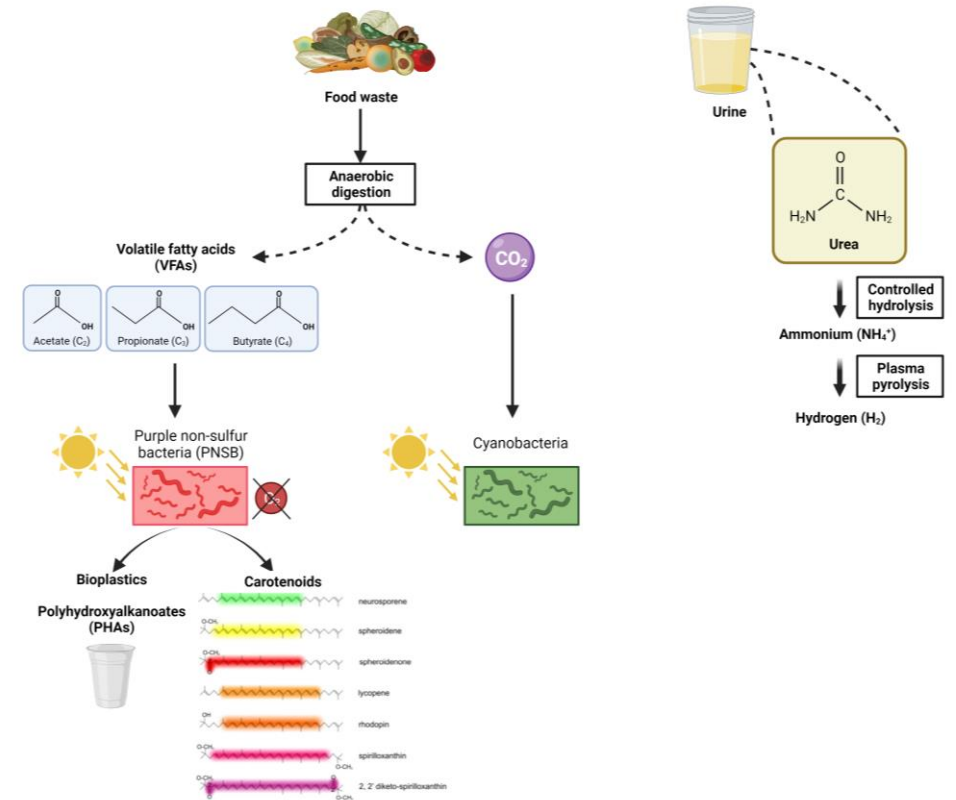


# WalBioPower: Valorisation of organic waste for the production of clean energy

Simone Krings, Ruddy Wattiez, Baptiste Leroy

