CHAPTER IV
RESULTS AND DISCUSSION

4.1 Biomass Characterizations

4.1.1 Chemical Composition of Corncob Waste

The quantity of cellulose, hemicelluloses, and lignin of corncob sample was shown in Table 4.1 as a dry weight unit.

**Table 4.1** Chemical composition of corncob waste

Chemical components	Dry solid (%)
Cellulose	41.27
Hemicellulose	46.00
Lignin	7.40
Others	5.33

Table 4.1 shows the composition of corncob waste, which compose of 41.27% cellulose, 46.00 % hemicelluloses, and 7.4% lignin, respectively. Normally, lignocellulosic biomass like corn, cassava, sugarcane etc. mainly composed of cellulose (38–50%), hemicellulose (23–32%), and lignin (15–30%) on dry basis along with smaller amounts of extractive and ash. Cellulose is a main structural component in plant cell walls. The structure of cellulose is a homopolymer consists of β–D–glucose repeating units that are linear connected by β–1–4 glycosidic bonds. The cellulose in a lignocellulosic biomass comprises of two parts with a crystalline structure and amorphous structure. Crystalline cellulose is a major proportion of cellulose while a small proportion is amorphous cellulose. Cellulose is a highly crystalline material which is a major effect to resist enzymatic hydrolysis accessibility. Hemicellulose acts as a connection between cellulose and lignin which give cellulose-hemicellulose-lignin network more rigidity. Hemicellulose is a heteropolymers of carbohydrate which consists of five-carbon sugars (e.g. xylose and

arabinose) and six-carbon sugars (e.g. mannose, glucose, and galactose). Hemicellulose has a lower molecular weight than cellulose. The structure of hemicellulose is random, amorphous, and branched that help hemicellulose is easily to hydrolyze. Lignin is a very complex molecule made up of three types of phenolic acids (p–coumaryl, coniferyl, and sinapyl alcohol) which linked in a three dimensional structures that make lignin particularly difficult to hydrolyzed (Kumar *et al.*, 2009).

4.1.2 Thermal Gravimetric Analysis (TGA)

TG-DTG, Perkin Elmer/Pyris Diamond is an equipment to analyze thermal decomposition of biomass. The thermal decomposition of corncob waste is shown in Figure 4.1
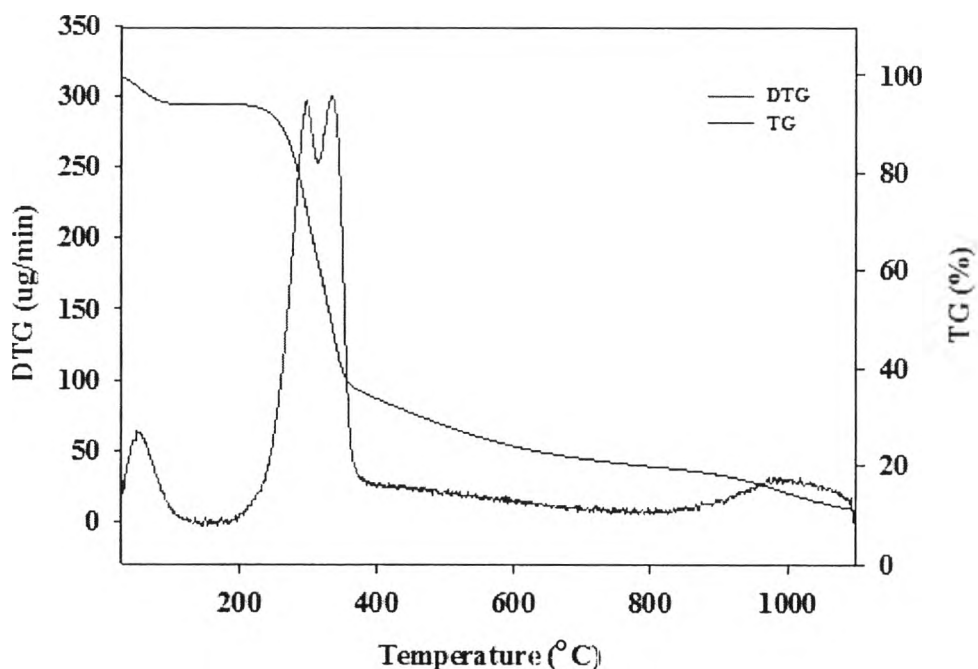
Figure 4.1 Thermal gravimetric analysis curve of corncob waste.

From the result of thermal gravimetric analysis, it has four decomposition temperature ranges, suggesting that corncob waste consisted of four components. The difference in decomposition temperature range depends on the

physical and chemical structure of each component in corn cobs. The first decomposition presented around 100 °C which can imply that in this decomposition temperature was moisture contained in the corncob waste. The second decomposition generated in the range of 200–315 °C which was a hemicellulose. Decomposition of hemicellulose presented in the low temperature range because its structure is a random, amorphous, and branches. Cellulose was the third decomposition that presented in the range of 315–380 °C. The structure of cellulose is mainly consisted of both crystalline cellulose and the small percentage is amorphous cellulose (Palmqvist and Hahn-Hagerdal, 2000). Therefore, the cellulose decomposition is higher than hemicelluloses. Among the four components in corncob waste, lignin was the most difficult component to decompose. The decomposition temperature range of lignin was higher than 900 °C, owing to its structure that consists of aromatic rings with various branches.

4.2 Optimization of Dilute Acid Pretreatment

4.2.1 Effect of Time and Temperature

The goals of the pretreatment process are removing lignin, separating hemicellulose, and improving enzymatic accessibility in order to increase sugar yield. There are various pretreatment techniques; however, this work focused on dilute acid pretreatment. Dilute acid pretreatment can break down cellulose and hemicellulose polymers in lignocellulosic materials to form individual sugar molecules, which ready to be fermented into biofuels (Wyman 1994). It is important to note that the structure of hemicellulose is branches with short lateral chains which help them are easily to hydrolyze than cellulose. Moreover, pretreatment can increase surface area and porosity of lignocellulosic structure in order to increase enzymatic digestibility of cellulose in enzymatic hydrolysis process. Therefore, the enzyme can easily to hydrolyze the polysaccharide to monosaccharide. The interesting point to use phosphoric acid pretreatment is that after sodium hydroxide (NaOH) neutralization of hydrolysate, the salt formed is sodium phosphate (Na_3PO_4). This salt can be used as a nutrient by micro-organisms in the fermentation process. Therefore, the filterable operation is not needed with the subsequent advantages:

