OPTIMIZATION OF ACID HYDROLYSIS OF PINEAPPLE LEAF RESIDUE AND BIOCONVERSION TO ETHANOL BY *SACCHAROMYCES CEREVISIAE*

SAOVANEE CHOOJIT,[*] TAWEESAK RUENGPEERAKUL[**] and CHAYANOOT SANGWICHIEN[*]

[*]*Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*
[**]*Department of Computer Engineering, Faculty of Engineering, Prince of SongklaUniversity, Hat Yai, Songkhla 90110, Thailand*
✉ *Corresponding author: C. Sangwichien, chayanoot.s@psu.ac.th*

Received March 21, 2017

In this study, response surface methodology (RSM) with central composite design (CCD) was employed to optimize the dilute acid hydrolysis of pineapple leaf residue pretreated by milling and drying in an oven at 110 °C overnight. The three manipulated variables were sulfuric acid concentration (0.2-5 M), temperature (110-130 °C), and hydrolysis time (30-120 min). The maximal 23.33 g/L RSM-predicted glucose yield was obtained at 0.24 M sulfuric acid concentration, 111 °C temperature, and 94 min hydrolysis time. A verification experiment indicated a highly reproducible glucose yield of 20.89 g/L (10.5% deviation from model prediction). The glucose resulting under optimal conditions was finally fermented to ethanol by using baker's yeast (*Saccharomyces cerevisiae*). The fermentation conditions were as follows: 1.5 g yeast per 50 mL substrate incubated at 30±2 °C. The highest ethanol yield of 9.75 g/L (0.47 g/g glucose) at 72 h was over 90% of the theoretical ethanol yield produced from glucose fermentation, which was 10.74 g (0.51 g/g glucose). The ethanol yield achieved appears quite attractive and demonstrates that pineapple leaves have excellent potential as an alternative feedstock for ethanol production.

Keywords: ethanol, lignocellulose, pineapple leaf, agricultural residue, acid hydrolysis, *Saccharomyces cerevisiae*

INTRODUCTION

Ethanol (ethyl alcohol or bioethanol) is a gasoline substituting fuel, an alternative fuel that has received special worldwide attention due to concerns about petroleum fuel shortages and global warming.[1] Generally, ethanol is produced from a variety of raw materials. Biomass is a modern source of renewable energy that requires proper management and technologies.[2] Agricultural biomass materials fall into three categories: sucrose-containing feedstock, starch materials and lignocellulosic materials. The current focus is on ethanol production from crops, such as corn, wheat and sugarcane, as well as from selected highly abundant agricultural wastes.[3] However, ethanol production from agricultural crops may conflict with other needs, because the limited agricultural land is also needed for food and feed production, especially for corn crops.[4] The economics of ethanol production by fermentation is significantly influenced by the cost of the raw materials, which accounts for more than half of the production costs.[5] To achieve cost-effective production, the agricultural supply of waste biomass is a good alternative substrate as it is inexpensive. Moreover, it does not demand separate land, water, or energy and does not have food value.[6] To avoid prohibitive transportation costs, specifically locally available agricultural residues should be used in bioethanol production.[7]

Pineapple leaf is an agricultural lignocellulose residue, which is not considered attractive for use as animal feed because of its high fiber content, high soluble carbohydrate and low protein content. After harvesting the fruit, the disposal of leaves is a big problem.[8] On the other hand, the high 70-82% cellulose content (by dry weight) of pineapple leaf[9] is appropriate for ethanol production, by hydrolysis of the cellulose to sugars, which are fermented to ethanol. In Thailand, during the past five years (2010-2014), the average annual pineapple fruit production amounted to two million tons, with a total production area of approximately 100,000 hectares spread over thirteen provinces. In each production cycle, fresh pineapple leaves are produced at over 4,000 kg/hectare and, in some areas, at up to 8,000-10,000 kg/hectare fresh weight.[10]

Lignocellulose consists of three main components: 30-60% cellulose, a glucose polymer; 25-35% hemicellulose, a sugar heteropolymer; and 15-20% lignin, a non-fermentable phenyl-propene unit. It also contains small amounts of minerals, oils, soluble sugars and other components.[11] Cellulose and hemicellulose, which typically make up two-thirds of the cell wall dry matter, are polysaccharides that can be hydrolyzed to sugars and then fermented to bioethanol. The lignin cannot be used for bioethanol production.[12] In particular, pentose sugars contained in hemicelluloses cannot be fermented into ethanol by conventional ethanologenic species like *Saccharomyces cerevisiae*. In general, baker's yeast *S. cerevisiae* has been traditionally used in the brewing industry to produce ethanol from hexoses.[13] The ethanol production process from lignocellulosic biomass has three major stages: delignification pretreatment is necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars; and fermentation of reducing sugars to ethanol.[14] Therefore, efficient and cost-effective pretreatment, hydrolysis and fermentation are needed to maximize sugar and ethanol productivities.[15] Dilute acids have been successfully used in the hydrolysis of a wide range of feedstocks, ranging from hardwoods to grasses and agricultural residues. Sulfuric acid (H_2SO_4) at concentrations usually below 4 wt% has been widely studied, as it is inexpensive, effective with low acid consumption, and gives high conversion of cellulose to glucose.[16,17]

Response surface methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the interactions between multiple manipulated experimental factors, and searching for their optimal set-point.[18] This methodology has been successfully applied to optimize the acid hydrolysis of several substrates, including cellulose.[19-21]

The aim of this research is to optimize the acid hydrolysis of pineapple leaf residue using RSM in order to maximize glucose selectivity and bioconversion to ethanol by *Saccharomyces cerevisiae*.

EXPERIMENTAL

Materials

Pineapple leaf residues

Pineapple leaves were sampled from residues in a pineapple farm after the fruit harvest, in Pattalung province, Thailand.

