Evolving microbes help Iowa State engineers turn bio-oil into advanced biofuels

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AMES, Iowa – Microbes are working away in an Iowa State University laboratory to ferment biofuels from the sugar and acetate produced by rapidly heating biomass such as corn stalks and sawdust.

But it's not an easy job for *E. coli* and *C. reinhardtii*.

The bacteria and microalgae, respectively, don't like something in the bio-oil produced by fast pyrolysis – the rapid heating of biomass without oxygen and with catalysts. The result of the thermochemical process is a thick, brown oil that smells like molasses.

A research team led by Laura Jarboe, an Iowa State assistant professor of chemical and biological engineering, is feeding the bio-oil (also known as "pyrolytic sugars") to the microbes. The *E. coli* are supposed to turn the levoglucosan in the sugar-rich fraction of bio-oil into ethanol and lactic acid; the *C. reinhardtii* are supposed to turn acetate-rich fractions into lipids for biodiesel.

It's part of the hybrid approach Iowa State researchers are using to produce the next generation of biofuels. They're combining two conversion paths – thermochemical and biochemical – to find efficient ways to produce renewable fuels and chemicals.

"The goal is to produce biorenewable fuels and chemicals in a manner that's economically competitive with petroleum-based processes," Jarboe said.

There are, however, contaminants and toxins in the bio-oil that are getting in the way of the fuel production. Jarboe and a research team are experimenting with pre-treatments of the bio-oil that could reduce the toxicity. And they're working to develop microbes that can tolerate the contaminants.

In addition to Jarboe, the research team includes Robert C. Brown, the Iowa Farm Bureau Director of Iowa State's Bioeconomy Institute, an Anson Marston Distinguished Professor in Engineering and the Gary and Donna Hoover Chair in Mechanical Engineering; Zhiyou Wen, an associate professor of food science and human nutrition; Zhanyou Chi, a post-doctoral research associate for Iowa State's Center for Sustainable Environmental Technologies; Tao Jin, a doctoral student in chemical and biological engineering; and Yi Liang, a doctoral student in food science and human nutrition. The project is supported by a three-year, $300,000 grant from the National Science Foundation and a three-year, $315,020 grant from the Iowa Energy Center.

The researchers are using a technique called directed evolution to produce microbes that are more tolerant of the contaminants in bio-oil. The microbes are grown with higher and higher concentrations of bio-oil and as they divide, they replicate their DNA. Sometimes there are replication mistakes that lead to mutations.

"It could be a mistake that's immediately lethal," Jarboe said. "Or it could be a mistake that helps the microbe tolerate the problematic compounds and it grows faster.

"At the end of the process, we want to say, 'Hey, I've got a great bug.'"

Every day researchers check the experiments for signs of progress. So far, Jarboe said the evolving bacteria and microalgae have been able to tolerate slightly higher concentrations of bio-oil.

When mutations eventually produce a better breed of microbe, the researchers will analyze genomic data to learn and understand the important mutations. That will allow researchers to duplicate the microbes for better biofuel production.

Jarboe said development of those hungry, robust microbes could lead to important advancements in biofuel production: a hybrid process that's biorenewable, fast, cheap and doesn't depend on food crops as a source of biomass.

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