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Effect of varying fermentation parameters in bioethanol production from pomelo peels

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ABSTRACT

Pomelo peels, a lignocellulosic material, exhibit potential in bioethanol production due to its high fermentable sugar content, lignin, and cellulose content. In this study, the researchers used pretreated pomelo peels using phosphoric acid and acetone to optimize the bioethanol yield through enzymatic hydrolysis and simultaneous saccharification and fermentation; where pH, amount of substrate, and yeast concentration are the parameters weighed. Optimal ethanol yield of 11.84% was obtained at pH 3.5, 10 mL of substrate, and a yeast concentration of 15%. A 2*k* factorial design was used to analyze the effect of varying parameters. Dilute acid pretreatment significantly impacted alpha cellulose, acid insoluble lignin, and moisture. At 75% acid dilution, acid insoluble lignin and extractives were affected, while hemicellulose and acid soluble lignin changed at 80% concentration. Amount variation of *Aspergillus niger* significantly affects the extraction of sugar content of pomelo peels. The optimum condition in reducing sugar analysis, 25.38%, was achieved after using a 75% acid pretreatment and 12 g of enzyme. The concentration of yeast has a significant effect on the percent ethanol yield after fermentation; in contrast, pH and amount of substrate have minor effects. Design-Expert application was used for statistical analysis, while Minitab 18® for analysis of variance. Using response surface methodology, a valid mathematical model with high desirability was generated.

Keywords: pomelo peels, phosphoric acid pretreatment, fermentation parameters, 2^k factorial

1. Introduction

Development of bioethanol continues to pique interest as an alternative to conventional fossil fuels for applications as single fuel dedicated for engine vehicles or fuel blends. Biofuels are renewable and carbon-neutral; they are considered sustainable in contrast to most of the liquid and gas fuels which are fossil-based with limited world reserves. In line with this, to arrive at a pure ethanol product, purification is achieved through various ways; distillation among which is most utilized. Though they pose economic issues in production costs, requiring repetitive vaporization and condensation, ethanol purification is critical for any kind of purpose.

The global bioethanol industry derives much of its growth from the rising need for renewable sources of energy. Bioethanol is essential transportation fuel, but its availability is limited and distributed unevenly across the world. After the transport sector, the best-known end-user of green ethanol is the food and beverage industry in the form of additives. But, in recent years, concerns about oil price hikes, rural growth, and anthropogenic climate change have contributed to the rapid growth of the biofuel industry. The so-called 'first-generation' technologies derived from food crops such as maize and sugarcane are currently dominating the global biofuels market; however, it is increasingly recognized that more advanced conversion options using non-edible biomass are required to extend the usage of biofuels while reducing adverse impacts on global food prices and the natural environment. The development of these so-called 'second' and 'third' generation biofuels hence received considerable government support including consumption mandates, research development and demonstration grants, and subsidies and tax credits in recent years.

Ethanol production from lignocellulosic biomass consists of three main steps: pretreatment of biomass, enzymatic hydrolysis, and fermentation. Pretreatment of biomass is performed to increase the accessibility of enzymes. This is a vital technique that increases the enzymatic hydrolysis of biomass. After pretreatment, enzymatic biomass hydrolysis is used to convert polysaccharides into monomeric sugars, such as xylose and glucose. In this process, enzymes enhance the bonding of molecules by adding the elements of water. The use of various microorganisms enables these sugars to be fermented into ethanol [1].

The optimization of pretreatment and enzymatic hydrolysis is one of the major challenges in the commercialization of second-generation feedstock bioethanol. Also, it is necessary to determine the chemical composition of the biomass involved to characterize specific pretreatment conditions; designing such ensures optimal bioethanol yield [2].

Citrus peel waste is a valuable lignocellulosic feedstock for bioethanol production due to its richness in fermentable sugars and low lignin content. Two main value-added items that include citrus peel are d-limonene and pectin. D-limonene functions as a microbial growth agent for yeast and must therefore be extracted before fermentation. Pectin, on the other hand, limits the hydrolysis of cellulose and hemicellulose since it acts as a physical barrier to restrict the access of enzymes to the substrate. It also demonstrated the feasibility of producing pomelo peel ethanol using acid

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pretreatment and a fed-batch SSF process [3].

The present study planned to optimize fermentation parameters using phosphoric acid-acetone for pretreatment. Innovative pretreatment is said to result in higher pretreated materials and ethanol content after fermentation compared to acidic pretreatment alone.

The study aimed to evaluate the effect of varying fermentation parameters in bioethanol production from pomelo peels. It sought to determine the significant difference in the physicochemical properties of the pomelo peels before and after pretreatment using varying phosphoric acid; assess the significant effect of the reducing sugar concentration extracted in enzymatic hydrolysis in the ethanol yield; and analyze the effect of the pH, amount of substrate, and yeast concentration as fermentation parameters on the bioethanol yield.