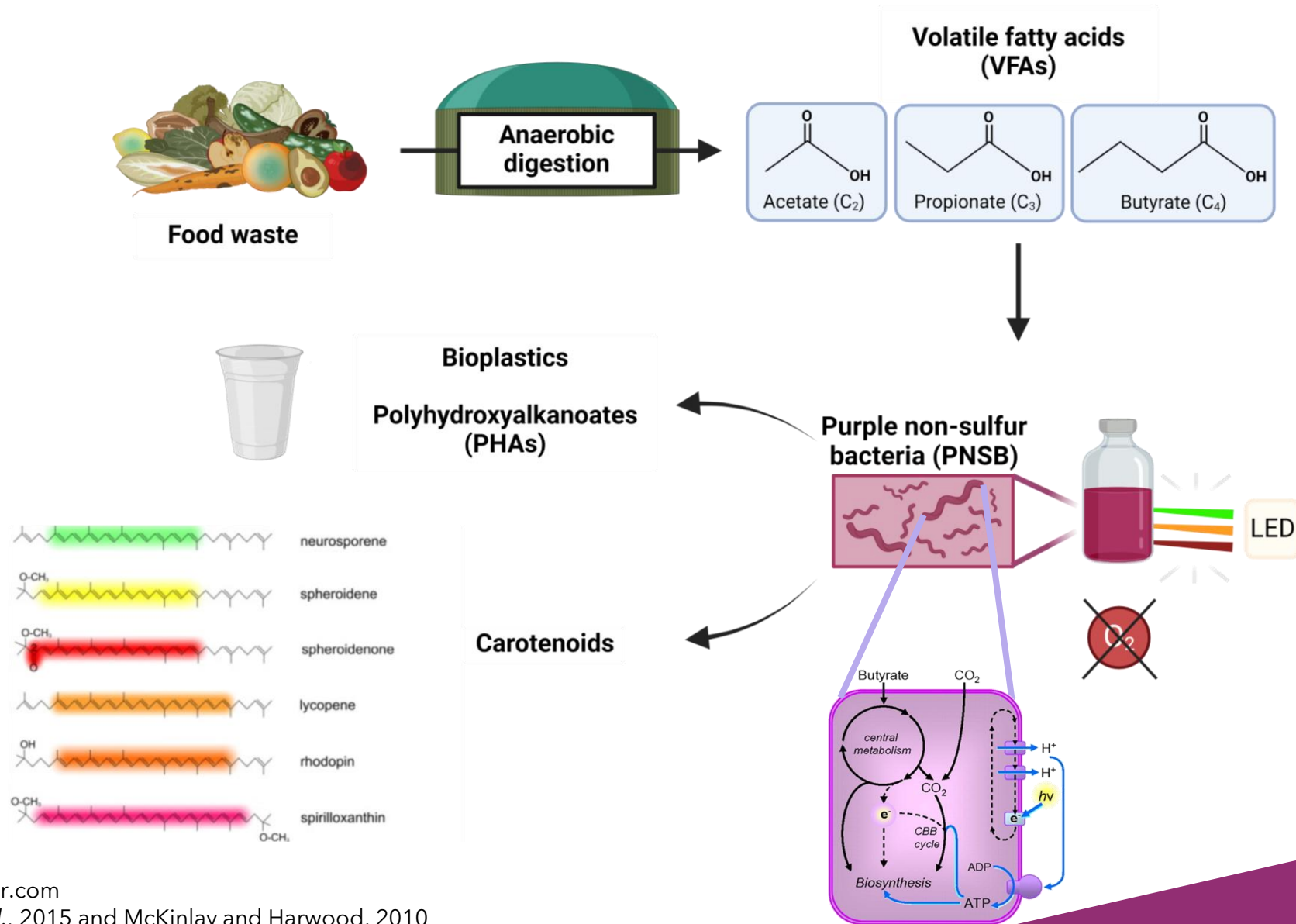
18/04/2024



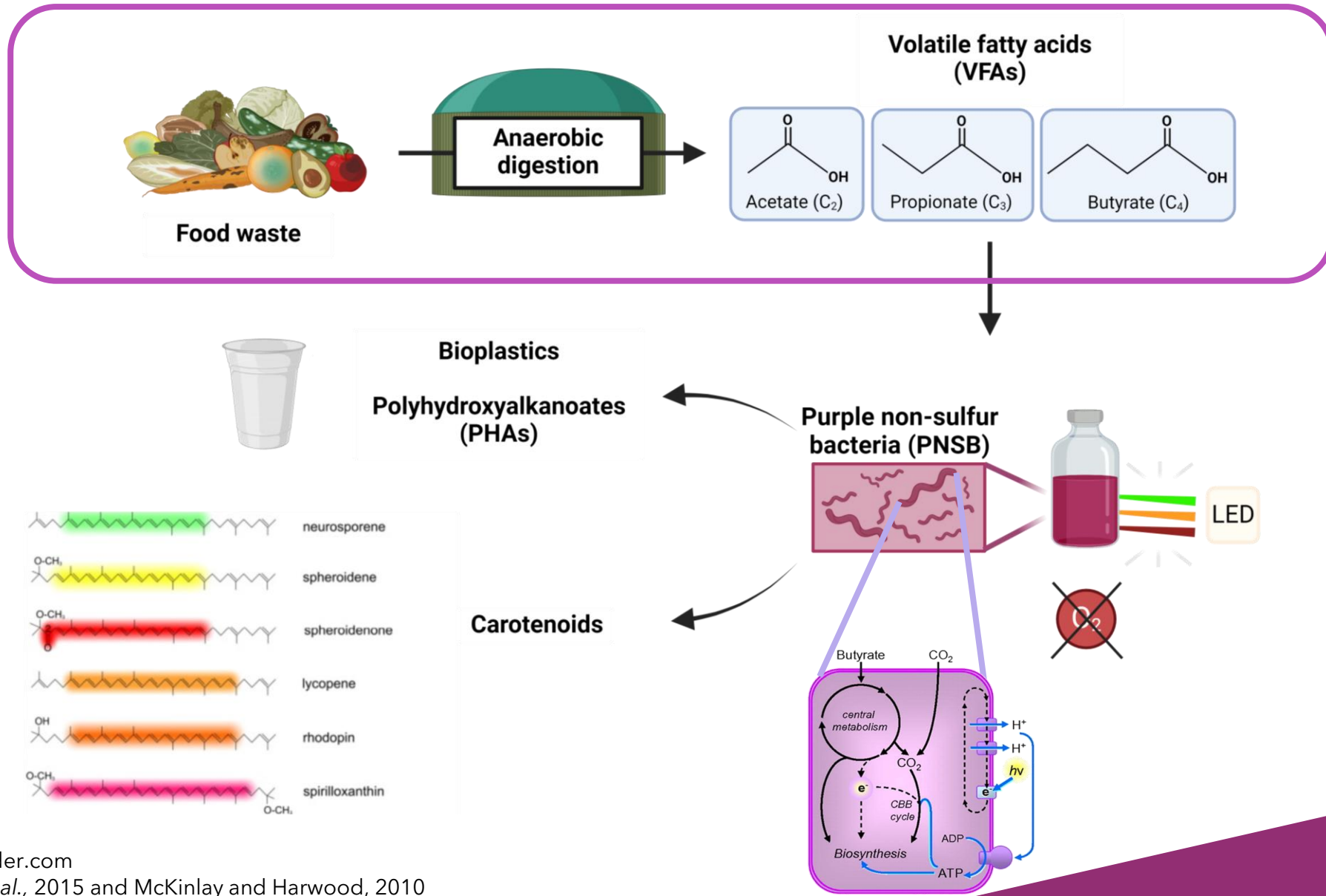
DEPARTMENT OF PROTEOMICS  
AND MICROBIOLOGY



