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# Cellulases Production and Application of Cellulases and Accessory Enzymes in Pulp and Paper Industry: A Review

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## Abstract:

Cellulases are produced from a variety of microorganisms; include both bacteria and fungi. Solid-state fermentation, liquid-state fermentation, and fed-batch fermentation techniques are utilized for the manufacture of cellulases. Cellulases have vast applications in almost every industry and are extensively used in fabric manufacturing. Enzymes have been used in the pulp for juice making, food processing, paper manufacturing, and pharmaceutical applications. The process of recycling in the paper industry had great importance and its value increased day by day in writing and printing papers. Many manufacturing services in the paper industry were integrated. Paper mills start with wood chipping at first, followed by pulping, bleaching, papermaking, and recycling of the past consumer products. Reduction of energy by cellulase enzyme and chemicals has been used to improve the quality of the paper and help to decrease the environmental influence of pulp production. In the near future, the need for cellulases will be strongly recommended for the commercial production of biofuels and bioenergy.

**Keywords:** Pulp, paper, cellulose, fungi, bacteria, bleaching.

## INTRODUCTION

Cellulase is a homo-polysaccharide which has beta 1-4 glycosidic linkage while cellulase complex along with synergistic action of its three enzymes brings about the hydrolysis of cellulose into monomeric units of glucose (Bhat, 2000). Cellulase complex is a complex of three enzymes and utilized in many industries for hydrolysis of cellulosic waste material of crops into glucose units for biofuel production (Mahmood *et al.*, 2013). Cellulases act on cellulose polysaccharides and break down the fiber and Alpha Amylases act on starch and improve its digestibility (Imran *et al.*, 2016). Microorganisms like bacteria and fungi are used for the production of cellulases and they may be aerobic, anaerobic, thermophilic or mesophilic. A wide diversity of microorganisms is utilized for decomposing cellulosic waste materials into many useful products (Jungebloud *et al.*, 2007). Species of *Trichoderma*, *Aspergillus*, *Fusarium*, and *Penicillium* are mostly used in cellulase production for different purposes (Iqbal *et al.*, 2011). Desired fungal species selections depend upon many factors like the type of cellulase production, fungal optimizing conditions, substrate and characterization (Malik *et al.*, 2010). Bacterial cellulases are complex than fungal cellulases. Bacterial cellulases now become the principal bio-crystals because of very multifaceted nature and wide range of industrial uses. Fungal cellulases typically have two separate domains; one is a cellulose-binding segment that connects by small polylinker regions to the catalytic domain at the terminal end which is second catalytic domain. Basically, the main difference between free cellulose enzyme and cellulosomes is that components of cellulosomes cohesion contain scaffolding and dockerin enzyme. Cellulase complex is a group of three enzymes, endoglucanase,

exoglucanase and the third one is beta-glucosidase. Endoglucanase randomly attacks the o-glycosidic bonds resulting in glycan chain of different length. The beta-glucosidase act on beta cellobiose disaccharide and produces glucose. The exoglucanase acts on the cellulose chain and releases beta cellobiose as the product (Kuhad *et al.*, 2011). Cellulases have major commercial and industrial uses. It is used in the processing of food, animal feed preparation, soap and detergent formation, pulp and paper industry and in the fabric industry. It has also been widely used in beverages industries to produce wine, beer, and different juices. The paper and pulp industry are the most important part of the Global economy. Lignocellulosic wastes are also used to produce ethanol which is an important laboratory solvent and has different industrial applications (Philippidis, 1994; Sukumaran *et al.*, 2005).

## Cellulases Production

Largest industrial enzymes had been used in various areas of life since 1995. Co-culture of *F. oxysporum* and *A. niger* utilize industrial and forest wastes to produce cellulases and xylanases. Cheap raw materials and improved bioprocess technologies are used for the production of cellulases (Reeta *et al.*, 2017). Group of cellulases and hemicellulases are utilized in various industries like; food, fuel, brewery, chemical wine, textile, agriculture, and fuel (Asghar *et al.*, 2013). This specifies its position as the most important industrial and commercial enzymes in the market (Yoon *et al.*, 2014). Fed-batch fermentation was used to improve the production of cellulase by *Trichoderma reesei*. Fed-batch fermentation had 2.11 times higher cellulase activity than batch fermentation. The cellulase from *T. reesei* trans formant C<sub>10</sub> with better cellobiohydrolase activity was used to the

