Targeted conversion of protein and glucose waste streams to volatile fatty acids by metabolic models

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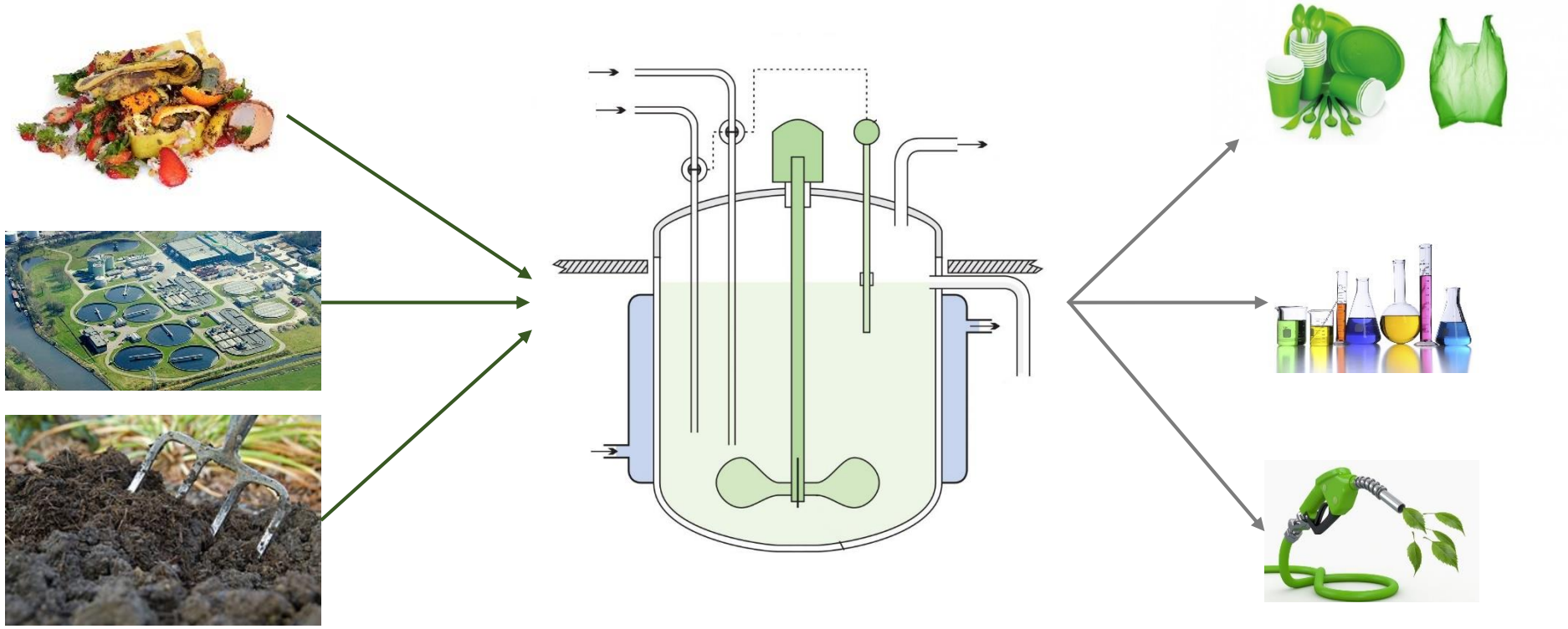
Circular economy: converting wastes to added-value products

- Anaerobic fermentations can turn organic wastes into high added-value products



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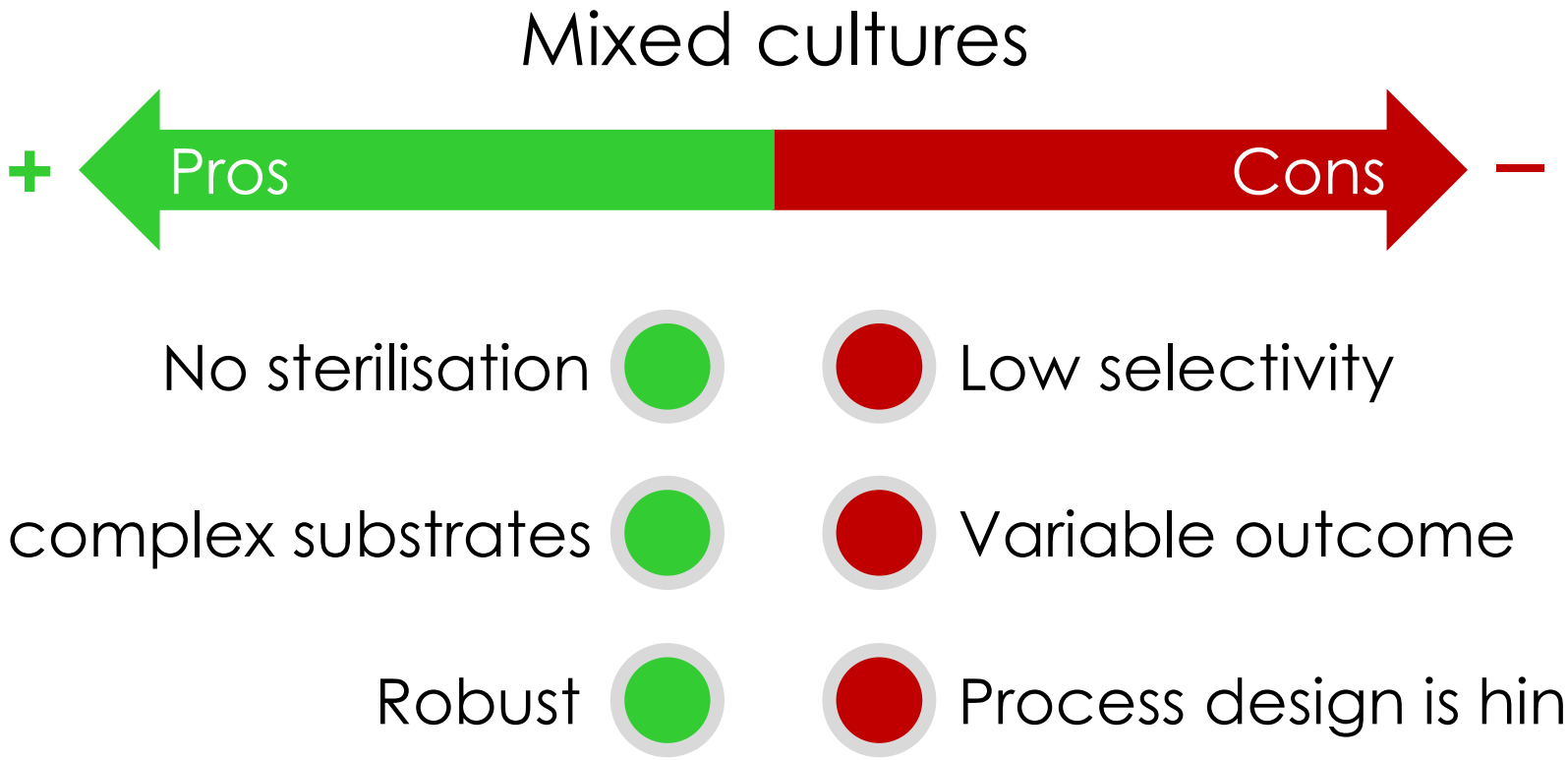
Mixed-culture fermentations to valorise organic wastes

- We need open mixed cultures of microorganisms (open fermentations) to make the process economically and technically viable



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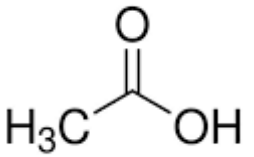
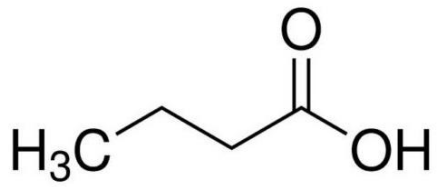
Organic waste can be converted to volatile fatty acids

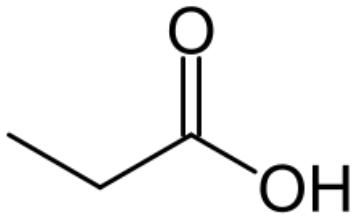
- Anaerobic fermentations produce a mixture of volatile fatty acids (VFA)
- It is not trivial which VFAs are produced
- We need to produce VFA in a selective way



