CHAPTER III
MATERIALS AND METHODS

3.1 Materials

The dry powder of cassava waste was obtained from cassava flour plant. Glucose, fructose, xylose, dimethyl sulphoxide were purchased from Wako Pure Chemical Company (Osaka). Sulfuric acid and naphthalene were purchased from Fluka and Merck, Singapore.

3.2 Preparation and characterization of catalyst

3.2.1 Preparation of catalyst

Naphthalene (20 g) was heated with concentrated sulfuric acid (>96%, 200 mL) in a 4-neck round bottom flask, at 523 K under a flow of nitrogen as shown in Figure 3.1. When heating naphthalene and sulfuric acid, 1000 ml flask containing about 300 g of activated carbon was connected to the heated flask to adsorb acid vapor. After heating for 15 hours, the nitrogen inlet flow was closed, the flask containing activated carbon was connected to a vacuum pump and the dark brown tar in the round bottom flask was heated at 523 K under vacuum for 8 hours to remove the excess sulfuric acid. It is noted that the round bottom flask and all the connections were made from glass of PYREX® tubings. The black solid occurred in round bottom flask was then ground to powder, and was washed repeatedly in boiling water until sulfate ions were no longer detected in the washing water.

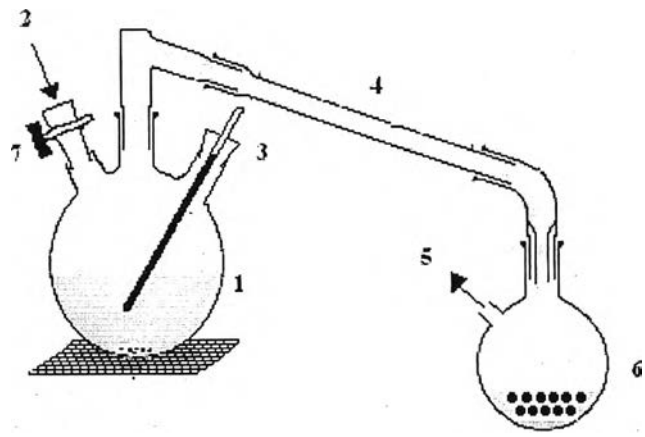

Figure.3.1 Apparatus setup for catalyst preparation instrument (1 : round bottom 4-neck flask, 2 : nitrogen inlet, 3 : thermometer, 4 : connection tube, 5 : vacuum pump, 6 : flask contained the activated carbon)

3.2.2 Catalyst Characterization

Boonnaul et al used the carbon based catalyst in reactive extraction to separate 1,3-PDO from a model solution of the fermentation broth and they characterized this catalyst as follow

3.2.2.1 Surface area, pore volume and sulfer content

The total surface area and pore volume of catalysts were determined using a Micromeritrics model ASAP 2020. The 0.5 g of sample cell was placed into Micromeritrics model ASAP 2020. After degassing, N_2 physisorption was carried out for measuring the surface area and pore volume of catalyst. The neutralization titration was applied in order to calculate the amount of the acidity. Here, mixture of iso-propyl-alcohol 12.5 ml and toluene 12.5 ml was replaced in 100 ml flask then added 1 gram of novel carbon based catalysts and 0.5 ml of phenopthalene. This solution was titrated with 0.25 molar of KOH [ASTM D6751]. The BET characterization results for the prepared carbon based catalysts and the amount of acide are shown in Table 3.1

Table 3.1 Physical properties of carbon based catalyst

BET surface area ($m^2\ g^{-1}$)	Pore volume ($cm^3\ g^{-1}$)	Sulfur content (mmol/g)
1.1	0.07	1.46

3.2.2.2 Concentration of acid sites

The sulfur content of sulfonated carbon based catalysts was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using 7500a ICP-MS (from Agilent, Japan). In detail, the catalyst was digested with 5 mL of HNO_3 (Suprapure, 65% v/v, Merck, Germany) and made up to 25 mL with ultrapure water at 18.2 mΩ, using Anton Paar Microwave Digester (Anton Paar, Austria) for testing. In addition, The XRD of sulfonated carbon-based catalyst was identified to confirm the sulfonated group by SIEMENS, D500 X-ray Diffractometer (Germany) using Ni-filtered CuK_α radiation. The measurements were carried out in the 2Θ range of 10-80 degree at the scan step of 0.04 degree. The result of the sulfur content of sulfonated carbon based catalysts was shown in Figure 3.2 and 3.3. Figure 3.2, showing the sulfonic group that was confirmed by the IR spectra. The strong absorption at 1600 - 1800 cm^{-1} which confirmed the existence of the S=O stretching and the absorption at

2600-3500 cm^{-1} confirmed the existence of OH functional group [Mo, 2008]. In addition, the XRD result of the catalyst shown in Figure 3.3 confirmed the sulfonated group of the carbon-based catalyst at 2Θ=12 and the carbon at 2Θ=25.

