Impact of substrate concentration on granular fermentation for caproic acid production

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HIGHLIGHTS:

- Lower substrate concentrations do not affect caproic acid selectivity.
- Higher substrate concentrations give rise to toxic caproic acid concentrations, which shifts production towards butyric acid.
- Lowering substrate concentrations induces biomass aggregation but the mechanism remains unclear.

BACKGROUND: Todays increasing organic waste production poses a challenge for the conversion of liquid waste streams into added-value products beyond non-profitable methane and ethanol-based medium chain carboxylic acids (MCCA). Over the past decade, lactic acid chain elongation to produce caproic acid (C6) has gathered an increasing amount of attention due to its potential in coupling lactic acid production from carbohydrates to chain elongation, thereby eliminating the need for exogeneous addition of electron donors[1,2]. To date these systems, however, still struggle with rate limitations, a key barrier that had been identified early on in the new wave of MCCA-research of the 2010s[3]. Carvajal-Arroyo et al. (2019) recently demonstrated the possibility of C6 production via chain elongation using granular reactor technology fed with thin stillage, a biorefinery side-stream containing approximately 20 $g \cdot L^{-1}$ of carbohydrates, without external addition of electron donors[4]. By retaining high biomass concentrations as granular biofilm, that study enabled an order of magnitude increase in C6-production rates, up to 13.7 $gC6 \cdot L^{-1} \cdot d^{-1}$, compared to a suspended biomass system on a similar stream[5]. Understanding the effects of the feedstock composition, concentration and load on the chain elongation and biomass aggregation in this novel system form the crucial next steps towards feedstock diversification. The aim of this study was to shed light on the effect of substrate concentration, with solids-free thin stillage as feedstock, on granular fermentation in expanded granular sludge bed (EGSB) reactors.

RESULTS & DISCUSSION: In a first part, diluting the thin stillage (44.15 ± 2.64 $gCOD \cdot L^{-1}$) to respectively 75%, 50% and 25% of the original COD content resulted in a decrease in C6 concentration (from 4.35 ± 0.25 $g \cdot L^{-1}$ at 100% to 1.59 ± 0.27 $g \cdot L^{-1}$ at 25%) in the effluent while the selectivity with which C6 was produced, remained constant at 48 ± 3% (COD of C6

relative to all produced carboxylic acids) (Figure 1). Additionally, lower substrate concentrations resulted in higher substrate conversion efficiencies (Figure 1). Furthermore, the highest amounts of total biomass (sum of planktonic and granular) were consistently found at the lowest substrate concentrations and substantial growth of the granular bed was observed at lower substrate concentrations. This suggests that substrate limitation and/or concomitant low C6 concentrations may have a positive impact on biomass aggregation, but the mechanism remains unclear.

In a second part, amending the solids-free thin stillage with D-glucose to achieve 110%, 125% and 200% of the original COD content did not result in a proportional increase in C6 concentration (Figure 1). Instead, butyric acid started accumulating up to similar concentrations (4.03 ± 0.48 $g \cdot L^{-1}$ at 125% compared to 1.78 ± 0.20 $g \cdot L^{-1}$ at 100%) as C6 (4.35 ± 0.49 $g \cdot L^{-1}$). We hypothesized that this was due to product toxicity exerted by C6. This hypothesis was supported by batch experiments, which yielded similar results when feeding three times the carbohydrate concentration. Additionally, lower substrate conversion rates were obtained at higher initial C6 concentrations. In terms of granular biomass behaviour, higher substrate concentrations resulted in visually larger granules.

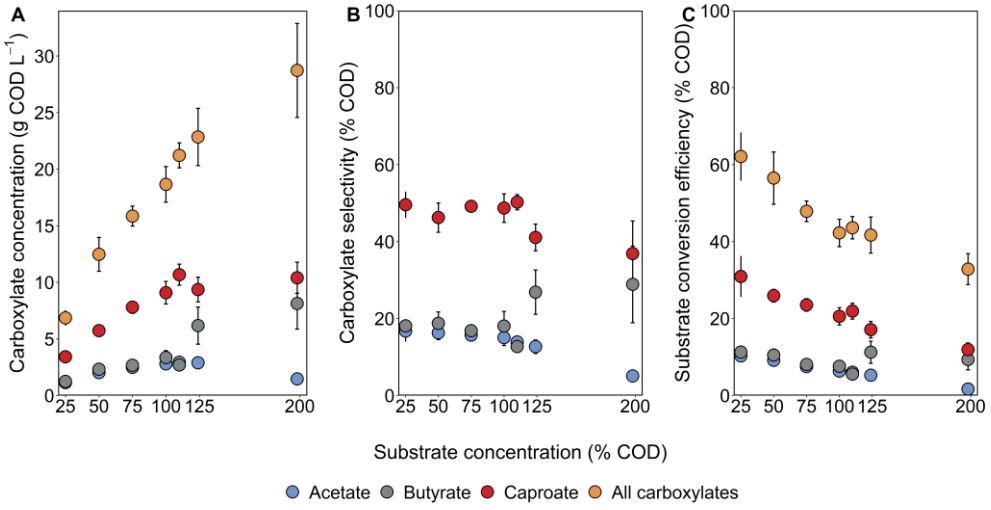

Figure 1. Carboxylic acid concentration (A), selectivity (B) and substrate conversion efficiency (C) in function of the substrate concentration.

CONCLUSION:

The constant selectivity and increased substrate conversion efficiencies at low substrate concentrations demonstrate that high COD concentrations are no prerequisite for caproic acid production in an EGSB. However, the low C6 concentrations may pose challenges for efficient product extraction. At higher substrate concentrations, selectivity for caproic acid decreases due to product toxicity, implicating in-situ product extraction is necessary to maintain high selectivities. Overall, this study demonstrates that chain elongation in expanded granular sludge beds offers untapped potential and could be expanded towards more dilute and concentrated waste streams.

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