



Biological Conversion of Thermochemical Aqueous Streams

WBS 2.3.2.301

2017 U.S. Department of Energy (DOE)

Bioenergy Technologies Office (BETO) Project Peer Review

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Biochemical Conversion

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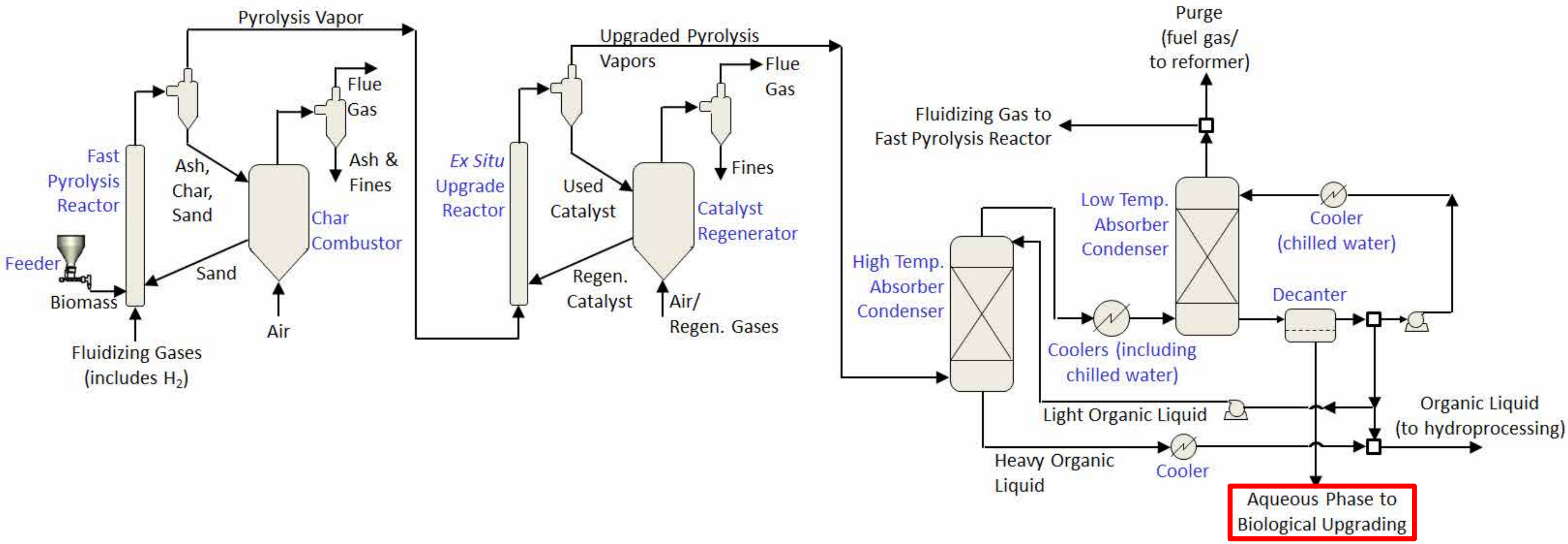
National Renewable Energy Laboratory (NREL)

Goal Statement

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

- Contribute to 2022 cost targets through valorization of TC waste streams to chemicals
- Waste streams can contain **up to 3%–10% of biomass-derived carbon** (**similar to lignin**)



Waste valorization will be a major economic benefit to TC biorefineries

- Collaborate with industry and universities to obtain streams and develop robust processes
- Contribute to other aqueous upgrading efforts by rigorous analytics
- **Outcome:** An integrated approach for converting TC aqueous streams to valuable compounds

Quad Chart

Timeline

- Start date: October 2016
- End date: September 2019
- Percent complete: 17%

Barriers

- **Ct-L Aqueous Phase Utilization and Wastewater Treatment**
  - Using biology to convert waste carbon
- Ct-J Process Integration
  - Working with process-relevant streams from multiple TC approaches

Budget

	FY14 Costs	FY15 Costs	FY16 Costs	Total Planned Funding (FY17-Project End Date)
DOE funded	$315k	$760k	$711k	$2,250k

Partners and Collaborators

- **Industry partner:** RTI International
- **NREL BETO projects**: Thermochemical Platform Analysis
  - NREL, Catalytic Fast Pyrolysis—NREL, Pilot-Scale Ex-Situ CFP with a DCR System, Lignin Utilization, **The Aqueous Project (NREL and PNNL)**, other BETO TC projects that produce aqueous waste
- **BETO-funded national lab projects:** Oak Ridge National Laboratory (ORNL) (A. Guss), PNNL
- **Office of Science funded centers:** Environmental Molecular Sciences Laboratory, BioEnergy Science Center
- **Academic collaborators:** Iowa State University, University of Georgia, University of Portsmouth, University of Stuttgart, RWTH Aachen

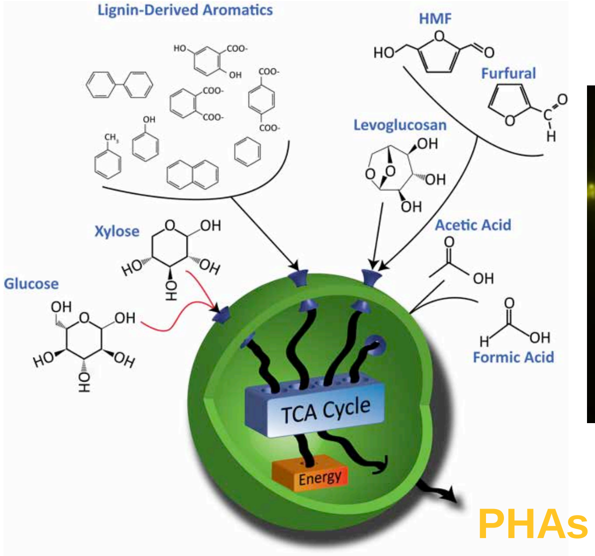
Project Overview

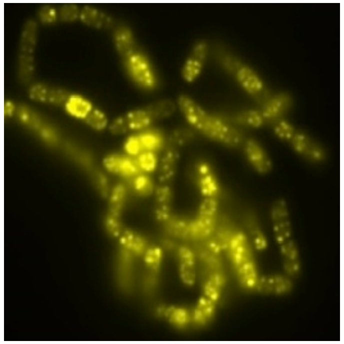
History: Valorization of waste identified as a key barrier that currently places a large cost burden on biorefineries

- Started as a seed project in FY14, major “go” decision in Sept 2015
- Apply learnings from Biochem. Conv. — **waste valorization can impart a major economic credit to the biorefinery ($2–$4/gge contribution in the case of lignin)**

Context: All TC processes produce aqueous waste

- Identify and capture “lost” carbon
- Reduce WWT cost **~$0.25/gge burden with large uncertainty**
- Enable a value-added co-product stream
- Adaptable to many biorefinery scenarios



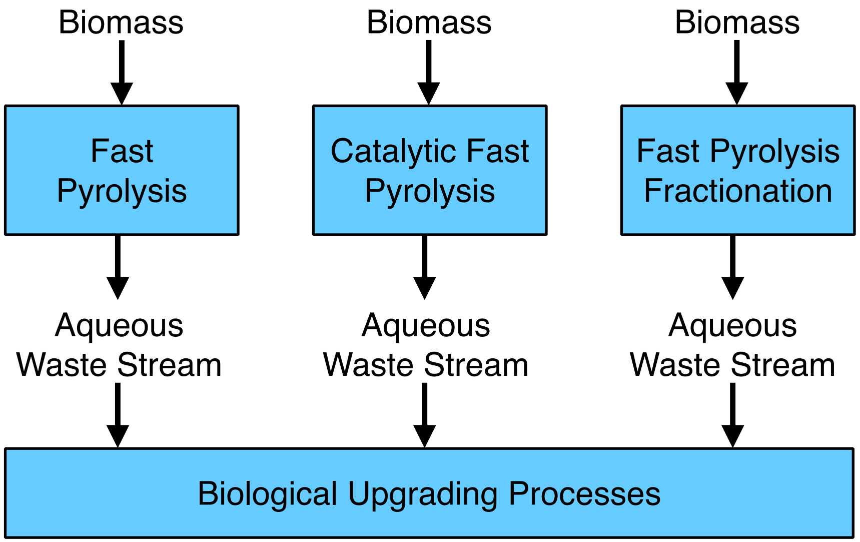


Project Objectives:

- Characterize composition of aqueous streams from TC processing
- Develop biological strategies for efficient valorization of TC waste streams to **PHAs**
- Conduct process development with “upstream” TC projects
- Work with TEA project to define targets and co-products for high-value chemicals

