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A review of enzymes and microbes for lignocellulosic biorefinery and the possibility of their application to consolidated bioprocessing technology

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HIGHLIGHTS

- Biorefinery approaches produce fuels and chemicals through biomass conversion.
- Consolidated bioprocessing of lignocellulose is desired for effective biorefinery.
- This review focuses on the development of microbes for consolidated bioprocessing.
- The production of bio-based chemicals and advances fuels is emphasized.

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ABSTRACT

The biorefinery manufacturing process for producing chemicals and liquid fuels from biomass is a promising approach for securing energy and resources. To establish cost-effective fermentation of lignocellulosic biomass, the consolidation of saccharification and fermentation processes is a desirable strategy, but requires the development of microorganisms capable of cellulose/hemicellulose hydrolysis and target chemical production. Such an endeavor requires a large number of prerequisites to be realized, including engineering microbial strains with high cellulolytic activity, high product yield, productivities, and titers, ability to use many carbon sources, and resistance to toxic compounds released during the pretreatment of lignocellulosic biomass. Researchers have focused on either engineering naturally cellulolytic microorganisms to improve product-related properties or modifying non-cellulolytic organisms with high product yields to become cellulolytic. This article reviews recent advances in the development of microorganisms for the production of renewable chemicals and advanced biofuels, as well as ethanol, from lignocellulosic materials through consolidated bioprocessing.

1. Introduction

To ensure a reliable future source of energy and raw materials, the utilization of sustainable biomass has considerable advantages over petroleum-based energy sources. A biorefinery is a concept that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass, instead of conventional oil refinery processes. Lignocellulosic biomass obtained as agriculture byproducts and industrial residues is an abundant, inexpensive, and renewable source of sugars, and is a desirable feedstock for the sustainable production of liquid fuels and chemical products through the biorefinery processes (Menon and Rao, 2012). After pretreatment of the biomass, cellulosic and hemicellulosic materials are enzymatically decomposed into simple sugars that can be metabolized by microorganisms and converted to desired chemical products, including alcohols, fatty acids, organic acids and amino acids in microbial fermentation. Bio-ethanol is currently one of the most promising alternatives to conventional petroleum-based transport fuels. However, the recalcitrant structure of lignocellulosic biomass makes the process involved in its bioconversion more complicated than that of starchy and sugary materials, because current sugar-platform technologies require enzymatic conversion of the substrate to fermentable sugars prior to initiating microbial fermentation (Mussatto et al., 2010).

Lignocellulosic materials are mainly composed of cellulose, hemicellulose, and lignin. In plant biomass, cellulose forms highly crystalline microfibrils consisting of homopolymers of β-1,4-linked glucose units embedded in a hemicellulose, pectin and lignin matrix, a confirmation that makes the structure resistant to saccharification by hydrolytic enzymes. In general, the chemical and physicochemical pretreatment of lignocellulose causes cellulose to swell, thereby increasing its accessibility to saccharification enzymes (Chandel et al., 2012; Hong et al., 2012; Menon and Rao,

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2012). However, the hydrolysis of cellulose remains a major limiting factor for the efficient utilization of lignocellulosic materials (Matano et al., 2012; Olson et al., 2011).

To release soluble sugars from cellulose, the activities of multiple enzymes, including endoglucanase, exoglucanase, and β-glucosidase, are required (Chandel et al., 2012). As cellulase reactions are inhibited by their intermediary and final products, such as cellooligosaccharides and glucose, microbial fermentation processes that combine enzymatic hydrolysis with sugar consumption are preferential for the alleviation of cellulase activity inhibition (van Zyl et al., 2007). However, the large difference in optimum temperatures between saccharification and fermentation during simultaneous saccharification and fermentation (SSF) is a drawback of bio-ethanol production (Hasunuma and Kondo, 2012). To overcome this limitation, large amounts of saccharification enzymes by fungi and bacteria are required, which severely impacts the cost effectiveness of biorefinery from lignocellulosic materials.

The recent development of microorganisms capable of efficient cellulose hydrolysis and fermentation represents a significant step in reducing the requirement for enzyme addition into the SSF processes (Matano et al., 2012). Such consolidation of enzyme production, saccharification, and fermentation into a single process is increasingly recognized as having potential for the low-cost production of biofuels and bio-based chemicals, as the high costs of capital investment, raw materials, and equipment associated with microbial enzyme production can be avoided (Menon and Rao, 2012; Mussatto et al., 2010; Olson et al., 2011). To develop such organisms, researchers have focused on either engineering naturally cellulolytic microorganisms to improve product-related properties or modifying non-cellulolytic organisms with high product yields to become cellulolytic (Hasunuma and Kondo, 2011; Olson et al., 2011), as there is still no ideal organism to use in one-step biomass conversion.

Although early efforts toward achieving efficient biorefinery processes from plant biomass have typically focused on ethanol, recent advances in microbial metabolic engineering have enabled to perform a challenge for producing renewable chemicals and advanced bio-fuels that are compatible with existing engines and fuel distribution infrastructure (Zhang et al., 2011a). This review will focus on a discussion of microbial strains for use in consolidated bioprocessing (CBP) technologies. In particular, approaches for the conversion of lignocellulosic materials into bio-based chemicals and fuels, as well as bio-ethanol, through microbial fermentation are emphasized.

