



## Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author: Lilis Hermida  
Assignment title: pdf files  
Submission title: Saccharification Kinetics at Optimise...  
File name: Agustian-lilis2019Saccharification.pdf  
File size: 1.38M  
Page count: 8  
Word count: 4,940  
Character count: 24,394  
Submission date: 21-Jan-2021 03:33PM (UTC+0700)  
Submission ID: 1491340976

Eurasian Chemical Journal 20 (2018) 911-918  
<https://doi.org/10.18321/ectj764>

**Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised on Mesoporous Cellular Foam Silica**

J. Agustian\* and L. Hermida  
Department of Chemical Engineering, Universitas Lampung, Bandar Lampung 35145, Lampung, Indonesia

**Article info**  
Received: 16 March 2018  
Received in revised form: 18 May 2018  
Accepted: 5 August 2018

**Abstract**  
As insoluble substrates such as tapioca can be used to make chemical compounds, saccharification of tapioca by glucoamylase immobilised on mesoporous cellular foam (MCF) silica using Box-Behnken Design of experiment was conducted to optimize this process so that the experimental results can be used to develop large-scale operations. The experiments gave dextrose equivalent (DE) values of 6.15–69.50% (w/w). Factors of pH and temperature affected the process highly. The suggested quadratic polynomial model is significant and considered acceptable ( $R^2 = 99.78\%$ ). Justification of the model confirms its validity and adequacy where the predicted DE shows a good agreement with the experimental results. The kinetic constants ( $V_{max}$ ,  $K_m$ ) produced by the immobilised enzyme differed highly from the values yielded by free glucoamylase indicating reduction of substrate access to enzyme active sites had occurred.

**1. Introduction**  
Mesoporous silicas, one of the important materials for enzymes' supports, have been used to immobilise free glucoamylase by applying various immobilisation methodologies. The materials prepared from tetraethoxysilane and phenyltriethoxysilane had successfully encapsulated this enzyme where the product was used for many cycles in soluble starch hydrolysis [1]. Functionalised ordered mesoporous silicas were also developed to support glucoamylase covalently to give the immobilised enzyme capable to hydrolyse soluble starch efficiently [2, 3]. Previously, Szymanska et al. [4] immobilised glucoamylase on functionalised mesoporous cellular foam (MCF) silica where the immobilised enzyme was used to saccharify the soluble starches. Saccharification of insoluble starches based on the silica supports is not found yet. As ethanol and other chemicals can be made from the insoluble substrates via fermentation, saccharification of tapioca starch using glucoamylase immobilised on the MCF silica is required as knowledge on the process are needed to develop large-scale operations. Variation of the operational factors simultaneously through a factorial experimental design is considered as the correct way to deal with the factors [5]. Since optimisation of process is considered to be a step to define the optimum conditions by evaluating the interactions of the operational factors [6], to optimize the tapioca saccharification using the glucoamylase immobilised on the MCF silica, a response surface methodology based on Box-Behnken design is herewith reported. The optimized results were used to measure the kinetics of the reaction. Reusability of the immobilised glucoamylase was also evaluated under the optimized conditions.

**2. Experimental**

**2.1. Chemicals and biochemical**  
Pluronic® P123 (435465), mesitylene (>98%), tetraethyl orthosilicate (>99%) and sodium acetate (>98%) were purchased from Sigma Aldrich. Ammonium fluoride (>98%), HCl (37%), di-sodium hydrogen phosphate dodecahydrate (>99%), D(+)-glucose monohydrate (>99%) and potassium dihydrogen phosphate (>99%) were supplied by Merck Indonesia. Glucoamylase LYPH170122 (30,000 U g<sup>-1</sup>) were imported from Xian Lijubar Biotech Co. Ltd. (China). Tapioca was bought from a supermarket.

Corresponding author: Email: joni.agustian@unila.ac.id  
©2018 Universitas Lampung

# Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised on Mesostructured Cellular Foam Silica

*by* Lilis Hermida

---

**Submission date:** 21-Jan-2021 03:33PM (UTC+0700)

**Submission ID:** 1491340976

**File name:** Agustian-lilis2019Saccharification.pdf (1.38M)

**Word count:** 4940

**Character count:** 24394

## Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised on Mesostructured Cellular Foam Silica

J. Agustian\* and L. Hermida

11

Department of Chemical Engineering, Universitas Lampung, Bandar Lampung 35145, Lampung, Indonesia

### Article info

Received:  
16 March 2018

Received in revised form:  
18 May 2018

Accepted:  
5 August 2018

### Abstract

As insoluble substrates such as tapioca can be used to make chemical compounds, saccharification of tapioca by glucoamylase immobilised on mesostructured cellular foam (MCF) silica using Box-Behnken Design of experiment was conducted to optimize this process so that the experimental results can be used to develop large-scale operations. The experiments gave dextrose equivalent (DE) values of 6.15–69.50% (w/w). Factors of pH and temperature affected the process highly. The suggested quadratic polynomial model is significant and considered acceptable ( $R^2 = 99.78\%$ ). Justification of the model confirms its validity and adequacy where the predicted DE shows a good agreement with the experimental results. The kinetic constants ( $V_{max}$ ,  $K_M$ ) produced by the immobilised enzyme differed highly from the values yielded by free glucoamylase indicating reduction of substrate access to enzyme active sites had occurred.

### 1. Introduction

Mesoporous silicas, one of the important materials for enzymes' supports, have been used to immobilise free glucoamylase by applying various immobilisation methodologies. The materials prepared from tetraethoxysilane and phenyltriethoxysilane had successfully encapsulated this enzyme where the product was used for many cycles in soluble starch hydrolysis [1]. Functionalised ordered mesoporous silicas were also developed to support glucoamylase covalently to give the immobilised enzyme capable to hydrolyse soluble starch efficiently [2, 3]. Previously, Szymanska et al. [4] immobilised glucoamylase on functionalised mesostructured cellular foam (MCF) silica where the immobilised enzyme was used to saccharify the soluble starches.

