Revamping the model CO-fermenting acetogen, *Clostridium autoethanogenum,* as a CO_2-valorisation platform

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HIGHLIGHTS:

- Phenotypic quantification of *C. autoethanogenum* CO_2 and H_2 chemostats, facilitated a modelling-based hypothesis for supplementation of the fermentation with CO.
- CO-supplemented CO_2 and H_2 chemostats indeed showed substantial performance improvements, including an increase in CO_2 uptake.
- *C. autoethanogenum* can be revamped from a chassis organism for CO clean-up, to a renewable CO_2-valorization platform additionally.

BACKGROUND: Acetogenic bacteria can convert waste gases into fuels and chemicals. There is potential to build on the success of commercial gas fermentation towards bacterial artificial-photosynthesis, wherein renewable substrates could serve as facilitators of CO_2-valorization (Claassens et al., 2016; Haas et al., 2018; Redl et al., 2017; Tizard and Sechrist, 2015). Steady state quantification of carbon flows greatly enhances cyclical-development of CO_2-utilizing bioprocesses.

RESULTS & DISCUSSION: CO_2 and H_2 chemostats had limitations, namely growth rate and stability (**Figure 1 A**). However, the fermentation revealed that captured carbon (460 ± 80 mmol/gDCW/day) was significantly distributed to ethanol (54 ± 3 C-mol% with a 2.4 ± 0.3 g/L titer; **Figure 1 B-D**). Quantification of the fermentation enabled flux balance analysis and comparison to previous datasets (Valgepea et al., 2018). This indicated CO-supplementation may lessen a potential constraint resulting from limited reduced ferredoxin at the pyruvate:ferredoxin oxidoreductase. Supplementation with a small amount of CO enabled co-utilisation with CO_2, and enhanced CO_2 fermentation performance significantly (9.7 ± 0.4 g/L ethanol with a 66 ± 2 C-mol% distribution, and 540 ± 20 mmol CO_2/gDCW/day; **Figure 1**).

CONCLUSION: We established a dataset quantifying steady-state of the model acetogen *C. autoethanogenum* during autotrophic-CO_2/H_2 growth in chemostat cultures. This enabled analysis *via* FBA, and highlighted CO as a potential supplement. CO supplementation successfully improved metabolic stability and CO_2 utilization. This was the first time that intracellular fluxes for net uptake of CO_2 (with enhancement) where characterized. Industry is actively developing gas fermentation to valorize CO_2 (Haas et al., 2018 & Tizard and Sechrist, 2015). Previously, genetic and process engineering of gas fermentation successfully developed the technology for industrial CO valorization (Liew et al., 2016). Therefore, progression to industrial CO_2 valorization is foreseeable, and CO supplementation may play a role in the continuing diversification of industrial gas fermentation.

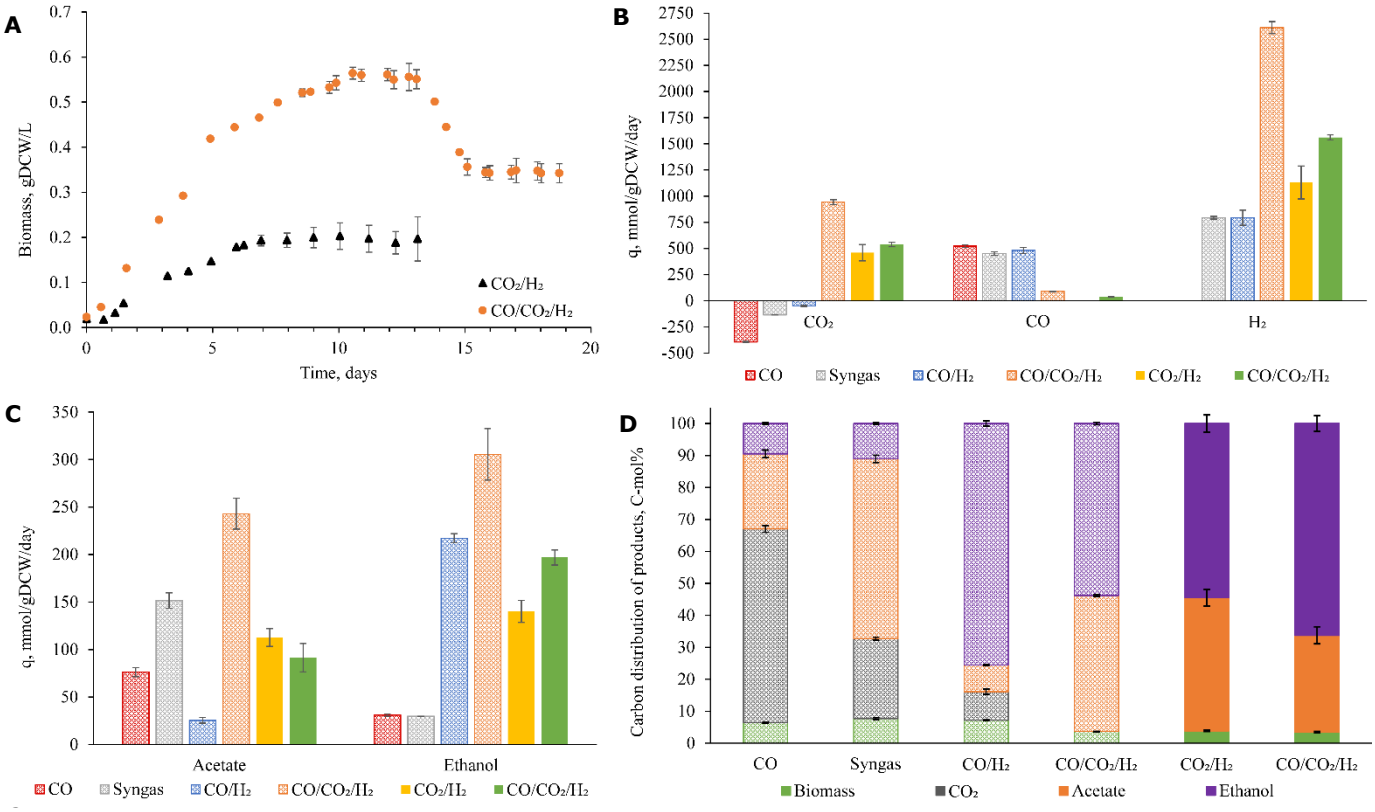

Figure 1. Important fermentation characteristics of *Clostridium autoethanogenum* in autotrophic chemostats. Growth curves of novel fermentations with standard deviation at steady-state (**A**) – following thirteen days of fermentation $CO/CO_2/H_2$ chemostats where switched to a dilution rate (D) of 1 day^{-1}. Specific rates of uptake (**B**) and production (**C**) for important metabolites. Product carbon balances (**D**). Results from Valgepea et al. (2018) are also displayed (**B**, **C** & **D**). Values represent the average ± standard deviation between biological replicates. Number of biological replicates, and detailed gas composition for each fermentation are available in Table 1. Patterned bars indicate a D of 1 day^{-1}, full bars indicate a D of 0.5 day^{-1} (**B**, **C** & **D**). Abbreviations: *q* –specific rate, *DCW* – dry cell weight.

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