Renewable and Sustainable Energy Reviews xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Applications of fungal cellulases in biofuel production: Advances and limitations

Neha Srivastava[a,b,1], Manish Srivastava[c,1], P.K. Mishra[b,*], Vijai K. Gupta[d,e], Gustavo Molina[f], Susana Rodriguez-Couto[g,h], Ambepu Manikanta[b], P.W. Ramteke[a,*]

[a] Department of Molecular and Cellular Engineering, Sam Higginbottom University of Agriculture Technology & Sciences (Formerly Allahabad Agricultural Institute), Allahabad 221007, Uttar Pradesh, India
[b] Department of Chemical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi, India, 221005
[c] Department of Physics & Astrophysics, University of Delhi, Delhi 110007, India
[d] Molecular Glyco-biotechnology Group, Discipline of Biochemistry, School of Natural Sciences, National University of Ireland Galway, Galway H91 CF50, Ireland
[e] Department of Chemistry and Biotechnology, ERA Chair of Green Chemistry, Tallinn University of Technology, 12618 Tallinn, Estonia
[f] Laboratoty of Food Biotechnology - Food Engineering, Institute of Science and Technology, UFVJM, Diamantina, Minas Gerais, Brazil
[g] Ceit-IK4, Water and Health Division, Paseo Manuel de Lardizábal 15, 20018 Donostia-San Sebastian, Spain
[h] IKERBASQUE, Basque Foundation for Science, María Díaz de Haro 3, 48013, Bilbao, Spain

ARTICLE INFO

Keywords:
Biofuel production
Cellulase
Fungi
Fermentable sugars
Solid-state fermentation
Submerged fermentation
Thermal dilution

ABSTRACT

Nonrenewable fossil fuels and their serious environmental impact have forced to develop renewable & sustainable energy sources. In this scenario, cellulases have found extensive applications in the biofuel industries. Three main components of the cellulase enzymatic system, namely endoglucanase, exoglucanase and β-glycosidase, effectively convert cellulosic substrates into fermentable sugars. The commercial production of cellulase is currently performed under submerged fermentation (SmF) conditions using mesophilic microbial strains which are non-economic and also non-sustainable. Although, production of fungal cellulases using solid-state fermentation (SSF) is economically advantageous and a preferable route for industrial purposes, it suffers from a few bottlenecks (*e.g.* scale-up, difficult to control process parameters). Therefore, the present review provides an overview of the cost-effective and present scenario of cellulase production in the biofuel industries including recent advancements. In addition, the current limitations hampering the cost-effective production of cellulase have also been discussed to resolve them in the near future.

1. Introduction

The enzymatic conversion of renewable lignocellulosic materials into biofuels is an environmentally friendly and sustainable alternative to fossil-derived fuels [1,2]. However, economic viability is one of the main constraints limiting its practical implementation so far. Therefore, research efforts on bioconversion of lignocellulosic biomass into biofuels are oriented towards the development of cost-effective processes which may able to compete with the existing ones [3,4].

Cellulases play a key role in the enzymatic hydrolysis of cellulosic polymers to release monomeric fermentable sugars to produce biofuels [5]. These enzymes hydrolyze β–1,4-d-glucan linkages in the cellulose structure to release glucose, cellobiose and cello-oligosaccharides. This is the most comprehensively studied enzymatic complex including endo-glucanases (EG; EC 3.2.1.4), cellobiohydrolases (CBH; EC 3.2.1.91) and β-glucosidases (BGL; EC 3.2.1.21) [6]. Additionally, endo-glucanases release nicks in the polymeric structure of cellulose, showing reducing and non-reducing ends while cellobiohydrolases produce cello-oligosaccharides as well as cellobiose units by acting on reducing and non-reducing ends. Meanwhile, β-glucosidases cleave cellobiose to release monomeric sugar molecules during the hydrolysis reactions [7]. Therefore, a complete cellulase system is required for the synergic action to further convert cellulose into monomeric sugars for the effective production of biofuels.

