

# Cellulase Production from Species of Fungi and Bacteria from Agricultural Wastes and Its Utilization in Industry: A Review

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## Abstract

In energy deficient world, cellulases play a major role for the production of alternative energy resources utilizing lignocellulosic waste materials for bioethanol and biogas production. This study highlights fungal and bacterial strains for the production of cellulases and its industrial applications. Solid State Fermentation (SSF) is more suitable process for cellulase production as compared to submerged fermentation techniques. Fungal cellulosomes system for the production of cellulases is more desirable and resistant to harsh environmental conditions. *Trichoderma* species are considered as most suitable candidate for cellulase production and utilization in industry as compared to *Aspergillus* and *Humicola* species. However, genetically modified strains of *Aspergillus* have capability to produce cellulase in relatively higher amount. Bacterial cellulase are more resistant to alkaline and thermophile conditions and good candidate in laundries. Cellulases are used in variety of industries such as textile, detergents and laundries, food industry, paper and pulp industry and biofuel production. Thermally stable modified strains of fungi and bacteria are good future prospect for cellulase production.

## Keywords

Cellulase, Bacteria, Lignocellulosic Wastes, Trichoderma, Solid State Fermentation

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## 1. Introduction

This review highlights the potential utilization of fungal and bacterial species for the production of cellulases and their applications in diverse fields and industries. Cellulases are utilized in textile, food, medical, laundries, agriculture, textile, enhancement of animal feed digestibility and paper and pulp industry. Cellulase is a synergistic enzyme which is accustomed to split cellulose into glucose and/or different oligosaccharide compounds [1]. Cellulase enzymes may be divided into 3 types: endoglucanase (endo-1, 4- $\beta$ -D-glucanase, EG, EC 3.2.1.4); cellobiohydrolase or exoglucanase (exo-1, 4- $\beta$ -D-glucanase, CBH, EC 3.2.1.91) and  $\beta$ -glucosidase (1, 4- $\beta$ -D-glucosidase, BG, EC 3.2.1.21) [2] [3], whereas EGs being the foremost economical enzyme [4].

Fungi are studied extensively among these organisms because of their elongated hyphae which produce mechanical pressure on the cellulose structure, inflicting them to supply massive amounts of cellulose. Subsequently, fungal strains have the capability to produce higher quantities of cellulases as compared to other organisms [5] [112].

Cellulases are required in potentially higher amount and its demands are expected to rise with passage of time [6]. Fungal cellulases have the potential to digest cellulose, hemicelluloses and lignin by secreting diverse set of hydrolytic and oxidative enzymes [7] [18]. Cellulases complex degrade cellulose in to fermentable sugars and play pivotal role in the conversion of biodegradable material in to ethanol. Cellulases have wide range of applications such as extraction of protein from soybeans and coconut, green tea compounds, unicellular vegetable production and formation of vinegar from citrus fruit pulp. Cellulases are widely used for the removal of seed coat of soybeans. It has potential use in the modification of glutinous rice, other food tissue and tensile strength of cellulosic material like paper quality improvement. Although, most important application of cellulases are the conversion of cellulosic wastes in to glucose but the microbial invasion make difficult the production of active extra cellular enzyme preparation [8] [15]. Lactose is amongst the well-known inducer of cellulase producer gene and is most economical additive in industry particularly in case of fermentation [9] [23]. Production of cellulase in microbial cultures is strictly concerned with growth and various factors affect the productivity [10] [22]. Various biomass inducing residues including; lignocellulosic material, paper waste, pulses cereals straw and bagasses have been widely used as carbon sources for commercial cellulase fermentations [11] [24]-[31]. Low yields over prolonged fermentation is the major limiting factor and for the production of cellulases; solid state fermentation (SSF) is gaining popularity being cost effective and equally useful for the bioconversion lignocellulosic material using cellulolytic microorganisms [12] [32]-[34].

