

DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

MegaBio: An integrated process for production of farnesene, a versatile platform chemical, from domestic lignocellulosic feedstock

March 6, 2019

Biochemical Conversion Session

Quinn Mitrovich, Amyris



- **MegaBio Goal: Develop a fully integrated and scalable process to produce farnesene from cellulosic sugars at \$2 per liter in the U.S.**
- A platform chemical like farnesene gives us flexibility to adapt to market demands
- Co-optimizing metabolic engineering and sugar purification strategies will enable improved renewables:
 - more competitively priced
 - reduced carbon footprint
 - produced in the U.S. with domestic feedstocks



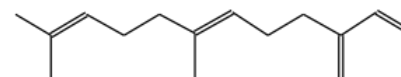
Pinus taeda
(loblolly pine)



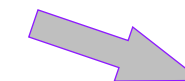
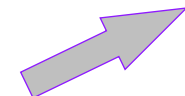
engineered
S. cerevisiae



amyris



(E)- β -farnesene



Higher value bio-products

e.g., squalane, vitamin E



2nd Gen Biofuels

e.g., farnesane jet and diesel fuel

Timeline

- Project start: October 1, 2016
- Project end: March 31, 2019 (early end date; original date was December 31, 2019)
- 67% complete

Barriers addressed

Improving Catalyst Lifetime (Ct-E):

Characterize the chemicals in cellulosic sugar feedstocks that inhibit biocatalyst performance, and improve biocatalyst tolerance of these inhibitors.

Increasing the Yield from Catalytic Processes (Ct-F):

Improve product yields by engineering biocatalyst to consume both C5 sugars and abundant organic acid growth inhibitors

Objective

Deliver an integrated, scalable manufacturing process for production of the platform chemical farnesene from a lignocellulosic feedstock

End of Project Goal

Fully integrated manufacturing cost of \$2 per liter of farnesene

	BP1 Costs (Q1 FY17)	BP2 Costs (Q2 FY17 – Q2 FY19)
DOE Funded	\$106,066	\$4,773,088
Cost Share (Amyris)	\$51,293	\$1,038,357
Cost Share (Renmatix)	\$15,273	\$221,149
Cost Share (Total S.A.)	\$3,731	\$133,858
•Partner funding: Amyris 77% (biocatalyst development), Renmatix 19% (feedstock development), Total S. A. 4% (engineering study, TEA, LCA)		

- Accessing higher value (but lower volume) markets has enabled the continued development of our biofuel molecule, farnesene.
- This aligns well with the philosophy behind the 2016 MegaBio FOA.
- We already have *established* markets if we can match our manufacturing costs on cane syrup.



Fuels

- Diesel fuel
- Jet fuel blend
- Meet ASTM standards



Tackifiers & sealants

- Wide variety of adhesives
- Tailored properties



Cosmetics

- Best-in-class emollients
- Shark-free squalane!



