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Bioethanol (biofuel) Production from Tea Waste: A Comparative Study Using Cellulase enzyme, Baker's Yeast, and Wild *Saccharomyces Cerevisiae* Cultivated from Rice bran in the context of Bangladesh

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ABSTRACT

Bangladesh ranks among the top twenty tea-producing nations, producing over 95 million kg of tea in 2021. This large-scale production generates considerable tea waste—infused leaves, branches, and discarded leaves posing environmental and economic challenges. Tea residues are abundant in cellulose, hemicellulose, lignin, and bioactive substances. This study was designed to evaluate three bioconversion routes like fermentation using wild Saccharomyces cerevisiae cultured from rice bran, commercial baker's yeast, and cellulase-mediated enzymatic hydrolysis methods. Bioethanol concentration (%v/v) from all the three media was measured using a double beam UV-Visible Spectrophotometer by absorption method. Enzymatic saccharification fermentation showed the highest ethanol yield with 4.10(%v/v) in cellulase enzyme containing treated samples, followed by wild yeast fermentation with 3.12(%v/v) in untreated samples. In case of commercial yeast extract, fermentation yield was 2.64(%v/v) in treated samples. The above results demonstrate that tea waste is available raw material for bioethanol production. The adoption of such processes could contribute to renewable energy regeneration and waste valorization in Bangladesh.

Keywords: Tea waste, Bioethanol, *Saccharomyces cerevisiae*, Enzymatic hydrolysis, Waste valorization.

1. Introduction

The history of beverages is deeply rooted with the history of human civilization. Beverages has been an integral part which have not only been consumed for quenching thirst but for nutrition, conservancy and cultural expressions. Different types of beverages are observed to be mentioned in historical documents which were prepared from various kinds of herbs and plant extracts. Historically, beverages can be classified into two categories- alcoholic and non-alcoholic. As far as non-alcoholic beverages are concerned, tea is the most recognized and widely consumed non-alcoholic beverage globally. Tea alone matches the total consumption of cold drinks, coffee, alcohol, and chocolate **[1]**. Bangladesh is no exception to this global phenomenon of consuming tea. Tea stalls are found in every corner of the country. The per capita yearly tea consumption was calculated to be 550g **[2]** (0.55Kg/Year), approximately 1.5 cup per day. Along with demand of tea leaves, the cultivation of tea leaves has also increased proportionately in the past decades. Tea cultivation in Bangladesh began during British rule, with the first commercial estates emerging in Sylhet in 1854 and later in Chittagong in the 1840s (Wikipedia .com, visited on 28/07/2025). Since then, Bangladesh is consistently ranked among the top twenty tea producers of the world. At present, there are approximately 168 tea estates in Bangladesh according to Bangladesh Tea Board (BTB). With some fluctuations, Bangladesh's tea production is generally on the rise; the biggest productions were in 2019 (96.07 million kg) and 2021 (96.50 million kg) **[3]**.

This huge production of tea also produces a huge amount tea waste. Spent tea leaves, immature tea leaves and branches are generally considered as tea wastes which are often disposed in landfills. This causes a detrimental effect on the environment, specially triggers soil and air pollution. In addition, waste tea leaves and branches are burned to produce energy which is not only ineffective but also releases greenhouse gases. However, effective valorization of these tea wastes can produce value added products as well as it can be processed to produce energy which can reduce dependency on traditional fossil fuels.

In addition to energy recovery, the application of tea waste promotes the overarching principle of a circular economy. Rather than being disposed of in landfills or burnt, which exacerbates pollution and greenhouse gas emissions, tea waste can be incorporated into a resource recovery system. Transforming this lignocellulosic material into bioethanol not only resolves waste management challenges but also fosters a value-added economy, wherein by-products of one sector serve as raw materials for another.