2. Materials and methods

2.1. Collection and preparation of pomelo peels

Pomelo peels were gathered from the local markets of Batangas City. It is important that the peels are to be manually separated and rinsed for the removal of remaining debris. Afterward, it was cut into smaller manageable pieces (approximately 1 cm thickness) which was then dried at 70°C in a hot-air oven. Drying takes place until all the moisture is removed or until a constant weight is reached.

2.2. Lignocellulose pretreatment

In a 500-mL glass beaker, each dry material of pomelo peels weighing 25 g was placed and mixed with 200 mL phosphoric acid with different concentrations, 75 wt% and 80 wt%. For an hour, the slurry was subjected to a rotary shaker at a speed of 120 rpm at 50 °C. Next, the slurry was thoroughly mixed with 300 mL acetone. Mixtures were then filtered and supernatants that formed were collected. The sediment collected was subjected to 300 mL acetone and underwent filtration thrice. Afterwards, the solid residue was again washed in 300 mL distilled water and filtered three times. For the last stage of washing, the pH was adjusted to 3.5 and 4.5 with 10 M NaOH.

2.3. Enzymatic hydrolysis

Aspergillus niger acts as the enzyme in enzymatic hydrolysis. Varying amount of the individual *A. niger* was added to the acid pretreated filtrate and was incubated at 50ºC. Using Whatman No. 1 filter paper, the media was filtered [4]. Samples for each setup were withdrawn after the specified time. The effects of varying the amount of enzymes (9 g and 12 g) on the reduction of the sugar concentration at a contact time of 72 h using enzyme hydrolysis were determined.

2.4. Simultaneous saccharification and fermentation (SSF)

The slurry used is determined by the highest reducing sugar content result provided by enzymatic hydrolysis. The hydrolysates were fermented with *Saccharomyces cerevisiae* using the *A. niger* to substrate ratio that produces the most reducing sugar. The SSF was situated in a 250 mL erlenmeyer flask containing the liquefied slurry of pomelo peels. The pH of the slurry was varied (3.5 and 4.5) then each was added with the *S. cerevisiae* having a varied dosing amount of substrate (10 mL and 20 mL) and yeast concentration (10% and 15% w/v). The Erlenmeyer flask needs to house an anaerobic environment for efficient fermentation. It was kept in an incubator at 35 °C for 72 h. Figure 1 shows the illustrative diagram of the methodology from the substrate preparation until the simultaneous saccharification and fermentation method.

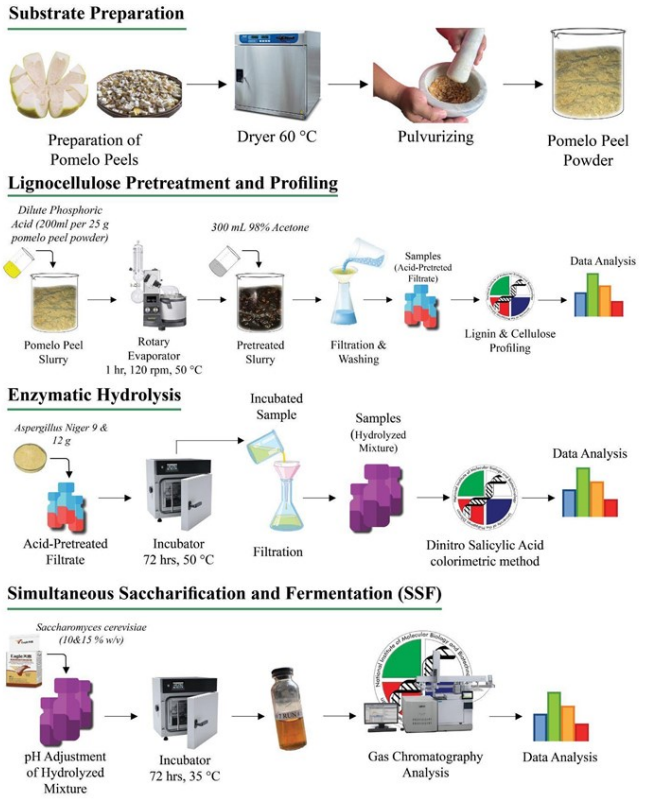


Figure 1. Schematic diagram of bioethanol production from pomelo peels in this study.

2.5. Effects analysis of parameters in bioethanol yield

A two factorial design was used to evaluate the effect of varying parameters to bioethanol yield (Table 1).

Table 1. Two factorial design factors and levels.

Factors	Unit	Low level (-)	High level (+)
pH	-	3.5	4.5
Amount of substrate	mL	10	20
Yeast concentration	%w/v	10	15

3. Results and discussion

Table 2 shows the physicochemical properties of pomelo peels before and after pretreatment at 75% and 80% concentration. These results are in agreement with the study of [5] which noted that enzymatic hydrolysis releases monomeric sugars from cellulose, hemicellulose and structural carbohydrates in biomass.

