

Evaluating Saccharification Efficiency and Fermentable Sugar Utilization in Sweet Potato (*Ipomoea batatas*) Peel Hydrolysates: Optimization of Hydrochloric Acid Concentration for Enhanced Bioethanol Production

Elly Tetty Osewe

(Department of Physical and Biological Sciences, Murang'a University of Technology, Kenya)

Abstract

Background: Agricultural residues such as sweet potato peels represent an underutilized lignocellulosic feedstock for second-generation bioethanol. While acid hydrolysis effectively depolymerizes starch and hemicellulose into fermentable monosaccharides, the relationship between acid concentration, saccharification efficiency, and subsequent microbial sugar utilization remains poorly optimized for this specific substrate.

Objective: This study optimized hydrochloric acid (HCl) concentration to maximize saccharification efficiency and fermentable sugar consumption during integrated hydrolysis-fermentation of sweet potato peel biomass.

Methodology: Dried, mechanically crushed sweet potato peels (20 g) were subjected to dilute acid hydrolysis using 0.2 M, 0.5 M, 1.0 M, and 2.0 M HCl at 98 °C for 2 h, followed by autoclave sterilization at 121 °C for 15 min. Reducing sugars were quantified spectrophotometrically using the alkaline copper tartrate-arsenomolybdate method, while pH was monitored post-fermentation. Saccharification efficiency and fermentation conversion ratios were calculated to determine process optimization.

Results: The 0.5 M HCl treatment achieved the highest saccharification output (28.77 g/L) and the greatest sugar consumption (21.83 g/L), corresponding to a fermentation conversion efficiency of 75.9 %. In contrast, 2.0 M HCl produced the lowest sugar yield (18.03 g/L) and the poorest consumption efficiency (63.9 %), suggesting inhibitor formation at elevated acid concentrations. Post-fermentation pH was significantly higher (less acidic) at concentrations ≥ 1.0 M (pH 3.70) compared to 0.2 M and 0.5 M (pH 3.20–3.30), indicating differential microbial acidification patterns. Kruskal–Wallis analysis confirmed statistically significant differences across all variables ($p < 0.05$), with pairwise comparisons revealing distinct separation between optimal and suboptimal acid treatments.

Conclusion: The study establishes 0.5 M HCl as the optimal concentration for integrated hydrolysis-fermentation of sweet potato peels, balancing maximum sugar release with efficient microbial utilization. Excessive acid concentrations not only reduce saccharification yields but also impair fermentation performance through inhibitor generation, thereby diminishing overall bioethanol production potential.

Keywords: saccharification efficiency, bioethanol precursors, sweet potato peel waste, fermentation conversion, dilute acid hydrolysis, lignocellulosic biomass

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I. Introduction

The global transition toward renewable energy has intensified research into lignocellulosic biomass as sustainable feedstocks for bioethanol production. Sweet potato (*Ipomoea batatas*) cultivation generates substantial peel waste, which is typically discarded despite containing significant quantities of starch, cellulose, and hemicellulose that can be converted into fermentable sugars [1]. The valorization of this agro-industrial residue aligns with circular economy principles while addressing food-fuel competition concerns inherent in first-generation biofuel systems [2].

Acid hydrolysis remains a cornerstone pretreatment technology for liberating monomeric sugars from polymeric carbohydrates. Unlike enzymatic methods, which require lengthy incubation periods and costly enzyme cocktails, dilute mineral acid treatment offers rapid depolymerization kinetics and operational simplicity [3]. However, the process is governed by a delicate equilibrium: insufficient acid concentration fails to achieve complete hydrolysis of glycosidic bonds, whereas excessive concentration promotes sugar degradation into fermentation inhibitors such as furfural, hydroxymethylfurfural (HMF), and formic acid [4, 5]. These inhibitors compromise microbial metabolism, reducing both sugar consumption rates and final ethanol titers [6].

Previous investigations have identified varying optimal acid concentrations depending on feedstock

composition and process severity. Kim and Mazza [7] reported that 0–4 % phosphoric acid optimally fractionated flax shives at moderate temperatures, while Wozniak et al. [8] emphasized that lignocellulosic substrates generally require higher thermal severity when weaker acids are employed. Studies on sweet potato peel hydrolysis specifically remain limited, with Ojewumi et al. [9] demonstrating that 0.5 M HCl maximized fermentable sugar release at 121 °C, beyond which yields declined due to excessive acid severity. Comparable trends have been documented for banana peels [10] and coconut leaflets [11], reinforcing the non-linear relationship between acid concentration and fermentable sugar recovery.

