Review Article

Microbial Cellulases in Industrial Applications

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ABSTRACT

Cellulose, most copious constituent of plant cell wall and a renewable resource, is of considerable economic importance due to its potential applications in production of various bioenergy and bio-based products. Cellulose is used as a food source by the wide range of microorganisms and animals. Cellulose degrading enzymes are utilized in numerous applications in several industries, such as biofuel production, food and feed industry, brewing, pulp and paper, textile, laundry, and agriculture. Cellulose degrading enzymes containing microbes are widely spread in nature and isolated from different environments. In this communication we are presenting an overview of microbial cellulases used in different industrial applications.

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Introduction

Cellulose is the most abundant organic molecule on the Earth and is the primary structural component of plants. It is fibrous, insoluble and high molecular weight homopolymer of anhydrous glucose units linked by the β-1, 4 glycosidic linkages. It is the major constituent of the lignocellulosic biomass which is an inexpensive and inexhaustible renewable organic material in the nature and has significant potential as alternative source of fuel and bio-based chemicals. In the lignocellulosic biomass, the cellulose fibers are embedded in a matrix of different biopolymers including hemicelluloses and lignin [1]. Structurally, long chains of cellulose are bundled together by numerous cross linkages, such as hydrogen and vander Waal interactions to pack cellulose into microfibrils. The parallel arrangement of cellulose chains result in the development of crystalline region whereas amorphous region of cellulose structure is due to less ordered arrangement of chains [2, 3]. The degree of polymerization of cellulose chain is highly variable ranging from 250 to 10000 and it influences the physiological, mechanical and biological properties of the cellulose. The length of cellulose chain or degree of polymerization depends on the source of material & treatment methods [4-7]. It is the chief constituent in the lignocellulosic biomass and also regarded as the strongest potential candidate for sustainable fuel production due to its environment friendly characteristics, such as renewability, biocompatibility and biodegradability [8, 9].

The cellulases are the third most significant commercial enzyme in the world market. These enzymes are important and essential for hydrolyzing the cellulose into fermentable sugars that can be used for further applications. The cellulose hydrolysis is chiefly carried out by a multi-enzyme system comprising endoglucanase, exoglucanase, and β-glucosidase [10, 11]. Endoglucanase (EC 3.2.1.4; endo-β-1,4-D-glucanase) acts in a random manner on internal but accessible sites in the cellulose chains and hydrolyze β-1,4 linkages to generate oligosaccharides of different lengths with new chain ends. In addition, endoglucanase also act on cellodextrin and convert them to cellobiose and glucose [12, 13]. Exoglucanase (EC 3.2.1.91; exo-β-1,4-D-glucanase, cellobiohydrolase) acts in a progressive manner on the reducing and non-reducing ends of the cellulose polysaccharide chain to release glucose or cellobiose as major products [13]. β-glucosidase (EC 3.2.1.21) hydrolyse the soluble cellodextrins and cellobiose to glucose units [13, 14]. These three proteins work synergistically and catalyze appropriate hydrolysis for obtaining glucose residues which are used for various applications including production of biofuel, feed stock, single cell protein, and chemicals etc. [3, 13, 15].

Cellulases are inducible enzymes, which are produced by a wide array of microorganisms including fungi and bacteria. Among the microbes, fungi are the major producers of cellulase and accounts for approximately 80% of the cellulose hydrolysis on the Earth [16]. Primarily, ascomycota, basidiomycota and deuteromycota members of the fungi are possessed with efficient cellulolytic activities. Cellulases derived from aerobic fungal microorganism are preferred widely for industrial applications as they are extracellular and secreted in bulk during growth [17]. *Trichoderma reesai* is the most widely studied fungus and has the ability to convert desired as well as native celluloseto glucose [18]. In addition, fungal species of *Aspergillus, Humicola, Penicillum* and *Sclerotium* are considered potential candidates for industrial production of cellulolytic enzymes [11, 19]. Actinomycetes genera such as *Cellulomonas, Streptomyces* and *Thermomonospora* have also been involved in the production of cellulolytic enzymes.