Flavors, Fragrances, Nutraceuticals

- Uniform quality
- Stable supply and competitive pricing

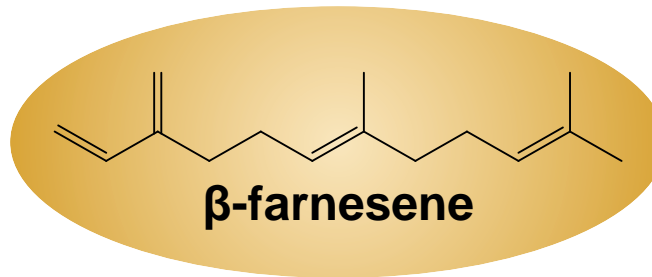


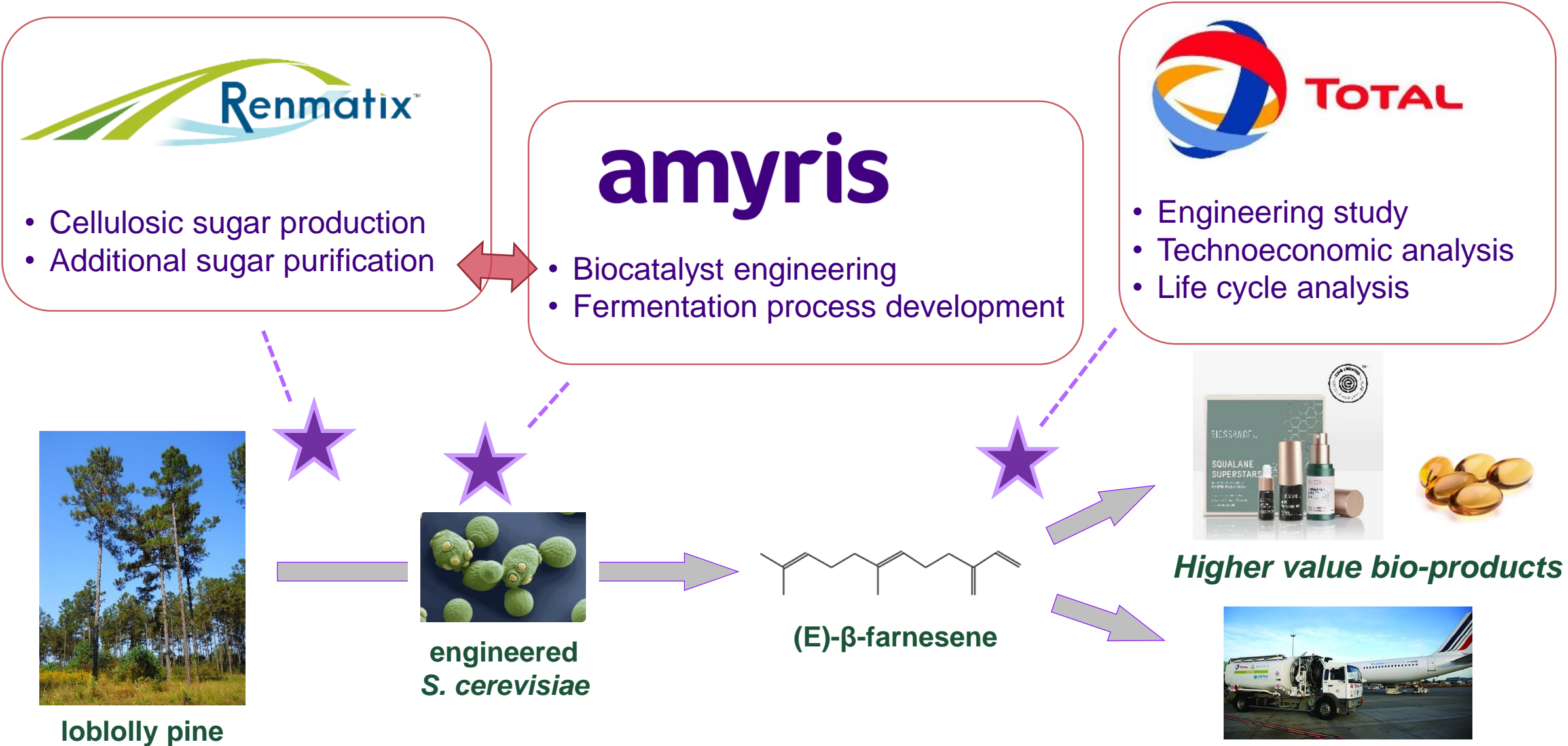
Solvents, Lubricants

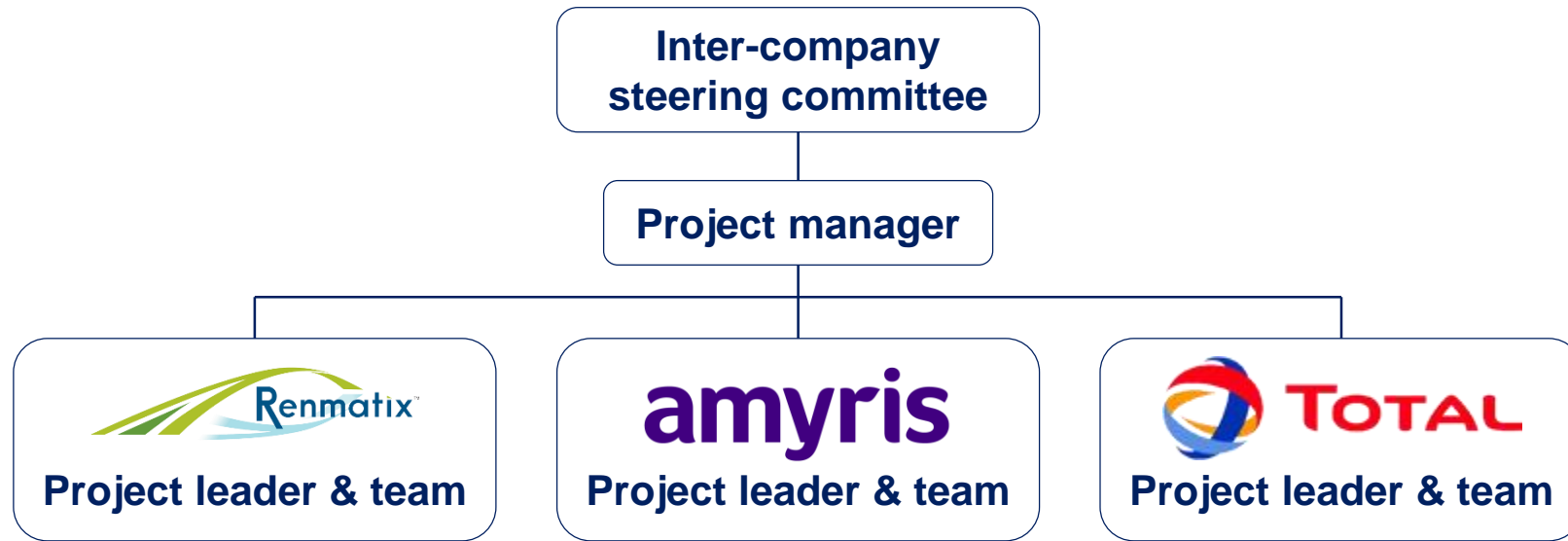
- Low VOC compliant for European regulations
- Biodegradable
- Better EH&S profile

Elastomers, Polymers, Rubber

- Cross-linking opportunities
- Tires with better gas mileage
- Tires with improved wet road grip



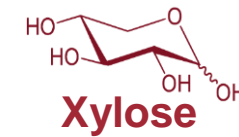




- **Renmatix:** Cellulosic feedstock production and development of feedstock purification methods
- **Amyris:** Biocatalyst engineering and scalable fermentation and recovery process development
- **Total:** Engineering study of an integrated plant, techno-economic analysis, and life cycle analysis
- For communication among the companies, we have held **monthly partner calls**, followed by monthly **check-ins with the DOE team**, with additional in-depth meetings as needed.

Goal: Through coordinated feedstock purification and strain engineering strategies, develop an integrated, scalable process for production of farnesene from cellulosic biomass at \$2 per liter.

- Enable robust consumption of **xylose** from hemicellulosic sugar streams.
- Deal with **growth inhibitors** present in feedstocks. We have three methods for addressing this:
 - Ideally, enable **consumption** of abundant inhibitors through strain engineering
 - Engineer (or evolve) **resistance** mechanisms into production strains
 - Develop feedstock **purification** methods that are compatible with final cost targets, tailored to the needs of our production strains
- Develop an integrated, scalable manufacturing process, with an **engineering study** and a **techno-economic model** to predict manufacturing costs at full scale, and a comprehensive **life cycle analysis** to ensure the sustainability of such a project.
- Annual Go/No-Go decision points based on progress against the above technical challenges, and a quantitative assessment of whether we will likely meet the final cost target by project end.



Pine hydrolysate samples (Hemicellulose and Cellulose)



Pine-based Hemicellulose and Cellulose Sugars for organism testing (shipped to Amyris)



Milestones B.4, J (complete): *Produce cellulosic sugars from Pine and deliver to Amyris for development work.*

Pine-Based Hemicellulose and Cellulose crude material reserved for future refining



- “**Standard**” refining did not allow robust fermentation performance with Amyris base production strains.
- Step 1: Tested **Modified Ion Exchange** method to remove organic acid inhibitors.
 - Improved fermentation performance, but not feasible for long-term continuous operation.
- Step 2: Tested use of **Simulated Moving Bed Chromatography (SMB)** on final product.
 - Insufficient separation of organic acids from sugars; large dilution.
- Step 3: Developed “**SMB 2.0**” strategy, integrated within Renmatix Refining process.
 - By implementing a 3-way split with recovery of oligomeric sugars, we might improve costs and sugar purities.
 - Work with an external vendor indicated:
 - Large yield losses (11%) of monomeric sugars to waste stream.
 - Very large water increase (6x)
 - More dilute sugar streams, requiring increased energy for concentration.
 - Increased capital deployment for SMB, water and evaporator units.
 - While promising, determined to be incompatible with final manufacturing cost target.

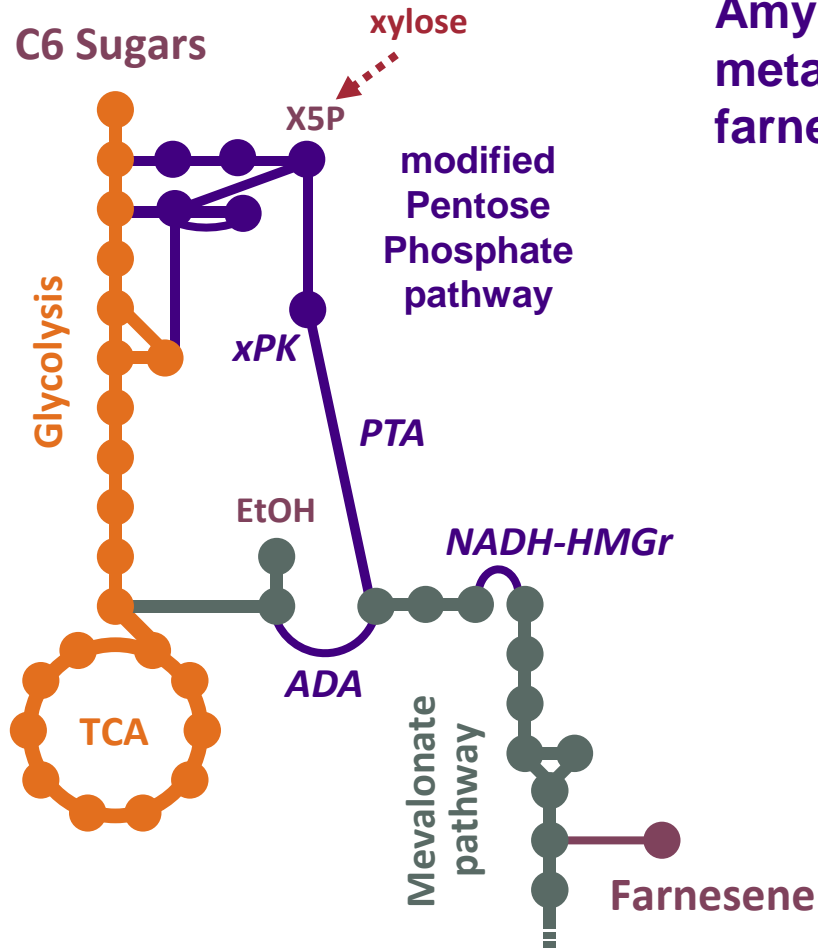
Milestones E.1, F, P (complete): *Decide on sugar purification method; set specifications.*

