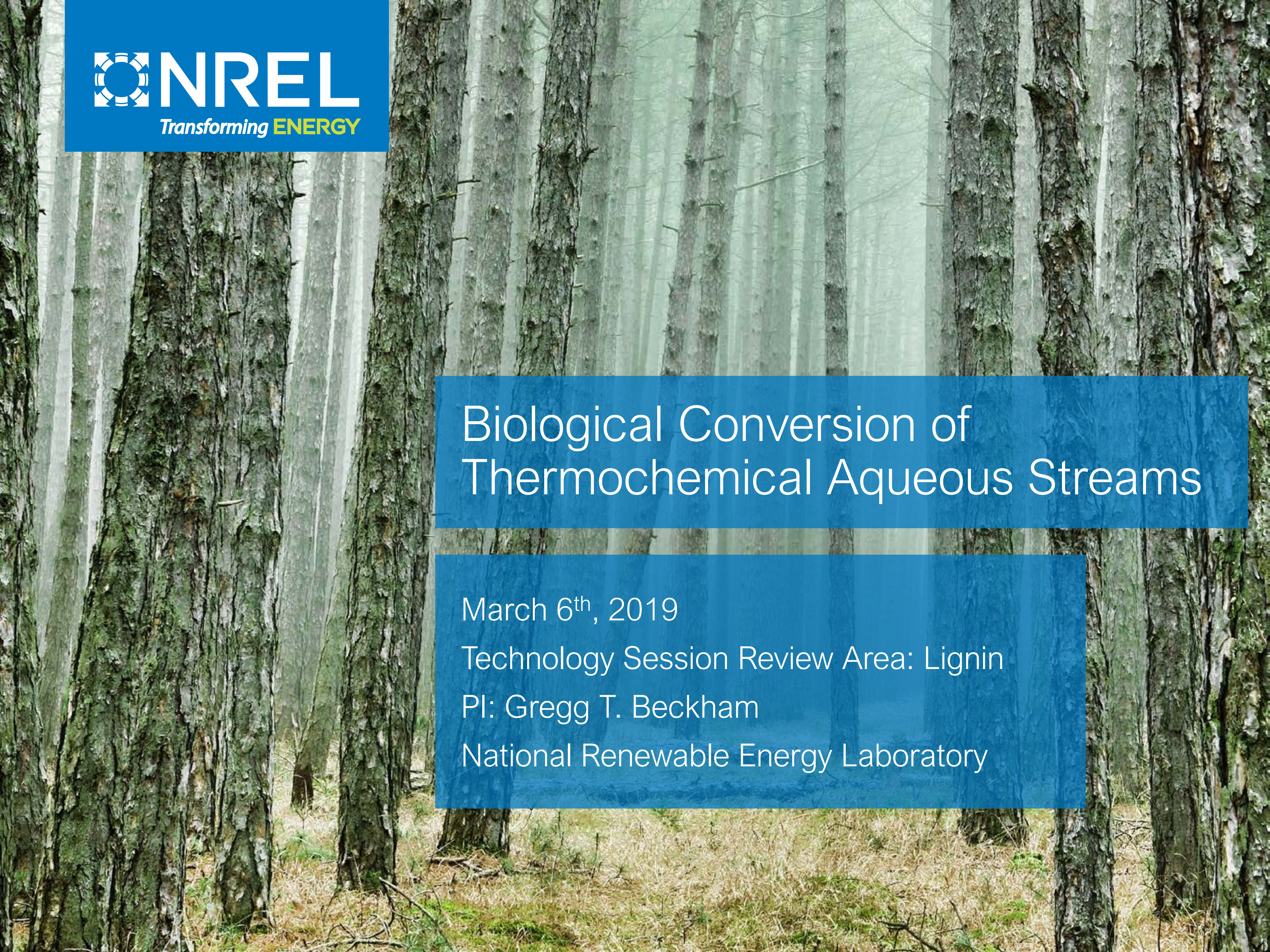


Biological Conversion of Thermochemical Aqueous Streams

March 6th, 2019

Technology Session Review Area: Lignin

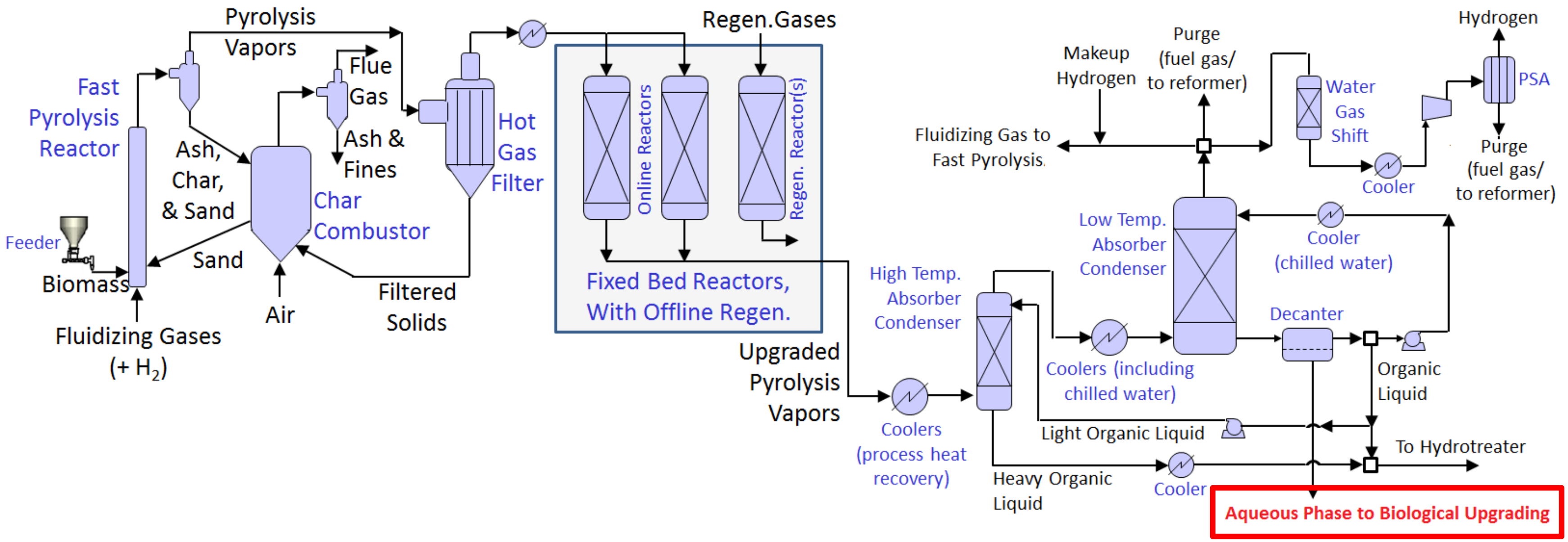
PI: Gregg T. Beckham

National Renewable Energy Laboratory

Goal statement

Goal: Adapt bio-funneling to wastewater valorization in thermochemical (TC) processes

- Contribute to cost targets through valorization of TC waste streams to chemicals
- Develop fractionation methods with Catalytic Upgrading of Pyrolysis Products
- Waste streams contain **between 3%–10% of biomass-derived carbon**



Relevance: waste valorization will be a major economic benefit to TC biorefineries

- Collaborate with Catalytic Fast Pyrolysis projects for integration
- Contribute to other aqueous upgrading efforts and TEA via rigorous analytics
- Understand strain robustness for broader synbio applications
- **Outcome:** An integrated approach for converting TC aqueous streams to valuable compounds

Quad chart overview

Timeline

- Start date: October 2016
- End date: September 2019
- Percent complete: 87%

	Total Costs Pre FY17	FY17 Costs	FY18 Costs	Total Planned Funding (FY19-Project End Date)
DOE funded	$663k	$573k	$366k	$400k

Partners:

BETO Projects: Thermochemical Platform Analysis, Catalytic Upgrading of Pyrolysis Products, Biological Lignin Valorization – NREL, Biochemical Processing Modeling and Simulation, Performance-Advantaged Bioproducts via Selective Biological and Chemical Conversion

Nat'l labs and universities: Iowa State University, Oak Ridge National Laboratory, University of Georgia, University of Portsmouth, UCLA, Montana State University, University of Stuttgart, RTWH Aachen University

DOE projects: Center for Bioenergy Innovation (ORNL)

Barriers addressed

- **Ct-I Development of process capable of processing high moisture feedstocks in addition to conventional AD**
  - Using biology to convert waste carbon
- **Ct-J Identification and evaluation of potential co-products**
  - Integration of co-products with drop-in fuels

Objective

Biologically upgrade aqueous waste streams from ex situ CFP processes to co-products (PHAs, muconates) that will result in a switch from a cost for wastewater treatment to a revenue stream to lower the MFSP

End of Project Goal

Develop a robust engineered microbe and associated cultivation process (in collaboration with other *ex situ* CFP TC projects) to **contribute at least $0.25/gge to the overall MFSP by producing a co-product**

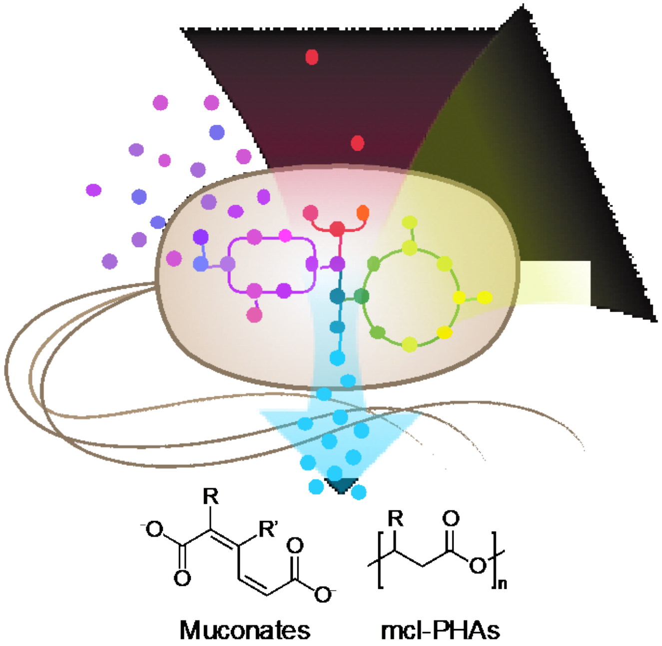
Project overview

History: Wastewater treatment is a barrier that places a cost burden on biorefineries

- Started as a seed project in FY14
- **Waste valorization can impart an economic credit** ($2–$4/gge in the case of lignin)
- Breakthrough in toxicity tolerance enabled a ’go’ decision for this project

Context: All TC processes produce wastewater

- Composition is often poorly characterized
- Current treatment is a **~$0.25/gge burden** via reverse thermal oxidation (SOT)
- This carbon from the feedstock is lost
- Multiple biorefinery paradigms exhibit this problem

