CHAPTER III

EXPERIMENTAL SECTION

3.1 Materials and Chemicals

3.1.1 Cassava starch

Cassava or Tapioca starch was used as a polymer matrix and was dried in an oven 60 °C and kept in a desiccators before use. The commercial cassava starch composed of 17% of amylose content.

3.1.2 Bagasse and Banana Stem

Bagasse and Banana Stem were used to prepare microcrystalline cellulose which was used as a reinforcement.

3.1.3 Glycerol

A commercial grade of glycerol, obtained from Siam Chemical Industry Co.,Ltd.,Thailand, was used as a plasticizer

3.1.4 Sodium hydroxide

An analytical grade of sodium hydroxide, purchased from Fluka Co,Ltd. Thailand, was used as a delignin reagent.

3.1.5 Hydrogen peroxide

A technical grade of 35 %hydrogen peroxide, provided by Peroxy Thai Co., Ltd.,Thailand, was used as a bleaching reagent .

3.1.6 Sodium silicate

A analytical grade of sodium silicate supplied by Fluka Co., Ltd.,Thailand, was used as stabilizer reagent.

3.1.7 Hydrochloric acid

An analytical grade of hydrochioric acid, purchased from Sigma Aldrich Co.,Ltd., Thailand, was used as a hydrolysis reagent.

3.1.8 Sulfuric acid

An analytical grade of sulfuric acid, perchased from Sigma Aldrich Co., Ltd., Thailand, was used as a hydrolysis reagent.

3.1.9 Cupriethylenediamine hydroxide solution

An analytical grade of cupriethylenediamine hydroxide solution supplied by Calo Elba Co., Ltd., Singapore, was used as a solvent for microcrystalline cellulose.

3.1.10 Commercial microcrystalline cellulose

A commercial grade of microcrystalline cellulose, purchase from Fluka Co., Ltd., USA. An average particle size is 20 μm. It was used as microcrystalline cellulose reference.

3.1.11 Perlite

A commercial grade of perlite supplied by Thiland Co.,Ltd., Thailand.

3.2 Instrument

Table 3.1 Experimental instruments

Instrument	Model	Manufacture
1. IKA® mechanical stirrer	D-38678	Germany
2. Ubbelohe viscometer glass capillary	Type A size 100	Germany
3. Acrylic mold, size 28×28×0.5 cm		
4. Speed mill	Firtch	Germany
5. Macbeth UV-Vis spectrophotometer,	Color-Eye 700,	Germany
6. Laser Particle Analyzer	materziser	Germany
7.Thermogravimetric analyzer	TGA 7	Perkin Elmer
8. Philips X-rays diffractometer	PW 3710	Japan
9. Differential scanning calorimeter	Dimon DSC	Perkin Elmer
10. Tensile testing machine	LR100k	LLOYD
11. Scanning electron microscope	JSM-5410 LV	Jeol

3.3. Experimental Procedure

The flow chart of the experimental process is shown in Figure 3.1

3.3.1 Crystalline Cellulose Preparation

The agricultural wastes, bagasse and banana stem, were dried in sunlight and then dried in an oven at 60-70 °C overnight. After that, they were cut into size 2 cm length before pre-treatment with NaOH solution. The cut plants were soaked in various NaOH solution concentrations (0.5, 1, 2 M) for 24 hrs, and then heated and stirred at 80-90 °C for 4 hrs to produce fibrils. The obtained fibrils were washed with distilled water prior to bleaching. Next, the fibrils were bleached at different H_2O_2 concentrations (2%, 4%, 6%, 8%, and 10 % in 15w/v NaOH solution) at 100 °C for 1 hr. This bleaching step was treated twice. Subsequently, the bleached pulp was washed

several times with distilled water until the pH of the pulp became neutral. After that, the bleached pulp was hydrolyzed with two types of acid, HCl and H_2SO_4, and stirred vigorously at 70–80 °C for various times (30, 60, 120, 180 min). Then, the suspension was washed several times with distilled water until the pH became neutral and then it was filtrated. After that, the microcrystalline was dried in an oven at 60–70 °C overnight. The final microcrystalline was ground for 4 min to decrease its agglomeration and kept drying in a desiccator until being used

