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Optimization of Glucose Recovery from Saccharomyces cerevisiae Waste via Hydrolysis Using RSM

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ABSTRACT

The liquid waste resulting from the regeneration of Saccharomyces cerevisiae contains a significant amount of starch, which has the potential to be optimized for glucose production. This study aims to optimize glucose levels from the liquid waste using hydrolysis with the Response Surface Methodology (RSM). The tested variables include the volume of Saccharomyces cerevisiae regeneration liquid waste (mL): 300; 600; 900; 1200; and 1500, as well as water volume (mL): 200; 400; 600; 800; and 1000. RSM with a Central Composite Design (CCD) requires 13 experimental data combinations of variables. The hydrolysis results were analyzed using RSM to determine the effect of variables and their interactions on glucose content. Optimization of glucose levels using RSM resulted in an optimal glucose content of 23.5% at a liquid waste volume of 1378.79 mL and a water volume of 1.5238 mL, based on an analysis using Minitab 19 software. Glucose content testing from 25 data points showed the highest level at 27% for a liquid waste volume of 900 mL and a water volume of 600 mL, while spectrophotometry recorded a peak glucose content of 29.7% under the same variables. The findings of this study can be the basis for developing a more efficient hydrolysis process that produces glucose which can help industry to make waste processing methods more efficient and increase the added value of by-products.

INTRODUCTION

PT. ENERO Mojokerto utilizes bioethanol waste as a raw material for biogas production. The bioethanol waste contains a high amount of glucose, originating from the sugarcane production process in Gempolkrep, Mojokerto. The byproduct of biogas production, known as vinasse biogas, is stored in reservoirs due to its high organic content. The organic content of vinasse biogas reaches 31,380 mg/L, indicating its potential for further biogas production enhancement (Enero, 2023). Starch, a major carbohydrate polymer in plants, consists of two main polysaccharides: amylose (linear structure) and amylopectin (branched structure), which contain α-(1,4) and α-(1,6) linkages in their molecular composition (Golachowski et al., 2020). As an energy reserve, starch is stored in plant organs such as seeds, roots, and peels. The composition of amylose and amylopectin influences its physical and chemical properties, including solubility and granule swelling capacity (Faridah et al., 2020). Meanwhile, glucose ($C_6H_{12}O_6$) is a monosaccharide that serves as the fundamental unit in starch structure. Glucose exists in linear or cyclic forms, with D-glucose being the dominant form under physiological conditions. Glucose is

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produced through the hydrolysis of polysaccharides or disaccharides using acid or enzymes and is commonly found in fruits, vegetables, and organic waste (Satoh et al., 2014).

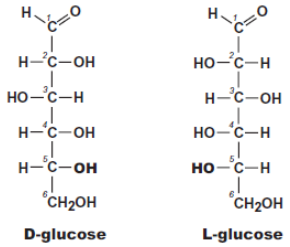

Figure 1. Formula of D-Glucose and L-Glucose According to Fischer Projection

Hydrolysis is a chemical reaction that utilizes water molecules (H_2O) to break chemical bonds into two parts, aiming to convert polysaccharides into simple monomers. This process involves the addition of -H and -OH groups from water to the broken molecule (Ellouze et al., 2023). The reaction is as follows:

$$2C_6H_{10}O_{5\,(s)} + 2H_2O_{\,(l)} \rightarrow 2C_6H_{12}O_{6\,(aq)} \quad \text{.....(1)}$$

(Starch) (Water) (Glucose)

In this process, starch molecules react with water to produce glucose molecules. Hydrolysis reactions typically occur slowly, necessitating catalysts such as acids or enzymes to accelerate the reaction rate (Praputri et al., 2018). The efficiency of acid hydrolysis is influenced by factors such as acid concentration, reaction temperature, and reaction time (Świątek et al., 2020). For glucose, acid hydrolysis involves breaking glycosidic bonds in polysaccharides, releasing glucose molecules (Chavan et al., 2015). Since the reaction is inherently slow, catalysts are required to enhance water activity, with sulfuric acid (H_2SO_4) being the preferred catalyst in this study (Iryani, 2020).

