*MAYFEB Journal of Environmental Science*
*Vol 1 (2016) - Pages 30-43*

# The Enzymatic Activities, Characterization, Properties and Applications of Cellulase

**K. Odelade**
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria, kennybramm@gmail.com
**O. Oladeji**
Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria, oladeji_oluwole@yahoo.com
**S. Aremu**
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria
**M. Adisa**
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Nigeria

***Abstract***

**Cellulosic waste may be converted to products of commercial interest such as glucose, soluble sugars, enzymes, alcohol, and single cell proteins and the key element in the saccharification process of lignocellulosics to these industrially useful products relies on participation of cellulolytic organisms and their cellulase enzymes. The production of cellulase enzyme is a major factor in the hydrolysis of cellulosic materials but it is usually produced in small quantities by the parent organisms. The spectacular successful examples of strain improvement in industry are mostly attributed to the extensive application of mutation and selection. Such improved strains can reduce the cost of the processes with increased productivity and may also possess some specialized desirable characteristics. Hence, there is need to study the production and application of cellulase to man and his environment.**

**Keywords: cellulosic waste, saccharification, cellulolytic, cellulase, fermentable sugar, bioethanol.**

## I. INTRODUCTION

The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose being the most abundant component. Plant biomass comprises an average of 23% lignin, 40% cellulose and 33% hemicelluloses by dry weight [1]. Cellulose is the most abundant renewable natural resource in the world and a potential source for the production of industrial useful materials such as fuels and chemical [2]. Its annual biosynthesis by both land plants and marine algae occurs at a rate of $0.85 \times 10^{11}$tonnes per annum [3]. Also, Lignocellulosic wastes are the largest group of wastes present on this plant causing environmental pollution [4]. It is estimated that the photosynthetic process produced 1.5 x 10 ton (150 billion tons) of dry material annually with respect to carbon of which about 50% is cellulose [5]. Cellulose, which is the most abundant renewable resource, is a polysaccharide composed of β-D-glucopyranosyl units joined by 1,4-glycosidic bonds. Because cellulose can be utilized to produce ethanol, it is a promising alternative energy source for the production of fossil fuels. Cellulose is degraded by cellulases to reducing sugars and fermented by yeast or bacteria to ethanol [6].

Important distinguishing features of cellulose biomass among potential feeds for biological processing include low purchasable price, potential for supply on very large scale, recalcitrance to reaction and heterogeneous composition. Cellulosic waste may be converted to products of commercial interest such as glucose, soluble sugars, alcohol, and single cell proteins. The key element in bioconversion process of lignocellulosics to these useful products is by some cellulosic microorganisms and their hydrolytic enzymes mainly cellulases.

Many microorganisms have been evaluated for the production of cellulase including bacteria such as *Pseudomonas fluorescens, Bacillus subtilis, Escherichia coli, Serratia marcescens*. Filamentous fungi have been used for more than 50 years in the production of industrial enzymes [7]. Many fungal strains secrete higher amounts of cellulases than bacterial ones. Cellulases from *Trichoderma* and *Aspergillus* species have been investigated in detail over the past few decades [8]. *Aspergillus sp* is an important commercial source of cellulases for food textile and pharmaceuticals industries [9]. In Pakistan, many cellulosic residues are produced to as much as 50 million tones every year [10] and could be utilized for bulk production of cellulases. Production of cellulases by fungi in SSF using agricultural wastes has been reported [11]. The lignocellulosic biomass, especially agricultural wastes, is known to be an excellent carbon sources for microbial enzyme production [12]. The utilization of cheaper and indigenous substrate for cellulase production has contributed somewhat to economical recovery [13], Various agricultural substrates, by products and microbial cultures have been used successfully in solid state fermentation for cellulase production [14]. *Aspergillus genus* is known to be a good producer of cellulases. In recent years, the interest in cellulases has increased due to many potential applications, for example, in the production of bio-energy and bio-fuel, in the textile industry and pulp and paper industry [15]. However, improvements in cellulase performance have been incremental, and no drastic activity enhancement has been reported to date. Therefore, further improvement on cellulase performance needs the better understanding of cellulose mechanisms as well as the relationship of cellulase molecular structure, function, and substrate characteristics.

### *1.1 Submerged State Fermentation*

Submerged State Fermentation (SmF) involves the production of enzymes by microorganisms in a liquid nutrient media. Fungal cultures adopt different growth patterns when cultivated in liquid and solid substrates. In liquid environment, fungi grow as pellets or free mycelia, depending on the genotype of the strain and culture conditions [16]. Submerged fermentation (SmF) systems can be defined as the cultivation of microorganisms in a liquid medium containing soluble carbon source and nutrients, maintained or not under agitation.

Solid state fermentation (SSF) holds great potential for the manufacture of enzymes. SSF has many returns over other fermentation. Processes in a sense that the culture media are simpler and many solid media upon being supplied with appropriate nutrients can be utilized directly as growth media [17]**.** Moreover the product of interest is obtained in a much higher concentration, which makes the purification process easier and cost effective. Contamination risks are also greatly decreased due to the minimal moisture content in the scheme. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source. A vast range of microbes, for instance bacteria, yeast and fungi make diverse types of enzymes, among which hydrolytic enzymes (cellulases, xylanases, pectinases, etc.) are secreted by fungal cultures because these enzymes are a part of their growth and metabolic reactions [18]. Solid substrates make a perfect supporting and nourishing environment to the microbial flora including bacteria, yeast and fungi. Filamentous fungi in particular are the best studied in relation to SSF because of their hyphal growth. These have the ability to grow on the surface as well as into the substrate particles. Aspergillus species have been experimentally proved to be very efficient in the production of cellulases, and many scientists have described cellulase synthesis by various species of Aspergillus [19].

