Borneo Journal of Resource Science and Technology (2025), 15(1): 189-197
DOI: https:doi.org/10.33736/bjrst.7718.2025

Impact of Various Pre-Treatments on the Lignocellulosic Compositions of Sarawak 'Paun' Pineapple Leaf Waste

ALEXANDRA CHERYL ANAK DENNIS, ROSMAWATI SAAT* & DIANA KERTINI MONIR

Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia
*Corresponding author: srosma@unimas.my
Received: 28 August 2024 Accepted: 15 April 2025 Published: 30 June 2025

ABSTRACT

Pre-treatment of lignocellulosic biomass is a crucial step in breaking down the complex structure of the plant`s cell wall to enhance the availability and digestibility of cellulose for bioconversion process of the biomass to value-added products. The overall efficiency of the process designed to convert lignocelluloses also lies on an accurate determination of compositions of the lignocellulosic substrate. In this study, local species of Sarawak 'Paun' pineapple leaves collected from Simunjan, Sarawak, were subjected to various pre-treatment methods including thermal treatment, acidic treatments and alkaline treatments. Compositional analyses of the raw and pre-treated leaves were conducted through a gravimetric method to study the effect of different methods of pre-treatments in altering the lignocellulosic compositions of the pineapple leaf wastes focusing on the hemicellulose, lignin and cellulose content. As the main purpose of a pre-treatment method focuses on its ability and efficiency to disintegrate the biomass complex structure, especially, in reducing lignin content for higher cellulose accessibility of enzymes in enzymatic hydrolyses. This study suggested that pre-treatment with 1.5% (v/v) hydrochloric acid solution displayed the most notable change to the lignocellulose contents of the leaves as highest cellulose content (51.5% w/w) and lowest amount of lignin (10.3% w/w) were recorded, compared to that of other pre-treatment methods. These findings may provide a better understanding for future research in designing a suitable biochemical process with enhanced enzymatic digestibility of cellulose present in the pineapple leaves to yield wealth-added products.

Keywords: Bioconversion, compositional analyses, hydrochloric acid, pineapple leaves, pre-treatments

---

INTRODUCTION

Lignocellulose or also known as lignocellulosic biomass is the most abundant type of biomass which includes a wide variety of distinct biomass types including grasses, woods, energy crops, agricultural and municipal wastes (Fadeyi *et al*., 2020). The term lignocellulosic is used to describe materials exist in the plant dry matter (biomass) that is mostly composed of three major components which consist of up to 60% of cellulose, 20 to 40% of hemicellulose, and 10 to 25% of lignin from the total dry basis of biomass (Sluiter *et al*., 2010, Maisyarah *et al*., 2019). These major components are bonded together through a covalent bonding, various intermolecular bridges and van der Waals' forces which contributed to their resistance towards enzymatic hydrolysis and their insolubility in water (Fadeyi *et al*., 2020). Cellulose is a glucose polymer linked by ß-1,4 glycosidic bonds with a basic building block of glucose-glucose dimer known as cellobiose (Ayeni *et al*., 2015). The structure of cellulose is organized as micro-fibrils and it possesses alternating regions of crystalline (well-ordered) and amorphous (disordered). On the other hands, hemicellulose, being the second major constituent of cell wall, is made up of short-chain hetero-polysaccharide with a lower degree of polymerization than cellulose (Lobo *et al*., 2021). The heteropolymer consists of different monosaccharide units such as glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts or traces dependent on the natural source (Hu *et al*., 2020). Lignin, a third major component of lignocellulosic biomass, is a highly cross-linked phenyl propylene polymer and the largest non-carbohydrate fraction of the lignocellulose (Ayeni *et al*., 2015). It acts significantly as the permanent binder in all plants by gluing the cellulose fibers together, thus, providing mechanical strength, rigidity and permeability of the plant's cell wall (Tumuluru *et al*., 2011; Lobo *et al*., 2021).

