MELISSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

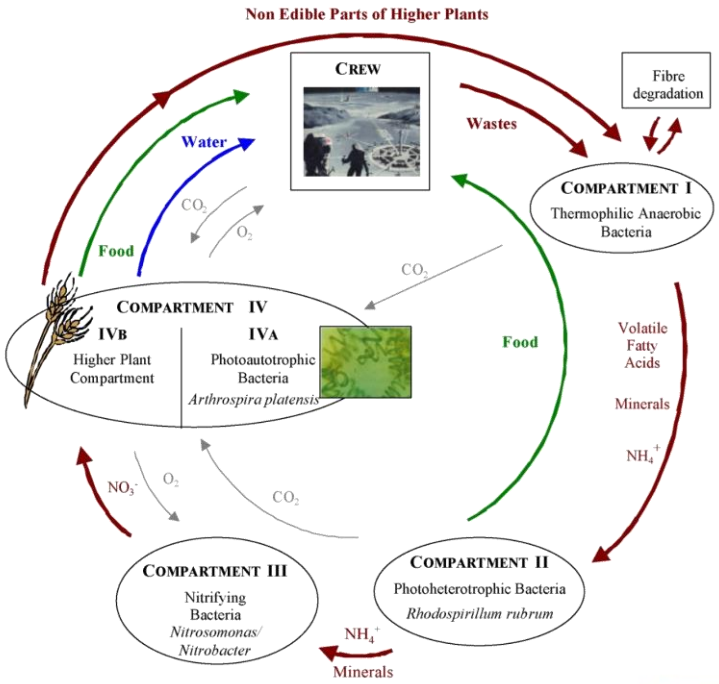
Bioenergetic modelling for predicting and steering VFA production in carbohydrates anaerobic fermentation

