STUDY OF PRETREATMENT IMPACTS ON SUGARCANE BAGASSE AND DURIAN PEEL BY USING RESPONSE SURFACE METHODOLOGY

JAKAPHAN RATANAPOOMPINYO

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DISSERTATION SUBMITTED IN PART OF FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD BIOTECHNOLOGY

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PAPER TITLE

STUDY OF PRETREATMENT IMPACTS ON SUGARCANE BAGASSE AND DURIAN PEEL BY USING RESPONSE SURFACE METHODOLOGY

ABSTRACT

Inappropriate combustion of lignocellulosic biomass plausibly generates environmental problems. Agricultural waste utilization does not only reduce air pollutions but also converts the biomass wastes into value-added products e.g. biofuels. However, the physical and chemical properties of agricultural wastes are the limiting determinants for utilization. Therefore, agricultural wastes e.g. sugarcane bagasse and durian peel were performed with diluted sulfuric pretreatment to break down the lignocellulosic fibrils and to enhance enzymatic saccharification. In this experiment, the optimum pretreatment parameters on sugarcane bagasse and durian peel were temperature (60–140ºC), time (20–100mins), and acid concentration (0.5–3.5%) and modified according to Response Surface Methodology (RSM) using Box-Behnken design. Pretreated lignocellulosic samples were enzymatically hydrolyzed after pretreatment and the efficiency of pretreatment were examined according to the reducing sugar concentration. The mathematical model demonstrated the correlation of each pretreatment factor and generated reducing sugars were used to optimize pretreatment conditions. At predicted optimum pretreatment conditions, the results revealed that the reducing sugar of pretreated sugarcane bagasse was obtained as 180.15 mg/g-sugarcane bagasse, 3.06 folds higher than unpretreated sugarcane bagasse, at pretreatment conditions; 136.08ºC, 75.36 minutes, 3.50% and the reducing sugar of pretreated durian peel was acquired as 551.07 mg/g-durian peel, 1.88 folds higher

compared to unpretreated durian peel, at pretreatment conditions; 127.14ºC, 74.13 minutes, 2.75%. However, during the pretreatment, some inhibitors obstructing the fermentation process were generated such as Acetic acid, Hydroxymethylfurfural (HMF), and Furfural. This study demonstrated about the pretreatment capability in agricultural waste utilization to further produce biofuels and value-added products.

KEY WORDS: Durian peel / Sugarcane bagasse / Pretreatment / Reducing sugar / Response Surface Methodology

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OBJECTIVE

To study the utilization of agricultural wastes such as sugarcane bagasse and durian peel by using mathematical models in order to not only reduce the problem of air pollution in Thailand but also generate value added products in downstream processing.

INTRODUCTION

Agricultural waste utilization could be one of the solutions for inappropriate combustion [1] - [2]. Sugarcane bagasse and durian peel are extensively recognized as agricultural biomass wastes. These biomass wastes have the capabilities to be converted into several products such as biofuels, absorbents, insulators, briquettes, medicines, food substances, platform chemicals, and biotechnological materials [3] - [7].

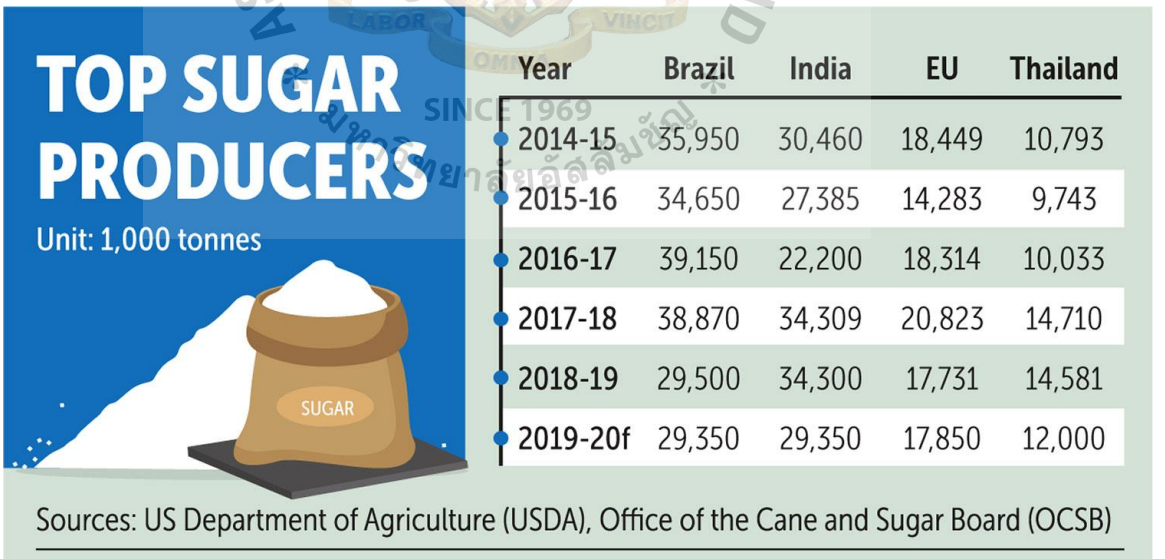


Figure 1. Comparison of various top sugar produced countries

According to the US Department of Agriculture (USDA) and Office of the Cane and Sugar Board (OCSB), Thailand has produced sugar around 12 million tons during the year of 2019-2020 but the amount of produced sugar is still less than other countries like Brazil, India and Europe as displayed in figure 1 [90]. However, the demand of other countries for Thai durians is steadily remained despite the coronavirus pandemic.

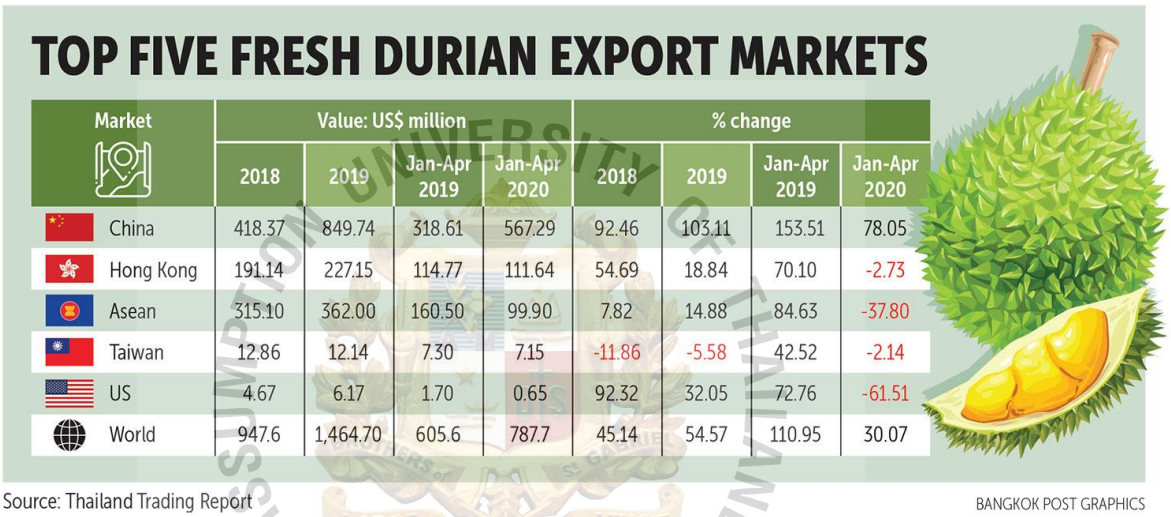


Figure 2. Top five markets of durian export from Thailand

The information of the Ministry of commerce revealed that, in the first four months of 2020, Thailand exported durians to China as $567.29 million, up 78% year-on-year from the same period of 2019. In 2019, Thailand also exported durians to oversea markets such as China, Hong Kong and Asian countries as $1.46 billion accounting for 98% of export volume which rises to 54.6% from the previous year as presented in figure 2 [91].

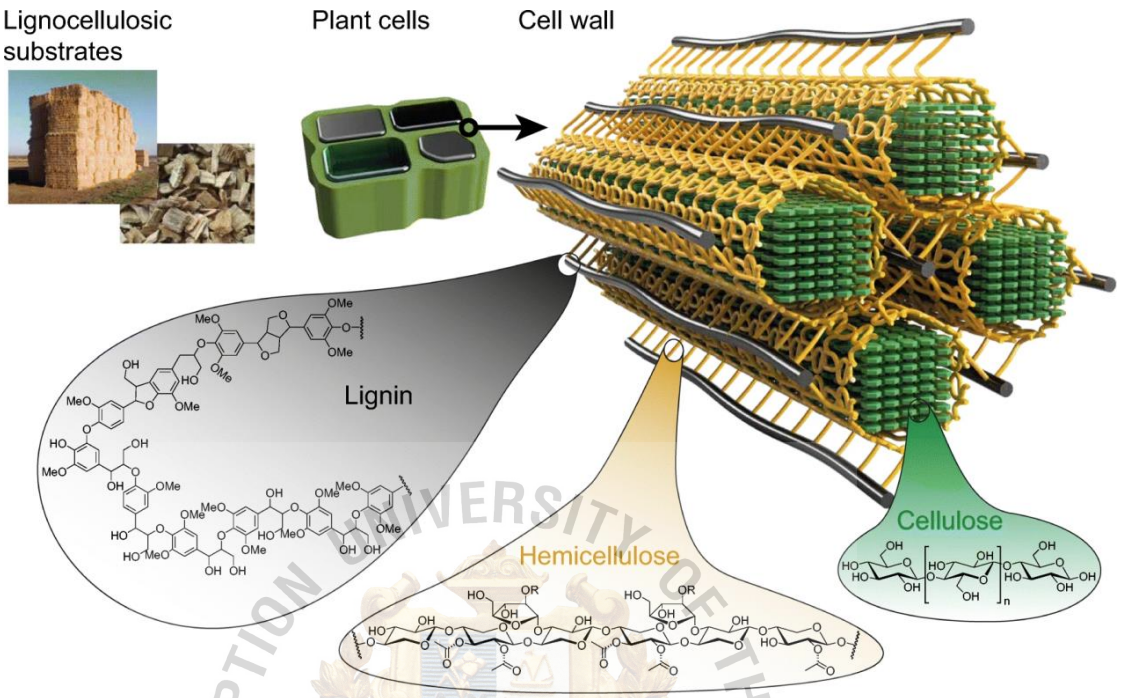


Figure 3. Components and structure of lignocellulosic plant cell walls (S. C. Yat, A. Berger and D. R. Shonnard, 2008)

Cellulose, hemicellulose, and lignin are united in the form of lignocellulosic biomass as shown in figure 3 [8]. The ratio of each composition might be varied according to the species of plants, for instance, a higher amount of hemicellulose is measured in wheat straws and leaves, while much quantity of cellulose is displayed in hardwood [9]. Furthermore, different ages, stages of growth, and others can affect the amount of each component in single plant species [10].

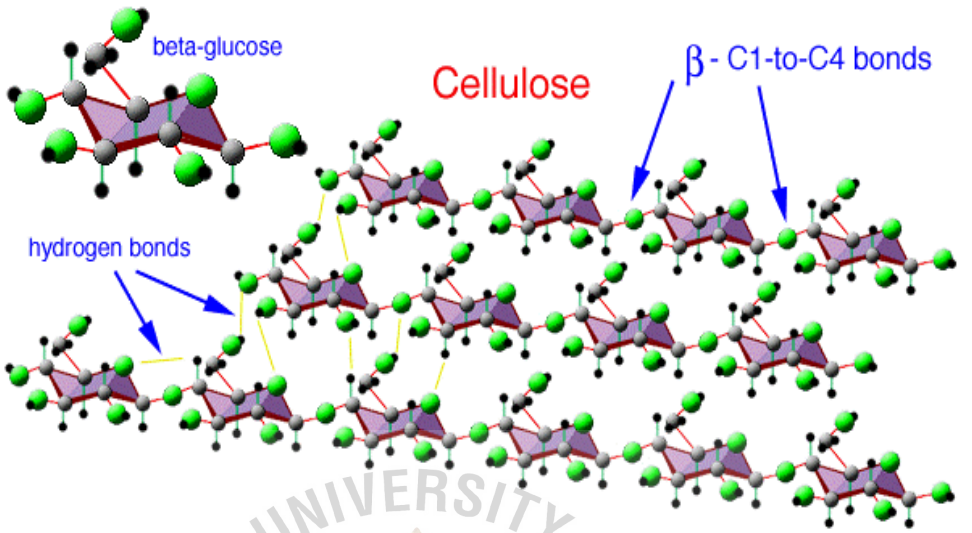


Figure 4. Long chain polymer of cellulose comprising D-glucose connected to others by β-(1,4)-glycosidic bonds (John Blamire, 2004)

In the plant cell wall, cellulose is the main composition assembling fibrous structure illustrated in the form of the linear polymer comprising D-glucose connected to others by β-(1,4)-glycosidic bonds as displayed in figure 4 [113]. Besides, Cellobiose is recognized as duplicate units of the linkage gathering cellulose chains. The long-chain polymers can be attached by hydrogen bonds and Van der Waals forces to transfer cellulose into microfibril concealed by lignin and hemicellulose. β-(1,4)-glycosidic linkages in cellulose can be broken by using acid or enzyme and further generate D-glucose via hydrolysis reaction.

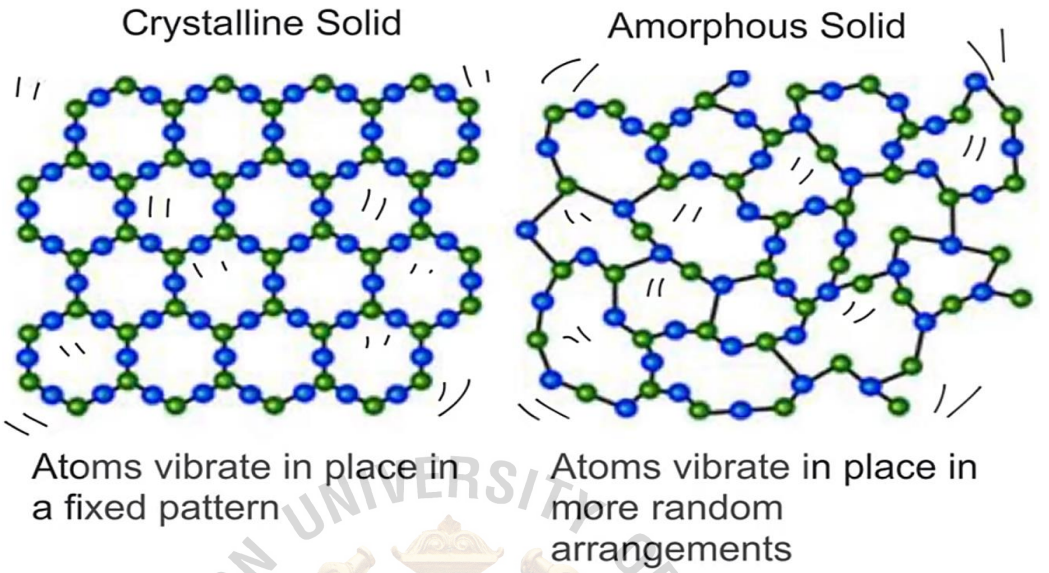
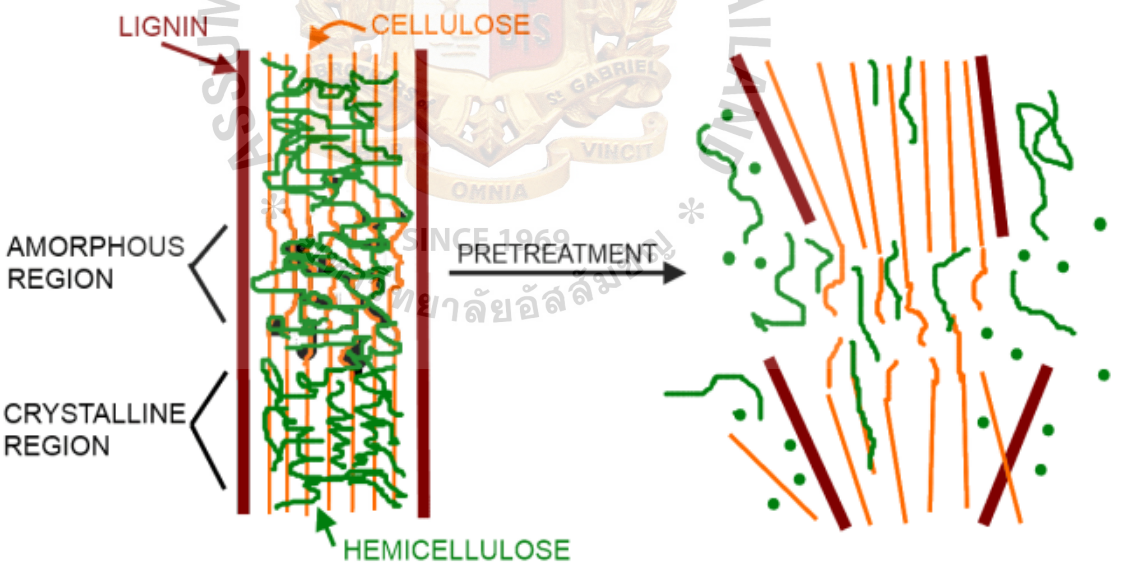


Figure 5. Comparison between the form of crystalline and amorphous (Brandis Stracuzzi, 2017)

Crystalline and amorphous are the ordinary forms displayed in cellulose as illustrated in figure 5 [114]. In cellulose linkage, the form of crystalline greatly appears, however, the little ratio occurs in the form of amorphous which is more susceptible to enzymatic hydrolysis [11] – [12]. Furthermore, the cellulose and hemicellulose should be initially disintegrated into proper monomers (e.g. sugars) for simplifying microorganism utilization during the conversion of lignocellulosic biomass. Consequently, the sugar monomers could be transformed into diverse value-added products of biofuels such as biodiesel, bioethanol, biomethane, and butanol based on microorganisms applied in the fermentation process. There are three important processes generally used for producing different sugars from lignocellulosic biomass as follows; diluted acid, concentrated acid, and enzymatic hydrolysis [13].

