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Bioconversion of Dragon Fruit Stem Waste into Bioethanol by Enzymatic Hydrolysis

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Abstract

Bioethanol represents a renewable energy source and a sustainable alternative to fossil fuels. In this study, dragon fruit (*Hylocereus* sp.) stem waste was assessed as a lignocellulosic substrate for bioethanol production through enzymatic hydrolysis and yeast fermentation. Cellulose was hydrolyzed using cellulase derived from *Trichoderma viride*, and subsequent fermentation was conducted with *Saccharomyces cerevisiae* for 96 hours. Bioethanol concentration, pH, and yeast growth were measured at 24-hour intervals. Results indicated a continuous increase in crude bioethanol concentration, reaching a maximum of 20% at 96 hours. The pH declined from 5 to 4, while yeast growth peaked at 72 hours and decreased at 96 hours. These results highlight the potential of dragon fruit stem waste as a sustainable substrate for bioethanol production.

Keywords: Bioethanol, Enzymatic saccharification, Lignocellulosic biomass, *Saccharomyces cerevisiae*, *Trichoderma viride*

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Introduction

Demand for fossil fuels such as gasoline, diesel, and coal continues increasing each year. Fossil fuels are non-renewable and drive pollution, with their extraction, transportation, and burning being key contributors to both air pollution and climate change (Rezaei *et al*., 2025). Therefore, environmentally friendly renewable energy alternatives are needed. An alternative solution to overcome the global energy source crisis problem is using bioethanol. Bioethanol is regarded as a leading alternative due to its renewability, biodegradability, and minimal environmental impact. Its elevated oxygen content facilitates more complete combustion, leading to reduced emissions of carbon monoxide (CO), hydrocarbons (HC), and particulate matter compared to conventional fossil fuels (Khan *et al*., 2021; Iodice & Cardone, 2021). Increasing the ethanol content in gasoline reduces carbon monoxide (CO) emissions by enhancing fuel oxidation and combustion efficiency due to the additional oxygen in ethanol molecules (Elshenawy *et al*., 2023).

Bioethanol is considered a clean energy as (Gupta & Verma, 2015) reported that bioethanol blending with 95% gasoline could reduce CO_2 emissions by about 90% and SO_2 emissions by 60–80%, highlighting its potential as a cleaner alternative fuel. Bioethanol feedstocks are typically classified as first-generation, comprising sugar- and starch-rich materials, and second-generation, consisting of lignocellulosic biomass. Recent developments include the use of algal and engineered biomass resources (Kazmi *et al*., 2025). Second-generation ethanol is produced from lignocellulosic biomass. According to Sun & Cheng (2002), for efficient lignocellulosic ethanol production requires feedstocks rich in cellulose and hemicellulose, which can be hydrolyzed to fermentable sugars with a low in lignin content. as lignin restricts enzymatic access to carbohydrates. Dragon fruit (*Hylocereus* sp.) stem (DFS) waste, also referred to as dragon fruit foliage by Anh *et al*. (2021), contains approximately 31% cellulose, 28% hemicellulose, and 14% lignin on a dry weight basis. With about 58% of its mass as

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polysaccharides, DFS is a promising substrate for bioethanol production and other biotechnological applications.

Previous studies have reported using various agricultural wastes as substrates for bioethanol production. For instance, Irhamni *et al*. (2017) successfully produced bioethanol from durian peel waste with a bioethanol purity of 96.99%. Meanwhile, Widyaningrum & Parahadi (2020) produced bioethanol from peeled dragon fruit of 2.46%. Mardawati *et al*. (2022) yielded 6.48 g/L ethanol after 96 h of fermentation from oil palm empty fruit bunch hydrolysate. Furthermore, Nasrun *et al*. (2017) obtained bioethanol of 6.234% from raw papaya skin. Dragon fruit stems so far is often discarded as agricultural residue or used as animal feed. Hawrot-Paw & Stańczuk, (2023) reported the production of 94 mL of distillate containing 65% (v/v) ethanol following separate hydrolysis and fermentation of sugars released from barley straw. To our knowledge the previous published study on DFS conversion to ethanol are lacking. This study therefore evaluates DFS as a new feedstock using enzymatic hydrolysis by *T. viride* cellulase and *S. cerevisiae*.

