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High-Throughput Microfluidic Electroporation (HTME): A Scalable, 384-Well Platform for Multiplexed Cell Engineering

Background

- Conventional electroporation is low-throughput and resource intensive.
- This creates a bottleneck for testing the many genetic constructs and delivery conditions required to solve complex biological problems.

Approach

- We built a 384-well "E-Plate" with independently addressable electrodes.
- This design allows for unique conditions in each of the 384 wells, enabling electroporation optimization studies and high-throughput workflows.

Results

- Successfully demonstrated robust, high-throughput electroporation.
- Characterized key performance differences between E-Plate and cuvettes.

Significance/Impacts

- Rapid optimization of electroporation conditions for difficult transformations.
- Resource optimization for high-throughput screening campaigns enabled by efficient, low-volume transformations.
- Generation of large, high-quality datasets to power Machine Learning/AI-driven workflows and enable self-driving labs.

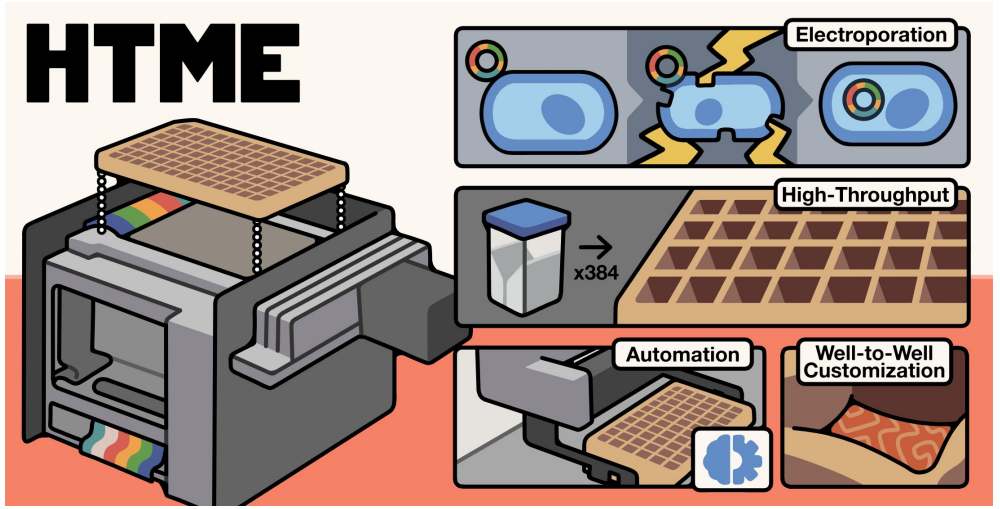

Figure A: High-Throughput Microfluidic Electroporation platform, E-Plate, and key system advantages.

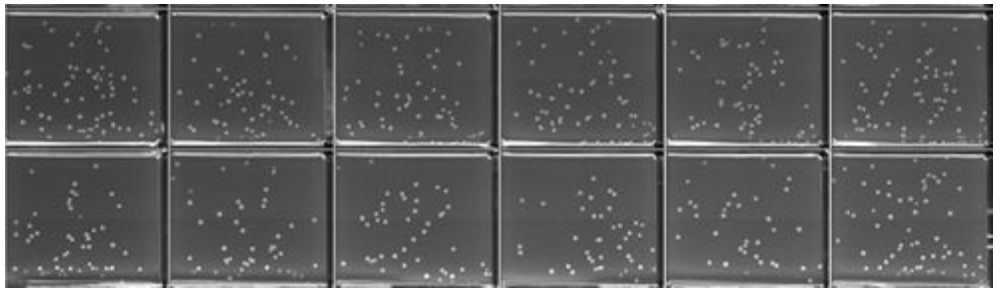

Figure B: Image of *E. coli* colonies expressing the selectable marker on a QTray following successful transformation using the HTME platform in a semi-automated workflow.

Gaillard, W. R., et al. Bioengineering. doi: 10.3390/bioengineering12080788 (JBEI #1245)

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Through the lens of bioenergy crops: advances, bottlenecks, and promises of plant engineering

Background

- This review piece was the outcome of discussions between the teams at all 4 of the BRCs as part of our shared research objective, identifying key technologies and bottlenecks from our view of agriculture, which is shaped by the bioeconomy, as opposed to more conventional markets e.g. food and feed.
- It formed part of a special issue of The Plant Journal on synthetic biology.

Findings

- The paper explored how to make use of emerging technologies, enormous volumes of data and extensive fundamental knowledge to generate the next generation of bioenergy crops.
- The promise of using rational design to rewrite natural pathways to the use of fully synthetic circuits were discussed, but require the solving of long-standing challenges around how to deliver crop-agnostic solutions.
- Open research questions were identified.

