PhD project: Construction and Analysis of a Genome Scale Metabolic Model of *Clostridium autoethanogenum*

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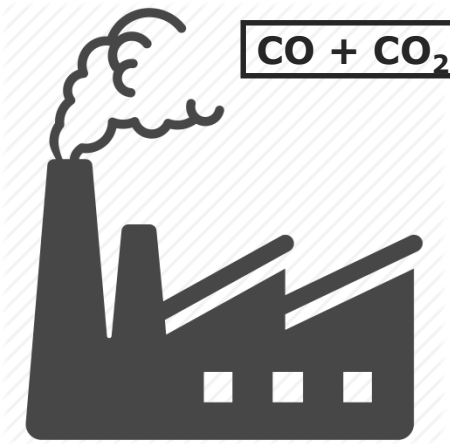


$CO + CO_2 + H_2$

metabolism

ethanol,
2,3-butanediol

Clostridium autoethanogenum

- Strictly anaerobic
- Gram-positive
- Spore forming
- Grows using CO as sole source of energy and carbon
- Products include: Acetate, ethanol, lactate, 2,3-butanediol & CO_2

Genome Scale Model

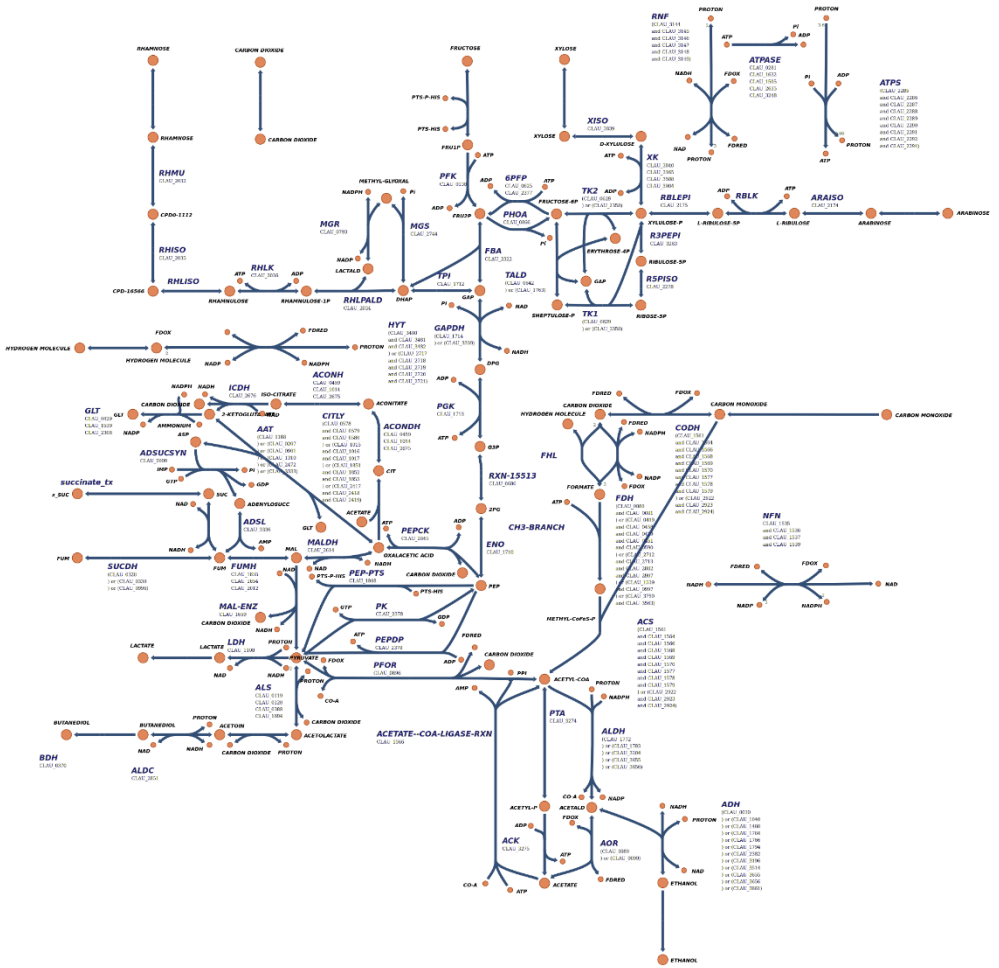
Construction

Methods:

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

Results:

