DOE Bioenergy Technologies Office (BETO)
2019 Project Peer Review (DE EE 0007104)

# Upgrading Biorefinery Waste for Bioplastics

03/06/2019
Biochemical Conversion
Biorefinery Upgrading

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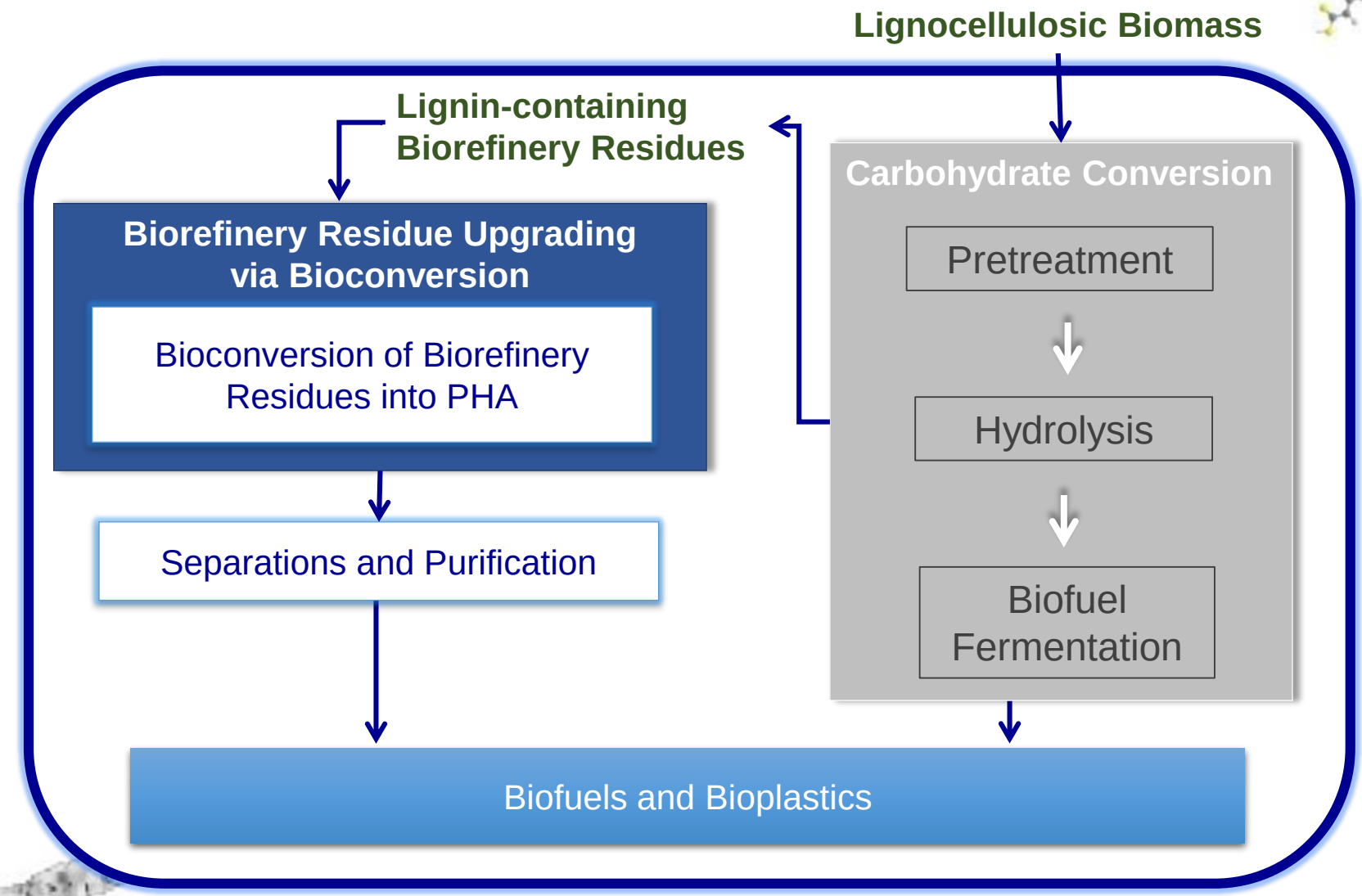
# Project Goal: Upgrading Biorefinery Waste to Fungible Products

- Address one of the most challenging issues in biofuel production: upgrading the lignin-containing biorefinery residues to fungible bioproducts.
- Develop a viable bioprocess to convert biorefinery waste to bioplastics at less than $5 dollar/Kg. – Project Outcome
- Overcome the key challenges for biorefinery cost-effectiveness and sustainability as laid out by BETO MYPP; Bring down the biofuel cost toward $3/GGE.

- BETO Missions:
  - Manage biorefinery waste
  - Reduce carbon emission by complete biomass usage
  - Improve biorefinery economics and sustainability

# Project Goal: Upgrading Biorefinery Waste for Fungible Products



# Quad Chart Overview

## Timeline

- Project start date: 07/01/2016
- Project end date: 12/31/2019
- Percent complete: 67%

| | Total Costs Pre FY17** | FY 17 Costs | FY 18 Costs | Total Planned Funding (FY 19-Project End Date) |
|---|---|---|---|---|
| **DOE Funded** | $127,408 | $742,872 | $656,049 | $973,664 |
| **Project Cost Share*** | $0 | $342,397 | $314,763 | $127,911 |

**Partners:**
**Texas A&M University: 50%**
**University of Tennessee/Oak Ridge National Lab: 30%**
**Washington State University: 15%**
**ICM inc.: 5%**

Barriers addressed:

- Ot-B. Cost of Production
- Ct-B. Efficient Preprocessing and Pretreatment
- Ct-C. Process Development for Conversion of Lignin
- Ct-D. Advanced Bioprocess Development
- Ct-J. Identification and Evaluation of Potential Bioproducts
- Ct-K. Developing Methods for Bioproduct Production
- Ct-L. Decreasing Development Time for Industrially Relevant Microorganisms
- At-D. Identifying New Market Opportunities for Bioenergy and Bioproducts
- At-E. Quantification of Economic, Environmental, and Other Benefits and Costs

## Objective

Develop a process to convert biorefinery waste to bioplastics at less than $5 dollar/Kg, which will reduce MESP and improve the sustainability of biorefinery.

## End of Project Goal

At the end of the project, we aim to reach:

- 8.4g/L PHA titer;
- 40% conversion of lignin to PHA;
- Less than $5/Kg of PHA price.

The new process will significantly reduce MESP and transform biorefinery design.

# Project Overview

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

**Project Title:**

**Upgrading lignin-containing biorefinery waste to bioplastics**

**Objectives:**

This project uniquely addresses BETO's mission and FOA's goals through process enablement, development, and optimization for the bioconversion of lignin-containing biorefinery residues into bioplastics.

(1) Process enablement by screening and engineering microorganisms to convert biorefinery waste streams to PHA for bioplastics;

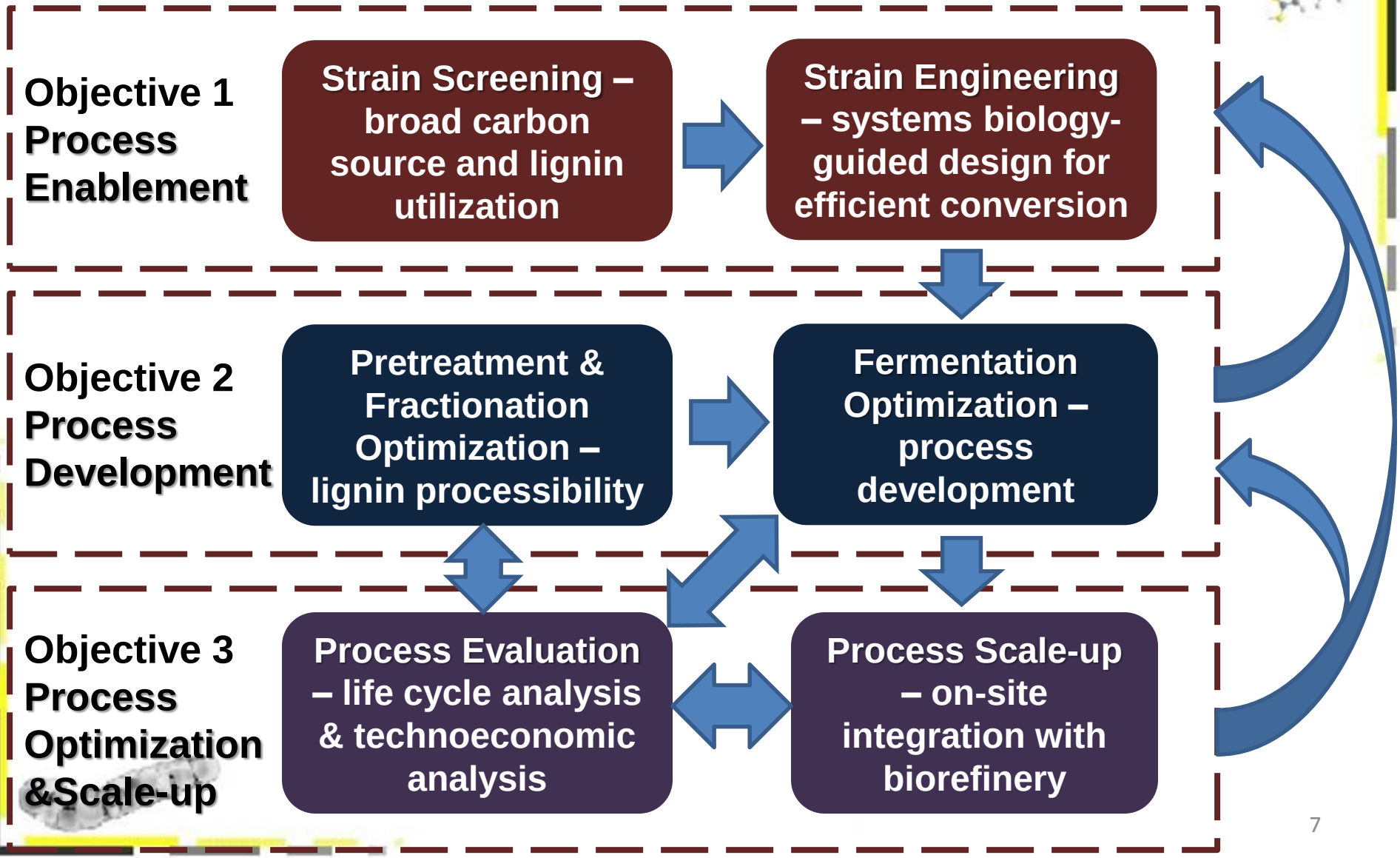
(2) Process development by characterizing biorefinery residues, optimizing pretreatment and lignin fractionation, enhancing fermentation, and designing the novel bioprocess;

(3) Process integration and optimization by biorefinery on-site scale-up, technoeconomic and life cycle analysis for the lignin-to-PHA up-grading process.

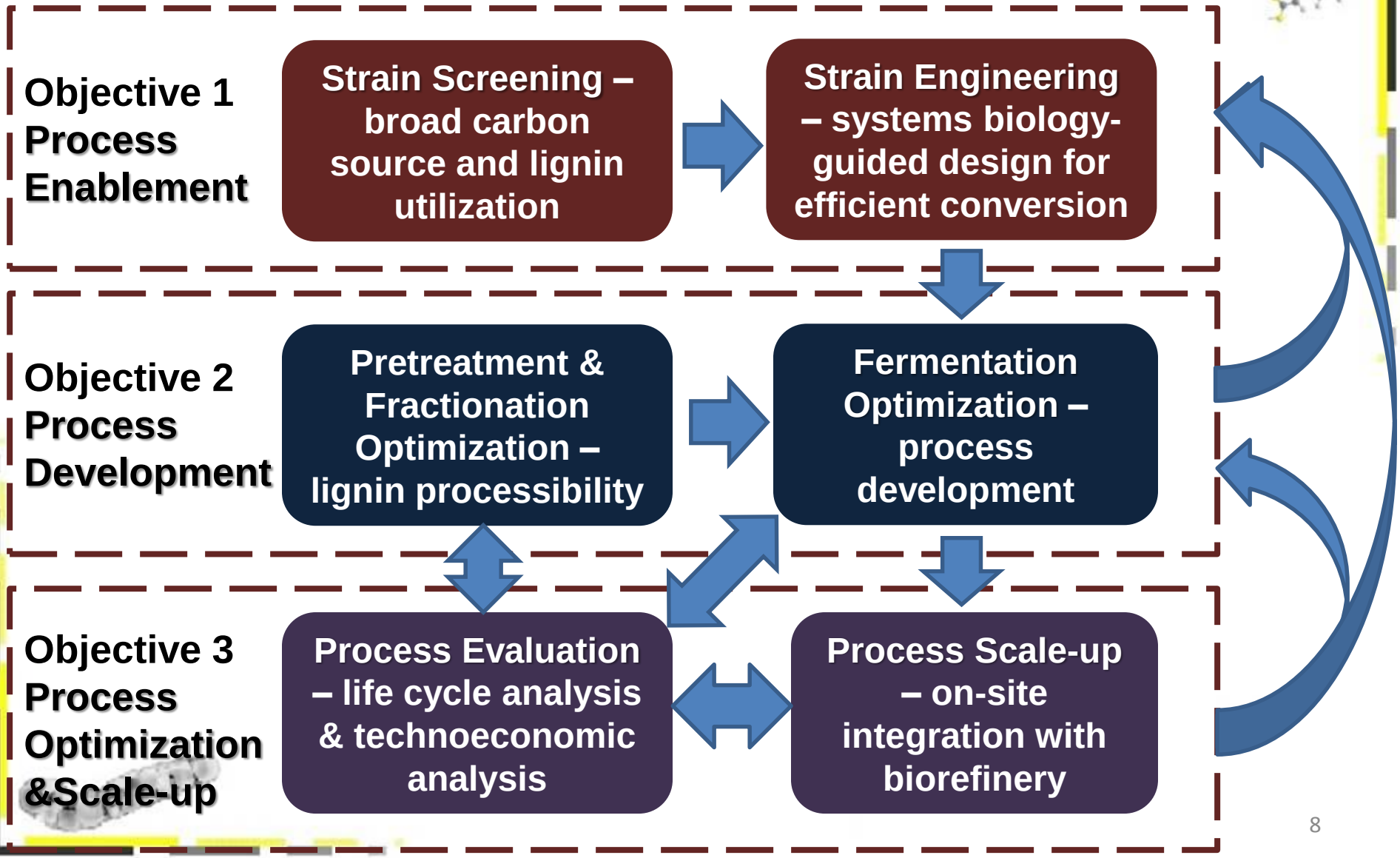
# Management Approach

- 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 and teleconference with program management were implemented to evaluate the progresses against milestones.
- Regular teleconferences between the PI and the program management are implemented to evaluate progresses, mitigate risks, and address management issues.
- Engage industrial partners including ICM inc. for deliverables relevant to EERE MYPP.
- Integrate TEA and LCA throughout the project to ensure the relevance of the project outcome.