Microorganism and culture conditions

Baker's yeast (*Saccharomyces cerevisiae*) was obtained from the Thailand Institute of Scientific and Technology Research (TISTR), Thailand. The culture of *S. cerevisiae* was maintained on YM agar slants (consisting of glucose, 20; yeast extract, 3; malt extract, 3; peptone, 5; and agar 1.5, all in g/l) at 4 °C. An inoculum was prepared by transferring a loopful of cells to 50 mL of YM medium broth, which was incubated and grown at 30±2 °C on a shaker at 150 rpm before inoculating the reactor.

Experimental methods

Pineapple leaves preparation and mechanical pretreatment

The sample of raw pineapple leaf residues was prepared by washing with distilled water to remove contaminants and then was fed through a roll nip to make thin sheets, and dehydrated by air-drying. For mechanical preparation of pretreated pineapple leaves, the dried sheets was mechanically ground with a hammer mill and sieved with mesh size 7. Then, the fraction that passed through the mesh was collected and the bigger particle size fraction was rejected. The pineapple leaf powder was dried in an oven at 110 °C overnight and analyzed for cellulose, lignin and moisture contents according to AOAC methods.[22]

Optimization of hydrolysis variables using response surface methodology

Response surface methodology (RSM) is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the

experimental data, which must describe the behavior of a data set with the objective of making statistical previsions. It can be well applied when a response or a set of responses of interest are influenced by several variables.[23] The central composite design (CCD) is one of the most commonly used response surface designs to study the effects of variables on the response, and subsequently in optimization studies.[24]

The optimization of fermentable glucose production from pineapple leaf residue was studied by using the Design Expert software (Trial version 10.0, Stat-Ease, Inc., Minneapolis, USA) with CCD design matrix. The hydrolysis reaction was carried out in a 150 mL Duran bottle containing 5 g of pretreated pineapple leaves per 50 mL of sulfuric acid (1:10 w/v) in triplicates. Three independent variables, namely sulfuric acid concentration (B, 0.2-5.0 M), hydrolysis temperature (C, 110-130 °C) and hydrolysis time (A, 30-120 min) were used at five coded levels (-α, -1, 0, +1, +α), as shown in Table 1. The 2^3 factorial central composite experimental designs with six start points and three replicates at the central point had 17 experimental runs in the design (Table 2).

The significance of each variable and their interactions, and fitting a predictive model to the experimental responses was based on the following second-order polynomial:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j \quad (1)$$

where Y is the observed response (xylose concentration, xylose yield, or digestibility of solid residue); β_0 is the constant term; i, j and k are integers (in this case, i is from 1 to 3, j is from 2 to 3, and k is the total number of factors, 3); β_i, β_{ii}, β_{ij} are, respectively, the coefficients for the linear, quadratic and interactive effects; and x_i and x_j are independent variables or factors, representing the acid concentration, hydrolysis temperature and hydrolysis time.

The statistical software package Design Expert (Trial version 10.0) was used to analyze the results. The fit of the models was assessed from the coefficient of determination R^2 and the adjusted R^2. Experimental validation of the model-based optimum set-point for acid hydrolysis was performed.

Fermentation process

The pineapple leaves acid hydrolyzed under optimum conditions provided glucose for ethanol fermentation by *S. cerevisiae* (commercial baker's yeast).

Table 1
Coded and real values of variables in the central composite design (CCD) – optimisation of pineapple hydrolysis

Variables	Code	Variable levels				
		-1.682	-1	0	1	1.682
Time (min)	A	30	48	75	102	120
Sulfuric acid (M)	B	0.2	1.2	2.6	4	5
Temperature (°C)	C	110	114	120	126	130

Table 2
Central composite design consisting of 17 experiments for the study of three experimental factors in coded units along with observed values

Run no.	Factor variables (code)			Coefficients assessed by
	A	B	C	
1	-1	-1	-1	
2	1	-1	-1	
3	-1	1	-1	
4	1	1	-1	Fractional 2^{3-1}
5	-1	-1	1	factorial design
6	1	-1	1	
7	-1	1	1	
8	1	1	1	
9	-1.682	0	0	
10	1.682	0	0	
11	0	-1.682	0	Star points
12	0	1.682	0	(6 points)
13	0	0	-1.682	
14	0	0	1.682	
15	0	0	0	
16	0	0	0	Central points
17	0	0	0	(3 points)

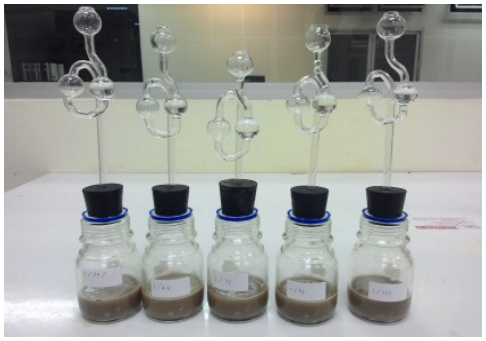

Figure 1: Hydrolysis products subjected to fermentation

Table 3
Components of pineapple leaf before and after mechanical pretreatment

Component	Content (%)	
	Unpretreated	Pretreated
Cellulose	29.85	35.14
Lignin	5.47	3.96
Others	64.68	60.9
Moisture (w.b.)	87.16	9.2

1% of inoculum was transferred into a 250 mL flask containing 50 mL of culture medium (containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose, at pH 5) and was subsequently incubated at 30±2 °C for 24 h. Then, the yeast cells were harvested by filtering with filter paper no. 1 and used as inoculum cells.

