

Efficient Delignification of Pineapple Waste by Low-Pressure Steam Heating Pre-Treatment

Norhafiza Nordin¹, Rosli Md Illias^{1,2}, Nor Hasmaliana Abdul Manas¹, Aizi Nor

Mazila Ramli^{3,4}, Nur Izyan Wan Azelee^{1,2,5*}

¹ School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

² Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

³ Faculty of Industrial Science and Technology, University Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia

⁴ Bio Aromatic Research Centre of Excellence, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Kuantan, Pahang Darul Makmur, Malaysia

⁵ Centre of Lipids Engineering and Applied Research (CLEAR), Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.

*Corresponding author: nur.izyan@utm.my

ABSTRACT

Fermentable sugars synthesized from pineapple waste (PW) biomass can be potentially used to supplement increasing demand for the application in fine chemicals, pharmaceuticals, agriculture, cosmetics, and many more. To produce fermentable sugars, pre-treatment process is required to break the complex structure of PW. This is a challenging process due to the inalcitrant structure of lignocellulosic material. With this regard, extensive research have been done to improve the efficiency of pre-treatment method for maximum fermentable sugars production. This project focuses on assessing the efficiency of low-pressure steam heating (LPSH) as a mean of pre-treating a mixture of pineapple waste using commercial pressure cooker without using harsh catalysts and at a potentially reduced energy expenditure. Optimized condition of LPSH pre-treatment, (60 kPa, 30 minutes) at 2.5% biomass loading successfully resulted in 60.89% lignin removal and retained as high 91% of hemicellulose and 77.6% of cellulose from the biomass. Due to the high lignin removal, it is projected to improve the cellulose and hemicellulose accessibility to enzyme hydrolysis and enhance the sugar recovery.

Keywords: Pineapple waste, lignin, cellulose, hemicellulose, low-pressure steam heating, pre-treatment

1. INTRODUCTION

Pineapple waste (PW) produced from industrial processing and cultivation comprises of crown, peel, leaf, and core of the fruit with about 30 - 50% of the total fruit weight is discarded after juice extraction. Many practices for sustainable development of pineapple in Malaysia have been documented in numerous publications in the past and recent years such as application for downstream value-added utilisation of biomass and waste for the further industrial process like fermentation, bioactive component extraction [1], biogas production [2] source of electricity [3], and many more. These alternatives are to avoid the improper treatment of the waste that can lead to environmental problems [4].

Physiochemical pre-treatment method such as steam explosion [5], ammonia fiber explosion [6], liquid hot water (LHW) [7], and superheat steam [8] have been suggested to minimize the formation of inhibitory compounds, reduced energy usage without the use of any chemical catalyst, and have resulted in increased fermentative sugar. Pre-treatment using pressurised vessel can open the bundles of lignocelluloses and make enzymatic reaction easily

accessible to the polymer chain of cellulose and hemicellulose due to the combination of heat and pressure which triggers the volatile material content for further breakdown [9]. These methods use different thermal stabilities for a different type of biomass. Severe degradation of the cellulose and hemicellulose structure occurs at a certain temperature and enough pressure to keep the water in a liquid state with the appropriate time so that the lignin degrade the most while the hemicellulose and cellulose remain intact [10].

Pre-treatment with a pressure cooker consume less energy compared to the steam explosion and autoclaving method [11]. The pressure is transmitted uniformly and instantaneously, to all parts of the materials. Previous study has observed a significant removal of hemicellulose in sugarcane bagasse after treated with high pressures (up to 50 MPa). There are also some modifications to cellulose fiber, which able to improve the porosity, allowing the amorphous regions of the cellulose more susceptible to cellulase [12]. High hydrostatic pressure easily break the weak intermolecular interactions, such as hydrogen bonds, hydrophobic and electrostatic interactions with no effect on covalent bond [13]. As a result, glucose yield significantly increased during the enzyme hydrolysis.