Technical Approach

Aim 1: Characterize streams from TC processes

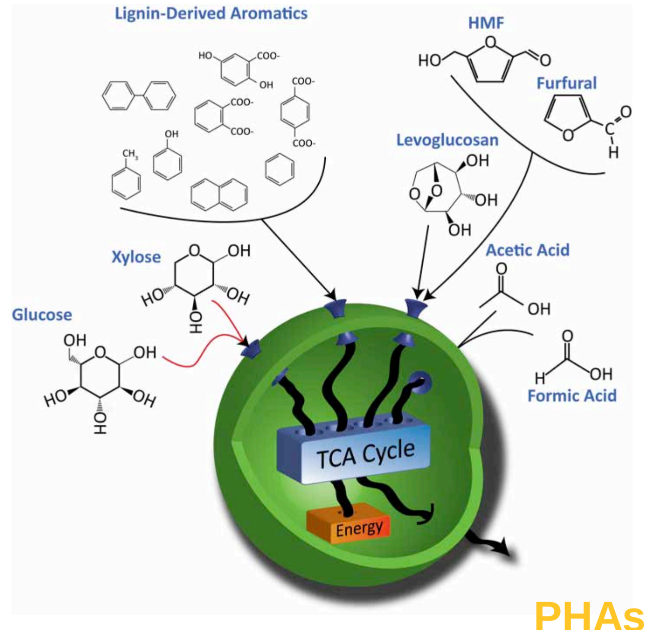


Approach:

- Develop new analytical approaches
- Tailor organism to process-relevant TC streams

Challenges: Sufficient/consistent substrate

Aim 2: Develop strains for a wide substrate range



Approach:

- Discover new catabolic capabilities (if needed)
- Engineer strains for target substrates
- Engineer strains for higher tolerance

Challenges: Substrate specificity and toxicity

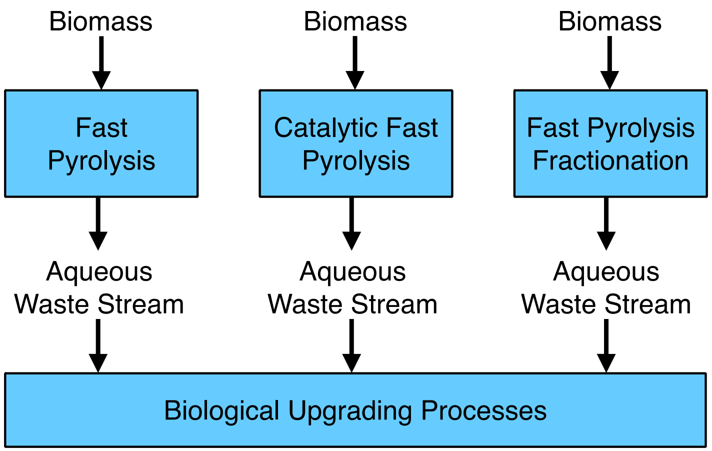
Critical Success Factors:

- Understand aqueous stream **composition** and tailor microbial strategy to stream(s) of interest
- Develop **organism tolerance** and **engineer pathways** to produce a value-added co-product.

Management Approach

- *Develop simple, integrated approaches and use TEA and Go/No-Go's to refine options*
- *Employ fundamentals-driven science/engineering approach with an interdisciplinary approach*

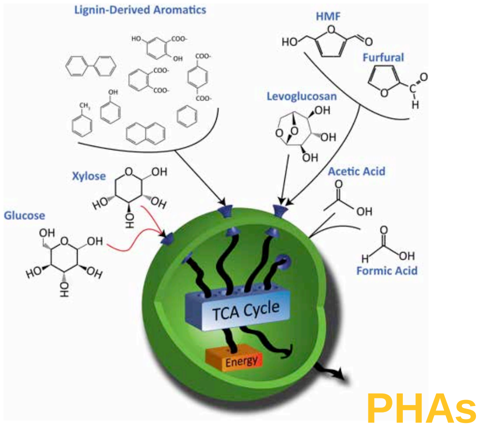
Aim 1: Characterize streams from TC processes



Collaborate with NREL, RTI, ISU and other TC groups to obtain process-relevant streams:

- Led by expert in analytics (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.

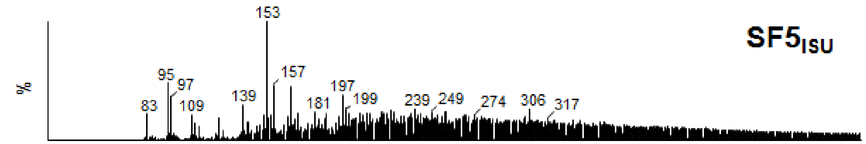
Aim 2: Develop strains for a wide substrate range



Assembled team of experts in metabolic engineering and pathway discovery:

- Led by *P. putida* expert (Chris Johnson)
- Milestones on substrate utilization and organism tolerance
- Employ KT2440 metabolic engineering expertise
- Strategic hire in microbial toxicity (L. Jayakody).

Outline—Technical Accomplishments and Progress

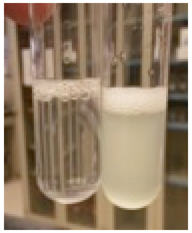


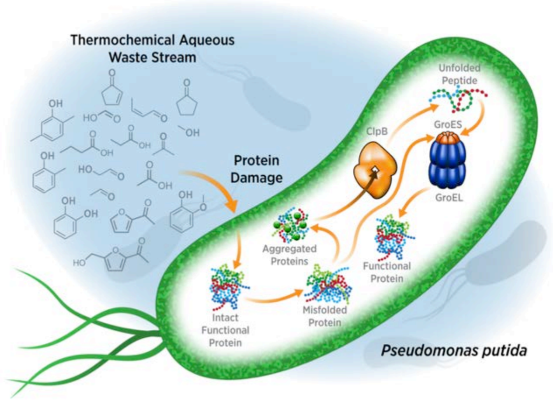
Aim 1

- Analytical method development
- Aqueous stream composition survey across multiple pyrolysis technologies
- Detailed analysis of multiple *ex situ* catalytic fast pyrolysis streams from NREL

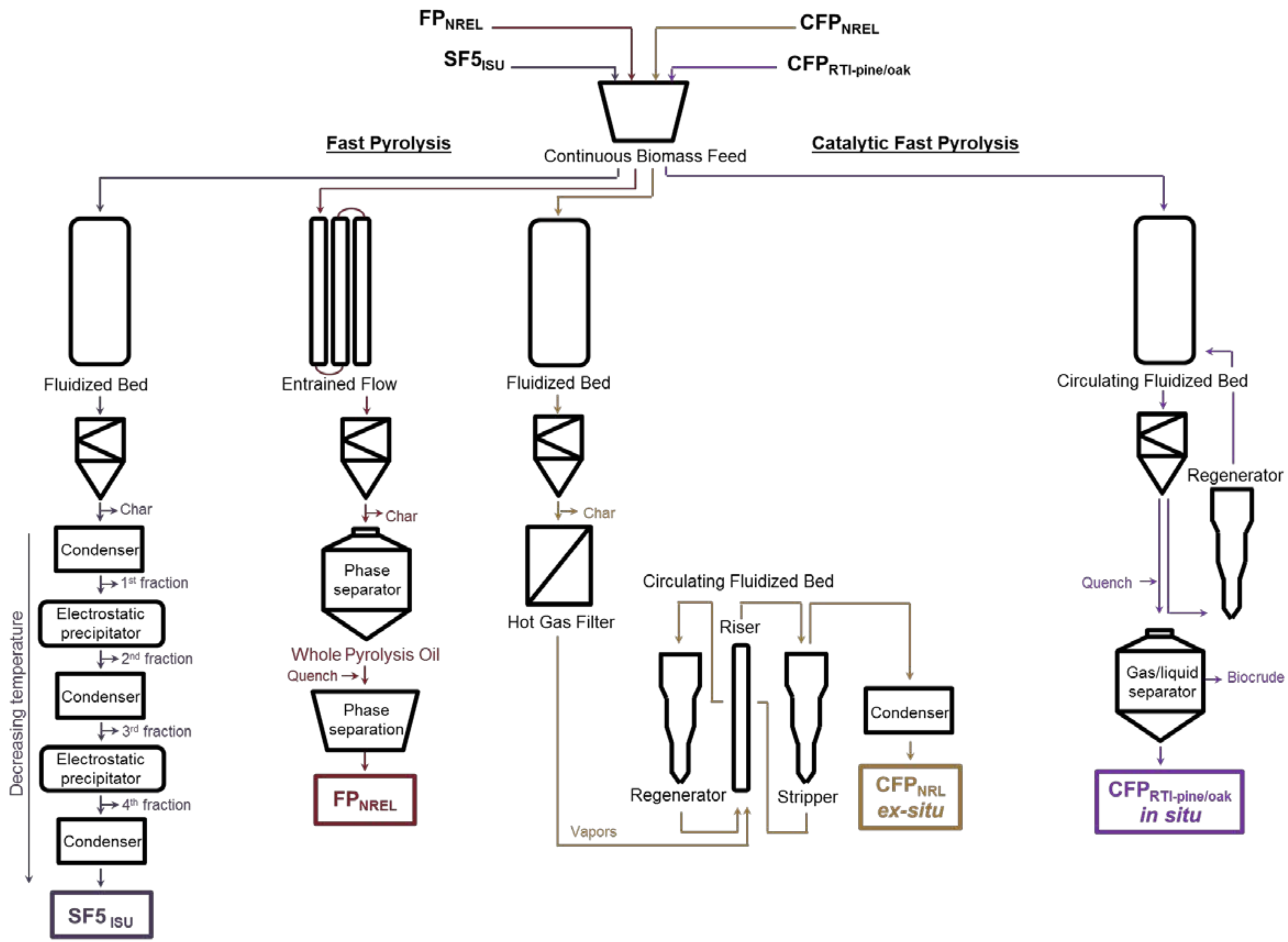
Aim 2

- Review progress in substrate utilization
- Improving organism tolerance
- Stream down-selection: Focus on PHAs (exemplary product) from *ex situ* catalytic fast pyrolysis (CFP).