Table 2. Physicochemical properties of untreated and treated pomelo peels.

Properties	Phosphoric acid concentration (v/v%)		
	Untreated	75%	80%
Alpha cellulose	20.15	24.50	25.29
Hemicellulose	8.82	15.61	14.82
Holocellulose	28.97	40.11	34.82
Acid soluble lignin	6.89	5.08	4.11
Acid insoluble lignin	2.52	9.7	7.85
Moisture	73.04	58.99	53.43
Extractivess	28.98	23.80	27.72

Reducing sugar is a simple sugar containing a hemiacetal functional group. It is a carbohydrate that undergoes oxidation through the use of a weak oxidizing agent in basic aqueous solution [6]. Table 3 shows that with an enzyme loading of 12 g on 75% pretreated substrate yields the highest reducing sugar content of 25.38% based on the alpha cellulose content of the biomass. The reducing sugar conversion was greatly improved upon the addition of enzymes in the enzymatic hydrolysis of pomelo peels. Increasing the concentration of enzymes translates to an increase in availability of enzymes per unit substrate. Enzymatic treatment liberates the monomeric sugars from the cellulose, hemicellulose, structural carbohydrates, and lignocellulosic biomass.

Table 3. Reducing sugar content upon varying enzyme loading and phosphoric acid concentration *(acid hydrolysis was done in duplicate)*.

Phosphoric acid concentration (%v/v)	Enzyme loading (g)	Reducing sugar (mg/ mL)	Conversion rate (%)
75	9	2.15 ± 0.09	13.28 ± 0.09
	12	4.11 ± 0.02	25.38 ± 0.02
80	9	4.03 ± 0.09	24.90 ± 0.09
	12	3.64 ± 0.00	22.49 ± 0.00

Figure 2 shows that the solution having 12 g enzyme loading for 75% phosphoric acid pretreatment has the highest yield of reducing sugar content. The results are similar to the study of [7] which indicate that the optimum amount of enzyme loading is achieved when 12 g of enzyme is added.

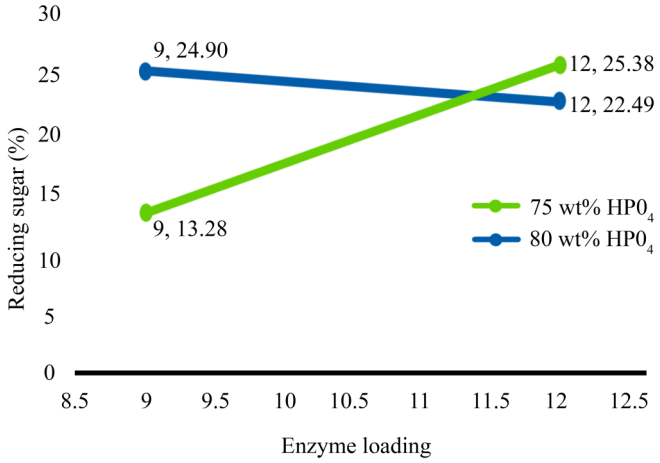


Figure 2. The effect on the reducing sugar content of pomelo peels after varying the enzyme loading.

Table 4 shows the difference in the percent reducing sugar upon varying the enzyme loading amount under the same pretreatment concentration. Paired t-test was used for the statistical treatment to determine and analyze the effect of varying the enzyme loading to the percent reducing sugar. Enzyme loading was varied using 9 g and 12 g. For 75% phosphoric acid pretreatment, p-value of 0.009, the enzyme loading indicates that the decision for the null hypothesis (Ho) should not be accepted since there is a significant difference in the percent reducing sugar upon varying the enzyme loading. Similarly, for the 80% pretreatment condition, there is also a significant difference in the enzyme loading amounts.

Table 4. Difference in the reducing sugar content upon varying enzyme loading with varying phosphoric acid concentration pretreatment.

Phosphoric acid concentration (%v/v)	Enzyme loading (g)	Reducing Sugar (%)	P-value	F-value	Decision on H_o	Verbal interpretation
75	9	13.28 ± 0.09	0.009	73.34	Reject	Significant
	12	25.38 ± 0.02				
80	9	24.90 ± 0.09	0.002	354.35	Reject	Significant
	12	22.49 ± 0.00				

Table 5 shows the percentage of ethanol produced at varying parameters. The yeast concentration of 15% with 10 mL of substrate at pH 3.5 yielded the highest percent ethanol of 11.84 while the yeast concentration of 10% with 20 mL of substrate at pH 4.5 yielded the lowest percent ethanol of 3.79.

