

## Comparison of Models for Ethanol Production Using Simultaneous Saccharification and Fermentation (SSF) Of Cassava Starch

Daniel Eke Ogboso, Kazeem Ajadi Ibraheem\*, Nasiru Idris, Abubakar Ahmed and Farouk Otaru Abdulmalik

Chemical Engineering Department, Ahmadu Bello University Zaria, Nigeria

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**\*Corresponding author:** Kazeem Ajadi Ibraheem, Chemical Engineering Department, Ahmadu Bello University Zaria, Nigeria, Email: kazeemolawale634@gmail.com

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### ABSTRACT

This study focused on comparing different models for ethanol production through simultaneous saccharification and fermentation (SSF) of cassava starch. In recent years, modeling has become an essential tool for advancing the bioethanol industry by optimizing fermentation processes and facilitating their integration into industrial applications. Within this framework, the present work examined the applicability of two mathematical models Andrews and Aiba, for both molasses fermentation and SSF. Kinetic parameters for the Aiba and Andrews models were derived from experimental data using MATLAB and Origin-Lab software. The models were then simulated and validated against an independent set of experimental results not used in the parameter estimation. The results of modelling showed that  $\mu_{max} = 2.469$  1/h and  $K_s = 8.509$  g. L<sup>-1</sup> for the Aiba model, whereas  $\mu_{max} = 0.9489$  1/h,  $K_s = 0.002314$  g. L<sup>-1</sup> and  $K_i = 0.1536$  g. L<sup>-1</sup> for the Andrews model, which are too close to the values of other models in this study. The validation of both models showed that the Andrew model is more suitable for batch fermentation and ssf modelling at a low concentration, where the highest R squared was observed at  $S_0 = 6.775$  g. L<sup>-1</sup> with an R squared equal to 0.9526 and 0.9051 for the biomass, substrate and product concentrations, respectively. In contrast, the Aiba model was more accurate at a high initial substrate concentration.

**Keywords:** Hydrolysis, Fermentation, Cassava Starch, Kinetic Modeling, Simultaneous Saccharification, MATLAB

### 1. Introduction

Over the past century, global energy consumption has risen sharply due to industrialization, population growth, economic expansion and modernization<sup>1</sup>. Meeting future energy requirements has therefore become a major focus of researchers. At present, fossil fuels remain the dominant energy source<sup>2</sup>. However, their extensive use has led to serious environmental and economic challenges, including resource depletion and climate change caused by greenhouse gas emissions<sup>3</sup>. Renewable energy sources such as biofuels, wind, solar and hydropower offer sustainable alternatives to conventional fossil fuels while

mitigating their negative impacts<sup>4</sup>. Although the adoption of renewable energy has grown, it still accounts for only about 4.4% of total primary energy consumption<sup>5</sup>. This highlights the urgent need to develop, promote and invest in new renewable technologies to address future energy demands.

Among renewable options, biofuels such as bioethanol and biodiesel—primarily derived from biomass stand out as promising alternatives due to their contributions to energy sustainability, greenhouse gas reduction and rural development<sup>6</sup>. Currently, bioethanol is largely produced from starch- and sugar-based feedstocks. However, concerns have arisen about the

long-term viability of using food crops, particularly in relation to food security in developing nations<sup>7</sup>. To overcome this challenge, researchers are exploring alternative raw materials for bioethanol production that do not compete with the food supply. Molasses has emerged as a particularly attractive feedstock, as it offers low production costs, high ethanol yields and no direct competition with staple crops<sup>8</sup>.

The alcoholic fermentation of molasses using *Saccharomyces cerevisiae* has been widely studied and considerable interest has been directed toward developing kinetic models to better describe microbial growth, substrate consumption and ethanol production<sup>9</sup>. Mathematical modeling is especially valuable in optimizing fermentation processes, as it helps improve process control, reduce production expenses and enhance product quality. Fermentation and simultaneous saccharification and fermentation (SSF) models are generally divided into two categories: unstructured models, which treat biomass as a single compound with an overall formula and structured models, which consider the organism's internal biochemical changes<sup>10</sup>.

Modeling batch bioprocesses, however, presents challenges due to their inherent nonlinearities, time-dependent dynamics and the complexity of biological systems. Factors such as multiple reactions organism adaptability and environmental variability make these systems particularly difficult to predict<sup>7</sup>. Nevertheless, batch reactors are commonly used to investigate the key mechanisms governing fermentation kinetics such as limitations, inhibition effects, cell death and maintenance requirements<sup>8</sup>. While a wide range of fermentation models have been proposed, the model introduced by<sup>11</sup> is among the most widely accepted.

