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Production of Bioethanol from Bamboo Powder Via Dilute Sulphuric Acid-Sodium Hydroxide Pre-Treatment and Simultaneous Saccharification and Fermentation (SSF)

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Abstract

Biofuel is a new type of fossil fuel that is usually derived from biomass waste. Studies found that lignocellulosic biomass (LCB) has the potential to become a new source for biofuel production. Even so, it is a challenging process to fully utilise the potential of cellulose in LCB due to the chemical structures of the lignocellulose biomass. Pre-treatment of LCB is one of the most crucial steps in converting LCB into bioethanol products. In this study, the potential of production for bioethanol from Bambusa Wray bamboo species via dilute sulphuric acid (H2SO4) and sodium hydroxide (NaOH) pre-treatment and simultaneous saccharification and fermentation (SSF) was explored. The effect of H2SO4 and NaOH pre-treatment on the chemical structure of Bambusa Wray was studied using Fourier-transform Infrared (FTIR) analysis. The impact of different concentrations of H2SO4 during pre-treatment towards the concentration of glucose produced was investigated using the dinitrosalicylic acid (DNS) method. The availability and the amount of bioethanol produced during the SSF were analysed using FTIR. It was found that, a noticeable decrease in the intensities of absorption peaks can be observed at 1604, 1462 and 1242 cm-1 after being treated with sequential acid-alkaline pre-treatment. It indicates the removal of lignin from the sample. It was reported that 4% H2SO4 + 5% NaOH produces the highest glucose concentration, 6.491 mg/ml, compared to untreated bamboo, which only produces 1.158 mg/ml. At the end of the study, bioethanol was absent in the sample due to the availability of yeast. Overall, it was proven that Bambusa Wray is capable of producing enough glucose for production of bioethanol.

1. Introduction

Biofuel is a new type of fossil fuel that is usually derived from biomass waste and has the potential to replace commercial fossil fuels. Unlike fossil fuels, biofuel is categorized as renewable energy because the feedstock material for biodiesel can be easily replenished. Other than that, biofuels also have the potential to become a new replacement for conventional fuels in transportation engines because it produces fewer emissions to the environment. It is found that most biofuels have the same characteristics and properties as conventional fuel [1]. Studies found that lignocellulosic biomass (LCB) has the potential to meet global demand and become a new source for biofuel production. LCB is one of the most abundant bio-renewable materials and the cheapest feedstock for the production of biofuel since it can be easily obtained from agricultural residues, forest products

and municipal solid waste [2]. LCB has a complex spatial structure and high composition of cellulose and hemicellulose which is suitable for the production of bioethanol [3]. There are various stages for producing bioethanol from LCB which are LCB pre-treatment, hydrolysis, fermentation and purification of the product. Pre-treatment of LCB is one of the most crucial steps in converting LCB into bioethanol products. This stage is responsible for preparing LCB for enzymatic hydrolysis and fermentation stages to maximize the production of bioethanol products. The sample was then undergoing simultaneous saccharification and fermentation (SSF). SSF is a process where enzymatic hydrolysis and fermentation is done at the same time in one reactor to achieve value-added products, which is ethanol [4]. The products are purified using a rotary evaporator to extract ethanol from the fermentation media. In this study, Bamboo from Bambusa Wray species has been used as the source for LCB. Bamboo is chosen due to its short maturity period, which is ready to harvest in 3-5 years, and has high cellulose content. The sequential dilute sulphuric acid-sodium hydroxide pretreatment was chosen for this study. In the first stage, the treated bamboo powder will be treated with a dilute sulphuric acid solution at different concentrations to solubilize hemicellulose. Then, the bamboo powder will be treated with sodium hydroxide solution to remove the lignin in the second stage. After that, the solid phase that obtains from the second stage will undergo enzymatic hydrolysis and fermentation stages for the production of bioethanol.

Thus, the objective of this study is to:

- To observe the effect of dilute sulphuric acid and sodium hydroxide pre-treatment on the chemical structure of Bambusa Wray.
- To investigate the impact of different concentrations of sulphuric acid during the pre-treatment process of Bambusa Wray towards the concentration of glucose produced throughout the simultaneous saccharification and fermentation process.
- To study the effects of different concentrations of sulphuric acid on the availability and amount of bioethanol produced during the simultaneous saccharification and fermentation process.

