



Determination of Optimum Reaction Velocity for Glucose Production from Cassava Starch Using Gluco Amylase Sourced from Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In an attempt to produce glucose from locally sourced materials to boost glucose syrup production and other applications, the optimum reaction velocity which enzyme becomes saturated with the substrate was investigated. In this study, cassava starch (substrate) and glucoamylase (enzyme) from rice were sourced and prepared. The time course of the enzyme hydrolysis was studied at pH 5 and temperature of 40⁰C as optimum conditions. The hydrolysis was carried out at various substrate concentrations. The time course of the reaction for glucose production was monitored at 10 minutes interval for a period of one hour. The glucose concentration produced was plotted against reaction time to evaluate the optimum glucose amount and its corresponding substrate concentration. The reaction velocity was then computed. Also, the experimental data were fitted into Michaelis-Menten model using Statistical Package for Social Scientists to compare the yield of

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glucose. Results showed optimum yield of glucose concentration was 0.253 g DE L⁻¹ achieved with 2.5 and 3.0% w/v substrate concentration at 50 and 40 minutes respectively. Further analysis gave the optimum reaction velocity range of 0.00506 to 0.00625 of g DE per litre-min. This range when compared with the model equation result (0.0052 to 0.0068 g DE litre-min) is reasonably close; hence the results from this study are valid. Technical evaluation to standardize the reaction velocity with respect to substrate concentration which may be referred to as specific reaction velocity gave 0.00205 ± 0.00005 g D per litre-min per substrate concentration %w/v.

Keywords: Kinetics; glucose; enzyme hydrolysis; cassava starch; rice amylase.

1. INTRODUCTION

Cassava starch is a white, fine powder obtained from pulverized cassava roots [1,2]. Its numerous applications include replacement for corn and potato starch. Production of glucose syrup requires pure starch, with low content of protein (especially, soluble protein). In this regard, cassava starch is preferable [3]. Cassava starch is made up of two types of polysaccharides namely amylose and amylopectin [4,5]. Hydrolysis of starch is used in various processes in many industries [6,7,3]. In the production of beer, the chemical transformation of starch to dextrines and maltose is a crucial part of the brewing process. The amylase enzymes are included in the grain mixture [8]. Hydrolysis of starch can be done in two ways, namely: enzymatic and acidic [9,10,11,12,13]. The acidic hydrolysis is old traditional technique which involves high acid medium of pH of 1 to 2. In enzymatic hydrolysis of starch, moderate conditions such as medium pH of 5 to 9, normal pressure and lower temperature (up to 70°C), are employed [14]. The basic parameters such as medium pH, temperature, substrate and enzyme concentrations which affect the hydrolysis process do change depending on the substrate and enzyme sources [15,16], (Alias-Rodinah, 2009); [17,18]. However, hydrolysis of starch using enzyme may yield glucose which may find application in various industries. The use of crystalline dextrose and glucose syrup, in food processing industries, has improved in recent years. They are employed in huge quantities in confectionaries, jam, fruit canning, ice cream, jellies, bakery products, alcoholic and beverages fermentation [1]. Glucose syrups from cassava are largely used in pharmaceutical application [19]. Fermentation and distillation aspect (food processing) require enzymes [20]. Amylases are enzymes that break down glycosidic linkages seen in starches. Amylases are found in all living organisms, but vary in specificity, activity, etc [21,22]. The enzyme activity is the rate in which a particular amount of the enzyme will change a

substrate to product. The activity of enzyme is influenced by concentrations of enzyme and substrate, temperature, pH and presence of cations of heavy metals (Alias-Rodinah, 2009). To follow an enzyme action, the test for starch (substrate) disappearance or the product appearance by reacting iodine with samples is usually employed [23]. Monosaccharides give positive test with Benedict solution between 2 - 3 minutes, while disaccharides may take about 10 or more minutes before yielding positive result [24]. The period of disappearance of hydrolysis of starch depends on enzyme activity [25]. The reaction rate could be modelled based on experimental data. The model that best fits the data generated is chosen and may be used as a guide in describing the process [26], (Owalude, 2004); [10,27,28]. In most cases, integral and differential methods may be used in finding reaction rate from experimental data. However, kinetic analysis of reaction involving enzymes may in some cases be based on Michaelis-Menten kinetics and/or Line Weaver-Burk models as shown in Equations 1 and 2, respectively.

$$V = \frac{V_{\max} [S]}{K_m + [S]} \quad (1)$$

Where, maximum velocity attained by the system is denoted as V_{\max} (at maximum [saturated] substrates concentrations), K_m (Michaelis constant) is the concentration of the substrate in which the reaction velocity is half of the V_{\max} , and $[S]$ is the concentration of the substrate S.

