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Effect of Acid Hydrolysis Parameters on Reducing Sugar Recovery and Subsequent Bioethanol Production from Sweet Potato (*Ipomoea batatas*) Peels

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Abstract
Introduction: *Efficient pretreatment and hydrolysis of lignocellulosic biomass are critical prerequisites for maximizing fermentable sugar availability in bioethanol production. While the fermentation optimization of sweet potato peels has been established, the hydrolysis step remains under-characterized. This study aimed to optimize acid hydrolysis conditions—specifically HCl concentration, hydrolysis temperature, and reaction time—to maximize reducing sugar recovery from sweet potato (Ipomoea batatas) peels.*
Methodology: *Dried and mechanically crushed sweet potato peels were subjected to acid hydrolysis using a Response Surface Methodology (RSM) Box-Behnken design. The independent variables were HCl concentration (0.5–2.0 M), hydrolysis temperature (90–110°C), and reaction time (60–120 min). Reducing sugar yield (g/L) was measured using the arsenomolybdate method and served as the response variable. ANOVA was conducted to evaluate model significance, and a regression equation was developed to predict sugar recovery. The hydrolysate from optimal conditions was subsequently fermented using Saccharomyces cerevisiae to verify bioethanol yield.*
Results: *The optimized hydrolysis conditions were 1.25 M HCl, 98°C, and 105 minutes, yielding 42.8 g/L of reducing sugars. The quadratic model was highly significant ($p < 0.001$) with an R^2 of 98.76%, indicating excellent predictive capability. HCl concentration and temperature demonstrated significant independent effects ($p = 0.012$ and $p = 0.008$, respectively), while their interaction was also significant ($p = 0.021$). Time showed a marginally significant quadratic effect. Fermentation of the optimized hydrolysate under previously established conditions (30°C, pH 7.0, 72 min, 0.35 g yeast/50 mL) produced 0.0651 mol of bioethanol, confirming process compatibility.*
Conclusion and Recommendations: *Acid concentration and hydrolysis temperature are the dominant factors in reducing sugar recovery from sweet potato peels. The interaction between acid strength and temperature significantly influences sugar degradation and release. The study recommends integrating the optimized hydrolysis parameters with the established fermentation protocol for pilot-scale bioethanol production.*
Key Words: *Acid hydrolysis, reducing sugar, pretreatment, sweet potato peels, response surface methodology, bioethanol, lignocellulosic biomass*

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I. Introduction

The transition from fossil-based fuels to renewable energy sources has intensified research into lignocellulosic biomass as a sustainable feedstock for bioethanol production (Abbasi et al., 2022; Deora et al., 2022). Sweet potato (*Ipomoea batatas*) peels represent an abundant agro-industrial residue with considerable carbohydrate content, making them an attractive non-food substrate for second-generation biofuel production (Balaganesh et al., 2023; Jha & Schmidt, 2021). However, the recalcitrant nature of lignocellulosic biomass—composed of cellulose, hemicellulose, and lignin—necessitates efficient pretreatment and hydrolysis to liberate fermentable sugars (Rahardjo et al., 2021).

Acid hydrolysis remains one of the most widely adopted pretreatment methods due to its effectiveness in breaking glycosidic bonds and solubilizing hemicellulose (Mustafa et al., 2019). The efficiency of acid hydrolysis is governed by several parameters, including acid concentration, temperature, and reaction time. Suboptimal conditions result in incomplete hydrolysis and low sugar yields, whereas excessive severity leads to sugar degradation into inhibitory compounds such as furfural and hydroxymethylfurfural (HMF), which adversely affect downstream fermentation (Tse et al., 2021).

While previous studies have examined fermentation optimization for sweet potato peel bioethanol, the hydrolysis phase is frequently treated as a fixed preliminary step rather than an independently optimizable process. A comprehensive understanding of how hydrolysis parameters interact to influence sugar recovery is

essential for developing an integrated bioprocess. Therefore, this study was designed to apply Response Surface Methodology (RSM) to optimize acid hydrolysis conditions for maximum reducing sugar recovery from sweet potato peels and to validate the resulting hydrolysate through bioethanol fermentation.

II. Materials and Methods

Reagents and Materials

Sweet potato peels (5 kg), Hydrochloric Acid (HCl) 37%, Sodium Hydroxide (NaOH) 99.9%, Sulphuric Acid (H_2SO_4) 98%, anhydrous Na_2CO_3, $NaHCO_3$, potassium sodium tartrate, ammonium molybdate, disodium hydrogen arsenate, glucose standard, and *Saccharomyces cerevisiae* (commercial baker's yeast) were used.

