CHAPTER 4

Delignification of Bana Grass Using Alkaline and Ozone.

This chapter is regarding method and result of lignin elimination using bana grass. The methods of elimination are alkaline solution and ozone pretreatment.The descriptions are as follows;

4.1 Materials and methods

4.1.1 Materials

Bana Grass used in this study was taken from Hang Chat District, Lampang Province, Thailand. The farming areas are approximately 1,200 rai (1,920,000 m^2). Land altitudes are 280 m. above sea level. This kind of grass is utilized as livestock feed at Thai Elephant Conservation Center in the number of 500 tons per year. Following TAPPI T 203 om-88, T 223 om-88 and T 222 om-88, the dry grass contains 60.2% cellulose, 23.8% hemicellulose and 8.2% lignin.

4.1.2 Sample preparation

Bana grass with the length of 3-4 m and age of 80-90 days was harvested from the land. It then was sliced into pieces smaller than 1 mm. Next, pieces of grass were washed by 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 the oven at 103°C for hours to achieve moisture content less than 10 wt.%. To keep the material for delignification, it was vacuumed and stored in sealed plastic bag in desiccator.

4.2 Condition for alkali pretreatment

4.2.1 NaOH Pretreatment

Prepare NaOH solutions with concentrations of 0.5, 3.0, 5.5, 8.0 and 10.5 wt.% 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.

4.2.2 NH_3 Pretreatment

Prepare NH_3 solutions with concentrations of 5, 10 and 20 wt.%. 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.

4.2.3 $Ca(OH)_2$ Pretreatment

Prepare $Ca(OH)_2$ solutions of 20, 60 and 100 wt.%. 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.

4.3 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 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.

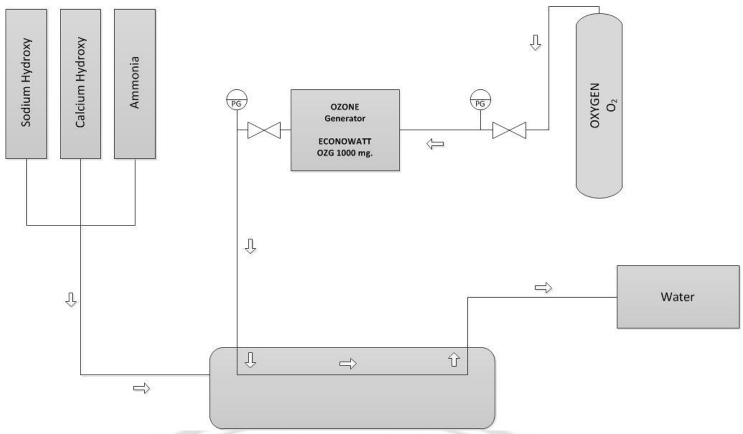

Figure 4.1 The treatment apparatus ozonolysis

Figure 4.2 ECONOWATT model OZG gas 1000 mg.

4.4 Results and Discussion

4.4.1 NaOH and Ozone Pretreatment

4.4.1.1 Effect of NaOH pretreatment

As described that lignin was removed from the sample by using NaOH solutions which concentrations and time were varied. Table 4.1 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 60.2% of cellulose, 8.2% of lignin, 23.8% of hemicellulose and 7.8% 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 alkaline concentration and longtime of reaction will destroy cell wall of the Bana Grass. At this condition, percentage of lignin at 6.49% is not the lowest, but percentage of cellulose at 69.9% is the highest. The lowest percentage of lignin at 5.2% 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 4.1 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 4.1, for example, 69.9% of cellulose is found to be very high for 10.5 wt.% NaOH and 3 hrs time, but only 45.01% of solid is remained and 52.2% of cellulose is recovered. This means 31.5% of an original cellulose is recovered and 68.5% of an original one is soluble in NaOH solution.

Figure 4.3-4.5 presents the composition of the sample after pretreating with alkaline 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 4.1 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 60.2% to 59.9%, 55.3%, 50.8%, 43.6% and 31.6% while the percentage of lignin decreased from 8.2% to 7.5, 6.6, 4.1, 3.6 and 3.2% for 0.5, 3.0, 5.5, 8.0 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 due to 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 4.6, 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 alkaline.

Looking at the effect of NaOH solution pretreatment by varying either concentration or time as shown in Figure 4.3-4.5 and 4.6, 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 Bana grass, high level of lignin removal and cellulose recovery are considered. Figure 4.7 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 84.3, 83.1 and 80.3 is recovery and lignin of 50.4, 51.5 and 52.6 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 4.7, it is found that lignin remover decreased dramatically from about 19-27% for 3.0 wt.% NaOH to 50–52% for 5.5 wt.% NaOH, while remaining cellulose decreased slightly from 91–90 for 3.0 wt.% NaOH to 84-80% 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 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 4.8 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 8, while pretreatment with 10.5 wt.% NaOH presents the medium ratio of around 10. The maximum ratio of cellulose is about 12 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 50% and about 80% of cellulose is remaining. It is concluded that the optimum condition for alkali pretreatment of Bana grass is 2 hours and 5.5 wt.% NaOH. The ratio of cellulose and lignin is 12.58 that is approximately 1.7 folds of untreated material.

Table 4.1 Characterization of untreated and pretreated materials with NaOH solutions pretreatment

NaOH solution (%)	Time (minute)	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	60.2	23.8	8.2	7.8	-	-	-	-	-
0.5	60	61.7	23.9	7.8	6.7	96.5	98.9	3.2	8.7	17.4
	120	61.9	24.0	7.8	6.3	96.0	98.8	3.3	8.8	22.5
	180	62.0	24.0	7.8	6.2	96.0	98.9	3.2	8.8	23.8
3.0	60	62.4	23.8	7.5	6.2	88.5	91.8	11.3	19.2	29.6
	120	63.2	24.1	7.5	5.2	87.0	91.3	11.9	20.4	42.0
	180	63.4	24.0	7.0	5.6	85.5	90.1	13.9	27.2	38.5

Table 4.1 (continued)

NaOH solution (%)	Time (minute)	Composition (wt.%, based on dry matter after pretreatment)				Solid remaining (%)	Cellulose recovery (%)	Remover (%)		
		Cellulose	Hemi-cellulose	Lignin	Other			Hemi-cellulose	Lignin	Other
5.5	60	64.9	25.2	5.2	4.7	78.2	84.3	17.2	50.4	53.3
	120	65.4	25.5	5.2	3.8	76.4	83.1	18.1	51.5	62.3
	180	65.9	26.1	5.3	2.7	73.4	80.3	19.5	52.6	74.6
8.0	60	65.1	24.0	5.5	5.5	67.1	72.5	32.4	55.3	52.8
	120	65.9	24.2	5.4	4.5	66.0	72.3	32.8	56.7	62.0
	180	66.9	24.8	5.4	3.0	64.0	71.1	33.4	58.0	75.5
10.5	60	67.2	21.3	6.7	4.8	47.0	52.5	58.0	61.5	71.1
	120	68.5	21.7	6.5	3.3	46.0	52.3	58.1	63.5	80.6
	180	69.9	21.0	6.5	2.7	45.0	52.2	60.3	64.3	84.7

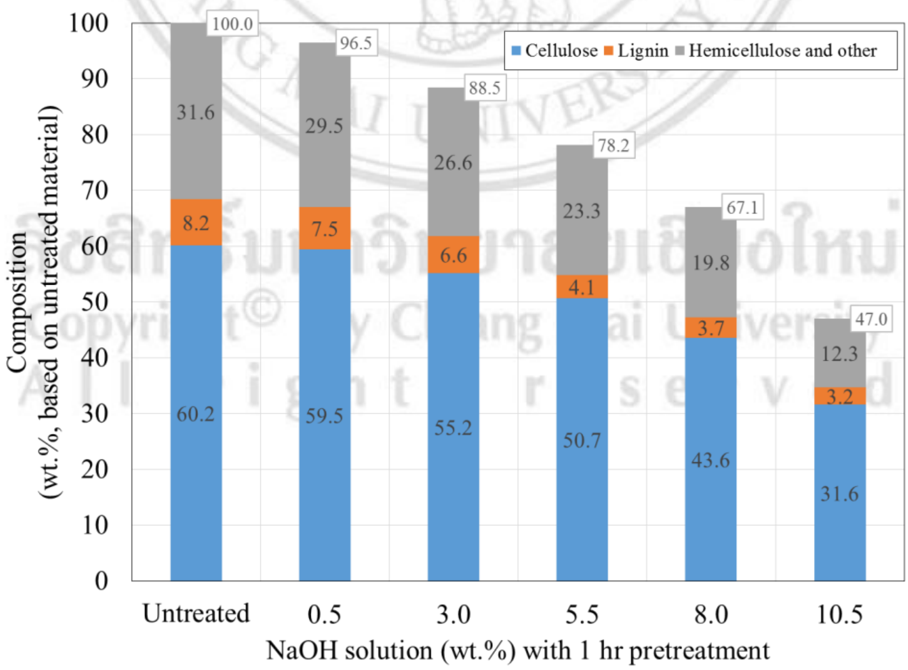

Figure 4.3 Remaining composition in NaOH solution with 1 hr pretreatment.