Cellulase is an enzyme of industrial significance and subsidizes around 20% overall market of enzyme around the world. [7] Further, it is expected that the demand for this enzyme will be highly motivated by the commercial biofuel production industries in the near future [8]. Therefore, production and efficiency of cellulase enzyme have become one of the main attentive points to be focused at industrial scale.

* Corresponding authors.
E-mail addresses: pkmishra.che@itbhu.ac.in (P.K. Mishra), pwramteke@gmail.com, pramod.ramteke@shiats.edu.in (P.W. Ramteke).
[1] These authors have equally contributed to this work.

http://dx.doi.org/10.1016/j.rser.2017.08.074
Received 1 June 2016; Received in revised form 5 August 2017; Accepted 21 August 2017

Microorganisms are known as efficient cellulase producers and bacteria as well as fungi are considered as potential sources for cellulase production. Furthermore, among different types of microorganisms, in bacteria (*e.g.* aerobic & anaerobic bacteria) and in fungi [*e.g.* soft-rot fungi (SRF), white-rot fungi (WRF) & brown-rot fungi (BRF)] are known to be potential cellulase producers [6,9]. However, for efficient cellulase production, fungi are always preferred over bacteria owing to their versatile substrate utilization and penetration ability. Though, fungi have the ability for better cellulase production, a single fungus having all the components of the cellulase system for the effective hydrolysis of biomass is very rare. Additionally, commercial cellulase production is carried out using different fungal strains in order to develop all the three main efficient enzymes of the cellulase system (exo, endo and β-g) *via* submerged fermentation (SmF). Nevertheless, SmF is a cost-intensive process due to the low concentration of the end products and additional purification steps involved therein [10,11]. Nowadays some of the commercial companies have started to produce cellulase from genetically modified organisms (GMO), but the developing process is still at bench scale because of the high production cost [12]. Therefore, cost-effective production of cellulase is essential to make the biofuel production process economically viable. In this context, the utilization of extreme thermophilic, hyper-thermophilic, thermophilic/thermotolerant fungi as vital sources for thermostable and thermoactive enzymes have been the subject of recent studies [13]. Thermoactive enzymes have been found to be highly stable under different environmental stresses, possessing high specific activities, longer half-life and more substrate solubility with higher diffusion rates,thus, making the overall process of enzyme production more economical [14]. In addition, biomass degradation at high temperatures diminishes the cooling costs after pre-treatment and reduces the possibility of microbial contamination.

Production of biofuels using lignocellulosic non-food waste biomass falls under the second-generation biofuels. Beside this, lignocellulosic biomass can be considered as the most abundant polymer found on the earth, reaching their annual production rate around 200 billion [15]. Also, lignocellulosic biomass has enormous importance as a renewable source of carbon and can be transformed into various high added-value products such as chemicals reagents and biofuels [8,16].

On the other hand, waste biomass is used for the fermentation process and the resulting by-products can be further used to increase the nutritional quality of the animal feeds. Lignocellulosic biomass is a complex structure which is made-up of three biopolymers namely; cellulose (~ 35–50%), hemicellulose (~ 25–30%) and lignin (~ 15–20%) [17]. The transformation efficiency of this biomass into biofuels relies on various factors including the total amount of lignin present in the biomass and the effective de-polymerization (DP) of cellulose and hemicellulose into sugars [7]. Therefore, cellulose and hemicellulose, which comprise nearly two-third of the lignocellulosic biomass, are preferred as the potential feed–stock for the second-generation of biofuel production [18]. To make cellulose and hemicellulose more accessible to cellulase enzymes, different pre-treatment methods are being applied to remove the lignin cover [19]. Subsequently, cellulose-degrading enzymes are used to release the fermentable sugars. In Fig. 1 the role of cellulases during the complete biofuel production process is shown.

