NREL
NATIONAL RENEWABLE ENERGY LABORATORY





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

Biochemical Process Modeling and Simulation (BPMS)

Michael Crowley
National Renewable Energy Laboratory (NREL)

Tuesday, March 7, 2017, 1 p.m.–1:30 p.m.
Conversion

Goal Statement

Reduce research time and cost, **increasing efficiency,** using **theory, modeling, and simulation** to examine experimentally inaccessible solution space for upgrading and deconstruction.

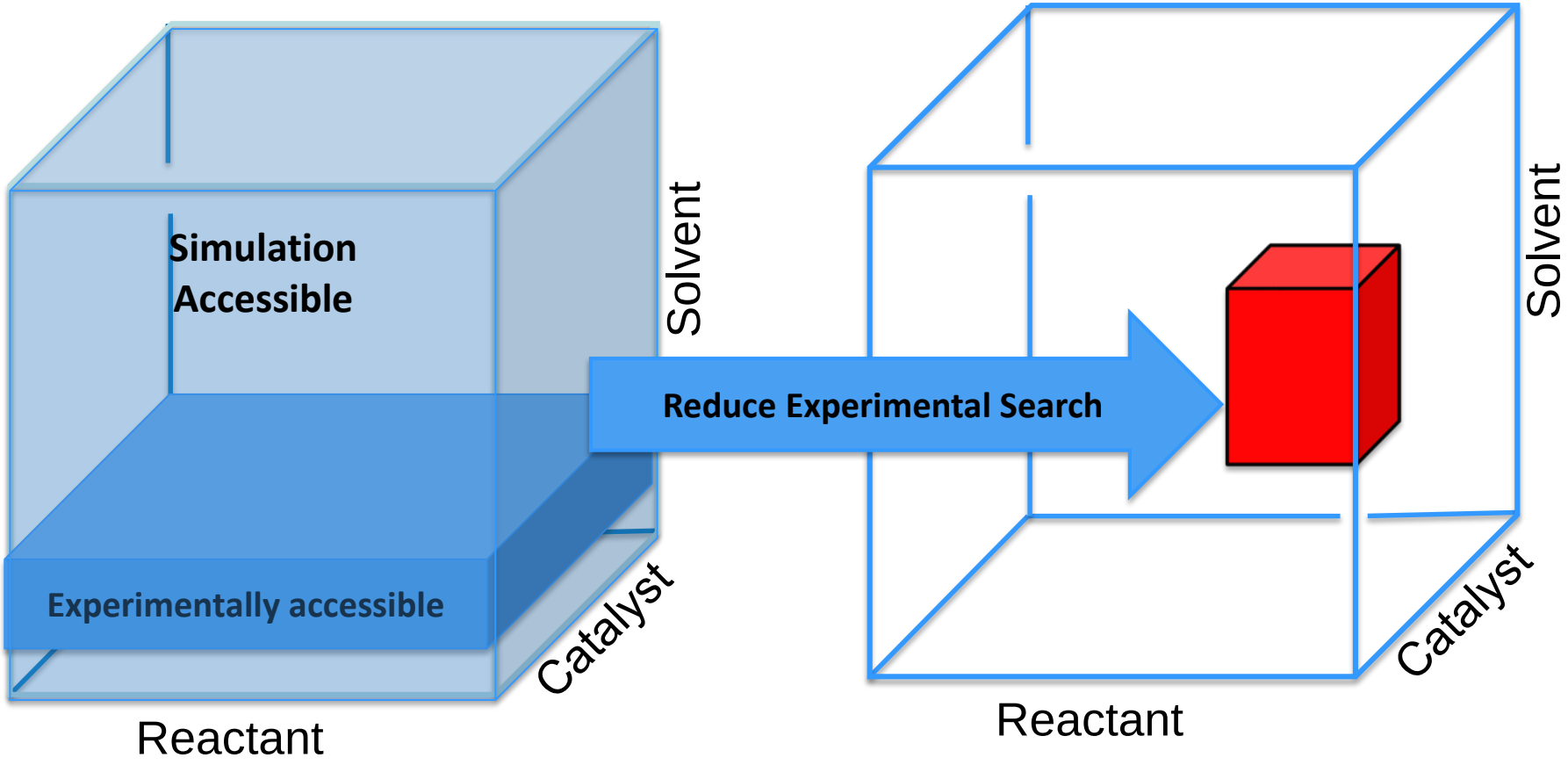
Outcomes:

- Greater productivity in fuels and products leading to reaching the 2022 $3 gasoline gallon equivalent (GGE) target
- More accurate techno-economic analysis (TEA) models for aerobic reactors
- Better carbon efficiency in conversion.

Relevance: Enable down-selection of the best pathways from the current four in time for the 2022 demonstration.

Modeling Relevance

Example of how modeling reduces experimental work and time.
Solution space is too big for experiment but accessible by modeling.

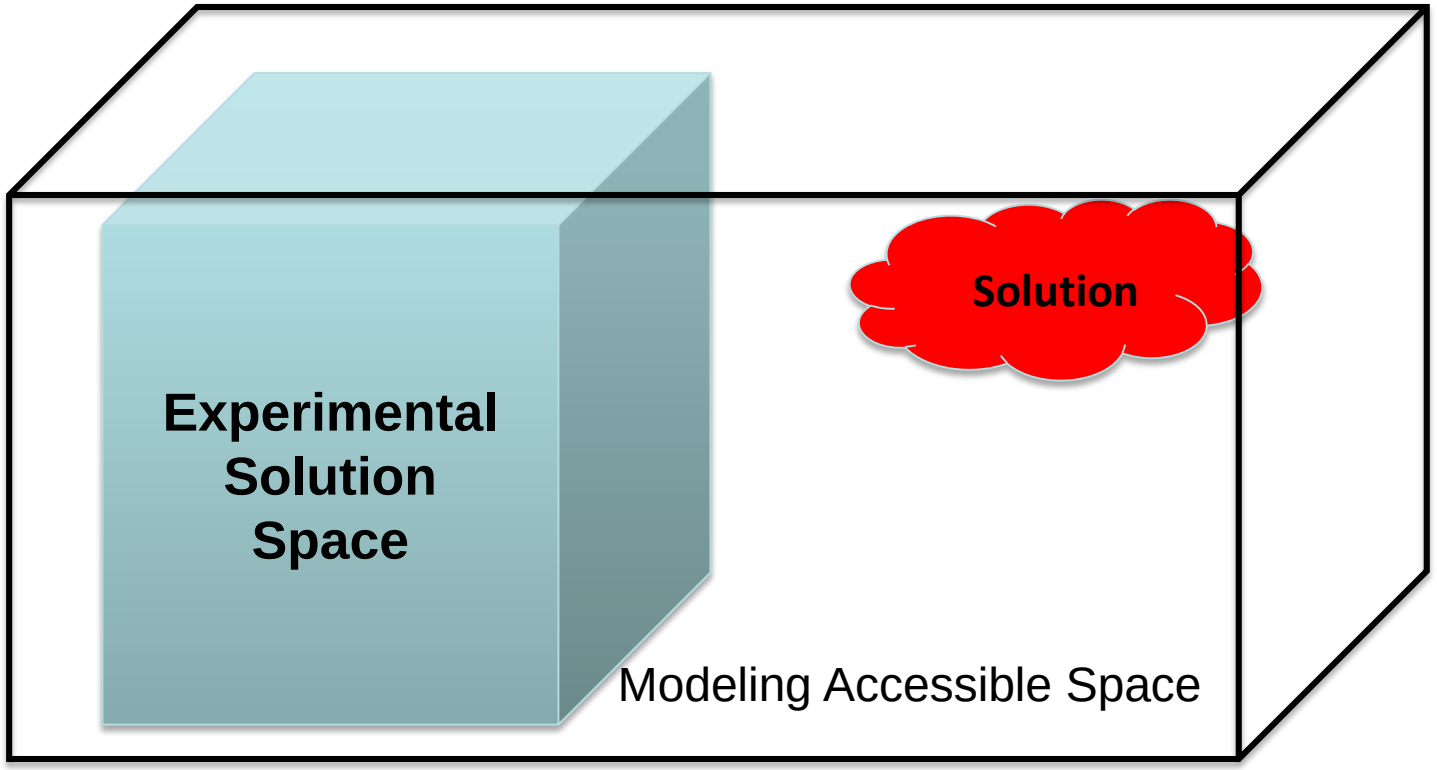


Example: Muconate upgrading by diels-alder reaction

Figure Credit: Mike Himmel, vision for theory/model and experiment synergy

Modeling Relevance

Modeling can find solutions unavailable to standard experimental search.

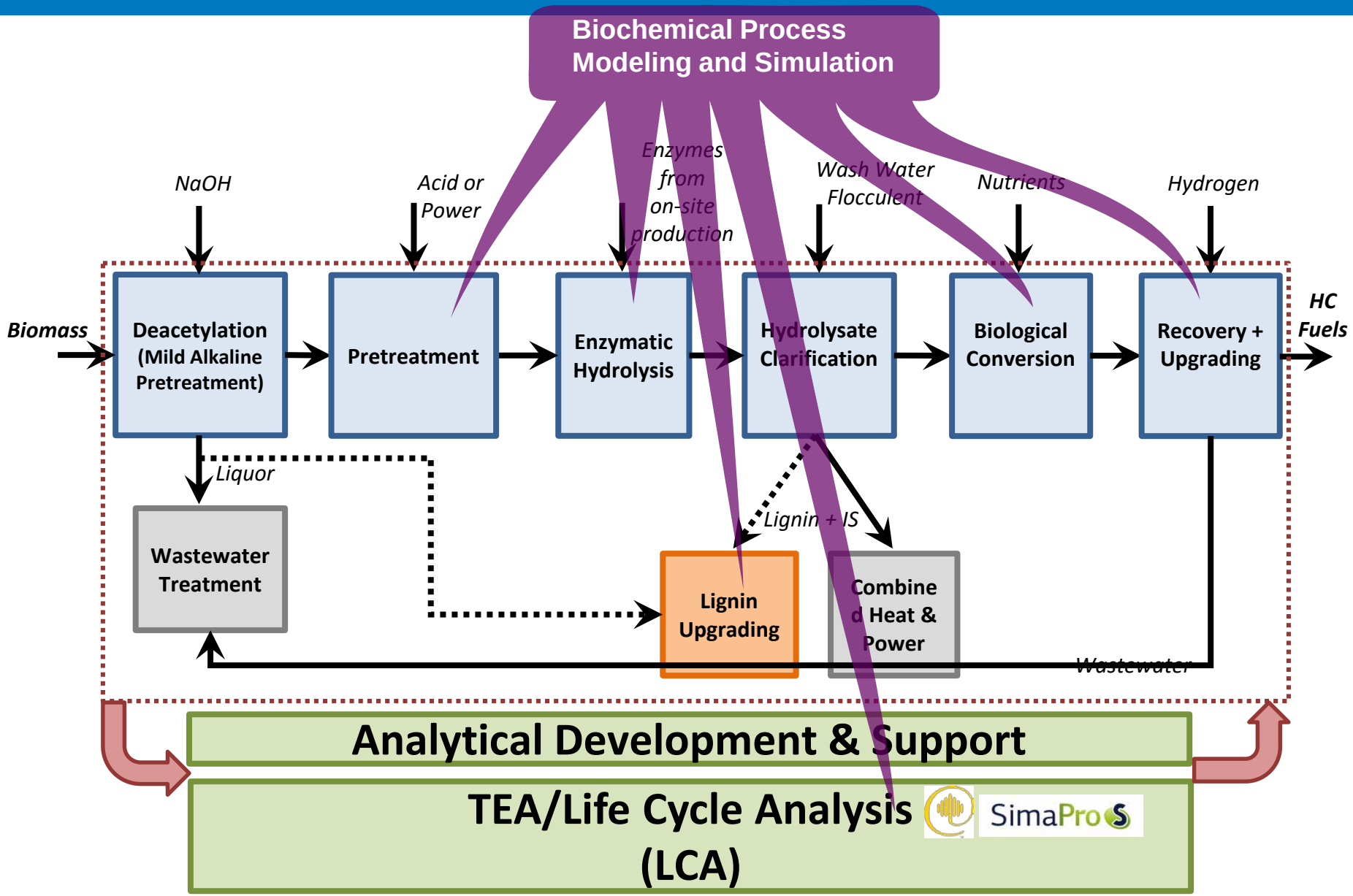


Examples:

