



## **Production, Characterization and Industrial Applications of Cellulase Derived from Agro-waste**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author RS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AS made necessary scientific incorporations for the final draft. Author SM assisted in carrying out the protocols for the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Agricultural wastes are widely available in the form of crop residues (residual stalks, straw, leaves, roots, husks, sheaths, bagasse etc.). Significant amount of these by-products derived from agro-processing industries goes to waste. Improper disposition of such agricultural waste contributes to environmental pollution and supports the growth of several micro-organisms. These wastes are lignocellulosic in nature and it could be recycled or further processed to extract or formulate value added products. Utilization and disposal of such agricultural waste is therefore essential and is progressively gaining attention as we drive towards a greener environment. The present review is focused on utilization of lignocellulosic biomass for cellulases production and its applications. Cellulose is considered one of the most important sources of carbon and its annual biosynthesis occurs in many tonnes per annum. Recycling of agricultural residue can be achieved naturally and artificially by microorganisms. Aerobic organisms such as fungi, bacteria, and some anaerobic organisms have been found to have the capability to degrade some or most of the constituents of these residues. Fungi play a significant role in the degradation of cellulose under aerobic conditions. Cellulases are important enzymes not only for their valuable applications in different sectors like

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food processing, animal feed production, pulp and paper production, detergent and textile industry, but also for the significant role in bioconversion of agriculture wastes to sugar and bio-ethanol.

*Keywords: Agricultural wastes; biomass; cellulase; fermentation.*

## 1. INTRODUCTION

Large amount of waste material is lodged into the environment by industries and agriculture every day. Approximately five billion metric tons of biomass has been produced annually from agriculture including ground nut cake, rice bran, rice straw, sugarcane bagasse, fruits and vegetable wastes, wheat bran, pea peels and many more. It is very crucial to convert these agriculture wastes effectively and economically into valuable products of industrial potential and also to reduce the harmful impact of these wastes on environment [1]. Lignocellulose and starch are the major content of agriculture wastes. About half of the plant matter is composed of lignocelluloses which is the most abundant renewable organic matter in soil [2]. A significant proportion of agricultural commodities in the developing countries go to waste because of lack of adequate transport and storage facilities. The waste thus generated, contributes to environmental pollution and is often left unattended. Utilization and disposal of such agriculture based waste is therefore essential and is progressively gaining attention as we drive towards a greener environment. Lignocellulose and starch are the major content of agro processing industrial wastes. It is composed of cellulose (35–50%), hemicellulose (20–35%), and lignin (15–25%) that is strongly connected together by variety of non-covalent and covalent linkages [3]. Cellulose is the most common carbohydrate in plants that consists of linear biopolymer of anhydroglucose units linked by the  $\beta$ -1,4 glycosidic bond. It is very difficult to degrade these components rapidly in the natural environment [4]. Lignin-degrading enzymes are widely used for the pretreatment of lignocellulosic biomass for bio-fuel production and also applied in textile, food, sugar, brewing, paper, cosmetic, pharmaceutical industries, organic synthesis, wastewater treatment, and bioremediation [5]. The utilization of biological wastes for growing microorganisms may constitute a better alternative for enzyme production with lower costs. Large number of commercial enzyme preparations from bacteria and fungi has limitations in their activity to degrade biomass with respect to several parameters including pH,

temperature, etc. Fungi and bacteria both have immense potential for producing bioactive molecules and is a promising source of industrially essential enzymes, owing to their greater metabolic diversity and higher adaptability to extreme environmental conditions [6].

## 2. PRODUCTION AND CHARACTERIZATION OF AGRO-INDUSTRIAL WASTE

Agro waste is lignocellulosic in nature which can be easily biodegraded by microorganisms the cellulosic matter in agro-waste is decomposed by a combination of physical, chemical and biological processes. Microorganisms degrade lignocellulosic and starchy materials due to their highly efficient enzymatic system. Agro-waste can be treated for enzyme production through fermentation under controlled conditions or is used for composting and bio-fuel. The natural decomposition of wastes by microbes generates products with high humus content. Prior researches have confirmed that this carbohydrate rich biomass can be used as a potential substrate for enzyme production.