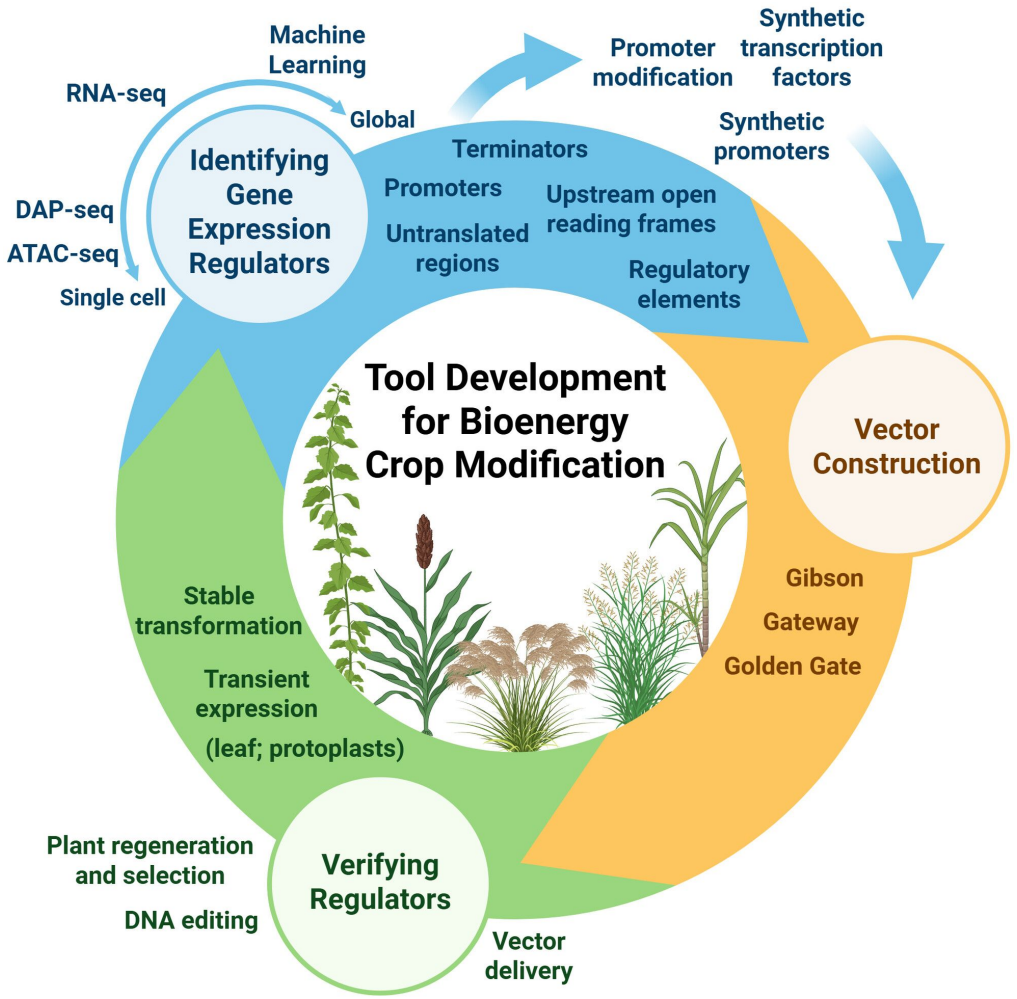

Figure caption: Roadmap of the integrated tools advancing bioenergy crop improvement.

Indibi, A., et al. The Plant Journal. doi: 10.1111/tpj.70294 (JBEI #1246)

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A substrate-multiplexed platform for profiling enzymatic potential of plant family 1 glycosyltransferases

Background/Objective

- Plants have expanded various biosynthetic enzyme families to produce a wide diversity of natural products; however, most enzymes encoded in plant genomes remain uncharacterized, highlighting the need for new functional genomic approaches.

Approach

- Using substrate-multiplexed reactions, mass spectrometry, and automated analysis, we screen 85 enzymes against a diverse library of 453 natural products, for a total of nearly 40,000 possible reactions.

Results

- The resulting dataset reveals a widespread promiscuity and a strong preference for planar, hydroxylated aromatic substrates among family 1 glycosyltransferases.

Significance/Impacts

- This work establishes a widely-applicable enzymatic screening pipeline, reflects the immense glycosylation capability of plants, and has implications in biocatalysis, metabolic engineering, and gene discovery.

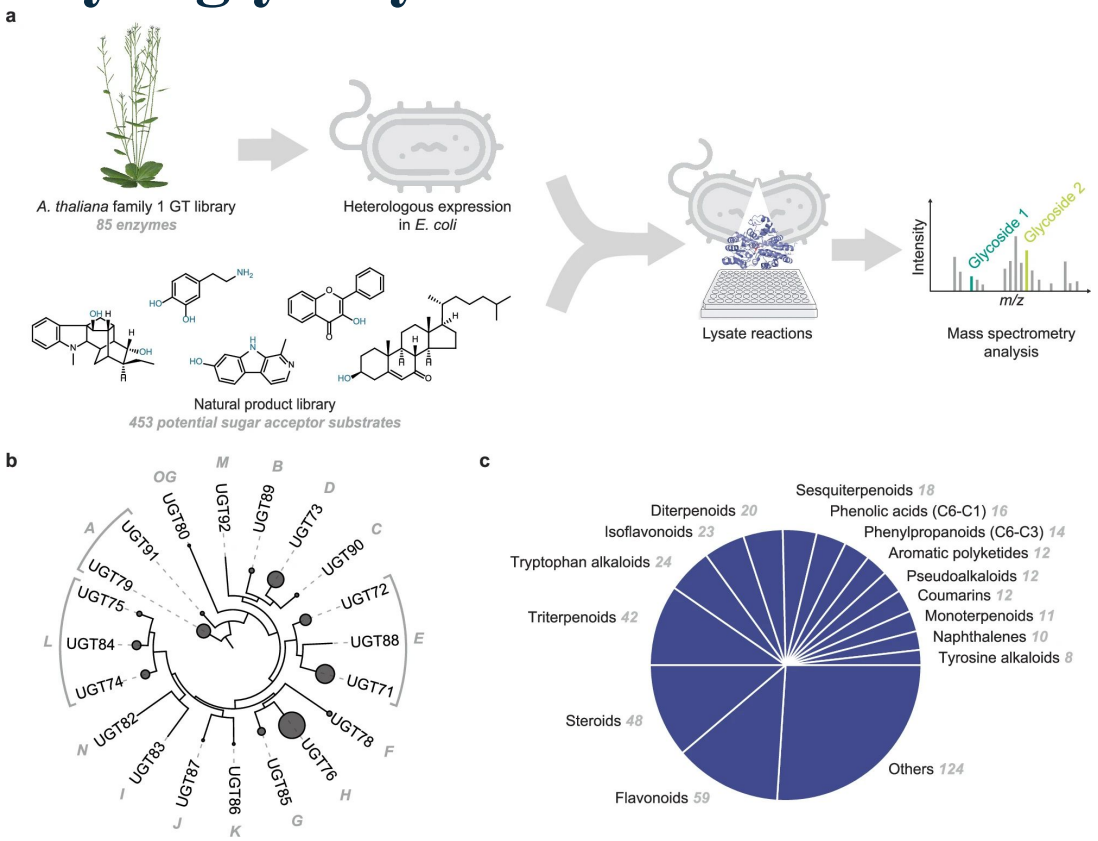

Figure A: Family 1 GT enzymes from Arabidopsis were expressed in E. coli, whose lysate was then used as the enzyme source to glycosylate 453 sugar acceptor substrate candidates selected from a natural product library. Lysate-based reaction mixtures were analyzed by high-resolution MS for glycosylation products without replicate (n = 1). Figure B: Arabidopsis family 1 GT enzymes are classified into 15 phylogenetic clades indicated in gray capital letters, each containing 1–3 families indicated with UGT family number. The size of the gray circle represents the number of enzymes in each family found in the Arabidopsis genome. c Sugar acceptor substrate candidate library spans 42 natural product superclasses, 14 of which were represented by at least ten molecules. The number of molecules in each superclass is shown in gray italics.