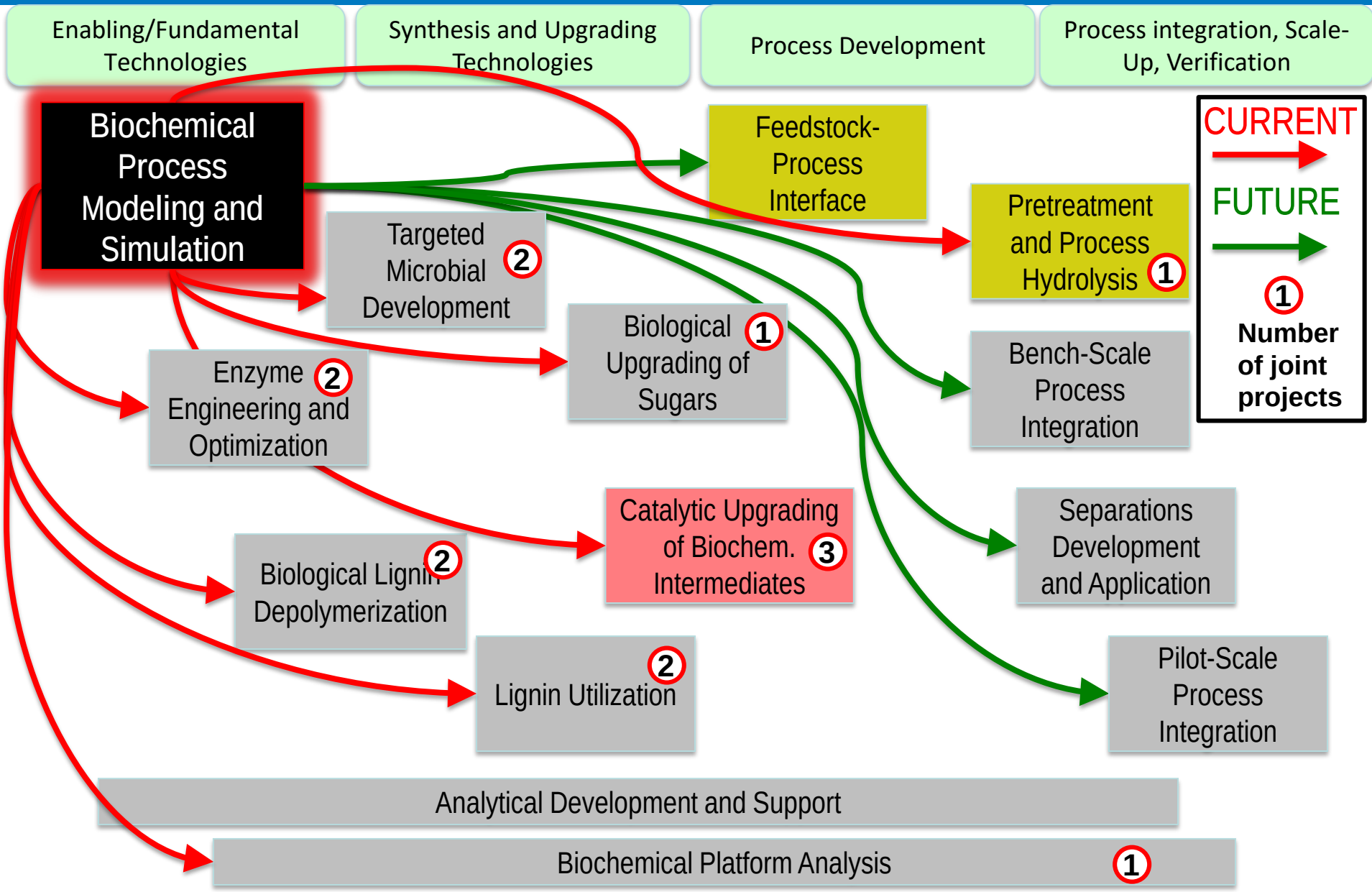
- Mutations/knockouts believed to be fatal to microbe
- Testing reactor designs at Industrial scale
- Exploring triple mutants.

Figure Credit: Mike Himmel, vision for theory/model and experiment synergy

BPMS Contribution to Process



BPMS Joint Projects



Quad Chart Overview

Timeline

Project Start: October 1, 2015
Project End: September 30, 2018
Percent Complete: 40%

Barriers

- Ct-D. Efficient **Pretreatment Process and Reactor Design**
- Ct-E. Efficient Low Temperature **Deconstruction Hydrolytic enzyme improvement, Hydrolysis Models**
- Ct-H. Efficient Catalytic **Upgrading** of Sugars/Aromatics, Gaseous and Bio-Oil Intermediates to Fuels and Chemicals **Catalyst Design, Reaction Mechanism, Metabolic Pathway Design, Aerobic Reactor Modeling, Microbe Enzyme Design**

Budget

	DOE
Total Costs FY12–FY14	$1.5 M FY14
FY15 Costs	$1.5 M
FY16 Costs	$1.5 M
Total Planned Funding (FY17–FY18)	$3.0 M

Partners

Subcontractors
UC San Diego 3%, U South Florida 3%, DWH Process Consulting 3%

Internal Partners
2.3.4.100 Lignin Utilization (LU)
2.5.4.100 Enzyme Engineering and Optimization (EEO)
2.3.2.105 Biological Upgrading of Sugars (BUS)
2.4.3.102 Targeted Microbial Development (TMD)
2.3.2.301 Biological Pyrolysis Oil Upgrading (BPOU)
2.3.2.104 Synthetic Metabolic Pathways for Bioconversion of Lignin Derivatives to Biofuels (SMPBLDB) **at ORNL.**
2.2.3.100 Pretreatment and Process Hydrolysis
2.1.0.100 Biochemical Platform Analysis

Other interactions/collaborations

NSF XSEDE Computers	U Portsmouth, UK	Purdue U
U. Kentucky	U. CO Boulder	Northeastern
ORNL	U. CO Denver	Penn State U
	U. South Florida	U. Michigan

1 – Project Overview

- **Improve hydrolytic and metabolic enzymes** through enzyme design
- **Design upgrading catalysts** and tune conditions for maximum productivity
- **Engineer and modify metabolic pathways;** increase yield, titer, and productivity
- **Determine best fermentation conditions**, media, gas sparging for microbes
- Predict best configurations and conditions for **industrial-scale reactors**
- Provide **reliable models for TEA** analysis where data or models are inadequate.

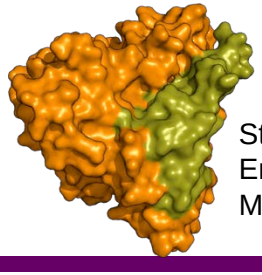
- ***Task 1 Molecular Modeling*** uses quantum and molecular simulation approaches to predict and design enzymes and catalysts for upgrading and deconstruction. A huge number of mutations, mechanisms, and molecular structures are screened saving large numbers of experiments.
- ***Task 2 Metabolic Modeling and Pathway Engineering*** develops new metabolic models and new algorithms able to dynamically calculate flux to increase productivity and also improve these models to include thermodynamic parameters therefore expanding the field of use and validity of these models.
- ***Task 3 Mechanistic Process Modeling*** develops and uses high-fidelity models at the unit-operation scale for modeling and predicting microscopic to macroscopic processes.
  - First principles and phenomenological models that represent the coupled dynamics of mass transport and reaction kinetics
  - Models used to gain insight into process dynamics, enable accelerated process development with fewer experiments and lower projected process costs.

2 – Approach (Management)

Project: Biochemical Process Modeling and Simulation
M. Crowley

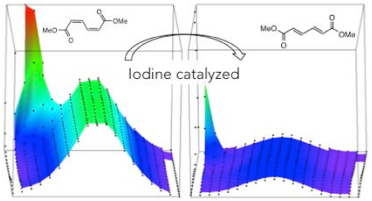
[Task 1] Molecular Modeling
M. Crowley

Molecular dynamics
Quantum mechanics



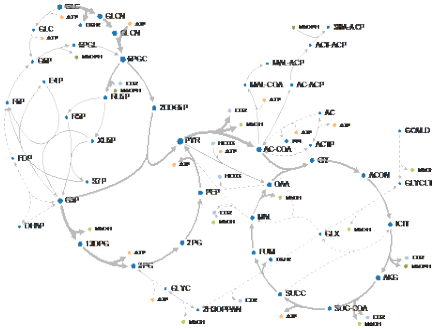
Structure/function
Enzyme design
Molecular processes

QM approaches to upgrading chemistry and catalysis0



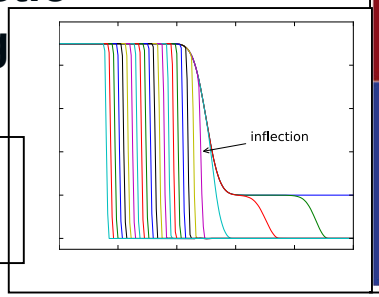
[Task 2] Metabolic Modeling and Pathway Engineering
Y. Bomble

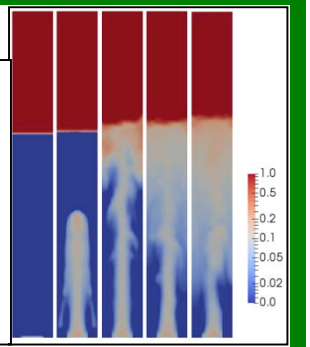
Metabolic models
Metabolic pathway
Flux analysis
Kinetic modeling
Sequence analysis
Rosetta design



[Task 3] Mechanistic Process Modeling
J. Stickel

Reaction-diffusion Modeling
Coupled CFD/Rxn-diffusion
Multi-scale modeling





Project is split into tasks by modeling type and managed by person with appropriate expertise.

Task Managers responsible for:

- AOP, Milestones, quarterly reporting according to the guidance of BETO.
- Communication with other projects
- Tracking go/no-go activities
- Budget management.

2 – Approach (Technical)

- **Approach:**
  - **Complement experiment and design** with **theory, simulation and modeling.**
  - Leverage EERE computer resource: **Peregrine (NREL)**
  - **Strong communication** between experimental and modeling efforts
  - Target most **relevant bottlenecks** in processes
  - Use molecular, metabolic/cellular, and macroscopic siimulation
  - **Go/no-go decisions** to stop ineffective approaches, replace with new approaches that will deliver needed insight in time for 2022 targets.
- **Objective:**
  - Gain **insight**, find new approaches and solutions
  - **Guide experiment and design**, select most promising directions
  - **Increase efficiency.**
- **Success Factors:**
  - Insights achieved, solutions found, unproductive efforts avoided
  - Reduced time to solution: increasing titer, efficiency, speed
  - New routes to advanced fuels and co-products.
- **Challenges:**
  - Software and methods need to be developed to meet the questions and necessary speed for timely answers (MD, CFD, QM/MM, FE, analysis)
  - Local computer hardware needs to stay at state-of-the-art.

