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A techno-economic assessment of carbon dioxide removal pathways via biochemical conversion of lignocellulose to biofuels and bioplastics

Background/Objective

- Biomass carbon removal and storage (BiCRS) can be used to both producing value-added products and fuels will directing additional carbon to long-term storage

Approach

- Modeled several fermentation technologies, producing a variety of bioproducts from lignocellulosic feedstocks, to understand their levelized cost of CO_2 removal under multiple scenarios.

Results

- Bioplastics that sequester bio-carbon at end-of-life have promise.
- Costs of carbon removal via bioplastic range from $61 to $195 per tCO2 removed.

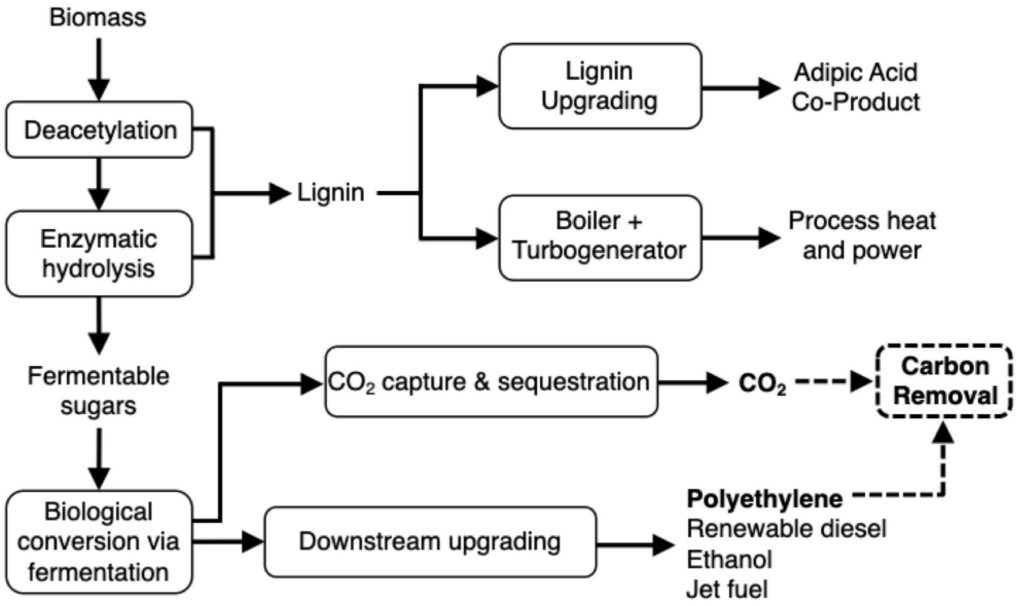

Figure 1: Simplified flow diagrams of eight bioprocesses assessed for biomass conversion and CO2 removal

Significance/Impacts

- Biorefineries can serve a dual purpose by directing biogenic carbon to long-term storage, which has a market value on its own, and deriving the highest value possible from important energy outputs and bioproducts

Clauser N., et.al. Renewable and Sustainable Energy Reviews. doi: 10.1016/j.rser.2025.115714 (JBEI #1225)

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In planta production of the nylon precursor beta-ketoadipate

Background/Objective

- The accumulation of coproducts *in planta* can increase the value of lignocellulosic biomass
- The goal was to demonstrate a pathway for beta-ketoadipate (βKA) production in model plant systems

Approach

- Potential βKA biosynthetic routes were tested in tobacco and Arabidopsis by expressing microbial enzymes from aromatic degradation pathways (Figure 1)
- Techno-economic analysis (TEA) was used to determine economically relevant βKA titers

Results

- A five-enzyme pathway led to βKA production in tobacco leaves (4 dwt%) and Arabidopsis stems (up to 0.25 dwt%, Figure 2)
- TEA indicated that βKA accumulated at titers of 4 dwt% could be competitively priced in the range of $0.47–2.12/kg

Significance/Impacts

- A pathway for efficient βKA in-planta production has been validated
- Introducing this pathway in bioenergy crops could improve the economics of advanced bioproducts