Over the years, lignocellulosic biomass has continued to be studied as a source of fermentable sugars for bioconversion of lignocelluloses to value-added products such as biofuels production, enzymes, and bio-based chemicals, due to its high availability in nature and cellulose as the major constituent of the feedstocks (Widihastuty *et al*., 2021; Saini *et al*., 2022). Pineapple leaves generated majorly from pineapple-related industry, are a potential feedstock and can be used as substrates in many biochemical processes such as microbial fermentation and anaerobic digestion due to the presence of fibre (Aili *et al*., 2021). Pineapple leaf wastes have extensively been used as lignocellulosic feedstock for the production of enzymes such as cellulases, by using *Trichoderma reesei* and *Aspergillus* sp. which are known as the most common and efficient producer of cellulases (Saravanan *et al*., 2013; Zhao *et al*., 2018). However, the overall efficiency of processes designed to convert lignocelluloses depends on the compositions of such materials which can be varied greatly due to the complex and heterogeneous nature of biomass (Fadeyi *et al*., 2020). Another fact that should be taken into account is the complex network of cellulose-hemicellulose-lignin present in the biomass. The hydrolysis of cellulose becomes highly difficult due to lignin which is covalently bound to the polysaccharides and the presence of complex lignin-hemicellulose shield, forming a protective covering surrounding the cellulose micro-fibrils and shields them from enzyme attack (Ariffin *et* al., 2020; Pendse *et al*., 2023). Hence, pre-treatment of the biomass is significantly required to deconstruct the lignocellulosic matrix especially in breaking down the lignified structure and degrading hemicellulose, making cellulose more available to enzymatic hydrolysis (Iroba *et al*., 2013; Pendse *et al*., 2023). The effectiveness of lignocellulosic biomass modification to useful fermentable sugars relies on the residues and type of pre-treatment that have been selected (Ariffin *et al*., 2020).

The present study aims to assess the impact of various pre-treatment methods on the compositional change of three major lignocellulosic components present in the local pineapple leaves. Prior to a gravimetric method by Maisyarah *et al.* (2019) adopted for the compositional analysis in this study, the local pineapple leaves were subjected to thermal treatment by autoclave, acidic treatment with dilute hydrochloric acid solutions, and alkaline treatment with sodium hydroxide solutions, separately. Changes in the cellulose, hemicellulose, lignin, and extractives contents of raw and pre-treated leaves evaluated in this present study may elucidate on the efficiency of various pre-treatment methods in breaking down the complex structure of the biomass. Subsequently, this may contribute to a better understanding for future studies in enhancing the enzymatic digestibility of cellulose present in the pineapple leaves as potential agro-based substrates for bioconversion processes in liberating valuable products.

MATERIALS AND METHODS

Collection and Preparation of Local Pineapple Leaves

Pineapple leaves from Sarawak 'Paun' cultivar used in this study were obtained locally from Sebangan, Simunjan, Sarawak, Malaysia. The leaves were rinsed with tap water and distilled water for the preliminary removal of unwanted impurities, followed by oven drying at 60 °C. The leaves were then cut into smaller pieces and ground by using an electrical blender, and sieved into particle size in between 0.5 mm to 1.0 mm. These ground samples were kept in a sterilised glass bottle prior to the pre-treatment process.

Pre-treatments of Pineapple Leaves

In this study, three different methods of pre-treatments were applied in treating the ground pineapple leaves in order to study the effect of different pre-treatments mainly on the lignocellulosic materials in the pineapple leaves.

Thermal Treatment

The method performed for thermal treatment was carried out according to Corbin *et al*. (2015). A ratio of 1:10 of solid to liquid was applied, in which distilled water was added to the lignocellulosic sample to prevent burning of the sample, followed by autoclaving at 121 °C for 30 minutes. Subsequently, the sample was let to cool down to room temperature prior to filtration. The sample was then dried in a drying oven at 60 °C and then, the dried sample was kept in an airtight container prior to the compositional analysis.

Acidic Treatment

Two different concentrations of hydrochloric acid (HCl) solutions were used in the acidic pre-treatment of pineapple leaves by adopting a method by Bansal *et al*. (2012) with a slight modification. A volume of 200 ml of 1% (v/v) and 1.5% (v/v) of HCl solution was poured separately into each Erlenmeyer flask. Then, an amount of 50 g of ground pineapple leaves was added, and followed with incubation for 2 hours at room temperature in a static condition. Upon completion of incubation, the sample was filtered and rinsed with distilled water for several times to discard any remaining acid residues, followed by drying process at 60 °C in a drying oven. Once dried, the treated leaves were kept in an airtight container prior to the compositional analysis.

Alkaline Treatment

The alkaline pre-treatment process was performed according to Bansal *et al*. (2012), with a slight modification. In this study, concentrations of 1% (w/v) and 1.5% (w/v) of sodium hydroxide (NaOH) solutions were used. An amount of 50 g of the ground leaves was added into an Erlenmeyer flask containing 200 ml of NaOH solution of each concentration, separately. The mixture was incubated for 2 hours at room temperature, statically. The sample was then filtered and thoroughly rinsed for several times with distilled water to remove any traces of base, followed by drying in a drying oven at 60 °C before the pre-treated sample was kept in an airtight container.

Compositional Analysis

A gravimetric method by Maisyarah *et al*. (2019) was performed on the dried raw sample (ground leaves) and dried pre-treated leaves for the compositional analysis in determining the amount of three major lignocellulosic components, namely, hemicellulose, lignin and cellulose.

Amount of Extractives

A mixture of 1 g of the sample (A) and 60 ml of acetone (as a solvent for the extraction) was refluxed at 90 °C for 2 hours on a hot plate. After 2 hours, the sample was dried in a drying oven at 105 – 110 °C until a constant weight (B) was obtained. By using the equation, Eq. (1), stated below, the amount of extractives was determined.

