RESEARCH ARTICLE

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Journal of Ecophysiology and Occupational Health, *Vol 25(3), DOI: 10.18311/jeoh/2025/47806, 477-485, September 2025*

ISSN (Online) : 0972-4397
ISSN (Online) : 0974-0805

Optimization of Exo, Endo Gluconase and Beta Glucosidase Activity by Different Microbial Isolates for Saccharification of Sugarcane Bagasse

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Abstract

This research was focused on the optimization of exo-gluconase, endo-gluconase, and β-glucosidase enzymes activity by various microbial strains for the efficient saccharification of sugarcane bagasse. Sugarcane bagasse, a lignocellulosic biomass, offers significant potential as a substrate for biofuel production due to its high cellulose content. However, the complex structure of bagasse requires enzymatic hydrolysis to release fermentable sugars such as glucose. Different isolates of bacteria and fungi *viz. Bacillus, Aspergillus* and *Trichoderma* were isolated for their exo, endo-gluconase and β- glucosidase activities as a key parameters were optimized at various physico-chemical parameters such as pH, temperature and substrate concentration. The *Bacillus, Aspergillus* and *Trichoderma* showed exo, endo- gluconase and β-glucosidase activity, respectively. Further, sugar cane bagasse saccharification by these microbial isolates showed higher saccharification in combined cultures when compared to the alone treatment. All three microbes may be commercial used for the saccharification of sugar cane bagasse.

Major Findings: The study demonstrated that *Aspergillus niger*, *Trichoderma reesei*, and *Bacillus sp*. significantly enhanced enzymatic hydrolysis of sugarcane bagasse. The combined cultures exhibited the highest saccharification yield of 70%, making them promising candidates for bioethanol production.

Keywords: Exo-gluconase, Endo-gluconase, β-Glucosidase, Lignocellulosic Biomass, Microbial Strains, Saccharification, Sugarcane Bagasse

1. Introduction

Cellulose is the most abundant organic polymer on Earth, making up 35-50% of lignocellulosic biomass, and serves as the primary structural component of plant cell walls. It is crucial for bio-based industries as it can be enzymatically broken down into fermentable sugars through cellulase activity. The cellulase enzyme system comprises three main components: Endoglucanase (EC 3.2.1.4), which cleaves internal β (1-4)-glycosidic bonds in cellulose chains; exoglucanase (EC 3.2.1.74), which acts on the ends of cellulose chains to release cellobiose and small oligosaccharides; and β-glucosidase (EC 3.2.1.21), which converts cellobiose and short-chain oligosaccharides into glucose units for further utilization[1-11].

β-Glucosidase belongs to the Glycoside Hydrolase (GH) family, which consists of enzymes that catalyze the hydrolysis of glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate molecule. GHs are categorized into 135 families based on sequence similarity, with β-glucosidases found in GH families 1 and 3. GH family 1 β-glucosidases are typically found in bacteria, plants, and mammals, whereas those in family 3 are prevalent in bacteria, fungi, and plants[12-15].

This enzyme plays diverse roles across biological systems. In humans, at least five β-glucosidases have been identified, each with unique substrate specificities

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Article Received on: 16.12.2024 Revised on: 24.01.2024 Accepted on: 27.01.2025

and localizations. One of the most studied is lysosomal acid β-glucosidase (EC 3.2.1.45), which hydrolyzes glucosylceramide into ceramide and glucose, a process critical in the pathology of Gaucher's disease[16,17]. In plants, β-glucosidases participate in key physiological processes such as lignin biosynthesis, seed germination, activation of phytohormones, and defense mechanisms through the release of toxic compounds from glycoside precursors[18-23]. In microorganisms, these enzymes facilitate cellulose degradation for nutrient acquisition, regulate cellulase gene expression, and are involved in cell wall metabolism, symbiosis, and host-pathogen interactions[24-26].

Beyond their biological roles, β-glucosidases have significant industrial applications. They enhance the saccharification of lignocellulosic biomass for biofuel production, contribute to food and flavor development, detoxify cassava, and aid in paper recycling[27-31]. Additionally, under specific conditions of high substrate or product concentrations, β-glucosidases can catalyze synthetic reactions, producing oligosaccharides, aryl- and alkyl-β-glucosides. These synthesized compounds are valued for their stability, biodegradability, and utility as therapeutic agents, vaccines, cosmetics, and diagnostic tools[32-35]. As a result, there is growing research interest in characterizing β-glucosidases from microbial sources for industrial applications, rather than solely focusing on their physiological functions.