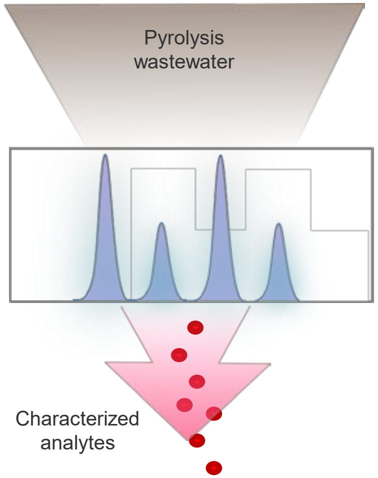


Project Goals:

- Characterize aqueous streams from *ex situ* CFP (and other TC processes)
- Use biological funneling in highly robust strains to convert aqueous waste streams to exemplary products, **PHAs** and **muconates**, **to enable $0.25/gge credit**
- Design new processes in collaboration with other TC project that lead to switch in cost burden to cost advantage for TC biorefinery

Management approach

Task 1: Characterization of CFP wastewater



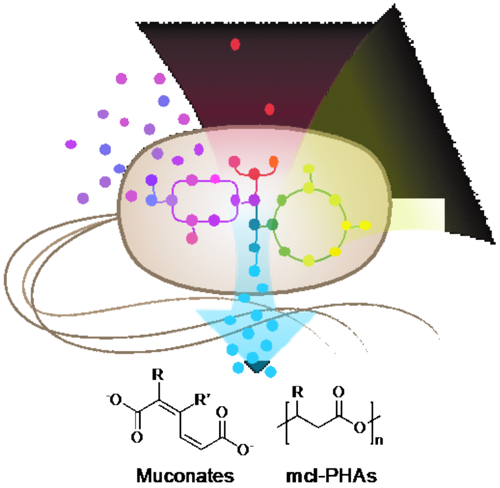
Interface with TC projects and other partners to characterize process-relevant streams:

- Led by analytics expert (Brenna Black)
- Milestones on **substrate analysis**
- Leverage new analytical techniques to fingerprint molecules in waste streams
- Aim for **full mass closure** in analysis
- Aids other aqueous waste valorization efforts

Dedicated project mgr. to track $$, progress

Collaborations with other BETO projects via frequent, *ad hoc* inter-project meetings

Task 2: Strain engineering for CFP wastewater



Assembled team of experts in metabolic engineering and microbial toxicity:

- Led by *P. putida* expert (Chris Johnson)
- Milestones on **substrate utilization** and **toxicity**
- Employ KT2440 metabolic engineering expertise
- Employ systems biology where applicable via collaborations with external partners
- Experts in microbial toxicity (R. Henson, L. Jayakody) and substrate expansion (A. Meyers)
- Aids other microbial toxicity efforts

Project interactions

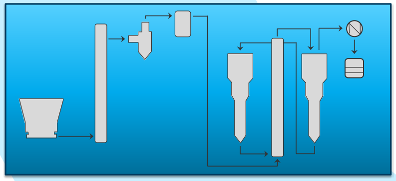
Biomass

Pyrolysis

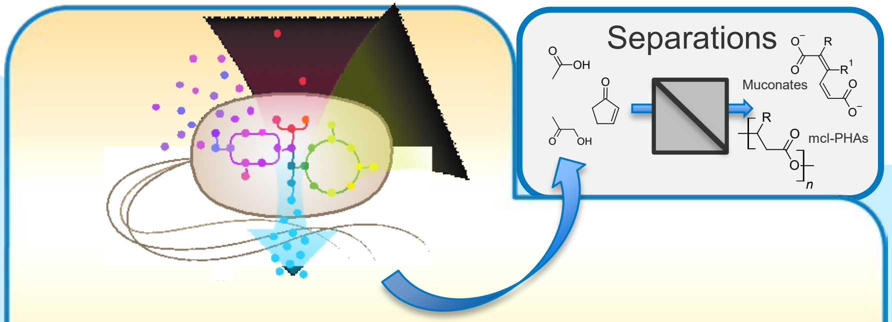
- Thermochemical Platform Analysis
- Catalytic Upgrading of Pyrolysis Products

PABP/ Separations

- Performance-Advantaged BioProducts via Selective Biological and Catalytic Conversion



Thermochemical Conversion



Biological Conversion of Thermochemical Aqueous Streams

Lignin

- Biological Lignin Valorization – NREL

Biochemical Conversion

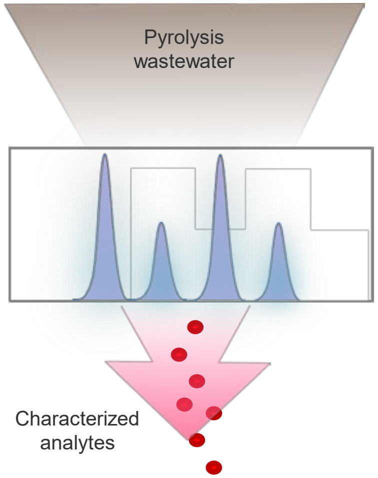
- Biochemical Process Modeling and Simulation

Technical approach

Critical Success Factors:

- Need to know stream **composition** and tailor microbial strategy to stream of interest
- Need incredibly robust **organism tolerance** to tolerate wastewater

Aim 1: Characterize streams from *ex situ* CFP



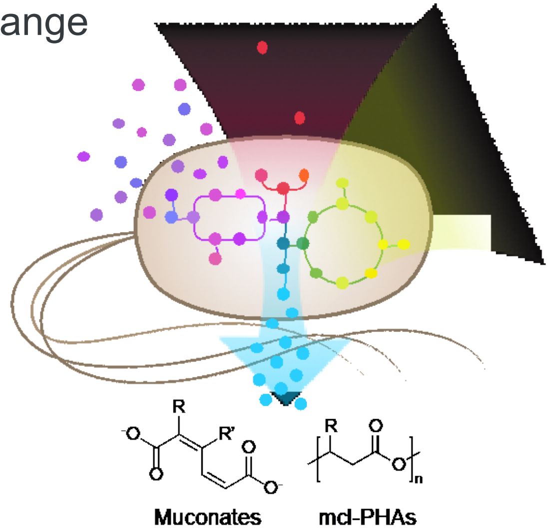
Approach:

- Develop/deploy aqueous-compatible analytics
- Provide analytics to TC teams

Challenges:

- Substrate changes (slightly) with upstream catalyst/process change
- Dilute nature of product streams

Aim 2: Develop robust strain for a broad substrate range



Approach:

- Add new catabolic capabilities (when needed)
- Multi-prong strategy for improved tolerance

Challenges:

- Substrate specificity needs change with upstream process/catalyst changes
- Toxicity tolerance remains a key challenge

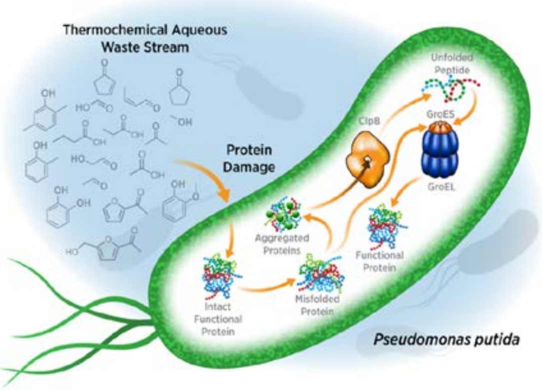
Outline of technical accomplishments

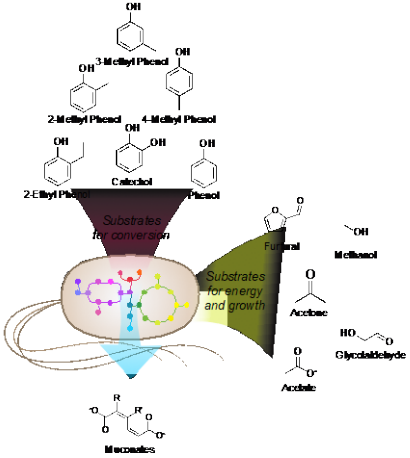
Aim 1: Characterize TC wastewater streams

- Characterization of *ex situ* CFP across scales
- Characterization of streams for State-of-Technology and techno-economic analyses
- Collaborative efforts in aqueous stream fractionation projects

Aim 2: Develop robust strain for a broad substrate range

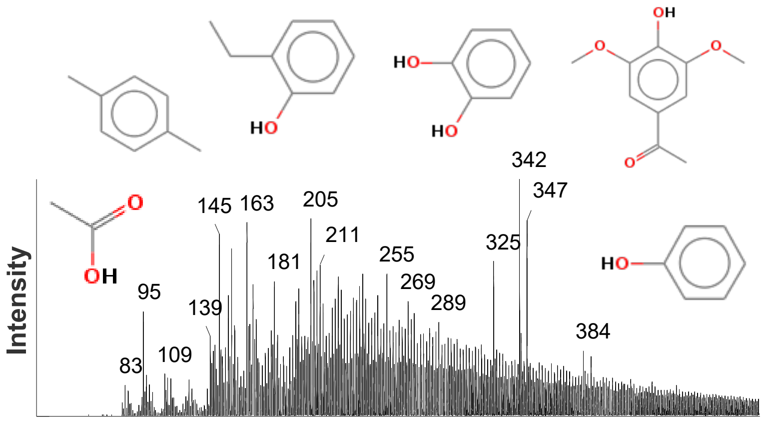
- Toxicity tolerance improvements through membrane engineering and chaperones
- Expansion to near-theoretical biological carbon conversion
- Discoveries of new catabolic capabilities along the way





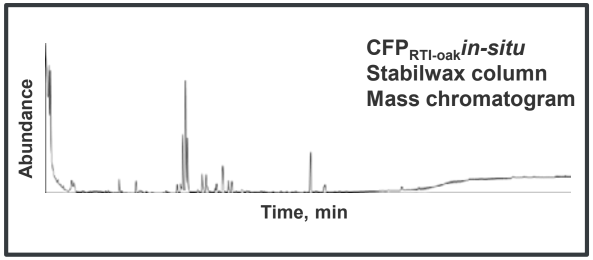
Aim 1: Aqueous-compatible wastewater characterization

LC-MS Identification



- High mass resolution MS with ion mobility
  - -2D LC
  - -Ion Mobility
  - -MS
  - -MS/MS
- Suitable for low and high molecular weight, thermally labile, non-volatile compounds