- 795 reactions
- 786 metabolites

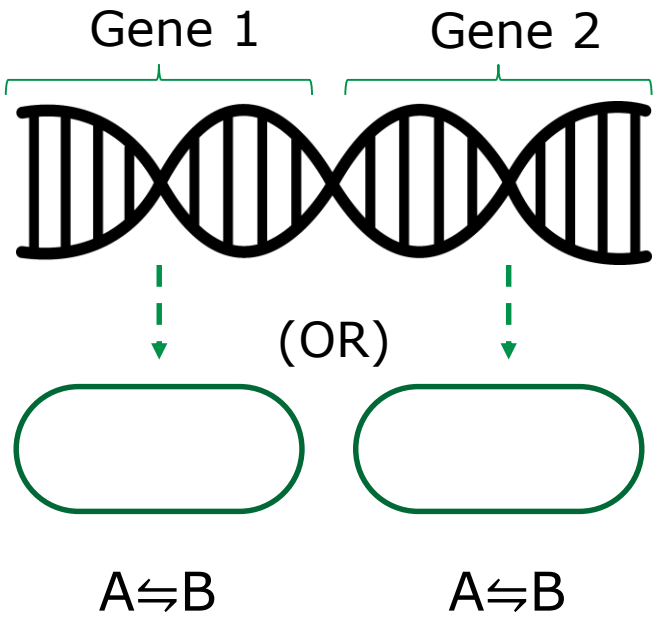


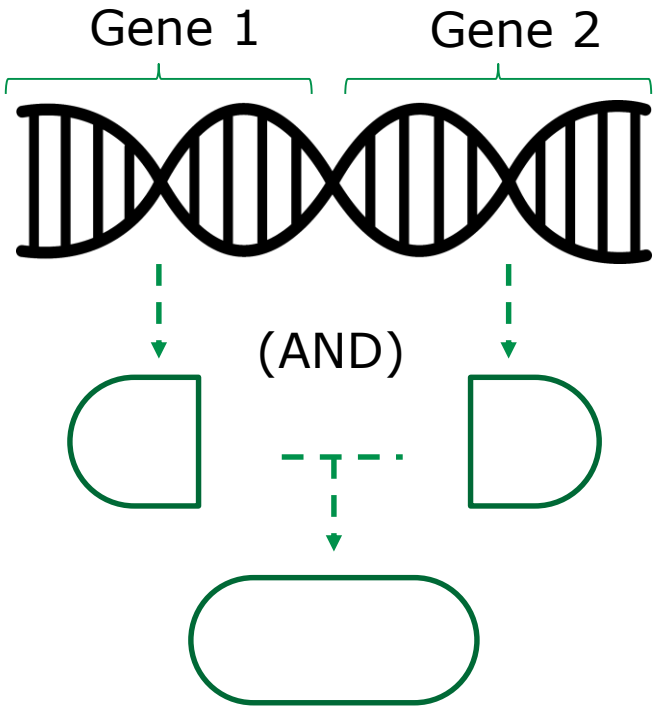
Acknowledgements:

David Fell, Mark Poolman, Hassan Hartman

Genome Scale Model

Gene-Protein-Reaction Relationships





Inference based on genomic information:

- Operon
- Annotated as subunit/isomer

(~500/795 inferred GPRs)

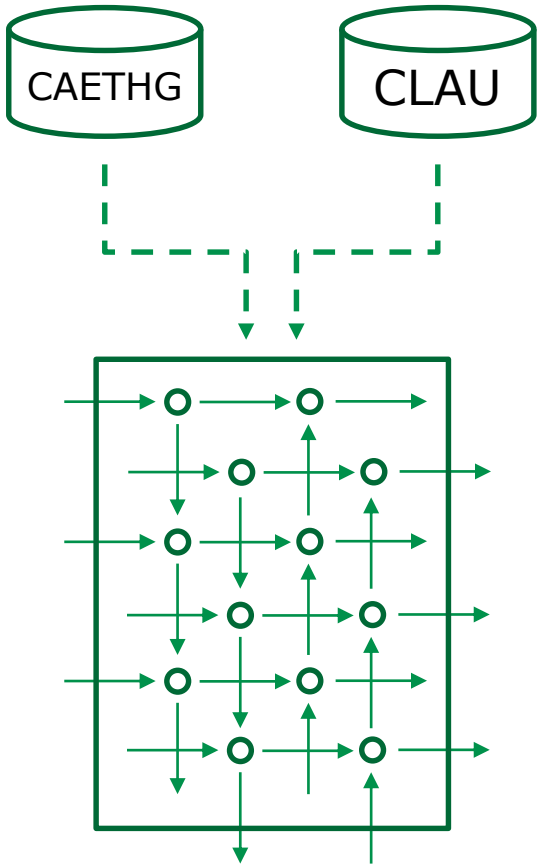
Genome Scale Model

Switching databases

Initial construction based on BioCyc genome annotation for *C. autoethanogenum*.

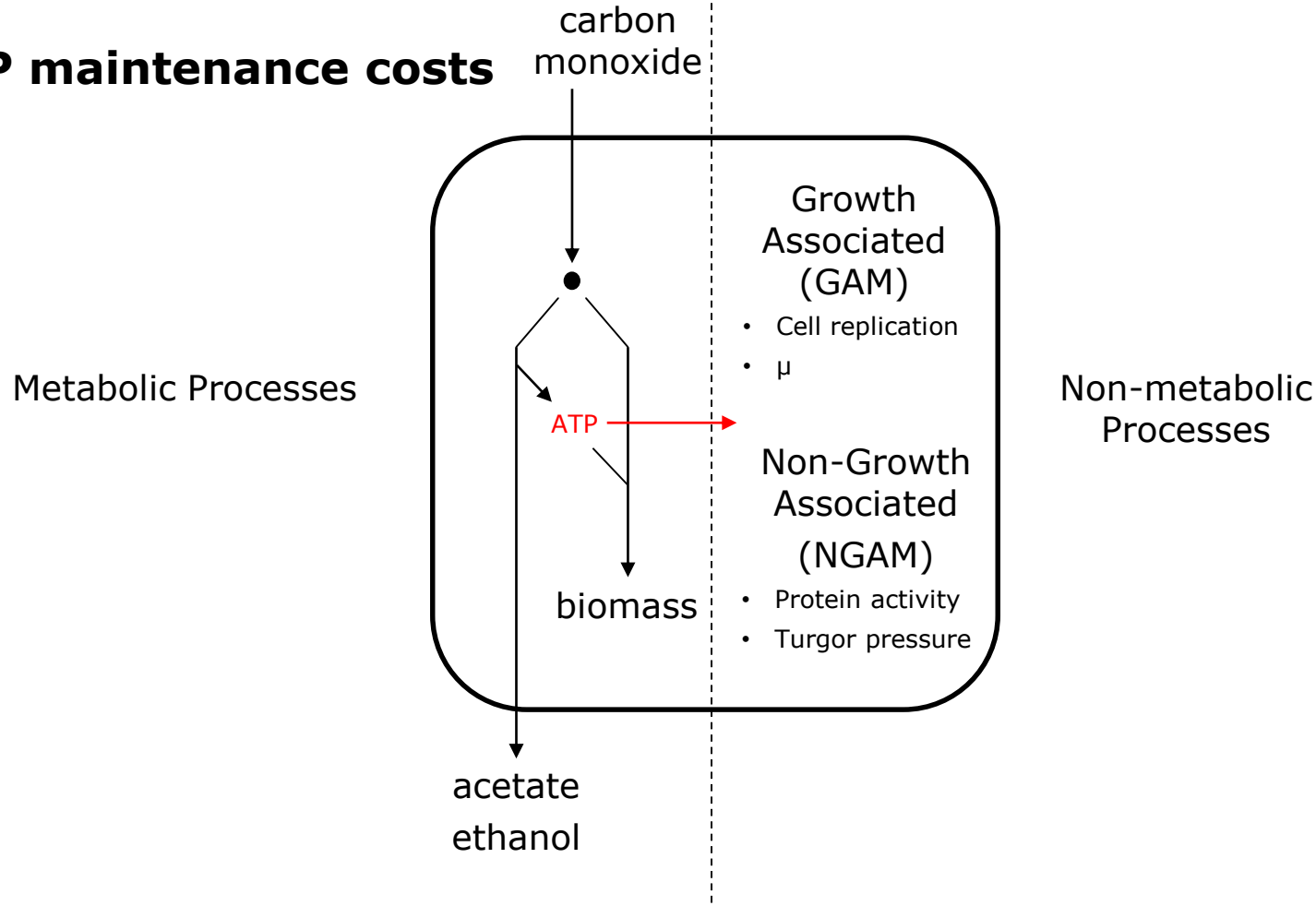
Switch to database generated from GASCHEM annotation (Humphreys *et al.,* 2015):

