BETO 2021 Peer Review: Biochemical Process Modeling and Simulation (BPMS)

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Biochemical Conversion & Lignin Utilization Session

March 9th, 2021

Project Overview

Why is the use of modeling important?

1. **Lower time to solution.**
2. **Explore solutions inaccessible experimentally.**

This project focuses on the following:

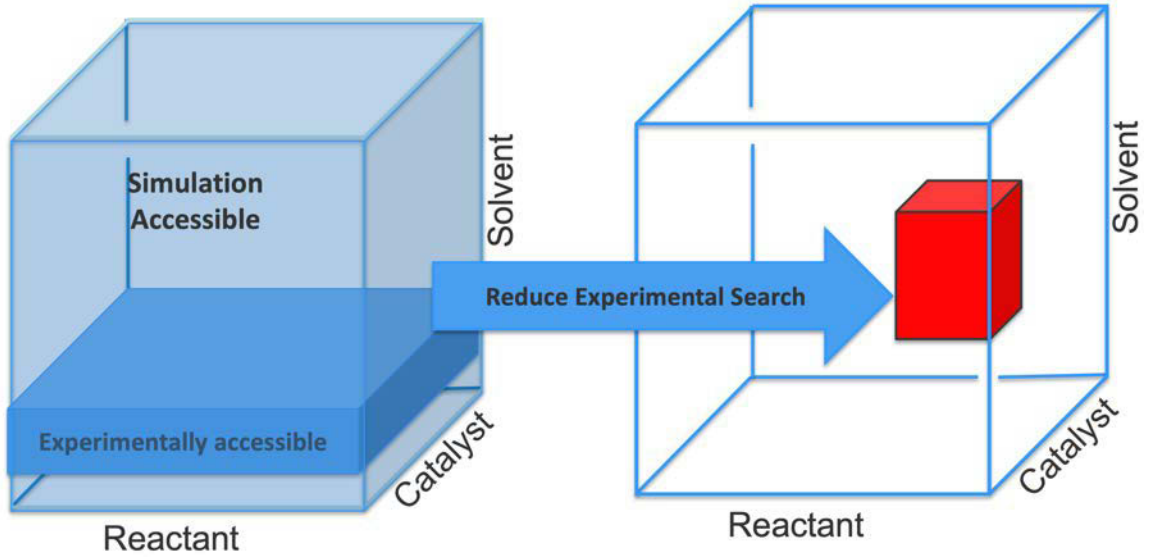
- Improving biomass, plastic degrading, and metabolic enzymes.
- Engineering and modifying metabolic pathways.
- Down selecting biochemical targets.
- Determining best fermentation conditions and providing reliable models for TEA

Heilmeier Catechism :

- **What:** Reducing research time and cost, **increasing efficiency,** using modeling and simulation to provide **actionable guidance to experimental efforts.**
- **Today:** Modeling often conducted without discussion and input from experimentalists.
- **Importance: Modeling can drastically reduce time to solutions and allow new breakthroughs.**
- **Risk:** Considering too many projects and not focusing on the ones where modeling could have impact.

Modeling Relevance

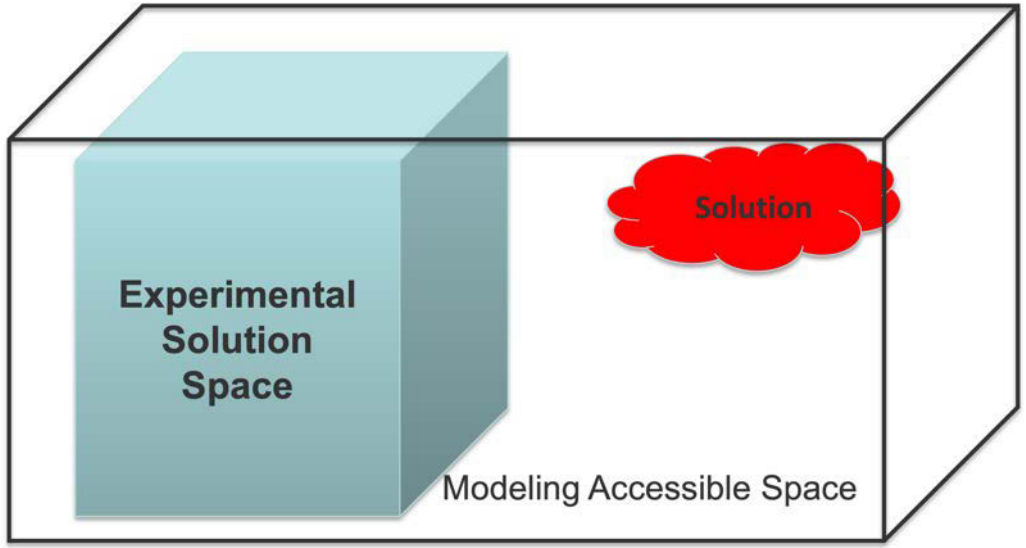
Modeling reduces experimental work and time.
Solution space is too big for experiment but accessible by modeling.



Example: Determine the right aliphatic compound to produce based on ease of extraction before experimental efforts are implemented.

Modeling Relevance

Modeling can find solutions unavailable to standard experimental search.



Examples: Risk too high

- Mutations/knockouts believed to be fatal to microbes.
- Testing reactor designs at industrial scale.
- Exploring mutants with very high number of mutations.