### *1.2 LIGNOCELLULOSIC MATERIALS*

Lignocellulosic materials are the most abundant organic compounds in the biosphere, representing 50% of terrestrial biomass which corresponds mainly by agribusiness materials, the urban waste, and the wood of angiosperms and gymnosperms. The lignocellulosic biomass is composed of three main polymer fractions: lignin, hemicellulose and cellulose, which are joined to each other by covalent bonds, forming a complex network resistant to microbial attacks [20]. The cellulose from natural materials is the world's most abundant biopolymer that is formed by residues of β-D-glucose bound together by β-1,4, bonds, and it maintains a linear and flat structure; cellulose figure 1, [21]. The disaccharide 4-O-β-D-glucopyranosyl-D-glucopyranose, is the repeating unit of the polymer that can be hydrolyzed to glucose with the help of acids [22].

Agro-industrial waste materials are rich in lignocellulosic materials that are inevitably produced by agricultural and industrial activities. The following can be cited as being among the lignocellulosic residues produced in bulk by the Brazilian agroindustrial activity: sugarcane bark, bagasse and straw, orange peel, rice straw and rice bran, corn cobs and straw, chaff and bran from wheat, banana straw, cotton waste, wood scraps, and waste based on paper. Most of these materials are either partly or entirely not taken, being transformed into pollutants from the environment. Agricultural wastes contain 20–60% cellulose, 20–30% hemicellulose, and 15–30% lignin. The available quantity of these materials in the world is very large [23]. Considering renewable resources, residues have a low cost as raw material for other processes and they can be purchased in regions that are located close to the local processing of the material. Due to the difficulty experienced by cellulose degradation in environmental conditions, cellulosic wastes accumulate, which makes them a nuisance to the environment [24].The use of lignocellulosic biomass derived from agriculture waste, forestry waste, and sewage can bring savings to the production of fuels and other products as well as reduce waste [25].

### 1.3 ENZYMES AND CELLULASES

#### *1.3.1 Enzymes*

Enzymes are proteins that exhibit catalytic activity. The enzyme complex molecular structure consists of one part of protein, but it can be connected to other molecules such as carbohydrates and lipids [26]. Enzymes are present in all living cells, which exercise the function of catalyst of the reactions that compose the anabolic and catabolic pathways of cellular metabolism [21].

The great interest in the use of enzymes can be explained by several factors, including the large variety of reagents in which the same act, the complex reactions which the enzymes are capable of catalyzing on routes where the generation of waste and by-products is reduced, and that they have the capacity to operate as catalysts at high speeds in conditions of reduced energy needs (mild conditions of pressure and temperature). The catalytic action of enzymes involves the delivery of a specific environment of the enzyme where the enzyme reaction is energetically more favorable, and this region where the reaction is called active site. The molecule that binds to the active site and acts on the enzyme is called the substrate. In general, the substrate binding site is a slot or groove on the surface of an enzyme, complementary to the shape of the substrates (geometric complementarily). In addition, the amino-acid residues that form the binding site are arranged to form specific interactions of attraction with the substrate (electronic complementarily). The reaction becomes more favorable, because the interaction between the amino-acid residues and the reactant molecules decreases the activation energy that is required for the rearrangement of covalent bonds and the performance of non-covalent interaction between the enzyme and the substrate [27].

