Design, Techno-Economic Analysis, and Life Cycle Assessment of Consolidated Bioprocessing for Ester Production

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Outline

- Part 1: Motivation and strategy for CBP ester production
- Part 2: Models of microbial cell physiology to constrain process simulation
- Part 3: Techno-economic analysis and life cycle assessment to inform R&D
- Summary

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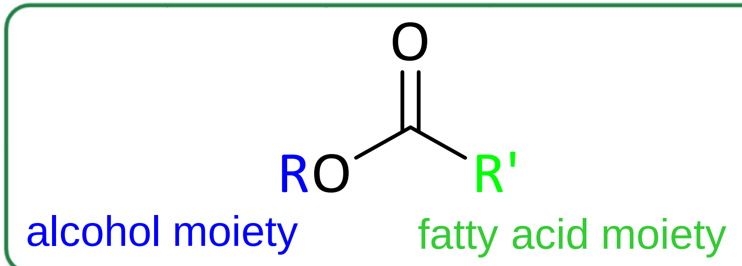
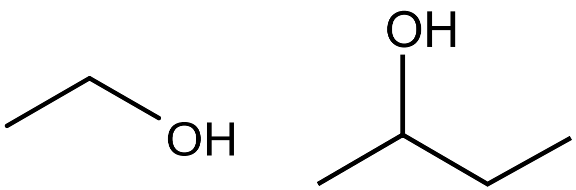
Importance of sustainable ester production in the emerging bioeconomy

	Long chain fatty acids (C14 +)	Medium chain fatty acids (C5 – C13)	Short chain fatty acids (C1 – C4)
Short and medium chain alcohols (C1-C9)	**Solvents, plasticizers and lubricants** Ethyl stearate **Biodiesel** Fatty acid methyl esters (FAME) Fatty acid ethyl esters (FAEE) **Vegetable oils and fats** Sunflower oil Soybean oil Rapeseed oil Cocoa butter Palm oil	**Solvents, plasticizers and lubricants** Hexanedioates (adipates) Decanedioates (sebacates) Ethyl heptanoate Dimethyl terephtalate **Drop-in fuels** Ethyl pentanoate, Isobutyl hexanoate **Aroma compounds and fragrances** Pentanoates	**Solvents, plasticizers and lubricants** Ethyl acetate Butyl propanoate Methyl acrylate Vinyl acetate Pentyl acetate Methyl lactate Ethyl acrylate **Drop-in fuels** Butyl butyrate **Aroma compounds and fragrances** Ethyl acetate Isoamyl acetate
Long chain alcohols C10+	**Waxes, coatings, adhesives, cosmetics** Dodecyl hexadecanoate Undecyl hexadecanoate Jojoba oil		



Kruis et al. Biotechnol. Adv. 2019

Expanding current CBP target molecules into esters

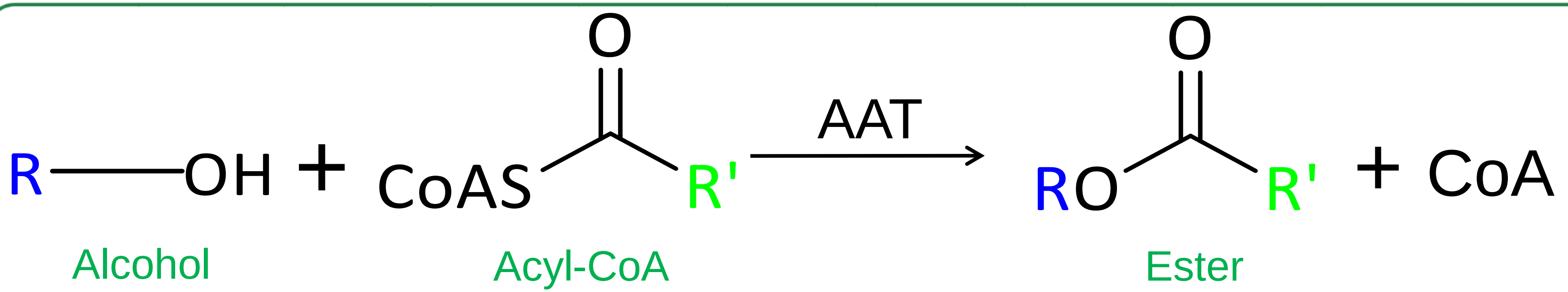


- Current CBP production targets are C2 and C4 alcohols using the bacterium *C. thermocellum*
- These alcohol pathways involve acyl-CoA and alcohols, enabling extension towards ester synthesis:
  - Acetyl-CoA, butyryl-CoA, isobutyryl-CoA
  - Ethanol, butanol, isobutanol
- C4-derived esters have low solubility (<5 g/L) as compared to C4 alcohols (~70 g/L):
  - Avoid biocatalyst inhibition associated with alcohols
  - Lower separation costs

C4-derived esters

- butyl acetate
- isobutyl acetate
- ethyl butyrate
- butyl butyrate
- isobutyl butyrate
- isobutyl lactate
- …