This study focuses on bioethanol production from molasses as a sustainable and cost-effective alternative to fossil fuels. By utilizing *Saccharomyces cerevisiae* in fermentation, the research seeks to optimize ethanol yields and minimize production costs. Furthermore, both structured and unstructured kinetic models will be applied and evaluated to better understand microbial behavior, substrate utilization and ethanol formation. Ultimately, mathematical modeling will serve as a key tool to enhance process efficiency, reduce costs and support the industrial-scale production of bioethanol as a cleaner energy source.

## 2. Methodology

### 2.1 Modelling

To better understand the reactor behaviour and to be able to simulate reactor operation, a flow conversion model must be developed using the well-established technique of combining reaction kinetics and Residence Time Distribution (RTD) information<sup>12</sup>. The model consists of the following, Aiba model<sup>13</sup> andrew model<sup>12</sup>, Aiba-andrews model<sup>12</sup>, Monod kinetics model, Tesier model and Webb model<sup>13</sup>.

**2.1.1. Monod's kinetics model:** The Monod equation is a mathematical model that describes microbial growth. It was introduced by Jacques Monod, who suggested that the rate of microbial growth in an aqueous system could be linked to the concentration of a limiting nutrient through this type of relationship. While it resembles the Michaelis-Menten equation in form, the Monod equation is empirical in nature, whereas the Michaelis-Menten model is derived from theoretical principles<sup>13</sup>.

In environmental engineering, the Monod equation is widely applied, particularly in modeling biological processes such as the activated sludge system used in wastewater treatment.

The Monod equation is:

$$\mu = \frac{\mu_m \cdot S}{K_s + S} \quad (1)$$

Where  $\mu$  is the specific growth rate of microorganisms,  $\mu_{max}$  is the maximum specific growth rate,  $S$  is the substrate concentration,  $K_s$  is the half-saturation constant (the substrate concentration at which the growth rate is half of  $\mu_{max}$ ).

### 2.2. Application

The rate of substrate utilization is related to the specific growth rate as follows:

$$r_{su} = -\mu X / Y \quad (2)$$

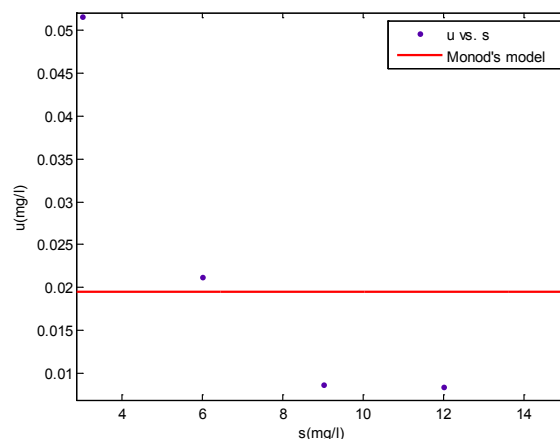
Where  $X$  is the total biomass (since the specific growth rate,  $\mu$  is normalized to the total biomass),  $Y$  is the yield coefficient,  $r_{su}$  is the rate of substrate utilization.

In some applications, multiple terms of the form  $[S/(K_s + S)]$  are multiplied together where more than one nutrient or growth factor has the potential to be limiting (e.g. organic matter and oxygen are both necessary to Heterotrophic bacteria). A very high yield coefficient, defined as the ratio of microbial biomass produced to the amount of substrate consumed, indicates that the available substrate is insufficient for proper utilization. (**Figures 1-5**) present the sample of plot obtained by the existing models.

#### 2.2.1. General model:

$$f(s) = \frac{\mu_m S}{K_s + S} \quad (3)$$

A negative R-square is possible if the model does not contain a constant term and the fit is poor (worse than just fitting the mean) (**Figure 1**).



**Figure 1:** Monod's model.

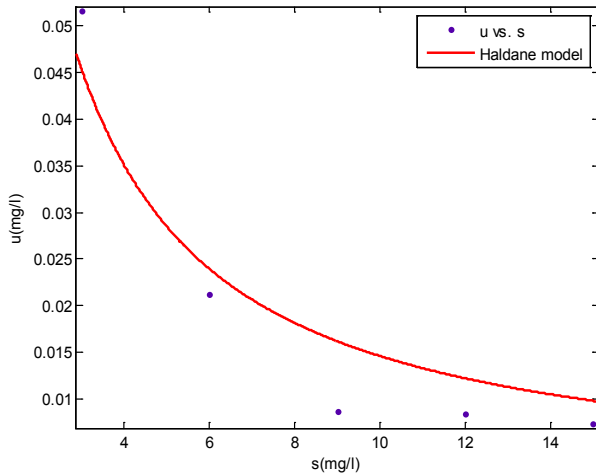
#### 2.2.2. Haldane model:

##### • General model:

$$f(s) = \frac{\mu_m \cdot S}{K_s + S + \frac{S^2}{K_i}} \quad (4)$$

- **Coefficients (with 95% confidence bounds):**  $K_i = 0.1095$  (-61.22, 61.44),  $K_s = 4.377$  (-3653, 3662),  $\mu_m = 1.355$  (-750.3, 753).