The fermentation was carried out in 150 ml Duran bottles, with the ratio of yeast cells to fermentation medium of 1.5:50 (w/v). The fermentation bottle was flushed with nitrogen gas for 1 min to create anaerobic conditions and then immediately capped with air-lock rubber stopper (Fig. 1). Three replicate fermentation bottles were incubated in the dark on a shaking incubator (100 rpm) at 30±2 °C for 5 days. Samples were harvested at the beginning and every 24 h during 5 days of fermentation to monitor cell growth, glucose concentration and ethanol productivity. Ethanol yield was calculated according to Chin *et al*.:[25]

$$Ethanol\ yield = \frac{Ethanol\ produced\ (g/L) \times 100}{Initail\ glucose\ (g/L) \times 0.51} \quad (2)$$

RESULTS AND DISCUSSION

Pineapple leaf composition

The raw and mechanically pretreated pineapple leaf samples were analyzed for chemical composition. Cellulose, lignin, moisture and other constituents in pineapple leaves before and after pretreatment are presented in Table 3.The pretreated pineapple leaves contained 35.14% cellulose, 3.96% lignin and 60.9% others, while the corresponding chemical components in the raw pineapple leaves had the following values: 29.85, 5.47 and 64.68%, respectively. Clearly, the pretreatment increased the content of cellulose by 17.72% and decreased that of lignin by 27.61%, which is beneficial for hydrolysis.

During the pretreatment, the lignocellulosic biomass was heated, which disrupted the crystalline structure of cellulose, broke down the lignin structure and hydrolyzed part of the hemicellulose.[26] Grous *et al*.[27] reported that 90% efficiency was achieved for 24 h enzymatic hydrolysis of poplar chips pretreated by steam explosion, whereas the corresponding yield from the untreated substrate was of only 15%. However, the biomass particle size reduction to below 40 mesh has little effect on the hydrolysis yield or rate.[28] Milling to reduce particle size increased the specific surface and reduced the degree of polymerization (DP),[29] and these factors can increase the total hydrolysis yield of lignocellulose in most cases by 5-25% (depending on the type of biomass, kind of milling and its duration), and can reduce the technical digestion time by 23-59% (reflecting an increase in the hydrolysis rate).[15] The effects of the pretreatment are believed to be primarily due to the increase in the surface area accessible to enzymes and measurements show a considerable increase in the

pore volume available to 5-9 nm solutes. Cara *et al*.[30] studied the production of fuel ethanol from olive-tree pruning biomass that was milled using a laboratory hammer mill, then subjected to steam-explosion pretreatment at 240 °C temperature. The results showed the maximum ethanol yield (7.2 g of ethanol/100 g of raw material) at particle size smaller than 10 mm. The power consumption in mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics. It has been proposed that, if the final particle size is held within the range of 3-6 mm, lower milling energy is used, while achieving highly increased specific surface area and reduced crystallinity.[31]

Hydrolysis of pretreated pineapple leaves

The optimal hydrolysis parameters, namely hydrolysis time, sulfuric acid concentration and hydrolysis temperature, for cellulose acid hydrolysis were determined experimentally using the central composite design (CCD). The observed and model-predicted values of glucose after 5 days are shown in Table 4. The maximum experimental glucose yield of 17.65 g/L was obtained with 0.2 M sulfuric acid at 120 °C for 75 min, while the value from the fitted model was 17.34 g/L (about 2% deviation). The significance and the effects of each variable on pretreated pineapple leaves and the glucose yield are presented in Table 5. Fitting the multiple regression model to the experimental data, the following second order polynomial model is obtained to describe the acid hydrolysis of pretreated pineapple leaves:

$$\text{Glucose (g/L)} = 383.30 + 0.52A - 6.92B - 6.10C + 0.28B^2 + 0.026C^2 - 4.94AC \quad (3)$$

where A, B and C are hydrolysis time, sulfuric acid concentration, and hydrolysis temperature, respectively. The statistical significance of this model was assessed by Fisher's statistical test (*F*-test) and by analysis of variance (ANOVA) of this response surface model (Table 5).

The model is highly significant, as is evident from the *F*-value 47.46 and the very low *P*-value < 0.0001. This indicates that there is only a 0.01% chance that an *F*-value this large could occur by random coincidence, as opposed to having an appropriate model. The value of R^2 = 0.9836 indicates that only 1.64% of the total variation remains not explained by the model, so the correlation of experimental and fitted values is excellent.

The adjusted coefficient of determination R^2_{Adj} = 0.9631 is also high and corroborates the high significance of the model. The predicted determination coefficient R^2_{Pred} =0.8777 points to good agreement of the experimental and the predicted values for acid hydrolysis (Fig. 2a). The R^2_{Pred} is also in reasonable agreement with the adjusted R^2 (R^2_{Adj}). This means that the data were well fit by the model, which gives good estimates of system response within the experimental range.

Table 4
Observed and predicted values of glucose yield from acid hydrolysis

Run no.	Glucose (g/L)		
	Observed value	Predicted value	Residual
1	15.37	16.21	-0.85
2	17.50	17.57	-0.07
3	9.05	9.58	-0.53
4	8.46	8.99	-0.52
5	14.54	14.57	-0.03
6	12.70	12.72	-0.02
7	8.55	9.03	-0.48
8	5.53	5.23	0.30
9	12.93	12.07	0.86
10	9.95	10.02	-0.08
11	17.65	17.34	0.31
12	5.93	5.46	0.47
13	15.62	14.71	0.91
14	10.04	10.17	-0.13
15	9.62	9.82	-0.20
16	9.71	9.82	-0.12
17	10.01	9.82	0.19

Table 5
Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Term	SS	DF	*F* value	Prob>*F*
A	5.08	1	9.92	0.0161*
B	170.32	1	332.68	< 0.0001**
C	24.91	1	48.66	0.0002**
A^2	2.12	1	4.13	0.0816
B^2	3.49	1	6.82	0.0348*
C^2	9.64	1	18.82	0.0034**
AB	1.91	1	3.72	0.0951
AC	5.13	1	10.02	0.0158*
BC	0.59	1	1.16	0.3175
Model	218.69	9	47.46	< 0.0001**
Residual	3.58	7		
Lack of fit	3.5	5	16.77	0.0572
Pure error	0.084	2		
Total	222.28	16		