Applications

Cellulases, solely or in a mixture with other enzymes, are involved in several industries including biofuel, food, feed, beverages, paper, textile, pharmaceutical, agricultural etc (Table 1.). Enzyme mediated hydrolysis of cellulosic biomass result in the generation of sugars that serve as the starting materials for the production of various value added products of commercial interest, such as bioethanol, organic acids, sugars and animal feeds [11, 20].

Table 1: Applications of cellulases in different industries [6, 10, 11, 21, 22].

Industry	Applications
Biofuel	Production of ethanol, solvents and organic acids; production of energy-rich animal feed and with improved nutritional value
Food/animal feed processing	Improved yield and extraction of fruit and vegetables juices, clarification of fruit juices, improved maceration, color extraction of fruit and vegetables
Textiles	Biopolishing, biostoning, biofinishing
Paper and pulp	Co-additive in pulp bleaching, improved draining, de-inking
Agriculture	Control of plant pathogens and disease, improved soil quality
Medical	Antibiofilm agent, treatment of phytobezoar
others	Reduction in biomass waste, extraction of olive oil and carotenoids

Food

Cellulolytic enzyme, solely or in combination with other enzymes, have find significant recognition in food industry. Cellulases are widely used in the preparation of fruit and vegetables juices, animal feed production and in alcohol based industries [20, 23]. The Macerating enzymes, a combination of cellulases, hemicellulases and pectinases, are added to improve process performance and yield to enhance cloud stability & texture and to reduce viscosity of nectars and puree obtained from fruits such as apricot, peach, plum, papaya. The macerization facilitates improved extraction, clarification, stabilization and yield of fruit and vegetables juices [10, 24, 25]. In juice processing, cellulases from *Aspergillus* and *Trichoderma* of fungal origin and *Bacillus* and *Paenibacillus* of bacterial origin are potentially used for juice clarification [26]. Cellulases are also utilized for extraction of the flavonoids from flowers and seeds. Cellulase mediated extraction is preferred over conventional extraction methods due to increased yield, low process time and less heat damage [11, 27]. They are also involved in the extraction of phenolic compounds from grape pomace [23, 28]. A combination of enzymes including cellulases is used to reduce bitterness of citrus fruits and, consequently, yield products with improved taste and aroma [10, 11]. β-glucosidase and pectinases are utilized in a combination to improve texture, flavour and other sensory properties of fruits and vegetables [23, 28].

Cellulases have also found application in animal feed industry. They are added to monogastric animal feed for improving the digestibility of cereal based food and nutritive value [29-31]. Cellulases from Bacillus subtilis can be used for soya bean hull degradation to enrich its nutritional value for monogasrtic animal feed animals [23, 32]. In wine and beer industry, cellulases are involved in fermentation processes to improve quality and yield of the products. Glucanase is used in mashing process that reduce viscosity of wort and enhance filterability. A mixture of cellulases, hemicellulases, and pectinases is used in wine industry for improving colour extraction, easy clarification & filtration and stabilization. β-glucosidase is utilized to improve the sensory property such as aroma of wine products [10].