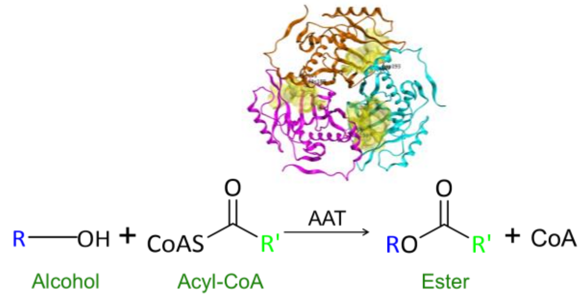
Ester Biosynthesis pathway



Ester CBP development approach

Enzyme engineering[1]

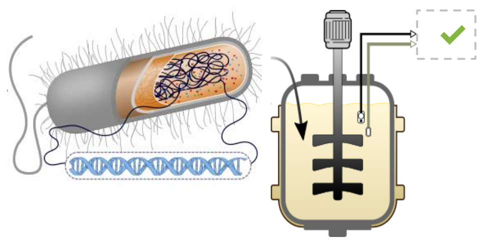
Engineer novel enzyme for most efficient ester condensation in the thermophile *C. thermocellum.*



Overcome pathway bottlenecks

Metabolic engineering[2]

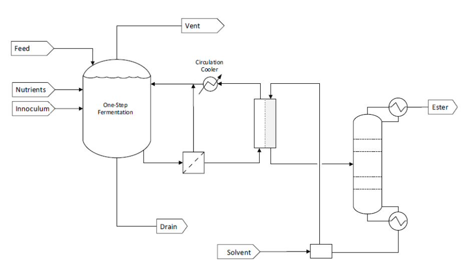
Engineer genetic and medium manipulations for high yield, rate, and titer ester production in *C. thermocellum.*



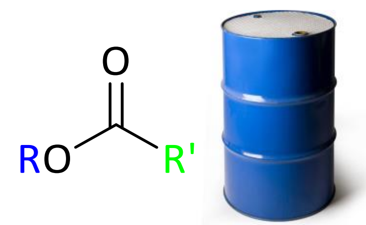
Narrow down R&D space

Process engineering

Engineer processes for economically feasible and environmentally sustainable ester production.

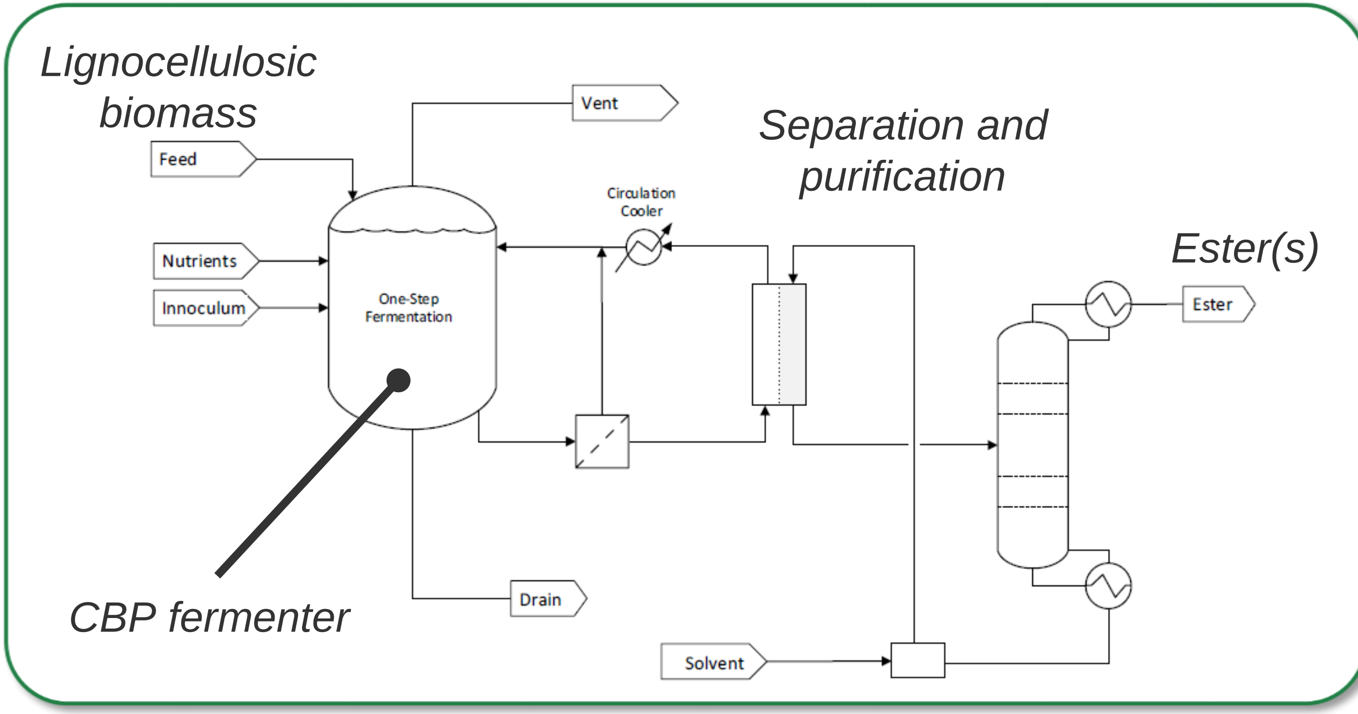


Ester CBP biorefinery



1. Seo, Lee, Garcia, and Trinh. Under review (see **Seo's #156 and Lee's #178 posters**)
2. Garcia, Dash, Maranas, and Trinh. In preparation (see **Garcia's poster #145**)

"One-step" CBP ester production process



- TEA will be used to determine what performance thresholds in the following metrics should the biocatalyst (*C. thermocellum*) meet:
  - Productivity: Rate of product synthesis (*g/L/hr*)
  - Yield: Fraction of substrate that is converted into product (*g product/ g substrate*)
  - Titer: Final concentration of product (*g/L*)
  - Toxicity tolerance: Maintain function upon high product concentration (*g/L*)
- What is needed to accurately simulate this process?

