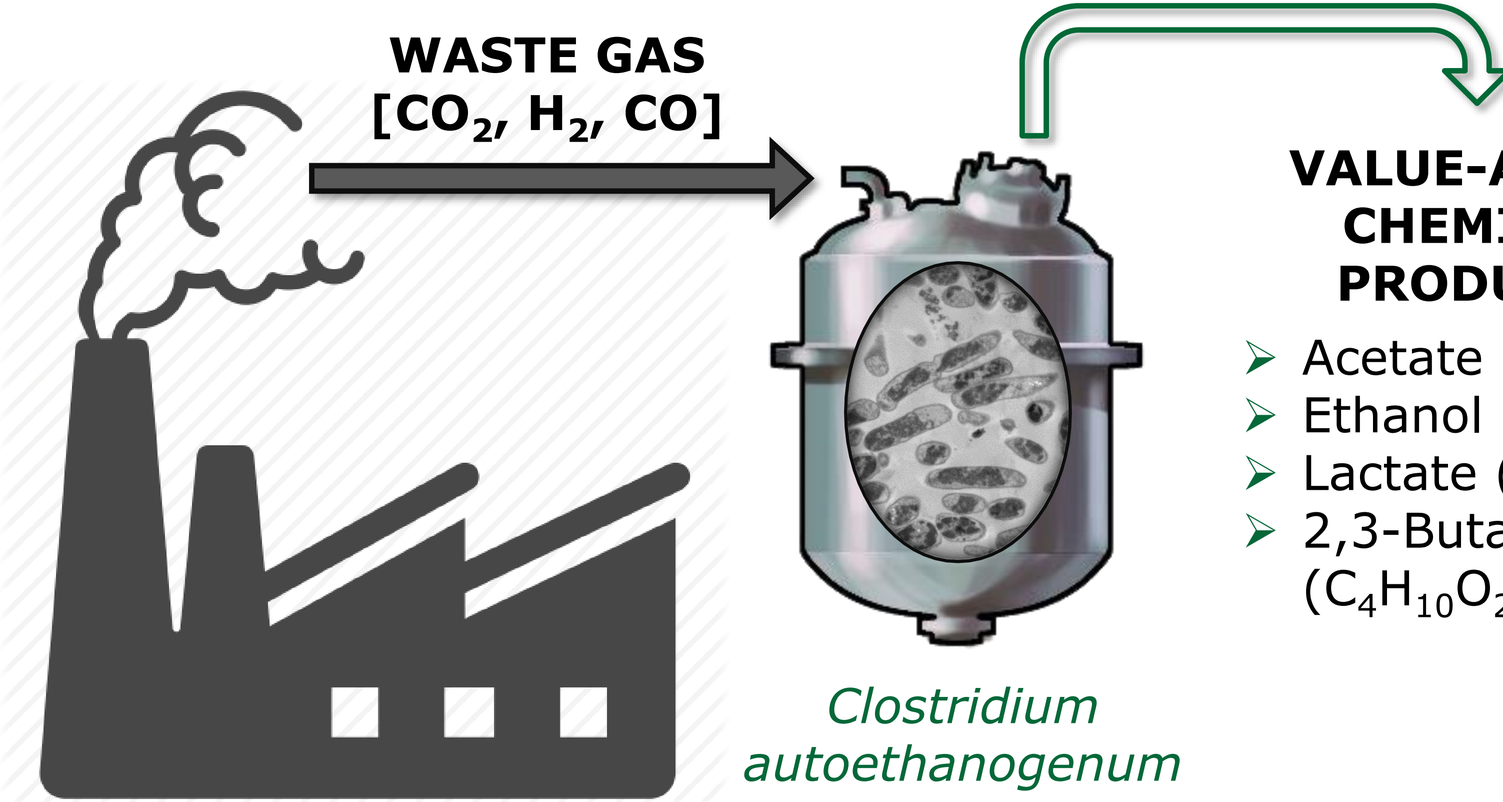
Construction and Analysis of a Genome-Scale Metabolic Model of *Clostridium autoethanogenum*

