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REVIEW

Fungal cellulases and their industrial importance: A Review

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Cellulose is a copious natural biopolymer on earth and most dominating agricultural waste. This cellulosic biomass is a renewable reserve with great potential for bioconversion to value added products. It can be degraded by cellulases, produced by various cellulolytic fungus like *Trichoderma sp* and *Aspergillus sp*. Basic and applied research on microbial cellulases, has not only generated significant scientific facts but has also revealed their enormous potential in biotechnology.This enzyme has various industrial applications and is considered as major group of industrial enzyme. The review discusses the production of cellulase, classification of cellulases, types of cellulolytic fungus, their screening and the application of the enzyme in various industries. The biotechnological facet of cellulase research and their future prospects are also discussed.

Key words: Cellulase, SSF, application,

INTRODUCTION

Cellulase is a significant enzyme for carrying out the depolymerization of cellulose into fermentable sugars. As a major resource for renewable energy and raw materials, it is widely used in the bioconversion of renewable lignocellulosic biomass (Shahzadi *et al*, 2014) . Biotechnological conversion of cellulosic biomass is potentially sustainable approach to develop novel bioprocesses and products. Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including both fungi and bacteria. Microbial cellulases have become the focal biocatalysts due to their complex nature and wide spread industrial applications. Bacteria which have high growth rate as compared to fungi have good potential to be used in cellulase production. However, the application of bacteria in producing cellulase is not widely used (Sethi *et al.,* 2013). Structurally fungal cellulases are simpler as compared to bacterial cellulase systems, cellulosomes (Bayer *et al.,* 1994, 1998; Zhang *et al.,* 2006). Fungal cellulases typically have two separate domains: a catalytic domain (CD) and a cellulose binding module (CBM), which is joined by a short polylinker region to the catalytic domain at the N-terminal. The CBM is comprised of approximately 35 amino acids, and the linker region is rich in serine and threonine. The main difference between cellulosomes and free cellulase enzyme is in the component of cellulosomes-cohesin containing scaffolding and dockerin containing enzyme. The free cellulase contains cellulose binding domains (CBMs), which are replaced by a dockerin in cellulosomal complex, and a single scaffolding-born CBM directs the entire cellulosomes complex to cellulosic biomass (Bayer *et al.,* 2004; Carvalho *et al.,* 2003). Cellulases catalyses cellulolysis which is basically the biological process controlled and processed by the enzymes of cellulase system. Cellulase enzyme system comprises three classes of soluble extracellular enzymes: 1, 4-β-endoglucanase, 1, 4-β-exoglucanase, and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase). Endoglucanase is responsible for random cleavage of β-1,4-glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of the nonreducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose, and β-1, 4-glucosidase hydrolyses cellobiose and water-soluble cellodextrin to glucose (Shewale 1982; Woodward *et al.,* 1983). Only the synergy of the above three enzymes makes the complete cellulose hydrolysis to glucose (Ryu *et al.,* 1980 ; Samdhu *et al.,* 1992; Wood, 1989).

Classification of cellulases

Microorganisms produced extracellular cellulases that are either free or associated with cells to hydrolyze and metabolize insoluble cellulose. The biochemical analysis of cellulose systems from aerobic and anaerobic bacteria and fungi has been comprehensively reviewed during the past three decades (Sadhu *et al.,* 2013)

Endoglucanases or Endo-1, 4-β-D-Glucan Glucanohydrolases (EC 3.2.1.4)

Endoglucanases cut at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends. It is generally active against acid-swollen amorphous cellulose, soluble derivatives of cellulose such as CMC, cellooligosaccharides (Wood, 1989).

Exoglucanase or 1, 4-β-D-Glucan Cellobiohydrolases (EC 3.2.1.91)

Exoglucanases act in a possessive manner on the reducing or non-reducing ends of cellulose polysaccharide chains, liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. These enzymes are active against crystalline substrat such as Avicel, amorphous celluloses and cellooligosaccharides. However, they are inactive against cellobiose or substituted soluble celluloses such as CMC (Sadhu *et al.,* 2013).

β - Glucosidases or β-D-Glucoside Glucohydrolases (EC 3.2.1.21)

β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose from non-reducing end. It is inactive against crystalline or amorphous cellulose (Sadhu *et al.,* 2013)

Production of cellulases

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms. It has been extensively used for the production of cellulase for their wide uses in industry. Over the years, fermentation techniques have gained huge importance due to their economic and environmental advantages. Two broad fermentation techniques have emerged as a result of this rapid development: Submerged Fermentation (SmF) and Solid State Cultivation (SSC) or Solid State Fermentation (Sadhu *et al.,* 2013; Zhuang *et al.,* 2007).

