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Breakthrough could help optimize capture of sugars for biofuels

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(Phys.org)—Scientists at the U.S. Department of Energy's National Renewable Energy Laboratory (NREL) and the BioEnergy Science Center (BESC) combined different microscopic imaging methods to gain a greater understanding of the relationships between biomass cell wall structure and enzyme digestibility, a breakthrough that could lead to optimizing sugar yields and lowering the costs of making biofuels.

A paper on the breakthrough, "How Does Plant Cell Wall Nanoscale Architecture Correlate with Enzymatic Digestibility?" appears in the current issue of *Science* magazine.

Principal Investigator Dr. Shi-You Ding of NREL said the imaging technologies allowed the interdisciplinary team of scientists to view the plants' architecture at scales ranging from millimeter to nanometer, a range of 1 million to one.

That allowed them to learn not just the plant cell wall architecture, but also the localization of the enzymes responsible for deconstruction of the cell wall polymers and the effects of enzyme action on the cell wall.

They didn't have to resort to wet chemistry, which ascertains the molecular makeup of a substance at the cost of destroying the spatial relationships. "The typical way to understand the structure of biomass is to break down all the individual components so they can be analyzed," Ding, a biologist, said. "The problem with that method is that then you don't know where all the components came from. You lose the structural integrity."

That's a crucial loss, because an understanding of how enzymes digest plants requires an understanding of where everything is inside the cell walls.

"Our imaging techniques gave us a deeper understanding of the cell wall structure and the process of enzyme hydrolysis of cell-wall carbohydrate polymers to release simple sugars," Ding said. "That allows us to optimize the process and reduce costs."

Dr. Paul Gilna, the director of the BESC, in which the project was conducted, added: "This work greatly improves our ability to closely examine the mechanisms behind the scientific improvements we have developed, all of which are targeted at enabling the emergence of a sustainable cellulosic biofuels industry." BESC is a multi-institutional Bioenergy Research Center supported by the Office of Biological and Environmental Research in the Department of Energy Office of Science.

The correlative imaging in real time allowed the team to assess the impact of lignin removal on biomass hydrolysis and to see the nanometer-scale changes in cell wall structure. And, that allowed them to see how those changes affected the rate at which enzymes from two different organisms digested the plant cell walls.

The aim in the biofuel industry is to access the plants' polymeric carbohydrate structures without damaging the basic molecules of which the polymers are constructed. "It's more like dis-assembling a building with wrenches, hammers and crowbars to recover re-useable bricks, wiring, pipes and structural steel than it is like using a wrecking ball or explosives," Gilna said. Enzymes, unlike typical harsh chemical catalysts, excel at this relatively gentle disassembly.

The NREL team examined two enzyme systems – one from a fungus, the other from a bacterium – both holding promise as biocatalysts for producing sugar intermediates for the biofuels industry.

The particular bacterial enzymes studied are organized through a large scaffolding protein into a multi-enzyme complex from which they make a coordinated attack on the cell walls. The separate fungal enzymes act more individualistically, although the ultimate result is cooperative in that case, as well.

The NREL team found that the easier the access to the cell walls, the better and faster the enzymes will digest the material.

In biofuels production, enzymes are needed to greatly speed up the chemical reactions that break down the biomass during fermentation.

The NREL scientists found that the gummy, poly-aromatic non-sugar lignin in plants interferes with enzymes' ability to access the polysaccharides in the cell wall – the stuff that both the enzymes and the industry want.

So, they concluded, ideal pre-treatment should focus on getting rid of the lignin while leaving the structural polysaccharides within the cell walls intact, thus leaving a relatively loose, porous native-like structure that allows easy access by the enzymes and rapid digestion, as opposed to pretreatments that remove some of the spongier carbohydrate polymers and allow the remainder to collapse into tighter and less-accessible structures. To continue the building dis-assembly and salvage analogy, removal of the lignin is like unlocking all of the doors in the building so that the workers can get in to pull out re-useable materials, but without collapsing the overall structure so that access is blocked.

By understanding the changing structure of the plant material, scientists can learn more about how enzymes work.

"The enzyme has evolved to deal with the real structure, not the pretreated, artificially decomposed one," Ding said. "So to understand how the enzyme goes about its business, it is really important to know where cell wall components are located, as well as the various modes of enzyme action."

"Then we can optimize the whole process," Ding said. "By observing where cellulase enzymes are localized and the nanostructural changes in the plant cell wall architecture that their actions produce, we hope to suggest rational strategies for more cost effective pretreatments and better enzymes."

More information: www.sciencemag.org/content/338/6110/1055.full

Provided by National Renewable Energy Laboratory

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