Cost-Effective Utilization of Agricultural Waste for the Production of Industrially Important Enzymes by *Aspergillus Niger*

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Abstract

To overcome the environmental and sustainability challenges caused by agro-wastes, such as wheat straw (Triticum aestivum L.), wheat bran (Triticum aestivum L.), and rice husk (Oryza sativa L.) biotechnological innovations played a vital role. This research focused on the use of lignocellulolytic fungus Aspergillus niger in solid-state fermentation for the cost-effective production of cellulase and pectinase enzymes with agricultural residues as substrates. The evaluation of wheat bran as a substrate for fungal growth revealed the highest fungal cellulase activity and pectinase activity at 6.47 ± 0.35 U/g and 4.03 ± 0.25 U/g, respectively. Wheat bran outperformed other substrates in terms of cellulose and pectinase activity, hence strengthening its position as a fermentation substrate. Moderate enzyme production was shown by rice husk (cellulase activity 3.27 ± 0.25 U/g and pectinase activity 1.90 ± 0.20 U/g). Wheat straw showed the least enzyme activity (1.03 ± 0.15 U/g cellulase and 0.73 ± 0.15 U/g pectinase), likely because high-cellulose fibrous materials slow down enzymatic breakdown of complex structures. These results showed the potential of agro-residues and agro-industrial waste, particularly wheat bran, as substrates for controlled bioprocesses, for the advancement of sustainable byproduct enzyme production and eco-friendly workflows.

Keywords: Agriculture Waste Substrates, Solid-State Fermentation, Enzyme Production.

Introduction

Crop residues, including wheat or rice straw, fruit peels, and sugarcane bagasse, are abundant sources of pectin and polysaccharides—plant cellular components. Cellulose, the most abundantly occurring organic polymer on Earth, is made up of long unbranched chains of glucose molecules linked by β-1-4 glycosidic bonds, which provide strength to plant tissues (Singh et al., 2016). Pectin, on the other hand, is a heteropolysaccharide that is primarily found in the middle lamella of plant cell walls. It has additional functions such as softening during ripening and aiding cell adhesion. These compounds are particularly abundant in agricultural residues from the harvesting

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of crops, fruit processing, and cereal milling. Often, such residues are burned or discarded, thereby undermining their potential in sustainable agriculture and rural biotechnology. Although indigestible to humans, cellulose, pectin, and other agro-residues can be transformed into fermentable sugars or bioactive compounds through solid-state fermentation. This bioconversion supports applications like the production of bioethanol, bio-fertilizers, and soil conditioners, reducing dependence on fossil fuels and synthetic agrochemicals. Furthermore, enzymatic treatment of cellulose- and pectin-rich material contributes to biodegradable product formation such as livestock feed additives and compost enhancers. Harnessing natural polymers in agro-waste supports circular agricultural practices and promotes the shift toward bio-based farming systems (Ravindran & Jaiswal, 2016).

Composition and Significance of Agro-Waste

Agro-waste includes the residual plant materials generated during agricultural and food processing activities—examples include wheat straw, rice husk, corn stalks, sugarcane bagasse, and fruit peels. These wastes contain valuable biochemical compounds like cellulose, hemicellulose, pectin, lignin, and nutrients. Among these, cellulose is the most abundant, offering structural integrity to plant cells, while pectin contributes to cell adhesion and fruit ripening (Pattnaik et al., 2020). Despite their richness in useful biomolecules, agro-wastes are frequently underutilized and often incinerated or landfilled, which harms the environment and squanders potential biotechnological resources. Given its composition, agro-waste is ideal for bioprocessing into enzymes, biofuels, organic fertilizers, and even bioplastics. Utilizing such residues helps reduce environmental pollution and adds agronomic income, supporting circular economy models. Transitioning to these practices offers cost-effective and environmentally sound alternatives aligned with sustainable development goals (Kumar et al., 2023).

Microbial Sources for Enzyme Production

Microorganisms are efficient producers of enzymes for cellulose degradation. Filamentous fungi such as *Trichoderma reesei, Aspergillus niger*, and *Penicillium* species are renowned for producing cellulases and pectinases (Kuhad et al., 2011). Bacteria such as *Bacillus* and *Clostridium thermocellum* also play critical roles. *C. thermocellum,* in particular, uses cellulosomes—multi-enzyme complexes that work efficiently even at elevated temperatures (Patel et al., 2019). These microbes are central to industrial-scale bioconversion of agro-waste.