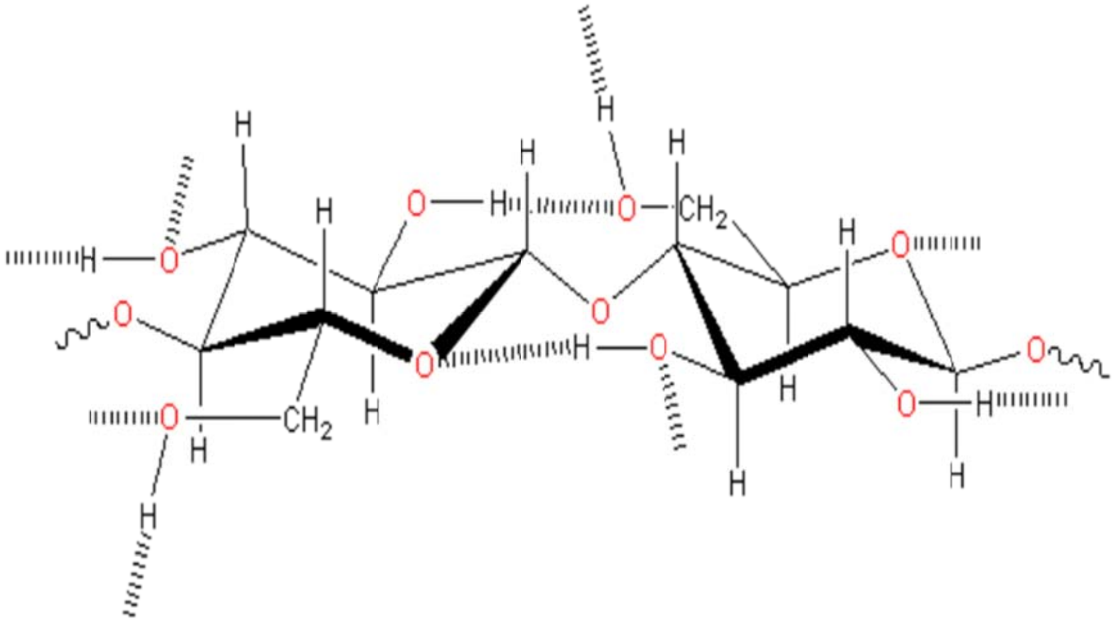

**Fig 1** Chemical structure of cellulose [21]

### *1.3.2a Endoglucanases*

Endoglucanases are also known as endo-β-1, 4 glucanase and carboxymethylcellulase. They catalyze the hydrolysis of internal bonds β-1, 4-D-glucosides of cellulose, randomly generating oligosaccharides of various sizes and, consequently, new chain terminals. Cellulose and xiloglicana serve as their natural substrate. They act only in the amorphous portion of cellulose, and their activity decreases along with shortening the length of the cellulose chain [28].

### 1.3.2b *Exoglucanases*

They act in a progressive way in reducers and nonreducer portions of the cellulose chains, liberating either glucose (glucanohidrolases) or cellobiose (celobiohidrolases) as the main products. They act on microcrystalline cellulose, thereby shortening the polysaccharide chains and have a limited effect on substrates such as carboxymethylcellulose (CMC) and hydroxyethylcellulose (HEC) [29].

### *1.3.3c β-glucosidase*

This is necessary to hydrolyze short-chain oligosaccharides and soluble cellobiose into glucose, and can also be called β-D-glucoside glucohidrolase. It loses activity with increasing the length of the cellulose chain and also performs the hydrolysis of terminal β-D-glucose oligosaccharides. When working together, the complex cellulolytic enzymes have a better yield than the sum of the individual income, that is, when acting in isolation from each other [30].

## 1.4 CELLULASES

To meet the challenge associated with degrading cellulose, cellulolytic microorganisms (table 1) produce a complex mixture of enzymes called cellulases. These enzymes, which collectively have links to specific β-1,4 cellulose, are necessary for the complete solubilization of cellulose, existing synergism between that [21]. The complex cellulolytic enzymes are hydrolases that cleave O-glycosidic bonds and are classified by the Enzyme Commission (EC) with the coding 3.2.1.x, where the value of x varies with the cellulase evaluated. The classification of cellulases, according to their site of action in the cellulosic substrate, allows them to be categorized into three groups: endoglucanases (EnG), which cleave internal cellulosic fiber bonds; exoglucanases (ExG) or celobiohidrolases (BNG), which work in the external region of the cellulose; and β-glucosidases (BG), which hydrolyze soluble glucose oligosaccharides [24]. Figure 2, illustrates the synergistic action between exoglucanases, endoglucanases, and β-glucosidases in the hydrolysis of cellulose fiber.

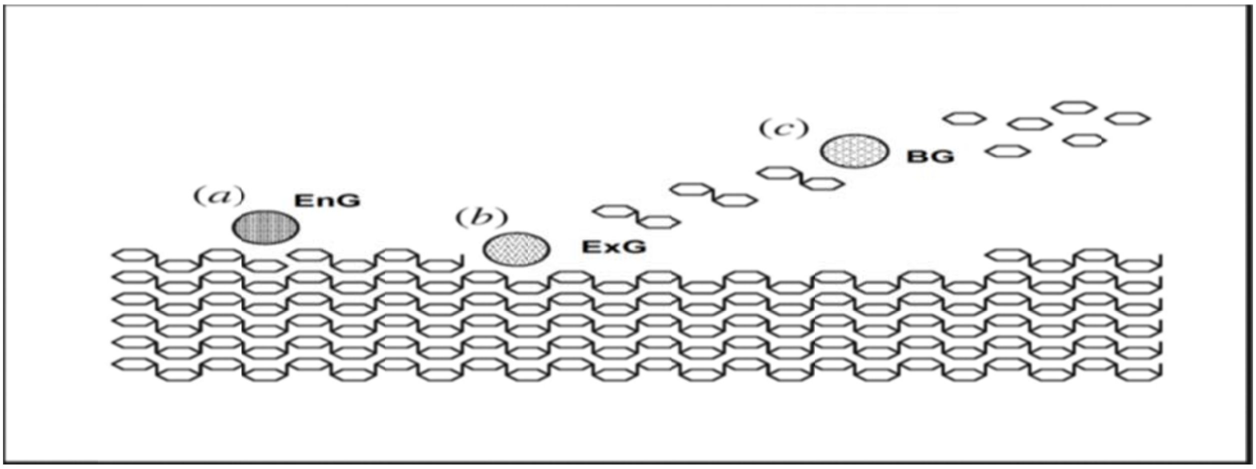

**Fig 2** Mode of action of enzymes of the cellulolytic complex [28](Lynd *et al.*, 2002).

Cellulase is considered as a huge reservoir of renewable energy source [31]. Plant wastes are chiefly composed of cellulosic and lignocellulosic materials, which are biodegradable polymers and can be converted into a vast range of valuable products [32]. Cellulase is a complex enzyme whose basic function is the conversion of complex carbohydrates which are present in lignocellulosic biomass efficiently into glucose monomers [33]. It also breaks down cellulose into other oligosaccharide compounds beside glucose monomers and the enzymatic hydrolysis of celluloses is a very effective alternative method for the generation of sugars [34], which claims cellulases extremely important from the commercial as well as industrial point of view.