Quad Chart Overview (for AOP Projects)

Timeline

- Project start date - 2018
- Project end date 2021

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020) $1,050,000	(negotiated total federal share over active project)

Project Partners*

Barriers addressed

Ct-N Multiscale computational framework accelerating technology

Ct-C Process Development for Conversion of Lignin

Ct-F Increasing the yield from catalytic processes

Ct-G Decreasing the cost to developing novel ind. relevant catalysts

Ct-K Developing methods for Co-product Production

Ct-L Decreasing devel. time for ind. relevant microorganisms

Ct-M Current reactors are not designed to handle many harsh conditions

Project Goal

Provide **actionable guidance** to experiments and TEA from mechanistic predictions and design principles:

- Mutations for enzymes
- Metabolic target products
- Chemical formulations for polymers
- Metabolic knockouts and insertions
- Reactor optimizations

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

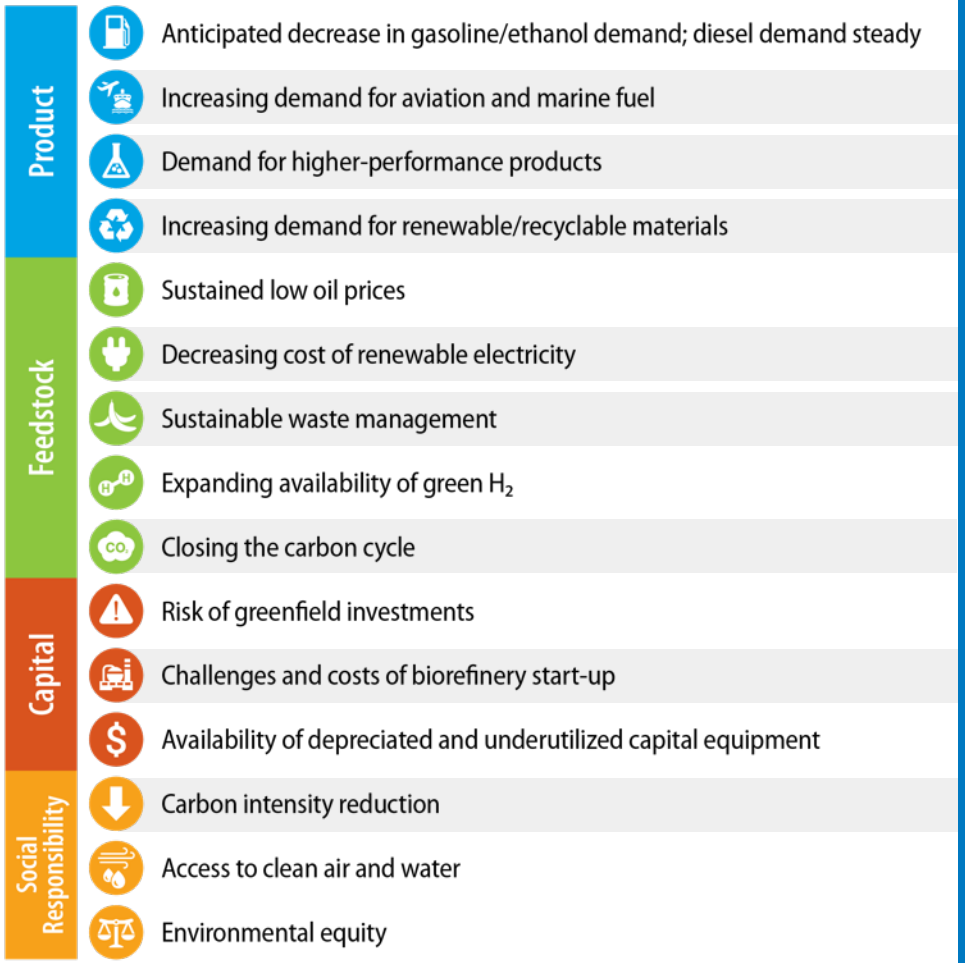
End of Project Milestone

Deliver complementary metabolic modeling and CFD methodologies (set of predicted metabolic pathway modifications, sugar feeding rates, oxygen sparging rates, reactor designs) leading to at least 20% increase in 2,3 BDO titer or 20% increase in yield of 2,3 BDO.

Funding Mechanism

AOP as WBS# - 2.5.1.100

Market Trends



NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- We aim to accelerate research, provide complementary insights and broaden research space to enable efficient processes for a new bioeconomy.

Key Differentiators

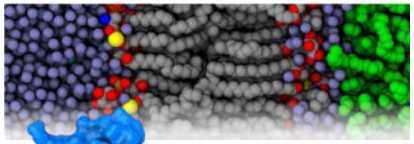
- We work directly with different experimental projects to ensure that we focus on the most impactful science.
- We have designed this project to be able to cover many time and length scales.

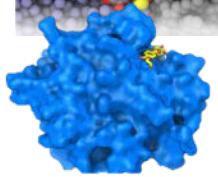
1. Management

Project: Biochemical Process Modeling and Simulation – Yannick Bomble

1- Molecular Modeling - Brandon Knott

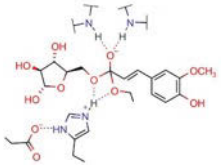
Molecular dynamics
Quantum mechanics

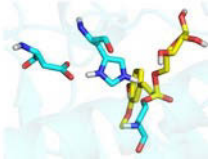




Structure/function
Enzyme design
Molecular processes
Specificity

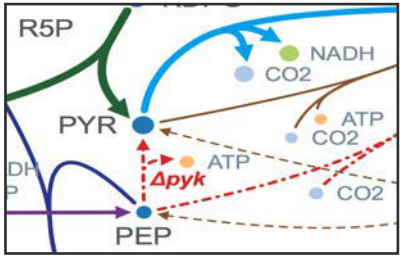
QM and QM/MM approaches to upgrading chemistry and catalysis





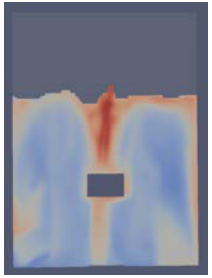
2 - Machine learning and Metabolic/Redox Potential Modeling - Yannick Bomble and Peter St. John

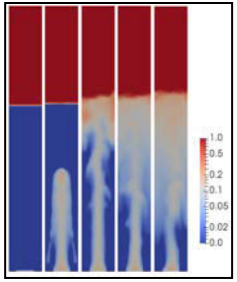
- Metabolic models
- Machine learning
- Kinetic modeling
- DBTL Learn efforts and omics analyses
- Redox enzyme / cofactor engineering



