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Assessment of Microwave-Assisted Pretreatments for Enhancing Pineapple Waste Delignification

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Abstract. Biological degradation of biomass for the production of fine chemicals is getting much interest nowadays. However, the complex and recalcitrance structure of the biomass hinders the success story of the degradation. Lignin is the main composition that impedes the bioconversion of biomass. Hence, an optimize delignification pretreatment need to be developed to enable the biological degradation process becomes much easier and thus higher production yield can be achieved afterwards. This study focuses on the assessment of pineapple waste (PW) delignification by applying microwave radiation on two different pretreated PW to facilitate the processes. The PW was initially pretreated with distilled water (dH_20) and peracetic acid (PAA) prior to the microwave radiation. Three main parameters (pretreatment time, min; temperature, °C; and microwave radiation power, W) were studied towards the effect of PW delignification. Lignin percentage before and after the pretreatments were compared and analysed. The results obtained revealed that the microwave-assisted PAA shows the best percentage of delignification compared to the microwave-assisted dH_20 pretreatment. The best delignification process obtained in this study is a key indicator for a better biomass degradation to achieve higher yield of products in the future.

INTRODUCTION

In recent years, the dependant uses of non-renewable energy resources, fossil fuels, such as crude oil, natural gas, lignite and coal have continuously polluted the environment due to their polluting nature. Moreover, these non-renewable energy resources are expected to decline after a certain amount of time in specific regions. The industrial society has taken its action to introduce a new renewable energy resources to prolong its economic and welfare growth. Bio-energy is currently the major contributor of renewable energy resources representing 10.3% of global primary energy requirement in 2014. The production and distribution of bio-energy from biomass or renewable waste materials could serve as a possible solution to the ongoing demands of energy.

Ananas comosus, or pineapple is a one of the most popular tropical and economic fruits in Malaysia with encouraging global market potential [1]. It is categorized in a group of major fruit because it has a great potential to generate incomes for farmers, as well as countries. Its popularity is due to its multi-forms in consumption. The global pineapple producers were led by Costa Rica, Brazil, Philippines, Thailand and Indonesia producing roughly 10 million tons of pineapple. Malaysia was ranked at 19th pineapple producing country in the world by the year 2013 [2]. Meanwhile the state of Johor is known to be the largest pineapple producer in Malaysia. Hence, due to the mass production of the fruit, it comes together with the waste. The pineapple waste from the pineapple company consist of the stem, crown, leaves, peel and core. Table 1 shows the type of pineapple varieties cultivated in Malaysia. Previous studies have shown that these pineapple waste can produce other beneficial products such as enzyme [3], biohydrogen [4] and sugars [5].

Microwave pretreatment is one of the alternatives for the conventional high cost with harsh and toxic chemicals. It has high energy-efficiency, fast heating and friendly operation. Microwaves changes the

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ultrastructure of biomass by the microwave irradiation. Additionally, microwave pretreatment can be easily combined with other chemical pretreatments to enhance the reaction efficiency [6]. In this case, the focal point of this research is to convert the abundantly available, renewable and non-edible lignocellulosic biomass, such as agricultural and food industry waste, into usable chemicals, fuels and energy. However, the main hindrance of this conversion is the incomplete hydrolysis of hemicellulose and cellulose molecules which are protected inside the complex structure of lignocelluloses (protected by lignin) and unavailable for fermentation into biofuels [7]. The use of efficient pre-treatment methods can improve the efficiency of the whole process by delignification (lignin removal) and thus increasing the yield of targeted product [8]. The goal of pre-treatment is to break down the lignin protective barrier, open-up the complex cellulose-hemicellulose structure, releasing free-cellulose and hemicellulose molecules followed by the breakdown of their compact crystalline and amorphous arrangement (depolymerisation) so as to enhance the production yield. The aim of this study is to investigate microwaves as an alternative pretreatment method for pineapple industrial waste to improve the enzymatic hydrolysis yield by removing as much lignin as possible.

