CHAPTER 5

Delignification of Corn Stover Using Alkaline and Ozone

This chapter is regarding the method and the result of lignin elimination for corn stover. The elimination methods are alkaline solution and ozone pretreatment in the same methods as Bana grass. The description is as follows;

5.1 Materials and methods

5.1.1 Materials

Corn stover used in the studied was obtained from field around Fang District, Chiang Mai, Thailand. Land altitude is 500 meters above sea level. Following TAPPI T 203 om-88, T 223 om-88 and T 222 om-88, the dry grass contains 44.5% cellulose, 32.4% hemicellulose and 16.9% lignin.

5.1.2 Sample preparation

Corn stover with the length of 1-1.5 m and age of 90-100 days was harvested from the land. It then was sliced into pieces smaller than 1 mm. Next, pieces of corn stover were washed using distilled water and immediately dried where air could flow in all directions. After that, the material was grinded to a particle size smaller than 1 mm and dried again in an oven at 103°C for hours to achieve moisture content of less than 10 wt.%. To keep the material for delignification, it was vacuumed and stored in a sealed plastic bag in desiccator.

5.2 Condition for alkaline pretreatment

5.2.1 NaOH Pretreatment

Prepare the NaOH solutions with concentrations of 0.5, 5.5 and 10.5 wt.% were used. 20.0 g of material was immersed in the solutions and incubated at 40, 65 and 90 ºC for 1, 2 and 3 hrs. Then, it was washed with distilled water until pH value was neutral and dried at 103 – 105 ºC for 10 hrs. The material was characterized to determine its composition. All contents were expressed on a dry basis throughout this work.

5.2.2 NH_3 Pretreatment

Prepare the NH_3 solutions with concentrations of 5, 10 and 20 wt.% were used. 20.0 g of material was immersed in the solutions and incubated at 40, 65 and 90 ºC for 12, 24 and 36 hrs. Then, it was washed with distilled water until pH value was neutral and dried at 103 – 105 ºC for 10 hours. The material was characterized to determine its composition.

5.2.3 $Ca(OH)_2$ Pretreatment

Prepare the $Ca(OH)_2$ solutions 20,60 and 100 wt.% were used. 20.0 g of material was immersed in the solutions and incubated at 40, 65 and 90 ºC for 12, 24 and 36 hrs. Then, it was washed with distilled water until pH value was neutral and dried at 103 – 105 ºC for 10 hrs. The material was characterized to determine its composition.

5.3 Condition for ozone pretreatment

Before delignification with ozone, a selected material was dried at 103 – 105 ºC for 4 hrs. 20.0 g of material was mixed with 160 g of distilled water in a reactor. 5 liters per minute of ozone was flowed into the reactor for 10, 20 and 30 mins. The ozone was generated from ECONOWATT model OZG gas 1000 mg. Then, the materials were washed with distilled water and dried at 103 – 105 ºC for 10 hrs. The material was characterized to determine its composition.

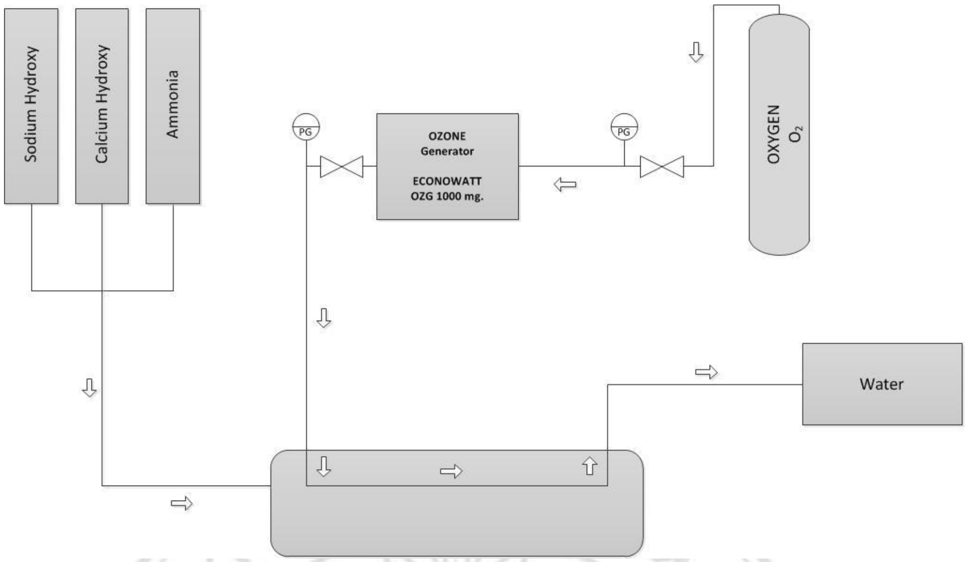

Figure 5.1 The ozonolysis treatment apparatus

Figure 5.2 ECONOWATT model OZG gas 1000 mg.

5.4 Results and Discussion

5.4.1 NaOH and Ozone Pretreatment

5.4.1.1 Effect of NaOH pretreatment

As described that lignin was removed from the sample by using NaOH solutions which concentrations and time were varied. Table 5.1 shows list of composition of each delignification condition. Since contents of lignin remover and remaining cellulose are interested in this study, the compositions of matter were divided into cellulose, lignin, hemicellulose, and others. It was found that the untreated material contains 44.6% of cellulose, 16.9% of lignin, 32.4% of hemicellulose and 6.2% of others. For pretreated material, it is found that percentage of cellulose is higher and percentage of lignin is lower than the untreated one as expected. At the highest concentration of 10.5 wt.% NaOH and the highest time of 3 hrs, it is expected that lignin must be at the lowest since high alkali concentration and longtime of reaction will destroy cell wall of the Corn stover. At this condition, percentage of lignin at 8.9% is not the lowest, but percentage of cellulose at 66.7% is the highest. The lowest percentage of lignin at 7.6% is found at 5.5 wt.% NaOH and 2 hrs treatment. This is because all materials can become solvent with NaOH solution, some of them are remaining. Also, characterization of the sample was measured and shown in Table 5.1 These are percentages of solid remain, cellulose recovery and percentage of substance remover, that are lignin, hemicellulose and others.

Solid remain is also the key point for selecting a pretreatment method for delignification. This is because NaOH solution can act as a solvent to any composition of the material. Lignin is not only removed but cellulose is also eliminated. Even though high percentage of cellulose is shown after lignification, its quantity may remain so little. As can be seen in Table 5.1, for example, 66.7% of cellulose is found to be very high for 10.5 wt.% NaOH and 3 hrs time, but only 38.4% of solid is remained and 57.5% of cellulose is recovered. This means 25.6% of an original cellulose is recovered and 74.4% of an original one is soluble in NaOH solution.

Figure 5.3-5.5 presents the composition of the sample after pretreating with alkali with concentrations of 0.5, 3.0, 5.5, 8.0 and 10.5 wt.% for 1, 2 and 3 hr. pretreatment. This figure differs from Table 5.1 due to showing the percentage which is based on the untreated material. This is because it is expected to present the percentage of remaining substance for 1 hr pretreatment, for example, the percentage of cellulose is reduced from 44.6% to 43.8%, 40.9%, and 26.3% while the percentage of lignin decreased from 16.9% to 15.9, 5.3 and 3.6% for 0.5, 5.5 and 10.5 wt.% NaOH, respectively. The patterns of reduction also occur for 2 hrs and 3 hrs of the pretreatment. The dramatic decrease of each substance and total substances, is found with increasing the concentration of NaOH solution. This is because NaOH solution destroys the cell-wall of the glass so not only lignin is eliminated but cellulose is also removed from the matter.

Considering the effect of pretreatment time of NaOH solution as shown in Figure 5.6, only NaOH solution concentration of 5.5% is presented in the figure. For example, it can be seen that each substance is dramatically decreased for 1 hr pretreatment but it is slightly reduced for 2 hrs and 3 hrs of the pretreatment. This is due to when the substance is solvent in the solution, it also reduces the concentration of the alkali.

Looking at the effect of NaOH solution pretreatment by varying either concentration or time as shown in Figure 5.3-5.5 and 5.6, it is necessary to find the optimum pretreatment condition for using with the alkaline. high removal of lignin is expected, but high content of cellulose remains in the intermediate for ethanol production.

In order to select the optimum condition for NaOH solution pretreatment of Corn stover, high level of lignin removal and cellulose recovery are considered. Figure 5.7 shows weight percent of remaining cellulose and lignin removal based on an untreated material. Increasing of pretreatment time makes a slight decrease in both lignin and cellulose. At 5.5 wt.% NaOH, for example, cellulose of 91.7, 91.5 and 90.4 is recovery and lignin of 68.7, 70.0 and 69.7 is removed. Even though increasing pretreatment time cannot affect the lignin removal and cellulose recovery, increasing alkali concentration affects both of them. Cellulose and lignin are dramatically reduced as described before. Again, high removal of lignin is expected, but high content of cellulose remains in the intermediate for ethanol production. Considering Figure 5.7, it is found that lignin remover decreased dramatically from about 5.6-6.3% for 0.5 wt.% NaOH to 68.7–70.0% for 5.5 wt.% NaOH, while remaining cellulose decreased slightly from 98.3 for 0.5 wt.% NaOH to 98.3% for 5.5 wt.% NaOH. Pretreatment with 5.5 wt.% NaOH should be the optimum concentration for Bana grass.

The other criteria for considering the optimum condition for alkali pretreatment is the ratio of cellulose and lignin since it presents high content of cellulose and low content of lignin. This also means that high percentage of lignin is removed, but cellulose is still the same or slightly less as before pretreatment or remaining maximum cellulose. Figure 5.8 presents the ratio of cellulose and lignin for NaOH solution pretreatment using in this study. Pretreatment with 0.5 wt.% NaOH shows the lowest ratio of around 2.7, while pretreatment with 10.5 wt.% NaOH presents the medium ratio of around 7.3. The maximum ratio of cellulose is about 8.1 for 5.5 wt.% pretreatment. It is found that high content of lignin still be in the material if low concentration of 0.5 is used. Using high concentration of 10.5, not only lignin is highly removed, but cellulose is also eliminated. Using pretreatment with 5.5 wt.% shows fair amount of lignin removal around 69% and about 91% of cellulose is remaining. It is concluded that the optimum condition for alkaline pretreatment of Corn stover is 2 hours and 5.5 wt.% NaOH. The ratio of cellulose and lignin is 8.07 that is approximately 3 folds of untreated material.