Types of Industrially Important Enzymes

Two major enzymes—cellulase and pectinase—are particularly valuable for industrial applications. Cellulase breaks down cellulose into glucose, facilitating processes like biofuel generation, enhanced digestibility in animal feeds, and fiber modification in textiles and paper production (Arshad et al., 2020; Saeed et al., 2025). Pectinases degrade pectin and are widely used in fruit juice clarification, oil extraction, and fiber treatment in textiles. In agriculture, these enzymes contribute to composting by breaking down plant cell walls and enhancing nutrient cycling in soil. Beyond cellulase and pectinase, enzymes like amylases, proteases, and lipases also serve key roles, but cellulase and pectinase are especially important for agro-waste bioconversion and sustainable bio-product generation (Jayani et al., 2005).

Biotechnological Applications of Cellulases

Cellulases are integral to the hydrolysis of lignocellulosic biomass. They are employed in various industries such as textile, pulp and paper, and animal feed (Sun & Cheng, 2002). The enzyme

complex includes endoglucanases, exoglucanases, and β-glucosidases that synergistically break down cellulose into fermentable sugars (Bhat, 2000). This efficiency makes cellulases essential in advancing bioeconomy strategies. Utilizing agro-waste supports circular agricultural systems. Residues are converted into biofuels, organic fertilizers, and enzymes creating value from waste and reducing environmental burden (Ravindran & Jaiswal, 2016). Biotechnological processing of such materials is not only sustainable but also economically viable for rural industries (Lynd et al., 2002). Transitioning to these practices is critical for achieving global sustainability goals.

Materials and Methods

Chemicals Used

The chemicals used in this study were of analytical reagent grade and were procured from reputable suppliers, including Sigma-Aldrich (USA)**,** Merck (Germany), and HiMedia Laboratories (India)**.**

Isolation of Fungi

The fungal isolate, identified as *A. niger*, was obtained from dead and decaying organic matter collected from the local environment. Samples were processed using standard serial dilution and plating techniques on Potato Dextrose Agar (PDA) to isolate the fungus. The isolate was selected based on its morphological characteristics and cellulase-producing capability for further enzyme production studies.

Culture Maintenance

PDA slants and glycerol stock solution of fungal isolate was made to keep the culture fresh and was regularly transferred to fresh agar plates or slants every two or three months to keep the strain vital.

Pretreatment Method for Lignin, Cellulose, and Hemicellulose Assessment

Dried and milled samples of rice husk (*Oryza sativa* L.), wheat straw (*Triticum aestivum* L.) and wheat bran (*Triticum aestivum* L.) underwent sequential chemical treatments to check the quantity of lignin, cellulose, and hemicellulose contents. Neutral detergent fiber (NDF) estimated total cell wall components, acid detergent fiber (ADF) measured cellulose and lignin, and hemicellulose was calculated as the value difference in between NDF and ADF. Acid detergent lignin (ADL) was determined by treating the ADF residue with 73 % sulfuric acid to check and isolate lignin (Van Soest et al., 1991).

Factor Studies

Factor studies were conducted to check the effects of temperature, pH, and incubation time on enzyme activities Temperature variations were tested to check the optimal conditions for maximum enzyme efficiency and thermal stability. Similarly, enzyme activity was assessed across a range of pH levels to identify the most favorable pH for catalytic performance. Incubation time was also varied to observe its impact on the extent of substrate degradation and to establish the time required to achieve peak enzyme activity**.**

Solid State Fermentation (SSF)

In the solid-state fermentation (SSF) method, three different solid substrates were used, each weighing 5 g and placed in separate Erlenmeyer flasks. A 1 cm diameter hole was cut out at the

center of the pure fungal culture to inoculate the substrate. The mineral salt medium prepared for SSF contained $(NH_4)_2SO_4$ (0.6 g), KH_2PO_4 (0.4 g), K_2HPO_4 (0.5 g), and $MgSO_4$ (0.2 g) to support fungal growth (Gummadi et al., 2007). The medium was sterilized by autoclaving at 121°C and 15 psi for 15 minutes.

