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# Optimization and Analysis of Bioethanol Production from Cassava Starch Hydrolysis

**Liew, E. W. T.**

*Department of Chemical Engineering, School of Engineering and Science, Curtin University, CDT 250 98009 Miri, Sarawak, Malaysia*

## ABSTRACT

Current ethanol production processes utilizing crops such as sugar cane and corn starch have been well established over the decade. Other crop such as cassava is a potential candidate in producing ethanol. However, thermal processes are required to hydrolyze starch for the production of fermentable sugars. The processes are energy intensive and could lead to undesirable by-products generation. In this work, the hydrolysis of cassava starch is studied following an experimental design as a statistical problem solving approach. Central composite design (CCD) is used in order to select the most important variables from the simultaneous study on the effect and influence of operating conditions of bioreactor utilized, namely, pH, temperature and substrate concentration, as well as to optimize the process of cassava starch hydrolysis. From the results obtained, it can be concluded that the cassava starch hydrolysis is enhanced by pH and temperature. Model validations show good agreement between experimental results and the predicted responses.

*Keywords*: Cassava, central composite design, hydrolysis, optimization, pH, temperature

## INTRODUCTION

During the last few decades, there has been an increasing demand for alternative sources of fuels due to the excessive consumption of fossil fuels globally. These alternative sources such as ethanol may reside in the production of renewable energies. Currently, ethanol is being produced commercially by using starch crops such as cassava (Cardona & SánchezÓ, 2007). Cassava is a potential candidate to produce ethanol in large scale since it can be easily cultivated and has high carbohydrate content. On the other hand, cassava is able to yield 3-15 tons/hectare in an agricultural environment and even 20-40 tons/hectare in an extensive cultivation area (Daubresse &

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*Email addresses:*
Liew, E. W. T. (emily.liew@curtin.edu.my)
*Corresponding Author

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Ntibashirwa, 1987). Due to its high drought tolerance and low demand for nutrients, it can produce acceptable amount of yield even under marginal environmental conditions (Cock, 1982; Stupak *et al*., 2006). It is suggested to utilize *Saccharomyces cerevisiae* as the cultivation microbe since this type of yeast is most commonly used for cell growth in fermentation. This is due to the fact that this type of yeast has an active glucose transport system, whereby it metabolizes glucose through the glycolytic pathway, a metabolic pathway to convert glucose to pyruvate and energy and subsequently to ethanol (Nath & Das, 2004).

Generally, very high ethanol performances in fermentation are affected by process conditions such as pH, temperature and substrate concentration (Aldiguier *et al*., 2004). These process conditions are crucial for optimizing the fermentation process so that high production could be attained with optimum settings of these process conditions. There are very few studies reported on the impact of temperature on the dynamic behaviour of *Saccharomyces cerevisiae* during fermentation processes (Torija, 2003). It is important to note that pH has a great impact on the microbial cell activities and can modify the chemical pathways of the biological reaction as well as the kinetics (Akin, 2008). The significance of these three combined process and operating conditions have yet to be studied in fermentation processes. Based on literature studies, most studies so far focused on combined conditions of pH and temperature.

Due to the diversity and importance of process conditions of an alcoholic fermentation process, it is vital to ensure that each condition are well operated in order to ensure good bioreactor operation and production rate of ethanol under optimum process conditions. Therefore, the objective of this study is to investigate the optimum conditions of the three conditions in achieving high ethanol productivity. It is of interest to develop a low energy requiring process to convert cassava starch to fermentable sugars in order to reduce the cost of bioethanol production.

## MATERIALS AND METHODS

### *Materials and Instruments*

The bioreactor used is in this study is the BIOSTAT A Plus 2L, MO-Assembly. Industrial Baker's yeast, i.e. *Saccharomyces cerevisiae*, is utilized as the inoculum culture. 1.5L of fermentation medium is prepared by adding 0.75L of solution medium and 0.75L of hydrolyzed cassava into the bioreactor tank.

### *Solution Medium*

The solution medium is prepared by adding the following components: 1.5g yeast extract, 3.75g $NH_4Cl$, 4.37g $Na_2HPO_4$, 4.5g $KH_2PO_4$, 0.38g $MgSO_4$, 0.12g $CaCl_2$, 6.45g citric acid and 4.5g sodium citrate.

### *Starch Hydrolysis*

150g of fresh cassava starch in powder form is added into a 0.75L of 0.1M sulphuric acid solution. Both are mixed evenly in a 1L beaker and sterilized at 121°C for 45 minutes to break

down the cassava starch into fermentable sugars. The hydrolyzed cassava starch is then cooled to room temperature.

