CHAPTER IV

CAPABILITY OF THAI MISSION GRASS (*PENNISETUM POLYSTACHYON*) AS A NEW WEEDY LIGNOCELLULOSIC FEEDSTOCK FOR PRODUCTION OF MONOMERIC SUGAR

4.1 Abstract

Mission grass (*Pennisetum polystachyon*) grown in Pakchong District, Nakornratchasima Province, Thailand, with high cellulose and hemicellulose contents were harvested to determine the fermentable monomeric sugars for bioethanol production by two-stage microwave/chemical pretreatment process. Microwave-assisted NaOH pretreatment effectively removed approximately 85 % lignin content in Mission grass, using 3 % (w/v) NaOH, 15:1 liquid-to-solid ratio (LSR) at 120 °C temperatures for 10 min. As a result, in the second stage, microwave-assisted H_2SO_4 pretreatment of an alkaline-pretreated Mission grass solid released an impressively high fermentable sugar content (34.3 ± 1.3 g per 100 g of dried biomass), consisting mainly of 31.1 ± 0.8 g of glucose per 100 g of dried biomass, using 1 % (w/v) H_2SO_4, 15:1 LSR at 200 °C temperature for a very short pretreatment time (5 min). The total monomeric sugar yield obtained via two-stage microwave/chemical process was 40.9 g per 100 g of dried biomass.

(**Keywords**: Two-stage pretreatment; Microwave irradiation; Bioethanol production; Lignocellulosic biomass)

4.2 Introduction

Industrialization and world population growth have continually increased the demand for energy even while the costs of the main energy sources, such as crude oil, coal, and natural gas, have also increased. Moreover, the shortage of energy sources and the increasing level of greenhouse gasses compounds (GHCs) has become a major world problem (Ballesteros *et al.*, 2006). Awareness of global climate change and the uncertainty of fossil fuel have thus led to the development of bioethanol as an alternative liquid transportation fuel. Currently, human and animal food chains are being disturbed because most of the world's bioethanol is produced from unsustainable and high-cost food crops, such as corn, barley, oat, rice, wheat, sorghum, and sugarcane (Balat, 2011). The utilization of non-food crops, such as weedy materials, as biofuel feedstock is an alternative route for bioethanol production (Chandel and Singh, 2011). For example, Bermuda grass (*Cynodondactylon*) found in Southern United State was specially considered to be a promising feedstock for bioethanol, biogas, and syngas production. It is a cheap feedstock applied for nutrient management in animal farms, thus could be used as biomass feedstock. However, the effective recalcitrance technique and genetic engineering for Bermuda grass and microorganism have still developed for biofuel feasibility (Xu *et al.*, 2011). Thailand also has inedible plants which are suitable for biomass feedstock in South East Asia region, and needs to develop cellulosic ethanol technology.

Mission grass (denoted as MG, *Pennisetum polystachyon*) is a common weed naturally grown in India, Sri Lanka, Malaysia, Philippines, Australia, and also many parts of Thailand. A tall, perennial weed, MG is normally found on road sides and in cultivated areas, where it displaces desirable plants and spreads easily (Miller and Darwin, 2006). Slashing, burning, and spraying with herbicide are the general methods used in eradicating the weed. Therefore, conversion of MG into bioethanol would be an effective process for both weed management and bioethanol production in Thailand, eliminating the need for reseeding and fertilizing.

The key step in converting the MG and other lignocellulosic biomass feedstocks into bioethanol is the effective pretreatment and hydrolysis process to

produce high amount of fermentable sugars. Several pretreatment processes are used to remove lignin, partially solubilize hemicelluloses, decrease cellulose crystallinity, and increase the porosity of material (McMillan, 1994). Two-stage microwave/chemical pretreatment is a promising pretreatment technique used to release high monomeric sugar yields with high heating efficiency, easy operation, and short reaction time (Boonmanumsin *et al.*, 2012).

MG is widely distributed throughout Thailand. To make use of it, this study focused on the production of monomeric sugars from Thai MG via microwave/NaOH pretreatment followed by microwave/ H_2SO_4 pretreatment at various temperatures for various times. Both acid and base concentrations were investigated to determine the optimum conditions of the two-stage pretreatment process that released the highest fermentable sugar yield. The structural changes in MG from the two-stage microwave/chemical pretreatment process were also characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM).

4.3 Experimental

4.3.1 Materials and Chemicals

Mission grass (*P. polystachyon*) was collected from Pakchong District, Nakhon Ratchasima Province, Thailand. Only stems and leaves were air and sun dried before being pulverized with a hammer mill to obtain 60 mesh size powders for the experiment. MG powders were then stored in plastic bags at room temperature for further uses.

Sodium hydroxide (NaOH, Labscan Asia Co.) and sulfuric acid (H_2SO_4 , Merck Co., Germany) were directly used in the pretreatment process without purification. D-(+)-Glucose (G5400), D-(+)-Xylose (X3877), and D-(-)-Arabinose (A6085) standards (Sigma–Aldrich Chemicals Co. Inc., USA) were used for sugar quantitative analysis.

