

# Validating High Throughput Kinetics Methods for Diverse Chain Elongating Microbes

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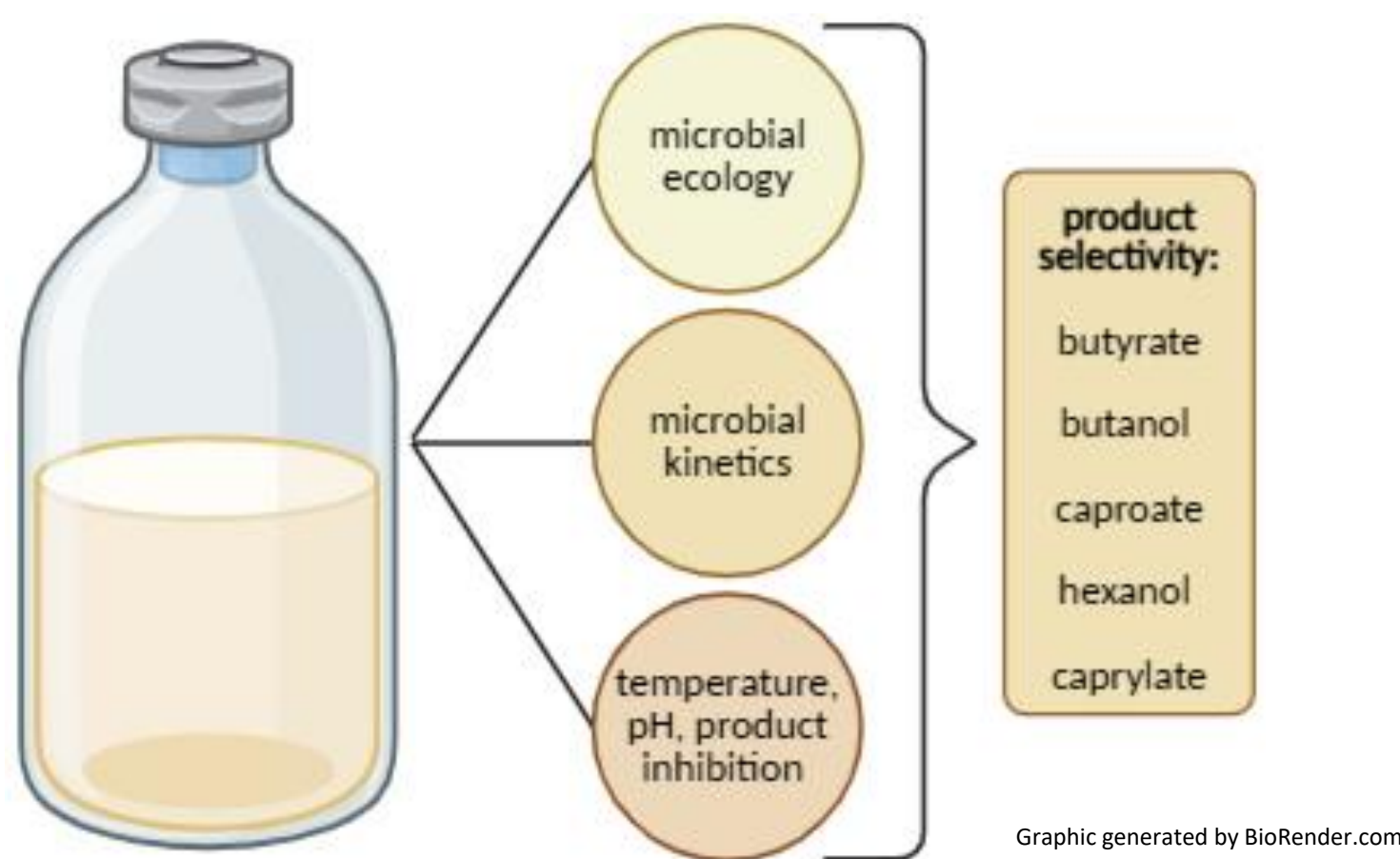
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## Introduction

- Microbial chain elongation may be a powerful tool towards a circular economy, as it can upgrade organic-rich wastes into commodity chemicals
- While chain elongation products are desirable to produce, they are toxic to cells. Understanding the mechanism of inhibition and differential impacts on microbial community members is key to optimizing chain elongation
- The impacts of product inhibition on pure vs. mixed cultures may vary based on temperature, inhibitor concentration, and pH
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## Research Questions

- How do different pure and mixed chain elongating cultures tolerate product inhibition?
- What is the impact of temperature on product inhibition and product selectivity?
- How does product inhibition impact the productivity and microbial community of mixed chain elongating cultures?