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Macroscopic kinetic model for bioreactor design

- Phenomenological model is formulated to describe the empirical relationships of key fermentation properties including:
  - Inhibition of microbial growth due to substrate or product concentrations
  - Product synthesis rates associated to growth or stationary phases
- More detailed models (e.g., genome-scale metabolic model*) will be added to integrate biocatalyst and process design.

Model

$$\mu = \begin{cases} \dfrac{\mu_m S}{S + K_S + (S^2/K_I)} \left(1 - \dfrac{P}{P_d}\right)^a, & \text{if } P \leq P_d \\ 0, & \text{otherwise} \end{cases} \quad (1)$$

$$\frac{dX}{dt} = \mu X \quad (2)$$

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}} \frac{dX}{dt} + \frac{1}{Y_{P/S}} \frac{dP}{dt} + \frac{Y_{B/P}}{Y_{P/S}} \frac{dP}{dt} + mX \quad (4)$$

- **(1-2)** Growth rate is limited and inhibited by substrate (K_s, K_I) and also inhibited by product (a). Above the toxic product concentration P_d growth does not occur.
- **(3)** Product synthesis rates occurs in two phases, growth (α) and non-growth (β).
- **(4)** Mass balance applies to biomass ($Y_{X/S}$), product ($Y_{P/S} = 1.03\ \text{gg}^{-1}$), and byproduct ($Y_{B/P}$).

Modeling of batch experimental kinetics and applications to fed-batch fermentation of Clostridium tyrobutyricum for enhanced butyric acid production. Song et al. Biochem. Eng. 2010.

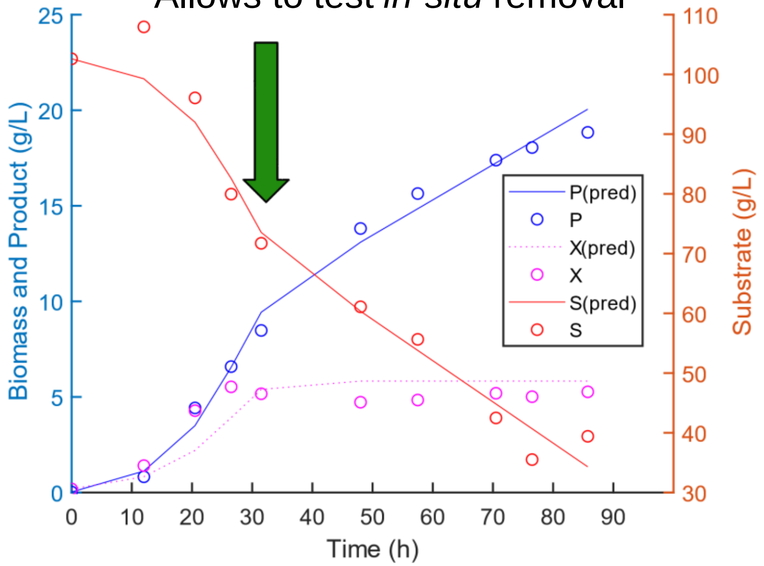
* See **Garcia's et al. poster #145**

Macroscopic kinetic model for bioreactor design

- Ester fermentation data is not currently available. So high-substrate loading fermentation data was used and all alcohols and acids were assumed to form esters.
- This is an optimistic assumption, thus if the process is infeasible we can be certain of such result, otherwise we can take a more pessimistic route
- The model, despite its simplicity, provides a good fit for the data! So we are ready for TEA/LCA.

Model fitting results

Critical product concentration, P_D =10 g/L, stops growth. Allows to test *in-situ* removal



Parameter	Description	Value
μ_m	Maximum growth rate (h^{-1})	0.28
K_s	Substrate saturation constant (gL^{-1})	2.08
K_I	Substrate inhibition constant (gL^{-1})	84
P_d	Critical product concentration (gL^{-1})	10
a	Degree of product inhibition	0.58
α	Growth-associated product formation ($gg^{-1}DCW$)	1.4
β	Non-growth-associated product formation ($gg^{-1}DCW\ h^{-1}$)	0.032
$Y_{X/S}$	Stoichiometric yield coefficient for biomass on cellulose substrate (gg^{-1})	0.82
$Y_{P/S}$	Stoichiometric yield coefficient for isobutyl acetate product, on cellulose substrate (gg^{-1}).	(1.03)
$Y_{B/P}$	Stoichiometric yield coefficient for biproducts, including CO2, amino acids, etc., on cellulose substrate (gg^{-1})	1.2
m	Maintenance coefficient (h^{-1})	0.052