enzymatic hydrolysis of the alkaline pretreated corn stover and rice straw. *T. reesei* ZU-02 showed enhanced yield in the enzymatic hydrolysis (C 10 cellulase) of both corn stover and rice straw. C10 cellulase and xylanase were used in the enzymatic hydrolysis of alkaline pretreated corn stover (20 FP IU/g substrate) and produced a high yield of 93.8% (Fang and Xia, 2015). *F. oxysporum* F8 and *A. niger* F7 co-culture were used in solid-state fermentation for the production of xylanase and cellulase (Kaushal *et al.*, 2012). *F. oxysporum* isolated from *Baccharis dracunculifolia* was used to produce cellulase. Sugarcane bagasse was pretreated to remove all the sugar contents and then *F. oxysporum* inoculated culture was incubated for 96 days at 28 °C for cellulase production. Dinitrosalicylic acid reagent accompanied by the indirect spectrophotometric method was used to quantify enzyme. The maximum yield of cellulase ( $55.21 \pm 10.54$  IU/g) was obtained at pH 5.96 after 55 days of a fermentation process using *F. oxysporum* (Onofre *et al.*, 2013). Sugarcane press mud was used as a lignocellulosic substance to produce cellulase by *Pleurotus sajor-caju* under solid-state fermentation. The maximum yield was obtained at 25°C, pH 5, pretreatment with NaOH + Hydrogen peroxide, press mud thickness 0.8 cm and 10 days of the incubation period (Pandit and Maheshwari, 2012). The study revealed *A. niger* has the capability of producing cellulase and pectinase using corn cobs under SSF.

Orange peel, a waste substrate was used for cellulase production using *Trichoderma viride* through solid-state fermentation under optimum conditions of 96 hours of incubation, 35°C, pH 5, producing maximum exoglucanase ( $412 \pm 4.3$  U/mL), endoglucanase ( $655 \pm 5.5$  U/mL) and  $\beta$ -glucosidase ( $515 \pm 3.5$  U/mL). Endoglucanase with 5.5-fold purification, 498 U/mg enzyme activity and molecular weight of 58kD

comparing to the crude enzyme was purified by SDS-PAGE and ammonium sulfate fractionation. This purified enzyme had long storage ability i.e. up to 45 days at 30°C (Irshad *et al.*, 2012; Narra *et al.*, 2012). Plackett and Burman design were used for the optimization of cellulase enzyme using a central composite rotary design (CCD). Higher initial moisture content for SSF showed a negative or inhibiting effect on cellulase production, employing lower moisture content for maximum yield. The maximum cellulase yield was raised up to 6.2 folds by a low level of moisture content and by adjusting the incubation period by CCD strategy (Damisa *et al.*, 2011). Ryu and Mandels (1980) specified that compounds of carbon obtained by enzymatic hydrolysis of cellulose could be utilized as fermentation and chemical feed stock. For this purpose, *T. reesei* and its mutants were a good source of cellulase for hands-on saccharification. The response surface methodology was a statistical tool of optimization and used for the optimization of cellulase production from different fungal strains (Zhang *et al.*, 2012; Imran *et al.*, 2017).

## Paper and pulp Industry

Paper and pulp are the biggest industries in the world. Total paper production in the world was an estimated 403 million tons in 2013. The major aspects of paper and pulp making processes were presented in most unique areas to uplift the status of the economy of any country (Bajpai, 2015). The grinding and refining of agro-industrial wood by special mechanical forces led the pulp to a high degree of acceptable stiffness and bulk quantity. Biomechanical grinding and refining using cellulases and hemicellulases is more economical and saved energies around 20-40% and provided more strength to paper sheets (Bayer *et al.*, 2004). Cellulases and hemicellulases were used in paper and pulp

industry to improve drainage aeration ability in different paper mills of the world (Dienes *et al.*, 2004). A profitable cellulase preparation on different fractions of Douglas fir Kraft pulp, cellulase treatment helped in reducing the fiber coarseness (Mansfield *et al.*, 1996). On the other hand, endoglucanases reduced the viscosity with a lower degree of hydrolysis (Pere *et al.*, 1995). Cellulases used in combination with xylanases or alone are beneficial for deinking of different types of

paper wastes (Kuhad *et al.*, 2010). The main advantages of enzymatic deinking were; reduced alkali usage, improved fiber brightness, enhanced strength properties, increased pulp freeness, cleanliness, and reduced fine particles in the pulp (Kuhad *et al.*, 2010). The history of paper and pulp industries is shown in Table 1.