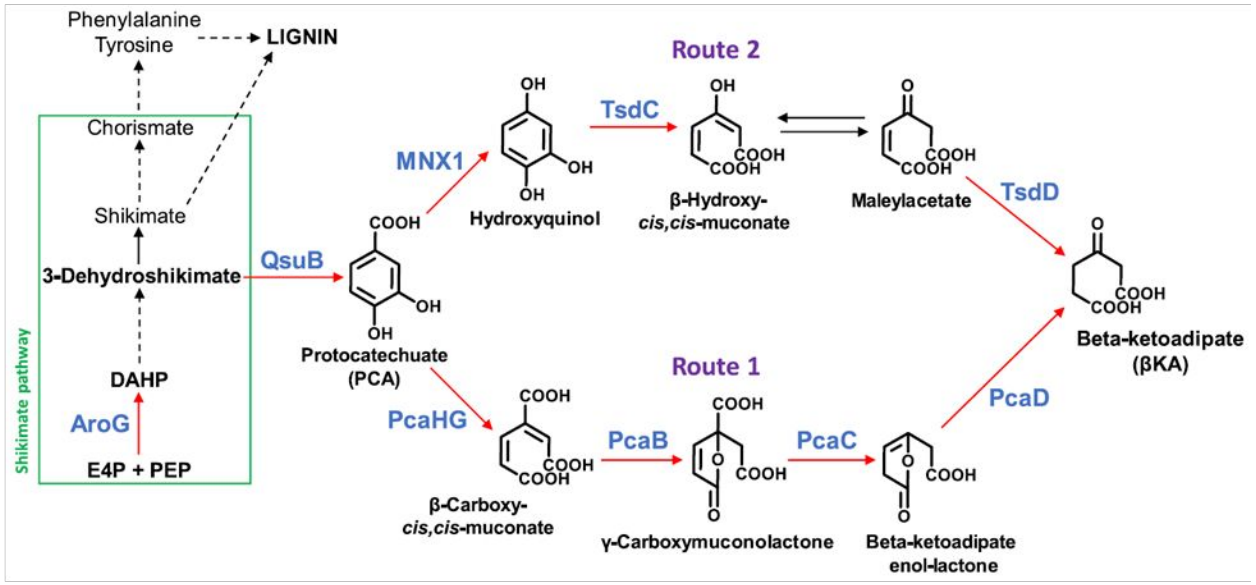

Figure 1: Tested βKA biosynthetic routes. Route 1 was the most effective for in-planta βKA production.

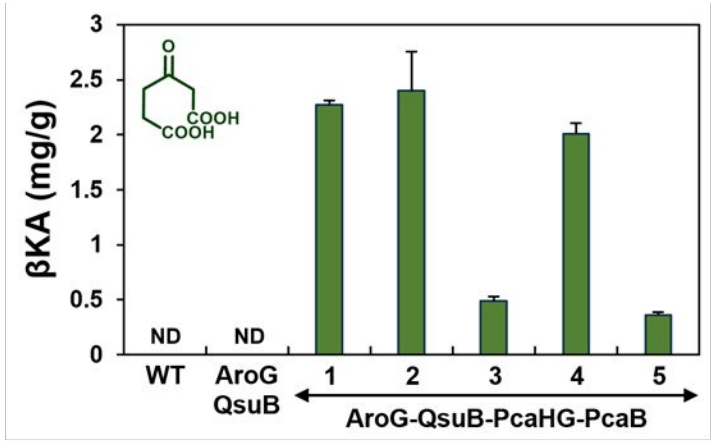

Figure 2: βKA titers in Arabidopsis co-expressing AroG-QsuB-PcaHG-PcaB. For each line, values are averages ± SE from four biological replicates.

Kazaz, S., et.al. Journal of Biotechnology. doi: doi: 10.1016/j.jbiotec.2025.04.008 (JBEI #1226)

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Engineering Pseudomonas putida for production of 3-hydroxyacids using hybrid type I polyketide synthases

Background/Objective

- Engineered type I polyketide synthases (T1PKSs) are a potentially transformative platform for the biosynthesis of small molecules.
- Recent efforts target faster-growing, industrial hosts for more efficient chemical production.

Approach

- We outline the assessment of *P. putida* as a host for the expression of engineered T1PKSs and production of 3-hydroxyacids.

Results

- CoA pool expansion of Pseudomonas putida to diversify PKS products.
- Functional genomics to improve acyl-CoA supply for polyketide production.
- Further engineered initial PKS design by acyl transferase (AT) swapping.
- 3-hydroxyacids production with varying chain lengths and branching patterns.