Amount of Extractives (g) = (A – B) Eq. (1)

Amount of Hemicellulose

A volume of 150 ml of 0.5 M of sodium hydroxide (NaOH) solution was added to 1 g of extractive-free sample (B). Then, the mixture was boiled on a hot plate at 80 °C for 3.5 hours. After boiling, the sample was washed multiple times with deionised water for the removal of excess basic solution. A pH meter was used to confirm complete removal of basic solution when the pH of the solution is closer to 7. The sample was then dried in a drying oven at 105 – 110 °C to a constant weight (C). The amount of hemicellulose was determined by using the following equation, Eq. (2).

Amount of Hemicellulose (g) = (B – C) Eq. (2)

Amount of Lignin

Prior to handling concentrated sulphuric acid and to prevent excessive evaporation of the acid, a reflux apparatus was prepared before hands which basically includes the setting up of heating mantle, round bottom flask (containing the sample mixture with concentrated acid), reflux condenser and rubber tubing as connecting glassware (Ferreira *et al*., 2013). A volume of 30 ml of 98% sulphuric acid (H_2SO_4) was added to 1 g of sample with extractives free (B), in which the mixture was then left at room temperature for 24 hours, prior to boiling at 100 °C for 1 hour. After boiling, the mixture was filtered by using Whatman filter paper no. 1 and the solid residue was washed multiple times by using deionised water until sulphate ion was undetectable. The detection of sulphate ion was performed through a titration with 10% of Barium chloride ($BaCl_2$) solution in which the disappearance of white precipitation indicated that the solid residue was free from acidic solution. The sample was then left to dry in a drying oven at 105 – 110 °C until a constant weight (D) was achieved. The final weight of the solid residue was recorded as lignin content, Eq. (3).

Amount of Lignin (g) = D Eq. (3)

Amount of Cellulose

The amount of cellulose was determined by calculating the difference between initial weight of the sample with the weight of three other components obtained from the previous steps, assuming that the only components of an entire sample are extractives, hemicellulose, lignin and cellulose. Thus, cellulose content (E) was determined by using the following equation, Eq. (4), in which 1 g refers to the total amount of sample used in the experiment.

(A – B) + (B – C) + D + E = 1 g Eq. (4)

RESULTS AND DISCUSSION

The basic lignocellulosic compositions of raw (ground leaves) and pre-treated 'Paun' pineapple leaves are as shown in Table 1 and Figure 1. Each experimental procedure was conducted in duplicates, thus, reported results below indicates the average values of the replicates.

Based on the results, it is observed that the lowest content of extractives was identified at 8.5% (w/w) when the sample was subjected to acid pre-treatment with 1.5% (v/v) of HCl solution, by which it was reduced by two-fold compared to the extractives observed in raw pineapple leaves. The extractives include non-structural components of biomass such as sucrose, nitrate/nitrite, protein, ash, chlorophyll, waxes, that are required to be removed because they have the potential to interfere with downstream analysis of biomass sample (Ayeni *et al*., 2015). Hemicelluloses, being one of the polysaccharides in the biomass, were identified to be the lowest at 13.1% (w/w) for raw pineapple leaves but recorded the highest hemicellulose content at 31.1% (w/w) of the total biomass dry mass when the Sarawak 'Paun' pineapple leaves were pre-treated with 1% (v/v) HCl solution. On the other hands, amount of lignin of raw and treated pineapple leaf wastes was observed to be in the range 10.3% (w/w) to 15.7% (w/w), with the lowest lignin content recorded when the samples were subjected to acid pre-treatment with hydrochloric solutions of 1% (v/v) and 1.5% (v/v). Cellulose, which is stated to be the main component in most lignocellulosic biomass, was also revealed to be the major constituent of the Sarawak 'Paun' pineapple leaves with 37.0% (w/w) of cellulose content recorded. The cellulose content displayed an increased amount across all the pre-treatments employed, compared to that of raw pineapple leaves (37.0% w/w). The highest content of celluloses recorded was at 51.5% (w/w) of its total dry mass when the local pineapple leaves were pre-treated with 1.5% (v/v) HCl solution. Based on the findings of this present study, different pre-treatment methods gave a notable impact in altering the lignocellulosic compositions present in the Sarawak 'Paun' pineapple leaf wastes. As the main purpose of a pre-treatment method focuses on its ability and efficiency to disintegrate the biomass complex structure, especially, in reducing lignin content for higher cellulose accessibility of enzymes in enzymatic hydrolyses, pre-treatment with 1.5% (v/v) of HCl solution is shown to be the most effective method in treating the pineapple leaves as the lowest lignin content at 10.3% (w/w) and the highest content of cellulose at 51.5% (w/w) were recorded.

