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Hybrid biological-chemical strategy for converting polyethylene into a recyclable plastic monomer using engineered Corynebacterium glutamicum

Background/Objective

- Converting polyethylene (PE) into valuable materials helps mitigate environmental issues caused by plastic waste
- PE decomposition conditions present significant challenges for downstream applications, especially for bioconversion

Approach

- We developed a hybrid biological-chemical conversion process for PE, converting its decomposition products, short-chain diacids, into a monomer, β-keto-δ-lactone (BKDL), for highly recyclable polydiketoenimine plastics

Results

- We employed an alternative biosynthesis pathway to produce malonyl-CoA
- We optimized the PE deconstruction process to produce C4 to C6 diacids
- We engineered *C. glutamicum* with a PKS to enable BKDL synthesis

Significance/Impacts

- This work demonstrates the potential of a chemo-biological hybrid strategy for recycling plastic waste, highlighting its promise in addressing environmental challenges and promoting sustainable materials

Zhan, C., et. al. Metabolic engineering. doi: 10.1016/j.ymben.2025.03.007 (JBEI #1221)

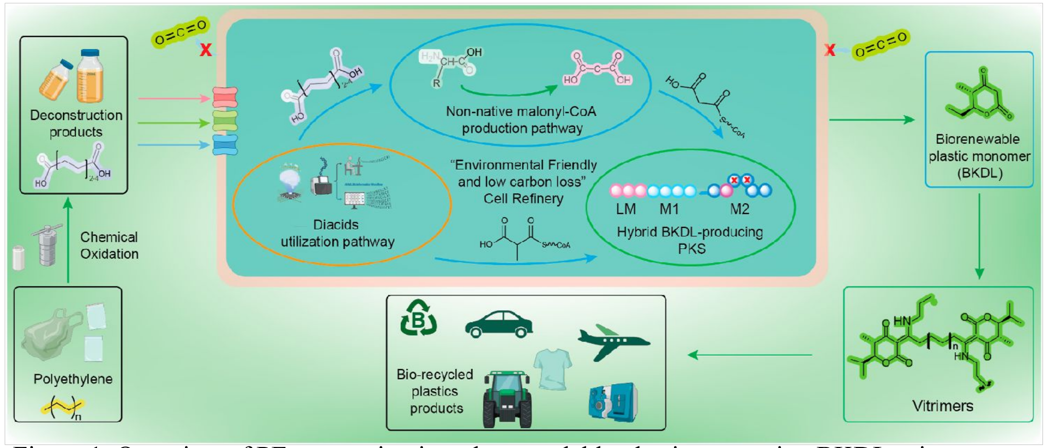

Figure 1. Overview of PE conversion into the recyclable plastic precursing BKDL using engineered *C. glutamicum*.

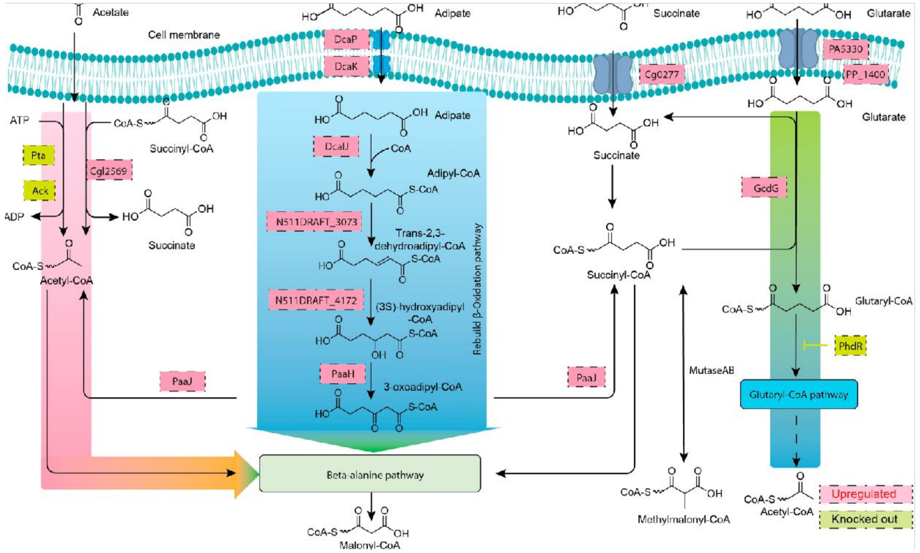

Figure 2. Pathways engineered to consume diacids and transform them into malonyl-CoA and methylmalonyl-CoA.