The Michaelis-Menten model equation 1 could be used to estimate reaction velocity as a function of concentration of substrate, with significant kinetic parameters (V_{\max} and K_m); and may be rearranged to give Equation 2 known as Line Weaver-Burk model:

$$\frac{1}{V} = \left(\frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} \right) + \frac{1}{V_{\max}} \quad (2)$$

Where, V = reaction velocity at any instant (g DE. Litre-min⁻¹), V_{\max} = maximum velocity (g DE.

Litre-min⁻¹) and K_m = Michaelis Menten's constant (g DE. per 100 ml).

Lineweaver-Burk plot of $\frac{1}{v}$ against $\frac{1}{[S]}$ generates a straight curve with an intercept = $\frac{1}{V_{max}}$ and slope = $\frac{K_m}{V_{max}}$.

In predicting the performance of an enzyme substrate system, V_{max} and K_m are useful for estimation of intracellular reaction rate and quantitative comparison of alternative substrates. The smaller the value of K_m , the higher the enzyme affinity for the substrate [29]. In this study, reaction velocity via the amount and rate of glucose produced from enzymatic hydrolysis of cassava starch using glucoamylase sourced from rice was investigated.

2. MATERIALS AND METHODS

2.1 Materials / Reagents and Equipment

The major materials used were TMS 30572 cassava tubers, rice paddy, spectrophotometer, standard glucose D and Benedict solution. Cassava (*Manihot esculenta*) roots, TMS 30572 tubers, and rice paddy were purchased in Nigeria from Research Institute, Umudike, Abia State and Afikpo, Ebonyi State, respectively.

2.2 Preparation of Rice, Gluco Amylase and Buffer Solutions

Powdered rice malt was produced according to the procedure reported by Onyenekwe [3]. 2 g of the powered rice malt was suspended in 100 ml of distilled water at 60°C for ten minutes. This was followed by removing the supernatant to leave behind gluco amylase solution. Buffer solution (pH range of 5.0) was prepared using mixing adjuster.

2.3 Preparation of Starch (Substrate) and Standard Curve of Glucose Concentration

Cassava starch (substrate) and standard glucose curve were prepared as described by Oyewole and Obieze [30]. Distilled water was used in preparing blank solution and six (6) concentrations (50, 100, 150, 200, 250 and 300 ppm) from standard glucose (D) solution (0.1% w/v) [31] Standard glucose curve produced using spectrophotometer readings of these concentrations.

2.4 Experimental Procedure

Six (6) substrate (cassava /starch) concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% [w/v]) were prepared using distilled water and the time course of reaction (0, 10, 20, 30, 40, 50 and 60 minutes) were used in studying the enzymatic hydrolysis based on glucose production. Each substrate concentration was hydrolyzed at pH 5 and temperature of 40°C (as optimum conditions) using glucoamylase, as described by Franco and Ciacco [32]. The presence of reducing sugar during the hydrolysis was conducted using Benedict test [33,34]. Spectrophotometer reading of glucose produced at each time course of the reaction and blank solution were recorded. These readings were then used to generate data from the standard glucose curve. The generated data were used to plot several curves of glucose concentrations produced against reaction times. Also, the experimental data obtained were fitted into model Equation 3 [35] and Michaelis-Menten model Equation 1 [36], for estimating amount of glucose produced and reaction rate respectively; and all unknowns determined by iteration using Statistical Package for Social Scientists [SPSS] Version 20.

$$P = \left(\frac{P_{max} \times t}{t + t_{1/2}} \right) \quad (3)$$

Where, P and P_{max} are glucose produced at any instant and maximum glucose produced in g DE.litre⁻¹ respectively, t = reaction time (minute) and $t_{1/2}$ = half reaction time (minutes).

$$V = \frac{V_{max} [S]}{K_m + [S]} \quad (4)$$

Where, V = rate of glucose produced or reaction velocity at any instant (g DE. Litre-min⁻¹), V_{max} = maximum reaction velocity, [s] = concentration of glucose produced (g DE. Litre) and K_m = Michaelis Menten constant.