Aim 1: Fast pyrolysis & catalytic fast pyrolysis streams



B.A. Black *et al. ACS Sust. Chem. Eng.* 2016

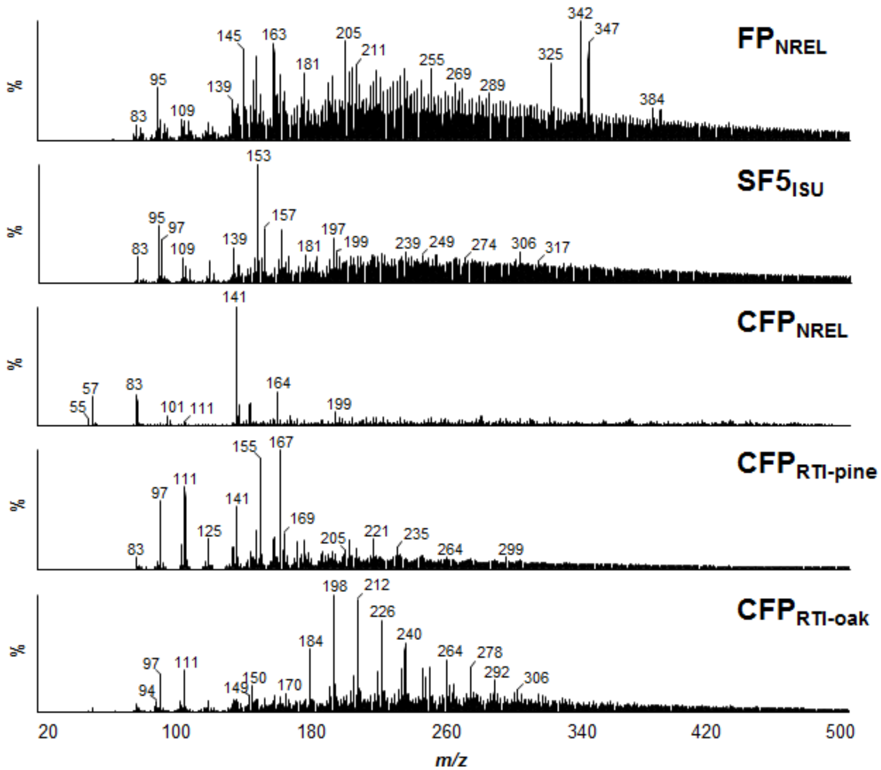
Aim 1: Employ aqueous-compatible analytics

Characterization steps:

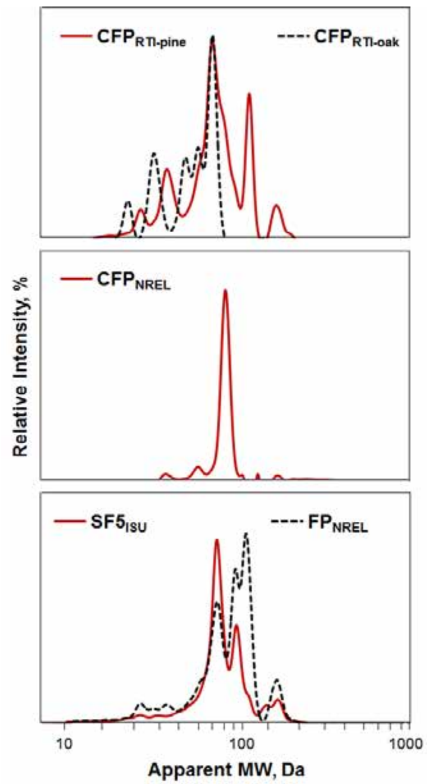
1) Identify which compounds are present in each sample

Tandem MS and identifying from fragmentation ions; NIST11 Library and manual identification used for GC-MS; Manual identification for LC-MS; Mass distribution focus confirmed via aqueous GPC

Infusion ESI-MS$_{positive\text{-}ion}$



Aqueous GPC



B.A. Black *et al. ACS Sust. Chem. Eng.* 2016

Aim 1: Employ aqueous-compatible analytics

Characterization steps:

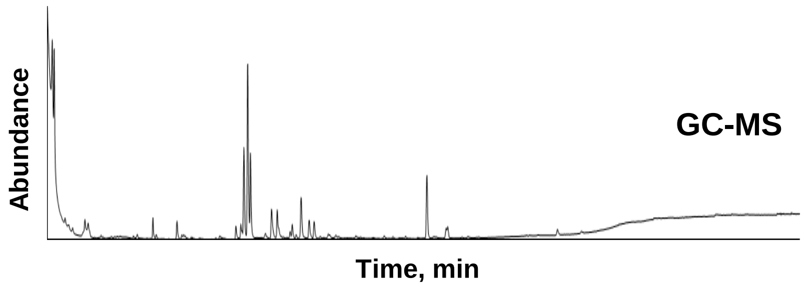
1) Identify which compounds are present in each sample

   Tandem MS and identifying from fragmentation ions; NIST11 Library and manual identification used for GC-MS; Manual identification for LC-MS; Mass distribution focus confirmed via aqueous GPC

2) Quantify compounds in each sample

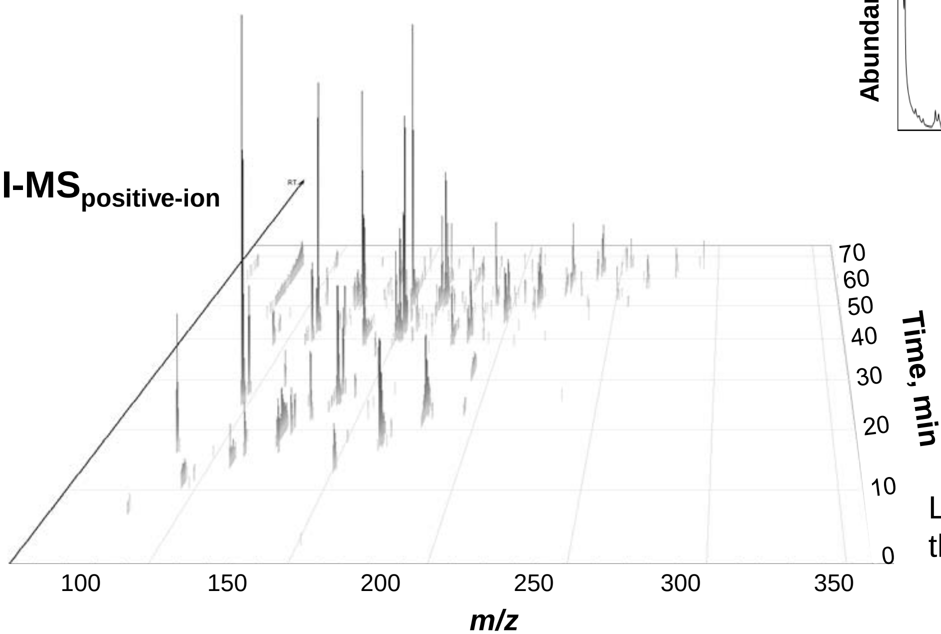
   Compare detector responses using authentic standards at known concentrations