Solid state cultivation

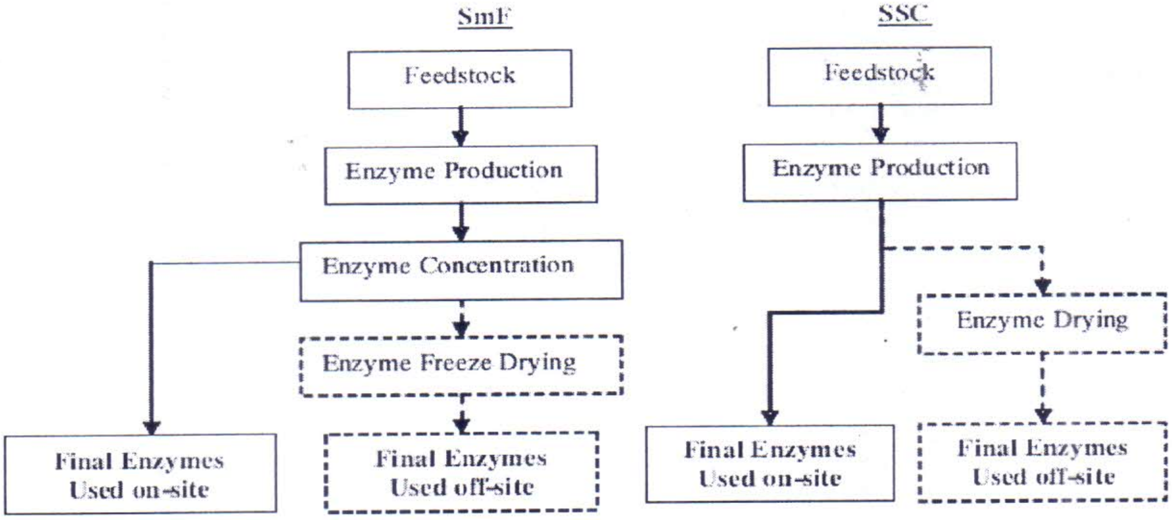
SSC utilizes solid substrates, like bran, bagasse, paddy straw, other agricultural waste and paper pulp (Subramaniyam *et al.,* 2013). The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as cheaper substrates. SSC is appropriate for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high water activity, such as bacteria (Babu *et al.,* 1996). As wastes are utilized as substrates for SSC it can serve as an effective method of reduction of environmental pollution. The SSC is generally preferred as it offers many advantages such as higher enzyme production as well as protein rate, higher concentration of the product in the medium, employment of natural cellulosic waste as substrate in contrast to the necessity of using pure cellulose in submerged fermentation (SmF) and the possibility of carrying out fermentation in non-aseptic conditions (Chahal, 1983).

Submerged fermentation

SmF utilizes free flowing liquid substrates, such as molasses and broth (Subramaniyam *et al.,* 2013). This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content. An additional advantage of this technique is that purification of products is easier (Samdhu *et al.,* 1992).

Fungal cellulases and their screening

Cellulase is one of the most useful enzymes in industry and can be produced by fungi, bacteria or actinomycetes, but the most common producer is



Straight (dashed) lines represent onsite (off-site) enzyme production process

Fig. 1 : Flow chart of enzyme production using the traditional SmF method compared to the SSC method. (Zhuang *et al.*, 2007).

fungi (Ariffin *et al.,* 2006). These organisms grow under suitable condition to produce cellulase for e.g. *Trichoderma viride* , *Trichoderma reesei* , *Aspergillus niger* produces active cellulases when grown in liquid media by both surface and submerged culture methods and recently by solid state fermentation (Haq *et al.,* 2005). The detailed study was made on production of cellulase using *Trichoderma reesei* (Muthuvelayudham *et al.,* 2003). Production of cellulase was also reported by using substrates like cellulose, xylose and lactose using *T.reesei* (Muthuvelayudham *et al.,* 2005). Some species of *Penicillium* i.e. *Penicillium iriensis* and *P. citriviride* produce significant quantities of cellulase, when grown under different conditions. *Penicillium funiculosum* is capable of dissolving cotton completely by cellulase production. *Chaetomium sp.NIOCC 36* was found better for production of cellulase (Ravindram *et al.,* 2010). For screening of fungal cellulase producer isolation of potential organism will have to done, and for screening of cellulase producing microorganism pure cultures of fungal isolates will have to be individually transferred in CMC agar plates. The isolates would be added as suspensions made with sterile water in cups made by cork borer on the solidified medium, the plates would then be incubated at 30°C for 72 hours (Stork *et al.,* 1996) and then again incubated for 18 h at 50°C which is the optimum temperature for cellulases activity. After incubation, 10 ml of 1% aqueous solution of congo–red would be added to the plates that were shaken at 50 rev/min for 15 minutes. The congo-red staining solution will then be discarded and 10 ml of 1 N NaOH will be added to the plates and shaken again at 50 rev/min for 15 minutes (Lakshmi *et al.,* 2012). Finally 1 N NaOH will also be discarded and the staining of the plates would be analyzed by noticing the formation of yellow zones around the fungal spore inoculated wells.

