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Effects of Different Pre-Treatments on Local Pineapple Leaf as a Potential Substrate for the Production of Value-Added Products

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Lignocellulosic biomass such as pineapple leaves can be applied as substrates in various biochemical processes such as solid-state fermentation to produce industrially important enzymes or other value-added products. However, recalcitrant nature of the biomass hinders the accessibility of cellulose for enzymatic hydrolysis in yielding high amounts of the intended products. In order to overcome this obstacle, pre-treatment is necessary as to loosen the lignocellulose structure to ease accessibility of enzymes and biodegradability of the biomass. In this study, local pineapple leaves were subjected to different methods of pre-treatments which are thermal pre-treatment by autoclaving, and chemical pre-treatments involving treatments with alkaline solutions (1% and 1.5% (w/v) sodium hydroxide) as well as acid solutions (1% and 1.5% (v/v) hydrochloric acid). Scanning Electron Microscopy was conducted to compare and study the effect of different pre-treatments on morphological changes of raw and pre-treated leaves. The obtained results revealed that the biomass was severely affected by 1.5% (v/v) hydrochloric acid solution as the surface structure was vigorously ruptured and more aggregated cracks were clearly visible in most parts, compared to the surface morphologies observed on raw samples and samples with other pre-treatment methods.

Keywords: pineapple leaves; pre-treatments; Scanning Electron Microscopy; hydrochloric acid solution; value-added products

I. INTRODUCTION

Pineapple (*Ananas comosus*) is a perennial plant and an edible fruit which belongs to the Bromeliaceae family (Upadhyay *et al.*, 2013). As it is considered as one of the famous tropical fruits in Malaysia, a total of 434,811 metric tonnes of pineapples were produced in year 2020 alone, covering around 13,433 hectares of the farmlands nationwide (Nor Mazila, 2020). Pineapple industry in the country has been growing from time to time, which benefits the livelihoods of both large-scale and smallholder pineapple producers as well as contributing to nation's income, with Johor and Sarawak being the top two pineapple producers (Noorlidawati, 2016). As the second-largest pineapple producer in Malaysia, Sarawak has a significant potential in pineapple farming as there are several cultivars that have been currently commercialised, which are known as Nanas Sarawak, Maspine, Yankee, Gandul, Moris Gajah, Josapine, N36, MD2, and Moris pineapple (Matius *et al.*, 2022). However, the agricultural practice of pineapple generates huge amounts of by-products such as pineapple leaves, pulps, stems and skins. In addition, in the pineapple processing industry, there is only 52% of the fruit utilised for the production of pineapple, while waste materials consist of fruit peels and leaves formed the remaining 48% (Rabiu *et al.*, 2018). These wastes, especially, pineapple leaves are usually left to decay, disposed on the ground or landfills, and even worse, burnt in an open field (Zainuddin *et al.*, 2014). In fact, improper disposal and management of agricultural wastes give negative impact on the environment and constitute an

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irritant to environmental sanitation (Awogbemi & Kallon, 2022). This poor waste management occurs in the field during harvesting phase due to limited technology and ignorance from farmers and local communities about the existence of commercial uses and sustainable utilisation of pineapple leaves as lignocellulosic biomass (Matius *et al.*, 2022).

Generally, lignocellulosic biomass mainly consists of up to 50% of cellulose, 25% to 30% of hemicellulose, 15% to 20% of lignin, and the rests made up to some amounts of pectin, nitrogen compounds and several inorganic compounds (Anu *et al.*, 2020). Pineapple leaves, being a major part of wastes generated from pineapple-related industry, are a promising feedstock for biodegradable material due to the presence of its fibre, and these wastes can be used as substrates in many biochemical processes such as microbial fermentation and anaerobic digestion (Aili *et al.*, 2021). A number of previous studies have been shown to utilise pineapple leaves as lignocellulosic substrates for the production of value-added products such as enzymes and biofuels or second-generation ethanol through fermentation processes (Widihastuty *et al.*, 2021; Saini *et al.*, 2022). A study by Chable-Villacis *et al.* (2021) utilised pineapple leaves as the substrate in producing laccases through solid state fermentation by using *Trameter hirsuta* strains as well as evaluating its enzymatic hydrolysis. Pineapple leaves 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 producers of cellulases (Saravanan *et al.*, 2012; Zhao *et al.*, 2018). In addition, filamentous fungi has the ability to synthesise and excrete a variety of hydrolytic enzymes such as xylanases, cellulases, amylases and pectinases that are used for breaking down polymeric carbohydrates in lignocellulosic biomass to soluble and simple sugars for direct bioconversion of biomass to valuable chemicals and bio-products (Mondala, 2014; Cho *et al.*, 2019). Hence, in order to release high enzyme activity, the nutrients present in the lignocellulosic biomass need to be easily accessible to the microorganisms for better mycelial penetration, thus, the enzymatic digestibility of the lignocellulosic substrate can be enhanced (Lee *et al.*, 2020).