Sample: **CFP$_{\text{RTI-oak}}$***$^{\text{in-situ}}$*



GC for low MW, thermally stable, volatile compounds

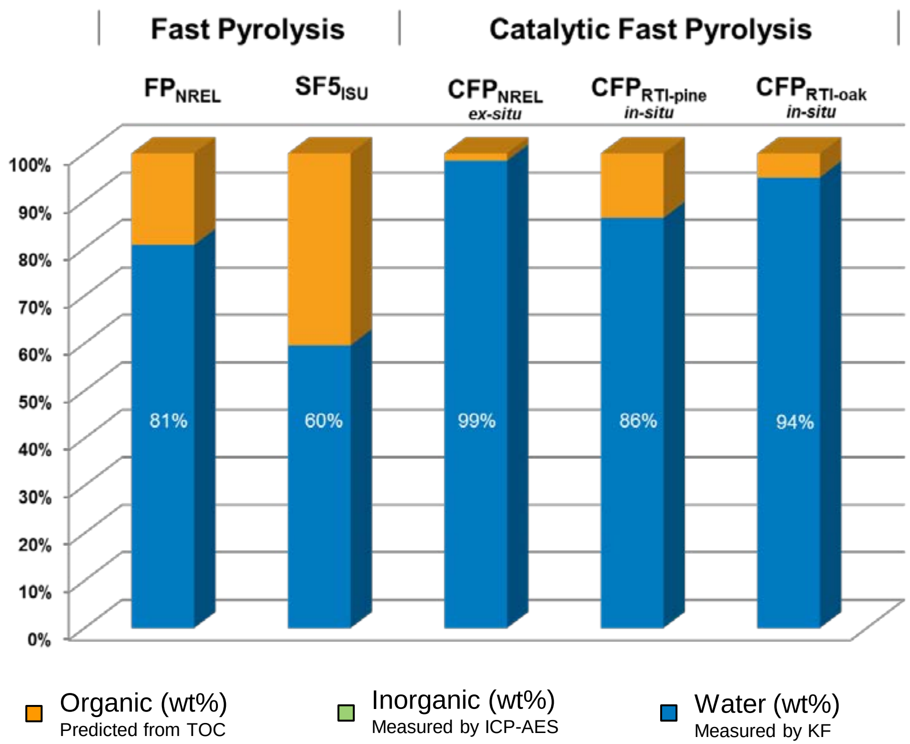
LC/ESI-MS$_{\text{positive-ion}}$

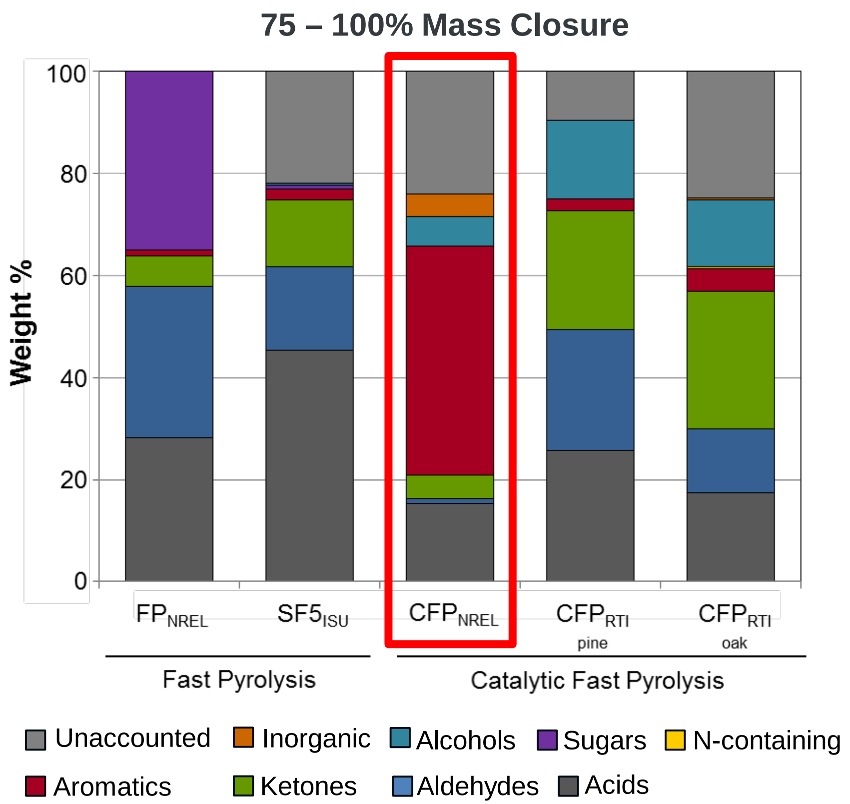


LC for low and high molecular weight, thermally labile, non-volatile compounds

B.A. Black *et al. ACS Sust. Chem. Eng.* 2016

Fast pyrolysis and catalytic fast pyrolysis characterization

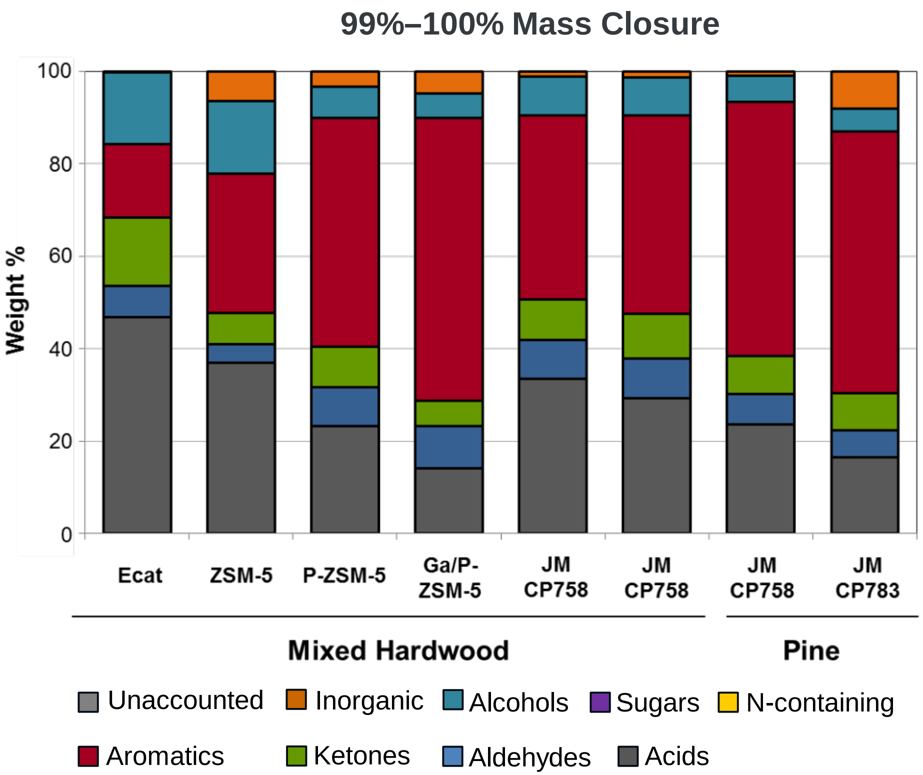




- **≥75% mass closure** (**100+ compounds quantified, 200+ identified**)
- Wide range of carbon in aqueous streams depending on upstream technology
- Thorough characterization can guide development of selective valorization strategies
- Multiple methods developed or optimized to characterize TC aqueous streams.

B.A. Black *et al. ACS Sust. Chem. Eng.* 2016

ex situ CFP aqueous streams are mostly aromatics, acids, aldehydes



g/kg Compound	Mixed Hardwood Feedstock Ecat	ZSM-5	P-ZSM-5	Ga/P-ZSM-5	JM CP 758
Acetic acid	16.48	6.35	4.79	2.87	6.73
Formic acid	7.73	3.78	0.78	0.45	2.10
Propanoic acid	1.28	0.13	0.10	0.05	0.07
2-Hydroxyacetic acid	3.49	ND	0.16	ND	0.46
Acetaldehyde	1.30	0.77	0.21	ND	0.39
2-Hydroxyacetaldehyde	1.53	ND	0.06	ND	0.16
Phenol	2.23	2.38	1.85	1.98	1.97
2-Methylphenol	1.03	1.05	0.65	0.65	0.70
3-Methylphenol	1.95	2.25	1.61	1.47	1.56
4-Methylphenol	0.79	0.76	0.58	0.54	0.61
2,5-Dimethylphenol	0.55	0.74	0.47	0.45	0.55
2-Ethylphenol	0.29	0.27	0.18	0.17	0.18
Benzene-1,2-diol	0.21	0.05	2.86	2.93	0.29
Benzene-1,4-diol	0.40	0.20	0.42	0.36	0.55
3-Methylbenzene-1,4-diol	0.74	0.12	1.85	2.33	1.47
4-Methylbenzene-1,2-diol	0.64	0.09	0.68	1.50	0.69
4-Ethylbenzene-1,2-diol	0.31	0.10	0.49	0.91	0.38
4-Ethylbenzene-1,3-diol	0.22	0.11	0.25	0.52	0.30
Methanol	7.87	3.57	1.19	0.82	1.68

ND = not detected

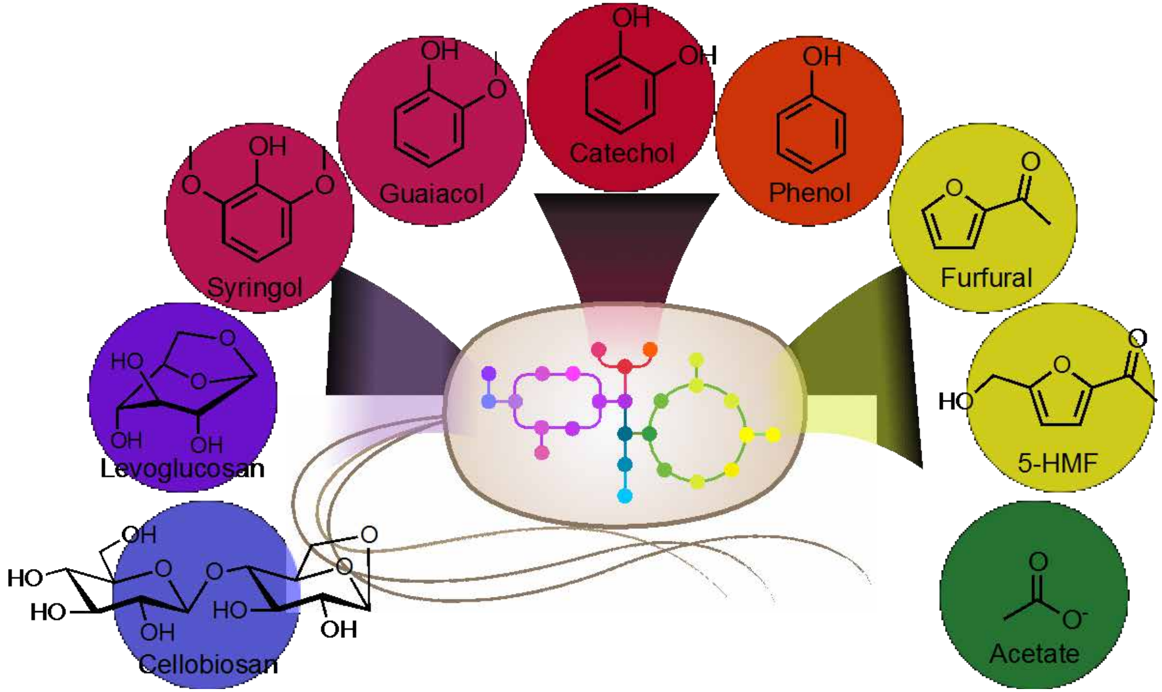
B.A. Black *et al.* in preparation; K. Magrini *et al.* in preparation