R^2 = 0.9839; adjusted R^2 = 0.9631; predicted R^2= 0.8777; CV (%)=6.3; adequate precision=22.49; SS, sum of squares; DF, degrees of freedom; *,** Significant at <0.05, <0.01, respectively

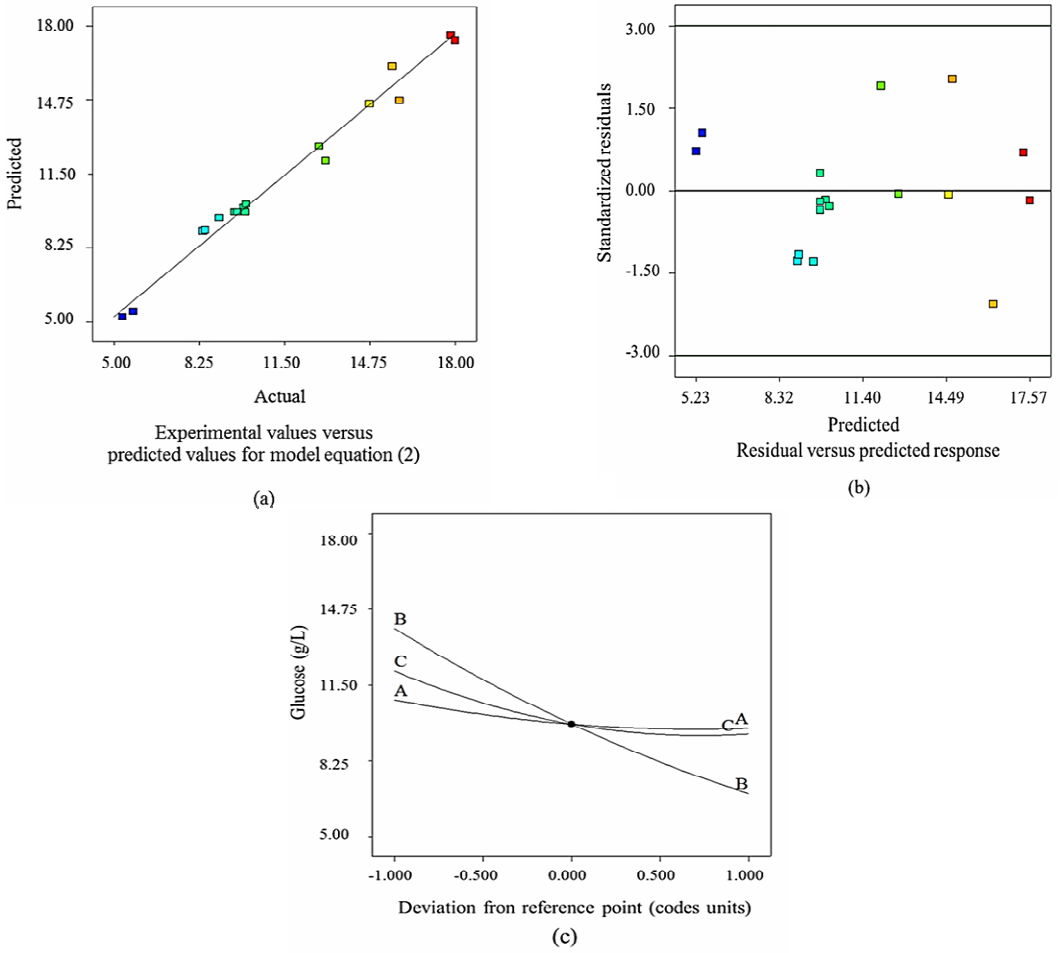

Figure 2: Validity of model equation (a, b) and perturbation plot (c)

Figure 2(b) is a plot of the residuals (the differences of fitted and observed values of the response variable studied) *versus* the predicted response. The quality of the fit is good because the residual distribution does not follow a trend with respect to the predicted values of the response variable, which indicates that the quadratic model adequately represents the glucose production over the studied experimental range. If, in contrast, a clear trend was present, the model would require additional terms to match that trend and correspondingly reduce the residuals. The perturbation plot (Fig. 2c) shows the comparative effect of each manipulated variable on glucose. The curvatures confirm the analysis of variance results (ANOVA, Table 5) in that the second order terms are significant. The signal-to-noise ratio is a measure of model precision, and a ratio greater than 4 is desirable.[32] The ratio of 22.49 for the acid hydrolysis model indicates an adequate signal, so the model can be used to navigate the design space. The coefficient of variation (CV) indicates the degree of precision with which the treatments are compared. Usually, the higher the CV, the less reliable is the experiment. In these experiments, the low CV (6.3%) indicates highly reliable experimental results. The lack of fit measures the failure of the model to represent the experimental data, and here the lack of fit of regression (Eq. (3)) is not significant (P = 0.0572). This indicates that the model equation was adequate for the experimental data on acid hydrolysis. The P-value is used as a tool to check the significance of each coefficient, which helps understand the interactions of factors. In this study, hydrolysis time (A), sulfuric acid concentration (B) and hydrolysis temperature (C) were highly significant in their individual effects. Representative response surface plots are shown in Figure 3a-3c.

In Figure 3a, the interaction plot of hydrolysis time and sulfuric acid concentration shows that the glucose production increased remarkably over time, but decreased with increasing sulfuric concentration. On the other hand, the glucose production decreased with sulfuric acid concentration and with temperature (Fig. 3b).

