



# INSIGHTS INTO THE EFFECT OF A BIOCATHODE ON DRIVING MIXED-CULTURE FERMENTATIONS UNDER LOW ELECTRON RECOVERY

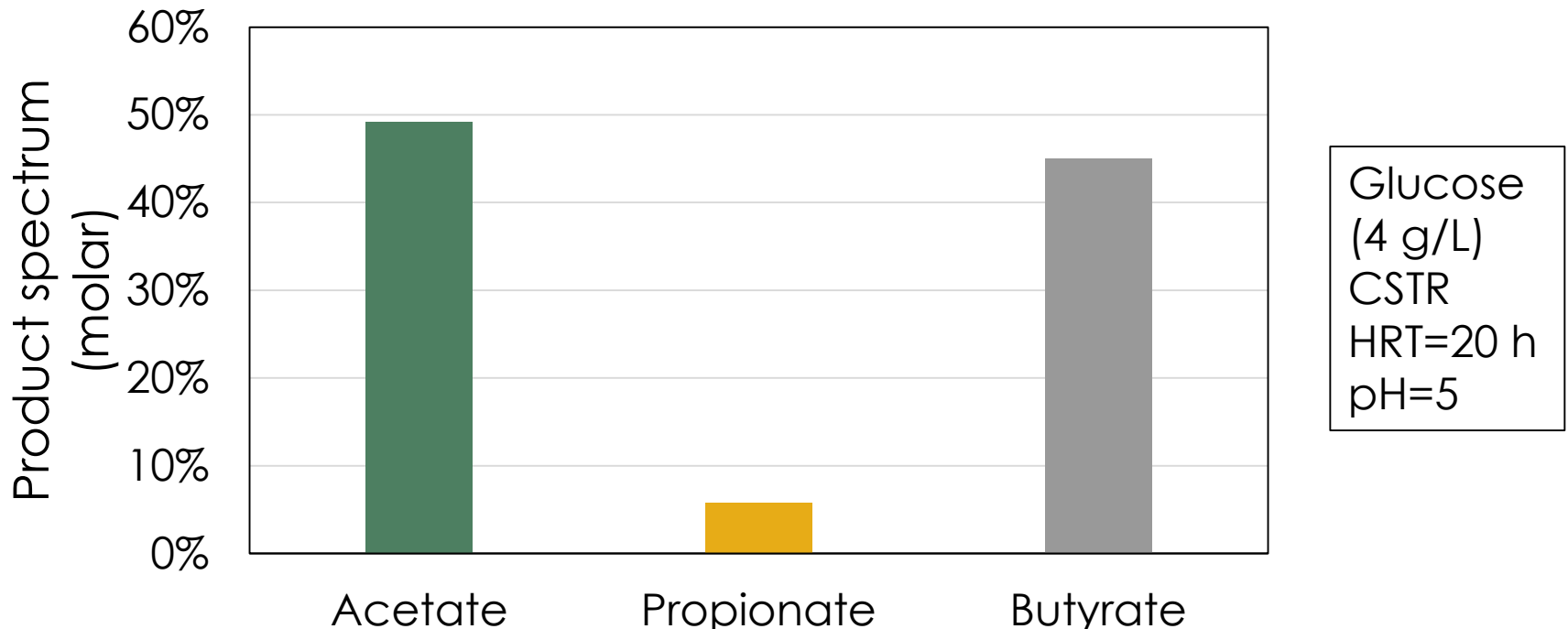
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A. Esteve-Núñez, J. M. Lema

Department of Chemical Engineering  
Universidade de Santiago de Compostela



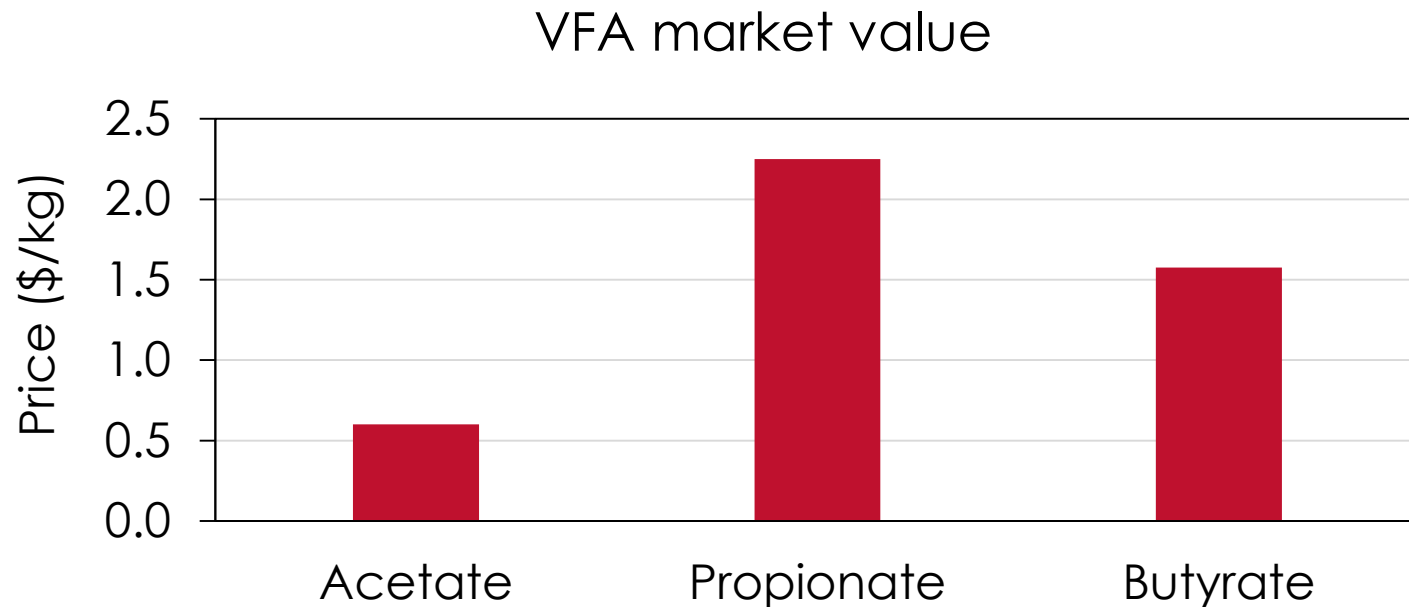
# Open fermentations produce valuable products...

- Volatile fatty acids (VFA) are produced in open fermentations.
- The product spectrum is heterogeneous and variable.