- Mainly **recalcitrant C1-C3 <u>acids</u>, <u>aldehydes</u>, <u>phenolics</u>—ideal substrates for *P. putida***
- Vary between 50-100 g/L organic carbon—**ideal range for biological conversion**
- 3-10% of organic carbon in original biomass – similar to lignin streams
- Characterization assists upstream processing and aqueous valorization strategies
  - **Upstream—**understand how changing conditions and reactor configurations affects WW composition
  - **Downstream—**inform catalytic or biological approaches for targeted valorization.

Aim 2: Strain development background

P. putida KT2440 chosen for broad catabolic capability and tolerance

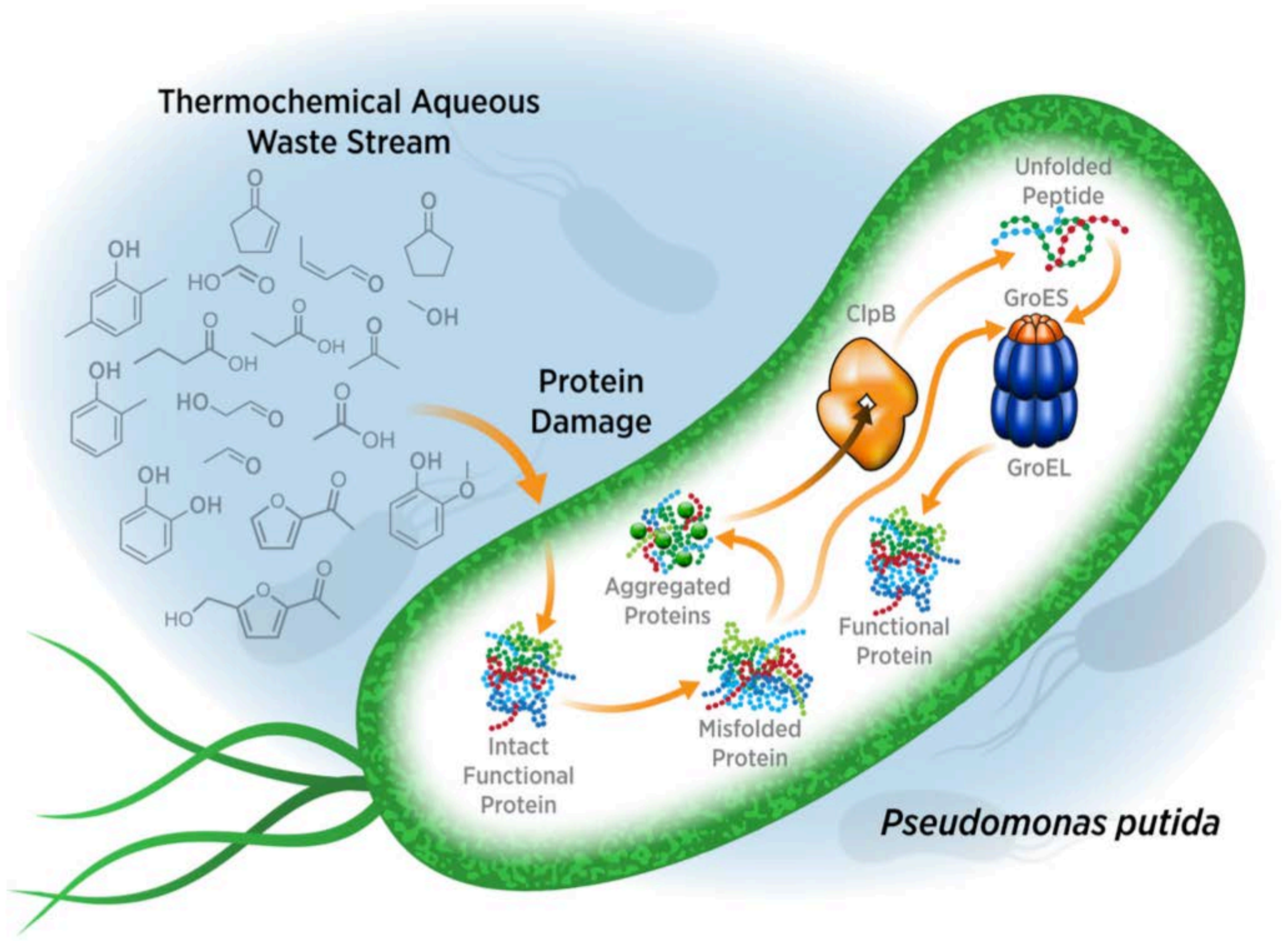
- Natively metabolizes catechol, formate, and acetate
- This concept will require a **“wider” funnel than lignin substrates**
- Integrated enzymes for cellobiosan, levoglucosan, phenol, furfural, and 5-HMF
- Discovered genes for guaiacol metabolism, isolated microbe capable of growth on syringol
- Produced **polyhydroxyalkanoates** (**PHAs**) from TC-derived aqueous streams



Microbial toxicity is the main challenge for this concept

Toxicity likely occurs via membrane, protein, and/or DNA damage

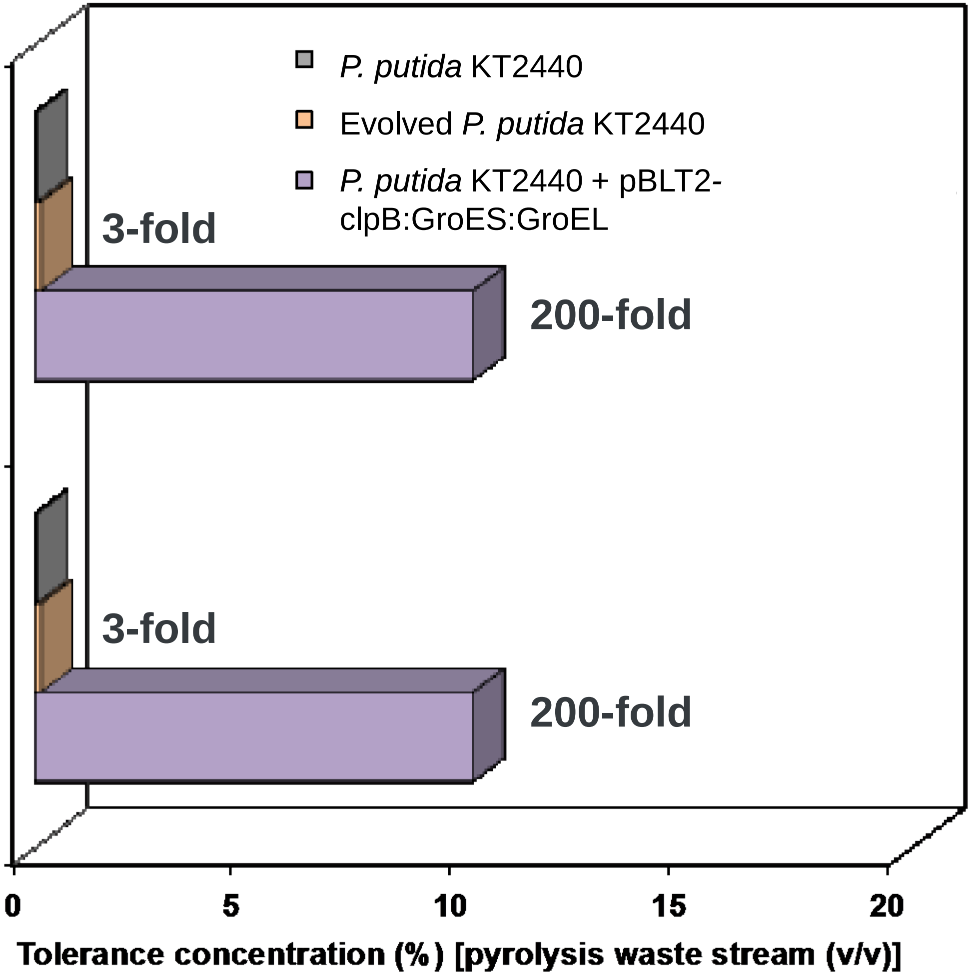
Hypothesized that protein damage may be the most prevalent toxicity mechanism