2. Methodology

The chronology of the experiment and the method use was further discussed in this chapter. The characterisation of the sample was also mentioned.

2.1 Preparation of Bamboo Powder from Bamboo Culm

Bambusa Wray culm was collected from Batu 7, Jalan Pahang, with an average age of 3 years. The bamboo culm was first converted into bamboo powder to increase the total surface area and increase the efficiency of the pre-treatment process. The bamboo culm was let be immersed in the tap water for 1 day so that the bamboo would absorb the water and become softer. Then, the wet bamboo culm was processed into bamboo fibrils by loading it into a fibre extraction machine. The bamboo fibre was then cut approximately 5 cm in length. Afterwards, the bamboo fibrils size was reduced to particle size using a planetary ball mill at 450 rpm for 10 minutes. The bamboo powder obtained was sieved with a 1 mm-sized siever.

2.2 Dilute Sulphuric Acid-Sodium Hydroxide Pre-Treatment of Bamboo

The bamboo pre-treatment was performed by using a double-stage pre-treatment which is sodium hydroxide and dilute sulphuric acid. In the first stage, the bamboo powder was treated with dilute sulphuric acid at 1%, 2%, 3% and 4% (v/v) concentrations. The ratio for the bamboo powder and dilute sulphuric acid would be 1:10 (w/v). The solution was processed in the autoclave for 90 min. The temperature of the autoclave reactor was set to 121°C. After that, the slurry solution was filtered and separated into two fractions which are solid and liquid. For the second stage, the bamboo powder was treated with a sodium hydroxide solution. The ratio for the bamboo powder and sodium hydroxide solution with a concentration of 5% (v/v) would be 1:10 (w/v). The slurry solution was then stirred at the rate of 120 rpm in the rotary air bath in the incubator at a temperature of 85°C for 1 hour. After that, the slurry solution was filtered and separated into two fractions which are solid and liquid fractions. The pre-treated solids were washed with distilled water 5 times until the pH value for the effluents was neutral. Next, the pre-treated solids were dehydrated in the oven at 85°C for 24 hours.

2.3 Preparation of 0.05M Citrate Buffer with pH 4.8.

In a beaker, 800 ml of distilled water was prepared as a first step to produce the citrate buffer solution. Next, 4.529 g of nitric acid and 7.771 g of sodium citrate dihydrate were added to the solution. 0.1 M HCl and 0.1 M NaOH were used to adjust the desired pH of 4.8. Distilled water was added up to 1 litre of volume. Preparation of 0.5% cellulase enzyme solution 0.5g of cellulase enzyme was weighed out by using an analytical balance. 80 mL of 0.05M citrate buffer at pH 4.8 was measured using a measuring cylinder and poured into a beaker containing cellulase

enzyme. The solution was stirred gently using a magnetic stirrer until the cellulase enzyme dissolved. Adjust the volume of the solution by using citrate buffer until it reaches 100 mL.

2.4 Preparation of Dinitrosalicylic Acid (DNS) Reagent Solution

1 gram of 3,5-dinitrosalicyclic acid was measured by using an analytical balance. 60 ml of distilled water was added to the beaker containing 3,5-dinitrosalicyclic acid. The mixture was stirred thoroughly with a magnetic stirrer until the powder was completely dissolved. Then, 1.6g of sodium hydroxide was added to the beaker. Gradually, 30 g of potassium sodium tartrate (Rochelle salt) was added for around 20 to 30 minutes. Next, the solution was heated at 45°C until the solution became clear. The solution was transferred to a 100 mL volumetric flask. Fill the solution until it reaches 100 mL with distilled water. Close the top of the flask to prevent the carbon dioxide from reacting with the solution.

2.5 Simultaneous Saccharification and Fermentation (SSF)

The media solution contains yeast extract of 5 g/L, ammonium sulphate of 7.5 g/L, dipotassium sulphate of 3.5 g/L, epsomite of 0.7 g/L and calcium chloride dihydrate of 1 g/L was prepared in the citrate buffer (pH 4.8). 15 g of pre-treated bamboo powder and prepared solution media were added into the Erlenmeyer with a ratio of 1:10 (w/v). The pH value of the mixed solution was kept at ± 5. This solution was then placed in the autoclave at a temperature of 121°C for 60 minutes. The bamboo pulp was left cold until the temperature dropped to 37°C. 1.5 ml of 0.5% cellulase enzyme and 2.5 g of dry yeast (Saccharomyces cerevisiae) were added to the Erlenmeyer. The fermentation process occurs at different residence times, which are 72 hours, 96 hours, 120 hours and 144 hours, by placing it in the incubator at a temperature of 37°C and a rate of 120 rpm. Ensure that all the glass tools are closed.