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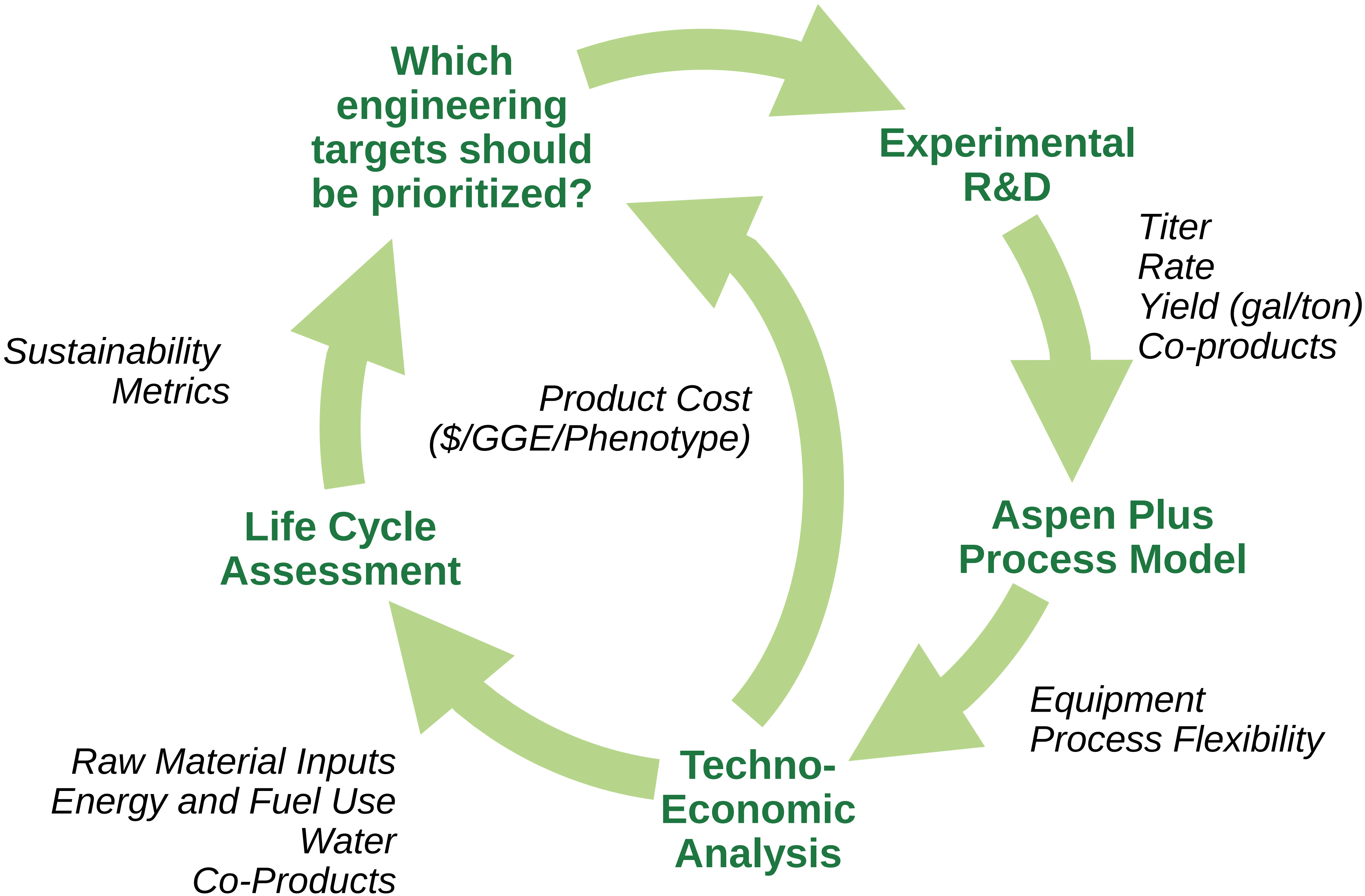
Informing R&D with TEA and LCA



Key Questions Being Explored

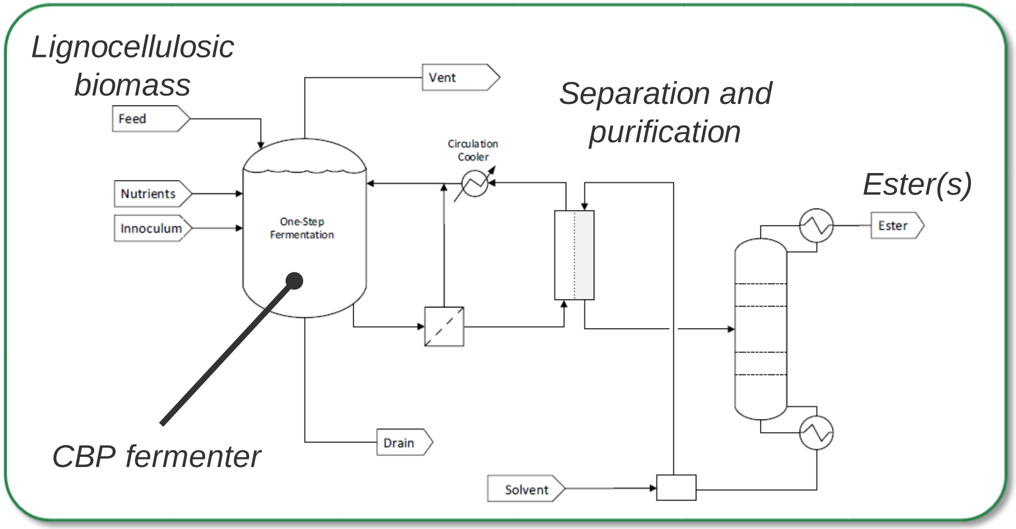
- How do **alternative strategies** for production impact overall cost and sustainability?

