

Optimization Of Ph, Time, Yeast Substrate Ratio And Temperature For Production Of Bioethanol From Sweet Potato (*Ipomoea Batatas*) Peels

Osumba Onyango Vitalis, Elly Tetty Osewe, Sylvia Awino Opiyo
(Department Of Physical And Biological Science, Murang'a University Of Technology, Kenya)

Abstract:

Introduction: The growing demand for renewable energy has led to increased interest in bioethanol production from non-food biomass. Sweet potato (*Ipomoea batatas*) peels, an agro-industrial waste, present a viable feedstock for bioethanol production using *Saccharomyces cerevisiae*. This study aimed to optimize fermentation conditions, specifically pH, temperature, fermentation time, and yeast-substrate ratio, to maximize bioethanol yield.

Methodology: Sweet potato peels were collected, dried, and then hydrolyzed using 0.5M, 1.0M, 1.5M and 2.0MHCl to release fermentable sugars. The fermentation process was carried out using *Saccharomyces cerevisiae*, with pH levels adjusted to 4.5, 5.0, 5.5, and 6.0. The effect of different fermentation conditions on bioethanol yield was assessed. ANOVA was used to determine the significance of independent factors and their interactions, and a regression model was developed to predict bioethanol yield.

Results: The optimized conditions for bioethanol production were temperature: 30°C, pH: 7.0, fermentation time: 72 minutes, and yeast amount: 0.35 g/50mL, yielding 0.0655 mol of bioethanol. ANOVA results indicated that pH and yeast amount significantly influenced bioethanol yield independently, while temperature and time were only significant in interaction with other factors. The quadratic effect of pH was strong, suggesting an optimal range for bioethanol production. The interaction of temperature with pH and temperature with yeast amount significantly influenced bioethanol yield. The regression model demonstrated high accuracy ($R^2 = 99.42\%$) in predicting bioethanol production.

Conclusion and Recommendations: pH and yeast amount have a significant independent effect on bioethanol yield, while temperature and time are only significant when considered in interaction with other factors. Quadratic effects show that pH has a strong curvature, suggesting an optimal range for bioethanol production, while time has a borderline effect. Interaction analysis reveals that temperature combined with pH, as well as temperature combined with yeast amount, significantly influence bioethanol yield. The study recommends that the optimized parameters should be tested at a pilot scale before full-scale implementation so as to assess feasibility for bioethanol production from sweet potato peels.

Key Words: Bioethanol, fermentation, optimization, pH, sweet potato peels, temperature, time, yeast-substrate ratio

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I. Introduction

The issue of energy resources' sustainability and the use of cleaner energy sources have become prominent due to the diminishing nature of fossil fuels. Energy demand for use in sectors such as transportation and industrial use has been increasing day-to-day. This has led to a strain in global energy supplies from non-renewable energy resources. In addition, global prices of petroleum have been on the rise (Abbasi *et al.*, 2022). The issue of having an environmental friendly source of energy has also raised concerns over the current sources of fuel (Deora *et al.*, 2022). As a result, attention has shifted towards the search for substitute renewable fuels. Advocacy on renewable fuels has been driven by the need for environmental protection and increasing energy supplies while reducing dependence on non-renewable sources of energy and petroleum (Balaganesh *et al.*, 2023).

In Sub-Saharan Africa, the first generation feedstocks, specifically cereal grains, are utilized as food for the increasing population. Further, some cereal grains that are rich in starch such as maize are also utilized as raw materials for animal feeds (Jha & Schmidt, 2021). Therefore, their demand implies that priority is not to utilize them for biofuels production. This means that research on the use of second generation feedstocks for biofuels production with minimal competition for domestic and farm use is timely in this era where crude oil has become scarce and costly. Wastes from agro-processing plants, the food industry such as hotels and farms have become of great interest in bioethanol production. Based on this, the current study sought to optimize temperature, pH,

time and yeast to substrate ratio for bioethanol production from Sweet potatoes (*Ipomoea batatas*) peels using *Saccharomyces cerevisiae*.

