CHAPTER 4 RESULT AND DISCUSSION

The purpose of this experiment is to study the influence of temperature, time and concentration on lignin removal and other products such as xylose, glucose, furfural, HMF and acetic acid. The 2 – levels of fractional factorial design (FFD) is applied to investigate the effect of temperature, time and concentration. Moreover, the most effective chemical reagent for removing lignin is revealed in this chapter.

4.1 Acid Pretreatment Result

Usually, the diluted acid catalyst is an attractive method that used in biorefinery industry to recover the sugar product from hemicellulose such as xylose (consisted 80% of the sugar content in hemicellulose fraction), arabinose, glucose, galactose and mannose while leaves the most cellulose and lignin in the solid fraction [46]. However, the further hydrolysis of monomeric sugar can produce inhibitor products such as furfural (product from xylose hydrolysis), HMF (product from glucose hydrolysis), weak acid, phenolic etc. Furfural and HMF affect cell growth and respiration of yeast in the sugar fermentation process; however, furfural is more toxic than HMF and the concentration in hydrolyzate is usually high. To keep further hydrolysis under temperature and acid catalyst, furfural and HMF will convert to levulinic and formic acid and the reaction is shown in Figure 4.1 [45]. Moreover, acetic acid can be generated from diluted acid pretreatment by hydrolyzed the acetyl groups in hemicellulose. [47].

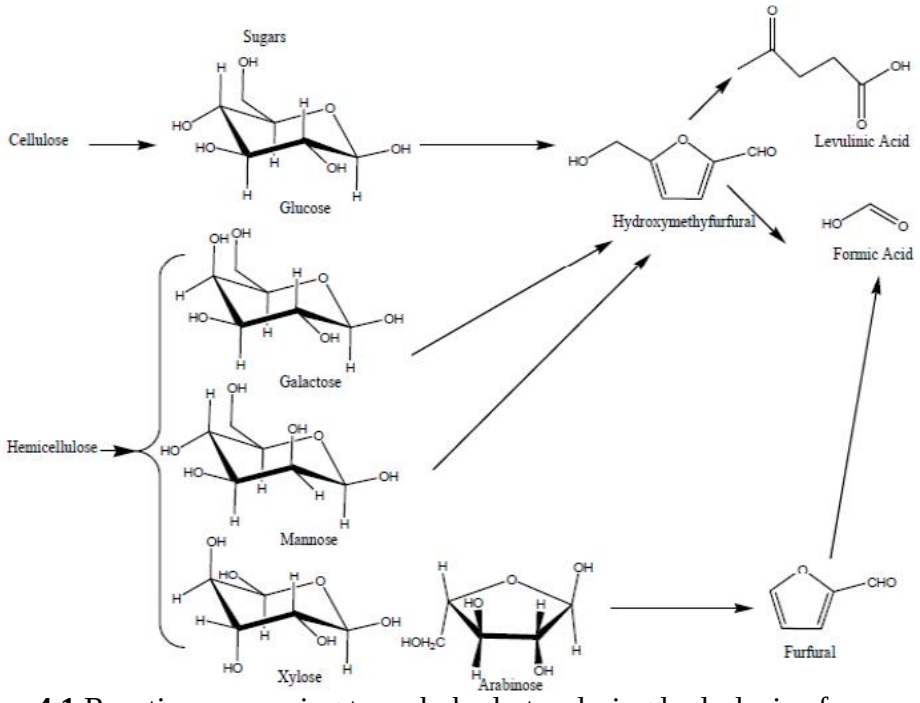


Figure 4.1 Reactions occurring to carbohydrates during hydrolysis of lignocellulosic material.

4.1.1 Sulfuric Acid (H_2SO_4) Pretreatment

1. The Result of Pretreatment product in Liquid Phase

Table 4.1 Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/l.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	HMF (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
A	-1,(1)	1,(130)	-1,(30)	22.2371	1.8786	0.1724	0.0000	0.4262	1.4341
B	-1,(1)	-1,(100)	1,(90)	11.8845	0.4695	0.0000	0.0000	0.3147	1.1294
C	1,(5)	-1,(100)	-1,(30)	16.8882	1.1773	0.0348	0.0000	0.3069	1.4399
D	1,(5)	1,(130)	1,(90)	14.1317	11.5518	2.6965	0.0729	0.4240	2.1166
E	0,(3)	0,(115)	0,(60)	19.4579	1.8323	0.2948	0.0000	0.3898	1.4782
F	0,(3)	0,(115)	0,(60)	21.3744	2.0205	0.3679	0.0000	0.3543	1.5609

Table 4.2 Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	HMF ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
A	-1,(1)	1,(130)	-1,(30)	0.0540	0.0046	0.0004	0.0000	0.0010	0.0035
B	-1,(1)	-1,(100)	1,(90)	0.0298	0.0012	0.0000	0.0000	0.0008	0.0028
C	1,(5)	-1,(100)	-1,(30)	0.0403	0.0028	0.0001	0.0000	0.0007	0.0034
D	1,(5)	1,(130)	1,(90)	0.0344	0.0281	0.0066	0.0002	0.0010	0.0052
E	0,(3)	0,(115)	0,(60)	0.0498	0.0047	0.0008	0.0000	0.0010	0.0038
F	0,(3)	0,(115)	0,(60)	0.0540	0.0051	0.0009	0.0000	0.0009	0.0039

The data from the table 4.1 and 4.2 are presented in the form of graph column shown in Figure 4.2 and 4.3. In Figure 4.2, the products from pretreated bagasse are shown in terms of concentration while in Figure 4.3; the product concentration is transformed into product content.

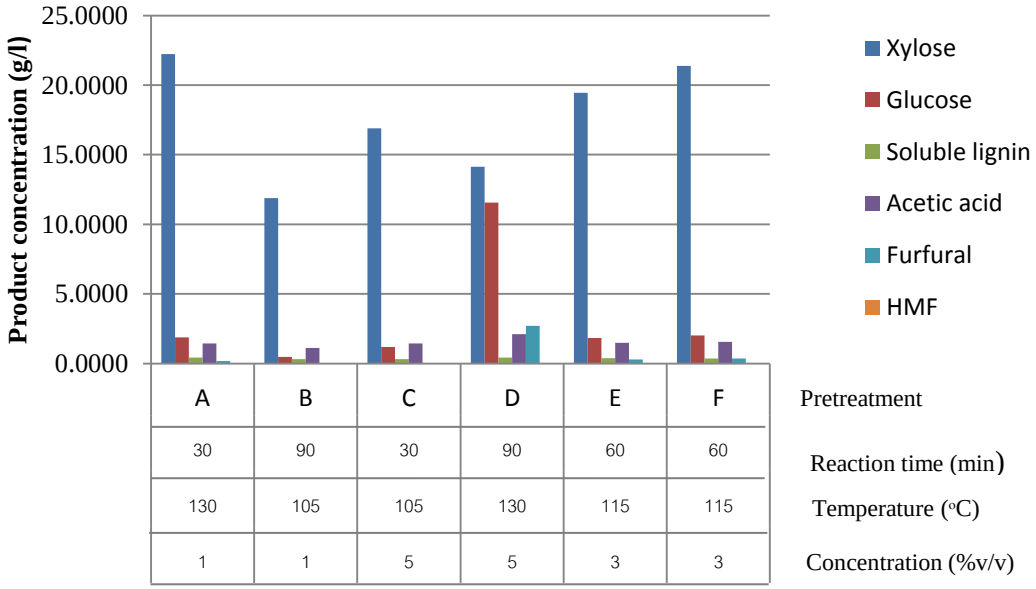


Figure 4.2 The product concentration (g/l) with different pretreatment condition by H_2SO_4 pretreatment.

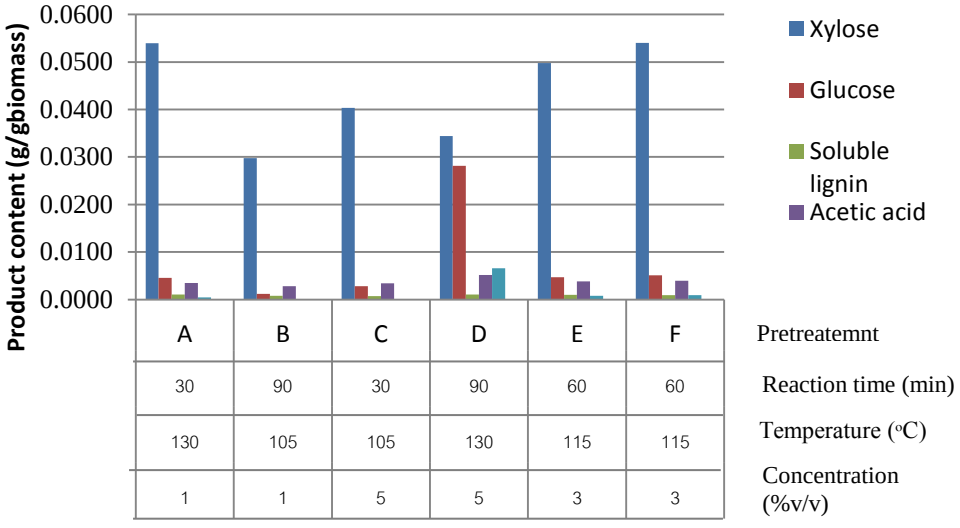


Figure 4.3 The product content ($g/g_{biomass}$) with different pretreatment condition by H_2SO_4 pretreatment.

Since hemicellulose is an amorphous structure that can be relatively hydrolyzed with diluted acid, the xylose concentration (major monomeric sugar component in hemicellulose structure) in hydrolyzate is higher than other products and the results are shown in Figure 4.2 and 4.3.

In Figure 4.2, the highest xylose concentration presents in pretreatment A (1% H_2SO_4 at 130 °C for 30 min). This condition produces the furfural product as a concentration of 0.1724 g/l while the maximum allowable concentration of bioethanol production of this product should be lower than 0.25 g/l [47]. The furfural is the decomposition product of monomeric sugar, such as arabinose and xylose. Then, when the process is further carried on under acid condition and temperature, five carbons sugar undergoes dehydration reaction. The monomeric sugars lost three water molecules to become a furfural. The formation of HMF is not reported, although this condition presents the glucose product. HMF is the product of decomposition of six carbons sugar (glucose) or the cellulose can be directly converted to HMF. The cellulose structure has crystalline region that makes it insoluble in water and non-dissolve in diluted acid at low temperature Therefore, the pretreatment under this condition is unsuitable for the formation of HMF although the glucose sugar is produced. Because of diluted acid pretreatment, the concentration of soluble lignin in hydrolyzate is consisted as low level (about 0.4262g/l). The 1.4341g/l of acetic acid is produced from the hydrolysis of acetyl groupon the side chain of hemicellulose. The concentration of acetic acid in pretreatment A is lower than the maximum allowable concentration that limited as 4 g/l [1].

The pretreatment D (5% H_2SO_4 at 130 °C for 90 min) presents 0.4240 g/l of soluble lignin and 14.1317 g/l of xylose together with furfural, HMF and acetic acid as a concentration of 2.6965 g/l, 0.0729 g/l and 2.1166 g/l, respectively. Although all of the parameters were maintained at highest condition, the xylose concentration is still consisted as low level. This condition produces the concentrated furfural and HMF. The furfural is produced when the pretreatment performs under acid condition and strongly increase with temperature and reaction time [47]. For this reason, when the pretreatment is carried out under longer temperature and time, the xylose is further hydrolyzed to form furfural product. So, the xylose concentration at condition D will decrease when the temperature and reaction time are increased. Furthermore, this condition presents the HMF that is the decomposition product of glucose. Since the pretreatment is maintained at strong condition, the highest glucose concentration is presented.

For the pretreatment E and F (3% v/v at 115 ºC for 60 min), these conditions show the center point in the fractional factorial design for estimating the error of the experiment. The concentration of the products of pretreatment E and F is different that can be occurred by the soaking time before autoclaving the sample or the poor mixing of samples.

For condition B (1% H_2SO_4 at 105 °C for 90 min), this condition shows the lowest concentration of xylose, glucose and acetic acid and the result is shown in Table 4.1.

This condition was performed under low level of acid concentration and temperature that is unsuitable to hydrolyze the lyxan chain in the backbone of hemicellulose composition.

According to pretreatment C (5 % H_2SO_4 at 105 °C for 30 min), this condition produces 16.8882 g/l and 1.1773 g/l of xylose and glucose, respectively. To compare with pretreatment B, this condition performs under the higher acid concentration but shorter reaction time. However, the concentrations of xylose, glucose and inhibitor products (furfural, HMF and acetic acid) are higher than pretreatment B.

The product concentration in Figure 4.2 is multiplied by the volume of hydrolyzate after filtration and then divided by the weight of bagasse in each condition. The product contents from Figure 4.3 have the meaning that "When using 1g of bagasse, how much of each product is created?"

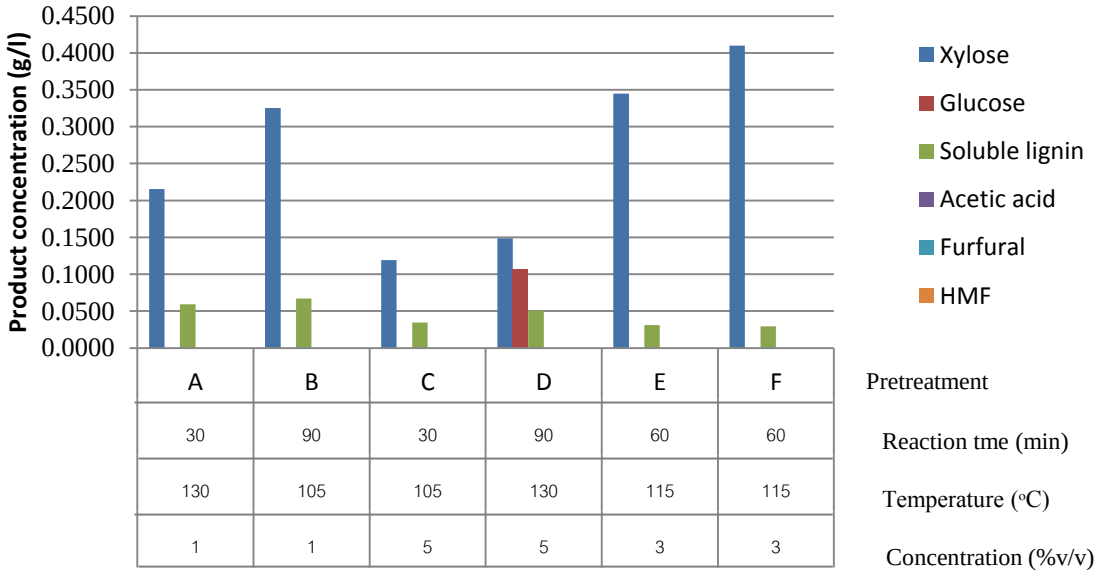


Figure 4.4 The product recovery (g/l) with different pretreatment condition by H_2SO_4 pretreatment.

After filtration, the pretreated solid was washed by 60 ºC distilled water until the pH of bagasse reaches the neutral value. After pretreatment, some decompositions of lignin are not hydrolyze into the filtrate, but still consist on the bagasse. To use the hot water can help to wash the broken down lignin and recovers it into washing water. The washing water for each condition would be sampled to determine the concentration of the recovered product and the result is shown in Figure 4.4. From the figure, the xylose shows the highest yield in the washing water, followed by a small portion of lignin, glucose, furfural, HMF and acetic acid. The highest yield of xylose derives from the pretreatment F as a concentration of 0.4100 g/l. For the glucose, this product presents as low concentration and the highest yield of this product presents in pretreatment D.

Other products present as low concentration because these compositions are produced in a small portion during the pretreatment step.

The concentration results of the products from the pretreatment process are analyzed to identify the significant parameters by using the analysis of variance (ANOVA) method and this step is carried out through the Unscramble X version 10.3 program.

2. The Analysis of Variance (ANOVA) of Product in Liquid Phase from H_2SO_4 Pretreatment

1. Xylose Content in Liquid Solution

According to FFD, the xylose concentrations vary from 11.8845 g/l to 22.2371 g/l and the data are shown in Table 4.1. The analysis of variance (ANOVA) result of the main effects (temperature, time and concentration) is shown in Table 4.3. The normal probability plot of the Y – residual for xylose content data is shown in Figure 4.5. The normal plot of Y - residual came up by default. If the distribution of Y - residual is normal, the plot will be a straight line [42].

Table 4.3 Effect estimates on xylose concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				4.2838	0.0443
Constant			17.6625		
H_2SO_4 concentration	C	-1.5500	-0.7550	0.5169	0.4927
Temperature	J	3.8000	-0.5860	3.1061	0.1160
Time	K	-6.5500	-5.7610	9.2284	0.0161

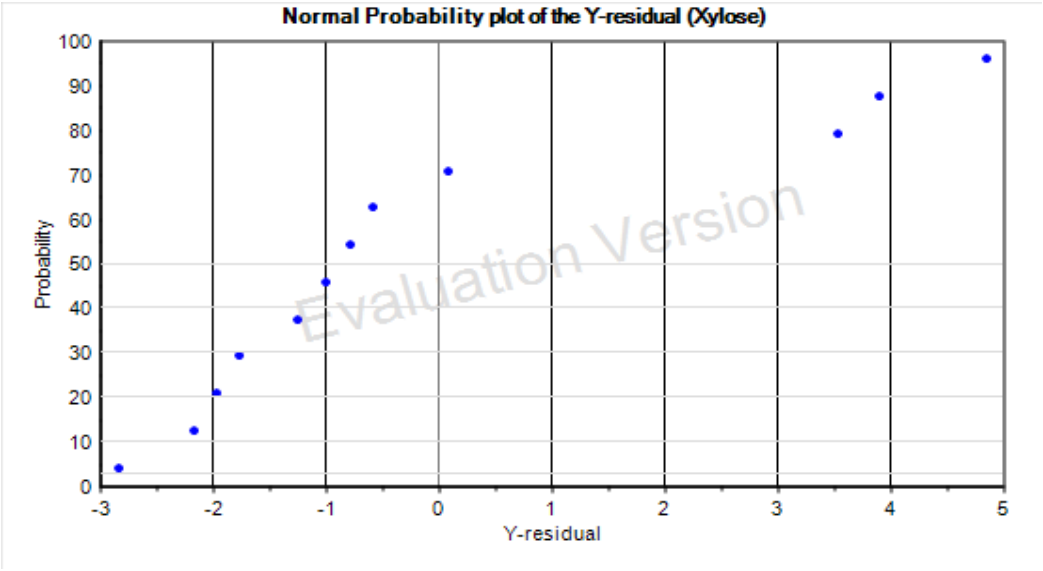


Figure 4.5 Normal plot of residuals of xylose content of H_2SO_4 pretreatment.

The ANOVA result of xylose could be concluded that only the reaction time significantly affected the xylose production. The estimated effect of reaction time was a negative value which means that the xylose product will increase when the reaction time is decreased. The coefficient of determination (R^2) was calculated to be 0.6163. This value indicated that the confidant level of statistics explains 62% of the variability in the data [48].

2. Glucose Content in Liquid Solution

The yields of glucose content vary from 0.4695 g/l to 11.5518 g/l and the data of glucose product are shown Table in 4.1. The analysis of variance result of the main effects is shown in Table 4.4. Moreover, the normal probability plot of glucose is shown in Figure 4.6.

Table 4.4 Effect estimates on glucose concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				43.2087	0.0000
Constant			3.1567		
H_2SO_4 concentration	C	5.1925	2.5963	42.7383	0.0002
Temperature	J	5.8925	2.9463	55.0381	0.0001
Time	K	4.4825	2.2413	31.8497	0.0005

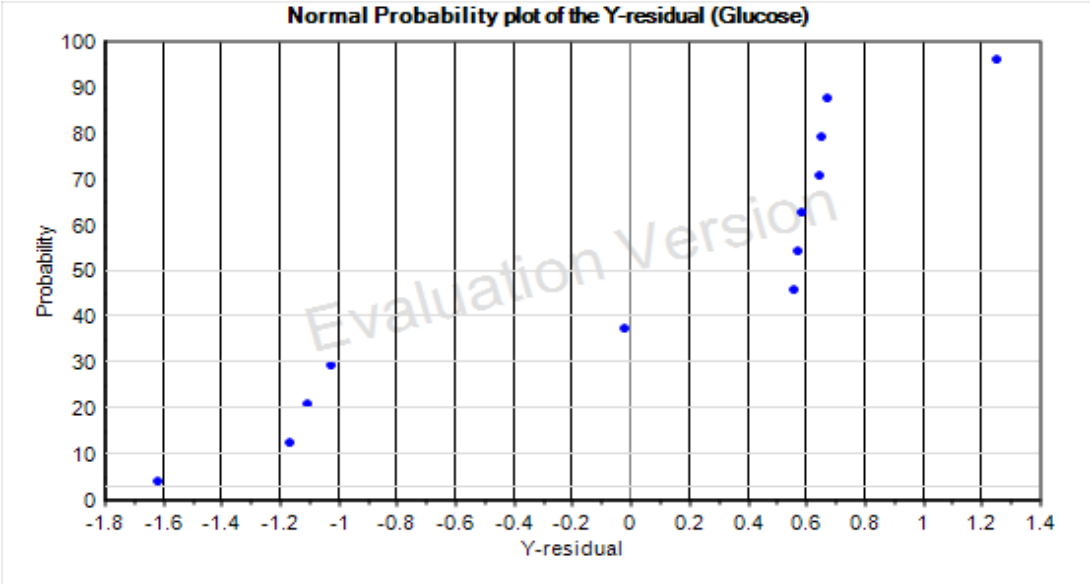


Figure 4.6 Normal plot of residuals of glucose content of H_2SO_4 pretreatment.