Even though a number of researches on the pre-treatment of pineapple leaves have been published, previous findings that reported mainly on the correlation between various pre-treatment methods applied on local pineapple leaf waste particularly from Sarawak pineapple cultivar and their impacts on lignocellulosic compositions are still very limited. Table 2 shows the amounts of major lignocellulosic constituents, mainly the hemicellulose, cellulose, lignin and extractives, in pineapple leaf wastes illustrated in previous literatures. In this study, cellulose contents in the Sarawak 'Paun' pineapple leaves regardless of treatment methods were the major constituent at the range of 37.0 – 51.5% (w/w), followed by hemicellulose and lignin contents at 13.1 – 31.1% (w/w) and 10.3 – 19.7% (w/w), respectively. Hemicellulose contents in Sarawak 'Paun' leaves were comparable with most of the literatures stated in Table 2, but, faintly lower than the hemicellulose value acquired by Maisyarah *et al*. (2019) which was at 37% (w/w). On the other hands, the amounts of cellulose in the present study were significantly lower than the cellulose contents obtained by Nashiruddin *et al*. (2020) and Reddy and Yang (2005), but, slightly higher than the cellulose amount by Maisyarah *et al*. (2019).

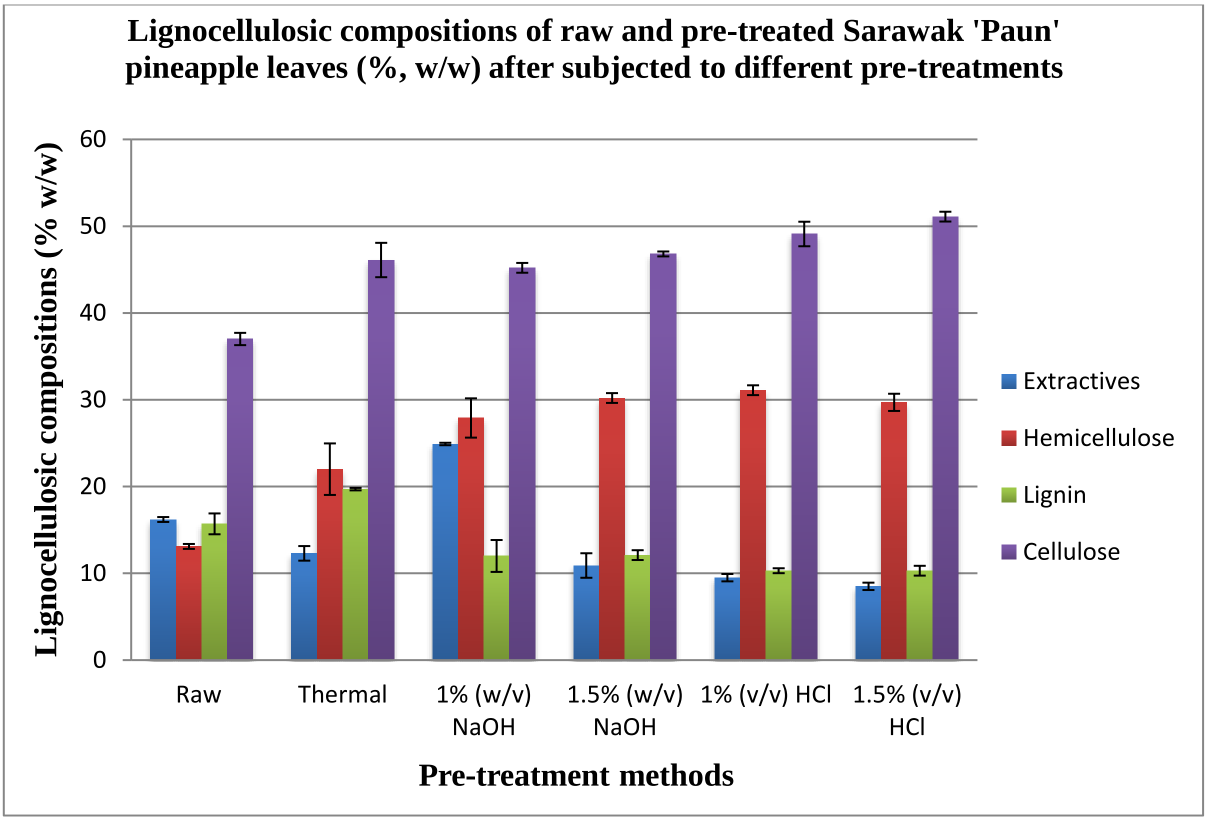

Figure 1. Bar chat interpretation of lignocellulosic compositions of raw and pre-treated Sarawak 'Paun' pineapple leaves (%, w/w)