Saccharification of insoluble starches based on the silica supports is not found yet. As ethanol and other chemicals can be made from the insoluble substrates via fermentation, saccharification of tapioca starch using glucoamylase immobilised on the MCF silica is required as knowledge on the process are needed to develop large-scale operations. Variation of the operational factors simultaneously through a factorial experimental design is consid-

ered as the correct way to deal with the factors [5]. Since optimisation of process is considered to be a step to define the optimum conditions by evaluating the interactions of the operational factors [6], to optimize the tapioca saccharification using the glucoamylase immobilised on the MCF silica, a response surface methodology based on Box-Behnken design is herewith reported. The optimized results were used to measure the kinetics of the reaction. Reusability of the immobilised glucoamylase was also evaluated under the optimized conditions.

### 2. Experimental

#### 2.1. Chemicals and biochemical

Pluronic® P123 (435465), mesitylene (>98%), tetraethyl orthosilicate (>99%) and sodium acetate (>98%) were purchased from Sigma Aldrich. Ammonium fluoride (>98%), HCl (37%), di-sodium hydrogen phosphate dodecahydrate (>99%), D(+)-glucose monohydrate (>99%) and potassium dihydrogen phosphate (>99%) were supplied by Merck Indonesia. Glucoamylase LYPH170122 (30.000 U g<sup>-1</sup>) were imported from Xi'an Lyphar Biotech Co. Ltd. (China). Tapioca was bought from a supermarket.

\*Corresponding author. Email: joni.agustian@ng.unila.ac.id

## 2.2. Synthesis of MCF silica (9.2T-3D)

A method developed by Hermida et al. [7] was used. 4 g Pluronic® P123 was dissolved in 70 mL of HCl (1.6 M) at ambient temperature in a flask. Then, 3.4 mL trimethylbenzene was added. The mixture was stirred at 750 rpm and 40 °C for 2 h. After 9.2 mL tetraethyl orthosilicate was put in. It was stirred at 1.000 rpm for 5 min and later kept in water bath for 20 h at 40 °C. 5 ml NH<sub>4</sub>F (46 mg per 5 mL) was inserted under slow stirring. The mixture was kept in water bath for 72 h at 80 °C. After 3 days, it was cooled to ambient temperature and filtered. The solids were dried at 100 °C for 12 h. They were calcined at 300 °C for 30 min and continued at 500 °C for 6 h in order to obtain the MCF silica.

## 2.3. Immobilisation process

Immobilization of glucoamylase was conducted directly without pre-treatment. 10 mg enzyme was dissolved in 30 mL Sorensen phosphate buffer 100 mM pH 5. Then, 500 mg MCF silica was added. The mixture was shaken at 100 rpm and 30 °C in Medline BS-31 unit. After 5 h, the mixture was filtered. Filtrate was kept in refrigerator before protein analysis was conducted, whilst residue was washed with Sorensen buffer (3×50 mL) based on the immobilisation pH. The filtrate from the washing was also kept in refrigerator before the protein analysis. Residue was dried in desiccator at room temperature overnight. The dried immobilised enzyme was stored in refrigerator. Amount of enzyme on support was calculated as follows:

$$\text{Immobilised Enzyme (\%)} = \frac{(C_0 - C_i)}{C_0} \times V \times 100 \quad (1)$$

where  $C_0$  is initial free enzyme concentration (mg mL<sup>-1</sup>),  $C_i$  is final free enzyme concentration (mg mL<sup>-1</sup>), and  $V$  is reactor working volume (mL).

## 2.4. Enzyme specific activity

Bernfeld method as described by Milosavic et al. [8] was used to determine activity of both free and immobilised enzyme. 4 g soluble starch was dissolved in 100 mL 0.05 M sodium acetate buffer pH 4.5 and homogenized. Then 25 mL of the solution was poured in two 100 mL Erlenmeyer flasks where 50 mg immobilised enzyme was put into the first flask and 25 mg free enzyme was added into the second Erlenmeyer. Both Erlenmeyers were placed in water bath shaker at 60 °C and 140 rpm. Samples were withdrawn every 5 min for 30 min. The samples were centrifuged at 1000 g for 3 min before DNS (dinitrosalicylic acid) analysis were conducted. One glucoamylase unit (U) was defined as the amount of enzyme, which released reducing carbohydrates equivalent to 1 μmole glucose from soluble starch in 1 min.

## 2.5. Design of experiment (DOE)

Saccharification of tapioca was optimised using Box Behn Design (BBD). The design was prepared and analysed using Design Expert® v. 6.0.6 software. Three reaction factors were studied as described in Table 1. Dextrose equivalent value (DE) was set as response.

## 2.6. BBD experimental procedure

30 mL 0.1 M sodium acetate buffer solution pH 4.3–4.9 were placed in 100 mL Erlenmeyer flasks. Then tapioca starch was dissolved in each flask at concentration of 3% (w/v). 400 mg immobilised glucoamylase was added into the tapioca solution. The flasks containing the mixture were placed in water bath shaker where temperature and agitation speed were set at 65–75 °C and 130–150 rpm, respectively, for 8 h. All experiments used operating conditions provided by DOE. Initial and final sample were collected and measured their DE value using DNS analysis.

**Table 1**

The experimental factors and their levels

Factor	Name	Unit	Type	Low Actual	High Actual	Low Coded	High Coded
A	Buffer pH	-	Numeric	4.30	4.90	-1	+1
B	Temperature	°C	Numeric	65.00	75.00	-1	+1
C	Agitation Speed	rpm	Numeric	130.00	150.00	-1	+1

### 2.7. Determination of kinetics

To determine kinetic constants of the saccharification process ( $V_{max}$ ,  $K_M$ ), the initial rate of reaction was investigated by varying the concentration of tapioca starch in the range of 0.5–5.0% (w/v) in 30 mL 0.1 M sodium acetate buffer pH 4.6. The process was set at 70 °C and 140 rpm for 4 h. 400 mg of enzyme was added into the starch solution. Samples were withdrawn every half an hour for the DNS analysis. The kinetic constants were evaluated using Chem SW Enzyme Kinetics!Pro.

