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

Synthetic Metabolic Pathways for Bioconversion of Lignin Derivatives to Biofuels

March 7, 2017

Biochemical Conversion Session

Adam Guss

Oak Ridge National Laboratory

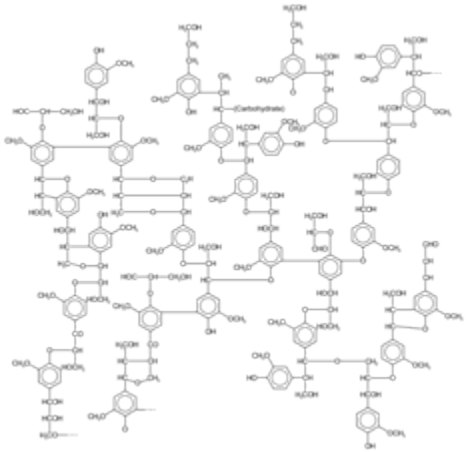
This presentation does not contain any proprietary, confidential, or otherwise restricted information

ORNL is managed by UT-Battelle
for the US Department of Energy

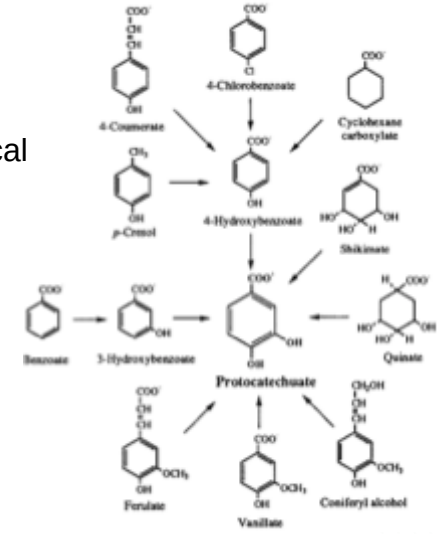
OAK RIDGE National Laboratory

Goal Statement

- The goal of this project is to develop biological routes to convert lignin-derived aromatic compounds into value-added fuels and chemicals.
- Lignin valorization to high value products is essential to meeting BETO's 2022 verification targets



Thermochemical
Catalytic
Biological



Biological

Fuels
+
Chemicals

Quad Chart Overview

Timeline

- 10/01/2015
- 9/30/2018
- 45% complete

Budget

	Total Costs FY 12 – FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 14-Project End Date)
DOE Funded	$205k	$270k	$402k	$1.53 MM

Barriers

- Ct-H. Efficient Catalytic Upgrading of Sugars/Aromatics
- Ct-E. Efficient Low-Temperature Deconstruction
- Ct-G. Efficient Intermediate Cleanup and Conditioning

Partners

- Collaboration and shared milestones with:
  - Beckham (NREL) “Lignin Utilization” WBS 2.3.4.100
  - Bomble (NREL) Metabolic modeling. WBS 2.5.1.100
  - Biddy (NREL) Techno-economic Analysis. WBS 2.1.0.100

1 - Project Overview

Context:

- Lignin accounts for ~25% of plant biomass by weight and 40% of the carbon
  - Underutilized during biofuel production
- Primary current use is for process heat and electricity

Project Objectives:

- Develop a biological platform for production of fuels and chemicals from lignin-rich streams
- Engineer *Pseudomonas putida* to convert aromatic compounds to value-added products
  - PHAs as initial product (proof of concept molecule for products made through fatty acid chain elongation pathway)
  - Now also targeting medium chain length alcohols (plasticizers, lubricants, surfactants and solvents; future possible fuel)
  - Beginning to target products that preserve the aromatic nature of lignin substrates

2 – Approach (Management)

Management approach

- Regular interactions with NREL collaborators (Beckham, Biddy, Bomble)
  - MTA and NDA in place
  - Standing monthly phone call to discuss progress
  - Site visits, data sharing, strain exchanges
- Go/No Gos
  - FY16 Q2 – dropped examination of other microbes for lignin conversion
  - FY17 Q4 – achieving sufficient titer to justify continuation