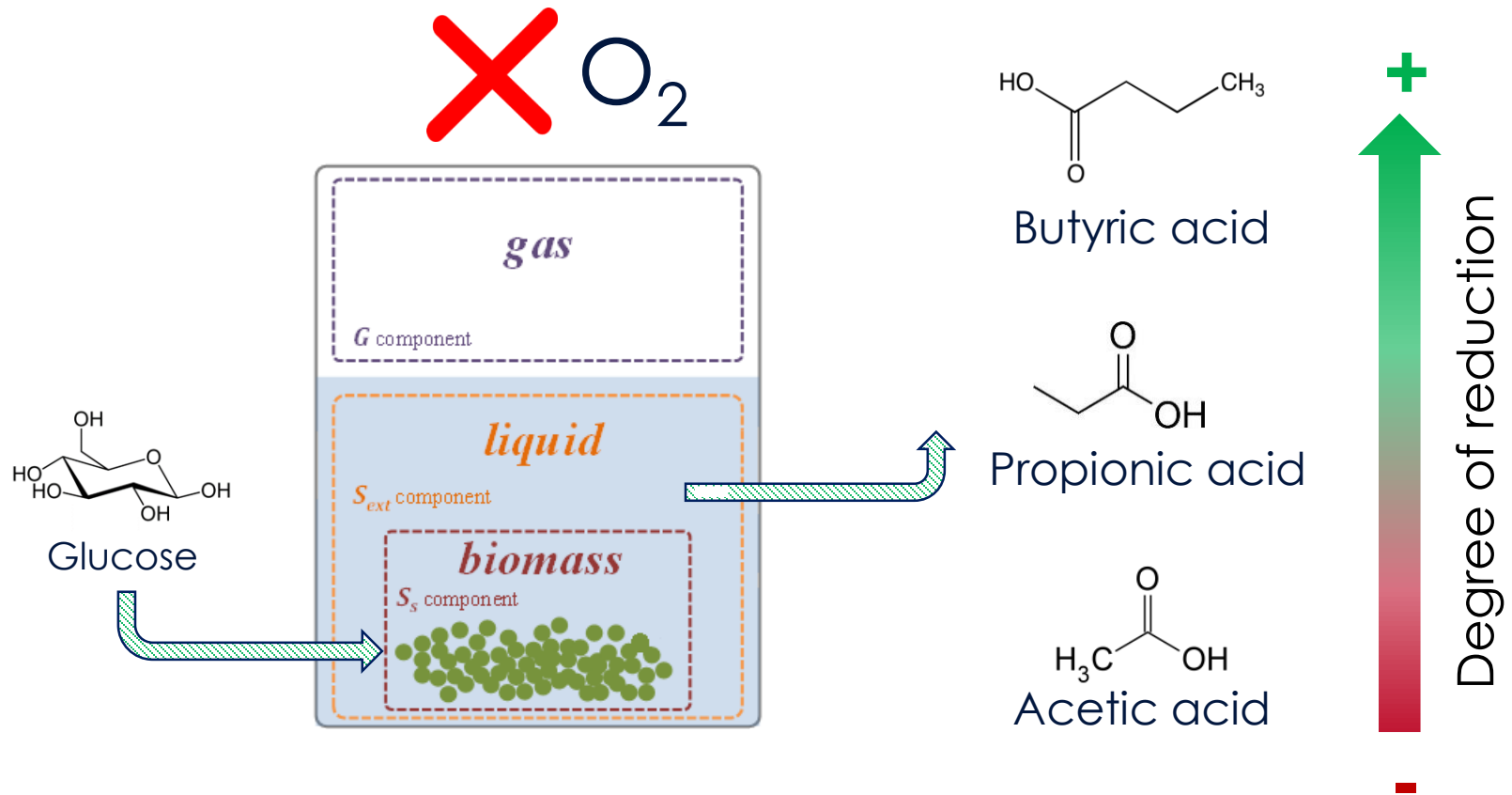


## ... but their opportunities are quite different

- Volatile fatty acids (VFA) are produced in open fermentations.
- The product spectrum is heterogeneous and variable.
- Directing the production towards more reduced products.



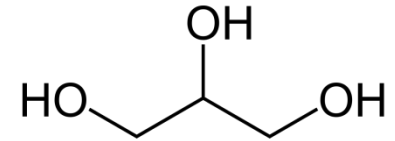
# Absence of external electrons acceptors constrains the process



- Production of reduced products is limited by how reduced the substrate is.

# Fermentation can be driven to more reduced products

- With a more reduced substrate: glycerol



- Injecting H<sub>2</sub> gas in the fermenter



or...

Why not provide a cathode as electron donor?! (Electrofermentation)



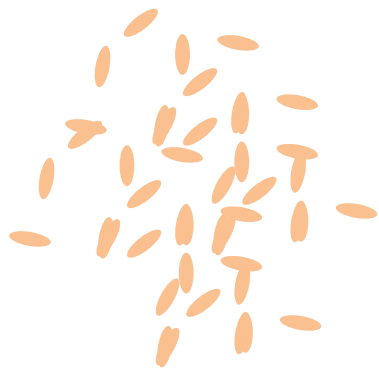
# Presentation outline

- Electrofermentation essays
- Improvements in the experimental design
- First promising results

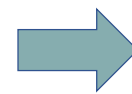
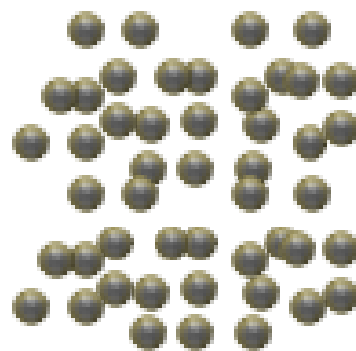
# Experimental setup

- Glucose (Initial concentration: 1 g/L)
- 2 chamber MET
- Mixed-culture inoculum enriched with a pure culture of *Geobacter sulfurreducens*
- Cathode simulating a fluidized electrode (glassy carbon).
- BES was added to avoid methanization

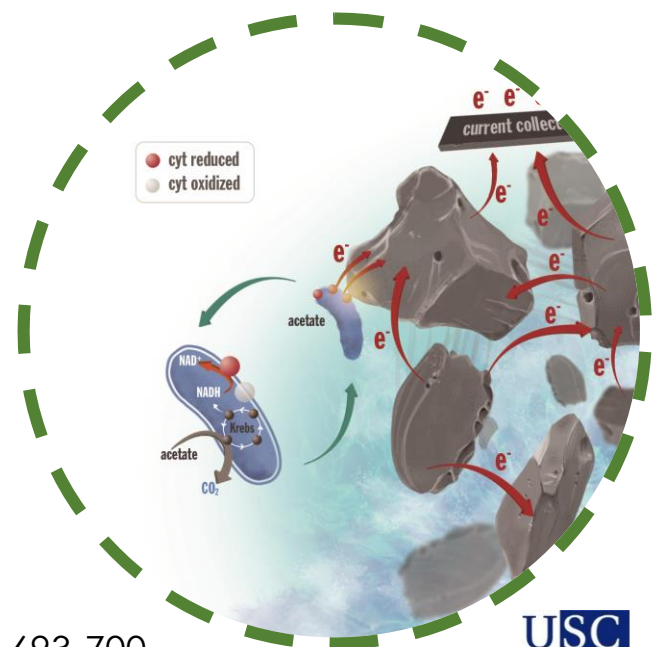
planktonic cells



'planktonic' cathode



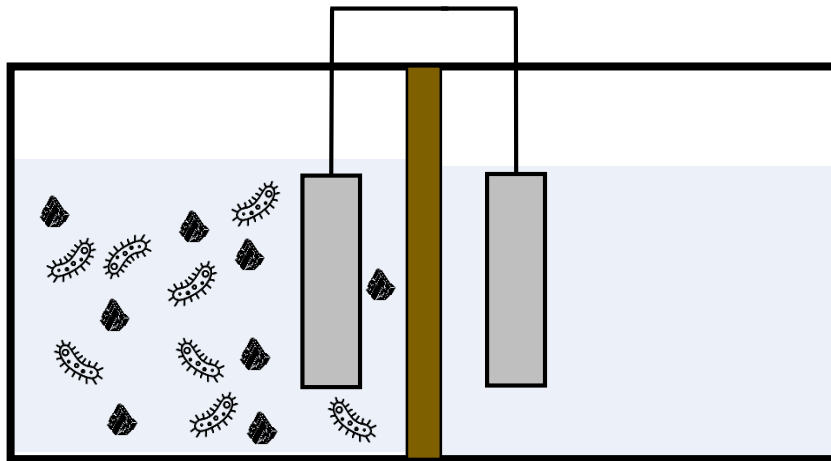
interaction in motion



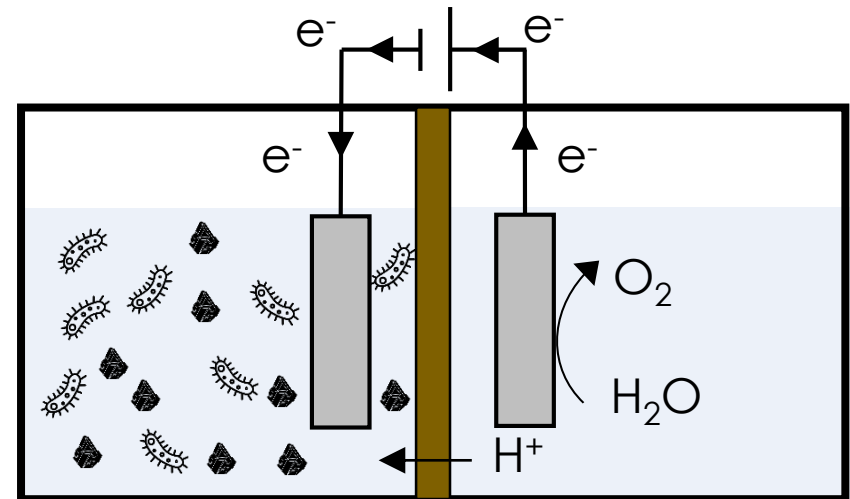
# Experimental setup

- Glucose (Initial concentration: 1 g/L)
- 2 chamber MET
- Mixed-culture inoculum enriched with a pure culture of *Geobacter sulfurreducens*
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## 1. CONTROL at open circuit potential (OCP)