3 - Mechanistic Process Modeling - James Lischeske

Coupled CFD/Rxn-diffusion
Multi-scale modeling



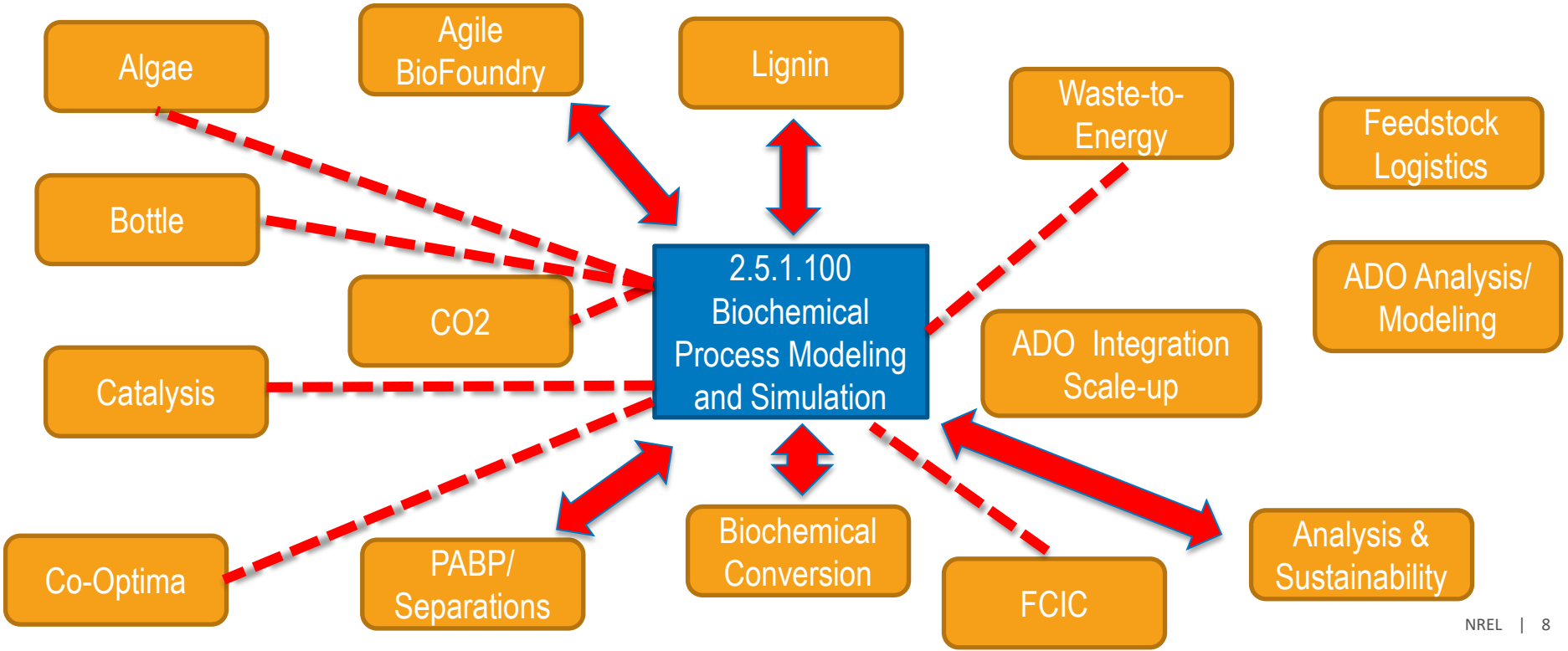


Project split into tasks by modeling type, managed by person with appropriate expertise
Task Managers responsible for:

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

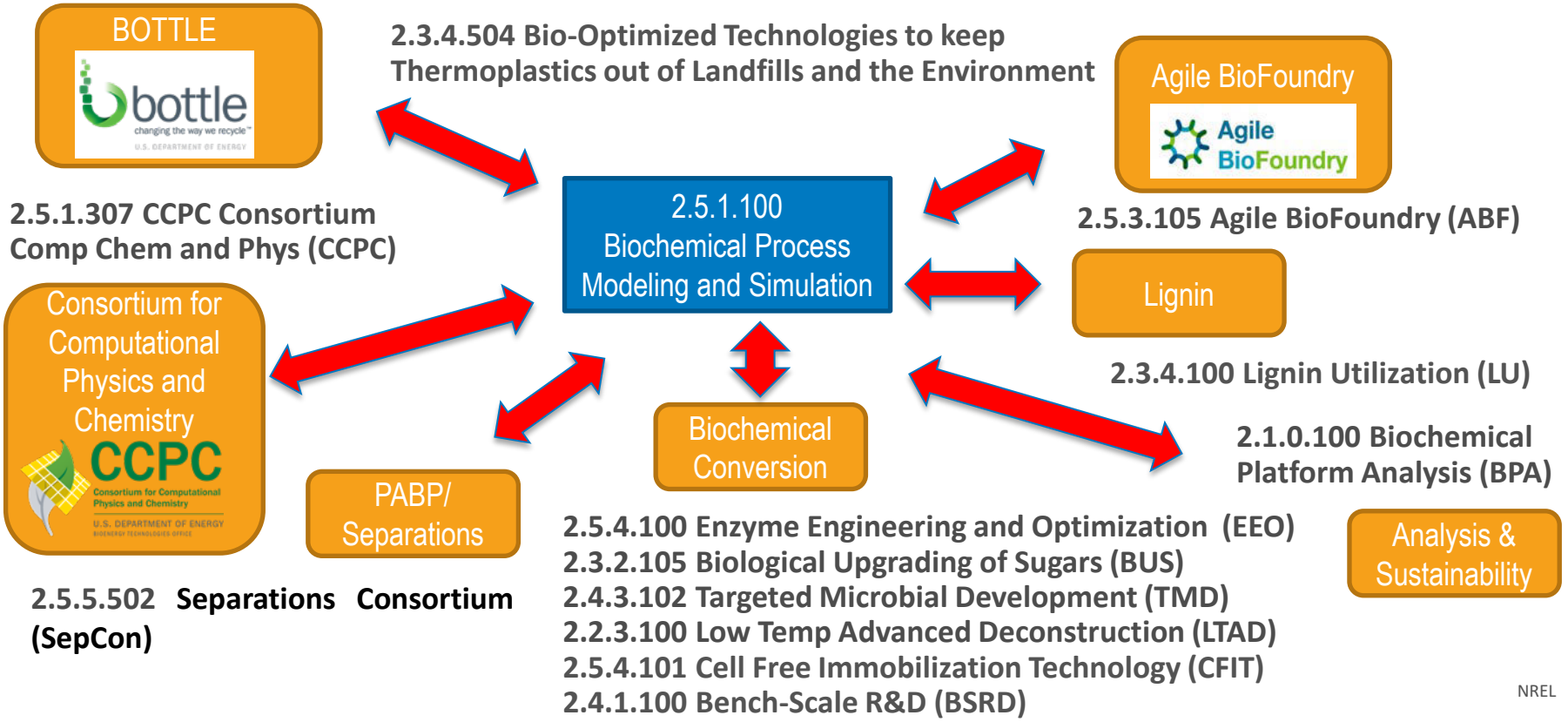
1. Management

The **most important** aspect in managing this project to **mitigate risks is the identification and prioritization of modeling activities** that are most meaningful and impactful to experimental projects.



