



Electron bifurcation mechanism and homoacetogenesis explain product yields in mixed culture anaerobic fermentations

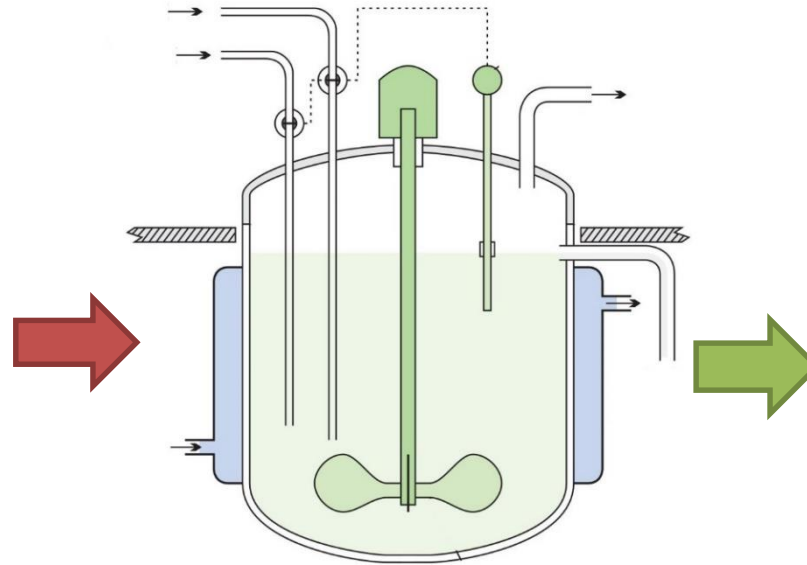
A. Regueira, R. González-Cabaleiro, D. Ofiteru, J. Rodríguez, J. M. Lema

*Department of Chemical Engineering
Universidade de Santiago de Compostela*

Mixed culture fermentations for a circular economy



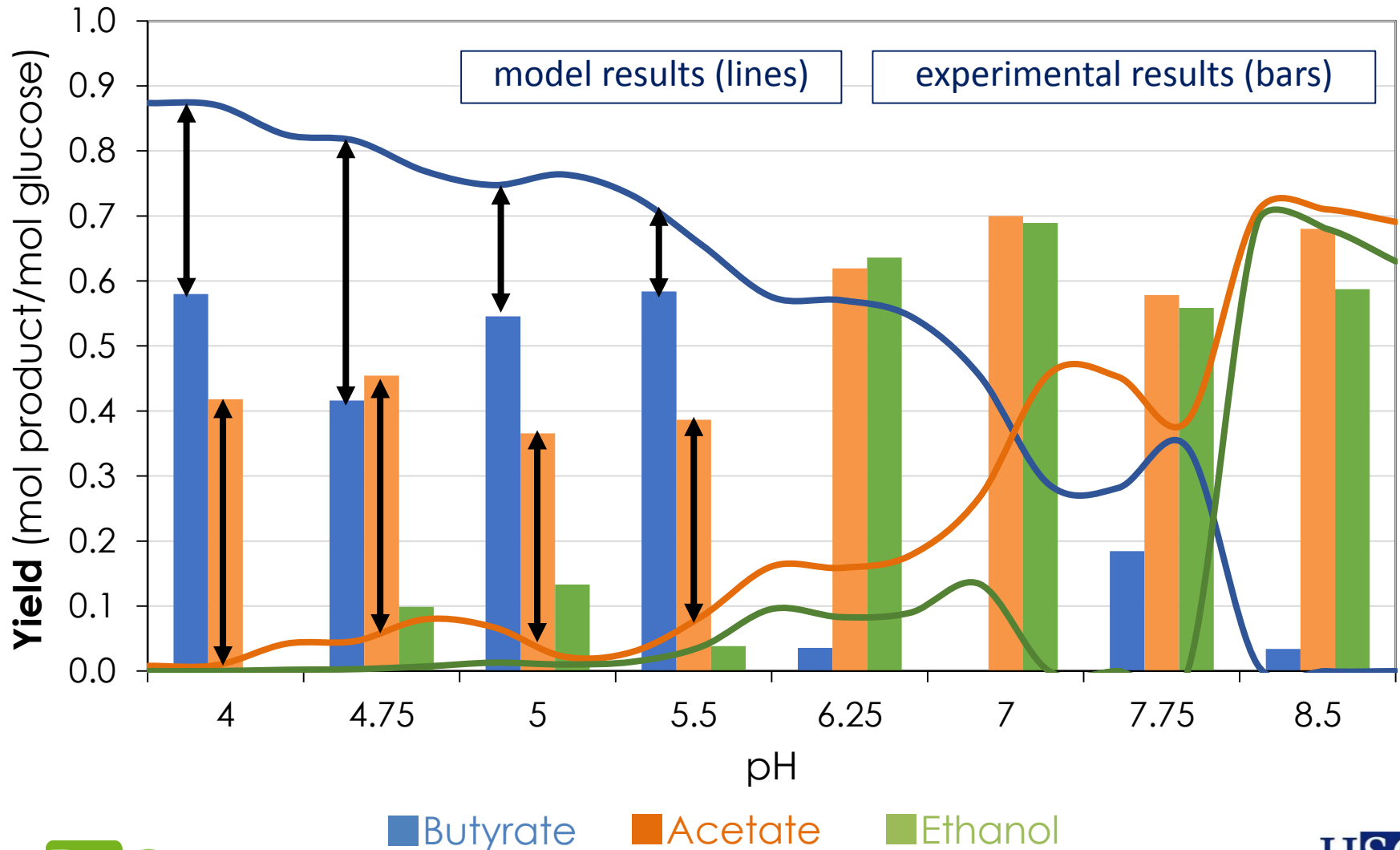
Wastes



Products with value

Product yield changes with pH

Model results: González-Cabaleiro et al. (2015). PLoS ONE
Experimental results: Temudo et al. (2007). Biotechnol. Bioeng.

