Biological Lignin Valorization – NREL

March 6th, 2019

Technology Session Review Area: Lignin

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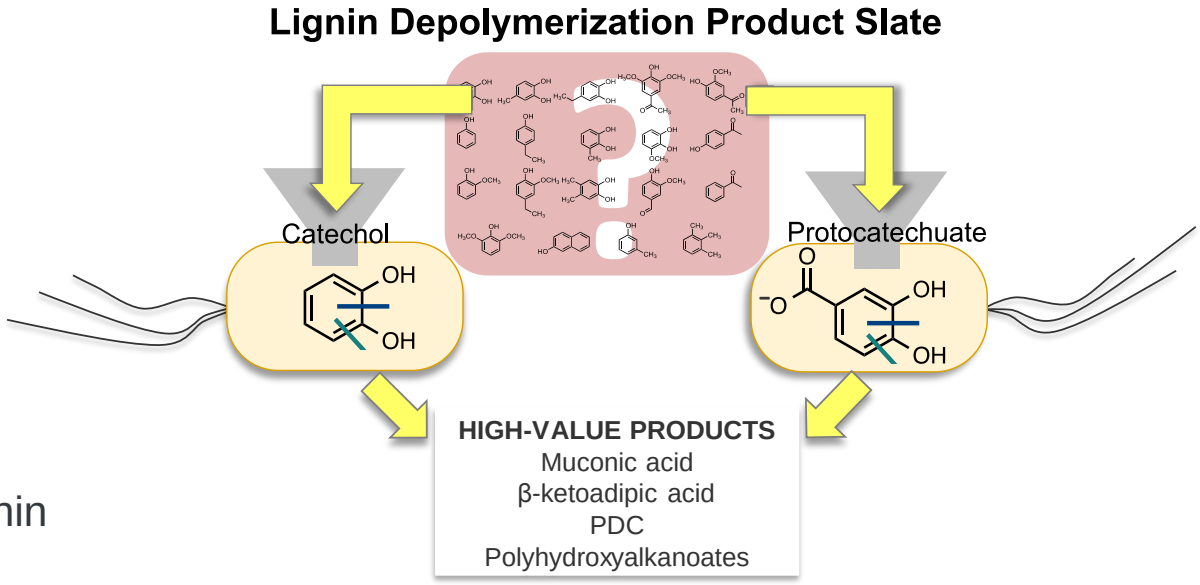
National Renewable Energy Laboratory

Goal Statement

Goal: Develop biological processes to produce co-products from lignin

- Contribute $2-3/gge for biofuel production
- Focus on products with sufficient market size to aid industry
- Collaborate with Lignin Utilization project for catalysis, analytics
- Develop foundational concepts to harness biological systems for lignin valorization



Outcome: Biological approach to convert lignin to co-products that make a positive contribution to the economic viability of an integrated biorefinery

- Target titer, rate, and yield (TRY) of co-products that can be converted into commodity monomers and performance-advantaged bioproducts

Relevance: lignin currently undervalued, but can be up to 40% of biomass carbon

Quad chart overview

Timeline

- Start date: October 2015
- End date: September 2019
- Percent complete: 87%

	Total Costs Pre FY17	FY17 Costs	FY18 Costs	Total Planned Funding (FY19- End Date)
DOE funded	$281k	$251k	$470k	$700k

Partners:

BETO Projects: Lignin Utilization, Metabolic Engineering for Lignin for Lignin Conversion, Biological Lignin Valorization – SNL, Low Temperature Advanced Deconstruction, Biological Process Modeling and Simulation, Performance-Advantaged Bioproducts

Nat'l labs and universities: Oak Ridge National Laboratory, Lawrence Lawrence Berkeley Laboratory, Sandia National Laboratory, University of Georgia, University of Portsmouth, Denmark Technical University

Other DOE projects: Center for Bioenergy Innovation (CBI, ORNL), Joint ORNL), Joint Bioenergy Institute (JBEI, LBNL)

Barriers addressed

Ct-C Process development for conversion of lignin

- Using biological funneling to overcome lignin heterogeneity

Ct-D Advanced bioprocess development

- Strategies to convert aromatics to value-added compounds at high TRY

Objective

Develop strains and bioprocesses to convert lignin-derived monomers and oligomers to exemplary products that enable ≥50% lignin conversion and a positive contribution to minimum fuel selling price

End of Project Goal

Demonstrate production of three compounds from lignin-derivable mixtures at titers ≥50 g/L and ≥90% yield. Develop ligninolytic enzymes able to cleave the 5 most relevant linkages in lignin that can be secreted by *P. putida*.

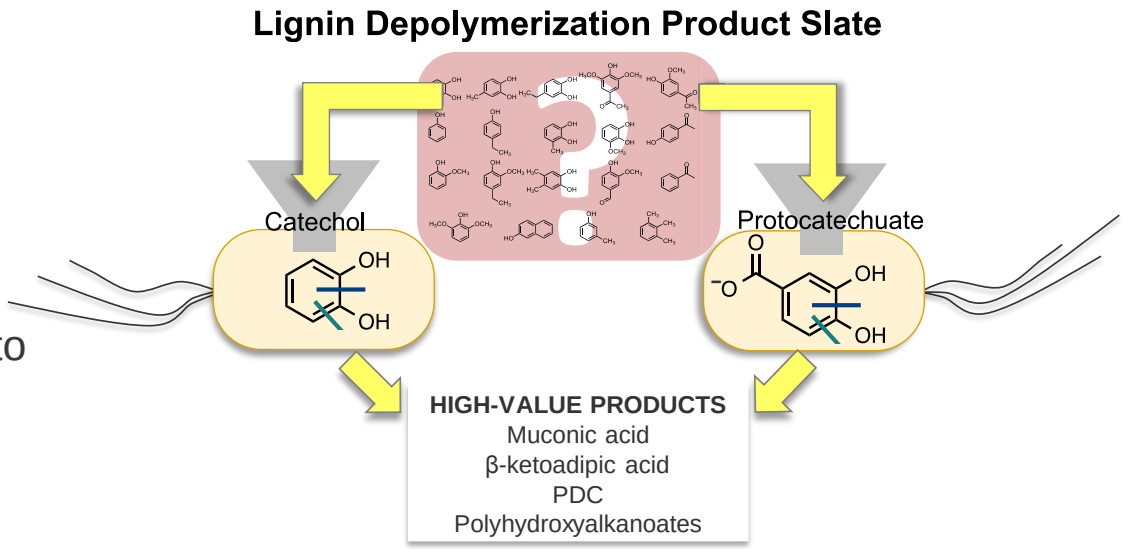
Project overview

History: Project started in FY18, preceding work also funded by BETO

- **TEA predicted lignin to adipic acid could provide ~$3/gge and major CO_2 offsets**
- Earlier subtasks in separate projects for oligomer deconstruction and metabolic engineering
- Combined into a single project in FY18; collaborate with similar projects at ORNL, SNL

Context: Lignin is undervalued in biorefineries

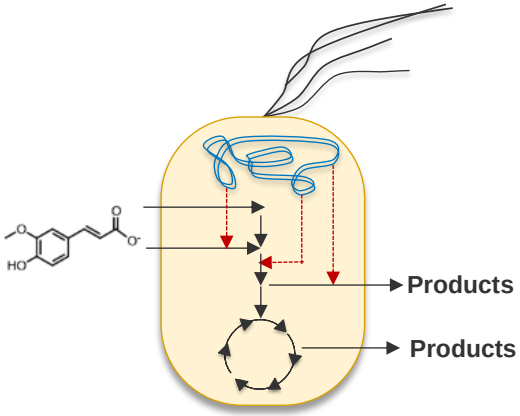
- Heterogeneity is the main barrier to lignin valorization
- Biological funneling offers approach to overcome heterogeneity

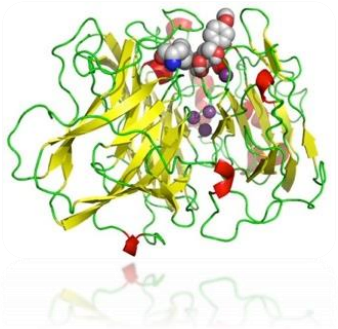


Project Goals:

- Develop *P. putida* as a robust host for monomer and oligomer conversion
- Conduct strain engineering and bioprocess development together to achieve targets:
  - Three compounds at ≥50 g/L, ≥90% yield
- Work with lignin catalysis efforts to tailor strains for efficient conversion

Management Approach





Biweekly meetings internal to project

Regular in-person meetings with external partners

Task 1: Strain Development

- Led by *P. putida* engineering expert (Chris Johnson)
- Milestones: **overcoming bottlenecks, expanding substrate specificity, improving yields/rates**
- Collaborate with systems biologists and BPMS project

