DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

2.2.2.501 Hybrid Conversion of Lignin and Bioconversion Intermediates

March 5th, 2019

Deconstruction and Fractionation

Jim Collett

PNNL

Goal Statement

The goal of this project is to achieve TRL-3 validation of a new "Hybrid Conversion" biorefinery concept for achieving diesel fuel production with a modeled minimum fuels selling price (MFSP) of <$3/GGE via coproduction of:

- a commodity chemical produced via bioconversion of lignocellulosic sugars, and
- a high-quality distillate fuel blendstock produced from biocrude oil from hydrothermal liquefaction (HTL) of corn stover lignin, microbial cell mass, and other residual streams that are currently burned for heat and power in contemporary biorefinery designs.

Quad Chart Overview

Timeline
- Project Start: 10/1/2018
- Project End: 9/30/2020
- Percent Complete:15%

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	0	0	0	$400 K
Project Cost Share*				

•**Partners: NREL, INL**

MYPP Barriers Addressed
- **Ot-B. Cost of Production**
- **Ct-C. Process Development for Conversion of Lignin**
- **Ct-K. Developing Methods for Bioproduct Production**

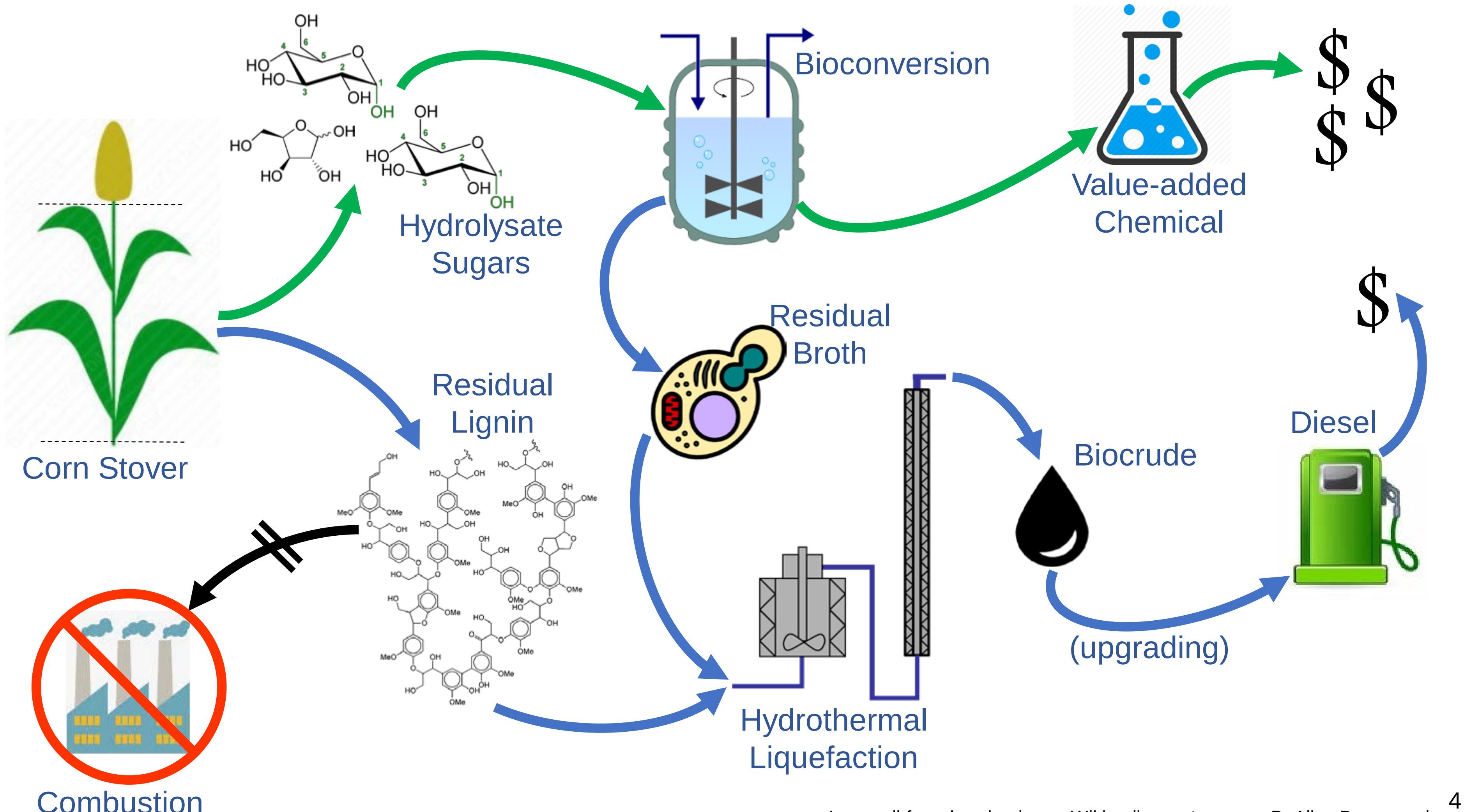
Objective
To achieve targeted bench-scale bioconversion titers of a model commodity chemical (at a titer > 80 g/L) from corn stover sugar hydrolysate while generating a sidestream of microbial cell mass that, when blended with corn stover lignin, serves as a suitable feedstock for the production of biocrude via hydrothermal liquefaction (HTL).

End of Project Goal
Experimental data from the bench scale bioconversion and HTL operations will be used for techno-economic analysis to project the cost and performance of a 2205 ton/day biorefinery that integrates these operations in a new Hybrid Conversion approach, and enables step change improvement (i.e. 2X) in the distillate fuel blendstock yield, and a modeled MFSP that is 20% lower than SOT MFSP determined during FY19.

1 – Project Overview

Hybrid Conversion maximizes value from biomass by:

- routing refined sugar streams into higher-value chemicals
- routing residual lignin and bioreactor broth streams into lower-priced fuels to increase overall system yield.



Icons: all-free-download.com, Wikipedia, pngtree.com, D. Allen Drummond

1 - Project Overview

Challenge

- Conventional biorefineries treat feedstock lignin, microbial cell mass, and black liquor (from biomass pretreatment) as **residual waste streams**, and route them to combustion and wastewater treatment operations for production of low-value steam and electricity.

- TEA indicates that about **60% of the feedstock dry mass** entering a conventional cellulosic biorefinery each day winds up **in residual waste streams**.

Solution

- **Route biorefinery residual streams to continuous hydrothermal liquefaction** (HTL) for the production of biocrude, which may be upgraded to a higher quality distillate fuel than that produced from direct HTL of unrefined biomass.

- **Route hydrolysate sugars into bioconversion for production of a commodity chemical** with a higher selling price than fuel.

- TEA indicates that this genuinely innovative approach for co-production of fuels and chemicals can could **significantly improve conventional cellulosic biorefinery profitability** in the near-term.

