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Alkali Pretreatment and Acid Hydrolysis of Coconut Pulp and Empty Fruit Bunch to Produce Glucose

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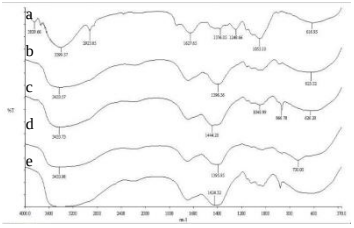
Graphical abstract



Abstract

Lignocellulose waste has great potential to be converted into value added products sustainably as it is readily available at low cost. The aim of this study is to examine the amount of glucose produced from coconut pulp and coconut empty fruit bunch using acid hydrolysis. Sodium hydroxide pretreatment is carried out at 70ºC using different concentrations of sodium hydroxide which are 5%, 10%, 15% and 20% v/v for a duration time of 2 hours. Optimum pretreatment is evaluated using Fourier Transform Infra-Red (FTIR) analysis. It is observed that optimum pretreatment is at 20% v/v sodium hydroxide. The optimum samples are then hydrolysed using concentrations of 5%, 10%, 15% and 20% v/v of sulphuric acid at different temperatures of 30, 50, 70 and 90ºC for 2 hours. Glucose concentration is analysed using an ultraviolet (UV) Spectrophotometer. The highest glucose concentrations obtained are 0.895 g/L and 0.550g/L for coconut pulp and coconut empty fruit bunch, respectively at 20% v/v acid concentration and a temperature of 90ºC.

Keywords: Lignocellulose; alkaline pretreatment; acid hydrolysis, glucose

1.0 INTRODUCTION

Lignocellulosic materials such as wood, grass and agricultural and forest residues have received considerable attention as a sustainable feedstock that can replace diminishing fossil fuels for the production of energy. Lignocellulosic material is mainly composed of three major components which are cellulose (33–50%), (23–32%), and lignin (10–25%) [1] arranged in a complex polysaccharide matrix as shown in Figure 1. Cellulose microfibrils contain crystalline, subcrystalline and noncrystalline chains packed together by hydrogen bonds in which the crystalline chains are the most resistant to degradation. The cellulose microfibrils are covered by hemicellulose which is hydrogen bonded to the microfibril surface [3] whereas lignin fills the spaces in the cell walls between the cellulose and hemicellulose and is covalently linked to hemicellulose acting as glue to hold the lignocellulose matrix together [4]. It binds to cellulose and hemicellulose by α-ether bonds, phenyl glycosidic linkages, acetal linkages and ester bonds. Lignin also has carbonyls, phenol hydroxyls, aromatic rings and methoxyl functional groups [5].

Cellulose can be biologically or chemically hydrolysed into glucose which subsequently can be converted into value added products such as biofuels, chemicals, medicines, nanocomposites and biomaterials in a sustainable and environmentally friendly system. Lignocellulosic materials are mostly hydrolysed by acids and enzymes such as cellulases.

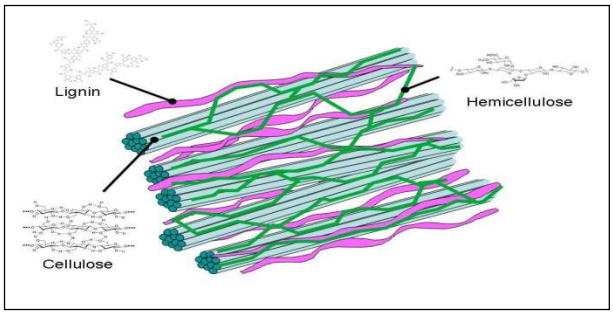


Figure 1 Lignocellulosic biomass polysaccharide matrix [2]

Acid hydrolysis can be divided into two groups which are concentrated acid hydrolysis and dilute acid hydrolysis. Concentrated acid hydrolysis can be operated at low temperature (e.g. 40ºC) compared to dilute acid processes that have to be operated at high temperatures. However, if the concentration of acid used is very high (e.g. 30-70%) it would be extremely corrosive during dilution and heating [6].

Sulphuric and hydrochloric acids are the most commonly used acids for hydrolysis of lignocellulosic biomass.