Table 1. Lignocellulosic compositions of raw and pre-treated Sarawak 'Paun' pineapple leaves (%, w/w) after subjected to different pre-treatments

Lignocellulosic component	Raw PL	Pre-treatment				
		Thermal by autoclave	1% (w/v) NaOH	1.5% (w/v) NaOH	1% (v/v) HCl	1.5% (v/v) HCl
Extractives	16.2±0.3	12.3±0.8	24.9±0.1	10.9±1.4	9.5±0.4	8.5±0.4
Hemicellulose	13.1±0.3	22.0±3.0	27.9±2.3	30.2±0.6	31.1±0.6	29.7±1.0
Lignin	15.7±1.2	19.7±0.1	12.0±1.8	12.1±0.6	10.3±0.3	10.3±0.6
Cellulose	37.0±0.7	46.1±2.0	45.2±0.6	46.8±0.3	49.1±1.4	51.5±0.6

Note: PL indicates pineapple leaves.

Table 2. Lignocellulosic compositions (cellulose, hemicellulose, lignin and exrractives) of pineapple leaves from various studies

Cellulose (% w/w)	Hemicellulose (% w/w)	Lignin (% w/w)	Extractives (% w/w)	References
37.0 - 51.5	13.1 – 31.1	10.3 – 19.7	8.5 – 24.9	This study
72.76	17.15	4.76	-	Nashiruddin *et al*. (2020)
30	37	22	11	Maisyarah *et al*. (2019)
56.0 – 82.0	-	4.4 – 4.7	26.21 – 28.47	Yuliasmi *et al*. (2017)
66.2	19.5	4.28	-	Zawawi *et al*. (2014)
70 – 82	18	5 – 12	3.6 – 7.0	Reddy and Yang (2005)

The ideal cellulose content of the biomass used in biochemical and bioconversion process is typically preferred to be in between 30% to 50% as the range allows efficient enzymatic hydrolysis and sugar extraction while sustaining a balanced composition with other major components, especially, hemicellulose and lignin (Vélez-Mercado *et al*., 2021). In addition, the high cellulose proportion of up to 37.0% (w/w) and low lignin content of 15.7% (w/w) present in the raw Sarawak 'Paun' pineapple leaves (refer Table 1) are comparable with several previous findings in Table 2 that suggested local pineapple leaf wastes from few Malaysian pineapple varieties used in their studies were majorly made up of high cellulose content up to 66.2% (w/w), as well as low lignin content ranging between 4% to 22% (w/w) of their total dry masses (Zawawi et al., 2014; Maisyarah et al., 2019). Cellulose component of the biomass is the most abundant renewable organic source and a high content of this compound contributes to the potential of the lignocellulose of being a valuable use in the production of various high value products through bioconversion process (Fadeyi *et al*., 2020). In most enzymatic hydrolyses occurring in biochemical processes, it is important to identify the cellulose contents of a lignocellulosic biomass used as it acts as a cellulolytic enzymes inducer and that, the production of cellulose is affected by the nature of the substrate (Lodha *et al*., 2020; Singh, 2021). Besides, knowing the lignocellulosic compositions of a biomass is vital prior to its use in biochemical and bioconversion process involving microbes, such as fermentation, as lignocellulosic substrate acts as a source of nutrients and that, a biomass which contains sufficient amount of nutrients to supplement the microbial growth is highly preferable (Yoon *et al*., 2014).

This present study has also shown that pre-treated Sarawak 'Paun' pineapple leaves displayed a substantial change in their major lignocellulosic compositions, compared to that of raw leaves. The changes in the cellulose and lignin contents of the pre-treated local pineapple leaves compared to that of raw leaves used in this study are similar with a previous study by Awoyale and Lokhat (2021). Awoyale and Lokhat (2021) mentioned that the values of the cellulose content in the pre-treated biomasses (corn cobs, rice husks, cassava peels, sugar cane bagasse and white yam peels) used in the study illustrated a significant difference from the amount of cellulose in raw biomass, with values ranging from 33.2 wt% to 43.8 wt% and, that of the lignin content decreasing significantly regardless of the pre-treatment methods. Utilisation of lignocellulosic biomass for the production of wealth-added products such as fuels, fine chemicals and industrially-important enzymes requires an effective pre-treatment method to enhance the available enzymes for enzymatic hydrolysis process by disrupting the hemicellulose-lignin complex structure (Nashiruddin *et al*., 2020).