*Fermentation Medium*

Both the solution medium (0.75L) and hydrolyzed starch (0.75L) is mixed evenly and sterilized again at 121°C for 45 minutes to avoid contamination of the fermentation medium. The fermentation medium cooled to room temperature after sterilization before fermentation starts.

*Sampling and Analysis*

Sampling is taken every 2 hours during the first 24 hours of the fermentation process. After 24 hours, sampling was taken in every 3 hours since it is observed that cell growth starts to decrease and plateau. Samples were analyzed straight away for the concentrations of glucose and ethanol in order to prevent contamination of the samples. Enzymatic test kits (R-Biopharm) and UV-VIS spectrophotometer were utilized to analyze the concentrations of glucose and ethanol.

*Response Surface Methodology (RSM) Optimization*

RSM is a statistical technique which is useful for modelling and analyzing problems in which a response of interest is influenced by several variables and to optimize the response (Aldiguier *et al*., 2004; Torija, 2003).

In this study, three independent variables are studied at three levels (-1, 0, +1) with eight ($2^3$) factorial points and three replicate central points. A central composite design (CCD) is employed to determine the effects of independent variables on the response, namely, glucose and ethanol concentrations, as well as factor interactions. Table 1 shows the input variables and levels employed.

TABLE 1
Input Variables and Their Levels Employed

| Factor | Variable | Units | Low Level (-) | Middle Level (0) | High Level (+) |
|---|---|---|---|---|---|
| X1 | pH | | 2 | 6 | 10 |
| X2 | Temperature | °C | 25 | 32.5 | 40 |
| X3 | Substrate Concentration | g/L | 30 | 40 | 50 |

CCD is one of the most commonly used response surface designs for fitting second-order models in fermentation studies (Akin, 2008). This design provides a solid foundation for the generation of a response surface map. The response pattern and synergy in the optimum region are investigated and to identify the optimum conditions for glucose and ethanol concentrations. The results of each CCD are analyzed using Design Expert® software version 8, from Statease, Inc., Minneapolis, USA. Their interactions and significance were evaluated by variance analysis (ANOVA) test. Three-dimensional surface plots are drawn to illustrate the effects of the

independent variables on the dependent variables, being described by a polynomial equation, fitted on experimental data. $R^2$ coefficient is used to evaluate the fit of the models.

## RESULTS AND DISCUSSION

### *Optimization by RSM*

The coded values of experimental variables in CCD and response values are shown in Table 2. Lack-of-fit tests were carried out for deriving the best correlation between independent variables and responses. It is indicated that the LFT is not significant which supports the fitness of the model. On the other hand, probability value ($p$-value < 0.05) indicated that the model is significant, as shown in Table 3.

TABLE 2
Central Composite Design (CCD) for Optimization and Values of Observed Responses

| Run | Block | X1 | X2 | X3 | Glucose Conc. (g/L) | Ethanol Conc. (g/L) |
|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 30 | 25 | 0.135 | 1.10 |
| 2 | 1 | 10 | 50 | 40 | 1.70 | 3.00 |
| 3 | 1 | 2 | 50 | 25 | 0.13 | 1.50 |
| 4 | 1 | 10 | 50 | 25 | 1.51 | 3.50 |
| 5 | 1 | 2 | 50 | 40 | 0.50 | 0.95 |
| 6 | 1 | 10 | 30 | 25 | 4.84 | 0.75 |
| 7 | 1 | 10 | 50 | 25 | 2.38 | 0.36 |
| 8 | 1 | 2 | 30 | 25 | 0.15 | 0.96 |
| 9 | 1 | 10 | 50 | 40 | 1.50 | 3.70 |
| 10 | 1 | 10 | 30 | 25 | 4.92 | 0.73 |
| 11 | 1 | 6 | 40 | 32.5 | 0.03 | 21.38 |
| 12 | 1 | 2 | 30 | 40 | 0.17 | 0.85 |
| 13 | 1 | 6 | 40 | 32.5 | 0.03 | 21.36 |
| 14 | 1 | 6 | 40 | 32.5 | 0.04 | 21.02 |
| 15 | 1 | 10 | 30 | 40 | 1.90 | 2.45 |
| 16 | 1 | 2 | 30 | 40 | 0.17 | 0.86 |
| 17 | 1 | 2 | 50 | 40 | 0.15 | 0.88 |
| 18 | 1 | 10 | 30 | 40 | 1.95 | 2.50 |
| 19 | 1 | 2 | 50 | 25 | 0.14 | 1.55 |
| 20 | 2 | 2 | 40 | 32.5 | 0.14 | 0.98 |
| 21 | 2 | 6 | 40 | 17.5 | 0.52 | 18.63 |
| 22 | 2 | 6 | 40 | 32.5 | 0.03 | 21.36 |
| 23 | 2 | 14 | 40 | 32.5 | 1.56 | 2.50 |
| 24 | 2 | 6 | 60 | 32.5 | 0.07 | 10.50 |
| 25 | 2 | 6 | 40 | 47.5 | 0.08 | 15.33 |
| 26 | 2 | 6 | 20 | 32.5 | 0.07 | 11.50 |
| 27 | 2 | 6 | 40 | 32.5 | 0.04 | 19.64 |
| 28 | 2 | 6 | 40 | 32.5 | 0.04 | 18.97 |