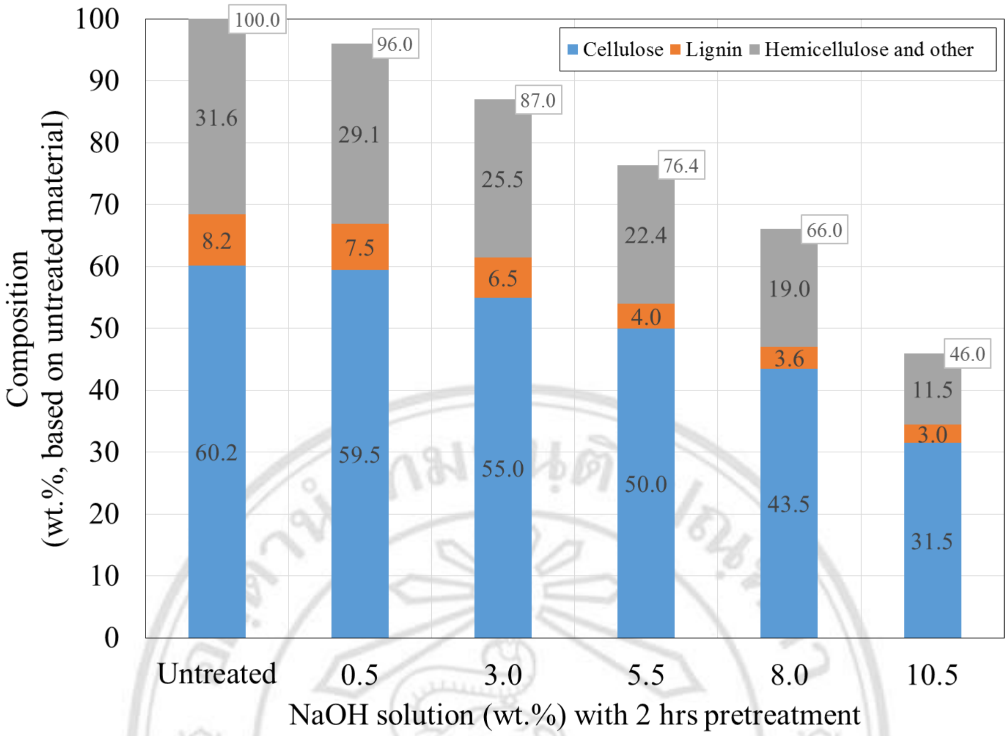

Figure 4.4 Remaining composition in NaOH solution with 2 hrs pretreatment.

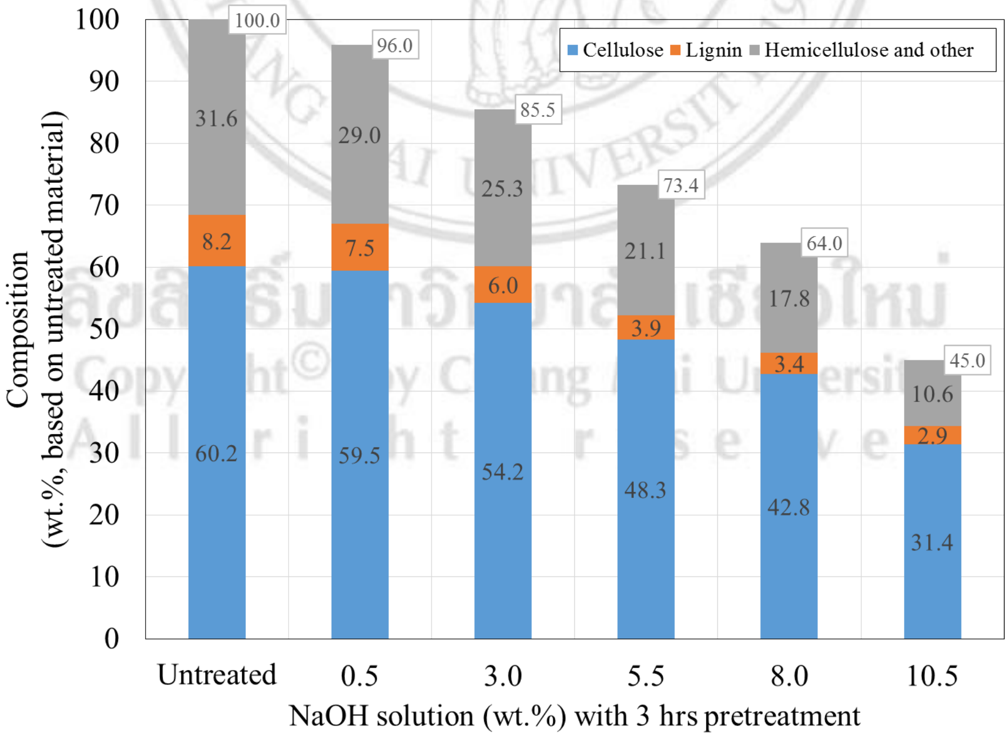

Figure 4.5 Remaining composition in NaOH solution with 3 hrs pretreatment.

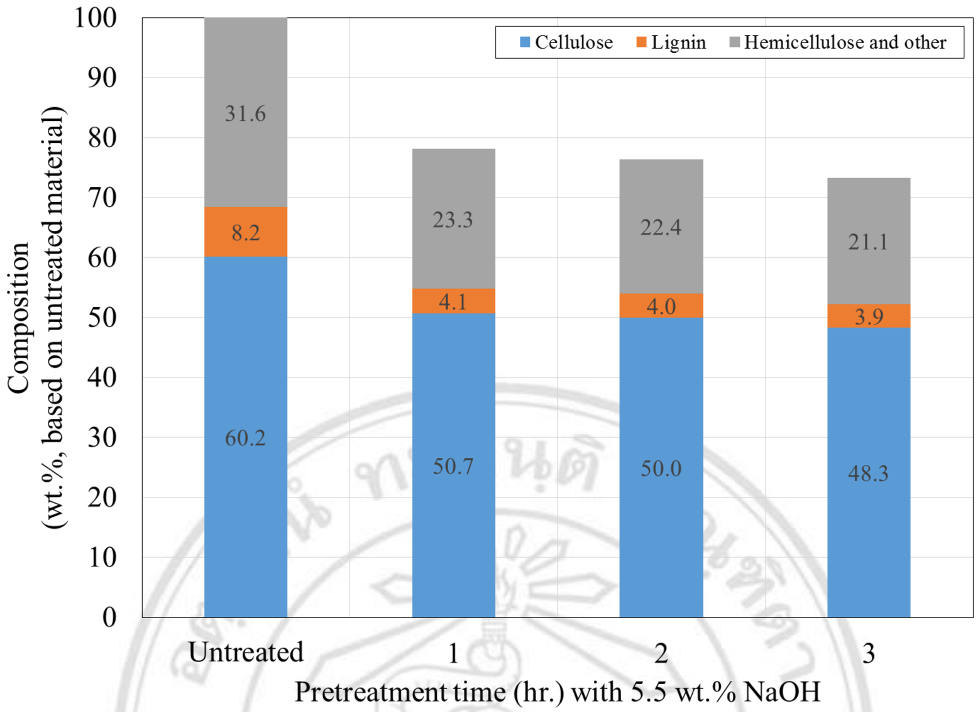

Figure 4.6 Effect of pretreatment time of NaOH solution.

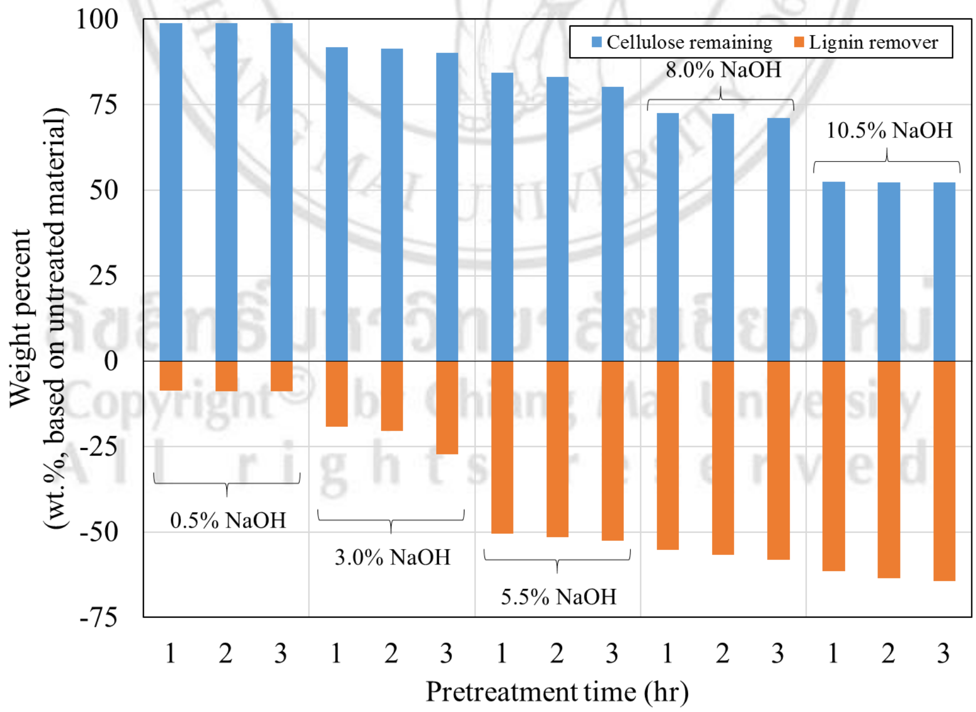

Figure 4.7 shows weight percent of remaining cellulose and lignin removal based on untreated material.

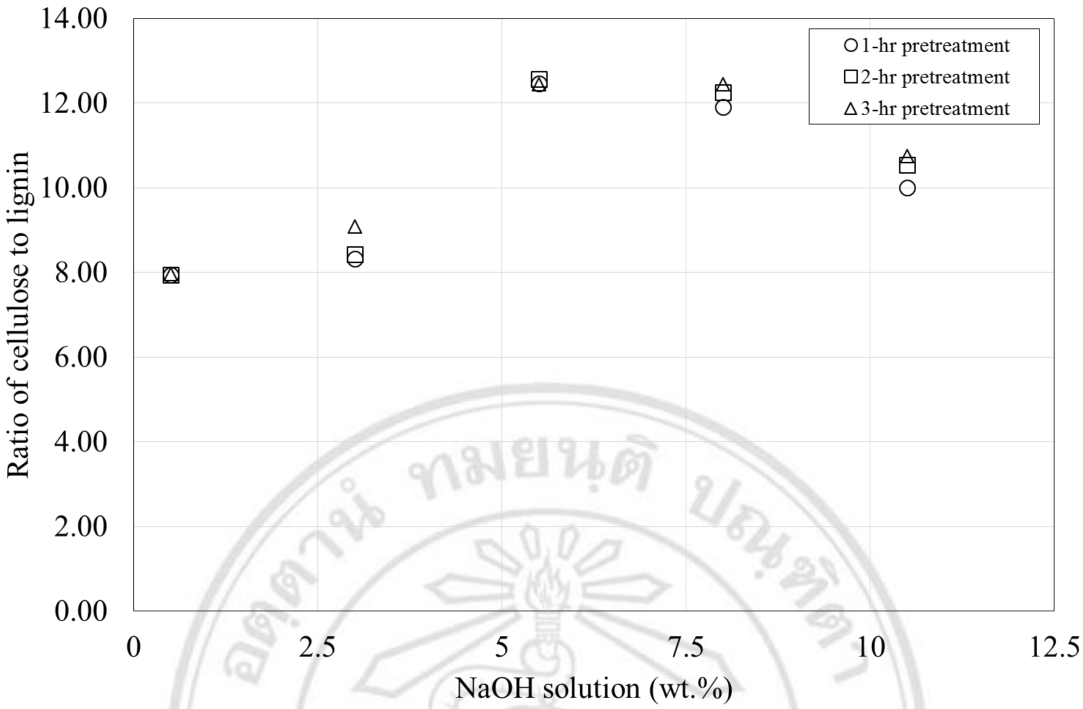

Figure 4.8 presents the ratio of cellulose and lignin for NaOH solution pretreatment.