Sirirungruang, S., et al. Nature Communications. doi: 10.1038/s41467-025-61530-6 (JBEI #1247)

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Fast growth and high-titer bioproduction from renewable formate via metal-dependent formate dehydrogenase in *Escherichia coli*

Objective

- We sought the improve the growth of formatotrophic bacteria and employ these improved strains for the production of JBEI-relevant products

Approach

- We introduced a fast, metal-dependent formate dehydrogenase to increase the rate of energy generation in a strain employing the reductive glycine pathway

Results

- The formatotrophic strain grew faster with the metal-dependent FDH
- The improved formatotrophic strain produced more mevalonate
- The strain produced isoprenol and mevalonate from biomass and CO_2-derived formate

Significance/Impacts

- This technology could enable to production of biochemicals from CO_2 and electricity or from deconstructed biomass

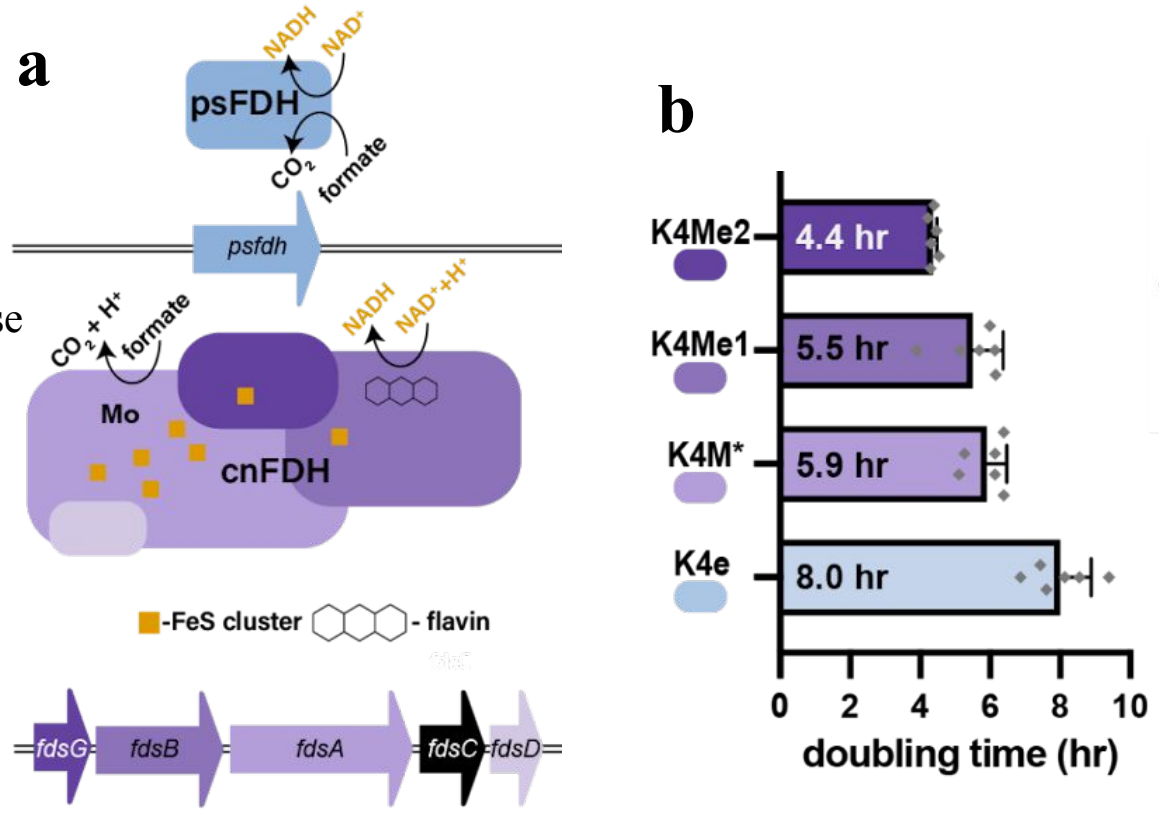

Figure caption: (a) Schematic diagram of FDH proteins and genes used in this study highlighting the structural complexity of cnFDH and cofactors required for functionality. (b) Doubling times of previous fastest formatotrophic e coli and improved versions developed at JBEI.

Cowan, A. E., et al. Nature Communications. doi: 10.1038/s41467-025-61001-y (JBEI #1248)

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Merging the computational design of chimeric type I polyketide synthases with enzymatic pathways for chemical biosynthesis

Background/Objective

- We built a computational synthesis planning software to enable synthetic biologists to design biosynthetic pathways to valuable commodity chemicals using both multifunctional chimeric type I polyketide synthases (PKSs) as well as regular monofunctional enzymes within primary metabolism.

Approach

- We used reaction rules (RetroTide) that encode for the enzymatic activities of PKS domains to first build the carbon scaffold for a target molecule.
- With reaction rules describing reactions catalyzed by monofunctional enzymes, this scaffold was then recursively expanded upon (DORAnet) to bridge the gap between the polyketide scaffold and the final target.

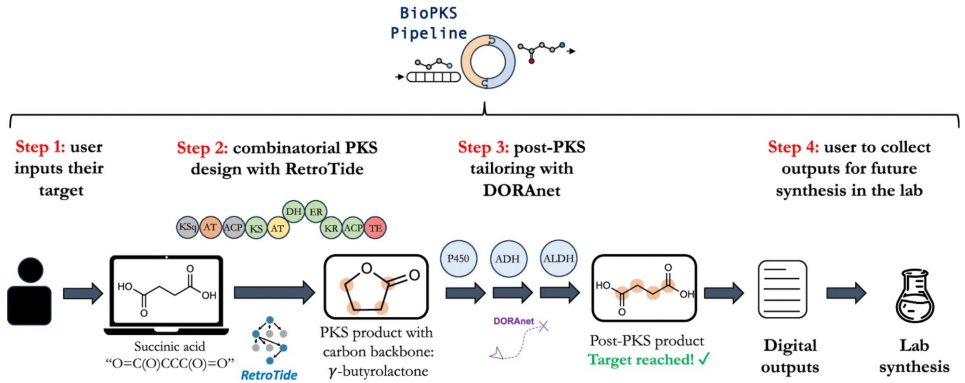

Figure caption: BioPKS pipeline uses both multifunctional type I polyketide synthases (PKSs) and monofunctional enzymes for chemical biosynthesis.

Results

- We successfully designed and released biosynthetic pathways to 93 molecules out of a list of 155 candidates that would be valuable for biomanufacturing.
- Recommended pathways to two natural products - cryptofolione and basidalin - whose natural biosynthetic pathways are currently unknown.