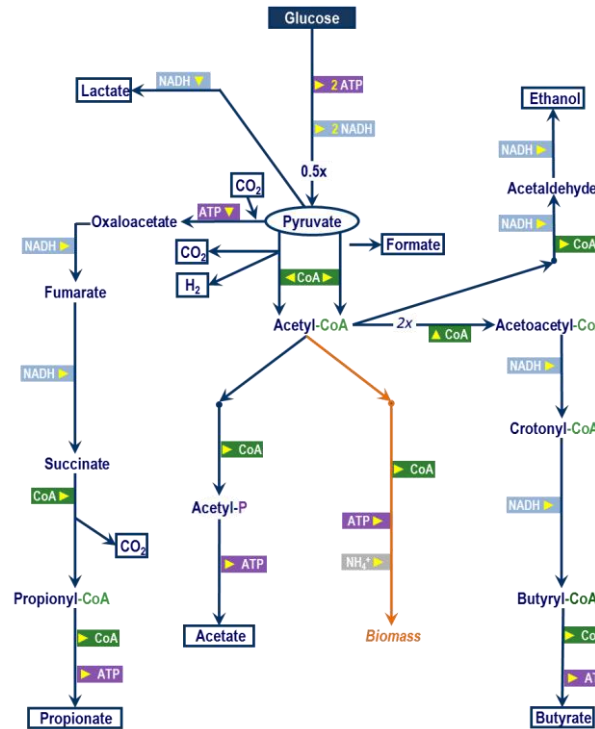


Maybe... the metabolic
network is incorrect?

How can we test the metabolic network?

Experimental yields of products
(Acetate, Propionate, Butyrate, Ethanol)

