steps in the delignification and conversion of lignocellulosic into biofuel. From several literatures, a few comparisons about the percentage of lignin removal are summarised in **Table 2**. Meanwhile, low-pressure steam heating pretreatment was used in this study to investigate the efficiency of this method on the delignification process. In this method, the pineapple wastes were treated in a pressurized condition using water-steam in a conventional pressure cooker. It was hypothesized that with the aid of this pretreatment method, the recalcitrant structure could be reduced, hence improved glucose recovery.

The goal of this screening procedure was to remove the most lignin from the pineapple wastes. By lowering condensed lignin, which can adsorb protein from aqueous solutions and lessen non-specific enzyme adsorption, lignin removal will enhance the efficiency of enzymatic hydrolysis (Yang and Wayman, 2004). Lignin residue on lignocellulose surfaces lowers hydrolysis rates and reduces digestibility (Zhang et al., 2007).

Table 2 Summary of the percentage of lignin removal from different biomass substrates and pretreatment methods

Substrate	**Pretreatment**	**Percent of lignin removal (%)**	**Reference**
Pineapple waste	Low-pressure steam heating	46.89	This study
Pineapple peel	Sulfuric acid and alkaline hydrogen peroxide	71	(Dahunsi, 2019)
Empty fruit bunch	Combination of low-pressure steam heating and dilute acid	29.30	(Hazirah, 2015)
Empty fruit bunch	High pressure steam	16.6	(Baharuddin et al., 2013)
Corn Stover	Combination of hot water and aqueous ammonia	79	(Kim & Lee, 2006)
Forest biomass	Alkaline-sodium chlorite	20-40	(Yu et al., 2011)
Waste Newspaper	Ethylene glycol	75	(Lee et al., 2011)

Effect of Pretreatment with different biomass loadings on the delignification of pineapple wastes

The effect of biomass loading on the delignification of pineapple wastes is shown in **Figure 1**. In this present study, increasing the biomass loading from 2.5 %w/v to 3.75 %w/v and from 5.0 %w/v to 6.25 %w/v with the fixed value of time which is 30 minutes with pressure 80 kPa significantly decreased the percentage of lignin removal. It means that lignin is easier to be removed at low biomass loading as compared to high biomass loading. Higher biomass loading does not help improve delignification but lowers the removal of lignin (Tian et al., 2017). Meanwhile, increasing the biomass loading from 1.25 %w/v to 2.5 %w/v and from 3.75 %w/v to 5.0 %w/v significantly increased the percentage of lignin removal. This observation occurred might be due to the sufficient severity of steam pretreatment to fully change the structure and degree of crystallinity (Hu et al., 2013). The results obtain in this study shows a slight fluctuation in the trend of % lignin removal. The % lignin removal drops drastically when 3.75% of biomass loading was used and sharply increased when 5% biomass loading was used. The trend remains the same even after the experiment has been repeated in triplicates. Further in-depth study needs to be done to investigate the reason for the sudden from in % lignin removal at 3.75% biomass loading.

A previous study regarding the enzymatic hydrolysis of the switchgrass samples with various initial biomass loadings from 50 to 200g/L was done by Ioelovich & Morag (2012). It was confirmed that increasing the biomass loading reduced the rate of delignification and thereby reduced the enzymatic digestibility. A similar finding was observed from another study done by Cruz et al. (2013). It was presumed that lignin removal efficiency was decreased as the biomass loading increased. In this present study, the highest percentage of lignin removal was achieved at biomass loading of 5.0 %w/v (46.89 %) while the lowest percentage of lignin removal achieved at biomass loading of 3.75 %w/v (21.6 %). As such, 5.0 %w/v was chosen as the best pretreatment biomass loading considering the percentage of lignin removal. This maximum 5% biomass loading is parallel with the previous study conducted by Azelee et al. (2014).

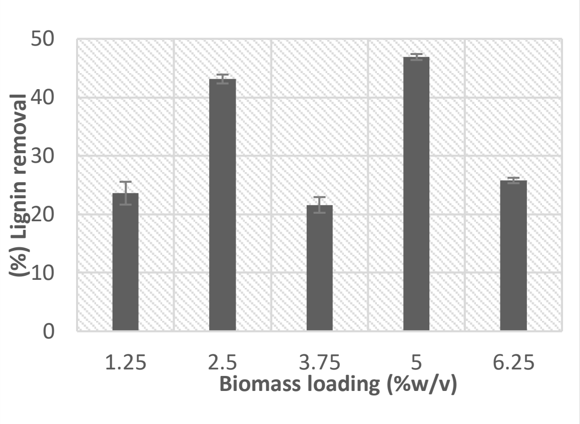

Figure 1 Screening for the effects of different biomass loadings on delignification of pineapple wastes at fixed pressure (80 kPa) and residence time (30 minutes).

Effect of pretreatment with different pressures on the delignification of pineapple wastes

The effect of pressure on the delignification of pineapple wastes is shown in **Figure 2** at fixed residence time of 30 minutes. It has been demonstrated that increasing the pressure during pretreatments for delignification increases the percentage of lignin removal (Azelee et al., 2014). In this study, at fixed values of biomass loading(5%w/v) with the time (30 minutes), showed increasing the pressure from 40 to 80 kPa significantly increased the percentage of lignin removal to more than 40 % in pineapple wastes. At 40 kPa, about 25.79 % of the lignin was removed from the pineapple wastes using low-pressure steam heating pretreatment. Furthermore, at 50 kPa, more than 33 % of the lignin was removed. The high pressure enhances the breakdown of the glycosidic bonds in cellulose and hemicellulose and cleavage of hemicellulose-lignin bonds (Chen & Liu, 2015). High pressure promotes swelling and collapse of the cells, causing the efficient breakdown of the fiber structure (Harun et al., 2011). Besides, high pressure disrupts the fibrous structure of biomass, reduces the crystallinity of cellulose, and thereby improves the accessibility of the enzyme.