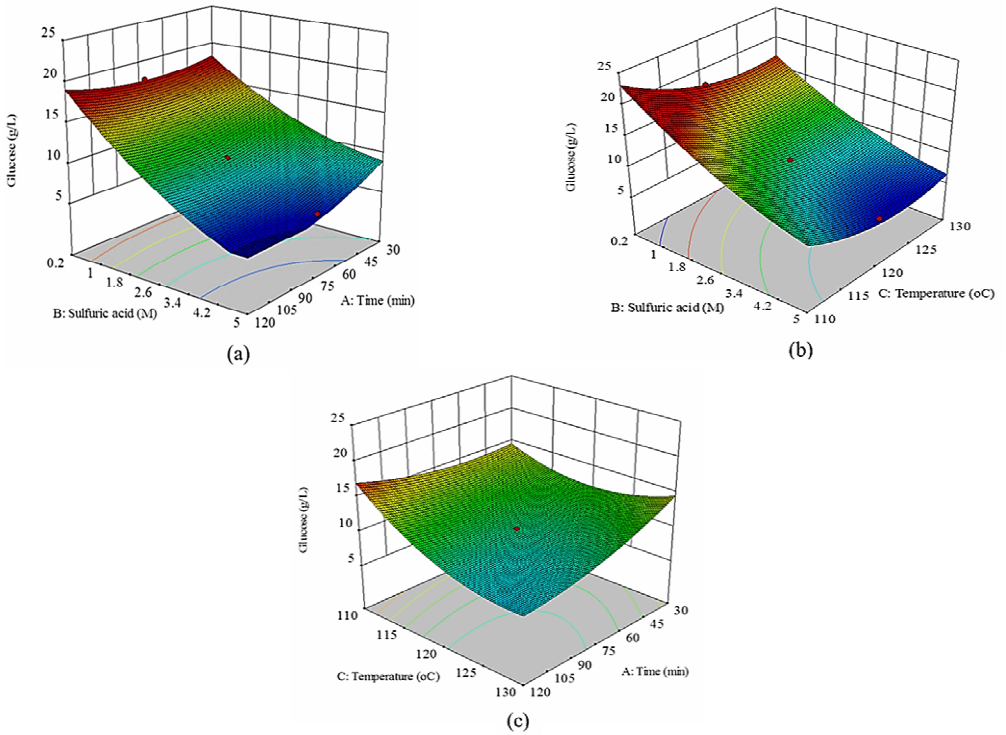

Figure 3: 3D response surface plots for glucose production showing the interaction between (a) hydrolysis time and sulfuric acid concentration; (b) sulfuric acid concentration and hydrolysis temperature; and (c) hydrolysis time and hydrolysis temperature

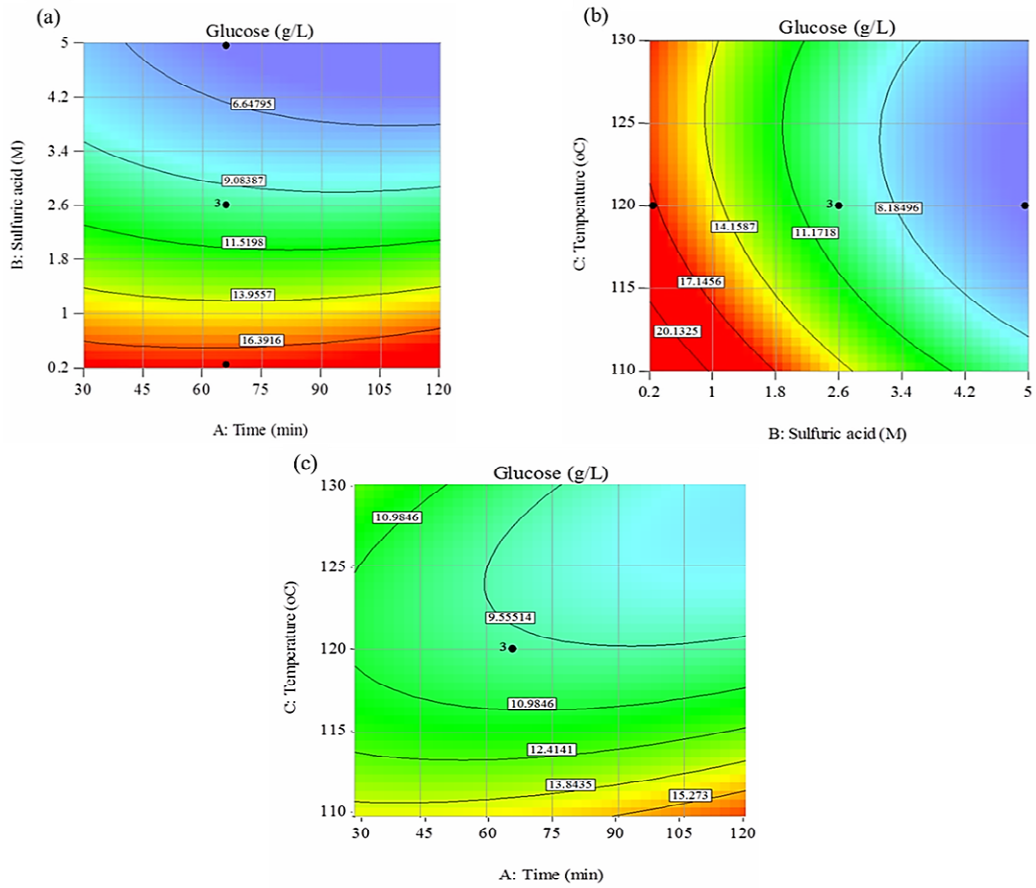

Figure 4: Contour plots for glucose production showing the interaction between (a) hydrolysis time and sulfuric acid concentration; (b) sulfuric acid concentration and hydrolysis temperature; and (c) hydrolysis time and hydrolysis temperature

However, the interaction of time and temperature was more dominant, so at low temperature glucose increased with increasing time, while at high temperature, it decreased with time (Fig. 3c). The mutual interactions of the factors can also be assessed from contour plots. If the interactions are negligible, the contours (if not straight lines) will be elliptical with principal axes parallel to the coordinates/factors (Fig. 4a, 4b). In case of significant interactions, the elliptical contours become tilted: the axes of the ellipsoid do not align with the coordinate axes (Fig. 4c).