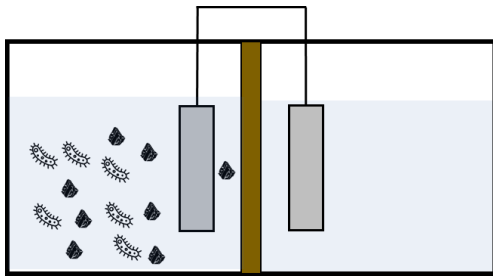


## 2. Cathode at -0.7 V (vs Ag/AgCl)

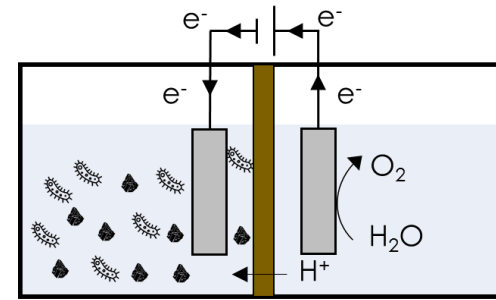
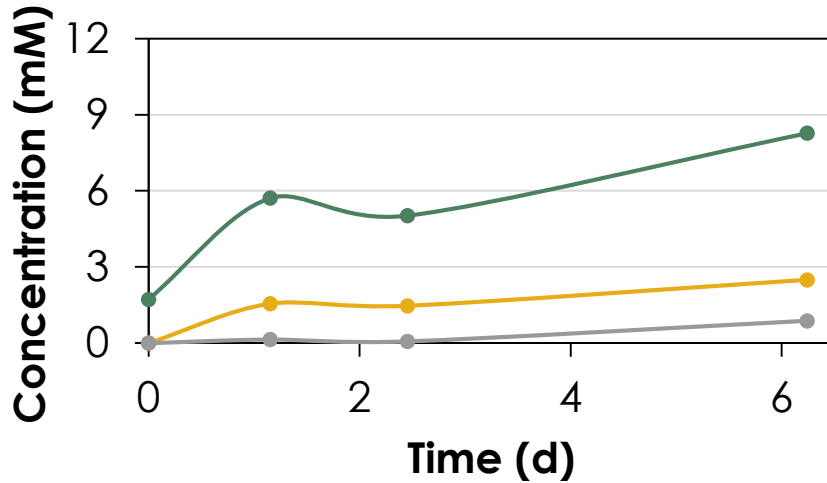




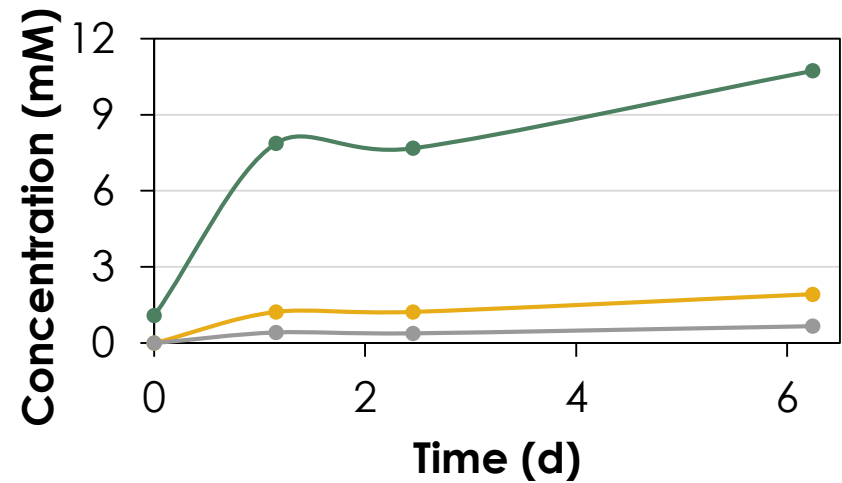
# No improvement in the cathodic fermentation



Control (OCP)



Cathode



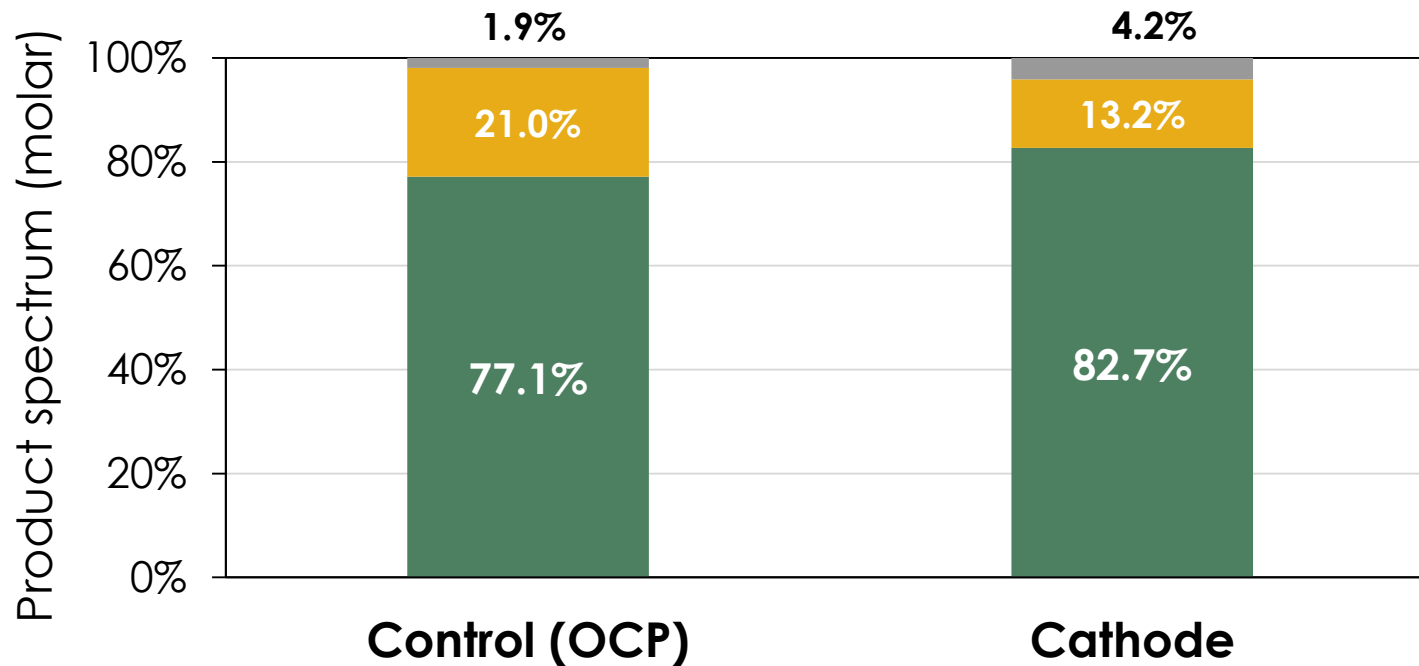
Initial glucose concentration **1 g/L**

Temperature

**30°C**

pH **7**

# No difference in product spectrum



Degree of reduction  
(mol e<sup>-</sup>/C-mol)