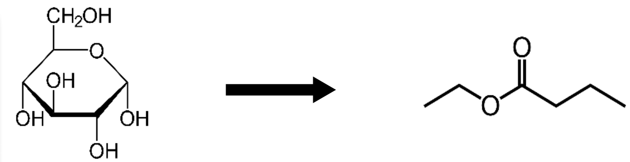
- How **does system robustness** (e.g., in conversion yield, product toxicity, other process variables) **affect costs, sustainability, and process risks**?

- What are the **current risk/limitations** in the integrated process and **how is the basic science tracking to overcome** these challenges?

- Key metrics: Minimum Fuel Selling Price (MFSP)(*$/GGE*); Greenhouse Gas Emissions (*kg* CO_2*-eq/ton*); Cumulative Energy Demand (*MJ/ton*), many others relevant to CBI goals

"One-Step" Process Configuration: Production of Ethyl Butyrate via Mono or mixed Culture



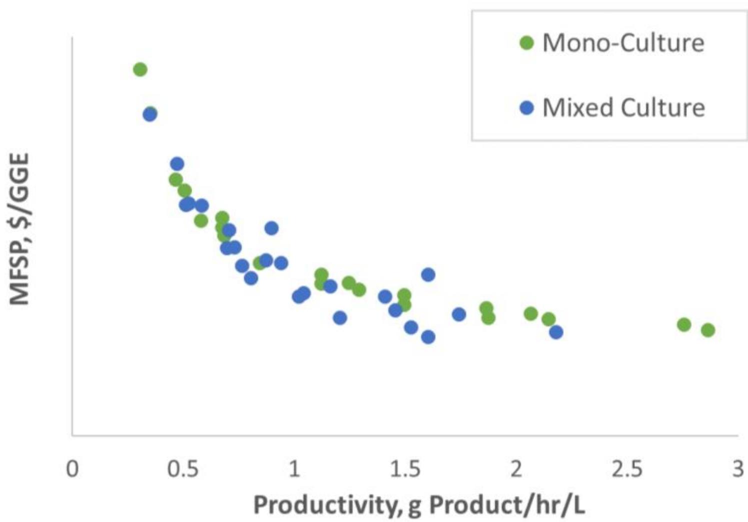


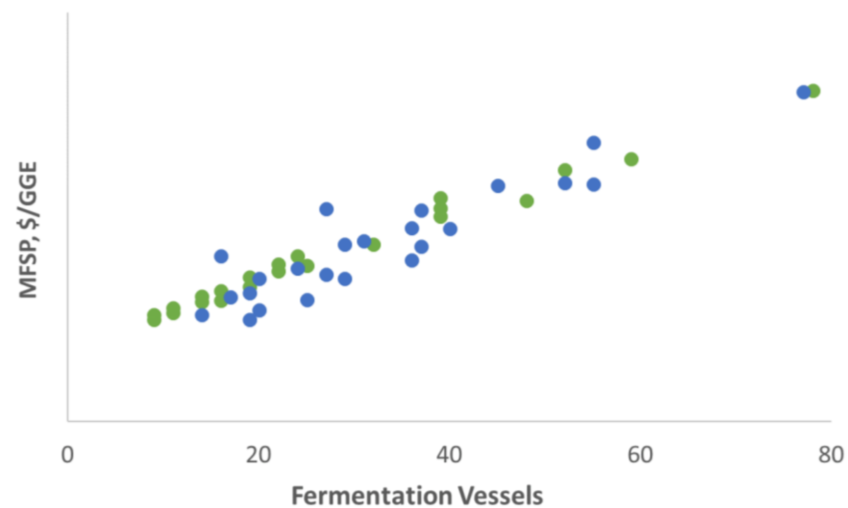
Mono culture: Lower yield, faster growth

1 Glu → **0.67** Ethyl-Butyrate + 2 CO_2 + 0.67 H_2O + **1.33 H_2**

Mixed culture: Higher yield, slower growth

1 Glu → **0.75** Ethyl-Butyrate + 1.5 CO_2 + 1.5 H_2O



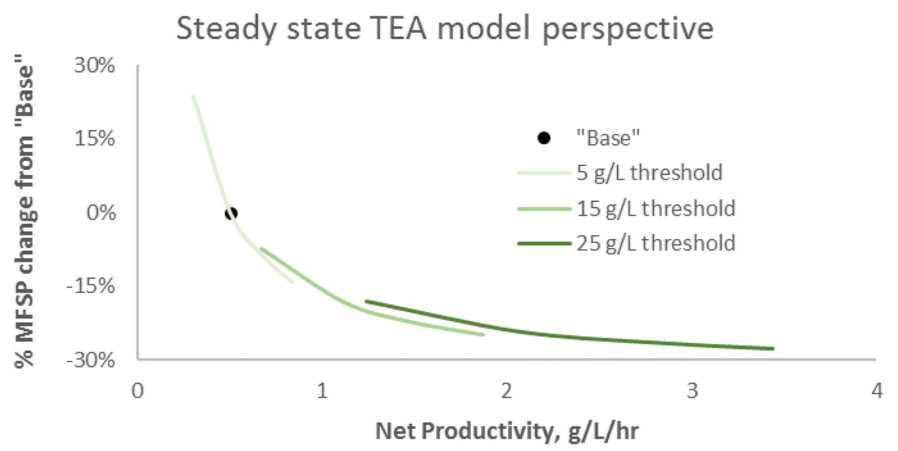


- *mono* and *mixed* cultures have similar cost drivers at a steady state level
- *mono* and *mixed* cultures are very different during fermentation/biologically