Rupert Norman

Synthetic Biology Research Centre

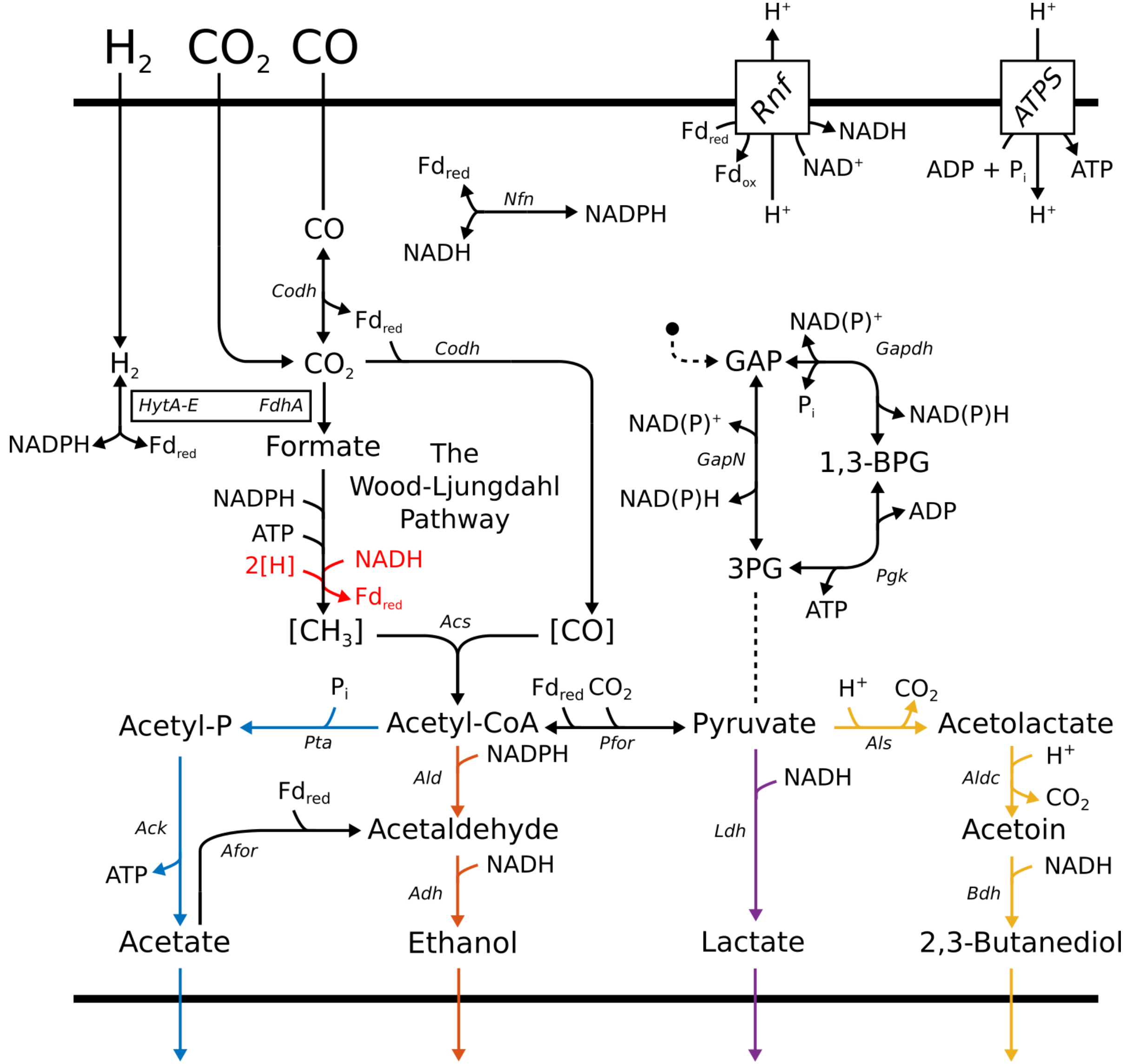
18th January 2018

Background – Research Setting



VALUE-ADDED CHEMICAL PRODUCTS

- Acetate ($C_2H_4O_2$)
- Ethanol (C_2H_6O)
- Lactate ($C_3H_6O_3$)
- 2,3-Butanediol ($C_4H_{10}O_2$)


H2 CO2 CO
Rnf
ATPS
H+
Fdred
Fdox
NADH
NAD+
ADP + Pi
ATP
Nfn
NADPH
CO
Codh
CO2
H2
HytA-E
FdhA
Formate
The Wood-Ljungdahl Pathway
ATP
2[H]
[CH3]
Acs
[CO]
GAP
NAD(P)+
Gapdh
Pi
NAD(P)H
GapN
1,3-BPG
ADP
3PG
Pgk
Acetyl-P
Pta
Acetyl-CoA
Pfor
Pyruvate
Als
Acetolactate
Ald
Aldc
Acetaldehyde
Afor
Ack
Acetoin
Adh
Ldh
Bdh
Acetate
Ethanol
Lactate
2,3-Butanediol

Genome Scale Metabolic Model

Construction

Methods:

- Pathway Tools
- *ScrumPy*
- Humphreys *et al*. (2015)

Results:

- 795 reactions
- 786 metabolites
- 84 transport reactions

University of Nottingham
UK | CHINA | MALAYSIA

SynBio
SBRC Nottingham
a BBSRC/EPSRC Synthetic Biology Research Centre

Parametrization

ATP maintenance costs

Marcellin *et al.* (2016):

- GAM = 41.257

Nagarajan *et al.* (2014):

