Factorial Design Analysis of a Tapioca Slurry Saccharification Process Using Encapsulated Enzymes

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A three-factor two-level (2^3) full factorial design analysis was conducted to identify the significant factors that influence glucose production from tapioca slurry with an encapsulated enzymatic saccharification process using a stirred bioreactor. The factors investigated were pH (5 to 7), temperature (40 to 60 °C), and agitation speed (80 to 160 rpm). From the statistical analysis, a mathematical model for tapioca slurry saccharification was derived, and the variance analysis resulted in a high determination coefficient (R^2=0.9993). The main effects and their interactions were also investigated. The results showed that all the main factors and the two-way interaction factors were statistically significant. The most significant factor in the tapioca slurry saccharification was found to be pH, while the interaction between pH and agitation speed was the most influential two-way interaction.

Keywords: Saccharification; Tapioca slurry; Encapsulated enzymes; Stirred bioreactor; Factorial design

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INTRODUCTION

Cassava roots are one of the major commercial sources of carbohydrates in tropical countries such as Malaysia, Thailand, and Indonesia. In addition to food, cassava roots can also be used as feedstock for renewable energy (Wang *et al.* 2012). To produce renewable energies such as bioethanol, cassava roots must be first converted into an intermediate sugar product using an enzymatic saccharification process. Owing to high starch and fibre contents, three types of enzymes are commonly used in the saccharification process of cassava roots namely alpha-amylase, glucoamylase, and cellulase. Alpha-amylase enzyme is able to hydrolyze alpha-1,4 glycosidic bonds in the starch to produce maltodextrin, while glucoamylase enzyme is able to cleave alpha-1,4 and alpha-1, 6 glycosidic bonds in starch to produce glucose. On the other hand, cellulase enzymes are together able to hydrolyze fibre by attacking beta-1,4 linkages to produce glucose. Because of the expensive price of enzymes, the saccharification process must be productive and preferably have an inexpensive operating cost. Enzyme encapsulation technology represents an inexpensive and effective method, permitting enzyme recovery and reuse. It also allows easier separation of enzymes from the reaction media (Abd Rahim *et al.* 2013a).

From our research investigation, a factorial experimental design is a widely applied statistical method and an alternative to the one factor at a time (OFAT) method. The purpose is to reduce the number of experiments, time, and cost (Rathinam *et al.* 2011). Factorial design involves changing all variables from one experiment to the next.

The design also determines which factors significantly affect the response as well as giving an understanding of how the effect of one factor varies with the levels of the other factors (Abdel-Ghani *et al.* 2009). A very recent study by Belle *et al*. (2014) demonstrated that factorial design can be a reliable method for determining the impacts of various factors on the wet web strength of manufactured paper. Therefore, it can be concluded that factorial experimental design has been demonstrated as a reliable screening method for the optimization study that allow measuring the main and interaction effects of factors.

In the present study, the saccharification of tapioca slurry into glucose was carried out in a stirred bioreactor using encapsulated enzymes. A 2^3 full factorial design was used to screen significant factors that influence the saccharification process, such as pH, temperature, and agitation speed.

EXPERIMENTAL

Materials

Alpha-amylase from *Bacillus subtilis*, glucoamylase from *Rhizopus niveus* Lyophilized, and cellulase from *Aspergillus niger* were purchased from MP Biomedicals, United States. Encapsulated enzymes within calcium alginate-clay beads were prepared following a previous method (Abd Rahim *et al.* 2013b).

For the tapioca slurry preparation, cassava roots were washed, hand peeled, cut to small pieces, and dried in an oven at 65 °C for 24 h before being ground to a powder. Then, 1% (w/v) of gelatinized tapioca slurry was prepared by boiling tapioca powder in a citrate phosphate buffer solution in a water bath. Due to the difficulty of the saccharification process using an encapsulated enzyme system at higher gelatinized tapioca slurry concentration (*i.e.* 5 and 10%), a lower concentration was selected for this study.

Methods

Bioreactor system

A stirred bioreactor with a capacity of 2.1 L made from borosilicate glass was used in this study (Fig. 1). The temperature was controlled using a water jacket system. The agitation system was operated by a propeller connected to a variable speed electrical motor. In the bioreactor, 27 g of alginate-clay beads was added to 1000 mL of 1% (w/v) tapioca slurry for the saccharification.