Agro-waste is a special group of biomass that needs to be characterized to understand its nature for application as raw material and to propose the best methodology for its proper utilization. A common feature of various forms of agro-waste includes high COD, richness in protein, carbohydrate and lipid bio-molecules. Waste composition also influences the overall yield and kinetics of the biological reaction during digestion. Characterization of this waste can be done physically, chemically or biologically. Physical characterization of solid waste include determination of volume, moisture, ash, total solid, volatile solid, color, temperature, odor etc., while dissolved and suspended solids are estimated for liquid wastes. Chemical studies include the measurement of cellulose, hemicellulose, starch, reducing sugar, protein, total organic carbon, phosphorus, nitrogen, BOD (Biological oxygen demand), COD (Chemical oxygen demand), pH, halogens, toxic metals, etc. Besides these biochemical parameters, carbon, potassium, phosphorus, sulfur, calcium,

magnesium etc. can also be tested. All these chemical and biochemical parameters provide an insight on the applicability of waste for employment in specific energy production. Agro-wastes have high BOD and are a rich source of several nutrients like fibers, minerals, polyphenols, vitamins etc. So, a detailed study of waste characteristics is essential for its application and determination of economic feasibility of the process.

### 3. CELLULASE PRODUCTION

The use of solid state and submerged fermentation is widely used for production of enzymes at industrial as well as lab-scale. In this section, a brief description of these methods followed by their application for production of cellulase using various substrates has been discussed.

#### 3.1 Solid-state Fermentation

Solid-state fermentation (SSF) is defined as the growth of microbes without free flowing aqueous phase. SSF is alternative to submerged fermentation for production of value added products like antibiotics, enzymes, organic acids, single cell protein, bio-fuel and aromatic compounds. The advantages of ssf in various processes are found to be greater than that of submerged fermentation. However, at the industrial scale, submerged fermentation has been found to be more economic than ssf due to lower specific energy consumption.

#### 3.2 Submerged Fermentation

Submerged fermentation is a process involving the development of bio-molecules and microorganisms in a liquid broth. This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products. The substrates in submerged fermentation are utilized quite rapidly and hence need to be constantly supplemented with nutrients. This fermentation technique is best suited for substrates which require high moisture.

#### 3.3 Reported Applications of Production Methods

Most cellulolytic microorganisms utilize carbohydrates for their energy but are unable to use proteins or lipids as energy source for their growth [7,8]. It has been found that high level of