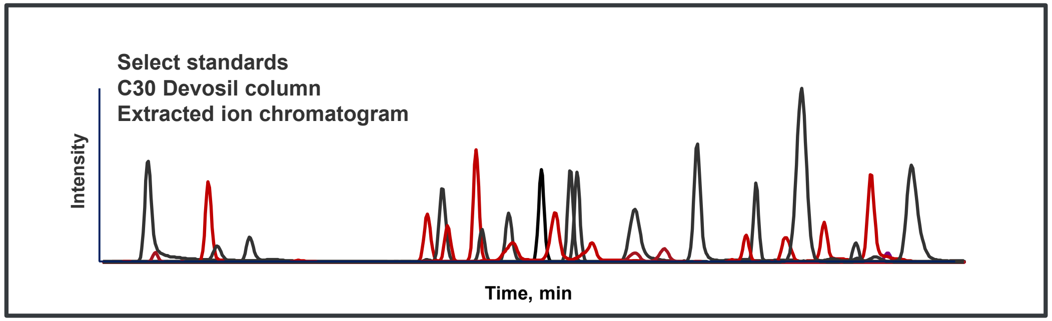
GC-MS



$CFP_{RTI\text{-}oak}$ *in-situ*
Stabilwax column
Mass chromatogram

- Suitable for low MW, thermally stable, volatile compounds
- Stabilwax® MS columns are compatible with water

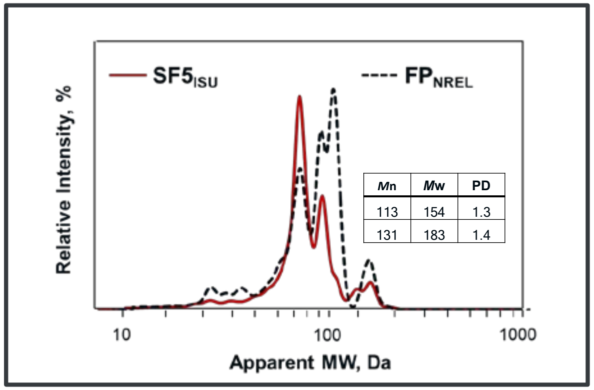
LC-MS Quantitation



Select standards
C30 Devosil column
Extracted ion chromatogram

- Low mass resolution MS - sensitive, specific, rapid, quantitation
- No derivatization
- Chromatographic resolution not necessary

Aqueous GPC

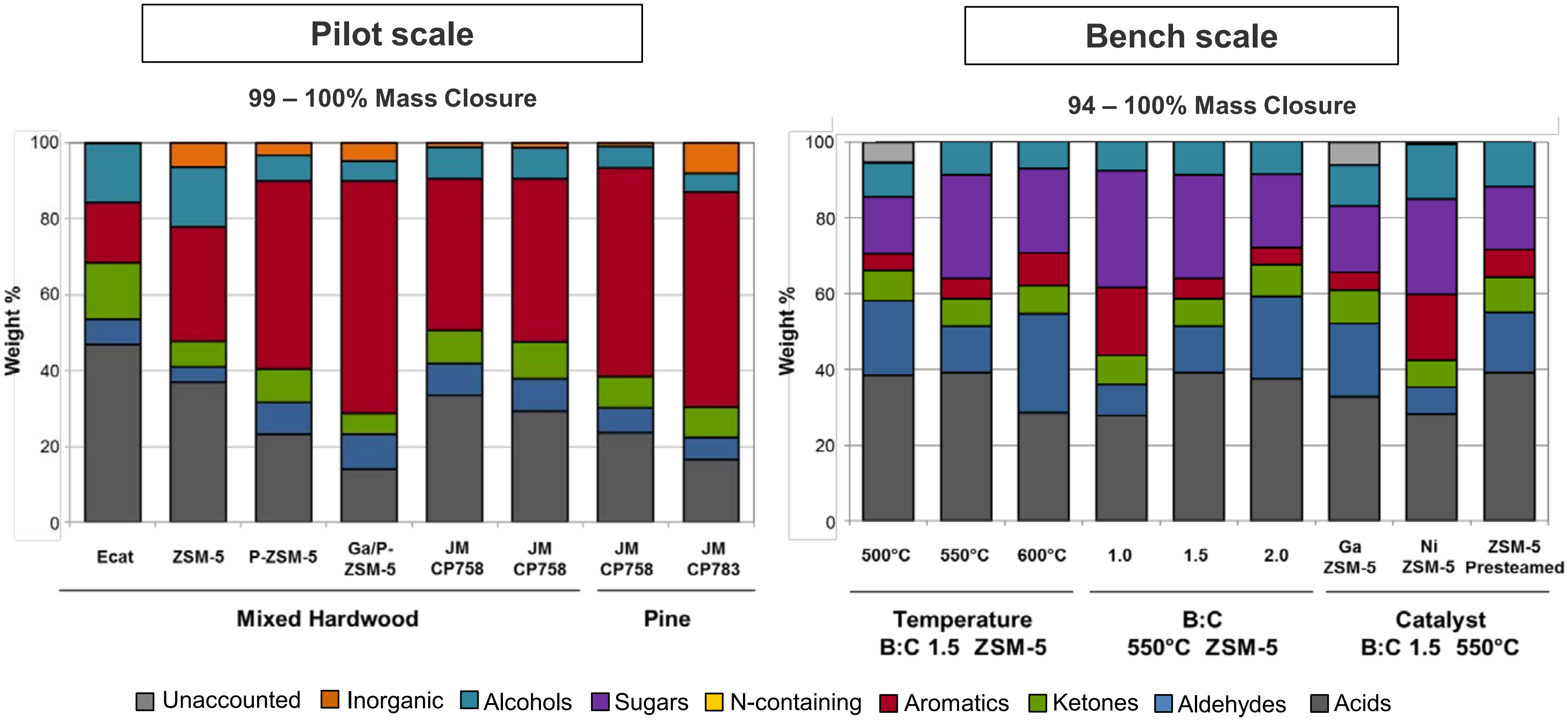


Mn	*Mw*	PD
113	154	1.3
131	183	1.4

- No derivatization; directly compatible analysis for molecular weight distribution

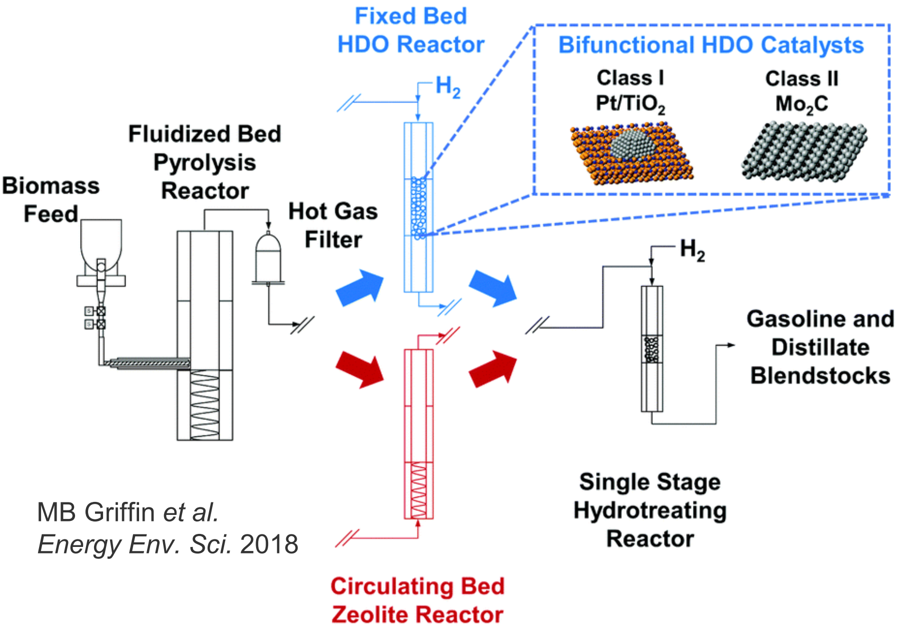
BA Black *et al. ACS SusChemEng* 2016, A Starace, BA Black *et al. ACS SusChemEng* 2017
L. Jayakody *et al. Energy Env Sci* 2018, AN Wilson, BA Black *et al.* in review

Aim 1: Multi-scale *ex situ* CFP stream characterization

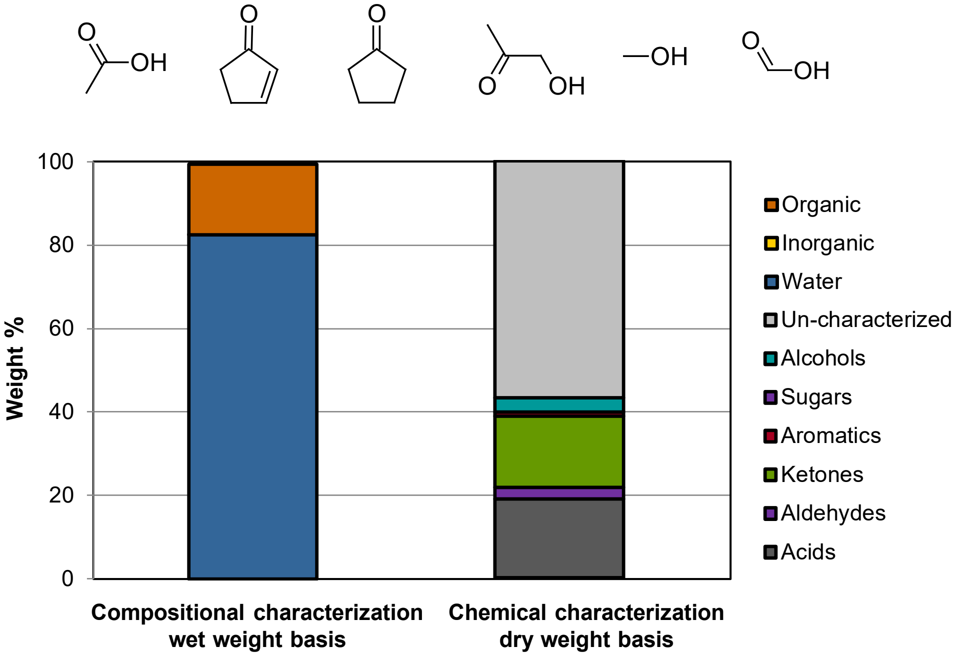


- Collaboration with Catalytic Upgrading of Pyrolysis Products and Catalytic Fast Pyrolysis
- **Outcome:** Aqueous stream characterization highlighted disparity between bench and pilot scale *ex situ* CFP. Results fed back to catalysis teams.