Milestone E.2 (complete): *Feasibility study to define sugar concentration and purification at external vendor.*

Milestone K (incomplete): *Produce 3 kg of purified and concentrated sugars for strain testing.*

Milestone O (incomplete): *Engineer a pilot sugar purification unit.*

Amyris 2nd Gen (Synthetic) Pathway re-routes central metabolism to increase maximum theoretical yield of farnesene from glucose by 25%, and from oxygen by 300%



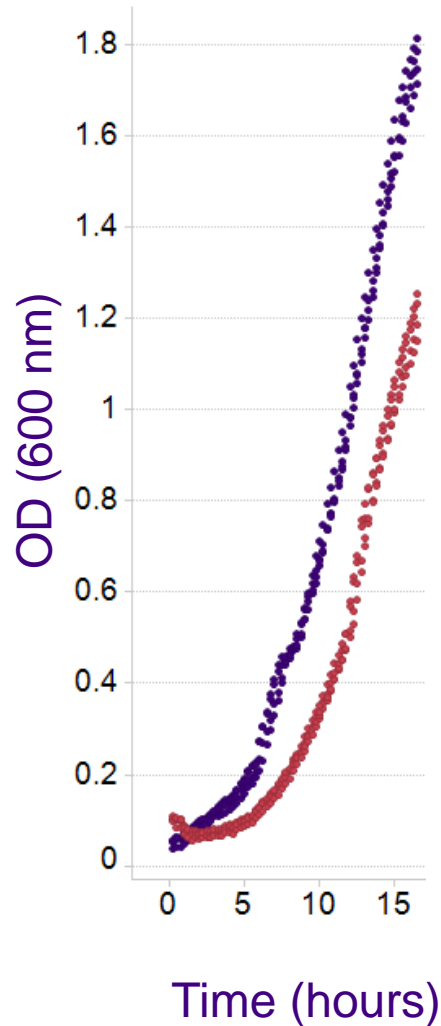
Current farnesene manufacturing strains:

- > 110,000 base pairs added
- > 40,000 base pairs removed
- > 500 single-nucleotide mutations

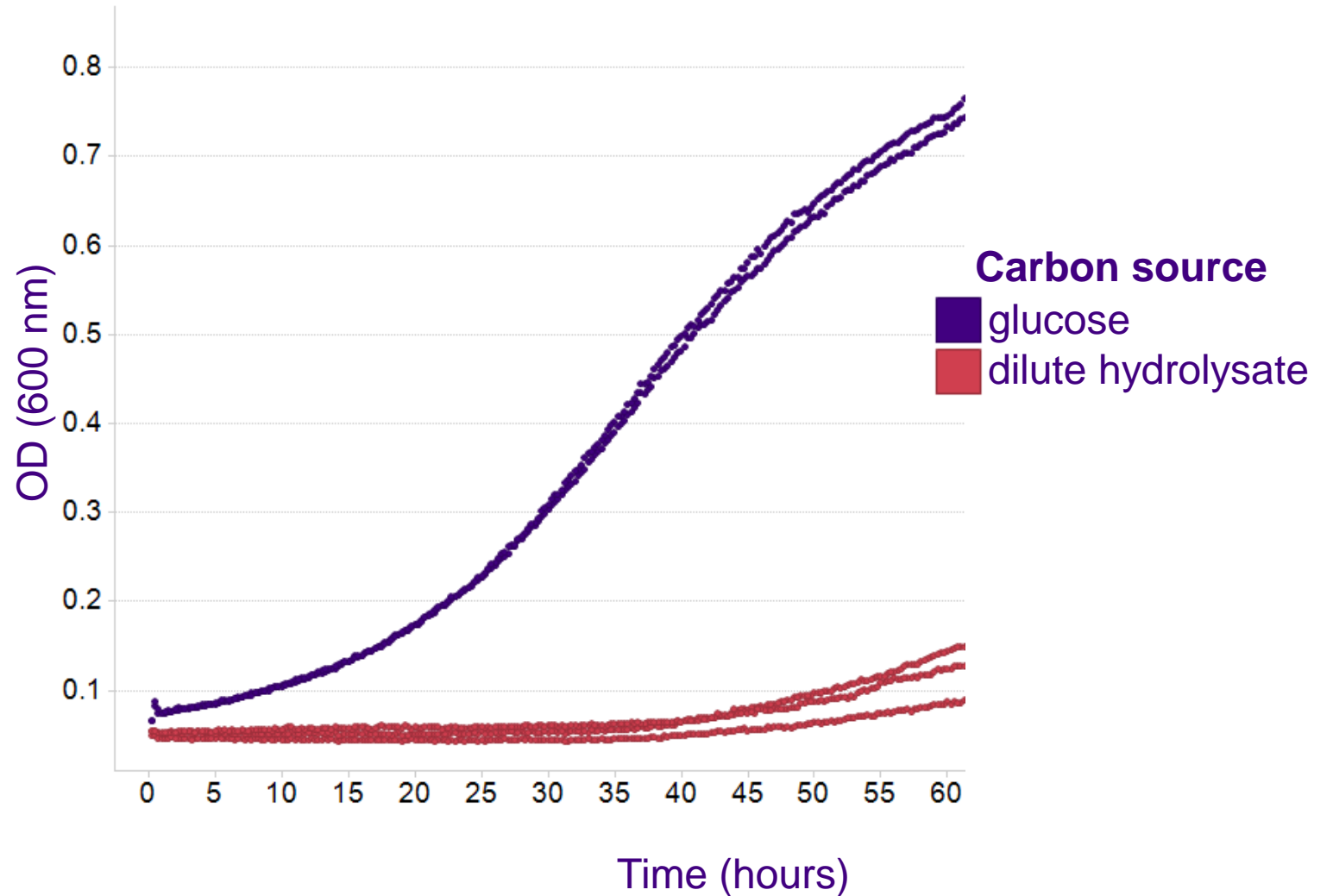
Possible challenges for cellulosic feedstocks:

- Accumulated mutations that could affect tolerance
- Changes in redox co-factors (e.g. NADPH) critical for inhibitor detoxification
- Substantial engineering around the xylose entry point
- Most incoming sugar used for product formation, not biomass growth and maintenance