Table 5. Percent ethanol yields upon varying different parameters *(yield determination was done in duplicate)*.

pH	Amount of substrate (mL)	Yeast concentration (%)	Ethanol yield (%)
3.5	10	10	3.95
3.5	10	10	4.11
4.5	10	10	5.68
4.5	10	10	5.37
3.5	20	10	4.58
3.5	20	10	4.58
4.5	20	10	5.05
4.5	20	10	3.79
3.5	10	15	11.84
3.5	10	15	6.16
4.5	10	15	6.63
4.5	10	15	8.37
3.5	20	15	7.89
3.5	20	15	6.13
4.5	20	15	6.79
4.5	20	15	6.79

Pomelo peels underwent pretreatment using 75 wt% and 80 wt% phosphoric acid. The enzymatic hydrolysis and SSF processes were employed. The pH, amount of substrate and yeast concentration were the parameters that varied in the bioethanol production. SSF was performed in a constant period of 72 h (3 days). The Minitab software was used to determine the number of runs and the combinations of the three parameters to be utilized in the study.

The varying parameters such as pH, amount of substrate and yeast concentration were compared during the SSF process to determine the best parameters and conditions for producing the highest percent ethanol yield. ANOVA was used and the results were presented in Table 6.

In the statistical analysis of data, three subsets of null hypothesis were tested (1) pH does not significantly affect the percent ethanol yield; (2) amount of substrate does not significantly affect the percent ethanol yield; and (3) yeast concentration does not significantly affect the percent ethanol yield.

Table 6. Difference in the bioethanol yield upon varying parameters during SSF.

Parameter	P-value	F-value	Decision on H_o	Verbal interpretation
pH	0.930	0.01	Accept	Not significant
Amount of substrate	0.446	0.61	Accept	Not significant
Yeast concentration	<0.05	16.97	Reject	Significant

The null hypothesis that the yeast concentration had no effect on bioethanol yield was rejected since the yeast concentration was significant at p-values less than 0.05 which means that there was a substantial effect on the percent ethanol yield. In addition, increasing the yeast concentration increased the percent ethanol yield, according to the table. It is suggested to reject the null hypothesis for the pH and the amount substrate because their p-value surpassed 0.05. This means that neither the pH nor the amount of substrate has any effect on the percent ethanol yield. Between the 3.5 and 4.5 pH, the ethanol yield did increase but not to a significant value. This is also true in increasing the substrate from 10 mL to 20 mL, where there is a decrease in ethanol yield but not significant. However, for yeast concentration variation there is a significant increase in ethanol yield when yeast is increased from 10% to 15%. The optimum values for the pH, amount of substrate and yeast concentration were 3.5, 10 mL, and 15% respectively. The highest ethanol yield obtained from these optimum conditions was 11.84% ethanol.

Figure 3 depicts the effect of varying pH on the percent ethanol yield. The percent ethanol yield increased and decreased in response to increasing the pH solution. When the pH solution was raised from 3.5 to 4.5, the ethanol output increased but not significantly. This can be explained by [8] who found that the pH of the solution is responsible for enzyme stability. At a certain point, enzyme stability is reached, and activity is at its highest. The enzyme is stable at the pH range of 3.5-4.5 that was employed in the study. In the pH range of 4-5, cellulase enzyme is highly stable, but it also has the unique ability to maintain its activity at low pH levels [9]. The pH in the range of 2.75-4.25 affects yeast growth and survival [10]. When the pH is less than 4.0, the incubation duration must be extended, although the ethanol concentration does not decrease much [11].

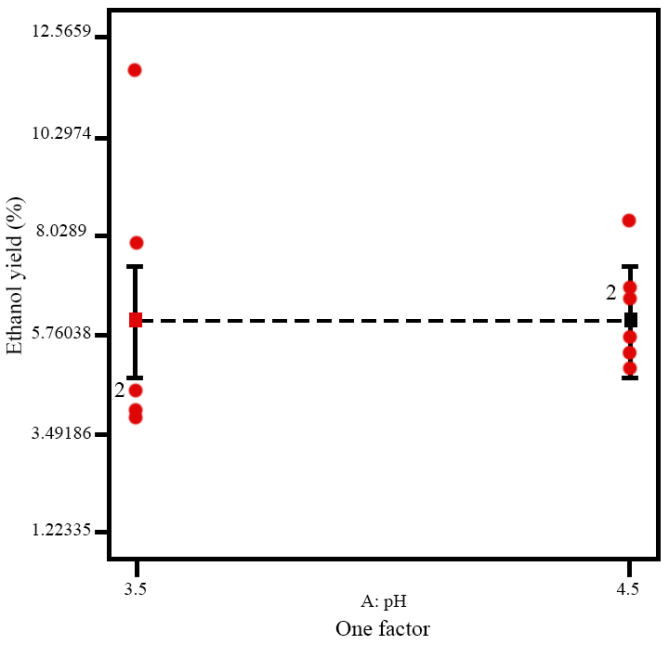


Figure 3. Effect of pH on the percent ethanol yield of pomelo peels by Minitab 18®.