The optimal set-point for manipulated variables was obtained numerically from the regression fit (Eq. (2)). To validate the predicted optimum, it was checked experimentally. The numerical optimal solution was the following: sulfuric acid concentration of 0.24 M, hydrolysis temperature of 111 °C, and hydrolysis time of 94 min, with the maximum glucose production of 23.33%. The verification experiments indicated a highly reproducible glucose yield at 20.89 g/L (90% of the theoretical yield), which is close to the model based yield prediction. According to the report of Prosen *et al.*,[33] when wood pyrolysate is hydrolyzed with sulfuric acid, levoglucosan is hydrolyzed to glucose and the toxic materials are converted to inactive materials. Furthermore, the acid-hydrolyzed pyrolysate (biomass) is utilized by microorganisms very well. This led us to investigate the application of sulfuric acid hydrolyzed cellulosic pyrolysate as substrate for fuel ethanol production.

In conventional practice, high temperature and long pretreatment give the most hydrolyzed hemicellulose for production of reducing sugars.[34] The amount of reducing sugars on hydrolysis of corn cobs using 1.75% (w/w) H_3PO_4 was least at short times or at low temperatures of pretreatment.[35] Sulphuric acid at high temperatures degrades xylose and glucose into furfural, while maleic acid degrades these sugars less. Sulfuric acid treatment at 1% concentration and at 130 °C for one hour produced 33.35 g/L of

reducing sugars, while phosphoric acid produced the most (35.21 g/L) and maleic acid produced 36.72 g/L of reducing sugars.[36]

Acid hydrolysis of cellulosic pyrolysate from cotton waste to glucose and its fermentation to ethanol has been investigated. The maximum glucose yield (17.4%) was obtained by hydrolysis with 0.2 mol/L sulfuric acid using autoclaving at 121 °C for 20 min. The fermentation by *S. cerevisiae* of a hydrolysate medium containing 31.6 g/L glucose gave 14.2 g/L ethanol in 24 h, whereas fermentation of the medium containing 31.6 g/L pure glucose gave 13.7 g/L ethanol in 18 h. These results showed that acid hydrolyzed pyrolysate could be used for ethanol production.[37] Hsu *et al*.[19] reported on dilute acid pretreatment of rice straw, and they found the glucose content to range from 53% to 58% in the pretreated solid residues, and to slightly increase when the operating temperature was changed from 160 °C to 180 °C. However, decreased glucose content was observed at 190 °C. The glucose yield decreased with H_2SO_4 concentration, while it slightly increased with hydrolysis time. Moreover, hydrolysis temperature interacted with hydrolysis time so that low temperature for long time (160°C and 25 min) gave similar glucose yield as high temperature for short time (180 °C and 1 min) with an approximate glucose yield of 57.4 g/100g rice straw.

Ethanol production from pineapple leaf hydrolysate

The feasibility of ethanol fermentation from pineapple leaf residues by baker's yeast (*S. cerevisiae*) was tested experimentally. The fermentation medium contained 20.89 g/L glucose from the acid hydrolyzed leaves, without otherwise added nutrients. Each batch fermentation was performed in a 150 ml Duran bottle, the ratio of *S. cerevisiae* to fermentation medium was 1.5:50 (w/v), and incubation was performed in the dark on a shaking incubator at 30±2 °C. Ethanol production was monitored by sampling at 24, 48, 72, 96 and 120 h. The ethanol concentration was 7.58 g/L after the first day of fermentation, and slightly increased after 24 h until the maximum concentration was observed at 72 h (9.75 g/L). After 3 days, the ethanol concentration decreased to the final 8.03 g/L at 120 h. The highest ethanol production of 9.75 g (0.47 g/g glucose) was over 90% of the theoretical ethanol yield produced from glucose fermentation with 10.74 g (0.51 g/g glucose)(Eq. (2)).

As regards the glucose concentration in the fermentation medium, the initial glucose was 20.83 g/L and it dramatically decreased by 67.7%(w/v) in 24 h and then slightly decreased until the remaining glucose concentration was 2.74% (w/v) after 120 h (Fig. 5).The pattern of ethanol production and sugar utilization by *S. cerevisiae* was elaborated by Tropea *et al*.[38]

In general, dilute acid hydrolysis of lignocellulose may result in sugars, along with other by-products from some serial and parallel reactions (Fig. 6).[39] The performance of *S. cerevisiae* in lignocellulosic hydrolysates was correlated to the contents of acetic acid, formic acid, 2-furfuraldehyde (furfural; from pentoses), 5-hydroxymethyl-2-furfuraldehyde (5-HMF; from hexoses) and phenol monomers. Concurrently, when the pH is not controlled, water acts as a weak acid and promotes rapid acid-catalyzed hydrolysis of polysaccharides to monosaccharides, which subsequently degrade to furfural, 5-HMF, and other inhibitors.[40]

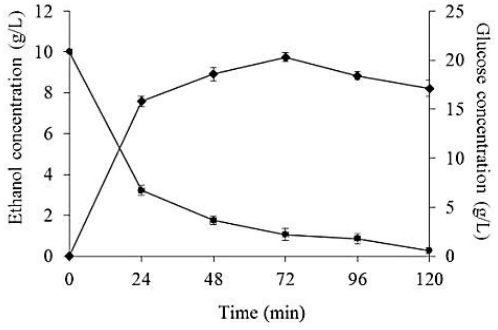

Figure 5: Time profile of ethanol concentration (♦) and glucose concentration (●) from acid hydrolyzed pineapple leaf residuals by *S. cerevisiae*

