Regulation of Metabolic Activity in *Clostridium autoethanogenum*

Thomas Millat, Rupert Norman,
Anne Henstra, Klaus Winzer, Nigel Minton

Synthetic Biology Research Centre

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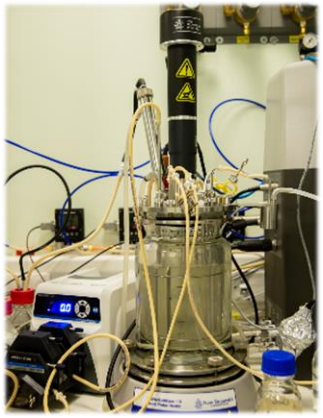
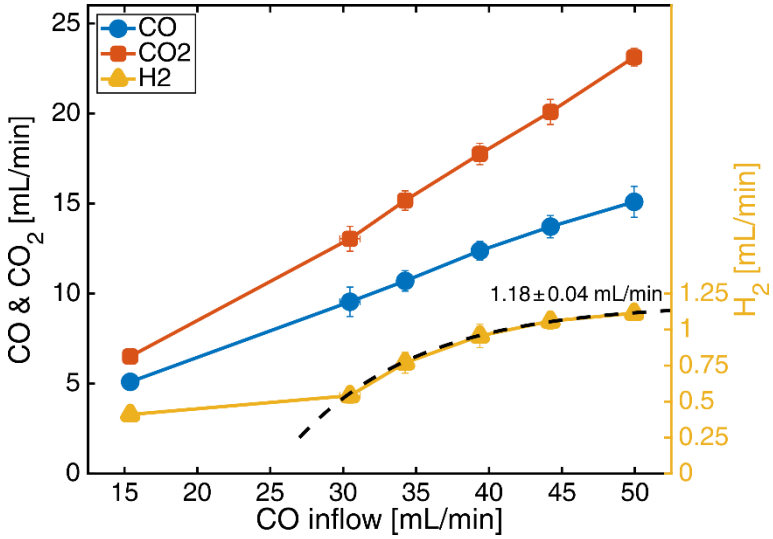
Gas-shift in Continuous Culture

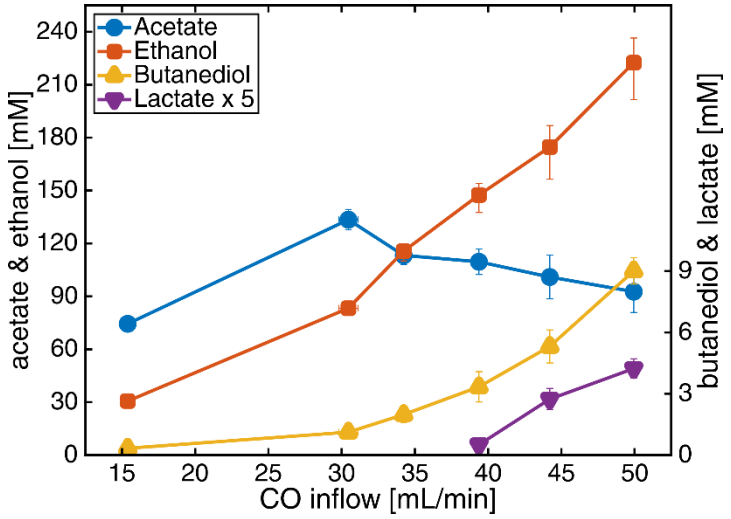
- Gradual increase of CO supply
- Constant pH 5.0
- Investigations at steady state
- Multi-omics approach



  - Transcriptome
  - Proteome
  - Metabolome
  - Product formation
  - Culture growth
  - Environome

- Metabolite analysis using Robust Statistics (Poster 2.6)
- Genome-scale Model (Poster 2.12)

