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# A Review: Microbial Production of Cellulase Enzyme and its Application

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**ABSTRACT**

The most abundant biomass on the planet is Cellulase. Cellulase-producing bacteria can be obtained from a garden soil sample, industrial area soil samples like the pulp and paper industry, the feed industry, textile industry and marine soil samples etc. Cellulase hydrolyzes Cellulose's 1, 4-linkage and is responsible for cellulose breakdown. Although aerobic and anaerobic microbes produce these enzymes, aerobic cellulolytic fungi such as Trichoderma viride and T. reesei have received much attention. Cellulase is also produced by bacteria such as Acidothermus, Bacillus, Clostridium, Pseudomonas, Rhodothermus and some actinomycetes such as Cellulomonas, Streptomyces, Thermononospora. The application of cellulase enzyme includes pulp and paper, feed, textile, wine and brewery, and food and detergent industries. Cellulase is used in agriculture to improve crop growth and as a disease control agent.

Keywords - Cellulase, Cellulase producing organisms, Production, Application

## 1. Introduction

Cellulose is a complex carbohydrate consisting of 3,000 or more glucose units in a polysaccharide. It is the most abundant of all organic molecules found in nature. Cellulose is the basic structural component of plant cell walls, accounting for around 33% of all vegetable matter (90% of cotton and 50% of Wood) [Britannica, 2019]. The chemical formula of Cellulose as a chain is $(C_6H_{10}O_5)_n$. Cellulose is an organic molecule that is both water-soluble and biodegradable.

Cellulases have been the focus of extensive investigation because they can convert cellulosic waste material into food to suit the needs of an ever-increasing population [Chandrakant S. et al., 2011, J. W. Bennet et al., 2002]. Cellulases are essential in both the industrial and natural worlds, as they play a critical part in the global carbon cycle by converting insoluble Cellulose into soluble sugars [Schaechter, 2009].

Environmental pollution requires increased attention, particularly in densely populated places, and concerns such as waste avoidance and recycling, as well as bioproduct development, are so timely [Block et al., 1999]. Because of the depletion of fossil fuel supplies and the growing pollution caused by their combustion, the development of biofuels is promoted. Many towns and countries struggle to manage this biodegradable component of domestic refuse, market garbage, yard waste, and animal and human waste, with about 80% of dry municipal solid waste classified as organic waste. The main component of these organic wastes is used paper, which contains Cellulose as a primary structural component. The conversion of waste cellulose materials to fermentable sugars would help with waste reduction, as well as the creation of sustainable bioenergy [Jeffries et al., 1999] and bioproduct such as lactic acid [Venkatesh, K. V., 1997]. Cellulase treatment of various waste cellulose sources has resulted in variable degrees of bioconversion [Wood et al., 1997]. It has been determined that used foolscap paper, filter paper, office paper, newsprint and microcrystalline Cellulose are susceptible to saccharification by *Pseudomonas funiculus* Cellulase [van Wyk et al., 2000].

In the structure of Cellulose, catalytic and non-catalytic modules can be found. Cellulase catalytic modules have been classified into several families based on their amino acid sequences and crystal structures. The N-or C-terminus of a catalytic module could contain non-catalytic carbohydrate-binding modules (CBMs) and other functionally known or unknown modules [Jayasekara et al., 2019]. Cellulase from bacteria and fungi typically includes two or more functional and structural domains connected by peptide linkers [Sakka et al., 2000]. In aerobic organisms, the cellulose-binding domain binds to a catalytic domain, but in anaerobic organisms, the dockerin domain attaches to the catalytic domain [Ohmiya et al., 1997]. The cellulose-binding module (CBM) and the catalytic domain (CD) of fungal Cellulases are connected by a brief polylinker region. The catalytic binding region of fungal Cellulases has fewerthan40amino acid residues, including three conserved aromatic residues [Ejaz et al., 2021, Kuhad et al., 2011].

