CHAPTER IV
RESULTS AND DISCUSSION

4.1 Raw material composition

Mission grass (*Pennisetum polystachyon*), Cogon grass (*Imperata cylindrica*), Guinea grass (*Panicum maximum*), Kans grass (*Saccharum spontaneum*), and Giant reeds (*Arundo donax*) were analyzed the chemical compositions by National Renewable Energy Laboratory (NREL) method, as summarized in Table 4.1. Comparing with the literatures and considering the contents of glucan, xylan, and lignin as phenolic polymer in those five weeds, Mission grass, Kans grass, and Giant weeds show the highest polysaccharide contents. Therefore, these three weeds were chosen for further study to produce monomeric sugarss as bioethanol feedstock.

Table 4.1 Chemical compositions of five Thai grass (Mission grass, Cogon grass, Guinea grass, Kans grass, and Giant reed)

Raw lignocellulosic biomass sample	Composition (% dry matter)						Reference
	Water extractive	Ethanol extractive	Lignin	Cellulose as glucan	Hemicellulose as xylan	Ash	
Mission grass	**5.7**	**10.9**	**14.6±0.5**	**39.8±1.5**	**29.2±1.0**	**3.3±0.5**	**This study**
	-	-	10.6	38.7	27.5	8.5	Supaporn *et al.*, 2003
Cogon grass	**3.2**	**7.6**	**14.5±1.9**	**36.7±0.5**	**25.1±0.6**	**2.0±0.5**	**This study**
	-	-	8.2	37.2	32.2	6.3	Supaporn *et al.*, 2003
	-	-	-	38.5	22.4	-	Lin and Lee (2011)
Guinea grass	**8.6**	**4.2**	**9.9±2.0**	**31.0±1.4**	**22.6±0.9**	**2.8±0.4**	**This study**
	-	-	10.7	39.4	28.3	8.2	Supaporn *et al.*, 2003
Kans grass	**8.5**	**3.9**	**14.5±1.8**	**35.4±0.9**	**26.7±0.4**	**1.1±1.0**	**This study**
	-	-	8.3	42.2	31.9	5.0	Supaporn *et al.*, 2003
	-	-	20.0	36.8	23.7	1.2	Scordia *et al.*, 2010
Giant reed	**5.5**	**1.9**	**19.2±3.3**	**39.1±0.3**	**24.4±0.5**	**4.2±0.7**	**This study**
	-	-	23.0	34.8	20.9	-	Franscisco *et al.*, 2010
	-	-	-	42.5	31.2	-	Y.J. Jeon *et al.*, 2010

4.2 Grass particle size analysis

Mechanical pretreatment is an important process for size reduction and lignocellulosic ethanol production. Five Thai weeds were milled until the particle sizes were about 60 meshes. The particle size of the grass samples were also confirmed by particle size analyzer and the results are shown in Table 4.2.

Table 4.2 The particle size of five Thai grasses (Mission, Cogon, Guinea, Kans, and Giant reed) after mechanical pretreatment.

Raw lignocellulosic biomass sample	Particle size (mean diameters) (μm)
Mission grass (*Pennisetum polystachyon*)	336.0
Cogon grass (*Imperata cylindrica*)	368.4
Guinea grass (*Panicum maximum*)	323.5
Kans grass (*Saccharum spontaneum*)	304.5
Giant reed (*Arundo donax*)	326.1

After cutting, chopping, milling, and grinding, the mean diameters of the grasses were found in a range of 300–370 μm. For acid hydrolysis step, the particle size was optimized to -20/+80 meshes since a larger size distribution will cause incomplete hydrolysis while a smaller size will lead to over hydrolysis (Sluiter *et al.*, 2011). Thus, the 60 mesh particle size is chosen for chemical composition analysis, pretreatment, and hydrolysis process.

4.3 Optimization of Microwave-assisted NaOH Pretreatment

NaOH pretreatment is an effective chemical pretreatment method to remove lignin and reduce cellulose crystallinity (Chen *et al.*, 2009). The main effect of NaOH pretreatment is to delignify by separating structural linkages between lignin and carbohydrates, and disrupt the lignin structure from solvation and saphonification of biomass (Fang *et al.*, 1987). In addition, NaOH can solubilize some part of hemicelluloses. Thus, in this study, Mission grass, Kans grass and Giant reed were first pretreated with microwave/NaOH pretreatment process. The optimum conditions to release the highest amount of monomeric sugars using the microwave-assisted NaOH pretreatment were investigated, and the results are shown in Table 4.3

Table 4.3 Optimum condition for Microwave/NaOH pretreatment of Mission grass, Kans grass, and Giant reed.

Raw lignocellulosic biomass	Temperature (°C)	Time (min)	NaOH (%w/v)	Total monomeric sugars yield (g/100g biomass)
Mission grass	120	10	3	6.6 ± 0.1
Kans grass	80	5	5	6.8 ± 0.3
Giant reed	120	5	5	6.8 ± 0.2

4.3.1 Effects of Time and Temperature

Mission grass, Kans grass, and Giant reed were pretreated with microwave/NaOH pretreatment at the temperature range of 40°-120 °C for 5–60 min using 0.5% (w/v) NaOH with 15:1 liquid-to-solid ratio (LSR) to find optimum temperature and time condition. Mission grass releases the highest monomeric sugars (6.0 g/100g biomass) at 120 °C for 10 min (Fig 4.1), while the maximum monomeric sugars from Kans grass and Giant reed were 4.8 g and 4.5 g/100g biomass at 80 °C for 5 min (Fig 4.2), and 120 °C for 5 min (Fig 4.3), respectively.

The effects of temperature and time on monomeric sugars released from Mission grass, Kans grass, and Giant reed were also compared (Fig 4.4). As can be seen from the results, both temperature and time not only affected the lignin removal, as described earlier, but also the monomeric sugar yield by degradation process when the temperature and time conditions were too high (Hu and Wen, 2008). Thus, the appropriate temperature and time in this pretreatment process are to obtain the highest monomeric sugar yield, those are 120 °C-10 min, 80 °C-5 min, and 120 °C-5 min for Mission grass, Kans grass, and Giant reed, respectively.

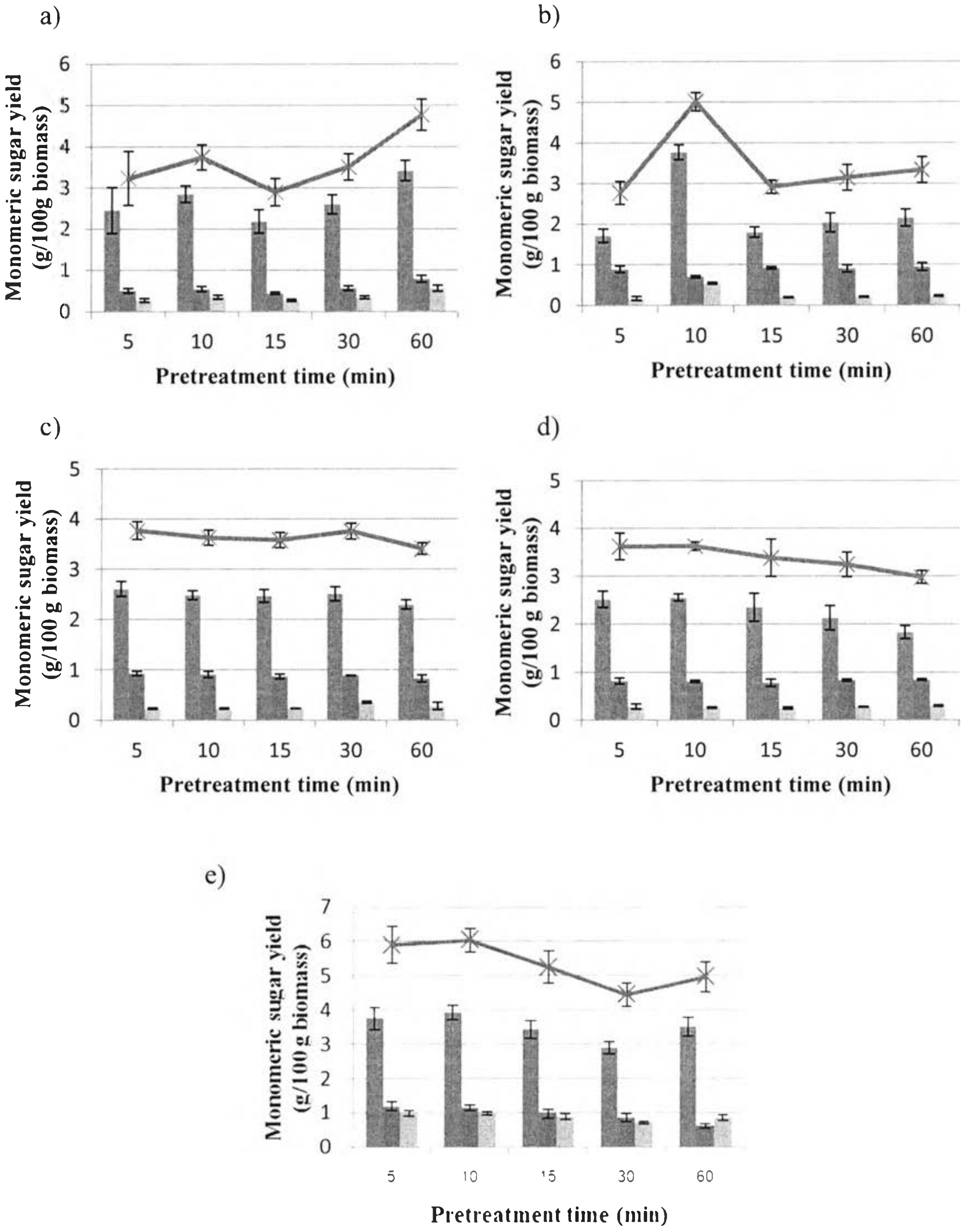


Figure 4.1 The glucose (▩), xylose (▩), arabinose (□) components and total monomeric sugar yield (-x-) of Mission grass (*Pennisetum polysta chyon*) using 0.5 % (w/v) NaOH, 15:1 LSR, different times, and temperatures: a) 40°, b) 60°, c) 80°, d) 100°, and e) 120 °C.

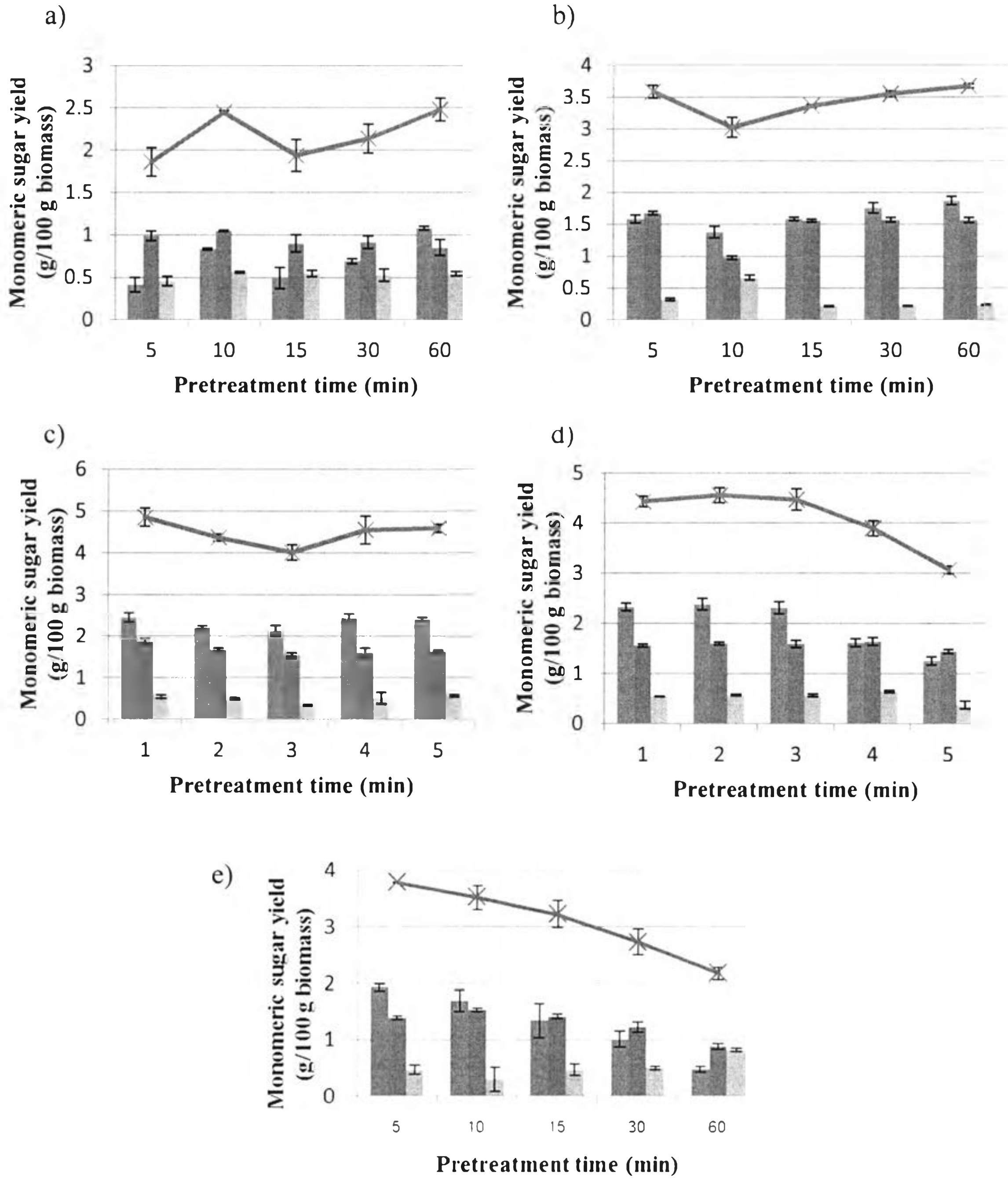


Figure 4.2 The glucose ( ), xylose ( ), arabinose ( ) components and total monomeric sugar yield (-x-) of Kan grass (*Saccharum spontaneum*) using 0.5 % (w/v) NaOH, 15:1 LSR, different times, and temperatures: a) 40°, b) 60°, c) 80°, d) 100°, and e) 120 °C.