Enabling substantial gains in microbial tolerance

ISU Stage Fraction 5

RTI *in situ* CFP pine

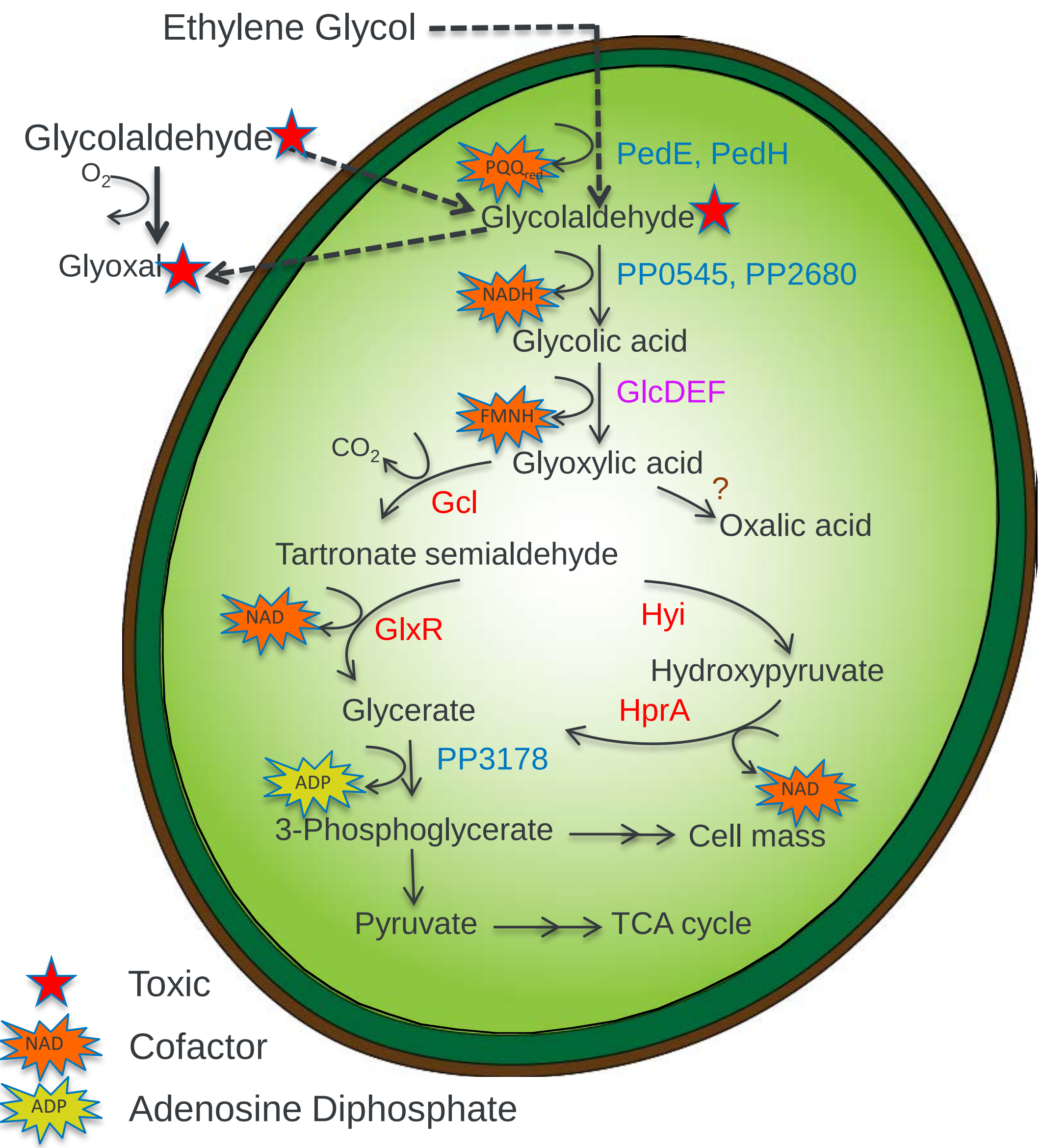


Over-expressed *P. putida* **protein QC** machinery

Similar toxicity profiles for DCR streams

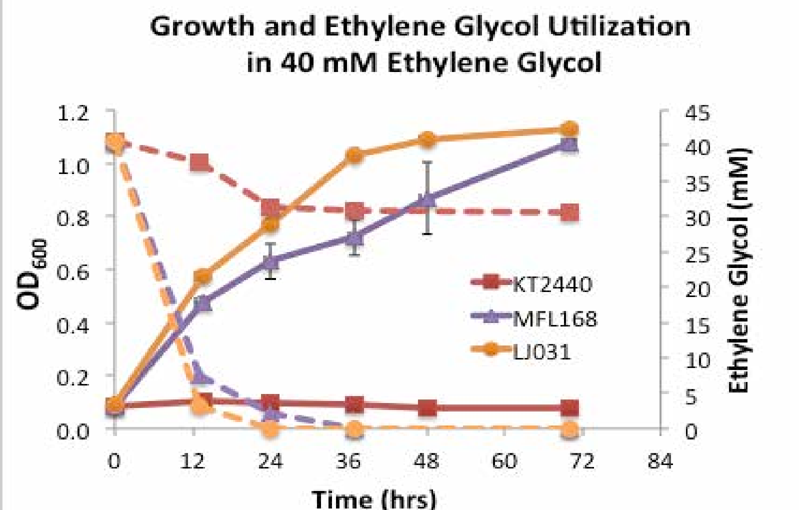
Outcome: Enabled **2 orders-of-magnitude gain** in tolerance

Continued substrate expansion example: Glycolaldehyde



Deregulation of key enzymes in red allows for metabolism of glycolaldehyde, a major inhibitor in TC aqueous streams and ethylene glycol

- *P. putida* KT2440 engineered to overexpress the genes in red (MFL168) allowing for efficient utilization of ethylene glycol and glycolaldehyde
- Further improvement was made with the overexpression of glycolate oxidase (shown in purple, GlcDEF) to create strain LJ031



OD_{600} (solid lines)
Ethylene Glycol (dashed lines)

Relevance

Valorization of aqueous waste streams will be a major contributor to 2022 HC cost targets

Highlighted in MYPP as a key barrier: **"The aq. phase from high-temperature deconstruction and upgrading may contain acids, ketones, and phenolics, which require conversion or removal before the water can be released. Research is needed to characterize organics in the aqueous phase and to convert these organics to … biochemicals ..."**

Key MYPP areas:

Aqueous Phase Utilization and Wastewater Treatment
- Enables selective, tunable route for upgrading "waste" carbon
- Potential for both fuels and chemicals production via a biological route
- Maximizing use of carbon

Process Integration
- Working with process-relevant streams from 4 TC approaches

Catalytic Upgrading of Bio-Oil Intermediates to Fuels/Chemicals
- Enables tuning upstream steps to reduce HDO costs

