DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review
(DE EE 0007104, WBS: 2.3.1.206)

Upgrading Biorefinery Waste for Bioplastics

03/09/2021
Technical Area Session
Biochemical Conversion and Lignin Utilization

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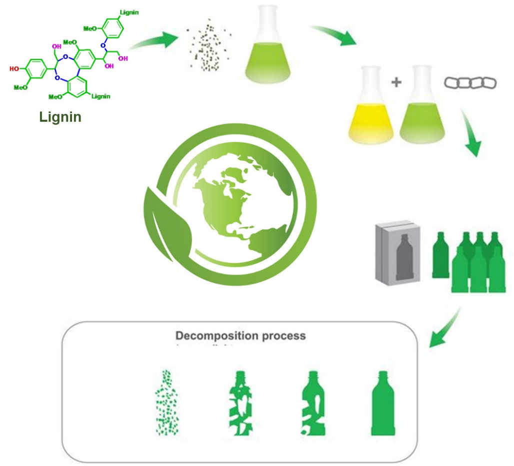
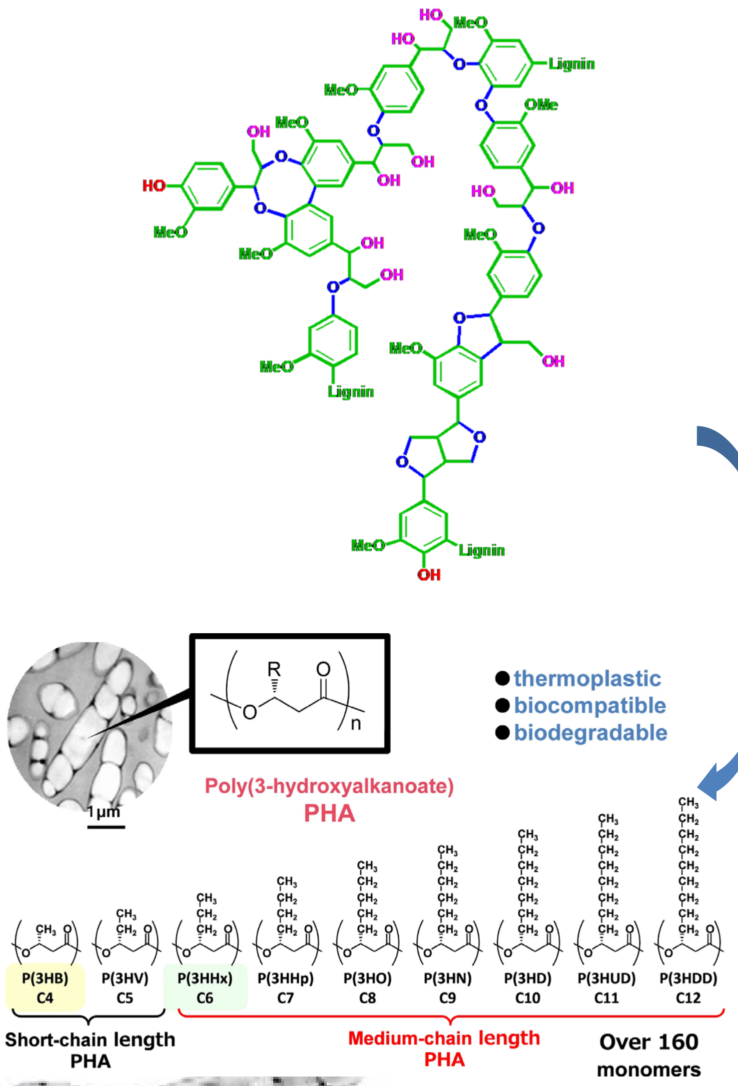
Project Overview

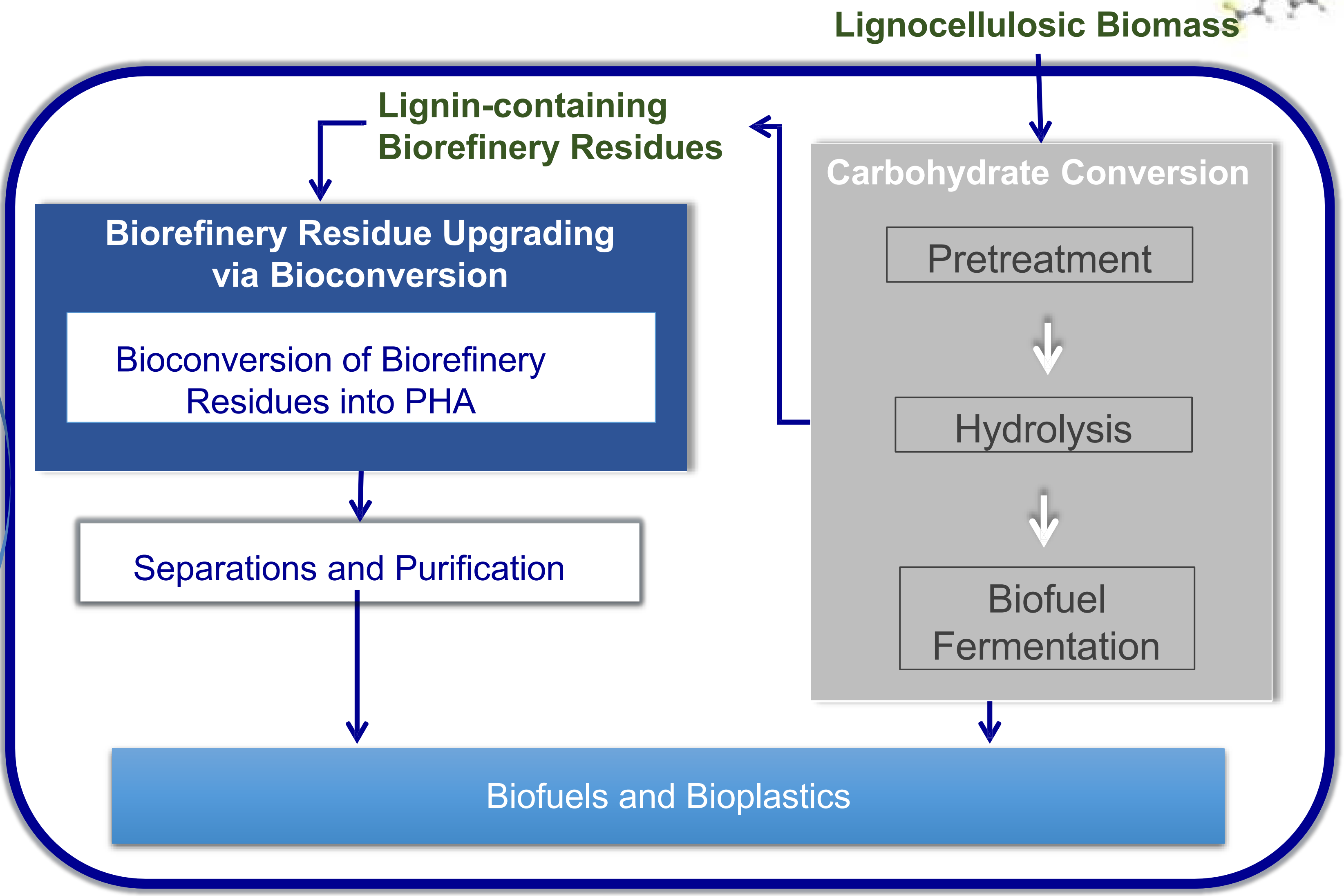
- History: FOA: DE-FOA-0001085; Topic 1: Process development and optimization of a single unit operation for the upgrading of chemically or biologically derived intermediates to fuels and products. The project successfully went through initial validation, intermediate verification, passing all milestones.
- Project Goal
  1. Develop a viable bioprocess to convert biorefinery waste to bioplastics at less than $5 dollar/Kg.
  2. Integrate technical advancements and TEA to evaluate the impact on lignocellulosic biorefinery.
  3. Overcome the key challenges for biorefinery cost-effectiveness and sustainability in the BETO MYPP. Bring down the biofuel cost to $3/GGE.
- BETO Missions Addressed:
  1. Manage biorefinery waste.
  2. Reduce carbon emission by complete biomass usage.
  3. Improve biorefinery economics and sustainability.
  4. Deliver biodegradable plastics with proper structure and properties.



Upgrading Biorefinery Waste for Fungible Products



Taguchi, *et al. Polymer J.* 53, 67–79



Liu, *et al. Renew Sustain Energy Rev, 105, 349-362.*

Project Overview: Heilmeier Catechism Summary

- What we are trying to do?
  - Transform biorefinery economics and sustainability with the value-added bioplastics production from lignin-containing waste stream.
  - Produce structure-beneficial and cost-effective bioplastics.
- What is the state of the art? What is the limit?
  - Part of the lignin waste are burned to power the operation, and the remaining were disposed as waste stream.
  - Limited value recovery and low overall carbon efficiency – The success of modern biorefinery depends on the value-added products such as DDGS.
- Why is the project important?
  - Bring down the biofuel price to competitive range to enable lignocellulosic biorefinery ($259 billion economic potential and 1.1 million jobs).
  - Address the fossil fuel plastics challenge with low cost and structurally controlled PHAs.
  - Improve biorefinery and environmental sustainability by turning waste to bioplastics ($10.5 Billion market size and 22% annual growth).
- What are the risks?
  - Lignin recalcitrance and chemical features not suitable for conversion.
  - Integration with biorefinery is challenging: the waste stream is insoluble and contains inhibitors

Management and Team

DOE BETO Program Manager

Institute Support
Patent application
Commercial partner engagement
Project management

Industrial Collaborators
Evaluate technology readiness
Identify commercialization risks
Make suggestions for directions
Promote commercialization

Leading PI
Coordinate report
Management
Facilitate communication
Implement Milestone

Process Enablement
Yuan, Pierson, Gross, Dai, TAMU

Process Development
Yuan, TAMU; Ragauskas, UTK; Yang, WSU
Emme, ICM

Process Scale-up & TEA
Emme, ICM
Yang, WSU
Yuan, TAMU
ABPDU

A|M
THE UNIVERSITY of TENNESSEE UT KNOXVILLE
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Management

- Defined and measurable milestones were laid out for technology development and commercialization.
- Go/No-Go milestones were set at the end of each year and each of the two budget periods. BP1 ends at 24 months.
- Monthly group teleconferences with the team were implemented to evaluate the progresses against milestones. Risk mitigation strategies were designed and technoeconomic analysis guided the development.
- Monthly teleconferences between the PI and the program management are implemented to evaluate progresses, mitigate risks, and address challenges.
- Engage industrial partners and advisors including ICM inc. and others for deliverables relevant to EERE MYPP.
- Integrate TEA throughout the project to ensure the relevance of the project outcome.

