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Review

Time dependence of enzyme synergism during the degradation of model and natural lignocellulosic substrates

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ABSTRACT

Cellulosic ethanol production relies on the biochemical (enzymatic) conversion of lignocellulose to fermentable sugars and ultimately to bioethanol. However, the cost of lignocellulolytic enzymes is a limiting factor in the commercialisation of this technology. This therefore necessitates the optimisation of lignocellulolytic enzyme cocktails through the elucidation of synergistic interactions between enzymes so as to improve lignocellulose hydrolysis and also lower protein loadings in these reactions. However, many factors affect the synergism that occurs between these lignocellulolytic enzymes, such as enzyme ratios, substrate characteristics, substrate loadings, enzyme loadings and time. This review examines the effect of time on the synergistic dynamics between lignocellulolytic enzymes during the hydrolysis of both complex (true) lignocellulosic substrates and model substrates. The effect of *sequential* and *simultaneous* application of the lignocellulolytic enzymes on the synergistic dynamics during the hydrolysis of these substrates is also explored in this review. Finally, approaches are further proposed for efficient and synergistic hydrolysis of both complex lignocellulosic substrates and model substrates. With respect to the synergistic enzymatic hydrolysis of lignocellulosic biomass, this review exposed knowledge gaps that should be covered in future work in order to fully understand how enzyme synergism works: e.g. elucidating protein to protein interactions that exist between these enzymes in establishing synergy; and the effect of lignocellulose degradation products of one enzyme on the behaviour of the other enzyme and ultimately their synergistic relationship.

1. Introduction

Lignocellulose is the most abundant biomass in the world and can be used to produce biofuels that are sustainable and give significant emission reductions [1]. Lignocellulose contains up to 75% polysaccharides such as cellulose and hemicellulose, which act as structural and storage compounds along with smaller amounts of pectin, protein, extractives and ash [1–4].

Degradation of the polysaccharides into their respective monomeric sugars within the lignocellulosic biomass is achieved biologically through the synergistic attack of glycoside hydrolases (GH), lignin modifying enzymes and other accessory enzymes [2,5]. This enzyme synergism facilitates an enhanced hydrolytic activity, where the resulting activity is greater than the theoretical sum of the individual enzyme activities. The released monomer sugars can subsequently be fermented into bioethanol by yeasts, e.g. *Saccharomyces cerevisiae*.

Due to the complexity of lignocellulose, various enzymes are required to degrade the different polysaccharides and polymers into their respective monomers (See Table 1 and Fig. 1). These enzymes include:

- Cellulases, namely endoglucanases (EG, EC 3.2.1.4), cellobiohydrolases (CBHs, EC 3.2.1.9 and EC 3.2.1.176) and β-glucosidases (BGLs EC 3.2.1.21) [6–10] (See Table 1 and Fig. 1.A).
- Hemicellulases such as xylanases and mannanases divided into two groups: depolymerising enzymes which cleave the backbone and side-chain cleaving enzymes that remove substituents which may pose steric hindrances to the depolymerising enzymes [11–13] (see Table 1 and Fig. 1B1 and B2).
- Ligninases [14] (see Table 1).
- Pectinases [15,16] (see Table 1 and Fig. 1.C).
- Accessory enzymes such as carbohydrate binding modules (CBMs) and or cellulose binding domains (CBDs), expansins and or swollenins, and Lytic polysaccharide mono-oxygenases (LPMOs) greatly enhance the activity of hydrolytic enzymes [17–19].

Organisms such as bacteria and fungi have evolved several mechanisms for the degradation of lignocellulosic material. The most common

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Table 1
The main enzymes required to degrade lignocellulose.

Polymer	Enzymes	References
1. Cellulose	Cellobiohydrolase; endoglucanase; β-glucosidase; lytic polysaccharide mono-oxygenase (AA9′s); carbohydrate binding modules (CBM's); expansins and swollenins	[6,12,86–88]
2. Hemicellulose	Endo-xylanase; β-xylosidase; α-arabinofurosidase; α-glucoronosidase; α-galactosidase; acetyl xylan esterase; glucoronoyl esterase; ferulolyl esterase	[11,86,88,89]
a. Xylan		
b. Mannan	β-mannanase; β-mannosidase; β-glucosidase; acetyl mannan esterase; α-galactosidase	
3. Pectin	Pectin lyases, pectate lyases; polygalacturonases; rhamnogalacturonan hydrolases; rhamnogalacturonan lyases and rhamnoglacturonan acetylesterases	[88,90,91]
4. Lignin	Peroxidases (laccases), oxidases (lignin, manganese, versatile) and esterases	[14,92]

mechanism for the degradation of lignocellulosic material is the free enzyme system, which mainly exists in fungi and aerobic bacteria [20,21]. Anaerobic bacteria, on the other hand, have evolved a different type of lignocellulolytic system that involves complex protein structures supporting enzymes for the hydrolysis of biomass, known as cellulosomes [22] or xylanosomes [23,24], depending on the enzymes supported on the complex. And finally, the least commonly studied system, multicatalytic enzyme systems, which combine various activities into a single gene product [25,26]. In addition to the production of a variety of hydrolytic enzymes, many microorganisms can produce multiple isozymes of the same enzyme. Examples of such instances are seen in fungi such as the genus *Aspergillus* which harbour multiple mannanases [27,28]. At least three endoglucanases [29,30] and fifteen extracellular xylanases are reported in *A. niger* [31], while *Trichoderma reesei* has up to eight endoglucanases and two cellobiohydrolases [21,32]. Also, up to six LPMOs are reported to be secreted by the bacterium, *Streptomyces coelicolor* – additionally, another bacterium reported to have a plethora of these enzymes is *Thermobifida fusca* [33]. Multiplicity of the same enzyme have been also reported in bacteria such as *Bacteroides ovatus,* which harbours two mannanases [34], and *Cellvibrio japonicus* which is reported to express five endo-acting mannanases [35].

