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ORIGINAL RESEARCH ARTICLE

PREPARATION OF YEAST *(SACCHAROMYCES CEREVISIAE)* BIOMASS FROM SUGARCANE BAGASSE

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ABSTRACT

In this study, the pretreatment by milling, dilute-acid hydrolysis of sugarcane bagasse, and subsequent fermentation of its glucose product was performed to investigate the effects of process conditions on the production of Saccharomyces cerevisiae. The hydrolysis was carried out using HCl at three substrate concentrations of 20, 25, and 30% (w/v) of bagasse to distilled water. Hydrolysis parameters (time, acid concentration, and temperature) were varied for each case of substrate concentration in full factorial experiments, and an optimum glucose yield of 1.907 g/L was obtained with the 20% (w/v) substrate concentration, at conditions of 10 min time, 0.5 M acid concentration, and 80°C temperature. Thereafter, fermentation experiment was performed with S. cerevisiae in the product of hydrolysis. An optimum ca. 207 yeast number of colonies (yield: 20, 700, 000 cfu/ml) was achieved in 40 h, and the growth of S. cerevisiae was governed by the kinetic equation, ln X_t = 8.4338 + 0.2943t.

1.0 Introduction

The yeast *Saccharomyces cerevisiae* is one of the most abundantly used yeasts in industry (Snoek, 2007), and the most utilized microorganism for the biosynthesis of ethanol due to its several advantages such as high fermentation efficiency, rapid growth, effective sugar use, osmotolerance, thermotolerance, and more (Reis et al., 2013). From economic point of view, lignocellulosic ethanol production using S. cerevisiae has been considered a viable route to commercialize the second-generation biofuel (Ask, 2013). The cost of bioethanol production would be reduced further if S. cerevisiae is prepared also from lignocellulosic biomass.

Sugarcane bagasse is an abundant lignocellulosic biomass and residue from industrial and agricultural processing (Jabasingh and Nachiyar, 2012). It is mainly used in the manufacture of pulp and paper products, building materials, fuel and cellulosic ethanol (Jabasingh and Nachiyar, 2012). Fermentation as an important industrial process of using microorganisms for several applications is affected by factors such as the concentration of microorganisms, cells, cellular components, enzymes, pH, and temperature (Yusuf, 1999; Rao, 2005). It is a suitable means of producing S. cerevisiae from lignocellulosic biomasses by either simultaneous hydrolysis and fermentation (SSF), or separate hydrolysis and fermentation (SHF). Both the SSF and SHF have been widely studied.

However, it has been difficult to identify one best method to hydrolyze lignocellulosic material into its building blocks, especially that this is affected by the source of the lignocellulose material and its geographic location (Kumar et al., 2010; Tomás-Pejó et al., 2011; Binod et al., 2011; Stephen, Mabee and Saddler, 2013). Generally, the hydrolysis methods have been extensively studied using pretreatment, chemical hydrolysis, enzymatic hydrolysis, or combinations thereof, with varying results (Olsson and Hahn-Hägerdal, 1993; Galbe and Zacchi, 2012). Pretreatment can affect the yields of sugar obtained during hydrolysis, and the techniques range from physical, physicochemical, chemical, and biological pretreatments (Rosalin and Amit, 2007).

The present study has adopted physical pretreatment by mechanical milling, followed by dilute acid hydrolysis under moderate heating of 80 – 97°C since acid hydrolysis is able to penetrate lignin without pretreatment of biomass, hence breaking down the cellulose and hemicellulose polymers into individual sugar molecules (Galbe and Zacchi, 2002). Sulphuric and hydrochloric acids in concentrated or dilute forms are the most studied for hydrolysis of lignocellulosic biomass (Lenihan et al., 2010). Again, dilute acid hydrolysis is preferred because equipment corrosion is lessened and less amount of acid is utilized, except that the high processing temperatures for achieving high cellulose conversion would need to be reduced.

The task of identifying the optimum conditions of dilute acid hydrolysis, and efficient sugar utilization during fermentation are optimization problems to be addressed (You et al., 2017). Nonetheless, this study investigated the effects of acid concentration, temperature, and time on the acid hydrolysis of milled sugarcane bagasse at different substrate concentrations in order to obtain the conditions of optimum glucose yield for use in preparation of S. cerevisiae by fermentation. Hydrolysis experiment was systematically carried out in a full factorial design of experiment.

2. Materials and Methods

2.1 Equipment, Materials and Chemicals

A 14-liter locally fabricated bioreactor with a working volume of 10.5 liters was used. Its suitability for metabolites synthesis has been investigated previously (Aransiola, 2016), by converting corn cob into bioethanol. It has a temperature controller, and the agitator speed varied from 200 to 250 rpm. An air compressor supplied air to the vessel, while a flow meter measured the air flow rate. Other apparatuses and materials used for the study include a Water bath, Weighing balance, Whatman filter papers, Test tubes, Conical flask, Beaker, Glass stirrer, Petri dish, Sugarcane bagasse, Glucose analysis kit (Cypress diagnostics), Colony counter, Drop pipette, and Sieve. Also used for the study were analytical grade Sodium hydroxide, Ammonium sulphate, Potassium hydrogen phosphate, Magnesium sulphate heptahydrate, Distilled water, Yeast extract agar, Cell biomass, concentrated ethanol, and HCl.

2.2 Pretreatment of Sugarcane bagasse

The sugarcane bagasse used for this study was obtained in Ilorin, Nigeria. It was air-dried, milled and screened to select the fraction of particles in the sub-150 µm size range. A sieve analysis was carried out on a sieve shaker equipped with sieves of pore diameters of 150 µm, 75 µm, 48 µm, and 38 µm, respectively. The stainless steel sieves were stacked with the sieve size increasing from top to bottom (38 µm, 48 µm, 75 µm, and 150 µm). About 75 g of the milled sugarcane bagasse was placed into the top sieve. The sieves were shaken until no more particles

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passed through. All the prepared samples were stored at room temperature in sealed polythene bags.

2.3 Acid Hydrolysis of Sugarcane bagasse

Acid hydrolysis of the bagasse particles was performed using HCl and water bath as heat source. Three sets of substrates with concentration of 20, 25, and 30% (w/v) were prepared by the addition of 20, 25, and 30 g of bagasse particles to 100 mL distilled water respectively. In order to optimize the hydrolysis process, for each case of substrate concentration, the effects of hydrolysis time (10 – 60 min), temperature (80 – 97°C), and acid concentration (0.1 – 0.5 M) were investigated by varying these parameters in 23 Factorial experiment (8 runs) as presented in Table 1.

Table 1: Levels of factors used in the factorial experimental design of acid hydrolysis

Parameter	Time (min)	Acid concentration (M)	Temperature (°C)
Upper Level	60	0.5	97
Lower Level	10	0.1	80

Each substrate sample previously prepared in 100 mL of distilled water was added to 100 mL of acid (0.1/0.5 M), maintaining (1:1 v/v) of acid to distilled water. It was then heated in water bath at set temperature (80/97°C) for the required time (10/60 min). At the completion of hydrolysis duration, the mixture was taken out of the water bath and then filtered. The filtrates (hydrolysate) were immediately kept in the Refrigerator for analysis of glucose concentration.