Unfortunately, xylose yield decreased when the pressure used is too high, which likely due to the solubilisation of hemicellulose during the hot water phase [8]. High energy input, extreme temperature and extended time can cause pyrolysis with significant degradation of hemicellulosic sugars (5-carbon sugars xylose) compared to cellulose and lignin. This is because hemicellulose is known as the thermosensitive and most reactive component [14]. The degradation of pentose sugar from the hemicellulose produces furfural while the degradation of hexose sugar produces toxic 5-hydroxymethylfurfural (HMF). Both degraded form can inhibit the enzyme activity during the enzymatic hydrolysis and affect the cell growth that will prolong the lag phase during fermentation [15]. However, there is a maximum allowable concentration for specific inhibitors that make it non-toxic to the process [16].

As an alternative to avoid carbohydrate hydrolysis and sugar loss, low pressure is used to weaken the lignin structure while increasing its chemical and biological reactivity and thus, help the subsequent pre-treatment and hydrolysis process to enhance the sugar recovery. Hence, this study focuses on optimizing the LPSH-pre-treatment parameters on PW for the highest lignin removal while retaining the most hemicellulose and cellulose so that it is highly available for the subsequent enzymatic hydrolysis.

2. EXPERIMENTAL

2.1. Preparation of Raw Materials

The raw mixture of N36 variety PW consisting of a mixed part such as the leaves, peels, crown, and other non-fruit parts was collected from Lee Pineapple factory located at Taman Tun Aminah, Johor. The PW mixture was oven-dried at 70°C to remove all the moisture and ground using an electrical grinder (Waring® Products Division, U.S.A.) to obtain particle sizes of 500µm. The biomass was air-dried overnight to remove the moisture left and then stored in an air-tight container.

2.2. Optimization of Pre-treatment Condition

The pre-treatment of the PW was conducted using a 6L Breville pressure cooker (Model BPR700BSSUSC) that can control its pressure up to a maximum of 11.6 psi (80 kPa). The parameters chosen for screening are biomass loading (% w/v), pressure (kPa), and treatment time (min). All the screening processes were carried out by one-factor-at-a-time (OFAT) in triplicates. Then the dried samples were analysed for their compositional analysis, which are the percentages of lignin, hemicellulose, and α -cellulose removals calculated in weight basis. The dried pineapple waste were weighted into different biomass loading (1.25, 2.5, 3.75, 5, 7.5 % w/v) in a total volume of 1L under different pressure level (40,50, 60, 70 and 80 kPa) and treatment time (15, 30, 45, 60, 75 minutes) inside the pressure cooker.

The solid samples were separated from the solution using a sieve and dried at 105°C to collect the pre-treated substrate. Then the dried samples were analysed for their compositional analysis, which are the percentages of lignin, hemicellulose, and α -cellulose retained in the biomass on weight basis.

2.3 Compositional Analysis of Pineapple Waste Biomass

The compositional analysis of the untreated and pre-treated PW biomass which is after the LPSH pretreatment were done in triplicates by using proximate, ultimate, and standard composition analysis methods. Proximate analysis includes the determination of acid-insoluble lignin, cellulose, hemicellulose, and extractives. The extractives, which are non-structural materials from biomass that mainly consist of fats, fatty acids, phenols, waxes, steroids, etc. were determined by soxhlet extraction in a well-ventilated fume hood for over 4 to 5 hour period according to Tappi [17]. Extractives are necessary to be removed to prevent any interference at the down-stream analysis of the biomass sample. The samples were dried at a vacuum oven at 45°C overnight and cooled down in a dessicator before the determination of other main compositions. The acid insoluble lignin (AIL) using Klason Lignin method were determine following the Rowell and Rowell [18] method, while the cellulose and hemicellulose composition were determine using centrifugation technique according to the improved method developed by Designer Energy Ltd (DE) [19].

2.4 Analytical Method

The glucose yield in the hydrolysate was read spectrophotometrically at 505 nm following the procedure stated in the glucose assay kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China). The xylose yield in the hydrolysate was read at 340nm following the assay procedure of xylose determination stated inside the Megazyme xylose kit (Ireland).