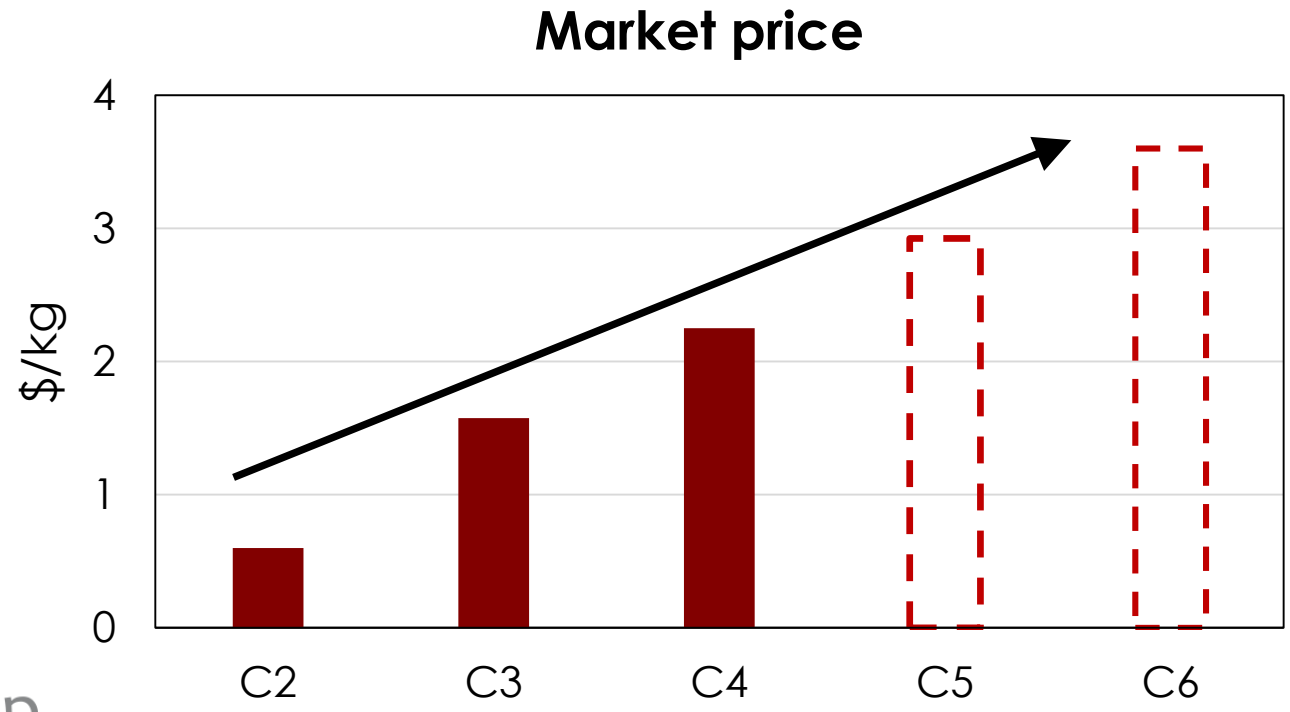
Acetate



Propionate

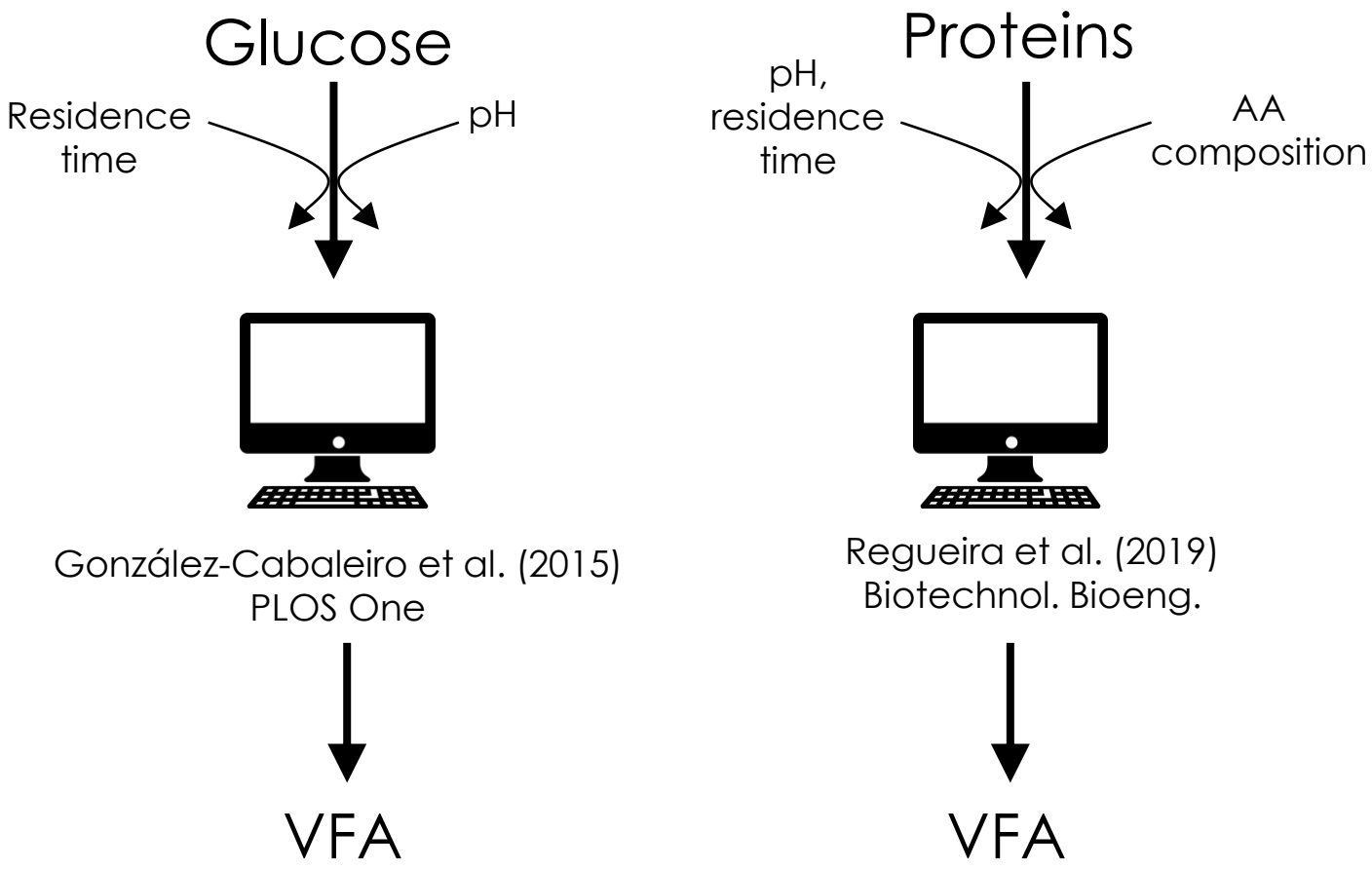


Butyrate



Source: Atasoy *et al.* (2018). Bioresource Technology

There are modelling tools for glucose or protein fermentations



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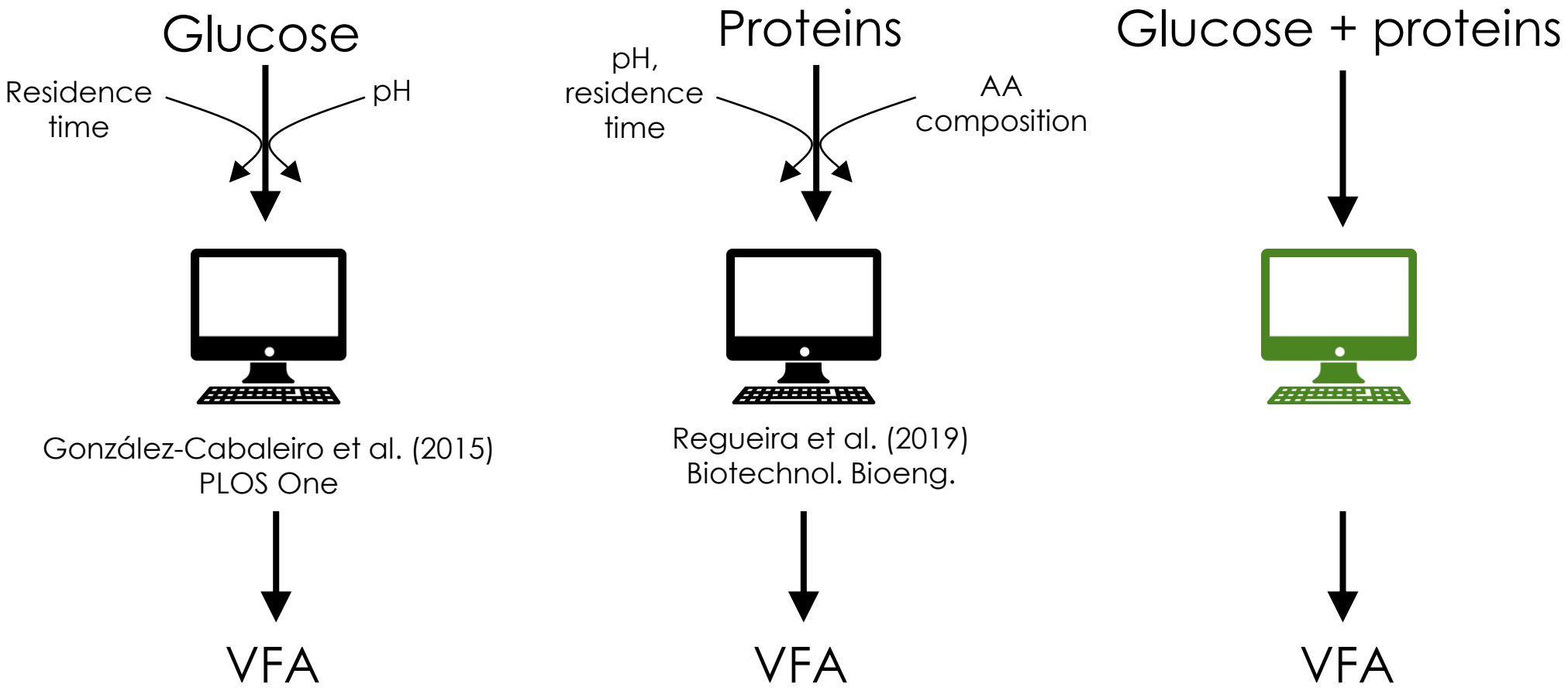
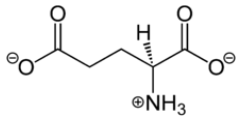
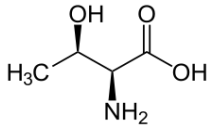
But no modelling tools for cofermentation scenarios



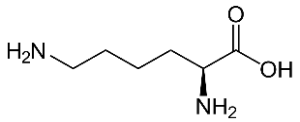
Objective: To model cofermentation processes