2.3.1 Glucose estimation

Glucose analysis was carried out using glucose analysis kit with wavelength of 505 nm, temperature 37 °C or 15 – 25 °C critical temperature, and the instrument was adjusted to zero with distilled water. The standard was prepared by measuring 10 µl of glucose standard and 1mL of working reagent into a test tube. Also, 10 µl each of the 8 samples of hydrolysate obtained from factorial experimental runs and 1 ml of working reagent were measured into a different test tube, the mixture was shaken and incubated for 15 – 20 min at room temperature and the absorbance for each was taken using a UV spectrophotometer. Absorbance recorded for each sample was used for the calculation of the glucose concentration using appropriate equations provided with the kit. The absorbance of the standard was observed to be 0.175.

2.4 Fermentation Experiment

2.4.1 Pre-Culture Medium

The Saccharomyces cerevisiae (Baker's yeast) was provided by the Department of Microbiology of University of Ilorin, and it was maintained on agar at room temperature. Afterward, 200 mL of distilled water was measured into a conical flask, followed by the addition of 4.6 g of yeast extract agar, and stirred until a homogenous mixture was obtained. The solution was then autoclaved at 121°C and 15 psi for 15 min. After autoclaving the solution, it was poured into a petri dish and then allowed to cool. Cells of S. cerevisiae were transferred into the petri dish aseptically in a sterile condition and incubated at 30°C for 6 h.

2.4.2 Sterilization of Fermenter

The bioreactor (fermenter) was sterilized using concentrated ethanol and using sterile distilled water at 90°C. Fermentation media was prepared by measuring 1 L of the hydrolysate into a

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conical flask, followed by the addition of 4 g of yeast extract, 2 g of ammonium sulphate, 2 g of potassium hydrogen phosphate, 0.75 g of magnesium sulphate heptahydrate, and the pH was adjusted to 5.5 using NaOH.

2.4.3 Sterilization of the Fermentation Medium

The fermentation media was autoclaved at 121°C, 15 psi for 15 min to maintain sterile condition.

2.4.4 Fermentation Proper

The yeast (biomass) was aseptically added into the sterile culture of glucose obtained at highest yield conditions. This was added to the fermenter with the agitation rate set at 160 rpm. The reactor temperature was set to 34°C and samples were withdrawn at a 4-hour interval for 48 h. Air was supplied to the reactor at a safe working pressure. Analysis was carried out on the sample immediately to determine the yield of S. cerevisiae.

2.4.5 Yeast estimation

Plate count was carried out to determine the yeast biomass produced in each run. Five (5) test tubes were filled with 9 mL of distilled water each, as they were all sterilized in an autoclave at 121°C, 15 psi for 15 min. These were all labeled 1 to 9. Then, 1 ml of the sample was measured into the first test tube using a drop pipette and was shaken; 1 ml was measured from the first test tube and added to the second test tube and so on until the desired dilution factor is reached (i.e. for the dilution factor of 10^{-5} it was stopped at test tube 5). Then, 1 ml was measured from the desired dilution level into the petri dish and the prepared yeast extract agar that has been allowed to cool was poured into the petri dish aseptically to avoid contamination. It was covered, taped with a paper tape and kept aside for 24 – 48 h after which the organisms were counted using colony counter. The essence of the dilution is to avoid overcrowding of the organism on the plate and to ensure proper counting of the organisms.

3. Results and Discussion

3.1 Particle Size Distribution of milled bagasse

The particle size distribution (PSD) of the milled bagasse as obtained after screening with the sub 150 µm sieves (150 µm, 75 µm, 48 µm, and 38 µm sieves) is shown in Figure 1. The PSD as presented is indicative of bagasse composed of 9.6% wt particles in the sub 38 µm size range, 13.7% wt in the 38 - 48 µm size range, 22.8% wt in the 48 - 75 µm size range, 17.6% wt in the 75 - 150 µm size range, and 36.3% wt above the 150 µm size.

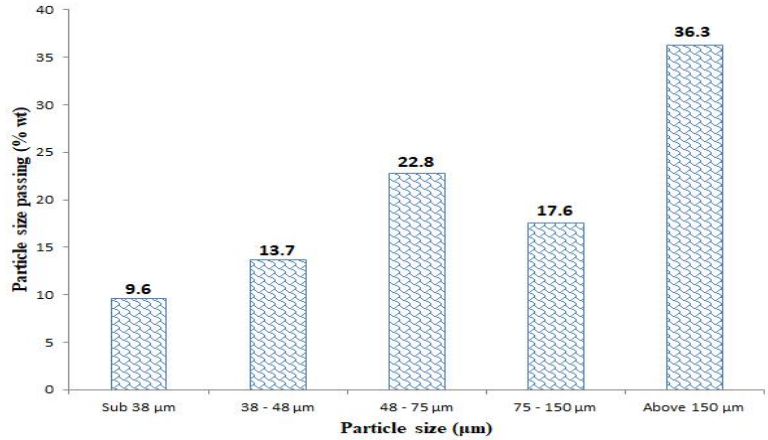

Figure 1: Particle size distribution of milled sugarcane bagasse in sieve pore diameters of 150µm, 75 µm, 48 µm, and 38 µm.

Previous studies on enzymatic hydrolysis of lignocellulosic biomass have shown that smaller particle sizes of the substrate resulted in lower crystallinity index, higher specific surface area, cellulose adsorption, cellulose adsorption per specific surface area, and larger average pore size (Li et al., 2016). That study also revealed a strong dependence of glucose yield on the crystallinity index and average pore size of sugarcane bagasse. Smaller particle sizes are desirable for high yield of glucose during enzymatic hydrolysis. However, acid hydrolysis could show contrary results to enzymatic hydrolysis since acids are able to penetrate the lignin without pretreatment. Thus, the effect of particle size was not considered in the present study. The bagasse biomass after milling exhibited particle size distribution dominated by particles above 48 µm size range.

3.2 Acid Hydrolysis

3.2.1 Acid Hydrolysis with 20% (w/v) Substrate concentration

Presented in Table 2 is the factorial experimental design matrix for the acid hydrolysis with 20% (w/v) substrate concentration, and glucose yield as the response.

Table 2: Experimental Design Matrix for Acid hydrolysis with 20% (w/v) Substrate concentration

Run	Time (min)	Acid conc. (M)	Temperature (°C)	Glucose yield (g/L)
1	60.00	0.10	80.00	1.406
2	60.00	0.50	80.00	1.321
3	10.00	0.50	97.00	1.583
4	60.00	0.50	97.00	1.068
5	10.00	0.10	97.00	1.096
6	60.00	0.10	97.00	1.532
7	10.00	0.10	80.00	1.017
8	10.00	0.50	80.00	1.907

Using the 20% (w/v) Substrate concentration, the results of glucose yield in Table 2 shows that the highest yield of 1.907 g/L was obtained under acid hydrolysis conditions of 10 min hydrolysis duration, 0.50 M acid concentration, and 80°C temperature (Run 8). The varying yields of glucose under different conditions also indicate that the hydrolysis parameters have influences on the process. Effects of these parameters on the glucose yield were investigated, and results are presented in Figures 2 to 4 for the hydrolysis temperature, acid concentration, and time respectively.