The models used were verified and validated using statistical computations and analyses reported by Antia and Assian [37,38]. Glucose concentration produced was converted into reaction velocity using Equation 5.

$$\text{Reaction velocity} = \frac{\text{Amount of glucose produced (g DEL}^{-1})}{\text{Reaction time (minutes)}} \quad (5)$$

It may be necessary that the reaction velocity should be standardized for uniform assessment.

In this regard, it is suggested the reaction velocity may be evaluated with respect to substrate concentration as Equation 5:

$$\frac{\text{Reaction velocity (g DE.L}^{-1}\text{.min)}}{\text{Substrate concntration (\% w/v)}} \quad (6)$$

The Equation 6 may be referred to a specific reaction velocity.

3. RESULTS AND DISCUSSION

3.1 Standard Glucose Calibration Curve and Test for Presence of Reducing Sugar

Spectrophotometer reading against standard glucose (D) concentration is presented in Fig. 1.

The standard calibration curve is seen to be polynomial function

$$Y = (-4.0 \times 10^{-11} \cdot G^4) + (1 \times 10^{-8} \cdot G^3) + (4 \times 10^{-6} \cdot G^2) + (0.0004 \times G) + 0.0021 \quad (7)$$

Where, 1 ppm = 1 mg.L⁻¹ = 0.001 g.L⁻¹, G = glucose concentration (ppm) as independent variable and Y = diluted value of spectrophotometer reading (nm) as dependent variable. From Fig. 1, the spectrophotometer reading increases as the glucose concentration is increased and has a strong direct relationship with coefficient of determination (R²) of 0.9955.

3.2 Production of Glucose with Time

The glucose concentration against reaction time is shown in Fig. 2.