Figure 4 depicts a slight decrease in the percent ethanol yield as the amount of substrate increases. The increase in percent ethanol yield from 10 mL to 20 mL was noticeable but not statistically significant. This is explained by a study conducted by [12] which states that the best substrate for producing the highest ethanol yield is based on the type of substrate extract rather than the amount of it.

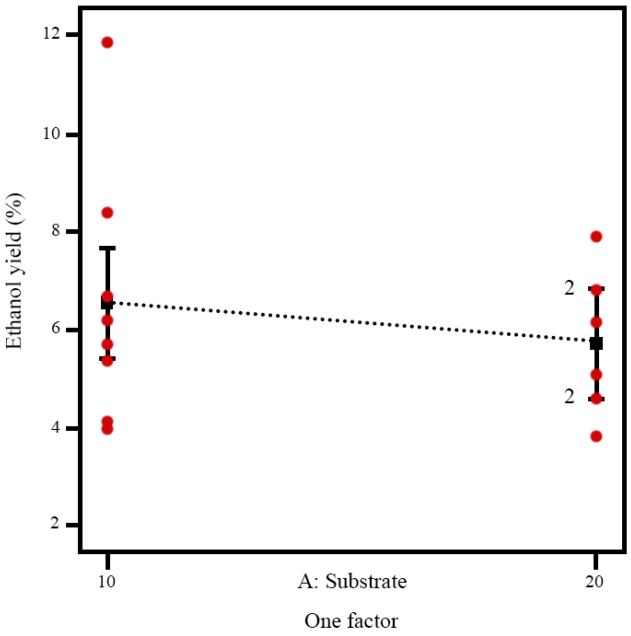


Figure 4. Effect of amount of substrate on the percent ethanol yield of pomelo peels by Minitab 18®.

Figure 5 shows that increasing the yeast concentration resulted in a significant increase in the percent ethanol yield. At a yeast concentration of 15%, the percent ethanol yield was found to be the highest. This is since yeast activity is at its peak when the amount of substrate available is limited.

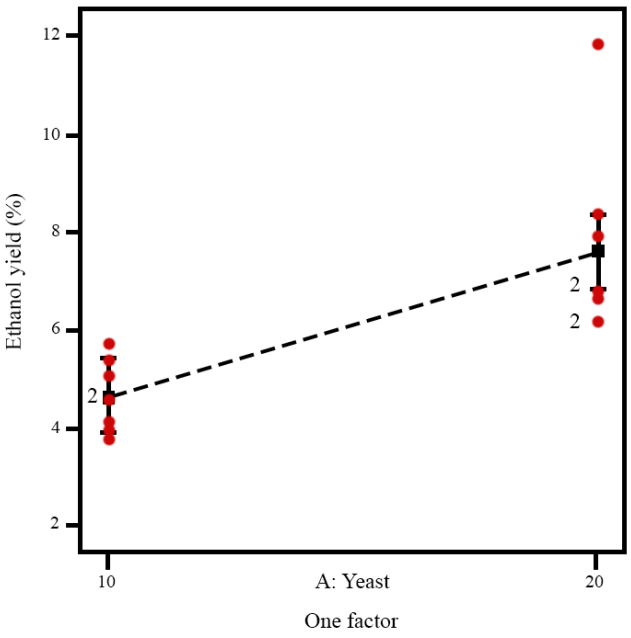


Figure 5. Effect of yeast concentration on the percent ethanol yield of pomelo peels by Minitab 18®

3.1 Effect of the interaction of two variable factors on the percent ethanol yield of pomelo peels

Contour plots can be used to easily understand the interpretation of the interaction of two variable factors. The 3D surface plot and contour plot from the model graphing option of Minitab 18® were used to navigate through the model design space. The effect of pH, substrate amount, and yeast concentration on percent ethanol yield are shown in Figures 6.