Info In



NADH balance

H₂ yield

CO₂ yield

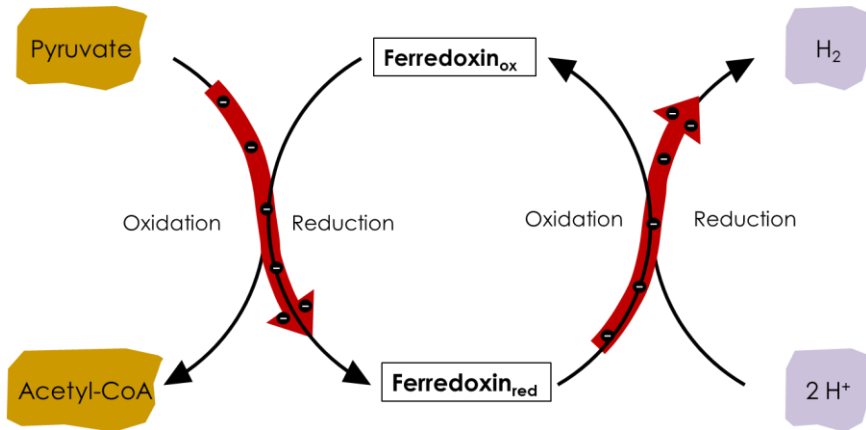


Info Out

Metabolic network

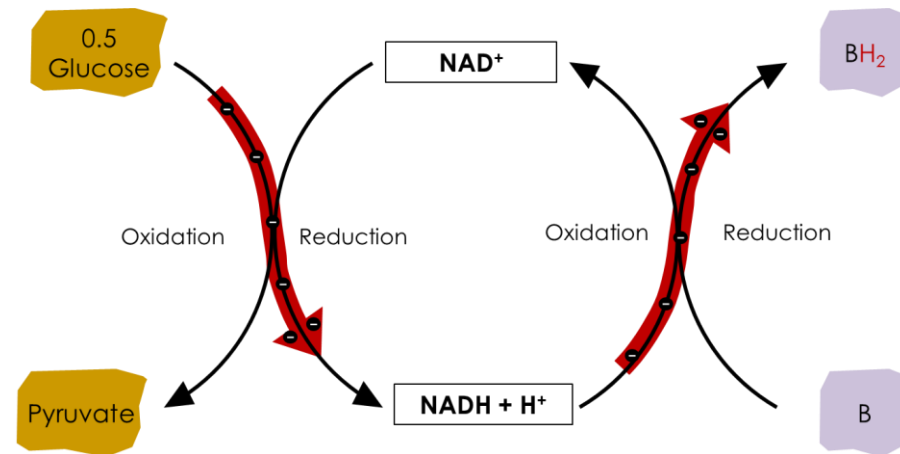
The electron carriers considered

Ferredoxin



$$E^{\circ'} \approx -400 \text{ mV}$$

NADH/NAD⁺



$$E^{\circ'} \approx -320 \text{ mV}$$

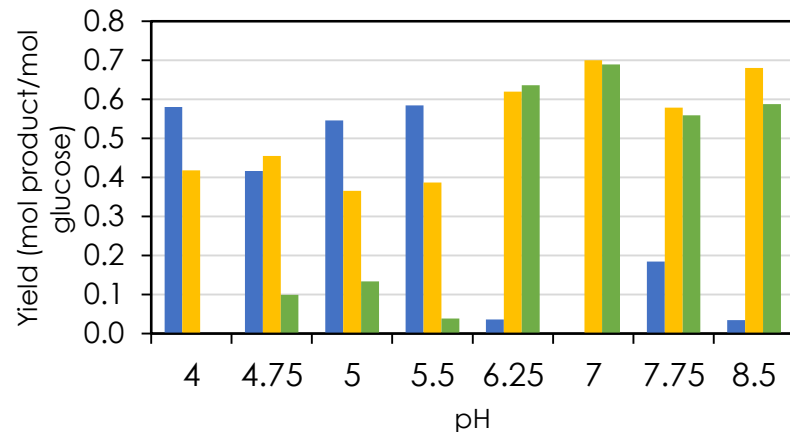
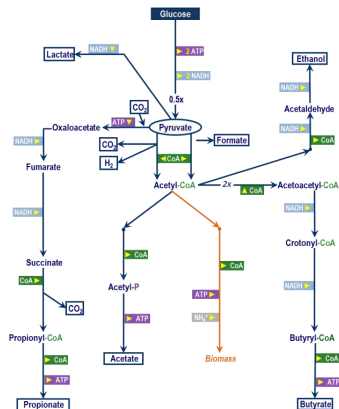
Why performing NADH balances?

- An accurate network will result in a neutral NADH balance:

$$\sum_{i=\text{products}} \vartheta_{NADH,i} \cdot y_i = 0$$

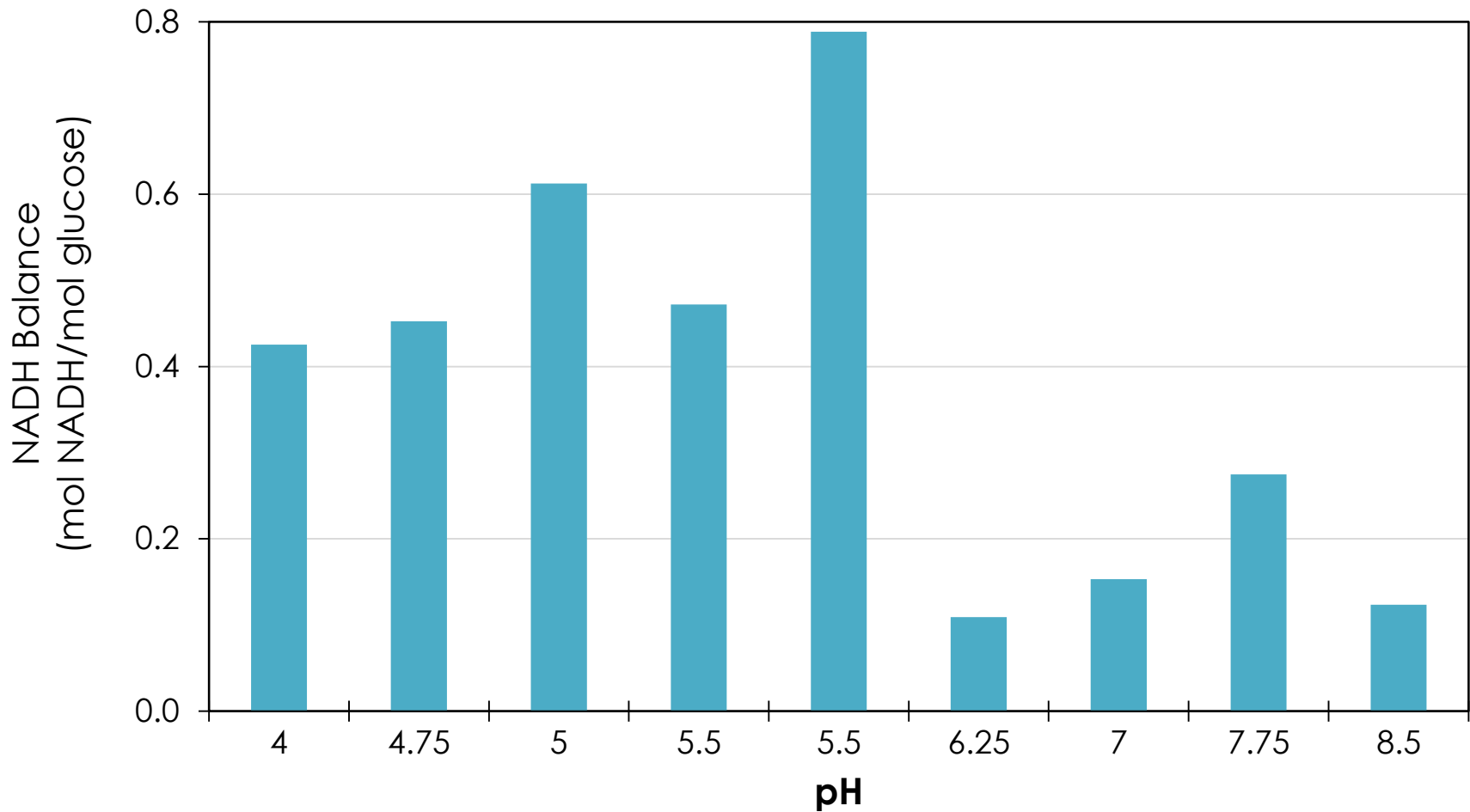
Stoichiometric coeffs.

