



CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chemical Composition of Agricultural Waste

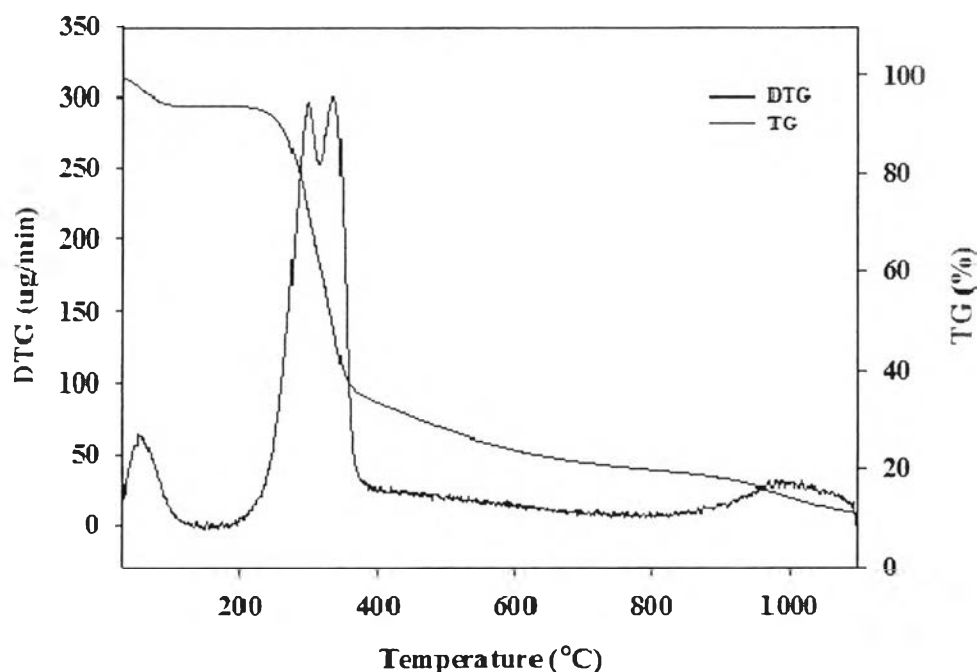
Lignocellulose is the primary building block of plant cell walls. Normally, the composition of corn cobs is 45% cellulose, 35% hemicellulose, and 15% lignin. The ratios between various constituents within a single plant vary with age, stage of growth, and other conditions (Kumar *et al.*, 2009). In this research, corn cobs were received from B&C Pulaski Corporation Limited, Thailand, the main component was 41.27% cellulose, 46% hemicellulose, and 7.4% lignin, as shown in Table 4.1. The others may include some organic compounds (uronic acid and acetyl groups) and other trace components such as minerals, waxes, fats, starches, resins, and gums (Wang *et al.*, 2010). Cellulose is the β -1,4-polyacetal of cellobiose, which is considered as the polymer of the glucose. It is present in both crystalline and amorphous forms. The long-chain cellulose polymers are packed into microfibrils by hydrogen and van der waals bonds. The microfibrils are covered by hemicelluloses and lignin. In this way cellulose can obtain a crystalline structure highly resisting to attack by enzymes (limited accessibility of cellulose chains) which is the major proportion of cellulose. The common polymer of hemicellulose is xylan that is mainly composed of five carbon sugar monomers such as xylose and arabinose, and six carbon sugar monomers such as glucose, mannose, and galactose. The structure of hemicellulose is the lack of crystalline structure due to the highly branched structure and amorphous forms that are easy to hydrolysis. Lignin is a complex polymer which consists of three types of phenolic acids: *p*-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol that links in a three dimensional structure making lignin difficult to hydrolyze. It plays an important role in the cell's endurance and development, as it affects the transport of water, nutrients and metabolites in the plant cell (Harmsen *et al.*, 2010).

Table 4.1 Chemical composition of corn cobs

Composition	Dry solid (% , w/w)
Cellulose	41.27
Hemicellulose	46.00
Lignin	7.40
Other	5.33

4.2 Thermal Gravimetric Analysis (TGA)

The thermal decomposition behavior of untreated corn cobs was investigated by thermal gravimetric analysis TG-DTG, as shown in Figure 4.1.

**Figure 4.1** TG-DTG curves of corn cobs.

The first step of decomposition begins with moisture which was removed by 5.5% mass loss occurs at about 100 °C. The second mass loss step was hemicellulose starting decomposition at 200–320 °C by 32.1% mass loss. Hemicellulose was

constructed an amorphous structure and linear polymer structure with short side chains, which were easier to remove from the main stem than cellulose and degraded to volatiles such as CO, CO₂, and some hydrocarbon at the lower temperature. The third thermal decomposition of cellulose took place from 320 °C to 700 °C by 56.6% mass loss. Since cellulose was mainly consisted of semicrystalline arrangement chains associated with other which its structure was strong and produced it thermally. As for lignin, it was steadily decomposed at 250 to 500 °C. However, some research groups reported that decomposition of heavier volatiles such as lignin occurs from temperature range 150 up to 900°C since it is more thermally stable in contrast to cellulose and hemicelluloses (Abdullah *et al.*, 2010). Lignin was the most difficult to decompose because it was complex structure of phenolic polymer covering the polysaccharides of the cell walls which made strong and durable composite material.

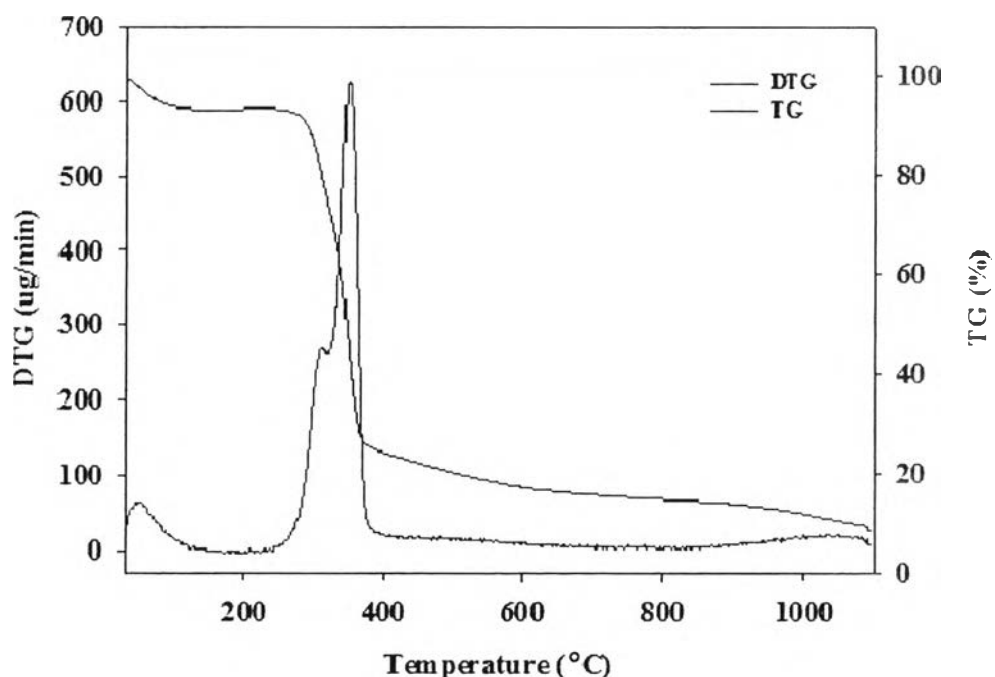


Figure 4.2 TG-DTG curves of the pretreated corn cobs.

The lignocellulosic material is packed with layer of lignin that protects cellulose and hemicellulose against enzymatic hydrolysis. The pretreatment method

is to break down the complex and resistant structure of lignocellulosic to increase the efficiency of subsequent enzymatic hydrolysis. Figure 4.2 shows that the microwave/KOH pretreatment could remove hemicellulose and lignin. The weight loss of hemicellulose decreased from 32.1% to 19.2 %. Due to the overlapped peak of lignin involved with another peak that could not prove the percentage of lignin removal.

4.3 Effect of Microwave and Potassium Hydroxide Pretreatment on Weight Loss

The conversion of biomass to fuel involves 4 steps: pretreatment, hydrolysis, fermentation, and separation/purification. The main objective of pretreatment process is to remove lignin and hemicellulose, and increase the surface area and porosity of the lignocellulosic materials. In the hydrolysis process, the sugars are released by breaking down the carbohydrate chains, before they are fermented for alcohol production (Kumar *et al.*, 2009). Cellulose chains can be broken down into individual glucose sugar molecules by enzymes known as cellulase. The presence of lignin and hemicellulose makes the accessibility of cellulase to cellulose more difficult, as a result, reducing the efficiency of the hydrolysis process.

The use of an alkali causes the degradation of ester and glycosidic side chains, resulting in structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose, and partial solvation of hemicellulose (Brodeur *et al.*). The mechanism of alkaline pretreatment is believed to be saponification of intermolecular ester bonds crosslinking hemicellulose and other components. Moreover, alkali pretreatments remove acetyl and various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface, as reported by Chang and Holtzapple, 2000. As a result, alkali pretreatment disturbed the cell wall of corn cobs by the solubilization of lignin and some hemicellulose in KOH solution, resulting in the enlarged pore size benefits the accessibility of corn cobs to hydrolysis cellulose and hemicellulose into glucose,

xylose, and arabinose. Thus, the weight loss was determined as an important parameter on pretreatment.

