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Biochemical Characterization of Cellulases: Review

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Abstracts

Biochemical characterization of cellulases for increasing cellulase production was one of the aims of this review paper, Biochemical characterization as this would lead to decreased production costs for enzyme systems derived from lignocellulosic materials and used in ethanol production. Overall economics of the biomass to ethanol process is largely determined by the efficiency of biomass hydrolysis. Performance of cellulase cocktails used for saccharification of cellulose in biomass is often limited by lower amounts of β-glucosidases present, which catalyse hydrolysis of cellobiose, the product of endocellulases and exocellulases to glucose. lignocellulosic materials would bring benefits to the local economy, environment, and national energy security.

Key words: - Catalytic Domain (CD), Cellulose Binding Module (CBM), Fluorescence Polarization Assay (FPA), Ammonia Fiber Expansion (AFEX)

Introduction

Biotechnological conversion of cellulosic biomass is potentially sustainable approach to develop novel bioprocesses and products. Microbial cellulases have become the focal biocatalysts due to their complex nature and wide spread industrial applications. Cellulases are composed of independently folding, structurally and functionally discrete units called domains or modules, making cellulases module. Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including both fungi and bacteria during their growth on cellulosic materials. These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. Among them, the genera of *Clostridium,Cellulomonas,Thermomonospora, Trichoderma,* and *Aspergillus* are the most extensively studied cellulase producer. Structurally fungal cellulases are simpler as

compared to bacterial cellulase systems, cellulosomes. 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.

Cellulose is the most abundant renewable biological resource and a low-cost energy source based on energy content ($3–4/GJ) (Lynd *et al.*, 2008; Zhang, 2009). The production of bio-based products and bioenergy from less costly renewable lignocellulosic materials would bring benefits to the local economy, environment, and national energy security (Zhang *et al.*, 2008). High costs of cellulases are one of the largest obstacles for commercialization of biomass biorefineries because a large amount of cellulase is consumed for biomass saccharification, for example, ~ 100 g enzymes per gallon of cellulosic ethanol produced (Zhang *et al.*, 2006 ; Zhu *et al.*, 2009). In order to decrease cellulase use, increase volumetric productivity, and reduce capital investment, consolidated bioprocessing (CBP) has been proposed by integrating cellulase production, cellulose hydrolysis, and ethanol fermentation in a single step (Lynd *et al.*, 2002, 2008).

Lignocellulose biorefinery

Current lignocellulosic ethanol technology focuses on integrated use of various component of lignocellulosic biomass to get chemicals, materials and energy. The term biorefinery owes its origin to petroleum refinery that involves processing of crude petroleum oil to get petrol and other products (e.g. plastic). At least, biomass can be processed to get bioethanol (from cellulose), xylose/xylitol/ethanol (from hemicelluloses) and lignin. Other products resulting from secondary conversion of lignin (phenolic compounds e.g. itaconic acid, syringaldehyde, etc) sugars (Furfural, HMF etc). A process that takes account of producing these and other compounds from plant biomass will be treated as biomass biorefinery.

Hydrolysis of celluloses

Hydrolysis of cellulose to glucose there are two techniques viz., steam explosion at high temperature/pressure and enzymatic one of these two techniques, enzymatic is given more focus as the other one is more energy intensive. The enzyme used for hydrolysis is cellulases which is a composite enzyme whose three basic components together to bring about complete hydrolysis of cellulose as follows-

a. Exo- β-1-4, glucanase: It acts on the non reducing end of the cellulose chain and successively removes single glucose units;
b. Endo-β-1-4, glucanase: It randomly attacks the internal β-1-4, linkages;
c. β-glucosidases or Cellobiases: It eventually breaks down cellobiose, the building unit of cellulose, to glucose.

Although from this basic concept we enter the realm of cellulase catalysed cellulose hydrolysis where we can find the involvement of a number of other enzymatic and non enzymatic proteins and considerable variations in strategies adopted by various microorganisms.

Enzymatic analyses of Endoglucanases

Endoglucanases were measured according to the method of Ghose (1987), and b-glucosidase activity was measured by salicin hydrolysis, as described by Chahal (1985). The FPA assay was performed as described by Ghose (1987), with some modifications. For this analysis, 100-ll aliquots of supernatant enzymatic solution, obtained after centrifuging, were transferred to test tubes containing 200 ll of 50 mol l)1, pH 4 Æ8 sodium citrate buffer, together with 0Æ5 · 6 cm (25 mg) strips of Whatman filter paper No. 1. The tests tubes were then incubated at 50C for 60 min. After wards, 600 ll of DNS solution was added and the tubes were incubated at 100C for 5 min. After cooling, 600 ll of water was added to each test tube and 300 ll of the solution containing reducing sugars was measured at 545 nm. One unit of FPA, endoglucanases or b-glucosidases was defined as the amount of enzyme that catalyses the release of 1l mol of reducing sugar, measured as glucose per minutes, under test conditions.

Enzymatic analyses of Exoglucanases

Exoglucanases act in a processive manner on the reducing or nonreducing ends of cellulose polysaccharide chains, liberating either cellobiose or glucose as major products. Exoglucanases can effectively work on microcrystalline cellulose, presumably peeling cellulose chains from the microcrystalline structure (Teeri, 1997). CBH is the most-studied exoglucanase. Different CBHs are produced by many bacteria and fungi, with catalytic modules belonging to families 5, 6, 7, 9, 48, and 74 glycoside hydrolases. Aerobic fungal CBHs are in families 6 and 7 only; aerobic bacterial CBHs are in families 6 and 48; anaerobic fungal CBHs are in family 48; and anaerobic

bacteria CBHs are in family 9 as well as 48. In other words, family 7 CBHs only originate from fungi, and family 48 CBHs mostly originate from bacteria.