2.6 Bioethanol Extraction by Using a Rotary Evaporator

After analysing glucose concentration using the DNS method, all the samples from the simultaneous saccharification and fermentation process underwent extraction using a rotary evaporator (1000162171, Switzerland). The rotary evaporator equipment was set up with the correct adjustment. The ethanol solvent was selected at the solvent library with a bath temperature of 42 °C, boiling point was 20 °C and pressure was 58 mbar. Note that the ethanol produced was not pure.

2.7 Testing and Characterization

Fourier-transform infrared spectroscopy (FTIR) was used to study the change in the chemical structure of the pre-treated bamboo powder. Thermo Electron Corporation spectrometer (Nicolet 380 FT-IR) was used in this study. The pre-treated bamboo powder is scanned between the wavelength of 400 cm-1 and 4000 cm-1 [5]. FTIR is also used for analysing the availability of bioethanol and estimates the amount of bioethanol produced by comparing the spectrum peak produced by bioethanol with pure ethanol. The signal changes in integration for pure ethanol are used to generate a standard curve using ethanol concentration and calculate the initial ethanol concentration of each sample. The carbon-oxygen bond that appears at 1044 cm-1 -1045 cm-1 was chosen for the calibration curve due to its distinction from other peaks and relative distance from the ample oxygen- hydrogen peak at ~3300 cm-1 [6]. The result obtained is then analysed by using OMNIC software.

The dinitrosalicylic acid (DNS) method is used to estimate the concentration of reducing sugar in the sample. In this study, DNS assay was conducted in the test tube containing 1 ml of samples and 1 ml of DNS to study the colour changes. The mixture was incubated in the water bath at a temperature of 95°C for 5 minutes. Ensure that the test tube is sealed with parafilm to prevent carbon dioxide exposure to the sample. The test tube was left cold for several minutes until the temperature of the test tube was around room temperature. 8 mL of distilled water was added to the test tube and mixed with the vortex mixture. Let the solution rest for 20 to 30 minutes. Fill the cuvette with the sample until it reaches 2/3 of the cuvette. The sample will be analysed by using an ultraviolet-visible (UV-VIS) spectrophotometer at the wavelength 540 nm. The same setup was done for the glucose with a different concentration (1-10 mg/mL) to obtain the absorbance reading of the glucose at a specific concentration. A graph of absorbance against glucose concentration was created to make a glucose standard curve. The standard curve was used to obtain the concentration of the sample.

3. Results and Discussion

In this chapter, the result of the production of bioethanol from simultaneous saccharification and fermentation (SSF) of pre-treated bamboo powder and the characterisation of the sample will be analysed and discussed.

3.1 Fourier-Transform Infrared (FTIR) Spectroscopy Analysis on Bamboo Powder

Fourier-transform Infrared (FTIR) spectroscopy was used to analyse chemical changes in the composition of bamboo powder before and after being treated with acid-alkaline pre-treatment. In general, bamboo has almost a similar basic structure to wood species due to the existence of lignin, hemicellulose and cellulose. Hence, the infrared (IR) peaks present during FTIR analysis for bamboo can be analysed and characterised by referring to other woody species [7]. In this study, the FTIR measurement range for the pre-treated bamboo samples was between 4000cm-1 and 400cm-1 with a spectral resolution of 0.5cm-1. Fig. 1 shows the effect of acid-alkaline pre-treatment on the FTIR spectra of bamboo composition.