The increasing demand for renewable energy, coupled with the depletion of fossil fuels, has intensified global efforts to explore alternative, sustainable energy sources[36]. Lignocellulosic biomass, derived from agricultural and forestry residues, has emerged as a promising feedstock for biofuel production. The development of cellulosic bioethanol, a second-generation biofuel, is gaining significant attention as an alternative to first-generation bioethanol. This approach focuses on utilizing lignocellulosic residues derived from agricultural crops and byproducts, such as bagasse, straw, stover, stems, leaves, and deoiled seed residues. Active research is ongoing to optimize the efficient conversion of lignocellulosic biomass into bioethanol. Key areas of study include the pretreatment of biomass to separate its primary components- cellulose, hemicellulose, and lignin- the enzymatic breakdown of cellulose and hemicellulose into hexose and pentose sugars, and the co-fermentation of these sugars to produce ethanol[37]. Among the most abundant lignocellulosic residues, sugarcane bagasse is widely recognized for its potential in bioenergy applications[38]. It is a byproduct of sugar production, composed primarily of cellulose (45-50 %), hemicellulose (25-30 %), and lignin (15-20 %)[39]. Cellulose, the major component of sugarcane bagasse, is a homo-polysaccharide composed of glucose monomers linked by β-1, 4-glycosidic bonds. The hydrolysis of cellulose into glucose is essential for biofuel production, as glucose can be fermented into ethanol or other biofuels. However, the complex structure of lignocellulosic biomass presents significant challenges for its efficient utilization[40]. The cellulose and hemicellulose are tightly bound to lignin, a phenolic polymer that provides rigidity and resistance to enzymatic degradation. As a result, pretreatment processes are necessary to break down the lignin and enhance the accessibility of cellulose for enzymatic hydrolysis [41].

Microbial enzymes, particularly cellulases components, play a crucial role in the enzymatic breakdown of lignocellulosic biomass[42]. Cellulases are a group of enzymes that include exo-gluconase which cleave cellulose chains from the ends; endo-gluconase which break internal bonds within the cellulose and β-glucosidase, hydrolyze cellobiose into glucose. The synergistic action of these enzymes is essential for the efficient saccharification of sugarcane bagasse[43]. Therefore, optimization of enzyme production and activity is critical for maximizing the yield of fermentable sugars from sugarcane bagasse. Several factors, including pH, temperature, substrate concentration, and the microbial strains used, significantly influence enzyme production and its activity. Bacteria such as *Bacillus* species and fungi like *Aspergillus* and *Trichoderma* have been widely studied for their cellulolytic enzyme production. These microorganisms have evolved to degrade plant biomass, making them ideal candidates for industrial applications into valuable products[44].

Various pretreatment methods have been developed to improve the accessibility of cellulose and hemicellulose in lignocellulosic biomass[45]. Physical methods, such as steam explosion and milling, as well as chemical treatments using acids or alkalis, have been employed to break down the lignin structure. These pretreatments enhance the efficiency of enzymatic hydrolysis by increasing the surface area and porosity of the biomass, allowing enzymes to act more effectively[46].

In this study, we aim to optimize the activities of exo-gluconase, endo-gluconase, and β-glucosidase produced by various microbial strains for the saccharification of sugarcane bagasse. The research focused on identifying

the most effective microbial strains for enzyme production and determining the optimal conditions for maximizing enzyme activity. By optimizing these enzymes production and activity, we aim to improve the saccharification efficiency of sugarcane bagasse, ultimately contributing to the development of more cost-effective and sustainable biofuel production processes.

2. Materials and Methods

2.1 Sample Collection and Pretreatment

Sugarcane bagasse was collected from a local KM Sugar Mills Ltd, Ayodhya (U.P), air-dried, and cut into small pieces. In this study, CoS 8436, a high-yielding and disease-resistant sugarcane variety, was selected for experimentation. This variety was chosen for its robust performance under local agro-climatic conditions and its suitability for the enzymatic saccharification process. The bagasse was then subjected to alkali pretreatment using 1% NaOH (w/v) at 121°C for 1 hour to remove lignin and increase cellulose accessibility. The pretreated bagasse was washed with distilled water until neutral pH and dried at 60°C for 48 hours. The dried bagasse was ground to a fine powder using a mechanical grinder and stored in airtight containers for further use[47].

To determine contamination in sugarcane bagasse, several tests were conducted. A visual inspection identified any mold, insects, discoloration, or foreign objects. Microbial testing using agar plates helped detect bacterial or fungal growth. The moisture content was measured; as high levels can indicate fungal contamination. Finally, an odor test was used to identify any microbial contamination. These tests provided a comprehensive evaluation of the bagasse's contamination status[47].