4.22

4.19

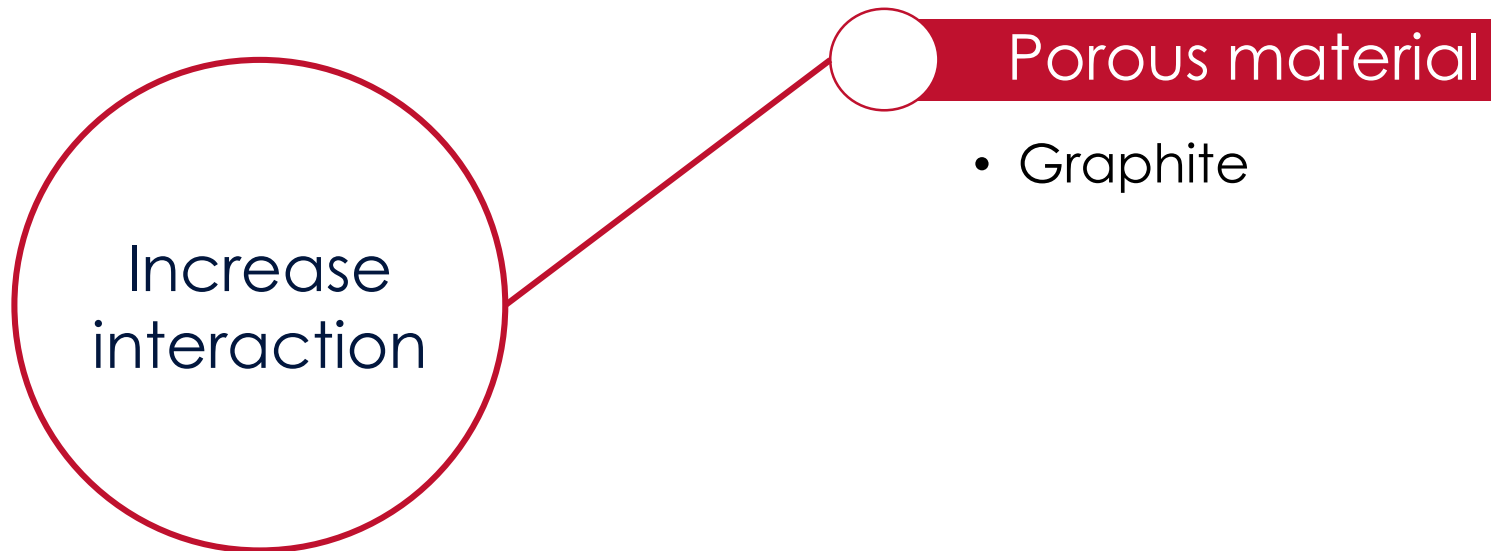
- Electron recovery <1% of electrons provided by glucose.

■ Acetate

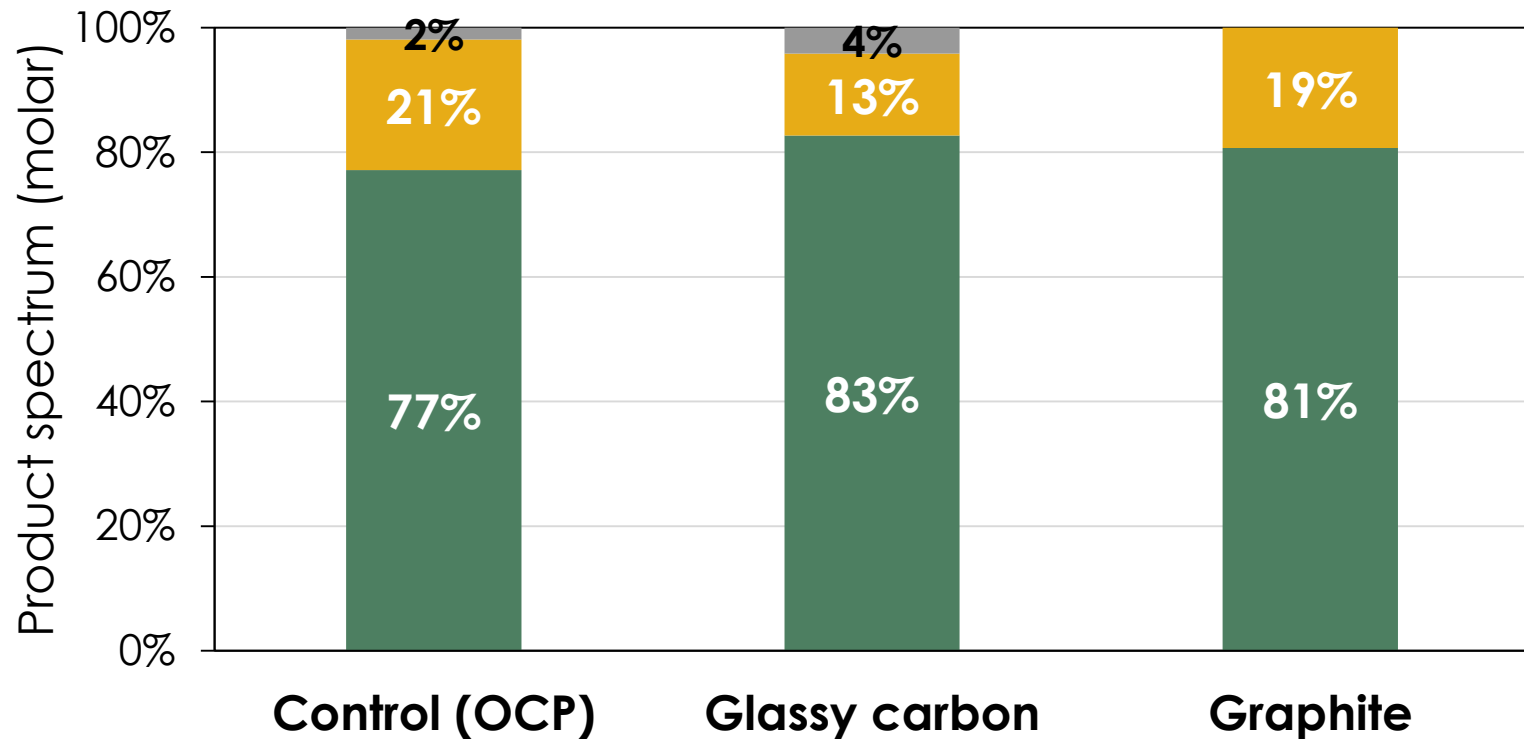
■ Propionate

■ Butyrate

# We want to increase the effect of the cathode



# Graphite does not influence product spectrum



Degree of  
reduction  
(mol e<sup>-</sup>/C-mol)