4.3.2   Characterization

The particle size of the MG powder was detected by a particle size analyzer (Malvern/Mastersizer X) with the 300 mm lens size in sample detection unit. The physical structure images of untreated and pretreated MG were obtained using scanning electron microscope (SEM, Hitachi/S-4800) at 2 kV accelerating voltages. The chemical structure change of MG from untreated sample to pretreated sample in the two-stage microwave/chemical pretreatment process was detected by Fourier transform infrared spectrometer (FTIR, Nicolet nexus 670) in a wavenumber range of 400–4000 cm^{-1} with a resolution of 1 cm^{-1} and 64 scans per sample. After each pretreatment, the liquid fraction was analyzed for monomeric sugars by high-performance liquid chromatography (HPLC, RID-10A, Shimadzu Corp., Kyoto, Japan) equipped with a refractive index detector, an Aminex-HPX 87H column (300 x 78 mm, Bio-Rad Lab, USA) using 0.005 M sulfuric acid (HPLC grade) as mobile phase.

4.3.3 Chemical Compositions Analysis

The chemical compositions (cellulose, hemicellulose, lignin and ash) of MG, microwave/NaOH-pretreated MG, and two-stage microwave/NaOH-pretreated MG were analyzed using the two step acid hydrolysis method developed by the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2011). All analytical determinations were repeated three times and the results averaged for both accuracy and precision.

4.3.4   Microwave-assisted Two-stage Microwave/Chemical Pretreatment Process

4.3.4.1   Microwave-assisted Dilute Sodium Hydroxide (NaOH) Pretreatment

NaOH pretreatment is an effective method for removing lignin and reducing cellulose crystallinity (Chen *et al.*, 2009). In other words, the main function of the NaOH pretreatment is to delignify the biomass by separating structural linkages between lignin and carbohydrates, and disrupting the lignin structure by salvation and saphonification of biomass (Fan *et al.*, 1987). In addition,

NaOH solubilizes some parts of hemicelluloses. Thus, in this study, MG was first pretreated with a microwave/NaOH pretreatment process. The optimum conditions for the release of the highest amount of monomeric sugars using the microwave-assisted NaOH pretreatment were investigated.

In microwave/NaOH pretreatment, MG powders were suspended in NaOH solution (0.5 % (w/v)) using 15:1 liquid-to-solid ratios (LSR) (ml of solution: g of MG samples), a suitable ratio for releasing high monomeric sugar yields (Boonmanumsin *et al.*, 2012). The mixture was stirred until homogeneous, and then transferred to a Teflon-vessel sealed with a Teflon cap. The microwave pretreatment was performed under various temperatures (40–120 °C) and for various times (5–60 min) by microwave-accelerated reaction system (CEM Corporation Mars 5 version 049104, 300–1200 W). The mixture was filtered to separate solid residues from filtrate fraction after the pretreatment step. After the optimum temperature and time were obtained, the MG sample was also suspended in NaOH solution (0.1–7 % (w/v) using 15:1 LSR at optimum temperature and time conditions to get the optimum condition in the microwave/NaOH pretreatment process to release the highest monomeric sugar yields.

The liquid fraction was collected for monomeric sugar analysis by HPLC. Measurement of pH of the liquid fraction after pretreatment was performed by pH meter (Hanna Instrument). 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 untreated samples to measure % solid loss. The pretreated solid at optimum conditions of microwave/NaOH pretreatment was neutralized, dried, and stored in valve bags for further study and characterization of dilute acid pretreatment in the two-stage pretreatment.

4.3.4.2   *Microwave-assisted   Dilute   Sulfuric   Acid   (H$_2$SO$_4$) Pretreatment*

Two-stage microwave/chemical pretreatment process is an effective method for producing high monomeric sugars from lignocellulosic biomass with high heating efficiency and short reaction time (Boonmanumsin *et al.*, 2012). After removing lignin and reducing cellulose crystallinity by a microwave/alkaline pretreatment stage, the microwave/acid pretreatment stage is required to hydrolyze

the polysaccharides and provides the maximum monomeric sugar yield for bioethanol production. In recent years, acid pretreatment by sulfuric acid at concentrations below 4 % was found to be the most effective pretreatment to improve hydrolysis and achieve a high reaction rate (Esteghlalian *et al.*, 1997). When compared to other types of dilute acid (phosphoric, hydrochloric, and maleic acids), sulfuric acid results in the highest total monomeric sugars (Feng *et al.*, 2011). Thus, sulfuric acid was adopted in this study.

The solid residues from the microwave/NaOH pretreatment with optimum conditions were treated with microwave/dilute H_2SO_4. The alkaline-pretreated MG samples were mixed with dilute acid solution (0.5 % (w/v)) using 15:1 LSR. The microwave/dilute H_2SO_4 pretreatment was conducted under various temperatures (80–200 °C) and for various times (5–60 min) to obtain optimum temperature and time conditions. Then, the microwave/H_2SO_4 pretreatment was conducted at optimum temperature and time condition under various H_2SO_4 concentrations (0.5–3 % (w/v)) to get the optimum conditions of this process. After the acid pretreatment, the liquid fraction was collected for monomeric sugar analysis by HPLC technique. 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. Measurement of pH of the liquid fraction after pretreatment was also performed.

