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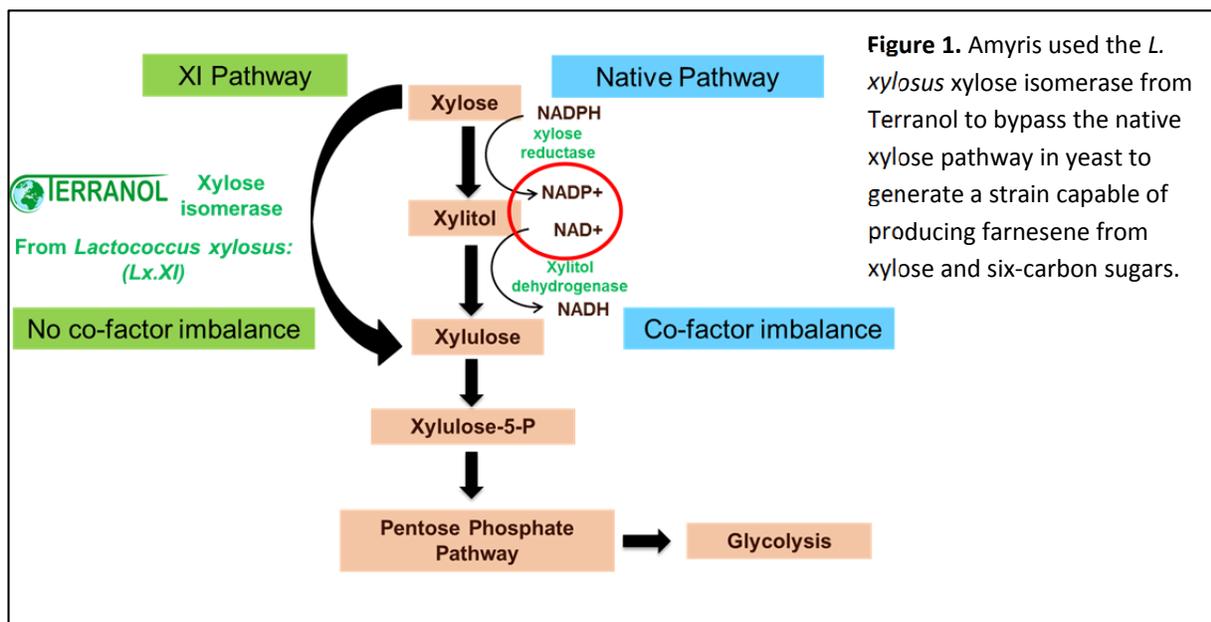
Amyris Successfully Makes Renewable Diesel from Cellulosic Hydrolysate

[Amyris](#), in collaboration with its partners in the National Advanced Biofuels Consortium (NABC), has produced a drop-in diesel fuel and chemical precursor by the fermentation of cellulosic sugars. Amyris has successfully integrated the enzyme xylose isomerase into its existing Biofene producing strains, thereby allowing for the simultaneous fermentation of glucose and xylose to farnesene. Biofene, Amyris's brand of renewable farnesene, is a long-chain, branched renewable hydrocarbon molecule that can be easily adapted to serve as an alternative to fossil fuel-derived products across a number of markets. Pilot scale fermentations are currently on-going using cellulosic corn stover hydrolysate produced by the U.S. Department of Energy's National Renewable Energy Laboratory (NREL). Amyris has demonstrated that Biofene can be produced by fermentation of a variety of sugar sources, including cellulosic sugars, at their California-based pilot plant. The resulting cellulosic Biofene is indistinguishable from Biofene produced using other sugar sources. Amyris has successfully chemically transformed this cellulosic Biofene into farnesane, our drop-in renewable diesel. Amyris is currently producing and selling Biofene from conventional sugars, and this work suggests that cellulosic sugars can and will be used in Biofene production when they become commercially available at industrial scale.

Within the NABC, Amyris is leading the Fermentation of Lignocellulosic Sugars (FLS) process strategy. The goal of the FLS strategy is to integrate the ability to utilize cellulosic sugars derived from non-food biomass into Amyris's existing Biofene-producing yeast. The process of breaking down plant biomass into monomeric sugars suitable for microbial fermentation results in the six-carbon sugars glucose, galactose, and mannose, as well as the five-carbon sugars xylose and arabinose. Yeast (*S. cerevisiae*) readily consume these six-carbon sugars but cannot consume the five-carbon sugars. Since most of the five-carbon sugar fraction in cellulosic hydrolysate is xylose (on average about 85%), the FLS strategy has focused on the fermentation of xylose, along with six-carbon sugars, derived from biomass to produce the isoprenoid farnesene.

Farnesene is a 15-carbon branched alkene hydrocarbon that can be hydrogenated to a diesel or jet fuel component (farnesane) or used as a chemical precursor to a wide variety of products including surfactants, polymers, cosmetics, and lubricants. Amyris Diesel fuel meets ASTM D975, the diesel fuel specification, and is approved by the EPA as a renewable fuel to a 35% blend level with ultra-low sulfur diesel in the United States. Farnesane is also currently undergoing ASTM validation to be used in aviation turbine fuel.