The ANOVA result of glucose could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the glucose production. The estimated effect of these variables was positive values which mean that the glucose product will increase when the effect of all parameters is increased. The coefficient of determination (R^2) was calculated to be 0.9419. This value indicated that the confidant level of statistics explains 94% of the variability in the data [48].

3. Furfural Content in Liquid Solution

The yields of furfural content vary from 0.0000 g/l to 2.6965 g/l and the data of furfural are shown in Table 4.1. The analysis of variance result of the main effects is shown in Table 4.5. Moreover, the normal probability plot of furfural is shown in Figure 4.7.

Table 4.5 Effect estimates on furfural concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	P - level
Model				65.4849	0.0000
Constant			0.5944		
H_2SO_4	C	1.2794	0.6397	61.9144	0.0000
concentration	J	1.4170	0.7085	75.9481	0.0000
Temperature	K	1.2446	0.6223	58.5921	0.0001
Time					

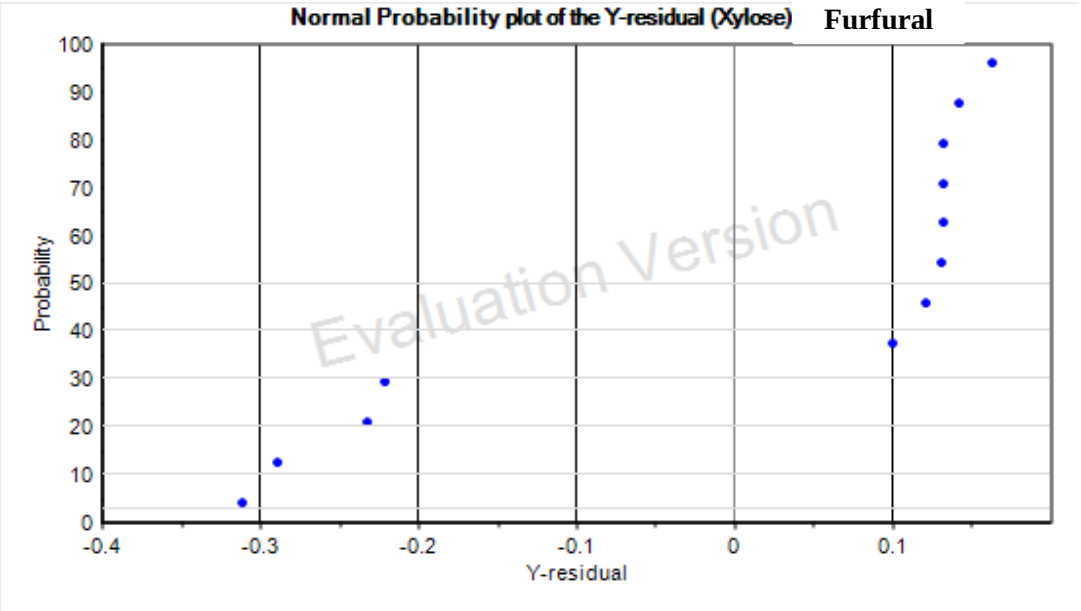


Figure 4.7 Normal plot of residuals of furfural content of H_2SO_4 pretreatment.

The result ANOVA result of furfural could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the furfural production. The estimated effect of these variables was positive values which mean that the furfural product will increase when the effect of all parameters is increased. The fitness of the model explains 96% of the variability in the data as discussed by R^2 of 0.9609 [48]

4. HMF Content in Liquid Solution

The HMF concentrations from pretreatment vary from 0.0000 g/l to 0.0729 g/l and the data of HMF are shown in Table 4.1. The analysis of variance result of the main effects is shown in Table 4.6. Moreover, the normal probability plot of HMF is shown in Figure 4.8.

Table 4.6 Effect estimates on HMF concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				23.7167	0.0002
Constant			0.0122		
H_2SO_4	C	0.0365	0.0182	23.7167	0.0012
concentration	J	0.0365	0.0182	23.7167	0.0012
Temperature	K	0.0365	0.0182	23.7167	0.0012
Time					

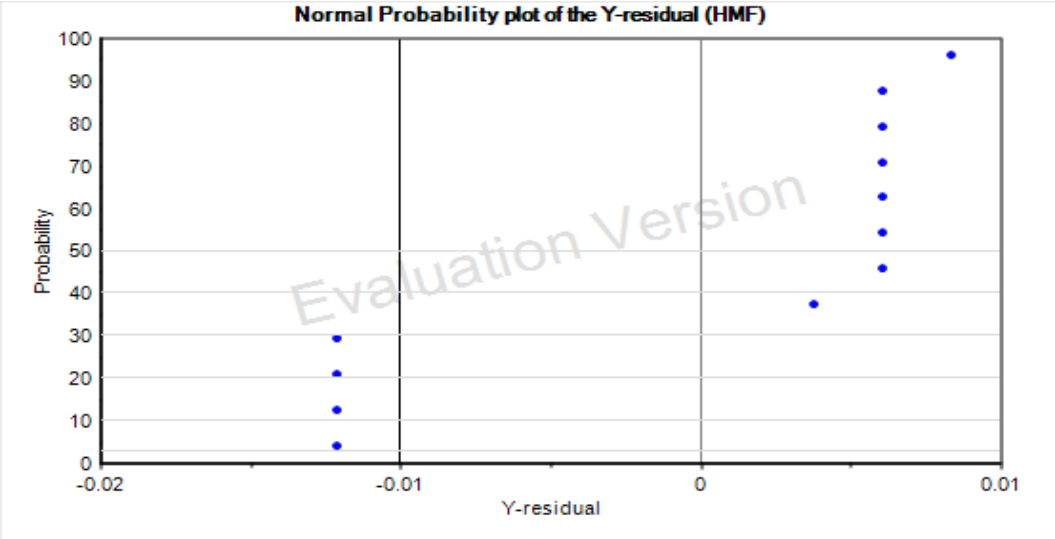


Figure 4.8 Normal plot of residuals of HMF content of H_2SO_4 pretreatment.

The ANOVA result of HMF could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the HMF production. The estimated effect of all parameters was positive values which mean that the HMF product will increase when the effect of all parameters is increased. The fitness of the model explains 90% of the variability in the data as discussed by R^2 of 0.8989 [48]

5. Soluble Lignin Content in Liquid Solution

Removing lignin from bagasse is the main objective of this experiment. From Table 4.1, soluble lignin concentrations vary from 0.3069 g/l to 0.4262 g/l. The analysis of variance result of the main effects is shown in Table 4.7. Moreover, the normal probability plot of soluble lignin is shown in Figure 4.9.

Table 4.7 Effect estimates on soluble lignin concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				18.7894	0.0006
Constant			0.3692		
H_2SO_4 concentration	C	-0.0050	-0.0025	0.1162	0.7419
Temperature	J	0.1100	0.0550	56.2518	0.0001
Time	K	0.0000	0.0000	0.0000	1.0000

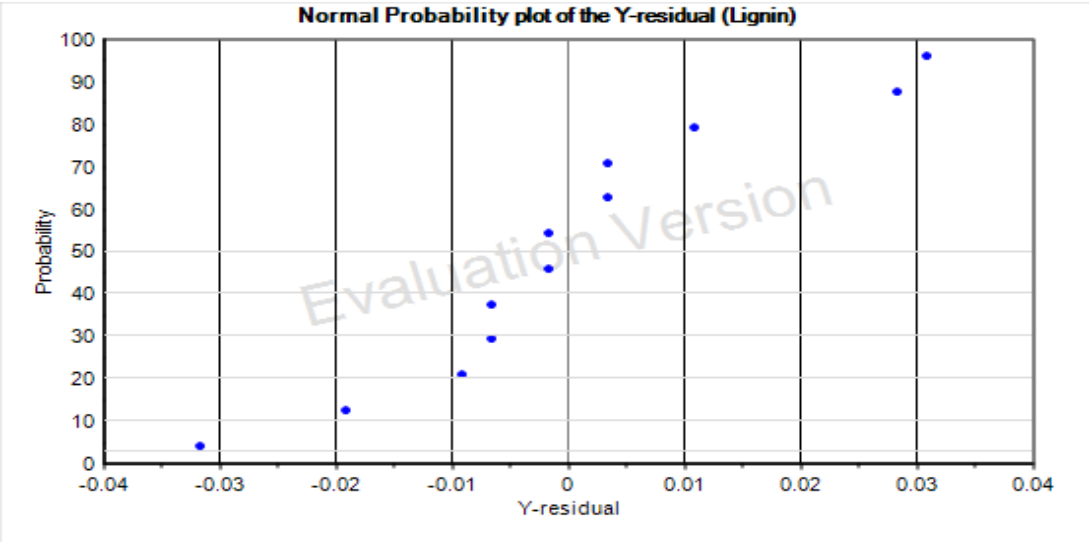


Figure 4.9 Normal plot of residuals of soluble lignin content of H_2SO_4 pretreatment.

According to The ANOVA result of soluble lignin revealed that only the temperature significantly affected the lignin removal and the estimated effect was a positive value. Moreover, the fitness of the model explains 88% of the variability in the data as discussed by R^2 of 0.8757 [48].

6. Acetic Acid Content in Liquid Solution

The yields of toxicity acetic acid production contents vary from 1.1294 g/l to 2.1166 g/l and the data of acetic acid are shown in Table 4.1. The analysis of variance result of the main effects is shown in Table 4.8. Moreover, the normal probability plot of acetic acid is shown in Figure 4.10.

Table 4.8 Effect estimates on acetic acid concentration from FFD of H_2SO_4 pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				15.2111	0.0011
Constant			1.5267		
H_2SO_4 concentration	C	0.5000	0.2500	21.5556	0.0017
Temperature	J	0.4950	0.2475	21.1266	0.0018
Time	K	0.1850	0.0925	2.9510	0.1242

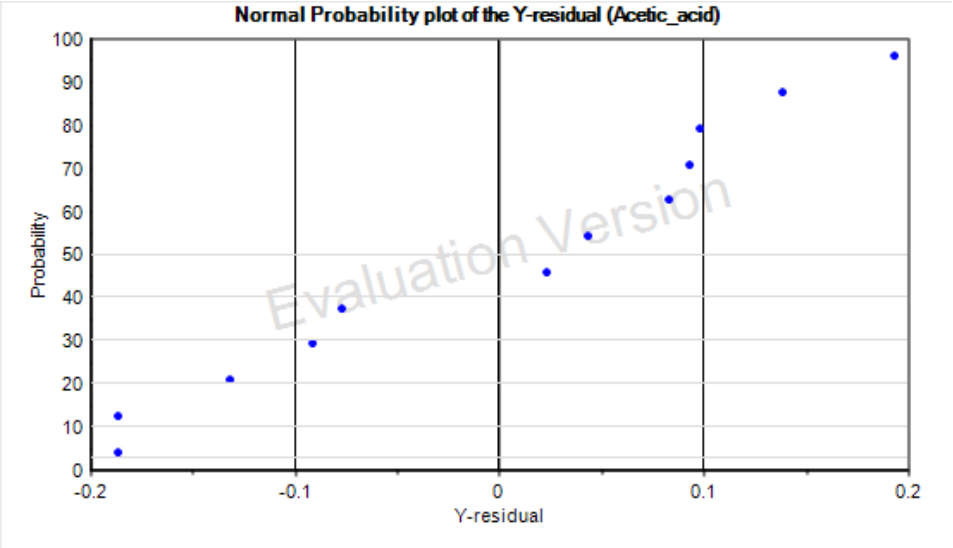


Figure 4.10 Normal plot of residuals of acetic acid content of H_2SO_4 pretreatment.

The ANOVA result of formation of acetic acid could be concluded that the H_2SO_4 concentration and temperature significantly affected the acetic acid production and the estimated effect was positive values. The coefficient of determination (R^2) was calculated to be 0.8508. This value indicated that the confidant level of statistics explains 85% of the variability in the data [48].

From the ANOVA result of xylose, the reaction time was the only parameter that significantly affected the xylose production and the estimate affected was a negative value which means that the change from low to high level will decrease the xylose production (Harry et al., 2010). If the xylose is the major product in pretreatment, the reaction time should be maintained at a low level. Candido.R.G el at., 2012 revealed that the concentrations of xylose increase relatively rapidly until 50 min because this period presents the hydrolysis of the easy-to-hydrolysis fraction of xylan. However, the hydrolysis rate will decrease after 50 min because this period presents the hydrolysis of hard-to-hydrolysis fraction of xylan.

For glucose production, the ANOVA result demonstrates that all of parameters significantly affected the glucose yield and the estimated effect values were positive. Therefore, to maintain the pretreatment at high condition means that the glucose concentration in hydrolyzate will increase. The glucose is derived from the hydrolysis of both hemicellulose and cellulose from lignocellulosic material. Since hemicellulose is a copolymer of C5 and C6, the monomeric glucose can be generated from this part or from the hydrolysis of the amorphous region in cellulose. However, the purpose of the pretreatment is to remove the physical barrier composition such as hemicellulose and lignin from the lignocellulosic biomass and retains as much as possible of cellulose composition in the solid phase. Therefore, the effective pretreatment should minimize the glucose concentration in hydrolyzate.

The amorphous three-dimensional polymer with phenylpropane unit leads the lignin to form the complex structure. For this reason, the hydrolyzate will be consisted with a dilute concentration of soluble lignin under the acid pretreatment condition. Because of acid condition, the condensation reactions are performed and these reactions are undesirable because they prevent lignin from the solubilization process (Candido.R.G el al., 2012). The ANOVA reveals that only the temperature significantly affected the lignin removal and the estimated effect was a positive value.

The diluted acid pretreatment can produce the toxic product such as furfural, HMF and acetic acid. These products are produced when the process is sustained at the longer pretreatment time and higher temperature [47]. From the ANOVA result, temperature, reaction time and acid concentration significantly affected furfural and HMF production with positive values of estimated effect. However, the H_2SO_4 concentration and temperature significantly affected the formation of acetic acid and the estimated effect values were positive. Then, to prevent the formation of furfural, HMF and acetic acid the process should maintain the level of temperature and reaction time as low as possible [47].

4.1.2 Hydrochloric Acid (HCl) Pretreatment

1. The Result of Pretreatment Product in Liquid Phase

Table 4.9 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of g/l.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	HMF (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
M	-1,(1)	1,(130)	-1,(30)	24.8159	2.2874	0.6797	0.0322	0.3578	1.9023
N	-1,(1)	-1,(100)	1,(90)	17.0364	0.8355	0.0498	0.0000	0.4351	1.6694
O	1,(5)	-1,(100)	-1,(30)	26.4780	1.9387	0.6486	0.0000	0.2369	2.5242
P	1,(5)	1,(130)	1,(90)	1.8267	17.8836	2.5310	0.2266	0.4908	2.4351
Q	0,(3)	0,(115)	0,(60)	20.6879	2.3575	1.2696	0.0257	0.2867	1.9723
R	0,(3)	0,(115)	0,(60)	23.5158	2.5563	1.2729	0.0000	0.2695	2.1296

Table 4.10 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	HMF ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
M	-1,(1)	1,(130)	-1,(30)	0.0566	0.0052	0.0015	0.0001	0.0008	0.0043
N	-1,(1)	-1,(100)	1,(90)	0.0475	0.0023	0.0001	0.0000	0.0012	0.0046
O	1,(5)	-1,(100)	-1,(30)	0.0692	0.0051	0.0017	0.0000	0.0006	0.0066
P	1,(5)	1,(130)	1,(90)	0.0046	0.0450	0.0064	0.0006	0.0012	0.0061
Q	0,(3)	0,(115)	0,(60)	0.0525	0.0060	0.0032	0.0001	0.0007	0.0050
R	0,(3)	0,(115)	0,(60)	0.0683	0.0074	0.0037	0.0000	0.0008	0.0062

The data from Table 4.9 and 4.10 can be presented in the form of graph column shown in Figure 4.11 and 4.12. In Figure 4.11, the products from pretreated bagasse are shown in terms of concentration while in Figure 4.12; the product concentration is transformed into product content.

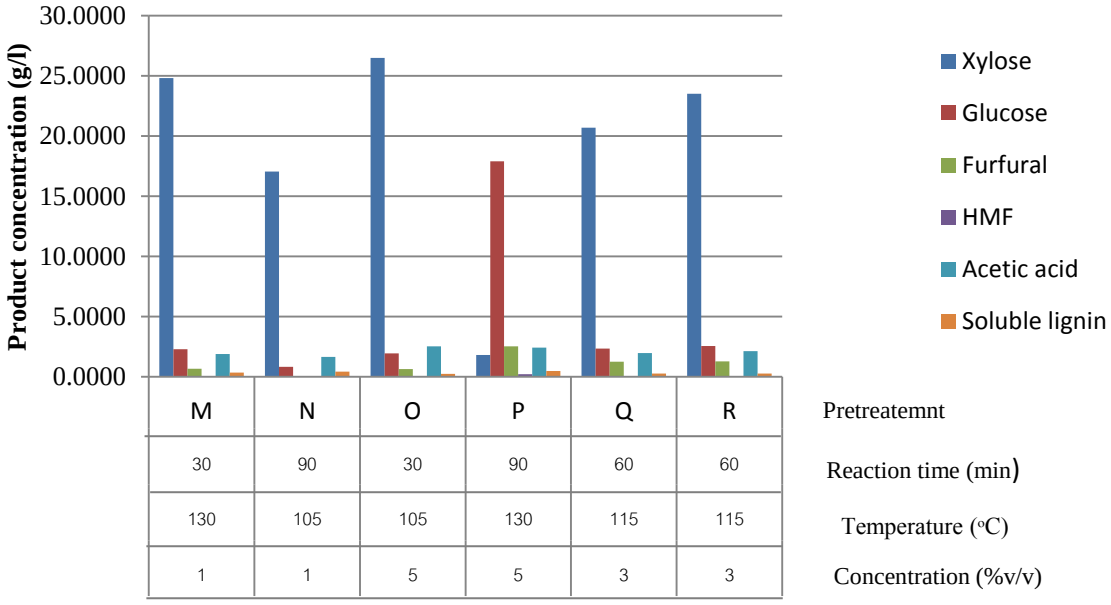


Figure 4.11 The product concentration (g/l) with different pretreatment condition by HCl pretreatment.

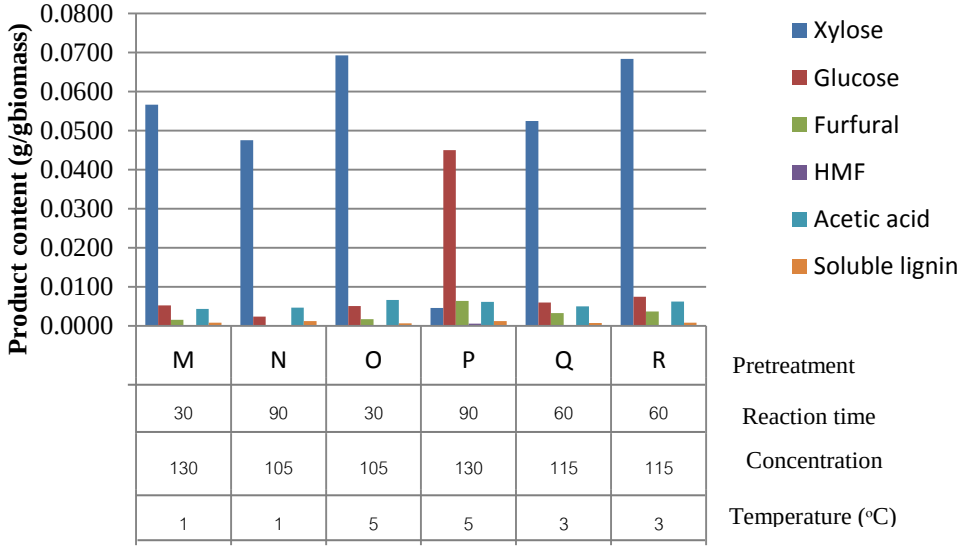


Figure 4.12 The product content ($g/g_{biomass}$) with different pretreatment condition HCl pretreatment.

The hydrochloric acid is the commercial chemical used in the diluted acid pretreatment Therefore, the main product of this pretreatment is the xylose sugar from hemicellulose hydrolysis. Moreover, the amorphous region of cellulose can be hydrolysed into glucose sugar and the further hydrolysis of xylose and glucose can be generated the inhibitor products such as furfural and hydroxymethylfurfural (HMF). The acetic acid is the production from hydrolysis of acetyl group on the side chain of hemicellulose. Since the pretreatment performs under mild condition of acid concentration and temperature, the hemicellulose can be extracted without affecting of cellulose and lignin [50]. For this reason, the lignin concentration in the hydrolyzate will consist as low level and the result is shown in Figure 4.11.