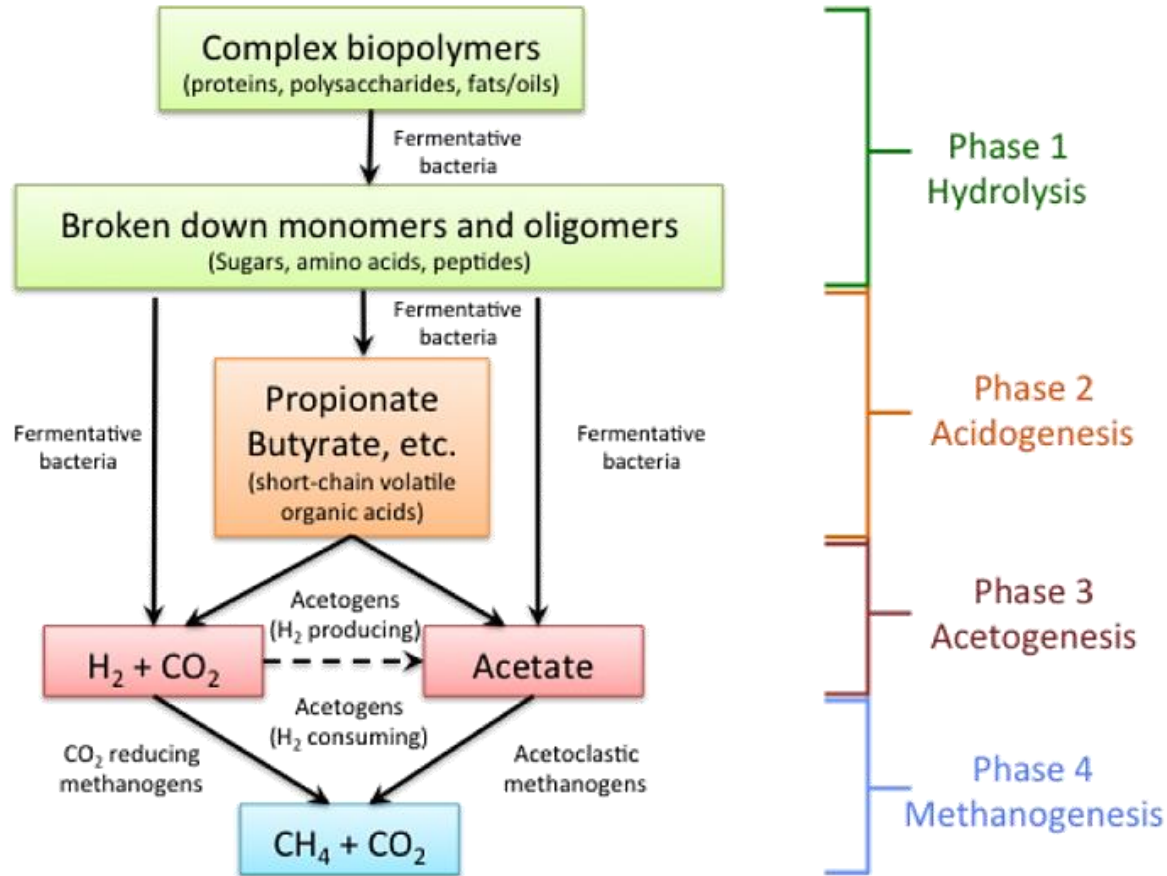
# Project overview



# Project overview



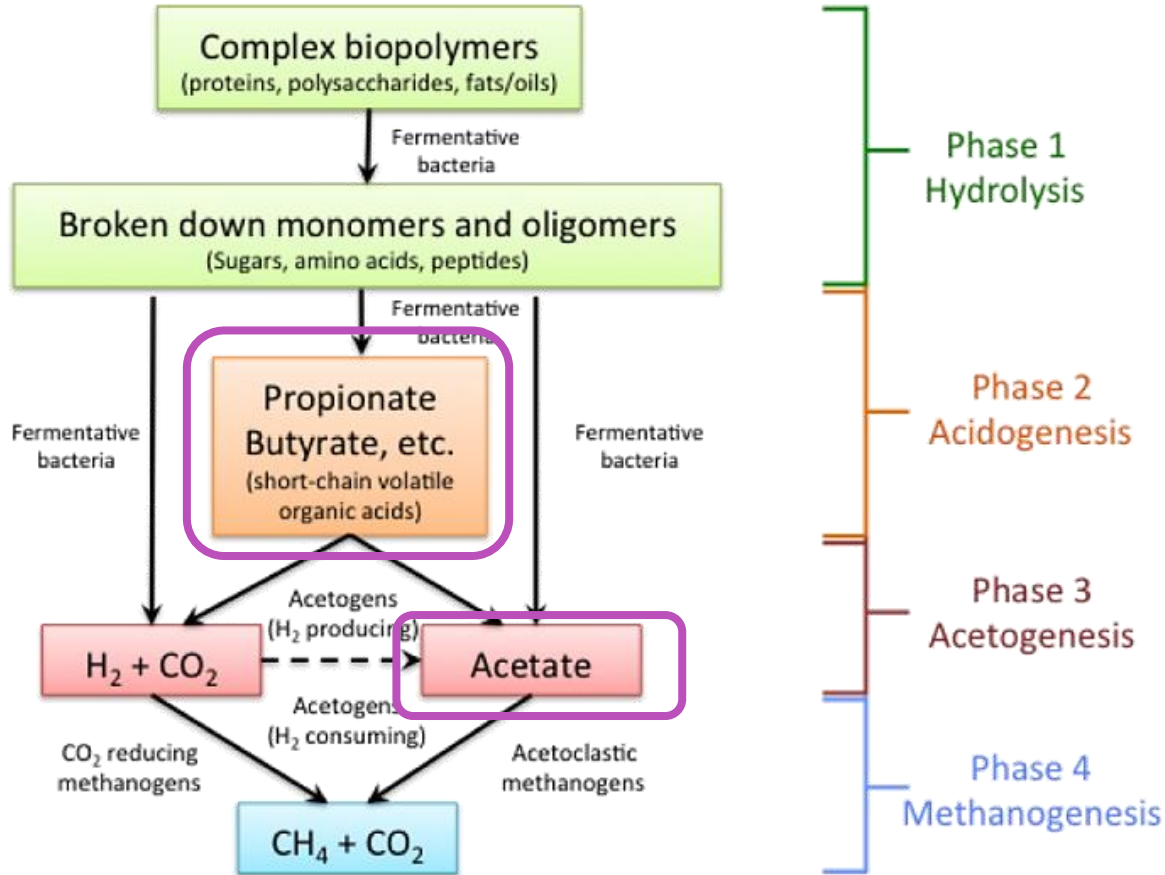
# Anaerobic digestion (AD)



## Waste streams

- Agriculture-based (manure and crops)
- Wastewater treatment plants
- **Food** and municipal waste

