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Optimizing Enzymatic Processes for Enhanced Sugarcane Bagasse Utilization in Ethanol Production through Fungal Solid-State Fermentation

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ABSTRACT: The impact of incubation time on enzyme production was examined using wheat bran as the sole carbon source in SSF. A time-dependent increase in enzyme production was observed, particularly between the 3rd and 6th days, indicating a substantial and stable rise. *T. viride* exhibited peak activities, with CMCase and FPase reaching 6.33 IU/g and 8.09 FPU/g, respectively, after 6 days. In contrast, *T. harzianum* demonstrated superior xylanase activity, peaking at 9104 IU/g after 7 days. This underscores the critical role of temporal dynamics in optimizing enzyme production for efficient wheat bran utilization. In temperature optimization results revealed 30 °C as the most favourable temperature for maximal enzyme secretion. Under SSF conditions, *T. viride* displayed peak activities of 8.76 IU/g, 9.13 FPU/g, and 1115.25 IU/g for CMCase, FPase, and xylanases, respectively. Similarly, *T. harzianum* exhibited maximum activities of 3.16 IU/g, 7.35 FPU/g, and 9088.43 IU/g for CMCase, FPase, and xylanases, respectively, under the same conditions. The enzymatic activity of *T. viride* and *T. harzianum* has increased the ethanol production process. These findings provide valuable insights into creating an optimal environment for maximizing enzyme productivity, enhancing the efficiency of sugarcane bagasse utilization in ethanol production.

Keywords: Sugarcane, Wheat Bran, Solid-State Fermentation, Ethanol production.

INTRODUCTION

Due to the finite storage capacity of fossil fuels globally, unrestrained human consumption of these resources has led to severe environmental pollution issues. Consequently, there is a growing global demand for renewable fuel and energy sources. Ethanol is widely recognized as the primary biofuel employed in diverse transportation modes on a global scale (Cantarella *et al.,* 2023). Lignocellulosic biomass has emerged as a highly valuable and sustainable renewable energy source on Earth, presenting an ideal substitute for fossil resources in the synthesis of bio-derived materials and chemicals (Arhin *et al.*,2023). The production of ethanol often relies on feedstocks such as sugar-containing, starch-containing, and lignocellulosic materials(Sharma *et al.,* 2020). Commonly used feedstocks encompass sugarcane, corn, cassava, sweet potato, and wheat. In comparison with alternative feedstock materials, lignocellulosic materials offer distinct and advantageous characteristics, including a dependable supply source, minimal conflict with land utilization for food and feed production, and reduced reliance on fossil fuel inputs (Singh *et al.,* 2023).

Saccharum officinarum L. constitutes primary source for human sugar consumption. The plant belongs to the Poaceae family and is recognized for its pivotal role in providing a substantial portion of the global sugar supply (Musavi *et al.,* 2015). Sugarcane (SC) serves as a fundamental crop in tropical and subtropical regions across the globe. The majority of sugarcane is directed towards the manufacturing of additional value-added goods, including ethanol, enzymes, and sugars (Prati *et al.,* 2005). Sugarcane bagasse (SCB) emerges as a substantial agro-industrial by-product, generated in the course of sugarcane sugar production (Ajala *et al.,* 2021a; Ameram *et al.,* 2019). Effective disposal of sugarcane bagasse (SCB) is crucial for both agricultural profitability and environmental protection. Unfortunately, a considerable portion of bagasse is still incinerated or discarded, primarily attributed to overproduction and the absence of economically viable disposal methods (Ajala *et al.,* 2021b). Due to its widespread availability and high content of cellulosic substances, sugarcane bagasse (SCB) holds the potential for producing ethanol and various other value-added products (Bensah & Mensah 2018).

Many studies on the enzymes production using lignocellulosic biomass, in solid-state bioprocess, have been carried out (Chakraborty *et al.,* 2016; Teigiserova *et al.,*2021). Recently, an overview on 487 enzymes productions and identified that xylanases, CMCases and filter paper activities (FPases) are the most studied; and the residues from wheat, rice and corn the most used (Teigiserova *et al.,* 2021). Wheat bran, a byproduct of

wheat milling, is rich in lignocellulosic materials and represents an abundant and underutilized resource (Soares *et al.,* 2022). Enzymes, particularly cellulases and hemicellulases, play a pivotal role in the hydrolysis of complex polysaccharides present in lignocellulosic biomass, facilitating the release of fermentable sugars(Chakraborty *et al.,* 2016).