Alberte Regueira, L. Ma, A. Ashraf, N. Kamot, R. Ganigué

CMET
Center for Microbial Ecology and Technology

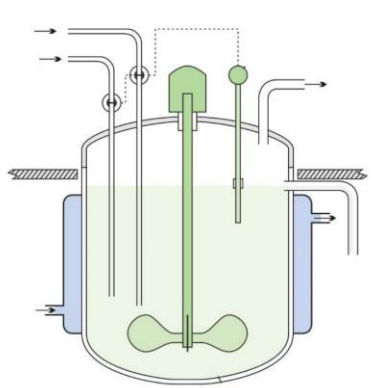
GHENT UNIVERSITY

The C1 compartment is an open-culture fermenter



Crew waste

Fibers



$CO_2 + H_2$

Volatile fatty acids

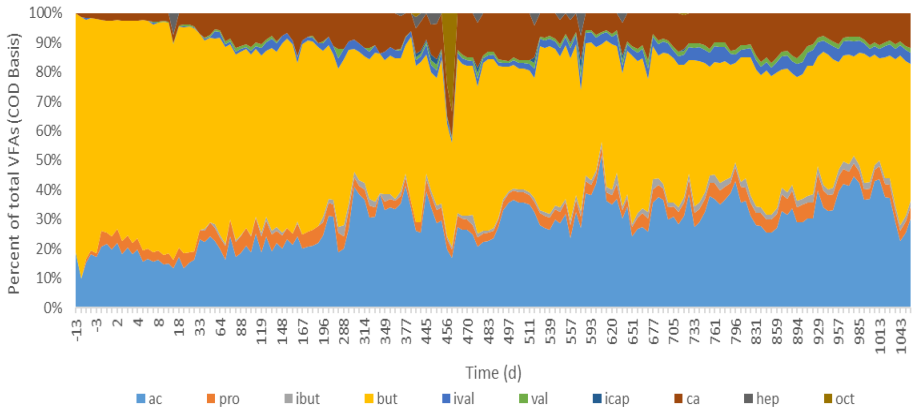
Sludge

C1 compartment

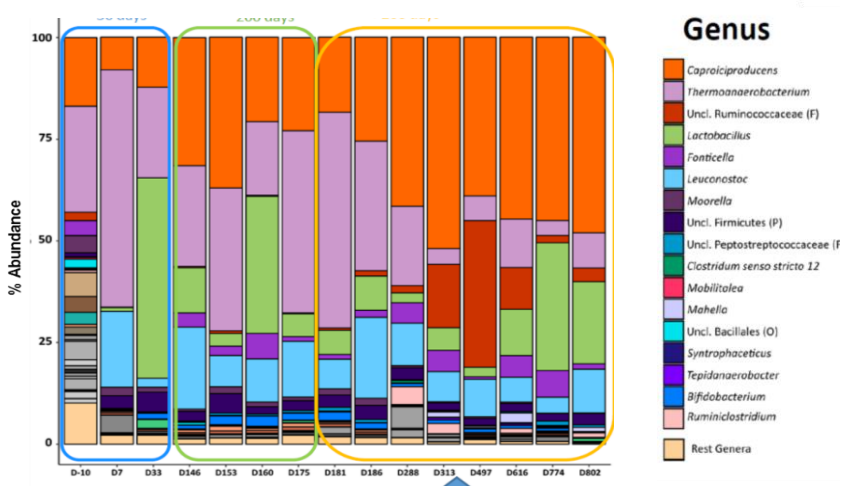
- Open-culture bioreactor
- pH 5.5
- Thermophilic temperature: 55°C

We know the overall product spectrum and the microbial community composition

Product spectrum



Microbial community composition



We need to expand our knowledge

$CO_2 + H_2$

Crew waste

Fibers

Volatile fatty acids

Synthetic microbial community

Protein hydrolyser

Fiber degrader

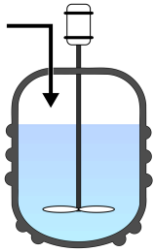
Carbohydrate fermenter

- ✓ **Mathematical modelling** can be a **powerful tool** for this knowledge mission
- ✓ **Bioenergetic** models for **stoichiometry** prediction

Different kinds of mathematical modelling for different purposes

Kinetic unstructured models

- ✓ Biomass is a black box
- ✓ Solve the macroscopic mass balances
- ✓ Variable selectivity is not addressed

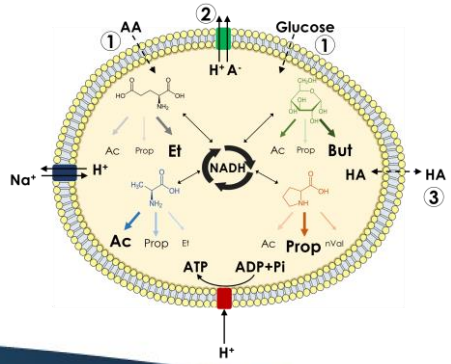


$$\frac{dC_i}{dt} = D \cdot \left(C_i^{IN} - C_i\right) + r_{j,i}$$

$$r_{j,i} = r_{max,j} \cdot \frac{C_i}{C_i + K_{j,i}} \cdot X$$

Bioenergetic models

- ✓ Intracellular processes are modelled
- ✓ Cell-environmental interactions
- ✓ Their task is limited to predict the process stoichiometry



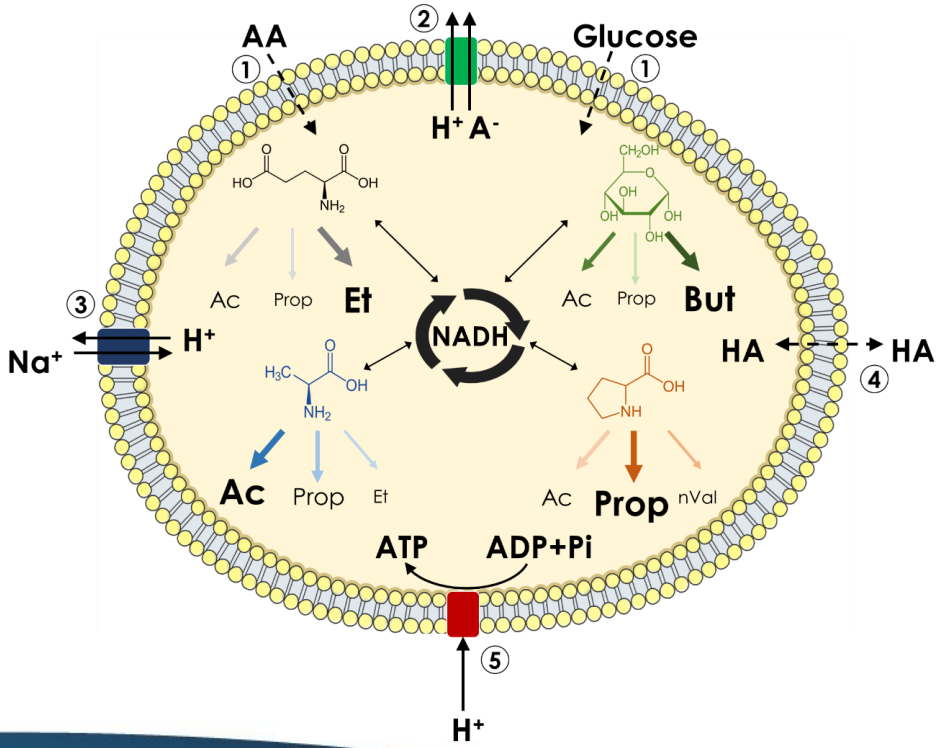
The microbial community is modelled as an enzyme soup