4.4.1.2 Effect of Ozone pretreatment

Table 4.2 lists the composition of untreated material and ozone pretreatment of 10, 20 and 30 mins. Cellulose increases from 60.2% to 60.3% and lignin decreases from 8.2% to 8.1% for 30 mins pretreatment. Thus, pretreatment with ozone cannot eliminate both cellulose and lignin.

Table 4.2 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	60.2	23.8	8.2	7.8
10	60.2	23.8	8.2	7.8
20	60.2	23.8	8.2	7.8
30	60.3	23.7	8.1	7.8

4.4.1.3 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 Bana grass, ozone can pass through the cell 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. In this condition the ratio of cellulose to lignin is 12.6 as described above.

Table 4.3 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 65.4% to 75.9% and lignin decreases from 5.2% to 0.8% 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 76.4% to 62.6%.

Table 4.3 Characterization of NaOH solution pretreated materials with ozonolysis process.

Time of ozone flow (minute)	Composition (wt.%, based on dry matter)				Solid remaining (%)	Celllulose recovery (%)	Remover (%)		
	Cellulose	Hemi-cellulose	Lignin	Other			Hemi-cellulose	Lignin	Other
0	65.4	25.5	5.2	3.8	76.4	83.1	18.1	51.5	62.3
10	69.8	23.2	4.2	2.8	70.9	82.2	30.8	63.6	74.6
20	74.8	21.4	1.5	2.3	65.5	81.4	41.1	88.0	80.7
30	75.9	20.8	0.8	2.4	62.6	78.9	45.3	93.8	80.5

Fig 4.9 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 76.4% of an ozone-untreated matter to 70.92, 65.5% and 62.6% for 10, 20 and 30 mins, respectively. At 30 minutes treatment, it is found that remaining ozone is the less one with remaining only 0.5%, while cellulose is 47.5%. This condition may be the optimum one for the combination of ozone and NaOH solution pretreatment for intermediate cellulosic material.

Fig 4.10 compares the ratio of cellulose and 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 12.6 to 16.6, 46.0 for 10 and 20 mins ozone and at the highest at 93.8 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.

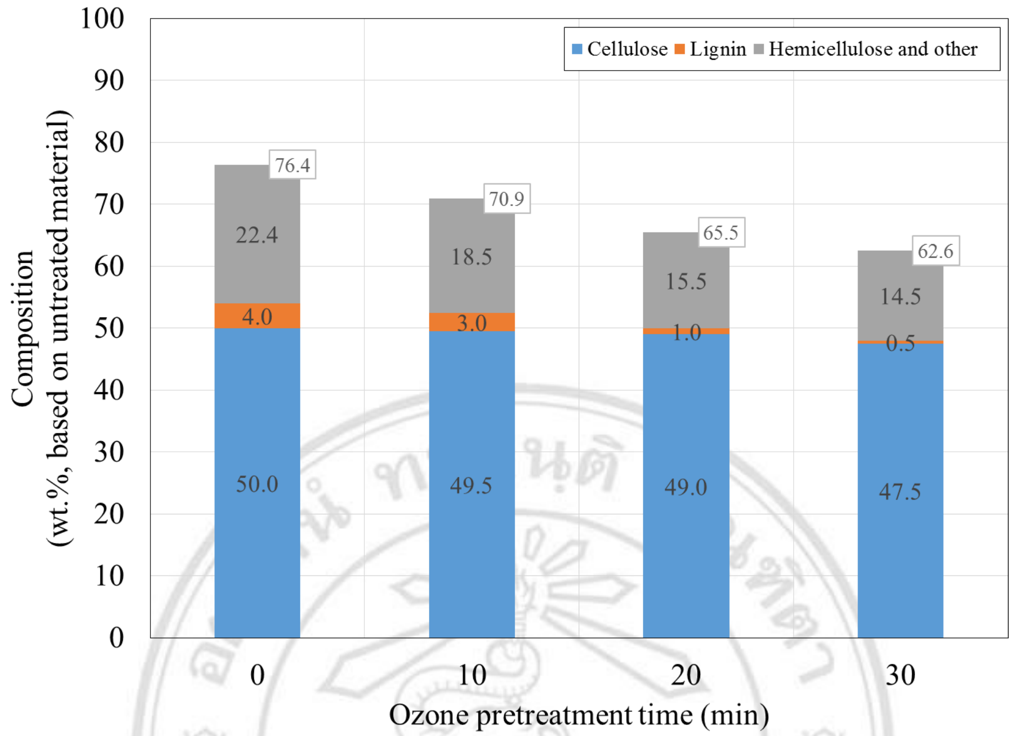

Fig 4.9 shows the composition of NaOH solution pretreatment matter with ozone.

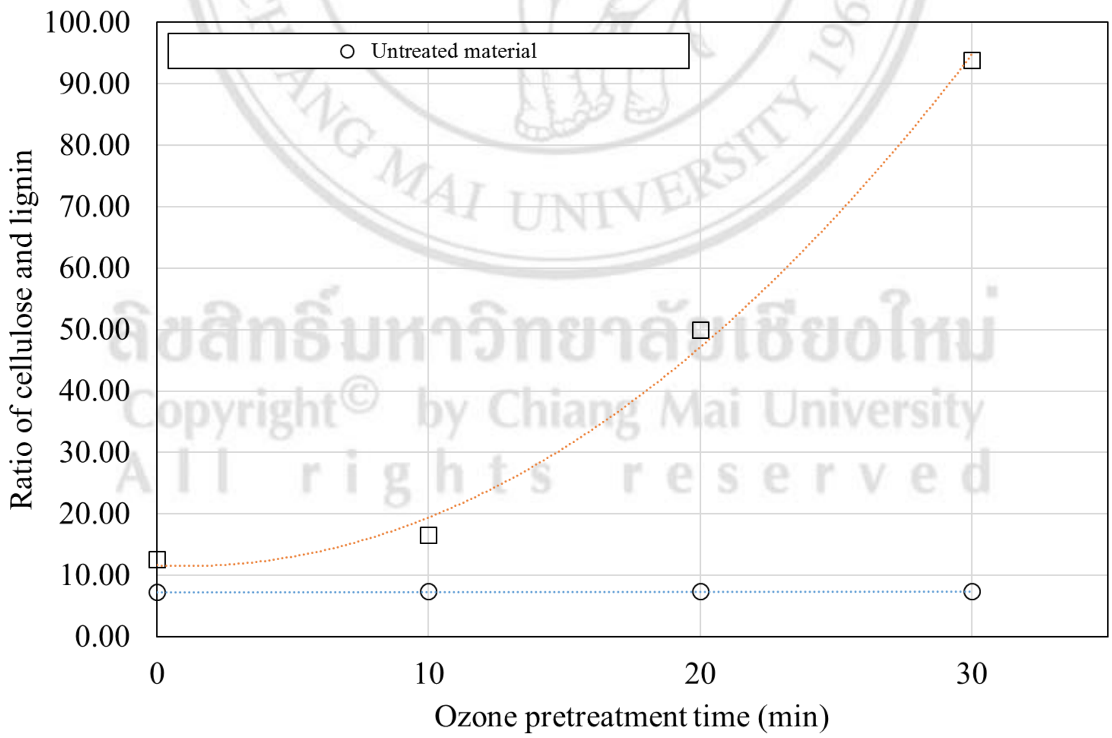

Fig 4.10 compares the ratio of cellulose to lignin of material with ozone pretreatment of untreated and NaOH solution pretreatment.

4.4.2 NH_3 solution and Ozone Pretreatment

4.4.2.1 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 4.4 shows list of composition of each delignification condition. Since contents of lignin remover and cellulose 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 60.2% of cellulose, 8.2% of lignin, 23.8% of hemicellulose and 7.8% 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 36 hrs, it is expected that lignin must be the lowest since high NH_3 solution concentration and longtime of reaction will destroy cell wall of the bana grass. At this condition, percentage of lignin at 5.4% is the lowest and percentage of cellulose at 64.7% is the highest.

Solid remain is also the keypoint for selecting a pretreatment method for delignification. This is because NH_3 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 4.4, for example, 64.7% of cellulose is found to be very high for 20.0 wt.% NH_3 solution and 36 hrs, but only 81.8 % of solid is remained and 87.9% of cellulose is recovered. This means 52.9% of an original cellulose is recovered and 47.1% of an original one is soluble in NH_3 solution.

Fig 4.11-4.13 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 4.4 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 60.2% to 59.8%, 57.3% and 53.4% while the percentage of lignin decreased from 5.2% to 7.6%, 6.2% and 4.5% 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 pretreat time of NH_3 solution as shown in Figure 4.14 only NH_3 concentration of 5.0% is presented 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 NH_3 solution pretreatment by varying either concentration or time as shown in Fig 4.11-4.13 and 4.14, 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 NH_3 solution pretreatment of Bana grass, high level of lignin removal and cellulose recovery are considered. Figure 4.15 shows weight percent of remaining cellulose and lignin removal based on an untreated material. Increasing of pretreatment time makes a slightly decrease in both lignin and cellulose. At 5.0 wt.% NH_3, for example, cellulose of 99.3, 99.4 and 99.4 is recovery and lignin of 7.0, 9.6 and 10.9 is removed. Eventhough increasing pretreatment time cannot effect 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 4.15, it is found that lignin remover decreased dramatically decreased from about 7 to 11% for 5.0 wt%NH_3 to 44 – 46% for 20.0 wt.% NH_3, while remaining cellulose decreased slightly from 99% for 5.0 wt% NH_3 to 88% for 20 wt.%

NH_3. Pretreatment with 20.0 wt.% NH_3 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 4.16 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 8, while pretreatment with 20.0 wt.% NH_3 solution presents the high ratio of around 12. To selected the condition for NH_3 solution pretreatment of bana grass is 36 hrs and 20.0 wt.% NH_3. The ratio of cellulose and lignin is 12.1 that is approximately 1.6 folds of untreated material.