Significance/Impacts

- Expands the chemical space of molecules that can be manufactured with biology and provides alternate routes to existing targets.

Chainani, Y., et al. Nature Communications. doi: 10.1038/s41467-025-61160-y (JBEI #1249)

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Life Cycle Assessment of Chemical Recycling of Polyethylene Terephthalate to Produce Aramid Polymers

Background/Objective

- Understanding the unintended consequences of chemically upcycling plastics like PET into polyamides is critical
- The study evaluates environmental impacts of converting PET to polyamides via aminolysis across 12 pathways

Approach

- Life cycle assessment (LCA) evaluated 12 aminolysis pathways using lab data per kg of aramid
- Variables analyzed included amine type, solvent use, energy input, and monomer yield efficiency

Results

- Eight of twelve recycling pathways suggesting lower impact than virgin Kevlar; BAHT-B (aminolysis with 1,6-hexadiamine) was lowest
- High chemical input and energy use, especially in low-yield processes, drove greater impacts

Significance/Impacts

- Identifies solvent choice and conditions to reduce environmental recycling burdens
- Provides LCA framework to guide low-impact waste conversion process development

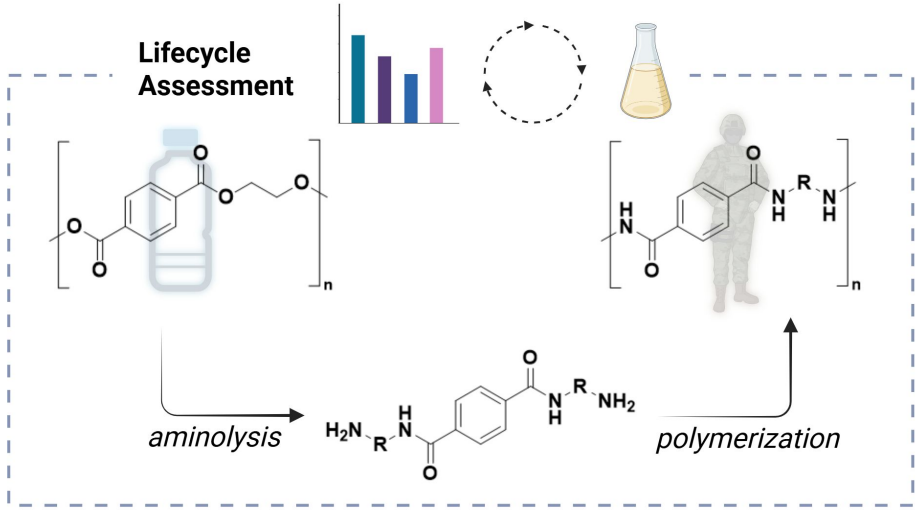

Figure caption: Schematic overview of PET chemical recycling via aminolysis for polyamide production, considering the LCA framework across the entire process chain

King, C., et al. ACS Sustainable Chemistry & Engineering. doi: 10.1021/acssuschemeng.5c01025 (JBEI #1250)

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Multi-scale Computational Screening and Mechanistic Insights of Cyclic Amines as Solvents for Improved Lignocellulosic Biomass Processing

Background/Objective

- A multi-scale computational approach to screen cyclic amines as solvents for improved lignocellulosic biomass deconstruction.
- Molecular interactions understanding between solvents and biopolymers.

Approach

- COSMO-RS model-based high-performing solvents are predicted for the deconstruction of different lignocellulosic biomass fractions.
- Understanding the mechanistic insights using DFT methods that control the biopolymer dissolution capacity of cyclic amines.

Results

- 1-Piperazineethanmaine achieved the highest lignin removal (97.1%) observed experimentally.
- The functional groups that determine biopolymer solvation, such as azines and oxazolines, can be tuned easily.

Significance/Impacts

- Identifies the new classes of solvents tailored for specific biomass components, potentially improving the overall efficiency of the process.
- Improving biopolymer solubilization and extraction could lead to more efficient and cost-effective biofuel production.

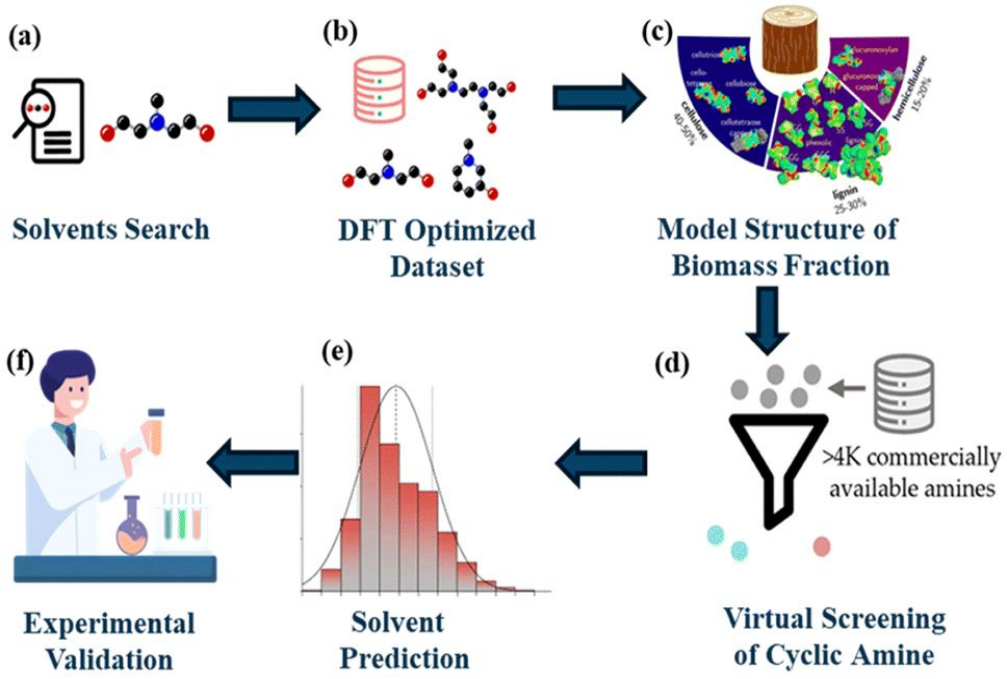

Figure caption: Workflow of the proposed methodology. (a) Data were collected from the literature on amines used as solvents; (b) the COSMO-RS model was applied to a diverse dataset containing 650 cyclic amine structures by first optimizing and generating the cosmo file; (c) the model structure of different biomass fractions; mainly cellulose, hemicellulose, and lignin were generated; (d) the activity coefficients of different biomass fractions were calculated for the different cyclic amines; (e) solvents were ranked based on the solute-solvent activity coefficient; and (f) solvents predicted to be the most effective were selected for further study and experimental testing.