Aim 1: Recent analytical results from new catalyst discoveries



MB Griffin *et al.*
Energy Env. Sci. 2018

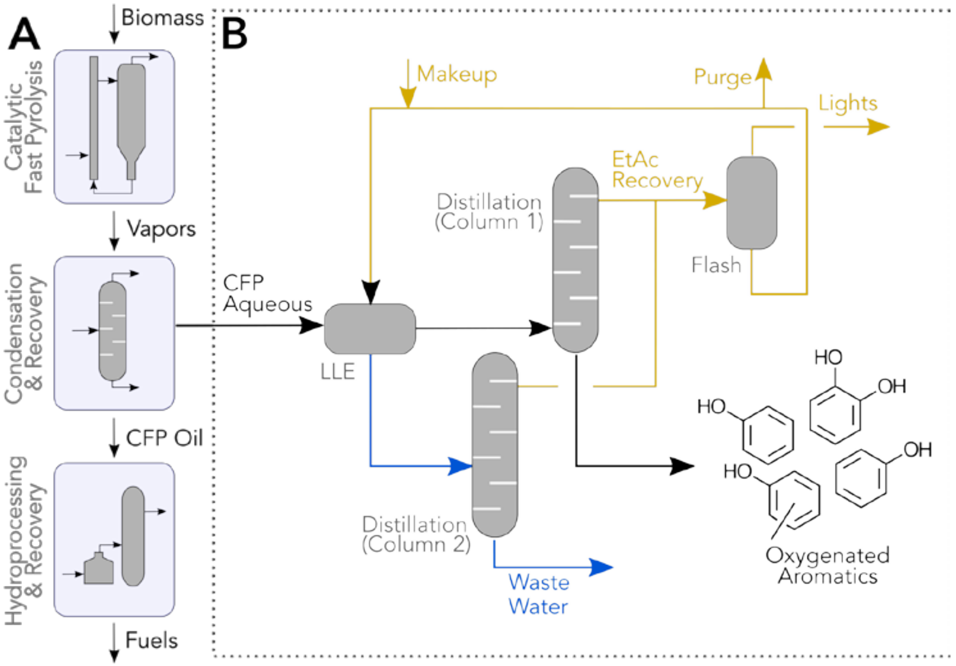


Work in progress

- Collaboration with BETO-funded **Catalytic Upgrading of Pyrolysis Products** project
- Upstream changes (Griffin *et al. Energy Env Sci* 2018) to the catalyst have changed the wastewater profile and content
- **Outcome:** Comprehensive characterization has directly informed strain engineering and other wastewater valorization efforts in other BETO projects as a result of upstream changes

Aim 1: Collaborative efforts for wastewater fractionation

Modelled process and analysis for separating organics from the CFP aqueous stream



- Collaboration with **Catalytic Upgrading of Pyrolysis Products**
- Many aromatics from *ex situ* CFP are better suited as co-products
- TEA predicts that oxygenated aromatics produced at $1.06/kg
- **Outcome:** Isolation of high-purity aromatics in an economically feasible way to increase atom efficiency of TC conversion
- **Future work:** Strategies to further add value could leverage strains from the BCTAS project

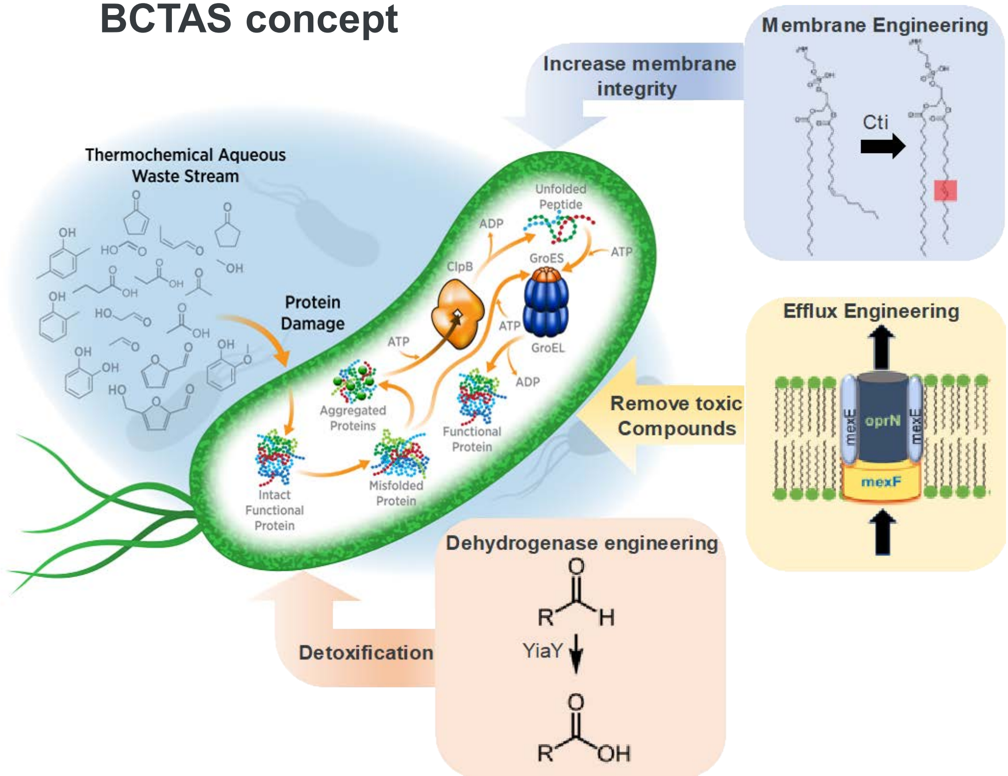
AN Wilson, BA Black, *et al.* in review at *Green Chemistry*

Aim 2: Robust strains for biological conversion of TC wastewater

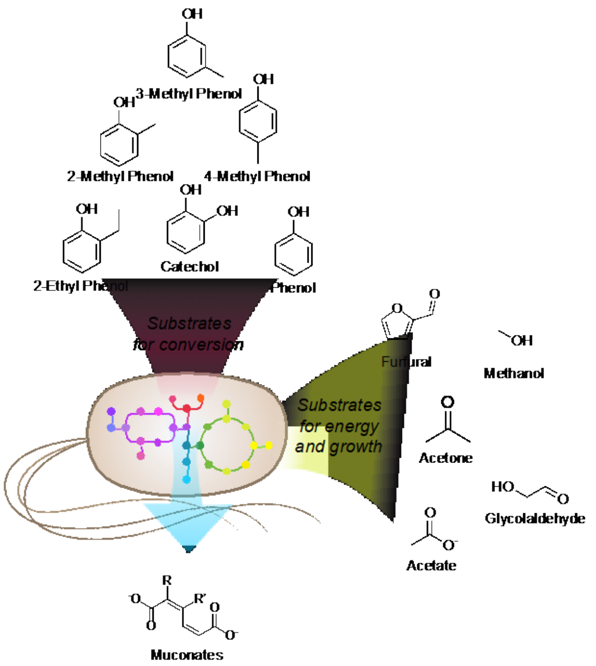
P. putida KT2440 chosen for broad catabolic capability and tolerance

- Metabolizes many aromatics and organic acids
- Robust microbe
- BCTAS concept requires a **“wider” funnel than lignin**
- All experiments are done with chromosomally-integrated strains
- Producing **PHAs** and **muconates** from TC aqueous streams

Improved toxicity is a key enabler of the BCTAS concept

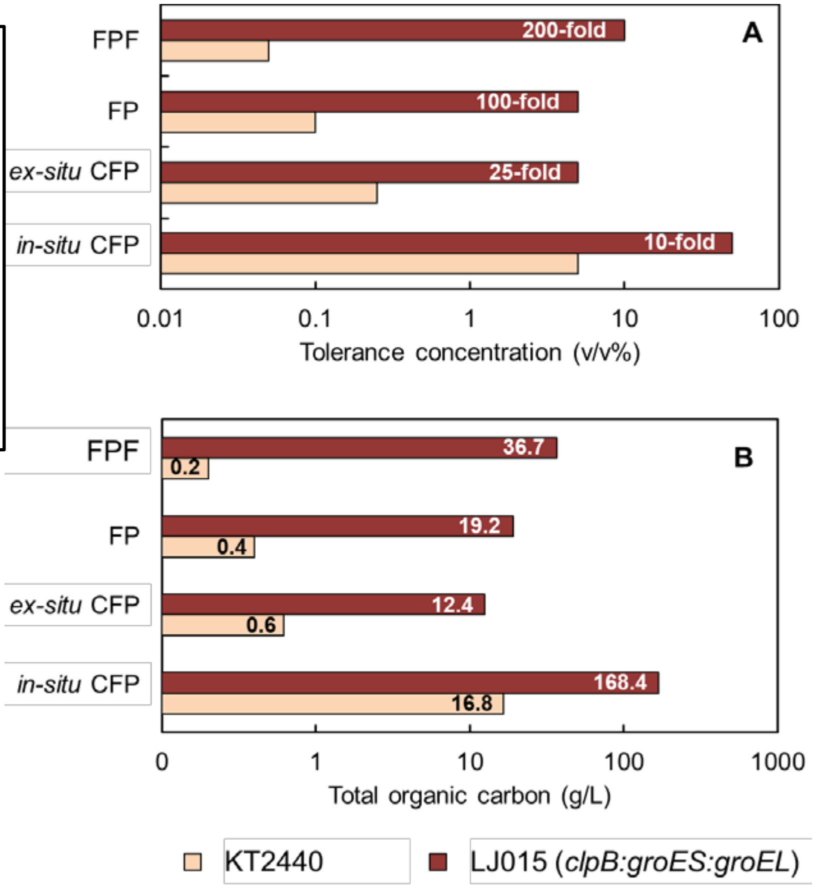


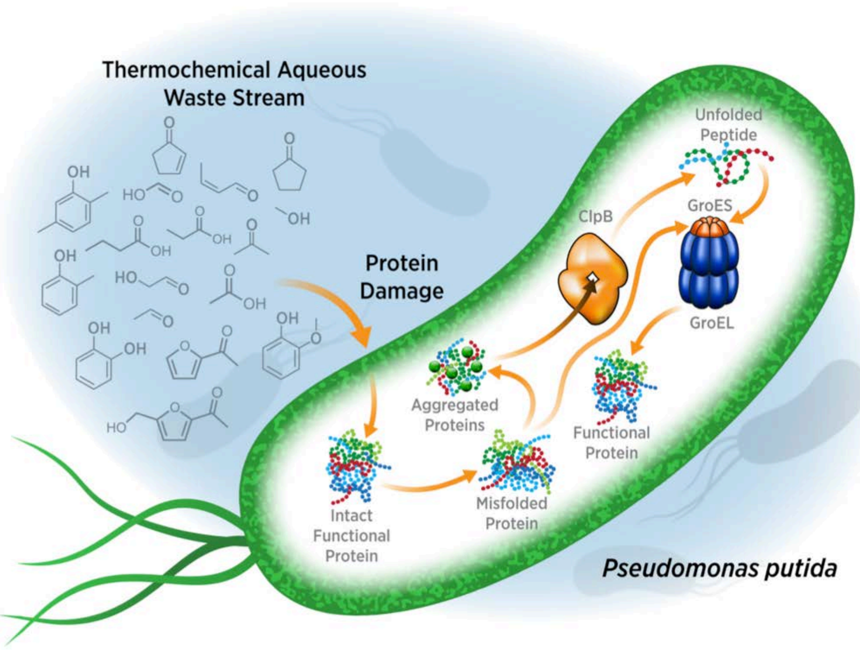
Expanded substrate specificity is required for high product yields and wastewater treatment