- **Goodness of fit:** SSE: 0.0001252, R-square: 0.9123, Adjusted R-square: 0.8246, RMSE: 0.007913 (**Figure 2**).



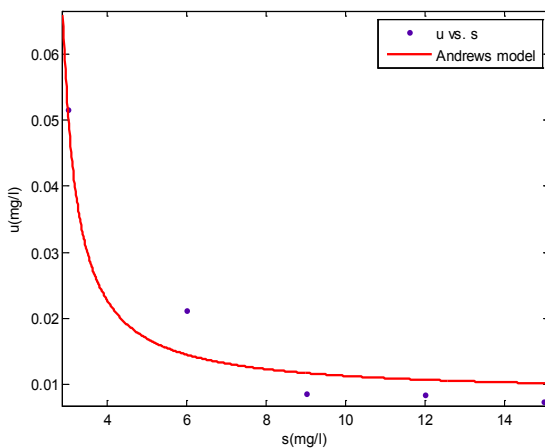
**Figure 2:** Haldane's model.

### 2.2.3. Andrews model:

According to<sup>12</sup> who developed an inhibition model for substrate-inhibited enzymatic kinetics:

$$\mu = \frac{\mu_m \cdot [S]}{K_s + [S] + \frac{[S]^2}{K_i}} \quad (5)$$

- **General model:** Coefficients (with 95% confidence bounds):  $K_i = 5023$  (-8.648e+006, 8.658e+006),  $K_s = 2.513$  (1.134, 3.891),  $\mu_m = 0.008412$  (-0.02297, 0.0398)
- **Goodness of fit:** SSE: 6.775e-005, R-square: 0.9526, Adjusted R-square: 0.9051, RMSE: 0.00582 (**Figure 3**).



**Figure 3:** Andrew's model.

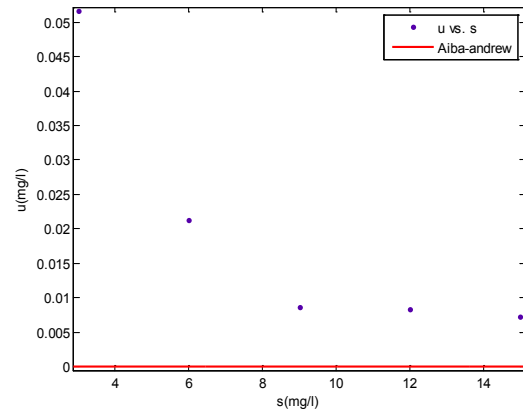
### 2.2.4. Aiba-andrews model:

- **General model:**

$$f(s) = \frac{\mu_m \cdot e^{-\frac{s}{k_i}}}{\left(1 + \frac{K_s}{s}\right) \left(1 - \frac{s}{K_i}\right)} \quad (6)$$

- **Coefficients (with 95% confidence bounds):**  $K_i = 0.4383$  (-8.052e+007, 8.052e+007),  $K_s = 0.5637$  (-5.466e+011, 5.466e+011),  $\mu_m = 0.01023$  (-1.585e+009, 1.585e+009)
- **Goodness of fit:** SSE: 0.003317, R-square: -1.323, Adjusted R-square: -3.647, RMSE: 0.04073

A negative R-square is possible if the model does not contain a constant term and the fit is poor (worse than just fitting the mean). Try changing the model or using a different Start Point (**Figure 4**).



**Figure 4:** Aiba-andrew's model.

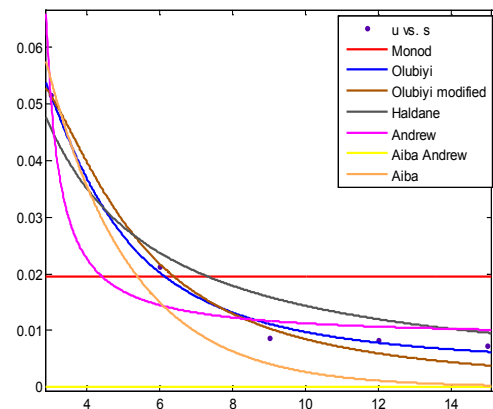
### 2.2.5. Aiba model

$$\mu = \frac{\mu_{\max} I}{T_k + I + \frac{I^2}{K_1}} \quad (7)$$

- **General model:**

$$f(s) = \frac{\mu_m \cdot s}{K_s + s + \frac{s^2}{K_i}} \quad (8)$$

- **Coefficients (with 95% confidence bounds):**  $K_i = 0.148$  (-62.01, 62.31),  $K_s = 2.973$  (-2149, 2155),  $\mu_m = 1.009$  (-417.3, 419.4)
- **Goodness of fit:** SSE: 0.0001274, R-square: 0.9108, Adjusted R-square: 0.8216, RMSE: 0.00798