- This project will **achieve TRL-3 validation of a "Hybrid Conversion" biorefinery concept** by demonstrating bioconversion of corn stover hydrolysate to a model commodity chemical, followed by hydrothermal co-conversion of residual microbial cell mass and corn stover lignin to a biocrude oil suitable for upgrading to a **high-quality distillate fuel blendstock with an MFSP of <$3**.

2 – Approach (Management)

- Standard Project Management Good Practices
  - Statement of work and how it relates to DOE goals
  - AOP Project Management Plan
  - Quarterly milestones
  - Go/No Go decision point
- Frequent project communications
  - Teleconferences with BETO platform leads
  - Quarterly formal reporting to BETO
  - Site visits by industrial collaborators
- Leverage expertise in related projects
  - 2.5.1.102 Process Analytical Technologies for Conversion Operations
  - 2.2.2.501 Fungal Biotechnology
  - 2.2.2.301 Hydrothermal Processing PDU
  - 2.1.0.301 Analysis and Sustainability Interface (TEA)

2 – Approach (Technical)

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This publication resulted from collaboration between these ongoing AOPs:

- 2.5.1.102 Process Analytical Technologies for Conversion Operations
- 2.2.2.501 Fungal Biotechnology
- 2.2.2.301 Hydrothermal Processing of Biomass
- 2.1.0.301 Analysis and Sustainability Interface (TEA)

In previous work, renewable diesel with a modeled yield of 63 GGE/dry ton feedstock was produced via HTL of oleaginous yeast and residual lignin from bioconversion of corn stover.

The modeled yield was approximately two times higher than previous biorefinery designs in which intracellular lipids are solvent-extracted for fuel production, residual lignin is routed to combustion for steam and power, and residual microbial cell mass is routed to anaerobic digestion for biogas production.

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Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover

Check for updates

James R. Collett*, Justin M. Billing, Pimphan A. Meyer, Andrew J. Schmidt, A. Brook Remington[1], Erik R. Hawley[2], Beth A. Hofstad, Ellen A. Panisko, Ziyu Dai, Todd R. Hart, Daniel M. Santosa, Jon K. Magnuson, Richard T. Hallen, Susanne B. Jones

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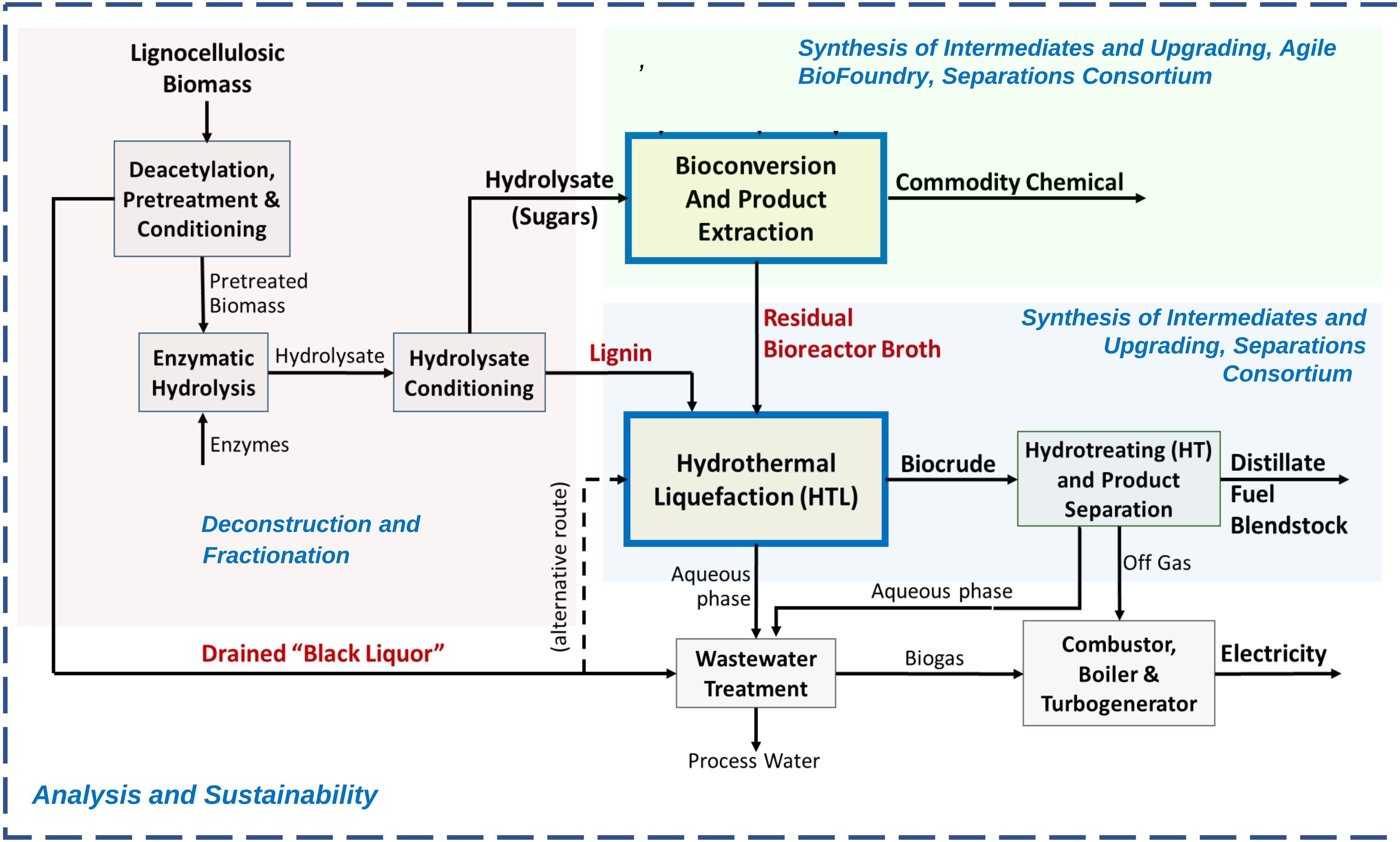
Collett, J.R. et al, Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover. Applied Energy, 2019. **233-234**: p. 840-853