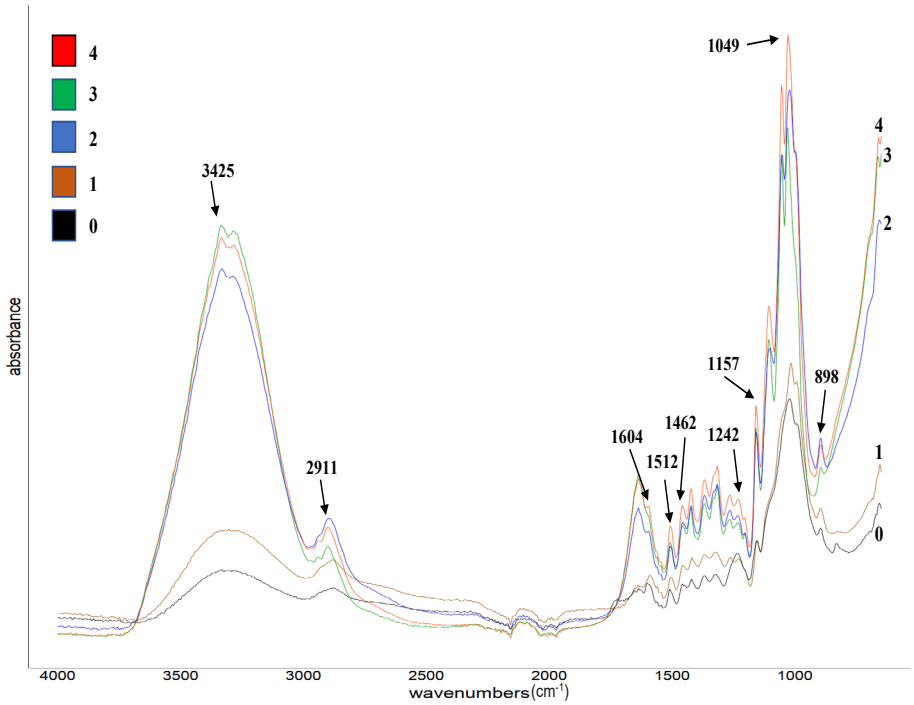

Fig. 1 *FTIR spectrum for bamboo powder after acid-alkaline pre-treatment: 0 = untreated bamboo; 1 = 1% H2SO4 + 5% NaOH bamboo; 2 = 2% H2SO4 + 5% NaOH bamboo; 3 = 3% H2SO4 + 5% NaOH bamboo; 4 = 4% H2SO4 + 5% NaOH bamboo*

Significant changes can be observed in the IR spectra of bamboo composition after undergoing the pre-treatment stage. There was an apparent formation of firm peaks around 3425 cm-1 and 2911 cm-1, indicating the O-H and C-H functional groups. Even so, the peak here can be ignored since it is due to the remaining water in the sample and is far from the bamboo fingerprint region. A noticeable decrease in the intensities of absorption peaks can be observed at 1604 cm-1, 1462 cm-1 and 1242 cm-1 after being treated with different concentrations of dilute sulphuric acid, significantly at 4% dilute sulphuric acid pre-treated bamboo. The peak at 1604 cm-1 and 1242 cm-1 is almost absent in this condition. This indicates the removal of lignin from the sample. However, the sharp peak at 1512 cm-1, which indicates the lignin group, remains constant even after the acid-alkaline pre-treatment. Additionally, the absorption waves between 1658 cm-1 reflect the presence of the alkene group, which has variable C=C bonds between atoms of moderate strength. A sharp peak around 1157 cm-1, 1049 cm-1 and 898 cm-1 was found after the pre-treatment which indicates carbohydrates group [7]. This shows the high intensities of hemicellulose and cellulose remains in the sample.

Kristiani (2013) states that acid pre-treatment can achieve a high reaction rate and considerably boost the amount of hydrolysed cellulose. Although acid pre-treatment is ineffective for dissolving lignin, it does have the potential to interfere with lignin and enhance the susceptibility of cellulose to enzymatic hydrolysis [8]. Another study by Song et al. (2016), who investigated the surface characterisation and chemical analysis of bamboo substrates pre-treated by alkali hydrogen peroxide, found that an alkaline solution can potentially cause the breakdown of a low-molecular-weight polymer composed of lignin and hemicellulose, as well as the breakdown and dissolution of extractives [9]. Hence, it is proven that the combination of acid and alkaline pre-treatment increases the accessibility of enzymes by reducing the amount of lignin in the bamboo.

