

Coculture with Hemicellulose-Fermenting Microbes Reverses Inhibition of Corn Fiber Solubilization by *C. Thermocellum* at Elevated Solids Loadings

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Abstract

Background The cellulolytic thermophile *Clostridium thermocellum* is an important biocatalyst due to its ability to solubilize lignocellulosic feedstocks without the need for pretreatment or exogenous enzyme addition. At low concentrations of substrate, *C. thermocellum* can solubilize corn fiber >95% in 5 days, but solubilization declines markedly at substrate concentrations higher than 20 g/L. This differs for model cellulose like Avicel, on which the maximum solubilization rate increases in proportion to substrate concentration. The goal of this study was to examine fermentation at increasing corn fiber concentrations and investigate possible reasons for declining performance. **Results** The rate of growth of *C. thermocellum* on corn fiber, inferred from CipA scaffoldin levels measured by LC-MS/MS, showed very little increase with increasing solids loading. To test for inhibition, we evaluated the effects of spent broth on growth and cellulase activity. The liquids remaining after corn fiber fermentation were found to be strongly inhibitory to growth on cellobiose, a substrate that does not require cellulose hydrolysis. Additionally, the hydrolytic activity of *C. thermocellum* cellulase was also reduced to less than half by adding spent broth. Noting that >15 g/L hemicellulose oligosaccharides accumulated in the spent broth of a 40 g/L corn fiber fermentation, we tested the effect of various model carbohydrates on growth on cellobiose and Avicel. Some compounds like xylooligosaccharides caused a decline in cellulolytic activity and a reduction in the maximum solubilization rate on Avicel. However, there were no relevant model compounds that could replicate the strong inhibition by spent broth on *C. thermocellum* growth on cellobiose. Cocultures of *C. thermocellum* with hemicellulose consuming partners - *Herbinix* spp. strain LL1355 and *Thermoanaerobacterium thermosaccharolyticum* – exhibited lower levels of unfermented hemicellulose hydrolysis products, a doubling of the maximum solubilization rate, and final solubilization increased from 67% to 93%. **Conclusions** This study documents inhibition of *C. thermocellum* with increasing corn fiber concentration and demonstrates inhibition of cellulase activity by xylooligosaccharides, but further work is needed to understand why growth on cellobiose was inhibited by corn fiber fermentation broth. Our results support the importance of hemicellulose-utilizing coculture partners to augment *C. thermocellum* in the fermentation of lignocellulosic feedstocks at high solids loading.