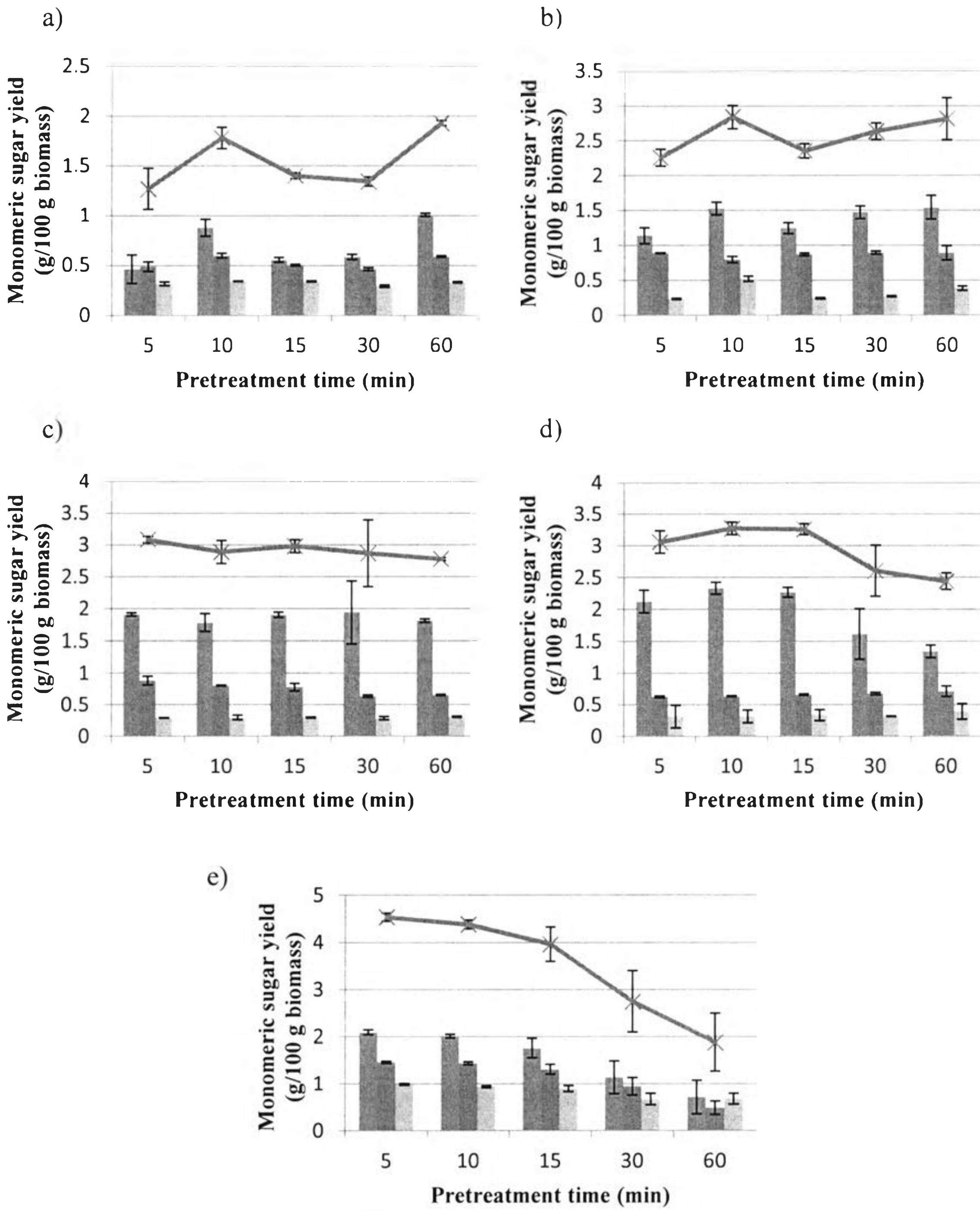


Figure 4.3 The glucose (■), xylose (■), arabinose (□) components and total monomeric sugar yield (-x-) of Giant reed (*Arundo donax*) using 0.5 % (w/v) NaOH, 15:1 LSR, different times, and temperatures: a) 40°, b) 60°, c) 80°, d) 100°, and e) 120 °C.

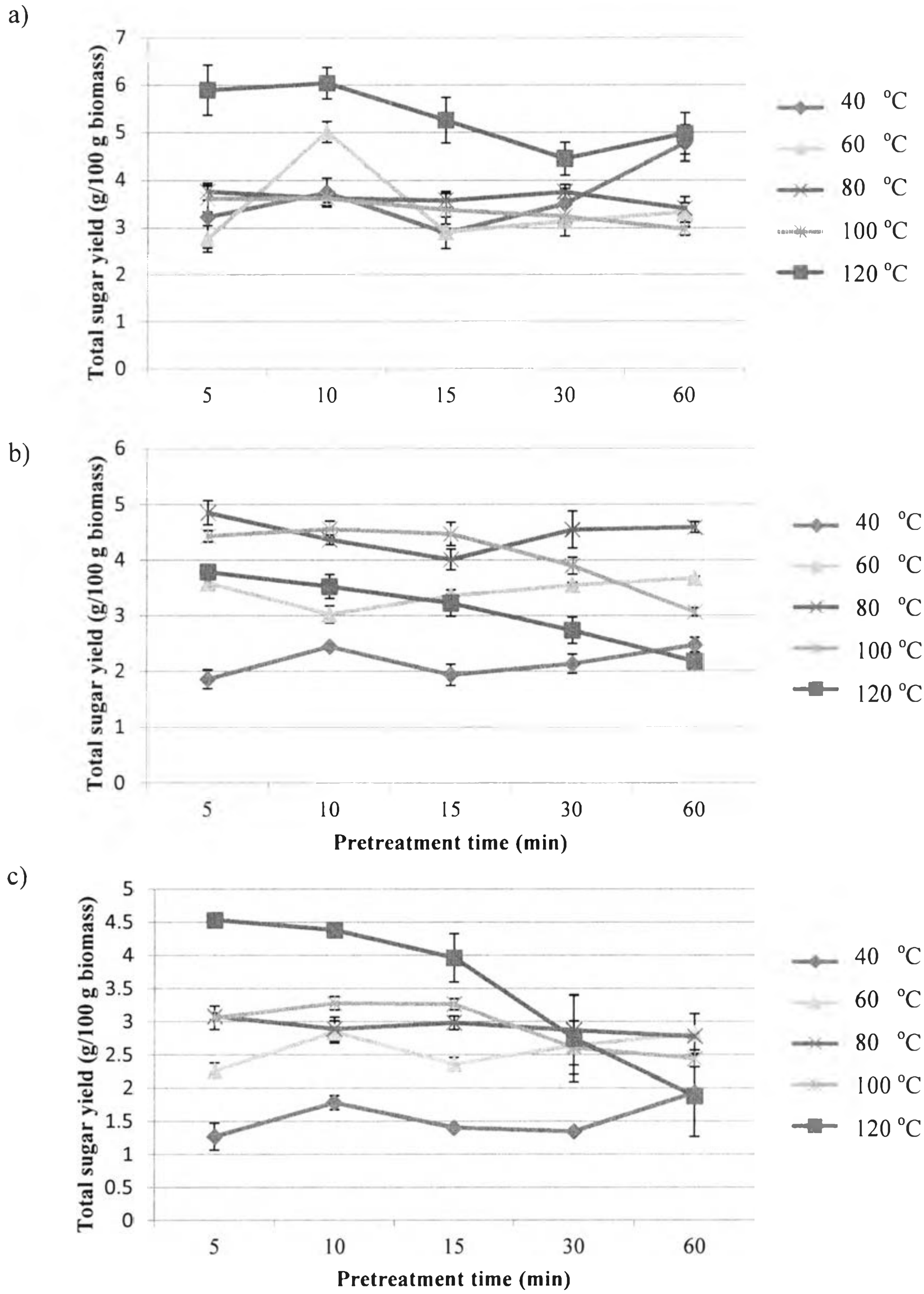


Figure 4.4 Comparison of the total yield of monomeric sugars of a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) NaOH and 15:1 LSR.

4.3.2 Effect of NaOH Concentration

The effect of NaOH concentration was studied by varying the NaOH concentration from 0.1 to 7 %w/v at the optimum conditions of temperature and time of Mission grass (120 °C,10 min), Kans grass (80 °C,5 min), and Giant reed (120 °C,5 min), see Fig. 4.5. The maximum monomeric sugar yields from those three grasses were 6.6, 6.8, and 6.8 g/100g biomass at 3, 5, 5 % (w/v) NaOH, respectively.

From Fig. 4.6, the stronger alkaline pretreatment caused the solubilization of hemicelluloses and cellulose and led to higher monomeric sugars yields. However, the monomeric sugar yields decreased when too high NaOH concentration was employed due to sugar degradation process (Hu and Wen, 2008). The acquired optimum condition for microwave/NaOH pretreatment confirmed the efficiency of this pretreatment method that short reaction time, low heating temperature, and low NaOH concentration are needed.

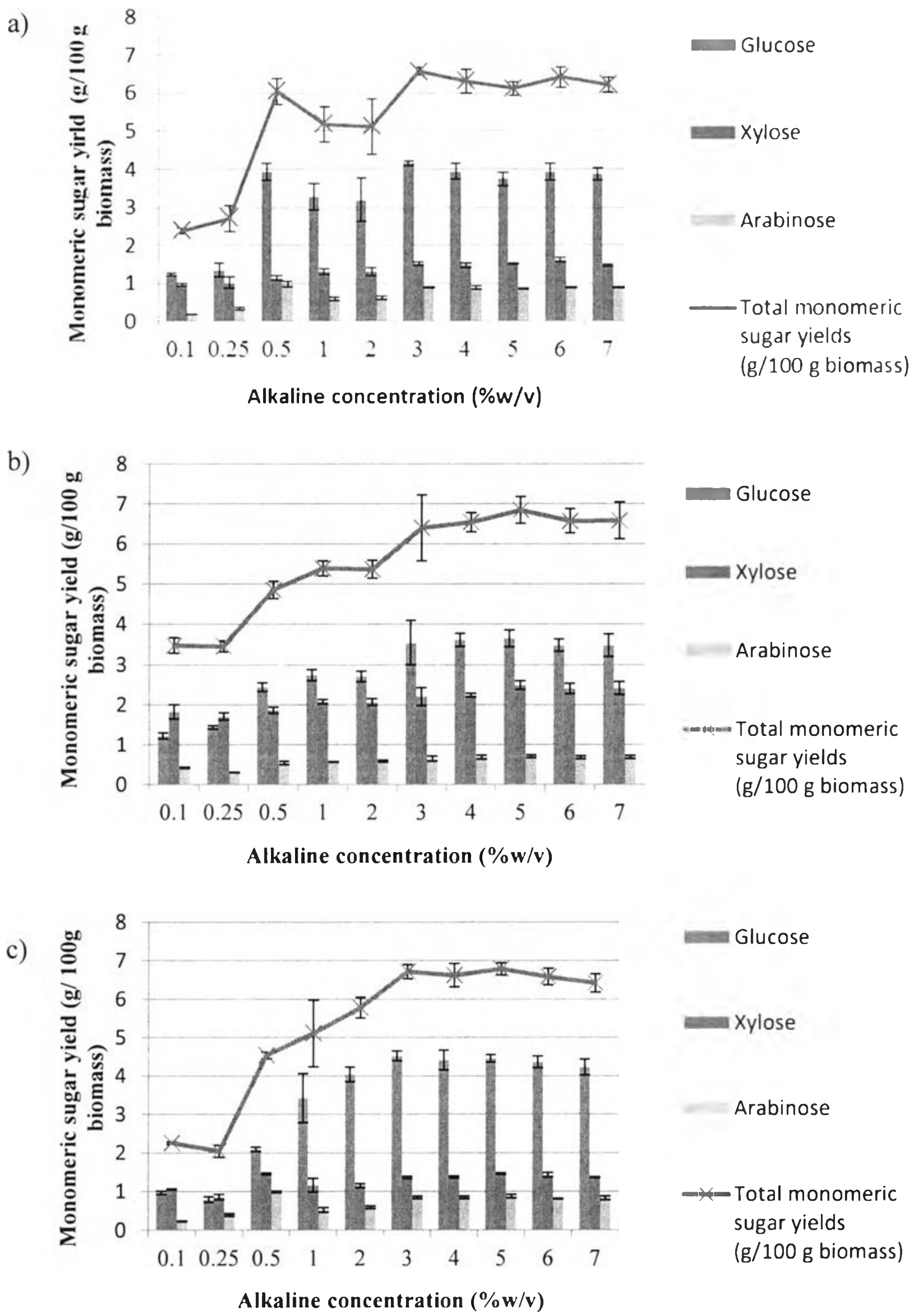


Figure 4.5 Effect of NaOH concentration (% w/v) on monomeric sugar yields of a) Mission grass at 120 °C-10 min, b) Kans grass at 80 °C-5 min, and c) Giant reed at 120 °C-5 min using 15:1 LSR

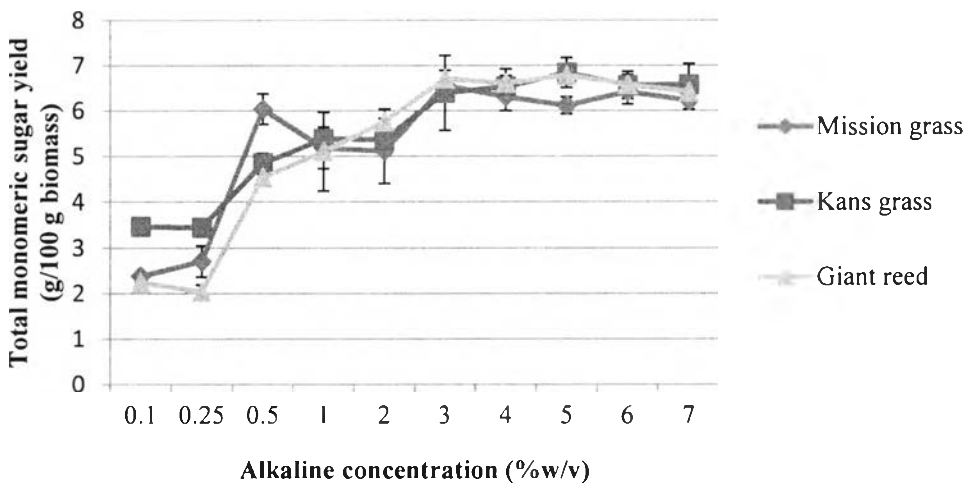


Figure 4.6 Comparison of the total yield of monomeric sugars of Mission grass at 120 °C-10 min, Kans grass at 80 °C-5 min, and Giant reed at 120 °C-5 min using 15:1 LSR and different NaOH concentrations (%w/v).