Enzyme Assays for Cellulase and Pectinase

The DNSA (3,5-dinitrosalicylic acid) method was employed to quantify the specific activities of both cellulase and pectinase enzymes. For the cellulase assay, a reaction mixture containing 1 mL of diluted enzyme, 1 mL of 1% carboxymethyl cellulose (CMC), and 1 mL of acetate buffer (pH 5.0) was incubated at 50 °C for 30 minutes. Following incubation, 3 mL of DNSA reagent was added, and the mixture was heated in a boiling water bath for 5–6 minutes (Mohun & Cook 1962). The development of a brick-red color indicated the presence of reducing sugars released by enzymatic hydrolysis. Similarly, for the pectinase assay, 1 mL of diluted enzyme was mixed with 1 mL of 1% pectin solution and 1 mL of acetate buffer (pH 5.0) 1mL of then incubated under the same conditions as cellulose but the substrate for pectinase will be glucuronic acid (Khan & Barate 2016) After incubation, DNSA reagent was added, and the tubes were boiled for 5–6 minutes to develop the color. The enzyme activities were then calculated and expressed in units per gram (U/g).

Statistical Analysis

Each assay was carried out three times and their results were expressed as the arithmetic mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess differences.

Results

Among the tested agro-waste substrates, wheat bran supported the highest production of both cellulase and pectinase enzymes. Rice husk showed moderate enzymatic activity, while wheat straw resulted in the lowest enzyme yields. The superior performance of wheat bran highlights its effectiveness as a substrate for solid-state fermentation with *Aspergillus niger*. These results underline the potential of agro-residues, particularly wheat bran, for eco-friendly enzyme production in sustainable bioprocessing applications.

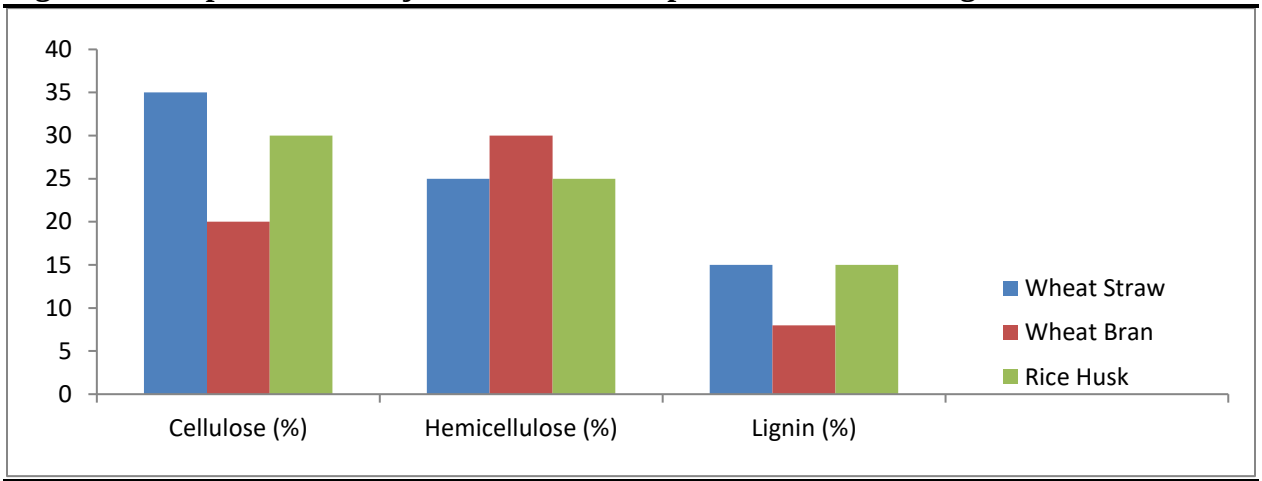
Lignin, Cellulose, and Hemicellulose Content

Wheat straw has the highest cellulose (35%) but also high lignin (15%), which may hinder enzyme access. Wheat bran contains less cellulose (20%) but more hemicellulose (30%) and lower lignin (8%), making it easier to degrade. Rice husk has moderate cellulose (30%) and hemicellulose (25%) with high lignin (15%) as shown in Table 1. likely requiring pretreatment for effective breakdown. Overall, wheat bran is the most readily degradable, while wheat straw and rice husk may need more processing**.**

Table 1: Composition of Major Structural Components in Selected Agro-Waste Substrates

Agro-Waste Substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Wheat Straw (*Triticum aestivum* L.)	35	25	15
Wheat Bran (*Triticum aestivum* L.)	20	30	8
Rice Husk (*Oryza sativa* L.)	30	25	15

Figure 1: Composition of Major Structural Components in Selected Agro-Waste Substrates