# Anaerobic digestion (AD)



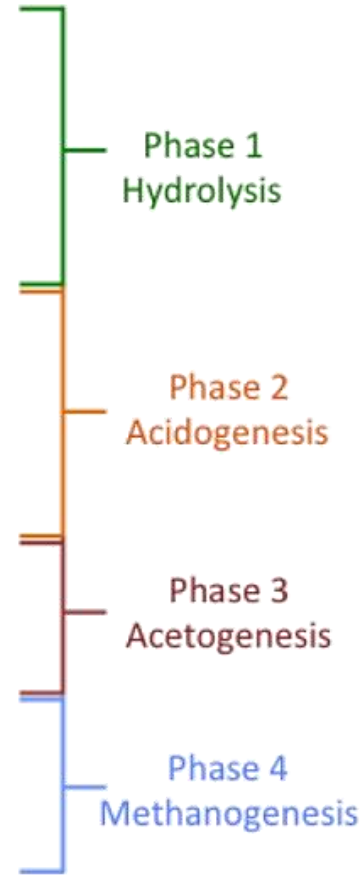
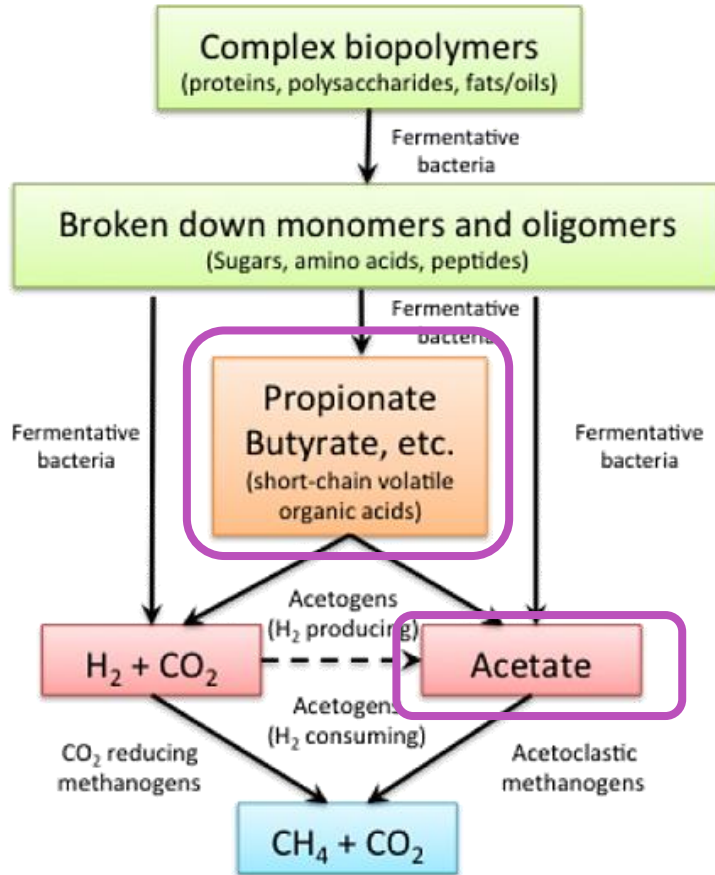
## Waste streams

- Agriculture-based (manure and crops)
- Wastewater treatment plants
- **Food** and municipal waste

## Short-chain fatty acids (SCFAs) = Volatile fatty acids (VFAs)

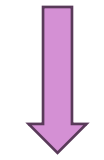
- Acetate
- Propionate
- (Iso)butyrate
- (Iso)valerate

# Anaerobic digestion (AD)



- Waste streams
- Agriculture-based (manure and crops)
  - Wastewater treatment plants
  - **Food** and municipal waste

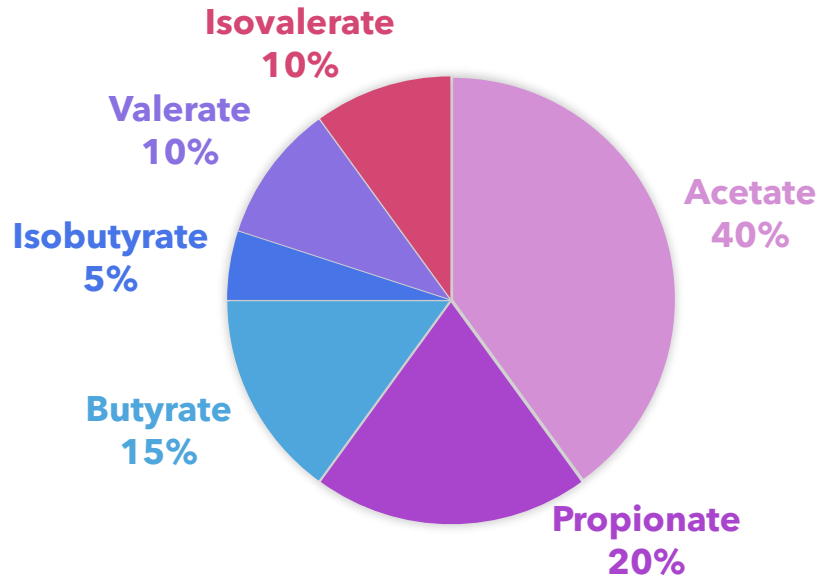
- Short-chain fatty acids (SCFAs) = Volatile fatty acids (VFAs)
- Acetate
  - Propionate
  - (Iso)butyrate
  - (Iso)valerate





Purple non-sulfur bacteria (PNSB)

# Culturing PNSB in Synthetic Digestate

## Synthetic Digestate (%)



- *Rhodospirillum rubrum* 
- Co-culture crs: *Rhodobacter capsulatus*, *Rhodospirillum rubrum* and *Cereibacter sphaeroides* 