3.3.2 Biocomposite Film Preparation

Biocomposite flims were prepared by casting technique [18]. 6 w/v of cassava starch and 2 w/v of glycerol were first mixed and dispersed in distilled water and stirred at 70 -90 °C for 15 min. After 15 min, the gelatinization of starch was obtained while keeping the temperature of plasticized starch solution at 70-80 °C. Subsequently, the microcrystalline was added (0–30 wt% of starch) and stirred for another 15 min then the mixture was poured on the acrylic mold and dried overnight in an oven at 60 °C. After drying, the film was removed.

3.4 Selection of condition for crystalline cellulose preparation

3.4.1 Deligninfication condition

Thermal behavior all of delignified bagasse and banana stem was investigated using TGA analysis was carried out under nitrogen atmosphere at a heating rate of 20 °C/min from 50 °C to 500 °C. Prior to do the experimental, the samples were dried in oven at 60 °C for 24 hours. The onset degradation temperature (T_d) for each sample was recorded.

3.4.2 Bleaching condition

3.4.2.1 Whiteness of Pulp

Macbecth color-eye 700 spectrophotometer was used to study whiteness and yellowness of pulp according to ASTM 1925 and ASTM E313,

respectively. Bleached sample was cut in to 5X5 cm. Each sample was measured three times and the average value was reported.

3.4.2.2 Thermal gravimetric analysis (TGA)

TGA analysis was carried out under nitrogen atmosphere at a heating rate of 20 °C/min from 50 °C to 500 °C. The samples were dried in oven at 60 °C for 24 hours before test. The onset degradation temperature (T_d) for each sample was recorded.

3.4.2.3 Average Particle Size

Average Particle size data of pulp powder was determined by the Laser Particle Analyzer. The powder was dispersed in 20 ml of distilled water and sonicated for 20 min before testing

3.4.2.4 Morphological Study

The size and shape of the pulps powder were investigated by scanning electron microscope (SEM). The accelerating voltage applied was 15 kV. The surface of the sample was coated with a thin layer of gold before being scanned.

3.4.3 Hydrolysis condition

3.4.3.1 Average Particle Size

Average Particle size data of microcrystalline cellulose powder was determined by the Laser Particle Analyzer. The microcrystalline powder was suspended in 20 ml of distilled water before testing

3.4.3.2 Morphological Study

The size and shape of microcrystalline cellulose was investigated by scanning electron microscope (SEM). The surface of the sample was coated with a thin layer of gold before being scanned that is to improve the conductivity of samples and the quality of the SEM image. The system was running at 15 kV.

3.4.4.3. Degree of Polymerization

Degree of polymerization (DP) of cellulose was determined by intrinsic viscosity method followed the ASTM D1795-96. This test method was examined the intrinsic viscosity of sample by using the relative viscosity of cellulose solution and solvent. Then the intrinsic viscosity was used to calculate the degree of polymerization. The details of the experimental procedures are described as follows.

3.4.4.3.1 Preparation of Stock Cellulose Solution

Microcrystalline cellulose was dried in an oven at 60 °C overnight. Stock of 2g/l of cellulose solution was prepared. 0.1 gram of microcrystalline was filled in 100 ml glass bottle and 50 ml of 0.5 M Cupriethylenediamine hydroxide solution was added . Nitrogen gas was flushed in the solution before closing. After that, the solution was stirred until the sample was completely dissolved.