4.4 Optimization of Two-stage Pretreatment (Microwave/dilute NaOH Followed by Microwave/dilute H_2SO_4 Pretreatment)

Two-stage microwave/chemical pretreatment process is one of effective methods to produce 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 microwave/alkaline pretreatment stage, the microwave/acid pretreatment stage is required to hydrolyze the polysaccharide and provides the highest monomeric sugars yield for bioethanol production. In recent years, acid pretreatment by sulfuric acid at concentration below 4% was found to be the most interestingly effective pretreatment to achieve high reaction rate and improve hydrolysis (Esteghlalian *et al.*, 1997). When comparing to other types of dilute acid (phosphoric acid, hydrochloric acid, and maleic acid), sulfuric acid results in the highest total monomeric sugars (Dawei *et al.*, 2011). Thus, sulfuric acid was adapted in this study. Solid residues obtained from microwave/NaOH pretreated Mission grass, Kans grass, and Giant reed at the optimum conditions were washed with distilled water until the solution pH became 7. The washed solids were dried in a vacuum oven and kept for the microwave/dilute H_2SO_4 Pretreatment. In the microwave/acid pretreatment, various temperatures, times, and acid concentrations were taken into account to obtain the optimum conditions, as shown the results in Table 4.4.

Table 4.4 Optimum conditions of microwave/H_2SO_4 pretreatments of microwave-assisted NaOH pretreated Mission grass, Kans grass, and Giant reed

Raw lignocellulosic biomass	Temperature (°C)	Time (min)	H_2SO_4 concentration (%w/v)	Total monomeric sugar yields (g/100g biomass)
Mission grass	200	5	1	34.3 ± 1.3
Kans grass	200	10	0.5	33.8 ± 3.9
Giant reed	180	30	0.5	31.9 ± 1.7

4.4.1 Effects of Time and Temperature

To study the effects of temperature and time, microwave/NaOH pretreated Mission grass, Kans grass, and Giant reed were treated with microwave/dilute H_2SO_4 pretreatment with a temperature range of 80°–200 °C for 5–60 min using 0.5% (w/v) H_2SO_4 and LSR was fixed at 15:1 because this ratio provides the highest monomeric sugar yield in the two-stage pretreatment process studied by Treebuubpha *et al.* in 2012. In the experiment, the pretreated Mission grass. Kans grass, and Giant reed released 33.7, 33.8, and 31.9 g/100 g biomass of the totals monomeric sugar yields at 200 °C-5 min, 200 °C-10 min, and 180 °C-30 min, see Fig 4.7-9, respectively. All three chosen weeds were compared the total monomeric sugar yield at various temperatures and times (Fig 4.10).

From the results, those grasses gave high contents of xylose and arabionose sugar at the temperature range of 80°–160 °C. When the temperature and the time increased, the amounts of xylose and arabionose also increased because of the hydrolysis of polysaccharide. These results confirm that hemicellulose was effectively hydrolyzed at these conditions. Unlike cellulose, hemicellulose differs by composition of sugar units, presence of shorter chains, a branching of main chain molecules, and being amorphous (Fengel *et al.*, 1989), making its structure easier to hydrolyze than cellulose.

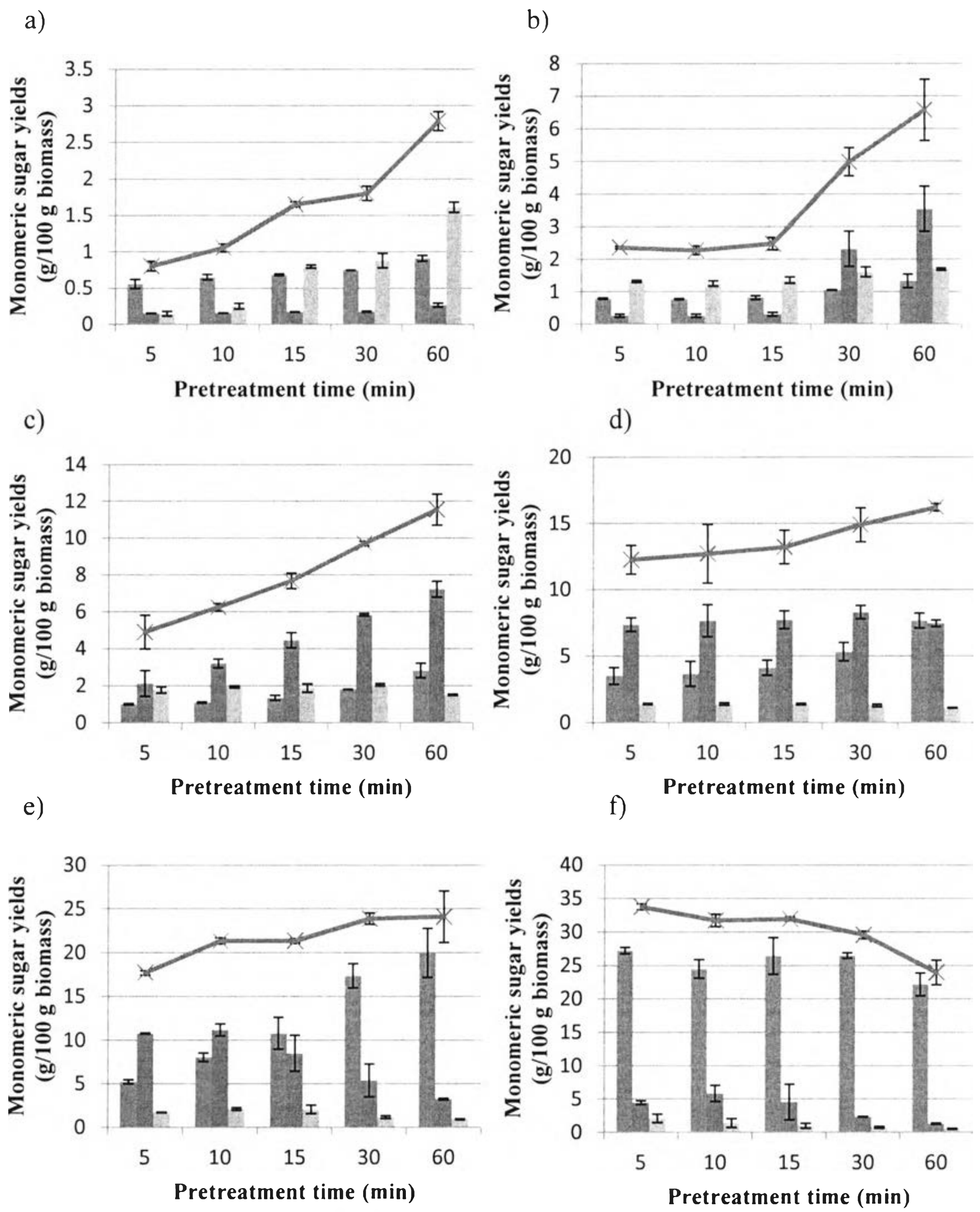


Figure 4.7 The glucose (■), xylose (▒), arabinose (□) components and total monomeric sugar yield (-x-) of microwave-assisted NaOH pretreated Mission grass (*Pennisetum polystachyon*) using 0.5 % (w/v) H_2SO_4, 15:1 LSR at different temperatures for different times: a) 80°, b) 100°, c) 140°, d) 160°, e) 180°, and f) 200 °C

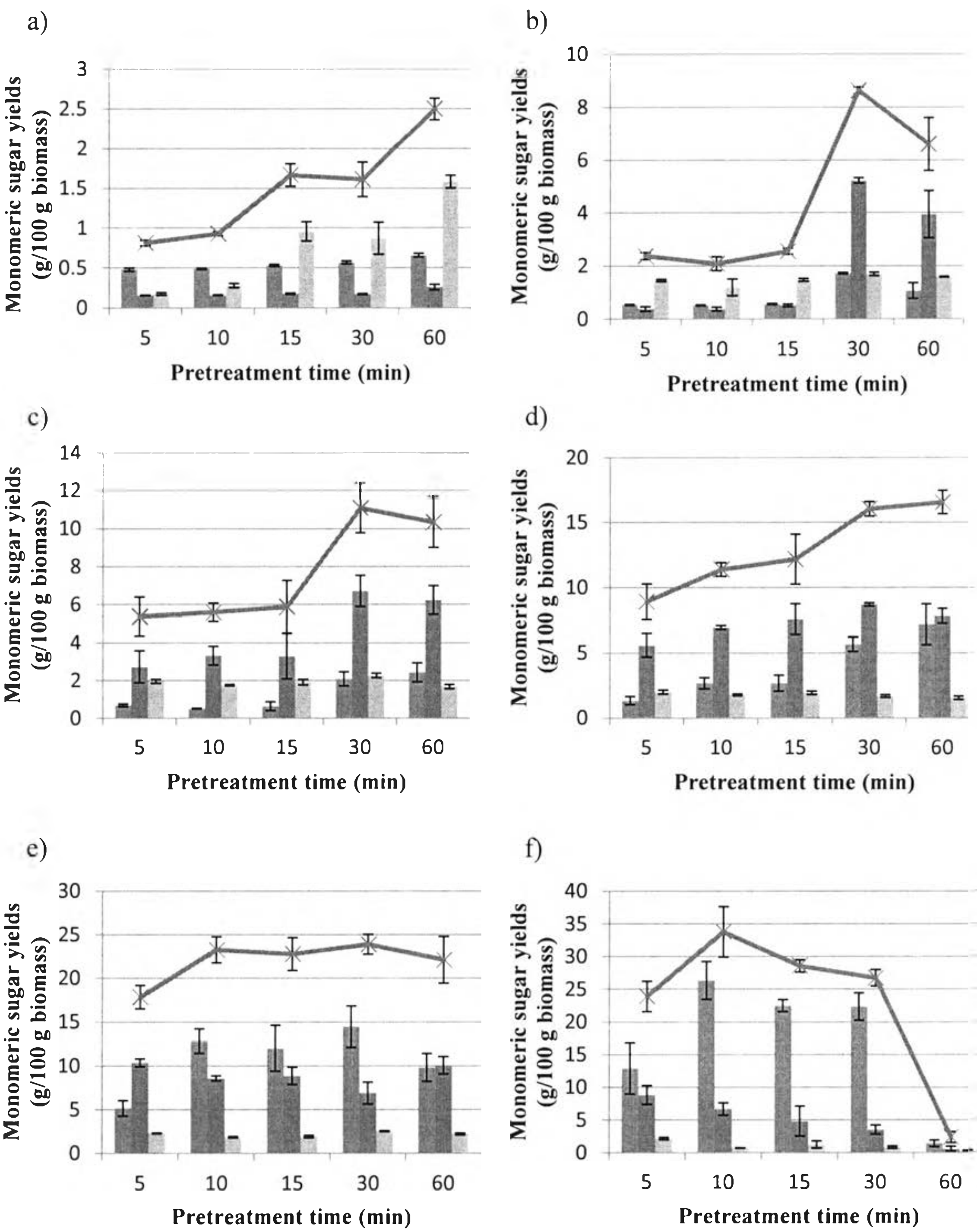


Figure 4.8 The glucose (■), xylose (■), arabinose (□) components and total monomeric sugar yield (-x-) of microwave-assisted NaOH pretreated Kans grass (*Saccharum spontaneum*) using 0.5 % (w/v) H_2SO_4, 15:1 LSR at different temperatures for different times: a) 80°, b) 100°, c) 140°, d) 160°, e) 180°, and f) 200 °C

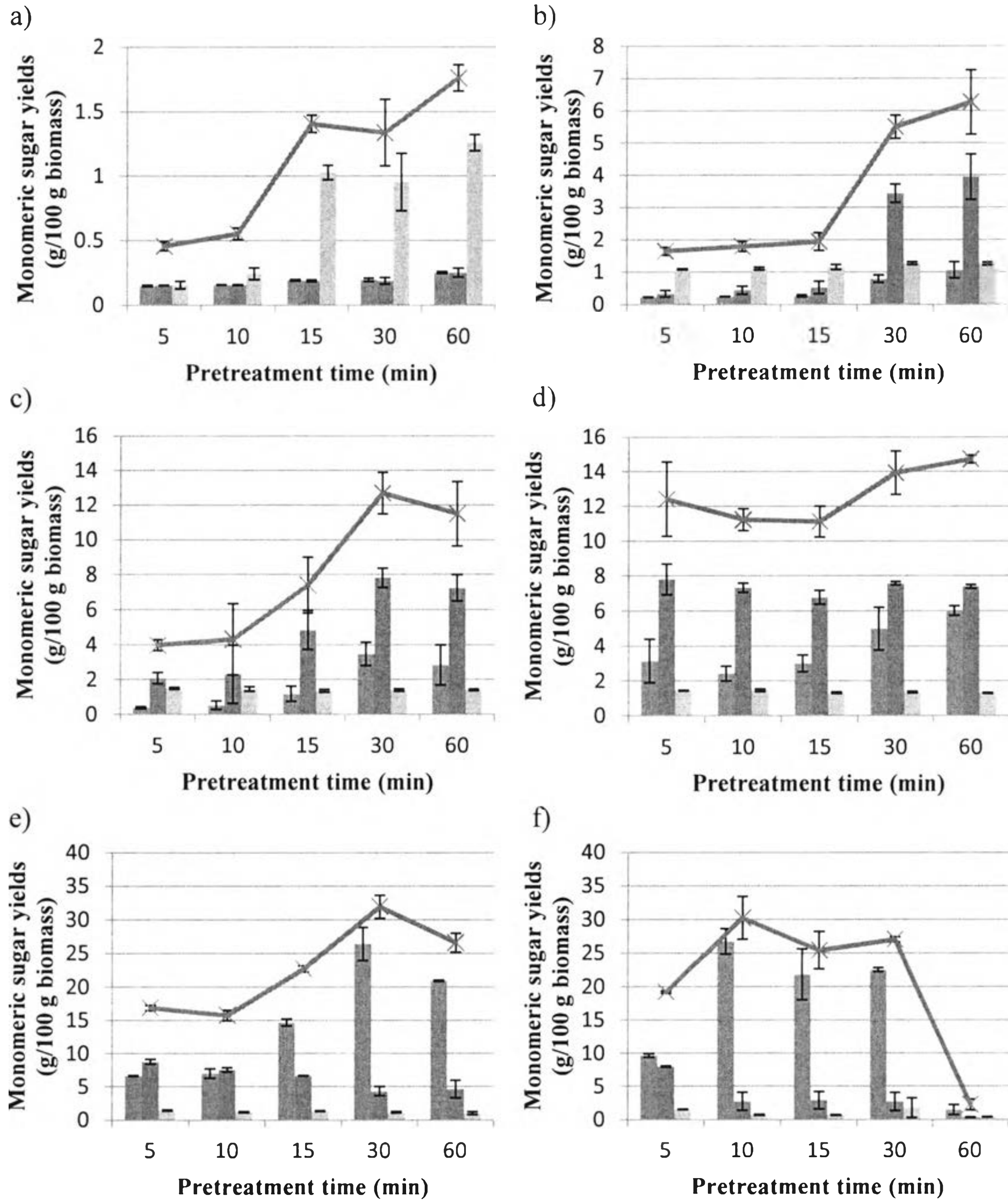
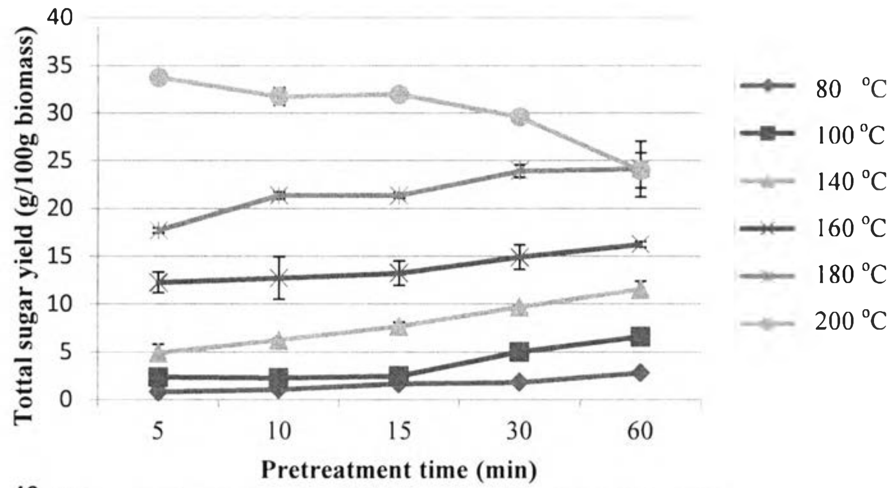
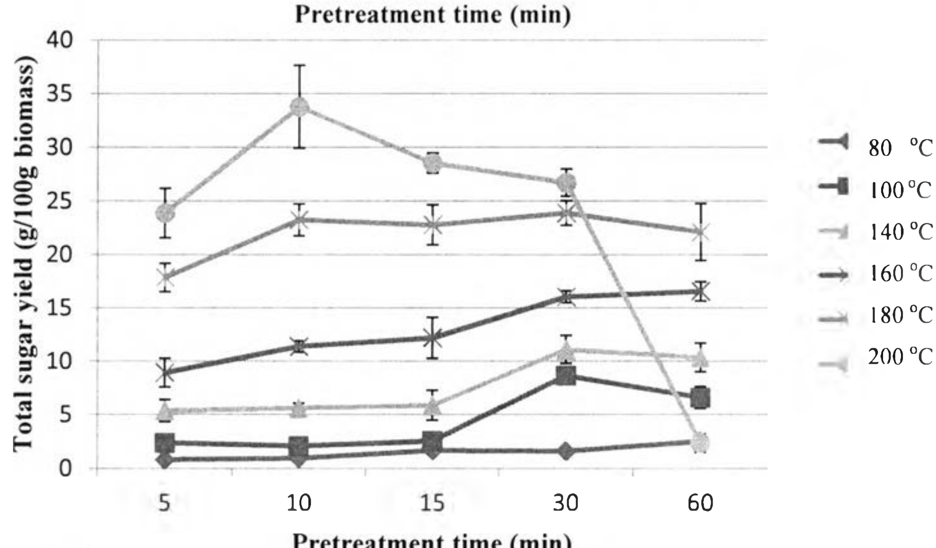