Glutamate

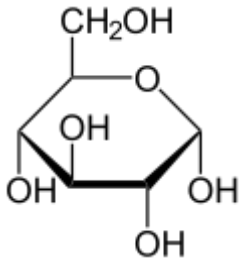


Serine

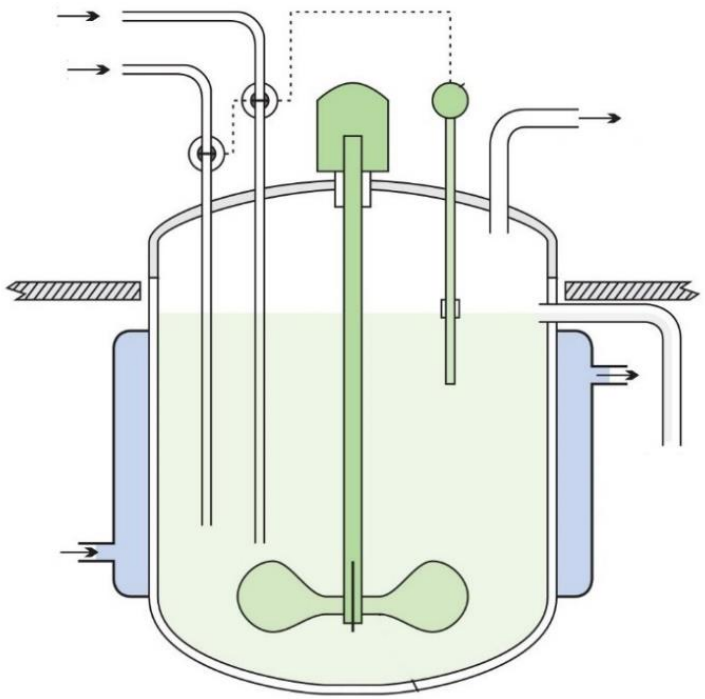


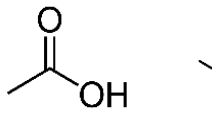
Lysine

+ 14 other aminoacids

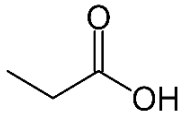


Glucose

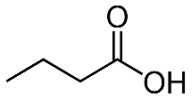




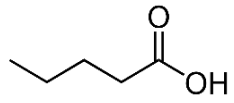
Acetate



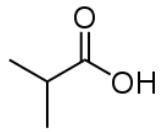
Propionate



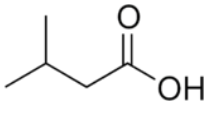
Butyrate



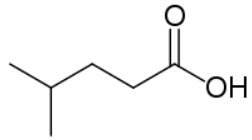
Valerate



Iso-butyrate



Iso-valerate



Isocaproate

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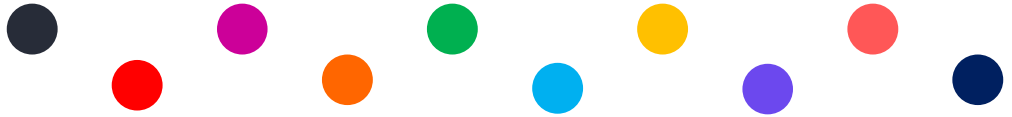
We model the microbial community as an enzyme soup

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



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

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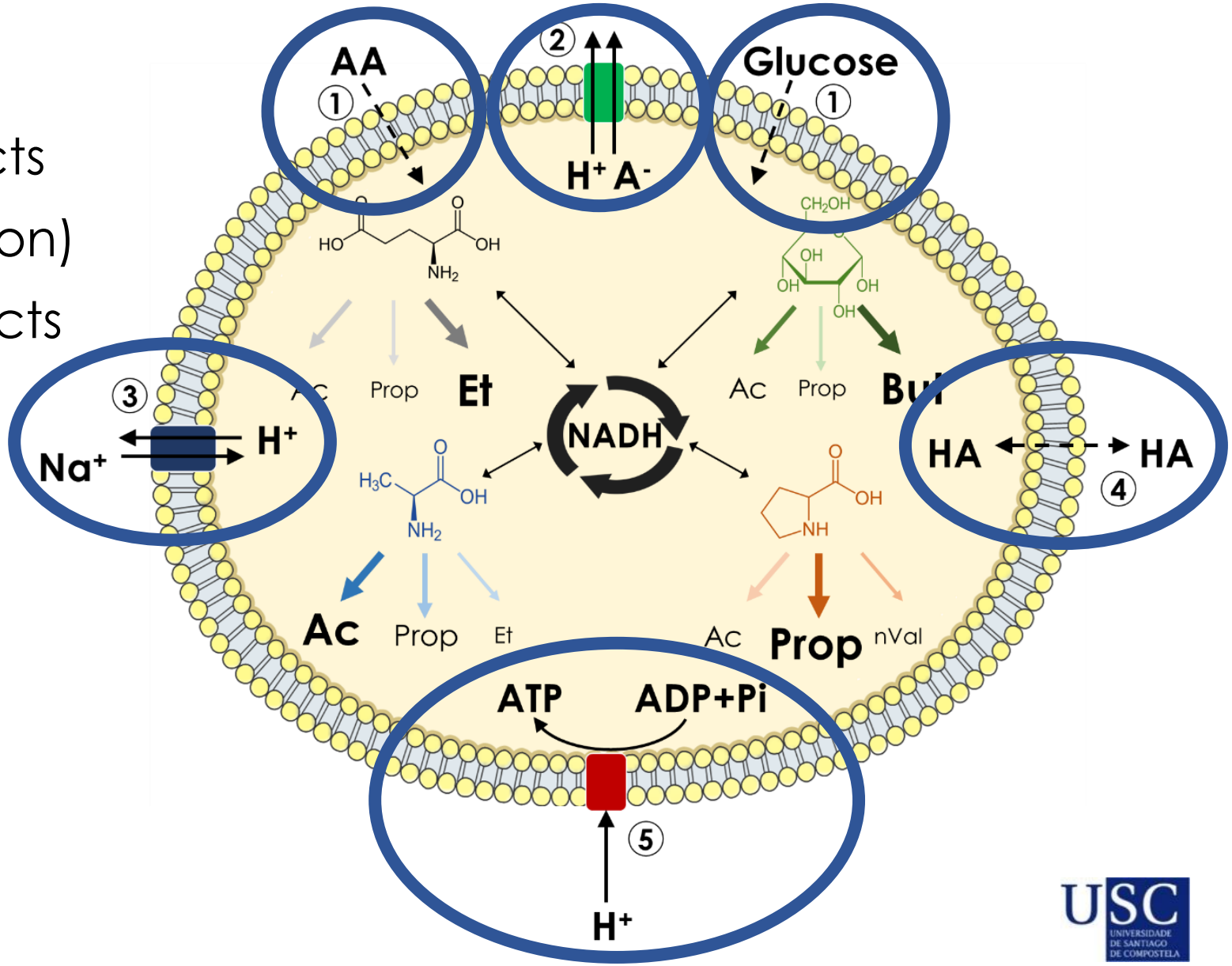
One virtual microorganism does all the possible processes

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



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Dynamic flux balance analysis

$$\frac{dC}{dt} = D \cdot \left(C^{in} - C\right) + r(z, C)$$

(68 states)

$$z(t) / \max_{z} r_{ATP}\left(C(t)\right)$$

Maximise ATP production

$$s.t. \quad r_{NADH}(z) = 0$$

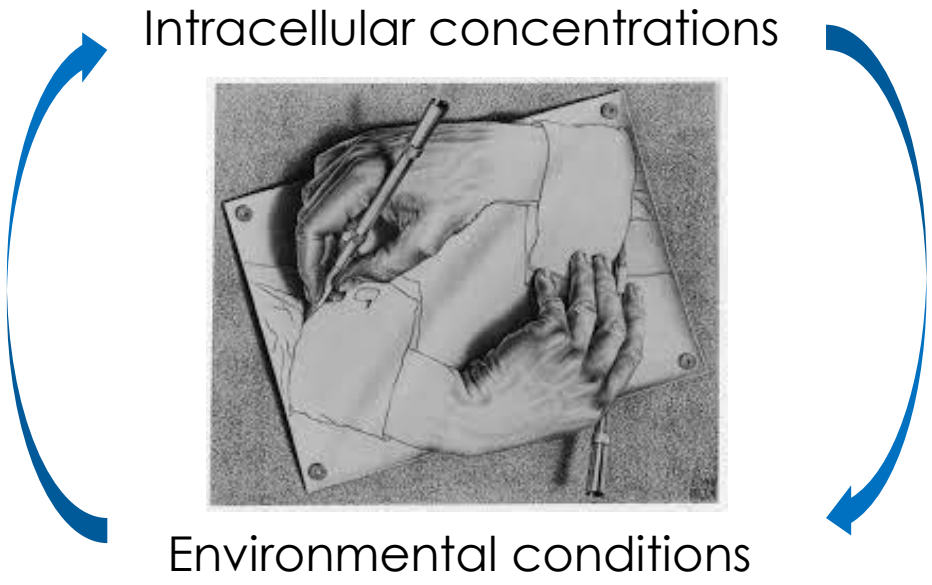
NADH is conserved

$$g(z) \leq 0$$

$$h(z) = 0$$

Environmental conditions

Intracellular concentrations vary with time and environmental conditions



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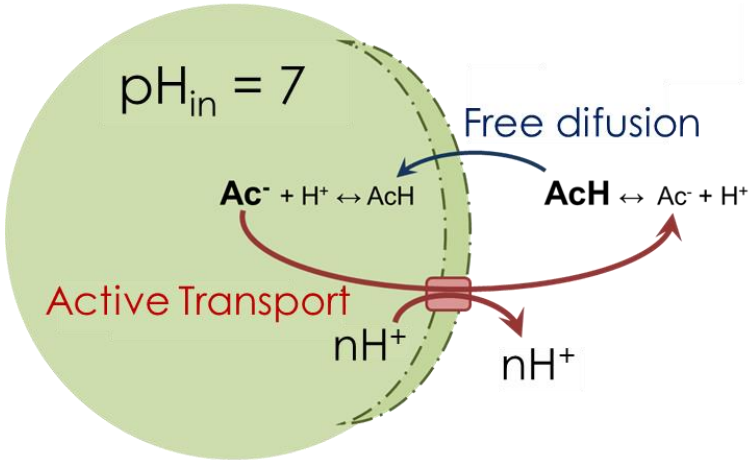
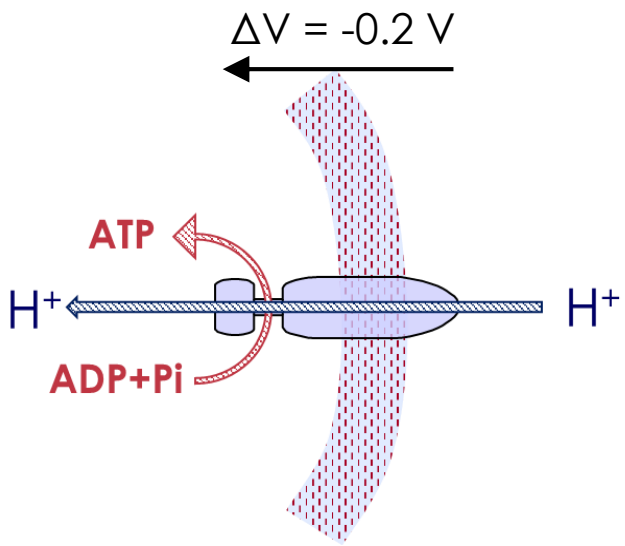
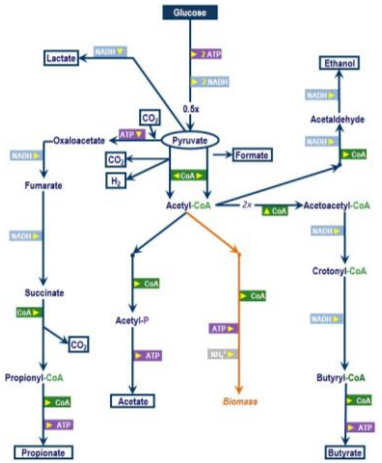
Pathway selection is determined by a linear program