4.3.5 Total Monomeric Sugar Analysis

Liquid fraction from the pretreatment of MG was analyzed for monomeric sugar (glucose, xylose, and arabinose) contents using HPLC and sugar standard curves. Sugar standard curves were plotted from the relationship between sugar concentrations of standard glucose, xylose, and arabinose and the peak area signals from HPLC to obtain standard equations. The obtained equations showing the relationship of the sugar contents and the peak areas of glucose, xylose, and arabinose were $Y = (4 \times 10^{-6})X + 0.0421$, $Y = (4 \times 10^{-6})X + 0.0952$, and $Y = (4 \times 10^{-6})X + 0.0396$, respectively ($Y$ = sugar concentration (g/l), X = peak area). The monomeric sugar yields in g/100 g biomass unit were calculated according to

Boonmanumsin *et al.* (2012). All sugar determination was analyzed three times and the results averaged for both accuracy and precision.

4.4 Results and Discussion

4.4.1 Chemical Composition of MG

The method developed by the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2011) was used to analyze the MG chemical composition, and the results are summarized in Table 1, comparing our results to those obtained by Premjet *et al.* (2013) who collected MG from other provinces in northern part of Thailand and used different analysis methods. Both results confirm that MG contains high polysaccharide content (39.8 ± 1.5 % cellulose, 29.2 ± 1.0 % hemicellulose); however, Thai MG from Nakhon Ratchasima Province seems to contain more lignin content, which is a major drawback in cellulose hydrolysis.

4.4.2 Particle Size Analysis

Because the size of a biomass sample can have an impact on monomeric sugar conversion (Vidal *et al.*, 2011), mechanical pretreatments by chopping, cutting, milling, and grinding were performed to reduce the size, to decrease cellulose crystallinity and to increase the biomass sample's surface area (Quintero *et al.*, 2011). The particle size analyzer was then used to confirm the exact sample size in this study, showing the average particle size (i.e. mean diameter) of MG powder after grinding with 60 mesh sieving size to be around 330 μm.

4.4.3 Optimization of Microwave-assisted NaOH Pretreatment

4.4.3.1 Effect of Temperature and Time

The optimal microwave temperature and time for Thai MG was first studied at the temperature and time ranges of 40–120 °C and 5–60 min, respectively, using 0.5 % (w/v) NaOH and 15:1 LSR. The results in Figure 4.1 show that MG released the highest monomeric sugar of 6.0 ± 0.3 g/100 g biomass, consisting of 3.9 ± 0.2, 1.1 ± 0.1, and 1.0 ± 0.1 g/100 g biomass of glucose, xylose,

and arabinose, respectively, at 120 °C for 10 min. The effects of temperature and time on monomeric sugars released from MG were also investigated, as shown in Figures 4.1a–f. Both temperature and time affected not only the lignin removal, as described earlier, but also the monomeric sugar yield. At low pretreatment temperature of 40 °C (Figure 4.1a), the monomeric sugar yield was increased at high reaction times due to more effective hydrolysis reaction. However, the monomeric sugar yields were slightly decreased when reaction times was increased at higher temperatures, 60–120 °C (Figures 4.1b–e). Figure 4.1f presents the comparison of the effects of temperature and time, clearly showing a decreasing trend of the monomeric sugar yield when increasing the temperature from 40 to 120 °C. The yield was decreased when the temperature and time conditions were too high due to the degradation process (Hu and Wen, 2008). Comparing between the reaction temperature and time, the reaction temperature gave more impact on the monomeric sugar releasing, consistent to the pretreatment results of switchgrass using microwave (Jackowiak et al., 2011). Thus, the appropriate temperature and time in this pretreatment process are required to obtain the highest monomeric sugar yields.

4.4.4 Optimization of Two-stage Pretreatment (Microwave/Dilute NaOH, Followed by Microwave/Dilute H_2SO_4 Pretreatments)

4.4.4.1 Effects of Pretreatment Temperature and Time

To study the effects of temperature and time, microwave/ NaOH-pretreated MG was treated with microwave/dilute H_2SO_4 pretreatment at a temperature range of 80–200 °C for 5–60 min using 0.5 % (w/v) H_2SO_4. The LSR used was the same as that studied by Boonmanumsin et al. (2012) because this ratio provided the highest monomeric sugar yield for the two-stage pretreatment processes. All results obtained from the microwave/dilute H_2SO_4 pretreatment (see Figure 4.2) show that the pretreated MG released 33.7 g/100 g biomass of the highest total monomeric sugar yields at temperature of 200 °C for 5 min time (Figure 4.2f). Interestingly, these results show that MG gave higher contents of xylose and arabionose at the other temperatures. At the temperatures of 80–160 °C (see Figures 4.2a–d), the total amount of xylose and arabionose increased with time, confirming that hemicellulose was easily hydrolyzed at lower temperatures. Hemicellulose

differs from cellulose in its composition of sugar units, presence of shorter chains, a branching of main chain molecules, and its amorphous structure (Fengel and Wegener, 1984), making it easier to hydrolyze than cellulose.