In the last year, Amyris focused on integrating the enzyme xylose isomerase into its Biofene-producing yeast strains. Like wild-type yeast, Amyris’s farnesene strains can consume the six-carbon (C6) sugars found in hydrolysates, but not the five-carbon (C5) sugars xylose and arabinose. Xylose isomerase (XI) is an enzyme found in bacteria and some fungal species that converts xylose to the intermediate metabolite xylulose. Xylose isomerase bypasses the native route of xylose consumption in yeast, which utilizes two enzymes and two different co-factors (Figure 1). This creates a co-factor imbalance, hampering the performance of yeast grown on xylose or mixed C5 and C6 sugars. For the NABC project, Amyris is using the xylose isomerase developed by the company Terranol in its C5 yeast development.



Amyris has successfully engineered xylose consumption in our Biofene-producing strains, using xylose isomerase and additional engineering steps (Figure 2). Notably, because Amyris’s fermentation process is aerobic, our yeast cells consume the acetic acid that is released during the conversion of lignocellulosic biomass to sugars. This is in marked contrast to anaerobic ethanol fermentations, where acetic acid is not consumed and inhibits robust growth (Figure 3).

Fermentations using Amyris’s C5-farnesene strains on concentrated, defined media (300 g/L glucose and 150 g/L xylose) consumed in excess of 95% of the xylose fed into the tanks. (Typical corn stover hydrolysates usually have sugar concentrations of approximately 100 g/L glucose and 50 g/L xylose.)

Figure 2. Co-consumption of glucose and xylose in Amyris’s C5-farnesene strains. Strains were grown in a 96-well plate containing defined media with 3% maltrin, amylase, and 4% xylose. Maltrin is a polysaccharide consisting of D-glucose units; yeast cannot consume maltrin. The enzyme amylase catalyzes the breakdown of the maltrin into glucose. This results in a constant, slow feed of glucose to the media, mimicking a fed-batch fermentation. Amyris’s latest C5-farnesene strains are capable of consuming 100% of the xylose in this assay (green strain).

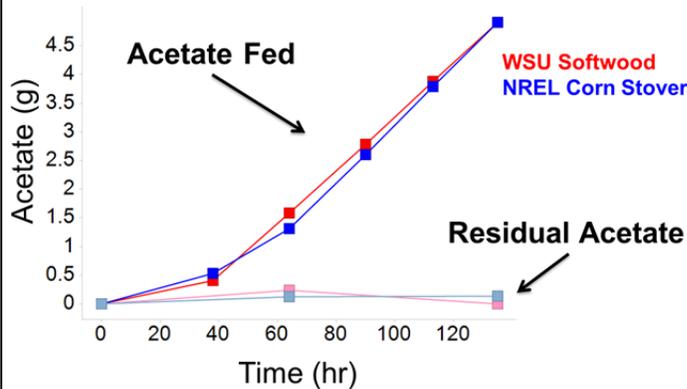
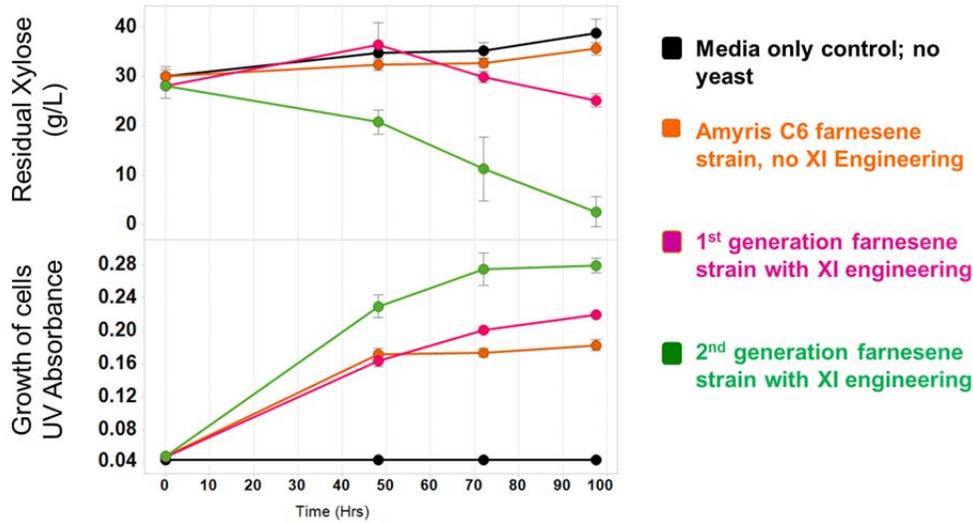


Figure 3. Amyris’s aerobic fermentation process allows for the consumption of acetic acid. The graph shows the amount of acetic acid fed into representative small scale fermentations using different cellulosic hydrolysates produced by the NABC partners NREL and Washington State University. The acetic acid is consumed by Amyris’s C5-farnesene producing yeast.

The first pilot scale demonstration (300 L fermentation) of farnesene production on cellulosic hydrolysate was performed in June of 2013 using corn stover hydrolysate supplied by NREL. The farnesene produced from this pilot fermentation was further distilled and hydrogenated into farnesane, which is Amyris’s renewable diesel product. There was no detectable difference observed between farnesene produced from cellulosic hydrolysate versus sugarcane syrup, and the renewable diesel produced from the cellulosic hydrolysate passed all quality control specs for diesel fuel. Additional 300 L pilot scale farnesene fermentations on concentrated cellulosic hydrolysate are planned before the end of the NABC program later this year.