Table 4.4 Characterization of untreated and pretreated materials with NH_3 solutions pretreatment

NH_3 solution (%)	Time (hr)	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	60.2	23.8	8.2	7.8	-	-	-	-	-
5.0	12	61.5	22.8	7.8	7.9	97.3	99.3	6.9	7.0	1.5
	24	61.7	22.7	7.6	7.9	96.9	99.4	7.7	9.6	1.3
	36	61.8	22.6	7.5	8.0	96.7	99.4	8.0	10.9	1.2
10.0	12	63.1	21.5	6.8	8.5	90.8	95.2	18.0	24.9	0.4
	24	63.3	21.4	6.6	8.6	89.8	94.5	19.0	27.1	0.8
	36	63.4	21.3	6.6	8.7	88.6	93.3	20.8	28.8	0.7
20.0	12	64.6	20.5	5.5	9.4	82.7	88.7	28.6	44.6	0.6
	24	64.7	20.5	5.4	9.4	82.3	88.4	29.1	45.7	0.7
	36	64.7	20.4	5.4	9.5	81.8	87.9	30.0	46.4	0.0

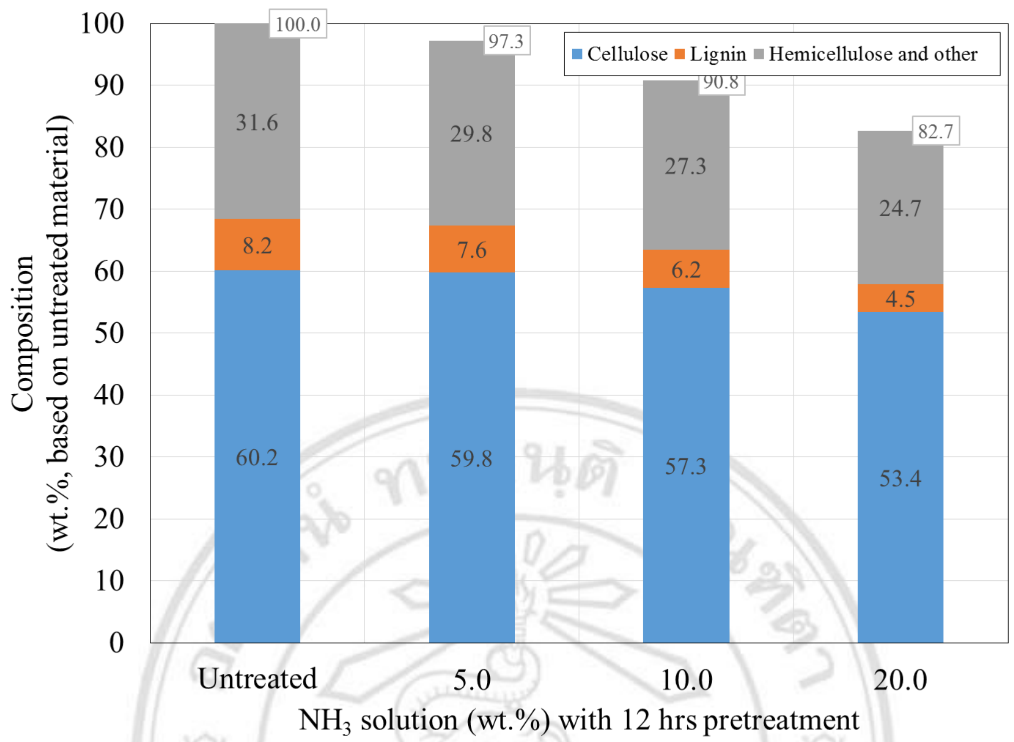

Figure 4.11 Remaining composition in NH_3 solution with 12 hrs pretreated material.

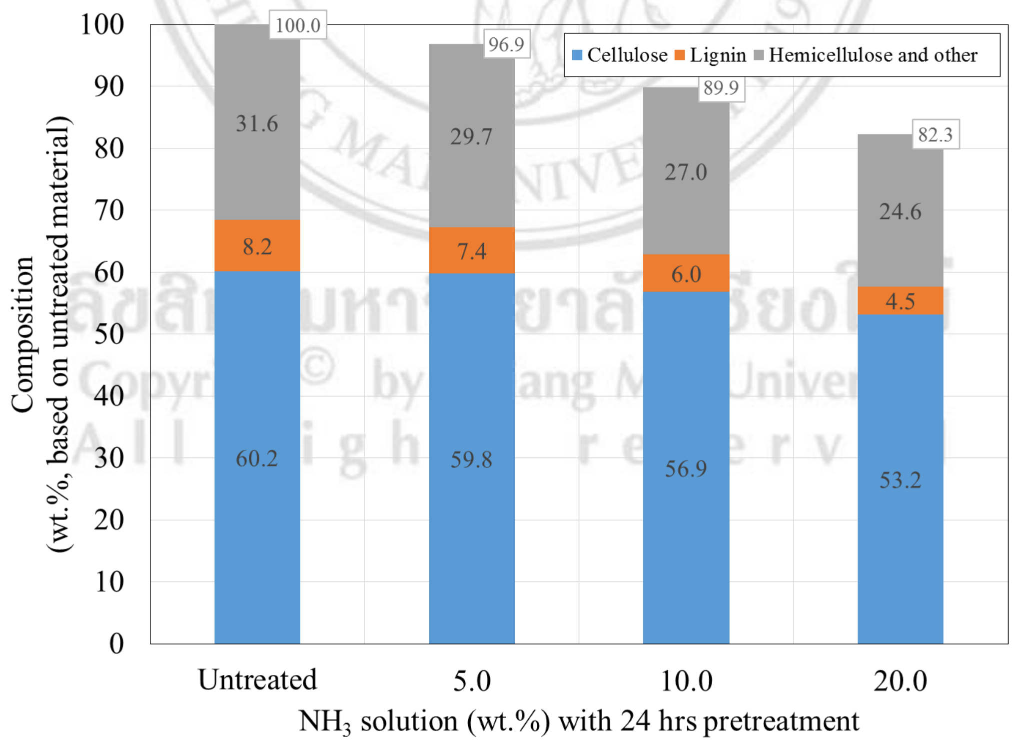

Figure 4.12 Remaining composition in NH_3 solution with 24 hrs pretreated material.

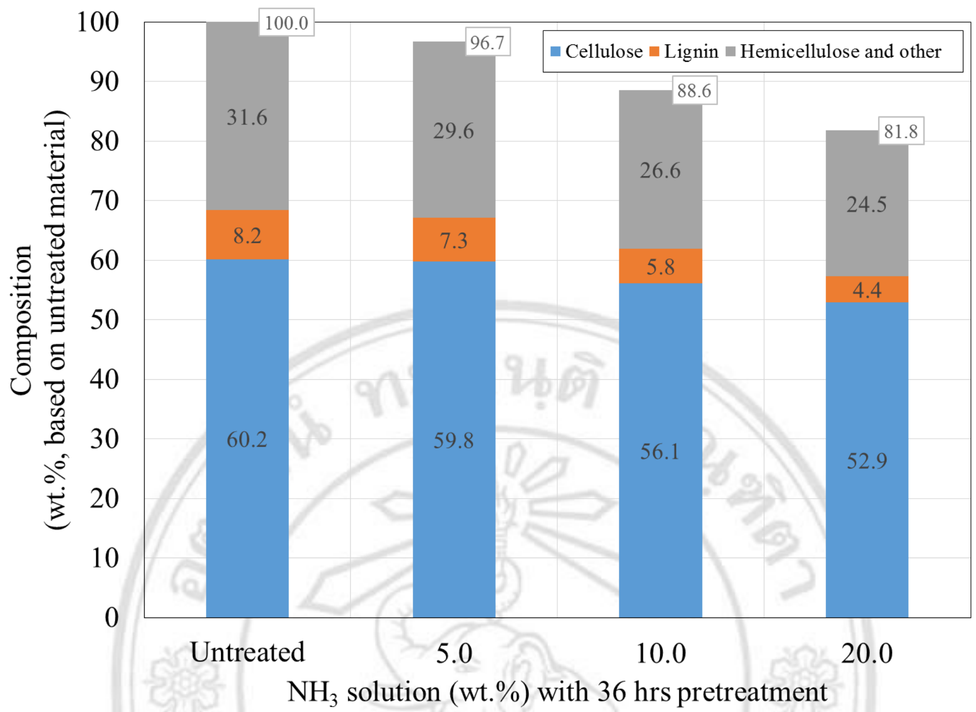

Figure 4.13 Remaining composition in NH_3 solution with 36 hrs. pretreated material.

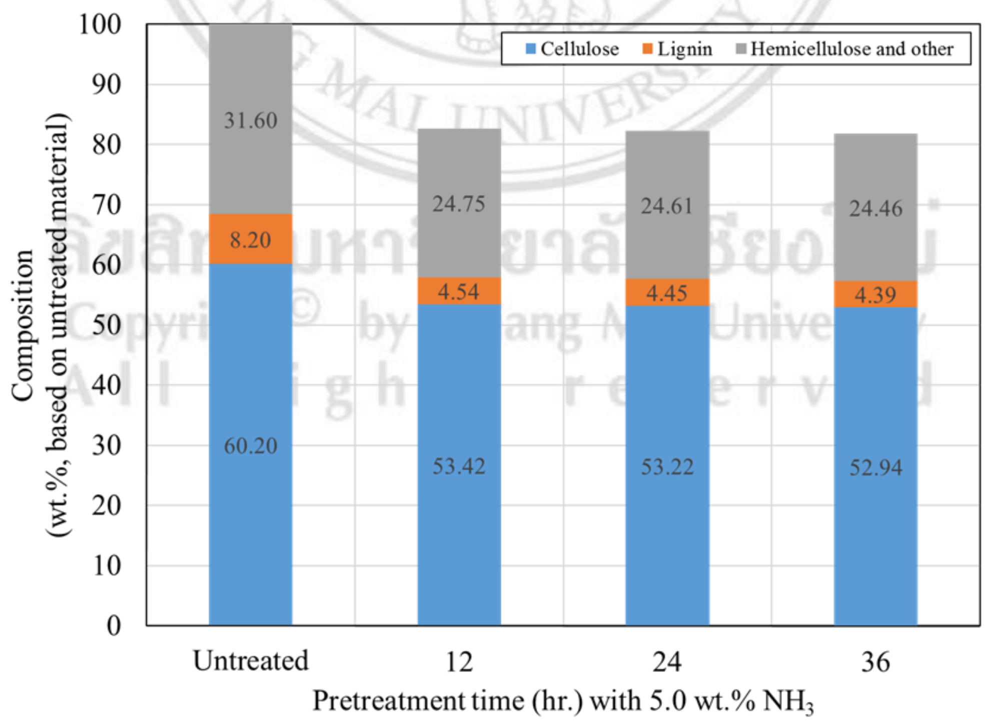

Figure 4.14 Effect of pretreatment time of NH_3 solution.