On the other hand, the amounts of xylose and arabionose clearly decreased at excessive temperatures 180 and 200 °C (see Figures 4.2e–f) because of the sugar degradation. At 180° and 200 °C, the main monomeric sugar released was glucose from the cellulose hydrolysis. Cellulose has a more ordered structure than hemicelluloses (O'Sullivan, 1997), thus it requires more severe conditions for hydrolysis to release glucose. However, the glucose degradation also occurred if extremely severe conditions were used. Similar to this study, Redding *et al.* (2011) studied the ANOVA output for the xylose releasing from high temperature dilute acids pretreatment of coastal Bermuda grass and found that both temperature and acid concentrations provided a great impact for monomeric sugar releasing. The effect of temperature and time on microwave/dilute acid pretreatment summarized in Figure 4.2g indicates that the reaction temperature highly influences on the monomeric sugar releasing while the reaction time slightly affected.

4.4.5 Effect of Chemical Concentration

4.4.5.1 Effect of NaOH Concentration

In 2008, Hu and Wen found that NaOH concentration was another factor not only in the removal of lignin, but also in degrading the sugars released from the pretreatment. This study thus varied the NaOH concentration from 0.1 to 7 % w/v at the optimum conditions of the pretreatment temperature and time (120 °C, 10 min) to maximize the monomeric sugar yields. The maximum monomeric sugar yield was obtained by using 3 % (w/v) NaOH to give 6.6 ± 0.1 g/100 g biomass, consisting of 4.2 ± 0.1 g glucose, 1.5 ± 0.0 g xylose, and 0.9 ± 0.0 g/100 g biomass arabinose, as can be seen in Figure 4.3a. As expected, the stronger alkaline concentration from 0.1 to 3 % w/v NaOH caused the solubilization of hemicelluloses and cellulose, leading to a higher monomeric sugar yield. However, the monomeric sugar yields decreased when an excessive NaOH concentration (4–7

% w/v NaOH) was employed, due to the sugar degradation process (Hu and Wen, 2008).

As a conclusion, the maximum total monomeric sugar yield of 6.6 ± 0.1 g/100 g biomass was released at 120 °C after 10 min pretreatment time using 3 % (w/v) NaOH. The obtained optimum conditions using the microwave/NaOH method confirmed the efficiency of the pretreatment, requiring a much shorter reaction time at low heating temperature with low NaOH concentration.

4.4.5.2 Effect of H_2SO_4 Concentration

The concentration of H_2SO_4 was another parameter studied in this work to optimize the monomeric sugars released using the two-stage microwave/chemical pretreatment process. The microwave/ NaOH-pretreated MG was treated with microwave/ H_2SO_4 pretreatment at the optimum temperature and time of 200 °C and 5 min, respectively, 15:1 LSR, using various H_2SO_4 concentrations (0.5–3 % (w/v)). The maximum monomeric sugar yields in Figure 4.3b from the microwave/NaOH-pretreated MG was 34.3 ± 1.3 g/100 g biomass using 1 % (w/v) H_2SO_4, and the yield obviously decreased when the H_2SO_4 concentration increased above 1 % (w/v) because at high temperature the increase of the H_2SO_4 concentration generally accelerates the sugar degradation process. Thus, the maximum monomeric sugar yields of the microwave/ NaOH-pretreated MG could be obtained using a low acid concentration at a high temperature for a shorter reaction time.

Conclusively, the maximum monomeric sugar yield of 34.3 ± 1.3 g/100 g biomass, consisting of 31.1 ± 0.8 g glucose, 2.2 ± 0.4 g xylose, and 1.0 ± 0.2 g arabinose per 100 g biomass, was obtained from the microwave/NaOH-pretreated MG at the optimum condition of 200 °C temperatures for 5 min pretreatment time, using 1 % (w/v) H_2SO_4. The investigated optimum conditions of the two-stage microwave/chemical pretreatment process of MG produced high glucose content, which shows many benefits for bioethanol production, because the six-carbon sugars are readily fermented to ethanol by many naturally occurring organisms (Mosier et al., 2005), while the fermentation of pentose (five-carbon sugar) is performed by a only few strains and usually results in relatively low yields.

4.4.6 Effect of Two-stage Microwave/Chemical Pretreatment Process on % Solid Loss and pH

The two-stage microwave/chemical pretreatment process has not only had an impact on monomeric sugars yield but has also affected % solid loss and the pH of liquid hydrolysate from the pretreatment process.

4.4.6.1 % Solid Loss

Both the microwave/NaOH (Figure 4.4a) and the microwave/NaOH/ H_2SO_4 pretreatments (Figure 4.4b) resulted in the same trend of the % solid loss. Increasing the temperature and time caused the % solid loss to increase due to the digestion of MG. The temperature was the main effect causing a higher % solid loss. Tucker *et al.* (2003) also reported similar trends in the % solid loss during the pretreatment process. The % solid loss found was in a range from 12.4 ± 0.8 % to 37.5 ± 0.3 % for the microwave/NaOH pretreatment. and from 7.7 ± 2.0 % to 76.0 ± 0.0 % for the two-stage microwave/ NaOH/ H_2SO_4 pretreatment at various temperatures for various times.