In bioethanol fermentation, *S. cerevisiae* functions as the primary ethanologenic microorganism, converting fermentable sugars such as glucose, fructose, and sucrose into ethanol and carbon dioxide through glycolytic and fermentative metabolism under anaerobic conditions (Walker & Stewart, 2016). Meanwhile, *T. viride* helps degrade cellulose into glucose by producing enzymes that function in the saccharification of lignocellulosic residues (Lima *et al*., 2024). The integration of cellulolytic fungi, such as *T. viride,* with ethanologenic yeast *S. cerevisiae,* establishes a synergistic system that improves the efficiency of enzymatic saccharification and alcoholic fermentation, thereby enhancing lignocellulosic biomass conversion from agricultural waste and increasing bioethanol yield (Duressa *et al*., 2026; Lima *et al*., 2024). The purpose of this research is to evaluate the feasibility of utilizing DFS (*Hylocereus spp.*) waste as a potential feedstock for bioethanol production thereby can contribute to the advancement of bioethanol production technologies and promotes the utilization of agricultural waste as an alternative energy resource.

Materials and Methods

Materials. Dragon fruit stems (*Hylocereus* sp). collected from Bantul Regency, Yogyakarta, Indonesia. region area; *S. cerevisiae* 3012 and *T. viride* 6013; potato dextrose agar and broth (PDA and PDB); chemical reagents (NaOH, HCl, NaH_2PO_4, KH_2PO_4, $CaCl_2$, $MgCl_2$, and urea) were supplied by Merck (Darmstadt, Germany); deionized water. *Equipment.* oven, blender, 100-mesh sieve, analytical balance, autoclave, magnetic stirrer, rotary shaker, vacuum filtration unit, laboratory centrifuge, incubator, pH meter, colony counter, and alcoholmeter.

Dragon Fruit Stem Powder Preparation

The *Hylocereus* sp. stem biomass (9.7 kg) was cleaned, cut into 1–2 cm fragments, and blended. It was then dried in a convection oven at 80 °C for 4 hours. The dried biomass was ground with a mortar and pestle, sieved through a 100-mesh sieve, and yielded 200 g of fine dragon fruit stem powder (Ariyani *et al*., 2013).

Microbial strain, maintenance, and cultivation

S. cerevisiae and *T. viride* isolates were subcultured on PDA medium and incubated at room temperature for 7 days. *S. cerevisiae* was prepared in PDB and incubated in a rotary shaker at 100 rpm for 48 hours at 30 °C (Widyaningrum & Parahadi, 2020).

Pretreatment

A total of 200 g of dragon fruit stem powder was mixed with 1 L of deionized water and 100 g of 10% (w/v) NaOH in an Erlenmeyer flask and left for 24 hours to disrupt lignin. The mixture was then vacuum-filtered, and the filtrate was rinsed with deionized water at 100°C until neutralized to pH 7 (Ariyani *et al*., 2013).

Cellulase Enzyme Production from T. viride

The medium was prepared by dissolving 4.7% sodium dihydrogen phosphate (NaH_2PO_4), 1.02% potassium dihydrogen phosphate (KH_2PO_4), 0.1% calcium chloride

($CaCl_2$), 0.02% magnesium chloride ($MgCl_2$), and 0.3% urea (CH_4N_2O) (w/v) in distilled water. The medium was then homogenized by a magnetic stirrer (Nathan *et al*., 2014). Approximately 40 g of pretreated DFS filtrate was transferred into a 250-mL Erlenmeyer flask, followed by the addition of 200 mL of the nutrient medium and sterilized by autoclaving at 121 °C for 20 min. After cooling, *T. viride* was aseptically inoculated and incubated at room temperature for 7 days (Widyaningrum & Parahadi, 2020). Following incubation, the culture was filtered, and the filtrate was centrifuged. The obtained supernatant served as the crude cellulase extract for subsequent enzyme activity assays (Fang & Chen, 2018; Legodi *et al*., 2019).

Bioethanol Production from Dragon Fruit Stems

Hydrolysis Stage

A total of 5 g of pretreated dragon fruit stem filtrate was transferred into a 250-mL Erlenmeyer flask. Subsequently, 25 mL of nutrient solution and 10 mL of crude cellulase enzyme extract from *T. viride* (10% v/v) were added aseptically. Distilled water was then added to obtain a final volume of 100 mL. The mixture was homogenized using a sterile glass stirrer and incubated at 37 °C for 24 h (Widyaningrum & Parahadi, 2020).