### *1.4.1 Types of Cellulase and Action of Cellulase*

There are five general types of cellulases based on the type of reaction catalyzed [35];

(i). Endocellulases (Ec 3.2.l.4) randomly cleave internal bonds at amorphous sites that create new chain ends

(ii). Exocellulases or Cellobiohydrolases (3.2.l.9l) cleave two or four units from the ends of the exposed chains produced by endocellulase resulting in tetra saccharide or disaccharides such as cellobiose

(iii). Exocellulases are further classified into type l, that work processively from the reducing end of the cellulose chain and type II, that work processively from the non-reducing end

(iv). Cello-biases (Ec 3.2.l.2l) or beta-glucosidases hydrolyse the exocellulases product into individual monosaccharides

(v). Oxidative cellulases depolymerize cellulose by radical reactions as for instance cellobiose dehydrogenase (acceptor)

(vi). Cellulose phosphorylases depolymerize cellulose using phosphate instead of water. Avicelase has almost exclusively exo-cellulase activity, since avicel is a highly microcrystalline substrate

Within the above types, there are also progressive (also known as processive) and non-progressive types. Progressive cellulase will continue to interact with a single polysaccharide strand, nonprogressive cellulase will interact once they disengage and engage another polysaccharide strand [36].

Cellulase action is considered to be synergistic as all three classes of cellulase can yield much more sugar than the addition of all three desperately. Aside from ruminants, most animals (including humans) do not produce cellulase in their bodies and can only partially break down cellulose through fermentation, limiting their ability to use energy in fibrous plant material [37].

**TABLE 1** Modular Architectures of Cellulases from different Bacteria

| Organism | Modular Architecture | Modular bank |
|---|---|---|
| *Anaerocellum thermophilum* | GH9-(CBM3)$_3$-GH48 | ACM60955 |
| *A. thermophilum* | GH9-(CBM3)$_3$-GH5 | ACM60953 |
| *Bacillus subtilis* | GH5-CBM3 | CAA82317 |
| *Clostridium phytofermentans* | GH48-Ig-CBM3 | ABX43721 |
| *C. phytofermentans* | GH9-CBM3-(Ig)$_2$-CBM3 | ABX43720 |
| *Clostridium thermocellum* | GH48-(Doc)$_2$ | AAA23226 |
| *C. thermocellum* | GH26-GH5-CBM11-(Doc)$_2$ | AAA23225 |
| *Clostridium cellulolyticum* | GH48-Doc | ACL75108 |

GH, glycoside hydrolase; CBM, carbohydrate-binding module; Ig, immunoglobulin-like domain; Doc, dockerin; Fn, fibronectin-like domain.

### *1.4.2 Structure of Cellulase*

Most fungal cellulases are a two-domain structure, with one catalytic domain and one cellulose binding domain that are connected by a flexible linker. This structure is adapted for working on an insoluble substrate and it allows the enzyme to diffuse two dimensionally on a surface in a caterpillar like fashion. However, there are also cellulases (mostly endoglucanases) that lack cellulose binding domains. These enzymes might have a swelling function [35].

### *1.4.3 Characterization and Properties of Cellulases*

With the aim of using cellulases in industrial processes under the conditions of their best performance, it is essential that their properties are determined, especially with regard to kinetic factors and physical chemists [24]. Another property of cellulolytic enzymes commonly reported in the literature is their ability to be influenced by other molecules, especially metals, and this characteristic is suffering from inhibitory or inductive effects at the moment. Among the ions studied, the ones that more often inhibit cellulases are those of mercury, copper, silver, and zinc ($Hg^{2+}$, $Cu^{2+}$, $Ag^{+}$, and $Zn^{2+}$), which even lead to the total loss of catalytic activity, and are present at concentrations as low as 2.0 mM. For the characterization of crude cellulase preparations with regard to their activity, different substrates of endoglucanases are used, stressing, however, that the synergy between the two types of enzyme prevents a precise quantification [21]. A substituted cellulose derivative such as carboxymethyl cellulose (CMC), which is soluble, is used as a substrate for endoglucanase activity.

The enzyme attacks the polymer in a random mode, producing a rapid change in the degree of polymerization. After the enzymatic reaction, the formation of reducer sugars is determined, which is known as CMCase activity [21].The Microcrystalline cellulose is one of the substrates used in the tests for measuring the activity of exoglucanases. The measurement of enzyme activity is commonly used as a reference for determining the activity in both cellulosic global academic work and for commercial enzyme preparations [38].

## 1.5 APPLICATION OF CELLULASES

Cellulases have been investigated mainly with regard to their potential as an additive in the detergent industry, textile industry, and also in the bioconversion of agricultural biomass into products with commercial value (table 2) [21]. In the food industry, cellulases are used in maceration processes, usually along with hemicellulases and pectinases, such as the extraction of fruit juice and oil seeds. They also have an important role in the filtration and clarification of fruit juices, which increases the effectiveness of the extraction of color and juices in the liquefaction of plant tissue, thereby allowing for a better extraction of pigments from fruits Cellulases have great potential use in the production of glucose syrups from cellulosic materials that compete with starch and sucrose in the production of alternative sweeteners which are used in food and beverage industries [39].