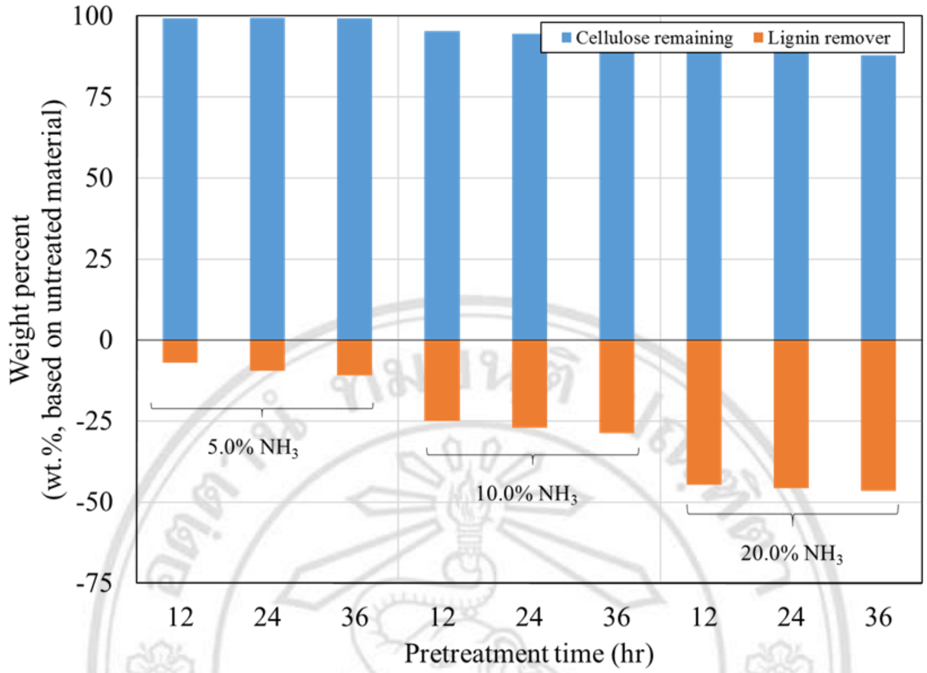

Figure 4.15 shows weight percent of cellulose remaining and lignin removal based on an untreated material.

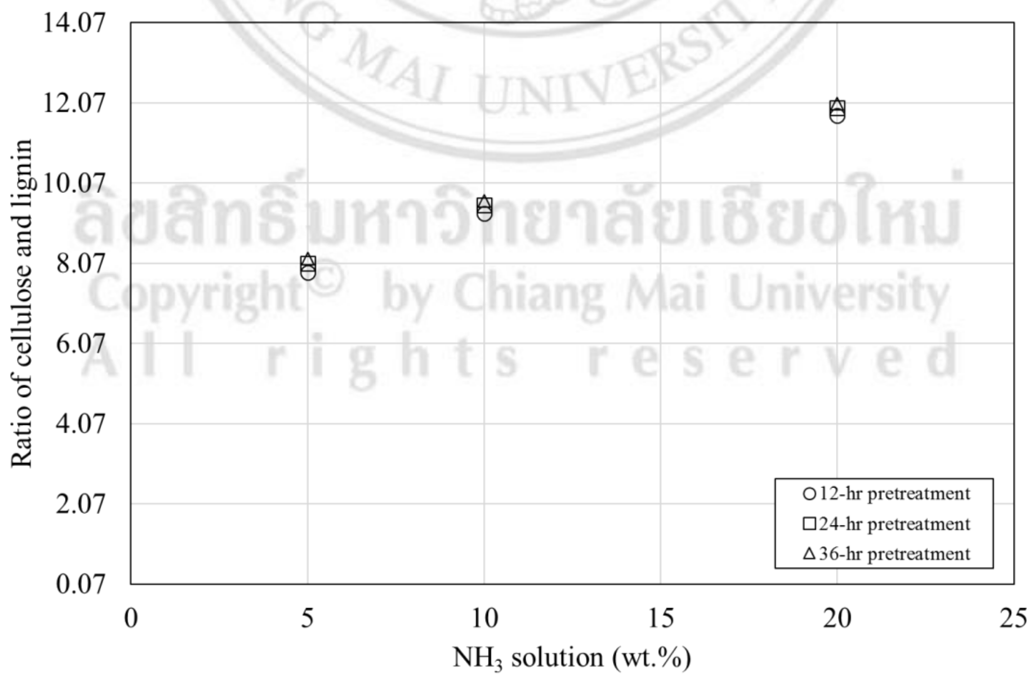

Figure 4.16 presents the ratio of cellulose to lignin for NH_3 solution pretreatment.

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

Table 4.5 presents the compositions of the NH_3 solution pretreatment material after ozone pretreatment. As shown in the table, content of cellulose increases and that of lignin decreases. Cellulose rises from 64.7% to 84.2% and lignin decreases from 5.4% 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 81.8% to 57.9%.

Table 4.5 Characterization of NH_3 solution pretreated materials with ozonolysis process

Time of ozone flow (min)	Composition ((wt.%, based on dry matter)				Solid remaining (%)	Celllulose recovery (%)	Remover (%)		
	Cellulose	Hemi-cellulose	Lignin	Other			Hemi-cellulose	Lignin	Other
0	64.7	20.4	5.4	9.5	81.8	87.9	30.0	46.4	0.0
10	68.7	19.2	4.4	7.7	76.2	87.0	36.6	59.4	24.4
20	79.3	16.3	1.6	2.8	64.6	85.1	55.8	87.6	76.8
30	84.2	12.3	1.0	2.6	57.9	81.0	70.0	93.8	80.5

Fig 4.17 shows the composition of NH_3 solution pretreatment matter with ozone. It can be seen that all compositions decrease slightly. The total composition is reduced from 81.8% of an ozone-untreated matter to 76.2, 64.6 and 57.9% for 10, 20 and 30 mins, respectively.

Fig 4.18 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 12.0 to 15.8, 48.9 for 10 and 20 mins ozone and at the highest at 96.6 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.

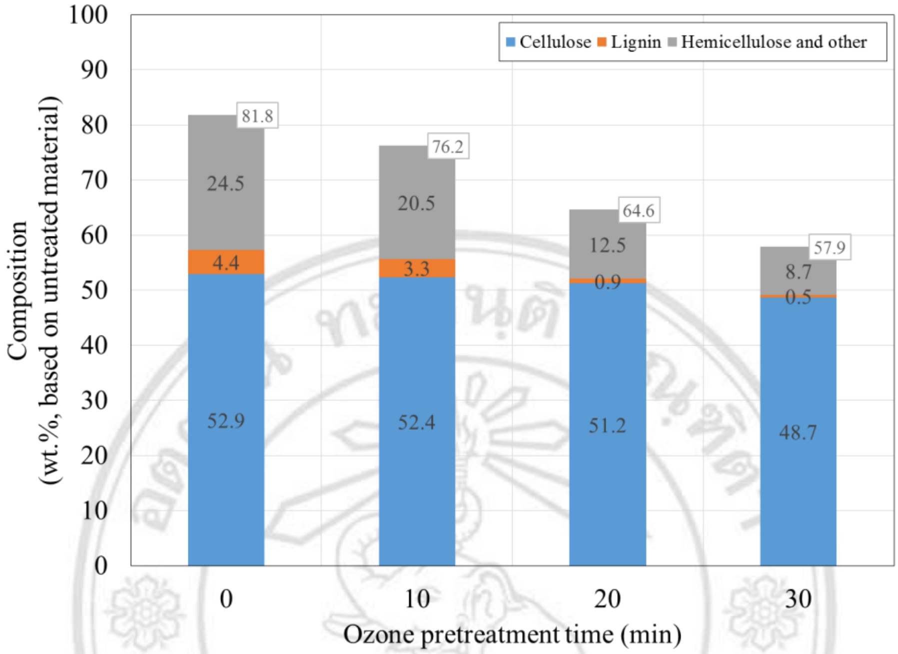

Figure 4.17 shows the composition of NH_3 solution pretreatment matter with ozone.

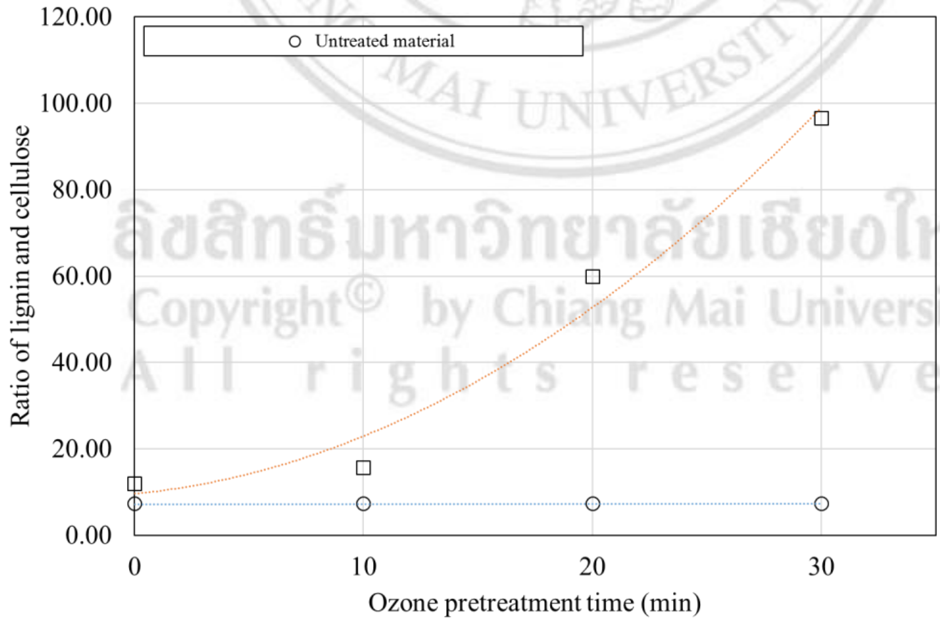

Figure 4.18 compare the ratio of cellulose to lignin of material with ozone pretreatment of untreated and NH_3 solution pretreatment.