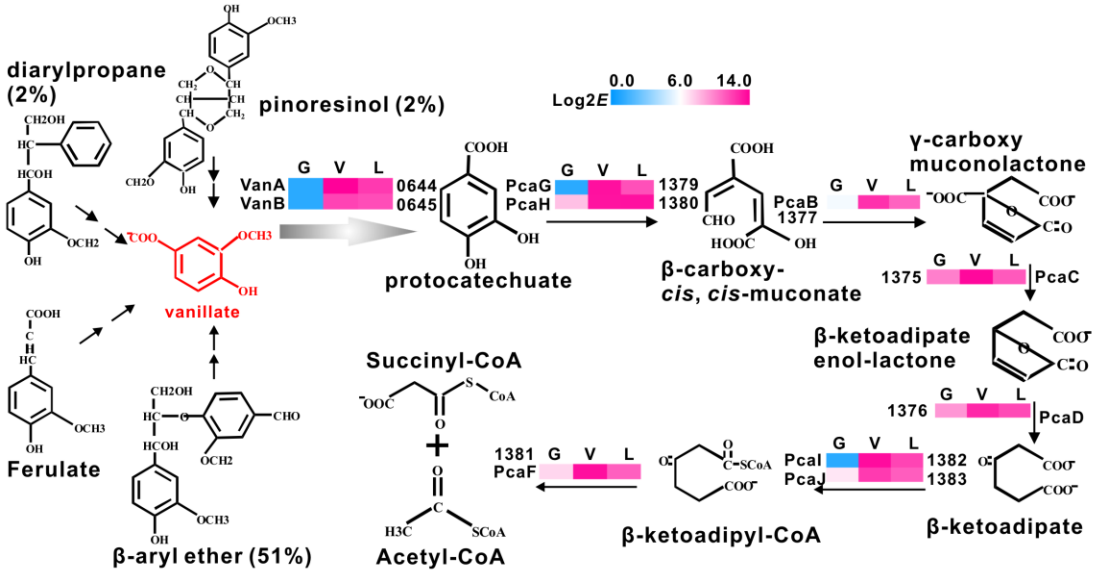
# Technical Approach

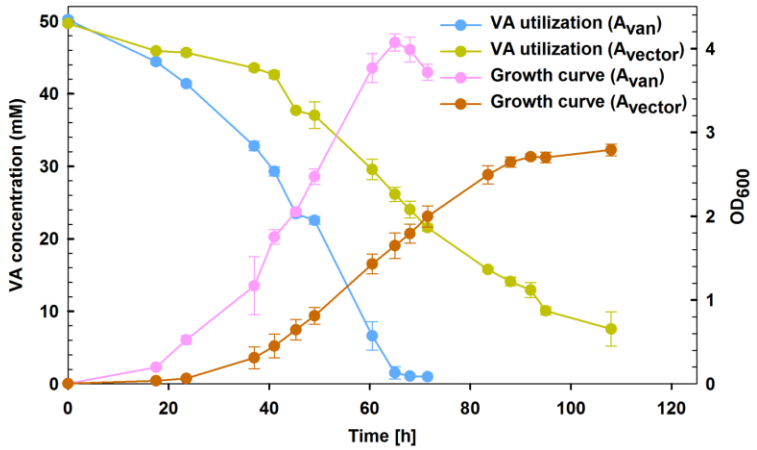


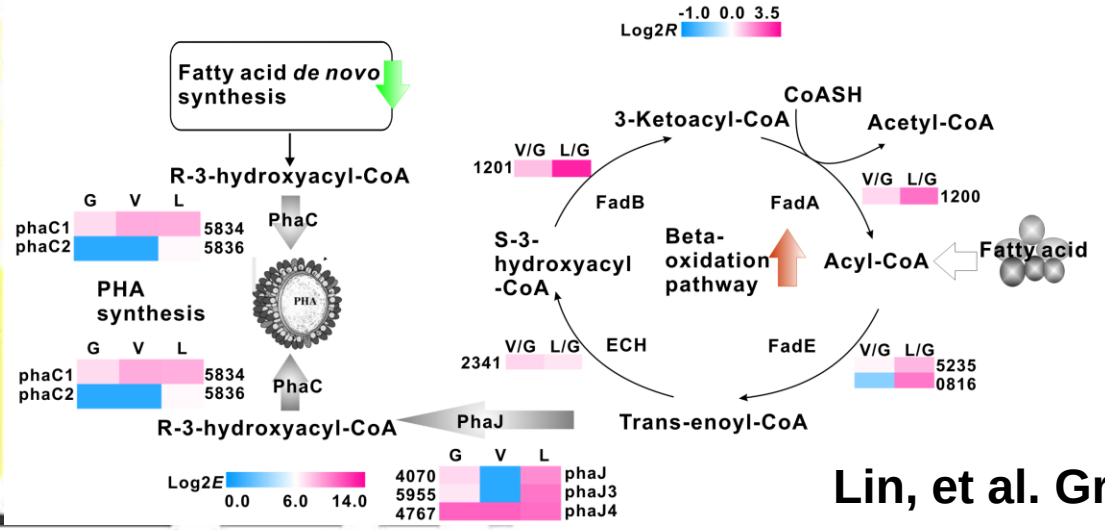
# Technical Progress

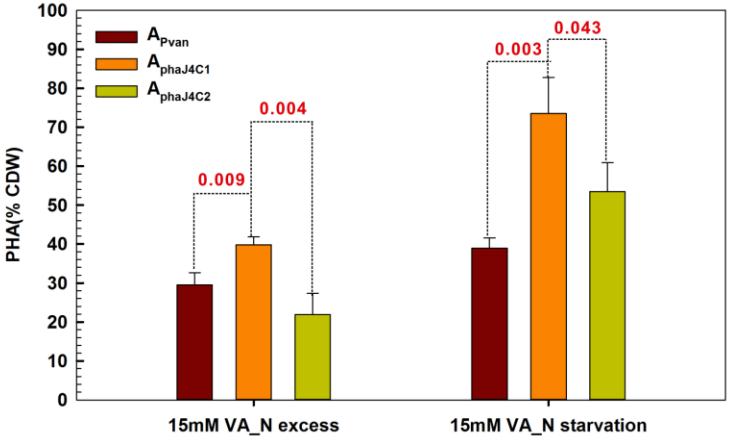


# Design of Aromatic Compound Catabolism and PHA Biosynthesis Modules to Maximize Carbon Flux

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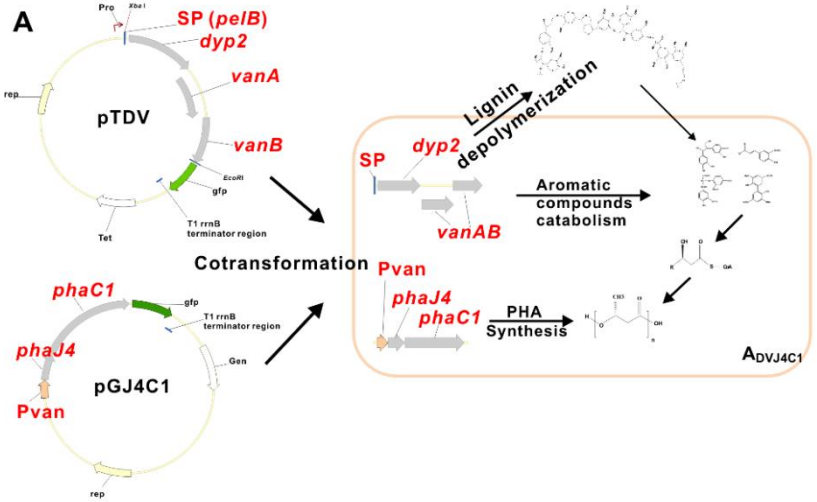
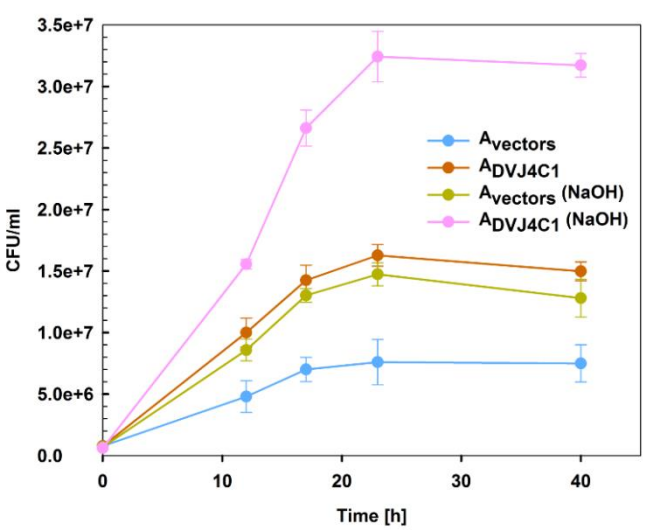
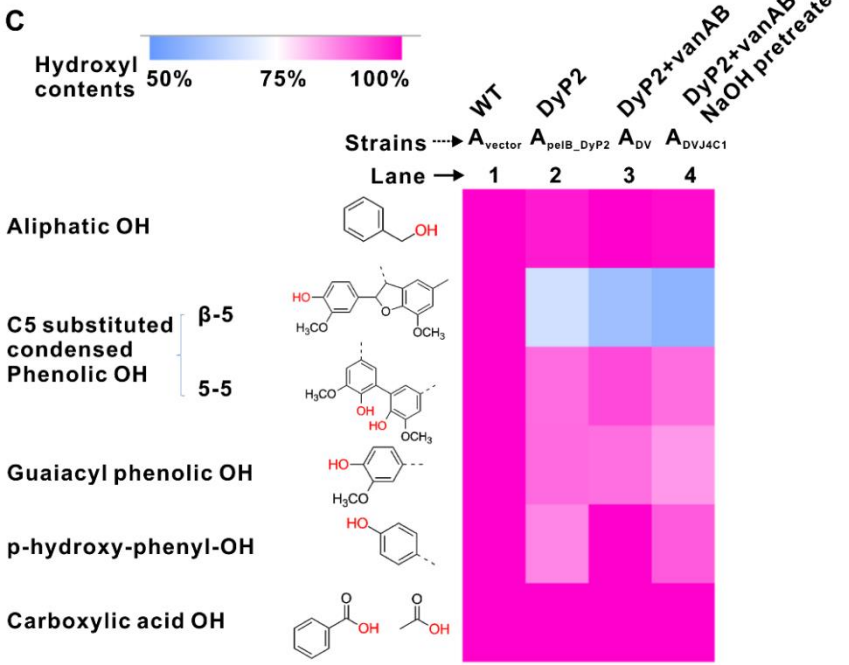
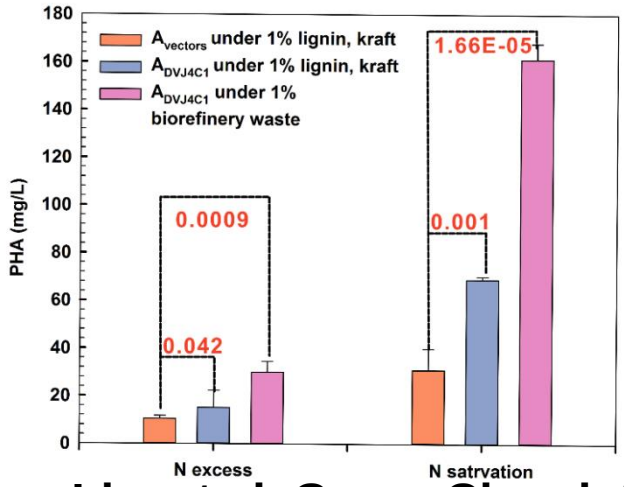
# Multiple Module Integration for Biorefinery Residue Upgrading to PHA

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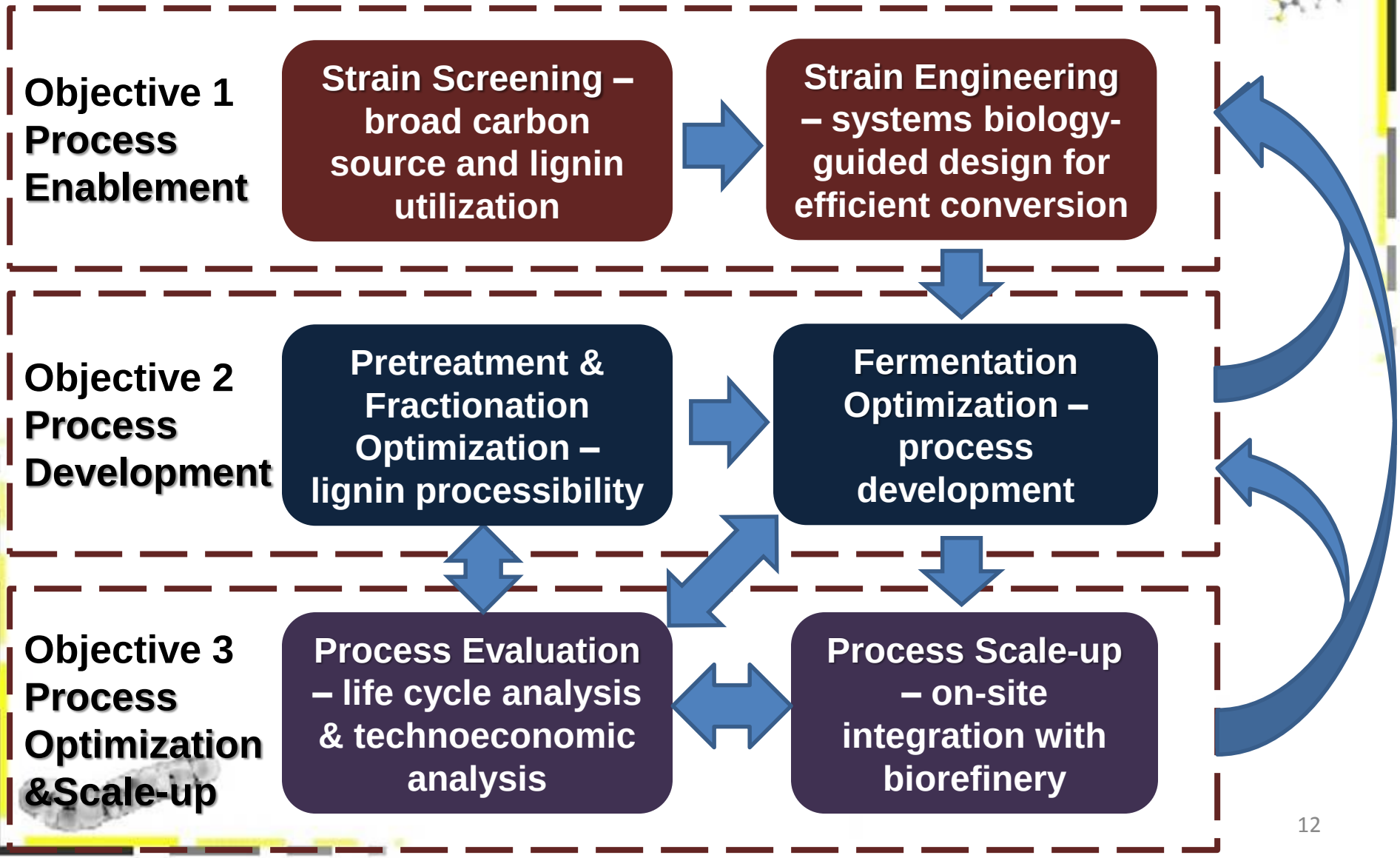
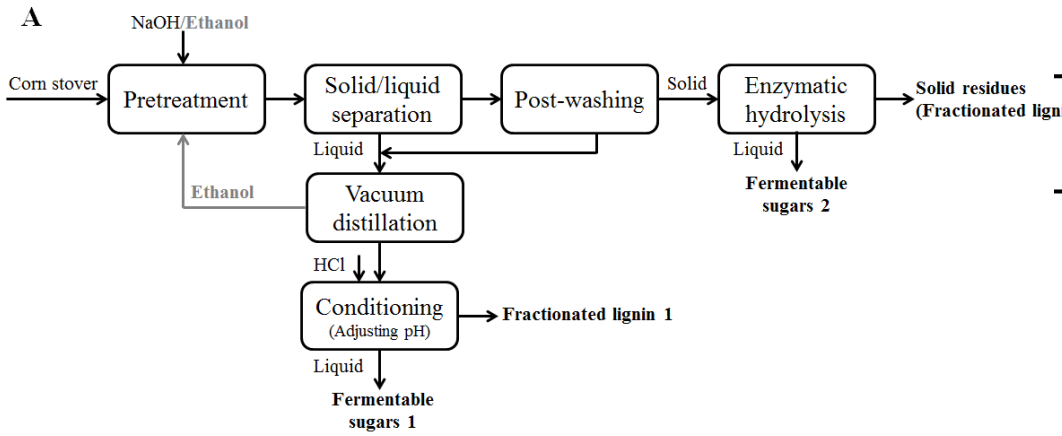
# Strain Screening and Engineering for Biorefinery Residue Upgrading

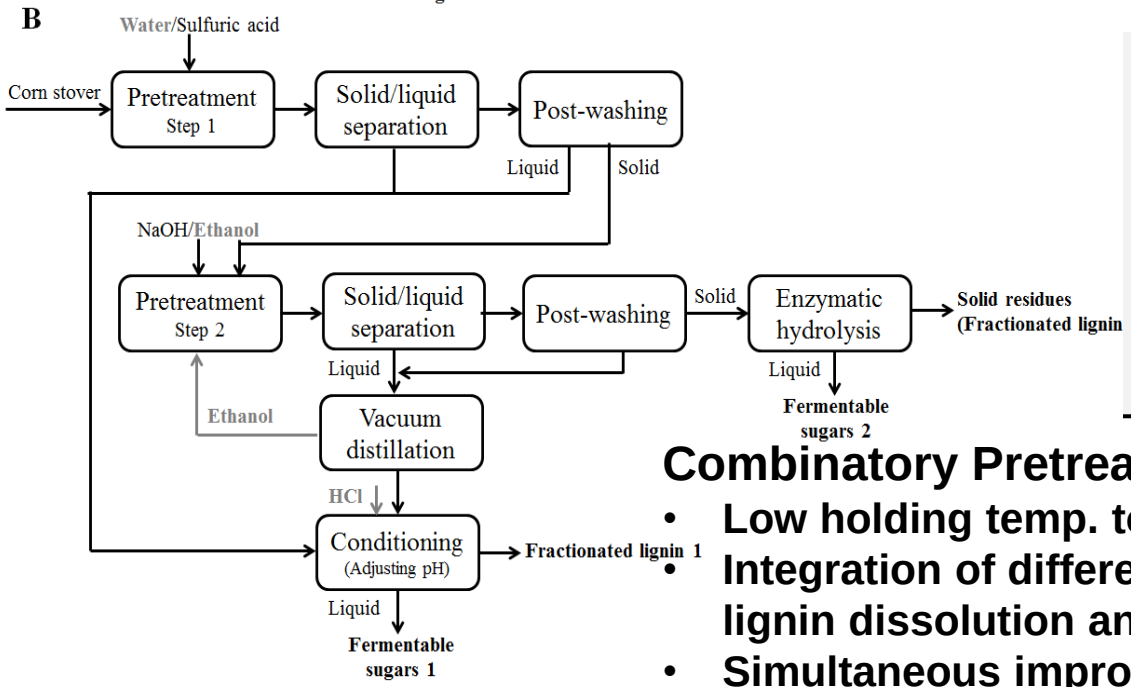
1. Comparative genomics revealed broad lignin and aromatic compound degradation mechanisms – coordinative pathways in *P. putida*.
2. Systems biology analysis revealed mechanisms for lignin degradation in *P. putida* and guided the design of lignin depolymerization module as well as fractionation technology development for biorefinery.
3. Systems biology-guided strain engineering is effective in guiding the design of three functional modules to enhance the upgrading of biorefinery waste to PHA.
4. On lignin medium, the fatty acid degradation pathway is up-regulated, which can be coupled with PHA biosynthesis. The knowledge guided further engineering.