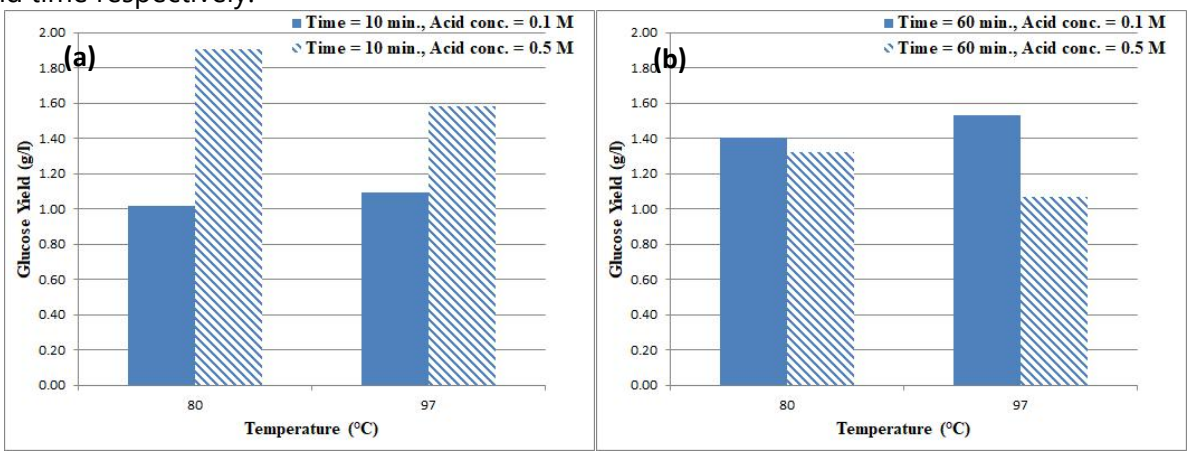

Figure 2: Effect of hydrolysis temperature on glucose yields at different acid conc. in (a) 10 min., and (b) 60 min.

As presented in Figure 2(a) and (b), the effect of hydrolysis temperature on the yield of glucose is dependent on acid concentration. Regardless of duration of hydrolysis (10 or 60 min), higher temperature favored glucose yield at 0.1 M acid concentration (Figure 2a). But, the reverse is the case with 0.5 M acid concentration where the glucose yield decreased with increase in temperature irrespective of hydrolysis duration (Figure 2b). This observation could be due to a combined effect of temperature and acid concentration on the yield of glucose. However, this supports the claim that hydrolysis with dilute acids requires high processing temperatures for achieving high cellulose conversions, and thus lower temperatures favor the hydrolysis with more concentrated acids.

In Figure 3, the influence of acid concentration on the yield of glucose is dependent on hydrolysis time. When 10 min hydrolysis time was employed (Figure 3a), an increase in acid concentration from 0.1 to 0.5 M caused the glucose yield to increase regardless of temperature (80 or 97°C). But, with 60 min hydrolysis time (Figure 3b) at either temperature of 80 or 97°C, an increase in acid concentration from 0.1 to 0.5 M caused the glucose yield to decrease. This indicates an interactive effect of acid concentration and duration of hydrolysis.

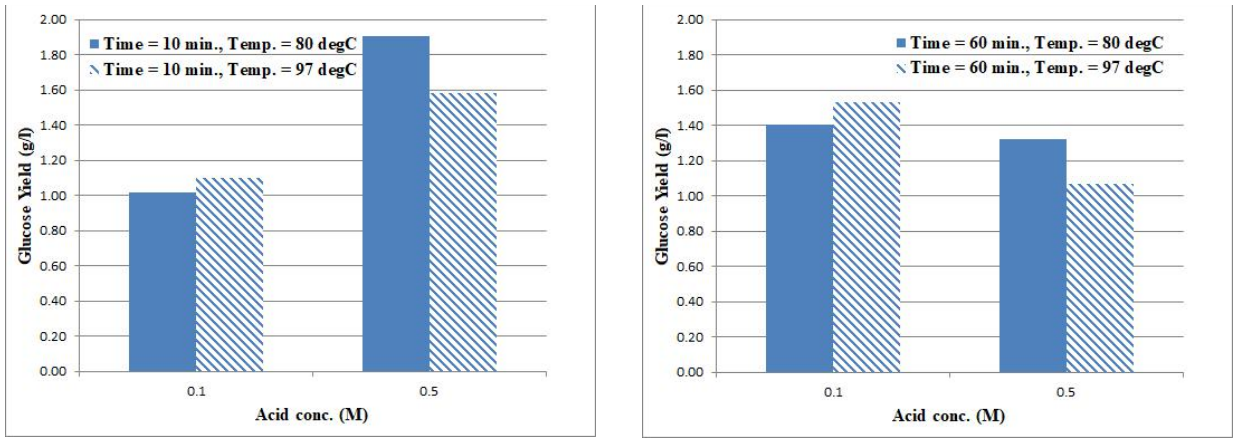

Figure 3: Effect of hydrolysis acid concentration on glucose yields at different temperatures in (a) 10 min., and (b) 60 min.

Again in Figure 4, the effect of hydrolysis time on the yield of glucose is also not independent of acid concentration. The more dilute acid of 0.1 M concentration was influenced positively by increase in hydrolysis time yielding more glucose at 60 min, regardless of temperature employed (Figure 4a). When 0.5 M acid concentration was used, the glucose yield was favored by shorter hydrolysis time and lower hydrolysis temperature (Figure 4b).

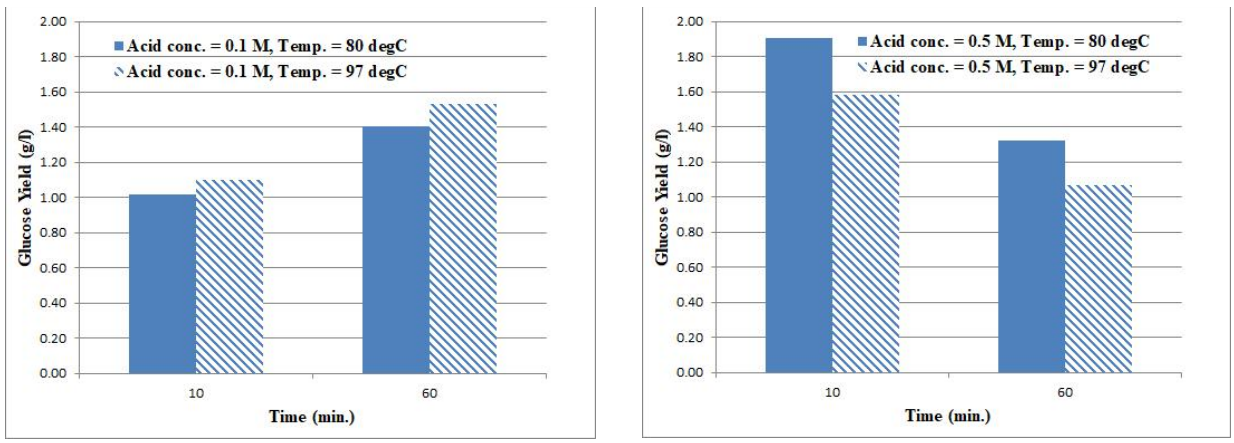

Figure 4: Effect of hydrolysis time on glucose yields at different temperatures and (a) 0.1 M, (b) 0.5 M acid concentrations.

Generally, the interactive effects of the hydrolysis parameters may not be disconnected with the molecular adsorption and liquid-solid mass transfer mechanisms that occur during the hydrolysis. It seems that when concentrated acids are used, longer hydrolysis time and higher temperatures encourage quick adsorptive mass transport of products on the surface of the lignocellulosic substrate which blocks the pores available for mass transfer. In this case, shorter hydrolysis time and mild temperatures are desirable to allow the hydrolysis to proceed further. Because such obstructions may not occur with the more dilute acids, the conversion process is able to proceed at higher temperatures and for longer duration of hydrolysis.