The cross-linked polysaccharide structure makes it resistant to enzymatic and chemical conversions. Pretreatment needs to be carried out in order to disrupt the naturally resistant structure of lignocellulose that limits the hydrolysis of cellulose and hemicellulose as shown in Figure 2. The pretreatment stage is comprised of both physical and chemical methods and is intended to delignify lignin in order to release free cellulose and hemicellulose, reduce the crystallinity of cellulose and increase the surface area and porosity of the lignocellulosic materials resulting in increased hydrolysis rate. Thus, a pretreatment stage is needed as only 20% of the native biomass is hydrolysed without pretreatment [7].

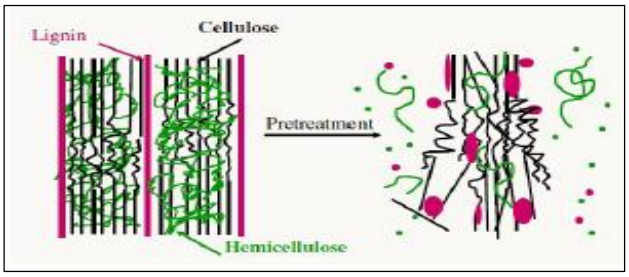


Figure 2 Simplified impact of pretreatment on lignocellulosic biomass [8]

Alkali pretreatment is one of the pretreatments mostly used by researchers especially for hardwood and agricultural residues. Alkali pretreatment refers to the application of alkali solution such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), or ammonia (NH_3) to the biomass to modify its structure and composition of lignin and hemicellulose in the biomass. Alkali pretreatment of lignocellulosic materials causes the disruption of lignin, the swelling and decrystallisation of cellulose thus increasing the accessibility of cellulose towards acid hydrolysis attack. Alkali pretreatment also extracts hemicelluloses from polysaccharides and produces organic acids that lower the pH [7].

Sodium hydroxide (NaOH) solution is usually preferred by many researchers due to its relative high alkalinity and many benefits. Pretreatment using sodium hydroxide solutions enhances polyionic character of the pretreated lignocellulose which is related to the diffusion of sodium ions into the lignocellulose which also acts as a counterchange to carboxylate ions and also promotes swelling [9].

This paper investigates the optimum pretreatment condition using different concentrations of sodium hydroxide and the effects of sulphuric acid concentrations and hydrolysis temperatures on glucose production on coconut empty fruit bunch and coconut pulp.

■2.0 EXPERIMENTAL

2.1 Materials and Chemicals

Coconut empty fruit bunch and pulp were obtained from the local market. Sodium hydroxide, sulphuric acid, dinitrosalicyclic acid, phenol, sodium sulphite, potassium sodium tartrate and potassium bromide were purchased from Merck.

2.2 Preparation of Coconut Empty Fruit Bunch and Coconut Pulp

Coconut empty fruit bunch and coconut pulp collected are washed with water and cut to an average size of 2 cm. Drying is carried out in an oven at 75ºC for 24 hours until a constant weight is obtained. The dried sample is further reduced in size by grinding using fiber grinder to get particle sizes that pass through sieve mesh 10 (2 mm). Then, it is stored in a sealed plastic bag at room temperature.

2.3 Alkali Pretreatment

10 g each of coconut empty fruit bunch and coconut pulp was weighed and soaked inside 150 ml of 5% wt/v sodium hydroxide solution separately for 2 hours. The mixture was then heated in a water bath of 70ºC.

The mixtures were then filtered using filter paper and vacuum filtration to separate the treated coconut wastes from solution before they were analysed for delignification using Fourier Transform Infra-Red (FTIR). These steps were repeated for different alkali concentrations of 10%, 15% and 20% wt/v of sodium hydroxide.

2.4 FTIR Analysis

Disc were prepared by mixing 3 mg of dried samples with potassium bromide (KBr) powder which were then pressed for 3 minutes. FTIR spectrum was then recorded between 4000 and 400 cm^{-1} by a spectrometer with detector at 4 cm^{-1} resolution and 25 scans per sample.

2.5 Acid Hydrolysis

10 g of dried pretreated samples were subjected to acid hydrolysis by using 150 ml of 5% v/v sulphuric acid separately. The mixture was then heated in water bath for 2 hours using four different temperatures of 30, 50, 70 and 90ºC.

2.6 Glucose Test

3 mL of the filtrate sample was added with 3 mL of DNS reagent in a test-tube covered with a piece of paraffin film in order to avoid any loss of liquid due to evaporation. The mixture was heated using a hot water bath at 110ºC for 10 minutes until it changed to red brown. 1mL of potassium sodium tartrate solution was added to stabilize the colour, and left for 20 minutes to cool down to room temperature. Glucose concentration was then determined using UV spectrophotometer at 540 nm.