However, due to their compositional make-up and complex physicochemical structure, some lignocellulosic biomasses are not easily degraded. Cellulose, hemicellulose and lignin are usually connected tightly in a macromolecular structure, thus causing a reduction in the pore size of the molecules (Awoyale & Lokhat, 2021). In addition, hemicellulose and lignin parts of the biomass are linked to the enzyme and act as an inhibitor that resists the digestibility of the biomass to its component sugars, thereby, hindering the enzyme accessibility to the cellulose (Awoyale & Lokhat, 2021). The crystalline structure and recalcitrant nature of the biomass makes it resistant to enzymatic hydrolysis due to low accessibility of cellulase to the cellulose (Mund *et al.* 2021). Therefore, pre-treatment is a necessary and significant step in order to overcome this obstacle, especially, in breaking down their complex structure to enhance more accessibility to the cellulose to be transformed into valuable products (Anu *et al.*, 2020). Numbers of research have been conducted for better understanding on the effects of different pre-treatments on lignocellulosic biomass in enhancing yields of fermentable sugars. The effective of lignocellulosic biomass modification to useful fermentable sugars depends on the residues and type of pre-treatment that have been selected (Ariffin *et al.*, 2020). Thermal pre-treatment is the simplest and cheapest method when low heat temperature heat is applied (Dumlu *et al.*, 2021). However, one of the impediments of this process is the possible circumstance of Maillard reaction when thermal pre-treatment is employed at a high temperature or at a low temperature with a long reaction time. Other pre-treatments, such as chemical pre-treatments by using acid or alkaline solutions are also widely studied. Chemical pre-treatment is a treatment method that entails chemical hydrolysis, which solubilises hemicellulose and lignin, consequently making the cellulose more open for enzyme action during the process of fermentation (Awoyale & Lokhat, 2021). Dilute or concentrated acids can be used in the treatment, but dilute acid is more favourable as it is less corrosive and forms lower numbers of inhibitors compared to concentrated acid. Chemical pre-treatment using alkaline solutions, on the other hand, involves the use of bases such as sodium and potassium, which mainly targets in the structural alteration and removal of lignin (Brodeur *et al.*, 2011; Wunna *et al.*, 2017).

The assessment of morphological properties of various lignocellulosic biomasses by Scanning Electron Microscopy (SEM) has been widely employed in numerous previous studies. However, SEM study of pineapple leaves for morphological structural changes caused by prior treatments are still limited. Thus, this present study was conducted as to evaluate the effect of different pre-treatments on the structural changes of untreated and pre-treated local pineapple leaves using SEM. Pineapple leaves throughout the study were locally collected from Sebangan, Simunjan, Sarawak, Malaysia and subjected to few pre-treatment methods, which include thermal treatment by autoclaving, alkaline treatment with sodium hydroxide solution as well as acid treatment with dilute hydrochloric acid solution. Subsequently, SEM was performed on the raw and pre-treated leaves to observe and analyse changes in the surface morphological structure of the biomass. Each pre-treatment method employed in this research is studied for its efficiency and effectiveness in altering and breaking down of the complex structure of the pineapple leaf biomass. Significantly, this study may shed light as reference for future studies involving the utilisation of pineapple leaf wastes as substrates for biochemical processes in generating valuable chemicals and bio-products.

II. MATERIALS AND METHOD

A. Collection and Preparation of Local Pineapple Leaves

Pineapple leaves 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 electrical blender, and sieved into particle size in between 0.5 mm to 1.0 mm.