- Reactions common to both databases unchanged
- Reactions unique to CAETHG tested for essentiality
- Reactions unique to CLAU added



Parametrization

ATP maintenance costs



Parametrization

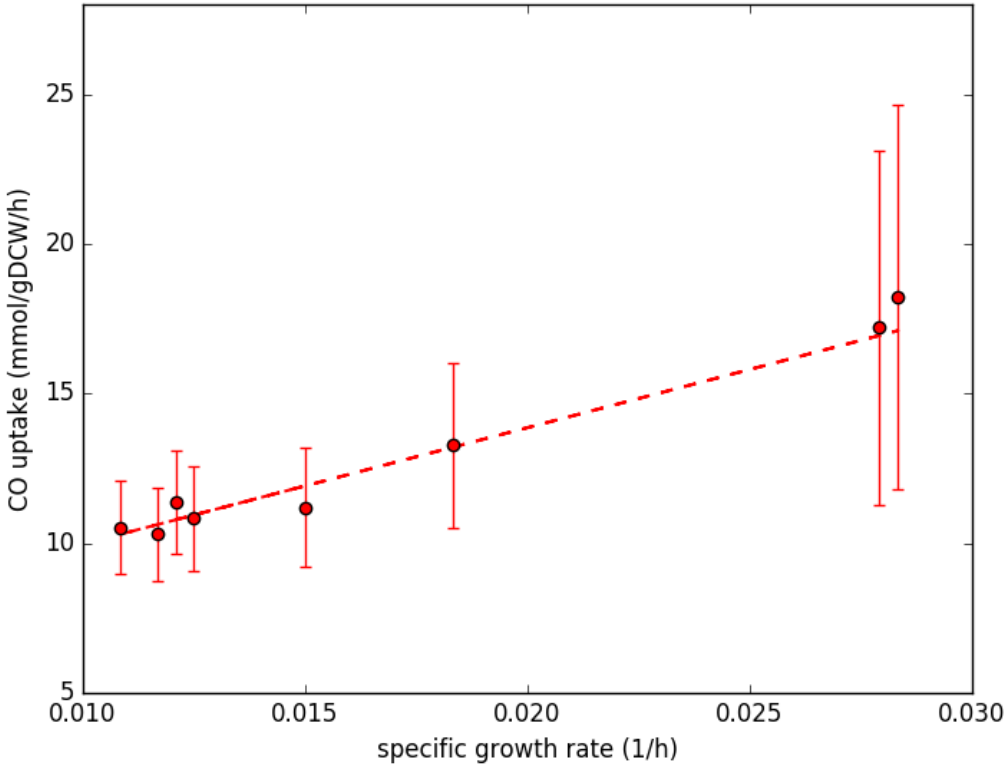
ATP maintenance costs

Methods:

- Vary dilution rate
- CO uptake
- Estimate ATP yields

Results:GAM = 108.9 mmol $gDCW^{-1}$
NGAM = 2.28 mmol $gDCW^{-1}$ h^{-1}
(gDCW/L)/OD = 0.340 ±0.015