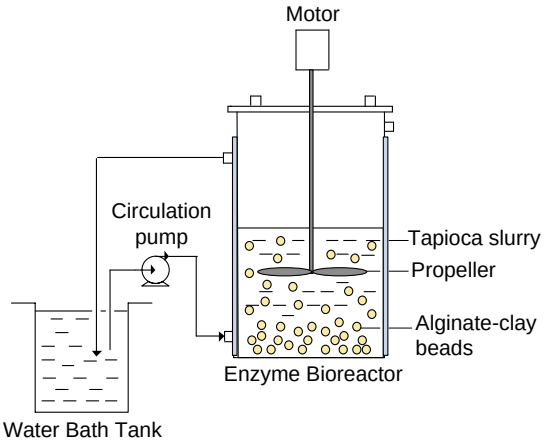

Fig. 1. Schematic diagram of stirred bioreactor

Determination of enzyme activity

The activity of enzymes was measured with a spectrophotometer at 540 nm with 3,5-dinitrosalicylic acid (DNS) as an indicator (Bernfeld 1955). Theoretically, one unit of alpha-amylase activity is defined as the quantity of enzyme that releases 1 mg of reducing sugar per min at pH 6.6 and 30 °C. One unit of glucoamylase is defined as the amount of enzyme releasing 10 mg of reducing sugar glucose per min at pH 4.5 and 40 °C. One unit of cellulase activity is defined as the amount of enzyme that produces 1 μmol of reducing sugar (glucose) at pH 5 and 37 °C in 1 min.

Design of Experiments

A 2^3 full factorial experiment was designed to observe the effect of factors that influence the concentration of glucose (response) from a tapioca slurry saccharification process in a bioreactor with encapsulated enzymes. Three factors, namely pH, temperature, and agitation speed, were chosen in the experimental design. The high and low levels of each factor are listed in Table 1.

Table 1. High and Low Levels of Factors

Factor	Low level (-1)	High level (+1)
pH	5	7
Temperature (°C)	40	60
Agitation speed (rpm)	80	160

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA)

The design matrix for coded values on factors and response in terms of glucose yield is shown in Table 2. Eight experiments with all possible combinations of factors were conducted in duplicate and the results were analyzed using Minitab 16 software. In this study, the main effects and interaction between factors were investigated.

Table 2. Factorial Arrangement and Responses for Saccharification Process

Run order	pH	Temperature (°C)	Agitation speed (rpm)	Glucose yield (mg/mL)
1	-1	-1	-1	4.88
2	-1	-1	+1	1.88
3	-1	+1	-1	2.43
4	-1	+1	+1	1.50
5	+1	-1	-1	4.70
6	+1	-1	+1	6.90
7	+1	+1	-1	3.66
8	+1	+1	+1	7.64

The effects, regression coefficients, and t and P values are presented in Table 3. In general, the larger magnitude of t and the smaller value of P indicate that the corresponding coefficient term is more significant (Bingol *et al.* 2010). It was observed that all effects were statistically significant with a 95% confidence level ($P < 0.05$), except for the interaction between pH, temperature, and speed ($P = 0.098$). Thus, the three-way interaction was neglected and the regression model equation for the 2^3 factorial design was expressed as:

$$Y = 4.1992 + 1.5273A – 0.3915B + 0.2817C + 0.3178AB + 1.2630AC + 0.4797BC$$

where Y is the glucose yield (mg/mL), A is pH, B is temperature (°C), and C is agitation speed (rpm).

Table 3. Estimated Effects and Coefficients for Glucose Production

Term	Effect	Coefficients	Standard error	t	P
Constant		4.1992	0.01925	218.19	0.000
pH	3.0546	1.5273	0.01925	79.36	0.000
Temperature	-0.7831	-0.3915	0.01925	-20.34	0.000
Speed	0.5634	0.2817	0.01925	14.64	0.000
pH x temperature	0.6357	0.3178	0.01925	-16.51	0.000
pH x speed	2.5260	1.2630	0.01925	65.62	0.000
Temperature x speed	0.9594	0.4797	0.01925	24.92	0.000
pH x temperature x speed	-0.0721	-0.0361	0.01925	-1.87	0.098

R-Sq = 99.93%, R-Sq (adj) = 99.88%

Fitting the model was necessary to ensure that it provided an adequate approximation to real systems. As can also be seen in Table 3, the model presents 99.93% of square correlation coefficient (R^2), which is very precise for a statistical model. In addition, the small difference between R^2 and the adjusted R^2 values indicates there is a small chance for insignificant terms to be included in the model.