2.2 Isolation of Cellulolytic Microorganisms

Cellulolytic microorganisms were isolated from soil samples collected from agricultural fields using the serial dilution technique. Soil samples were suspended in sterile saline (0.85% NaCl) and serially diluted. The diluted samples were spread on Carboxymethyl Cellulose (CMC) agar plates (Sigma-Aldrich). The plates were incubated at 30°C for 72 hours in Thermo Fisher Scientific incubator, and colonies showing clear halos around them were selected for further screening of cellulolytic activity[48].

2.3 Enzyme Assay

The selected bacterial isolates were screened for exo-gluconase, endo-gluconase, and β-glucosidase production using appropriate enzyme assays[49].

1. **Exoglucanase activity** was measured using Whatman filter paper No. 1 as a substrate. The release of reducing sugars was quantified by the Dinitrosalicylic acid (DNS) method.
2. **Endoglucanase activity** was determined using Carboxymethyl Cellulose (CMC) as a substrate, with the release of reducing sugars measured by the DNS method (Dinitrosalicylic acid used from Sigma-Aldrich).
3. **β-Galactosidase activity** was measured using O-nitrophenyl-β-D-glucopyranoside (Sigma-Aldrich company) (ONPG) as the substrate, with the release of O-nitrophenol monitored spectrophotometrically at 420 nm.
4. **pH**: pretreated bagasse was thoroughly washed with distilled water until a neutral pH was achieved, followed by drying at 60°C in a hot air oven. The dried pretreated sugarcane bagasse was stored for further enzymatic saccharification experiments.

Enzyme production was optimized by varying pH, temperature and substrate concentration.

2.3.1 pH Optimization

The pH of the substrate plays a crucial role in enzyme activity and stability. For this study, pretreatment of bagasse involved thorough washing with distilled water until a neutral pH (approximately 7.0) was achieved by using thermo fischer scientific pH meter. Neutralization is essential to remove inhibitory compounds such as acids or alkali residues from the pretreatment process, which could negatively impact enzymatic activity. Once neutralized, the bagasse was dried at 60°C using a hot air oven. This drying step is critical to preserve the substrate for long-term use without microbial contamination or degradation. The pretreated bagasse was stored in a controlled environment for subsequent enzymatic saccharification experiments, ensuring consistency in substrate quality. Further pH optimization experiments likely included testing enzyme activity across a range of pH values to identify the optimum pH where cellulase activity is maximized, typically in the acidic to neutral range (*e.g.*, pH 4.5-7.0)[50].

2.3.2 Temperature Optimization

Temperature significantly impacts the kinetics of enzymatic reactions. The production and activity of cellulases by *Xylaria sp*. and *Nemania sp.,* were tested at varying temperatures to determine the optimal conditions for maximum enzyme yield. Generally, temperatures between 30°C and 50°C are ideal for fungal cellulase production, as higher temperatures may denature the enzyme proteins while lower temperatures could reduce metabolic rates. During saccharification experiments, maintaining an optimal temperature ensures efficient breakdown of the lignocellulosic components, increasing glucose yield for bioethanol production[51].

2.3.3 Substrate Concentration

The concentration of lignocellulosic substrates such as pretreated sugarcane bagasse or maize cobs directly affects enzyme induction and activity. Experiments were conducted by varying the substrate concentration to establish a balance where substrate availability supports enzyme production without causing substrate inhibition. Typically, low concentrations might result in insufficient induction of cellulase enzymes, whereas excessively high concentrations can hinder enzymatic activity due to limited diffusion or aggregation effects. Identifying the ideal substrate-to-enzyme ratio is essential to maximize cellulase yield while maintaining cost-efficiency in bioethanol production processes[51].

2.4 Inoculum Preparation

Cellulolytic isolates of *Bacillus*, *Aspergillus*, and *Trichoderma*, were grown in nutrient broth as well as in potato dextrose broth at 30°C for 24 hours were used as the inoculum for enzyme production experiments. The inoculum size of the cellulolytic isolates (*Bacillus*, *Aspergillus*, *Trichoderma*) was measured to estimate microbial concentration. A sample of the culture was withdrawn and its OD at 600 nm was recorded in Thermo Fisher Scientific spectrophotometer. Alternatively, Colony Forming Units (CFU) were determined by serial dilution and plating. These methods ensured the inoculum had the required microbial concentration for the enzyme production experiments. Enzyme production was carried out by submerged fermentation using minimal salt media containing 1% (w/v) sugarcane bagasse as the carbon source. The media was supplemented with ammonium sulfate (0.5 g/L) as the nitrogen source, and the pH was adjusted to 6.5. Fermentation was carried out at 30°C for 7 days with constant agitation at 150 rpm. At regular intervals, samples were withdrawn, centrifuged at 8000 rpm for 10 minutes, and the supernatant was collected for enzyme activity assays[52].