This study focuses on the production of enzymes from wheat bran and explores their potential application in the saccharification process, specifically targeting pretreated sugarcane bagasse (Ladeira-Ázar *et al.,*2019). Pretreatment of lignocellulosic materials is a crucial step to enhance the accessibility of enzymes to cellulose and hemicellulose components, thus improving the efficiency of enzymatic hydrolysis (Bensah & Mensah 2013). First, wheat bran is a low-cost and readily available agricultural byproduct, contributing to the economic feasibility of the enzymatic saccharification process (Chugh *et al.,* 2022). Second, the enzymatic machinery produced from wheat bran possesses the potential to exhibit a diverse range of activities necessary for the effective breakdown of complex lignocellulosic structures. Third, the exploration of enzyme applications in the saccharification of pretreated sugarcane bagasse aligns with the broader goal of developing sustainable and economically viable processes for biofuel production (Valladares-Diestra *et al.,* 2022).

Understanding the enzymatic capabilities of wheat bran-derived enzymes and their efficacy in the saccharification of pretreated sugarcane bagasse can provide valuable insights into optimizing bioconversion processes. This research contributes to the growing body of knowledge aimed at developing environmentally friendly and economically viable strategies for the production of biofuels from renewable resources, ultimately advancing the transition towards a more sustainable bio-based economy.

MATERIALS AND METHODS

A. Chemicals and reagents

In the present study, all reagents and chemicals employed were of analytical grade and commercially accessible. Specific chemicals and substrates, such as beech wood xylan, carboxymethyl cellulose (CMC), 3,5-dinitrosalicylic acid, and ethanol, were sourced from Sigma-Aldrich (US). The growth medium, potato dextrose agar, and additional medium components, including yeast extract, peptone, dextrose, and agar, were obtained from Hi-Media (India). Sugar standards and other chemicals like glucose, xylose, mannose, sodium hydroxide, sulfuric acid, hydrochloric acid, glacial acetic acid, sodium chlorite, phloroglucinol, cellobiose, arabinose, galactose, xylitol, sucrose, sorbitol, and maltose were also acquired from Hi-Media (India). Every other chemical and reagent employed in the ongoing investigation adhered to the highest purity grade commercially available.

B. Microbial culture and condition

The fungal strains used in current research, namely *Trichoderma harzianum* and *Trichoderma viride*, were obtained from the Department of Plant Pathology at CCS Haryana Agricultural University in Hisar, India. All ascomycetes fungal strains were stored on PDA slants at a temperature of 4 ºC. This method of storage on PDA slants was selected to maintain the longevity and viability of the fungal strains for future experimental use. The basidiomycetous yeast, *Saccharomyces* sp., was collected from the Bioenergy Laboratory, Department of RBEE, CCSHAU, Hisar, and kept on a YEPDA slant at 4 ºC.

C. Wheat bran Substrate

Wheat bran, a plentiful agricultural by-product rich in cellulose, hemicellulose, and lignin, proves to be a cost-effective substrate for industrial enzyme production. This study highlights its crucial role in stimulating the production of key enzymes, such as cellulases and xylanases. The utilization of locally sourced wheat bran ensures a sustainable and economical approach, emphasizing its contribution to a nutrient-rich environment that supports microorganism growth. Thorough cleaning and tunnel-drying processes have been employed to effectively remove contaminants, preserving the integrity of the enzymes. Utilizing wheat bran as a substrate underscores a commitment to sustainability in enzyme production.

D. Production of cellulases and xylanases

(i) Inoculum preparation. A spore suspension was uniformly prepared from recently cultured Trichoderma harzianum and Trichoderma viride by introducing sterile normal saline with 0.5% Tween-80. Spores were harvested by delicately scraping the surface, resulting in a 1×10^6 spores/ml concentration suspension, serving as the inoculum. For cultivation, a 250 ml Erlenmeyer flask containing 10.0 g of wheat bran was humidified with a mineral salt solution in a 1:3 (w/v) ratio. After sterilization, the medium was inoculated with fungal spores at a concentration of 1×10^6 spores/ml/g. The spores were then filtered using a laboratory-designed assembly, ensuring a controlled and standardized environment for subsequent experimental procedures.