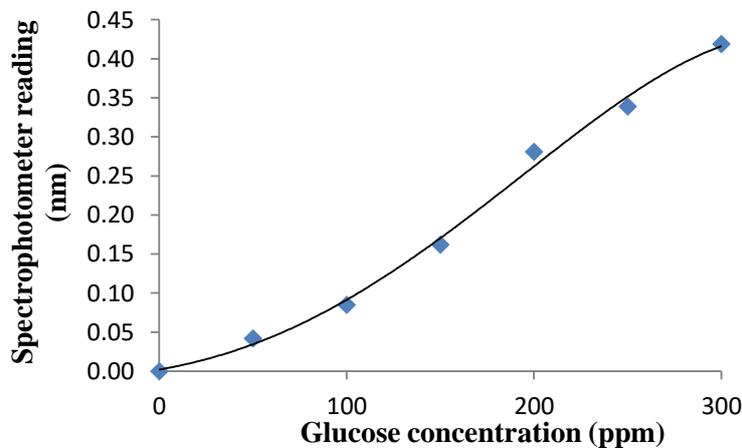


Fig. 1. Standard glucose calibration curve

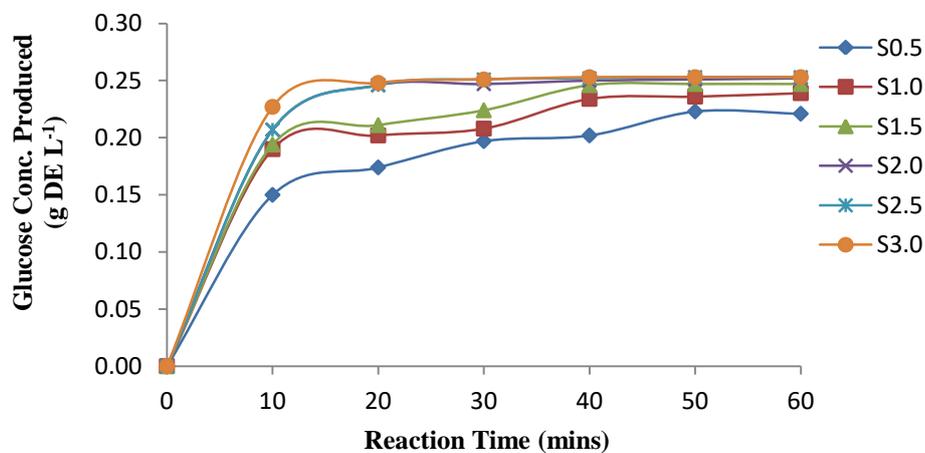


Fig. 2. Glucose concentration produced against reaction time

In Fig. 2, initially, the amount of glucose produced at reaction time, $t = 0$ minute was 0 g DE.L^{-1} . The amount of glucose started increasing rapidly until 10 mins. Thereafter, a gradual increase in glucose concentration from 10 minutes to 50 minutes reaction time was observed. Beyond this period, there was a decrease in glucose concentration. It is observed that the production of glucose was optimal ($0.253 \text{ g DE.L}^{-1}$) at 40 and 50 minutes corresponding to 3.0% w/v and 2.5% w/v substrate concentrations. This trend suggests that as the concentration of substrate is increased, the active site available for enzyme hydrolysis is increased due to increase in surface area of the substrate. Thus, expose the enzyme to be quickly saturated with the substrate; causing the rate of glucose production to be higher than at low substrate concentration of which active site available for the enzyme is limited. More so, at 20 minutes, the production of glucose may be discontinued and viewed as being economical when compared with increase of $0.003 \text{ g DE.L}^{-1}$ between 20 to 50 minutes. However, the extension of time for enzyme hydrolysis is suggested to accommodate any effect of pH and temperature on the production of glucose.

3.3 Estimating Maximum Glucose Produced

The model describing glucose concentration produced (P) with reaction time (t) is shown as Equation 3 whereas values of its parameters /constants in the six (6) substrate concentrations are given in Table 1.

From Table 1, the maximum estimated glucose produced ($0.269 \text{ g DE.L}^{-1}$) could be found in substrate concentration of 2.5% (w/v). The experimental value of maximum glucose production was $0.253 \text{ g DE.L}^{-1}$ from Fig. 2 at substrate concentration of 2.5 and 3.0% w/v. The amount of glucose got in this study may be due to the quantity of enzyme and substrate used. The longest estimated half reaction time ($t_{1/2}$) was found as 6.991 minutes for substrate concentration of 0.5% (w/v). The least estimated $t_{1/2}$ value (1.412 min) is suggested to be the fastest assay to produce reasonable amount of glucose within a given reaction time coupled with high substrate concentration.

Plots of predicted and experimental values of glucose concentration produced for each substrate concentration are presented in Figs. 3 to 8.

Table 1. Values of parameters /constants for model Equation 3

Substrate concentration	P_{\max}	$t_{1/2}$	Coefficient of determination (R^2)
$S_{0.5}$	0.245	6.991	0.994
$S_{1.0}$	0.248	3.596	0.991
$S_{1.5}$	0.263	3.946	0.995
$S_{2.0}$	0.266	2.562	0.997
$S_{2.5}$	0.269	2.687	0.997
$S_{3.0}$	0.261	1.412	0.999

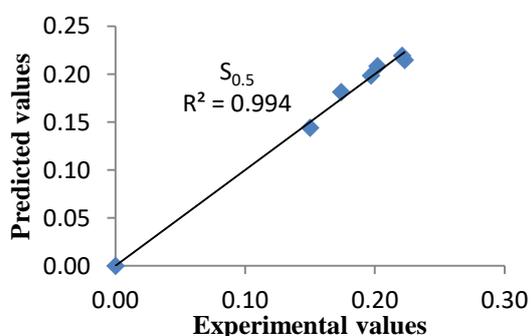


Fig. 3. Predicted against experimental values of glucose produced for 0.5% (w/v) substrate

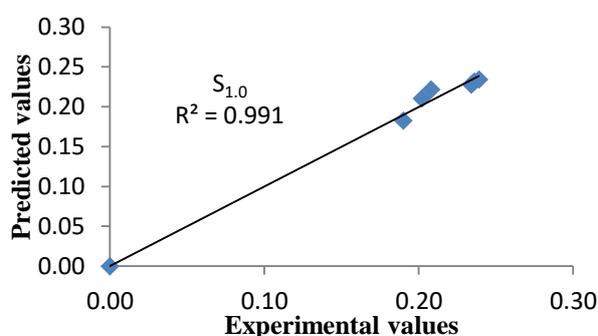


Fig. 4. Predicted against experimental values of glucose produced for 1.0% (w/v) substrate

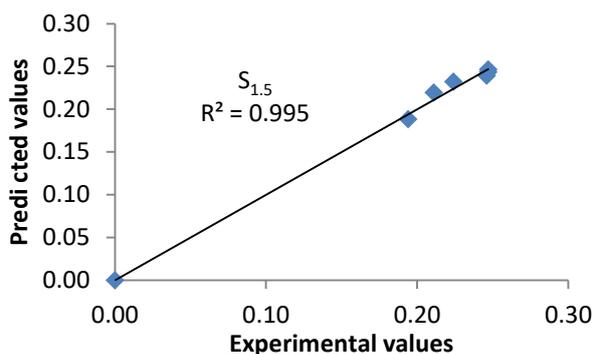


Fig. 5. Predicted against experimental values of glucose produced for 1.5% (w/v) substrate