Full Text

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Figures

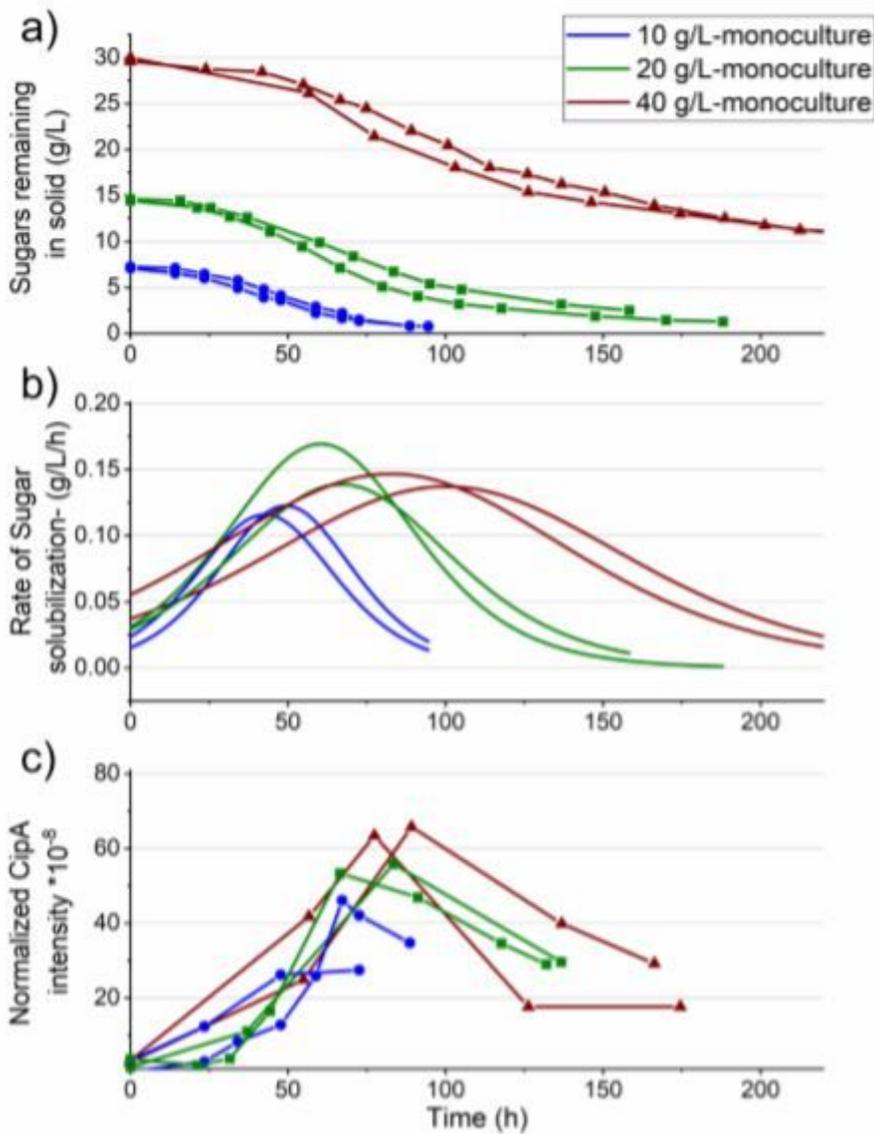


Figure 1

C. thermocellum fermentations on corn fiber. Fermentation data for *C. thermocellum* monoculture fermentations with increasing solids loading of corn fiber: a) sugar-equivalent carbohydrate remaining in the solid fraction; b) rate of solubilization; c) normalized CipA protein amount with increasing solids loading.

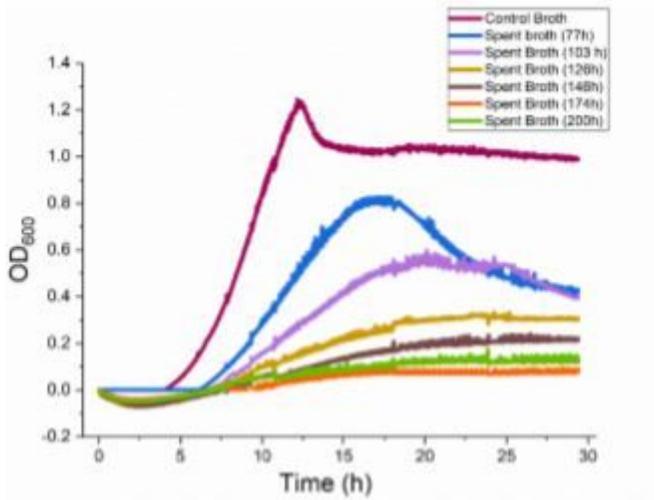


Figure 2

Inhibition of *C. thermocellum* growth on cellobiose. Growth of *C. thermocellum* on cellobiose with the addition of spent broths sampled at various times from a 40 g/L monoculture fermentation on corn fiber. Cultures and measurements of Optical Density at 600 nm (OD₆₀₀) were conducted in a plate reader. Spent broth constituted 75% of the cultures by volume, with the remainder being cellobiose, media components and inoculum.

