

APPENDIX C

Analytical Methods

C.1 Analysis of Liquid Fraction

1. Determination of carbohydrate (Monosugar)

The levels of sugar from chemical pretreatment (acid, alkaline and organic solvent) such as glucose, cellobiose, xylose, arabinose and acetic acid presented in filtrated were analyzed through High Liquid Performance Chromatography (HPLC). A chromatograph Shimadze applying CR 7A model refraction index detector Shimadzu RID- 6A model, a column was Aminex HPX 87H (300 mm x 7.8 mm, BIO-RAD). The mobile phase was 0.005 mol/L of H₂SO₄ with a flow of 0.6 mL/min, the operating temperature was 45 °C. The glucose and cebilose were converted into cellulose by using conversion factor such as 0.90 and 0.95, respectively. In addition, xylose and arabinose were converted to hemicellulose by using the conversion factor 0.88. Acetic acid was converted to acetyl group of hemicellulose and the conversion factor was 0.72. The concentration of the compounds were determined by using calibration carve [8].

2. Determination of Furfural and Hydroxymethylfurfural (HMF)

The furfural is the derivative of hemicellulose by hydrolysis of arabinose and xylose, hydroxymethylfurfural (HMF) is the product of glucose degradation. The samples of furfural and HMF from chemical pretreatment (acid, alkaline and organic solvent) were analyzed through HPLC by using chromatograph Shimadze model C-R7A and detector with an UV visible Shimadzu SPD model, column was RP 10A-18 (C-18) of 125 mm x 4 mm (Hawlett-Packard), the mobile phase was acetonitrile-water 1:8 (v/v) with 1% acetic acid, a flow of 0.8 mL/min and operating temperature of 25 °C. The furfural was converted to cellulose by using conversion factor of 1.37 and HMF was converted to hemicellulose by conversion factor of 1.29 [8].

3. Determination of soluble lignin

The soluble lignin in filtrated from chemical analysis of bagasse was determined by measuring absorbance at 280 nm in UV-visible spectrophotometer Perkin Elmer model Lambda 25. 25 mL of sample was filled in 100.00 mL of volumetric flask with 50.0 mL of distilled water and 6.50 mol/L of NaOH 2.00 mL (pH near 12.5). Then the sample was transferred into cuvet and analyzed in the spectrophotometer [8]. The level of soluble lignin was calculated follow this equation [5]:

$$C_{lig} = \frac{A_{lig280} - A_{pd280}}{\epsilon_{lig}}$$

Where; C_{lig} = concentration of soluble lignin (gL⁻¹)

A_{lig280} = absorbance of solution 280 nm

A_{pd280} = absorbance of sugar degradation product (furfural and HMF)

$$A_{pd280} = C_1\varepsilon_1 + C_2\varepsilon_2$$

Where; C_1 = furfural concentration (gL^{-1})

C_2 = HMF concentration (gL^{-1})

ε_1 = furfural absorptivity at 280 nm ($\text{cm}^{-1}\text{g}^{-1}\text{L}$) 14,685 $\text{cm}^{-1}\text{g}^{-1}\text{L}$

ε_2 = HMF absorptivity at 280 nm ($\text{cm}^{-1}\text{g}^{-1}\text{L}$) 114 $\text{cm}^{-1}\text{g}^{-1}\text{L}$

ε_{lig} = lignin absorptivity at 280 nm ($\text{cm}^{-1}\text{g}^{-1}\text{L}$) 23.7 $\text{cm}^{-1}\text{g}^{-1}\text{L}$

C.2 Analysis of Solid Fraction

1. Extractives [37]

The extractives were determined by using Soxhlet extractor. 300 mL of acetone was used for the solvent extractives (5 g of dry biomass, w_0 , g) with residence time for boiling and rising stage 70 °C and 25 min for 4 h. The sample was leaved in dried air for few minute in the room temperature and then was dried in conventional oven at 105 °C until constant weight (w_1 , g). The percent of extractive weight was calculated follow equation (1). Mineral components were determined by calcination at 575 °C for 6 h.

$$\% (w/w) = \{(w_0 - w_1) / w_0\} \times 100\% \quad (1)$$

2. Determination of hemicellulose [37]

1 gram of dried biomass from extractives was transferred in to 250 mL Erlenmeyer flask and then 150 mL of NaOH solution was added. The mixture was boiled with the distilled water. After cooling mixture, the sample was filtrated through vacuum filtration and washed (until pH value of solution approached neutral pH). The sample was dried until constant weight at 105 °C. The residue was cooled in a desiccator and weighted. The difference between sample weight before and after this treatment is the hemicellulose.

3. Determination of insoluble lignin [37]

300 mg of dried sample was weight and 3 mL of 72% H_2SO_4 was added and the sample was carried out at room temperature for 2 h with mixing of sample every 30 min. After 2 h, 84 mL distillate water was added into the sample and this step bringing the total volume to 87 mL. The samples were autoclaved for 1 h at 121 °C. After the second weak acid hydrolysis step, the samples were cooled to room temperature and filtered through the vaccum filtration. The acid insoluble lignin was determined by drying the residue at 105 °C and accounting for ash by incinerating the hydrolyzed samples at 575 °C. The acid insoluble lignin was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm.

4. Determination of cellulose [37]

The cellulose content was calculated by assuming that extractives, hemicellulose, lignin, ash and cellulose are only components of the entire biomass.

$$\% W_{\text{cellulose}} = 100 - (\% W_{\text{ash}} + \% W_{\text{extractives}} + \% W_{\text{hemicellulose}} + \% W_{\text{lignin}})$$

Where; % W is the % (w/w) of lignocellulosic components analyzed