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Sustainable Bioprocessing of Pineapple Peel Hydrolysate: Optimized Fermentation for Xylitol Production Using *Candida tropicalis*

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Abstract

Pineapple peel, a readily available agricultural waste, can be converted into xylitol, a widely used sugar substitute, via microbial fermentation. This study evaluates the fermentation capability of *Candida tropicalis* in utilizing pineapple peel hydrolysate as a substrate for xylitol production. Two fermentation stages were optimized: pH adjustment in synthetic media and substrate preparation using hydrolysis techniques. The fermentation process was assessed under different conditions to determine the most efficient approach for maximizing xylitol yield. The results demonstrated that adjusting the pH and minimizing acetic acid concentration significantly improved fermentation efficiency, resulting in a 30% increase in xylitol production. The highest yield of xylitol production was 0.42 g/g in synthetic media and 0.39 g/g using pineapple peel hydrolysates. This study highlights the effectiveness of bioconversion strategies for agricultural waste valorization, demonstrating the potential of pineapple peel as a cost-effective and sustainable feedstock for xylitol production. The findings contribute to the development of an economically viable bioprocess, reducing dependence on chemical synthesis methods while promoting sustainable waste management practices. Future research should focus on optimizing fermentation conditions at an industrial scale to improve yield and process efficiency.

Keywords: Xylitol; *Candida tropicalis*; pineapple peel; immobilization; bioconversion

Introduction

Malaysia's pineapple industry produces over 300,000 metric tonnes annually, with a substantial portion consisting of pineapple peel, a non-edible lignocellulosic residue (Mardawati et al. 2023). This biomass is often underutilized, typically discarded or composted, leading to resource inefficiencies and environmental burdens (Nasoha et al. 2023). The valorization of pineapple peel into value-added biochemicals aligns with sustainable development goals (SDGs), particularly SDG 12 (responsible consumption and production).

Xylitol, a five-carbon sugar alcohol, is industrially valued for its health-promoting characteristics, particularly in oral hygiene and diabetic-friendly food formulations. Conventional xylitol production involves catalytic hydrogenation of pure xylose derived from hardwood hemicellulose, a process that is both energy-intensive and cost-prohibitive (Bianchini et al. 2023).

Biotechnological alternatives, using yeast strains like *Candida tropicalis*, provide an avenue for milder, more sustainable conversion of hemicellulose-rich agro-wastes into xylitol (Júnior et al. 2019). However, commercial implementation faces challenges such as fermentation inhibition due to acetic acid and furfural, nutrient cost, and inefficient sugar conversion (Rao et al. 2016).

This study proposes a bioprocessing framework that uses dilute nitric acid hydrolysis for selective xylose release, optimizes fermentation parameters including pH and nitrogen source, and evaluates performance using real pineapple peel hydrolysates. The outcome lies in demonstrating efficient xylitol biosynthesis without detoxification, relying instead on strategic bioprocess tuning.

Materials and methods

Raw Material and Pretreatment

Fresh pineapple peel (PP) was sourced from a local fruit processing facility in Selangor. The peel was washed to remove residual sugars and soil, dried at 60 °C for 48 hours, and milled to pass through a ~2 mm sieve. The composition of pineapple peel was characterized using the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (Sluiter et al., 2013). Acid hydrolysis was conducted using 5% v/v nitric acid (HNO₃) at 105°C for 20 min. The filtrates were neutralized to pH 7.0 and stored at 4°C.

Microorganism and Inoculum Preparation

Candida tropicalis FTI20037 was obtained from the Thailand Institute of Scientific and Technological Research. It was maintained on YPD agar slants at 4°C and cultured in YPD broth for 24 hours at 30°C with 200 rpm shaking to achieve a logarithmic-phase inoculum with an OD600 of ~1.0 (Queiroz et al. 2025).

Fermentation Protocol

Batch fermentations were performed in 250 mL Erlenmeyer flasks containing 50 mL of either synthetic medium (50 g/L xylose) or hydrolysate supplemented with 5 g/L nitrogen source. Variables tested included pH (4.0, 5.5, 7.0), and nitrogen types (yeast extract, soy peptone, urea, and a yeast extract/urea blend). All cultures were incubated at 30°C, 200 rpm for 96 hours. Samples were withdrawn at regular intervals.