- Optimal pathways → Highest ATP production rate

$$r_{ATP} = r_{TRANSPORT} + r_{PMF} + r_{CAT}$$







Glucose + amino acids metabolic network
99 possible reactions

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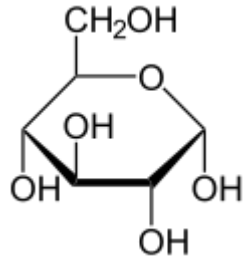
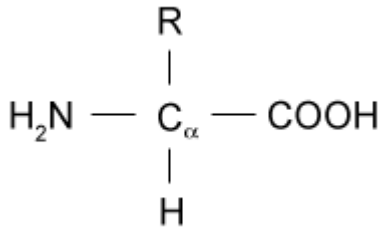
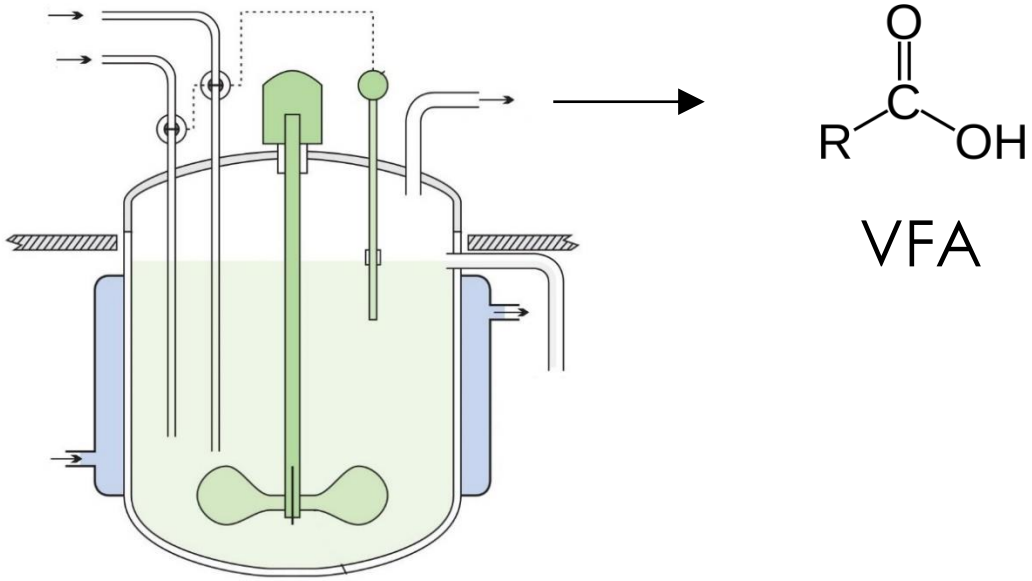
Modelling the cofermentation of gelatine and glucose



Glucose



Gelatine
(17 aminoacids)



VFA

pH 4-8.5

D=0.1 h^{-1}

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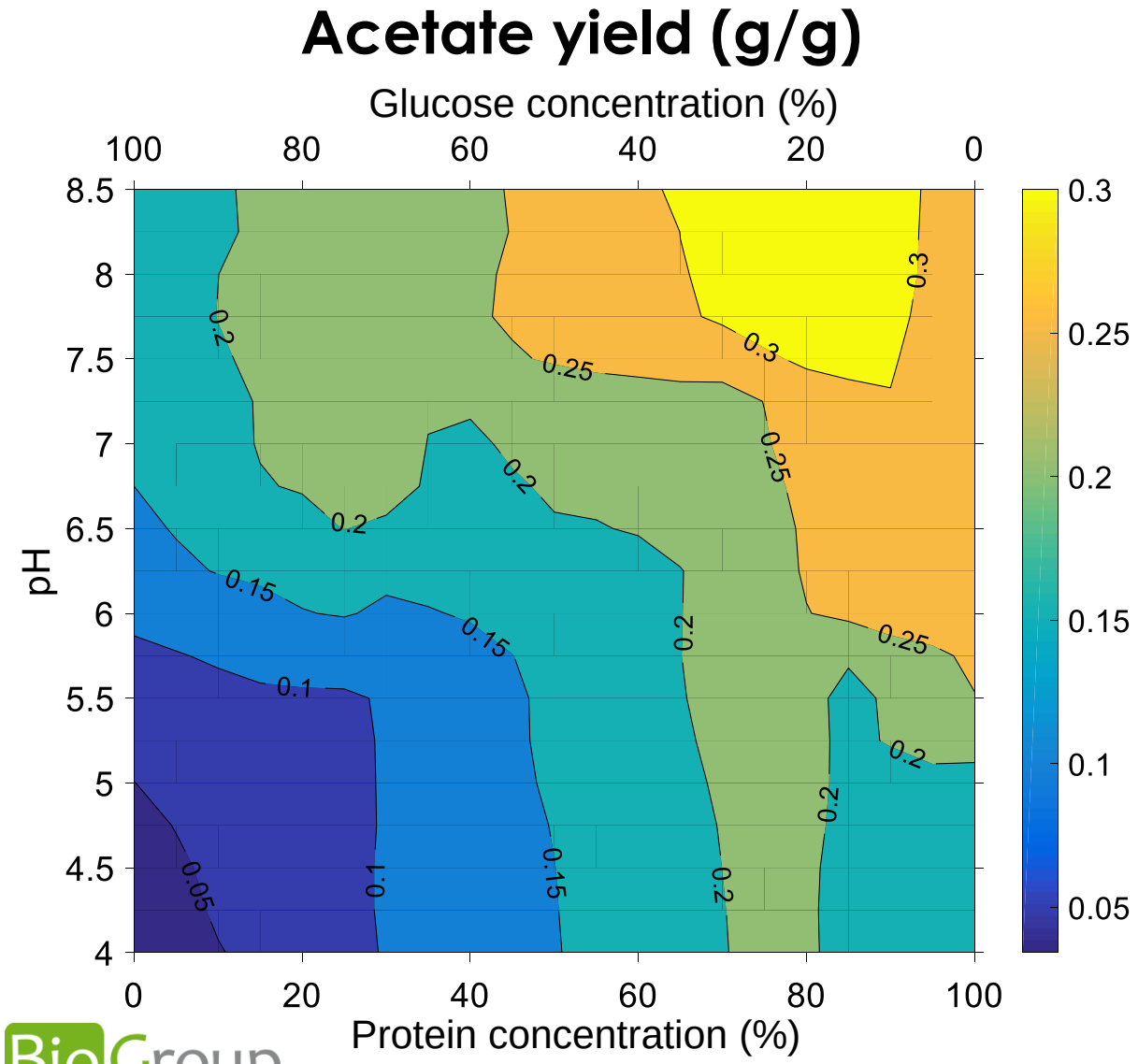
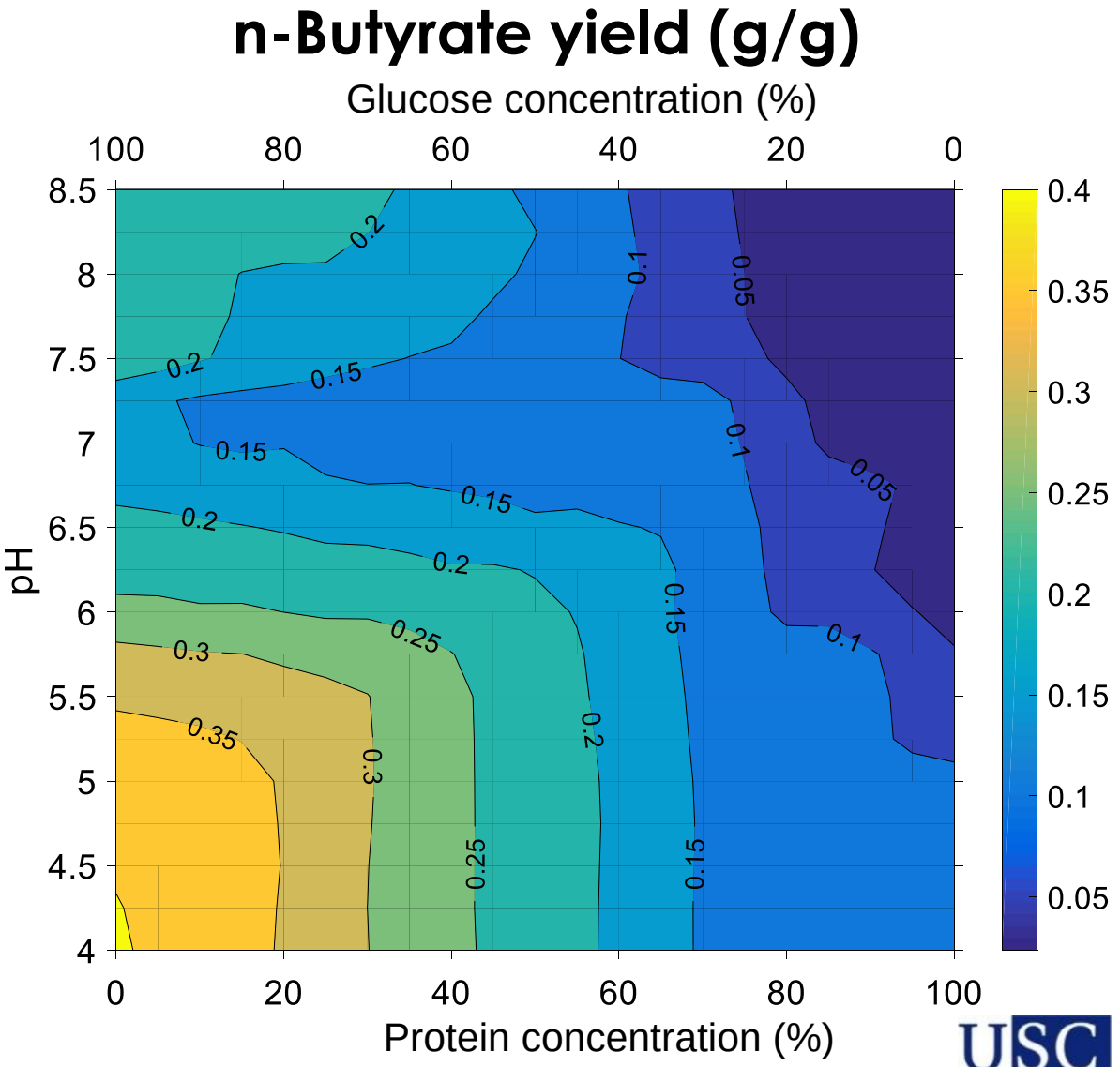
Changing operational conditions favour different VFA