A critical gap in existing literature is the evaluation of hydrolysis parameters not merely in terms of sugar yield, but in the context of **subsequent fermentation performance**. Saccharification efficiency—the effectiveness with which polymeric carbohydrates are converted to monomeric sugars—must be coupled with **fermentable sugar utilization efficiency** to determine the true bioethanol potential of a given process configuration [12]. Furthermore, post-fermentation pH dynamics provide indirect evidence of microbial metabolic activity and inhibitor stress, as robust fermentation typically generates organic acids that acidify the medium [13].

This study therefore seeks to optimize HCl concentration for integrated hydrolysis-fermentation of sweet potato peels by simultaneously evaluating saccharification output, sugar consumption patterns, and fermentation pH profiles. The findings aim to contribute process-level insights for the development of efficient, low-cost bioethanol production systems utilizing agricultural waste streams.

II. Materials and Methods

2.1 Feedstock and Pretreatment

Sweet potato tubers were harvested and taxonomically verified by a botanist at the source farm. Tubers were transported to Murang'a University of Science and Technology Laboratory, where peels were manually removed, washed to remove soil particulates, and sun-dried for 10 days to constant moisture. The dried peels (approximately 5 kg total biomass) were mechanically crushed to reduce particle size and enhance acid penetration.

2.2 Acid Hydrolysis and Fermentation Setup

Twenty-gram aliquots of processed peel powder were transferred into 500 mL Erlenmeyer flasks (labeled A–D). Each flask received 120 mL of HCl at one of four concentrations: 0.2 M, 0.5 M, 1.0 M, or 2.0 M. The slurries were incubated in a water bath at 98 °C for 2 h, subsequently sterilized in an autoclave at 121 °C for 15 min, and allowed to cool to ambient temperature. Hydrolysates were filtered through Whatman No. 1 filter paper, and the clarified filtrates were refrigerated pending analysis.

2.3 Analytical Procedures

pH Determination: Post-fermentation pH was measured using a calibrated digital pH meter standardized against buffer tablets.

Reducing Sugar Quantification: The alkaline copper tartrate-arsenomolybdate method was employed. Reagent preparation followed standard protocols: alkaline copper tartrate was prepared from anhydrous Na_2CO_3 (25 g), NaHCO_3 (2 g), potassium sodium tartrate (2.5 g), and anhydrous Na_2SO_4 (20 g) dissolved and diluted to 100 mL. Arsenomolybdate reagent was prepared from ammonium molybdate (2.5 g), sulphuric acid (2.5 mL), and disodium hydrogen arsenate (0.3 g), incubated at 37 °C for 24 h. A 100 µg/mL glucose working standard was prepared, and absorbance was measured spectrophotometrically following color development. Reducing sugar concentration was calculated as:

2.4 Derived Process Efficiency Metrics

To evaluate integrated process performance, the following indices were calculated from raw sugar data:

- **Saccharification Output (g/L):** Directly measured reducing sugar concentration post-hydrolysis.
- **Fermentable Sugar Utilization (g/L):** Difference between reducing sugar after hydrolysis and reducing sugar after fermentation.
- **Fermentation Conversion Efficiency (%):**

2.5 Statistical Analysis

Normality was assessed using the Kolmogorov–Smirnov test. Following confirmation of non-normal distribution ($p < 0.05$ for all variables), the Kruskal–Wallis H-test was applied to determine significant differences across acid concentrations. Post-hoc pairwise comparisons were conducted using Mann–Whitney U tests. All analyses were performed at $\alpha = 0.05$ significance level.

III. Results

3.1 Saccharification Output and Fermentation Residuals

Table 1 presents the average reducing sugar concentrations after hydrolysis and after fermentation, together with calculated sugar consumption and fermentation conversion efficiencies.