Experimental yields



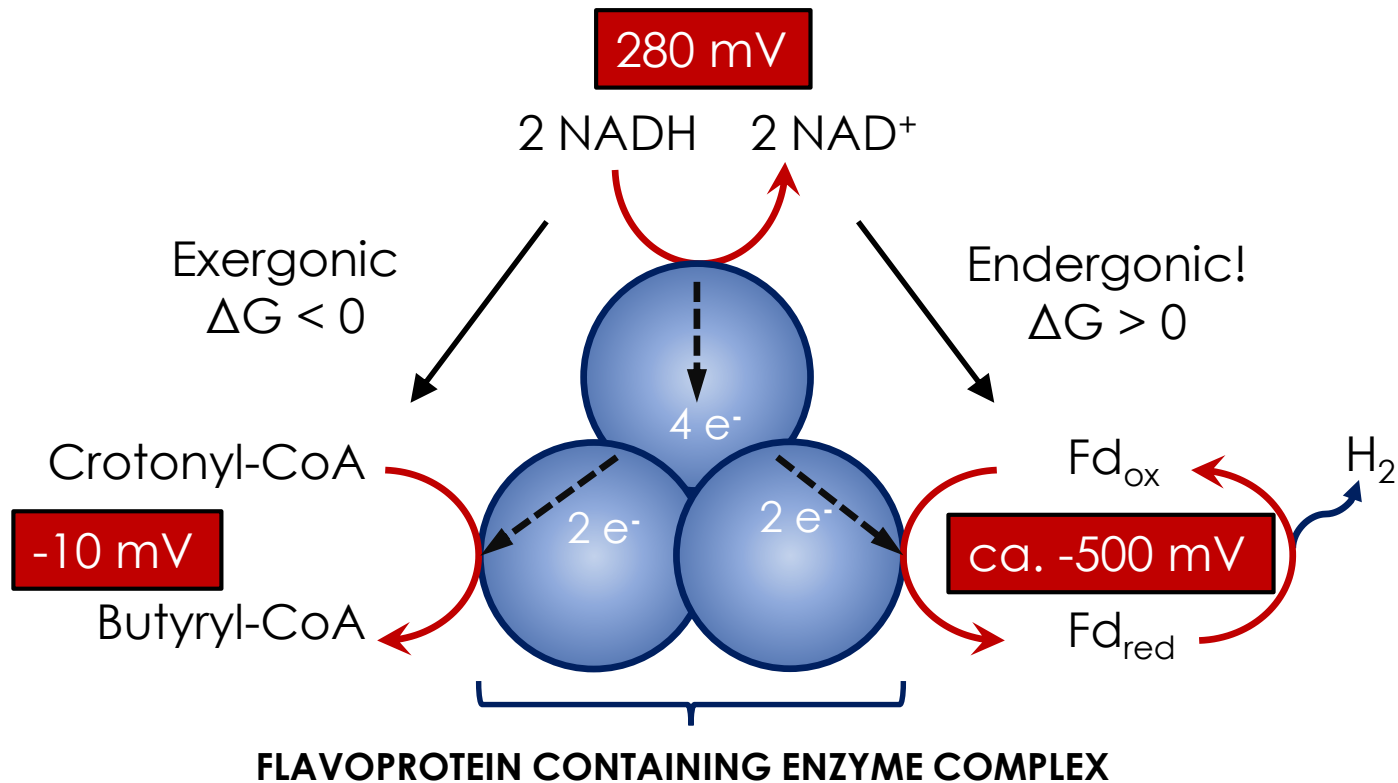
■ Butyrate (exp) ■ Acetate (Exp) ■ Ethanol (exp)