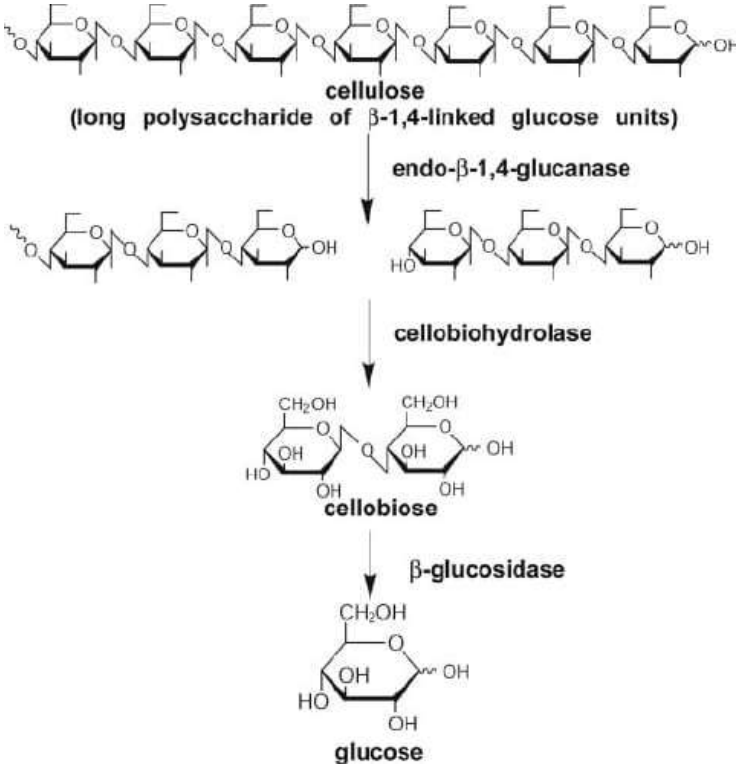


Figure: 1 Reaction of Cellulase enzyme [Xie, Gary, et al., 2007]

Cellulase is the most diversified class of enzymes that catalyze the hydrolysis of a single substrate. Cellulase, which hydrolyzes cellulose's1,4-linkages, is responsible for cellulose breakdown [Zverlov VV et al., 2005]. Cellulose exists in crystalline and amorphous topologies; despite being chemically homogeneous, no one enzyme can hydrolyze it.

### *1.1 Classification of Cellulase*

There are five general types of Cellulases based on the type of reaction they catalyzed.

1) Endocellulases (EC 3.2.1.4): -Cleave internal bonds at amorphous sites at random to create new chain ends [Baht et al., 2000].
2) Exocellulases or Cellobiohydrolases (3.2.1.91): -Endocellulase produces tetrasaccharides or disaccharides, such as cellobiose, by cleaving two to four units off the exposed chain's ends.

Exocellulases are divided into two types once more [Baht et al., 2000].

a. Type1: -Works processively from the reducing end of the cellulose chain.
b. Type2: -Works processively from the nonreducing end.

3) Cellobiases (EC 3.2.1.21) or beta glycosidase: -Hydrolyze the exocellulase product into individual monosaccharides [Baht et al., 2000].
4) Oxidative Cellulases: - Depolymerize Cellulose by the radical reaction.
5) Cellulose phosphorylases: -Use phosphates instead of water to depolymerize Cellulose.