4.4.3 $Ca(OH)_2$ solution and Ozone Pretreatment

4.4.3.1 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 4.6 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 60.2% of cellulose, 8.2% of lignin, 23.8% of hemicellulose and 7.8% 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 Bana grass. At this condition, percentage of lignin at 7.0% is the lowest and percent of cellulose at 63.3% is the highest.

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 4.6, for example, 63.3% of cellulose is found to be very high for 100 wt.% $Ca(OH)_2$ solution and 36 hrs time, but only 87.4 % of solid is remained and 91.9% of cellulose is recovered. This means 55.2% of an original cellulose is recovered and 44.7% of an original one is soluble in $Ca(OH)_2$ solution.

Figure 4.19-4.21 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 4.6 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 hour pretreatment, for example, the percentage of cellulose is reduced from 60.2% to 59.7%, 57.9% and 55.3% while the percentage of lignin decreased from 8.2% to 7.7%, 7.0% and 6.2% 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 substances, is found with increasing the concentration of alkali. This is due to 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 4.22, only $Ca(OH)_2$ concentration of 20.0% is presented 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 $Ca(OH)_2$ solution pretreatment by varying either concentration or time as shown in Figure 4.29-4.21 and 4.22, 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 $Ca(OH)_2$ solution pretreatment of bana grass, high level of lignin removal and cellulose recovery are considered. Figure 4.23 shows weight percent of remaining cellulose and lignin removal based on an untreated material. Increasing of pretreatment time makes a slightly decrease in both lignin and cellulose. At 20.0 wt.% $Ca(OH)_2$ solution, for example, cellulose of 99.2, 99.0 and 98.9 is recovery and lignin of 5.4, 6.0 and 7.1 is removed. Eventhough increasing pretreatment time cannot affect the lignin removal and cellulose recovery, increasing alkaline concentration affects both if 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 4.23, it is found that lignin decreased dramatically from about 5 to 7% for 20.0 to 24% for 100 wt.% $Ca(OH)_2$, while cellulose decreased slightly from 99% for 20.0 to 92% for 100 wt.% $Ca(OH)_2$ solution .

Pretreatment with 100 wt.% $Ca(OH)_2$ solution should be the optimum concentration for Bana grass.

The other criteria for considering the optimum condition for alkaline pretreatment is the ratio of cellulose to 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 4.24 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 8, while pretreatment with 100 wt.% $Ca(OH)_2$ solution presents the high ratio of around 9. To selected the condition for $Ca(OH)_2$ solution pretreatment of bana grass is 36 hrs and 100 wt.% $Ca(OH)_2$ solution. The ratio of cellulose and lignin is 8.9 that is approximately 1.1 folds of untreated material.

Table 4.6 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	Hemi-cellulose	Lignin	other			Hemi-cellulose	Lignin	Other
0.0	0	60.2	23.8	8.2	7.8	-	-	-	-	-
20.0	12	60.8	23.5	7.9	7.8	98.2	99.2	2.9	5.4	1.9
	24	60.8	23.5	7.9	7.8	98.0	99.0	3.3	6.0	2.0
	36	61.0	23.3	7.8	7.8	97.5	98.9	4.4	7.1	2.2
60.0	12	62.0	22.3	7.5	8.2	93.4	96.2	12.6	14.5	1.4
	24	62.0	22.2	7.4	8.3	92.8	95.6	13.6	15.7	0.9
	36	62.3	21.9	7.5	8.4	91.9	95.0	15.5	16.3	1.5
100	12	62.9	21.2	7.1	8.8	87.9	91.9	21.8	24.2	0.6
	24	63.1	21.1	7.1	8.8	87.9	92.0	22.2	24.2	0.8
	36	63.3	20.8	7.0	8.9	87.4	91.9	23.5	24.9	0.7

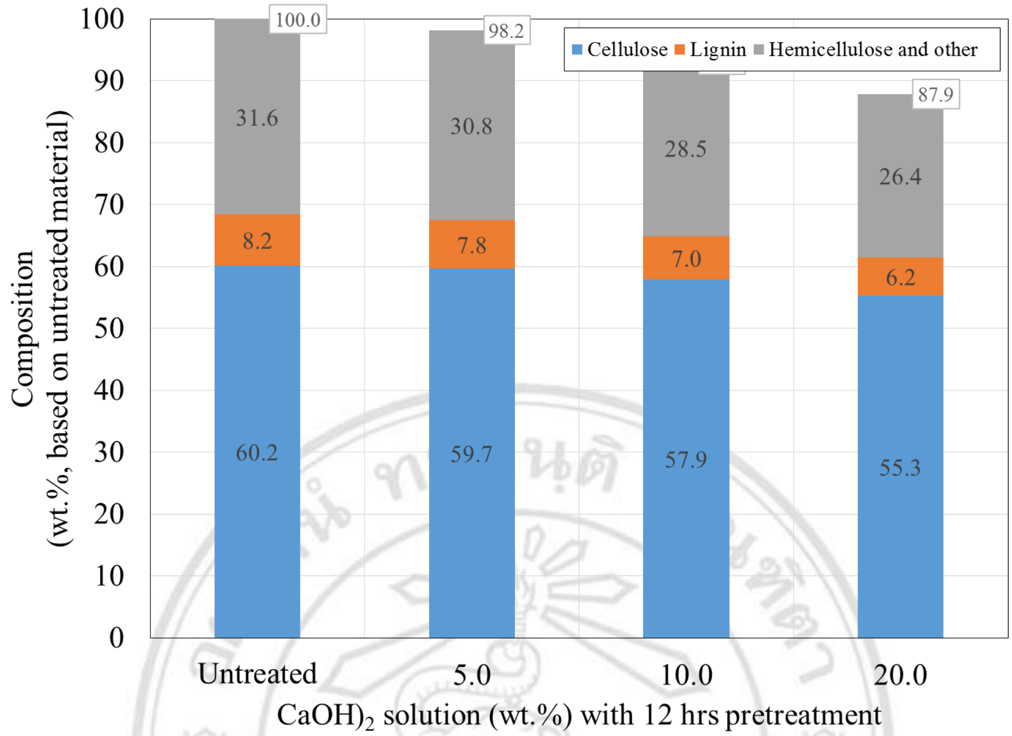

Figure 4.19 Remaining composition in Ca(OH)2 solution with 12 hrs pretreated material.

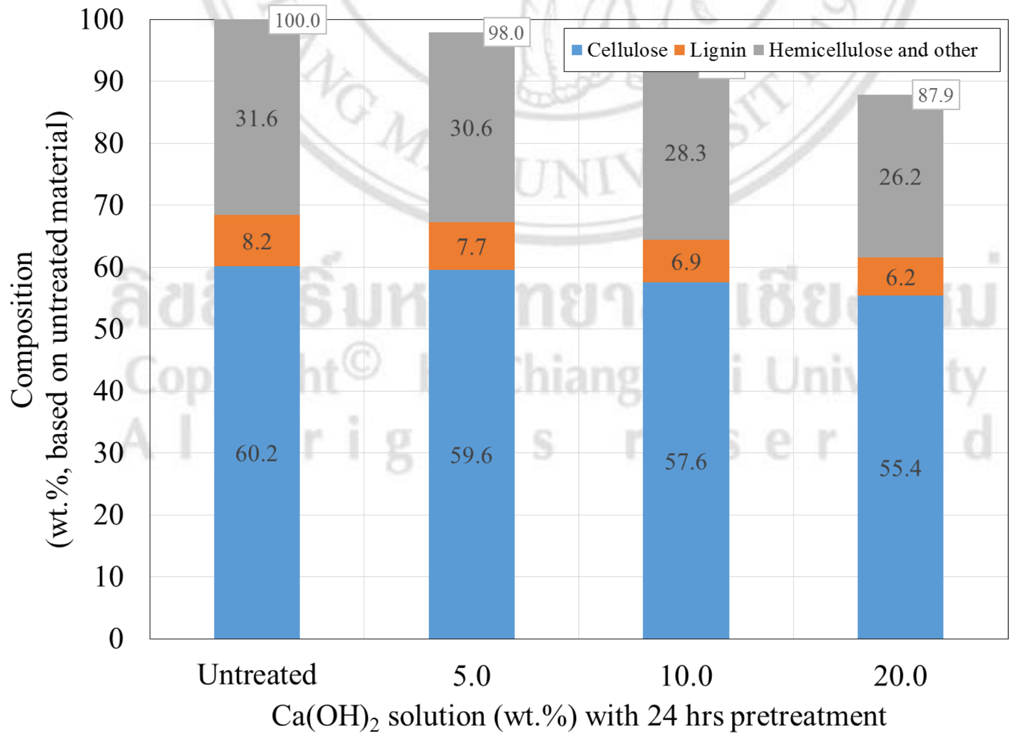

Figure 4.20 Remaining composition in Ca(OH)2 solution with 24 hr pretreated material.