Recent development on biochemistry, genetics and protein as well as on the structure function relationships of cellulases including cellulosomes and related enzymes from bacteria and fungi has led to speculation and anticipation of their enormous commercial potential in bio - technology and research. Cellulases have been a potential candidate for research by both academic and industrial research groups. Biotechnological conversions of cellulosic biomasses are potentially sustainable approach to develop novel bioprocesses and products. Cellulase have become the focal biocatalysts due to their complex nature and wide spread industrial applications. These include:

### *1.5.1 Waste Management*

Lignocellulose is the most abundant plant cell wall component of the biosphere and the most voluminous waste produced by our society. Biomass is the only domestic, sustainable and renewable primary energy resource that can provide liquid transportation fuels. The conversion of cellulosic waste to useful by – products has long been recognized as a desirable endeavor. Disposal of cellulosic municipal solid waste through processes that would also desire energy production are of the particular interest. The benefits would be ‘Two fold’: Firstly, the amount of cellulose waste would be diminished and its effects on our environment will be reduced and Secondly, the pollutant would be converted to an alternative source of energy to help displace our growing dependence on fossil fuels.

**TABLE 2** Comparisons of Potential CBP Microorganisms for Production of Industrial Biocommodities

| **Key Feature** | ***Saccharomyces cerevisiae*** | ***Escherichia coli*** | ***Clostridium thermocellum*** | ***Bacillus subtilis*** |
|---|---|---|---|---|
| C5 sugar utilization | -/+ | +++ | - | +++ |
| Oligosaccharide utilization | - | -/+ | +++ | +++ |
| Protein secretion capacity | + | -/+ | +++ | +++ |
| Easiness of genetic modifications | +++ | +++ | -/+ | ++ |
| Medium cost benefits | ++ | - | ++ | +++ |
| Resistant to product inhibition | +++ | - | -/+ | +++ |
| Value of cell residues | ++ | - | - | +++ |
| Growth rate | ++ | +++ | + | +++ |
| Anaerobic fermentation | +++ | ++ | +++ | + |
| Culture temperature | -37$^{0}$C | -37$^{0}$C | -60$^{0}$C | 30-45$^{0}$C |

The biological decomposition of organic matter principally to methane and carbon dioxide by ‘Anaerobic’ digestion is a natural process that occurs readily in solid waste landfills. In natural anaerobic digestion processes some members of the microbial consortia collectively produce fermentable sugars from polysaccharides and others specialize in converting sugars to methane and carbon dioxide. Such mixed fermentations are notoriously difficult to establish and maintain at large scale. The anaerobic biological conversion of the major polymerize components of solid waste requires appropriate microorganisms and hydrolytic enzyme systems.

### *1.5.2 Bio–Ethanol Industry*

Enzymatic saccharification of lignocellulosic materials such as rice bran, sugarcane bagasse, corncob, rice straw, green gram husk, saw dust, and forest residues by cellulases for bioethanol production is perhaps the most popular application currently being investigated. Bioconversion of lignocellulosic materials into useful and higher value products normally requires multistep processes. These processes include; pretreatment (mechanical, chemical, or biological), hydrolysis of the polymers to produce readily metabolizable molecules (e.g., hexose and pentose sugars), bioconversion of these smaller molecules to support microbial growth and/or produce chemical products, and the separation and purification of the desired products. The utility cost of enzymatic hydrolysis may be low compared with acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4–6 and temperature 45–50°C) and does not have corrosion issues.

Technologies are currently available for all steps in the bioconversion of lignocellulosics to ethanol and other chemical products. However, some of these technologies must be improved to produce renewable bio-fuel and other byproducts at prices, which can compete with more conventional production systems. To reduce the enzyme cost in the production of fuel ethanol from lignocellulosic biomass, two aspects are widely addressed: optimization of the cellulase production and development of a more efficient cellulase-based catalysis system.

### 1.5.3 *Animal Feed Industry*

Applications of cellulases and hemicellulases in the animal feed industry have received considerable attention because of their potential to improve feed value and performance of animals. Pretreatment of agricultural silage and grain feed by cellulases or xylanases can improve its nutritional value. The enzymes can also eliminate anti-nutritional factors present in the feed grains, degrade certain feed constituents to improve the nutritional value, and provide supplementary digestive enzymes such as proteases, amylases, and glucanases. For instance, the dietary fiber consists of non starch polysaccharides such as arabinoxylans, cellulose, and many other plant components including resistant dextrins, inulin, lignin, waxes, chitins, pectins, β-glucan, and oligosaccharides, which can act as anti-nutritional factor for several animals such as swine. In this case, the cellulases effectively hydrolyze the anti-nutritional factor, cellulose; in the feed materials into easily absorbent ingredient thus improve animal health and performance.

β-Glucanases and xylanases have been used in the feed of monogastric animals to hydrolyze non starch polysaccharides such as β-glucans and arabinoxylans. Cellulases, used as feed additives alone or with proteases, can significantly improve the quality of pork meat. Glucanases and xylanases reduce viscosity of high fiber ryeand barley-based feeds in poultry and pig. These enzymes can also cause weight gain in chickens and piglets by improving digestion and absorption of feed materials.

Cellulases can be used to improve silage production for cattle feeding, which involves enhancement of the digestibility of grasses containing large amounts of potentially total digestible nutrients and energy values together with only small amounts of water-soluble carbohydrates.