4.4.6.2 pH Value

Because of the chemical reaction taking place in the lignocellulosic structure, the final hydrolysate pH was affected by the pretreatment temperatures and times, as demonstrated in Fig. 4.4c. The pH decreased from 12.7 ± 0.0 to 10.4 ± 0.1 when the temperature and time increased because more hydroxide ions were used to cleave the lignin ether bond in alkaline delignification process (Gierer, 1985; Harmsen *et al.*, 2010). Moreover, acetic acid could also be generated in highly severe conditions from the sugar degradation process, and thus lower the pH (Pedersen and Meyer, 2010). The final pH of the microwave/ H_2SO_4 pretreatment on MG was also measured at various temperatures and times (see Figure 4.4d). At the same pretreatment time. the pH value mostly increased with high temperature conditions. The pH of the acid hydrolysate increased with temperature because acid protons acting as a catalyst were used to protonate the oxygen atom linkage between cellulose and hemicellulose in the hydrolysis process. The pretreatment time slightly changed the pH value of the mixture. However, the pH value fluctuated because acetic acid generated at highly severe conditions by the degradation process could lower the hydrolysate pH value.

4.4.7 Effect of Pretreatment on Chemical Composition

The chemical compositions of MG, including cellulose, hemicellulose, lignin, and ash, were investigated using the NREL method. After MG was treated with the microwave/NaOH and the two stage microwave/chemical pretreatments, the chemical compositions were analyzed and summarized in Table 4.2. It is worth noting that the pretreatment of MG by microwave/NaOH provided higher total content of cellulose, hemicelluloses, and lignin (104 %) than 100 %. This is due to the standard deviation from experimental repeatability. These results confirm the above results and discussion of the two-stage pretreatment process and explain the obtained monomeric sugar yield. The higher cellulose content in the MG sample results in a higher released glucose content. On the other hand, the higher hemicellulose content should release higher xylose and arabinose contents.

After the microwave/NaOH pretreatment, the solid residues contained cellulose as the main component, giving a high glucose content after treating with the two-stage pretreatment at the optimum conditions. This pretreatment step also solubilized some part of hemicelluloses, lowering its component from 29.2 ± 1.0 % to 20.0 ± 0.3 %. Most importantly, this alkali pretreatment stage effectively removed and lowered the lignin content in the MG samples, leaving most reactive biomass with high cellulose content up to 82.4 ± 1.6 %.

When the microwave/NaOH-treated MG solid residues were further treated with the microwave/H_2SO_4 pretreatment at the optimum conditions, the polysaccharide contents in MG were significantly reduced, retaining only 16.1 ± 3.5 % cellulose content and a small amount of hemicellulose. Because the acid pretreatment was efficiently performed at 200 °C, the main cellulose component was hydrolyzed, degraded, and burned. The ash content rarely remained in the two-stage pretreated samples. Therefore, these results assure that the two-stage pretreatment process effectively converts MG with high cellulose content into high monomeric sugar yields.

4.4.8 Fourier Transform Infrared Spectrometer (FTIR) Analysis

The chemical structure change of MG after treating with the NaOH/microwave and the two-stage (NaOH/ H_2SO_4) pretreatments was detected using FTIR, comparing it with the untreated MG. It was found that after the microwave/NaOH pretreatment the FTIR peak at 1734 cm^{-1} disappeared from the MG sample because the complex linkages between lignin and hemicellulose, such as ester-linked acetyl, feruloyl, and p-coumaroyl groups, were broken. The peaks at 1515 (aromatic C=C stretching from aromatic ring of lignin) and 1248 cm^{-1} (aromatic C–O stretching of lignin) (Wang et al., 2010) also decreased. In addition, the other polysaccharide peaks (898, 1108, 1164, 1260, 1325, and 1378 cm^{-1}) became sharper, as compared with the untreated MG. All FTIR results also indicate no lignin peaks, confirming the efficient pretreatment method for the lignin removal and giving mostly reactive biomass of cellulose contents for the dilute acid pretreatment step. The spectra obtained from the two-stage pretreatment at the optimum conditions were different from the microwave/NaOH pretreatment, such that the two-stage pretreated MG clearly shows a peak at 1722 cm^{-1}, corresponding to lignin component (carbonyl bond unconjugated to aromatic ring) because most cellulose contents were hydrolyzed, leaving only the lignin still remaining in the residues.

4.4.9 Scanning Electron Microscopy (SEM) Characterization

SEM images of the untreated, the microwave/NaOH-pretreated, and the two-stage treated samples were characterized to study the physical appearance changes of MG. The morphology of the untreated MG shows a highly fibril and intact structure covered with a thin film layer, probably the wax layer commonly found in herbaceous biomass (Hu and Wen, 2008) or the lignin, and lignin-carbohydrate complexes condensed on the surface of the cellulose fiber (Zhu et al., 2009). SEM image of the microwave/NaOH-pretreated MG still shows a fibril structure, but the thin film on the surface has disappeared. Moreover, the surface became smooth and thinner with ridges and was perforated by the microwave/ NaOH pretreatment process (Hu and Wen, 2008). The generated pores would increase the surface area, causing the hydrolysis step to be more effective. This morphology

confirms the FTIR results that the lignin was destroyed, easing the exposure of the cellulose and the hemicellulose structures. On the contrary, the fibril structure of the two-stage pretreated MG was distorted by the hydrolysis process at high temperature. The SEM image shows that the two-stage pretreatment effectively breaks down the lignocelluloses structure for monomeric sugar releasing.