In Figure 4.11, the highest concentration of xylose is shown in the pretreatment O (5% v/v at 105 ºC for 30 min). The concentration of xylose, furfural and acetic acid were 26.4780 g/l, 0.6486 g/l and 2.5242 g/l, respectively. However, the maximum allowable concentration of furfural for bioethanol production is 0.25 g/l. The HMF concentration under this pretreatment is not reported although the glucose is produced as a concentration of 1.9387 g/l. Generally, the HMF product is hardly formed when compare with furfural.

For the pretreatment P (5% v/v at 130 ºC for 90 min), this condition presents the highest yield of glucose, furfural, HMF and acetic acid. Since the temperature, acid concentration and reaction time are maintained at the highest levels, this condition can hydrolyze the cellulose composition in bagasse. For the lowest xylose concentration might be caused by the hydrolysis of xylose sugar to furfural product.

For the pretreatment Q and R (3% v/v at 115 ºC for 60 min), these conditions show the center point in the fractional factorial design for estimating the error of the experiment. The concentration of the products of pretreatment Q and R is different that can be occurred by the soaking time before autoclaving the sample or the poor mixing of samples.

Pretreatment M carries out under 1% v/v at 130 ºC for 30 min which produces the xylose, glucose, furfural, HMF and acetic acid products as a concentration of 24.8159 g/l, 2.2874 g/l, 0.6797 g/l 0.0322 g/l and 1.9023 g/l, respectively. Although this condition is performed under the lowest acid concentration and reaction time, the xylose concentration presents as high concentration. The furfural content is higher than the maximum allowable concentration while HMF and acetic acid concentration are lower.

For pretreatment N (1% v/v at 105 ºC for 90 min), this condition presents 17.0364 g/l of xylose, 0.8355 g/l of glucose, 0.0498 g/l of furfural, 0.0000 g/l of HMF and 1.6694 g/l of acetic acid. Because of the low concentration of xylose, the furfural content is lower than the maximum allowable concentration.

Figure 4.12 presents the product content in $g/g_{biomass}$ unit. The product concentration in Figure 4.11 is multiplied by the volume of hydrolyzate after filtration and then divided by the weight of bagasse in each condition. The product contents from Figure 4.12 have the meaning that "When using 1g of bagasse, how much of each product is created?"

From Figure 4.13, the xylose product presents the highest recovered yield. For the glucose, the highest recovered yield shows in the pretreatment P because this pretreatment produces the highest yield of glucose product during the pretreatment step. To apply the hot water, the decomposition of glucan can be washed into the washing water. For the furfural, HFM, soluble lignin and acetic acid, the recovered yields in the washing water are lower than 0.1000 g/l. From the figure, the concentration of recovered xylose was less than 0.8000 g/l. Since each pretreatment is performed as the different condition, the volumes of the hot water that uses to wash the pretreated solid are different. The chemical concentration is the most important factor that affects the hot water consumption. For this reason, the concentrated acid concentration will consume the volume of hot water higher than the dilute acid.

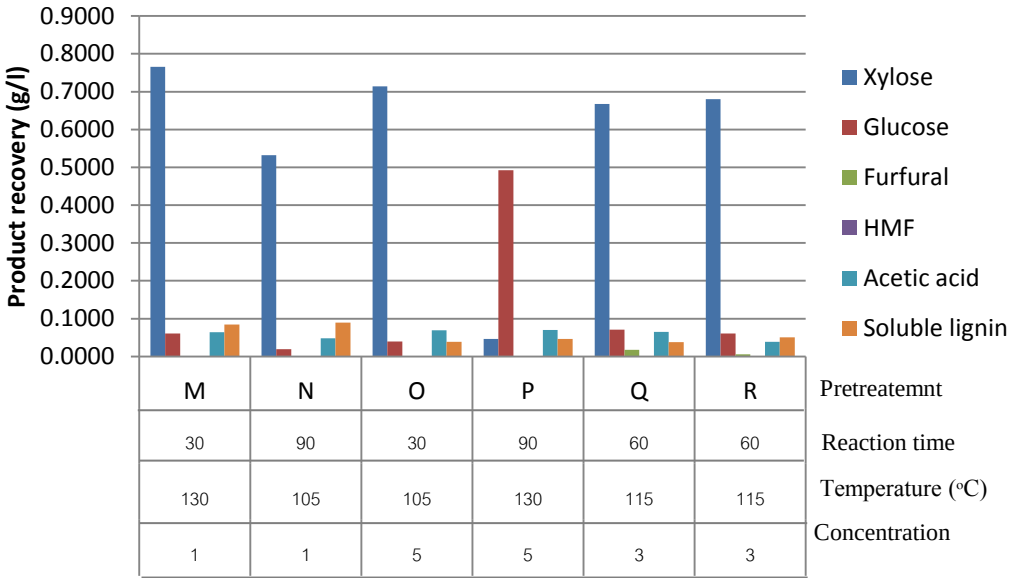


Figure 4.13 The product recovery (g/l) with different pretreatment condition HCl pretreatment.

The concentration results of the products from the pretreatment process are analyzed to identify the significant parameters by using the analysis of variance (ANOVA) method and this step is carried out through the Unscramble X version 10.3 program.

2. The Analysis of Variance (ANOVA) of Product in Liquid Phase from HCl Pretreatment

1. Xylose Content in Liquid Solution

According to FFD, the xylose concentrations vary from 1.8267 g/l to 24.8159 g/l and the data are shown in Table 4.9. The analysis of variance (ANOVA) result of the main effects (temperature, time and concentration) is shown in Table 4.11. The normal probability plot of the Y – residual for xylose content data is shown in Figure 4.14. The normal plot of Y - residual came up by default. If the distribution of Y - residual is normal, the plot will be a straight line [42].

Table 4.11 Effect estimates on xylose concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				23.7063	0.0002
Constant			19.0601		
HCl concentration	D	-6.7738	-3.3869	8.5877	0.0190
Temperature	J	-8.4360	-4.2180	13.3193	0.0065
Time	K	-16.2155	-8.1077	49.2120	0.0001

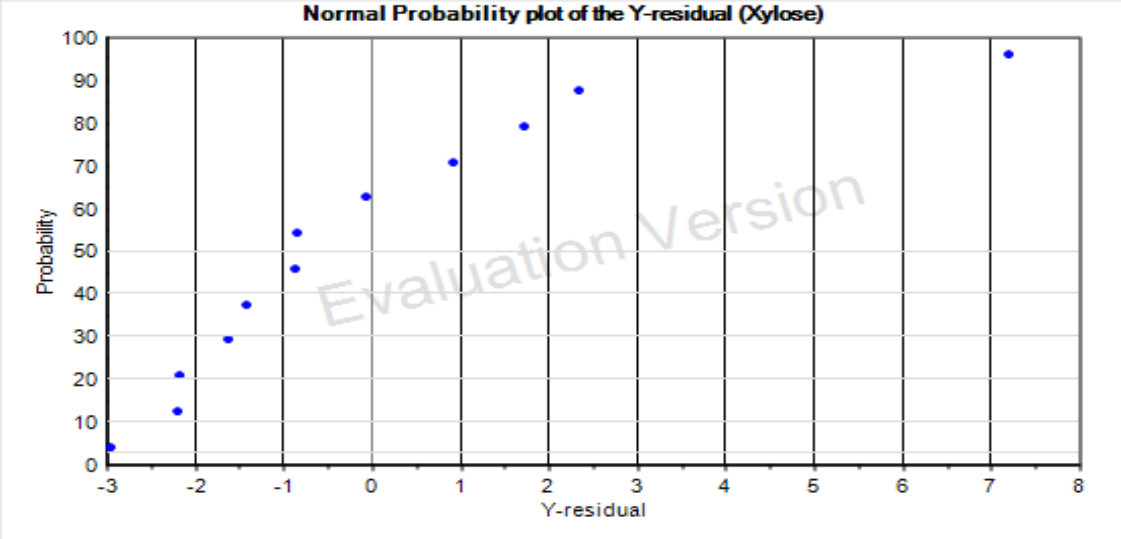


Figure 4.14 Normal plot of residuals of xylose content of HCl pretreatment.

The ANOVA result of xylose could be concluded that all of the parameters significantly affected the production of xylose. The estimated effect of reaction time, HCl concentration and temperature was negative values which mean that xylose product will increase when the effect of all parameters is decreased. The coefficient of determination (R^2) was calculated to be 0.8988. This value indicated that the confidant level of statistics explains 90% of the variability in the data [48].

2. Glucose Content in Liquid Solution

The yields of glucose contents vary from 0.8355 g/l to 17.8836 g/l and the data of glucose product are shown in Table 4.9. The analysis of variance result of the main effects is shown in Table 4.12. Moreover, the normal probability plot of glucose is shown in Figure 4.15.

Table 4.12 Effect estimates on glucose concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F – value	p - level
Model				33.9434	0.0001
Constant			4.6432		
HCl concentration	D	8.3497	4.1748	35.8747	0.0003
Temperature	J	8.6984	4.3492	38.9341	0.0002
Time	K	7.2465	3.6233	27.0214	0.0008

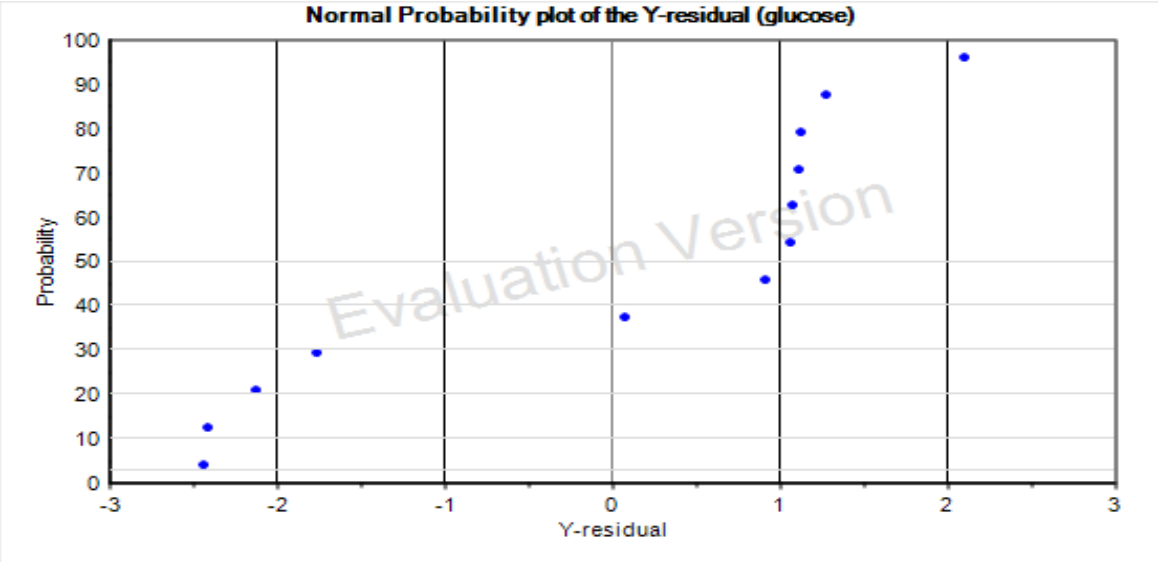


Figure 4.15 Normal plot of residuals of glucose content of HCl pretreatment.

The ANOVA result of glucose could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the glucose production. The estimated effect of these parameters was positive values which mean that the glucose product will increase when the effect of all parameters is increased. The coefficient of determination (R^2) was calculated to be 0.9271. This value indicated that the confidant level of statistics explains 93% of the variability in the data [48].

3. Furfural Content in Liquid Solution

The yields of furfural contents vary from 00498 g/l to 2.5310 g/l and the data of furfural are shown in Table 4.9. The analysis of variance result of the main effects is shown in Table 4.13. Moreover, the normal probability plot of furfural is shown in Figure 4.16.

Table 4.13 Effect estimates on furfural concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	P - level
Model				70.8280	0.0000
Constant			1.0753		
HCl concentration	D	1.2251	0.6125	91.8814	0.0000
Temperature	J	1.2561	0.6280	56.5903	0.0000
Time	K	0.6263	0.3131	24.0122	0.0012

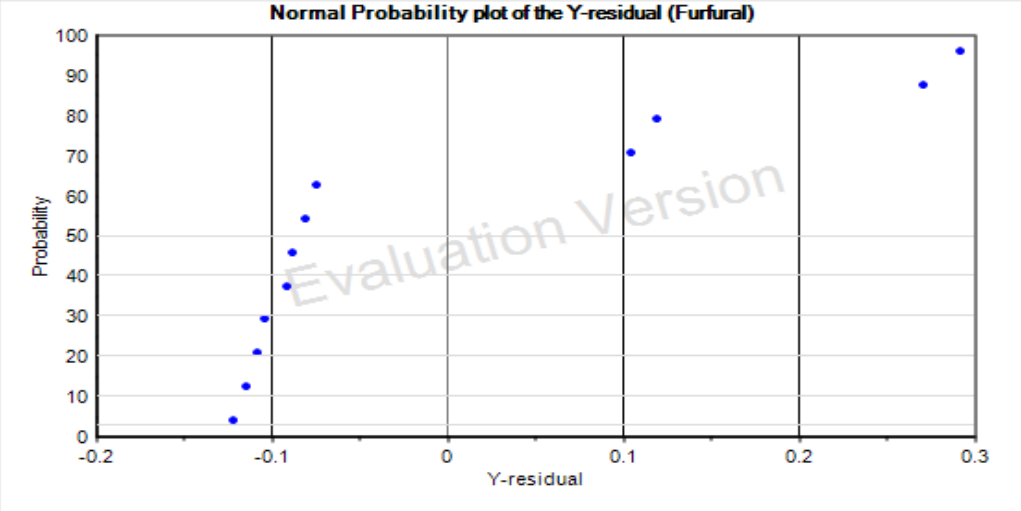


Figure 4.16 Normal plot of residuals of furfural content of HCl pretreatment.

The ANOVA result of furfural could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the furfural production. The estimated effect of reaction time, temperature and concentration was positive values which mean that the furfural product will increase when the effect of all parameters is increased. The fitness of the model explains 26% of the variability in the data as discussed by R^2 of 0.2598 [48]

4. HMF Content in Liquid Solution

The HMF contents vary from 0.0000 g/l to 0.226 g/l and the data of HMF are shown in Table 4.9. The analysis of variance result of the main effects is shown in Table 4.14. Moreover, the normal probability plot of HMF is shown in Figure 4.17.

Table 4.14 Effect estimates on HMF concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				23.0675	0.0003
Constant			0.0474		
HCl concentration	D	0.0972	0.0486	19.3449	0.0027
Temperature	J	0.1294	0.0647	32.5126	0.0005
Time	K	0.0972	0.0486	18.3449	0.0027

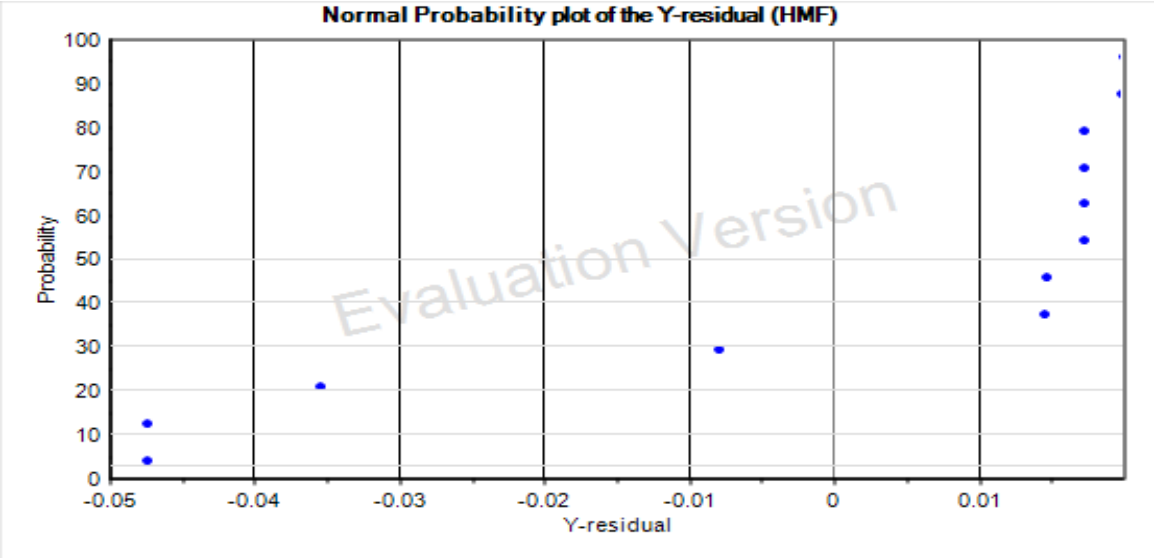


Figure 4.17 Normal plot of residuals of HMF content of HCl pretreatment.

The result of HMF production could be concluded that all of the independent variables such as temperature, H_2SO_4 concentration and reaction time significantly affected the formation of HMF. The estimated effect of these variables was positive values which mean that the HMF product will increase when the effect of all parameters is increased. The fitness of the model explains 90% of the variability in the data as discussed by R^2 of 0.8964 [48]

5. Soluble Lignin Content in Liquid Solution

Removing lignin from bagasse is the main objective of this experiment. From Table 4.9, soluble lignin concentrations vary from 0.02369 g/l to 0.4908 g/l. The analysis of variance result of the main effects is shown in Table 4.15. Moreover, the normal probability plot of soluble lignin is shown in Figure 4.18

Table 4.15 Effect estimates on soluble lignin concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				6.2632	0.0171
Constant			0.3461		
HCl concentration	D	-0.0326	-0.0163	0.5504	0.4794
Temperature	J	0.0883	0.0442	4.0377	0.0794
Time	K	0.1656	0.0826	14.2016	0.0055

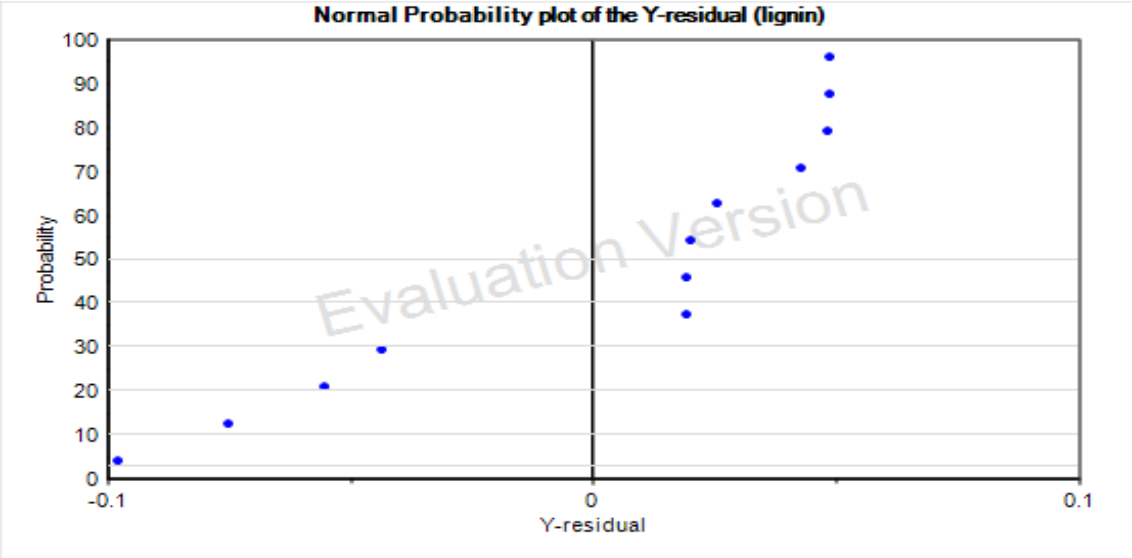


Figure 4.18 Normal plot of residuals of soluble lignin content of HCl pretreatment.

The ANOVA result of soluble lignin revealed that only the reaction time significantly affected the lignin removal and the estimated effect was a positive value. Moreover, the fitness of the model explains 70% of the variability in the data as discussed by R^2 of 0.7014 [48].

6. Acetic Acid Content in Liquid Solution

The yields of toxicity acetic acid production contents vary from 1.6694 g/l to 2.5242 g/l and the data of acetic acid are shown in Table 4.9. The analysis of variance result of the main effects is shown in Table 4.16. Moreover, the normal probability plot of acetic acid is shown in Figure 4.19.