### *1.2 Mode of Action of the Enzyme*

Beta-glycosidase, endo-1, 4-D-glucanase (endoglucanase), and exo-1, 4-D-glucanase are the three enzymes that makeup Cellulase (exoglucanase). These three enzymes work together to hydrolyze Cellulose in a synergetic manner for complete and effective cellulose hydrolysis [Patel et al., 2019]. Trichoderma reesei, the best-studied cellulolytic fungus, produces seven-glucosidase components, eight endo-1, 4-glucanase components, and two cellobiohydrolase components [Sakka, K. et al., 2000]. Endoglucanase attacks oligosaccharides' inner sites in carboxymethyl cellulose, cello oligosaccharides, and amorphous Cellulose. Exoglucanase hydrolyzes the nonreducing ends of crystalline Cellulose to produce cellobiose or glucose. Cellobiose and cello dextrin's nonreducing ends are attacked by beta-glucosidase [Ohmiya, K. et al., 1997, Ejaz et al., 2021]. Several novel proteins involved in cellulose hydrolysis have recently been discovered. GH-61 genes are found in most cellulolytic fungi, and a few of them encode proteins with limited cellulolytic activity [Harris et al., 2010]. When magnesium ions were present, a protein from the GH-61 family stimulated cellulose hydrolysis of pretreated biomass by crude T. reesei Cellulase. Still, it did not stimulate hydrolysis of pure Cellulose [Harris et al., 2010]. When growing on Cellulose,

Thermobifida fusca generates and secretes substantial amounts of two families of 33 CBM proteins. One is a family of 33 CBM attached to a family of 2 CBM, while the other is a family of 33 CBM with a family of 2 CBM. These proteins have been shown to bind to Cellulose and chitin and stimulate T. Fusca cellulase to hydrolyze Cellulose [Moser, Felix, et al., 2008]. Swollen, a T. reesei protein similar to expanding has been shown to loosen crystalline Cellulose [Saloheimo, Markku, et al., 2002]. T. reesei cellulase activity was significantly increased after a Bacillus subtitles homolog was cloned [Kim et al., 2009, Wilson et al., 2011].

### *1.3 Catalytic mechanism of Cellulase*

Glucosidic hydrolases use acid-base catalysis to break down glycosidic bonds. Two catalytic residues of the enzyme accomplish the hydrolysis of a general acid (proton donor) and a nucleophile/base. Hydrolysis occurs via retention or inversion of the anomeric configuration, depending on the spatial position of these catalytic residues. After double displacement hydrolysis with two crucial glycosylation/deglycosylation stages, the anomeric C bearing the glucosidic target bond preserves the same (substituent) configuration. In contrast, following single nucleophilic displacement hydrolysis, the anomeric C of "inverting" Cellulases inverts its (substituent) structure [Vocadio and Davies, 2008].

## 2. Microorganisms Produce the Cellulase

Archaea has 35 genera with hyperthermophilic organisms that use a variety of carbon and energy sources. Only Desulfurococcus fermentans, a hyperthermophilic archaeon, have grown on crystalline Cellulose (filter paper) at an optimal temperature of 81°C [Graham, Y.M.et al., 1998, Perevolova et al., 2005].

Table-1 Diversity of Cellulase producing microorganisms

| **Diversity of Cellulase producing microorganisms** | **Reference** |
|---|---|
| **Fungi**<br>*Aspergillus Niger, Aspergillus nidulans, Aspergillus oryzae, Penicillium brasilianum, Penicillium Occitania, Penicillium decumbens, Penicillium janthinellum, Fusarium solani, Fusarium oxysporum, Fusarium chlamydospores, Humicola insolens, Humicola grisea, Melanocarpus albomyces, Trichoderma logibrachiatum, Trichoderma harianum, Trichoderma reesei.* | Sukumaran et al., 2005; Qin, Yongling et al., 2010; Adsul M.G. et al., 2004; Reczey, K. et al., 1996 |
| **Bacteria**<br>*Acidothermus cellulolyticus, Pseudomonas cellutosa, Bacillus subtilis, Clostridium acetobutylicum, Clostridium thermocellum, Rhodothermus Marinus* | Tomme et al., 1995, Wachinger et al., 1989 |
| **Actinomycetes**<br>*Cellulomonas fimi, Cellulomonas bioazotea, Thermononospora fusca, Thermononospora curvata, Streptomyces reticuli, Streptomyces drozdowiczii, Cellulomonas uda, Streptomyces lividans.* | Rajoka, M. et al.,1997, de Lima, et al., 2005, Théberg et al., 1992, |

The Congo red overlay method was used to conduct a qualitative screening of Cellulase production bacteria [Ariffin et al., 2006]. The use of Congo red as a marker for Beta-D glucan breakdown in an agar medium offers the foundation for a fast and sensitive cellulolytic bacteria screening test [Teather et al., 1982]

### *2.1 Fermentation Process for Production:*

There are two types of fermentation.