Besides, lignin is often regarded as the main parameter that limits the biodegradation of lignocellulosic biomass due to its major mechanism involving the covalent cross linking of lignin with other cell wall compounds which imparts the predominant role in the biomass recalcitrance (Rajesh *et al*., 2021). Thus, a pre-treatment method is significantly required to remove lignin for higher cellulose accessibility of enzymes in enzymatic hydrolyses occurring in the bioconversion and biochemical process (Iram *et al*., 2020). Another fact that should be highlighted is the effectiveness of dilute HCl solution in pre-treating local pineapple leaves from this present study, which can be supported with earlier findings that also applied similar method of chemical treatment in pre-treating lignocellulosic agro-substrates as the promising feed stocks in bioconversion and biochemical processes (Cho *et al*., 2019; Ariffin *et al*., 2020; Usino *et al*., 2020). It was suggested that pre-treatment of agro-based substrates with dilute acid solution such as HCl have particularly improved cellulose contents and reduced lignin composition in pre-treated samples, compared to those in raw samples (Cho *et al*., 2019; Ariffin *et al*., 2020; Usino *et al*., 2020). Unlike alkaline pre-treatment that primarily interacts with lignin for lignin removal, acid pre-treatment is more suitable for biomass with low lignin content as its core reaction involves hydrolysis of hemicellulose and solubilisation of small fractions of lignin (Kim *et. al.,* 2016; Oriez *et al.*, 2019). As alkaline pre-treatment is better suited for biomass with high lignin content, the alkaline pre-treatment in the current study might not be effective enough in pre-treating the Sarawak 'Paun' leaves due to the nature of the leaves of having low lignin content as it might slow down the primary interaction between the alkaline solution and lignin (Kim *et. al.*, 2016; Oriez *et al.*, 2019). In addition, during pre-treatment of biomass with dilute acid solution, the hydrolysis of hemicelluloses might highly occur as acid interrupts the hydrogen bonds that linked hemicelluloses and celluloses, as well degrading the covalent bonds between hemicelluloses and lignin (Amin *et al*., 2017).

Consequently, the chemical reaction caused by acid pre-treatment of the biomass can lead to high solubilisation of hemicellulose, thereby increasing the accessibility to cellulose to be transformed into valuable sugars (Amin *et al*., 2017; Ariffin *et al*., 2020). In fact, the glycosidic bonds present in hemicelluloses are susceptible to acid, leading to the removal of hemicellulose which subsequently increases the pore size of the biomass and enhances the digestibility of cellulose (Shi *et al*., 2020). Thus, this explains the results of this present study by which pre-treatment with dilute HCl solution, especially, 1.5% (v/v) HCl solution, works better and gave more impacts on the lignocellulosic compositions of Sarawak 'Paun' pineapple leaves, compared to the leaves treated with sodium hydroxide solutions. In addition, acid solutions have been used as reagents in enhancing degradation efficiency of the glycosidic bonds in lignocellulose to promote the release of fermentable sugars, but, only diluted acids are recommended to minimize the risk of toxicity, corrosion and handling (Awogbemi & Kallon, 2022).

CONCLUSION

Efficiency of process designed for bioconversion processes lies on the effectiveness of a pre-treatment method in causing compositional changes in the lignocelluloses by breaking down its complex compositional make-up and physicochemical structure. Removal of lignin and solubilisation of hemicellulose are taken as a significant consideration for an efficient method of pre-treatment in enhancing availability of cellulose to enzymatic hydrolysis for higher conversion yields. Compositional analysis of the Sarawak 'Paun' pineapple leaves by gravimetric method in this present study indicated that acid pre-treated leaves with 1.5% (v/v) hydrochloric solution displayed a notable change in its lignocellulose contents with the highest value of cellulose at 51.5% (w/w), hemicelluloses at 29.7% (w/w) and lowest lignin content of 10.3% (w/w), compared to that of raw leaves and other pre-treatment methods applied in this study. These findings may contribute for a better understanding in designing a biochemical process in converting lignocellulosic materials to valuable products, with greater efficiency and effectiveness.

ACKNOWLEDGMENTS

Our gratitude to Tun Zaidi Chair Grant (F07/TZC/2162/2021), UNIMAS for funding this project and the Faculty of Resource Science & Technology, UNIMAS, Malaysia in providing the equipment.

REFERENCES

Aili Hamzah, A.F., Hamzah, M.H., Che Man, H., Jamali, N.S., Siajam, S.I. & Ismail, M.H. (2021). Recent updates on the conversion of pineapple waste (*Ananas comosus*) to value-added products, future prespectives and challenges. *Agronomy*, 11: 2221. DOI: 10.3390/agronomy11112221

Amin, F.R., Khalid, H., Zhang, H., Rahman, S.U., Zhang, R., Liu, G. & Chen, C. (2017). Pre-treatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express*, 7(72): 1-12. DOI: 10.1186/s13568-017-0375-4

Ariffin, K.K., Masngut, N., Seman, M.N.A., Saufi, S.M., Jamek, S. & Sueb, M.S.M. (2020). Dilute acid hydrolysis pretreatment for sugar and organic acid production from pineapple residues. *IOP Conference Series: Materials Science and Engineering*, 991: 1-9. DOI: 10.1088/1757-899X/991/1/012057