Management Approach – Go/No-Go Milestones

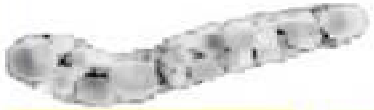
Time Point	Benchmark	End of BP1	End of the Project
Metrics	Milestones	Milestones	Milestones
Titer	0.04g/L	2.5g/L	8.4g/L
Efficiency	N.A.	30%	40%
MPSP[1]	N.A.	N.A.	$5/Kg
MESP[2]	N.A.	N.A.	N.A.
~$/GGE[3]	N.A.	N.A.	~$3/GGE

Technical Advance-ments Derive Economic Output

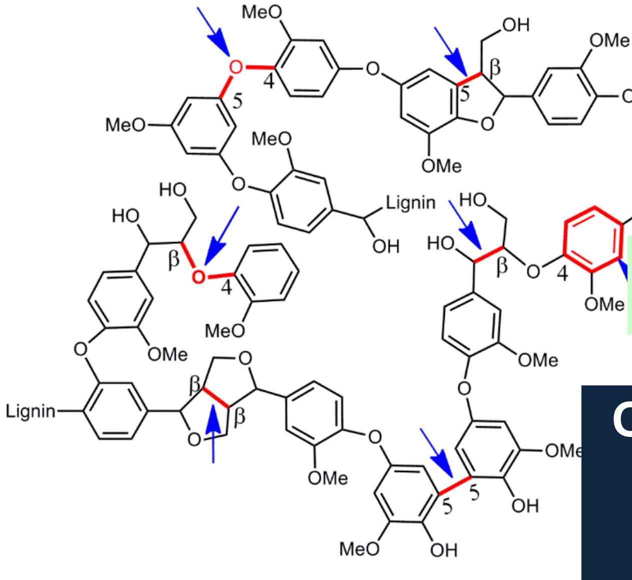
TEA Informs Technology Develop-ment

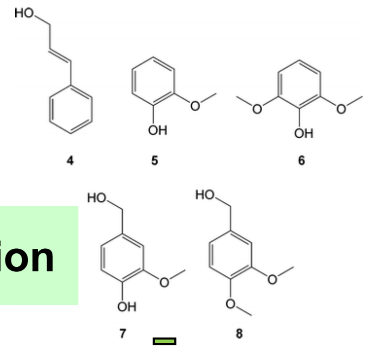
1. Minimal PHA Selling Price
2. Minimal Ethanol Selling Price
3. Gasoline Gallon Equivalent

- **Defined S.M.A.R.T. Go/No-Go milestones were set and implemented to ensure project progresses.**
- **The technical milestones were designed in a way to ensure that the economic targets can be achieved. Full ASPEN model was built.**
- **Frequent communications within the team and with BETO project management to measure against the milestones.**
- **StageGate and Verification to gain inputs for project enhancement.**



Technical Approach – Advancing the State-of-the-Art

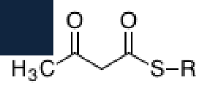




Lignin depolymerization

Obj. 2. T1: Pretreatment and fractionation advancement to depolymerize lignin

Aromatic compound conversion



Obj. 2. T2: Ferm. Opt. to improve conversion efficiency

Obj. 3. Process scale-up, integration, and evaluation: Techno-economic analysis to derive MPSP and MESP

Obj. 1: Strain screening (Task 1) and engineering (Task 2) to achieve efficient conversion of lignin and aromatics into PHA

PHA production And accumulation

PHA Production

Challenges for efficient lignin bioconversion – Recalcitrance of lignin.

- Redox reactions instead of hydrolysis.
- Diverse chemical linkages.
- Diverse aromatic compound monomers
- Diverse chemical intermediates

Zhao, et al. *Green Chem.* 18:1306-12.
Xie, et al. *Ind. Biotech.* 12: 161-167.
Xie, et al. *ACS Sust. Chem. Engin.*, 5:2215-2223

Technical Approach – Holistic Efforts to Achieve Deliverables

Time Point	Benchmark	End of BP1	End of the Project
Metrics	Milestones	Milestones	Milestones
Titer	0.04g/L	2.5g/L	8.4g/L
Efficiency	N.A.	30%	40%
MPSP[1]	N.A.	N.A.	$5/Kg
MESP[2]	N.A.	N.A.	N.A.
~$/GGE[3]	N.A.	N.A.	~$3/GGE

Process Evaluation – technoeconomic analysis

Objective 3
Process Optimization &Scale-up

Objective 1
Process Enablement

Strain Screening – broad carbon source and lignin utilization

Strain Engineering – systems biology-guided design for efficient conversion

Process Scale-up – integration with biorefinery

Objective 2
Process Development

Pretreatment & Fractionation Optimization – lignin processibility

Fermentation Optimization – process development

Risk Mitigation:

1. Holistic approach to address all technical barriers.
2. Go/No-Go milestones directly tied to the deliverables.

Technical Approach – Task Integration

Objective 1 Process Enablement

- Strain Screening – broad carbon source and lignin utilization
- Strain Engineering – systems biology-guided design for efficient conversion

Objective 2 Process Development

- Pretreatment & Fractionation Optimization – lignin processibility
- Fermentation Optimization – process development

Objective 3 Process Optimization &Scale-up

- Process Evaluation – technoeconomic analysis
- Process Scale-up – integration with biorefinery

Responses to Previous Review:

1. Complete ASPEN model to integrate the process with biorefinery.
2. More emphasis on biorefinery design than enzyme deploymerization.
3. More refined mass balance and conversion ratio to integrate with TEA.

Integration for deliverables:

1. Technical advancements were evaluated in TEA in relevance to achieving milestones, MPSP and GGE targets.
2. TEA identifies the economic drivers to guide the R&D.
3. Holistic integration of metabolic and process engineering to achieve the economic targets.

Broad Scientific Impact and Transformative Industrial Impact

- Tangible and Transformative Industrial Impact
  1. Develop a viable bioprocess to convert biorefinery waste to bioplastics at less than $5 dollar/Kg.
  2. If the PHA is sold at $5/Kg, the project will bring down the MESP to below $3/GGE range.
  3. Constantly engage biorefinery companies like ICM, ADM, and POET.
  4. We have scaled up the technology with Altex for whole biomass conversion to bioplastics.
  5. We have engaged with Danimer Scientific to work on additional funding to produce bioplastics composite for packaging industries, addressing the concerns from previous review.
  6. We obtained one licensing agreement.
- Broad Energy and Environmental Impacts– Well Addressing BETO Missions
  1. Improve biorefinery sustainability and cost-effectiveness with value-added products from waste.
  2. Address the plastics challenges by providing structure-controlled performance-enhancing PHA.
- Broad Scientific Impacts
  1. 44 publications with total impact factors at 271.
  2. One PCT patent application, and one patent disclosure filed.
  3. Numerous scientific presentations and special events to engage companies.

Progresses and Outcomes

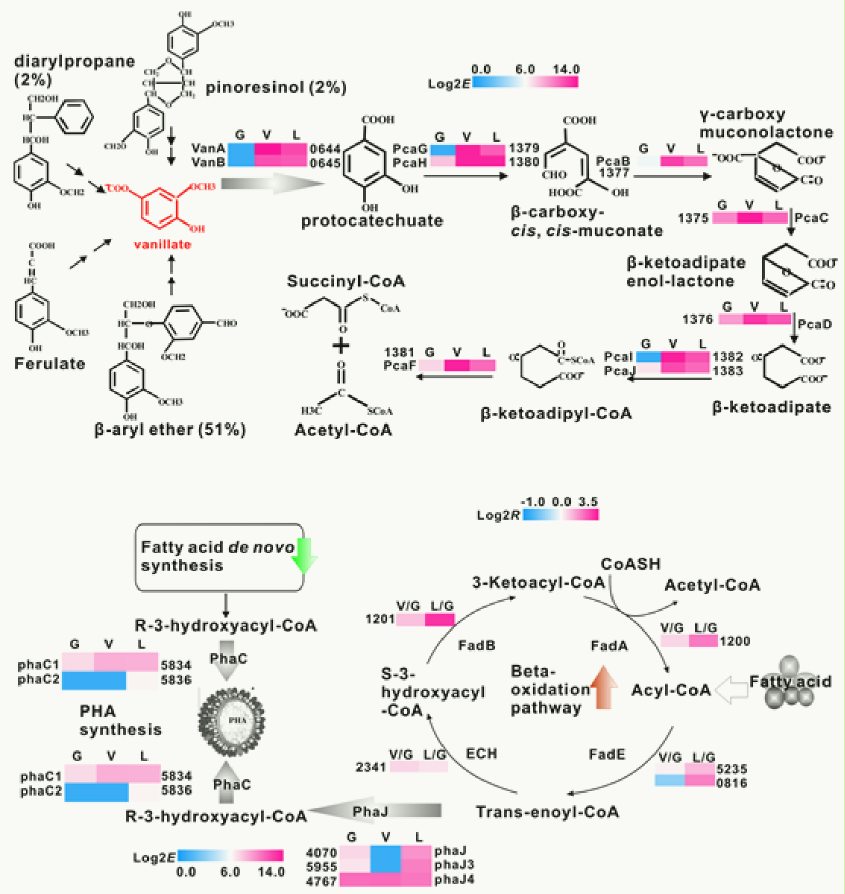
Time Point	Benchmark		End of BP1		End of the Project	
Metrics	Milestones	Actual	Milestones	Actual	Milestones	Actual
Titer	0.04g/L	0.1g/L	2.5g/L	4.6g/L	8.4g/L	10 g/L
Efficiency	N.A.	2.5%	30%	>40%	40%	>40%
MPSP[1]	N.A.	$240	N.A.	$6.07	$5/Kg	$2.06/Kg
MESP[2]	N.A.	N.A.	N.A.	N.A.	N.A.	$1.62/Gal
~$/GGE[3]	N.A.	N.A.	N.A.	N.A.	~$3/GGE	$2.43/GGE

40 times increases of titer, 20 times of efficiency, and 40 times decrease of MPSP.