II. Materials And Methods

Reagents

Sweet potato peels (5 Kg), Sodium Hydroxide (NaOH) 99.9%, Sulphuric Acid (H₂SO₄) 98%, Sodium Phosphate buffer (pH 4 and 7), 0.2M acetate buffer, *Saccharomyces Cerevisiae* (purchased locally).

Sample Collection

The species of the sweet potatoes were identified by a botanist using the leaves in the farm before harvesting the tubers for fermentation. The sweet potatoes were then transported to Murang'a University of Science and Technology Laboratory for fermentation.

Sample Preparation

The sample of sweet potatoes was washed to remove the soil and other dirt from farm. The sweet potatoes were then be peeled off. The peels were dried for about 10 days. The quantity of the sweet potato peels was about 5 Kilograms. The sweet potato peels underwent physical pretreatment. This was achieved through mechanical crushing of the dried peels so as to reduce them into small particle sizes.

Preparation of Reagents

Acid and base required was achieved through mixing acid and NaOH pellets in certain amount of water in ratio that was obtained through calculations. To obtain 1M HCl 86 ml HCl was mixed with distilled water then made up to 1litre, for sulphuric acid 55 ml was mixed with water and made up to 1 litre while for sodium hydroxide 40 grams pellets was mixed with 1 litre of water to make 1M NaoH. Acid hydrolysis was adopted for the study. This was achieved through the use of 0.2M, 0.5M, 1.0M and 2.0M HCl at temperatures of 98°C.

Hydrolysis of the sample

20g of samples was weighed into five different conical flasks (500ml Erlenmeyer) and labeled A, B, C, D and E (Okoro *et al.*, 2022). 120ml of 0.2M, 0.5M, 1.0M, 1.5M and 2.0M of Hydrochloric acid was added to flasks respectively. The mixture in the flasks was heated in water bath for 2hours at 98°C, sterilized in the autoclave at 121°C for 15mins and the flask allowed to cool. The samples were filtered through Whatman filter paper No.1 and stored in refrigerator to be used later.

Determination of Sugar Content

The sugar content in the hydrolyzed sample was determined using refractive index, a drop of hydrolyzed sample was applied on the glass of refractometer and sugar content determined using light (Mustafa *et al.*, 2019).

Determination of PH

The sample's pH was determined by use of well calibrated pH meter. The calibration of pH meter was done using a specific buffer tablet.

Determination of Reducing Sugar

Reducing sugar in sweet potatoes peels, hydroyzed peels, cellulose degraded sweet potatoes peels and fermented peels was determined. 25 grams anhydrous Na₂CO₃, 2 g NaHCO₃, 2.5 g potassium sodium tartrate and 20 g anhydrous sodium was weighed into 80 ml and made up to 100 ml. Arsenomolybdate reagent was prepared by dissolving 2.5 g ammonium molybdate in 45 ml water and 2.5 ml sulphuric acid added, then mixed with 0.3 g disodium hydrogen arsenate dissolved in water. The solution was incubated at 37°C for 24 hours.

Standard glucose solution was made by dissolving 1g of glucose in 100ml distilled water. Working standard of 100 µg/ml was prepared by adding 1ml of glucose solution to 100ml of water. 0.2 ml, 0.4 ml, 0.6 ml, 0.6 ml and 1.0 ml of working standard was measured into five well-labeled test tubes. 0.1g of each test sample was measured into three beakers then 100ml of water added to each beaker. 1 ml of test samples was measured into four test tubes. Distilled water was then added followed by 1ml of alkaline copper tartrate reagent and shaken for proper mixing. Test tubes were then boiled for 10 minutes then cooled in water followed by addition of arsenomolybdate reagent and shaken well for proper mixing. 7 ml of distilled water was added to each test tube to make up to 10 ml. The absorbance of the blue color in each sample was determined by spectrometer, and then graph of absorbance against concentration plotted to obtain the amount of reducing sugar. The following quantities were determined:

% reducing sugar = Concentration of sugar in test tube

Concentration of sugar = $\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard sample}}$

Fermentation Process

Preparation of *Saccharomyces Cerevisiae*

Saccharomyces Cerevisiae was cultured at Potato Dextrose Agar Medium (PDA). The culture was incubated at a temperature of 30°C for duration of 24 hours.