### 1.1. Fungal Cellulosome System

The adherence and digestion of lignocellulosic biomass by microbes is not such an easy understanding, in fact, it requires very specific molecular binding sites which are made to facilitate this purpose. These kind of specific molecular structures are known as cellulosomes and have a complex of varied enzymatic domains. The cellulosomes work in an efficient way in which they have proper mechanism of actions *i.e.*, they attach to the biomass in the first step and in the next step; they degrade the biomass resulting in components which are further absorbed by the microbes to fulfill their food requirements [13].

As compared to bacterial cellulase systems, fungal cellulases are structurally less complicated. Fungal cellulases usually consist of 2 separate domains: cellulose binding module (CBM) and a catalytic domain (CD), which has a short polylinker region to its N-terminal to join cellulose binding module (CBM) with it. The CBM has 35 amino acids, and the linker region has a plenty of Serine and Threonine. The major differentiating character between cellulosomes *i.e.*, bound cellulase and free cellulase is that cellulosomes-cohesion has scaffolding and dockerin containing enzyme. Cellulose binding domains (CBMs) replaced by a dockerin in cellulosomal complex in free cellulase, and one scaffolding-born CBM directs the complete cellulosomes complex to cellulosic biomass [14] [19].

### 1.2. Cellulase Bio-Production through Fermentation Using Agriculture Waste Materials

Fermentation technique has been mostly used for the production of cellulases and *T. reesei* has been widely used in bioprocessing for cellulase production. Solid substrate fermentation could be preferred over liquid media in case of aerobic microorganisms [15].

The production of cellulase and pectinase using *Aspergillus niger* on corn cobs as a carbon source is examined.

Different parameters are implied to check the optimization including temperature, pH, biomass production and activity of enzyme. The maximum activity of cellulase ( $1.9 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ) is produced on 4<sup>th</sup> day while pectinase shows maximum activity ( $1.5 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ) on 4<sup>th</sup> and 5<sup>th</sup> day. The temperature range of 50°C is found to be optimum for cellulase activity ( $1.3 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ) while activity of pectinase ( $1.6 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ) shows 60°C as optimum temperature [16]-[19]. The pH 4 is optimum for cellulase activity ( $2.70 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ) and pH 6 is for activity of pectinase ( $1.5 \times 10^{-4}$   $\mu\text{g/mL/sec}$ ). The study reveals that *Aspergillus niger* has the capability of producing cellulase and pectinase using corn cobs under SSF [20].

Bagasse powder is used as a substrate for cellulase production employing novel thermo-stable yeast. Maximum cellulase yield obtains at 50°C, medium of bagasse powder 4% (w/v) +  $(\text{NH}_4)_2\text{SO}_4 = 0.1\%$  (w/v), pH 5.5 and incubation time of 72 hours. Moreover the isolated yield is tolerant to wide ranges of substrate concentration, temperature and pH expressing higher productivity of enzyme [21]. Additionally, C1 exo-gluconase and endo-gluconase using a crude lignocellulosic material are also produced and hence can even be used for ethanol production [21]. Solely, fungi naturally manufacture the require titers of cellulases needed for the entire saccharification (30 - 50 mg enzyme/g of crystalline cellulose) [6] [22]. Many cellulase producing fungi including *Trichoderma*, *penicillium*, *Botrytis neurospora* [7] [23] genera *Aspergilli* [8] [24] *Aspergillus niger* and *Aspergillus terreus*, *Rhizopus stolonifer* [9] [25], *Fusarium oxysporum* [10] [26] are suitable for bioprocessing.