TABLE 1 Type of pineapple cultivated in Malaysia.

Type	Consumption
Mauritus	Eaten fresh
Sarawak	Eaten fresh
Gandol	Canned and turned into juice form
Maspine hybrid	Fulfilling both usage
N36	Fulfilling both usage
Josapine	Eaten fresh

MATERIALS AND METHOD

Sample Collection

The pineapple wastes from the N36 variety were freely taken from the Lee Pineapple Co. Pte. Ltd. factories in Skudai, Johor, located in the southern part of Malaysia. Only the waste parts such as their leaves, skins and other non-fruit parts were used in this experiment. The amount of fruit wastes used for the experiment were approximately less than 1kg.

Chemicals

Sulphuric acid reagent was purchased from *My Lab Supplier* located in Universiti Teknologi Malaysia. Peracetic acid, PAA was house-made from the combination of pure hydrogen peroxide and acetic acid glacial reagents which were purchased from Sigma–Aldrich, respectively. All reagents used are analytically graded.

Sample Preparation

The fruit wastes were carefully washed with tap water in order to remove undesired particles. Then, the samples were air dried for 2-5 days at an open and sunny area with active ventilation. Final moisture content was determined by drying the samples in an oven at 60°C (*Memmert, Germany*) for 24 h or until constant weight to reduce its moisture content below 10 %(w/v). The initial and final weight of samples were recorded. The difference in weight represents its moisture content. The air-dried samples were cut into smaller pieces by grinding using a commercial electric grinder *(Waring® Products Division, U.S.A.).* The powdered samples were sieved using a mechanical siever to obtain small and even particle sizes. The final samples were kept in a sealed container for further usage. Figure 1 details

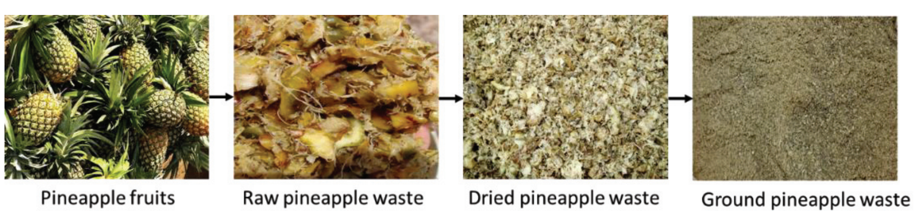

FIGURE 1 The preparation process of the mixed pineapple waste as the substrate

Preparation of peracetic acid (PAA)

PAA was prepared by the reaction of acetic acid glacial and 30% hydrogen peroxide, with a volume ratio of 1.5:1 at room temperature for 72 h. To hasten the reaction, 1.5% (w/w) of sulphuric acid was added as a catalyst [9].

Pretreatment

The pretreatment of the samples were conducted using a microwave (*Sineo*, *Shanghai*) at different parameters. Firstly, 500mg of raw dried sample was placed into a 100ml beaker. A total of 50ml distilled water were added into the beakers. The samples were then pre-treated in the microwave using several parameters. After pretreatment, the samples were filtered off to collect the pre-treated substrate. Any residues were washed with hot water. The samples were dried in an oven at 60°C overnight or until constant weight. After drying, the dried samples were moved to a desiccator and let them sit for an hour before weighing.

Lignin Determination

A 100 mg of pre-treated dried sample was placed into a 100ml Scott bottle. Next, 1ml of 72% (w/w) H_2SO_4 was added and stirred. The bottles were incubated in a water bath at 30°C for 60min to enable the total delignification process to occur. Then, 56ml of distilled water were added. The bottles were autoclaved at 121°C, 15psi for 60min. The lignin was then filtered off. Any residues were washed with hot water. The samples were dried in an oven at 105°C overnight. After drying, the dried samples were moved to a desiccator and let them sit for an hour before weighing. The percentages of lignin removal were calculated using the final weight of samples. The acid insoluble lignin content (Klason Lignin) was determined by a modified version of the method described in TAPPI T222, for acid-insoluble lignin in wood and pulp [10] by two-stage sulphuric acid hydrolysis. The percentage lignin content of the pineapple waste was determined before and after each pretreatment process. All analyses were carried out on a dry weight basis [11].