Reality: **Multiple** species performing different or similar metabolic functions

Model: **One** virtual species is able of performing **all** the metabolic functions of the community

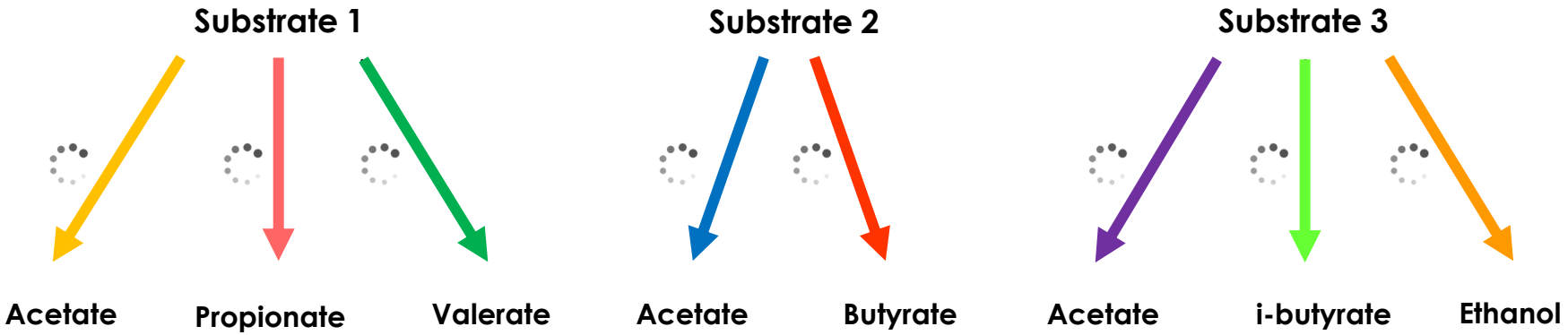
One virtual microorganism does all the possible processes

1. Substrate transport
2. Active transport of products
3. Na^+/H^+ pump (pH regulation)
4. Passive transport of products
5. ATP production (ATPase)



Flux balance analysis to determine product selectivity

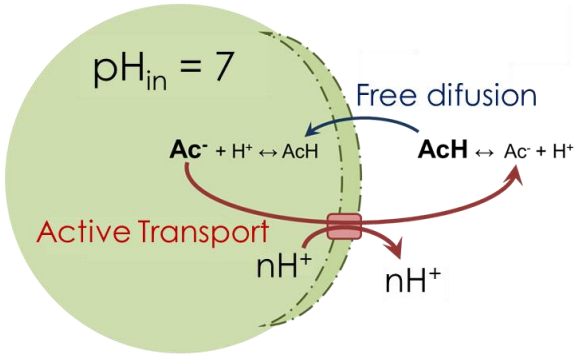
- FBA determines the metabolite flow throught the pathways of the metabolic network
- The flow distribution maximises a given objective (e.g. maximum growth rate)



Fermentative microbes maximise energy production

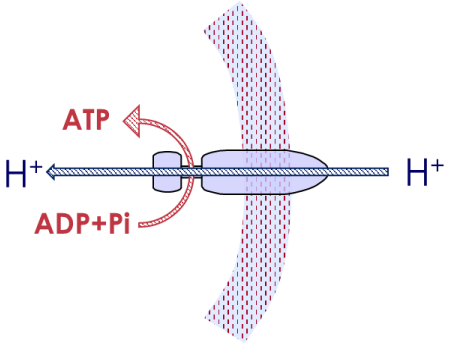
ATP production from the substrate is **maximised**

Pathway selection $\longrightarrow$ $r_{ATP} = r_{TRANSPORT} + r_{PMF} + r_{CAT}$