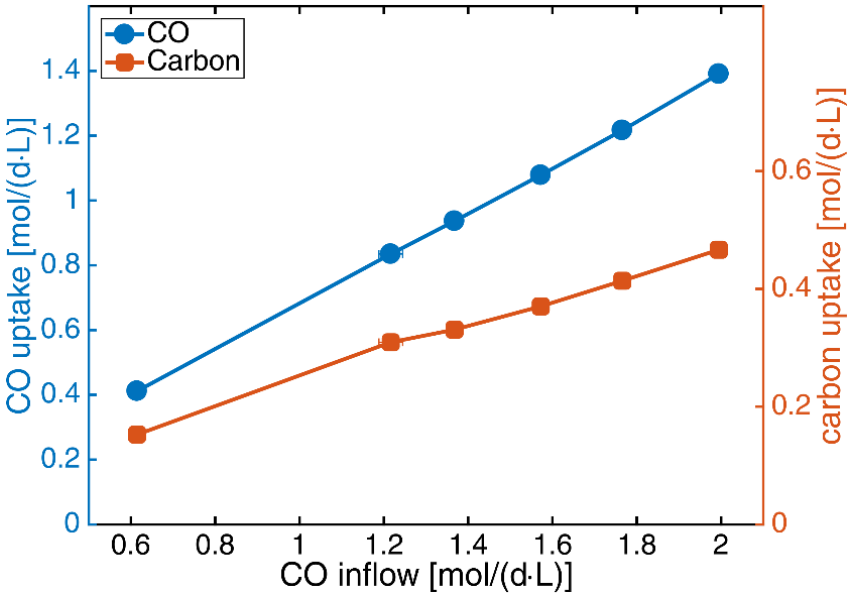




CO Uptake and Culture Growth

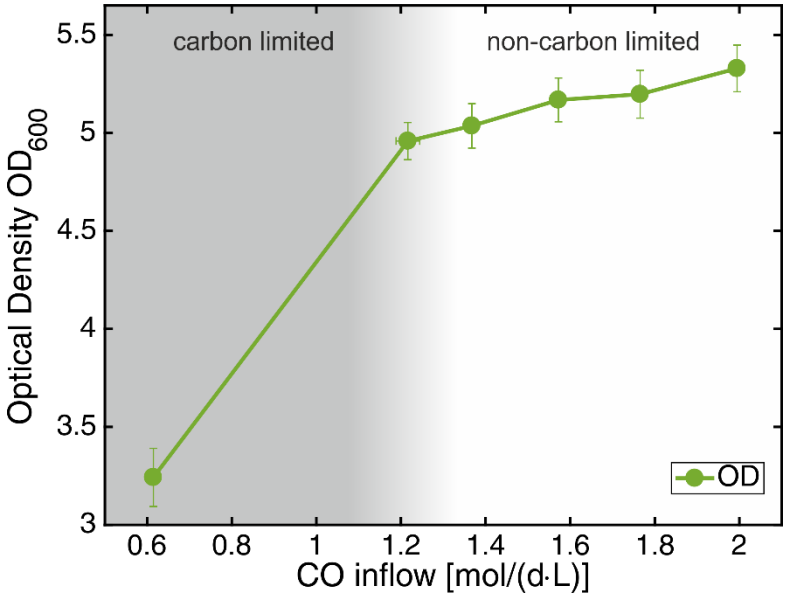
CO Uptake

- 70% of supplied CO is assimilated, irrespective the actual supply rate