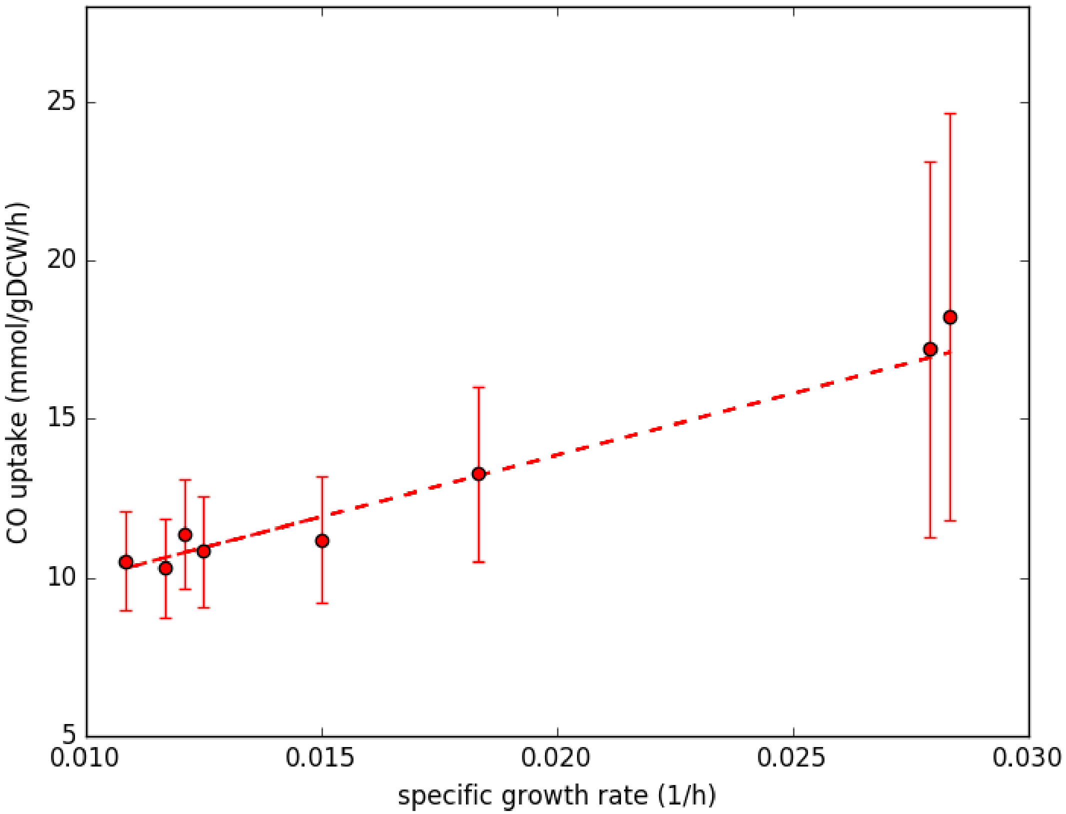
- GAM = 46.666
- NGAM = 0.45

Experimental Methods:

- Vary dilution rate
- CO uptake
- Estimate ATP yields

Results:

- GAM = 100.0 mmol $gDCW^{-1}$
- NGAM = 2.28 mmol $gDCW^{-1}$ h^{-1}



Parametrization

Biomass composition

Biomass Component	g/g (%)	±
Protein	26.250	2.278
DNA	14.569	7.532
RNA	17.949	4.202
Lipid	22.002	1.716
Polysaccharide	07.625	0.033
Teichoic acid	10.197	7.833
Others	09.270	–

Validation

Substrate testing

CO	$CO_2 + H_2$	Fructose	Fumarate	Glucose
☑	☑	☑	☑	☒

Growth Rate Prediction

Methods:

- Flux Balance Analysis (FBA)
  - Objective: Maximize growth rate
  - Constraint: CO as sole carbon and energy source

Results:

- Predicted growth rate = 0.026 h^{-1}
- Acetate forms sole product
- Measured growth rate = 0.027 ± 0.001 h^{-1}
- Measured uptake rate = 16.57 ± 0.002 mmol $gDCW^{-1}$ h^{-1}

Validation

Product spectrum

Compound	Y_{ATP}	Nett stoichiometry
Acetate	0.344	$4\ CO + 2\ H_2O \rightarrow C_2H_4O_2 + 2\ CO_2$
Ethanol	0.313	$6\ CO + 3\ H_2O \rightarrow C_2H_6O + 4\ CO_2$
Lactate	0.146	$6\ CO + 3\ H_2O \rightarrow C_3H_6O_3 + 3\ CO_2$
Hydrogen	0.125	$CO + H_2O \rightarrow H_2 + CO_2$
2,3-butanediol	0.11	$11\ CO + 5\ H_2O \rightarrow C_4H_{10}O_2 + 7\ CO_2$

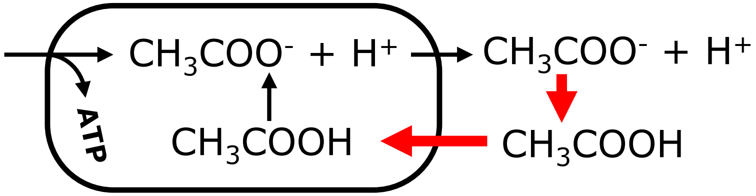
"…the **ATP yield for ethanol production from CO is higher than for acetate production** from CO. And indeed, some acetogens like *C. autoethanogenum* produce ethanol when growing on CO."

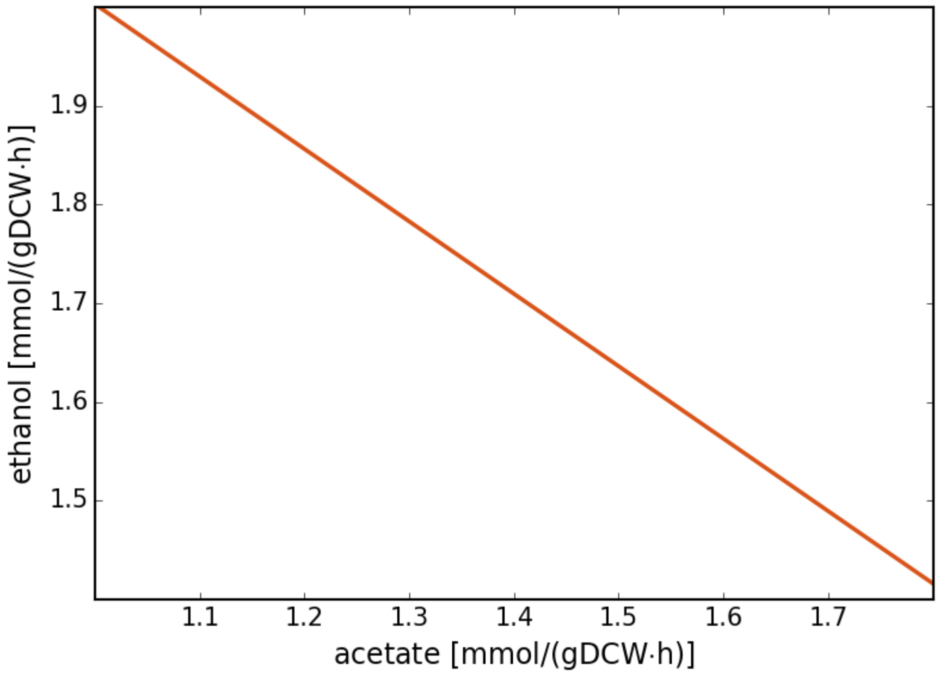
- *Bertsch & Müller (2015)*

Hypothesis testing

pH-induced transport restriction

- *C. auto* maintains a constant transmembrane pH gradient, ΔpH ≈ 1
- External pH level affects dissociation of acetic acid (pK_a = 4.76)





