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Review

Improvement of lignocellulosic biomass *in planta*: A review of feedstocks, biomass recalcitrance, and strategic manipulation of ideal plants designed for ethanol production and processability

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ABSTRACT

Plant biomass, or lignocellulosic biomass, is evaluated worldwide as a potential feedstock for the sustainable production of bioenergy in the near future due to its abundance, availability and renewability. Promising sources of plant biomass include agricultural residues and energy crops; however, the natural recalcitrance of this material is a major bottleneck for lignocellulose-derived ethanol production. The current process requires pre-treatment with severe conditions to disrupt the plant cell wall structures and remove hemicellulose and lignin components so that cellulose is more accessible to cellulases. However, the generation of enzyme inhibitors/deactivators and toxic substances during pre-treatment may subsequently affect enzymatic saccharification and fermentation processing. The pre-treatment and saccharification processability can be simplified if the plant biomass resistance to biochemical or enzymatic treatment is reduced. While there are many developed pre-treatment technologies and formulated enzyme cocktails that match pre-treated substrates, there has been attempt to design ideal energy crops via plant genetic manipulation. Cellulose engineering is aimed at reducing the crystallinity of cellulose structures. Expression of cellulose-disrupting proteins, including carbohydrate-binding modules, expansins, and swollenins, produces irregular forms of cellulose fibrils, which change from tightly packed fibrils to splayed ribbons with a high sugar release after enzymatic treatment. In addition, modifying genes and proteins involved in cellulose synthesis resulted in an unusual secondary cell wall deposition and composition and a lower crystallinity index. Reducing lignin content though engineering lignin biosynthesis pathways improves the saccharification process; however, abnormal growth and plant fitness remain problematic when improper genes are selected for manipulation. Lignin composition can be modified by introducing phenolic derivatives or peptide cross-links upon lignification, and these approaches might minimise the interference with plant growth and development. Hemicellulose biosynthesis is a complicated process. Currently, the reduction of hemicellulose content relies mostly on enzymes involved in xyloglucan/glucoarabinoxylan synthesis and the arrangements of those polymers in developing wood. Additionally, several

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glycosyltransferase and glycoside hydrolases are believed to be involved in hemicellulose modification in relation to loosened cell walls. Importantly, the expression of foreign glycoside hydrolases in plants may facilitate the reduction of enzyme loadings, thus making lignocellulosic ethanol production economically viable.

1. Introduction

Ethanol produced from sugars and starch from corn is the first generation of ethanol production. In Brazil, ethanol from sugarcane has been utilised in the transportation sector since 1975, and it has been used as a gasoline blend (ethanol mixed with gasoline) or as pure ethanol in vehicles [1,2]. Due to rising prices and decreasing oil supplies, many countries such as the USA, European Union (EU) and Asian countries like Thailand have adopted this platform to substitute for pure gasoline; partial substitution—blends of 5–20% ethanol by volume in gasoline—is common in vehicles at the present time [2,3]. The procedure for starch-based ethanol production typically includes saccharification and fermentation. By using starch-degrading enzymes and chemicals under mild conditions, saccharification converts high molecular weight polymers (starch) to short oligomers and finally to monomers (glucose). Starch is a homogenous polymer of glucoses linked by α-1,4-glycosidic bonds, comprising both linear (amylose) and branched (amylopectin) forms in its structure. The molecular arrangements of α-1,4 bonds give starch a helical structure, and α-1,6-glycosidic linkages of amylopectin cause branches [4], resulting in loosely-packed amorphous structures that are easy for weak chemicals or enzymes to hydrolyse [5]. The product resulting from saccharification, glucose, can be directly taken up by common brewer's yeasts for ethanol fermentation. However, ethanol production from sugars and starches is unsustainable because these materials are foods for humans. In addition, there are some conflicts in food/fuel production, land use and plant diversity [6]. Therefore, lignocellulosic raw materials, with no competition in food production, are good alternative solutions.

2. Plant biomass as a promising bioenergy feedstock

Plants fix atmospheric carbon via photosynthesis to synthesise carbon-based polymers such as cellulose. Plant biomass refers to lignocellulosic materials available largely in the form of agricultural residues (i.e. corn stover, sugarcane bagasse, and rice straw) and crops (i.e. switchgrass, miscanthus, poplar, and willow). It is a promising resource for biorefineries because it is an abundant, low-cost, non-food material. Plant biomass is primarily composed of plant cell walls; about 75% of this material is polysaccharides that are a major source of potential sugars [7]. In Asia, many crop residues such as rice straw [8,9], cassava pulp [10], sugarcane bagasse [11], and palm residues [12,13] are promising feedstocks, whereas the USA and EU countries rely on corn stover, soybean and wheat straw [14].

Besides agricultural residues, non-food crops are also in demand for raw material supplies. Energy crops are grown for biofuel production (Table 1). The characteristics of these plants include the following: high biomass yield, high sugar content, are easy-to-grow with broad cultivation in a short period of time, tolerance to drought, high temperatures, flooding and salt stress, and low input requirements [15,16] (Table 1). Most candidates for energy crops are C4, C3, and perennial herbaceous plants. In the USA, miscanthus and switchgrass are the leading dedicated energy crops that have been selected for ethanol production [17–19]. In Asian countries, sweet sorghum and miscanthus are grown in China [20], whereas wild sugarcane is a promising plant in India [21]. In Thailand, bitter cassava varieties have been promoted for ethanol production due to their minimal inputs for growth, all-year round plantation and harvest and high carbohydrate content. In addition, the bitter type contains high levels of hydrocyanic acid, which is toxic [22]. Other promising dedicated energy crops include poplar [23], willow [24], alfalfa [25], hemp [26] and water hyacinth, which as a water-growing plant has a potential advantage over plants competing for land use [27,28]. Therefore, the selection of bioenergy crops likely depends on quantity and agronomic considerations including seasons, environment, and regional geography.

3. Recalcitrance of plant biomass to enzymatic digestion

Regardless of the lignocellulosic materials used (residues or energy crops), plant cell walls are very difficult to hydrolyse. Cell walls surround all plant cells and define their form and functions [29]. In young plant cells, the walls are typically thin and flexible, thus allowing the cells and organs to expand while the cell is dividing and growing; in contrast, the walls become considerably more rigid and reinforced by secondary cell wall deposition as older tissues mature [30]. The cell walls provide strength and resilience and form woody tissues in the plant. In addition, the plant cell walls are the first shields against the environment and natural enemies, such as pests and pathogens; thus, they have evolved into complex structures that are resistant to enzymatic degradation and biochemical attacks by living organisms [7,31]. This resistance contributes to the natural recalcitrance of plant biomass [32].

The plant cell walls are composed of three major components, namely, cellulose, hemicellulose and lignin. In nature, these biopolymers are organised into complex structures [32]. Bundles of cellulose microfibrils are associated with hemicellulosic polysaccharides. Hemicelluloses