Application of cellulases

Cellulases have a wide range of enormous prospective applications in biotechnology and many thermo stable endoglucanase appeared to have a great potentiality for industrial use (Karmakar *et al.,* 2010).

Food processing, wine and brewery industries

Enzyme infusion has the potential of producing fruit and vegetable juices which is imperative from commercial standpoint. The production of fruit and vegetable juices requires methods for extraction, clarification and stabilization. Macerating enzymes are generally used after crushing, to macerate the fruit pulp for partial or complete liquifaction, which increases the juice yield, reduces the processing time and improves the extraction of valuable fruit components. 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 including olive oil extraction, that has attracted the world market because of its numerous health claims. Application of macerating enzymes could increase both production 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 (Sukumaran *et al.* 2005; Singh *et al.,* 2007; Bhat *et al.,* 2000; Calvalho *et al.,* 2008). Bioconversion of cellulosic materials to bioalcohol involves a multistep process which first uses cellulolytic enzymes for hydrolysis of polymers to pentose or hexose sugars and fermentation followed by distillation of these sugars into ethanol. In wine production, enzymes such as pectinases, glucanases, and hemicellulases play an important role by improving color extraction, skin maceration, must clarification, filtration, and lastly the wine quality and stability (Singh *et al.,* 2007; Galante *et al.,* 1998). β-Glucosidases can perk up the aroma of wines by modifying glycosylated precursors. Macerating enzymes also improve pressability, settling, and juice yields of grapes used for wine fermentation. A number of commercial enzyme preparations are now available to the wine industry. 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 (Galante *et al.,* 1998).

Textile industries

Enzymes have been used in the leather industry for many years and more recently have been introduced into modern textile industries. 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 separate 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 cellulase-based treatment include less damage of fibers, increased efficiency of the machines, and less work-intensive environment. (Sukumaran *et al.* 2005; Singh *et al.,* 2007; Galante *et al.,* 1998; Uhlig *et al.,* 1998). The acidic cellulases improve softness and water absorbance property of fibres and provide a cleaner surface structure with less fuzz (Ibrahim *et al.,* 2011).

Pharmaceutical industries

Since, humans inadequately digest cellulose fiber, taking a digestive enzyme product, like Digestin, that contains cellulase enzymes is not only necessary, but also vital for healthy cells (Karmakar *et al.,* 2010).This can do away with digestive problems such as malabsorpsion(Sharada *et al.* 2014).

Detergent industries

Use of cellulases along with protease and lipase in the detergents is a more recent innovation in this industry (Singh *et al.,* 2007). Removal of oil from inter fibre space by selective contraction of fibres by the alkaline cellulase increases the cleansing ability of a detergent. 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 interfibril spaces in the presence of the more conventional detergent ingredients (Sukumaran *et al.,* 2005; Singh *et al.,* 2007).

Pulp and paper industries

Attention in the application of cellulases in the pulp and paper industry has increased considerably during the last decade (Mai *et al.,* 2004). The mechanical pulping processes such as refining and grinding of the woody raw material lead to pulps with elevated content of bulk, fines and stiffness. Biopulping with the help of cellulases and allied enzymes is a better alternative for mechanical pulping process as the former provides major energy savings as these enzymes require lower energy input to achieve the required freeness and strength and check the problem of pollution. Refining, of primary or secondary fibers, can generate fines that can reduce the drainage rate of pulps during paper making operations. Cellulases seem to preferentially attack and hydrolyze the fines produced during the refining operation and therefore, improve the pulp's drainage property. Cellulase and hemicellulases helps in modification of coarse mechanical pulp and handsheet strength properties, partial hydrolysis of carbohydrate molecules and the release of ink from fibre surfaces which results into deinking of recycled paper. Cellulases have also been used to remove ink from papers and to enhance papermaking properties of recycled fibers. Enzymatic deinking can lower the need for deinking chemicals and reduce the adverse environmental impacts of the paper industry (Stork *et al.,* 1996)