Table 4.16 Effect estimates on acetic acid concentration from FFD of HCl pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				6.1053	0.0183
Constant			2.1055		
HCl concentration	D	0.6938	0.3469	17.2043	0.0032
Temperature	J	0.0720	0.0360	0.1851	0.6784
Time	K	-0.1610	-0.0805	0.9266	0.3693

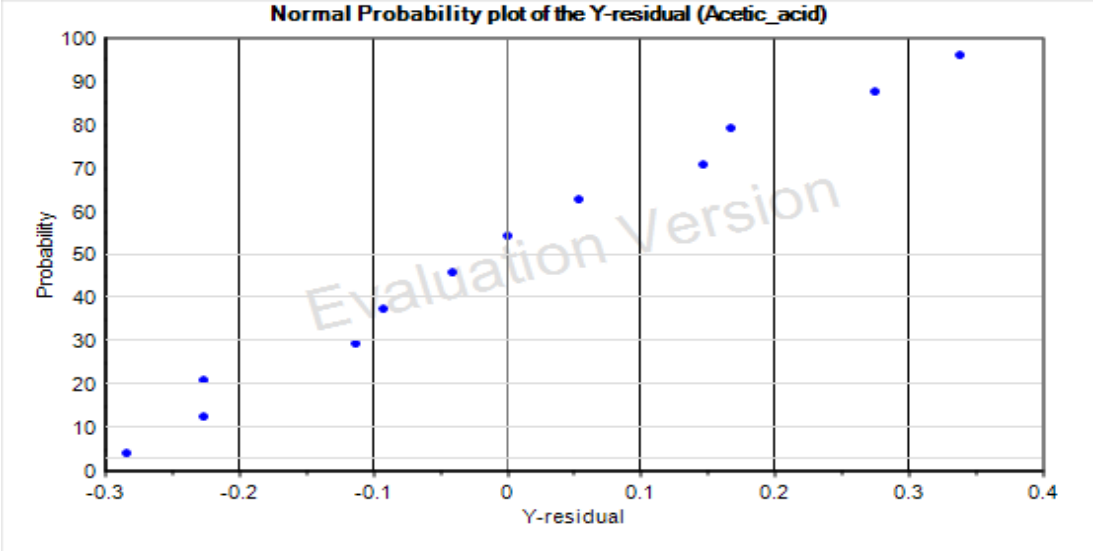


Figure 4.19 Normal plot of residuals of acetic acid content of HCl pretreatment.

The ANOVA result of formation of acetic acid could be concluded that only the HCl concentration significantly affected the acetic acid production and the estimated effect was a positive value. The coefficient of determination (R^2) was calculated to be 0.6906. This value indicated that the confidant level of statistics explains 69% of the variability in the data [48].

From the ANOVA result of xylose, all of the parameters (temperature, reaction time and HCl concentration) significantly affected the xylose production and the estimated effect was negative values which mean that the change from low to high level will decrease the production (Harry et al., 2010). The estimated effect results shown in Table 4.11 reveal that the reaction time was the most effective parameter for xylose production,

followed by temperature and HCl concentration. Candido.R.G el at., 2012 presented the effect of reaction time on the lignocellulose hydrolysis by using the 10% v/v H_2SO_4 at the temperature about 100 ºC. The result showed that the concentrations of xylose increase relatively rapidly until 50 min because this period presents the hydrolysis of the easy-to-hydrolysis fraction of xylan. After that the hydrolysis rate was slower, but measured rate due to this period composed of the hydrolysis of hard-to-hydrolysis fraction of xylan. Moreover, to increase the temperature and reaction time under strong acid condition, the xylose concentration will decrease because the xylose is further hydrolyzed to form the furfural product.

For glucose production, the ANOVA result demonstrates that all of parameters significantly affected the glucose yield and the estimated effect values were positive. The glucose product derives from both hemicellulose and cellulose from lignocellulosic material. Since hemicellulose is a copolymer of C5 and C6, the monomeric glucose can be generated from this part or from the hydrolysis of the amorphous region in cellulose. The main purpose of pretreatment method would like to retain as much as possible of cellulose in the pretreated solid, so the effective pretreatment should minimize the concentration of glucose in the hydrolyzate.

The diluted acid pretreatment can produce the toxic product such as furfural, HMF and acetic acid. These products are produced when the pretreatment is maintained at the longer pretreatment time and higher temperature [47]. The acetic acid is generated from the hydrolysis of acetyl group on the backbone of hemicellulose chain. From the ANOVA result, the temperature, reaction time and acid concentration significantly affected furfural and HMF production and the estimated effect values were positive. For acetic acid, only the HCl concentration significantly affected this product and the estimated effect was a positive value. Acetic acid is the enzyme inhibitor that its concentration should be controlled lower than 4 g/l. Then, to prevent the formation of furfural, HMF and acetic acid, the process should maintain the level of temperature, reaction time and HCl concentration as low as possible [47].

The three-dimensional amorphous with phenylpropane unit leads the lignin to form the complex structure. For this reason, the hydrolyzate will be consisted with a dilute concentration of soluble lignin under the acid pretreatment. Because of acid condition, the condensation reactions are performed that lead to the low concentration of soluble lignin. From the ANOVA result, only the reaction time significantly affected lignin removal and the estimated value was a positive value. Generally, under the mild condition of diluted acid pretreatment, the hemicellulose can be extracted without affecting to the cellulose and lignin content in biomass.

4.2 Alkaline Pretreatment Result

4.2.1 Sodium Hydroxide (NaOH) Pretreatment

1. The result of pretreatment product in liquid phase

Table 4.17 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of g/l**.**

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	HMF (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
G	-1,(1)	1,(130)	-1,(30)	0.0000	0.0000	0.0000	0.0000	22.6631	3.7897
H	-1,(1)	-1,(100)	1,(90)	0.0000	0.0000	0.0000	0.0000	19.1956	3.1420
I	1,(5)	-1,(100)	-1,(30)	0.0635	0.0000	0.0000	0.0000	42.9598	3.1555
J	1,(5)	1,(130)	1,(90)	0.0710	0.0000	0.0000	0.0000	71.7200	2.7068
K	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	60.8362	3.6858
L	0,(3)	0,(115)	0,(60)	0.0672	0.0000	0.0000	0.0000	64.8665	3.6502

Table 4.18 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	HMF ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
G	-1,(1)	1,(130)	-1,(30)	0.0000	0.0000	0.0000	0.0000	0.0270	0.0045
H	-1,(1)	-1,(100)	1,(90)	0.0000	0.0000	0.0000	0.0000	0.0215	0.0035
I	1,(5)	-1,(100)	-1,(30)	0.0001	0.0000	0.0000	0.0000	0.0922	0.0068
J	1,(5)	1,(130)	1,(90)	0.0001	0.0000	0.0000	0.0000	0.1495	0.0057
K	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	0.1165	0.0071
L	0,(3)	0,(115)	0,(60)	0.0002	0.0000	0.0000	0.0000	0.1483	0.0083

The data from Table 4.17 and 4.18 can be presented in the form of graph column shown in Figure 4.20 and 4.21. In Figure 4.20, the products from pretreated bagasse are shown in terms of concentration while in Figure 4.21; the product concentration is transformed into product content.

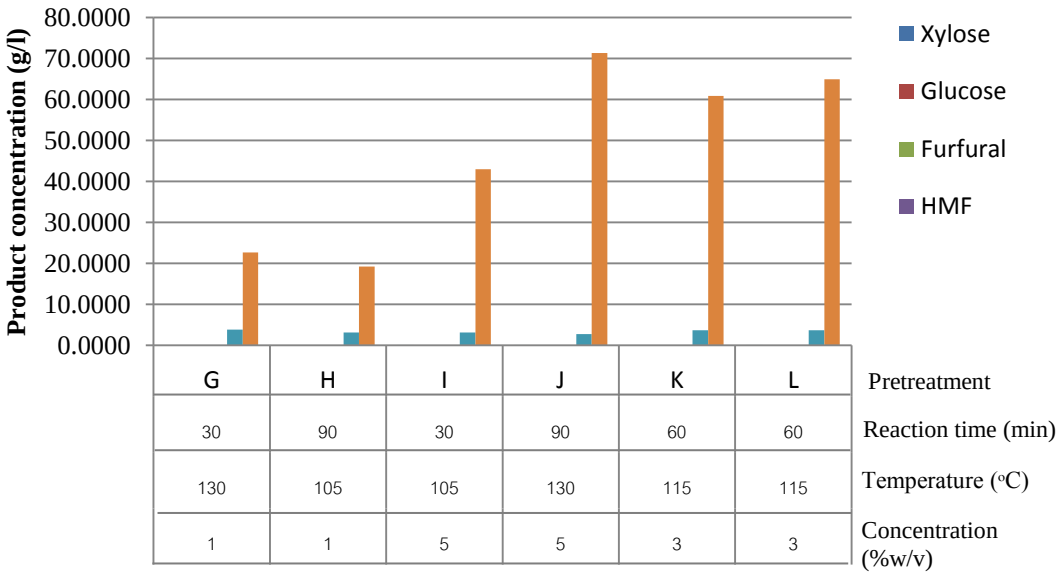


Figure 4.20 The product concentration (g/l) with different pretreatment condition NaOH pretreatment.

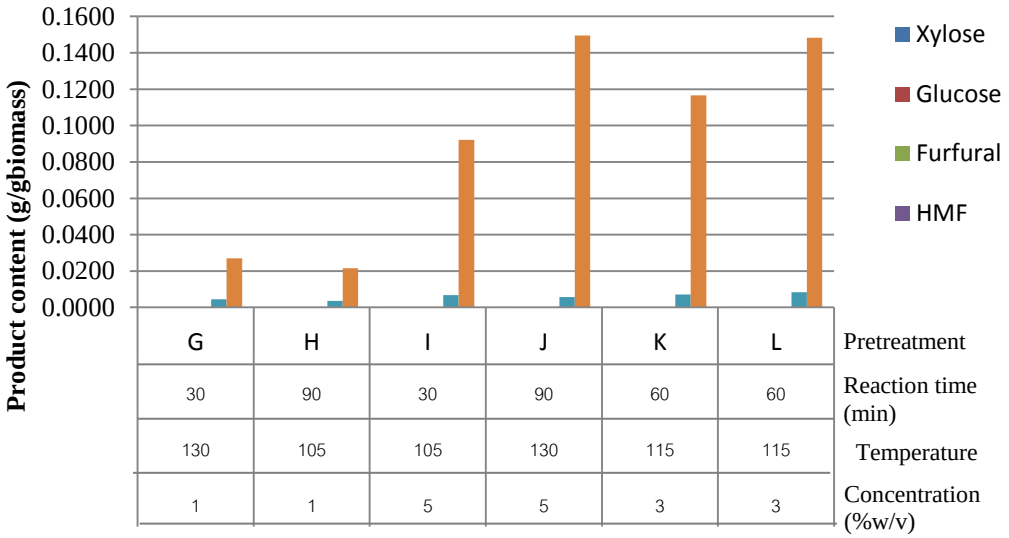


Figure 4.21 The product content ($g/g_{biomass}$) with different pretreatment condition NaOH pretreatment.

In the biofuels industry, the purpose and challenge of cellulosic material pretreatment is to remove lignin from the selective manner while cellulose and hemicellulose are preserved in the substrate. Moreover, to open the cellulose structure for enzymatic accessibility and break down the cellulose and hemicellulose to monomeric sugar are also the aims of pretreatment. Lignin is considered to be a major physical barrier to the enzymatic hydrolysis of cellulosic material. Since lignin provides the surface onto the nonproductive and irreversible adsorption of enzymatic, the effectiveness of enzymatic hydrolysis is decreased. To remove lignin will create the porosity that allows cellulose and hemicellulose to be more accessible. The swelling of cellulose can help to increase the reactivity of hydrolysis because the cellulose surface will be more contacted by enzyme [49]. The diluted alkaline pretreatment is applied to disturb the lignocellosic cell wall by dissolving lignin, hemicellulose and silica and break down the uronic, acetic acid ester and also swelling cellulose. Under diluted alkaline solution, lignin decomposition occurs via the cleavage of α – aryl ether bonds from polyphenolic monomers while the hemicellulose is dissolved and the hydrogen bond weakening help to the swelling of cellulose. Using sodium hydroxide is presented the most effective chemical for lignin degradation and fragment fermentation yield when compared to other alkaline chemicals [11]. To apply the diluted alkaline solution, lignin is the major component dissolved in the filtrate and the result presents in Figure 4.20.

The pretreatment J (5% NaOH at 130 °C for 90 min) shows the greatest of lignin removal from the bagasse (71.7200 g/l) with xylose concentration of 0.0710 g/l. This condition doesn't present the production of glucose, furfural and HMF. This result demonstrates that the hemicellulose is hardly hydrolyzed by NaOH solution that leads to the dilute concentration of xylose in hydrolyzate. The lignin removal occurs via the cleavage of the ester bonds between hydroxycinnamic acids and the α-benzyl ether linkages of the cell wall (Mosier et al,. 2005; Silerstien, Chen, Sharma-Shivappa, Boyette, & Osborne, 2007; Zhao et al,. 2008). Under diluted alkaline condition, the OH ion hydrolyzes acetyl group on polysaccharide chain of hemicellulose, so the process can detect the formation of acetic acid. The acetic acid is harmful to yeast when its concentration is located between 4 and 10 g/l.

For the pretreatment K and L (3% w/v at 115 ºC for 60 min), these conditions show the center point in the fractional factorial design for estimating the error of the experiment. The concentration of the products of pretreatment K and L is different that can be occurred by the soaking time before autoclaving the sample or the poor mixing of samples.

For pretreatment H (1% NaOH at 105 °C for 90 min) presents the lowest concentration of soluble lignin in the filtrate. This condition maintains the temperature and alkaline concentration at a low level while the reaction time is settled at the longest period. This result shows that only a long reaction time is not enough to remove the lignin composition in lignocellulosic material. Lignin is the three-dimensional amorphous and has several functional groups that lead it difficult to break down the complex structure.

So, to enhance the efficiency of lignin removal, the process should be performed under a higher temperature and alkaline concentration.

The pretreatment G (1% NaOH at 130 °C for 30 min) presents a higher level of soluble lignin concentration when compared with pretreatment H although this pretreatment performed under a shorter reaction time. For this reason, the temperature is more effective parameter for the lignin removal than reaction time. The concentration of xylose, glucose, furfural and HMF are not detected in pretreatment H and G.

For pretreatment I (5% NaOH at 105 °C for 30 min), the concentration of lignin removal was 42.9598 g/l. Pretreatment I is performed under the highest concentration, but the lowest temperature and reaction time. However, this condition shows the level of lignin removal higher than pretreatment G and H. Then the alkaline concentration is the most effective factor for lignin removal. Under the high level of alkaline solution, the OH group (hydroxide group) is generated, and then the various functional groups (α – aryl ether bonds, aryl glycerol β- aryl ether bong) in lignin composition are more increasable hydrolyzed by OH. The acetic acid concentration from pretreatment G, H and I were 3.7897 g/l, 3.1420 g/l and 3.1555 g/l, respectively and the levels of this product are lower than the maximum allowable value for bioethanol production.

Figure 4.21 presents the product content in $g/g_{biomass}$ unit. The product concentration in Figure 4.20 is multiplied by the volume of hydrolyzate after filtration and then divides by the weight of bagasse in each condition. The product contents from Figure 4.21 have the meaning that "When using 1g of bagasse, how much of each product is created?"

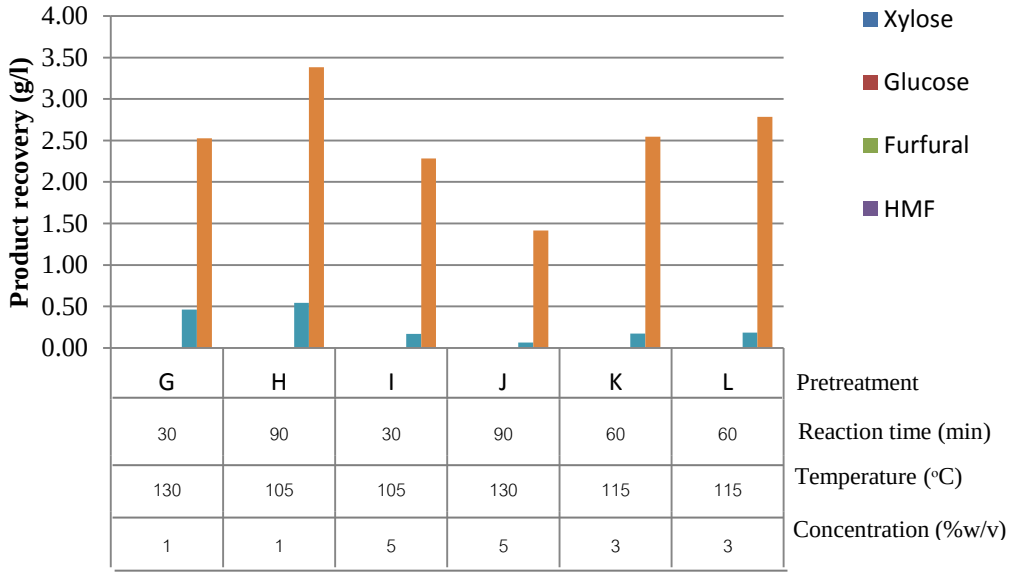


Figure 4.22 The product recovery (g/l) with different pretreatment condition NaOH pretreatment.

Alkaline pretreatment is different from acid pretreatment because the main product of acid pretreatment is monomeric sugar, especially, xylose; however, the soluble lignin is

the major product for alkaline pretreatment. In Figure 4.22 shows the product recovery of soluble lignin and acetic acid while the concentration of xylose, glucose, furfural and HMF product are not reported. From the result, the highest recovery of soluble lignin product presents in the pretreatment H as the concentration of 3.4000 g/l; however, the lignin concentration from this pretreatment shows the lowest level in hydrolyzate. On the contrary, the highest yield of soluble lignin in the filtrate derives from the pretreatment J; however, this pretreatment presents the lowest lignin concentration in the washing water. This result can be explained that the broken down of lignin linkages are not hydrolyzed into the liquid phase and still consists on the solid phase during the pretreatment. Therefore, the decomposition lignin in the solid phase will be recovered in the hot water during the washing step, so the lignin concentration in pretreatment H is higher than others. Since each the pretreatment is performed as the different condition, the volume of the hot water that is used to wash the pretreated solid also different. The chemical concentration is the most important factor that affects the hot water consumption because the concentrated chemical will consume a high volume of washing water. The extracted lignin is many useful, such as it will employ as generation of electricity, process heat, lignin-based adhesive and other products [15]. The trend of acetic acid is similar to the soluble lignin that the highest yield derives from pretreatment H and the lowest concentration presents in pretreatment J.

The concentration results of the products from the pretreatment process are analyzed to identify the significant parameters by using the analysis of variance (ANOVA) method and this step is carried out through the Unscramble X version 10.3 program.

2. The Analysis of Variance (ANOVA) of Product in Liquid Phase from NaOH Pretreatment

1. Soluble Lignin Content in Liquid Solution

According to FFD, the soluble lignin concentrations vary from 19.1956 g/l to 71.7200g/l and the data are shown in Table 4.17. The analysis of variance (ANOVA) result of the main effects consisted of temperature, reaction time and concentration is shown in Table 4.19. The normal probability plot of the Y – residual for soluble lignin content data is shown in Figure 4.23. The normal plot of Y - residual came up by default. If the distribution of Y - residual is normal, the plot will be a straight line [42].

Table 4.19 Effect estimates soluble lignin concentration from FFD of NaOH Pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p – level
Model				5.6689	0.0222
Constant			46.9650		
NaOH concentration	A	36.1850	18.0925	12.9757	0.0070
Temperature	J	15.8900	7.9450	2.5022	0.1523
Time	K	12.2400	6.2100	1.5287	0.2514

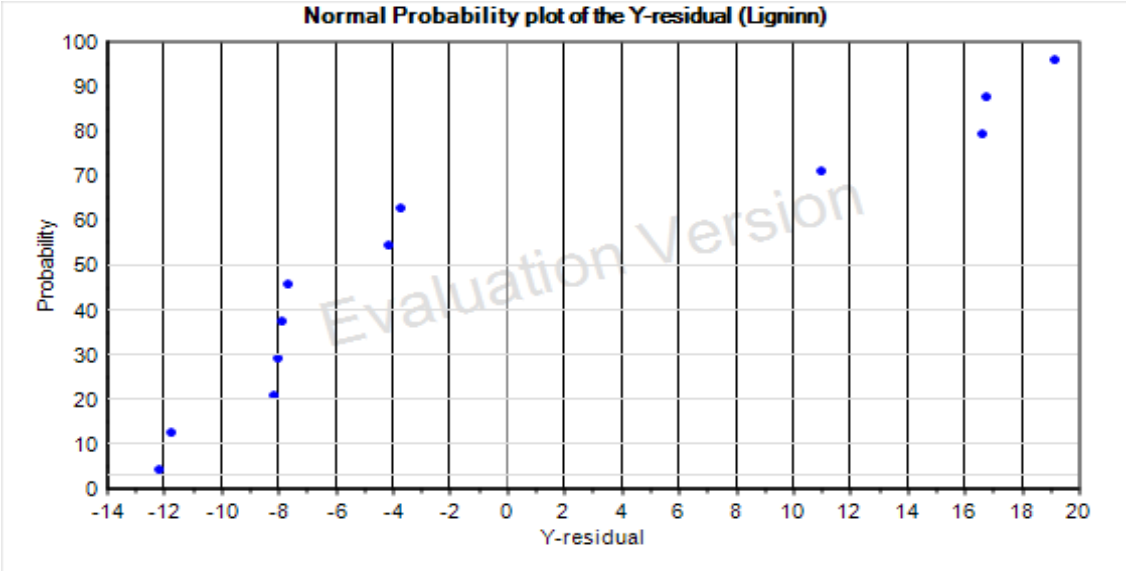


Figure 4.23 Normal plot of residuals of soluble lignin content of NaOH pretreatment.

