Mildly acidic pH selects for chain elongation over propionic acid production in lactic acid fermentation

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HIGHLIGHTS:

- Lactic acid-fed microbial communities were exposed to varying pH
- pH below 6 resulted in caproic acid production by Caproiciproducens
- pH above 6 led to production of propionic acid by Aminobacterium and Veilonella

BACKGROUND: Lactic acid-mediated chain elongation technologies offer a highly promising route for production of medium-chain carboxylic acids (MCCA; e.g. caproic, caprylic acid). Carbohydrates can be relatively easily converted to lactic acid by – among others – Lactobacillus and Olsenella. In a second metabolic step (physically separated, or joined in one reactor stage), this lactic acid can then be elongated to caproic acid. This approach has been demonstrated repeatedly in literature1-4, and is currently part of at least one pilot-scale approach5. However, nearly all reports show the persistent presence of odd-chain products, i.e. propionic acid (C3), valeric acid (C5) and heptanoic acid (C7), in the obtained product profile. Propionic acid bacteria (such as Propionibacterium) can convert lactic acid to a mixture of acetic and propionic acid6, lowering product selectivity. So far, no study has explicitly investigated which parameters control the competition between these two functional guilds. Here, we present a set of long-term bioreactor experiments, along with short-term pH-controlled batch incubations, investigating the role of pH in steering this competition. Based on pH preferences of known propionic acid producers6 and chain elongators7, we hypothesized that chain elongators prefer low pH, whereas propionic acid producers prefer high pH.

RESULTS & DISCUSSION: Two bioreactor communities were fed with a synthetic lactic acid medium. Initial enrichment pH were pH 5.5 (R1) and pH 5 (R2). Conversion of lactic acid was low at pH 5.5 (38.7± 18.4%), whereas pH 5.5 showed nearly complete conversion, with only transient lactic acid accumulation (Figure 1A). To test our hypothesis, pH in R2 was increased with 0.5 pH unit increments, allowing stabilisation with each
increment. Product profiles at pH 5.5 (Phase II) in R2 were similar to those in R1. Further increasing pH to 6 (Phase III) did not affect caproic acid concentrations but did result in increasing propionic acid concentrations. After some operational issues (Phase IV), the reactor stabilized at pH 6.5 (Phase V), leading to a product profile made up nearly completely by acetic and propionic. To confirm this observation, pH was then decreased in the same way, in 0.5 pH unit increments. While pH 6 (Phase VI) showed a butyric acid-dominated product profile, Phases VII (pH 5.5) and VIII (pH 5) showed caproic acid dominated profiles.

Community characterization enabled us to further characterize these interactions (Figure 1B). The initial community in R2 was initially made up mostly of Caproiciproducens. As pH increased, propionic acid producers (Veilonella, Aminobacterium) overtook the community, mirroring the observed shifts in product profile. These communities subsequently lost terrain to Caproiciproducens as pH lowered again. Based on these observations, we conclude that pH is a key factor driving the interaction between chain elongating bacteria and propionic acid bacteria. We failed to completely eradicate propionic acid producers from the community and further research should investigate pH-based approaches to properly control this undesirable guild.

Figure 1. Product profile and community composition as a function of pH in R1 (varying pH) and R2 (constant pH 5.5).

**CONCLUSION:** We demonstrate here that pH is a key selecting factor during lactic acid-fed fermentations, where low pH select for chain elongating communities, whereas high pH favour propionic acid producers. This study provides a mechanistic understanding of this competitive interaction, which could enable better control of undesirable lactic acid consumption during future technology development.
REFERENCES