Acknowledgements:
Anne Henstra, Louise Sewell



Validation

Product Spectrum

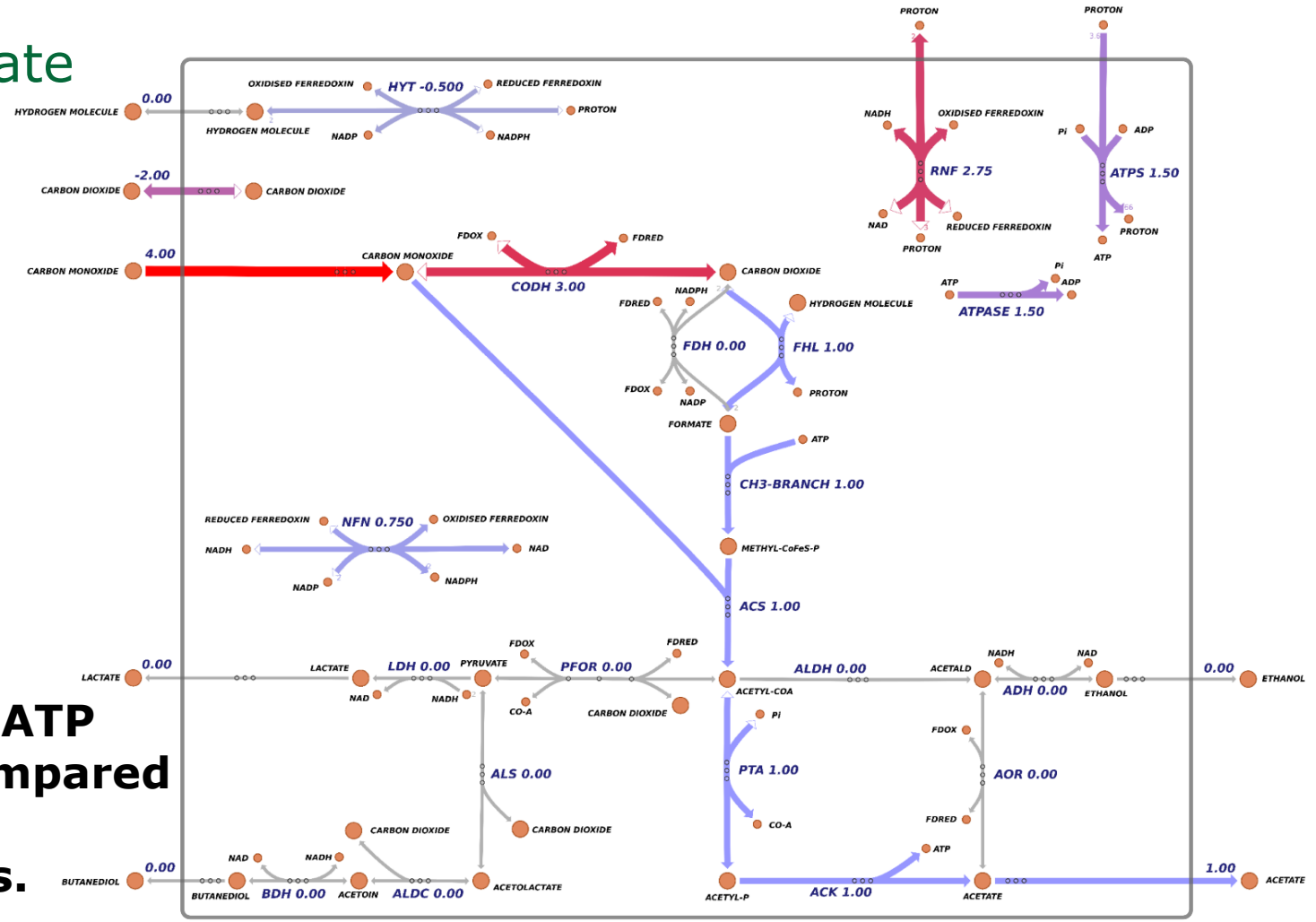
Methods:

- Elementary Flux Modes Analysis
- ATP yields (Y_{ATP}): v_{ATPase}/v_{CO}

Results:

- Valid net stoichiometries (Mock *et al*., 2015)
- Steady states exist for full range of expected products (Acetate, Ethanol, Lactate, 2,3-Butanediol)

Acetate



Highest ATP yield compared to other products.

Y_{ATP}: 0.375

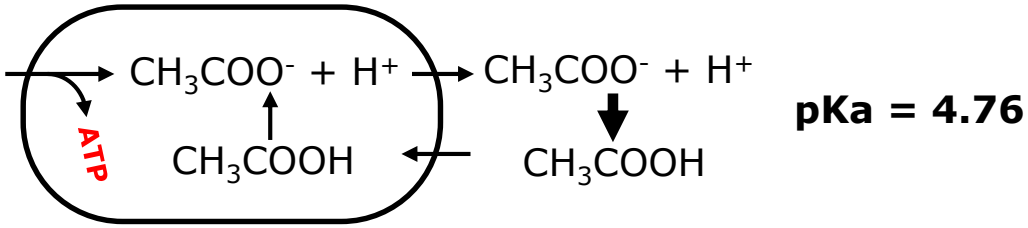
$$CO + 1/2\ H_2O \rightarrow 1/4\ CH_3COO + 1/4\ H^+ + 1/2\ CO_2$$

Bertsch & Müller, 2015:

"…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."

Alternative hypothesis:

pH-induced shift (5.0-4.5)