### *1.5.4 Laundry and Detergent Industry*

Use of cellulases along with protease and lipase in the detergents is a more recent innovation in this industry. Cellulase preparations capable of modifying cellulose fibrils can improve color brightness, feel, and dirt removal from the cotton blend garments. The industrial application of alkaline cellulases as a potential detergent additive is being actively pursued with a view to selectively contact the cellulose within the interior of fibers and remove soil in the inter fibril spaces in the presence of the more conventional detergent ingredients. Nowadays, liquid laundry detergent containing anionic or nonionic surfactant, citric acid or a water-soluble salt, protease, cellulose, and a mixture of propanediol and boric acid or its derivative has been used to improve the stability of cellulases. As most of the cellulose fibers in the modern textile industry enzymes are used increasingly in the finishing of fabrics and clothes are arranged as long, straight chains of some small fibers can protrude from the yarn or fabric. The cellulases are applied to remove these rough protuberances for a smoother, glossier, and brighter-colored fabric.

### *1.5.5 Textile Industry*

Cellulases are the most successful enzymes used in textile wet processing, especially finishing of cellulose-based textiles, with the goal of improved hand and appearance. Cellulases have been successfully used for the biostoning of jeans and biopolishing of cotton and other cellulosic fabrics. During the biostoning process, cellulases act on the cotton fabric and break off the small fiber ends on the yarn surface, thereby loosening the dye, which is easily removed by mechanical abrasion in the wash cycle. The advantages in the replacement of pumice stones by a cellulose-based treatment include less damage of fibers, increased productivity of the machines, and less work-intensive and environment benign.

The bio-polishing is usually carried out during the wet processing stages, which include desizing, scouring, bleaching, dyeing, and finishing. The acidic cellulases improve softness and water absorbance property of fibres, strongly reduce the tendency for pill formation, and provide a cleaner surface structure with less fuzz. Cellulase preparations rich in endoglucanases are best suited for biopolishing enhancing fabric look, feel, and color without needing any chemical coating of fibers. The action of cellulases removes short fibers, surface fuzziness, creates a smooth and glossy appearance, and improves color brightness, hydrophilicity and moisture absorbance, and environmentally friendly process.

### *1.5.6 Paper and Pulp Industry*

Interest in the application of cellulases in the pulp and paper industry has increased considerably during the last decade. The mechanical pulping processes such as refining and grinding of the woody raw material lead to pulps with high content of fines, bulk, and stiffness. While in contrast, biomechanical pulping using cellulases resulted in substantial energy savings (20–40%) during refining and improvements in hand-sheet strength properties.

Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for biomodification of fiber properties with the aim of improving drainage and beatability in the paper mills before or after beating of pulp. Mansfield et al. studied the action of a commercial cellulase preparation on different fractions of Douglas fir kraft pulp and observed that the cellulase treatment decreased the defibrillation reducing the fibre coarseness. While endoglucanases have the ability to decrease the pulp viscosity with a lower degree of hydrolysis, cellulases have also been reported to enhance the bleachability of softwood kraft pulp producing a final brightness score comparable to that of xylanase treatment.

### *1.5.7 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 yields of the fermented products. Glucanases are added either during mashing or primary fermentation to hydrolyze glucan, reduce the viscosity of wart, and improve the filterability.

In wine production, enzymes such as pectinases, glucanases, and hemicellulases play an important role by improving color extraction, skin maceration, must clarification, filtration, and finally the wine quality and stability. β-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. The main benefits of using these enzymes during wine making include better maceration, improved color extraction, easy clarification, easy filtration, improved wine quality, and improved stability.

### *1.5.8 Food Processing Industry*

Cellulases have a wide range of potential applications in food biotechnology as well. The production of fruit and vegetable juices requires improved methods for extraction, clarification, and stabilization. Cellulases also have an important application as a part of macerating enzymes complex (cellulases, xylanases, and pectinases) used for extraction and clarification of fruit and vegetable juices to increase the yield of juices. The use of macerating enzymes increases both yield and process performance without additional capital investment. The macerating enzymes are used to improve cloud stability and texture and decrease viscosity of the nectars and purees from tropical fruits such as mango, peach, papaya, plum, apricot, and pear. Texture, flavor, and aroma properties of fruits and vegetables can be improved by reducing excessive bitterness of citrus fruits by infusion of enzymes such as pectinases and β-glucosidases. Enzyme mixtures containing pectinases, cellulases, and hemicellulases are also used for improved extraction of olive oil. Use of macerating enzymes not only improves the cloud stability and texture of nectars and purees, but also decreases their viscosity rapidly. Thus, the macerating enzymes, composed of mainly cellulase and pectinase, play a key role in food biotechnology and their demand will likely increase for extraction of juice from a wide range of fruits and vegetables. Furthermore, infusion of pectinases and β-glucosidases has also shown to alter the texture, flavor, and other sensory properties such as aroma and volatile characteristics of fruits and vegetables.

### *1.5.9 Pharmaceutical and Medical Sciences*

Considerable research in this area is being done and newer applications are being discovered, such as the treatment of enzyme deficiencies. Enzymes are also used as anti- tumor or anti -microbial agents, treatment of blood cloth and herniated discs. Effect of enzyme cellulase as digestive aid i.e. cellulase digests fiber. It helps in the remedy of digestive problems such as 'malabsorption'. Since, humans poorly digest cellulose fiber, taking a digestive enzyme product, like Digesting, that contains cellulase enzymes is not only necessary, but also vital for healthy cells.