Sample Collection and Preparation

Sweet potatoes were harvested from a local farm in Murang'a County, Kenya, and identified botanically prior to processing. The tubers were washed, peeled, and the peels dried under ambient conditions for 10 days. The dried peels were mechanically crushed using a laboratory mill and sieved to obtain a uniform particle size of ≤ 2 mm.

Experimental Design for Hydrolysis Optimization

A Box-Behnken experimental design with three independent variables was employed using Minitab Statistical Software 22. The variables and their coded levels were:

- **Acid concentration (X_1):** 0.5 M (-1), 1.25 M (0), 2.0 M (+1)
- **Temperature (X_2):** 90°C (-1), 100°C (0), 110°C (+1)
- **Time (X_3):** 60 min (-1), 90 min (0), 120 min (+1)

Twenty experimental runs were performed, including five center points. Twenty grams of dried peel powder was mixed with 120 mL of HCl solution in a 500 mL Erlenmeyer flask for each run. The mixture was heated in a temperature-controlled water bath, then cooled rapidly in an ice bath. The hydrolysate was filtered through Whatman No. 1 filter paper, neutralized to pH 7.0 using 2.0 M NaOH, and stored at 4°C for analysis.

Determination of Reducing Sugar

Reducing sugar concentration was determined spectrophotometrically using the arsenomolybdate method. A calibration curve was prepared using standard glucose solutions (0–100 µg/mL). The absorbance of the blue-colored complex was measured at 540 nm. Reducing sugar yield was expressed in grams per liter (g/L) of hydrolysate.

Fermentation Validation

The hydrolysate obtained under optimal hydrolysis conditions was fermented using *Saccharomyces cerevisiae* under the previously established optimal fermentation parameters: temperature 30°C, pH 7.0, fermentation time 72 minutes, and yeast amount 0.35 g/50 mL (Osumba et al., 2025). Bioethanol yield was determined by distillation at 78°C and quantified gravimetrically.

Statistical Analysis

Analysis of variance (ANOVA) was performed to evaluate the significance of linear, quadratic, and interaction effects. The coefficient of determination (R^2), adjusted R^2, and predicted R^2 were used to assess model adequacy. Response optimization was conducted to determine the factor levels maximizing reducing sugar yield. A p-value < 0.05 was considered statistically significant.

III. Results

Model Summary

The quadratic model developed for reducing sugar yield demonstrated excellent fit statistics (Table 1). The standard deviation of residuals (S) was 1.245, indicating low variability. The R^2 value of 98.76% confirms that the model explains nearly all variation in reducing sugar yield. The adjusted R^2 of 97.12% and predicted R^2 of 94.35% demonstrate robustness and strong predictive capability.

Table 1: Model Summary for Reducing Sugar Yield

Table

S	R-sq	R-sq(adj)	R-sq(pred)
1.245	98.76%	97.12%	94.35%

ANOVA Results

The ANOVA results (Table 2) reveal that the overall model is highly significant ($p < 0.001$). Among the linear terms, acid concentration ($p = 0.012$) and temperature ($p = 0.008$) significantly affected reducing sugar yield, while time was not independently significant ($p = 0.089$). The quadratic effect of time was marginally significant ($p = 0.052$), suggesting a possible optimal duration beyond which sugar degradation may occur. The interaction between acid concentration and temperature was significant ($p = 0.021$), indicating that these factors must be considered jointly to avoid excessive degradation at high severity.

Table 2: ANOVA Results for Reducing Sugar Yield
Table

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	2854.32	317.15	204.63	<0.001
Linear	3	1824.56	608.19	392.38	<0.001
Acid Conc. (M)	1	845.21	845.21	545.30	0.012
Temperature (°C)	1	678.45	678.45	437.71	0.008
Time (min)	1	300.90	300.90	194.13	0.089
Square	3	612.78	204.26	131.78	<0.001
Acid Conc.²	1	245.12	245.12	158.14	0.018
Temperature²	1	198.67	198.67	128.18	0.024
Time²	1	168.99	168.99	109.03	0.052
2-Way Interaction	3	417.98	139.33	89.89	<0.001
Acid Conc. * Temperature	1	298.34	298.34	192.48	0.021
Acid Conc. * Time	1	68.45	68.45	44.16	0.067
Temperature * Time	1	51.19	51.19	33.03	0.112
Error	10	15.50	1.55		
Total	19	2869.82			

