Butyric acid production from delignified corn stover using thermophilic bacterial co-cultures

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Introduction

Butyric acid (BA) is a valuable chemical that can be used as platform intermediate for diesel and sustainable aviation fuel as well as for diverse commodity chemicals such as monomers for polymers, fibers, solvents, and preservatives. This work presents a co-culture-based solids-to-acids bioprocess using two thermophilic bacteria, Clostridium thermocellum (Ctc), one of the world's most efficient degraders of cellulosic substrates, and Clostridium thermobutyricum (Ctb), a highly efficient producer of butyric acid.

Objective

To understand the fundamental synergy and limitations between the two proposed organisms to enable optimal production of butyric acid from solid biomass under process relevant conditions.

Materials and Methods

Process configuration

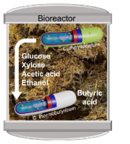


Fig. 1. Proposed process configuration for this technology. Co-cultures at 350 mL working volume are inoculated with Ctc and Ctb. Ctc will carry out the primary biomass deconstruction while Ctb will utilize solubilized sugars (including those not used by Ctc) and by-products to produce butyric acid. Cultivations were performed under anaerobic conditions, at pH 7, and 55˚C.

Biomass substrate: Deacetylated and mechanically refined corn stover (DMR)

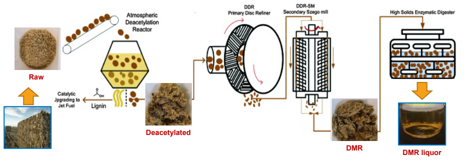


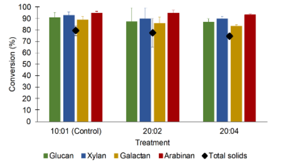
Fig. 2. Different corn stover substrates obtained from NREL pilot plant DMR pre-treatment process. ***Modified from Chen, X., et al. (2016).***

Results

Inoculation size and ratio test

To investigate the effect of different bacterial loadings on the deconstruction of DMR (Fig. 2) and by-products production by clostridial co-cultures.

Biomass deconstruction

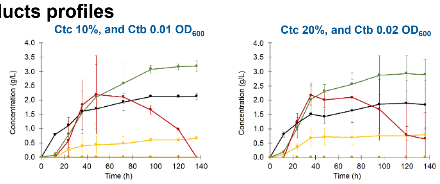


Treatments:
Ctc 10%, and Ctb 0.01 OD_{600}
Ctc 20%, and Ctb 0.02 OD_{600}
Ctc 20%, and Ctb 0.04 OD_{600}

Cultivation conditions were as described in Fig. 1.

Fig. 3. Solids conversion by clostridial co-cultures at different bacterial loadings

Products profiles



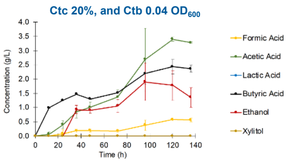


Fig. 4. By-products production by clostridial co-cultures at different bacterial loadings

Sugars profiles

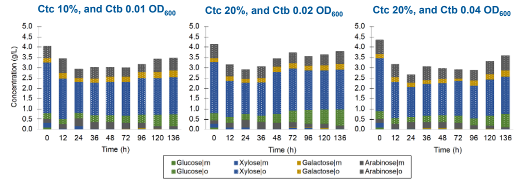


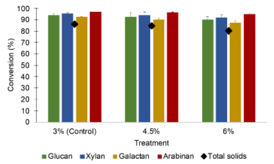
Fig. 5. Sugars profiles in clostridial co-cultures at different bacterial loadings

- ✓ Solids and carbohydrates conversions (%) did not differ among treatments ($p<0.05$) (Fig. 3).
- ✓ Maximum BA content was observed from 96h in all treatments. It ranged between 2.1 and 2.4 g/L (Fig. 4).
- ✓ Xylose moiety most accumulated at all times in the cultures (Fig. 5).

Solids loadings test

To investigate the effect of different DMR solids loadings on the deconstruction of solids materials and by-products production by clostridial co-cultures.

Biomass deconstruction

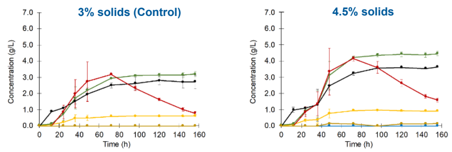


Treatments: DMR solids concentration at 3, 4.5, and 6%

Cultivation conditions were as described in Fig. 1.