A further increase in pressure to 80 kPa caused a further increase in lignin removal up to 43.13 %. The maximum pressure was set up to only 80 kPa due to the maximum limit of the pressure setting by the pressure cooker. Thus, high pressure (80 kPa) was chosen as the best pretreatment pressure considering the percentage of lignin removal in this study. Previous study regarding the high-pressure homogenization pretreatment of four different lignocellulosic biomasses (grass clipping, corn straw, catalpa sawdust, and pine sawdust) for enhancing enzymatic digestibility was done by Jin et al. (2015). With a high working pressure of 10 MPa, all the four lignocellulosic biomasses were significantly changed, such as a decrease in particle size and lignin content, structure destruction, and crystallinity change. Moreover, results showed that lignocellulosic biomass pretreated with high-pressure homogenization yielded more reducing sugar, suitable for subsequent biofuel production. The high pressure used provided better results than those in work performed by Hazirah (2015), where the highest degree of delignification obtained in empty fruit brunch was around 29 % by using high pressure (10 psi) with acid (3 % v/v H_2SO_4 concentration) pretreatment.

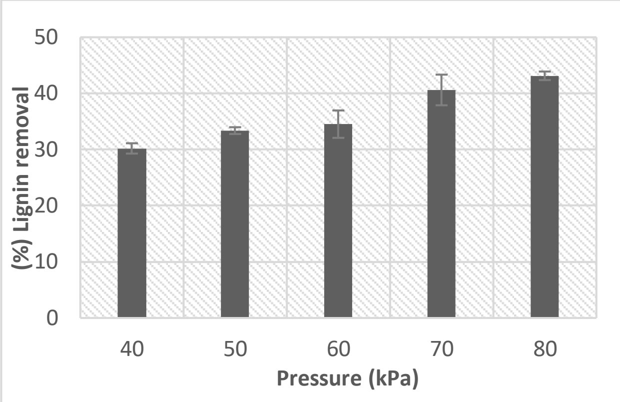

Figure 2 Screening for the effects of different pressures on delignification of pineapple wastes at fixed biomass loading (5%) residence time (30 minutes).

Effect of pretreatment with different residence times on the delignification of pineapple wastes

The effect of residence time on the delignification of pineapple wastes is shown in **Figure 3**. At fixed values of biomass loading and pressure which are 5%w/v and 80kPa, it showed that the percentage lignin removal was between 19.65 to 34.52 %. At 15 minutes, only 19.65 % lignin removal was observed. This observation occurred might due to the shorter residence time for the treatment process was not enough for the severity of treatment (Iroba et al., 2014). Meanwhile, increasing residence time from 30 minutes to 75 minutes also showed a negative resulted on the percentage removal of lignin. According to the study by Amnuaycheewa et al. (2017), longer pretreatment time was not able to enhance delignification and enzymatic hydrolysis but led to more reduction in the obtained sugars. In this present study, the highest percentage of lignin removal was achieved at 30 minutes residence time (34.52 %), while the lowest percentage of lignin removal achieved at a residence time of 15 minutes (19.65 %). As such, 30 minutes was chosen as the best pretreatment residence time considering the lignin removal percentage.

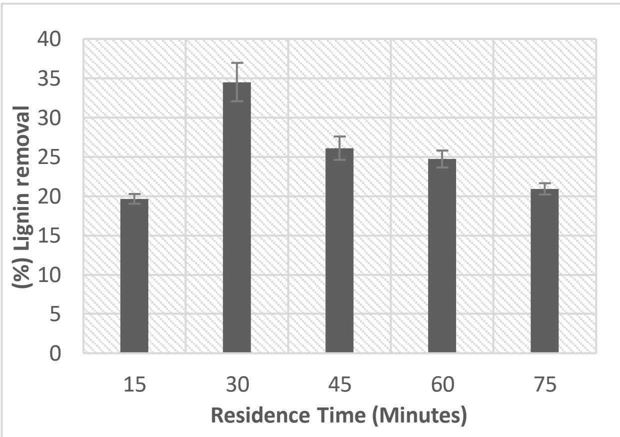

Figure 3 Screening for the effects of different residence times on delignification of pineapple wastes at fixed biomass loading (5%) and pressure (80 kPa).

Previous study regarding the effect of organic acid pretreatment on Napier grass straw biomass conversion was done by Amnuaycheewa et al. (2017). Organic acid pretreatment on Napier grass straw was performed in a range of pretreatment residence time from 30 minutes to 120 minutes. The residence time of 30 minutes was the most favourable in terms of lignin removal and sugar yield. A similar finding was observed from another study done by Alam et al. (2013). It was presumed that shorter pretreatment time will achieve higher rate of delignification and yield of glucose.

CONCLUSION

In this study, the process of low-pressure steam heating pretreatment was successfully developed. This pretreatment method had successfully removed more than 40 % of the lignin components from pineapple wastes. The data obtained from the composition analysis was used to compare the percentage of lignin removal after treatment. Screening of low-pressure steam heating pretreatment parameters (biomass loading, pressure, and residence time) were carried out in this study to determine the best condition for this pretreatment. Findings from this screening showed that the best condition using low-pressure steam heating pretreatment was at biomass loading of 5 %w/v, at 80 kPa pressure and 30 minutes residence time with a maximum of 46.89 % lignin removal has successfully achieved using these pretreatment condition. Some variation in the % lignin removal occurs most probably due to the variations in the different biomass batch used. In conclusion, the low-pressure steam heating pretreatment proposed in this study has been demonstrated as a suitable pretreatment method for pineapple wastes due to the significant destruction of the lignin layer.

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