Hemicellulose is more vulnerable to dilute-acid hydrolysis than cellulose. Dilute-acid hydrolysis is commonly conducted under high temperature and pressure and low glucose concentration from hydrolysis therefore generate a low yield of by-product. Nevertheless, concentrated acid-hydrolysis could ameliorate the amount of by-product because of better hydrolysis capability [14]. Many enzymes from microorganisms could disintegrate lignocellulose into smaller molecules but require a longer retention time.

Enzymatic hydrolysis is extensively applied in lignocellulosic biomass utilization and there are various physicochemical, structural, and compositional factors obstructing the cellulose digestibility. Lignocellulosic biomass required pretreatment for making cellulose fibril more accessible and facilitate the interaction of cellulase with further steps [15].



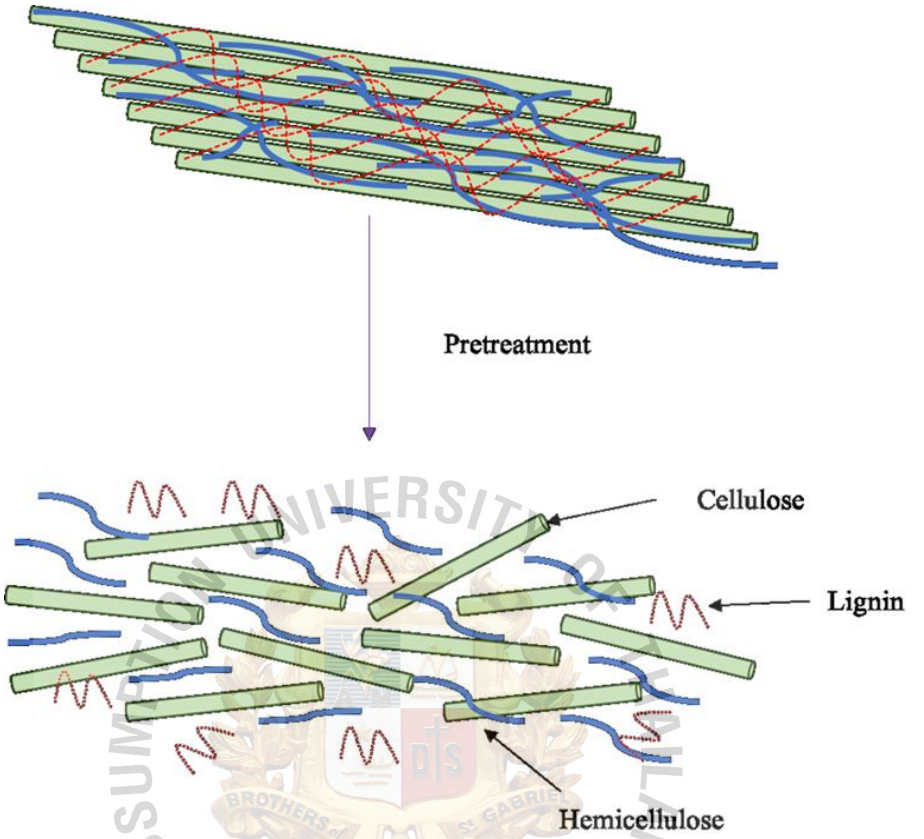


Figure 6. Schematics of lignocellulosic biomass pretreatment (N. S. Mosier et al., 2005)

One of the crucial barriers of sugarcane bagasse and durian peel utilization was its tight structure, therefore, it needs some strategies to break down these sturdy structures into smaller particles. Pretreatment is recognized as a necessary process for converting biomass and the major objective of pretreatment is disintegrating lignin structure, decreasing the crystallinity of hemicellulose and cellulose, and enlarging the porosity of the lignocellulose to allow acids or enzymes simply enter and hydrolyze cellulose as shown in figure 6 [16].

Pretreatment could be fundamentally classified into different categories as follows; physical (milling or grinding) [17], physicochemical (autohydrolysis or hydrothermolysis) [17], chemical (alkali, dilute acid, oxidizing agents and organic solvents, ionic liquid) [18] – [23],

biological [24] – [27] and electrical [28]. Concentrated acid is regularly used as a pretreatment agent, therefore, it is meticulous for applications because of toxicity, corrosion, and hazard [29]. Dilute-acid hydrolysis has been applied in lignocellulosic biomass pretreatment, for instance, dilute sulfuric acid (<4wt%) was utilized as a prudent and productive solvent for the cellulosic biomass industry [30]. Diluted sulfuric pretreatment potentially catalyzes the reaction rates and cellulose hydrolysis [31], furthermore, it could be capably used for hydrolyzing and digesting hemicellulose to be xylose and small molecule of sugars [32]. Diluted acid pretreatment with high temperature has capability in cellulose hydrolysis [33].

In this experiment, dilute sulfuric acid (H_2SO_4) was used for the pretreatment of sugarcane bagasse and durian peel to enzymatically enhance lignocellulosic saccharification, Response Surface Methodology (RSM) was also performed to optimize the pretreatment conditions and escalate the amount of reducing sugars, which could be further converted to value-added products or biofuels in downstream processing.

RSM is recognized as an effective method in optimization of process conditions determining the influence of various factors and their interactions on the interested measures under investigation during technological operation and applying in an absolute quadratic polynomial model through central inclusive experiments which presents an outstanding experiment design and result expression as displayed in figure 7 [60 , 62]. This method is frequently used for empirical modeling and prediction [61].

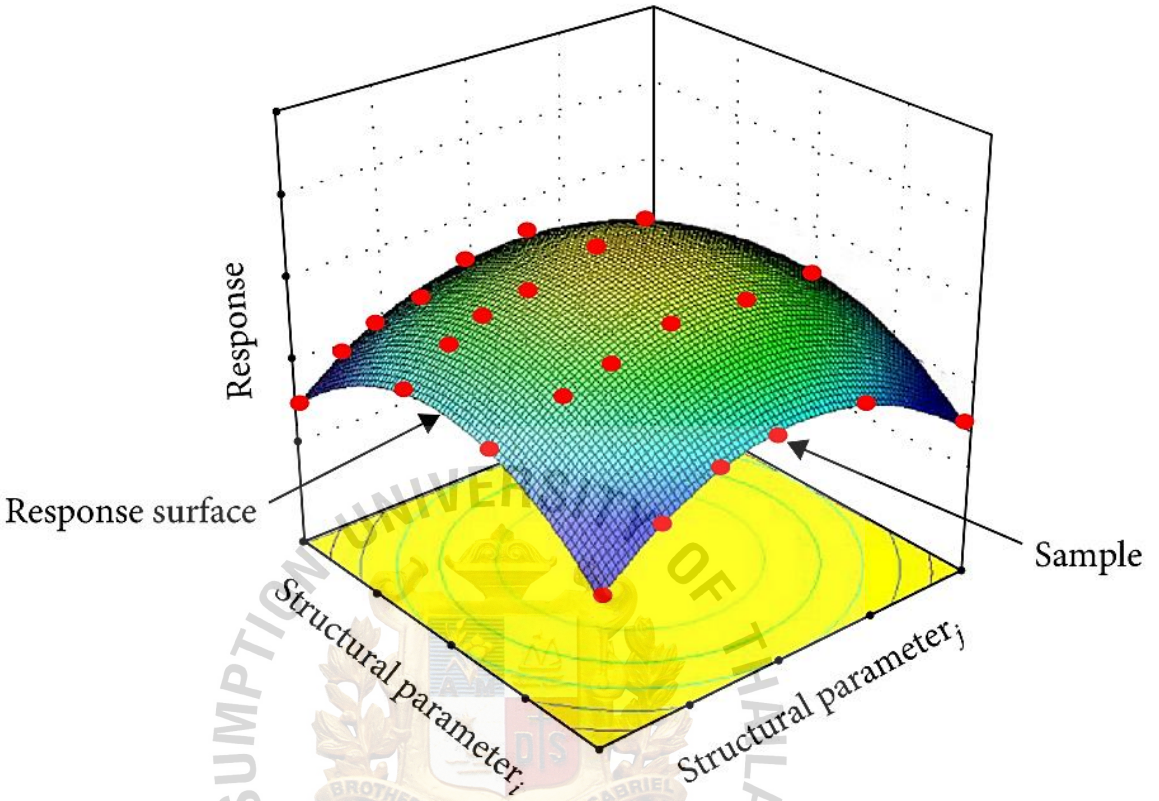


Figure 7. The diagram of response surface representing the explicit relationship between response value and structural parameters (Huang, W. et al., 2020)

Moreover, Fourier Transform Infrared Spectrophotometer (FTIR) was used for analyzing the types of components and differentiating the functional groups of compounds in samples by absorbing the light at a wavelength of middle infrared region 2.5 - 50 mm and wave number range 4000 - 400 cm^{-1}, the advantage of this technique is a non-destructive sample, short time measurement and safety for both liquid and solid samples [34]. FTIR is also beneficial for monitoring and characterizing unknown compounds, examining contaminants, finding additives, and inspecting decomposition and oxidation.

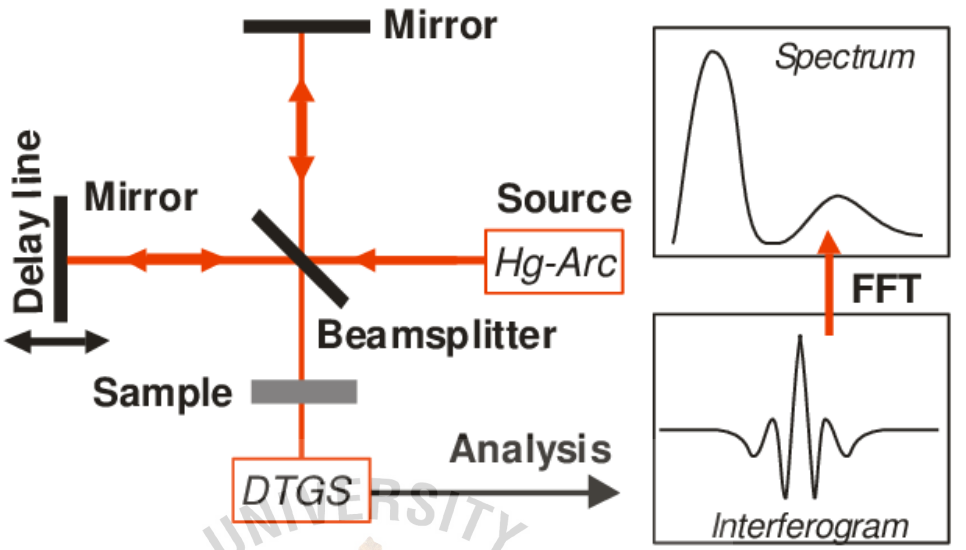


Figure 8. A schematic diagram of FTIR (Panowicz, R. et al., 2011)

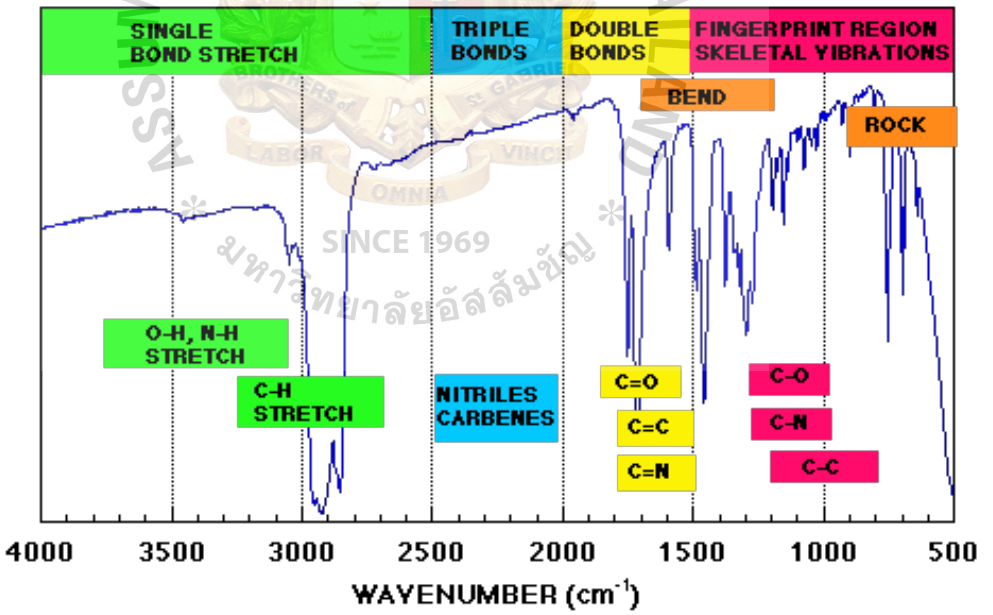


Figure 9. Fourier transform infrared spectroscopy (FTIR) spectrum analyzing qualitative and quantitative of many compounds (Photometrics, n.d.)

FTIR instruments generally compose of a source, sample cell, detector, amplifier, A/D convertor, and a computer. FTIR mechanisms start with radiation from the source arrives to the detector after passing through interferometer then the signal is amplified and converted to digital signal by A/D convertor and amplifier after that signal is transferred to the computer and finally processed by Fourier transform as shown in Figure 8,9 [88] – [90].

Finally, this experiment could at least motivate some readers to utilize and convert the agricultural wastes to be value-added products and alleviate environmental pollutions from inappropriate combustion of biomass waste.

MATERIALS AND METHODS

A. Preparation of raw materials

Sugarcane bagasse was obtained from a sugar factory in Thailand and durian peel was also acquired from Trat Province, Eastern Thailand. Sugarcane bagasse was processed by a sugar factory and then transferred to the laboratory in the form of chopped and dried samples.

Durian peel was firstly chopped into small size as 2x2 cm^2, further blended with water at a ratio of 1:4 (kg/L), and ground by using Moulinex A643. Durian peel paste was eventually received and subsequently dried at 60°C using a tray dryer until the sample was dried and the weight was stable. Thereafter, the durian peel sample was then ground once more into smaller pieces, 10-mesh sized aluminum sieve filtered, and sealed in a plastic bag, at last, to be ready for experimental usage.

B. Pretreatment of biomass

The samples of both sugarcane bagasse and durian peel weighed as 0.8 g were separately pretreated with 40 ml of diluted sulfuric acid (H_2SO_4) at various times and concentrations. Both Agri-waste samples were adjusted with acid and base until pH equal to 7.0 and they were then heated at 60°C until the weight was stationary. Each sample from both of them was weighed as 0.1 g and further translocated into a 15-ml test tube. The range of pretreatment conditions (temperature, time, and %concentration of H_2SO_4) for both sugarcane bagasse and durian peel samples were achieved by using RSM optimization (Box-Behnken Design, Design-Expert version 7.0). These pretreatment condition ranges for both sugarcane bagasse and durian peel were determined by using RSM as followings pretreatment temperature (X_1: 60-140°C), pretreatment time (X_2: 20-100 mins), and acid concentration (X_3:0.5-3.5%), and they were simultaneously filled

in the RSM design table. In the RSM table, each pretreatment factor on both of them was adjusted with three levels, i.e. high (+1), mid (0), and low (-1). The experiments of pretreatment were performed based on each designed condition. After pretreatment, each sample was enzymatically hydrolyzed and the amount of reducing sugar liberated from each pretreated sample was then determined and filled in the RSM table. X_1, X_2, X_3, and Y variables were recorded into the RSM table and finally examined after assembling all experimental results [35]. ANOVA was used to evaluate these experimental results to discover the pretreatment factors that effectively affect the amount of reducing sugars. The mathematical model was designed according to RSM calculation with a significant pretreatment factor. Moreover, not only the optimum pretreatment conditions but also the predicted equation of reducing sugar for sugarcane bagasse and durian peel were also revealed.

C. Measurement of sugar concentration and analysis of biomass composition

After pretreatment, pretreated biomass weighed as 100 mg was filled into hydrolysis buffer comprising 4 ml of 0.05M citrate buffer, 40 µL of 2M sodium azide, 35 µL of CelluClast 1.5L, and 10 µL of the β-glucosidase enzyme. The incubated condition on each test tube was performed at 45°C, 150 rpm for 72 hours. After incubation, the supernatant fraction was stored as a sample for DNS analysis [36] to examine the concentration of reducing sugar acquired from each sample. 50 µL of the hydrolyzed sample was mingled with 150 µL of DNS solution and thoroughly blended with vortex, heated at 95°C for 5 minutes, and cooled off with an iced bath for 5 minutes, respectively. 1 ml of distilled water was consecutively added into the sample and shaken again with vortex before transferring into a cuvette and finally translocated into a spectrophotometer (540 nm) to measure the reducing sugar concentration. The effect of each pretreatment factor on the amount of released reducing sugar was statistically analyzed by ANOVA using the SPSS

program (Version 26.0). Measurement of cellulose, hemicellulose, and lignin were executed based on Goering & Van Soest, (1970) [37] to examine the amount of cellulose, hemicellulose, and lignin.

D. Measurement of inhibitors (GC-MS)

Gas Chromatograph and Mass Spectrometer; SHIMADZU, Kyoto, Japan: GCMS-QP2020 NX (Capillary columns) was used to determine the concentration of potential inhibitors e.g. Furfural, Hydroxymethylfurfural (HMF), and Acetic acid. Three sections of samples for sugarcane bagasse and durian peel were prepared as a section of control, a section of no enzyme addition, and a section of enzyme addition. After that, all prepared samples were then filtered by passing through a syringe filter (obstructing large particles that could be clogged inside the GC column during analysis) and kept into a 2-ml GC-MS vial. 0.001 ml of each sample inside the 2-ml GC-MS vial was further transferred into GC-MS by using a 0.01 ml-microliter syringe. GC-MS was continually operated and the peak area vs. retention time of each chromatograph from sugarcane bagasse and durian peel samples was monitored and finally recorded to interpret these data into the concentration of inhibitors by using the standard curve of each inhibitor afterward. The GC-MS conditions of HMF was shown as followings; Injector temperature at 250°C, 50°C (holding for 2 minutes), 110°C (70°C/min), 250°C (15°C/min and holding for 10 minutes) and the GC-MS conditions of Furfural and Acetic acid were presented as followings; Injector temperature at 250°C, 50°C (holding for 1 minute), 120°C (4°C/min and holding for 2 minutes), 170°C (6°C/min and holding for 1 minute), 200°C (10°C/min and holding for 1 minute).

