# CHAPTER V

# COMPARATIVE POTENTIALITY OF KANS GRASS (*SACCHARUM SPONTANEUM*) AND GIANT REED (*ARUNDO DONAX*) AS LIGNOCELLULOSIC FEEDSTOCKS FOR THE RELEASE OF MONOMERIC SUGARS BY MICROWAVE/ CHEMICAL PRETREATMENT

## 5.1 Abstract

Two-stage microwave (microwave/ NaOH pretreatment followed by microwave/$H_2SO_4$ pretreatment) was used to release monomeric sugars from Kans grass (*Saccharum spontaneum*) and Giant reed (*Arundo donax*). The optimum pretreatment conditions were investigated, and the maximum monomeric sugar yields were compared. The microwave-assisted NaOH and $H_2SO_4$ pretreatments with a 15:1 liquid-to solid ratio were studied by varying the chemical concentration, reaction temperature, and reaction time to optimize the amount of monomeric sugars. The maximum amounts of monomeric sugars released from microwave-assisted NaOH pretreatment were 6.8 g/100 g of biomass [at 80 °C/5 min, 5 % (w/v) NaOH for *S. spontaneum* and at 120 °C/5 min, 5 % (w/v) NaOH for *A. donax*]. Furthermore, the maximum amounts of monomeric sugars released from microwave-assisted $H_2SO_4$ pretreatment of *S. spontaneum* and *A. donax* were 33.8 [at 200 °C/10 min, 0.5 % (w/v) $H_2SO_4$] and 31.9 [at 180 °C/30 min, 0.5 % (w/v) $H_2SO_4$] g/100 g of biomass, respectively. The structural changes of *S. spontaneum* and *A. donax* were characterized using Fourier transform infrared spectroscopy and scanning electron microscopy.

(**Keywords**: *Saccharum spontaneum*; *Arundo donax*; Lignocellulosic biomass; Two-stage Pretreatment; Microwave irradiation)

## 5.2 Introduction

The growth of the world population and industrial prosperity has brought an inevitable increase in energy consumption. Fossil fuels have been the major resources used to respond to the rising energy demand (Sun and Cheng, 2002). The use of these resources, however, has been accompanied by many problems regarding the cost, petroleum drilling technology, and global warming. Thus, researchers are endeavoring to find another source of energy. Ethanol presents one feasible source of alternative energy to replace fossil fuels (Jeffries, 2006).

Bioethanol is a good alternative for transportation fuel because of its economic, environmental, and strategic attributes (Bai *et al.*, 2008). Presently, sugar and starch-based raw materials are utilized in the production of bioethanol. However, the increasing human population and fuel demand make these raw materials insufficient for bioethanol production. Thus, researchers are attempting to use lignocellulosic biomass as a resource for bioethanol (Li *et al.*, 2010). Lignocellulosic biomass is the most abundant and cheapest biomass available (Sassner *et al.*, 2008). Polysaccharides present in the lignocellulosic materials can be depolymerized to monosaccharides and converted to ethanol (Hsu *et al.*, 2010). The major components of lignocellulosics are cellulose (polymers of hexose sugars, 35–50 %), hemicellulose (polymers of pentose sugars, 20–35 %), and lignin (polyphenols, 10–25 %) (Taherzadeh and Karimi, 2007).

*Saccharum spontaneum* (wild sugarcane or Kans) is one of the most promising future biomass feedstocks for fuel ethanol production because of its ability to grow quickly without requiring any economic input (Chandel *et al.*, 2009). *Saccharum spontaneum*, long considered a wasteland weed, is a tall perennial grass with deep roots and rhizomes. The plant can be as tall as 6 m. It infests millions of acres of land as a weed in agricultural fields (Tai and Miller, 2001; Chandel *et al.*, 2009). The cell wall of the plant stem and sheath of *S. spontaneum* contains a high carbohydrate content (67.9 % by wt.), proving its capacity to serve as an appropriate substrate for bioconversion of fuel ethanol (Scordia *et al.*, 2010).

*Arundo donax L.* (Giant reed) is a fast-growing Gramineae. The rapid spread of this species could potentially contribute to high ethanol productivity. *A. donax* is a

tall perennial grass. The stem can reach a height of 8–9 m (Perdue, 1958). It is native to Asia and countries surrounding the Mediterranean Sea.

The productivity of liquid biofuels from lignocellulosic biomass depends on the ability to separate and/ or break down the tight bonding among the polymeric constituents: cellulose, hemicellulose, and lignin (Howard *et al.*, 2003). Pretreatment of lignocelluloses is the key step to efficient utilization of biomass for ethanol production. The objective of pretreatment is to allow cellulose and hemicellulose to become more accessible and susceptible to enzymatic hydrolysis, hence providing a high monosaccharide yield for the subsequent fermentation (Mosier *et al.*, 2005).

Microwave irradiation has been widely used because of its high heating efficiency and easy operation. Some studies have shown that microwave irradiation could change the ultra structure of cellulose and degrade lignin and hemicellulose, which increase the cellulose's susceptibility to enzymes. Moreover, microwave irradiation could be easily combined with chemical solutions in order to accelerate the chemical reaction rate (Xiong *et al.*, 2000; Intanakul *et al.*, 2003).

The objective of this work is to study the release of monomeric sugars of Kans grass (*S. spontaneum*) and Giant reed (*A. donax*) via microwave/NaOH pretreatment followed by microwave/$H_2SO_4$ pretreatment with respect to temperature, holding time of treatment, and alkaline/acid concentrations. The structural changes in *S. spontaneum* and *A. donax* from the two-stage microwave/chemical pretreatment process were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

## 5.3 Experimental

### 5.3.1 Materials and Chemicals

Kans grass (*S. spontaneum*) was obtained from Bangkok Metropolis, Thailand. Giant reed (*A. donax*) was obtained from Nakhon Pathom Province, Thailand. Before any pretreatment, Kans grass (*S. spontaneum*) and Giant reed (*A. donax*) (leaves and stems only) were air- and sun-dried before being chipped into small pieces. The grasses were then milled to obtain 60 mesh-size powders. The

ground biomass was then stored in sealed plastic bags at room temperature for further use.

Sodium hydroxide (NaOH, Labscan Asia Co., Thailand) and sulfuric acid ($H_2SO_4$, Merck Co., Germany) were utilized in the pretreatment process. D-(+)-Glucose (G5400), D-(+)-xylose (X3877), and D-(-)-arabinose (A6085) standards (Sigma Aldrich Chemical Co., Inc., USA) were used for sugar quantitative analysis. Acetic acid ($CH_3COOH$), 5-(hydroxymethyl) furfural, and furfural standards (Sigma Aldrich Chemical Co., Inc., USA) were used for inhibitory chemicals for quantitative analysis.

### 5.3.2 Chemical Composition Analysis

The chemical compositions [cellulose, hemicelluloses, lignin, extractive, acetic acid, 5-(hydroxymethyl) furfural, furfural, and ash] of *S. spontaneum* and *A. donax*, microwave/NaOH-pretreated *S. spontaneum* and *A. donax*, and two-stage microwave (dilute NaOH followed by dilute $H_2SO_4$)-pretreated *S. spontaneum* and *A. donax* were analyzed using the method of the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2011).

### 5.3.3 Particle Size Analysis

Mechanical pretreatment is a fundamental method for reducing cellulose crystallinity. Reduction in particle size leads to an increase of the available specific surface and reduction of the degree of polymerization (Leustean, 2009). The particle size of *S. spontaneum* and *A. donax* after mechanical pretreatment was detected by a particle size analyzer (Malvern/Mastersizer X) with a 300-mm lens in the sample detection unit.

### 5.3.4 Microwave-assisted Pretreatment: Two-stage Microwave/Chemical Pretreatment Process

#### *5.3.4.1 Microwave-assisted Dilute Sodium Hydroxide (NaOH) Pretreatment*

Before microwave pretreatment, *S. spontaneum* and *A. donax* powders were suspended in NaOH solution (0.5 % (w/v)) using 15:1 liquid-to-

solid ratios (LSR) (ml of NaOH solution: g of *S. spontaneum* or *A. donax* grass powder). The ratio is reported to be optimal for the maximum release of 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) at various times (5–60 min). After the optimum temperature and time had been obtained, the *S. spontaneum* and *A. donax* samples were suspended in different concentrations of NaOH solution [0.1–7 % (w/v)] using 15:1 LSR at the optimum temperature and time to obtain suitable conditions for the maximum release of monomeric sugars.

After the pretreatment, the mixture was filtered. The liquid fractions were collected for monomeric sugars and pH analysis; the solid residues were dried in an oven. The oven-dried solid samples were weighed to compare percent solid loss with that of the untreated samples. The amount of monomeric sugars (glucose, xylose, and arabinose) was quantitatively determined using high-performance liquid chromatography (HPLC, RID-10A, Shimadzu Corp., Kyoto, Japan) equipped with a refractive index detector and an Aminex-HPX 87H column (300 x 78 mm, Bio-Rad Lab, USA) under the following conditions: 0.005 M of $H_2SO_4$ as the mobile phase with a flow rate of 0.60 ml/min. The pretreated solids were neutralized, dried, and stored in sealed containers for further pretreatment studies.