Enzymes may also differ with regards to chemical tolerance (particularly biomass pre-treatment by-products), which may occur as a result of the microorganisms from which the particular enzyme is derived. For instance, Ximenes and co-workers have demonstrated that phenolics inhibit β-glucosidase from *T. reesei* about twice as much as that from *A. niger* [36,37]. Malgas et al. [38] have also demonstrated mannanolytic enzymes (mannanases, mannosidases and galactosidases) from different sources to interact differently with pre-treatment by-products. A better understanding of the interactions between pre-treatment by-products such as phenolics or lignin and individual enzymes will contribute to the rational design of a better enzymatic consortium (that which would be composed of enzymes resistant to pre-treatment by-products) to effectively break down the lignocellulose. However, this review does not cover this aspect about hydrolytic enzymes – reviews on the effects of pre-treatment by-products on hydrolytic enzymes can be found elsewhere [39,40].

Most enzyme synergy studies have described synergistic application of GH enzymes and other lignocellulolytic enzymes to combat lignocellulose recalcitrance and improve biomass saccharification, but have mainly focused on elucidating the components and or ratios of these

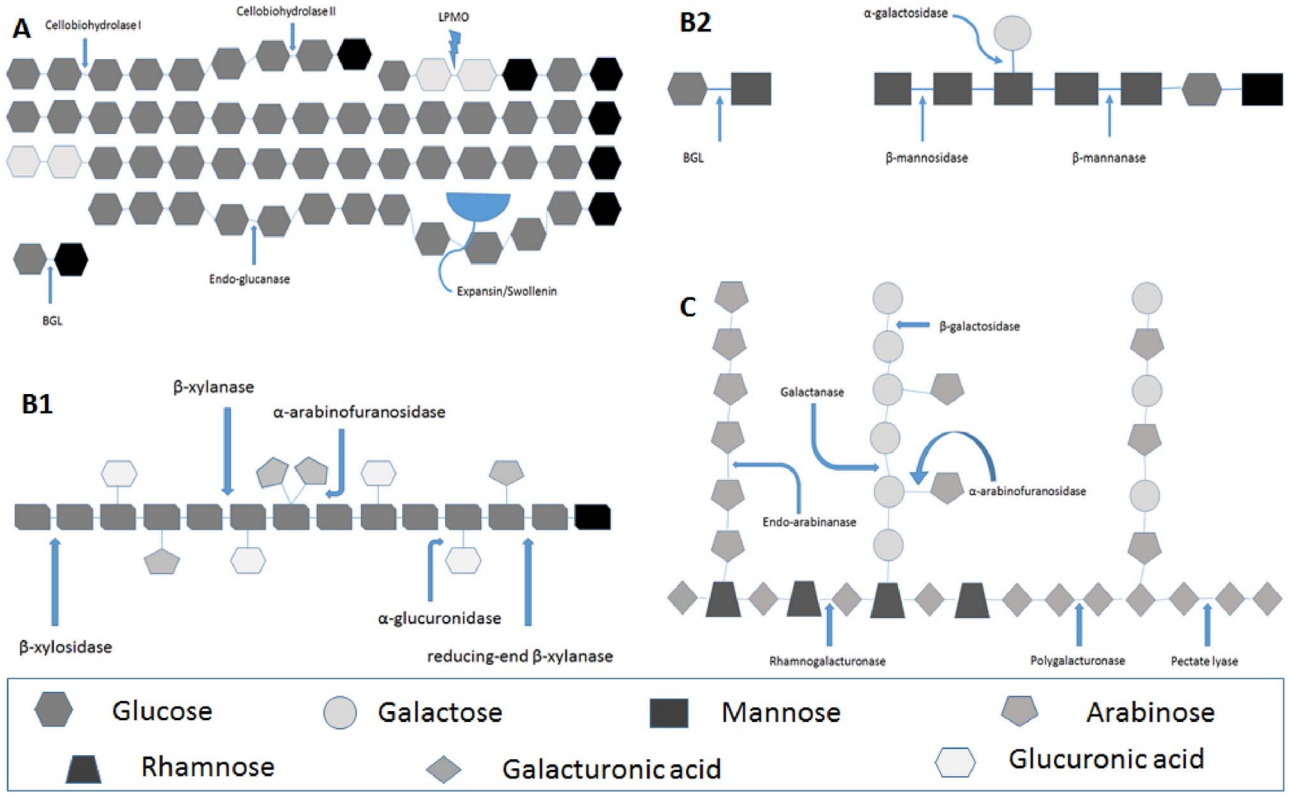

Fig. 1. Schematic representation of plant cell wall polysaccharides and selected corresponding polysaccharide-degrading enzymes. Enzymatic degradation of: (A) Cellulose; (B1) glucuronoarabinoxylan; (B2) galactoglucomannan; and (C) pectin/arabinogalactan. Black squares represent reducing ends.