3 – Progress—Muconate Production (metabolic)

Increasing production of Muconate from non-lignin source (sugar)

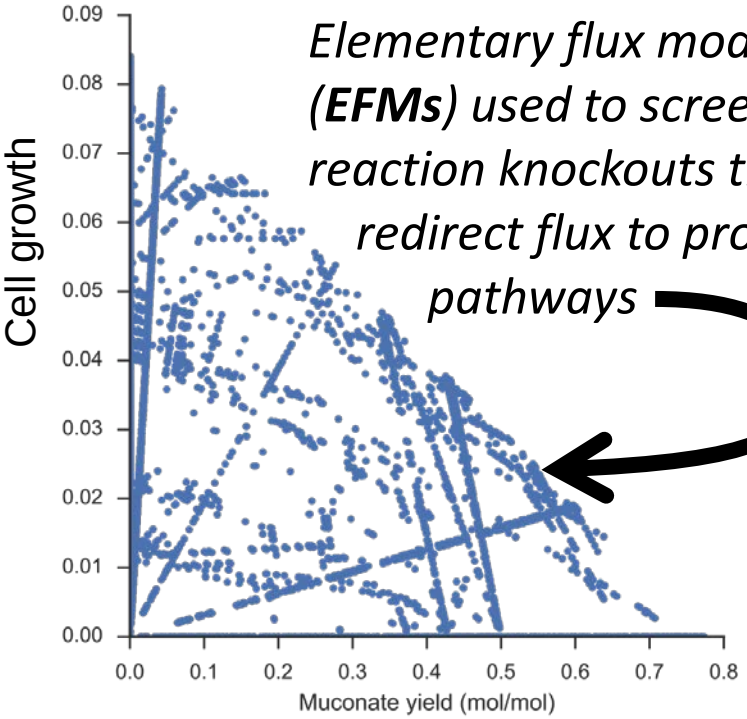
- Initial engineering approaches gave **muconate yields ~4-5%**
- Developed new model of central carbon metabolism in *P. putida.*

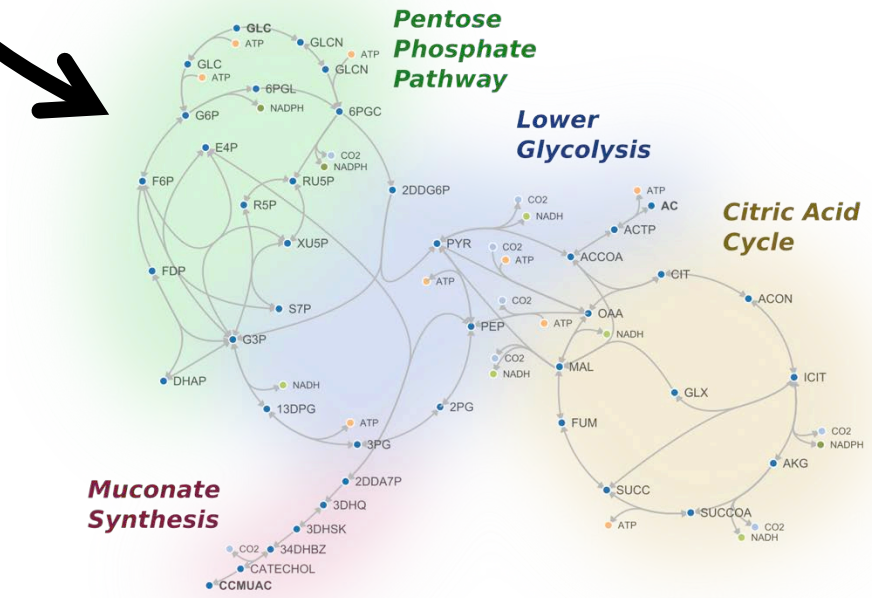
RELEVANCE: Enable muconate from non-lignin sources (sugar) → NECESSARY (TEA) for $5/gge routes (fatty acid)

Increase muconate production 10-fold

Relevant to other non-lignin sources

*Elementary flux modes (**EFMs**) used to screen for reaction knockouts that redirect flux to product pathways*

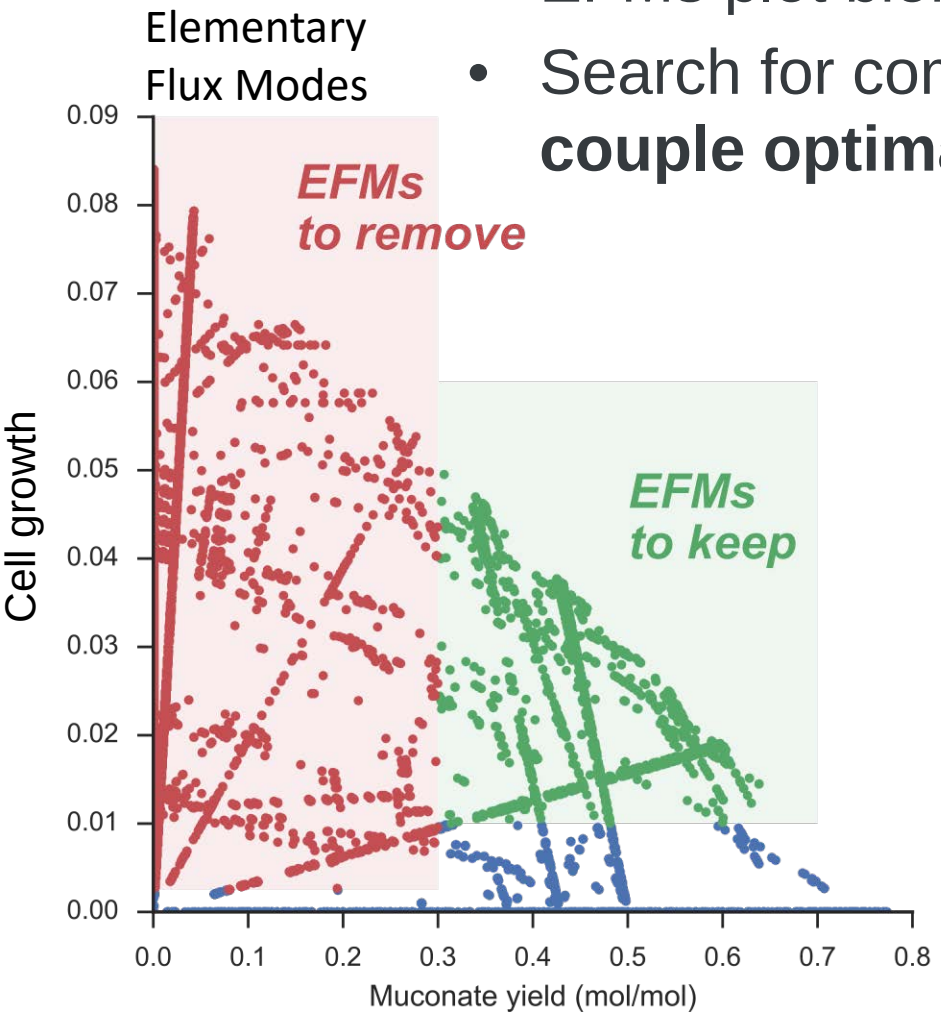


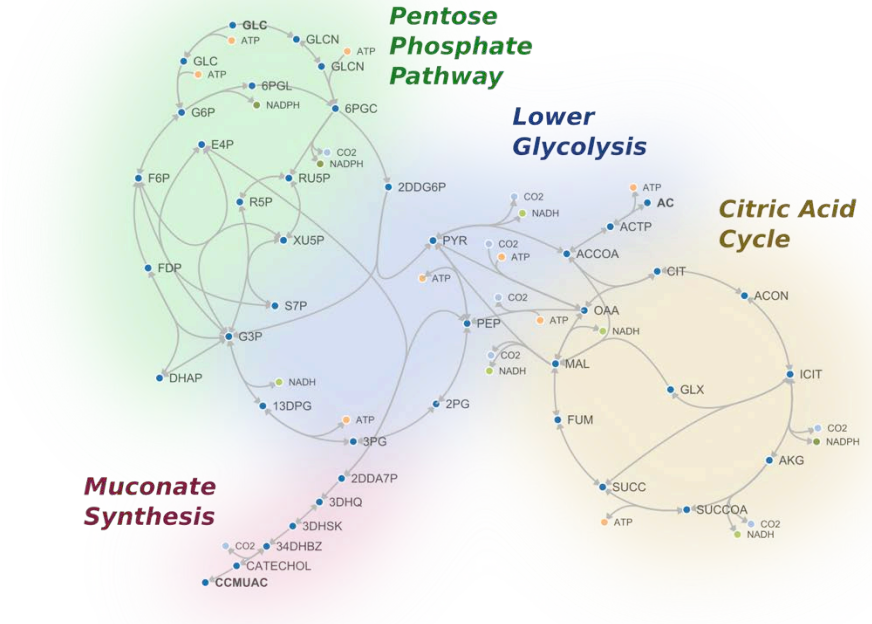


3 – Progress—Muconate Production (metabolic)

Increasing production of Muconate from non-lignin sources

- EFMs plot biomass yield vs. product yield.
- Search for combinations of knockouts to **couple optimal growth to product formation**.