Significance/Impacts

- This work demonstrates the potential of T1PKSs in *P. putida* as a production platform for the sustainable biosynthesis of unnatural polyketides.

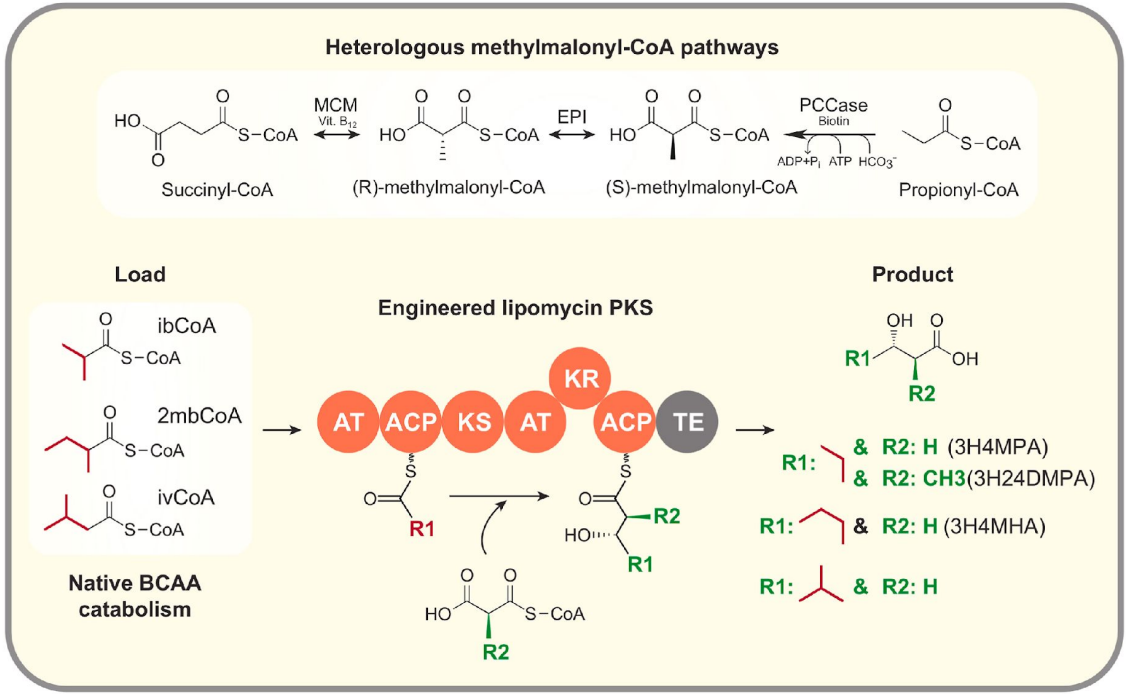

Figure 1: Design of the engineered LipPKS pathway and its precursors in *P. putida*. Methylmalonyl-CoA (mmCoA) supply routes included MCM/EPI from *Sorangium cellulosum* or PCCase from *Streptomyces coelicolor*. Preferred loading substrates derive from *P. putida*'s native BCAA catabolism, yielding enantiopure 3-hydroxyacids dependent on the loaded substrate.

Schmidt, M., et.al. Metabolic engineering communications. doi: 10.1016/j.mec.2025.e00261 (JBEI #1227)

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Machine learning-led semi-automated medium optimization reveals salt as key for flaviolin production in Pseudomonas putida

Background/Objective

- Media optimization is a critical, and often overlooked, process which is essential to obtain the titers, rates and yields needed for commercial viability.

Approach

- We created an active learning process able to optimize the culture media for production of a desired metabolite.
- We created a highly reproducible, semi-automated pipeline to provide the high-quality data the active learning process needed.

Results

- The active learning process guided by ART produced increases of 60% and 70% in titer, and 350% in process yield in three different campaigns.
- Surprisingly, the salt NaCl emerged as the most critical feature overall influencing flaviolin production.

Significance/Impacts

- This work illustrates how machine learning and automation can change the paradigm of current synthetic biology research to make it more effective and informative.

