FLUORESCENCE MICROSCOPY TECHNIQUES TO STUDY ENZYMES DYNAMICS INSIDE PLANT CELL WALLS

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Biomass deconstruction using lignocellulolytic enzymes in order to produce added value compounds is considered as a promising alternative to the use of fossil carbon resources. However, cellulose, hemicelluloses and lignin, the main constituents of the plant cell walls, form a complex tridimensional structure that impedes enzymes' action either by physical obstruction or by non-specific interactions with lignin [1]. Pretreatment processes are thus mandatory in order to disorganize the plant cell walls and enhance enzymes efficiency. Many studies have focused on optimizing the pretreatment processes by analyzing the substrate modifications or enzymes' activity enhancement, but little is known about how pretreatment impacts enzymes dynamics inside the plant cell walls.

To address this question, we have submitted 2 cm-long poplar fragments to different pretreatment known to have contrasted effects both on structure and composition of the plant cell walls [1]. Pretreatments efficiency was assessed by analyzing chemical composition modifications and by following glucose releasing during the enzymatic hydrolysis over 96h

Fluorescence techniques in confocal microscopy were used to assess accessibility and interactions of enzymes inside the plant cell walls. Two kinds of rhodamine-labelled probes were used for these experiments: polyethylene glycol probes known to mimic enzymes non-specific interactions [2] with lignin, and dextran probes that are considered as inert. Probes of different sizes (hydrodynamic radii of 2.3, 3.5 and 4.7nm) were used to cover the size range of the lignocellulolytic enzymes. Fluorescence Recovery After Photobleaching experiments were performed in order to measure probes mobility inside the plant cell walls and so physical accessibility of the different biopolymers. Probes interactions with the plant cell walls compounds, especially lignin, were assessed by Fluorescence recovery Energy Transfer, thus providing information on the chemical accessibility to the biopolymers.

This work shows that fluorescence confocal microscopy provide powerful techniques to unravel biomass recalcitrance. The use of fluorescent probes displaying similar properties to lignocellulolytic enzymes can help understand enzymes dynamics inside the plant cell walls, thus providing new clues to improve biomass valorization.

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