

Ethanol Production From *Clostridium thermocellum*

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Introduction

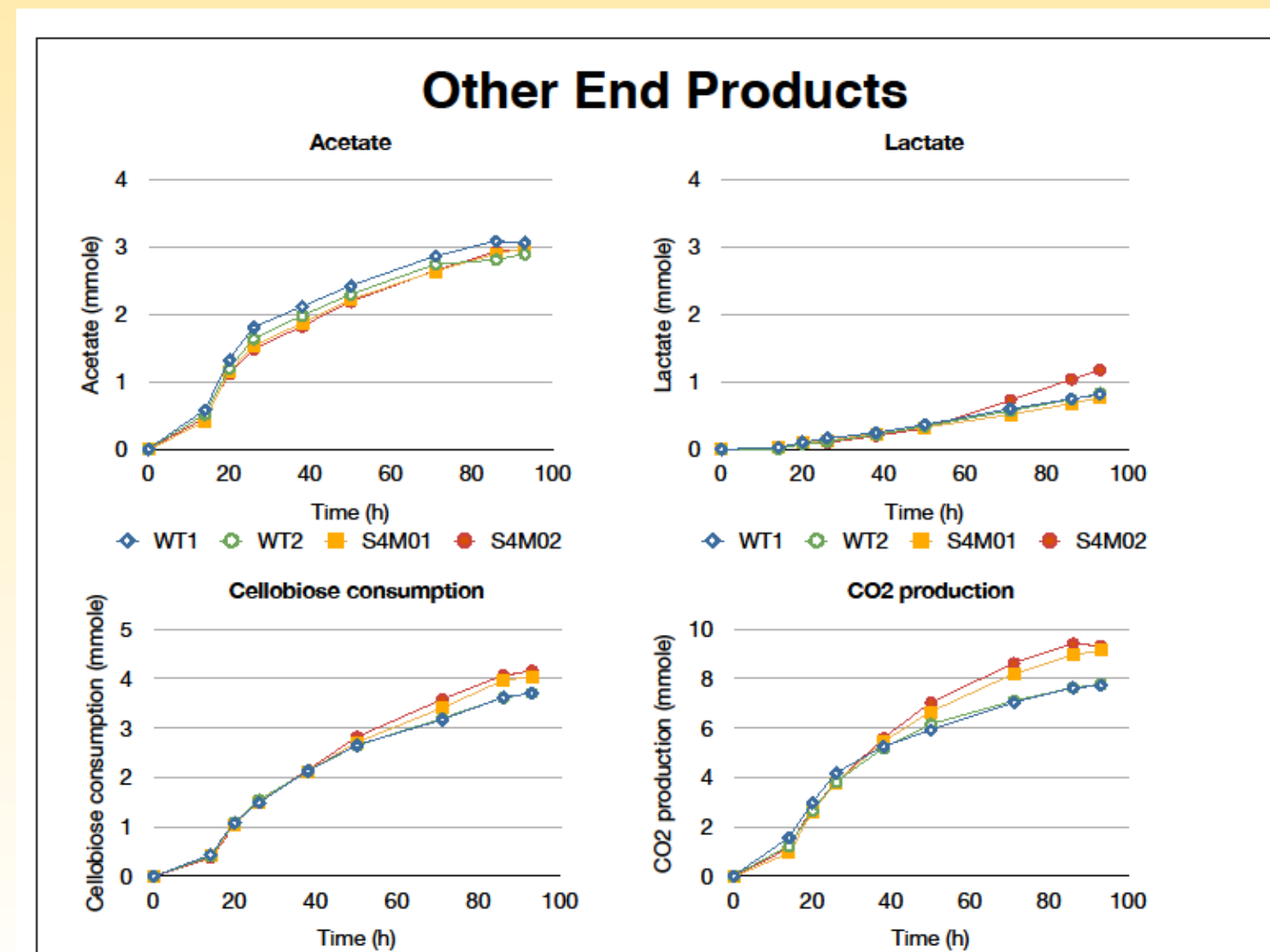
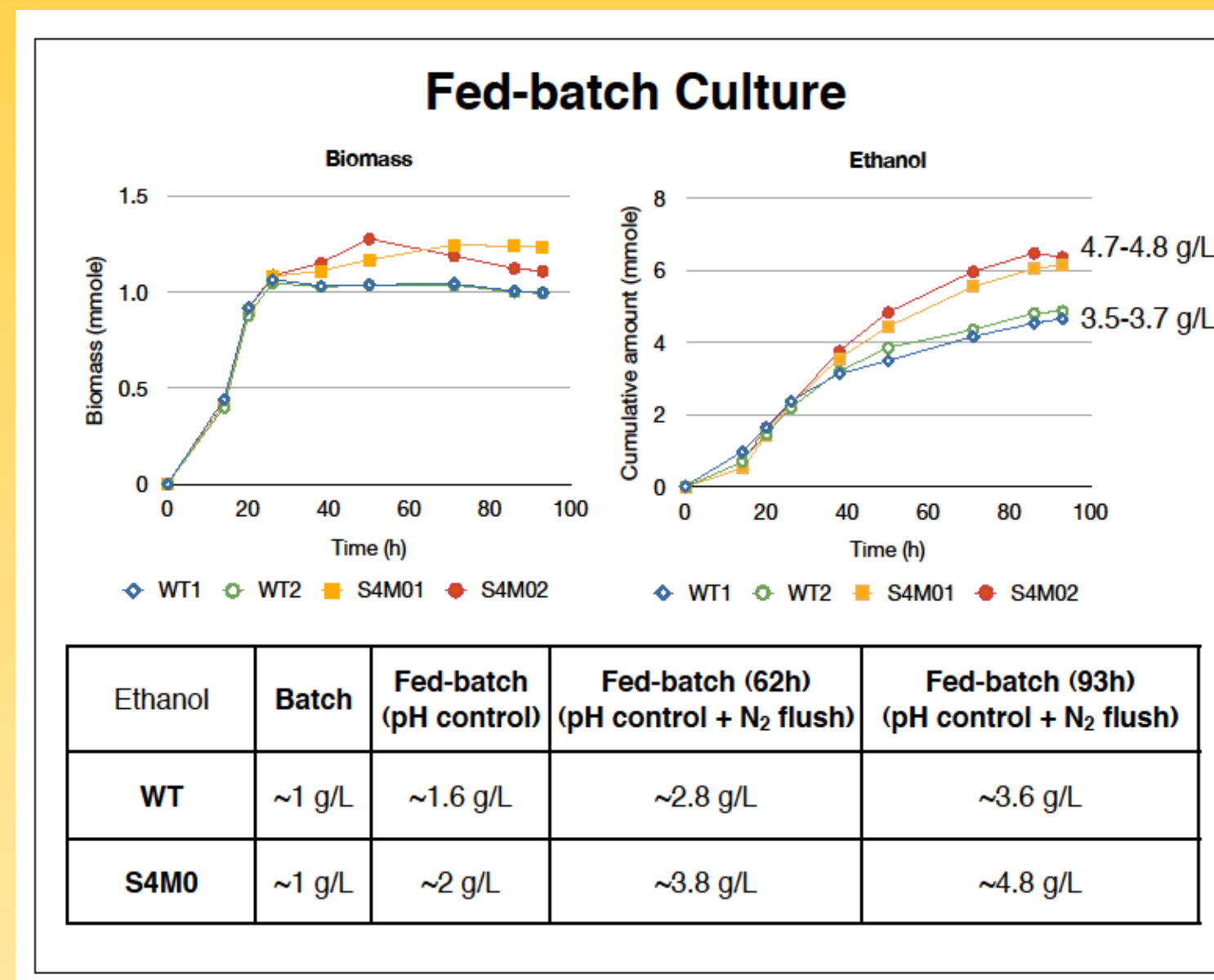
The goal of this research project was to increase the total ethanol yield of *Clostridium thermocellum*. *C. thermocellum* is an anaerobic, thermophilic, cellulolytic and ethanogenic bacterium that utilizes cellobiose as a substrate to convert to ethanol. The advantage of growing these cells under high temperatures (60 °C) is that contamination factors are lessened as well as facilitating ethanol production.