1. Management

Many interactions across the BETO project portfolio that are **revised at each AOP cycle by meeting with project PIs** to see if modeling is appropriate/tractable and if we have the proper resources.



2. Approach

Objectives:

- Gain **insight**, discover new approaches and solutions.
- **Guide and stimulate design, experiment, and engineering;** select most promising directions.
- **Accelerate research, provide complementary insight and broaden research space.**

Approach:

- Use MultiScale Approach:
  Molecular (Task 1), Metabolic/Cellular (Task 2), and Macroscopic (Task 3) simulation.
- Leverage EERE computer resource: **Eagle (NREL).**
- Leverage **CCPC** (Consortium for Computational Physics and Chemistry) collaborations using all theory and modeling expertise **across laboratories.**
- **Strong and regular communication and joint metrified milestones with other experimental projects.**
- Target most **relevant bottlenecks and barriers** in **most BETO-relevant processes.**
- **Go/no-go decisions:** Produces at least a 10% increase in 2,3 BDO titer or 10% increase in yield of 2,3 BDO over current strains and scale-up strategies from either glucose, xylose, arabinose, or all of these C5/C6 sugars (3/31/2020).

2. Approach

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.
- Project and time management given the number of projects.

Success Factors:

- Insights achieved, solutions found, unproductive efforts avoided.
- Reduced time to solution: increasing titer, efficiency, speed, performance.
- New routes to advanced fuels and co-products.

3. Impact

Reduce Cost of Research and Time-to-Solution

- **Lignin-upgrading enzymes designed for more diverse substrates, increasing carbon efficiency.**
- **Plastic-degrading enzymes** designed for circular economy.
- **New Omics methods developed to improve DBTL cycles – more accurate and efficient from large omics set.**
- Reactor models predict outcomes outside of experiment, **lowering uncertainties of TEA.**
- Reactor modeling to predict the **effect of different oxygen sparging** on fermentations.

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.

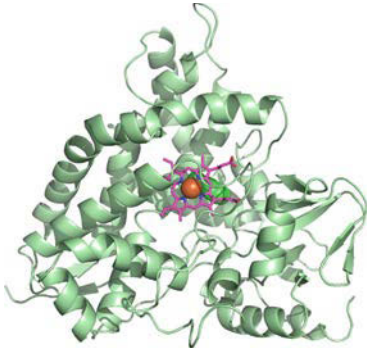
Technology Transfer

- Lignin Force Field and Builder publicly available for all lignin-related molecular modeling.
- Omics methods and metabolic modeling visualization tool released for public use.
- Reactor models are publicly available for industrial use.
- Record of Inventions and publications (24 since last peer review).

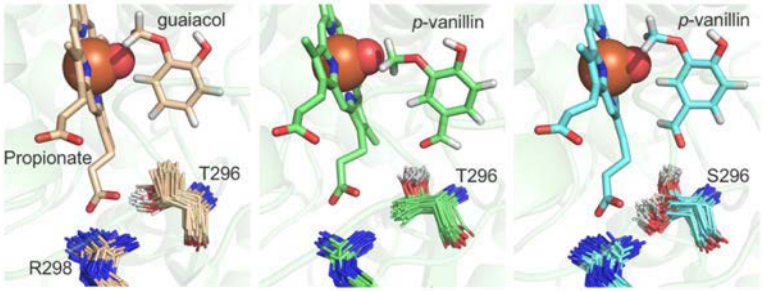
Biological funneling for lignin valorization

RELEVANCE: Driving the engineering of bottleneck enzymes for the upgrading of lignin degradation products to biofuels and products.

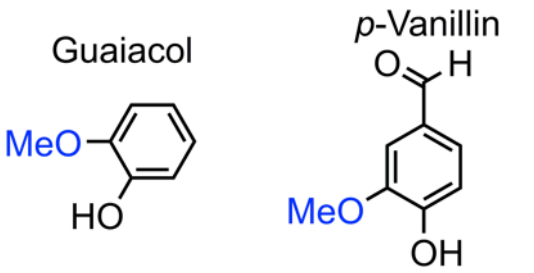
OUTCOME: Enzymes with broader substrate specificity for lignin upgrading.



- Engineering microbes to convert a broad slate of lignin degradation products is a promising strategy for lignin valorization.
- GcoA is capable of natively demethylating guaiacol to catechol.
- Two rationally-designed mutants of GcoA now able to use *o*- and *p*- vanillin.
- MD simulations of the WT and mutants reveal that the stability of *p*-vanillin substrate at the active site and restored hydrogen bonding patterns enable non-native catalytic action.



Native substrate **New substrate**



Progress and Outcomes

Modular computational tool for lignin analytics

RELEVANCE: Rapid identification of dimeric and oligomeric compounds in lignin-derived samples coupling computation, experiments, and analytics