Microwave irradiation had been widely used in many processes because of its high heating efficiency and easy operation. Some studies had demonstrated that microwave irradiation can change the structure of lignocellulosic materials so that it disintegrated the lignin and hemicellulose, changed the ultrastructure of cellulose and enhanced the enzymatic hydrolysis of biomass materials (Kashaninejad and Tabi, 2011 and Zhu *et al.*, 2006). Combination of microwave irradiation and dilute alkali treatment was an alternative method for pretreatment of lignocellulosic materials at lower temperatures.

Table 4.2 Effect of pretreatment of corn cobs on weight loss

%KOH	Time(min)	% weight loss			
		60 °C	80 °C	100 °C	120 °C
0.75%	10	20.43	26.75	31.76	35.74
	20	25.30	27.97	32.44	37.85
	25	26.22	28.51	32.76	37.88
	30	27.84	30.48	34.58	38.91
1%	10	24.06	28.35	34.59	41.25
	20	26.81	31.06	35.48	42.05
	25	26.88	31.53	36.09	42.77
	30	27.81	33.87	37.14	44.93
2%	10	25.33	43.95	46.53	49.17
	20	27.11	46.60	47.51	54.38
	25	29.11	47.35	51.50	55.31
	30	30.06	49.21	52.73	55.82
3%	10	35.60	45.34	50.08	52.33
	20	36.90	46.80	51.45	55.21
	25	37.60	47.09	51.97	55.96
	30	38.92	48.97	54.57	56.55

Table 4.2 shows the weight loss of pretreated corn cobs with microwave and KOH. The result showed that KOH concentration, temperature, and time strongly affected on the weight loss. The percentage of weight loss was 20.43% at mild condition (0.75% (w/v) KOH for 10 min at 60 °C) up to 56.55% in pretreated corn cobs by 3% (w/v) KOH for 30 min at 120 °C. The percentages of weight loss of sample pretreated with 1–3% KOH at 100 °C and 120 °C were much higher than that of 0.75% KOH, compared with other conditions. The big difference between the weight loss of the two samples at 100 °C and 120 °C, the sample were analyzed by TG-DTG. The sample was hold for 30 min to remove moisture first and then the TG-DTG was performed, which explained in the previous topic. The same results were also observed and there is no significant different in the composition of corn cobs before and after remove moisture. The lignin was removed with an increased temperature, retention time, and KOH concentration. In addition, increasing in temperature can effectively release hemicellulose in alkaline substances which caused by sugar release from the pretreatment process.

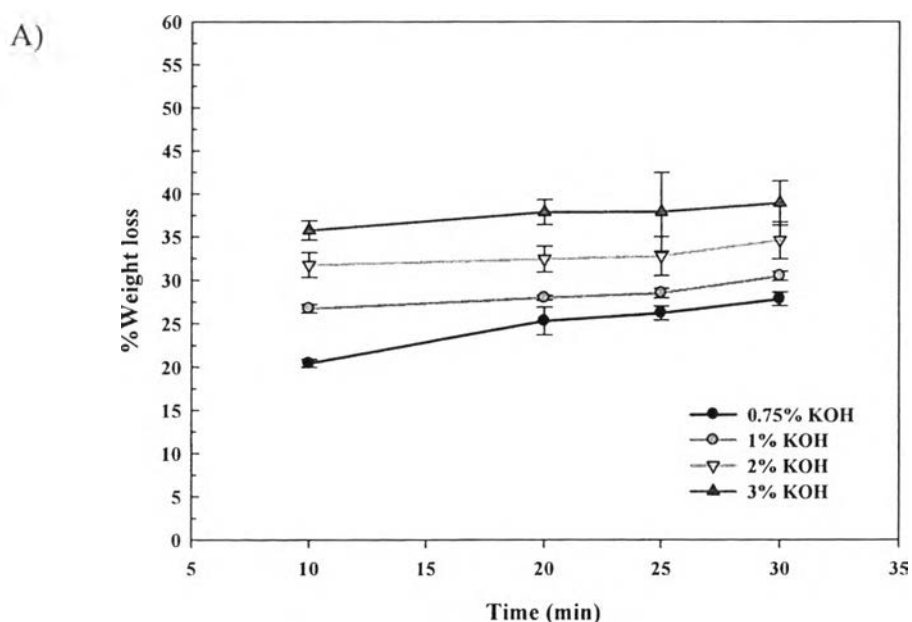


Figure 4.3 Percentage of weight loss of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

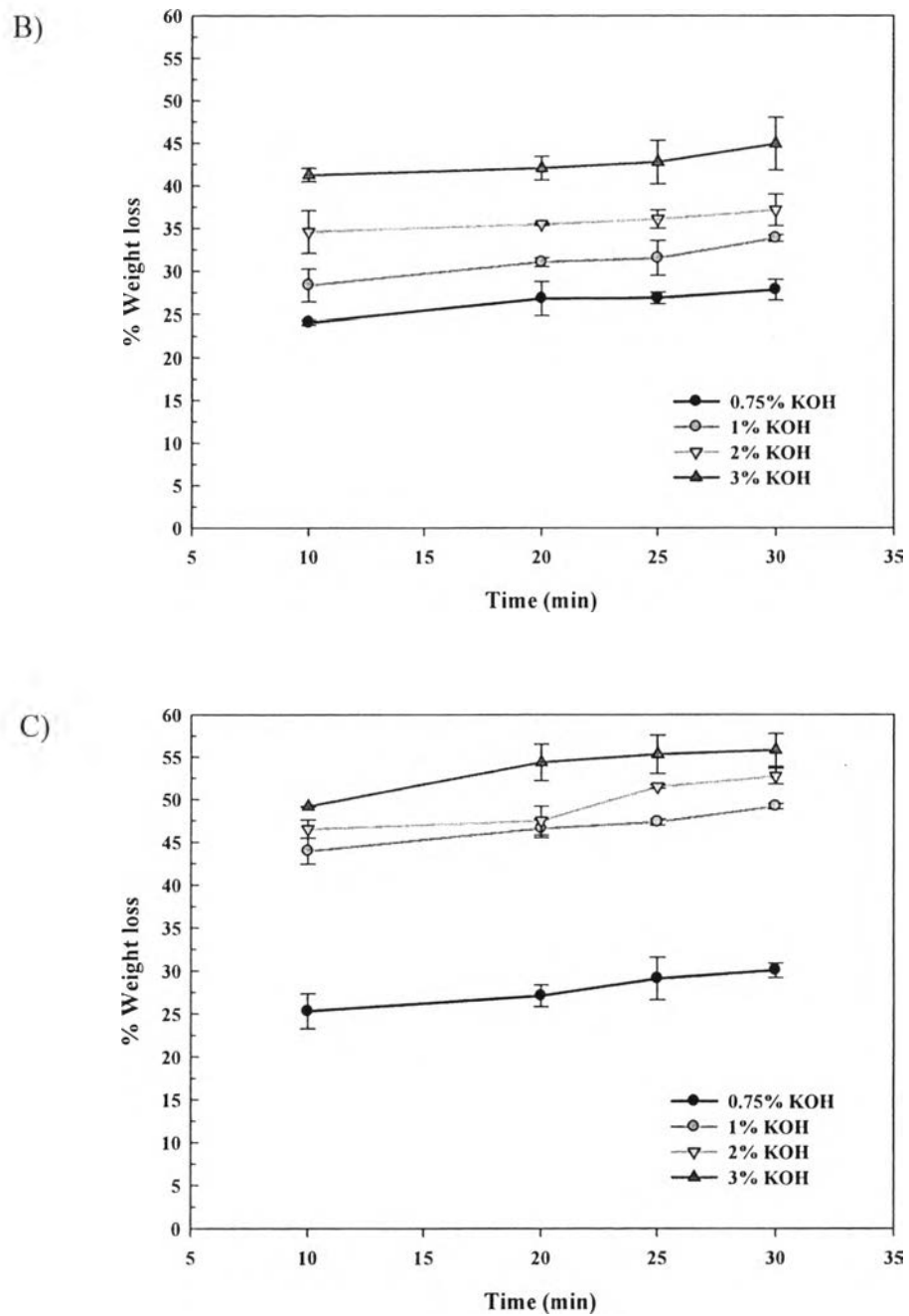


Figure 4.3 (Cont.) Percentage of weight loss of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

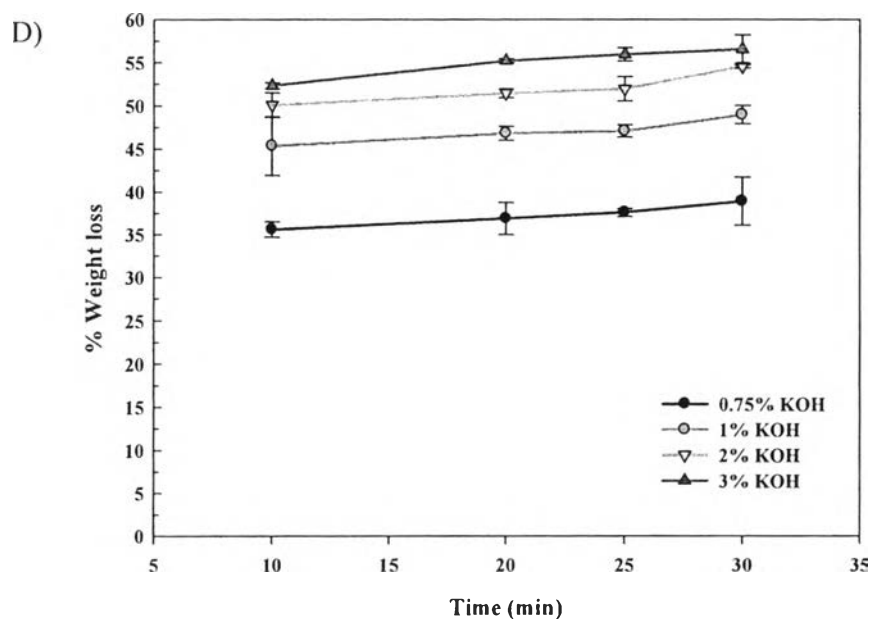


Figure 4.3 (Cont.) Percentage of weight loss of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

4.4 Total Sugar in Enzymatic Hydrolysis

After pretreatment step, the enzymatic hydrolysis is the following step to hydrolyze hemicellulose and cellulose into five carbon sugars and six carbon sugars by using enzyme. The treated corn cobs with microwave and KOH solution were used as the substrates. In order to investigate the optimal condition of the pretreatment with microwave and KOH, glucose and total sugar concentrations were analyzed.