Animal feed industry

Applications of cellulases and hemicellulases in the feed industry have received substantial attention because of their potential to improve feed value and performance of animals (Dhiman *et al.,* 2002). Pretreatment of agricultural silage and grain feed by cellulases or xylanases can improve its nutritional value (Godfrey *et al.,* 1996). Nevertheless, the successful use of these enzymes in animal diet is to: eliminate Anti-Nutritional Factors (ANF) present in grains or vegetables; degrade certain cereal components in order to improve the nutritional value of feed. Moreover, Cellulases and hemicellulases are responsible for partial hydrolysis of lignocellulosic materials, hydrolysis of b-glucans and better emulsification and flexibility of feed materials which results in the improvement in the nutritional quality of animal feed (Chesson, 1987; Cowan, 1996 ; Galante *et al.,* 1998)

Table 1 : Fungus having cellulolytic abilities (Kuhad *et al.* 2011)

Fungi
Soft rot fungi
Aspergillus niger; A. nidulans; A. oryzae; A. terreus; Fusarium solani; F. oxysporum; Humicola insolens; H. grisea; Melanocarpus albomyces; Penicillium brasilianum; P. occitanis; P.decumbans; Trichoderma reesei; T.longibrachiatum; T. harzianum; Chaetomium cellulyticum; C. thermophilum; Neurospora crassa; P. fumigosum;Thermoascus aurantiacus;Mucor circinelloides; P. janthinellum; Paecilomyces inflatus; P. echinulatum; Trichoderma atroviride
Brown rot fungi
Coniophora puteana; Lanzites trabeum; Poria placenta; Tyromyces palustris; Fomitopsis sp.
White rot fungi
Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes versicolor; Agaricus arvensis; Pleurotus ostreatus; Phlebia gigantean

Agricultural industries

Various enzyme preparations consisting of different combinations of cellulases, hemicellulases, and pectinases have prospective applications in agriculture for enhancing growth of crops and controlling plant diseases (Bhat *et al.,* 2000; Chet *et al.,* 1998). Cellulases and related enzymes from certain fungi are capable of degrading the cell wall of plant pathogens and in a way controls the plant disease (Bhat *et al.,* 2000). Many cellulolytic fungi including *Trichoderma* sp., *Chaetomium* sp., and *Penicillium* sp. 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 (Bailey *et al,* 1998; Harman *et al,* 1998a, 1998b). Cellulases have also been used for the improvement of the soil quality. Many studies have attempted to hasten straw decomposition via microbial routes. Cellulolytic fungi applications such as *Aspergillus, Chaetomium*, and *Trichoderma*, (Bowen *et al,* 1990; Tiwari *et al,* 1987), and actinomycetes (Abdulla *et al,* 2007) have shown promising results.

Waste utilization

The wastes generated from forests, agricultural fields, and agroindustries contain a large amount. of unutilized or underutilized cellulose, causing environmental pollution (Milala *et al,* 2005; Abu *et al,* 2000). Cellulose is the major part of plant biomass. Therefore, the wastes generated from forests, agricultural fields and agro industries contain a large amount of unutilized or underutilized cellulose. Agricultural and industrial wastes are among the causes of environmental pollution (Milala *et al,* 2005) These wastes generally accumulate in the environment causing pollution problem (Abu *et al,* 2000). Nowadays, these so called wastes are judiciously converted into valuable products such as enzymes (Ray *et al,* 1994) biofuels, chemicals, cheap energy sources for fermentation (Howard *et al,* 2003).

Conclusion

The conversion of cellulosic biomass by microorganisms is a prospective sustainable approach to develop novel bioprocesses and products. Fungal cellulases are now commercially produced by several industries globally and are being widely used in various industries like food, animal feed, fuel, paper industry, textile industry and also various chemical industries. In near future newer knowledge of excellent cellulolytic and hemi-cellulolytic systems and implementation of different biotechnological strategies will certainly bring great prospect in the field of industrial greenchemistry. The development of rapid and reliable methods for the screening of cellulases from microorganisms within inhospitable environments will allow a greater number of novel fungal cellulases to be isolated with purpose for industrial use. With modern biotechnology tools, especially in the area of microbial genetics, molecular biology novel enzymes and new enzyme applications will become available for the various industries. Improvements in cellulase activities or imparting of desired features to enzymes by protein engineering are probably other areas where cellulase research has to advance. More research works are resulting into improved scientific knowledge along with the success of meeting the growing demands of the cellulase and related enzymes for generation of environment friendly textiles, detergents, bio-pulping and bio-alcohols. Moreover, it is opening new avenues for utilization of various agro-wastes and organic pollutants as a source of renewable energy as a substitute of dumping them to cause dreadful environmental conditions.

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