### 2.8. Reusability of immobilised enzyme

Reusability of immobilized glucoamylase was performed in a batch reactor. The process was conducted as follows: 3% (w/v) tapioca was dissolved in a 100 mL Erlenmeyer flask containing 30 mL 0.1 M sodium acetate buffer pH 4.6. Then, 400 mg immobilised glucoamylase was added in. The flask was placed in water bath shaker set at temperature of 70 °C and agitation speed of 140 rpm for 8 h. Initial and final sample were collected for DE value measurements. After the saccharification process, the immobilised enzyme was filtered-off and washed with 0.1 M sodium acetate buffer pH 4.6 (50 mL). The filtered immobilised enzyme was reintroduced into a fresh reaction medium and checked its performance. Activity of the immobilised enzyme in the 1st run was identified as 100%. Activities obtained in other runs were compared with that of the 1st run.

### 2.9. Estimation of protein and glucose

Pierce™ BCA protein assay was used to estimate protein content by the standard method provided by manufacturer. Hydrolysis of starch to glucose was determined by the DNS analysis method. The enzyme contents and glucose samples were observed using spectrometer Shimadzu UV-VIS 1800.

### 2.10. Characterization (FTIR, SEM)

Average cell pore size was evaluated using Barrett-Joyner-Halenda (BJH) method from the adsorption branch of the isotherm data. Average window pore size was evaluated using BJH method from the desorption branch.  $S_{BET}$  was calculated using Brunauer-Emmett-Teller (BET) method. Samples (support and enzyme) were analyzed

using Zeiss EVO field emission scanning electron microscope (SEM), equipped with an Oxford IN-CAX act, energy dispersive X-ray (EDX) micro-analysis system, to obtain SEM images and chemical compositions. Further samples were observed using Frontier Perkin Elmer FTIR unit.

## 3. Results and discussion

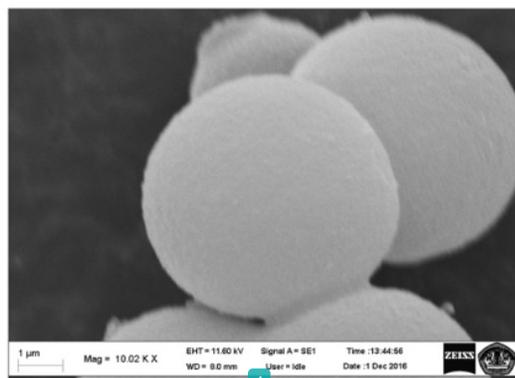
### 3.1. Immobilised glucoamylase

Adsorption of the free glucoamylase on MCF silica (9.2T-3D) ( $S_{BET}$ : 378 m<sup>2</sup> g<sup>-1</sup>;  $V_{pore}$ : 2.12 cm<sup>3</sup> g<sup>-1</sup>;  $d_{cell}$ : 235 Å;  $d_{window}$ : 158 Å) gave the immobilised enzyme with 82.06% (w/w) load. The load was quite high as the enzyme has lower size than the support (~80 Å). Compared with other enzyme supports, the immobilisation of glucoamylase on the MCF silica (9.2T-3D) produced a good result. Milosavic et al. [8–9] immobilised 16–19% (w/w) the free glucoamylase on poly(GMA-co-EGDMA). Later, Guo et al. [10] obtained 2.7–12% (w/w) loads of the glucoamylase on carboxyl magnetic nanoparticles (CMNPs). Other researchers found less than 30% (w/w) loads of the glucoamylase of polypropylene fibers, magnetic clay, magnetic chitosan and magnetic carbon nanotubes supports [11–14].

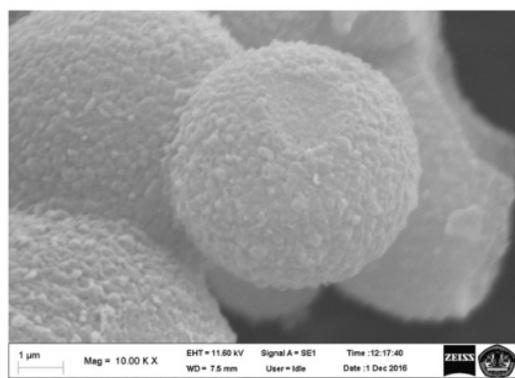
SEM analysis on the MCF silica (9.2T-3D) confirmed changes of the support surfaces from smooth (Fig. 1a) to rough (Fig. 1b). The figures comparison proved presence of the enzyme on the support. A further observation on the FT-IR spectrum of the MCF silica (9.2T-3D) as described in Fig. 2 showed its vibrations at 1300 cm<sup>-1</sup> and 850 cm<sup>-1</sup> with peak at 1061 cm<sup>-1</sup> to indicate Si–O–Si stretching vibration. These vibrations were also observed on the immobilized enzyme, however, additional vibrations at 2020 cm<sup>-1</sup> and 1900 cm<sup>-1</sup> were found. The vibration observed at 1962 cm<sup>-1</sup> confirmed the C≡N or C≡C bounds on the support surfaces [23]. Because glucoamylase is a protein consisting of a single polypeptide chain of amino acids, the 1962 cm<sup>-1</sup> vibration indicated the presence of protein on the support.

The free and immobilised glucoamylase used in the experiments were found to have the specific activity of 30.335.17 U g<sup>-1</sup> and 1.856.78 U g<sup>-1</sup>, respectively. The specific activity reduction after the immobilisation process was frequently found because conformational changes on enzyme structures occurred so that some active sites are difficult to be accessed by substrates [16, 17].