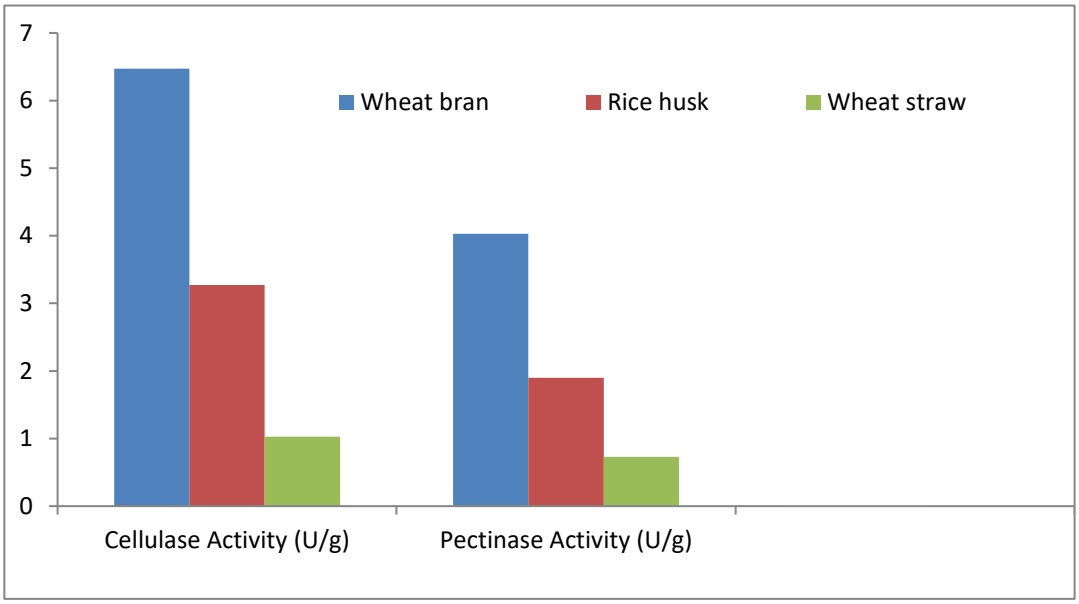
Enzyme Activity

Pectinase and cellulase enzyme activities were measured to assess their efficiency in breaking down plant cell wall components. Units per gram (U/g) of enzyme activity were calculated using different substrates as the carbon source, as shown in Table 2 and Figure 2. The results are based on triplicate experiments, and the mean values with standard deviations are presented below.

Table 2: Enzyme Activity of cellulose and Pectinase

Agro-Waste Substrate	Cellulase Activity (U/g)	Mean ± SD (Cellulase)	Pectinase Activity (U/g)	Mean ± SD (Pectinase)
Wheat bran (*Triticum aestivum* L.)	6.8		4.3	
	6.5	6.47 ± 0.35	4.0	4.03 ± 0.25
	6.1		3.8	
Rice husk (*Oryza sativa* L.)	3.5		2.1	
	3.3	3.27 ± 0.25	1.9	1.90 ± 0.20
	3.0		1.7	
Wheat straw (*Triticum aestivum* L.)	1.2		0.9	
	1.0	1.03 ± 0.15	0.7	0.73 ± 0.15
	0.9		0.6	

Figure 2: Mean values of Cellulase and Pectinase activity



Optimization of Parameters for Enzyme Activity

The findings showed that the optimal time for cellulase and pectinase activity was 96 hours, with an optimal pH range of 5.5 to 6. The highest activity of pectinase was recorded at 50 °C, while for cellulase it was 40 °C, as shown in Figures 3, 4, and 5, respectively.