Hydrolysis experiment contained a mixture of buffer solution and pretreated corn cobs. The commercial enzyme solution consisted of three components: endo- β -glucanase (EG) which attached to regions of low crystallinity in the cellulose fiber, generating free chain ends; exo- β -glucanase or cellobio-hydrolase (CBH) which degraded cellulose molecules further by removing cellobiose units from the free chain; and, β -glucosidase that hydrolyzed cellobiose to produce glucose (Champagne and Li, 2009).

The glucose and total sugar concentration obtained from enzymatic hydrolysis of pretreated corn cobs are shown Figures 4.3 and 4.4, respectively. It is clearly seen that the major composition in hydrolysis process was glucose (6–20 g/L) and the minor composition were xylose and arabinose. Owing to cellulose was more difficult to degrade than hemicellulose so that hemicellulose was easier to be lost in the pretreatment than cellulose, which might contribute to the fact that glucan conversion rate was much higher than xylan conversion rate.

Other types of sugar that were released from enzymatic hydrolysis process were xylose and arabinose. The xylose yield obtained was in the range of 2–8.9 g/L. The xylose yield was gradually increased with increasing KOH concentration and temperature; however, its yield was not much different as pretreated time increased. When KOH concentration increased to 3% at 120 °C, the xylose yield decreased with pretreated time. This was probably due to the partial degradation of hemicellulose at high KOH concentrations and pretreatment times. The arabinose yield is not significantly different with increasing KOH concentration, temperature, and pretreated time, as shown in Table 4.3.

In the case of glucose, it was found that the corn cob treated by 0.75% (w/v) KOH at 60 °C for 10 min produced the lowest glucose concentration (6.63 g/L). At low temperature (60 °C and 80 °C), the glucose concentration slightly increased when pretreatment time and KOH concentration increased. It can be concluded that lower temperatures for KOH pretreatment was not favorable to enhance total sugar released because the crosslink between lignin and carbohydrates were not interrupted sufficiently to reach high sugar production (Ploypradith, 2010). At high temperature (100 °C and 120 °C), the glucose concentration of sample pretreated by 0.75%, 1%, and 2% (w/v) KOH increased as pretreatment time increased. Increasing the concentration of KOH solution increased the swelling cellulose containing creates a very large internal surface area for accessible enzymatic hydrolysis. On the other hand, a trend of glucose concentration of sample pretreated by 3% (w/v) KOH was lower than that of 2% (w/v) KOH with increased pretreatment time. Because high concentration of KOH solution can form more nonreversible salts, they were unfavorable for cellulose hydrolysis (Cheng *et al.*, 2011). As a result of higher

pretreatment time and higher KOH concentration during pretreatment, resulted in higher solid loss which leading to less total sugar released. As shown in Figures, 4.4(D) and 4.5(D) there was no significant different in glucose and total sugar concentration between 25 and 30 min of 2% (w/v) KOH.

Table 4.3 Monomeric sugar and total sugar concentration from enzymatic hydrolysis of pretreated corn cobs at different concentrations and pretreated times. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C

A)	%KOH	Time(min)	Glucose	Xylose	Arabinose	Total sugar
			(g/L)	(g/L)	(g/L)	(g/L)
0.75		10	6.63	2.02	6.03	14.68
		20	7.27	3.26	6.04	16.57
		25	7.66	2.99	6.15	16.81
		30	9.63	3.31	6.34	19.28
1		10	7.08	2.52	6.42	15.02
		20	9.14	3.23	7.09	19.46
		25	9.31	3.14	7.33	19.78
		30	10.27	4.19	6.79	21.24
2		10	10.45	4.10	6.44	20.99
		20	11.20	4.07	6.15	21.43
		25	11.69	5.04	6.85	23.58
		30	12.50	5.75	5.77	24.02
3		10	14.54	6.88	5.89	27.31
		20	15.07	7.32	6.28	28.68
		25	15.12	7.41	6.67	29.21
		30	15.16	7.42	6.90	29.48

Table 4.3 (Cont.) Monomeric sugar and total sugar concentration from enzymatic hydrolysis of pretreated corn cobs at different concentrations and pretreated times. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C

B)		Glucose	Xylose	Arabinose	Total sugar
%KOH	Time(min)	g/L	g/L	g/L	g/L
0.75	10	7.45	2.14	6.69	16.27
	20	7.70	2.12	7.81	17.63
	25	8.32	3.06	6.45	17.83
	30	8.64	3.08	8.06	19.78
1	10	8.86	3.57	7.49	19.92
	20	10.08	4.05	7.68	21.81
	25	11.30	4.88	6.41	22.59
	30	11.63	4.94	7.35	23.92
2	10	15.81	6.91	6.44	29.15
	20	16.23	7.12	6.21	29.55
	25	15.66	7.73	6.67	30.07
	30	14.55	8.04	7.57	30.17
3	10	15.02	6.40	4.38	25.80
	20	15.93	6.50	4.83	27.25
	25	17.47	6.61	5.78	29.86
	30	16.36	7.11	6.44	29.91
C)		Glucose	Xylose	Arabinose	Total sugar
%KOH	Time(min)	g/L	g/L	g/L	g/L
0.75	10	10.44	4.35	5.34	20.13
	20	11.03	4.70	5.38	21.11
	25	11.52	3.93	6.01	21.47
	30	11.66	4.79	6.12	22.58

Table 4.3 (Cont.) Monomeric sugar and total sugar concentration from enzymatic hydrolysis of pretreated corn cobs at different concentrations and pretreated times. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C

C)					
%KOH	Time(min)	Glucose g/L	Xylose g/L	Arabinose g/L	Total sugar g/L
1	10	12.25	5.60	5.28	23.13
	20	12.26	5.41	5.78	23.45
	25	12.93	5.75	5.04	23.72
	30	13.33	5.86	5.70	24.89
2	10	16.84	6.93	5.43	29.20
	20	17.41	6.88	5.27	29.56
	25	17.98	6.99	5.34	30.31
	30	18.07	7.32	6.76	32.16
3	10	17.25	6.94	6.15	30.33
	20	16.73	6.55	4.98	28.26
	25	15.95	6.43	5.10	27.48
	30	15.95	6.54	5.03	27.53
D)					
%KOH	Time(min)	Glucose g/L	Xylose g/L	Arabinose g/L	Total sugar g/L
0.75	10	10.49	6.48	5.25	22.23
	20	11.29	6.50	4.91	22.70
	25	11.73	6.46	5.60	23.79
	30	12.12	6.66	5.53	24.32
1	10	12.81	6.65	5.58	25.03
	20	13.53	6.18	5.68	25.39
	25	13.79	6.03	6.54	26.37
	30	14.80	6.65	5.59	27.04

Table 4.3 (Cont.) Monomeric sugar and total sugar concentration from enzymatic hydrolysis of pretreated corn cobs at different concentrations and pretreated times. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C

D)		Glucose	Xylose	Arabinose	Total sugar
%KOH	Time(min)	g/L	g/L	g/L	g/L
2	10	16.39	7.49	5.59	29.48
	20	18.28	8.35	5.89	32.53
	25	19.49	8.92	6.38	34.79
	30	19.48	8.93	6.43	34.84
3	10	18.23	7.42	6.00	31.65
	20	18.74	7.56	5.71	32.01
	25	18.98	7.50	6.09	32.57
	30	16.72	6.85	4.63	28.20