B. Pre-Treatments of Pineapple Leaves

In this study, three methods of pre-treatments were employed in treating the pineapple leaves to study and compare the effect of different pre-treatments, mainly on the morphology of lignocellulosic biomass structure. The pre-treatments involved were thermal pre-treatment, alkaline pre-treatments and acid pre-treatments.

1. Thermal treatments

Thermal treatment method applied was adopted from Corbin *et al.* (2015). Pineapple leaves were pre-treated thermally by autoclaving the lignocellulosic substrate added with distilled water, at 121 °C for 30 minutes. The 1:10 ratio of solid to liquid was applied by which liquid is added prior to the treatment to prevent burning of the substrate throughout the process. After autoclaving, the mixture was allowed to cool down to room temperature, followed by filtration, and the filtrated substrate was dried in a drying oven at 60 °C. The thermally-treated substrate was kept in an airtight container until further use.

2. Alkaline treatment

A method from Bansal *et al.* (2012) was adopted and modified, by which sodium hydroxide (NaOH) solution of two different concentrations were used to treat the local pineapple leaf. Two separate Erlenmeyer flasks containing 200 ml of 1% (w/v) and 1.5% (w/v) NaOH solutions, respectively, were added with 50 g of pineapple leaves in each flask. The mixture was then incubated at room temperature, and static condition, for 2 hours. After 2 hours, the mixture was thoroughly rinsed with distilled water to remove any traces of the base, followed by drying in an oven at 60 °C. Then, the dried leaves were kept in an airtight container prior to use.

3. Acid Treatment

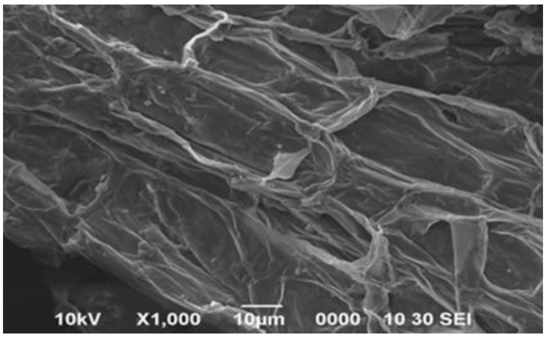
A method by Bansal *et al.* (2012), with a slight modification was applied. Two separate 500 ml Erlenmeyer flasks were prepared. An amount of 50 g of ground pineapple leaves was dispensed into each 500 ml Erlenmeyer flasks which contained 200 ml of hydrochloric acid (HCl) solution of 1% (v/v) and 1.5% (v/v) separately. After 2 hours of incubation at room temperature in a static condition, each mixture was rinsed a few times with distilled water to discard any acid residues, followed by drying in an oven at 60 °C. The dried leaves were then kept in an airtight container until further use.

C. Scanning Electron Microscopy (SEM)

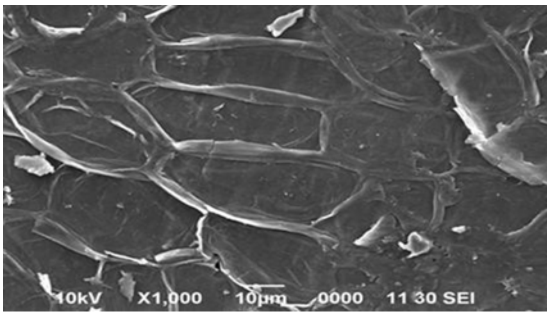
Scanning Electron Microscopy (SEM) is an analytical procedure that scans a sample with an electron beam to create magnified image for assessment (Awoyale & Lokhat, 2021). Thus, in this research study, SEM is mainly applied for the comparison analysis on the morphological changes occurred between raw (prepared mechanically through cutting and grinding) and pre-treated samples. Raw and pre-treated pineapple leaves were prepared beforehand based on the standard procedure and each sample was observed under SEM (JEOL (MALAYSIA) SDN. BHD, JSM-6390LA, MP 1440035). Prior to SEM observation, the samples were coated with gold for 100 seconds by using a coater machine (JEOL, Model JEC-3000FC).