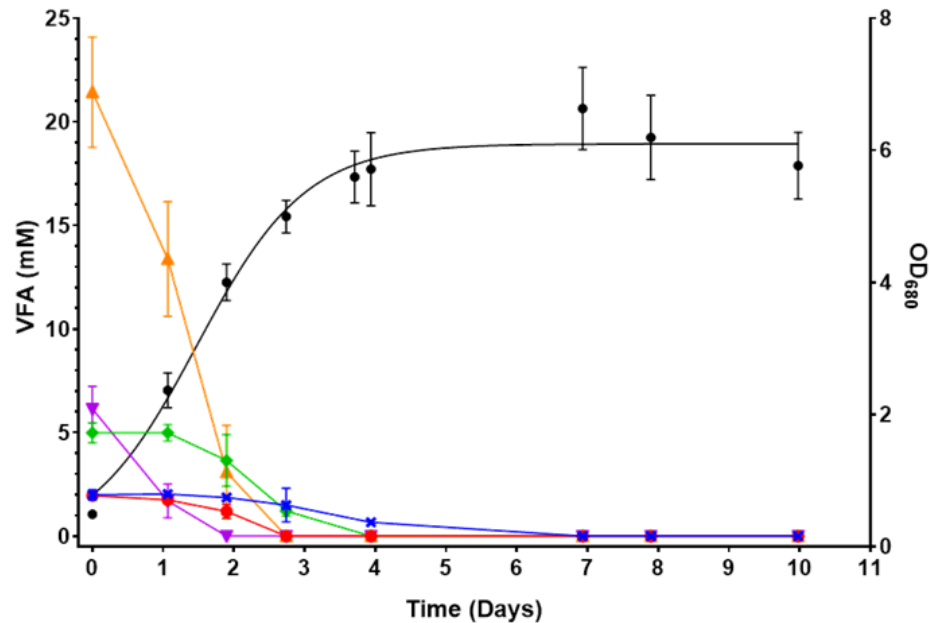
- Culture in SMN broth
- Preculture in MELiSSA medium + VFA (Acetate, Propionate and Butyrate (1:1:1) + 50 mM NaHCO<sub>3</sub> + Thiamine and Niacin
- Experiment in MELiSSA with synthetic digestate



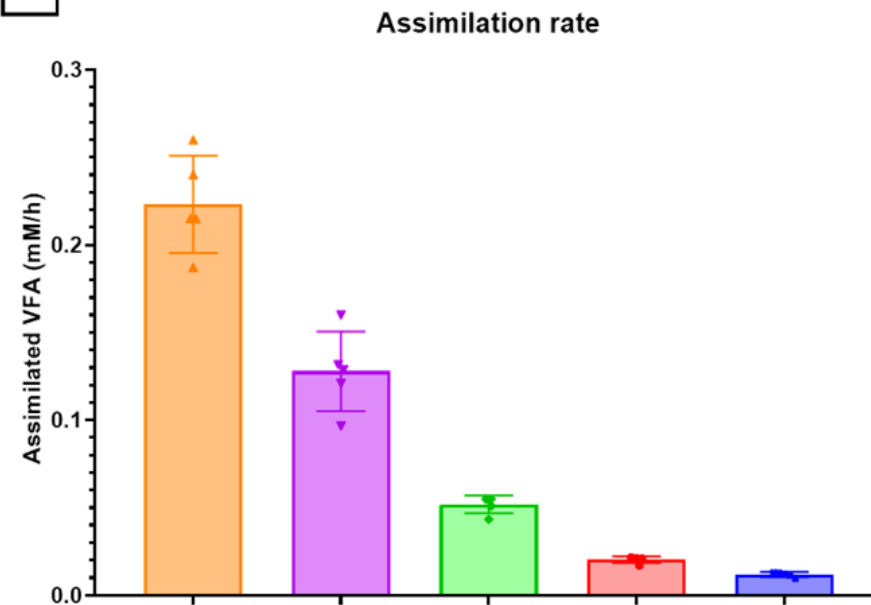
# Culturing PNSB in Synthetic Digestate

- LC-MS analysis of VFA assimilation

**A**



**B**



▲ Acetic acid    ▼ Propanoic acid    ◆ (Iso)butanoic acid    ● Valeric acid    \* Isovaleric acid