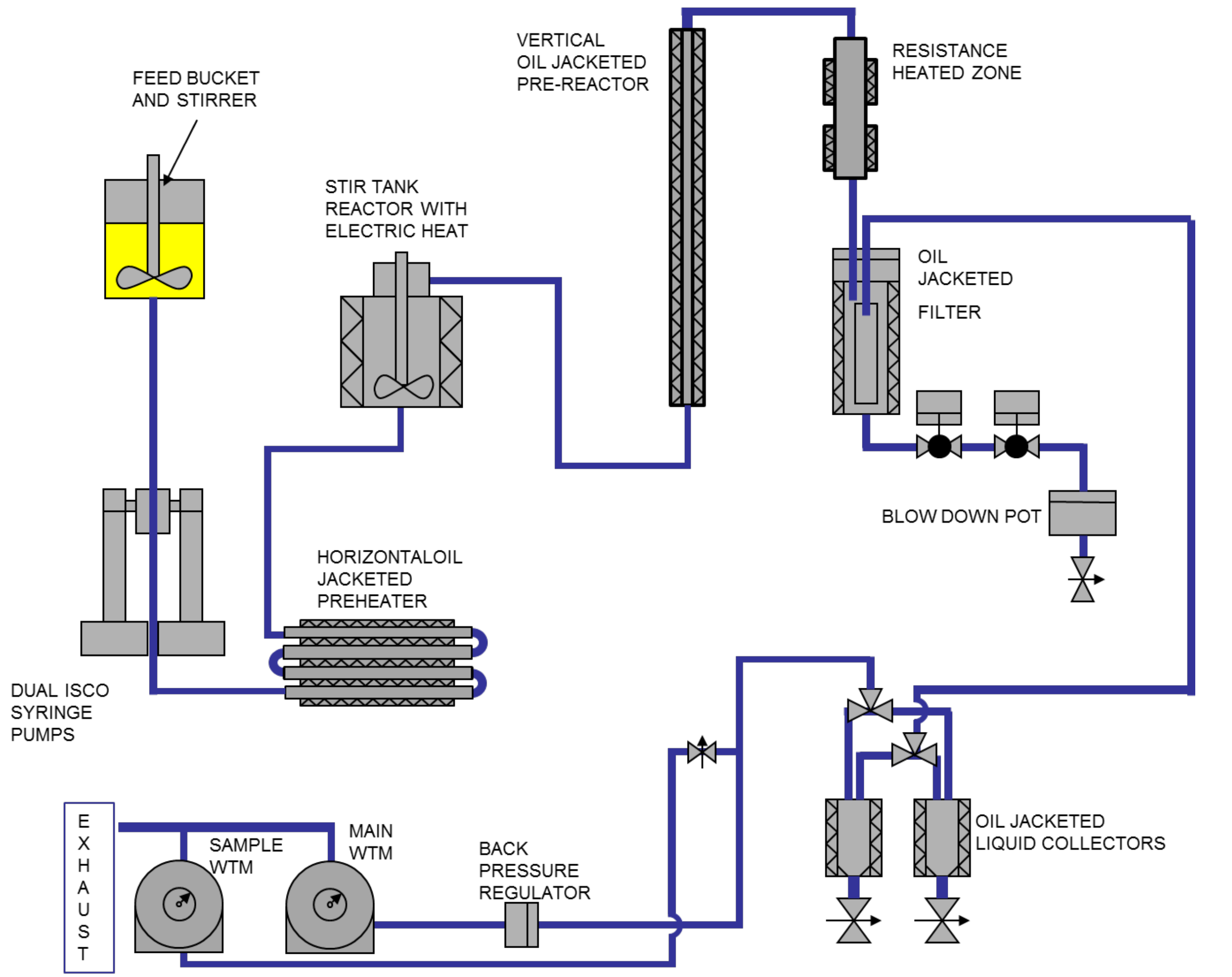
2 – Approach (Technical)

Hybrid Conversion integrates existing BETO technologies in a new and genuinely innovative way.



2 – Approach (Technical)

Continuous Hydrothermal Liquefaction Process Flow Diagram

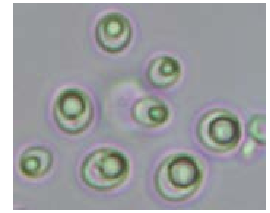


2 – Approach (Technical)

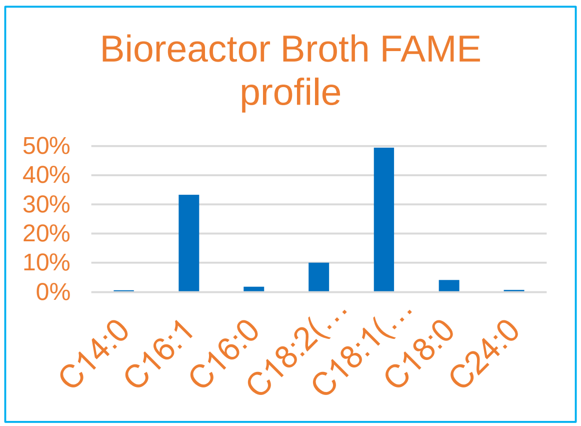
The HTL feedstock was composed of corn stover lignin mixed with whole oleaginous yeast bioreactor broth (previous work).

Lignin

Bioreactor Broth



Lipomyces starkeyi



Major lipid profile of *L. starkeyi* cell mass (measures as fatty acid methyl ester – FAME)

Composition of HTL feedstock

Stream	Wet mass of material (kg)	Dry solids fraction (wt/wt)	Dry solids mass (kg)	Lipid fraction (wt/wt)	Lipid mass (kg)
Bioreactor broth	17.50	0.16	2.79	0.31	0.87
Lignin paste	6.00	0.29	1.74	0.00	0.00
Broth + lignin mixture	23.50	0.19	4.53	0.19	0.87

