CHAPTER III
EXPERIMENTAL

3.1 Materials

1. Sodium hydroxide (NaOH, Labscan Asia Co.)
2. Sulfuric acid (H_2SO_4, Merck Co., Germany)
3. D-(+)-Glucose (G5400) (Sigma Aldrich Chemicals Co. Inc., USA)
4. D-(+)-Xylose (X3877) (Sigma Aldrich Chemicals Co. Inc., USA)
5. D-(-)-Arabinose (A6085) (Sigma Aldrich Chemicals Co. Inc., USA)
6. Ethanol (C_2H_5OH, J.T. Baker)

3.2 Equipment

1. CEM Corporation Mars 5 version 049104 : Microwave Accelerated reaction Systemsusing power 300 W, 600 W, and 1200 W at requied temperature and time
2. Shimadzu Corporation: High Performance Liquid Chromatography (HPLC) with a refractive index detector(RID-10A, Shimadzu Corp., Kyoto, Japan) using an Aminex-HPX 87H column (300 mm x78 mm, Bio-Rad Lab, USA) under 20 μl injection volume, 0.005 M sulfuric acid (HPLC grade) as mobile phase, 0.6 ml/min flow rate, 65 °C column temperature, and 20 min run time condition
3. Scanning Electron Microscope (SEM) (Hitachi/S-4800)
4. Fourier transform infrared spectrometer (FTIR,Nicolet nexus 670)
5. UV-Visible spectrophotometer (Shimadzu/ UV-1800)
6. Particle size analyzer (Malvern / Mastersizer X)
7. pH meter (Hanna Instrument)
8. Analytical balance
9. Oven
10. Water bath
11. Autoclave

12. Muffin furnace using 8 step ramping programme [1. 105 °C/30 min, 2. 105 °C/12 min, 3. 250 °C/15 min, 4. 250 °C/30 min, 5. 575 °C/17 min, 6. 575 °C/180 min, 7. 30 °C/180 min, 8. 30 °C/24 h]
13. Vacuum oven at 105 °C
14. Soxhlet extraction
15. Rotary evaporation (Heidolph WB 2001)
16. Filtration setup

Software:

1. Omnic
2. LCsolution

3.3 Methodology

3.3.1 Biomass Preparation

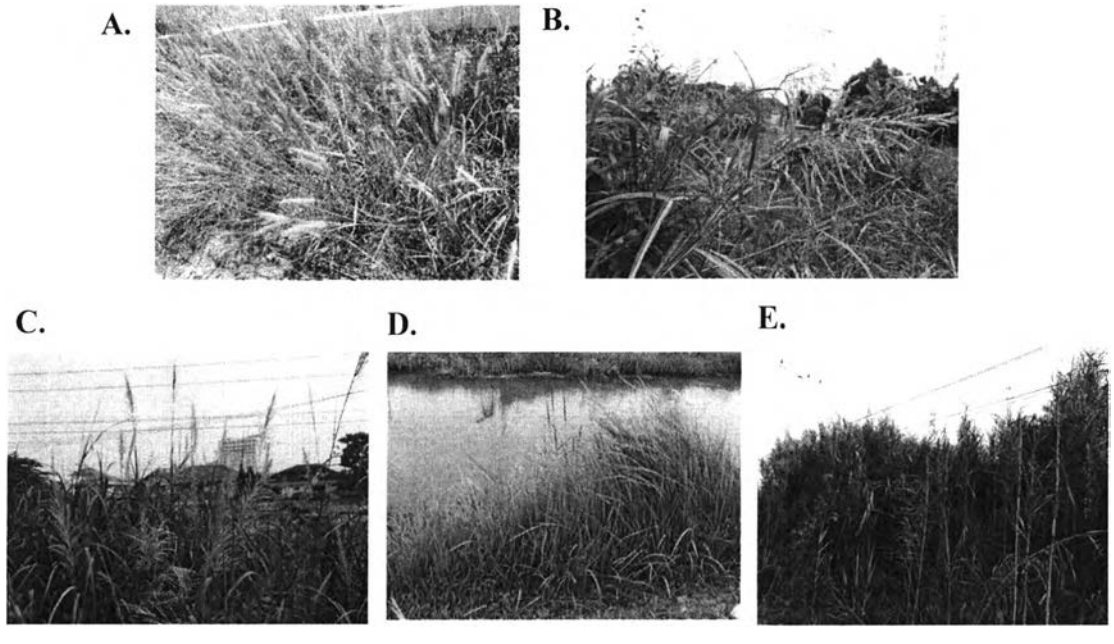


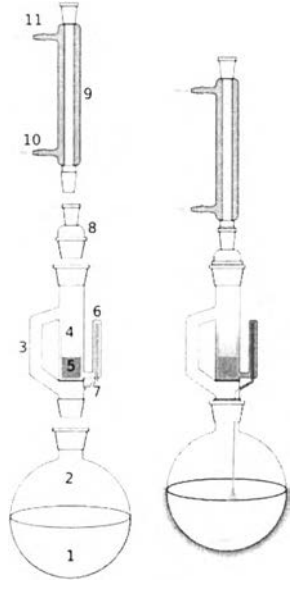
Figure 3.1 Grass samples grown in Thailand a.)Mission grass (*Pennisetumpolystachyon*), b.)Guinea grass (*Panicum maximum*), c.)Kans grass (*Saccharumspontaneum*), d.)Cogon grass (*Imperetacylindrica*), and e.) Giant reed (*Arundodonax*)

Cogon grass (*Imperetacylindrica*) and Giant reed (*Arundodonax*) were collected at road-side from Nakornpathom province, Guinea grass (*Panicum maximum*) and Kans grass (*Saccharumspontaneum*) were obtained from wasteland in Bangkok, and Mission grass (*Pennisetumpolystachyon*) was collected from Pakchong distinct, NakornRatchasima province. All biomass samples (only leaves and stem part) were cut into small pieces and air dried under sunlight. The dried samples were milled to get small particles using hammer mill machine until 60 mesh size. All of them were stored in plastic bags at room temperature for further uses.

3.3.2 Composition analysis of raw biomass

Raw biomass samples were determined the chemical compositions using a two step acid hydrolysis method developed by the National Renewable Energy Laboratory (NREL) (Sluiter*et al.*, 2006). All analytical determinations were repeated for 3 times and averaged the results for both accuracy and precision.

Before performing chemical composition determination, the biomass raw materials were extracted consecutively with water and ethanol (two-step extraction procedure) to determine extractive in biomass by Soxhlet extractor (Fig. 3.2). Sample (≈ 2 g) was added into an extraction thimble, before inserting the thimble into the Soxhlet tube. Water (HPLC grade, 190 ml) was added to the receiving flask equipped with the Soxhlet apparatus. A minimum 4-5 siphon cycles per hour was set before refluxing for 24 h. Then, water extracted solution was collected beforeethyl alcohol (190 ml) was added and continued refluxing for another 24 h. The extracted solid was collected when the reaction completed and dried with air dry for further composition analysis. Solvent (water and EtOH) was evaporated off using a rotary evaporator. After all solvent was removed, the flask was heated in a vacuum oven at 40°C for 24 h andweighed to determine water extractive and EtOH extractive.



1: Stirrer bar
2: Receiving flask
3: Distillation path
4: Thimble
5: Solid
6: Siphon top
7: Siphon exit
8: Expansion adapter
9: Condensor
10: Cooling water in
11: Cooling water out

Figure 3.2 A schematic representation of a Soxhlet extractor (http://en.wikipedia.org/wiki/Soxhlet_extractor_)

In two step hydrolysis process, the 72% sulfuric acid (3 ml) was added into the extracted sample (300mg) and stirred at 30 °C for 60 min, followed by a second 4% sulfuric acid hydrolysis at 121 °C for 60 min using autoclave. The autoclaved hydrolysis solution was vacuum filtered through a Buchner funnel. The filtrate was collected for further analysis (Fig 3.3).

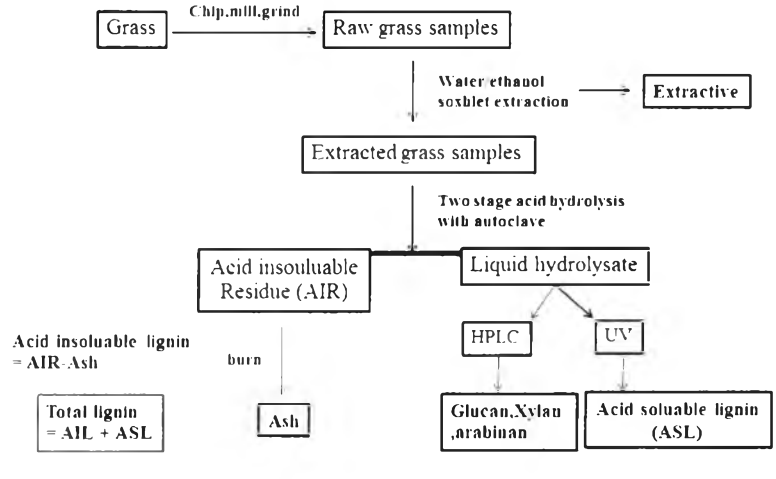


Figure 3.3 A schematic representation of chemical composition analysis methods for biomass feedstocks from National Renewable Energy Laboratory.