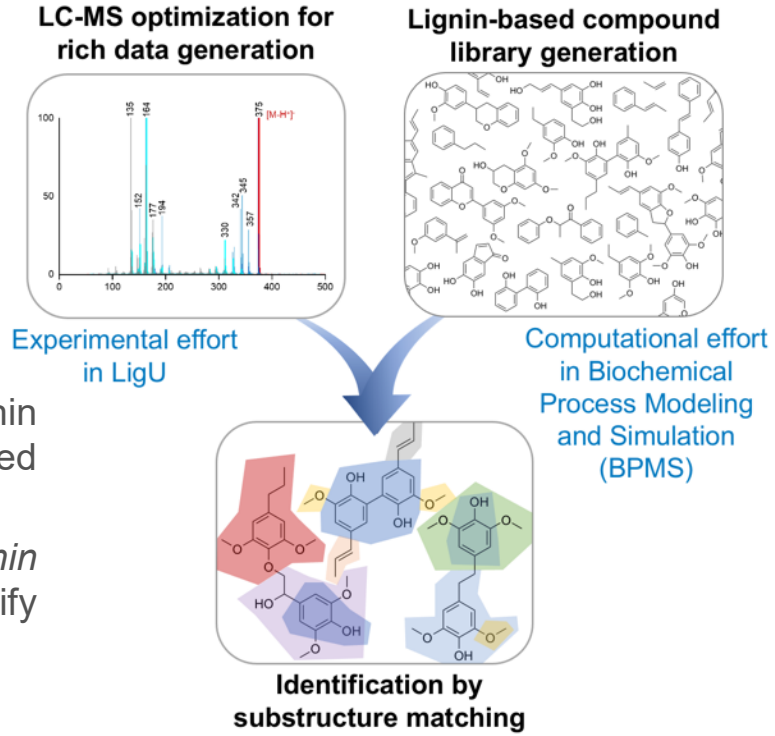
OUTCOME: Better characterization of lignin streams.

Challenges:

- Existing analytics methods depend on standards to develop fragmentation patterns that do not include lignin-derived compounds.
- Current methods thus limited for high-fidelity compound ID.

Approaches:

- Build on previous computational efforts and knowledge of lignin chemistry to generate a comprehensive lignin-based compound library.
- Pattern match experimental fragmentation data (from *Lignin Utilization)* with computational library to rapidly identify compounds.
- Validating this approach with lignin-derived catalysis samples

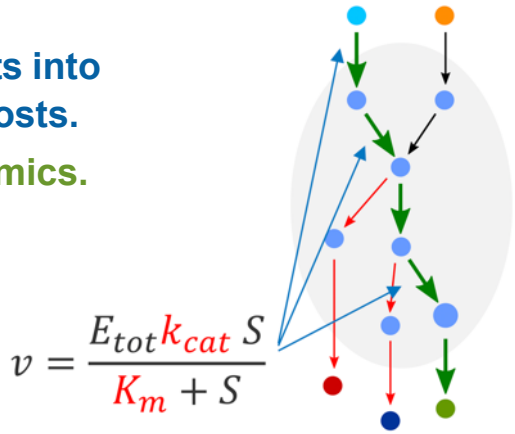


Progress and Outcomes

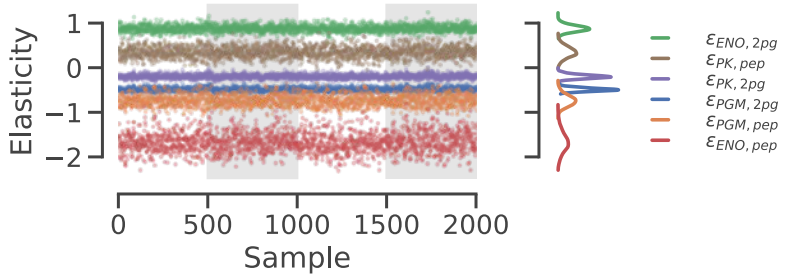
Bayesian metabolic control analysis

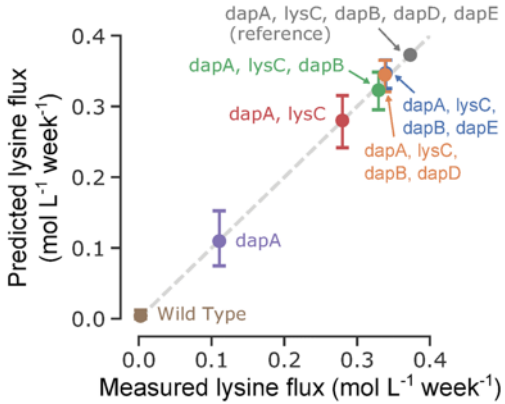
RELEVANCE: Convert 'Omics data from high-throughput experiments into actionable strategies to improve processes in industrially-relevant hosts.

OUTCOME: More informed models and predictions extracted from Omics.



- Linking enzyme activity to metabolic fluxes & concentrations requires kinetics of every enzyme in a pathway.
- These parameters are difficult to obtain from experimental data.





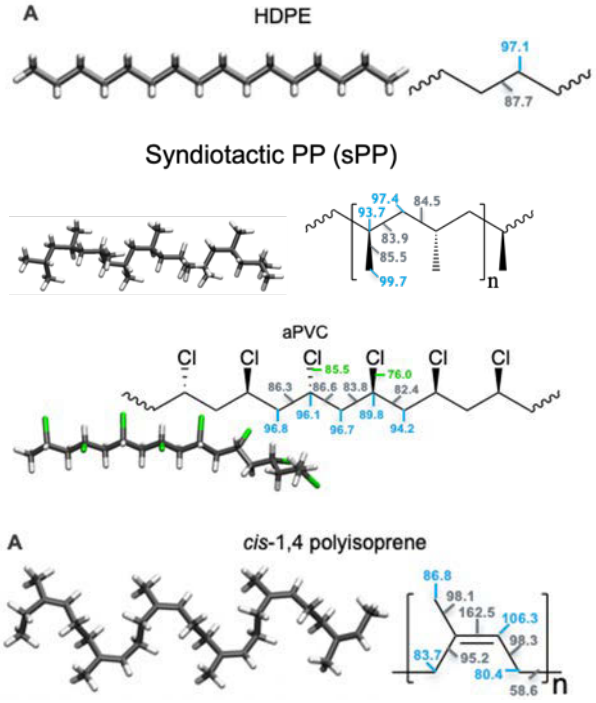
Developed computational approach to **infer probability distributions** in kinetic parameters, which can then be used to **guide optimal strain design.**

Bond strengths in commodity plastics

RELEVANCE: Reducing design space to the most promising targets for catalytic cleavage of plastics and subsequent upcycling.

OUTCOME: List of most labile bonds and likely degradation products.

- Polymer bond dissociation enthalpies (BDE) calculated by density functional theory (DFT) can predict how different plastics will decompose in analytical instruments and in recycling efforts, giving critical guidance to plastics upcycling efforts.
- BDEs were determined with DFT calculations of oligomers of more than 25 of the most common plastics:
  - polyolefins, rubbers, polyesters, nylons, halides,
- These results will serve as a roadmap for experimental efforts to functionalize and degrade commodity plastics to monomers for efficient upcycling.