cellulase can be produced in media when supplemented with carbohydrate rich waste such as rice straw by *Penicillium* sp., and at 65°C and pH 4–5. Tallapragada et al. observed that biological wastes are organic and is easily degraded by microorganisms mainly fungi, which make these wastes very appropriate for enzyme production under submerged fermentation [9]. They also reported that submerged fermentation is finding increased application in the production of value added products from wastes mostly from lignocelluloses agro wastes. Verma et al. utilized pea peel, whey and wheat bran as a carbon source for *Trichoderma reesei* for the production of cellulase and protein [10]. It was observed that maximum cellulase activity of 2.86 U/mL was obtained by using pea peel as a substrate for *Trichoderma reesei*. Dhillon et al. evaluated the potential of agricultural biomass for the production of microbial enzyme production [11]. They reported that maximum cellulase activity of 13.57 IU/g dry substrate (gds), CMCase activity of 35.8 IU/gds and Xylanase activity of 33.71 IU/gds were obtained using wheat bran and rice straw medium at 96 h incubation period with *Aspergillus niger*, *Trichoderma reesei* and mixed culture of *Trichoderma reesei* and *Aspergillus niger*. Jhadhav et al. investigated the productivity of cellulase enzyme by using different substrates [12]: Rice husk, millet husk, banana peels, wheat bran, coir waste and saw dust. They found that banana peels gave the higher production of the cellulase enzyme i.e. 12.4 U / mL after 4 days of incubation. Annamalai et al. reported higher activities of cellulase enzyme (4040.45 U/mL) with 6.27 g/L rice bran, 2.52 g/L of yeast extract, an initial pH 9.0 and temperature 50°C in *Bacillus carboniphilus* CAS 3 [13]. *Trichoderma reesei* and its mutant were co-cultured with *Aspergillus phoenicis* QM329 for cellulase production on bagasse. Parent *Trichoderma* strain and the *Aspergillus* revealed a synergistic effect, resulting in enhanced production of cellulase, endoglucanase, and  $\beta$ -glucosidase activities [14]. Liu et al. cultured *Aspergillus fumigates* Z5 under solid-state fermentation (SSF) for thermostable cellulase production to degrade agricultural wastes for ethanol production. It yields 0.112 g bio-ethanol/g dry substrate suggesting that it is a promising fungus in the bio-ethanol production process [15]. Waghmare et al. investigated the potential of various agricultural wastes such as sugarcane bagasse, sugarcane barbojo, sorghum husks, grass powder, corn straw, and paddy straw for cellulolytic enzyme production by *Klebsiella* sp. PRW-1 [16]. Grass powder and sugarcane barbojo were found to be the best

carbon sources for enzyme [17]. For cellulase production by *Trichoderma harzianum*, temperature, pH, inoculum concentration, agitation rate, substrate (domestic wastewater sludge), and co-substrate (wheat flour) were optimized using two-level fractional factorial design. They obtained highest cellulase activity (10.2 Filter Paper Unit (FPU)/mL) during the fermentation process which was 1.5-fold higher than the cellulase produced from the results of design of experiment (6.9 FPU/mL). Bhavna et al. used different substrates: sorghum, groundnut, wheat and cotton for the production of cellulase in submerged and solid state fermentation [18]. They observed that the maximum cellulase activity in submerged fermentation was 0.28 U/mL to 0.77 U/mL as compared to solid state fermentation 0.01 U/mL to 0.36 U/mL. A previous study by the author on production of cellulase with pea hull substrate using *Trichoderma reesei* showed specific enzyme activity of 13.8 U/mL under submerged fermentation [19]. Literatures reported by various researchers indicate that agro-waste provides an attractive source for the production of cellulase enzyme and other by-products with better results in submerged fermentation. Table 1 shows the compilation of findings related to the utilization of lignocellulosic biomass for cellulase production.

## 4. APPLICATIONS

Cellulases were initially investigated several decades back for the bioconversion of biomass which gave way to research leading to industrial applications of the enzyme in animal feed, food textiles, and detergents and in the paper industry. With the shortage of fossil fuels and the rising need to find alternative source for renewable energy and fuels, there is a renewed interest in the bioconversion of lignocellulosic biomass using cellulases and other enzymes. In other fields, however, the technologies and products using cellulases have reached the stage where these enzymes have become indispensable.

### 4.1 Textile Industry

Cellulase has become the third largest group of enzymes used in the industry since their introduction a decade ago [1]. They are used in the bio-stoning of denim garments for producing softness and for producing the faded look, replacing the use of pumice stones which were traditionally employed in the industry. They act

on the cellulose fiber to release the indigo dye used for coloring the fabric, producing the faded look of denim. *Humicola insolens* cellulase is most commonly employed in the bio-stoning. Cellulases are also utilized for digesting off the small fiber ends protruding from the fabric resulting in a better finish. Cellulases have been used in defibrillation, bio-polishing [20] and in processes for providing localized variation in the color density of fibers.