# Project Progresses



# Low Holding Temperature Combinatorial Pretreatment to Enhance Lignin Processibility



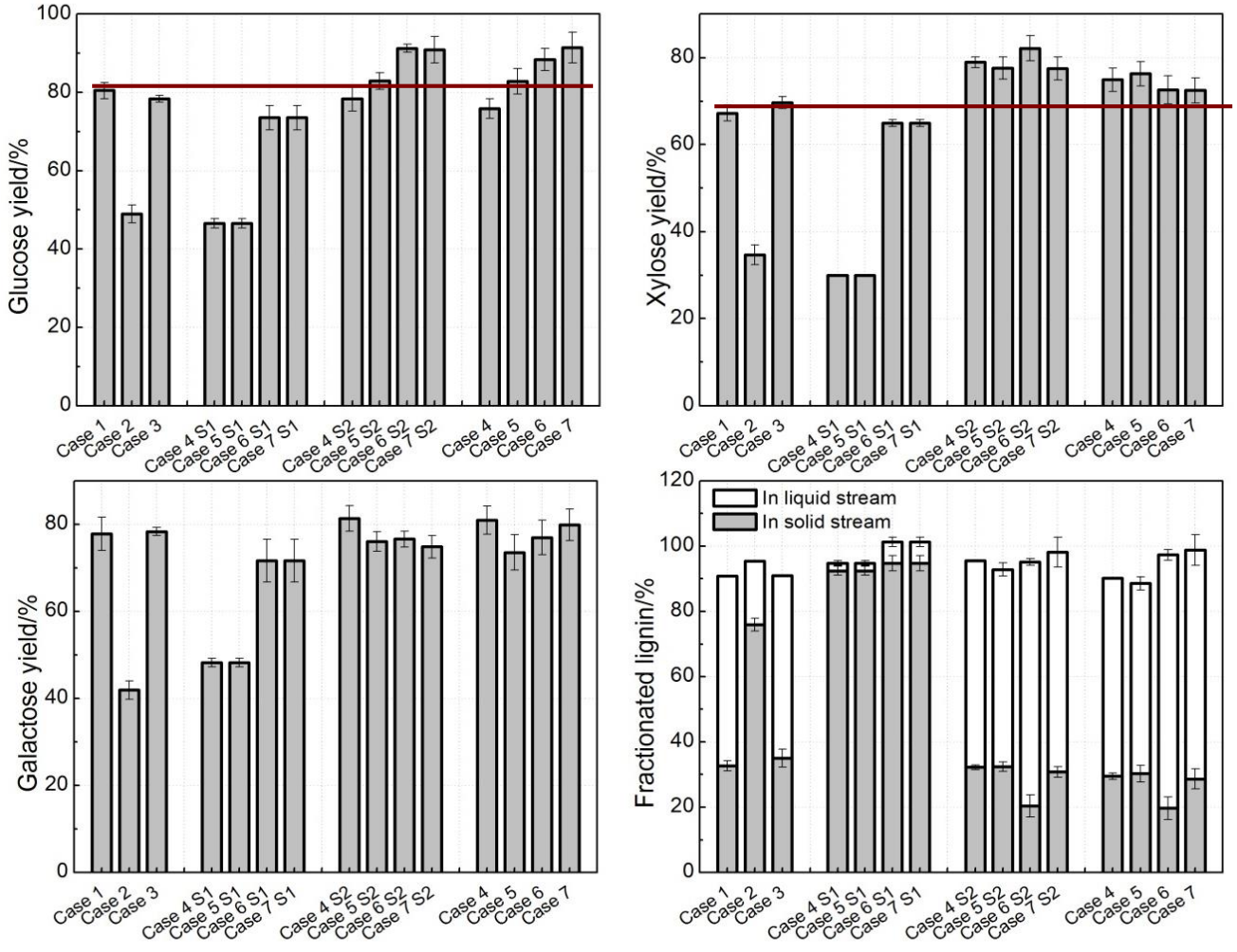

| Case | Step 1 | | Step 2 | |
|---|---|---|---|---|
| | Chemicals | Conditions | Chemicals | Conditions |
| 1 | 1% NaOH | 120 ˚C, 60 min | | |
| 2 | 50% Ethanol | 120 ˚C, 60 min | | |
| 3 | 50% Ethanol+1% NaOH | 120 ˚C, 60 min | | |
| 4 | Liquid hot water | 120 ˚C, 30 min | 1% NaOH | 120 ˚C, 60 min |
| 5 | Liquid hot water | 120 ˚C, 30 min | 50% Ethanol+1% NaOH | 120 ˚C, 60 min |
| 6 | 1% $H_2SO_4$ | 120 ˚C, 30 min | 1% NaOH | 120 ˚C, 60 min |
| 7 | 1% $H_2SO_4$ | 120 ˚C, 30 min | 50% Ethanol+1% NaOH | 120 ˚C, 60 min |

## Combinatory Pretreatment:

- Low holding temp. to reduce inhibitor generation
- Integration of different fractionation strategies to improve lignin dissolution and processibility
- Simultaneous improvement of carbohydrate processibility

Liu et al. Green Chemistry, 2017, 19, 4939-4955

# Co-optimization of Carbohydrate and Lignin Conversion by Combinatorial Pretreatment




**Fermentable sugar yields**

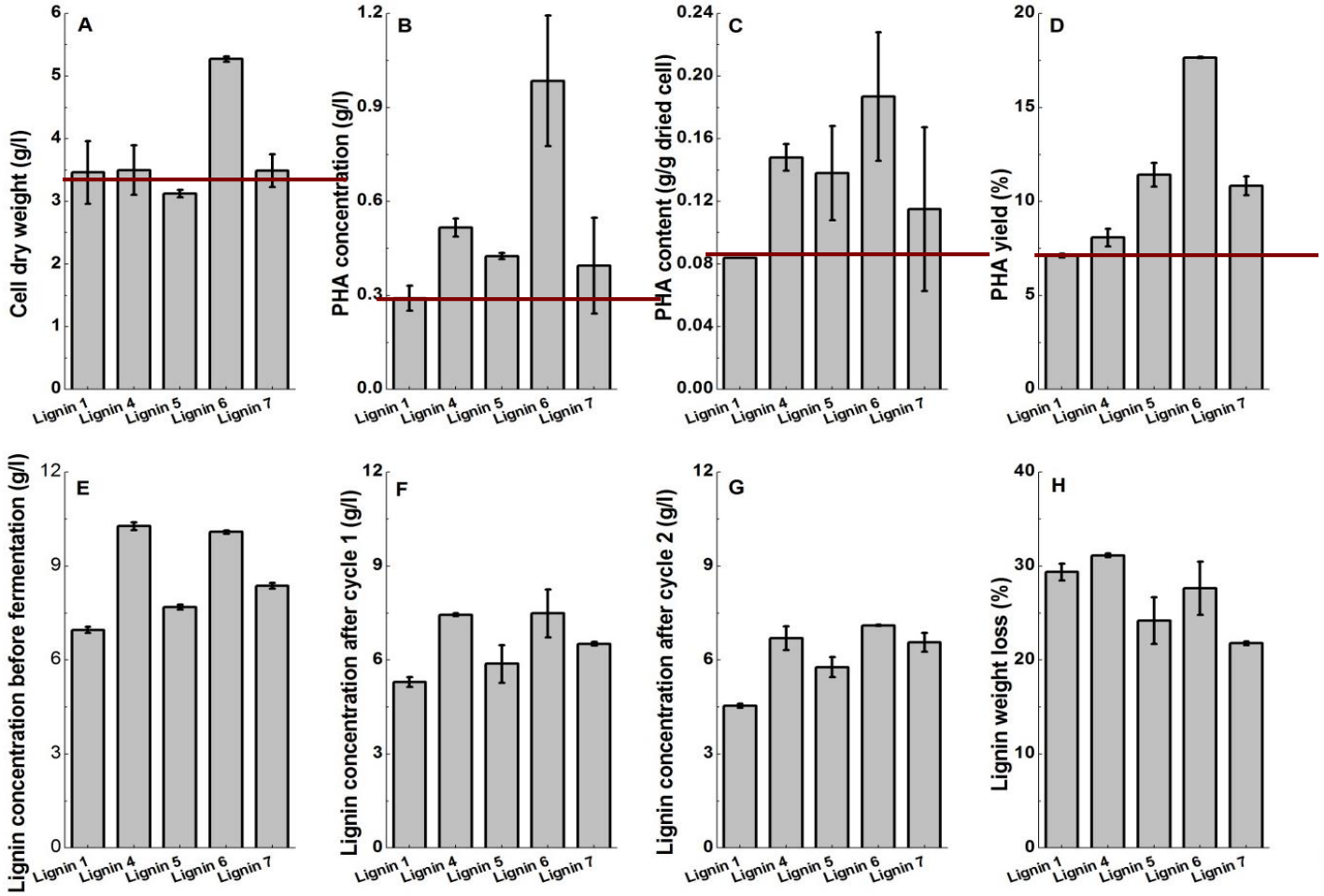
**Fractionated lignin yields**

**Combinatory Pretreatment improved both carbohydrate hydrolysis and lignin fractionation and dissolution.**



**Liu et al. Green Chemistry, 2017, 19, 4939-4955**

# Significant Increase of Lignin Processibility by Combinatorial Pretreatment




**Combinatory Pretreatment improved the total cell dry weight, as well as the concentration, content, and yield of PHA for *P. putida* fermentation of waste.**

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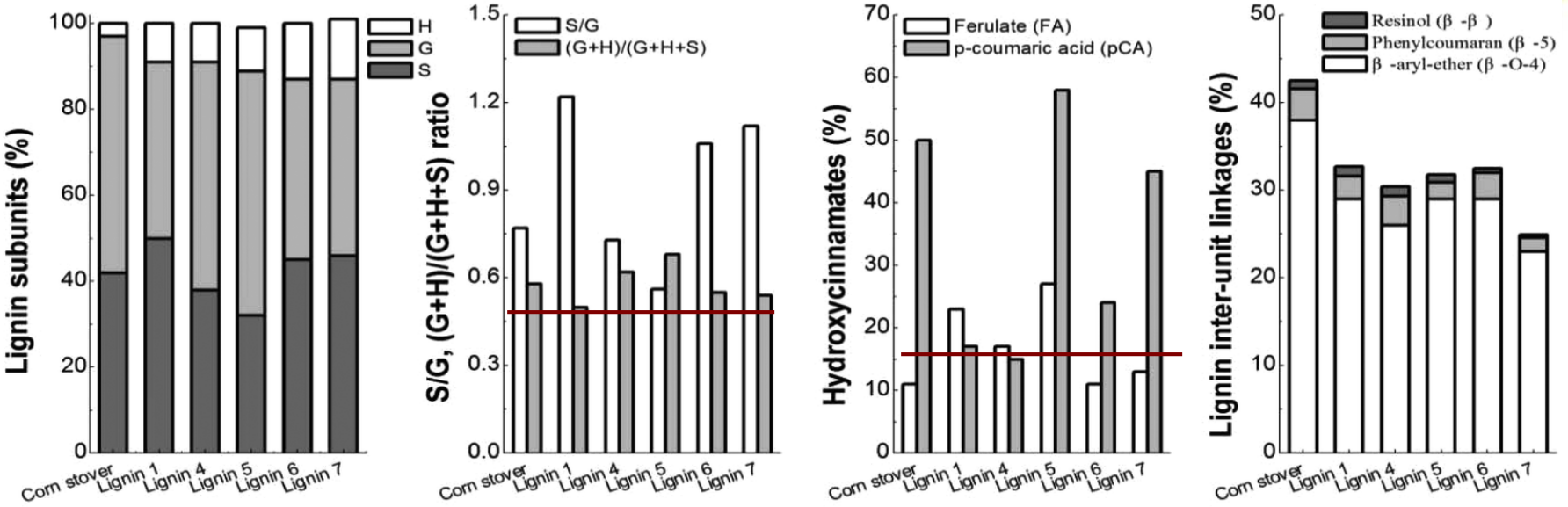
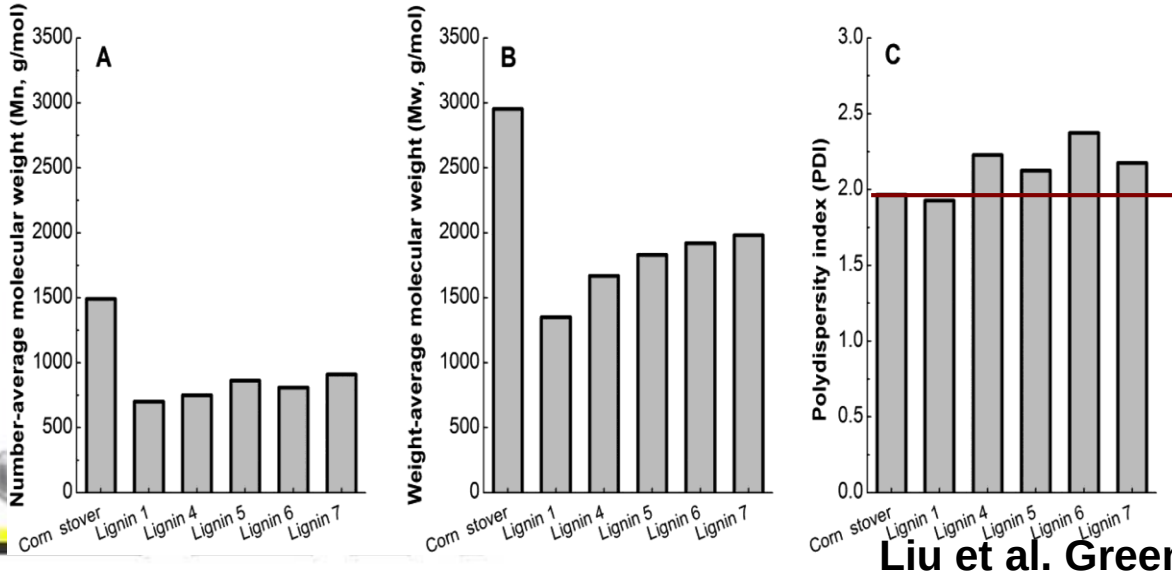
# Composition, Linkage, and Molecular Weight Analysis of Fractionated Lignin




**Mechanisms for Better PHA Yield:**

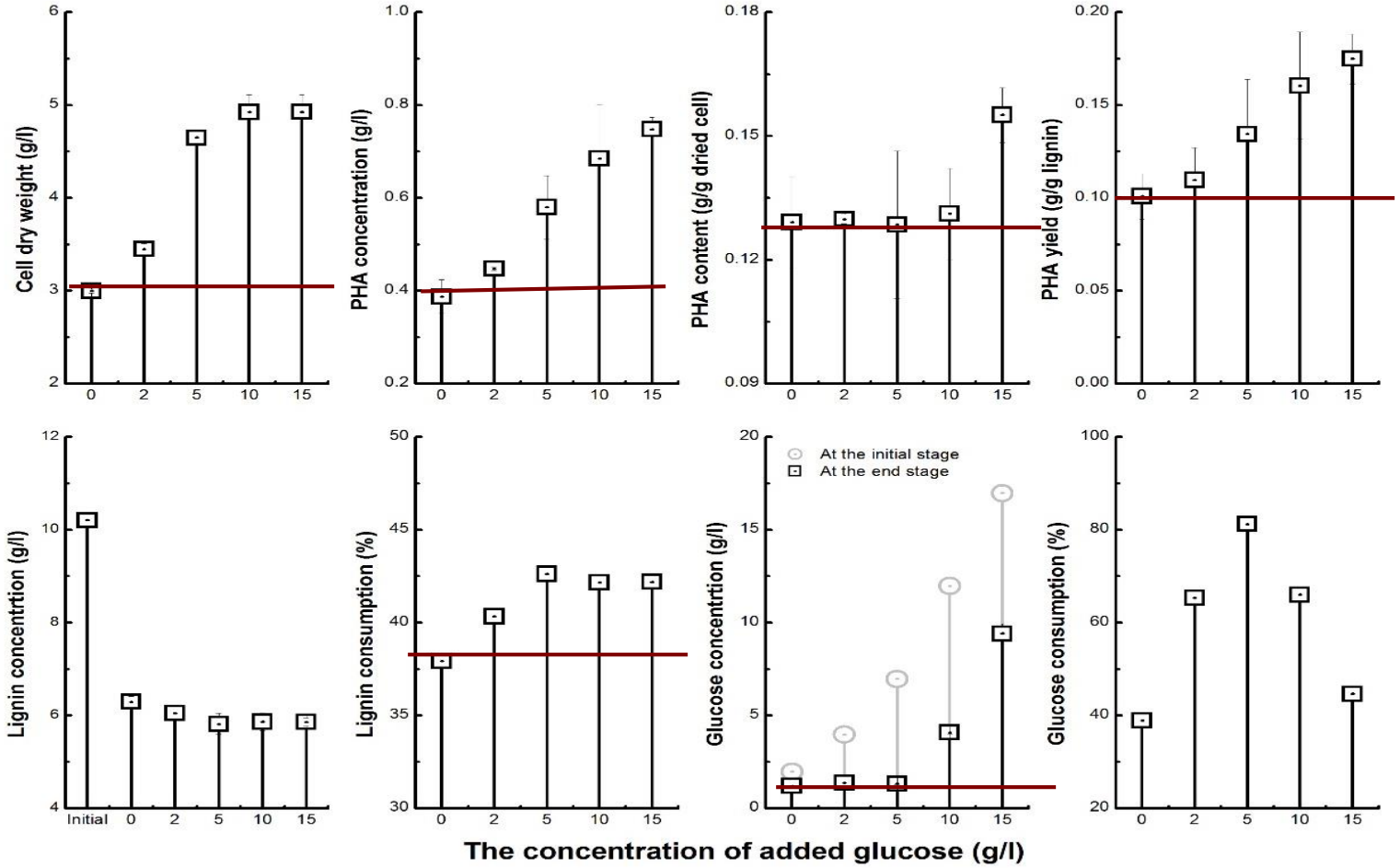
- **Composition: More G+H Unit**
- **Linkages: Better Lignin Degradation**
- **More Lignin Monomers: pCA and FA**
- **Molecular Weight: Decreased MW**
- **<u>Molecular Uniformity: Less Uniform Lignin, with More Small Molecules</u>**

**Liu et al. Green Chemistry, 2017, 19, 4939-4955**

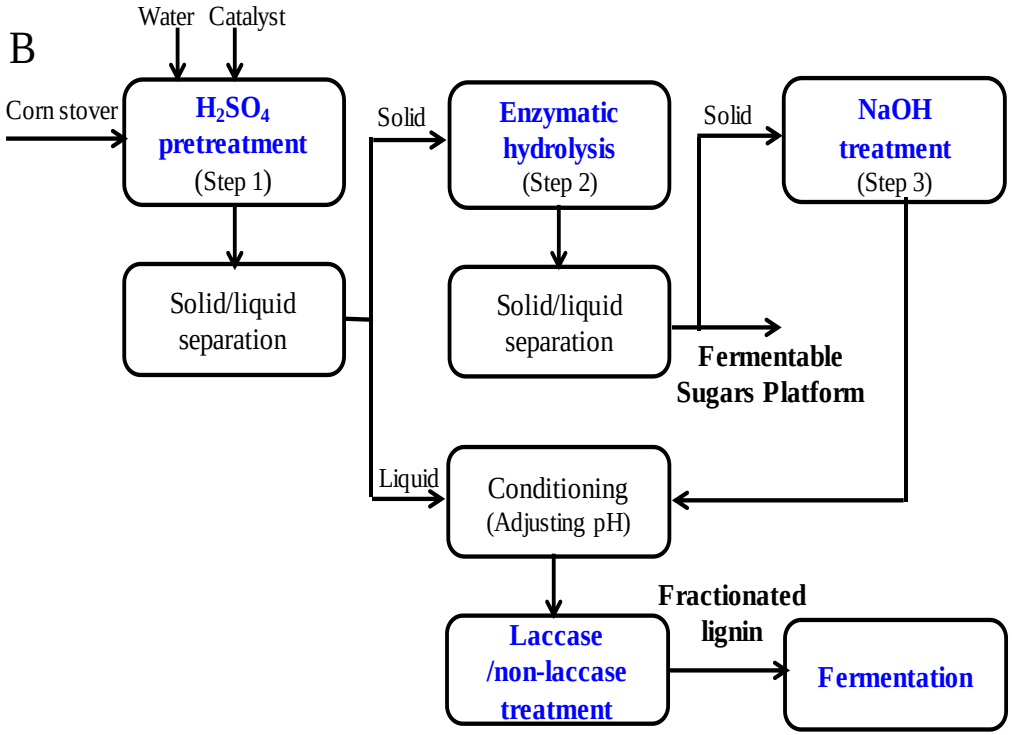
# Conclusion for Combinatorial Pretreatment

1. Combinatorial pretreatment can achieve the simultaneous optimization of carbohydrate and lignin processibility, where both carbohydrate scarification efficiency and lignin bioconversion were improved.
2. More lignin were solubilized by combinatorial pretreatment.
3. The record level of lignin to PHA titer was achieved at the time. A high PHA content and conversion rate were also achieved.
4. The increased lignin conversion to PHA is due to the better fractionation of lignin, lower level of inhibitor, and more free aromatic compounds released. In particular, PDI indicated that the less uniform (more small molecular weight lignin) lignin can be better converted to PHA.

