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Hemicellulase for pretreatment of lignocellulosic biomass: Mini Review

Dewina Syahrifa *, Mico Batara Tohab Situmorang, Nengah Dwianita Kusywytasari and Endry Nugroho Prasetyo

Department of Biology, Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology, Surabaya 60111, Indonesia.

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Abstract

Pretreatment of lignocellulosic biomass is an important stage in bioethanol production and other bioconversions. One method that can be used is the enzymatic method. This method has a selective, efficient, and environmentally friendly approach. Hemicellulolytic enzymes such as xylanase and mannanase play an important role in hydrolyzing hemicellulose components, thereby opening up cellulase enzymes access to cellulose. The use of xylanase has been shown to increase sugar release from biomass such as rice straw and bagasse, while mannanase shows high effectiveness on mannan rich substrates such as palm kernel meal and coffee grounds. The combination of these two enzymes with cellulases accelerates saccharification and increases fermentable sugar conversion yields, making it a potential strategy in industrial scale biomass processing.

Keyword: Hemicellulase; Xylanase; Mannanase; Lignocellulosic Biomass; Pretreatment

1. Introduction

Lignocellulose derived from natural materials is generally composed of cellulose, hemicellulose, and lignin. Molecules that make up lignocellulose form a strong structure from the bonds between existing molecules [1]. Lignocellulose structure is composed of fifteen to twenty percent lignin, twenty five to thirty percent hemicellulose, and forty to fifty percent cellulose [2].

Hemicellulose is composed of various hexoses, pentoses, and uronic acids connected in both linear and branched models derived from heteropolymeric structures. The hydroxyl groups of these individual sugars can be acetylated or methylated. The backbone sugars that make up the bulk of hemicellulose are bound by 1,4-β bonds. Xylan is the main hemicellulose component that makes up 20-30% of the stems of hard woody (dicot) and herbaceous plants while in certain grasses and cereals (monocots), the amount reaches 50%. Xylan consists mostly of D-xylose, accounting for 25% of the total sugars in lignocellulosic biomass, with glucose occupying the major portion of fermentable sugars [3]. To optimize the conversion process of lignocellulosic biomass into fermentable sugars, enzymatic saccharification methods are gaining increasing attention due to their environmentally friendly and selective nature. The complexity of the lignocellulosic structure; which is composed of cellulose, hemicellulose, and lignin; makes the specific role of hemicellulolytic enzymes such as xylanase and mannanase even more important. Both enzymes have unique contributions in breaking down hemicellulose components, thereby increasing the efficiency of hydrolysis and fermentation processes on various types of biomass.

* Corresponding author: Dewina Syahrifa

2. Xylanase

Xylanase is a hydrolyzing enzyme capable of hydrolyzing polysaccharides from biomass into xylooligosaccharides, also known as saccharification. This enzyme can be produced by microorganisms such as fungi and bacteria. Examples of microorganisms capable of producing xylanase are *Aspergillus foetidus*, *Aspergillus tubingensis*, *Bacillus aerophilus*, and *Bacillus mojavensis* [4]

The work of xylanase is divided into several types. First, the back bone of xylan is randomly cut by endo-1,4-β-d-xylanase then the xylose polymer is broken down into its monomeric form by the action of β-d-xylosidases. Acetyl and phenolic side branches are removed by the action of α-glucuronidase and acetylxylan esterase. α-l-Arabinofuranosidases catalyze the removal of side groups. The ester bond present in xylan is broken by the action of p-coumaric esterase and ferulic acid esterase [2].

Xylanase holds an important role in improving the hydrolysis efficiency of lignocellulosic biomass by removing hemicelluloses that block the access of cellulases to cellulose. The combination of xylanase with ammonia pretreatment on corn straw and rice straw reported improved the saccharification and ethanol fermentation yields, proving the synergistic effect between xylanase and chemical pretreatment processes [5]. Furthermore, the application of xylanase to bagasse pretreated with alkali and hydrogen peroxide improved the efficiency of enzymatic hydrolysis, with glucose conversion approaching 99% in the early stages of hydrolysis, confirming its effectiveness in facilitating cellulase access to the substrate [6].