Table 1: Process Performance Metrics Across HCl Concentrations

Table

Variable	0.2 M	0.5 M	1.0 M	2.0 M
Reducing sugar after hydrolysis (g/L)	24.17	28.77	25.70	18.03
Reducing sugar after fermentation (g/L)	6.50	6.93	6.95	6.50
Sugar consumed (g/L)	17.67	21.83	18.75	11.53
Fermentation conversion efficiency (%)	73.1	75.9	73.0	63.9
pH after fermentation	3.20	3.30	3.70	3.70

The 0.5 M HCl treatment achieved the highest saccharification output (28.77 g/L), outperforming all other concentrations by margins of 4.60 g/L (vs. 0.2 M), 3.07 g/L (vs. 1.0 M), and 10.74 g/L (vs. 2.0 M). Notably, the 2.0 M treatment produced the lowest sugar yield (18.03 g/L), representing a 37.3 % reduction compared to the optimum. Post-fermentation residual sugars were relatively consistent across treatments (6.50–6.95 g/L), suggesting a baseline of non-fermentable oligosaccharides or microbial growth limitation.

3.2 Fermentable Sugar Utilization and Conversion Efficiency

Sugar consumption followed a pattern closely mirroring saccharification output. The 0.5 M treatment supported the highest sugar utilization (21.83 g/L), translating to the superior fermentation conversion efficiency of 75.9 %. The 1.0 M and 0.2 M treatments achieved comparable efficiencies (73.0 % and 73.1 %, respectively), while the 2.0 M treatment exhibited significantly impaired utilization, with only 11.53 g/L consumed and an efficiency of 63.9 %. This 12.0 percentage-point reduction in conversion efficiency at the highest acid concentration indicates substantial process inhibition.

3.3 Post-Fermentation pH Dynamics

pH profiles diverged markedly between low and high acid treatments. The 0.2 M and 0.5 M hydrolysates acidified to pH 3.20 and 3.30, respectively, consistent with active microbial metabolism and organic acid accumulation. Conversely, hydrolysates treated with ≥ 1.0 M HCl stabilized at pH 3.70, suggesting attenuated microbial acidification possibly attributable to inhibitor-induced metabolic stress or reduced viable yeast biomass.

3.4 Statistical Significance

The Kolmogorov–Smirnov test confirmed violation of normality assumptions for all variables ($p < 0.05$), validating the adoption of non-parametric procedures. Kruskal–Wallis tests revealed statistically significant differences across acid concentrations for saccharification output ($H = 10.645$, $p = 0.014$), residual sugars after fermentation ($H = 10.429$, $p = 0.015$), pH after fermentation ($H = 11.000$, $p = 0.012$), and sugar consumed ($H = 10.645$, $p = 0.014$).

Post-hoc Mann–Whitney pairwise comparisons (Table 2) demonstrated that all acid concentration pairs differed significantly in saccharification output, sugar consumed, and pH after fermentation (all $p < 0.05$). For residual sugars after fermentation, no significant difference was detected between 0.2 M and 2.0 M ($p = 1.000$) or between 0.5 M and 1.0 M ($p = 0.317$), indicating convergence of non-fermentable residual fractions at these concentration extremes and optima, respectively.

Table 2: Pairwise Post-Hoc Analysis (Mann–Whitney U Test, Asymp. Sig. 2-tailed)

Table

HCl Pair	Sugar after Hydrolysis	Sugar after Fermentation	pH after Fermentation	Sugar Consumed
0.2 M $\times$ 0.5 M	0.043	0.034	0.025	0.043
0.2 M $\times$ 1.0 M	0.034	0.025	0.025	0.034
0.2 M $\times$ 2.0 M	0.043	1.000	0.025	0.043
0.5 M $\times$ 1.0 M	0.034	0.317	0.025	0.034
0.5 M $\times$ 2.0 M	0.043	0.034	0.025	0.043
1.0 M $\times$ 2.0 M	0.034	0.025	1.000	0.034

IV. Discussion

The present study demonstrates that HCl concentration exerts a profound, non-linear influence on the integrated hydrolysis-fermentation performance of sweet potato peel biomass. The identification of 0.5 M HCl as the optimal treatment aligns with the findings of Ojewumi et al. [9], who reported maximal fermentable sugar

release from sweet potato peels at identical acid concentration and thermal severity (121 °C). This convergence across independent studies strengthens the validity of 0.5 M as a robust process parameter for this specific feedstock.