Awogbemi, O. & Kallon, D.V.V. (2022). Pretreatment techniques for agricultural waste. *Case Studies in Chemical and Environmental Engineering*, 6: 1-9. DOI: 10.1016/j.cscee.2022.100229

Awoyale, A.A. & Lokhat, D. (2021). Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production. *NatureResearch*, 11(2021): 557. DOI: 10.1038/s41598-020-78105-8

Ayeni, A.O., Adeeyo, O.A., Oresegun, O.M. & Oladimeji, T.E. (2015). Compositional analysis of lignocellulosic materials: evaluation of an economically viable method suitable for woody and non-woody biomass. *American Journal of Engineering Research (AJER)*, 4(4): 14-19.

Bansal, N., Tewari, R., Soni, R. & Soni, S.K. (2012). Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. *Waste Management*, 32(7): 1341–1346. DOI: 10.1016/j.wasman.2012.03.006

Cho, E.J., Trinh, L.T.P., Song, Y., Lee, G.Y. & Bae, H.J. (2019). Bioconversion of biomass waste into high value chemicals. *Bioresource Technology*, 2019: 1-48. DOI: 10.1016/j.biortech.2019.122386

Corbin, K.R., Hsieh, Y.S.Y., Betts, N.S., Byrt, C.S., Henderson, M., Stork, J., DeBolt, S., Fincher, G.B. & Burton, R.A. (2015). Grape marc as a source of carbohydrates for bioethanol: chemical composition, pre-treatment and saccharification. *Bioresource Technology*, 2015: 1-34. DOI: 10.1016/j.biortech.2015.06.030

Fadeyi, A.E., Akiode, S.O., Emmanuel, S.A. & Falavi, O.E. (2020). Compositional analysis and characterization of lignocellulosic biomass from selected agricultural wastes. *Journal of Science and Mathematics Letters*, 8(1): 48-56. DOI: 10.37134/jsml.vol8.1.6.2020

Ferreira, S.L.C., Silva, L.O.B., de Santana, F.A., Junior, M.M.S., Matos, G.D. & dos Santos, W.N.L. (2013). A review of reflux systems using cold finger for sample preparation in the determination of volatile elements. *Microchemical Journal*, 106(2013): 307-310. DOI: 10.1016/j.microc.2012.08.015

Hu, L.S., Fang, X.Z., Du, M.H., Luo, F. & Guo, S. (2020). Hemicellulose-based polymers processing and application. *American Journal of Plant Sciences*, 11: 2066-2079. DOI: 10.4236/ajps.2020.1112146

Iram, A., Cekmecelioglu, D. & Demirci, A. (2020). Ideal feedstock and fermentation process improvements for the production of lignocellulolytic enzymes. *Process*, 9(38): 1-26. DOI: 10.3390/pr9010038

Iroba, K.L., Tabil, L.G., Dumonceaux, T. & Baik, O. (2013). Effect of alkaline pretreatment on chemical composition of lignocellulosic biomass using radio frequency heating. *Biosystems Engineering*, 116: 385-398. DOI: 10.1016/j.biosystemseng.2013.09.004

Kim, J.S., Lee, Y.Y. & Kim, T.H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology*, 2015: 1-7. DOI: 10.1016/j.biortech.2015.08.085

Lobo, F.C.M., Franco, A.R., Fernandes, E.M. & Reis, R.L. (2021). An overview of the antimicrobial properties of lignocellulosic materials. *Molecules*, 26: 1749. DOI: 10.3390/molecules26061749

Lodha, A., Pawar, S. & Rathod V. (2020). Optimised cellulase production from fungal co-culture of *Trichodetma reesei* NCIM 1186 and *Penicillium citrinum* NCIM 768 under solid state fermentation. *Journal of Environmental Chemical and Engineering*, 8(5): 1-6. DOI: 10.1016/j.jece.2020.103958

Maisyarah, A., Lim, J.S., Ani, F.N., Hashim, H. & Ho, W.S. (2019), Characteristics of cellulose, hemi-cellulose and lignin of MD2 pineapple biomass. *Chemical Engineering Transaction*, 72: 79–84. DOI: 10.3303/CET1972014

Nashiruddin, N.I., Mansor, A.F., Rahman, R.A., Ilias, R.M. & Wan Yussof, H. (2020). Process parameter optimization of pretreated pineapple leaves fiber for enhancement of sugar recovery. *Industrial Crops and Products*, 152(112514): 1-8. DOI: 10.1016/j.indcrop.2020.112514

Oriez, V., Peydecastaing, J. & Pontalier, P. (2019). Lignocellulosic biomass fractionation by mineral acids and resulting extract purification processes: conditions, yields, and purities. *Molecules*, 24(2019): 4273. DOI: 10.3390/molecules24234273