#### *5.3.4.2 Microwave-assisted Dilute Sulfuric Acid ($H_2SO_4$) Pretreatment*

The solid residues from the microwave/NaOH pretreatment were treated with microwave/dilute $H_2SO_4$. The alkaline-pretreated *S. spontaneum* and *A. donax* were mixed with dilute acid solution [0.5 % (w/v)] using 15:1 LSR. The pretreatment temperature was varied between 80 and 200 °C at a 5–60-min time range. After the optimum temperature and time conditions had been obtained, the microwave/dilute $H_2SO_4$ pretreatment was conducted at different concentrations of $H_2SO_4$ solution [0.5–3 % (w/v)] to maximize the release of monomeric sugars.

After the two-stage pretreatment process, the liquid fractions were collected for monomeric sugar analysis by HPLC. All liquid fractions

collected were measured for pH values. The solid residues were oven-dried and weighed to measure % solid loss. Then, the residues were kept in sealed bags for further characterization.

5.3.5 Total Monomeric Sugar Analysis

Monosaccharides were measured using HPLC with a pulsed refractive index detector. Sugar standard curves were plotted to calculate the sugar concentrations. The relationship between sugar concentrations and their peak areas is represented by the following equations: $y = 4E^{-06} x + 0.0421$ for glucose, $y = 4E^{-06} x + 0.0952$ for xylose, and $y = 4E^{-06} x + 0.0396$ for arabinose [y = sugar concentration (g/l), x = peak area]. The monomeric sugar yields in the unit of g/100 g biomass could be calculated from the following equation (Boonmanumsin *et al.*, 2012).

Monomeric sugar yields (g/100g biomass)

= [Sugar concentration (g/l) x **V**(ml) x 100 g] / [1000 (ml) x **M** (g)]

where **V** is the volume of acid or base added (ml), and **M** is the amount of biomass added (g) in the retreatment process.

5.3.6 Characterization

The chemical structures of *S. spontaneum* and *A. donax* before and after the pretreatment process were compared and characterized using a Fourier transform infrared spectrometer (FTIR, Nicolet nexus 670). For each sample, 64 scans were recorded with a resolution of 1 $cm^{-1}$. The spectra were run at the range 400–4,000 $cm^{-1}$. The physical structures of untreated and pretreated *S. spontaneum* and *A. donax* were obtained using a scanning electron microscope (SEM, Hitachi/S-4800) at 2 kV accelerating voltages.

## 5.4 Results and Discussion

### 5.4.1 Chemical Compositions of *S. spontaneum* and *A. donax*

*S. spontaneum* and *A. donax* were initially characterized according to their chemical compositions by the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2011). The chemical compositions of *S. spontaneum* and *A. donax* are summarized in Table 1. Our results were comparable to those of Scordia *et al.* (2010) (with *S. spontaneum* collected from Southern Italy) and Francisco *et al.*, (2010) (with *A. donax* collected from Spain). Cellulose and hemicelluloses together make up the total carbohydrates of *S. spontaneum* and *A. donax* (62.1 and 63.6 %, respectively). These carbohydrates are the potential sugars in the pretreated substrates. Moreover, *S. spontaneum* from Bangkok Province and *A. donax* from Nakornpathom Province contain less lignin content, which further enhances the cellulose hydrolysis step.

### 5.4.2 Particle Size Analysis

Particle size affects lignocellulose conversion. Smaller particle sizes correlate to higher sugar conversion (Vidal *et al.*, 2011). The combination of chipping, grinding, and milling can reduce particle size and cellulose crystallinity. The reduction in particle size leads to an increase of the available specific surface and decrease of degree of the polymerization (Leustean, 2009). The particle size analyzer was used to confirm the average sample size of *S. spontaneum* and *A. donax* powders after they had been milled with 60-mesh sieving size. The particle size of the grass samples was about 300 μm.

### 5.4.3 Optimization of Microwave-assisted Alkali Pretreatment

#### *5.4.3.1 Effect of Time and Temperature*

Dilute NaOH pretreatment effectively removes lignin and consequently increases the surface area and pore size of the substrate, resulting in an improvement of enzymatic digestibility. Moreover, NaOH pretreatment can decrease the crystallinity of cellulose, separate structural linkages between lignin and carbohydrates, and disrupt the lignin structure (Fan *et al.*, 1987). Thus, in this study,

*S. spontaneum* and *A. donax* were first pretreated with the microwave/NaOH pretreatment process. The effects of temperature and time on *S. spontaneum* and *A. donax* pretreatment were investigated at 40–120 °C under 5–60 min, using 0.5 % (w/v) NaOH and 15:1 LSR. The result shows that the maximum yield of monomeric sugars released from *S. spontaneum* is 4.8 g/100 g biomass at 80 °C for 5 min (Figure 5.1), while the maximum yield of monomeric sugars from *A. donax* is 4.5 g/100 g biomass at 120 °C for 5 min (Figure 5.2). Both temperature and time affected not only lignin removal, but also the monomeric sugar release. At low pretreatment temperatures of 40 °C (Figures. 5.1, 5.2a) and 60 °C (not shown), the increasing yields of monomeric sugar as the reaction time increased were caused by the structural disruption of the crystalline cellulose (Hu and Wen, 2008). However, the decreasing monomeric sugar yields as the reaction time increased to higher pretreatment temperatures (80–120 °C), as seen in Figures. 5.1, 5.2b, c, were due to the degradation process (Lu and Mosier, 2007). Figures 5.1d and 5.2d illustrate the comparison of the effects of temperature and time. The graphs clearly show a decreasing trend in the monomeric sugar yields at increased temperatures. When the reaction time and temperature are compared, reaction temperature is the more significant factor in the release of monomeric sugars. These results are consistent with the pretreatment results for switch grass using the microwave (Jackowiak *et al.*, 2011). Thus, the suitable reaction time and temperature were investigated to obtain the maximum monomeric sugar yields.

*5.4.3.2 Effect of Alkali Concentration*

The release of monomeric sugars is influenced by the alkali concentration (Scordia *et al.*, 2010). The effect of the NaOH concentration was studied by varying the NaOH concentration from 0.1 to 7 % (w/v) at the optimum temperature and time conditions of *S. spontaneum* (80 °C/5 min) and *A. donax* (120 °C/ 5 min). The results in Figure 5.3 show that the maximum monomeric sugar yields occurred when 5 % (w/v) NaOH was used. The highest sugar yields obtained from *S. spontaneum* and *A. donax* were both 6.8 g/ 100 g biomass. The monomeric sugar release increased with an increasing alkali concentration because stronger alkaline pretreatment caused the solubilization of hemicellulose and cellulose. This solubilization led to higher monomeric sugar yields. However, severe NaOH

concentrations caused a decrease in monomeric sugar yields due to a high degree of sugar degradation (Hu and Wen, 2008). From the alkali pretreatment process, the amounts of monomeric sugar released from *S. Spontaneum* and *A. donax* were similar using the microwave/NaOH method.

### 5.4.4 Optimization of Two-stage Pretreatment (Microwave/Dilute NaOH, Followed by Microwave/Dilute $H_2SO_4$ Pretreatment)

The two-stage microwave/chemical pretreatment process is an effective method to obtain high monomeric sugar yields because of the high heating efficiency and short reaction time (Boonmanumsin *et al.*, 2012). Wang *et al.*, (2010) also found that the two-stage pretreatment significantly enhanced the hydrolysis rate because hemicellulose and lignin were partially removed in the first pretreatment.