E. Fourier Transform Infrared Spectroscopy (FTIR) Determination

Fourier Transform Infrared Spectrophotometer (Perkin Elmer; FTIR-1000, Waltham, Massachusetts, United States) with the range of wavenumbers between 400 to 4,000 cm^{-1} (14 cycles) was used to analyze the compositions on unpretreated and pretreated of both sugarcane bagasse and durian peel samples. Potassium bromide (KBr) Disk, Infrared Radiation (IR) and Fourier Transformation were respectively used as a sample preparation method, application and mathematical technique providing full coverage of the mid-IR spectral region and good refractive index match with these samples.

F. Statistical Data Analysis

ANOVA Single factor, T-test dependent (Paired sample test) and T-test independent supported with IBM SPSS Statistics Program and XLMiner Analysis ToolPak (Microsoft Excel) were used to analyze on statistical data analysis in this study. In Appendix E., ANOVA Single factor and T-test dependent (Paired sample test) were used to make a statistical analysis on the comparison of %Cellulose, %Hemicellulose and %Lignin (before and after pretreatment) because of the same type of sample but different treatment conditions. In the same way, for Appendix F., the weight of sugarcane bagasse and durian peel sample (before and after pretreatment) were statistically analyzed by ANOVA Single factor and T-test dependent (Paired sample test) because using similar type of sample but different treatment conditions. However, on Appendix G., the comparison of quantity on fermentative inhibitors on each experimental section (control, no enzyme addition and enzyme addition) between two cellulosic raw materials (sugarcane bagasse and durian peel) were statistically analyzed by ANOVA Single factor and T-test independent because sugarcane bagasse and durian peel were categorized as independent factors and the focus were upon the concentration of inhibitor on each experimental

section comparing between two different treatments. The criteria of consideration for ANOVA Single factor were the p-value should be lower than at 95% confident level ($\alpha = 0.05$) in order to be labelled as significant difference. Moreover, for both T-test dependent (Paired sample test) and T-test independent; H_0 = No significant difference and H_a = Significant difference, if | T-stat | was higher than T-critical, it would deny main hypothesis (H_0) and accept secondary hypothesis (H_a), when df = 2 (T-test dependent); at 95% confident level, T-table = 2.920 and df = 4 (T-test independent); at 95% confident level, T-table = 2.132. According to Appendix E. – F., T-stats (Paired sample test) were all higher than T-critical at 95% confident level ($\alpha = 0.05$), it could therefore indicate that %Hemicellulose, %Cellulose and %Lignin of both sugarcane bagasse and durian peel samples before pretreatment is significantly higher than after pretreatment and the weight of sugarcane bagasse and durian peel samples after pretreatment were also significantly lower than before pretreatment, respectively. In addition, according to Appendix G., | T-stats | (T-test independent) were also higher than T-critical ($\alpha = 0.05$), therefore, it could display that the concentration of fermentative inhibitors like acetic acid and furfural on sugarcane bagasse sample in each experimental section is significantly different rather than on durian peel sample.

RESULTS AND DISCUSSION

In this experiment, Goering & Van Soest method was used to measure the amount of cellulose, hemicellulose, and lignin on sugarcane bagasse and durian peel before and after pretreatment as shown in Table 1 indicating that cellulose (%), hemicellulose (%), and lignin (%) in sugarcane bagasse were all decreased after optimum conditions of pretreatment. Moreover, durian peel compositions after optimal pretreatment conditions were also decreased associating with the reducing amount of cellulose (%), hemicellulose (%), and lignin (%).

It could be implied that optimum pretreatment conditions potentially affected the composition amount of both sugarcane bagasse and durian peel as other researchers have studied that the process of pretreatment could not only break down and disintegrate hemicellulose and lignin [55] but also reduce the cellulose crystallinity and increase the porosity (surface area) of lignocellulosic structure in sugarcane bagasse and durian peel [56] , [58] - [59].

This is a reason why the amount of cellulose, hemicellulose, and lignin after optimum pretreatment conditions became lower than before pretreatment as similar to other studies; the amount of cellulose in sugarcane bagasse was decreased after pretreatment with 8%NaOH, 40°C for 24 hours [57], 58% and 77% of lignin and hemicellulose were respectively removed after sequential acid-base pretreatment [53] and the amount of lignin in sugarcane bagasse was reduced when using sodium hydroxide as alkaline pretreatment agent [54]. Moreover, this phenomenon reasonably complied with the weight after pretreatment decreased in both sugarcane bagasse and durian peel illustrated in Table 2.

Table 1. The contents of cellulose, hemicellulose and lignin in sugarcane bagasse and durian peel before and after pretreatment

	Cellulose (%)	**Hemicellulose (%)**	**Lignin (%)**
Before pretreatment (Sugarcane bagasse)	48.72 ± 1.45	23.28 ± 2.01	20.72 ± 1.16
After pretreatment (Sugarcane bagasse)	32.49 ± 1.53	1.67 ± 0.79	11.46 ± 1.09
% Reduction	33.30 (p-value = 0.000408)	92.84 (p-value = 0.000146)	44.66 (p-value = 0.001213)
Before pretreatment (Durian peel)	57.64 ± 1.17	15.22 ± 1.83	18.45 ± 0.86
After pretreatment (Durian peel)	40.44 ± 2.41	1.20 ± 0.36	10.52 ± 1.71
% Reduction	29.83 (p-value = 0.000817)	92.09 (p-value = 0.000444)	42.99 (p-value = 0.004279)

Table 2. The weight of sugarcane bagasse and durian peel before and after optimum pretreatment

	Sugarcane bagasse	**Durian peel**
Before pretreatment (g)	0.81 ± 0.00070	0.81 ± 0.00064
After pretreatment (g)	0.58 ± 0.0047	0.54 ± 0.018
Difference (%)	28.45 (p-value = 2.71993E-07)	33.98 (p-value = 2.93478E-05)

So far, a lot of scientific papers have revealed the use of RSM for determining the pretreatment factors and condition ranges to suitably optimizing their experiments [38] – [42] because RSM is an efficient method for optimizing the process conditions and examining the relationship between response value and structural parameters on the interested factors under investigation during technological operation and applying in an absolute quadratic polynomial model through central inclusive experiments showing an outstanding experiment design and result expression which is frequently applied in empirical modeling and prediction [60] – [61].

In this experiment, the pretreatment condition ranges performing RSM on sugarcane bagasse and durian peel were adjusted and optimized according to other studies as shown in Table 3 then the experiments were proceeded until the final pretreatment conditions were concluded and conformed to this study [59], which were determined at pretreatment temperature (X_1) of 60-140^{o}C, pretreatment time (X_2) of 20-100 mins, and acid concentration (X_3) of 0.5- 3.5%.

Table 3. Comparison of pretreatment conditions from other studies

No.	Temperature (°C)	Time (mins)	Concentration (%)	Reference
1	60 - 140	20 – 100	0.5 – 3.5	This study
2	60 - 140	20 – 100	0.5 – 3.5	[59]
3	112.5 – 157.5	5 - 35	0 - 3	[63]
4	130	15 - 180	0.5 – 3.5	[64]
5	135 - 195	5 - 25	0.5 – 2.5	[65]
6	121	30 - 90	0 - 4	[66]
7	120	120	0.1 M	[67]

The concentration of reducing sugars from each pretreated sample on sugarcane bagasse and durian peel after hydrolysis was determined and recorded as the dependent factor in Table 4 and Table 5, respectively. After implementing RSM on each pretreatment condition, the enzyme cocktails of cellulase and β-glucosidase enzymatically hydrolyzed each pretreated sample afterward and the reducing sugar concentration of sugarcane bagasse and durian peel hydrolysates were then measured by the DNS method and recorded in Table 4 and Table 5, respectively.

Table 4. RSM with Box-Behnken Design of sugarcane bagasse representing the relationship between pretreatment factors (X_1 Pretreatment temperature, X_2 Pretreatment time, and X_3 Concentration of H_2SO_4) and Reducing sugar concentration (Y)

Run	Temp. (ºC) X_1	Time (mins) X_2	Conc. of H_2SO_4 (%) X_3	Concentration of reducing sugar (mg/ml), Y
1	140	100	2.0	4.774
2	100	20	3.5	1.917
3	60	60	3.5	1.418
4	100	60	2.0	2.788
5	60	20	2.0	1.157
6	100	20	0.5	1.428
7	140	60	0.5	3.215
8	100	60	2.0	3.056
9	60	100	2.0	1.390
10	100	60	2.0	3.079
11	140	60	3.5	4.328
12	100	100	0.5	2.788
13	60	60	0.5	1.193
14	140	20	2.0	2.128
15	100	60	2.0	2.977
16	100	60	2.0	2.798
17	100	100	3.5	4.131

Table 5. RSM with Box-Behnken Design of durian peel representing the relationship between pretreatment factors (X_1 Pretreatment temperature, X_2 Pretreatment time, and X_3 Concentration of H_2SO_4) and Reducing sugar concentration (Y)

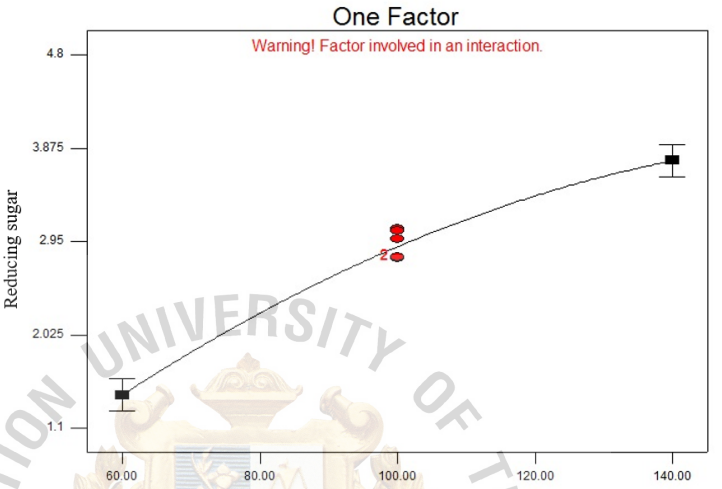
Run	Temp. (°C) X_1	Time (mins) X_2	Conc. of H_2SO_4 (%) X_3	Concentration of reducing sugar (mg/ml), Y
1	60	60	0.5	6.638
2	140	60	3.5	15.016
3	100	60	2.0	14.628
4	60	20	2.0	6.182
5	100	60	2.0	12.652
6	100	100	0.5	10.155
7	100	20	3.5	9.003
8	100	60	2.0	13.496
9	140	60	0.5	12.354
10	140	100	2.0	13.437
11	100	60	2.0	13.754
12	100	20	0.5	6.907
13	100	100	3.5	13.248
14	100	60	2.0	12.831
15	60	100	2.0	8.039
16	140	20	2.0	8.503
17	60	60	3.5	7.761

The model summary statistics suggested that the correlation model between pretreatment factors and reducing sugars of sugarcane bagasse and durian peel were Quadratic model with correlation efficiency (R^2) as 0.9896 and 0.9585, respectively which significantly supported the model fitting. Independent (X value) and dependent (Y value) factors in the RSM table were further examined as fitness to the suggested model by using ANOVA analysis.

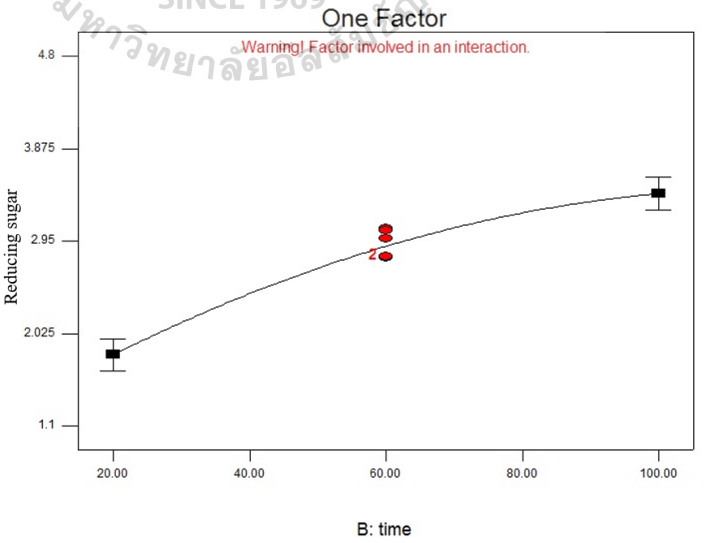
The ANOVA results showed that model fitting of sugarcane bagasse (P-value < 0.01) and durian peel (P-value < 0.001) were statistically significant and implying that the Quadratic vs 2FI model were the dependable models to express the correlations between pretreatment factors and amounts of reducing sugars. Likewise, according to the Lack of fit test (ANOVA for Response Surface Reduced Quadratic Model) on sugarcane bagasse and durian peel were shown as 0.2259 and 0.2861, respectively representing to insignificant Lack of Fit model. Consequently, these RSM models were greatly assured and trustworthy. Moreover, the variables of the pretreatment model were examined with the same basis displaying the pretreatment temperature (model term-A), pretreatment time (model term-B) and acid concentration (model term-C) of sugarcane bagasse and durian peel were also significant with P-value < 0.001.

Refer to the RSM analysis (ANOVA), the effects of each pretreatment factor on reducing sugar yield of sugarcane bagasse and durian peel could be forecasted through the fit model and demonstrated in one coordinating factor plot (Figure 10,11) and contour plot (Figure 12,13). The relationship between pretreatment factors (e.g. pretreatment temperature, pretreatment time, and acid concentration) and reducing sugar yield of sugarcane bagasse and durian peel represented the same direction as illustrated in Figures 10 and 11; increasing the level of pretreatment factor could enhance the concentration of reducing sugar.

(A)



(B)



(C)

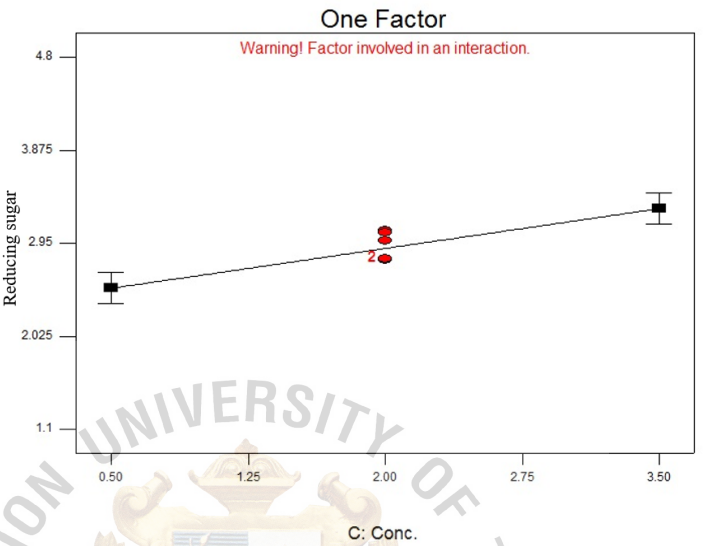
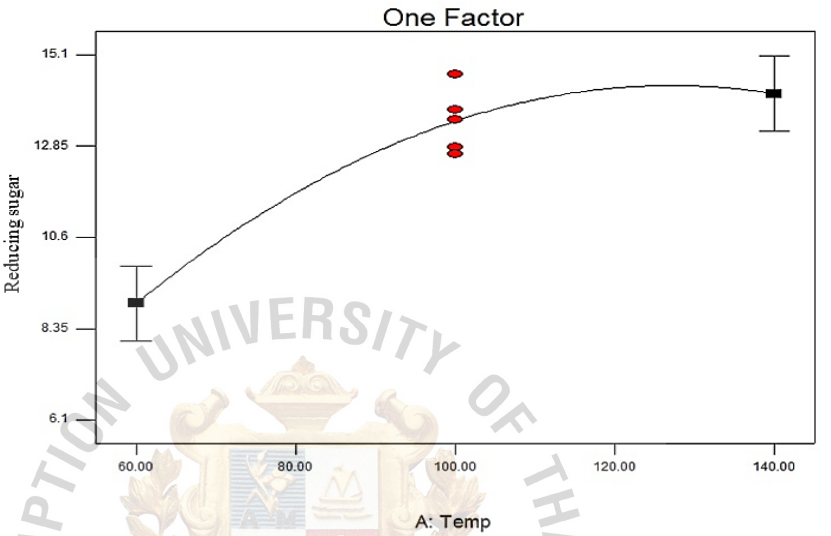


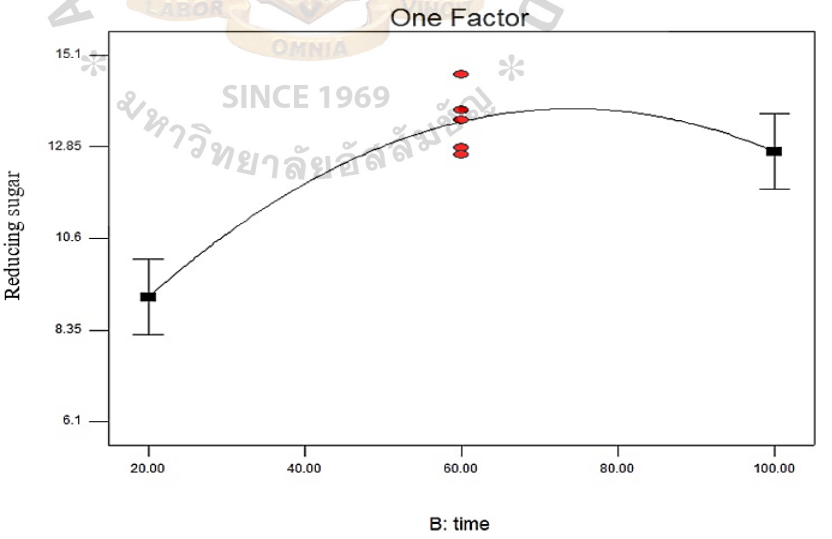
Figure 10. The relationship between each pretreatment factor, including (A) pretreatment temperature (ºC), (B) pretreatment time (min) and (C) acid concentration (%) and reducing sugar concentration (mg/ml) obtained from pretreated sugarcane bagasse.