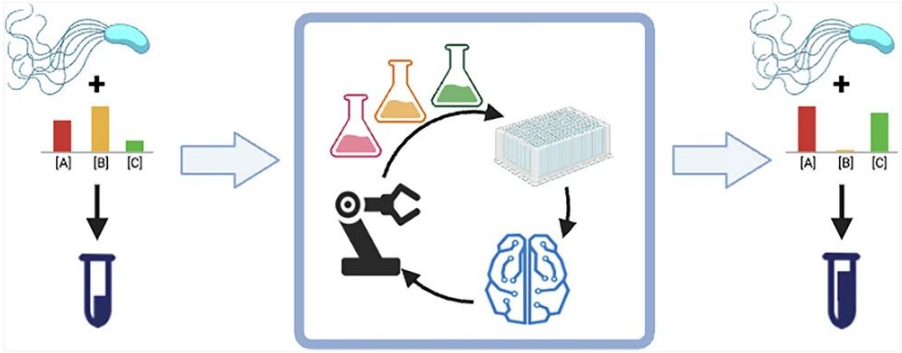

Figure 1: Our semi-automated pipeline leverages automation and machine learning to find the combination of media component concentrations that optimizes final production.

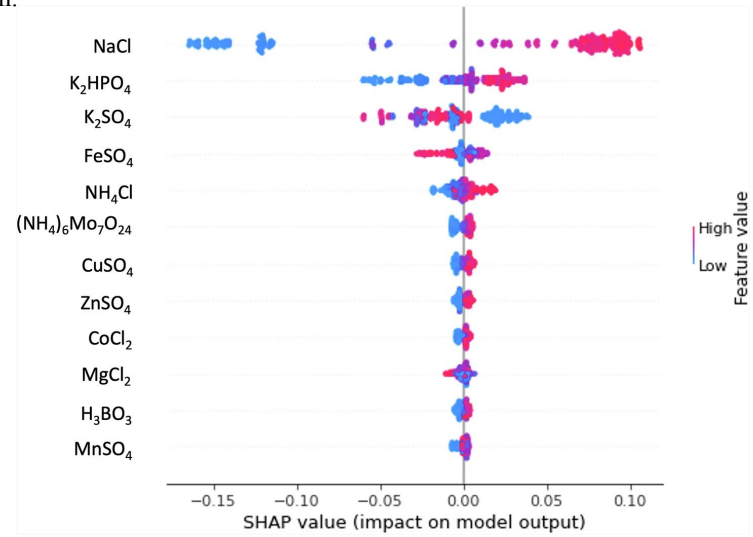

Figure 2: Surprisingly, NaCl (common salt) concentration has the highest impact on the predicted production, opening up the possibility of considering brackish water for biomanufacturing.

Zournas, A., et.al. Communications biology. doi: 10.1038/s42003-025-08039-2 (JBEI #1228)

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Retrobiosynthesis of unnatural lactams via reprogrammed polyketide synthase

Background/Objective

- Engineered polyketide synthases (PKSs) are capable of synthesizing molecules that are either not amenable to biosynthesis or are extremely challenging to access chemically.

Approach

- We employed a retrobiosynthesis approach to design and construct PKSs to produce δ-valerolactam (VL) and its analogues in P. putida

Results

- We produced VL and three enantiopure α-substituted analogues that have no known biosynthetic route.
- We employed proteomics, metabolomics, and culture optimization to boost production of the target compounds.
- These α-substituted VLs were polymerized into nylon-5 or converted to N-acryloyl derivatives, enabling RAFT polymerization of bio-based polymers for biomedical use.

Significance/Impacts

- This interdisciplinary effort highlights the versatility and effectiveness of a PKS-based retrobiosynthesis approach in exploring and developing innovative biomaterials.

