

Delignification and Exploitation of Lignocellulosic Biomass for biofuel Production

Lakshmi K and Sheelamary S.

Division of Crop Improvement, ICAR- Sugarcane Breeding Institute, Coimbatore,
Tamil Nadu, India.

ARTICLE ID: 013

Introduction

The world energy consumption is expected to increase multi fold by the year 2025. Biofuels produced from high biomass producing non-grain crops are called lignocellulosic biofuels, is currently the only alternative and potential energy source for future. These plant biomass-derived biofuels have great potential in countries that have limited oil resources because they reduce the dependence on fossil fuel, mitigate air pollution by cutting down greenhouse gas emissions (Matsuoka *et al.*, 2009). The primary limit of lignocellulosic biomass usage is its high degree of complexity due to its mixed composition of cellulose, hemicelluloses and lignin, in which the lignin is the compound most resistant to degradation. To effectively hydrolysis cellulose and hemicellulose into ethanol, it is essential to release them from lignin bonds. Therefore, removal of the lignin from the complex by pre-treatment is the most important step in bioethanol production.

Depolymerisation of Lignin

Lignin depolymerization is very promising process which can generate value added products from lignin raw materials. The primary purpose of lignin depolymerization is to convert the complex lignin compound into small molecules for fuels and basic chemicals or oligomers for further application. The depolymerization process can be achieved under 2 MPa pressure and 250 °C for 1 h. The results show that using sulphuric acid as catalyst and 1:1 water-ethanol mixture as the solvent can produce 70 wt% depolymerized lignin.

Enzymes involved in Lignin Degradation

Lignin is a polymer comprised of chemically distinct monolignols, the abundance of which can vary among species, among individuals, and even among cell types within an organism. Lignin is a complex, three-dimensional polymer of aromatic compounds such as p-coumaryl, coniferyl, and sinapyl alcohols, which is randomly held together by strong C–C and C–O bonds making lignin depolymerization an intriguing task. There are several types of linkages in lignin and their type and ratio is dependent on the plant source. Indeed, in nature complete degradation of lignin is mainly assured by fungi and few bacteria. White-rot fungi produce a number of extracellular enzymes that include laccases and peroxidases, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP) that degrade lignin, cellulose, and hemicellulose of the plant cell wall.

These enzymes could therefore be of a big value for further improvements for the biofuel production and make the biomass conversion less laborious. Laccases possess relatively low redox potentials (0.48–0.78 V) that restrict their action to the oxidation of the phenolic lignin components. However, a typical lignin polymer consists of 10–15% phenolic and up to 80–90% of the non-phenolic polymer. Although Lac mediator systems can oxidase non-phenolic lignin model compounds through a common mediator, 1-hydroxybenzotriazole (HBT). Lignin modifying peroxidases contain protoporphyrin IX as a prosthetic group and a newly identified enzymes called dye-peroxidases (DyP), may also participate in degradation of lignin. The application of secretomics to thermophilic fungal species exhibiting thermostable ligninolytic enzymes community will help in identification of novel ligninolytic enzymes, their characterization for deconstruction of biomass and their application in lignin valorization in the near future.

Lignin degradation strategy

Selective delignification with cellulose preservation is regarded to be the best lignin degradation strategy for reducing the saccharification recalcitrance of lignocellulosic biomass. Extensive research efforts have been dedicated towards biological lignin depolymerisation. Partial depolymerization of high-lignin content bio-refinery stream using fungal secretomes containing high laccases and peroxidase activity in the presence of an aromatic-catabolic

