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Microbial fermentation for production of cellulases and their industrial applications

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Abstract

Lignocellulosic agro-wastes being one of the most plentiful renewable biomass are perceived to be potent feedstocks for production of biofuels and biochemicals, providing a sustainable alternative to depleting fossil derived energy sources. However, for the transformation of these recalcitrant agro-wastes, a consolidated one-pot bioprocess of efficient pretreatment, saccharification and fermentation is required. The cellulases, belonging to glycoside hydrolases (GH) are the key enzymes responsible for the hydrolysis of glycosidic linkages in cellulose to release reducing sugars, which can be fermented to different valuable products viz. butanol, ethanol, lactic acid, succinic acid etc. Therefore, their cost effective production by utilizing different agro-wastes as substrates becomes a desirable pursuit. The present review summarizes some of the notable examples of their production by valorisation of different agro-wastes.Further, the importance of robust cellulases which are stable in presence of ionic liquids have also been encompassed. Besides the realm of cellulases in biorefinery development, their other commercialapplications have also been incorporated.

Keywords:*Cellulases,biorefinery, pretreatment, saccharification, agro-wastes, solid-state fermentation.*

Introduction

To secure energy sources for future, the utilization of abundant lignocellulosic biomass as renewable feedstock for generation of biofuels and biochemicals is a promising approach, as it does not compete with edible human food resources. The annual generation of 5.4×10^2 million tons of lignocellulosic biomass potentiates its role as sustainable source of energy [1]. The generic composition of biomass is constituted by 35-50% cellulose, 25–30% hemicellulose and 15–20% lignin, which are intertwined together to make a compact and rigid structure which isn't easily prone to hydrolysis[2]. The main component i.e. cellulose, is the most abundant polysaccharide on this earth and is constituted by monomeric glucose units joined together by β(1–4) glycosidic linkage. These polysaccharide chains strengthened by hydrogen bonds are arranged in compact layers, which imparts crystallinity and recalcitrance to cellulose. Thus, the transformation of this cellulosic biomass requires release of constituting monosaccharides as free fermentable sugars, which can be achieved by action of cellulases[3, 4]. Cellulases hold ~20% share in enzyme market and their demand is going to escalate further as all governmental policies have laid emphasis on replacement of 30% of petro-based fuels by biomass derived fuels by 2025 [5].

Cellulases are member of glycoside hydrolases (GH) families and belong to EC.3.2.1 class of hydrolytic enzymes. These complex hydrolytic systems are constituted by three main enzymatic complexes i.e. cellobiohydrolases or exoglucanases (E.C. 3.2.1.91), endo-glucanases (E.C. 3.2.1.4) and β-glucosidases (BGL; EC 3.2.1.21), which differ in their substrate specificities and mode of enzymatic action. The complete hydrolysis of cellulose into monomeric sugars requires their synergistic action and thus, the generic term of cellulases may collectively refer to these cellulolytic systems [1, 2]. Endoglucanases and exoglucanases acting on amorphous and crystalline parts of cellulose respectively perform synergistic hydrolysis to break glycosidic bonds and finally release cellobiose, a disaccharide. The cellobiose is then acted upon by β-glucosidase or cellobiase to liberate free monomeric reducing sugars, which can be fermented to various high-value products by appropriate microbial fermentation approaches [6, 7].

Though microbial cellulases can be sourced from aerobic, anaerobic bacteria, fungi and actinomycetes, only few are able to produce cellulases with all the three hydrolytic activities in high titre. Thus, the quest for efficient cellulase producers capable of completely hydrolysing crystalline cellulose into monomeric sugars is still in progress. Nevertheless, fungi genera are the most preferred sources due to their versatility in utilizing various substrates as well as production in high titre, as compared to other microbial sources [2, 7]. The production of cellulases is mostly carried out by submerged fermentation at commercial level, which leads to escalated costs in production process. The cellulase production constitutes ~40% cost of bioethanol production process from lignocellulosic biomass and thus, the production of cellullase in a cost-effective manner has garnered a lot of attention. In this context, the solid state fermentation (SSF) has been suggested as an effective approach for economical production of cellulase with dual benefit of valorisation of agro-wastes and contributing to solid-waste management [5]. This aspect has been elaborately encompassed in the present review. Further, the applications of cellulases in biorefinery as well as in other sectors have also been discussed.

