Extremozymes for wood-based building blocks: from pulp mill to board and insulation products – WoodZymes project

Susana Camarero[1], David Rodríguez-Escribano[1], Rocío Pliego[1], David Talens-Perales[2], Julio Polaina[2], Petri Ihalainen[3], Alex Michine[3], Valérie Meyer[4], Michel Petit-Conil[4], Nazaré Almeida[5], Susana Pereira[5], Paula Pinto[6], Sandra Tapin-Lingua[7], Denilson da Silva Perez[7], Michael Lecourt[7], Jorge Rencoret[8], Gisela Marques[8], Ana Gutiérrez[8], François Privat[9], Gerard Deroubaix[9], Alexandru Dan Sarbu[10], Rémi Perrin[10], Jérémy Boucher[11], Thierry Sanguina[12], Marta Pérez-Boada[1] and Angel T. Martínez[1]

[1]CIB-CSIC, Madrid, Spain; [2]IATA-CSIC, Valencia, Spain; [3]MetGen Oy, Kaarina, Finland; [4]CTP, Grenoble, France; [5]RAIZ, Aveiro, Portugal; [6]The Navigator Company, Setubal, Portugal; [7]FCBA, Grenoble, France; [8]IRNAS-CSIC, Seville, Spain; [9]FCBA, Paris, France; [10]Soprema, Strasbourg, France; [11]Fibre Excellence, Saint Gaudens, France, [12]FINSA France, Morcenx, France.

ABSTRACT

Enzymes can substitute harsh and energy-demanding chemical treatments for production of bio-based building blocks and products from wood processing. However, their properties need to be adapted to the extreme operation conditions (such as high T and pH) commonly used by these industries. Here, we summarize the main results obtained during the WoodZymes European Project (www.woodzymes.eu), which aimed to provide tailor-made extremozymes and extremozyme-based processes never assayed before in wood biorefineries. Novel extremophilic enzymes active on kraft lignin (laccases) and xylan (xylanases) were developed and produced at pilot or industrial scales. The enzymatic fractionation of kraft lignins using the METNIN™ lignin refining technology, and the extremozyme-aided delignification and bleaching of kraft pulps were demonstrated at pilot scale. The resulting lignin and hemicellulose derived compounds were chemically characterized and applied as components of phenol-(lignin)-formaldehyde resins for wood panels and of polyurethane foams, or as papermaking additives. The new extremozymes were also applied to improve some of the latter applications. The techno-economic and environmental assessment of the new materials and processes, developed in WoodZymes project, showed that extremozyme-based processes led to clear benefits in energy savings during the refining of pulp or wood fibres, enabled lower addition of harsh chemicals (e.g. ClO_2 during pulp bleaching), and resulted in a lower carbon footprint of the new bio-based products by substitution of fossil-derived components.

Keywords: *Extremophilic enzymes, laccases, xylanases, kraft cooking, pulp bleaching, lignin, hemicelluloses, bio-based building blocks, polyurethane foam, fibreboard, paper pulp.*

INTRODUCTION

One of the main challenges faced by the wood industry today is to attain the full exploitation of all wood components. During kraft pulping, the predominant technology to produce paper pulp from wood, only cellulose is exploited whereas the majority of lignin and many hemicelluloses are released into the black liquors and burnt in the recovery boiler to generate heat and energy for the mill and to recover the cooking chemicals. In particular, kraft lignins account for about 85% of the total lignin production in the world, but there is no established catalytic technology to achieve their depolymerization into aromatic chemicals and building blocks, despite their great potential as bio-based substituents of fossil-based counterparts in many applications. On the other hand, hemicelluloses recovery could enable their integration in the kraft process as pulp refining aids and papermaking additives.

Biotechnology is expected to make an important contribution in the wood conversion sector. However, the integration of enzyme biocatalysts in wood processing requires enzymes adapted to the extreme operating conditions of the current industrial processes, either found in extremophilic organisms or developed by protein engineering such as directed molecular evolution.