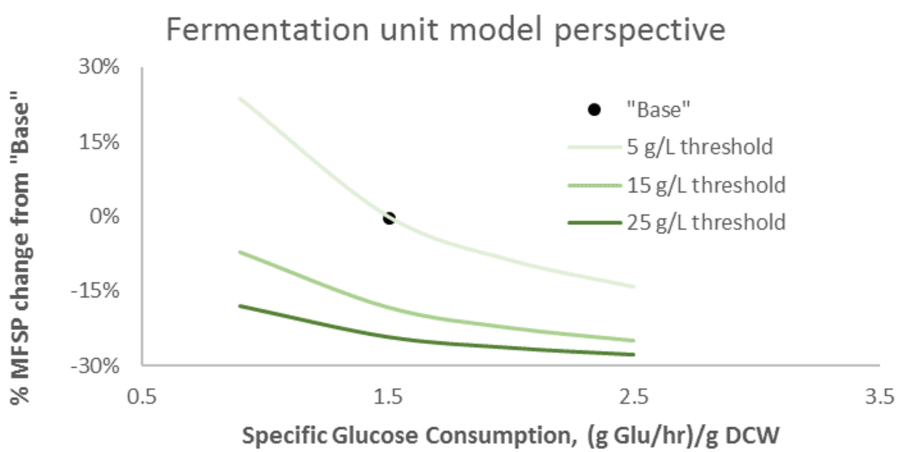
Economic Sensitivity of Mono Culture

Goal:

- Explore MFSP sensitivity to product toxicity and specific glucose uptake rate while all other fermentation parameters constant



Higher productivity lowers MFSP



Multiple factors affect "net productivity"

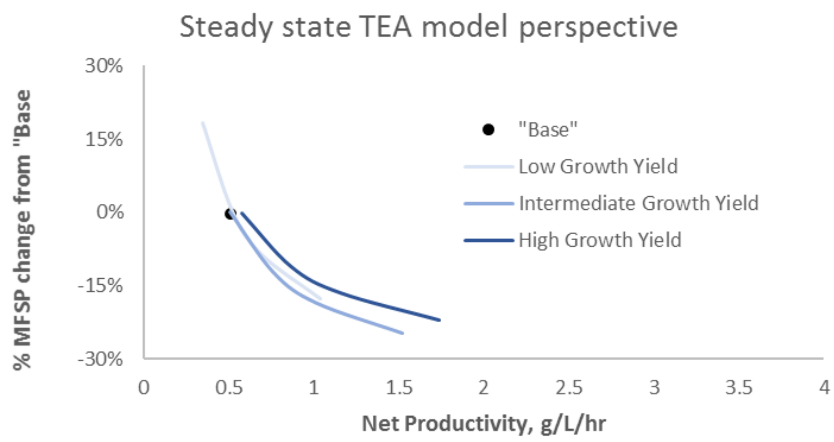
-Is a further increase in specific consumption rate feasible?

-Is a further increase in stress tolerance feasible?

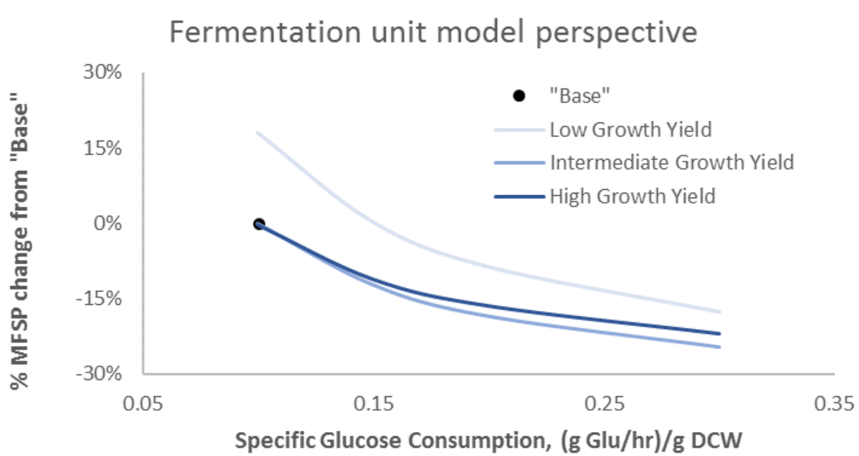
Economic Sensitivity of Mixed Culture

Goal:

- Explore MFSP sensitivity to product toxicity and specific glucose uptake rate while all other fermentation parameters constant