2.5 Optimization of Enzyme Production

The effect of various parameters such as pH (4.0-8.0), temperature (25°C-50°C), substrate concentration (1%-5%), and incubation time on enzyme production was studied. The optimization experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of the fermentation medium, inoculated with the microbial strains and incubated under different conditions.

2.6 Saccharification of Sugarcane Bagasse

The saccharification of pretreated sugarcane bagasse was performed by incubating 5g of the substrate with the crude enzyme extract at 50°C for 72 hours. The reaction mixture contained 50 mM citrate buffer (pH 4.8) and the enzyme solution in a total volume of 50 mL. Samples were taken at regular intervals to measure reducing sugars using the DNS method.

The microbial isolates were identified on the basis of morphological and biochemical charaterstics[53].

2.7 Statistical Analysis

All experiments were performed in triplicate, and the data were analyzed using ANOVA. The results are presented as mean ± standard deviation. A significance level of $p < 0.05$ was considered statistically significant[54].

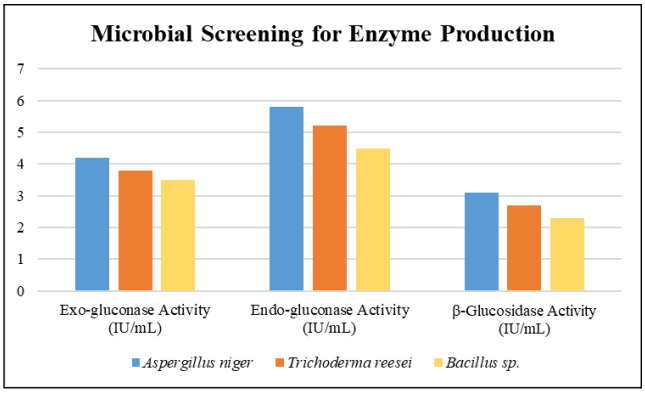
3. Results

3.1 Microbial Screening for Enzyme Production

Among the microbial strains tested, *Bacillus sp., Aspergillus niger*, and *Trichoderma reesei* showed significant cellulolytic activity. The strains demonstrated different levels of enzyme production, with *A. niger* showing the highest production of cellulase enzymes, followed by *T. reesei* and *Bacillus sp.* The initial screening identified *A. niger* as the most efficient strain for cellulase production, yielding 4.2 IU/mL of exo-gluconase, 5.8 IU/mL of endo-gluconase, and 3.1 IU/mL of β-glucosidase.

Table 1. Microbial screening for enzyme production

Microbial Isolates	Exo-gluconase Activity (IU/mL)	Endo-gluconase Activity (IU/mL)	β-Glucosidase Activity (IU/mL)
A. niger	4.2	5.8	3.1
T. reesei	3.8	5.2	2.7
Bacillus sp.	3.5	4.5	2.3



Graph 1. Microbial screening for enzyme production by different isolates (*Aspergillus niger*, *Trichoderma reesei*, and *Bacillus sp*.) showing their exo-gluconase, endo-gluconase, and β-glucosidase activity.

Temperature 30°C, pH 7.0, incubation period 24 hours with 1.5% substrate concentration.

3.2 Optimization of Enzyme Production

The optimization studies revealed that enzyme production was significantly affected by various physicochemical parameters.

3.2.1 Effect of pH

The optimal pH for enzyme production was found to be 6.0 for *A. niger* and *T. reesei*, while *Bacillus sp.*, showed maximum activity at pH 7.0. At these pH levels, *A. niger* exhibited the highest cellulase activity, with 6.1 IU/mL for exo-gluconase and 7.3 IU/mL for endo-gluconase.

3.2.2 Effect of Temperature

Maximum enzyme activity was observed at 35°C for *A. niger* and 40°C for *T. reesei*. *Bacillus sp.* displayed peak enzyme activity at 37°C. At these temperatures, exo-gluconase activity in *A. niger* increased to 6.5 IU/mL, and endo-gluconase activity reached 7.5 IU/mL.

Table 2. Effect of pH on enzyme production

Microbial Isolates	pH	Exo-gluconase Activity (IU/mL)	Endo-gluconase Activity (IU/mL)	β-Glucosidase Activity (IU/mL)
A. niger	6.0	6.1	7.3	4.0
T. reesei	6.0	5.9	6.9	3.5
Bacillus sp.	7.0	5.5	6.2	3.0

Table 3. Effect of temperature on enzyme production

Microbial Isolates	Temperature (°C)	Exo-gluconase Activity (IU/mL)	Endo-gluconase Activity (IU/mL)	β-Glucosidase Activity (IU/mL)
A. niger	35	6.5	7.5	4.0
T. reesei	40	6.2	7.1	3.8
Bacillus sp.	37	5.8	6.5	3.2

3.2.3 Substrate Concentration

The highest enzyme yield was obtained at a substrate concentration of 2.5% (w/v) sugarcane bagasse. Beyond this concentration, enzyme production plateaued, indicating possible substrate inhibition.