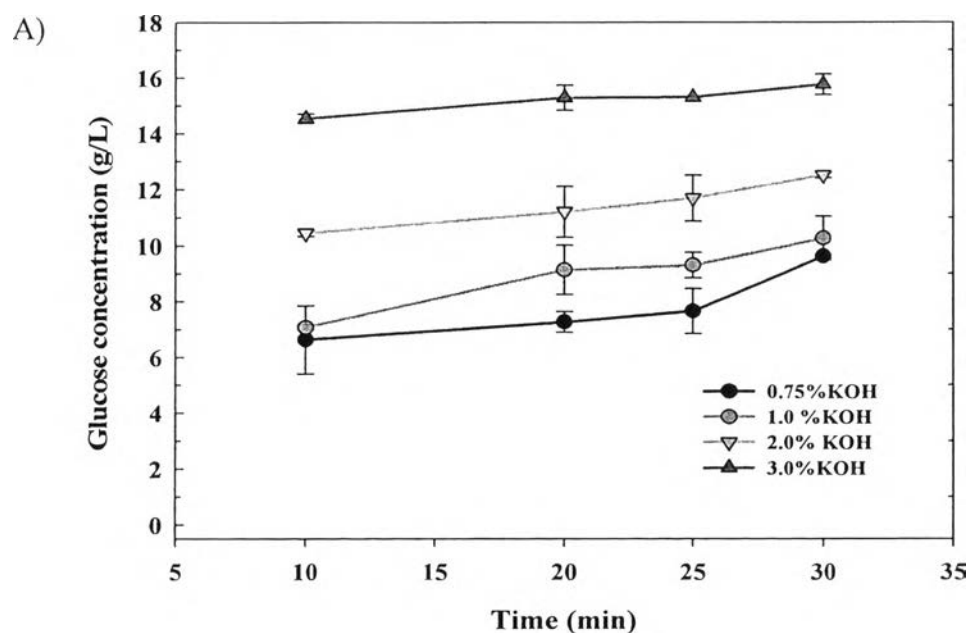


Figure 4.4 Glucose concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

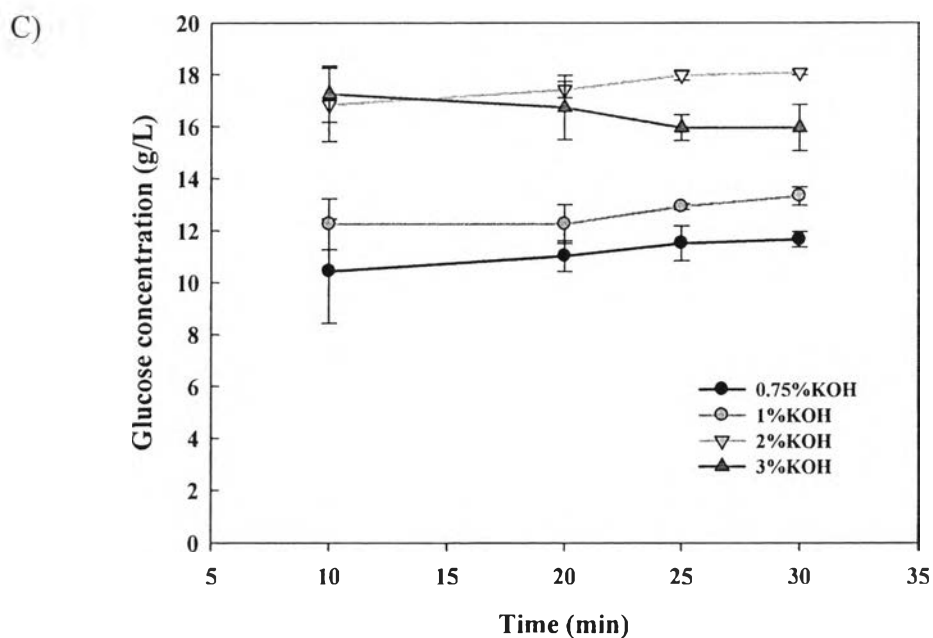
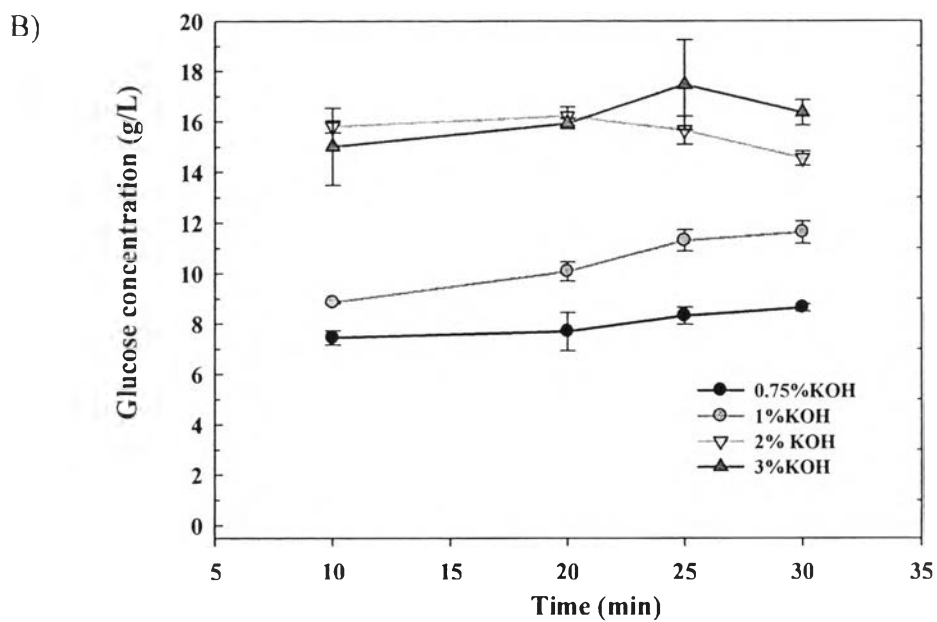


Figure 4.4 (Cont.) Glucose concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

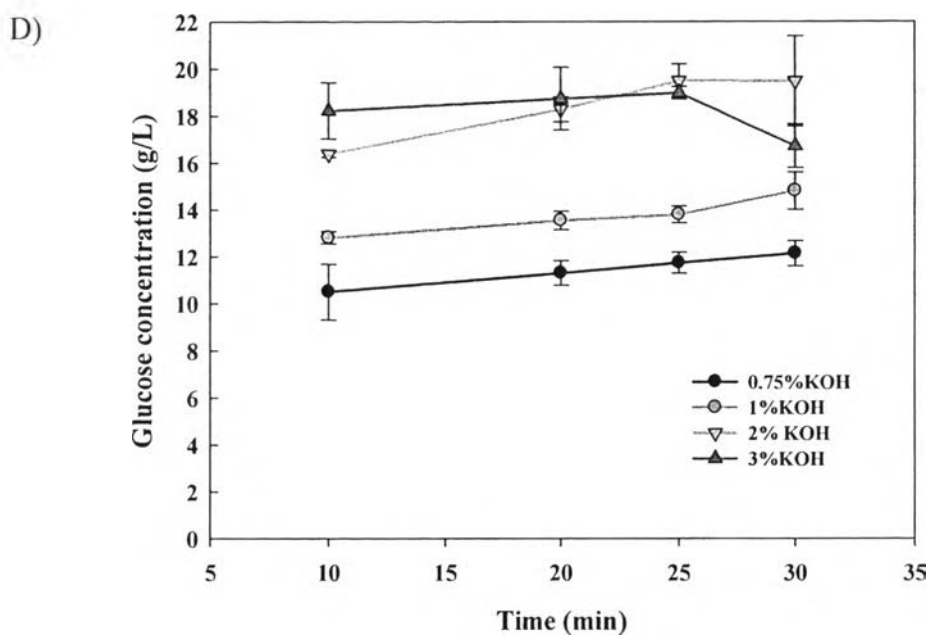


Figure 4.4 (Cont.) Glucose concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

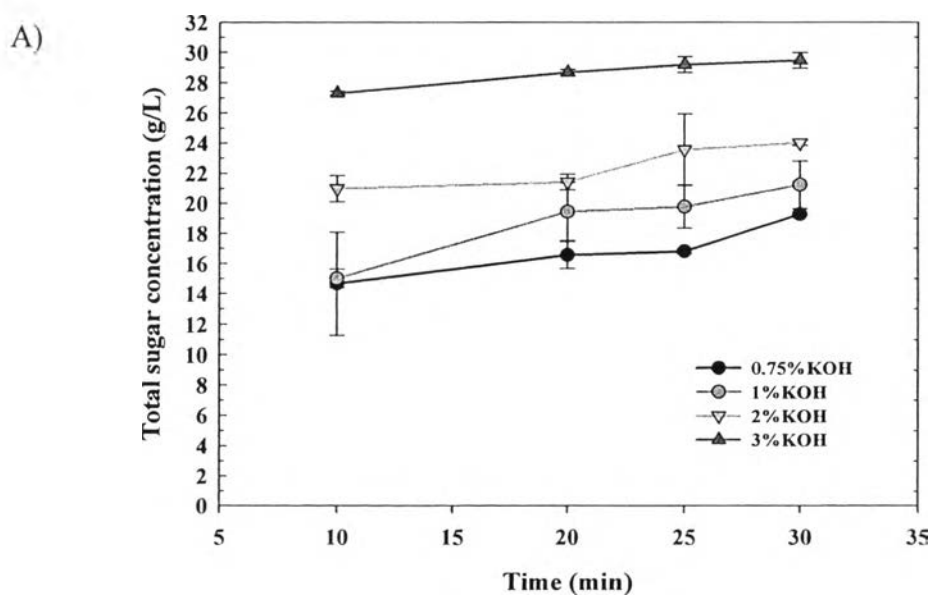


Figure 4.5 The total sugar concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

