BIOCATALYSIS FOR THE TREATMENT OF LIGNOCELLULOSIC BIOMASSES

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State of the art

The lignocellulosic biomass of the grape stalks is principally composed by three biopolymers whole cellulose, hemicellulose and lignin (**Figure 1**). These three polymers are organized in a complex structure where they are linked by a dense network of ethereal and hydrogen bonds with hemicellulose and lignin which cover cellulose. For this reason this biopolymer is trapped and protected within this structure and it is very difficult to break it into the monosaccharides.

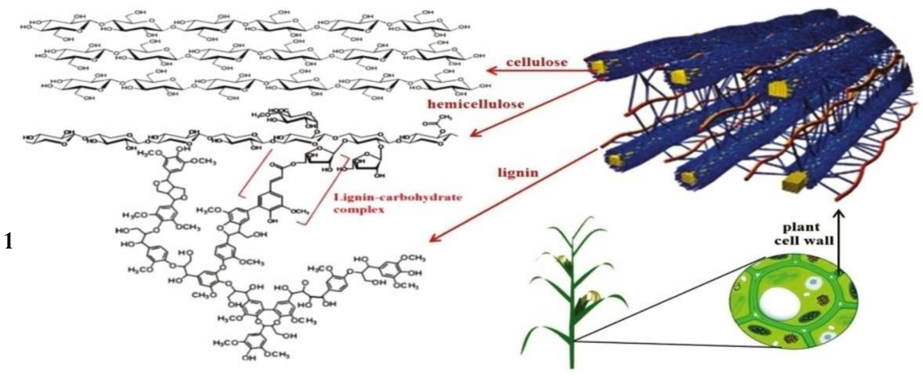
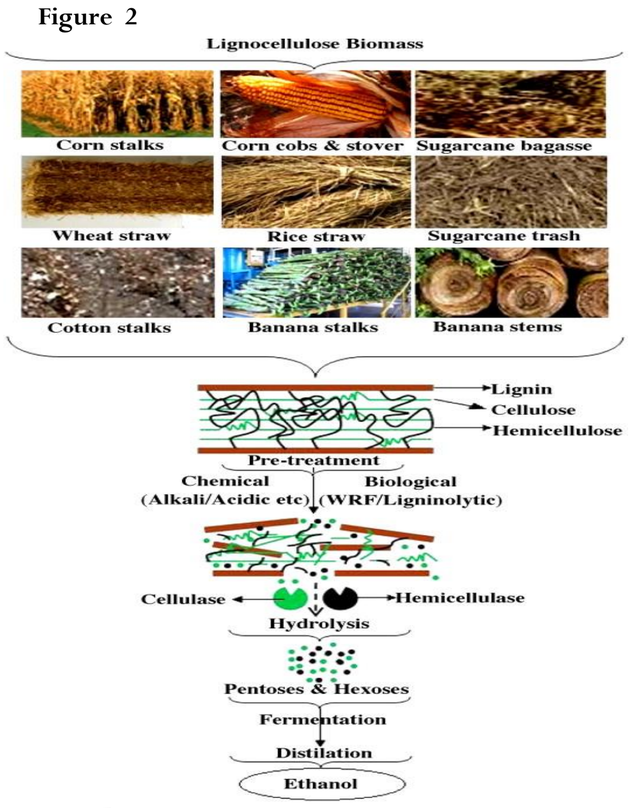


Figure 1

Figure 2



Thus it is necessary to break and separate these three biopolymers to obtain the phenolic aromatic moieties from lignin and monosaccharides from hemicellulose and cellulose.

The approach of biocatalysis with lignocellulotyc enzymes based on the first use of laccase and then hemicellulase and cellulase represents a valid alternative to the chemical methods of hydrolysis with acids and bases and it allows to obtain the same products, but in a cleaner way (**Figure 2**). The laccase enzyme, in the pretreatment phase, causes the break of lignin structure to yield aromatic phenolic compounds some with potential industrial interest as buiding blocks for the fine chemistry (e.g vanillin). Hemicellulase and cellulase allow to obtain sugars as glucose and xylose from cellulose and hemicellulose for microbial fermentations, biofuels and biopolymers.

Materials & Methods

The biocatalytic activity on the lignocellulosic biomass of the grape stalks was made with the enzymes laccase, hemicellulase and cellulase during 24 and 48 hours with 100, 200 Units of enzymatic activity, respectively. The grape stalks were dried at the 60°C temperature and subsequently milled. The powder was then suspended in a citrate buffer at pH 4.6 and treated with enzymes for 24 and 48 hours, respectively at 45 °C. The carbohydrate analysis, for the qualitative determination of sugar oligomers and monosaccharides derived from the biocatalytic break of cellulose and hemicellulose, was carried out using phenol/sulfuric acid colorimetric method. The formation of phenolic compounds and monosaccharides was monitored with the UV-VIS spectrophothometry at 280 and 500 nm.

