U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO)
2017 Project Peer Review

MegaBio

Integrated process for production of farnesene, a versatile platform chemical, from domestic lignocellulosic feedstock

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Biochemical Conversion

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Goal Statement

- Develop a scalable process to produce farnesene from cellulosic sugars at $2 per liter in the U.S.
- 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

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Quad Chart Overview

Timeline

- Project start: October 1, 2016
- Project end: December 31, 2019
- (10% complete)

Budget

	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$7,000,000
Amyris Cost Share	$1,325,619
Renmatix Cost Share	$349,367
Total Cost Share	$160,591

Barriers

- ***Efficient Intermediate Cleanup and Conditioning (Ct-G):*** Identify a sugar purification method that meets cost targets and reduces biocatalyst inhibition.
- ***Efficient Catalytic Upgrading of Sugars... to Fuels and Chemicals (Ct-H):*** Engineer biocatalysts for consumption of C5 sugars and resistance to inhibitors in sugar feedstocks.

Partners

- 75% **Amyris** (biocatalyst development)
- 20% **Renmatix** (feedstock development)
- 5% **Total** (engineering study of the integrated plant, cost estimates and life cycle analysis)

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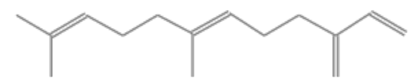
1 - Project Overview

- Farnesene, a 15-carbon terpene, is used as a precursor for a wide variety of commercial molecules, including renewable diesel and jet fuels.
- Amyris currently produces farnesene at its manufacturing facility in Brazil, using engineered microbes that consume a sugarcane syrup feedstock.
- By taking advantage of Renmatix's Plantrose® technology for cost-effective production of lignocellulosic sugars, we hope to enable commercial production of farnesene from woody feedstocks. This will require further optimization of Amyris production microbes.



(E)-β-farnesene





2 – Approach (Management)

Inter-company steering committee

Project manager

Renmatix	Amyris	Total
Project leader & team	**Project leader & team**	**Project leader & team**

- **Renmatix**: Cellulosic feedstock production and development of feedstock purification methods
- **Amyris**: Strain engineering and scalable process development
- **Total**: Engineering study of an integrated plant, techno-economic analysis (TEA), and life cycle analysis (LCA)

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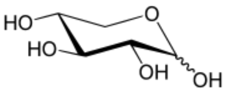
2 – Approach (Technical)

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

- Enable consumption of **xylose** from hemicellulosic sugar streams.



- Deal with **cellular inhibitors** present in feedstocks. We have three methods for addressing this:
  - Enable **consumption** of these inhibitors through strain engineering
  - Engineer (or evolve) **resistance** mechanisms into production strains
  - Develop feedstock **purification** methods that are compatible with final cost targets
- Develop a scalable fermentation 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.

3 – Technical Accomplishments/ Progress/Results

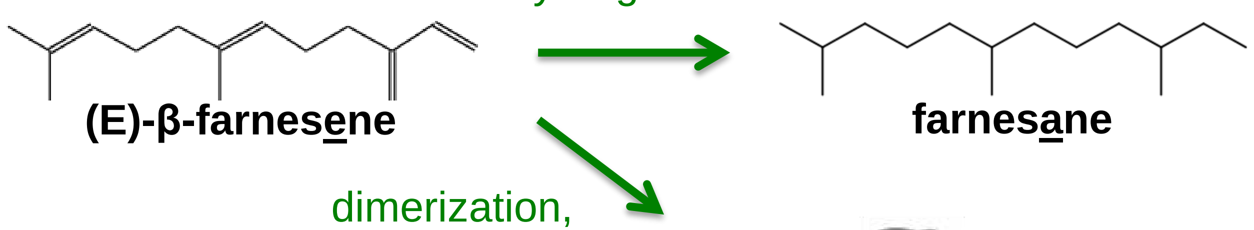
- In the first project quarter (Q4 2016), we successfully completed our validation stage:
  - Renmatix demonstrated production of C6 hardwood-derived hydrolyzates produced using its supercritical water-based Plantrose® process.
  - Total demonstrated a Renmatix C6 sugar upgrade process that is compatible with farnesene fermentations.

  - Amyris demonstrated efficient conversion of upgraded sugars into farnesene using its scalable fermentation process.