Alternate routes to acetate tolerance lead to varied isoprenol production from mixed carbon sources in Pseudomonas putida

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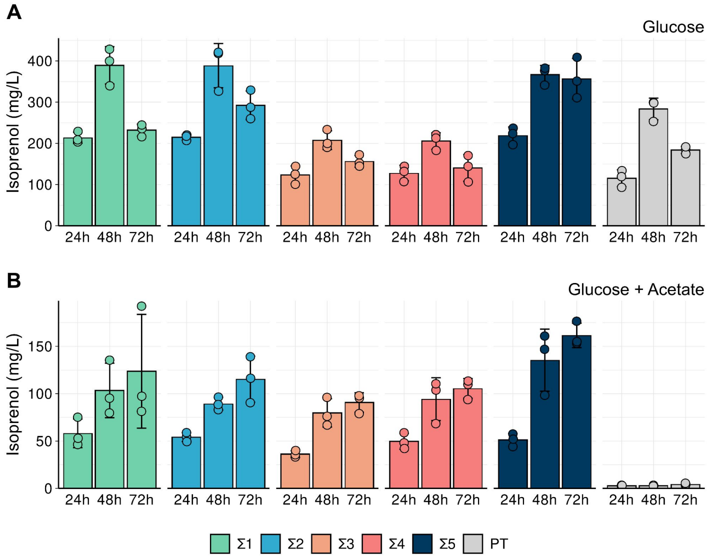
Background/Objective

- In *Pseudomonas putida*, growth and isoprenol production using mixed carbon sources, particularly glucose and acetate, remain limited due to toxicity and metabolic incompatibilities
- While glucose is a well-utilized sugar feedstock, co-occurring compounds such as acetate in hydrolysates are available but impair microbial growth and productivity



Approach

- We used co-cultivation and mutant selected to find mutants that are tolerant to acetate and engineered these for isoprenol production
- We used omics analysis to examine underlying mechanisms of tolerance and production

Results

- Recovered strains demonstrated robust growth and enhanced isoprenol production from mixed glucose-acetate media
- Tolerized strains showed different metabolic rewiring, including upregulation of succinyl-CoA:acetate CoA-transferase, differential expression in gluconate assimilation pathways, correlating with higher isoprenol titers
- HemN (coproporphyrinogen-III oxidase) was consistently upregulated and found to be essential for acetate tolerance in at least one isolate

Significance/Impacts

- In this study we further expand the use of all components of lignocellulosic biomass is a promising renewable source and produce a platform chemical like the biofuel isoprenol
- Different strain lines showed different routes to tolerance and a different final phenotypes for isoprenol production

de Siqueira, G. M. V., et. al. Applied and environmental microbiology. doi: 10.1128/aem.02123-24 (JBEI #1222)

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Enzymatic cleavage of model lignin dimers depends on pH, enzyme, and bond type

Background/Objective

- Lignin-modifying enzyme (LME) studies are analytically challenging
- We created "LigNIMS:" NIMS* + robotics + synthetic lignin substrates

Approach

- Our method was bond-specific and high-throughput
- We applied our novel pipeline to 4 different lignin bond types, 10 LMEs, and pH 3-10

Results

- The novel pipeline provided detailed data at a range of conditions
- We observed that the type of bond and the type of enzyme determined pH range and reactivity (oxidation vs. bond cleavage)

Significance/Impacts

- Enzyme/pH/bond type data informs future lignin valorization efforts
- Our LigNIMS platform is now being used by 6+ projects within and outside of JBEI