Pendse, D.S., Deshmukh, M. & Pande, A. (2023). Different pre-treatments and kinetic models for bioethanol production from lignocellulosic biomass: A review. *Heliyon*, 9: 1-15. DOI: 10.1016/j.heliyon.2023.e16604

Rajesh, B.J., Sugitha, S., Kavitha, S., Yukesh, K.R., Merrylin, J. & Kumar, G. (2021). Lignocellulosic biomass pretreatment for enhanced bioenergy recovery: Effect of lignocelluloses recalcitrance and enhancement strategies. *Frontiers in Energy Research*, 9: 1-17. DOI: 10.3389/fenrg.2021.646057

Reddy, N. & Yang, Y. (2005). Biofibers from agricultural byproducts for industrial applications. *TRENDS in Biotechnology*, 23(1): 22-27. DOI: 10.1016/j.tibtech.2004.11.002

Saini, R., Chen, C., Patel, A.K., Saini, J.K., Dong, C. & Singhania, R.R. (2022). Valorization of pineapple leaves waste for the production of bioethanol. *Bioengineering*, 9(557): 1-9. DOI: 10.3390/bioengineering9100557

Saravanan, P., Muthuvelayudham, R. & Viruthagiri, T. (2013). Enhanced production of cellulase from pineapple waste by response surface methodology. *Journal of Engineering*, 2013: 1-8. DOI: 10.1155/2013/979547

Shi, F., Wang, Y., Davaritouchaee, M., Yao, Y. & Kang, K. (2020). Directional structure modification of poplar biomass- inspired high efficacy of enzymatic hydrolysis by sequential dilute acid-alkali treatment. *ACS Omega*, 5: 24780-24789. DOI: 10.1021/acsomega.0c03419

Singh, A., Bajar, S., Devi, A. & Bishnoi, N.R. (2021). Evaluation of cellulase production of *Aspergillus niger* and *Aspergillus heteromorphus* under submerged and solid-state fermentation. *Environmental Sustainability*, 4: 437-442. DOI: 10.1007/s42398-021-00173-x

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D. & Templeton, D.W. (2010). Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. *Journal of Agricultural Food Chemistry*, 58: 9043-9053. DOI: 10.1021/jf1008023

Tumuluru, J.S., Sokhansanj, S., Wright, C.T. & Boardman, R.D. (2011). A review on biomass torrefaction process and product properties for energy applications. *Industrial Biotechnology*, 7(5): 384-401. DOI: 10.1089/ind.2011.0014

Usino, D.O., Sar, T., Ylitervo, P. & Richards, T. (2023). Effect of acid pretreatment on the primary products of biomass fast pyrolysis. *Energies*, 16: 2377. DOI: doi.org/10.3390/en16052377

Vélez-Mercado, M.I., Talavera-Caro, A.G., Escobedo-Uribe, K.M., Sánchez-Muñoz, S., Luévanos-Escareño, M.P., Hernández-Terán, F., Alvarado, A. & Balagurusamy, N. (2021). Bioconversion of lignocellulosic biomass into value added products under anaerobic conditions: Insight into proteomic studies. *International Journal of Molecular Sciences*, 22(12249): 1-26. DOI: 10.3390/ijms222212249

Widihastuty, Y.R., Sutini & Nur Ramadhani, A. (2021). Optimization of fermentable sugar production from pineapple leaf waste (*Ananas comosus [L.] Merr*) by enzymatic hydrolysis. *Jurnal Presipitasi*, 18(1): 73-80. DOI: 10.14710/presipitasi.v18i1.73-80

Yoon, L.W., Ang, T.N., Ngoh, G.C. & Adeline, S.M.C. (2014). Fungal solid-state fermentation and various methods of enhancement in cellulase production. *Biomass and Bioenergy*, 67: 319-338. DOI: 10.1016/j.biombioe.2014.05.013

Yuliasmi, S., Nerdy & Husnita, A. (2017). Characterization of microcrystalline from pineapple leaf (*Ananas comosus* L. Merr). *IOP Conference Series: Materials Science and Engineering*, 180: 1-7. DOI: 10.1088/1757-899X/180/1/012267

Zawawi, D., Mohd Zainuri, M.H., Angzzas S.M.K., Halizah, A. & Ashuvila, M.A. (2014). Exploring of agro waste (pineapple leaf, corn stalk, and napier grass) by chemical composition and morphological study. *BioResources*, 9(1): 872-880. DOI: 10.15376/biores.9.1.872-880

Zhao, C., Deng, L. & Fang, H. (2018). Mixed culture of recombinant *Trichoderma reesei* and *Aspergillus niger* for cellulase production to increase the cellulose degrading capability. *Biomass and Bioenergy*, 112(2018): 93-98. DOI: 10.1016/j.biombioe.2018.03.001