Production of cellulases by utilizing agro-wastes as substrates in SSF

Since ancient times, microbial fermentation has been used for production of food products with high nutritive value. Nonetheless, SSF has proved to be an effective process for production of high-value microbial products viz. enzymes, organic acids, antibiotics, aroma compounds etc. The fermenting microbes in SSF utilize the agro-wastes as source of their nutrients for growth and in turn produce desirable products [8]. The utilization of a variety of agro-wastes as substrates in SSF helps in cutting the production cost by providing a viable alternate to use of synthetic media ingredients. Besides cost cutting, other advantages of using SSF for cellulase production include lower catabolic repression, low risk of contamination, lower energy consumption, production with high titre, better robustness of produced enzymes as well as enrichment in nutritive quality of spent substrate [5]. Sousa et al.[9] reported increase in protein content by 6.1 times, after fermentation of vine-shoots trimming waste by *Aspergillusibericus*with simultaneous cellulaseproduction of 20 U/g. The fermented wastes could be used as protein source for feeding ruminant animals as well as fishes.

Various studies have reported use of variety of agro-wastes viz. oil palm residues, cottonseed cake, wheat straw, rice straw, corn straw, sugarcane bagasse by various microbes for economical production of cellulase. Some of the characteristic examples of valorisation of agro-wastes for cellulase production have been summarized in Table 1.

Table 1 Cellulase production by utilizing different agro-wastes as substrates in SSF

S. No.	**Agro-wastes used as substrate**	**Microbes used for fermentation**	**β-glucosidase (IU/g)**	**CMCase (IU/g)**	**FPase (IU/g)**	**Reference**
1.	Raw oil palm frond leaves	*Trichodermaasperellum* UC1	130.09	136.16	26.03	[10]
2.	Cottonseed cake	Consortium of *Aspergillusniger, Trichodermareesei*and*Phanerochaetechrysosporium*	29.73	155.41	21.62	[11]
3.	Oil palm frond	*A.niger* DWA8	-	2.38	2.47	[12]
4.	Steam pretreated sugarcane bagasse and wheat bran supplemented with lactose	Mixed culture of *A.niger*3T5B8 and *T.reesei*RUT-C30	64.50	85.80	-	[13]
5.	Brewer’s spent grain	*A.ibericus*	-	50.00	-	[9]
6.	Hydrogen peroxide-alkalinepretreated Carnauba (*Coperniciaprunifera*) straw	*T.reesei*CCT276	-	13.00	0.90	[14]
7.	Sorghum supplemented with 0.5% peptone	*A.niger* SCBM1	63.61	41.47	2.11	[15]
8.	Ultrasound pretreated olive pomace	*A.niger*CECT 2915	-	35.00	-	[16]
9.	Wheat bran	*T.reesei* CBS 836.91	-	15.6–17.8	8–10	[17]
10.	Hot water pretreated sugarcane bagasse	*A.niger*	-	14.90	0.40	[18]

11.	Corn straw	*P. chrysosporium*	110.70	114.00	11.23	[19]
12.	Rice straw	*P. chrysosporium*	46.68	33.60	5.32	[19]
13.	Soybean bran	*A.niger* P47C3	6.50	136.70	5.60	[20]
14.	Wheat straw	*A.niger*NS-2	5.50	11.20	2.80	[21]
15.	Rice straw	Mixed culture of *A.niger* BC-1 and *T.reesei* RUT C-30 (ATCC 56765)	22.47	74.00	17.58	[22]
16.	Steam pretreatedhorti-cultural waste powder (HWP)	*T.reesei* RUT-C30	61.60	90.50	15.00	[23]

*Carboxymethylcellulase (CMCase), Filter paperase (FPase)

Nevertheless, the production level of cellulolytic enzymes achieved varies widely with the nature of agro-wastes and the fermenting microorganisms used. Some agro-wastes may require pretreatment or supplementation with another substrate before they can used be for production of cellulases effectively viz. stream pretreated sugarcane bagasse mixed with wheat bran was found to be suitable substrate for production of 85.80 U/g cellulase by co-cultivation of *A.niger*3T5B8 and *T. reesei*RUT-C30[13]. Further, the main challenge is in production of cellulase, which has all the three activities i.e. cellobiohydrolase (FPase), endoglucanase (CMCase) and β-glucosidase in high titre for efficient applications in biomass saccharification. Thus, various strategies have been employed for achieving all the three cellulolytic activities viz. selecting appropriate fermenting microrganism by natural or genetic engineering, co-cultivating different microbes as well as utilizing appropriate combination of substrates. A study reported the statistical optimization of all experimental variables to ensure all three cellulolytic activities by *A. niger* i.e. 31.5 U/g FPase, 655.9 U/g CMCase and 540.6 U/g β-glucosidase, utilizing a mixture of wheat bran and wheat straw (1.64:1.36) as substrate in SSF [24].

Some of the most important applications of these microbial cellulases are discussed below.