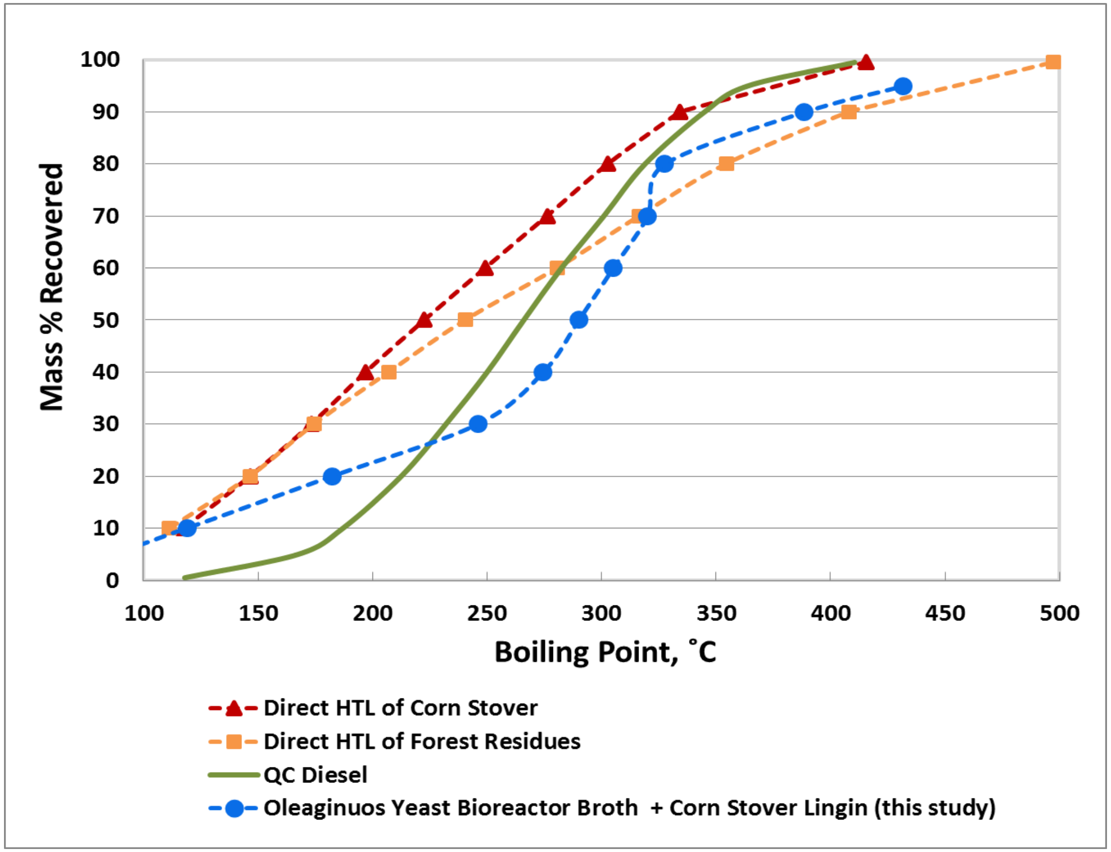
Element	Carbon	Hydrogen	Nitrogen	Oxygen	Sulfur	Ash	Total
Weight %	49.8	6.5	1.7	32.6	0.9	8.8	100.3

Collett, J.R. et al, Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover. Applied Energy, 2019. **233-234**: p. 840-853

2 – Approach (Technical)

Biocrude produced from HTL of oleaginous yeast and lignin was upgraded via hydrotreating to an excellent candidate distillate fuel blendstock (previous work).

75% of the product boiled within the diesel range (150-380ºC) as shown by comparison with the diesel quality control (QC) sample.



Boiling point distribution by simulated distillation of hydrotreated biocrude

Biocrude from HTL of corn stover lignin mixed with lipid-rich bioreactor broth

Finely ground forest residue

Mechanically pulverized, rehydrated unhydrolyzed corn stover

Diesel QC sample

Collett, J.R. et al, Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover. Applied Energy, 2019. **233-234**: p. 840-853

4 – Relevance

Hybrid Conversion (modeled SOT, fuels only case) produces twice as much fuel per ton of biomass than comparable, conventional biorefinery designs.

The installed cost of a Hybrid Bioconversion biorefinery for coproduction of fuels and chemicals is expected to be much lower than other recent biorefinery designs in the BETO portfolio.

Biorefinery Designs for Distillate Fuel Production	**Installed Cost** ($ MM)	**Fuel Yield** (GGE/dry ton)	**Co-Product Yield** (ton/dry ton)	**MFSP** ($/GGE)
This Project Baseline SOT - Distillate fuel only via hydrotreating of biocrude produced from **HTL of whole oleaginous yeast and lignin**	**445**	**63**	-	5.00
SOT - Distillate fuel only via hydrotreating of **solvent-extracted lipids from oleaginous yeast grown on hydrolysate sugars**[2]	488	30-34	-	9.50
SOT - Distillate fuel and coproduct via hydrotreating of **solvent-extracted lipids from oleaginous yeast** grown on C6 hydrolysate sugars with **coproduction of succinic acid** via bioconversion C5 hydrolysate sugars[2]	643	19	0.16	5.28
BETO 2030 Target Case - Distillate fuel and coproduct via **catalytic upgrading of carboxylic acid or BDO** from bioconversion of hydrolysate sugars, with coproduction of **adipic acid via bioconversion of lignin**[3]	697-758	43-45	0.13	2.47-2.49
This Project Target - Distillate fuel and coproduct via hydrotreating of biocrude from **HTL of lignin and residual bioreactor solids** with bioconversion of hydrolysate for **commodity chemical production.**	Experimental and modeling objectives of this project			

1. Collett, J.R. et al, Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover. Applied Energy, 2019. **233-234**: p. 840-853
2. Biddy, M.J., et al., *Techno-Economic Basis for Coproduct Manufacturing To Enable Hydrocarbon Fuel Production from Lignocellulosic Biomass.* ACS Sustainable Chemistry & Engineering, 2016. **4**(6): p.3196-3211
3. Davis, et al, Process Design and Economics for the Conversion of Lignocellulosic Biomass to Hydrocarbon Fuels and Coproducts: 2018 Biochemical Design Case Update, 2018. NREL/TP-5100-71949

4 – Relevance

- Hybrid Conversion addresses this March 2016 MYPP Performance Goal:
  - "Develop technologies that *enable a reduction in…cost of converting lignocellulosic biomass* to hydrocarbon fuels while *maximizing the renewable carbon* in the products."

- Hybrid Conversion contributes to achieving this Conversion R&D Milestone:
  - By 2021, complete the R&D necessary to set the stage for a 2022 verification that produces both fuels and high-value chemicals to enable a biorefinery to achieve a positive return on investment.

- Hybrid Conversion leverages BETO's successful reinvention of hydrothermal liquefaction (HTL) for wet waste valorization to solve the long-standing challenge of converting the lignin component of terrestrial biomass to fuel.

- Hybrid Conversion increases cellulosic biorefinery revenues by routing all feedstock lignin into HTL for the production of renewable distillate fuels, instead of burning it for heat and power.

- Hybrid Conversion, when fully developed, may be readily deployed at existing cellulosic ethanol biorefineries by simply adding HTL and hydrotreating unit operations.

- Hybrid Conversion decreases risk in the BETO portfolio by providing a new, less complex route for lignin valorization in cellulosic biorefineries with lower installed costs.

5 – Future Work – Task 1 - Bioconversion

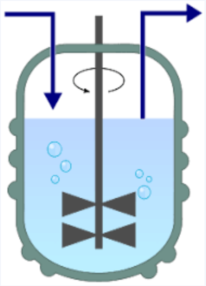
Bioconversion Critical Material Attributes (CMAs), Critical Process Parameters (CPPs), and Critical Quality Attributes (CQAs) will be identified and ranked in a series of 30-liter bioreactor runs to generate residual microbial broth for HTL and a model commodity chemical.