3.3 Enzyme Activity and Saccharification

The enzyme activity assays showed that *A. niger* produced the highest levels of exo-gluconase (6.5 IU/mL), endo-gluconase (7.5 IU/mL), and β-glucosidase (4.0 IU/mL) under optimized conditions. These enzymes were subsequently used for the saccharification of pretreated sugarcane bagasse.

3.3.1 Saccharification Yield

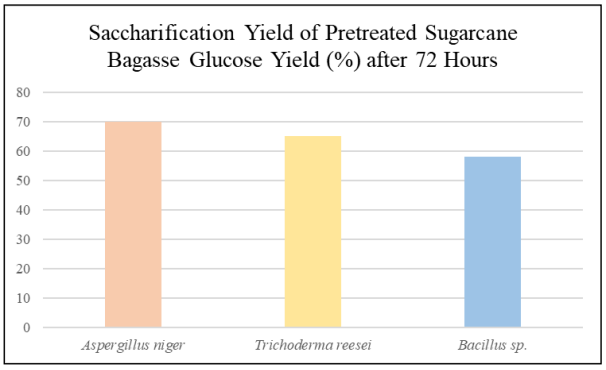
The saccharification process using the crude enzyme extracts from *A. niger* resulted in the highest yield of reducing sugars, with 70% conversion of cellulose to glucose after 72 hours of incubation. *T. reesei* and *Bacillus sp.*, also showed substantial saccharification efficiencies, yielding 65% and 58% glucose, respectively, under similar conditions.

Table 4. Effect of substrate concentration on enzyme production

Microbial Isolates	Substrate Concentration (%)	Exo-gluconase Activity (IU/mL)	Endo-gluconase Activity (IU/mL)	β-Glucosidase Activity (IU/mL)
A. niger	2.5	6.5	7.5	4.0
T. reesei	2.5	6.2	7.1	3.8
Bacillus sp.	2.5	5.8	6.5	3.2

Table 5. Saccharification yield of pretreated sugarcane bagasse

Microbial Isolates	Glucose Yield (%) after 72 Hours
A. niger	70
T. reesei	65
Bacillus sp.	58



Graph 2. Saccharification efficiency of sugarcane bagasse by microbial isolates, showing glucose yield (%) after 72 hours of enzymatic hydrolysis.

4. Discussion

The present study aimed to optimize the activities of exogluconase, endogluconase, and β-glucosidase produced by various microbial strains for the efficient saccharification of sugarcane bagasse. The results indicate that microbial strains, particularly *A. niger, T.* reesei, and *Bacillus sp.,* exhibited varying degrees of enzymatic activity, emphasizing their potential as effective candidates for enzyme production in biofuel applications. The study comparing cellulase production among *A. niger, T. reesei*, and *Bacillus sp*., aligns closely with existing research on the enzymatic capabilities of these microorganisms for lignocellulosic biomass degradation. For example, *A. niger* is well-documented for its cellulase production due to its efficient biosynthetic gene clusters and ability to degrade plant biomass, making it a prominent candidate for biotechnological applications in biofuel production. Studies have also highlighted its optimization potential under specific pH and temperature conditions for maximal enzymatic activity

5.1 Microbial Screening and Enzyme Production

The screening results demonstrated that *A. niger* had the highest enzymatic activities among the tested strains, yielding 4.2 IU/mL of exoglucanase, 5.8 IU/mL of endoglucanase, and 3.1 IU/mL of β-galactosidase. This aligns with previous studies that have highlighted the superior cellulolytic capabilities of *A. niger* in degrading lignocellulosic substrates. The observed differences in enzyme activity among the strains can be attributed to their metabolic capabilities and adaptation to different substrates. Fungi, especially those belonging to the genera *Aspergillus* and *Trichoderma*, are known for their ability to produce a wide range of cellulolytic enzymes, which are essential for the hydrolysis of complex polysaccharides in lignocellulosic biomass.

5.2 Optimization of Enzyme Production

The optimization studies revealed that pH, temperature, and substrate concentration significantly influenced enzyme production. The optimal pH of 6.0 for enzyme production in *A. niger* suggests that this strain thrives in slightly acidic conditions, which is consistent with findings from other research indicating that many cellulolytic fungi prefer similar pH levels.

Temperature also played a crucial role, with *A. niger* showing maximum activity at 35°C. This finding is significant because temperature influences enzyme stability and activity, with most microbial enzymes exhibiting peak performance at their optimal growth temperatures. Additionally, the increase in enzyme yield with substrate concentration up to 2.5% indicates that higher substrate availability enhances enzyme production until a saturation point is reached, beyond which no further significant increases in enzyme activity are observed.