NADH is not conserved

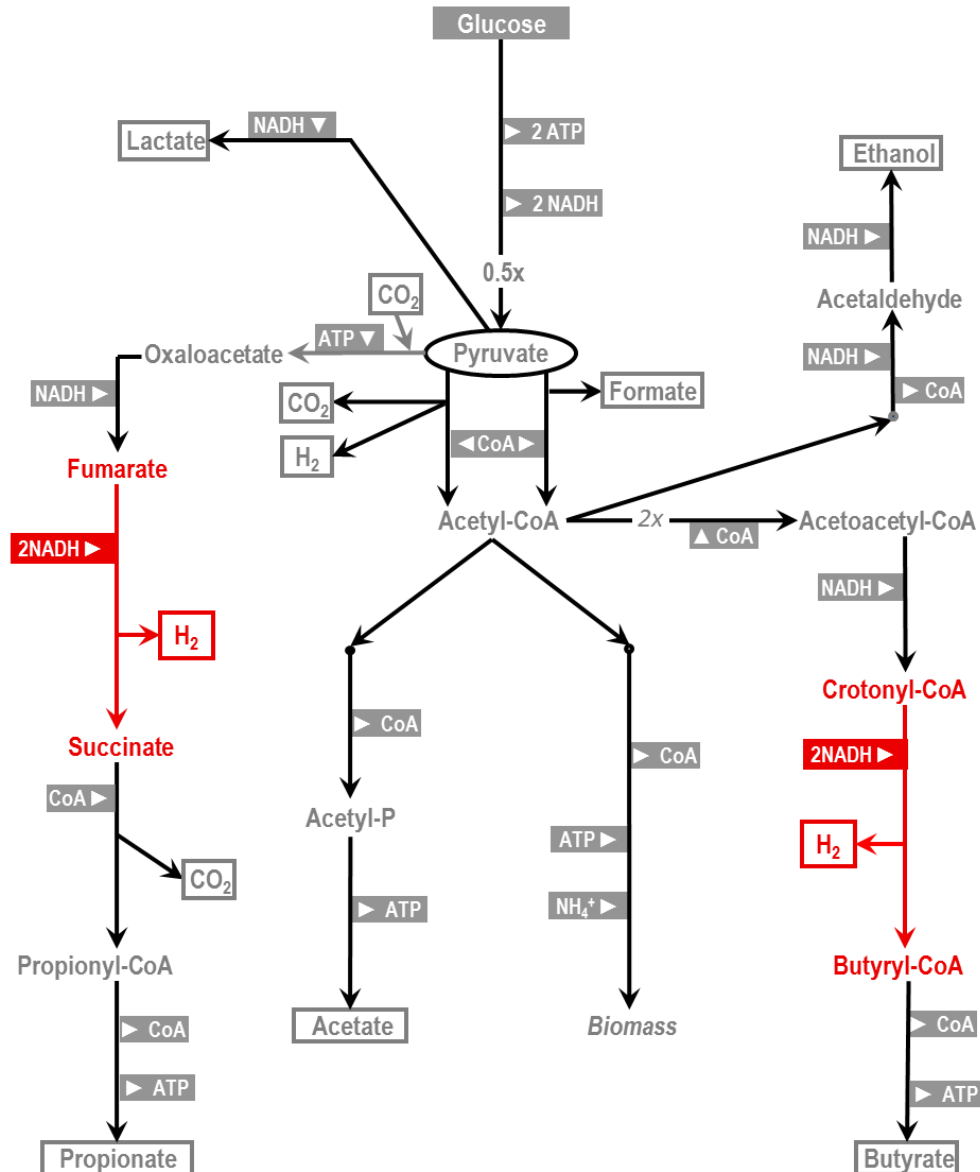


Electron bifurcation: a novel biochemical mechanism

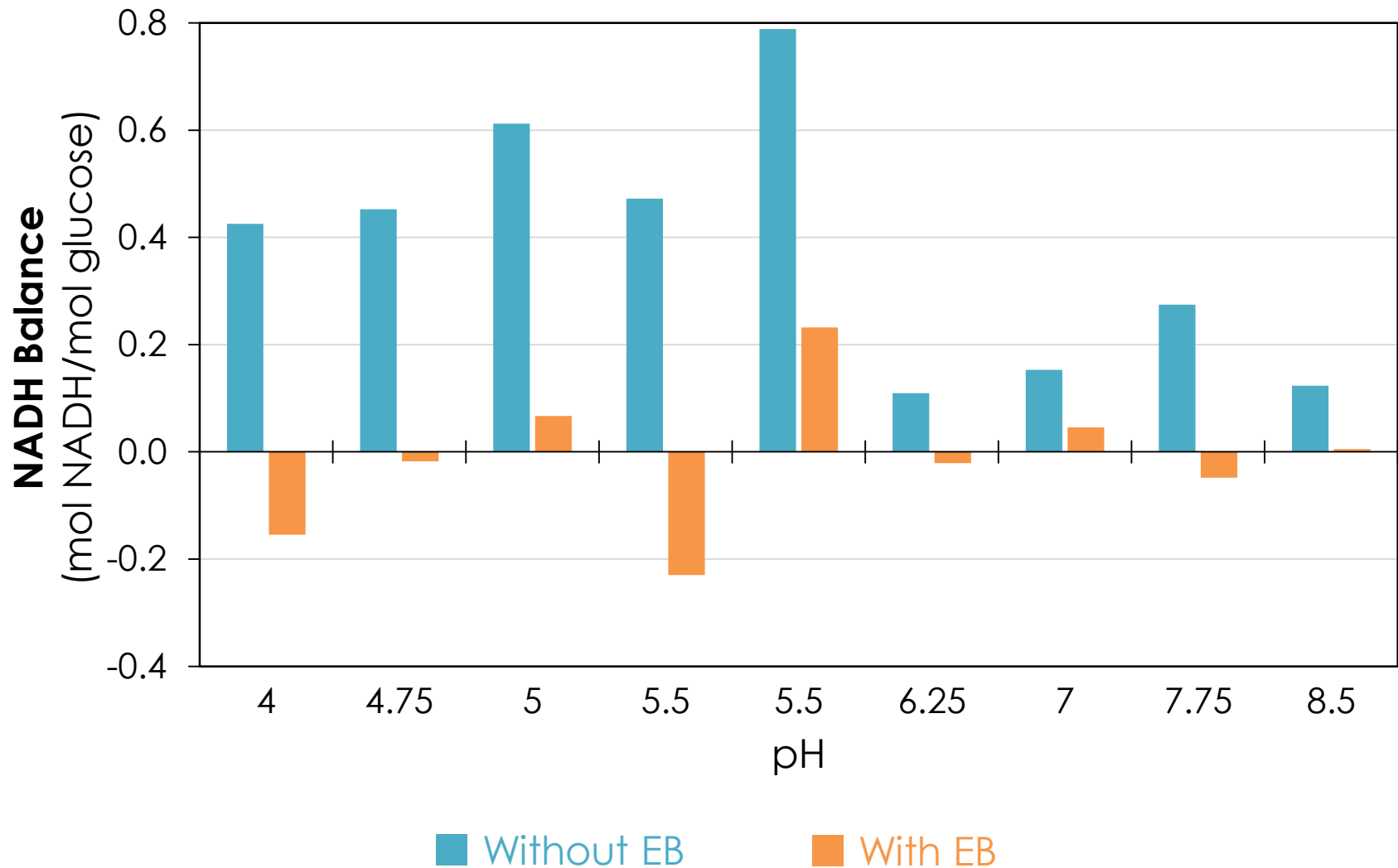
- Only observed *in vitro* (*Clostridium pasteurianum*)