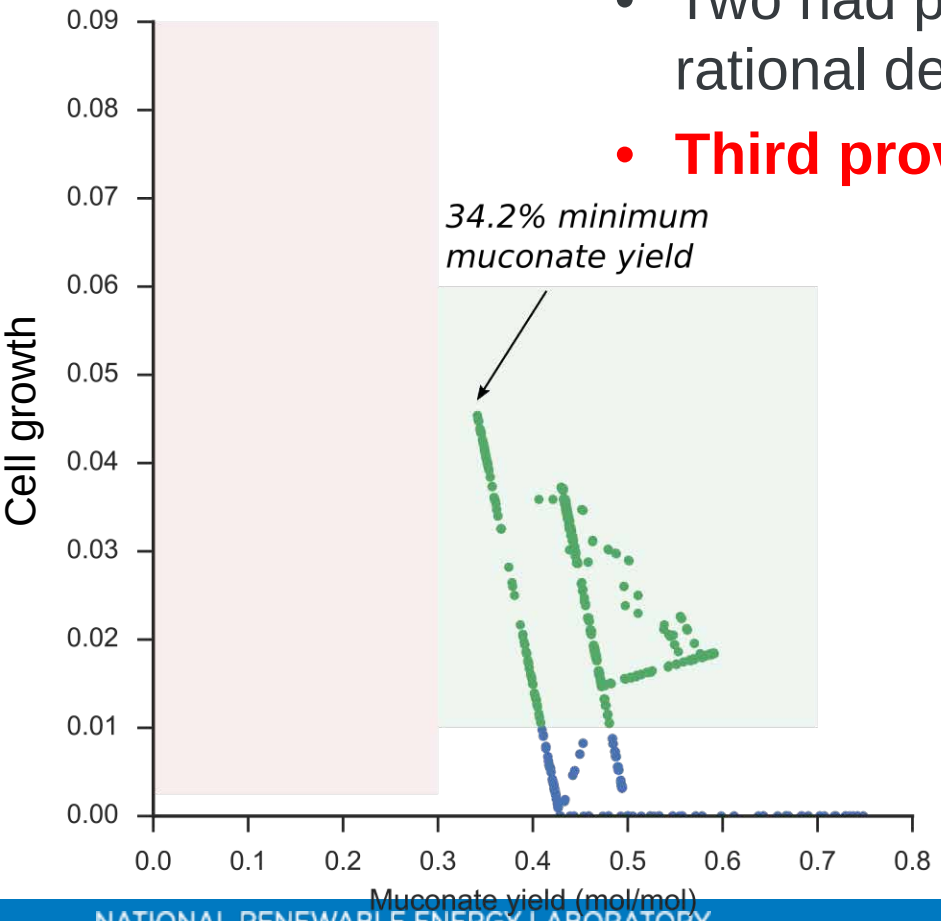


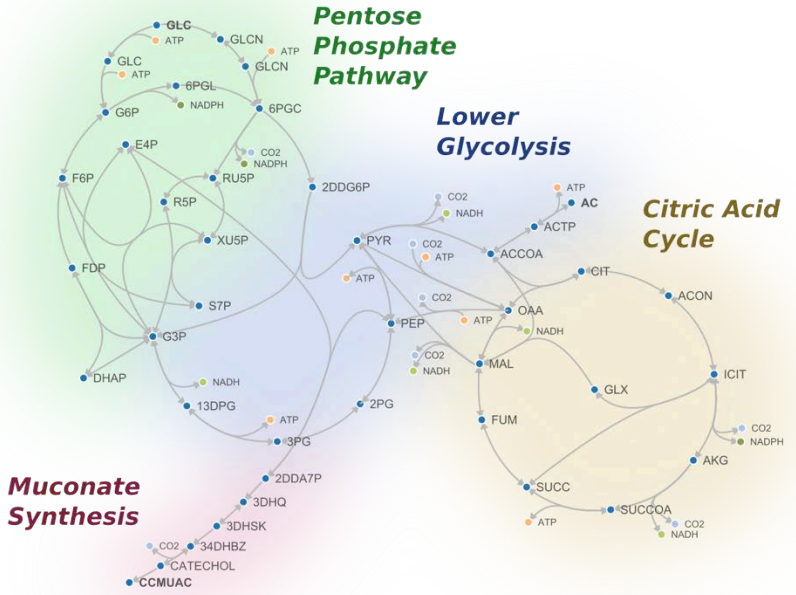
3 – Progress – Muconate Production (metabolic)

Increasing production of Muconate from non-lignin sources

Three knockouts were revealed for optimal muconate production

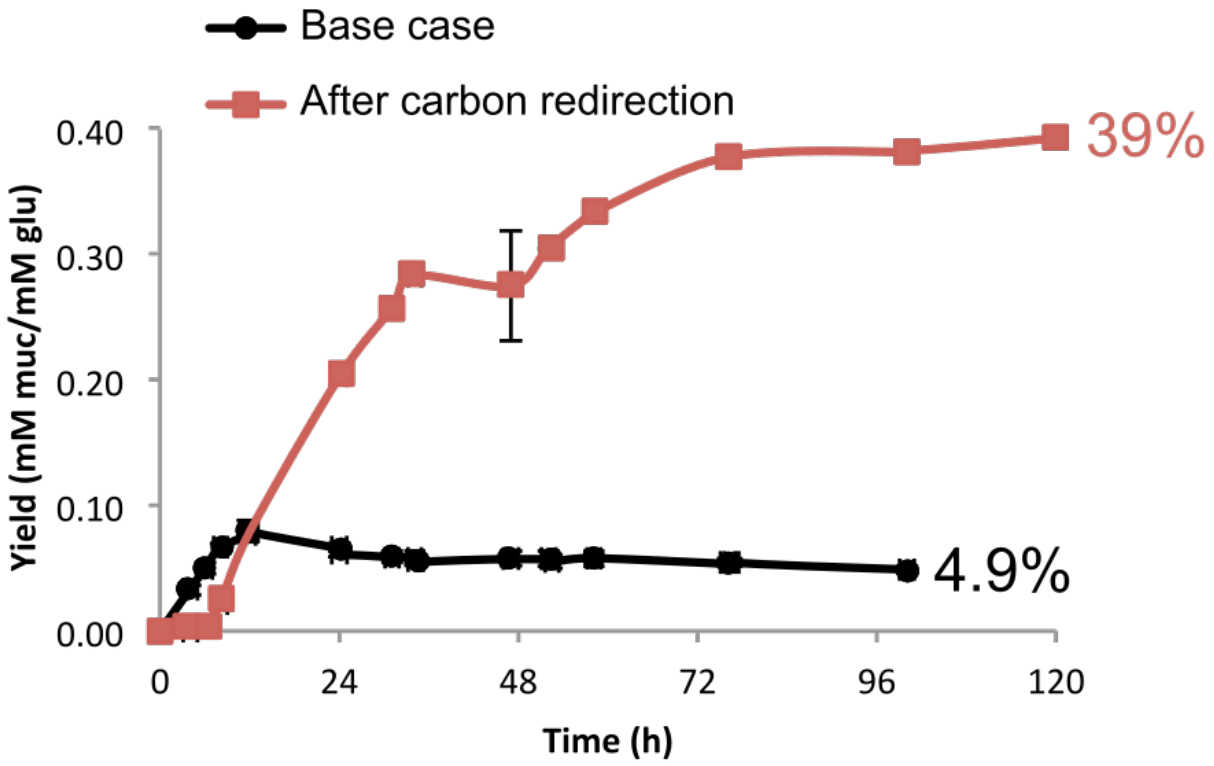
- Two had previously been removed through rational design by experimentalists
- **Third proved to be crucial**





3 – Progress—Muconate Production (metabolic)

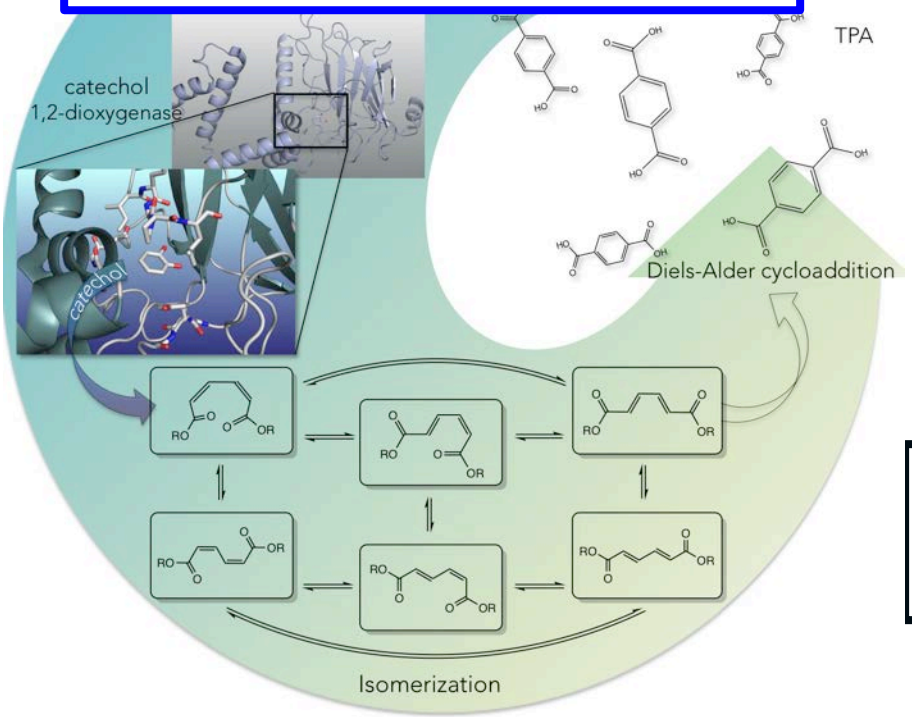
Computational design reached nearly 10x improvement in yield over the top-producing rational strain design.



Experimental work led and conducted by Chris Johnson & Gregg Beckham as part of the work impacting both the Agile BioFoundry and Lignin Utilization projects

3 – Progress Muconate Conversion (step 1)

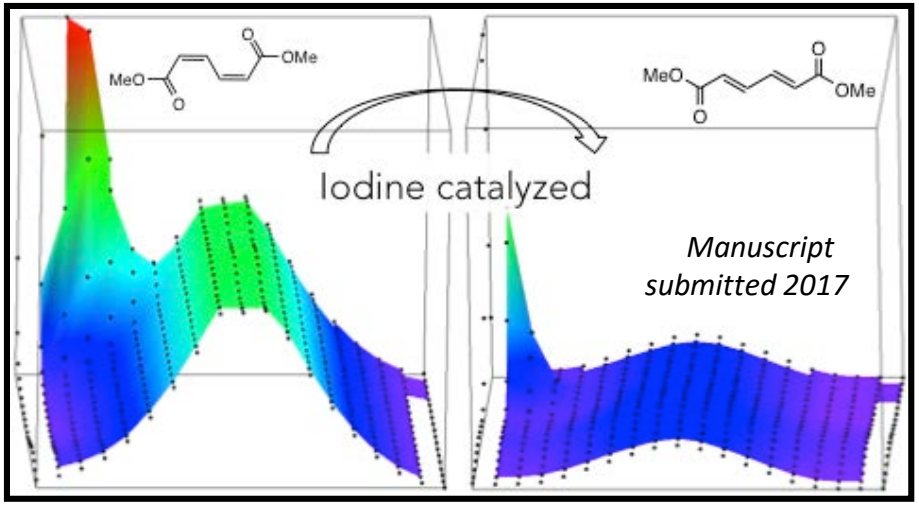
Muconate Conversion



RELEVANCE: Enabling valuable product terephthalic acid → large polymer market
SOLVED Isomerization Problem—improvement from 10% to 90% conversion to trans-trans

Key Results:

- Conditions for efficient catalysis with iodine radicals
- novel parallel pathway mechanism
- feedback to experiments → achieve full conversion to *trans,trans* muconate
  - catalyst (re)activation with UV.



- Recent advances enable large production of muconate
- **Upgrading Step 1:** isomerize *cis,cis* muconate to reach the *trans,trans* muconate isomer
- **Collaboration with 2.3.4.100 Lignin Utilization**
  - experimental hurdles encountered
    - cis,trans → trans,trans challenge
    - non-standard conversion rates.

3 – Progress Muconate Conversion (step 2)

Muconate Conversion

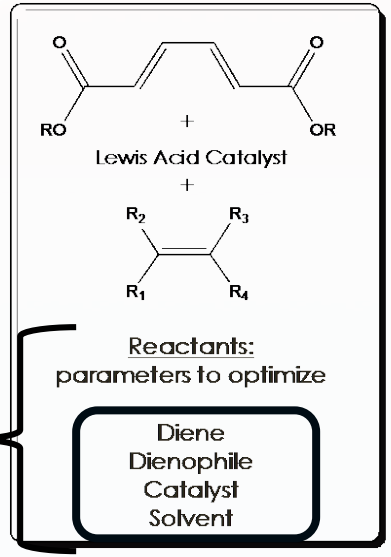
Upgrading Step 2: Catalyzed Diels-Alder cycloaddition with *trans-trans* muconate

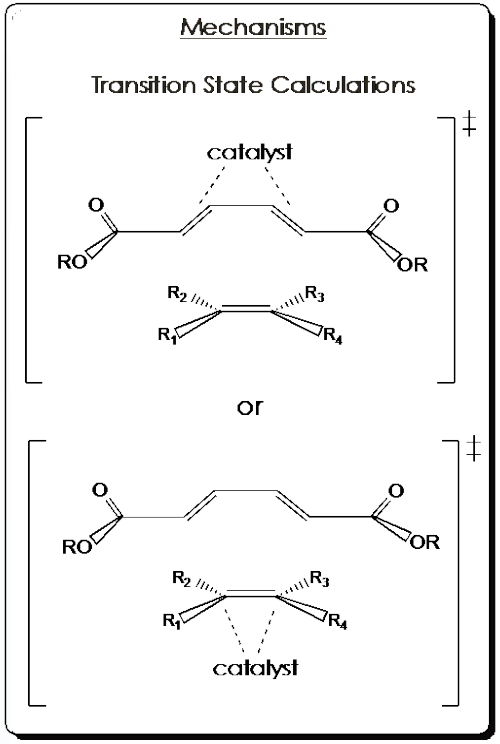
- High cost & time to optimize many variables via experiments
- Calculations inexpensively & extensively scan reaction parameters and mechanism.