## Methods

### Culturing Pure and Mixed Chain Elongators

- Cultures were grown in reduced anaerobic media in glass serum bottles
- Cultures of interest include ethanol-elongating *Clostridium kluyveri* (pure) and Bozeman (mixed) as well as lactate-elongating *M. elsdenii* (pure) and Hudson (mixed)
- The mixed lactate-elongating culture, Hudson, was enriched from soil and monitored

### Assessing Product Inhibition

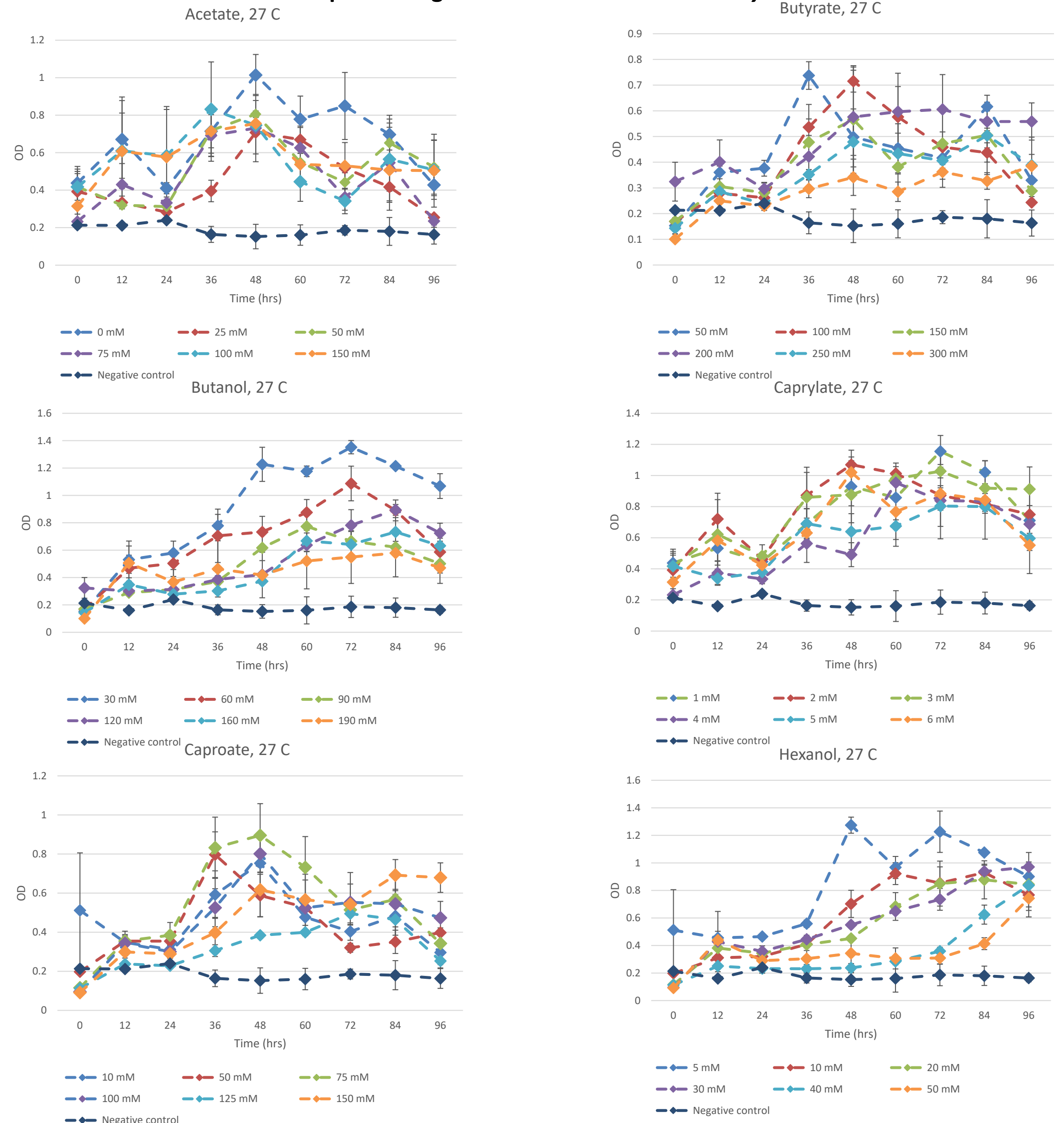
- Differential concentrations of inhibitory products were tested in 96-well plates; growth was monitored via optical density measurements
- Butyrate, caproate, caprylate, butanol, and hexanol tested as product inhibitors