Onley, J. R., et. al. Scientific reports. doi: 10.1038/s41598-025-88571-7 (JBEI #1223)

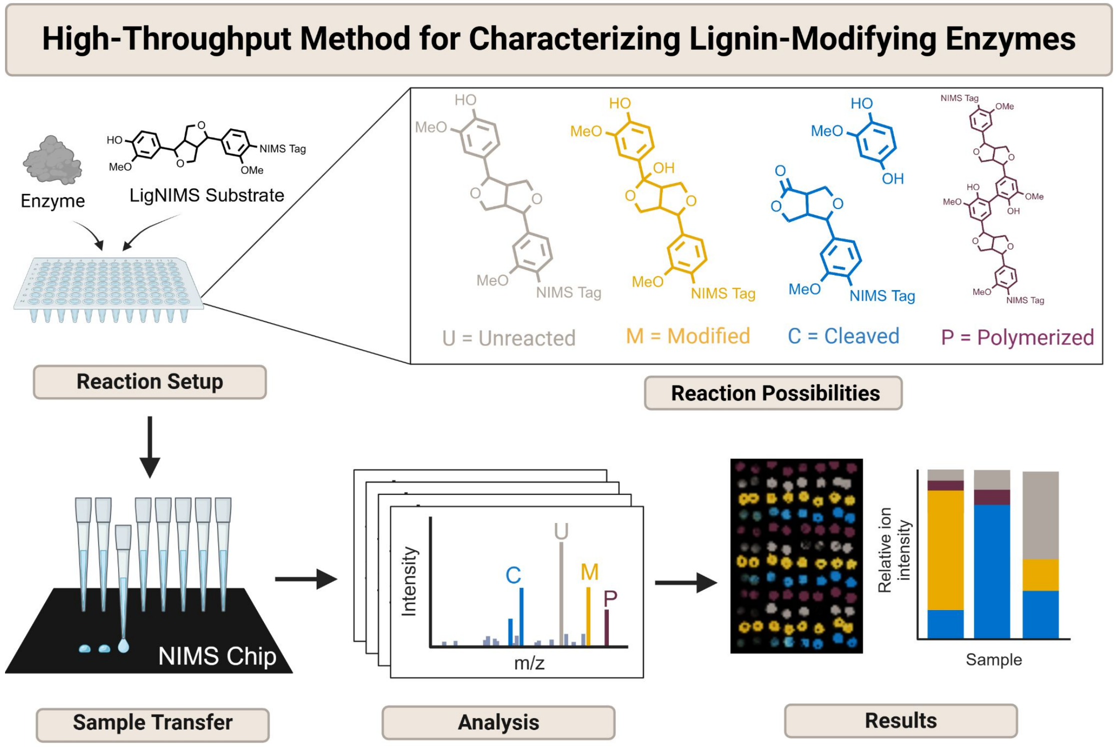

Figure. LigNIMS pipeline for high-throughput analysis of LMEs. LigNIMS was used to test the effects of enzyme type and pH on 4 different lignin bond types (1 type is shown above). *NIMS = nanostructure-initiator mass spectrometry

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A WRKY transcription factor confers broad-spectrum resistance to biotic stresses and yield stability in rice

Background/Objective

- Few genes are known to confer broad-spectrum resistance to pathogens and pests
- It is often challenging to balance stress tolerance and yield

Approach

- We overexpressed and knocked out, using CRISPR/Cas9, the WRKY36 transcription factor in rice

Results

- The WRKY36 transcription factor represses the phenylpropanoid pathway in rice, which suppresses resistance
- Lignin biosynthesis increased in knockout plants and lead to enhanced resistance to both pathogens and pests
- Plants also had increased number of spikelets and tillers

Significance/Impacts

- The approach offers a strategy to breed grasses with broad-spectrum resistance while maintaining yield
- The approach also provides a strategy to fine-tune lignin content, which is important for biomass recalcitrance