develop the economic process (avoid the operation by filtration to remove the salts and decrease the amount of nutrient needed for fermentation process) and improve environmental friendly (the salt formed is not a waste) (Gamez *et al.*, 2006). Corn cobs was pretreated with dilute phosphoric acid under these following conditions: temperature in the range of 100–160 °C, 5–60 min pretreatment time, 1.75 % (w/w) H_3PO_4, and at a 15:1 liquid-to-solid ratio (LSR) After this process was completed, the product in liquid phase, prehydrolysate, was measured the monomeric sugar yield.
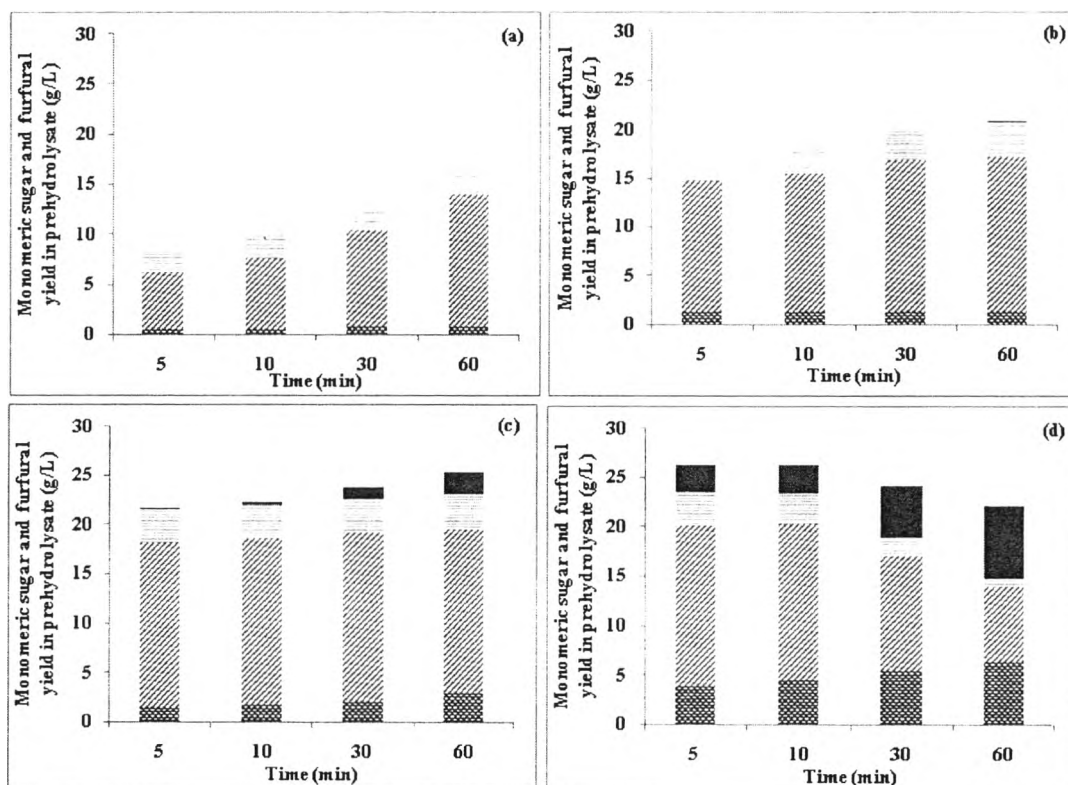
Table 4.2 and Figure 4.2 show monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, and different pretreatment times and temperatures. The glucose yield was found to be further increase along with increasing pretreatment times and temperatures. This result was supported by cellullose structure, as mentioned in chapter 2. The structure of cellulose is crystalline and less amorphous which made cellulose is quite difficult to pretreat. Therefore, severity pretreatment temperature and time can enhance a high glucose yield. The maximum glucose yield in prehydrolysate, 6.51 g/L, was measured after pretreated at condition of 160 °C, 60 min pretreatment time. The major component in prehydrolysate from pretreatment process was xylose. Owing to the structure of hemicellulose which is a random, amorphous, and branches, therefore, xylose presented as the part that can be readily to hydrolyze than glucose. In term of xylose yield, a clear trend is visible, showing that it increased with pretreatment time from 5 min to 60 min and pretreatment temperature from 100 °C to 120 °C. These results were supported by previous works, which reported that the hemicelluloses's solubility increased with increasing pretreatment temperatures (Gray *et al.*, 2003). Under severity pretreatment conditions (> 120°C), the xylose yield decreased owing to xylose degradation into furfural which can obstacle the micro-organism growing in fermentation process (Ezeji *et al.*, 2007). Ezeji *et al.*, 2007 suggested that furfural level upper than 1 g/L can inhibit micro-organism growing in fermentation process. The mechanism and toxicity of furfural was reported by Weil *et al.*, 2002. The result from Tables 4.2 showed that the xylose lost related with the furfural formation. Moreover, the result showed that increasing pretreatment temperatures and times can drive xylose

degradation into furfural. At the highest pretreatment temperatures and times, there is faster furfural formation which is a good agreement with the previous results (Vazquez *et al.*, 2007). The highest xylose yield of 17.10 g/L was obtained at 140 °C for 30 min pretreatment time. The arabinose production was obtained as a function of pretreatment temperatures and times that is similar to the xylose trend under mild pretreatment conditions. The highest arabinose yield of 3.48 g/L was obtained at 140 °C for 60 min pretreatment time, the arabinose yield decreased since the mass of solid residue was burned under severity pretreatment conditions.

Table 4.2 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, and different pretreatment times and temperatures

Temperature (°C)	Pretreatment time (min)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Average furfural (g/L)
100	5	0.69	5.59	1.75	N/A
	10	0.75	6.86	2.45	N/A
	30	0.81	9.72	2.90	N/A
	60	0.89	13.13	3.25	N/A
120	5	1.23	13.45	3.08	N/A
	10	1.25	14.29	3.12	N/A
	30	1.37	15.49	3.32	N/A
	60	1.43	15.77	3.46	0.13
140	5	1.60	16.61	3.36	0.22
	10	1.82	16.79	3.37	0.35
	30	2.12	17.10	3.39	1.22
	60	3.02	16.56	3.48	2.35
160	5	3.92	16.30	3.38	2.66
	10	4.52	15.76	3.08	2.97
	30	5.60	11.50	1.79	5.27
	60	6.51	7.49	0.83	7.32

N/A; Not Available

Figure 4.2 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment by using 1.75% (w/w) H_3PO_4, at a 15:1 LSR, and different pretreatment times and temperatures: (a) 100°C, (b) 120°C, (c) 140°C, and (d) 160°C. Symbols; ⊠ glucose, ⋰ xylose, ▭ arabinose, and ■ furfural.
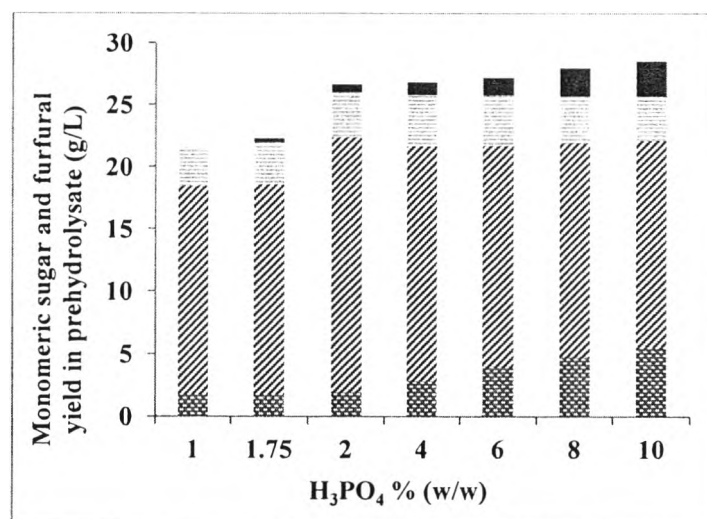
4.2.2 Effect of Acid Concentration

To investigate the effect of acid concentration, corn cobs was pretreated at 140 °C for 10 min pretreatment time, at a 15:1 LSR, and different phosphoric acid concentrations in the range of 1–10% (w/w) H_3PO_4. Table 4.3 and Figure 4.3 show monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140 °C for 10 min pretreatment time, and at a 15:1 LSR under various acid concentrations. High acid environment can disrupt structure of crystalline cellulose to an amorphous form, as the result showed that glucose yield increased with increasing acid concentrations. Under higher acid concentrations, the hemicellulose was hydrolyzed faster, easier, and more completely, conducting the cellulose to pretreat as well. The maximum glucose yield in prehydrolysate, 5.60 g/L, was measured after pretreated at condition of 10% (w/w) H_3PO_4. In part of xylose, it was still the major component in prehydrolysate. From previous work (Fengel and Wegener, 1984) reported that xylose can be extracted well in acid environment than glucose. Therefore, it can imply that the solubility of hemicelluloses to be xylose not only depend on temperature but also depends on pH (paper pretreatment to enhance digestibility). The maximum xylose yield of 20.43 g/L was obtained under pretreatment condition at 2% (w/w) H_3PO_4. At high concentration of H_3PO_4, the xylose degradation occurred due to high level of furfural formation. The first presence of furfural concentration of 0.35 g/L was obtained as shown in Table 4.3. The result from Tables 4.3 showed that furfural level up to 0.66 g/L can decrease the xylose yield in prehydrolysate. In part of arabinose, arabinose yield increased up from 3.14 g/L at 1% (w/w) H_3PO_4 to 4.11 g/L at 4% (w/w) H_3PO_4 and then decreased to 3.61 g/L at 10% (w/w) H_3PO_4. From the result, it can imply that the maximum capacity of arabinose decomposition occurred at 4% (w/w) H_3PO_4.

Table 4.3 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140 °C, 10 min pretreatment time, at a 15:1 LSR, and different acid concentrations

Acid concentration %(w/w)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Average furfural (g/L)
1	1.76	16.70	3.14	N/A
1.75	1.82	16.79	3.37	0.35
2	1.95	20.43	3.66	0.66
4	2.86	18.84	4.11	1.03
6	3.97	17.75	4.06	1.46
8	4.76	17.16	3.82	2.26
10	5.60	16.51	3.61	2.84

N/A; Not Available

Figure 4.3 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140 °C, 10 min pretreatment time, at a 15:1 LSR, and different acid concentrations. Symbols; ⌗ glucose, ⌐ xylose, arabinose, and ▪ furfural.

