Evaluation of Fermentation Parameters of Elephant foot Yam *(Amorphophalluspaeoniifolius) f*or Bioethanol Production

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Rejie C. Magnaye*, Sheenamay S. Alagar, Christian M. Pagsinohin and Ma. Lourdes Yvette A. Magnaye
Chemical and Food Engineering Department, Batangas State University, Alangilan, Batangas City, Philippines
**rmagnaye635@gmail.com*

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Abstract - *Bioethanol successfully finds its role in the development of renewable energy sources to supplement the world's increasing demand in energy supply. In this study, elephant foot yam (Amorphophalluspeoniifolius), a starch-based crop, abundantly grown in tropical countries like Philippines, was used for the evaluation of the effect of substrate concentration and yeast loading (Saccharomyces cerevisiae) in Simultaneous Saccharification and Fermentation (SSF). In SSF, the optimum condition was observed at10% w/v substrate concentration in 20 mL yeast loadingwith an ethanol yield of 12.02 ± 0.21 %. As substrate concentration decreases and yeast loading increases, percent ethanol yield increases. Best mathematical model was generated to describe the relationship of the substrate concentration and yeast loading to ethanol yield. The generated quadratic model,* $Y^{2.47} = 80.63 - 84.46\,A + 126.73\,B - 17.49AB + 65.59A^2 + 91.02B^2$*,can explain 99.96 % (*$R^2$*) of the variability in the yield. The statistical significance of the model was evaluated by F-test for analysis of variance (p<0.05). The results showed that the production of ethanol was more strongly affected by the variation of yeast loading. Using the best substrate concentration and yeast loading, ethanol yield was determined in SSF coupled with Acid Hydrolysis (SSF-AH) having an ethanol yield of 19.1952%. The results revealed that subjecting first the substrate to acid hydrolysis could increase the ethanol yield for it increased the reducing sugar of the substrate.*

Keywords: *Amorphophalluspaeonifolius, bioethanol, Elephant foot yam, fermentation, substrate concentration*

INTRODUCTION

With the increasing world's population and industrial development, the need for energy supply greatly increases, too. However, there is also an inevitable depletion of world's energy supply, which makes everything difficult. In fact, the worldwide energy consumption has increased 17-fold in the last century. However, conventional energy resources, like fossil fuels, cannot meet the increasing energy demand. The quantities of non-renewable (conventional) energy resources are limited and they have a considerable negative environment impact (*e.g.* increased greenhouse gas emissions). This results to an increasing interest worldwide in alternative sources that are eco-friendly at the same time. Therefore, one of the challenges for the society is to meet the growing demand for energy for transportation, heating and industrial processes; also to provide raw materials for the industry in a sustainable way and to reduce greenhouse gas emissions. Our energy systems will need to be renewable and sustainable, efficient and cost-effective, convenient and safe. And one of the alternatives that has a potential to resolve the following issues mentioned is the biofuel [1].

Biofuels are the best alternative known for their ease in production for commercial products. It is a renewable fuel source produced from plants that process and store energy from the sun. In a sustainable cropping system, plant feedstock can be produced year after year. Regions of the world without crude oil deposits could consider 'fuel farming' as a long-term solution to offset their energy needs and foreign oil

dependency [2]. Indeed, the alternatives being looked for are those coming from plants.

Bioethanol has become an immediate viable alternative in rapidly exhausting fossil fuel deposits, and increasing concerns over environmental pollution. In this study, Elephant foot yam (*Amorphophalluspaeoniifolius*) was used in the bioethanol production. It is locally known as *Pungapung*, a starch-based crop, abundantly grown in tropical countries like Philippines. Being an irritant of the mouth and throat once consumed, it is not thoroughly paid attention by the Filipinos, which means the competition between the fuel, and food sector is less compared to other feedstocks such as cassava and corn.

Due to these issues, the researchers would like to evaluate the fermentation parameters of Elephant foot yam (*Amorphophalluspaeoniifolius)* starch for bioethanol production in terms of substrate concentration and yeast loading. Three levels of fermentation parameters were used for the study: substrate concentration (10, 20 and 30 %) and yeast loading (10, 15 and 20 mL). Moreover, mathematical model was generated to describe the relationship of the substrate concentration and yeast loading to ethanol yield. Also, the effect of acid hydrolysis in ethanol yield was assessed. This study would also probably introduce Elephant foot yam (*Amorphophalluspaeoniifolius)* as viable bioethanol feedstock.