**Figure 5:** Aiba's model.

## 3. Results and discussion

### 3.1. Experimental result

The experimental results associated to the processing set up of each independent variable are listed in (**Table 1**). Five level central composite design matrix and the experimental responses of the dependent variable (ethanol concentration) are listed. The regression equation coefficients were calculated and the data is fitted to a second order polynomial (pure quadratic) equation. The (ethanol concentration) by *saccharomyces cerevisiae* can be expressed in terms of the following regression equation which can be used for future prediction.

**Table 1:** Table for the models.

MODEL	Ki	Ks	Um	SSE	R <sup>2</sup>	ADJ R <sup>2</sup>	RSME
MONOD	-	1.022	0.01944	0.001428	-2.297	-2.297	0.01889
HALDANE	0.1095	4.377	1.355	0.0001252	0.9123	0.8246	0.007913
ANDREW	5023	2.513	0.008412	6.775	0.9526	0.9051	0.00582
AIBA-ANDREW	0.4383	0.5637	0.01023	0.003317	-1.323	-3.647	0.04073
AIBA	0.148	2.973	1.009	0.0001274	0.9108	0.8216	0.00798

$$Y = 45.9069 + 0.3852X_1 - 4.4712X_2 - 1.7669X_3 - 0.1264X_4 - 0.0163X_5 - 0.0132X_1^2 + 0.4151X_2^2 + 0.0268X_4^2 - 0.0032X_5^2 \quad (9)$$

Besides the linear effect of the ethanol concentration Y, the response surface method gives an insight about the parameter's quadratic and combined effects. The analysis was done by using both Fiser's f-test and student t-test statistical tools. The statistical significance of the ratio between residual error and the mean square residual error was tested using analysis of variance (ANOVA) was presented in (Table 2).

**Table 2:** Analysis of variance for ethanol production.

Variable	Coefficient	Se	T-stat	Pval
Constant	45.9096	15.1852	3.0231	0.0029
Substrate conc.	0.3852	0.1957	1.9684	0.0505
Ph	-4.4712	3.3654	-1.3286	0.1856
Temperature	-1.7669	0.5779	-3.0577	0.0026
Enzyme conc.	-0.1264	0.1162	-1.0872	0.2784
Time	-0.0163	0.0323	-0.5040	0.6149
(Substrate conc.) <sup>2</sup>	-0.0132	0.0229	-0.5778	0.5641
pH <sup>2</sup>	0.4151	0.3056	1.3582	0.1761
Temperature <sup>2</sup>	0.0268	0.0085	3.1558	0.0019
Enzyme conc.) <sup>2</sup>	0.0048	0.0048	1.0069	0.3153
Time <sup>2</sup>	-0.0032	0.0013	-2.4768	0.0142

Anova is a statistical technique that subdivides the total variation of a set of data into components associated to specific sources of variation. The regression equation obtained from the ANOVA shows that the R<sup>2</sup> (coefficient of determination) was 0.951 (a value > 0.75 indicates fitness of the model). This is an estimate of the fraction of the overall variation in the data accounted by the model and thus the model can explain 95.1% of the variation in the response. The adjusted R<sup>2</sup> is 0.907, which indicates that the model is good for a good statistical model, the R<sup>2</sup> value should be in the range of 0-1.0 and the nearer to 1.0 the value is the more fit the model is deemed to be.

The response surfaces can be used to predict the optimum range for different values of the test variables and the major interactions between the test variables can be identified from the circular or elliptical nature of the contours.

Since saccharification occurred simultaneously with fermentation, a certain amount of glucose was expected to be released during the process. In this study, however, the glucose derived from starch was rapidly consumed by fermentation and therefore rarely detected during SSF. Similar early glucose depletion has been observed by other researchers when using substrates such as soluble starch<sup>14</sup> or raw cassava starch<sup>15</sup> in combination with immobilized yeast. Nutrient limitation has been suggested as a factor influencing saccharification efficiency<sup>16</sup>. Another important consideration is the temperature difference between the optimal activity of amyl glucosidase (55°C) and the optimal growth of yeast (35°C). Lower temperatures are generally preferred, as they enhance yeast metabolism and accelerate the

completion of fermentation<sup>17</sup>. A potential alternative highlighted in the literature is the use of thermo-tolerant yeast strains, which allow fermentation at around 42°C while achieving higher ethanol yields<sup>18</sup>. Additional factors may also explain the reduced saccharification performance, including nutrient depletion and the limited amylolytic activity of *S. cerevisiae*, which may contribute to saccharification only to a small extent<sup>16</sup>.