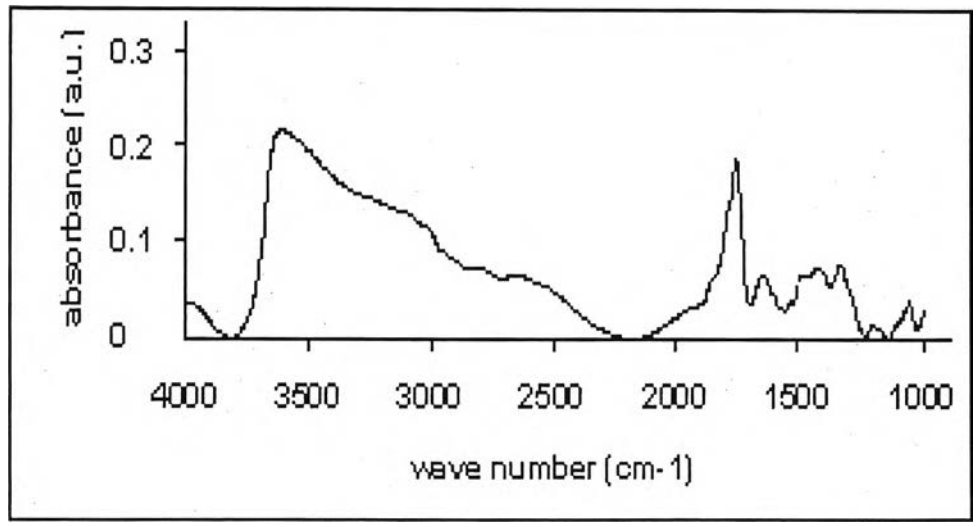

Figure 3.2 FTIR spectra of sulfonated carbon based catalyst.

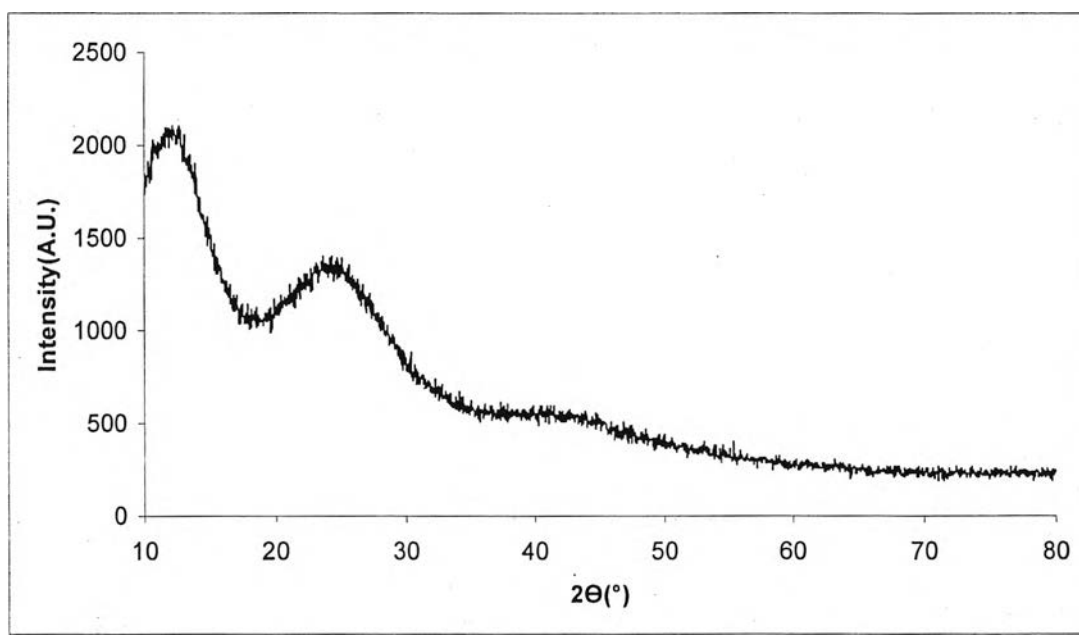

Figure 3.3 XRD of sulfonated carbon-based catalyst.

3.3 Experiment

3.3.1 Determination of suitable conditions for cassava waste conversion to HMF and furfural

The apparatus used for this experiment is shown in Figure 3.4, which consists of a 8.5 ml SS 316 stainless steel reactor, a furnace heater, and a temperature controller. The reaction was carried out in the reactor, into which 0.1 g of the dry powder of cassava waste and the reaction medium (2 wt % dry cassava waste) were charged. The reaction system was heated by a furnace heater to the desired temperature, and was controlled at a constant temperature by a controller connecting to it. The reaction was allowed to take place for a specified reaction time, after which the reaction was quenched in a water bath. The effects of reaction temperature, reaction time, type of reaction medium, types and dose of catalysts (carbon-based catalyst and conventionally used catalysts) were determined on the production yield. The ranges of different variables studied are summarized in Table 3.2.