4.22

4.19

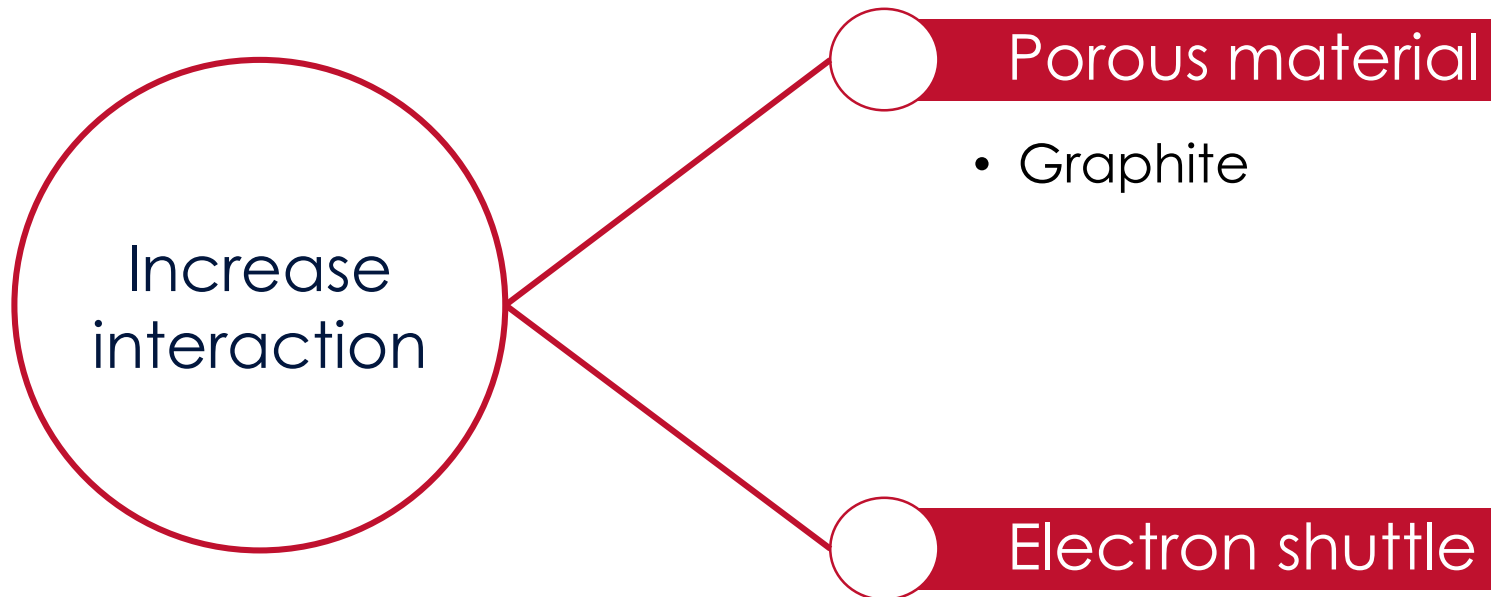
4.17

■ Acetate

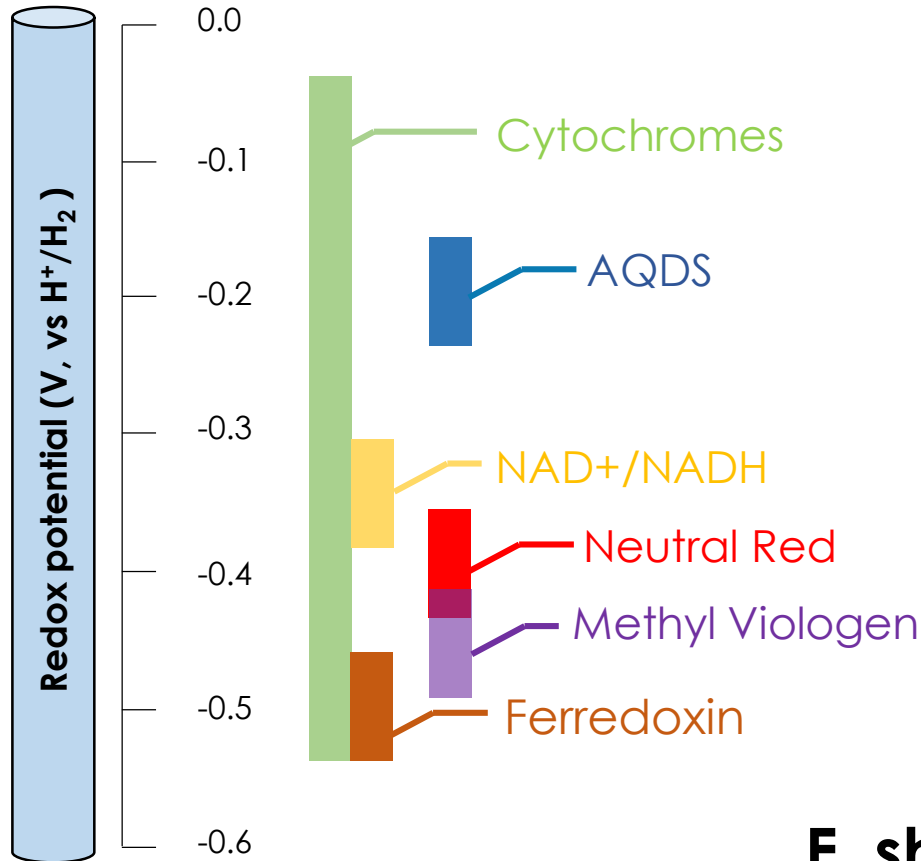
■ Propionate

■ Butyrate

# We want to increase the effect of the cathode

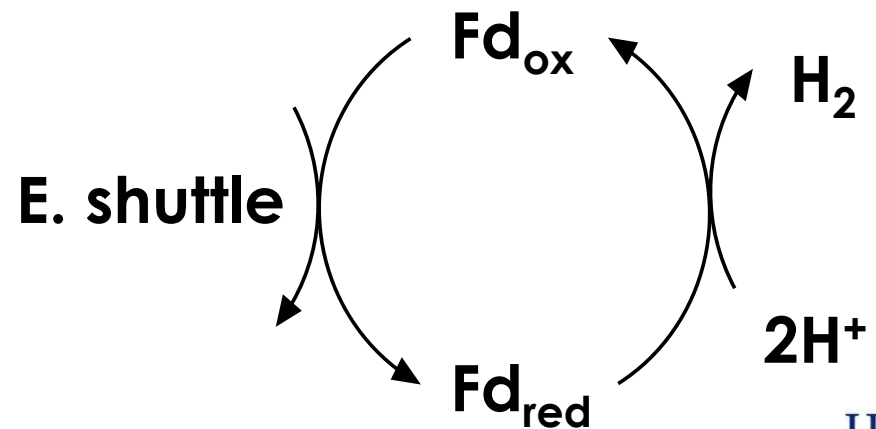


# Neutral Red is the best candidate as electron shuttle



## Requirement list:

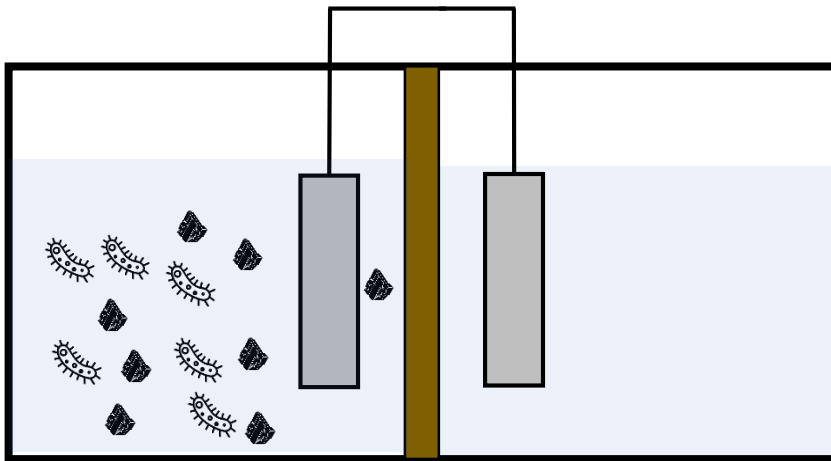
- Compatible with *G. sulfurreducens*
- Reversible reaction
- A lower redox potential than NADH
- A higher redox potential than Ferredoxin