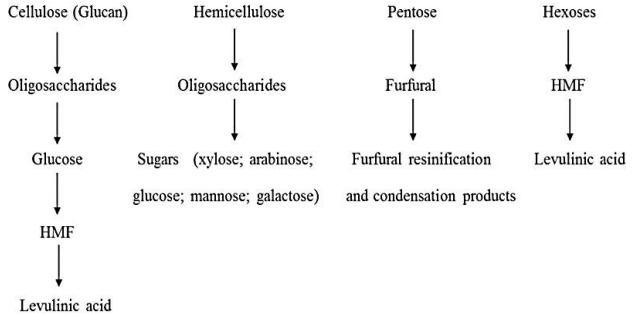

Figure 6: Dilute-acid hydrolysis of lignocelluloses may result in sugars and other by-products in some serial and parallel reactions; furfural and hydroxymethylfurfural (from pentoses and hexoses, respectively), levulinic and condensation products (modified from Karimi *et al*.[39])

The poor fermentability of dilute acid bagasse hydrolysates by *S. cerevisiae* is related to the high concentrations of fermentation inhibitors formed during severe acidic pretreatment.[41] However, Duangwang *et al*.[42] found that *S. cerevisiae* could ferment the hydrolysate of oil palm empty fruit bunches to ethanol 56 fold better than a mixed culture starter. When oil palm trunk (OPT) sap was utilized to produce sugar and bioethanol using *S. cerevisiae*, the highest ethanol content achieved was 8.49 g/L. The amount of bioethanol produced from OPT is quite high and an interesting traditional process is used to convert waste biomass to feedstock for efficient biofuel production.[43] Moreover, pineapple shells contain a high amount of cellulose (37.68±6.97%) and, after conversion to sugar (36.25±2.87 g/L), the maximum yield of ethanol (9.69 g/L) was achieved after 72 h with *S. cerevisiae*, but lower ethanol production (1.38 g/L) was observed after 72 h with *E. aerogenes*, according to Choonut *et al*.[44] So, a high sugar yield resulting from the pretreatment facilitates ethanol production from biomass. On the other hand, a low ethanol yield may indicate that the microorganism is sensitive to inhibitory compounds in the fermentation medium.

CONCLUSION

The present study investigated obtaining fermentable glucose from pineapple leaves by acid hydrolysis, and demonstrated experimentally that pineapple leaves can be a potential raw material for bioethanol production. The leaves contained a high fraction of cellulose (35.14%) after the mechanical milling pretreatment. The optimum conditions for diluted acid hydrolysis were obtained by response surface methodology: sulfuric acid concentration of 0.24 M, hydrolysis temperature of 111 °C, and hydrolysis time of 94 min. The maximum model predicted glucose yield was 23.33 g/L, while a verification experiment gave the highly reproducible glucose yield of 20.89 g/L (over 90% of the model predicted yield). The under optimal conditions, acid hydrolyzed pineapple leaves were utilized in fermentation by *S. cerevisiae* without added nutrients to produce ethanol. The maximum yield of ethanol (9.69 g/L) was achieved after 72 h at 30±2 °C. This is approximately 92% of the theoretical ethanol yield. The ethanol yield achieved appears quite attractive and demonstrates that pineapple leaves have excellent potential as an alternative feedstock to the production of fuel ethanol.

The production of fermentable glucose from this underutilized agro-waste has commercial application potential, which can add value to pineapple cultivation, generate extra income for farmers, and also help in agribusiness diversification. Moreover, in light of the high cost of petroleum fuels, the production of bioethanol could be an economically attractive possibility. In addition, the use of agricultural residues allows significant reduction in the volume of dumped waste in the environment. It is important to compare technical alternatives each at their optimal performance on making decisions about industrial applications, while comparison between optimized and non-optimized alternatives would be inherently flawed. The experimental optimization in this study serves as a basis for fair comparisons.

ACKNOWLEDGEMENT: This work benefited from financial, facilities and laboratory support

from the Department of Chemical Engineering, Faculty of Engineering, the Prince of Songkla University. The authors are grateful to Mr. Arnon Phanthong and Mr. Jetda Chuchuen for kindly performing some parts of this study. Also thanks to the PSU Research and Development Office (RDO) and Assoc. Prof. Seppo Karrila, Ph.D. (Chem. Eng.). The first author was granted additional support by the Postdoctoral Fellowship from the Prince of Songkla University.

REFERENCES

[1] C. Bloyd and N. Foster, "An Update on Ethanol Production and Utilization in Thailand- 2014", DOE. Ed. USA,accessed on 9/2014,https://www.pnnl.gov /main/publications/external/PNNL-23673.pdf

[2] S. Preechajarn and P. Ponnarong, "Thailand Biofuels Annual". USDA, F.A.S., Bangkok, EIA, Thailand, accessed on 7/2016, https://gain.fas.usda. gov/Recent/Publication/Biofuels/

[3] M. Balat, *Energ. Convers. Manage.*, **52**, 858 (2011).

[4] J. Sun, L. Chen and H. L. Wang, *Renew. Energ.*, **112**, 5 (2003).

[5] P. Redding, Z. Wang, D. R. Keshwani and J. J. Cheng, *Bioresour. Technol.*, **102**, 1415 (2011).

[6] N. Sarkar, S. K. Ghosh, S. Bannerjee and K. Aikat, *Renew. Energ.*, **37**, 19 (2012).

[7] L. Mojovic, D. Pejin, O. Grujic, J. Penjin, M. Rakin *et al.*, *Chem. Ind. Chem. Eng. Q.*, **15**, 211 (2009).