Main Effects

The main effects represent deviations of the average between the high and low levels for each factor. When the effect of a factor is positive, the glucose concentration increases as the factor changes from low to high level. In contrast, if the effect is negative, a decrease in glucose concentration occurs for the high level of the same factor (Chowdhury *et al.* 2011). As shown in Fig. 2, the effect of pH was positive, indicating that the glucose production increased when the factor changed from a low to high level. This is because alginate-clay beads as a supporting material have both cationic and

anionic charges that affect the nature of the active enzyme (Abd Rahim *et al.* 2013a). Therefore, when the pH is reduced to more acidic conditions, it reduces the activity of the enzyme as well.

The second most important main effect regarding the saccharification process was temperature. The negative sign of temperature means that the glucose concentration was favored at a low temperature level. This is probably due to enzymes that are highly sensitive to changes in temperature. When the temperature increases, the enzyme molecule vibrates, which can affect the structure of the enzyme as well. Eventually, it will reduce the catalytic power of the enzymes and the enzymes will be denatured (Abd Rahim *et al.* 2013a).

The agitation speed also had a considerable effect on the saccharification process. The plot revealed that the speed had a similar effect to the pH where the glucose concentration was higher at a high rpm. This behavior can be attributed to the surface contact between the tapioca slurry and enzymes. According to Yadav and Trivedi (2003), when the speed is high, the tapioca slurry more easily diffuses from the bulk liquid to the external surface of the particles and from there into the interior pores of the enzymes.

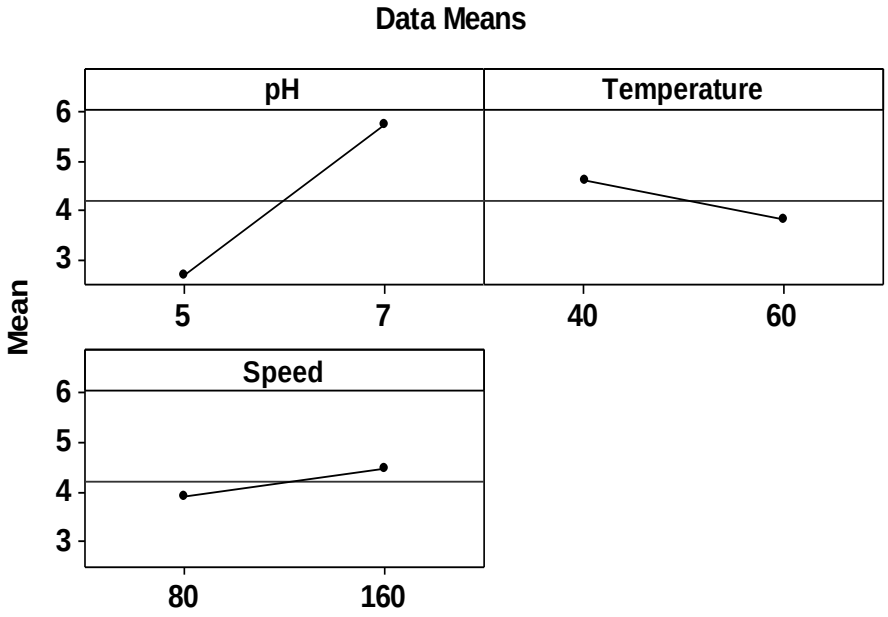

Fig. 2. Main effects plot for glucose production

Interaction Effects

The interaction plot provides the mean response of two factors at all possible combination of their settings. If the lines are not parallel, this indicates that an interaction between the two factors occurred (Chowdhury *et al.* 2011). The interaction plot provided in Fig. 3 clearly shows that all interactions were statistically significant. The wider cross line of pH and speed showed that this interaction had a strong influence on glucose production.

