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Review

Fungal solid-state fermentation and various methods of enhancement in cellulase production

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ABSTRACT

Cellulase serves vast applications in the industries of biofuel, pulp and paper, detergent and textile. With the presence of its three components i.e. endoglucanase, exoglucanase and β-glucosidase, the enzyme can effectively depolymerize the cellulose chains in lignocellulosic substrate to produce smaller sugar units that consist of cellobiose and glucose. Fungi are the most suitable cellulase producers attributing to its ability to produce a complete cellulase system. Solid state fermentation (SSF) by fungi is a preferable production route for cellulase as it imposes lower cost and enables the production of cellulase with higher titre. This article gives an overview on the major aspects of cellulase production via SSF by applying white-rot fungi (WRF) and brown-rot fungi (BRF), which include the type of lignocellulosic substrates for cellulase production, inoculum preparation and process conditions applied in SSF. The parameters that affect SSF production of cellulase such as fermentation medium, duration, pH, temperature and moisture content are highlighted. In addition, potential methods that can improve cellulase production, namely genetic modification, co-culture of different fungal strains, and development of bioreactors are also discussed.

1. Introduction

Cellulase is the enzyme that plays a key role in hydrolyzing β-1,4-glycosidic linkage in cellulose, a dominant component in plant cell wall. Cellulase which contributed to a large proportion in the global market of industrial enzymes signifies its status as an important enzyme class in the market. In fact, cellulase is the third largest industrial enzymes by dollar volume [1] and accounts for approximately 20% of the total enzyme market in the world [2]. The strong demand of cellulase is attributed to its major applications in the pulp and paper, textile, food and beverages, detergent and animal feed industries [1,3]. It has been forecasted that the demand of cellulase will be strongly driven by the commercial production of biofuel in near future [1]. This will further boost the production of cellulase due to the skyrocketing demand from the biofuel industry.

Products obtained from hydrolysis of lignocellulosic substrate by cellulase consist of mainly glucose, cellobiose and cello-oligosaccharides [1]. Among them, glucose is the most desirable product as this basic subunit of cellulose could serve as valuable feedstock for a large variety of specialty chemicals such as ethanol, organic acid and single cell protein [4,5]. To facilitate a complete hydrolysis of cellulose into glucose, a cellulase system consists of endoglucanase, exoglucanase and

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β-glucosidase are required to be present in an appreciable amount [6].

At present, cellulase can be produced via biological route by means of bacterial or fungal fermentation. There are a wide range of microorganisms capable of producing cellulase such as aerobic and anaerobic bacteria, anaerobic fungi, soft rot fungi, white rot fungi (WRF) and brown rot fungi (BRF) [2,7,8]. Most of the fungi are able to produce a complete cellulase system as compared to bacteria [9]. The commercial cellulase is most commonly produced from two strains of soft rot fungi (SRF), namely *Trichoderma reesei* and *Aspergillus niger* [1], via submerged fermentation [10]. Although fungi are able to produce a complete cellulase system, cultivation of either *T. reesei* or *A. niger* resulted in deficiency on a particular cellulase components. For example, *T. reesei* is not capable of producing substantial amount of β-glucosidase, meanwhile endoglucanase and exoglucanase are found to be lacking in the cellulase system of *A. niger* [2,11]. Besides that, submerged fermentation suffers from a major drawback that is associated with the low concentration of end products [12], and thus, further purification is needed. The additional downstream processes required for the submerged fermentation contribute to higher cost of cellulase production.

Due to the shortcomings mentioned, researchers are focusing on how to improve the titre of cellulase as well as to reduce the production cost of cellulase. One of the solutions is by applying solid state fermentation (SSF) as an alternative production route for various industrial enzymes [12] because it closely resembles the conditions of the natural habitat of filamentous fungi. Besides, the titre of enzymes produced from SSF is more superior compared to the titre produced via submerged fermentation [13,14]. Several successful cases have been reported for cellulase production by *A. niger* and *T. reesei* via SSF [15–20]. As an example, when *A. niger* was cultivated on wheat bran, corn bran and kinnow peel in the ratio of 2:1:2, a higher cellulase activity of 10.81 $U \cdot g^{-1}$ was recorded from SSF compared to 5.54 $U \cdot g^{-1}$ from submerged fermentation [18]. Similar result was also obtained through the cultivation of *T. reesei* via SSF whereby cellulase activity in the range of 250–430 $IU \cdot g^{-1}$ was obtained compared to 160–250 $IU \cdot g^{-1}$ obtained from liquid state fermentation [15].