Table 1 Composition of Spent Tea Leaves (Dry Weight Basis) **[4]**

Component	**Content (% w/w)**
Catechins, Theaflavins, Rutin	25–30
Carbohydrates	5–7
Cellulose	18–20
Hemicellulose	6–8
Proteins	15–20
Fibers	26
Lignin	6.5
Minerals (Ca, P, K, Mg, Mn)	5
Amino acids	1–4
Lipids	2
Organic acids	1.5
Chlorophyll	0.5
Carotenoids	0.5
Vitamins	Trace

Tea trash typically has similar components to ordinary tea in roughly identical levels **[5]**. Tea is rich in poly-phenolic compounds which have beneficial health impacts. A number of valuable compounds, including activated carbon **[6–8]**, nanoparticles **[9-10]**, biochar **[11–12]**, and others, can be produced from tea waste.

Energy is the backbone of this modern industrial world. Traditionally, burning fossil fuels is the principal source of energy for decades. As the amount of fossil fuels reserve is decreasing rapidly day by day, necessity of finding alternative sources of renewable energy has become a reality. Bio-ethanol is being seen as a potential candidate to meet the rising energy demand. Bio-ethanol is a renewable, environment friendly and clean energy which can be derived from agricultural biomass. Burning Bio-ethanol emits less CO_2 than traditional fossil fuels and Bio-ethanol can be blended with gasoline to reduce dependency on fossil fuels. Fermentable sugars from lignocellulosic biomass have the potential to be effectively transformed into biofuel **[13]**. Tea waste, a common by-product in Bangladesh, can be used to produce bio-ethanol which will not only reduce environmental pollutions but ensure a circular economy by utilizing tea waste to curtain reliance on fossil fuels.

Achieving sustainability goals, especially those set forth in the United Nations Sustainable Development Goals (SDGs), has become more and more important to the world community in recent years. The need to investigate biomass resources for the production of renewable energy is specifically highlighted by SDGs 7 (Affordable and Clean Energy) and 12 (Responsible Consumption and Production). By turning a plentiful agro-industrial byproduct into a clean fuel, tea waste valorization supports these goals by lowering the strain on traditional energy sources and minimizing environmental impacts.

Bangladesh, akin to several emerging countries, confronts a dual predicament: a growing energy demand and a substantial dependence on imported fossil fuels. The Bangladesh Power Development Board (BPDB) reports that over 70% of energy generation relies on natural gas and imported coal. The volatility of global fuel prices and rising worries regarding supply security have rendered the development of domestic renewable alternatives essential. Tea trash, readily accessible in substantial quantities, provides a strategic benefit by enhancing energy security and alleviating the import load.

Tea waste, being a lignocellulosic substance, has cellulose, hemicellulose, and lignin, which can be transformed into bio-ethanol using several biochemical methods. Cellulose and hemi-cellulose are converted to fermentable sugars (i.e., glucose) using enzymatic hydrolysis and then are fermented by microorganisms to produce bio-ethanol.

While numerous studies have reported the conversion of lignocellulosic biomass such as corn stover, rice husk, and sugarcane bagasse into bioethanol, limited research has focused on tea waste, particularly in the Bangladeshi context. Moreover, existing studies often employ a single conversion route and commercial yeast strains, providing limited insight into route efficiency and the potential of local microorganisms. To the best of our knowledge, no study has conducted a comparative evaluation of three distinct bio-conversion routes for tea-waste bioethanol production using rice-bran-derived wild yeast. Hence, the present study aims to (i) develop bioethanol from spent tea leaves using three different conversion routes, (ii) compare the efficiency of these routes under local conditions, and (iii) evaluate the suitability of rice-bran wild yeast for fermentation. This approach not only identifies the most promising route for sustainable bioethanol production in Bangladesh but also contributes to circular economy practices and renewable energy development.

2.Methodology

2.1 Chemicals and reagents used

Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Media were obtained from Microbiology and Biotechnology Research Laboratory, Department of Microbiology. Sodium Acetate and Sulfuric Acid were collected from Applied Chemistry Lab, University of Rajshahi. Commercial Dry Bakers' Yeast (*S. cerevisiae*) was purchased from local market of Rajshahi and was used without further purification. Cellulase enzyme (Himedia, India) was bought from Selim Traders, Rajshahi and black tea leaves in form of small beads were obtained from local market in Rajshahi. All the chemical and reagents were Laboratory grade.