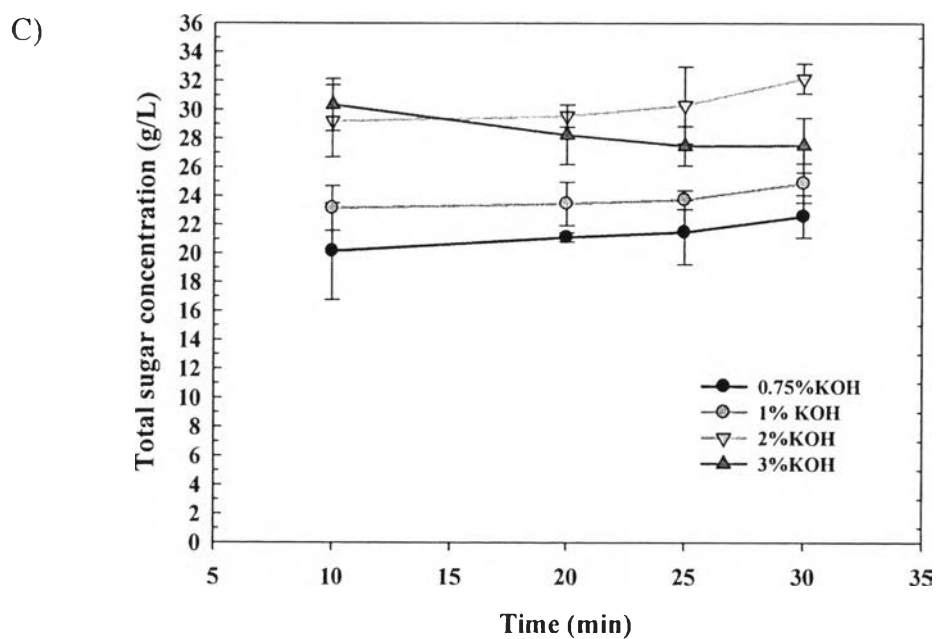
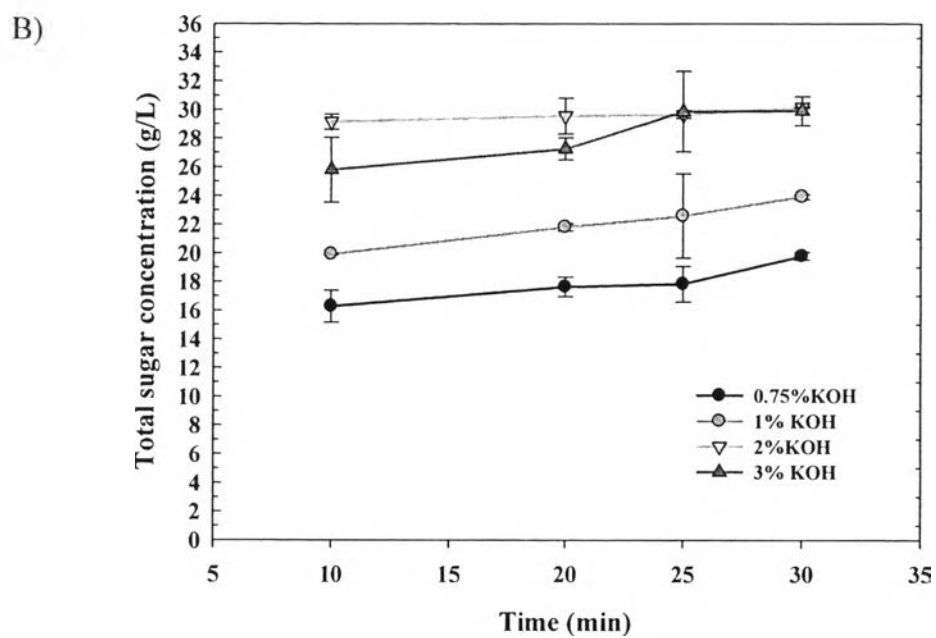


Figure 4.5 (Cont.) The total sugar concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

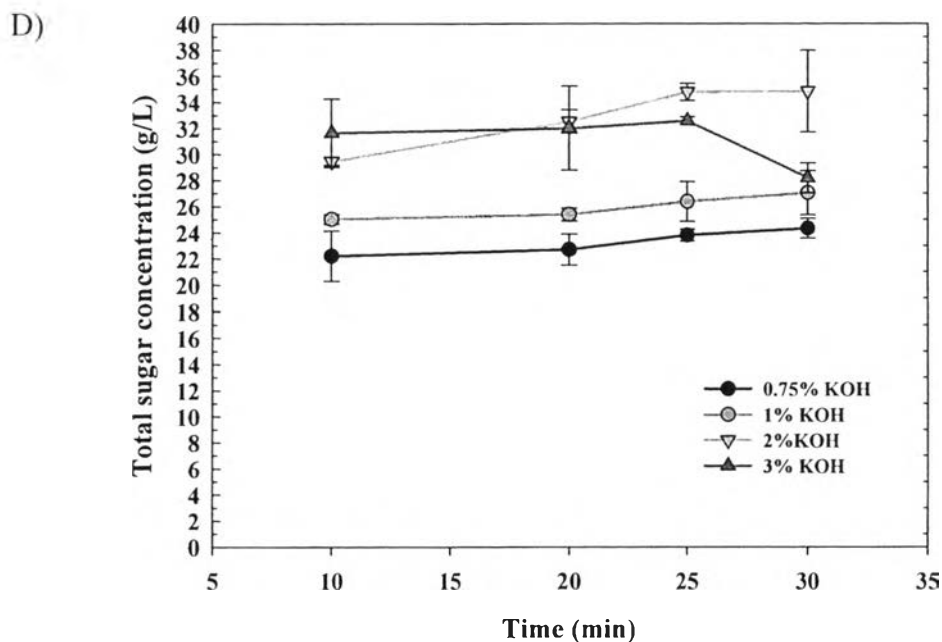


Figure 4.5 (Cont.) The total sugar concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions. (A) 60 °C; (B) 80 °C; (C) 100 °C; (D) 120 °C.

As mentioned above, the pretreatment at high KOH concentration, temperature, and longer pretreated time gave a higher glucose concentration and consequently the glucose concentration in enzymatic hydrolysis step was also improved. The highest glucose concentration of 19.49 g/L and total sugar concentration of 34.79 g/L, which were obtained from the samples pretreated by 2% (w/v) KOH at 120°C for 25 min.

The effect of pretreatment temperature on the sugar yield was investigated in the range of 60 °C to 140 °C. From previous section, it was found that 2% (w/v) KOH was the suitable concentration and gave the maximum glucose yield. At 2% (w/v) KOH, the trend of glucose concentration increased when pretreated temperature was increased from 60 °C to 120 °C. After pretreated at high temperature (140 °C – 160 °C), the glucose concentration was slightly decreased because pretreated at harsh temperature resulted in higher weight loss, suggesting

that mean cellulose may be loss and leading to glucose concentration decreased, as shown in Figure 4.6. Moreover, further increasing in temperature might trigger significant sugar degradation and resulted in a significant increase in lignin removal (Hu and Wen, 2008). Thus, the optimal condition was 2% (w/v) at 120 °C.

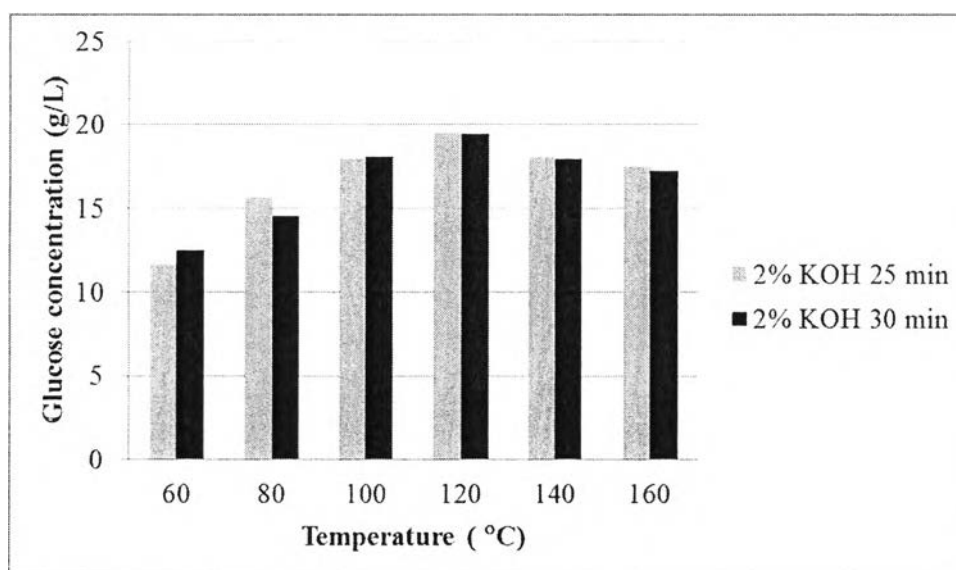


Figure 4.6 Glucose concentrations from pretreated corn cobs with 2% (w/v) KOH for 25 and 30 min with different pretreatment temperatures.