# Low-level of Sugar in Biorefinery Waste Promotes Lignin Utilization




- Addition of low-level of sugar increases cell dry weight, PHA concentration, content, and yield in biorefinery waste utilization by *P. putida.*
- Addition of sugar (<5g/L) actually promotes lignin utilization.
- At less than 5g/L, most of the sugar were utilized when co-fermented with lignin.

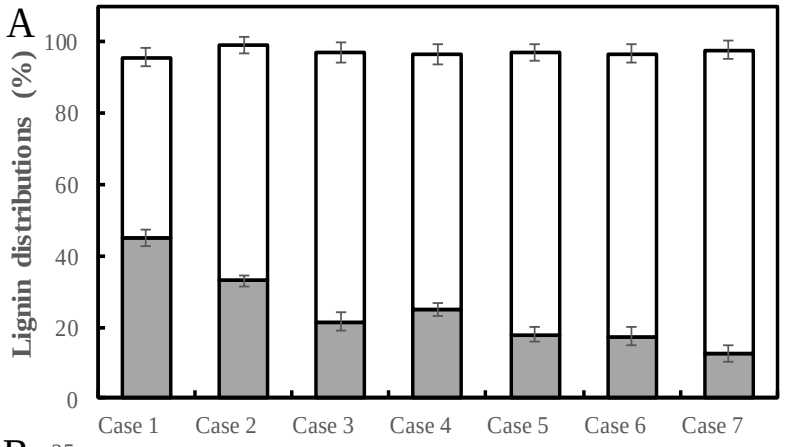


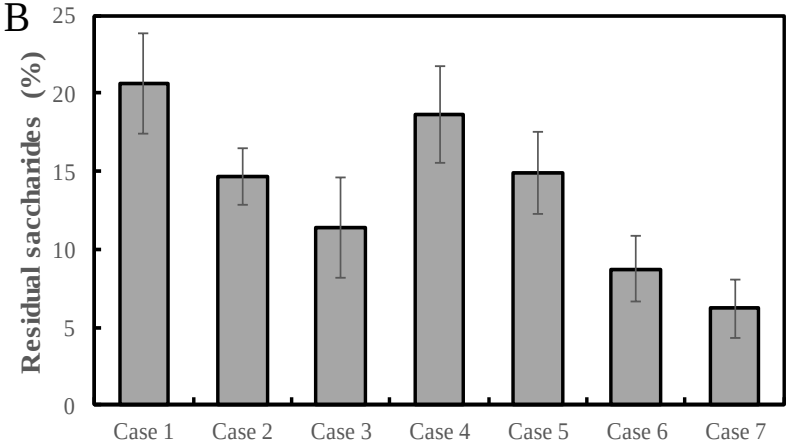

# Biorefinery Design to Release Residual Sugars

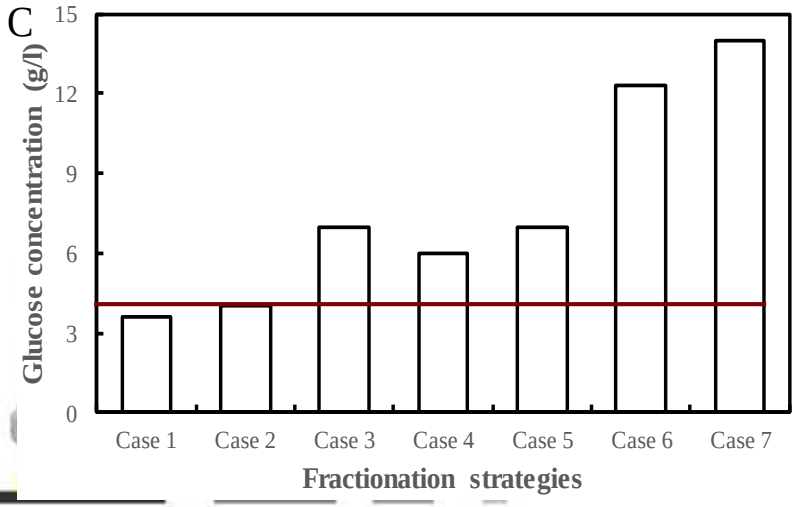
**Based on the sugar-lignin synergy, we have designed biorefinery configuration to release residual sugar from biorefinery waste:**

- **Alkaline treatment to release residual sugars.**
- **Alkaline treatment to fractionate and solubilize lignin.**
- **Laccase-mediator treatment to further fractionate lignin.**

| Case | Step 1 | | Step 2 | | Step 3 | | Laccase treatment |
|---|---|---|---|---|---|---|---|
| | Chemicals | Conditions | Chemicals | Conditions | Chemicals | Conditions | |
| 1 | 1% NaOH | 120° C, 60 min | EH | 10 FPU/g solid, 168 h | - | - | No |
| 2 | 1% $H_2SO_4$ | 120° C, 30 min | 1% NaOH | 120° C, 60 min | EH | 10 FPU/g solid, 168 h | No |
| 3 | 1% $H_2SO_4$ | 120° C, 60 min | 1% NaOH | 120° C, 30 min | EH | 10 FPU/g solid, 168 h | No |
| 4 | 1% $H_2SO_4$ | 120° C, 30 min | EH | 10 FPU/g solid, 168 h | 1% NaOH | 120° C, 60 min | No |
| 5 | 1% $H_2SO_4$ | 120° C, 60 min | EH | 10 FPU/g solid, 168 h | 1% NaOH | 120° C, 60 min | No |
| 6 | 1% $H_2SO_4$ | 120° C, 30 min | EH | 10 FPU/g solid, 168 h | 1% NaOH | 120° C, 60 min | Yes |
| 7 | 1% $H_2SO_4$ | 120° C, 60 min | EH | 10 FPU/g solid, 168 h | 1% NaOH | 120° C, 60 min | Yes |



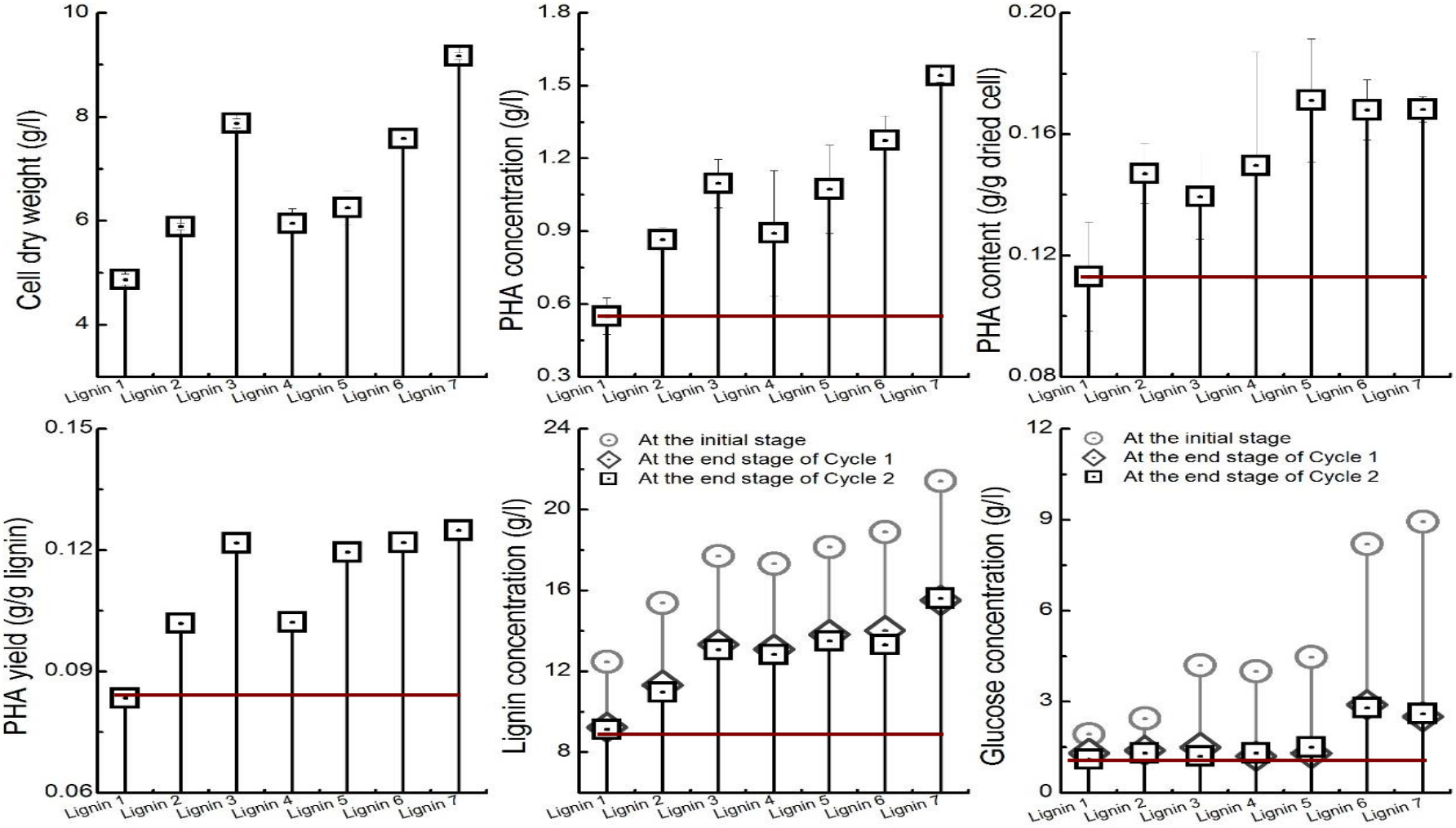




# Biorefinery Design to Best Release Lignin and Residual Sugars

**The Lignin Derived from Co-processing of Lignin and Residual Sugar (CLARS) Has the Following Features:**

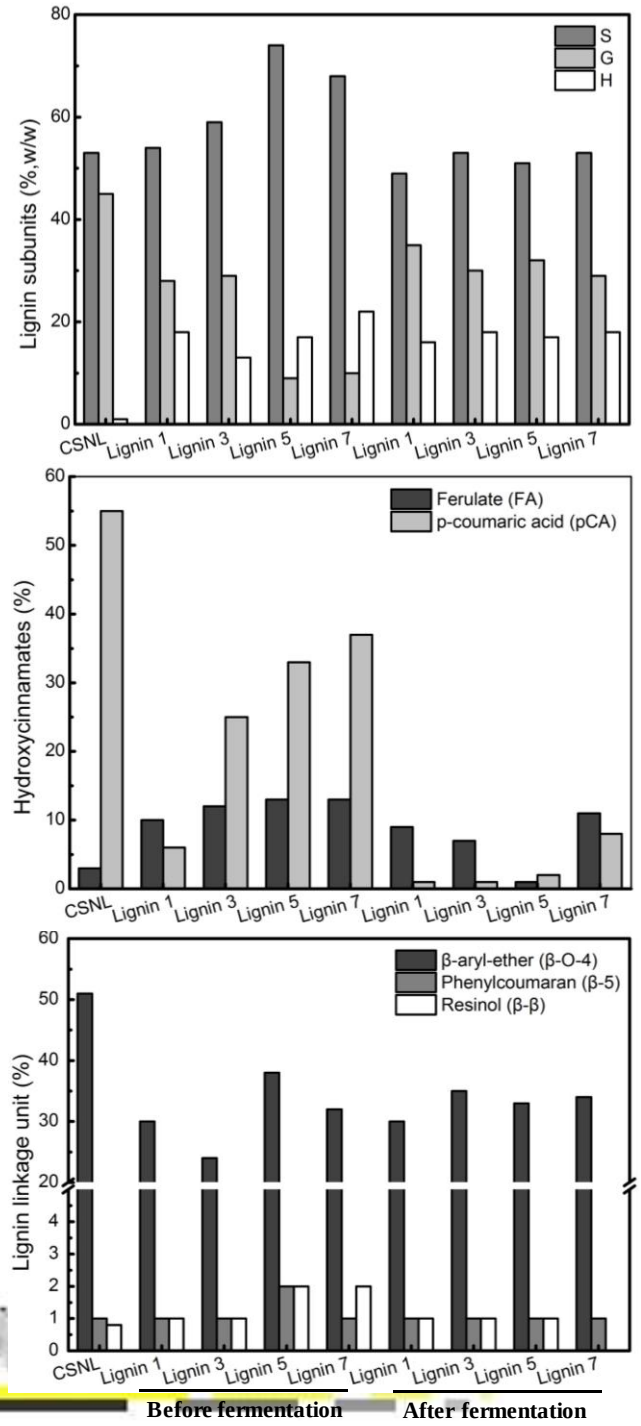
- **More lignin dissolution (A: Case 4-7).**
- **Higher residual sugar content in lignin stream (C: Case 5-7).**
- **Less residual sugar content in the waste stream (B).**

# Residual Sugar Promotes Lignin Utilization to a Record Yield




**Biorefinery waste from CLARS process enabled record PHA concentration and yield from lignin. Both lignin and sugar were consumed synergistically.**

# HSQC Analysis and GPC Analysis




| Samples | Before cultivation | | | After cultivation | | |
|---|---|---|---|---|---|---|
| | Mn | Mw | PDI | Mn | Mw | PDI |
| CSNL | 1371 | 6241 | 4.5 | | | |
| Lignin 1 | 435 | 2669 | 6.1 | 370 | 1116 | 3 |
| Lignin 3 | 400 | 2463 | 6.1 | 603 | 2172 | 3.6 |
| Lignin 5 | 311 | 2260 | 7.3 | 487 | 2385 | 4.9 |
| Lignin 7 | 267 | 1396 | 5.2 | 477 | 2833 | 5.9 |

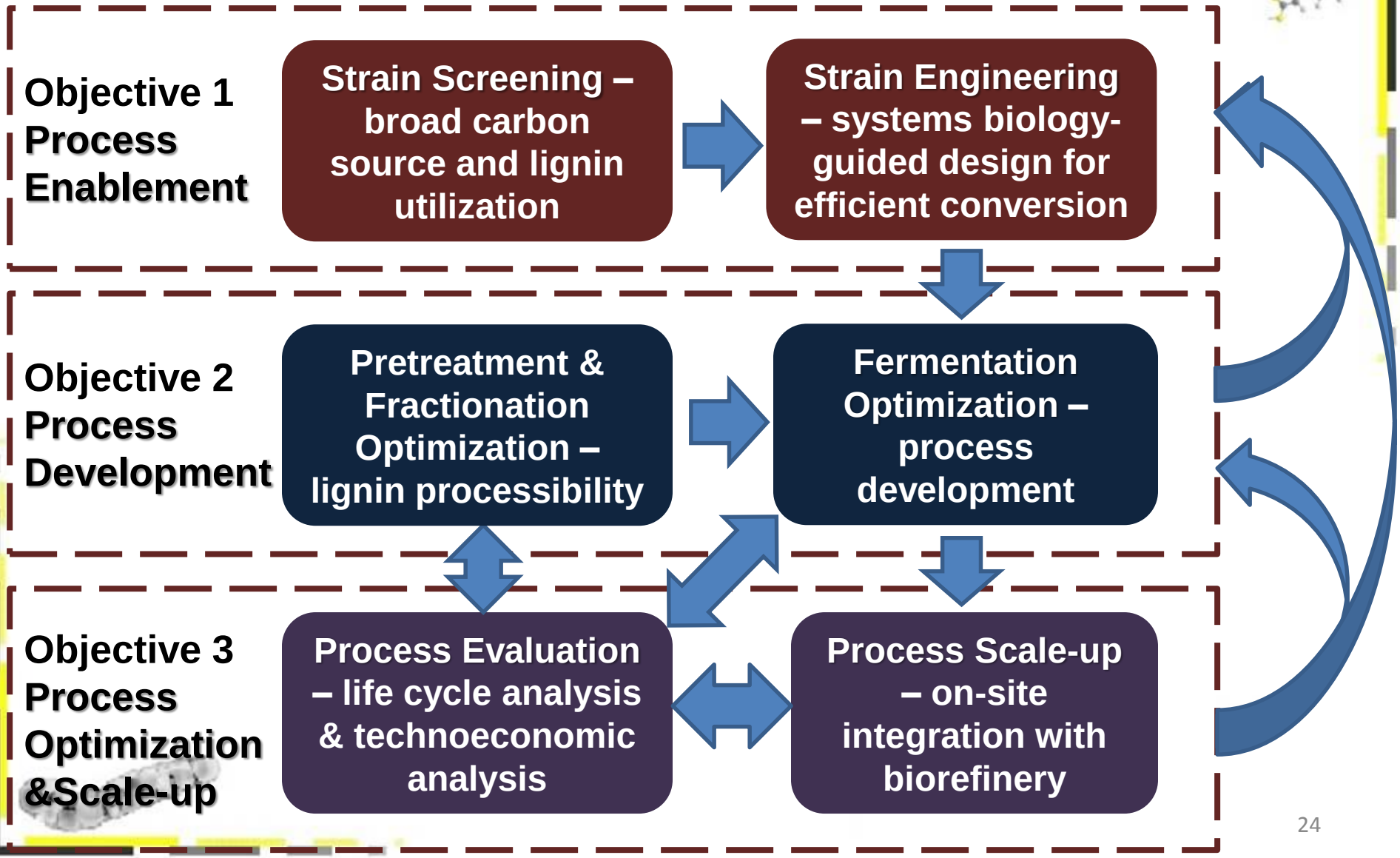
**Mechanisms for more efficient lignin utilization for CLARS with sugar synergy:**

