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Review

The most stirring technology in future: Cellulase enzyme and biomass utilization

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In recent years, fundamental and applied researches on cellulase enzyme have not only generated significant scientific knowledge but also have revealed their enormous potential in biotechnology. Growing attention has been devoted to its bioconversion of biomass into fuel ethanol, considered the cleanest liquid fuel alternative to fossil fuels. Significant advances have been made towards the production and alteration technology of cellulase enzyme. This review simply introduces cellulose and cellulase enzyme, gives a broad overview of the current research status of cellulase enzyme, briefly refers to its applied fields, and lastly summarizes its promising prospects.

Key words: Cellulose, cellulase enzyme, fuel ethanol, biomass.

INTRODUCTION

Currently, the problems of energy and environment are large obstacles to the development of human civilization. The world's energy demand is increasing steadily as the human population grows and economic development. However, the current predominant energy resource, which is the fossil fuel supply, is limited. In addition, since an accelerated release of fossil entombed CO_2 due to human activities, the global greenhouse effect is more and more serious. Therefore, continuous efforts have to be emphasized towards the solution of the energy supply depletion problem and the environmental impacts caused by the human activities. There has been also an increasing worldwide interest in alternative sources of energy (Aristidou and Penttila, 2000; Jeffries and Jin, 2000; Zaldivar et al., 2001), such as agricultural biomass, to substitute fossil-fuel-based energy resources. The use of biomass for energy can complement solar, wind, and other intermittent energy resources in the renewable energy mix of the future, reduce fossil fuel greenhouse gas emissions, and contribute to social sustainable development.

One of the most immediate and important applications of biomass energy systems could be in the fermentation of ethanol from biomass (Lin and Tanaka, 2006). Each year, photosynthetic fixation of CO_2 yields more than 10^{11} tons of dry plant material worldwide, and almost half of this material consists of cellulose (Leschine, 1995). In addition, agronomic residues arised from human activities, such as corn stover (corn cobs and stalks), sugarcane waste, wheat or rice straw, forestry, and paper mill discards, the paper portion of municipal waste and dedicated energy crops, also have plentiful cellulose, which can be converted into fuel ethanol. Nowadays, nearly all fuel ethanol is produced by fermentation of corn glucose in America or sucrose in Brazil, and any countries advanced in agriculture can use current technology for fuel ethanol fermentation (Lin and Tanaka, 2006). But this technology consumes a lot of food materials, costs too much, and under the pressure of world's food crisis at present, there is even a serious competition between human food and fuel ethanol. Thus, there is need to find another better way to solve this problem, and now it is mostly concentrated in the field of cellulase enzymes,

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which can hydrolyze cellulose in biomass with high efficiency.

This review will introduce some background knowledge of cellulase enzyme and its hydrolyzation mechanism, focus on its current status in research and application, and give insight into some promising prospects for its future.

CELLULOSE AND CELLULASE

Cellulose is a fibrous, insoluble, crystalline polysaccharide. It is a major polysaccharide constituent of plant cell walls, composed of repeating D-glucose units linked by β-1,4-glucosidic bonds (Jagtap and Rao, 2005) and being the most abundant carbohydrate polymer on earth (Guo et al., 2008).