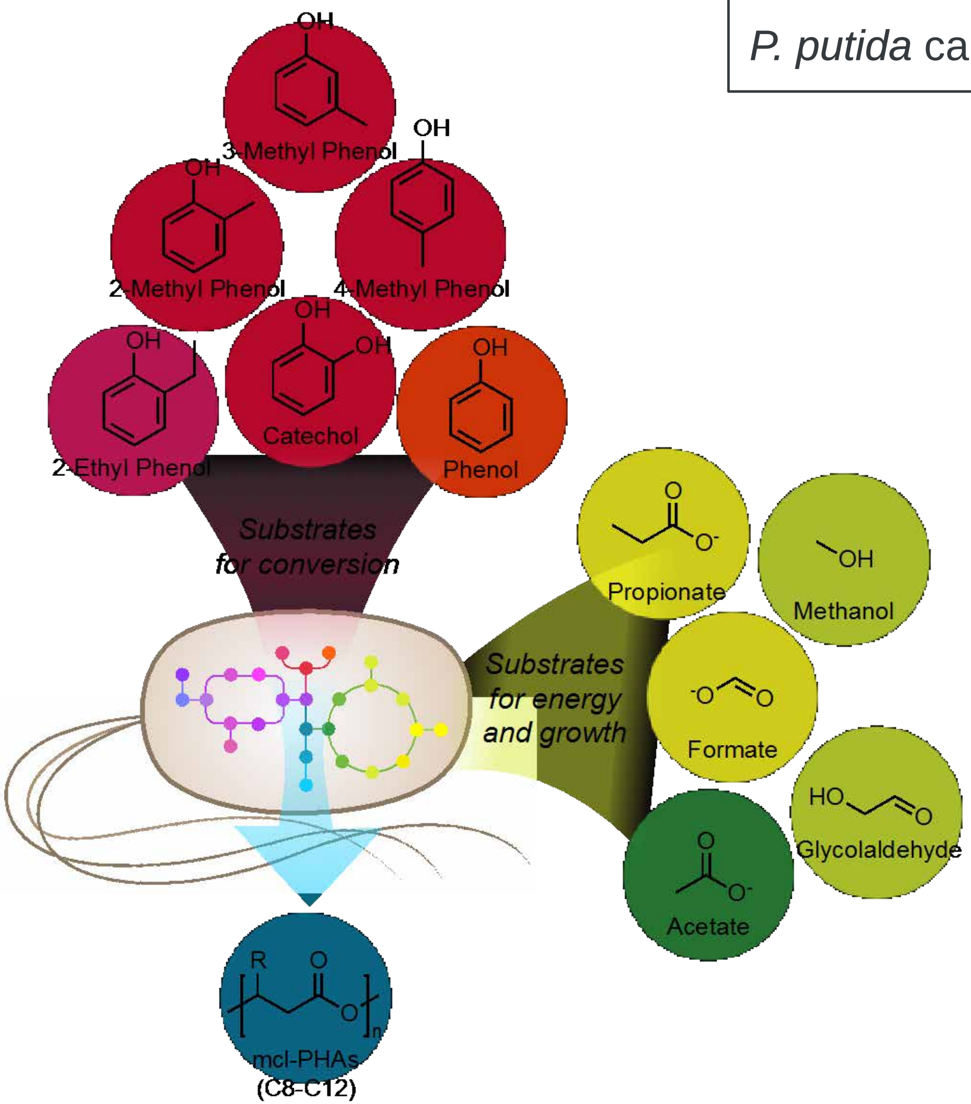
Key Stakeholders and Impacts:
- **Economics and sustainability of TC processes will benefit from co-products and reducing WWT costs—incorporating into TEA models ($2-$4/gge for lignin)**
- Enabling chemicals from waste in TC biorefineries
- Impacts the "**Whole Barrel of Oil**" initiative
- **Portfolio of chemicals from waste will diversify and accelerate development of TC biorefineries**
- Significant peer-reviewed science and IP
- Methods to upgrade heterogeneous intermediates can be adapted by other platforms

Future Work (Aim 1)

Work with "upstream" process development to understand how conditions affect aqueous composition

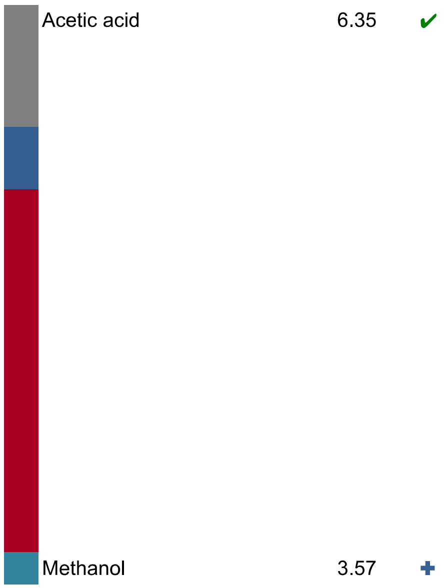
- Continue to **increase analytical method capabilities and throughput** to support multiple projects
- Continue characterization of DCR aqueous streams
- Understand environmental impacts of WWT
- **Incorporate biological valorization into core TEA model for *ex situ* CFP**

Future Work (Aim 2)



Conversion of aromatics to value-added co-products

P. putida can convert aromatics in DCR streams to **PHAs**



✓=Native pathway

✚=Known pathway

- Currently can use ~50% of C
- Theoretically can utilize 100% of C

Utilization of C1-C3 substrates in DCR for energy and growth

- *P. putida* metabolizes C1-C3 acids
- Engineered KT2440 to metabolize glycolaldehyde
- Engineering for methanol ongoing

Summary

- Approach
  - Characterize TC aqueous streams using a wide range of aqueous-compatible analytical methods
  - Develop strategies for bioconversion of aqueous streams via toxicity improvement and substrate expansion
  - Collaborate widely with academic, national lab, and industrial partners including TC platform tasks
- Technical accomplishments
  - Achieved complete mass closure characterization for *ex situ* CFP aqueous waste stream characterization
  - Demonstrated incorporation of multiple genes into a robust host, *P. putida* KT2440
  - Dramatically improved toxicity tolerance of *P. putida* via simple protein QC machinery changes
- Relevance
  - Reduce economic and sustainability burden on wastewater treatment in TC processes
  - Co-products essential to meet DOE hydrocarbon cost targets
  - Addresses Whole Barrel of Oil Initiative and bolsters the biomass value chain
- Critical success factors and challenges
  - Stream toxicity, **economic** and **sustainable** production of co-products, high yields of **PHAs**
- Future work
  - Continue development of analytical approaches for aqueous waste stream valorization efforts
  - Ramp up efforts on **PHA** production in NREL DCR streams and substrate expansion
  - **Begin developing process targets using TEA modeling**
- Technology transfer
  - Working with **industry** to build commercialization path to wastewater valorization in TC processes

Acknowledgments

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NREL contributors

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- Calvin Mukarakate
- Mark Nimlos
- Jessica Olstad
- Kelsey Ramirez
- Michelle Reed
- Josh Schaidle

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy BIOMASS PROGRAM

External collaborators

- Dave Dayton, RTI International
- Adam Guss, Oak Ridge National Laboratory
- Ellen Neidle, University of Georgia
- R. Robinson, E. Zink, Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory
- Robert Brown, Laura Jarboe, Marjorie Rover, Ryan Smith, Xianglan Bai, Iowa State University
- John McGeehan, University of Portsmouth
- Nick Wierckx, RWTH Aachen
- Janosch Klebensberger, University of Stuttgart

Additional slides

- Publications and patent applications
- Acronyms
- Previous reviewer comments
- Supplementary slides

Publications

Publications in press:

1. J.G. Linger, S.E. Hobdey, M.A. Franden, E. Fulk, G.T. Beckham, "Conversion of levoglucosan and cellobiosan by *Pseudomonas putida* KT2440", *Metab. Eng. Comm*. (2016) 3, pp. 24-29.
2. B.A. Black, W.E. Michener, K.J. Ramirez, M.J. Biddy, B.C. Knott, M.W. Jarvis, J. Olstad, O. Mante, D.C. Dayton, G.T. Beckham, "Aqueous stream characterization from biomass pyrolysis and catalytic fast pyrolysis", *ACS Sust. Chem. Eng.* (2016) 4(12), pp. 6815–6827.
3. M.T. Guarnieri, M.A. Franden, C.W. Johnson, G.T. Beckham, "Conversion and assimilation of furfural and 5-(hydroxymethyl)furfural by *Pseudomonas putida* KT2440", in revision at Metabolic Eng. Comm.

Publications in preparation:

1. B.A. Black *et al.*, Characterization of aqueous streams from a 2" fluidized bed *ex situ* catalytic fast pyrolysis reactor
2. L. Jayakody *et al*., Enabling biological conversion of highly toxic biorefinery wastewater streams by tuning protein quality control machinery
3. M. Tumen-Velasquez, C.W. Johnson, A. Ahmed, G. Dominick, E. Fulk, P. Khanna, S. Lee, J.G. Linger, M. Eiteman, G.T. Beckham, E.L. Neidle, "Accelerating pathway evolution through genome amplification", in preparation.
4. K. Magrini *et al.,* Reforming Biomass Derived Catalytic Fast Pyrolysis Aqueous Waste Streams to Process Hydrogen

Patent Applications:

1. Methods for cellobiosan utilization, US Patent Application 15/063,197

Acronyms

- CFP: Catalytic Fast Pyrolysis
- DCR: Davison Circulating Reactor
- FP: Fast Pyrolysis
- LCA: Life-Cycle Analysis
- LGK: Levoglucosan Kinase
- PHAs: Polyhydroxyalkanoates
- QC: Quality Control
- TC: Thermochemical
- TEA: Techno-Economic Analysis
- WWT: Wastewater Treatment

Previous Reviewer Comments

Project Overview

- Focusing on capturing carbon-containing molecules from wastewater, which fits with the mission of increasing carbon efficiency.
- Pyrolysis systems produce aqueous streams than need to be treated or converted. The project could be useful in doing that. The presentation does not make clear what has been done previously by others in this area or what the state of the art is.
- The project name would be more accurate if it were something like "Biological Treatment of TC Wastewaters".
- Clearly presented objectives and interesting approach for capturing aqueous phase carbon
- The aims of the project were clearly defined, although targets were not.

Project Approach:

- Convert carbon in water waste to products. Develop biological substrate for converting target molecules. Need some scoping economics to identify how much capital can be supported on converting some of the products in this stream and recovering them.
- The approach is very broad-based. The presentation does not make clear what types of products are being focused on or which molecules will be converted. There is not clear focus on what types organisms are needed. There is little focus on which end-products are of interest or how those might help improve process economics. The rationale for selecting organisms is unclear if the end-products are not known
- New catalysts for waste water upgrading, well coordinated
- The investigators are focusing on potential toxic entities in pyrolysis effluent in selection of organisms. Move to real py-oils is important to insure success since there will always be unanticipated potential poisons. Use of a GMO is likely to increase capital costs significantly. Good collaboration to obtain waste streams. The investigators need to identify targets for materials to be produced by organisms. The question of separating produced materials from dilute solutions may be a show stopper for the revenue generating aspect of this project and needs to be addressed.