#### *5.4.4.1 Effect of Time and Temperature*

A hydrolysis process assisted by dilute sulfuric acid has been successfully developed to improve cellulose hydrolysis (Sánchez and Cardona, 2008). The process converts hemicelluloses into fermentable monomeric sugars and makes cellulose more accessible to hydrolytic enzymes. Dilute sulfuric acid leads to optimal monomeric sugar release, hydrolysis efficiency, and cellulose digestibility (Hendriks and Zeeman, 2009). To investigate the effects of temperature and time, microwave/NaOH-pretreated *S. spontaneum* and *A. donax* were treated with microwave/0.5 % (w/v) $H_2SO_4$ at 15:1 LSR. The range of temperature used in the experiment was 80–200 °C and that of time was 5–60 min. In the experiment, the total sugars released from the pretreated *S. spontaneum* and *A. donax* were 33.8 and 31.9 g/100 g biomass, respectively. The optimal conditions for *S. spontaneum* were 200 °C/ 10 min (Figure 5.4), whereas the ideal conditions for *A. donax* were 180 °C/30 min (Figure 5.5). According to the results in Figures. 5.4a, b and 5.5a, b, the grasses released high contents of xylose and arabinose at 80–160 °C and 100–140 °C, respectively. The amounts of xylose and arabinose increased with increasing temperature and time because of better hydrolysis of hemicellulose. These results confirmed that hemicellulose was effectively hydrolyzed at mild conditions. Hemicellulose differs from cellulose because of the sugar composition units, shorter chains, branching of main chains, and amorphous structure (Fengel and Wegener,

1984). Therefore, hemicellulose is easier to hydrolyze than cellulose. However, the amount of xylose and arabinose decreased at severe temperatures (180–200 °C) because of sugar degradation. At 180° (not shown) and 200 °C, glucose was the main monomeric sugar released from the hydrolysis process. Due to its crystalline structure, cellulose is not as easily hydrolyzed as hemicelluloses. Thus, more severe conditions are required in order to release glucose. However, further increases of temperature and time also result in glucose degradation (Figures. 5.4d, 5.5d). Sugar degradation contributes to the formation of inhibitors for both enzyme and fermenting microorganisms (García-Aparicio *et al.*, 2006). Figures 5.4d and 5.5d illustrate the effects of temperature and time on the two-stage pretreatment. The optimum conditions of the two stage microwave/chemical pretreatment of the grasses were obtained by the highest glucose content. The amount of glucose released from the pretreated *S. spontaneum* and *A. donax* was 26.3 and 26.4 g/100 g biomass, respectively. High glucose content offers many benefits to bioethanol production. The six-carbon sugars (from cellulose) are readily fermented into bioethanol by a variety of microorganisms, while the five-carbon sugars (from hemicellulose) can only be fermented by a few strains of microorganisms and usually the yield is relatively low (Mosier *et al.*, 2005).

*5.4.4.2 Effect of Acid Concentration*

The grasses were pretreated using various concentrations of $H_2SO_4$ (0.5–3 % (w/v)), 15:1 LSR at 200 °C/ 10 min for *S. spontaneum*, and at 180 °C/30 min for *A. donax*. The results in Figure 5.6 show that at $H_2SO_4$ concentrations higher than 0.5 % (w/v), the monomeric sugar yields decreased because strong acidic conditions accelerated sugar degradation (Pedersen and Meyer, 2010). Thus, the maximum monomeric sugar yields from *S. spontaneum* and *A. donax* were 33.8 and 31.9 g/100 g biomass, respectively, using 0.5 % (w/v) $H_2SO_4$. Moreover, after the two-stage pretreatment (microwave/dilute NaOH, followed by microwave/dilute $H_2SO_4$), *S. spontaneum* released monomeric sugars more than *A. donax* because *S. spontaneum* possesses higher xylose contents than *A. donax*. However, the release of glucose, which is the preferred sugar because of the ease of bioethanol conversion, is insignificant between the two grasses. In comparison to Saha *et al.* (2005) the

maximum monomeric sugar yield from wheat straw after the conventional heating pretreatment was 19.8 g/100 g biomass using 1 % (w/v) $H_2SO_4$ at 121 °C/60 min.

5.4.5 Effect of Pretreatment on Chemical Composition

The change in chemical composition of *S. spontaneum* and *A. donax* is a significant factor for the effectiveness of the microwave/chemical pretreatment. The composition of *S. spontaneum* and *A. donax* treated with the microwave/NaOH and the two stage microwave/ chemical pretreatment is summarized in Tables 5.2 and 5.3, respectively. The cellulose, hemicellulose, and lignin content for *S. spontaneum* is 108 %, and the content for *A. donax* is 109 %. The values are higher than 100 % because of the standard deviation from experimental repeatability.

The microwave-assisted NaOH pretreatment of *S. spontaneum* and *A. donax* improved the cellulose concentration while reducing hemicellulose and lignin concentrations. The decrease of hemicellulose and lignin concentration was due to their solubilization in NaOH (Zhu *et al.*, 2005). Moreover, acetic acid and furfural concentrations decreased after the hydrolyzate had been treated with the microwave/NaOH. The decrease could possibly be due to chemical transformation at high pH (Martinez *et al.*, 2000).

After the microwave/NaOH pretreatment, the solid residues of *S. spontaneum* and *A. donax* were treated with the microwave/ $H_2SO_4$ pretreatment at optimal conditions. At optimal conditions, the polysaccharide content in *S. spontaneum* was significantly reduced. The remaining 13.6 % of polysaccharide content belonged to cellulose chains because the pretreatment was performed at 200 °C. The cellulose component was hydrolyzed, degraded, and/or burned. The optimal conditions for *A. donax* were also obtained. At 180 °C, the higher cellulose content remained (48.8 %). The acetic acid concentrations from *S. spontaneum* and *A. donax* decreased (0.9 and 1.0 %, respectively), while untraceable quantities of furfural were produced under these conditions. However, the increase in temperature and/or time resulted in the degradation of these cellulose chains. Furthermore, acetic acid and furfural are generated from hemicellulose degradation at high sulfuric acid concentration (Mansilla *et al.*, 1998). In comparison to Scordia *et al.* (2012) acetic acid and total phenolic compounds were 44.3 %, furfural and 5-(hydroxymethyl)

furfural, respectively, for 5.6 and 5.7 % of the total inhibitor compounds released in the giant reed hydrolysate fraction after the conventional heating pretreatment using 4 % (w/v) $H_2SO_4$ at 121 °C/60 min.

These results demonstrate that the two-stage pretreatment process effectively converts cellulose into monomeric sugars. The optimization of the method did not produce much of the inhibitory compounds including acetic acid and furfural, which are detrimental to fermentative microorganisms.

### 5.4.6 Effect of Pretreatment on % Solid Loss

The % solid loss of *S. spontaneum* and *A. donax* is another important factor for effective pretreatment. The results in Figure 5.7 indicate that the microwave/ NaOH (Figures 5.7a, c) and the microwave/NaOH/ $H_2SO_4$ pretreatments (Figure 5.7b, d) of *S. spontaneum* and *A. donax* have similar trends in % solid loss. The increase in reaction time and temperature caused an increase in % solid loss because the % solid loss came from the NaOH-soluble components such as lignin and hemicelluloses (Zhu *et al.*, 2005). Temperature was the main cause for higher % solid loss. The % solid loss during the microwave/NaOH pretreatment was found to be in a range of 13.3 ± 0.5 to 40.5 ± 0.9 for *S. spontaneum* and 10.2 ± 4.9 to 32.5 ± 2.2 for *A. donax*. The % solid loss during the two-stage microwave/ NaOH/ $H_2SO_4$ pretreatment was between 9.7 ± 1.3 to 77.7 ± 3.8 for *S. spontaneum* and 7.1 ± 0.8 to 72.5 ± 1.0 for *A. donax* at various temperatures and times.

### 5.4.7 Effect of Pretreatment on the Hydrolysate pH

The variation of temperature and time plays a role in the final pH of the hydrolysate because of the change in lignocellulosic structure. The final pH values of alkaline pretreatment of *S. spontaneum* and *A. donax* at various temperatures and times are shown in Figure 5.8. As the reaction temperature and time increased, the pH decreased from 12.6 to 9.9 for *S. spontaneum* and 12.7 to 9.9 for *A. donax*. During the alkaline pretreatment, hydroxide ions attacked lignin by splitting ether bonds in the delignification process (Gierer, 1985). Moreover, acetic

acid could be created at severe conditions because of sugar degradation, which caused the lowering of pH (Pederson and Meyer, 2010) (Figures 5.8 a, c).

For the microwave/ $H_2SO_4$ pretreatment, the final pH values were also measured at various temperatures and times, as shown in Figures 5.8b, d. The pH values increased with increasing temperature. As the temperature increased, the acid proton acted as a catalyst to protonate the oxygen atom linkage between cellulose and hemicellulose. However, the pH value could fluctuate at severe conditions because of the formation of acetic acid by the degradation process. The formation of acetic acid caused the lowering of the pH value of the hydrolysate.

### 5.4.8 Fourier Transform Infrared Spectrometer (FTIR) Analysis

FTIR was used to investigate the influence of the microwave/chemical pretreatment on the chemical structure of *S. spontaneum* and *A. donax*. After the microwave/NaOH pretreatment, the disappearance of the peak at 1,734 $cm^{-1}$ in Figures 5.9a, b signifies the broken linkages between lignin and hemicelluloses such as ester-linked acetyl, feruloyl, and p-coumaroyl groups. The lignin peaks around 1,515 $cm^{-1}$ (aromatic C=C bonds from aromatic rings of lignin) and 1,248 $cm^{-1}$ (aromatic C–O bonds of lignin) were diminished because of partial removal of the lignin. In addition, other polysaccharide peaks (898, 1,108, 1,164, 1,260, 1,325, and 1,378 $cm^{-1}$) after the pretreatment process became sharper compared to that of the untreated weeds. They corresponded to the increase of polysaccharide content after the microwave/ NaOH pretreatment (Wang *et al.*, 2010). FTIR results confirmed the efficiency of the lignin removal pretreatment method to improve the sugar yield for the microwave/ $H_2SO_4$ pretreatment step. The spectrum of the two-stage pretreatment of *S. spontaneum* was remarkably changed compared to the spectra of untreated and microwave/NaOH-pretreated samples. The peaks generated at 593, 1,207, 1,309, 1,512, and 1,703 $cm^{-1}$ may be due to lignin and other degraded residues (Figure 5.9a). Unlike *S. spontaneum*, the FTIR spectrum of the two-stage pretreated *A. donax* in Figure 5.9b was similar to that of the microwave/NaOH pretreatment because the optimum conditions of *A. donax* caused less chemical composition change. The conditions used in *A. donax* were less severe compared to those in *S. spontaneum*, thus causing less sugar degradation.