Solid state fermentation is the cheapest way of cellulase production from agro industrial wastes [11] [27]. Interestingly, recent studies report that SSF provides an additional adequate environment for fungi [12] [28]-[30] for various enzymes production. SSF commercialization has been used for production of enzymes (~3.5 billion tons per year). The advantage of exploiting SSF to attain the low cost fermentation system needed and the likelihood of getting it administrated on farms [13] [31]. Moreover, it is environmentally favorable, low energy demanding, inhibiting waste water release and economically feasible [14] [32]. The roles of Cellulases are inevitable in paper, pulp and textile industry of the world. Cellulose has a higher level of crystallinity and thus becomes difficult to be broken down into sub particles. To overcome this problem, Cellulolytic enzymes are used synergistically because the combination of cellulases expresses much more activities than the activity of individual cellulases. The action of these enzymes has been more elaborately explained by a most common and most accepted endo-exo energy model. This model suggests that there is proper mechanism of action employ by such enzymes in which endo-glucanases attack on random sites of the lignocellulosic chains exposing some new sites for cellobiohydrolases to attack. Cellobiohydrolases further performs its function as exoenzymes liberating two main products; one of the products is cellobiose which is the major product of this degradation process. On the other hand,  $\beta$ -glucosidases, which aren't considered legitimate cellulases, play a vital role within the breakdown of cellobiose and some other short oligosaccharides to final product is glucose [16] [33]. Cellulase activity is described as the capability of cellulase to digest crystalline cellulose extensively [17] [34].

*Aspergillus terreus* ion for cellulase production using rice straw as a substrate under solid state fermentation is reported and Response surface methodology implicating Box-Behnken-design apply to optimize temperature and pH. The filter paper activity which is predicted under optimized conditions is 9.73 U/g and the approved activity is 10.96 U/g. The study shows the use of pretreated rice straw with mild alkali to produce fermentable sugars with 74.19% adequacy [35].

Rice grass (*Spartina* spp.) use lignocellulosic material by implicating *Aspergillus* species under solid state fermentation process. The study reveals the efficacy of using rice grass (*Spartina* sp.) as the major substrate for yielding cellulase through a novel isolated strain of *Aspergillus* sp. (SEMCC-3.248) in solid-state fermentation. The parameters which are optimized for cellulase production, are rice grass 2.5 g, 1.5 g of wheat bran, 4 mL of nutrient medium ( $(\text{NH}_4)_2\text{SO}_4$  14 g/L,  $\text{CaCl}_2$  4 g/L,  $\text{KH}_2\text{PO}_4$  2 g/L, soluble starch 2.62 g/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/L and peptone 1.51 g/L), 1 mL of inoculum, moisture level 70%, pH 5.0, temperature 32°C and 5 days of incubation period. Following these optimum conditions, the total cellulase activity is 1.14 FPIU/gds [36].

*Aspergillus niger* HQ-1 is studied for cultivation and optimization using solid state fermentation process. Plackett-Burman design (PBD) is used to identify optimum incubation temperature, moisture content and culture pH for cellulase activity. The optimal regions containing three significant factors, is determined. Furthermore, response surface analysis and Box-Behnken design (BBD) are employed to find interactive effect between the three variables on the activity of cellulases. The optimum conditions are expressed to be temperature 33.5°C - 33.7°C, moisture level 70.3% - 70.6% and pH 4.626 - 4.662. Moreover, the activity of cellulase or hydrolysis of chitosan is high at 50°C and pH 5.6. This cellulase hydrolyzing activity is further improved by some metal ions  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  while is inhibited by  $\text{Ba}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Ag}^+$  [37].

Corn cobs are used for the production of cellulase enzyme using *Alternaria alternata* through solid state fermentation. Different optimizing parameters are implied to check the maximum yield of cellulase including incubation period (1 - 7 days), pH (3.0 - 9.0) and temperature (25°C - 40°C). The optimal cultural conditions are like incubation period of 96 hours, pH 6.0 and incubation temperature as 35°C expressing cellulase activity as 15.06 µg/mL, 26.4106 µg/mL and 31.2406 µg/mL respectively [38].

*Eichhornia crassipes* (water hyacinth) is used for cellulase production and the growth medium is enriched with water hyacinth mixture in ratio 1:05 (V/V) as energy source. Maximum cellulase is produced after incubation time of 6 days, temperature 30°C, 150 rpm shaking speed at pH 5.0. Cellulases show maximum activity at optimum conditions including 40°C temperature and pH 5.0. Vmax and Km are observed to be 58.3 µmol/mL/min and 4.7 mg/mL respectively [39].