Analytical Techniques

Xylose, xylitol, acetic acid, furfural, and HMF were quantified by HPLC equipped with an Aminex HPX-87H column and refractive index detector using 5 mM H₂SO₄ as mobile phase. Cell biomass was determined gravimetrically after centrifugation and oven drying at 80°C for 24 hours. Statistical significance (p<0.05) was assessed using ANOVA.

Results and discussion

Hydrolysis Performance and Sugar Recovery

The aim of hydrolysis is to release xylose from the hemicellulose fraction of pineapple peel. This study found that untreated pineapple peel contained 42.9% cellulose, 20.7% hemicellulose, 9.4% lignin, 2.7% ash, 4.6% crude protein, and 18.8% total extractives. For xylitol production, the availability of xylose is critical, which is derived primarily from hemicellulose degradation. At a suitable hydrolysis condition (5% HNO₃, 105°C, 20 min), the xylose concentration reached 20.3 ± 0.6 g/L with low formation of inhibitory byproducts i.e., acetic acid (4.8 ± 0.2 g/L), furfural (0.6 ± 0.1 g/L), and HMF (0.3 ± 0.05 g/L). These results indicate that nitric acid selectively enhanced hemicellulose degradation while minimizing lignin-derived by-products. The hydrolysate was directly usable for fermentation without detoxification, supporting a low-cost, environmentally safer biorefinery approach (Júnior et al. 2019).

Effect of Initial pH in Hydrolysate Medium on Xylitol Fermentation

The investigation of pH is important in regulating fermentation efficiency and directing xylose metabolism towards either biomass formation or xylitol production (Figure 1). In this study, fermentations at neutral pH (7.0) outperformed those at acidic conditions, producing higher biomass (2.8 g/L compared to 1.9 g/L at pH 5.5) and greater xylitol production (0.39–0.42 g/g). Although maximum biomass was observed at pH 5.0, further increases in pH enhanced xylitol yield while reducing biomass.

This trend is likely due to reduced weak acid toxicity, improved membrane transport, and enhanced cofactor (NAD(P)H) availability for xylose reductase activity under alkaline conditions. Studies have shown that undissociated acetic acid, more prevalent at low pH, inhibits xylitol biosynthesis. Increasing pH lowers this inhibition, favoring product formation overgrowth. Additionally, higher pH levels accelerated xylose consumption, shortening fermentation time from 48 to 24 hours.

While biomass continued to increase slightly post- xylose depletion, likely due to available nitrogen, xylitol levels plateaued. Therefore, optimizing initial pH is key to shifting metabolic flux towards desired outcomes in fermentation.