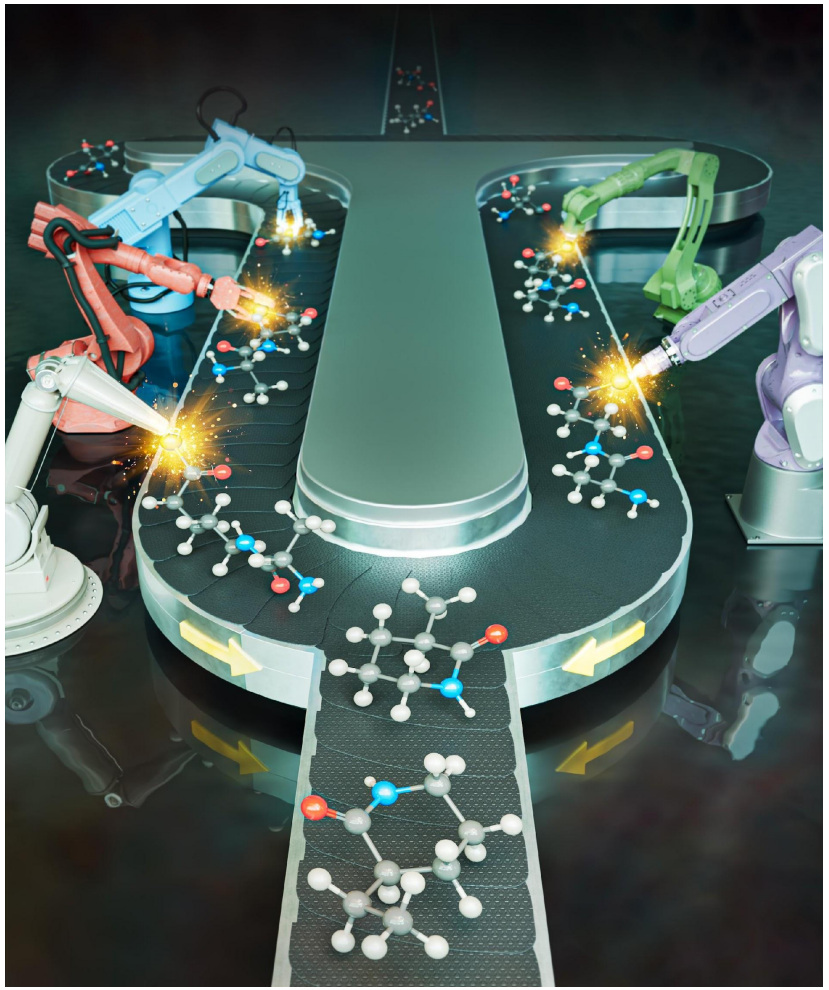

Figure 1: Schematic of PKS for production of VL and its analogues

Lee N., et.al. Nat Catal. doi: 10.1038/s41929-025-01325-6 (JBEI #1229)

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Identification of an a-factor-like pheromone secreted by the heterothallic ascomycete *Aspergillus fumigatus*

Background/Objective

- The fungal genera, *Aspergillus* and *Penicillium*, contain several important cell factory species
- The ability to perform traditional forward genetics in these cell factories is lacking

Approach

- A functional screen developed for the a-factor mating pheromone in *Aspergillus fumigatus*
- A bioinformatics approach in the JBEI/JGI whole Aspergillus genus genome sequencing project was implemented

Results

- A candidate a-factor pheromone was identified in *Aspergillus fumigatus*
- Syntenic genome regions in *Aspergillus* and *Penicillium* were found to harbor a-factor pheromones across each genus, respectively

Significance/Impacts

- Most cell factory species from *Aspergillus* and *Penicillium* are only known to reproduce asexually
- Identifying functional a-factor pheromone genes for each species opens up the possibility of using traditional genetic approaches to strain improvement

