

A thermotolerant mannanase improves lignocellulose degradation for bioethanol production

Renewable energy sources, such as 2nd generation biofuels, could serve as an alternative to fossil fuels and alleviate environmental issues caused mainly by greenhouse gas emissions. Lignocellulosic biomass is considered to be a profuse and inexpensive feedstock for bioethanol production, and is turned into biofuels through the fermentation of sugars that are present in the form of polysaccharides such as cellulose and hemicellulose. However, the release of such sugars using cellulolytic enzymes is severely restrained by the complex nature of plant cell wall. The main structural components of lignocellulosic biomass, such as agricultural or forestry residues, are cellulose, hemicellulose, and lignin. Degradation of cellulose may be restricted not only by lignin, but also by hemicellulose. The fact that hemicelluloses, such as mannans, hinder the enzymatic hydrolysis of various lignocellulosic substrates is reported in several studies. Hence, a possible strategy that is being suggested involves the use of hemicellulose-degrading enzymes such as mannanases and xylanases in order to design more efficient ethanol production processes.

Forest residues & sawdust

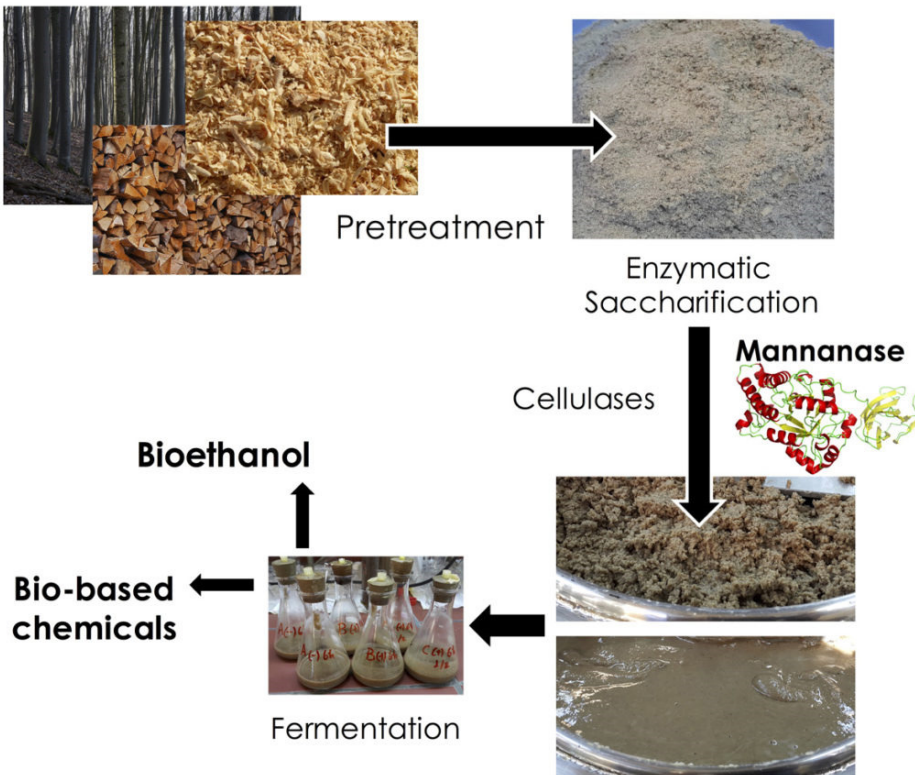


Fig. 1.

In hardwoods and grasses, the main hemicellulose component is xylan, while mannans are more common in softwoods and other structures such as plant seeds and fruits. Enzymes capable of degrading mannan

comprise β -mannanases, β -mannosidases, and β -glucosidases. Furthermore, mannanases responsible for the random hydrolysis of linkages in β -1,4 mannans have found applications in pharmaceutical, pulp and paper, food, feed, oil, and textile industries.

A desirable trait of such hemicellulolytic enzymes is high thermal stability at temperatures typical of those used during enzymatic hydrolysis of lignocellulosic biomass, which enables its incorporation in commercial enzymatic mixtures. Moreover, thermophilic enzymes are of industrial interest as they exhibit robustness and, thus, are more stable under harsh process conditions. A profuse source of genes encoding thermophilic enzymes with the potential to break down cell wall polysaccharides such as mannan is the fungus *Myceliophthora thermophila*.

A mannanase gene from the thermophilic fungus *M. thermophila*, was functionally expressed in the methylotrophic yeast *Pichia pastoris*. *P. pastoris* can produce and secrete very high levels of recombinant enzymes, which is a prerequisite for their use in industrial applications and also has been successfully used as expression system for other recombinant fungal mannanases. The recombinant enzyme was purified, biochemically characterized, and a homology model was constructed with the aim to understand better its structure–function relationships. The optimum activity of recombinant mannanase was observed at 60 °C, pH 6, and its stability was significant at temperatures up to 60 °C, retaining 72 and 30 % of its initial activity after 8 and 24 h, respectively.

Finally, with respect to incorporation of the produced mannanase into an ethanol production process, it has demonstrated capacity to enhance cellulose hydrolysis during the enzymatic saccharification of complex lignocellulosic substrates. A positive effect on the release of fermentable sugars from pretreated beech wood was observed when a commercial enzyme mix was supplemented with the recombinant mannanase. It seems that solubilization of mannan by the added recombinant enzyme renders cellulose more vulnerable to cellulolytic enzymes. Additionally, the supplementation of enzymatic mixtures with hemicellulolytic enzymes could possibly reduce enzyme loadings contributing to an overall process cost reduction for the sustainable production of biofuels and valuable chemicals, in the frame of the biorefinery concept that currently appeals significant scientific and industrial interest.

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