Flux Balance Analysis

Scan

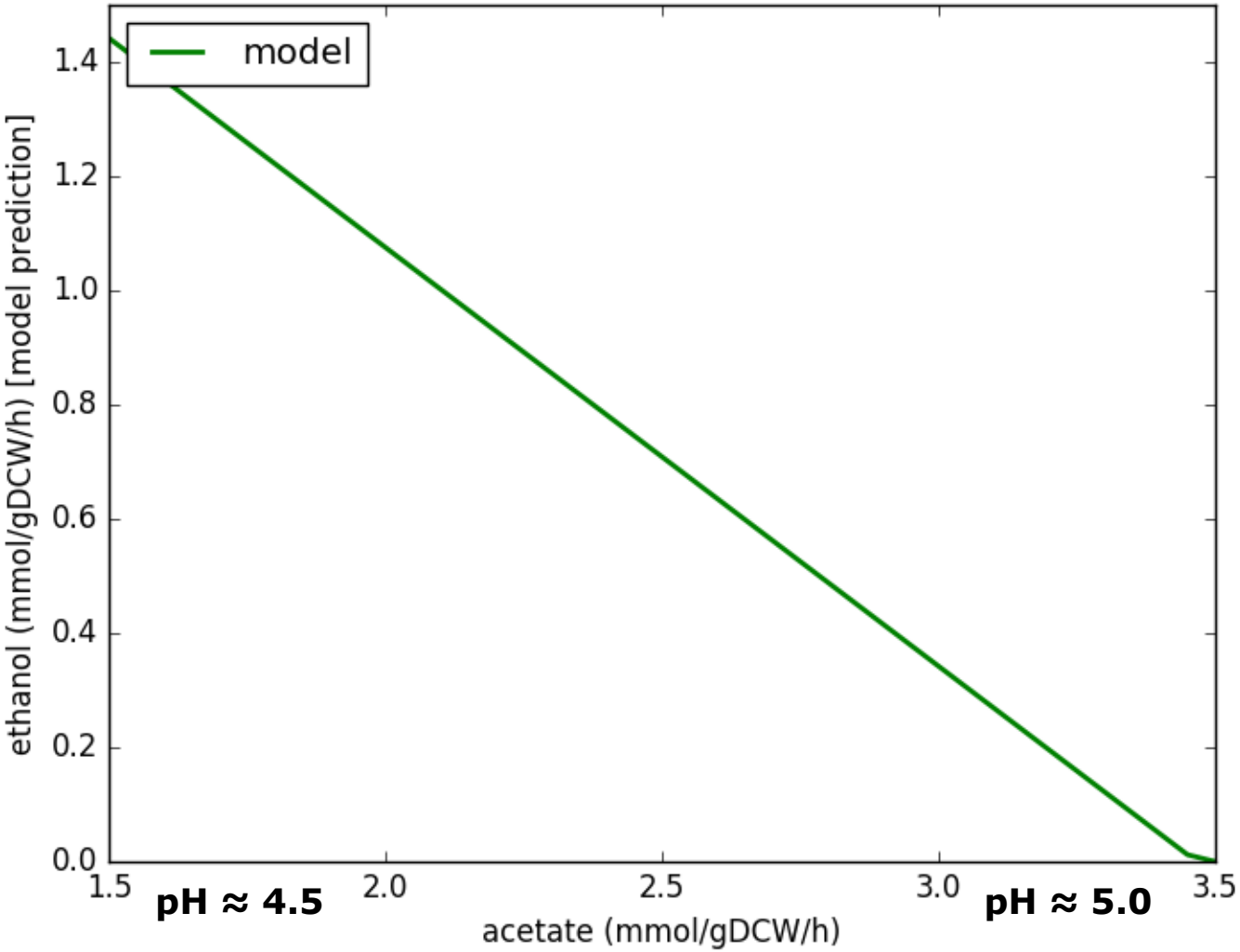
Minimize: |v| (minimize absolute value of all network reactions)
Steady state constraint (Sv = 0)
Fixed growth rate (μ)
Fixed GAM & NGAM maintenance costs
Range of fixed product flux values:

$$v_{min} \leq v_{product} \leq v_{max}$$

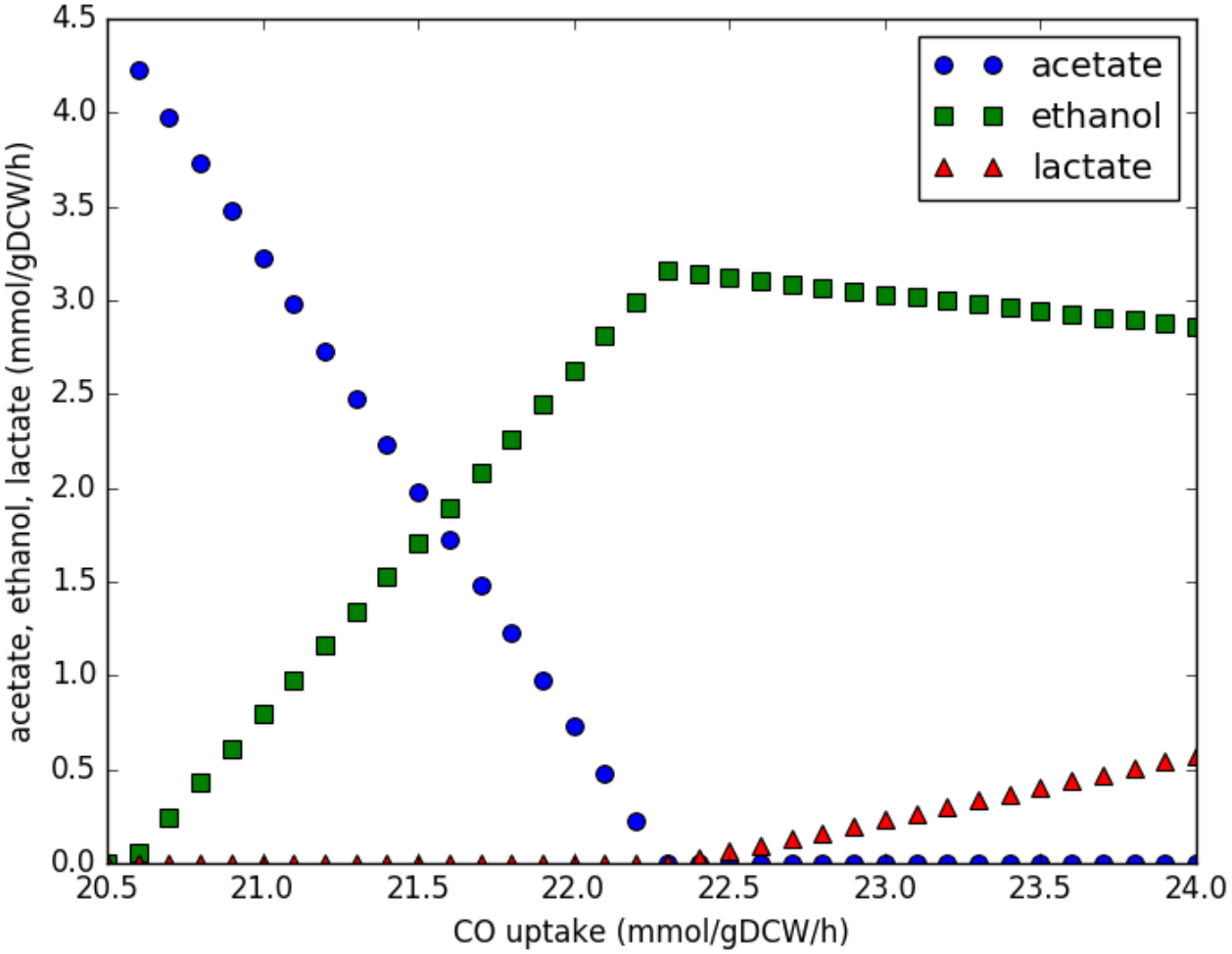
flux distribution for each value of $v_{product}$