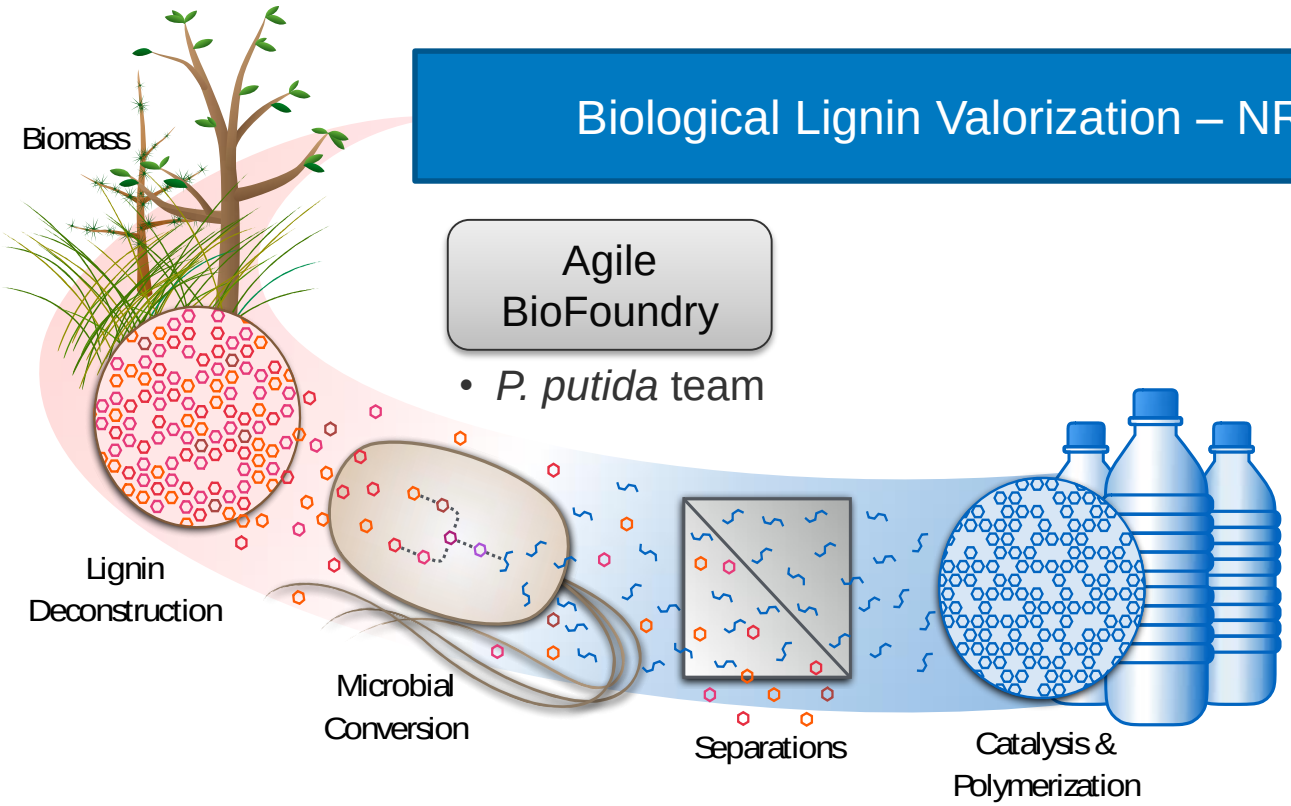
Task 2: Bioprocess Development

- Led by *P. putida* microbiology expert (Davinia Salvachúa)
- Milestones: **TRY improvements, evolution to higher tolerance**
- Collaborate with Denmark Tech. Univ. on adaptive laboratory evolution
- Deploy processes to strains from other BETO projects (e.g., ORNL, ABF)

Task 3: Deconstruction of Soluble Oligomers

- Led by ligninolytic enzyme expert (Davinia Salvachúa)
- Milestones: **expanding *P. putida* oligomer catabolism**
- Collaborate with SNL and ORNL on oligomer cleavage
- Leverage discoveries from academic and Office of Science projects

Project interactions



Biological Lignin Valorization – NREL

Agile BioFoundry

- *P. putida* team

Lignin

- Lignin Utilization
- Metabolic Engineering for Lignin Conversion – ORNL
- Biological Lignin Valorization – SNL

Biochemical Conversion

- Biochemical Platform Analysis
- Low Temperature Advanced Deconstruction
- Biochemical Process Modeling and Simulation

PABP/ Separations

- Separations Consortium
- Performance-Advantaged Bioproducts via Selective Biological and Catalytic Conversion

Beckham *et al. Curr Opin Biotech* 2016

Technical Approach

Critical Success Factors:

- Need to achieve industrial TRY of lignin products
- Availability of high yields of bio-available substrate is “make or break” for this concept

Task 1: Strain Development

Approach:

- *P. putida* chassis, chromosomal integration
- Modeling with BPMS project
- Monomer conversion to muconic acid (first product)
- Hydroxycinnamic acids as initial substrates

Challenges:

- Metabolic bottlenecks

Task 2: Bioprocess Development

Approach:

- Optimize bioprocesses with models and lignin
- Evolve strains for substrate/product tolerance
- Conduct cultivations for other lignin efforts

Challenges:

- Substrate solubility and stability
- Toxicity of substrate and product

Task 3: Deconstruction of Soluble Oligomers

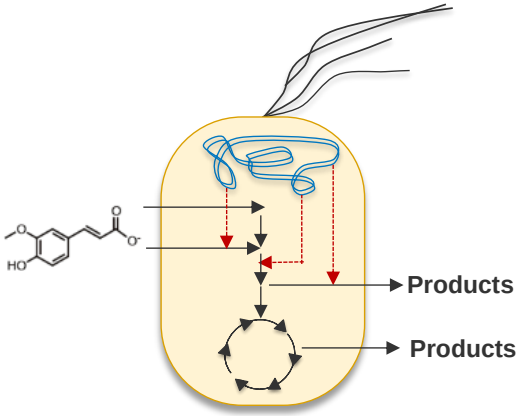
Approach:

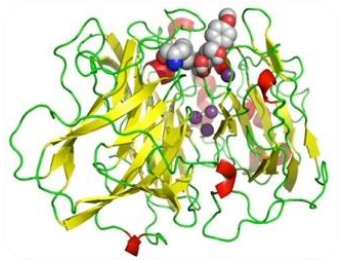
- Engineer ligninolytic enzymes into *P. putida*
- Analytics development for enzyme analyses

Challenges:

- Enzyme expression levels, efficient secretion
- Lignin repolymerization

Outline of technical accomplishments





Task 1: Strain Development

- Engineered strain to convert *p*-coumarate and ferulate to muconate
- Overcame multiple bottlenecks from H and G compounds
- Engineered strain to utilize S-lignin compounds
- Engineered biological syringol conversion for the first time

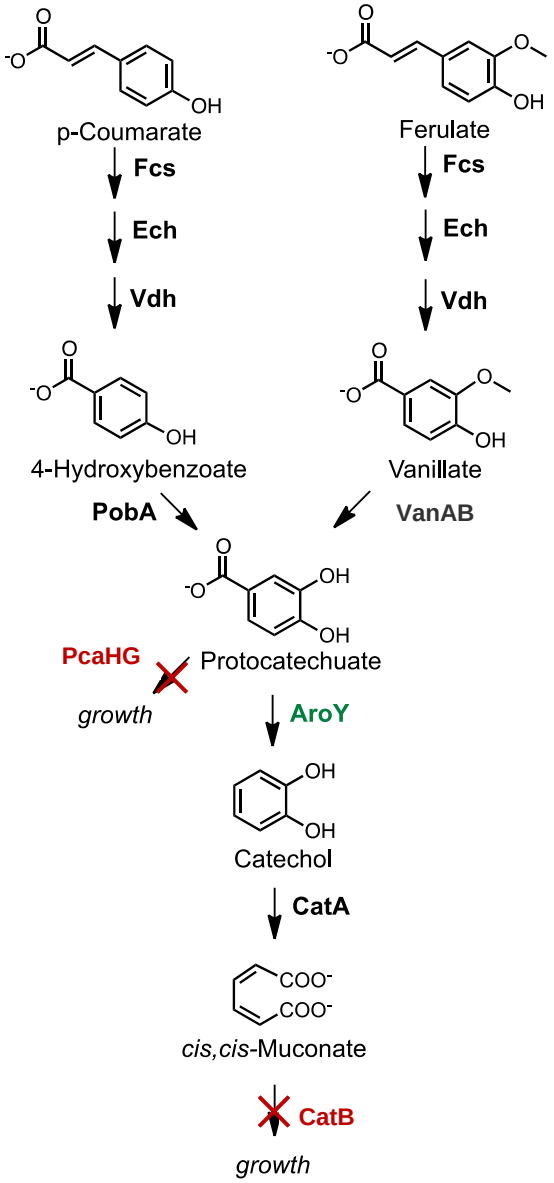
Task 2: Bioprocess Development

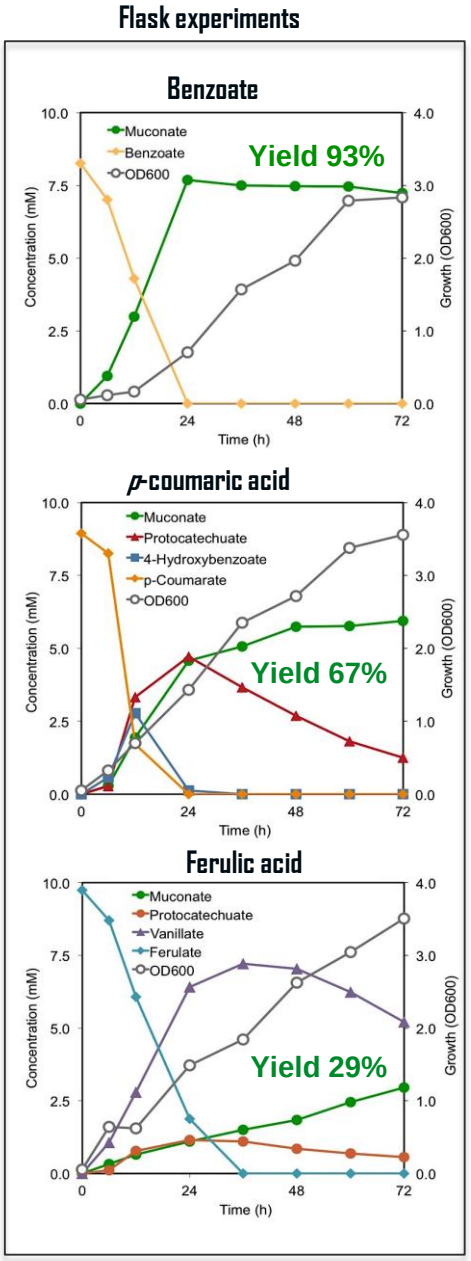
- Developed cultivations to achieve ~50 g/L muconate, at >90% yields, and productivities from 0.2-0.5 g/L/hr
- Evaluated lignin streams for muconate production
- Evolved and screened strains for substrate/product toxicity
- Ran cultivations with collaborators for itaconic acid, PHAs, etc.