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3.2 Simultaneous Saccharification and Fermentation Study Via Dinitrosalicylic (DNS) Acid Method

In this subtopic, the effect of different concentrations of sulphuric acid during the acid-alkaline pre-treatment stage and the residence times of fermentation towards the production of glucose were observed. The dinitosalicylic acid (DNS) method was used to estimate the glucose produced during SSF. The DNS solution was added to the sample to study the colour reaction of the solution. This calorimetric technique relies on a redox reaction between 3,5-dinitrosalicylic acid and the reducing sugars present in the sample [10]. Since the colour of the sample is proportional to the amount of the reducing sugar, these colour changes can be quantified using the UV-Vis spectrophotometer at wavelength 540 nm. In this study, it was found that the colour of the media solution for 4% dilute sulphuric acid pre-treated bamboo has the darkest colour compared to the colour of the media solution for untreated bamboo. Visually, it can be concluded that 4% dilute sulphuric acid pre-treated bamboo produces more glucose than untreated bamboo. However, the exact amount of glucose concentration in the sample can only be determined using a calibration curve of absorbance versus glucose concentration. Hence, several samples with a known glucose concentration were used to plot this graph. Fig. 2 shows the calibration curve of absorbances against glucose concentration.

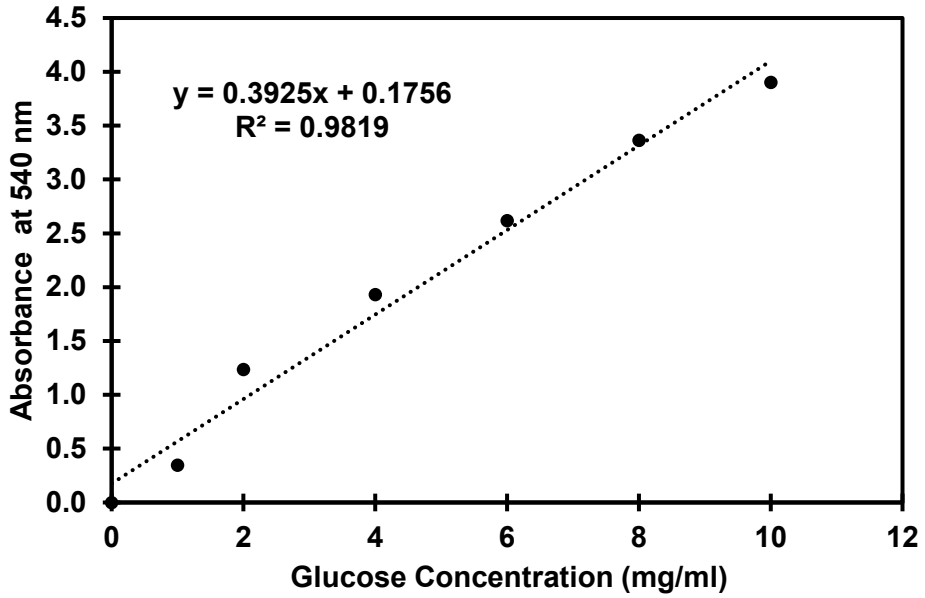

Fig. 2 *Calibration curve of absorbances against glucose concentration*

The equation derived from the Fig. 2 is as follows:

$$y = 0.3925x + 0.1756 \quad (1)$$

Where y = absorbance (nm) and x = glucose concentration (mg/ml). Equation 1 was rearranged so the glucose concentration would be on the Left-Hand Side (LHS). The equation was derived as follows:

$$x = \frac{(y - 0.1756)}{0.3925} \quad (2)$$

The effect of different concentrations of sulphuric acid during the acid-alkaline pre-treatment stage and the residence times of fermentation towards the production of glucose were investigated by calculating the glucose concentration for each sample's absorbance reading using Equation 2. The results are shown in Table 1. The effect of different concentrations of sulphuric acid during the acid-alkaline pre-treatment stage and the residence times of fermentation on glucose concentration is simplified in Fig. 3.

Table 1 *Glucose concentration for different sulphuric acid and days of fermentation*

Concentration of Sulphuric Acid (v/v)	Days	Average Absorbance	Standard Deviation	Standard Error	Glucose Concentration (mg/ml)
Untreated Bamboo	3	0.630	0.0281	0.0162	1.158
	4	0.625	0.0125	0.0072	1.145
	5	0.612	0.0095	0.0055	1.111
	6	0.593	0.0096	0.0055	1.063
1% H2SO4 + 5% NaOH Bamboo	3	1.966	0.0155	0.0090	4.561
	4	1.865	0.0085	0.0049	4.304
	5	1.745	0.0106	0.0061	3.998
	6	1.666	0.0131	0.0075	3.796
2% H2SO4 + 5% NaOH Bamboo	3	2.364	0.0076	0.0044	5.575
	4	2.261	0.0118	0.0068	5.313
	5	2.212	0.0135	0.0078	5.188
	6	2.132	0.0136	0.0078	4.985
3% H2SO4 + 5% NaOH Bamboo	3	2.469	0.0120	0.0069	5.843
	4	2.424	0.0197	0.0114	5.728
	5	2.384	0.0085	0.0049	5.627
	6	2.329	0.0157	0.0091	5.486
4% H2SO4 + 5% NaOH Bamboo	3	2.723	0.0125	0.0072	6.491
	4	2.695	0.0055	0.0032	6.420
	5	2.678	0.0103	0.0059	6.375
	6	2.649	0.0161	0.0093	6.302