3.4.4.3.2 Measurement of Solvent Flow time (t_0)

10 ml of 0.5 M cupriethylenediamine hydroxide solution was poured into viscometer then it was flushed nitrogen before closing with aluminium foil previously placed in the water bath at 25 °C for 5 minutes. After 5 minutes, the pressure was appiled with a rubber bulb to push solvent into the upper of the viscometer. Beginning of record time was the meniscus of the solvent passes the upper line located the top of viscometer. And it was stopped when the solvent meniscus passes the lower line located at the bottom of viscometer then the solvent flow time was

measured. The measurement was repeated at three times and then the average solvent flow time was calculated.

3.4.4.3.3 Measurement of Cellulose Solution Flow time (t_n)

The stock of cellulose solution in section 3.5.4.1 was diluted into five concentrations (1, 0.8, 0.6, 0.4, 0.2 g/dl) by adding distilled water and mixing well. The diluted solution was flushed with nitrogen gas before closing. The flow time of cellulose solution was then measured by the same procedure as those previously describe for measuring the solvent flow time. This process was repeated three times and the flow time of each dilute cellulose solution was measured and average.

3.4.4.3.4 Calculation the Degree of Polymerization

The degree of polymerization of cellulose sample was determined as follows. First, the relative viscosity (η_{rel}) was calculated from the ratio of the cellulose solution flow time (t_n) and solvent flow time (t_0) as shown in the equation (3.2):

$$\eta_{rel} = t_n/t_o \qquad \text{(Equation 3.2)}$$

where:

η_{rel} = relative viscosity

T_n = flow time of diluted cellulose solution

t_o = flow time of solvent

The data was plotted with relationship of log $[(\eta_{rel}-1)/C]$ and C follows Martin equation as shown in equation (3.3). to plot the straight line. The interception of the extrapolated line was obtain the intrinsic viscosity, $[\eta]$ and the intrinsic viscosity was calculated.

$$\log [(\eta_{rel}-1)/C] = \log [\eta] + K[\eta]C \quad \text{(Equation 3.3)}$$

where:

$[\eta]$ = intrinsic viscosity

η_{rel} = relative viscosity

K = constant

C = concentration of diluted cellulose solution

Then, the degree of polymerization of microcrystalline cellulose was calculated by multiplying the intrinsic viscosity by 190 as shown in equation 3.4

$$DP = [\eta]x190 \quad \text{(Equation 3.4)}$$

3.5 Characterization of microcrystalline cellulose

3.5.1 Crystallinity study

A Philips X-ray diffractometer (model PH 3710) was employed to characterize Crystallinity of the microcrystalline. The samples were laid on the glass sample holders and analyzed under plateau conditions. The scan scope was between 2° and 50°.

3.5.2 Thermal Properties

TGA was carried out under nitrogen atmosphere at a heating rate of 20 °C/min from 50 °C to 500 °C. Before doing the experiment, the sample was dried in an oven at 60 °C for 24 hours and weighed about 4 mg. The onset of degradation temperature (T d) for each sample was recorded

3.6 Evaluation of Biocomposites Films

3.6.1. Physical Appearance

3.6.1.1 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) was used to investigated the surface and cross section of biocomposite films. The surface of the sample was coated with a thin layer of gold prior to being scanned in order to prevent the sample was destroyed.

3.6.1.2 Haze

The haze of biocomposite films was measured by Macbeth Color-Eye 700 spectrophotometer. The percent transmittance of calibrated white standard and biocomposite films sample was measured in renge of 400-750 nm.

3.6.2 Mechanical Properties

Mechanical properties of the biocomposite films, such as tensile strength, elongation at break and Young's modulus, were determined according to ASTM D882 using a crosshead speed of 50 mm/min and a gauge length of 10 cm. The average thickness of the films was less than 0.25 mm. At least five specimens of each rectangular sample (1.5x2.5 cm) were tested and averaged.

3.6.3 Thermal Properties

3.6.3.1 Thermogravimetric Analyzer (TGA)

TGA analysis was performed under nitrogen gas at a heating rate of 10 °C/min from 50 °C to 800 °C. Before doing the experiment, the sample was dried in an oven at 60 °C for 24 hours and weighed 0-10 mg The onset of degradation temperature (T d) for each sample was recorded.