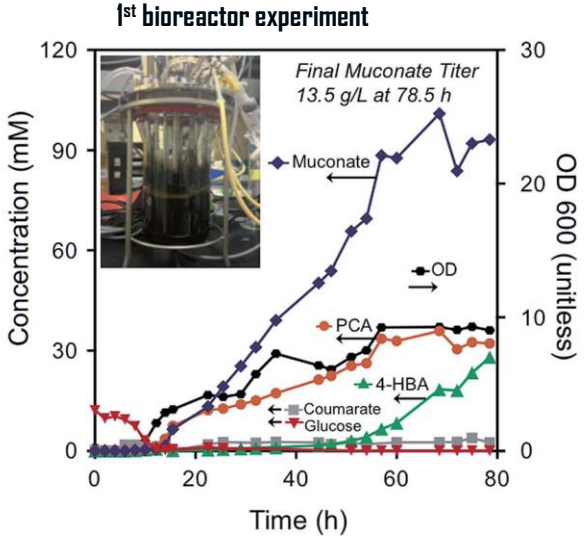
Task 3: Deconstruction of Soluble Oligomers

- Discovered new mechanism for lignin catabolism in *P. putida*
- Screened enzymes for ß-O-4 cleavage and flavonoid catabolism
- Worked closely with Lignin Utilization Characterization team

Muconate production via strain and bioprocess development



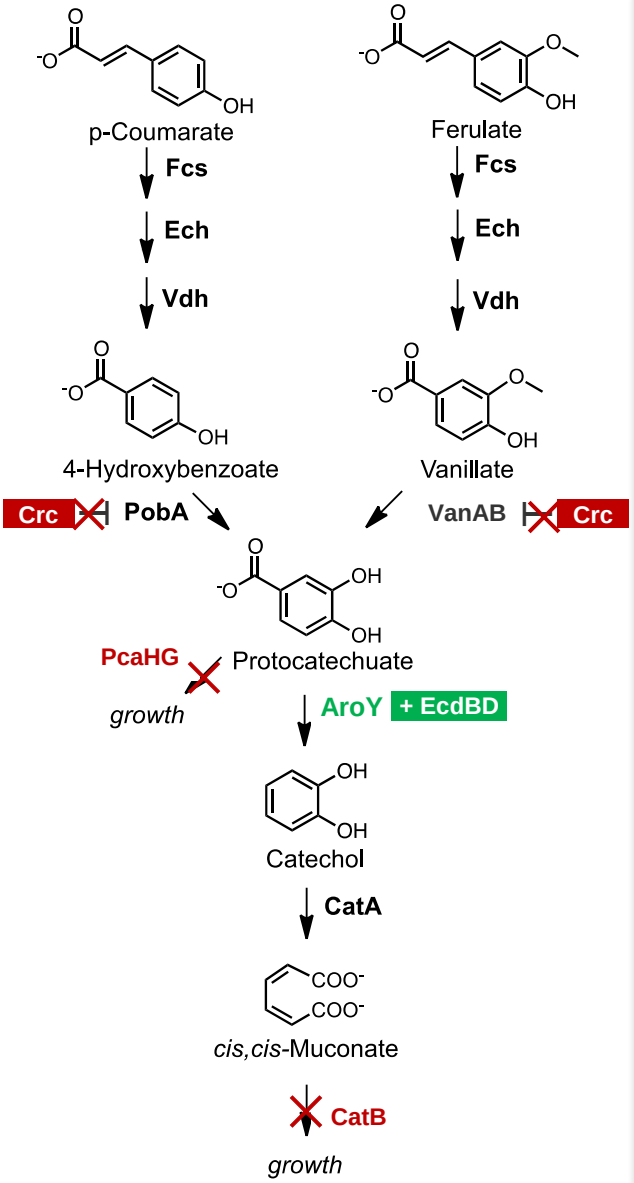


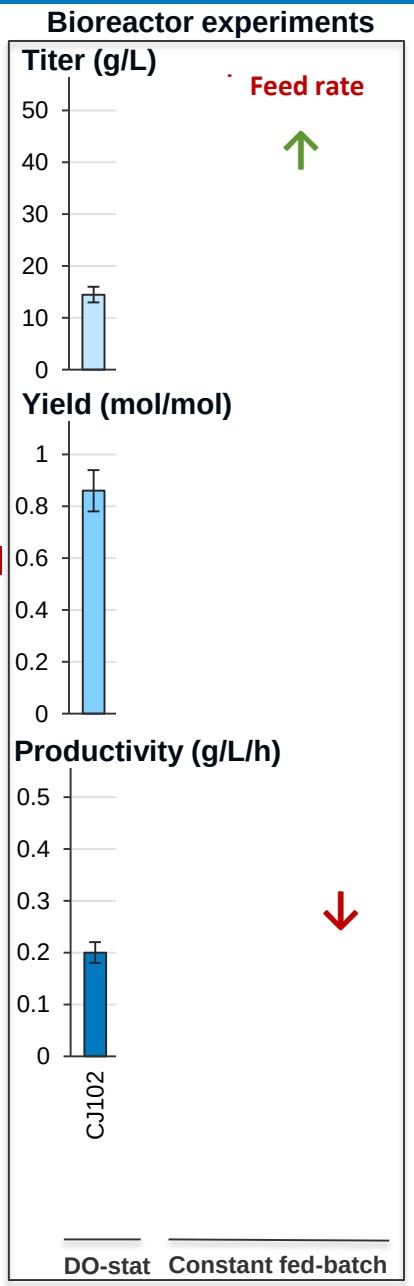


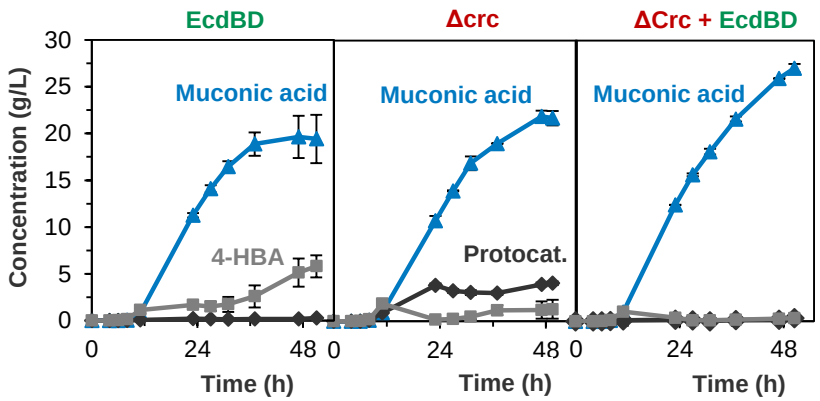
- Initial strain for muconate production from lignin monomers, *p*-coumarate and ferulate
- Yields limited by bottlenecks in enzymes that metabolize protocatechuate, 4-hydroxybenzoate, and vanillate
- **Outcome**: **muconate demonstration from model compounds, observations of 3 key bottlenecks**