Compared with other results, the immobilised glucoamylase on MCF silica (9.2T-3D) has a better specific activity since the specific activity of this enzyme on poly(GMAco-EGDMA) was only 1.100 U g<sup>-1</sup> [8, 9], while the glucoamylase on Si-bunit gave the value of 420–540 U g<sup>-1</sup> [18].



(a)



(b)

Fig. 1. SEM images of supporting material (a) and glucoamylase on support (b).

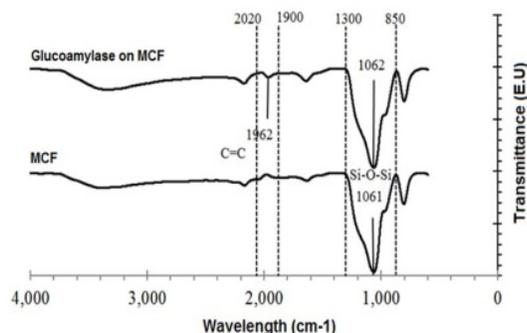


Fig. 2. The FTIR spectra.

### 3.2. Statistical DOE

Saccharification of tapioca was designed by combining 3 experimental factors with 3 levels based on Box-Behnken Design (BBD). This design required 17 experimental runs as described in Table 2. Dextrose Equivalent (DE) were found in the range of 6.15–69.50% (w/w) where the highest result was produced at pH 4.3, temperature of 70 °C and agitation speed of 130 rpm, whilst the lowest DE was obtained at conditions of pH 4.9, 65 °C and 140 rpm.

The sequential model sum of squares analysis concludes that the developed statistical quadratic model is significant in explaining relationship between the response and factors including the factorial interactions during the process where the P>F value is < 0.0001. The model is not aliased and has a low standard deviation (i.e. 1.75%). It is defined as follows:

$$\begin{aligned} \text{DE} = & 9.655.35 + 702.28 (\text{pH}) + 191.51 \\ & (\text{temperature}) + 18.97 (\text{agitation speed}) - \\ & 55.55 (\text{pH})^2 - 1.21 (\text{temperature})^2 - 0.05 \\ & (\text{agitation speed})^2 - 2.42 (\text{pH} \times \text{temperature}) - \\ & 0.37 (\text{pH} \times \text{agitation speed}) - 0.04 \\ & (\text{temperature} \times \text{agitation speed}) \end{aligned} \quad (2)$$

Table 2  
Box-Behnken Design matrix

Run	Independent Variables			DE (% (w/w))	
	x <sub>1</sub> : Buffer pH	x <sub>2</sub> : (°C)	x <sub>3</sub> : Agitation Speed (rpm)	Observed	Predicted
1	4.6	65	130	8.77	9.61
2	4.6	70	140	68.41	68.41
3	4.6	70	140	68.41	68.41
4	4.3	65	140	15.03	14.99
5	4.9	65	140	6.15	3.87
6	4.9	75	140	43.82	43.86
7	4.9	70	150	44.61	45.41
8	4.3	75	140	67.25	69.52
9	4.6	70	140	68.41	68.41
10	4.3	70	130	69.50	68.70
11	4.6	70	140	68.41	68.41
12	4.6	70	140	68.41	64.41
13	4.6	75	150	52.82	51.97
14	4.9	70	130	51.12	52.55
15	4.3	70	150	67.47	66.04
16	4.6	65	150	7.31	8.76
17	4.6	75	130	62.42	60.95

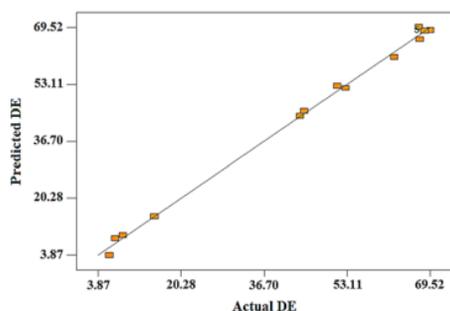


Fig. 3. The parity plot of the actual versus predicted DE.

The analysis of variance indicates that the model fits well and satisfactory. Coefficient of determination ( $R^2$ ) is 99.78%, while the adjusted and predicted  $R^2$  are 99.49% and 96.41%, respectively. As the  $R^2$  are more than 95%, hence more than 95% of the actual data can be explained by the model [5]. Only 0.22% of the total variations were not described by the model. The  $R^2$  is acceptable as the closer the  $R^2$  value to unity, the better the models fits the data [19, 20]. The predicted responses matched the experimental results reasonably well with the  $R^2$  of more than 95% (Fig. 3). payang et al. [21] obtained the  $R^2$  of 99.14% in optimization of reducing sugar production from *Manihot glaziovii* starch using BBD. The optimization of polysaccharides hydrolysis using Central Composite Design (CCD) gave the  $R^2$  of 86.36–96.94% [22–25]. Therefore, this model is applicable and reliable and can be used to simulate the process.

3.3. Mutual effects of factors

Effects of the operational factors ( $x_1$ - $x_3$ ) are given in Table 3. Individually, pH ( $x_1$ ) and temperature ( $x_2$ ) showed significant effects on the process by changing the response highly as described in Fig. 4. A slight reduction (<5%) was found when pH was increased from 4.30 to 4.45, but further increases reduced the DE quickly. The buffer pH that produced the highest DE was similar to the previous observations conducted by Milosavic et al. [8, 9, 26] who concluded the optimum operational pH for the immobilised glucoamylase were 4.5. Temperature factor increased the DE rapidly when it was changed from 65 °C to around 72 °C, however, reduction of DE values were obtained when further temperature elevations were made. Although the agitation speed ( $x_3$ ) tended to decrease the response, its effect was considered low and not significant. The speeds up to 150 rpm were frequently found in the starches hydrolyses [21, 27, 28].