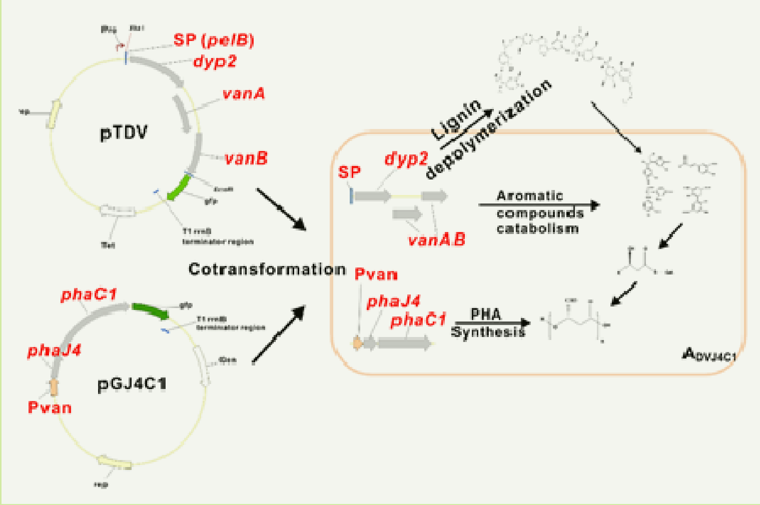
2 times increase of titer, further efficiency increase, and 3 times decrease of MPSP.

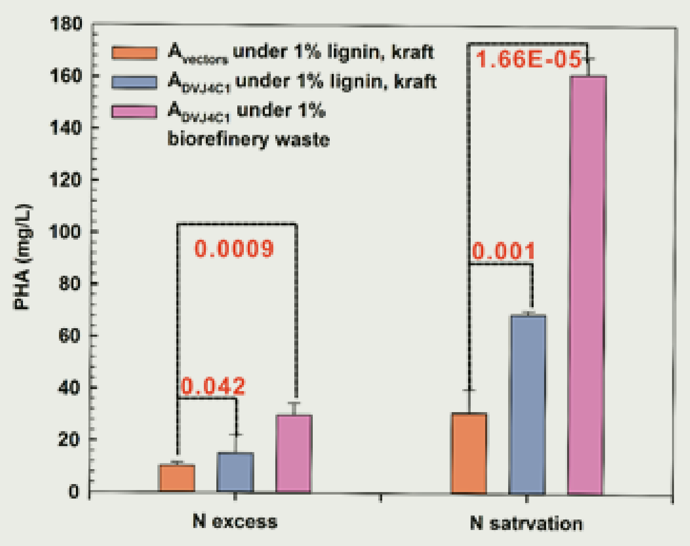
1. The project has achieved all milestones including both the technical and economic ones.
2. The project has led to 44 publications, one patents, and one disclosure.
3. The technical achievements have enabled the economic targets.
4. The project is being scaled up to 50 Liter scale.

Systems Biology-guided Strain Screening and Engineering for Better Conversion



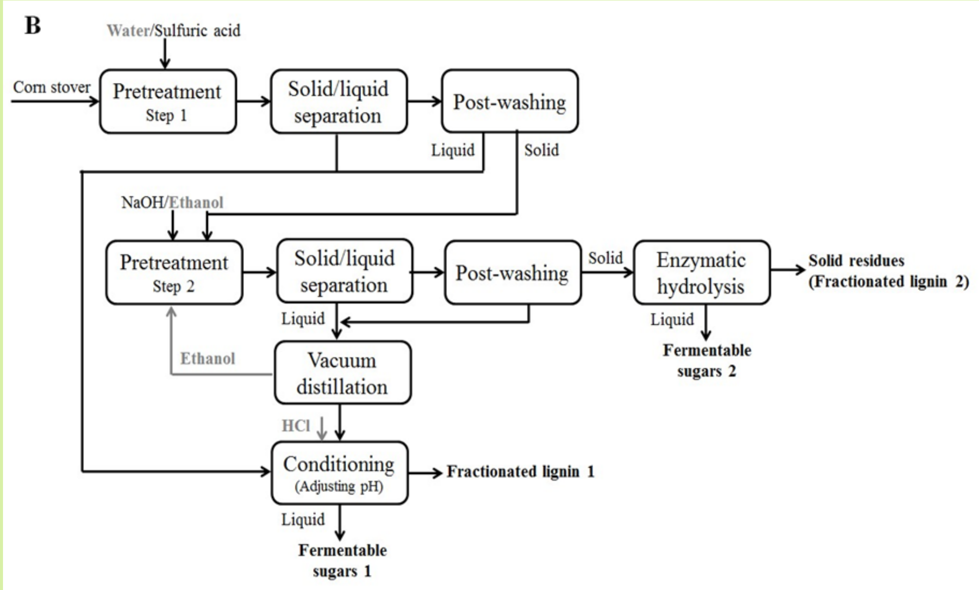
Eight times of increase in lignin-to-PHA conversion titer using AFEX derived lignin.

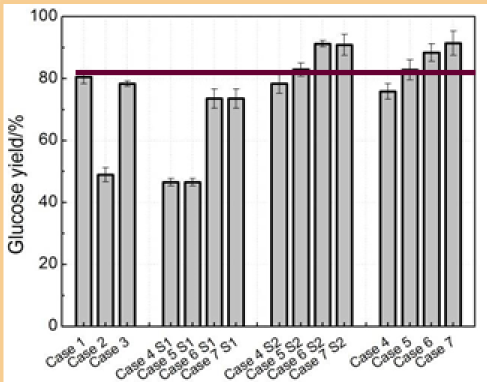


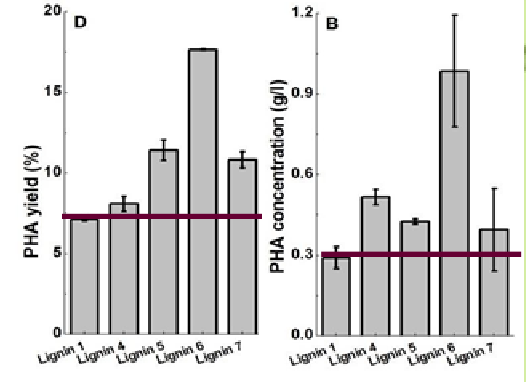


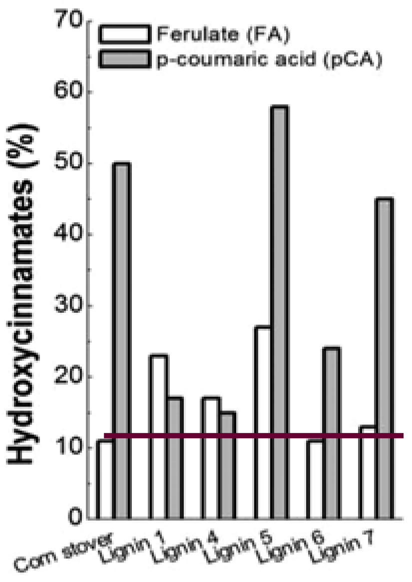
Lin, et al., *Green Chemistry*, 18: 5536-5547.

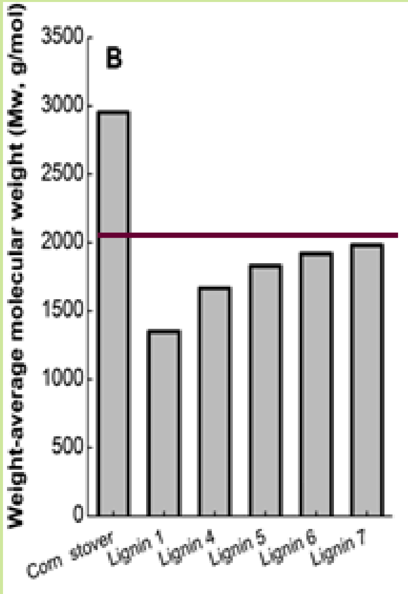
Bioconversion Improvement through New Pretreatment and Fractionation Techs