Kumar, N., et al. Green Chemistry. doi: 10.1039/D4GC05891G (JBEI #1251)

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Cost Impact of Hexose-to-Pentose Sugar Ratios for Biomanufacturing

Background/Objective

- TEA models largely ignore glucose inhibition effects
- Objective: quantify the cost benefit of increasing glucan to xylan ratios in bioenergy feedstocks

Approach

- Modeled ethanol and bisabolene production using empirical time series data on sugar consumption and product titers
- Explored cost impacts across different residence times

Results

- At 24 hr residence times for ethanol, increasing glucan-to-xylan ratios can make a feedstock worth up to $77/tonne more
- Increasing the ratio benefits bisabolene production for all residence times

Significance/Impacts

- Highlights the importance of feedstock engineering to increase glucan-to-xylan ratios
- Indicates that TEAs should not overlook sugar consumption rate differences

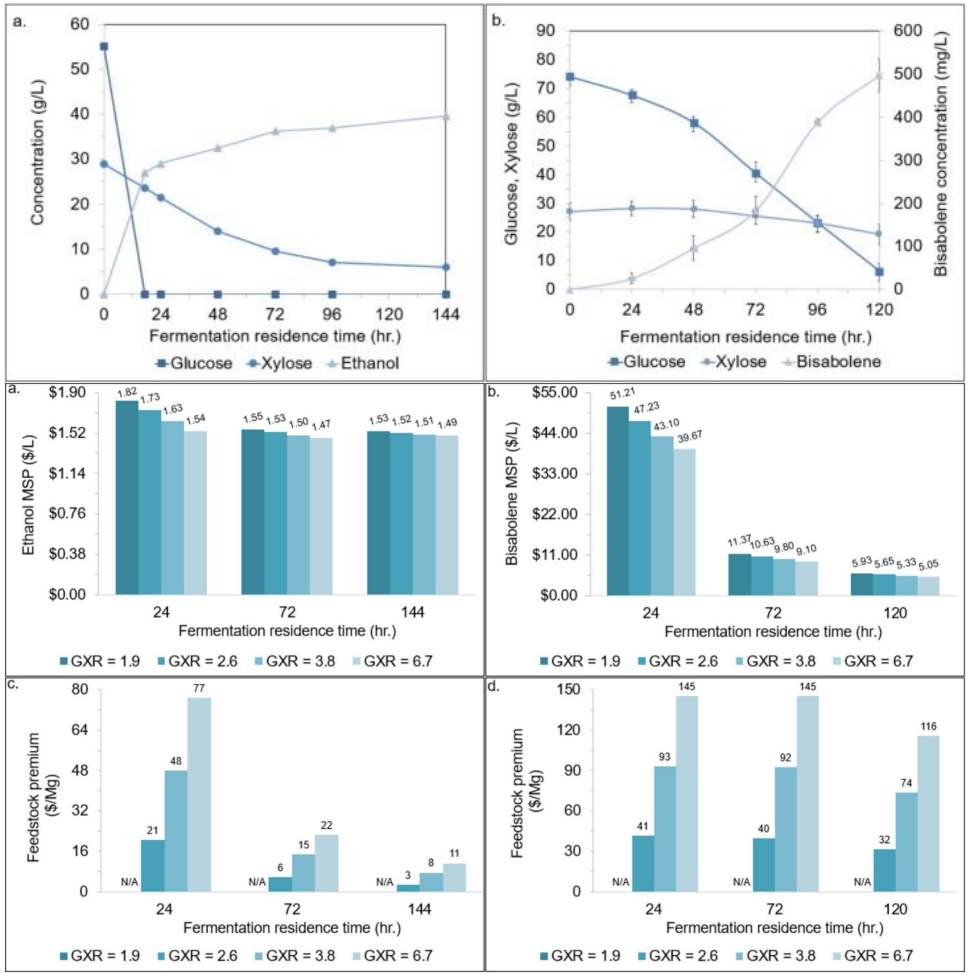

Figure caption: Top: Consumption of glucose and xylose, and accumulation of product used for TEA modeling. Bottom: MSP and feedstock premium based on glucan-to-xylan ratios.

Tripathi, J., et al. Bioresource Technology. doi: 10.1016/j.biortech.2025.133051 (JBEI #1252)

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Rational Modulation of Plant Root Development Using Engineered Cytokinin Regulators

Background/Objective

- Achieving precise control over quantitative developmental phenotypes is a key objective in plant biology.

Approach

- We leverage Type-B response regulators to modulate the expression of genes involved in cytokinin-dependent growth and development processes.
- We rationally engineered these regulators to modulate their transcriptional activity (i.e., repression or activation) and potency while reducing their sensitivity to cytokinin.

Results

- By localizing the expression of these engineered transcription factors using tissue-specific promoters, we can predictably tune cytokinin-regulated traits.
- As a proof of principle, we deployed this synthetic system in *Arabidopsis thaliana* to either decrease or increase the number of lateral roots.

Significance/Impacts

- The simplicity and modularity of our approach makes it an ideal system for controlling other developmental phenotypes of agronomic interest in plants.

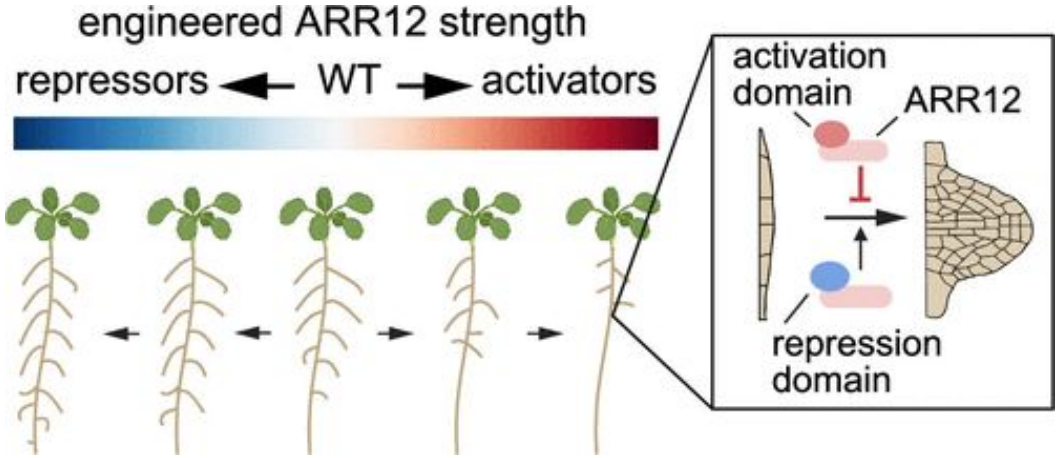

Figure caption: This shows the predicted effects of repressor and activator phosphomimetic Type-B ARRs on lateral root initiation, where an activator phosphomimetic ARR acts as a negative regulator and repressor phosphomimetic ARR as a positive regulator of lateral root development. (D) Cartoon illustrating the expected outcomes of deploying an engineered phosphomimetic ARR library to root cells in A. thaliana, with the lateral root phenotype scaling in accordance with ARR strength.