bacterium as a 'microbial sink' has been reported by Salvachua *et al.*, 2013. Most of the molecular methods developments are driven to find microorganisms or enzymes for cellulosic/hemicellulosic fermentation, while limited efforts are towards the biological selective delignification. Dong *et al.*, 2013 has investigated lignin biodegradation of sugarcane bagasse (SCB) by three lignin degrading fungi, *Phanerochaete chrysosporium* PC2, *Lentinula edode* LE16 and *Pleurotus ostreatus* PO45. They have identified a *Lentinula edodes* LE16 as the best fungus for selective delignification with high Polyphenol oxidase (PPO) and MnP activities on sugarcane bagasse. Role of esterase in degrading the hemicellulose–lignin matrix was confirmed and PPO as one of the primary ligninolytic enzymes. Machado *et al.*, 2017 evaluated sugarcane bagasse pre-treatment with selective white-rot fungi *Ceriporiopsis subvermispora*, and showed that forty-seven percent of potential glucose was recovered after *C. subvermispora* bio-treatment. Two filamentous fungi *Aspergillus niger* and *Trichoderma reesei*, were grown on sugarcane biomass with two levels of cell wall complexity, culm in nature and pre-treated bagasse. The production of enzymes related to biomass degradation was monitored using secretome analyses after 6, 12 and 24 hours by Borin *et al.*, during 2015. The sugars from the polysaccharides such as arabinoxylan and β -glucan were released first and assimilated by both species of fungi. The most important enzymes related to biomass degradation, including cellobiohydrolases, endoglucanases, β -glucosidases, β -xylosidases, endoxylanases, xyloglucanases, and α -arabinofuranosidases, were identified in both secretomes. They also noticed that the both fungi produce more enzymes when grown in culm as a single carbon source.

The same group of researchers conducted a RNA-sequence comparative transcriptome profiling of both fungi growing on SEB as carbon source. Particular attention was focused on CAZymes, sugar transporters, transcription factors (TFs) and other proteins related to lignocellulose degradation. The expression of these enzymes differed between *A. niger* and *T. reesei* and were regulated during transcription. Several sugar transporters were upregulated in these fungal strains which might be the potential players for delignification of the biomass besides their role in sugar uptake. Interestingly, they found that in both strains several genes that code for proteins of unknown function and pro-oxidant, antioxidant, and detoxification enzymes were induced during growth in steam exposed bagasse (SEB) as carbon source, but their specific roles on lignocellulose degradation remain to be elucidated. This was the first



report of a time-course experiment monitoring the degradation of pretreated bagasse by two important fungi using the RNA-sequence technology (Borin *et al.*, 2017).

Conclusion

Converting lignocellulosic biomass into value added chemicals and biofuels is the most potential opportunity to produce sustainable economies. However, this is greatly affected by the need for pre-treatment, effective enzymatic hydrolysis of cellulose, and fermentation. Moreover, extent of delignification solely depends on the type of the enzymes, mediators, solvents and their reaction conditions. The near future goal is to overcome all these problems and produce the cost-effective biofuel.

References

1. Dong, X. Q., Yang, J. S., Zhu, N., Wang, E. T., and Yuan, H. L. (2013). Sugarcane Bagasse Degradation and Characterization of Three white-rot Fungi. *Bioresour. Tech.* 131, 443–451. doi:10.1016/j.biortech.2012.12.182
2. Gustavo Pagotto Borin, Camila Cristina Sanchez, Eliane Silva de Santana, Guilherme Keppe Zanini, Renato Augusto Corrêa dos Santos, Angélica de Oliveira Pontes, Aline Tieppo de Souza, Roberta Maria Menegaldo Tavares Soares Dal'Mas, Diego Mauricio Riaño-Pachón, Gustavo Henrique Goldman² and Juliana Velasco de Castro Oliveira (2017). *Comparative transcriptome analysis reveals different strategies for degradation of steam-exploded sugarcane bagasse by Aspergillus niger and Trichoderma reesei.* BMC Genomics 18:501 DOI 10.1186/s12864-017-3857-5
3. Matsuoka S, Ferro J, Arruda P (2009) The Brazilian experience of sugarcane ethanol industry. *In Vitro Cell. Dev. Biol.-Plant* 45, 372– 381.
4. Salvachúa D, Prieto A, Martinez AT, Martinez MJ: Characterization of a novel DyP-type peroxidase from *Irpex lacteus* and its application in the enzymatic hydrolysis of wheat straw. *Appl Environ Microbiol.* 2013, 79: 4316-4324. 10.1128/AEM.00699-13.