5.4.9 Scanning Electron Microscopy (SEM) Characterization

The changes in the structural appearance of the untreated, microwave/NaOH pretreated, and two stage treated samples were characterized by SEM, as shown in Figures. 5.10 and 5.11. The morphology of untreated *S. spontaneum* (Figure 5.10a) and *A. donax* (Figure 5.11a) showed the existence of cellulose in the forms of fibers, macrofibrils, and microfibrils. The cellulose was surrounded by lignin and hemicelluloses (Somerville *et al.*, 2004). Figures 5.10a and 5.11a show that the textures of *S. spontaneum* and *A. donax* were covered with thin films, which may be the wax layer found in the herbaceous biomass (Hu and Wen, 2008). The images of the pretreated microwave/NaOH for *S. spontaneum* (Figure 5.10b) and *A. donax* (Figure 5.11b) still left an indication of a fibril-like structure. However, the waxy layer on the surface could no longer be observed (Hu and Wen, 2008). The rough surface generated from the pretreatment increased the surface area, causing the hydrolysis step to be more effective.

In the two-stage pretreatment of *S. spontaneum* (Figure 5.10c), the fibril structure was disrupted by the hydrolysis process at high temperature. In contrast, the image of *A. donax* after the two-stage pretreatment (Figure 5.11c) still showed a fibril-like structure. The fibril structure of *A. donax* was partially intact because it was hydrolyzed at lower temperature. The SEM images demonstrated the effectiveness of microwave pretreatment on enhancing the digestibility of *S. spontaneum* and *A. donax*.

## 5.5 Conclusions

Both Kans grass (*S. spontaneum*) and Giant reed (*A. donax*) possess the capability to become lignocellulosic feedstock for bioethanol production using two stage microwave/chemical pretreatment. *S. spontaneum* and *A. donax* were found to contain high cellulose and hemicellulose contents. Under the microwaveassisted NaOH pretreatment, the highest monomeric sugar yield for both *S. spontaneum* and *A. donax* was 6.8 g/100 g biomass. After conducting the two-stage pretreatment (microwave/NaOH pretreatment followed by microwave/ $H_2SO_4$ pretreatment), the highest monomeric sugar yields were 33.8 g/100 g biomass for *S. spontaneum* and

31.9 g/100 g biomass for *A. donax*. At optimal conditions, the maximum glucose amounts released from the pretreated *S. spontaneum* and *A. donax* were 26.3 and 26.4 g/ 100 g biomass, respectively. This two-stage pretreatment is an effective technique to remove lignin, disrupt the structure of lignocellulose, and release high monomeric sugars yields, especially glucose.

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**Table 5.1** Chemical composition of kans grass (*S.spontaneum*) and giant reed (*A.donax*)

| **Composition (%)*** | Kans Grass (*S.spontaneum*) | | Giant Reed (*A.donax*) | |
|---|---|---|---|---|
| | **This study** [1] | **Scordia *et al.*, 2010** [2] | **This study** [1] | **Franscisco *et al.*, 2010** |
| Cellulose | 35.4±0.87 | 36.8±0.13 | 39.1±0.25 | 34.8 |
| Hemicellulose | 26.7±0.37 | 23.7±0.04 | 24.4±0.52 | 20.9 |
| Lignin | 14.5±1.82 | 20.0±0.12 | 19.2±3.25 | 23.0 |
| Ash | 1.1±0.95 | 6.6±0.21 | 4.2±0.67 | - |

* = Composition percentages are on dry weight basis

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

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

**Table 5.2** Chemical composition of kans grass (*S.spontaneum*) solid residues from each pretreatment stage

| Composition (% Dry Matter) | Kans Grass (*S.spontaneum*) | | |
|---|---|---|---|
| | **Untreated** | **Microwave/NaOH** | **Microwave/NaOH / $H_2SO_4$** |
| **Glucan** | 35.4±0.87 | 78.8±0.96 | 13.6±1.14 |
| **Xylan** | 23.1±0.32 | 17.0±0.19 | 0 |
| **Arabinan** | 3.6±0.06 | 3.3±0.11 | 0 |
| **Lignin** | 14.5±0.82 | 6.4±0.92 | 2.1±0.29 |
| **Acetic acid** | 4.4±0.09 | 1.2±0.07 | 0.9±0.03 |
| **5-(Hydroxymethylfurfural)** | 0 | 0 | 0 |
| **Furfural** | 1.5±0.04 | 1.1±0.09 | 0 |
| **Ash** | 1.1±0.95 | 0.1±0.07 | 0 |
| **Other** | 16.4 | - | 83.4 |

**Table 5.3** Chemical composition of giant reed (*A.donax*) solid residues from each pretreatment stage

| Composition (% Dry Matter) | Giant Reed (*A.donax*) | | |
|---|---|---|---|
| | **Untreated** | **Microwave/NaOH** | **Microwave/NaOH / $H_2SO_4$** |
| **Glucan** | 39.1±0.25 | 79.2±0.93 | 48.8±2.67 |
| **Xylan** | 21.7±0.43 | 14.9±0.14 | 7.6±0.16 |
| **Arabinan** | 2.8±0.09 | 2.4±0.07 | 0 |
| **Lignin** | 19.2±3.25 | 10.2±1.36 | 0.4±0.02 |
| **Acetic acid** | 4.8±0.08 | 1.2±0.06 | 1.0±0.11 |
| **5-(Hydroxymethylfurfural)** | 0 | 0 | 0 |
| **Furfural** | 1.2±0.09 | 0.8±0.05 | 0 |
| **Ash** | 4.2±0.67 | 0.1±0.08 | 0.26 |
| **Other** | 7.0 | - | 41.9 |