Rattan, R., et al. ACS Synthetic Biology. doi: 10.1021/acssynbio.5c00051 (JBEI #1253)

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Evaluating the industrial potential of emerging biomass pretreatment technologies using transgenic sugarcane

Background/Objective

- The selection of pretreatment methods is critical to achieving high product yields during bioconversion of lignocellulosic biomass. Hydrothermal, soaking-in-aqueous ammonia, and ionic liquid pretreatment methods are viable candidates for releasing high concentration of fermentable sugars and industrially relevant ethanol titers.

Approach

- The effects of these three pretreatment methods were investigated and compared in terms of lipid recovery, sugar yield, and ethanol yields on non-modified sugarcane cultivar CP88-1762 and two transgenic lipid-accumulating sugarcane lines, oilcane 1565 and oilcane 1566.

Results

- Commercially viable ethanol titers of 100.62 g/L, 64.47 g/L, and 52.95 g/L were achieved from ammonia, hydrothermal, and ionic liquid pretreated hydrolysates with corresponding ethanol productivities of 2.08 g/L/h, 0.53 g/L/h, and 0.36 g/L/h.

Significance/Impacts

- The observed differences in titer, productivity, and lipid content indicate that careful selection and validation of upstream processing methods can contribute to improved economic and environmental outcomes.

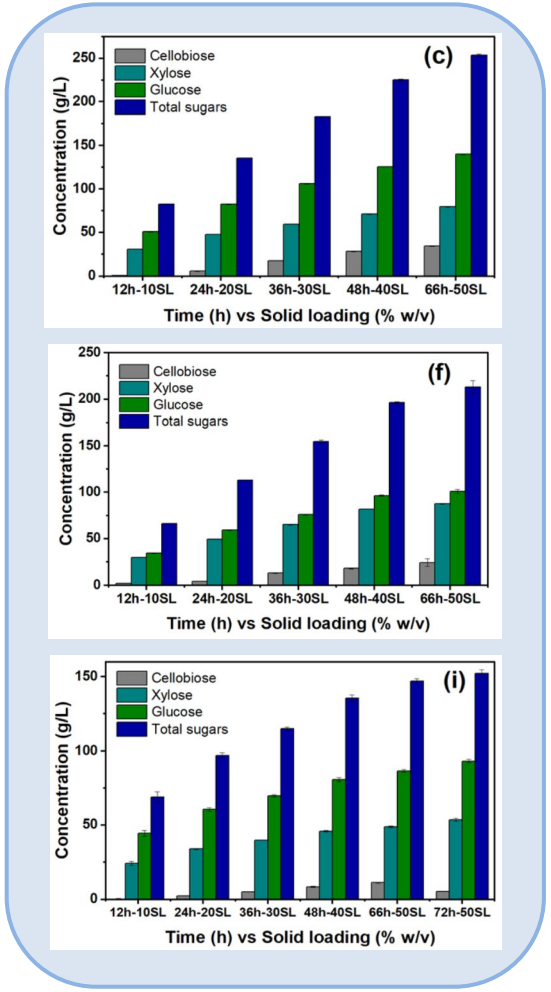

Figure caption: Sugar release from fed-batch high solid loading enzymatic hydrolysis on ammonia pretreated (c), hydrothermally pretreated (f) and ionic liquid pretreated (i) oilcane 1566 biomass.

Deshavath, Narendra, et.al. Sustainable Energy and Fuels. DOI: 10.1039/d5se00519a (JBEI #1254)

Enabled Publications

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Chelator-mediated Fenton post-treatment enhances methane yield from lignocellulosic residues via microbial community modulation

Background/Objective

- Enhance methane yield during anaerobic digestion by chemically treating lignocellulosic residues (i.e. manure digestate, poplar lignin)
- Aiming to access the residual energy potential through improved biomass degradability and conversion efficiency

Approach

- Novel application of chelator-mediated Fenton chemistry (CMF) to 1) deconstruct the recalcitrant biomass and 2) recover untapped biomethane in a subsequent lab-scale anaerobic digestion

Results

- CMF treatment à greater than 50% delignification and a significant increase in dissolved organic carbon available for microbial metabolism
- Anaerobic Digestion à increase in relative abundance of cellulolytic microbes and up to ten-fold increase in biomethane potential

Significance/Impacts

- Unlocking more energy through CMF offers a potential pathway to improve performance of AD systems and enhance the availability of downstream fuels and chemicals for the industrial and transportation sectors