Hydrolysate CMAs
- Sugar content
- Non-sugar, insoluble solids
- Inhibitor content
- Cost

Biocatalyst CMAs
- Coproduct titer
- Coproduct productivity
- Growth rate
- Residual lipids

Nutrient CMAs
- Composition stability
- Cost
- Complexity

Potential Bioreactor CPPs
- Aeration
- Temperature
- pH
- Agitation rate
- Residence time



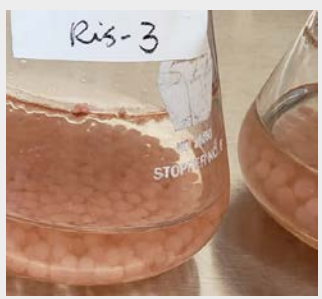
Residual Broth CQAs
- Lipid content
- N & S content
- Pumpability (rheology)
- Lubricity
- Miscibility with lignin

Coproduct CQAs
- Market size
- Sale price
- Separations Cost

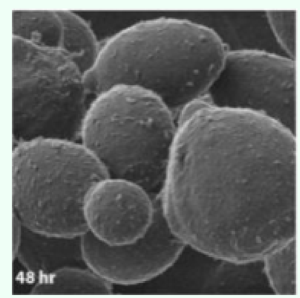
5 – Future Work – Task 1 - Bioconversion

To advance the Hybrid Conversion concept, model biocatalyst microbes will be grown on corn stover hydrolysate in a 30-liter bioreactor to optimize production of a model commodity chemical and a sidestream of residual cell mass as an HTL feedstock.

Organism	Morphology	Product	Compound Class	Applications	SOT Titer	SOT Lipid %	Xylose Utilization	Market Potential
A. terreus & pseudoterreus	filamentous	itaconic acid	carboxylic acid	plasticizer, chelant	160 g/L	up to 20%	WT	$0.1-0.5 B/year [1,2] 400 K tons/year [1]
Yarrowia lipolytica	unicellular	citric acid	carboxylic acid	preservative, solvent	80 g/L	up to 70%	GMO	$3.3 B/year[5] 2.4 MM tons/year
		triacetic acid lactone[3]	polyketide	platform compound for sorbic acid, acetylacetonate, and other chemicals[4]	36 g/L	up to 70%	GMO	TBD

Aspergillus terreus (filamentous fungus)



Yarrowia lipolytica (oleaginous yeast)

There are many other potential Hybrid Conversion biocatalysts such as *R. toruloides* or *R. opacus*

1. USDA, U.S. Biobased Products: Market Potential and Projections Through 2025, OCE-2008-1, February 2008
2. Kuenz, A. & Krull, S. Appl Microbiol Biotechnol (2018) 102: 3901.
3. Markham KA, et al. Rewiring Yarrowia lipolytica towardtriacetic acid lactone for materials generation. Proc Natl Acad Sci U S A. 2018 Feb 27;115(9):2096-2101.
4. Chia, M. et al. Green Chemistry 14 (7), 1850-1853, 2012.
5. https://www.grandviewresearch.com/press-release/global-citric-acid-market

5 – Future Work – Task 2 – Hydrothermal Liquefaction

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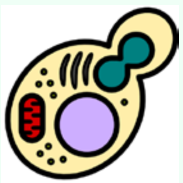
Critical Material Attributes (CMAs), Critical Process Parameters (CPPs), and Critical Quality Attributes (CQAs) for production of biocrude from residual bioreactor broth and lignin will be determined in a series bench-scale HTL runs.



Residual Broth CMAs

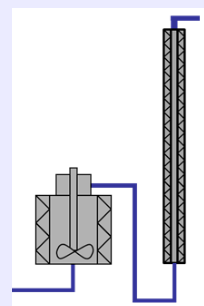
- Lipid content
- N & S content
- Pumpability (rheology)
- Lubricity
- Miscibility with lignin

Lignin Cake CMAs

- Ease in handling
- Thermochemical reactivity
- Particle size distribution

HTL CPPs

- Residence time
- Temperature
- Pressure
- Solids loading
- pH
- Buffer salts



Biocrude CQAs

- Carbon yield
- C15-C18 content (for diesel cetane)
- Naphtha content
- Ease of partitioning from aqueous phase

5 – Future Work – Task 2 – Hydrothermal Liquefaction

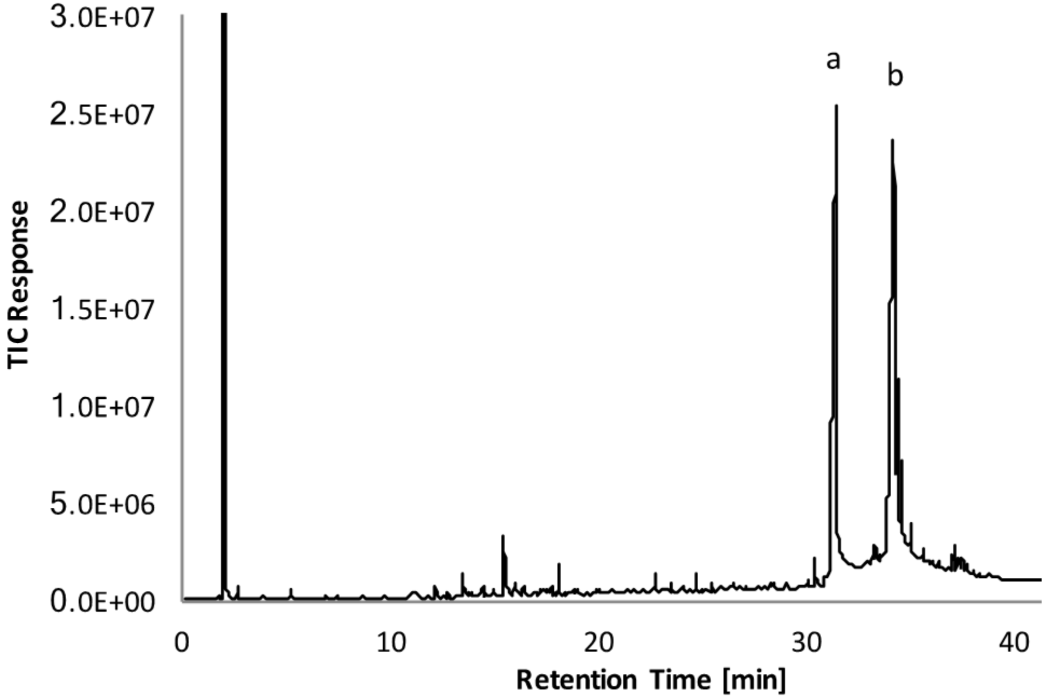
Biocrude from HTL co-conversion of residual cell mass and lignin will be compared to the previously published, fuel-only benchmark case.

Biocrude benchmark properties & yield (fuel-only case)

Balance and Yields	Wt. %
Overall HTL mass balance	102
Elemental balances	
Carbon	103
Hydrogen	105
Oxygen	103
Nitrogen	72
Mass yield (normalized)	
Biocrude (non-optimized separation)*	40/56*
Solids	9
Gas	23
Aqueous	29
Carbon yield (normalized)	
Biocrude (non-optimized separation)*	56
Solids	11
Gas	12
Aqueous	21

*GC-MS analysis of the HTL aqueous phase revealed that 16 wt% of biocrude components reported to the aqueous phase. Optimized separation will greatly improve the biocrude yield.