4.2.3 Effect of Liquid-to-Solid Ratio

The effect of LSR on monomeric sugar yield was investigated by using different LSRs at a 10:1, 15:1, and 20:1 at 140 °C for 10 min pretreatment time, and 2% (w/w) H_3PO_4. Table 4.4 and Figure 4.4 show the monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140 °C for 10 min pretreatment time, 2% (w/w) H_3PO_4, and different LSRs. Table 4.4 shows that the highest monomeric sugar yield was presented under the lowest LSR. The highest monomeric sugar yield was obtained at a 10:1 liquid-to-solid ratioLSR containing 2.03 g/L of glucose, 21.83 g/L of xylose, and 3.76 g/L of arabinose respectively. This is the fact that lowest LSR is a suitable ratio which phosphoric acid can well-mixed with corncob so at this ratio got highest monomeric sugar yield. However, at lowest LSR generated highest furfural levels. Furfural formation at a10:1 LSR highest because of high amount of xylose yield which presented in prehydrolysate. Table 4.4 shows that at a 10:1 LSR is containing 0.68 g/L of furfural.

Table 4.4 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and different LSRs

Liquid to solid ratio	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Average furfural (g/L)
10:1	2.03	21.83	3.76	0.68
15:1	1.95	20.43	3.66	0.66
20:1	1.93	19.83	3.60	0.63
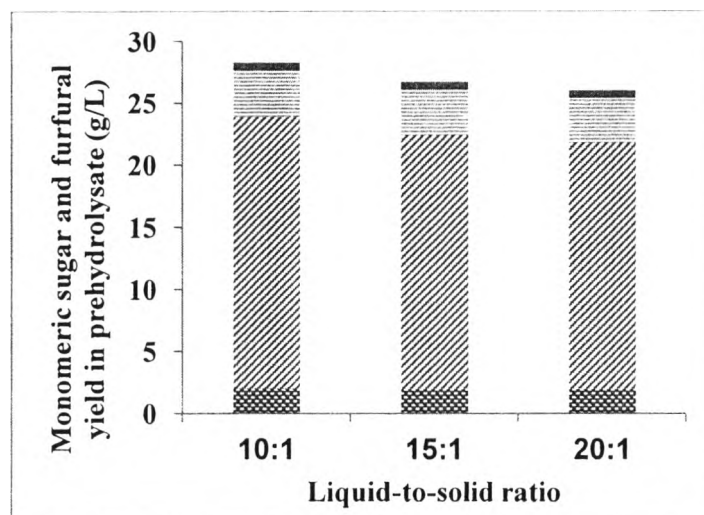
Figure 4.4 Monomeric sugar and furfural yield of corncob in prehydrolysate after dilute phosphoric acid pretreatment at 140°C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and different LSRs. Symbols; ▨ glucose, ⁄ xylose, arabinose, and ▪ furfural.
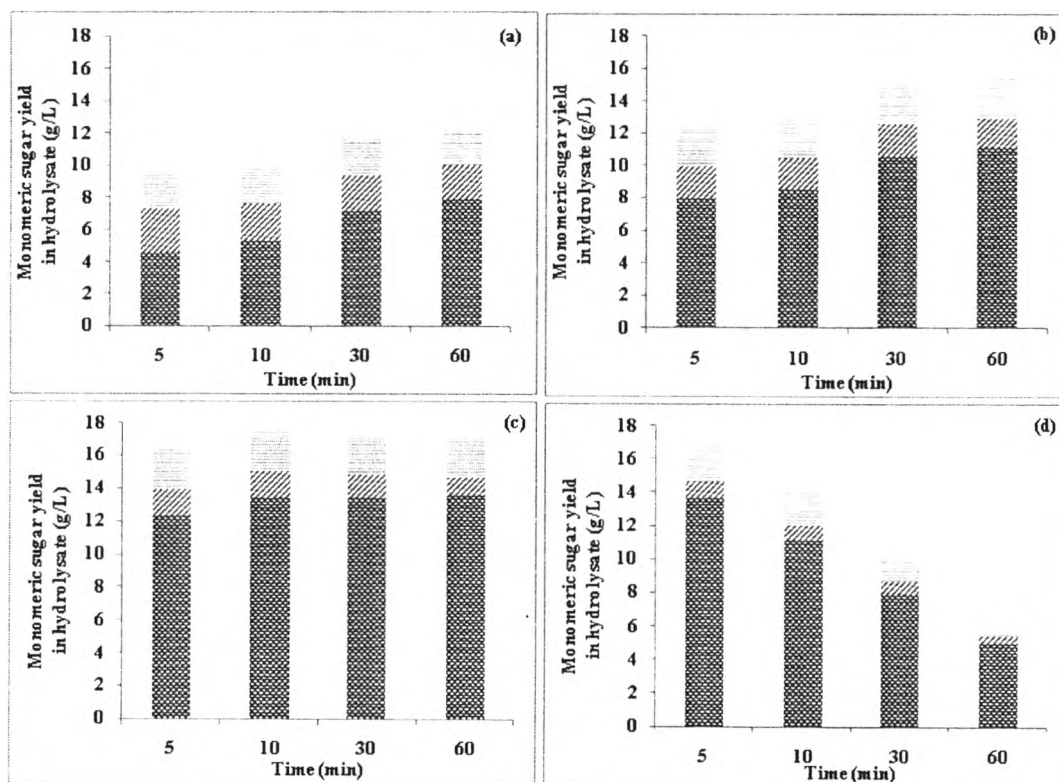
4.3 Enzymatic Hydrolysis

4.3.1 Effect of Time and Temperature

The enzymatic hydrolysis of dilute acid pretreated corncob was performed in enzymatic hydrolysis step. After pretreatment process, the dilute acid pretreated corncob which is a substrate in enzymatic hydrolysis step was mixed with citrate buffer at a 1:30 LSR and 100 µL of enzyme. The condition in enzymatic hydrolysis was fixed in every experiment. After this step was completed, the monomeric sugar yield in liquid phase, hydrolysate, was detected. The major component in hydrolysate was glucose and others were xylose and arabinose. In pretreatment process, hemicellulose was removed in order to maximize glucose yield in hydrolysate after enzymatic hydrolysis step. Table 4.5 and Figure 4.5 show the monomeric sugar and furfural yield in the hydrolysate after enzymatic hydrolysis of corncob under pretreatment condition at 1.75% (w/w) H_3PO_4, at a 15:1 LSR under different pretreatment temperatures and times. The maximum glucose yield contained in the hydrolysate was 13.65 g/L under the pretreatment condition at 160

°C, 5 min pretreatment time, 1.75% (w/w) H_3PO_4, at a 15:1 LSR. The glucose yield increased with increasing pretreatment temperature and time which resulted from hemicelluloses removal during the pretreatment step, as mentioned previously. In addition, the pretreatment process can disorganize the crystalline cellulose in dilute acid pretreated corncob to an amorphous form. The damaged structure of dilute acid pretreated corncob has high surface area which can increase enzymatic accessibility, as shown in Figure 4.6. Figure 4.6 shows the structure of dilute acid pretreated corncob after pretreatment process at condition of 1.75% (w/w) H_3PO_4, at a 15:1 LSR under different pretreatment temperatures and times by using scanning electron microscope (SEM). Figure 4.6 (a) shows the SEM image of fresh corncob. Figure 4.6 (b), (c) show the SEM images of dilute acid pretreated samples with different temperatures and Figure 4.6 (c), (d) illustrate the SEM images of dilute acid pretreated samples with different pretreatment times. The SEM pictures show that fresh corncob had smooth and uniform surface, and non-porous. In contrast, SEM images of the dilute acid pretreated corncob shows significant collapse and destruction structures. The dilute acid pretreated corncob had a rough surface and more porous than fresh corncob. The sample which was pretreated in severity pretreatment temperature and time had deeply crack surface. This kind of cracks can increase surface area and porosity of lignocellulose which was prerequisite for enzymatic hydrolysis of cellulose (Gabhane *et al.*, 2011). After pretreated sample at 160 °C and 5 min pretreatment time, the glucose yield in hydrolysate significantly decreased. The minor products in hydrolysate were xylose and arabinose. In part of xylose yield, increasing pretreatment times and temperatures in pretreatment process decreased the yield of xylose in hydrolysate due to hemicellulose removal in pretreatment step. In part of arabinose, its trend is similar to xylose. Increasing pretreatment temperatures and times in pretreatment process decreased the arabinose yield in hydrolysate owing to the amount of arabinose yield that was explored during pretreatment step.
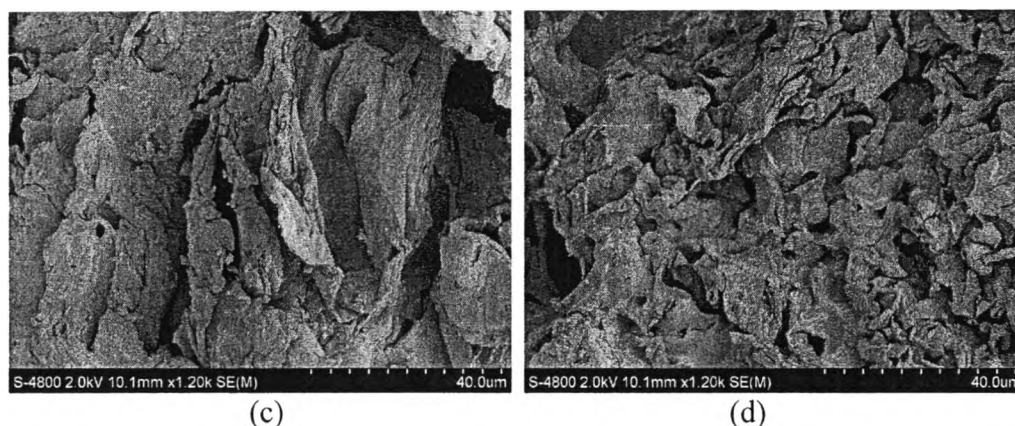
Table 4.5 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions by using 1.75% (w/w) H_3PO_4 at a15:1 LSR, and different pretreatment temperatures and times