Applications of microbial cellulases

Biomass saccharification for production of biofuels and biochemicals

Due to depleting petro-reserves, the current research focus is on utilization of renewable lignocellulosic biomass as feedstock for production of biofuels and biochemicals. Primarily, the bioconversion involves three steps i.e. pretreatment, saccharification and fermentation [3]. The complex and recalcitrant lignocellulosic biomass is not easily accessible to enzymatic action by hydrolases i.e. cellulases and hemicellulases and therefore, requires pretreatment. The conventional modes of pretreatment include alkali or acid treatment, microwave, steam explosion and ammonia fibre expansion (AFEX) but recently, ionic liquids (ILs) have emerged as promising green solvents for biomass dissolution and reduction in recalcitrance of lignocellulosic biomass. After pretreatment, the action of cellulases is required to release free sugars, which can be fermented to bioethanol or other biochemicals by appropriate fermenting microorganisms. Most of the pretreatments including IL treatment are harsh enough to denature cellulases and thus, extensive washing of pretreated biomass is required before cellulases can be added for the second step of saccharification. Therefore, the need for robust cellulases is in high demand. For example, the IL stable cellulases are being extensively probed for carrying out one-pot pretreatment, *in-situ* saccharification and fermentation, which can be highly cost effective and prevent effluent generation [4, 25-27].

The cellulases from many microbial species viz. *Paenibacillus* sp., *Aspergillus terreus, Pseudoalteromonas* sp., *Fusarium oxysporum*, *Trichoderma aureoviride* have been reported to be stable in presence of various ILs and, thus highly effective for generation of maximum amount of reducing sugars [4]. Similar thermostable cellulases, able to withstand harsh conditions of high temperatures, salinity and wide pH range are reported from various thermophilic microbes belonging to genus of *Clostridium, Bacillus, Caldibacillus, Geobacillus, Caldocellum* and*Acidothermus.* These areconsidered very attractive for use in various biorefinery applications [28]. Numerous high value biochemicals viz. xylitol, sorbitol, glucaric acid, levulinic acid, lactic acid, itaconic acid, 3-hydroxypropionic acid and succcinic acid can be produced by fermentation of sugars released from lignocellulosic biomass by enzymatic action of cellulases [29-31].

Food processing and beverage industries

Cellulases, along with other macerating enzymes such as xylanases, pectinases are in high demand for easy clarification, reduction of viscosity, improving aroma, sugar yield, color extraction and cloud stability of several beverages [32, 33]. Similarly, the improvement in dough texture, loaf volume, dough stability and developmental time by addition of cellulases has been reported, which makes cellulases an important enzyme for varius bread and baking industries [6].

Textile industries and detergent formulations

Biopolishing and biostoning are the two major processes, wherein cellulases are extensively used in textile industry to produce glossy, smooth textures, imparting color brightness along with prevention of fuzz formation and defibrillation of lyocell fabrics[34].The cellulases from *Humicola insolens* and *Trichoderma reesei* are most commonly used for enzymatic washing, finishing and processing in cellulose based textiles [6]. The enzymatic cocktail of proteases, lipases and cellulases is being extensively used for detergent formulations for achieving improved cleansing properties with effective removal of strains viz. the endoglucanase from *Thermonosporafusca*was efficiently used for stonewashing of denim jeans [6].

Feed industries

The cellulases in combination with xylanases, phytases are very important for improving the nutritive quality of feed. Their enzymatic action helps in easier digestibilty of complex and recalcitrant macromolecules, allowing better accessibility of nutrients to feeding animals. Many studies have reported that the addition of cellulases as additives to feed, helps in reduction of anti-nutritional factors along with improvement in nutritional quality which leads to benefical health effects and improved growth performance of feeding animals [6, 33, 35].

Besides above applications, cellulases have been found to extremely useful in improving soil fertility, removal of bacterial biofilm constituted by exopolysaccharides and acting as bio-control agent and help in protecting against plant pathogens by degrading their cell wall[6, 36]. Thus, considering their wide spectrum of applications, the economical production of cellulases becomes highly relevant.

Conclusions

The current scenario of depleting fossil reserves and escalating energy demands has focused the shift from petroleum to biofuels and biochemicals derived from abundant lignocellulosicfeedstocks. Cellulases, the biocatalyst, degrading recalcitrant cellulose into fermentable free sugars play the pivotal role in biotransformation of biomass to high-value products. Apart from their central role in biorefinery applications, they find extensive use in pharmaceuticals, textile industries, animal feeds, food processing and beverage industries.Thus, the research efforts are focussed towards the cost effective production of robust cellulases which are stable at high temperature, wide range of pH and in presence of ILs.The utilization of various agro-wastes as substrates for cellulase production has proved to be an effective strategy for economical production of cellulases. However, further tailoring of these robust cellulases by mutagenesis, genetic and metabolic engineering needs to be actively pursued to harness their optimal potential in various diverse applications.

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