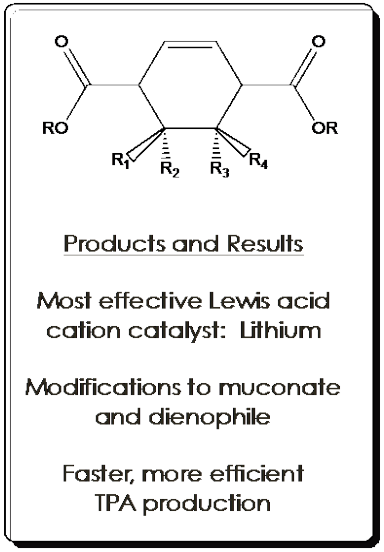
RELEVANCE:

- **Enabling lignin upgrading to TPA**
- **Vastly reduce parameter space for experimental investigation**
- **From 1,000s to 10s of combinations.**

Finding the best process from1,000s of possible combinations **Narrowed down to 10s**







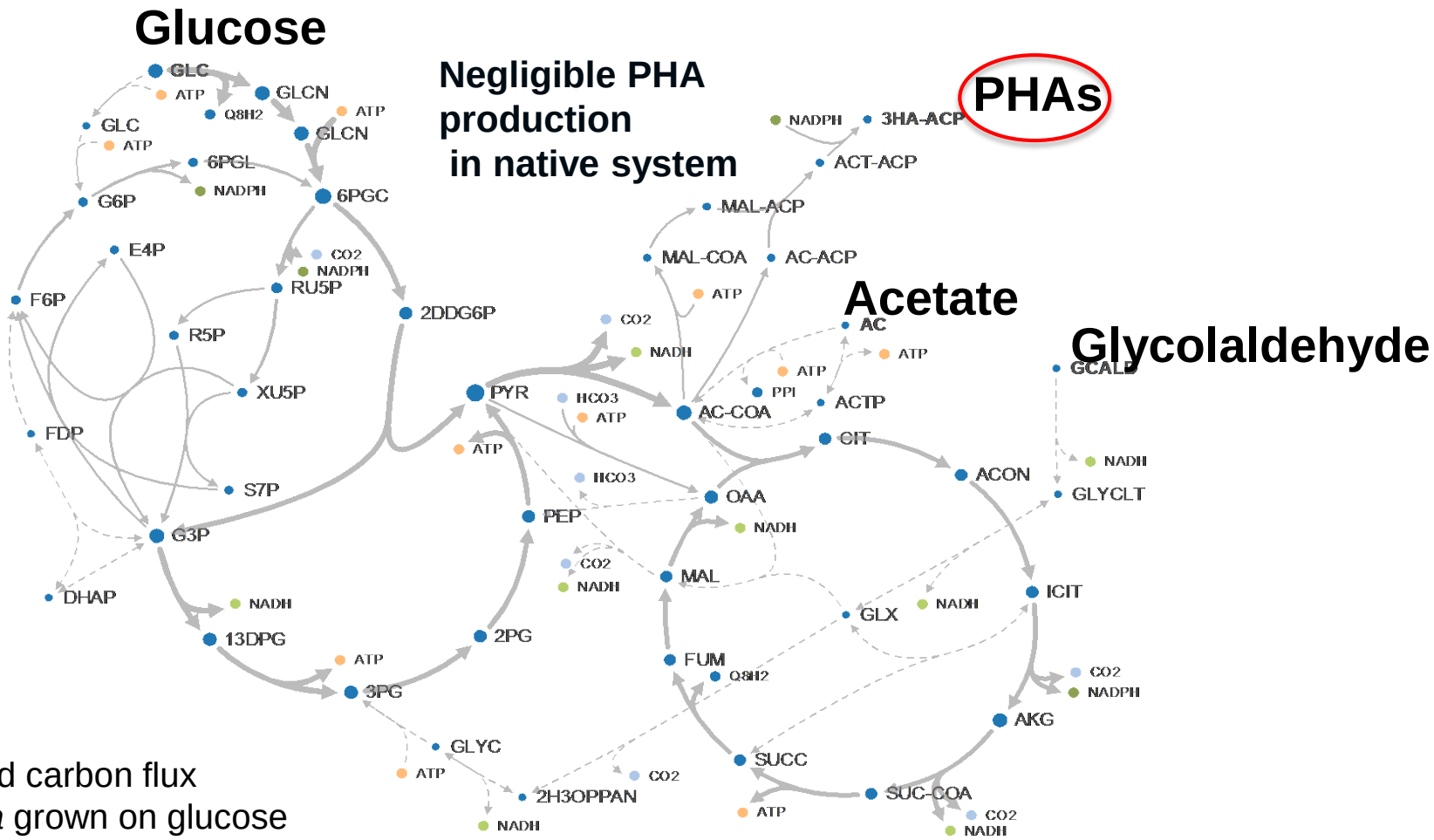
Project output

- Cost-effective improvements to TPA production from lignocellulosic biomass
- Integral to cost-effective technical advances in laboratory experiments.

3 – Progress – Metabolic Design – Upgrading

Pyrolysis aqueous waste stream upgrading

RELEVANCE: Increasing carbon utilization, production of high-value product, prevent toxin buildup

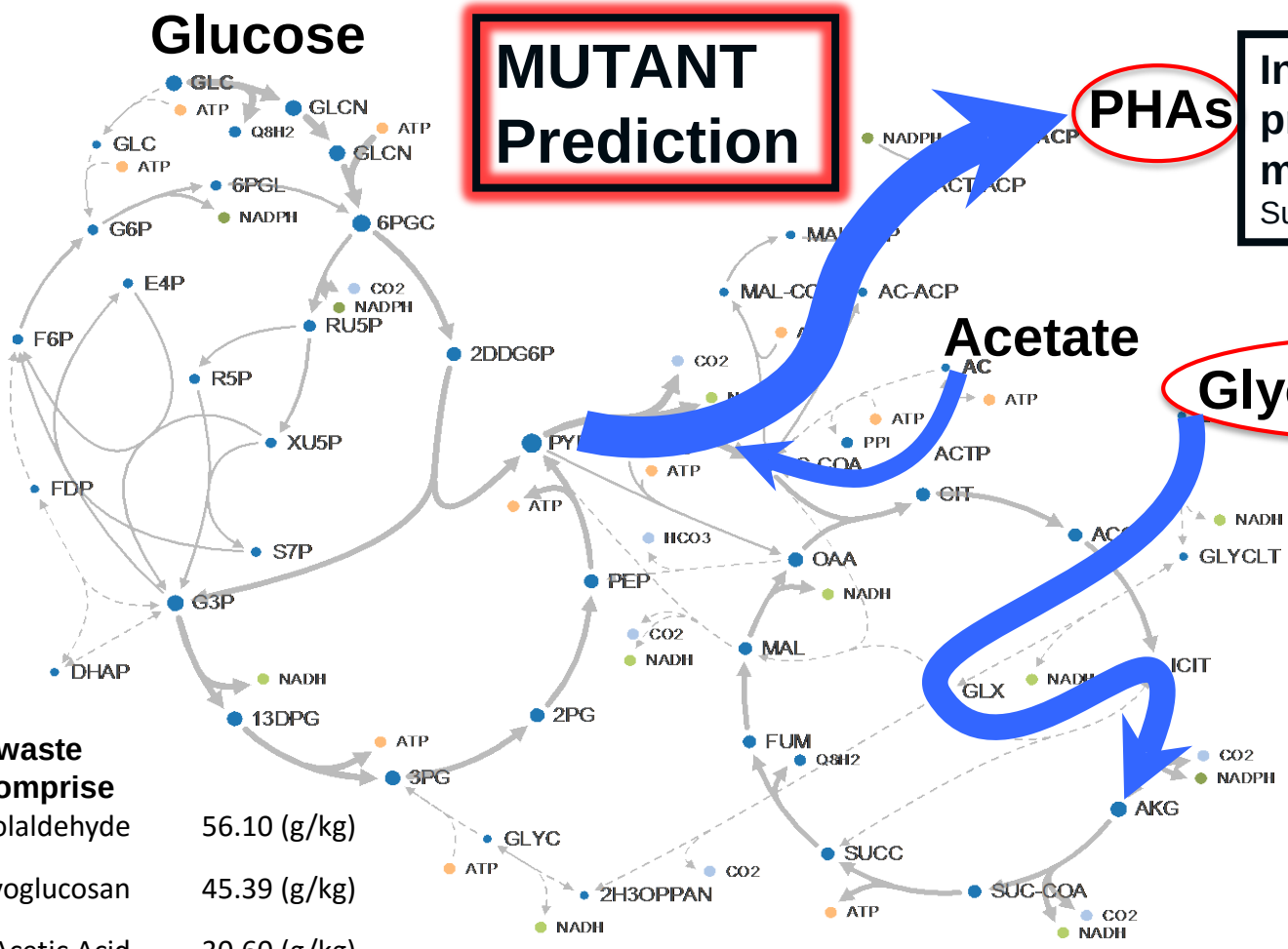


Expected carbon flux
P. putida grown on glucose

3 – Progress – Metabolic Design – Upgrading

Pyrolysis aqueous waste stream upgrading

RELEVANCE: Increasing carbon utilization, production of high-value product, prevent toxin buildup



Increased PHAs production in the mutant

Sugars go to producing PHAs

Forced consumption of glycolaldehyde

Toxic glycoaldehyde does not build up.
Glycoaldehyde goes to cell growth
Increased C utilization

Pyrolysis waste streams comprise

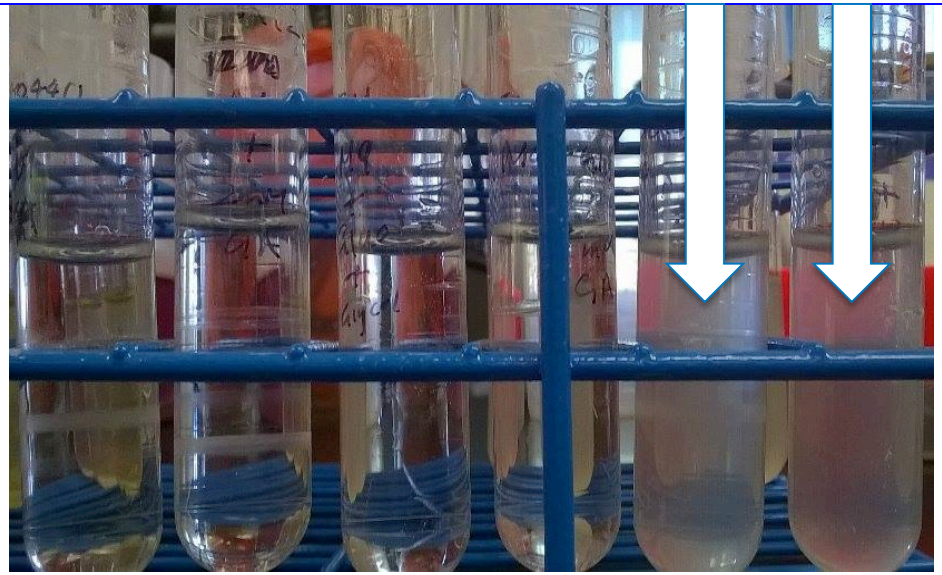
Glycolaldehyde	56.10 (g/kg)
Levoglucosan	45.39 (g/kg)
Acetic Acid	30.60 (g/kg)