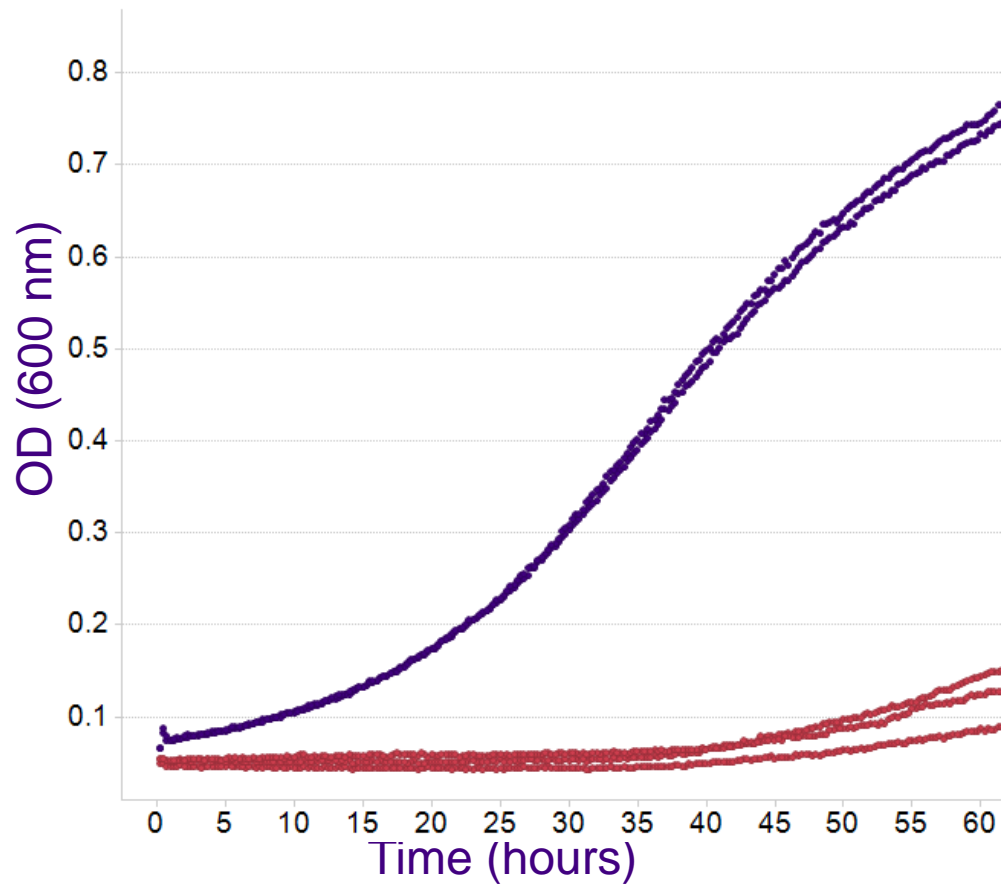
wild-type strain



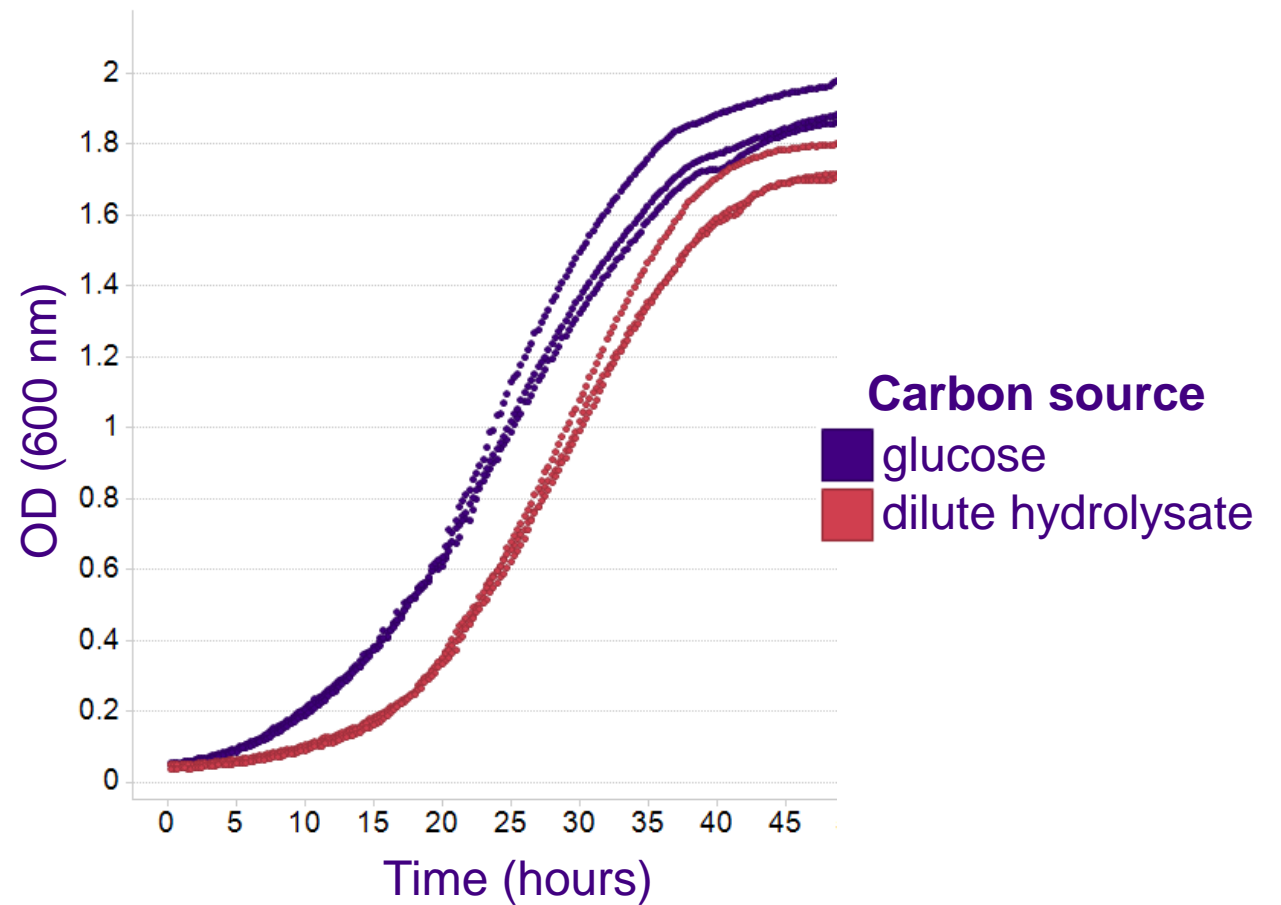
commercial farnesene production strain



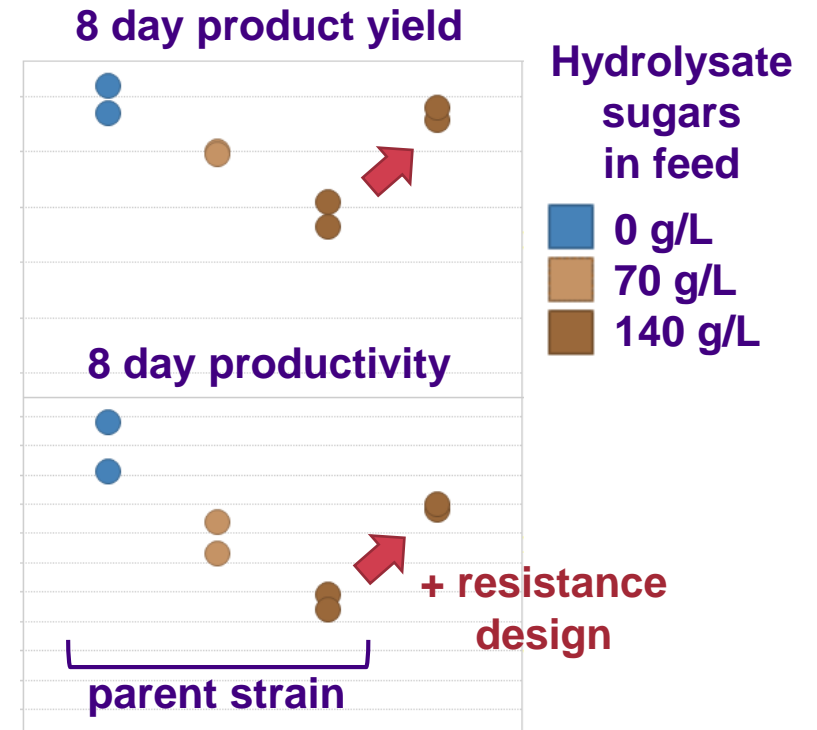
commercial farnesene production strain



production strain with reverted mutations



- Biocatalyst evolution
 - Growth selection on hydrolysates has led to substantial tolerance improvements
 - Unfortunately, these improvements often don't persist once the production pathway is re-activated
- Rational engineering
 - We've identified many designs that improve tolerance
 - Some designs negatively affect product formation, but others look great!