2. Hydrolysis of cellulosic materials by cellulases

2.1. Cellulase enzymes

The hydrolysis of insoluble cellulose by microorganisms requires the production of either free or cell-associated extracellular cellulases. The biochemical analyses of cellulase systems from aerobic and anaerobic bacteria and fungi performed during the past two decades have revealed that multiple enzymatic activities are needed to hydrolyze cellulose into soluble sugar monomers that can be metabolized by microorganisms (van Zyl et al., 2007; Zhang and Lynd, 2004). At least three major types of enzymes are required for hydrolyzing cellulose: (i) endoglucanase (EG) or 1,4-β-D-glucan-4-glucanohydrolase; (ii) exoglucanase, including 1,4-β-D-glucan glucanohydrolase (also known as cellodextrinase) and 1,4-β-D-glucan cellobiohydrolase (cellobiohydrolase; CBH); and (iii) β-glucosidase (BGL) or β-glucoside glucohydrolase.

EG randomly hydrolyzes the β-glycoside linkages of internal amorphous regions in cellulose to produce oligosaccharides of various degrees of polymerization and generate new chain ends. Exoglucanases hydrolyze cellulose in a processive manner from the reducing or non-reducing ends of cellulose chains to generate either glucose or cellobiose as major products. Exoglucanases can also hydrolyze microcrystalline cellulose, conceivably by peeling cellulose chains from the microcrystalline structure. BGL cleaves soluble cellodextrins and cellobiose into glucose. The correct combination of the activities and production level of each cellulase enzyme is critical for efficient lignocellulosic biomass utilization (Chandel et al., 2012).

2.2. Non-complexed cellulase systems

Fungi belonging to the genus *Trichoderma* have received intensive attention due to their high level production of secreted cellulases. In particular, the cellulase system of *Trichoderma reesei* has been the focus of research for more than 50 years. The high-level production of complex mixture of cellulases produced by *Trichoderma* species, which often exceeds 100 g L^{-1}, is the current gold standard for commercialized cellulase production (Cherry and Fidantsef, 2003). *T. reesei* produces at least two exoglucanases (CBHI and CBHII), five endoglucanases (EGI, EGII, EGIII, EGIV, and EGV), and two β-glucosidases (BGLI and BGLII) (Zhang and Lynd, 2004). The cellulase activity of *T. reesei* is predominantly attributed to CBHI, CBHII, and EGII (Nidetzky and Claeyssens, 1994). Although *T. reesei* secrets BGL, the production level of this enzyme is significantly lower than that found in other fungi, such as *Aspergillus* species. The expression levels of the *T. reesei* BGL gene are presumably sufficient to permit cell growth on cellulose, but not to allow the industrial use of BGL as a cellulase reagent. Furthermore, BGL from *T. reesei* displays product (glucose) inhibition (van Zyl et al., 2007), whereas those of *Aspergillus* species are more glucose tolerant. Sakamoto et al. (1985) isolated BGL1 and BGL2 from *Aspergillus aculeatus* and found that the two enzymes were potently active not only on soluble cellooligosaccharide substrates, from cellobiose to cellohexaose, but also on insoluble cellooligosaccharides with an average degree of polymerization of 20. Nakazawa et al. (2011) constructed a recombinant *T. reesei* strain expressing *A. aculeatus* BGL1. The supernatant from the recombinant strain grown on Avicel and xylan efficiently hydrolyzed NaOH-pretreated rice straw at a low enzyme dose. Based on these findings, cellulase cocktails from *T. reesei* engineered to express BGL from the genus *Aspergillus* appear most suitable for cellulose saccharification. *Acremonium cellulolyticus*, which efficiently produces both cellulase and β-glucosidase in addition to carboxymethyl cellulose-hydrolyzing enzyme and small amounts of xylanase, β-1,3-glucanase and amylase because of its high accessibility to cellulose, is an alternative cellulase producer (Park et al., 2011). Park et al. (2011) noted that the level of cellulase produced from pretreated waste milk pack in cultures of *A. cellulolyticus* is similar to those obtained with pure cellulose.

2.3. Complexed cellulase systems (cellulosome)

Cellulosome systems are multi-enzymatic complexes produced by anaerobic bacteria that efficiently degrade plant biomass (Fontes and Gilbert, 2010). In these systems, different types of cellulose-degrading enzymes are assembled on the structural scaffoldin subunits through strong non-covalent protein–protein interactions between the docking modules (dockerin) and complementary modules (cohesins). In addition, scaffoldin contains a carbohydrate-binding module, which binds the entire enzymatic complex to the cellulose surface. As the scaffoldin subunits are covalently bound to the cell walls of microbes by their anchoring proteins, microbes expressing cellulosomes can utilize cellulose as a source of carbon and energy. The efficient synergistic degradation of plant biomass results from the combination of targeting the

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