3. RESULTS AND DISCUSSION

3.1. Pineapple Waste Composition

The proportion of the three main components, cellulose, hemicellulose, and lignin, as well as the extractives in the PW were identified and listed in Table 1. The amount of original lignin that exists in the PW mixture in this study was approximately 17.70 % per dry weight basis, this significant amount creates a recalcitrance barrier to the biomass and hinders further degradation towards the biomass. The lignin composition slightly higher compared to the previous analysis that has been done by Azelee, et al. [20] using the same source of PW from the N36 variety. From that previous analysis, percentage of holocellulose,

hemicellulose, cellulose, and lignin are 63.53%, 28.9%, 34.6%, and 12.37%, respectively. Compared to this current composition analysis, the amount of holocellulose, which is total of hemicellulose and cellulose of raw PW were lower. It takes about 36% of the dry weight of PW biomass.

Table 1 Chemical composition of pineapple waste mixture in % total dry weight (w/w).

Chemical composition	Percentage (% dry weight basis)
Ash	5.56
Extractives	20.03
Acid Insoluble Lignin	17.70
Holocellulose	36.36
Cellulose	26.81
Hemicellulose	9.55

The differences in the chemical composition and fiber properties of plant tissue might be because of the different age and harvesting period of the biomass. As the biomass becomes mature, the lignin content increases while holocellulose composition decreases, especially for the amorphous hemicellulose [21].

Extractives, which are the hydrophobic and hydrophilic non-structural components including inorganic content that is known to be able to decrease the yield of sugars from cellulose [22, 23] from the raw PW shows a high content (20.03 %) in this study. Thus, it needs to be removed before the analysis of polymer composition

3.2. Low-Pressure Steam Heating Pre-treatment of Pineapple Waste

The performance of this pre-treatment was measured by evaluating the chemical composition of the pre-treated biomass, which mainly focused on lignin, cellulose, and hemicellulose content, as well as the glucose, xylose released to the hydrolysate. The untreated PW was labelled as the control. The aim for the best pre-treatment condition to be selected during screening process are the one that gives i) the lowest percentage of lignin content (indicating the highest delignification while maintaining the highest percentage of both hemicellulose and cellulose in the pineapple waste) and ii) minimize the loss of xylose and glucose and avoid the condition that favour their degradation.

3.3 Effect of Pre-treatment with Different Biomass Loading (%)

Optimizing solid biomass loading from low-, moderate- and high solid loadings is significant as it could affect the efficiency of the pre-treatment, including cellulose crystallinity, increasing viscosity, delignification rate, and

hydrolysis rate kinetics and saccharification products [24]. Increased in solid loading will increase the viscosity of the biomass slurries and make it paste-like nature. Previous research used high solid loading to get higher sugar concentration, but there might be risks in producing a high concentration of inhibitors, which also limit mass and heat transfer during the pre-treatment and inhibit the downstream process [25]. High solid loading also needs a particular reactor that gives an effective mixing to avoid the inhibitors formed being localized. In the enzymatic saccharification process, Ioelovich and Morag [26] confirmed that an increase of initial loading of the pre-treated biomass leads to a decrease of enzymatic digestibility.

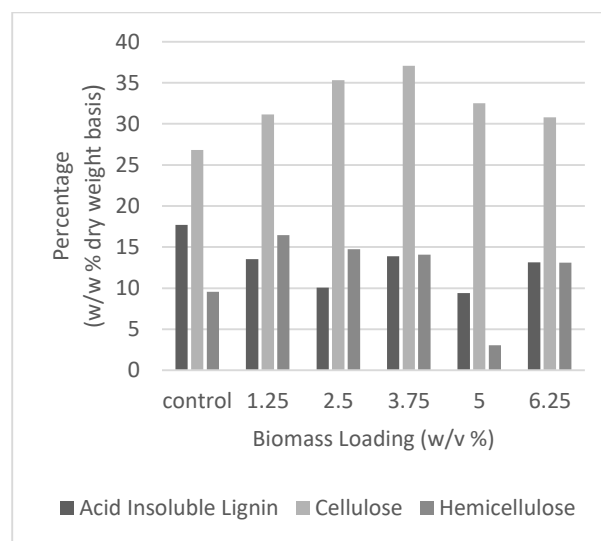


Figure 1 Composition (% dry weight basis) of the LSPH-pre-treated PW at different biomass loading (1.25, 2.5, 3.75, 5, 6.25 w/v %), with fixed pressure level (80 kPa) and moderate reaction time, 30 min.