3 – Progress—Metabolic Design Upgrading

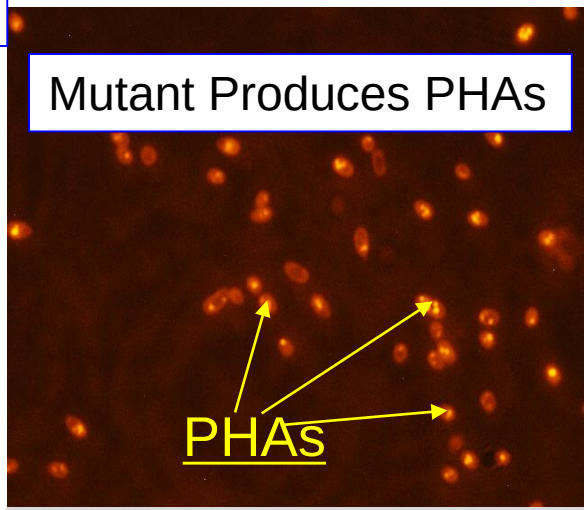
Strain design for pyrolysis waste stream upgrading

Experimental Confirmation

Mutant only grows with Glycolaldehyde and Glucose



Glucose	+	+	-	-	+	+
Glycolaldehyde	-	-	+	+	+	+



M9 + 1.5 g/L of acetic acid + 1. 2 g/L of glyoxylic acid

Experimental work led and conducted Lahiru Jayakody, Chris Johnson & Gregg Beckham in Biological Pyrolysis Oil Upgrading (2.3.2.301)

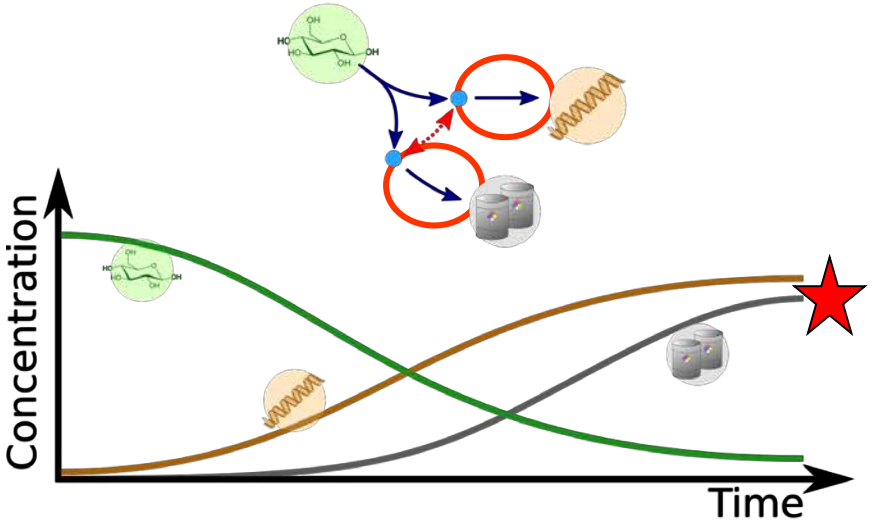
Subject of ROI 16-117: "Engineering the TCA Cycle in Pseudomonas Putida KT2440 to Produce PHAs from C2-carbon Sources" by Gregg Beckham, Lahiru Jayakody, Yannick Bomble, and Peter St. John.

3 – Progress—Dynamic Design

Maximum theoretical productivity

RELEVANCE: Higher yield and productivity in microbial upgrading

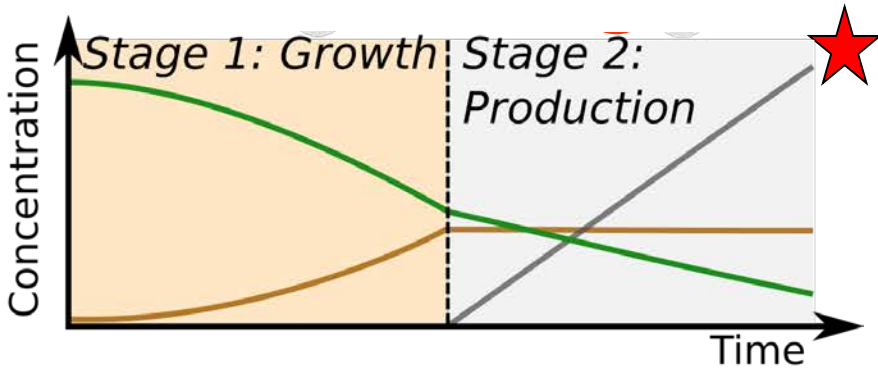
Static strain design



Typical knockout approaches must balance between growth and product production

1 stage of growth and production

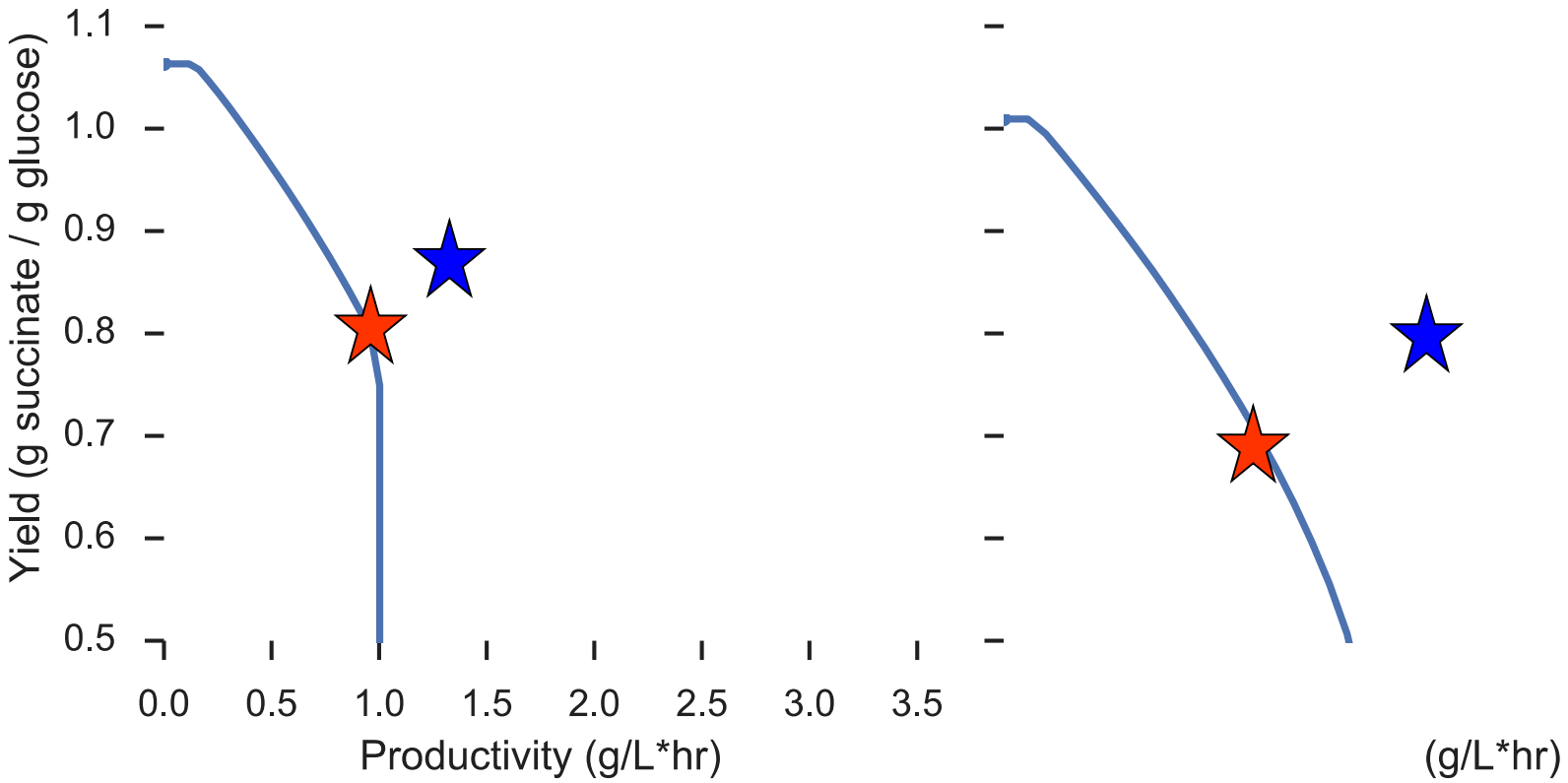
Dynamic strain design



Higher productivities are possible with dedicated cell growth and product formation stages

2 stages: growth then production

Maximum theoretical productivity



- Developed method to compute max productivity from dynamic strain designs
  - Uses assumptions on maximum growth and substrate uptake rates
- Productivity vs. yield surfaces show potential gains from experimental effort.

Peter St. John, Michael Crowley, & Yannick Bomble, *Biotech For Biofuels* 2016 (in press)

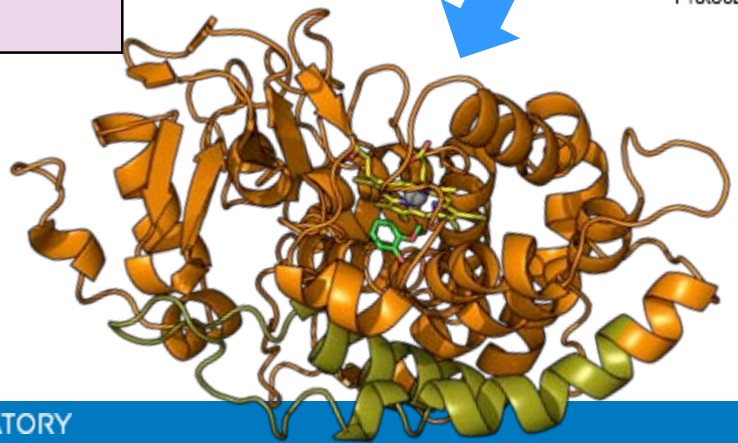
3 – Progress—Muconate Production(enzymes)