■3.0 RESULTS AND DISCUSSION

3.1 Pretreatment Analysis

FTIR spectroscopy analysis as shown in Figure 3 shows obvious changes in the functional group of lignin in coconut empty fruit bunch sample after pretreatment. The peaks located at 3433 cm^{-1} and 2923 cm^{-1} correspond to –OH stretching and CH_2 stretching, respectively. Both peaks indicate the distinguished features of cellulose.

It can be seen from Figure 3 that the spectra of coconut empty fruit bunch for untreated and treated sample have the same profile but different intensities of the absorption band. The

peak located at 3433 cm^{-1} is diminished in the spectrum of pretreated sample with 5, 10, 15 and 20% wt/v sodium hydroxide. However, the intensities of the absorption band were different. It clearly shows that the spectra of pretreated sample with 20% wt/v sodium hydroxide have the lowest intensity compared to the other pretreated samples.

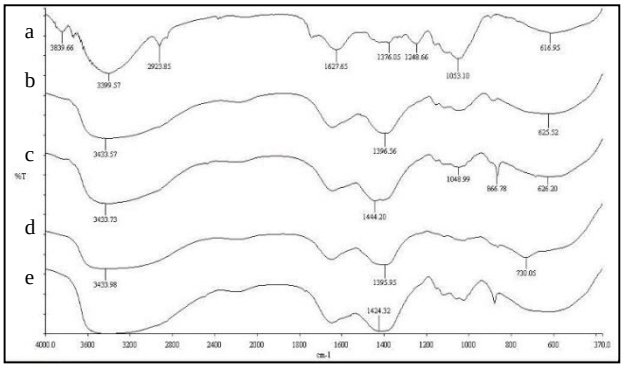


Figure 3 FTIR analysis of coconut empty fruit bunch at 70ºC. a) untreated sample b) 5% NaOH c) 10% NaOH d) 15% NaOH e) 20% NaOH

This is suggesting that the lignin barrier in this pretreated sample is highly degraded since every lignin infrared spectrum has a strong wide band between 3500 and 3100 cm^{-1} assigned to –OH stretching vibrations. This is in agreement with He [5] that stated the absorption peak in the range 3500–3100 cm^{-1} for rice straw pretreated with sodium hydroxide was diminished, suggesting that the partial hydrogen bond of cellulose was destroyed enhancing the accessibility of cellulose to reagents.

The peak located at 2923 cm^{-1} corresponding to the C−H stretching diminishes in the spectrum of all pretreated samples especially for the pretreated sample with 20% wt/v sodium hydroxide. The peak is greatly diminished in this spectra which means there is some rupture in methyl and methylene of cellulose in the pretreated sample of 20% wt/v sodium hydroxide.

The absorption peak located at 1396 cm^{-1} is associated with aromatic hydroxyl groups. The cleavage of ether bonds within the lignin may have caused the peak to be reduced [10]. A peak located at 1248 cm^{-1} in the untreated sample corresponds to C-O stretching of ether linkage. It is diminishing in the spectrum of pretreated sample. According to Kubo and Kadla [11], the decrease of this band indicates that lignin is diminishing after the substrate was pretreated. It can be seen that the peak is completely diminished in the spectra of pretreated sample with 20% wt/v alkali as at this band, a smooth line is formed. The prominent band observed at 1053 cm^{-1} is typically related to the structural characteristics of cellulose and hemicellulose.

While, in the anomeric region (950–700 cm^{-1}), a small sharp band at 866 and 730 cm^{-1} is observed. This band corresponds to the C1 group frequency or ring frequency and is indicated of β-glycosidic lingkages between sugar units [12]. This band indicates the presence of glucose in the sample. In pretreated sample of 20% wt/v sodium hydroxide (NaOH), both of these bands are obviously increased greatly compared to other samples, indicating that the linkages between the sugar units are changed and intermolecular degradation occurred in the hemicellulose structure.

FTIR spectroscopy analysis of coconut pulp as shown in Figure 4 shows obvious changes in the functional group of lignin after alkali pretreatment. The changes in the spectrum of the samples could be classified into the disappearance of bands, the decrease of functional group contents and the appearance of bands.

It clearly shows the diminishing of peak located at 3434, 2924 and 2854 cm^{-1} for all pretreated coconut pulps. The peak located at 3434 cm^{-1} corresponds to –OH stretching in the hemicellulose. The intensity of the band decreased after sodium hydroxide treatment due to the disruption and breakage of hydrogen bond.