OBJECTIVES OF THE STUDY

The objective of the study is to evaluate the effect of substrate concentration and yeast loading (Saccharomyces cerevisiae) in ethanol yield when varied in Simultaneous Saccharification and Fermentation (SSF). Three levels of fermentation parameters were used for the study: substrate concentration (10, 20 and 30 %) and yeast loading (10, 15 and 20 mL). Moreover, mathematical model was generated to describe the relationship of the substrate concentration and yeast loading to ethanol yield. Also, the effect of acid hydrolysis in ethanol yield was assessed.

MATERIALS AND METHODS

Collection and Preparation of Raw Materials

Elephant foot yam *(Amorphophalluspaeoniifolius)* tubers were collected at Sitio Palawon, Tabangao, Batangas City, washed and then brought to Batangas State University. The collected Elephant foot yam *(Amorphophalluspaeoniifolius)* tubers were secured for initial testing. The edible portion was washed with running water to remove impurities and cut into small pieces.

Starch Extraction

Edible portion of Elephant foot yam (100g) was homogenized with 1 M NaCl (900.00 mL) using a blender. The mixture was filtered through triple-layered cheesecloth and the starch was washed with distilled water. The granules were allowed to settle and water was decanted. The settled starch was air-dried overnight. Further removal of moisture present in the starch was carried out by oven drying [3].

Acid Hydrolysis

Fifty grams of Elephant foot yam *(Amorphophalluspaeoniifolius)* starch as substrate, with 100 mL of 0.6 M sulfuric acid (H_2SO_4, analytical grade) was pressurized (15 psi) in an autoclave at 120^{O}C for 30 minutes. Then, it was cooled down to room temperature [4].

Liquefaction

Starch slurry was heated in an autoclave with a working condition as stated in acid hydrolysis. Then, heated starch slurry was allowed to cool at room temperature. Enzyme hydrolysis at pH of 5.5 and temperature of 40^{O}C were performed using liquefying enzyme, *α-amylase* from *Bacillus subtilis.* With an enzyme activity level of 400 U/g of starch, *α-amylase* was added at 40 ^{O}C for two hours at constant agitation rate of 100 rpm. Under the conditions above given by Enzyme Laboratory of UP - Los Baños, Laguna, where enzyme was purchased, the pH of the liquefied starch was maintained at 5.5. One molar (1M) NaOH and 1 M HCl were used to stabilize the pH.

Simultaneous Saccharification and Fermentation (SSF)

Substrate concentration was varied (10, 20 and 30% w/v) and prepared from 5 g, 10 g and 15 g of starch in 50 mL of solution, respectively. Varying yeast loading were 10, 15 and 20 mL. The reaction of SSF under this mode was carried out at 30^{O}C, pH of 4.5 for 48 hours in order to undergo both saccharification and fermentation simultaneously. One molar (1M) NaOH and 1 M HCl were used to stabilize the pH.

Simultaneous Saccharification and Fermentation with Acid Hydrolysis (SSF-AH)

In this process, before liquefaction, starch slurry using the optimized substrate concentration in SSF was first treated with 0.6 M H_2SO_4 under the above conditions specified for acid hydrolysis. The conditions for the enzymatic hydrolysis and fermentation were same as the previous one. During fermentation, the optimized yeast loading and substrate concentration in SSF were used.

Statistical Analyses

All the experiments were performed in triplicates, and the results presented as mean ± standard deviation. T-test was used to compare Simultaneous Saccharification and Fermentation with Acid Hydrolysis (SSF-AH) and without Acid Hydrolysis (SSF). Results were statistically treated at 5 % level of significance. Statistical analysis was performed by *SigmaPlot v12.3©2011 Systat Software Inc*.

Experimental design was performed according to Response Surface Methodology (RSM) using *Design-Expert*® Software (Trial version 8.0.7.1, Stat-Ease Inc., Minneapolis, 2010). Mathematical modeling was used to critically study the effects of substrate concentration and yeast loading to percent ethanol yield and represent the experimental results. A *Box-Cox Plot* was used to diagnose first the correct power law transformation (λ) of the generated model to increase the higher predictability of the model. Equation for percent ethanol yield was generated as a function of substrate concentration and yeast loading and was based in the form:

$$\boldsymbol{Y^{\lambda} = f(A, B)} \qquad \text{Eq. (1)}$$

Where Y- percent ethanol yield

λ -power for transformation

A -substrate concentration

B -yeast loading

Different polynomial fits and different power law transformations were compared in terms of different statistics such as regular, adjusted and predicted R-squared and adequate precision to assess the most appropriate model for the data gathered in the experiment. Predicted and actual values were graphed to see the goodness of fit. The best polynomial fit was graphed to easily understand the effects of the varied parameters to percent ethanol yield.