### ***1.5.10*** *Agricultural Industry*

Various enzyme preparations consisting of different combinations of cellulases, hemicellulases, and pectinases have potential applications in agriculture for enhancing growth of crops and controlling plant disests produced using microbial hydrolases can be used to produce hybrid strains with desirable properties. Cellulases and related enzymes from certain fungi are capable of degrading the cell wall of plant pathogens in controlling the plant disease. Fungal β-glucanases are capable of controlling diseases by degrading cell walls of plant pathogens. Many cellulolytic fungi including Trichoderma sp., *Geocladium* spp, *Chaetomium* spp, and *Penicillium* spp. Cellulases are known to play a key role in agriculture by facilitating enhanced seed germination, rapid plant growth and flowering, improved root system and increased crop yields. Although these fungi have both direct (probably through growth-promoting diffusible factor) and indirect (by controlling the plant disease and pathogens) effects on plants, it is not yet clear how these fungi facilitate the improved plant performance.

Cellulases have also been used for the improvement of the soil quality. Traditionally straw incorporation is considered an important strategy to improve soil quality and reduce dependence on mineral fertilizers. Cellulolytic fungi applications such as *Aspergillus*, *Chaetomium*, and *Trichoderma*, and *Actinomycetes* have shown promising results. Exogenous cellulase supplementation accelerated decomposition of cellulose in soil. Therefore, using exogenous cellulase may be a potential means to accelerate straw decomposition and increase soil fertility.

## 1.6 CONCLUSION

Cellulose is the most abundant renewable biological resource and a low-cost energy source based on energy content. The production of bio-based products and bioenergy from less costly renewable lignocellulosic materials would bring benefits to the local economy, environment, and national energy security. The study show that cellulases are of substantial industrial interest because of their applications in industries of starch processing, grain alcohol fermentation, malting, brewing and extraction of fruit and vegetable juices and pulp and paper industries. Therefore, studies should be carried out on how to improve the applications of cellulases through certain factors such as kinetic factors and inductive effects.

# REFERENCES

[1]. Ahmed, S., Bashir, A., Saleem, H. (2009). Production and purification of cellulose degrading enzymes from a filamentous fungus Trichoderma harzianum. Pakistan Journal of Botany, 41, 1411- 1419.

[2]. Facchini, F. D., Vici, A. C., Reis, V. R., Jorge, J. A., Terenzi, H. F., Reis, R. A. Polizeli Mde, L. (2011). Production of fibrolytic enzymes by Aspergillus japonicus C03 using agro-industrial residues with potential application as additives in animal feed. Bioprocess Biosyst. Eng. 34: 347-55.

[3]. Niranjane, A. P., Madhou, P. Stevenson, T. W. (2007). The effect of carbohydrate carbon sources on the production of cellulase by Phlebia gigantean. Enzyme Microbial. Technol. 40: 1464-1468.

[4]. Rani, D. S. and Nand, K. (2000). Production of thermostable cellulase-free xylanase by Clostridium absonum. Process Biochem., 36: 355- 362.

[5]. Persson, I., Tjerneld, F. and Hahn-Hägerdahl, B. (1991). Fungal cellulolytic enzyme production part of: Persson, I. Production and utilization of cellulolytic enzymes in aqueous two-phase systems. Thesis University of Lund, Sweden.

[6]. Duff, S. J. B. and Murray, W. D. (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresour. Technol. 55: 1-33.

[7]. Silva, L. A. D. (2008). Produção e caracterização de enzimascelulásicaspor Aspergillus phoenicis. Master's Thesis, Universidade Federal do Rio Grande do Sul, pp 153-156.

[8]. Tao, Y. M., Zhu, X. Z., Huang, J. Z., Ma, S. J., Wu, X. B., Long, M. N. Chen, Q. X. (2010). Purification and properties of endoglucanase from a sugar cane bagasse hydrolyzing strain, Aspergillus glaucus XC9. J. Agric. Food Chem. 58(10): 6126-6130.

[9]. Naika, G. S. Tiku, P. K. (2010). Characterization of functional intermediates of endoglucanase from Aspergillus aculeatus during urea and guanidine hydrochloride unfolding. Carbohydr. Res. 345(11): 1627-31.

[10]. Irshad, M., Ahmed, S., Latif, F. Rajoka, M. I. (2008). Regulation of Endo- β-Dxylanase and β Xylosidase synthesis in Humicola lanuginosa. J. Chem. Soc. Pak. 30: 913-918.

[11]. Abo-State, M. A. M., Hammad, A. I., Swelim, M. and Gannam, R. B. (2010). Enhanced Production of Cellulase(S) By Aspergillus spp. Isolated From Agriculture Wastes by Solid State Fermentation. American-Eurasian J. Agric. & Environ. Sci. 8(4): 402-410.

[12]. Gao, J. H., Weng, D., Zhu, G., Yuan, F., Guan, and Xi, Y. (2007). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal Aspergillus terreus M11 under solid- state cultivation of corn stover. Bioresource Technol., 99: 7623-7629.

[13]. Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R. and Roussoss, S. (2000). Biotechnology potential of agro-industrial residues, part II. Cassava bagasse.Bioresource Technology 78:81-87.

[14]. Deunas, R., Tengerdy, R. P. and Gutierrez, M. (1995). Cellulase production by mixed fungi in solid state fermentation of bagasse. World J. Microbiol. Biotechnol., 11: 333-337.

