Anaerobic microbiomes possess powerful enzymatic strategies to maintain lignocellulose deconstruction at high biomass solids

Background

- Because substantial titers of liquid fuels are required to avoid high costs for product recovery and fermentation, biological processes for conversion of lignocellulose need to operate at high solids loadings—typically 15 wt% or more.
- Defined cultures of thermophilic anaerobic cellulolytic bacteria can reach high solubilization levels of lignocellulosic carbohydrates. However, solubilization diminishes with increasing solids loading. Biomass-degrading microbial consortia can effectively solubilize carbohydrates with increasing biomass solids.

Approach

- Metaproteomics was used to identify key enzymes and functions employed by microbial consortia to maintain carbohydrate solubilization from low to high switchgrass loading concentrations (30, 75, 120, and 150 g/L respectively).
- Fractional carbohydrate solubilization, and production of methane and carbon dioxide, were measured as direct performance metrics of comparison between the different conditions.
- Microbial and enzymatic material was measured from three different fractions: supernatant, planktonic and substrate bound.

Results

- Carbohydrate solubilization and the rate of methane and overall gas production was linear with solids loading indicating non-diminishing metabolic processes even at higher solids loadings.
- Metaproteomics revealed beta-glucosidases are quite prevalent and oligomer degrading enzymes and hemicellulose debranching enzymes are more abundant at high solids. Pectin-acting enzymes increase in substrate-associated fractions.
- Bacterial AA6 proteins increase dramatically at higher solids. Little is known about these bacterial AA6 enzymes other than they are benzoquinone reductases and are thought to be involved in generation of Fenton reagents.
- Microbiomes make relatively more enzymes to remove potential inhibitors at high solids.

Significance

- The thermophilic, lignocellulose-fermenting, methanogenic anaerobic microbiome reported here exhibits a key feature desired for an industrial process: undiminished fractional carbohydrate solubilization with increasing substrate loading.
- Metaproteomics revealed enzymes associated with carbohydrate solubilization at high solids loading These indicate metabolic engineering strategies for improving performance in defined cultures at high solids.

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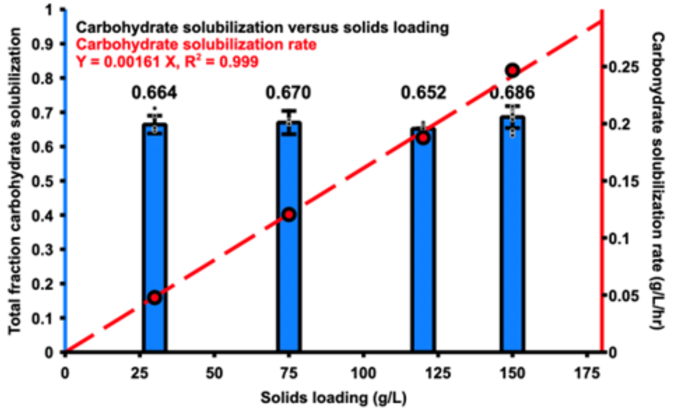
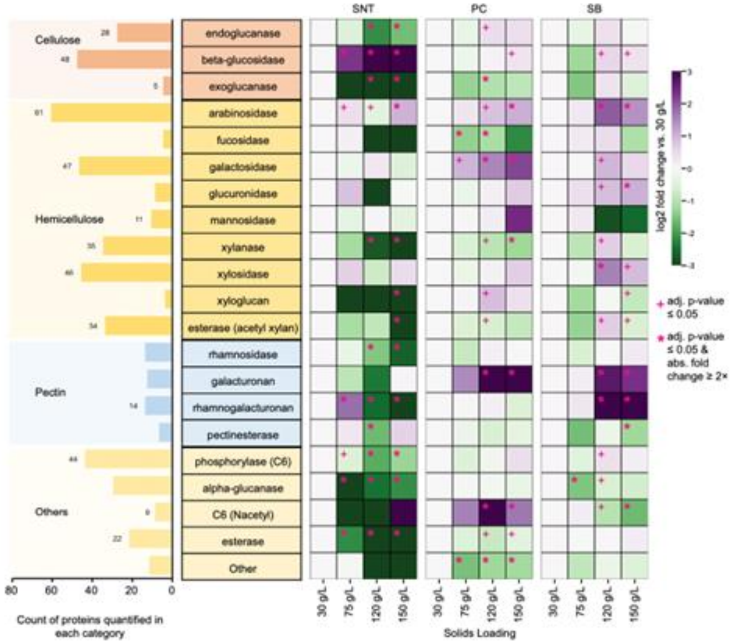


Stable carbohydrate solubilization (blue bars) at ~66.8% was obtained, with the rate of substrate solubilization (red circles) increasing linearly with switchgrass solids loading.



Enumeration of quantified proteins across different CAZyme families (GH, CE, PL). Heatmap depicting the change in aggregate abundance of CAZymes in each functional category across solids loadings for each fraction. Log2 differences from 30 g/L solids in the respective fraction are shown.