

Simulating chain elongation with constraint-based metabolic modelling

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HIGHLIGHTS:

- We reconstructed the metabolism of six functional guilds involved in chain elongation: sugar-elongating organisms (SEOs), sugar fermenting organisms (SFOs), hydrogenic sugar fermenters (HSFs), lactate-elongating organisms (LEOs), ethanol-elongating organisms (EEOs), and homoacetogenic organisms (HAOs).
- We simulated a well-studied chain elongating bioreactor to test the predictive power of the model and to diagnose bottlenecks to medium-chain fatty acid (MCFA) production.
- Simulation results suggest that MCFAs are produced by a single guild in the bioreactor by converting sugars to MCFAs.
- Lactate is predicted to be produced as an intermediate but only used to produce butyrate rather than MCFAs.

BACKGROUND: Chain elongation has been proposed as a process to recover valuable products from complex organic waste streams.¹ Of particular interest is the production of medium-chain fatty acids (MCFAs) containing 6 to 12 carbon atoms. The targeted production of MCFAs over short-chain products remains a challenge. While strategies to increase MCFA specificity have been explored, including modifying ratios of electron donor to electron acceptor and real-time extraction of products,^{2, 3} we used metabolic models to investigate potential physiological drivers for MCFA production. From simulation results, we hypothesize several strategies to increase MCFA production.

RESULTS & DISCUSSION: Two metabolic models were constructed, expanding the range of substrates, products, and biochemical pathways of existing mixed-culture fermentation models.⁴⁻⁶ First, a single cell metabolic model (iFermCell215) representing the combined metabolic capabilities of all functional guilds within a single unit was used to investigate substrates that may favour MCFA production. iFermCell215 allows for the free exchange of metabolic intermediates and cofactors between pathways and represents an organism that can perform the metabolic activities of all the functional guilds. It can be used to identify sets of reactions that may favor MCFA production. Modelling results suggested that ethanol, but not lactate, increased MCFA production. Modelling results also suggest that an ideal ratio of acetate to ethanol is 0.56 mol acetate per mol ethanol which aligns

with recent observations by others.² Second, a guild-based metabolic model (iFermGuilds789) was used to simulate the behaviour of a well-studied bioreactor that contained four functional guilds.^{7, 8} The guilds were constrained according to their relative abundance in the bioreactor. When constraining this model to produce the products observed in the bioreactor, it was predicted that all of the MCFAs were produced by sugar-elongating organisms. While lactate-elongating organisms were present in the reactor, they were predicted to convert lactate into butyrate as a sole product. Third, we used the models to explore specific metabolic features and their impacts on MCFA production. We found that while energy conserving mechanisms can improve growth, they can be detrimental to MCFA production. For instance, the models predicted that energy conserving hydrogenases (ECH, HydABC) decreased MCFA production compared to the use of non-energy conserving hydrogenase. In these cases, the presence of alternative energy-conserving mechanisms obviate the need to conserve energy via reverse β -oxidation coupled to proton translocation with the RNF complex. These results demonstrate that metabolic features outside of reverse β -oxidation impact the primary fermentation products.

CONCLUSION: This study demonstrates the value of metabolic modelling to augment other omics techniques to assess chain elongation bioreactors. Modelling results suggest that in carbohydrate-based platforms, conversion of sugars to lactate may be undesirable. Instead, organisms that directly convert sugars to MCFAs may be desirable. Further, the model results highlight differences between ethanol and lactate as intermediates. While ethanol is predicted to increase MCFA production, lactate may not. In addition to the modelling results, we also propose a guild-based framework for analysing and assessing chain elongation bioreactors and the models described in this work are available to the chain elongation research community (<https://github.com/mscarbor/Mixed-Culture-Fermentation-Models>).

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