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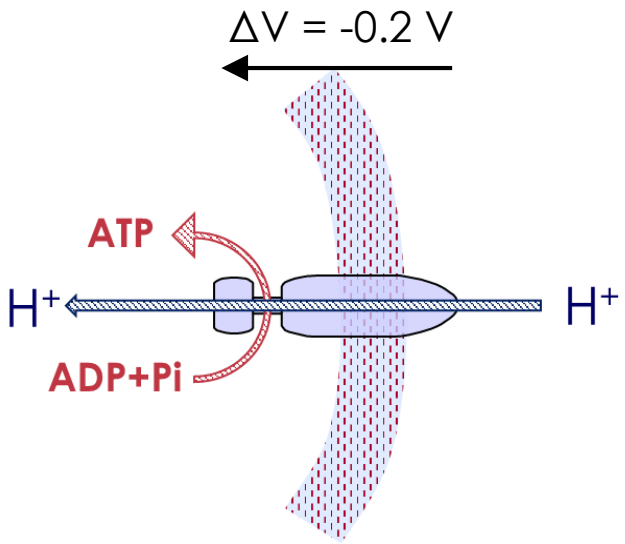
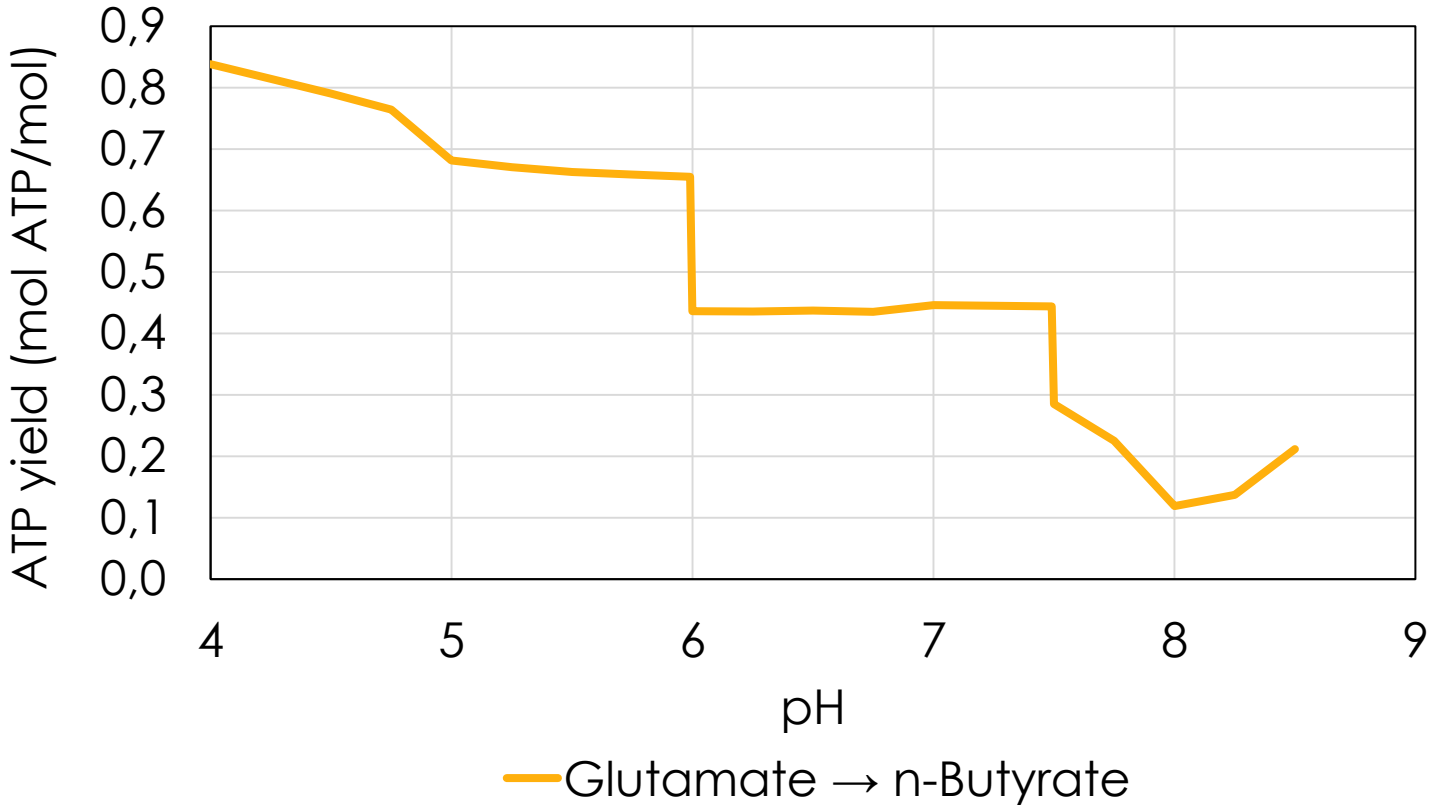
The pH has a strong non-linear effect on pathway selection

- Proton motive force



Glutamate ⟶ n-Butyrate + $\Delta\mu_{H+}$



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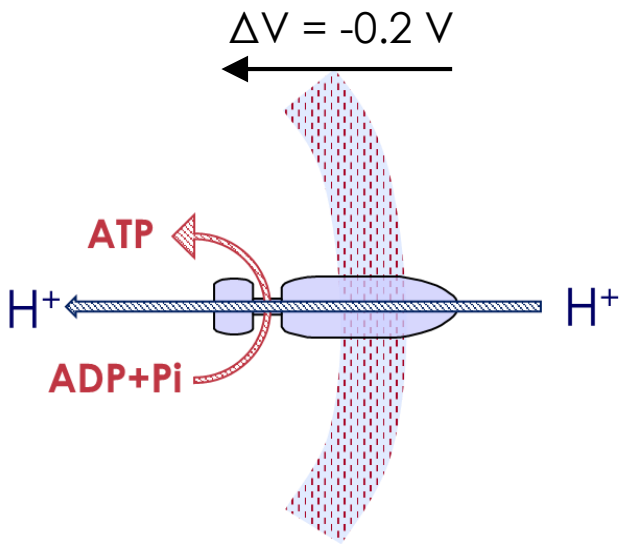
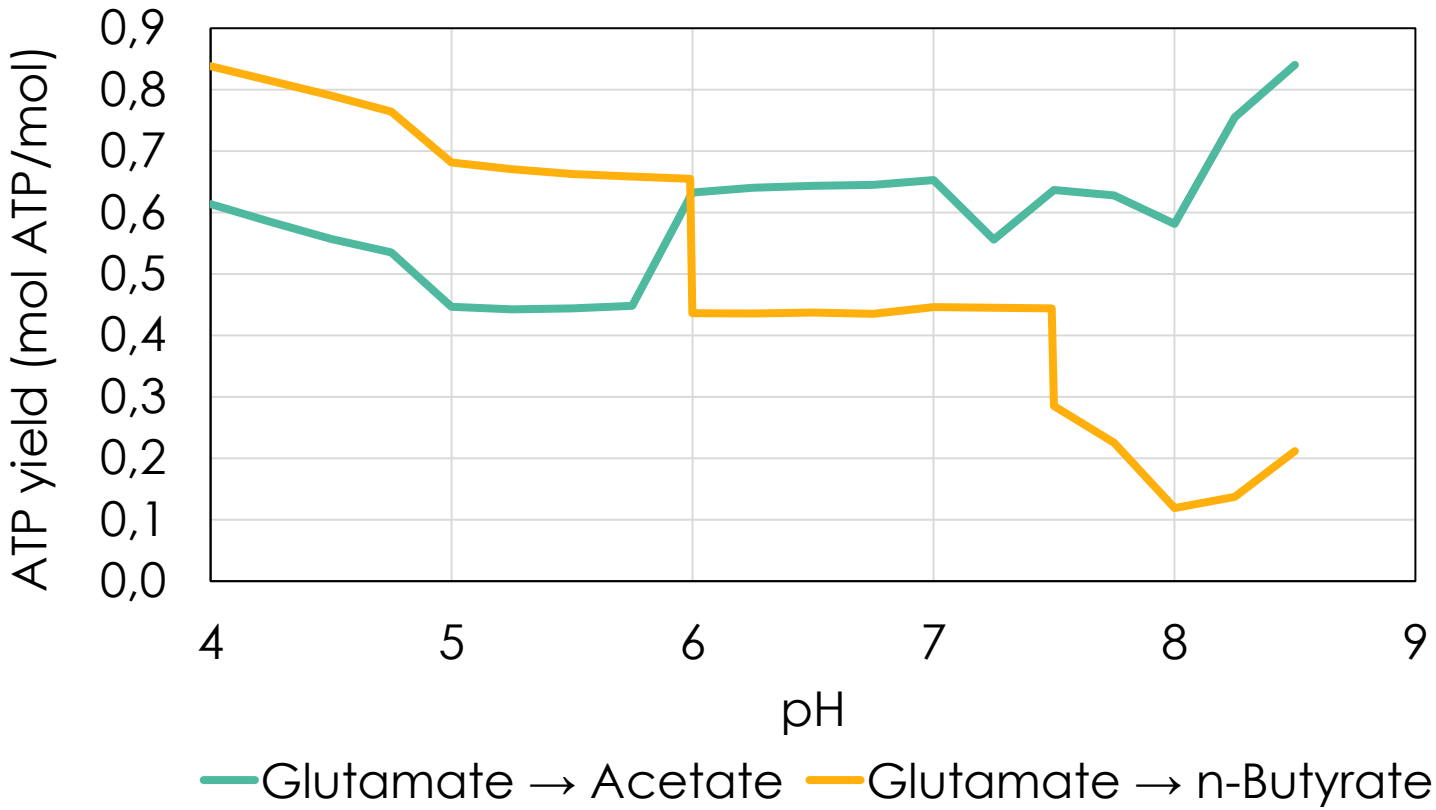
The pH has a strong non-linear effect on pathway selection