Table 5.1 Characterization of untreated and pretreated materials with NaOH solutions pretreatment

NaOH solution (%)	Time (min)	Composition (wt.%, based on dry matter after pretreatment)				Solid remaining (%)	Cellulose recovery (%)	Remover (%)		
		Cellulose	Hemi-cellulose	Lignin	other			Hemi-cellulose	Lignin	Other
0.0	0	44.6	32.4	16.9	6.2	-	-	-	-	-
0.5	60	45.1	32.2	16.4	6.3	97.1	98.3	3.4	5.6	1.4
	120	45.1	32.2	16.3	6.3	97.1	98.3	3.4	5.9	1.2
	180	45.3	32.2	16.3	6.2	96.8	98.3	3.7	6.2	3.2
5.5	60	60.9	23.7	7.8	7.6	67.1	91.7	50.9	68.7	17.5
	120	61.1	23.6	7.6	7.7	66.7	91.5	51.3	70.0	17.1
	180	61.0	23.6	7.7	7.7	66.0	90.4	52.0	69.7	17.8
10.5	60	67.5	17.9	9.3	5.3	38.9	58.9	78.5	78.6	66.3
	120	66.1	18.0	8.9	6.9	38.7	57.5	78.5	79.4	56.6
	180	66.7	17.8	8.9	6.7	38.4	57.5	79.0	79.7	58.5

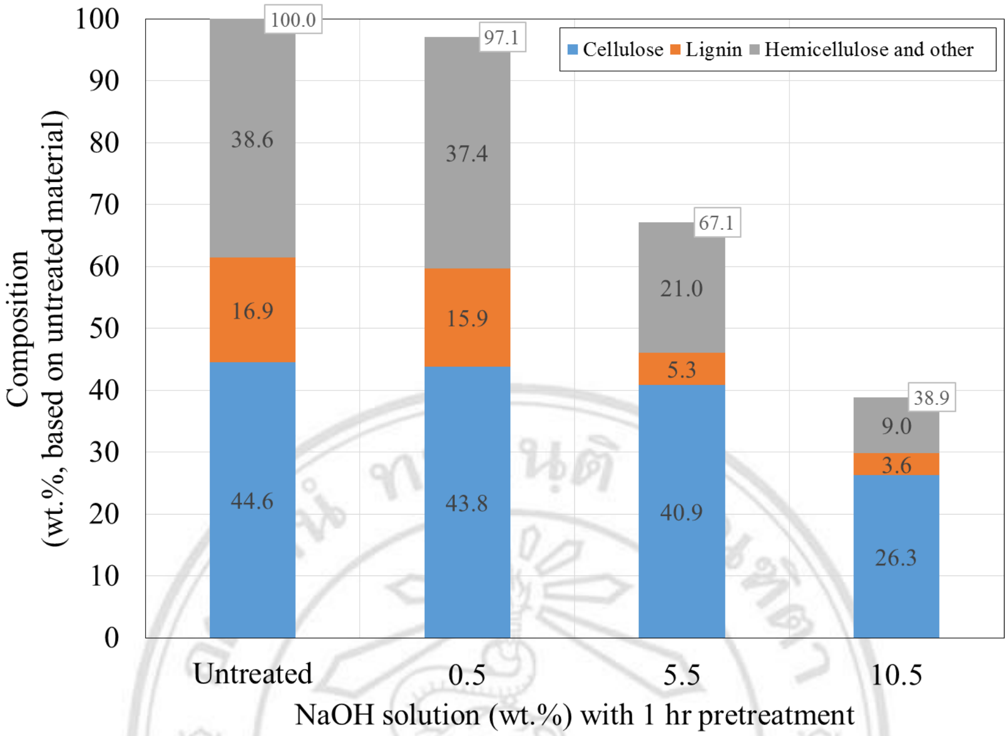

Figure 5.3 Remaining composition in NaOH solution with 1hr preatment.

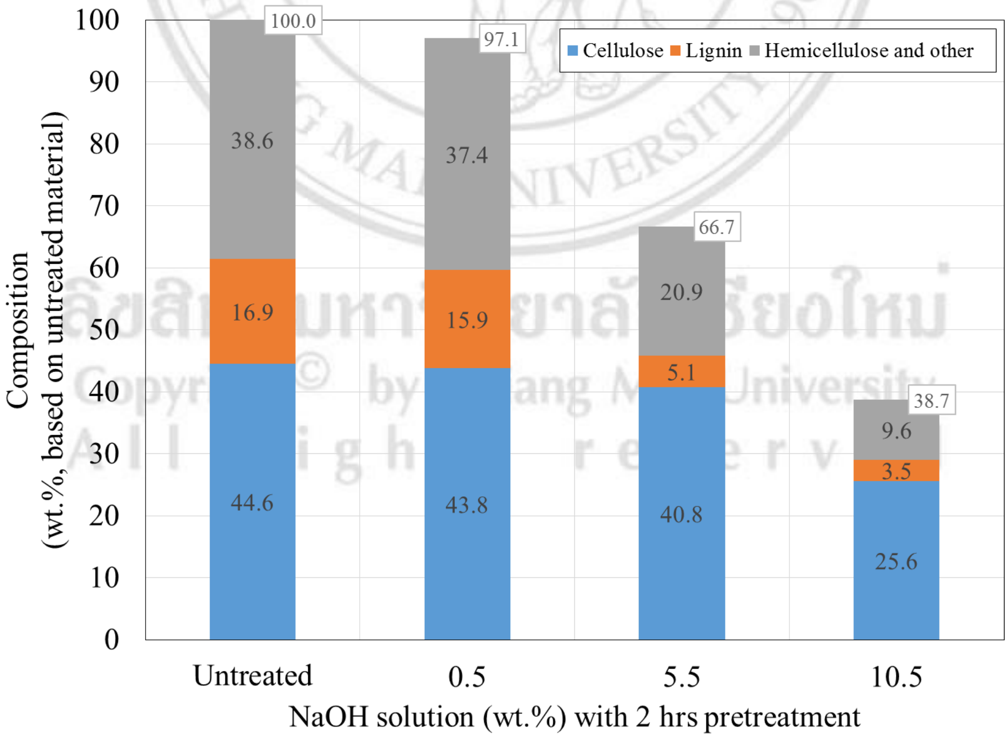

Figure 5.4 Remaining composition in NaOH solution with 2 hrs preatment.

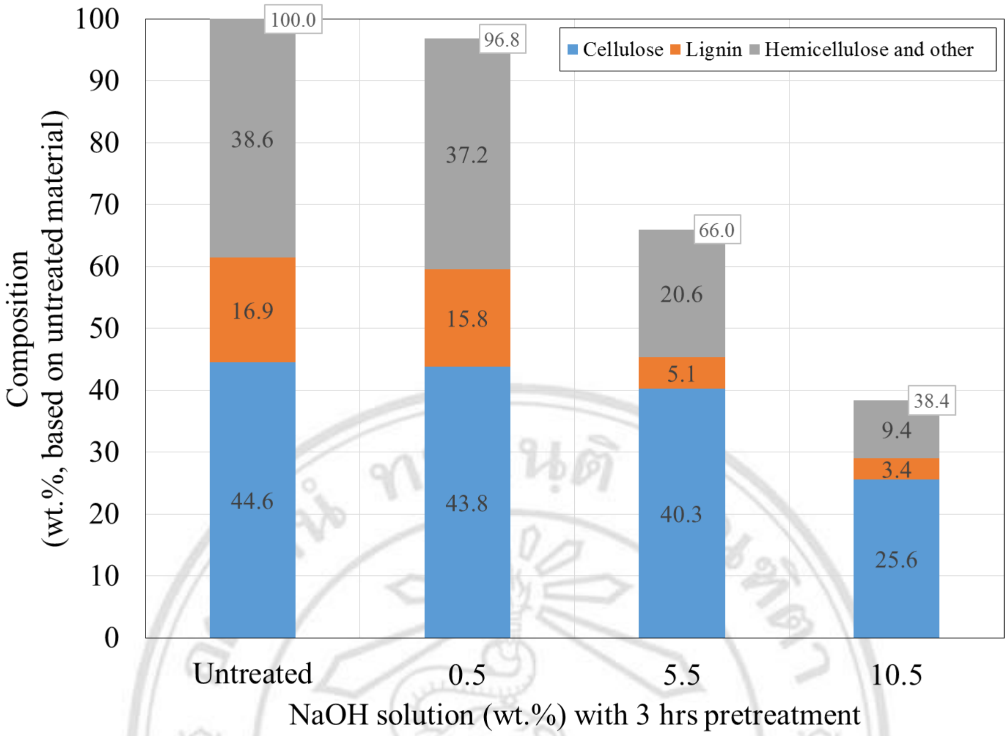

Figure 5.5 Remaining composition in NaOH solution with 3 hours preatment.

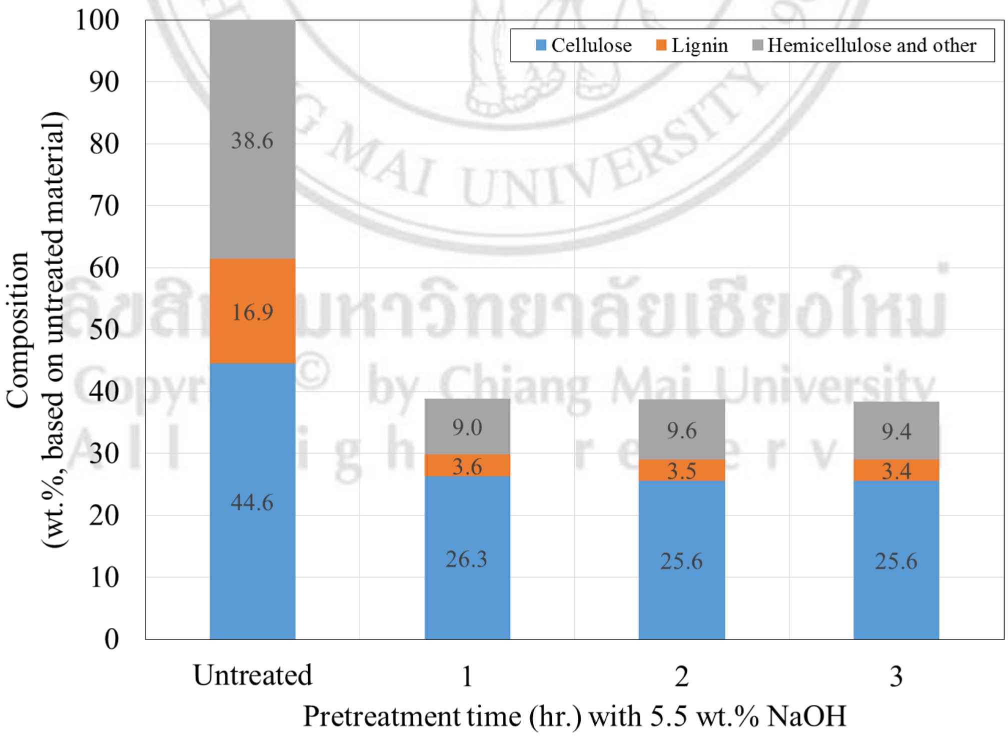

Figure 5.6 Effect of pretreatment time of NaOH solution.