Electron bifurcation changes the stoichiometry



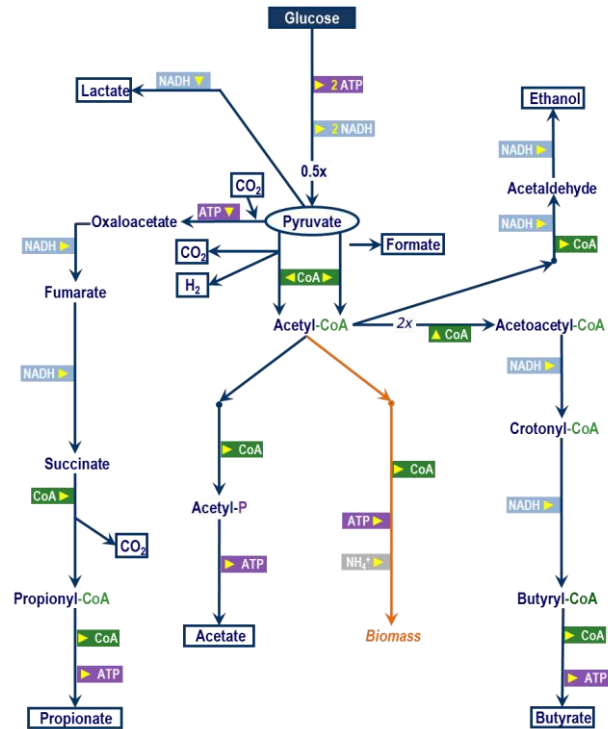
Electron bifurcation reduces the error in the e⁻ balances



How can we test the stoichiometry?

Experimental yields of products (Acetate, Propionate, Butyrate, Ethanol)