TABLE 3
Lack-of-Fit Test and Model Summary Statistics

| Source | SS[a] | df[b] | MS[c] | *p*-value[d] | |
|---|---|---|---|---|---|
| Glucose concentration | | | | | |
| Model | 34.42 | 6 | 5.74 | 0.0019 | Significant |
| Lack-of-Fit | 13.97 | 8 | 1.75 | 0.0503 | not significant |
| Ethanol concentration | | | | | |
| Model | 1767.61 | 6 | 294.60 | 0.0111 | significant |
| Lack of Fit | 967.52 | 8 | 120.94 | 0.0771 | not significant |

[a]SS, sum of squares; [b] df, degree of freedom; [c] MS, mean squares; [d] Statistically significant at 95% of confidence level.

The ANOVA results on the models are shown in Table 4. The *p*-values are less than 0.05, indicating that models and their terms are significant. In all cases, the insignificant model terms (*p*-value > 0.05) have been omitted to give a better fit. The fitness of the model is subsequently examined by the coefficient of determination $R^2$. The $R^2$ value for glucose concentration is 92.46% and for ethanol concentration, the $R^2$ value is 95.30%. Meanwhile, the adjusted $R^2$ value of glucose and ethanol concentrations are 90.56% and 93.40%, respectively. $R^2$ and adjusted $R^2$ values of regression model higher than 90% are considered to be high correlated (Bao, 2011). The models are denoted by Equations [1] and [2], as follows:

$$\text{Glucose concentration} = f(X_1.X_2.X_3) = -0.41 + 0.84X_1 + 0.09X_2 - 0.07X_3 - 0.02X_1X_2 \quad [1]$$

$$\text{Ethanol concentration} = f(X_1.X_2.X_3) = 19.37 - 5.72X_1 - 0.39X_2 - 0.11X_3 + 0.2X_1X_2 \quad [2]$$

TABLE 4
Analysis of Variance (ANOVA) Results

| Source | df[a] | Glucose conc. | | Ethanol conc. | |
|---|---|---|---|---|---|
| | | SS[b] | *p*-value[c] | SS[b] | *p*-value[c] |
| $X_1$ | 1 | 11.15 | 0.0042 | 172.50 | 0.0015 |
| $X_2$ | 1 | 1.25 | 0.0096 | 855.58 | 0.0055 |
| $X_3$ | 1 | 12.18 | 0.0030 | 28.00 | 0.0034 |
| $X_1X_2$ | 1 | 5.97 | 0.0282 | 565.12 | 0.0139 |
| $X_1X_3$ | 1 | 2.78 | 0.1221 | 60.56 | 0.3882 |
| $X_2X_3$ | 1 | 1.09 | 0.3234 | 85.86 | 0.3061 |

[a]df, degree of freedom; [b] SS, sum of squares; [c] Statistically significant at 95% of confidence level.