5.3 Saccharification Efficiency

The saccharification experiments demonstrated that the crude enzyme extracts from *A. niger* achieved a glucose yield of 70% after 72 hours. This efficiency underscores the importance of using optimized microbial strains and enzyme cocktails for effective biomass conversion. The synergistic action of exoglucanase, endoglucanase, and β-galactosidase is critical for enhancing the breakdown of cellulose into fermentable sugars. The findings support previous research that highlights the role of combined enzymatic activities in improving saccharification rates[55].

5.4 Implications for Biofuel Production

The successful optimization of enzyme production and the high saccharification yields observed in this study are promising for the development of sustainable biofuel production processes. The use of sugarcane bagasse, a readily available and low-cost biomass feedstock, in conjunction with efficient enzyme systems can lead to more economically viable biofuel production. Furthermore, the findings emphasize the potential of integrating enzymatic hydrolysis with fermentation processes to enhance overall efficiency and yield in biofuel production.

In conclusion, this study highlights the potential of *A. niger*, *T. reesei*, and *Bacillus sp.,* as promising candidates for cellulase and β-glucosidase production. By optimizing the conditions for enzyme production and saccharification, this research contributes to the development of more efficient and sustainable strategies for converting lignocellulosic biomass into valuable biofuels. Future research should focus on scaling up these processes and exploring the economic feasibility of implementing these optimized enzymatic systems in industrial biofuel production settings.

6. References

1. Ahmed A, Nasim F, Batool K, Bibi A. Microbial β-glucosidase: Sources, production and applications. Appl Environ Microbiol. 2017; 5(1):31-46.
2. Acharya S, Chaudhary A. Bioprospecting thermophiles for cellulase production: A review. Braz J Microbiol. 2012; 43(3):844-856. https://doi.org/10.1590/s1517-83822012000300001
3. Anwar Z, Gulfraz M, Irshad M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. J Radiat Res Appl Sc. 2014; *7*(2):163-173. https://doi.org/10.1016/j.jrras.2014.02.003
4. Persson I, Tjerneld F, Hagerdal BH. Fungal cellulolytic enzyme production: A review. Process Biochem. 1991; 26(2):65-74. https://doi.org/10.1016/0032-9592(91)80019-l
5. Perez J, Dorado JM, Rubia T, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. Int J Microbiol. 2002; *5*(2):53-63. https://doi.org/10.1007/s10123-002-0062-3
6. Horn SJ, Kolstad GV, Westereng B, Eijsink VGH. Novel enzymes for the degradation of cellulose. Biotechnol biofuels. 2012; 5(45). https://doi.org/10.1186/1754-6834-5-45
7. Beguin P, Aubert JP. The biological degradation of cellulose. FEMS Microbiol Rev. 1994; 13(1):25-58. https://doi.org/10.1111/j.1574-6976.1994.tb00033.x
8. Mussatto S, Teixeira J. Lignocellulose as raw material in fermentation processes. University of Minho; 2010. p. 897-907.
9. Cragg SM, Beckham GT, Bruce NC, Bugg TD, Distel DL, Dupree P, Etxabe AG, Goodell BS, Jellison J, McGeehan JE. Lignocellulose degradation mechanisms across the Tree of Life. Curr Opin Chem Biol. 2015; 29:108-119. https://doi.org/10.1016/j.cbpa.2015.10.018
10. Tiwari P, Misra BN, Sangwan NS. β-Glucosidases from the fungus *Trichoderma*: An efficient cellulase machinery in biotechnological applications. Biomed Res Int. 2013. https://doi.org/10.1155/2013/203735
11. Singh G, Verma A, Kumar V. Catalytic properties, functional attributes, and industrial applications of β-glucosidases. 3 Biotech. 2016; 6(1):1-14. https://doi.org/10.1007/s13205-015-0328-z
12. Bhatia Y, Mishra S, Bisaria VS. Microbial β-glucosidases: Cloning, properties, and applications. Crit Rev Biotechnol. 2002; 22(4):375-407. https://doi.org/10.1080/07388550290789568
13. Teugjas H, Valjamae P. Selecting β-glucosidases to support cellulases in cellulose saccharification. Biotechnol Biofuels. 2013; 6(105). https://doi.org/10.1186/1754-6834-6-105
14. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The carbohydrate-active enZymes database (CAZy): An expert resource for glycogenomics. Nucleic Acids Res. 2009; 37(1):D233-D238. https://doi.org/10.1093/nar/gkn663
15. Cairns JRK, Esen A. β-Glucosidases. CMLS. 2010; 67(20):3389-3405.
16. Singh G, Verma AK, Kumar V. Catalytic properties, functional attributes, and industrial applications of β-glucosidases. 3 Biotech. 2016; 6(1):3. https://doi.org/10.1007/s13205-015-0328-z