Apart from the commonly applied soft rot fungi (SRF), white rot fungi (WRF) and brown rot fungi (BRF) have also been applied in cellulase production by means of solid state fermentation. In view of the lack of compiled literatures related to fungal solid-state fermentation in cellulase production, this review highlights the potential of WRF and BRF in cellulase production, the selection of suitable lignocellulosic substrate and the fungal inoculum preparation for SSF. Furthermore, the major process parameters affecting SSF are presented. The enhancement of cellulase production via genetic modification, co-culture of fungi and improvement of bioreactor designs are also discussed in detail.

2. Wood rotting fungi and their ability in degrading lignocellulose

Wood rotting fungi can be classified into three categories, namely white-rot fungi (WRF), brown-rot fungi (BRF), and soft-rot fungi (SRF). These fungi have the ability to depolymerize lignocellulosic materials. Among them, the SRF especially *T. reesei* and *A. niger* are the prominent cellulase producers that possess a complete cellulase system. Besides SRF, some of the WRF and BRF such as *Phanerochaete chrysosporium* and *Gloeophyllum trabeum* are also competent cellulase producers [21,22]. Most of the WRF are categorized under the phylum of Basidiomycota while only a few are from the phylum of Ascomycota [23]. On the other hand, all the BRF belongs to the phylum of Basidiomycota [23]. The WRF and the BRF that belong to the class of Basidiomycetes are reported to be able to degrade cellulose by releasing cellulase enzymes [24]. However, the fungi act differently in the degradation of lignocellulosic substrates in terms of the decay pattern and the structural changes of the degraded substrates. The WRF tend to degrade all the components namely lignin, cellulose and hemicellulose in lignocellulosic substrate once they colonize the lignocellulosic substrate [8] whereas BRF preferentially degrade the cellulose and hemicellulose contents with the lignin content being modified but not degraded [23,25].

Based on the degradation pattern, the WRF can be classified into two categories, according to their ability to either undergo selective delignification or simultaneous degradation of lignin and cellulose [8,23]. Under selective delignification, lignin and hemicellulose in the substrate are broken down before cellulose [25]. In contrast to the selective delignification, all the components in the substrate including cellulose are degraded by the WRF in accordance with the simultaneous degradation mechanism [25,26]. *P. chrysosporium* and *Ceriporiopsis subvermispora* are the examples of WRF that experience simultaneous degradation and selective delignification mechanisms respectively [27].

The relationship between the degradation pattern of WRF and BRF with the yield and activity of cellulase remains unknown. The degradation pattern as deduced from lignocellulosic components loss was found to have no apparent correlation with cellulase production [28,29]. Degradation of lignocellulosic substrate might not be taking place readily though a significant amount of cellulase enzymes has been produced [29]. Despite this, the production of enzymes might be dependent on the initial content of cellulose, hemicellulose and lignin in the lignocellulosic substrate. Liu et al. [30] has reported that cellulase was preferably secreted from lignocellulosic substrate with a higher initial cellulose content and lower lignin content. Similar observation was also stated by Philippoussis et al. [31] whereby endoglucanase production was affected by the initial hemicellulose content of the lignocellulosic substrate.

3. Cellulose hydrolysis

Cellulose is the major structural polysaccharides found in plant cell wall of lignocellulosic substrate [32]. It is commonly present together with hemicellulose and lignin in lignocellulosic substrate such as sugarcane bagasse, rice husk and wheat straw [33]. Cellulose chains are single-type polymers made up of glucose monomers and these monomers are linked together by β-1,4-glycosidic bond [2]. The chains contain highly ordered crystalline regions and some amorphous regions at an irregular interval [33]. Due to the presence