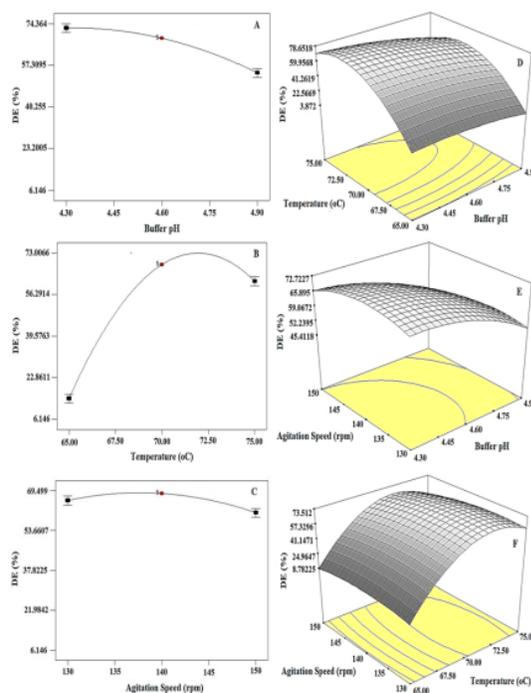


Fig. 4. The individual factors and their interactions effects.

Table 3  
Summary of the factorial effects

Factor	Effects		
$x_1$	High	Antagonist	Significant
$x_2$	High	Synergist	Significant
$x_3$	Low	Antagonist	Not Significant
$x_1x_2$	High	Antagonist	Significant
$x_1x_3$	Low	Antagonist	Significant
$x_2x_3$	High	Synergist	Significant

Three interactions were found. They are weighed significant and show relatively high effects except the second interaction ( $x_1x_3$ ) (Fig. 4). The first interaction, pH and temperature ( $x_1x_2$ ), gave the response as high as 66.19% at pH of 4.30–4.75 and temperature of 69 to 75 °C. This interaction produced the lowest DE of 16.33% obtained at the lowest operational temperature (65 °C) for the all employed pHs. The pH and agitation speed interaction ( $x_1x_3$ ) resulted the maximum DE of 66.19% at pH of 4.30–4.65 and agitation speed of 130–150 rpm. The minimum DE given by this interaction was 53.72%. The last interaction, the agitation speed and temperature ( $x_2x_3$ ), developed the DE values the same as the first interaction.

The highest DE was obtained at the combination of the operating conditions at temperatures of around 69.5–74.5 °C and agitation speed of 130–150 rpm, whilst the lowest result was produced at temperature of less than 66 °C and agitation speed of 130–150 rpm.

### 3.4. Model Verification

Examination must be performed to conclude that the predicted and experimental results produced at the optimum conditions are the same [29]. From the above description, the constraints used to obtain the optimum values for the saccharification process using immobilised glucoamylase on MCF silica were pH (level: 4.6; range: 4.3–4.9), temperature (level: 70 °C; range: 65–75 °C), agitation speed (level: 140 rpm; range: 130–150 rpm), and DE (range: 6.15–69.50%). To verify the model, some proposed solutions for the optimum conditions were tested. The results are described in Table 4. The experimental DE are reasonably closed to the predicted values where most of them are lower than the modelling results. 3 out of 5 experimental data show the relative errors of less than 5% (i.e. 0.204–3.107%), which are acceptable as they are in the range of 95% confidence level. The other results have the errors of 8.226% and 9.297%, which are tolerable as errors around 10–15% are considered good in the optimization processes [30]. These descriptions confirmed validity and adequacy of the predicted model.

**Table 4**  
Predicted and experimental results  
at the optimum conditions

pH	Temp. (°C)	Agitation Speed (rpm)	DE (%)		
			Predicted	Experimental	Error
4.6	70	140	68.41	68.55	0.14
4.3	70	150	66.04	64.05	-1.99
4.6	65	130	8.78	8.63	-0.15
4.6	75	150	51.97	48.02	-3.95
4.9	70	130	52.55	48.08	-4.47

### 3.5. Steady State Kinetics

Formation of reducing sugars during initial reaction rate observations at various tapioca starch concentrations (1–5 mg mL<sup>-1</sup>) is illustrated in Fig. 5. All employed concentrations almost have the same reaction rates during 4 h. DE values were less than

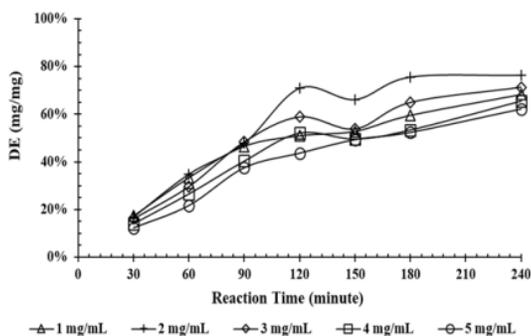


Fig. 5. Progress of DE during initial rate measurements.

20% (w/w) (all concentrations) in the first 30 min. After 90 min, DE were tripled to 40.34–48.43% (w/w) proving that the active sites of the enzyme was not difficult to be accessed by the starch. Slow increases in DE were found after the process run for 2 h. Approximately 50% (w/w) DE was obtained after 150 min.

The kinetics constants,  $K_M$  and  $V_{max}$ , were determined by using the Michaelis-Menten model as described in Eq. 2. Both constants were analysed using Hanes-Woolf plot (Eq. 3) because this plot gives low deviations from true  $K_M$  and  $V_{max}$  [31]. The errors resulted by the plot are distributed more evenly than Lineweaver-Burk and Eadie-Hofstee plot [32].

$$V_0 = \frac{V_{max} [S]}{K_M + [S]} \quad (3)$$

$$\frac{[S]}{V_0} = [S] \left( \frac{1}{V_{max}} \right) + \frac{K_M}{V_{max}} \quad (4)$$

where  $V_0$  is rate of reaction,  $K_M$  is the Michaelis-Menten constant,  $V_{max}$  is the maximum reaction rate and  $[S]$  is concentration of substrate. Regression of Eq. (4) by plotting  $([S]/V_0)$  versus  $[S]$  gave  $(1/V_{max})$  as the slope and  $(K_M/V_{max})$  as the intercept. The  $K_M$  and  $V_{max}$  were determined from the slope and intercept. Figure 6 describes the Hanes-Woolf plot for both glucoamylase types.