Previous Reviewer Comments

Technical Progress and Accomplishments

- Presented functional and compositional data in waste water to highlight initial slate of components, which is very useful. But approach appears to be synthetic biology to target molecules. Need to develop a compelling economic case of what are the target feedstock molecules, the product and the potential volume.
- Substantial technical progress has been made. There is a good appreciation of the toxic nature of the aqueous effluents. There is not a clear rationale for the need for synthetic biology to modify organisms -- there is no clear discussion of why the base-line performance of known species is inadequate or how much improvement could potentially be made. The project needs to rapidly move to whole bio-oil to have relevance.
- Well organized and leveraged program
- Investigators have done a good job of modifying organisms for poisoning resistance. The workers have made significant progress in a short time. The project needs accompanying techno-economic analysis to determine whether waste treatment products can have a significant impact.
- (Also look at lipid bilayer (P. putida) surface impact due to activity of changes with phenol and other compounds.) This is a cross-over of biochem (maybe even algae) to utilize bio- or py-oil. If oil is "made" why is it then be used as a carbon source for organisms - it seems like a wastewater treatment approach instead of py-oil upgrading. It is unclear exactly what the "upgrade" oil looks like as a result of this approach. I suggest that future reporting and research keep in mind highlighting the ultimate goal - it's easy to get lost in the excitement of details of the biological system and not move the analysis to the "product recovery" stage.

Previous Reviewer Comments

Relevance

- Techno-economics need to be done upfront. The prize is unclear. How much carbon ends up in the aqueous phase? Product recovery from dilute streams will be an issue, let alone any purification or further upgrading that will be required.
- Improving conversion of aqueous wastes is important and could have significant impact. The presentation does not make clear how the tool-box approach will actually accomplish that for effluents from whole bio-oil, or how much economic impact would be realized if successful. The project needs to simply put a high-level "box" around the economics and compare that to current approaches such as AD to power.
- Relevance goes way beyond biomass, insofar as water purification could be more important than energy
- Since the project focuses on remediation of waste streams, it's economic impact may be minimal. The investigators need to identify the current costs of waste treating and show how significantly their developments can lower costs. The project needs accompanying techno-economic analysis to determine whether waste treatment products can have a significant impact.

Previous Reviewer Comments

Future Work

- Future work should consider organic acids--the most prevalent molecules in the stream (and potentially problematic for microorganisms), then do the techno-economics, and finally the biology. Need to compare against anaerobic digestion for electricity production or even methane for CNG--although compression cost will likely hurt the economics.
- Future work is described. Improved focus on critical issues is needed as noted previously, and potential economic impacts need to be very rapidly determined. Improved decision points based on performance parameters are also needed
- Future work objectives are fine, but more importantly this project could lead to many applications in industry
- The workers have made significant process and have outlined a reasonable plan to move forward. Techno-economic analysis will help to clarify the value of the process.
- Big project not enough focus. Fundamental research in nature.
- For immediate project targets, this is on track. Please address the TEA for a higher level TRL objective.

Previous Reviewer Comments

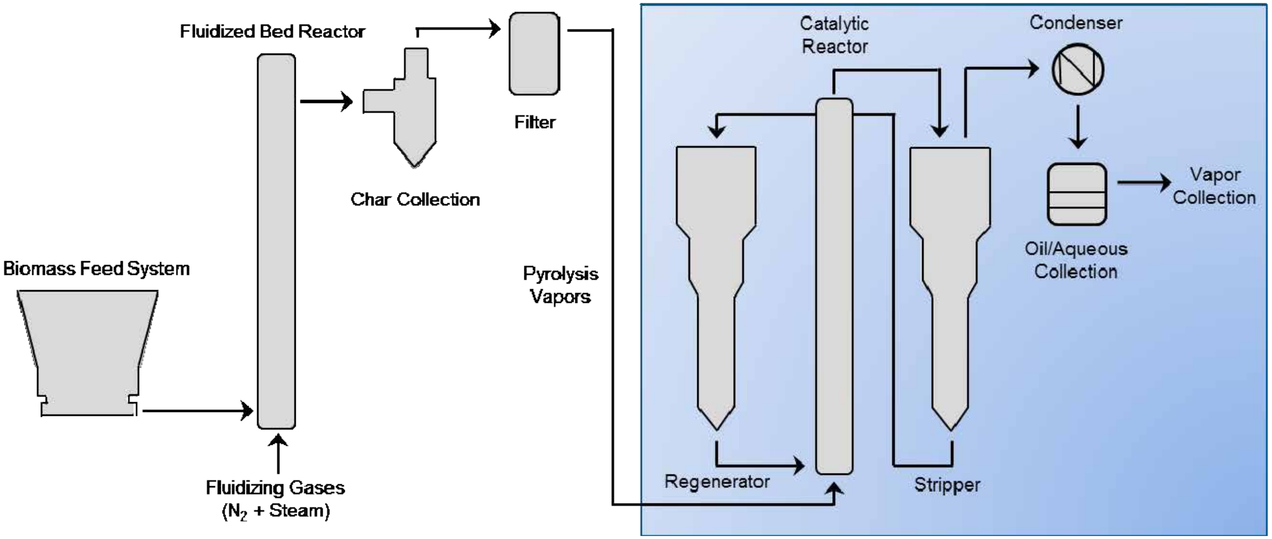
Overall Impressions

- Organism tolerance to acetic acid is an issue. This will be a huge challenge given that this is the largest oxygenated compounds in the aqueous stream. Working with actual aqueous bio-oil stream is critical. Need to demonstrate techno-economics. Separation of products will also be a huge issue. Not even sure what the target molecules are. Way too much focus on synthetic biology at this point.
- The project deals with an important area in cleaning up aqueous streams from pyrolysis and potentially using the carbon in those wastes more efficiently. This is a worthwhile goal. However, the project is currently very unfocused. The project needs to rapidly (2-3 months) identify priority process streams, the main constituents of those, and determine which products might improve economics using a quick, high level TEA. The project should then appropriately focus on a few relevant organisms and have the most promise. The project should also be more closely coordinated with other aqueous effluent characterization/valorization projects funded by BETO.
- The investigators have made good progress on engineering organisms to metabolize some of the major organics in waste streams. Selection of target "upgraded molecules" will be critical. The volumes and values of potential products must be quickly assessed to define what success might look like.
- Probably doing what was funded but not answering the high level economics questions.

Focus on *ex-situ* CFP aqueous streams (NREL)

Aim to contribute to 2022 TC cost targets through value-added co-product

Pilot-scale pyrolysis/Davison circulating riser system



B.A. Black *et al.* in preparation

Detailed compositional analysis

Example of a partial table

g/kg	Fast Pyrolysis		Catalytic Fast Pyrolysis		
Compound	**FP_{NREL}**	**$SF5_{ISU}$**	**CFP_{NREL} *ex-situ***	**$CFP_{RTI\text{-}pine}$ *in-situ***	**$CFP_{RTI\text{-}oak}$ *in-situ***
Acetic acid	30.60	104.86	1.97	19.92	2.84
Formic acid	12.51	55.22	ND	6.26	ND
Butanoic acid	0.71	1.50	ND	0.64	0.11
But-2-enoic acid	0.20	0.90	ND	0.48	ND
Propanoic acid	4.07	3.11	0.51	2.10	5.68
Prop-2-enoic acid	0.19	6.86	ND	0.66	ND
Pentanoic acid	0.03	0.10	ND	0.07	ND
2-Methylidenebutanedioic acid	7.38	6.52	ND	0.73	ND
2-Hydroxyacetic acid	T	3.76	ND	4.18	0.15
Acetaldehyde	ND	3.99	ND	14.71	3.94
2-Hydroxyacetaldehyde	56.10	47.07	ND	2.72	0.29
But-2-enal	0.05	4.01	ND	5.77	0.23
2-Methylbut-2-enal	0.02	0.05	ND	0.07	0.09
Furan-2-carbaldehyde	1.92	9.79	ND	6.11	1.15
Furan-3-carbaldehyde	0.01	0.26	ND	0.19	0.08
5-Methylfuran-2-carbaldehyde	0.21	0.96	ND	2.41	0.39
5-(Hydroxymethyl)-2-furancarbaldehyde	0.28	0.49	0.18	0.22	0.14
D-Xylose	7.48	T	ND	ND	ND
D-Glucose	1.90	ND	ND	ND	ND
D-Galactose	1.43	ND	ND	ND	ND
D-Cellobiose	T	ND	ND	ND	ND
1,6-Anhydro-β-D-glucopyranose	45.39	3.37	ND	ND	ND
1,4:3,6-Dianhydro-β-D-glucopyranose	8.12	T	ND	ND	ND
1,6-Anhydro-β-D-cellobiose	4.92	ND	ND	ND	ND

T = Trace; ND = Not detected

200+ compounds identified

100+ compounds quantified