3.6.3.2 Differential Scanning Calorimeter (DSC)

Sample size with an average of mg encapsulated in a hermetically sealed aluminum pan was prepared for each sample. The sample temperature history was applied to all samples| first heating from -50 to200, followed by quenching the sample to 200 to -50 °C to remove any previous thermal history, and finally heating again to 200 °C at a heating and cooling rate of 20 °C/min. The melting temperature (T_m) and heat of fusion (ΔH_f^*) of the sample were obtained from the maximum peak and the area under the peak, respectively.

3.6.2 Water Absorption

The water absorption test was performed followed the ASTMD570-98 The specimen size was 1.2x7.5x0.25 dried in an oven at 60°C for 1 h. Immediately upon cooling, the specimens were weighed. After that, the specimens were immersed in distilled water at ambient temperature and weighed at 2 and 24 hour. After 24 hours, specimens were gently wiped dry using clean, lint-free tissue paper and allowed to stand free at ambient environment for 2 min. The weight gain of this specimen was compared to the weights of both dried and water absorbed. The water gain percentage, M%, was determined from the equation:

$$M\% = \frac{(W - Wd)}{Wd} \times 100$$

Follow :

M% = water gain percentage

W = weight of the water absorbed specimen

Wd = initial weight of the dry specimen

3.6.5. Biodegradation Test

Soil burial test was performed to evaluate biodegradation of the biocomposite films. The samples of each biocomposite films were cut in to size 1.5x15 cm and then placed in a basket containing a multi-layer substrate that is the films were

sandwiched between two layers of a mixture of 100 g of milled perlite and of 200g of soil, moistened with 100 ml of distilled water. The bottom and top layers were contained with 60g of perlite moistened with 120 ml of distilled water. The biodegradation of biocomposite films was followed during soil burial test for three months and were removed for testing their biodegradation at every three days. After removal, the samples were brushed softly, washed with distilled water and dried under vacuum at 60 °C for 24 hours before testing. Measurement of biodegradable was evaluated as the percentage weight loss and SEM analysis.