Minitab includes tools for Design of Experiments (DOE), regression analysis, analysis of variance (ANOVA), control charts, and reliability analysis (Basset et al., 1996). Response Surface Methodology (RSM) integrates mathematical and statistical approaches to develop and optimize processes. Predictions in RSM are made using a second-order polynomial model to describe the impact of variables on responses (Hadiyat et al., 2022). The relationship between variables can be expressed in the following equation:

$$y = f(x_1, x_2) + \varepsilon \quad \text{.....(2)}$$

Where:
y = Response variable
x_1, x_2 = Independent variables (factors)
ε = Error term

(Montgomery, 2009)

Validation of responses is performed using ANOVA, a statistical method in RSM to identify significant independent variables affecting response variables. The optimal value is obtained from a model that exhibits curvature, typically a second-order model:

$$y = \beta_0 + \sum\beta_i x_i + \sum\beta_{ii} x_i^2 + \sum\beta_{ij} x_i x_j + \varepsilon \quad \text{.....(3)}$$

Where:
Y = Predicted response
x_i, x_j = Independent variables
β_0 = Intercept
β_i = Linear regression coefficient
β_{ii} = Quadratic regression coefficient
β_{ij} = Interaction regression coefficient
ε = Error term

(Nabilah, 2023)

A study by Mardina (2014) on rice straw yielded 5.44% furfural at 1% H_2SO_4 concentration for 1 hour, while cassava produced 5.7% (b/v) bioethanol with a 30.5% (b/b) yield at acid concentrations of 0.1–1.0 M for 5–20 minutes (Susmiati et al., 2011). Wheat flour wastewater produced 11% glucose with 5% H_2SO_4 in 1 hour (Bachtiar et al., 2021), whereas rotten potatoes generated 40.545 g of glucose in 6 hours of hydrolysis (Suryati et al., 2017). Meanwhile, *Saccharomyces cerevisiae* liquid waste yielded 17.96% glucose using RSM optimization with HCl (Sari et al., 2024). This study highlights the importance of optimizing process parameters to enhance starch utilization efficiency.

Hydrolysis-based research focusing on waste volume and sulfuric acid (H_2SO_4) concentration in the hydrolysis process of Saccharomyces cerevisiae regeneration waste aims to extract glucose levels above 30%, approaching molasses-based raw materials. These findings offer a potential solution for fermentation-related industries, such as PT. Enero, in managing Saccharomyces cerevisiae regeneration waste and transforming it into a valuable reusable product.

RESEARCH METHODS

Materials and Equipment

The main materials used in this study include the liquid waste from the regeneration of *Saccharomyces Cerevisiae*, sulfuric acid (H_2SO_4) 98%, distilled water (Aquadest), and sodium hydroxide (NaOH). The primary equipment used in this research includes a series of hydrolysis apparatus, as shown in Figure 2. The research equipment consists of a storage tank to collect waste before being pumped into the pre-treatment tank to measure and adjust pH using sulfuric acid or sodium hydroxide. The waste is then directed to the filtration tank to remove impurities, which are discarded in the filtration waste tank. The filtered waste flows into the hydrolysis tank for a hydrolysis process lasting 3 hours at a stirring speed of 200 rpm, resulting in a product that is then tested for its glucose content.