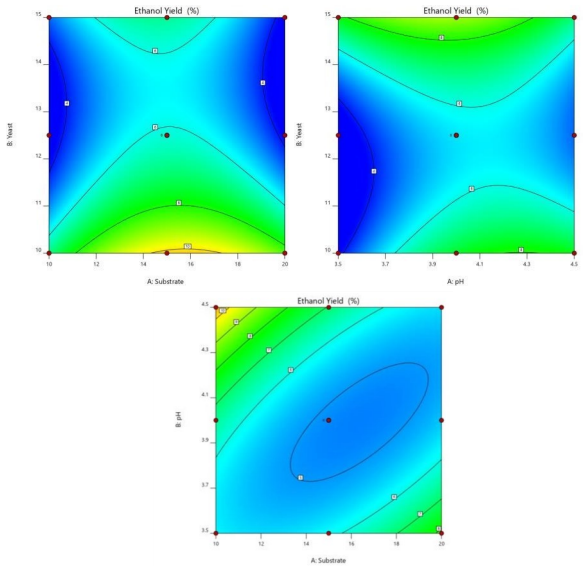
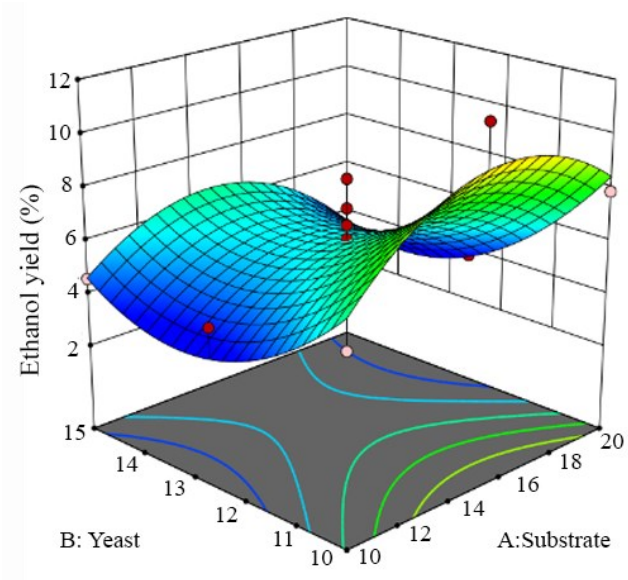
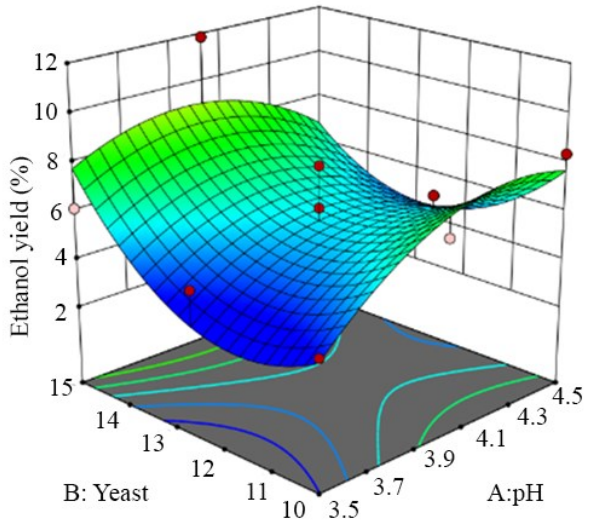


Figure 6. Contour plot of the effect of pH, amount of substrate and yeast concentration to percent ethanol yield.

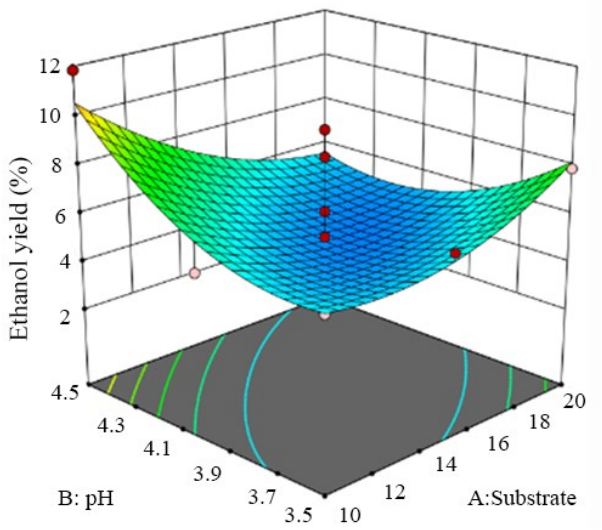
The 3D surface plot from the model graphing option of Minitab 18® was used to navigate through the model design space. The effect of pH, substrate amount, and yeast concentration on percent ethanol yield, as well as the interpretation of the interaction of two variable factors amongst the three factors, are shown in Figures 7a, 7b, and 7c.



a) Effect of the amount of substrate and yeast concentration to percent ethanol yield.



b) Effect of pH and yeast concentration to percent ethanol yield.



c) Effect of the amount of substrate and pH to percent ethanol yield.

Figure 7. The interaction effect of pH, substrate amount, and yeast concentration on percent ethanol yield.

With the adjusted fit statistic, there is a reasonable agreement between Predicted R2 and Adjusted R2 values (Table 7). This was proven by using Box-Cox, which was graphed to select the correct power law transformation of the fit. Power (λ) of 0.71 was found in the graph indicating that it is the best power for the model to make better descriptions of the experimental results as shown in Figure 8.

Table 7. Modified fit model.

