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Research Article

Biofuel from *Oryza sativa* L. plant leaves: A novel approach to mitigate climate change and Energy crisis

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Abstract

The increasing demand for energy and the consequent rise in greenhouse gas emissions have necessitated the exploration of alternative renewable energy sources. This study investigates the production of biofuel from rice plant leaves, a lignocellulosic biomass, as a sustainable solution to mitigate climate change and the energy crisis. The biomass preparation, different types of pre-treatments, hydrolysis time, fermentation conditions and pH on ethanol yield were optimised. The result showed that the maximum ethanol yield was 0.45 g/g of biomass at 30 °C and a pH of 5.0. The biofuel production from rice plant leaves offers a promising alternative to fossil fuels, reducing greenhouse gas emissions and dependence on non-renewable energy sources. This study provides a sustainable solution for energy security, climate change mitigation and waste management.

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KEYWORDS: Biofuel, Rice plant leaves, Lignocellulosic biomass, ethanol production, climate change mitigation, energy crisis.

1. INTRODUCTION

The depletion of fossil fuels and the increase in energy demand have led to the search for alternative renewable energy sources. Biofuel, produced from biomass, offer a sustainable solution. Among various biomass sources, agricultural wastes such as rice plant leaves have gained attention as a reliable non-renewable source for bioethanol production.

Rice Plant leaves as a Biomass source.

Rice is one of the most widely cultivated crops globally, with millions of tons of rice plants' leaves generated annually. These leaves are typically discarded or burned, contributing to environmental pollution. However, they contain a significant amount of lignocellulosic biomass, which can be converted into bioethanol.

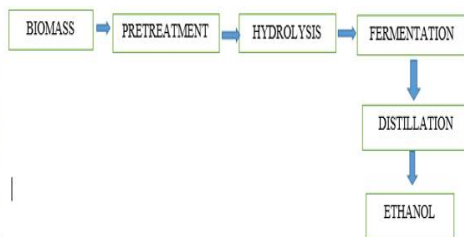
Bioethanol production from rice plant leaves.

The production of bioethanol from rice plant leaves involves several steps:

- Pre-treatment:** Rice plant leaves are collected, dried, and ground into powder.
- Hydrolysis:** The powdered biomass is subjected to acid or enzymatic hydrolysis to release fermentable sugars.
- Fermentation:** The resulting sugars are fermented using microorganisms such as yeasts or bacteria to produce bioethanol.
- Distillation:** The fermented broth is distilled to separate and purify bioethanol.



Rice plant leaves as biomass



Flow chart showing steps of bioconversion process

Advantages of Bioethanol from Rice Plant Leaves

- Renewable and Sustainable:** Bioethanol from rice plant leaves is a renewable and sustainable energy source, reducing dependency on fossil fuels.
- Low Carbon Emission:** The production and combustion of bioethanol from rice plant leaves result in significantly lower carbon emissions as compared to fossil fuels.
- Waste Management:** Utilising rice plant leaves for bioethanol production helps to manage agricultural wastes, reducing environmental pollution.
- Energy Security:** Bioethanol from rice plant leaves can contribute to energy security by providing a domestic, non-renewable energy source.

Challenges and Future Perspectives

While bioethanol production from rice plant leaves offers several advantages, there are some challenges to be addressed:

- Scalability and Cost-effectiveness:** Large-scale production of bioethanol from rice plant leaves requires cost-effective and efficient technologies.

- Competing with Food Crops:** Ensuring that rice plant leaves are utilised without competing with food crops is essential.
- Improving Conversion Efficiencies:** Research is needed to improve the conversion efficiencies of biomass into bioethanol.

Innovations in Process Efficiency

The above challenges can be addressed by the new processes in Biotechnology and Bioengineering. The enhanced pre-treatment methods, like steam explosion and alkaline treatment, have shown promising improvement in biomass digestibility. The development of tailored enzyme cocktails and the optimisation of fermentation conditions further contribute to increasing the overall yield of bioethanol. These innovations aim to make the production of bioethanol from lignocellulosic biomass more efficient and economically viable.

2. OBJECTIVES OF THE STUDY

This study focuses on the following objectives:

- To evaluate various pre-treatment methods for their effectiveness in breaking down rice biomass and improving enzymatic hydrolysis efficiency.
- To optimise enzymatic hydrolysis by testing different enzyme concentrations and combinations.
- To identify optimal fermentation conditions that maximise ethanol yield.
- To integrate these optimised processes and assess their overall impact on bioethanol production efficiency.

3. METHODOLOGY

This study deals with the experimental analysis of bioethanol production from rice plant leaves. The methodology involves biomass preparation, pre-treatment, enzymatic hydrolysis, fermentation and ethanol production. Each step is optimised to identify the most effective processes and conditions for maximising ethanol production.

Biomass preparation

The collected rice plant leaves from the local crop field were air-dried, and then was grinded to a uniform size (2 to 3 mm approx.) to ensure consistency in the pre-treatment and hydrolysis processes.