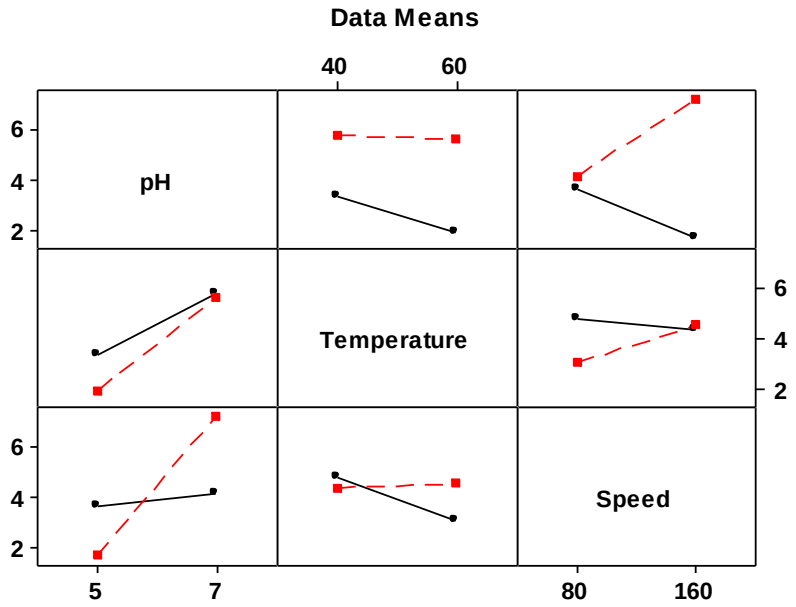

Fig. 3. Interaction plot for glucose production

Pareto Chart

The main effects and their interactions can also be visualized using a Pareto chart (Fig. 4). With a 95% confidence level and 8 degrees of freedom, the t-value was equal to 2.31. The vertical line in the chart indicates the minimum significant effect magnitude for 95% confidence level (Bingol *et al.* 2010). It was observed that all the main and two-way interaction effects extending beyond 2.31 are significant. Nonetheless, the three-way interaction is not significant in term of glucose production. Clearly from Fig. 4, pH had the largest effect on the saccharification process because it had the longest bar (Abdel-Ghani *et al.* 2009). It can also be stated that the effects were in decreasing order as A > AC > BC > B > AB > C > ABC.

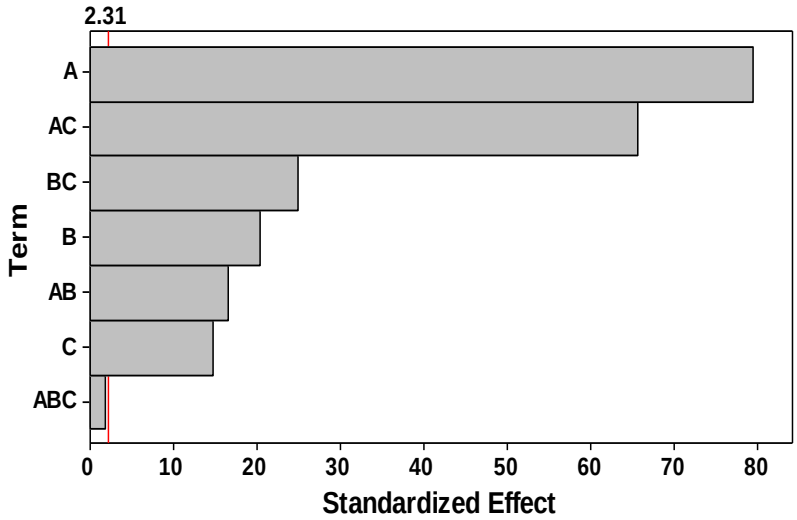

Fig. 4. Pareto chart of the standardized effects

CONCLUSIONS

This study demonstrated the effectiveness of factorial experimental design to identify the significant factors that influenced the tapioca slurry encapsulated enzymatic saccharification process using a stirred bioreactor. The effects of pH, temperature, and agitation speed were investigated using a 2^3 full factorial design. We can conclude that:

1. The main effects and their two-way interactions were statistically significant.

2. Agitation speed and pH had a positive effect, while the temperature had a negative effect.

3. The interaction between pH and speed was found to be the most influential interaction. The pH had the most significant impact on the glucose production.

4. This study indicates the suitability of factorial design as a screening tool to evaluate the effect of factors.

5. For further study, pH, temperature, and agitation speed can be used in the optimization study using response surface methodology.

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