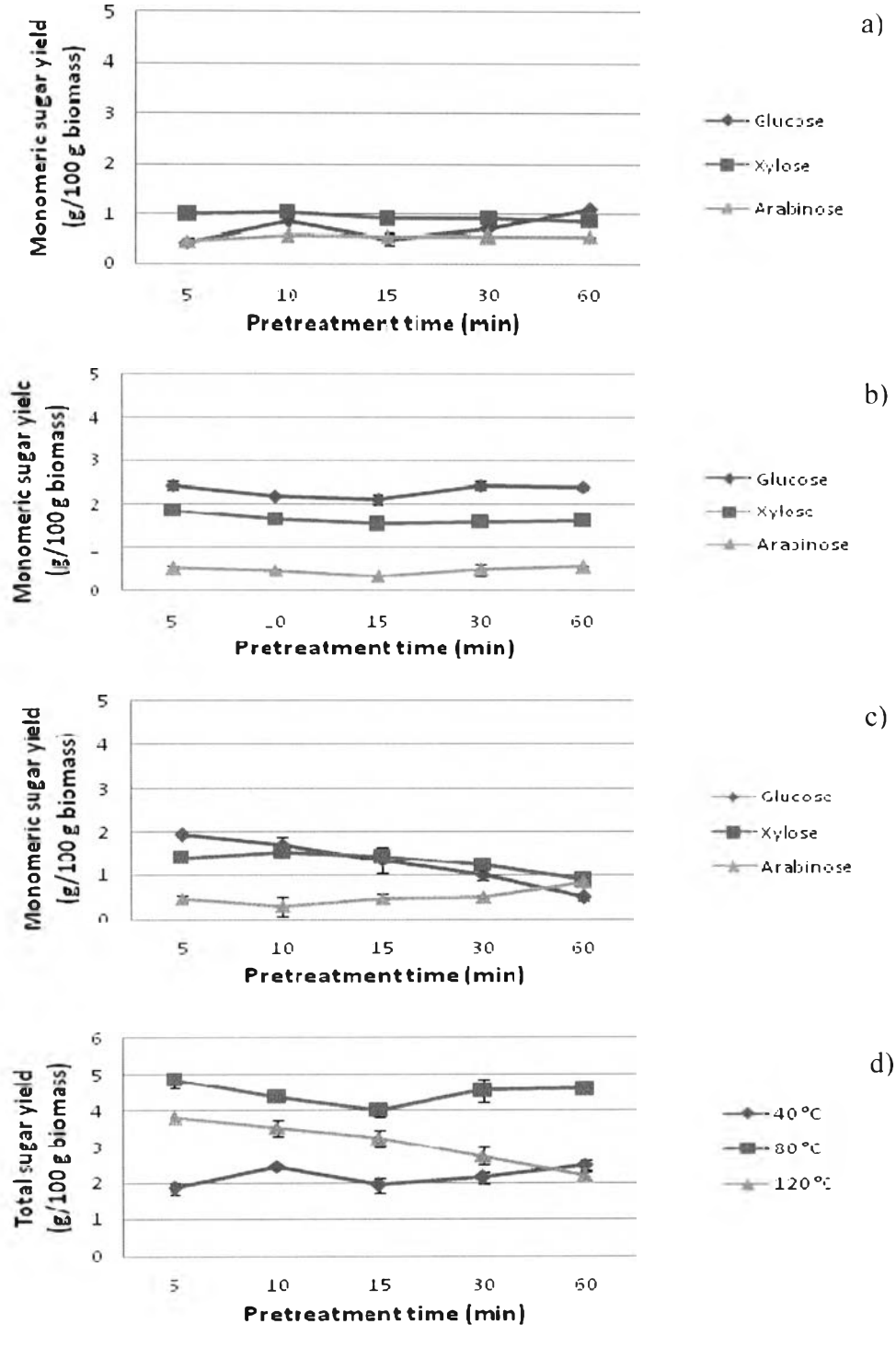



**Figure 5.1** The yields of glucose, xylose, and arabinose of untreated *S.spontaneum* using 0.5 % (w/v) NaOH, 15:1 liquid to solid ratio (LSR) at different times and temperatures: a) 40°, b) 80°, c) 120 °C, and d) comparison of total monomeric sugar yields.

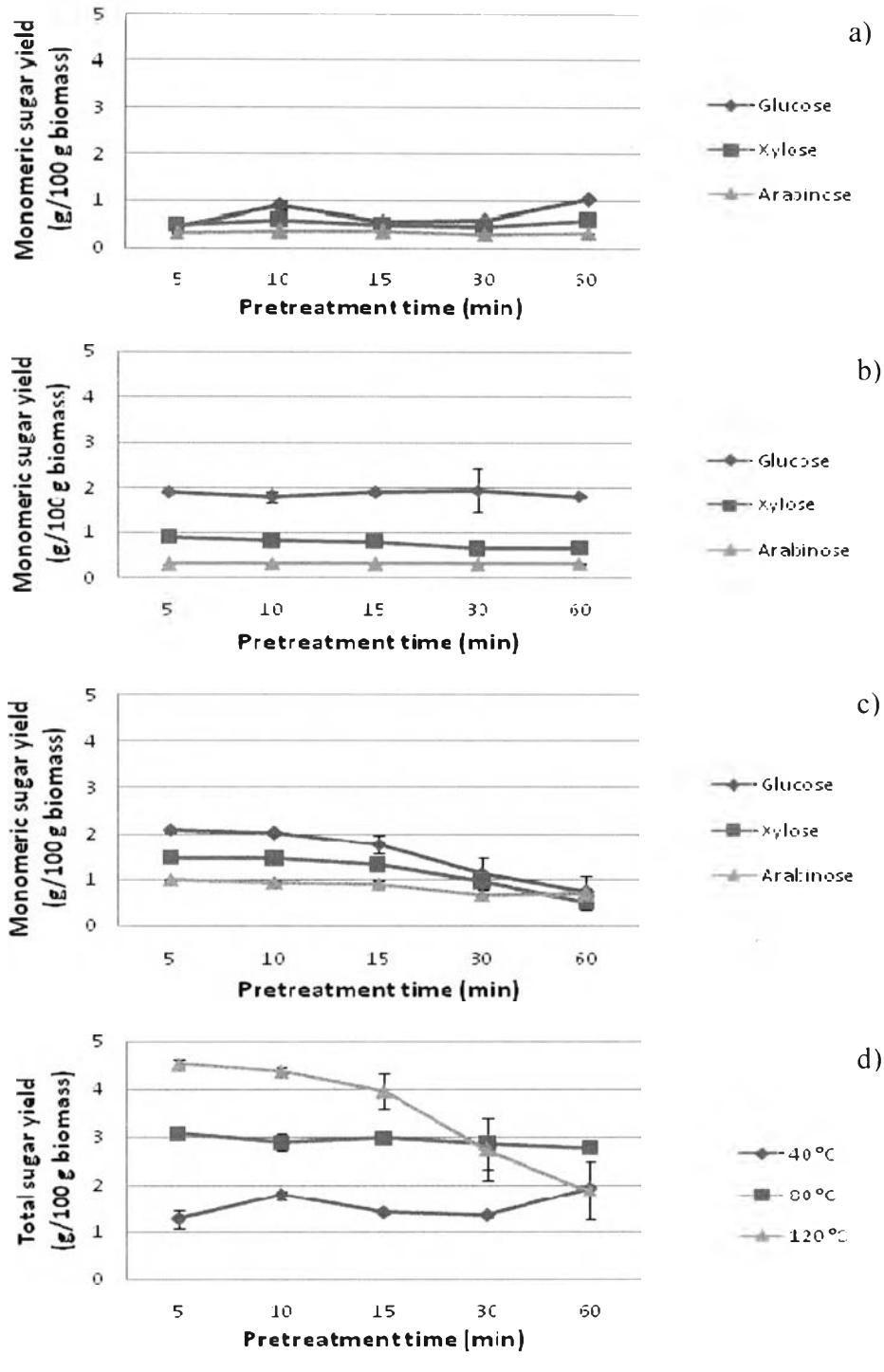



**Figure 5.2** The yields of glucose, xylose, and arabinose of untreated *A.donax* using 0.5 % (w/v) NaOH, 15:1 liquid to solid ratio (LSR) at different times and temperatures: a) 40°, b) 80°, c) 120 °C, and d) comparison of total monomeric sugar yields.

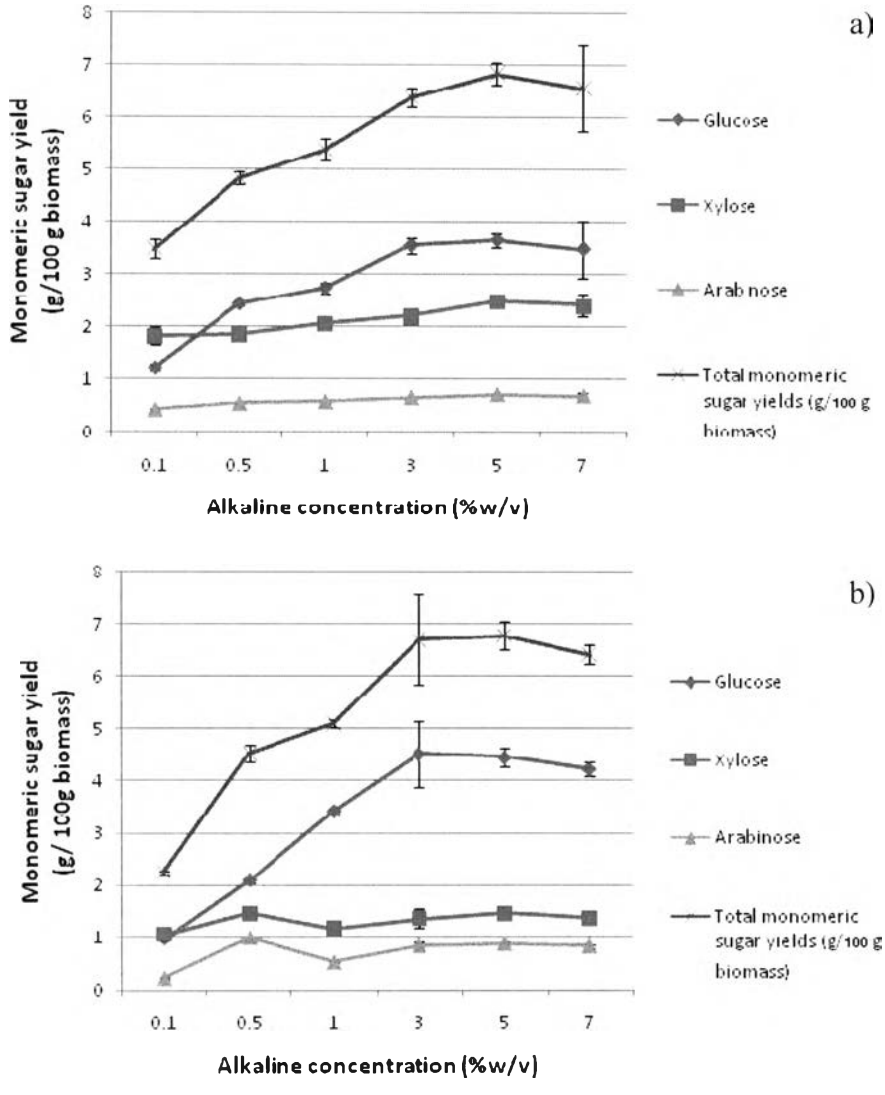



**Figure 5.3** Effect of NaOH concentration (% w/v) on monomeric sugar yields of a) *S.spontaneum* at 80 °C, 5 min, using 15:1 LSR and b) *A.donax* at 120 °C, 5 min, using 15:1 LSR.