Figure 4.9 The glucose (■), xylose (■), arabinose (□) components and total monomeric sugar yield (-x-) of microwave-assisted NaOH pretreated Giant reed (*Arundo donax*) using 0.5 % (w/v) H_2SO_4, 15:1 LSR at different temperatures for different times: a) 80°, b) 100°, c) 140°, d) 160°, e) 180°, and f) 200 °C

a)



b)



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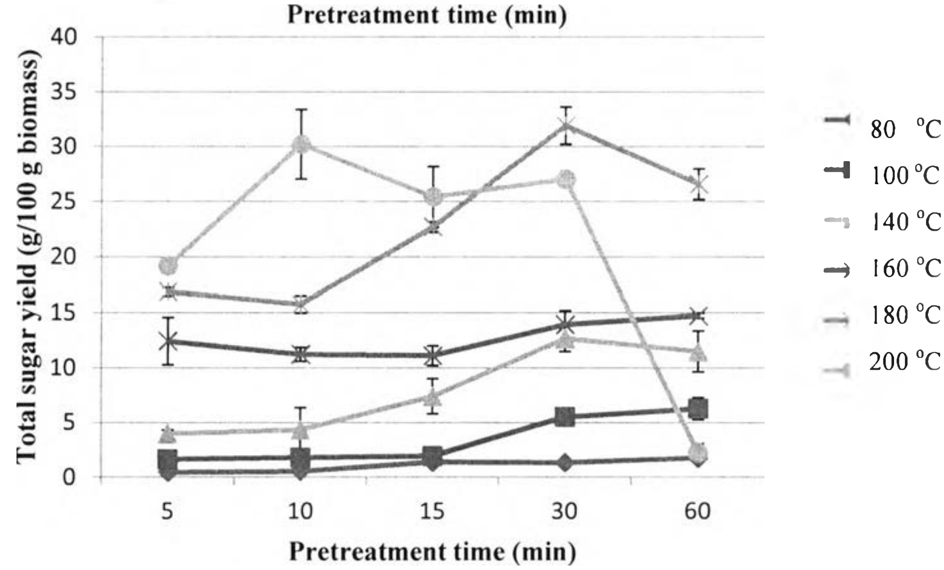


Figure 4.10 Comparison of the total yields of monomeric sugars of microwave-assisted NaOH pretreated a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) H_2SO_4 and 15:1 LSR.

On the other hand, the amount of xylose and arabionose clearly decreased at too high temperature (at 180°, 200 °C) because of the sugar degradation. At 180° and 200 °C, the main monomeric sugar released was glucose from the cellulose hydrolysis. Cellulose has more ordered structure than hemicellulose, so it requires more severe condition to release glucose. However, the glucose degradation also occurs at too high severity. The optimum conditions of the two-stage microwave/chemical pretreatment of those three grasses were to produce high glucose content. The glucoses released from the pretreated Mission grass, Kans grass, and Giant reeds were 31.1, 26.3, and 26.4 g/100 g biomass, respectively. The release of high glucose content shows many benefits on 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 only done by a few strains and usually results in relatively low yields.

4.4.2 Effect of H_2SO_4 concentration

The concentration of H_2SO_4 was another paramerter studied in this work to optimize the monomeric sugars released using the two-stage microwave/chemical pretreatment process. The microwave/NaOH pretreated Mission grass, Kans grass, and Giant reeds were treated with microwave/H_2SO_4 pretreatment at optimum temperature and time condition, 15:1 LSR, and various H_2SO_4 concentrations (0.5–3% (w/v)). The maximum monomeric sugar yields (Fig.4.11) from Mission grass, Kans grass, and Giant reed were 34.3, 33.8, and 31.9 g/100g biomass using 1, 0.5, and 0.5 %(w/v) H_2SO_4, respectively.

The effect of H_2SO_4 concentrations on the grasses are summarized in Fig 4.12. The monomeric sugar yield obviously decreased when the H_2SO_4 concentration increased from 0.5 to 3% (w/v) due to the sugar degradation at high temperature. The increase of the H_2SO_4 concentration generally accelerates the sugar degradation process. Thus, the maximum monomeric sugar yield on three chosen weed could be obtained using and low acid concentrations at high temperature for shorter reaction time.

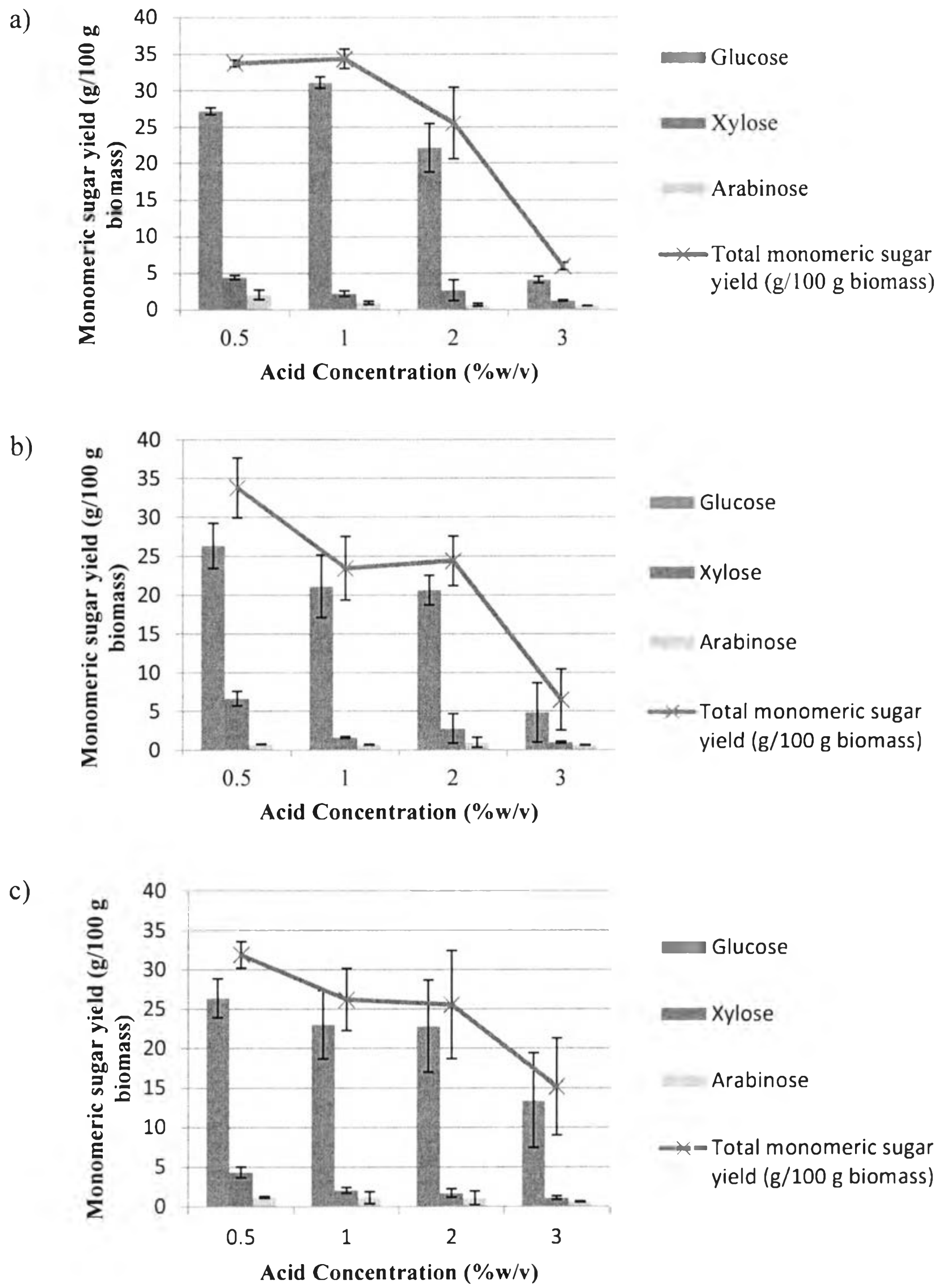


Figure 4.11 Effect of H_2SO_4 concentration (% w/v) on monomeric sugar yields of microwave-assisted NaOH pretreated a) Mission grass at 200 °C-5 min, b) Kans grass at 200 °C-10 min, and c) Giant reed at 180 °C-30 min with 15:1 LSR

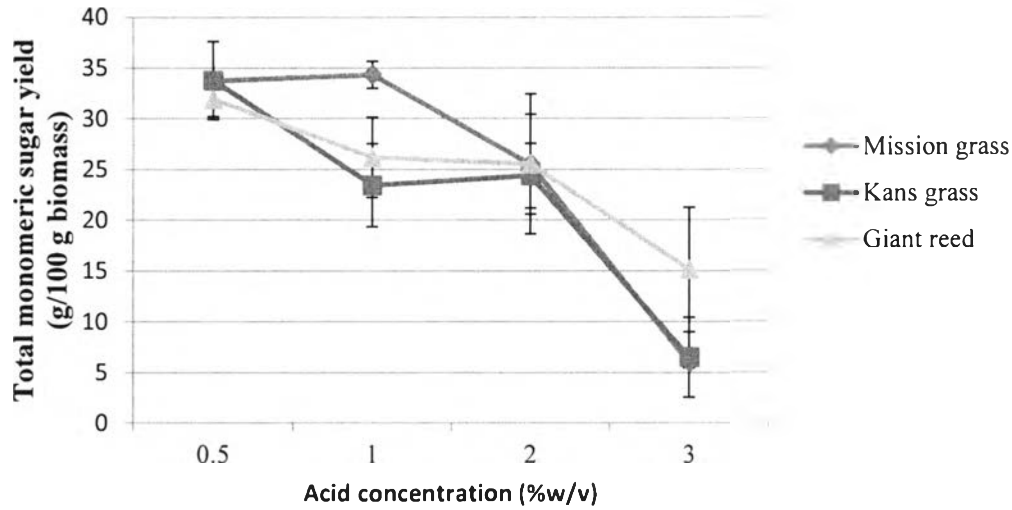


Figure 4.12 Comparison of the total yields of monomeric sugars of microwave-assisted NaOH pretreated a) Mission grass at 200 °C-5 min, b) Kans grass at 200 °C-10 min, c) Giant reed at 180 °C-30 min using 15:1 LSR and different H_2SO_4 concentrations (%w/v)

4.5 Effect of two-stage microwave/chemical pretreatment process on % Solid loss and pH

The two-stage microwave/chemical pretreatment process has not only impact on monomeric sugars yield but also effect on physical appearance of liquid hydrolysate and solid sample, solid loss, and hydrolysate pH. The color of the liquid hydrolysate from the microwave/NaOH pretreatment was dark brown while the liquid hydrolysate color from the microwave/H_2SO_4 was yellow. The intensity of the hydrolysate color in both pretreatment stages increased with severity of the pretreatment because of the increase of the lignin content and the product degradation.