Aim 2: Microbial toxicity tolerance via multiple mechanisms

- Omics showed chaperone expression under stress
- Chaperones are well known to repair proteins
- Hypothesized protein damage is a toxicity driver
- Chaperone overexpression improves toxicity tolerance up to 10-200 fold across multiple streams
- **Outcome:** base strain for engineering additional toxicity tolerance and expanded substrate specificity





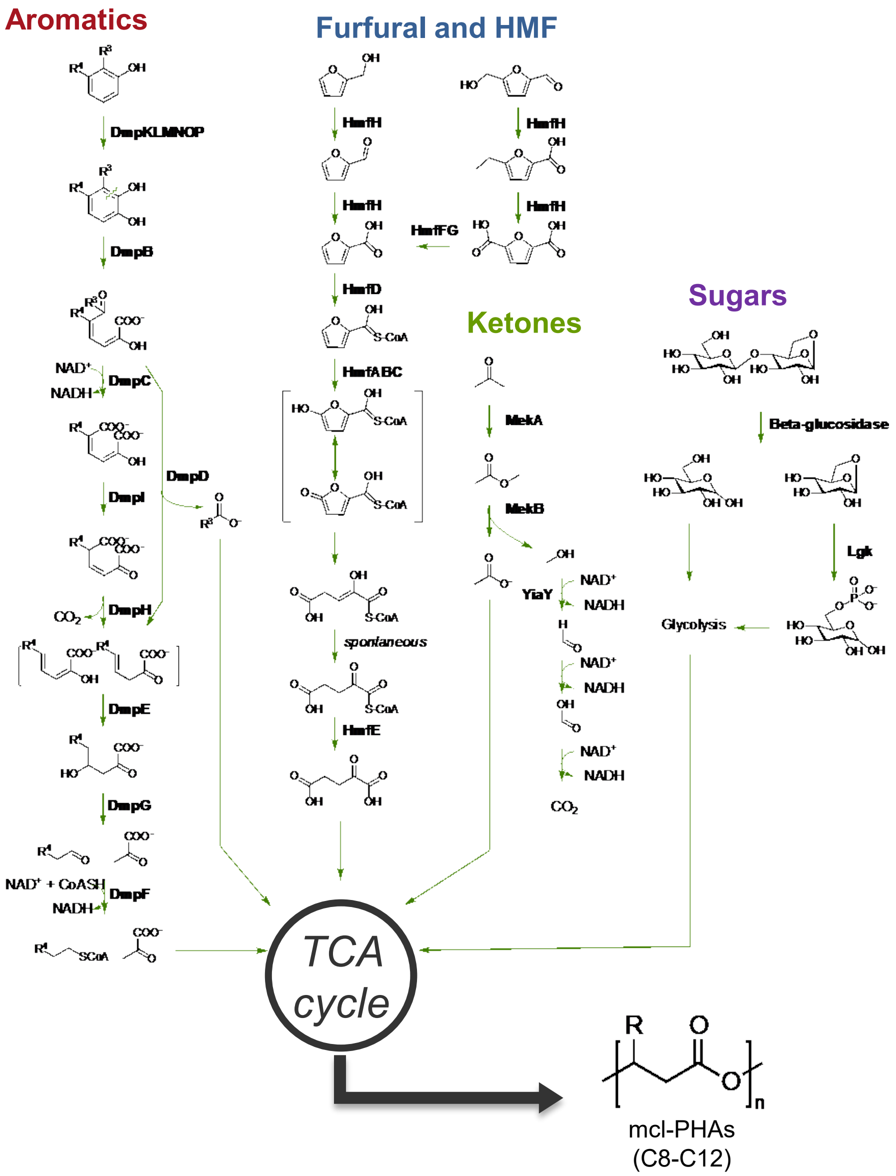
Stacking strategies include:

- Efflux pumps, membrane engineering, dehydrogenase engineering
- Synergy studies ongoing now

L Jayakody *Energy Env Sci* 2018

Aim 2: Substrate specificity of engineered *P. putida* for *ex situ* CFP streams

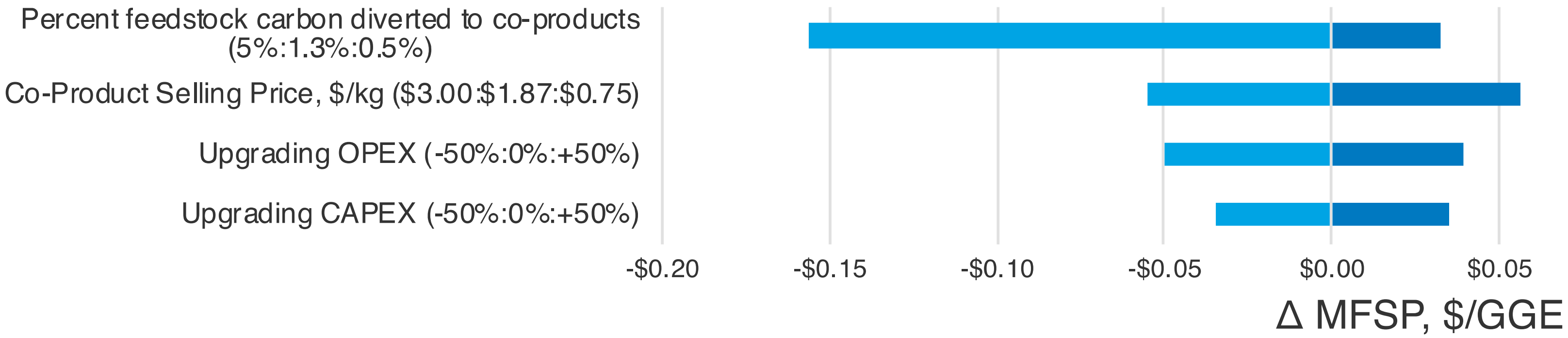
Substrate	g/kg	Native	Engineered
Acetic acid	4.14	✓	✓
Formic acid	0.98	✓	✓
Propanoic acid	0.1	✓	✓
2-Itaconic acid	0.65		
2-Hydroxyacetic acid	0.43	✓	✓
Acetaldehyde	0.51	✓	✓
2-Hydroxyacetaldehyde	0.16	✓	✓
Formaldehyde	1.07	✓	✓
Furfural	1.39		✓
Acetone	2.06		✓
Cyclopent-2-en-1-one	0.19		
Catechol	4.57	✓	✓
Phenol	2.94		✓
o-cresol	0.92		✓
m-cresol	2.14		✓
p-cresol	0.72		✓
3-Methyl catechol	1.14		✓
4-Methyl catechol	0.96		✓
2,5-Dimethylphenol	0.75		
2,6-Dimethoxylphenol	0.24		
2-Ethylphenol	0.28		✓
3-Ethylphenol	0.03		✓
Hydroquinone	0.7		✓
2-Methylbenzene-1,4-diol	0.22		
2,5-Dimethylbenzene-1,4-diol	0.29		
4,5-Dimethylbenzene-1,3-diol	0.09		
3-Ethyl catechol	0.1		✓
4-Ethyl catechol	0.46		
4-Ethylbenzene-1,3-diol	0.33		
Methanol	1.14		✓
Propan-1-ol	0.25	✓	✓
Butan-1-ol	0.29	✓	✓
Total mass	30.24	12.5	27.02
Percent utilized		41.34	89.35

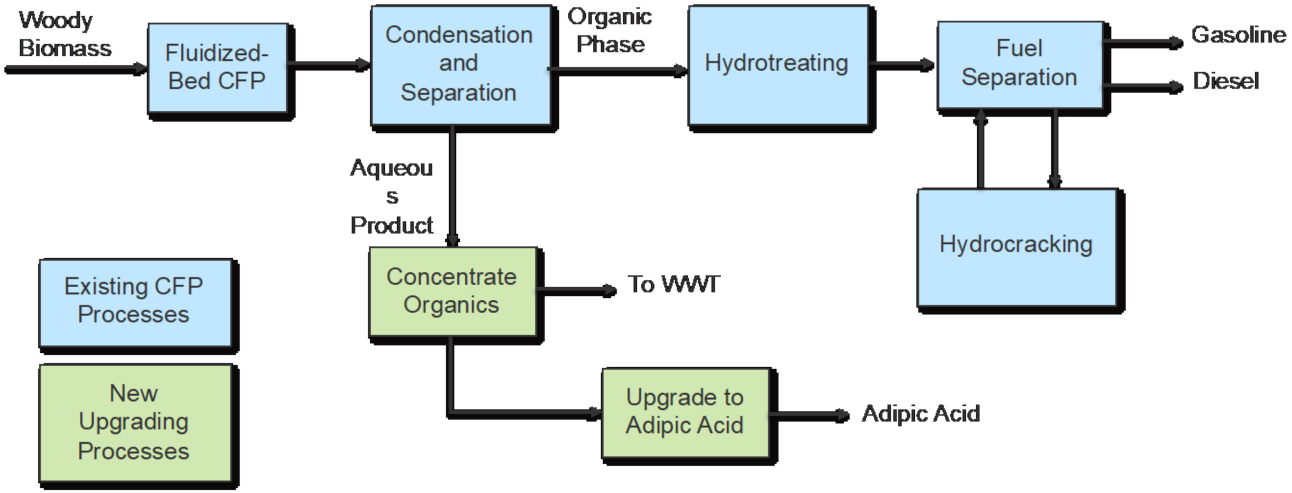


Outcome: 89% carbon utilization in DCR streams

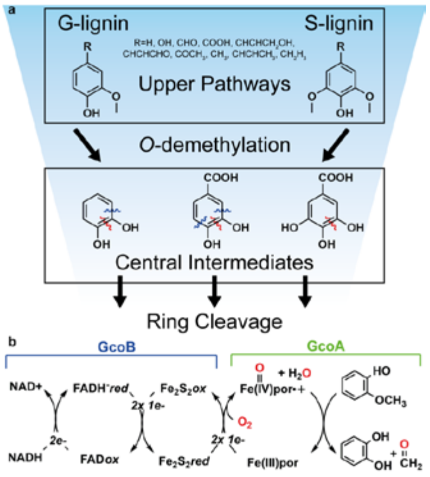
Aim 2: Techno-economic analysis

- Modeled an adipic acid production process as a product to have a direct comparison to
- **Outcome:** Potential for cost savings in MFSP, but dependent on carbon amount in aqueous phase and product selling price
- Potential for performance-advantaged bioproducts to have higher selling prices (e.g., methyl muconates)