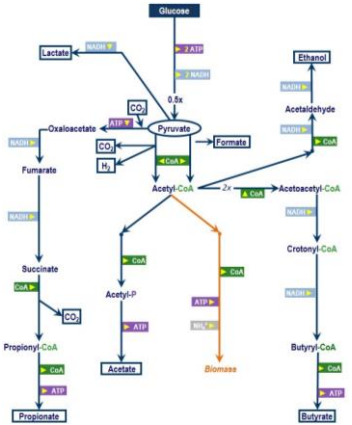


Transport

$\Delta V = -0.2$ V

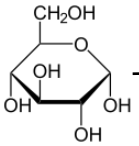


Proton translocations

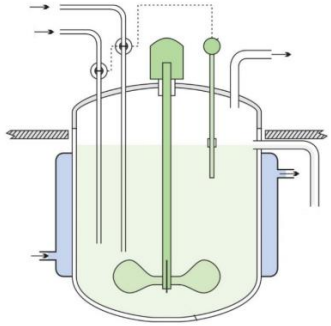


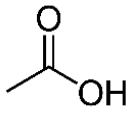
Catabolic ATP

Developing a bioenergetic model for the C1 compartment

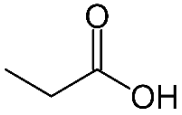


Glucose

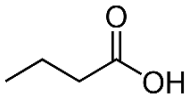




Acetate



Propionate



Butyrate

Ethanol

55°C

4.5 – 5.5 – 6.5

Objectives

- Develop bioenergetic thermophilic model for fermentation
- Study the effect of the pH on product spectrum
- We will first focus on glucose

Adaptations for thermophilic conditions

Thermodynamics

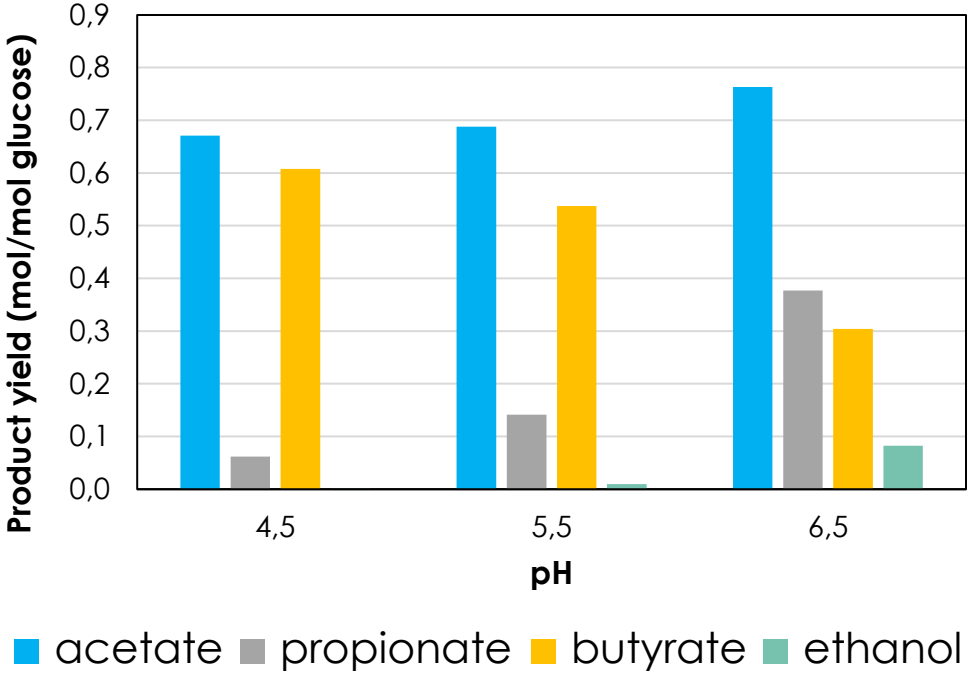
- Gibbs-Helmholtz equation to account for temperature effect on ΔG determination