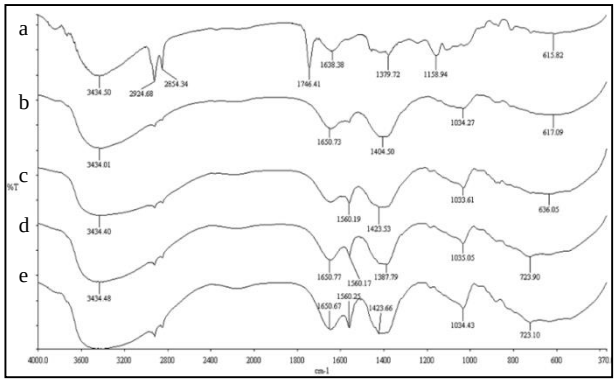


Figure 4 FTIR analysis of coconut pulp at 70ºC. a) untreated sample b) 20% NaOH c) 15% NaOH d) 10% NaOH e) 5% NaOH

Both peaks located at 2924 and 2854 cm^{-1} correspond to C−H stretchings are diminished in the spectrum of all pretreated coconut pulp sample especially pretreated sample of 20% wt/v sodium hydroxide. The peak is completely diminished in this spectra, producing smooth curve line. There may be some rupture in methyl and methylene of cellulose in this pretreated sample which leads to the diminishing of C−H peak.

It obviously shows that the peak at 1746 cm^{-1} is completely disappeared after sodium hydroxide pretreatment especially in pretreated sample of 20% wt/v sodium hydroxide (NaOH). The band at 1746 cm^{-1} is assigned to the carbonyl (C−O) stretching unconjugated ester. Carbonyls mainly exist in the side chains of lignin structural units and are also an important functional group in the side chains. The disappearance of such bands indicates that the side chain of lignin is broken down during sodium hydroxide (NaOH) treatment

The peak at 1638 cm^{-1} is assigned to the C−C stretching of aromatic group. The disappearance of this peak in all the pretreated coconut pulp sample indicates that the carbon chain of aromatic group is degraded during alkali pretreatment. The band near 1379 cm^{-1}, associated with C−H bending in cellulose and hemicellulose, is reduced after pretreatment. The cleavage of ether bonds within the lignin may have caused the peak to be reduced [10].

The prominent band at 1365 cm^{-1} corresponds to methoxyl stretching. It is observed that the content of methoxyl in the lignin of pretreated sample is reduced compared to untreated coconut pulp. In the pretreated sample of 20% wt/v sodium hydroxide (NaOH), the peak has completely disappeared. This is mainly attributed to the nucleophilic reaction of methoxyl with sodium hydroxide which is in agreement with He *et al.* [5].

The peak at 1158 cm^{-1} is representative of ester bond (C−O−C) stretching in cellulose and hemicellulose. From Figure 4, it clearly shows that the peak is reduced in all pretreated coconut pulp spectrums especially for the pretreated coconut pulp of 20% wt/v sodium hydroxide (NaOH). This finding implied that the saponification reaction occurs during pretreatment as stated by Sekiguchi *et al.* [13] in his study. Such a reaction damaged the ester bond linkage between lignin and

carbohydrate and released cellulose from the bonding of lignin, making more cellulose to be exposed and available for hydrolysis into glucose.

Some bands such as the band at 1034 cm^{-1} appear after sodium hydroxide (NaOH) pretreatment. This band represents aromatic ring deformation in the C−H plane [14]. Its appearance implies that the content of aromatic compounds had increased. In the anomeric region (950–700 cm^{-1}), a small sharp band at 898 and 723 cm^{-1} is observed. This band corresponds to the C1 group frequency or ring frequency and is indicated of β-glycosidic linkages between sugar units [12]. This band indicated the glucose presence in the sample. In pretreated sample of 20% wt/v sodium hydroxide (NaOH), both of these bands increased sharply compared to other samples, indicating that the linkages between the sugar units are changed and intermolecular degradation occurred in the hemicellulose structure.

Based on the above result, the study on the effects of sulphuric acid concentrations and hydrolysis temperatures on glucose production is subsequently carried out at 20% wt/v sodium hydroxide (NaOH) with 70°C of water bath temperature and retention time of 2 hours.