Microstructure Analysis

The electron micrograph of the raw and pretreated and unpretreated kenaf samples was taken by a scanning electron microscope (SEM). All the samples were initially pre-coated with a thin layer of nanosized gold powder to enhance the contrast of the images.

RESULTS AND DISCUSSION

Table 2 shows the chemical compositional analysis of raw pineapple waste. The amount of original lignin exist in the raw pineapple waste was approximately 12.37 % per dry weight basis which is indicated by the Klason Lignin (acid insoluble lignin). This significant amount creates a recalcitrance barrier to the biomass and hinders further degradation towards the biomass. Hence, this 12% of lignin need to be greatly removed to ease further processes.

TABLE 2 Chemical compositional analysis of the raw pineapple waste

Chemical Composition	Percentage (% dry weight basis)
Holocellulose	63.53
α-cellulose	34.60
Hemicellulose	28.94
Klason Lignin (Acid insoluble lignin)	12.37

Effect of Pretreatment Time

Time of pretreatment is one of the important variables affecting the efficiency of lignin removal from biomass. Different biomass may require different pretreatment time for the maximum lignin removal depending on the chemical composition and the biomass structure, respectively. In this study, the pineapple waste was pretreated with two different methods which are microwave-assisted dH_20 and microwave-assisted PAA. The pattern between the two graphs seem similar whereby up to a certain time, highest lignin removal can be achieved. Further extend in the pretreatment time resulted in less lignin removal. From Figure 2 below, the microwave-assisted PAA pretreatment shows shorter time is required to remove greater lignin composition compared to the microwave-assisted dH20. This may be due to the effect of PAA which is also known as a strong oxidizing agent. PAA oxidized the lignin chemical structure and eventually releasing it from the biomass structure. The highest lignin removal was achieved at 10 min with the microwave-assisted PAA pretreatment. The optimum time taken is similar to what had been achieved by Satimanont *et al.* (2012) where the same 10 min was required to achieve the highest xylose yield at temperature of 140 °C for corn cobs [12]. However, the time may vary depending on the type of substrate used and the type of pretreatment method chosen. Using acid or alkali in the pretreatment may help to reduce the time taken compared to when only dH$_2$0 is used. This is explained by the different charge in the acid and alkali solution which helps in interfering with the chemical bond inside the lignin structure [12, 13].

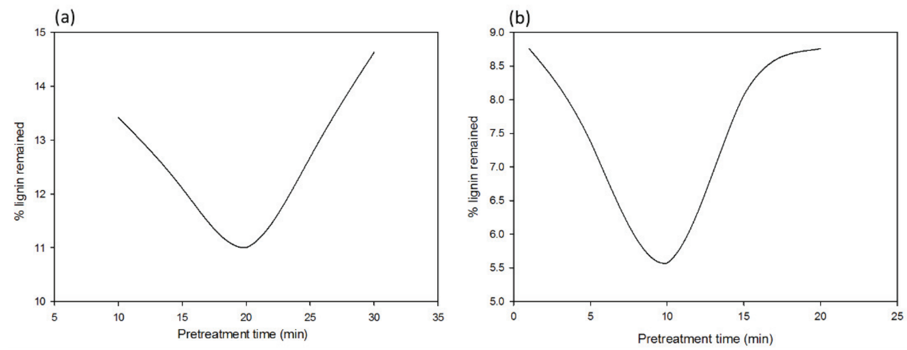

FIGURE 2 Screening of pretreatment time for (a) microwave-assisted dH20 pretreatment and (b) microwave-assisted PAA pretreatment