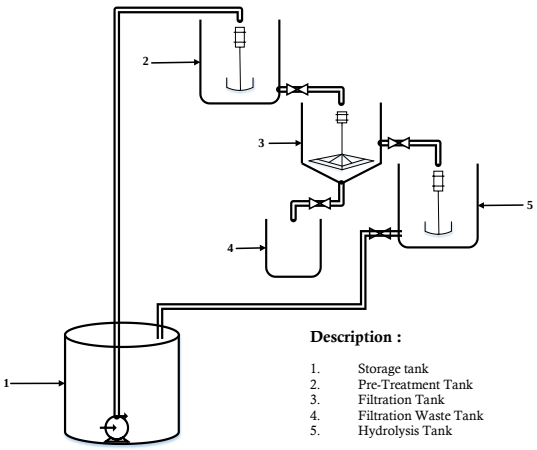

Figure 2. Hydrolysis Equipment Setup

Procedure

This research consists of several stages, starting with the pre-treatment phase using NaOH, followed by the acid hydrolysis phase with H_2SO_4 98%, and concluding with glucose content optimization using Minitab-19 software and the Response Surface Methodology (RSM).

Hydrolysis Stage

The hydrolysis process begins with the preparation of liquid waste from the regeneration of *Saccharomyces Cerevisiae*. The liquid waste (volume in mL): 300; 600; 900; 1200; and 1500 is hydrolyzed with H_2SO_4 98% solution 1,5 N with the addition of water volume (volume in mL):

200; 400; 600; 800; 1000 at 200 rpm for 180 minutes at approximately 30°C. After cooling, the filtrate is analyzed for glucose content using a glucose refractometer (Bachtiar et al., 2021).

Data Optimization Procedure (RSM)

First, the independent variables are entered, namely liquid waste volume (mL): 300; 600; 900; 1200; and 1500, and water volume (mL): 200; 400; 600; 800; and 1000. Then, the Response Surface Method (RSM) design is created. For second-order response surfaces, the Central Composite Design (CCD) method is used. The experimental data are then input, and a Pareto Chart is created. The regression values obtained from the study are analyzed. Optimization graphs are generated to obtain contour plots, determining the optimal glucose concentration based on waste volume and water volume. The contour plot identifies the optimal region, while the surface plot visualizes the optimal glucose concentration and the best values for each variable.

RESULTS AND DISCUSSION

Analysis of Liquid Waste Composition from Saccharomyces Cerevisiae Regeneration

The analysis of the liquid waste from Saccharomyces cerevisiae regeneration at PT. Enero was conducted using a glucose refractometer. The results are presented in Table 1.

Table 1. Analysis of Glucose Content of Liquid Waste from SC Regeneration

Waste Volume (mL)	Water Volume (mL)	Glucose Content (%)
300	200	12
	400	14
	600	16
	800	15
	1000	14,5
600	200	16
	400	15
	600	20
	800	19
	1000	17
900	200	18
	400	22
	600	24
	800	27
	1000	22,5
1200	200	22
	400	23
	600	23,5
	800	21
	1000	20
1500	200	20
	400	21
	600	22
	800	21,5
	1000	21

Based on the table above, it is known that the glucose levels obtained increase along with the increase in water volume in the hydrolysis process with the highest glucose levels of 27% and there is a decrease in glucose levels after reaching the optimum point due to the hydrolysis process, namely with the lowest glucose levels of 12%. In this study, after the concentration reached its peak, the glucose concentration decreased due to several factors, such as glucose degradation due to long reaction time, the effect of temperature causing caramelization or other side reactions, and the possibility of glucose consumption by residual microorganisms or active enzymes in the system. In addition, excess hydrolysis agents can trigger further reactions that convert glucose into other compounds, thereby reducing its concentration in solution.