Studies on raw wheat flour as the substrate for SSF have shown that glucose production increases during the initial stages of the process, closely linked to a rapid decrease in maltose consumption, with maximum ethanol yields of about 69 g/L<sup>19</sup>. Some authors have reported even greater ethanol concentrations 93 g/L or 140 g/L when using yeast strains engineered for enhanced ethanol productivity<sup>18,20</sup>. Since conventional yeasts lack amylolytic enzymes and cannot directly convert starch into ethanol, genetic modification has been explored. As summarized in (Table 3), ethanol-fermenting microorganisms have been developed by engineering yeasts to express enzymes such as  $\alpha$ -amylase and amyloglucosidase<sup>21</sup> or to ferment xylose, a major pentose sugar present in cellulosic biomass, which is a commonly used feedstock for bioethanol production<sup>22</sup>.

**Table 3:** F-stat analysis ethanol production.

F-stat					
Sse	Dfe	Dfr	Ssr	F	Pval
186.96	185	10	177.89	17.698	0

### 3.2. Optimization of process variables on ethanol production

The factors affecting the Simultaneous saccharification and fermentation of Cassava starch with Glucoamylase enzyme and *S. cerevisiae* culture was studied using CCD experiments. The substrate concentration (X1, g/l), the pH (X2), the temperature (X3, °C) and the Glucoamylase enzyme concentration (X4, IU) were chosen as the independent variables as shown in (Table 4). Ethanol Concentration (Y) was chosen as the dependent output variable. Thirty-six experiments based on the CCD were carried out with different combinations of variables and the results were presented in (Table 4). The data obtained from the four-level central composite design matrix were used to develop models in which each dependent variable (Ethanol Concentration, Y) was obtained as the sum of the contributions of the independent variable through second order polynomial equation and interaction terms. The actual ethanol concentration obtained in the experiments and the yields predicted by the model equation (2).

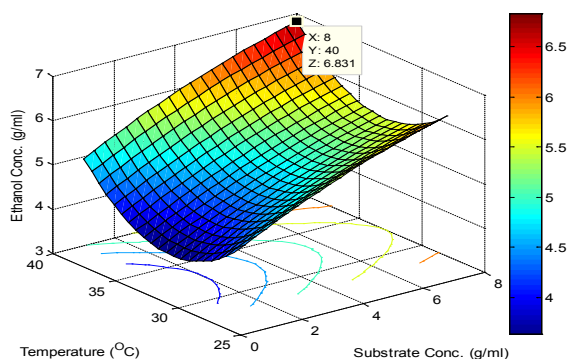
It showed that the regression coefficients of all the linear term and all quadratic coefficients of X1, X2, X3 and X4 were significant at < 1% level. The individual effect of all the four parameters studied quadratic effects and interaction effects between the dependent variables were found to be significant from the response surface plots shown in Figures 6 to 15. The clear elliptical shape of the curve shown in (Figures 6-15) indicates the interaction effect between all the four independent



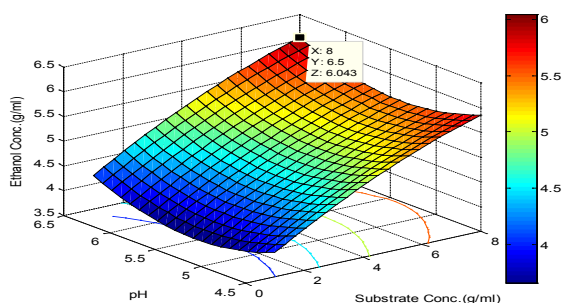
variables were significant. Hence optimum combinations of substrate concentration, pH, pretreatment temperature, with the enzyme concentration play a major role to get maximum bioconversion of cassava starch to ethanol. The ANOVA result of quadratic regression model for Y is described in Table 4. ANOVA of the regression model for Y demonstrated that the model was significant due to an F-value of and a very low probability value ( $P < 0.005$ ). The P-values are used as a tool to check the significance of each of the coefficients, which in turn indicate the pattern of the interactions between the variables. Smaller value of P then it was more significant to the corresponding coefficient. It showed that the experimental yields fitted the second order polynomial equation well as indicated by high  $R^2$  values (0.907).