Temperature (°C)	Pretreatment time (min)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
100	5	4.58	2.66	2.15
	10	5.20	2.32	2.18
	30	7.10	2.25	2.23
	60	7.84	2.10	2.29
120	5	7.97	2.08	2.33
	10	8.51	2.02	2.39
	30	10.71	1.90	2.40
	60	11.18	1.77	2.43
140	5	12.30	1.64	2.48
	10	13.49	1.58	2.49
	30	13.56	1.30	2.46
	60	13.60	1.11	2.38
160	5	13.65	1.01	2.35
	10	11.10	0.90	2.21
	30	8.02	0.64	1.19
	60	5.02	0.41	0.16

Figure 4.5 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, and different pretreatment temperatures and times: (a) 100°C, (b) 120°C, (c) 140°C , and (d) 160°C. Symbols; ⊠ glucose, ▨ xylose, and ▧ arabinose.
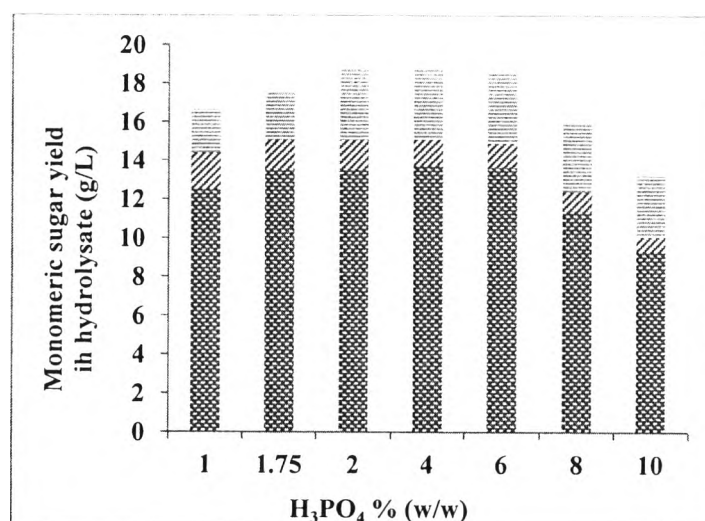

(a)   (b)
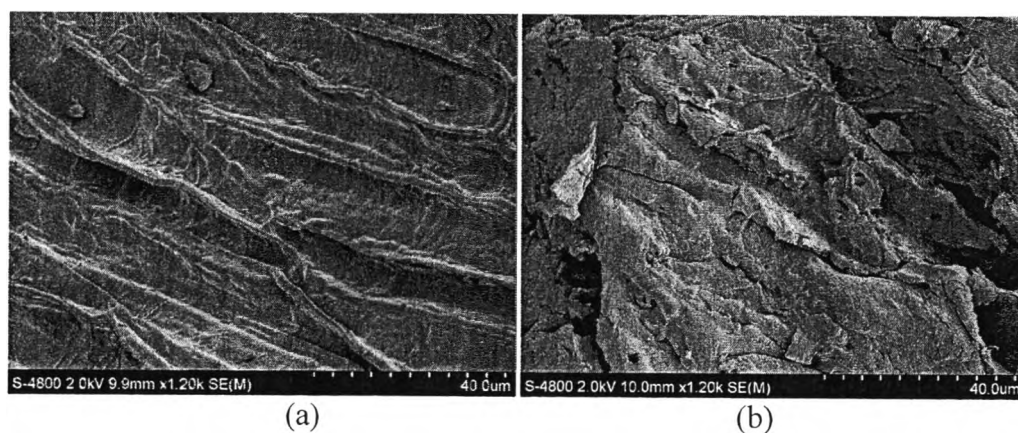
Figure 4.6 SEM images of samples after pretreated with dilute phosphoric acid under the condition of 1.75% (w/w) H_3PO_4 at 15:1 LSR: (a) fresh corncob, (b) 100 °C 10 min, (c) 140 °C 10 min, and (d) 140 °C 60 min.

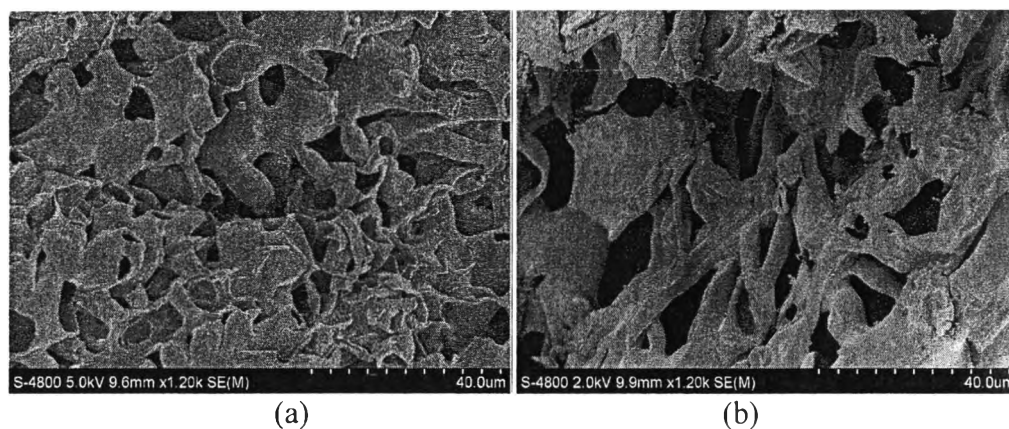### 4.3.2. Effect of Acid Concentration

Effect of dilute phosphoric acid concentrations on the sugar yield of hydrolysate after enzymatic hydrolysis under pretreatment conditions at 140 °C for 10 min pretreatment time, at a 15:1 LSR, and different acid concentrations is shown in Table 4.6 and Figure 4.7. The glucose yield increased from 12.59 g/L at 1 % (w/w) H_3PO_4 to 13.80 g/L at 4% (w/w) H_3PO_4 and then decreased along with higher acid concentration. Acid environment can rapidly break down crystalline in lignocelluloses structure to amorphous form which has higher surface area shown in Figure 4.8. Figure 4.8 shows the structure of dilute acid pretreated corn cobs after pretreatment process under different acid concentrations by using SEM. Figure 4.8 (a), (b) display the SEM images of dilute acid pretreated samples at 2 % (w/w) H_3PO_4, and 10 % (w/w) H_3PO_4 respectively. It was found that the high damaged and cracked surface area of dilute acid pretreated corncob structure was presented at high acid concentration pretreatment. Another component in hydrolysate was xylose. Increasing acid concentration decreased the xylose yield significantly due to the hemicelluloses removal in pretreatment step. The highest yield of arabinose in hydrolysate was 3.74 g/L at 4% (w/w) H_3PO_4. After that, the arabinose yield decreased along with higher acid concentration.

Table 4.6 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions at 140°C, 10 min pretreatment time, at 15:1 LSR, and different acid concentrations

Acid concentration %(w/w)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
1	12.59	1.80	2.29
1.75	13.49	1.58	2.49
2	13.58	1.50	3.69
4	13.80	1.30	3.74
6	13.68	1.21	3.69
8	11.42	0.97	3.55
10	9.35	0.74	3.18

Figure 4.7 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions at 140 °C, 10 min pretreatment time, at a 15:1 LSR, and different acid concentrations. Symbols; ⬚ glucose, ⬚ xylose, and arabinose.

(a)   (b)

Figure 4.8 SEM images of samples after pretreated with dilute phosphoric acid under the condition at 140 °C, 10 min pretreatment time, at a 15:1 LSR: (a) 2% (w/w) H_3PO_4, and (b) 10% (w/w) H_3PO_4.