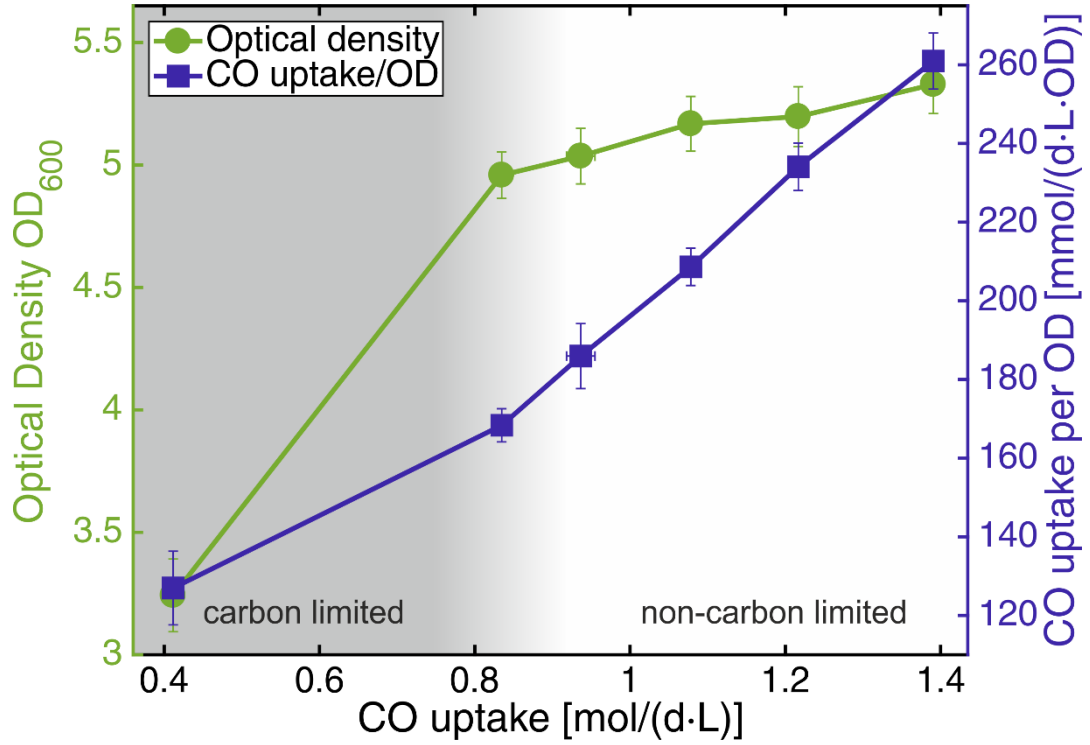


Culture Growth

- Culture size increases with CO
- Transition from carbon-limited to non-carbon-limited growth