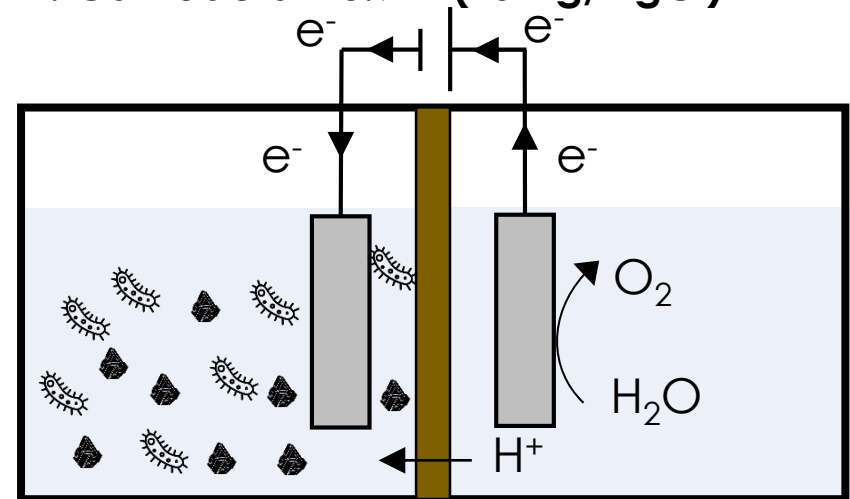
# Experimental setup

- Glucose (1 g/L)
- 2 chamber MET operated in batch mode.
- Mixed-culture inoculum enriched with a pure culture of *Geobacter sulfurreducens*
- Cathode simulating a fluidized electrode (glassy carbon).
- BES was added to avoid methanization
- **100  $\mu\text{M}$  Neutral Red added**

## 1. CONTROL at open circuit potential (OCP)



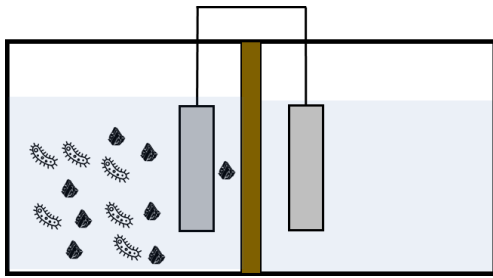
## 2. Cathode at -0.7 V (vs Ag/AgCl)



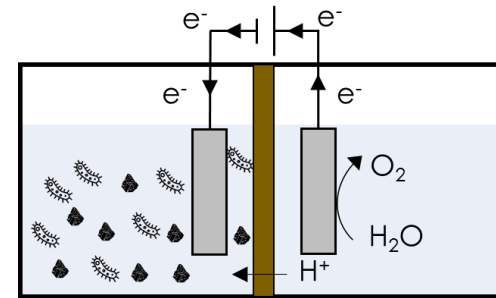
Cathode

Anode

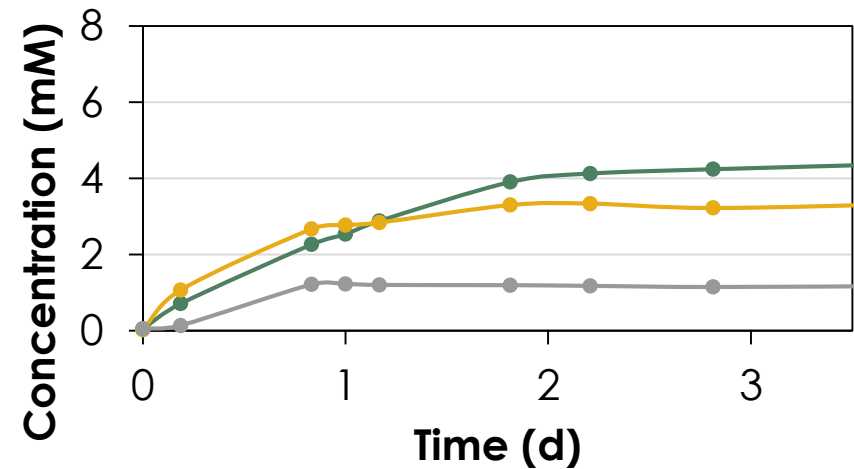
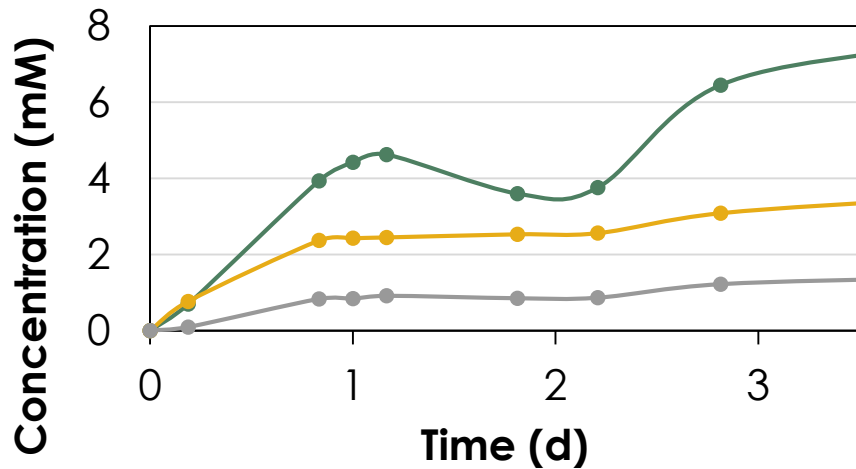
# Neutral Red addition does influence product spectrum



Control (OCP)