Solid state fermentation methodology is employed for endo-cellulases production of by *Aspergillus japonicus* C03. The temperature for maximum production is observed to be 50°C - 55°C for cellulase production with the optimum pH of 4.0. Moreover, this enzyme is capable to bear the pH change of 4.0 - 7.0. It is also observed that Manganese and Copper enhanced the cellulase activity up to 64% [40].

Soybean hulls are used to yield cellulolytic enzymes under solid state fermentation by *Aspergillus oryzae* and *Trichoderma reesei* cultures. It is observed that crystallinity is extremely increased by mild acid, alkali and steam pretreatments. The steam pretreated hulls is first inoculated with *T. reesei* and shows more cellulase activities (4 filter paper units (FPU)/g-ds, 45 IU/g-ds endo-cellulase and 0.6 IU/g-ds β-glucosidase) than untreated soybean hulls (0.75 FPU/g-ds, 7.29 IU/g-ds endocellulase and 0.06 IU/g-ds β-glucosidase). In case of *A. oryzae*, the pretreated hulls produce more endo-cellulases (47.10 IU/g-ds) than the untreated hulls (30.82 IU/g-ds). The work shows an interrelationship between enzymatic production and physiochemical characteristics [11] [41].

Castor bean is used as a substrate for cellulase production using *Aspergillus japonicus* URM5620 under solid state fermentation. A full factorial design (2<sup>4</sup>) is used to study the effects of different parameters like substrate concentration, pH, moisture level, incubation period and temperature on enzyme yield. The optimum conditions are observed to be substrate concentration 5.0 g, pH 6.0, moisture level 15%, 120 h of incubation period and 25°C temperature. The optimization processes describes clearly the impact on enzyme production [41].

Some mutant strains of *Aspergillus* sp. SU14 for the production of cellulase are employed. *Aspergillus* sp. SU14 spores are frequently treated with ultraviolet irradiation, (Co60) γ-rays and N-methyl-N'-nitro-N-nitrosoguanidine. *Aspergillus* sp. SU14-M15 is a mutant strain with cellulase production 2.2-fold more than that of wild type. The optimum requirements for growth are examined to be medium containing wheat-bran enriched with urea 1% (w/w), rice starch 1% (w/w), Tween 80 0.05% (v/w) and MgCl<sub>2</sub> 2.5 mM, moisture 50% (v/w), pH 3.5 with aeration area of 3/100. When, 25% of 48 hours seeding culture is inoculated for 3 days at 35°C, the resultant cellulase production is 8.5 times more than the conventional type of cellulase production [42].

Cellulase production on carboxymethyl cellulose by *Aspergillus niger* which is isolated from various sources of soil, is also studied with shaking flasks incubation with ambient temperature. All isolated strains show cellulase activity with the maximum yield at day 4 (0.07162 IU/mL/min) produce by *Aspergillus niger* isolated by rice growing field whereas minimum activity (0.02911 IU/mL/min) is by *Aspergillus niger*, isolated from street soil. The experiment reveals the cellulolytic capability of *Aspergillus niger* in almost all soil environments. Moreover, the results display that this robust strain can be isolated from rice growing fields for the production of commercial cellulase [43].

## 2. Cellulase Producing Organisms

Cellulolytic microorganisms mostly degrade carbohydrates and cannot utilize lipids and proteins as source of energy for metabolism and growth [44]. Among them, most important microorganisms are bacteria, cytophaga, cellulomonas can degrade carbohydrates other than cellulose [45] [46]. Anaerobic microbial species have limited cellulolytic activity restricted to cellulose and its hydrolytic products [15] [47].