(ii) Temp optimization and time-course study for cellulases and xylanases production. In a 250 ml Erlenmeyer flask, 10.0 g of wheat bran was moistened with a mineral salt solution in a 1:2.5 (w/v) ratio. After sterilization at 125 °C for 20 minutes, the medium was uniformly inoculated with freshly acquired fungal spores (1 × 106 spores/ml/g). The incubation took place at 25°C, 30°C, and 35°C under static culture conditions. Enzymes were extracted on the third day of incubation at 24-hour intervals using a 0.5 M sodium acetate buffer (pH 5.0). The crude culture sample extract underwent vigorous vortexing for 2 hours at 150 rpm and 30 °C. Enzymes were then extracted by filtering through an eight-layer muslin cloth and centrifuging for 12 minutes at 9,800g in a cooled centrifuge. Cellulases and xylanases activity was determined in the biomass-free culture filtrate.

E. Analytical methods

(ii) Sugars estimation. The formulation of the stock solution comprised the following ingredients (g/L): 3,5-

dinitrosalicylic acid (10.0), potassium sodium tartrate (200), crystalline phenol (2.0), and sodium hydroxide (10.0). Subsequently, these components of the DNSA (3,5-dinitrosalicylic acid) reagent were meticulously combined in 1.0 L of distilled and deionized water (DDW) under continuous stirring. The resulting solution underwent careful filtration through Whatman filter paper no. 1 under subdued lighting conditions. To safeguard the stability and integrity of the reagent, it was stored in an amber-coloured bottle at room temperature until its utilization, ensuring optimal conditions for its subsequent application.

RESULTS AND DISCUSSION

A. Effect of incubation time on cellulases and xylanases production

Cellulases and xylanases were produced through solid-state fermentation (SSF) using wheat bran as the sole carbon source by two ascomycetous fungi, namely *T. harzianum* and *T. viride*, at 75% moisture content. The time-course analysis revealed a direct correlation between fermentation duration and enzyme production. As the fermentation time increased from the 3rd to the 6th day, enzyme production showed a consistent rise and remained stable thereafter. The peak activities of CMCase (6.33 IU/g) and FPase (8.09 FPU/g) were achieved after 6 days of incubation with *T. viride* under SSF conditions (see Fig. 1a-b). In contrast, *T. harzianum* exhibited the highest xylanase activity, reaching 9104 IU/g after 7 days of incubation under SSF culture conditions (see Figure 1c).

B. Effect of incubation temperature on cellulases and xylanases production

Cellulases and xylanases production from the ascomycetous fungi *T. harzianum* and *T. viride* underwent optimization at varying temperature conditions. The findings revealed that the optimal temperature for maximal enzyme secretion from both fungi was 30 °C. Under solid-state fermentation (SSF) culture conditions, *T. viride* exhibited peak activities of 8.76 IU/g, 9.13 FPU/g, and 1115.25 IU/g for CMCase, FPase, and xylanases, respectively (refer to Fig. 2). Similarly, *T. harzianum*, under SSF culture conditions, displayed maximum activities of 3.16 IU/g, 7.35 FPU/g, and 9088.43 IU/g for CMCase, FPase, and xylanases, respectively (Fig. 2).