Cellulose has attracted worldwide attention as a renewable resource that can be converted into biobased products and bioenergy. But nowadays, enormous amounts of agricultural, industrial and municipal cellulose wastes have been accumulating or used inefficiently due to the high cost of their utilization processes (Kim et al., 2003). Therefore, it has become of considerable economic interest to develop processes for the effective treatment and utilization of cellulosic wastes as cheap carbon sources. Cellulose is used as a food source by a wide variety of organisms including fungi, bacteria, plants and protists, as well as a wide range of invertebrate animals, such as insects, crustaceans, annelids, molluscs and nematodes (Watanabe and Tokuda, 2001; Davison and Blaxter, 2005). These organisms possess cellulases and the complete enzymatic system of them include three different types, that is, exo-β-1,4-glucanases(EC 3.2.1.91), endo-β-1,4-glucanases(EC3.2.1.4), and β-1,4-glucosidase(EC 3.2.1.21) (Wilson and Irwin, 1999). These enzymatic components act sequentially in a synergistic system to facilitate the breakdown of cellulose and the subsequent biological conversion to an utilizable energy source, glucose (Beguin and Aubert, 1994). The endo-β-1,4-glucanases randomly hydrolyzes the β-1,4 bonds in the cellulose molecule, and the exo-β-1,4-glucanases in most cases release a cellobiose unit showing a recurrent reaction from chain extremity. Lastly, the cellobiose is converted to glucose by β-1,4-glucosidase (Bhat and Bhat, 1997). This whole enzymatic process to hydrolyze cellulosic materials could be accomplished through a complex synergistically reaction of these various enzymatic components in an optimum proportion (Tomme et al., 1995).

Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen et al., 2005). But currently, two significant points of these enzyme-based bioconversion technologies are reaction conditions and the production cost of the related enzyme system. Therefore, there has been much research aimed at obtaining new microorganisms producing celllullase enzymes with higher specific activities and greater efficiency (Subramaniyan and Prema, 2000).

RESEARCH STATUS OF CELLULASE ENZYME

Cellulase is an important and essential kind of 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 cellulosic biomass. Glucose, from appropriate hydrolysis of this cellulosic biomass under the treatment of advanced biotechnology can be used in different applications such as production of fuel ethanol, single cell protein, feed stock, industrially important chemicals and so on (Gawande and Kamat, 1999; Fujita et al., 2002; Lynd et al., 2002). Given the importance of this enzyme to these so many industries, extensive interest and considerable research efforts are focused on the understanding of reaction mechanism and industrial application of cellulase, which began in the early 1950s. Nature has evolved a number of cellulases for the hydrolysis of cellulose, including exoglucanase enzymes that depolymerize cellulose from the reducing and non-reducing ends processively, and endoglucanase enzymes that cleave along the cellulose chains randomly (Warren, 1996; Coughlan, 1985; Wilson and Irwin, 1999).

Prior studies for natural cellulose hydrolysis have revealed many cellulolytic microorganisms and their complex cellulases (Lowe et al., 1987; Lynd et al., 2005). A number of fungi and bacteria capable of utilizing cellulose as a carbon source have been identified (Kim et al., 2003). Among the cellulolytic fungi, *Trichoderma reesei* has the strongest cellulose-degrading activity, and its cellulase has been widely investigated (Penttila et al., 1986; Tomme et al., 1988). Meanwhile, cellulases produced by other fungi such as the *Aspergillus* and *Rhizopus* species have also been extensively studied by several researchers (Murashima et al., 2002; Saito et al., 2003). Interestingly, recent works confirmed the production of cellulases from insect for themselves (Watanabe et al., 1998; Girard and Jouanin, 1999; Tokuda et al., 1999) along with reports on the production from symbiotic organisms harboring in the insect gut and both (Ohtoko et al., 2000; Scharf et al., 2003). These new findings challenged the traditional view of cellulase activity that cellulose digestion in insects was mediated by microbial cellulase activity in their gut.

Current understanding of the modes of catalysis and the role of various structural domains has been gained from protein engineering, X-ray crystallography and fluorescence studies, which evaluate cellulase–cellulose interactions in bulk (Pilz et al., 1990; Chapon et al., 2001; Violot et al., 2005; Pinto et al., 2007). To date, a battery of cellulase genes have been found and their gene structures and functions have also been studied (Mae et al., 1995; Murray et al., 2003; Lee et al., 2005). The purification and properties of cellulases have been described in many papers (Whitaker, 1951; Kanda et al., 1976;