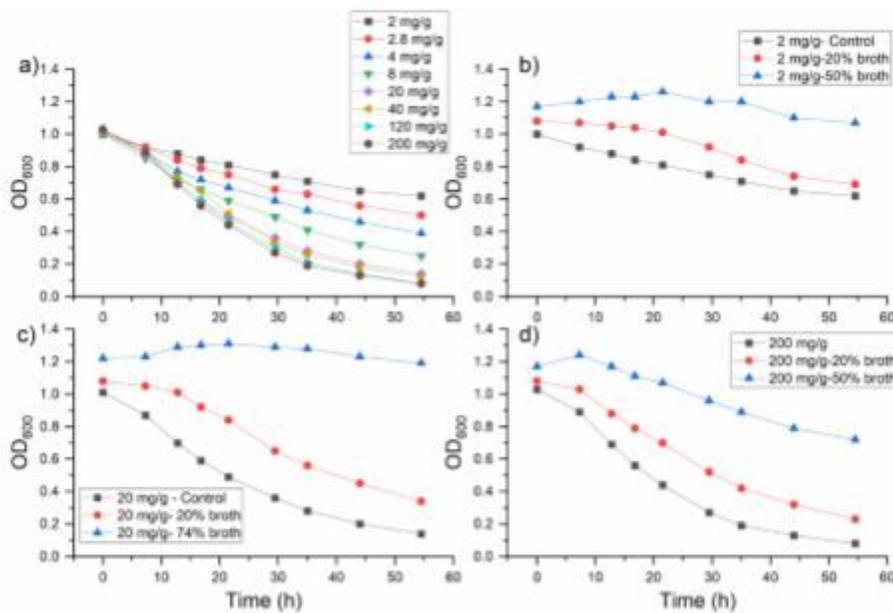


Figure 3

Inhibition of *C. thermocellum* cellulase. Cellulase enzyme activity assay with starting concentration of 1 g/L Avicel and reaction volume of 5 mL. Legend shows amount of enzyme in mg (cellulase)/g (Avicel) and the amount of spent broth added. a) Dependence of activity on amount of added enzyme; b), c), d) inhibition of cellulase activity with different amounts of added enzyme and broth.

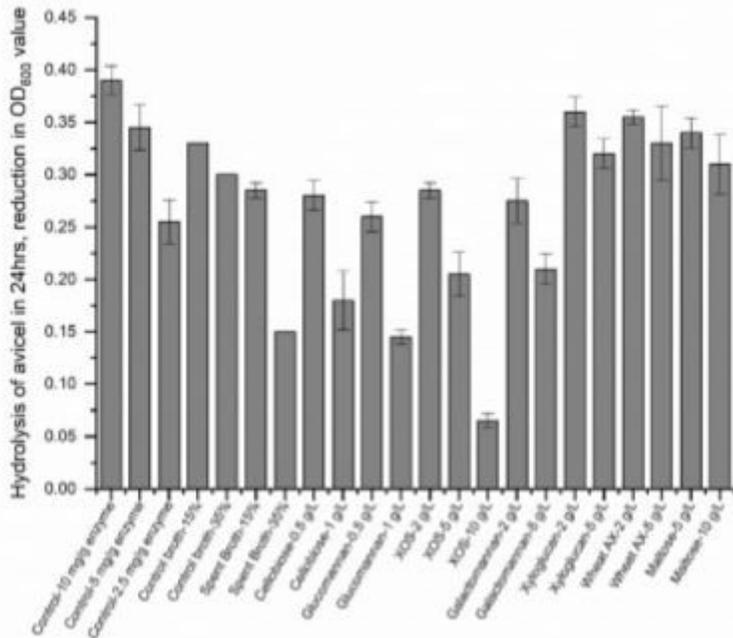


Figure 4

Effect of different carbohydrates on *C. thermocellum* cellulase activity. 0.5 g/L initial Avicel was incubated with 10 mg/g *C. thermocellum* cellulase and activity was estimated by measuring the decrease in OD600 after 24h. The error bars show the SD for n=2. XOS = xylooligosaccharides, AX = arabinoxylan, Control Broth - see Materials and Methods. Glucosaminan, galactomannan and xyloglucan used here are all oligosaccharide mixtures, prepared by partial enzymatic hydrolysis of the respective carbohydrate - see Materials and Methods.