Interestingly, the straight positive trend of reducing sugar on durian peel was saturated when the pretreatment level reached a certain point and the sugar concentration was gradually converted to a negative point afterward. However, the saturated point of reducing sugar from sugarcane bagasse was indistinctly demonstrated when compared to the saturated point of durian peel. Besides, the slope of each graph, not only sugarcane bagasse but also durian peel, was varied depending on the pretreatment factor. These conditions could be described as the reducing sugar deterioration at high temperature, long pretreatment time, and high acid concentration could continually lead to the hydrolysis of sugar [21], [43], [44].

(A)



(B)



(C)

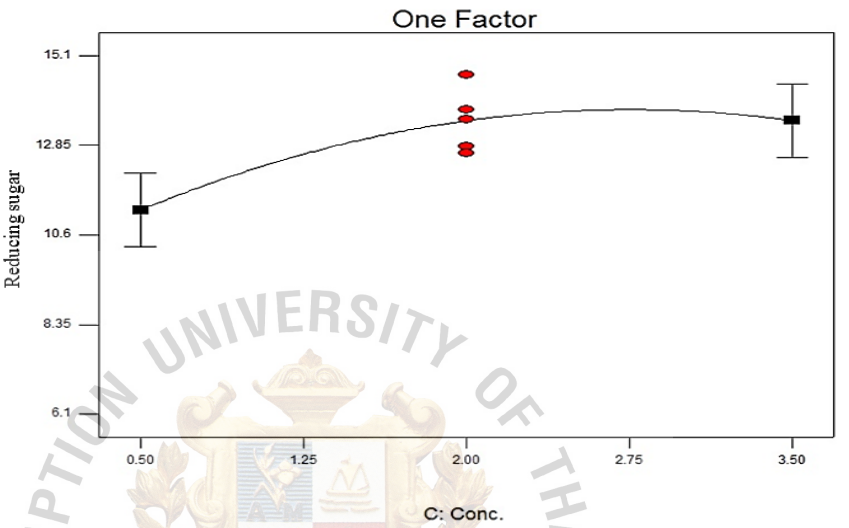
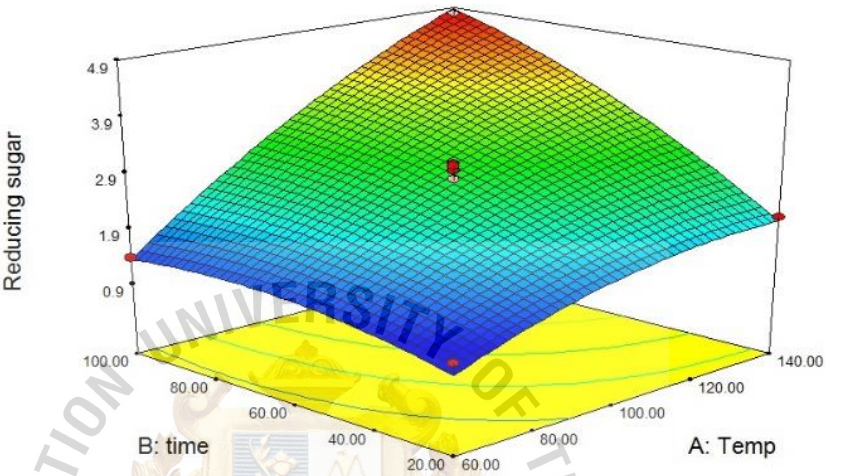


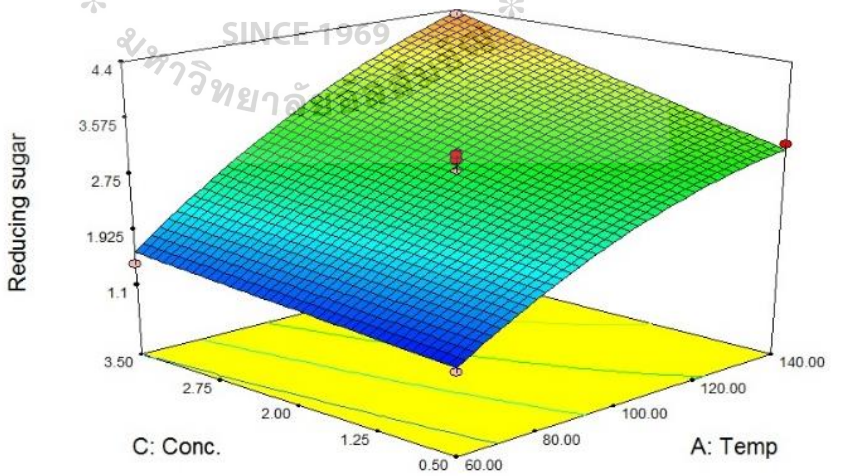
Figure 11. The relationship between each pretreatment factor, including (A) pretreatment temperature (ºC), (B) pretreatment time (min) and (C) acid concentration (%) and reducing sugar concentration (mg/ml) obtained from pretreated durian peel.

Moreover, contour plots from RSM indicating the interacting effects of two factors at a time on the amount of reducing sugar on sugarcane bagasse and durian peel were illustrated in Figures 12 and 13, respectively. These plots could enhance the determination of the synergistic effect or inhibitory effect of two interacting factors. For instance, Figures 12A showed that increasing pretreatment temperature and pretreatment time could increase the amount of reducing sugar similar to the single factor plot displayed in Figures 10A and 10B.

(A)



(B)



(C)

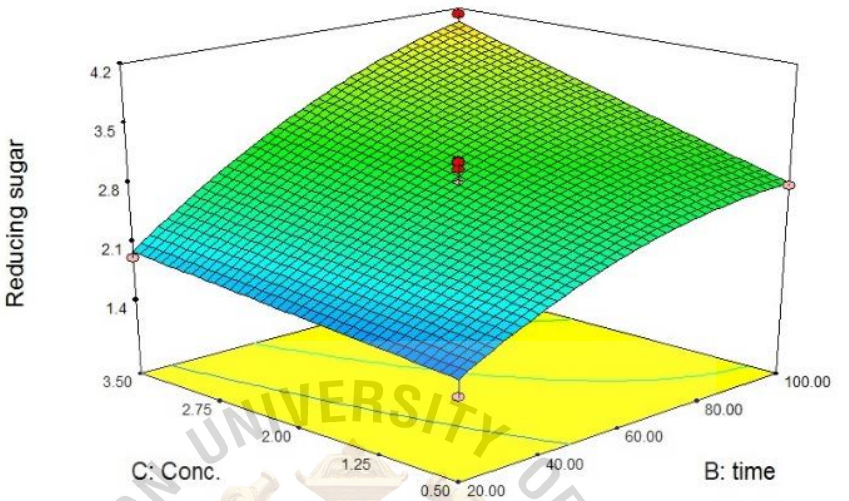
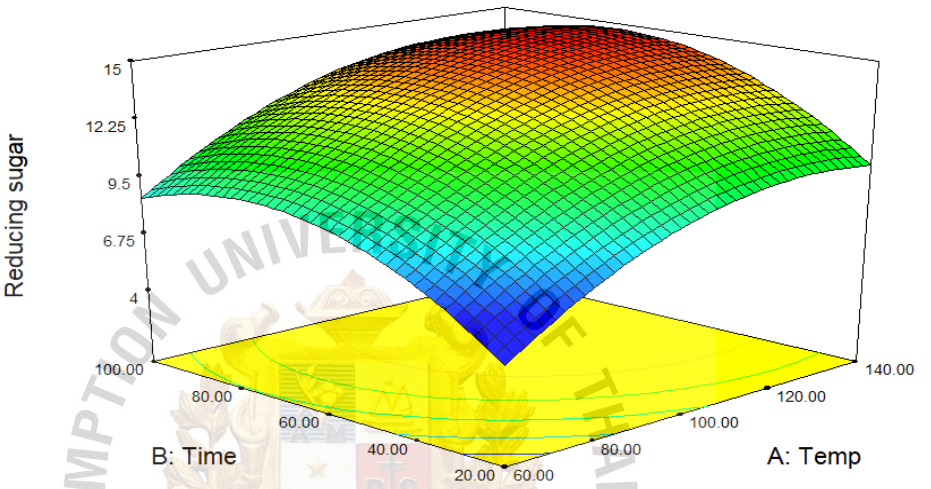


Figure 12. Contour plots of sugarcane bagasse representing the effects of pretreatment factors including (A) pretreatment time vs. pretreatment temperature, (B) acid concentration vs, pretreatment time and (C) pretreatment temperature vs. acid concentration on the concentration of reducing sugars (mg/ml).

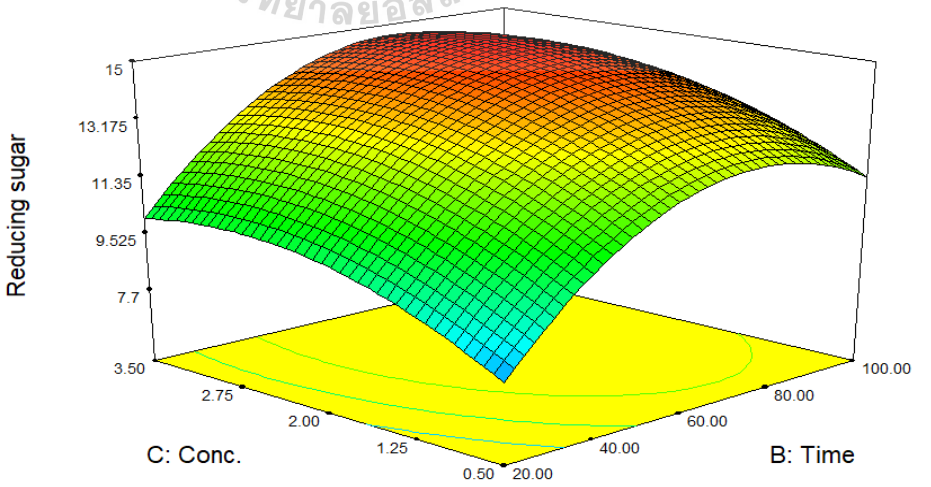
Nevertheless, at the point of high temperature and long pretreatment time, the highest amount of reducing sugar could be achieved as illustrated in the red-colored zone of the contour plot. These patterns were similarly revealed in the contour plots, for sugarcane bagasse, pretreatment temperature vs. pretreatment time demonstrated in Figure 12A and for durian peel, pretreatment time vs. acid concentration and pretreatment temperature vs. acid concentration illustrated in Figure 13B and 13C. The contour plots were consequently used as tools for evaluating the optimum pretreatment conditions and observing their interacting effects. The RSM with Box-Behnken Design experiment could be explained in the mathematical models as presented in Table

4,5 displaying the relationship between pretreatment factors (X_1, X_2, and X_3) and reducing sugar concentration (Y).

(A)



(B)



(C)

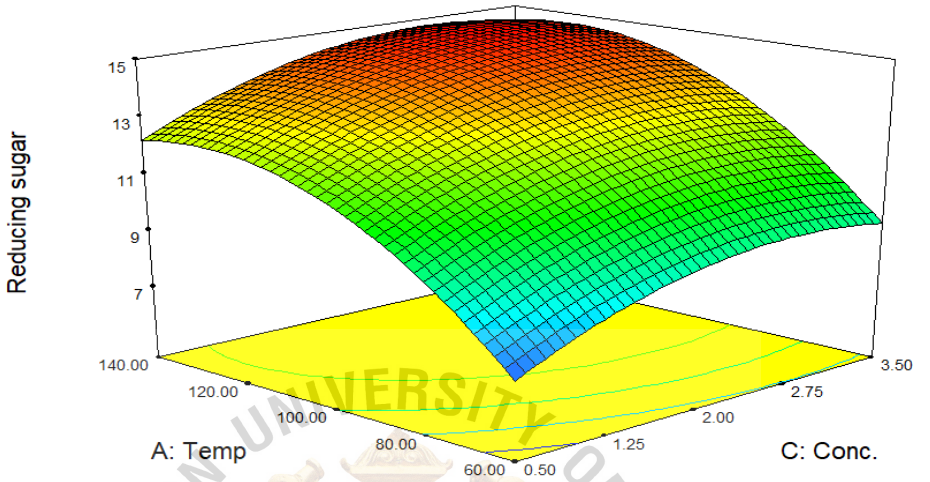


Figure 13. Contour plots of durian peel representing the effects of pretreatment factors including (A) pretreatment time vs. pretreatment temperature, (B) acid concentration vs. pretreatment time and (C) pretreatment temperature vs. acid concentration on the concentration of reducing sugars (mg/ml).

According to the predicted equations of agricultural samples calculated by RSM (Table 6 and Table 7), the predictions on optimum conditions of pretreatment for sugarcane bagasse were pretreatment temperature at 136.08ºC, pretreatment time at 75.36 minutes, the acid concentration at 3.50%, with predicted optimum yield at 4.85 mg/ml, and for durian peel, pretreatment temperature at 127.14ºC, pretreatment time at 74.13 minutes, the acid concentration at 2.75%, with predicted optimum yield at 14.95 mg/ml, respectively.

Model accuracy verification was executed by experimenting with pretreatment at optimum conditions again to validate the amount of reducing sugar. Based on the validation results, the concentration of reducing sugar obtained from the sugarcane bagasse and durian peel at optimum

conditions were 4.41 mg/ml and 13.49 mg/ml which were different from the predicted value as 9.07% and 9.76%, respectively.

Compared to other studies as shown in Table 8, the range of reducing sugar concentration for sugarcane bagasse was observed to be around 197.4 to 537 mg/g biomass but in this study, the sugar concentration result after enzyme addition was 180.15 mg/g biomass, which is lower than other experiments. At the same time, the concentration range of reducing sugar on durian peel sample was monitored to be around 560.7 to 905.5 mg/g biomass but the sugar concentration of durian peel sample after adding enzyme in this experiment was 551.07 mg/g biomass displaying that the sugar concentration of durian peel in this experiment is lower than other studies. These situations could possibly be happened because the pretreatment agent, experimental condition, age, strain, quality and characteristic of biomass samples on both sugarcane bagasse and durian peel in this experiment were different from other experiments.

Interestingly, the reducing sugar concentrations of both sugarcane bagasse and durian peel samples labeled as control and no enzyme addition are lower than the concentrations of reducing sugar after adding enzyme because enzyme addition effectively influences on the reducing sugar concentration in this experiment, for example, CelluClast enzyme has properties to not only catalyze the degradation of the glucose polymers comprising of cellulose to be glucose, cellobiose (e.g. pairs of glucose units) and longer chains of glucose units but also break down cellulosic materials into fermentable sugars [69] and β-Glucosidase enzyme also has a potential to catalyze the hydrolytic breakage of β-glycosidic linkages, presented in either disaccharides, oligosaccharides, or so-called conjugated glucosides, between two glycone residues or between glucose and an alkyl or aryl aglycone in order to convert cellobiose as well as short cellodextrins

into glucose [70] – [72]. This is a reason why the amount of reducing sugar after adding enzyme is higher than no enzyme addition.

In this experiment, the total reducing sugar yield obtained from pretreated sugarcane bagasse and pretreated durian peel at optimum conditions were 180.15 mg/g sugarcane bagasse and 551.07 mg/g durian peel that was 3.06 folds and 1.88 folds compared to unpretreated sugarcane bagasse and unpretreated durian peel, respectively.