Figure 3: Effect of Incubation time on activity of Cellulase and Pectinase

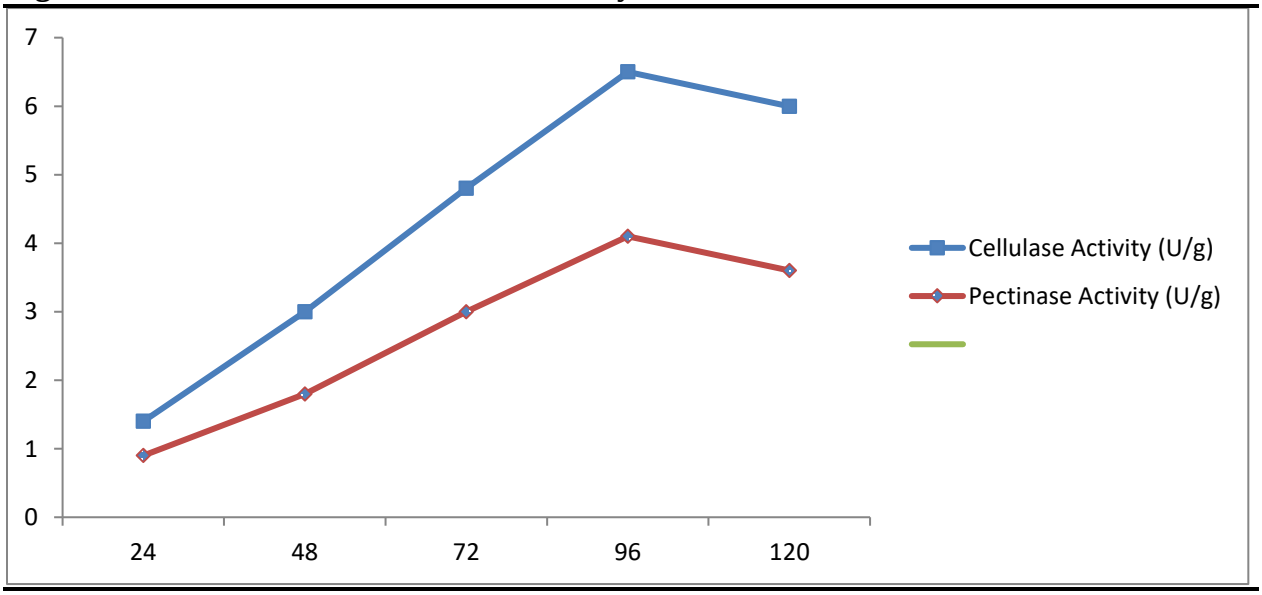


Figure 4: Effect of pH on activity of Cellulase and Pectinase

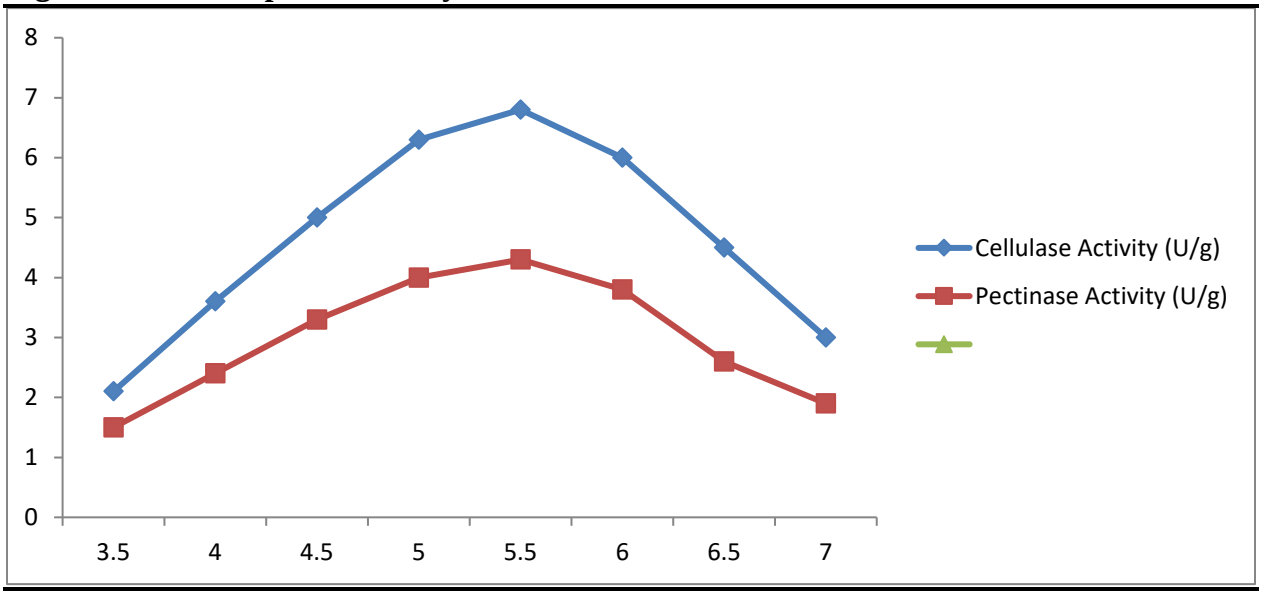
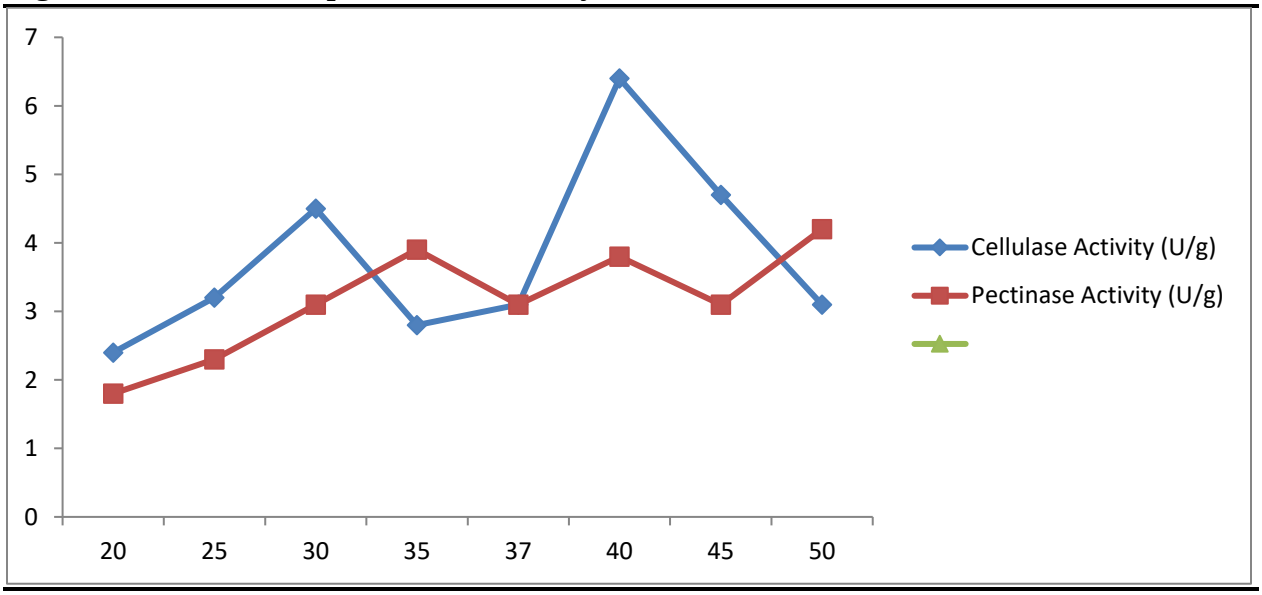


Figure 5: Effect of Temperature on activity of Cellulase and Pectinase



Conclusion

To sum up, this research demonstrates the great promise of agro-waste materials, especially wheat bran, for use as economical and environmentally-friendly substrates for enzyme cellulase and pectinase produced by As*pergillus niger* via solid-state fermentation. It is evident that the elevated yields of enzymes obtained with wheat bran as a substrate attest to its greater nutritive and structural value that helped fungal propagation along with enzymatic secretion. Although rice husk and wheat straw were less effective because of their dense and fibrous nature, they still offer moderate potential for enzyme production, thereby underscoring the myriad uses of agricultural by-products.

The retreatment of these agro residues not only addresses the problem of agricultural waste in an eco-friendly manner, but also enhances the value of wasted biomass, thereby contributing to a circular economy in the agricultural industry. This approach is biotechnologically innovative as it directly meets sustainable development objectives through reduced dependence on expensive commercial substrates and lowered production costs, which increase the feasibility of industrial scale enzyme production.

Further doors are open for integration of such bioprocesses into agribusiness models. Agro-industries can leverage these results to produce enzyme cost effectively units that convert can serve valuable bio products in industries creating new income streams and employment opportunities. Moreover, further research into optimizing fermentation conditions, scaling up production, and exploring and isolation of enzyme-producing microorganisms could enhance efficiency and broaden applications, including in biofuel production, food processing, and waste management industries. Overall, the valorization of wheat bran and other agro-residues as fermentation substrates exemplifies a sustainable innovation with the potential to transform agricultural waste management, foster green biotechnological solutions, and drive economic growth in rural and industrial communities moreover other waste substrates like potato peel orange peel and sugarcane bagasse can be used to check the enzyme production by these substrates.

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