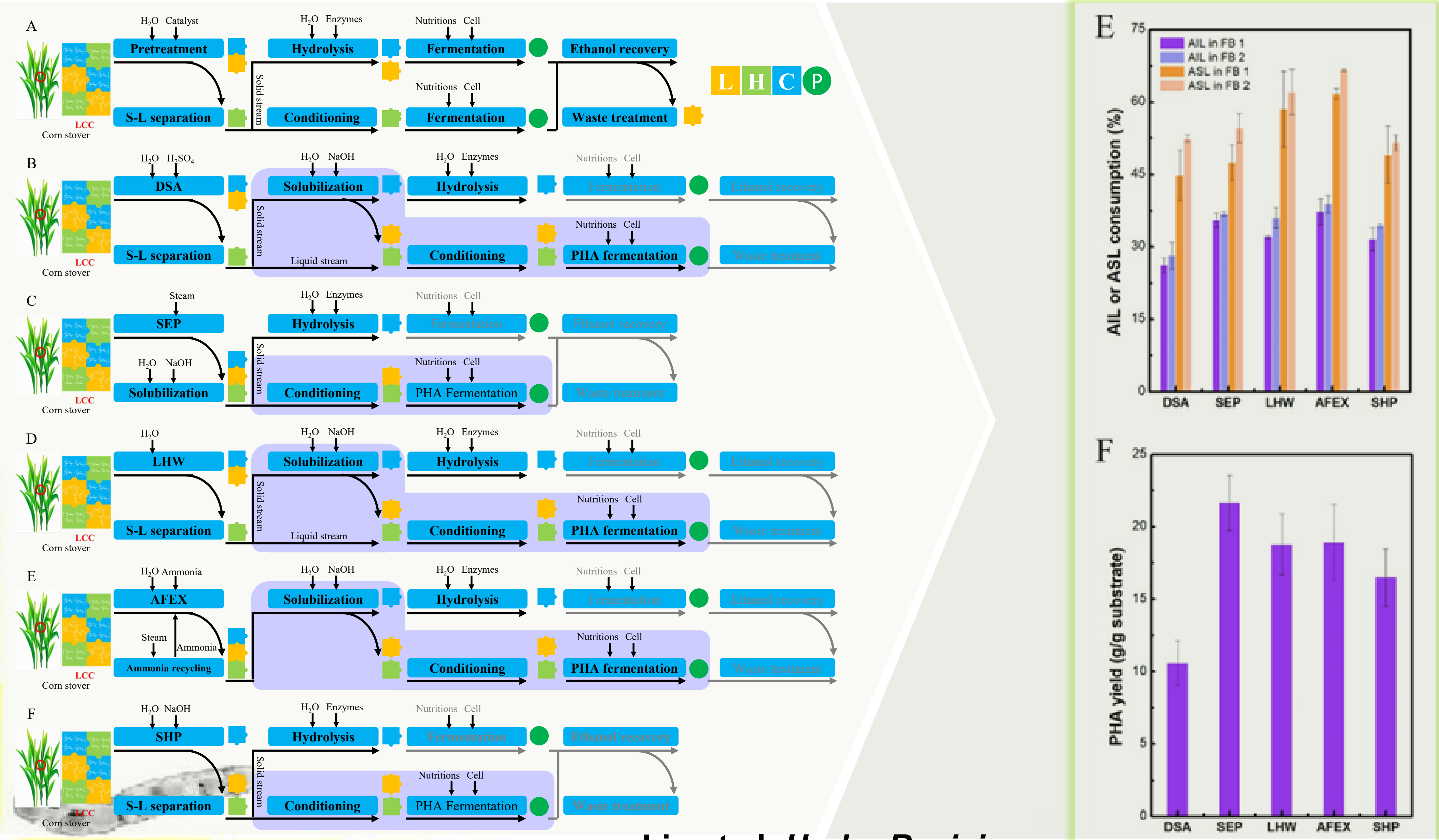


Technical Achievement: Simultaneous improve carbohydrate and lignin conversion. >2 times increase of efficiency and >3 times increase of titer in lignin-to-PHA routes.

Scientific Discovery: Less uniform, low molecular weight lignin are more bioprocessible.

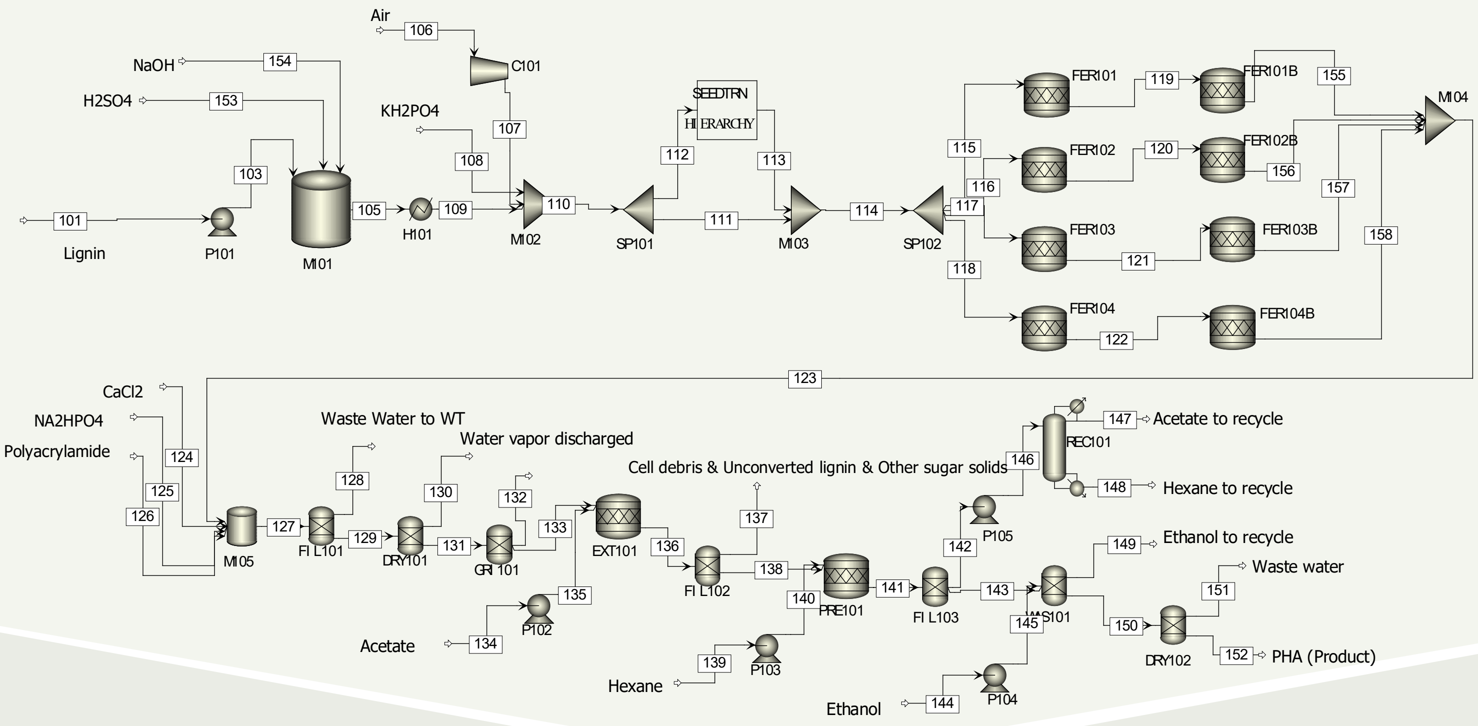
Liu et al. *Green Chemistry*, 19, 4939-4955
Xie et al. *ACS Sust. Chem. Engin.*, 5:2215-2223.
Liu et al. *Biotech. Biofuel*, 11: 21
Liu et al. *Sust. Energy Fuel*, 3 (8), 2024-2037.

Plug-in Process of Lignin (PIPOL) to Transform Current Biorefinery Designs



Liu et al. *Under Revision*

A Complete ASPEN Model for PIPOL and Lignin Processing



	Scenario 1 DSA-PIP	Scenario 2 SEP-PIP	Scenario 3 LHW-PIP	Scenario 4 AFEX-PIP	Scenario 5 SHP-PIP
Annual production/MMkg	2.19	3.21	2.6	3.54	2.02
Total capital cost/MM$	44.98	42.64	42.13	39.85	42.12
Total operation cost/MM$/yr	11.2	12.42	12.26	13.24	14.65
Raw material/MM$/yr	4.88	5.9	5.77	7.4	8.12
Utilities/MM$/yr	0.268	0.267	0.266	0.27	0.266
Unit cost/$/kg	7.52	5.49	6.73	5.05	9.9
Rate of return/%	10	10	10	10	10
Minimum selling price/$/kg	9.58	6.82	8.32	6.18	11.99

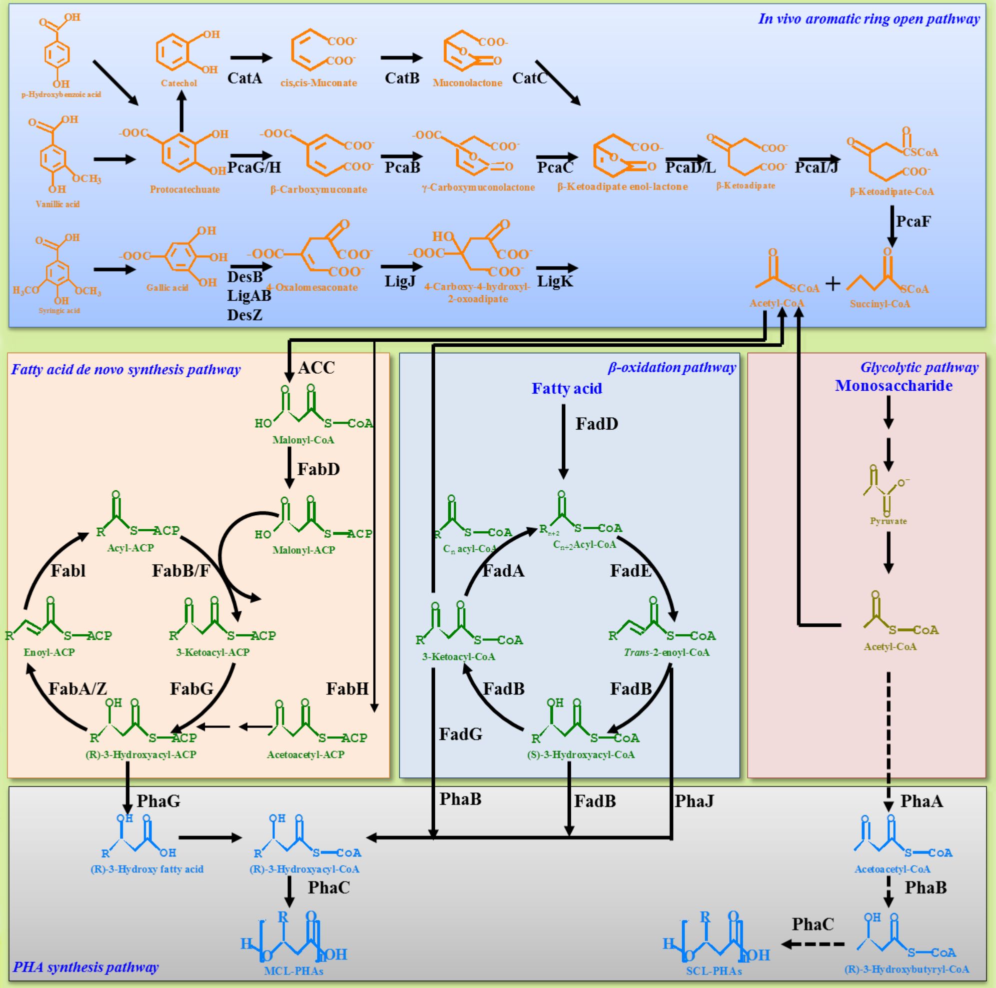
Technical Achievement: PIPOL has improved carbohydrate conversion in leading pretreatments and enabled the lignin streams from these pretreatments to be used for bioplastics production.