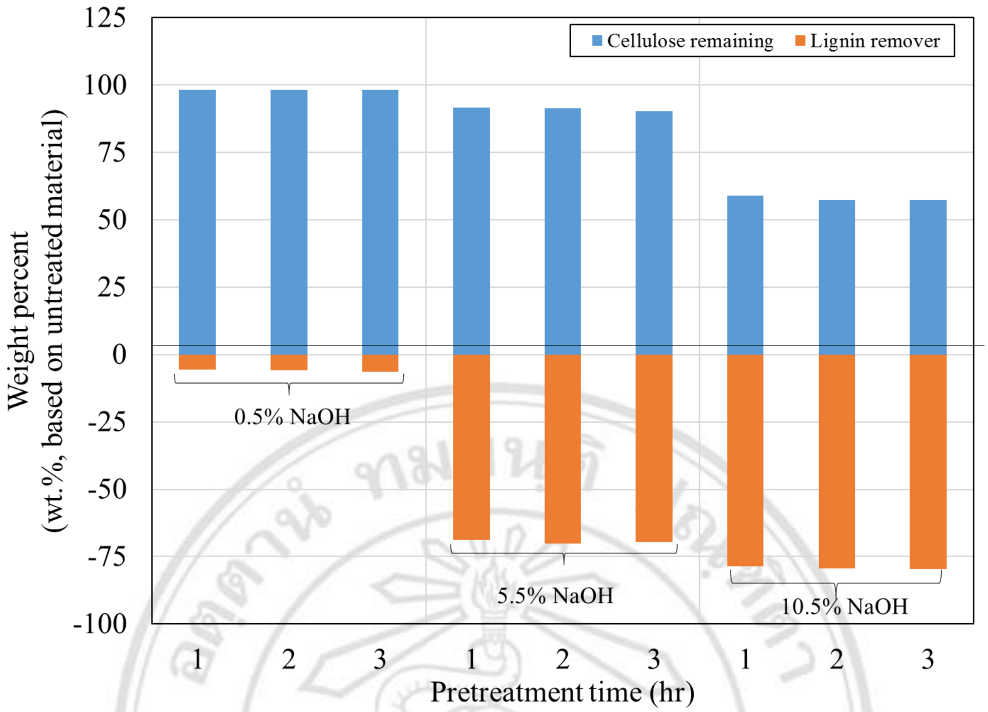

Figure 5.7 shows weight percent of remaining cellulose and lignin removal based on an untreated material.

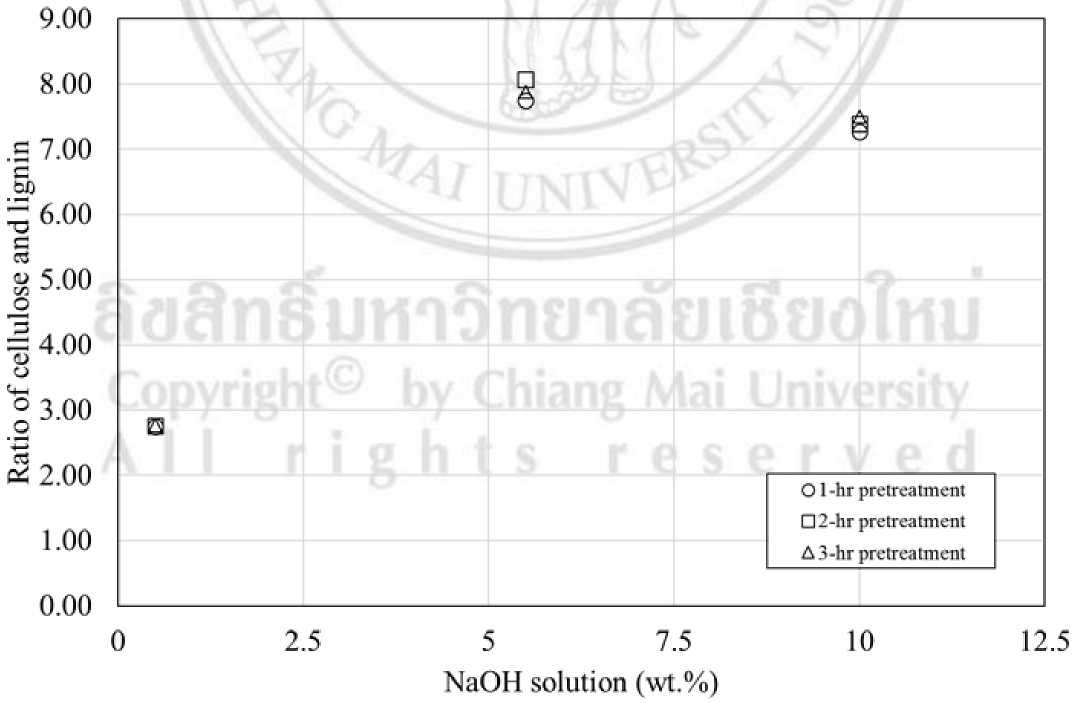

Figure 5.8 presents the ratio of cellulose and lignin for NaOH solution pretreatment.

5.4.1.2 Effect of Ozone pretreatment

Table 5.2 lists the composition of untreated material and ozone pretreatment of 10, 20 and 30 mins. Cellulose is increases from 44.5% to 44.6 and lignin decreases from 16.9% to 16.8% for 30 mins pretreatment. Thus, pretreatment with ozone cannot eliminate both cellulose and lignin.

Table 5.2 Major compositions of untreated and pretreated materials with ozonolysis process

Time of ozone flow (min)	Composition (wt.%, based on dry matter)			
	Cellulose	Hemicellulose	Lignin	Other
0	44.5	32.4	16.8	6.2
10	44.5	32.4	16.9	6.2
20	44.6	32.4	16.8	6.2
30	44.6	32.4	16.8	6.2

5.4.1.3 Improvement of lignin removal with NaOH solution and ozone pretreatments.

Ozone pretreatment is further used with the material with NaOH solution pretreatment. This is because it is expected that NaOH destroys the cell wall of Corn stover, ozone can pass through the cell wall and remove lignin from the NaOH pretreated one. Then, the material with the optimum NaOH solution pretreatment with 2 hrs and 5.5 wt.% NaOH is selected. At this condition the ratio of cellulose to lignin is 8.1 as described above.

Table 5.3 presents the compositions of the NaOH solution pretreatment material after ozone pretreatment. As shown in the table, content of cellulose increases and that of lignin decreases. Cellulose rises from 61.1% to 83.4% and lignin decreases from 7.6% to 1.0% for 30 mins ozone. However, the percentage of lignin and cellulose which based on dry matter does not present the optimum one, since the solidity of matter is also eliminated. For 30 mins ozone, solidity is reduced from 66.7% to 45.1%

Table 5.3 Characterization of NaOH solution pretreated materials with ozonolysis process.

Time of ozone flow (min)	Composition ((wt.%, based on dry matter)				Solid remaining (%)	Celllulose recovery (%)	Remover (%)		
	Cellulose	Hemicellulose	Lignin	Other			Hemicellulose	Lignin	Other
0	61.1	23.6	7.6	7.7	66.7	91.5	51.3	70.0	17.1
10	66.2	22.0	6.2	5.6	61.0	90.5	58.6	77.4	44.4
20	76.2	18.6	1.8	3.4	51.9	88.7	70.2	94.4	71.7
30	83.4	13.6	1.0	2.0	45.1	84.4	81.0	97.4	85.3

Fig 5.9 shows the composition of NaOH solution pretreatment matter with ozone. It can be seen that all compositions decrease slightly. The total composition is reduced from 66.7% of an ozone-untreated matter to 61.0, 51.9 and 45.1% for 10, 20 and 30 mins.

Fig 5.10 compares the ratio of cellulose to lignin of material between ozone pretreatment of untreated and NaOH solution pretreatment. It is found that without alkaline, the ratio remains the same, but with alkaline first and then ozone, the ratio rises sharply from 8.1 to 10.6, 41.9 for 10 and 20 mins ozone and at the highest at 85.9 for 30 mins ozone. This is because ozone pretreatment should be the method for further pretreatment with other one that destroy cell wall so that ozone can pass though the cell wall easier.

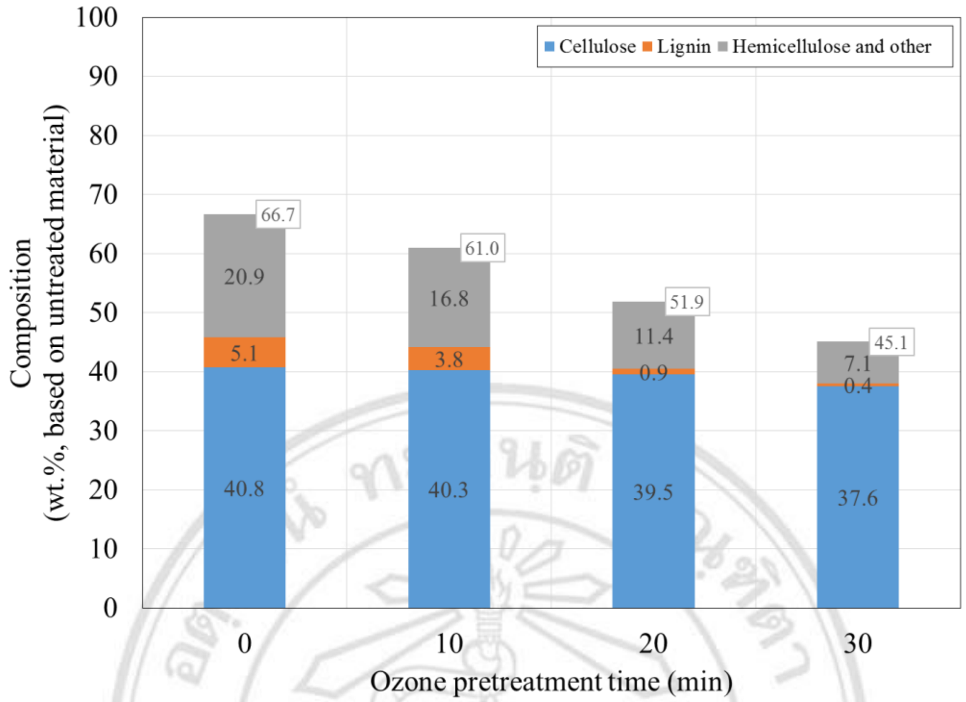

Fig 5.9 shows the composition of NaOH solution pretreatment matter with ozone.

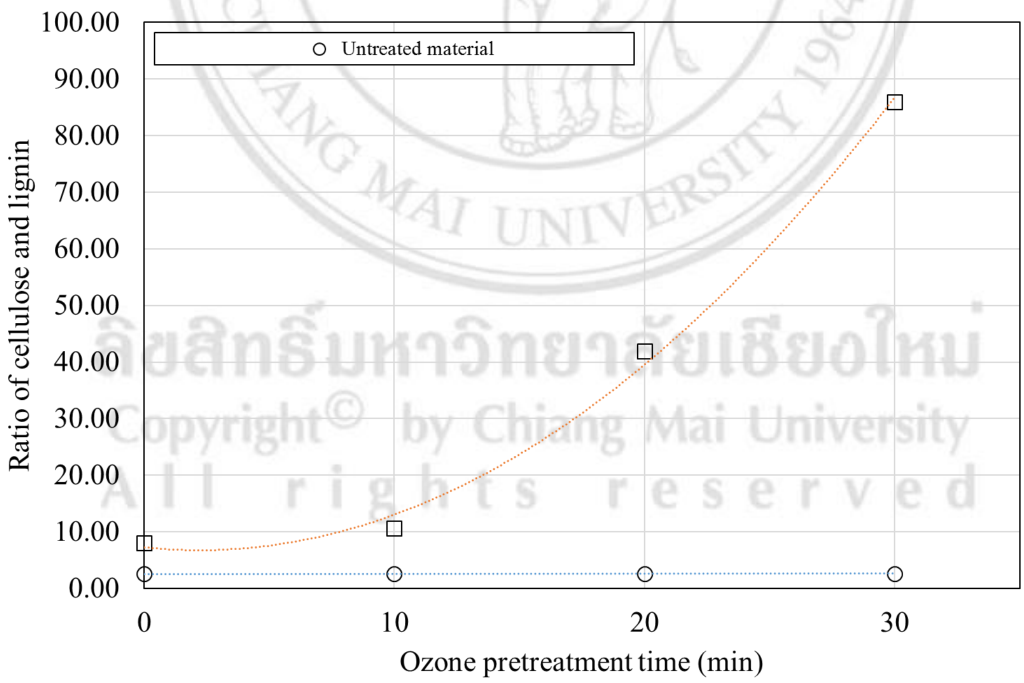

Fig 5.10 compares the ratio of cellulose and lignin of material with ozone pretreatment of untreated and NaOH solution pretreatment.