In the washing industry, cellulases are commonly used in detergents for leaning textiles. Several reports disclose that endoglucanase III variants, in particular from *Trichoderma reesei*, are suitable for use in detergents. Cellulase preparations, mainly from species of *Humicola*, are active under mild alkaline conditions and at elevated temperatures, and are commonly added in washing powders and detergents.

### 4.2 Food and Animal Feed

In the food industry, cellulases are used in extraction and clarification of fruit and vegetable juices, production of fruit nectars and purees, and in the extraction of olive oil. Glucanases are added to improve the malting of barley in beer manufacturing and in wineries, better maceration and color extraction is achieved by use of exogenous hemicellulases and glucanases. Cellulases are also used in carotenoid extraction in the production of food coloring agents. Enzyme preparations containing hemicellulase and pectinase in addition to cellulases are used to improve the nutritive quality of forages. Improvements in feed digestibility and animal performance are reported with the use of cellulases in feed processing. The feed additive use of *Trichoderma* cellulases in improving the feed conversion ratio and increasing the digestibility of a cereal-based feed has also been reported.

### 4.3 Pulp and Paper Industry

In the pulp and paper industry, cellulases and hemicellulases have been employed for biochemical pulping for modification of the coarse mechanical pulp and hand sheet strength properties, de-inking of recycled fibers and for improving drainage and run-ability of paper mills. Cellulase, xylanase, laccase, and lipase are the most important enzymes that can be used in the pulp and paper processes [35]. The main advantages of enzymatic de-inking are reduced

**Table 1. Utilization of lignocellulosic substrate for cellulase production**

<b>Substrate used for cellulase production</b>	<b>Enzyme producing microbial strain</b>	<b>Enzyme production</b>	<b>Fermentation type</b>	<b>References</b>
Banana stalk	<i>Bacillus subtilis</i>	Exoglucanase	SSF	[21]
Agricultural waste	<i>Aspergillus niger</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SmF	[22]
Wheat bran	<i>Aspergillus niger</i> and <i>Trichoderma reesei</i>	FP-ase, Endoglucanase, $\beta$ -glucosidase	SmF	[23]
Waste paper	Mixing of <i>Aspergillus niger</i> and <i>Trichoderma viride</i>	FP-ase, Endoglucanase, Exoglucanase,	SSF	[24]
Cassava waste	<i>Aspergillus niger</i> , <i>Aspergillus terreus</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[25]
Paper, wood, litmus paper	<i>Fusarium oxysporum</i>	$\beta$ -glucosidase		[26]
Pine apple waste	<i>Bacillus pumilus EB3</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SmF	[27]
Palm fruit	<i>Bacillus coagulans</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[28]
Citrus limonium and papaya waste	<i>Pleurotoso streatus</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[29]
Freshly ripe tomato fruit	<i>Aspergillus flavus</i> Linn	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[30]
Olive processing residue	<i>Trichoderma reesei</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase		[31]
Apple pomace	<i>Trichoderma species</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[32]
Molasses	<i>Bacillus subtilis</i>	Endoglucanase	SmF	[33]
Sawdust, Bagasse	<i>Aspergillus flavus</i>	Endoglucanase, Exoglucanase, $\beta$ -glucosidase	SSF	[34]

or eliminated alkali usage, improved fiber brightness, enhanced strength properties, higher pulp freeness and cleanliness, and reduced fine particles in the pulp. Moreover, de-inking using enzymes at acidic pH also prevents the alkaline yellowing, simplifies the de-inking process, changes the ink particle size distribution, and reduces the environmental pollution. Enzymatic de-inking can lower the need for de-inking chemicals and reduce the adverse environmental impacts of the paper industry. However, the excessive use of enzymes must be avoided, because significant hydrolysis of the fines could reduce the bondability of the fibers. Cellulases are employed in the removing of inks, coating and toners from paper. Bio characterization of pulp fibers is another application where microbial cellulases are widely used. Cellulases are also used in preparation of easily biodegradable cardboard. The enzyme is employed in the manufacture of soft paper including paper towels and sanitary paper and preparations containing cellulases are used to remove adhered paper.