Project structure

- Adam Guss, PI
- Postdoc Joshua Elmore leads strain design and construction
- Post-MS Annette DeCapite leads product detection and quantification
- Postdoc Jason Bouvier leads targeting aromatic products

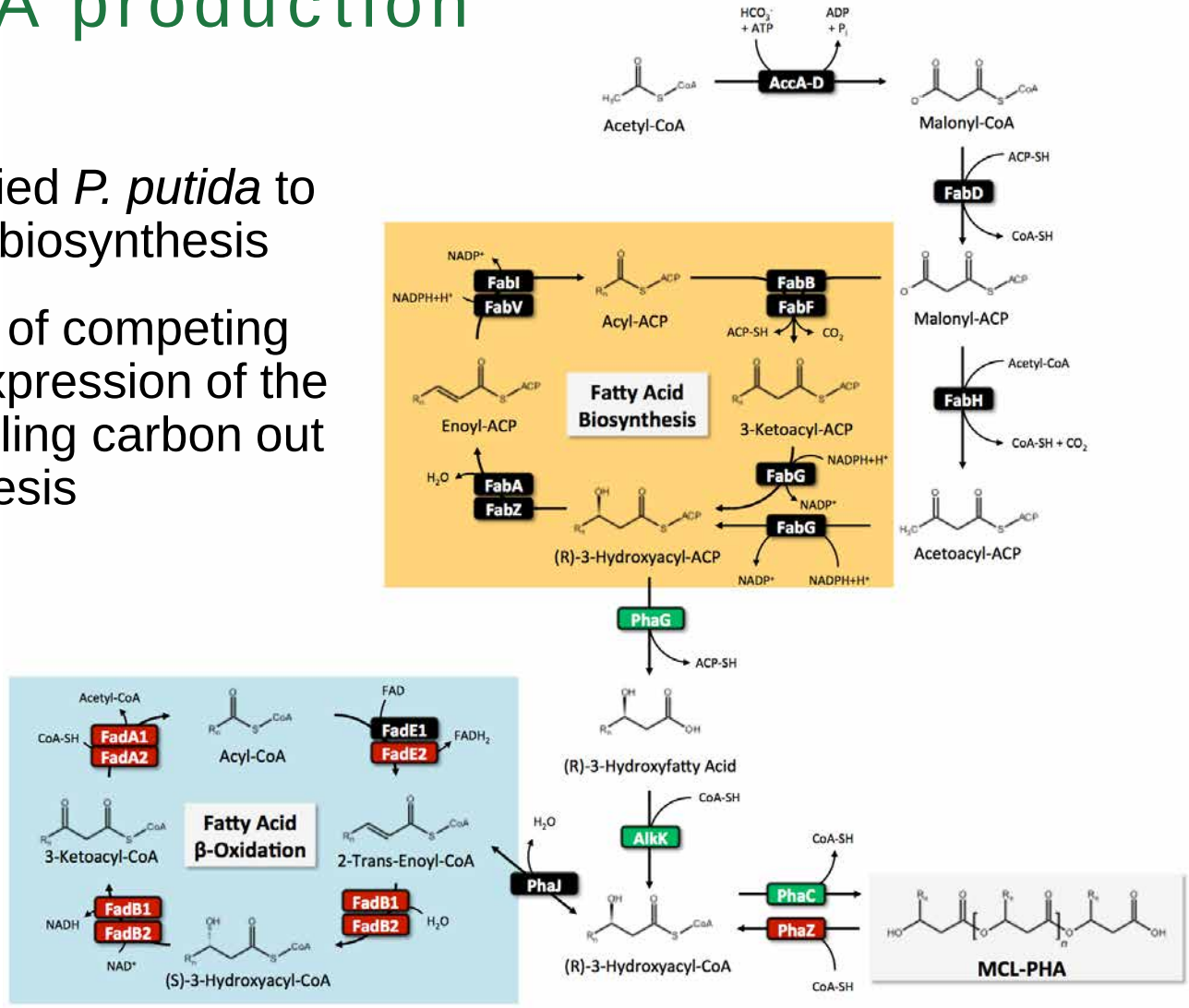
2 – Approach (Technical)

