

Scientists develop efficient methods to turn woody biomass into fuels

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Carpita (pictured) and Maureen McCann have developed and refined methods for efficiently converting cellulosic biomass into fuels. Their findings could be used with gene-editing technology to make fuel from biomass sorghum, seen here, or other bio feedstock plants. Credit: Purdue Agricultural Communication photo/Tom Campbell



Increasing production of second-generation biofuels—those made from non-food biomass such as switchgrass, biomass sorghum, and corn stover—would lessen our reliance on burning fossil fuels, which contributes to climate change.

Several barriers have prevented the efficient conversion of that biomass. Lignin, a complex compound in cell walls, blocks access to plant carbohydrates that could be cleaved into sugars and then fermented into biofuels. The compounds that hold plant <u>cells</u> together, as well as their tightly packed cell clusters, also block access to sugars for fermentation into fuels.

Now, a team led by Purdue University has built on success in removing the lignin barrier to solve other cellular obstacles. Their findings, reported in the journals *Plant Biotechnology Journal* and *Biotechnology for Biofuels*, offer opportunities to significantly increase renewable biofuel production from crop waste products and biofeedstocks that could be grown on marginal lands.

"Lignin is no longer a problem. We have a way of removing it and making useful products from it, as well as getting access to plant carbohydrates for production of biofuels," said Nick Carpita, a Purdue professor in the Department of Botany and Plant Pathology.

Purdue's C3Bio Energy Frontier Research Center has worked for more than a decade to tailor bioenergy crop species for chemical conversion to liquid hydrocarbon fuels like gasoline or jet fuel. Led by Maureen McCann, a Purdue professor of biological sciences, the C3Bio team has explored the obstacles besides lignin that must be overcome to make the carbohydrates more accessible for fuel production.

"Removing lignin didn't eliminate all the issues of biomass recalcitrance," McCann said. "We needed to look at factors that made



woody biomass difficult to degrade beyond lignin, and in its absence."

Former Purdue chemist Mahdi Abu-Omar, a professor and Mellichamp Chair of Green Chemistry at UC Santa Barbara, had discovered that using a nickel-carbon catalyst was an inexpensive and effective method for removing lignin without degrading the plant's carbohydrates. Even with lignin removed, however, the Purdue team had to find ways to break the tightly connected plant cells apart so that chemical catalysts or yeasts used in the biofuel refining process could do their jobs.

Plant biologists Clint Chapple, a Purdue distinguished professor of biochemistry, and Rick Meilan, Purdue professor of molecular tree physiology, developed genetically modified poplar tree with altered lignin structure. Lignin is made of three basic building blocks called monolignols—guaiacyl (G), p-hydroxyl phenol (H), and syringyl (S). One of the trees developed by Chapple and Meilan contains greater than 90 percent S-lignin, which has weaker bonds with plant carbohydrates.

Other poplar trees were also genetically modified to allow for easy breakdown of rhamnogalacturonan, a pectin-like substance in the middle lamella, the zone that glues the walls of plant cells together. Meilan and McCann overexpressed genes that control production of rhamnogalacturonan lyase (RG-lyase), an enzyme that breaks down rhamnogalacturonan, removing the connections between cells.

"Although rhamnogalacturonan is present at only 2 percent of the mass of the <u>cell wall</u>, removing it allows you to deconstruct the biomass particles into smaller clusters of cells, and that can have real energy savings when trees are being shredded to particles for any conversion process," McCann said. "Lignin is also deposited in the middle lamella, but removing only the lignin using the nickel-carbon catalysis, didn't allow the cells to become unglued."



With all the lignin removed from Chapple and Meilan's poplar through the nickel-carbon catalysis, the team treated poplar wood particles with trifluoroacetic acid to loosen the tightly packed crystalline cellulose and its aggregation into large bundles in plant cell walls.

The trifluoroacetic acid causes the cellulose to swell, making it easier to access the glucose molecules present in the cell walls for fermentation to ethanol. Or, using other chemical catalysts discovered by the C3Bio team, the cellulose and other carbohydrates can be converted to platform chemicals, such as hydroxymethylfurfural and levulinic acid, which are substrates or precursors for liquid hydrocarbon fuels.

For now, the engineered poplars cannot be grown commercially as a bio feedstock because they're genetically modified organisms. They would need costly and difficult-to-obtain federal government approvals to grow these trees for any purpose other than research. But the knowledge that he, McCann and Carpita gained from them could be used in other crops modified through gene-editing CRISPR technology.

"We now know how to disassemble the cell walls to produce various products, including transportation fuel," Meilan said. "What we're doing with poplar can help inform what's being done with other cellulosic feedstocks derived from corn stalk residues, or biomass sorghum and switchgrass."

Carpita added that biofuels may be the main product produced, but certainly not the only one.

"It would work for something like sorghum where you could use CRISPR to modify these <u>plants</u> to generate not only biofuels, but also chemicals from <u>lignin</u> and other compounds that we remove from <u>plant</u> <u>cell</u> walls," Carpita said.



More information: Haibing Yang et al. Overcoming cellulose recalcitrance in woody biomass for the lignin-first biorefinery, *Biotechnology for Biofuels* (2019). DOI: 10.1186/s13068-019-1503-y

Provided by Purdue University

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