The effects of duration of KOH pretreatment on the sugar concentration at 120 °C were investigated. From the optimal condition at 2% (w/v) KOH, 120 °C, when pretreatment time increased from 25 to 30 min, glucose concentration was slightly decreased from 19.49 to 19.48 g/L. When pretreatment time further increased to 40 min, glucose concentration dramatically decreased to 10.75 g/L, as shown in Figure 4.7. Less pretreatment time was inadequate for lignin destroying and cellulose hydrolysis, but more pretreatment time will result in nonreversible salts, which were unfavorable for cellulose hydrolysis (Cheng *et al.*, 2011). It is evident that the effect of pretreatment time is more pronounced at 25 min. From the previous part, higher temperature, higher pretreatment time and higher KOH concentration during pretreatment, resulted in higher solid loss, leading to less total sugar released.

Therefore, the maximum glucose concentration was 19.49 g/L and total sugar concentration of 34.79 g/L were obtained from 2% (w/v) KOH at 120 °C for 25 min.

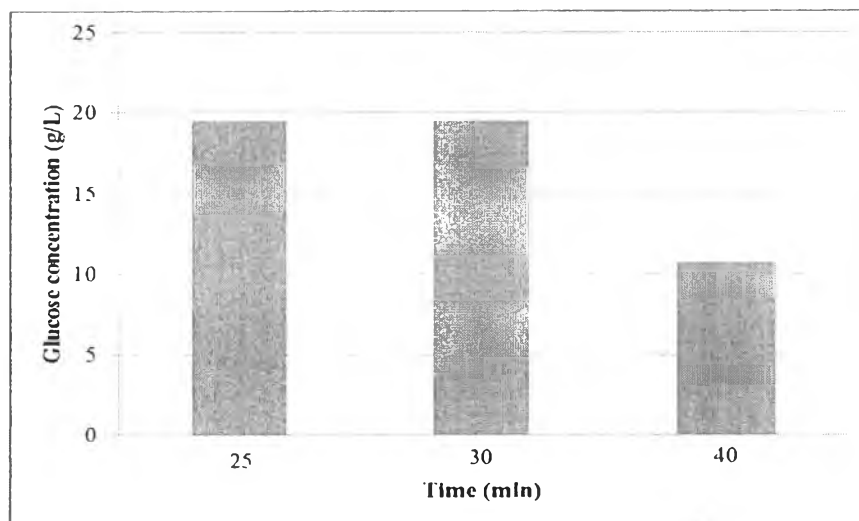


Figure 4.7 Glucose concentration from pretreated corn cob with 2% (w/v) KOH for 25, 30, and 40 min at 120 °C.

In this work, no furfural or hydroxymethyl furfural (HMF) were observed, which normally was detected in hydrolyzates obtained from enzymatic hydrolysis process. Generally, a neutralizing step to remove lignin and inhibitors (salts, phenolic acids, furfural and aldehydes) is required before enzymatic hydrolysis. This work agrees well with the work reported by, Alvira and co-worker (2010). They found that pretreatment with alkali had no inhibitor in the solution that favored the fermentation step in an ethanol production process.

4.5 Composition of Corn Cobs After Pretreatment and Enzymatic Hydrolysis

Pretreated corn cobs by 2% KOH at 120 °C for 25 min were analysed for chemical components. The characterization of untreated and pretreated corn cobs was conducted according to ADF-NDF-ADL method. The untreated consisted of 0.82 g cellulose, 0.92 g hemicelluloses, and 0.15 g lignin. As seen in Figure 4.8, pretreatment reduced hemicellulose and lignin by 82.93% and 89.79%, respectively,

compared with the chemical components of pretreated corn cobs. This implied the swelling of lignocellulosic structure; therefore, their solubilization in the KOH aqueous was improved. The removal of lignin content resulted in an increase internal surface area and helped in exposing more cellulose to the enzymatic accessibility. The structural linkage between lignin and carbohydrate were separated and lignin structure was also disrupted by KOH pretreatment. The effect of microwave assisted KOH pretreatment enabled and accelerated the breaking down the lignin-hemicellulose complex and increased the exposure of cellulose surface to cellulase (Ding *et al.*, 2012).

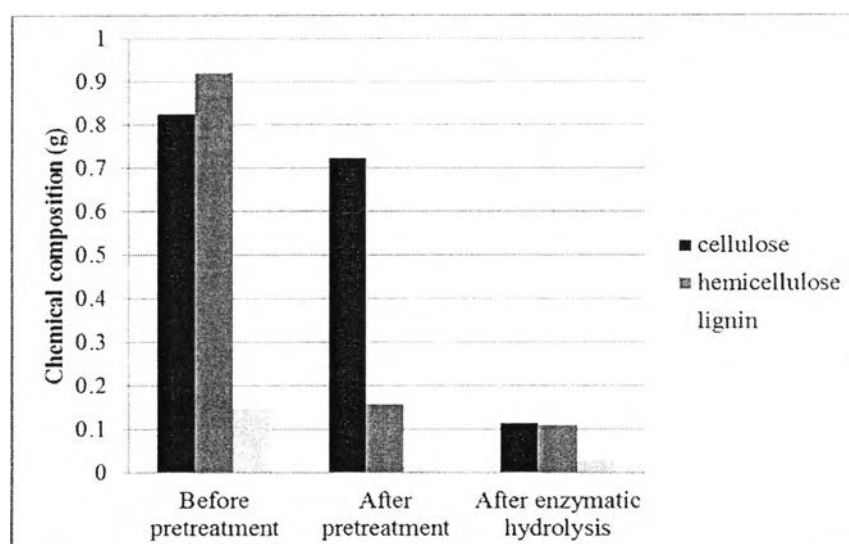


Figure 4.8 Content of cellulose, hemicellulose, and lignin in corn cobs.

On the other hand, cellulose was decomposed by 62.00% after enzymatic hydrolysis. The result showed that cellulose content substantially decreased compared with after pretreatment because the cellulase enzyme complex adsorbed onto the surface of the cellulose structure and converted it to glucose. Hendriks and Zeeman (2009) mentioned that removal of hemicellulose increases the mean pore size of the substrate and therefore increases the probability of the cellulose to hydrolyse.

4.6 Surface Morphology by SEM

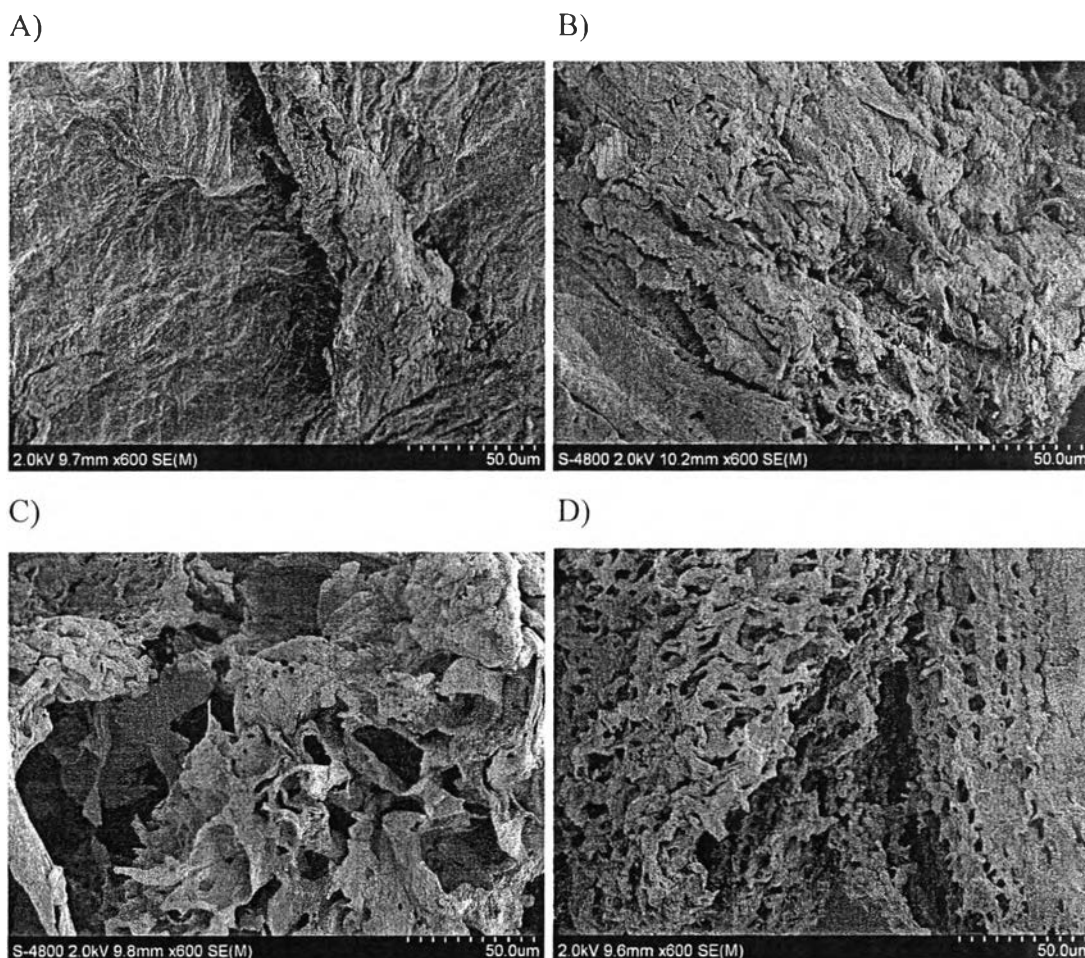


Figure 4.9 Scanning electron microscope images of corn cobs. (A) Raw corn cobs without treatment; (B) Corn cobs after pretreatment with 0.75% (w/v) KOH at 60°C for 25 min; (C) Corn cobs after pretreatment with 0.75% (w/v) KOH at 120°C for 25 min; (D) Corn cobs after pretreatment with 2% (w/v) KOH at 120°C for 25 min.