5.4.2 NH_3 solution and Ozone Pretreatment

5.4.2.1 Effect of NH_3 solution pretreatment

As described that lignin was removed from the sample by using NH_3 solutions which concentrations and time were varied. Table 5.4 shows list of composition of each delignification conditions. Since contents of lignin remover and remaining cellulose are interested in this study, the compositions of matter were divided into cellulose, lignin, hemicellulose, and others. It was found that the untreated material contains 44.6% of cellulose, 16.9% of lignin, 23.4% of hemicellulose and 6.2% of others. For pretreated material, it is found that percentage of cellulose is higher and percentage of lignin is lower than the untreated one as expected. At the highest concentration of 20 wt.% NH_3 solution and the highest time of 24 hrs, it is expected that lignin must be at the lowest since high NH_3 solution concentration and longtime of reaction will destroy cell wall of the corn stover. At this condition, percentage of lignin at 13.1% is the lowest , and percent of cellulose at 49.6% is the highest.

Solid remain is also the keypoint for selecting a pretreatment method for delignification. This is because NH_3 solution can act solvent to any composition of the material. Lignin is not only removed but cellulose is also eliminated too. Even though high percentage of cellulose is shown after lignification, its quantity may remain so little. As can be seen in Table 5.4, for example, 49.6% of cellulose is found to be very high for 20.0 wt.% NH_3 solution and 24 hrs time, but only 80.5 % of solid is remained and 89.7% of cellulose is recovered. This means 44.5% of an original cellulose is recovered and 55.5% of an original one is soluble in NH_3 solution.

Fig 5.11-5.13 presents the composition of the sample after pretreating with NH_3 solution with concentrations of 5.0, 10.0 and 20.0 wt.% for 12, 24 and 36 hrs pretreatment. This figure differs from Table 5.4 due to showing the percentage which is based on the untreated material. This is because it is expected to present the percentage of remaining substance for 12 hrs pretreatment, for example, the percentage of cellulose is reduced from 44.6% to 43.1%, 41.0% and 40.0% while the percentage of lignin decreased from 16.9% to 14.8%, 12.5% and 10.6% for 5.0, 10.0 and 20.0 wt.% NH_3, respectively. The patterns of reduction are also occur for 24 hrs and 36 hrs of the pretreatment. The dramatic decrease of each substance and total substance, is found with increasing the concentration of alkali. This is because alkaline destroys the cell-wall of the glass so not only lignin is eliminated but cellulose is also removed from the matter.

Considering the effect of pretreatment time of alkaline as shown in Figure 5.14 only NH_3 concentration of 5.0% is presented in the figure. For example, it can be seen that each substance are dramatically decreased for 12 hrs pretreatment but it is slightly reduced for 24 hrs and 36 hrs of the pretreatment.

Looking at the effect of NH_3 solution pretreatment by varying either concentration or time as shown in Fig 5.11-5.13 and 5.14, it is necessary to find the optimum pretreatment condition for using with the alkali. High removal of lignin is expected, but high content of cellulose remains in the intermediate for ethanol production.

In order to select the optimum condition for NH_3 solution pretreatment of corn stover, high level of lignin removal and cellulose recovery are considered. Figure 5.15 shows weight percent of remaining cellulose and lignin removal based on an untreated material. Increasing of pretreatment time makes a slight decrease in both lignin and cellulose. At 5.0 wt.% NH_3, for example, cellulose of 97.7%, 96.6% and 95.6% is recovery and lignin of 10.4%, 12.4% and 14.8% is removed. Even though increasing pretreatment time cannot affect the lignin removal and cellulose recovery, increasing alkaline concentration affects both of them. Cellulose and lignin are dramatically reduced as described before. Again, high removal of lignin is expected, but high content of cellulose remains in the intermediate for ethanol production. Considering Figure 5.15, it is found that lignin decreased dramatically from about 10.4% to 14.8% for 5.0 wt% NH_3 to 36.1% to 38.3% for 20.0 wt.% NH_3, while cellulose decreased slightly from 95.6%-97.7% for 5.0 wt% NH_3 to 89.5%-89.7% for 20 wt.% NH_3. Pretreatment with 20.0 wt.% NH_3 should be the optimum concentration for corn stover.

The other criteria for considering the optimum condition for alkaline pretreatment is the ratio of cellulose and lignin since it presents high content of cellulose and low content of lignin. This also means that high percentage of lignin is removed, but cellulose is still the same or slightly less as before pretreatment or remaining maximum cellulose. Figure 5.16 presents the ratio of cellulose and lignin for NH_3 solution pretreatment using in this study.

Pretreatment with 5.0 wt.% NH_3 solution shows the lowest ratio of around 2.9-3.0., while pretreatment with 20.0 wt.% NH_3 solution presents the high ratio of around 3.7-3.8 To selected the condition for NH_3 solution pretreatment of corn stover is 20 hour and 20.0 wt.% NH_3. The ratio of cellulose and lignin is 3.8 that is approximately 1.4 folds of untreated material.

Table 5.4 Characterization of untreated and pretreated materials with NH_3 solution pretreatment

NH3 solution (%)	Time (hr)	Composition (wt.%, based on dry matter after pretreatment)				Solid remaining (%)	Cellulose recovery (%)	Remover (%)		
		Cellulose	Hemicellulose	Lignin	other			Hemicellulose	Lignin	Other
0.0	0	44.6	32.4	16.9	6.2	-	-	-	-	-
5.0	12	45.8	32.0	15.9	6.2	94.9	97.7	6.3	10.4	4.1
	24	46.0	31.9	15.8	6.3	93.6	96.6	7.9	12.4	4.7
	36	46.1	31.9	15.5	6.4	92.4	95.6	9.2	14.8	3.5
10.0	12	47.3	31.1	14.6	7.0	86.6	92.0	16.8	25.2	2.5
	24	47.6	31.0	14.5	7.0	86.2	92.0	17.6	25.8	2.8
	36	47.8	30.9	14.4	6.9	85.1	91.2	18.8	27.2	5.0
20.0	12	49.3	30.0	13.3	7.4	81.1	89.7	25.0	36.1	2.4
	24	49.6	30.0	13.1	7.2	80.5	89.7	25.5	37.3	5.5
	36	49.4	29.5	13.7	7.5	80.8	89.7	26.5	34.5	2.4

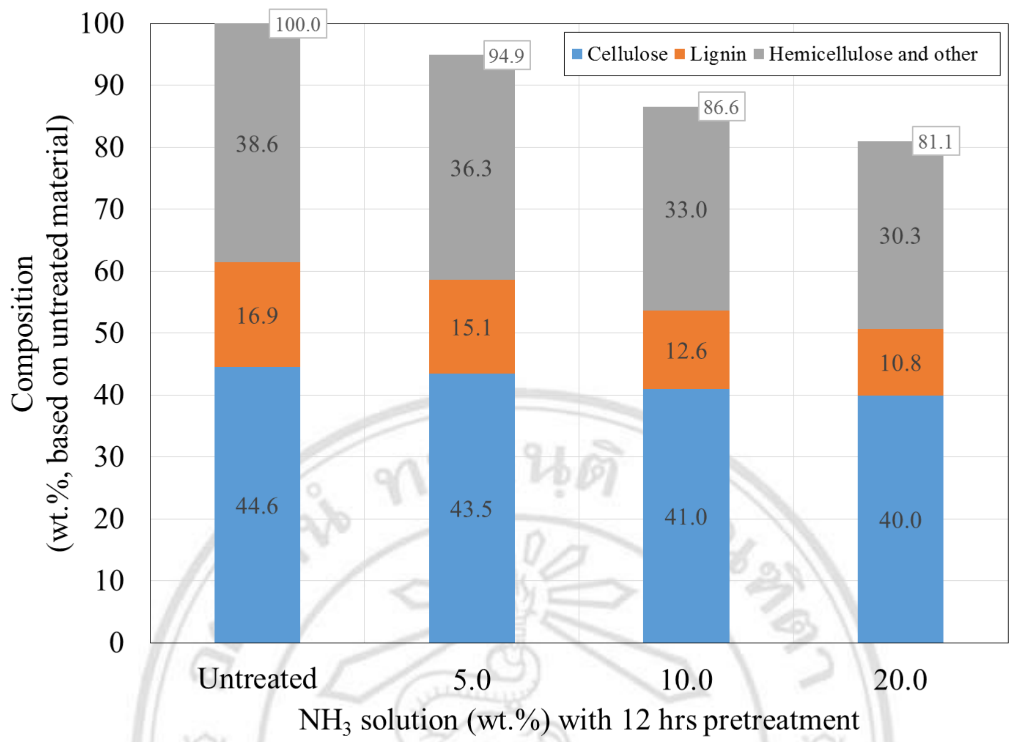

Figure 5.11 Remaining composition in NH_3 solution with 12 hrs pretreated material.

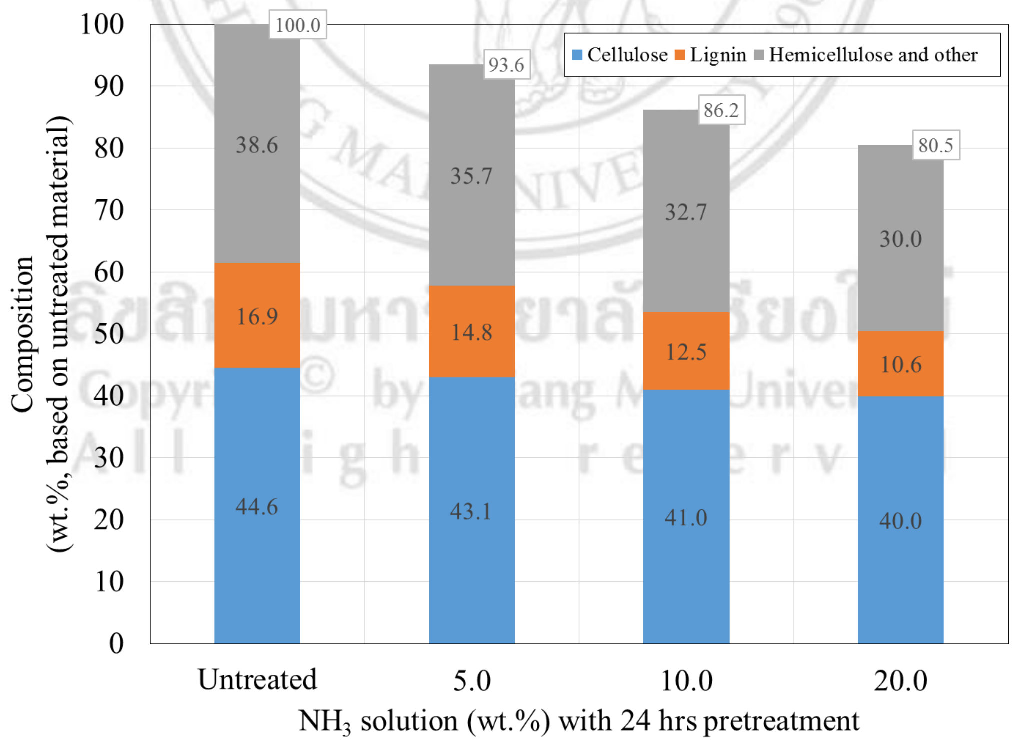

Figure 5.12 Remaining composition in NH_3 solution with 24 hrs pretreated material.