4.3.3 Effect of Liquid-to-Solid Ratio

Table 4.7 and Figure 4.9 show the monomeric sugar yield of hydrolysate after enzymatic hydrolysis of dilute acid pretreated corn cobs under different pretreatment conditions at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and various LSRs. The result showed that the highest monomeric sugar yield was presented from the dilute acid pretreated corncob, which was pretreated at the highest LSR. However, the glucose, xylose, and arabinose concentration were not much different at different LSRs. The structures of dilute acid pretreated corncob were shown in Figure 4.10. Figure 4.10 (a), (b) show the SEM images of dilute acid pretreated corncob were pretreated at 10:1 and 20:1 LSR respectively. It was found that dilute acid pretreated corncob which was pretreated under higher LSR was disorder than that lower LSR.
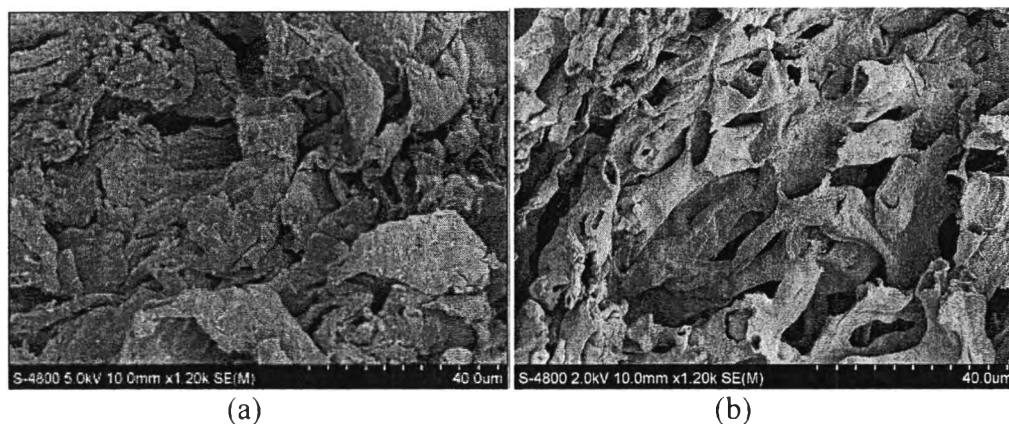
Table 4.7 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions at 140 °C, 10 min pretreatment time, 2% (w/w) H₃PO₄, and at different LSRs

Liquid-to-solid ratio	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
10:1	13.38	1.49	3.65
15:1	13.58	1.50	3.68
20:1	13.60	1.51	3.71
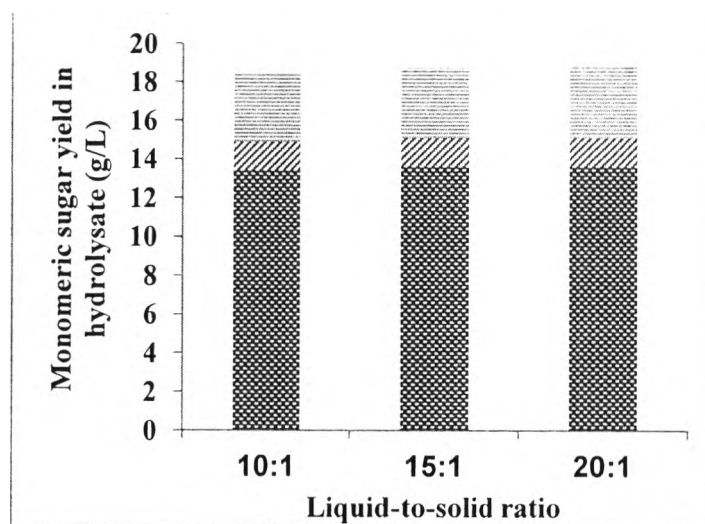
Figure 4.9 Monomeric sugar yield of corncob in hydrolysate after enzymatic hydrolysis under the pretreatment conditions at 140 °C, 10 min pretreatment time, 2% (w/w) H₃PO₄, and at LSRs. Symbols; ⌘ glucose, ⌐ xylose, and □ arabinose.

(a)   (b)

Figure 4.10 SEM images of samples after pretreated with dilute phosphoric acid under the condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4: (a) 10:1 LSR, and (b) 20:1 LSR.
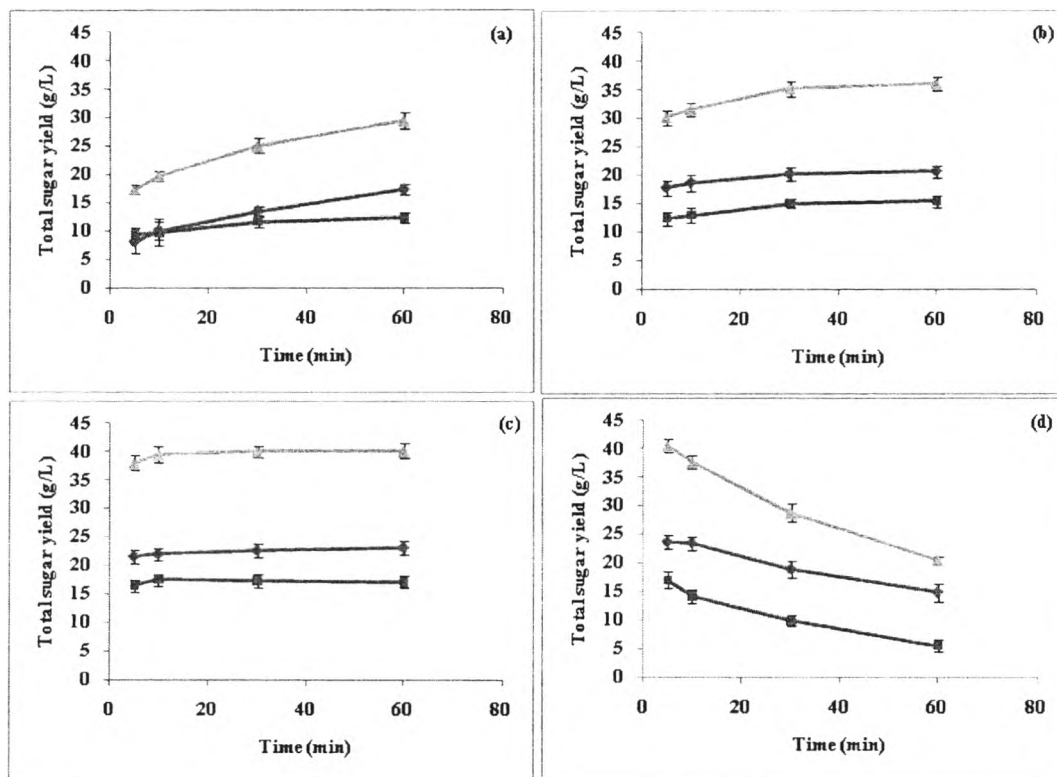
4.4 Total Sugar Yield

4.4.1 Effect of Temperature and Time

The monomeric sugar yield in prehydrolysate and hydrolysate obtained from pretreatment and enzymatic hydrolysis step were combined in order to determine the optimum condition that can give the highest overall monomeric sugar yield. Table 4.8 and Figure 4.11 show the total monomeric sugar yield in prehydrolysate, hydrolysate, and combined two-stage under the pretreatment conditions by using 1.75% (w/w) H_3PO_4, at a 15:1 LSR, and different pretreatment temperatures and times. The highest yield of total monomeric sugar in the prehydrolysate, 23.60 g/L, was obtained under a pretreatment condition at 160 °C, 5 min pretreatment time, 1.75% (w/w) H_3PO_4, and at a 15:1 LSR. The maximun yield of total monomeric sugar in the hydrolysate, 17.56 g/L, was measured after enzymatic hydrolysis under pretreatment condition at 140 °C, 10 min pretreatment time, 1.75% (w/w) H_3PO_4, at a 15:1 LSR. In contrast, the maximum release of total sugar, 40.61 g/L, was obtained at pretreatment condition at 160 °C, and 5 min pretreatment time, 1.75% (w/w) H_3PO_4, and at a 15:1 LSR. However, the previous works found that the furfural formation can inhibit the microorganism growing in fermentation process. They found that furfural level > 1.0 g/L was an inhibitor in

fermentation process (Navarro, 1994; Delgenes *et al.*, 1996; Taherzadeh *et al.*, 1997; Palmqvist and Hahn-Hagerdah, 2000; Almedida *et al.*, 2009). Therefore, the pretreatment condition at 140 °C, and 10 min pretreatment time, 1.75% (w/w) H_3PO_4, and at a 15:1 of LSR seem to be the appropriate condition in this work.