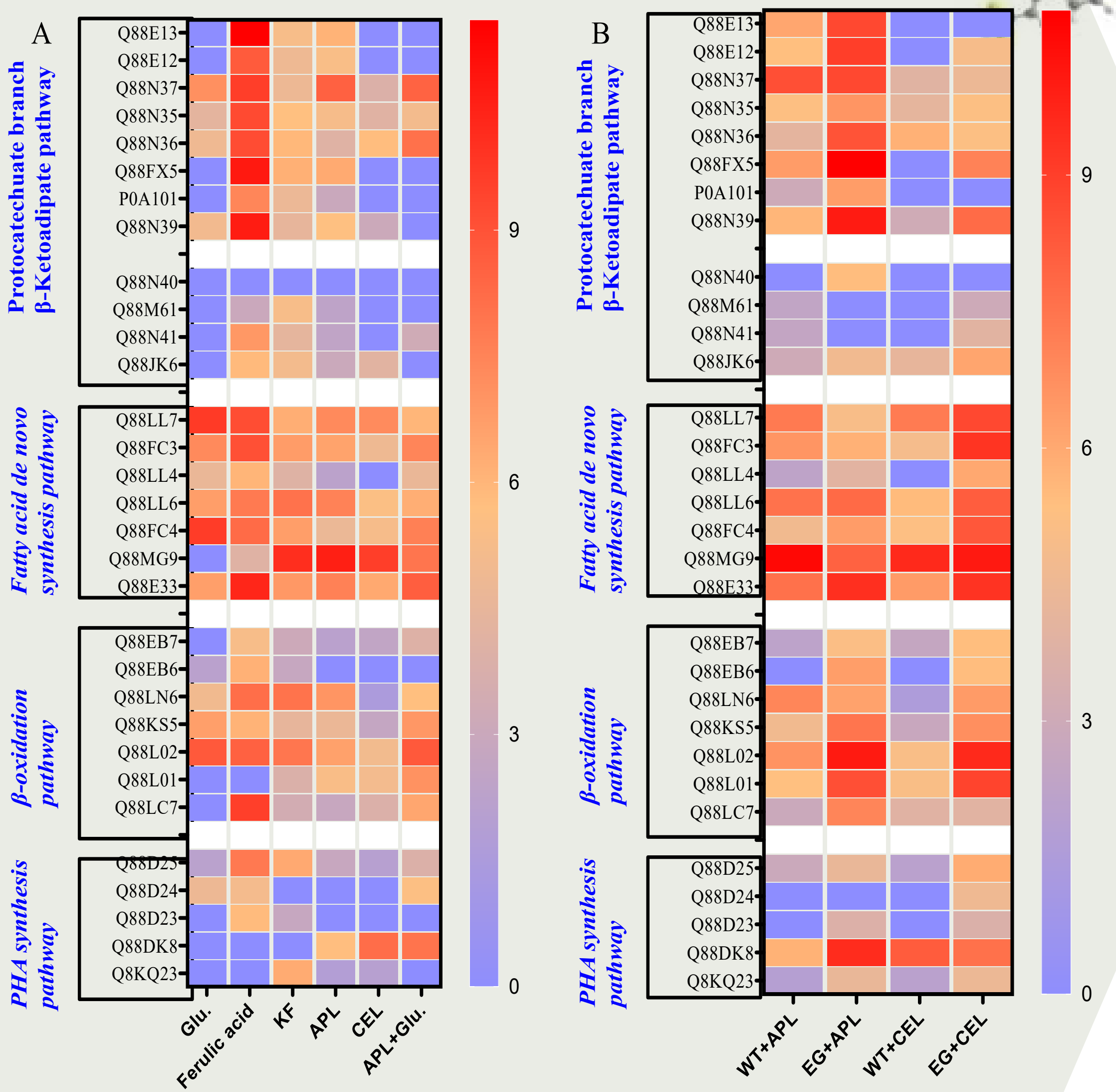
Economic Relevance: A full ASPEN model revealed that the AFEX and SEP can be integrated with PIPOL to produce market competitive bioplastics

Scientific Discovery: Fractionation of lignin residues to low molecular and solubilized components will improve bioconversion.

Liu et al. *Under Revision*

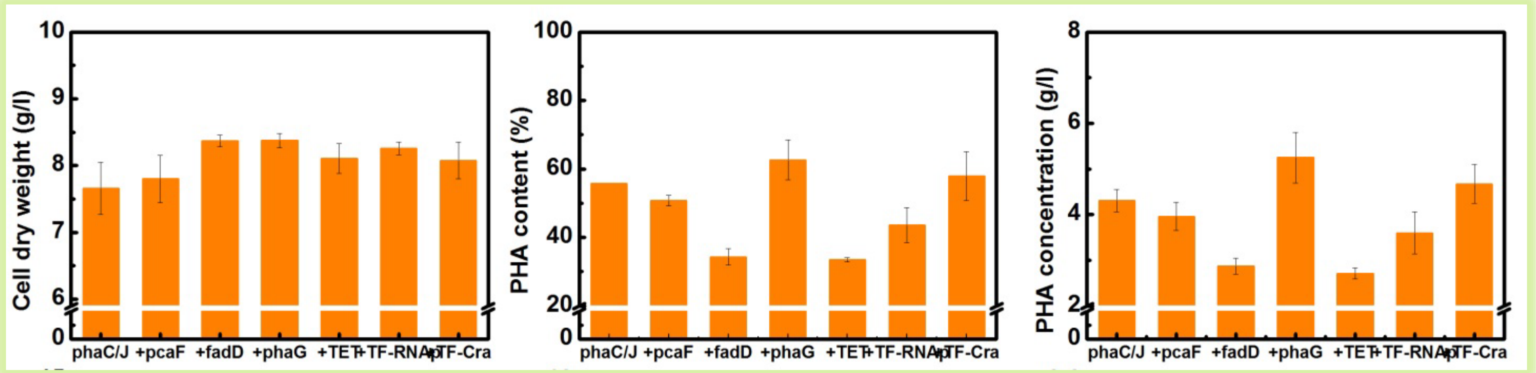
Reiterative Systems Biology Analysis to Improve PHA Conversion