Amount of monomeric sugars (glucose,xylose, and arabinose) was determined by HPLC (Shimadzu) equipped with refractive index (RI) detector using the following condition: 20 μl injection volume, 0.005 M sulfuric acid (HPLC grade) as a mobile phase, a flow rate of 0.60 ml/min, 65 °C column temperature, and 20 min run time.

Amount of acid soluble lignin (ASL) was determined by UV-visible spectroscopy. The absorbance of the filtrate was measured at 205 nm, using a 1 cm light path cuvette and distilled water as a reference blank. Amount of acid insoluble lignin (AIL) content was defined as the difference between the weight of filter cake (oven-dried at 105 °C to constant weight) and the weight of ash.

Amount of the ash content was determined by placing the dried solid residue in the muffle furnace at 575 °C for 8 h. Total lignin content is the sum of acid soluble lignin (ASL) and acid insoluble lignin (AIL).

In addition, the chemical composition of biomass sampleswas characterized on FTIR with a resolution of 1 cm^{-1} over the wavelength range of 400–4000 cm^{-1}. For FTIR sample preparation, the solid samples were dried and pressed into a disc with KBr. Moreover, SEM was also used to characterize the physical structure of biomass sample. From the composition analysis results, the 3 types of weed were chosen to examine the ethanol production potential.

3.3.3 Microwave-assisted two stage pretreatment process of raw biomass sample

Microwave-assisted two-stage pretreatment process was used to release monomeric sugar from lignocellulosic biomass. The schematic diagram of the optimum condition determination in this process is shown below.

Stage 1: Microwave-assisted dilute alkaline pretreatment

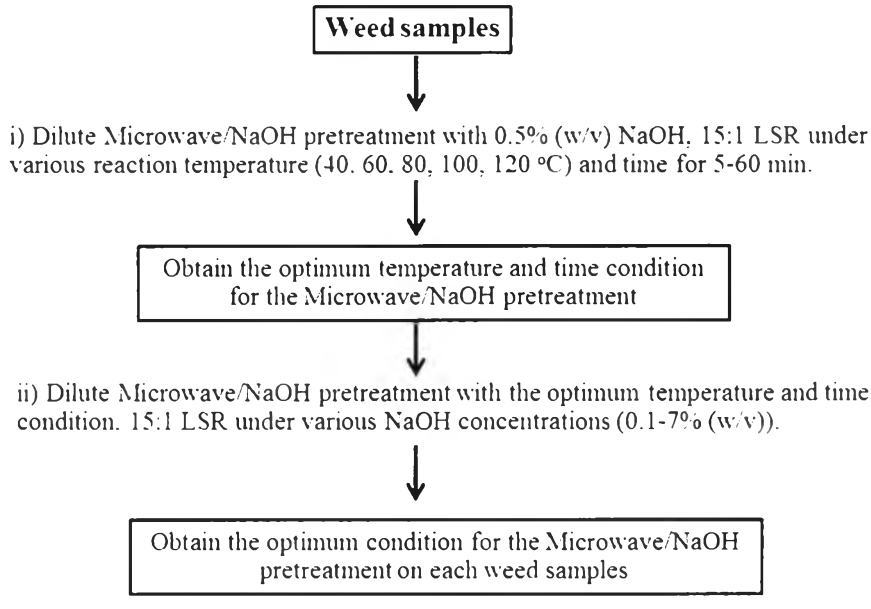


Figure 3.4 A schematic representation ofmicrowave-assisted dilute NaOH pretreatment process

Microwave-assisted dilute alkaline pretreatment

In microwave pretreatment, each of the weed samples was suspended in NaOH solution (0.5% (w/v)), using 15:1 liquid-to-solid ratios (LSR) (ml of solution: g of weed sample). The mixture was stirred until homogeneous before transferring to a Teflon-vessel sealed with a Teflon cap. The microwave pretreatment was performed under various temperatures (40–120 °C) and times (5–60 min). The mixture was filtered to separate solid residues from filtrate fraction after the pretreatment step. After the optimum temperature and time were obtained, weed samples was also suspended in NaOH solution (0.1-7 % (w/v) using 15:1 liquid-to-solid ratios at optimum temperature and time condition to get the optimum condition in microwave/NaOH pretreatment process (Fig. 3.5).