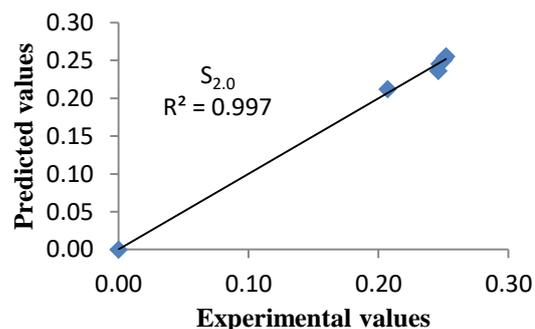


Fig. 6. Predicted against experimental values of glucose produced for 2.0% (w/v) substrate

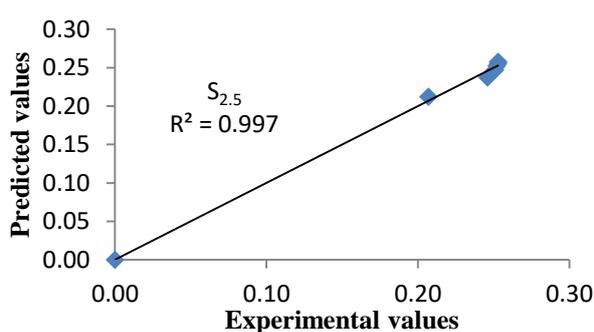


Fig. 7. Predicted against experimental values of glucose produced for 2.5% (w/v) substrate

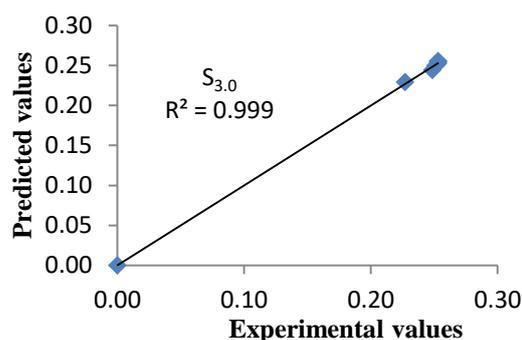


Fig. 8. Predicted against experimental values of glucose produced for 3.0% (w/v) substrate

The summary of statistical parameters for goodness of fit for model Equation 3 at different substrate concentrations is presented in Table 2.

Generally, the plots showed that the points for experimental and predicted values have positive correlation. The line for the slope equal one is the one for which predicted values would equal experimental values. All the values of R^2 for model Equation 3 were higher than the values of reduced Chi-square (χ_c^2), root mean square error (RMSE) and mean bias error (MBE). These are properties of satisfactory quality fit. Therefore,

the empirical Equation 3 is considered to be reasonably good for estimating glucose concentration produced.

The Michaelis Menten model was fitted into the experimental data based on Equation 2 which is a Linearized form of Equation 1. The slopes and intercepts of the regression were obtained for each reaction time, and used in determining values of maximum velocity (V_{max}), Michaelis Menten's constant (K_m) and coefficient of determination (R^2). These are presented in Table 3.

Table 2. Summary of statistical parameters for goodness of fit for model equation 3 for different substrate concentrations

Parameters	Values					
	$S_{0.5}$	$S_{1.0}$	$S_{1.5}$	$S_{2.0}$	$S_{2.5}$	$S_{3.0}$
Coefficient of correlation, r	0.9970	0.9955	0.9975	0.9985	0.9985	0.9995
Coefficient of determination, R^2	0.9940	0.9910	0.9950	0.9970	0.9970	0.9990
Reduced Chi-square, χ_c^2	0.0000	0.0000	0.0000	-0.0011	-0.0014	-0.0007
Mean bias error, MBE	0.0000	0.0000	0.0000	-0.0008	-0.0010	-0.0005
Root mean square error, RMSE	0.0054	0.0075	0.0057	0.0016	0.0020	0.0010

Table 3. Values of Michaelis Menten's constant for reaction velocity against substrate concentrations for various reaction times