Standard deviation	Mean	CV%	R^2	Adjusted R^2	Predicted R^2	Adeq. precision
0.5727	6.11	9.38	0.9635	0.9218	0.7472	18.5625

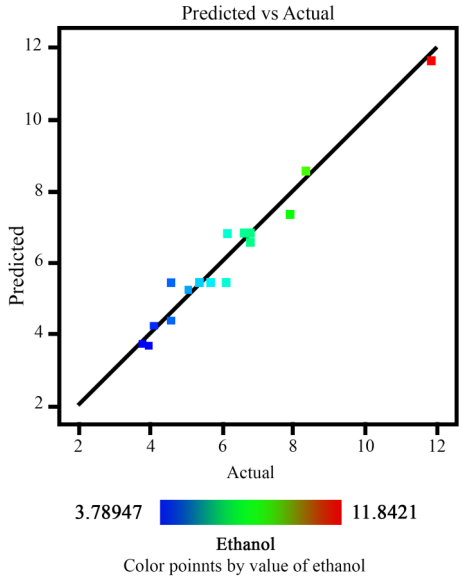


Figure 8. Parity plot of predicted vs actual values of polynomial fit.

Using experimental data obtained, predictions about the percent ethanol yield upon varying parameters such as pH, amount of substrate, and yeast concentration. Table 8 presents the data generated using the multiple response optimization feature of Design Expert 12®. This identifies the combination of input variable, the fermentation parameter, settings that optimize a single response, ethanol yield. Amongst the one hundred response iterations provided by the software, the responses that are predicted to produce high ethanol yield were ranked; at run 7, optimal parameters of pH 4; 20 mL substrate; and 15% yeast concentration.

Table 8. Optimized numerical response of fermentation parameters using Design Expert 12®.

Run	pH	Amount of substrate (mL)	Yeast concentration (%)	Ethanol yield (%)
7	4	20	15	11.66

Table 9 provides the constant that will be used. A simulated equation was utilized to determine the factor's dependent significance in relation to the factor coefficients. When the variance inflation factor (VIF) is equal to 1, the factors are orthogonal.

Table 9. Coefficients used in quadratic fit model based on coded variables.

Factor	Coefficient estimate	df	SE (σ)	95% CI low	95% CI high	VIF
Intercept	5.44	1	0.29	4.76	6.12	
pH (A)	0.28	1	0.20	-0.20	0.76	
Amount of substrate (B)	2.41	1	0.29	1.73	3.09	1
Yeast concentration (C)	1.56	1	0.20	1.08	2.04	2
AB	-0.16	1	0.29	-0.84	0.52	1
A^2	-0.92	1	0.29	-1.60	-0.25	1
B^2	1.25	1	0.29	0.57	1.92	1
C^2	1.01	1	0.29	0.33	1.69	1
A^2B	-1.46	1	0.41	-2.41	-0.50	1

The table also shows that the factors A, B, C, AB, A2, B2, C2, and A2B are all orthogonal, which means they are statistically independent of all other factors in the model and are not overly correlated, which could lead to multi-collinearity.

A coded equation was generated based on the coefficients from Table 6 through the aid of Design Expert 12 ® and presented below:

$$Y = 5.44 + 0.2763A + 2.41B + 1.56C - 0.1579AB - 0.9237A^2 + 1.25B^2 + 1.01C^2 - 1.46A^2B \quad (1)$$

where Y is the percent ethanol yield and A, B and C are coded variables for the pH, amount of substrate and yeast concentration respectively.

It can be inferred that all the variables A, B, and C have a positive or increasing effect on the percent ethanol independently with a coefficient of 0.28, 2.41 and 1.56 respectively. Based on the coefficients, it can be indicated that the amount of substrate has the greatest effect on the percent ethanol yield while pH has the least effect on the response.

Based on the different statistic criteria in mathematical modelling, the actual equation was generated as shown:

$$Y = 242.21447 - 109.15263A - 19.45737B - 3.41842C + 9.28421AB + 13.83158A^2 + 0.049895B^2 + 0.161684C^2 - 1.16842A^2B \quad (2)$$

This equation in terms of actual factors can be used to make predictions about the percent ethanol yield upon varying parameters such as pH (A), amount of substrate (B), and yeast concentration (C). To validate the reliability of the model, it is suggested to conduct a confirmation run which determines the actual percent ethanol and compare with the predicted values computed by the model generated.

The normality test is one of the statistical analyses to be first considered in mathematical modeling. The findings of the experiment are subject to error, which implies that the errors do not vary significantly. If the errors have a normal distribution, it is easy to infer that the errors in the results of the experiment were caused by a single source. The normal probability plot of internally studentized residuals is shown in Figure 9. Based on the graph, data points less than 20 cannot be considered as normal. The normality test will determine whether the internally studentized residuals will have a normal distribution. The plot shows no abnormalities, indicating a successful outcome.