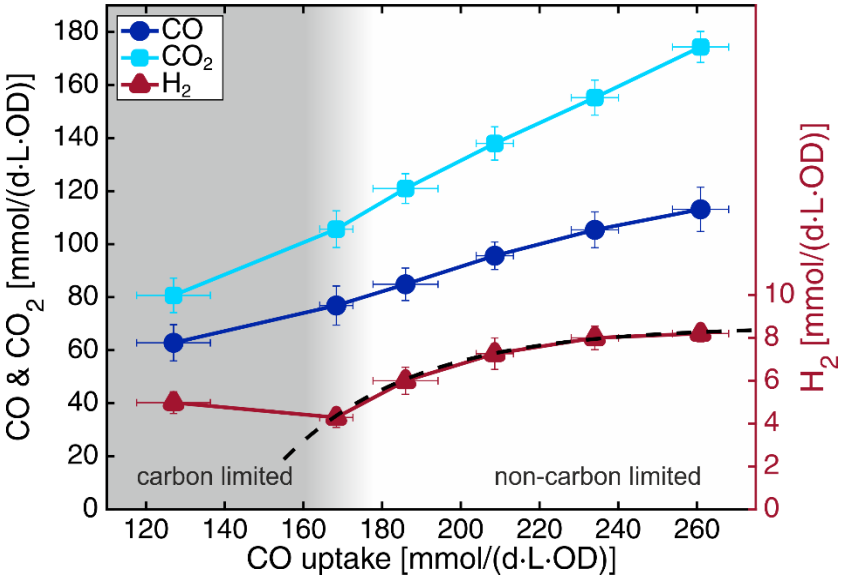


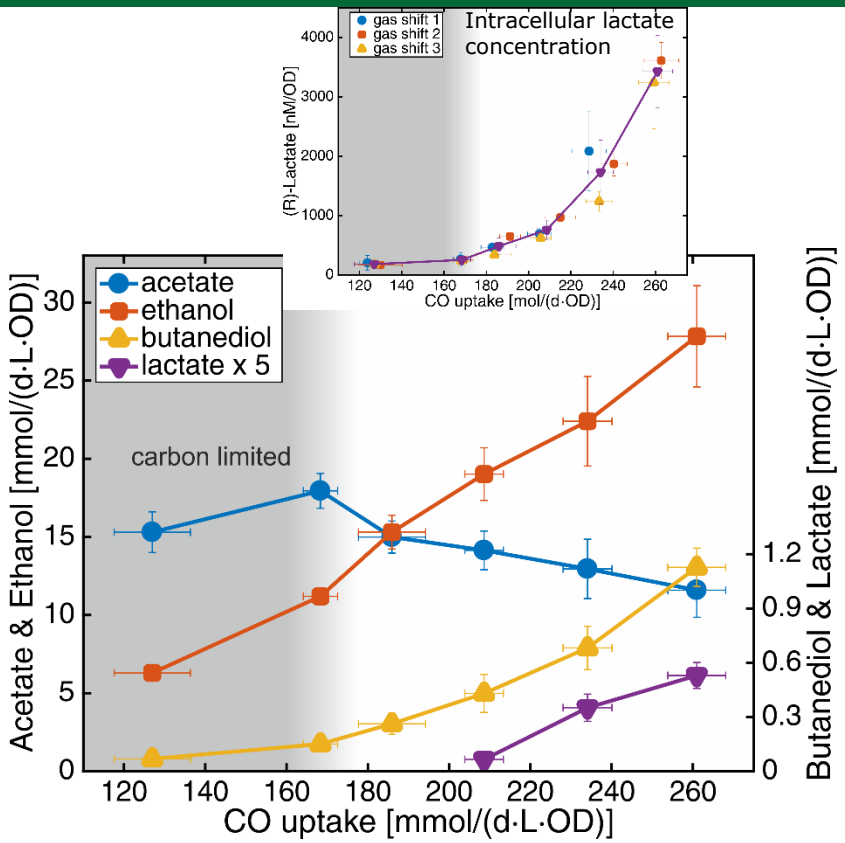
CO Uptake & Culture Growth



With increasing CO supply, the cells assimilate and convert rising amounts of CO.

Cellular Product Formation





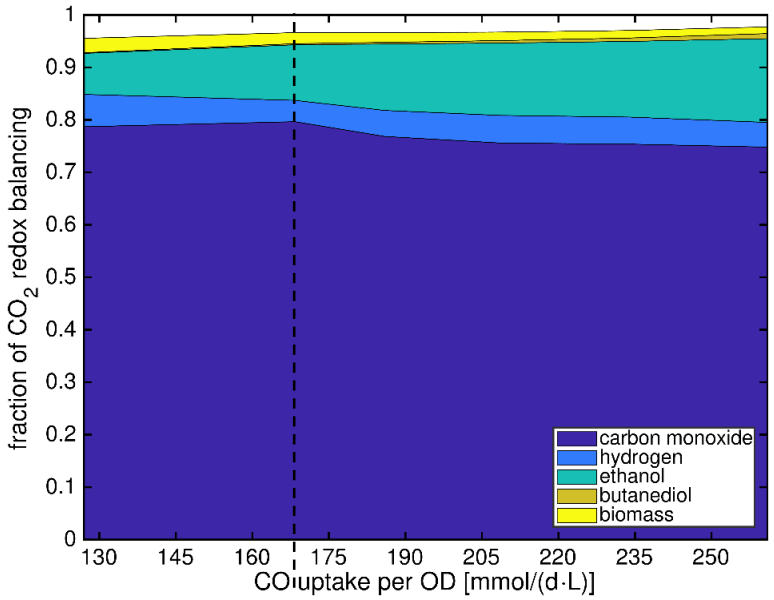
Hydrogen formation approaches its thermodynamic limit for high CO supply rates.