Optimization of Fermentable Condition

The pH of the filtrate was brought down to 4.5, 5.0, 5.5, and 6.0 by use of 2.0M NaOH (Okoro *et al.*, 2022). Reducing sugar was determined by use of refractometer. 50ml of each sample was placed in the conical flask, *Saccharomyces Cerevisiae* was added. A balloon was tightened on the opening of each flask for collection of carbon (IV) oxide. The flask was shaken and incubated at different temperatures in a water bath.

Distillation

The mixture was added to distilling pot where it was heated to the boiling point, where the lower boiling component vaporized first and last the component with higher boiling point. Ethanol was collected at 78°C. Different properties of the ethanol produced were determined.

Determination of Quantity of Ethanol Produced

The distillate collected was measured using measuring cylinder and expressed as g/L by multiplying the volume of the distillate by the density of ethanol.

Data Analysis Procedures

Optimization of fermentation conditions was done using design of experiments (DOE) using Minitab Statistical Software 22. This helped determine optimal values for temperature, pH, fermentation time and yeast-to-substrate ratio for bioethanol production from sweet potato peels. A response surface methodology (RSM) with a custom DOE design was used to evaluate the influence of fermentation factors and their interactions. Analysis of variance (ANOVA) was conducted to determine the significant independent factors (temperature, pH, time and yeast amount) and their interactions on bioethanol yield. Factors with p-values below 0.05 were considered significant. A regression model was developed to describe the relationship between process parameters and bioethanol yield, incorporating linear, quadratic and interaction effects. Response optimizer tool in Minitab was used to determine the optimal levels of fermentation conditions that maximize bioethanol yield.

III. Results

The results for bioethanol yield maximization included model summary findings shown in Table 1. The column S (or standard deviation of the residuals) show a standard deviation = 0.0014573, which is very low. This suggests low variability in the residuals, meaning that the model prediction closely align with actual data. R-Square = 99.42% is the coefficient of determination. This result shows that 99.42% of the variation in bioethanol yield is explained by the model. This represents an excellent fit. High value of adjusted R-Square, that is, R-Square (Adjusted) – 95.64%, further confirms that the model is a good fit and remains robust even after adjusting for the number of terms

Table 1: Model Summary for Bio-Ethanol Production

S	R-square	R-sq(adj)	R-sq(pred)
0.0014573	99.42%	95.64%	*

The ANOVA results are as shown in Table 2. The ANOVA table provides insights into which factors significantly affect bioethanol yield.

Table 2: ANOVA Results on Significant Factors

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	13	0.000727	0.000056	26.33	0.037
Linear	4	0.000176	0.000044	20.67	0.047
Temperature (oC)	1	0.000022	0.000022	10.17	0.086
pH	1	0.000067	0.000067	31.66	0.030
Time(min)	1	0.000021	0.000021	10.09	0.086
Amount of yeast (g/50mL)	1	0.000062	0.000062	29.34	0.032
Square	3	0.000165	0.000055	25.88	0.037
Temperature (oC)*Temperature (oC)	1	0.000002	0.000002	0.76	0.475
pH*pH	1	0.000079	0.000079	37.18	0.026
Time(min)*Time(min)	1	0.000030	0.000030	13.99	0.065
2-Way Interaction	6	0.000543	0.000090	42.60	0.023

Temperature (oC)*pH	1	0.000185	0.000185	87.14	0.011
Temperature (oC)*Time(min)	1	0.000004	0.000004	1.86	0.306
Temperature (oC)*Amount of yeast (g/50mL)	1	0.000255	0.000255	120.06	0.008
pH*Time(min)	1	0.000072	0.000072	33.87	0.028
pH*Amount of yeast (g/50mL)	1	0.000018	0.000018	8.57	0.100
Time(min)*Amount of yeast (g/50mL)	1	0.000011	0.000011	5.08	0.153
Error	2	0.000004	0.000002		
Total	15	0.000731			

The overall fit of the model is presented by p-value (model) = 0.037 which is significant since it is less than 0.05. This shows that the overall model is statistically significant. This means that at least one of the factors (temperature, pH, time and yeast amount) or their interactions significantly affects bioethanol yield. Since the model is a good fit, the study proceeds with analyzing the individual factors and interactions.