Fig. 6. Solids conversion by clostridial co-cultures at different solids loadings in batch operation

Products profiles



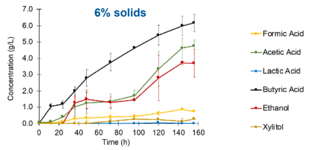


Fig. 7. By-products production by clostridial co-cultures at different solids loadings in batch operation

Sugars profiles

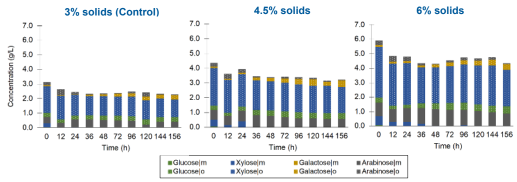


Fig. 8. Sugars profiles in clostridial co-cultures at different solids loadings in batch operation

- ✓ Solids and carbohydrates conversions (%) did not differ among treatments ($p<0.05$) (Fig. 6).
- ✓ BA production increased significantly with solids loadings ($p<0.05$). Maximum values were 2.8 g/L, 3.6 g/L, and 6.2 g/L in the 3%, 4.5%, and 6% treatments, respectively (Fig. 7).
- ✓ Increased amounts of sugars in cultures were observed at higher solids loadings (Fig. 8).

Fed-batch test

To assess the capability of the co-culture to deconstruct DMR and to produce by-products in a fed batch fermentation.

Biomass deconstruction

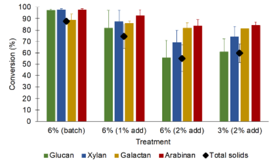


Fig. 9. Solids conversion by clostridial co-cultures at different solids loadings in fed-batch operation

Treatments	Initial solids (%)	Solids per feeding (g)	Total solids loaded (g)
6% batch	6	0.0	21.0
6%, 1% add	6	3.5 (1%)	31.5
6%, 2% add	6	7.0 (2%)	42.0
3%, 2% add	3	7.0 (2%)	31.5

Factors tested: Initial solids concentration and solids amount fed at once

Feeding times: 44, 94, and 137 hours.

Cultivation conditions were as described in Fig. 1.

Products profiles

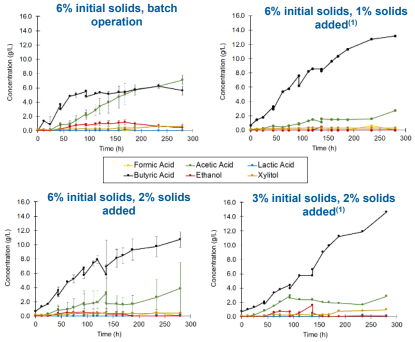


Fig. 10. By-products production at different solids loadings in fed-batch operation. [(1)]**Data shown are from singular experimental runs.**

Sugars profiles

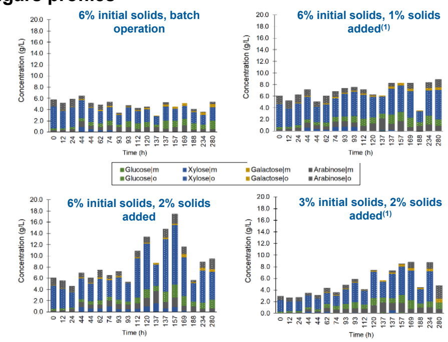


Fig. 11. Sugars profiles in clostridial co-cultures at different solids loadings in fed-batch operation. [(1)]**Data shown are from singular experimental runs.**

- ✓ Co-cultures' capacity to deconstruct solids was reduced at higher solids loaded. (Fig. 9).
- ✓ Increased sugars utilization and BA titers values were measured in fed-batch compared to batch operations (Figs. 10 and 11).

Conclusions

BA production did not differ with the inoculum size, but it was significantly increased at higher solids loadings in batch operation. Fed-batch approach resulted in approximately double the amount of BA observed in the most productive batch condition.

References: Chen, X., et al. (2016). "DMR (deacetylation and mechanical refining) processing of corn stover achieves high monomeric sugar concentrations (230 g L−1) during enzymatic hydrolysis and high ethanol concentrations (>10% v/v) during fermentation without hydrolysate purification or concentration." Energy & Environmental Science 9(4): 1237-1245.

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