Table 4.8 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, and different pretreatment temperatures and times

Temperature (°C)	Pretreatment time (min)	Total sugar in prehydrolysate (g/L)	Total sugar in hydrolysate (g/L)	Total monomeric sugars (g/L)
100	5	8.03	9.39	17.42
	10	10.06	9.7	19.76
	30	13.43	11.58	25.01
	60	17.27	12.23	29.50
120	5	17.76	12.38	30.14
	10	18.66	12.92	31.58
	30	20.18	15.01	35.19
	60	20.66	15.38	36.04
140	5	21.57	16.42	37.99
	10	21.98	17.56	39.54
	30	22.61	17.32	39.93
	60	23.06	17.09	40.15
160	5	23.60	17.01	40.61
	10	23.36	14.21	37.57
	30	18.89	9.85	28.74
	60	14.83	5.59	20.42

Figure 4.11 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions by using 1.75% (w/w) H_3PO_4 at 15:1 LSR, and different pretreatment temperatures and times (a) 100°C, (b) 120°C, (c) 140°C, and (d) 160°C. Symbols; ● total sugar yield in prehydrolysate, ■ total sugar yield in hydrolysate, △ total sugar yield in two stage.
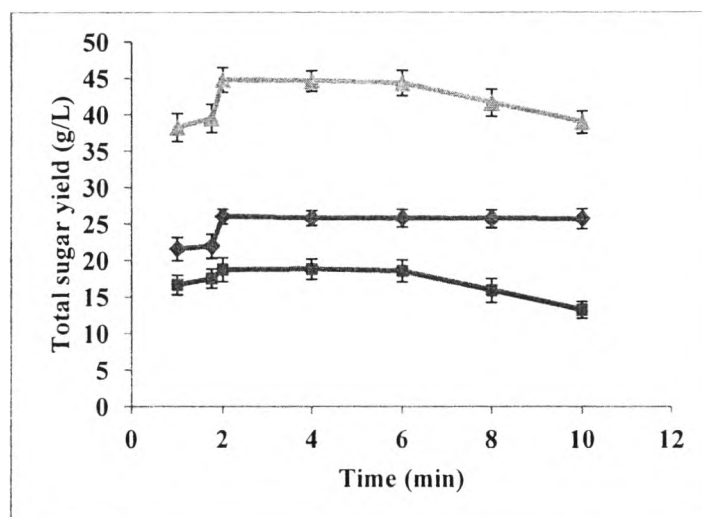
4.4.2 Effect of Acid Concentration

Table 4.9 and Figure 4.12 show the total monomeric sugar yield in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions at 140 °C, 10 min pretreatment time, at a 15:1 LSR, and various acid concentrations. The highest total monomeric sugar yield in prehydrolysate was 26.04 g/L under the pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 and at a 15:1 LSR. While the maximum total monomeric sugar yield in hydrolysate was 18.84 g/L under the pretreatment condition at 140 °C, 10 min pretreatment time, 4% (w/w) H_3PO_4 and at a 15:1 LSR. The highest overall total monomeric sugar yield of two-stage was 44.81 g/L at the pretreatment condition at 140 °C, and 10 min

pretreatment time, 2% (w/w) H_3PO_4, and at a 15:1 LSR and the furfural level in this condition was 0.66 g/L.

Table 4.9 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions at 140 °C, 10 min pretreatment time at a 15:1 LSR, and different acid concentrations

| Acid concentration % (w/w)	Total sugar in prehydrolysate (g/L)	Total sugar in hydrolysate (g/L)	Total sugar (g/L)
1	21.60	16.68	38.28
1.75	21.98	17.56	39.54
2	26.04	18.77	44.81
4	25.81	18.84	44.65
6	25.78	18.58	44.36
8	25.74	15.94	41.68
10	25.72	13.27	38.99

Figure 4.12 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions at 140 °C, 10 min pretreatment time at a 15:1 LSR, and different acid concentrations. Symbols; —●— total sugar yield in prehydrolysate, —■— total sugar yield in hydrolysate, and —▲— total sugar yield in two-stage.

4.4.3 Effect of Liquid to Solid Ratio

Table 4.10 and Figure 4.13 show the total monomeric sugar yield in prehydrolysate, hydrolysate, and combined two-stage under pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at different LSRs. The highest total monomeric sugar yield in prehydrolysate was 27.62 g/L under the pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 at a 10:1 LSR. However, the highest total monomeric sugar yield in hydrolysate was 18.82 g/L under the pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 at a 20:1 LSR. Low liquid-to-solid ratio showed that the overall highest total monomeric sugar yield of 46.14 g/L was obtained under pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 10:1 LSR. The furfural level under this condition was 0.68 g/L which has been found that it has no effect on micro-organism growing in fermentation process (Navarro, 1994).

Table 4.10   Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 and at different LSRs

Liquid-to-solid ratio	Total sugar in prehydrolysate (g/L)	Total sugar in hydrolysate (g/L)	Total sugar yield in two-stage (g/L)
10:1	27.62	18.52	46.14
15:1	26.04	18.77	44.81
20:1	25.36	18.82	44.18
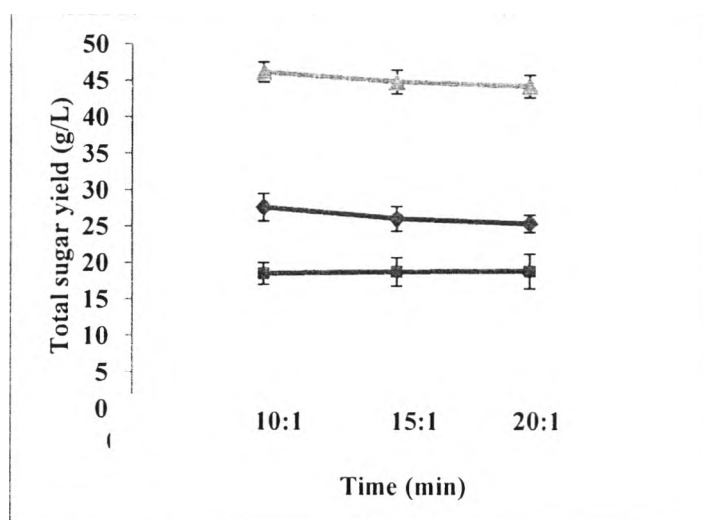
Figure 4.13   Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under pretreatment conditions at 140°C, 10 min pretreatment time, 2% (w/w) H_3PO_4 and at different LSRs. Symbols; ━◆━ total sugar yield in prehydrolysate, ━■━ total sugar yield in hydrolysate, and ━▲━ total sugar yield in two-stage.

4.5 A Comparison between Conventional Heating and Microwave Irradiation Method at an Optimal Pretreatment Condition

Most pretreatment process, conventional heating method, can improve the total sugar yield in both pretreatment and enzymatic hydrolysis process. However, the previous works claimed that there are several factors such as digestion rate, ordor generation, corrosion problems, long reaction time limit, and supply of heat, which be concerned in pretreatment process (Gabhane *et al.*, 2011). Therefore, another interesting method which can avoid these problems is microwave radiation method. Microwave radiation has been used for lignocellulosic materials pretreatment long time ago (Azuma *et al.*, 1984). Microwave radiation has high heating efficiency and easy to operate. Thermal energy of microwave generates through dielectric heating and the energy is transferred into the reactor directly without any contact between the energy source and the reaction mixture. Microwave irradiation has been extensively used in many processes. Some studies have been reported that microwave can reduce pretreatment time, consume less energy, and smaller hazardous emission when compared with other methods. The frequency of microwave radiation is between common radio and infrared frequencies. The microwave frequency is usually of 2.45 gigahertz (GHz) and the wavelength is 122 millimeters. While industrial scale, the microwave frequency and wavelength are 915 Magahertz (MHz) and 328 milimeters, respectively. However, not all frequencies can be used for lab chemistry. A suitable frequency of 2.45 gigahertz (GHz) was claimed to use for scientific works to avoid interference with other signals such as radar and telecommunications. In order to increase the efficiency of microwave, some researchers have combined microwave treatment with alkaline.
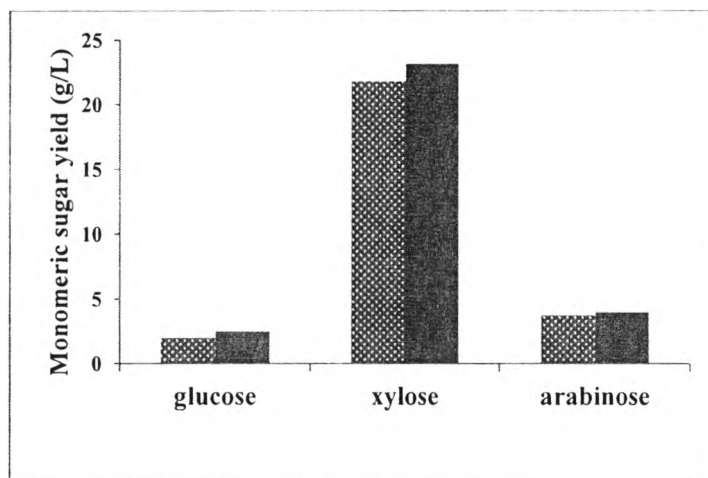
The monomeric sugar yield in prehydrolysate and hydrolysate under an optimal condition (140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 10:1 LSR) by using conventional heat and microwave radiation methods are compared in Table 4.11 and Figure 4.14. The result showed that all monomeric sugar yield in prehydrolysate of sample pretreated with microwave radiation was higher than that of sample pretreated by conventional heat method due to thermal energy direction. In case of conventional heat, thermal energy generates through heating coils and