From the ANOVA table, each factor's p-value shows whether it independently affects bioethanol yield. From the results in Table 2, pH (p-value = 0.030) and yeast amount (p-value = 0.032) significantly affect bioethanol yield independently. Temperature (p-value = 0.086) and time (p-value = 0.086) are not independently significant. However, to examine whether they have a role in bioethanol yield, the interactions are examined.

The square results also known as the quadratic or curvature effects check whether the effect of a factor changes at different levels. For example, it checks whether an increase in pH, time and temperature initially improves bioethanol yield and later leads to a reduction in yield. The results indicate that pH² (p-value = 0.026) have a significant curvature effect. Time² (p-value = 0.065) shows a marginally significant curvature effect, meaning that the borderline effect may still influence bioethanol yield. Temperature² (p-value = 0.475) shows that the curvature effect is not significant. The interpretation for these findings is that pH has a strong quadratic effect. This implies that there is an optimal pH level for bioethanol yield. Time also demonstrates some curvature, suggesting that there may be an optimal fermentation time.

The interaction effects (2-way interactions) results show that temperature * pH (p-value = 0.011), temperature * Yeast amount (p-value = 0.008) and pH * Time (p-value = 0.028) have a statistically significant effect on bioethanol yield. However, the interactions: Temperature * Time (p-value = 0.306), pH * Yeast amount (p-value = 0.100) and Time * Yeast amount (p-value = 0.153) do not have a statistically significant effect on bioethanol yield. This means that temperature and pH together have a significant effect on bioethanol yield; temperature and yeast amount together have a strong influence on bioethanol yield; pH and time together also play a significant role on bioethanol yield. The other interactions do not have a significant effect on bioethanol yield.

Analysis of the error term helps assess the model fit and variability. The residual of the error = 0.000004 is very small. This indicates that the model explains most of the variability. The total sum of squares = 0.000731 while the model sum = 0.000727 shows that the model explains almost 100% of the variation in bioethanol yield. On overall, the model is an excellent predictor of bioethanol yield and very low error suggests that the results are highly reliable.

The regression equation below provides an insight into how the independent variables (Temperature, pH, Time, and Amount of Yeast) influence bioethanol yield.

$$\begin{aligned} \text{Avg. bioethanol yield (m)} = & -0.1475 + 0.00555 \text{ Temperature (oC)} + 0.03177 \text{ pH} \\ & - 0.001398 \text{ Time(min)} + 0.2667 \text{ Amount of yeast (g/50mL)} \\ & - 0.000014 \text{ Temperature (oC)*Temperature (oC)} \\ & - 0.001561 \text{ pH*pH} + 0.000004 \text{ Time(min)*Time(min)} \\ & - 0.000566 \text{ Temperature (oC)*pH} \\ & - 0.000007 \text{ Temperature (oC)*Time(min)} \\ & - 0.006458 \text{ Temperature (oC)*Amount of yeast (g/50mL)} \\ & + 0.000205 \text{ pH*Time(min)} \\ & - 0.00862 \text{ pH*Amount of yeast (g/50mL)} \\ & + 0.000697 \text{ Time(min)*Amount of yeast (g/50mL)} \end{aligned}$$

The interpretation of the regression model is as outlined herein:

1. Intercept (-0.1475)

This shows the estimated bioethanol yield when all independent variables are at zero. However, this value has no practical meaning since such conditions (e.g., 0°C, pH = 0) are unrealistic for fermentation.

2. Linear Effects

Each coefficient of temperature, pH, time and amount of yeast represents change in bioethanol yield per unit increase in the corresponding variable while other variables remain constant. The coefficient for temperature = 0.00555 show that increase in temperature results to a slight increase in bioethanol yield. The coefficient for

pH = 0.03177, show that higher levels of pH increase bioethanol yield. The coefficient for time = - 0.001398 shows that longer fermentation time slightly reduces bioethanol yield; this suggests that excessive fermentation time may not be beneficial. The coefficient for yeast amount = 0.2667 shows that increasing yeast concentration strongly increases the yield of bioethanol.