The liquid fraction was collected for monomeric sugar analysis by HPLC. Measurement of pH of liquid fraction after pretreatment was performed. The collected solid residues were thoroughly washed with distilled water to neutral pH and dried in the oven. Finally, the oven-dried samples were weighed to compare with unpretreated sample (measure % weight loss). The pretreated solid at optimum

condition was stored in valves bags for further dilute acid pretreatment in the two-stage pretreatment study and characterization.

Stage 2: Microwave-assisted dilute acid pretreatment

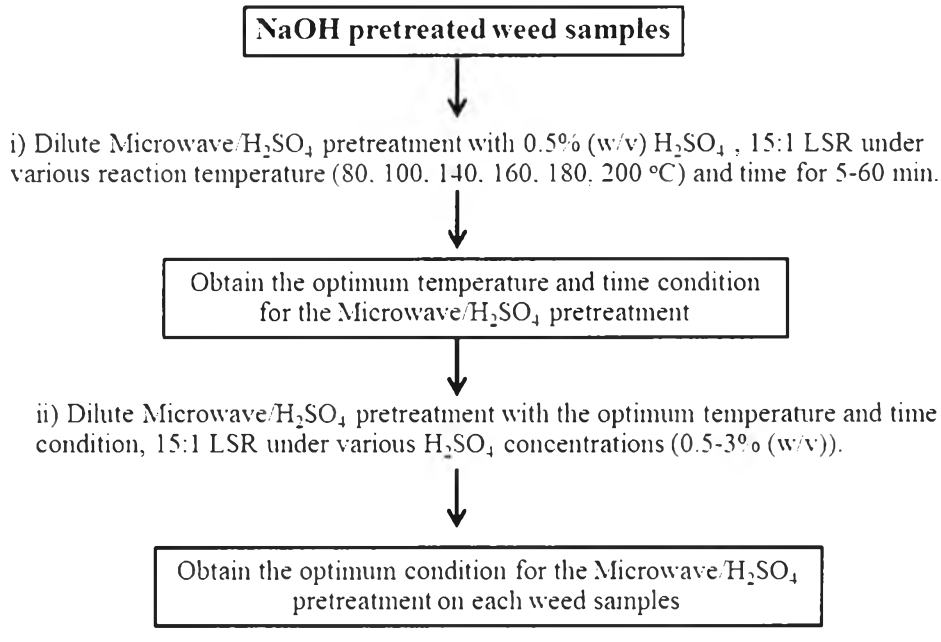


Figure 3.5 A schematic representation of Microwave assisted dilute H_2SO_4 pretreatment process.

Microwave-assisted dilute acid pretreatment

The solid residues from the NaOH pretreatment with optimum condition (giving the highest monomeric sugar release) were treated with microwave/dilute H_2SO_4. The pretreated weed samples were mixed with dilute acid solution (0.5%(w/v)) using 15:1 LSR.The microwave pretreatment was conducted under various temperatures (80–200 °C) and times (5–60 min) to obtain optimum temperature and tine condition. Then, the microwave/H_2SO_4pretreatment was conduct at optimum temperature and time condition under various H_2SO_4concentrations (0.5-3% (w/v)) to get the optimum condition of this process. After the acid pretreatment, the liquid fraction was collected for monomeric sugar analysis by HPLC and solid residues were thoroughly washed with distilled water to neutral pH, and dried in the oven for further characterization. The oven-dried

samples were weighed for comparison. Measurements of pH of the liquid fraction after pretreatment were also performed.

3.3.4 Compositional analysis of pretreated biomass

Pretreated biomass composition was analyzed for glucose, hemicelluloses sugar, acid soluble lignin (ASL), and acid insoluble lignin (AIL) contents by using the same procedure as that used for the raw biomass, except that no extraction step was needed. All analytical determinations were performed in triplicate and averaged results for both accuracy and precision. Chemical characteristics of the pretreated solid fraction were also determined by FTIR technique using the same procedure as that used in the raw material, as well. The physical structures of pretreated samples were studied by SEM.