**Table 1.** History of pulp and paper industry.

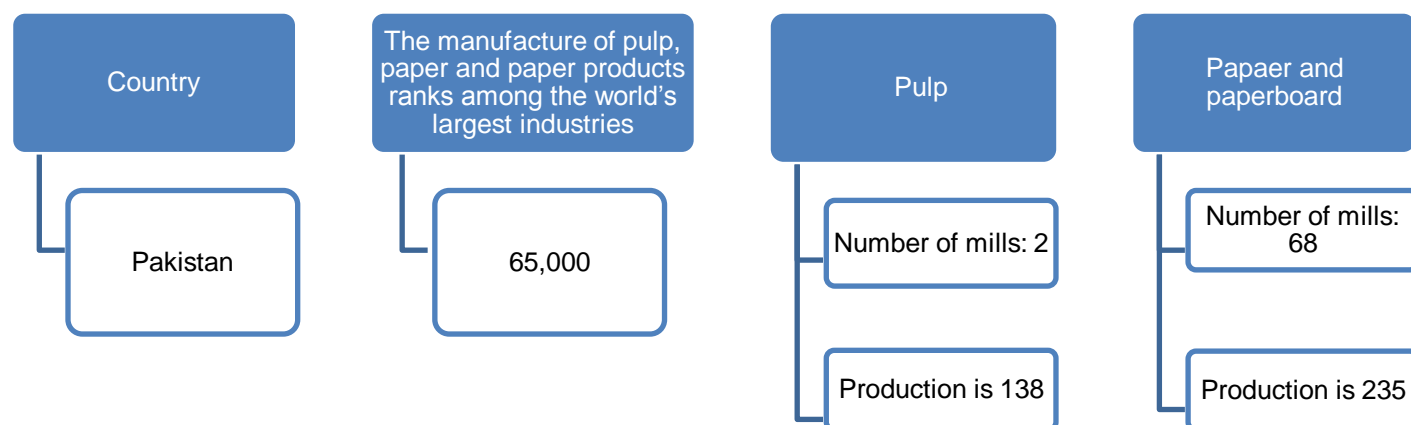
Country	History of pulp and paper	Reference
Egypt	Paper made up from cellulose. In Egypt in 3000 BC, people discovered tall reeds growing along the river of Nile. Egyptians took soft fibrous part, beat, and soaked them for making papyrus. Tall papyrus reeds grew only in a warm environment. Tall papyrus reeds grew in Egypt about four millennia which were thick enough for making writing material.	(Philippidis, 1994; Pelach <i>et al.</i> , 2003)
China	Real paper made from the pulp was recorded in China. Chinese used different raw materials like a bamboo tree, tree bark, and fishnet. For preventing from insects, Chinese coated it with dye and then this technique spread across many countries. In the 15 <sup>th</sup> and 16 <sup>th</sup> centuries, paper mills spread across Europe.	(Philippidis, 1994)
Greek and Romans	Around 800 B.C, Greeks and Romans used waxed tablets as a writing material.	(Ansari, 1990)
Europe	Europe started paper making techniques at the end of the 15 <sup>th</sup> century. In the 19 <sup>th</sup> century, wood was used as raw material for paper production.	(Pelach <i>et al.</i> , 2003; Brady, 2001)
India	Leaves of the palm tree were used for writing in ancient South-East Asia and India. Leaves were first boiled then dried to use as a paper.	(Ansari, 1990; Pelach <i>et al.</i> , 2003)
Pakistan	In Past, barks of Himalayan trees were used as material for writing in the northern part of Pakistan. In Indus Valley, copper plates used for writing material almost 5,000 years ago.	(Ansari, 1990)

## Economic Importance

The paper and pulp mills were found all over the world. Manufacturing of paper, pulp products included the world's biggest industries and these industries were providing a source of employment to 3.5 million people (Pelach *et al.*, 2003; Adrio and Demain, 2014).

## Status of pulp and paper industry in Pakistan

The first mill was established in 1971 and it started production in 1972. Mill imported 151-ton long fiber pulp, 5,000 tones straw pulp (Figure 1) and 20,000-25000 tons bagasse for the production of various grades of paper (Ansari, 1990). Paper mills were existing in more than 100 countries in the world and provided employment for the 3.5 million people (Ansari, 1990).