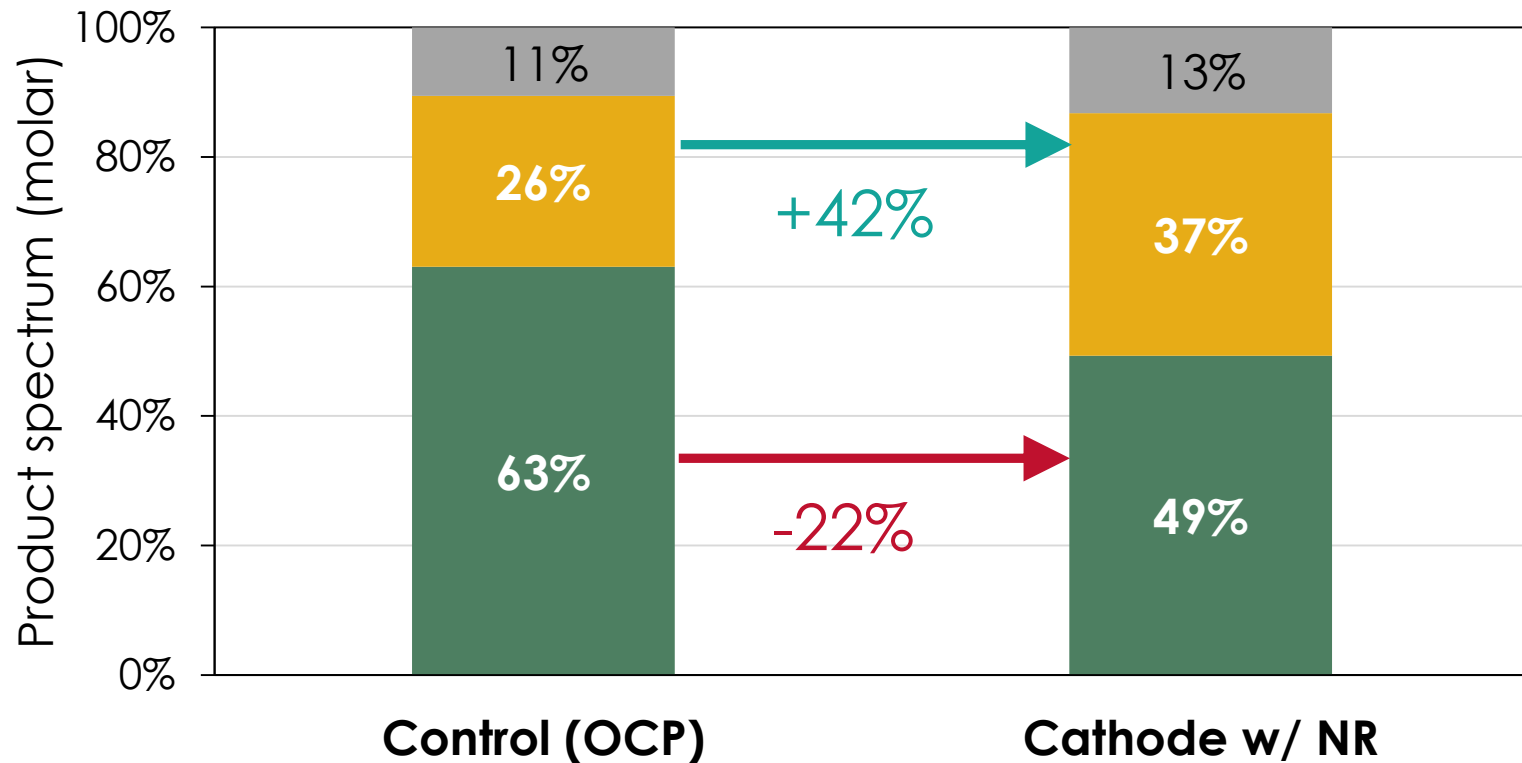
Cathode w/ NR



Initial glucose concentration **1 g/L**    pH **7**  
 Temperature **30°C**    Neutral Red **100 μM**



# Neutral Red addition does influence product spectrum



Degree of reduction  
(mol e<sup>-</sup>/C-mol)

4.42

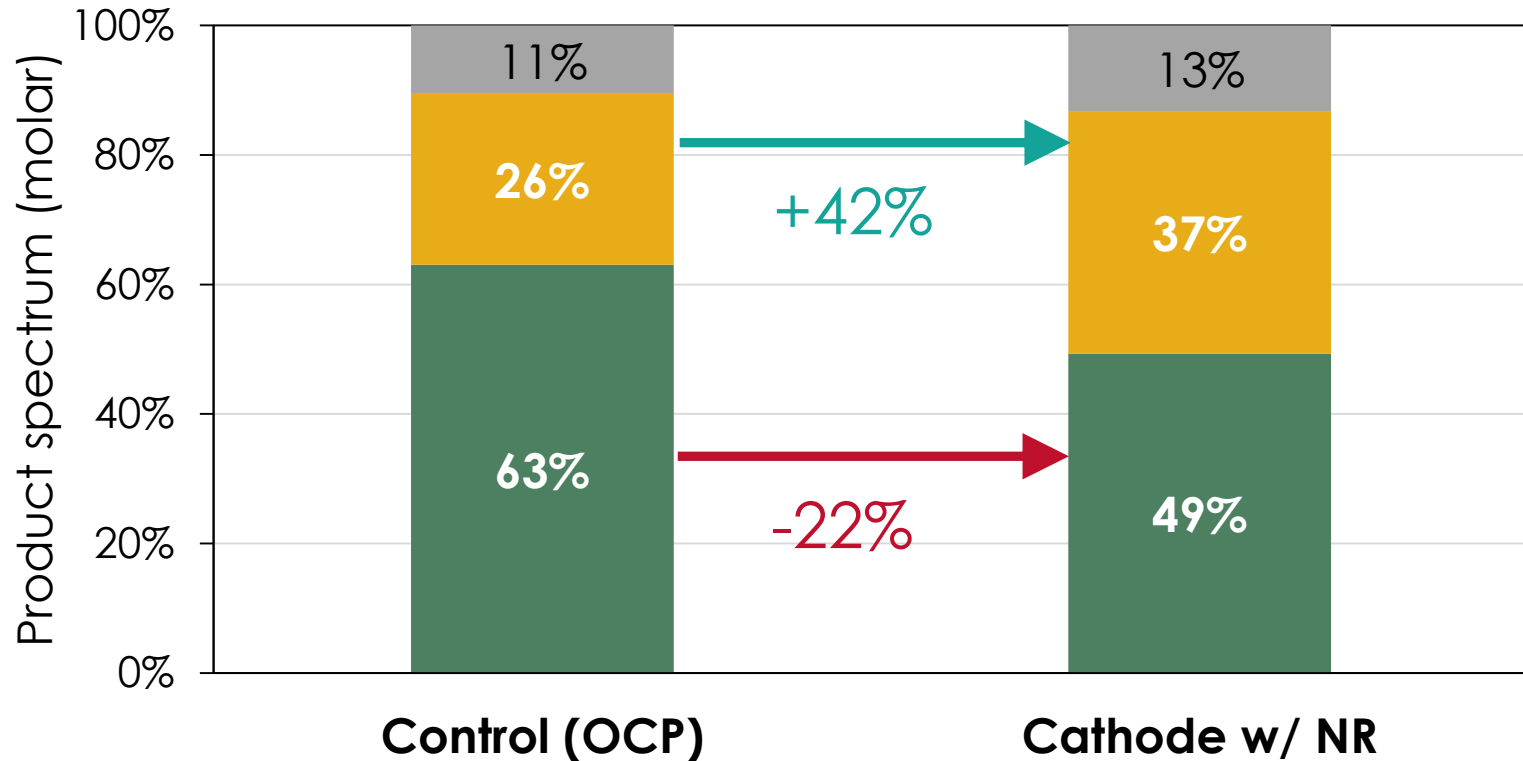
4.48

■ Acetate

■ Propionate

■ Butyrate

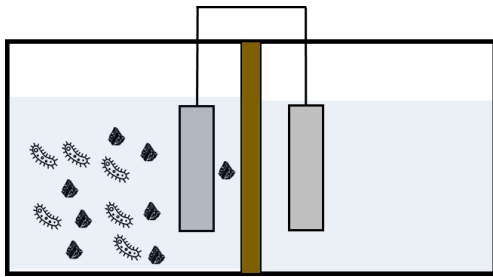
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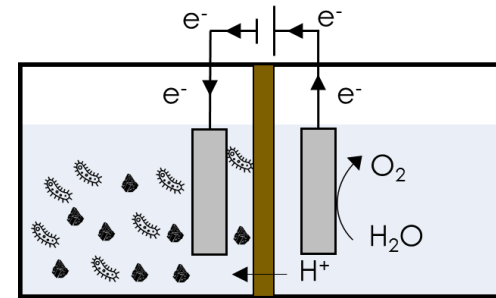
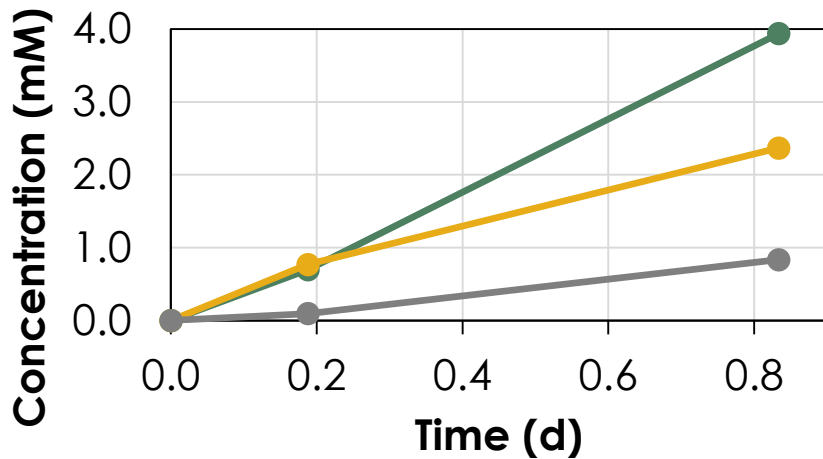
<b>Biomass concentration</b>	Control (OCP)	Cathode
(g VSS/L)	0.41	0.18