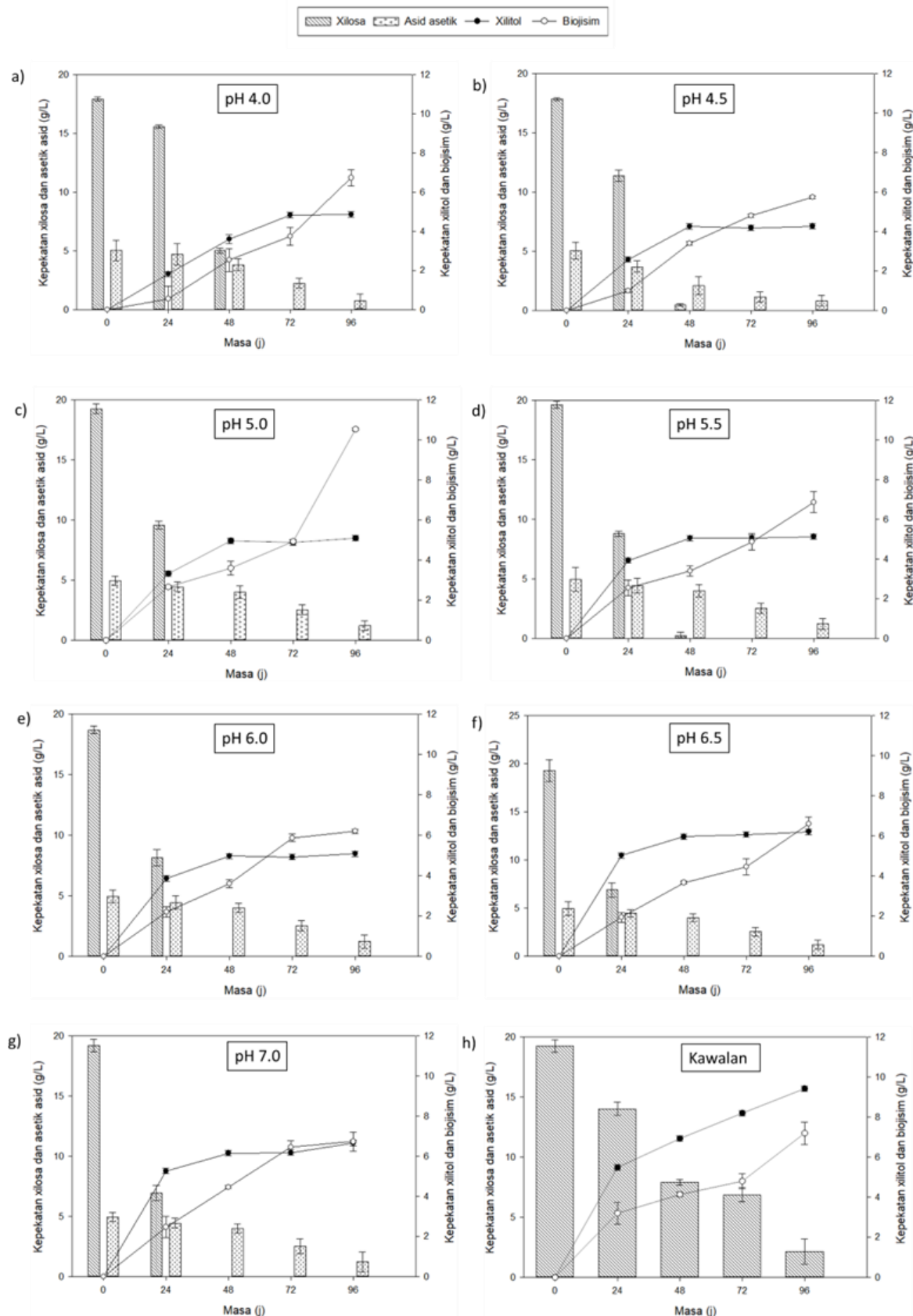
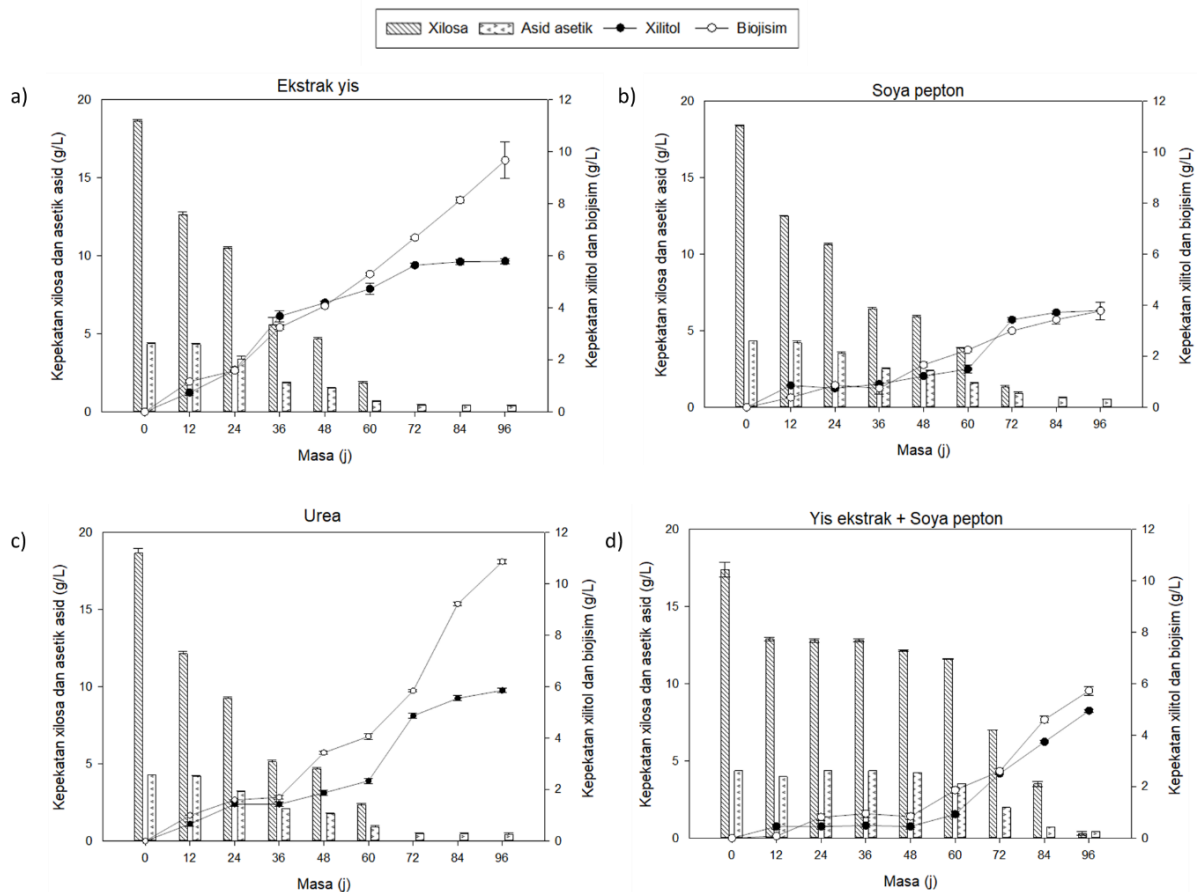