Scanning electron microscope (SEM) images of the untreated and the pretreated corn cobs in different conditions are shown in Figure 4.9. Figure 4.9 A shows the SEM picture of the untreated corn and it was clearly seen that, no pore was observed on compact surface structure and intact morphology. After the pretreatment with 0.75% (w/v) KOH at 60 °C for 25 min, the structure was damaged, broken down and some crack also appeared on the particle surface, looked soft, and the

pores were appeared (Figure 4.9B). When the pretreatment temperature was increased to 120 °C, the structure was damaged and become more porous (Figure 4.9C). An increase of porosity in pretreatment processes can significantly improve the hydrolysis (Alvire *et al.*, 2010). It was found that when the sample was treated with the KOH, the surface of corn cobs had many micropores which were favorable for cellulase enzyme to hydrolyze the cellulose into reducing sugar.

From SEM results, corn cobs were highly disrupted with increased surface area and porosity when corn cobs was treated with microwave and KOH that was consistent with BET analysis, as shown in Table 4.4. At optimal pretreatment condition in both surface area and total pore volume of corn cobs were increased from 3.926 m²/g to 5.719 m²/g and 0.0078 cm³/g to 0.0096 cm³/g, respectively. An average pore diameter was also increased from 7.930 nm to 8.158 nm.

Table 4.4 BET surface area, total pore volume, and average pore diameter of samples

Sample	Surface area (m ² /g)	Total pore volume (cm ³ /g)	Average pore diameter (nm)
Untreated corn cobs	3.926	0.0078	7.930
Pretreated corn cobs with 2% (w/v) KOH at 120 °C for 25 min	5.719	0.0096	8.158

4.7 X-ray Diffraction Analysis

The degree of crystallinity in the crystal structure of cellulose has been well established as the main determining factor in the efficiency of the enzymatic hydrolysis of biomass. Several factor related to higher crystalline index of the substrate may contribute to the reaction of digestibility at the lower enzymatic

loading; the decrease in the reactivity of the substrates during hydrolysis; difference kinds of inactivation; and non-specific adsorption enzyme into lignin (Xiao *et al.*, 2011). Figure 4.9 shows the XRD patterns of untreated and pretreated corn cobs at different conditions. In the past studies (Cao and Tan, 2005 and Xiao *et al.*, 2011), it was reported that the crystalline index of cellulose samples were calculated from the X-ray diffraction patterns of untreated and pretreated corn cobs by the following equation:

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100\%$$

Where I_{002} is the intensity for the crystalline portion of biomass (i.e., cellulose) at about $2\theta = 22.5^\circ$ and $I_{amorphous}$ is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at about $2\theta = 18.7^\circ$.

The major effect on the KOH pretreatment was the removal of hemicellulose and lignin which were amorphous structure. The crystallinity index was influenced by composition of biomass (Kim *et al.*, 2005). The cellulose crystallinity value of untreated sample was 24.5 % while the pretreated sample was increased to 57.28 %. As illustrated in Figure 4.10 (A), (B) and (C), the peaks did not show much difference in each condition compared with untreated corn cobs. The crystallinity index of the pretreated corn cobs with water for 25 min at 120°C was higher than that of untreated sample; therefore, temperature and pretreated time have a significant effect on the degree of crystallinity. The trend of crystallinity index number increased with increasing KOH concentration, as shown in Table 4.5. When concentration increased from 0.75% to 2% (Figure 4.10 (C) and (F)), the crystalline peak at $2\theta = 22.5^\circ$ of sample became sharper than untreated corn cobs and the shoulder of the peak around $2\theta = 20^\circ$, which was the another crystalline peak of cellulose, had a tendency to increase in intensity by pretreatment. In addition, the peaks became sharper as the reaction temperature and pretreated time increase, as shown in Figure 4.10 (D), (E) and (F). Figure 4.10 (F) which have the highest cellulosic crystallinity structure at optimal condition.

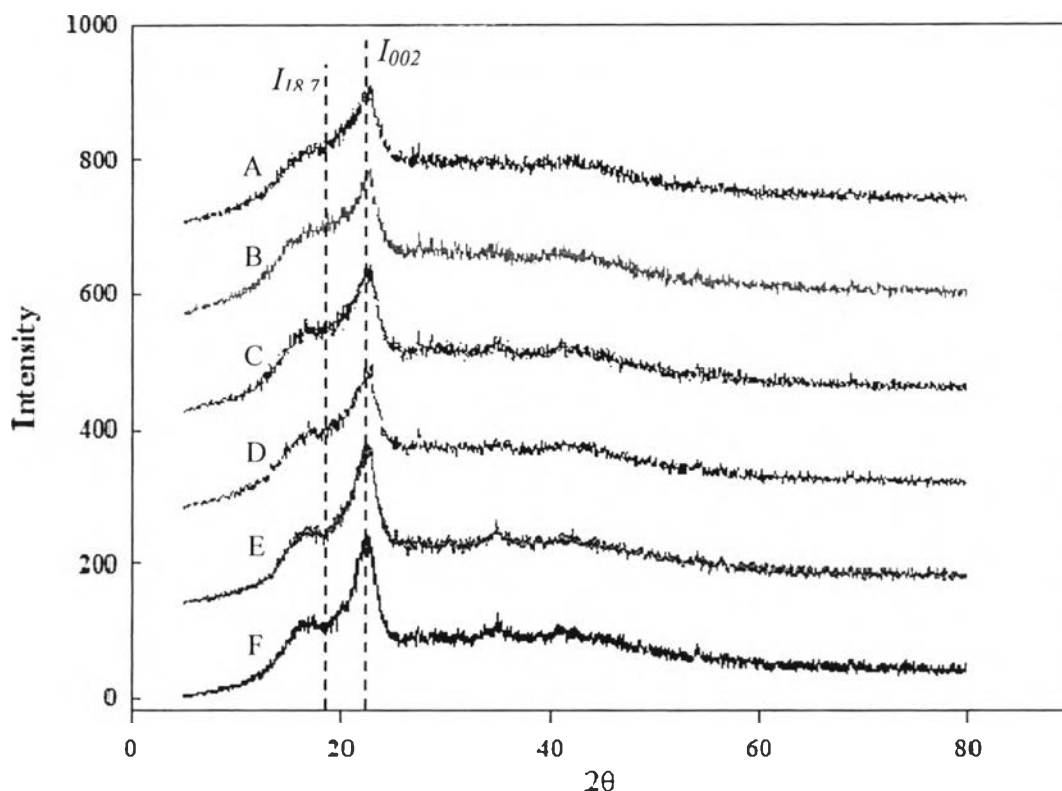


Figure 4.10 XRD patterns of untreated and pretreated corn cobs. (A) Untreated corn cobs; (B) Pretreated corn cobs with water for 25 min at 120 °C; (C) Pretreated corn cobs with KOH of 0.75% (w/v) for 25 min at 120 °C; (D) Pretreated corn cobs with KOH of 2% (w/v) for 25 min at 60 °C; (E) Pretreated corn cobs with KOH of 2% (w/v) for 10 min at 120 °C; (F) Pretreated corn cobs with KOH of 2% (w/v) for 25 min at 120 °C.

The crystallinity index of the pretreated corn cobs with KOH and microwave increased due to lignin and hemicellulose removal, which was a good evidence that the amorphous portion of the corn cobs were more removed than the crystalline portion. It had been suggested that hemicellulose was eliminated from the surface by pretreatment and cellulose undergoes changed in crystallinity by chemical and physical treatment, particularly heating (Xiao *et al.*, 2011). In addition, Yu and Wu (2011) demonstrated that in the course of conversion processes the amorphous portion and segments with short chains that were more reactive may be reacted first,

leaving a residual that had large sizes and was more inert. Xiao and co-worker (2011) investigated that the solubilization of hemicellulose and lignin together with less ordered cellulose was probably the main reason for the increase in cellulose crystallinity. Moreover, higher KOH concentration, pretreatment temperature, and time led to higher lignin removal, resulting in more crystallinity index which raised the enzymatic accessibility.

Table 4.5 Crystallinity index (%) of untreated and treated corn cobs

Sample	Crystallinity index
Untreated corn cobs	24.50
Pretreated corn cobs with water for 25 min at 120 °C	39.78
Pretreated corn cobs with 0.75% (w/v) KOH for 25 min at 120 °C	40.07
Pretreated corn cobs with 2% (w/v) KOH for 25 min at 60 °C	45.39
Pretreated corn cobs with 2% (w/v) KOH for 10 min at 120 °C	56.06
Pretreated corn cobs with 2% (w/v) KOH for 25 min at 120 °C	57.28

4.8 Comparison of Total Sugar Concentration Obtained from Different Pretreatment Methods

In this part, the pretreatment process by microwave and KOH solution was compared with other pretreatment methods. From Table 4.6 showed that the total sugar obtained from enzymatic hydrolysis of pretreated corn cobs with 2% (w/v) KOH with microwave 120 °C for 25 min was 34.79 g/L, which was higher than total sugars of pretreated corn cobs with 2% KOH with autoclave 121 °C for 60 min and 2% (w/v) KOH with conventional heating 120°C for 25 min. Because microwave radiation could heat uniformly inside the sample, it broke down the lignin and hemicellulose structure. The microwave/chemical pretreatment resulted in a more

effective pretreatment than the conventional heating chemical pretreatment by accelerating reactions during the pretreatment process (Zhu *et al.*, 2005, Zhu *et al.*, 2006). Microwave radiation supplied internal heat passing through the biomass. The molecules in the material acted like small magnets attempting to arrange themselves with the electrical field. Under the influence of this high frequency alternating electrical field, the particles oscillated about their axes creating intermolecular friction which demonstrated itself as heat accelerating chemical processes. Microwave also saved the pretreatment time and increased both its accessibility to hydrolytic enzymes and product yields.