What will be produced if acetate efflux is restricted, as it would be at lower pH?

pH shift


model
ethanol (mmol/gDCW/h) [model prediction]
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.5
2.0
2.5
3.0
3.5
pH ≈ 4.5
pH ≈ 5.0
acetate (mmol/gDCW/h)

Predicted effect of CO uptake on product spectra



Knock Out Prediction

Target Products (from CO) for Increased Yield

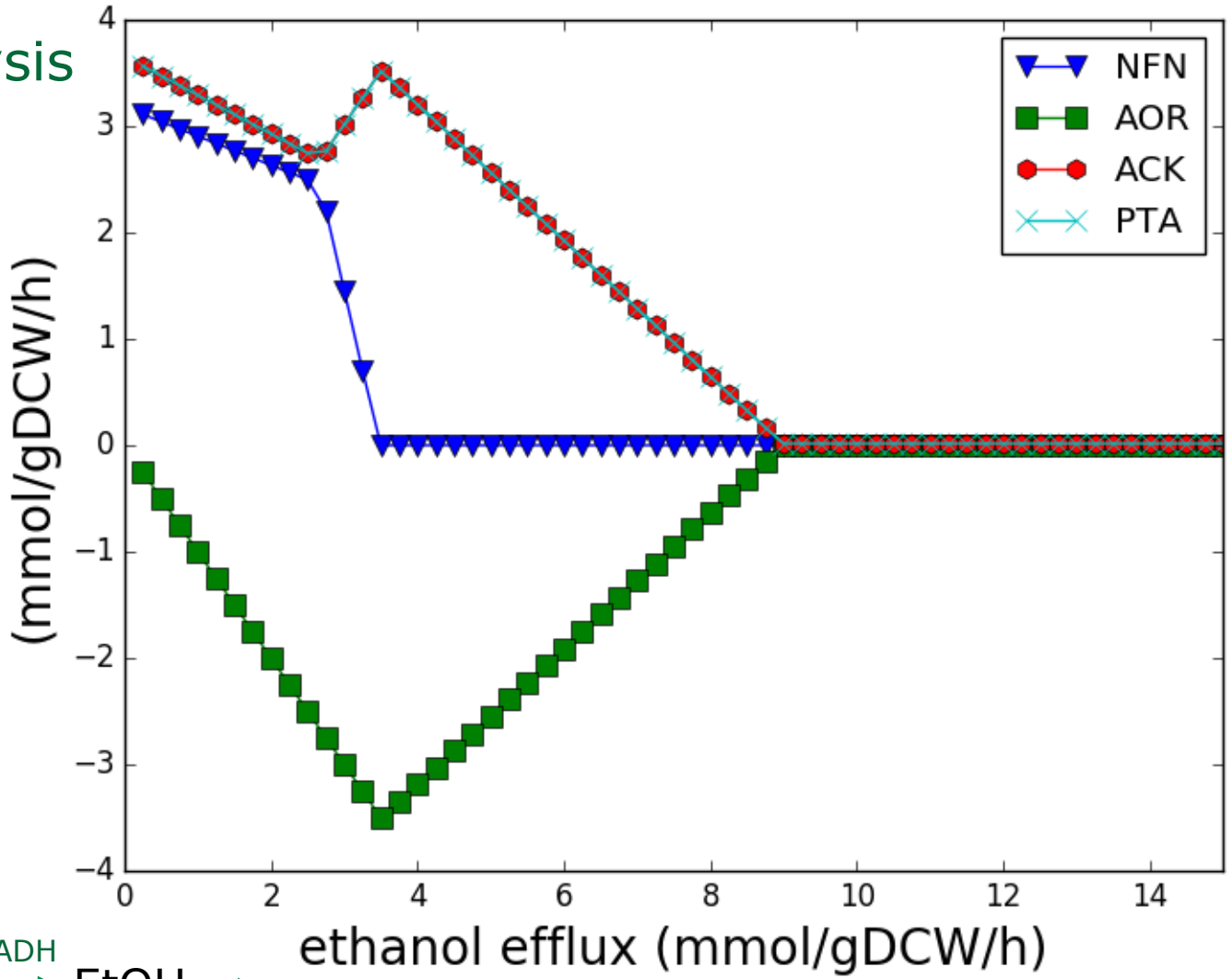
- Ethanol
- Hydrogen