Info In



NADH balance ✓

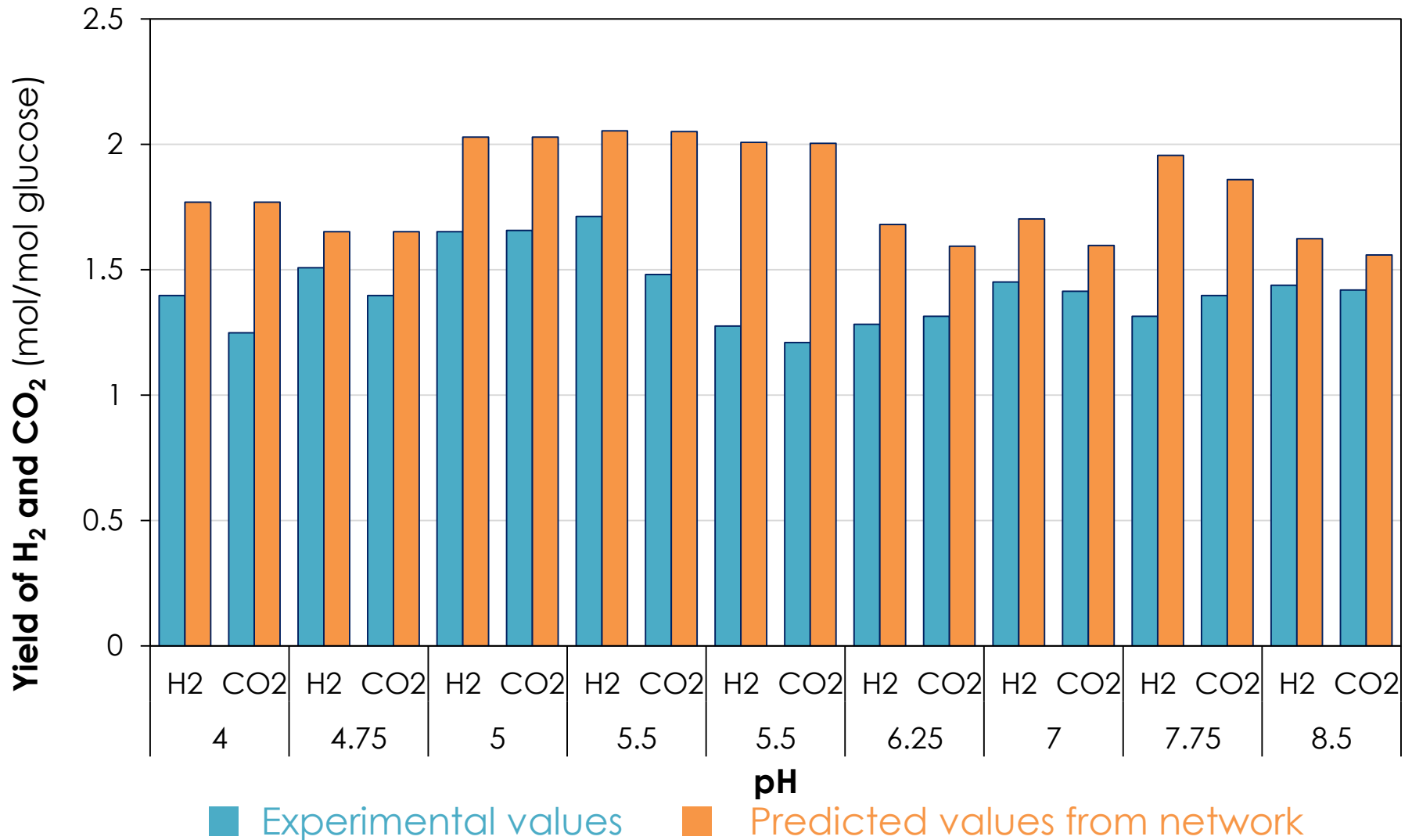
H₂ yield

CO₂ yield

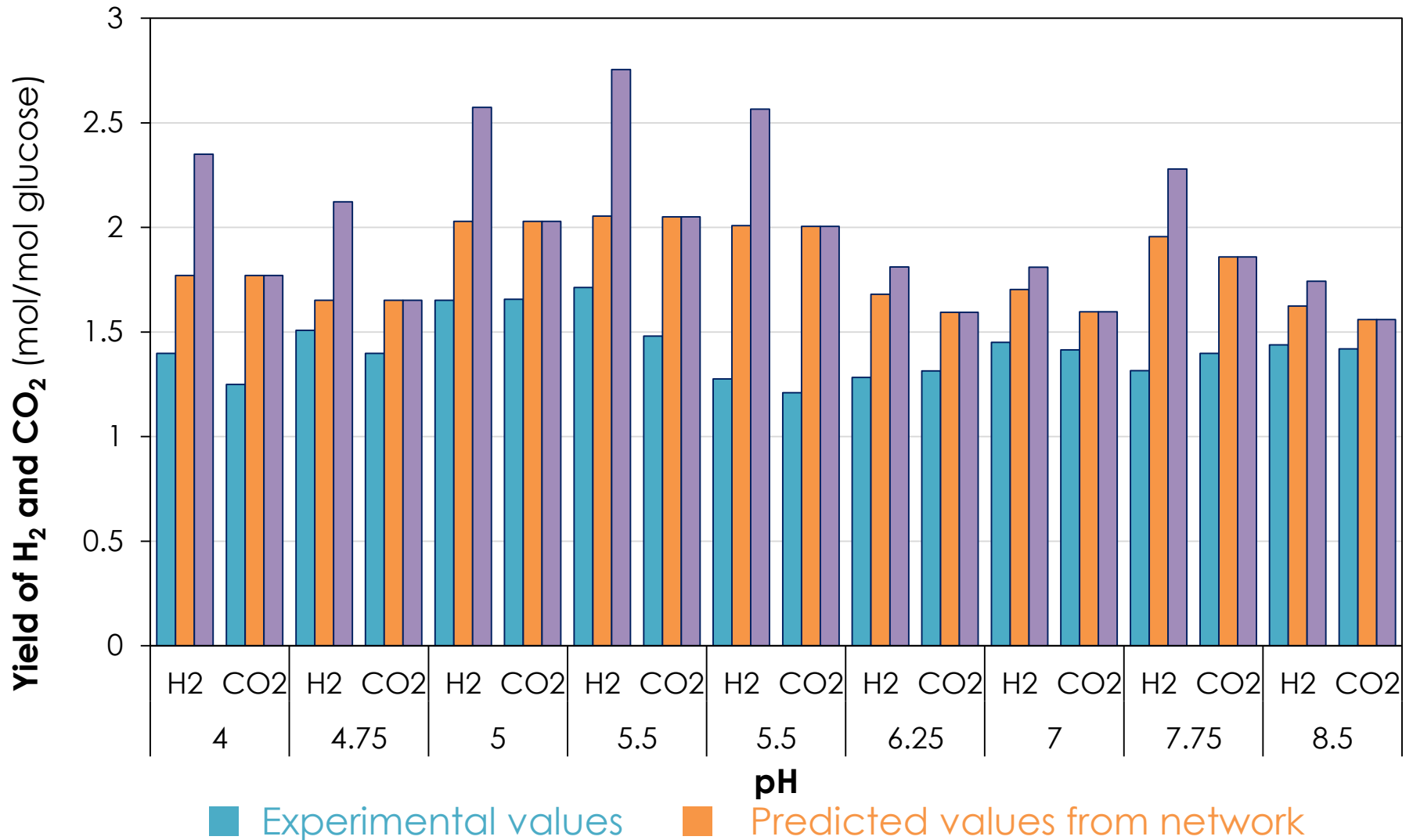
Info Out

Stoichiometry

H₂ and CO₂ yields do not fit either



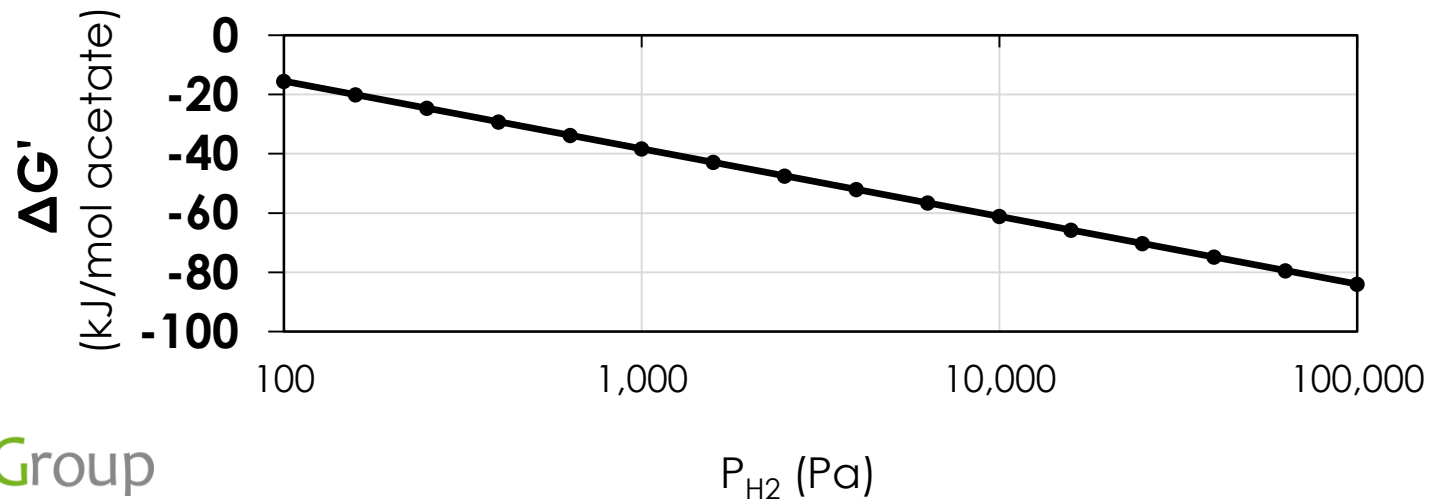
Electron Bifurcation does not solve the problem