Vardon, Franden, Johnson, Karp *et al. Energy Env Sci* 2015

Debottlenecking via co-factor engineering and regulatory changes



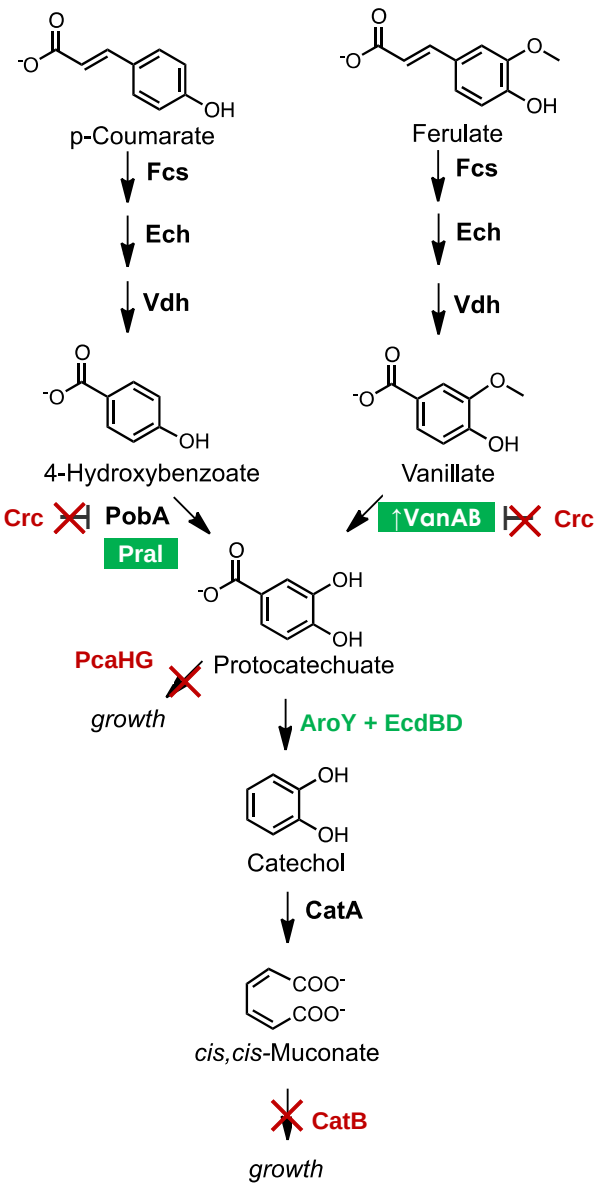


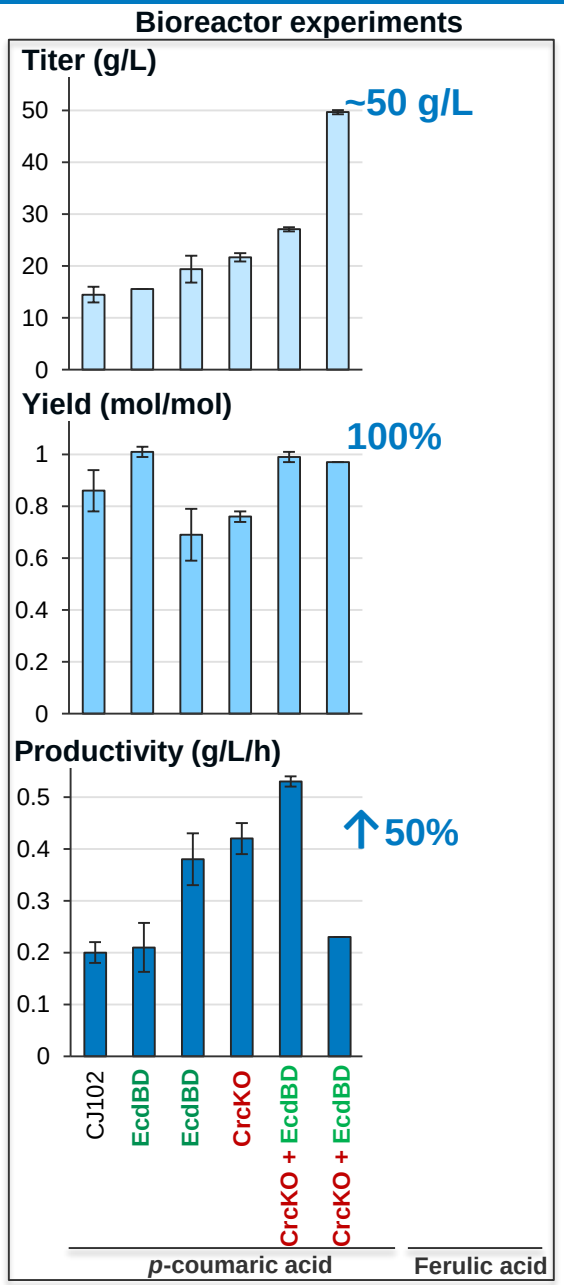


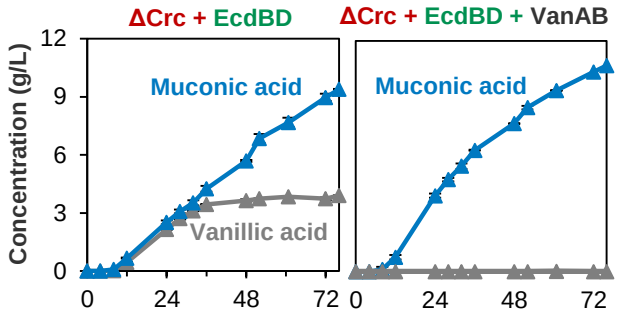
- EcdBD generates a prenylated co-factor for AroY
- Crc knockout improves substrate utilization rates and product yields via increased expression of PobA and VanAB
- **Outcome: Improved selectivity of protocatechuate decarboxylase, reduced buildup of 4-hydroxybenzoate and vanillate in presence of additional carbon source**

Johnson *et al. Met Eng Comm* 2016
Johnson *et al. Met Eng Comm* 2017
Salvachúa et *al. Green Chem* 2018

Debottlenecking via endogenous and heterologous gene expression



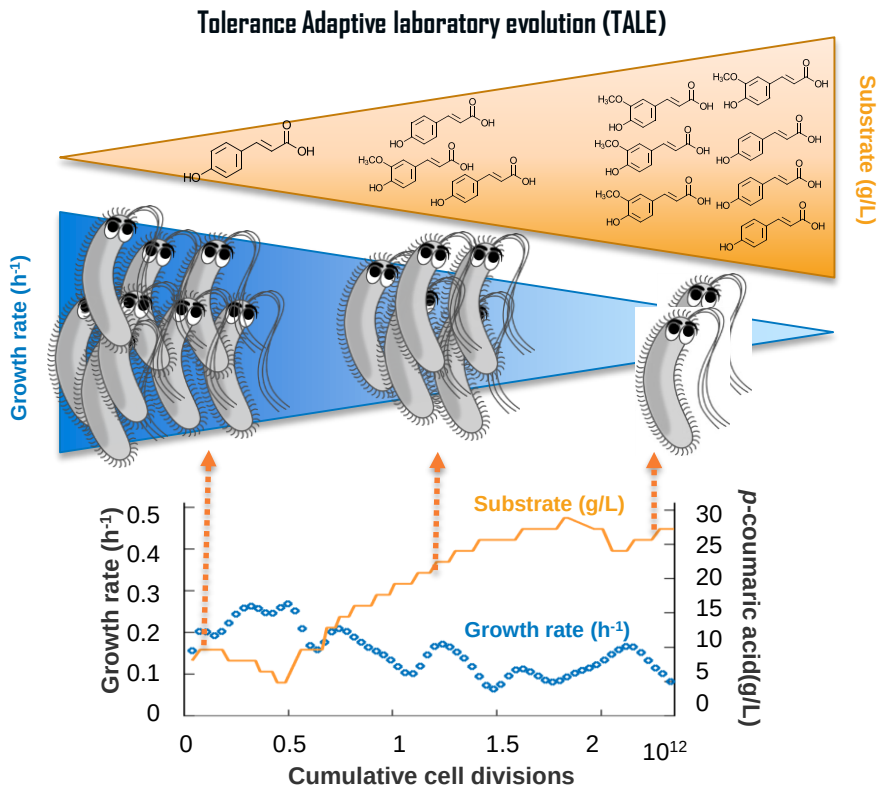


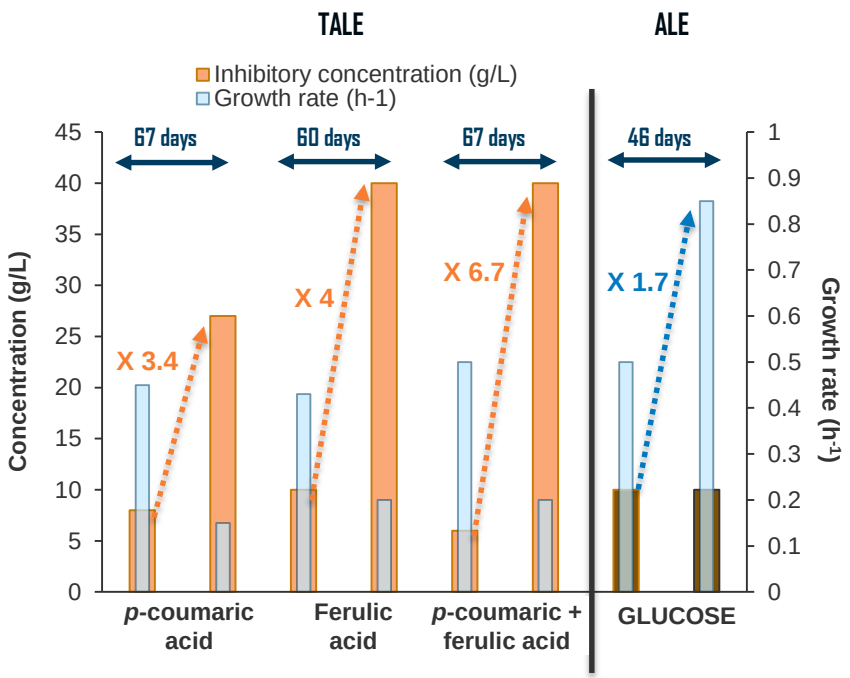


- Overexpression of VanAB alleviates vanillate bottleneck
- Heterologous expression of PraI from *A. baylyi* ADP1 alleviates 4HB bottleneck
- **Outcome: Muconate titer, rate, and yield substantially improved through multiple engineering strategies**
- Beyond higher rates, improved substrate and product toxicity tolerance is needed
- Ultimate target metrics: 1 g/L/h, 50 g/L, ≥90% yield