Enhancement in β-glucosidase Activity by Enzyme Immobilisation

Immobilisation of β-glucosidase enzyme is an important tool for enhancement in its activity as immobilised enzyme facilitates efficient recovery and reuse of costly enzymes besides providing increased stability over wider ranges of temperature, pH and organic solvent. Immobilisation of enzyme has been tried on various inorganic compounds and organic polymers like chelated magnetic metal ion nanoparticles, magnetic chitosan, alginate, polyacrylamide gel, agarose and silica. Immobilisation of the enzyme has been tried by both physical adsorption and covalent modification method, the main drawback being enzyme leakage and reduced activity, respectively. Besides, use of nanoparticles for enzyme immobilisation are in vogue these days as it offers additional advantage of high surface area to volume ratio which facilitates higher enzyme loading and enhanced biocatalytic efficiency for industrial application. Immobilisation of β-glucosidase enzyme on magnetic Fe3O4 nanoparticles coupled with agarose showed enhanced activity as well as superior usability with more than 90% of enzyme activity retained even after 15 successive cycles. A 10% increase in saccharification efficiency has been observed on supplementation of immobilised T. reesei β-glucosidase enzyme on synthetic super paramagnetic magnetite to cellulases than supplementation of free β- glucosidase enzyme. Pretreatment of the immobilised β-glucosidases with cellobiose and glucose has been found to increase the activity of the enzyme but would surely add to the cost of fuel production and affect the economy of the process. It has been also observed that immobilized enzymes differ in their physiochemical properties and increased thermo stability and different pH optima have been observed as compared to free enzyme. In most of the cases, an increase in Km and decrease in Vmax value have been reported on enzyme immobilisation but the advantage of being used multiple times with enhanced stability at extreme range of temperature and pH makes the process economically feasible. The use of nanoparticles for enzyme immobilisation has been found to improve biochemical properties of the entrapped enzyme. Therefore, enzyme immobilisation can be used as an efficient tool to combat the crisis faced due to high cost of the various hydrolytic enzymes and to economise biofuel production.

Cellulase production in submerged culture

The enzyme-mediated hydrolysis of the cellulosic and hemicellulosic biomass components to release soluble and fermentable sugars is the central step of any biorefinery based on the sugar platform. Several microorganisms possess the native ability to deconstruct lignocellulosic biomass and to utilize the sugar products as sole carbon source for growth. For digestion of native cell-wall materials, three categories of enzymes are considered necessary: cellulases, hemicellulases, and accessory enzymes the hydrolytic activities of these enzymes not only make them suitable for biofuel production but also for production of aglycone moiety (antitumor agent) and low viscosity gellan. The enzyme can also be used to remove bitterness from cooked soybean syrup citrus fruit juices, and unripe olive and even to detoxify cassava. The enzymes are also involved in various biological pathways like degradation of structural and storage polysaccharides, host-pathogen interactions, cellular signalling and oncogenesis. β- glucosidases can also cause synthesis of surfactant, o-alkyl-glucoside, by reverse hydrolysis. This surfactant is suitable for biological degradation and can be used as detergent in food industry, in cosmetic and pesticide formulation, and extraction of organic dyes. However, application of β- glucosidases in biomass refining has gained unprecedented importance. All these applications require large scale production of the β-glucosidase enzyme in a cost effective manner.

Production of Ethanol from Lignocellulosic Biomass

The conversion of lignocellulosic biomass to ethanol as target product has received most research and development efforts. Ethanol is a widely applied biofuel comprising about 10% of the fuel mix in the US and 30% of the mixin Brazil, corresponding to a yearly ethanol consumption of 75 billion liter in the setwocountries. Up to 10% of anhydrous ethanol can be blended into gasoline to be utilized in standard combustion engines. Flexiblefuel vehicles, which are very common in Brazil, can be run with any gasoline ethanol mixture up to an ethanol concentration of 85% (called E85). An interesting but far less known option is fueling adapted heavy duty diesel vehicles such as trucks or buses with ED95 consisting of 95% hydrous ethanol supplemented with an ignition improver, a lubricant and a corrosion protection. ED95 produces very low emissions of particulates, nitrogen oxides and hydrocarbons compared to equivalent diesel usage. Nowadays fuel ethanol is mainly produced by fermentation of sugars derived from

first-generation feedstocks such as sugar cane or corn. Due to ethical (food vs. fuel) and environmental reasons, second-generation ethanol produced from lignocellulosic biomass is the better choice for the future and the technology for its production is reviewed in the following sections.

Conclusion

The Biochemical characterization is biological aspects of processing for cellulosic biomass becomes the crux of future research involving cellulases and cellulolytic microorganisms. Cellulases are being commercially produced by several industries globally and are widely being used in food, animal feed, fermentation, agriculture, pulp and paper, and textile applications with modern biotechnology tools, especially in the area of microbial genetics, novel enzymes and new enzyme applications will become available for the various industries.

Discussion

Biochemical characterization is Improvements in cellulase activities or imparting of desired features to enzymes by protein engineering are probably other areas where cellulase research has to advance. Overall economics of the biomass to ethanol process is largely determined by the efficiency of biomass hydrolysis. Performance of cellulase cocktails used for saccharification of cellulose in biomass is often limited by lower amounts of β-glucosidases present, which catalyse hydrolysis of cellobiose, the product of endo and exocellulases to glucose.

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