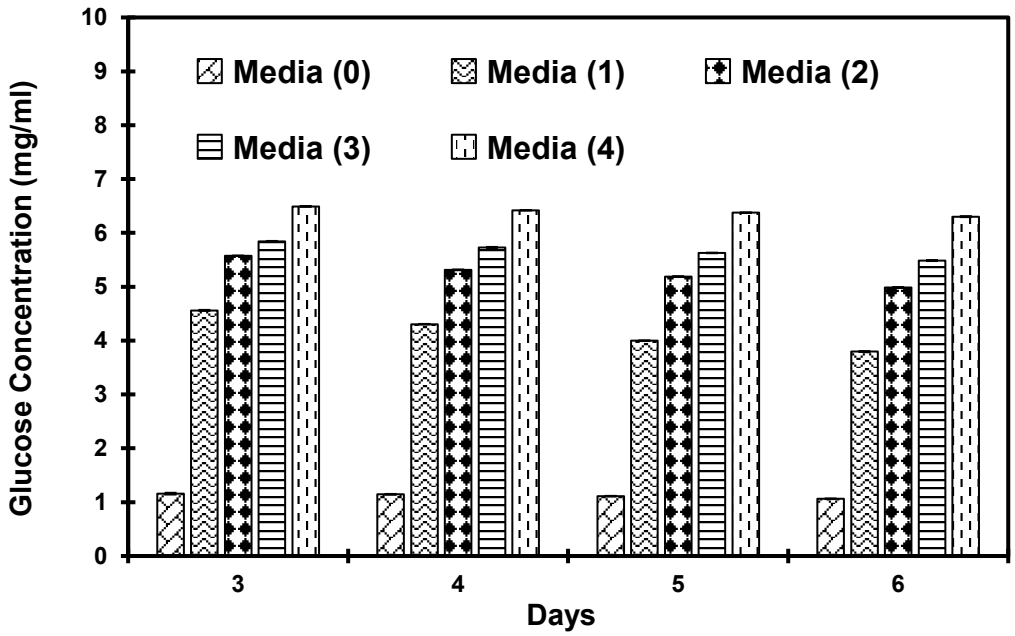

Fig. 3 *Glucose concentration for different sulphuric acid and days of fermentation: Media (0) = Untreated bamboo; Media (1) = 1% H2SO4 + 5% NaOH bamboo; Media (2) = 2% H2SO4 + 5% NaOH bamboo; Media (3) = 3% H2SO4 + 5% NaOH bamboo; Media (4) = 4% H2SO4 + 5% NaOH bamboo*

From Fig. 3, it can be seen that the trend of the glucose concentration increases as the concentration of sulphuric acid during acid-alkaline pre-treatment increases. Untreated bamboo powder produces the lowest

concentration of glucose which is 1.158 mg/ml on day 3. There was a huge difference in glucose concentration, around 3.4 mg/ml, between untreated bamboo powder and treated bamboo powder, even at 1% sulphuric acid. The high presence of lignin in untreated bamboo reduces the enzyme hydrolysis activities in the sample. Lignin acts as a protector for cellulose and hemicellulose, resulting in low contact of cellulose and hemicellulose to enzymes [11]. This statement was supported with discovery of Xiao during 2011. He found that even though the enzymatic hydrolysis yield was only around 20% after 12 hours, no considerable accumulation of reducing glucose was found in untreated woody biomass at temperatures below 140 °C [12]. Thus, low glucose was produced for untreated bamboo powder during the fermentation process.