Time, t (mins)	V_{max} (g DE per litre-min)	K_m (g DE per 100 ml)	R^2
10	0.0239	0.2927	0.9652
20	0.0134	0.2815	0.9251
30	0.0087	0.1793	0.8457
40	0.0068	0.1655	0.9910
50	0.0052	0.0872	0.9748
60	0.0044	0.0948	0.9953

From Table 3, the low value of K_m (0.0872) indicates greater enzyme affinity for substrate, and hence, forms the ES complex more quickly. This value corresponds to substrate concentration of 2.5% w/v. This is in line with the experimental data of which the maximum glucose produced was 0.253 g DE.L⁻¹ based on Fig. 2. On the other hand, the high value of the K_m (0.2927) portrays lesser enzyme affinity for substrate, and hence, forms ES complex more slowly.

Plots of predicted and experimental values of reaction velocities of glucose produced for each reaction time are presented in Figs. 9 to 14.

Generally, the plots in Figs. 9 to 14 showed that the points for predicted and experimental values have positive correlation. The line for the slope = 1 is the one for which predicted values would equal experimental values. The statistical parameters for goodness of fit for model Equation 1 for different reaction times showed good quality fit as presented in Table 4.

The values of reduced Chi-square (χ_c^2), root mean square error (RMSE) and mean bias error (MBE) were found to be lesser than the values of R^2 of the model Equation 1. Therefore, Equation 1 is considered to be reasonably good for

estimating the reaction velocity of the glucose concentration produced.

3.4 Optimum Reaction Velocity

The optimum reaction velocity range for the production of glucose may be estimated to be about 0.005060 – 0.006325 g DE per litre-min based on Fig. 2 and Equation 5. This implies that the enzyme was able to consume a given substrate concentration (2.5 and 3.0% w/v) and convert it at 50 and 40 minutes, respectively to maximum glucose production of 0.253 g DE.L⁻¹. Moreso, the reaction velocity obtained from the experiment is reasonably close to the values obtained from model Equation as 0.0052 – 0.0068 g DE.L⁻¹.min presented in Table 3. Hence, the experimental result is valid. Applying Equation 6, the values obtained for 2.5 and 3.0% w/v substrate concentrations are 0.00202 and 0.00208 g DE per litre-min per %w/v substrate concentration, respectively. These values are very close and may be approximated within a range. Thus, the optimum reaction velocity for the production of glucose using cassava starch and glucoamylase from rice may be taken as 0.00205 ± 0.00005 g DE per litre-min per % w/v substrate concentration. This implies that reaction velocity may best be evaluated in terms of reaction velocity per substrate concentration which may be referred to as specific reaction velocity.

Table 4. Summary of statistical parameters for goodness of fit for model Equation 1 for different reaction times

Parameters	Values					
	10 Mins	20 Mins	30 Mins	40 Mins	50 Mins	60 Mins
Coefficient of correlation, r	0.9678	0.9419	0.8927	0.9938	0.9878	0.9965
Coefficient of determination, R^2	0.9367	0.8871	0.7970	0.9877	0.9750	0.9931
Reduced Chi-square, χ_c^2	-0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mean bias error, MBE	-0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Root mean square error, RMSE	0.0006	0.0004	0.0003	0.0000	0.0000	0.0000

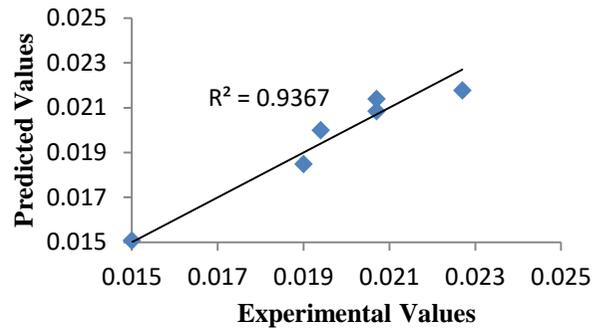


Fig. 9. Predicted against experimental values of reaction velocity after 10 minutes of hydrolysis