Using the glucose yield results presented in Table 2, a statistical analysis of variance (ANOVA) was carried out on the data, with result presented as in Table 3.

Table 3: ANOVA Table for Acid hydrolysis with 20% (w/v) Substrate concentration

Source	SS	DF	MS	F Value	p – value (Prob > F)
Model	0.65	6	0.11	1577.31	0.0193
X_1	0.009554	1	0.009554	138.23	0.0540
X_2	0.085	1	0.085	1235.14	0.0181
X_3	0.017	1	0.017	252.24	0.0400
X_1X_2	0.46	1	0.46	6711.45	0.0078
X_1X_3	0.001695	1	0.001695	24.53	0.1268
X_2X_3	0.076	1	0.076	1102.27	0.0192
Residual Error	0.00006912	1	0.00006912		
Corrected Total	0.65	7			

X_1 = Time (min.), X_2 = Acid conc. (M), and X_3 = Temp. (°C)

SS: sum of squares, DF: Degree of freedom, MS: Mean Square

The ANOVA results in Table 3 indicate the model F-value is 1577.31, which implies that the model is significant, and there is only a 1.93% chance that it could occur due to noise. The R^2 value of 0.9999, and the difference between the Adjusted R^2 (0.9993) and the Predicted R^2 (0.9932) shows that the model is reliable. Also, using the p-value (< 0.05) criteria, the model terms with significant effects are the main effects from acid concentration (p-value = 0.0181) and temperature (p-value = 0.0400), and the interactive effects of (time and acid concentration, p-value = 0.0078), and (acid concentration and temperature, p-value = 0.0192). Among all the model terms, the combined effect of time and acid concentration has the most significant effect on glucose yield.

A linear regression model Equation (1) was fitted to represent the parametric effects of the acid hydrolysis process variables on the yield of glucose:

$$Y = 1.37 - 0.035X_1 + 0.10X_2 - 0.047X_3 - 0.24X_1X_2 + 0.015X_1X_3 - 0.098X_2X_3 \quad (1)$$

where Y is the yield of glucose (g/L), and X_1, X_2 and X_3 are the coded values of time, acid concentration and temperature respectively.

Based on the model Equation (1), the graph of 'Predicted' against 'Actual' for glucose yield as presented in Figure 5 indicates a good correlation between the predicted and the actual values, and hence the model can be used to predict other hydrolysis process conditions.

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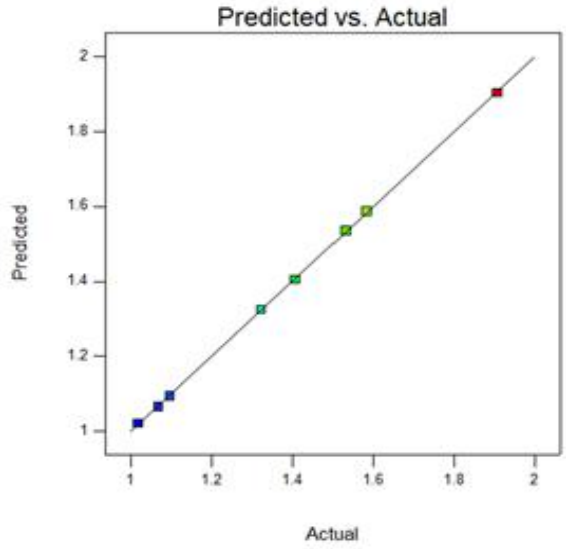

Figure 5: Predicted vs. Actual values for glucose yield in the acid hydrolysis with 20% Substrate concentration.

With the experiment on 20% substrate concentration, numerical optimization was performed which gave optimal glucose yield of 1.907 g/L (corresponding to experimental run 8) at hydrolysis conditions of 10 min time, 0.5 M acid concentration, and 80oC temperature with a resulting desirability of 0.999, out of a total number of 35 solutions.

3.2.2 Acid Hydrolysis with 25% (w/v) Substrate concentration

The factorial experimental design matrix for the acid hydrolysis with 25% (w/v) substrate concentration with glucose yield as the response is presented in Table 4.

Table 4: Experimental Design Matrix for Acid hydrolysis with 25% (w/v) Substrate concentration

Run	Time (min)	Acid conc. (M)	Temperature (°C)	Glucose yield (g/L)
1	60.00	0.10	80.00	1.42857
2	60.00	0.50	80.00	1.29143
3	10.00	0.50	97.00	1.61143
4	60.00	0.50	97.00	1.28000
5	10.00	0.10	97.00	1.35429
6	60.00	0.10	97.00	1.66286
7	10.00	0.10	80.00	1.05714
8	10.00	0.50	80.00	1.57143

With 25% (w/v) Substrate concentration, the results of glucose yield in Table 4 shows that the highest yield of 1.66286 g/L was obtained under conditions of 60 min hydrolysis time, 0.10 M acid concentration, and 97°C temperature (Run 6). The effects of hydrolysis parameters on the glucose yield were also investigated and results are presented as shown in Figures 6 to 8.

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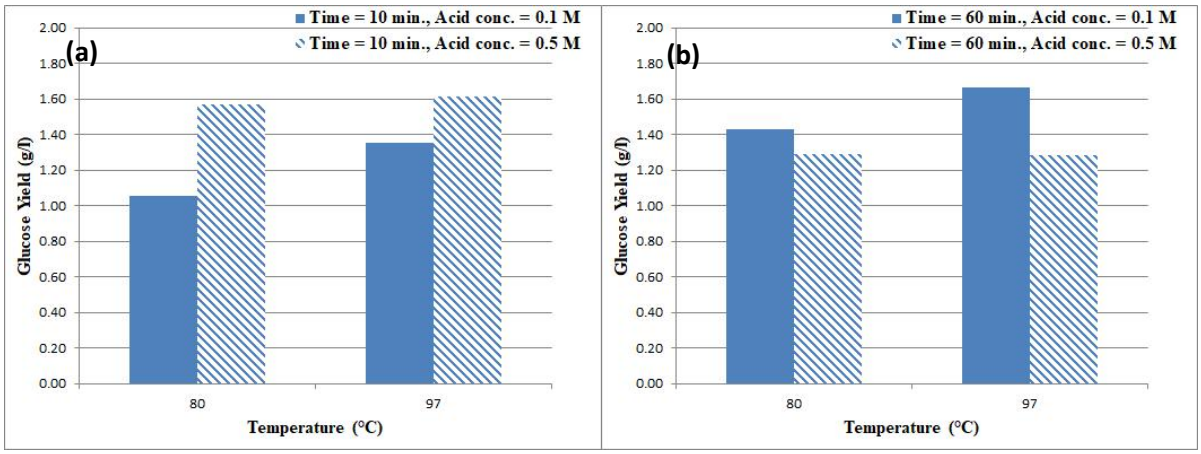

Figure 6: Effect of hydrolysis temperature on glucose yields at different acid conc. in (a) 10 min., and (b) 60 min.