Textile Industry

Cellulolytic enzymes are utilized in textile processing for effective substitution of conventional chemical methods. Enzyme based processing are more economic, eco-friendly and energy saver. In textile industry, cellulases are employed for scouring, polishing, carbonization and stone wash. Biotechnological development extended the range of application in acid, alkali and neutral medium during different phase of fabric finishing. Scouring i.e. removal of impurities within raw cotton is carried out more effectively using cellulases in combination with pectinases, proteases and lipases where cellulases hydrolyze the cellulosic surface components and facilitate other enzymes to get in and react on their specific non cellulosic components for fabric finishing [20, 33]. Cellulase mediated wool scouring/pretreatment of wool replaces the conventional use of sulfuric acid or hydrochloric acid and requirement of high temperature (100-110°C). Enzyme mediated scouring helps in less emission of CO_2 and saving of approximately 20,000 liters of water per ton of fabric [34]. Endocellulases and acid cellulases are used for biopolishing and carbonization of fabrics, respectively. Cellulases utilization for denim finishing results in minimizing use of stone, damage to denim and water pollution. Enzyme mediated carbonization is non-corrosive, non-hazardous and eco-safe. Acid cellulases are employed for shade correction attained by disperses or reactive blends of cotton with polyester [33]. The enzyme based processing in textile industries are economic, eco-friendly, non-hazardous with minimal water consumption. The applications of cellulases have been recognized widely in cellulose based textiles for their advantages over conventional methods as well as for quality improvement and fabric care [10]. Cellulases from microorganisms are efficiently involved for substituting pumice stones for bio-stoning and removal of excess dye to provide softness and faded look to denim. In addition, these cellulolytic enzymes are also used to degrade protruding fiber ends from the fabric for an improved finishing, softening and for colour gradient [11, 35]. Most commercially utilized cellulases in textile industry are derived from fungal microbes, *Aspergillus niger* and *Trichoderma reseei* [23]. Cellulases are utilized in detergents and added to laundry detergents to improve fabric softness and brightness. In many formulations, cocktail of different enzymes including protease, amylase, cellulase and lipase are also used for improved washing effect for household purposes [36]. Thermostable cellulases produced by *Aspergillus, Trichoderma* and *Humicola* are generally added to enzyme detergent formulations [11].

Paper

Cellulose degrading enzymes are versatile and can be utilized effectively in paper and pulp industry to replace non eco-friendly conventional methods. Cellulases are used for a wide variety of uses, such as in biomechanical pulping, de-inking, and drainage management, manufacturing biodegradable paper towels, sanitary paper and cardboards. In addition, cellulase mediated processes are more economical, energy saving and low chlorine consuming. They are used as co-additive in pulp bleaching and for biomechanical pulping [10, 22].

The mechanical pulping methods include grinding and refining of the plant materials resulting in the pulp with high bulk and stiffness whereas cellulase mediated pulping is considerably energy saver and with improved physical properties including inter-fibre bonding and mechanical strength of the product [21, 37, 38].

The recycling of waste papers results in less solid waste and reduces deforestation required to make new paper products. A combination of cellulase and hemicellulase is used for deinking of waste papers that improve the quality of recycled papers [39-41]. The enzymatic deinking leads to minimal or negligible use of alkali, enhanced brightness and strength properties. In addition, enzyme mediated deinking prevent alkaline yellowing and adverse effect on the environment. The cellulase treatment is used to remove fibrils and colloidal substance to improve the drainage problems is paper mills. Besides, cellulases have also find applications in the manufacturing of soft papers and biodegradable cardboards [10, 42-44].

Conversion of Lignocellulosic Biomass into Ethanol

Continuous and increased use of fossil fuel will leads eventually to the depletion of fossil fuel reserves as well as increase environmental pollution by releasing toxic gases including greenhouse gases. In the light of fuel crisis and environmental concern, biotechnological development in search of alternative fuels have been accepted and recognized widely. Naturally occurring starch and cellulose have been strongly considered as the potential source of alternative biofuels [11, 36]. Lignocellulosic biomass is available in huge amount in many forms, such as agricultural and forestry residues and wastes generated from different industries including solid municipal wastes. Whereas the principal sources of starch for fuel production are cultivated crops such as maize, tapioca, potato, wheat, oats, barley etc. In addition to huge cultivation cost, utilization of starch containing crops may also lead to the competition with starch based food supply to continuously growing population [3, 9, 45]. Use of lignocellulosic biomass to obtain biofuels is more advantageous than starch due to its easy and low cost availability. The wastes generated every year in huge amount can be utilized for the production of value added products. The production of lignocellulosic biomass is cost effective as well as faster in comparison to other agricultural feedstock, such as corn, sugar cane and soybeans [46-48]. Being abundant and outside the human food chain, makes lignocellulosic materials relatively inexpensive feedstock for ethanol production [49, 50]. The degradation of lignocellulosic biomass is an expensive process and it requires three steps: physiochemical pretreatment, enzymatic hydrolysis and fermentation [51].

