

A Comparison of Lignocellulose Solubilization by Cellulolytic Monocultures and a Mixed Enrichment Yields Surprising Results

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

The question “What biocatalysts are most effective at mediating biomass deconstruction?” is of considerable fundamental interest and is foundational for developing processes for biological conversion of cellulosic biomass. Yet controlled comparative studies are scarce. Recently, substantial differences in solubilization capability have been demonstrated with respect to the ability of various biocatalysts to solubilize untreated switchgrass,¹ and *Clostridium thermocellum* has been shown to be several fold more effective than a commercial fungal cellulase preparation at solubilizing several feedstocks under a broad range of conditions.²

Mixed lignocellulose-fermenting enrichments (aka microbiomes, microbiota) have repeatedly been shown to contain a diversity of cellulolytic microbes and cellulase genes. It is generally thought that lignocellulose solubilization by mixed enrichments is enabled by synergistic interactions and more effective than solubilization by pure cultures. However, few, if any, studies have quantitatively compared the relative rates and extents of solubilization by pure cultures and mixed enrichments. We sought to provide such a comparison in this study.

In an initial experiment carried out in batch culture, we compared solubilization of 30 g/L mid-season harvested switchgrass by *Clostridium thermocellum* and an inoculum from a mixed enrichment (thermophilic microbiome) maintained for over 2 years on this feedstock. Both cultures were incubated at 55°C under anaerobic conditions. Based on

16S rRNA and metagenomic analysis, the mixed enrichment inoculum appeared to have substantial diversity with respect to both the microorganisms and glycosyl hydrolase genes present. The change in diversity during solubilization was followed using relative abundance for 16S rRNA genes. Total carbohydrate solubilization (TCS) was greater for *C. thermocellum* than the mixed enrichment for early time points, and essentially equal for later time points.

In a second experiment we cultivated *C. thermocellum* and mixed enrichments on the same switchgrass feedstock in intermittently-fed continuous cultures maintained at various residence times. The extrapolated concentration of inaccessible carbohydrate was the same for *C. thermocellum* and the mixed enrichment, which also agreed well with results from batch culture. For both the pure and mixed culture, the rate of TCS was first order in accessible substrate. To our surprise, the first order rate constant was over 2-fold higher for the pure culture compared to the mixed culture.

Our results contradict the understanding that mixed enrichment cultures achieve more complete and more rapid deconstruction of lignocellulosic biomass, but will need further confirmation.

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