Effect of Pretreatment Temperature

Next is the screening of pretreatment temperature. Different pretreatment temperatures were applied onto the pineapple waste ranging from room temperature (25 °C) up to 70 °C. The microwave-assisted dH$_2$0 pretreatment required higher temperature (50 °C) to achieve maximum lignin removal of only 32 % compared to the pretreatment with the PAA. Higher temperature exerted using the microwave-assisted dH$_2$O reduced the efficiency of the lignin removal. Meanwhile, a different pattern had been seen by the microwave-assisted PAA pretreatment. The lower the pretreatment temperature gave a better result than higher temperature. From the study, room temperature (25 °C) was the best temperature for lignin removal which resulted in 61% delignification. According to report by Zhao *et al* (2007), PAA worked effectively under mild condition which was less than 70 °C. A temperature of 70 °C was required to achieve the best condition for the reaction of sugarcane bagasse using PAA pretreatment alone [9]. Higher temperature was needed when the PAA was replaced with either H_2SO_4 or NaOH. In the study, it was assumed that some interaction might occur between PAA and the microwave irradiation which causes bad lignin removal at higher temperature (Figure 3b). However, in-depth information on this phenomenon was still scarce and need further study.

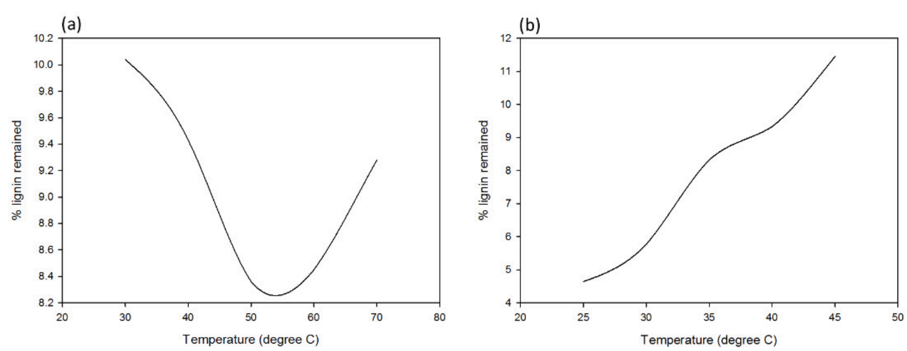

FIGURE 3 Screening of pretreatment temperature for (a) microwave-assisted dH2O pretreatment and (b) microwave-assisted PAA pretreatment

Effect of Pretreatment Microwave Power

Another parameter been studied was the effect of microwave irradiation power towards delignification of the PW. The microwave power was set in the range of 450 to 850 W for the microwave-assisted dH_20 pretreatment and 250 to 650 W for the microwave-assisted PAA pretreatment. Similar study was conducted by Akhtar *et al*. (2015) but was performed on oil palm empty fruit bunch as the biomass [13]. The optimum power achieved in the previous study by Akhtar *et al.* was 900 W. Interestingly in this study, lower microwave powers were needed for both pretreatment methods. Maximum delignification was reached at 750 W for the microwave-assisted dH_2O pretreatment while much lower power, 550 W was needed for the microwave-assisted PAA pretreatment. The addition of acid in the microwave-assisted pretreatment had manage to reduce the power needed with greater lignin removal.