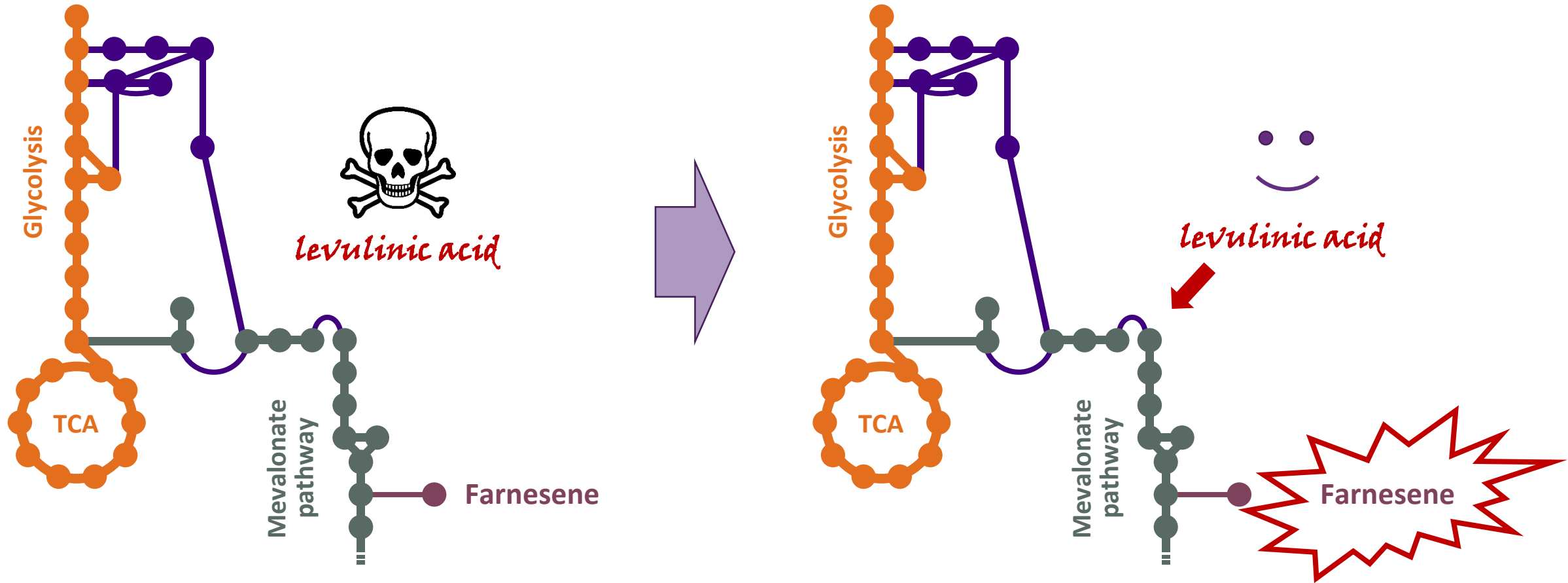
Milestone C.4 (complete): *Baseline testing of Renmatix C6 sugar from the standard process (no inhibitor removal) versus defined glucose media in 0.5L fermentation.*

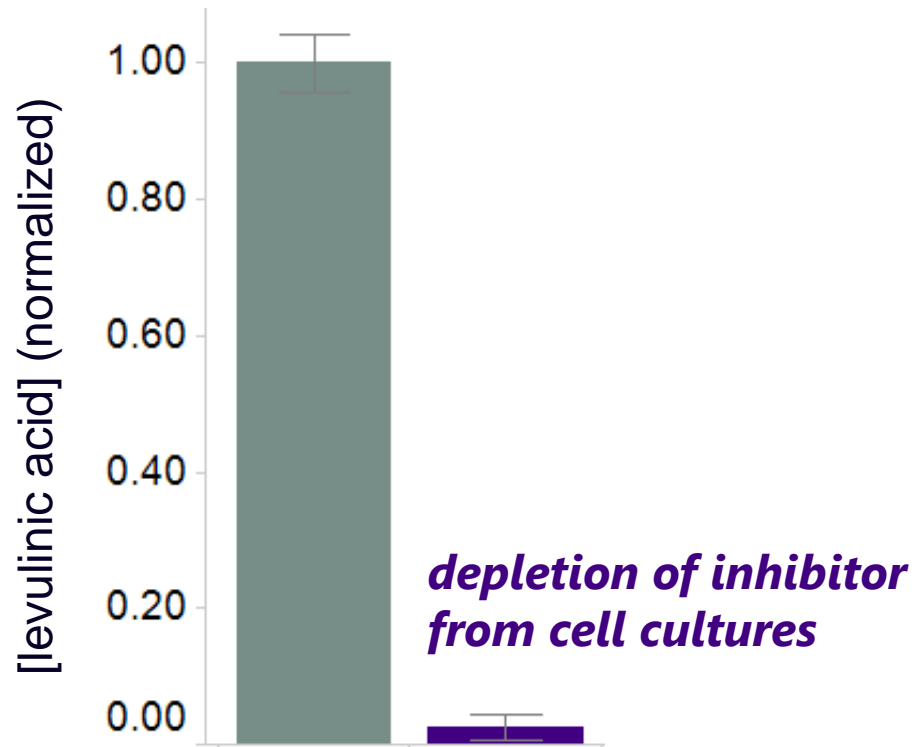
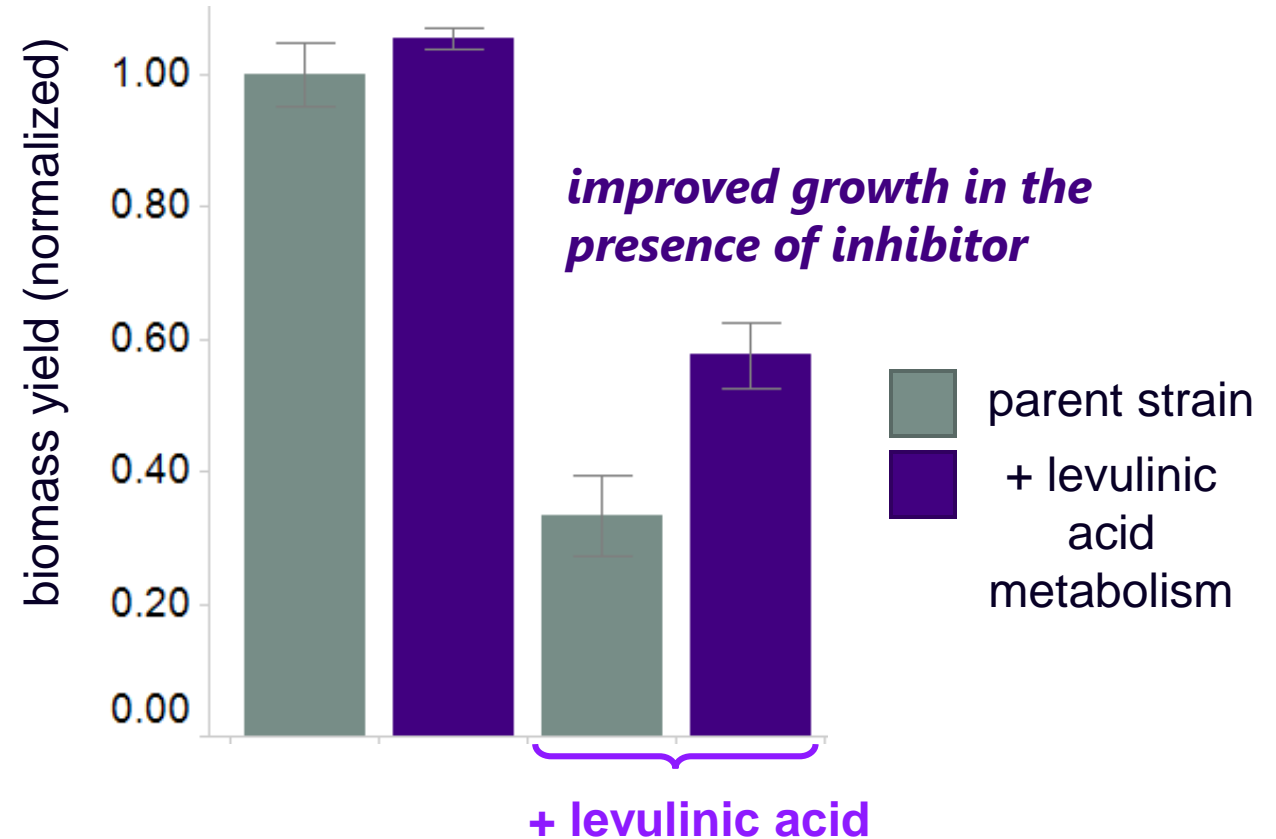
2017 Q4 Decision Point (complete) includes: *>25% consumption of, or >20% increase in tolerance to, the organic acid inhibitors in Renmatix sugars.*