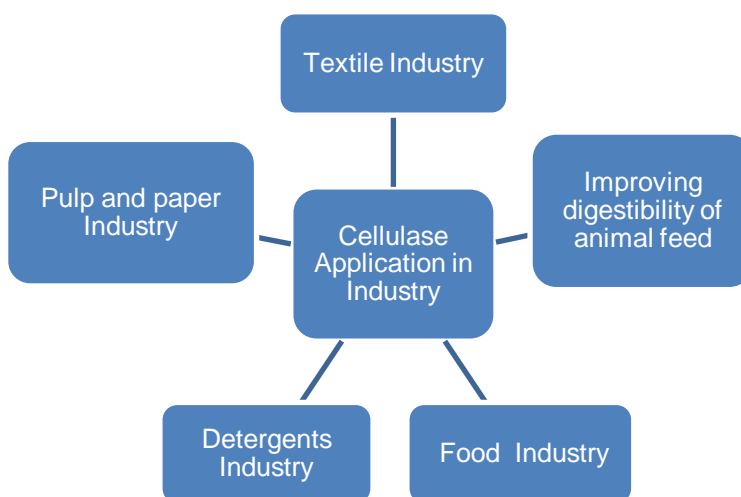
**Fig. 1.** Pulp and paper industries in Pakistan.

## Paper and Pulp Process

### Bleaching

To improve paper properties and texture, bleaching employed to decolorize the colored compounds that might affect the aesthetic quality of the paper. Bleaching is defined as a process in which heavy amounts of toxic chemicals like chlorine are used. These compounds proved harmful and might accumulate in biological systems and caused serious mutagenic disorders. To avoid chemical poisoning, chlorine-free methods could be adopted which might include oxygen delignification,

prolonged cooking, and replacement of chlorine oxides, hydrogen peroxides, and ozone. Alternatively, biological enzymes including cellulase and xylanase could be used to improve the paper and pulp quality (Figure 2). The commercial use of biological enzymes in the paper industry was a big leap towards environment protection. The use of additional decolorizing chemicals could be minimized by the application of enzymes which in turn was a modest way to avoid climate changes and hazardous environmental disorders (Jeffries, 1992; Imran *et al.*, 2016).



**Fig. 2.** Application of cellulase in paper and pulp industry.

## Hemicellulases Enzymes

These enzymes had excellent decolorizing properties. Hemi cellulolytic and xylanase enzymes had been extensively used for quality enhancement. During a period of 1988 to 1991, the experts had developed a single-step method for xylanase pre-bleaching and considered to be the most suitable way to fulfill biological needs. This can be the economical and environment friendly method to overcome the pressure imposed by the environment regulating authorities using harmful decolorized chemical compounds in western countries (Schulein *et al.*, 1998). In Canada and Northern Europe, several industries used to employ a totally chlorine-free process (TCF) much lower limits on absorbable organohalogens (AOX) level. In North American mills, enzymatic bleaching was carried out permanently. Dissimilar paper products, tissue papers as well as magazine paper mass-produced from enzymatically preserved pulps had been successfully produced (Jeffries, 1992).

## Hemicellulases origin

Organic bleaching idea with xylanase enzyme was developed to selectively eliminate the hemicellulose from biochemical mashes to harvest the cellulose acetate for papermaking (Pelach *et al.*, 2003).

## Role of cellulase in paper de-inking

The process of de-inking began with the breakdown of paper materials which was also known as recycling. For this purpose, steps were involved by the addition of different chemicals in the alkaline medium in demand to support the ink particles free and de-fibrin. Consequently, technologies of washing were used in mild alkaline media that helped to remove ink from suspension (Moerbak and Zimmermann, 1998). For the recovery of wastepaper de-inking and many secondary fibers, cellulases and hemicellulases were used. For different types of newsprints and office waste papers, hemicellulases and cellulases could be utilized. Brightness and ink subtraction efficiency reported in several cases and



were more reliable to the conventional de-inking procedure. Those papers printed by vegetable oils ink-based must be followed the de-inking procedure which included a type of neutral de-inking combination with xylanases, lipases, and cellulases (Bajpai, 1999).

### **Caustic Replacement**

To improve the paper potential and swelled fibers, caustics are used in pulp recycling. Cellulase enzyme could be used as a substitute or in conjunction for swelled caustic fiber. Neutral de-inking also had some advantages like less COD at the lower cost (Buzby and Evans, 1992).