# Bacterial strain proportions

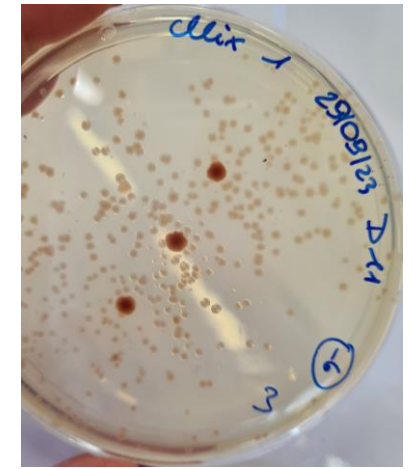
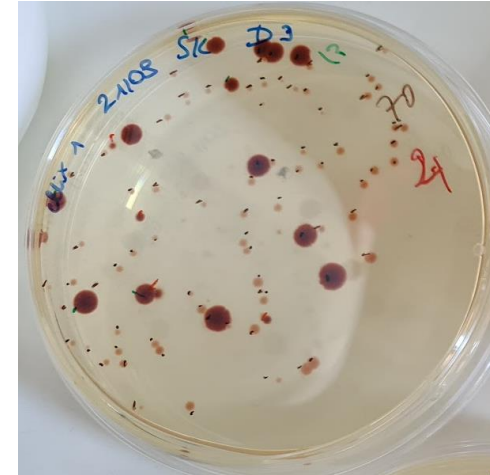
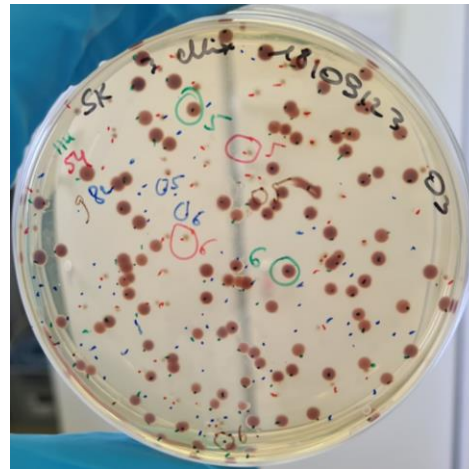
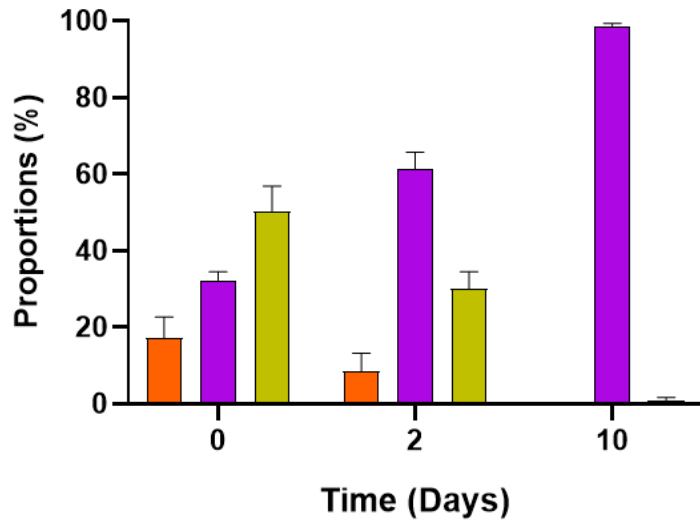
- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$