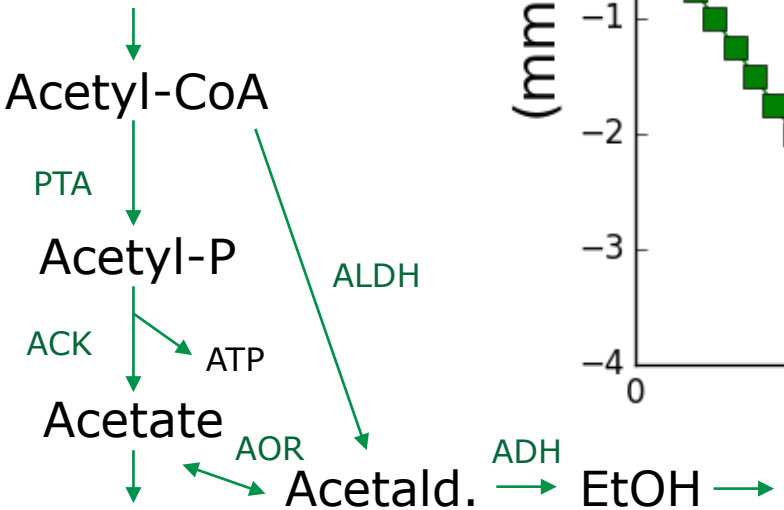
FBA scanning technique can be used to identify knock-out/-down targets.

Scanning Analysis

Ethanol

- Decreasing reactions
- Distinct phases





Scanning Analysis

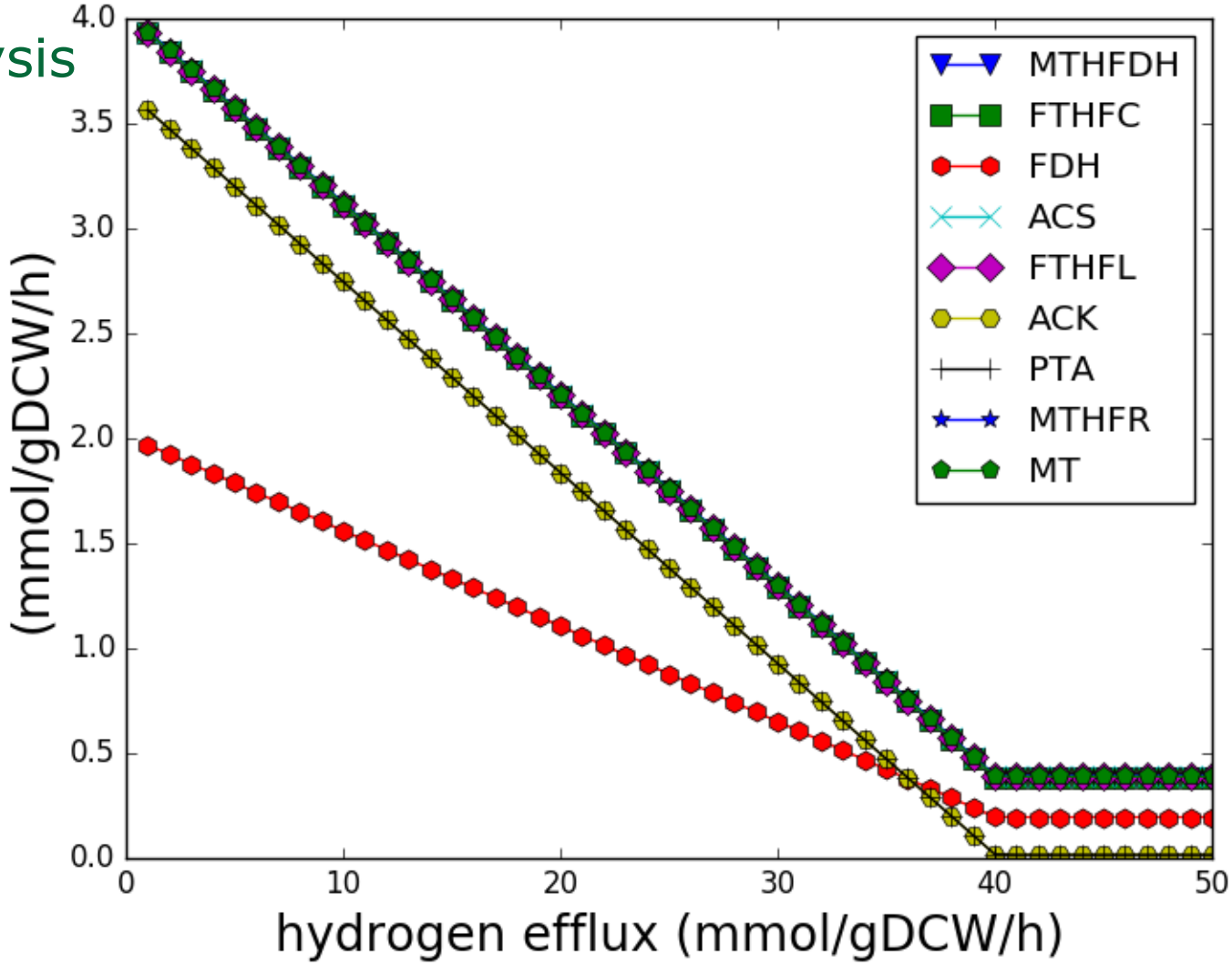
Ethanol

- Nfn: NADH + H^+ + 2 $NADP^+$ + $\mathbf{Fd_{red}}$ ⇌ NAD^+ + 2 NADPH + $\mathbf{Fd_{ox}}$
- Aor: ACETATE + $3H^+$ + $\mathbf{Fd_{red}}$ ⇌ ACETALDEHYDE + H_2O + $\mathbf{Fd_{ox}}$