Effect Waste Volume and Water Volume at Glucose Level

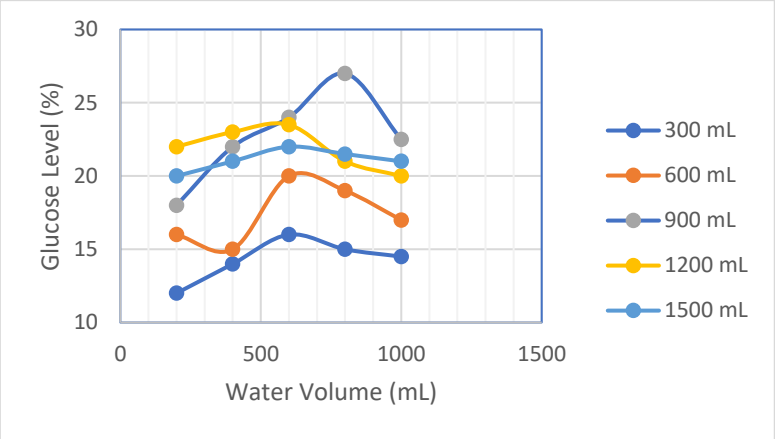

Figure 3. Graph of the Relationship Between Water Volume and Glucose Levels at Various Waste Volumes

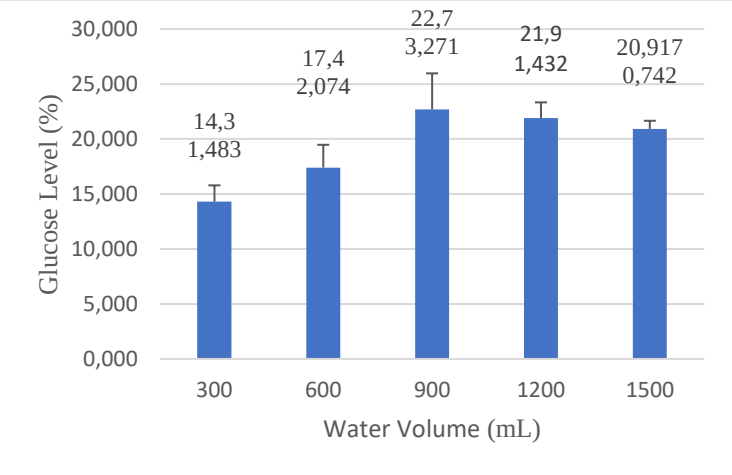

Figure 4. Error bar graph of the effect of waste volume on glucose yield

Based on figure 3 and 4 there is a relationship between water volume and glucose concentration, where glucose concentration increases, peaking at 27% at 800 mL, before decreasing at higher volumes. Similarly, the relationship between waste volume and glucose concentration follows a similar trend, reaching 22.7% at 900 mL before slightly declining at higher volumes. The error bar indicates the highest data variation at 900 mL (standard deviation of 3.271%) and greater stability at 1500 mL (0.742%), suggesting that glucose production becomes more consistent at larger waste volumes.

Optimization of Glucose Yield Using (RSM)

Based on the analysis using the CCD method, 13 experimental data points were required, incorporating the following two factors:

Table 2. Factor and Response Data for RSM Analysis (13 Data Points)

Factor 1	Factor 2	Response
Waste volume (mL)	Water Volume (mL)	Glucose Levels (%)
300	400	14
300	600	16
300	1000	14,5
600	200	16
600	600	20
600	1000	17
900	200	18
1200	600	23,5
1200	800	21
1200	1000	20
1500	200	20
1500	800	21,5
1500	1000	21

The optimal condition aimed to maximize glucose content in a single treatment using experimental design data from 25 trials. The equation obtained is as follows:

$$Y1=3.47+0.01768_{x1}+9.38_{x2}-0.000006x_1^2-2.681_{x2}^2-0.00087_{x1x2}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Description:

$y1$ = Glucose Content
$x1$ = Wastewater Volume
$x2$ = Water Volume

Graphs and Discussion

Results for the Optimization Results for Glucose Content (13 Data Points)

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,811588	95,57%	92,41%	85,67%

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Glucose Level (%)	Maximum	14	23,5		1	1

Solution

Solution	Waste Volume (mL)	Water Volume (mL)	Glucose Level (%) Fit	Composite Desirability
1	1378,79	609,353	22,8303	0,929508

Multiple Response Prediction

Variable	Setting
Waste Volume (mL)	1378,79
Water Volume (mL)	609,353

Response	Fit	SE Fit	95% CI	95% PI
Glucose Level (%)	22,830	0,497	(21,654; 24,006)	(20,579; 25,081)

Based on Table 3, the optimization results indicate that the most optimal condition occurs at a wastewater volume of 1378.7879 mL and a Water Volume of 1.5234 mL, yielding a glucose content of 22.8303% with a desirability value of 0.929508, reflecting a high level of accuracy in meeting the established criteria (Ramadhani et al., 2017).