transfers to the lignocellulosic materials by convective mode, while microwave radiation uses dielectric polarization to collide molecule directly. The highest total monomeric sugar yield in prehydrolysate was 29.67 g/L obtained by pretreatment with microwave radiation. The microwave-assisted H_3PO_4 pretreatment resulted in a higher total monomeric sugar yield than the conventional heating. This result was supported by SEM images, as shown in Figure 4.16. Figure 4.16 shows the SEM images of dilute acid pretreated corncob under an optimal pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 at a 10:1 LSR by using conventional heat and microwave radiation. The SEM images show that the sample pretreated by microwave radiation has more rough surface and deeply crack than that pretreated by conventional heat method. It would expect that an increase of surface area can increase enzymatic accessibility in enzymatic hydrolysis step.

Table 4.11 Monomeric sugar yield of corncob in prehydrolysate and hydrolysate under an optimal condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 15:1 LSR by using conventional heat and microwave radiation method

| Method	Monomeric sugar yield in prehydrolysate (g/L)			Monomeric sugar yield in hydrolysate (g/L)		
	Glucose	Xylose	Arabinose	Glucose	Xylose	Arabinose
Conventional heat	2.03	21.83	3.76	13.38	1.49	3.65
Microwave radiation	2.50	23.16	4.01	14.65	2.32	3.81

Figure 4.14 Monomeric sugar yield of corncob in prehydrolysate and hydrolysate under an optimal condition at 140°C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 15:1 LSR by using different pretreatment reactor. Symbols; ▨ conventional heat, and ■ microwave radiation.

Table 4.12 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under an optimal condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4 at a 15:1 LSR by using conventional heat and microwave radiation method.

Method	Total sugar yield in prehydrolysate (g/L)	Total sugar yield in hydrolysate (g/L)	Total sugar yield in two-stage (g/L)
Conventional heat	27.62	18.52	46.14
Microwave radiation	29.67	20.78	50.45
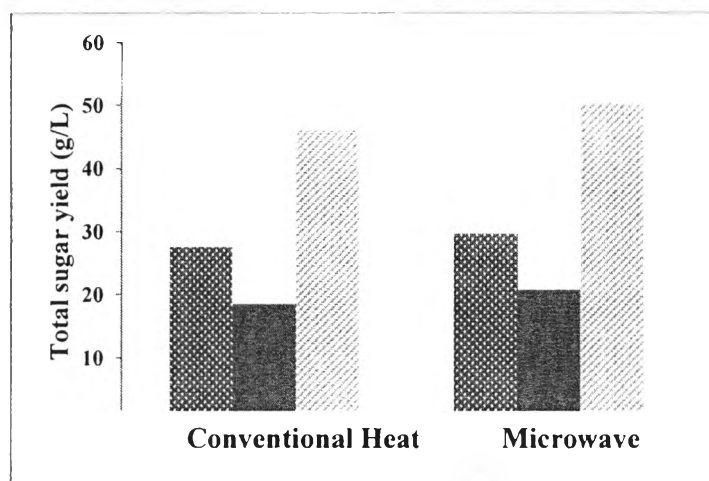
Figure 4.15 Total sugar yield of corncob in prehydrolysate, hydrolysate, and combined two-stage under an optimal condition at 140°C, 10 min pretreatment time, 2% (w/w) H₃PO₄ at a 15:1 LSR by using conventional heat and microwave radiation method. Symbols; ⊠ total sugar yield in prehydrolysate, ■ total sugar yield in hydrolysate, and total sugar yield in two-stage.

(a)   (b)
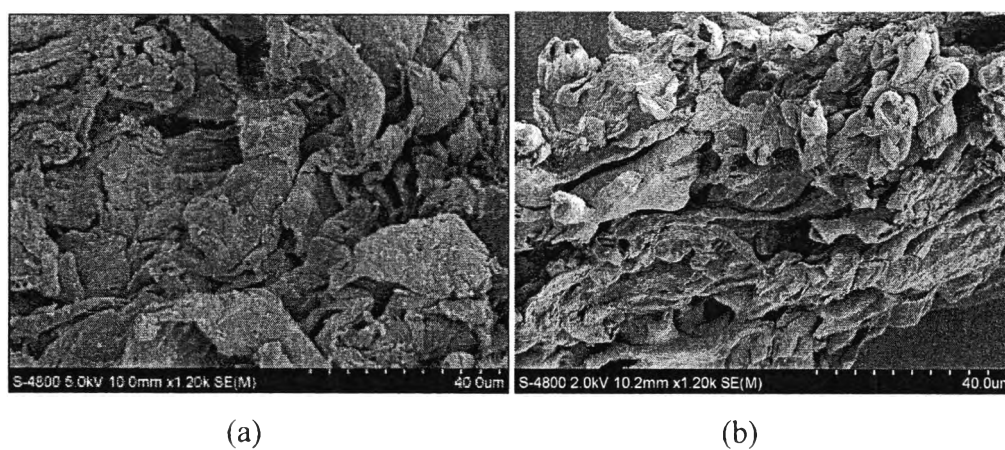
Figure 4.16 SEM images of samples pretreated with dilute phosphoric acid under the condition at 140°C, 10 min pretreatment time, 2% (w/w) H₃PO₄ at a 10:1 LSR by using different pretreated reactor; (a) conventional heat, (b) microwave radiation.

4.6 Physical Characterization of Pretreated Corncob at an Optimal Condition

The physical properties of the samples such as surface area and average pore diameter were characterized by BET. The results in Table 4.13 show the physical properties of dilute acid pretreated corncob (at an optimal condition) compared with untreated corncob. After dilute phosphoric acid pretreatment, it was found that the surface area and total pore volumn of pretreated corncob were higher than the untreated corncob while the average pore diameter of pretreated corncob was lower. This result is corresponding with pretreatment's principle that this process can increase surface area and porosity of lignocellulosic materials (Grethlein, 1992).

Another physical property characterized by x-ray diffraction (XRD) is the crystallinity index (CrI). The untreated and pretreated corncob under various pretreatment conditions were characterize and the results are shown in Table 4.14 and Figure 4.17. Crystallinity index was defined as follows:

$$CrI = (I_{002} - I_{am})/ I_{002} \times 100$$

where I_{002} and I_{am} are the intensity of crystal peak and intensity of diffraction at $2\theta = 18.7°$, respectively (Yoshida et al., 2008). The concept of CrI is based on the assumption that there is only single crystal peak along with amorphous peak (Cheng et al., 2011). CrI were calculated taking into consideration the most prominent peak and the amorphous peak. CrI refers to the fraction of crystalline material in the sample. CrI value depends on the compositions in lignocellulosic materials. Figure 4.17 shows the XRD pattern of pretreated corncob with dilute phosphoric acid under various pretreatment conditions and untreated corncob. There are three peaks of cellulose at the 2θ of $16.82°$, $22.76°$, and $34.97°$, respectively, as shown in Figure 4.17 (a). Which the middle peak at $22.76°$ is sharp peak, while the other two are shoulder peaks. XRD patterns showed an increase in crystallinity with changing in pretreatment temperature, time, acid concentration, and LSR, as compared to the untreated corncob. With increasing pretreatment temperature biomass crystallinity was increased, as shown in Figure 4.17 (b), (c). In addition, an increase in biomass

crystallinity can be observed with increasing pretreatment time and acid concentration, as shown in Figure 4.17 (c), (d) and Figure 4.17 (b), (e), respectively. On the other hand, the opposite trend was observed when increasing LSR. There are two research groups presented the causes of crystallinity changing. Cheng *et al.*, (2011) reported that pretreatment can modify structure of cellulose from crystalline into amorphous. Zhang *et al.*, (2010) reported that the cellulose crystallinity will decrease after dilute phosphoric acid pretreatment. Phosphoric acid esterified hydroxyl groups of cellulose forming cellulose phosphate. And amorphous cellulose was formed when cellulose phosphate converted back to free phosphoric acid and cellulose without significant recrystallization. These two research groups suggested that cellulose crystallinity enhance the enzymatic digestibility. While, Yoshida *et al.*, (2008) discovered the crystallinity will be increased after pretreatment process owing to the removal of lignin which is considered to be amorphous covering cellulose in lignocellulosic biomass.