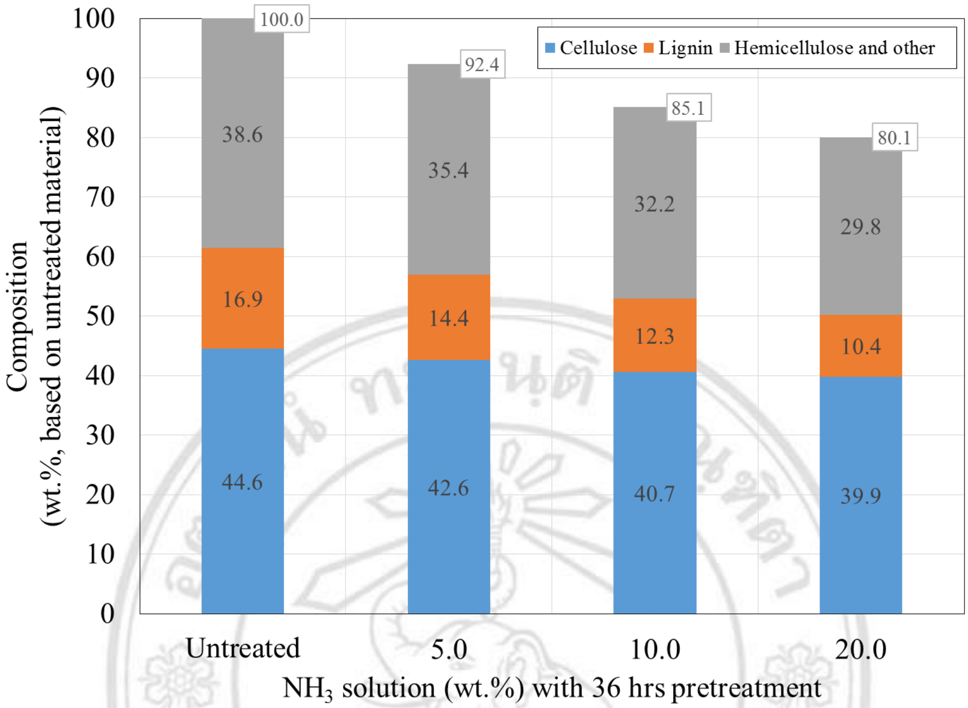

Figure 5.13 Remaining composition in NH_3 solution with 36 hrs. pretreated material.

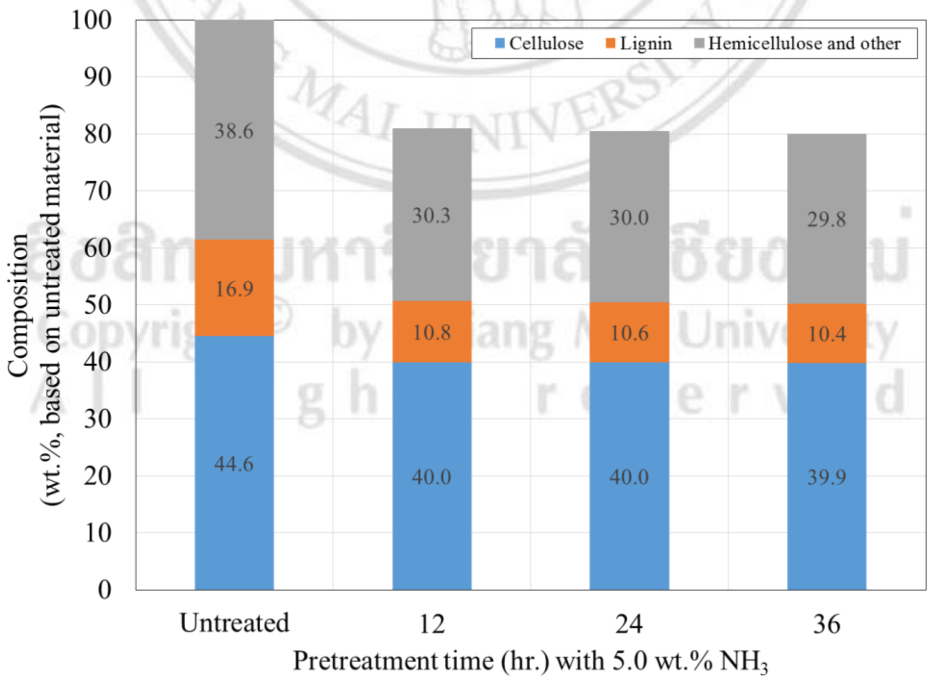

Figure 5.14 Effect of pretreatment time of NH_3 solution.

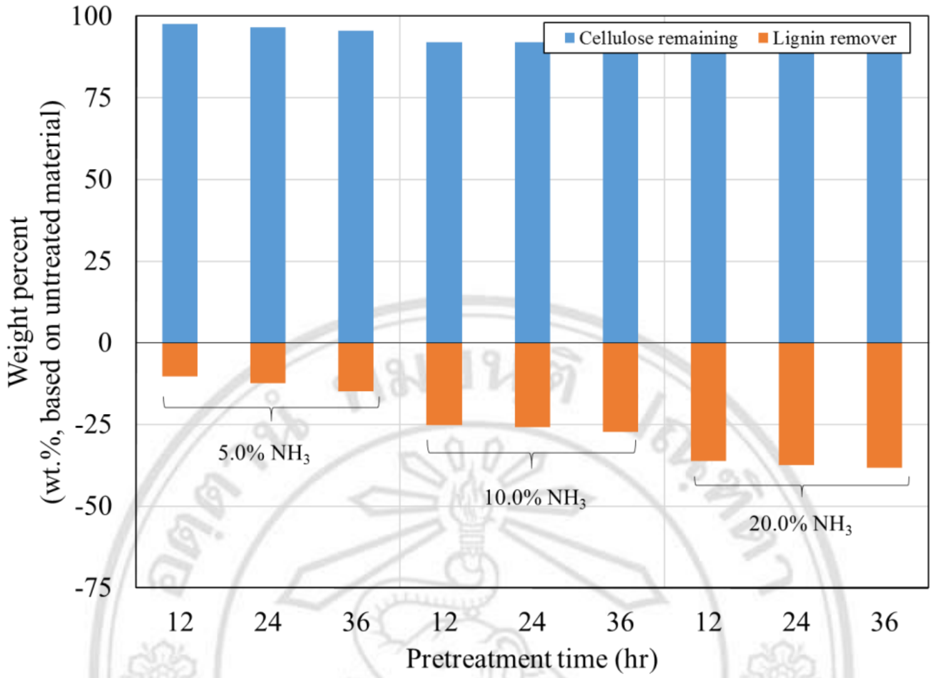

Figure 5.15 shows weight percent of remaining cellulose and lignin removal based on an untreated material.

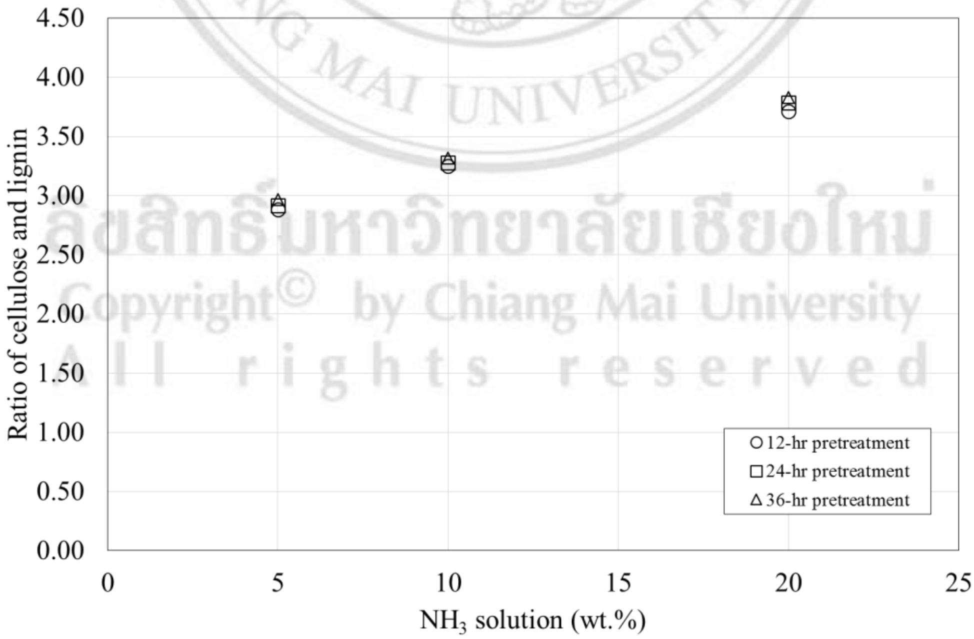

Figure 5.16 presents the ratio of cellulose and lignin for NH_3 solution pretreatment.

5.4.2.2 Improvement of lignin removal with NH_3 solution and ozone pretreatments.

Table 5.5 presents the compositions of the NH_3 pretreatment material after ozone pretreatment. As shown in the table, content of cellulose increases and that of lignin decreases. Cellulose raises from 49.6% to 73.4% and lignin decreases from 13.1% to 2.1% for 30 mins ozone. However, the percentage of lignin and cellulose which based on dry matter does not present the optimum one, since the solidity of matter is also eliminated. For 30 mins ozone, solidity is reduced from 80.5% to 50.1%.

Table 5.5 Characterization of NH_3 solution pretreated materials with ozonolysis process

Time of ozone flow (minute)	Composition ((wt.%, based on dry matter)				Solid remaining (%)	Celllulose recovery (%)	Remover (%)		
	Cellulose	Hemicellulose	Lignin	Other			Hemicellulose	Lignin	Other
0	49.6	30.0	13.1	7.2	80.5	89.7	25.5	37.3	5.4
10	54.1	28.8	10.8	6.2	72.9	88.6	35.1	53.1	26.2
20	66.5	25.9	3.6	4.0	58.0	86.6	53.7	87.6	62.6
30	73.4	20.3	2.1	4.2	50.1	82.4	68.7	93.6	65.9

Fig 5.17 shows the composition of NH_3 solution pretreatment matter with ozone. It can be seen that all composition decrease lightly. The total composition is reduced from 80.5% of an ozone-untreated matter to 72.9%, 58.0% and 50.1% for 10, 20 and 30 mins, respectively.

Fig 5.18 compares the ratio of cellulose and lignin of material between ozone pretreatment of untreated and NH_3 solution pretreatment. It is found that without alkaline, the ratio remains the same, but with alkaline first and then ozone, the ratio rises sharply from 3.8 to 5.0, 18.5 for 10 and 20 mins ozone and at the highest at 34.3 for 30 mins ozone. This is because ozone pretreatment should be the method for further pretreatment with other one that destroy cell wall so that ozone can pass though the cell wall easier.