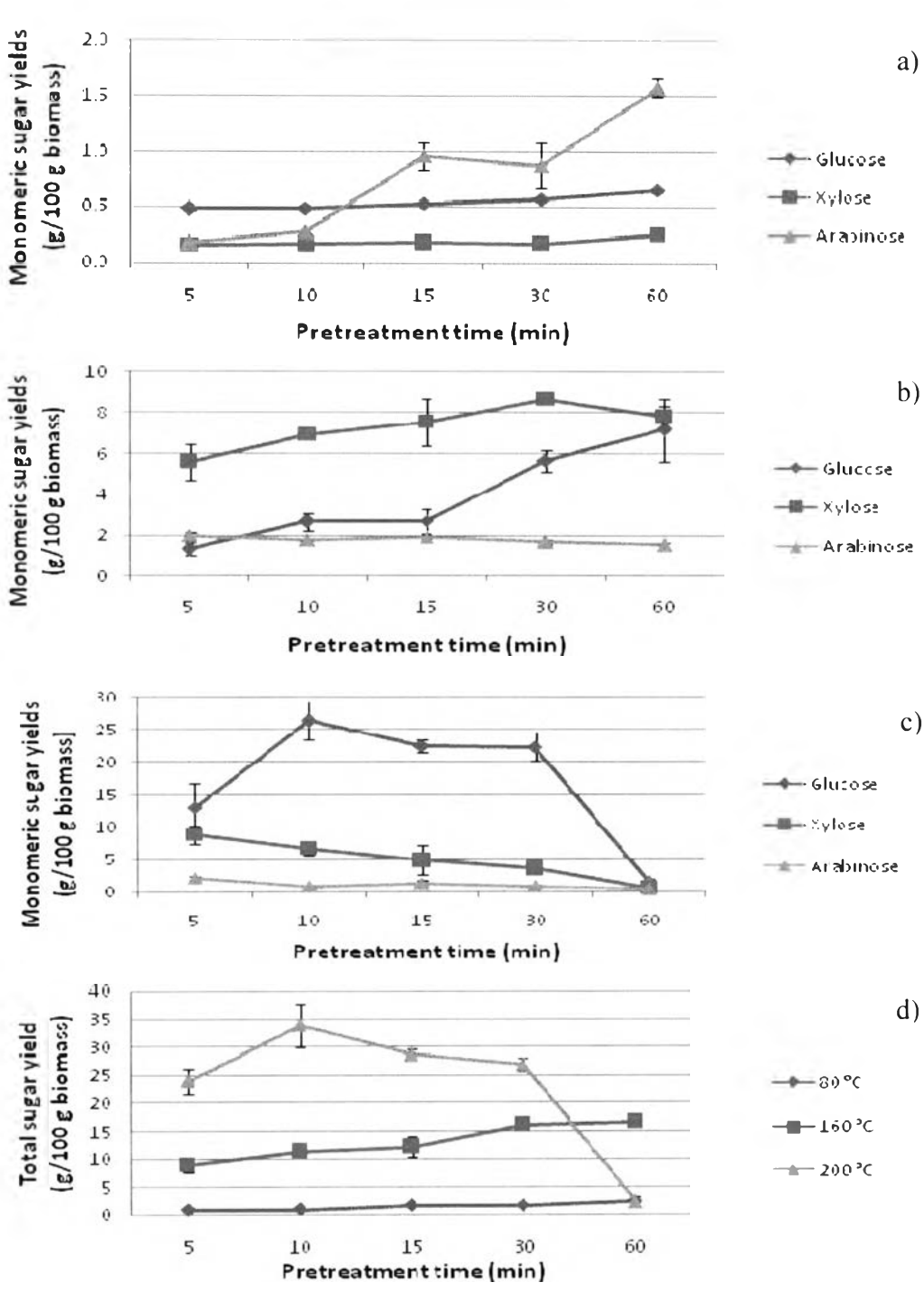



**Figure 5.4** The yields of glucose, xylose, and arabinose of microwave-assisted NaOH pretreated *S.spontaneum* using 0.5 % (w/v) $H_2SO_4$, 15:1 LSR at different times and temperatures : a) 80°, b) 160°, c) 200 °C, and (d) comparison of total monomeric sugar yields.

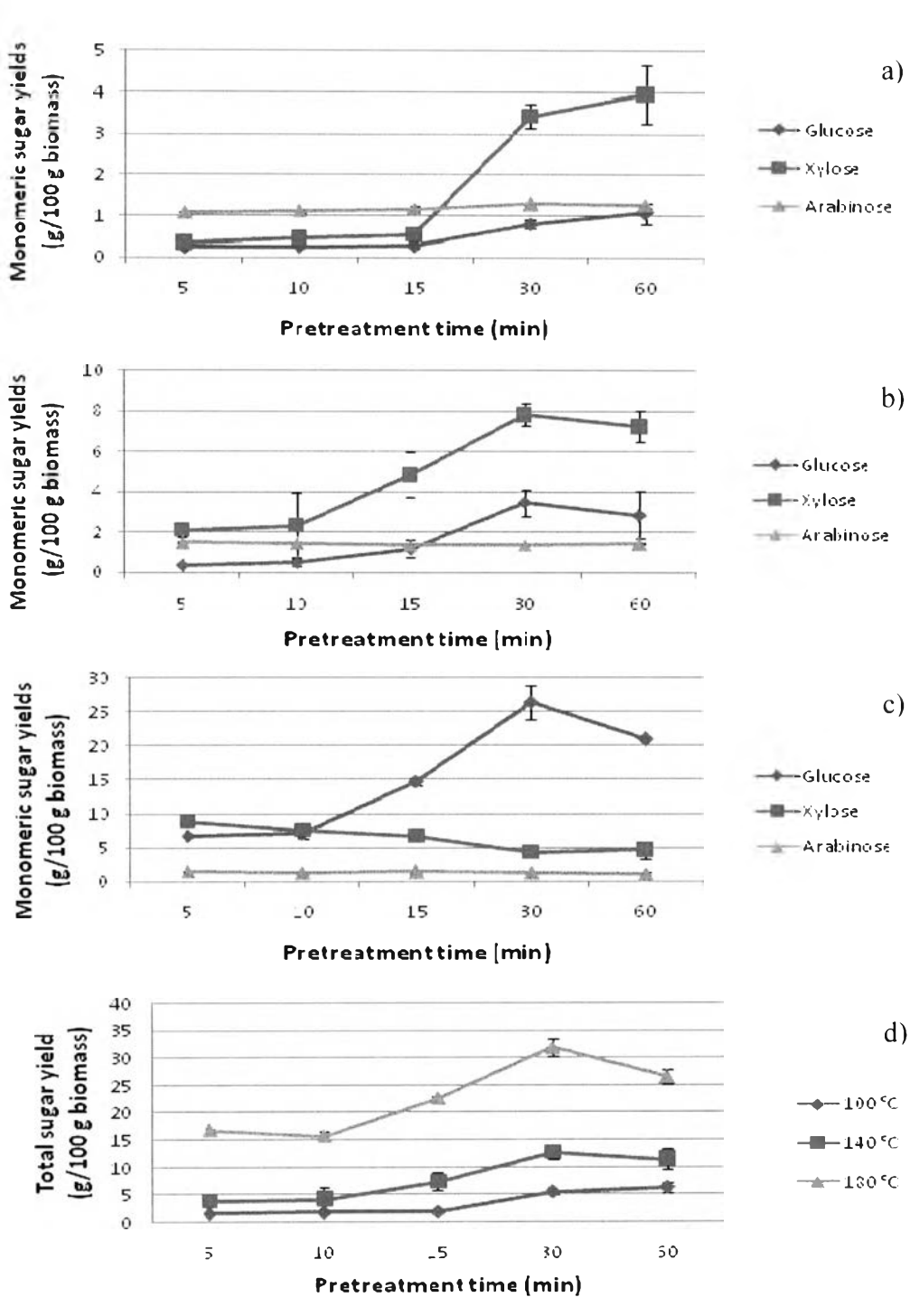



**Figure 5.5** The yields of glucose, xylose, and arabinose of microwave-assisted NaOH pretreated *A.donax* using 0.5 % (w/v) $H_2SO_4$, 15:1 LSR at different times and temperatures: a) 100°, b) 140°, c) 180 °C, and (d) comparison of total monomeric sugar yields.