The verification of results was conducted by comparing the model's predicted values with experimental results using Confidence Interval (CI) and Prediction Interval (PI) at a 95% confidence level. The CI range was 21.654–26.006, while the PI range was 20.579–25.081. The CI measures the accuracy of the model estimation, whereas the PI accounts for observations and model errors, resulting in a wider range.

Model Analysis Using ANOVA

Table 3. Analysis of Variance (ANOVA) Results

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	99,582	19,9	30,24	0
Linear	2	83,523	41,8	63,4	0
Waste Volume	1	79,36	79,4	120,48	0
Concentration	1	2,219	2,22	3,37	0,109
Square	2	23,539	11,8	17,87	0,002
Volume*Volume	1	8,961	8,96	13,6	0,008
Water Vol * Water Vol	1	16,841	16,8	25,57	0,001
2-Way Interaction	1	1,012	1,01	1,54	0,255
Volume* Water Vol	1	1,012	1,01	1,54	0,255
Error	7	4,611	0,66		
Total	12	104,192			

A P-value < 0.05 indicates a significant effect on bioethanol content. Significant variables include the regression model, linear time and pH, and the quadratic pH term, whereas the

quadratic time term and pH-time interaction are not significant. The model's validity is supported by its significance and a coefficient of determination (R^2) of at least 80% (Ramaraj, 2019).

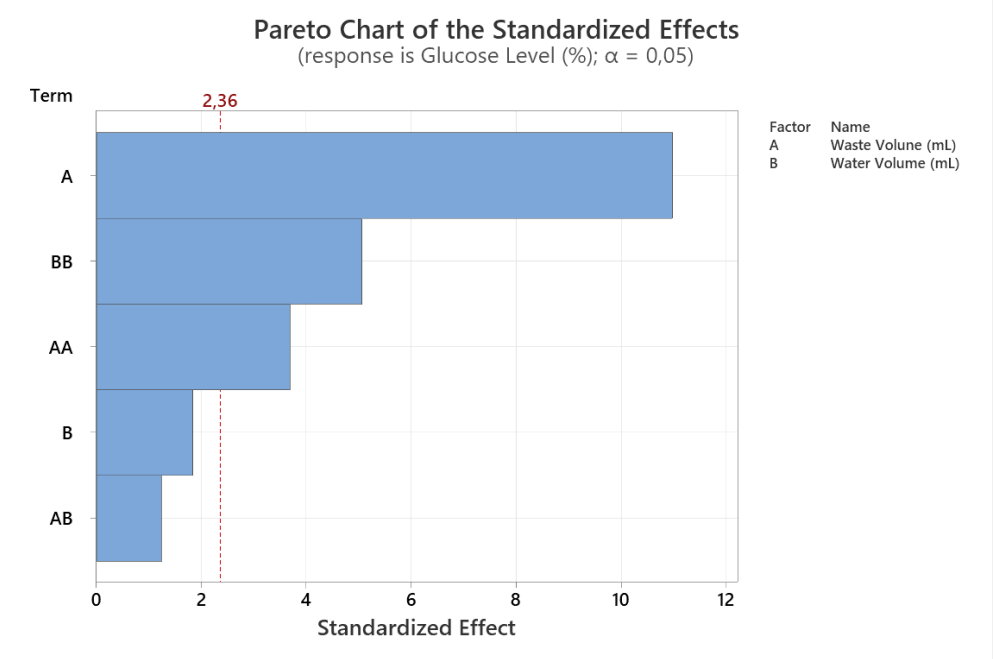

Figure 7. Pareto Chart

The Pareto chart is used to identify independent variables that contribute most significantly to the dependent variable. As shown in **Figure 7**, **wastewater volume (A)** has the longest bar, indicating its dominant influence in reducing glucose content.