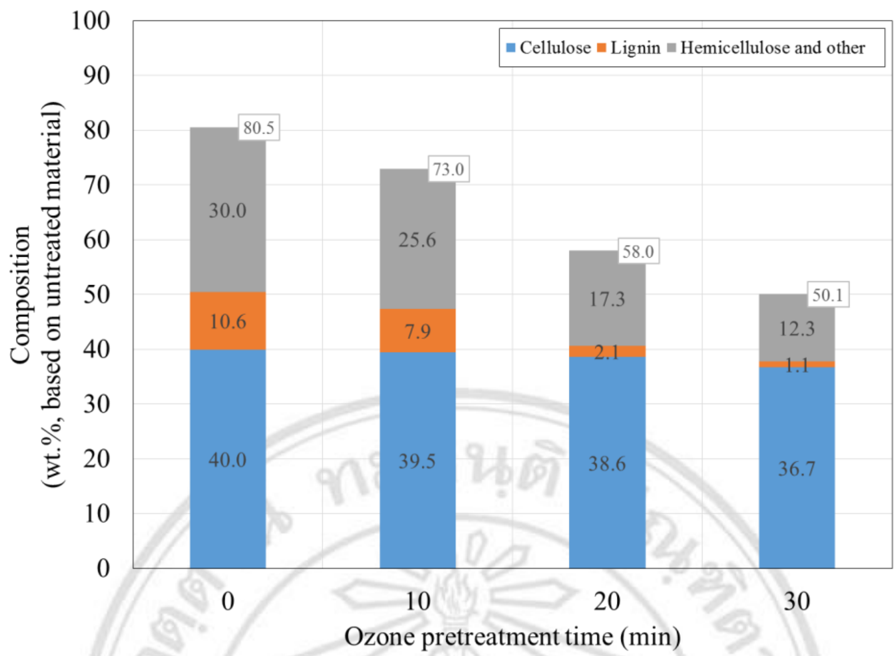

Figure 5.17 shows the composition of NH_3 solution pretreatment matter with ozone.

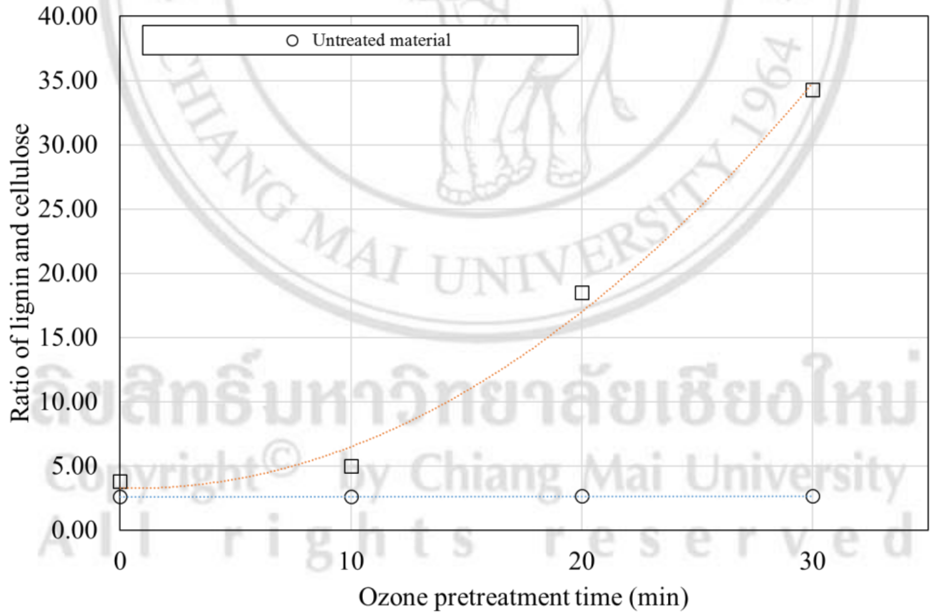

Figure 5.18 compares the ratio of cellulose to lignin of material with ozone pretreatment of untreated and NH_3 solution pretreatment.

5.4.3 $Ca(OH)_2$ solution and Ozone Pretreatment

5.4.3.1 Effect of $Ca(OH)_2$ solution pretreatment

As described that lignin was removed from the sample by using $Ca(OH)_2$ solutions which concentrations and time were varied. Table 5.6 shows list of composition of each delignification condition. Since contents of lignin remover and remaining cellulose are interested in this study, the compositions of matter were divided into cellulose, lignin, hemicellulose, and others. It was found that the untreated material contains 44.6% of cellulose, 16.8% of lignin, 32.4% of hemicellulose and 6.2% of others. For pretreated material, it is found that percentage of cellulose is higher and percentage of lignin is lower than the untreated one as expected. At the highest concentration of 100 wt.% $Ca(OH)_2$ solution and the highest time of 36 hrs, it is expected that lignin must be at the lowest since high $Ca(OH)_2$ solution concentration and longtime of reaction will destroy cell wall of the corn stover. At this condition, percentage of lignin at 13.9% is the lowest one, but percent of cellulose of 48.3% is the highest one.

Solid remain is also the key point for selecting a pretreatment method for delignification. This is because $Ca(OH)_2$ solution can act as a solvent to any composition of the material. Lignin is not only removed but cellulose is also eliminated too. Eventhough high percentage of cellulose is shown after lignification, its quantity may remain so little. As can be seen in Table 5.6, for example, 48.3% of cellulose is found to be very high for 100 wt.% $Ca(OH)_2$ solution and 36 hrs time, but only 83.9 % of solid is remained and 91.0% of cellulose is recovered. This means 44.0% of an original cellulose is recovered and 56.0% of an original one is soluble in $Ca(OH)_2$ solution.

Figure 5.19-5.21 presents the composition of the sample after pretreating with $Ca(OH)_2$ solution with concentrations of 20.0, 60.0 and 100 wt.% for 12, 24 and 36 hrs pretreatment. This figure differs from Table 5.6 due to showing the percentage which is based on the untreated material. This is because it is expected to present the percentage of remaining substance for 12 hrs pretreatment, for example, the percentage of cellulose is reduced from 44.6% to 43.7%, 41.9% and 40.7% while the percentage of lignin decreased from 16.9% to 15.3%, 13.2% and 11.8% for 20.0, 60.0 and 100 wt.% $Ca(OH)_2$, respectively. The patterns of reduction are also occur for 24 hrs and 36 hrs of the pretreatment. The dramatic decrease of each substance and total substance, is found with increasing the concentration of $Ca(OH)_2$ solution. This is because alkaline destroys the cell wall of the glass so not only lignin is eliminated but cellulose is also removed from the matter.

Considering the effect of pretreatment time of $Ca(OH)_2$ solution as shown in Figure 5.22, only $Ca(OH)_2$ concentration of 20.0% is present in the figure, For example, it can be seen that each substance is dramatically decreased for 12 hrs pretreatment but it is slightly reduced for 24 hrs and 36 hrs of the pretreatment. Looking at the effect of alkali pretreatment by varying either concentration or time as shown in Figure 5.19-5.21 and 5.22, it is necessary to find the optimum pretreatment condition for using with the alkali high removal of lignin is expected, but high content of remains in the intermediate for ethanol production.

In order to select the optimum condition for $Ca(OH)_2$ solution pretreatment of corn stover, high level of lignin removal and cellulose recovery are considered. Figure 5.23 shows weight percent of remaining cellulose and lignin removal based on an untreated material. Increasing of pretreatment time makes a slight decrease in both lignin and cellulose. At 20.0 wt.% $Ca(OH)_2$ solution, for example, cellulose of 98.1, 97.9 and 97.5 is recovery and lignin of 8.9, 10.7 and 12.1 is removed. Eventhough increasing pretreatment time cannot affect the lignin removal and cellulose recovery, increasing alkali concentration affects both of them. Cellulose and lignin are dramatically reduced as described before. Again, high removal of lignin is expected, but high content of cellulose remains in the intermediate for ethanol production. Considering Figure 5.23, it is found that lignin decreased dramatically from about 8.9-12.1% for 20.0 wt% $Ca(OH)_2$ to 29.9-30.8% for 100 wt.% $Ca(OH)_2$, while remaining cellulose decreased slightly decreased from 97.5-98.1% for 20.0 to 91.0-91.4% for 100 wt.% $Ca(OH)_2$ solution. Pretreatment with 100 wt.% $Ca(OH)_2$ solution should be the optimum concentration for corn stover.

The other criteria for considering the optimum condition for alkali pretreatment is the ratio of cellulose and lignin since it presents high content of cellulose and low content of lignin. This also means that high percentage of lignin is removed, but cellulose is still the same or before pretreatment or remaining maximum. Figure 5.24 presents the ratio of cellulose and lignin for $Ca(OH)_2$ solution pretreatment using in this study. Pretreatment with 20.0 wt.% $Ca(OH)_2$ solution shows the lowest ratio of around 2.8-2.9, while pretreatment with 100 wt.% $Ca(OH)_2$ solution presents the high ratio of around 3.4-3.5 To selected the condition for $Ca(OH)_2$ solution pretreatment of corn stover is 36 hrs and 100 wt.% $Ca(OH)_2$ solution. The ratio of cellulose and lignin is 3.5 that is approximately 1.3 folds of untreated material.

Table 5.6 Characterization of untreated and pretreated materials with $Ca(OH)_2$ solution pretreatment

$Ca(OH)_2$ solution (%)	Time (hr)	Composition (wt.%, based on dry matter after pretreatment)				Solid remaining (%)	Cellulose recovery (%)	Remover (%)		
		Cellulose	Hemicellulose	Lignin	other			Hemicellulose	Lignin	Other
0.0	0	44.6	32.4	16.8	6.2	-	-	-	-	-
20.0	12	45.3	32.4	15.9	6.3	96.4	98.1	3.5	8.9	1.5
	24	45.5	32.5	15.7	6.3	95.8	97.9	4.0	10.7	2.3
	36	45.7	32.3	15.6	6.4	95.1	97.5	5.1	12.1	1.6
60.0	12	46.8	31.5	14.8	6.9	89.5	93.9	13.1	21.4	0.0
	24	47.1	31.4	14.6	6.8	89.3	94.5	13.4	22.5	1.3
	36	47.3	31.2	14.5	7.0	88.7	94.2	14.5	23.7	0.0
100	12	48.2	30.6	14.0	7.3	84.6	91.4	20.2	29.9	0.0
	24	48.3	30.5	13.9	7.3	84.3	91.4	20.7	30.3	0.7
	36	48.3	30.5	13.9	7.3	83.9	91.0	21.1	30.8	0.9