The non-linear (quadratic) effects indicate whether the effect of a factor changes at higher or lower values. The coefficient for Temperature² = - 0.000014 shows a negative coefficient that indicates that increasing temperature beyond a certain point reduces bioethanol yield. This implies an optimal temperature range. The coefficient for pH² = - 0.001561 show a strong negative effect; an indication that too high or too low pH reduces yield. This is a suggestion that an optimal range of pH exists. The coefficient for Time² = 0.000004 is a very small coefficient. This suggests that time does not have a strong quadratic effect.

The interaction effects coefficients show how two factors together influence bioethanol yield. The coefficient for Temperature * pH = - 0.000566, a negative coefficient meaning that high temperature combined with high pH decreases bioethanol yield. The coefficient for Temperature * Time = - 0.000007, a very small negative effect meaning that temperature and time together have minimal interaction. The coefficient for Temperature * Yeast = - 0.006458 shows a negative effect suggesting that very high temperature with high yeast concentration negatively affects bioethanol yield. This is possibly due to thermal yeast inactivation. The coefficient for pH * Time = 0.000205 shows a small positive effect; this means that adjusting pH and time together can slightly increase bioethanol yield. The coefficient for pH * Yeast = - 0.00862 shows a negative effect; an indication that high pH with high yeast concentration may have a negative effect on bioethanol yield. The coefficient for Time * Yeast = 0.000697 shows a slight positive effect' this means that longer fermentation with increased yeast amount might lead to slight improvement in bioethanol yield.

To determine the optimal levels of the factors, Minitab's Response Optimizer was used to find the optimal pH, temperature, time and yeast amount that maximized bioethanol yield. The optimization results are as shown in Table 3.

Table 3: Optimal Levels of Temperature, pH, Time and Yeast Amount for Optimal Bioethanol Production

Solution	Temperature (oC)	pH	Time(min)	Amount of yeast (g/50mL)	Bioethanol yield (m Fit)	Composite Desirability
1	30	7	72	0.35	0.065525	1

The results in Table 3 show that the optimal conditions for maximum bioethanol yield are: Temperature = 30^o C, pH = 7, Time = 72 Minutes, Yeast amount = 0.35 g/50 mL. The predicted bioethanol yield at these conditions is 0.0655 mol. The Composite Desirability (D-Value) = 1 means that the optimization model is highly reliable. Therefore, the best conditions for maximum bioethanol yield temperature (30°C), high pH (7), fermentation time (72 min), and yeast amount (0.35 g/50mL).

These results are further confirmed by the following Optimizer Graph (Figure 1).

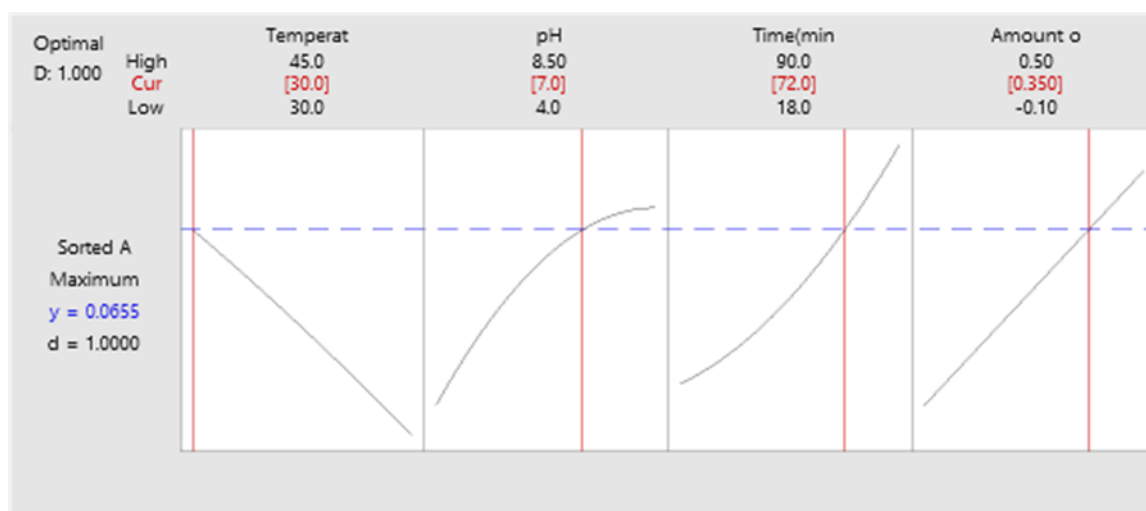


Figure 1: Optimizer Graph for Optimal Factors

From the graph in Figure 1, Composite Desirability (D-Value) = 1.000; this is an indication that the model has found an optimal solution that meets bioethanol yield maximization criteria. The optimal factor levels