Figure 1: Effect of pH on xylitol production.**Effect of Nitrogen Source on Xylitol Fermentation**

Nitrogen source evaluation highlighted its critical role in supporting microbial metabolism and coenzyme function during xylitol fermentation (Mo et al. 2013). As can be seen in Figure 3, urea proved to be a cost-effective alternative to yeast extract, achieving a comparable xylitol yield (0.31 vs. 0.32 g/g) and even higher biomass (10.92 vs. 10.22 g/L), likely due to its rapid assimilation and intracellular ammonium release. The combination of urea and a neutral initial pH (7.0) created an optimal metabolic environment that enhanced xylose uptake, reduced by-product formation, and supported NAD(P)H-dependent xylose reduction to xylitol (Sasaki et al. 2012).

Blending nitrogen sources (e.g., yeast extract and soy peptone) did not improve performance, thus simplifying media formulation for scalable applications. Fermentations using pineapple peel hydrolysates achieved xylitol yields around 0.39 g/g, affirming the process efficiency. Over 96 hours, fermentation kinetics showed a biphasic trend: an initial exponential phase (0–36 h) characterized by rapid biomass growth and xylose consumption, followed by a deceleration phase (36–72 h) where most xylitol was produced. Specific growth rates reached 0.22 h^{-1} in synthetic medium and 0.19 h^{-1} in hydrolysate medium. Beyond 72 hours, xylitol production plateaued, with peak productivities of $0.11 \text{ g/L}\cdot\text{h}$ and $0.09 \text{ g/L}\cdot\text{h}$. HPLC analysis confirmed minimal ethanol and glycerol formation, indicating efficient carbon channeling towards xylitol (Chen et al. 2010).

**Figure 2:** Effect of nitrogen source on xylitol production.**Conclusion**

This study highlights the feasibility of converting pineapple peel, an abundant agro-waste, into xylitol using a simple, scalable, and non-detoxified fermentation process. The integration of nitric acid hydrolysis and optimized fermentation parameters, neutral pH and urea supplementation, achieved high xylitol productivity without requiring cell immobilization or complex nutrient systems. These findings contribute to the development of sustainable biorefineries that valorize local biomass into value-added chemicals.

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