Fermentation Stage

Following hydrolysis, the solution was adjusted to pH 5 by adding 0.1 M HCl. *S. cerevisiae* was then added at a concentration of 10% (v/v), and distilled water was added to achieve a final volume of 200 mL. The flasks were incubated at room temperature for fermentation periods of 24, 48, 72, and 96 h. Each treatment was performed in triplicate to facilitate the production of bioethanol. After fermentation, the mixture was filtered through filter paper, and the filtrate was collected for analysis of ethanol concentration (Widyaningrum & Parahadi, 2020). Ethanol content was determined using an alcoholmeter. The filtrate was transferred to a measuring cylinder, and the alcoholmeter was lowered gently into the filtrate until it floated freely. The reading was taken at eye level at the bottom of the meniscus after the instrument had settled, and ethanol content was expressed as % (v/v).

pH Measurement and Yeast Growth Dynamics

The pH of the fermentation broth was measured at 0, 24, 48, 72, and 96 h using a pH meter. Measurements were performed in triplicate.

The growth dynamics of *S. cerevisiae* were monitored at the same time points. At each time point, 1 mL of the fermentation broth was transferred into 9 mL of sterile distilled water and vortexed until homogeneous. Serial tenfold dilutions were prepared up to 10^{-6}. An aliquot (0.5 mL) of each dilution was aseptically dispensed into a sterile Petri dish, and molten PDA at 45 °C was poured over it. The plates were then incubated at 37 °C for 48 h. Colonies were counted using a hand counter, and yeast abundance was expressed as colony-forming units per mL (CFU/mL).

Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed descriptively to evaluate the effect of fermentation time on bioethanol production, pH changes, and yeast growth dynamics.

Result and Discussion

The fermentation of dragon fruit stems was conducted for 96 hours and physical changes observed with a progressive increase in turbidity of the fermentation substrate as shown in Fig. 1.

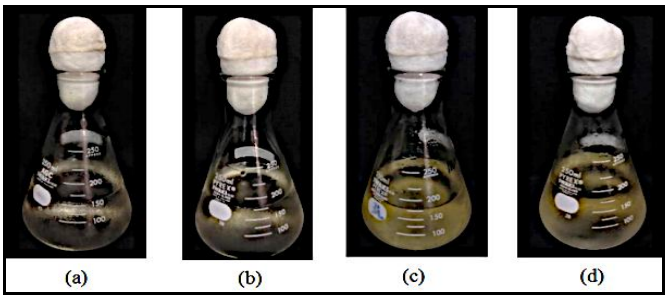


Figure 1. Temporal physical changes in dragon fruit stem liquid during a 96-hour fermentation process: (a) 24h, (b) 48h, (c) 72h, and (d) 96h.

This physical changes indicated the positive correlation between turbidity measurement and microbial growth during fermentation period as similarly observed by Mira *et al*. (2022). This finding supports the use of turbidity as a reliable method for estimating yeast growth in anaerobic fermentation substrates. Yeast growth was quantified by plate counting of viable cells (Fig. 3), which reflects the population actually responsible for fermentation. Culture of *S. cerevisiae* cell in fermentation substrates growth increased over 96-hour incubation period. Yeast assimilated the nutrient contain in the dragon fruit stems such as glucose and fructose as primary carbon sources, as well as essential vitamins and amino acids functions as essential nutrients supporting yeast growth and metabolic activity during fermentation (Walker & Stewart, 2016; Wang *et al*., 2024).

The pH of the fermented substrates was determined every 24 h during the fermentation period using pH indicator kit. Each treatment was performed in triplicate, and the results are presented in Figure 1.

Significant changes in pH during the fermentation process were observed (Fig 2). The initial pH at 0 h was 5 and gradually decreased to 4 after 96 h of fermentation, with a notable decline observed at 48 h. This decrease in pH is associated with the metabolic activity of *S. cerevisiae*, which converts fermentable sugars into ethanol and other metabolites during alcoholic fermentation. These metabolic processes may lead to the accumulation of organic acids and other by-products, contributing to the acidification of the fermentation medium. Similar observations have been reported in previous studies (Walker & Stewart, 2016), where yeast fermentation resulted in the production of ethanol, glycerol, and organic acids that influence the pH of the medium and fermentation performance. In addition, *S. cerevisiae* is known to tolerate and adapt to acidic environments during ethanol production through various metabolic regulation mechanisms, including amino acid metabolism and stress response pathways (Wu *et al*., 2022).

The growth dynamics of *S. cerevisiae* were monitored at 24-hour intervals (Fig 3). This allowed evaluation of the relationship between microbial growth and physicochemical changes in the fermentation substrate.