Upgrading Lignin Fragments → Value added by simulations

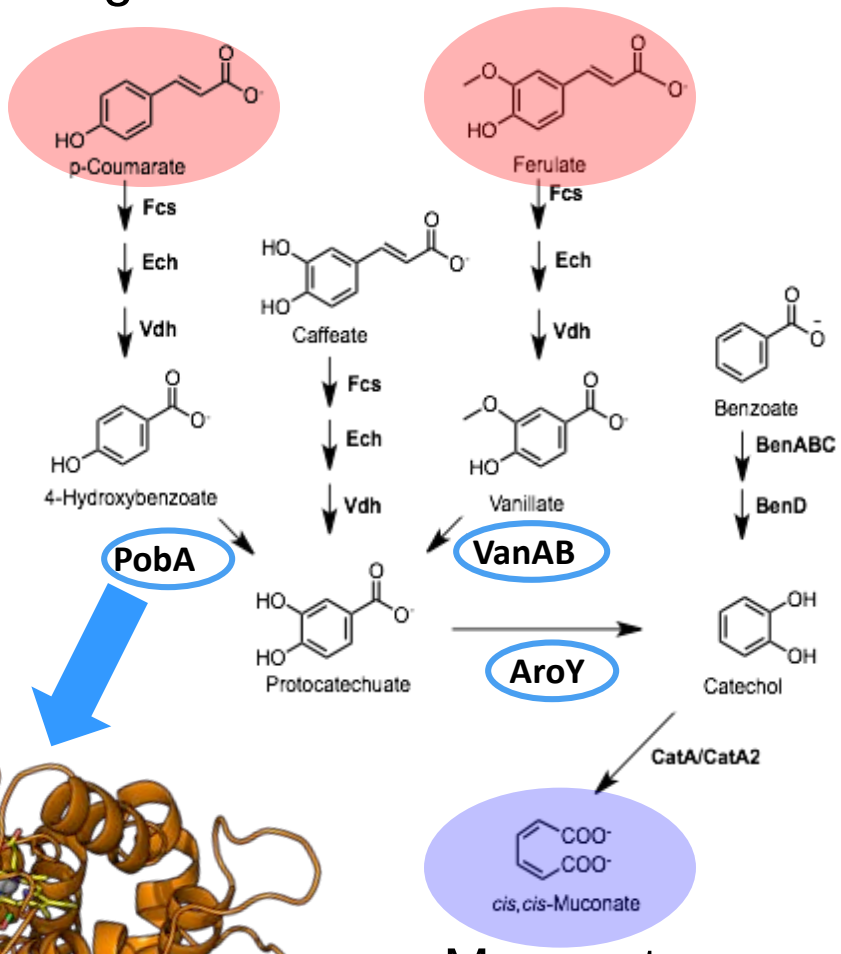
RELEVANCE: Enabling lignin upgrading to valuable products

- **Induced fit** mechanism
- How does substrate binding translate to loop closing?
- How does product expulsion translate to loop opening?
- What are the dynamical roles for the P450-conserved residues?
- **CAN THIS ENZYME CONVERT OTHER FRAGMENTS?**
- Simulations are constructed to answer these questions.

And there are a *host* of other products within the **biological funneling concept** that are future targets.
McGeehan group (UK) is currently targeting AroY for crystallization.



Lignin monomer reactants



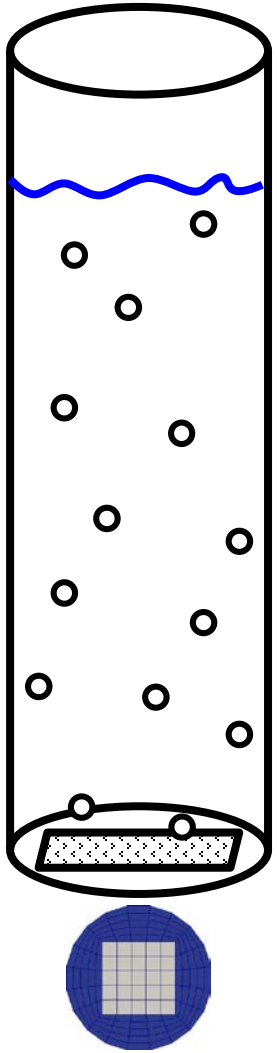
Muconate Product

3 – Progress – Aerobic Reactor Design

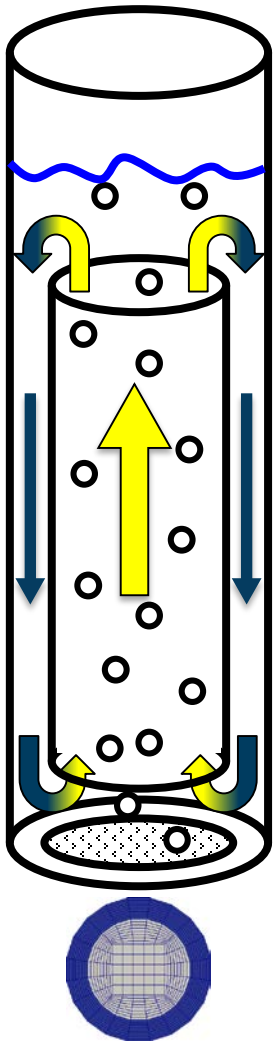
Aerobic Bioreactor CFD

RELEVANCE: Enable aerobic pathways and inform techno-economic analysis

- **Adapted OpenFOAM two-phase solver**
  - Gas-liquid mass transfer
  - Oxygen depletion in the liquid (mimicking microorganism metabolism)
- **Tested bubble-column reactor types**
  - Central inlet reactor
  - Draft-tube air-lift reactor
- **Simulations of aeration rates** needed to achieve specific oxygen-transfer rates—important to determine reactor costs
  - Commercial-scale reactors have improved OTR because higher head pressures increase O_2 saturation
  - CFD results confirmed empirical-engineering calculations for costs.



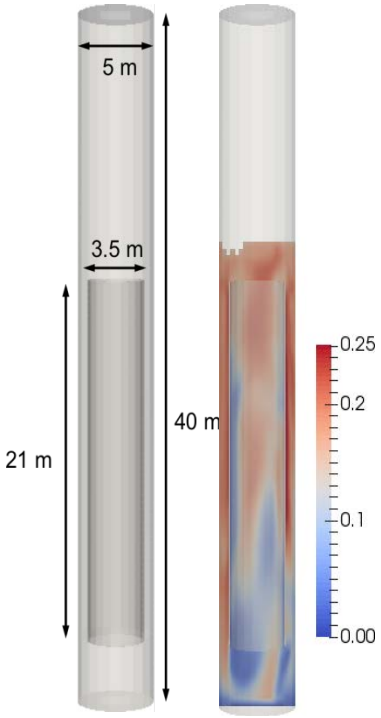
Central inlet reactor



Draft-tube air-lift reactor

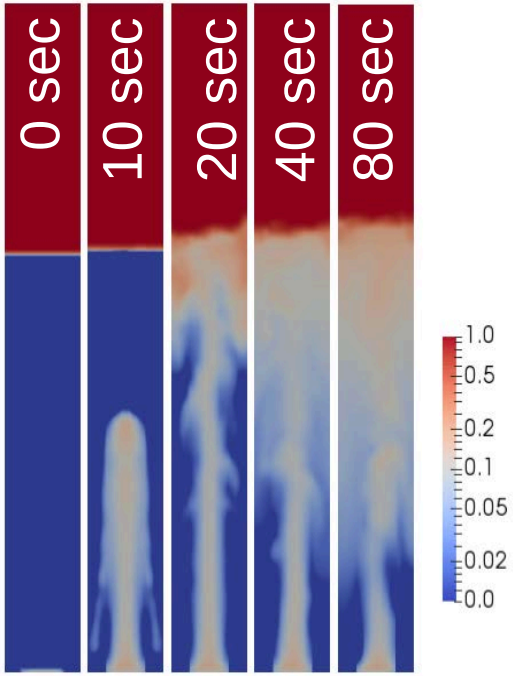
3 – Progress—Aerobic Reactor Design

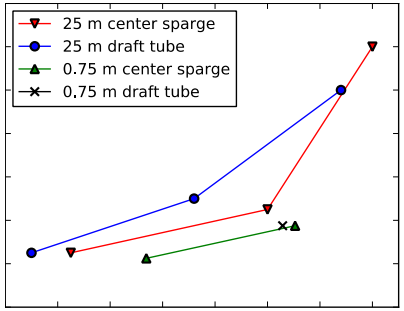
Aerobic Bioreactor CFD



Dynamic startup of aeration

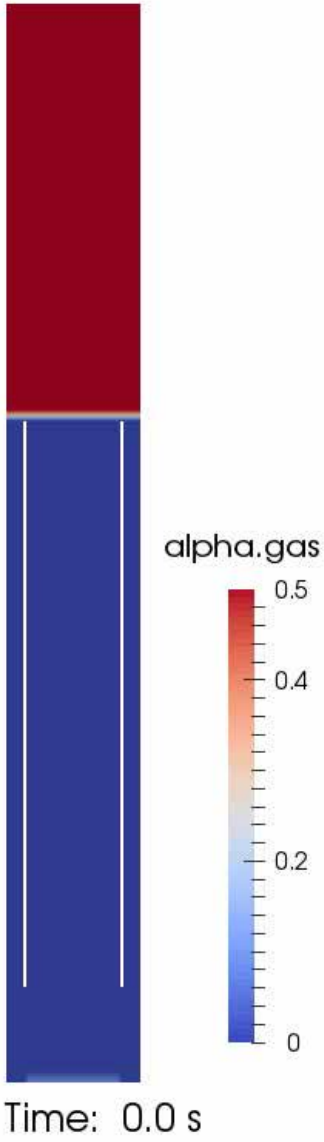
Air fraction is shown in a slice from 3D column.





Maximum oxygen transfer rate (OTR function of superficial gas velocity

- Gas velocity does not matter for s columns, matters a lot for industr
- Best OTR with **draft tube**
- Model informs process design.



3 – Progress—Completed

- Muconate Isomerization
- Lignin structure and depolymerization
- New catalysts for muconate upgrading
- Decarboxylase mechanism and prediction of mutations for selectivity (FY15 milestone met)
- Thioesterase mechanism and selectivity study for lipid chain length selectivity (FY16 milestone met)
- *T. reesei* metabolic model completed (milestone achieved)
- *A. succinogenes* complete metabolic model (milestone completed)
- Optimize succinate production from hydrolysate in *A. succinogenes*
- Develop a dynamic approach to maximize productivity of batch processes with microbes
- Bubble Column Reactor Model designed, implemented, and tested (go/no-go milestone achieved with go determination)
- Enzymatic hydrolysis model (milestone achieved)

3 – Progress—Work In Progress

- Lignin engineering
- **Lignin bond energy determinations (Lignin Utilization)**
- Chain termination
- Cellulose bond energetics
- Expansin-GH45
- ***P.fun-T.reesei* domain swaps (Enzyme Engineering and Optimization)**
- Cel7A linker glycosylation (EEO)
- PDC tuning (Targeted Microbial Development)
- **BDO pathway design for eliminating need for oxygen by developing strategies for more efficient cofactor recycling**
- Maximum productivity analysis

3 – Progress—Work In Progress (cont.)

- Codon Harmonization for higher success with heterologous expression, upcoming **go/no-go with enzyme engineering and optimization (EEO)**
- Pyrolysis waste stream utilization
- *P. putida* metabolic model refinement
- **FY17 milestone:** Construct a core carbon model of *Clostridium butyricum* to maximize carboxylate production of from glucose, xylose, arabinose, and galactose
- **FY17 Milestone:** Model lignin-derived aromatic compounds conversion to muconic acid and mixed medium chain length alcohols in *Pseudomas putida*
- Bubble column model enhancement and refinement
- Coupling aerobic reactor model with TEA

4 – Relevance

Reduce Cost of Research and Time-to-Solution

- Scanned 1000s of lignin bonding regimes to down-select to 10s for experimental examination, **enhance lignin deconstruction**.
- Explain mechanism of action for enhancing deconstruction enzymes, **reducing cost of hydrolysis** steps
- Reactor models predict outcomes outside of experiment, **lowering uncertainties of TEA**
- Reactor models quantify **viability of aerobic pathways**

Discover NEW solutions to bottlenecks

- Found solutions to muconate upgrading through iodine-catalyzed isomerization, **increasing yield of muconate derivatives, → $3/gge**
- Discovered knockout for **10-fold increase in muconate production from non-lignin sources → enable fatty acid pathway at $5/gge**

Provide NEW insights

- **TEA** enhanced by accurate models; can now accurately include many reactor design variables at full industrial scale
- Knockouts considered lethal provide **higher productivity**.