The result of soluble lignin removal could be concluded that only the sodium hydroxide concentration significantly affected the removing of lignin from lignocellulose (p- level = 0.0070) and the estimated effect was a positive value. The coefficient of determination (R^2) was calculated to be 0.6800. This value indicated that the confidant level of statistics explains 68% of the variability in the data [48]

2. Acetic acid content in liquid solution

The yields of toxicity acetic acid production contents vary from 2.7068 g/l to 3.7897 g/l and the data of acetic acid are shown in Table 4.17. The analysis of variance result of the main effects is shown in Table 4.20. Moreover, the normal probability plot of acetic acid is shown in Figure 4.24.

Table 4.20 Effect estimates on acetic acid concentration from FFD of NaOH pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p - level
Model				2.5611	0.1279
Constant			3.3558		
NaOH concentration	A	-0.5350	-0.2675	3.6790	0.0914
Temperature	J	0.0950	0.0475	0.1160	0.7422
Time	K	-0.5500	-0.5966	3.8882	0.0841

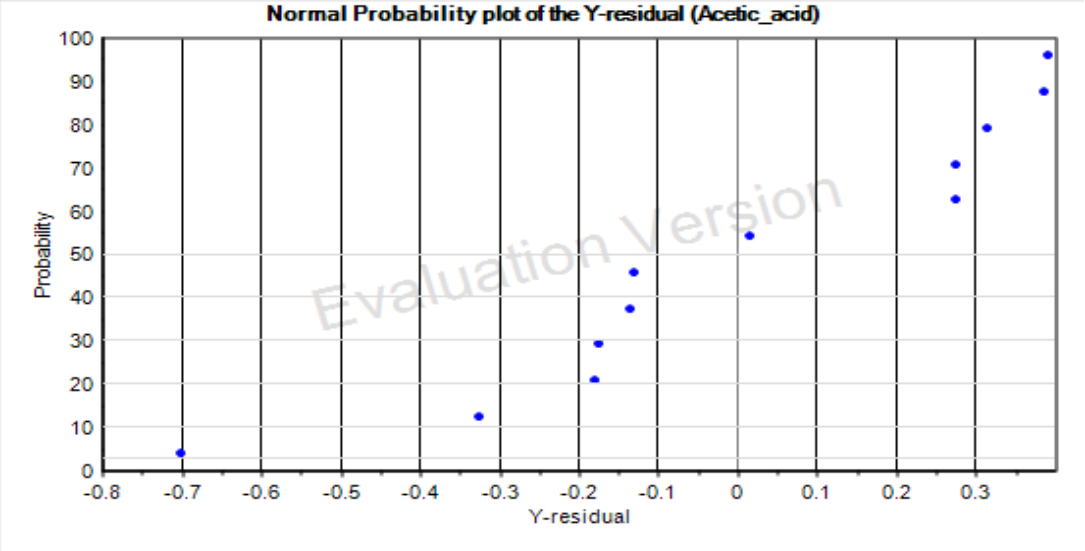


Figure 4.24 Normal plot of residuals of acetic acid content of NaOH pretreatment.

The ANOVA result of acetic acid production could be concluded that none of the independent variables such as temperature, NaOH concentration and reaction time significantly affected the formation of acetic acid. The p – level of all parameters were more than 0.05 ($p > 0.05$). The coefficient of determination (R^2) was calculated to be 0.3051. This value indicated that the confidant level of statistics explains 30% of the variability in the data [48].

For the ANOVA result of soluble lignin, the NaOH concentration was the only parameter that significantly affected the lignin removal ($p = 0.0070$) and the estimate effected was a positive value which means that the change from low to high level will increase the removing of lignin (Harry et al., 2010). From Table 4.20, the estimated effect of NaOH concentration was the highest value, followed by temperature and reaction time, which means that the NaOH concentration is the most effective parameter that affected the lignin removal. Silverstein et al., 2011 found that the highest level of delignification (65% of lignin removal) was 2% NaOH in 90 min for 121 °C with cellulose conversion of 60.8%. Mirahmadi et al., 2011 revealed that the low concentration of alkaline pretreatment, with the concentration of 0.5% - 4% NaOH at high temperature and pressure, the structure of cellulose was destroyed under this condition while NaOH pretreatment at high temperature, the majority of lignin and hemicellulose composition could be removed from the lignocellulosic material. The p – value of soluble lignin model was equal to 0.0222 ($p < 0.05$) which means that the model for lignin removal is rather reliable in these studied ranges (temperature 105 ºC – 130 ºC, reaction time 30 min – 90 min and NaOH concentration 1 % w/v – 5% w/v).

According to the ANOVA result of acetic acid, all of the parameters were not significant to the formation of acetic acid. Since the acetic acid is the inhibitory of yeast in the fermentation process, the pretreatment should control the concentration of this product to be lower than 4 g/l [1]. From the experimental result, the concentrations of acetic acid for all of pretreatments are lower than the maximum allowable value.

4.2.2 Sodium Hydroxide (KOH) Pretreatment

1. The Result of Pretreatment Product in Liquid Phase

Table 4.21 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of g/l.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	HMF (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
S	-1,(1)	1,(130)	-1,(30)	0.0000	0.0000	0.0000	0.0000	12.9146	1.4261
T	-1,(1)	-1,(100)	1,(90)	0.0000	0.0000	0.0000	0.0000	17.0876	2.6261
U	1,(5)	-1,(100)	-1,(30)	0.0000	0.0000	0.0000	0.0000	55.1179	2.7834
V	1,(5)	1,(130)	1,(90)	0.0000	0.0000	0.0000	0.0000	82.8774	2.5064
W	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	65.1357	2.5144
X	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	51.9707	2.6863

Table 4.22 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Run	**Variable**			**Response**					
Pretreatment	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	HMF ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
G	-1,(1)	1,(130)	-1,(30)	0.0000	0.0000	0.0000	0.0000	0.0122	0.0014
H	-1,(1)	-1,(100)	1,(90)	0.0000	0.0000	0.0000	0.0000	0.0205	0.0032
I	1,(5)	-1,(100)	-1,(30)	0.0000	0.0000	0.0000	0.0000	0.0825	0.0042
J	1,(5)	1,(130)	1,(90)	0.0000	0.0000	0.0000	0.0000	0.1232	0.0037
K	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	0.0956	0.0037
L	0,(3)	0,(115)	0,(60)	0.0000	0.0000	0.0000	0.0000	0.0794	0.0041

The data from Table 4.21 and 4.22 can be presented in the form of graph column shown in Figure 4.25 and 4.26. In Figure 4.25, the products from pretreated bagasse are shown in terms of concentration while in Figure 4.26; the product concentration is transformed into product content.

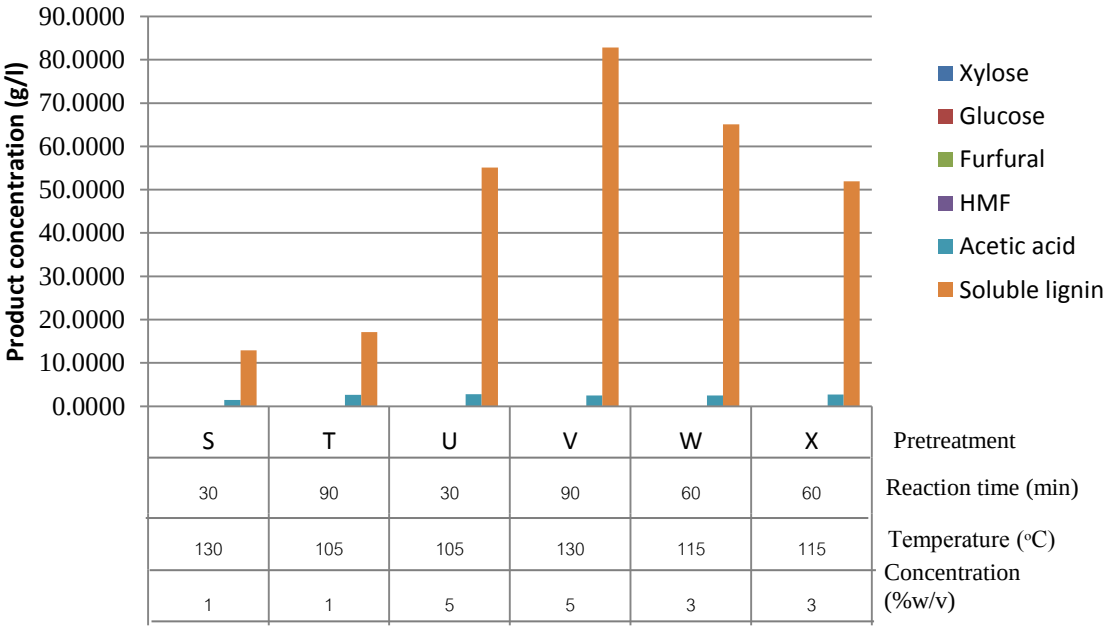


Figure 4.25 The product concentration (g/l) with different pretreatment condition KOH pretreatment.

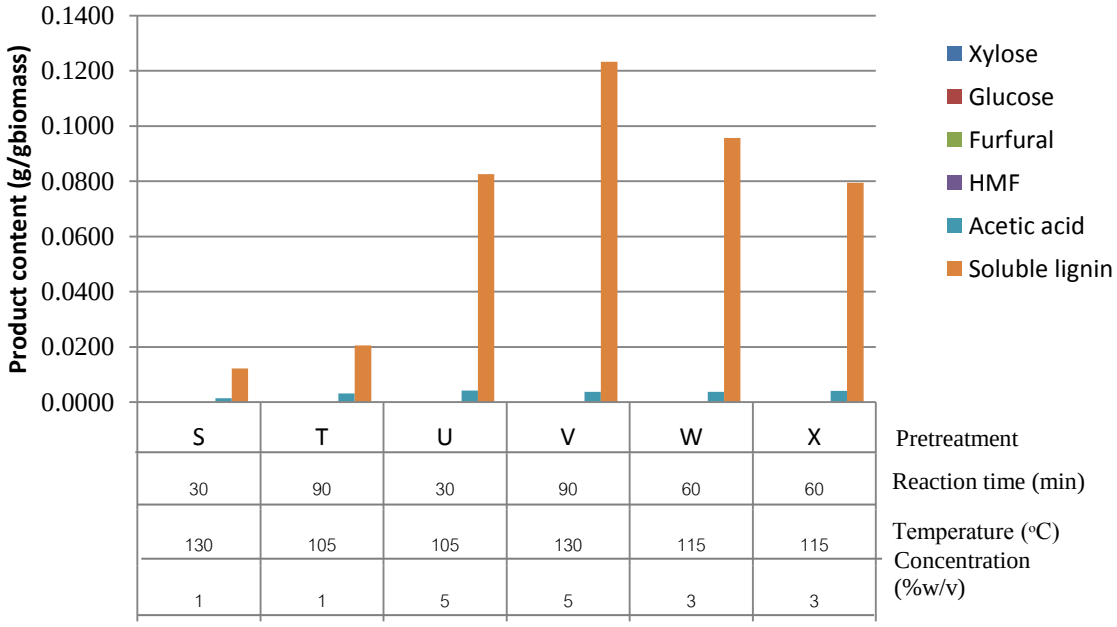


Figure 4.26 The product content ($g/g_{biomass}$) with different pretreatment condition KOH pretreatment.

Potassium hydroxide (KOH) is relatively less explored (Ong et al., 2010) but this chemical can be used for lignocellulose pretreatment. KOH is reported for the reactivity with carbon nano tube and carbon nano structure and the capability to deacetylate biomass. Raymundo-Pinero et al., 2005 used the carbon activating agent for investigating the structural pattern of carbon activation on carbon nano tube and this experiment was studied by using NaOH and KOH. The result revealed that NaOH could degrade the tubular structure of disoriented structure while KOH cloud degrades highly ordered tubular. Based on the difference in its reactivity with carbon nano fibers and carbon nano structure, it is believed that KOH can be effective for lignin-carbon removal to increase the efficiency of enzymatic hydrolysis. Since the KOH is classified for the alkaline chemical type, the reaction on lignin removal is similar to NaOH. The diluted alkaline pretreatment is applied to disturb the lignocellosic cell wall by dissolving lignin, hemicellulose and silica and break down the uronic, acetic acid ester and also swelling cellulose. Under diluted alkaline solution, lignin decomposition occurs via the cleavage of α – aryl ether bonds from polyohenolic monomers, while the hemicellulose is dissolved and the hydrogen bond weakening leads to the swelling of cellulose. Using the KOH, the major product from this chemical pretreatment is soluble lignin, followed by a small portion of acetic acid and the result is shown in Figure 4.25.

The pretreatment V (5% KOH at 130 °C for 90 min) shows the most effective condition for lignin removal from the bagasse and the concentrations of other products are not detected. This result demonstrates that the hemicellulose and cellulose are not hydrolyzed by OH^- from KOH solution. The acetic acid concentration (2.5064 g/l) can be detected which means that under dilute alkaline condition the OH group hydrolyzes acetyl group in polysaccharide chain of hemicellulose. The acetic acid is inhibitory to yeast when its concentration is located between 4 and 10 g/l.

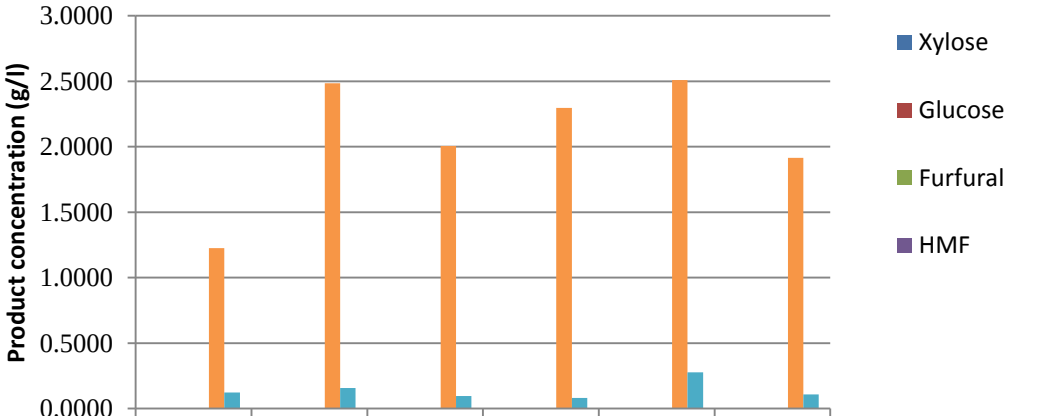
For the pretreatment W and X (3% w/v at 115 ºC for 60 min), these conditions show the center point in the fractional factorial design for estimating the error of the experiment. The concentration of the products of pretreatment W and X is different that can be occurred by the soaking time before autoclaving the sample or the poor mixing of samples.

For pretreatment S (1% KOH at 130 °C for 30 min) presents the lowest concentration of the soluble lignin in the black liquor. This condition is maintained the reaction time and KOH concentration at a low level while the temperature is settled as the highest level. This result shows that only highest temperature is insufficient to remove the lignin composition from lignocellulosic material.

The pretreatment T (1% KOH at 105 °C for 90 min) presents a higher level of soluble lignin concentration when compared with pretreatment S although this pretreatment performs under a lower temperature. For this reason, the reaction time is more effective parameter for the lignin removal than temperature. The concentration of xylose, glucose, furfural and HMF are not detected in pretreatment S and T.

For pretreatment U (5% KOH at 105 °C for 30 min), the concentration of lignin removal was 55.1179 g/l. The pretreatment U performs under the concentrated concentration, but lowest level of temperature and reaction time. However, this condition shows to be more effective for lignin removal than pretreatment G and H. So, the alkaline concentration is the most effective factor for lignin removal. The concentrations of acetic acid from pretreatment S, T and U were 1.4261 g/l, 2.6261 g/l and 2.7834 g/l, respectively and these values are lower than the maximum allowable concentration.

The product concentration in Figure 4.25 is multiplied by the volume of hydrolyzate after filtration and then divides by the weight of bagasse in each condition. The product contents from Figure 4.27 have the meaning that "When using 1g of bagasse, how much of each product is created?"



	S	T	U	V	W	X
Pretreatemnt						
Reaction time (min)	30	90	30	90	60	60
Temperature (°C)	130	105	105	130	115	115
Concentration (%w/v)	1	1	5	5	3	3

Figure 4.27 The product recovery (g/l) with different pretreatment condition KOH pretreatment.

Figure 4.27 presents the product recovery in washing water. The 60 °C of distillated water was applied for washing the decomposition product that is consisted in the pretreated solid and to neutralize pretreated bagasse until the pH value equal to 7.0. The major composition in washing water is the lignin, followed by acetic acid. The concentrations of xylose, glucose, furfural and HMF are not reported. The highest concentration of lignin presents in the pretreatment W as a concentration of 2.5000 g/l. The extracted lignin is many useful, such as it uses as the generation of electricity, process heat, lignin-based adhesive and other products [15]. For the acetic acid, the concentration of this product presents in a small portion in washing water.

The concentration results of the products from the pretreatment process are analyzed to identify the significant parameters by using the analysis of variance (ANOVA) method and this step is carried out through the Unscramble X version 10.3 program.

2. The Analysis of Variance (ANOVA) of Product in Liquid Phase from KOH Pretreatment

1. Soluble Lignin Content in Liquid Solution

According to FFD, the soluble lignin concentrations vary from 12.9146 g/l to 82.8774 g/l and the data are shown in Table 4.21. The analysis of variance (ANOVA) result of the main effects consisted of temperature, reaction time and concentration is shown in Table 4.23. The normal probability plot of the Y – residual for soluble lignin content data is shown in Figure 4.28. The normal plot of Y - residual came up by default. If the distribution of Y - residual is normal, the plot will be a straight line [42].

Table 4.23 Effect estimates soluble lignin concentration from FFD of KOH pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p – level
Model				17.1774	0.0008
Constant			47.5173		
KOH concentration	B	53.9966	26.9983	45.3975	0.0001
Temperature	J	11.7933	5.8967	2.1656	0.1793
Time	K	15.9663	7.9831	3.9692	0.0815

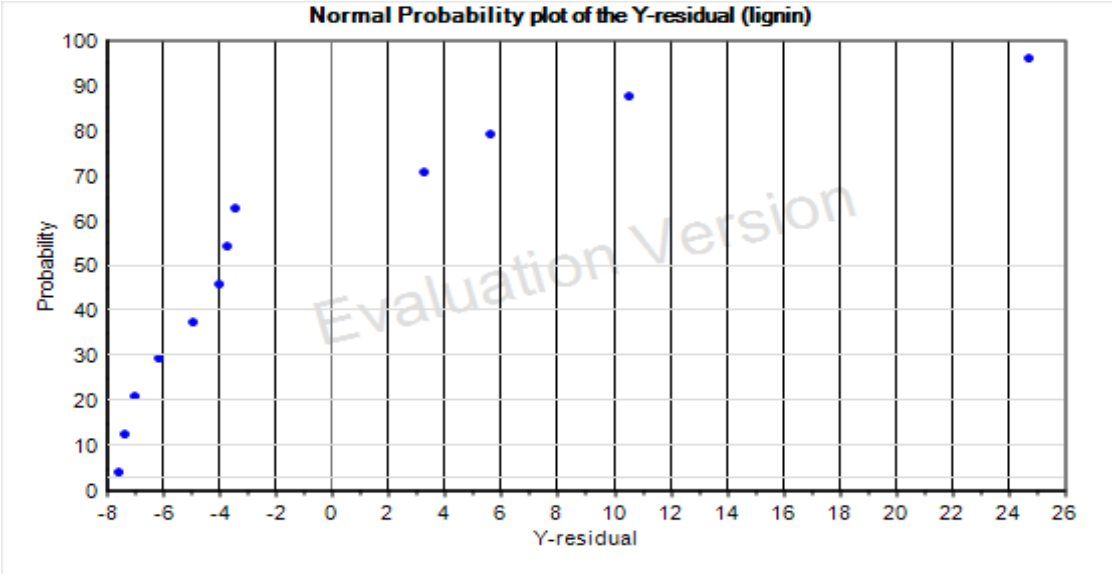


Figure 4.28 Normal plot of residuals of soluble lignin content of KOH pretreatment

The result of soluble lignin removal could be concluded that only the potassium hydroxide concentration significantly affected the removing of lignin from lignocellulose (p- level = 0.0001) and the estimated effect was a positive value. The coefficient of determination (R^2) was calculated to be 0.8656. This value indicated that the confidant level of statistics explains 87% of the variability in the data [48].

2. Acetic Acid Content in Liquid Solution

The yields of toxicity acetic acid production contents vary from 1.4261 g/l to 2.7834 g/l and the data of acetic acid are shown Table 4.21. The analysis of variance result of the main effects is shown in Table 4.24. Moreover, the normal probability plot of acetic acid is shown in Figure 4.29.

Table 4.24 Effect estimates on acetic acid concentration from FFD of KOH pretreatment.