4.5.1 % Solid loss

Firstly, the physical appearance of Mission grass, Kans grass, and Giant reeds were changed (Fig. 4.13) when increasing the severity of the pretreatment conditions. Both the first (microwave/NaOH) and the second (microwave/H_2SO_4) stages also resulted in the same trend of %solid loss. Increasing the severity conditions (temperature, time, and chemical concentration) caused the %solid loss to increase due to the degradation of grasses. Among these severity factors, the temperature was the main effect to cause higher %solid loss than the others. Other works also reported similar trend in %solid loss during the pretreatment process (Chen *et al.*, 2004). The %solid loss found was in the range of 7–86%, based on type of grass and severity of the pretreatment condition, as shown in Fig. 4.14-4.17.

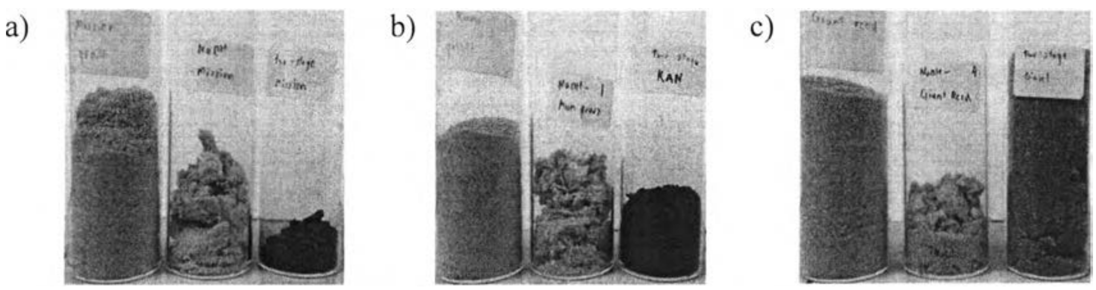


Figure 4.13 The physical appearance of a) Mission grass, b) Kans grass, and c) Giant reed from untreatment (left bottle), microwave/NaOH pretreat ment (middle bottle), and two-stage, microwave/NaOH followed by microwave/H_2SO_4, pretreatment (right bottle).

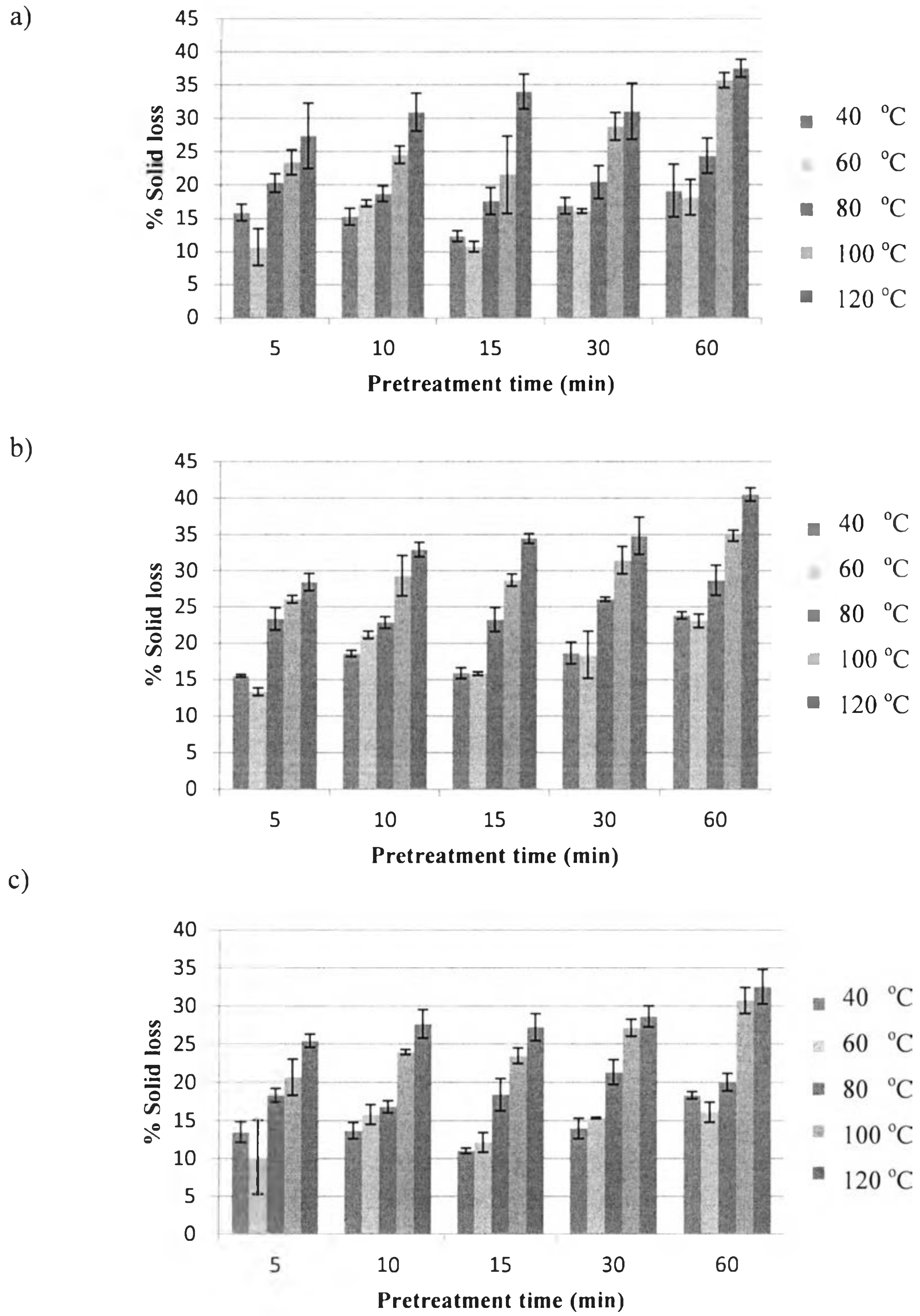
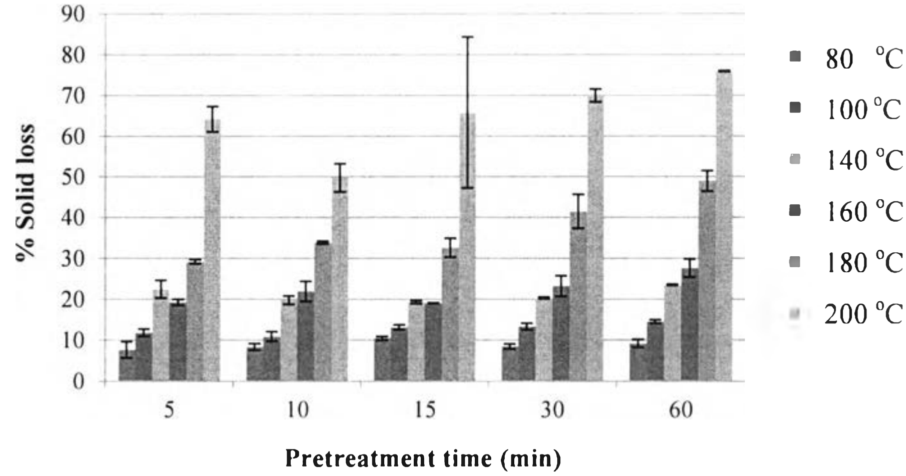


Figure 4.14 %Solid loss of untreated a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) NaOH and 15:1 LSR

a)



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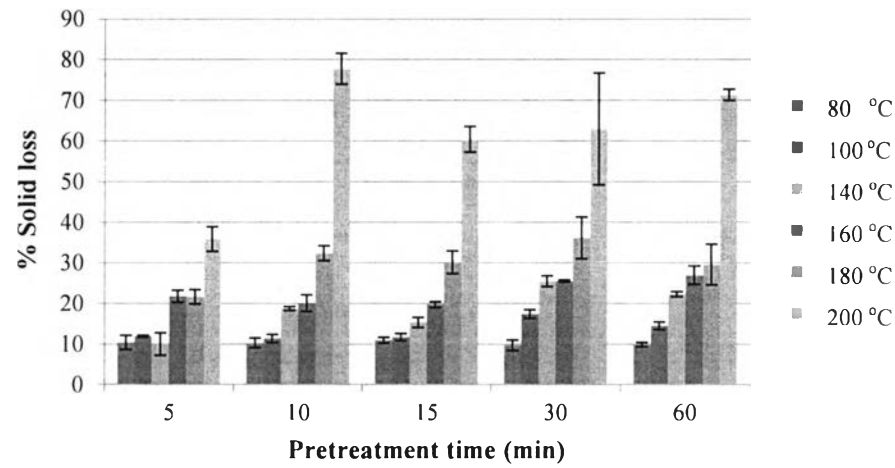

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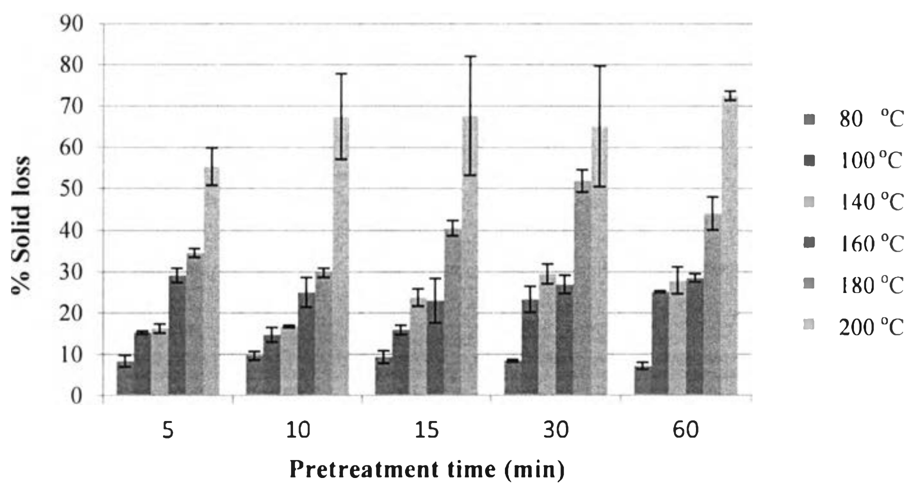


Figure 4.15 %Solid loss of microwave-assisted NaOH pretreated a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) H_2SO_4 and 15:1 LSR

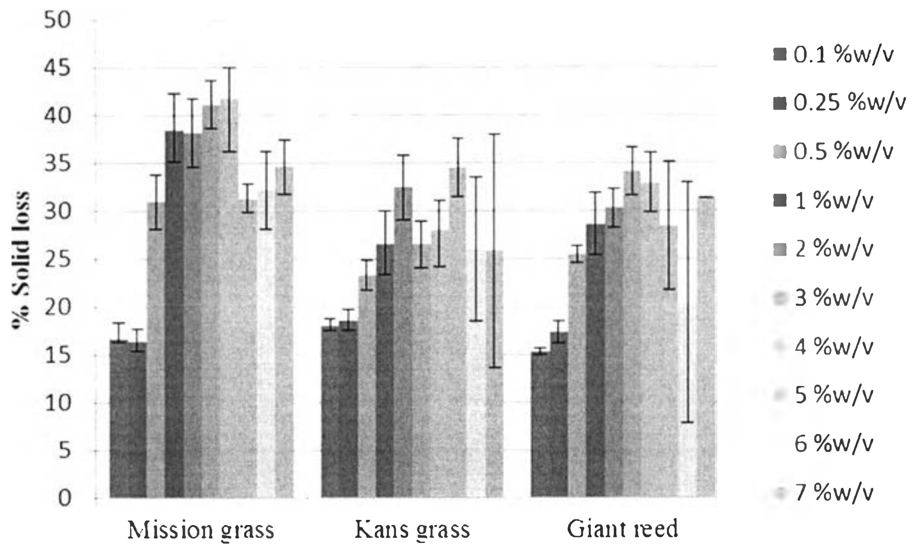


Figure 4.16 %Solid loss of Mission grass at 120 °C-10 min, Kans grass at 80 °C-5 min, c) Giant reed at 120 °C-5 min using 15:1 LSR and different NaOH concentrations (%w/v)

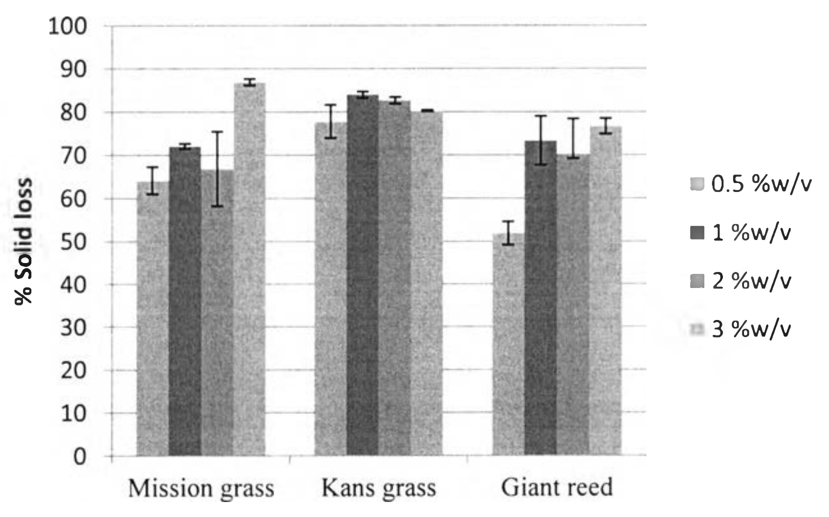


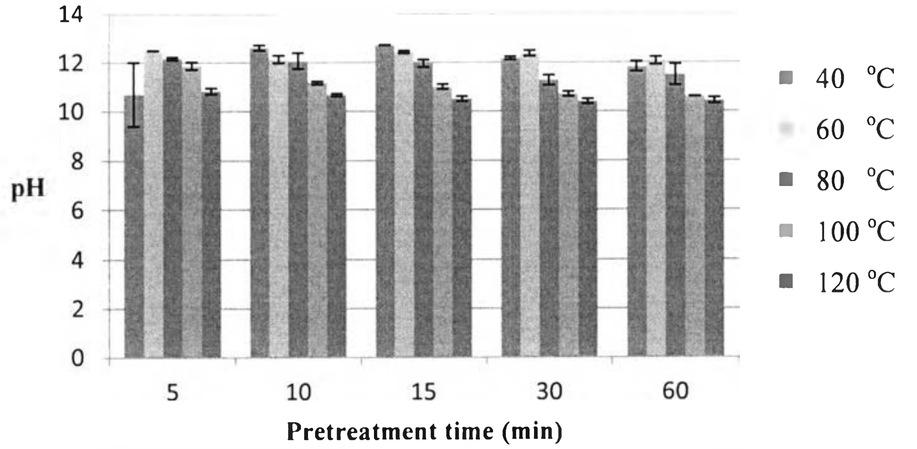
Figure 4.17 %Solid loss of microwave-assisted NaOH pretreated Mission grass at 200 °C-5 min, Kans grass at 200 °C-10 min, and Giant reed at 180 °C-30 min using 15:1 LSR and different H_2SO_4 concentrations (%w/v)