The  $K_M$  value for the immobilised glucoamylase is higher than the free enzyme. The immobilised enzyme has the  $K_M$  of 14.23 mg mL<sup>-1</sup>, whilst the  $K_M$  for the free glucoamylase is 4.83 mg mL<sup>-1</sup>. The increase of the  $K_M$  value after immobilisation indicates that the enzyme has low affinity on the substrate, which could be caused by change of the enzyme structure that decreased the accessibility of the enzyme

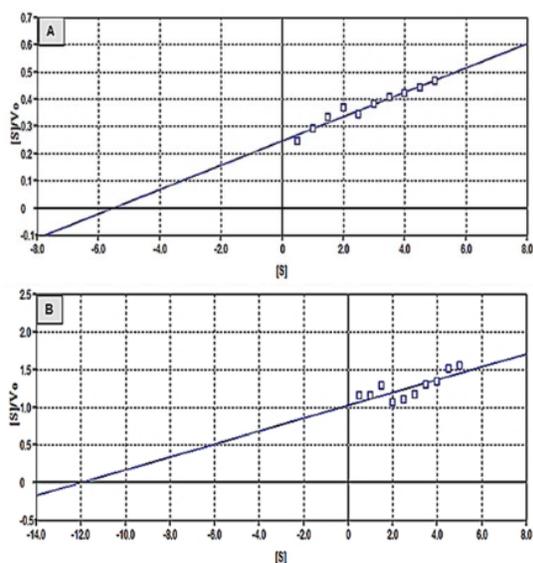


Fig. 6. The Hanes-Woolf plot (A: Free enzyme; B: Immobilised enzyme).

active sites [33]. From the plot, it is found that the maximum reaction rates of the saccharification process catalyzed by the immobilised enzyme ( $V_{\max}$ ) is  $0.07 \text{ mg mL}^{-1} \text{ min}^{-1}$ . However, the free enzyme gives the  $V_{\max}$  of  $0.10 \text{ mg mL}^{-1} \text{ min}^{-1}$ . The lower  $V_{\max}$  emphasizes that reduction of the enzyme activity relates to the change of the enzyme structure and difficult access to the enzyme active sites [3].

### 3.6. Reusability study

Kalburcu et al. [34] described that reusability of an enzyme immobilised on a matrix is a must for large-scale operations. Hence, a study on repeated uses of the immobilised glucoamylase on MCF silica (9.2T-3D) was conducted to know its activity after many saccharification processes as shown in Fig. 7. It was observed that the immobilised enzyme activity decreased rapidly to 68.83% after the second cycle. The gradual decreases continued where the enzyme had activity of  $\pm 50\%$  after 5 cycles. The last observation found that its activity decreased to 21.52% after 10 cycles. Demirkan et al. [35] found the enzyme activity as high as 38% after 6 cycles. Later, Ashly et al. [36] summarized that the immobilised amylase had 20% activity after 10 times used in the process. Reductions in the immobilised glucoamylase activity were mainly caused by treatment of the enzyme after the reaction was conducted where the immobilised enzyme must be

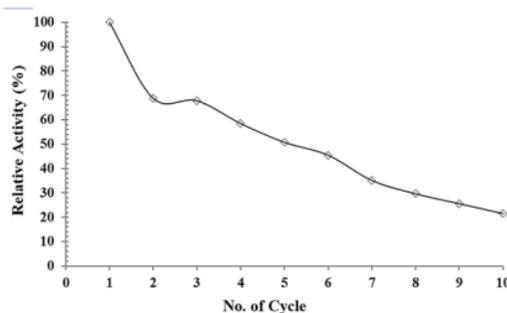


Fig. 7. Reusability of glucoamylase immobilised on MCF silica (9.2T-3D).

washed several times with the buffer solutions that caused enzyme leaching from the support [35, 36].

## 4. Conclusions

In conclusion, the enzymatic saccharification of tapioca starch using glucoamylase adsorbed on surfaces of MCF silica (9.2T-3D), which had the specific activity of  $1.856.78 \text{ U/g}$ , was investigated. The statistical design of experiment using Box-Behnken response surface method with 3 operational factors gave the DE values in the range of 6.15–69.50% (w/w). The proposed polynomial quadratic model is acceptable where the  $R_2$  is 99.78%. The justification confirmed the model validity and adequacy. Its predicted values matched the observed values reasonably well. pH and temperature are considered significant and give high effects on the DE. All interactions show their significance on the developed model. The saccharification using the immobilised enzyme gave the  $K_M$  and  $V_{\max}$  of  $14.23 \text{ mg mL}^{-1}$  and  $0.07 \text{ mg mL}^{-1} \text{ min}^{-1}$ , respectively, which differed highly with the kinetic constants developed by the free glucoamylase and indicated the reduction of the substrate access to the enzyme active sites.

## Acknowledgements

Both authors thank deeply for the financial support for this research provided by Universitas Lampung via The Superior Research Grant No. 270/UN26.21.PN/2017.

## References

- [1]. B. Vlad-Oros, G. Preda, Z. Dudas, M. Dragomirescu, A. Chiriac, *Proc. Appl. Ceram.* 1 (2007) 63–67. DOI: 10.2298/PAC0702063VR.