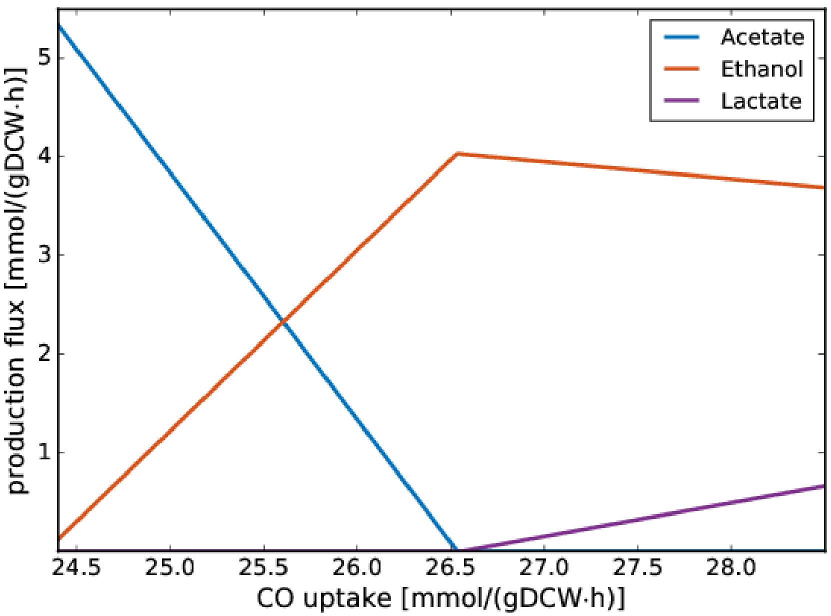
- Restriction on acetate efflux

pH-induced efflux restriction of acetic acid favours routes for the formation of ethanol.