2018 Q4 Decision Point (complete) includes: *strains can tolerate 40% more organic acids in feedstock.*

- We used both **non-targeted LC-MS/MS** and **candidate LC/GC-MS** to identify and quantify non-consumable components of hydrolysates, and tested these for **biological inhibition**
- In addition to two organic acids identified previously, three additional molecules showed growth inhibition individually
- By *combining* 26 non-consumable components, we can fully replicate the inhibition seen from fermentation-spent hydrolysates

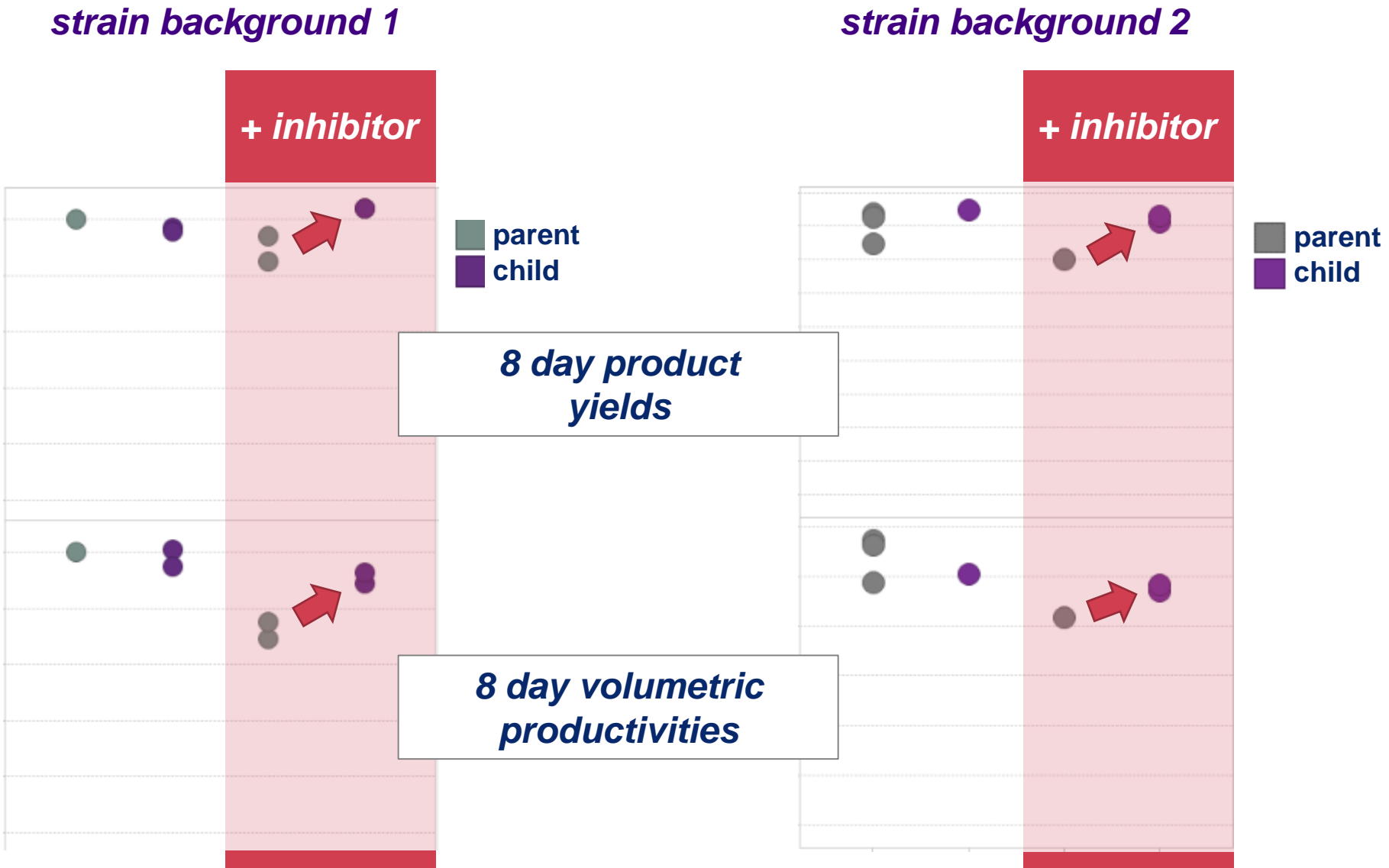
Milestone C.3 (complete): *Determine the primary inhibitors in both Renmatix C5 and C6 sugar streams on a farnesene manufacturing strain.*