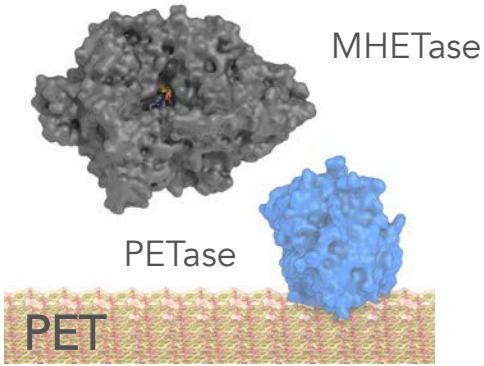
Progress and Outcomes

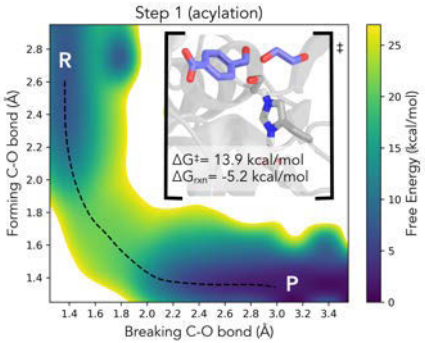
Guiding enzyme engineering for plastics recycling

RELEVANCE: Plastics (a major MSW component) represent a carbon waste product and major environmental problem.

OUTCOME: Better enzymes for plastic degradation.

- We are driving enzyme and cocktail engineering to enable cost-efficient upcycling of commodity plastics (e.g. PET).
- Leveraging BETO investment in biomass recalcitrance and cellulase cocktail development.
- Enzyme engineering by partners driven by structural analysis, QM/MM simulations.
- Published in PNAS
  - Altmetric top 100 (#37 overall, #2 in *Chemical Science* category)





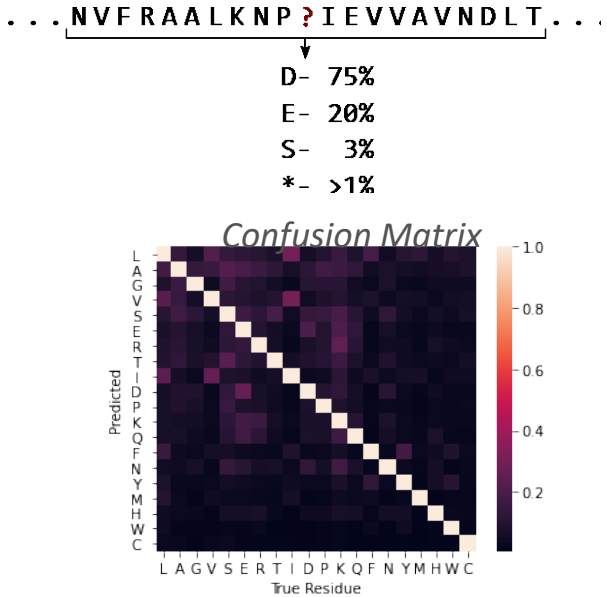
MHETase reaction energetics

Progress and Outcomes

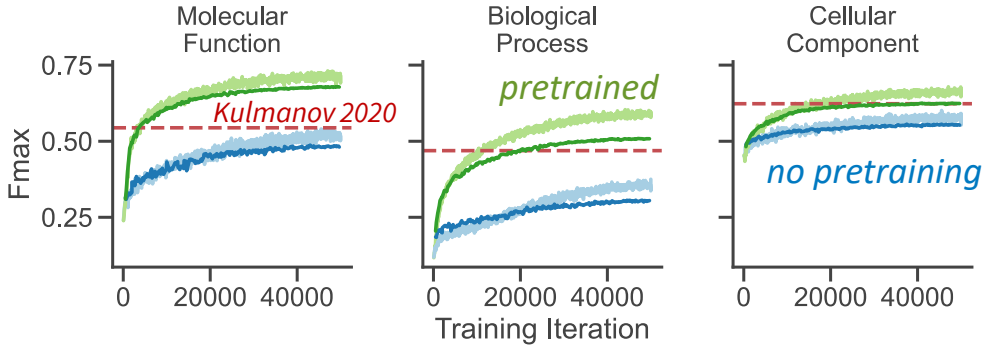
Prediction of enzyme promiscuity and improvements through sequence-only machine learning

RELEVANCE: Developing computational approaches to predict new protein function and guide enzyme engineer for a variety of applications in BETO.

OUTCOME: *Natural language processing* based techniques to improve accuracy of sequence -> function prediction.



- **Pretraining** on 261M unlabeled protein sequences allows large ML models (>100M parameters) to learn relationships between residues.
- Pretrained models show state-of-the-art performance in predicting protein function.



With these sequence->function models, we can do enzyme engineering in new ways that (1) gives us alternative suggestions to 3D models or (2) allows engineering on proteins without 3D structures (membranes, etc.)

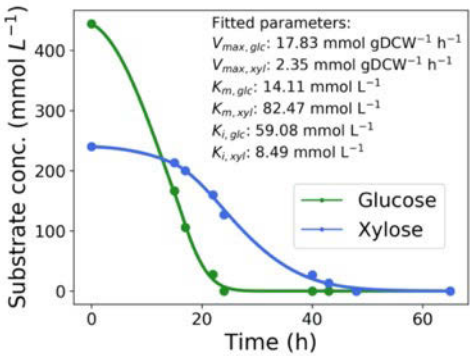
Progress and Outcomes

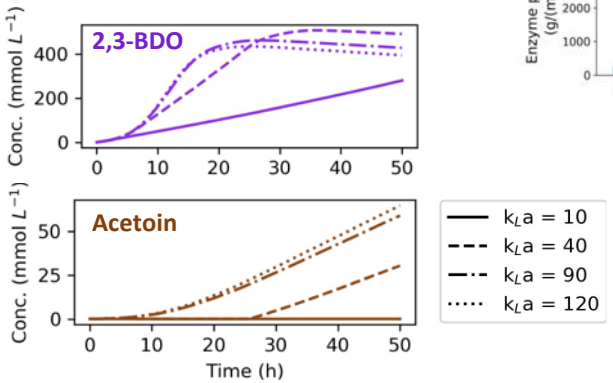
Thermodynamic and Kinetic Modeling of 2,3-BDO production by *Z. mobilis*