Metabolic shift coincides with the transition between carbon and non-carbon limited growth.

Redox Balance

- In anaerobic organisms reduction and oxidation reactions must be balanced

Compound	Sum formula	Reduction value		
Carbon monoxide	CO			+1
Carbon dioxide	CO_2	-2		
Hydrogen	H_2			+1
Acetic acid	$C_2H_4O_2$		0	
Ethanol	C_2H_6O			+2
2,3-butanediol	$C_4H_{10}O_2$			+3
Lactic Acid	$C_3H_6O_3$		0	
Biomass	$C_4H_8O_2N$			+2



Hydrogen (limited) and ethanol (flexible) are the major compounds to maintain the redox balance at steady state.

Only ethanol formation can compensate the increasing CO flow.

Redox Balance

- Kirchhoff's law

$$\sum_n I_n = 0$$

$$0 = -2n_{CO_2}\,CO_2 + n_{CO}\,CO + n_{H_2}\,H_2 + 2n_{BM}\,BM + 2n_{Eth}\,Eth + 3n_{BDH}\,BDH$$

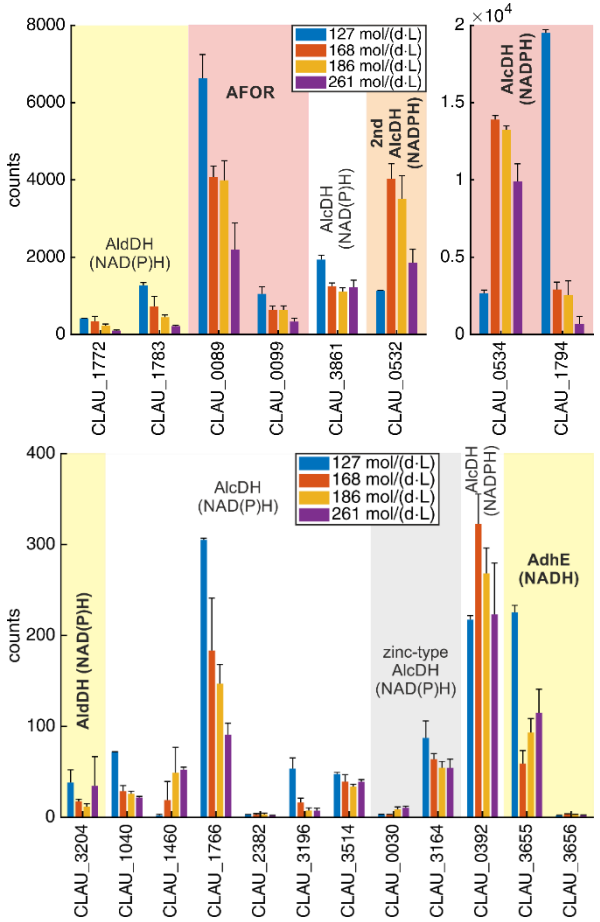
- Defines a space of feasible solutions
- Some fluxes might be restricted by
  - Thermodynamic & Kinetic limitations
  - Cellular conditions
- Some can be regulated or limited externally
  - Nutrient limitations
  - Acid excretion due to variation in pH (acetate)
  - (Partial) Pressure of gaseous products, e.g. H_2
- Cellular capabilities & regulation

Ethanol Formation – Transcriptomic Profile

- AFOR-mediated route dominates ethanol formation
- NADPH-dependent AlcDH preferred