4.5 Conclusions

Mission grass (*P. polystachyon*) in Thailand shows its potential as a biofuel feedstock by two-stage microwave/chemical pretreatment process. The lignin content of MG was effectively removed by microwave/NaOH pretreatment under the optimum conditions and the monomeric sugar yield of 6.6 ± 0.1 g/100 g biomass was released. The optimum conditions of the next microwave/ H_2SO_4 stage were found, and released the maximum monomeric sugar yield of 34.3 ± 1.3 g/100 g biomass, consisting of high glucose content for the fermentation process. The effects of the two-stage pretreatment were also confirmed by MG chemical composition, SEM and FTIR results.

4.6 Acknowledgements

4.7 References

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Table 4.1 The Chemical compositions of mission grass (*Pennisetumpolystachyon*)

Composition (%)*	Mission Grass (*Pennisetumpolystachyon*)	
	This study [1]	Premjet *et al.*, 2013 [2]
Cellulose	39.8±1.5	40.0±0.0
Hemicellulose	29.2±1.0	23.3±0.1
Lignin	14.6±0.5	6.2±0.2
Ash	3.3±0.5	7.5±0.3

* = Composition percentages are on dry weight basis

[1] = Value represent the mean, n = 3, ±SD

[2] = Value represent the mean, n = 2, ±SD

Table 4.2 Chemical composition of mission grass solid residues from each pretreatment stages

| Composition (% Dry Matter)	Mission Grass (*Pennisetumpolystachyon*)		
	Untreated	Microwave/NaOH	Microwave/NaOH/ H_2SO_4
Cellulose	39.8±1.5	82.4±1.6	16.1±3.5
Hemicellulose	29.2±1.0	20.0±0.3	3.4±0.1
Lignin	14.6±0.5	2.3±2.0	0.4±0.0
Ash	3.3±0.5	0.1±0.1	0.1±0.0