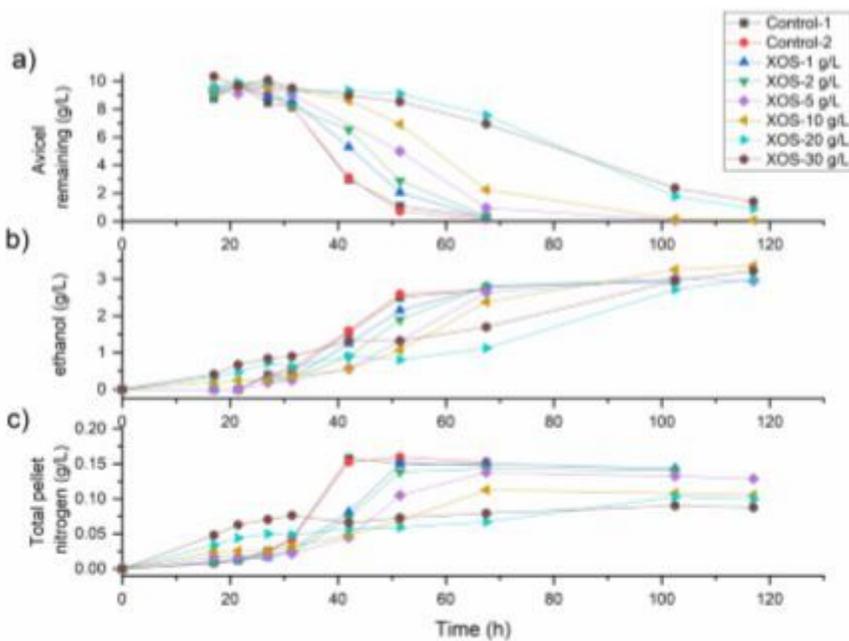


Figure 5

Effect of XOS addition on growth of *C. thermocellum* on Avicel. Fermentations were done in serum bottles with starting concentration of 10 g/L Avicel. a) Avicel remaining (g/L), b) ethanol produced (g/L) and c) cell growth estimated by total pellet nitrogen (g/L).

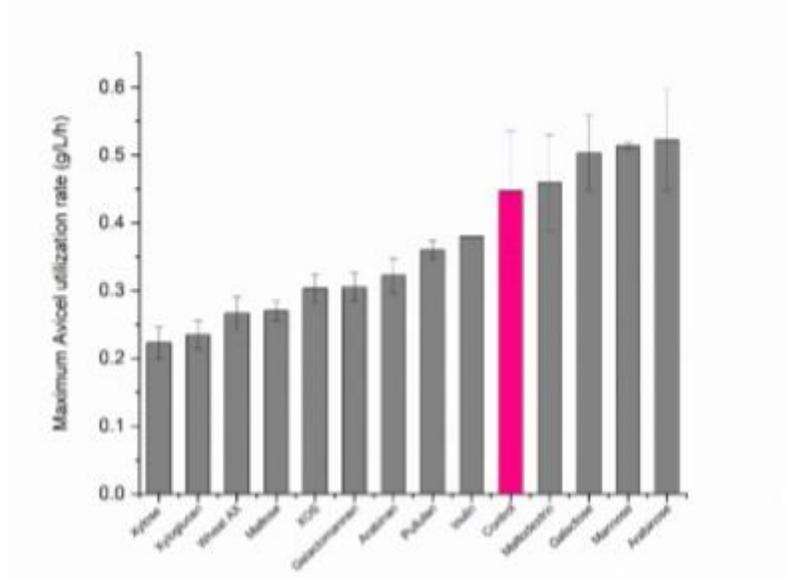


Figure 6

Effect of various carbohydrates on *C. thermocellum* growth on Avicel. *C. thermocellum* grown on 10 g/L Avicel with the addition of 10 g/L of various carbohydrates. Rate was calculated by differentiating a Boltzmann Sigmoidal fit curve on the Avicel utilization timecourse data. R² values of >0.98 were observed in all cases. Error bars show the standard deviation (n≥2). For the control n = 6. XOS = Xylooligosaccharides, AX = arabinoxylan.