- *Main approach*
  - Microbial genetics and synthetic biology: Combination of gene deletion and heterologous gene overexpression to optimize the metabolic pathways
- *Potential challenges to be overcome for achieving successful project results*
  - Genetic modifications can be toxic or lethal
  - Flux through heterologous pathways may not be sufficient
- *Critical success factors (technical, market, business) that will define technical and commercial viability*
  - Titer, rate, and yield of target molecules
  - Availability of aromatic streams capable of supporting bioconversion

3 – Technical Accomplishments/ Progress/Results

P. putida metabolic engineering for increased PHA production

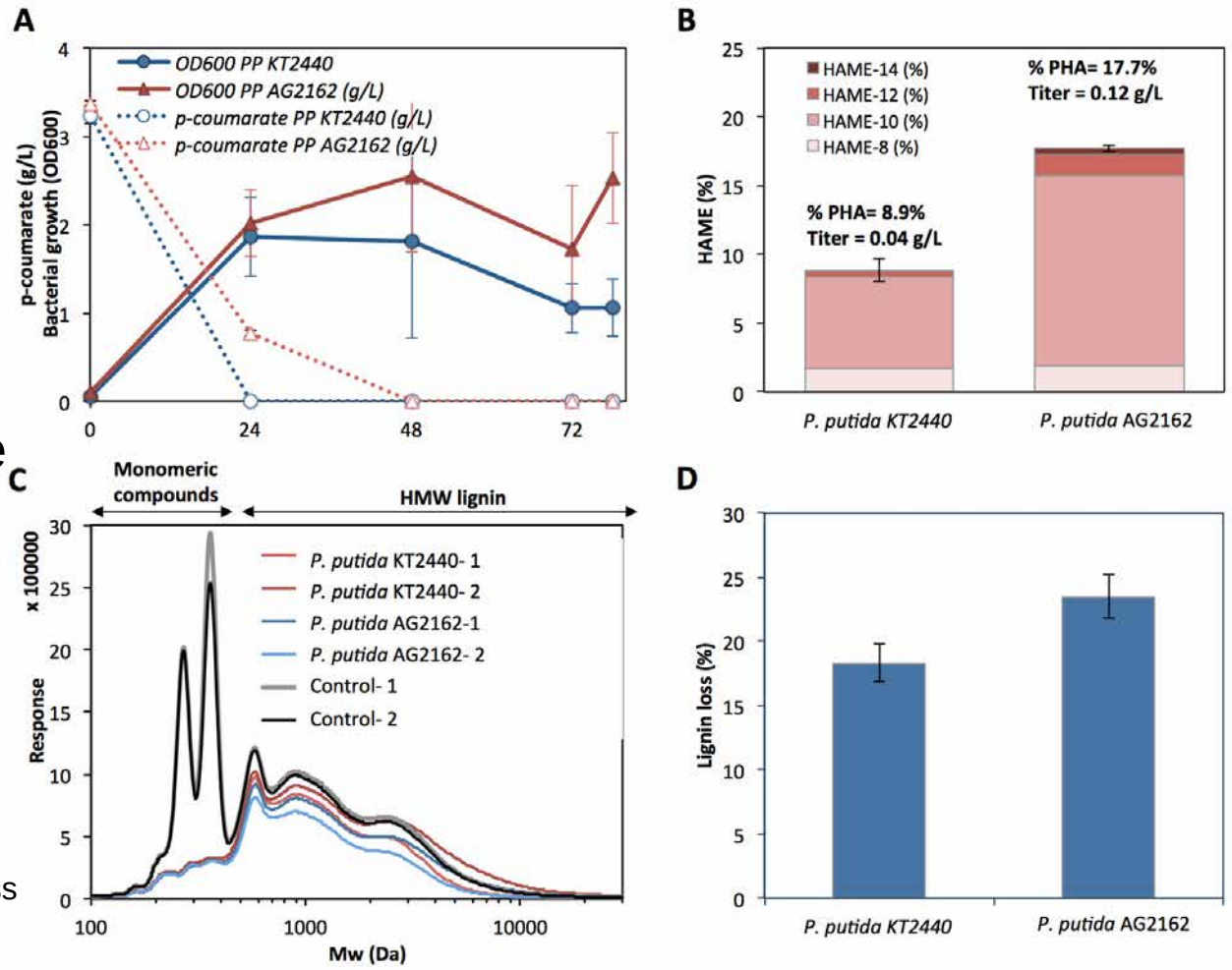
- We genetically modified *P. putida* to increase flux to PHA biosynthesis
- We targeted deletion of competing pathways and overexpression of the genes involved in pulling carbon out of fatty acid biosynthesis