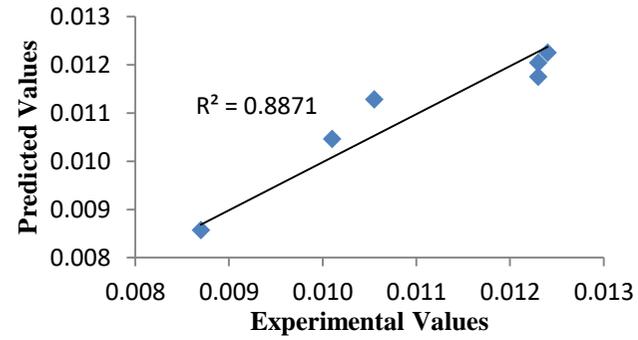


Fig. 10. Predicted against experimental values of reaction velocity after 20 minutes of hydrolysis

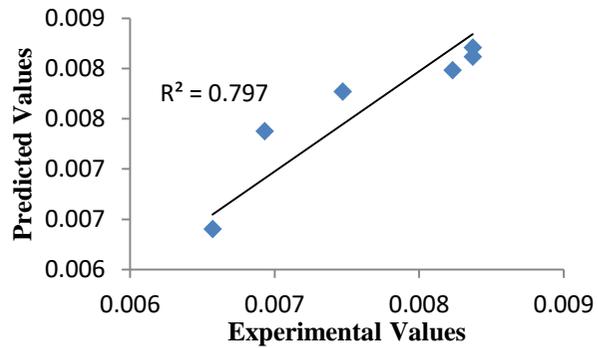


Fig. 11. Predicted against experimental values of reaction velocity after 30 minutes of hydrolysis

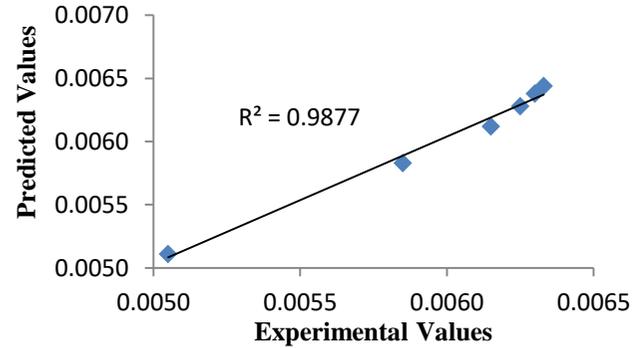


Fig. 12. Predicted against experimental values of reaction velocity after 40 minutes of hydrolysis

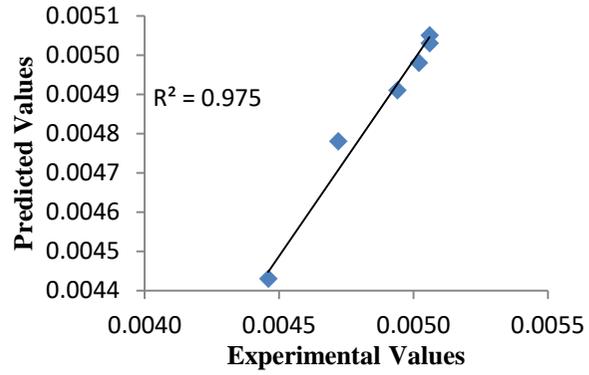


Fig. 13. Predicted against experimental values of reaction velocity after 50 minutes of hydrolysis

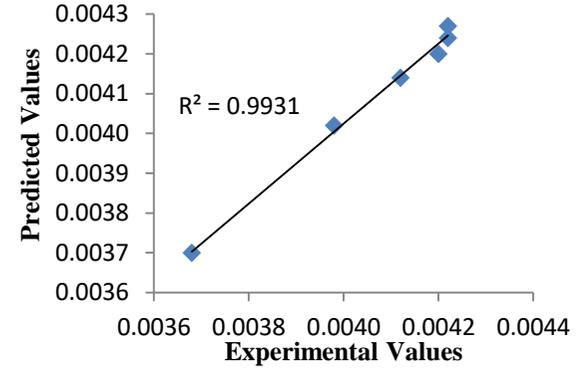


Fig. 14. Predicted against experimental values of reaction velocity after 60 minutes of hydrolysis

4. CONCLUSION

The models Equations 3 and 1 obtained could be used in estimating glucose concentration produced and the rate of glucose produced during enzymatic hydrolysis of starch. The optimum reaction velocity may be standardized with respect to substrate concentration. Hence, for maximum glucose production using cassava starch and glucoamylase from rice, the optimum reaction velocity range is 0.005060 to 0.006325 g D per litre-min (0.00205±0.00005 g D per litre-min per substrate concentration % w/v). Boosting of glucose syrup production is possible because the rice paddy used as a source of enzyme hydrolysis is in abundance locally.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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