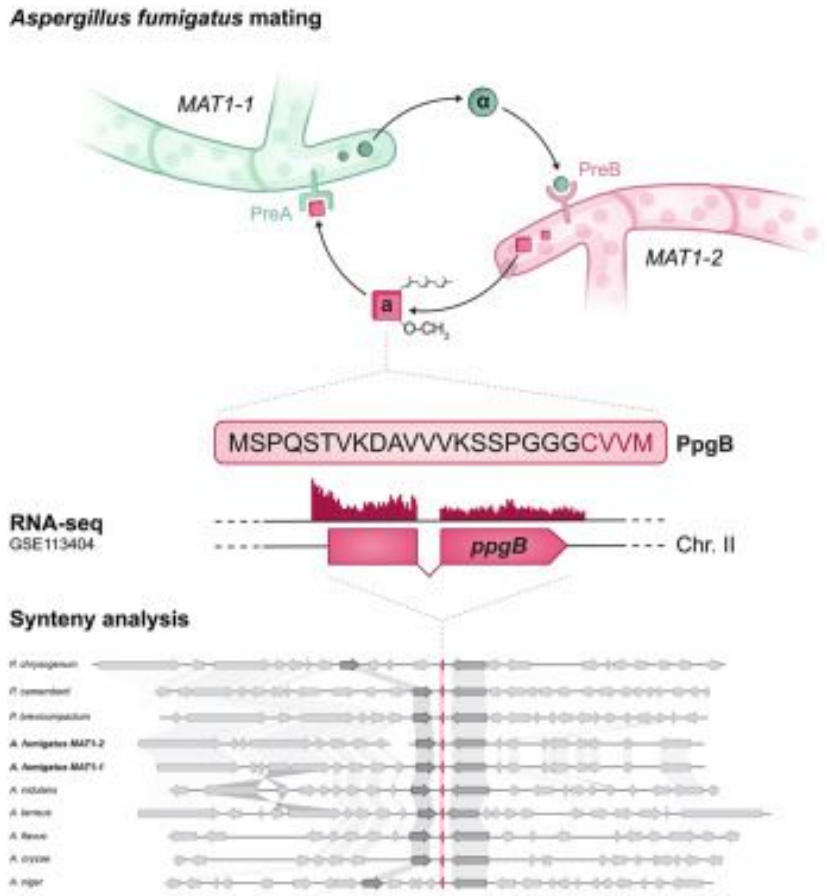

Figure 1: We identified a candidate pheromone precursor gene B (*ppgB*) to encode the elusive and conserved Eurotiomycete a-factor pheromone. Its deduced peptide is 24 aa in length and features a canonical CaaX farnesylation motif.

Krappmann, S.,, et.al. Current Biology. doi: 10.1016/j.cub.2025.03.080 (JBEI #1230)

Enabled Publications

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Harnessing organelle engineering to facilitate biofuels and biochemicals production in yeast

Background/Objective

- Conventional metabolic engineering approaches in yeast focus on biosynthetic pathways in the cytoplasm
- Eukaryotic cells contain subcellular organelles with distinct physicochemical properties, and repurposing these organelles as specialized microbial cell factories for enhanced production of valuable chemicals is an emerging strategy.

Approach

- We review recent progress and significant outcomes of harnessing organelle engineering for biofuels and biochemicals production in both conventional and non-conventional yeasts
- We highlight key engineering strategies for the compartmentalization of biosynthetic pathways within specific organelles such as mitochondria, peroxisomes, and endoplasmic reticulum

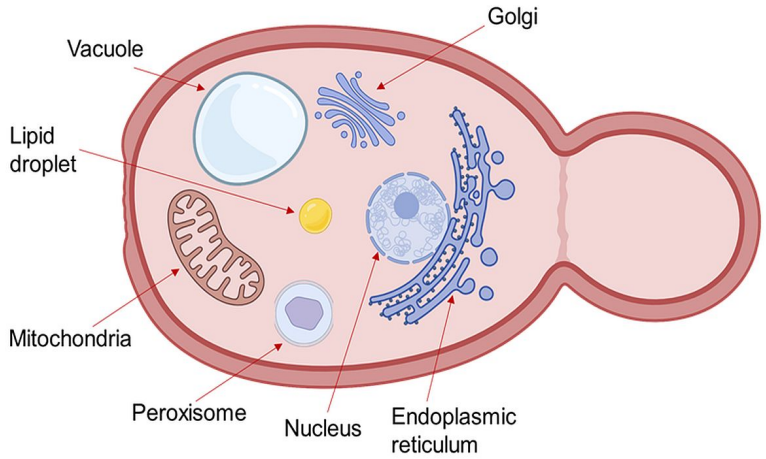

Figure 1. Primary cellular organelles found in yeasts.

Results

- In this review, we summarize the advances of leveraging organelle engineering to improve biofuel and biochemical production in both conventional and non-conventional yeasts.
- We also discuss the opportunities and challenges of metabolic engineering strategies on each targeted organelle.

Significance/Impacts

- Subcellular compartmentalization of desired biosynthesis pathways within the interested organelles substantially improves the production of the target products.
- Our review envisions that organelle engineering can be coupled with automation pipeline and AI/ML to maximally support the compartmentalized pathways towards the high-level production.

Tran, P. H. N., et.al. Journal of Microbiology. doi: 10.71150/jm.2501006 (JBEI #112)