Results

The following figures (**from 3 to 6 fig.)** show the graphs of absorbance according to the biocatalytic activity of the these enzymatic groups during 24 and 48 hours. The absorbance at 280 nm (then also phenolic compounds) increase in both 24 and 48 when the laccase ranges from 25 to 100 Units. In the 24 hours, the enzymatic systems that give higher absorbance values and therefore allow a greater release of phenolic compounds are the combination of 100 Units of laccase and 200 U cellulase, and the combination triple of 100 U laccase, 200 U hemicellulase and 100 U cellulase. At 48 hours, the results are confirmed for laccase but higher effect it is observed by binary system formed by 100 U laccase and 200 U cellulase. The absorbance at 500 nm undergo a strong increase, particularly pronounced within 48 hours, keeping the concentration of laccase constant (100 Units) and increasing those of hemicellulase and cellulase (200 Units). The enzymatic system that provides the highest absorbance value at 500 nm in both the 24 and above all within 48 hours and hence a best release of sugar oligomers and monosaccharides is that constituted by the laccase 100 and hemicellulase and cellulase 200 Units of enzymatic activity.

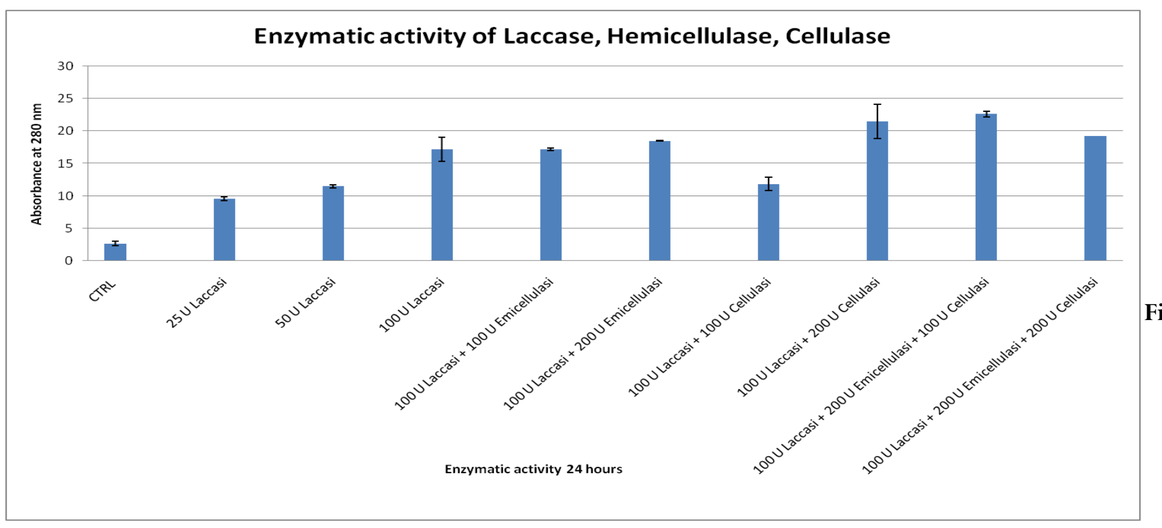


Figure 3

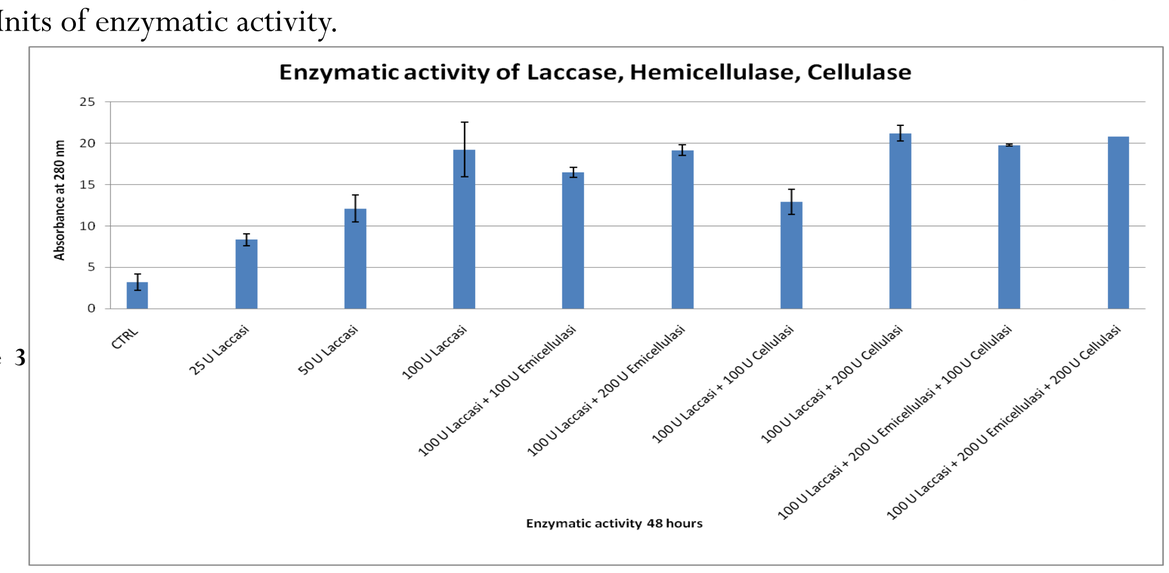


Figure 4

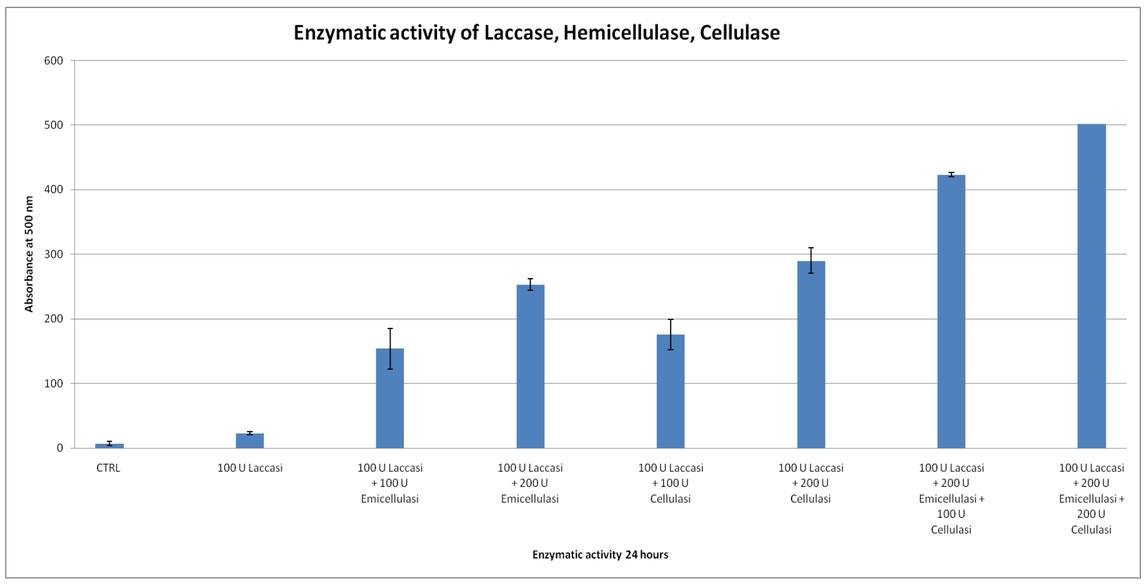


Figure 5

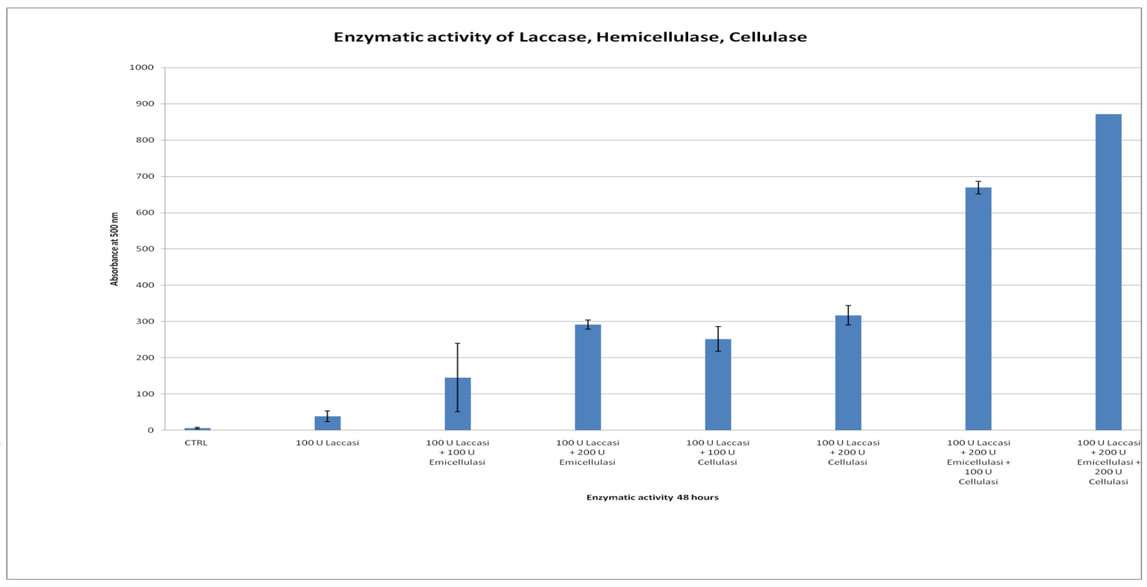


Figure 6

Conclusion

The combination of the laccase, hemicellulase and cellulase enzymes improve the release of the sugar oligomers and monosaccharides and the use of a growing concentration of laccase enzyme leads to a better release of the biophenols resulting from the splitting of the lignin complex structure. This biocatalytic approach can be improved with the use of the green chemistry solvents ionic liquids to dissolve the lignocellulosic biomass and to increase the accessibility to the biopolymers for the enzymes in the field of biocatalysis in ionic liquids.

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