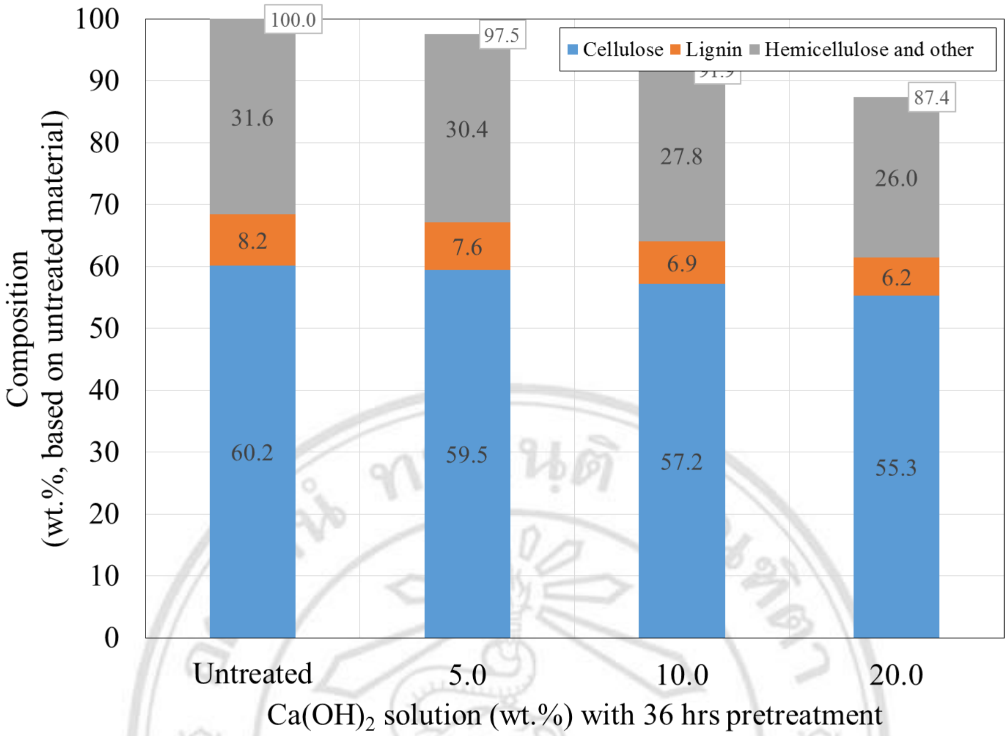

Figure 4.21 Remaining composition in Ca(OH)2 solution with 36 hrs. pretreated material.

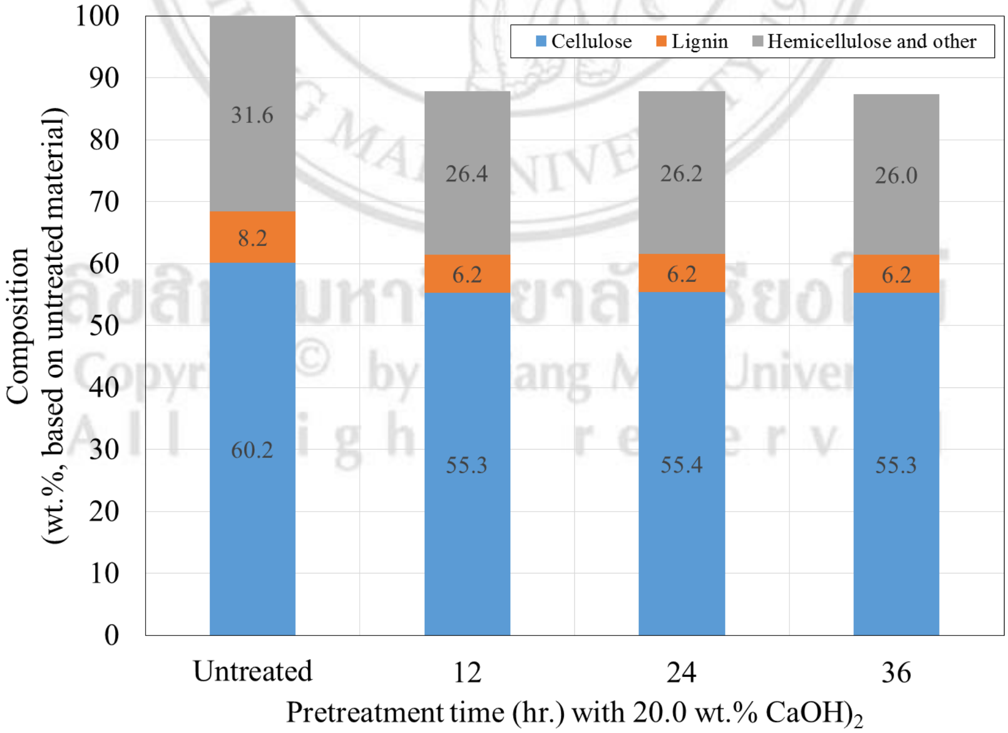

Figure 4.22 Effect of pretreatment time of Ca(OH)2 solution.

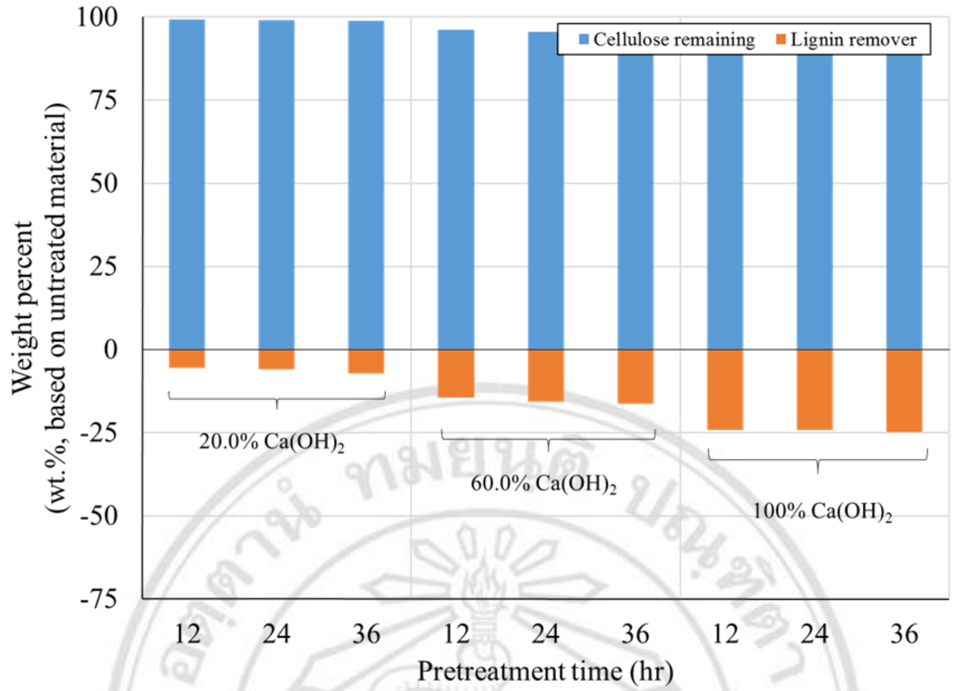

Figure 4.23 shows weight percent of cellulose remaining and lignin removal based on an untreated material.

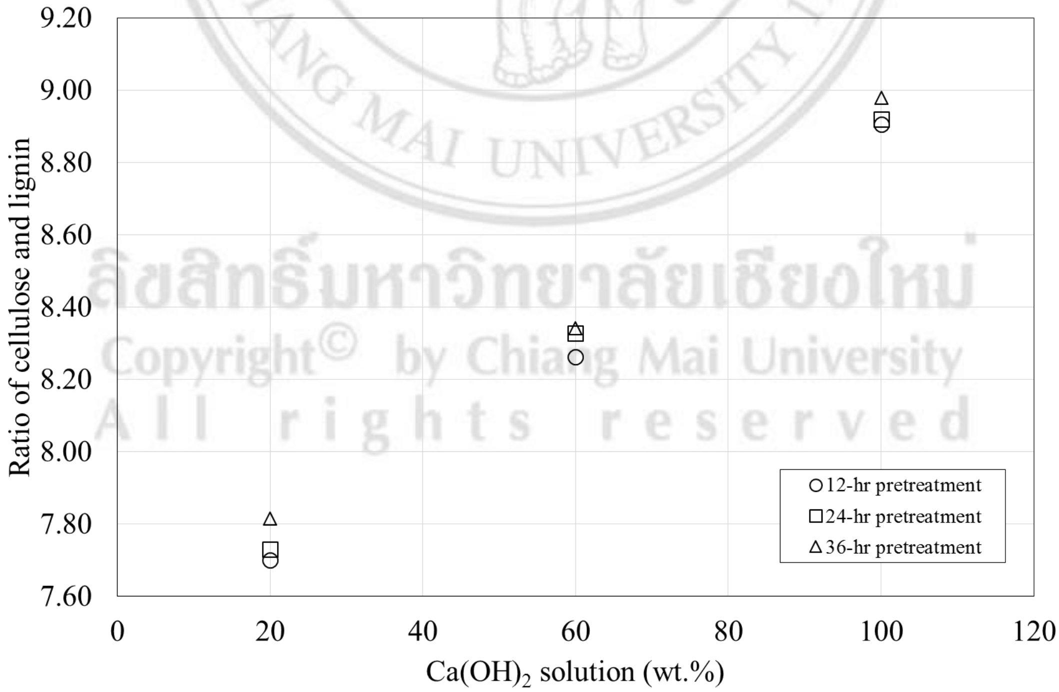

Figure 4.24 presents the ratio of cellulose to lignin for $Ca(OH)_2$ solution pretreatment.

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

Table 4.7 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 rose from 63.2% to 63.6% and lignin decreases from 7.0% to 7.0% 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 87.5% to 84.9%.

Table 4.7 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	Hemi-cellulose	Lignin	Other			Hemi-cellulose	Lignin	Other
0	63.2	21.1	7.0	8.6	87.5	91.9	22.2	24.9	3.3
10	63.3	21.1	7.0	8.5	87.0	91.5	22.9	25.7	4.7
20	63.5	21.0	7.0	8.5	86.0	90.8	23.9	26.6	6.6
30	63.6	21.0	7.0	8.4	84.9	89.8	25.1	27.6	8.7

Figure 4.25 shows the composition of $Ca(OH)_2$ pretreatment matter with ozone. It can be seen that all composition decrease slightly. Figure 4.26 compares the ratio of cellulose to lignin of material between ozone pretreatment of untreated and $Ca(OH)_2$ pretreatment. It is found that without alkaline and $Ca(OH)_2$ pretreated the ratio remains the same because $Ca(OH)_2$ pretreated can not reduce lignin for the material.