Benchmark biocrude hydrocarbon profile (fuel-only case)



Primary peaks (a) and (b) are hexadecanoic acid (at 31.429 min) and octadecanoic acid (34.391 min), respectively.
TIC = total ion chromatogram.

Collett, J.R. et al, Renewable diesel via hydrothermal liquefaction of oleaginous yeast and residual lignin from bioconversion of corn stover. Applied Energy, 2019. **233-234**: p. 840-853

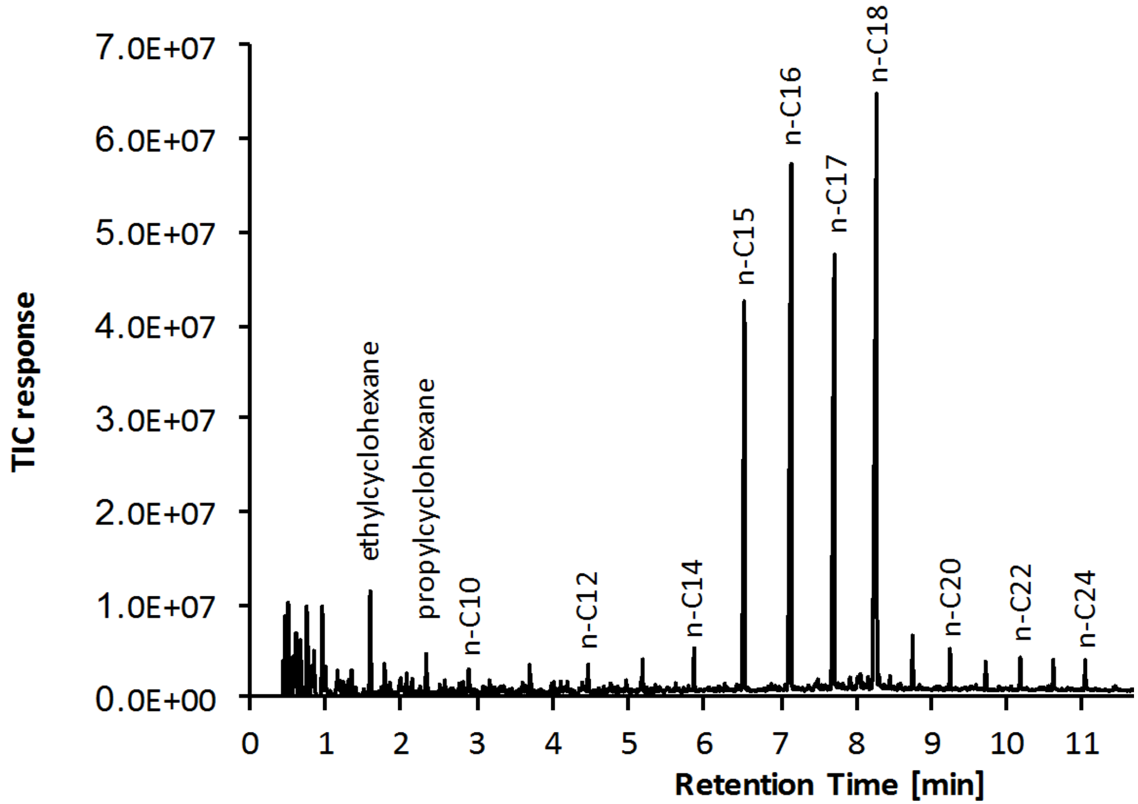
5 – Future Work– Task 2 – Hydrotreating

Distillate fuel from hydrotreating biocrude produced via HTL will be compared to the previously published, fuel-only benchmark case.

Hydrotreating benchmark properties & yield (fuel-only case)

Consumption and Yields	Units	Value
Weight hourly space velocity	Wt. dry biocrude/wt. catalyst/h	0.39
Liquid hourly space velocity (LHSV)	Vol. wet biocrude/vol. catalyst/h	0.21
Hydrogen consumption	g H_2/g dry biocrude	0.040
Total mass balance	%	98
Hydrocarbon volumetric yield	ml/ml wet biocrude	0.97
Hydrocarbon mass yield	g/g dry biocrude	0.84
Aqueous mass yield	g/g dry biocrude	0.14
Gas mass yield	g/g dry biocrude	0.04

Distillate product benchmark hydrocarbon content (fuel-only case)



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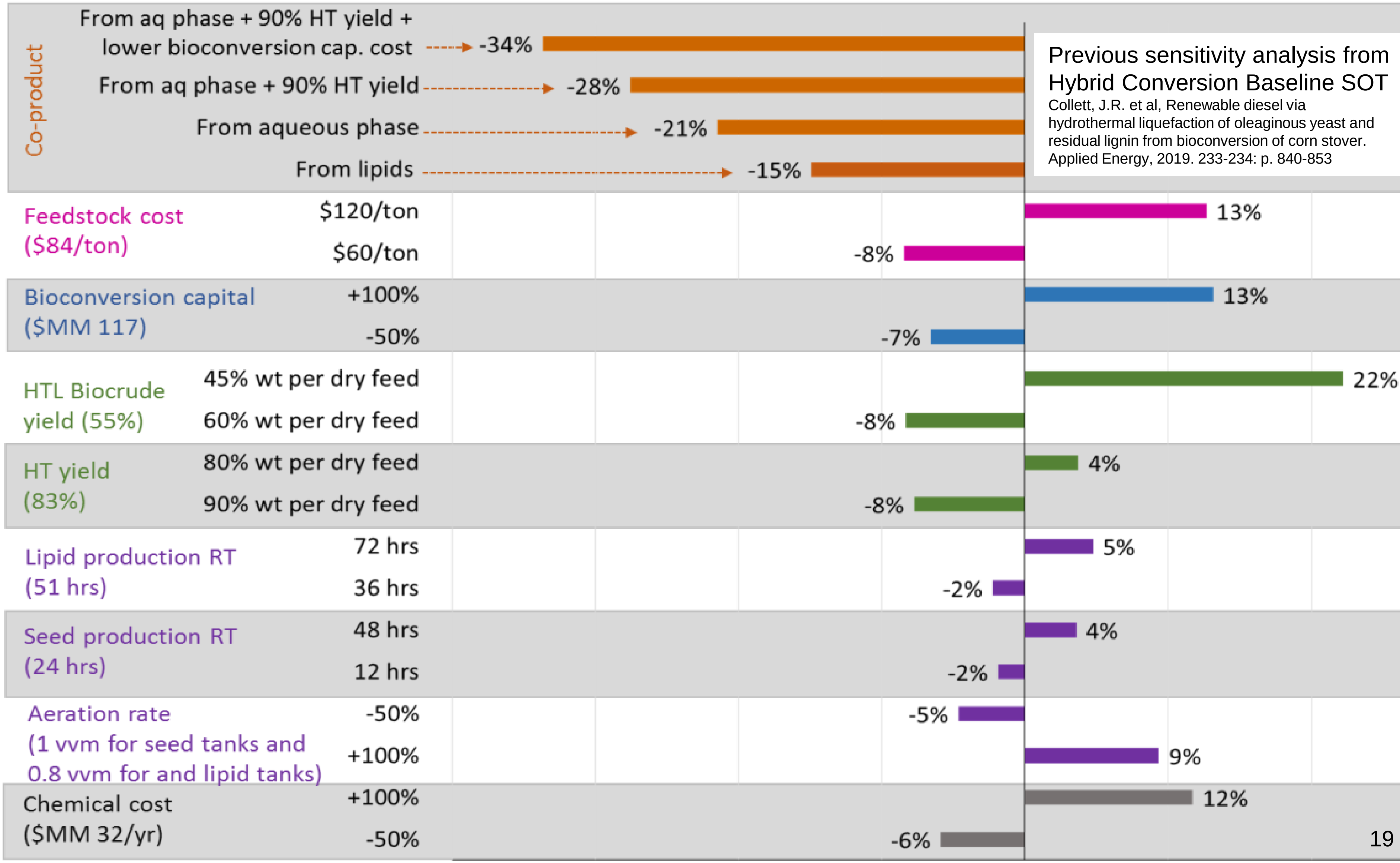
5 – Future Work – Task 3 – Techno-economic Analysis (TEA)