In this context, the main objective of the WoodZymes project was to supply the wood and paper pulp industries with new extremophilic enzymes (extremozymes) able to deconstruct the lignin and hemicellulose polymers at the conditions of high temperature and alkalinity typical of the kraft process, aiming to valorise these underutilized wood fractions. The goal was to obtain bio-based building blocks through the extremozyme-assisted depolymerization of kraft lignin and delignification and bleaching of kraft pulps. The lignin-derived phenols were tested as resin precursors in the manufacture of fibreboards or as components of rigid polyurethane (PU) foams to replace petroleum-derived chemicals, whereas the hemicellulose-derived sugars were applied as additives in papermaking. Moreover, the addition of extremozymes was evaluated in some of the latter applications.

The WoodZymes consortium covered the whole value chain from feedstock suppliers to end-users, comprising: four world-leading companies of the pulp & paper (The Navigator Company and Fibre Excellence), fibreboard (FINSA) and PU foam (Soprema) sectors; one biotech SME commercializing industrial enzymes (MetGen); and six reputed research institutes (CSIC: CIB, IATA and IRNAS) and technological centres (CTP, FCBA and RAIZ) of the wood, cellulose, lignin and enzyme sectors.

EXPERIMENTAL

Kraft lignins were prepared at semi-pilot scale from industrial black liquors of The Navigator Company eucalyptus mills and Fibre Excellence hardwood and softwood mill, by CO_2 bubbling neutralization, filtration and washing **(**InTechFibres platform protocol at CTP).

Kraft lignin phenolic compounds were produced through METNIN™ enzymatic lignin refining technology developed by MetGen and chemically characterized by GPC, 2D-NMR and ^{31}P-NMR (CSIC-IRNAS).

Novel extremophilic enzymes adapted to industrial operation conditions were obtained by *in silico* screening of protein databases of extremophilic bacteria by CSIC-IATA, or protein engineering to confer extremophilic properties to in-house fungal (CSIC-CIB) or bacterial (MetGen) enzymes. The production of the best candidates was up-scaled to pilot or industrial scale using MetGen's ENZINE® technology platform.

Extremozyme-aided delignification and bleaching assays of kraft pulps were carried out by CTP and RAIZ, first at lab scale for evaluation of different conditions, and then at pilot scale for the optimized extremozyme-aided bleaching sequence. Hemicelluloses were isolated from bleached eucalyptus kraft pulp and chemically modified by FCBA and evaluated as papermaking additives by CTP.

Fibreboard manufacture trials and formulation of resole resins were carried out at FCBA facilities in collaboration with FINSA. Lignin oxypropylation and PU foaming tests were carried out by SOPREMA.

RESULTS AND DISCUSSION

New thermophilic and alkaliphilic xylanases and laccases, from bacteria and fungi, were discovered by *in silico* screening of protein databases of extremophiles or designed by enzyme engineering. The extremozymes were produced in heterologous hosts and fully characterised. Best performing ones reached high-pilot or industrial scale production using ENZINE® technology platform to be used in the application tests. Extremophilic fungal laccases able to depolymerise kraft lignin at alkaline pH were developed by directed evolution (1). The most extremophilic bacterial laccase (max. activity at 40ºC, pH 10.6) whose impact on kraft lignin depolymerisation and demethylation was demonstrated, reached industrial scale production. New bacterial xylanases with remarkable extremophilic properties were discovered through *in silico* analyses (2,3). One in particular, Xyn11, presented outstanding activity on xylan at pH 10.5 and 90ºC (3,4). The enzyme was produced at 400 L pilot scale.

Kraft lignins were obtained from the black liquors of eucalyptus, softwood and hardwood kraft pulp mills and chemically characterized. Enzymatic fractionation of these lignins was carried out at pilot scale by using METNIN™ technology that combines the oxidation and depolymerisation of lignin by laccase at alkaline conditions, with a cascading membrane operation to limit repolymerisation of lignin products. The resulting lignin fractions exhibited varied molecular weight distribution, with different structural and physicochemical properties, phenolic and carboxylic groups being the most relevant functional groups evaluated.

The integration of extremozymes in the production of bleached kraft pulps was investigated. Extremophilic laccases had a positive effect on pulp bleachability and delignification, resulting in lignin enrichment in the effluent. This positive effect on pulp bleaching efficiency could result in 11% ClO_2 savings compared to the reference bleaching sequence. The most extremophilic xylanase (Xyn11) enabled to save 25% of ClO_2 during bleaching of eucalyptus kraft pulp at pilot scale, without affecting the pulp mechanical properties, with concomitant reduction of organochlorinated compounds in the effluent.