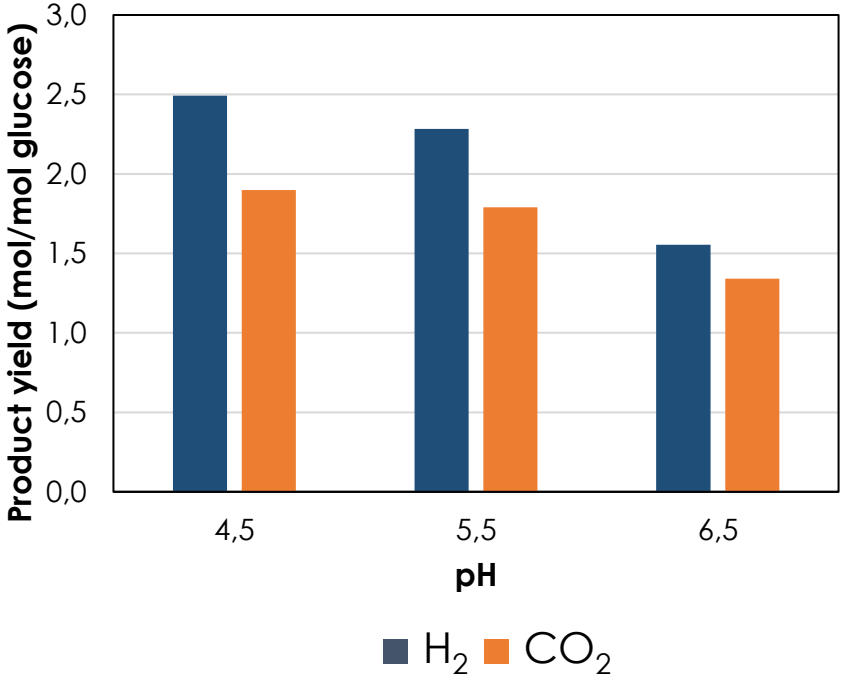
$$\Delta G^0_{r,T_{act}} = \Delta G^0_{r,T_{ref}} \cdot \frac{T_{act}}{T_{ref}} + \Delta H^0_{r,T_{ref}} \cdot \frac{T_{ref} - T_{act}}{T_{ref}}$$

Microbial diversity

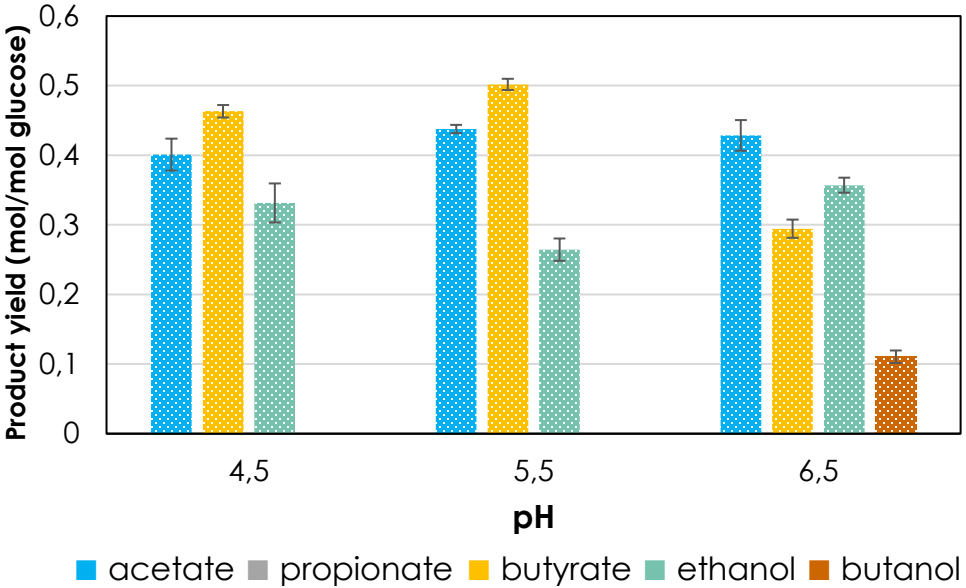
- Diversity is expected to be reduced at thermophilic conditions
- Adapting the model is an iterative process