3.2 Effects of Temperature and Acid Concentration on Glucose Production

Figure 5 shows the changes in glucose concentration of coconut empty fruit bunch using different concentrations of 5%, 10%, 15% and 20% v/v sulphuric acid. The plots for all samples treated with sulphuric acid follow the same trend. The lowest glucose concentration for coconut empty fruit bunch of 0.07 g/L was obtained when the sample was hydrolysed with 5% v/v sulphuric acid at 30°C. This is due to the very low rate of reaction between sulphuric acid and cellulose which is in agreement with Yoon *et al.* [15] that stated crystalline structure of microfibril bundles in lignocellulosic material cannot be penetrated by acid in acid hydrolysis at lower temperature, which resulted in lower glucose yield.

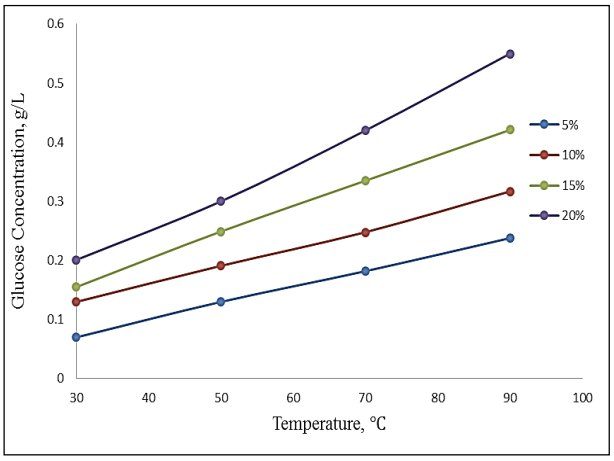


Figure 5 Effect of concentration of sulphuric acid on glucose concentration of coconut empty fruit bunch

The results show a higher concentration of sulphuric acid produces a higher concentration of glucose at a higher temperature. The highest glucose concentration of 0.550 g/L is obtained when the acid concentration is 20% v/v at 90°C.

This is in agreement with Botha and Blottnitz [16] that stated the uses of concentrated acid at moderate temperatures (90–150°C) will achieve high glucose yield. High temperature will initiates the rate of reaction between sulphuric acid and cellulose. However, too high a temperature will degrade the glucose into organic acid. Yoon *et al.* [15] stated that the reaction time, temperature and acid concentration control the degradation of glucose to organic acids.

In all the treated coconut pulp samples as shown in Figure 6, there is a general increase in glucose yield as the acid concentration and temperature increased. Initially, at 30°C, the glucose concentration of the sample was low with the lowest concentration of 0.09 g/L being obtained when coconut pulp was treated with 5% v/v sodium hydroxide, indicating the low rate of reaction.

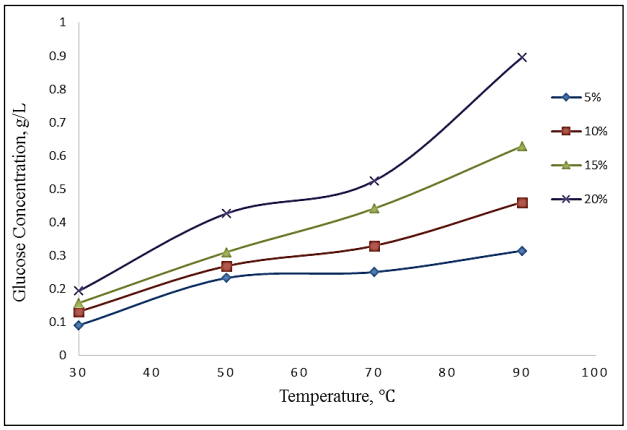


Figure 6 Effect of concentration of sulphuric acid on glucose concentration of coconut pulp

The glucose concentration increases with increasing temperature and acid concentration for all samples. The highest glucose concentration of 0.895 g/L for coconut pulp was obtained at 90°C.

4.0 CONCLUSION

It is concluded that the optimum delignification of the pretreated samples of coconut empty fruit bunch and pulp is 20% wt/v sodium hydroxide (NaOH) at 70°C with a retention time of 2 hours as it gives the lowest intensity of lignin. Upon hydrolysis of all the samples at the optimum pretreatment conditions, the maximum glucose concentration obtained for coconut pulp and coconut empty fruit bunch are 0.895 g/L and 0.55 g/L, respectively at 20% v/v sulphuric acid and at 90°C.

Both coconut empty fruit bunch and pulp show great potential to produce glucose which could serve as an alternative feedstock for future energy and value added production especially on their availability at considerably low cost.

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