Hemicelluloses of great purity were isolated from fully bleached eucalyptus pulp by combining extremophilic enzymes and alkaline treatments. These sugars were chemically modified and evaluated as refining aids and paper binders. Cationised hemicelluloses allowed up to 80% energy savings during pulp refining, and notably improved the mechanical and physical properties of paper sheets when added as paper binders, Moreover, the paper sheets can be recycled preserving the enhanced properties and without increase in effluent pollution.

One end-use case of WoodZymes project was the production of fibreboards using extremozymes and lignin-derived phenols. The application of extremophilic laccases during the defibration of wood chips reduced the energy required during defibering without affecting board performances. Lignin fractions from METNIN™ process were assayed as phenol substituent for the preparation of lignin-phenol-formaldehyde (LPF) resole resins aiming to reduce the content on fossil-derived chemicals. A deep chemical characterization of the samples provided valuable knowledge on the linkages formed during the resin formation process. It was shown that the LPF resins met the target adhesive properties in the boards by adjusting the process conditions, and that the use of the lignin-derived phenols can significantly decrease not only the phenol content of resole resins but also the formaldehyde content. Besides, addition of laccase to resin formulation allowed to further reduce the amount of formaldehyde in the resin, thus decreasing the environmental impact of the fibreboard production process.

Another WoodZymes project end-use case was to use lignin and lignin-derived phenols as substituents of fossil polyols in the formulation of rigid PU foams. Liquid polyols compliant with foaming requirements were obtained by oxypropylation of the lignin samples. Up to 50% substitution of fossil-derived polyols by these renewable polyols could be attained. However, due to the heterogeneous reactivity profiles of the different lignin samples, deeper studies are required before scaling up the lignin oxypropylation reaction to produce PU foams.

Finally, Life Cycle Assessment analyses showed that WoodZymes products entail an environmental benefit, with all impact categories analysed being not changed or positively affected. The main improvement concerns the use of the LPF resins for fibreboard manufacture, resulting in > 40% reduction of fossil carbon emissions.

CONCLUSIONS

These results illustrate the potential of extremozymes in the global bio-based economy, contributing to the sustainability and competitiveness of cellulose pulp, fibreboard and polyurethane industries, and establishing a novel direct link between the pulp and wood sectors. The new extremozymes and extremozyme-aided technologies enable the valorisation of residual biomass streams of kraft pulp mills into bio-equivalents of fossil-based components in several products, while open new scenarios for the industrial processes. WoodZymes biotech approach assists to overcome kraft mills' bottleneck (i.e. the limited capacity of the recovery boiler) through the integrated production of lignin from black liquors in the mill. Moreover, it enables smooth incorporation of biomass by-products into the production process (e.g. use of hemicelluloses as pulp refining aids and paper binders), closing the loop of the kraft process. The adoption of clean and environmentally sound technologies based on extremozymes leads to energy and chemical savings in the industrial processes, while increasing the process efficiency. Moreover, the sustainability of the WoodZymes products is enhanced by turning from fossil to bio-based components, reducing the dependence on fossil resources with cost benefits (e.g. through the use of competitive renewable lignin) and less environmental impact (e.g. reduced carbon footprint), as shown by life-cycle assessment.

ACKNOWLEDGMENTS

WoodZymes project was funded by the Bio-based Industries Joint Undertaking (BBI JU) under GA 792070. The BBI JU received support from the EU's H2020 research and innovation programme and the Bio Based Industries Consortium.

REFERENCES

1. Rodríguez-Escribano, D., Pliego R., de Salas, F., Aza, P., Molpeceres, G. Camarero, S. (2021) Tailor-made extremophilic fungal laccases. EP21382978
2. Talens-Perales D, Sánchez-Torres P, Marín-Navarro J, Polaina J (2020) *In silico* screening and experimental analysis of family GH11 xylanases for applications under conditions of alkaline pH and high temperature. Biotechnol. Biofuels, 13:198
3. Talens-Perales D, Jiménez-Ortega E, Sánchez-Torres P, Polaina J (2021) Phylogenetic, functional and structural characterization of a GH10 xylanase active at extreme conditions of temperature and alkalinity. Comput. Struct. Biotechnol. J., 19:2676
4. Polaina, J., Talens-Perales, D., Sanchez-Torres, P. (2020) Xylanase enzyme with extreme thermostability and alkaline stability. EP20382849