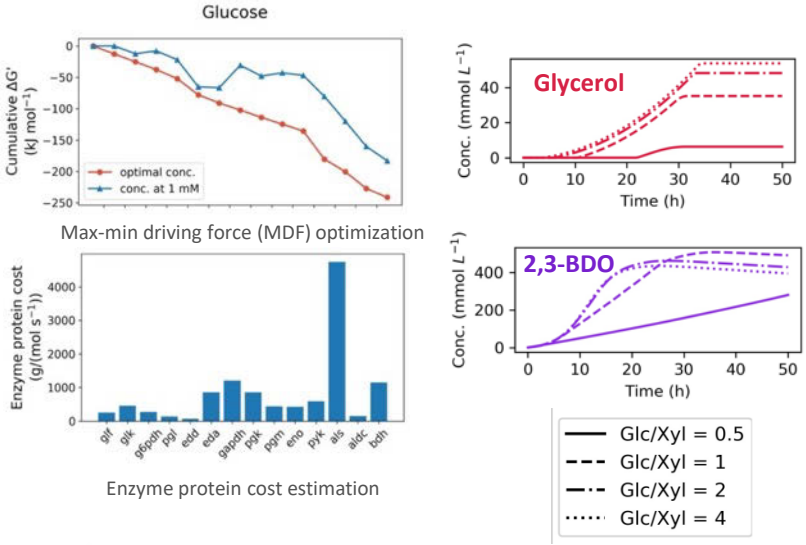
RELEVANCE: Developing new computational approaches to guide improvements in 2,3-BDO production and biochemicals in general.

OUTCOME: Models to predict product distribution based on substrate composition and aeration rates

- Not surprisingly, experimental data and kinetic modeling show diauxic growth of *Z. mobilis* on glucose and xylose.
- Thermodynamic analysis shows enzyme costs associated with glucose metabolism are 58% lower than for xylose.
- These models can be used to determine 2,3 BDO production as well as the production of by products one different feedstocks.







These models can also help us determine that aeration rate (k_La) must be carefully tuned for maximum 2,3-BDO production

Progress and Outcomes

Process-scale Fermentation Methods Development

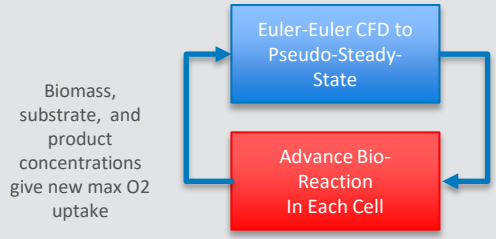
RELEVANCE: Enable (micro-)aerobic pathways and inform techno-economic analysis

Challenge:

Fermentation time scales (hours to days) does not match computationally accessible CFD time scales (seconds to minutes).

Solution:

Developed a novel reactor subcycling methodology to capture batch dynamics over a long batch reaction.

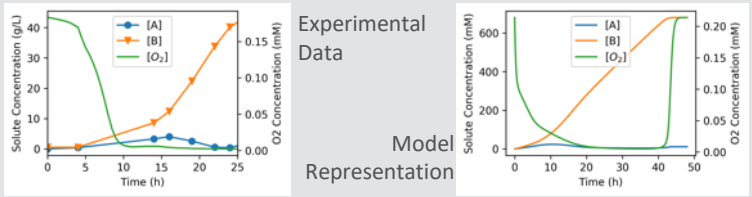


Challenge:

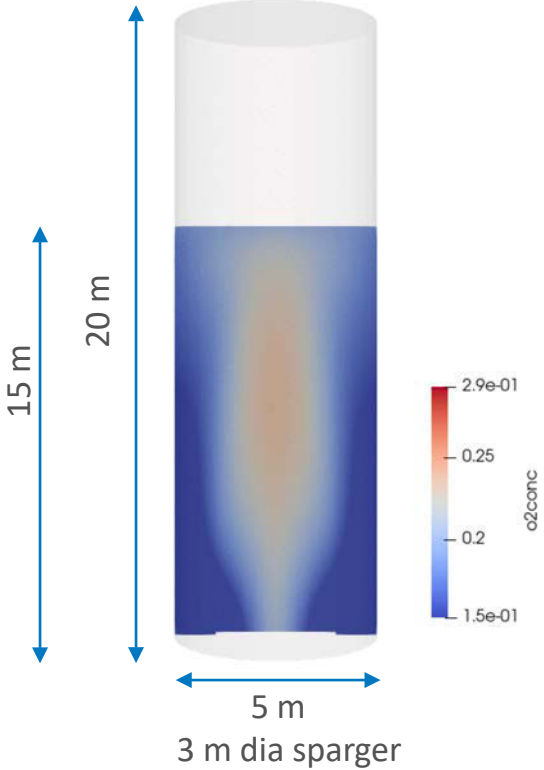
Metabolic models are complex and computationally expensive, whereas subcycling requires a compact model.

Solution:

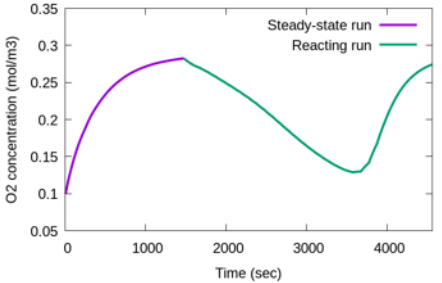
Developed a light-weight phenomenological model to describe aerobic-environment-dependent product partitioning.



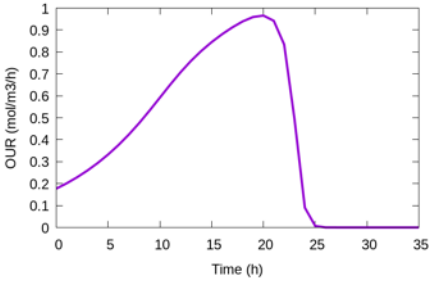
400,000L Bubble Column



Mean O2 across startup phase and all CFD cycles.