1) **Submerged fermentation:** In a submerged fermentation method, cellulolytic species were evaluated for Cellulase enzyme production using a basal salt medium containing carboxymethylcellulose and sterilized. Fermentation took place in flasks containing sterile production medium and standard inoculums. The flasks were incubated on a rotary shaker for microorganisms [Lokhande, Shipa et al., 2017, Shaikh, N.M. et al., 2013].
2) **Solid-state fermentation:** In a flask containing coir waste and distilled water, solid-state fermentation was carried out. The flasks were sterilized before being allowed to cool to room temperature. The inoculum was added and thoroughly mixed before being incubated in a humidified incubator. Regularly, gentle shaking was used to mix the flasks [Mrudula, Soma et al., 2011].

Table 2 shows the various bacteria fermentation and production medium.

| No. | Bacteria | Production medium | Type of fermentation | Author |
|---|---|---|---|---|
| 1. | *Bacillus subtilis* | Glucose | SMF | Sethi, Sonia et al., 2013 |
| 2. | *Paenibacillus sp.* | CMC | SMF | Islam, et al., 2018 |
| 3. | *Cellulomonas sp. YJ5* | CMC | SMF | Yin, et al., 2010 |
| 4. | *Bacillus subtilis CY5* | Seeded with tryptone soya broth | Ssf | Ray, Arun K., et al., 2007 |
| 5. | *Bacillus circulans TP3* | Seeded with tryptone soya broth | Ssf | Ray, Arun K., et al., 2007 |
| 6. | *Bacillus pumilus EWBCM1* | Sugarcane bagasse | SMF | Shankar, T., et al., 2011 |
| 7. | *Achromobacter xylosoxidans BSS4* | Basal salt medium | SMF | Sreedevi et al., 2013 |
| 8. | *Bacillus sp. BSS3* | Basal salt medium | SMF | Sreedevi et al., 2013 |
| 9. | *Pseudomonas sp. BSS2* | Basal salt medium | SMF | Sreedevi et al., 2013 |

Smf: Submerged fermentation. Ssf: Solid-state fermentation

Submerged fermentation is better than solid-state fermentation because it requires moisture for the growth of the bacteria, and solid-state fermentation does not have enough moisture for the growth of bacteria [Sethi, Sonia et al., 2013, Bohair Peter et al., 2008]. *Aspergillus niger* was used to produce Cellulase in both submerged (SMF) and solid-state fermentation (SSF). After 72 hours in ssf and 96 hours in SMF, the maximal amount of Cellulase was produced. CMCase and FPase activity were 8.89 and 3.56U per gramme of dried mycelial bran (DBM) in ssf, respectively. The activity of CMCase and FPase were determined to be 3.29 and 2.3U per ml of culture broth, respectively, in SMF [Mrudula, Soma et al., 2011].

### *2.2 Enzyme Assays:*

Two methods are used for enzyme assays.

1) Activity of carboxymethyl cellulase (CMCase)
2) Activity of filter - papers (FPase)

The phase method is similar to the CMCase approach, except the FPase method uses Whatman as the substrate. Sodium citrate buffered No. 1 filter paper strip [Mandels, M. et al., 1969, Sohair Peter et al., 2008].