Hypothesis testing

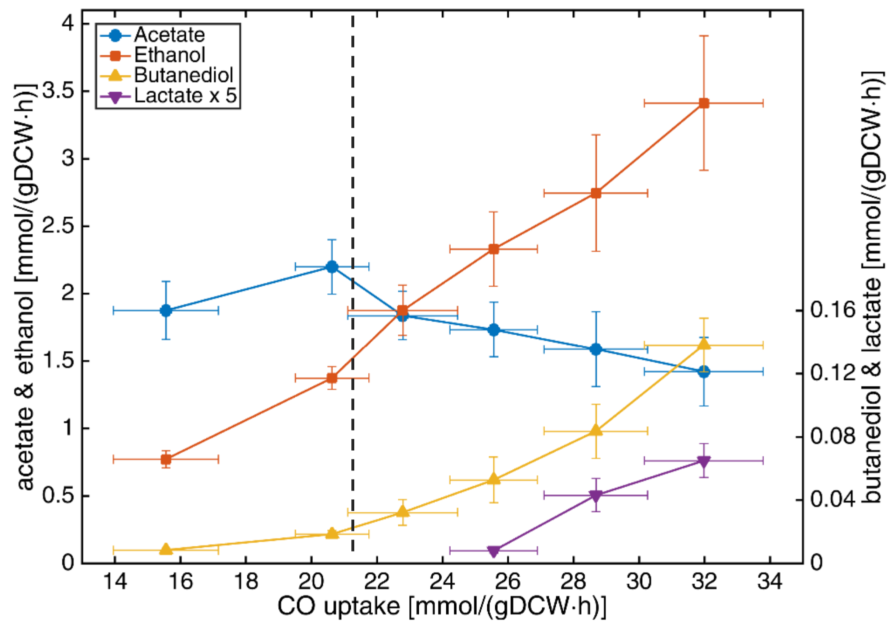
Gas Shift

carbon limited
non-carbon limited



carbon limited

non-carbon limited



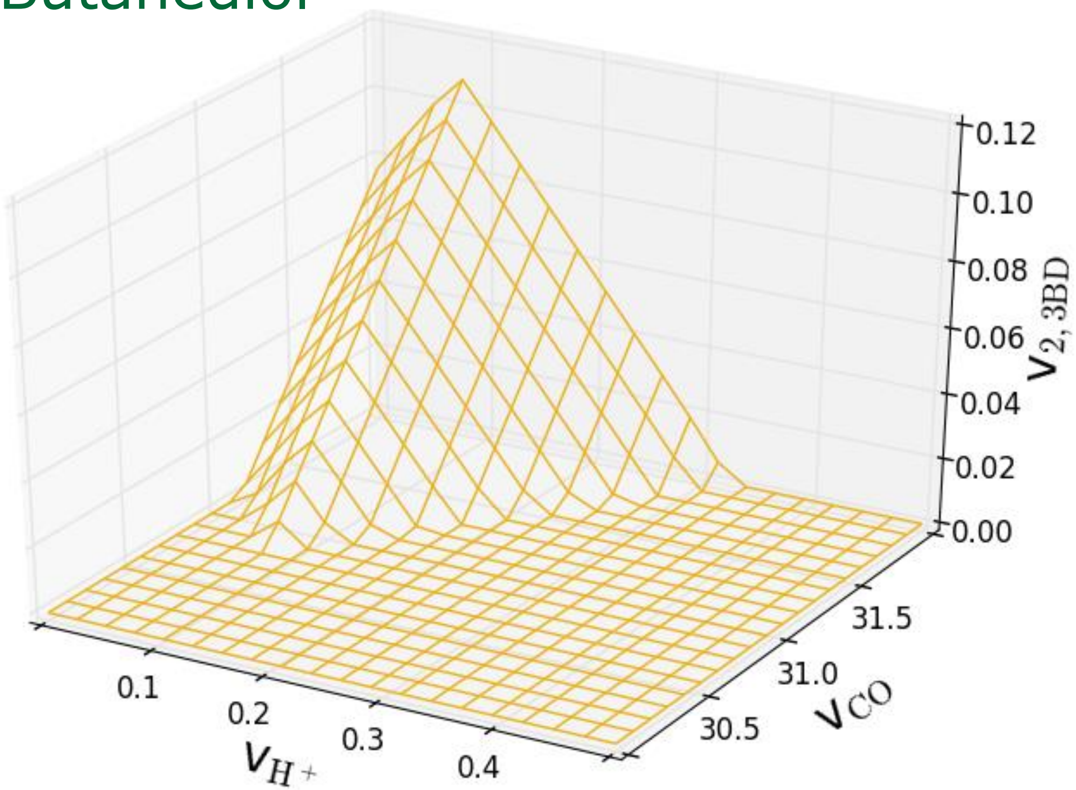
Product shift seen with CO uptake rates beyond v_{CO}^{μ}.

Non-carbon growth limitation is required for a product shift.

Hypothesis testing: 2,3-Butanediol

Acid stress response

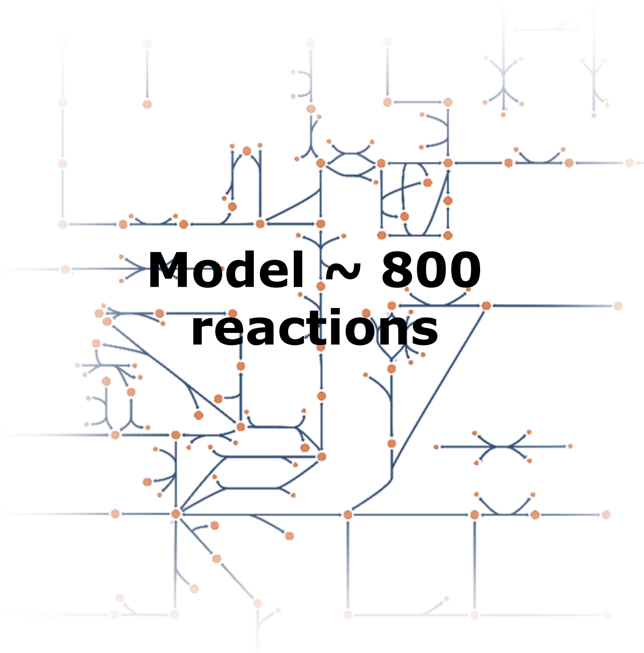
- 2,3-Butanediol (BD) production is associated with culture crash
- Acidification occurs with acetate production
- Intercellular pH adjusted through 'consumption' of protons
- $\frac{d[H^+]}{dt} < 0$



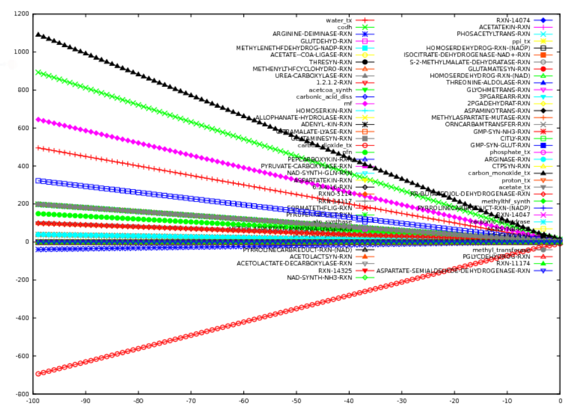
Proton consumption flux associated with BD production at high v_{CO}.
Production of BD may become most favourable at non-steady states.

2,3-Butanediol

Elementary Modes Analysis

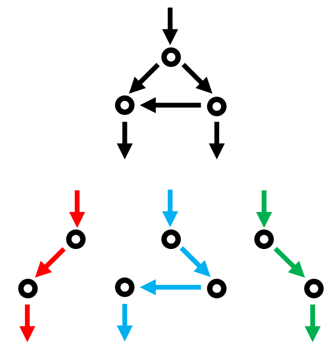


1. Sub-Network Extraction (FBA)



Reactions	52
Transporters	8
Metabolites	55

2. Elementary Modes Analysis



Elementary Modes	75
2,3-BD Producers	**6**

2,3-Butanediol

Elementary Modes Analysis

Mode	Y_{ATP}	# reactions
1	0.114	20
2	**0.0**	25
3	**0.0**	30
4	**0.0**	31
5	**0.0**	31
6	**0.0**	32