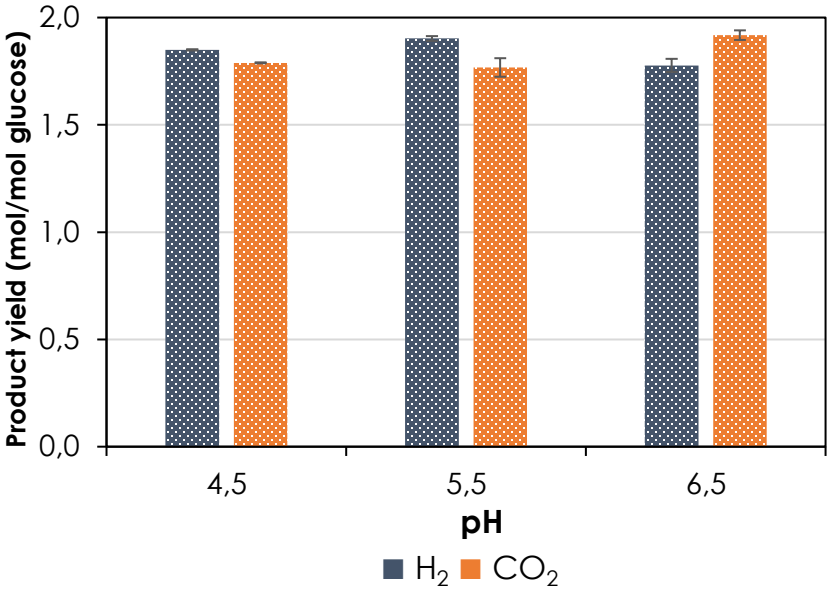
Model predictions





Experimental results





No propionate
Significant ethanol production

H_2 and CO_2 in 1:1 ratio

Changes implemented

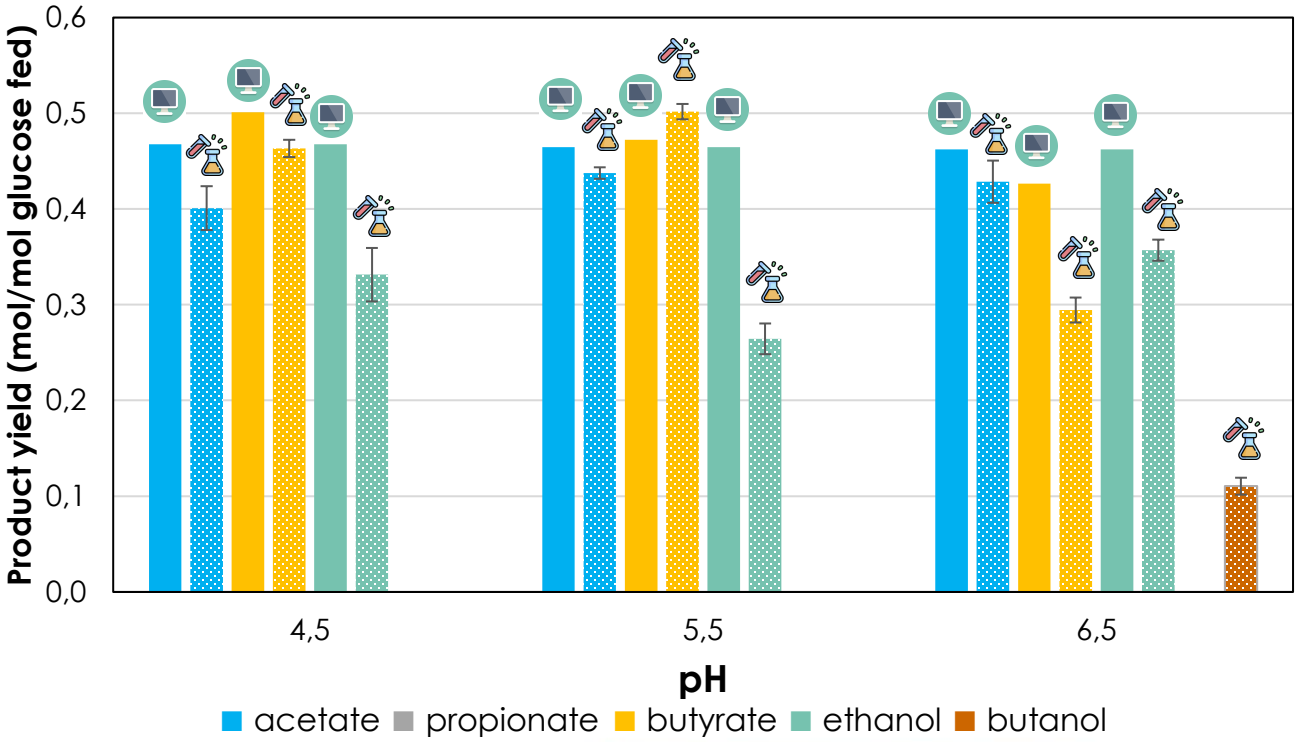
Propionate production

- Transcriptomic analyses of the C1 compartment show that there are **no active propionate producers**
- **Propionate** production pathway **is excluded** from the new version of the metabolic network