Table-3 Enzyme activity

| No. | Bacteria | Source | CMCase activity (U/ml) | FPase activity (U/ml) | Author |
|---|---|---|---|---|---|
| 1. | *B.velesensis* strain P3-1 | Soil | 0.015 | ND | Akaracharanya, Ancharida, et al., 2014 |
| 2. | *B.safensis* strain PJI-24S | Oil palm meal | 0.233 | ND | Khainngam, Saowapar, et al., 2014 |
| 3. | *B.methylotrophicus* strain R4C01101* | Bovine feces | 0.230 | 0.080 | Chantarasiri, Aiya et al., 2014 |
| 4. | *Bacillus subtilis* AS3 | Cow dung | 0.070 | 0.020 | Akhtar, Nadeem et al., 2013 |
| 5. | *B.licheniformis* JK7 | Rumen fluid | 0.750 | ND | Seo, J. K., et al., 2013 |
| 6. | *B.amyloliquefacien* SS35 | Dung sample | 0.080 | ND | Singh, Shuchi, et al., 2013 |

ND denoted “not determined”

### *2.3 Application*

**1) Pulp and Paper industry:** Biomodification of fibre properties with Cellulases and hemicellulases mixtures has also been used in paper mills to improve drainage and beat ability before or after pulp beating [Dienes, et al., 2004]. Cellulases are also used to make biodegradable cardboard, soft paper-like towels and sanitary paper and remove the stuck-on paper and pulp industries to increase production rates and improve mill performance [Singh, Shalini et al., 2016]. Deinking various types of paper waste are aided by Cellulases alone or combined with xylanases. The majority of applications proposed thus far involve partial hydrolysis of carbohydrate molecules by Cellulases and hemicellulases to release ink from the fibre [kuhad R.C. et al.,

2010]. Fewer or eliminated alkali usage, improved fibre brightness, improved strength qualities, higher pulp freeness and cleanliness, and reduced fine particles in the pulp are the key benefits of enzymatic deinking [kuhad, R.C. et al., 2010].

**2) Textile industry:** Hydrolases and, to a lesser extent, oxidoreductase is commonly used in the textile industry. Furthermore, Cellulases and hemicellulases are utilized in various pre-treatment and finishing processes for cotton [Mojsov, Kiro 2012]. Cellulase is a stone substitute that prevents harm to washing machines and clothing. It removes the need for spent stone disposal and enhances the wastewater quality. It also minimizes the effluent load by eliminating the need for many rinses to remove dust from the clothing [Doshi et al., 2001]. Acidic Cellulase from *T. reesei* and neutral cellulases from *Humicola insolens* are commonly used in the enzymatic washing of cotton fabrics.

**3) Wine and Brewery industry:** Cellulases, hemicellulases, and pectinases are used in winemaking to improve skin maceration and colour extraction, which is especially significant in the creation of red wine. Furthermore, it increases the wine's clarity, filtration, and general quality and stability [Chandrakant S. et al., 2011, M. Leisola et al., 2006]. Oksanen et al. found that the *Trichoderma* Cellulase system's endoglucanase two and exoglucanase 2 were responsible for the greatest reduction in the degree of polymerization and worth viscosity [Oksanen, j., j et al., 1985]. Using beta-glucosidases to hydrolyze glycosylated precursors into glucose and aglycone could improve the aroma of wine [Raveendran, S. et al., 2018]. Xue et al. [Xue, D.S. et al., 2018] isolated ethanol-tolerant endoglucanase from *A. niger* from a wine fermenter; the enzyme was stable at high temperatures and acidic pH.

**4) Detergent industry:** The combination of Cellulase, protease and lipase is a recent innovation [Singh, A. et al., 2007]. The cleansing capability of a detergent is increased by removing oil from interfiber space by selective contraction of fibers by alkaline Cellulase. Nowadays, liquid laundry detergents include anionic or nonionic surfactants, citric acid or a derivative thereof. Before adding the citric acid/salt to the mixture, it is necessary to add the diol and boric acid. The Cellulase's stability is improved by this order of addition [Boyer, et al., 1995].