Salvachúa *et al. Green Chem.* 2018
Johnson, Salvachúa *et al.* in preparation

Strain evolution for higher substrate and product tolerance

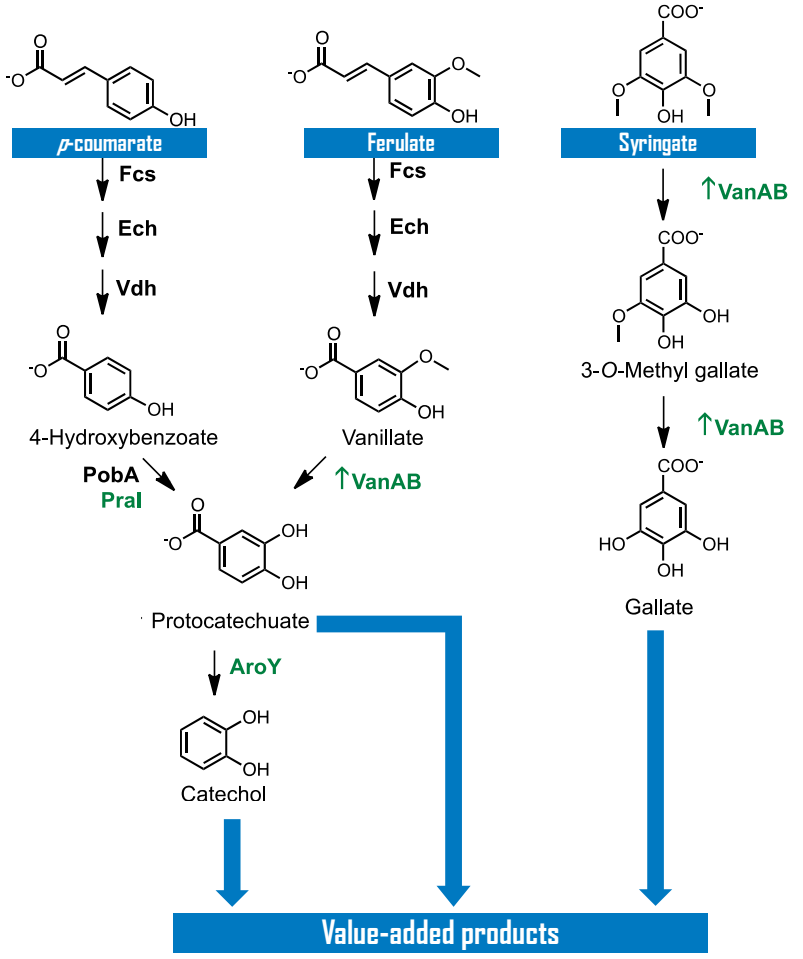




- Collaboration with Adam Feist at Denmark Technical University
- Demonstrated improved tolerance to key substrates, *p*-coumarate and ferulate
- Analysis of re-sequenced genomes ongoing, muconate adaptive laboratory evolution ongoing
- **Outcome: Improved strains for substrate/product tolerance will enable TRY targets**

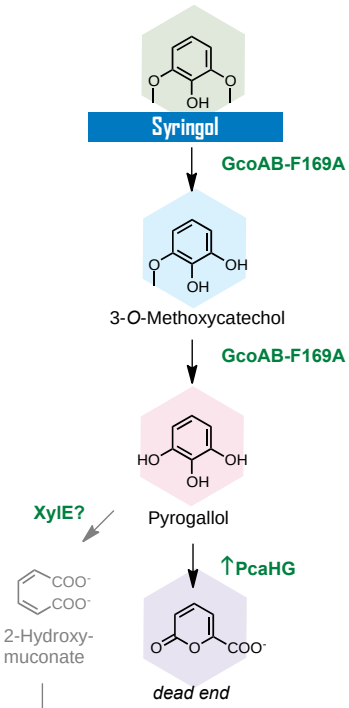
Feist, Salvachúa *et al.* in preparation

Expanding the funnel of *P. putida* to consume S-lignin

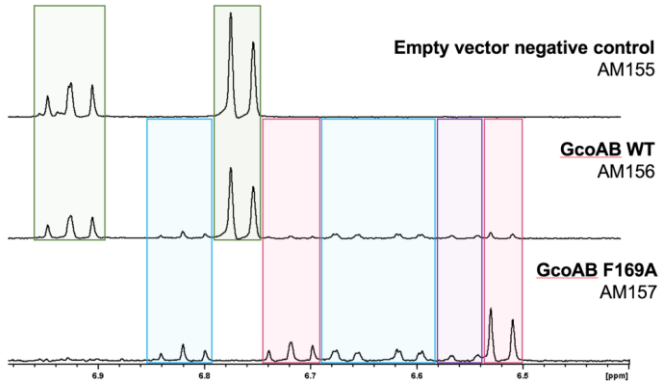


- The native O-demethylase VanAB enables syringate utilization in *P. putida*
- **Outcome**: **Demonstration of S-lignin (a major component of lignin) bioconversion**

Notonier *et al.* in preparation



- Engineered GcoAB enables syringol conversion
- Cleavage of pyrogallol by PcaHG leads to metabolic dead end, but growth may be enabled via XylE
- **Outcome**: **First demonstration of biological syringol turnover**



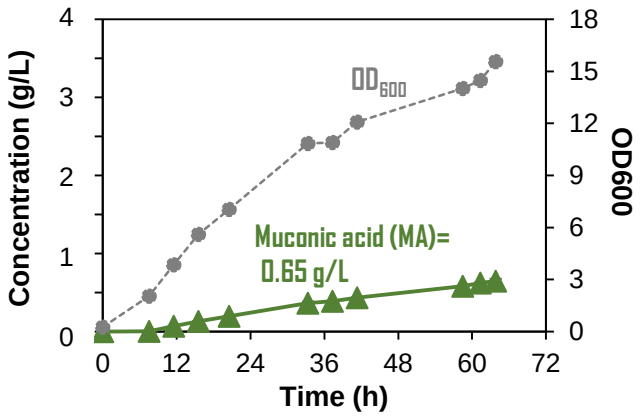
Machovina, Mallinson, Knott, Meyers, Garcia, *et al. PNAS* 2019

Feeding real lignin streams to engineered strains

Black liquor (pre- EH)

Lignin feed at high pH
pH= 9
***p*-coumaric = 0.9 g/L**
Ferulic acid = 0.3 g/L

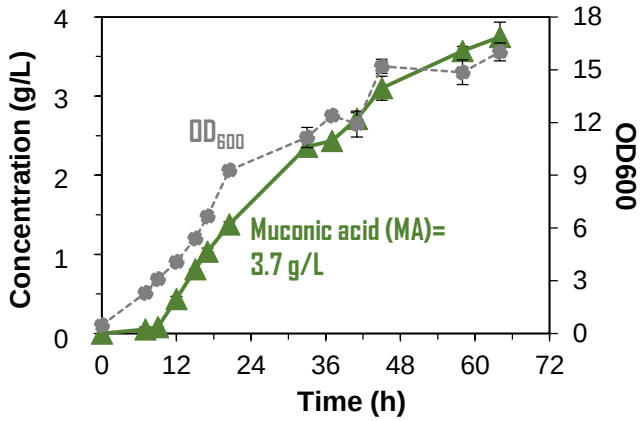
Yield (mM MA/mM pCA+FA)= **135%**
Yield (g MA/g lignin liquor)= **15%**



Base catalyzed depolymerization of solid lignin (post- EH)

Lignin feed at high pH
pH= 10.5
***p*-coumaric acid = 5.6 g/L**
Ferulic acid = 0.5 g/L

Yield (mM MA/mM pCA+FA)= **137%**
Yield (g MA/g solid lignin) = **15.4%**

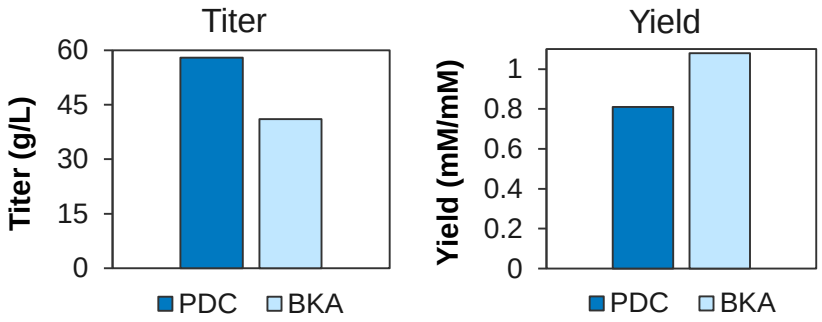


- Base-catalyzed depolymerization from collaboration with SNL BLV project
- Demonstrated 4 g/L muconate production from lignin
- Work with **Lignin Utilization** project/**Separations Consortium** to obtain streams with **higher extent of bio-available carbon** to improve muconate yields
- **Outcome**: Proof-of-concept demonstration of muconate production from process streams

Rodriguez, Salvachúa *et al. ACS SusChemEng* 2017
Salvachúa, *Green Chem.* 2018

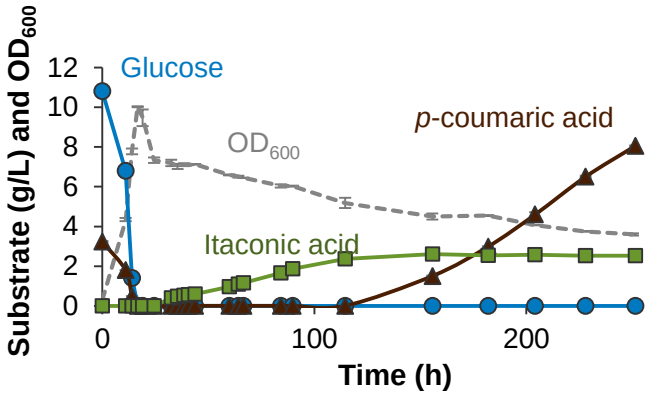
Collaborative efforts, progress towards additional targets