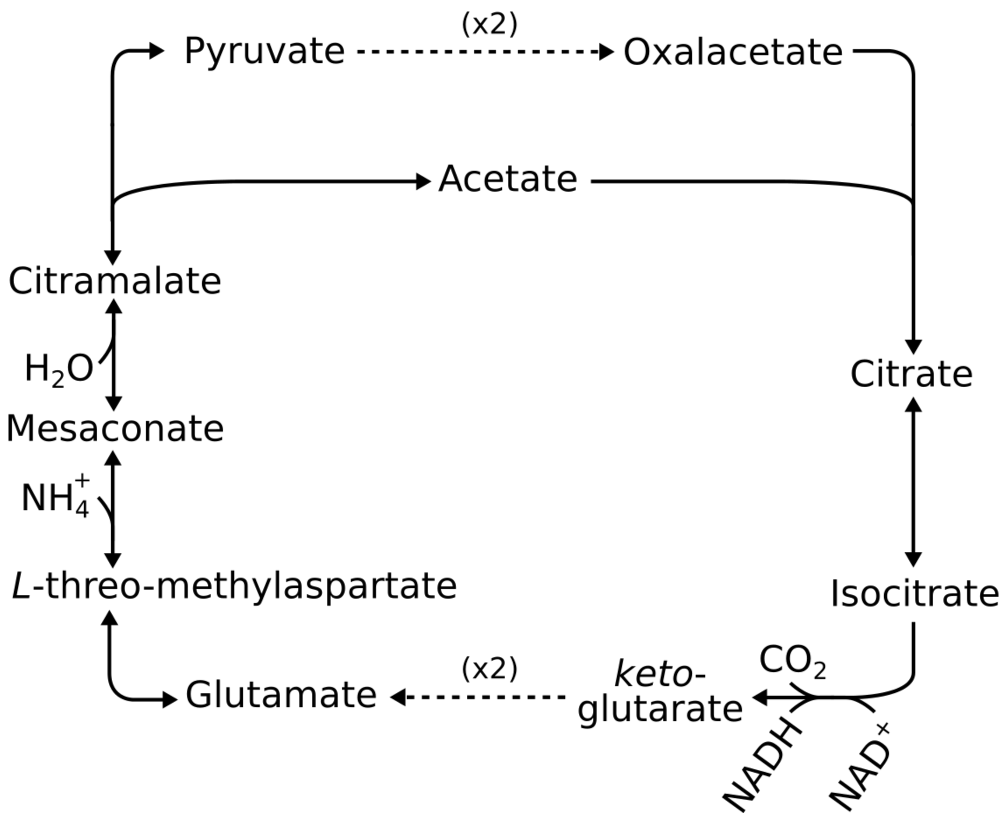
5 elementary modes of 2,3BD production are **ATP neutral.**

What advantage could be gained from these modes?

2,3-Butanediol

Elementary Modes Analysis

- Cyclic structure coupled to expected pathway
- Involves central carbon metabolism (TCA cycle)
- 4 permutations
  - Pyr ⇌ Oxa (× 2)
  - K'Glu ⇌ Glt (× 2)
- Nett conversion represents transhydrogenase reaction

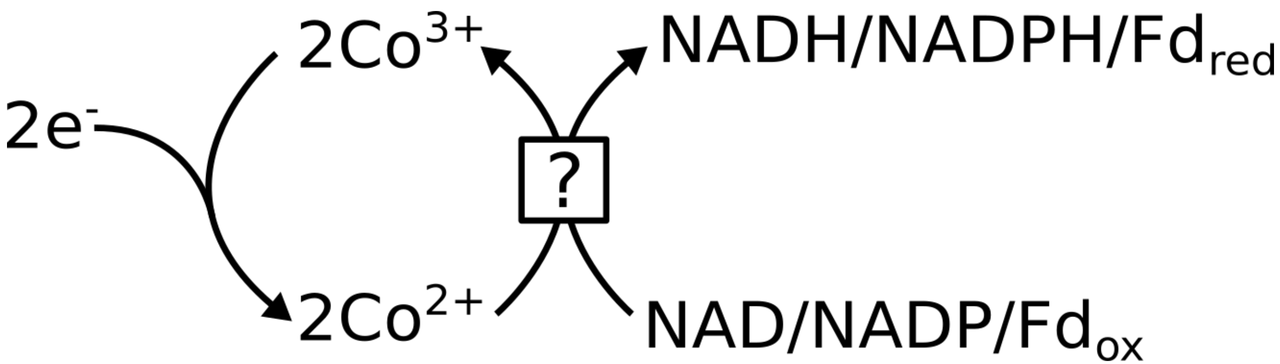


The following conversions are available to the network:

$$NADPH + NAD^+ + k\,ATP \longrightarrow k\,ADP + k\,Pi + NADP^+ + NADH$$

Where $k \in [0,1,2]$

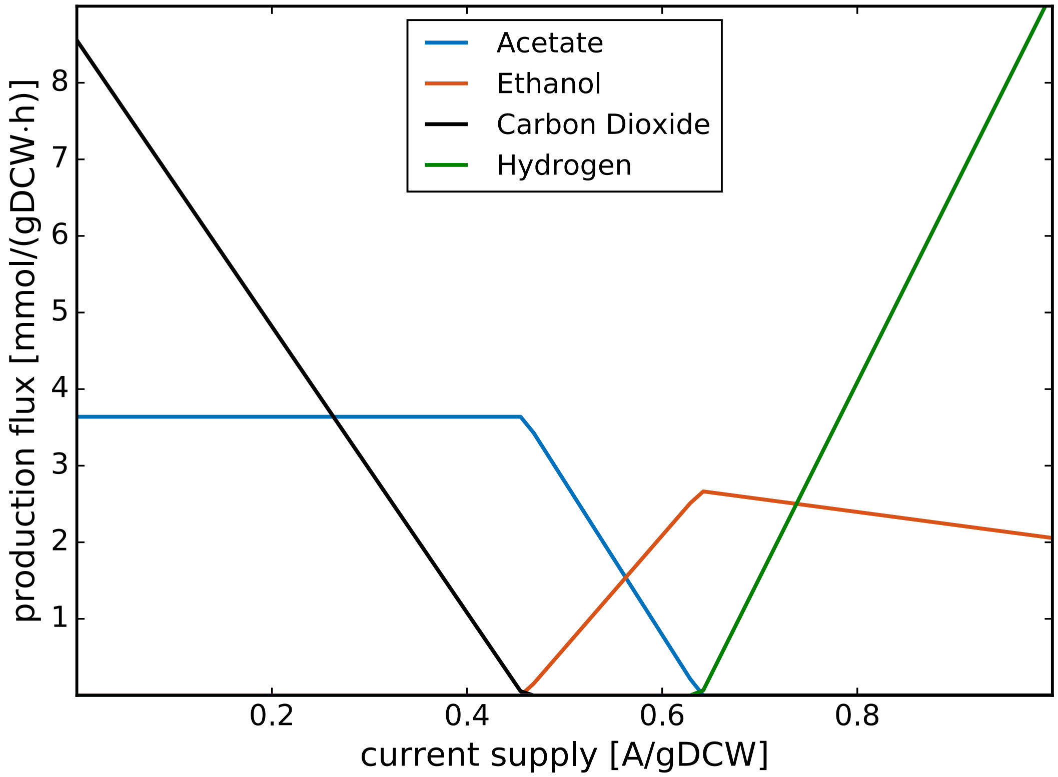
Microbial Electrosynthesis



Kracke *et al*. (2016)

Microbial Electrosynthesis

Product profile in optimal solutions



Acknowledgements

Supervisors

Charlie Hodgman
Thomas Millat
Sarah Schatschneider
Klaus Winzer
Nigel Minton
David Fell
Mark Poolman
Hassan Hartman

Experimentalists

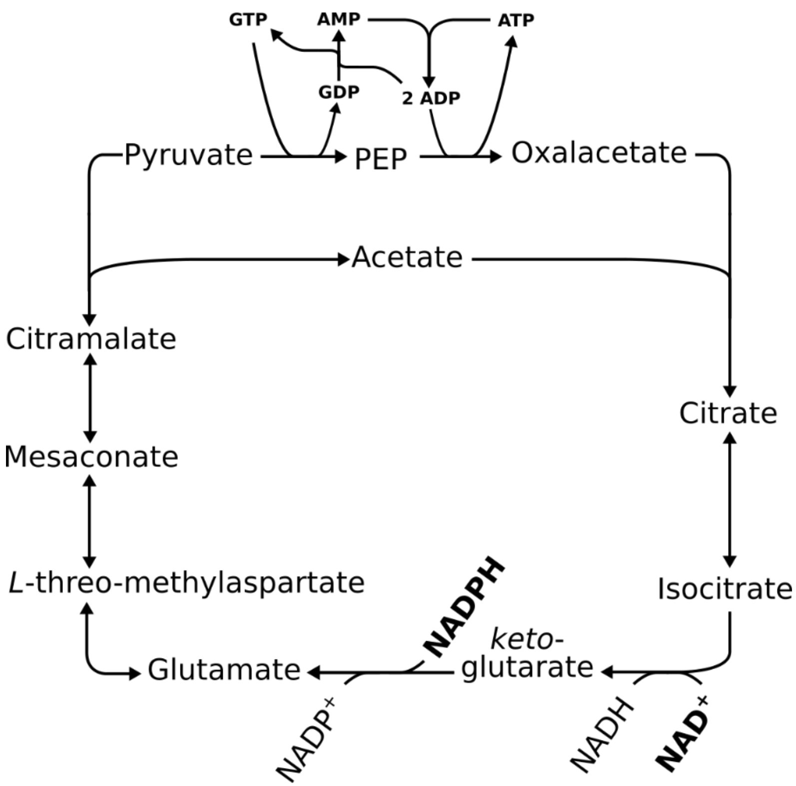
Anne Henstra
Louise Sewell
Bart Pander
Florence Annan
Pawel Piatek
Ronja Brietkopf
Dave Barrett
Salah Abdelrazig
Laudina Safo

BBSRC
bioscience for the future

LanzaTech

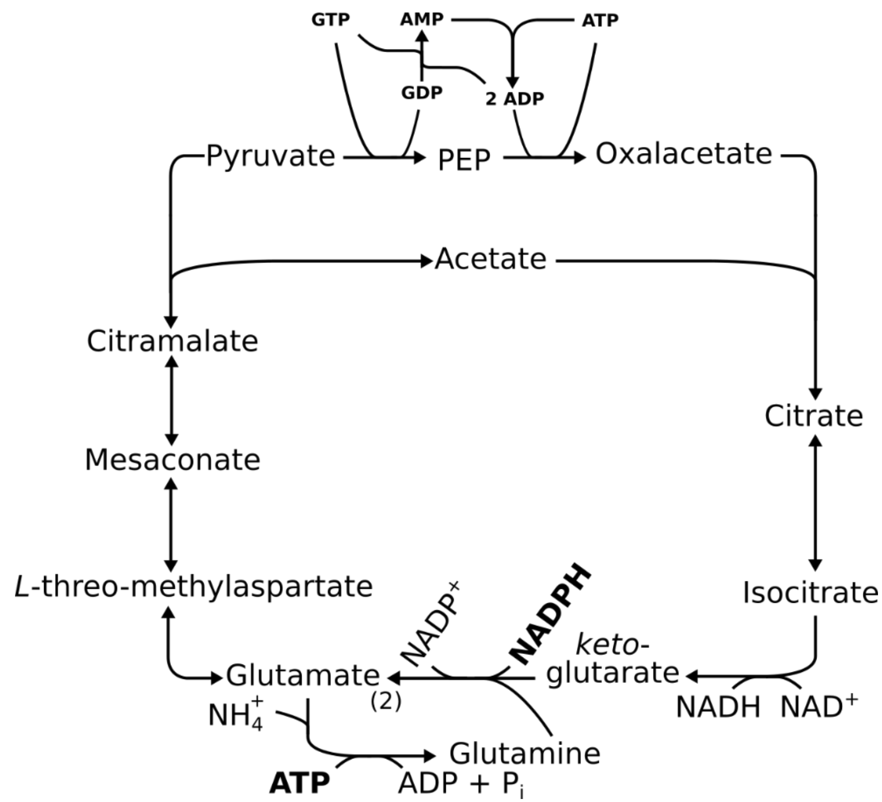
i)