Table 6. Predicted equation and predicted optimal pretreatment conditions for production of reducing sugars obtained from sugarcane bagasse

Predicted model equation; **Reducing sugar concentration (mg/ml) =** -0.86616 + 0.037451 x Temp – 3.65380E-003 x time – 0.31926 x Conc. + 3.77076E-004 x Temp x time + 3.69853E-300 x Temp x Conc. + 3.55887E-300 x time x Conc. – 1.92255E-004 x $Temp^2$ – 1.74990E-004 x $time^2$		
Predicted optimal conditions of the highest reducing sugar **(Sugarcane bagasse)**		
Temperature (ºC)	***Time (mins)***	***H_2SO_4 Concentration (%)***
136.08	75.36	3.50
Predicted sugar concentration (mg/ml)	***Actual sugar concentration (mg/ml)***	***Difference (%)***
4.85	4.41 ± 0.0057	9.07

Table 7. Predicted equation and predicted optimal pretreatment conditions for production of reducing sugars obtained from durian peel

Predicted model equation; **Reducing sugar concentration (mg/ml)** = -16.76690 + 0.30325 x Temp + 0.23388 x time + 2.74082 x Conc. - 1.19301E-003 x Temp2 - 1.57703E-003 x time2 - 0.49827 x Conc.2		
Predicted optimal conditions of the highest reducing sugar **(Durian peel)**		
Temperature (ºC)	***Time (mins)***	***H_2SO_4 Concentration (%)***
127.14	74.13	2.75
Predicted sugar concentration (mg/ml)	***Actual sugar concentration (mg/ml)***	***Difference (%)***
14.95	13.49 ± 0.0079	9.76

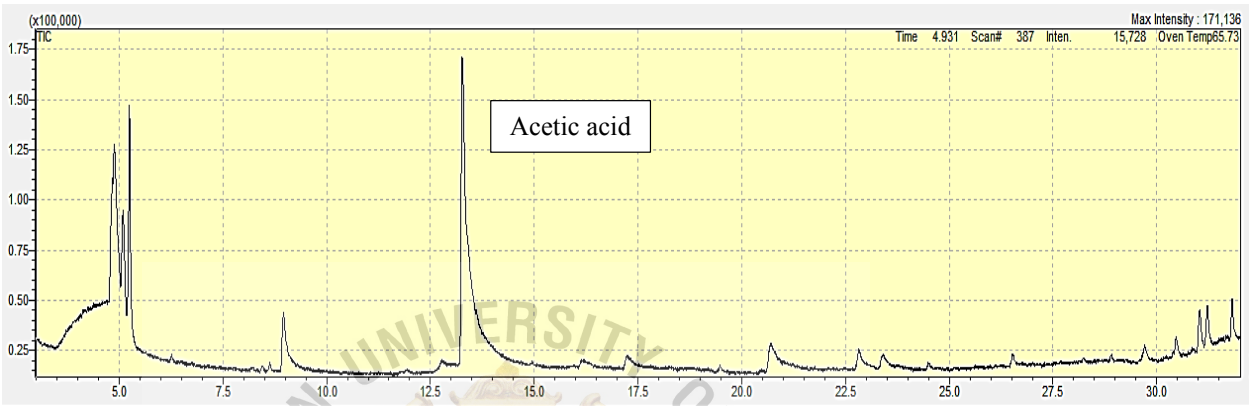
Table 8. Comparison of reducing sugar concentration in experimental sections of pretreatment on Agri-waste samples

REDUCING SUGAR CONCENTRATION	SUGARCANE BAGASSE (MG/G BIOMASS)	DURIAN PEEL (MG/G BIOMASS)
CONTROL	58.82 ± 0.81	293.30 ± 0.75
NO ENZYME ADDITION*	59.23 ± 0.64	340.28 ± 0.59
ENZYME ADDITION**	180.15 ± 0.77	551.07 ± 0.86
[63]	197.4	-
[65]	350	-
[66]	537	-
[68]	-	560.7
[58]	-	905.5

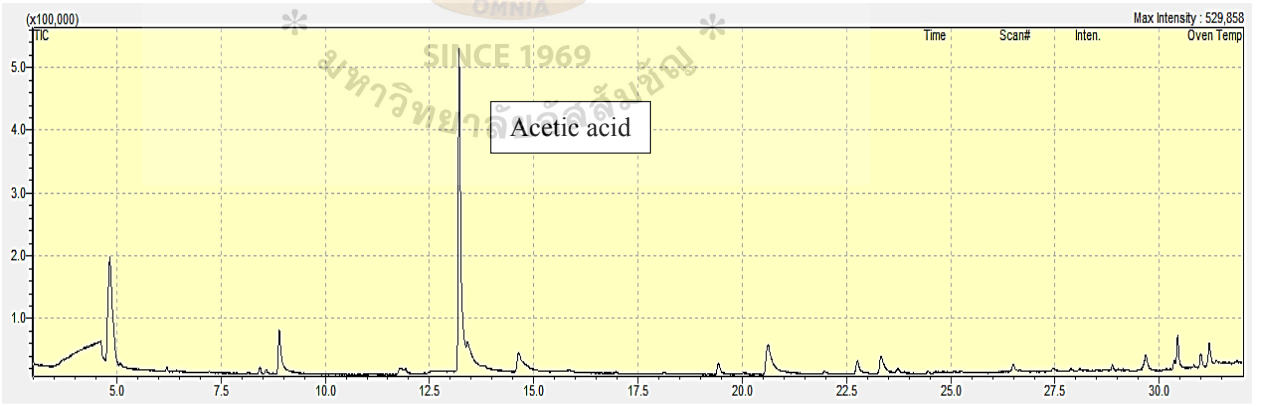
*Applying optimum pretreatment conditions but no enzyme addition

**Applying both optimum pretreatment conditions and enzyme addition

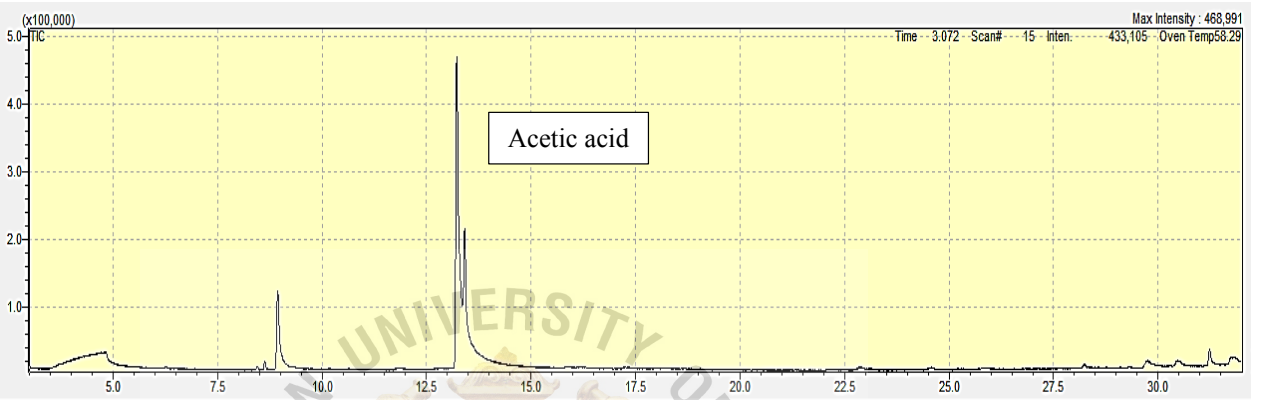
(a) Detection of acetic acid on sugarcane bagasse (control)



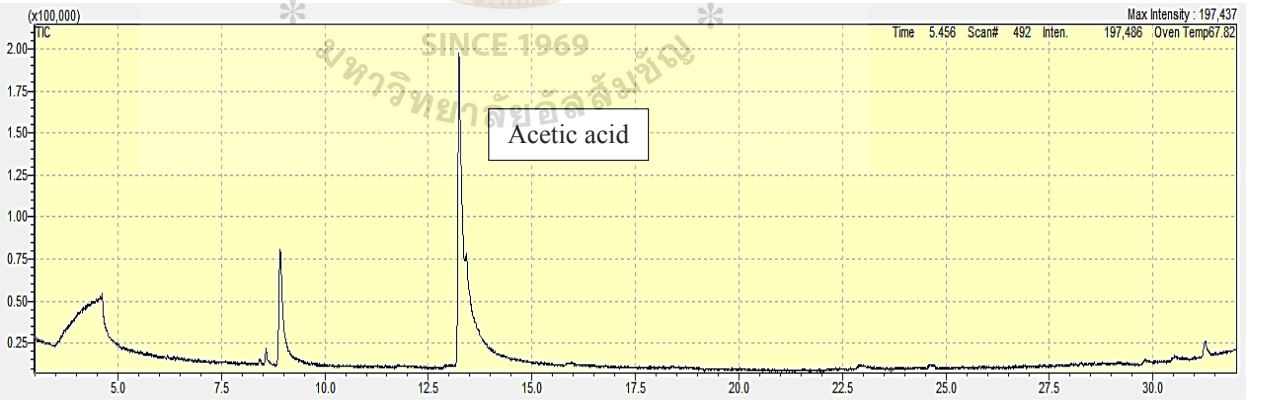
(b) Detection of acetic acid on durian peel (control)



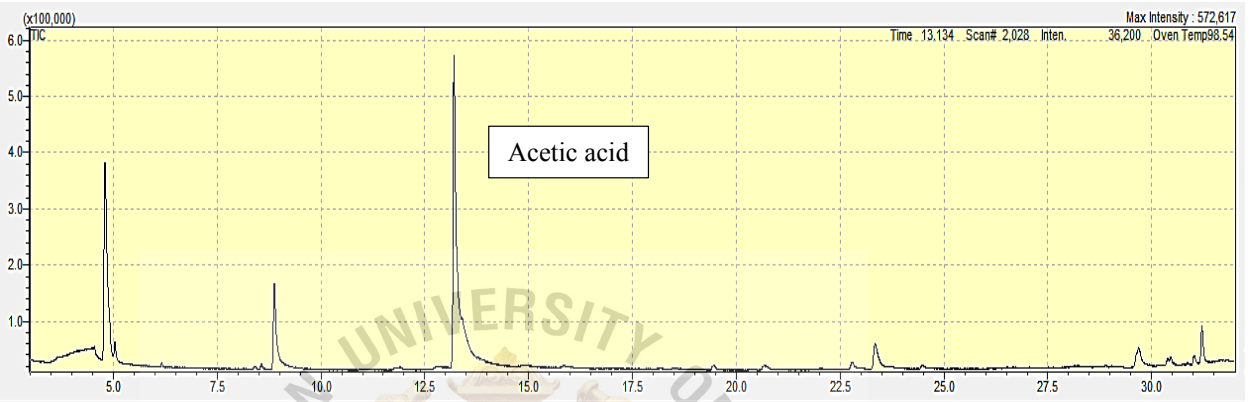
(c) Detection of acetic acid on sugarcane bagasse (liquid)



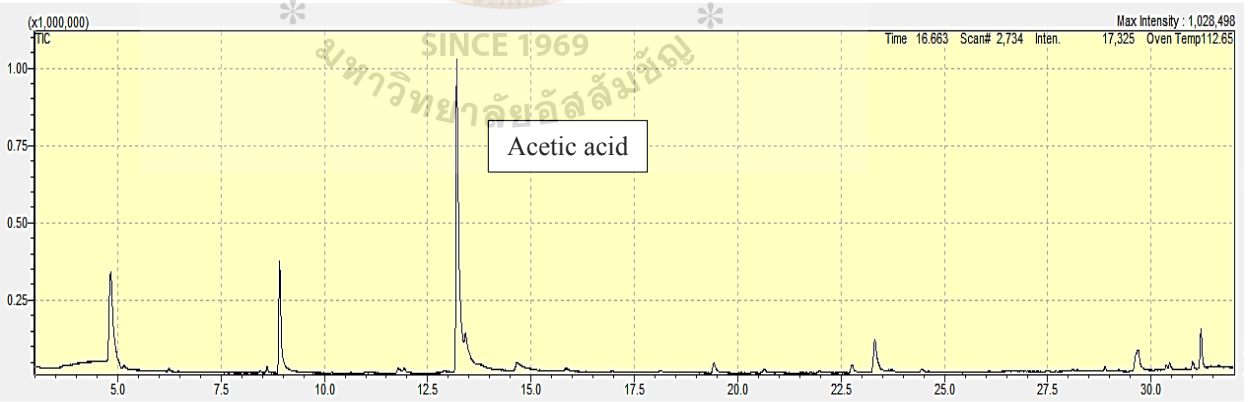
(d) Detection of acetic acid on durian peel (liquid)



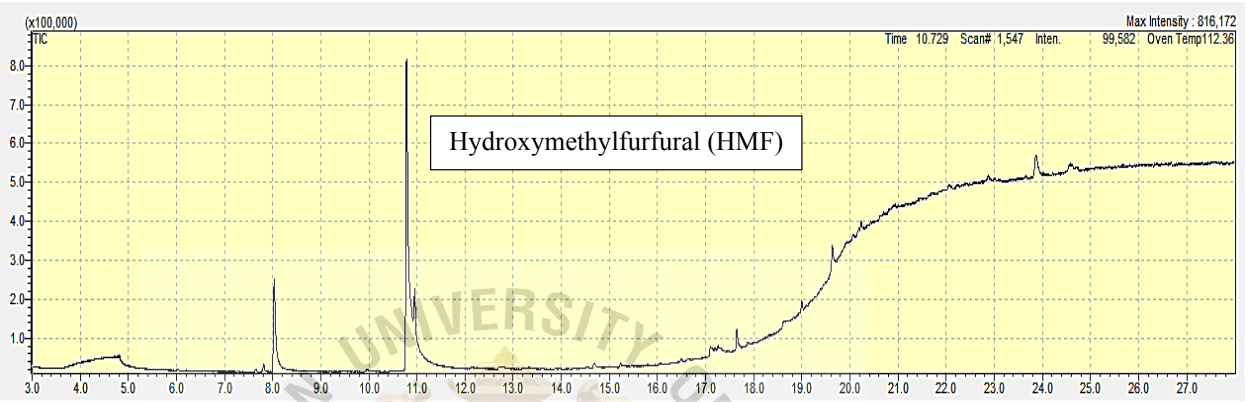
(e) Detection of acetic acid on sugarcane bagasse (RSM)



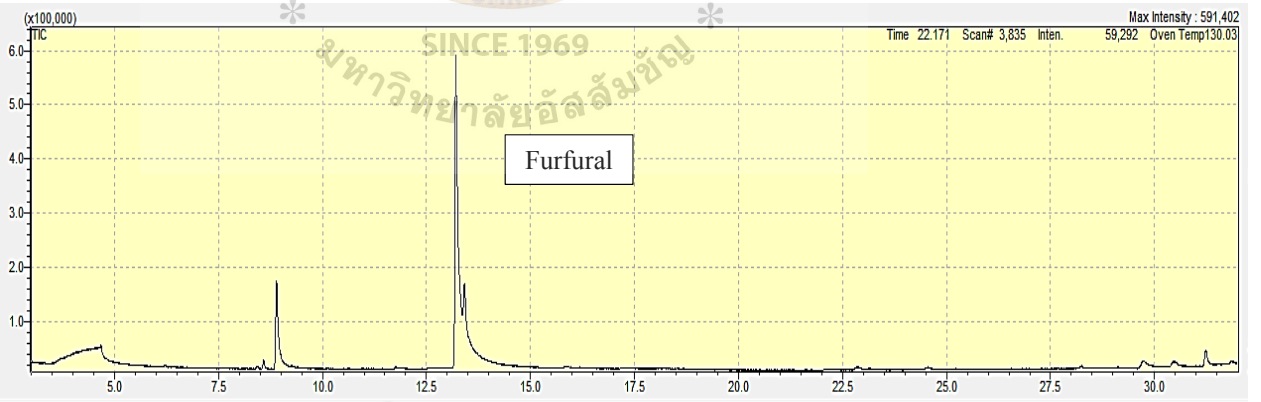
(f) Detection of acetic acid on durian peel (RSM)



(g) Detection of hydroxymethylfurfural (HMF) on sugarcane bagasse (liquid)



(h) Detection of furfural on sugarcane bagasse (liquid)



(i) Detection of furfural on durian peel (liquid)

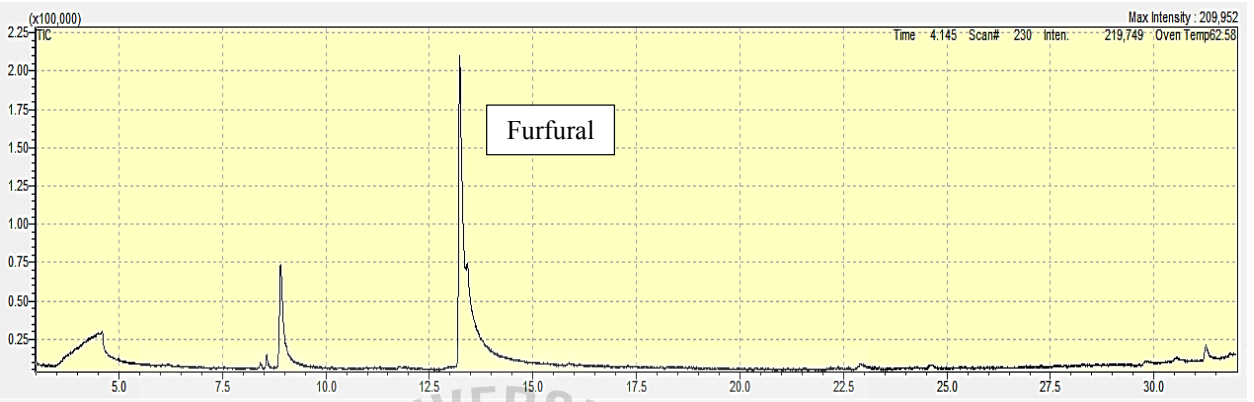


Figure 14. GC-MS Chromatograms displaying various peaks of substances including inhibitors (acetic acid, hydroxymethylfurfural (HMF) and furfural) in experimental sections of pretreatment on Agri-waste samples (a) – (i)

During the pretreatment of sugarcane bagasse and durian peel, various inhibitors were potentially manifested such as Acetic acid, Hydroxymethylfurfural (HMF), and Furfural. These inhibitor substances potentially impeded the fermentation process because pretreatment process can generate potent fermentative inhibitors interfering subsequent fermentation and bioconversion such as furfural and 5-hydroxymethylfurfural (HMF) that are furaldehyde and degraded products from pentoses and hexoses, respectively. These inhibitors can also inhibit and delay the growth and metabolism of yeast cells and subsequent fermentation (causing a lag-phase) in a dose-dependent manner. Even though both HMF and furfural can perform synergistically but yeast cells are more susceptible to the growth inhibition by furfural rather than by HMF at the same concentration.

Moreover, there are reports showing that furfural not only influences on glycolytic activities and tricarboxylic acid cycle, lessens the activities of various dehydrogenases and leads to oxidative stress but also weakens the bulk translation activity and disperses cytoplasmic mRNP granules in yeast cells. These various inhibitory by-products can be generated by lignocellulosic pretreatment depending on both biomass and pretreatment conditions such as temperature, time, pressure, pH, redox conditions and addition of catalysts. For example, at high temperature, fermentable carbohydrate formation and product degradation are depended on pH and time. By-products from sugar degradation e.g. furfural (from pentoses) and 5-hydroxylmethyl furfural (from hexoses) are generated in high concentration during harsh acidic pretreatment conditions. These inhibitor formations and sequential toxic compounds can provide a negative effect on the enzymatic hydrolysis rate.

There are different performances for microorganism to grow in hydrolysates which depend on the types of raw material and pretreatment. Therefore, discharge of these inhibitors is required to efficiently convert sugars to ethanol. However, these inhibitors can be reduced by optimizing the conditions of pretreatment on each sample. Several physical, chemical and biological methods have been developed to remove these fermentative inhibitors from lignocellulosic hydrolysates such as over-liming, ion exchange, enzymatic conversions, active charcoal adsorption, vacuum evaporation, precipitation and hydrolysate neutralization [73] – [78].