3 – Technical Accomplishments/ Progress/Results

Conversion of DMR-EH lignin to PHAs by engineered *P. putida*

- Modified *P. putida* was grown on minimal medium with BCD-DMR-EH lignin
- Demonstrated 100% increase in PHA abundance per dry cell weight, and an increase in PHA titer by 200%
- Will look at fed-batch approaches to increase available carbon for PHA production



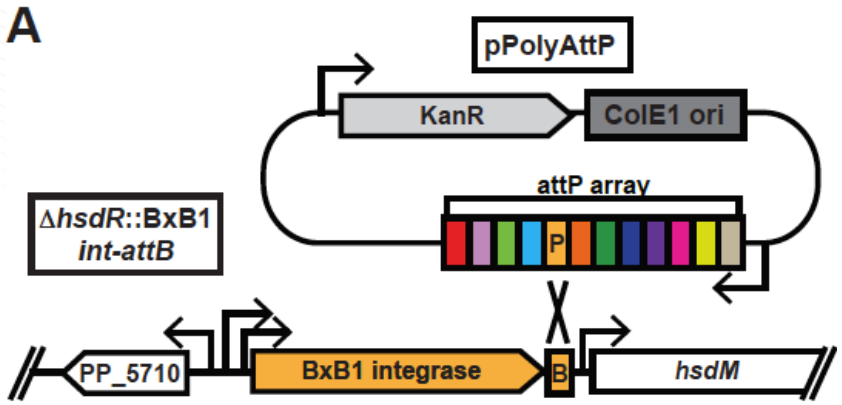
*DMR-EH = Deacetylated/Mechanical-Refined, Enzymatically Hydrolyzed biomass