Variables	Variable code	Effect (Ex_i)	Coefficient	F - value	p – level
Model				7.3293	0.0111
Constant			2.4238		
KOH concentration	B	0.6108	0.3094	7.3769	0.0264
Temperature	J	-0.7385	-0.3692	10.5070	0.0119
Time	K	0.4615	0.2308	4.1039	0.0774

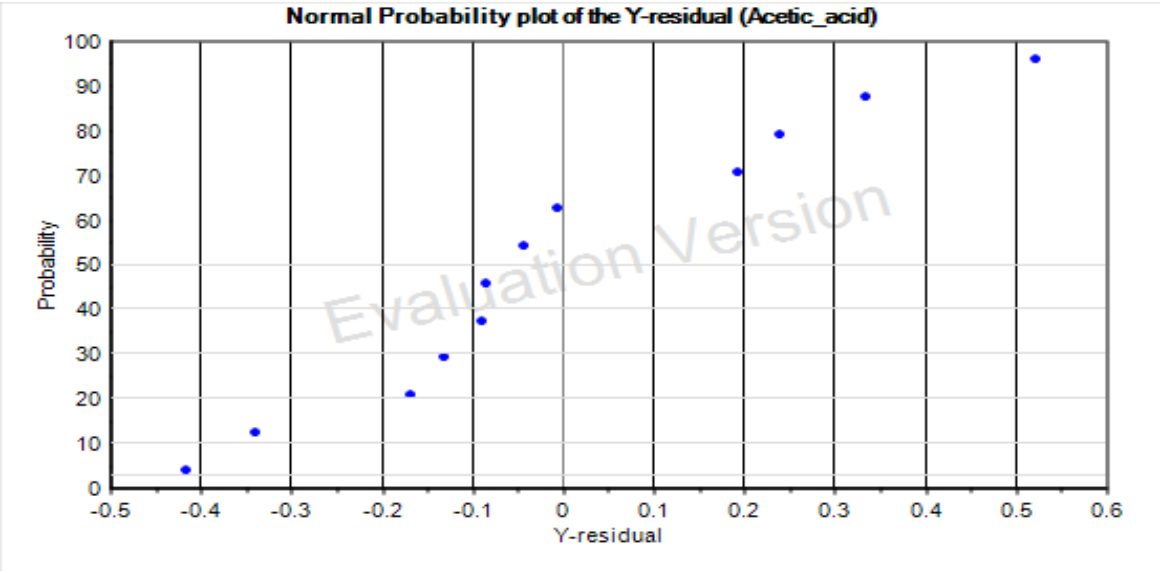


Figure 4.29 Normal plot of residuals of acetic acid content of KOH pretreatment.

The ANOVA result of acetic acid production could be concluded that the KOH concentration and temperature significantly affected the formation of acetic acid and the estimated effect of KOH concentration was a positive value while the effect value of temperature was negative. The coefficient of determination (R^2) was calculated to be 0.7332. This value indicated that the confidant level of statistics explains 73% of the variability in the data [48].

For the ANOVA result of soluble lignin, the KOH concentration was only the parameter that significantly affected the lignin removal ($p = 0.0001$) and the estimate effected was a positive value which means that the change from low to high level will increase the removing of lignin (Harry et al., 2010). From Table 4.23, the estimated effect value of KOH concentration is the highest, followed by temperature and reaction time which means that the KOH concentration is the most effective parameter that affects the lignin removal. The p – value of soluble lignin model was equal to 0.0008 ($p < 0.05$) which means that the model for lignin removal is rather reliable in these studied ranges (temperature 105 ºC – 130 ºC, reaction time 30 min – 90 min and KOH concentration 1 % w/v – 5% w/v).

According to the ANOVA result of acetic acid, KOH concentration and temperature significantly affected the formation of acetic acid. Since the acetic acid is the inhibitory of yeast in the fermentation process, the pretreatment should minimize the concentration of this chemical to be lower than 4 g/l [1]. From the experimental result, the concentrations of acetic acid for all of pretreatments are lower than the maximum allowable value.

4.3 Distillated Water Pretreatment (Blank)

1. The Result of Pretreatment Product in Liquid Phase

Table 4.25 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of g/l.

Run	**Variable**			**Response**					
Blank	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	HMF (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
1	-1,(1)	1,(130)	-1,(30)	1.2780	0.1169	0.0000	0.0000	2.7138	0.0257
2	-1,(1)	-1,(105)	1,(90)	1.0766	0.0966	0.0000	0.0000	1.8630	0.0000
3	1,(5)	-1,(105)	-1,(30)	0.5758	0.0474	0.0000	0.0000	3.3427	0.0000
4	1,(5)	1,(130)	1,(90)	0.4454	0.0000	0.0000	0.0000	1.4376	0.0000
5	0,(3)	0,(115)	0,(60)	0.9184	0.0818	0.0000	0.0000	2.8053	0.0000
6	0,(3)	0,(115)	0,(60)	1.3103	0.2200	0.0000	0.0000	1.8318	0.0000

Table 4.26 Influence of temperature, time and concentration on monomeric sugar, acetic acid, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Run	**Variable**			**Response**					
Blank	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	HMF ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
1	-1,(1)	1,(130)	-1,(30)	0.0020	0.0002	0.0000	0.0000	0.0043	0.0000
2	-1,(1)	-1,(100)	1,(90)	0.0013	0.0001	0.0000	0.0000	0.0023	0.0000
3	1,(5)	-1,(100)	-1,(30)	0.0010	0.0001	0.0000	0.0000	0.0055	0.0000
4	1,(5)	1,(130)	1,(90)	0.0008	0.0000	0.0000	0.0000	0.0025	0.0000
5	0,(3)	0,(115)	0,(60)	0.0015	0.0001	0.0000	0.0000	0.0046	0.0000
6	0,(3)	0,(115)	0,(60)	0.0023	0.0004	0.0000	0.0000	0.0032	0.0000

The data from Table 4.25 and 4.26 can be presented in the form of graph column shown in Figure 4.30 and 4.31. In Figure 4.30, the products from pretreated bagasse are shown in terms of concentration while in Figure 4.31; the product concentration is transformed into product content.

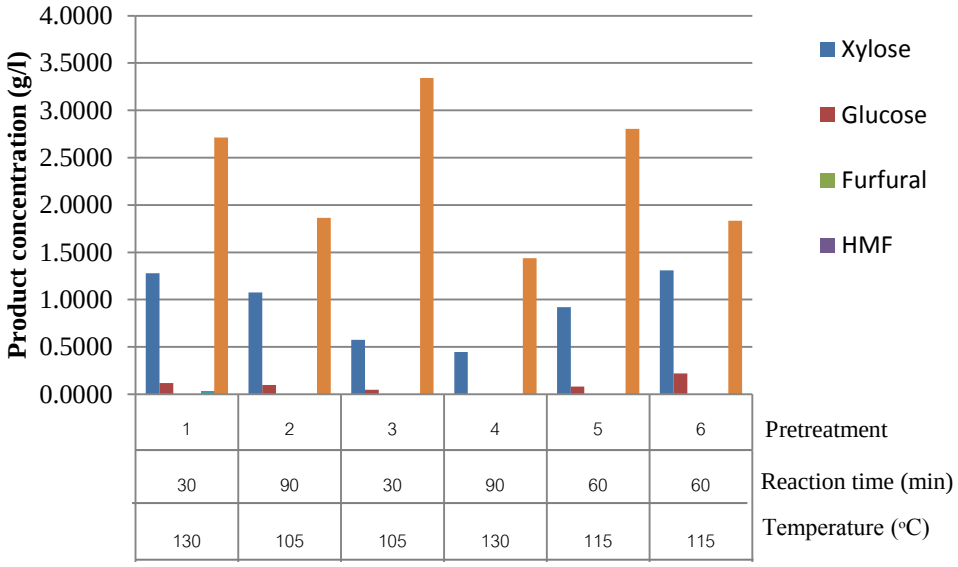


Figure 4.30 The product concentration (g/l) with different pretreatment condition by distillated water pretreatment.

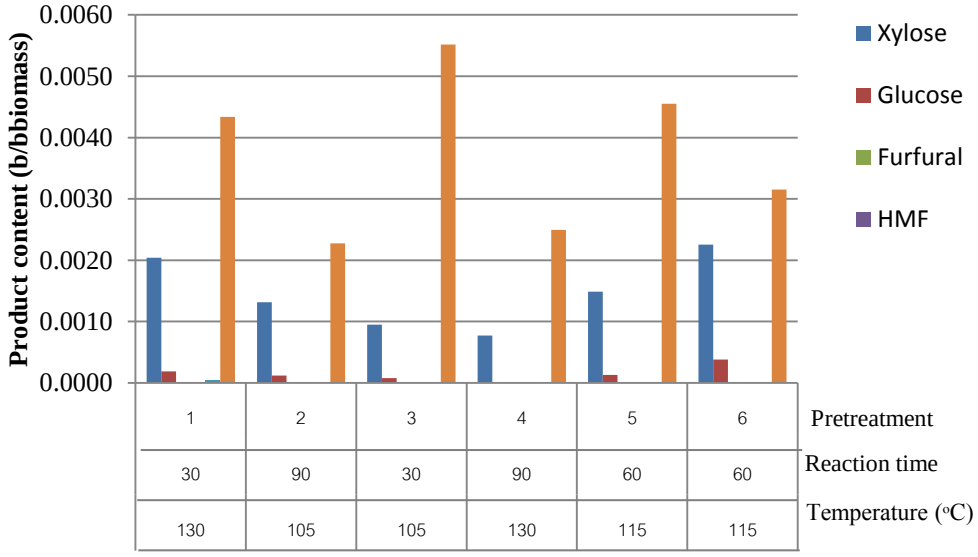


Figure 4.31 The product content ($g/g_{biomass}$) with different pretreatment condition by distillated water pretreatment.

The blank pretreatment is the pretreatment using the distillated water instead of acid/alkaline chemical. However, the operating conditions are as the same as chemical pretreatment. The blank pretreatment is similar to the liquid hot water pretreatment (LHW) but the temperature and pressure are lower. The liquid hot water pretreatment (LHW) is carried out under high pressure to keep the water in the liquid phase at the elevated temperature (160 ºC – 240 ºC) [50]. However, the blank pretreatment is conducted at mild condition (temperature 105 ºC – 130 ºC under atmospheric pressure). The catalyst of the blank pretreatment is the hydronium ion (H_3O^+) that can be generated from the de-ionized of the water and gives the process similar to the acid pretreatment. From this reason, the hydrolysis of acetyl group on the side chain of hemicellulose will generate the acetic acid product that can lead the system to be acid condition [52]. Therefore, under the acid condition that generated from acetic acid and other organic acids, the xylan in the backbone of hemicellulose is hydrolyzed together with the removal of lignin composition and the result is shown in Table 4.25.

To compare the chemical pretreatment result with the blank pretreatment is found that the xylose and glucose concentration of acid pretreatment is higher than the blank pretreatment for all conditions because the acidity of acid chemical is higher than distillated water pretreatment. Then under H_2SO_4 and HCl condition, the lyxan in hemicellulose and the amorphous region in cellulose can be easier hydrolyzed than under blank condition. However, the lignin removal of acid pretreatment is lower than the blank pretreatment. Because of the acid condition, the degradation of the lignin occurs via the substitution reactions and broken links. Unfortunately, these reactions are accompanied by the condensation reaction that prevents the dissolution of lignin [8]. From this result, the lignin concentration under the acid condition is lower than blank pretreatment.

On the other hand, the xylose concentration and glucose concentration of blank pretreatment are higher than the alkaline pretreatment. Under the alkaline condition, the OH^- is generated and this molecule does not hydrolyze the linkage in the backbone and on the side chain of hemicellulose and cellulose. Nonetheless, the soluble lignin in hydrolyzate of alkaline pretreatment is higher than the distillated water pretreatment because the alkalinity of NaOH and KOH is higher than water. From this result, the ester linkage between lignin and hemicellulose can be easier hydrolysed by alkaline chemical.

Figure 4.31 presents the product content in $g/g_{biomass}$ unit. The product concentration in Figure 4.30 is multiplied by the volume of hydrolyzate after filtration and then divides by the weight of bagasse in each condition. The product contents from Figure 4.31 have the meaning that “When using 1g of bagasse, how much of each product is created?”

For the blank pretreatment, the main product in the hydrolyzate is the soluble lignin, followed by xylose and glucose. During the washing step, the broken down lignin composed in the solid phase will be recovered into the washing water and the result is shown in Figure 4.32. Since the blank pretreatment uses the distillated water as the

solution, the pH of the process is the neutral pH (pH = 7.0). During the pretreatment, the acetyl groups of the side chain of hemicellulose are hydrolysed to form the acetic acid and other organic acids then the pH of the hydrolyzate will drop below than 7.0. However, the pH of the mixture is higher than the pH of acid pretreatment. For this reason, the consumption of hot water used to wash the pretreated solid of blank condition is less than acid/alkaline pretreatment. The extracted lignin is useful, such as it uses as generation of electricity, process heat, lignin-based adhesive and other products [15].

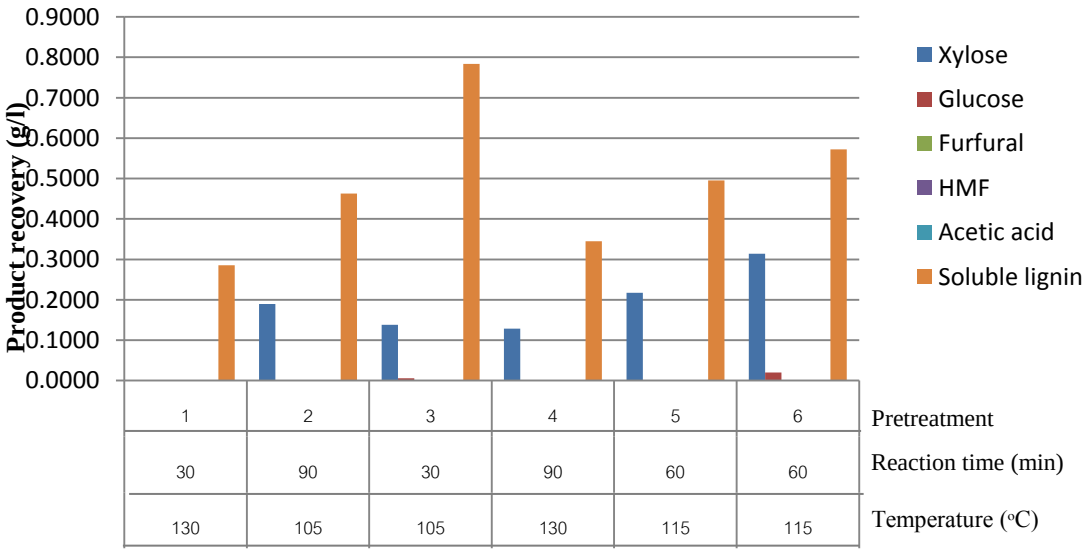


Figure 4.32 The product recovery (g/l) with different pretreatment condition by distillated water pretreatment.

Since the significant level of this experiment set equal to 0.05 ($\alpha = 0.05$) or 95% confidence. If the p-level of the independent variable is higher than 0.05, the variable does not significantly affect the dependent variable (products). The non-significant variable also affects the dependent variable, but the percent of confidence of the result is less than 95% ($\alpha > 0.05$). Then, the result in this case ($\alpha > 0.05$) is considered to be not-significantly affect. Generally, 99% ($\alpha = 0.01$), 95% ($\alpha = 0.05$) and 90% ($\alpha = 0.10$) confidence are used as the level of significance for performing the analysis of variance (ANOVA). Nevertheless, the 95% confidence is commonly used for scientific experiment. Moreover, the three dimensional diagrams or RSM graph (the relation between a dependent variable and two independent variables) can be generated from the equation of the ANOVA analysis and the result of RSM graph will be discussed in the topic 4.6 – 4.9.

4.4 The Comparison of the Effective of the Chemical Pretreatment for Xylose Production

(A) **(B)**

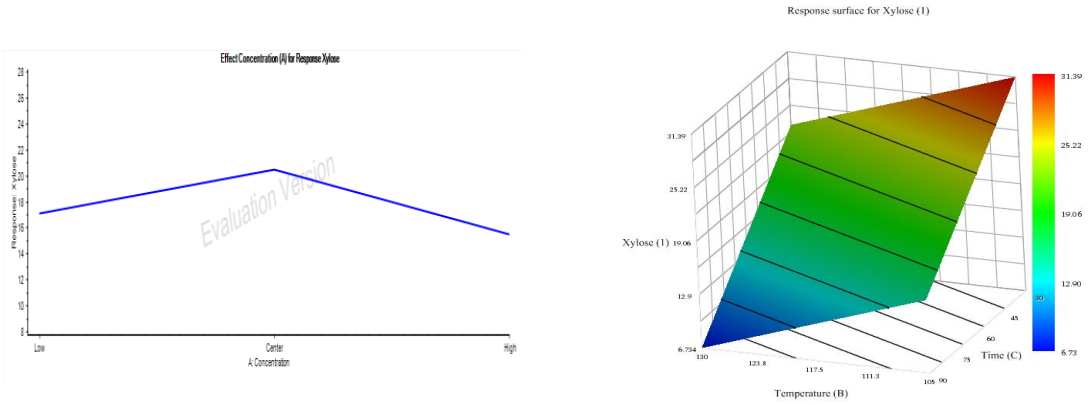


Figure 4.33 the response surface plot on xylose production. A- Effect of reaction time at H_2SO_4 concentration of 3% v/v and temperature of 115 ^oC. B - Effect of temperature and reaction time at HCl concentration of 3% v/v.

Xylose is the major product of hydrolysis of the xylan chain in the backbone of hemicellulose. The diluted acid hydrolysis is allowed deconstruction the lignocellulosic material and produced the pentose monomeric sugar especially xylose sugar. Since the hemicellulose is an amorphous structure and more accessible surface area than cellulose, this structure allows for diffusion in acid. For this reason, the diluted acid hydrolysis is relatively applied for the destruction of hemicellulose. The most of glycosidic bonds in carbohydrates and other polysaccharides are acetal linkages and these bonds can be cleavage by using acid catalysis. Because of the acetal linkage in the xylan chain, to use the diluted alkaline solution to be the catalyst cannot cleave these bonds in the hemicellulose. Therefore, the xylose product does not present in alkaline pretreatment and the acetal formation reaction is shown in Figure 4.34.

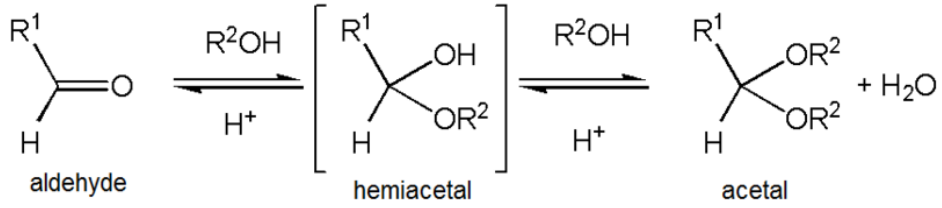


Figure 4.34 The acetal formation reaction.

From Figure 4.35 (A), the H_2SO_4 concentration is only the variable that significantly affects the xylose product while this graph the temperature and reaction are maintained at the center point (115 ^oC and 60 min, respectively). From the figure reveals that before 60 min, the concentration of xylose product will increase and the maximum xylose concentration is about 20.50 g/l. However, after this time the xylose product is relatively decreasing. From this result, it is clearly that the maximum point of xylose is located at 60 min of reaction time. From Figure 4.33 (B), the reaction time is the most effective parameter for xylose production, followed by temperature and HCl concentration. However, the RSM 3-dimensional graph presents only the relation between reaction time and temperature while the relation of temperature vs. HCl concentration and HCl concentration vs. reaction time will be shown in the APENDIX D. The maximum point of xylose product is 31.39 g/l at 3%v/v for 30 min of 105 ^oC. From this result, the maximum yield of xylose from HCl pretreatment is more than H_2SO_4 pretreatment. These results are the maximum yield that present in these studied ranges (1%v/v -5% v/v of acid concentration, 30 min-60 min of reaction time and 105 ^oC – 130 ^oC of temperature), the maximum yield can vary when the studied ranges are changed.

4.5 The Comparison of the Effective of the Chemical Pretreatment for Glucose Production

(A) **(B)**

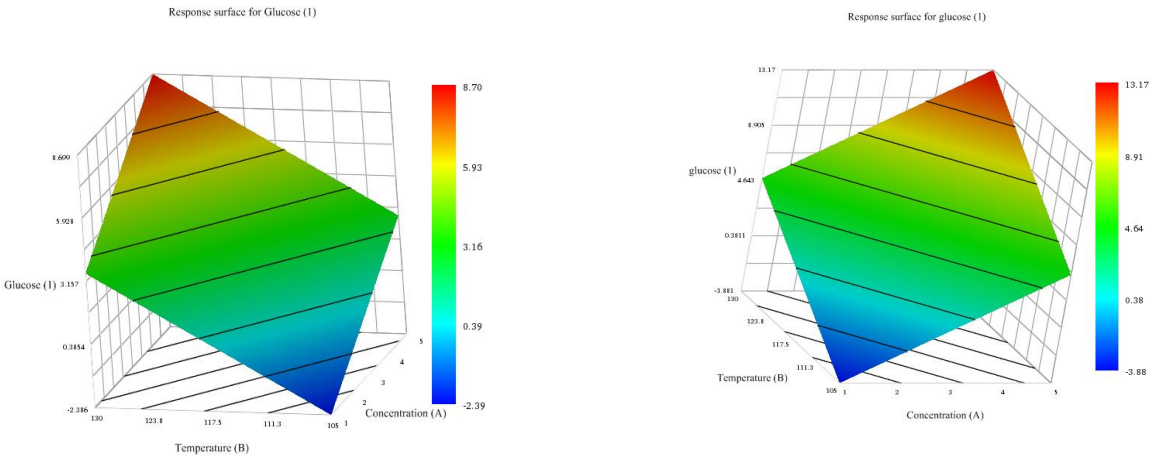


Figure 4.35 the response surface plot on glucose production. A-Effect of temperature and H_2SO_4 concentration at reaction time of 60 min. B-Effect of temperature and HCl concentration at reaction time of 60 min.