■ Acetate    ■ Propionate    ■ Butyrate

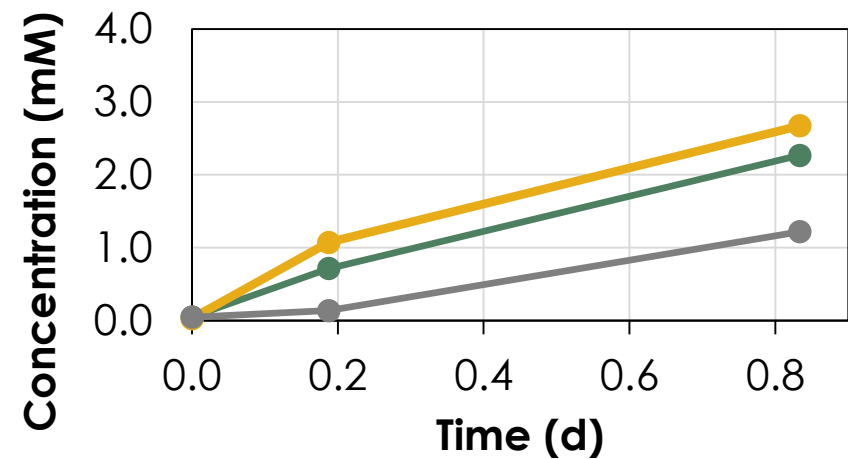
# Initial production rates are different



Control



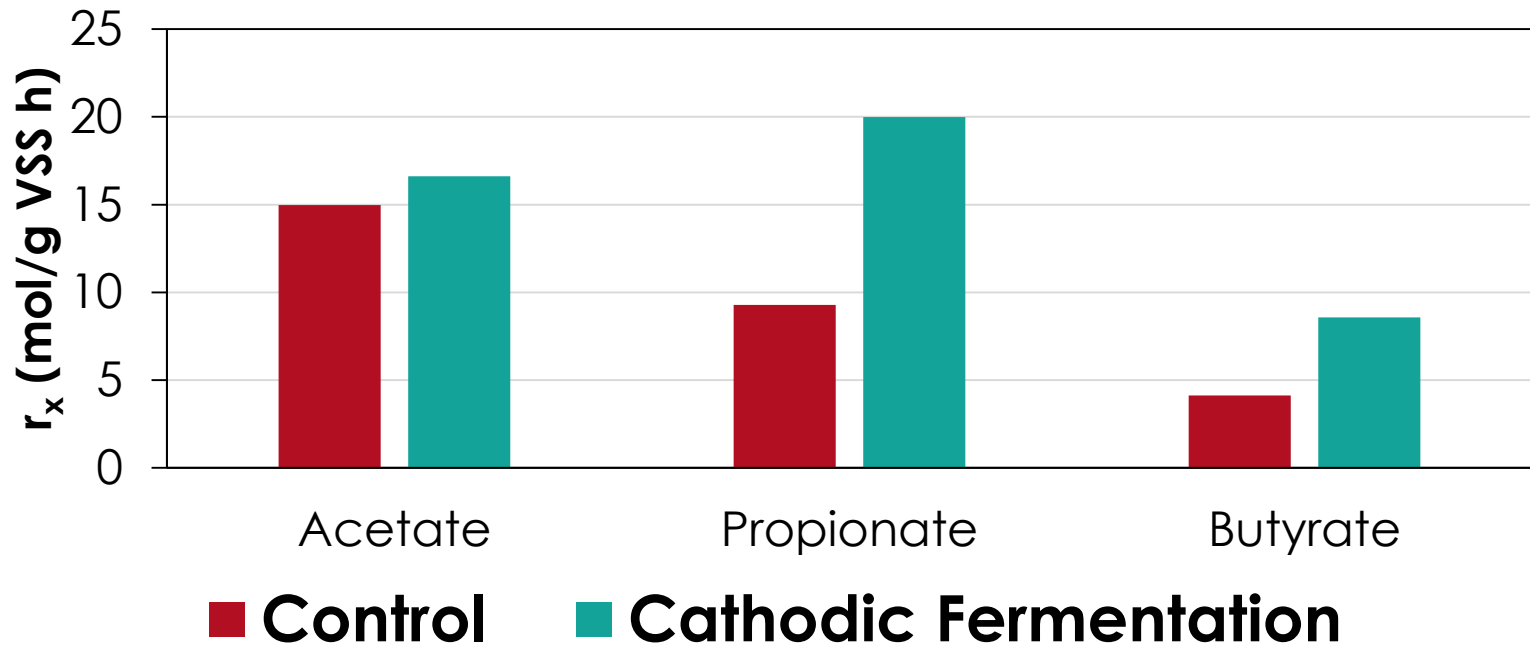
Cathode



Initial glucose concentration **1 g/L**    pH **7**  
 Temperature **30°C**    Neutral Red **100 μM**

# Propionate and butyrate specific productivities doubled

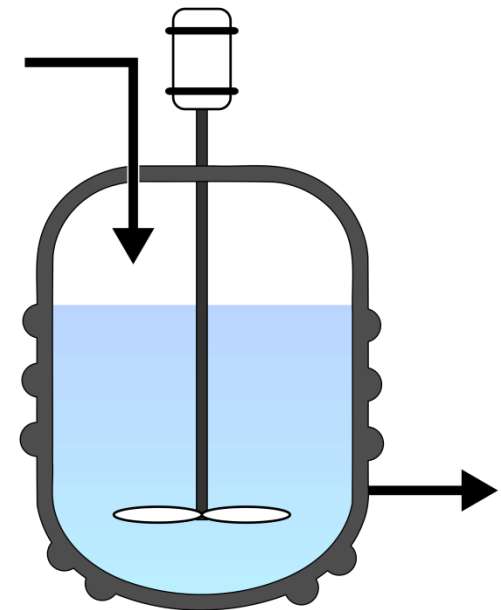
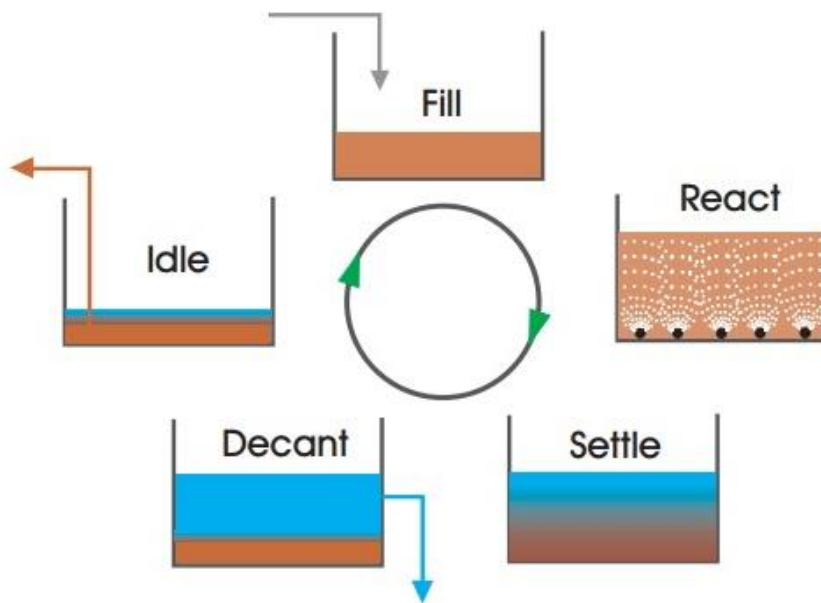
Specific productivity (0-20 h)



- <1% of electrons provided by glucose.

# Next step

- Towards a process that takes advantage of higher initial productivities



## Take home message

- We have promising results on directing open fermentations towards more reduced products.
- An electron shuttle is needed to provoke changes in product spectrum under the conditions tested.
- The increase of reduced products is over the stoichiometric contribution of the cathodic electrons.



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