RESULTS AND DISCUSSION

The ethanol yield in SSF at varying substrate concentration and yeast loading is presented in Table 1. The results showed maximum ethanol yield of Elephant foot yam starch at 10 % w/v of substrate with 20 mL of yeast loading, while the lowest was 30 % w/v of substrate with 10 mL of yeast loading.

It can be deduced from the result that substrate concentration as shown in Figure 1.a is inversely proportional to the percent ethanol yield. This was similar to the obtained trend from the study of *Neves* and *Abara et al.* It was expected from the result that further increase in the substrate concentration would reach a limiting value wherein it would no longer affect the percent ethanol yield that was observed in the study of *Abara, et al.* The results from this study revealed that the percent ethanol yields were dependent upon the substrate concentration until all the enzyme was saturated with the substrates at the active sites at maximum yield. This relationship suggested that at very low substrate concentrations, most of the active sites of the enzyme were unoccupied. Decreasing the substrate concentration allowed more active sites to be occupied, thus, resulting in increased yield. On the other hand, at higher concentrations of substrate molecules, most of the active sites of the enzyme were occupied and the observed yield depended only on the concentration at which the bound substrates were converted to products [6].

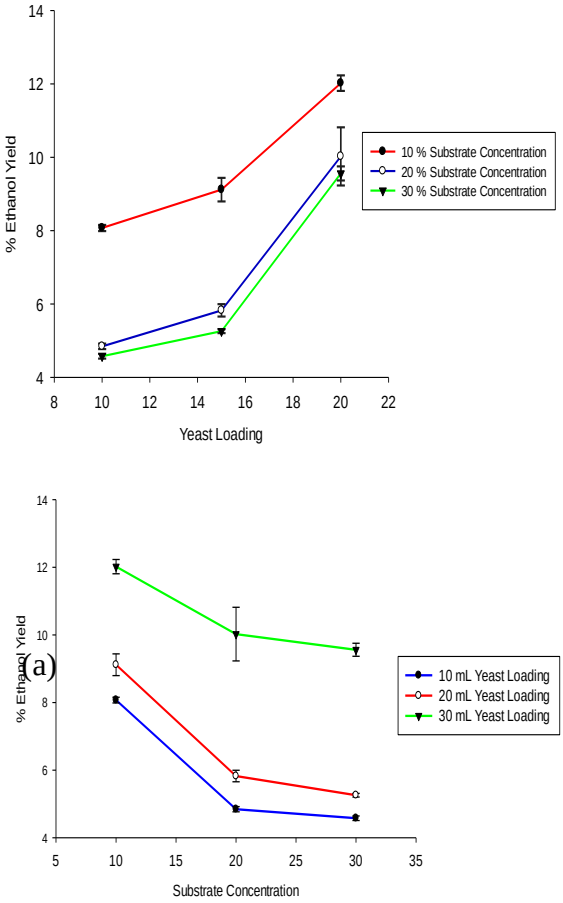
Table 1. Percent Ethanol Yield at Varying Substrate Concentration and Yeast Loading

Substrate Concentration (%w/v) A	Yeast Loading (mL) B	Percent Ethanol Yield (g of ethanol/g starch)
10	10	8.07 ± 0.08
	15	9.12 ± 0.32
	20	12.02 ± 0.21*
20	10	4.84 ± 0.08
	15	5.83 ± 0.17
	20	10.03 ± 0.79
30	10	4.58 ± 0.07
	15	5.26 ± 0.05
	20	9.56 ± 0.19

**maximum percent ethanol yield*

On the contrary, yeast loading behaved differently as shown in Figure 1.b. As yeast loading increased,

the percent ethanol yield also increased in the range selected. Similar trend was noticed in the study of *Abara, et al.* and *Togarepi E., et al. (2012).* It was found out that for the yeast concentration the ethanol production rates increased rapidly with the increase for yeast added, but up to the certain yeast content. Beyond that point, the rates no longer significantly increased. Additionally, at that point, the substrate (fruit pulp) becomes limiting and increasing the yeast amount does not increase the rate of reaction [5].Increasing the amount of yeast increased the ethanol yield but at certain amount of yeast, ethanol yield was in its peak value and more yeasts made yield to decrease[6]. In the present study, the limiting amount of yeast was not known, further increase in the amount was necessary to compare with or to prove some related studies mentioned above.



(a)

Figure 1. (upper) Percent Ethanol Yield vs. Substrate Concentration and (lower) Percent Ethanol Yield vs. Yeast Loading

To analyze the effect of varying the parameters in a deeper manner and to represent the experimental results, mathematical model was generated from the given mean percent ethanol yields as presented in Table 1. Quadratic Fit Model is the suggested model by *Design Expert® 8.0.7.1* (2012).