Figure 6 shows that at the substrate concentration of 25% (w/v) and employing 0.1 M acid concentration, the effect of hydrolysis temperature on glucose yields presented a similar trend as observed with 20% (w/v) substrate concentration: higher temperature favored glucose yield irrespective of hydrolysis time. But, when the 0.5 M higher concentration of acid was used, the effect of hydrolysis temperature depended on hydrolysis time: higher temperature favored glucose yield at 10 min, while it caused the glucose yield to decrease at 60 min. More so, the changes in hydrolysis temperature had no significant effect on glucose yields at 0.5 M acid concentration. Again with 0.5 M concentration, the molecular mass transfer could have affected the hydrolysis process by reaching equilibrium at the early time; longer time only decreased the conversion to glucose or affected selectivity by forming undesired products. However, Figures 7 and 8 showing the effects of acid concentration and hydrolysis time present similar relationship between the process variables and yield of glucose as observed with 20% (w/v) substrate concentration.

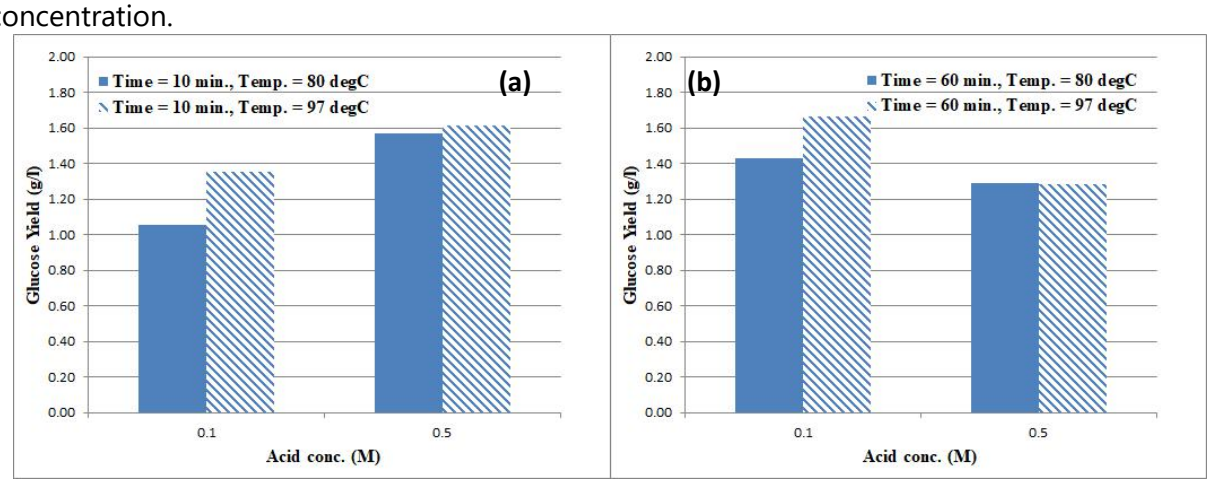

Figure 7: Effect of hydrolysis acid concentration on glucose yields at different temperatures in (a) 10 min., and (b) 60 min.

In Figure (7a), when 10 min hydrolysis time was employed, higher concentration of acid caused the glucose yield to increase regardless of temperature employed (80 or 97°C). With 60 min hydrolysis time (Figure 7b) lower acid concentration favored the yield of glucose at either temperature of 80 or 97°C. Again, this shows that the effect of acid concentration is dependent on duration of hydrolysis. Further, the change in substrate concentration of 20 to 25% (w/v) did

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not influence the effect of hydrolysis time on the yield of glucose (Figure 8): hydrolysis time affected the glucose yield in a way that depended on acid concentration.

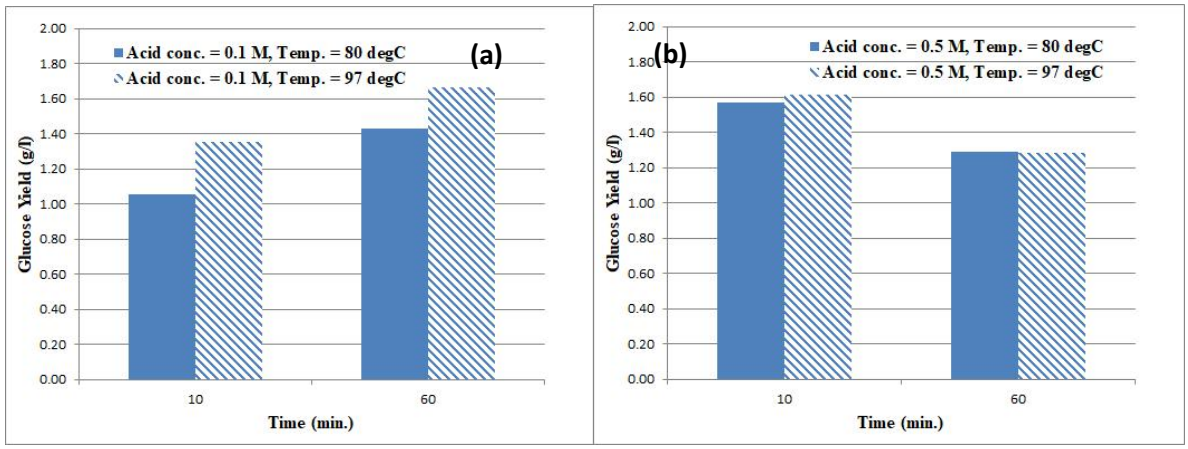

Figure 8: Effect of hydrolysis time on glucose yields at different temperatures and (a) 0.1 M, (b) 0.5 M acid concentrations.

In Figure 8, when 0.1 M acid concentration was used, longer duration of hydrolysis of 60 min yielded more glucose regardless of temperature employed (Figure 8a). But, with 0.5 M acid concentration, the glucose yield was favored by shorter hydrolysis time and lower hydrolysis temperature (Figure 8b). Table 5 shows the result of ANOVA performed on Table 4 experimental results of glucose yield for 25% (w/v) substrate concentration.

Table 5: ANOVA Table for Acid hydrolysis with 25% (w/v) Substrate concentration

Source	SS	DF	MS	F Value	p – value (Prob > F)
Model	0.29	6	0.048	2954.33	0.0141
X_1	0.0005878	1	0.0005878	36.00	0.1051
X_2	0.007902	1	0.007902	484.00	0.0289
X_3	0.039	1	0.039	2401.00	0.0130
X_1X_2	0.21	1	0.21	12769.00	0.0056
X_1X_3	0.001633	1	0.001633	100.00	0.0635
X_2X_3	0.032	1	0.032	1936.00	0.0145
Residual Error	0.00001633	1	0.00001633		
Corrected Total	0.29	7			

X_1 = Time (min.), X_2 = Acid conc. (M), and X_3 = Temp. (°C)

SS: sum of squares, DF: Degree of freedom, MS: Mean Square

The ANOVA results in Table 5 indicating the model F-value of 2954.33 and p – value of 0.0141 imply that the model is significant and there is only a 1.41% chance that it could occur due to noise. The model is shown to be reliable based on R^2 value of 0.9999, and the close agreement between the Adjusted R^2 (0.9996) and the Predicted R^2 (0.9964). Using the p-value (< 0.05) criteria, the model terms with significant effects are the acid concentration (p-value = 0.0289), temperature (p-value = 0.0130), and the interactive effects of time and acid concentration (p-value = 0.0056), and acid concentration and temperature (p-value = 0.0145). The combined effect of time and acid concentration has the most significant effect on glucose yield.