Other inhibitor occurred during fermentation is acetic acid, which is generated during acidic hydrolysis of hemicellulose. Increment of acetic acid concentration can also increase its inhibitory effect. Cellular growth and ethanol production were significantly affected when increase of acetic acid concentration. Simultaneously, the lag phase of fermentation was extended and delayed when increasing amount of acetic acid. Ethanol production was totally inhibited in excess

amount of acetic acid. Therefore, low concentration of acetic acid has no significant effect on ethanol production but high concentration of acetic acid can significantly affect to ethanol productivity [77].

Gas Chromatograph-Mass Spectrometer (GC-MS) was consequently applied in the analysis of these inhibitors and different peaks of substances on sugarcane bagasse and durian peel samples, as shown in Figure 14, were displayed as chromatograms indicating peak areas which could be further converted into different inhibitor concentrations using standard curves of inhibitors.

Table 9. The concentrations of (A) Acetic acid, (B) Hydroxymethylfurfural (HMF) and (C) Furfural found in experimental sections of pretreatment on Agri-waste samples

(A) ACETIC ACID	***Sugarcane bagasse (g/L)***	***Durian peel (g/L)***
Control	0.86 ± 0.050	1.32 ± 0.024
No enzyme addition *	1.20 ± 0.042	0.81 ± 0.045
Enzyme addition **	1.14 ± 0.090	1.54 ± 0.056
(B) HMF	***Sugarcane bagasse (g/L)***	***Durian peel (g/L)***
Control	-	-
No enzyme addition *	0.000022 ± 0.0000027	-
Enzyme addition **	-	-
(C) FURFURAL	***Sugarcane bagasse (g/L)***	***Durian peel (g/L)***
Control	-	-
No enzyme addition*	0.55 ± 0.034	0.22 ± 0.025
Enzyme addition**	-	-

*Applying optimum pretreatment conditions but no enzyme addition

**Applying both optimum pretreatment conditions and enzyme addition

The concentrations of various inhibitors found in different experimental sections of pretreatment on sugarcane bagasse and durian peel were demonstrated in Table 10. Acetic acid was found in every experimental pretreatment section on sugarcane bagasse and durian peel and the highest amount of acetic acid on sugarcane bagasse was shown in the section of no enzyme addition (1.20 g/L), however, on durian peel, it was displayed in a section of enzyme addition (1.54 g/L). Interestingly, hydroxymethylfurfural (HMF) only occurred on the sugarcane bagasse sample in the section of no adding enzyme (0.0000186 g/L). Furthermore, the furfural concentration on sugarcane bagasse (0.545 g/L) was higher than on durian peel (0.224 g/L) and they happened in the section of no enzyme addition on both of them. Therefore, the concentration of acetic acid (inhibitor) on sugarcane bagasse and durian peel in the section of enzyme addition (1.14 and 1.54 g/L) was higher than in the section of control (0.86 and 1.32 g/L). These results were also similar to other studies revealing that the pretreatment of polysaccharides could generate fermentative inhibitors [45] – [46].

For example, Luo, C. *et al.*, (2002) found that by-products (more than 35 potential inhibitors e.g. 5-hydroxymethylfurfural (HMF), Furfural and Acetic acid) from dilute acid hydrolysis and fermentation process were identified to inhibit *Saccharomyces cerevisiae* fermentation in dilute nitric acid hydrolysates of hybrid poplar by comparing the anion exchange fermentable treatment and untreated hydrolysate samples with their chemical compositions, and by chemical regenerative analysis eluate from the ion exchange resin saturated by hydrolysate [79]. Dogaris, I. *et al.,* (2012) discovered that increment of pretreatment temperature and acid concentration could increase the number of inhibitors such as acetic acid, formic acid, furfural, and 5-hydroxymethyl furfural (HMF) [80]. Palmqvist *et al.,* (1996) reported that furfural has inhibitory capability more than HMF on fermentation by *Saccharomyces cerevisiae* [81]. Xiros *et*

al., (2011) also observed that the inhibitory effect of furfural is greater than HMF on the growth of fungus; *Fusarium oxysporum F3* and ethanol fermentation was inhibited around 20–50% at concentrations above 3.0 g/L furfural and 3.2 g/L HMF [82]. The growth of yeast was ordinarily inhibited when the concentration of acetic acid is between 4 and 10 g/L [83]. Ethanol production with 2.1% theoretical ethanol yield was completely inhibited when the medium containing 4 g/L of acetic acid [84]. Complete inhibition was provided on ethanol production using *Pichia stipitis* in synthetic medium containing 3.9 g/L of acetic acid at pH 4 [85].

Fourier transform infrared spectroscopy (FTIR) is a technique that use mathematical process (Fourier transform) to translate the raw data (interferogram) into the actual spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas. FTIR method obtains infrared spectrum of transmission or absorption of a fuel sample then identifies the presence of different functional groups not only for organic and inorganic compounds but also for polymeric materials of the sample. FTIR spectrum range is frequently recorded between 4000 and 400 cm^{-1} by utilizing infrared light for scanning the samples and the specific molecular groups prevailing in the sample will be monitored through spectrum data in the automated software of spectroscopy. The change of material composition is generally observed by the modification of characteristic pattern on absorptive bands [86] – [88].

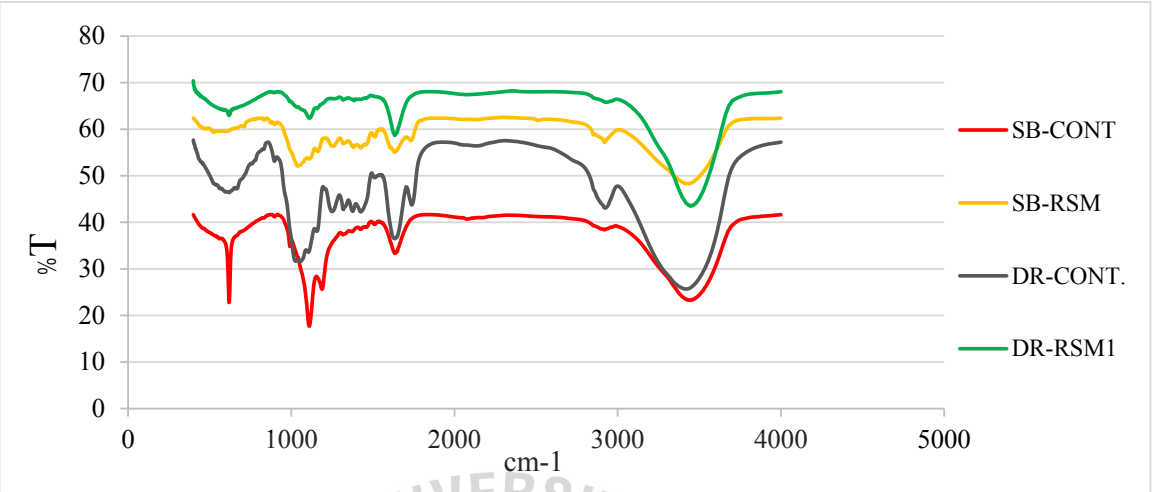


Figure 15. FTIR results of sugarcane bagasse and durian peel samples

The results of FTIR measurement on optimum pretreatment of sugarcane bagasse and durian peel comparing with their controls were illustrated as graphs in figure 15 and the compound compositions in each sample were also displayed in Table 10. In this experiment, FTIR results implied that, in sugarcane bagasse samples, some identical compounds were detected as conjugated alkene and alcohol in both SB-CONT. and SB-RSM, moreover, other compounds could be transformed into other configurations, for example, alcohol from SB-CONT. could be transformed into secondary alcohol and tertiary alcohol found in SB-RSM which were the same type of compound but different configuration. Moreover, in durian peel samples, similar compounds were monitored as halogen compound, alkene, and alcohol in both DR-CONT. and DR-RSM.

Table 10. FTIR results indicating the compound compositions in sugarcane bagasse and durian peel samples

Sample	%T (Transmittance)	Wave number (cm-1)	Functional Group	Details
SB-CONT.	22.89, 17.78, 25.75, 33.52, 23.38	620, 1112, 1191, 1643, 3460	C-S linkage, C-O-C group, Ester carbonyl, Diketones, Hydroxyl compound	Halogen compound, Secondary alcohol, Tertiary alcohol, Conjugated alkene, Alcohol
SB-RSM	52.27, 55.14, 57.76, 57.34, 48.45	1055, 1637, 1739, 2924, 3451	C-O-C group, Diketones, Carbonyl compounds, Methyl group, Hydroxyl compound	Sulfoxide, Conjugated alkene, Amine, Cyclic alkene, Aldehyde, Alkane, Alcohol
DR-CONT.	46.48, 31.69, 36.66, 43.88, 43.16, 25.73	622, 1061, 1643, 1742,	C-S linkage, C-O-C group, Diketones,	Halogen compound, Sulfoxide, Alkene,

		2929, 3432	Carbonyl compounds, Methyl group, Hydroxyl compound	Esters, Alkane, Alcohol
DR-RSM	62.91, 62.39, 58.68, 43.68	619, 1114, 1635, 3463	C-S linkage, C-O-C group, Diketones, Hydroxyl compound	Halogen compound, Aliphatic ether, Alkene, Conjugated alkene, Amine, Cyclic alkene, Alcohol

*Meaning of Abbreviations

SB-CONT. = Control of sugarcane bagasse

SB-RSM = Optimum pretreatment of sugarcane bagasse

DR-CONT. = Control of durian peel

DR-RSM = Optimum pretreatment of durian peel

Additionally, other forms of alkene could also be displayed as conjugated alkene and cyclic alkene in DR-RSM. From these results, it was shown that, after optimum pretreatment, some compounds were stable and others could be also remodeled into other forms.

Interestingly, the fluctuated FTIR spectrum trends of both sugarcane bagasse and durian peel were quite similar indicating that they have identical chemical structures and functional groups. However, the distinct spectrums of C-S linkage, C-O-C group (Ether) and Ester carbonyl at 620 cm^{-1}, 1112 cm^{-1} and 1191 cm^{-1} on unpretreated sugarcane bagasse (control) and 1061 cm^{-1} and 1742 cm^{-1} on unpretreated durian peel (control) were stronger than pretreated sugarcane bagasse (RSM) and pretreated durian peel (RSM), respectively. The weaken signal of both pretreated sugarcane bagasse and durian peel spectrums mean that the disruption of linkages on ether and ester carbonyl group between lignin and biomass carbohydrates or the lignin reduction were happened.

Moreover, the β-glycosidic linkages between sugar units in cellulose and hemicellulose were changed demonstrating that the alteration of linkages between sugar units and intermolecular degradation in hemicellulose structure leads to the removal of hemicellulose facilitating the enzymatic digestion of pretreated biomass like other studies [93] – [95].

As well, it can be briefly implied that there was a conformity between Table 1 showing the weight before and after pretreatment on each sample and %transmission comparing between control and after RSM pretreatment of samples in Figure 15. It was found that %transmittance of sample after RSM pretreatment in both sugarcane bagasse and durian peel would be higher because after pretreatment, some compositions of samples such as cellulose, hemicellulose or lignin could possibly leak out then the infrared light in FTIR machine could easily transmit through the sample and the results were then measured as higher %Transmittance for RSM pretreated samples.

Therefore, it was implied that not only sugarcane bagasse but also durian peel has been plausibly used as raw materials in the biorefining process for generating a great amount of reducing sugars which could be fermented by various microorganisms afterward to generate many by-products such as ethanol and biobutanol.

CONCLUSION

In this experiment, the optimization on the pretreatment conditions of sugarcane bagasse and durian peel (e.g. temperatures, times, and acid concentrations) was performed to reach the highest quantity of reducing sugars. These sugars potentially transformed into various beneficial products, such as bioethanol and biobutanol. After conducting the RSM, the optimum pretreatment conditions of sugarcane bagasse and durian peel were finalized as 136.08^{o}C (pretreatment temperature), 75.36 minutes (pretreatment time), 3.50% (concentration of H_2SO_4) and 127.14^{o}C (pretreatment temperature), 74.13 minutes (pretreatment time), 2.75% (concentration of H_2SO_4), respectively. Furthermore, the reducing sugar concentration after optimum pretreatment conditions were 180.15 mg/g sugarcane bagasse, which was 3.06 folds to unpretreated sugarcane bagasse and 551.07 mg/g-durian peel, which was 1.88 folds higher compared to unpretreated durian peel. However, the inhibitors such as acetic acid, hydroxymethylfurfural (HMF), and furfural, which possibly obstructed the fermentation process, could also be occurred during the pretreatment. Hence, this experiment revealed the efficiency of RSM and mathematical modeling in the optimization of pretreatment conditions, which could potentially perform in the biorefining process of sugarcane bagasse and durian peel to generate biofuels and value-added products. Furthermore, the demand for biofuels usage in Thailand needs to be carefully determined and this study could persuade some readers utilizing lignocellulosic biomass to not only manipulate the unexpected situations of energy consumption in the future but also diminish inappropriate agricultural waste combustion.

FEASIBILITY OF THIS STUDY IN COMMERCIAL SCALE

According to this study, pretreatment processes was applied and lignocellulosic biomasses were used as raw materials and the fermentation processes could be mainly classified into 4 types. Firstly, SHF or Separate Hydrolysis & Fermentation, in this process, it would start with enzyme production then hydrolysis and for hexose and pentose fermentation, it would be separated and finally go to distillation and separation. Secondly, SSF or Simultaneous Saccharification & Fermentation, in this process, the hydrolysis process would be included with hexose fermentation process then go to pentose fermentation, distillation and separation, respectively. Thirdly, SSCF or Simultaneous Saccharification & Co-Fermentation, all hydrolysis, pentose and hexose fermentation would be together operated at the same time. Finally, CBP or Consolidated Bioprocessing, all enzyme production, hydrolysis, pentose and hexose fermentation process would be together operated at the same time and all of these processes would finally generate the final product like ethanol [95] or other feasibly lucrative byproducts such as food flavoring agents.

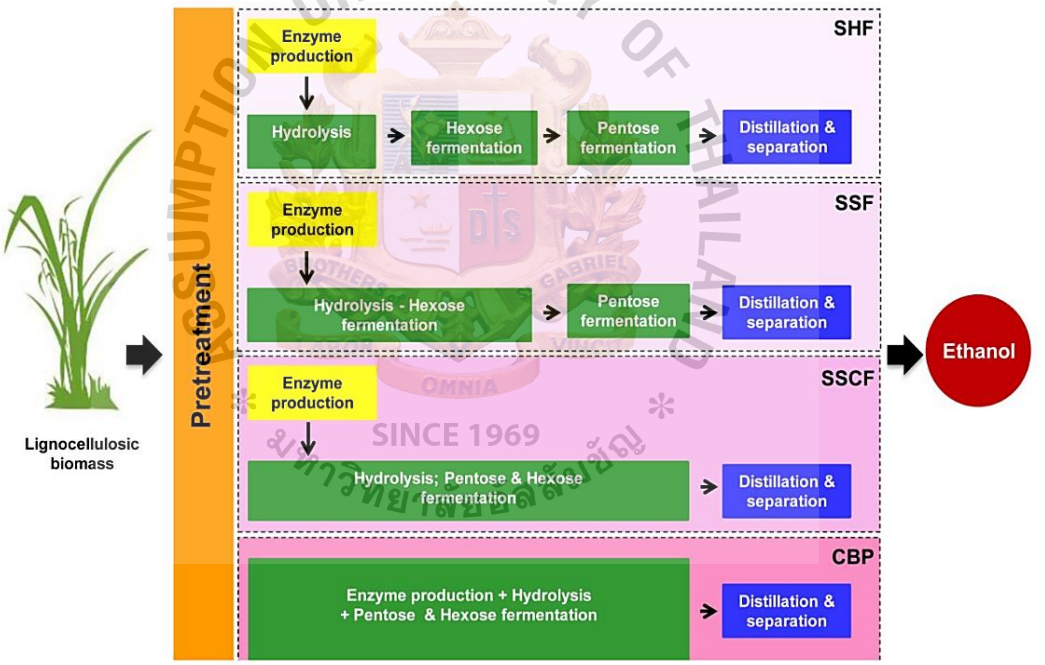


Figure 16. Lignocellulosic biomass process configurations (i) Separate Hydrolysis & Fermentation (SHF) (ii) Simultaneous Saccharification & Fermentation (SSF) (iii) Simultaneous Saccharification & Co-Fermentation (SSCF) (iv) Consolidated Bioprocessing (CBP) (Adapted from Hamelinck et al., 2005)

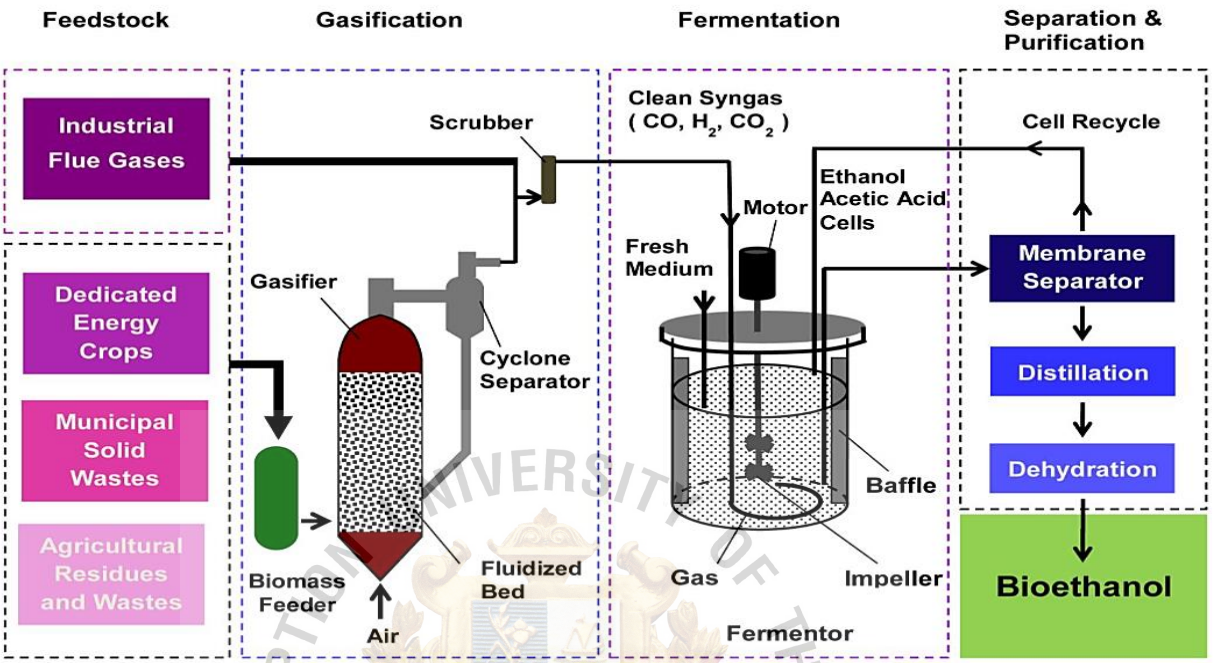


Figure 17. Hybrid gasification-syngas fermentation conversion process for the production of ethanol from various feedstock (Devarapalli, M., & Atiyeh, H. K., 2015)

In order to produce ethanol or other byproducts like food flavoring agent from agricultural waste, the main processes like fermentation, separation and purification are inevitably operated and some procedure diagrams could be illustrated in figure 17.