residual levulinic acid*relative cell biomass yield*

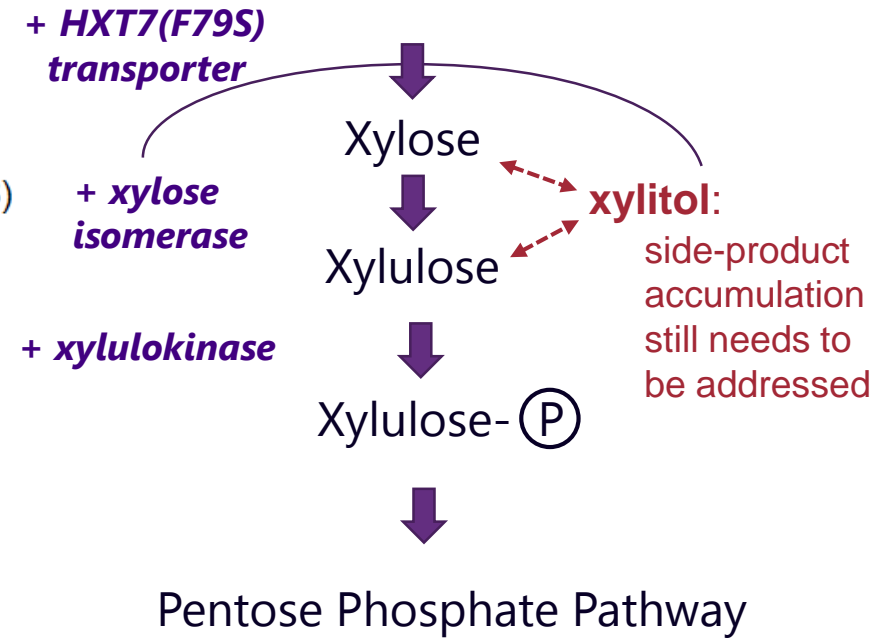
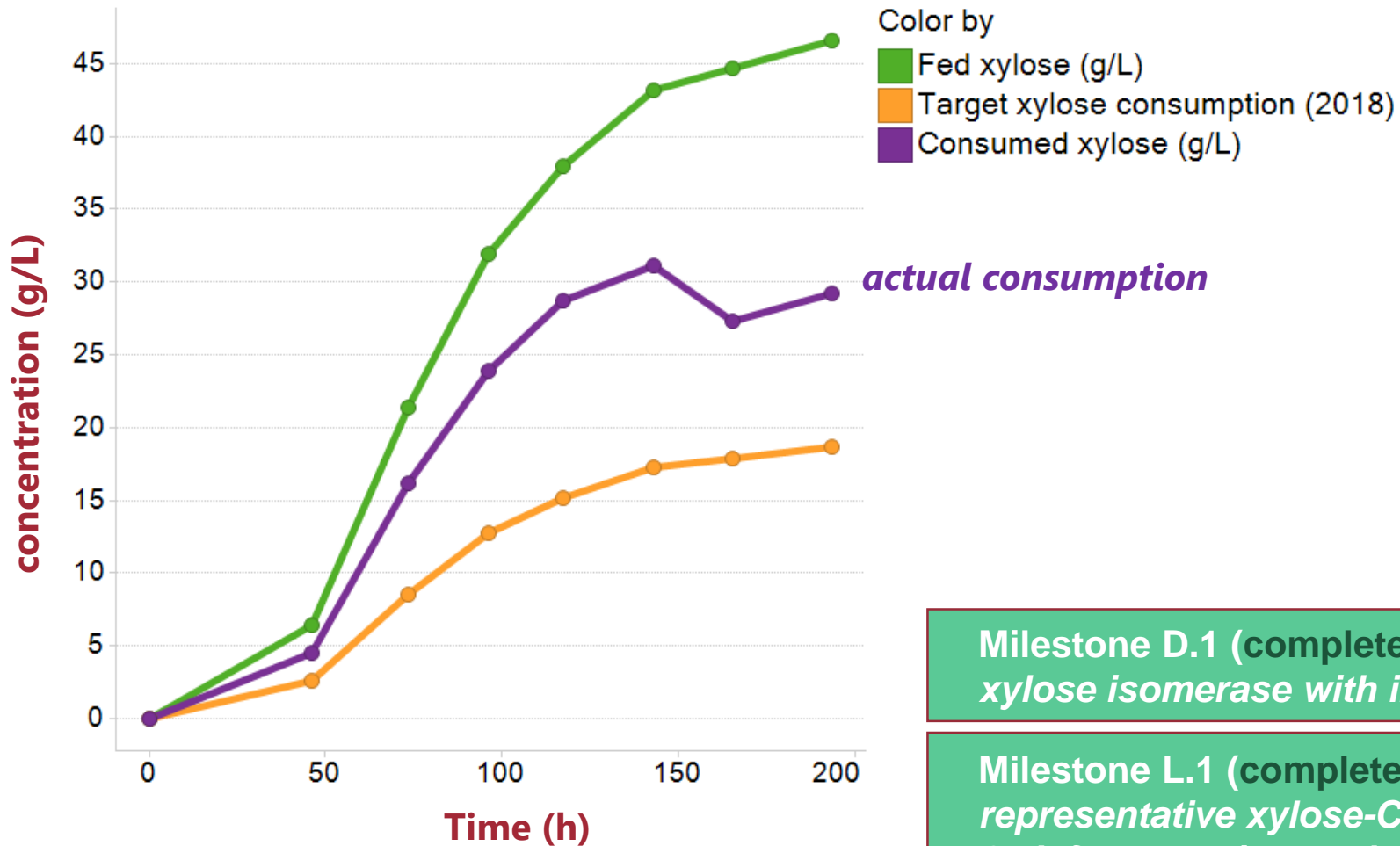
- In lab cultures, levulinic acid consumption pathways are beneficial.
- In fermentors, however, we've seen problems with strain performance. This is likely due to incomplete pathway balancing.

Engineered consumption of another organic acid growth inhibitor rescues fermentation performance



A heterologous pathway enables xylose consumption

Fed-batch fermentation with defined xylose/C6 sugars mimicking the distribution in hydrolysates (xylose = 9% of total sugars)

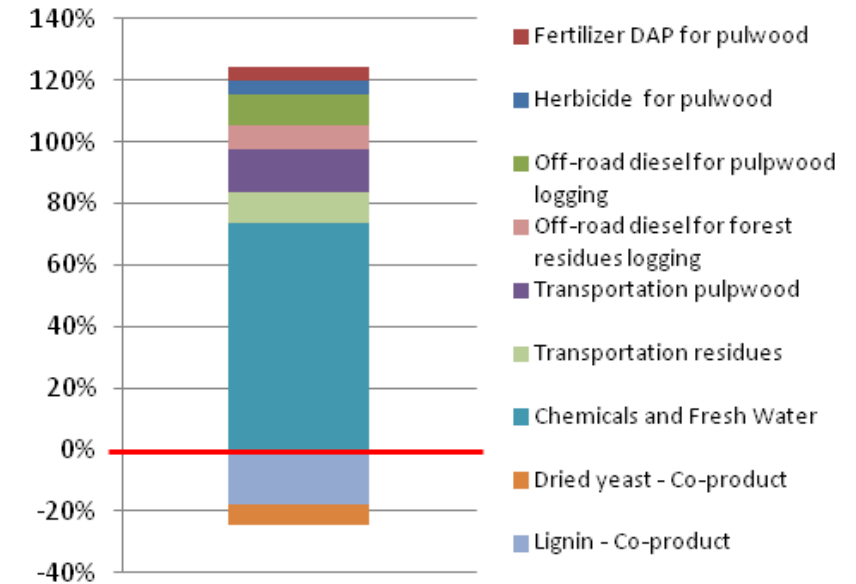


Milestone D.1 (complete): Identify (and license if necessary) a xylose isomerase with in vitro flux capacity ≥ 1 mmol/gdcw/h.

Milestone L.1 (complete): Strains consume >40% of xylose in a representative xylose-C6 defined media (without inhibitors) at 0.5L fermentation scale.

- **Goal : selection of two potential sites to be used for LCA and TEA**
- Plant size : 300,000 dry tons of pine wood per year (pulpwood quality).
- Selection criteria : price, logistics, competition, and LCA.
- The selection process encompassed four phases, narrowing down nationwide data to regional/county data. The Southeastern region of US was found to be the most favorable in terms of Pine availability and pricing, and the “Sub regional Timber Supply (SRTS) model” was used to predict pulpwood inventory, growth/ removals, and price change for sub regions in the Southeast through 2025.
- After weighing each sub region along all the relevant demand and supply scenarios, including a demand scenario with twice the plant demand as indicators for durability, two final procurement areas were determined: **Fulton, Mississippi** and **Winona, Mississippi**.
- Pine wood costs would be \$50 – \$60/dry ton delivered (stumpage price + logging + transport).

- Using data from pine plantation owners, a preliminary LCA performed using GREET showed that fertilizers would represent 5% of the net carbon emissions. The two sites were not differentiated by LCA.
- Fulton, MS had planned to host a cellulosic ethanol and pellet facility, but construction was halted after site preparation. Using this prepared site could save up to \$1.3M in plant construction, and so we decided to select **Fulton, MS**.