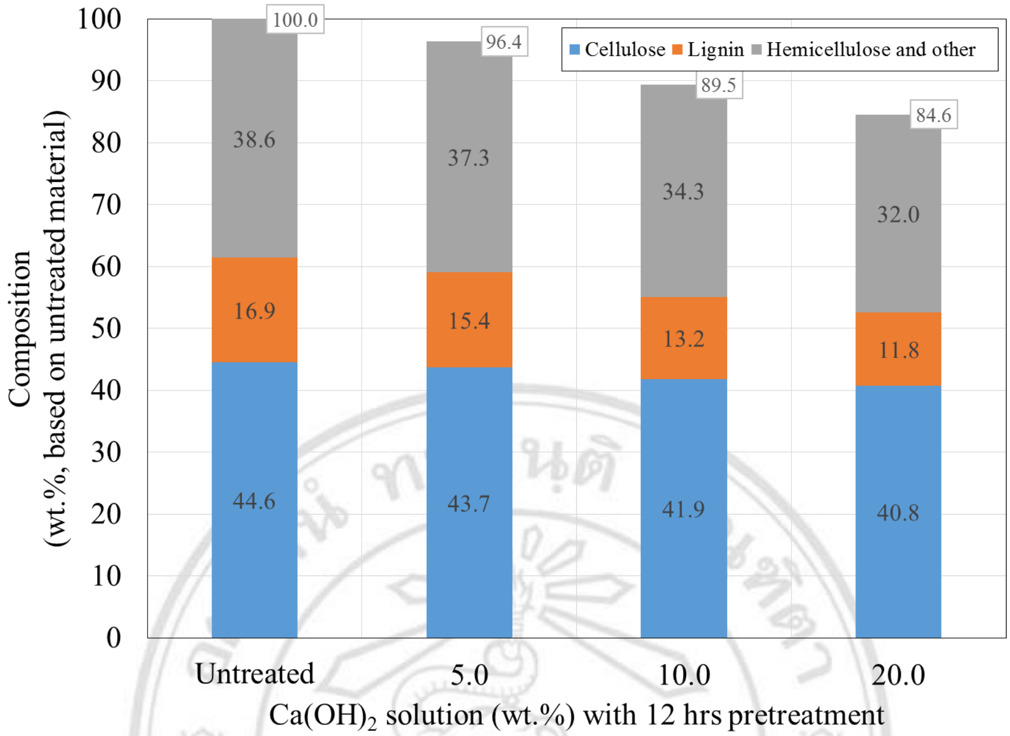

Figure 5.19 Remaining composition in $Ca(OH)_2$ solution with 12 hrs pretreated material.

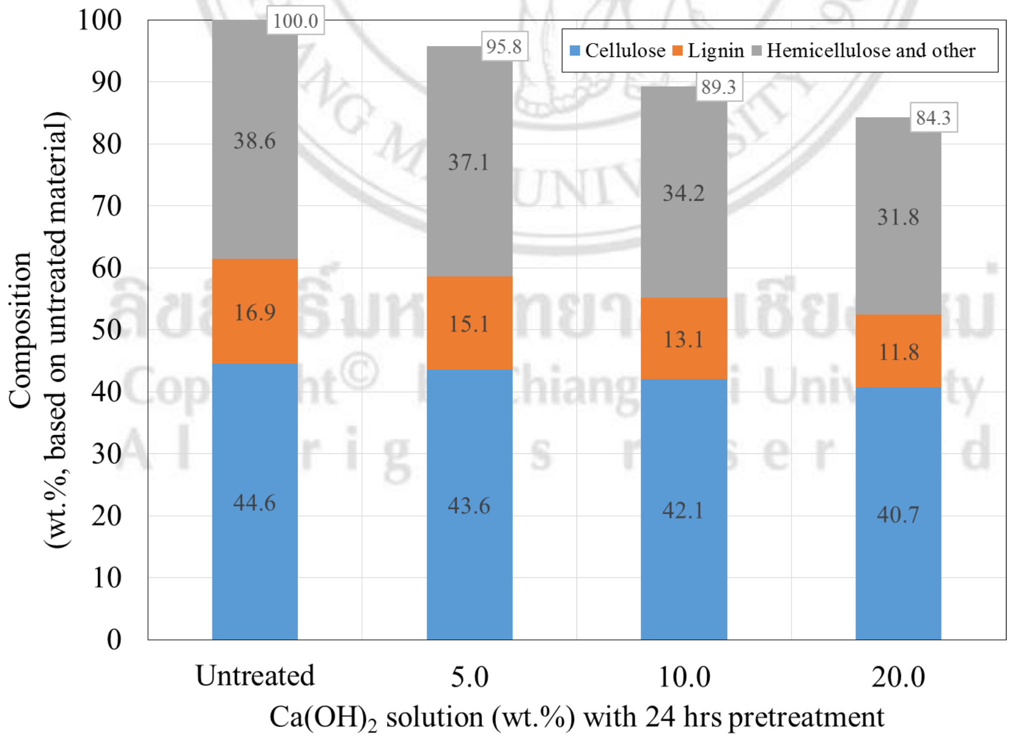

Figure 5.20 Remaining composition in $Ca(OH)_2$ solution with 24 hr pretreated material.

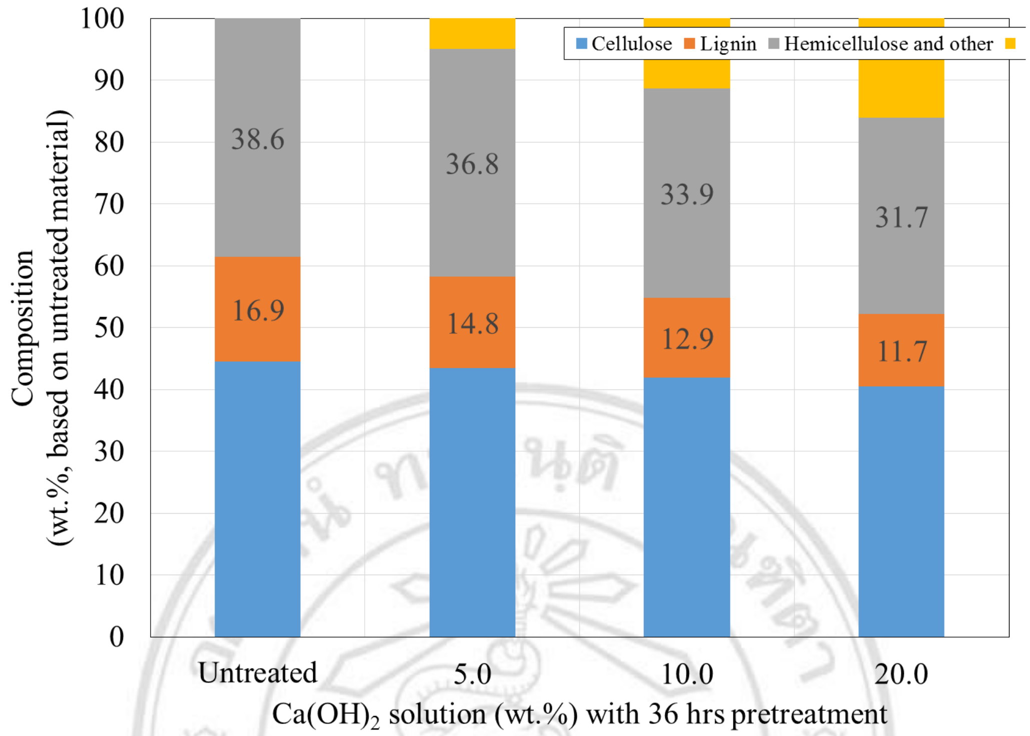

Figure 5.21 Remaining composition in $Ca(OH)_2$ solution with 36 hrs. pretreated material.

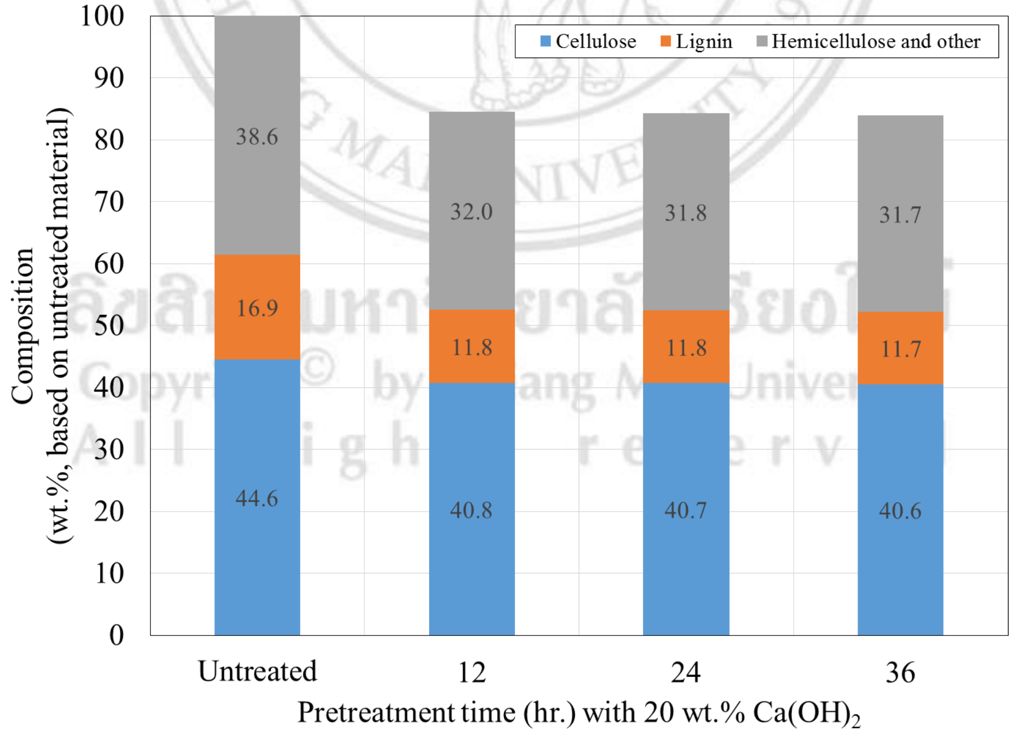

Figure 5.22 Effect of pretreatment time of $Ca(OH)_2$ solution.

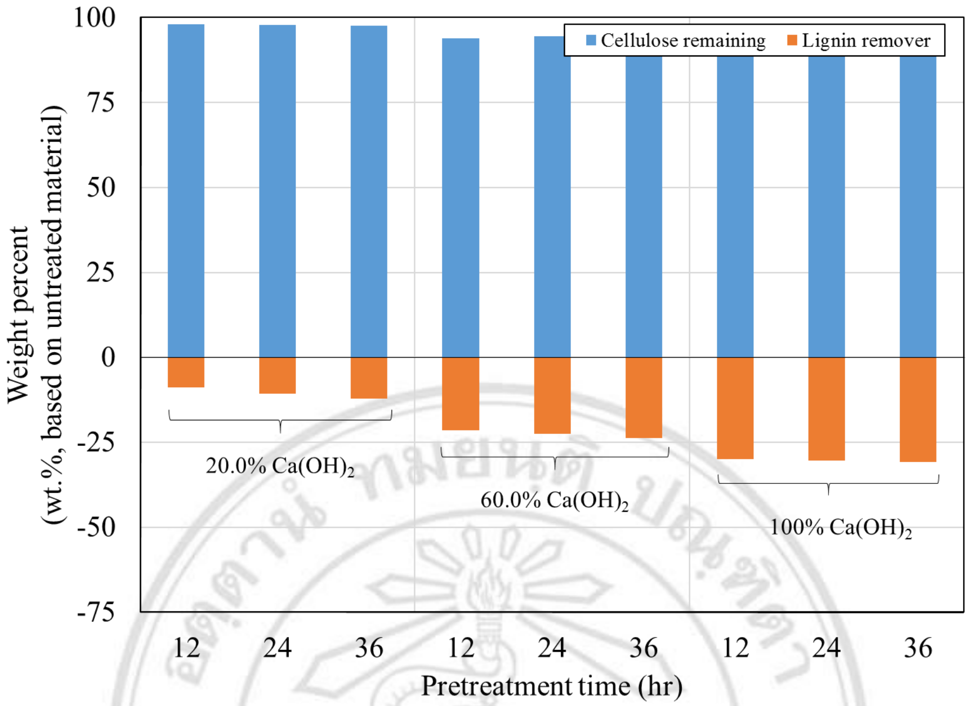

Figure 5.23 shows weight percent of cellulose remaining and lignin removal based on an untreated material.