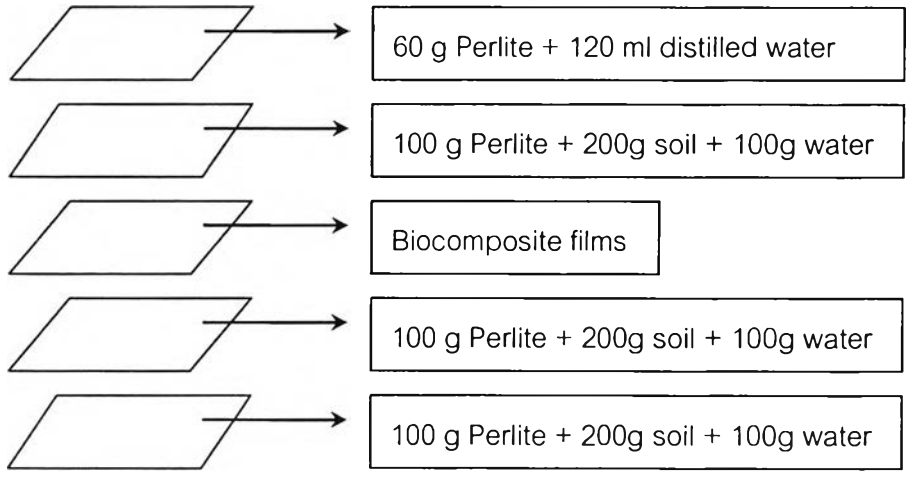

Figure 3.1 Schematic of the multilayer of soil burial test

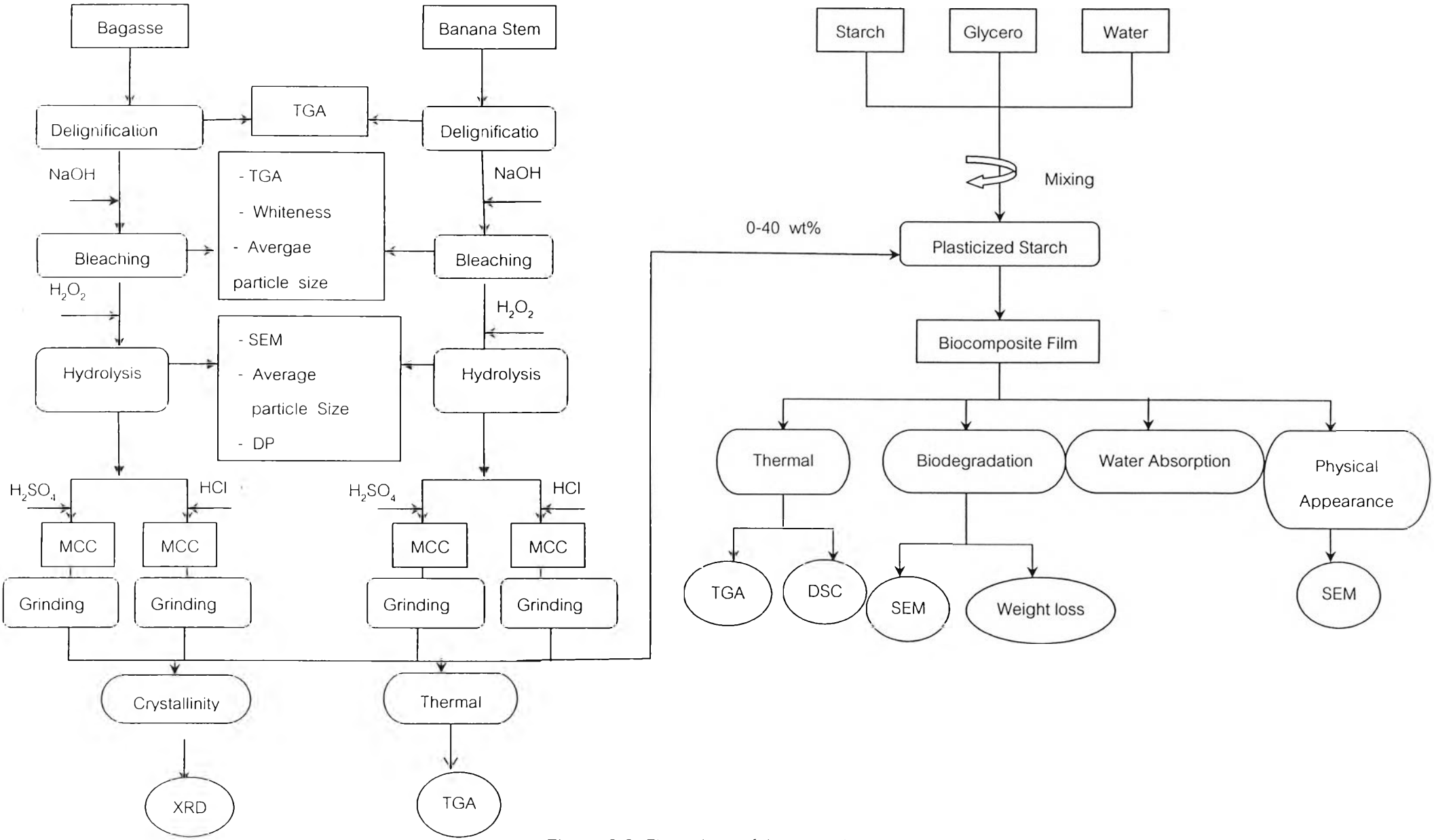

Figure 3.2 Flow chart of the experimental