Two images illustrating cell-to-cell heterogeneity in the Miscanthus stem, with and without fluorescence

Participants

- Synchrotron SOLEIL (DISCO and SMIS Beamlines)
- UMR BBF, Marseille

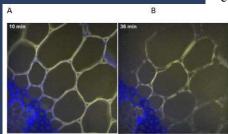
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Action of lytic polysaccharide monooxygenase on plant tissue is governed by cellular type

(2017) Scientific Reports, 7 Chabbert B *et al.*

Synchrotron Time-Lapse Imaging of Lignocellulosic Biomass Hydrolysis: Tracking Enzyme Localization by Protein Autofluorescence and Biochemical Modification of Cell Walls by Microfluidic Infrared Microspectroscopy

(2018) Frontiers in Plant Science 9 Devaux M F *et al.*



Deep-UV fluorescence time-lapse images of degradation kinetics in a maize stem

Fig A. © Inra, Devaux et al. Fig. B

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Synchrotron Soleil sheds real-time light on the enzymatic deconstruction of lignocellulosic biomass

The enzymatic conversion of lignocellulosic biomass is an attractive route to bio-based substitutes for fossil-fuel compounds as it can mobilize highly-selective enzymes in 'soft chemistry' processes. However, if not pretreated, the lignocellulosic cell walls often prove recalcitrant to enzymatic digestion.

► RESULTS

An ambitious strategy coupling microscopy imaging with the SOLEIL synchrotron's deep-UV and infrared sources made it possible to track the action of cellulases on lignocellulosic biomass in real time. On the DISCO beamline, the deep-UV source served to visualize the enzymes and cell walls using their autofluorescence properties. On the SMIS beamline, a microfluidics device purpose-engineered for FTIR microspectroscopy served to track subtle shifts in chemical composition of the cell walls. Chemometric spectral and image analysis served to compare reactivities according to cell-wall type.

UV fluorescence imaging on the grass stems showed a contrasted cell-type-dependent distribution of commercial cellulases, right

from the start of the reaction. On non-pretreated samples, the enzymes swiftly colonized the non-lignified parenchyma cell walls but not the lignified cell walls. FTIR microspectroscopy revealed that different cell types degrade at different rates, and that hemicelluloses degrade before cellulose. Adding a cellulose-oxidizing enzyme (LPMO) to the cellulase cocktail boosted the progress of the enzymes and the degradation of pretreatment-delignified cell walls. This LPMO boost, like for cellulases alone,

was cell-type-dependent, indicating contrasting recalcitrance levels of plant cell walls.

► FUTURE OUTLOOK

Harnessing these innovative approaches offers an insightful strategy for tracking the dynamics of grass cell wall deconstruction at microscopic scale and how different kinds of enzymatic cocktails work on cell-type-dependent recalcitrance levels. Moving forward, we now plan to investigate the action of enzyme mixtures designed with specific purpose-optimized activities for cell wall variability and cell wall responsiveness to physico-chemical pretreatments, to gain deeper insight into enzyme-mixture synergies.