An ethanol tolerant strain (S4M0) was developed in the laboratory with a 4% ethanol tolerance and was compared to the wild type strain (WT) under fed-batch conditions. Fed batch culture involves injecting the cells with medium during the stationary growth phase to prolong cell growth and produce more ethanol. Under normal batch conditions, the WT and S4M0 cells both produced around 1 g per liter of ethanol.

Methods

The quantification of cell growth was obtained by measuring the optical density using a spectrophotometer at 600 nm. Ethanol, acetate, and lactate produced by the cells were quantified by following the procedures of an enzymatic kit.

The medium (GS-2) that was prepared for the fed batch culture contained necessary ingredients to prolong cell growth such as urea, cellobiose, MOPS, vitamins, and FeSO₄. The pH of the medium was adjusted to 6.8 and then purged with nitrogen.



Results

- Ethanol tolerant strain (S4M0) produced more ethanol than the wild type (WT).
- S4M0 had 20% more biomass than WT, indicating greater cell growth.
- S4M0 also had 35% more ethanol production and 9% more cellobiose consumption.
- Both the S4M0 and WT produced similar amounts of acetate and lactate with S4M0 having a greater ethanol to acetate ratio.
- Ethanol produced by both cells increased when using pH controls and nitrogen purging.

Conclusions

The results obtained indicate that the ethanol tolerant strain produced more ethanol than the wild type as well as having increased cell growth and biomass.

The data collected in this experiment could be used for future DNA/RNA analysis to determine key genes and gene sets that affect the ethanol production of the *C. thermocellum* cells. By targeting certain genes within the cell, it is possible to further increase the ethanol produced.

References

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- Demain, Arnold L., et al. Cellulase, Clostridia, and Ethanol. *Microbiol. Mol. Biol. Rev.* 2005 (69): 124-54.

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