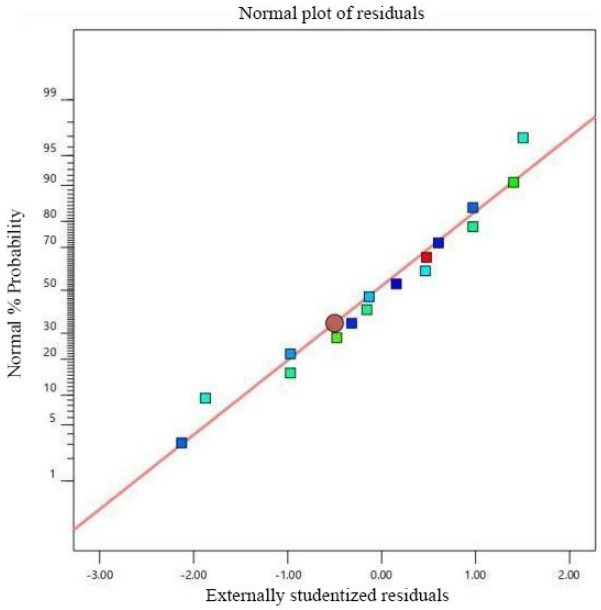


Figure 9. Normal probability plot residuals.

4. Conclusions

After the phosphoric acid-acetone pretreatment alpha cellulose, hemicellulose, and holocellulose were optimized and exhibited significant differences, proving that treatment of the raw materials is effective. Pomelo peels contain high amounts of lignin and cellulose, which is favorable in the bioethanol production process.

Reducing sugar conversion is significantly affected by the amount variation of enzyme loading. As enzyme loading increases, the reducing sugar yield also increases; 75% pretreatment condition is favorable for the ethanol yield. Yeast to pomelo peels ratio have significant effects on the percent ethanol yield. In contrast, pH and the amount of substrate has considerable to no significant effect on the percent ethanol yield. It was observed that under these parameters: pH of 3.5, substrate dose of 10 mL, and 15% yeast concentration; highest ethanol yield, 11.84%, was obtained.

References

[1] Goshadrou A, Karimi K, Taherzadeh MJ. Bioethanol production from sweet sorghum bagasse by Mucor hiemalis. Ind Crop Prod [Internet]. 2011 Jul;34(1):1219-25. Available from: https://doi.org/10.1016/j.indcrop.2011.04.018

[2] Rivas B, Torrado A, Torre P, Converti A, Domínguez JM. Submerged citric acid fermentation on orange peel autohydrolysate. J Agric Food Chem [Internet]. 2008 Apr;56 (7):2380-7. Available from: https://doi.org/10.1021/ jf073388r

[3] Huang R, Cao M, Guo H, Qi W, Su R, He Z. Enhanced ethanol production from pomelo peel waste by integrated hydrothermal treatment, multienzyme formulation, and fed-batch operation. J Agric Food Chem [Internet]. 2014 May 13;62(20):4643-51. Available from: https://doi.org/10.1021/ jf405172a

[4] Kousar H, Navitha K. Bioethanol production using Aspergillus niger and Saccharomyces cerevisiae from coffee pulp by separate hydrolysis and fermentation process. Int J Sci Technol Res. 2020 Jan;9(1):2853-5.

[5] Arcan I, Yemenicioğlu A. Effects of controlled pepsin hydrolysis on antioxidant potential and fractional changes of chickpea proteins. Food Res Int [Internet]. 2010 Jan;43 (1):140-7. Available from: https://doi.org/10.1016/ j.foodres.2009.09.012

[6] Garriga M, Almaraz M, Marchiaro A. Determination of reducing sugars in extracts of Undaria pinnatifida (harvey) algae by UV-visible spectrophotometry (DNS method). Actas Ing [Internet]. 2017;3:173-9. Available from: http:// fundacioniai.org/actas

[7] Ilao C, Mame H, Velasco C. Process parameter optimization of bioethanol production from Cyperus rotundus (nutgrass) [undergraduate thesis]. Batangas City: Batangas State University; 2019.

[8] Worthington CC, Worthington V, Worthington A. Worthington Biochemical [Internet]. Introduction to enzymes; 2019. Available from: https://www.worthington-biochem.com/tools-resources/intro-to-enzymes

[9] Røjel N, Kari J, Sørensen TH, Borch K, Westh P. pH profiles of cellulases depend on the substrate and architecture of the binding region. Biotechnol Bioeng

[Internet]. 2019 Dec 10;117(2):382-91. Available from: https://doi.org/10.1002/bit.27206
[10] Mohd Azhar SH, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Mohd Faik AA, Rodrigues KF. Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep [Internet]. 2017 Jul;10:52-61. Available from: https://doi.org/10.1016/j.bbrep.2017.03.003
[11] Staniszewski M, Kujawski W, Lewandowska M. Ethanol production from whey in bioreactor with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product – Kinetic model predictions. J Food Eng [Internet]. 2007 Oct;82(4):618-25. Available from: https://doi.org/10.1016/j.jfoodeng.2007.03.031
[12] Faustine AS, Rustini AD. Bioethanol production from various agricultural waste substrate using Saccharomyces cerevisiae. IOSR J Pharm Biol Sci (IOSR JPBS). 2021;16 (1):7-13.