- **CLARS process increased the lignin monomer concentrations (FA and pCA).**
- **CLARS process decreased molecular weight, in particular, after laccase-mediator treatment.**
- **Fermentation consumed most of the monomer lignin.**
- **Fermentation increased molecular weight of lignin, consistent with the consumption of low molecular weight lignin.**

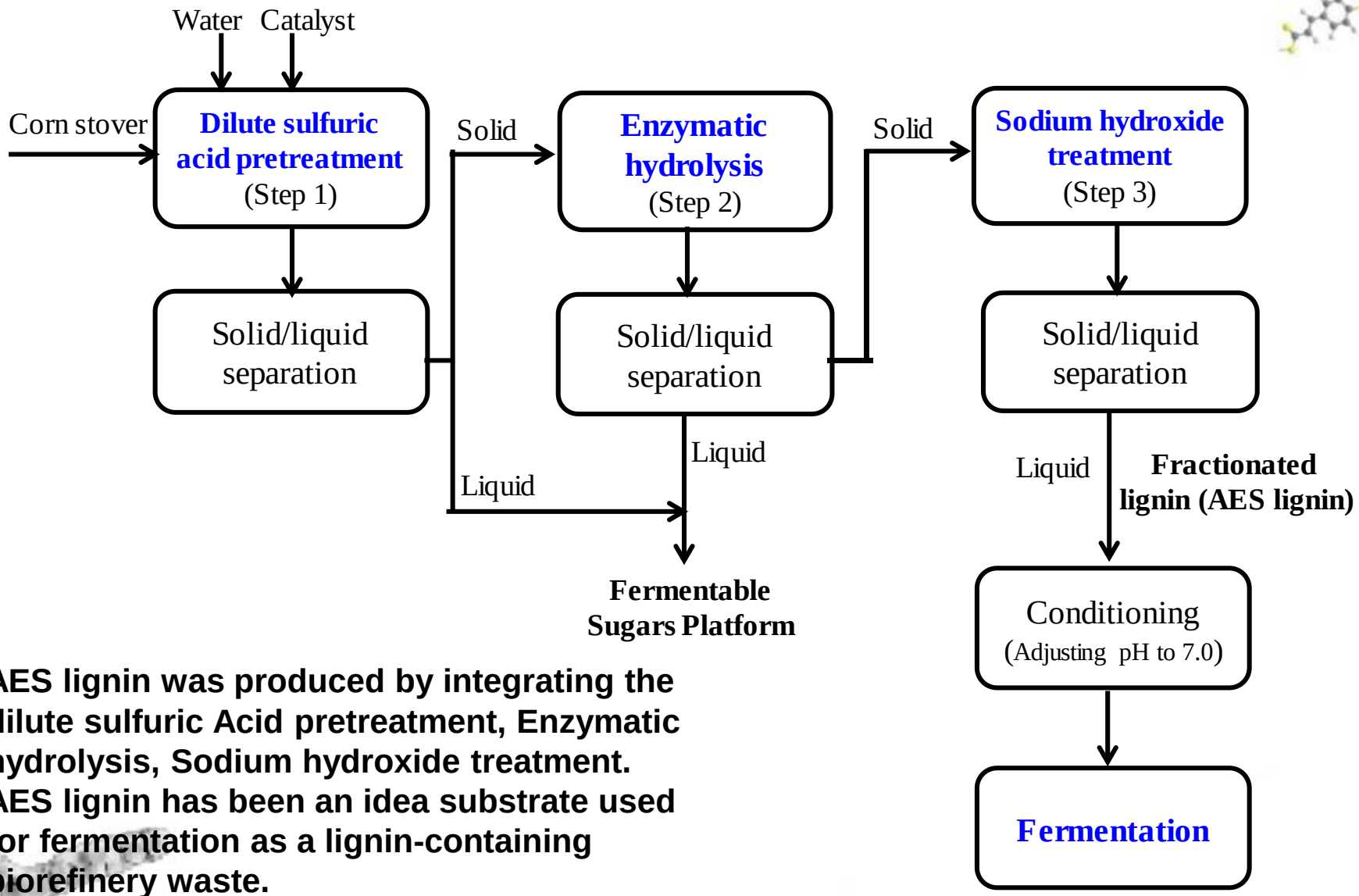
# Conclusion for Residual Sugar in Promoting Lignin Utilization

1. **Low-level of residual sugar can promote lignin consumption by *P. putida* and the conversion to PHA, which guided the development of new biorefinery configuration for Co-processing of Lignin and Residual Sugar (CLARS)**
2. **Based on this discovery, CLARS were developed so that lignin can be better fractionated and residual sugars can be better released.**
3. **The resultant lignin stream have both more residual sugar and more lignin dissolution.**
4. **The resultant lignin stream enabled a record titer of PHA conversion from lignin, as well as high dry cell weight, lignin conversion efficiency, and PHA content.**
5. **The improved lignin conversion is both due to the residual sugar and better lignin fractionation (more aromatic compound released, and lower molecular weight).**

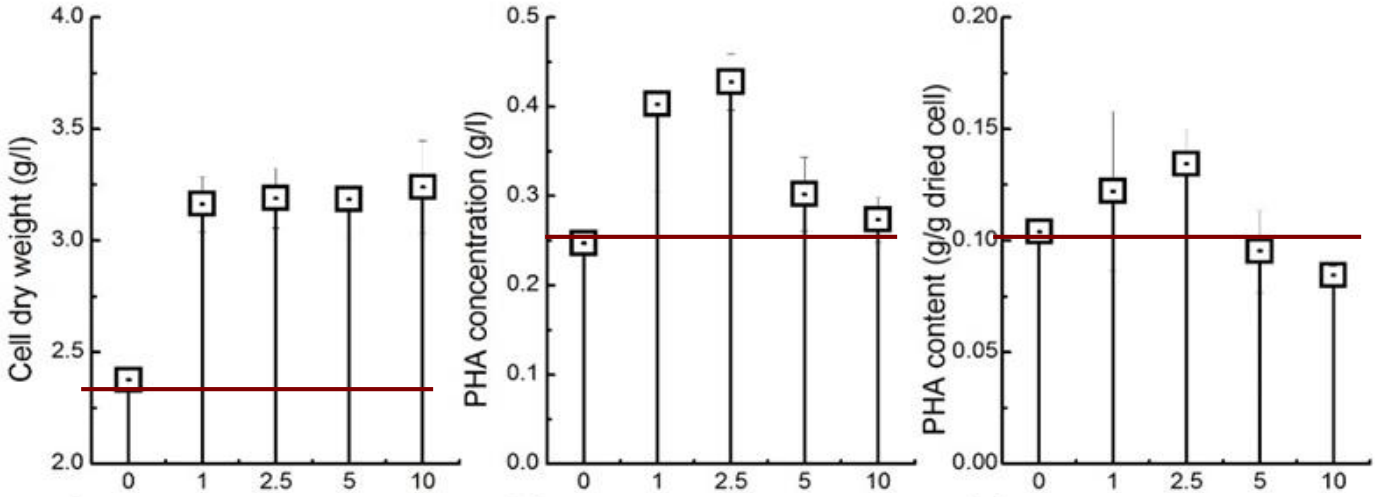
# Project Progresses

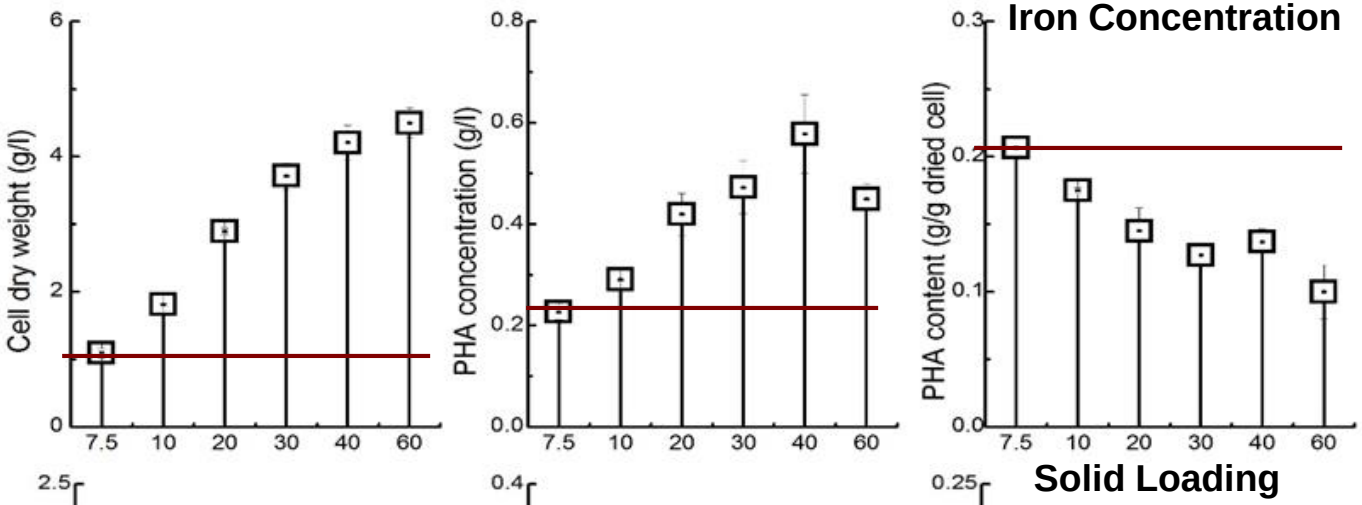


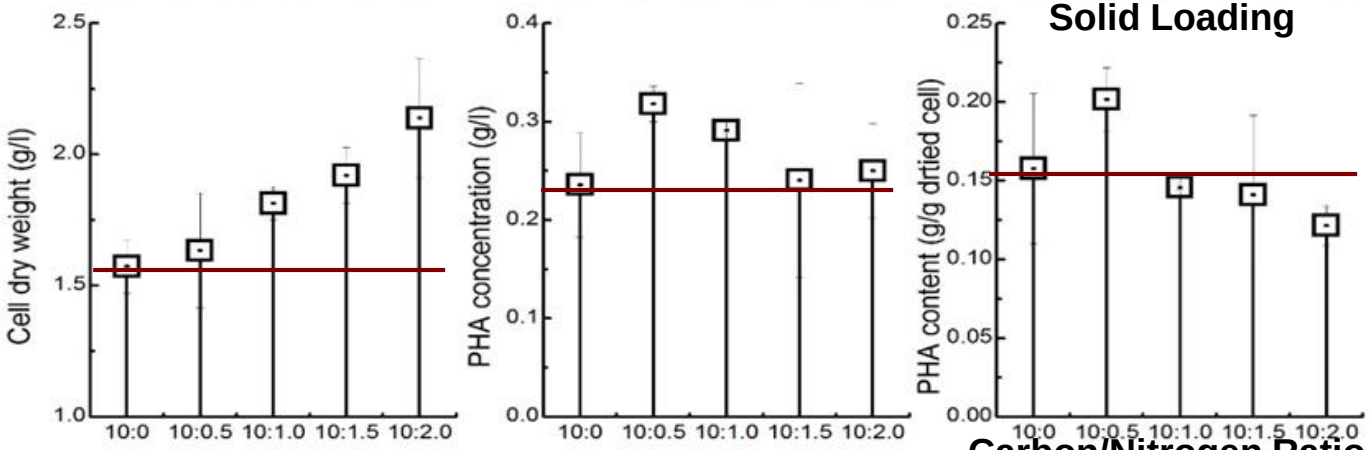
# Process Flow Diagram of Fermentable Sugar and AES Lignin




**AES lignin was produced by integrating the dilute sulfuric Acid pretreatment, Enzymatic hydrolysis, Sodium hydroxide treatment. AES lignin has been an idea substrate used for fermentation as a lignin-containing biorefinery waste.**



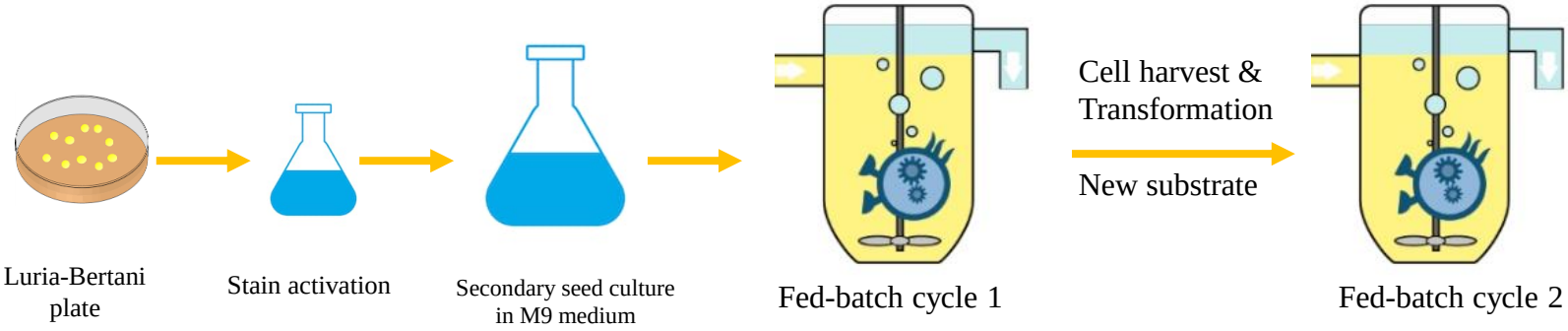

# Optimization of Iron, Nitrogen, and Solid Loading

**Optimal fermentation conditions was identified by fermentation optimization:**

- **Fe Con.: 2.5mg/L**
- **Solid Loading: 40g/L**
- **C:N Ratio: 10:0.5**

**These conditions were used for fed-batch fermentation optimization**

# Fed-batch Fermentation in 2-L Fermentor




| Fed-batch cycle 1 | Fed-batch cycle 2 |
|---|---|
| • EG *P. putida* KT2440 | • EG *P. putida* KT2440 |
| • AES | • AES |
| • 30 g/l SSC | • 30 g/l SSC |
| • 1.0 g/l NH4Cl | • 0 g/l NH4Cl |
| • 1000 ml | • 1000 ml |
| • 18 hours | • 8 hours |
| • pH 7.0 | • pH 7.0 |
| • 28 ° C | • 28 ° C |
| • 180 rpm | • 180 rpm |

**Fermentation conditions**

# Conclusion 1: The conversion efficiency of ferulic acid to PHA is over 30%.

| Parameter | PHA Production (g) | FA Consumption (g) | Conve±rsion Rate (% |
|---|---|---|---|
| Results | 2.94±0.19 | 9.65±0.41 | 30.5% |

Considering the complex substrate of biorefinery waste, Ferulic acid was used to evaluate the conversion efficiency. We have achieved a 30.5% efficiency for FA to PHA conversion. The result met the intermediate milestone.

# Conclusion 2: The Engineered Strain is More Efficient than Wild-type Strain

# Conclusion 3: The PHA Titer Reached 2.5g/L

# Conclusion 4: For 1Kg Lignin Consumed, about 770g of PHA is Produced.

| | Cell Dry Weight (g/L) | PHA Production (g/L) | PHA Content (%) |
|---|---|---|---|
| Wild-Type Strain | 12.59±0.30 | 3.23±1.02 | 25.7% |
| Engineered Strain | 12.30±0.74 | 5.77±0.60 | 46.9% |

The engineered strain produced about the same amount of dry biomass, yet much higher PHA yield and content. The results fits very well with the hypothesis that carbon are redirected to PHA production from lipid oxidation pathway. All intermediate milestones were met.

# Conclusion 5: The Process has been Successfully Scaled up to 1 Liter

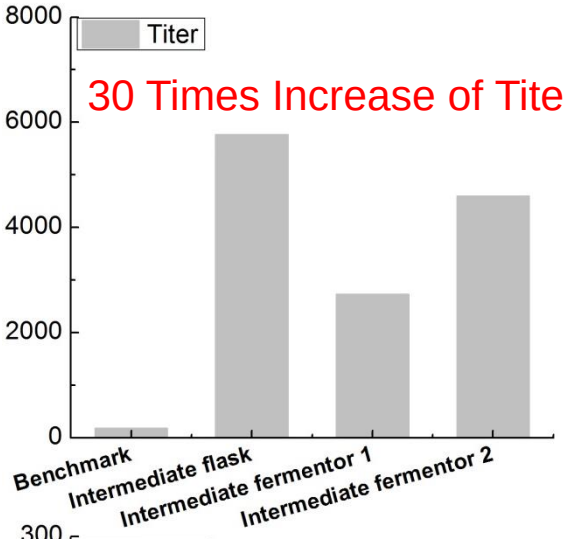
| | Cell Dry Weight (g/L) | PHA Produc-tion (g/L) | Lignin Consum-ption (g/L) | Estimated Conver-sion Rate (%) | MSP ($) |
|---|---|---|---|---|---|
| Batch Pre-Verification | 10.04±0.21 | 4.60±0.35 | 8.75 | 52.6% | $6.07 |
| Batch Verification | 10.01±0.24 | 2.73±0.31 | 8.59 | 31.7% | $10.88 |

The process has been successfully scaled up to the 1 Liter reaction. The conditions are still being optimized, as the yield is still lower than the level in flasks. The scale-up technology can produce PHA between $6.07/Kg and $10.88/Kg.