### Analytical Methods

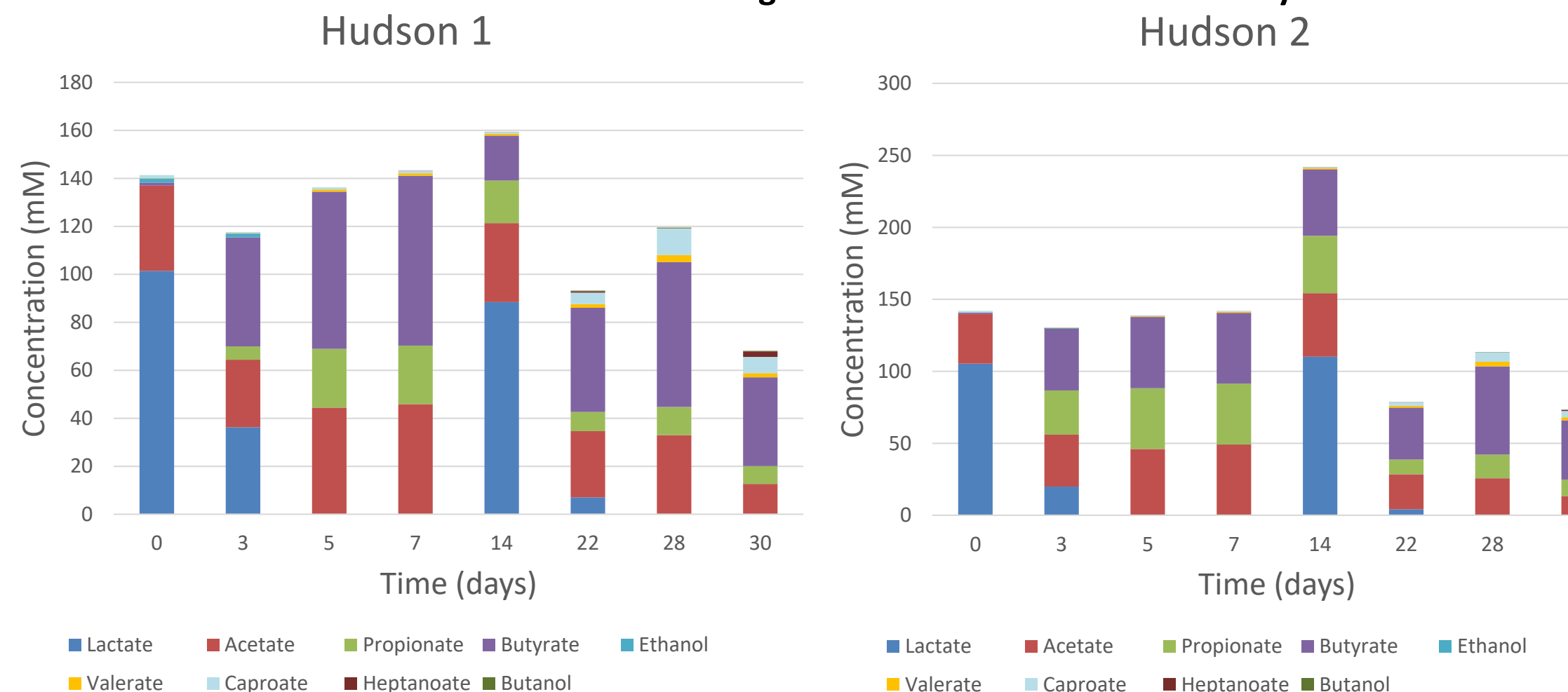
- Gas composition was analyzed on a gas chromatograph (GC) equipped with a total conductivity detector
- Liquid composition was analyzed via high-performance liquid chromatography (HPLC)

## Results

### Product Inhibition impacts the growth rate of *Clostridium kluyveri* as measured with OD



### Hudson enrichment cultures elongate lactate to medium-chain fatty acids



## Conclusions and Next Steps

### Conclusions:

- The pure culture *C. kluyveri* showed great resilience against several product inhibitors even at high concentrations, including caproate, butanol, and hexanol
- Overall, dose-dependent product inhibition was observed
- Inhibition increased dramatically with an increase in temperature: growth was completely inhibited at 37 degrees Celsius
- Successful chain elongation from enriched soil culture, Hudson, was achieved
- Hudson was producing butyrate as early as day 3, and caproate production increased dramatically after a transfer

### Next Steps:

- Testing pure lactate elongating cultures
- Testing mixed lactate- and ethanol-elongating cultures
- Repeating kinetic experiments to validate results

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