NABC National Advanced Biofuels Consortium
Biofuels for Advancing America
Developing cost-competitive, sustainable biofuels for today's transportation infrastructure.

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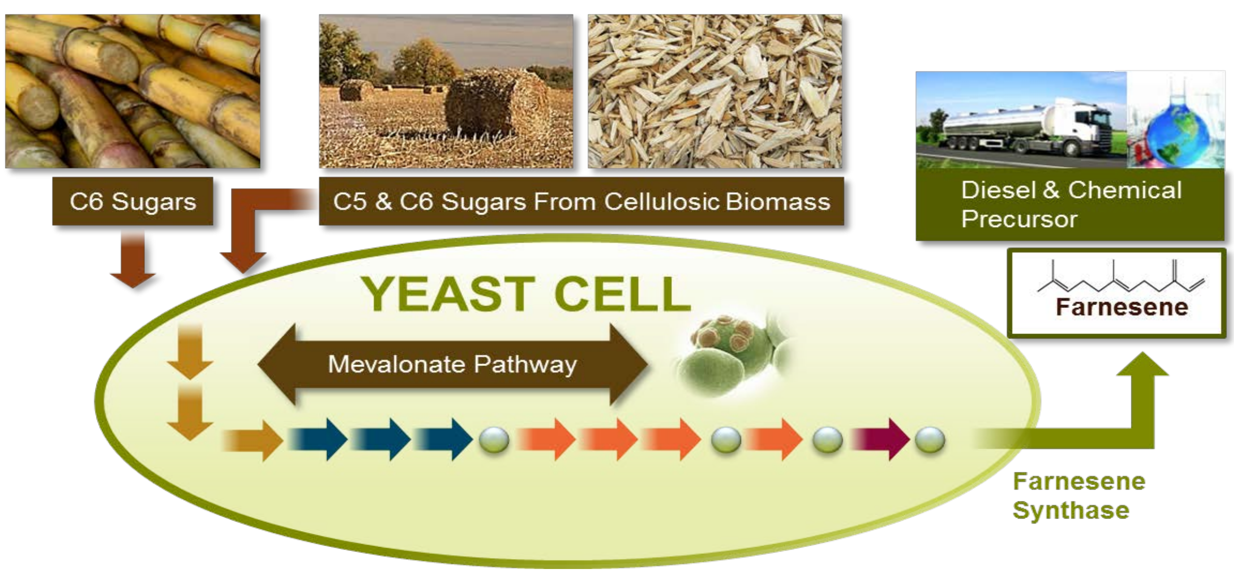
Amyris Successfully Makes Biofene from Cellulosic Hydrolysate

Amyris, in collaboration with its partners as part of the National Advanced Biofuels Consortium (NABC), has produced renewable farnesene (Biofene®), a diesel fuel and chemical precursor, by the simultaneous fermentation of ligno-cellulosic sugars including xylose.

Within the NABC, Amyris is leading the Fermentation of Ligno-cellulosic Sugars (FLS) process strategy. The goal of the FLS strategy is to merge Amyris' isoprenoid-yeast platform with the ability to utilize cellulosic sugars derived from non-food biomass. 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 is focusing on the fermentation of xylose, along with six-carbon sugars, derived from biomass to produce the isoprenoid farnesene. Farnesene is a hydrocarbon that can be hydrogenated to a diesel or jet fuel component or used as a chemical precursor in a wide variety of applications.



In order to meet the NABC FLS Stage I goals within the one-year time frame, Amyris has used Microbiogen's non-GMO *S. cerevisiae* strains that were previously evolved for efficient xylose consumption and tolerance to traditional hydrolysate inhibitors as base strains for further engineering. Amyris has engineered its farnesene technology into Microbiogen's yeast to create strains capable of producing farnesene from cellulosic hydrolysates. Based on the success of producing farnesene from a mixed glucose and xylose sugar stream, Amyris was selected to continue onto Stage II in the NABC, which will continue through September of 2013.

Amyris will be evaluating the effect of a variety of hydrolysate pretreatment processes and biomass sources on our C5-farnesene strains in cellulosic hydrolysates generated by our NABC partners: Catchlight Energy, Iowa State University, Washington State University, and NREL. One of the goals of Stage II of the FLS is to work with our partners to define the optimal cellulosic hydrolysate for the fermentation of farnesene. To date, engineering of the farnesene pathway into the xylose-consuming and hydrolysate-resistant yeasts, coupled with the fermentation process developed at Amyris, has delivered new strains proven to be highly tolerant of the traditional inhibitors in cellulosic hydrolysate. For example, the high levels of acetate in cellulosic hydrolysates are a known inhibitor of ethanol fermentation, but Amyris has found that hydrolysates containing as high as 13 g/L acetate have no observable impact on our fermentations (Hydrolysate 2 in Figure 1).

Figure 1 shows the performance of Amyris' C5-farnesene strains in cellulosic hydrolysate relative to strain performance in a glucose/xylose defined media. Product yields in all hydrolysates tested are equivalent to or better than the performance in defined media. Xylose conversion was equivalent to defined media in two out of three hydrolysates.

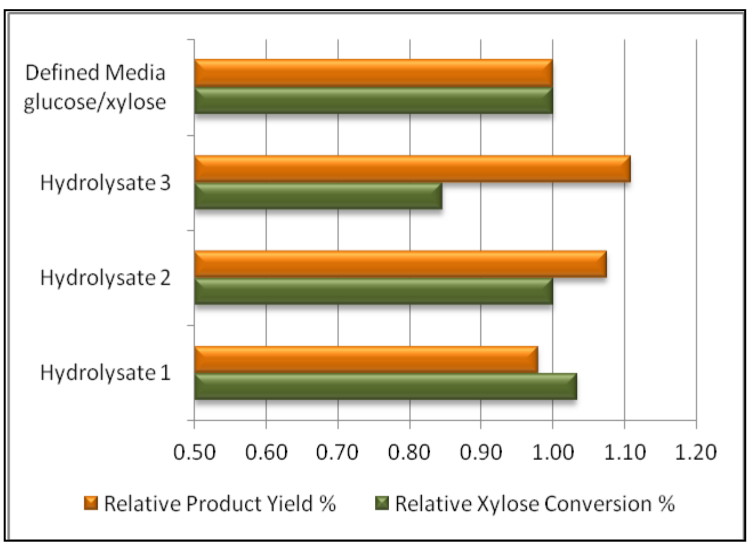


Figure1. Relative performance of Amyris' C5-farnesene strain in defined vs. cellulosic hydrolysate.

The defined media contains 100 g/L glucose and 50 g/L xylose. Hydrolysates were generated from final harvest residual chips (predominately loblolly pine) or corn stover using either a dilute acid or wet oxidation pretreatment process, followed by enzymatic hydrolysis. Product yields are based on total usable sugars in the media.

Also as part of the FLS Stage II, Amyris will scale up production of farnesene to the 300 L scale using the identified optimum cellulosic hydrolysate and work with our partners to develop the techno-economic analysis of producing Biofene from cellulosic material as part of the greater goal to establish a biomass-derived biofuel industry in the United States.