Predicted ethanol efflux increased from 0.0 to 5.08 mmol $gDCW^{-1}$ h^{-1}

Scanning Analysis

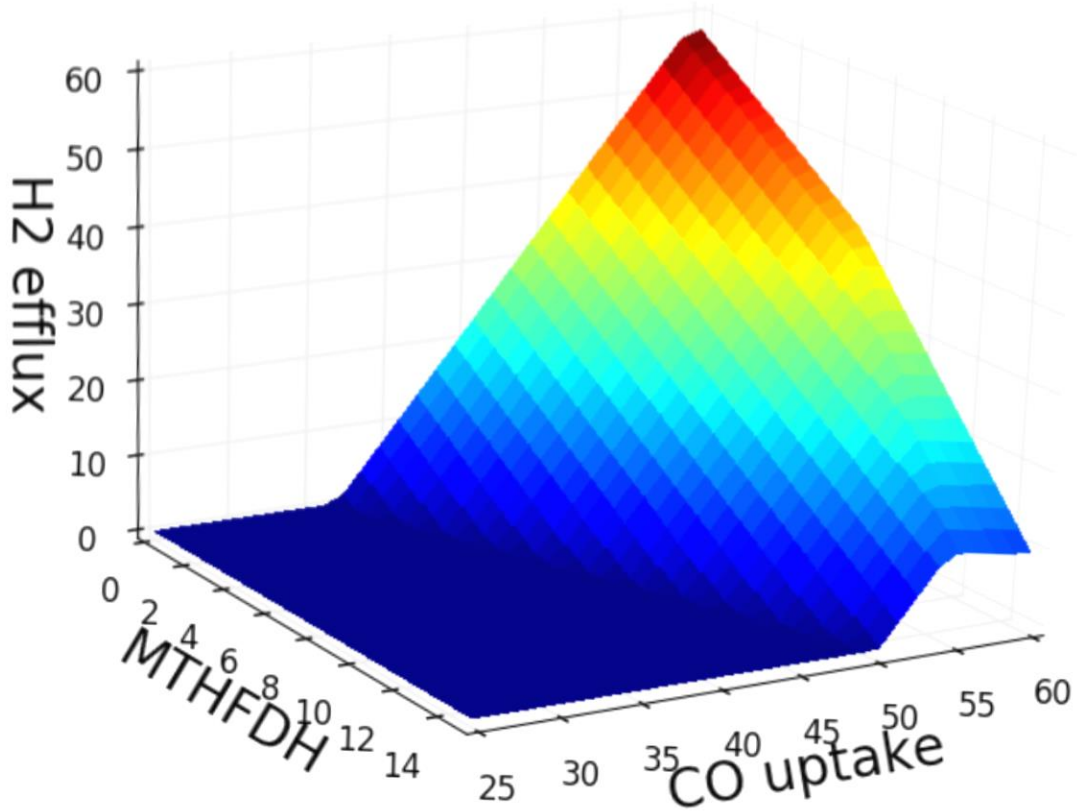
Hydrogen



WL pathway enzymes do not work as knock-out/knock-down targets in standard FBA.

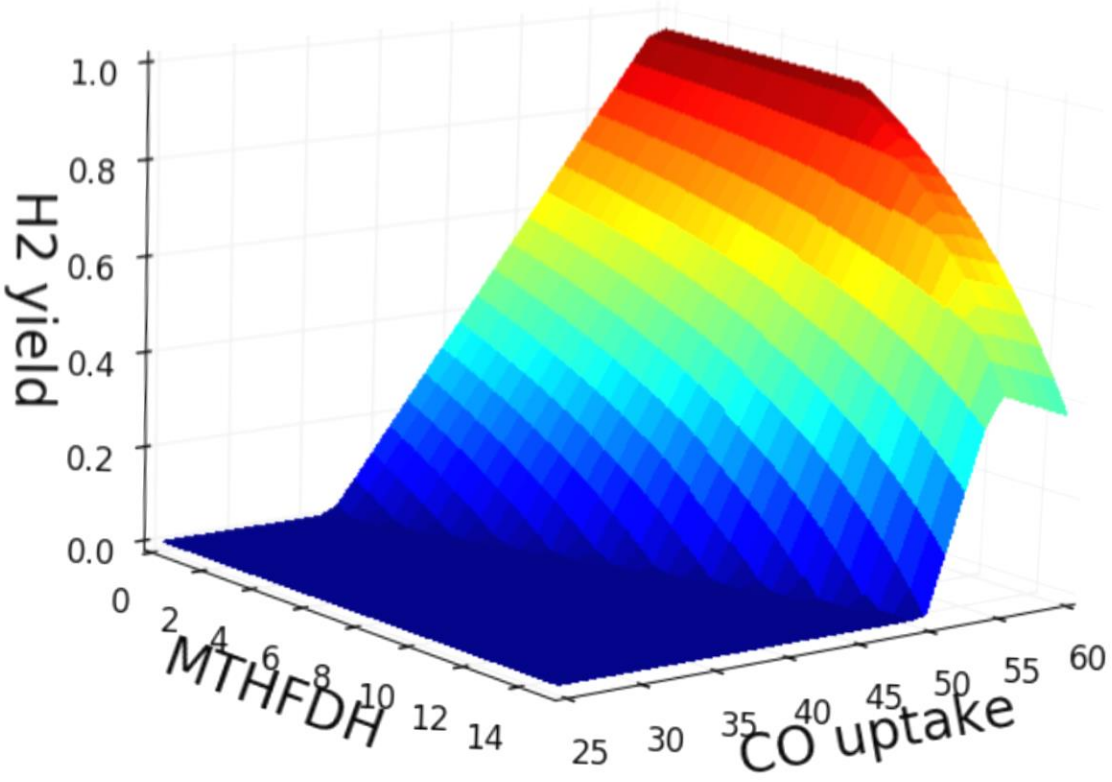
Scanning Analysis

Hydrogen



Scanning Analysis

Hydrogen



Conclusions

- Multi-disciplinary approach has produced a predictive model for platform chemical production in *C. autoethanogenum*
  - Experimentally derived parameters
  - Metabolic shifts
  - Knock-out/down targets