**Table 4:** Anova analysi independent variable.

Source	Sum sq	Df	Mean sq	F
X1	59.625	3	19.8751	21.99
X2	0.712	3	0.2372	0.26
X3	6.529	3	2.1762	2.41
X4	0.852	3	0.2841	0.31
X5	119.723	6	19.9539	22.08
Error	159.088	176	0.9039	
Total	363.848	196		



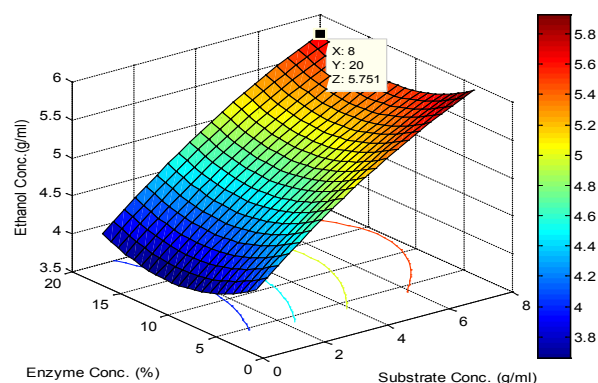
**Figure 6:** 3d plot showing the effect of substrate concentration and temperature on ethanol concentration.



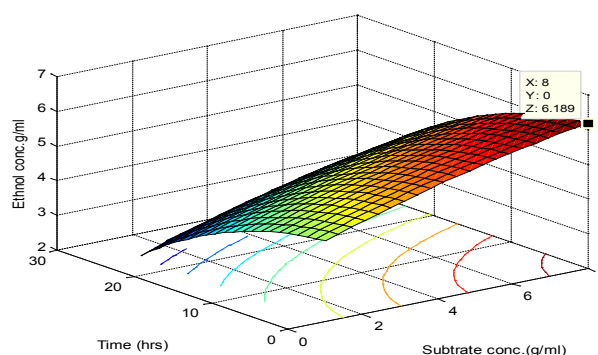
**Figure 7:** 3d plot showing the effect of substrate concentration and ph on ethanol concentration.

The orientation of the principal axes of the contour plots between the variable's substrate concentration and temperature, substrate concentration and pH, Substrate Concentration and enzyme concentration pH and temperature, pH and enzyme concentration and temperature and enzyme concentration indicated that the mutual interactions between these set of variables had a significant effect on the ethanol Concentration. The values of P less than 0.005 in Table 2 also indicate the significance of interaction effects of all the four chosen independent variables. Based on the model, the optimal working

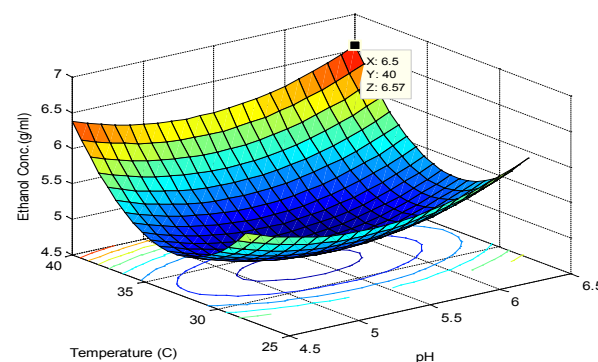
conditions were obtained to attain high percentage conversion of starch. The optimum values of the parameters X1, X2, X3 and X4 were found to be 160 g/l, 5.5, 30°C and 50 IU respectively and were obtained by solving the regression equation (2) using the experimental data with square MATLAB version 7.0.



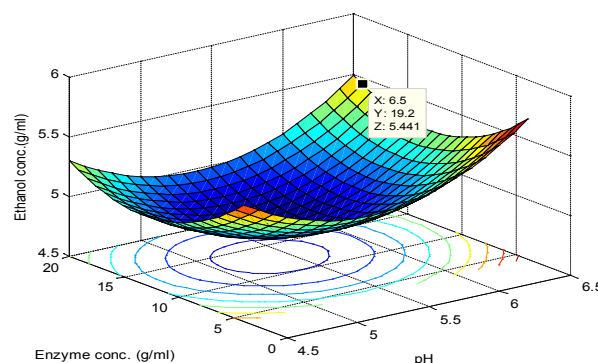
**Figure 8:** 3d plot showing the effect of substrate concentration and temperature on ethanol concentration.