**5) Food industry:** In the food industry, enzyme plays a critical role in achieving several of the above goals. Using Cellulase in the food industry improves the nutritional value of products. Cellulase is used with pectinase and hemicellulase in the food industry [Shiv Kumar 2015, Bhat 2000]. The macerating enzymes are made up of a combination of these three enzymes. In the extraction of olive oils, macerating enzymes play a significant role. Increased extraction, greater quality, more antioxidants, lesser risk of rancidity, and less wastage are all benefits of their natural state; turning them to juice or puree might extend their shelf life. Adding macerating enzymes to these procedures improves product quality and reduces browsing [Anoop Kumar, 2019].

**6) Feed industry:** The usage of Cellulase in the animal feed industry is another use. It can be used to improve the nutritional value of animal feed by pretreating grain feed and agricultural silage [kuhad, Ramesh 2011]. Cellulases also destroy anti-nutritional components like oligosaccharides, beta-glucan, pectin, lignin, inulin, dextrin, Cellulose and arabinoxylans, improving feed nutritiolue and animal health [Rastogi, Gurdeep 2010, Mori, Toshio 2014, Asmare, Bimrew 2014, Ejaz, Uroosa 2021]. During silage and fodder preservation, Cellulases, hemicellulase and pectinases can cause partial hnal vaydrolysis of plant cell walls. They are responsible for ruminant and monogastric animals' expression of selected genes for high feed conversion efficiency. These economically essential enzymes can create and preserve high-quality ruminant feed while enhancing grass silage quality [Ali, Simi 1995, Hall, Judith 1993, Selmer-Olsen 1993, Karmakar, Moumita 2011].

Table-4 Enzyme application and function in various industries

| No. | Industry | Application process | Function of enzyme | Author |
|---|---|---|---|---|
| 1. | Pulp and Paper | Thermo mechanical pulping | Softens wood chips | Skals, Peter, et al., 2008 |
| | | Deinking | Acts on recycled fibers & facilitates ink loosening | Skals, Peter, et al., 2008 |
| 2. | Detergent | Laundry washing | Removes stains from laundry | Nielsenet al., 2007 |
| 3. | Textile | Bio stoning of denim fabrics | Removal of excess dye mdenin | Galante, et al., 1998. &Mojsov, Kiro et al., 2012 |
| | | Production of high-quality and environmentally friendly washing powders | Fabrics soften the cotton fabrics without damaging the fiber | Galant, et al., 1998. & Mojsov, Kiro et al., 2012 |
| 4. | Feed | Ruminants feeding | Increase the rate and scope of fiber digestion by stimulating fiber digestion and digesting feeds | McAllister T. A. et al., 1999 & Murad, H. A.et al., 2010 |
| 5 | Food | Macerating enzyme complex (Cellulases, xylanases& Pectinases) | Increase the output of juices by extracting and clarifying fruit and vegetable juices | RameshChander Kubad, et al., 2011, Minussi et al., 2002, De Carvalho et al., 2008 |

Table 4: Shows that the Cellulase enzyme is used for various purposes in various industries.

According to a 2018 Global Cellulase Market Research Report, Asia-Pacific is the largest consumer of Cellulase, with roughly 32.84% revenue market share in 2016. Furthermore, in 2016, animal feed accounted for 29.71% of the Cellulase market demand, food and drinks accounted for 26.37%, and the textile industry accounted for 13.77%. According to the same report, Cellulase applications will reach 2300 million USD by the end of 2025, rising at a

compound annual growth rate (CAGR) of 5.5% from 2018 to 2025. These figures indicate that the use of Cellulases in industries is rapidly increasing [Jayasekara et al., 2019].

## Conclusion

It is now believed that enzyme-based methods for biomass conversions are the most successful, economic, and environmentally friendly after decades of research on the exploitation of lignocellulosic biomass. Although there has been a lot of progress in the quest for extremophiles, their entire diversity has not yet been thoroughly investigated. Future challenges for producing Cellulases include technologies for pretreating cellulosic biomass for a better microbial attack, methods for producing Cellulases economically, and finally, strategies for organism development to enhance the properties of enzymes to increase their specific activities, process tolerance, and thermal stability.

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