First-of-a-kind study of thermophilic CBP with cotreatment

**Background**
Milling during lignocellulosic fermentation (or “cotreatment”) is an alternative to thermochemical pretreatment to enhance biological solubilization of lignocellulose.

**Approach**
Carbohydrate solubilization was measured in consolidated bioprocessing with *Clostridium thermocellum* with cotreatment or with hydrothermal pretreatment.

**Outcome**
- Total fractional carbohydrate solubilization achieved after fermentation of senescent switchgrass by *C. thermocellum* for 5 days was 0.45 without cotreatment or pretreatment, 0.81 with hydrothermal pretreatment (200°C, 15 minutes, severity 4.2 – not shown), and 0.88 with co-treatment with ball milling (Figure 1). This corresponded with fermentation gas production (mostly CO₂).
- Milling had little effect on soluble substrate fermentation by *Clostridium thermocellum* whereas yeast fermentation was completely arrested (likely due to the impact of milling shear forces on the yeast and not the bacteria).
- Characterization of residual solids using molecular beam mass spectrometry and solid-state nuclear magnetic resonance spectroscopy indicated little change in lignin structure following CBP with cotreatment, and substantially larger changes for CBP following hydrothermal pretreatment (Figure 2).

**Significance**
- High carbohydrate solubilization is demonstrated without thermochemical pretreatment and added saccharolytic enzymes
- *C. thermocellum* appears able to attack all the major linkages in cellulosic biomass provided that these linkages are accessible.
- The ability of *C. thermocellum* to withstand high intensity milling supports the feasibility of cotreatment.
- Less modified lignin may foster production of value-added coproducts.