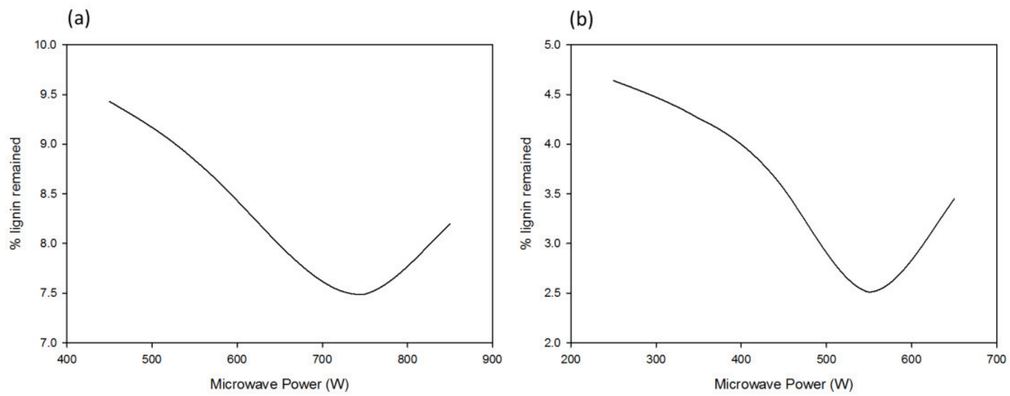

FIGURE 4 Screening of microwave power for (a) microwave-assisted dH20 pretreatment and (b) microwave-assisted PAA pretreatment

From the results obtained by the screening of three types of parameter above, Table 3 shows the difference in lignin percentage which remained in the biomass after the pretreatment. Microwave-assisted PAA pretreatment shows a tremendous removal of lignin composition, leaving with only 2.51% of lignin in the biomass. Approximately of 80% of the lignin inside the pineapple waste were successfully been removed.

Table 3 Percentage of lignin content in three different biomass conditions

Biomass (Pineapple waste)	% lignin
Raw biomass	12.37
Microwave-assisted dH20 pretreated biomass	7.49
Microwave-assisted PAA pretreated biomass	2.51

Microscopic Structure Analysis

Figure 2 shows the microscopic image of different types of pineapple waste pretreatments. Figure 2(a) shows the packed and almost-smooth surface of the raw pineapple waste. The surface was seen to be tightly covered with lignin layer with no existence of pores. The biomass structure was seen to be in hard and crystalline form. Figure 2(b) shows the partially opened-up structure of the pretreated pineapple waste using microwave-assisted distilled water pretreatment. The lignin outer layer was seen to be partially ruptured and many pores can be observed. The biomass structure was seen to be in partially amorphous form. Figure 2(c) shows the widely opened-up structure with many inner-layer granules could be observed. Most of the lignin layer had successfully been removed by the pretreatment leaving with an amorphous structure. Hence, it could be concluded that different pretreatments applied on the biomass will greatly affect its microscopic structure and thus would influence further hydrolysis.

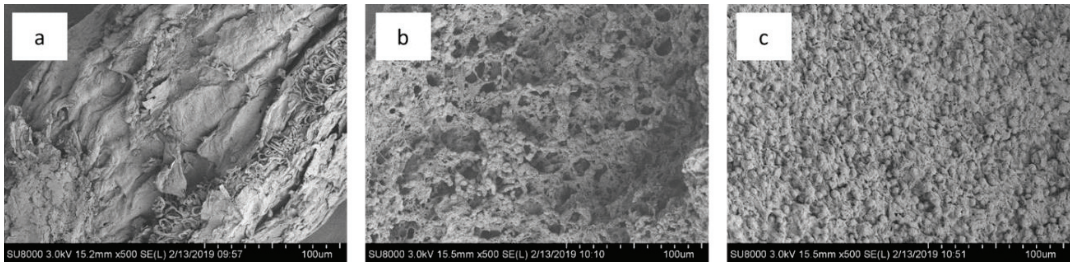

FIGURE 5 SEM images of the (a) raw pineapple waste, (b) microwave-assisted distilled water pretreatment of pineapple waste and (c) microwave-assisted PAA pretreatment of pineapple waste. The images were taken at 500x magnification.

CONCLUSIONS

The best type of pretreatment for the delignification of pineapple waste using microwave-assisted technology was the microwave-assisted PAA pretreatment. The results obtained show that the minimum percentage of lignin remained after the microwave-assisted PAA pretreatment was only 2.51 % (w/w) compared to the microwave-assisted dH_20 pretreatment which was 7.49 %(w/w). The optimum conditions for the microwave-assisted PAA pretreatment were t=10 min, T= 25 °C and P= 550 W which were shorter in time, less temperature and less microwave power setting needed. This achievement was an initial passport for a better biomass degradation to achieve higher production yield in future studies.

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