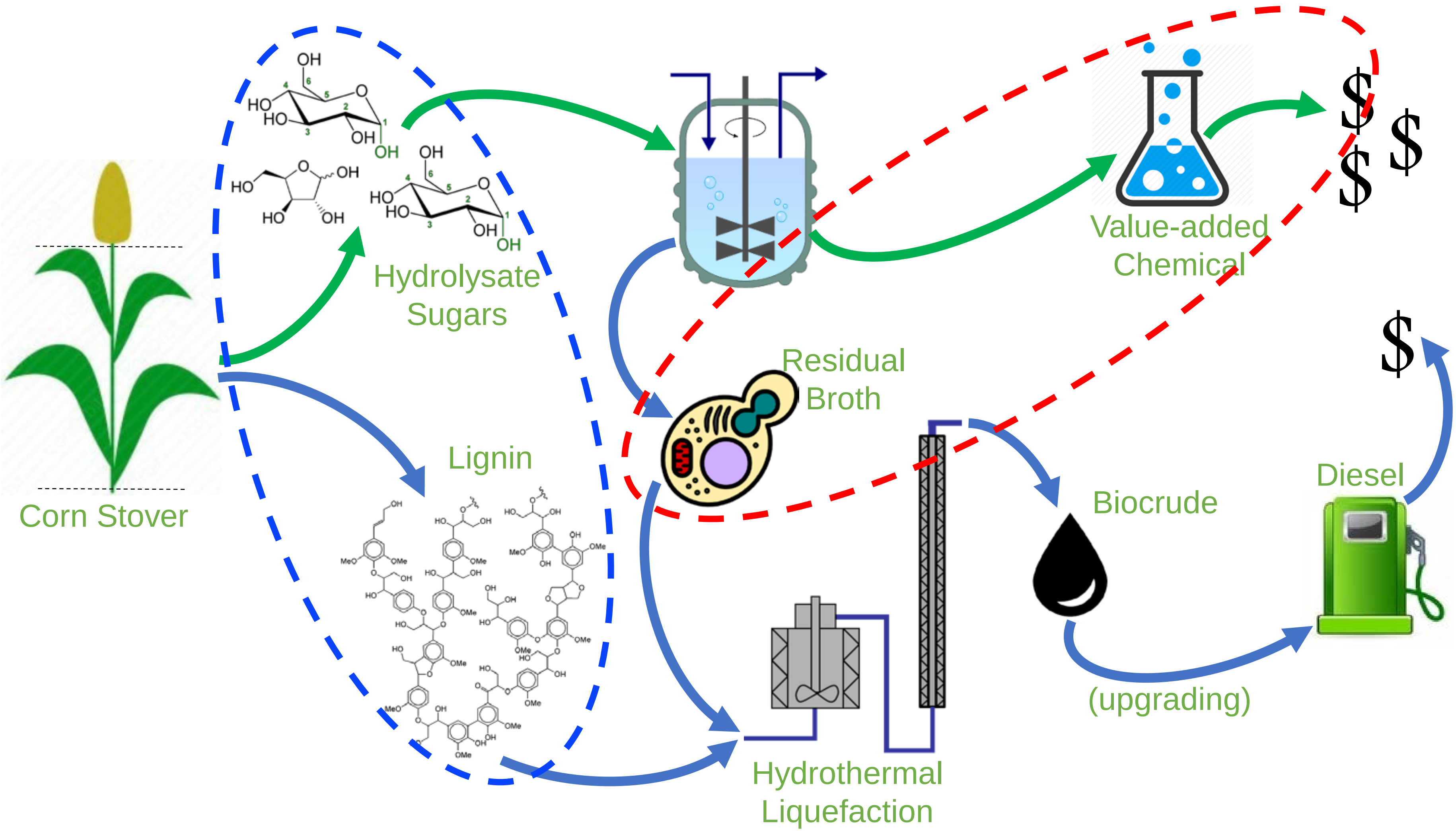
A TEA model previously developed for the fuel-only Hybrid Conversion SOT will be updated with new data to identify S&T gaps and to define R&D objectives for achieving <$3/GGE distillate fuels. *(support from 2.1.0.301 Analysis and Sustainability Interface)*



5 – Future Work – Task 3 – Techno-economic Analysis (TEA)

The new Hybrid Conversion paradigm invites consideration of:

- optimal allocation of carbon to chemical product vs. fuel
- cost/benefit of stringent enzymatic hydrolysis