2.2 Pre-treatment process of tea leaves

Spent black tea leaves were first subjected to hydrothermal pretreatment. Approximately 50 g of dried tea leaves were boiled in 500 mL of distilled water for 8 minutes to remove soluble impurities and polyphenols. After boiling, the leaves were washed 4–5 times with tap water to ensure removal of surface contaminants. The washed leaves were drained and dried in an air-circulating oven at 60°C for 24 hours until reaching a constant moisture content of ~10% (w/w). These leaves were stored in airtight containers and labeled as untreated tea waste (UTW).

For the microwave-assisted thermal pretreatment, a portion of the untreated tea waste (20 g) was soaked in 100 mL of distilled water and subjected to microwave heating in a domestic microwave oven at 300 W (corresponding to ~149°C) for 10 minutes. The sample temperature was monitored using an infrared thermometer, and the leaves were allowed to cool to room temperature before drying in an oven at 60°C for 12 hours to a moisture content of ~10% (w/w). These leaves were labeled as treated tea waste (TTW).

The microwave pretreatment was designed to disrupt the lignocellulosic matrix, enhance cellulose accessibility, and partially solubilize polyphenols, thereby improving enzymatic hydrolysis efficiency **[14]**.

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2.3 Tea waste fermentation by rice bran-cultured wild *Saccharomyces Cerevisiae*

The rice-bran cultured wild Saccharomyces cerevisiae strain was propagated on a medium containing 10 g/L rice bran, 5 g/L SDA, 4 g/L PDA, and 5 g/L malt extract at 25 °C for 24 h. Cells were harvested by centrifugation (2000 rpm, 15 min),[15] washed, and resuspended to an inoculum density of OD600 ≈ 1.0 (~1×10^7 CFU/mL), with viability confirmed by Trypan Blue staining. Fermentation was performed in 100 mL Erlenmeyer flasks with 50 mL working volume under limited anaerobic conditions at 27 °C for up to 7 days, using rice-bran supplemented medium as nutrients. Initial pH was ~5.0, and all experiments were carried out in triplicate biological replicates. The wild yeast strain was morphologically characterized (oval budding cells) and molecularly confirmed by ITS and 26S rDNA sequencing. Fermentation performance was benchmarked against a commercial Baker's yeast strain under identical conditions to evaluate ethanol yield and growth efficiency.

Then the cells resuspended were inoculated with both the tea wastes and incubated at 27 °C for fermentation up to 7 days. Then the fermented liquid was filtered using Whatman qualitative filter paper (diam. 150 mm) and the filtrate was collected. Finally, bioethanol concentration (%v/v) in filtrate was measured by double beam UV-Vis Spectrophotometer (Infitek, USA).

2.4 Tea waste fermentation using Baker's yeast

Initially both treated and untreated tea wastes were mixed separately with 2(%w/v) Baker's yeast solution in an aqueous phase. The experiment was maintained at 30 ˚C for 24 h under agitating conditions at 150 rpm **[15]**. Then the aqueous solution was poured into a small wire screen to separate the solid and liquid phase. Later, the mixture was centrifuged at a 5000 rpm for 15 minutes for further separation, following the procedure adapted from **[14]** with modifications in rotation speed. Finally, bioethanol concentration ((%v/v)) was measured as mentioned above.

2.5 Cellulase enzyme treated tea wastes fermentation by *Saccharomyces cerevisiae*

Enzymatic hydrolysis of tea wastes was carried out to release fermentable sugars prior to fermentation. Cellulase (Sigma-Aldrich, Type: Celluclast 1.5 L) with an activity of 70 FPU/mL was used. The enzyme loading was 15 FPU/g of dry substrate.

A 0.1 M citrate buffer was prepared by dissolving sodium citrate and adjusting the pH to 4.8, which is optimal for cellulase activity. In a typical hydrolysis, 5 g of dried tea waste was mixed with 50 mL of citrate buffer (solid-to-liquid ratio 1:10 w/v). The enzyme solution was added to achieve the target enzyme loading, and the mixture was incubated in an agitating incubator at 50 °C and 150 rpm for 48 hours **[14]**. Finally liberated bio-ethanol concentration ((%v/v)) was measured using a double beam UV-Visible spectrophotometer. General conversion of ethanol using yeasts are given in Figure 1.