The composition of pretreated and untreated corn cobs was examined by Nakhonratchasima Animal Nutrition Research and Development Center (Nakhonratchasima province, Thailand). The methods to determine cellulose, hemicelluloses, lignin, and ash in solid residue after pretreatment and enzymatic hydrolysis process were amylase neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose acid detergent lignin (ADL). Amylase neutral detergent fiber (NDF) method used to measure the quantity of cellulose, hemicelluloses, and lignin. After that, the hemicelluloses were detected by using acid detergent fiber (ADF) method. Then, cellulose was measured by using cellulose acid detergent lignin (ADL) method. The quantity of cellulose was measured when the sample wassuspended with sulfuric acid. After this process was completed, the sample was burned at 500 °C for 2 h in order to determine lignin's quantity. The solid residue after this process is ash. Figure 4.18 shows the composition of untreated corn cobs and pretreated after pretreatment and enzymatic hydrolysis step under an optimal pretreatment condition at 140 °C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 10:1 LSR. Composition of hemicelluloses in pretreated sample under an optimal condition was lower than the untreated sample. Figure 4.18 also shows the composition of sample after enzymatic hydrolysis step. The result showed that

cellulose removal during enzymatic hydrolysis step occured. This result is corresponding with the pretreatment concept that pretreatment can solubilize hemicelluloses in order to improve enzymatic accessibility in enzymatic hydrolysis step (Sun and cheng, 2002).

Table 4.13 Characterization of untreated corncob and pretreated corncob at an optimal condition; 140°C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 10:1 LSR by BET

| Sample	Surface area [a] (m²/g)	Pore diameter [a] (nm)	Total pore volumn [a] (cm³/g)
Untreated corncob	1.187	11.260	0.0041
Pretreated corncob	2.032	8.158	0.0053

[a] Estimate by BET

Table 4.14 Crystallinity index (CrI) of untreated corncob and pretreated corncob under various pretreatment conditions

Sample	Crystallinity index (CrI)
(a) Untreated corncob	38.51
(b) Pretreated corncob at 100°C, 10 min, 1.75% (w/w) H_3PO_4, 15:1 LSR	52.90
(c) Pretreated corncob at 140°C, 10min, 1.75% (w/w) H_3PO_4, 15:1 LSR	61.37
(d) Pretreated corncob at 140°C, 60 min, 1.75% (w/w) H_3PO_4, 15:1 LSR	65.22
(e) Pretreated corncob at 140°C, 10 min, 10% (w/w) H_3PO_4, 15:1 LSR	69.64
(f) Pretreated corncob at 140°C, 10 min, 2% (w/w) H_3PO_4, 20:1 LSR	61.87
(g) Pretreated corncobs at 140°C, 10 min, 2% (w/w) H_3PO_4, 10:1 LSR	68.88
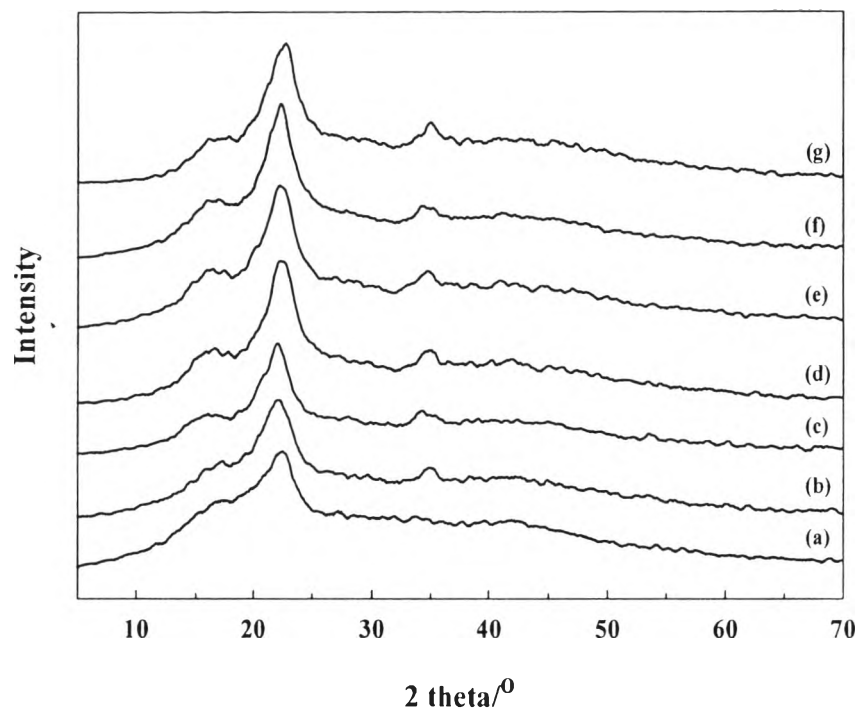
Figure 4.17 X-ray diffraction patterns of the corncob. Symbols; (a) fresh corncob, (b) pretreated corncob at 100 °C, 10 min of pretreatment time by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, (c) pretreated corncob at 140 °C, 10 min pretreatment time by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, (d) pretreated corncob at 140 °C, 60 min pretreatment time by using 1.75% (w/w) H_3PO_4 at a 15:1 LSR, (e) pretreated corn cob at 140 °C, 10 min pretreatment time by using 10% (w/w) H_3PO_4 at a 15:1 LSR, (f) pretreated corncob at 140 °C, 10 min pretreatment time by using 2% (w/w) H_3PO_4 at a 20:1 LSR, and (g) pretreated corncob at 140 °C, 10 min pretreatment time by using 2% (w/w) H_3PO_4 at a 10:1 LSR
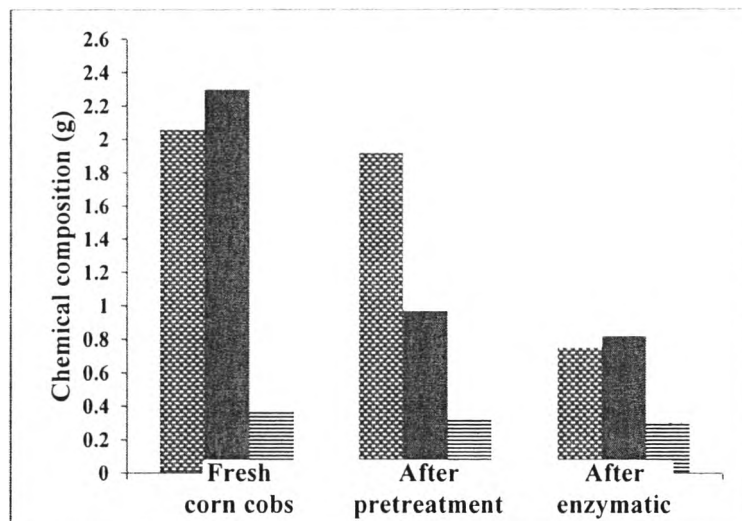
Figure 4.18 Composition of untreated corncob and pretreated corncob after pretreatment and enzymatic hydrolysis under an optimal pretreatment condition; 140°C, 10 min pretreatment time, 2% (w/w) H_3PO_4, and at a 10:1 LSR. Symbols; ∝ cellulose, ■ hemicellulose, and ≡ lignin.

4.7 Ethanol production

Ethanol is an one of the important biofuels. An ethanol production from corn cobs consists of four major steps: pretreatment, enzymatic hydrolysis, fermentation, and product separation. First, corn cobs was pretreated by dilute phosphoric acid. For pretreated hemicellulose, it is ready to be fermented to produce ethanol because fermentable sugars such as five carbon sugars like xylose and arabinose and six carbon sugars like glucose are gained right after pretreatment process. After pretreatment process, cellulose will be less crystalline, allowing enzyme to hydrolyze it into fermentable sugar which mainly consists of six carbon sugar like glucose in hydrolysis step. Then, the prehydrolysate hemicellulose and hydrolysate cellulose were fermented to ethanol by using active yeast (*Saccharomyces Cerevisiae*). Ethanol production from prehydrolysate hemicellulose and hydrolysate cellulose were 7.0 g/L and 9.2 g/L respectively.