D. Liu, et. al. Proc. Natl. Acad. Sci. U.S.A. doi.org/10.1073/pnas.2411164122 (JBEI #1224)

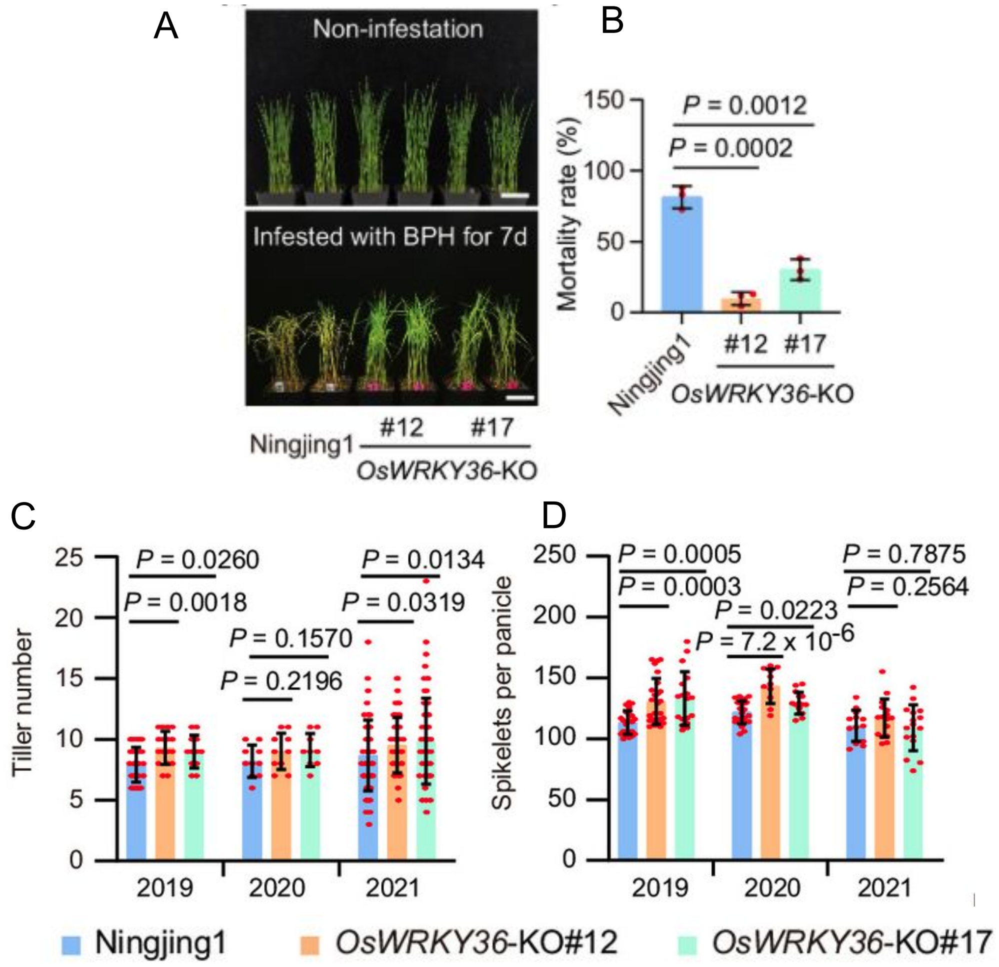

Figure. Genome edited OsWRKY36 loss-of-function plants showed increased resistance to brown leafhopper (A, B). The plants also had increased tiller numbers (C) and spikelets per panicle (D).