III. RESULT AND DISCUSSION

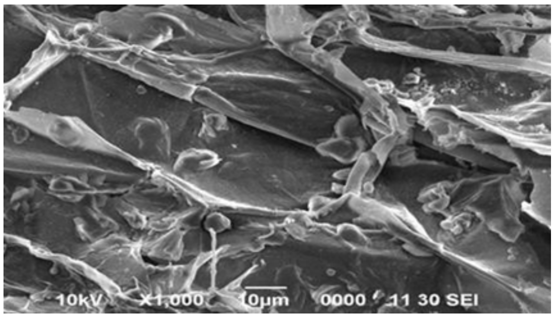
SEM images for morphological changes on the surface of local pineapple leaf wastes before and after pre-treatment are presented in Figure 1 and the images were observed and captured under magnification of X1000.



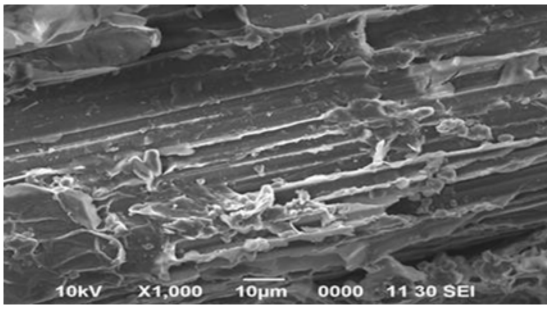
(a)



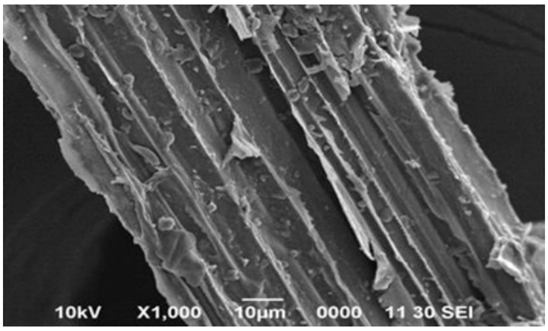
(b)



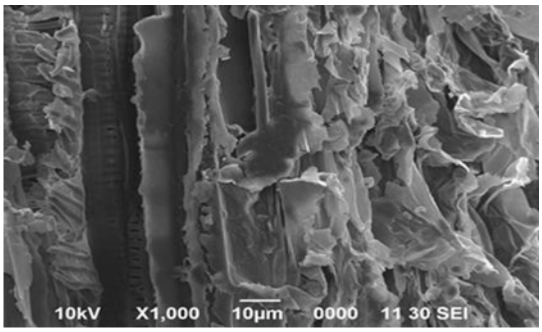
(c)



(d)



(e)



(f)

Figure 1. SEM images of morphology of lignocellulosic surface of local pineapple leaves (PL) (X1,000 magnification): (a) raw PL (prepared mechanically through cutting and grinding), (b) thermal-treated PL, (c) PL treated with 1% (w/v) NaOH, (d) PL treated with 1.5% (w/v) NaOH, (e) PL treated with 1% (v/v) HCl, and (f) PL treated with 1.5% (v/v) HCl

Based on the results, Figure 1(a), which represents raw pineapple leaves clearly revealed rough and intact structure with rigid shape which has not been damaged through crushing and grinding of the biomass during the sample preparation. Thermal-treated pineapple leaves (Figure 1(b)) showed less significant changes on its surface structure compared to raw sample as the rigid shape of the surface structure remains visible in most part, despite having slightly loosened pores. However, large differences on the morphological changes were observed when the pineapple leaves were chemically treated with different concentrations of alkaline and acid solutions. The pineapple leaf wastes which were subjected to alkaline treatment with 1.5% (w/v) NaOH solution (Figure 1(d)) displayed more of loosened and irregular structure, compared to when it was treated with 1% (w/v) of NaOH solution (Figure 1(c)). Figure 1c has less significant different in its morphological change compared to that of raw pineapple leaf (Figure 1(a)) as the regular shape of its surface structure remains clear, indicating that lower concentration of sodium hydroxide solution is inefficient in breaking down the complex surface structure of the biomass used. On the other hand, more vigorous morphological alteration can be seen when the pineapple leaf samples were subjected to diluted hydrochloric acid treatments of 1% (v/v) HCl solution (Figure 1(e)) and 1.5% (v/v) HCl solution (Figure 1(f)). Both SEM images of samples treated with HCl solutions showed relatively unorganised and ruptured surface structure. However, the biomass was observed to be more severely affected by 1.5% (v/v) HCl solution (Figure 1(f)) as aggregated cracks were clearly visible compared to the samples treated with 1% (v/v) HCl solution (Figure 1(e)) as well as other pre-treatments. This indicates that 1.5% (v/v) HCl solution is the most efficient method for the pre-treatment of pineapple leaves based on the severity of surface morphological structure alteration displayed.