4.5.2 pH value

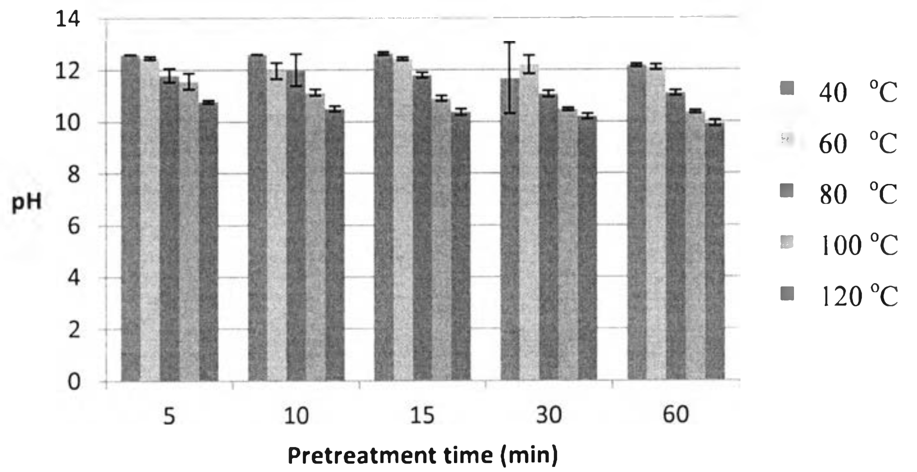
The pretreatment temperature and the time also affect the final hydrolysate pH because of the chemical reaction taken place to the lignocellulosic structure. The final pH values of each alkaline pretreatment condition of Mission grass, Kans grass, and Giant reeds at various temperatures and times were demonstrated in Fig. 4.18. The pH was decreased from 12.5 to 10.4, 12.6 to 9.9, and 12.7 to 9.9 for Mission grass, Kans grass, and Giant reed, respectively. The reason for this decreased pH is because more hydroxide ions were used to cleave the lignin ether bond in alkaline delignification process (Fig. 4.19) (Gierer *et al.*, 1985, Lin *et al.*, 2002, Harmsen *et al.*, 2010). Moreover, acetic acid could be generated at highly severe conditions from the sugar degradation process, and thus lower the pH (Larson *et al.*, 2008).

The final pH of the microwave/H_2SO_4 pretreatment on three chosen weeds was also measured at various temperature and time conditions. At the same pretreatment time, the pH value mostly increase with high temperature condition. (Fig 4.20). Treebubpha *et al*, in 2012 proposed that the pH of the acid hydrolysate increased with temperature because proton acting as a catalyst protonated the oxygen atom linkage between cellulose and hemicellulose in the hydrolysis process. The pH value is slightly change by time at the same pretreatment temperature. However, the pH value can be fluctuated because acetic acid formed at high severity condition by degradation process could lower the hydrolysate pH value.

a)



b)



c)

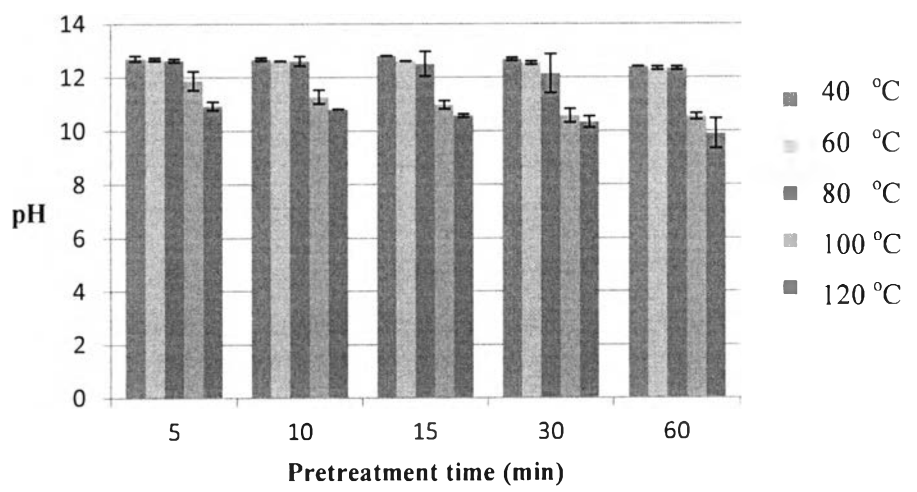
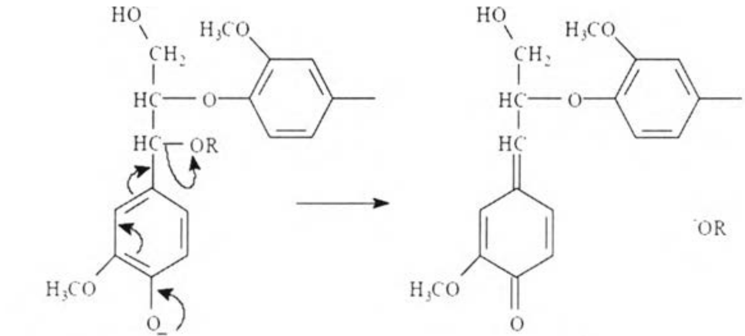


Figure 4.18 The solution pH of a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) NaOH and 15:1 LSR

a)



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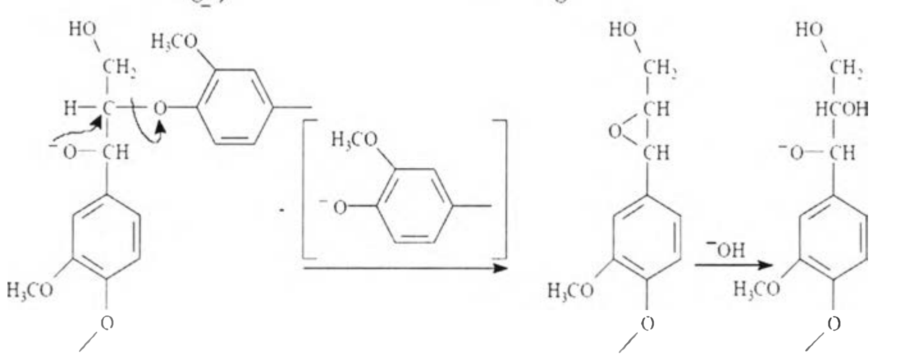

Figure 4.19 Alkaline cleavage of a) α-aryl ether bonds and b) β-aryl ether bonds (Gierer *et al*., 1985).

a)

b)

c)

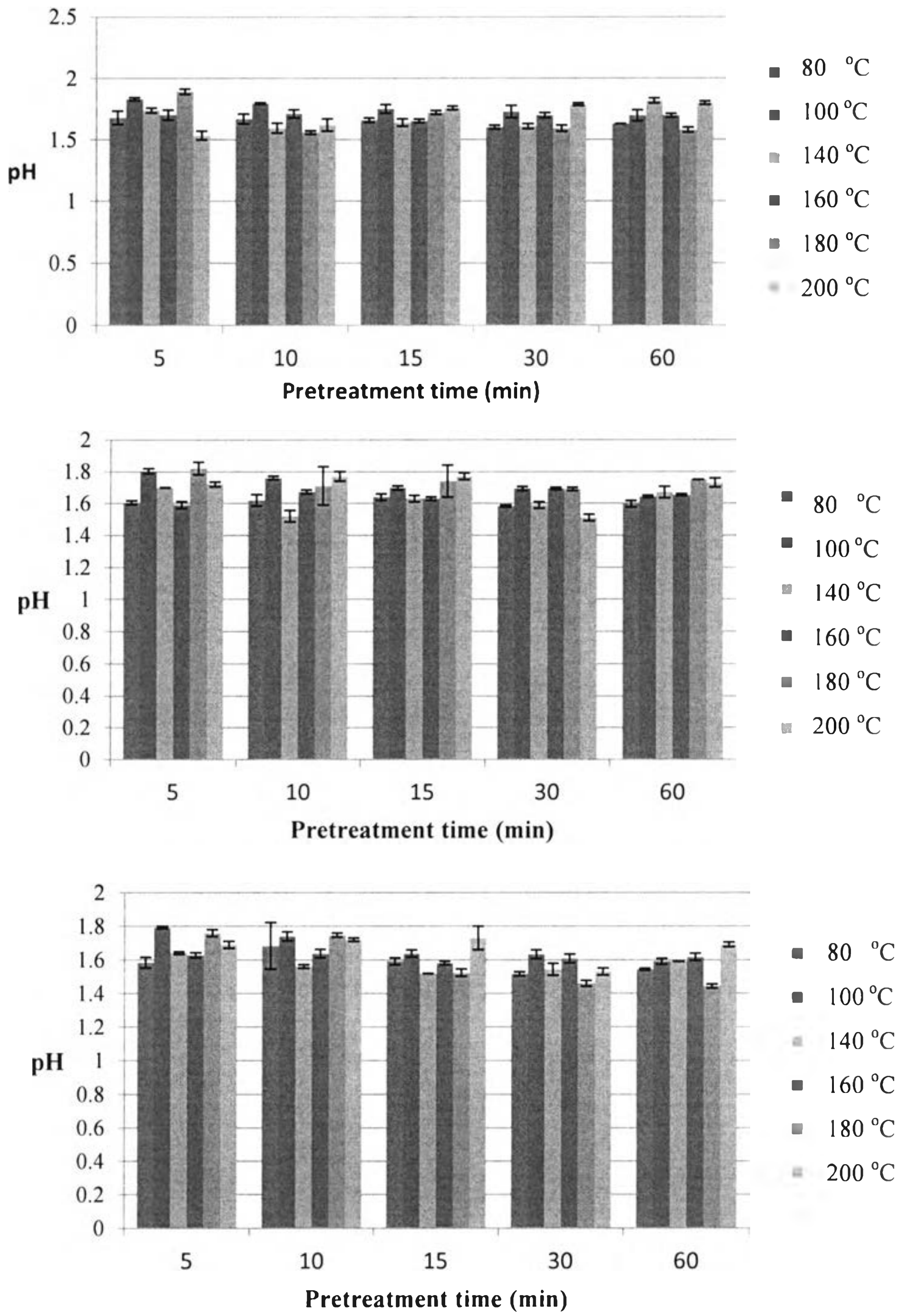

Figure 4.20 The solution pH of microwave-assisted NaOH pretreated a) Mission grass, b) Kans grass, c) Giant reed at different temperatures for different times using 0.5% (w/v) H_2SO_4 and 15:1 LSR

When changing NaOH/H_2SO_4 concentrations in the pretreatment process, the final pH of the hydrolysate was found that the higher NaOH concentrations led to the higher pH while the higher H_2SO_4 concentration caused the lower pH. The pH results at various NaOH and H_2SO_4 concentrations are shown in Fig 4.21 and 4.22, respectively.

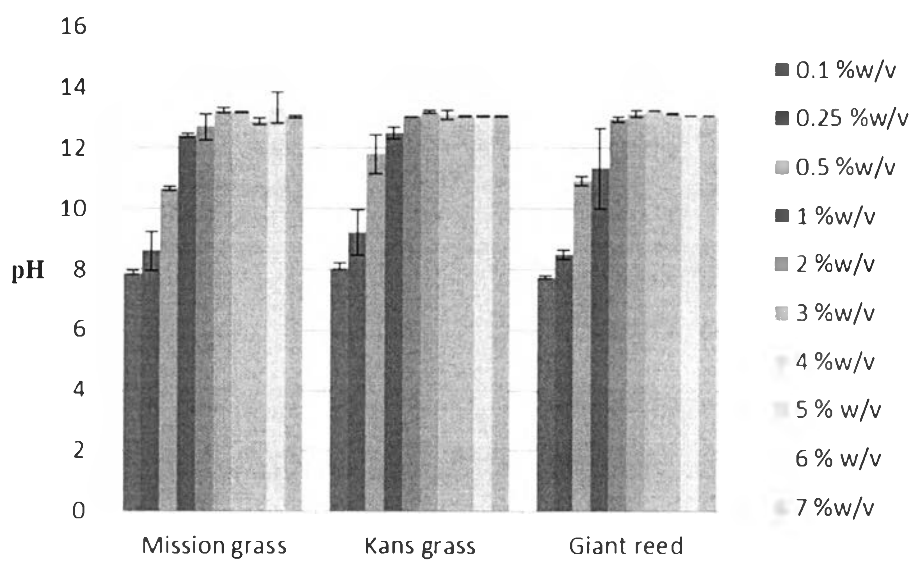

Figure 4.21 The solution pH of Mission grass at 120 °C-10 min, Kans grass at 80 °C-5 min, c) Giant reed at 120 °C-5 min using 15:1 LSR and different NaOH concentrations (%w/v)

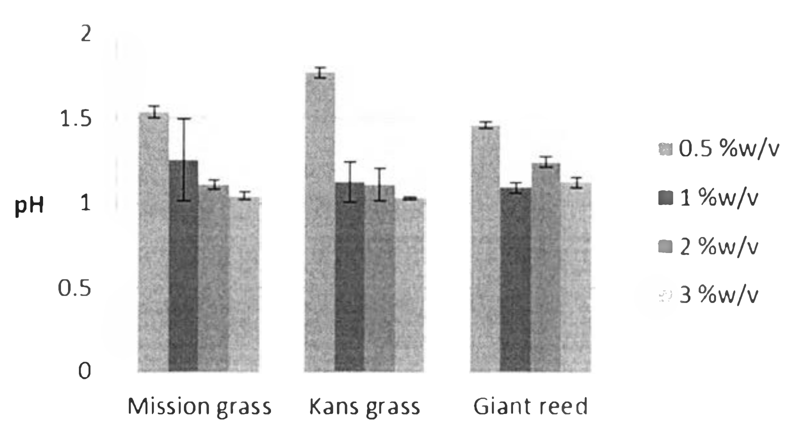

Figure 4.22 The solution pH of microwave-assisted NaOH pretreated Mission grass at 200 °C-5 min, Kans grass at 200 °C-10 min, and Giant reed at 180 °C-30 min using 15:1 LSR and different H_2SO_4 concentrations (%w/v)

Both $NaOH/H_2SO_4$ concentrations and temperature affect the pH and the lignocellulose structure, as schematically sketched in Fig 4.23. The lignin is degraded, liberating phenolic lignin monomer at alkaline condition whereas spherical lignin droplets are deposited onto the solid fractions under acid condition. For cellulose and hemicellulose, this polysaccharide is hydrolyzed in a strong acidic pretreatment (Pedersen and Meyer, 2010).