The mathematical equation (*pseudo* or coded) that would best describe the behavior and trend of the percentage ethanol yield upon varying substrate concentration and yeast loading was :

$$Y^{2.47} = 80.63 - 84.46\,A + 126.73\,B - 17.49AB + 65.59A^2 + 91.02B^2 \qquad \text{Eq. (2)}$$

and the mathematical equation (actual) that could represent the experimental results that can be used in predicting the percent ethanol yield was:

$$Y^{2.47} = 845.96805 - 29.43553\,A - 76.88261B - 0.34976AB + 0.65590A^2 + 3.64079B^2 \qquad \text{Eq. (3)}$$

where Y – Percent Ethanol Yield**,** A – Substrate concentration (%w/v)and B – Yeast loading (mL).

Table 2 presents the Analysis of Variance for Response Surface Methodology of the model with a power transformation of 2.47. Based on ANOVA results, the quadratic model f-value of 1424.25 implies that the model is statistically significant. It means that the independent variables (substrate concentration and yeast loading) of the generated model have significant effect in the dependent variable, for this case, the ethanol yield. The goodness of the model can be checked by different criteria. Fischer's F-test indicates the overall significance of model, in fact, a value greater than one means that the null hypothesis that the model cannot represent the data is false. Moreover, coefficient of determination R^2measures the goodness of fit of regression model.

Table 2. Regression Analysis (ANOVA) for Response Surface Quadratic Model

Source	f-value	p-value	Interpretation
Model	1424.25	<0.0001	Significant
A	1840.94	<0.0001	
B	4144.89	<0.0001	
AB	52.62	0.0054	
A^2	370.10	0.0003	
B^2	712.70	0.0001	`

R^2=0.9996Adj R^2=0.9989Pred R^2=0.9954
Adeq Pre=107.287

The fit of the model where response surface plot are shown in Figure 2 was expressed by the coefficient of determination (R^2) was found to be 0.9996 indicating that 99.96 % of the variabilty in the response can be explained by the model. The difference between adjustedR-squared of 0.9989 and predictedR-squared of 0.9954 is less than 0.2 which indicates that there is a reasonable agreement between these two measures of R-squared. The value of adjusted R-squared is high (0.9989) so as to advocate high significance of the model. The value of coefficient of variation (CV=2.61) was low due to the small residue between the predicted and experimental percent ethanol yields. Adequate precision, a measure of signal to noise ratio (107.287) indicates a better precision and reliability of the experiments carried out. A ratio greater than 4 is desirable. In this case, the ratio of 107.287 indicates an adequate signal to use the model for prediction purposes (Montgomery, 2001). P-values, which are less than 0.05, indicate that model terms are significant. Smaller p-value indicates higher significant effect. In this case, A, B, AB, A^2 and B^2 are significant model terms as shown in Table 2. In addition, Table2shows that the interaction effect of these two variables is the least significant (0.0054); its effect is not so high compared to the other sources of variation. Yeast loading (B) had the greatest effect with f-values of 4144.89 and 712.70 in linear and quadratic, respectively.

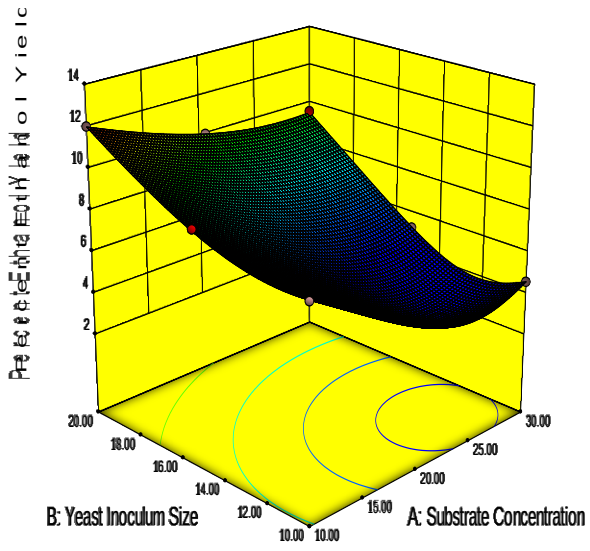


Figure 2. Response Surface Plot of Percent Ethanol Yield

The analysis of variance (ANOVA) of the quadratic regression model with power transformation of 2.47 demonstrated that equation is highly statistically significant predictor of percent ethanol yield, which was evident from the Fisher's F-test with a very low probability value of accepting the null hypothesis that the model cannot be used to explain the experimental results .