  - Total provided an updated preliminary techno-economic analysis.

4 – Relevance

- **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*.



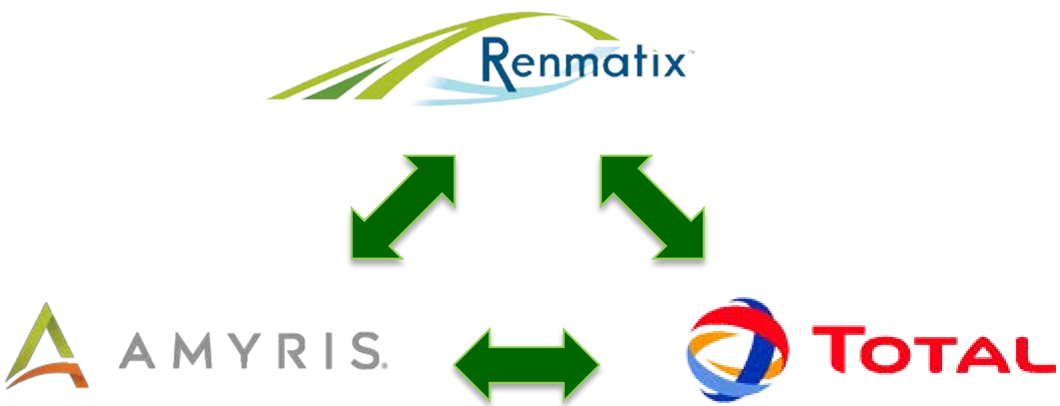
diesel & jet fuels

cosmetics, solvents, elastomers, flavors and fragrances, etc.

4 – Relevance (continued)

Our three companies have existing, commercially-relevant capabilities that will be co-optimized to develop a viable cellulosic fermentation process:

- **Renmatix** will coordinate sugar stream upgrading with **Amyris** biocatalyst improvement strategies to avoid over-engineering each step.
- **Total** will apply its expertise in techno-economic modeling and life cycle analysis to ensure that an integrated manufacturing plant design will meet cost targets and life-cycle objectives.



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5 – Future Work

We have now assembled project teams and are optimistic about meeting the requirements of our next go/no-go decision point (end of 2017). *Activities for this coming year:*

- **1) Enable xylose utilization in manufacturing strains**.
  - *Project goal*: >95% consumption of xylose in fermentation feedstocks.
  - *End of 2017 goal*: Amyris will obtain/license enzymes compatible with final flux requirements.
  - *Current efforts*: Amyris has identified candidate enzymes, and strain engineering efforts have begun.

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5 – Future Work (continued)

We have now assembled project teams and are optimistic about meeting the requirements of our next go/no-go decision point (end of 2017). *Activities for this coming year:*

- **2) Mitigate against cellular inhibitors in cellulosic feedstocks**.
  - *Project goal*: Overcome biocatalyst inhibition through strain engineering (Amyris) or cost-effective sugar purification (Renmatix).
  - *End of 2017 goal*: Amyris meets intermediate milestone for inhibitor tolerance or consumption; *or* Renmatix demonstrates path for cost-effective purification.
  - *Current efforts*: Amyris has initiated strain engineering; Renmatix has begun sugar purification technology option development.

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5 – Future Work (continued)

We have now assembled project teams and are optimistic about meeting the requirements of our next go/no-go decision point (end of 2017). *Activities for this coming year:*

- **3) Identify locations in the U.S. suitable for an integrated plant for conversion of wood to farnesene, and update TEA by end of 2017.**
  - Based on the retained locations, Total will conduct an LCA of the feedstock sites and prepare a preliminary block flow diagram with optimized energy integration.
  - Together with experimental progress, the TEA will be updated to evaluate if the project is on track to meet the $2/L target.

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Summary

- Having successfully completed our validation stage, we are now beginning the main work of the project.
- We are optimistic about addressing the key project challenges over the next three years, and delivering:
  - Cost-competitive sugar feedstocks derived from wood (cellulose and hemicellulose)
  - A production strain and scalable process capable of effectively converting this feedstock into farnesene
  - A detailed engineering study and TEA supported by a comprehensive LCA for achieving the sustainable manufacture of farnesene from U.S. lignocellulosic sugars at $2 per liter

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