NADPH + NAD$^+$ → NADP$^+$ + NADH



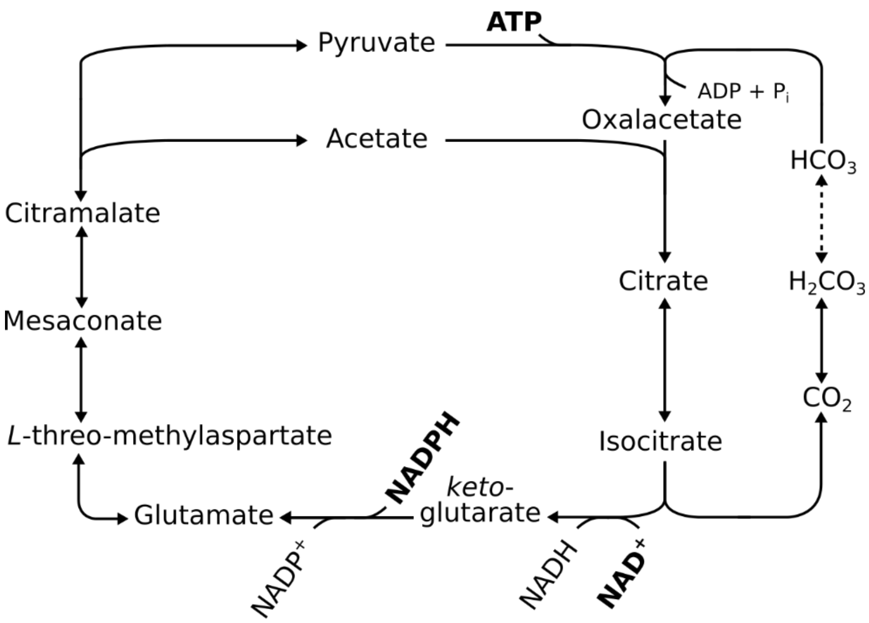
ii)

ATP → ADP + Pi
NADPH + NAD$^+$ → NADP$^+$ + NADH



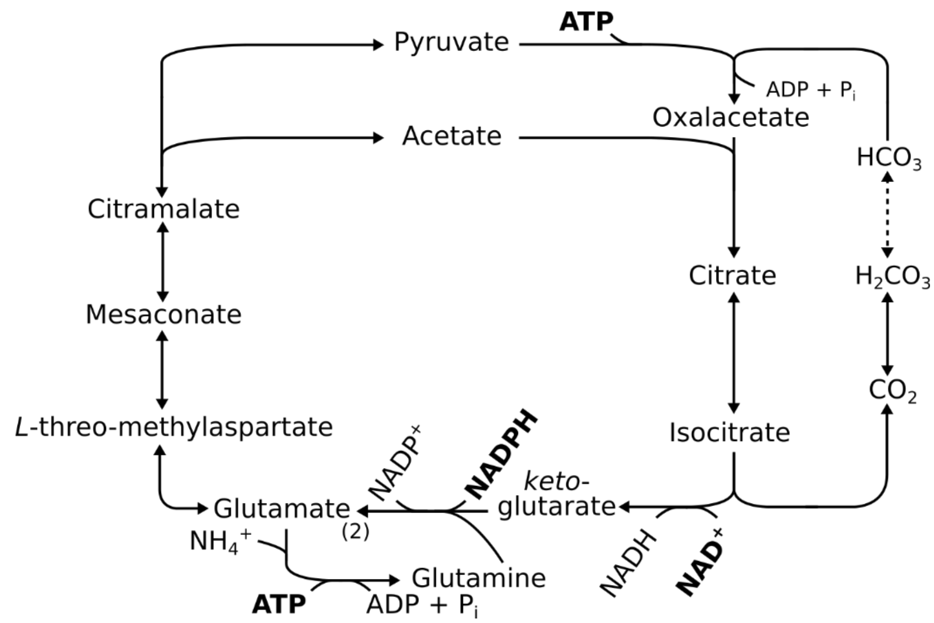
iii)

ATP → ADP + Pi
NADPH + NAD⁺ → NADP⁺ + NADH



iv)

2 ATP → 2 ADP + 2 Pi
NADPH + NAD⁺ → NADP⁺ + NADH



Thus, the following conversions are available to the network:

$$\text{NADPH} + \text{NAD}^+ + k\,\text{ATP} \longrightarrow k\,\text{ADP} + k\,\text{Pi} + \text{NADP}^+ + \text{NADH}$$

Where $k \in [0,1,2]$