*Trichoderma reesai* is the most widely studied fungus and has ability to convert desired as well as native cellulose to glucose. Among most widely studied organisms having notably high cellulolytic activity, include various fungal species like *Humicola*, *Trichoderma*, *Penicillium* and *Aspergillus*. Some bacterial species include; *Pseudomonas*, *Bacilli*, *Actinomycetes*, *streptomycetes*, *Cellumonas*, *Streptomyces* and *Actinomucor* [48]-[50]. Because of the ability of fungi to consume cellulose for energy consumption, only certain species could be used practically for cellulose hydrolysis. Despite of *T. reesai*, other fungal species include *Aspergillus*, *Penicillium*

and *Humicola* have practical implementation to produce high yields of cellulases [51]-[53].

Certain aerobic bacterial species such as *Cytophaga*, *Cellulomonas* and *Cellovibrio* have ability to degrade cellulose in pure culture [44] [54]. The most accepted commercially applicable microbes are *A. niger recombinant*, *T. reesai*, *H. insolens*, *Thermomonasporafusa*, *Bacillus* species and some other organisms (Table 1).

**Table 1.** Microorganisms used in cellulase production from microorganisms.

Group	Genus	Species	References
Bacteria	<i>Bacillus</i>	<i>Bacillus species</i>	[54]
	<i>Acidotherrmus</i>	<i>A. Cellulyticus</i>	[55]
	<i>Pseudomonas</i>	<i>P. cellulosa</i>	[56]
	<i>Ruminococcus</i>	<i>R. albus</i>	[57]
	<i>Clostridium</i>	<i>C. thermocellum</i>	[58]
	<i>Clostridium</i>	<i>C. acetobutylium</i>	[59]
	<i>Rodothermus</i>	<i>R. marinus</i>	[60]
Fungi	<i>Fusarium</i>	<i>F. solani</i>	[61]
	<i>Aspergillus</i>	<i>A. niger</i>	[53] [62]
	<i>Aspergillus</i>	<i>A. oryzae (recombinant)</i>	[62]
	<i>Aspergillus</i>	<i>A. fumigatus</i>	[63]
	<i>Aspergillus</i>	<i>A. acculeatus</i>	[64]
	<i>Aspergillus</i>	<i>A. nidulans</i>	[65]
	<i>Melanocarpus</i>	<i>M. albomyces</i>	[66]
	<i>Humicola</i>	<i>H. grisea</i>	[67]
	<i>Humicola</i>	<i>H. insolens</i>	[49] [67]
	<i>Trichderma</i>	<i>T. reesai</i>	[68]
	<i>Trichderma</i>	<i>T. koningii</i>	[69]
	<i>Trichderma</i>	<i>T. viride</i>	[64] [69]
	<i>Trichderma</i>	<i>T. harjianum</i>	[70]
	<i>Trichderma</i>	<i>T. branchiatum</i>	[71]
	<i>Sclerotium</i>	<i>S. rolfsii</i>	[72]
	<i>Acremonium</i>	<i>A. Cellulyticus</i>	[73]
	<i>Fusarium</i>	<i>F. solani</i>	[74]
	<i>Sporotrichum</i>	<i>S. cellulophilum</i>	[75]
<i>Irpex</i>	<i>I. lacteus</i>	[63] [75]	
<i>Penicillium</i>	<i>P.fumiculosum</i>	[76]	
<i>Talaromyces</i>	<i>T. emersonii</i>	[77]	
Actinomycetes	<i>Streptomyces</i>	<i>S. lividans</i>	[78]
	<i>Streptomyces</i>	<i>S. drozdowiejii</i>	[79]
	<i>Cellulomonas</i>	<i>C. uda</i>	[80]
	<i>Cellulomonas</i>	<i>C. fimi</i>	[81]
	<i>Cellulomonas</i>	<i>C. bioajotea</i>	[46] [81]
	<i>Thermonospora</i>	<i>T. curvata</i>	[82]
	<i>Thermonospora</i>	<i>T. fusca</i>	[83]

### 3. Cellulases Applications

For the past few decades, cellulases has been widely studied for their importance in the conversion of biomass and other cellulosic materials which are otherwise consider as waste material. This is an important research tool in paper industry, textile industry, bio-fuel as renewable energy source, animal feed and detergents.