Data are mean value ± S.D. of three replicates
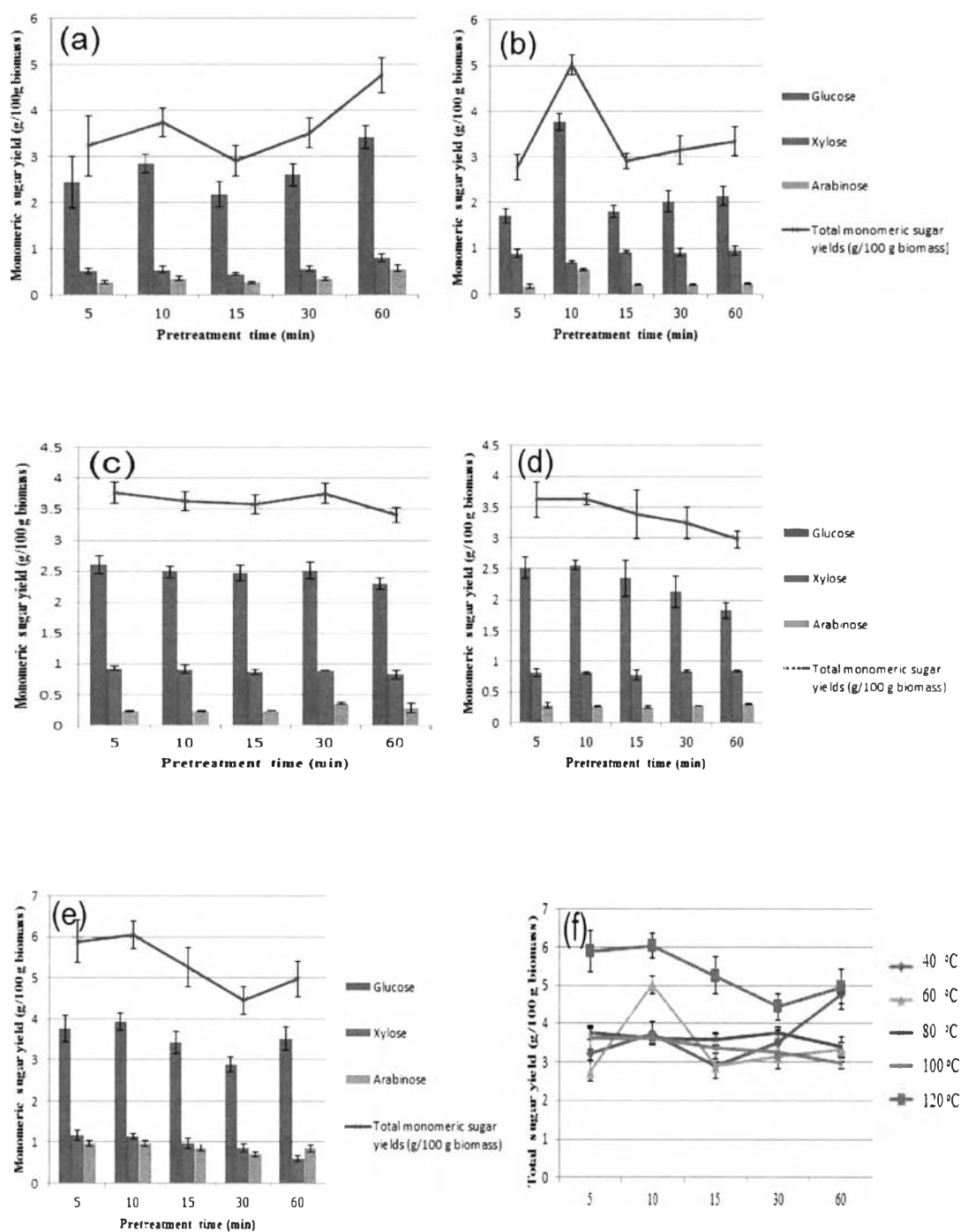
Figure 4.1 The glucose, xylose, arabinose components and the total monomeric sugar yield of untreated mission grass (*Pennisetumpolystachyon*) using 0.5 % (w/v) NaOH, 15:1 LSR, different times and temperatures: a) 40°, b) 60°, c) 80°, d) 100°, e) 120 °C, and f) comparison of the total monomeric sugar yields.
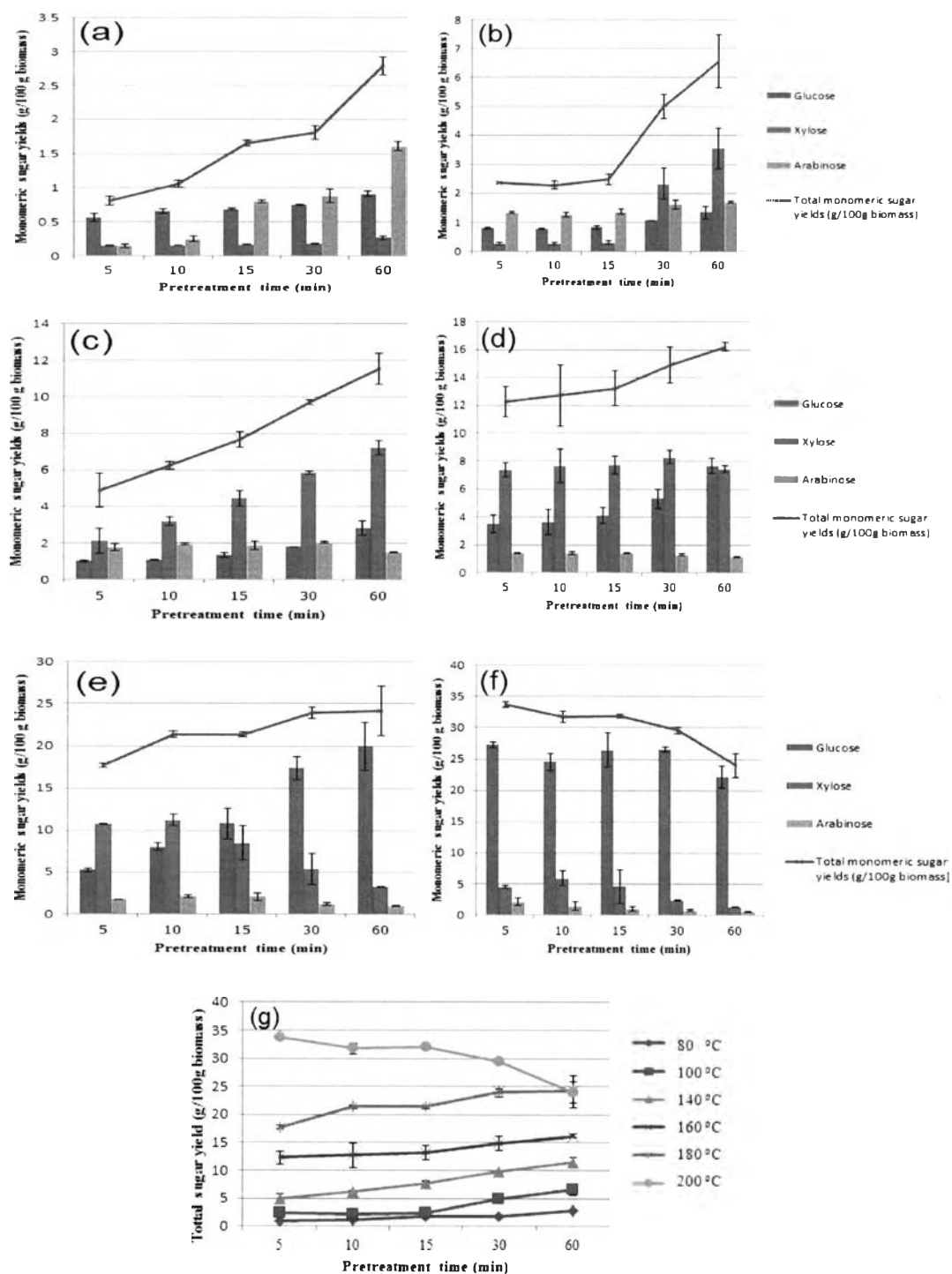
Figure 4.2 The glucose, xylose, arabinose components and the total monomeric sugar yield of microwave-assisted NaOH pretreated mission grass using 0.5 % (w/v) H_2SO_4, 15:1 LSR at different temperatures for different times: a) 80°, b) 100°, c) 140°. d) 160°, e) 180°, f) 200 °C, and (g) comparison of the total monomeric sugar yields.