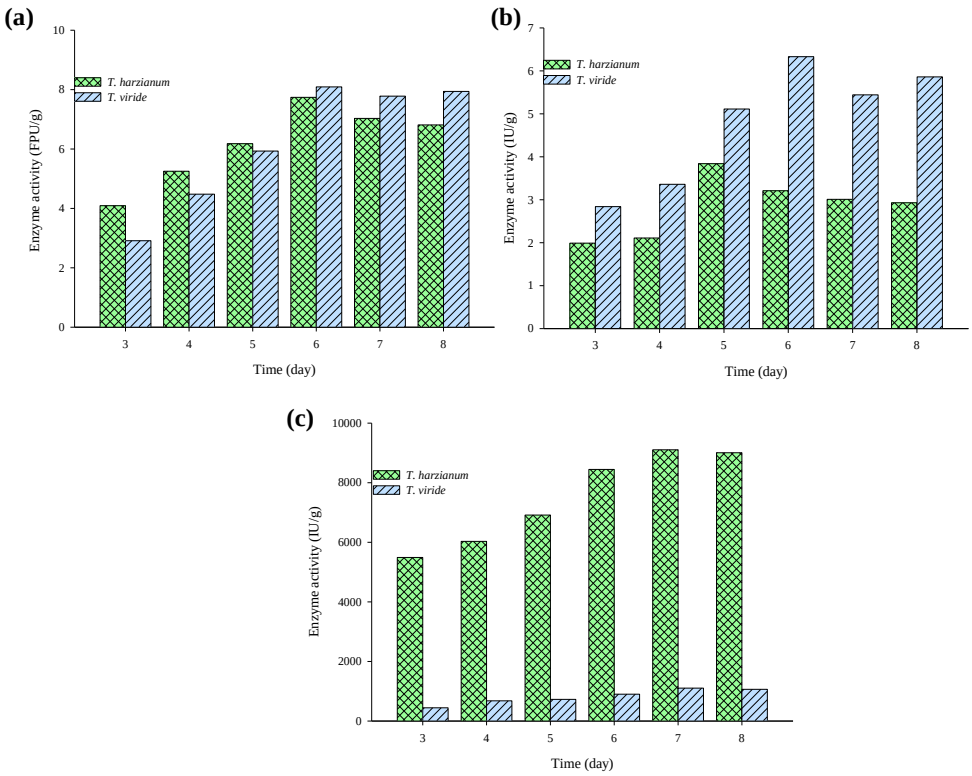

Fig. 1. Time course study for the production of cellulases and xylanases from ascomycetes fungi using wheat bran as a production medium under SSF culture condition. Production of (a) FPase, (b) CMCase, and (c) Xylanase.

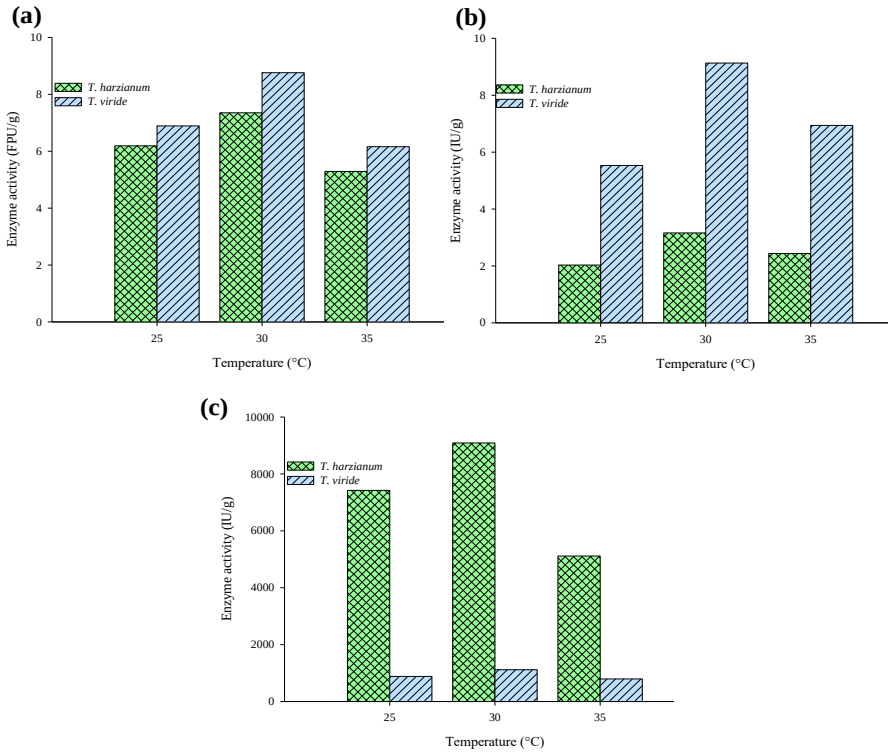

Fig. 2. Effect of incubation temperature on cellulases and xylanases production from ascomycetes fungi using wheat bran as a production medium under SSF culture condition. Production of (a) FPase, (b) CMCase, and (c) Xylanase.

CONCLUSIONS

This study on cellulases and xylanases production via solid-state fermentation with *T. harzianum* and *T. viride* revealed a direct correlation between fermentation time and enzyme yield, peaking at 6 days for *T. viride* and 7 days for *T. harzianum*. Temperature optimization showed 30°C as the ideal condition for maximal enzyme secretion, with *T. viride* and *T. harzianum* exhibiting peak activities at 8.76 IU/g, 9.13 FPU/g, and 1115.25 IU/g, and 3.16 IU/g, 7.35 FPU/g, and 9088.43 IU/g, respectively. These findings advance our understanding of the temporal and temperature dynamics in fungal solid-state fermentation, offering valuable insights for industrial enzyme production.

FUTURE SCOPE

This research lays the groundwork for continued exploration in optimizing enzyme production for improved utilization of sugarcane bagasse in ethanol production. Subsequent studies could centre on scaling up these refined processes to industrial levels, thereby enhancing overall efficiency in biofuel production. Moreover, exploring the applicability of these findings to diverse lignocellulosic biomass sources holds the potential to expand the horizons of sustainable bioenergy production.

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Conflicts of Interest. None.

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