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

Candry, Pieter[*,a,b,c]. Radić, Ljubomir[a,b]. Favere, Jorien[a,b]. Carvajal-Arroyo Jose Maria[a,b]. Rabaey, Korneel[a,b]. Ganigué, Ramon[a,b].

* presenter, pcandry@uw.edu
[a] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium, [b] Center for Advanced Process Technology for Urban Resource Recovery (CAPTURE), Coupure Links 653, 9000 Ghent, Belgium, [c] Civil and Environmental Engineering, University of Washington, 201 More Hall, Box 352700, Seattle, WA 98195-2700, USA

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 literature[1–4], and is currently part of at least one pilot-scale approach[5]. 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 acid[6], 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 producers[6] and chain elongators[7], 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.

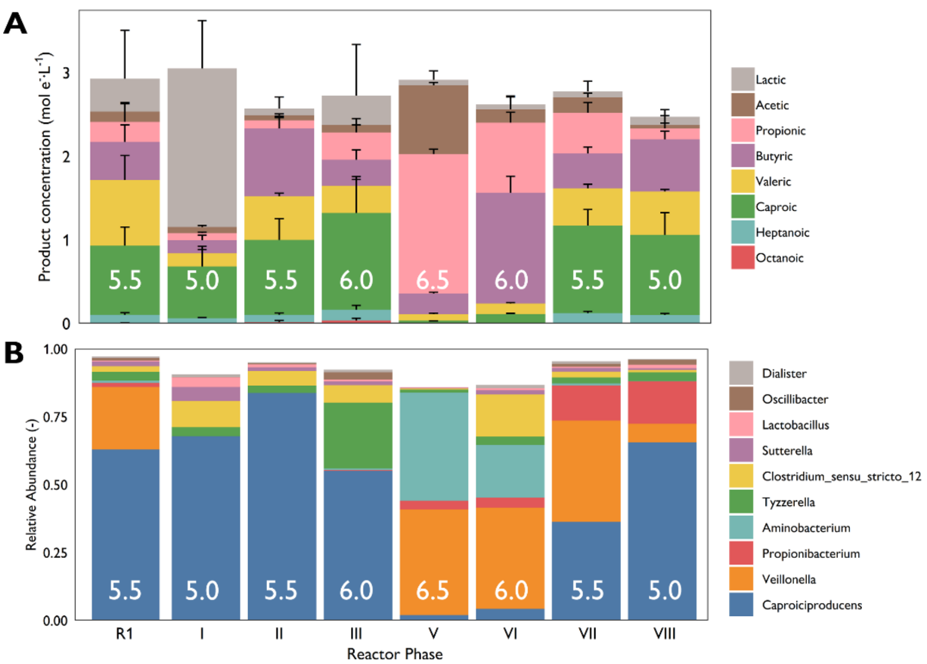

Figure 1. Product profile and community composition as a function of pH in R1 (varying pH) and R2 (constant pH 5.5).

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

1. Zhu, X. *et al.* The synthesis of n-caproate from lactate: a new efficient process for medium-chain carboxylates production. *Sci. Rep.* **5**, (2015).

2. Xu, J. *et al.* Temperature-Phased Conversion of Acid Whey Waste Into Medium-Chain Carboxylic Acids via Lactic Acid: No External e-Donor. *Joule* **2**, 280–295 (2018).

3. Khor, W. C., Andersen, S., Vervaeren, H. & Rabaey, K. Electricity-assisted production of caproic acid from grass. *Biotechnol. Biofuels* **10**, 1–11 (2017).

4. Andersen, S. J. *et al.* A Clostridium Group IV Species Dominates and Suppresses a Mixed Culture Fermentation by Tolerance to Medium Chain Fatty Acids Products. *Front. Bioeng. Biotechnol.* **5**, 1–10 (2017).

5. Capro-X. Capro-X: The Sustainable Way for Whey. (2020). Available at: http://juanandonly.com/capro-x/. (Accessed: 10th July 2020)

6. Lewis, V. P. & Yang, S. Propionic acid fermentation by Propionibacterium acidipropionici: effect of growth substrate. *Appl. Biochem. Biotechnol.* **37**, 437–442 (1992).

7. Zhu, X. *et al.* Production of high-concentration n-caproic acid from lactate through fermentation using a newly isolated Ruminococcaceae bacterium CPB6. *Biotechnol. Biofuels* **10**, 1–12 (2017).