[8] R.T. P. Correia, P. Mccue, M. M. A. Magalhaes, G. R. Macedo and K. Shetty, *Process Biochem.*, **39**, 2167 (2004).

[9] P. Omojasola, O. Jilani and S. Ibiyemi, *Natur. Sci.*, **6**, 64 (2008).

[10] Ministry of Agriculture and Cooperatives (Thailand), accessed on 12/2015. http://www.oae.go.th/download/ document_tendency/ journalofecon2558.pdf.

[11] C. N. Hamelinck. V. G. Hooijdonk and A. P. C. Faaij, *Biomass Bioenerg.*, **28**, 384 (2005).

[12] C. Karunanithy, K. Muthukumarappan and J. L. Julson, in *Procs. ASABE Conference*, Rhode Island, June 29-July 2, 2008, pp. 3603-3611.

[13] J .H. V. Vleet and T. W Jeffries, *Curr. Opin. Biotechnol.*, **20**, 300 (2009).

[14] M. Balat, H. Balat and C. Oz, *Prog. Energ. Combust. Sci.*, **34**, 55 (2008).

[15] P. Kumar, D. M. Barrett, M. J. Delwiche and P. Stroeve, *Ind. Eng. Chem. Res.*, **48**, 3713 (2009).

[16] Q. A. Nguyen, M. P. Tucker, F. A. Keller and F. P. Eddy, *Appl. Biochem. Biotechnol.*, **84**, 561 (2000).

[17] N. Bujang, M. N. M. Rodhi, M. Musa, F. Subari, N. Idris *et al.*, *Procedia Eng.*, **68**, 372 (2013).

[18] D. C. Montgomery, "Design and Analysis of Experiments", 5th ed., John Wiley & Sons, Inc., 2001, pp. 427-510.

[19] T. C. Hsu, G. L. Guo, W. H. Chen and W. S. Hwang, *Bioresour. Technol.*, **101**, 4907 (2010).

[20] B. S. Baboukani, M. Vossoughi and I. Alemzadeh, *Biosyst. Eng.*, **111**, 166 (2012).

[21] P. Noparat, P. Prasertsan, S. O.-Thong and X. Pane, *Energ. Procedia*, **79**, 924 (2015).

[22] W. Horwitz and G. W. Latimer, in "Official Methods of Analysis of AOAC International", 18th ed., AOAC International Inc., USA, 2005, pp. 856-857.

[23] M. A. Bezerra, R. E. Santelli, E. P. Oliveiraa, L. S. Villar and L. A. L. Escaleira, *Talanta*, **76**, 965 (2008).

[24] Y. L. Gao, X. R. Ju and H. H. Jiang, *Process Biochem.*, **41**, 362 (2006).

[25] K. L. Chin, P. S. Hing, L. J. Wong, B. T. Tey and M. T. Paridah, *Bioresour. Technol.*, **101**, 3287 (2010).

[26] M. J. Taherzadeh and K. Karimi, *Int. J. Mol. Sci.*, **9**, 1621 (2008).

[27] W. R. Grous, A. O. Converse and H. E. Grethlein, *Enzyme Microb. Technol.*, **8**, 274(1986).

[28] V. S. Chang and M. T. Holtzapple, *Appl. Biochem. Biotechnol.*, **84**, 5 (2000).

[29] M. Galbe and G. Zacchi, *Adv. Biochem. Eng. Biotechnol.*, **108**, 41 (2007).

[30] C. Cara, C. Ruiz, M. Ballesteros, P. Manzanares, M. J. Negro *et al.*, *Fuel*, **87**, 692 (2008).

[31] L. Cadoche and G. D. Lopez, *Biol. Wastes*, **30**, 153 (1989).

[32] J. K. Kim, B. R. Oh, H. J. Shin, C. Y. Eom and S. W. Kim, *Process Biochem.*, **43**, 1308 (2008).

[33] E. M. Prosen, D. Radlein, J. Piskorz, D. S. Scott and R. L. Legge, *Biotechnol.Bioeng.*,**42**, 538 (1993).

[34] M. G. O. Ogawa, Y. U. Ishida and U. Naoto, *Afr. J. Microbiol. Res.*, **2**, 110 (2008).

[35] S. Satimanont, A. Luengnaruemitchai and S. Wongkasemjit, *World Acad. Sci. Eng. Technol.*, **6**, 531 (2012).

[36] M. J. Kootstra, H. H. Beeftink, E. L. Scott and J. P. M. Sanders, *Biochem. Eng.*, **46**, 126 (2006).

[37] Z. Yu and H. Zhang, *Bioresour. Technol.*, **93**, 199 (2004).

[38] D. Tropea, L.Wilson, G. L. Torre, R. B. L. Curto, P. Saugman *et al.*, *J. Food Res.*, **3**, 60 (2014).

[39] K. Karimi, S. Kheradmandinia and M. J. Taherzadeh, *Biomass Bioenerg.*, **30**, 247 (2006).

[40] J. R. Weil, B. Dien, R. Bothast, R. Hendrickson, N. S. Mosier *et al.*, *Ind. Eng. Chem. Res.*, **41**, 6132 (2002).

[41] C. Martin, M. Galbe, C. F. Wahlbom, B. Hahn-Hagerdal and L. J. Jonsson, *Enzyme Microb. Technol.*, **31**, 274 (2002).

[42] S. Duangwanga, Ethanol production from cellulose in oil palm empty fruit bunch by separation hydrolysis and fermentation, http://kb.psu.ac.th/psukb/bitstream/ 2010/9142/1/365504.pdf.

[43] N. Hossain and R. Jalil, *Asia Pac. Energ. Environ.*, **2**, 81 (2015).

[44] A. Choonut, M. Saejong and K. Sangkharak, *Energ. Procedia*, **52**, 242 (2014).