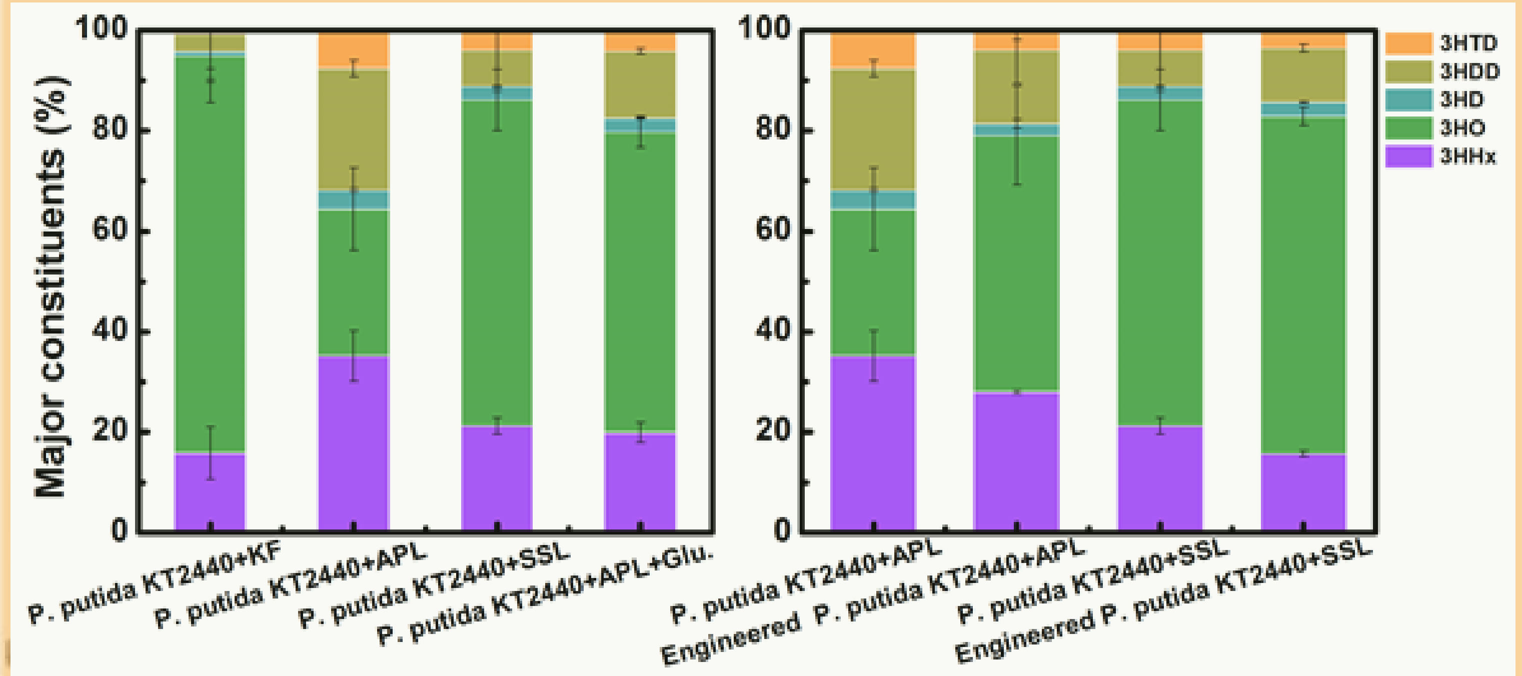




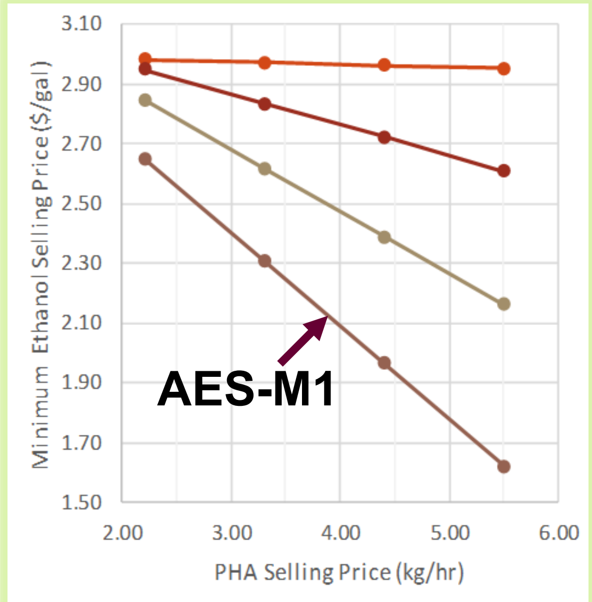
Liu et al. *In Preparation*

Reiterative Systems Biology Analysis to Improve PHA Conversion





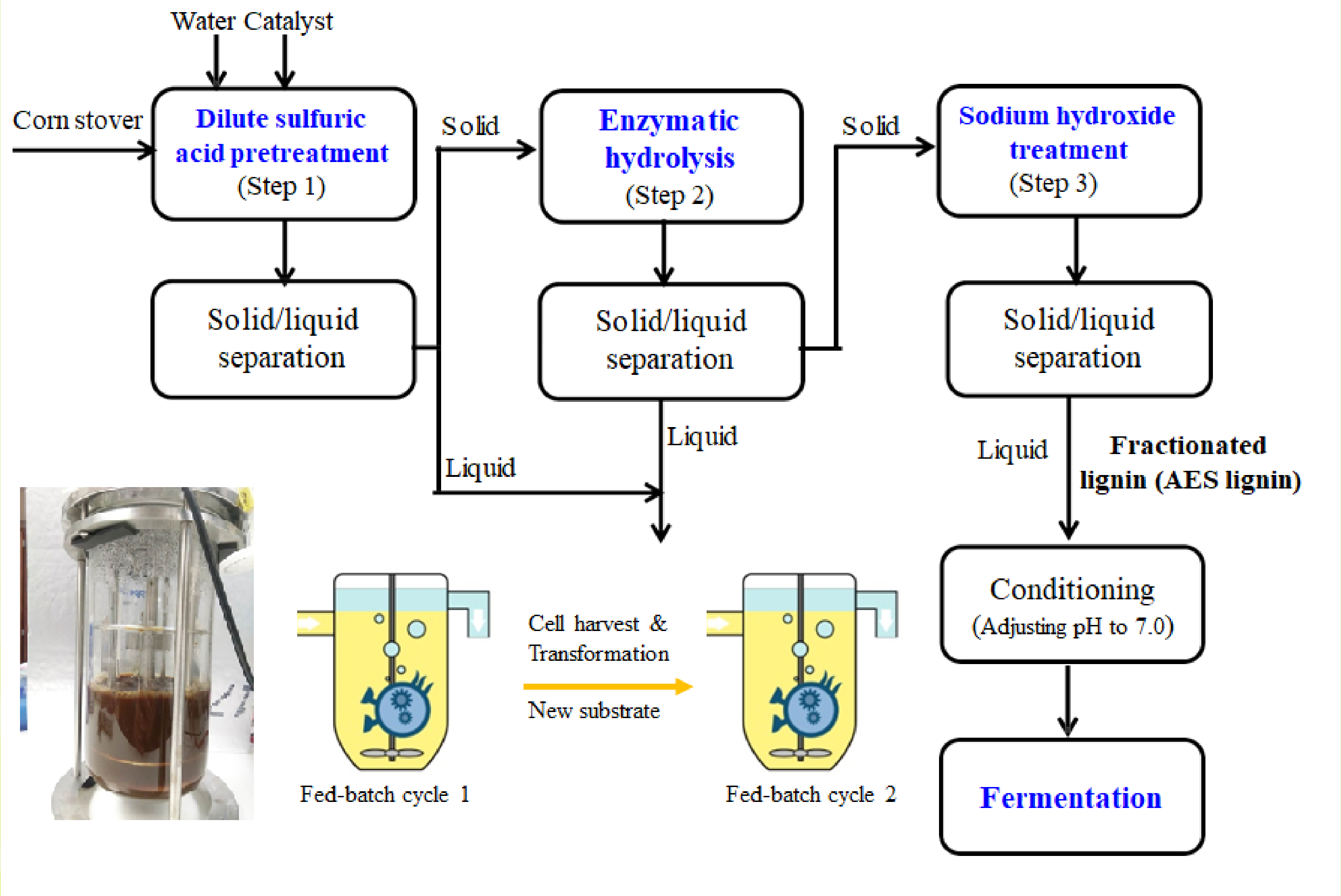
	UNITS	AES-M1	AFEX-PIPOL-M2
Annual production	MMkg	11.425	12.191
Total Capital Cost	MM$	45.10	45.12
Total Operating Cost	MM$/yr	11.79	14.93
Raw Material	MM$/yr	5.63	8.35
Utilities	MM$/yr	0.32	0.32
Unit cost	$/kg	1.55	1.71
Rate of return	%	10	10
MPSP (PHA)	$/kg	1.94	2.08

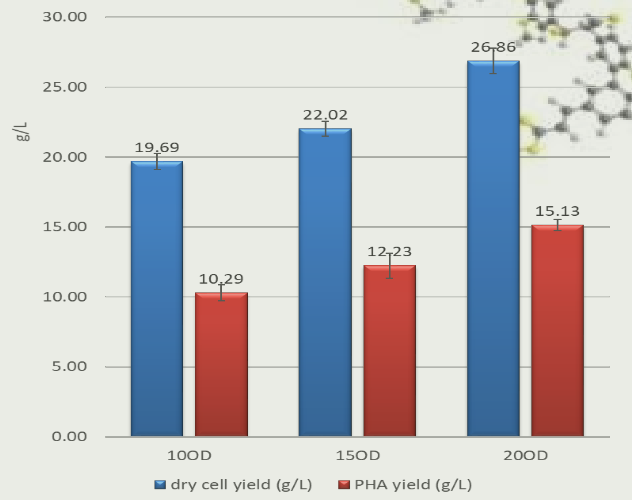


Reiterative biodesign delivers two strains to bring PHA price down to $2 dollar range with uniform structure, enabling MESP at $1.6 range

Liu et al. *In Preparation*

Process Scale-up at 1 Liter and 50 Liter Scale





MPSP ($/kg)	If sold at :	NEW MESP	MESP reduction
2.06	2.2	2.65	-0.18
	5.5	1.62	-1.21

Scale-up at 50L scale at ABPDU, LBNL

The technology has been scaled up at 1 liter scale to achieve targeted performance and all milestones. We are working on scale up at 50 Liter scale in ABPDU.

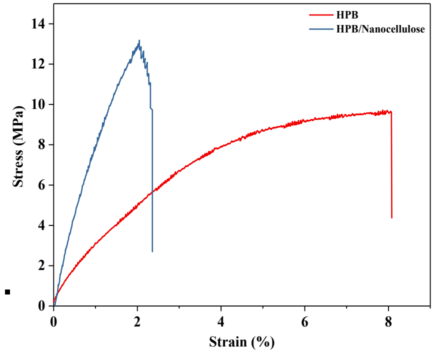
Summary of Progresses

- The project takes a holistic approach to integrate microbial strain screening, engineering, biorefinery design, process integration and scale up to achieve record level of efficiency, titer, and productivity for converting biofinery waste lignin into PHA.
- The technical advances allows us to achieve all milestones, including the Go/No-Go Milestones. Current technology validation is at 1 Liter scale. The technical metrics includes >40% conversion efficiency, >10g/L titer, <$2.5/Kg PHA, and approximate $1.6/gallon ethanol, and approximately $2.5/GGE.
- The technology integration with both current biorefinery configurations and new biorefinery designs were evaluated and the economic drivers were identified.
- The project emphasizes on biorefinery integration, instead of model compounds or other lignin sources. The deliverables thus can directly translate into biorefinery solutions.
- The fundamental understanding on lignin chemistry, microbial systems biology, and biorefinery design can guide the future biorefinery advancements.

Summary

The project delivered innovative solutions for converting biorefinery waste to bioplastics, transforming biorefinery sustainability and cost-effectiveness, along with low-cost bioplastics.

1. Approach and Team
   - Process enablement by microbial engineering
   - Process development by fractionation and fermentation improvement
   - Process integration with biorefinery by TEA and scale-up.
   - Vigorous management to ensure Go/No-Go milestones are met, and these milestones integrates technology and economics to ensure the project deliverables is relevant to biorefinery industry.
2. Impact
   - The project is directly addressing MYPP goals.
   - Aspen Plus model showed that the process can significant reduce MESP and produce bioplastics at ~$2.
   - The project produces cost-effective bioplastics with more uniform structure, potentially for improved performance.
3. Technical Accomplishments/Progress/Results
   - Innovative engineered microbial strain for efficient conversion of lignin to PHA
   - Innovative pretreatment/fractionation design to improve lignin processiblity and enable high PHA yield by co-conversion of lignin and Residue sugar.
   - >40% conversion efficiency, >10g/L titer, ~$2/Kg PHA, and ~$1.6 MESP ($2.5 GGE).
   - The project achieved all milestones at 1 Liter scale with 44 publications, and 2 patents.
   - The project is being scaled up at ABBPDU with 50 Liter scale.



Quad Chart Overview

Timeline

Project start date: 07/01/2016
Project end date: 12/31/2021
Percent complete: 95%

	FY 19 Costed	FY20 Costed	Total Award
DOE Funding	$832,720	$547,572	$2,353,955
Project Cost Share	$265,122	$395,644	$788,747

Project Partners*

Texas A&M University: 50%
University of Tennessee/Oak Ridge National Lab: 30%
Washington State University: 15%
LNBL.: 5%
ICM inc.: Initial Partner; ABPDU: Scale up.

Project Goal

1. Develop a viable bioprocess to convert biorefinery waste to bioplastics at less than $5 dollar/Kg.
2. Integrate technical advancements and TEA to evaluate the impact on lignocellulosic biorefinery.
3. Overcome the key challenges for biorefinery cost-effectiveness and sustainability in the BETO MYPP. Bring down the biofuel cost to $3/GGE.

End of Project Milestone

At the end of the project, we achieved:
8.4g/L PHA titer;
40% conversion of lignin to PHA;
Less than $5/Kg of PHA price.