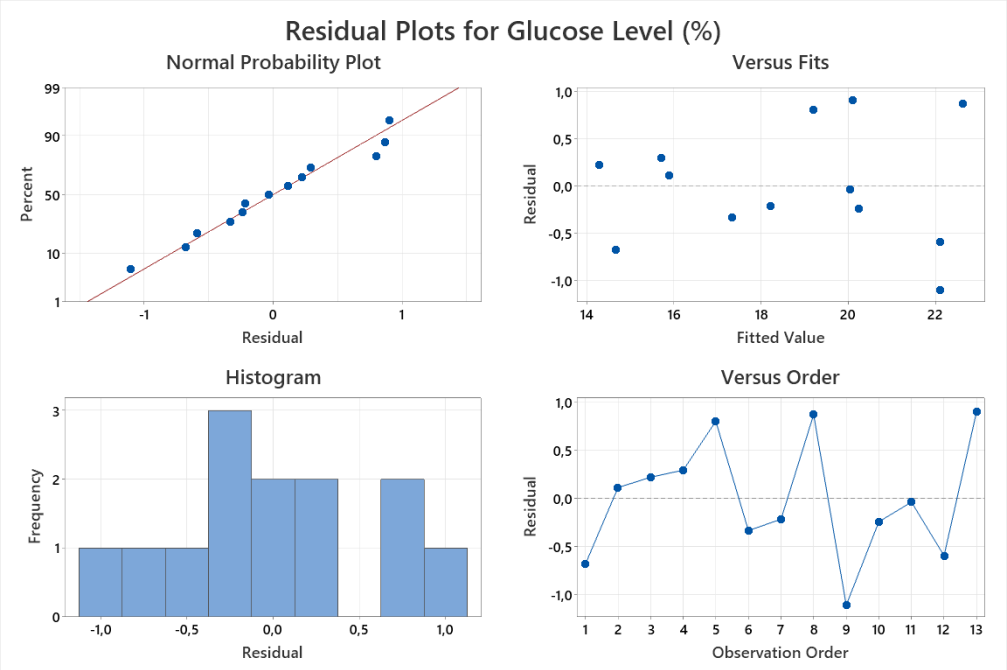

Figure 8. Residual Plots

Residual plots include a normal probability plot, histogram, residuals versus fits, and residuals versus order. The normal probability plot indicates normally distributed data if the points form a straight line. The histogram displays the residual distribution and requires at least 20 data

points for accuracy. The residuals versus fits plot confirms model accuracy if the residuals are randomly scattered around zero. The residuals versus order plot tests residual independence, indicated by random scatter without a specific pattern. Figure 8 shows that the residuals are randomly distributed, suggesting a valid model.