2.6 Ethanol concentration determination

To separate the treated and untreated samples into two phases, they were centrifuged for 15 minutes at 5000 rpm. The lower phase was opaque whereas the upper phase was transparent. After that, 1 mL of the upper layer was placed in a test tube along with 1 mL of dichromate reagent (10% w/v $K_2Cr_2O_7$ in 5 M H_2SO_4) and the mixture was vortexed vigorously for one minute. To allow the oxidized sample in the lower phase to develop a blue-green colour, the mixture was left undisturbed at room temperature for about ten minutes. Afterwards, 500 µL of the oxidized product was diluted with 500 µL of deionized water. Finally, the optical density of the tested sample was measured at 650 nm using a spectrophotometer.

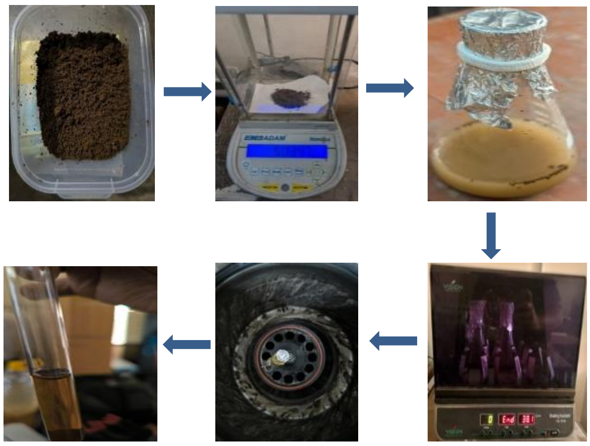

Fig 1: Ethanol conversion steps using Baker's yeast.

3. Results and discussions

The highest ethanol yield was 4.10(%v/v) obtained using treated tea waste with enzymatic saccharification followed by yeast fermentation, highlighting the role of pretreatment in improving cellulose accessibility. The results for ethanol concentration ((%v/v)) obtained from different fermentation conditions are presented in Table 1. The analysis gives preliminary insight into the ability of tea waste for bioethanol production, consistent with globally researched strategies on renewable energy and waste valorization. Among the three fermentation processes, the highest ethanol concentration was produced after treatment with multi-cellulase enzyme. The treated tea waste achieved 4.1(%v/v) ethanol, and the untreated sample achieved 3.8(%v/v) ethanol. Our work confirms that enzymatic saccharification efficiently converts the complex carbohydrates in tea waste into fermentable sugars, resulting in higher ethanol yield. Boiling and microwave thermal treatment prior to enzymatic hydrolysis enhanced cellulose accessibility, explaining the slightly higher yield in the treated sample.

When using commercial baker's yeast, ethanol levels were lower than with the cellulase enzyme. The treated sample showed 2.64(%v/v) ethanol, while the untreated showed 2.18(%v/v). Pretreatment positively affected baker's yeast fermentation, likely through structural modification or partial removal of inhibitors. However, baker's yeast was less efficient than the optimized cellulase treatment in degrading the tea waste matrix.

Fermentation using *S. cerevisiae* cultured from rice bran showed a different trend: the untreated tea waste yielded 3.12(%v/v), slightly higher than the treated sample (2.86% v/v). This may indicate either the wild yeast's better adaptation to raw tea waste or that pretreatment inadvertently produced compounds that inhibited this strain. Further studies on the metabolic response of the wild yeast to pretreated substrates are needed.

All experiments were conducted in triplicate (n=3). Values are reported as mean ± standard deviation. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed that enzymatic saccharification of pretreated tea waste produced significantly higher ethanol than other treatments ($p < 0.05$).