Enabled Publications

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The oleaginous yeast Rhodosporidium toruloides engineered for biomass hydrolysate-derived (E)-α-bisabolene production

Background/Objective

- The oleaginous yeast *R. toruloides* is a promising host to produce a wide range of biofuels and bioproducts
- We optimized *R. toruloides* for high bisabolene production

Approach

- We optimized the flux towards bisabolene biosynthesis via extensive pathway engineering
- Multiomic analysis was performed to identify pathway bottlenecks

Results

- Literature-high titer of 20.8 g/L bisabolene was obtained from 300 g/L total sugar from corn stover hydrolysate
- Three pathway bottlenecks were identified via multi-omic analysis

Significance/Impacts

- The results further establishes R. toruloides as a promising host for the production of bisabolene and other biofuels and bioproducts

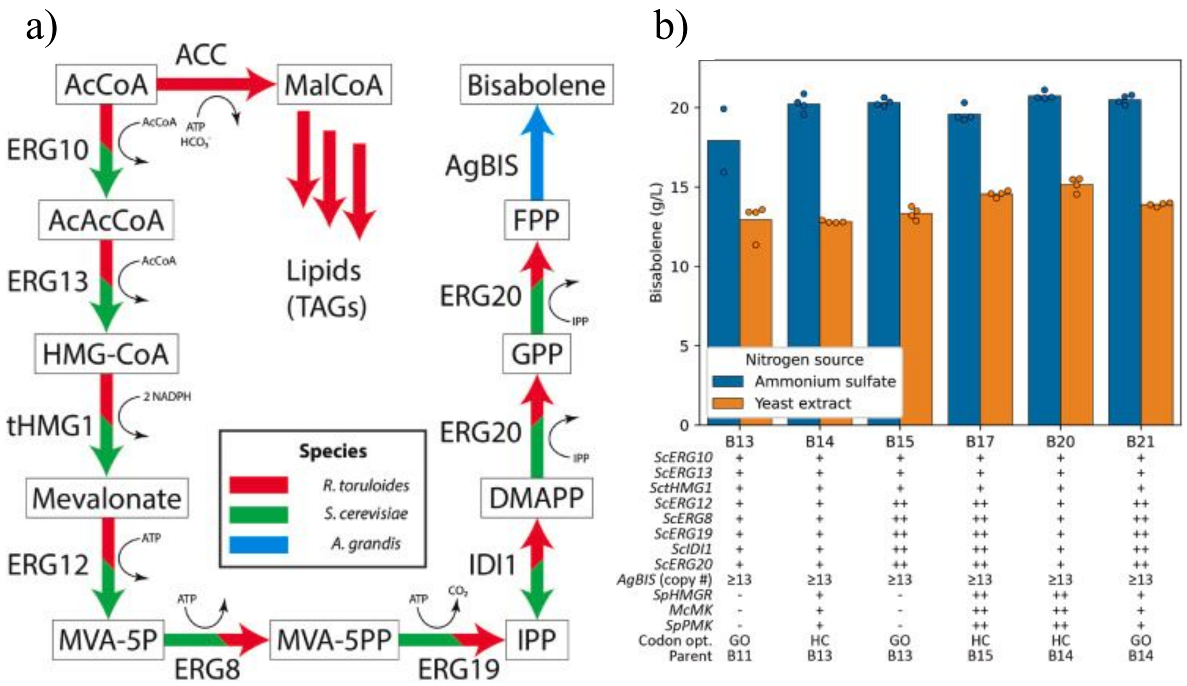

Figure. a) Map of native *R. toruloides* metabolism overlaid with heterologous MVAP and bisabolene synthase integrated in this study. b) Production of bisabolene in engineered strains with different nitrogen sources.

Adamczyk, P. A., et. al. Metabolic engineering. doi: 10.1016/j.ymben.2025.02.014 (JBEI #110)

Dynamic Accounting of Carbon Uptake in the Built Environment

Background/Objective

- Buildings can transition to being net CO_2e emitters to net carbon sinks
- This can be accomplished in part by using bio-based materials

Approach

- We developed a time-adjusted GWP approach for calculating the impact over the life of a building
- We applied the approach for cross-laminated timber and concrete

Results

- Long storage time of biogenic carbon in CLT results in substantial time-adjusted GWP benefits relative to concrete

Significance/Impacts

- Biomaterials offer, in many cases, temporary (but multi-decade) C storage and better accounting methods need to be developed to value this