Enzyme mediated processes are specific, low energy consuming and environmental friendly and hence, Cellulases mediated hydrolysis due to its enzymatic nature is preferred over acid/alkali chemical methods. The conversion of biomass to fuel involves hydrolysis of constituents of biomass to fermentable sugars, followed by sugar fermentation to ethanol using appropriate microorganism. The first step involves degradation of the lignocellulosic polymer, delignification to release cellulose and hemicellulose content, followed by hydrolysis of carbohydrate polymers to produce free sugars [46, 52]. According to an evaluation, there is about 40% cost reduction if cellulases are used for bioprocessing and pretreatment [1]. An anaerobic thermophilic bacterium, *Clostridium thermocellum*, has significant potential to hydrolyze cellulose and, at the same time a potential to ferment sugar to ethanol. This microbe offers many advantages, such as higher growth rate, improved enzyme stability, and the recovery of products is also easier [46, 53]. *Saccharomyces cerevisiae* has been conventionally utilized for the fermentation of sugars, obtained by cellulosic hydrolysis, to produce ethanol [54, 55].The hexose sugars, such as glucose, galactose, and mannose are readily fermented to alcohol whereas pentoses, such as xylose and arabinose require additional efforts for their hydrolysis to achieve higher production of cellulosic biofuels and other products. In recent years, metabolic engineering of microorganisms has shown significant progress in the efficiently fermentation of hydrolysates containing xylose and arabinose [56-58]. Cellulases produced by various filamentous fungi including *Aspergillus*, *Trichoderma*, and *Penicillium* have been used widely for bioconversion of lignocellulosic biomass into biofuel and others derivatives [11, 59].

Other Applications

Carotenoids, organic pigments, are preferred and used as food colorants owing to natural origin, high versatility, lipo- as well as hydrophilic and negligible toxicity. Solvent extraction method may dissociate pigments from the proteins and may cause water insolubility and their oxidation. Whereas enzyme mediated extraction of pigments, using a mixture of cellulases and pectinolytic enzymes, keep them in the natural state and attached to proteins. Natural state pigments are equipped with desired properties to be used as food colorants [10, 60, 61].

A combination of cellulases, hemicellulases and pectinases has also been used during olive oil extraction to improve oil yield, and maintaining high level of antioxidant and vitamin E content [62]. They are also used to reduce viscosity of oil paste and strengthen the extraction of polyphenolic substances from the olive fruits [10, 63].

Cellulolytic enzymes from fungal microbe *Aspergillus, Chaetomuim* and *Trichoderma* and actinomycetes have also been used to improve soil quality by decompositing soil cellulose and consequently, reducing the dependency on the mineral fertilizers [10, 64, 65]. Enzyme mixtures containing cellulases, hemicellulases and pectinases have applications in enhancing growth of crops and controlling plant diseases by killing plant pathogens [21, 66].

In medicinal field, cellulases are used for the treatment of phytobezoars and as antibiofilm agents. Fungal cellulases are utilized to cure phytobezoars, a trapped concretion of indigestible plant materials in the gastrointestinal tract. Bacterial cellulases are potentially degrading cell wall of pathogenic *Acanthamoeba*, which is responsible for blinding keratitis as well as granulomatous amoebic encephalitis. Cellulase can potentially degrade cellulose component of biofilms and consequently, restrict distribution of pathogenic organisms and drug accessibility to them [23, 67, 68].

Conclusion

Cellulolytic enzymes are potentially utilized in a wide range of industries due to hydrolytic action on cellulosic biomass. Their activity on biomass lead to the production of fermentable sugars which are further used as a raw material for the production of several value added products, such as biofuel including ethanol, organic acids, sugars etc. Though a wide range of microorganisms have been reported for cellulase production but their application in industries is hindered by the production cost and the low yield. Hence, biotechnological developments to improve production efficiency at a competitive cost are needed to utilize microbial enzyme systems that can have significant industrial impact.

Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

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None

Competing Interests

None Declared

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