Tea polyphenols, tannins, and other phenolic compounds present in the tea waste may act as fermentation inhibitors. Boiling and microwave pretreatment partially reduced polyphenol content, which is likely beneficial for commercial baker's yeast and cellulase-assisted fermentation. The wild *S. cerevisiae* from rice bran appeared less sensitive to residual polyphenols, as evidenced by its higher ethanol yield from untreated tea waste. Future studies should include quantitative inhibitor analysis and evaluate detoxification strategies to further optimize yields.

Ethanol production under different treatments, calibration curve, and concentrations are presented in Table 1 and Figures 2–3, respectively. Reporting ethanol as g ethanol per kg dry tea waste, volumetric productivity, and conversion efficiency provides a normalized metric for comparing fermentation strategies. Although the dichromate colorimetric method coupled with UV–Vis spectrophotometry was employed for ethanol quantification, it is acknowledged that this approach may be prone to minor interferences from residual tea phenolics. Due to limited access to reference analytical equipment such as GC-FID or HPLC, cross-validation of ethanol concentration could not be performed at this stage. Therefore, the reported ethanol values should be interpreted as indicative rather than absolute. Nevertheless, the calibration curve used in this study showed strong linearity ($R^2 > 0.99$), ensuring reasonable comparative accuracy among treatments. Future work will aim to validate these measurements using chromatographic methods and to determine parameters such as the limit of detection (LOD) and limit of quantification (LOQ) for enhanced analytical reliability.

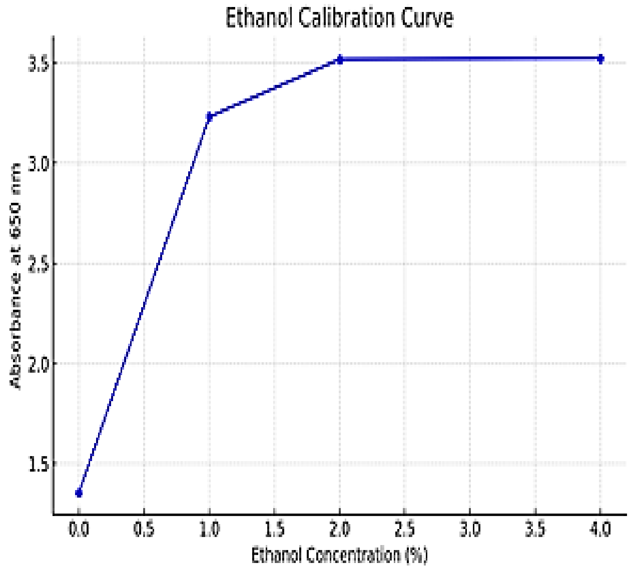

Fig 2: Ethanol calibration curve

Table 2. Ethanol production under different treatments

Fermentation Method	Sample Type	Concentration (% v/v)	Ethanol Yield (g/kg DW)	Volumetric Productivity (g/L•h)	Sugar-to-Ethanol Conversion Efficiency (%)
Cellulase Enzyme + S. cerevisiae	Treated	4.10	32.4 ± 0.8	0.027 ± 0.001	78
Cellulase Enzyme + S. cerevisiae	Untreated	3.80	30.0 ± 0.9	0.025 ± 0.001	72
Baker's Yeast	Treated	2.64	20.8 ± 0.7	0.017 ± 0.001	60
Baker's Yeast	Untreated	2.18	17.2 ± 0.6	0.014 ± 0.001	55
S. cerevisiae (from rice bran)	Treated	2.86	22.5 ± 0.8	0.018 ± 0.001	63
S. cerevisiae (from rice bran)	Untreated	3.12	24.6 ± 0.9	0.020 ± 0.001	65