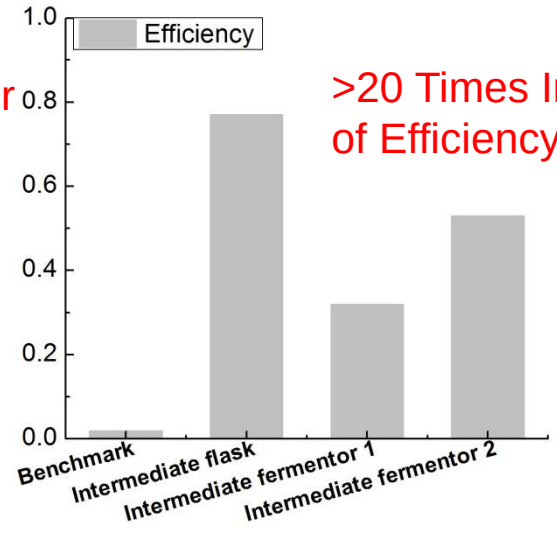
# Conclusion

1. The Fe, SSC, and nitrogen conditions all impact fermentation performance, all of which are optimized in our study.
2. By combining strain engineering, fermentation optimization, and pretreatment/fractionation optimization, we have consistently reached the intermediate target for PHA titer (2.5g/L).
3. The scale up of the process from 50mL to 1Liter reaction was successful.
4. The conversion rate of Ferulic acid to PHA is over 30%.
5. The project meets all of the intermediate milestones, and thus moves to the BP2.

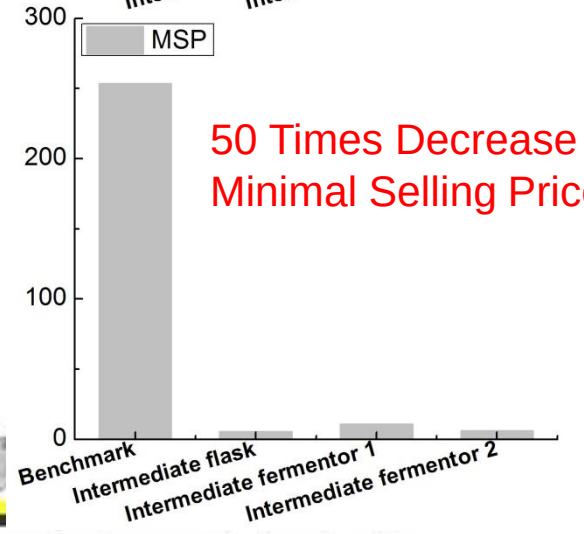
# The Project Has Made Significant Technical Progresses, Enabled Commercialization, and Represented the State-of-the-Art




30 Times Increase of Titer




>20 Times Increase of Efficiency




50 Times Decrease of Minimal Selling Price

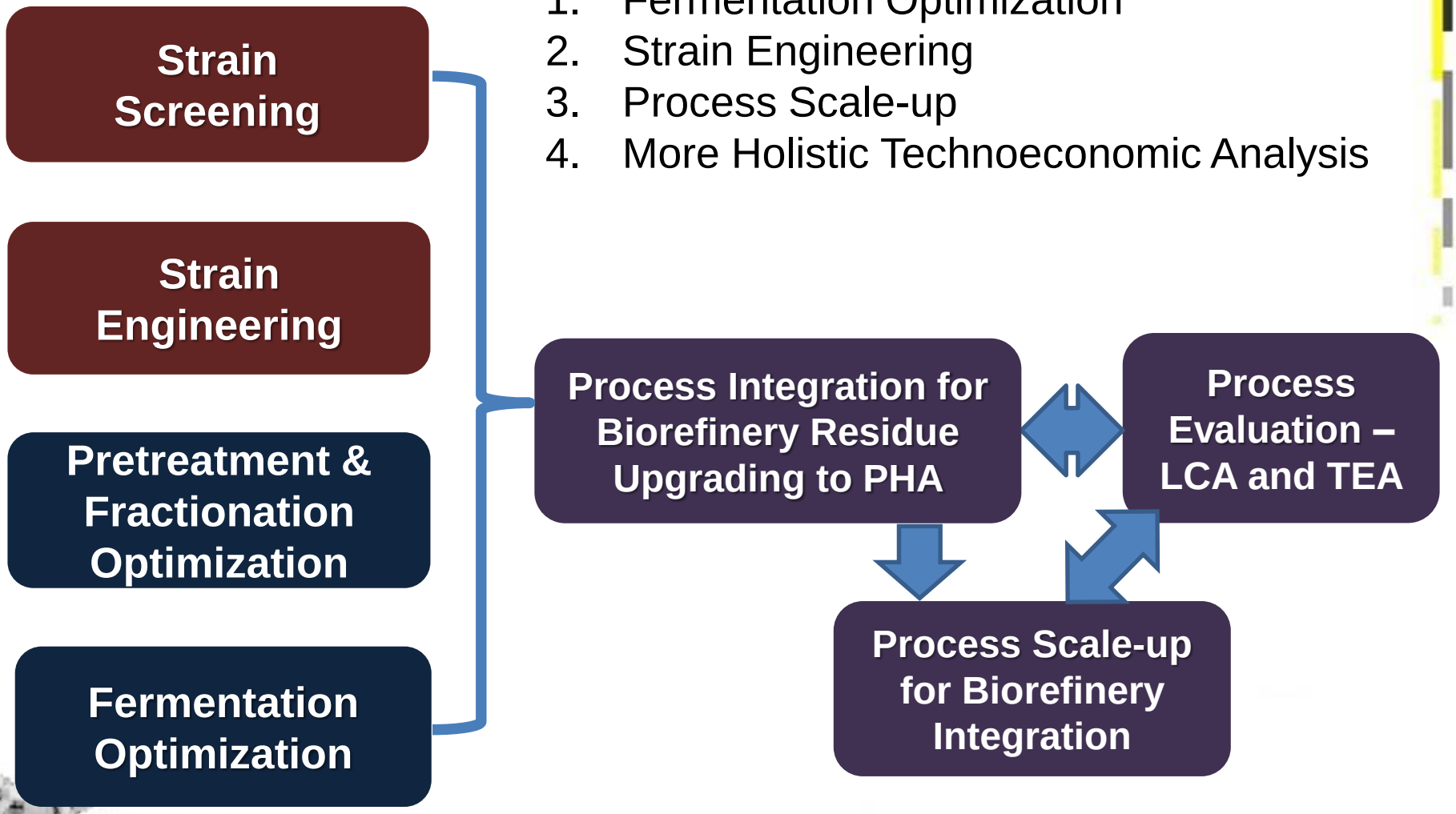
**Key Technical and Economical Progresses:**

- **30 Times increase of PHA titer from biorefinery waste**
- **>20 Times increase of conversion efficiency for biorefinery waste-to-PHA conversion**
- **>50 times decrease of PHA cost**

# Future Work

The future work will focus on:

1. Fermentation Optimization
2. Strain Engineering
3. Process Scale-up
4. More Holistic Technoeconomic Analysis



# Future Work

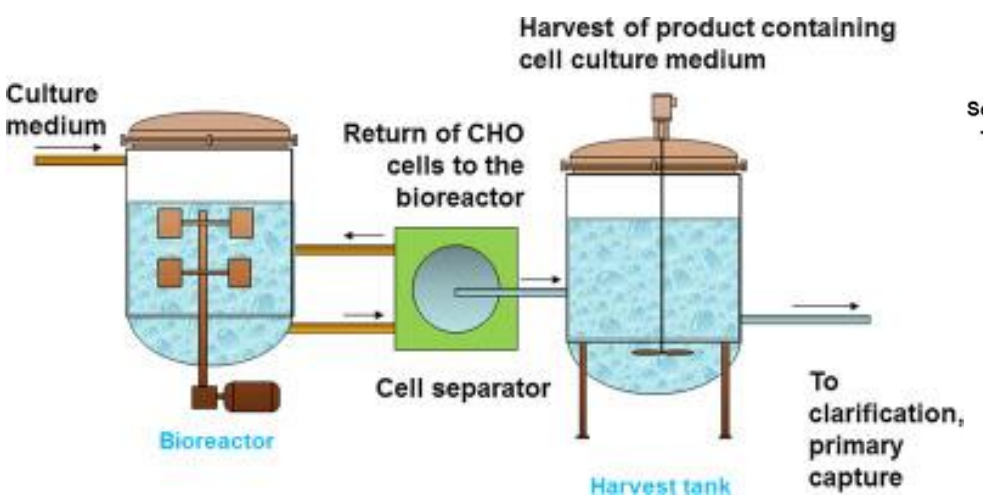
**The goal of future work is to continue to improve PHA conversion, decrease PHA production cost, reduce MESP, and eventually scale up the technologies toward commercialization:**

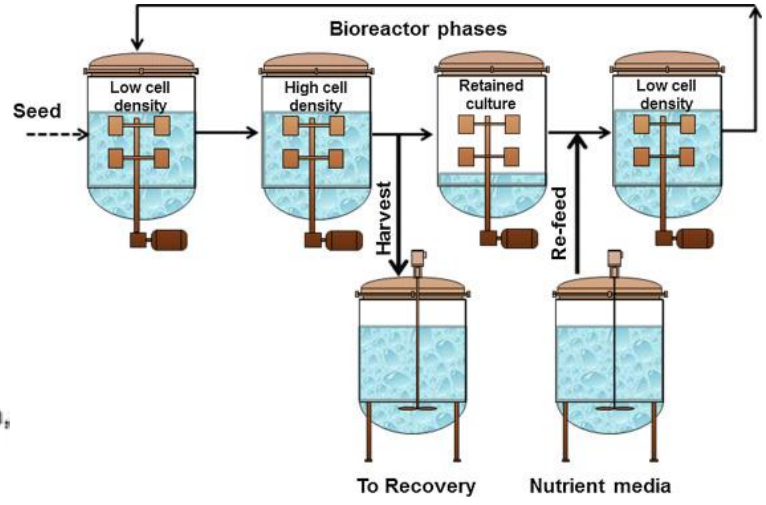
1. **Continue the strain engineering based on omics analysis from the biorefinery waste. In particular, we will focus on blocking the PHA degradation pathways to enhance PHA yield at high cell loading.**
2. **We will work with ICM inc. to scale up the technology to 50 Liter scale.**
3. **We will build more robust TEA model to evaluate various scenario to integrate the lignin-to-PHA process with biorefinery. We will evaluate how to best reduce MESP.**
4. **We will evaluate the effects of inducing reagents, such as acetic acid, glycerol, fatty acid, under low concentration on the PHA yield and the monomer constituents of PHA , which will produce mcl-PHA and will further increase the value of PHA.**

# Future Work

**5. We will continue the fermentation optimization, in particular, to improve oxygen level, introduce defoaming, increase stirring speed, and prepare lignin stream with high SSC for fermentation. All of these will increase PHA yield.**

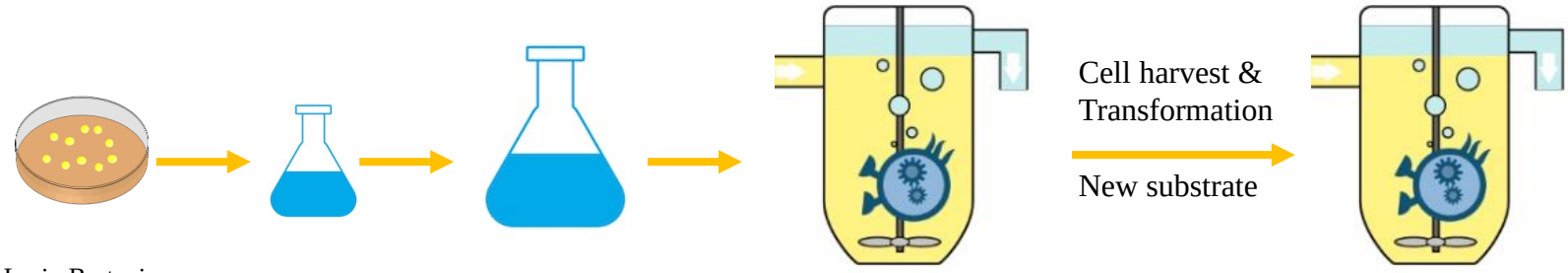
**6. We will try to conduct the semi-continuous culture for PHA fermentation**



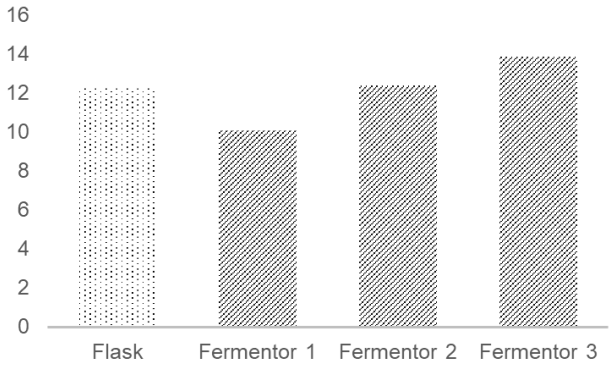




**7. We will evaluate the synergistic utilization of lignin and limited sugars: substrate concentration, ratio of lignin and sugar, fermentation time**

# On-going Work Prove the Effective Future Approach




Luria-Bertani plate | Stain activation | Secondary seed culture in M9 medium | Fed-batch cycle 1 | Fed-batch cycle 2

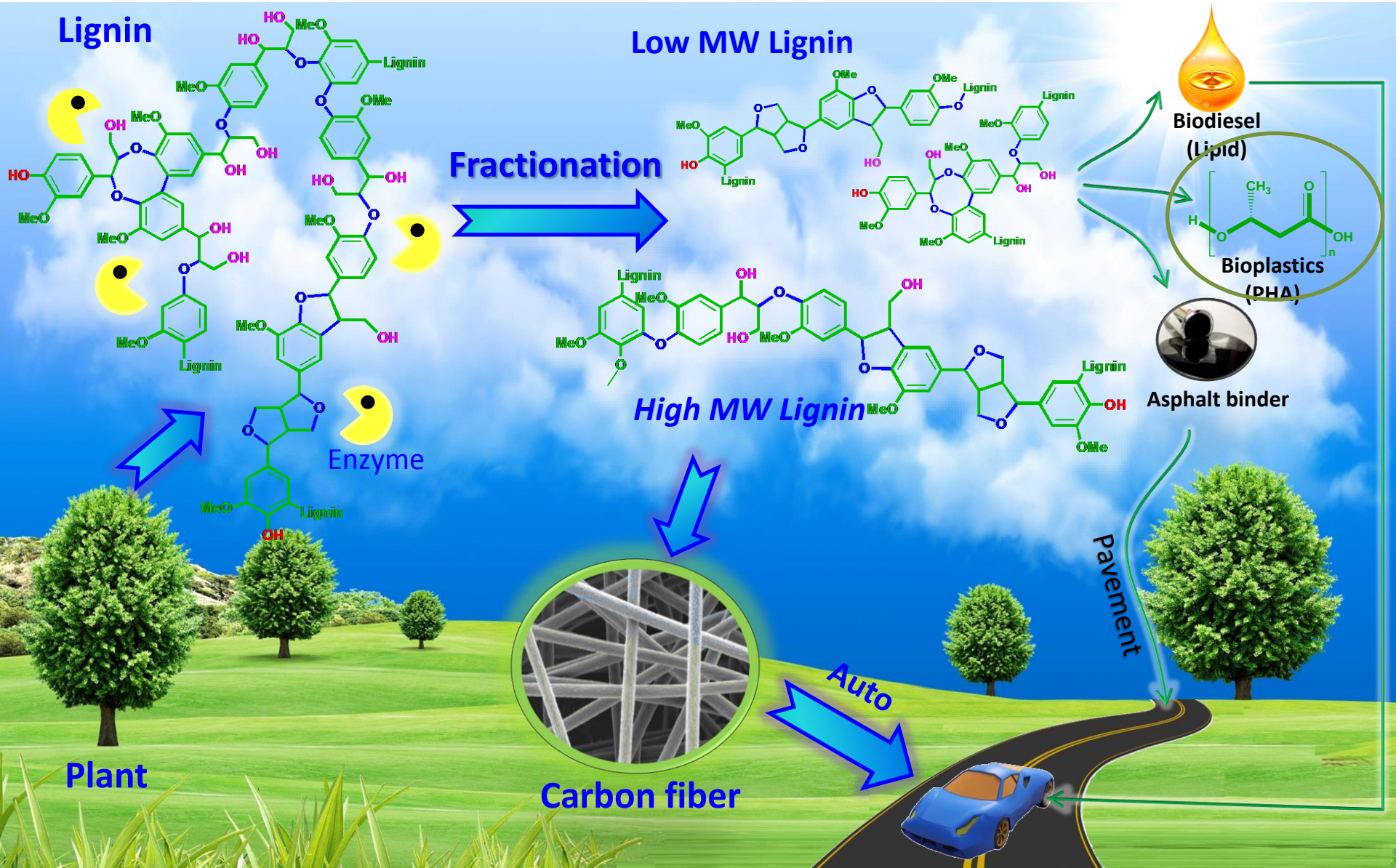



- EG *P. putida* KT2440
- AES
- 1.0 g/l NH4Cl
- 1000 ml
- pH 7.0
- 28 ° C
- 180 rpm