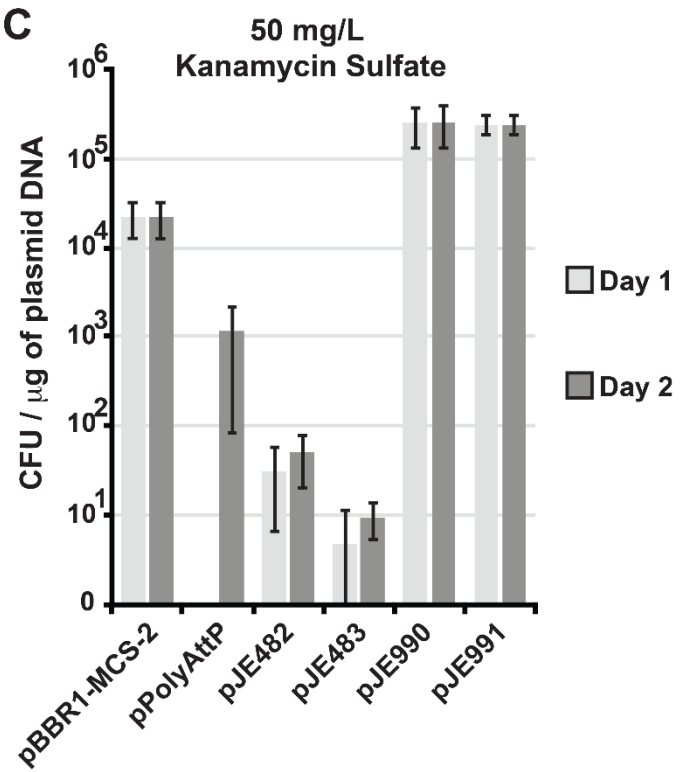
*BCD = Base-Catalyzed Depolymerization

In collaboration with Salvachua and Beckham, NREL, project 2.3.4.100 (Lignin Utilization)

3 – Technical Accomplishments/Progress/Results

Development of a high throughput DNA integration system in *P. putida*





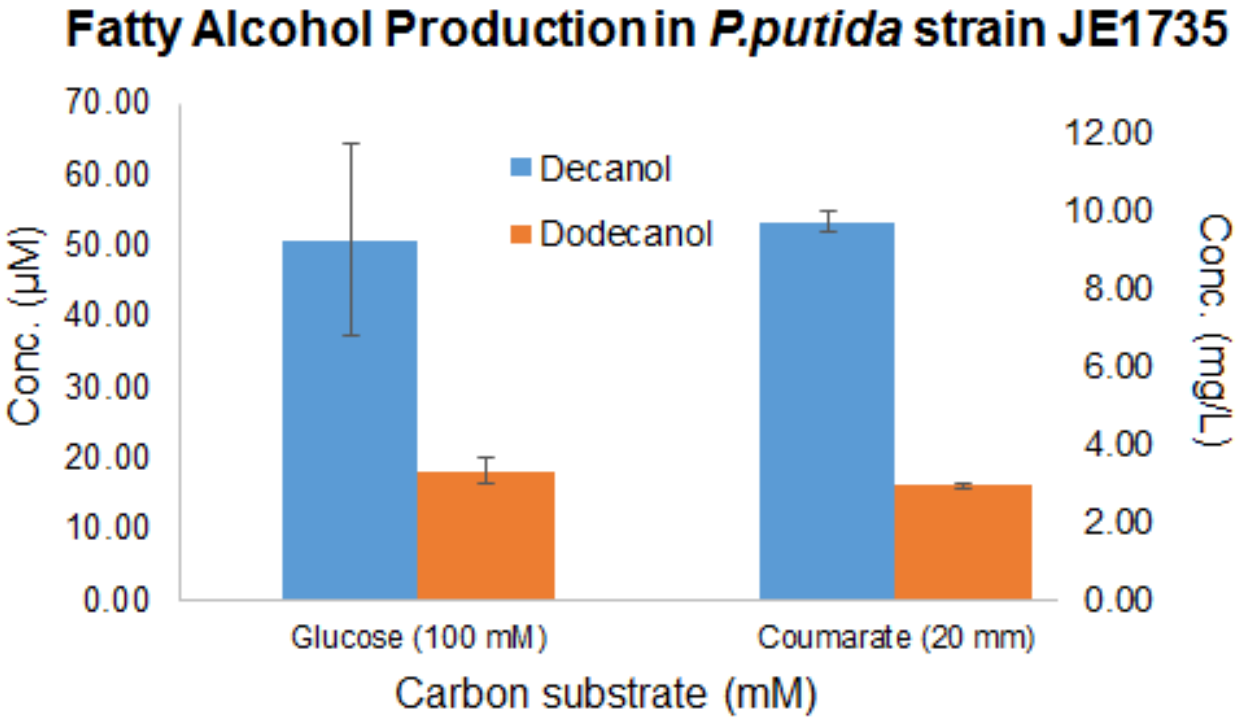
- We needed a method to rapidly introduce DNA stably into the chromosome, so we developed a phage integrase-based site specific recombination system
- DNA inserts into the chromosome, transforming *P. putida* as efficiently (or more so) than a replicating plasmid.

