Direct conversion of delignified biomass into non-volatile products using thermophilic bacteria

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Introduction

Butyric acid can be used as a platform intermediate for diesel and sustainable aviation fuels as well as a precursor for diverse commodity chemicals for the production of polymers, fibers, solvents, and preservatives. This work presents a co-culture-based bioprocess for the production of butyric acid from corn stover by two anaerobic thermophilic bacteria, *Clostridium thermocellum* (Ctc), a well-known efficient degrader of insoluble and oligomeric cellulosic substrates, and *Clostridium thermobutyricum* (Ctb), a highly efficient butyric acid producer from monomeric sugars.

Hypothesis

By co-cultivating Ctc and Ctb, their distinct metabolic properties can be harnessed to efficiently solubilize and convert corn stover substrates (Fig. 1) into various organic byproducts, including 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

Biomass substrate: corn stover

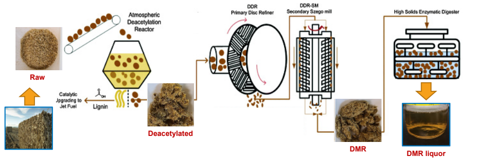
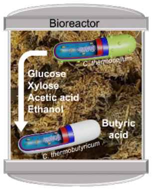


Fig. 1. Various corn stover substrates obtained from NREL pilot plant deacetylated and mechanically refined (DMR) pre-treatment process (modified from Chen et al. 2016).

Cultivation conditions

Fig. 2. Ctc will carry out the primary biomass deconstruction while Ctb will utilize solubilized sugars (including those not used by Ctc) and byproducts to produce butyric acid. Fermentations were carried out at 500 mL scale for 5 days in controlled bioreactors in duplicate. Cultures were maintained under anaerobic conditions at 55ºC, pH 7.0.



Results

Biomass conversion and metabolic performance of Ctc

- Goal: Assess various corn stover feedstocks for initial co-culture optimization.
- Factors: Three solid corn stover streams (at initial 30 g/L solids loading): raw, deacetylated, and DMR.
- Responses: Biomass deconstruction (Fig. 3) and product formation (Fig. 4 and Fig. 5).

Biomass deconstruction

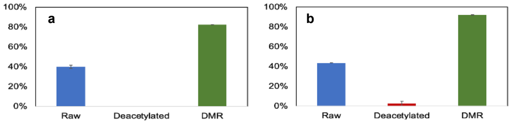


Fig. 3. Solids (a) and carbohydrates (b) conversion % by Ctc

Soluble sugar formation

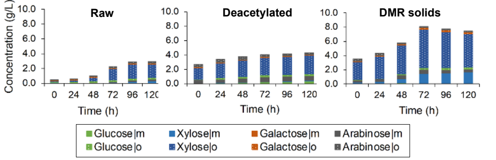


Fig. 4. Monomeric (m) and oligomeric (o) soluble sugar formation by Ctc on corn stover substrates

Product profiles

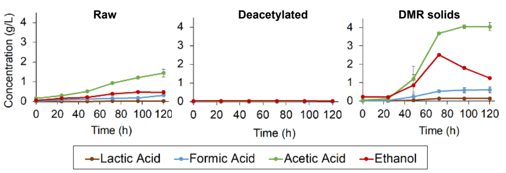


Fig. 5. Metabolic product formation by Ctc on corn stover substrates

✓ Corn stover DMR selected as substrate for co-culture fermentations.

Butyric acid production capabilities by Ctb

- Goal: Determine carbon utilization bottlenecks
- Factors: Three liquid carbon sources (at initial 30 g/L total sugars): Glucose, xylose, and DMR liquor
- Inoculation size for Ctb: Initial optical density (OD_{600}) of 0.01
- Responses: Carbon utilization and product formation (Fig. 6)

Sugar utilization and product profiles

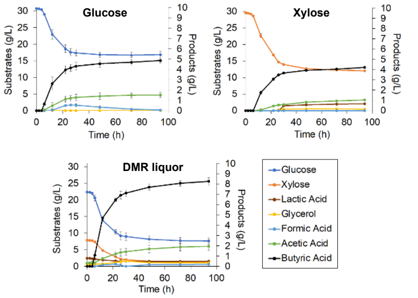


Fig. 6. Sugar utilization and metabolic product formation by Ctb on DMR liquor and model carbon sources.