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5 – Future Work

Hybrid Conversion Milestones FY19-20

12/31/2018	Establish 30-L bioreactor batch cultivation of a fungal biocatalyst on PCS hydrolysate for coproduction of a model carboxylic acid and fungal cell mass for HTL processing.
6/30/2019	Demonstrate 30-L bioreactor fed-batch cultivation of a fungal biocatalyst on DDA-PCS for coproduction of a model carboxylic acid with a titer of >40 g/L and fungal cell mass with a titer of 20 g/L to define a State of Technology. This information will be the baseline performance for Year 1 and end of project targets.
9/30/2019	Demonstrate 30-L bioreactor fed-batch cultivation of a fungal biocatalyst on DDA-PCS for coproduction of a model carboxylic acid with a titer of >60 g/L and a fungal cell mass titer of >40 g/L. Establish residual cell mass critical quality attributes for optimal blending with lignin for conversion to biocrude via HTL.
9/30/2019	Complete tests of HTL co-conversion of residual cell mass and lignin - Complete tests of HTL co-conversion of residual cell mass and lignin. Upgrade at least one sample to a finished oil product. Complete TEA for coproduction of chemicals and fuels via Hybrid Conversion to establish an FY19 SOT MFSP, and Sensitivity Analysis to prioritize R&D for attaining <$3/gge MFSP target.
9/30/2020	Demonstrate Hybrid Conversion via bioreactor coproduction of a model carboxylic acid at a titer of >80 g/L and residual cell mass stream that may be blended with lignin for HTL production of biocrude that may be upgraded to a distillate blendstock with a TEA-modeled MFSP that is 20% lower than SOT MFSP determined at the end of FY19.
9/30/2019	*Go/No-Go:* Can a bioreactor carboxylic acid titer of 60 g/L be achieved while generating residual cell mass that when mixed with lignin and converted to biocrude may be upgraded to an oil of which 75% boils in the jet to diesel range?

Summary

The goal of this project is to achieve TRL-3 validation of a new “Hybrid Conversion” biorefinery concept for achieving diesel fuel production with a minimum fuels selling price (MFSP) of <$3/GGE via coproduction of:

- a commodity chemical produced via bioconversion of lignocellulosic sugars

  and

- a high-quality distillate fuel blendstock produced from biocrude oil from hydrothermal liquefaction (HTL) of corn stover lignin, microbial cell mass, and other residual streams that are currently burned for heat and power in contemporary biorefinery designs.

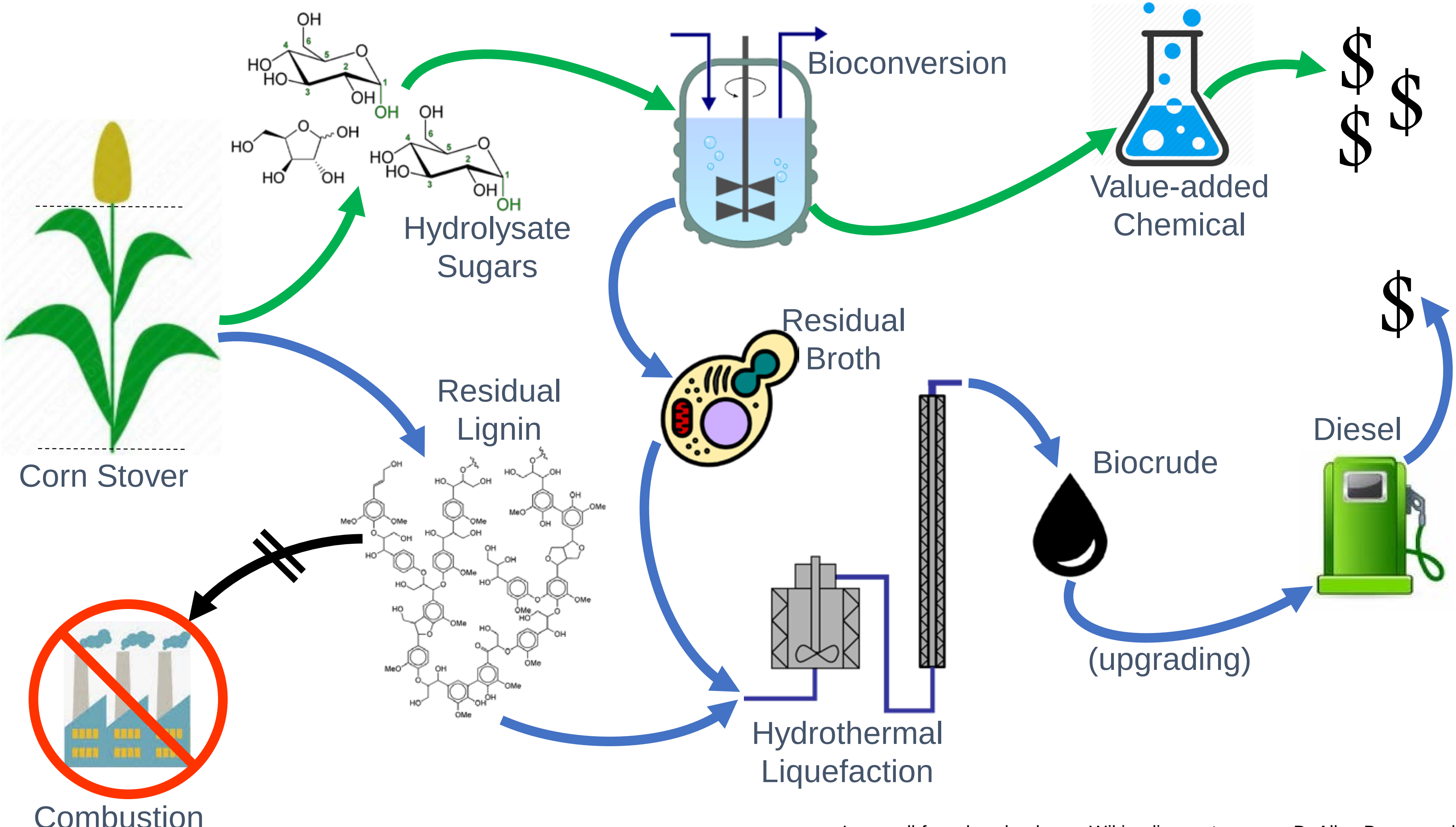
This new seed project builds upon a previous proof-of-concept study on Hybrid Conversion which projected a distillate fuel yield of 63 GGE/ton of dry corn stover without coproduction of a commodity chemical. It decreases risk in the BETO portfolio by providing a new, less complex route for lignin valorization in cellulosic biorefineries with lower installed costs compared to other current biorefinery designs.

Future work will focus on selecting on optimizing bioconversion of hydrolysate sugars to a valuable commodity chemical while producing a side stream of oleaginous residual cell mass with critical material attributes for maximizing biocrude yield and quality via HTL.

Summary

Hybrid Conversion maximizes value from biomass by:

- routing refined sugar streams into higher-value chemicals
- routing residual lignin and bioreactor broth streams into lower-priced fuels to increase overall system yield.



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Thank you

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Fungal Biotechnology Team: Beth Hofstad, Kyle Pomraning, Ziyu Dai, Diana Rodriguez, Shuang Deng, Ellen Panisko, Jon Magnuson

TEA Team: Aye Meyer, Yunhua Zhu, Sue Jones

Analytical Team: Marie Swita, Teresa Lemmon

Additional Slides

Responses to Previous Reviewers' Comments

- This is a new start for FY19

Publications, Patents, Presentations, Awards, and Commercialization

- This is a new start for FY19