Rewiring Metabolism of Clostridium thermocellum for Consolidated Bioprocessing of Lignocellulosic Biomass Poplar to Produce Short-Chain Esters

Background

- Esters are platform chemicals with broad use as flavors, fragrances, solvents, and fuels.
- Consolidated bioprocessing (CBP) of lignocellulosic biomass (e.g., poplar) uses cellulolytic microorganisms with enzyme production, biomass hydrolysis, and fermentation to produce biofuels, biochemicals, and biomaterials in a single step; CBP is economically attractive.
- A Co-solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment is a mild biomass preprocessing step to recover glucan-rich solids.
- However, redirecting metabolism of CBP-microorganisms (e.g. *C. thermocellum*) to produce non-native metabolites (e.g., short-chain esters) is currently limited.

Approach

- We redirected electron and carbon metabolism of *C. thermocellum* for production of short-chain (C4-C8) esters from CELF-pretreated woody poplar (**Panel A**).
- We analyzed the impact of different severity of CELF pretreatment conditions on the metabolism of *C. thermocellum* and its CBP-conversion of CELF-pretreated poplar to esters.

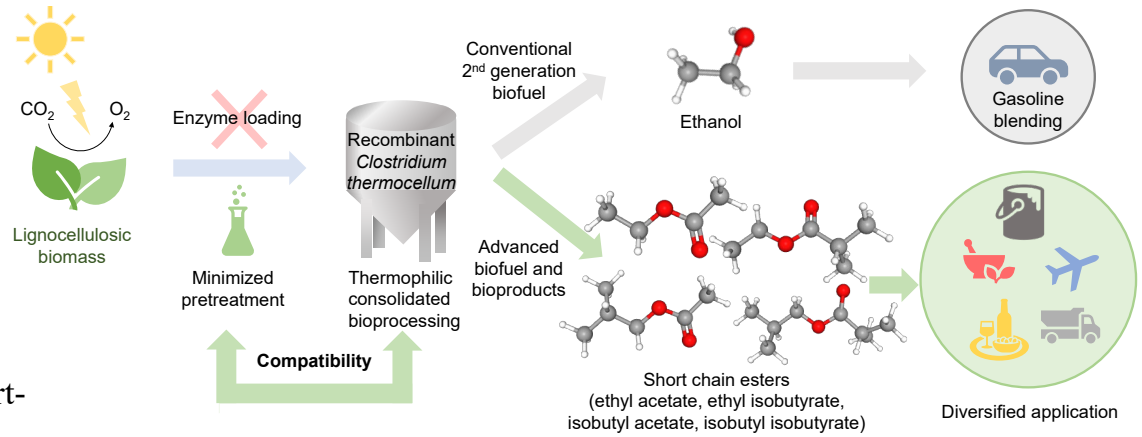
Results

- Engineered *C. thermocellum* can be used for CBP-production of short-chain (C4-C8) esters from CELF-pretreated woody poplar (**Panel A**).
- Deleting promiscuous thioesterase activities of carbohydrate esterases lowered ester degradation and enhanced ester production as did deletion of lactate biosynthesis.
- Identified a positive correlation between severity of CELF pretreatment of woody poplar and short chain ester production (**Panel B**).
- Ester production was enhanced up to 80-fold and composition was controlled by manipulating either the carbon and electron metabolisms (**Panel C**) or severity of CELF pretreatment.

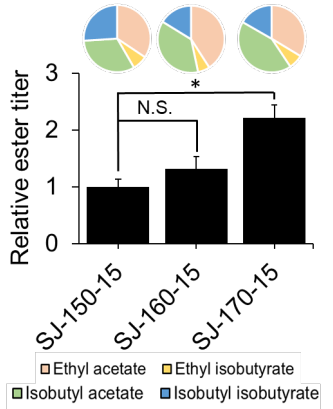
Significance

- We demonstrated for the first time that engineering the endogenous metabolism of *C. thermocellum* allows for de novo biosynthesis of short-chain esters directly from pretreated cellulosic biomass.
- This demonstrates the potential of CBP for more than simple alcohol production and to produce valued-added products.

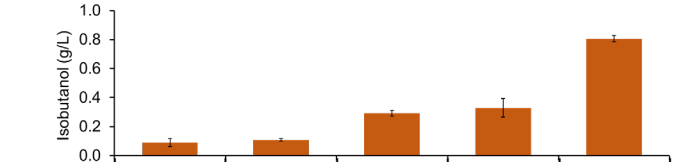
Seo, H., et al. *Bioresource Technology*, 2023. 10.2139/ssrn.4441470.



A. A scheme of CELF-CBP for short-chain ester production.



B. Ester production vs. severity of CELF pretreatment



	M1354	HSCT0102	HSCT2105	HSCT0103	HSCT2108
Esterases			Δ		Δ
CAT		CATsa F97W	CATsa F97W	CATec3 Y20F	CATec3 Y20F
Fold change	1.0	1.2	3.2	3.7	9.0
Pellet protein (mg/L)	818.5 ± 27.5	542.2 ± 42.8	484.9 ± 35.1	*n.d.*	412.8 ± 38.9

C. Carbohydrate esterase (CE) deletion and overexpression of thermostable chloramphenicol acetyltransferases (CATs) enhanced production of isobutanol and short-chain ester biosynthesis