There is an optimal balance of rate and yield in a consortia

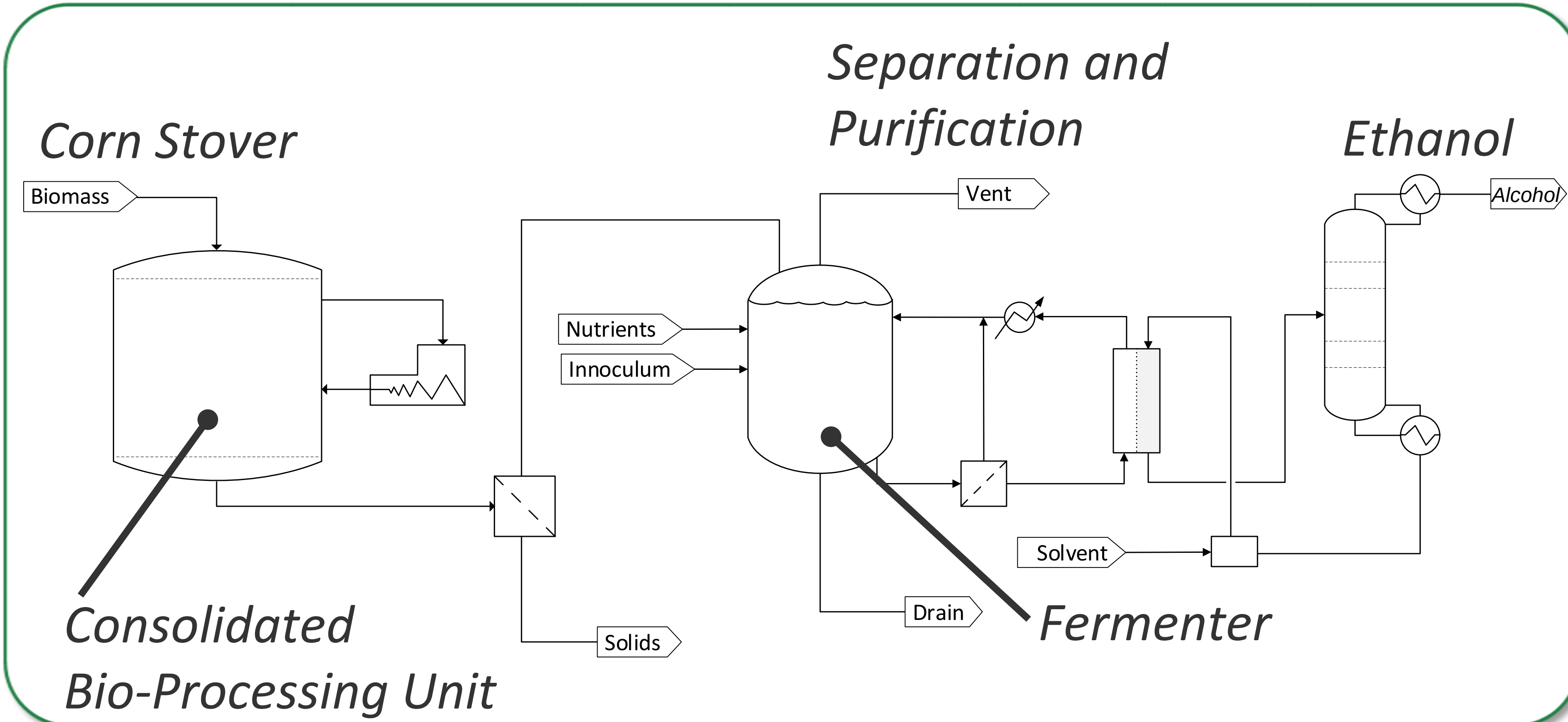


How can we target an improved "consortia"?

-Can we control the growth and product balance of one consortia better than another (e.g. pH effects)?

-When is a long lag phase detrimental?

Proof of Concept TEA+LCA Analysis
Impact of Engineering Targets



- Note: This analysis is not for the target ester product, but it is used to illustrate the workflow and potential insights.