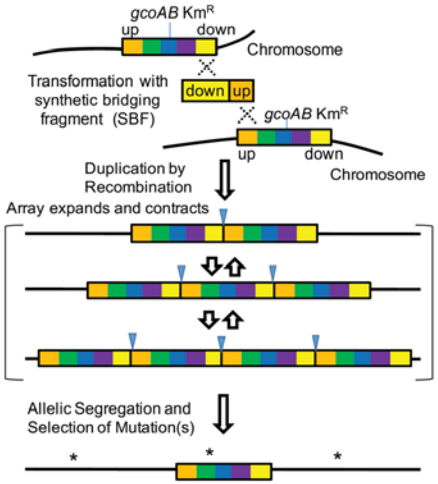




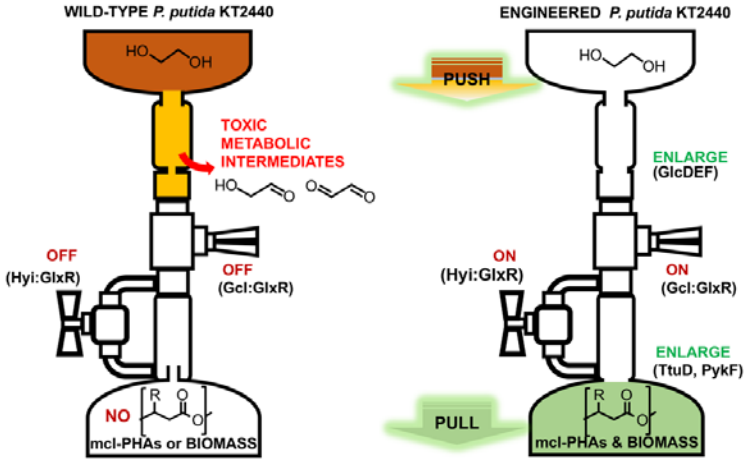
Impactful discoveries from BCTAS project



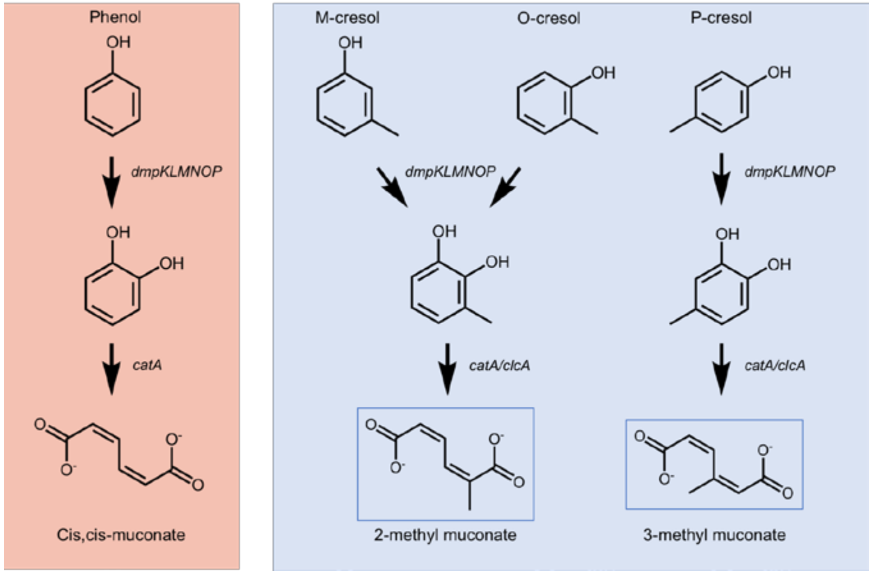
Mallinson, Machovina *et al*. *Nat. Comm.* 2018



Tumen, Johnson *et al*. *PNAS*. 2018



Franden *et al*. *Metabolic Eng*. 2018

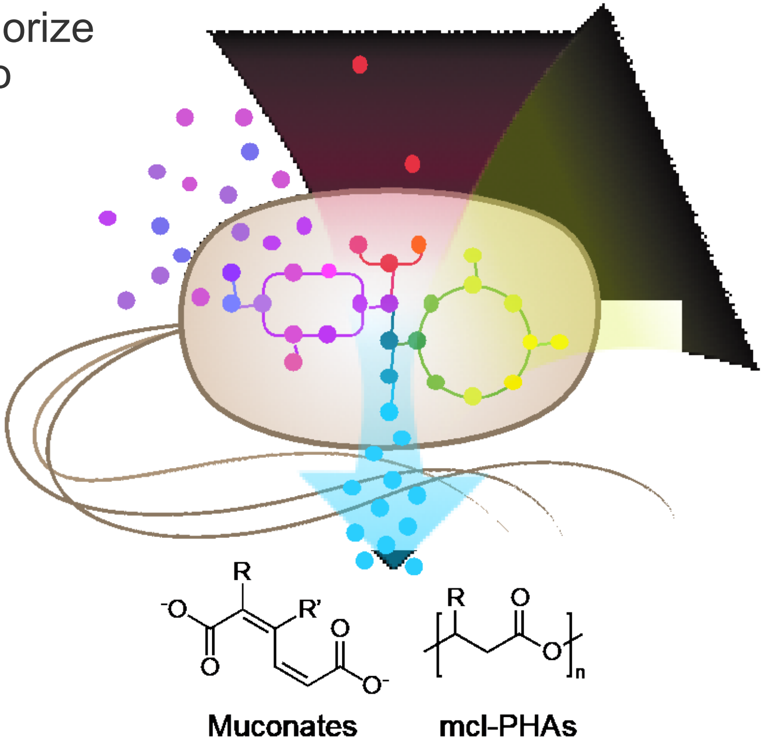


Meyers, Rorrer, Hoyt, *et al*. in preparation

- New aromatic O-demethylation strategy
- Novel method for rapid strain evolution developed
- Ethylene glycol and glycolaldehyde metabolism engineered into *P. putida*
- New class of alkylated nylons discovered (collaboration with **Performance-Advantaged Bioproducts** project)
- **Outcome**: **BCTAS has enabled discoveries that can be leveraged across other BETO and external projects**

Relevance

Goal: Develop biological, process-relevant solutions to valorize carbon in aqueous wastewater from pyrolysis processes to contribute at least $0.25/gge to MFSP



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

- **Increase atom efficiency of pyrolysis** via co-products
- Advances in **aqueous analytics**, microbial **robustness**, **synthetic biology for funneling**
- **Contribute to cost targets** from *ex situ* CFP
- **Develop robust strains of *P. putida* KT2440**

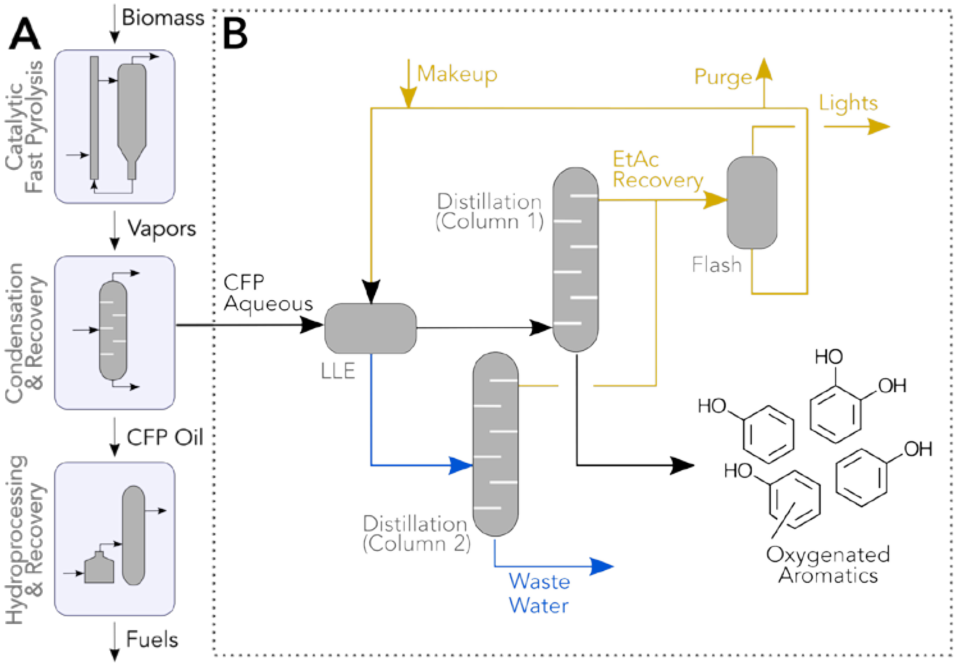
How does this project advance the SOT, contribute to commercial viability of biofuels production?

- Process solutions for waste carbon in pyrolysis
- Developed **concept of engineered, aerobic monocultures for wastewater valorization**
- Change process cost (WWT) to revenue
- Could be used for HTL or other WWT valorization

Technology transfer activities

- Patent applications, publications on strains, pathways, analytics
- Working with industry on WW characterization
- Procedures (LAPs) for WW characterization for industry

Future work in Aim 1



TC aqueous waste characterization as upstream conditions evolve

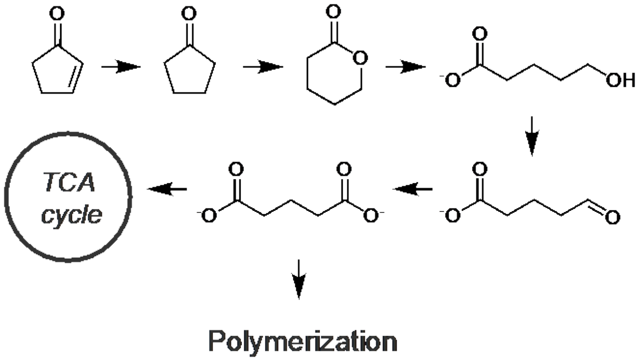
- **Increase analytical throughput**
- Continue DCR aqueous streams characterization and fractionated streams from the **Catalytic Upgrading of Pyrolysis Products**
- **Provide *ex situ* CFP results to TEA team for improved process models**