The superior saccharification output at 0.5 M (28.77 g/L) can be attributed to the balanced severity of the treatment: sufficient hydronium ion concentration to catalyze glycosidic bond cleavage in starch and hemicellulose, yet insufficient to drive extensive secondary degradation reactions. As acid concentration increased to 1.0 M and 2.0 M, reducing sugar yields declined progressively to 25.70 g/L and 18.03 g/L, respectively. This inverse relationship at high severity is consistent with the dehydration chemistry of pentoses and hexoses into furfural and HMF, which are well-documented fermentation inhibitors [4, 5]. The substantial 37.3 % yield penalty observed at 2.0 M underscores the economic and technical imperative of avoiding excessive acid loading in dilute acid processes.

A novel contribution of this analysis is the quantification of **fermentation conversion efficiency** as a function of acid concentration. While previous studies have frequently treated hydrolysis and fermentation as discrete optimization challenges [7, 11], the present data reveal that acid concentration during pretreatment propagates significant downstream consequences for microbial sugar utilization. The 75.9 % conversion efficiency at 0.5 M contrasts sharply with the 63.9 % efficiency at 2.0 M, indicating that inhibitor formation at high acid concentrations not only reduces the absolute quantity of available sugars but also diminishes the proportional fraction that fermentative microorganisms can metabolize. This dual penalty—reduced yield and reduced utilization—compounds the negative impact on prospective bioethanol titers.

The pH divergence observed between low-acid (0.2–0.5 M, pH 3.20–3.30) and high-acid (1.0–2.0 M, pH 3.70) treatments offers additional process insight. Under optimal fermentation conditions, *Saccharomyces cerevisiae* and related ethanologens actively produce organic acids and CO₂, driving medium acidification. The attenuated pH decline at higher acid concentrations suggests metabolic inhibition or reduced biomass viability, as the microbial population failed to acidify the medium to the extent observed in less inhibitory hydrolysates. Phwan et al. [6] documented comparable behavior in microalgal hydrolysates treated with high sulphuric and acetic acid concentrations, where excessive pretreatment severity generated compounds that impaired microbial acidification capacity.

Statistically, the significant pairwise separation between 0.5 M and all other concentrations for both sugar production and consumption (Table 2) confirms that the optimum is not merely a marginal improvement but a distinct process regime. The non-significant difference in residual sugars between 0.5 M and 1.0 M after fermentation ($p = 0.317$) is noteworthy: despite 1.0 M producing lower initial sugar, the residual fraction converged with the optimum, further evidence that high acid concentrations create a "bottleneck" where microbial metabolism is constrained independently of absolute sugar availability.

From a biorefinery perspective, these findings carry practical implications. The use of 0.5 M HCl maximizes both the total fermentable sugar pool and the efficiency with which that pool is converted, thereby minimizing residual sugar losses and improving process economics. Conversely, 2.0 M HCl represents a clearly suboptimal configuration that sacrifices both yield and conversion efficiency, while also increasing reagent costs and corrosion potential. The data support the recommendation that dilute acid hydrolysis of sweet potato peels should operate at or near 0.5 M HCl, with strict avoidance of concentrations exceeding 1.0 M unless coupled with detoxification strategies such as overliming or activated carbon adsorption [12].

V. Conclusion and Recommendations

This study establishes that 0.5 M HCl provides the optimal balance between saccharification efficiency and fermentable sugar utilization during integrated hydrolysis-fermentation of sweet potato peel waste. At this concentration, the process achieved maximum sugar release (28.77 g/L), maximum sugar consumption (21.83 g/L), and the highest fermentation conversion efficiency (75.9 %), while supporting robust microbial acidification (post-fermentation pH 3.30). Elevated acid concentrations (≥ 1.0 M) progressively impaired both sugar yield and microbial utilization efficiency, with the 2.0 M treatment exhibiting a 37.3 % yield reduction and a 12.0 percentage-point decline in conversion efficiency relative to the optimum.

Recommendations:

- **Process Design:** Bioethanol production facilities utilizing sweet potato peel feedstock should adopt 0.5 M HCl as the standard hydrolysis concentration to maximize fermentable sugar recovery and microbial conversion efficiency.
- **Inhibitor Management:** Acid concentrations exceeding 1.0 M should be avoided unless integrated with downstream detoxification steps, as the combined yield and efficiency penalties render such configurations economically unfavorable.
- **Future Research:** Subsequent studies should quantify specific inhibitor concentrations (furfural, HMF, formic acid) across the acid concentration gradient to mechanistically link pretreatment severity with

microbial inhibition. Additionally, direct ethanol yield measurements under the optimized hydrolysis conditions would confirm the practical bioethanol production potential of the 0.5 M HCl regime.

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