Additionally, it was found out that at 10% substrate concentration and 20 mL of yeast loading, the ethanol yield is in its maximum predicted value of 12.0306 %. The mean result from three replications was coincident with the predicted value; the average actual percentage ethanol yield was 12.02267 % and a difference of 0.00793 revealed the high accuracy of the model which can be used to predict the percent ethanol yield given the substrate concentration and yeast loading. However, the minimum percent ethanol yield (3.09909%) was observed at 25.58 % substrate concentration and 11.79 mL yeast loading and this can be seen at Figure, which was the center of the ellipse.

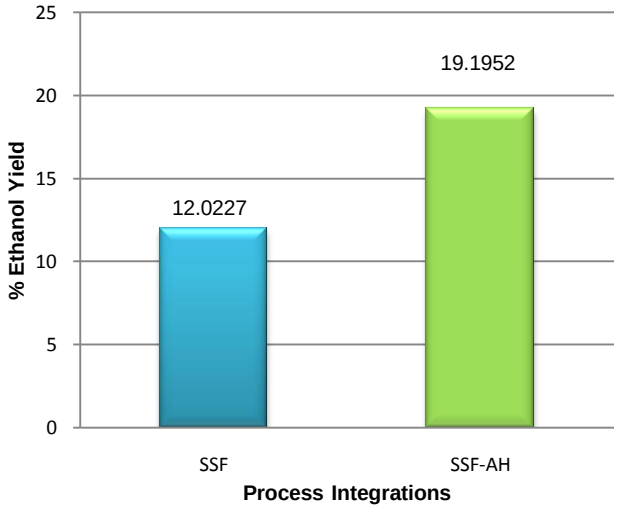


Figure 3.Percent Ethanol Yield during SSF and SSF-AH

T-test result done by *SigmaPlot® v.12.3.0.36 (2011* confirms that there was a significant difference between the percent ethanol yield during SSF and SSF-AH. The absolute value of correlated t-value of -58.7392 was greater than the two-tailed critical t-value of 2.7764 with four degrees of freedom, which supports that the difference of means was significant. An obtained p-value of 0.000000503 was less than the level of significance of 0.05, which can be interpreted as highly significant.

Figure 3 shows that percent ethanol yield is higher in SSF-AH than SSF. This was because acid

hydrolysis served as pre-treatment method prior to liquefaction. Indeed, it was a combination of dilute acid hydrolysis and heat pretreatment. It was observed during the experimentation that after the acid hydrolysis of starch, the starch solution was slightly in its liquid form, with lesser suspended solids. It was far different from the non-acid-hydrolyzed solution, where larger particles were very visible. Accordingly, from these observations, it can be deduced that after acid hydrolysis, the complex molecules of starch were broken down to simpler molecules or it can be inferred that acid hydrolyis removed the other impurities in the solution that cannot be easily broken down by the enzymes.

In general, acid hydrolysis is a useful pre-treatment method in solubilizing the residual components of starch biomass such as lignin, cellulose and other extractives to make the starch component susceptible to further treatments. It can be deduced that the cellulose content or other materials in the starch suspension were converted to fermentable sugar through acid hydrolysis. Likewise, it made the job of *alpha-amylase* easier since *alpha-amylase* can simply convert the starch into dextrins by breaking easily the α(1-4) linkages of starch. This also helped the *glucoamylase* to cut easily the glucose unit from dextrin molecule since impurities were lessened by acid hydrolyis.

CONCLUSION AND RECOMMENDATION

This study generalizes that the ethanol yield varies directly with yeast loading and varies inversely with substrate concentration. Also, the study has established the maximum ethanol yield from Elephant foot yam starch at 10 % w/v of substrate with 20 mL of yeast loading, while the lowest was 30 % w/v of substrate with 10 mL of yeast loading. The optimum process integration can be achieved by subjecting the substrate to acid hydrolysis first before SSF. Quadratic model is the best model that fits the gathered data from ethanol yield as a function of the substrate concentration and yeast loading. The generated quadratic model, $Y^{2.47} = 80.63 - 84.46\,A + 126.73\,B - 17.49AB + 65.59A^2 + 91.02B^2$, can explain 99.96 % ($R^2$) of the variability in the yield. It is suggested to further decrease the substrate concentration and increase the yeast loading to establish more evidences on the drawn inferences from the result obtained in the present study. Also, varying the substrate concentration and yeast loading in Simultaneous Saccharification and Fermentation coupled with Acid Hydrolysis (SSF-AH) and finding the best condition are also endorsed.

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