Therefore, considering the above issues, this review is devoted to present the production and application of fungal cellulase enzymes into the biofuel production process. The mechanism and efficiency of the cellulase enzyme system on cellulose have also been discussed with the specific classification of each cellulase enzyme. Additionally, limitation of cellulases in term of their production process, efficiency and practical applications to biofuels are also explained. Finally, various approaches to improve the production and efficiency of the cellulase enzyme system have been deliberated.

2. Mechanism of cellulose hydrolysis by cellulases

The enzymatic hydrolysis of cellulosic biomass into sugars requires the synergistic action of endoglucanase (1,4-β-D-glucan-4-glucanohydrolase), exoglucanase (1,4-β-D-glucan cellobiohydrolase; cellobiohydrolase) and β-glucosidase (β-glucosideglucohydrolase; cellobiase) [20–22]. The endoglucanases initiate hydrolysis by slicing the cellulose structure, uncovering reducing and non-reducing ends, whereas cellobiohydrolases act upon both ends to release cello-oligosaccharides and cellobiose units. Subsequently, β-glucosidases chop cellobiose to release glucose molecules. These all three steps lead to the hydrolysis process [23]. Further, during the hydrolysis reaction, the endoglucanases act on the amorphous region which is more soluble in the cellulose structure meanwhile cellobiohydrolases are active to cleave β−1,4-glycosidic bonds from the chain ends [24] to release oligosaccharides which consequently are converted into monomeric sugars by the action of theβ-glucosidases. In Fig. 2a schematic diagram of the synergic action of cellulases on cellulosic biomass hydrolysis is depicted [25].

3. Classification of cellulases

3.1. Cellulases

Cellulases belong to the O-glycoside oxidases (EC 3.2.1.) which are a broad group of enzyme systems. They hydrolyze the glycoside bond which is present between two or more carbohydrates molecules or between non-carbohydrate and carbohydrate molecules. Based on the description of carbohydrate-active enzyme database (CAZy), endoglucanases belong to the GH families (5–8, 12, 16, 44, 45, 48, 51, 64, 71, 74, 81, 87, 124 and 128). On the other hand, exoglucanases or cellobiohydrolases are related to the GH families (5–7 and 48) and β-glucosidases are found in the GH families (1, 3, 4, 17, 30 and 116) [26,27]. Generally, both endoglucanases and exoglucanases are called cellulase enzymes [28]. Because of having the carbohydrate-binding modules (CBM), endoglucanases are regarded as the primary cellulases, accountable for breaking the crystalline structure of the cellulosic substrate.

Trichoderma and *Aspergillus* sp. are known as the model fungi among a variety of fungal organisms known for their potential to produce cellulases. Nevertheless, in view of the lack of a complete cellulase system offered by these fungi, researchers are focusing on the isolation and screening of novel fungi with improved cellulase systems capable of acting synergically on the cellulosic biomass.

3.2. Endoglucanases and exoglucanases

Endoglucanase is also known as CMcase and participates in the production of cellobiose from cellulose. As discussed above, it is related to the GH family-5. Since, endoglucanases are capable of slicing β−1,4-glycosidic bonds from the internal structure and possess a cleft-shaped open active site, they are categorized as endo-acting cellulases. In addition, they are more active on the extra soluble amorphous region of the cellulosic substrate and degrade the polymerization rate by increasing the concentration of chain ends [29]. Moreover, the structure of endoglucanase can be explained in depth based on the crystalline structure of *Thermoascus aurantiacus* [27].

Further, CBHs are categorized as the exo-acting cellulases because of their ability to cut β−1,4-glycosidic bonds from the region of the chain ends and having a tunnel-shaped structure at the active site. This tunnel shaped structure avoids the re-adhering of the separated molecules to the cellulosic crystalline structure [24]. Additionally, exoglucanases or cellobiohydrolases are found in the GH family-7 and the detailed structure of this enzyme can be understood based on the crystalline structure of *Phanerochaete chrysosporium* cellobiohydrolase (Cel7A) (PDBID:1GPI). Therefore, endoglucanase plays an