### **Fiber Modification**

Enzymatic fiber modification has altered the thermo-mechanical processes for pulp production. Fiber structural changes synthesized during whipping, purifying, fiber swelling and fibrillation which provided the flexibility of fiber and that had bonding ability. The xylanases extracted from *Dimorphosporum sporotrichum* showed reasonable decolorized properties. Using an electron microscope, external fibrillation could be visualized. Water retaining value that defined the swelling of fiber noticeably increased. Process of enzymatic finishing of cotton fabric had been presented based on the T-EG and used cellulases for denim washing to get softness for cotton fabrics and denims (Anish *et al.*, 2007). Using the paper machine, the elimination of water like the outcome of incomplete hydrolysis of the fibers in the reprocessed paper was carried out. A mixture of xylanases and cellulases at a very short concentration found to increase liberation of the recycled fibers which reduced yield. Hemi-cellulases

and cellulases enzymes found to improve desiccation. Unsuitably, crude enzyme mixtures also reduced the strength properties. Mill tribunals accepted successfully using Pergalase A40. Selective eliminating on the surface mechanism, water concentration condensed, and drainage increased without clear alterations in the final quality of pulp. If large amounts of unpolished enzymes used, then the length of normal fiber reduced, and strength properties of fibers lost. It also described that drain ability of pulp might be enhanced by hemicellulases addition. According to the authors, xylanases improved desiccation of de-inked recycling pulp and has no damaging effect on fiber tensile properties. This study suggested that xylanases were much more active than cellulases. Increase in viscosity was shown due to treatment of pulps with xylanases enzymes (Bajpai, 2010). Results of cellulases obtained from the fungus *Acrophialophora nainiana* and *Penicillium echinulatum* in finishing of knitted cotton fabrics found comparable with enzymes obtained from *Trichoderma reesei*. *P. echinulatum* cellulases had considerable influence on cellulose textile processing results (Rau *et al.*, 2008). Cellulases used for denim finishing and synthetic fibers were the recent commercial advances. Recent advances in enzymes production techniques gave a better way to use enzymes in the textile industry (Araujo *et al.*, 2008).

### **Controlling Stickies in pulp and papermaking**

In pulping and papermaking, machinery was used for stickies accumulation and papermaking processes and produced a final paper. Recycled paper material was controlled by the addition of organo-titanium (IV)

that was a water-soluble compound for pulping and papermaking processes. It was also effective to reduce the deposition of stickies on machinery paper (Buzby and Evans, 1992). Many interesting enzymatic applications had been proposed to control stickies and had also been controlled by enzyme cellulase, so enzymes had great potential in solving a lot of problems like recycling fibers, de-inking, hornification, and refining. The potential of cellulase enzyme had also been demonstrated for lowering the energy needs in pulping, and stickies control when scientists used recycled fiber (Buzby and Evans, 1992; Jeffries, 1992).

### **Pitch in the paper industry**

The sticky materials that caused pitch trouble like wood in paper and pulp industry were extracted from wood. Traditional techniques of monitoring pitch problems had seasoning of the wood before development. Thus, this technique was frequently intolerable especially in those areas where space was partial. So, cellulases were used to resolve this problem (Roberta *et al.*, 2006; Trivedy and Pathak, 2015).

### **Removal of fines**

On the surface of fibers, some fines and peel of fibrils were damaged due to enzymatic treatments that dissolved the colloidal materials and caused a serious drainage problem in paper mills. But the extreme use of enzymes should be avoided (Kuhad *et al.*, 2011).

### **Using enzymes making pulp**

Treatment of bio-pulping of the wood chips with lignin damaging fungi was used to the mechanical

treating wood for pulping which enhanced the efficiency of the chemicals through wood chips. The microorganisms were genetically engineered and enzymes that were used to transfer many environmental opposing practices were utilized in the pulp industry. A fungus-like *Ophiostoma piliferum* was used in the fermentation process to damage the lignin. It decreased the energy requirements for the chemicals for the chemical pulping (Ansari, 1990).

### **The performance of paper mills and bio-improvement of drainage properties**

Many wood components like pitch, hemicellulose, and lignin dissolved and released into the drainage. These components were collectively known as colloidal and dissolved substances. During mechanical pulp peroxide bleaching, other components like pectin were released. All these components caused severe difficulties in paper mills including pitch accumulation, paper speck, and reduced de-watering. Cellulases improved the overall performance and paper mills quality (Yoon *et al.*, 2014).

### **Treatment of cellulase to reduce vessel picking**

The tropical wood was used for pulp manufacture because trees were rapidly grown and inexpensive. Tropical hardwood contained vessel elements which were large hard and did not fibrillate. Presence of vessels reduced the value of pulps. Cellulases increased the elasticity of vessels (Bhat, 2000).

### **CONFLICT OF INTEREST**

There is no conflict of interest.



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