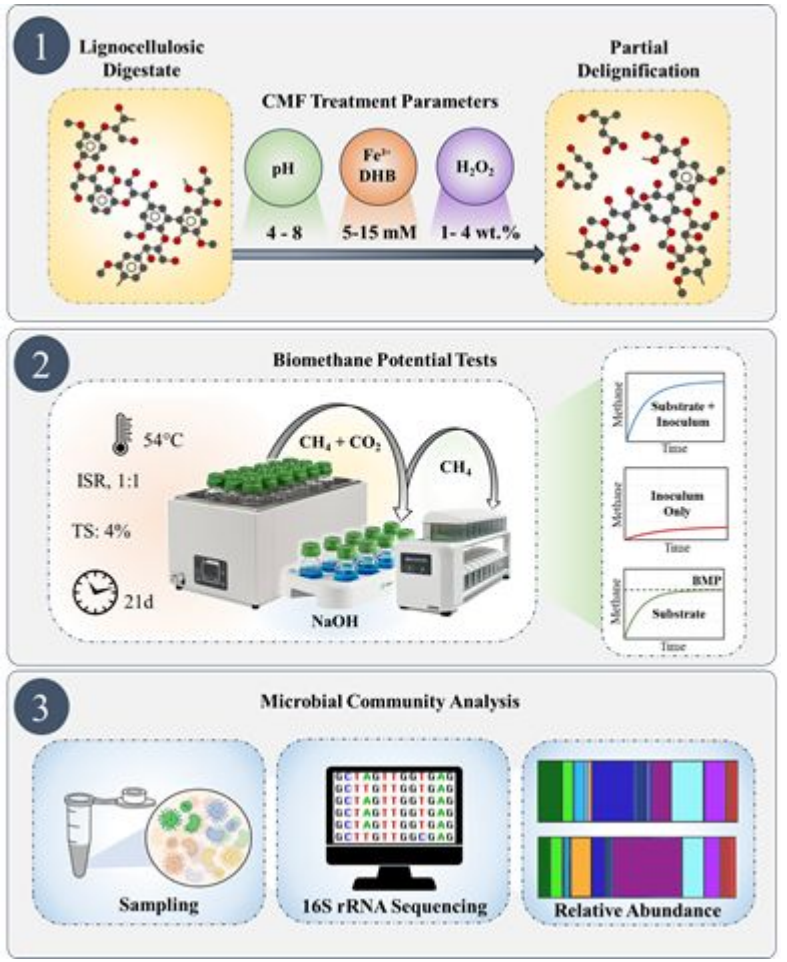

Figure caption: Experimental workflow for assessing the effects of CMF treatment parameters on delignification efficiency, biomethane potential, and microbial composition.

Martinez, D. V., et al. Biotechnology for Biofuels and Bioproducts. doi: 10.1186/s13068-025-02672-z (JBEI #117)

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Resin-to-Resin Circularity in Chemical Recycling of Dicyclopentadiene-Based Cycloolefin Resins

Background/Objective

- Widely used DCPD thermosets have few recycling options
- Deconstruct DCPD thermosets through reformation of the cyclopentene ring in DCPD, enabling reuse thereafter

Approach

- Ring-closing metathesis depolymerization screens to reform cyclopentenes as pendants on linear DCPD recyclates
- Establish grounds for recycling efficiency and reuse potential

Results

- 2nd-generation Hoveyda–Grubbs ruthenium(II) alkylidene produced reusable recyclates in high yield and purity
- Up to 30% recyclate incorporation produced materials with first-generation attributes

Significance/Impacts

- DCPD is a building-block of the future, likely to outcompete (meth)acrylates in additive manufacturing, thus motivating efforts to identify effective recycling processes
- Recycling also applicable to bio-based cycloolefin resins

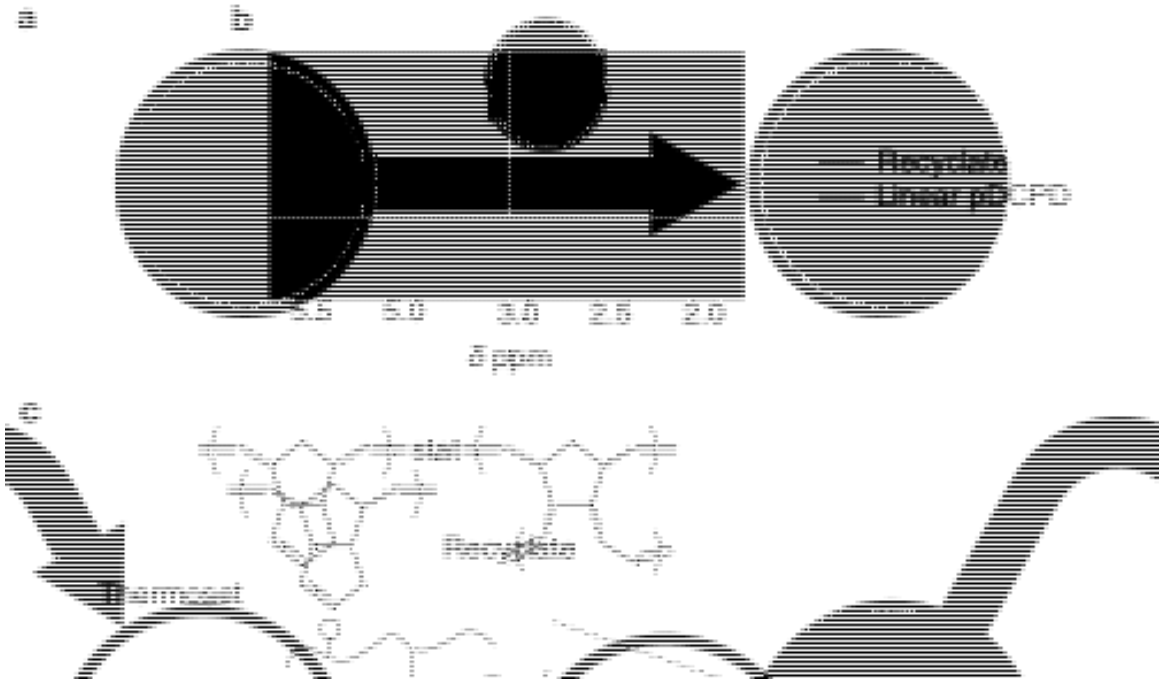

Figure caption: (a) Deconstruction of a commercial 3-D printed cycloolefin resin-based fullerene (polySpectra) into a polymeric recyclate through the action of a ring-closing metathesis depolymerization catalyst. (b) ^{1}H NMR spectrum of the polymeric cycloolefin resin recyclate alongside that for an authentic sample of linear polydicyclopentadiene (polyDCPD). (c) Schematic of the envisioned and realized resin-to-resin circularity, leveraging the chemistry of DCPD. DCPD is a widely available crosslinker for advanced manufacturing. Its commercial sources are jointly from petrochemical refining and biosynthesis in a variety of microbial hosts.

Xu, Z., et al. Journal of the American Chemical Society. doi: 10.1021/jacs.5c06626 (JBEI #118)

LC-MS-qTOF analysis and biological evaluation of Chrozophora tinctoria extracts: A novel attempt at integrating in vitro and in silico approaches

Background/Objective

- Phytochemical composition and biological potential of Chrozophora tinctoria extracts obtained with solvents of different polarities.

Approach

- The effects of different solvents on the phytochemical composition, antioxidant activity, enzyme inhibitory potential, and cytotoxicity of Chrozophora tinctoria extracts were investigated. A combination of in vitro and in silico analyses was used to evaluate their potential as future nutraceutical candidates comprehensively.