Elmore J, Furches A, Wolff G, Gorday K, and **Guss AM***. Accepted for publication at Metabolic Engineering Communications

3 – Technical Accomplishments/Progress/Results

P. putida engineering: medium chain alcohols

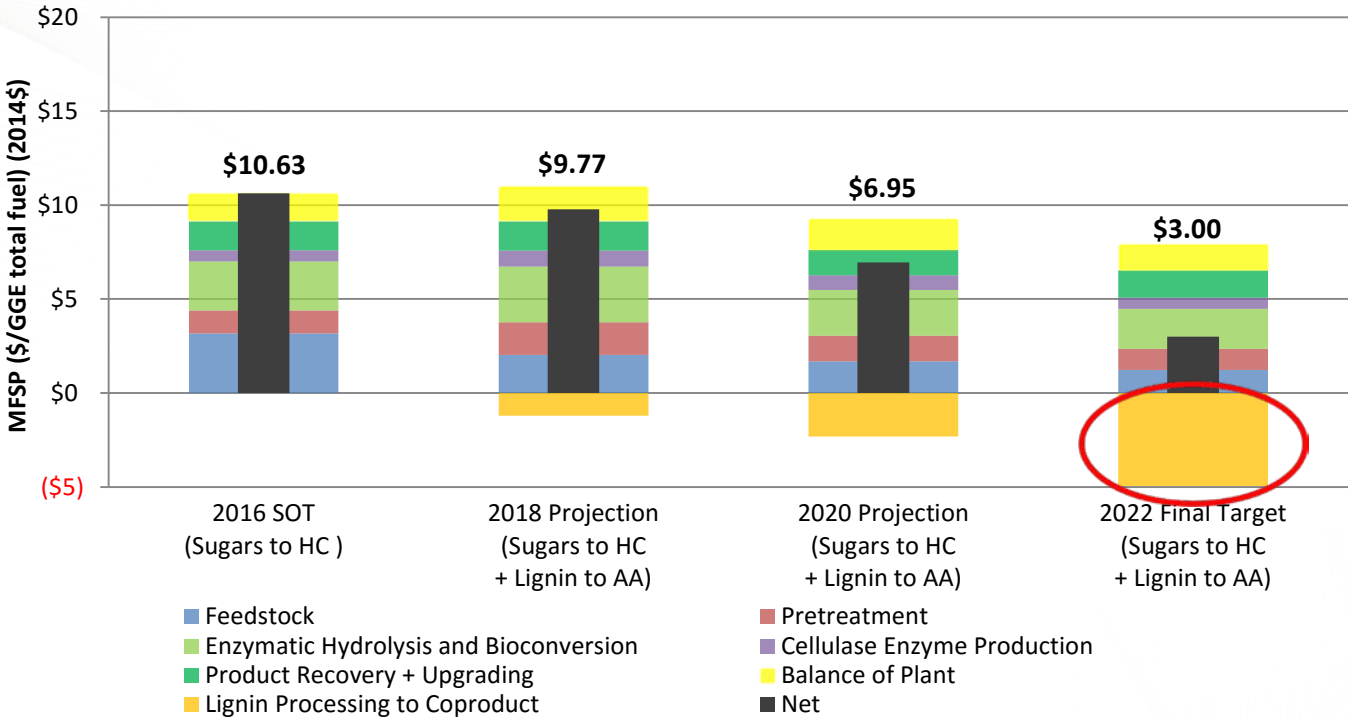
- Used integration system to test first round of designs
- Multiple producing strains
- Using this data to design the next round of pathways



Milestone Name/Description
FY17Q1 - Measure fatty alcohol production by GC/MS from at least 20 heterologous pathways in a *P. putida* strain modified for at least three other loci (e.g., deletions to eliminate competing pathways or overexpression of other genes).