Currently, we have achieved:
>10g/L PHA titer;
>40% conversion of lignin to PHA;
~$2/Kg PHA
~$1.6/Gallon Ethanol and $2.5GGE

Funding Mechanism

FOA: DE-FOA-0001085; Topic 1: Process development and optimization of a single unit operation for the upgrading of chemically or biologically derived intermediates to fuels and products.

Acknowledgement

Project Management:

Joshua Messner   Marykate O'Brien   Jay Fitzgerald

CoPIs:

Dr. Art Ragauskas
Dr. Jeremy Javers
Dr. Dennis Gross
Mr. Brandon Emme

Dr. Bin Yang
Dr. Susie Y. Dai
Dr. Betsy Pierson
Dr. Ning Sun

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Responses to Reviewers’ Previous Comments

- We appreciate the reveiwer's comment that "significant progress has been made under lab- and benchscale conditions." In response to Reviewers’ request, we have built a complete ASPEN Model for TEA. The technology has been scaled up to 1Liter scale and the 50Liter scale-up plan is finalized. The integration with both current biorefinery configurations and our new biorefinery designs were evaluated using the TEA model, and the results highlighted the potential of the project to bring down MESP to about $1.6/Kg and the ethanol fuel to $2.5/GGE.
- We appreciate the reviewer's acknowledgement of "very nice results” in a “challenging project”. Regarding the two times of differences to achieve final outcome. We have achieved the goal and all milestones at 1 Liter level, thanking partly to the inputs from peer review, stage-gate, and intermediate verification. We also agreed with the reviewer on the challenges in scale up and is working on the 50Liter scale up. Moreover, we have worked with Altex Technologies to scale up the technology at large scale for full biomass conversion into PHA. In addition, for addressing the concern over PHA price point, we have built an ASPEN model with sensitivity analysis of MEPS’ dependence on PHA price. We are also exploring the biodegradable material design to produce composites for broad packaging applications. In working with Danimer Scientific, we believe such broad applications will avoid PHA market saturation.

Responses to Reviewers’ Previous Comments

- We appreciate the reveiwer's acknowledgement of the success of the CLARS platform. We agree with the reviewer on calculating the detailed conversion. We have obtained the detailed mass balance for the processes and integrate with ASPEN models. All current conversion rates are based on mass balance. Ferulic acid serves as a simplified model compound reference only. In any scaled up process of 1 Liter or 50 Liter, no laccase is used as it is not economic. We have developed non-enzyme-based lignin fractionation technologies.
- We appreciate the reviewer acknowledged a ‘scientifically interesting’ proposal with ‘novel concept of biochemical lignin conversion.’ In response to the reviewer’s suggestion, we have built a complete ASPEN model and obtained detailed mass balance to identify the economic driver and platform performance. The comprehensive analysis allows the better ‘quantification of active substrates’. PHA is a perfect target for biorefinery products due to: (1) higher value, (2) large market potential due to the recent plastics waste management crisis, (3) serving as one of the many products for lignin utilization, and (4) funneling diverse aromatics into single products to reduce the separation challenge.
- We appreciate the reviewer acknowledging the holistic approach and ‘a range of accomplishment’. In response to the reviewer’s comment, we have carry out detailed mass balance analysis for each process. The study highlighted that lignin, especially the fractionated lignin’ utilization for PHA is the major driver for low cost of PHA. Scientifically, the ring opening of lignin monomers will produce organic acid-type compounds, which are preferred substrates for *P. putida* to produce PHA.

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A total of 44 publications were published with total Impact Factor at over 270.

Patent and Commercialization

- **The project has led to two patent applications.**

1. J.S. Yuan, et al., "Conversion of lignin into bioplastics and lipid fuels", PCT/US2016/024579, WO 2016154631 A1 – The PCT patent is at US and EU application stage.
2. B. Yang, and Z. Xu, "Method for Production of Polyhydroxalkanoates and Uses Thereof", PCT /US2017 /057092, April 11, 2019.

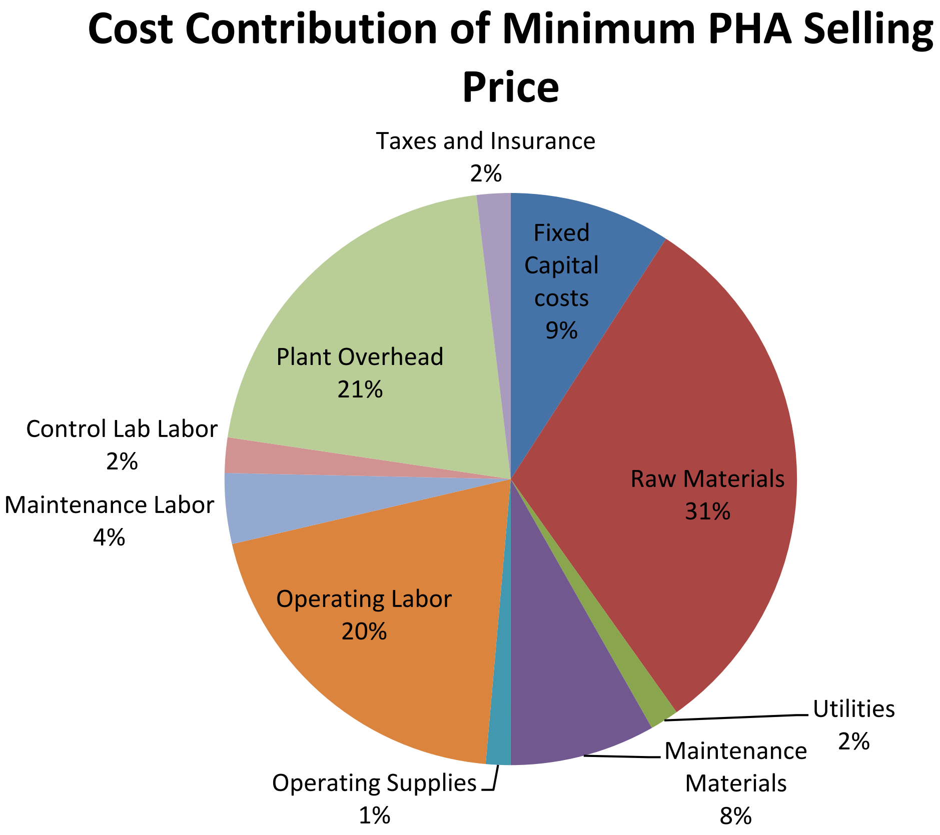
- **Commercialization efforts -- We have actively engaged with three industries.**

1. For lignocellulosic biorefineries, we have worked closely with ICM inc. We also had dialogue with POET for lignin utilization.
2. For bioplastics industry, we have been working with Danimer Scientific in obtaining funding for the development of rapidly biodegradable composites with enhanced performance to allow broad applications and larger market, while addressing the environmental challenges by petrochemical plastics.
3. We are also working with Altex Technologies to scale up a relevant platform to convert full biomass into PHA. We also explored the opportunities to work with Earth Energy Renewables to develop waste to bioplastics platform.

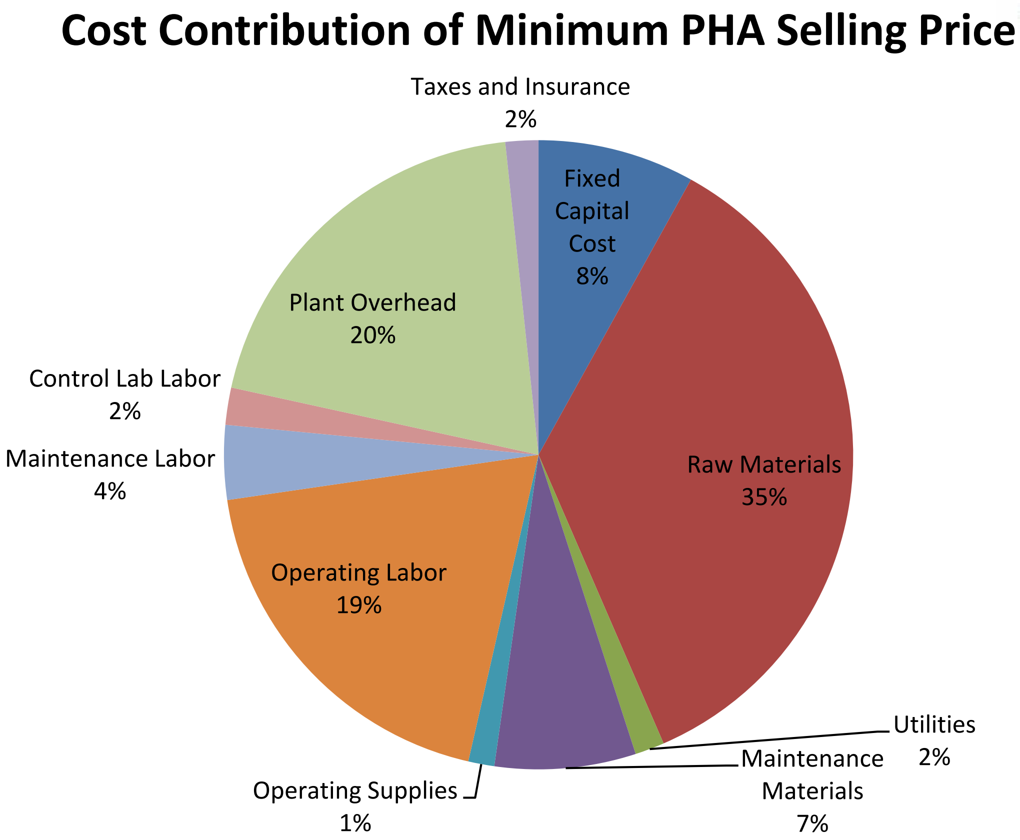
Techno-Economic Analysis of PHA

	UNITS	Case B	Case C	Case D	Case E	Case F
Annual production	MMkg	2.19	3.21	2.60	3.54	2.02
Total Capital Cost	MM$	44.984	42.636	42.128	39.845	42.121
Total Operating Cost	MM$/yr	11.208	12.420	12.264	13.237	14.648
Raw Material	MM$/yr	4.875	5.904	5.767	7.400	8.124
Utilities	MM$/yr	0.268	0.267	0.266	0.270	0.266
Unit cost	$/kg	7.52	5.49	6.73	5.05	9.90
Rate of return	%	10	10	10	10	10
Minimum selling price	$/kg	9.58	6.82	8.35	6.18	11.99