- EG *P. putida* KT2440
- AES
- 0 g/l NH4Cl
- 1000 ml
- pH 7.0
- 28 ° C
- 180 rpm

| | SSC/g/l | Fermentation time | Cell dry weight (g/l) | PHA yield (g/l) | DE-foaming | Control oxygen |
|---|---|---|---|---|---|---|
| Flask | 40 | 18+8 hours | 12.3 | 5.77 | - | - |
| Fermentor 1 | 40 | 18+8 hours | 10.1 | 2.73 | - | - |
| Fermentor 2 | 40 | 18+8 hours | 12.4 | 5.70 | Yes | Yes |
| Fermentor 3 | 50 | 24+24 hours | 13.9 | 6.11 | Yes | Yes |

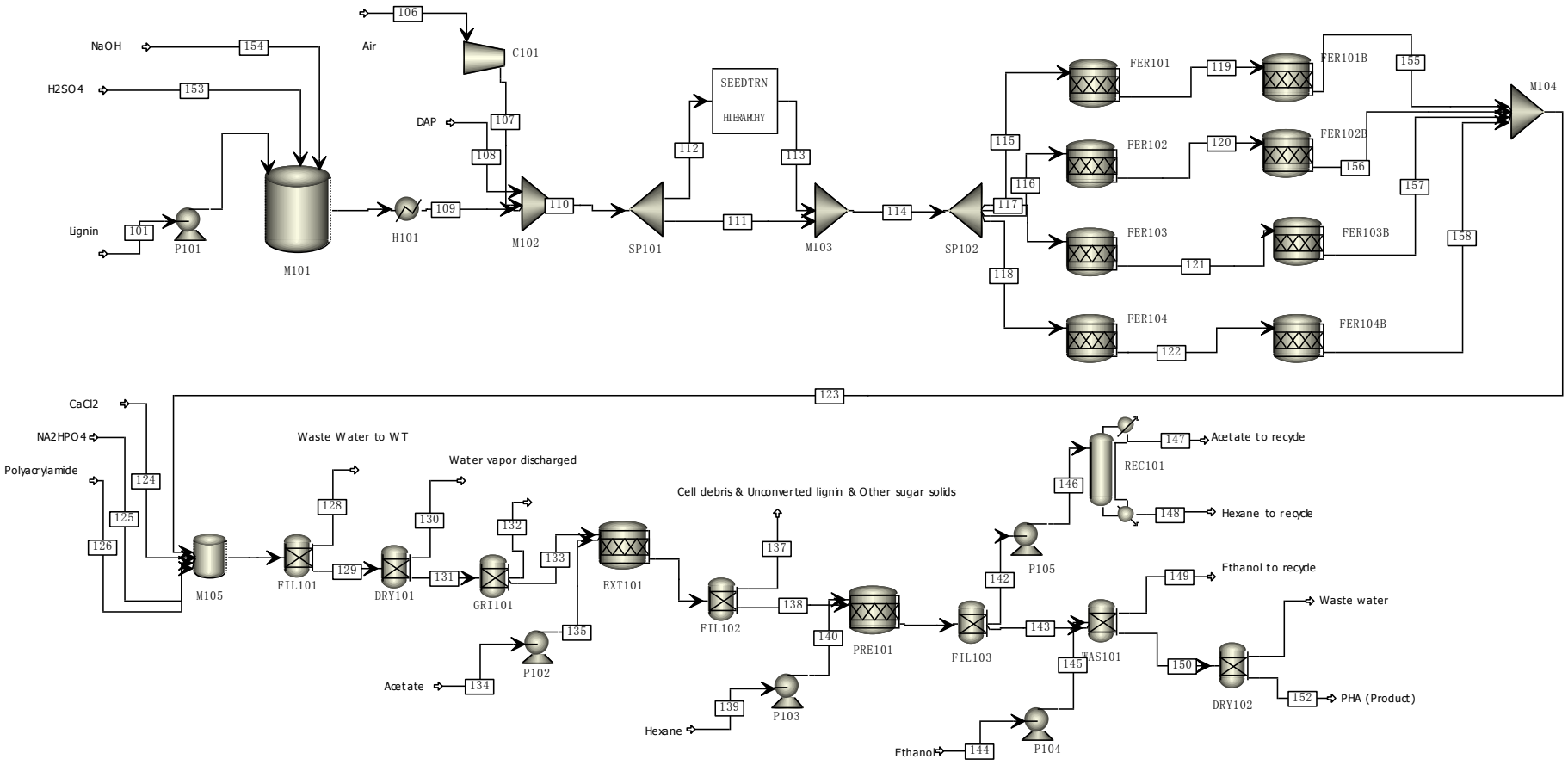
# Relevance – Enabled Multistream Integrated Biorefinery (MIBR)



# Relevance – Direct Relevance to MYPP and Challenges in Biorefinery

- The project will deliver a bioprocess to convert biorefinery residues to PHA at less than $5 per kg. The new biorefinery stream will add significant value to the lignocellulosic biofinery and reduce MEPS.
- With the multiple product stream and the maximized yield for both biofuels and bioproducts, the project well address the MYPP goal to achieve $3/GGE fuels. The project will improve the overall cost-effectiveness of biorefinery and reduce the fuel production cost.
- With more complete utilization of biorefinery residues, the project will address the mission of BETO, the MYPP goals, and the challenges in biofuel industry by improving biorefinery efficiency and sustainability.
- The project will enable the multi-stream integrated biorefinery (MIBR) to maximize the yield of both carbohydrate-based biofuels and lignin-based bioproducts, which will improve the sustainability of biorefinery and enable bioeconomy with value-added products.
- The research will significantly advance the current state-of-the-art in biorefinery residue upgrading. The technical breakthrough can be integrated with different platforms to produce valuable compounds from waste stream. Certain part of the technologies were licensed and we are working with commercial partner for scale up and commercialization.

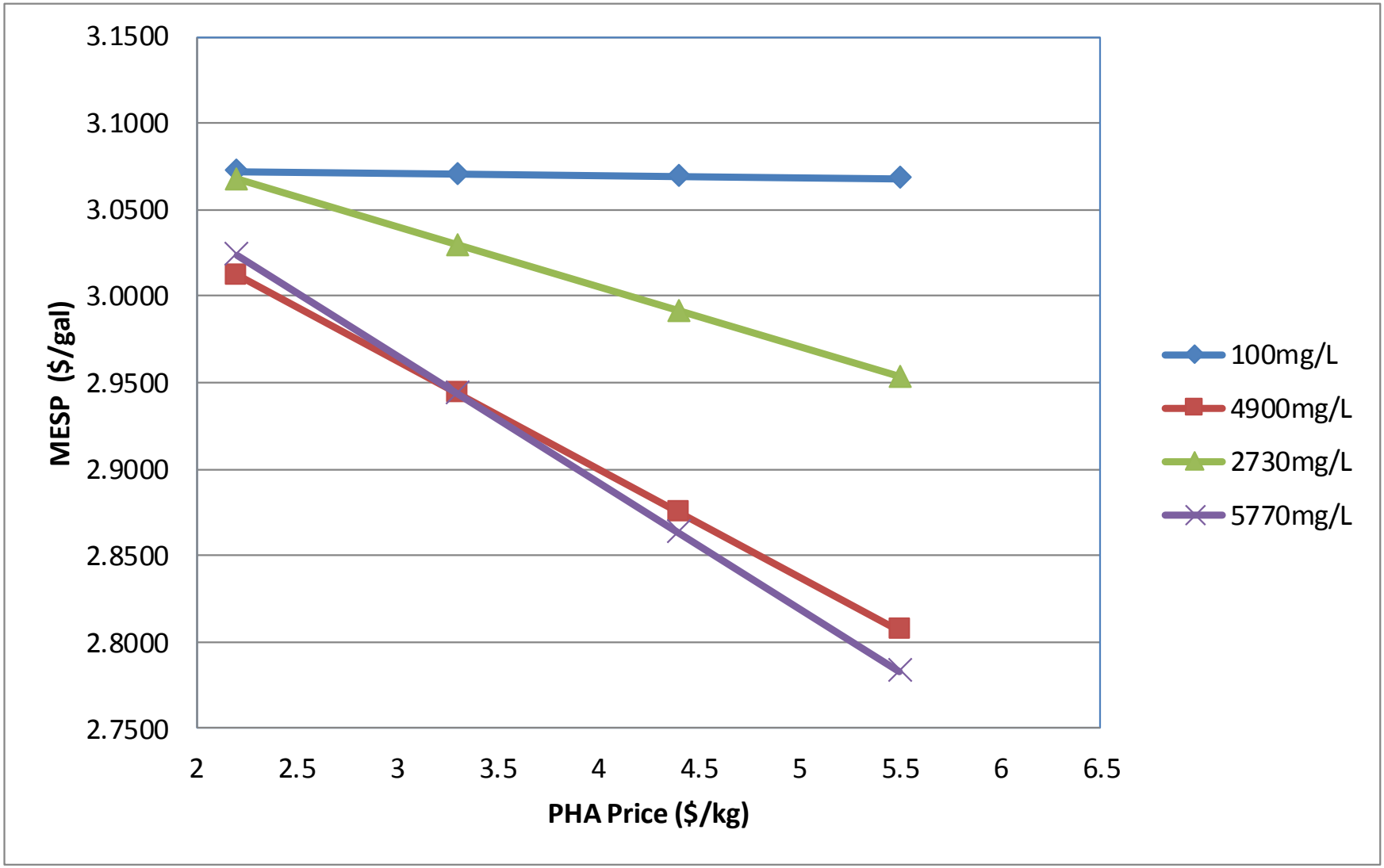
# TEA – Aspen Plus Model of Lignin-to-PHA Process



# Techno-Economic Analysis of PHA

| | UNITS | Proposed Data (100mg/L) | Before Intermediate Validation (4900 mg/L) | Intermediate Validation 1 (2730mg/L) | Intermediate Validation 2 (5770mg/L) |
|---|---|---|---|---|---|
| Annual production | MMkg | 0.74 | 3.56 | 1.98 | 4.18 |
| Total Capital Cost | MM$ | 41.1 | 41.6 | 41.9 | 42.1 |
| Total Operating Cost | MM$/yr | 9.08 | 12.06 | 12.12 | 13.3 |
| Raw Material | MM$/yr | 2.59 | 6.08 | 5.96 | 6.61 |
| Utilities | MM$/yr | 0.21 | 0.28 | 0.29 | 0.28 |
| Unit cost | $/kg | 197.74 | 5.81 | 8.76 | 4.52 |
| Rate of return | % | 10 | 10 | 10 | 10 |
| Minimum selling price | $/kg | 253.53 | 6.07 | 10.88 | 5.54 |

# Effects of PHA Price on the MESP



# TEA Summary – Direct Relevance of the Research to BETO and Bioeconomy

- Coproduction of PHA from waste lignin can improve the overall economic viability of an integrated process for lignocellulosic ethanol production by increasing revenue from fuels and co-product chemicals;
- The reduction of MESP is directly affected by the PHA selling price, which is a direct function of plant productivity (i.e. lignin utilization and conversion);
- Results indicate that the projected minimum PHA selling price will decrease as the technologies developed in this project mature over the life time;
- The original proposed bench mark selling price was $253.53/kg corresponded with fermentation titer at 100 mg/L. The Intermediate Validation selling price is around $ 10.88/kg (PHA titer at 2730 mg/L) since the titer is significantly increased.

# Summary

The project develops innovative solution for biorefinery waste utilization, transforming waste stream into profitable stream.

1. Overview – New biorefinery design to enable biorefinery waste upgrading, which will improve both cost effectiveness and sustainability of biorefinery.
2. Approach
   - Process enablement by microbial engineering
   - Process development by fractionation and fermentation improvement
   - Process optimization by TEA and scale-up.
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.
   - Efficient fed-batch fermentation to further improve PHA conversion
   - The project achieved all intermediate milestone goals and resulted in >20 publications and patent applications
4. Relevance
   - The project is directly addressing MYPP goals.
   - Aspen plus model indicated that the process has potential to significant reduce MESP and benefit the biorefinery and biofuel industry.
5. Future work
   - We will continue the process optimization with more fermentation optimization and strain engineering
   - We will scale up the technology and evaluate the impact on biorefinery

# Publications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12. Xie S, Li Q, Karki P, Zhou F, Yuan JS. Lignin as Renewable and Superior Asphalt Binder Modifier. ACS Sustainable Chemistry & Engineering. 2017 Mar 22;5(4):2817-23.
13. Yuan JS, Li Q, Ragauskas AJ. Lignin carbon fiber: The path for quality. Tappi Journal. 2017
14. Xie S, Sun Q, Pu Y, Lin F, Sun S, Wang X, Ragauskas AJ, Yuan JS. Advanced chemical design for efficient lignin bioconversion. ACS Sustainable Chemistry & Engineering. 2017 Jan 31;5(3):2215-23.
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- **33 papers have been published for the project.**
- **A comprehensive IP for lignin to bioplastics, carbon fiber and pavement materials have been filed for PCT**
- **Numerous industrial contacts have been initiated regarding commercialization.**