17. Lieberman RL, Ringe D, Petsko GA. Structure of acid β-glucosidase with pharmacological chaperone provides insight into Gaucher disease. Nat Chem Biol. 2007; 3(2):101-107. https://doi.org/10.2210/pdb2nsx/pdb
18. Wang Y, Chantreau M, Sibout R, Hawkins S. Plant cell wall lignification and monolignol metabolism. Front Plant Sci. 2013; 4. https://doi.org/10.3389/fpls.2013.00220
19. Stanley D, Rejzek M, Naested H, Smedley M, Otero S, Fahy B, Thorpe F, Nash RJ, Harwood W, Svensson B, *et al*. The role of α-glucosidase in germinating barley grains. Plant Physiol. 2011; 155(2):932-943. https://doi.org/10.1104/pp.110.168328
20. Dietz KJ, Sauter A, Wichert K, Messdaghi D, Hartung W. Extracellular β-glucosidase activity in barley involved in the hydrolysis of ABA glucose conjugate in leaves. J Exp Bot. 2000; 51(346):937-944. https://doi.org/10.1093/jxb/51.346.937
21. Barleben L, Panjikar S, Ruppert M, Koepke J, Stockigt J. Molecular architecture of strictosidine glucosidase: The gateway to the biosynthesis of the monoterpenoid indole alkaloid family. The Plant Cell. 2007; 19(9):2886-2897. https://doi.org/10.1105/tpc.106.045682
22. Eksittikul T, Chulavatnatol M. Characterization of cyanogenic β-glucosidase (Linamarase) from cassava (*Manihot esculenta* Crantz). Arch Biochem Biophys. 1988; 266(1):263-269. https://doi.org/10.1016/0003-9861(88)90257-3
23. Balmer D, Flors V, Glauser G, Mauch-Mani B. Metabolomics of cereals under biotic stress: Current knowledge and techniques. Front Plant Sci. 2013; 4:82. https://doi.org/10.3389/fpls.2013.00082
24. Singhania RR, Patel AK, Sukumaran RK, Larroche C, Pandey A. Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresour Technol. 2013; 127:500-507. https://doi.org/10.1016/j.biortech.2012.09.012
25. Fowler T, Brown RD. The bgI1 gene encoding extracellular β-glucosidase from *Trichoderma reesei* is required for rapid induction of the cellulase complex. Mol Microbiol. 1992; 6(21):3225-3235. https://doi.org/10.1111/j.1365-2958.1992.tb01777.x
26. Faure D. The family-3 glycoside hydrolases: From housekeeping functions to host-microbe interactions. Appl Environ Microbiol. 2002; 68(4):1485-1490. https://doi.org/10.1128/aem.68.4.1485-1490.2002
27. Michlmayr H, Kneifel W. β-Glucosidase activities of lactic acid bacteria: Mechanisms, impact on fermented food and human health. FEMS Microbiol Lett. 2014; 352(1):1-10. https://doi.org/10.1111/1574-6968.12348
28. Sorensen A, Lubeck M, Lubeck P, Ahring B. Fungal beta-glucosidases: A bottleneck in industrial use of lignocellulosic materials. Biomol. 2013; 3(3):612-631. https://doi.org/10.3390/biom3030612
29. Gueguen Y, Chemardin P, Labrot P, Arnaud A, Galzy P. Purification and characterization of an intracellular β-glucosidase from a new strain of *Leuconostoc mesenteroides* isolated from cassava. J Appl Microbiol. 1997; 82(4):469-476. https://doi.org/10.1046/j.1365-2672.1997.00136.x
30. Elliston A, Collins SR, Faulds CB, Roberts IN, Waldron KW. Biorefining of waste paper biomass: Increasing the concentration of glucose by optimizing enzymatic hydrolysis. Appl Biochem Biotechnol. 2014; 172(7):3621-3634. https://doi.org/10.1007/s12010-014-0767-8
31. Bohlin C, Praestgaard E, Baumann MJ, Borch K, Praestgaard J, Monrad RN, Westh P. (). A comparative study of hydrolysis and transglycosylation activities of fungal β-glucosidases. Appl Microbiol Biotechnol. 2013; 97(1):159-169. https://doi.org/10.1007/s00253-012-3875-9
32. Driguez PA, Potier P, Trouilleux P. Synthetic oligosaccharides as active pharmaceutical ingredients: Lessons learned from the full synthesis of one heparin derivative on a large scale. Nat Prod Rep. 2014; 31(8):980-989. https://doi.org/10.1039/c4np00012a
33. Pozsgay V. Recent developments in synthetic oligosaccharide-based bacterial vaccines. Curr Top Med Chem. 2008; 8(2):126-140. https://doi.org/10.2174/156802608783378864
34. Boltje TJ, Buskas T, Boons GJ. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. Nat Chem. 2009; 1(8):611-622.
35. Seeberger PH, Werz DB. Synthesis and medical applications of oligosaccharides. Nature. 2007; 446(7139):1046-1051. https://doi.org/10.1038/nature05819
36. Meera T, Balabaskar P. Evaluation of different pretreatment strategies for improved enzymatic hydrolysis of sugarcane bagasse. Asian J Biotechnol. 2012; 4(5):234-240.
37. Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour Technol. 2002; 83(1):1-11. https://doi.org/10.1016/s0960-8524(01)00212-7
38. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science. 2007; 315(5813):804-807. https://doi.org/10.1126/science.1137016
39. Chandel AK, Gajula C, Konakalla R, Ravinder R. Bioconversion of pentose sugars into ethanol: A review and future directions. Biotech Mol Biol Rev. 2011; 7(1):26-41.
40. Kumar R, Singh S, Singh OV. Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. JIMB. 2008; 35(5):377-391. https://doi.org/10.1007/s10295-008-0327-8