PDC and ß-ketoadipate from 4-HBA

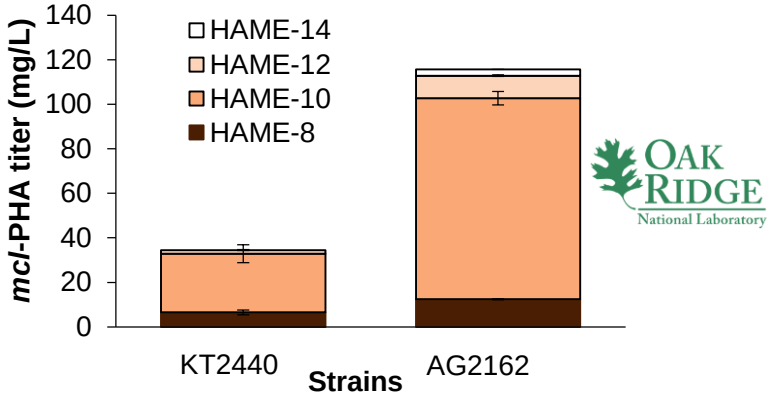


Johnson, Salvachúa *et al.* in review

Itaconic acid from *p*-Coumaric acid

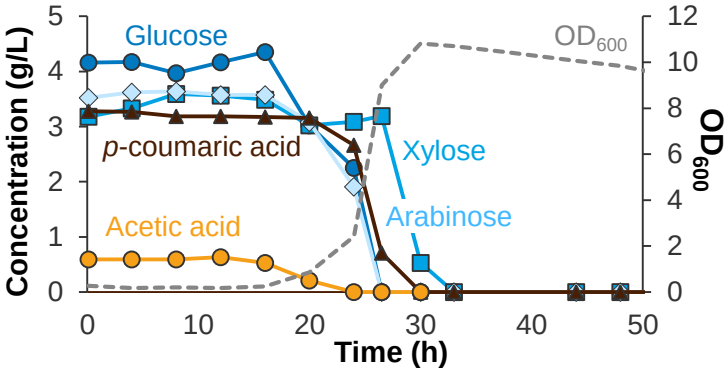


mcl-PHA production from BCD-lignin



Rydzak, Salvachúa *et al.* in review

DMR-EH streams (sugar + lignin) co-consumption

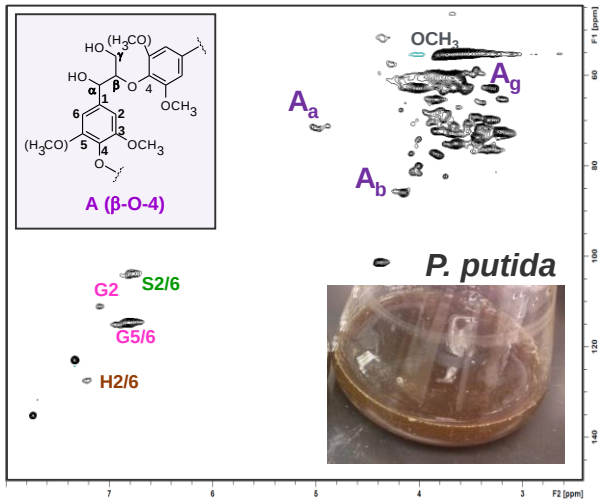


Elmore, Salvachúa *et al.* in review

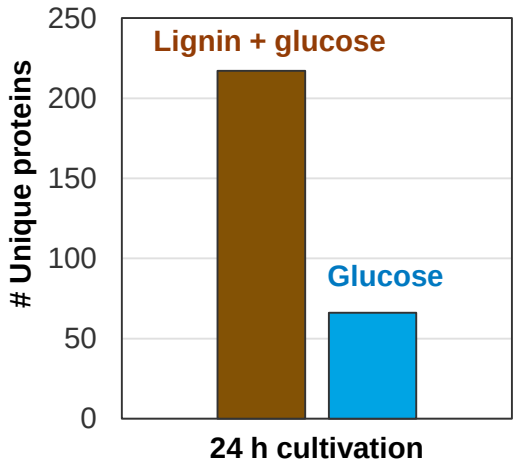
- Evaluated PHA, mcl-alcohols, itaconic acid, sugar/aromatic consumption (with A. Guss, ORNL)
- Demonstrated production of two additional targets, PDC, ß-ketoadipate
- **Outcome**: **Bioreactor cultivation development for a wider slate of lignin-derived products**

P. putida cleaves β-O-4 linkages

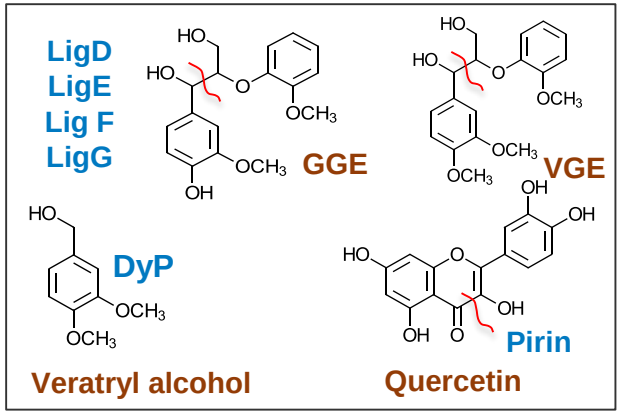
2D-NMR analysis



Differential exoproteome analysis



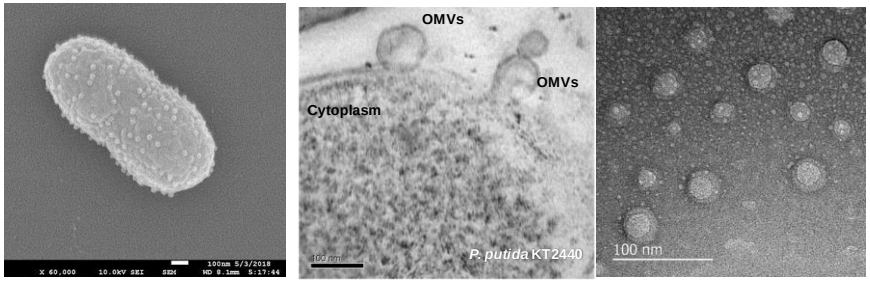
Chemical synthesis, development of analytical tools, and enzyme assays



- 2D-NMR shows β-O-4 cleavage after bacterial treatment
- Exoproteomics reveals a large pool of enzymes, exclusive in lignin cultures, that may beinvolved in oligomeric lignin breakdown
- Developed analytics for relevant lignin dimers, flavonoids, and metabolic intermediates
- *P. putida* releases outer membrane vesicles that mediate extracellular lignin catabolism
- **Outcome: demonstration of oligomeric lignin breakdown in *P. putida.***

P. putida releases outer membrane vesicles

SEM | TEM | TEM (isolated vesicles)



Extracellular lignin catabolism?

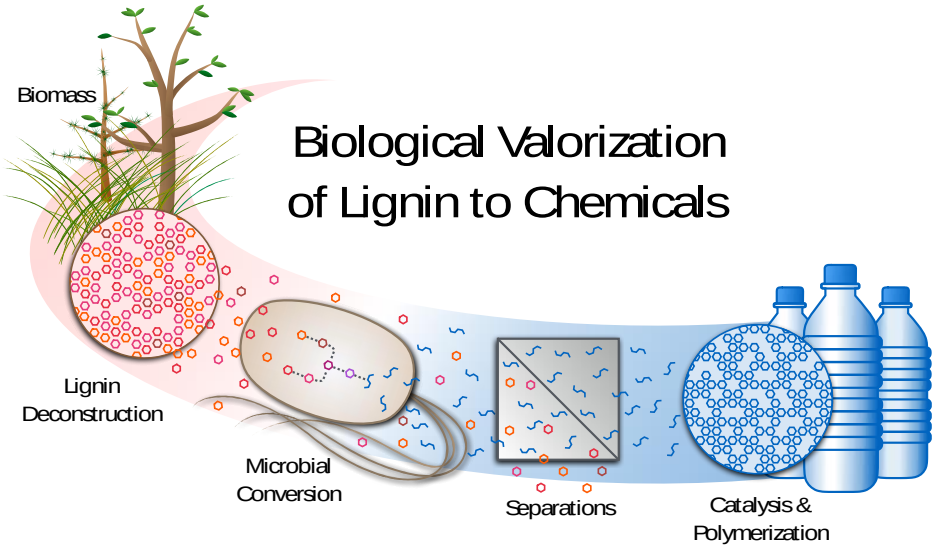
Salvachúa, Werner *et al.* in preparation

Relevance

Project goal: harness the ability of aromatic-catabolic microbes to funnel heterogeneous lignin streams – *in concert with catalysis* – to a single intermediate to lower the MFSP of a bioprocess by $2-3/gge

Why is this project important, what is the relevance to BETO and bioenergy goals?

- Lignin utilization is critical to the **bioeconomy** and **BETO cost targets**
- Up to $2-3/gge credit to MFSP
- **Biology can play a major role in lignin valorization**



Beckham *et al. Curr Opin Biotech* 2016

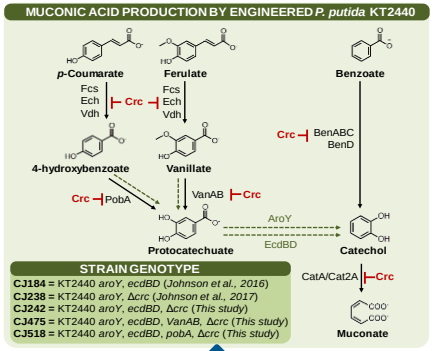
How does this project advance the SOT, contribute to biofuels commercialization?