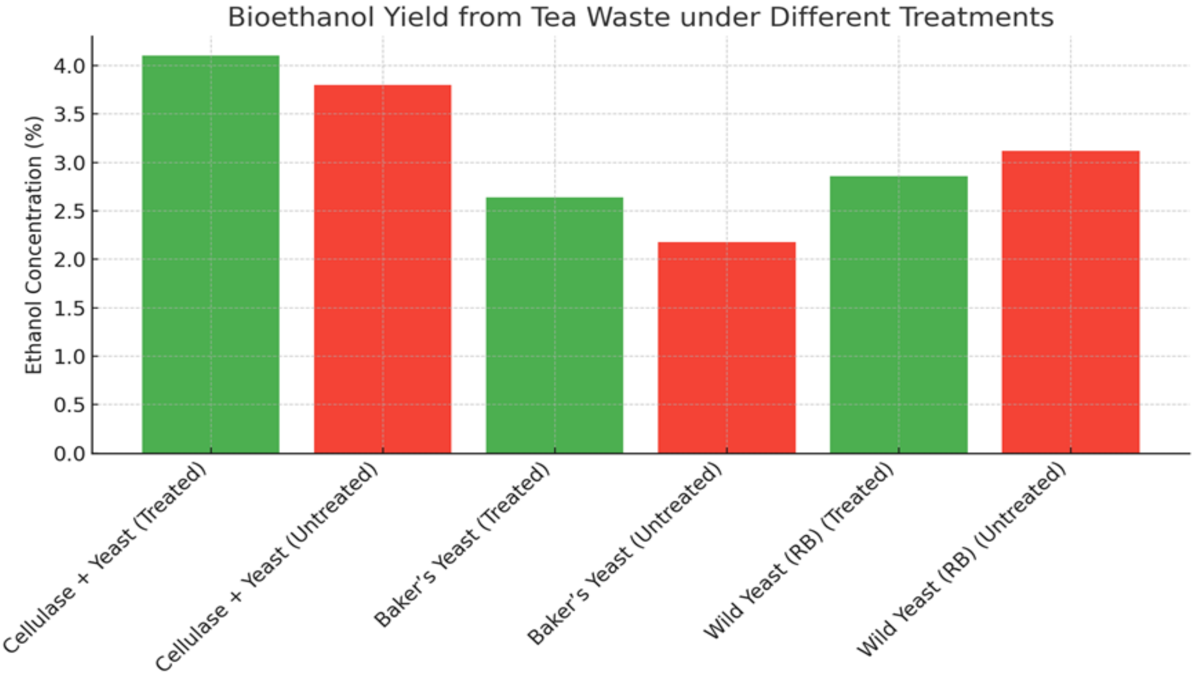

Fig 3: Bioethanol production (yields) from different media and fermentation

4. Conclusion

This comparative study on the feasibility of using three different bio-conversion processes to produce bioethanol from tea waste offered a new horizon of opportunities for renewable energy generation in Bangladesh. Among the three bio-conversion techniques tested, enzymatic saccharification followed by fermentation yielded the best results in terms of ethanol production (4.1 (%v/v) in treated samples), clearly indicating the potential of enzymatic hydrolysis for the conversion of lignocellulosic biomass into fermentable sugars. But, the use of wild S. cerevisiae obtained from rice bran, resulted in relatively high ethanolic fermentation yields, even higher than that of commercial baker's yeast. This is a low-cost microbial substitute that can be very practical for rural and underdeveloped areas. Interestingly though, wild yeast seemed to thrive better when unprocessed materials were utilized, indicating that pre-treatment shows varying effects on strains and demands further research.

The abundant waste tea from Bangladesh has potential as an alternate sustainable feedstock for bioethanol production, allowing for both energy production and waste disposal. The process is also economically viable for rural applications as it is based on materials that are available locally. However, these findings are based on laboratory-scale observations, and broad claims of large-scale economic feasibility should be made cautiously until validated by techno-economic and life-cycle assessments. At industrial scale, challenges such as process scalability, enzyme and yeast costs, and energy-intensive pretreatment steps must be carefully optimized. Integrating waste-heat recovery systems, solar-assisted drying, or using residual solids for energy generation could help balance energy input and output, improving overall process sustainability.

Future studies to enhance commercial applicability should focus on low-cost enzymes, optimized microbial doses, and piloting at a larger scale while assessing energy balance and cost-effectiveness through detailed techno-economic analysis. To wrap up, this study demonstrates the promise of a circular bioeconomy for Bangladesh, with a potential bioconversion pathway for the upcycling of agro-industrial waste into a clean biofuel.

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