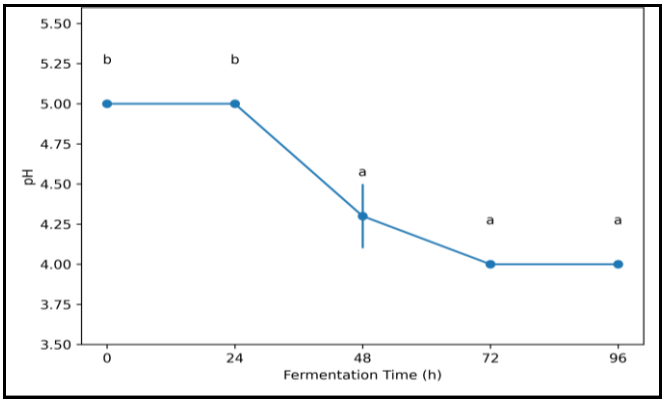


Figure 2. Changes in pH during fermentation of dragon fruit stem substrate over 96 h. Different letters above the data points indicate significant differences among fermentation times ($p < 0.05$)

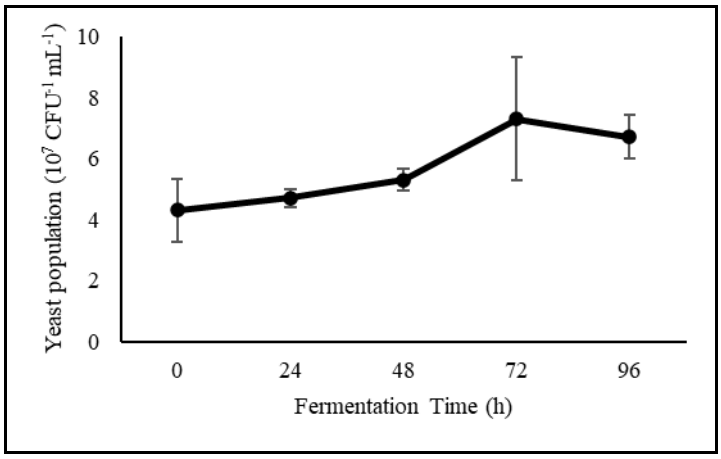


Figure 3. Growth dynamics of S. cerevisiae during fermentation of dragon fruit stem substrate over 96 h. Values represent mean ± SD. Different letters indicate significant differences among fermentation times according to one-way ANOVA followed by the least significant difference (LSD) test ($p < 0.05$).

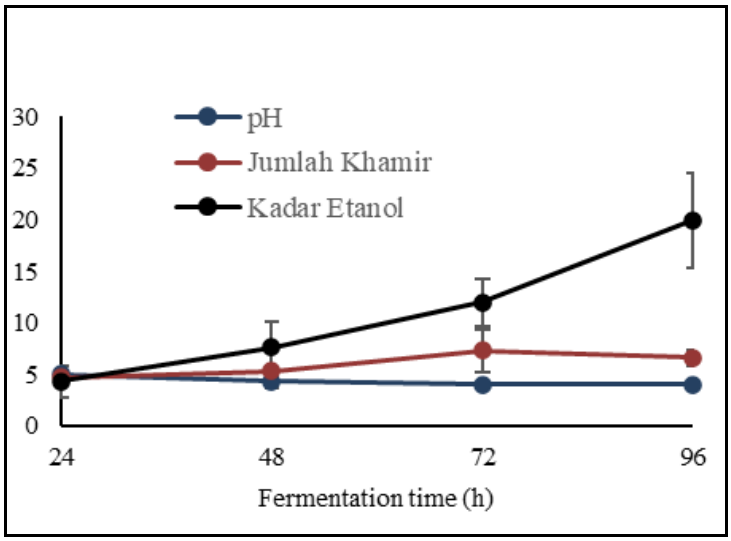


Figure 4. Correlation among pH, yeast growth dynamics, and bioethanol production during the fermentation process. Active fermentation is characterized by decreasing pH and increasing bioethanol concentration, with yeast growth reaching its peak during the mid-incubation phase.

As the yeast population increased from 0 to 72 hours, it entered the logarithmic phase and actively metabolized fermentable sugars to generate energy for cell growth and ethanol production. Dragon fruit (pitaya) stems contain polysaccharides comprising approximately 70–80% of their dry biomass, primarily consisting of various monosaccharides (Zheng et al., 2024). These carbohydrate polymers can be hydrolyzed into simple sugars, which serve as carbon sources for microbial metabolism. During fermentation, yeast converts simple sugars such as glucose and fructose through the glycolytic pathway, producing ethanol and carbon dioxide as primary metabolic products.