# Acknowledgement


**Project Management:**

Jay Fitzgerald  Joshua Messner

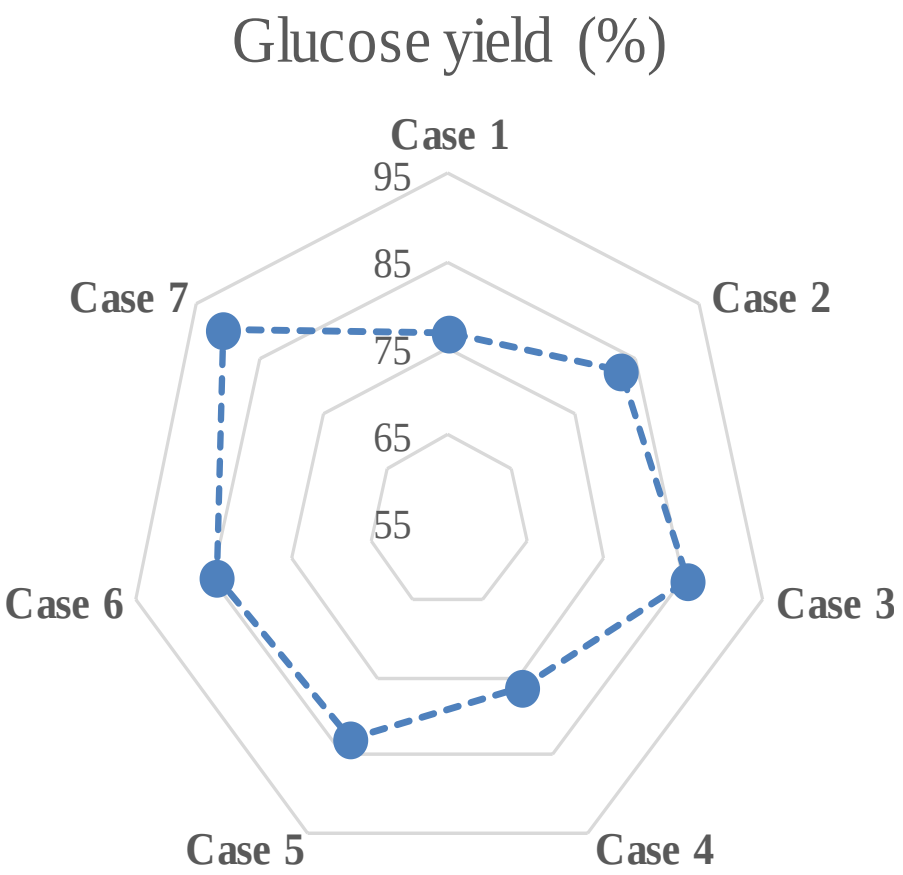
**CoPIs:**

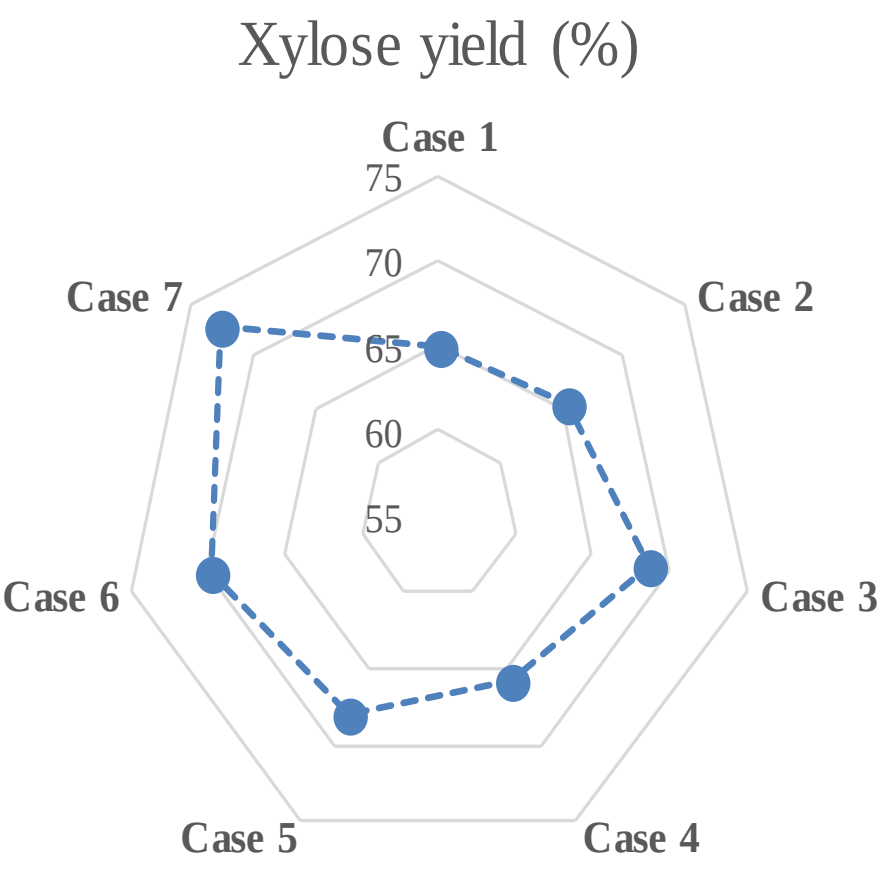
Dr. Art Ragauskas  Dr. Bin Yang

Dr. Jeremy Javers  Dr. Susie Y. Dai

Dr. Dennis Gross  Dr. Betsy Pierson

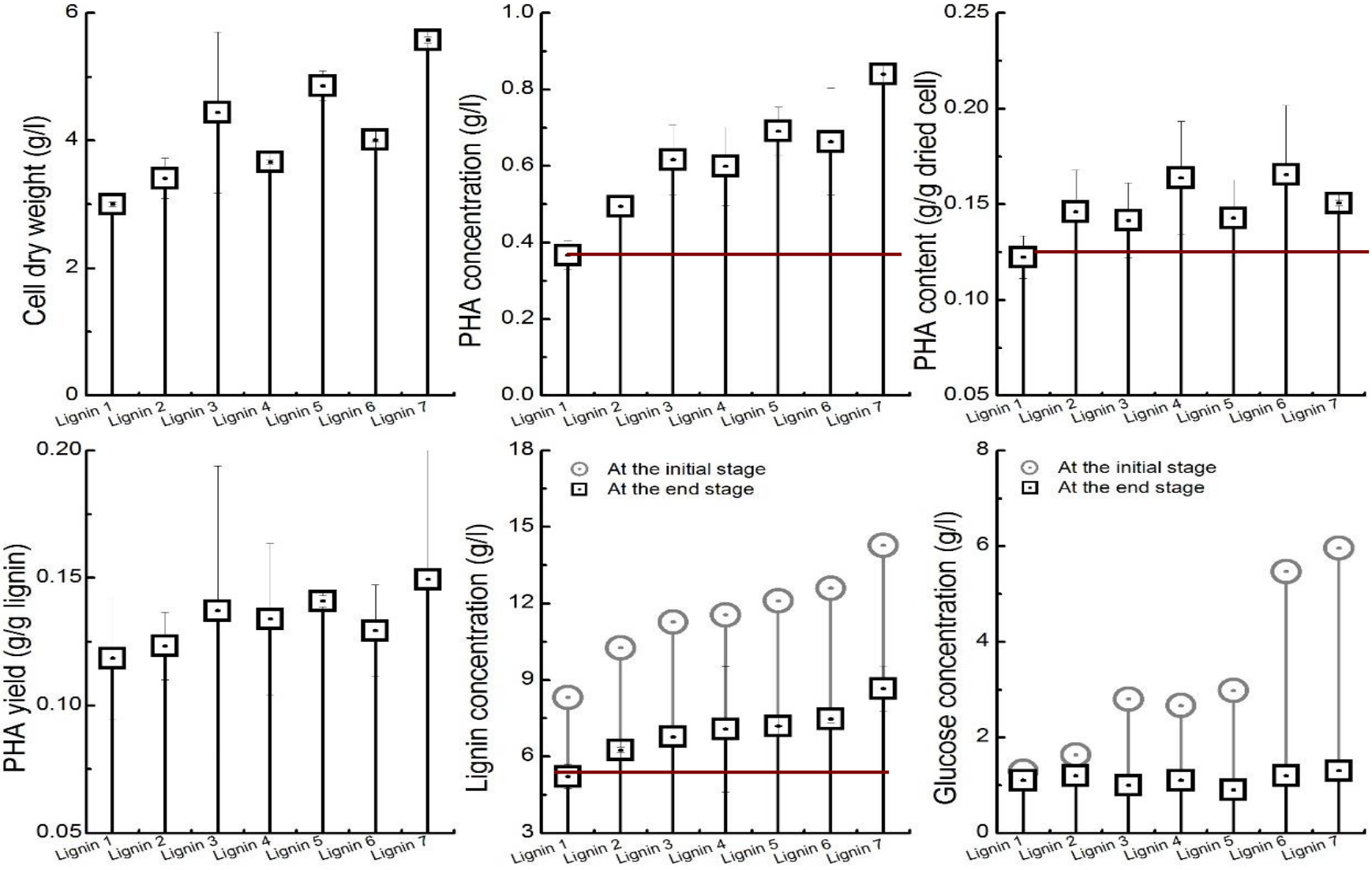

# Total Sugar Releases



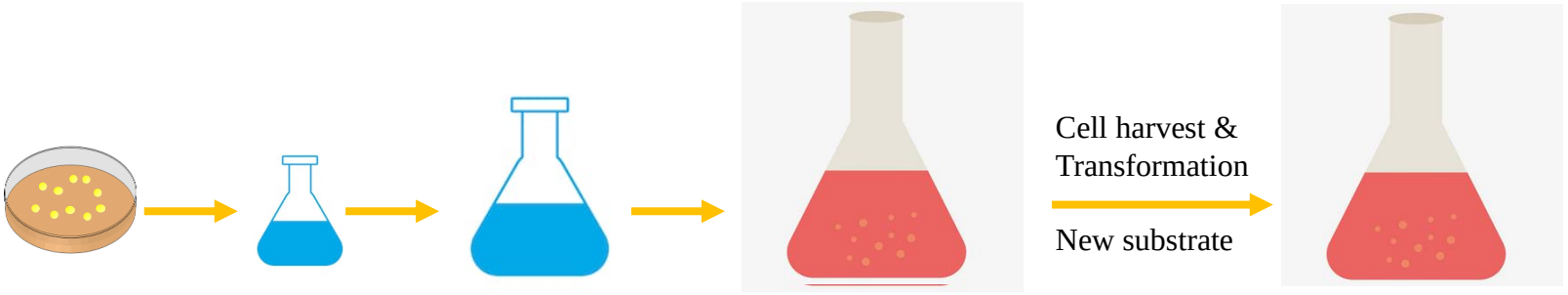




Get rid of it

# Residual Sugar Promotes Lignin Utilization



# Conversion Rate of Lignin (Aromatics) to PHA




Luria-Bertani plate | Stain activation | Secondary seed culture in M9 medium | Fed-batch culture cycle 1 | Fed-batch culture cycle 2

**Get one slide**

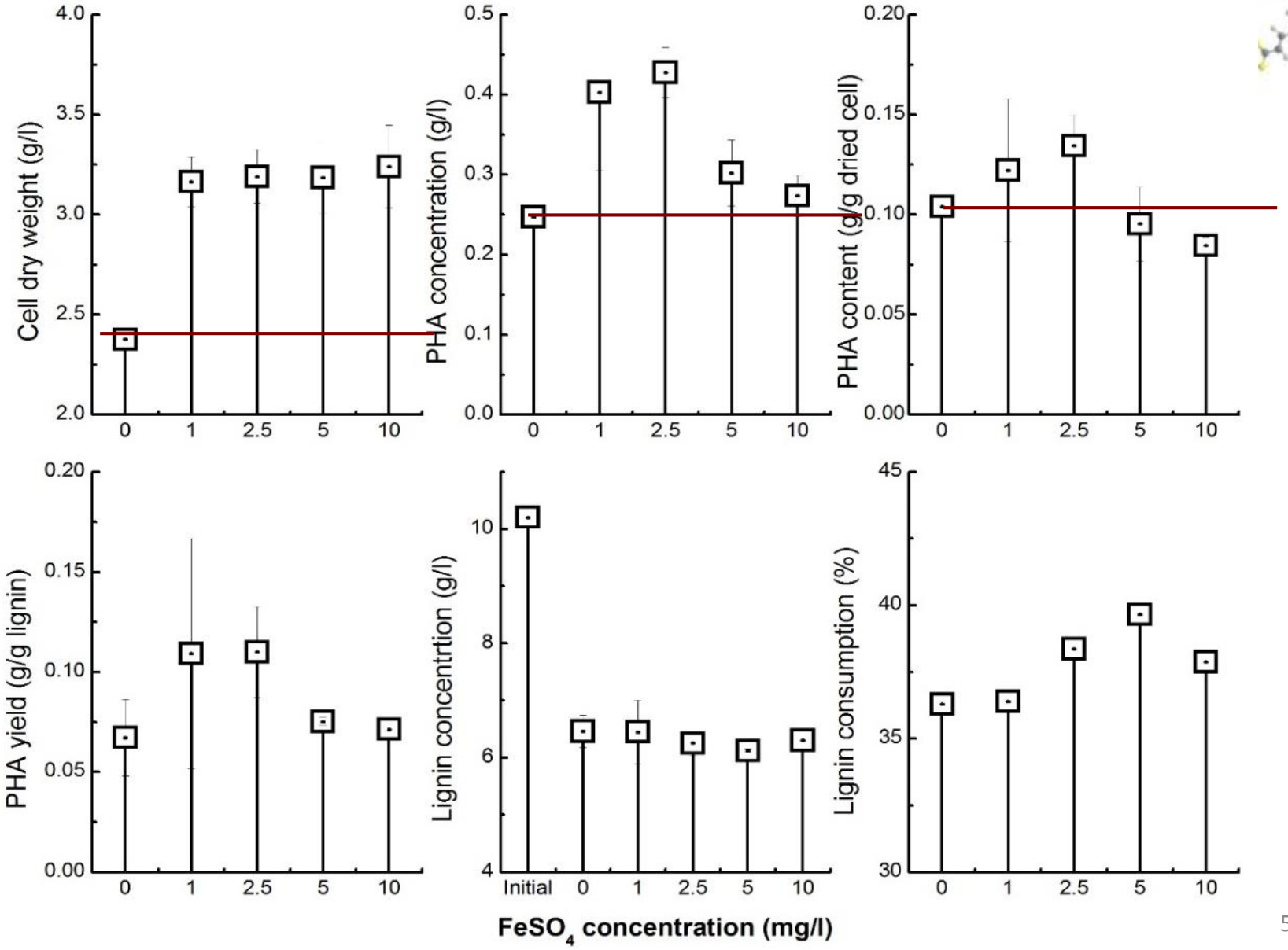
**Define AES lignin, It is better put a process here**

- **Ferulic acid**
- EG *P. putida* KT2440
- 5 g/l substrate
- 1 g/l NH4Cl
- 50 ml
- 15 hours
- pH 7.0
- 28 ° C
- 180 rpm

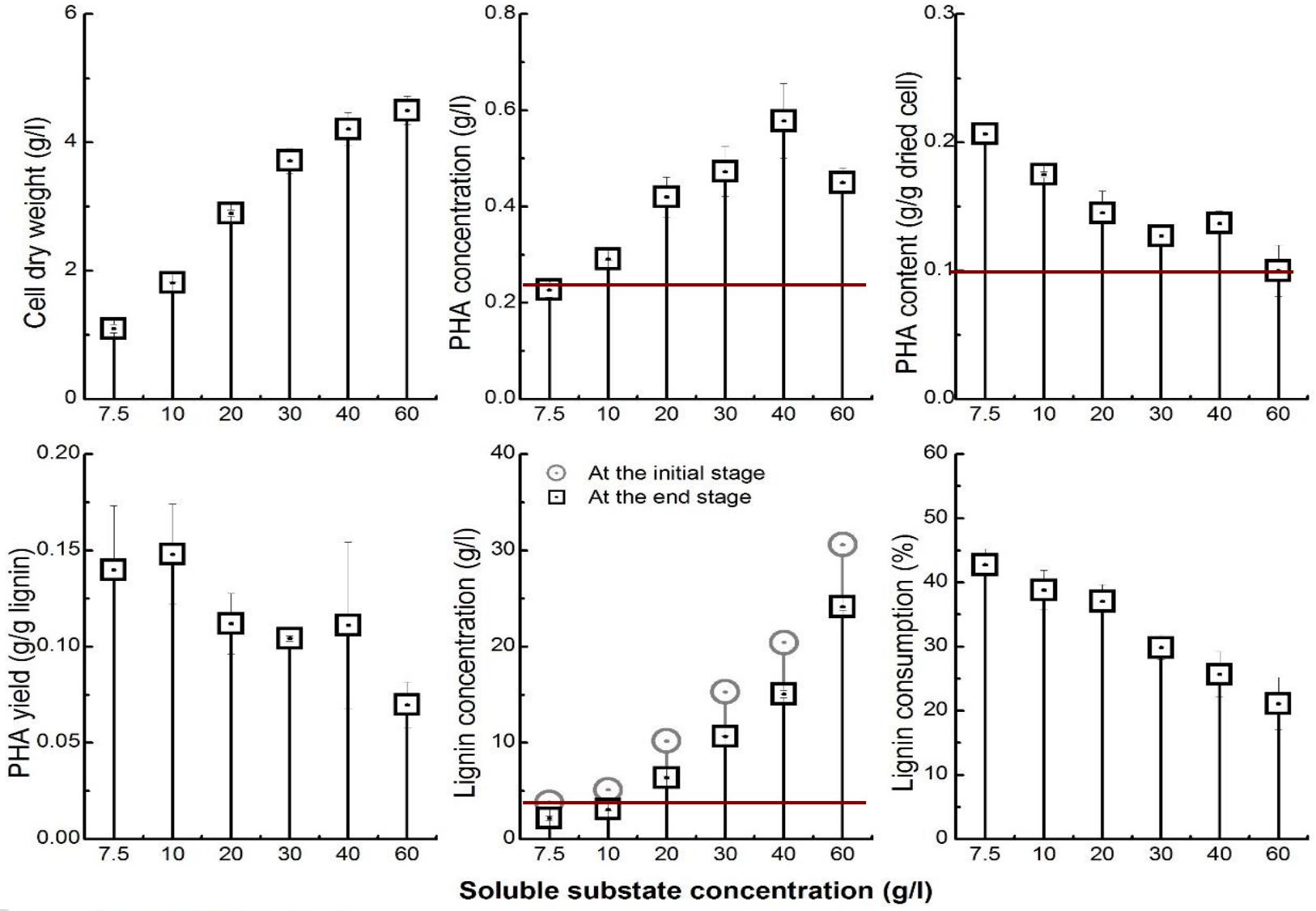
- **Ferulic acid**
- EG *P. putida* KT2440
- 5 g/l substrate
- 0 g/l NH4Cl
- 50 ml
- 5 hours
- pH 7.0
- 28 ° C
- 180 rpm

**Fermentation conditions**

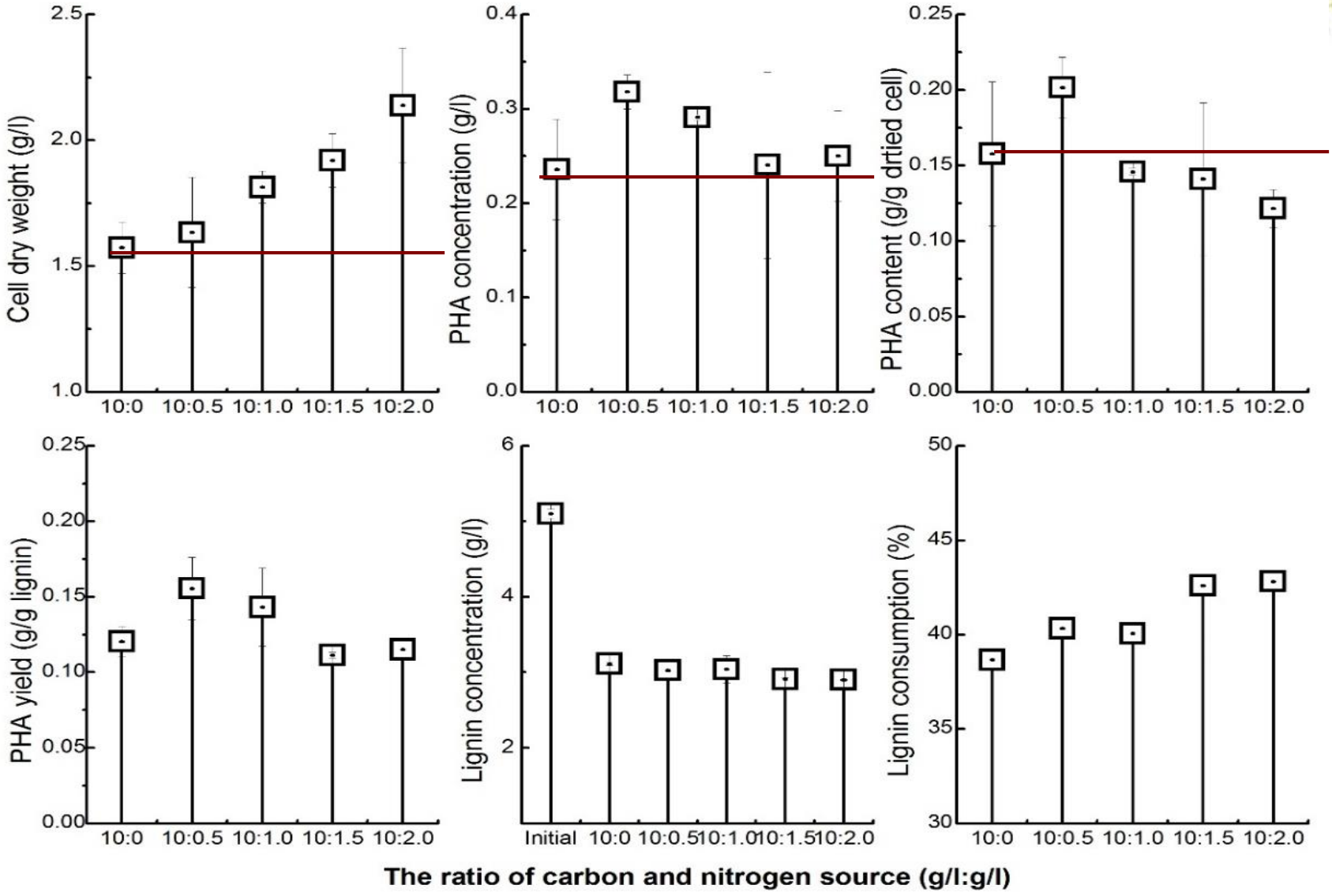
# Optimization of Iron Concentration



# The Impact of Soluble Substrate Concentration on Lignin Fermentation



# Optimization of Carbon Nitrogen Ratio



# Mass Balance of the Process