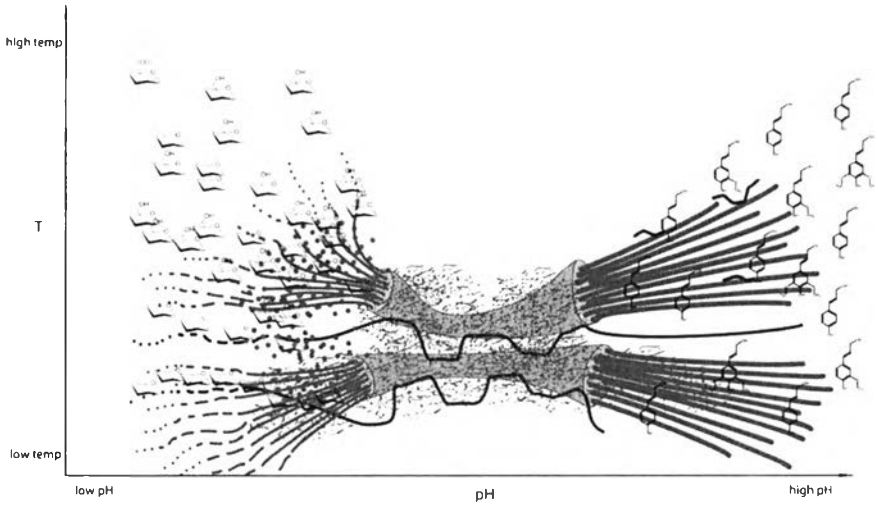

Figure 4.23 Sketch of lignocellulose pretreatment by temperature and pH. Gray 'veil' indicates lignin sheath; orange and red tubes illustrate cellulosic fibrils and microfibrils, respectively; black curved lines illustrate hemi cellulose (xylan); the gray dots on the cellulose microfibrils in the low pH region illustrate redeposited lignin (Pedersen and Meyer., 2010)

4.6 Effect of Pretreatment on Chemical Composition

The chemical compositions of lignocellulosic biomass, including cellulose, hemicellulose, lignin, and ash, were investigated using National Renewable Energy Laboratory (NREL) method. After Mission grass, Kans grass, and Giant reed were treated by the microwave/NaOH and the two-stage pretreatments, the chemical compositions were analyzed and are summarized in Tables 4.5–4.7. These results confirm the effect of the two-stage pretreatment process and explain the obtained monomeric sugar yield. The chemical composition with high polysaccharide and low lignin content can easily release monomeric sugar in the hydrolysis step. The cellulose contents in untreated Mission grass, Kans grass, and Giant reeds were 39.8, 35.4, and 39.1 % dry matter, respectively, and the glucose contents from the optimum condition obtained from the microwave/NaOH pretreatment of those untreated grasses were 4.2, 3.6, and 4.4 g/100 g biomass, respectively. These data confirm that the higher cellulose content in lignocellulosic biomass resulted in the higher released glucose content. On the other hand, the higher hemicellulose content should release the higher xylose and arabinose contents. However, some part of xylose and arabinose sugar can be degraded at the optimum condition. The lignin content in the untreated Giant reed (19.2 % dry matter) was higher than those in the untreated Mission grass and Kans grass, as a result, to release high monomeric sugar yields the optimum conditions of the Microwave/NaOH pretreatment for the Giant reed (at 120 °C, 5 min, 5%(w/v) NaOH) was more severe. In conclusion, the chemical composition in the raw biomass can predict the type of the monomeric sugar and the pretreatment condition.

Table 4.5 Chemical composition of Mission grass solid residues from each pretreatment stage

Composition (% dry matter)	**Mission grass (*Pennisetum polystachyon*)**		
	Untreated	**Microwave/NaOH**	**Microwave/NaOH/ H_2SO_4**
Glucan	39.8±1.5	82.4±1.6	16.1±3.5
Xylan	25.0±0.9	17.2±0.2	3.4±0.1
Arabinan	4.2±0.1	2.8±0.2	0
Lignin	14.6±0.5	2.3±2.0	0.4±0.0
Ash	3.3±0.5	0.1±0.1	0.1
Other	16.6	-	87.6±1.3

Table 4.6 Chemical composition of Kans grass solid residues from each pretreatment stage

Composition (% dry matter)	Kans grass (*Saccharum spontaneum*)		
	Untreated	Microwave/NaOH	Microwave/NaOH/ H_2SO_4
Glucan	35.4±0.9	78.8±1.0	13.6±1.1
Xylan	23.1±0.3	17.0±0.2	0
Arabinan	3.6±0.1	3.3±0.1	0
Lignin	14.5±0.8	6.4±0.9	2.1±0.3
Ash	1.1±1.0	0.1±0.1	0
Other	12.5	-	76.1±2.3

Table 4.7 Chemical composition of Giant reed solid residues from each pretreatment stage

Composition (% dry matter)	Giant reed (*Arundo donax*)		
	Untreated	Microwave/NaOH	Microwave/NaOH/ H_2SO_4
Glucan	39.1±0.3	79.2±0.9	48.8±2.7
Xylan	21.7±0.4	14.9±0.1	7.6±0.2
Arabinan	2.8±0.1	2.4±0.1	0
Lignin	19.2±3.3	10.2±1.4	0.4±0.0
Ash	4.2±0.7	0.1±0.1	0.3
Other	7.4	-	38.7±7.5

After the microwave/NaOH pretreatment, the Mission grass, Kans grass, and Giant reed solid residues contained cellulose as the main component in the range of 78–82 %. These results, corresponding to the monomeric sugar yield at the optimum condition of the two-stage pretreatment, thus showed a high glucose content. This pretreatment step also solubilized some part of hemicelluloses, lowering its component in the weeds. Most importantly, this alkali pretreatment stage effectively removed and lowered the lignin content in the samples, leaving most reactive biomass with high cellulose content.

When the treated microwave/NaOH Mission grass, Kans grass, and giant reed solid residues were further treated with the microwave/H_2SO_4 pretreatment at the optimum conditions, the polysaccharide contents in both Mission grass and Kans grass were significantly reduced, remaining only 13–16 % cellulose content. The results can be explained that the pretreatment was performed at 200 °C, the main component was degraded and burned. However, the Giant reeds was performed at 180 °C, lower temperature, higher cellulose content was remained (48.8 %). The ash content rarely remained in the two-stage pretreated samples. Therefore, these results assure that the two-stage pretreatment process effectively converts high cellulose content into monomeric sugars.

4.7 FT-IR Analysis

The chemical structure change of lignocellulose in the two-stage pretreatment process was detected by FTIR. The FTIR spectra of the untreated, the microwave/NaOH pretreated, and the two-stage pretreated Mission grass, Kans grass, and Giant reed are illustrated in Fig. 4.24–4.26, respectively. The untreated grasses show similar FTIR spectra to the other herbaceous biomass.

After the microwave/NaOH pretreatment, the FTIR peak at 1734 cm^{-1} disappeared from those grasses because the complex linkage between lignin and hemicellulose, such as ester-linked acetyl, feruloyl, and p-coumaroyl groups, were broken. FTIR 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 FTIR peaks (898, 1108, 1164, 1260, 1325, and 1378 cm^{-1}) became sharper, as compared with the untreated weeds. The FTIR results confirm the efficiency of the pretreatment method for lignin removal to give reactive biomass with high cellulose contents for the dilute acid pretreatment step.

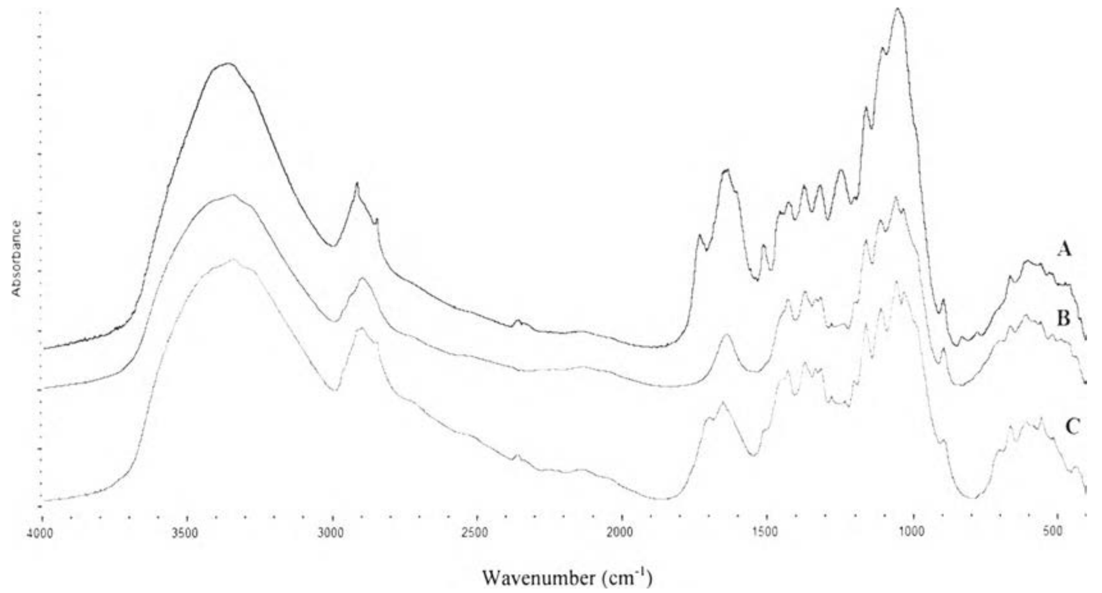

Figure 4.24 FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated Mission grass

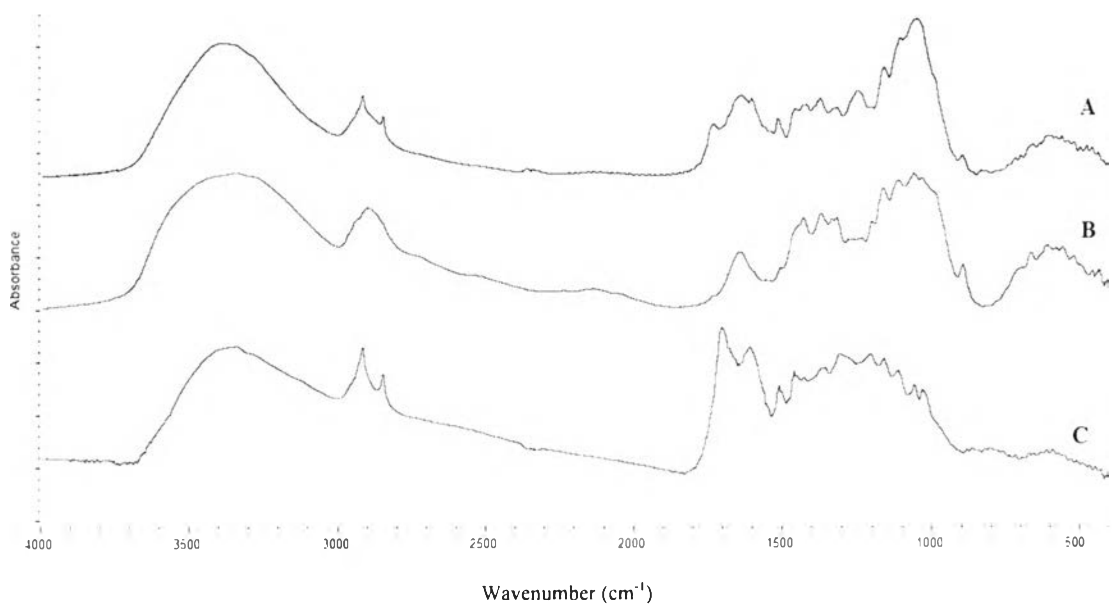

Figure 4.25 FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated Kans grass

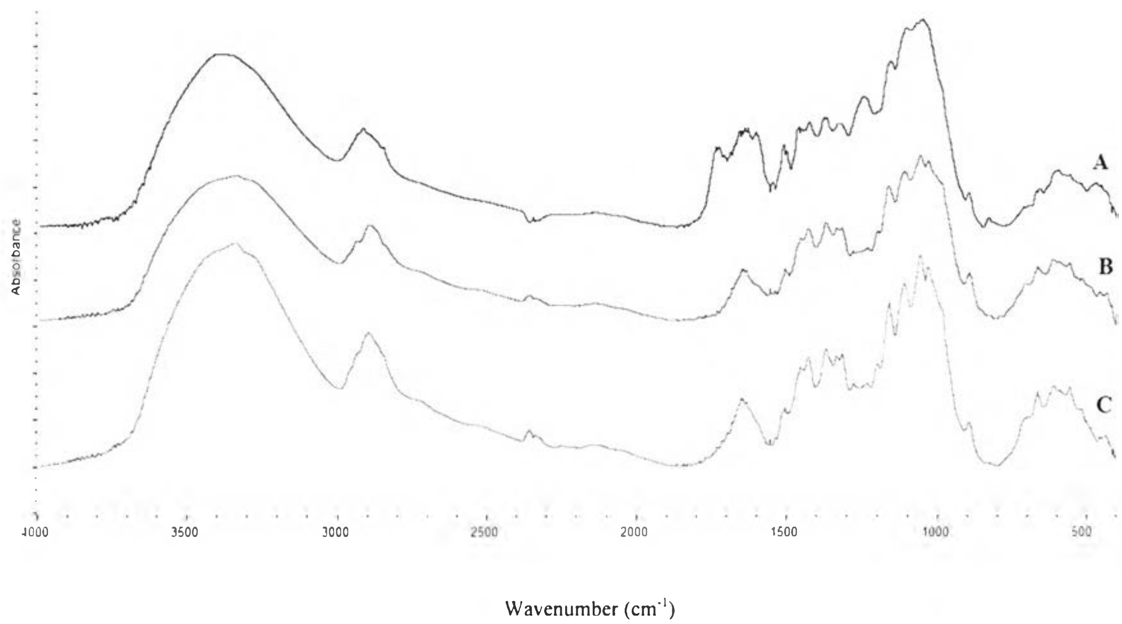

Figure 4.26 FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated Giant reeds

FTIR spectra obtained from the two-stage pretreatment at the optimum conditions were different. The FTIR spectrum of the two-stage pretreated Giant reed was similar to that of the microwave/NaOH pretreatment because the optimum conditions of Giant reed caused less chemical composition change, unlike Mission grass and Kans grass. Both grasses used more severe conditions, thus causing some sugar degradation. The two-stage pretreated Mission grass clearly show FTIR peak at 1722 cm^{-1} (carbonyl bond unconjugated to aromatic ring) because the lower cellulose content can emphasize the lignin signal. Differently, the FTIR pattern of the pretreated two-stage Kans grass was remarkably changed from the pretreated microwave/NaOH sample, generating FTIR peaks at 593, 1207, 1309, 1512, and 1703 cm^{-1} that maybe caused from the lignin and degraded residues.