- [2]. R. George, S. Gopinath, S. Sugunan, *Bull. Chem. React. Eng. Cat.* 8 (2013) 70–76. DOI: 10.9767/bcrec.8.1.4208.70-76]
- [3]. R. George, S. Sugunan, *J. Mol. Catal. B: Enzym.* 106 (2014) 81–89. DOI: 10.1016/j.molcatb.2014.04.016
- [4]. K. Szymanska, J. Bryjak, J. Mrowiec-Białon, J.B. Jarzebski, *Micropor. Mesopor. Mat.* 99 (2007) 167–175. DOI: 10.1016/j.micromeso.2006.08.035
- [5]. D.C. Montgomery, “Design and analysis of experiments”. Fifth ed., John Wiley & Sons Inc., London, 2001.
- [6]. A. Soyer, E. Bayraktar, U. Mehmetoglu, *Prep. Biochem. Biotech.* 40 (2010) 389–404. DOI: 10.1080/10826068.2010.525433
- [7]. L. Hermida, A.Z. Abdullah, A.R. Mohamed, “Nickel functionalized mesostructured cellular foam (MCF) silica as a catalyst for solventless deoxygenation of palmitic acid to produce diesel-like hydrocarbons”, *Materials and processes for energy: communicating current research and technological development*, 2013, pp. 312–319.
- [8]. N.B. Milosavić, R.M. Prodanović, S.M. Jovanović, Z.M. Vujčić, *Enzyme Microb. Tech.* 40 (2007) 1422–1426. DOI: 10.1016/j.enzmictec.2006.10.018
- [9]. N. Milosavić, R. Prodanović, S. Jovanović, I. Novakovic, Z. Vujčić, *J. Serb. Chem. Soc.* 70 (5) (2005) 713–719.
- [10]. C. Guo, M. Yunhui, S. Pengfei, F. Baishan, *Biochem. Eng. J.* 67 (2012) 120–125. DOI: 10.1016/j.bej.2012.06.002
- [11]. H. Kamal, G.M. Sabry, S. Lofty, N.M. Abdallah, J. Rosiak, A.H. El-Sayed, *J. Macromol. Sci. A* 45 (2008) 65–75. DOI: 10.1080/10601320701683322
- [12]. G. Zhao, Y. Li, J. Wang, H. Zhu, *Appl. Microbiol. Biot.* 91 (2011) 591–601. DOI: 10.1007/s00253-011-3299-y
- [13]. G. Zhao, J. Wang, Y. Li, X. Chen, Y. Liu, *J. Phys. Chem. C* 115 (2011) 6350–6359. DOI: 10.1021/jp200156j
- [14]. J. Wang, G. Zhao, Y. Li, Y. Liu, H. Hou, *Appl. Microbiol. Biot.* 97 (2013) 681–692. DOI: 10.1007/s00253-012-3979-2
- [15]. C.R. Baiz, M Reppert, A. Tokmakoff. An Introduction to Protein 2D IR Spectroscopy. In: Fayer MD, editor. *Ultrafast Infrared Vibrational Spectroscopy*. CRC Press; New York: 2013. pp. 361–404.
- [16]. D.A. Butterfield, D. Bhattacharyya, S. Dannert, L. Bachas, *J. Membr. Sci.* 181 (2001) 29–37. DOI: 10.1016/S0376-7388(00)00342-2
- [17]. A. Dwevedi, Basics of Enzyme Immobilization. In: *Enzyme Immobilization*. Springer, Cham, 2016. DOI: 10.1007/978-3-319-41418-8\_2
- [18]. G.A. Kovalenko, L.V. Perminova, T.G. Terent'eva, G.V. Plaksin, *Appl. Biochem. Micro.* 43(4) (2007) 374–378. DOI: 10.1134/S0003683807040023
- [19]. W. Cao, C. Zhang, P. Hong, H. Ji, *Food Chem.* 109 (2008) 176–183. DOI: 10.1016/j.foodchem.2007.11.080
- [20]. I.A.W. Tan, A.L. Ahmad, B.H. Hameed, *J. Hazard. Mat.* 153 (2008) 709–717. DOI: 10.1016/j.jhazmat.2007.09.014
- [21]. A.H. Sebayang, M.H. Hassan, H.C. Ong, S. Dharma, A.S. Silitonga, F. Kusumo, T.M.I. Mahlia, A.H. Bahar, *Energies* 10 (2017) 35–40. DOI: 10.3390/en10010035
- [22]. D. Mudgil, S. Barak, B.S. Khatkar, *J. Food Sci. Technol.* 51 (2012) 1600–1605. DOI: 10.1007/s13197-012-0678-z
- [23]. R.M. Collares, L.V.S. Miklasevicius, M.M. Bassaco, N.P.G. Salau, M.A. Mazutti, D.A. Bisognin, L.M. Terra, *J. Zhejiang Univ.-Sc. B* 13 (2012) 579–586. DOI: 10.1631/jzus.B1100297
- [24]. N. Nadir, M. Mel, M.I.A. Karim, R.M. Yunus. The Institution of Engineers Malaysia 71 (3) (2010) 26–34.
- [25]. N. Peatciyammal, B. Balachandar, M.D. Kumar, K. Tamilarasan, C. Muthukumar, *IJBET* 4 (2010) 126–130. DOI: 10.1999/1307-6892/11992
- [26]. N.B. Milosavić, R.M. Prodanović, S.M. Jovanović, Z.M. Vujčić, *Acta Periodica Technologica* 35 (2004) 207–214. DOI 10.2298/APT0435207M
- [27]. Y.N. Shariffa, A.A. Karim, A. Fazilah, I.S.M. Zaidul, *Food Hydrocolloid.* 23 (2009) 434–440. DOI: 10.1016/j.foodhyd.2008.03.009
- [28]. G. Zhao, J. Wang, Y. Li, H. Huang, X. Chen, *Biochem. Eng. J.* 68 (2012) 159–166. DOI: 10.1016/j.bej.2012.04.009
- [29]. M.J. Anderson, P.J. Whitcomb, “DOE Simplified: Practical tools for effective experimentation”, Second ed., Productivity Press, New York, 2007.
- [30]. M.S. Montilha, M.F. Sbroggio, V.R.G. Figueireido, E.I. Ida, L.E. Kurozawa, *Int. Food Res. J.* 24 (2017) 1067–1074.
- [31]. R.A. Copeland, “Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis”, Wiley-VCH Inc., New York, 2000.
- [32]. H. Bisswanger, “Enzyme Kinetics: Principles and Methods”, Wiley-VCH Verlag GmbH, Weinheim, 2002.
- [33]. G. Bayramoglu, M. Yilmaz, M.Y. Arica, *Food Chem.* 84 (2004) 591–599. DOI: 10.1016/S0308-8146(03)00283-8
- [34]. T. Kalburcu, M.N. Tuzmen, S. Akgol, A. Denizli, *Turk. J. Chem.* 38 (2014) 28–40. DOI: 10.3906/kim-1301-87
- [35]. E. Demirkan, S. Dincbas, N. Sevinc, F. Ertan, *Romanian Biotech. Lett.* 16 (2011) 6690–6701. cf15529aefcc18768d33a60049832e0fb310.pdf
- [36]. P.C. Ashly, M.J. Joseph, P.V. Mohanan, *Food Chem.* 127 (2011) 1808–1813. DOI: 10.1016/j.foodchem.2011.02.068

# Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised on Mesostructured Cellular Foam Silica

## ORIGINALITY REPORT

9%

SIMILARITY INDEX

5%

INTERNET SOURCES

7%

PUBLICATIONS

1%

STUDENT PAPERS

## PRIMARY SOURCES

1 123dok.com 2%  
Internet Source

2 www.spandidos-publications.com 1%  
Internet Source

3 Y. Zhou, , Y. Li, C. Wan, D. Li, and Z. Mao. 1%  
"Effect of Hot Water Pretreatment Severity on the Degradation and Enzymatic Hydrolysis of Corn Stover", Transactions of the ASABE, 2010.  
Publication

4 Rabah Boukherroub, Sabine Szunerits. 1%  
"Chapter 5 Wet Chemical Approaches for Chemical Functionalization of Semiconductor Nanostructures", Springer Science and Business Media LLC, 2009  
Publication

5 Submitted to University of Al-Qadisiyah <1%  
Student Paper

6

Internet Source

&lt;1%

7

Abdullah, A.Z.. "Optimization of mesoporous K/SBA-15 catalyzed transesterification of palm oil using response surface methodology", Fuel Processing Technology, 200907/08

Publication

&lt;1%

8

Smail Mostefaoui. "Discovery of Abundant In Situ Silicate and Spinel Grains from Red Giant Stars in a Primitive Meteorite", The Astrophysical Journal, 10/01/2004

Publication

&lt;1%

9

Jijiao Zeng, Deepak Singh, Difeng Gao, Shulin Chen. "Effects of lignin modification on wheat straw cell wall deconstruction by Phanerochaete chrysosporium", Biotechnology for Biofuels, 2014

Publication

&lt;1%

10

Gülay Bayramoğlu, M. Yakup Arıca. "Immobilization of laccase onto poly(glycidylmethacrylate) brush grafted poly(hydroxyethylmethacrylate) films: Enzymatic oxidation of phenolic compounds", Materials Science and Engineering: C, 2009

Publication

&lt;1%

11

Agustian, Joni, Azlina Harun Kamaruddin, and Hassan Y. Aboul-Enein. "Enantio-conversion

&lt;1%

and -selectivity of racemic atenolol kinetic resolution using free *Pseudomonas fluorescens* lipase (Amano) conducted via transesterification reaction", RSC Advances, 2016.

Publication

---

12

Deniss I. Díaz-Blanco, Jesús R. de La Cruz, Juan C. López-Linares, Thelma K. Morales-Martínez et al. "Optimization of dilute acid pretreatment of Agave lechuguilla and ethanol production by co-fermentation with *Escherichia coli* MM160", Industrial Crops and Products, 2018

Publication

---

13

Fatemeh Mahdizadeh, Mohammadreza Eskandarian, Javad Zabarjadi, Ali Ehsani, Amirhossein Afshar. "Silver recovery from radiographic film processing effluents by hydrogen peroxide: Modeling and optimization using response surface methodology", Korean Journal of Chemical Engineering, 2013

Publication

---

14

[repositorium.sdum.uminho.pt](http://repositorium.sdum.uminho.pt)

Internet Source

---

15

[pubs.rsc.org](http://pubs.rsc.org)

Internet Source

---

16

Optical Spectroscopy and Computational Methods in Biology and Medicine, 2014.

<1%

<1%

<1%

<1%

<1%

- 
- 17 [royalsocietypublishing.org](http://royalsocietypublishing.org) <1 %  
Internet Source
- 
- 18 [www.freepatentsonline.com](http://www.freepatentsonline.com) <1 %  
Internet Source
- 
- 19 [lib.ugent.be](http://lib.ugent.be) <1 %  
Internet Source
- 
- 20 [www.researchsquare.com](http://www.researchsquare.com) <1 %  
Internet Source
- 
- 21 Papita Das Saha, Apurba Dey, Pritam Marik. "Batch removal of chromium (VI) from aqueous solutions using wheat shell as adsorbent: process optimization using response surface methodology", Desalination and Water Treatment, 2012 <1 %  
Publication
- 
- 22 Ashim Kumar Khan, Ranjana Chowdhury. " Parametric Sensitivity of Municipal Solid Waste Integrated Power Plant: CO Footprint and Energy Analysis ", Chemical Engineering & Technology, 2020 <1 %  
Publication
- 
- 23 Dominic W. S. Wong. "Food Enzymes", Springer Science and Business Media LLC, 1995 <1 %  
Publication
-

25

Delcheva, G.. "Performance of *Aspergillus niger* B 03 @b-xylosidase immobilized on polyamide membrane support", *Journal of Molecular Catalysis. B, Enzymatic*, 200808

Publication

&lt;1%

26

Nisha Gaur, Korrapati Narasimhulu, Pydisetty Y. " Biochemical and kinetic characterization of laccase and manganese peroxidase from novel strains and their application in bioethanol production ", *RSC Advances*, 2018

Publication

&lt;1%

Exclude quotes      On

Exclude matches      Off

Exclude bibliography      On