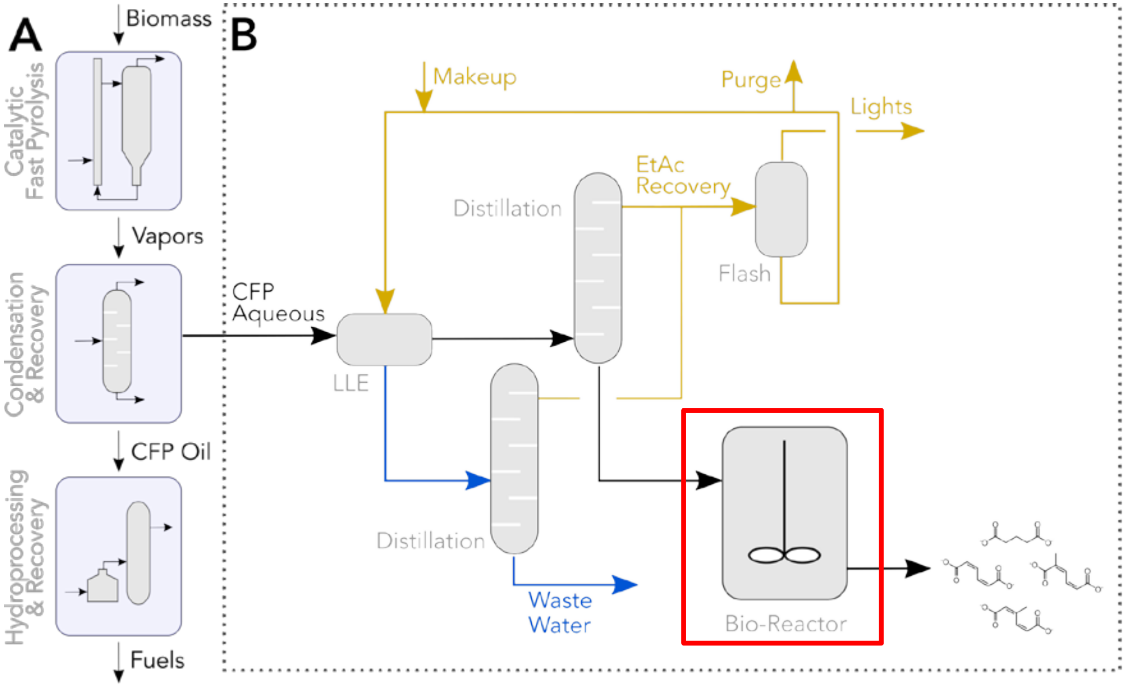
Future work in Aim 2

Develop a bench-scale bioprocess to enable cost savings for *ex situ* CFP

- Adapt current strains to new substrates from upstream catalyst changes
  - Cyclopentenone as a primary new carbon source
- Work with **Cat. Upgrading of Py. Prod.** to combine biological and chemical process developments towards valuable co-products (Q3 FY19 milestone)
- Bioprocess development **to achieve $0.25/gge credit** (Q4 FY19 milestone)

Proposed pathway for cyclopentenone degradation





Summary

Overview

- Aim to valorize carbon in pyrolysis wastewater via detailed analytics and strain/bioprocess engineering

Approach

- Characterize TC aqueous streams using aqueous-compatible analytics
- Enable bioconversion via tolerance improvement and substrate expansion

Technical accomplishments

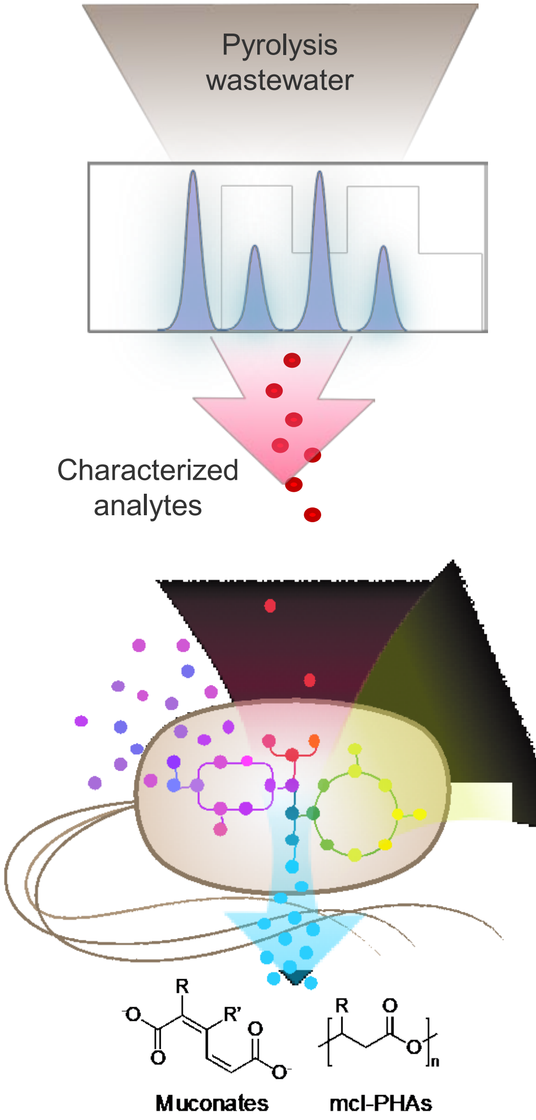
- Comprehensive characterization for aqueous waste streams in TC processes, including in emerging fractionation processes
- Incorporation of multiple catabolic genes into a robust host, *P. putida*
- Improved tolerance of *P. putida* via multiple toxicity mechanisms
- Discovered new genes, evolution approaches, and new advantaged monomers

Relevance

- Reduce economic and sustainability burden on wastewater treatment in TC processes including and beyond pyrolysis
- Co-products essential to meet DOE hydrocarbon cost targets

Future work

- Continue deployment of analytics for aqueous waste stream **fractionation** and **DCR** efforts
- Accelerate **bioprocess development with TEA, adapt strain to new substrates from upstream catalyst changes**



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- Nolan WIlson

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- Ellen Neidle, University of Georgia
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- Janosch Klebensberger, University of Stuttgart

Collaborators on BETO projects:

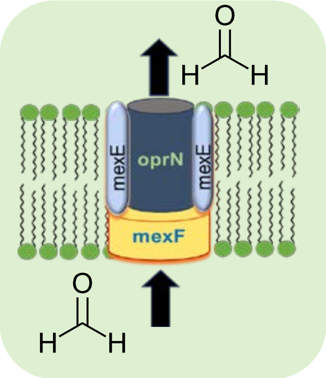
- Chris Kinchin, Mary Biddy, Abhijit Dutta, Thermochemical Platform Analysis
- Caroline Hoyt, Nicholas Rorrer, Performance-Advantaged Bioproducts
- Nolan Wilson, Mark Nimlos, Catalytic Upgrading of Pyrolysis Products

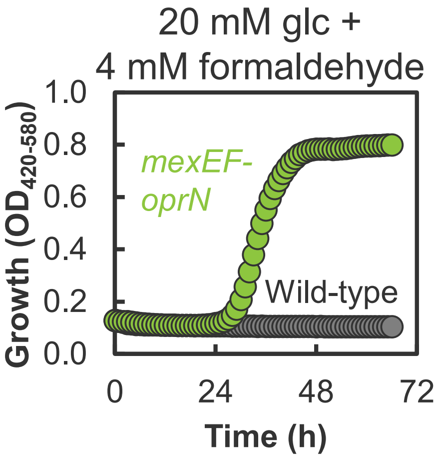
Aim 2: Additional targets for improved toxicity tolerance being pursued now

Strategies include:

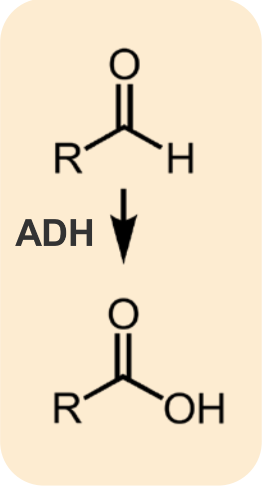
- Efflux pumps
- Membrane engineering
- Dehydrogenase engineering

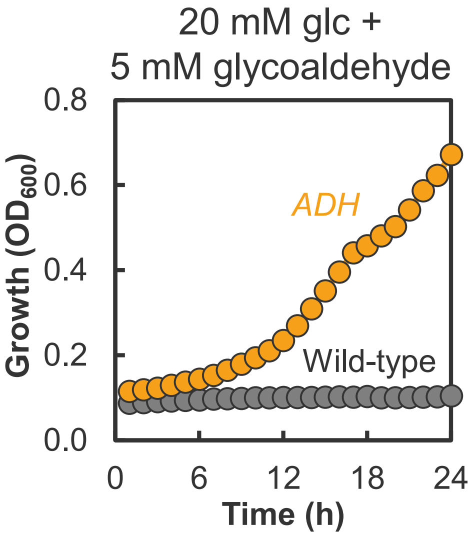
Efflux pumps



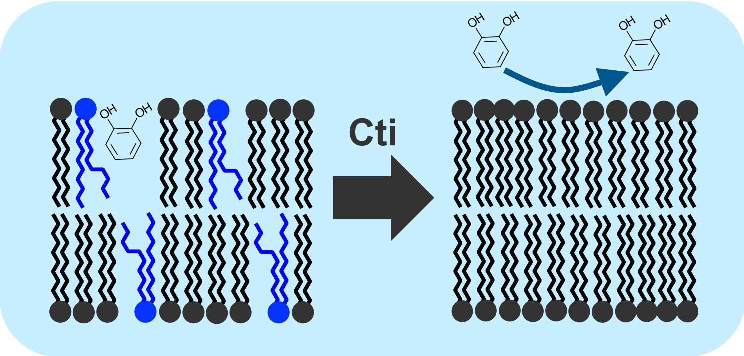


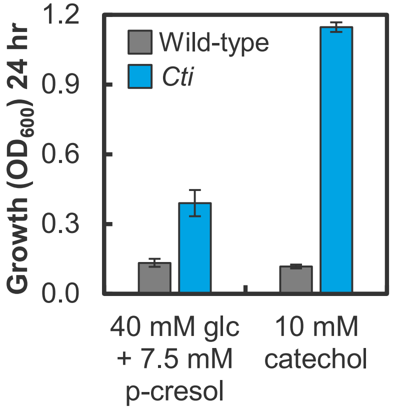
Dehydrogenase engineering



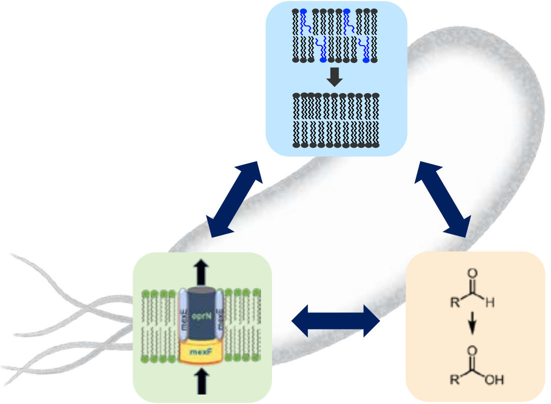


Membrane Engineering





Future Work: Synergy



Synergy tests ongoing now

Meyers, Jayakody *et al.*, in preparation

Response to Previous Reviewer Comments

Reviewer Comments

- The project looks well-managed, has a good and economically relevant target, and has made great progress so far. The chemical analysis of the waste streams and reaching the degrees of mass closure seen is in itself a tour de force. The pathway engineering strategy in P. putida looks very sound, with pathways identified to address the molecules seen in the waste streams. Only the biology will tell us what the limits of adding several new pathways to a single host may be, in terms of robustness or performance.