Distribution of CO₂ emissions per category

Milestone G (complete): *Selection of two potential feedstock sites to be used for LCA analysis.*

Milestone H (complete): *LCA assessment of feedstock sites.*

- 24 blocks modeled
- 122 streams considered
- Power generation sized to fulfill 100% of power requirements
- Turbines, supercritical steam generation and heat integration fully modeled under Aspen (60 block units)
- SMB technology considered for purification block of sugar
- Deliverable: general block diagram showing the inlets and outlets of the overall plant

Milestone I (complete): *Preliminary Block Flow Diagram for the integrated plant with Heat and Mass Balance and a utility flow diagram.*

- A conceptual engineering study has been performed (still under review among partners) and concluded in a CAPEX estimate at +/- 50%.
- The estimated production cost (OPEX + CAPEX depreciation at 7% discount rate over 20 years) are estimated to be in the range of **\$4-5/L farnesene.**
- One of the conclusions of this study was that the level of CAPEX associated with the OSBL part of the plant (Waste Water Treatment, Power Generation, Steam generation, Cooling units, etc.) was substantially underestimated initially.
- In the light of this study, the partners don't believe they would be able to reach the project cost target of \$2/L farnesene and collectively decided on a No-Go for the 3rd year of the project.

Milestone M (complete): *Conceptual engineering study based on information from both Renmatix and Amyris.*

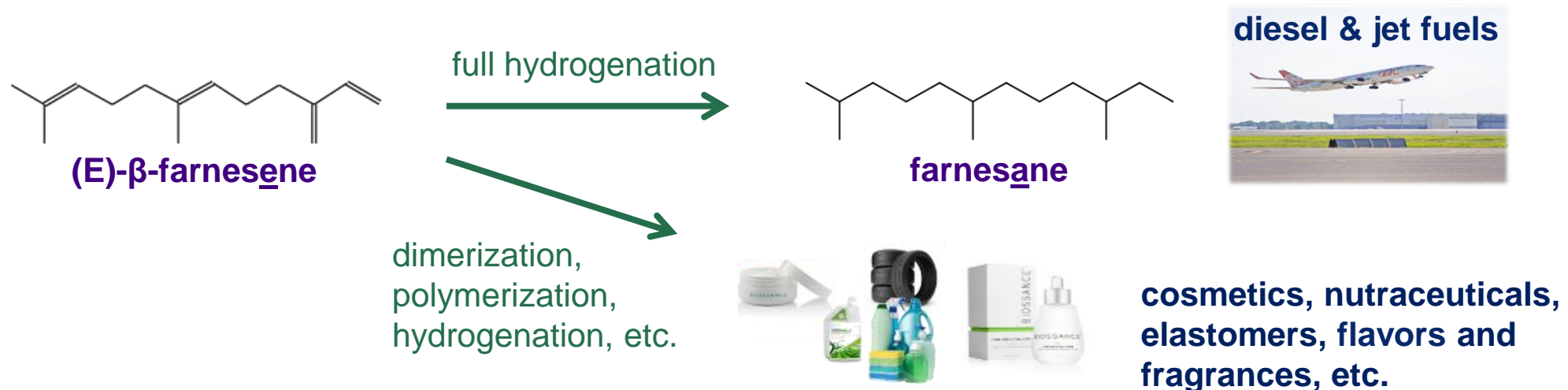
Milestone N (incomplete): *Preliminary LCA analysis based on conceptual study results.*


Our three companies have existing, commercially-relevant capabilities that we co-optimized in development of a cellulosic fermentation process:

- **Renmatix** coordinated sugar stream purification with **Amyris** biocatalyst improvement strategies to avoid over-engineering each step.
- **Total** applied its expertise in techno-economic modeling and life cycle analysis to assess whether our integrated manufacturing plant design met cost targets and life-cycle objectives.



- **Production of a platform chemical** that can earn high margins in specialty markets, and is easily upgraded (via hydrogenation) to a fuel replacement
 - Farnesane has ASTM approval for use in jet fuel as a 10% blend
 - Pure farnesane meets ASTM standards for Diesel #2, and currently has EPA approval as a 35% blend
- **Demonstrated markets for products** if the cost of cellulosic feedstocks can match historical average cane sugar costs
- **Dramatically expanded market potential** if the cost of the feedstock can be lower.



- **Biocatalyst development:** Amyris farnesene manufacturing strains were engineered for use of Renmatix cellulosic hydrolysates
 - A xylose consumption pathway meets our fermentation milestone
 - Strain clean-up and rational engineering improves tolerance to hydrolysates in fermentors
 - Organic acid consumption pathways are functional, and improve strain tolerance
 - Analytical methods were developed to identify, quantify and characterize new potential growth inhibitors in cellulosic hydrolysates
 - Fermentation process development improved performance on non-syrup feedstocks (not discussed)
- **Further feedstock purification:** Explored various options, and worked with an external vendor to develop a modified SMB process.
- **Process modeling:** Selected a favorable production site in Fulton, MS, and carried out a conceptual engineering study with full energy integration for techno-economic and life cycle analyses.
- Technoeconomic modeling of a fully integrated plant design shows us that unanticipated OSBL CAPEX costs make final cost targets infeasible, and the partners have decided with the DOE to suspend this three-year program after Year 2. 

Additional slides for reviewers

- Partner statement for 2017 EOY Go/No-Go:

Total, Renmatix and Amyris have jointly agreed that our MegaBio program remains on track to deliver on our final program goal of \$2.00/L farnesene from Loblolly pine feedstock. Specifically, as it pertains to the criteria laid out in the Statement of Project Objectives for Project Decision Point #2:

- 1) Although revision of CAPEX and OPEX estimates for the TEA are not scheduled until next year, Total has performed a Heat and Mass Balance analysis for the integrated plant design, and did not identify any issues of significant concern. Based on a review of assumptions that went into the initial TEA (original grant proposal), Total believes we can reach our program goal provided that the yield and productivity targets for bioconversion of Renmatix sugars into farnesene remain feasible, and Amyris believes that strain engineering efforts are on track to meet these bioconversion targets.
- 2) Conversion of [one organic acid inhibitor] into a native yeast metabolite exceeded our year-end consumption target by >7-fold in a scaled-down batch plate model. Conversion of levulinic acid is lower, at 70-100% of our consumption target in a scaled-down model, but for this inhibitor we exceeded our resistance target by >2.5-fold. Finally, a revised purification strategy for removing inhibitors prior to fermentation looks promising, though a more detailed cost analysis is still pending.
- 3) Xylose isomerase enzymes that meet our flux requirements have been licensed from two independent companies in Q3 2017.

Based on these criteria, Total, Renmatix and Amyris intend to move forward with the MegaBio program in 2018.

In addition, Amyris will move forward with subtask L.2 (acid consumption engineering), having demonstrated at least 10% [organic acid] consumption in a scaled-down batch plate model.

- 2018 EOY Go/No-Go:

- Strain improvement efforts have generally been successful, and have met end-of-year specifications.
- A feasible strategy for further sugar purification (if required) was established.
- Technoeconomic modeling of a fully integrated plant design, however, shows us that OSBL CAPEX costs make final cost targets infeasible, and partners have decided with the DOE not to move forward with Year 3 of the program.

- Public presentations (SBFC 2017, SBFC 2018, SMB 2018)
 - Amyris presented the Megabio project at the Symposium on Biotechnology for Fuels and Chemicals (SBFC) in San Francisco in May 2017 (Quinn Mitrovich, “Getting serious about cellulosic-derived products and fuels”)
 - Amyris presented the Megabio project at the Symposium on Biotechnology for Fuels and Chemicals (SBFC) in Clear Water, FL, in May 2018 (Yi Xiong, “Metabolic engineering of farnesene manufacturing strains to enable commercial production on cellulosic feedstocks ”)
 - Amyris presented the Megabio project at the Society for Industrial Microbiology and Biotechnology (SIMB) Annual Meeting in Chicago in August 2018 (Quinn Mitrovich, “Developing a commercially-viable bio-manufacturing process for cellulose-derived products and fuels”)