As shown in Figure 1, at biomass loading of 5%, acid-insoluble lignin content was in the lowest percentage. However, the percentage of hemicellulose content at that condition was also at the lowest, indicating that these constituents are highly solubilized during pre-treatment, where this condition is not preferred. As the biomass increased to 6.25%, lignin removal efficiency was observed to decrease, where lignin percentage increase back to 13.14%. This is comparable to previous researches by Wu, et al. [27] and Cruz, et al. [24] that investigated the chemical composition of different type of biomass with ionic liquid pre-treatment. They obtained a high extracted lignin at lower solid loadings (3-5%) and at increasing solid loadings the lignin dissolution is low which leads to high recovery of lignin with cellulose and hemicellulose at the solid fraction of recovered biomass after the pre-treatment. This proven that further increasing the biomass loading during pre-treatment impacts the dissolution of biomass, where it limits the solubilisation of some composition. Hence, 2.5% biomass loading was chosen as the best condition considering the percentage of lignin remained on

the biomass were low but high hemicellulose and cellulose retained at the biomass compared to other biomass loading condition.

3.4 Effect of Pre-treatment with Different Pressure Level

Pressure play an essential role in the delignification and hydrolysis of hemicellulose and partial disruption of cellulose. A sudden increase in pressure will trigger thermal expansion inside the pressure cooker and thus, increasing the temperature. During heating, the acetyl group of hemicellulose will cleave into acetic acid, which makes the process become acidic in condition and improve the hydrolysis rate of cellulose and hemicellulose into valuable sugar. Severe condition pre-treatment such as long reaction time and too high pressure need to be avoided because to prevent further dehydration of cellulose and hemicellulose to their degradation products, which are 5-hydroxymethyl-2-furaldehyde (HMF) and furfural respectively [28]

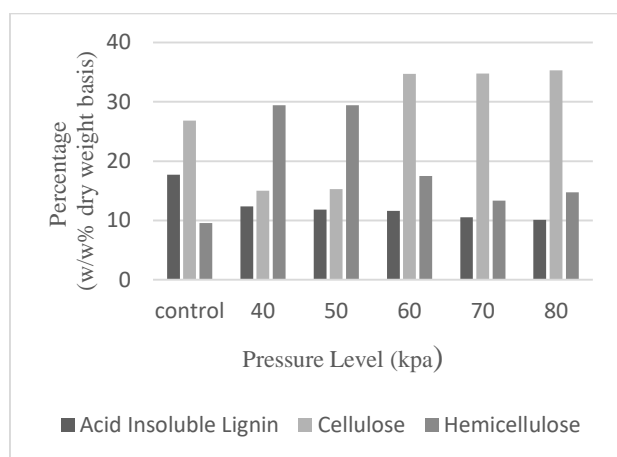


Figure 2 Composition (% dry weight basis) of the LSPH-pre-treated PW at different pressure levels (40, 50, 60, 70, 80 kPa), with fixed chosen biomass loading, 2.5% and moderate reaction time, 30 min.

As shown in Figure 2, the lignin content on biomass decreases as the pressure level increases. At the highest pressure level, which is 80 kPa, even though the lignin removal and cellulose content are highest than other pressure levels, but the hemicellulose content has shown at a low percentage, which is 14.75% per dry weight basis. Similar to 70 kPa, it shows the lowest hemicellulose content. This shows that at increasing pressure level, where the temperature inside the vessel are also increased, the depolymerization rate of xylose oligomers of hemicellulose to xylose into the liquid fraction also high [29]. The trend of increasing percentage of cellulose and decreasing lignin and hemicellulose content due to the solubilisation are similar to the previous research that investigated the pre-treatment of oil palm empty fruit bunch using different temperature of high-pressure steam [30].