Table 4.6 Comparison of total sugar concentration obtained from different pretreatment methods

Method	Glucose (g/L)	Xylose (g/L)	Total Sugar (g/L)
2% (w/v) KOH with microwave 120 °C for 25 min	19.49	8.71	34.79
2% (w/v) KOH with autoclave 121 °C for 60 min	15.88	7.29	27.64
2% (w/v) KOH with conventional heating 120 °C for 25 min	15.59	7.67	27.24

In conventional heating, the heat source caused the molecules to react from the surface toward the center so that successive layers of molecules heated in turn. The product surfaces might be in danger of overheating by the time heat penetrated the material. However, microwaves produced a volume heating effect that make all molecules being set in action at the same time. It also evens temperature gradients and offered other important benefits. Microwave pretreatment had better efficiency (more than 20% improvement in sugar yield) on biomass than autoclave and conventional heating. Clark and co-works (2004) reported that the total cost of microwave processing equipment usually was greater than conventional equipment

designed to perform the same tasks. Certainly, as microwave energy became more widely used for manufacturing, the costs would come down. In addition, the hydrolyzate from microwave/KOH process had higher glucose content but low xylose content, which was more suitable for subsequent fermentation process (Zhu *et al.*, 2005).

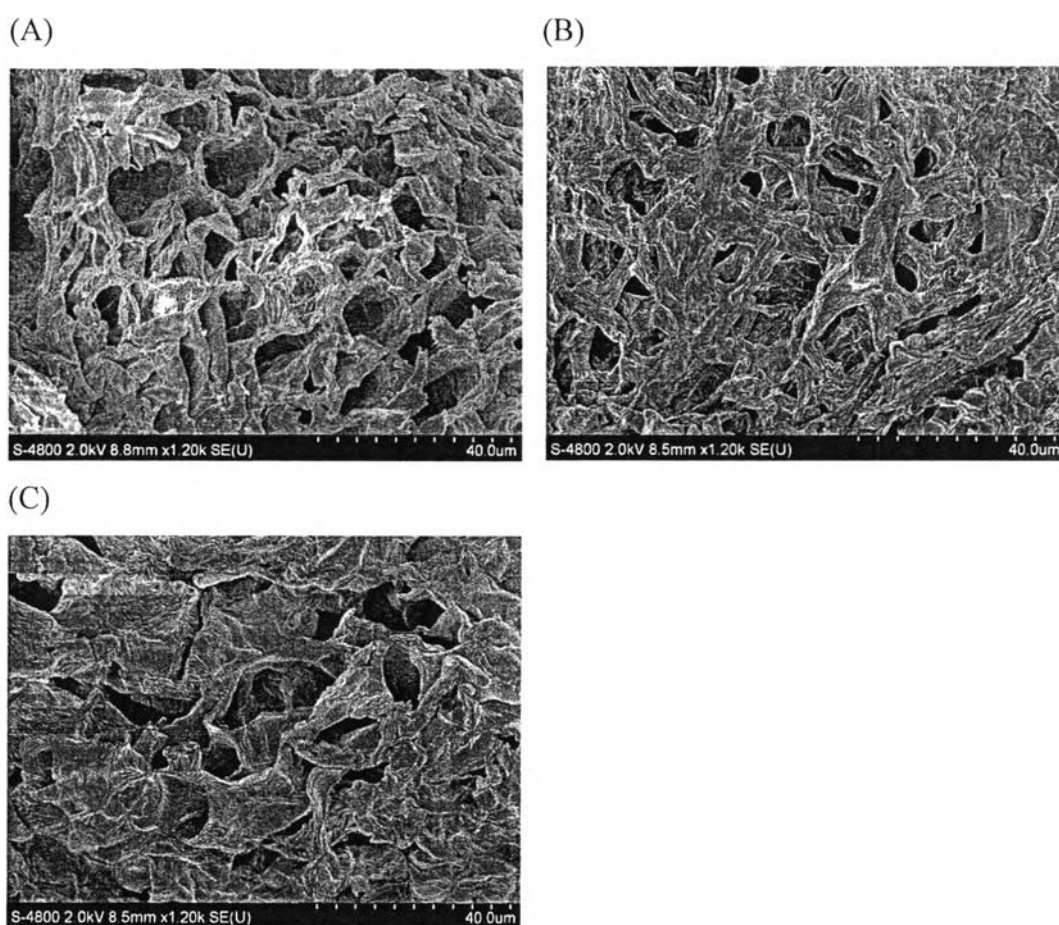


Figure 4.11 Scanning electron microscope images of corn cobs. (A) 2% (w/v) KOH with microwave 120 °C for 25 min; (B) 2% (w/v) KOH with autoclave 121 °C for 60 min and (C) 2% (w/v) KOH with conventional heating 120 °C for 25 min.

Figure 4.11 shows scanning electron microscope images of corn cobs. The untreated corn cobs were damaged to a sufficient extent, which surface of the pretreated corn cobs by autoclave and conventional heating became roughed and loosed (Figure 4.11 (B) and (C)). The microwave pretreatment had successfully

altered the initially organized morphology of corn cobs into more porous and uniform structure compare with other method (Figure 4.11 (A)). Furthermore, microwave pretreatment had lost the most morphological detail compared with autoclave pretreatment and conventional heating making them less available for enzyme access.

4.9 Ethanol Production

The production of ethanol from lignocellulosic material consists of mainly five different steps; pretreatment, (enzymatic) hydrolysis, fermentation, product separation, and post-treatment of the liquid fraction (Hendriks and Zeeman, 2009). The pretreatment was necessary to improve the rate of production and the total yield of monomeric sugars in the hydrolysis step. The conversion of cellulose and hemicellulose to monomeric sugars can be done enzymatically by addition of cellulases (enzymes responsible for the hydrolysis of cellulose). The produced monomeric hexoses (six carbon sugars) can be fermented to ethanol quite easily, while the fermentation of pentoses (five carbon sugars) is only done by a few strains. After the fermentation the ethanol had to be recovered from the fermentation broth by distillation.

A sugar solution from enzymatic hydrolysis step was adjusted with sodium hydroxide to neutral pH before using as a substrate in fermentation step. Then, the 5 ml of sugar solution was mixed 0.5 mL of active yeast (*Saccharomyces cerevisiae*) and transferred to water bath at 37 °C for 1 day to 3 days. After that, the solution was then centrifuged for 5 min at 8000 rpm and collected to analyze the ethanol concentration by a GC instrument.

The previous work found that at 24 h the highest ethanol production was obtained (Ploypradith, 2010). The hydrolyzate of corn cobs pretreated under optimal conditions, which contained total sugar 34.79 g/L, can produce the maximum ethanol of 10.19 g/L, as shown in Figure 4.12. In addition, the fermentation time was further increased to 48 h; the ethanol yield was decreased to 9.17 g/L. The longer fermentation time leads to less concentration of ethanol, which caused by some inhibitors during fermentation. Another problem occurred during the fermentation

was that the formed product ethanol is an inhibitor for the yeasts that perform the fermentation (Hendriks and Zeeman, 2009).

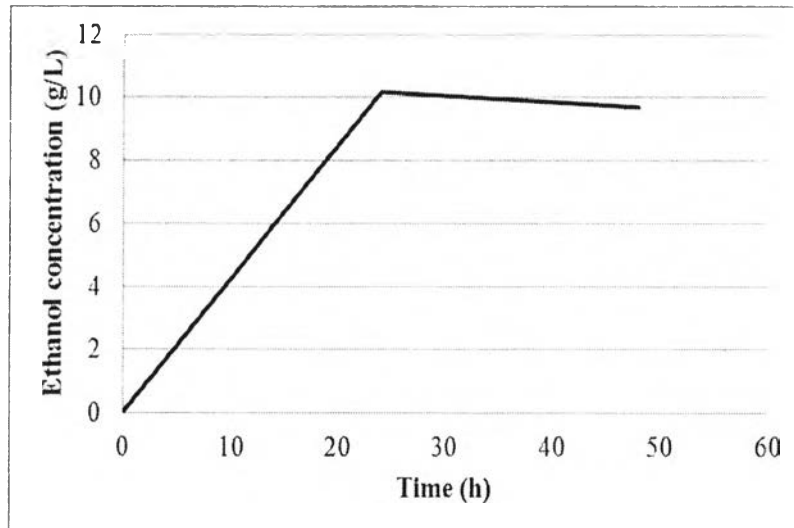


Figure 4.12 Ethanol production from hydrolyzate of corn cobs pretreated under optimal conditions.