#### 3.1. Textile Industry

Cellulases are amongst the most important group of enzyme in industry [84] and they have been employed to reduce the faded look and protruding fibers in fabrics and garments and to give them softness. Before that, pumice stone was used traditionally for this purpose [24] [85]-[87].

Cellulases from *H. insolens* are frequently used in bio-stoning along with proteases and *trichoderma* [88]. Cellulases give better finish and digest small fibers that cause roughness of the fabrics [70] [89]. They have been employed for defibrillation and softening of fabrics [89]. Cellulases are good localizing agents and are used to eliminate color variation of fibers [89] [90].

#### 3.2. Detergents and Laundries

Cellulase CBH I and EG III have excellent cleansing properties and are used in textile cleaning. It has been reported that *T. reesai* producing EG III variants are suitable for the modification detergents. Similarly, *T. harzianum* and *T. viride* are also used as naturally producing sources of cellulases like *A. niger* [91] [111].

Cellulase production from Humicola species (*H. grisea* and *H. insolens*) is effective under mild alkaline conditions and at elevated temperatures. So, they are mostly used as additives in detergents and washing powders [92] [93]. Cellulases are added to detergents for the breakdown of hydrogen bonding under harsh environmental conditions such as alkaline or thermophile conditions [94] [111].

#### 3.3. In Food Industry

Cellulases are employed in food industry to extract and clarify olive oil, fruit and vegetable juices, in the production purposes and fruit nectars [70] [94]. In brewing industry, glucanases are used as additives for the improvement of malting of barley. Decent color extraction and maceration could be achieved using glucanase and hemicellulose [70] [94]. Carotenoids, which have been used as food coloring agents can be extracted through cellulases [94]. Cellulases along with pectinases and hemicellulases have been used to modify nutritive quality of forages [95]. Digestibility and performances of animal feed has been reported to improve using cellulases [96]. Bedford *et al.* [97] reported that better digestibility and feed conversion ratio of cereal feed could be achieved through the addition of *Trichoderma* cellulases [97] [111].

#### 3.4. In Paper and Pulp Industry

In paper and pulp industry, hemicellulases and cellulases have been used to modify the biochemical pulping of coarse pulp and to improve strengthening [97] [98]. These are equally useful for the depolarization of recycled pulp and for the efficiency improvement and drainage of paper mills [99] [100]. Cellulases have been employed to remove toners and coatings from paper [101] [102]. Microbial cellulases have also been used for the characterization of fiber pulp. Manufacturing of biodegradable cardboard can be easily done using cellulases [103] and also used to improve the soft texture of paper, manufacturing of sanitary paper and paper towels [104]-[106].

#### 3.5. Biofuel Production

Production of biofuel is one of the most recently investigated applications of cellulases in the bioconversion of lignocellulosic wastes. Although, abundant cellulosic residues are available but major disadvantage of this biodegradation is cost effectiveness of the process. Cellulases have ability to convert lignocellulosic material into fermentable sugars like glucose, maltose, used as substrates to form bioethanol and other products. Certain microorganisms have been reported which have ability of direct conversion of biomass to various alcohols [107]-[109], but they are not used as efficient source commercially. This technique involves multistep process to convert lignocellulosic material into bioethanol. In the pretreatment process, fraction of hemicellulose and lignin is improved for further processing. Then the residues are hydrolyzed at 50°C to produce fermentable sugars and

in the final step, microorganisms have been employed to convert cellulosic wastes into alcohol [110] [111].

#### 4. Conclusion

Biotechnological applications of cellulases make future prospect for the hyper-production of cellulases by genetically modifying fungal and bacterial strains. In future, thermo-stable, alkaline resistant cellulases are made for applications in industries to attain high degradable yield.

#### 5. Conflict of Interest

Authors have no conflict of interest with any scientist or department.

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