[15]. Zhou, J., Wang, Y. H., Chu, J., Zhuang, Y. P., Zhang, S. L. Yin, P. (2008). Identification and purification of the main components of cellulases from a mutant strain of Trichoderma viride T 100-14. Bioresour. Technol. 99: 6826-6833.

[16]. Papagianni, M. (2006). Fungal morphology and metabolite production in submerged mycelia processes. Biotechnology Advances , pp. 606-615.

[17]. Perez-Guerra, N., Torrado-Agrasar, A., Lopez Macias, C. and Pastrana, L. (2003). "Main Characteristics and Applications of Solid Substrate Fermentation." Electronic Journal of environmental, Agricultural & Food Chemistry. 2 (3): 343-350.

[18]. Pandey, A., Maciel, G. M., Vandenberghe, L. P., Windson, C., Haminiuk, I., Fendrich, R. C., Bianca, D. E., Brandalize, T. Q. and Soccol, S. R. (1999). Xylanase production by Aspergillus niger 1pb 326 in solid state fermentation using statistical experimental designs. Food Technol. Biotechnol., 46: 183-189.

[19]. Gao, J. (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal Aspergillus terreus M11 under solid state cultivation of corn stover. Bioresource Technology, 99, 7623-7629. doi:10.1016/j.biortech.2008.02.005

[20]. Carvalho, W. R. (2004). Caracterização bioquímica da endoxilanase recombinante (HXYN2r) do fungo termofílico Humicola grisea var. thermoidea e sua aplicação na sacarificação de resíduos agrícolas. Doctoral thesis, Universidade Federal de Goiás, vdpp. 625-827.

[21]. Bon, E. P. S., Ferrara, M. A., Corvo, M. L., Vermelho, A. B., Paiva, C. L. A. M., De Alencastro, R. B. and Coelho, R. R. R. (2008). Enzimasembiotecnologia: Produção, aplicações e Mercado. Rio de Janeiro. Pp 120-129.

[22]. Bayer, E. A. and Lamed, R. (1992). The cellulose paradox: pollutant par excellence and/or a reclaimable natural resource. Biodegradation. pp. 171-188.

[23]. Pandey, A. (2003). "Solid-state fermentation." Biochemical Engineering Journal. 13: 81-84.

[24]. Hölker U, Höfer M, and Lenz J. (2004): "Biotechnological advantages of laboratory-scale solid-state fermentation with fungi." Applied Microbiology and Biotechnology. 64(2):175-186.

[25]. Coelho, M. A. Z., Leite, S. G. F., Rosa, M. F. and Furtado, A. A. L. (2001). Aproveitamento de resíduosagroindustriais: produção de enzimas a partir da casca de coco verde. Boletim CEPPA. pp 189-259

[26]. Alberton, L. R. (2004). Produção de xilanaseemresíduosagroindustriaispor Streptomyces viridosporus t7a e aplicação do extratobrutoemveterinária. pp 375-384.

[27]. Filho, U. C. (2006). Cinéticaenzimática e uso e produção de enzimas; Universidade Federal de Uberlândia, pp 170-174.

[28]. Lynd, L. R., Weimer, P. J., Zyl, W. H. V. Pretorius, I. S. (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiology and Molecular Biology Reviews, pp. 506-577.

[29]. Singh and Hayashi, K. (1995). Microbial cellulases: Protein architeture, molecular properties, and biosynthesis. Advances in Applied Microbiology, pp. 1-44.

[30]. Lima, U. A., Schimdell, W., Aquarone, E. and Borzani, W. (2001). Biotecnologia industrial: Processos Fermentativos e Enzimáticos. (São Paulo, Edgar Blüncher), pp. 627-726

[31]. Coral, G., Arikan, B., Unaldi, M. N. Guvenmes, H. (2002). Some properties of crude carboxymethyl cellulase of Aspergillus niger Z10 wild-type Strain. Turk. J. Biol, 26: 209-213.

[32]. Howard RL, Abotsi E, JansenVREL, Howard S. (2003): "Lignocellulose Biotechnology: Issue of Bioconversion and Enzsyme production." African Journal of Biotechnology. 2: 602-619.

[33]. Hölker U, Höfer M, and Lenz J. (2004): "Biotechnological advantages of laboratory-scale solid-state fermentation with fungi." Applied Microbiology and Biotechnology. 64(2):175-186.

[34]. Carere, C.R., Sparling, R., Cicek, N., Levin, D.B. (2008). Third generation Biofuels via direct cellulose fermentation.Int. J.Mol. Sci9: 1342-1360.

[35]. Saleem, F., Ahmed, S. Jamil, A. (2008). Isolation of a xylan degrading gene from genomic DNA library of a thermophilic fungus Chaetomium thermophile ATCC 28076. Pak. J. Bot. 40: 1225 1230.

[36]. Zhang, Y. H. P., Himmel, M. E. and Mielenz, J. R. (2006). Outlook for cellulase improvement: Screening and selection strategies. Biotechnol. Adv. 24: 452-481.

[37]. Escobar, J. C., Lora, E. S., Venturini, O. J., Yanez, E. F., Almazan, O. (2009). Biofuels: environment, technology and food security. Renew. Sustain. Energy Rev. 13: 1275- 1287

[38]. Ghose, T. K. (1987). Measurement of cellulase activities. Pure & Applied Chemistry, pp. 257-268.

[39]. Bhat, M. K. (2000). Cellulase and related enzymes in biotechnology. Biotechnology Advances. pp. 355-383.