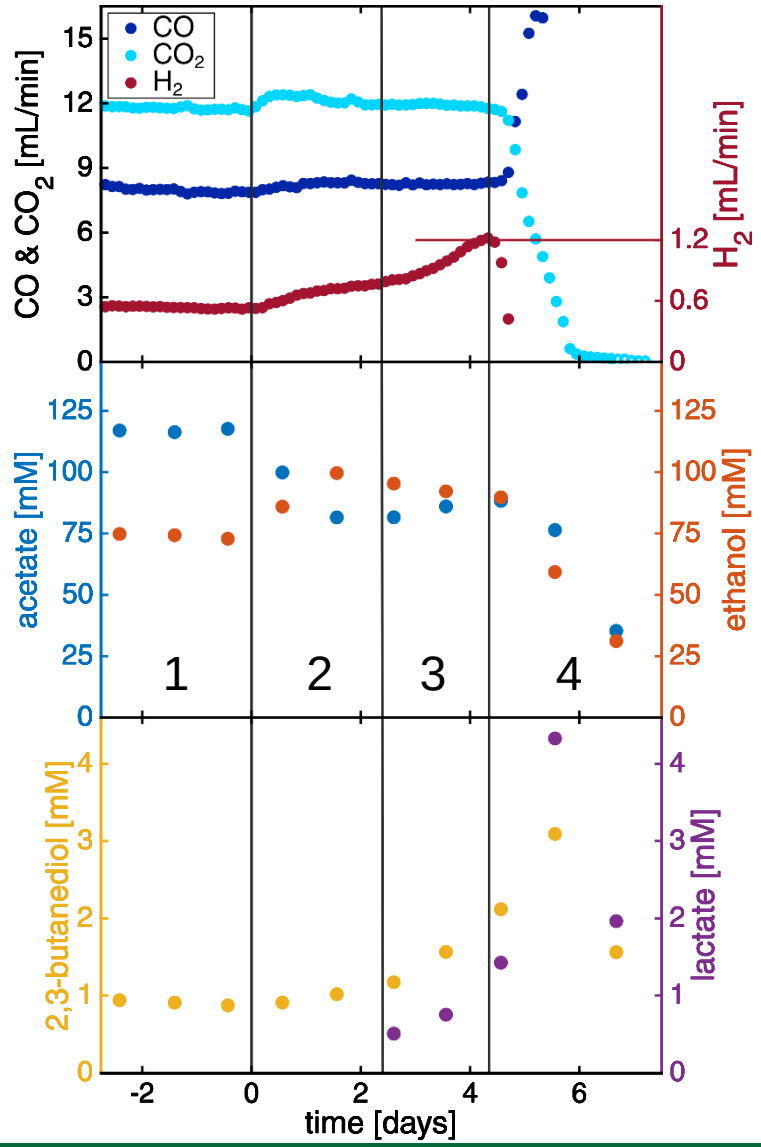
- Genes encoding for AFOR are strongly downregulated with CO
- Kinetic and thermodynamic regulation

- Bifunctional AdhE's and AldDH are much less expressed
- Significant role in ethanol formation at steady state is unlikely



Ethanol Formation & Culture Crash

1. Carbon-limited steady state at pH5
2. pH-shift at t=0
   - Increase in ethanol, hydrogen and 2,3-BDH and decrease in acetate
3. Shut down of ethanol formation
   - Increasing activity towards the remaining products
   - Stable CO uptake and cellular growth
4. Hydrogen formation at thermodynamic limit
   - No feasible solution can be achieved
   - Culture crash



Summary

- Gas-induced metabolic shift from acetogenesis to ethanologenesis in *C. autoethanogenum* using CO as solely carbon, energy and electron source reverses the ethanol/acetate ratio.
- Caused by thermodynamic and biological regulation
  1. Increasing CO uptake per cell
  2. Transition from carbon-limited growth phase to a non-carbon-limited growth phase
  3. Thermodynamically limited hydrogen formation
  4. Ethanol becomes the dominant measure to fulfil the redox balance
- Despite increasing ethanol formation, gene expression of ethanol genes is strongly downregulated.
- Shutting down of ethanol formation eventually results in the crash of the culture, because no feasible solution can be achieved by the cells.

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