While at a lower pressure level (40 and 50 kPa), even though the hemicellulose content was at the highest percentage compared to other pressure levels, the cellulose content showed the lowest percentage. This might be due to the lignin and hemicellulose structure are still highly available, which can hinder the cellulose exposure. This is comparable to the previous research that stated that solubilization and destruction of ester linkage of amorphous hemicellulose and lignin cross-linked cellulose solubilization will increase cellulose content, which this indicates the exposes of cellulose fibers [31]

As too low and too high-pressure level have their drawbacks, the most moderate pressure level (60kPa) was chosen after considering the percentage of lignin, cellulose, and hemicellulose removal. At 60 kPa, it has resulted in 34.5% lignin removal compared to the original lignin content. The cellulose content showed the second-highest percentage, which is 34.73%, and the hemicellulose percentage showed a moderate value, which is 17.50%. Hence, the pressure level of 60 kPa was chosen as the best condition considering the lowest percentage of lignin remained but still able to retain cellulose and hemicellulose.

3.5 Effect of Pre-treatment with Different Reaction Time

Time of pre-treatment is one of the critical variables affecting the efficiency of lignin removal from biomass. Different biomass may require different pre-treatment times for the maximum lignin removal depending on the chemical composition and the biomass structure, respectively. The longer the residence time, the more severe the pre-treatment. The structure and degree of crystallinity of the biomass also entirely changed after each pre-treatment time, which also contributed to fiber loss [32].

From Figure3, 30 minutes of pre-treatment recorded the lowest lignin content remained in the biomass, which means there was a high removal of lignin content. However, lignin content increased after treated for more than 45 minutes, which is an undesirable condition. [33] suggests that an increment of lignin could be due to the re-condensation reaction of lignin and melted onto the surface of cellulose microfibrils. Previous study also states that undesirable reactions such as repolymerization of lignin could occur during acid pre-treatment if the reaction time too long [34]. This led to increasing heterogeneity of the lignin, where the very reactive and unstable lignin fragments undergo condensation reactions instead of forming monomeric products [35].

As for cellulose and hemicellulose content, as time increases at the chosen constant pressure (60kPa) for pre-treatment, the cellulose content slightly decreases while for the hemicellulose, there is no significant change. This result suggests that in a similar pressure level, the retention time does not play a substantial role in the strength of cellulose–hemicellulose association. This can be proven by comparing with Figure4, where the yields of glucose and xylose remain similar (~30mg/g and ~2mg/g respectively), which this shows the insignificant effect to the

solubilization of cellulose and hemicellulose throughout the 30 to 75 minutes pre-treatment time.

After the actual weight of compositional change was calculated, as shown in Figure 5, 30 minutes pre-treatment time was chosen as it able to remove the most lignin content as much as 60.89% and able to retain as high as 91% of hemicellulose and 77.6% of cellulose from the biomass. Interestingly, lignin shows a significant weight loss from 4.42g to 1.74g per 25g of biomass, which these results indicate that this proposed pre-treatment method is effective in lignin de-polymerization.

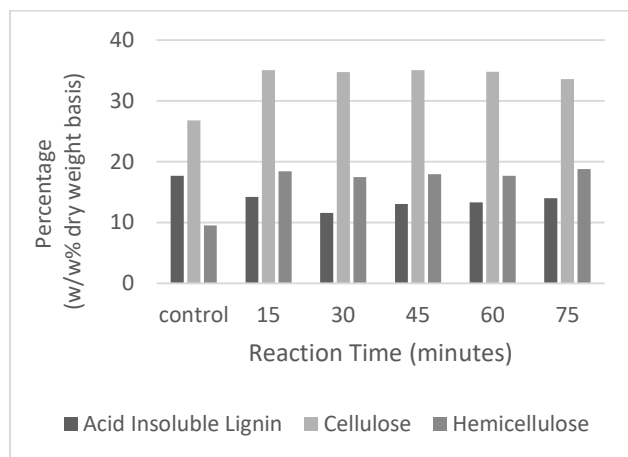


Figure 3 Composition (% dry weight basis) of the LSPH-pre-treated PW at different reaction time (15, 30, 45, 60 and 75min), with fixed chosen biomass loading, 2.5% and chosen pressure level, 60 kPa.