On the other hand, bamboo that has been treated with different concentrations of sulphuric acid shows an increasing pattern. At 1% dilute sulphuric acid, 4.561 mg/ml of glucose was produced. The amount of glucose keeps increasing, which is 5.575 mg/ml, 5.843 mg/ml and 6.491 mg/ml, as the concentration of sulphuric increases from 2%, 3% and 4%, respectively. Sindhu et al. (2014) carried out an experiment of bioethanol production from dilute sulphuric acid pre-treated bamboo to study the effect of concentration sulphuric acid towards production of bioethanol. An optimized condition of 15% (w/w) biomass loading, 5% acid concentration and 30min pre-treatment time was found, which yielded 0.319 g/g of reducing sugar [13]. Hence, this proven that production of glucose is affected by the concentration of dilute sulphuric acid.

The effect of residence times for fermentation towards the concentration of glucose in the sample has also been discussed. It was discovered that there was a slight decrease in the amount of glucose. The change in glucose concentration for all the samples was too small, which is less than ~0.5 mg. Since the changes were too few, it was concluded that there were no changes in glucose levels for all the samples. Supposedly, the glucose level in the sample should be decreased tremendously as the residence time of the fermentation increase. Glucose will be changed to ethanol and carbon dioxide by an enzymatic process with the assistance of an enzyme in yeast. Phwan et al. (2019) reported that after an 84-hour fermentation stage, the reducing sugar content in the sample, which was pre-treated with 5% (v/v) H2SO4 and 5% (v/v) CH3COOH, was extensively consumed. It drops from 32.04 g/L to 6.95 g/L and 30.05 g/L to 5.63 g/L, respectively [14]. Hence, it is expected that the glucose in the sample did not convert to bioethanol by dry yeast.

3.3 Analyzation of Bioethanol Content Using Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform Infrared (FTIR) spectroscopy was used to analyse the presence of ethanol in the extraction solution for untreated and treated bamboo powder. The infrared (IR) peaks formed by the bioethanol were compared with pure ethanol since both solutions have a similar functional group. Fig. 4 shows the FTIR spectrum of bioethanol for untreated and treated bamboo with different concentrations of sulphuric acid.

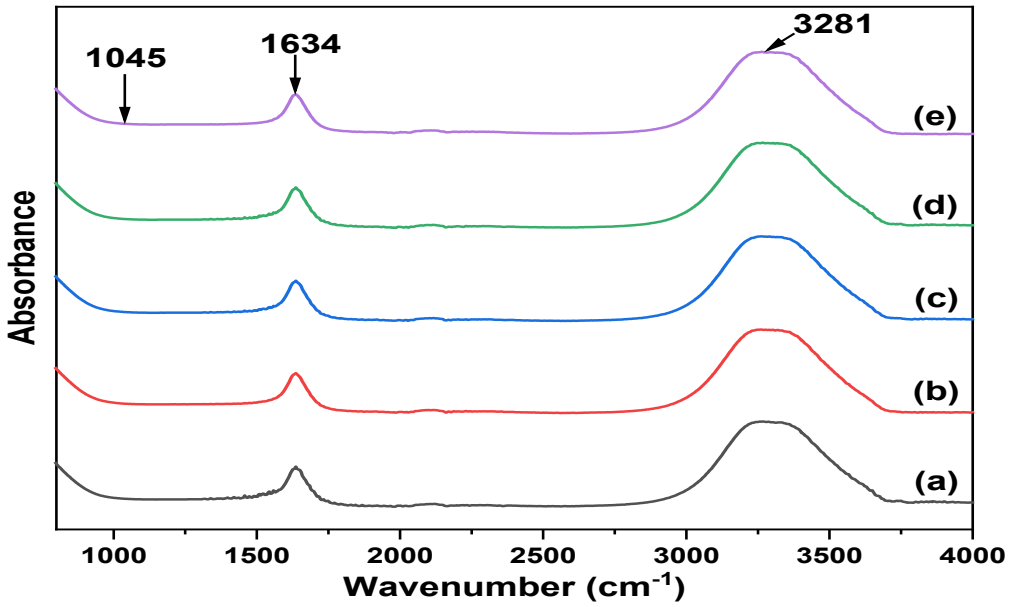

Fig. 4 *FTIR spectrum for bioethanol untreated and treated bamboo: (a) Untreated bamboo powder; (b) 1% H2SO4 + 5% NaOH treated bamboo; (c) 2% H2SO4 + 5% NaOH treated bamboo; (d) 3% H2SO4 + 5% NaOH treated bamboo; (e) 4% H2SO4 + 5% NaOH treated bamboo*