4 – Relevance

Need for lignin valorization to reach cost targets



- Mcl-alcohol market is similarly sized to adipic acid (3MM metric tons/year)
- Current mcl-alcohol selling price is ~ $1.20 per pound
- TEA is ongoing to determine needed performance metrics for bioconversion
  - Joint NREL/ORNL FY17Q2 milestone, will update as needed

5 – Future Work

- Complete metabolic engineering to maximize PHA production
- Continue engineering to produce >1 g/L medium chain alcohols
- Demonstrate pathways to convert aromatics into products that have high carbon yield and preserve aromatic ring
- TEA on each product being targeted

- Upcoming key milestones:
  - FY17 Q2 – Develop preliminary TEA analysis for production of mixed medium chain length alcohols produced from deconstructed lignin
  - FY17 Q2 – Develop and employ a core-carbon model of Pseudomonas putida KT2440 to model production of up to two target products from lignin-derived aromatic compounds
- Upcoming Go/No-Go Points
  - Demonstrate production of mcl-PHAs at a titer of at least 1 g/L or another product (e.g., mcl-alcohols or aromatic compounds) at a titer of at least 0.1 g/L from aromatic substrates
- The remaining budget is sufficient to complete this work.

Summary

- Summarize the key points you wish the audience and reviewers to take away from your presentation in the following categories:
  1. Lignin valorization will be critical to enable a bioeconomy
  2. Genetic modification of *P. putida* is an attractive approach to engineering an organism to produce lignin-derived fuels and chemicals
  3. We have increased PHA production, demonstrated medium chain alcohol production, and developed a genetic tool that will greatly accelerate progress in the future.
  4. Industry needs approaches to convert lignin into value-added products, which will enable more economical production of sugar-based fuels
  5. Future work will focus on increasing rate/yield/titer of our target molecules, including ones with high carbon efficiency

Additional Slides

Responses to Previous Reviewers' Comments

- The only concern is whether or not the project should also be looking at some lignin that is coming from current producers (POET-DSM, Abengoa, or DuPont), as well as the new lignin that NREL is generating from a new pretreatment.
  - We do not currently have plans to pursue lignin-rich streams from lignocellulosic biofuels companies. However, we are pursuing conversion of lignin streams generated by our colleagues at NREL. Initially, we focused on Alkaline Pretreated Liquor (APL), and we are now also looking at the upgrading of Base-Catalyzed Depolymerization (BCD) lignin from Deacetylated/Mechanical-Refined, Enzymatically Hydrolyzed (DMR-EH) biomass. As research on that pretreatment advances, we will also examine the resulting lignin as an alternate feedstock for our engineered microbes, typically in collaboration with colleagues at NREL.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

Responses to Previous Reviewers' Comments

Go/No-Go Description – FY16Q2

- Identify a lignin-degrading organism that demonstrates 15% greater lignin depolymerization and catabolism than Pseudomonas putida. Joint with NREL "Lignin Utilization" 2.3.4.100. We will screen a large set of lignin-degrading and catabolizing organisms together with ORNL and quantify how much lignin can be degraded by GPC and Klason lignin measurements.

Go/No-Go Criteria

- 15% greater lignin depolymerization and catabolism in APL over *Pseudomonas putida* in four days. If a 15% greater organism is found, develop tools for that organism. If not, drop work on other organisms and focus on *P. putida* engineering.

Results

- We examined >30 phylogenetically diverse microbes for lignin and aromatic catabolism. By all measures, *Pseudomonas putida* was either best or amongst the best at both lignin depolymerization and catabolism, including Klason lignin analysis, Gel Permeation Chromotography, and tested substrate range.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

Publications, Patents, Presentations, Awards, and Commercialization

- Elmore J, Furches A, Wolff G, Gorday K, and **Guss AM***. Development of a high efficiency integration system and promoter library for rapid modification of *Pseudomonas putida* KT2440 Accepted for publication at Metabolic Engineering Communications.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.