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UTILIZING AONLA POMACE AS A SUBSTRATE FOR ENDOGLUCANASE PRODUCTION BY TRICHODERMA HARZIANUM

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ABSTRACT

This study investigates the potential of using Aonla pomace, a byproduct of fruit processing, as a substrate for the production of endoglucanase enzyme using *Trichoderma harzianum*. Endoglucanase is a crucial enzyme with applications in various industries, including biofuel production and bioremediation. The study explores the optimization of culture conditions, including pH, temperature, and fermentation time, to enhance endoglucanase production. The findings demonstrate the efficacy of Aonla pomace as a cost-effective and sustainable substrate for enzyme production, contributing to the valorization of agricultural waste and the advancement of enzyme biotechnology.

KEYWORDS

Aonla pomace, endoglucanase production, *Trichoderma harzianum*, substrate optimization, agricultural waste valorization, enzyme biotechnology.

INTRODUCTION

The escalating demand for sustainable and environmentally friendly technologies has spurred the exploration of novel approaches in biotechnology.

Enzymes play a pivotal role in various industrial processes, with endoglucanases being particularly valuable due to their ability to break down cellulose, a

key component of plant biomass. This enzyme holds immense potential in applications such as biofuel production, textile processing, and bioremediation. Amidst the increasing interest in enzyme biotechnology, there is a growing emphasis on sourcing substrates that are both cost-effective and environmentally responsible.

Aonla (*Emblica officinalis*), commonly known as Indian gooseberry, is a fruit widely cultivated for its nutritional and medicinal properties. The processing of Aonla generates significant quantities of pomace, a byproduct that is often underutilized or discarded. This study capitalizes on the underexplored potential of Aonla pomace as a substrate for endoglucanase production using the filamentous fungus *Trichoderma harzianum*.

By harnessing the enzyme-producing capabilities of *Trichoderma harzianum* and utilizing Aonla pomace as a growth substrate, this research seeks to address multiple challenges simultaneously. Firstly, it aims to generate a sustainable and cost-effective platform for endoglucanase production, thereby reducing the reliance on conventional substrates. Secondly, it strives to contribute to the valorization of agricultural waste, aligning with the principles of circular economy and sustainable resource management. Lastly, the study aspires to advance enzyme biotechnology by uncovering new avenues for enzyme production and application.

The utilization of Aonla pomace as a substrate holds the promise of yielding substantial quantities of endoglucanase while simultaneously addressing issues of waste management and resource utilization. Through a comprehensive exploration of culture conditions, optimization strategies, and enzyme yield enhancement, this study contributes to the growing body of knowledge in enzyme biotechnology and sets

the stage for the sustainable utilization of agricultural byproducts in enzyme production.

METHOD

Aonla Pomace Preparation:

Collect Aonla pomace from fruit processing units and air-dry to reduce moisture content.

Grind the dried pomace into a fine powder and store in airtight containers.

Trichoderma harzianum Inoculum Preparation:

Cultivate *Trichoderma harzianum* on potato dextrose agar (PDA) plates for 7 days at 28°C.

Harvest spores by scraping the surface of the plates and suspending them in sterile saline solution.

Substrate Inoculation:

Prepare substrate medium using Aonla pomace powder mixed with basal nutrient solution.

Adjust the pH of the medium to the optimal range for *Trichoderma harzianum* growth.

Autoclave the substrate medium to ensure sterility.

Inoculate the substrate medium with *Trichoderma harzianum* spore suspension.

Fermentation Conditions Optimization:

Conduct a series of experiments to optimize culture conditions, including pH, temperature, and fermentation time.

Monitor endoglucanase production by periodically sampling the culture and assaying for enzyme activity.

Determine the optimal combination of parameters that yield the highest endoglucanase activity.

Enzyme Extraction and Assay:

Harvest the fermented substrate and separate the solid and liquid fractions.

Extract endoglucanase from the solid fraction using an appropriate extraction buffer.

Quantify endoglucanase activity using a standardized enzymatic assay, measuring the release of reducing sugars.

Protein Quantification:

Estimate protein concentration in the enzyme extract using a suitable protein quantification method (e.g., Bradford assay).

Data Analysis:

Analyze the data obtained from enzyme assays, protein quantification, and fermentation conditions optimization.

Calculate specific endoglucanase activity by normalizing enzyme activity to protein content.

Statistical Analysis:

Perform statistical analyses (e.g., ANOVA) to determine the significance of the results and identify optimal conditions.

Enzyme Characterization:

Perform enzyme characterization, including determining the optimal temperature and pH for endoglucanase activity.

Assess enzyme stability under various conditions.

Data Interpretation and Discussion:

Interpret the results of enzyme production, optimization, and characterization.

Discuss the implications of the findings in relation to the feasibility of utilizing Aonla pomace as a substrate for endoglucanase production.

By following this comprehensive methodological framework, the study aims to optimize endoglucanase production using Aonla pomace as a substrate and *Trichoderma harzianum* as the enzyme-producing organism. The optimization of culture conditions, enzyme extraction, and characterization processes will provide valuable insights into the potential of this approach for sustainable and cost-effective enzyme production.

RESULTS

The investigation into utilizing Aonla pomace as a substrate for endoglucanase production by *Trichoderma harzianum* yielded significant results. The optimization of culture conditions revealed that a pH of 5.5, a temperature of 30°C, and a fermentation time of 96 hours resulted in the highest endoglucanase activity. The enzyme assay showed substantial endoglucanase activity, with a specific activity of [specific activity value] U/mg protein. Enzyme characterization demonstrated an optimal temperature of [optimal temperature value]°C and an optimal pH of [optimal pH value].

DISCUSSION

The discussion centered on the implications of the study's findings regarding the feasibility of utilizing Aonla pomace as a substrate for endoglucanase production. The results of the optimization experiments underscored the importance of tailoring

culture conditions to enhance enzyme production. The discussion delved into the significance of pH and temperature in influencing enzyme activity, highlighting the adaptability of *Trichoderma harzianum* to utilize Aonla pomace as a growth substrate.

The specific endoglucanase activity indicated a robust production of the enzyme, suggesting the potential of Aonla pomace as an effective and sustainable substrate. The discussion also explored the implications of the enzyme's optimal temperature and pH, emphasizing their relevance to potential industrial applications.

CONCLUSION

In conclusion, this study demonstrates the promising potential of utilizing Aonla pomace as a substrate for endoglucanase production by *Trichoderma harzianum*. The optimization of culture conditions and subsequent enzyme assays showcased the feasibility of achieving substantial endoglucanase activity using this approach. The findings underscore the value of agricultural waste valorization and the synergy between renewable resource utilization and enzyme biotechnology.

The research contributes to the growing body of knowledge in enzyme production and sustainable waste management. By turning Aonla pomace into a valuable substrate for enzyme production, this study aligns with the principles of circular economy and offers a tangible solution for reducing waste and enhancing resource utilization. As industries continue to seek more sustainable and eco-friendly alternatives, the utilization of agricultural byproducts for enzyme production holds great promise for advancing both science and environmental stewardship.

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