H_2 and CO_2 ratio prediction

- Butyrate pathway includes **electron bifurcation**, which results in increased H_2 production.
- The new version of the metabolic network **does not include electron bifurcation**

Anaerobic fermentations are energy-limited processes

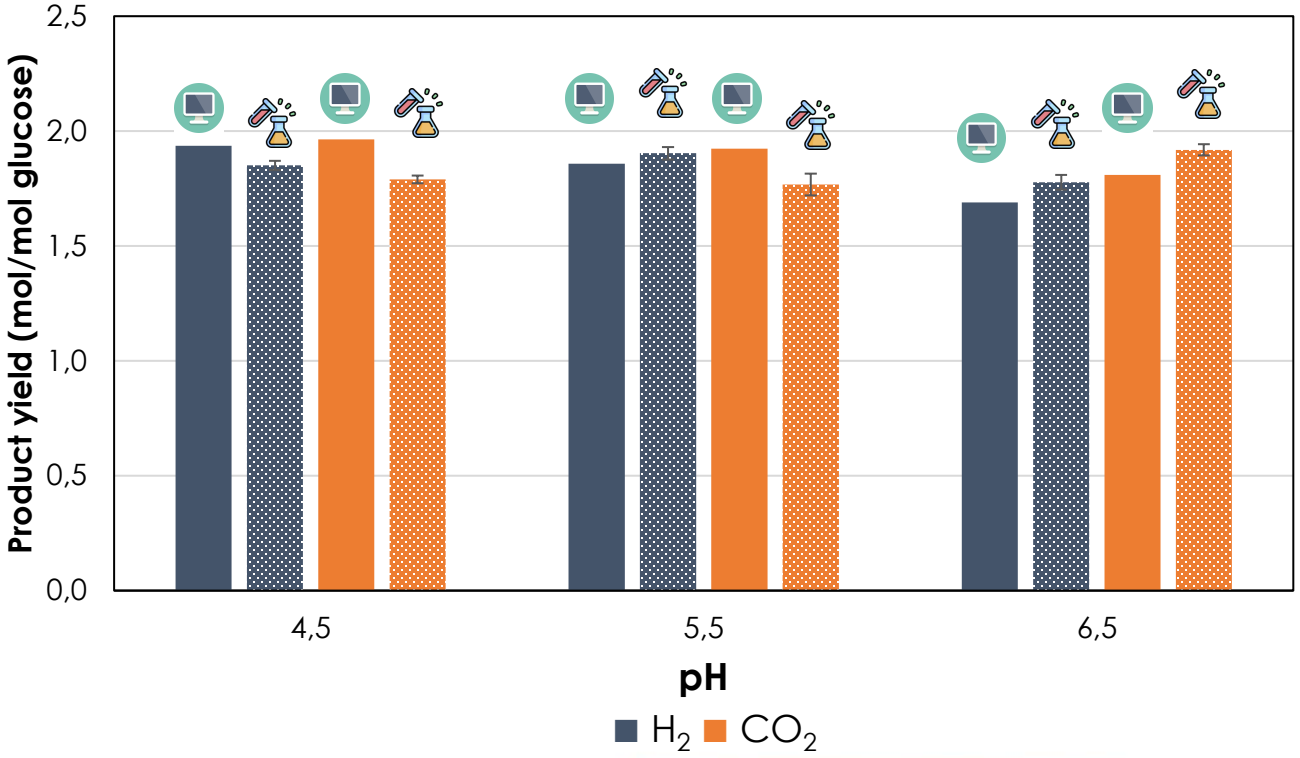


Acetate
Butyrate

Ethanol

Butanol

Anaerobic fermentations are energy-limited processes



Hydrogen

Inorganic carbon

Take home message

A **bioenergetic model** for thermophilic glucose fermentation was **developed** and **validated**

The **pH** did **not** show to have **potential** to steer the product spectrum

Propionate is not a product in glucose thermophilic fermentations

Have **other** molecules the **same behavior**?

MELISSA

MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

THANK YOU.

Alberte Regueira

@AlberteRegueira

Alberte.regueiralopez@ugent.be

www.**melissafoundation**.org

Follow us on social networks