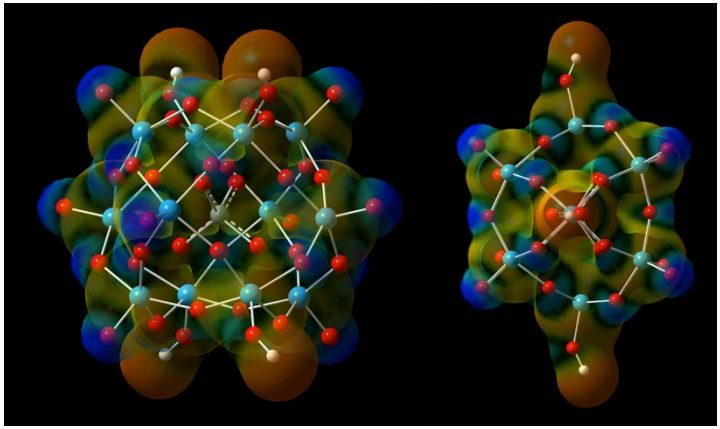
Technology Transfer

- Models are publicly available for industrial use (metabolic, reactor, lignin, cellulose)
- Return on investment, publications

5 – Future Work

- **Catalyst design, POM**, for enhanced lignin and sugar upgrading to existing fuel and chemical targets, and upgrading to new targets.
- **Lignin termination** linkage determinations (QM) for control of polymer length, enhancing utilization

Polyoxometalate (POM) Catalyst



- **Expansin and GH45** enzyme design.
- **Lignin-carbohydrate bonding** determinations (QM) for biomass design and process design for upgrading
- **Extraction solutions**: Lysis mechanisms, product transport across membranes
- **Optimize 2,3 butanediol fermentations** in *Zymomonas mobilis* by enzyme design, tuning specific enzyme performance to give experimental work a dial for altering product ratios. FY18 milestone.
- Determine knockout strategies and targeted products in *Pseudomas putida* for alternative streams of **waste pyrolysis oil upgrading**.
- Develop method for screening of **anaerobic fermentation pathways.**

5 – Future Work (continued)

- Methodology improvements in **metabolic ensemble modeling** (MEM)
  - MEM will enable the explicit modeling of enzyme kinetics to determine rate-limiting branch-points and enzymes.
  - Additional data needed to estimate kinetic parameters can be provided by cell-free pathway engineering and characterization.
- Model the introduction of **other pathways in *P. putida*** to produce new products with physical properties allowing for easier separations.
- Go/no-go FY17 Q3 milestone: Joint Milestone EEO 2.5.4.100: BPMS will design, implement, and optimize the software for **codon harmonization** and iterate on best codon optimization strategies.
- **Aerobic bioreactor CFD**: Evaluate Oxygen Transfer Rate with varying **liquid viscosity** and novel reactor designs.
- Include **lignin and xylan in enzymatic hydrolysis** (EH) model and validate with experiments. Use models in novel EH processes.
- Adapt previously developed pretreatment model to **state-of-the-art pretreatment technologies** and full-scale reactors.

Summary

Overview: Using state-of-the-art computational and modeling methods, find solutions to barriers and bottlenecks that are crucial to meeting BETO targets.

Approach: Very tight collaboration and iterative refinement between experimental projects and modeling/simulation. Three major tasks in molecular, metabolic, and process modeling. Leveraging EERE computer, Peregrine, for high productivity.

Accomplishments: New and innovative solutions for lignin and sugar upgrading increasing experimental productivity. Many examples of gains in efficiency, understanding, and new solutions.

Relevance: Significant contributions to co-product cost and value, reducing the cost of experimental research, fast and cheap down-selecting of experimental scans.

Future work: Increased catalyst design and condition tuning, increase scope of lignin and sugar upgrading chemistry and catalysis, more detailed design of reactor and process design to contribute to both the down-select of the four 2022 pathways and to focus on the final selection processes.

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Collaborators outside BETO
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Additional Slides

Responses to Previous Reviewers' Comments

Responses in blue

- This is a great project and significant progress has been made in this area, both in the scientific world and its application and in-house at NREL. Development and application of these models in-house at NREL are much better than simply collaborating with the many universities active in these areas. Because the applications are somewhat different at NREL, building an in-house expertise does just that; it builds up and gets better.
- Understanding the enzyme mechanism is relevant research and being able to **model and translate to the macro process world is huge.**
  The major effort of this project is to have impact on the most important bottlenecks which span from the molecular to the macro process. We agree with this comment and will continue to direct our efforts to having significant impact on all levels of the design and implementation of the process.
- It is great to see modeling and simulation as part of the portfolio. As possible, the project should consider **making available to the public versions** of the models at different stages. Continuous support of the models is of far less importance than availability, and much of **the industry needs a starting point for detailed R&D analysis (especially TEA)**.
  Besides solving barriers, we are committed to releasing all code developed and publishing all models for public and industrial use. We believe that our expertise and funded development is an asset to the industry and make all possible efforts within the limits of our resources and legal restrictions to make available full and usable models, methods, codes, and data through publications especially in supplemental information, web pages, and releases.
- This project takes a sophisticated approach to modeling at different scales. The project seems well connected with other activities and focused on key areas where modeling can provide actionable in- sights. It would be good to see some more examples where models have led to optimization strategies that were verified experimentally.
  This comment addresses the major thrust we have been undertaking for the last 1.5 years to insure that we are working on the most relevant problems and working directly with experimental projects to 1) verify theoretical predictions, 2) inform and improve simulation and modeling, and 3) remain on target with the experimental efforts as experimental directions move and down-select.
- This is an extremely important activity to guide the researchers and predict changes that need to be made. A challenge will be how to model real world substrates and situations such that accurate predictions can be made.
  The above response applies here, too.

Publications 2015-2017

5 publications for 2017 in preparation or submitted, not listed.

St. John, P.C., Crowley, M.F. and Bomble, Y.J., (**2017**). Efficient estimation of the maximum metabolic productivity of batch systems. *Biotech for Biofuels*, 10, 28.

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Haarmeyer, C.N., Smith, M.D., Chundawat, S.P., Sammond, D. and Whitehead, T.A., (**2016)**. Insights into cellulase-lignin non-specific binding revealed by computational redesign of the surface of green fluorescent protein. *Biotechnology and Bioengineering*. doi:10.1002/bit.26201

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Davinia Salvachúa, Holly Smith, Peter C. St. John, Ali Mohagheghi, Darren J. Peterson, Brenna A. Black, Nancy Dowe, Gregg T. Beckham (**2016**). Succinic acid production from lignocellulosic hydrolysate by Basfia succiniciproducens. *Bioresource Technology* 214 558–566.

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Berstis, Laura, Beckham, Gregg T., and Crowley, Michael F. (**2015**). Electronic coupling through natural amino acids. *The Journal of chemical physics* 143.22 : 12B618_1.

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Sitaraman H., Kuhn E.M., Nag A., Sprague M.A., Tucker M.P., and Stickel J.J. (**2015**). Multiphysics modeling and simulation of high-solids dilute-acid pretreatment of corn stover in a steam-explosion reactor. *Chem. Eng. J.*, 268:47–59.

Presentations 2015-2017

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Peter St. John, Michael F. Crowley and Yannick J. Bomble. Metabolic Modeling for Improved Bioprocess Efficiency, November 13th, **2016** AIChE Annual Meeting, San Francisco, CA

Peter St. John, Michael F. Crowley and Yannick J. Bomble. Efficient Estimation of Maximum Theoretical Productivity from Batch Cultures Via Dynamic Optimization of Flux Balance Models, November 13th, **2016** AIChE Annual Meeting, San Francisco, CA.

Peter St. John, Christopher Johnson, Payal Khanna, Yannick J. Bomble and Gregg T. Beckham, Metabolic Modeling and Pathway Engineering of an Industrially Relevant Pseudomonas Putida KT2440 Strain to Produce Muconate from Glucose, November 14th,. **2016** AIChE Annual Meeting, San Francisco, CA

Stickel J.J., Humbird D.W., Sitaraman H., Sprague M.A., and McMillan J.D. CFD study of full-scale aerobic bioreactors: Evaluation of dynamic oxygen distribution, gas-liquid mass transfer, and reaction. Poster presentation at the 38th Symposium on Biotechnology for Fuels and Chemicals, Balitmore, MD, April, **2016**.

Lischeske J.L., Nag A., and Stickel J.J.. Examining cell-wall transport effects on enzymatic hydrolysis by mechanistic modeling. Presented at the AIChE Annual Meeting, Salt Lake City, UT, November, **2015**.

Lischeske J.L., Nag A., and Stickel J.J.. Mechanistic modeling of enzymatic hydrolysis of lignocellulosic biomass with detailed structural and morphological considerations. Poster presented at the Symposium on Biotechnology for Fuels and Chemicals, San Diego, April, 2015.

Berstis, L., Beckham, G.T., Crowley, M.F., "Computational models to advance lignocellulosic biomass valorization"; Green Chemistry Gordon Research Conference, August **2016**

Berstis, L., Beckham, G.T., Crowley, M.F., “Computational models to advance the valorization of lignocellulosic biomass”, ACS Green Chem Eng, June **2016**

Presentations 2015-2017

Phillip Hudson, Ben Pollard, Michael Crowley, Henry Woodcock, “Strategies for improving accuracy in carbohydrate NMR chemical shifts computations via free energy simulation”, ACS **2016**, Philadelphia

Crowley, M.F., “Molecular Modeling of Cellulose, Cellulases, and Hemicellulose for Making Biofuels and Biomaterials”, Northwest Regional Meeting ACS, Anchorage, **2016**.

Michael Crowley, Antti-Pekka Hynninen, Gregg Beckham, Brandon Knott, Lintao Bu, “Milestones in simulation of plant cell-wall carbohydrates and biofuel-related enzymes”, ACS **2016**, San Diego

Laura Berstis, Thomas Elder, Gregg Beckham, Michael Crowley, “Coupling and reactions of catechol monolignols”, ACS **2016**, San Diego

Berstis, L., Beckham, G.T., Crowley, M.F., "Computational models to advance lignocellulosic biomass valorization”; National Engineering Day **2016**

Crowley, M.F., “Cellulose and Structure, Connecting Molecular Structure to Measurements”, 5th International Symposium on Diffraction Structural Biology, Knoxville, **2016**.

Berstis, L., Beckham, G.T., Crowley, M.F., "Theoretical models of electron transfer processes in LPMOs and model peptide systems” ACS 249th Annual Meeting March **2015**

Crowley, M.F., “Cellulase and Cellulose modeling and methods for biofuels production”, CHARMM Developers Meeting, Vienna, **2015**.

ROI 2015-2017

ROI 16-117: Engineering the TCA Cycle in Pseudomonas putida KT2440 to Produce PHAs from C2-Carbon Sources