The yeast S. cerevisiae is widely recognized for its ability to efficiently ferment a broad range of sugars into ethanol, making it one of the most extensively utilized microorganisms in industrial bioethanol production (Zhang et al., 2023). This efficiency is due to its ability to tolerate various environmental stresses such as temperature fluctuations, osmotic pressure, and fermentation conditions, which contribute to stable large-scale fermentation processes (Hawaz et al., 2024). The decline in yeast population observed at 96 hours is highly likely due to nutrient depletion and the accumulation of inhibitory metabolites such as ethanol and organic acids during prolonged fermentation. Compared to the ethanol production, this trend aligns with the findings reported by (Hawaz et al., 2024), in which cell

growth declines following prolonged incubation as a result of substrate depletion and stress conditions in the fermentation medium. The efficiency of Saccharomyces cerevisiae in bioethanol production is associated with its ability to tolerate various environmental stresses such as temperature fluctuations, osmotic pressure, and fermentation conditions, which contribute to stable large-scale fermentation processes (Hawaz et al., 2024).

According to this study, the pH, yeast growth dynamic and bio-ethanol production has a relevant interaction. As shown in Fig 4. illustrates the relationship between pH variation, yeast growth dynamics, and ethanol production during fermentation. After 24 hours, the pH remained close to the initial value (pH 5) and ethanol production was low (4.3%), indicating that S. cerevisiae was in the lag phase, characterized by cellular adaptation and limited metabolic activity. Between 48 and 72 hours, the yeast entered the exponential phase, as evidenced by an increase in viable yeast count and a corresponding rise in ethanol concentration (7.6–12% v/v), consistent with active sugar metabolism through glycolysis and alcoholic fermentation. The highest ethanol concentration (20% v/v) was recorded at 96 h, even though the viable yeast population had begun to decline. Ethanol is cumulative and can still form in non-dividing cells (Walker & Stewart, 2016).

The decline in viable count was likely caused by nutrient depletion and ethanol inhibition. Over the same period, pH decreased to approximately 4.0, which is likely related to organic acid accumulation and dissolved CO_2, typical of yeast fermentation (Wu et al., 2022). The highest ethanol yield (20%) was recorded at 96 hours, despite a decline in yeast population, which was highly likely due to substrate depletion and ethanol inhibition. Concurrently, pH decreased to approximately 4.0, reflecting the accumulation of organic acids and CO2, a typical feature of yeast-driven fermentation systems (Wu et al., 2022)

The fermentation process was performed at room temperature, within the optimal range for *S. cerevisiae* ethanol fermentation (approximately 28–32 °C). Previous studies have demonstrated that ethanol production increases significantly after approximately 72 h of incubation under suitable fermentation conditions (Derman *et al*., 2022; Lin *et al*., 2012). Similar findings were reported by Narindri *et al*. (2016), who observed increasing ethanol accumulation during bioethanol production from sorghum leaves using *S. cerevisiae* FNCC 3012.

The results indicate that dragon fruit stem is a promising feedstock for bioethanol production, supporting the growth and fermentative activity of *S. cerevisiae*. The concurrent decline in pH, increase in viable yeast population, and accumulation of ethanol are consistent with active substrate utilization and fermentation. However, sugar content and CO_2 evolution were not analyzed, so sugar release and consumption were inferred only indirectly. Future studies should monitor reducing sugars and CO_2 during hydrolysis and fermentation to establish a mass balance and calculate ethanol yield.

Conclusions and Suggestions

This study demonstrates the feasibility of using dragon fruit (*Hylocereus* sp.) stem waste as a sustainable lignocellulosic feedstock for bioethanol production through enzymatic hydrolysis and fermentation. Achieving a maximum crude bioethanol concentration of 20% at 96 hours indicates efficient saccharification by *T. viride* and effective bioconversion by *S. cerevisiae*. The observed decrease in pH and distinct yeast growth phases indicate active metabolic processes during fermentation. These findings support the conversion of agricultural waste into renewable bioenergy, thereby advancing environmentally sustainable bioethanol production systems.

Future studies should focus on optimizing key environmental and process parameters, such as pH, temperature, substrate concentration, and enzyme added, to improve bioethanol yield and fermentation efficiency. Also, measure reducing sugar and CO_2 production to confirm saccharification efficiency and fermentation yield. Investigating more robust or genetically engineered yeast strains with increased ethanol tolerance may further enhance process performance. Furthermore, scale-up experiments and techno-economic analyses are crucial for assessing the industrial feasibility

and sustainability of utilizing dragon fruit stem waste for bioethanol production.

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