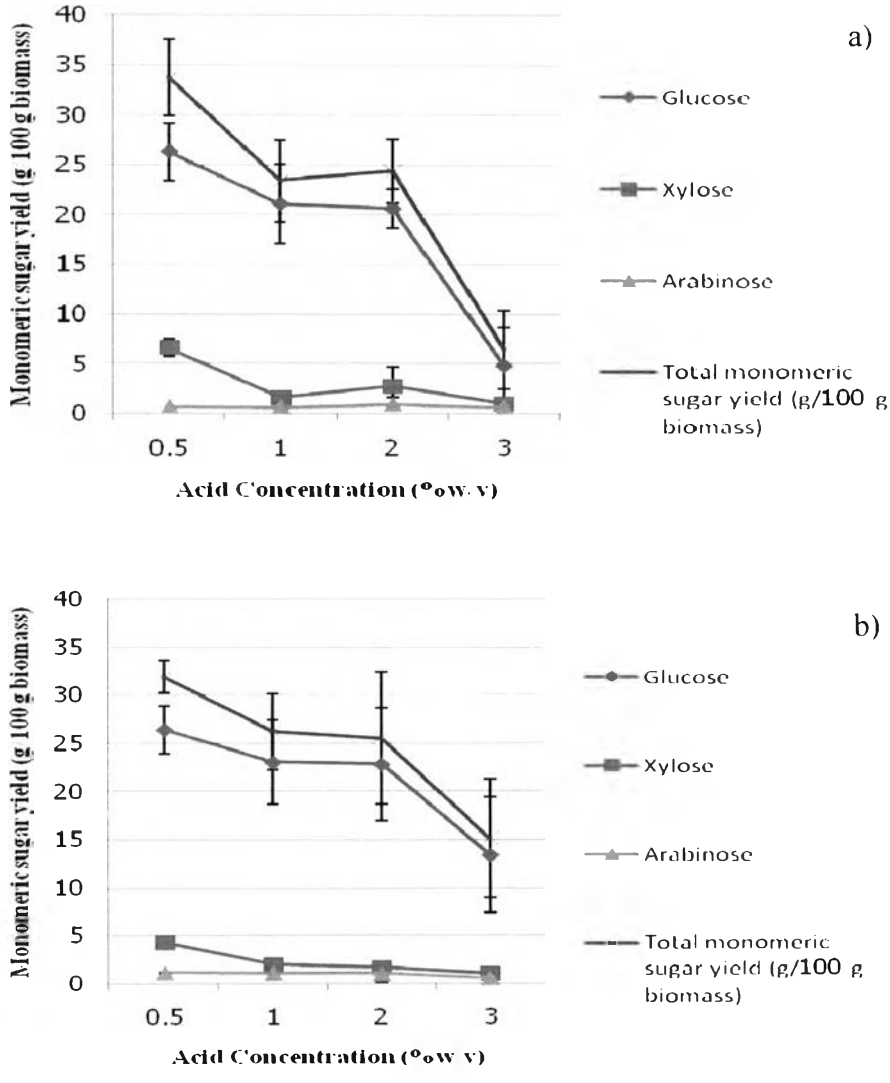



**Figure 5.6** Effect of $H_2SO_4$ concentration (% w/v) on monomeric sugar yields from microwave-assisted NaOH pretreated of a) *S.spontaneum* at 200 °C, 10 min, using 15:1 LSR and b) *A.donax* at 180 °C, 30 min, using 15:1 LSR.

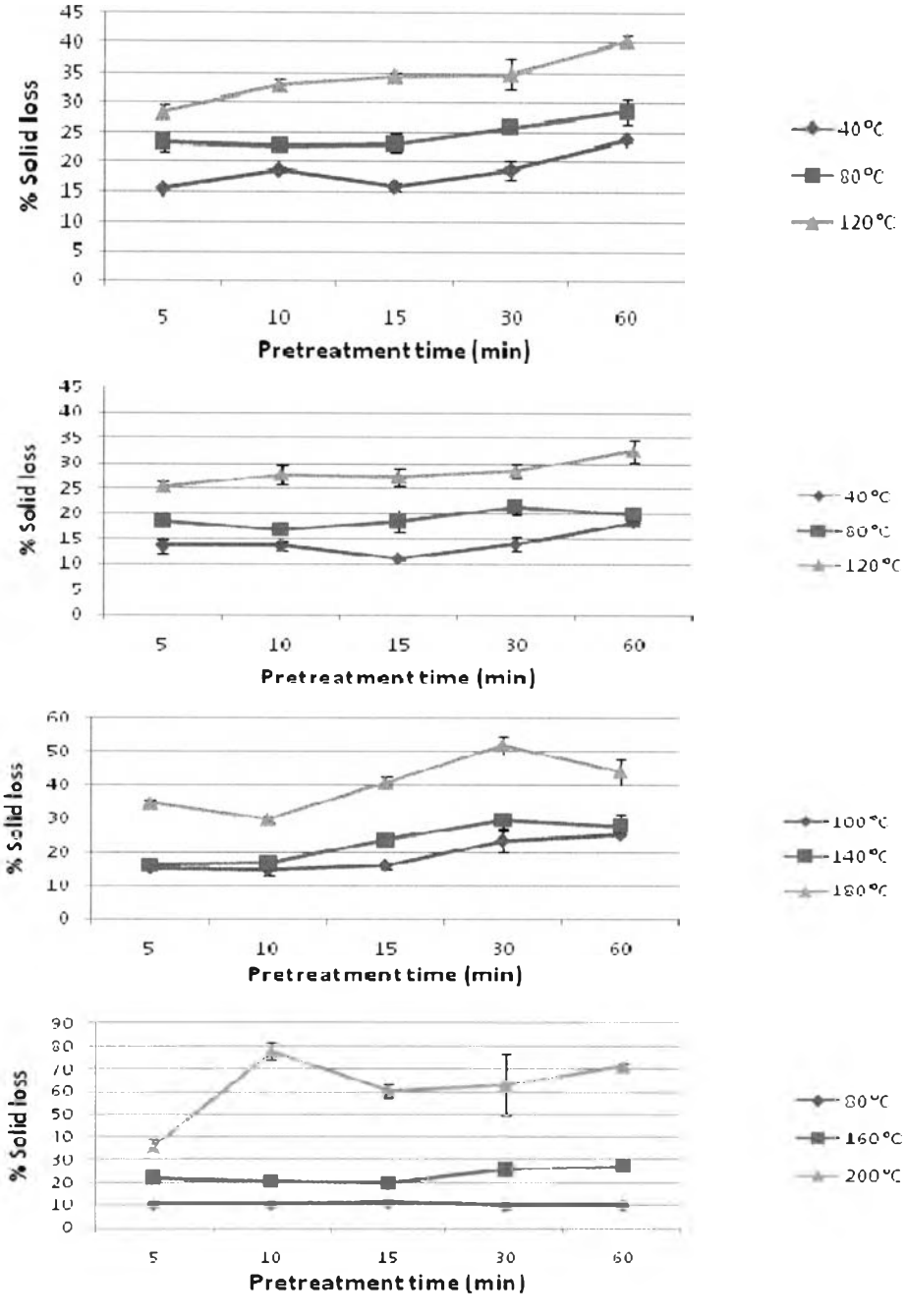



**Figure 5.7** a) and c) % Solid loss of untreated *S.spontaneum* and *A.donax*, respectively, at various temperatures and times using 0.5 % (w/v) NaOH and 15:1 LSR. b) and d) % Solid loss of microwave-assisted NaOH pretreated of *S.spontaneum* and *A.donax* , respectively, at various temperatures and times using 0.5 % (w/v) $H_2SO_4$ and 15:1 LSR.