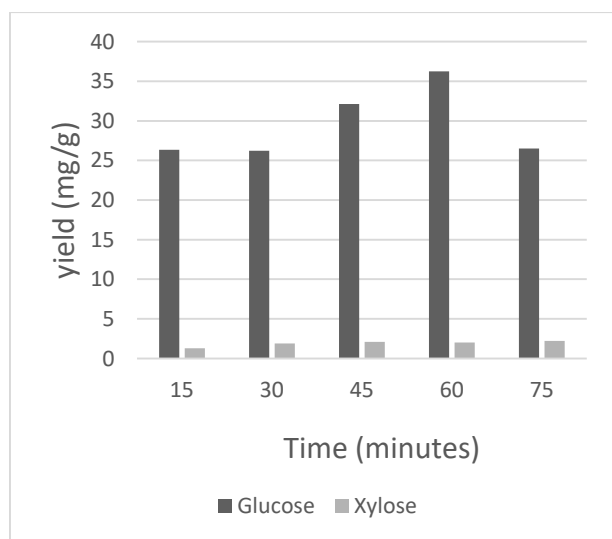


Figure 4 Glucose and xylose yield at different pre-treatment time at different reaction time (15, 30, 45, 60 and 75min), with fixed chosen biomass loading, 2.5% and moderate reaction time, 30 min.

According to Shimizu, et al. [36], ~15% of lignin removal and ~10% of hemicellulose removal will results at least

60% of glucose yield during the enzymatic hydrolysis steps. The glucose yield of the biomass is inversely proportional to the lignin content [37]. However, different biomass has different physicochemical characteristics, different cellulose content, so it might be giving different responses to the pre-treatment method used and also to the subsequent enzymatic hydrolysis.

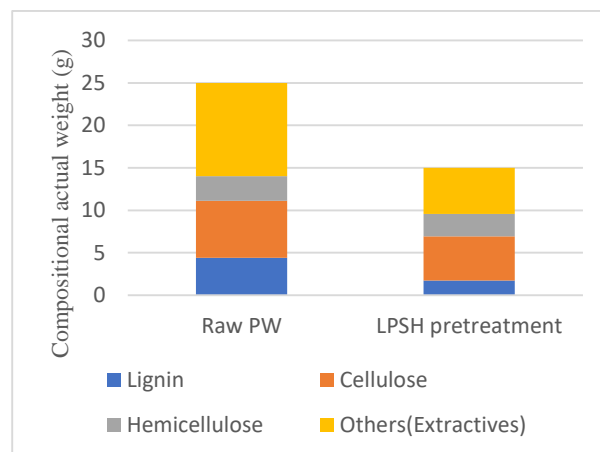


Figure 5 Actual weight (g) of lignin, cellulose, and hemicellulose before and after the pre-treatment with low-pressure steam heating at the optimized condition (2.5% biomass loading, 60 kPa, 30 minutes).

The final percentage of delignification of this current study is comparable to the previous research that used autoclaving method (121°C for 1 hour), which the pressure level is approximately 2 times higher (~110kPa) than this study (60kPa), and it able to remove lignin as much as 63%. Compared to this current study, at 60 kPa, the valve that regulates the pressure inside the pressure cooker able to boil the water up to temperature 114 °C. The percentage delignification also comparable which is (60.85%). However, autoclaved method is not preferable because of the high severity condition, because there was high removal of hemicellulose and degradation of sugar into the liquid fraction [38].

4. CONCLUSION

Based on the results of this works, it appears that the best condition for pre-treating PW with the aim of high delignification and retaining the most cellulose and hemicellulose would be pre-treatment at the pressure level of 60 kPa for 30 minutes using 2.5% biomass loading. This chosen condition of LPSH pre-treatment also contributed to some glucose and xylose yield that can be recovered from their liquid fraction. The pre-treated PW will be further evaluated for the released of sugar by enzymatic hydrolysis by optimizing the most efficient enzyme mixture and optimum reaction conditions for the maximum sugar yield.

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