The absorption spectrum with peaks between 3400 cm-1 to 3200 cm-1 and 1658 cm-1 to 1638 cm-1, representing hydroxyl and alkene groups, respectively were found. The FTIR results obtained were compared to other studies [14,15]. The broad peak produced between 3400 cm-1 to 3200 cm -1 indicates the presence of a hydroxyl group in the samples. In addition, IR peak forms between 1658 cm-1 and 1638 cm-1 show the presence of an alkene group with variable C=C bonds between atoms with moderate intensity. The data obtained shows that there is no formation of a peak, which represents the existence of ethanol in the sample. Supposedly, there will be a formation of an absorption wave between 1060 cm-1 and 1001 cm-1 that indicates the appearance of ethanol in the sample. The presence of an IR peak in that region is due to the absorption band of C-O stretch vibration [15]. Hence, it can conclude that there is no bioethanol in untreated and treated bamboo sample. Thus, several hypotheses were made to investigate the reason behind the failure of ethanol production during simultaneous saccharification and fermentation. Three potential factors were list out that may cause no bioethanol production in the sample: the concentration of glucose, the pre-treatment used and the condition of dry yeast. As for the first factor, the lowest glucose obtained during the experiment was 1.158 mg/ml. Theoretically, 1 g of glucose would produce 0.514 g of ethanol and 0.488 g of carbon dioxide. Nonetheless, in actual practice, 100% yield glucose cannot be obtained since microorganisms only consume some of the glucose for growth. Palniandy (2022) found that 16% of ethanol yield was obtained for pre-treated empty fruit bunches with 1.914 mg/ml glucose presented in the media [16]. From these studies, the concentration of the glucose is not a reason for the failure of the bioethanol for this experiment. As for the second factor, sequential dilute sulphuric acid and sodium hydroxide pre-treatment was conducted for this study. According to Lorenci Woiciechowski (2020), the combination of acid and alkaline pre-treatment removes the hemicellulose and lignin in bamboo powder, which enhance the enzymatic digestibility and fermentability [17]. Tan et al (2021) discovered that the combined method of acid-alkaline with the optimal parameters obtained the highest ethanol concentration of 1.55% with the bioethanol conversion of 55.75%. It is higher than single treatments, which only achieved 28.51% and 45.62% of bioethanol conversion for the acid and alkaline solutions, respectively [18]. Hence, the hypothesis of acid-alkaline pre-treatment causing no ethanol to be produced can be rejected.

The last factor was the condition of dry yeast before the experiment. Since the dry yeast was not checked before the experiment, a small experiment was conducted to check the condition of the yeast. A 5% glucose solution was prepared and heated up to 37°C. The yeast was then put in warm water for 15 minutes. If there was bubble form in the solution, it indicates the yeast was alive. From this experiment, it was observed that there was no bubble form after 15 minutes. Thus, it assumes that it died before the experiment was started. Due to the limitation of time, the experiment cannot be repeated. Hence it can be concluded that there is no presence of bioethanol in the sample is due to the yeast inactive before the experiment started.

4. Conclusion

This research study has successfully characterised the chemical structure of Bambusa Wray after been treated with dilute sulphuric acid and sodium hydroxide using FTIR. The infrared (IR) spectrum peak of lignin for treated bamboo powder was gradually flattened. Next, the glucose concentration produces during simultaneous saccharification and fermentation process was analysed using the DNS method. 4% dilute sulphuric acid pre-treated bamboo has the highest production glucose, which is 6.491 mg/ml, compared to un-treated bamboo, which only produces 1.158 mg/ml. It shows that acid-alkaline pre-treatment increases enzyme accessibility towards cellulose. However, this study did not achieve the third objective: to study the effect of different concentrations of sulphuric acid on the amount of bioethanol produced. The extraction sample from the fermentation media was analysed using FTIR to check the availability of ethanol in the sample. In spite that, no peak representing ethanol was formed in the IR spectrum. It is due to the yeast dying before the fermentation process. It can be concluded that the best model of experiment for the pre-treatment of Bambusa Wray is at 4% H_2SO_4 + 5% NaOH due to high glucose production. This research has brought forward new option for raw materials that could be used for bioethanol production and reduce the industry's problems in using bamboo as a biofuel feedstock.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

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