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A linear regression model Equation (2) was fitted to represent the parametric effects of the acid hydrolysis process variables on the yield of glucose (Y):

$$Y = 1.41 + 0.008571X_1 + 0.031X_2 + 0.070X_3 - 0.16X_1X_2 - 0.014X_1X_3 - 0.063X_2X_3 \quad (2)$$

Based on the model Equation (2), the graph of 'Predicted' against 'Actual' for glucose yield as presented in Figure 9 also indicates a good correlation between the predicted and the actual values.

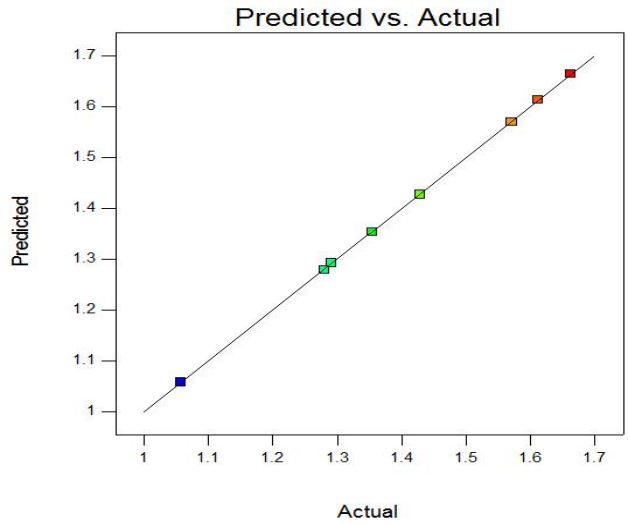

Figure 9: Predicted vs. Actual values for glucose yield in the acid hydrolysis with 25% Substrate concentration.

With the experiment on 25% substrate concentration, numerical optimization produced an optimal glucose yield of 1.663 g/L at 60 min hydrolysis time, 0.1 M acid concentration, and 97oC hydrolysis temperature (corresponding to Run 6), having a desirability of 0.999.

3.2.3 Acid Hydrolysis with 30 % (w/v) Substrate concentration

Table 6 shows the factorial experimental design matrix for the acid hydrolysis with 30% (w/v) substrate concentration, using glucose yield as the response.

Table 6: Experimental Design Matrix for Acid hydrolysis with 30% (w/v) Substrate concentration

Run	Time (min)	Acid conc. (M)	Temperature (°C)	Glucose yield (g/L)
1	60.00	0.10	80.00	1.37714
2	60.00	0.50	80.00	1.21714
3	10.00	0.50	97.00	1.44000
4	60.00	0.50	97.00	1.27429
5	10.00	0.10	97.00	1.23429
6	60.00	0.10	97.00	1.61714
7	10.00	0.10	80.00	1.01714
8	10.00	0.50	80.00	1.40000

For the experiment results with 30% (w/v) substrate concentration as presented in Table 6, the highest yield of 1.61714 g/L was obtained under conditions of 60 min hydrolysis time, 0.10 M acid concentration, and 97°C temperature (Run 6). The effects of the three hydrolysis parameters on the yield of glucose are discussed using Figures 10 to 12.

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In Figure 10, an increase in hydrolysis temperature (80 – 97°C) favored glucose yield at all conditions of hydrolysis time and acid concentration.

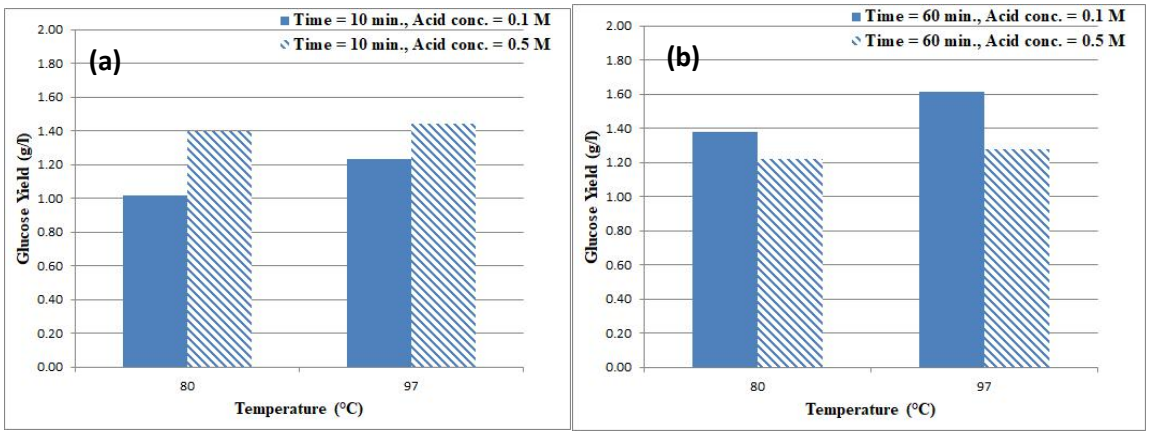

Figure 10: Effect of hydrolysis temperature on glucose yields at different acid conc. in (a) 10 min., and (b) 60 min.

By careful look at Figure 10, and following the observations made in sections 3.2.1 (Figure 2) and 3.2.2 (Figure 6) on the effects of hydrolysis temperature using experimental results with 20 and 25% (w/v) substrate concentrations, it can be inferred that substrate concentrations play an important role in the hydrolysis especially at higher acid concentrations. Particularly using 0.5 M acid concentration and substrate concentration of: (a) 20% (w/v), glucose yield decreased with increase in temperature irrespective of hydrolysis time of 10 or 60 min; (b) 25% (w/v), the effect of hydrolysis temperature depended on hydrolysis time where higher temperature favored glucose yield at 10 min and decreased the glucose yield at 60 min; (c) 30% (w/v), glucose yield increased with increase in temperature irrespective of hydrolysis time of 10 or 60 min. There was a gradual migration from negative to positive effect of hydrolysis temperature on glucose yield as substrate concentration increased from 20 to 30% (w/v). Generally, in these three cases the variation in hydrolysis temperature from 80 to 97°C caused insignificant changes in the glucose yield.

Using 30% (w/v) substrate concentration in the hydrolysis experiment, the trends of effects of acid concentration and hydrolysis time on yield of glucose as presented in Figures 11 and 12 are similar to the observations made with experimental results of 20 and 25% (w/v) substrate concentrations. The change in substrate concentration from 20 to 30% (w/v) did not influence the effect of acid concentration or hydrolysis time on the yield of glucose.

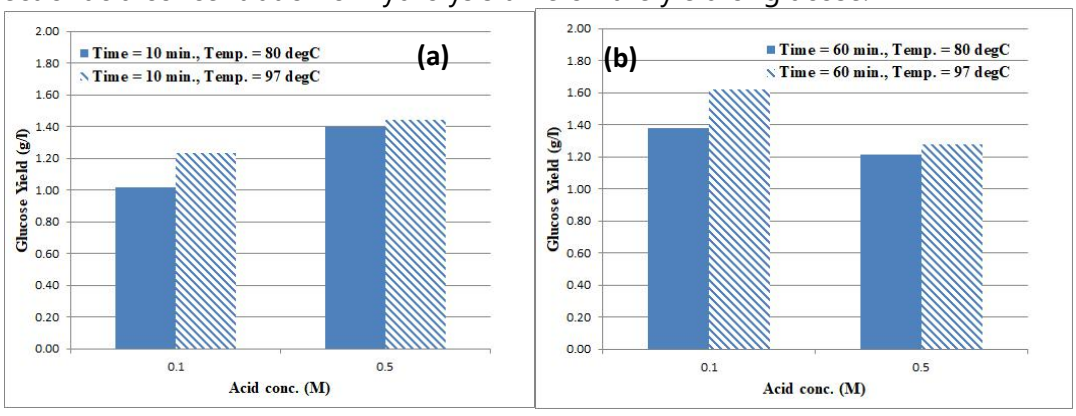

Figure 11: Effect of hydrolysis acid concentration on glucose yields at different temperatures in (a) 10 min., and (b) 60 min.