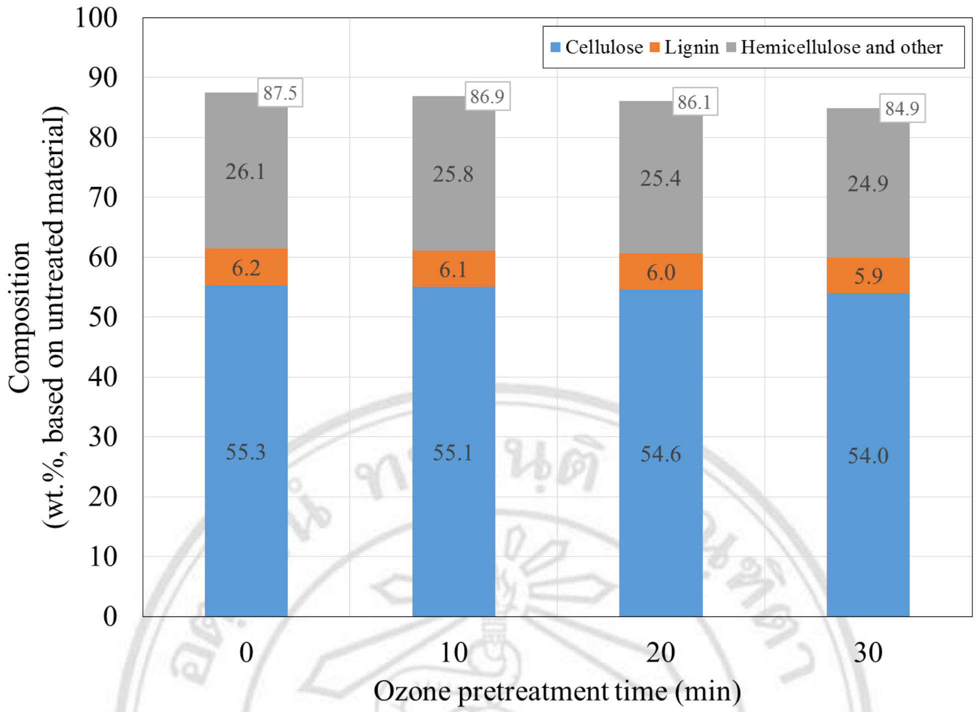

Fig 4.25 shows the composition of $Ca(OH)_2$ solution pretreatment matter with ozone.

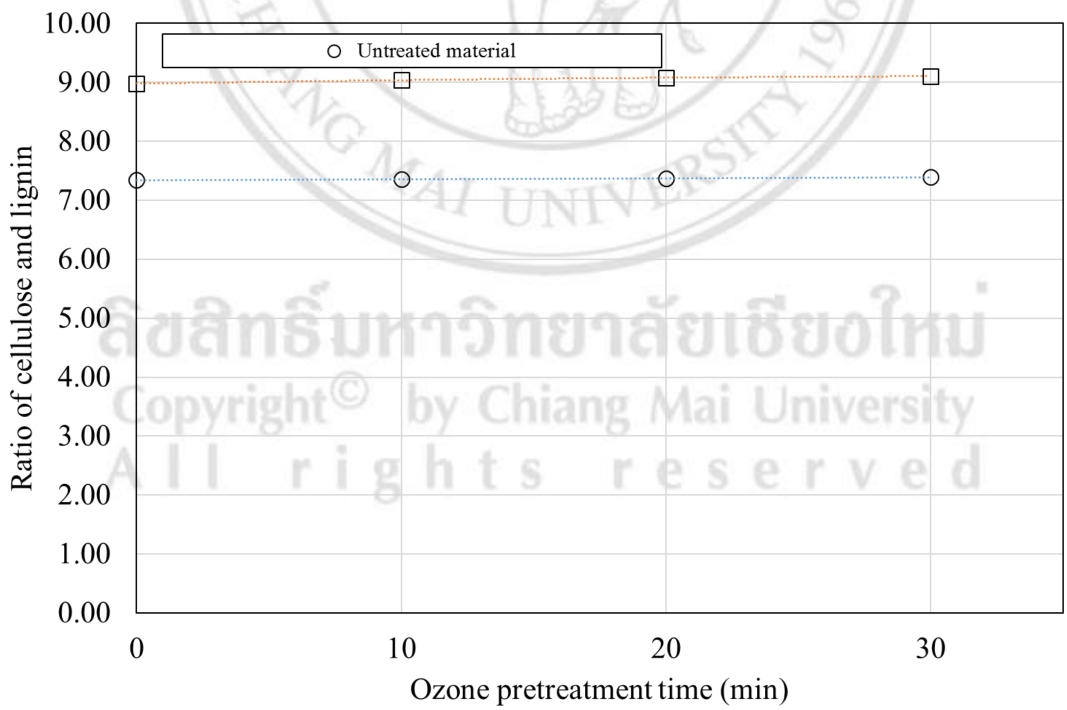

Fig 4.26 compare the ratio of cellulose to lignin of material with ozone pretreatment of untreated and $Ca(OH)_2$ solution pretreatment

4.5 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. Spectrophotometer is the method for Total phenolic analysis. The result is shown as table 4.8 and 4.9. 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 25.35±0.79, 9.83±0.03 and 3.06±0.03 respectively.

Table 4.8 Result of Acetaldehyde analysis.

No.	Pretreat	Result , mg/l
1	NaOH + O_3	Not Detected
2	NH_3 + O_3	Not Detected
3	$Ca(OH)_2$ + O_3	Not Detected

Table 4.9 Result of Total phenolic analysis.

No.	Pretreat	Result , mg/l
1	NaOH + O_3	25.35 ±0.79
2	NH_3 + O_3	9.83 ±0.03
3	$Ca(OH)_2$ + O_3	3.06 ±0.03

4.6 Mass flow diagram of ozonolysis

This topic illustrates mass flow diagram of ozonolysis which is the final step in delignification. This mass flow diagram is show the sample of reacted ozone calculation of bana grass which is pretreated with NaOH. The descriptions are as follows;

4.6.1 To calculate the amount of ozone generated from ECONOWATT machine model OZG that determines the flow of O2 in 5 per minute in 10, 20 and 30 minutes. The amount of ozone is analyzed by Iodometric method for the determination of ozone in a process gas. (Internatiomal ozone association, Quality Assurance Committee, Revised Standardized Procedure 001/96) as shown in Appendix D

4.6.2 Revise the experiment in 4.4.1.2 Effect of Ozone pretreatment and measure the remaining amount of Ozone from the reactivity (repeat this step 3 times)

4.6.3 Calculate the reacted ozone which react with bana grass by equation

Generated ozone – Remained ozone = Reacted ozone

The result is shown in table 6.1

4.6.4 The data of table 4.10 which is performed in 6.1.3 are shown as mass flow diagram of ozonolysis at 10,20 and 30 min. by fig 4.28, 4.29 and 4.30

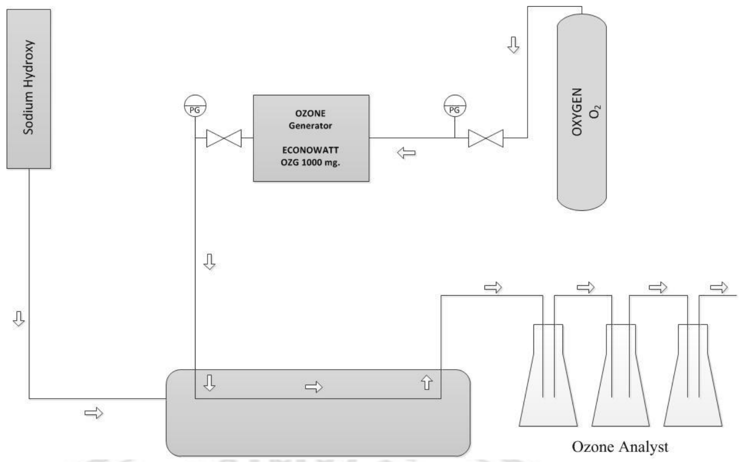

Fig 4.27 The ozonolysis treatment and ozone analysis apparatus.

Table 4.10 Mass flow data ozonolysis

Time of ozone flow (minute)	Generated ozone (mg.)	Remained ozone (mg.)	Reacted ozone
10	160	127	33
20	330	232	98
30	490	382	108

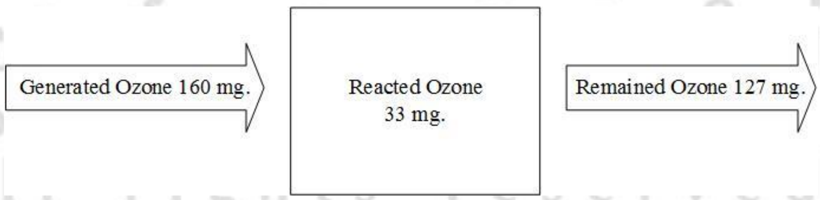

Fig 4.28 Mass flow diagram ozonolysis of 10 min.

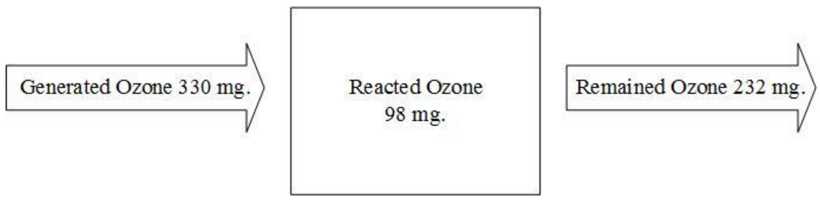

Fig 4.29 Mass flow diagram ozonolysis of 20 min.

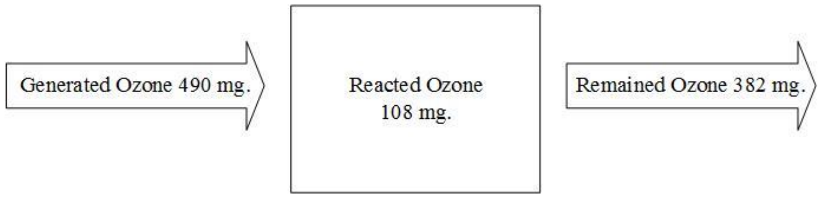

Fig 4.30 Mass flow diagram ozonolysis of 30 min.

From the result of ozonolysis, it is found that at 10 min of ozonolysis, there are 33 mg. of reacted ozone. At 20 min of ozonolysis, the amount of reacted ozone increases dramatically to 98 mg. Finally, at 30 min of ozonolysis, the amount of reacted ozone increases slightly to108 mg.

4.7 Conclusion

Lignin can be removed from Bana Grass 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 hr of pretreatment which is consider at ratio of cellulose to lignin. Ozone pretreatment only cannot eliminate lignin. But ozone can use to pretreat with alkaline solution to increase the efficiency of lignin elimination. The result of inhibitor examination found that it is not detect for Acetaldehyde inhibitor of NaOH with O_3, NH_3 with O_3 and $Ca(OH)_2$ with O_3 pretreated. But Total phenolic detected all of pretreatment condition.