Various feedstocks could be possibly converted into byproducts such as bioethanol (or food flavoring agents) via hybrid gasification-syngas fermentation conversion process. It could be mainly classified into 4 main sections like feeding, gasification, fermentation, separation and purification. More details about the important functions of each section are described as followings; Firstly, feeding section, it can start from industrial flue gases, dedicated energy crop, municipal solid waste, or from agricultural residue and their wastes. All of these biomass feeders would enter into gasification process. During processing, the heat would burn the biomass and transform biomass energy into combustible gas or fuel by using the phenomenon like fluidized bed or gasifier then it would transfer into cyclone separator and scrubber in order to filter and finally receive syngas like carbon monoxide, carbon dioxide and hydrogen gas. After that, fermentation process would operate inside the fermenter and there was a fresh medium supporting fermentation process in order to produce by-products including ethanol, acetic acid, other cells and some contaminants. However, this part could be separated and purified by using separation and purification process. In these processes, they comprise of sub processes like membrane separator, distillation and dehydration. All these processes, the contaminated section would be gradually purified, for example, membrane would filter the only desired part and keep that part to continually

transfer to distillation part. At this part, the heat would make a solution to be evaporated and distilled then the clearer solution would be provided and finally dehydrated and the purest part was only separated such as bioethanol or food flavoring agents [96]. There is a scientific review about food flavouring agent production from agricultural wastes like in the publication of "Production of Food Flavouring Agents by Enzymatic Reaction and Microbial Fermentation [97].

The demand of natural flavoring agent is feasibly increasing as the rising trend of sustainable consumer behavior (green consumption). The products labelled as natural are gradually and extensively accepted by successive generation of customer focusing more on their health and concerning about plausible side effect of excessive consumption of artificial ingredients. According to the information of REPORTS AND DATA, it was shown that the natural flavor market is forecasted to expeditiously grow at a Compound Annual Growth Rate (CAGR) of 6.3% from 2019 to reach USD 20.04 Billion by 2027 [98]. Therefore, this is a golden opportunity for food flavor industry to be more involved in natural flavor market and apportion this market share.

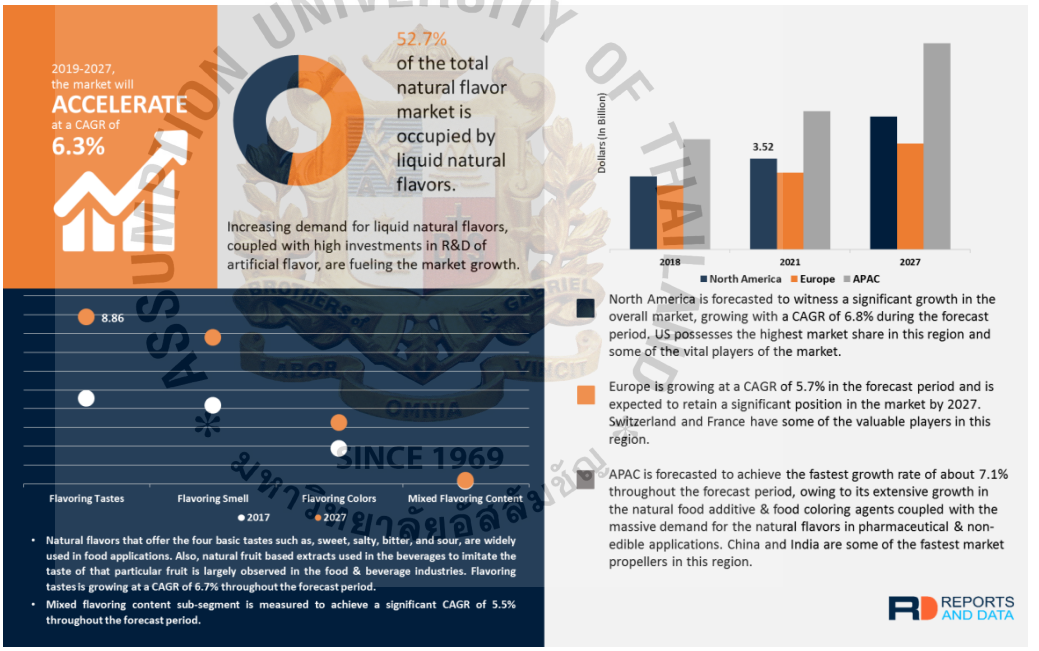


Figure 18. Forecast of Overall Natural Flavor Market in North America, Europe and APAC between 2017 – 2027 (REPORTS AND DATA, 2020)

According to this experiment, lignocellulosic waste like durian peel was studied and converted to reducing sugar which could be further modified into other byproducts in downstream processing such as bioethanol or food flavoring agents. However, there was no any reports pertaining about food flavoring agent production from durian peel utilization. Therefore, the details of cost or conversion process have not been provided in deeper calculation yet. However, the cost and conversion process details could be approximately predicted by other publication that used to apply other lignocellulosic waste as raw material in order to generate food flavoring agent.

Interestingly, there was an example publication that used Solid State Fermentation (SSF) cultivating *Rhizopus oligosporus USM R1* with the support of soya bean meal and rice husks as the substrate in order to produce lucrative byproduct like natural flavoring agent called as "Benzaldehyde", a bitter cherry almond flavor [99].

According to the certain composition of soya bean meal and rice husks that potentially compose of cellulose and hemicellulose as similar to other general agricultural wastes [100], [101], therefore, this study could be possibly used for demonstrating the calculation of food flavoring agent production from lignocellulosic waste and their feasibility in commercial scale.

The main cost of waste utilization of this mentioned study is mainly and approximately composed of fixed cost; Industrial Grinder = 98,580 Baht [102], 10 Industrial Fermenters (318.2 L) = 141,300 Baht [103] and variable cost as described in followings, respectively;

1. Soya bean meal and rice husks (raw materials) = Free
2. *Rhizopus oligosporus USM R1* = 650 Baht/Kg [104]
3. Sodium Acetate = 940 Baht/Kg [105]
4. Tween 80 = 265 Baht/Kg [106]
5. Water = 0.01 Baht/Kg [107]

In this experiment, the quantity is used as 10 g mixed soya bean meal (5 g) and rice husks (5 g) in order to generate benzaldehyde (bitter cherry almond flavor) equal to 38.69 mg/g substrate as followings;

1. Soya bean meal and rice husks = 10 g = Free
2. *Rhizopus oligosporus USM R1* = 1 g for 10 g of raw materials = 0.65 Baht
3. Sodium Acetate = 0.3 g for 10 g of raw materials = 0.282 Baht
4. Tween 80 = 0.025 ml for 0.1 g of raw materials = 0.00663 Baht
5. Water = 30 ml for 10 g of raw materials = 0.0003 Baht

Therefore, the variable cost of main ingredients for producing 38.69 mg benzaldehyde from 1 g of substrate is around 0.0938925 Baht. But if we want to produce 1,000 g of benzaldehyde, the variable cost will be 2,426.79 Baht.

Fixed cost = Industrial Grinder (98,580 Baht) + 10 Industrial Fermenters for 318.2 L (141,300 Baht) and Variable cost/Kg of product = Cost of Ingredients (2,426.79 Baht) + Maintenance Cost (48.61 Baht) [108] + Labor Cost (10.06 Baht) [109] + Operational Cost (47 Baht) [110] = 2,532.46 Baht/Kg of product

Hence, the total variable cost of production will be 2,532.46 Baht/Kg of product. However, the trading price of this byproduct is around 2,398.47 Baht/Kg, which is lower than the principal of production (2,532.46 Baht/Kg of product) as 5.29% [111].

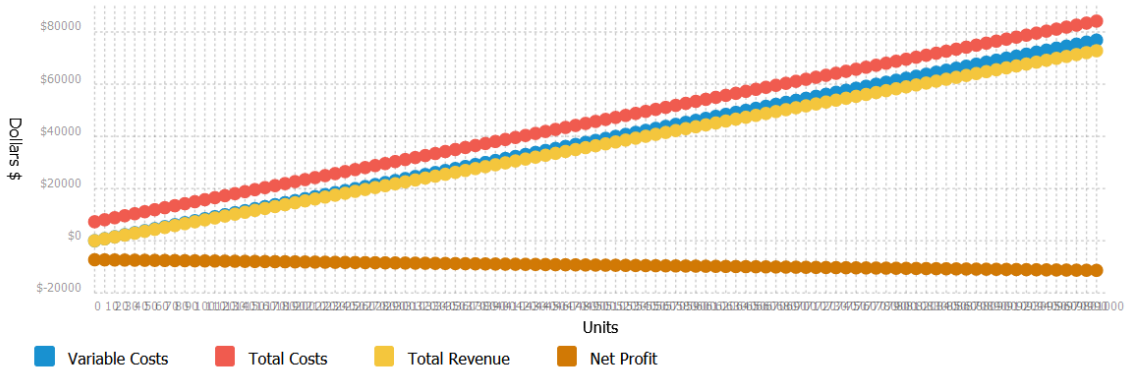


Figure 19. Break-Even Analysis Chart of benzaldehyde production from soya bean meal and rice husks at expected unit equal to 2,000 tons of benzaldehyde (Total Revenue: $72,810, Total Costs: $84,162, Net Profit: -$11,352, Break Even Units: -1,789, 1 US = 32.94 Baht) (Good Calculators, 2021)

Even though, this lucrative byproduct (benzaldehyde) could almost generate the profit in commercial scale but the selling price of this byproduct is still higher than the principal (~5.29%) as illustrated in figure 19 [112]. However, it needs 'Economies of scale' and other marketing strategies like creating unique or storytelling products specifically for niche market (possibly setting higher selling price) and finding other suppliers providing good quality of ingredients with reasonable and affordable price to not only reduce the cost of production but also to feasibly make this byproduct be available for price competition in commercial scale.

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%E0%B8%82%E0%B8%B2%E0%B8%A2%E0%B8%AA%E0%B9%88%E0%B8%87(2-4%E0%B8%96%E0%B8%B8%E0%B8%87)%E0%B8%96%E0%B8%B8%E0%B8%87%E0%B8%A5%E0%B8%B085%E0%B8%9A-1-2%E0%B8%81%E0%B8%81375%E0%B8%9A-1%E0%B8%81%E0%B8%81650-i.175220621.2782523110

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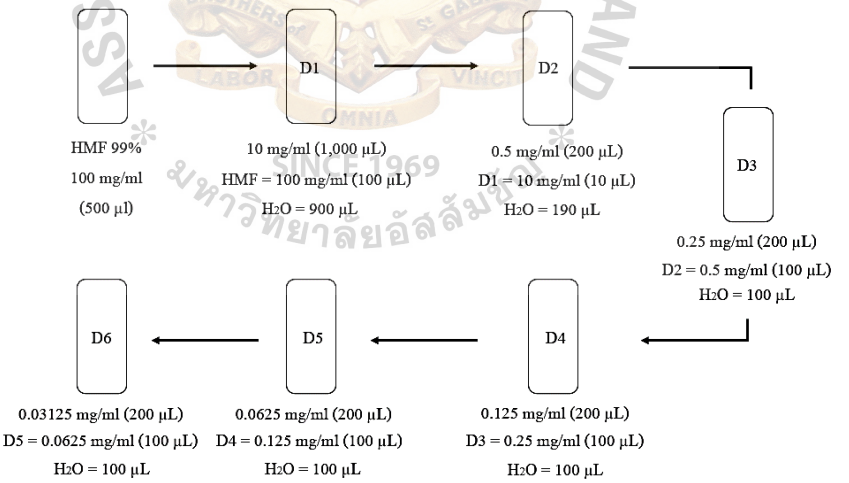
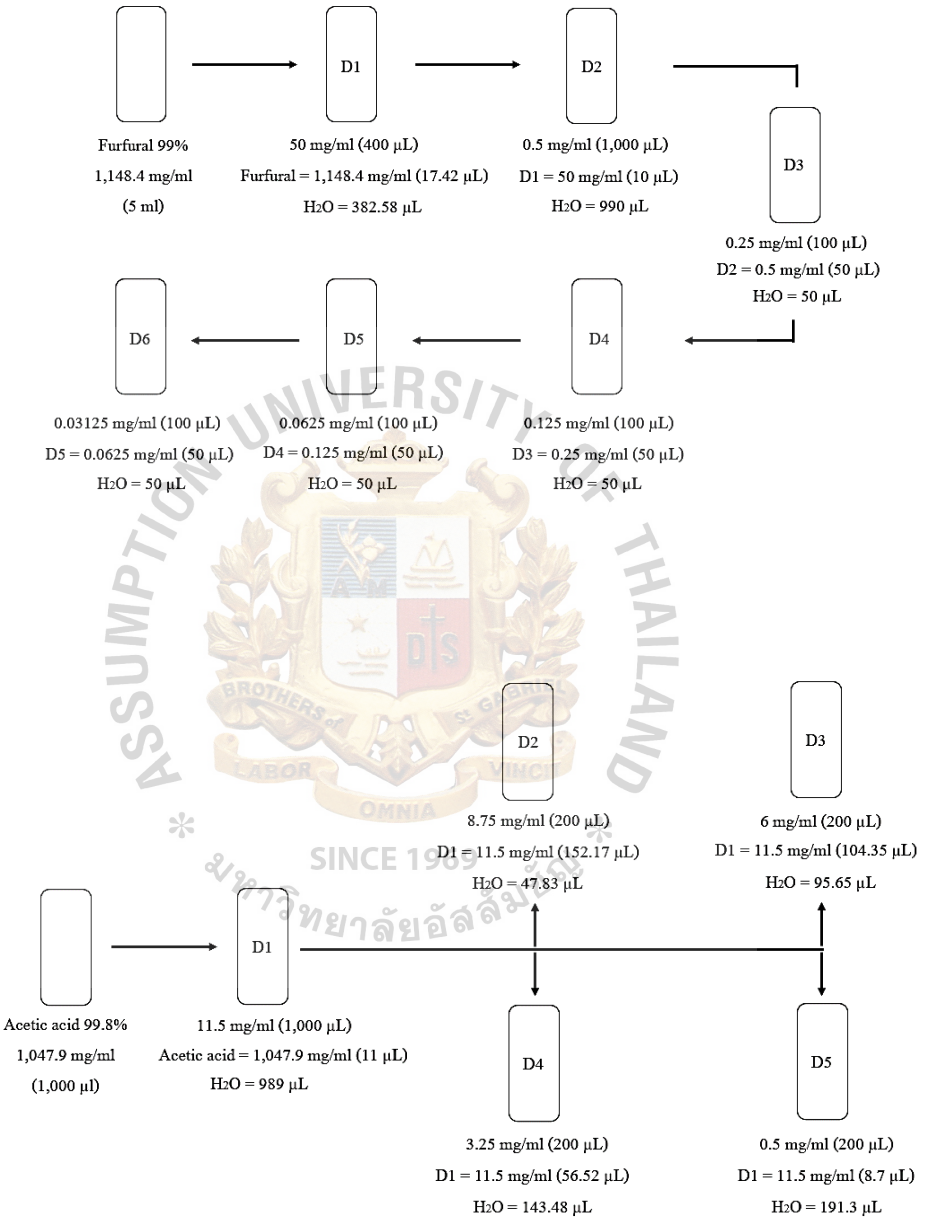
APPENDICES

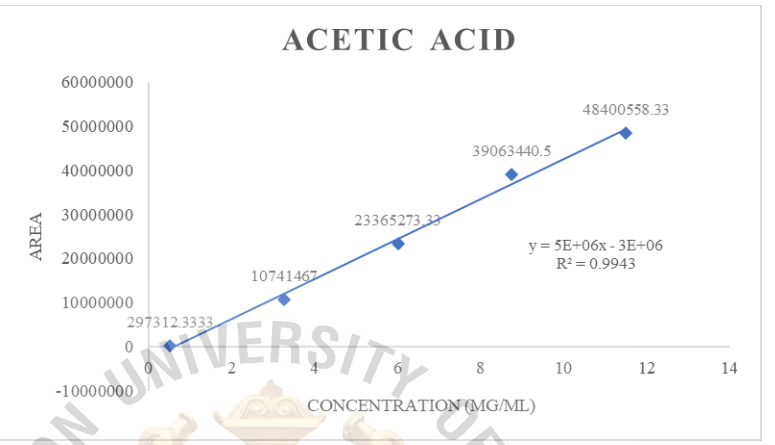
Appendix A. Preparation of standard curve with the details of inhibitor standard curves preparation diagram