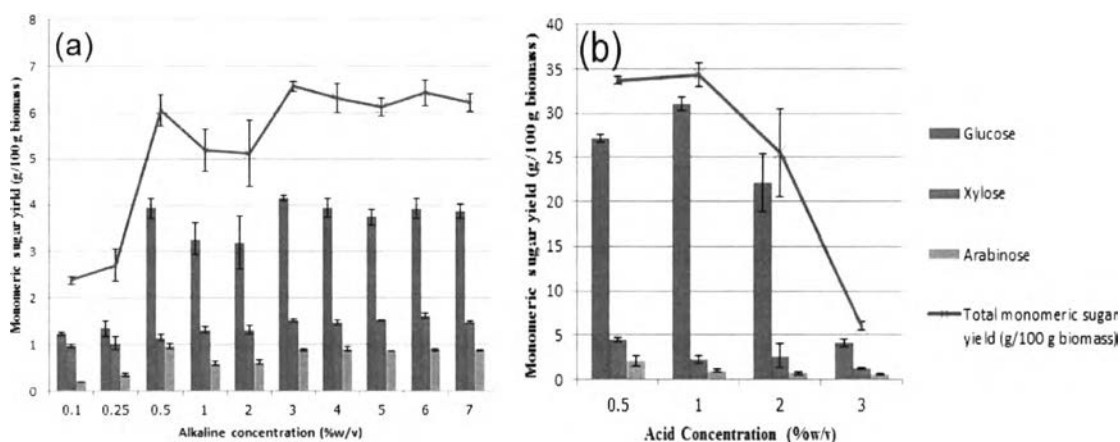
Figure 4.3 a) Effect of NaOH concentration (% w/v) on monomeric sugar yields at 120 °C, 10 min, using 15:1 LSR and b) effect of H_2SO_4 concentration (% w/v) on monomeric sugar yields of microwave-assisted NaOH pretreated mission grass at 200 °C, 5 min, using 15:1 LSR.
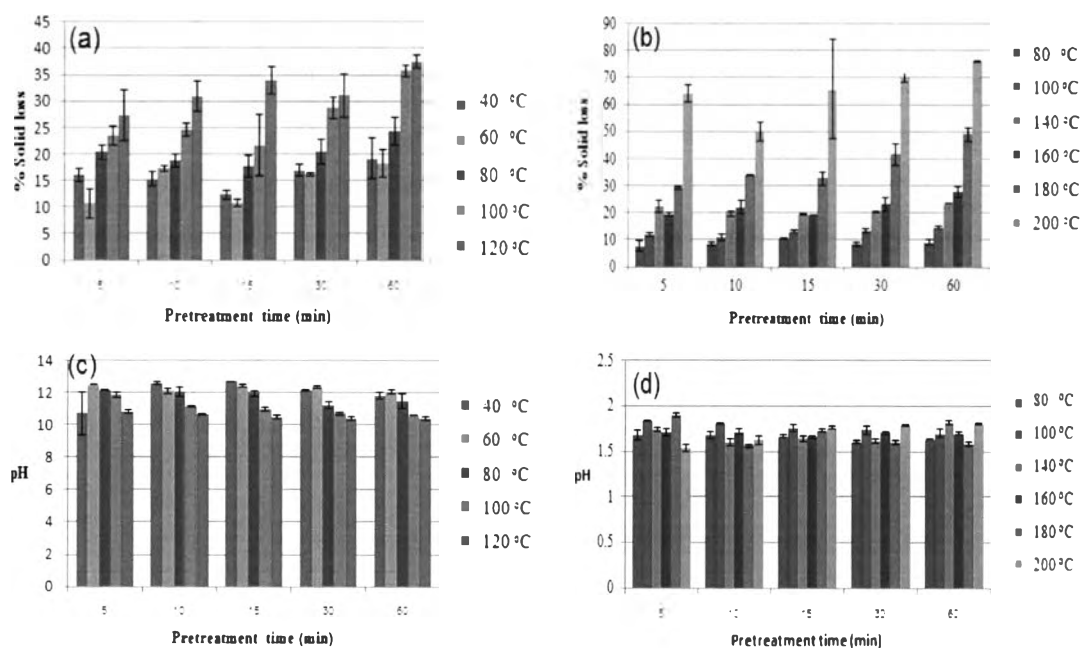
Figure 4.4 a) %Solid loss and c) solution pH of untreated mission grass at different temperatures for different times using 0.5% (w/v) NaOH and 15:1 LSR, b) % solid loss and d) solution pH of the microwave-assisted NaOH pretreated mission grass at different temperatures for different times using 0.5% (w/v) H_2SO_4 and 15:1 LSR.