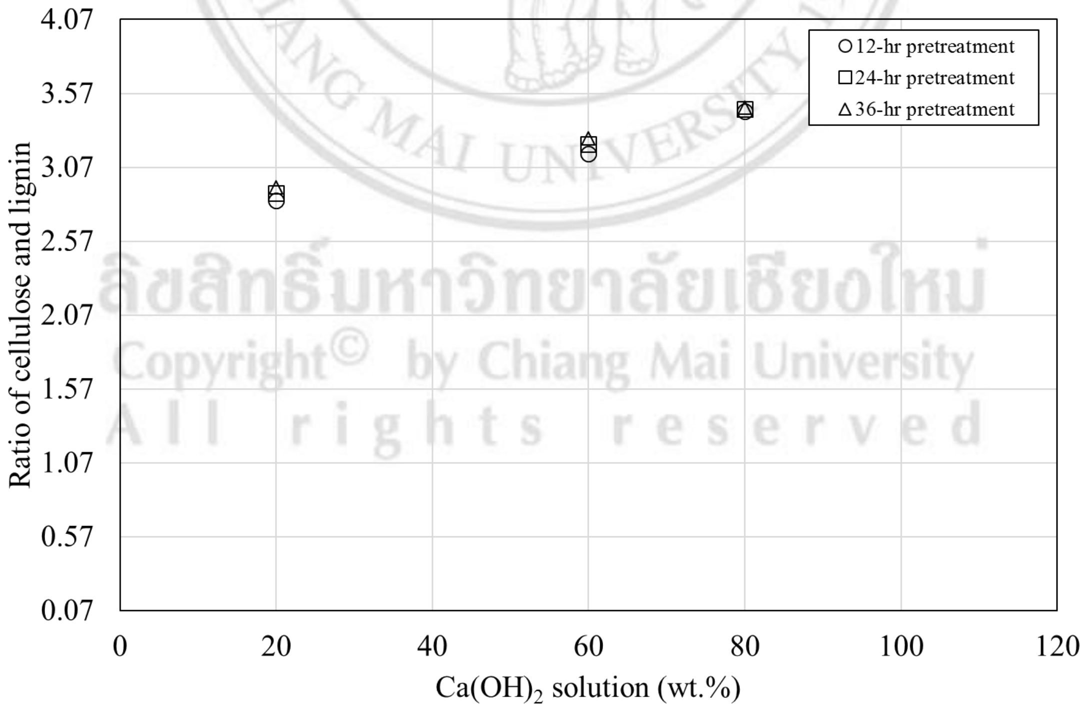

Figure 5.24 presents the ratio of cellulose to lignin for $Ca(OH)_2$ solution pretreatment.

5.4.3.2 Improvement of lignin removal with $Ca(OH)_2$ solution and ozone pretreatments.

Table 5.7 presents the compositions of the $Ca(OH)_2$ solution pretreatment material after ozone pretreatment. As shown in the table, content of cellulose increases and that of lignin decreases. Cellulose raises from 48.33% to 48.61% and lignin decreases from 7.3% to 7.1% for 30 mins ozone. However, the percentage of lignin and cellulose which based on dry matter does not present the optimum one, since the solidity of matter is also eliminated. For 30 mins ozone, solidity is reduced from 83.9% to 81.7%.

Table 5.7 Characterization of $Ca(OH)_2$ solution pretreated materials with ozonolysis process

Time of ozone flow (min)	Composition ((wt.%, based on dry matter)				Solid remaining (%)	Celllulose recovery (%)	Remover (%)		
	Cellulose	Hemicellulose	Lignin	Other			Hemicellulose	Lignin	Other
0	48.3	30.5	13.9	7.3	83.9	91.0	21.1	30.8	0.9
10	48.4	30.6	13.9	7.2	83.4	90.6	21.3	31.4	2.8
20	48.5	30.5	13.9	7.2	82.6	89.9	22.2	32.1	4.1
30	48.6	30.4	13.9	7.1	81.7	89.1	23.3	32.9	5.6

Figure 5.25 shows the composition of $Ca(OH)_2$ pretreatment matter with ozone. It can be seen that all composition decrease slightly change, because the amount of lignin is not change.

Figure 5.26 compares the ratio of cellulose to lignin of material betaween ozone pretreatment of untreated and $Ca(OH)_2$ pretreatment. It is found that alkaline untreated and $Ca(OH)_2$ pretreated the ratio remains the same because $Ca(OH)_2$ pretreated can not reduce lignin.

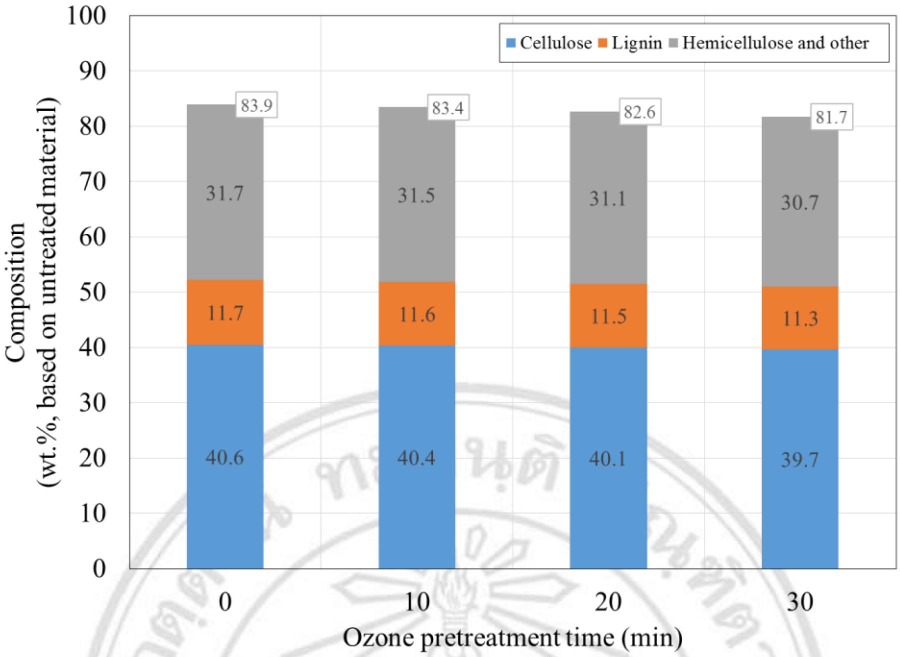

Fig 5.25 shows the composition of $Ca(OH)_2$ solution pretreatment matter with ozone.

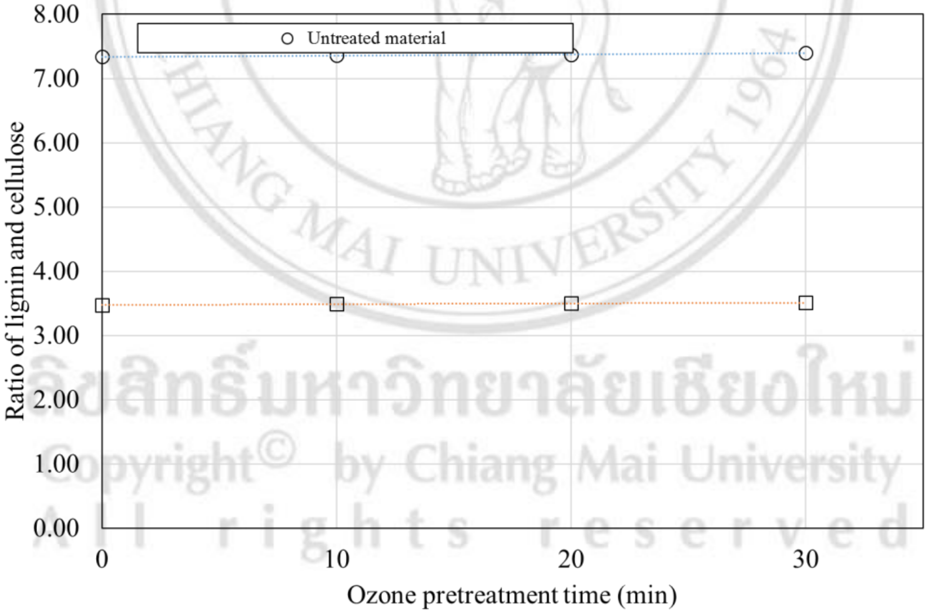

Fig 5.26 compares the ratio of cellulose to lignin of material with ozone pretreatment of untreated and $Ca(OH)_2$ solution pretreatment

5.5 Inhibitor examination

Inhibitors which occur during ozone pretreatment process are Acetaldehyde and Total phenolic. These inhibitors are toxic to yeast which is used in ethanol and sugar reducing production. Hence, it should be tested after ozone pretreatment. The inhibitors examination of this research are HS-GC-FID and Spectrophotometer. HS-GC-FID is the method for Acetaldehyde analysis. And Spectrophotometer is the method for Total phenolic analysis. The result is shown as table 5.8 and 5.9. The result shows that acetaldehyde is not detected, while total phenolic quantity from NaOH with ozone, NH_3 with ozone and $Ca(OH)_2$ with ozone are 18.87±0.56, 8.72±0.26 and 2.95±0.14 in order.

Table 5.8 Result of Acetaldehyde analysis.

No.	Pretreat	Result , mg/l
1	NaOH + O_3	Not Detected
2	NH3 + O_3	Not Detected
3	Ca(OH)2 + O_3	Not Detected

Table 5.9 Result of Total phenolic analysis.

No.	Pretreat	Result , mg/l
1	NaOH + O_3	18.87 ±0.56
2	NH3 + O_3	8.72 ±0.26
3	Ca(OH)2 + O_3	2.96 ±0.14

5.6 Conclusion

Lignin can be removed from corn stover by using alkaline solution pretreatment, and alkaline solution with ozone pretreatment. The alkaline solution are NaOH, NH_3 and $Ca(OH)_2$ .The result found that NaOH solution can eliminate the lignin of material more than NH_3 and $Ca(OH)_2$. But, $Ca(OH)_2$ has the worst efficiency for lignin elimination. The best condition of lignin elimination is 5.5% wt. of NaOH, 2 hours of pretreatment which is consider at ratio of cellulose and lignin. Ozone pretreatment only cannot eliminate lignin. But ozone can be used to pretreat with alkaline solution to increase the efficiency of lignin elimination. The result of inhibitor examination found that there is no detection for Acetaldehyde inhibitor of NaOH with O_3 , NH_3 with O_3 and $Ca(OH)_2$ with O_3 pretreated but total phenolic is detected all of pretreatment conditions.