Xylanase works by breaking the glycosidic bonds in xylan and disrupting the attachment between hemicellulose and lignin, thus enlarging the pores of the cell wall and making it easier for other enzymes to work. Xylanase treatment resulted in significant changes in biomass morphology, such as the formation of pores and voids, which accelerated enzymatic degradation by cellulases. However, a major challenge is the non-specific adsorption of xylanase on the lignin surface, which decreases its effectiveness. Certain pretreated lignin can unproductively adsorb xylanase, and this becomes a bottleneck in the efficient biomass saccharification process [7].

3. Mannanase

Mannanases are systematically classified into several glycoside hydrolase families based on their amino acid sequence, catalytic domain structure, and enzymatic mechanism of action [8]. Some of the major families containing mannanases are GH5, GH26, GH113, and GH134. The GH5 family is multispecific and includes enzymes that can hydrolyze various substrates, including mannan, cellulose, and xylan [9]. Meanwhile, GH26 is a more specific mannanase family and is mostly found in bacteria, with a high ability to hydrolyze substrates such as galactomannan and confirmed activity against linear mannan main chains [10]. GH113 and GH134, although relatively less studied, show specific activity towards different mannan structures, such as unbranched β-mannan or short chains of mannan-derived oligosaccharides [11].

Mannanase structure consists of several main components that work together to support its function in catalyzing the breakdown of the β-1,4 bond in mannan. Mannanase has a distinctive structure, generally consisting of a catalytic domain, a carbohydrate-binding module (CBM) domain, and a linker region. The catalytic domain serves as the center of enzyme activity, while the CBM increases the affinity of the enzyme to its substrate by binding to the mannan chain, allowing for a more efficient hydrolysis process, the CBM domain also helps direct the substrate to the active site of the enzyme [12].

The mechanism of action of mannanase involves several stages, starting from the binding of the substrate to the active site of the enzyme through non-covalent interactions such as hydrogen bonding. Once the substrate is bound, the enzyme forms a stable enzyme-substrate complex. In the catalysis process, the enzyme uses amino acid residues, such as glutamic acid or aspartic acid, to facilitate the cleavage of glycosidic bonds [13]. There are two main mechanisms in mannanase catalysis: retention mechanism and inversion mechanism. In the retention mechanism, the enzyme maintains the anomeric configuration of the substrate after bond breaking, while in the inversion mechanism, the anomeric configuration changes [14]. After the cleavage of the β-1,4 bond in mannan, the final product in the form of oligosaccharide or mannose is released, and the enzyme is ready for the next catalytic cycle [15].

Mannanase (particularly β-mannanase) play an important role in the pretreatment of mannan-containing biomass, such as corn straw, bagasse, and palm kernel meal; by breaking down mannan-type hemicelluloses that are a barrier to cellulase access. Recombinant β-mannanase (TaMan5) from *Trichoderma asperellum*, produced using *Pichia pastoris*,

could synergistically increase the release of soluble sugars from corn stover and bagasse when combined with other enzymes in one enzymatic pretreatment system[16]. Meanwhile, the combination of mannanase GH5 or GH26 together with cellulase enzyme (CTec2) has been shown to effectively decompose biomass such as sugarcane bagasse and pineapple pulp, with GH5 expressing high affinity towards bagasse (86.5%)[17]. This approach demonstrates the potential of mannanases in improving the efficiency of the early stages of hydrolysis before cellulases kick in.

The main challenges are enzyme stability and substrate specificity. The study of [18] introduced a thermostable modification of mannanase (ManB085M) that is highly stable at pH 7-12 and temperatures up to 50°C and having the ability to produce mannose from coffee grounds. In addition, the development of a recombinant from *Aureobasidium pullulans* showed the acid-resistant and thermostable mannanase could accelerate the hydrolysis of galactomannan from coffee grounds, yielding up to 16.27 mg mannobiose per 100 mg substrate [19].

4. Conclusion

The utilization of hemicellulolytic enzymes such as xylanase and mannanase in the pretreatment of lignocellulosic biomass is proven to be effective in increasing the hydrolysis efficiency and the release of fermentable sugars. With their specific ability to break down hemicellulose, these two enzymes are able to open up the complex structure of biomass, facilitate the work of cellulase enzymes in the next step of saccharification, also supporting environmentally friendly bioconversion process. This enzymatic combination is a promising approach to be applied in industrial scale processing of bioenergy and other biobased products.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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