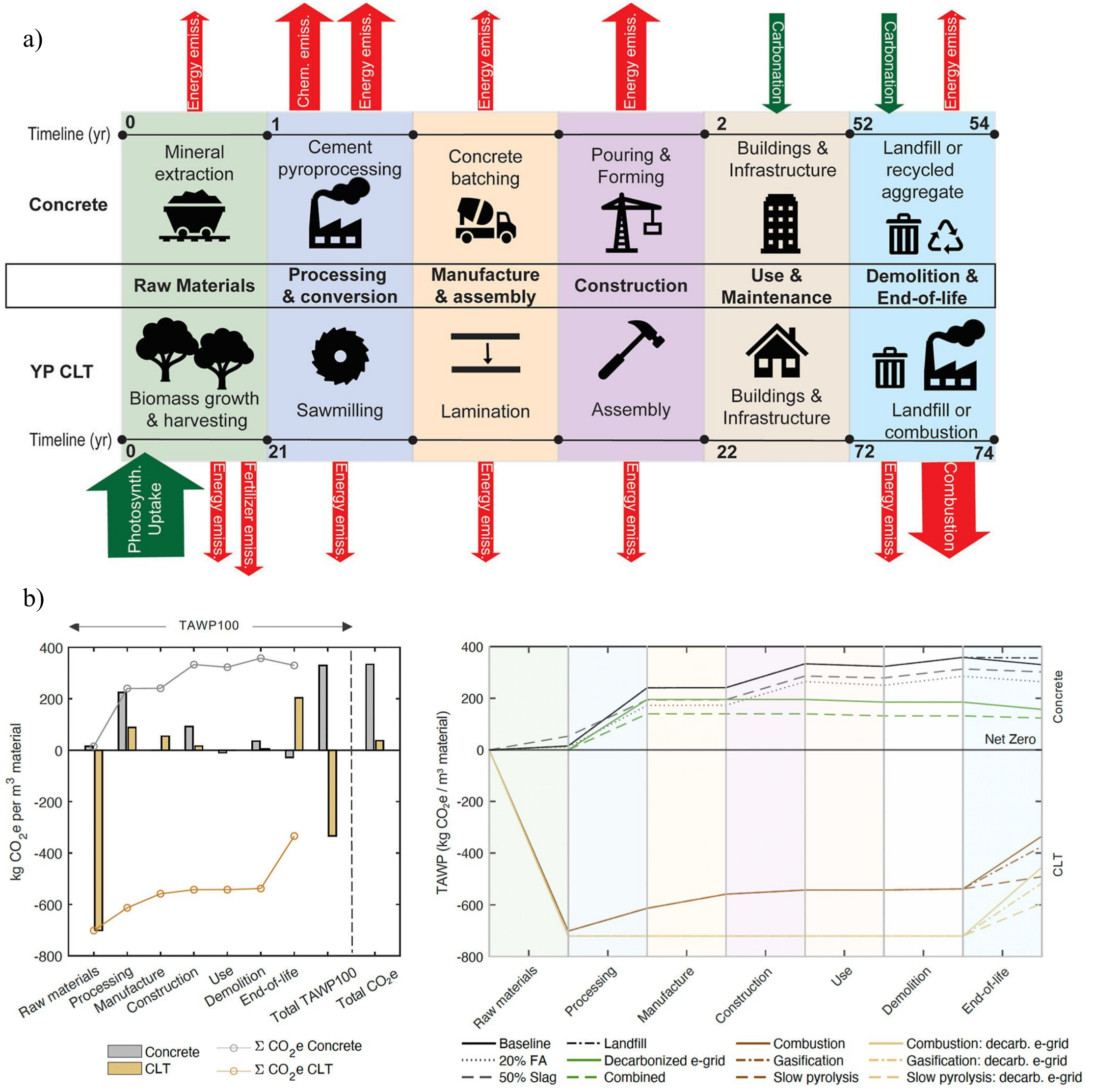

Figure. a) Modeling system boundary, b) Time-adjusted GWP for building materials

Van Roijen, E., et. al. Environmental science & technology. doi: 10.1021/acs.est.5c0008 (JBEI #111)