- The routes taken to increasing tolerance to protein damage have produced very good results (a 200x improvement in tolerance is impressive and suggests application to other systems). It would be good to bear in mind that, given that the strains may be able to actively digest the toxins, a fed-batch process may help the strains tolerate much higher levels of toxins than can be handled in batch.

- It would be interesting to think through the economics of applying the system to actively detoxifying other process streams, leaving behind sugars, for example, that can be readily utilized by a yeast system. Overall, this a great effort by the team, producing novel technical paths and excellent results.
- The PIs are developing one of the more exciting processes described, and the number of questions are a result of probing the potential of this approach. The ability for a single organism to navigate a huge range of functionalities and structures has the potential to be applicable in a wide range of areas. There are clear challenges, but the PIs recognize what is needed. Although it is a significant challenge to find an organism that can deal with a mixture of hundreds of materials, the PIs have found routes to make the funnel bigger. The presentation tells a nice, positive story regarding a concept that is quite straightforward.

- Aqueous waste streams account for 3%–10% of biomass carbon, currently sent to wastewater treatment. This project aims to create a monoculture that can not only remediate this stream, but turn it into a value-added product. One of the biggest accomplishments is the analytical characterization of these streams. P. putida was engineered to broaden substrate range, and a surprising increase in tolerance was obtained by overexpression of just GroES/EL (heat shock 10 kilodalton protein 1 (Hsp10)/another protein of 57 kilodaltons).

- The tolerance improvement here is likely sufficient. The organism has been engineered for utilization of various substrates, but the biggest challenge ahead is improving the utilization rates so that they will match the waste production rate without needing an enormous bioreactor. Also, if the waste treatment step is to be avoided, the organics have to be removed to an extremely low level. This is often challenging as catabolic pathways shut off at low substrate concentrations.

Response to Previous Reviewer Comments

- The tolerance improvement here is likely sufficient. The organism has been engineered for utilization of various substrates, but the biggest challenge ahead is improving the utilization rates so that they will match the waste production rate without needing an enormous bioreactor. Also, if the waste treatment step is to be avoided, the organics have to be removed to an extremely low level. This is often challenging as catabolic pathways shut off at low substrate concentrations.

- Waste valorization is one of the keys to cost-effective biorefineries, both bio- and thermo-conversion processes. The use of bio-funneling is a clever approach to upgrading myriad waste carbon components into a value-added product, and possibly saving catalyst cost by decreasing the severity of the thermochemical conversion (allowing bioconversion to “mop up”). Since the 2015 review, PHA has been identified as an exemplary product, and a TEA is in progress and should lead to quantitative metrics moving forward.

- This is a good example of high-risk/high-gain project. It is a very ambitious project with dynamic moving targets of variability of waste stream composition and multiple toxicities (e.g., membrane fluidity, protein generation and repair, global stress response, acid tolerance, etc.). These challenges make it difficult to judge if this project will make it to the end goal, which is transfer to industry to develop a fermentation process for the complete use of wastewater from thermochemical processes to a biobased product. If successful, it will make a big impact on the economics of the thermochemical processes.

Responses

- We thank the reviewers for the positive feedback and constructive comments. We completely agree that the approaches being developed here could potentially be useful for hydrolysate cleanup.

Milestones

FY17		
Q1	QPM	Overexpress the protein recycling machinery in *Pseudomonas putida* (GroEL, GroES, Clpb) in genome-integrated strains to achieve higher toxicity tolerance to pyrolysis-derived streams.
Q2	QPM	Demonstrate effects of overexpressing the protein-recycling machinery on the baseline toxicity tolerance to individual components (up to 25) in aqueous streams in *Pseudomonas putida*.
Q3	QPM	Demonstrate the baseline toxicity tolerance of protein-recycling genome-integrated strains of *Pseudomonas putida* on at least 6 different aqueous pyrolysis streams from FP, FP with fractionation, *in situ* CFP, and *ex situ* CFP. Demonstrate an improvement of at least 10-fold over the wild-type strain.
Q4	Annual	Report comprehensive, quantitative characterization of at least 10 streams across catalyst, feedstock, and reaction conditions for NREL ex situ CFP waste streams. Identify and quantify the top 25-50 compounds in each. Impact: Enable tailored strategies for bio-conversion and target selection.

Milestones

FY18		
Q1	QPM	Integrate genes to convert at least 50% of meta, ortho, and para-cresol in P. putida. Impact: increase carbon conversion efficiency in DCR-relevant waste streams.
Q2	QPM	Integrate genes and demonstrate conversion of alkylated phenolics in P. putida to methyl muconates. Impact: understand potential for producing value-added, atom efficient intermediates useful for biodegradable polymer production.
Q3	QPM	Identify the key drivers of toxicity for alkylated phenolics and design genetic strategies to overcome them (e.g., membrane engineering).
Q4	Annual	Demonstrate at least 25% carbon conversion efficiency in a representative mock DCR stream (including all compounds that are commercially available at the levels determined by detailed analytics conducted in FY17) to produce muconates or PHAs. Identify mechanism(s) of toxicity of the DCR stream for further toxicity tolerance engineering, and suggest at least two engineering strategies to further improve toxicity tolerance.

Milestones

FY19		
Q1	QPM	Demonstrate heterologous utilization of methanol and acetone in an engineered strain of P. putida with genome-integrated genes. Measure and report methanol and acetone utilization rates. These compounds together can represent up to 10% of the carbon in TC aqueous waste streams. Taken together with previous catabolic engineering studies, this should enable catabolism of >75% of the carbon in most ex situ CFP streams examined in detail to date.
Q2	G/NG	Develop a techno-economic model in collaboration with the Thermochemical Platform Analysis project that demonstrates the potential to save at least $0.25/gge on the MFSP for ex situ catalytic fast pyrolysis. Identify key cost drivers for the BCTAS project concept, both in the upstream (ex situ CFP) and downstream (conditioning, biological conversion, and product recovery) steps. We will examine multiple potential products, including muconates and PHAs.
Q3	QPM	In collaboration with the Catalytic Upgrading of Pyrolysis Products, demonstrate a combined thermochemical-biocatalytic production pathway to diacids by producing a CFP bio-oil, fractionating a phenolics stream from the oil having a >60 wt% purity, and biocatalytically ring-opening the purified phenolics using existing engineered-strains to produce diacids.
Q4	QPM	Measure synergy between membrane engineering, efflux pump, and chaperone toxicity tolerance strategies in the presence of mock wastewater streams that comprise up to 90% of the carbon in characterized streams.

Publications

1. A. Nolan Wilson, Abhijit Dutta, Brenna A. Black, Calvin Mukarakate, Kim Magrini, Joshua A. Schaidle, William E. Michener, Gregg T. Beckham, Mark R. Nimlos*, “Valorization of aqueous waste streams from biomass catalytic fast pyrolysis”, in review at ***Green Chem***.
2. Kirsten Davis, Laura R. Jarboe, Davinia Salvachua, Gregg T. Beckham, Zhiyou Wen, Robert C. Brown, Ryan G. Smith, Marjorie R. Rover*, “Preparation of bio-available phenolic substrates from bio-oil”, in review at ***J. Ind. Microbiol. Biotech.***
3. 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***.
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. Melissa Tumen-Velasquez‡, Christopher W. Johnson‡, Alaa Ahmed‡, Graham Dominick, Emily M. Fulk, Payal Khanna, Sarah A. Lee, Alicia L. Schmidt, Jeffrey G. Linger, Mark A. Eiteman, Gregg T. Beckham*, Ellen L. Neidle*, “Accelerating pathway evolution by increasing the gene dosage of chromosomal segments”, in press at ***PNAS***.
6. Mary Ann Franden, Lahiru Jayakody, Wing-Jin Li, Neil J. Wagner, Bernhard Hauer, Lars Blank, Nick Wierckx*, Janosch Klebensberger*, Gregg T. Beckham*, “Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization”, ***Metabolic Eng**.* (2018) 48, 197-207.
7. Lahiru Jayakody, Christopher W. Johnson, Jason M. Whitham, Richard J. Giannone, Brenna A. Black, Nicholas S. Cleveland, Dawn M. Klingeman, William E. Michener, Jessica L. Olstad, Derek R. Vardon, Robert C. Brown, Steven D. Brown, Robert L. Hettich, Adam M. Guss, Gregg T. Beckham*, “Thermochemical wastewater valorization via enhanced microbial toxicity tolerance”, ***Energy Env. Sci***. (2018) 11, 1625-1638.
8. Anne K. Starace, Brenna A. Black, David D. Lee, Elizabeth C. Palmiotti, Kellene A. Orton, William E. Michener, Jeroen ten Dam, Michael J. Watson, Gregg T. Beckham, Kimberly A. Magrini, Calvin Mukarakate*, “Characterization and catalytic upgrading of aqueous stream carbon from catalytic fast pyrolysis of biomass”, ***ACS Sust. Chem. Eng***. (2017) 5, 11761-11769.
9. Brenna A. Black, William E. Michener, Kelsey J. Ramirez, Mary J. Biddy, Brandon C. Knott, Mark W. Jarvis, Jessica Olstad, Ofei Mante, David C. Dayton, Gregg T. Beckham*, “Aqueous stream characterization from biomass pyrolysis and catalytic fast pyrolysis”, ***ACS Sust. Chem. Eng**.* (2016) 4(12), pp. 6815–6827.

Presentations

1. Biological valorization of aqueous waste carbon from catalytic fast pyrolysis, ACS National Meeting, August 22nd, 2018
2. Developing new processes to valorize lignin and sugars to building-block chemicals and materials, RWTH Aachen University, May 28th, 2018
3. Adventures in engineering *Pseudomonas putida* for expanded substrate specificity and improved tolerance, RWTH Aachen University, May 28th, 2018
4. Biological conversion of thermochemical waste streams, ACS National Meeting, March 18th, 2018
5. Biological conversion of thermochemical aqueous streams, AIChE Annual Meeting, November 1, 2017
6. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, University of Delaware, October 5, 2017
7. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, USDA-ARS Western Regional Research Center, June 14, 2017
8. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, University of Minnesota, March 27, 2017
9. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, École polytechnique fédérale de Lausanne, March 24, 2017
10. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, Michigan State University, February 2, 2017
11. Hybrid biological and catalytic processes to produce chemicals and materials from biomass, UT Austin, November 22, 2016
12. Lignin conversion by biological funneling and chemical catalysis, AIChE Annual Meeting, November 16, 2016
13. Developing hybrid biological and catalytic processes for biofuels and biochemicals, NIST, November 1, 2016