Proof of Concept TEA+LCA Analysis

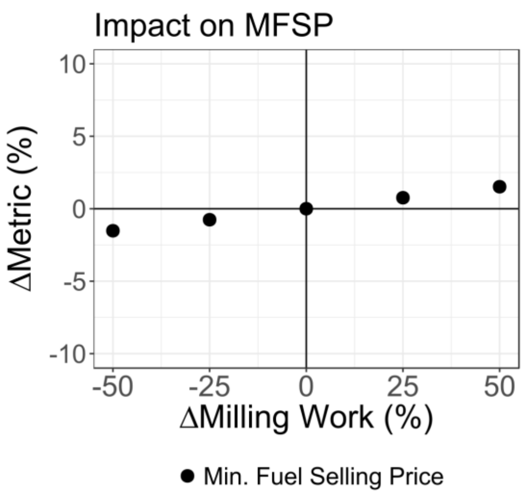
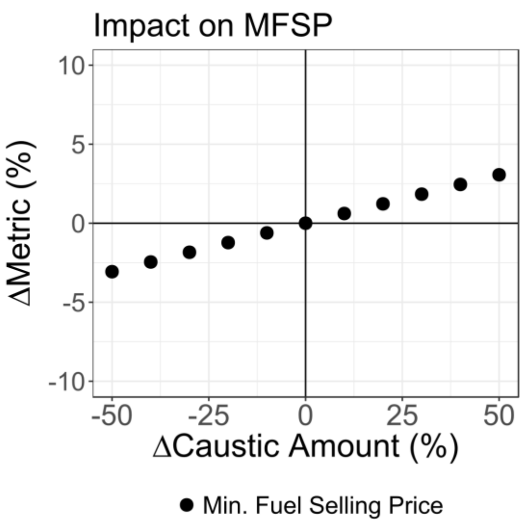
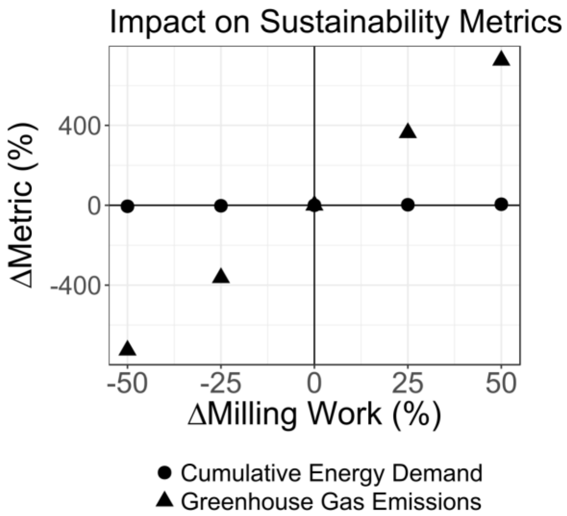
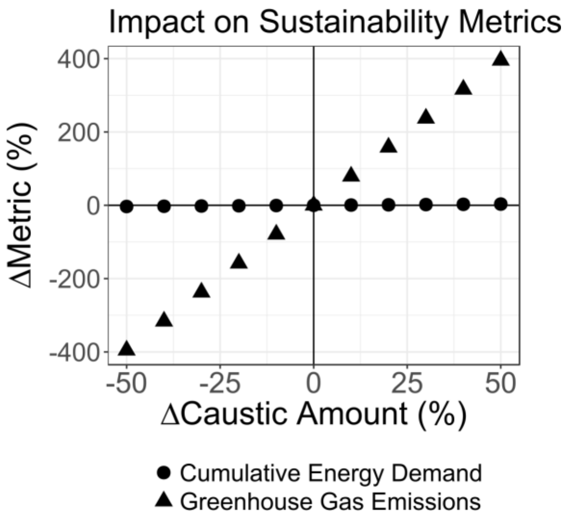
Impact of Engineering Targets

CBP/CT researchers with CBI have identified two promising engineering targets to pursue in the two-step process configuration.

1. Reduce corn stover milling required → Reduce process energy demand.
2. Improve microorganism pH tolerance → Reduce the amount of NaOH added to the CBP vessel.

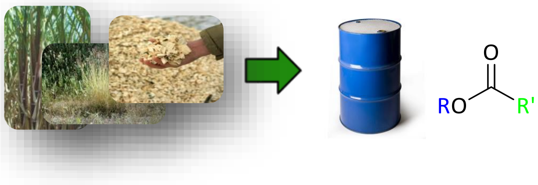
Both targets have the potential to improve economics and sustainability, but are they high-impact enough to make the targets worth pursuing?

Proof of Concept TEA+LCA Analysis
Impact of Engineering Targets

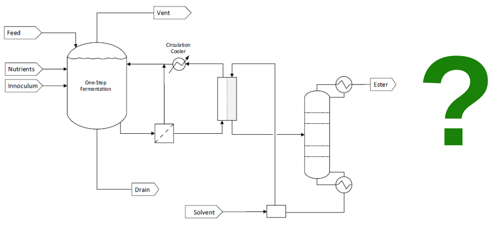
	Milling Energy	pH Tolerance
Economic Sensitivity		
Sustainability Metric Sensitivity		

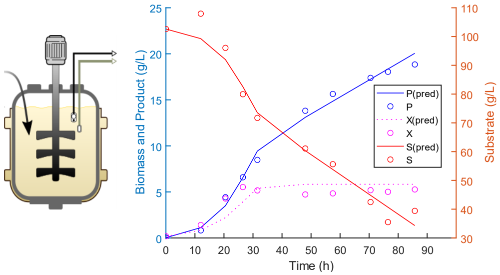
Summary

- Research question:
  - Can we extend C2 and C4 alcohol pathways in CBP organisms to produce esters in an economically feasible and environmentally sustainable manner?

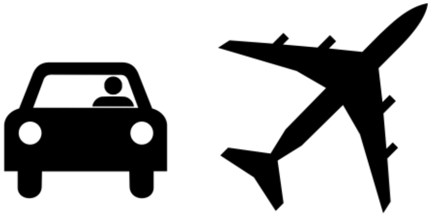


- Current status:
  - We have formulated a “one-step” process for CBP ester production and built a microbial fermentation model.
  - Preliminary TEA results indicate that a productivity above ~0.6 g/L/hr at a toxicity threshold of 5 g/L (same as ester solubility) reduces MFSP.





- Future work:
  - Integrate both metabolic and kinetic models together with process modelling.
  - Perform parameter sensitivity for all models.
  - Couple TEA and LCA to assess both the economic feasibility and sustainability of esters used as fuels. Esters may be more economically viable if sold as chemical products.
  - Explore alternative configurations based on different biocatalyst microbes to inform metabolic engineering goals.



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- Gregg Beckham[2]

[1] Oak Ridge National Laboratory (ORNL)

[2] National Renewable Energy Laboratory (NREL)

Thank you for your attention!

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