- Proton motive force

2 Acetate + 1 ATP ⟵ Glutamate ⟶ n-Butyrate + $\Delta\mu_{H+}$





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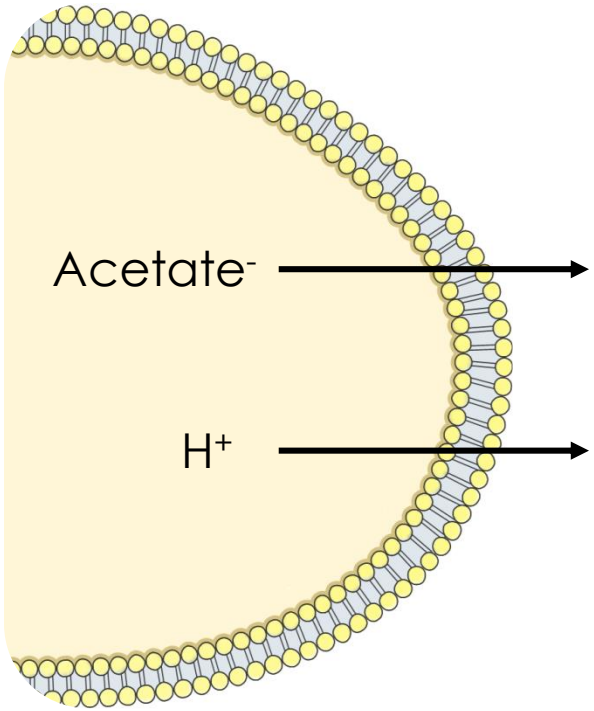
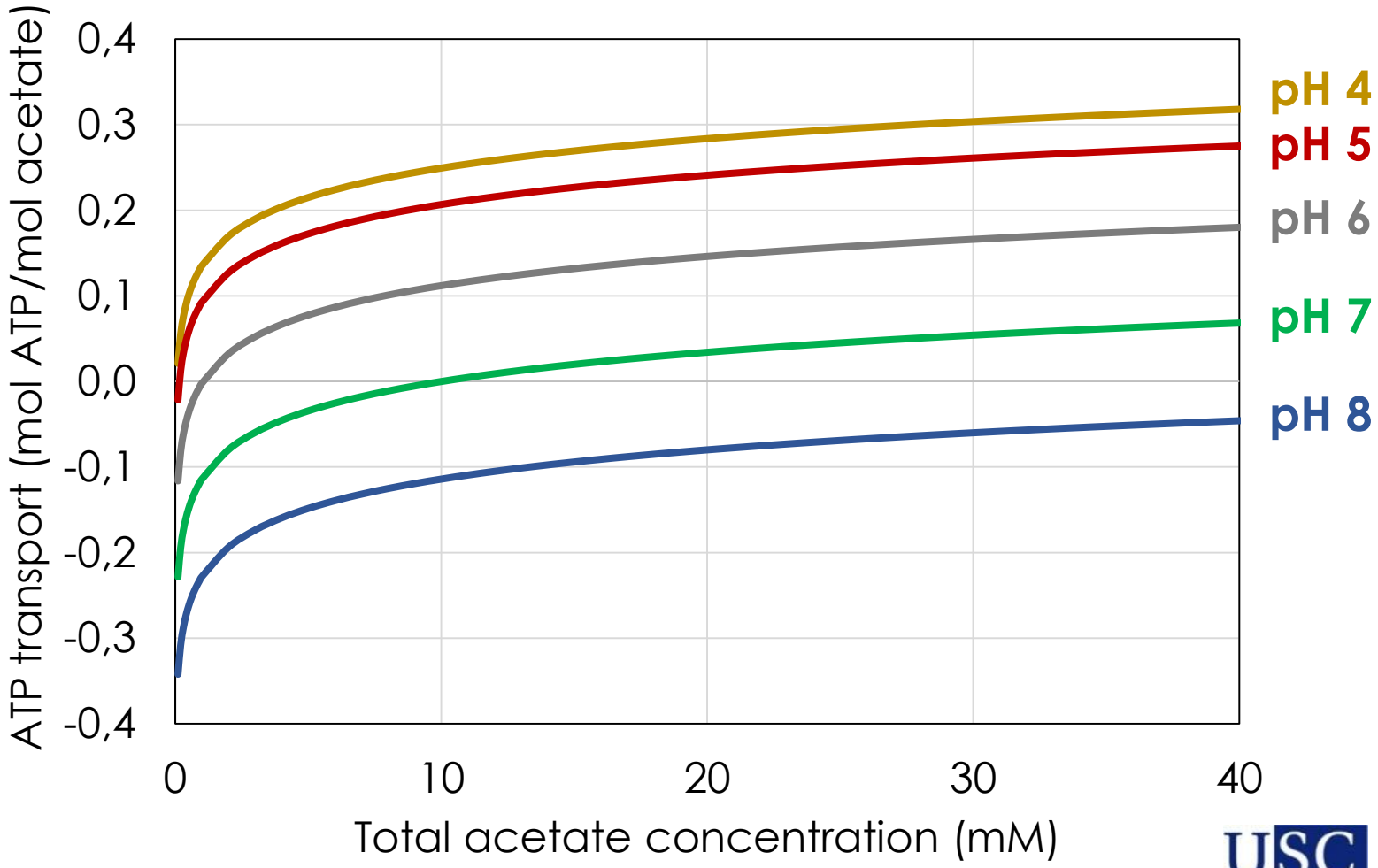
The energy required for transport also changes with pH

- Active transport energy





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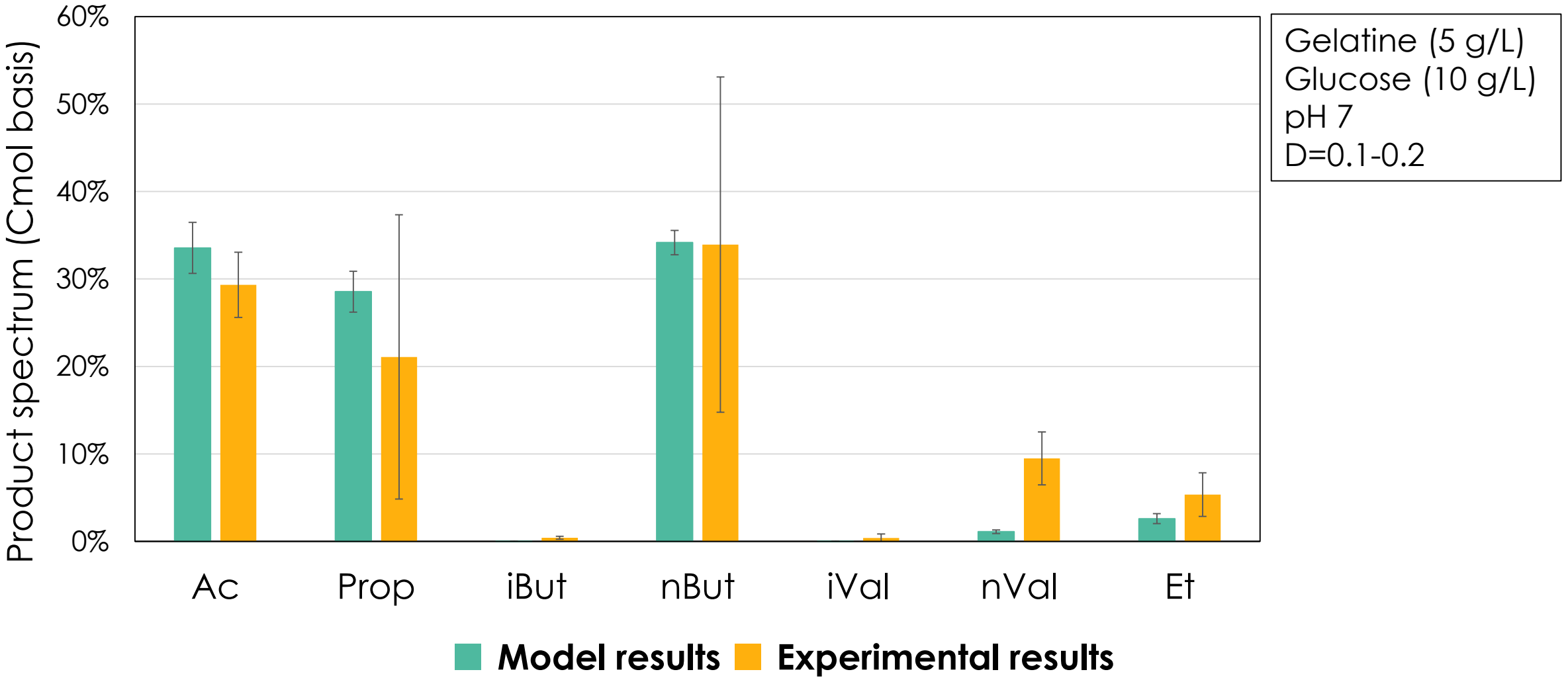
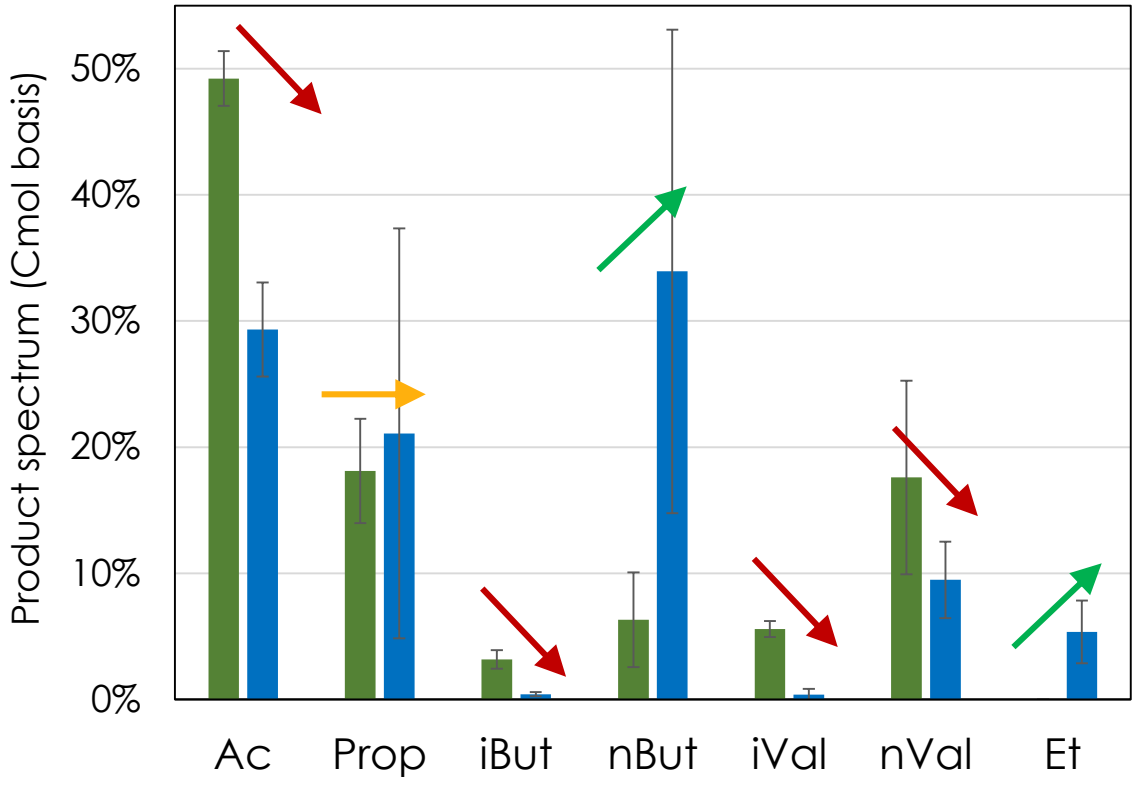
Validation: the model predicts well the product spectrum…