Glucose is the product of cellulose hydrolysis. Generally, cellulose has 2 regions in its structure that are crystalline and amorphous. The cellulose is composed of glucose unit that combined together with β-1, 4 glucosidic linkages allows the polymer to be arranged in the long straight chains. Along glucan chain, hydroxides which, composed in glucose structure distribute on both side and allows for the formation of hydrogen bonds between the cellulose. The hydrogen bonds cause the apparel chains attached to each other and to break these bonds will make the cellulose swelling. Usually, cellulose is insoluble in water and diluted acid solution at low temperature; however, during treatment with concentrated acid at high temperature, cellulose can be degraded into glucose molecules. However, the main purpose of the pretreatment would like to remain most of cellulose composition in the holocellulosic material while most of lignin and hemicellulose are removed. Therefore, the pretreatment which can minimize the degradation of cellulose composition, is considered to be the effective method.

From Figure 4.35 (A), the temperature is the most effective parameter for glucose production, followed by H_2SO_4 concentration and reaction time. However, the RSM 3-dimensional graph presents only the relation between temperature and H_2SO_4 concentration while the relation of temperature vs. reaction time and H_2SO_4 concentration vs. reaction time will be shown in the APENDIX D. The maximum point of glucose product is 8.70 g/l at 5 %v/v H_2SO_4 for 60 min at 130 ^oC. From Figure 4.35 (B), the temperature is the most effective parameter of glucose product, followed by HCl concentration and reaction time. However, the RSM 3-dimensional graph presents only the relation between temperature and HCl concentration while the relation of temperature vs. reaction time and HCl concentration vs. reaction time will be shown in the APENDIX D. The maximum point of glucose product is 13.17 g/l at 5 %v/v HCl for 60 min at 130 ^oC. Then, the maximum concentration of glucose from HCl pretreatment is higher than H_2SO_4 pretreatment. To minimize the degradation of cellulose is the most important objective of pretreatmen; therefore, H_2SO_4 acid is the chemical solution that can eventually prevent the degradation of cellulose. Because of the acetal linkage in the glucan chain, to use the diluted alkaline solution as the catalyst cannot cleave these bonds in the cellulose. Therefore, the glucose product does not present in alkaline pretreatment and the acetal formation reaction is shown in Figure 4.34. This result is the maximum yield that presented in the studied ranges (1%v/v -5% v/v of acid concentration, 30 min-60 min of reaction time and 105 ^oC – 130 ^oC of temperature), the maximum yield can be varied when the studied ranges are changed.

4.6 The Comparison of the Effective of the Chemical Pretreatment for Inhibitors Production

Furfural, HMF and acetic acid are the product inhibitors that are produced under acid/alkaline pretreatment. These inhibitors have toxic effects on the organism fermenting that causes the downstream products contain in lower productivity. The furfural is produced from the further hydrolysis of xylose and pentose sugars under heat

and acid condition. The xylose and other five carbons sugar undergo dehydration and lost three molecules of water to become furfural. Similarly to the furfural, HMF is the product of the hydrolysis of glucose and six carbons sugar under heat and acid condition. The furfural and HMF affect the cell growth respiration. However, HMF is less toxic than furfural and the concentration in hydrolyzate is usually low. The last one of the common inhibitor product which found from chemical pretreatment is acetic acid. This product is produced from the hydrolysis of acetyl group on the side chain of hemicellulose composition. The inhibitory effect of these compounds is higher when they are presented together because of the concomitant effect.

4.6.1. Furfural

(A) **(B)**

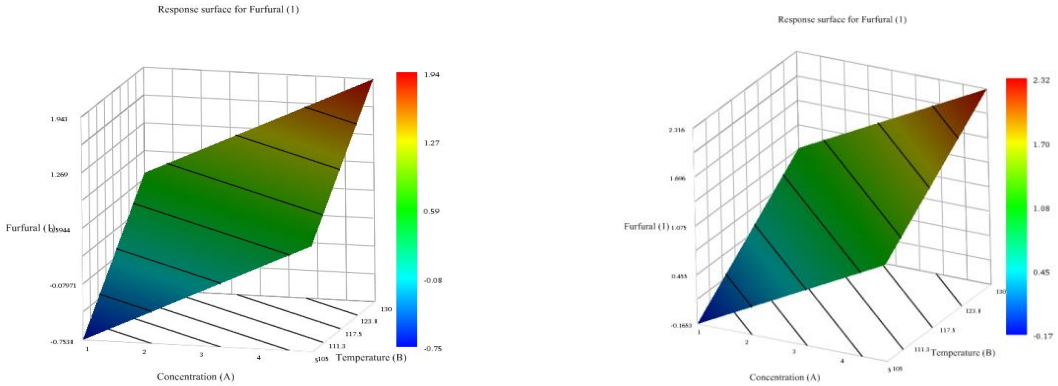


Figure 4.36 the response surface plot on furfural production. A-Effect of temperature and H_2SO_4 concentration at reaction time of 60 min. B- Effect of temperature and HCl concentration at reaction time of 60 min.

From Figure 4.36 (A) the temperature and H_2SO_4 concentration are the most effective parameters for furfural product, followed by reaction time. However, the RSM 3-dimensional graph is presented only the relation between temperature and H_2SO_4 concentration while the relation of temperature vs. reaction time and H_2SO_4 concentration vs. reaction time will be shown in the APENDIX D. From this graph, the maximum concentration of furfural is 1.94 g/l at 5% v/v H_2SO_4 for 60 min of 130 ^oC. For Figure 4.36 (B) the temperature, HCl concentration and reaction time have equal effect values. However, the RSM 3-dimensional graph presents only the relation between temperature and HCl concentration while the relation of temperature vs. reaction time and HCl concentration vs. reaction time will be shown in the APENDIX D. This graph presents the maximum of HMF concentration is 2.32 g/l at 5% v/v HCl for 60 min of 130 ^oC. Since the formation of furfural continuously performs under acid catalyst, this product cannot detect when the process is under alkaline solution

4.6.2. HMF

(A) **(B)**

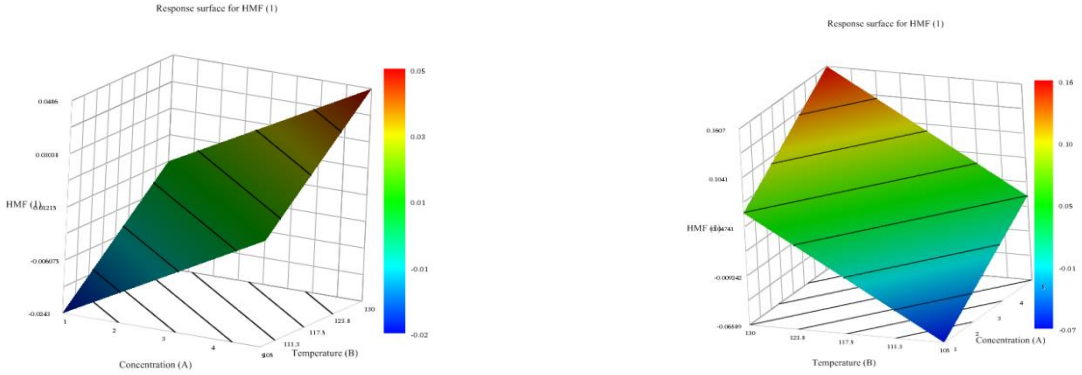
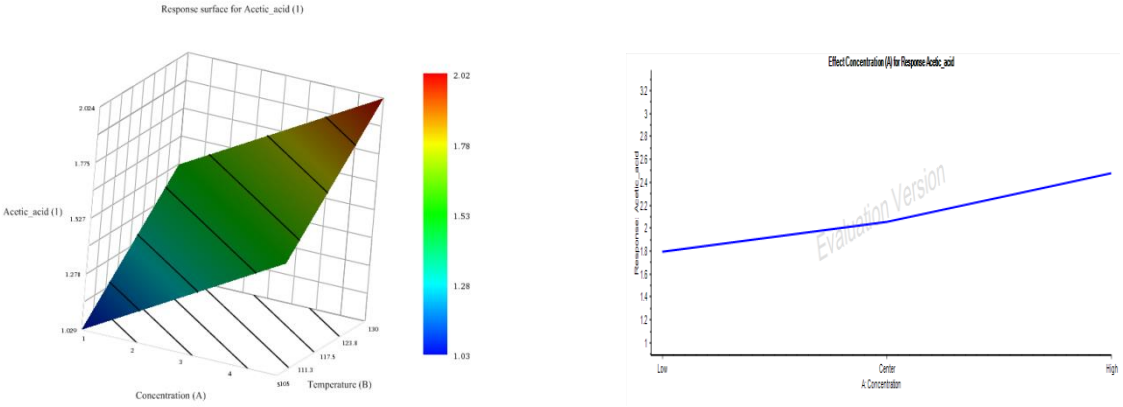


Figure 4.37 the response surface plot on HMF production. A-Effect of temperature and H_2SO_4concentration at reaction time of 60 min. B – Effect of temperature and HCl concentration at reaction time of 60 min.

From Figure 4.37 (A) the temperature, H_2SO_4 concentration and reaction time are effective parameters for HMF production. However, the RSM 3-dimensional graph presents only the relation between temperature and H_2SO_4 concentration while the relation of temperature vs. reaction time and H_2SO_4 concentration vs. reaction time will be shown in the APENDIX D. From this graph, the maximum concentration of furfural is 0.05 g/l at 5% v/v H_2SO_4 for 60 min of 130 ^oC. In Figure 4.37 (B) the temperature is the most effective parameter of HMF product, followed by HCl concentration and reaction time. However, the RSM 3-dimensional graph presents only the relation between temperature and HCl concentration while the relation of temperature vs. reaction time and HCl concentration vs. reaction time will be shown in the APENDIX D. This graph presents the maximum of HMF concentration is 0.16 g/l at 5% v/v HCl for 60 min of 130 ^oC. Since the formation of HMF continuously performs under acid catalyst, this product cannot detect when the process is under alkaline solution.

4.6.3. Acetic Acid

(A) **(B)**



(C)

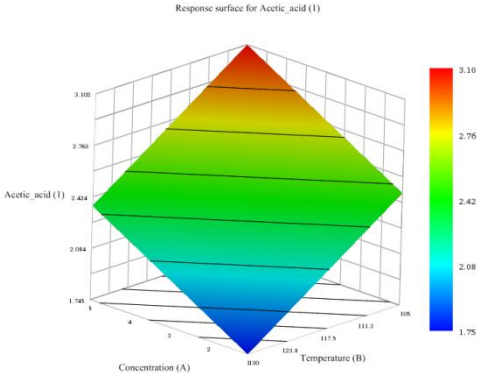


Figure 4.38 the response surface plot on acetic acid production. A-Effect of temperature and H_2SO_4 concentration at reaction time of 60 min. B- Effect of HCl concentration at reaction time of 60 min and temperature of 115 ^oC. C- Effect of KOH concentration and temperature at reaction time of 60 min.

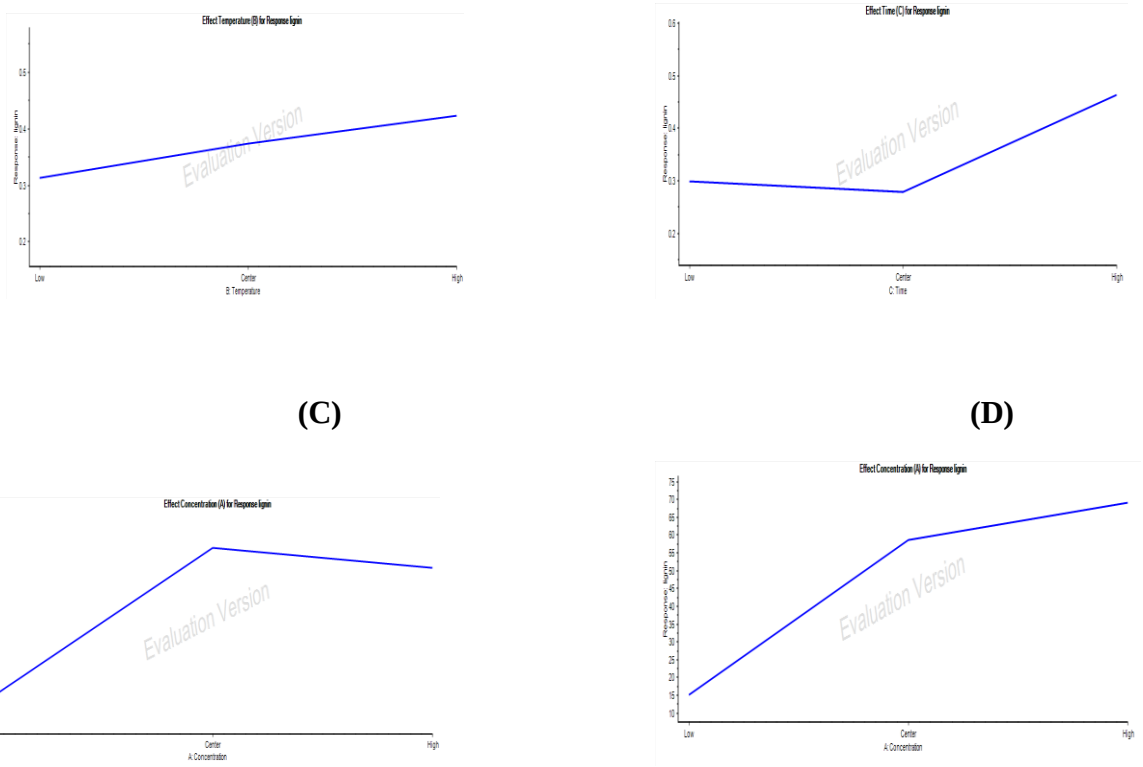
From Figure 4.38 (A) the H_2SO_4 concentration and reaction time are the most effective parameters for acetic acid production, followed by temperature then the RSM 3-dimensional graph presents only the relation between temperature and H_2SO_4 concentration. From this graph, the maximum concentration of acetic acid is 2.02 g/l at 5% v/v H_2SO_4 for 60 min of 130 ^oC. In Figure 4.38 (B), the HCl concentration is only the variable that significantly affects the formation of acetic acid while the temperature and reaction time are maintained at the center point (105 ^oC and 60 min, respectively). From the figure, the concentration of acetic acid will enhance when the temperature of

the process increases and the maximum yield of acetic acid concentration is 2.50 g/l. However, the trend of acetic acid also increases although the HCl concentration is over the studied range (1% v/v – 5% v/v) which means that the actual maximum acetic acid concentration maybe found over 5% v/v HCl.

In Figure 4.38 (C) the temperature is the most effective parameter for acetic acid production, followed by KOH concentration then the 3-dimensional graph presents only the relation between temperature and KOH concentration. This graph presents the maximum acetic acid concentration is 3.10 g/l at 5% w/v NaOH for 60 min of 105 ^{o}C. For the NaOH pretreatment, none of the parameters significantly affect the acetic acid production then there is no 3- dimensional diagram of this product from NaOH pretreatment. From these results, the highest concentration of acetic acid presents in the KOH pretreatment, followed by HCl and H_2SO_4 pretreatment. However, the acetic acid becomes toxicity when the concentration in hydrolyzate is located between 4 g/l to 10 g/l. Since the acetyl group is linked to the backbone chain of hemicellulose by ester linkages and these bonds can be hydrolyzed by alkaline solution via saponification reaction, the acetyl groups under alkaline solution are easier dissolved than consisted in acid condition.

4.7 The Comparison of the Effective of the Chemical Pretreatment for Lignin Removal

(A) **(B)**



(C) **(D)**

Figure 4.39 the response surface plot on lignin removal. A- Effect of temperature at 3% v/v of H_2SO_4 and reaction time of 60 min. B- Effect of temperature at 3% v/v of HCl and reaction time of 60 min. C- Effect of NaOH concentration at reaction time of 60 min and temperature of 115 ^oC. D- Effect of KOH concentration at temperature of 115 ^oC and reaction time of 60 min.

Lignin is a three dimensional polymer with phenylpropane units that are composed of three types of monomer; p-coumary alcohol, coniferyl alcohol and sinapyl alcohol. Because of the several types of monomer in lignin structure, the chemical interaction for both intermolecular and intramolecular are different that makes it very complicate structure. Lignin provides the surface onto irreversible and nonproductive adsorption of enzymes. For this reason, lignin will be considered as the physical barrier for enzyme hydrolysis.The previous research revealed that the greatest number of pores is created by lignin removal that allows cellulose and hemicellulose to be more accessible and easily contact with enzymes. Under the acid pretreatment, the condensation reactions are performed. These reactions are undesirable because they prevent lignin solubilization under acid condition (Candido.R.G el at.). From this reason, the lignin

removal concentration in hydrolyzate from acid concentration is very low when compared with alkaline pretreatment.

From Figure 4.39 (A), the temperature is only the variable that significantly affects the lignin removal while the H_2SO_4 concentration and reaction time are maintained at the center point (3% v/v and 60 min, respectively). From the figure reveals that the concentration of soluble lignin will enhance when the temperature of the process is increased and the maximum yield of soluble lignin concentration is 0.42 g/l. However, the trend of soluble lignin is also increased, although the temperature is over the studied range (115 ^oC- 130 ^oC) which means that the actual maximum concentration of soluble lignin maybe found over a temperature of 130 ^oC. From Figure 4.39 (B), the temperature is only the variable that significantly affects the lignin removal while the HCl concentration and reaction time are sustained at the center point (3% v/v and 60 min, respectively). From the figure shows that the concentration of soluble lignin decreases when the temperature of the process is lower than 105 ^oC. However, the lignin composition can be further hydrolyzed into hydrolyzate when the temperature of the system is increased more than 105 ^oC and the maximum yield of soluble lignin concentration is 0.46 g/l. Nevertheless, the trend of soluble lignin also increase, although the temperature is over the studied range (105 ^oC- 130 ^oC) which means that the actual maximum soluble lignin concentration maybe found over a temperature of 130 ^oC. In Figure 4.39 (C), the NaOH concentration is only the variable that significantly affects the lignin removal while the temperature and reaction time are maintained at the center point (105 ^oC and 60 min, respectively). This figure presents the clearly maximum soluble lignin concentration about 62.5 g/l at the NaOH concentration of 3 % w/v. After 3% w/v of NaOH concentration, the concentration of soluble lignin will decrease. In Figure 4.39 (D), the KOH concentration is only the variable that significantly affects the lignin removal while the temperature and reaction time are maintained at the center point (115 ^oC and 60 min, respectively). This figure demonstrates that the concentration of soluble lignin will enhance when then KOH concentration is increased and the maximum concentration is 70 g/l. However, the trend of soluble lignin is increased, although the KOH concentration is over the studied range (1% w/v – 5%w/v) which means that the actual maximum concentration of lignin removal maybe found over concentration of 5% w/v.

Note: The significant level of this design experiment is fixed at 0.05 ($\alpha = 0.05$) or 95% confidence; however, $\alpha = 0.10$ (90% confidence) is another value that is used in the general statistical analysis. From the ANOVA result of soluble lignin from KOH pretreatment, the reaction time has a significant level equal to 0.0815 ($\alpha < 0.10$) then the 3-dimensional diagram of soluble lignin will be plotted with KOH concentration and reaction time as the temperature of 115 ^oC and the picture is shown in the APPENDIX D. From the RSM result, the maximum yield of the soluble lignin concentration is 82.50 g/l at 5% w/v KOH for 90 min at 115 ^oC.

4.8 The validation of RSM result

1. The Result of Pretreatment Product in Liquid Phase

Table 4.27 Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/l.

Chemical	Variable							
	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose (g/l)	Glucose (g/l)	Furfural (g/l)	Soluble lignin (g/l)	Acetic acid (g/l)
KOH (≥ 180 µm)	1,(5)	0,(115)	1,(90)	0.298	0.316	0.025	73.562	1.540
KOH	1,(5)	0,(115)	1,(90)	0.000	0.402	0.015	65.841	1.571
HCl	0,(3)	-1,(105)	-1,(30)	20.756	1.894	0.214	0.207	1.551

Table 4.28 Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of $g/g_{biomass}$.