Previous studies have also shown similar results with other substrates such as research finding by Chen *et al.* (2019), which suggested that there was a great change on the morphological structure of sweet sorghum bagasse (SSB) when treated with diluted HCl solution by which SEM image of HCl-treated SSB revealed disorderly and unsystematic components on the cell walls surface compared to untreated SSB. Another study by Bharathiraja *et al.* (2014) which analysed different pre-treatment techniques on various types of agro wastes via SEM indicated that dilute acid was sufficient for removal of the inhibitory hydrocarbons and development of cracks on the lignocellulosic fibre, thus, increasing the porosity of biomass. The property of lignocellulosic biomass, especially its chemical compositions, is one of the key conditions to be taken into consideration in choosing most effective pre-treatment method (Refaat, 2012). Acid pre-treatment is more suitable for biomass with low lignin content as its main reaction involves hydrolysis of hemicellulose and solubilisation of small fractions of lignin, while alkaline pre-treatment interacts primarily with lignin for lignin removal, thus it is better suited for biomass with high lignin content (Kim *et al.*, 2016; Oriez *et al.*, 2019). In research finding by Imman *et al.* (2021) for chemical compositions analysis of pineapple leaves obtained from Phare Province, Thailand, it is revealed that based on the dry basis weight, the leaves are made up of 62.37% cellulose, 22.38% and 5.45% lignin. The low percentage of lignin in the pineapple leaf biomass is also supported by other several previous findings, which suggested that local pineapple leaves have high cellulose and hemicellulose content as well as low lignin content of between 4 to 22% (Zawawi *et al.*, 2014; Maisyarah *et al.*, 2019). The hydrolysis of hemicelluloses might highly occur as acid disrupts the hydrogen bonds that linked hemicelluloses and celluloses, as well breaks down 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 enhancing 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). As a result, from the breaking down and disruption of the complex structure of the leaves by acid pre-treatment, the chemical reactions occurred during the process caused rougher and aggregated cracks on the surface structure as illustrated in Figure 1(e) and Figure 1(f) were observed. The loosened and rougher surface structure of the substrate may enhance its specific area for better susceptibility to enzymatic hydrolysis in certain bioconversion processes (Zhou *et al.*, 2019). In contrast with the alkaline pre-treated pineapple leaves (Figure 1(c) and Figure 1(d)), NaOH solution was not effective enough for significant and vigorous morphological structural changes to take place due to the nature of the leaves of having low lignin content which slows down the primary interaction between alkaline solution and lignin.

IV. CONCLUSION

Based on the morphological structural images observed under SEM, pre-treatment of local pineapple leaves with 1.5% (v/v) hydrochloric acid solution was shown to be the most effective method in altering and breaking down complex structure makeup of the leaves, compared to other pre-treatments (thermal, alkaline treatments and treatment with 1% (v/v) HCl solution) employed in this study. The changes on surface morphology was observed to be more obvious, vigorous and severe when interacted with higher concentration of dilute HCl solution, indicating severe disruption on covalent bonds that hold together the constituents of cellulose, hemicellulose and lignin. Thus, a pre-treatment process of lignocellulosic residue is significant to be conducted in order to enhance its digestibility and cellulose accessibility for enzymatic hydrolysis, leading to higher yields of value-added products. Besides that, the result of this finding may contribute in reducing problems or issues arisen from inappropriate disposal and management of agro waste by utilising pineapple wastes as substrates in biochemical and bioconversion processes.

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