Mean Oxygen Uptake Rate in the kinetic time domain.

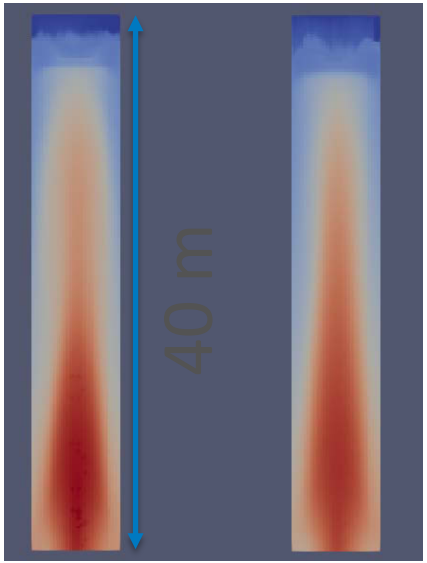


Oxygen consumption increases with biomass growth, then decreases at the end of the batch process due to substrate depletion.

Progress and Outcomes

Process-scale Fermentation Methods Development

OUTCOME: Enables simulation of batch fermentation at scale.



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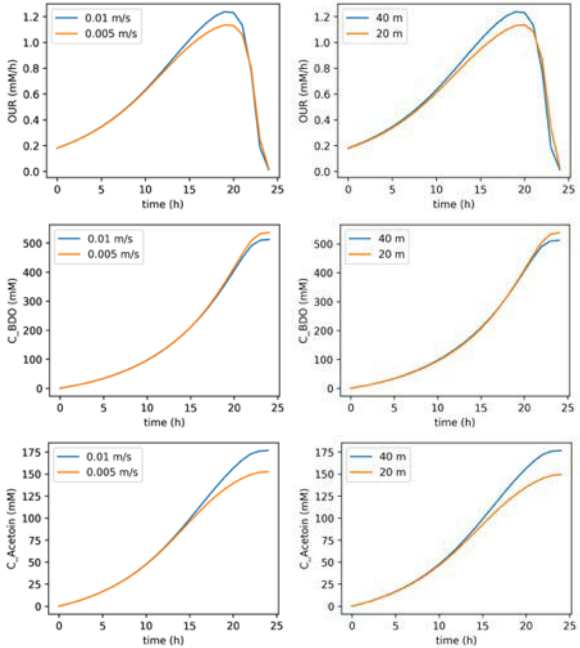

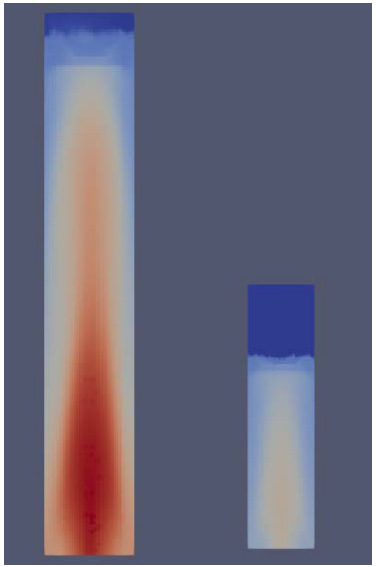

40 m | 20 m

- Sparge rates and column size don't seem to impact BDO production much but still allow to push selectivity toward BDO.
- Thinking of other reactor designs *e.g* loop reactors or shallow channel sparged vessels.

Summary

Management:

- **Active management** with reevaluation of efforts every year done by communicating with other projects to **ensure that we are working on the most tractable and impactful projects**.

Approach:

- Target **most relevant bottlenecks and barriers** in most BETO-relevant processes.
- Use a **MultiScale Approach**: Molecular (Task 1), Metabolic/Cellular (Task 2), and Macroscopic (Task 3) simulation and leverage EERE computer resource

Impact:

- **Reduced cost of research and time-to-solution**
- **Provided NEW insights**
- Enabled technology transfer and scientific dissemination

Progress and Outcomes (highlights):

- Enzymes with broader substrate specificity for lignin upgrading.
- More informed models and predictions extracted from Omics.
- List of most labile bonds and likely degradation products in commodity plastics.
- Natural language processing-based techniques to improve accuracy of sequence -> function prediction.
- Enabled simulation of batch fermentation at scale.

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Collaborations with other BETO Projects

- Biological Conversion of Thermochemical Aqueous Streams (BCTAS)
- Biological Lignin Valorization (BLV)
- Enzyme Engineering and Optimization (EEO)
- Biological Upgrading of Sugars (BUS)
- Lignin Utilization (LU)
- Targeted Microbial Development (TMD)
- Low Temperature Advanced Deconstruction (LTAD)
- Biochemical Platform Analysis (BPA)
- Cell Free and Immobilization Technologies (CFIT)
- Bench-Scale R&D (BSRD)
- Separations Consortium (SepCon)
- BOTTLE Consortium
- Agile BioFoundry
- CCPC Consortium Comp Chem and Phys

Thank you!

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Responses to Previous Reviewers' Comments

Q: Bringing together metabolic model dynamic process modeling and reactor design is a great ideas and will help reduce the timeline from concept to product. The project will benefit from focusing on one of the 3 tasks instead of working on all 3 together?

A: Our history has shown that the combination has been very successful in all three tasks and allows us to provide a multiscale approach to experimental bottlenecks from reaction mechanism to industrial scale reactors. Often these scales overlap in a question and require expertise at each scale to work together such as in the BDO work. This project does not require the direct attention of the PI in all three tasks. Each task lead has the responsibility of concentrating on achieving the goals of each task and in collaborating with experimental efforts in BETO.

Q: The models are impressive but there seems to be a lack of validation of the models maybe because the data necessary for direct validation is difficult to obtain.

A: This is a good point. It is always the most satisfying and constructive to get full validation in an effort to refine models. In BETO efforts, time is often at a premium so we find the fastest way to get the best answer we can to down-select out the worst candidates and to provide design principles at the cost of rigorous, validated, refined models. Since this approach has proven useful, we will be refining and validating as time and experimental effort permit. Also, direct validation may be difficult in some cases but several of the predictions have proven valid or lead to new thinking that improved the process (*e.g.* amino acid mutations, knockouts, sparging rate).

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