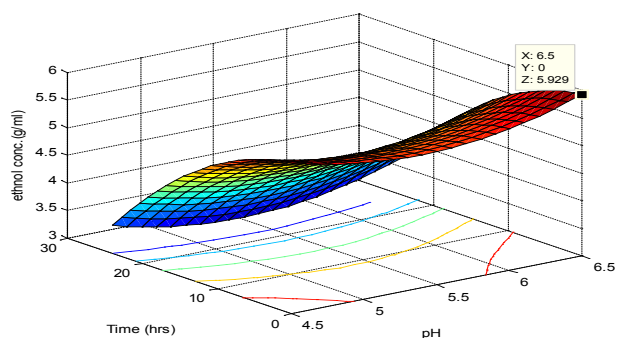
**Figure 9:** 3d plot showing the effect of substrate concentration and time on ethanol concentration.



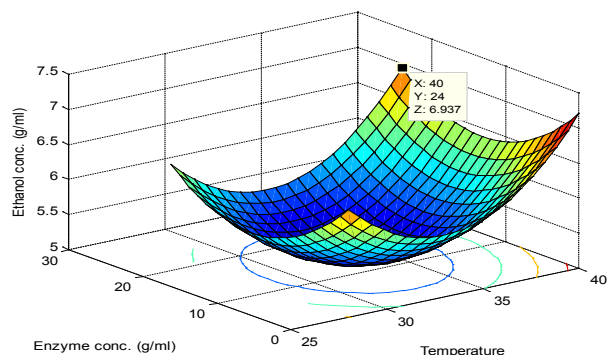
**Figure 10:** 3d plot showing the effect of ph concentration and temperature on ethanol concentration.



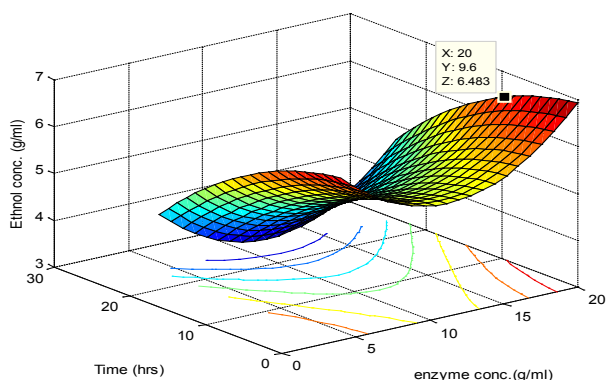
**Figure 11:** 3d plot showing the effect of enzyme concentration and ph on ethanol concentration.



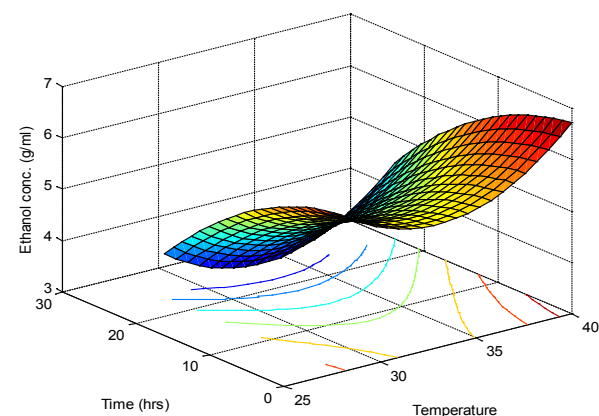
**Figure 12:** 3d plot showing the effect of time and ph on ethanol concentration.



**Figure 13:** 3d plot showing the effect of enzyme concentration and temperature on ethanol concentration.



**Figure 14:** 3d plot showing the effect of time and enzyme concentration on ethanol concentration.



**Figure 15:** 3d plot showing the effect of time and temperature on ethanol concentration.

### 3.3. Starch liquefaction optimization

The experiment was carried out for up to 2 hours, which is the reaction time reported in the literature as necessary for complete

starch hydrolysis<sup>19</sup>. Specifically, a 2-hour liquefaction step was required to achieve full hydrolysis when raw wheat flour was used as the substrate. Shorter liquefaction periods (0.5-1 hour) resulted in a wort with higher viscosity, which hindered the efficient breakdown of glucose polymers. In contrast, performing the process at a lower temperature (55°C) with increased enzyme activity produced the highest liquefaction yield. However, when liquefaction was conducted at 55°C with an enzyme dosage of 100 U/g-cassava starch, the yield was the lowest.