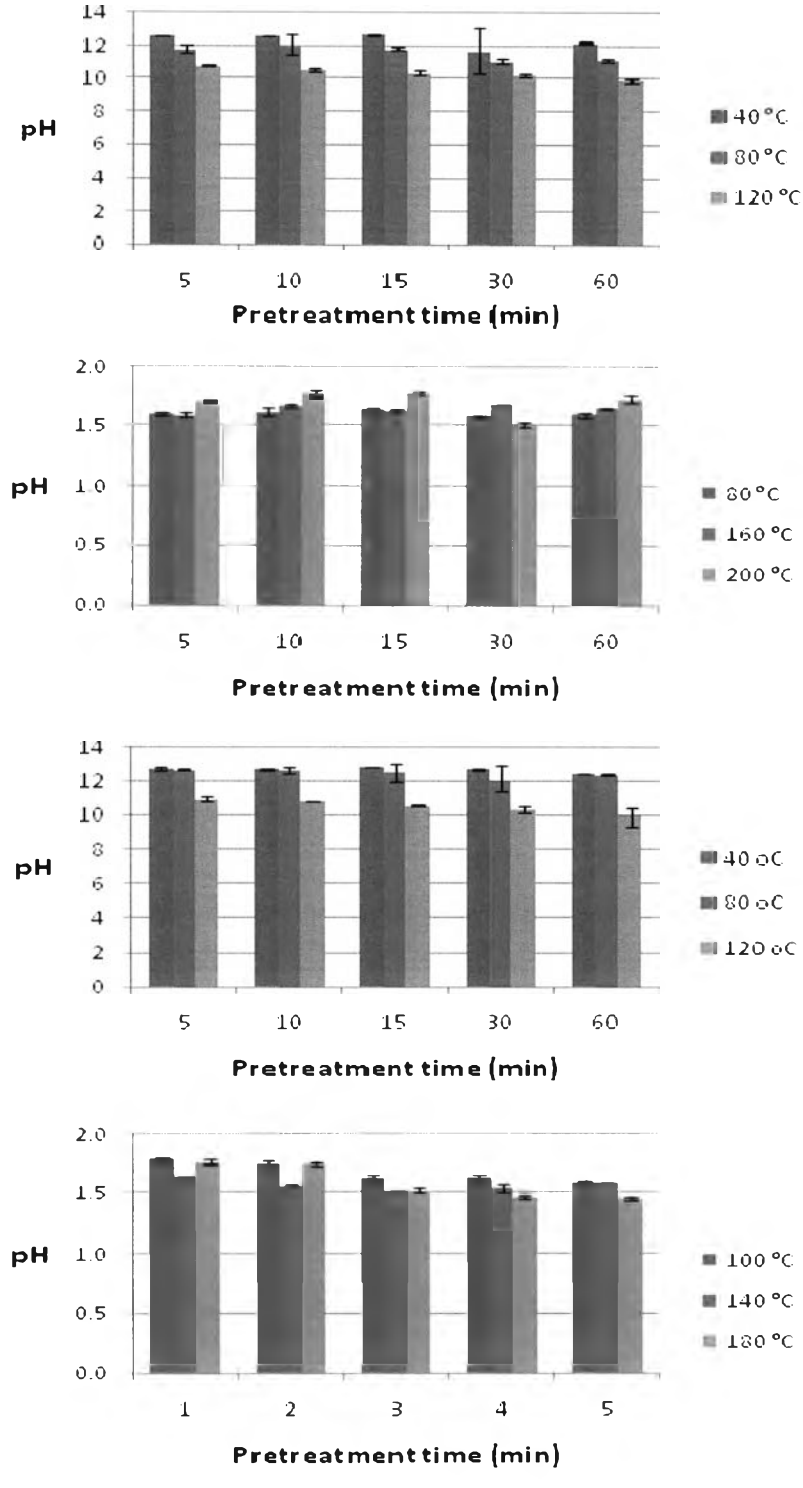
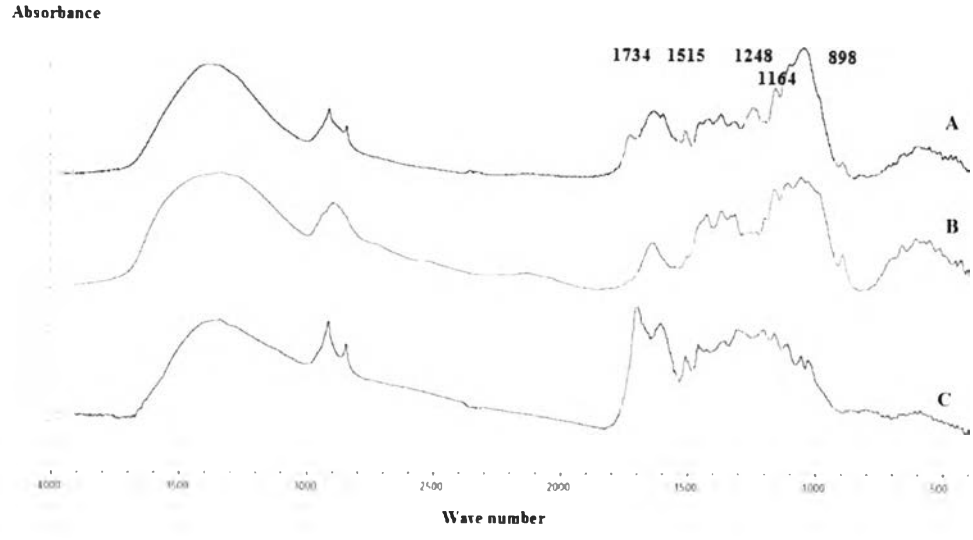



**Figure 5.8** a) and c) pH of untreated *S.spontaneum* and *A.donax*, respectively, at various temperatures and times using 0.5 % (w/v) NaOH and 15:1 LSR, b) and d) pH of microwave-assisted NaOH pretreated of *S.spontaneum* and *A.donax* , respectively, at various temperatures and times using 0.5 % (w/v) $H_2SO_4$ and 15:1 LSR.

a)




b)

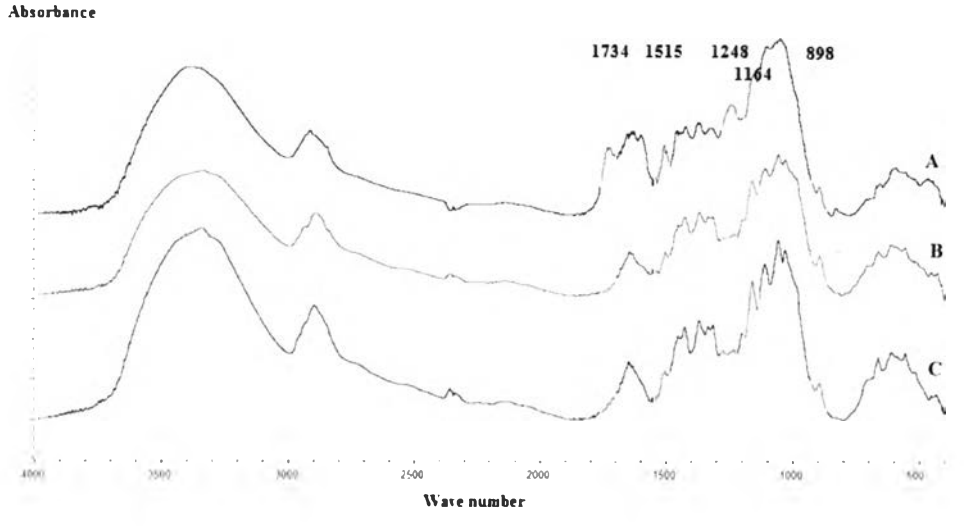



**Figure 5.9** a) FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated *S.spontaneum* and b) FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated *A.donax*.

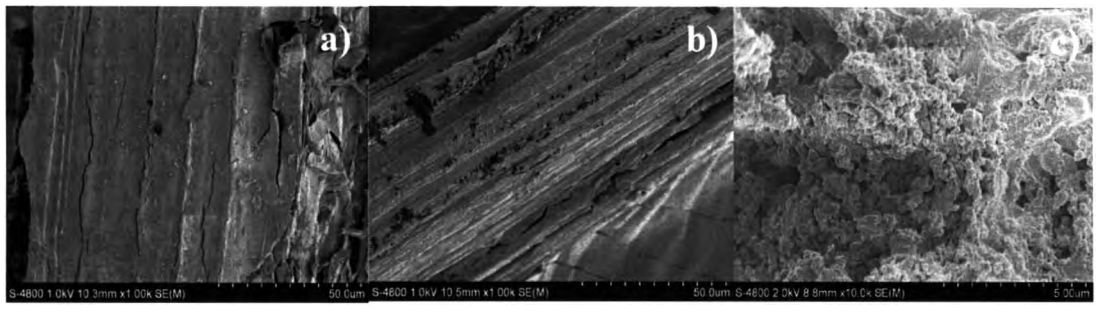



**Figure 5.10** SEM images of (a) raw, (b) microwave-assisted NaOH pretreated, and (c) two-stage pretreated *S.spontaneum* at magnification 1000x, 1000x, and 10000x respectively.

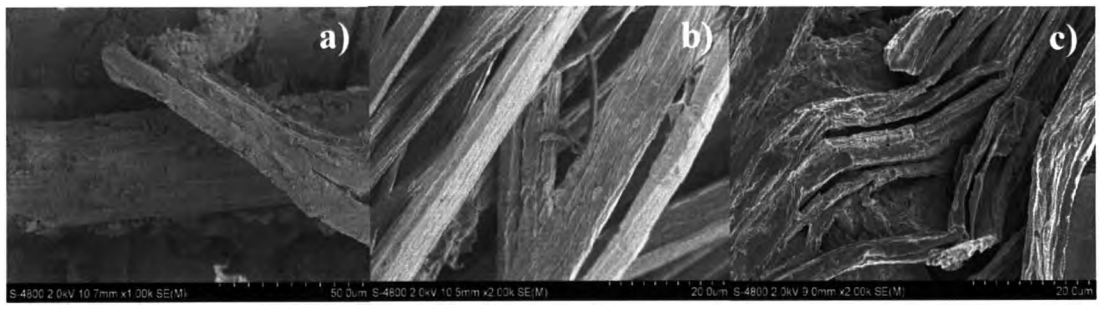



**Figure 5.11** SEM images of (a) raw, (b) microwave-assisted NaOH pretreated, and (c) two-stage pretreated *A.donax* at magnification 1000x, 2000x, and 2000x, respectively.