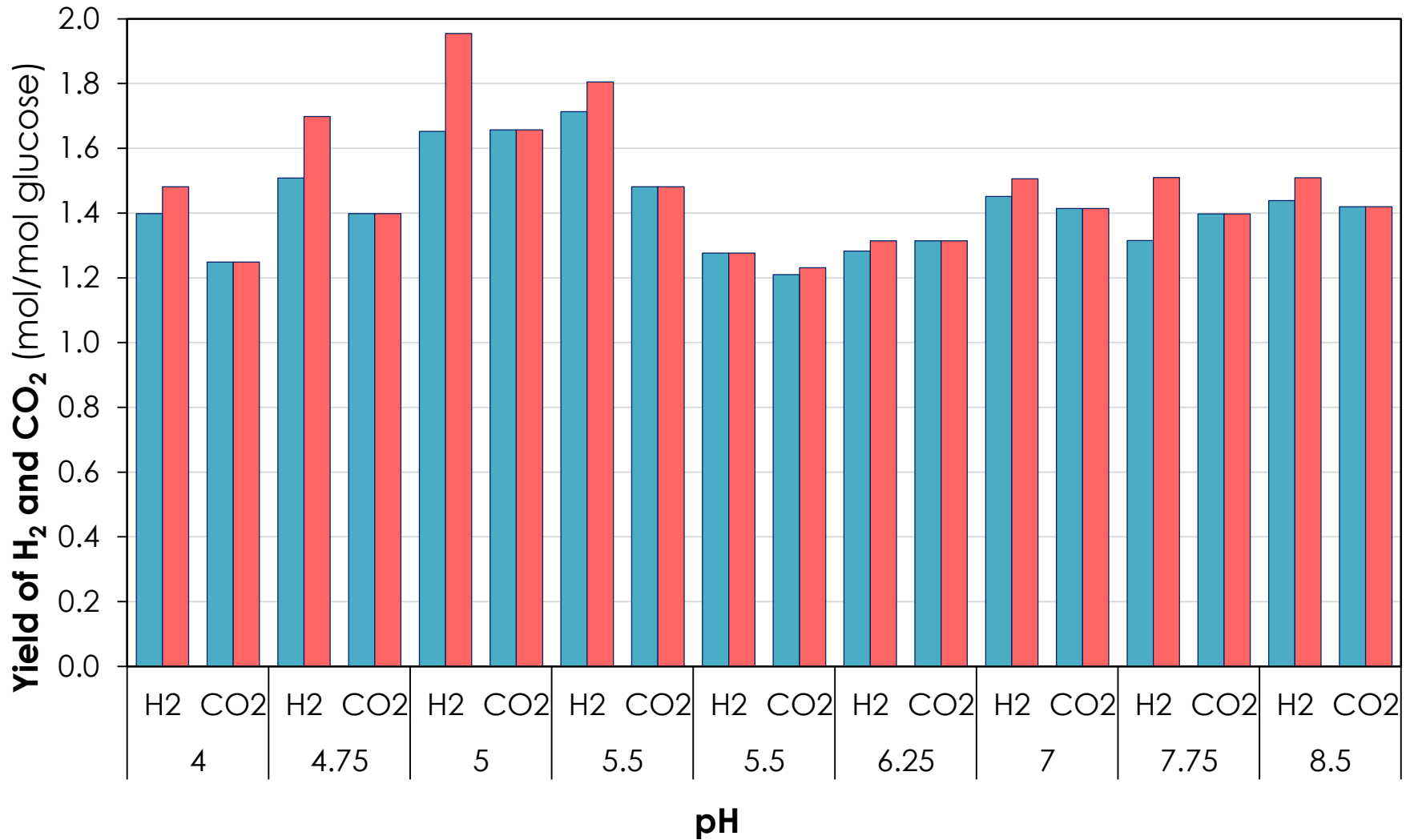
Possibilities to explain the H₂ and CO₂ yields observed

- Reduced Ferredoxin does not yield H₂
 - Could be oxidized in highly endergonic reactions in the anabolic process.
 - Could participate in other catabolic reactions as electron donor instead of NADH
- Homoacetogenesis $4\text{H}_2 + 2\text{HCO}_3^- + \text{H}^+ \rightarrow \text{Acetate} + 4\text{H}_2\text{O}$

Homoacetogenesis is exergonic in fermentations

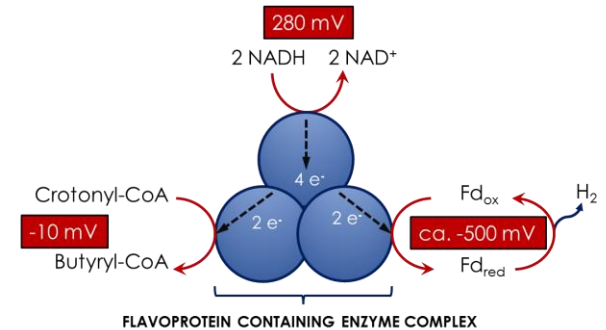


Homoacetogenesis explains the H₂ and CO₂ yields observed



Conclusions

- Electron Bifurcation is likely a mechanism commonly used by fermenters
- Homoacetogenesis could possibly occur and explain the H_2 and CO_2 yields observed
- The new network will help to improve model predictions and bioprocess design.



Acknowledgements

- Spanish Ministry of Education through FPU scholarship (FPU14/05457)



- Smart green gas



- Biogroup and CRETUS Strategic Partnership (AGRUP2015/02)





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alberte.regueira@usc.es

*Department of Chemical Engineering
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