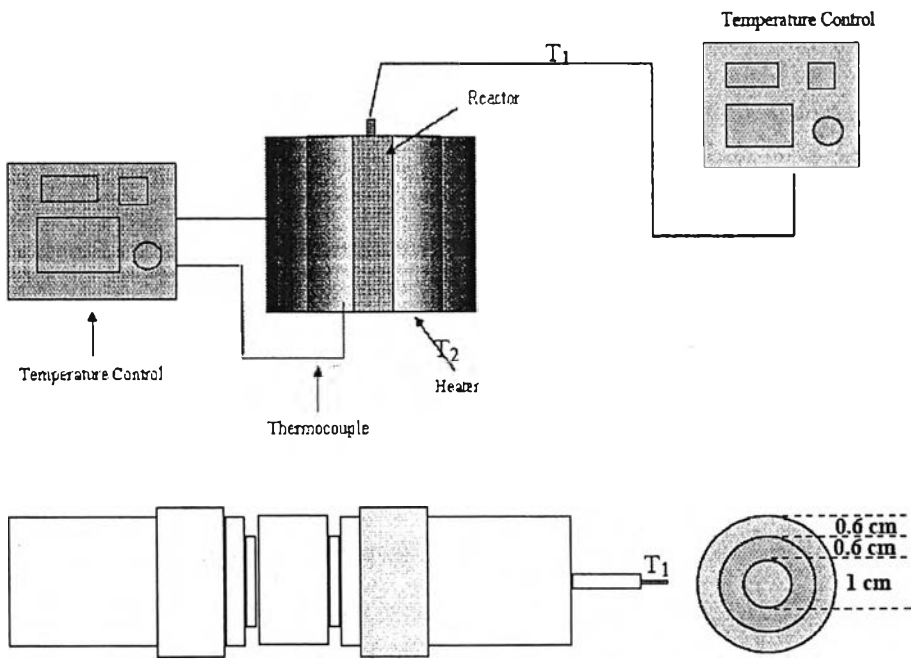

Figure 3.4 Experiment setup

Table 3.2 Experimental ranges of different conditions for cassava waste conversion

	Fixed variable	**variables**	**Range**	
1. To determine the composition of medium	Temperature at 250 °C	Composition of medium	Acetone/DMSO (70:30 w/w) to water 100:0, 90:10, 80:20, 70:30 to 0:100 (pure water)	
		Catalyst	Without catalyst	0.1 g of carbon based catalyst
2. To determine the suitable temperature	Composition of medium and catalyst from section 1	Temperature	180 ,190, 200, 210 and 220°C	
3. To determine the suitable time	Composition of medium catalyst and temperature from section 1 and 2	Time	0, 2, 5, 7, 10, 12 min	
4. To determine the dose of Catalyst	Suitable condition from section 1,2 and 3	Dose of carbon based catalyst	0.05 g, 0.1 g and 0.15 g	
5. To compare the activity of catalyst.	Suitable condition from section 1 2, 3 and 4	Kind of catalyst	Carbon based catalyst, sulfate zirconia and H_2SO_4	

3.3.2 Determination of the reaction pathway of HMF and furfural production using carbon based catalyst

To understand the reaction pathway, the reactions of fructose, glucose, xylose, hemicelluloses and cellulose were examined in the reaction medium at the most suitable composition determined previously. 0.1 g of each substrate and medium at suitable composition (2 w % of substrate) were charged in reactor. Fructose, glucose and xylose were reacted at 200°C while hemicelluloses and cellulose are reacted at 230°C. The reactions were carried out without and with 0.1g of carbon based catalyst for various times (0, 2, 5, 7, 10 and 12 min).

3.3.3 Determination of catalyst recycling

For possibility of recycling the catalyst, the reaction was carried out 4 more times after the initial use. The reaction of 0.1 g of fructose and medium at suitable composition (2 wt % of fructose) was carried out at 230 °C with carbon based catalyst at suitable dose.

3.4 HPLC Analysis

3.4.1 HMF and furfural

The quantification of HMF and furfural in the liquid product were conducted by using a high performance liquid chromatography (HPLC, Summit, Dionex Co., Germany) which consist of a Dionex PDA-100 photodiode array detector, a Dionex P680 pump system, a Dionex STH585 column oven and a Dionex ASI-100 automated sample injector equipped with a Shodex RSpak KC-811 (8.0mmID*300mm) column. The concentration of HMF and furfural are analysed based on UV absorbance at 280 nm by comparing to the corresponding standard curves. Phosphoric acid (H_3PO_4) was used as the eluent at a flow rate of 0.4 ml/min. Injection volume was 20 µl. The retention time for HMF and furfural was 49.5 and 80.5 min, respectively.

3.4.2 Glucose, fructose, xylose and 1,6-anhydroglucose

The liquid products were analyzed by HPLC to quantify the amount of glucose, fructose, xylose and 1, 6-anhydroglucose in the liquid product. The reversed phase HPLC analyses were carried out using Lichrocart NH2 250×4 mm column, a Diode Array Detector Module 335 and an automatic injector. The mobile phase was 98% acetonitrile in water, and the flow rate was at 1 ml/min. The sample injection volume was 20 µl. The retention time for AHG, xylose, fructose and glucose are 3.3, 4.9, 7.2 and 8.1, respectively.