#### 4.4 Bio-fuel

The most important application currently being investigated is in the utilization of lignocellulosic wastes for the production of bio-fuel. The lignocellulosic residues represent the most abundant renewable resource available to mankind but their use is limited only due to lack of cost effective technologies. Narra et al. reported the production of cellulase and bio-ethanol in a single reactor from water hyacinth [36]. A potential application of cellulose is the conversion of cellulosic materials to glucose and other fermentable sugars, which in turn can be used as microbial substrates for the production of single cell proteins or a variety of fermentation products like ethanol. Organisms with cellulose systems, which are capable of converting biomass to alcohol directly, are already reported. But none of the systems described are effective alone to yield a commercially viable process. The strategy employed currently in bio-ethanol production from lignocellulosic residues is a multi-step process involving pre-treatment of the residue to remove lignin and hemicellulose fraction. Cellulose is then treated at 50°C to hydrolyze the cellulosic residue to generate fermentable sugars, and finally use of a fermentative microorganism to produce alcohol from the hydrolyzed cellulosic residue to generate fermentable sugars. The cellulose preparation needed for the bio-ethanol plant is

prepared in the premises using same lignocellulosic residue as substrate, and the organism employed is always *Trichoderma reesei*. To develop efficient technologies for bio-fuel production, significant research have been directed towards the identification of efficient cellulose systems and process conditions. Besides studies directed at the biochemical and genetic improvement of the existing organisms utilized in the process, the use of pure enzymes in the conversion of biomass to ethanol or to fermentation products is currently uneconomical due to the high cost of commercial cellulases. Effective strategies are yet to resolve and active research has to be taken up in this direction. Overall, cellulosic biomass is an attractive resource that can serve as a substrate for the production of value added metabolites and cellulases as such.

#### 4.5 Wine and Brewery Industry

Microbial glucanases and related polysaccharides play important roles in fermentation processes to produce alcoholic beverages including beers and wines. These enzymes can improve both quality and yield of the fermented products. Glucanases are added either during mashing or during primary fermentation to hydrolyze glucan, reduce the viscosity of wort, and improve the filterability.

In wine and beer production, enzymes such as pectinases, glucanases, and hemicellulases play an important role by improving color extraction, skin maceration, must clarification, filtration, malting of barley and finally the quality and stability of wine and beer.  $\beta$ -Glucosidases can improve the aroma of wines by modifying glycosylated precursors. Macerating enzymes also improve pressability, settling, and juice yields of grapes used for wine fermentation. A number of commercial enzyme preparations are now available to the wine industry. Malting of barley depends on the biosynthesis and activation of  $\alpha$ - and  $\beta$ -amylases, carboxypeptidase, and  $\beta$ -glucanase which hydrolyze the seed reserves. The main benefits of using these enzymes during wine and beer making include better maceration, improved color extraction, easy clarification, easy filtration, improved wine quality, and improved stability. A range of improved enzymes like cellulase, pectinase and glucanase that would be exogenously added to the process are expected to enhance the productivity of existing brewing and wining processes in future.

## 5. CONCLUSION

Waste utilization is an alternative approach for waste management. Keeping in view the importance of enzymes in various process industries, effective and economical production of these enzymes is of utmost importance. Enzyme production by utilizing cheap lignocellulosic waste as agro waste would be a novel approach, which can allow cost-effective enzyme production. The utilization of all agro waste material, not only provides a cost effective and environmental friendly technology, but is also helpful up to some extent in the solid as well as liquid waste bio-resource management. With modern biotechnological tools, especially in the area of microbial genetics; novel enzymes applications will become available for various industries. Based on the literary works reported in this article, it is suggested that a collaborative research and development sector between food industries and institutes working on waste utilization be established. The proposed sector may benefit research institutions to better identify the domain for waste-utilization whilst generating higher income opportunities for industries through value-added products.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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