Breure *et al. (1986).* Appl Microbiol Biotechnol

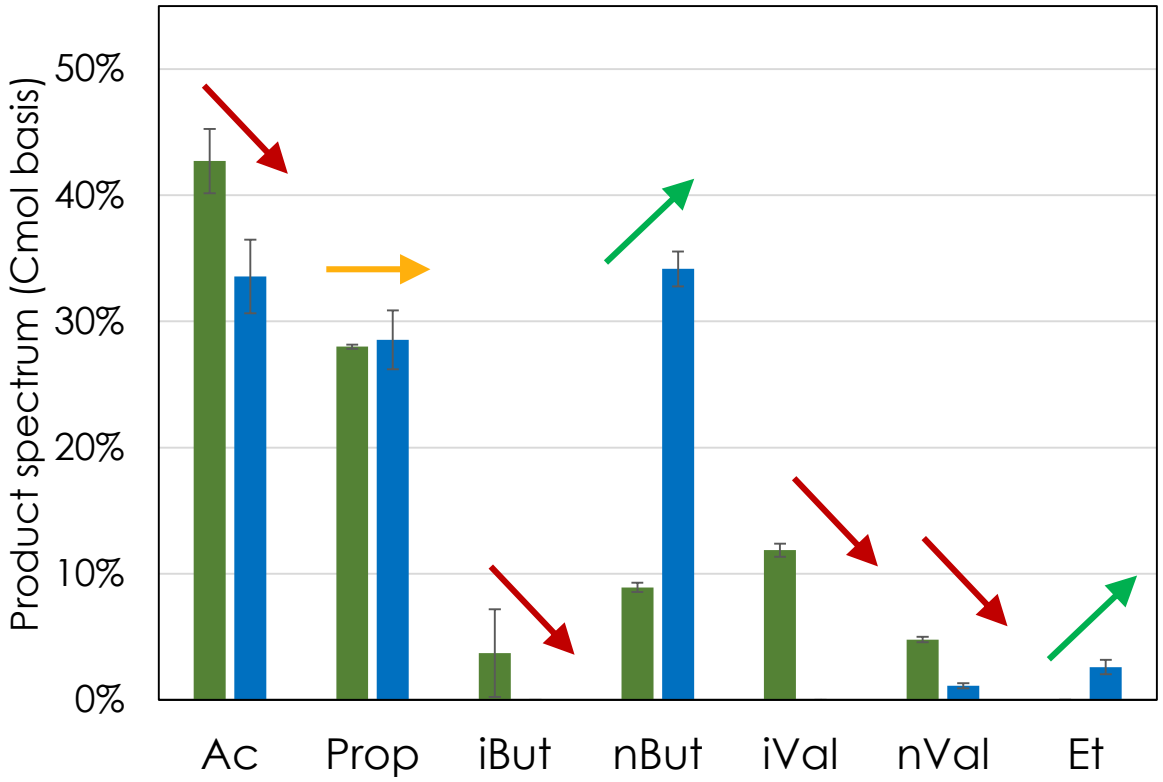
…as well as the trends of adding glucose to the fermentation

Experimental data



Breure *et al. (1986).* Appl Microbiol Biotechnol

Simulations



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Gelatine monofermentation (5 g/L) **Cofermentation w/ glucose (10 g/L)**

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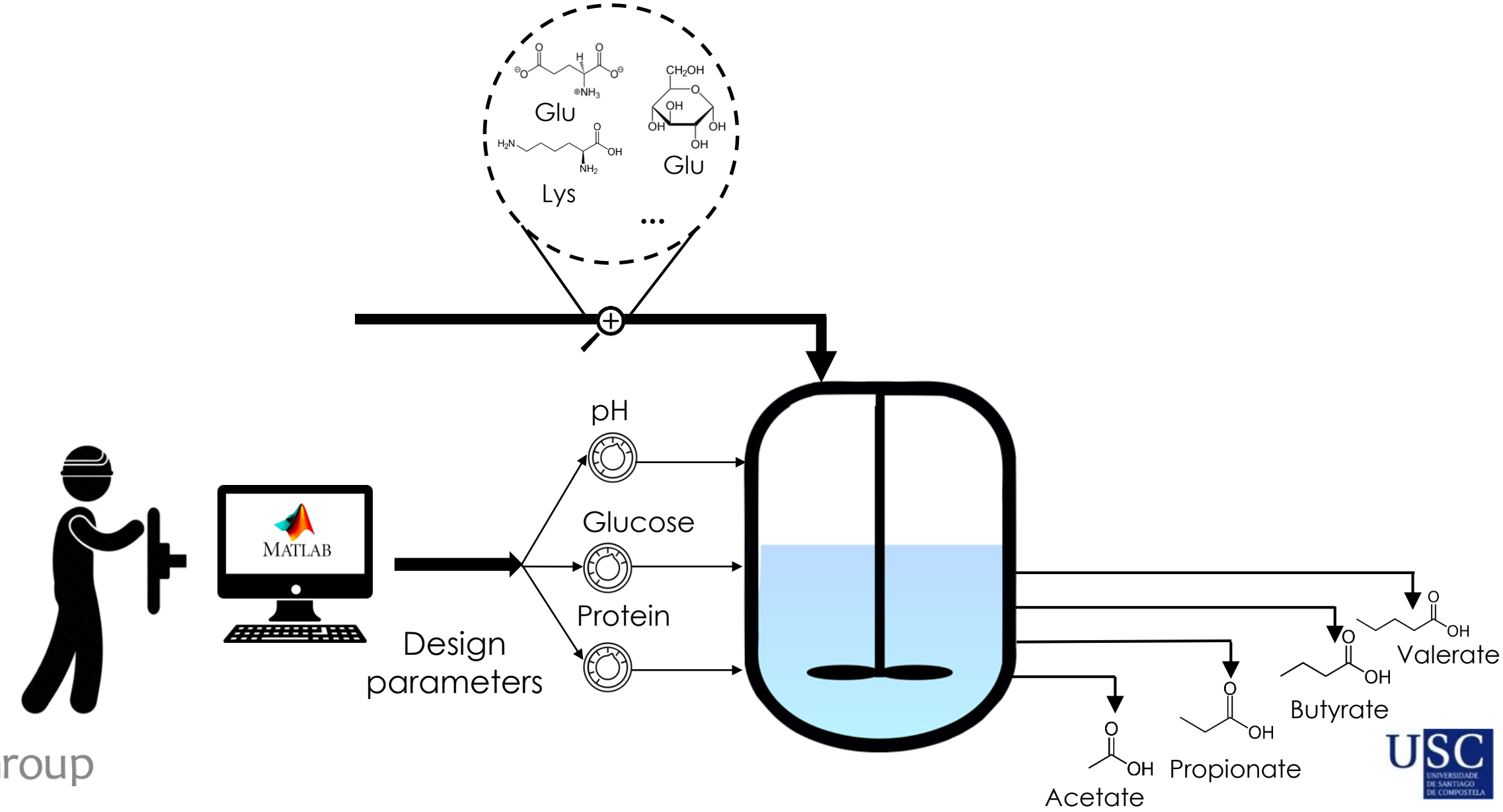
Application: the model can be used as a design tool



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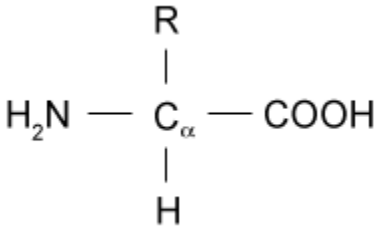
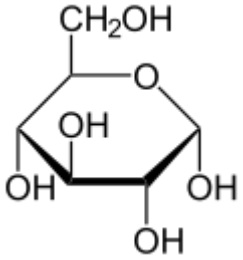
Targeting the substrate for bioplastic production

- VFA can be the substrate for producing polyhidroxyalkanoates (PHA)



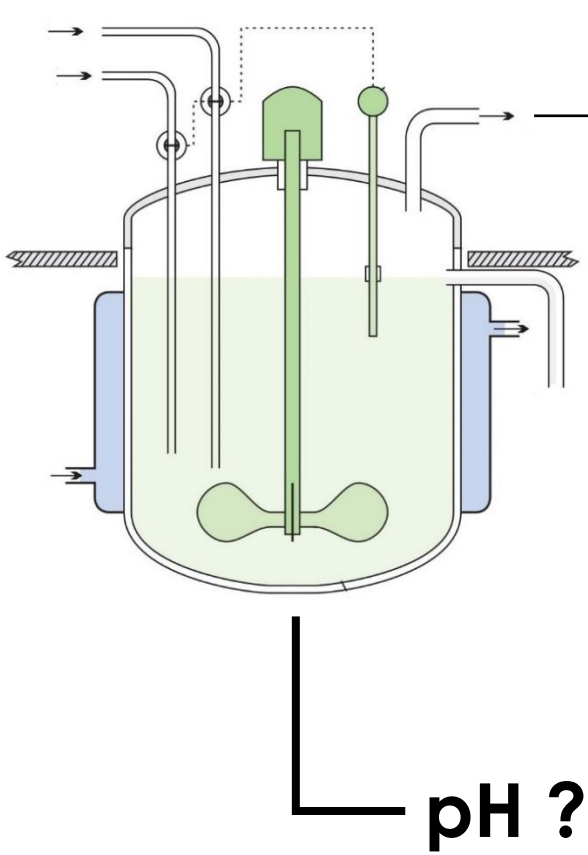
Gelatine

(? g/L)

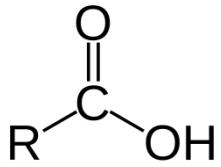


Glucose

(? g/L)



pH ?