✓ Sugar utilization: 13, 18, and 21 g/L of glucose, xylose, and sugars from DMR liquor, respectively

✓ Maximum butyric acid production of 4.9, 4.2, and 8.3 g/L in glucose, xylose, and DMR liquor, respectively

Co-cultivation on corn stover biomass

- Goal: Evaluation of co-culture synergies to perform a solid-to-acids bioprocess to produce butyric acid.
- Factors: Inoculation time of Ctb: 0, 36, and 72 h
- Inoculation size: Ctc: 10% v/v , Ctb: initial OD_{600} 0.01
- Responses: Biomass deconstruction (Fig. 7), carbon utilization (Fig. 8), and metabolic products formation (Fig. 9).

Biomass deconstruction

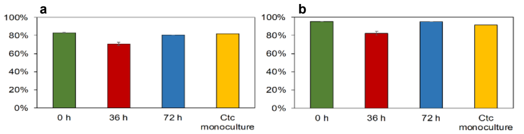


Fig. 7. Solids (a) and carbohydrates (b) conversion % by clostridia co-cultures

✓ Solids and carbohydrate conversions were higher in the 0-h treatment (83.1% and 95.6%, respectively), compared to the 36-h (70.8% and 82.4%) and 72-h (80.7% and 95.2%) treatments

Soluble sugar formation

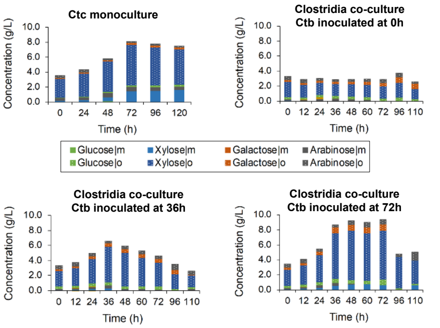


Fig. 8. Monomeric (m) and oligomeric (o) sugar formation by clostridia co-cultures on DMR corn stover

✓ Xylose species accumulating during Ctc fermentation disappear when Ctb is inoculated, indicating uptake and conversion.

✓ Lowest levels of carbohydrate accumulation when Ctb was inoculated at 0h.

Product profiles

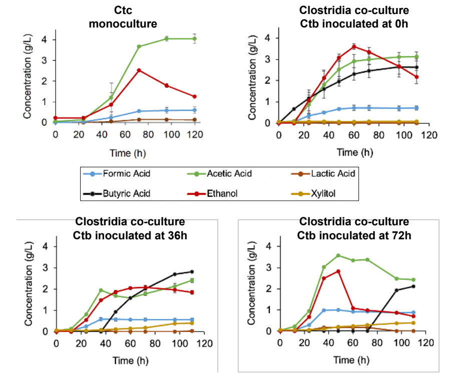


Fig. 9. Metabolic products formation by clostridia co-cultures in DMR corn stover

✓ Production of butyric acid is observed at the time of Ctb inoculation with final concentrations ranging from 2.3 – 2.8 g/L.

Conclusions

- Ctc monocultures can solubilize 82.3% and 91.7% of DMR solids and carbohydrates, respectively with acetic acid and ethanol as the primary byproducts.
- Co-cultivations of Ctc and Ctb on DMR corn stover resulted in increased carbohydrate conversion to 95.6% when compared to Ctc monocultures, with butyric acid formation occurring upon inoculation with Ctb.
- Hemicellulose fractions that accumulate and are unmetabolized during Ctc monocultures are successfully utilized by Ctb during co-cultivation.

Future work

- Test co-culture performance at increased initial solids concentrations
- Apply a fed-batch approach to solids addition for continuous fermentation
- Complete carbon mass balance closure on co-culture fermentation
- Develop methods for microbial biomass growth detection
- Test co-cultures on different biomass substrates

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