207. Degradation of High Loads of Crystalline Cellulose and of Unpretreated Switchgrass by Caldicellulosiruptor bescii

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Deconstruction of plant biomass traditionally involves thermochemical pretreatment in order to overcome its recalcitrant nature. The anaerobic bacterium *Caldicellulosiruptor bescii* degrades switchgrass biomass without prior chemical treatment. Use of high substrate loads decreases the costs of biofuel generation, and will in fact be necessary to generate fuel titers required for a viable industrial process. Here we show that *C. bescii* grows at 78°C without inhibition on crystalline cellulose and on unpretreated switchgrass of concentrations up to 200 g L^{-1}. In contrast, it grew poorly on acid-pretreated switchgrass on concentrations as low as 20 g L^{-1}. Indeed, the presence of acid-pretreated switchgrass (50 g L^{-1}) inhibited the growth of the organism on the unpretreated biomass (10 g L^{-1}). Growth of *C. bescii* and degradation of crystalline cellulose, but not that of switchgrass, were limited by changes in pH, nitrogen and vitamin (folate) availability. Under optimal conditions *C. bescii* solubilized ~60% of the cellulose and ~30% of the unpretreated switchgrass using 50 g L^{-1} substrates. Further fermentation of cellulose, but not switchgrass, was inhibited by the organic acids generated as fermentation products, while prolonged degradation of high loads of switchgrass led to accumulation of an uncharacterized inhibitor of *C. bescii* growth. However, we present evidence here that *C. bescii* is much more salt tolerant than previously assumed. Soluble sugars, acetate, lactate, carbon dioxide and biomass, quantitatively accounted for the cellulose and plant biomass carbon utilized even using 50 g L^{-1} substrates.

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