6x scale-up while maintaining stable production of *n*-caprylic acid

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HIGHLIGHTS:

- *n*-Caprylic acid has certain advantages compared to *n*-caproic acid, including a reduced odour and a higher bactericidal activity.
- Stable production of *n*-caprylic acid was possible with a 4.2-L wet volume bioreactor that included pertraction, which is a 6x scale-up from our previous work at Cornell University (1).
- Stable production occurred at an ethanol–to–acetate substrate ratio of 6:1.

BACKGROUND:

To increase the product portfolio of microbial chain elongation, we are developing a stable bioprocess system with membrane-based liquid-liquid extraction (pertraction) to include *n*-caprylic acid (C8; *n*-octanoic acid) in addition to other medium-chain carboxylic acids (MCCAs) such as n-caproic acid (C6; *n*-hexanoic acid) and *n*-heptanoic acid (C7). Certain advantages of C8 compared to C6 exist. This includes a higher bactericidal activity, a higher heat capacity (297.9 J/K mol), a 10x lower maximum solubility concentration, and a less unpleasant odour. At Cornell University, we had already achieved a C8–to–C6 productivity ratio of more than 20:1 by feeding a mixture of ethanol and acetate into an anaerobic filter (AF) as an open-culture system (1). However, this had been accomplished with a relatively small wet volume of 0.7 L. Here, we scaled up the process 6x to a 4.2-L wet volume AF. We investigated whether we could repeat this result from Cornell University with a stable production of a considerably higher amount of C8 than C6 when a mixture of ethanol and acetate was fed as substrate. Such substrate mixture is present in the effluent of syngas fermentation systems.

RESULTS & DISCUSSION:

The C8-producing AF was filled with K1-filter media (Kaldnes) in a 5-L glass upflow bioreactor with an active volume of 4.2 in the presence of the filter media. The fermentation broth was recirculated continuously through a forward membrane contactor together with a solvent as part of

the pertraction system. Continuous extraction of MCCAs was achieved by recirculating this solvent and an alkaline stripping solution through a backward membrane contactor. We verified the molecular structure of the produced C8 through GC/MS. In addition, we monitored the produced metabolites by GC/FID. The C8 production performance was evaluated within an experimental design by changing the substrate ratio of ethanol to acetate. When the substrate ratio of ethanol and acetate was 6:1, the C8–to–C6 productivity ratio was higher than 2.5:1. This can be seen by the ~2.5x steeper slope of the increase in the concentration of C8 compared to C6 in the alkaline stripping solution (**Figure 1A**), and also by the productivity ratio bar (**Figure 1B**). However, when the substrate ratio of ethanol to acetate was increased to 10:1, the overall C8–to–C6 productivity ratio decreased to ~1.5:1 due to lower production of C8, while the production of C6 remained constant (**Figure 1B**). By reversing the substrate ratio back to 6:1, we again achieved a stable production of C8, which was ~2.5x higher than C6 at a 6:1 substrate ratio of ethanol and acetate. Nevertheless, we have not been able to achieve the C8–to–C6 productivity ratio of 20:1, which we observed at Cornell University, and we are now trying to understand why.

CONCLUSION:

The bioreactor system for this study accumulated C8 up to 8000 mM C in the stripping solution (**Figure 1A**). We achieved a stable production of C8 at a 6:1 substrate ratio of ethanol to acetate.

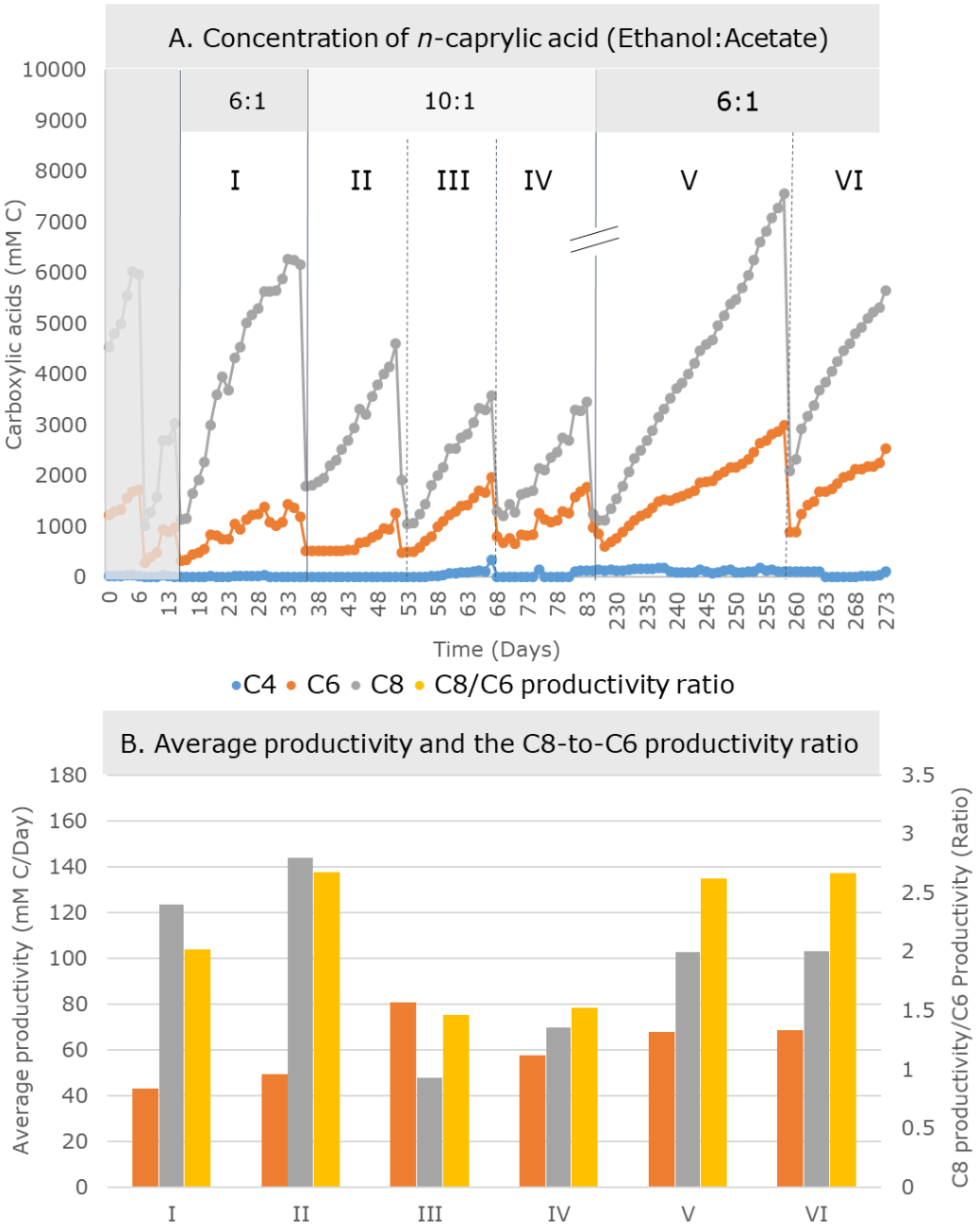

Figure 1: A. Concentration of *n*-caprylic acid in the alkaline stripping solution during the operating period (after each period the stripping solution was exchanged); **B.** Average productivity and the C8–to–C6 productivity ratio.

REFERENCES

1. Leo A. Kucek, Catherine M. Spirito and Largus T. Angenent. High n-caprylate productivities and specificities from dilute ethanol and acetate: chain elongation with microbiomes to upgrade products from syngas fermentation. Energy & Environmental Science, 2016,9, 3482-3494