Cost Contribution of Minimum PHA Selling Price

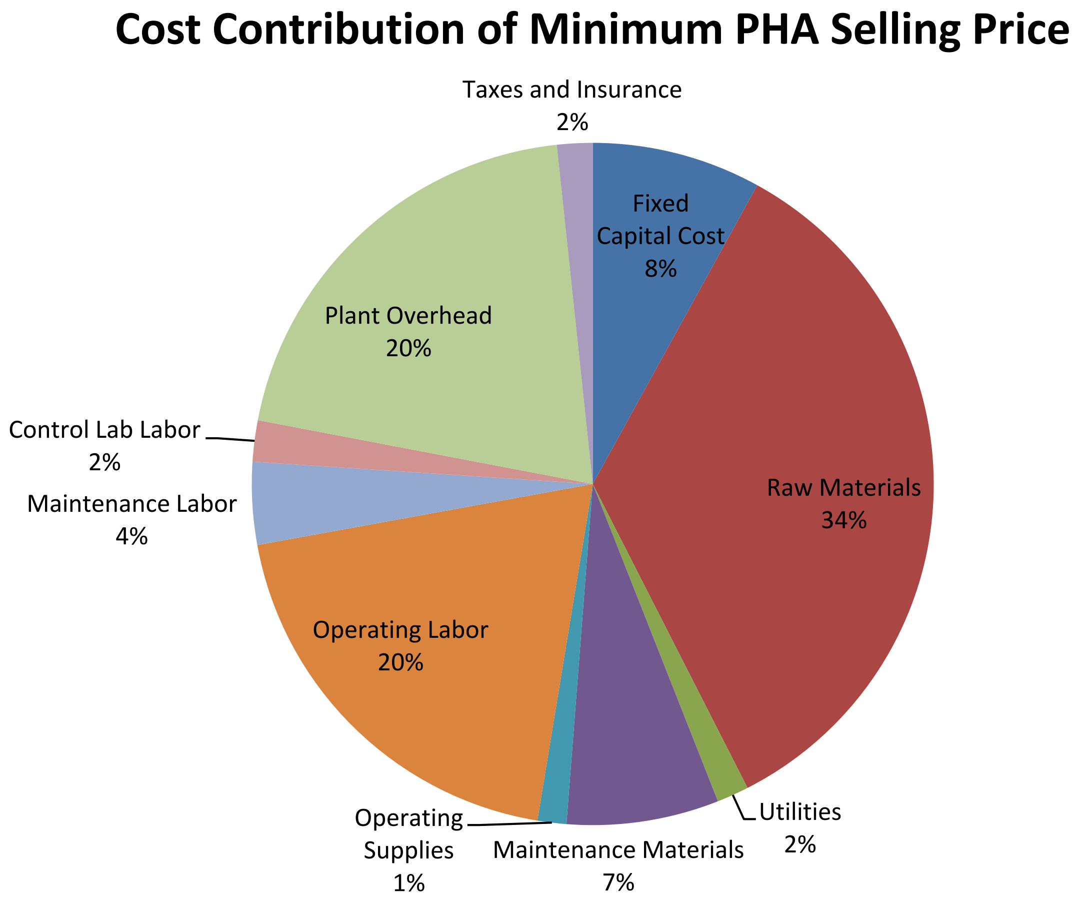


Case B

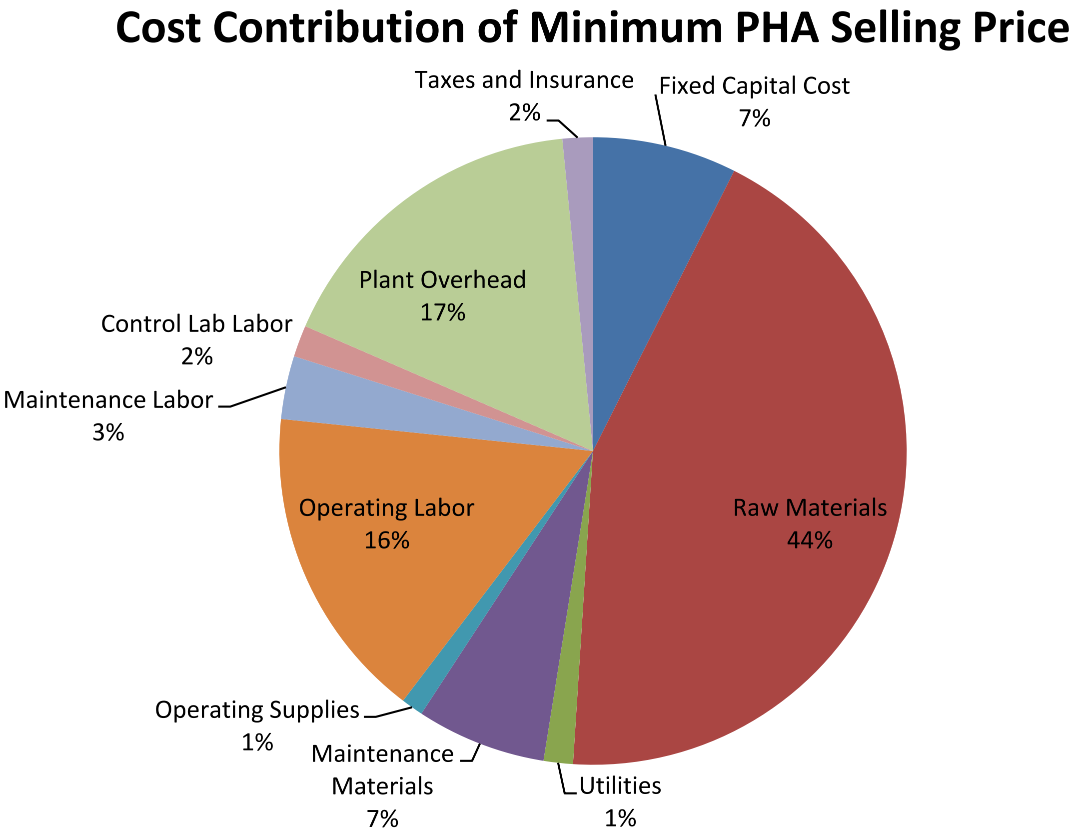


Case C

Cost Contribution of Minimum PHA Selling Price

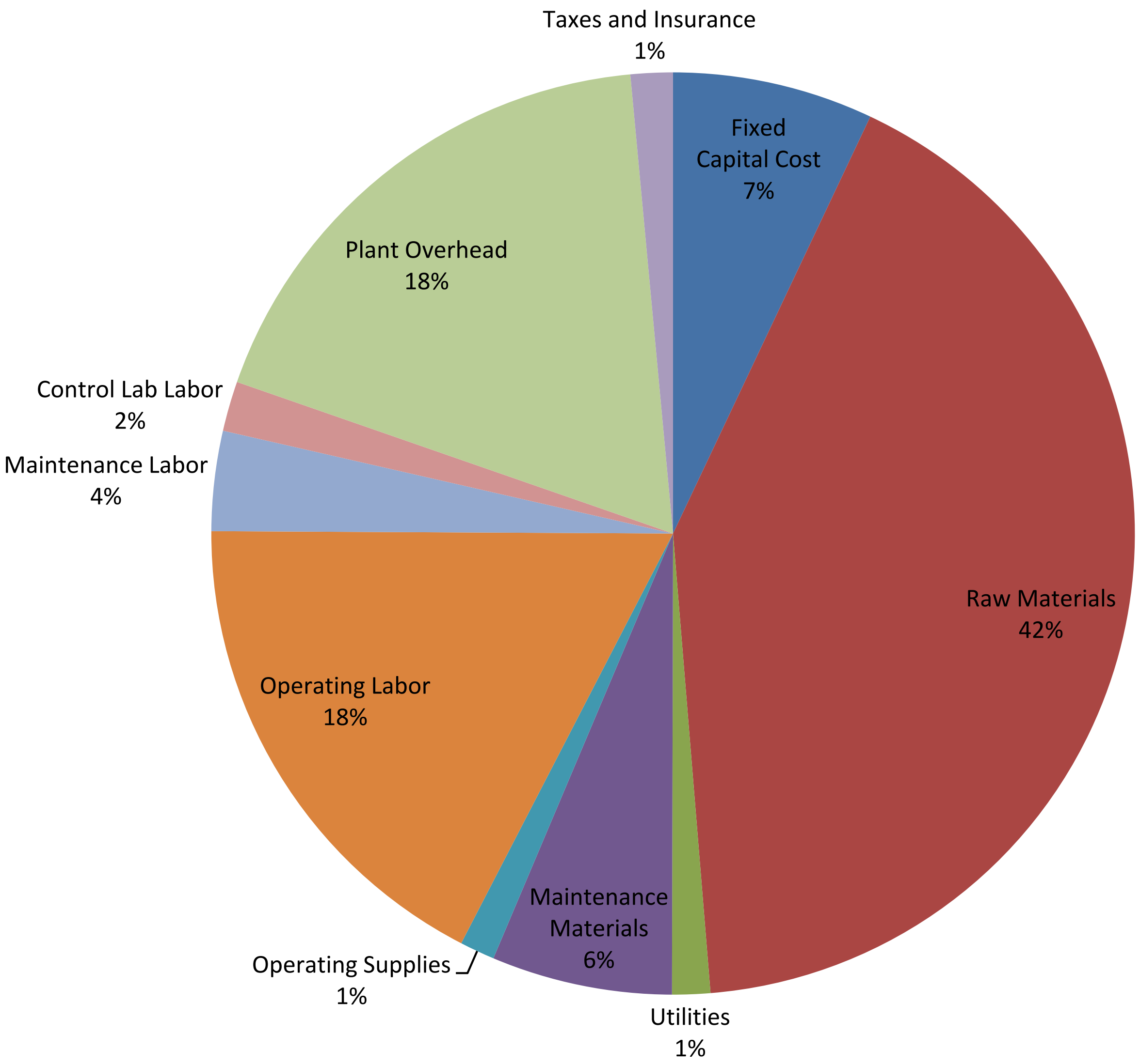


Case D



Case E

Cost Contribution of Minimum PHA Selling Price



Case F

Example of Mass Balance