Rhamnogalacturonan I Limits Microbial Deconstruction of Switchgrass During Consolidated Bioprocessing

Background/Objective

Efficient conversion of lignocellulosic biomass to biofuels is essential for advancing the growing bioeconomy. However, the structural complexity of plant cell wall pectic and hemicellulosic polymers represents a barrier to efficient degradation of biomass by microbes. Here we identify specific cell wall polymers that are not solubilized during consolidated bioprocessing (CBP) of switchgrass by the thermophilic bacterium *Clostridium thermocellum*.

Approach

- Compositional analysis of switchgrass subjected to 120-hour CBP fermentation by *C. thermocellum* was performed using mass spectrometry-based analytical tools.
- Yields of cellulosic, hemicellulosic, and pectic polysaccharides were quantified across the soluble fractions, solid residues, and washates from both CBP and control fermentations.
- Enzyme-linked immunosorbent assays (ELISA) employing glycan-specific monoclonal antibodies were used to identify cell wall polysaccharides most resistant to solubilization.

Results

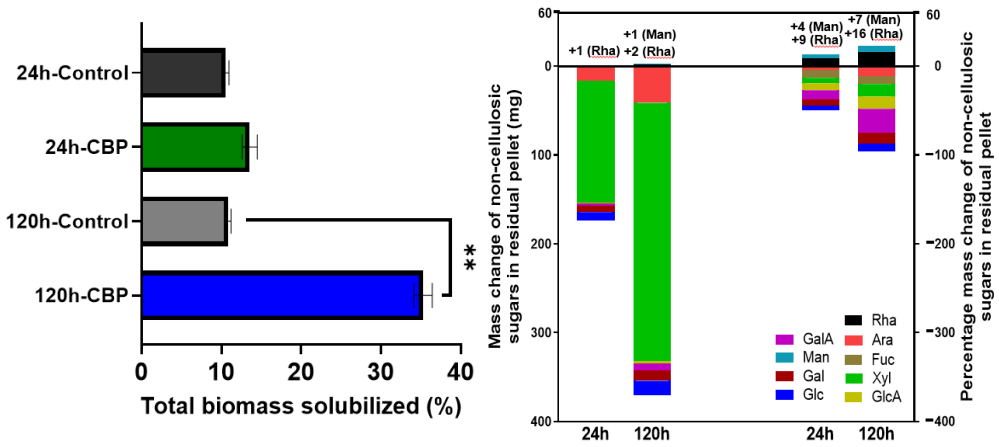
- *C. thermocellum* solubilized 24% of total switchgrass biomass and 49% of cellulose after 120 h of CBP fermentation.
- Hemicellulosic sugars (arabinose, xylose, galactose, and glucose) accumulated in the fermentation liquor, confirming partial solubilization but incomplete utilization.
- Solid residues were enriched in rhamnose and mannose, and ELISA analyses confirmed significant enrichment of the pectic polymer rhamnogalacturonan I (RG-I) and the hemicellulosic polymer galactomannan in post-CBP solid residues.

Significance/Impacts

This study reveals that RG-I and galactomannan are key recalcitrant components in switchgrass cell walls during CBP. These findings provide targets for feedstock engineering to alter RG-I and galactomannan content or structure as well as microbial/enzyme engineering targets to improve pectin- and mannan-degrading activities.

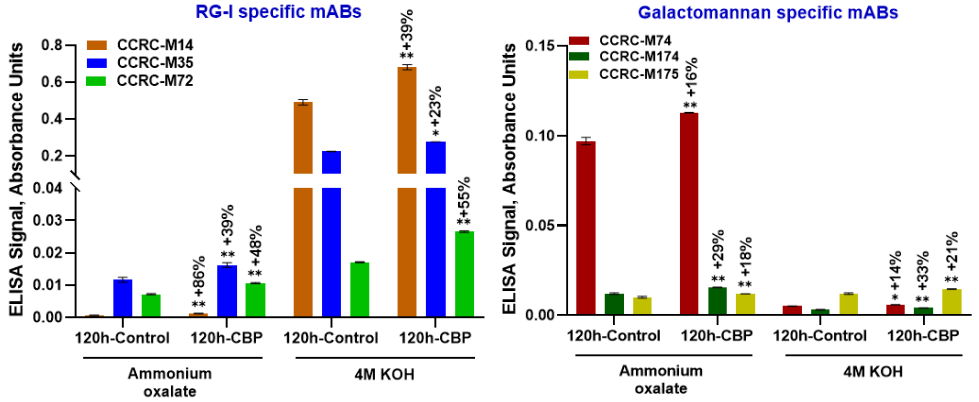
Biswal A.K. et al., *Biotechnol. Biofuels Bioprod.* (2025). doi: 10.1186/s13068-025-02703-9

Biomass solubilization and compositional changes during CBP by *C. thermocellum*



C. thermocellum solubilized 24% of switchgrass biomass after 120 h of CBP (left). Enrichment of rhamnose and mannose residues indicates persistence of RG-I and galactomannan (right).

RG-I and galactomannan resist microbial deconstruction



ELISA analyses showed significant enrichment of RG-I (left) and galactomannan (right) epitopes in post-CBP residues.