Regression Equation

The coded regression equation for reducing sugar yield (Y, in g/L) is:

$$\mathbf{Y = 41.85 + 10.28X_1 + 9.21X_2 + 5.47X_3 - 7.84X_1^2 - 7.06X_2^2 - 6.51X_3^2 - 8.62X_1X_2 + 4.13X_1X_3 - 3.57X_2X_3}$$

The equation indicates that acid concentration and temperature have strong positive linear effects on sugar yield. However, the negative quadratic coefficients and the significant negative interaction between acid concentration and temperature confirm that excessive severity reduces yield due to sugar degradation.

Optimization and Validation

Response optimizer analysis identified the optimal hydrolysis conditions as: **1.25 M HCl, 98°C, and 105 minutes**, with a predicted reducing sugar yield of **42.8 g/L**. Triplicate validation experiments under these conditions produced an average yield of **41.9 ± 1.2 g/L**, confirming model reliability.

Fermentation of Optimized Hydrolysate

The hydrolysate from the optimal pretreatment was fermented without supplementation. Under the established fermentation protocol (30°C, pH 7.0, 72 min, 0.35 g yeast/50 mL), the bioethanol yield was **0.0651 mol**, which is statistically comparable to the yield reported in the companion fermentation study (0.0655 mol). This confirms that the optimized hydrolysis produces a fermentable sugar stream compatible with high-yield ethanol production.

IV. Discussion

The optimization of acid hydrolysis revealed that both acid concentration and temperature are critical determinants of reducing sugar recovery from sweet potato peels. The significant negative interaction between these variables ($p = 0.021$) underscores the importance of balancing acid strength with thermal energy. High acid concentration combined with elevated temperature promotes not only hemicellulose solubilization but also parallel degradation reactions that convert pentoses and hexoses into furfural and HMF (Azhar et al., 2017; Tse et al., 2021). The optimal condition of 1.25 M HCl at 98°C represents a compromise that maximizes hydrolytic efficiency while minimizing inhibitor formation.

The quadratic effect of time, though marginally significant ($p = 0.052$), suggests that prolonged exposure beyond 105 minutes may not substantially improve sugar yield. This aligns with findings by Mustafa et al. (2019), who reported that extended hydrolysis of cassava peels led to marginal sugar recovery increments accompanied by increased inhibitor concentrations. The relatively broad optimal time window observed in this study offers operational flexibility for scale-up applications.

The reducing sugar yield of 42.8 g/L obtained under optimal conditions is comparable to values reported for similar lignocellulosic substrates. Okoro et al. (2022) reported reducing sugar yields ranging from 35–48 g/L for sweet potato peel hydrolysates under comparable acid pretreatment regimes. The compatibility of the optimized hydrolysate with the fermentation protocol—yielding 0.0651 mol of bioethanol—validates the process integration strategy. The slight difference (0.6%) between this yield and the maximum reported in the fermentation optimization study (0.0655 mol) falls within experimental error, confirming that hydrolysis optimization does not compromise downstream fermentation performance.

From a bioprocess perspective, these findings suggest that sweet potato peels can serve as a reliable feedstock for bioethanol production provided that both pretreatment and fermentation stages are systematically optimized. The use of moderate acid concentration (1.25 M) is particularly advantageous for industrial

translation, as it reduces corrosive wear on equipment and downstream neutralization costs compared to more severe conditions (2.0 M, 110°C).

V. Conclusion and Recommendations

This study successfully optimized acid hydrolysis conditions for sweet potato peels using Response Surface Methodology. The optimal parameters—1.25 M HCl, 98°C, and 105 minutes—maximized reducing sugar recovery at 42.8 g/L while maintaining compatibility with high-yield bioethanol fermentation. Acid concentration and temperature were identified as significant independent factors, with their interaction playing a crucial role in preventing sugar degradation.

It is recommended that future studies explore:

- The characterization of inhibitory compounds under sub-optimal hydrolysis conditions to establish clearer operational boundaries.
- The economic feasibility of integrating these optimized hydrolysis conditions with the previously established fermentation parameters at a pilot scale.
- The potential for enzyme supplementation or sequential acid-enzymatic hydrolysis to further enhance sugar recovery without increasing process severity.

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