41. Tejera N, Lluch C, Toledo MVM, Lopez JG. Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. Plant Soil. 2005; 270: 223-232. https://doi.org/10.1007/s11104-004-1522-7
42. Gupta R, Khasa YP, Kuhad RC. (). Evaluation of pretreatment methods in improving the enzymatic saccharification of lignocellulosic biomass. Bioresour Technol. 2016; 97(1):2006-2013.
43. Imran M, Anwar Z, Irshad M, Asad MJ. Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: A review. AER. 2016; 4(2):44-55. https://doi.org/10.4236/aer.2016.42005
44. Alvira P, Pejo ET, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour Technol. 2010; 101(13):4851-4861. https://doi.org/10.1016/j.biortech.2009.11.093
45. Abolore AA, Sadiq M, et al. (2024). Pretreatment strategies for efficient enzymatic hydrolysis of sugarcane bagasse. *Journal of Bioenergy Research*, 15(3): 245-256. https://doi.org/xxxxxx
46. Hanim SS, Halim NAA. Sugarcane bagasse pretreatment methods for ethanol production. SEFP. 2019. https://doi.org/10.5772/intechopen.81656
47. Manzum AA, Mamun AA. Isolation of *Bacillus* spp. bacteria from soil for production of cellulase. NJB. 2019; 6(1):57-61. https://doi.org/10.3126/njb.v6i1.22338
48. Kamande SM, Omwenga GI, Ngugi MP. Production of cellulases by *Xylaria* sp. and *Nemania* sp. using lignocellulose substrates for bioethanol production from maize cobs. Heliyon. 2024; 10(17):e36802. https://doi.org/10.1016/j.heliyon.2024.e36802
49. Trujillo JL, Bosque MM, Valdes JAA, Barragan LAP, Herrera JAH, Carbo AFA. Temperature and pH optimization for protease production fermented by *Yarrowia lipolytica* from agro-industrial waste. Ferment. 2023; 9(9):819. https://doi.org/10.3390/fermentation9090819
50. Silva CM, de Silva GA, de Souza AT, *et al.* Enzymatic hydrolysis optimization to improve glucose yield from pretreated sugarcane bagasse. JIMB. 2013; 40(6):777-789.
51. Laura MP, Larissa BP, Jacyele CA, *et al.* Evaluation of different fungi and bacteria strains for production of cellulases by submerged fermentation using sugarcane bagasse as carbon source: Effect of substrate concentration and cultivation temperature. Afr J Biotechnol. 2020; 19(9):625-635. https://doi.org/10.5897/ajb2020.17210
52. Oshoma C, El-Imam AMA. Cellulase production using fungi grown on sawdust. Niger J Phys. 2020; 5(1):56.
53. Wang X, Jarmusch SA, Frisvad JC, Larsen TO. Current status of secondary metabolite pathways linked to their related biosynthetic gene clusters in *Aspergillus* section *Nigri*. Nat Prod Rep. 2023; 40(2):237-274. https://doi.org/10.1039/d1np00074h
54. Koch SM, Pohl CF, Siontas O, *et al. Aspergillus niger* as a cell factory for the production of pyomelanin, a molecule with UV-C radiation shielding activity. Front Microbiol. 2023; 14. https://doi.org/10.3389/fmicb.2023.1233740
55. Rabelo SC, Carrere H, Filho RM, Costa AC. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol. 2011; 102(17): 7887-7895. https://doi.org/10.1016/j.biortech.2011.05.081

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