are: Temperature: 30°C (Lower end of the tested range); pH: 7.0 (Slightly higher than mid-range but within tested values); Time: 72 min (Longer fermentation time favors higher yield); Yeast Amount: 0.35 g/50mL (Higher yeast concentration promotes ethanol production). The predicted maximum bioethanol yield is 0.0655 mol under these conditions. An observation of the graph trends suggests the effect of each factor on bioethanol yield. Temperature graph shows a decreasing trend implying that lower temperatures (30°C) improve yield. The graph of pH show an increasing trend; an implication that higher pH increases yield, but the optimum is 7.0 and not beyond. Time graph shows an increasing trend; an implication that longer fermentation (of up to 72 minutes) improves bioethanol yield. The graph of yeast amount shows an increasing trend; an implication that higher amount of yeast (up to 0.35 g/50 mL improves bioethanol yield).

IV. Discussion

The optimal factor levels are: Temperature: 30°C (Lower end of the tested range); pH: 7.0 (Slightly higher than mid-range but within tested values); Time: 72 min (Longer fermentation time favors higher yield); Yeast Amount: 0.35 g/50mL (Higher yeast concentration promotes ethanol production). These results that optimal temperature level for bioethanol production is 30°C falls within the ranges proposed by Williams (2017) who demonstrated that optimal temperature for fermentation ranges from 28°C to 37°C based on the strain of yeast used. Temperature has a direct influence on micro-organisms growth. According to Azhar *et al.*, (2017), most enzymes that control microbial activities and the process of fermentation are sensitive to temperatures that are high.

The findings that optimal pH level was 7.0 is in agreement with Zakaria *et al.*, (2020) who established that environment's pH should not exceed the operational pH range of 2.0-7.0. Level of pH exceeding this range greatly affects enzymes' activity and intracellular pH. Level of pH also affect metabolism of enzymes and cells, bacteria contamination, yeast growth, fermentation rate and by-product formation. According to Tse *et al.*, (2021), entry of some nutrients that are essential into the cells is affected by H⁺ concentration in the fermentation broth. However, Zakaria *et al.*, (2020) claim that for *Saccharomyces cerevisiae* the best range of PH for fermentation of ethanol is 4.0-5.0; however, our optimal pH is outside this range.

Longer fermentation time was found to favour bioethanol production. According to Rahardjo *et al.*, (2021), shorter time of fermentation results to fermentation that is ineffective due to inadequate microbes' growth. Longer time of fermentation leads to toxicity on growth of microbes in batch mode since there is high ethanol concentration in the fermented broth.

Higher yeast to substrate ratio was found to optimize bioethanol production. According to Tse *et al.*, (2021) higher yeast levels imply faster consumption of sugar and ethanol production. When more yeast cells are added initially, the consume sugar rapidly leading to a higher yield of ethanol in a shorter time. This means that a higher yeast concentration speeds up fermentation. However, after a certain threshold, more yeast addition does not further increase ethanol yield because to many yeast cells compete for nutrients and space leading to depletion of nutrients, toxic byproducts accumulation and shorter fermentation time.

V. Conclusion And Recommendations

It can be concluded that pH and yeast amount have a significant independent effect on bioethanol yield, while temperature and time are only significant when considered in interaction with other factors. Quadratic effects show that pH has a strong curvature, suggesting an optimal range for bioethanol production, while time has a borderline effect. Interaction analysis reveals that temperature combined with pH, as well as temperature combined with yeast amount, significantly influence bioethanol yield. The study recommends that the optimized parameters should be tested at a pilot scale before full-scale implementation so as to assess feasibility for bioethanol production from sweet potato peels.

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