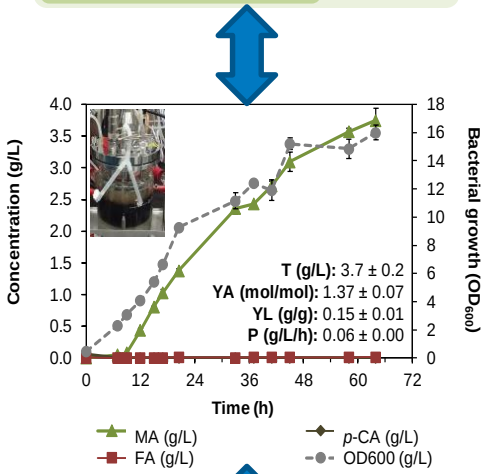
- Strains and bioprocesses for high TRY
- Demonstrated approaches to funnel lignin to products
- Work with polymer projects to show product value

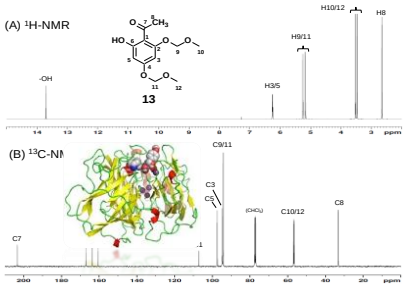
Technology transfer activities:

- Patent applications for engineered strains
- Publications and strains for dissemination to broader community
- Bioprocess development for many biological funneling efforts
- Work with Spero Energy and Sustainable Fiber Technologies via two TCF projects

Future work







Task 1: Strain Development

- Further debottleneck strains to achieve 1 g/L/hr
- Incorporate learnings from evolution to improve tolerance and TRY
- Expand to other products (PDC, ß-ketoadipate, etc.)

Task 2: Bioprocess Development

- Evolve muconate tolerance, expand evolution to dimer catabolism
- Work with Lignin Utilization to on-board new lignin streams
- **Achieve 90% yield, 50 g/L targets on model compound mixtures (informed by Lignin Utilization) by end-of-project**
- Support BETO lignin bio-centric projects with bioreactor cultivations

Task 3: Deconstruction of Soluble Oligomers

- Elucidate OMV-mediated conversion of lignin monomers and oligomers
- Identify new catabolic capacities for dimer conversion
- Engineer needed dimer catabolism into *P. putida*

Summary

Overview

– Develop industrially-relevant biological conversion processes for lignin

Approach

– Strain, bioprocess, and enzyme development for lignin bioconversion
– Muconate as an exemplary product from lignin
– Enzyme discovery to expand oligomer catabolism and tailor to lignin streams from catalysis project

Technical accomplishments

– Developed strains, bioprocesses to convert G and H monomers to muconate at high TRY
– Engineered S-lignin utilization and discovered new paradigm for extracellular lignin catabolism

Relevance

– Synthetic biology could potentially play a critical role in lignin valorization
– Lignin must be valorized to reduce the cost of biofuels for the BETO portfolio and the bioeconomy

Future work

– Improve productivity of muconate strains to achieve TEA targets of 50 g/L, 1 g/L/hr, >90% yield from lignin monomers
– Evolve strains for higher toxicity tolerance to muconate product, incorporate S-lignin utilization

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BETO projects

- Lignin Utilization
- Metabolic Engineering for Lignin Conversion
- Biological Lignin Valorization – SNL
- Separations Consortium
- Biological Process Modeling and Simulation
- Low Temperature Advanced Deconstruction
- Performance-Advantaged Bioproducts

Response to Previous Reviewer Comments

(Relevant) Reviewer Comments from Targeted Microbial Development

- The project has identified what seem to be relevant target pathways for co-products in order to improve overall economics. The metabolic engineering strategies selected are generally reasonable and reflect the expertise present at NREL in metabolic engineering. However, in the context of the current SOT with regard to synthetic biology in both industry and academia, the scope of the metabolic engineering strategies laid out here seem very limited. It is hard to think that progress towards both the TRY for each task and the understanding gained would not be faster and a more efficient use of resources with high-throughput strain generation approaches.
- One of the NREL strengths is developing a clear understanding of how biochemical systems work and then linking that understanding to processes that meet BETO's strategic direction. **This knowledge-based approach is proving itself through the identification of systems that support BETO's goals, for example, the discovery of organisms that can attain nearly theoretical yields of muconate from lignin derivatives.** This capability will be critical for current projects and ongoing efforts to engineer organisms that can selectively and rapidly convert both lignin and carbohydrates to high-value products.
- The Targeted Microbial Development program addresses three separate strain engineering projects. All three use different organisms and represent different product/co-product opportunities**. The use of P. putida to convert lignin monomers to muconic acid is especially exciting, since relatively little work has been published to address this challenge**. Progress has been very impressive on all efforts. The use of metabolic models in conjunction with experiments was shown to be essential to this work. However, the challenges that lie ahead may be even greater, and the team should make sure to use all resources available to guide strain and process development. This includes omics analysis, diagnostic experiments to identify bottlenecks, and adaptive evolution to overcome tolerance challenges.
- Three pathways to upgrading or co-products are being pursued in this project. The mixed ethanol/diol product is the most advanced, and BDO titers have dramatically improved with metabolic engineering and fermentation optimization. This is a nice, original, co-product proof of concept. The team has done a good job of recognizing the complexity of the separation and fermentation scale-up, and they are considering their options. **Lignin upgrading to fatty alcohols and muconate are successfully demonstrated concepts.** They are further from their target titers. Two of the tasks are considering scope changes due to their understanding of the economics and/or robustness of scalability. This project is doing a good job of using TEA to make decisions.

Response to Previous Reviewer Comments

- This is a good team with ambitious targets, which are relevant in aiding and expediting metabolic engineering of various hosts and are aligned with the MYPP goals. The overall TEA of fatty alcohols should be evaluated as this might be too challenging of a target for the oleaginous yeast program. However, thorough benchmarking of progress toward the TEA goal will likely keep things in spec, and the team, together with Hal Alper's collaboration, has the skill set to tackle the technical challenges.

Responses:

- We thank the Review Panel for the supportive comments and helpful feedback. In terms of high-throughput synthetic biology–based strain engineering, **we are attempting to actively deploy a rapid genome-scale editing tool in P. putida KT2440 currently in collaboration with a world-leading synthetic biology group**. **In addition, we are leveraging modern systems biology tools (proteomics, transcriptomics, and metabolomics) to identify bottlenecks and adaptive laboratory evolution to improve both exogenous and endogenous pathways. If these approaches are successful, we will be able to very rapidly modify and improve flux through aromatic-catabolic pathways in this robust host.**

(Relevant) Reviewer Comments from Biological Lignin Depolymerization

- The idea of a microbial sink to drive forward lignin degradation by pulling in low-molecular-weight compounds was a good one. This could have presented the opportunity to make a thorough survey of ligninases in the presence of a production organism. Given the source of many ligninase sequences in the public domain, heterologous expression in a fungal host, like T. reesei, would have perhaps allowed a more comprehensive survey.
- The project has identified issues and developed improved techniques, but it doesn't feel like the CBP idea of expressing ligninases in a Gram-negative host is great direction to go in. Certainly, there are multiple options for mixing chemical catalysis and depolymerization conditions with biological solutions, but I think these would be more complete with a more robust approach to enzyme diversity.
- The project presents a novel idea of combining fungal enzymes with microbial systems to realize both lignin deconstruction and simultaneous inhibition of repolymerization. However, the current process runs the risk of becoming overly complex, as additional treatments of lignin are necessary for its solubilization, while a large amount of organism development still appears to be necessary. Further, a better understanding of the lignin composition and the amounts actually converted will strengthen the project. The project would benefit from settling on one or two routes for deeper examination and optimization.

Response to Previous Reviewer Comments

- This program takes an alternate approach to lignin depolymerization, which the team has shown to be extremely challenging. After challenges getting enzymes to robustly solubilize lignin, the team decided to refocus on using chemical solubilization followed by microbial depolymerization of the soluble lignin. This was a good strategic decision, and it makes the project more viable and synergistic with other work.
- There were many encouraging reviews in previous years. Utilizing the lignin cake has high industrial relevance. The concept of biological depolymerization for lignin conversion—and even as a cleanup for chemical oxidative depolymerization—still has a very long way to go. The enhanced understanding of lignin structure and bonds that this and related lignin projects contribute is valuable. Linking this knowledge to key enzymes with the specificity to break those bonds is also valuable. Producing a CBP organism that can depolymerize/solubilize/monomerize lignin and “bio-funnel” the myriad aromatics to a product like muconic acid would be a home run, if it were possible. It’s a really long stretch goal because the proof-of-concept hasn’t worked, and maybe this is not the best use of these talented resources.
- The project is very relevant to BETO goals, the management plan is sound, and the technical plan (given the challenges of this topic) is reasonable. The starting from scratch approach, given the poor reproducibility of existing knowledge from prior academic laboratory work, puts and additional hurdle before the project team. This is very low-budgeted project for the task at hand, and it is impressive to see how much work to de-convolute challenges and identify opportunities was carried. Capitalizing on the Environmental Molecular Sciences Laboratory at PNNL and the experts at the Biological Research Center of the Spanish National Research Council is highly encouraged for prospecting of good candidates. It might be useful to partner with enzyme providers to identify good starting points to enzymes expressed by P. putida, as the CBP approach, while it has potential and value in some aspects, is still not technologically ready. The project team and BETO should evaluate this project in the bigger project context of lignin de-polymerization and conversion to products.