In Figure 11a, when 10 min hydrolysis time was employed, increase in acid concentration favored glucose yield regardless of temperature employed (80 or 97°C) but with 60 min hydrolysis time (Figure 11b) decrease in acid concentration favored the yield of glucose at either temperature of 80 or 97°C. Based on the graph presented in Figure 12, hydrolysis time affected the glucose yield in a way that depended on acid concentration.

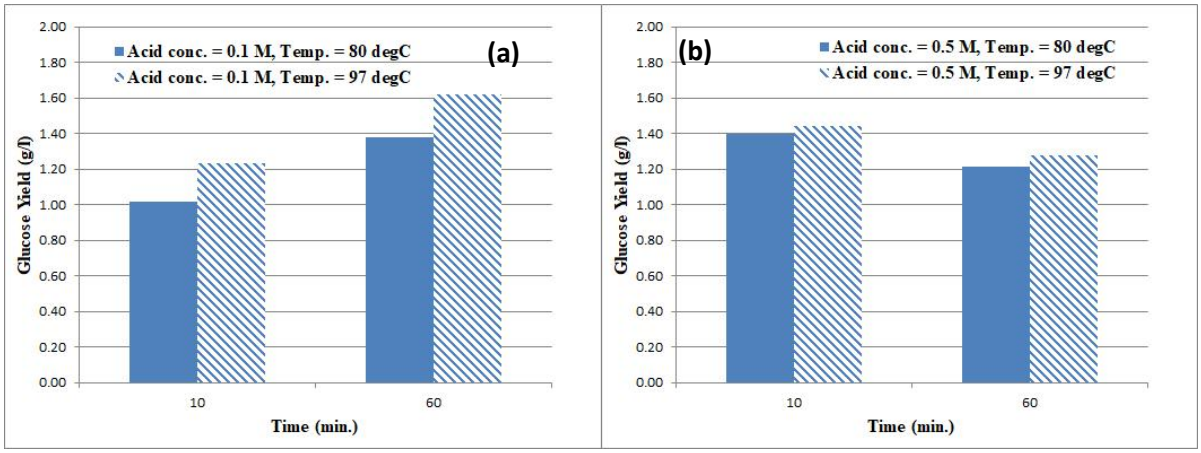

Figure 12: Effect of hydrolysis time on glucose yields at different temperatures and (a) 0.1 M, (b) 0.5 M acid concentrations.

In Figure 12, when 0.1 M acid concentration was used, longer duration of hydrolysis of 60 min yielded more glucose regardless of temperature employed (Figure 12a). But, with 0.5 M acid concentration, the glucose yield was favored by shorter hydrolysis time and higher hydrolysis temperature (Figure 12b). Presented in Table 7 is the result of ANOVA performed on Table 6 experimental yields of glucose for 30% (w/v) substrate concentration.

Table 7: ANOVA Table for Acid hydrolysis with 30% (w/v) Substrate concentration

Source	SS	DF	MS	F Value	p – value (Prob > F)
Model	0.22	6	0.037	9149.00	0.0080
X_1	0.019	1	0.019	4761.00	0.0092
X_2	0.0009184	1	0.0009184	225.00	0.0424
X_3	0.038	1	0.038	9409.00	0.0066
X_1X_2	0.15	1	0.15	36481.00	0.0033
X_1X_3	0.0002	1	0.0002	49.00	0.0903
X_2X_3	0.016	1	0.016	3969.00	0.0101
Residual Error	0.000004082	1	0.000004082		
Corrected Total	0.22	7			

X_1 = Time (min.), X_2 = Acid conc. (M), and X_3 = Temp. (°C)

SS: sum of squares, DF: Degree of freedom, MS: Mean Square

The ANOVA results in Table 7 indicating model F-value of 9149.00 and p – value of 0.0080, implies that the model is significant and there is only a 0.80% chance that it could occur due to noise. In addition, the R^2 value of 0.9999 and the close agreement between the Adjusted R^2 (0.9996) and the Predicted R^2 (0.9964) show that the model is reliable. By the p-value (< 0.05) criteria, all model terms have significant effects except the interactive effects of time and temperature (p-value = 0.0903). The combined effect of time and acid concentration (X_1X_2) has

the most significant effect on glucose yield. A regression model equation for the experimental results, following ANOVA, can be represented as given in Equation (3):

$$Y = 1.32 + 0.049x_1 + 0.011x_2 + 0.069x_3 - 0.14x_1x_2 + 0.005x_1x_3 - 0.045x_2x_3 \quad (3)$$

The graph of 'Predicted' against 'Actual' for glucose yield using 30% (w/v) substrate concentration is presented in Figure 13 indicating a good correlation between the predicted and the actual values.

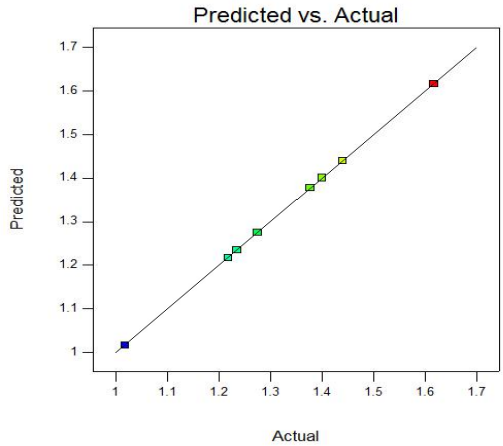

Figure 13: Predicted vs. Actual values for glucose yield in the acid hydrolysis with 30% Substrate concentration.

An optimal glucose yield of 1.617 g/L was obtained at 60 min hydrolysis time, 0.1 M acid concentration, and temperature of 97°C (corresponding to experimental run 6).

3.3 Fermentation in S. cerevisiae

Following the fermentation in S. cerevisiae, of the glucose product obtained at highest-yield condition of hydrolysis (20% substrate conc., 0.5 M acid conc., 80°C temp., and 10 min) and estimation of S. cerevisiae by the plate count method, results of the 4-hourly observation up to 48 h is presented in Table 8.

Table 8: Experimental Result of the Analysis of Yeast (S. cerevisiae) biomass production

Run	Time (hours)	Dilution Factor	Vol. of H_2O plate (ml)	No. of colonies counted	No. of colonies counted	Ave. No. of colonies counted	Yield (cfu/ml)
1	0	10^{-2}	1	42	50	46	4600
2	4	10^{-4}	1	165	163	164	1640000
3	8	10^{-5}	1	37	37	37	3700000
4	12	10^{-5}	1	59	61	60	6000000
5	16	10^{-5}	1	79	85	82	8200000
6	20	10^{-5}	1	91	91	91	9100000
7	24	10^{-5}	1	108	112	110	11000000
8	28	10^{-5}	1	145	135	140	14000000
9	32	10^{-5}	1	176	164	170	17000000
10	36	10^{-5}	1	194	202	198	19800000
11	40	10^{-5}	1	209	205	207	20700000
12	44	10^{-5}	1	200	194	197	19700000
13	48	10^{-5}	1	195	189	192	19200000

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Table 8 shows that optimum yield of 20, 700, 000 cfu/ml was attained in 40 hrs. Beyond this period, the number of S. cerevisiae counted reduced with further time. Figure 14 shows an image of the S. cerevisiae taken under a compound light microscope at X40 magnification. The organism as presented on yeast extract agar plate shows a flat and smooth morphology which is moist, dull, and has creamy color.