4.8 SEM Characterization

SEM images of the untreated, the pretreated microwave/NaOH, and the treated two-stage samples were characterized to study the physical appearance changes of Mission grass, Kans grass, and Giant reed, as shown in Fig. 4.27– 4.29.

The morphology of the untreated Mission grass, Kans grass, and Giant reed (Fig 4.27A–4.29A) show highly fibril and intact structure, and the major cellulose structure is maintained. The untreated weed structures were covered with thin film layer, probably the wax layer, commonly found in herbaceous biomass (Hu and Wen, 2008) or the lignin, and lignin carbohydrate complexes condense on the surface of the cellulose fiber (Zhu *et al.*, 2009).

In SEM images of the pretreated microwave/NaOH Mission grass, Kans grass, and Giant reed (4.27B–4.29B), the samples had still an indication of fibril structure, but the thin film on the surface was disappeared. Moreover, the surface became smooth and thinner with ridge and has been perforated by the microwave/NaOH pretreatment process (Hu and Wen, 2008). The generated pores would increase the surface area, causing the hydrolysis step more effective. This morphology confirms the FTIR results and that the lignin was destroyed to ease the cellulose and the hemicellulose structures better exposed.

On the contrary, the fibril structure of the two-stage pretreated samples (Fig 4.27C–4.29C) was distorted by hydrolysis process at high temperature. Only Giant reeds still maintained fibril-like structure because it was hydrolyzed with lower temperature than the others. These SEM images show that the two-stage pretreatment effectively break down the lignocellulose structure for monomeric sugar releasing. Another word, high severity pretreatment conditions can distort the biomass structure more than a low severity pretreatment condition.

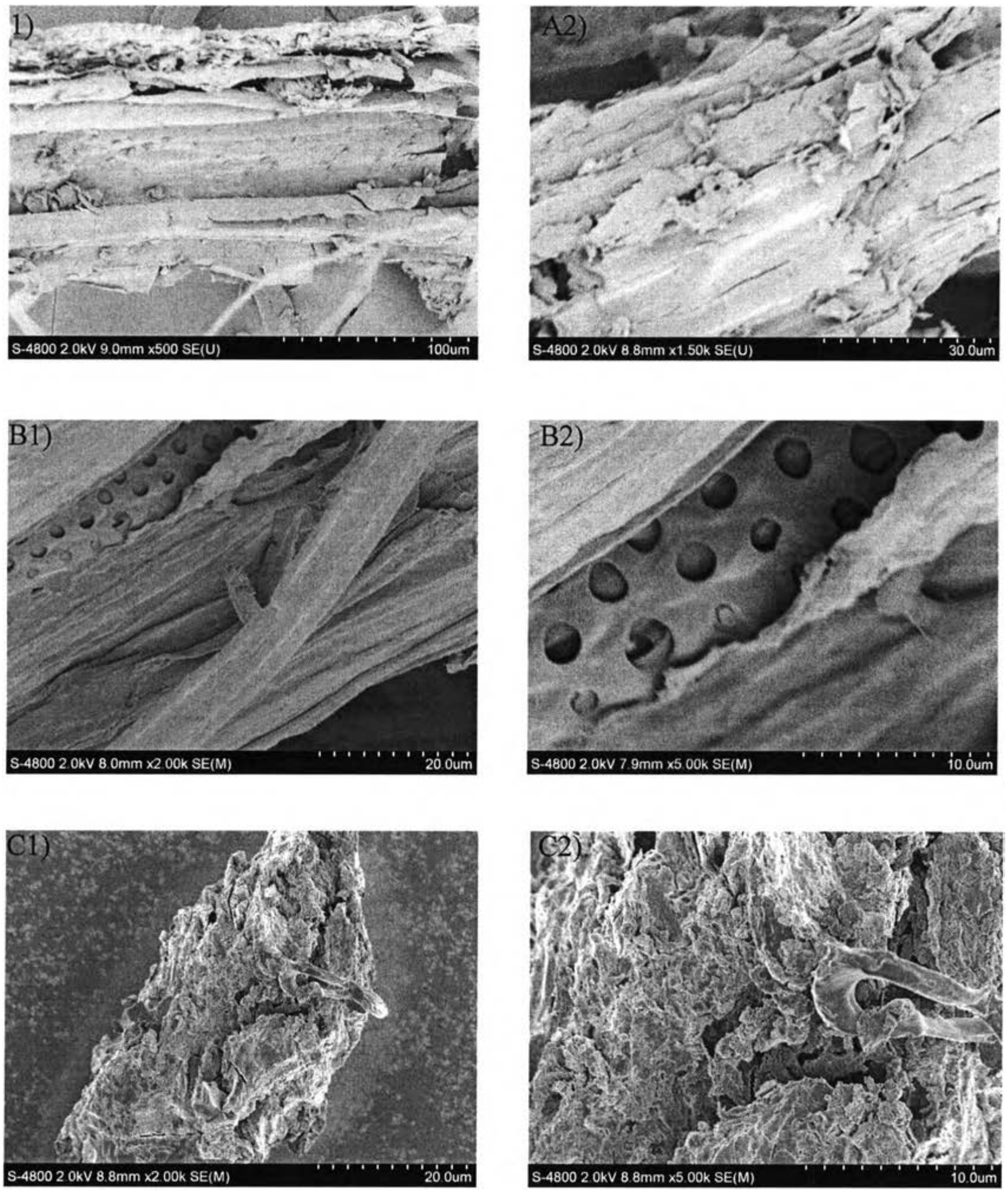

Figure 4.27 Scanning electron microscope images of A) unteated [(A1)(500x), (A2)(1500x)], B) microwave/NaOH pretreated (at 120 °C,10 min, 3% (w/v) NaOH, 15:1 LSR) [(B1)(2000x), (B2)(5000x)], C) two-stage pre treated Mission grass (at 200 °C,5 min, 1% (w/v) H_2SO_4, 15:1 LSR) [(C1)(2000x), (C2)(5000x)]

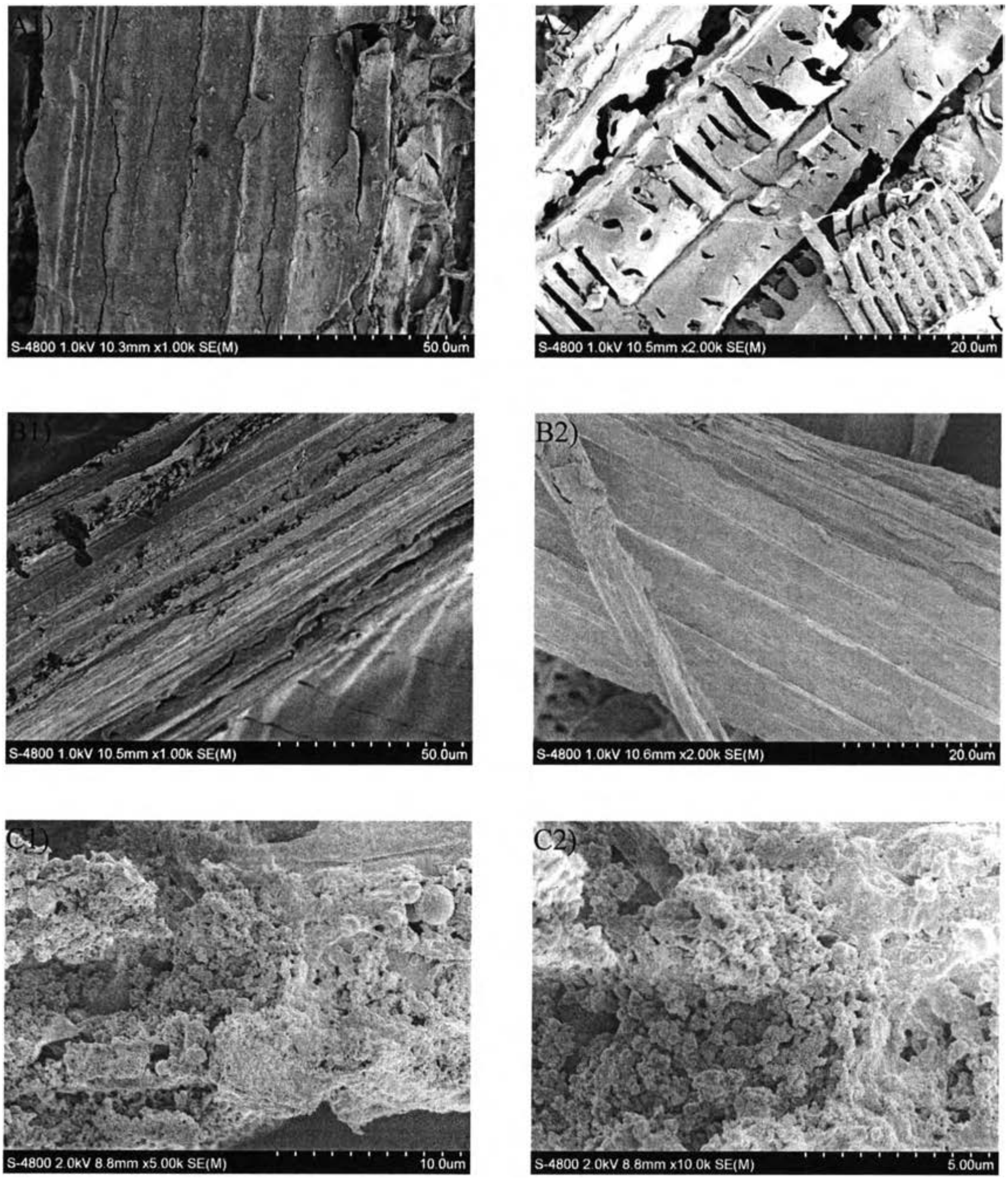

Figure 4.28 Scanning electron microscope images of A) unteated [(A1)(1000x), (A2)(2000x)], B) microwave/NaOH pretreated (at 80 °C,5 min, 5% (w/v) NaOH, 15:1 LSR) [(B1)(1000x), (B2)(2000x)], C) two-stage pre treated Mission grass (at 200 °C,10 min, 0.5% (w/v) H_2SO_4, 15:1 LSR) [(C1)(5000x) ,(C2)(10000x)]

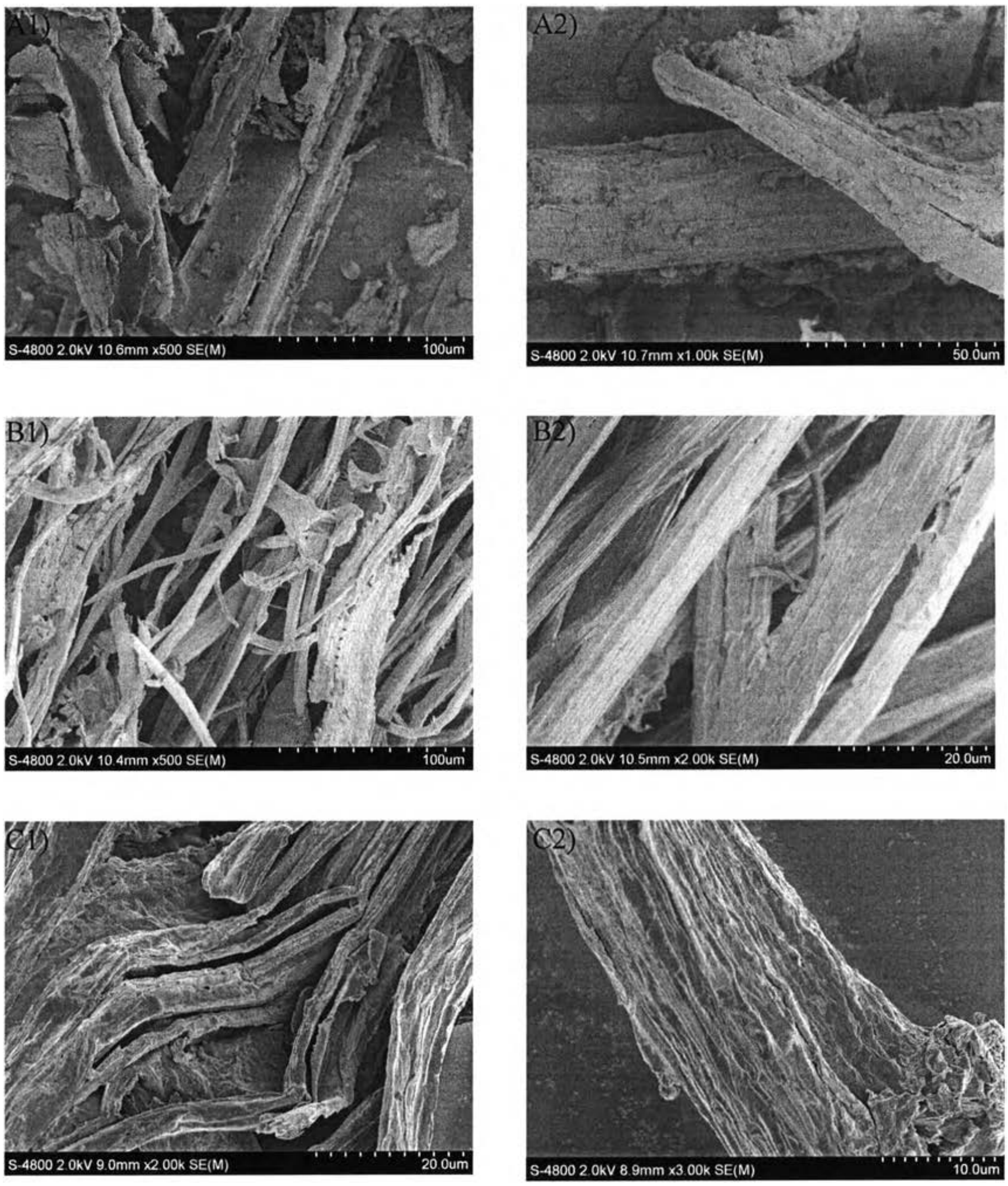

Figure 4.29 Scanning electron microscope images of A) unteated [(A1)(500x), (A2)(1000x)], B) microwave/NaOH pretreated Giant reed at 120 °C,5 min, 5% (w/v) NaOH, 15:1 LSR) [(B1)(500x), (B2)(2000x)], C) two-stage pretreated Giant reed (at 180 °C,30 min, 0.5% (w/v) H_2SO_4, 15:1 LSR) [(C1)(2000x), (C2)(3000x)]