Chemical	Variable							
	Concentration %(v/v)	Temperature (°C)	Time (min)	Xylose ($g/g_{biomass}$)	Glucose ($g/g_{biomass}$)	Furfural ($g/g_{biomass}$)	Soluble lignin ($g/g_{biomass}$)	Acetic acid (g/gbiomass)
KOH (≥ 180 µm)	1,(5)	0,(115)	1,(90)	0.001	0.001	0.000	0.140	0.003
KOH	1,(5)	0,(115)	1,(90)	0.000	0.001	0.000	0.118	0.003
HCl	0,(3)	-1,(105)	-1,(30)	0.049	0.004	0.001	0.000	0.004

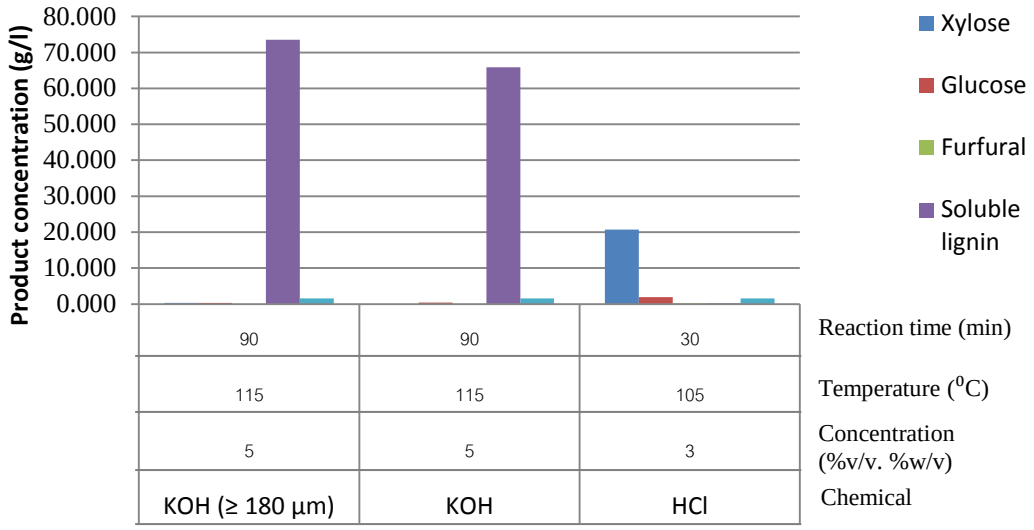


Figure 4.40 The product concentration (g/l) with different pretreatment condition.

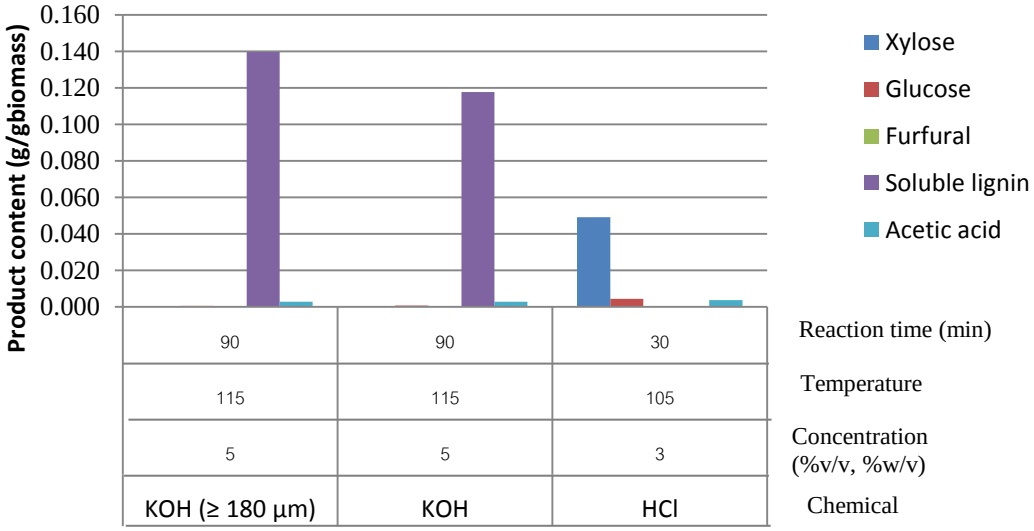


Figure 4.41 The product content ($g/g_{biomass}$) with different pretreatment condition.

This section will discuss about the repeating result of the best condition of chemical pretreatment. The RSM three dimensional graphs show the highest concentration of xylose and soluble lignin together with their conditions. For xylose product, 3% HCl at 105 ºC for 30 min considers the best condition that can produce the highest xylose concentration while 5% KOH at 130 ºC for 90 min is the best condition of soluble lignin product. Then, these two conditions were selected to perform the experiment and then the experimental result will be compared with RSM result. The result is shown in Table 4.27-4.28 and in Figure 4.37- 4.38. This result is clearly that the major product from KOH pretreatment is soluble lignin (65.841 g/l) while xylose (20.756 g/l) is derived from HCl pretreatment. Moreover, this experiment studies the effect of particle

size on lignin removal by using the different size of bagasse (≥ 425 μm and ≥ 180 μm). The result reveals that the smaller particle size can be more effectively remove lignin than a larger particle size and lignin concentration that derives from bagasse pretreatment at a particle size of ≥ 180 μm was 73.562 g/l. The smaller particle size has more surface area to contact with alkaline solvent than a large size, for this reason, the lignin composition can be more easier removed from bagasse material. However, the results from repeated experiments are different from the result from RSM graph. From the RSM result, the highest xylose and soluble lignin concentration were 31.39 g/l and 82.50 g/l whereas the result of the two products from experiment was 20.756 g/l for xylose and 65.841 g/l for soluble lignin. The concentration of these two products from repeated experiment is lower than the RSM program calculation. The accuracy of Response Surface Methodology (RSM) result depends on the degree of a polynomial equation. However, this study is designed for screening the significant parameters from the multivariable; moreover, the equation used in this study is linear. Therefore, the result from RSM calculation can consist of an error due to the accuracy of the RSM model. For this reason, the concentration results from RSM calculation and repeated experiment will have an error between these two processes.

4.9 Enzymatic Hydrolysis

The hydrolysis of cellulose is the process that carried out by using cellulases enzyme for converting this composition into the monomeric sugars especially glucose and xylose product. The utility cost of enzyme hydrolysis is lower than acid/alkaline hydrolysis because the process performs at mild condition (pH 4.8-5.0 and the temperature of 45 ^{o}C – 50 ^{o}C). Since the purpose of this project would like to study the effectiveness of chemical pretreatment for sugars production from bagasse, the enzymatic hydrolysis is the direct pointer that uses to determine the effective chemical pretreatment. The dried pretreated bagasse was continually hydrolyzed by using cellulases and β-glucosidase (cellobias) enzyme in sodium citrate buffer as pH of 4.8. The pretreated bagasse for each chemical type was added to mix with enzyme solution and the process was carried out for 48 hours at the temperature of 50 ^{o}C. The result of each chemical pretreatment is shown in Figure 4.39 and 4.40

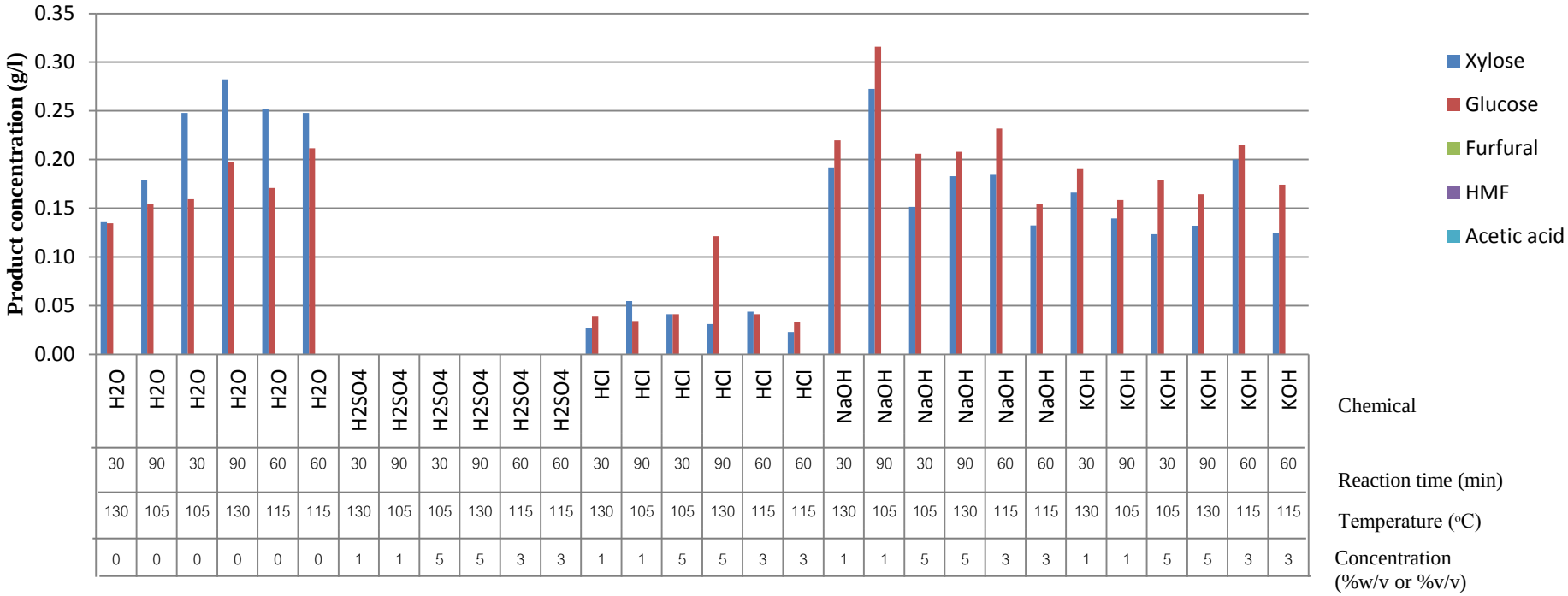


Figure 4.42 The product concentration (g/l) with different pretreatment condition from enzymatic hydrolysis at starting point (reaction time = 0).

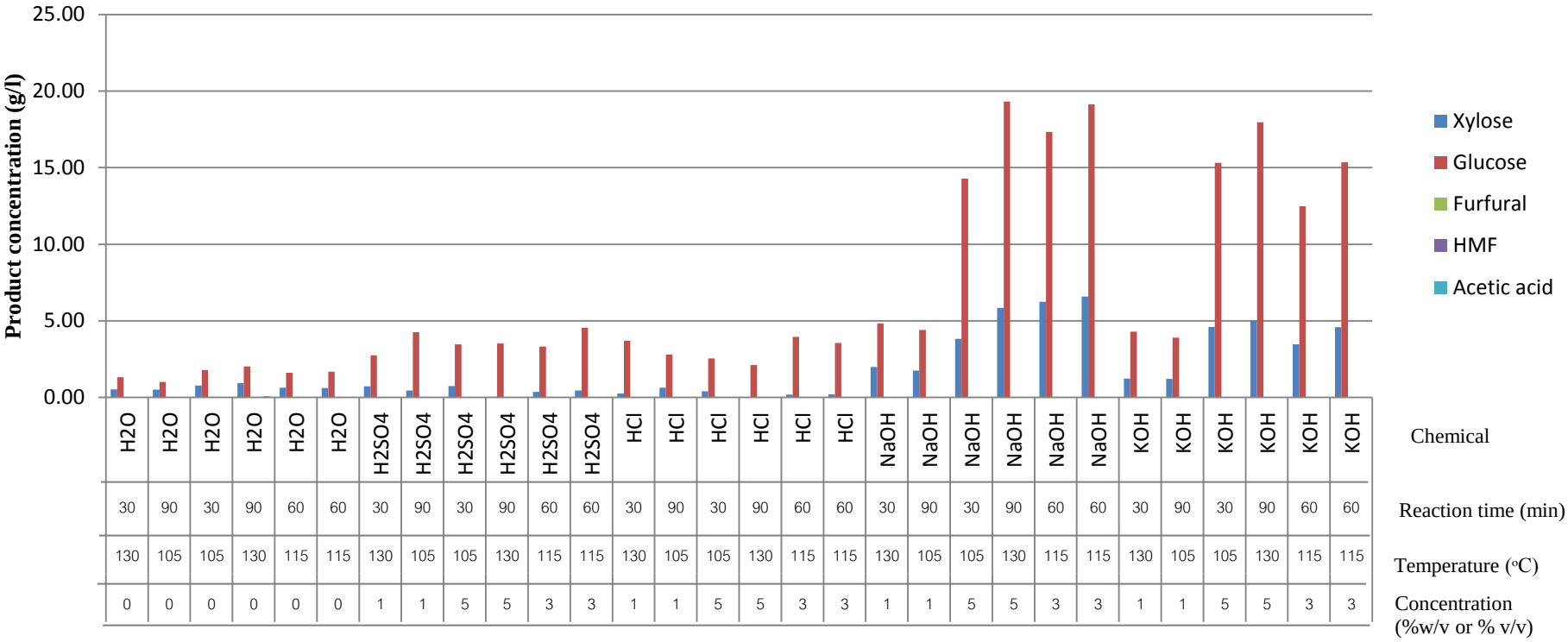


Figure 4.43 The product concentration (g/l) with different pretreatment condition from enzymatic hydrolysis for 48 hours.

Figure 4.39 and 4.40 are the result of enzymatic hydrolysis shown in the form of column graph. Figure 4.39 is the product concentration at the reaction time of 0.0 min. The results from these experiments are used to determine the product concentration at the starting point (reaction time = 0). The cellulases hydrolysis was carried out under the temperature of 50 ^{o}C for the reaction time of 48 hours and the result is shown in Figure 4.40. From the result, the highest yield of glucose concentration was presented under the pretreatment condition of 5% NaOH 130 ^{o}C for 90 min as the concentration of glucose about 19.31 g/l. This pretreatment can remove the lignin from the lignocellulose about 71.72 g/l that is the highest concentration from NaOH pretreatment. Although the highest lignin removal for all chemical pretreatments is presented in the condition of 5% KOH at 130 ^{o}C for 90 min as the concentration of 82.8774 g/l but the glucose concentration from this condition is 17.97 g/l. From this result, the NaOH solution is the most effective chemical that can swell and disturb the crystalline region of cellulose and leads its structure to be more accessible for enzymatic hydrolysis.

For the acid pretreatment, xylose is the major product then the hemicellulose is the main composition which is dissolved from acid solution while most of lignin and cellulose are composed in the pretreated bagasse. The previous research revealed that the greatest number of pores is created by lignin removal that allows cellulose and hemicellulose to be more accessible and easily contact with enzymes, Therefore, the lignin is considered to be the major physical barrier for enzyme hydrolysis. For this reason, the acid pretreatment shows a low concentration of glucose product in the hydrolyzate.

The distilled water pretreatment (Blank) presents the lowest concentration of glucose product from hydrolysis. The lignin removal from blank pretreatment is higher than acid pretreatment. However, when compares the xylose concentration after pretreatment, the acid solution can hydrolyze the hemicellulose from bagasse better than the distillated water. This result can conclude that removal of the hemicellulose is the alternative method that can enhance the effectiveness of enzymatic hydrolysis. These results can assure that the lignin is the major physical barrier for enzymatic hydrolysis process then to remove this composition can improve the efficiency of cellulases enzyme. Therefore, to obtain the high yield of monomeric sugars product, especially glucose, from cellulases enzyme hydrolysis, to remove lignin from the material (bagasse, corn crop, and others) is the best alternative way that can complete this purpose. For this experiment, the NaOH is the best chemical that can remove the lignin composition from bagasse and presents the highest yield of glucose product from enzymatic hydrolysis. Moreover, the physical parameters such as temperature and reaction time are the important factors that affect the lignin removal together with suitable conditions of these parameters.

In conclusion, the highest yield of glucose concentration from cellulases hydrolysis can obtain from the pretreatment that can remove the most of lignin from bagasse. The alkaline solution is the chemical which can effectively remove this composition especially, NaOH solution. The acid solution only removes a small portion of lignin, then the glucose concentration from enzymatic hydrolysis is lower than alkaline

solution However, acid chemical can effectively hydrolyze the hemicellulose composition from bagasse.

The summary of the % recovery of hexose sugar, pentose sugar and soluble lignin in liquid phase, including the % content of cellulose, hemicellulose and insoluble lignin in solid phase are shown in Table 4.29.In the liquid phase, the recovery of cellulose is presented in the form of hexose sugar which includes glucose and HMF whereas hemicellulose product such as xylose, arabinose and furfural are reported in term of pentose sugar. From the table, in the liquid phase is divided into 3 parts that are the % recovery of product after chemical pretreatment, product after hydrolysis and product in washing water. According to the solid phase, this part reports the % product content of cellulose, hemicellulose and insoluble lignin after chemical pretreatment. The pretreated solid is analysed to determine the % of composition content and the result is reveals that the alkaline pretreatment can remove the lignin composition better than acid pretreatment and also retains the cellulose composition in solid phase. The acid pretreatment can remove the most of hemicellulose from bagasse; however, this kind of pretreatment is inappropriate for using to hydrolyse the lignin composition and the results of the % recovery of each product for all pretreatment conditions are shown in Table 4.29.

Table 4.29 The % Recovery of cellulose, hemicellulose and lignin product in the filtrate after chemical pretreatment

Run	Liquid Phase								Solid Phase		
	% Recovery in washed water			% Recovery of filtrate after pretreatment			% Recover of hydrolyzate		% Composition content		
	Hexose	Pentose	Soluble lignin	Hexose	Pentose	Soluble lignin	Hexose	Pentose	Cellulose	Hemicellulose	Insoluble Lignin
A	0.00	2.06	0.43	0.98	21.34	0.38	14.17	6.46	49.37	18.34	32.88
B	0.00	1.34	0.37	0.25	11.78	0.29	22.00	4.10	48.20	18.31	34.41
C	0.00	1.47	0.43	0.61	16.03	0.27	17.18	3.25	48.39	18.64	34.70
D	1.31	1.83	0.62	6.06	16.35	0.38	23.56	4.02	49.24	18.27	34.32
E	0.00	3.84	0.35	1.01	19.87	0.37	17.94	6.66	47.34	19.92	33.83
F	0.00	4.84	0.35	1.11	21.93	0.33	18.18	0.36	34.45	26.94	40.04
G	0.00	0.53	10.58	0.01	0.02	9.97	25.06	17.95	48.81	25.34	21.19
H	0.00	0.43	10.46	0.02	0.03	5.29	22.74	15.84	50.18	25.16	21.89
I	0.00	0.36	16.51	0.01	0.07	34.15	89.71	56.57	55.96	31.64	7.55
J	0.00	0.17	10.72	0.01	0.07	55.45	99.07	59.65	57.93	29.90	9.16
K	0.00	0.62	15.82	0.01	0.02	42.93	73.92	34.67	66.68	22.01	8.97
L	0.00	0.89	19.17	0.01	0.08	54.70	99.94	52.89	67.05	24.12	8.62
M	0.42	5.55	0.58	1.13	22.77	0.30	19.18	2.26	51.86	15.88	33.52
N	0.13	3.88	0.62	0.50	18.82	0.45	14.51	5.63	51.71	16.93	31.50
O	0.30	5.53	0.29	1.10	27.85	0.23	20.40	1.57	48.38	18.33	34.31
P	3.68	0.35	0.35	9.70	5.27	0.46	18.38	1.72	51.31	16.64	33.19
Q	0.66	5.15	0.27	1.29	22.21	0.27	13.20	3.55	53.55	14.63	25.82
R	0.49	5.28	0.37	1.61	27.50	0.29	10.89	0.00	**	**	57.52
S	0.00	0.31	6.09	0.01	0.02	4.61	22.24	11.05	47.51	23.26	27.84
T	0.00	0.44	8.00	0.01	0.02	6.79	20.18	10.86	46.82	23.46	27.85
U	0.00	0.67	20.02	0.01	3.07	30.42	64.62	31.42	59.45	22.90	16.95
V	0.00	1.07	23.61	0.01	2.62	45.41	79.43	41.52	59.72	23.17	16.47
W	0.00	0.00	21.65	0.01	2.96	36.70	79.30	41.66	60.69	25.35	10.99
X	0.00	0.59	14.14	0.01	3.02	28.06	93.00	45.44	61.57	27.53	8.38

Table 4.29 The % recovery of cellulose, hemicellulose and lignin product in the filtrate after chemical pretreatment (Cont.)

Run	Liquid Phase								Solid Phase		
	% Recovery in washed water			% Recovery of filtrate after pretreatment			% Recover of hydrolysate		% Composition content		
	Hexose	Pentose	Soluble lignin	Hexose	Pentose	Soluble lignin	Hexose	Pentose	Cellulose	Hemicellulose	Insoluble Lignin
Blank G	0.00	0.00	0.40	0.17	1.52	1.60	6.81	4.73	44.91	21.62	25.07
Blank H	0.00	0.33	0.61	0.11	1.15	0.84	5.20	4.57	45.35	24.90	24.96
Blank Q	0.01	0.29	1.10	0.09	1.67	2.03	8.33	5.69	43.74	21.94	31.54
Blank L	0.00	0.29	0.51	0.08	2.38	0.92	8.68	5.51	45.07	20.91	26.50
Blank I	0.00	0.28	0.45	0.11	1.74	1.68	9.24	7.01	44.16	21.32	24.72
Blank C	0.03	0.67	0.93	0.18	1.82	1.16	10.50	8.44	45.88	22.64	28.79

Undetected component **