Response to Previous Reviewer Comments

Responses:

- We thank the reviewers overall for the constructive feedback and comments. Expression of heterologous enzymes in filamentous fungi is one route to produce ligninolytic enzymes, but this would require a very significant amount of time and resources, which we do not currently have bandwidth for in this project. This is an interesting approach, perhaps meriting its own AOP. In addition, this approach has been tried extensively in the peer-reviewed literature with known enzymes, and it has not yielded tangible results for the depolymerization of insoluble lignin, to our knowledge. More work on discovering novel lignolytic enzymes (primarily nucleophilic enzymes) that cleave specific lignin linkages is needed. As such, we are shifting focus to identify and engineer nucleophilic enzymes that are able to cleave dimers and small oligomers that result from chemical catalysis.
- We also completely agree with the reviewer that secretion of oxidoreductases will be challenging; as such, we have stopped work on expressing oxidoreductases to be secreted in bacteria and are focused solely on nucleophilic enzymes that are able to break down dimers and oligomers.
- In terms of process complexity, we stress that this project going forward will be solely focused on identifying and engineering enzymes that are able to cleave dimers and oligomers in tandem with detailed lignin analytics, directly in line with the reviewer feedback. We are also focusing on the catalytic streams being produced in the Lignin Utilization project and by industrial conversion processes.
- Regarding the CBP concept, this was simply a proof-of-concept study. In this study, we identified that many aromatic-catabolic microbes are able to depolymerize oligomers. This finding in itself is valuable, when taken with the analytical and proteomics work that is ongoing, to understand what linkages in oligomers are being broken by which enzymes and—just as importantly—what linkages are not being broken. This will enable us to understand the interplay between chemical catalysis (what linkages remain in dimers and oligomers) and the microbial engineering going forward.
- In terms of the larger lignin portfolio and the collaborative efforts, we thank the reviewer for the positive comments. We also note that this project closely collaborates with the Lignin Utilization and Targeted Microbial Development projects, and indeed, in many cases, the same staff members are working between these projects. The Biological Lignin Depolymerization project keeps the “big picture” in mind throughout the development of biological lignin depolymerization strategies.

Milestone Table

FY18		
Q1	QPM	Demonstrate conversion of, and toxicity tolerance to, an S-lignin monomer, syringate, in an engineered P. putida KT2440 strain. Measure toxicity tolerance of other S-lignin monomers including syringaldehyde and gallate to P. putida KT2440.
Q2	QPM	Synthesize at least 4 phenolic ß-O-4 linked dimer model compounds, and at least one flavonoid for further in vivo testing for lignin bond cleavage or modification. Develop robust analytical methods for monitoring chemical modifications in these lignin model compounds. Dimers will be identified, prioritized, and synthesized in collaboration with the analytics efforts in the Lignin Utilization project to ensure that the compounds studied here are relevant to lignin fractionated streams.
Q3	QPM	Demonstrate >50% conversion or modification of either a ß-O-4 linkage or a common C-C linkage in an in vitro enzyme system to identify enzyme combinations for engineering into an aromatic-catabolic microbe. The impact of this will be to increase the carbon conversion efficiency by pursuing dimers that are found in abundance in lignin-derived streams.
Q4	Annual	Demonstrate the simultaneous utilization of p-coumaric and ferulic acid in a bioreactor with a concomitant production of muconic acid at >25 g/L and at > 80% yield.

Milestone Table

FY19		
Q1	QPM	Conduct rational enzyme engineering to produce a cytochrome P450 enzyme to be able to demethylate syringol to 3-methoxycatechol or pyrogallol to enable utilization of this substrate at least at 50% conversion efficiency in an engineered strain of P. putida.
Q2	G/NG	Develop a P. putida KT2440-derived strain either through adapted evolution or metabolic engineering and/or bioreactor processes to increase at least 50% the current maximum muconic acid productivity (from 0.25 g/L to 0.37 g/L/h) while maintaining a titer of 50 g/L and yields > 90% from aromatic compounds.
Q3	QPM	Demonstrate 50% reduction in 4-HB accumulation during conversion of 20 mM p-coumarate to muconate relative to CJ242 (P. putida KT2440 Ptac:aroY:ecdBD Δcrc) in a shake flask culture using genetic intervention such as overexpression of the native 4-hydroxybenzoate hydroxylase, PobA, or the introduction of an exogenous 4-hydroxybenzoate hydroxylase.
Q4	Annual	Demonstrate catalytic processes able to generate >40% yield of usable monomers from lignin in biomass, either biologically convertible or separable. Demonstrate, in collaboration with the Lignin Utilization project, that the monomers can be assimilated by either a native or engineered strain of Pseudomonas putida KT2440 (joint with the Lignin Utilization project).

Publications

1. Thomas Rydzak, Davinia Salvachúa, Annette De Capite, Derek R. Vardon, Nicholas S. Cleveland, Joshua R. Elmore, Rui Katahira, Darren J. Peterson, William E. Michener, Brenna A. Black, Holly Smith, Jason T. Bouvier, Jay D. Huenemann, Gregg T. Beckham*, Adam M. Guss*, “Metabolic engineering of Pseudomonas putida for increased polyhydroxyalkanoate production from lignin”, in review at ***Microbial Biotech***.
2. Melodie M. Machovina‡, Sam J.B. Mallinson‡, Brandon C. Knott‡, Alexander W. Meyers‡, Marc Garcia-Borràs‡, Lintao Bu, Japheth E. Gado, April Oliver, Graham P. Schmidt, Daniel Hinchen, Michael F. Crowley, Christopher W. Johnson, Ellen L. Neidle, Christina M. Payne, Kendall N. Houk*, Gregg T. Beckham*, John E. McGeehan*, Jennifer L. DuBois*, “Enabling microbial syringol utilization via structure-guided protein engineering”, in revision at ***PNAS***.
3. Davinia Salvachúa*, Christopher W. Johnson, Christine A. Singer, Holly Rohrer, Darren J. Peterson, Brenna A. Black, Anna Knapp, Gregg T. Beckham*, “Bioprocess development for muconic acid production from aromatic compounds and lignin”, ***Green Chem***. (2018) 20, 5007-5019.
4. Sam J. B. Mallinson‡, Melodie M. Machovina‡, Rodrigo L. Silveira‡, Marc Garcia-Borràs‡, Nathan Gallup‡, Christopher W. Johnson, Mark D. Allen, Munir S. Skaf, Michael F. Crowley, Ellen L. Neidle, Kendall N. Houk*, Gregg T. Beckham*, Jennifer L. DuBois*, John E. McGeehan*, “A promiscuous cytochrome P450 aromatic *O*-demethylase for lignin bioconversion”, ***Nature Comm***. (2018) 9, 2487.
5. Wouter Schutyser, Tom Renders, Sander Vanden Bosch, Stef Koelewijn, Gregg T. Beckham, Bert Sels*, “Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading”, ***Chem. Soc. Rev***. (2018) 47, 10-20.
6. Christopher W. Johnson, Payal Khanna, Jeffrey G. Linger, Gregg T. Beckham*****, “Eliminating a global regulator of carbon catabolite repression enhances the conversion of aromatic lignin monomers to muconate in *Pseudomonas putida* KT2440”, ***Metabolic Eng. Comm***. (2017) 5, 19-25.
7. A Rodriguez‡, D Salvachúa‡, R Katahira‡, B Black, N Cleveland, M Reed, H Smith, E Baidoo, J Keasling, BA Simmons, GT Beckham*, J Gladden*. Base-Catalyzed Depolymerization of Solid Lignin-Rich Streams Enables Microbial Conversion. ***ACS Sus. Chem. Eng***. (2017) 5, 8171–8180.

Presentations

1. Deconstructing plants and plastics with novel enzymes and microbes, The Novo Nordisk Center for Biosustainability, DTU, August 24th, 2018
2. Production of renewable chemical building blocks by recombinant *Pseudomonas putida* KT2440 from lignin and further upgrading to polymers. SIMB Annual meeting, Aug 2018. Chicago, USA.
3. Hybrid biological and catalytic processes to manufacture and recycle plastics, University of British Columbia, June 20th, 2018
4. Developing new processes to valorize lignin and sugars to building-block chemicals and materials, RWTH Aachen University, May 28th, 2018
5. Recent adventures in the deconstruction of cellulose and lignin, LBNet3 Meeting (UK), May 16th, 2018
6. Production of renewable chemical building blocks by recombinant *Pseudomonas putida* KT2440 from lignin and further upgrading to polymers. 40th Symposium on Biotechnology for Fuel and Chemicals, SIMB, April 2018. Clearwater (FL), USA.
7. Synergy between white-rot fungal enzymes and aromatic-catabolizing bacteria during lignin decay. DOE-JGI User Meeting: Genomics of Energy & Environment, April, 2018. San Francisco, USA Lignin conversion by biological funneling and chemical catalysis, COST FP1306 Workshop, **Plenary** Invited Lecture, March 12th, 2018
8. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, University of Delaware, October 5, 2017
9. Lignin valorization to chemicals, Bioeconomy 2017, July 12, 2017
10. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, USDA-ARS Western Regional Research Center, June 14, 2017
11. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, University of Minnesota, March 27, 2017
12. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, École polytechnique fédérale de Lausanne, March 24, 2017
13. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, Michigan State University, February 2, 2017
14. Lignin valorization via biological funneling, January 23, 2017, NMBU Norway