Results

- C. tinctoria different polarities exhibited significant phytochemical richness and various in vitro biological activities. Ethyl acetate and ethanol extracts contained the highest levels of total phenols and flavonoids, correlating with a strong antioxidant potential. The ethanol extract also showed the most effective enzyme inhibition.

Significance/Impacts

- Integrating in vitro and in silico approaches provides a comprehensive understanding of the pharmacological significance of C. tinctoria and supports its potential application as a natural source for developing pharmaceutical and cosmetic products.

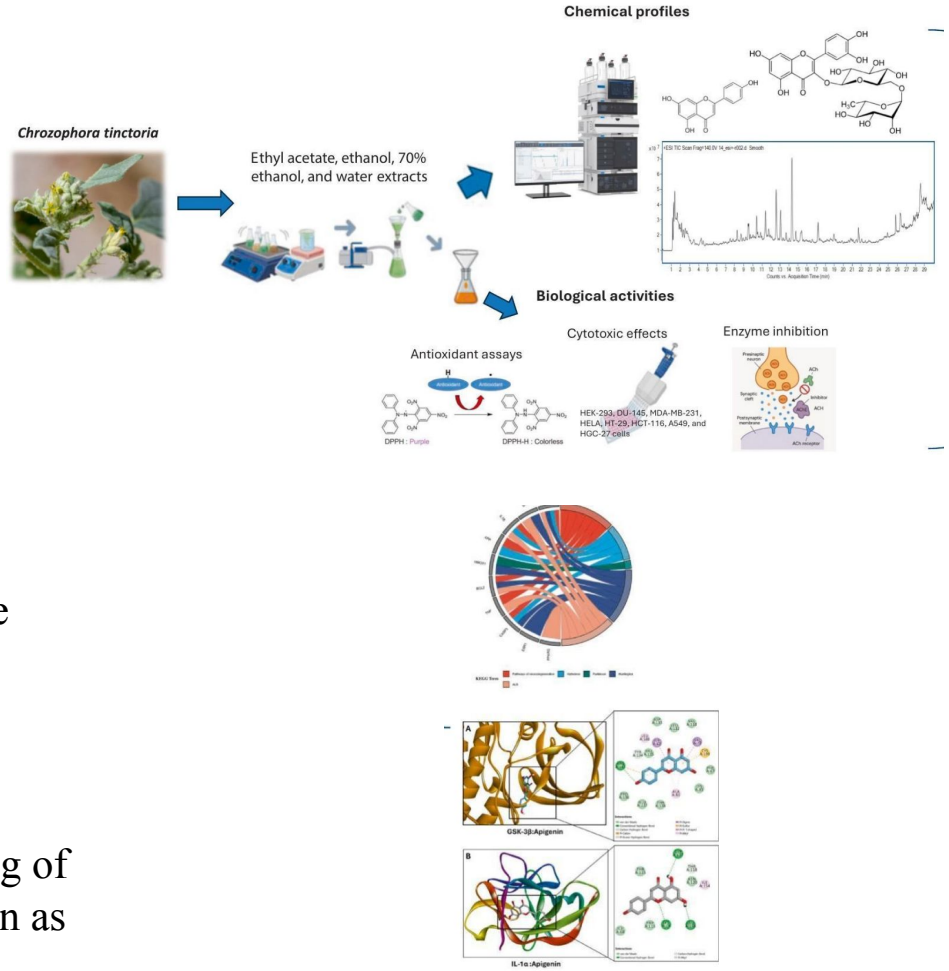

Figure caption: Experimental workflow for extracting, analyzing, and evaluating the chemical and biological properties of Chrozophora tinctoria extracts.

Terzic, M., et al. Fitoterapia. doi: 10.1016/j.fitote.2025.106740 (JBEI #119)

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Advances and Emerging Issues in Life-Cycle Assessment for Biofuel Policy

Background/Objective

- Financial incentives for biofuel production have a major impact on industry behavior and market outcomes
- TEAs typically exclude these because they are complicated to get right

Approach

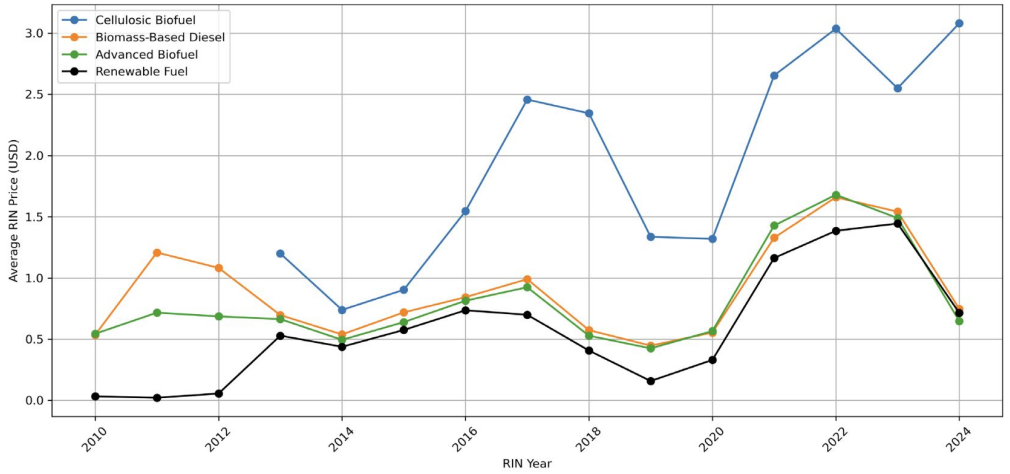
- This review lays out how financial incentives are calculated, and what goes into the emissions footprints tied to these monetary values

Results

- Counterfactual emissions calculations and forest carbon fluxes are areas where there continues to be evolution in implementation
- N_2O emissions quantification requires improved data and accounting

Significance/Impacts

- In some cases, financial incentives for biofuels exceed the base market value for fuels
- Understanding how they are calculated helps to understand commercialization potential and explain market dynamics for next-generation biofuels



Climate zone	Direct[a]	Indirect[b]	Total
Aggregated	1.00%	0.374%	1.374%
Wet	1.00%	0.418%	1.418%
Dry	0.50%	0.055%	0.555%

Figure caption: Top: RIN prices by year. Bottom: N2O emission rates differentiated by local climates as used in emissions calculations done in GREET.

Nematian, M., et al. GCB Bioenergy. doi: 10.1111/gcbb.70061 (JBEI #120)