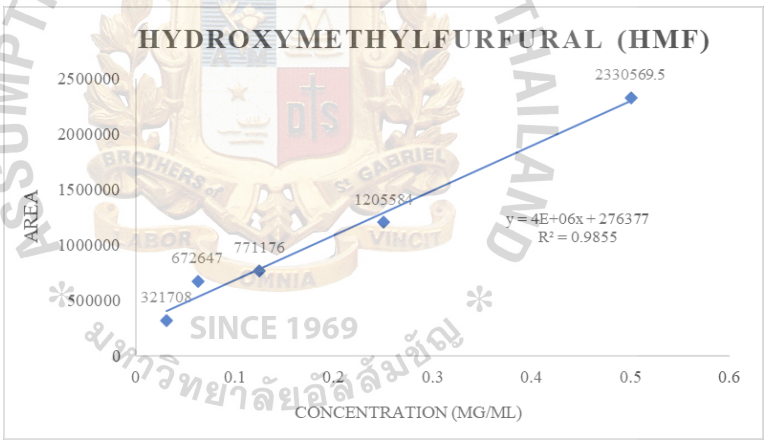
The ranges of inhibitor concentration for making standard curves were different depending on the type of inhibitor inspection. For example, the concentration range for Hydroxymethylfurfural (HMF) and Furfural were 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg/ml, however, the concentration range for Acetic acid was 0.5, 3.25, 6, 8.75 and 11.5 mg/ml. The details of preparation on each point of concentration were illustrated below;

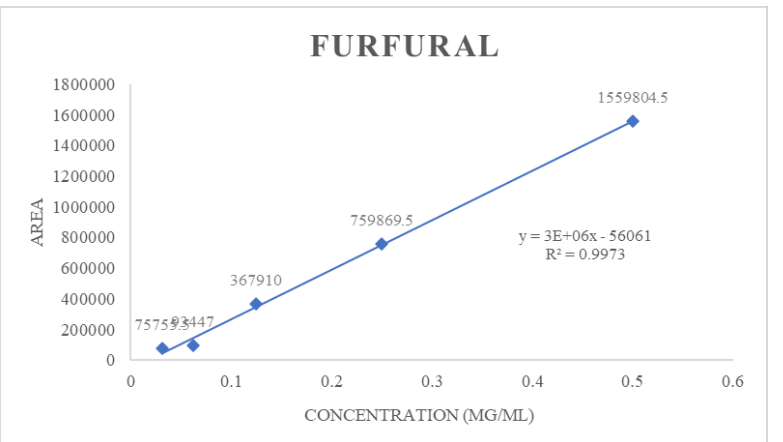



Furfural 99%
1,148.4 mg/ml
(5 ml)
D1
50 mg/ml (400 µL)
Furfural = 1,148.4 mg/ml (17.42 µL)
H2O = 382.58 µL
D2
0.5 mg/ml (1,000 µL)
D1 = 50 mg/ml (10 µL)
H2O = 990 µL
D3
0.25 mg/ml (100 µL)
D2 = 0.5 mg/ml (50 µL)
H2O = 50 µL
D4
0.125 mg/ml (100 µL)
D3 = 0.25 mg/ml (50 µL)
H2O = 50 µL
D5
0.0625 mg/ml (100 µL)
D4 = 0.125 mg/ml (50 µL)
H2O = 50 µL
D6
0.03125 mg/ml (100 µL)
D5 = 0.0625 mg/ml (50 µL)
H2O = 50 µL
Acetic acid 99.8%
1,047.9 mg/ml
(1,000 µl)
D1
11.5 mg/ml (1,000 µL)
Acetic acid = 1,047.9 mg/ml (11 µL)
H2O = 989 µL
D2
8.75 mg/ml (200 µL)
D1 = 11.5 mg/ml (152.17 µL)
H2O = 47.83 µL
D3
6 mg/ml (200 µL)
D1 = 11.5 mg/ml (104.35 µL)
H2O = 95.65 µL
D4
3.25 mg/ml (200 µL)
D1 = 11.5 mg/ml (56.52 µL)
H2O = 143.48 µL
D5
0.5 mg/ml (200 µL)
D1 = 11.5 mg/ml (8.7 µL)
H2O = 191.3 µL

Appendix B. Standard curve preparation for detecting inhibitors (acetic acid, hydroxymethylfurfural (HMF) and furfural) on lignocellulosic samples







Appendix C. ANOVA results of Response Surface Reduced Quadratic Model of sugarcane bagasse

Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)
Model	19.85	8	2.48	79.06	< 0.0001*
A-Temp	10.78	1	10.78	343.42	< 0.0001*
B-Time	5.21	1	5.21	165.92	< 0.0001*
C-Conc.	1.26	1	1.26	40.00	0.0002*
AB	1.46	1	1.46	46.38	0.0001*
AC	0.20	1	0.20	6.27	0.0367*
BC	0.18	1	0.18	5.81	0.0425*
A^2	0.40	1	0.40	12.73	0.0073*
B^2	0.33	1	0.33	10.54	0.0118*
Residual	0.25	8	0.031		
Lack of fit	0.17	4	0.043	2.25	0.2259
Pure Error	0.077	4	0.019		
Cor Total	20.11	16			

*statistically significant with P-value < 0.05

Appendix D. ANOVA results of Response Surface Reduced Quadratic Model of durian peel

Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)
Model	141.39	6	23.57	24.90	< 0.0001*
A-Temp	53.50	1	53.50	56.54	< 0.0001*
B-Time	25.50	1	25.50	26.95	0.0004*
C-Conc.	10.06	1	10.06	10.64	0.0086*
A^2	15.34	1	15.34	16.21	0.0024*
B^2	26.81	1	26.81	28.33	0.0003*
C^2	5.29	1	5.29	5.59	0.0396*
Residual	9.46	10	0.95		
Lack of fit	6.96	6	1.16	1.86	0.2861
Pure Error	2.50	4	0.63		
Cor Total	150.86	16			

*statistically significant with P-value < 0.05

Appendix E. ANOVA (Single factor) Analysis and T-test dependent (Paired sample test) of %cellulose, %hemicellulose and %lignin contents on sugarcane bagasse and durian peel samples before and after pretreatment

E.1) Comparison of % Hemicellulose on sugarcane bagasse (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	69.83317	23.27772	6.089237
After pretreatment	3	4.996889	1.66563	0.930449

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	700.6238	1	700.6238	199.6169	0.000146	7.708647
Within Groups	14.03937	4	3.509843			
Total	714.6632	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Hemicellulose Before pretreatment (SB)	23.2777221	3	2.46763791	1.42469141
Hemicellulose After pretreatment (SB)	1.6656296	3	.96459795	.55691088

Paired Samples Correlations

	N	Correlation	Sig.
Hemicellulose Before pretreatment (SB) & Hemicellulose After pretreatment (SB)	3	-.982	.121

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Hemicellulose Before pretreatment (SB) – Hemicellulose After pretreatment (SB)	21.61209249	3.41979298	1.97441840	13.116855	30.107329	10.946	2	.008

E.2) Comparison of % Hemicellulose on durian peel (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	45.64688	15.21563	5.023817
After pretreatment	3	3.61306	1.204353	0.192347

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	294.4736	1	294.4736	112.9081	0.000444	7.708647
Within Groups	10.43233	4	2.608082			
Total	304.9059	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Hemicellulose Before pretreatment (DP)	15.2156252	3	2.24138729	1.29406556
Hemicellulose After pretreatment (DP)	1.2043532	3	.43857388	.25321075

Paired Samples Correlations

	N	Correlation	Sig.
Hemicellulose Before pretreatment (DP) & Hemicellulose After pretreatment (DP)	3	-.399	.739

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Hemicellulose Before pretreatment (DP) – Hemicellulose After pretreatment (DP)	14.011 27204	2.44950649	1.414 22323	7.92636058	20.0961835	9.907	2	.010

E.3) Comparison of % Cellulose on sugarcane bagasse (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	146.1516	48.71721	3.179122
After pretreatment	3	97.48055	32.49352	3.516029

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	394.8124	1	394.8124	117.9398	0.000408	7.708647
Within Groups	13.3903	4	3.347575			
Total	408.2027	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Cellulose Before pretreatment (SB)	48.7172115	3	1.78300930	1.02942090
Cellulose After pretreatment (SB)	32.4935164	3	1.87510764	1.08259390

Paired Samples Correlations

	N	Correlation	Sig.
Cellulose Before pretreatment (SB) & Cellulose After pretreatment (SB)	3	.852	.351

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Cellulose Before pretreatment (SB) – Cellulose After pretreatment (SB)	16.22369505	.99977021	.57721760	13.7401281	18.7072619	28.107	2	.001

E.4) Comparison of % Cellulose on durian peel (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	172.9077	57.63589	2.043871
After pretreatment	3	121.3277	40.44255	8.722032

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	443.4165	1	443.4165	82.37422	0.000817	7.708647
Within Groups	21.53181	4	5.382952			
Total	464.9483	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Cellulose Before pretreatment (DP)	57.6358913	3	1.42964012	.82540311
Cellulose After pretreatment (DP)	40.4425504	3	2.95330872	1.70509359

Paired Samples Correlations

	N	Correlation	Sig.
Cellulose Before pretreatment (DP) & Cellulose After pretreatment (DP)	3	-.837	.369

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Cellulose Before pretreatment (DP) – Cellulose After pretreatment (DP)	17.193 34098	4.22306199	2.4381 8598	6.7026734	27.684008	7.052	2	.020

E.5) Comparison of %Lignin on sugarcane bagasse (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	62.14891	20.7163	2.034549
After pretreatment	3	34.39222	11.46407	1.797321

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	128.4056	1	128.4056	67.01983	0.001213	7.708647
Within Groups	7.663741	4	1.915935			
Total	136.0694	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Lignin Before pretreatment (SB)	20.7163043	3	1.42637629	.82351874
Lignin After pretreatment (SB)	11.4640740	3	1.34064211	.77402008

Paired Samples Correlations

	N	Correlation	Sig.
Lignin Before pretreatment (SB) & Lignin After pretreatment (SB)	3	-.892	.298

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Lignin Before pretreatment (SB) – Lignin After pretreatment (SB)	9.2522 3034	2.69150973	1.5539 4387	2.56614952	15.9383111	5.954	2	.027

E.6) Comparison of %Lignin on durian peel (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	55.33867	18.44622	1.11859
After pretreatment	3	31.54893	10.51631	4.407972

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	94.32523	1	94.32523	34.13523	0.004279	7.708647
Within Groups	11.05312	4	2.763281			
Total	105.3784	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Lignin Before pretreatment (DP)	18.4462220	3	1.05763393	.61062524
Lignin After pretreatment (DP)	10.5163111	3	2.09951711	1.21215677

Paired Samples Correlations

	N	Correlation	Sig.
Lignin Before pretreatment (DP) & Lignin After pretreatment (DP)	3	-.938	.226

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Lignin Before pretreatment (DP) – Lignin After pretreatment (DP)	7.92991086	3.11306545	1.79732918	.19662757	15.6631941	4.412	2	.048

Appendix F. ANOVA (Single factor) Analysis and T-test dependent (Paired sample test) of sugarcane bagasse and durian peel weights before and after pretreatment

F.1) Comparison of Sugarcane bagasse weight (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	2.4147	0.8049	7.3E-07
After pretreatment	3	1.7277	0.5759	3.28E-05

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.078662	1	0.078662	4693.407	2.71993E-07	7.708647
Within Groups	6.7E-05	4	1.68E-05			
Total	0.078729	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Weight Before pretreatment (SB)	.8049000	3	.00085440	.00049329
Weight After pretreatment (SB)	.5759000	3	.00572626	.00330606

Paired Samples Correlations

	N	Correlation	Sig.
Weight Before pretreatment (SB) & Weight After pretreatment (SB)	3	-.972	.151

Paired Samples Test (Cont.)

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Weight Before pretreatment (SB) – Weight After pretreatment (SB)	.22900000	.00655973	.00378726	.21270474	.24529526	60.466	2	.000

F.2) Comparison of Durian peel weight (Before-After pretreatment)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Before pretreatment	3	2.4333	0.8111	6.1E-07
After pretreatment	3	1.6066	0.535533	0.000507

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.113905	1	0.113905	448.8237	2.93478E-05	7.708647
Within Groups	0.001015	4	0.000254			
Total	0.114921	5				

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
Weight Before pretreatment (DP)	.8111000	3	.00078102	.00045092
Weight After pretreatment (DP)	.5355333	3	.02251585	.01299953

Paired Samples Correlations

	N	Correlation	Sig.
Weight Before pretreatment (DP) & Weight After pretreatment (DP)	3	.494	.671

Paired Samples Test (Cont.)

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Weight.Before pretreatment (DP) - Weight.After pretreatment (DP)	.27556667	.02214054	.01278284	.22056653	.33056681	21.558	2	.002

Appendix G. ANOVA (Single factor) analysis on concentrations of Acetic acid, Hydroxymethylfurfural (HMF) and Furfural found in experimental sections of lignocellulosic waste samples pretreatment

G.1) Comparison of acetic acid conc. (control) between sugarcane bagasse and durian peel

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Acetic acid Control (SB)	3	2.57311	0.857703333	0.002456243
Acetic acid Control (DP)	3	3.961285	1.320428333	0.000591779

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.321171638	1	0.321171638	210.7410402	0.00013093	7.708647
Within Groups	0.006096044	4	0.001524011			
Total	0.327267682	5				

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Acetic acid (Control)	Sugarcane bagasse	3	.8577033	.04956050	.02861377
	Durian peel	3	1.3204283	.02432650	.01404491

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Acetic acid (Control)	Equal variances assumed	.836	.412	-14.517	4	.000	-.46272500	.03187487	-.55122382	-.37422618
	Equal variances not assumed			-14.517	2.911	.001	-.46272500	.03187487	-.56594564	-.35950436

G.2) Comparison of acetic acid conc. (no enzyme) between sugarcane bagasse and durian peel

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Acetic acid No enzyme (SB)	3	3.601662	1.200554	0.00172715
Acetic acid No enzyme (DP)	3	2.435625	0.811875	0.002039697

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.226607048	1	0.226607048	120.3165643	0.000392475	7.708647
Within Groups	0.007533694	4	0.001883424			
Total	0.234140742	5				

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Acetic acid (No enzyme)	Sugarcane bagasse	3	1.2005540	.04155900	.02399410
	Durian peel	3	.8118750	.04516300	.02607487

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Acetic acid (No enzyme)	Equal variances assumed	.014	.912	10.969	4	.000	.38867900	.03543467	.2902965	.4870614
	Equal variances not assumed			10.969	3.973	.000	.38867900	.03543467	.2900288	.4873291

G.3) Comparison of acetic acid conc. (enzyme) between sugarcane bagasse and durian peel

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Acetic acid Enzyme (SB)	3	3.405912	1.135304	0.008158064
Acetic acid Enzyme (DP)	3	4.631056	1.543685333	0.0031346

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.25016297	1	0.25016297	44.30539573	0.002645817	7.708647
Within Groups	0.022585328	4	0.005646332			
Total	0.272748298	5				

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Acetic acid (Enzyme)	Sugarcane bagasse	3	1.1353040	.09032200	.05214743
	Durian peel	3	1.5436853	.05598750	.03232440

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Acetic acid (Enzyme)	Equal variances assumed	.418	.553	-6.656	4	.003	-.40838133	.06135325	-.57872526	-.23803740
	Equal variances not assumed			-6.656	3.339	.005	-.40838133	.06135325	-.59287949	-.22388318

G.4) Comparison of furfural conc. (no enzyme) between sugarcane bagasse and durian peel

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Furfural No enzyme (SB)	3	1.63488	0.54496	0.001138389
Furfural No enzyme (DP)	3	0.67142	0.223806667	0.000617523

ANOVA (Single Factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.154709195	1	0.154709195	176.2152604	0.000186127	7.708647
Within Groups	0.003511823	4	0.000877956			
Total	0.158221018	5				

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
Furfural (No enzyme)	Sugarcane bagasse	3	.5449600	.03374002	.01947981
	Durian peel	3	.2238067	.02485000	.01434716

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Furfural (No enzyme)	Equal variances assumed	.181	.692	13.275	4	.000	.32115333	.02419305	.25398265	.38832402
	Equal variances not assumed			13.275	3.676	.000	.32115333	.02419305	.25158619	.39072047

Appendix H. Enhanced Enzymatic Conversion of Durian Peel by Sulfuric Pretreatment for Biofuel Production was published in 2020 International Conference and Utility Exhibition on Energy, Environment and Climate Change (ICUE)

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Enhanced Enzymatic Conversion of Durian Peel by Sulfuric Pretreatment for Biofuel Production

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Burning of agricultural waste after harvesting seasons leads to persistent environmental pollution, especially PM2.5 and PM10. Utilization of agricultural waste by conversion to value-added product or biofuel is a solution for this problem, however, breaking down of lignocellulosic biomass in agricultural waste has a limiting factor due to its inappropriate physical and chemical properties. In this work, durian peel, as lignocellulosic biomass, was pretreated with diluted sulfuric acid to disintegrate the lignocellulosic fibrils and to promote enzymatic saccharification. To optimize this acid pretreatment, three pretreatment parameters, including temperature (60-140°C), time (20-100mins), and acid concentration (0.5-3.5%) were designed and varied based on Response Surface Methodology (RSM) using Box-Behnken design. After pretreatment, pretreated biomass was enzymatic hydrolyzed, and pretreatment efficiency was determined based on amounts of reducing sugars. The mathematical model representing the correlation of each pretreatment factor and reducing sugars was generated to calculate the optimized pretreatment condition. At predicted optimal pretreatment condition, 127.14°C, 74.13 minutes, 2.75%, the result showed that the reducing sugar was obtained at 553.1 mg/g-durian peel, which was 1.88 folds higher compared to unpretreated durian peel. This work suggested the necessity of pretreatment in bio-conversion of agricultural waste to produce biofuels and value-added products. Collapse