VFA

$$\frac{[Odd-carbon\ VFA]}{[Even-carbon\ VFA]} = \frac{[Pro]+[Val]}{[Ac]+[But]}$$

Mechanical properties of the bioplastic (PHA)

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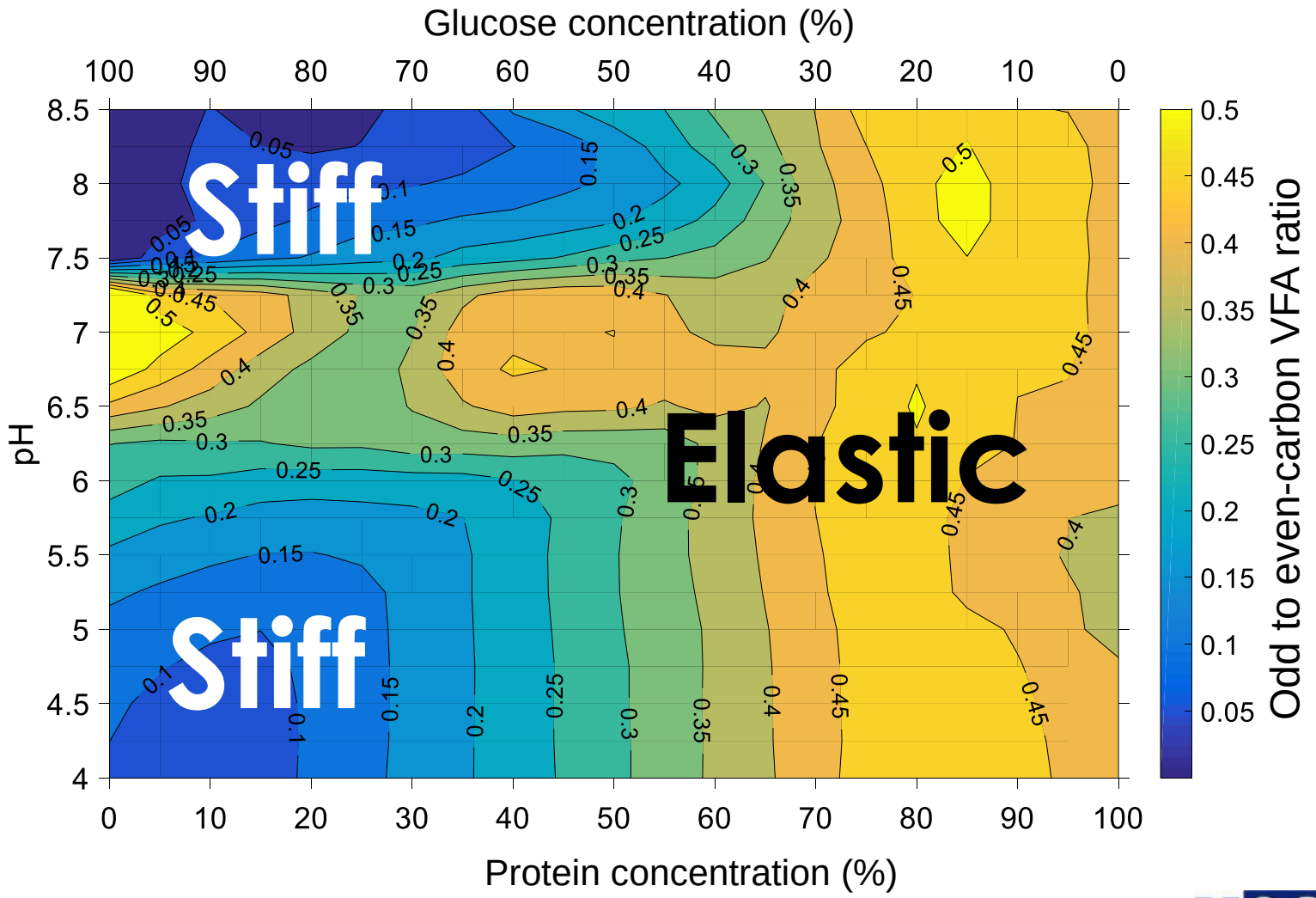
Targeting the substrate for bioplastic production

$$\frac{[Odd-carbon\ VFA]}{[Even-carbon\ VFA]} = \frac{[Pro]+[Val]}{[Ac]+[But]}$$

Mechanical properties of the bioplastic (PHA)



Conclusions

- We can simulate cofermentations at different pH values and proportions of glucose and protein.
- We envision this model as a process design tool to design processes targeting specific VFA

Take home message: we can target now specific VFA in cofermentations

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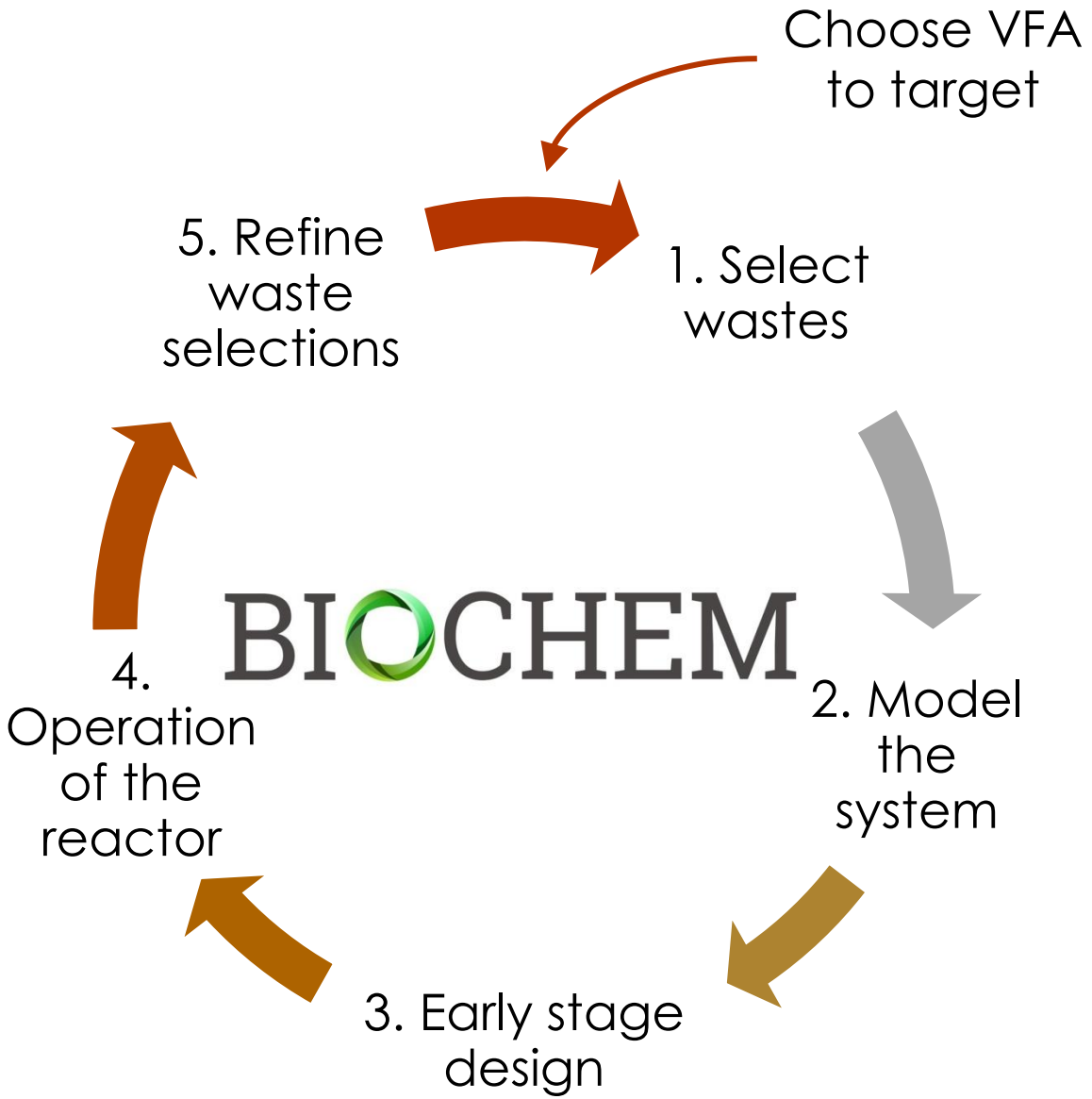

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Acknowledgements

- ✓ Spanish Ministry of Education (FPU14/05457)
- ✓ This research is funded by the Spanish Government through BIOCHEM project (PCIN-2016-102, ERA-IB-2 7th call, ERA-IB-16-052).
- ✓ Authors belong to CRETUS Strategic Partnership (AGRUP2015/02) and to Galician Competitive Research Group (GRC ED431C 2017/29). All these programs are co-funded by FEDER (EU).

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