### 3.4. Kinetic study

The essential feature of the model developed by<sup>23</sup> was that starch was structured into susceptible and resistant fractions that differed in the rate constants of hydrolysis. There are as many kinetic models for kinetic study but the best of them all is the monod kinetics as it can study the gradual growth and decline of the cell (yeast). In this work, the kinetic study was based on the stage of the experiment at which the greatest ethanol concentration was obtained. At this stage, the glucose concentration becomes the substrate concentration (as it is the substance that is being converted to ethanol) and its being plotted against time. The monod kinetic was linearized using the Line-weaverburk scheme as follow;

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (10)$$

$$\text{Linearalise; } \frac{1}{\mu} = \frac{K_s}{\mu_{\max}} \cdot \frac{1}{S} + \frac{1}{\mu_{\max}} \quad (11)$$

A plot of  $\frac{1}{\mu}$  against  $\frac{1}{S}$  will give a slope of  $\frac{K_s}{\mu_{\max}}$  and an intercept of  $\frac{1}{\mu_{\max}}$ . From the graph, the values of  $\mu_{\max}$  and  $K_s$  were determined as 0.3428hr<sup>-1</sup> and 0.275g/ml respective

### 3.5. Liquefaction with $\alpha$ - and $\beta$ -amylase

Maximizing ethanol productivity requires optimizing the amount of cassava starch available for saccharification in order to generate sufficient glucose for fermentation. To improve liquefaction efficiency,  $\beta$ -amylase was employed for starch hydrolysis and its performance compared with that of  $\alpha$ -amylase. Typically,  $\beta$ -amylase is expected to produce greater quantities of maltose during starch breakdown than  $\alpha$ -amylase<sup>24</sup>, as it is capable of converting polysaccharides containing  $\alpha$ -1,4 glycosidic bonds entirely into maltose. This enzyme is also widely applied in industrial ethanol production<sup>25</sup>. However, in this study,  $\alpha$ -amylase demonstrated considerably higher maltose production than  $\beta$ -amylase.

Ethanol production through SSF of wheat-based substrates using *Saccharomyces cerevisiae* was previously reported by<sup>18</sup>, with yields of 44.2 g/L from fine wheat flour and 34.1 g/L from damaged wheat flour. In comparison, ethanol production obtained from LG1 in this experiment (38.6 g/L) was significantly higher than that from damaged wheat. Similarly, ethanol fermentation of sago starch slurries with *Zymomonas mobilis* produced 100 g/L starch conversion and 40 g/L ethanol<sup>26</sup>, which was comparable to the ethanol yield achieved with LG1.

The ethanol yield from LG1 (0.49 L ethanol/kg flour) was nearly 61% greater than that from LG2 and closely matched the average yield from sugarcane (0.50 L ethanol/kg dry biomass)<sup>27</sup>. Furthermore, yields obtained from both LG1 and LG2 in this study were markedly higher than those reported for other agricultural residues such as wheat straw (0.29 L/kg) and

sugarcane bagasse (0.28 L/kg)<sup>28</sup>. Due to the superior performance of LG1, subsequent SSF experiments were conducted using this substrate. During SSF of LG1, both glucose and maltose generated in the liquefaction step were completely consumed within 12 hours, after which reducing sugar levels remained constant, confirming the completion of fermentation<sup>18</sup>.

A one-step ethanol production process from soluble starch using a co-culture of amylolytic yeast (*Saccharomyces diastaticus*) and *Saccharomyces cerevisiae* was reported by<sup>29</sup>. In that study, a maximum of 24.8 g/L ethanol was achieved from 60 g/L soluble starch, though yields declined with increasing starch concentrations in the medium. In another study, *Candida tropicalis* YMEC14, a low-rate amylolytic yeast, was used for ethanol production from corn soluble starch in the presence of  $\alpha$ -amylase<sup>30</sup>. Results showed that higher ethanol concentrations were obtained when  $\alpha$ -amylase was included, compared with fermentation by the yeast alone. The highest ethanol concentration reported was 43.1 g/L from 9% soluble starch, while 24 g/L was obtained from 6% starch. Additionally, fed-batch fermentation produced the highest yield, reaching 56 g/L.

#### 4. Conclusion

The review clearly describes the concept and potential of cost effective microbial SSF process for starch-based bio-ethanol production in the background of the feasibility of other processes. The process so-called Simultaneous Saccharification and Fermentation (SSF) was incited with expensive enzymatic hydrolysis with simultaneous fermentation and was virtually similar as for the separate process. The process has been modified by combining two separate processes in one vessel to reduce the time and increase the efficiency of the overall process. The presence of yeast or bacteria along with enzymes or enzyme containing microorganism minimizes substrate inhibition effects by reducing the sugar accumulation in the vessel. The presence of ethanol in the broth makes the mixture less susceptible to unwanted microorganisms' contamination and hence helped in increasing the overall ethanol yield and productivity using the SSF process. Such developed process with improved hydrolysis-fermentation efficiency could help in significant reduction of ethanol production costs. Such technological advancement towards green and clean bio-ethanol production will contribute to reducing the fossil fuels dependency for future energy needs and hence eliminating the chances of air pollution caused due to combustion of petroleum-based derivatives.

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