Understanding oscillations in gas fermentation

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HIGHLIGHTS:

- Metabolism in a biological system is ultimately determined by the organisms' ability to self-organise metabolism for maintaining cellular homeostasis.
- Quantification of the limits of metabolic robustness is essential for understanding cellular behaviour
- This work can contribute towards advancing the understanding of an industrially relevant cell factory used in gas fermentation for sustainable production of fuels and chemicals from waste feedstocks.

BACKGROUND: Gas fermentation can play a crucial role in the circular economy. However, oscillations have been observed in gas fermentation. Oscillatory behaviour provides a unique opportunity for quantitating the robustness of metabolism, as cells respond to changes by inherently compromising metabolic efficiency.

RESULTS & DISCUSSION: Here, we quantify the limits of metabolic robustness in self-oscillating autotrophic continuous cultures of the gas-fermenting acetogen *Clostridium autoethanogenum*. On-line gas analysis and high-resolution temporal metabolomics showed oscillations in gas uptake rates and extracellular by-products synchronised with biomass levels. We found that the intrinsict nature of gas fermentation allows for an

initial growth phase on CO, followed by growth on CO and H_2, after which a downcycle is observed in synchrony with a loss in H_2 uptake. Intriguingly, oscillations are not linked to translational control as no differences were observed in protein expression during oscillations. Intracellular metabolomics analysis revealed decreasing levels of redox ratios in synchrony with the cycles. We used a thermodynamic metabolic flux analysis (tMFA) model to investigate if regulation in acetogens is controlled at the thermodynamic level. By incorporating endo and exo-metabolomics data into the model we could show that the thermodynamic driving force of critical reactions collapsed as H_2 uptake is lost.

CONCLUSION: The oscillations are coordinated with redox. The data indicate that metabolic oscillations in gas fermentation acetogens are controlled at the thermodynamic level. The work presented will show that thermodynamic control of metabolism, potentially contributing to metabolic efficiency in gas fermentation.

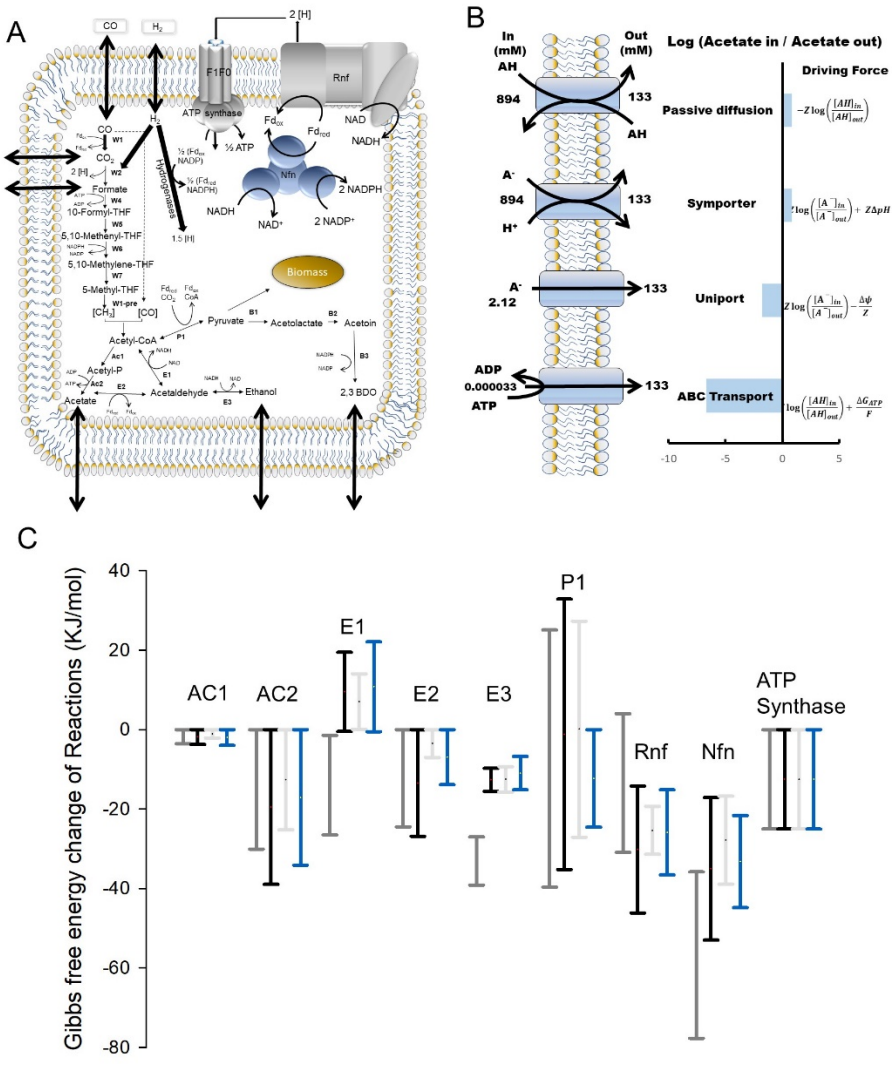

Figure 1. Thermodynamic metabolic flux analysis (tMFA) of *Clostridium autoethanogenum*. (*A*) Schematic representation of our tMFA model of *C. autoethanogenum*. Arrows across the cell membrane denote product transport. (*B*) Modelling four mechanisms for acetate transport which were then used in our tMFA model. See text and Excel datasheet. 2,3-BDO, 2,3-butanediol; THF, tetrahydrofolate; Fd, ferredoxin; A^-, acetate; AH, acetic acid; H^+, proton. (C) The plot shows the maximum allowable range of Gibbs free energy for respective reactions at given conditions calculated using thermodynamic variability analysis (TVA); Grey and black show the three phases from this work and blue shows data from the steady state. See text for details; Each bar denotes mean and error bars standard deviation of that allowable range. * Positive reactions are thermodynamically not feasible at given conditions (Have positive Gibbs free energy change)

REFERENCES

1. Mahamkali, et. al. Redox controls metabolic robustness in the gas-fermenting acetogen *Clostridium autoethanogenum*. PNAS 2020, 117 (23) 13168-13175; DOI: 10.1073/pnas.1919531117