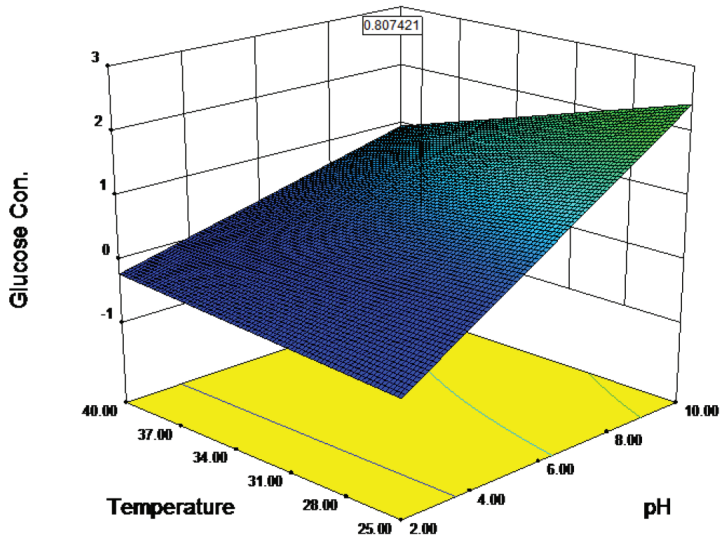


Fig. 1: Response Surface Plot for The Effect of pH and Temperature on Glucose Concentration. Substrate Concentration is Constant at Zero Level.

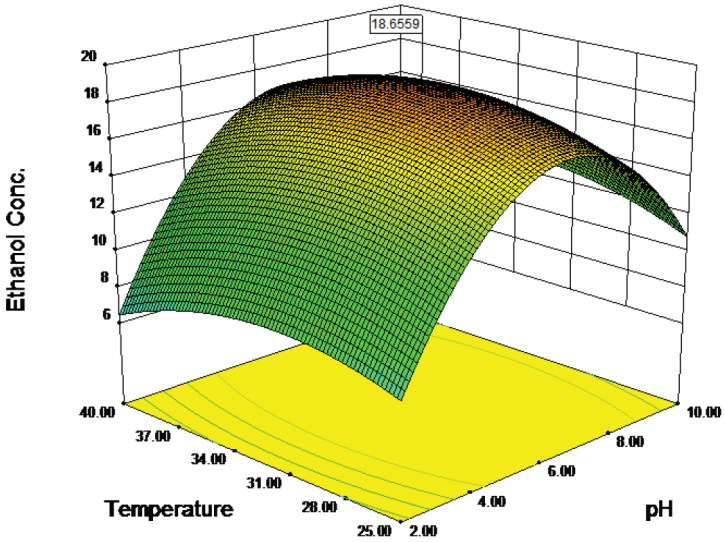


Fig. 2: Response Surface Plot for The Effect of pH and Temperature on Ethanol Concentration. Substrate Concentration is Constant at Zero Level.

It is observed that the linear terms for both pH and temperature have significant effects on glucose and ethanol concentrations. This shows that both pH and temperature highly affect the amount of glucose and ethanol concentrations produced during the fermentation process. At the same time, the interaction terms, i.e. $X_1X_2$ between pH and temperature show significant effect than the rest of the interaction terms since *p*-value is less than 0.05, as shown in

Table 4. Therefore, it is of interest to investigate the relationship between pH and temperature by visualizing the response surface plot. The polynomial equations to experimental data, i.e., Equations [1] and [2] can be described by the response surface plots as a function of pH and temperature, maintaining substrate concentration fixed at level zero, as shown in Fig.1 and Fig.2.

Based on the response surface plot, maximum ethanol concentration is obtained under high pH and high temperature conditions. The optimum values suggested to achieve high ethanol concentration is under the operation of pH 6.5, substrate concentration of 40.25g/L and temperature of 32.4°C.Under these conditions, it is predicted to achieve a maximum of 18.65g/L of ethanol and minimum of 0.81g/L of glucose.

Confirmation runs were conducted to validate the predicted ethanol concentration with respect to the optimum values suggested. The experimental response was 19.58g/L of ethanol and 0.85g/L of glucose. These values are in good agreement with the predicted values, considering a range of 95% confidence level. This shows the adaptation of the model to experimental data, confirming the validity of the models.

## CONCLUSION

Several studies have evaluated the influence of pH and temperature in the production of ethanol. It is recommended in several studies that yeast generally grows well in pH range of 4 to 4.5, whereas for temperature, it is suggested to be operated in the range of 20 to 30°C (Stanbury *et al*., 2006). It is important to ensure that both pH and temperature are well conditioned so as to allow good yeast growth and achieve desirable amount of ethanol. In addition, different amounts of substrate do make a difference in achieving desirable amount of ethanol. Low substrate level will not be able to achieve maximum amount of ethanol. Substrate level which is too high will result in substrate inhibition. Therefore, it is vital to ensure that the substrate concentration used is optimum as well in order to ensure good production of ethanol.

From the results, it can be concluded that pH and temperature highly affect the amount of ethanol compared to substrate concentration. Both pH and temperature play important roles in ensuring optimum yeast growth since it is statistically proven that both have significant interactions within each other. The model shows good predictions between experimental results and predicted responses.

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