Churilova et al., 1980; Anzai et al., 1984; Mori, 1992; Ye et al., 2001; Kim et al., 2005; Li et al., 2005; Ogura et al., 2006; Lee et al., 2008; Thongekkaew et al., 2008). These studies have allowed scientists to further understand the cellulase molecular mechanisms at the fundamental scale of cellulose, expand their understanding of microscale heterogeneous kinetics and identify essential amino acids in the catalytic site of various enzymes (Wilson and Irwin, 1999; Tomme et al., 1995; Henrissat et al., 1998; Jung et al., 2002; Jung et al., 2003; Zhang and Lynd, 2004). By the way, although there have been many papers dealing with more efficient cellulose degrading enzyme from various organisms such as *Trichoderma reesei*, *Trichoderma viride*, *Trichoderma lignorum*, *Chrysosporium lignorum*, *Chrysosporium pruinosum* and *Fusarium solani* (Selby and Maitland, 1967; Toyama and Ogawa, 1975; Tong et al., 1980), only limited research has identified the yeast as cellulase producer Oikawa et al., 1998; Hong et al., 2007). In addition, in the last decades, the high production cost and low yields of this enzyme are the major problems for industrial application. Therefore, investigations on ability of microbial strains to utilize inexpensive substrate (Griffin, 1973; Hurst et al., 1978; Liaw and Penner, 1990; Ju and Afolabi, 1999; Stenberg et al., 2000) and improvement of enzyme productivity (Kumakura et al., 1984; Chadha and Garcha, 1992; Hayward et al., 2000; Bailey and Tahtiharju, 2003; Villena and Gutierrez-Correa, 2006) have been done. However, by far, although the cellulase enzyme cost has dropped due to improvements in expression vectors and on-site production (Barros and Thomson, 1987; Din et al., 1990; Sahasrabudhe and Ranjekar, 1990; Harkki et al., 1991; Okamoto et al., 1994; Kobayashi et al., 2003; Kashima and Udaka, 2004), there is still a necessity of engineering a new generation of cellulase cocktails that would further reduce cellulase cost.

APPLICATION OF CELLULASE ENZYME

Along with the extensive fundamental researches of cellulases for decades of years, their application studies have also developed at a tremendous speed. Biotechnology of cellulases began in early 1980s, first in animal feed followed by food applications (Voragen et al., 1980). Subsequently, they were used in the textile, laundry as well as in the pulp and paper industries (Godfrey et al., 1996; Ito, 1997; Bajpai, 1999). Through developing for several decades, the use of cellulases has increased considerably, demonstrating their huge biotechnological potential in various industries, including above-mentioned industrial fields, brewery and wine, agriculture, as well as in research and development (Bhat and Bhat, 1997; Mandels, 1985; Bayer et al., 1994; Ohmiya et al., 1997; Bhat, 2000). These have been reviewed in many papers (Mandels, 1985; Coughlan, 1985a, 1985b; Godfrey and West, 1996; Harman and Kubicek, 1998; Uhlig, 1998).

PROSPECTS

Enhancing the activity of cellulase enzyme and reducing its production cost are two key issues in the enzymatic hydrolysis of cellulosic materials. Genetic techniques will be used to clone the cellulase coding sequences into bacteria, yeasts, fungi, plants and animals to create new cellulase production systems with possible improvement of enzyme production and activity. In addition, using cellulosic materials such as agricultural residues, grasses, forestry wastes, and other low-cost biomass can significantly reduce the cost of raw materials for ethanol production compared to corn. It is also predicted that the use of genetically engineered raw materials with higher carbohydrate content combined with the improvement of conversion technology could reduce the cost of ethanol a lot. All those will give a great help for solving the problems of energy and food in the world. In a word, the cellulase enzymes will be commonly used in many industrial applications, and the demand for more stable, highly active and specific enzymes will be also growing rapidly. So, cellulase enzyme will be as the most stirring technology in 2009, gaining the whole world attention.

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