A whole new look at lignin recalcitrance

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Real time and quantitative imaging of lignocellulosic films hydrolysis by Atomic Force Microscopy reveals lignin recalcitrance at nanoscale.

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- PhD thesis defended in December 2018 by Eléonore Lambert

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Context

To optimize the processing of lignocellulosic biomass, the way forward hinges on imaging, which gathers approaches drilling down on a gradient of scales–from mm to nm–via photon, electron and atomic force microscopy techniques. These are powerful tools for understanding the recalcitrance of the biomass and the role played by lignin which is widely recognized as a bottleneck to more efficient bioprocesses. Atomic force microscopy (AFM) images have so far provided essentially qualitative characterization but have failed to capture nanoscale structure changes.

Results

Here, to devise a working protocol for dynamic AFM-enabled observation of lignocellulolytic enzymes at work, we prepared polymer films containing cellulose and varying amounts of lignin. The lignin content translates into less-efficient enzymatic hydrolysis. To study the impact of lignin at nanometre-scale, we developed an in-situ time-lapse AFM imaging system to visualize the hydrolysis process over a time-window of several hours. We were able to observe differences in lignin node and plaque arrangements with different lignin concentrations and to see how these structures changed patterns over the course of hydrolysis. The real breakthrough was that we managed to quantify the structural time–course change in film surface by developing a new marker–our 'relative deconstruction index'–that proves sharper than conventional topographic imaging measurements. This index helped us to learn that recalcitrance to hydrolysis is explained not just by net lignin content but also by lignin distribution down at polymer scale. This pivotal finding enabled us to establish, for the first time with quantitative nanometre-scale AFM-enabled evidence, the barrier effect of lignin-packed structures against enzymatic action.

Future Outlook

The next step is a quantitative dynamic deconstruction study on samples of the far more structurally complex and challenging wood lignocellulose. As this approach is already firmly methodized at cellular scale, we will ultimately be equipped to perform dynamic multi-scale observations (along the cellular-scale to polymer-scale gradient) of enzymatic action at work.