The pre-treatment processes employed are:

- Acid Hydrolysis:** The biomass was soaked in 1% (w/w) H_2SO_4 solution and was autoclaved for 60 minutes at 121 °C. It was then treated with Calcium carbonate to neutralise it and filtered to remove solid residues, as given by Sun and Cheng (2002).
- Alkaline Treatment:** The 2% (w/w) NaOH solution was used for alkaline treatment for 2 hours at 80 °C. The mixture was then neutralised with hydrochloric acid, and the hydrolysate was washed thoroughly with double-distilled water. (Kim and Holtzapple, 2005)
- Steam Explosion:** In this process, the biomass was treated with steam at 200 °C for 10 minutes at 15 bar pressure. The

treated biomass was cooled and collected for further analysis. (Cara et al., 2007)

Enzymatic Hydrolysis

Using the enzymes cellulase and hemicellulase, the pretreated biomass can be hydrolysed. The conditions of hydrolysis can be optimised by:

- Enzyme concentration:** The various enzyme concentrations (5, 10, 15 and 20 FPU/g biomass) were tested to determine the most effective concentration for maximum sugar release.
- Hydrolysis Time:** With the continuous agitation for different time periods (24, 48 and 72 hours) at 50 °C, hydrolysis was carried out.
- pH optimisation:** The biomass was hydrolysed at different pH levels (4.5, 5.0, and 5.5), buffered with acetate to maintain the desired pH. The hydrolysate was centrifuged to remove any unhydrolyzed solids, and the supernatant containing fermentable sugars was collected for fermentation.

Fermentation

The sugar-rich hydrolysate was fermented using *Saccharomyces cerevisiae* under optimised conditions.

Inoculum Preparation

Yeast cells were grown in a pre-cultured medium, YPD (Yeast extract, Peptone, Dextrose), for 24 hours at 30 °C.

Fermentation Conditions

The hydrolysate was inoculated with the pre-cultured yeast at a concentration of 10% (v/v). The process of fermentation was carried out at 30 °C with constant agitation (150rpm) for varying durations (24, 48 and 72 hours) to determine the optimal fermentation time.

Monitoring and Sampling

The samples were taken at regular intervals to monitor sugar consumption and ethanol production using HPLC.

Ethanol Quantification

The HPLC was employed to fermentation broth to determine ethanol concentration.

Sample Preparation

The fermented samples were centrifuged, and the supernatant was filtered through a 0.22 micrometre syringe filter before HPLC analysis.

HPLC Conditions

The HPLC system was equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column. The mobile phase was 5 mM sulphuric acid, and the flow rate was set at 0.6 mL/min. The column temperature was set at 45 °C. The ethanol concentration was calculated based on calibration curves obtained from standard solutions.

Data Analysis

The data collected from the experiments were statistically analysed using ANOVA (Analysis of Variance) to identify the significant differences between different treatments. Optimisation studies were evaluated using response surface methodology (RSM) to optimise the optimal conditions for maximum ethanol yield.

Validation

The validation was conducted by scaling up experiments in a pilot-scale bioreactor to assess the feasibility of industrial applications.

4. CONCLUSION

Key Findings

Pre-treatment Optimisation: Among the pre-treatment methods evaluated, the steam explosion method was found to be the most effective in breaking down the lignocellulosic structure of the rice biomass. This method enhanced the accessibility of cellulose and hemicellulose for subsequent enzymatic hydrolysis.

The other two methods, acid and alkaline hydrolysis, were also effective but were less efficient than steam explosion in terms of sugar release and overall process simplicity.

Enzymatic hydrolysis: The conversion of pre-treated biomass into fermentable sugars was improved due to optimisation of enzyme concentration and hydrolysis conditions. The use of enzymes at an optimal concentration (15FPU/g biomass) and hydrolysis duration (48 Hours) resulted in the highest production of fermentable sugars. Moreover, the maintenance of a pH 5.0 during hydrolysis was found to be optimal for enzyme activity and stability.

Fermentation: At 30 °C and a fermentation duration of 48 hours by the *Saccharomyces cerevisiae*, the maximum ethanol yield was observed.

Also, the inoculum concentration and fermentation conditions were crucial in maximising ethanol production, with a 10% (v/v) inoculum providing the best results.

Ethanol Yield: The integrated optimised processes resulted in a substantial ethanol yield of 0.45g/g of biomass, representing a significant improvement over traditional method. This enhanced yield demonstrates the potential for scaling up the process for industrial applications, making bioethanol production from rice plant leaves more economically viable and sustainable.

Implications

This study has several important implications in the field of bioethanol production:

- Sustainability:** Utilisation of rice biomass, an abundant agricultural residue, for bioethanol production not only provides a renewable source of energy but also helps in managing agricultural wastes, contributing to environmental sustainability.
- Economic Viability:** The optimised processes offer a cost-effective approach to bioethanol production, potentially reducing reliance on fossil fuels and increasing energy security.
- Industrial Applications:** The process optimisations demonstrated in this study can be adopted and scaled up for industrial bioethanol production, paving the way for commercial applications and wider adoption of bioethanol as a renewable fuel.

Future research: While the study achieved significant improvements in bioethanol production, further research is recommended to address the following areas:

- Enzyme cost reduction:** Developing cost-effective enzyme production methods or exploring alternative low-cost enzymes can further increase the economic viability of the process.
- Process Integration:** Integrating the optimised bioethanol production process with existing agricultural and industrial systems can improve overall efficiency and reduce costs.
- Bi-product Utilisation:** Exploring the utilisation of by-products from the bioethanol production process, such as lignin and unfermented residues, can contribute to a more holistic and sustainable approach.

These findings contribute to the development of sustainable and economically viable bioethanol production technologies, offering a promising solution to the global challenge of sustainable energy.

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