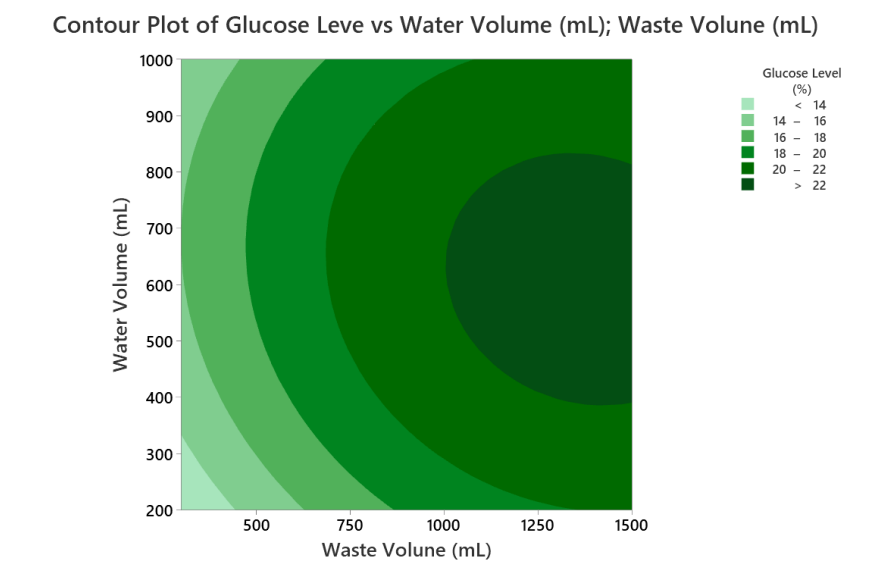

Figure 9. Contour Plot

Response surface and contour plots are 2D and 3D visualizations used to describe stationary points or optimal glucose content values based on the equation model (Zani et al., 2019). Figure 9 illustrates glucose content distribution in six layers: The first layer represents glucose levels below 14%, The levels increase progressively, The sixth layer contains glucose levels above 22%, which corresponds to the stationary point, indicating the optimal condition for maximum glucose content. This suggests that the selected process variables have successfully achieved optimal conditions.

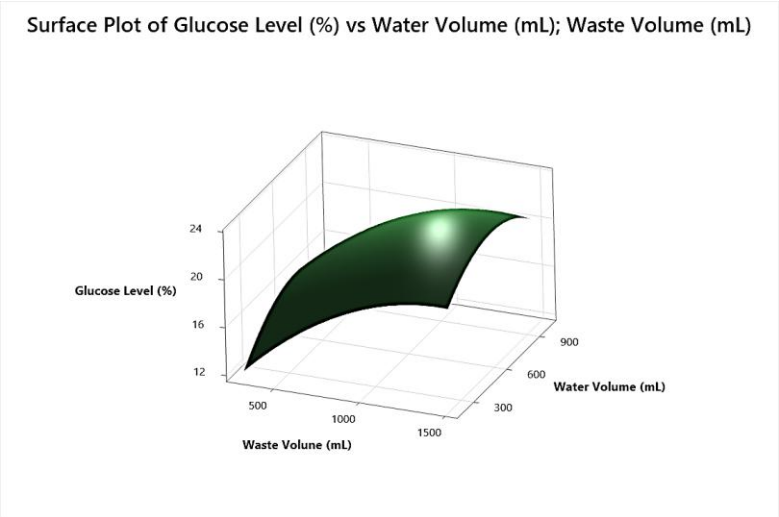

Figure 10. Surface Plot

Figure 10**.** presents a response surface graph illustrating glucose concentration based on waste volume and water volume. The 3D graph shows a peak bioethanol concentration of 23.5% at 1200 mL waste volume and 600 mL water volume. These graphs depict the interaction between

the two variables, where incomplete hydrolysis or excessive dilution can reduce process efficiency, leading to lower glucose concentrations.

CONCLUSION

From the research findings, it can be concluded that the optimization of glucose levels from the regeneration wastewater of Saccharomyces Cerevisiae using the Response Surface Methodology (RSM) yielded several key results. Glucose content testing from 25 data points showed the highest glucose level of 27% at a wastewater volume of 900 mL with a Water Volume of 600 mL, while spectrophotometric analysis recorded the highest glucose level of 29.7% under the same conditions. Optimization results using Minitab 19 software with RSM from 13 experimental data points indicated an optimal glucose level of 23.5% at a wastewater volume of 1378.79 mL and a Water Volume of 1.5238 mL. For further research, it is better if the sulfuric acid catalyst is in solid form to obtain maximum glucose concentration results. waste storage should also be maintained properly so as not to cause more impurities.

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