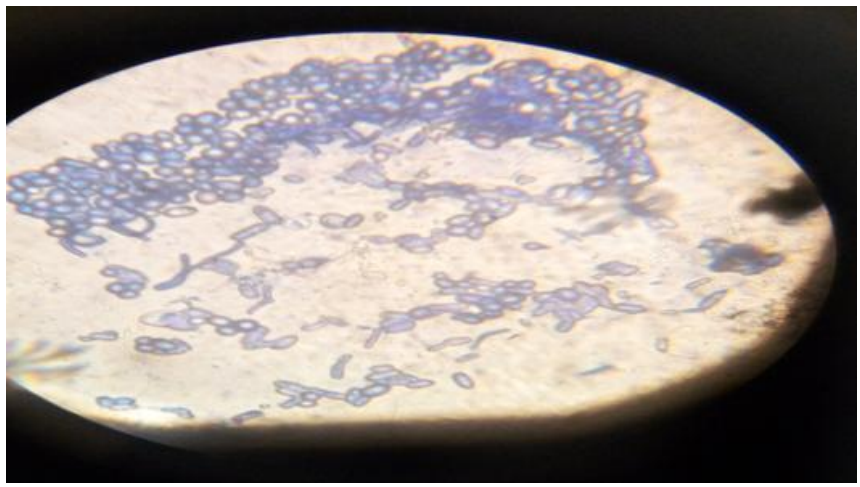

Figure 14: Colonies of S. cerevisiae as observed under a compound light microscope

3.4 Kinetics of Growth

Figure 15 shows a growth curve which was obtained to investigate the rate of production of S. cerevisiae in the fermentation experiment. Again, the growth curve indicate that there was a steady increase with time in the average number of colonies counted until the maximum was observed at 40 hr. Afterwards, there was a decline in the rate of S. cerevisiae growth.

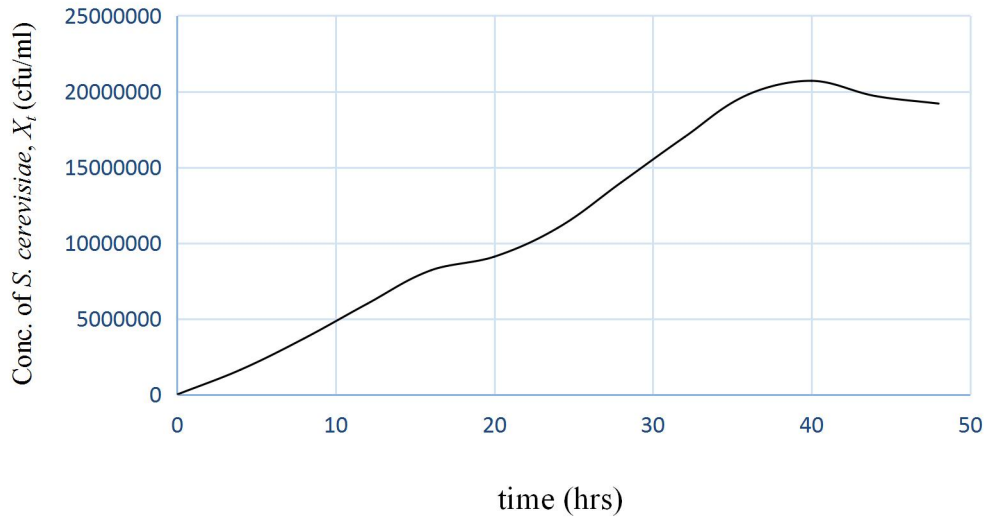

Figure 15: Variation of the concentration of S. cerevisiae with time

The kinetic equation for the growth of S. cerevisiae can be represented by the general Equation (4):

$$\ln X_t = \ln X_0 + \mu t \quad (4)$$

where X_t is the concentration/yield of S. cerevisiae at time t, X_0 is the concentration at time zero (0), and μ is the specific growth rate represented by the slope in the straight line graph of ln X_t against time t (Figure 16).

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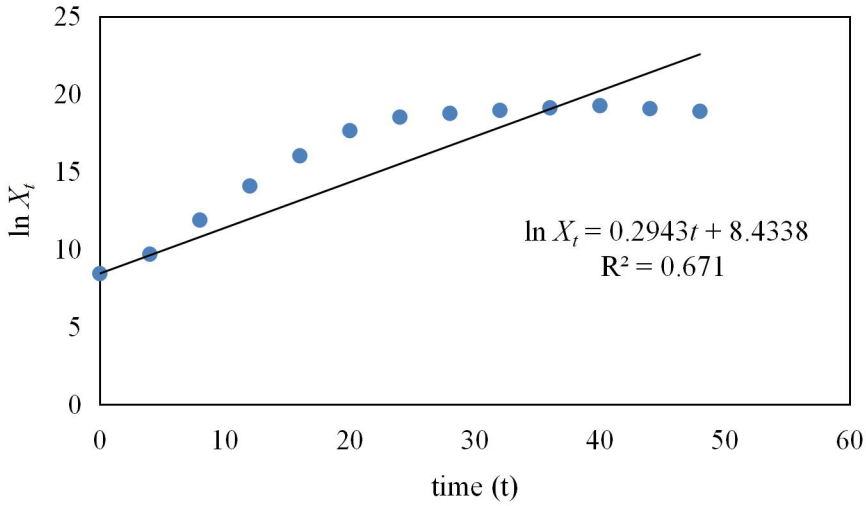

Figure 16: Plot of ln X_t against t

As presented in Figure 16, the plot gave a slope μ of 0.2943 hour $^{-1}$, and ln X_t at time t of zero is found at ln X_0 = 8.4338. Thus, the kinetic equation for S. cerevisiae growth becomes ln X_t = 8.4338 + 0.2943t, with R^2 value of 0.671.

4. Conclusion

Particles of sugarcane bagasse prepared into three different concentrations of 20, 25, and 30% (w/v) were hydrolyzed with HCl under various conditions of time (10 – 60 min), acid concentration (0.1 – 0.5 M) and temperature (80 – 97°C). Using ANOVA, the interactive effect of hydrolysis time and acid concentration showed the most significant influence on glucose yield. It was clear from the acid hydrolysis that the role of acid concentration in achieving high conversion into glucose is dependent on hydrolysis duration and temperature; high acid concentrations require low temperature and minimum time, while low acid concentrations require high temperature and maximum time. Also, substrate concentration tends to determine how acid concentration of 0.5 M particularly affected the yield of glucose. An optimum glucose yield of 1.907 g/L was attained by employing 20% (w/v) substrate concentration at hydrolysis conditions of 10 min, 0.5 M acid concentration and 80°C temperature. Fermentation of the glucose obtained was able to produce an optimum yeast (S. cerevisiae) biomass of 20, 700, 000 cfu/ml within 40 h. A kinetic equation for the growth was obtained as ln X_t = 8.4338 + 0.2943t, having a correlation coefficient, R^2 value of 0.671.

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