Optimum caproic acid production initiated from homogenised and acidified granular sludge is influenced by lactic:butyric acid ratio and concentration

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HIGHLIGHTS:

- The concentration of caproic acid significantly improved using lactic acid as ED and butyric acid supplementation.
- When optimum substrate concentration and ratio ($r_{Lac/But}$) are used, the initial acetic acid to butyric acid elongation cycle, reported in the literature could be bypassed, resulting in efficient caproic acid synthesis.
- Period of high caproic acid production dominated by species belonging to the *Ruminococcaceae* family.

BACKGROUND: Engineered biotechnological conversion of lactic acid to caproic acid is still in the early stages of development. A key feature of an efficient process management will be the development of a microbial community capable of efficient, stable and high-yielding conversions. In this study, the impact on inoculum development of physical and/or chemical pre-treatments of anaerobic granular sludge was evaluated. Additionally, the effect of the lactic acid and butyric acid concentration and ratio ($r_{Lac/But}$) on caproic acid yield was investigated

RESULTS & DISCUSSION: Granular sludge, homogenised in a food blender for 30 seconds before being acidified to either pH 3 or 5.5 yielded higher caproic acid concentrations than crushed or intact granules. Indeed, the pH 3, acidified and blended sludge yielded the highest caproic acid concentration. Moreover, substrate concentrations of 250 mM ($r_{Lac/But}$ = 1.5:1) and 300 mM ($r_{Lac/But}$ = 1:1) were optimal to efficiently produce

caproic acid using the pH 3, acidified and blended sludge. However, when using an enriched culture, which has been cultivated on a lactic acid/butyric acid-containing medium, the highest yields of caproic acid were achieved at a reduced substrate concentration of 200 mM ($r_{Lac/But}$ =1:1). With both sludge and enriched cultures, the lactic acid to butyric acid consumption (C rlac/but) under optimum conditions was 2:1. We report for the first time a lactic acid to butyric acid threshold concentration, below and above which the selectivity toward caproic acid is reduced. The highest caproic acid selectivity (≥ 90%) was achieved with substrate concentrations of 250 mM ($r_{Lac/But}$ = 1.5:1) and 300 mM ($r_{Lac/But}$ = 1:1) using the pH 3, acidified and blended sludge as inoculum. Caproate production was completely inhibited when the substrate concentration was increased to 400 mM ($r_{Lac/But}$ = 1:1). With these optimum substrate concentrations and ratios, the feasibility of a simplified pathway for efficient caproic acid production using lactic acid as electron donor and butyric acid as electron acceptor was demonstrated. The initial acetic acid to butyric acid elongation cycle, reported in the literature is bypassed when optimum substrate concentration and lactic acid to butyric acid ratio are used. Finally, species affiliated with *Ruminococcaceae* were likely involved in the synthesis of caproic acid.

CONCLUSION: The findings of this study have strong application potential, specifically in the design of a process that will allow for the continuous and sustainable production of caproic acid.