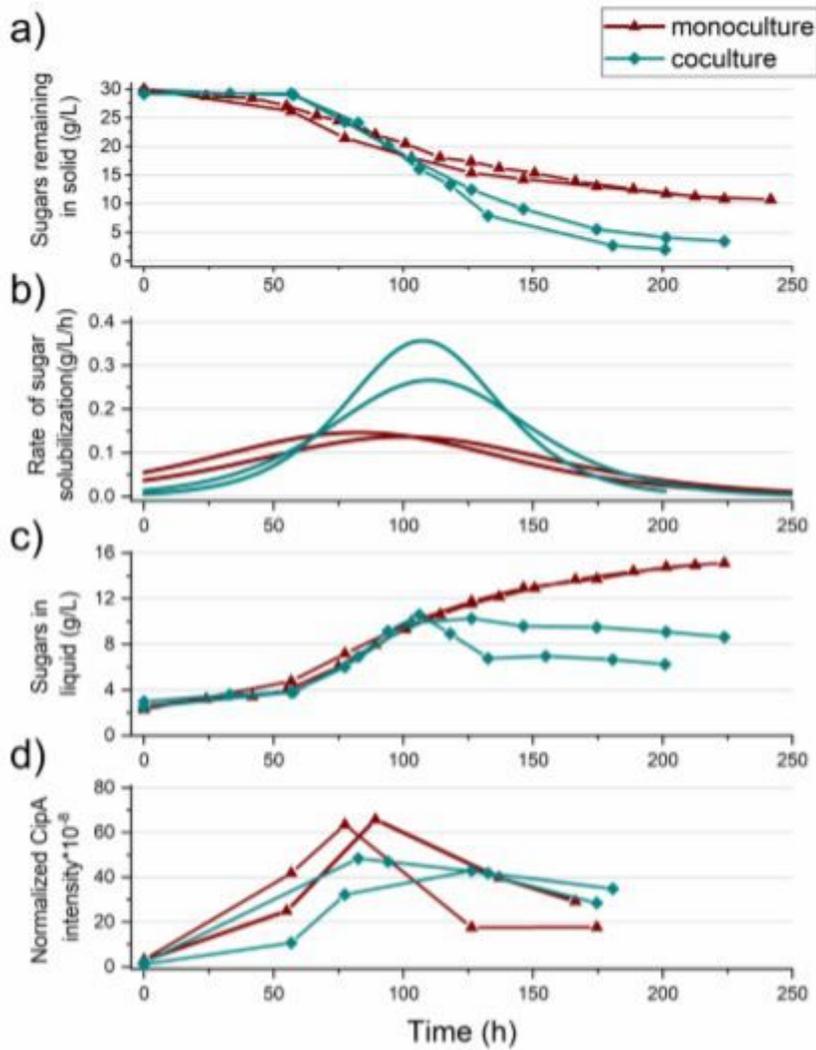


Figure 7

Comparison of monoculture and coculture in fermenting corn fiber. Fermentation data for *C. thermocellum* monoculture and its coculture with LL1355 and LL1548 on 40 g/L corn fiber in bioreactors: a) Sugars remaining in solid (g/L); b) Rate of solubilization (g/L/h); c) Sugars in liquid (g/L); d) biocatalyst as determined by normalized CipA intensity. Cocultures were inoculated with LL1355 and LL1548 at 0 and again at 50 hours.

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