# Bacterial strain proportions

- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$

Based on CFU/mL



 *Rb. capsulatus*

 *Rs. rubrum*

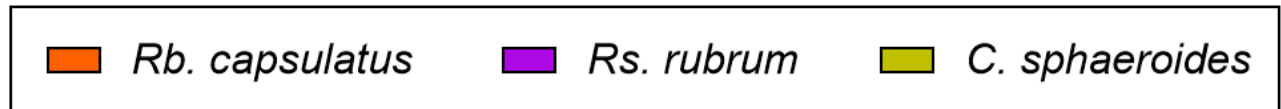
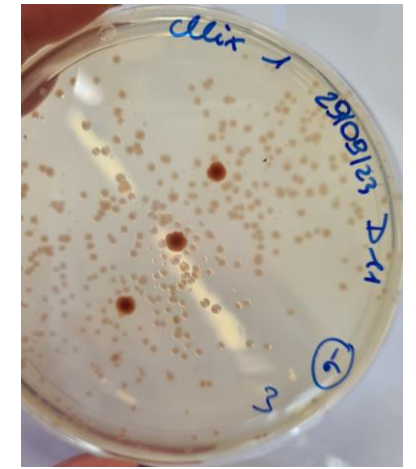
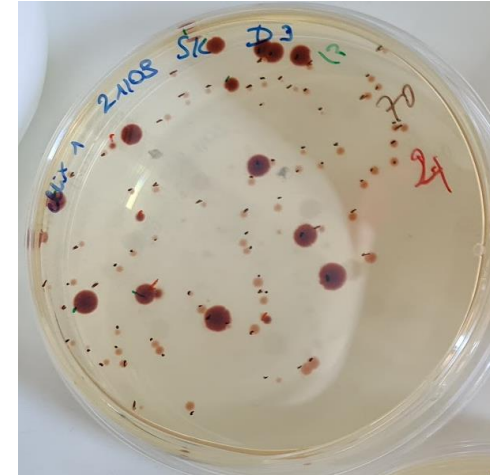
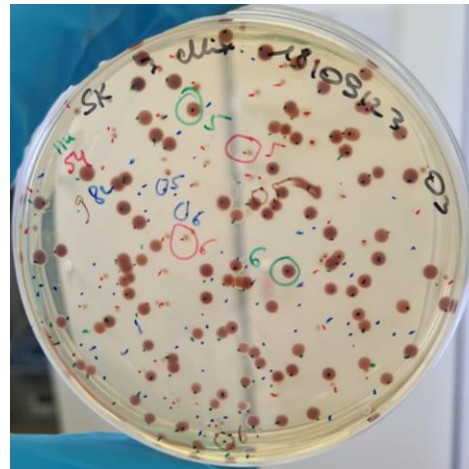
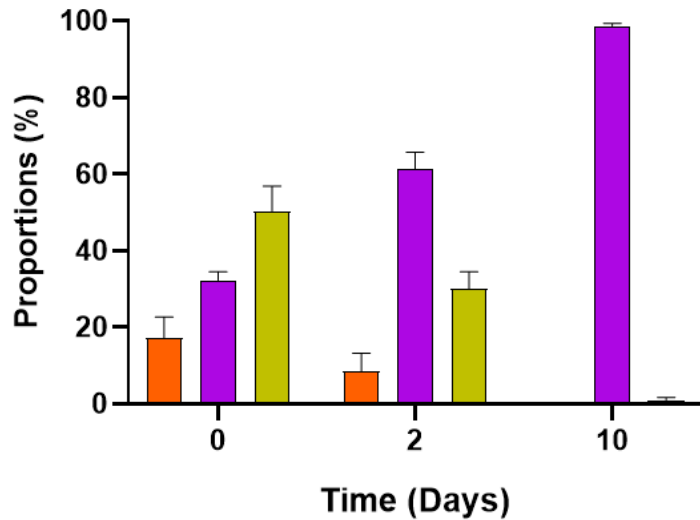
 *C. sphaeroides*



# Bacterial strain proportions

- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$

Based on CFU/mL

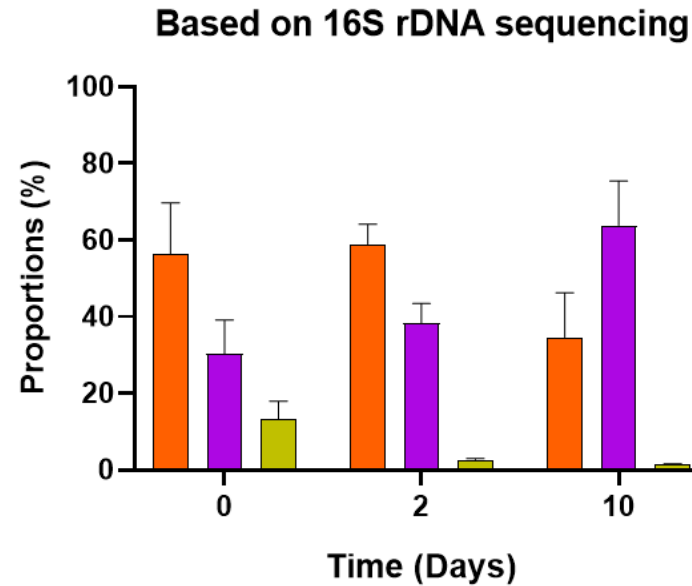
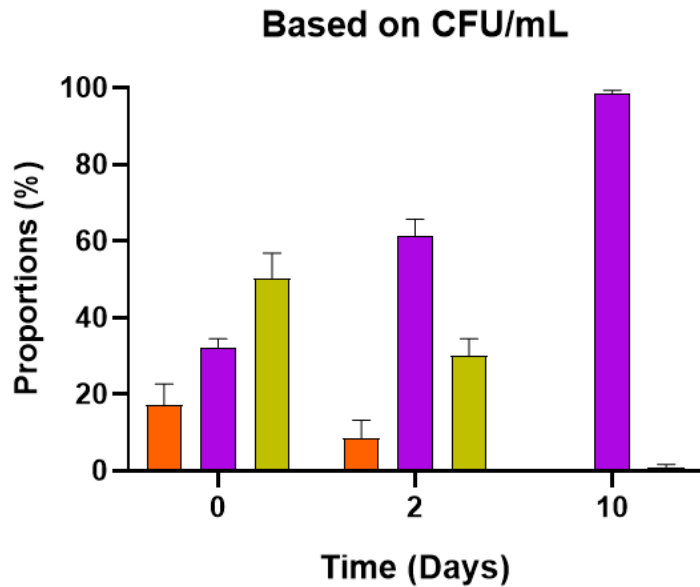


Similarity of colonies on agar plates






# Bacterial strain proportions

- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$



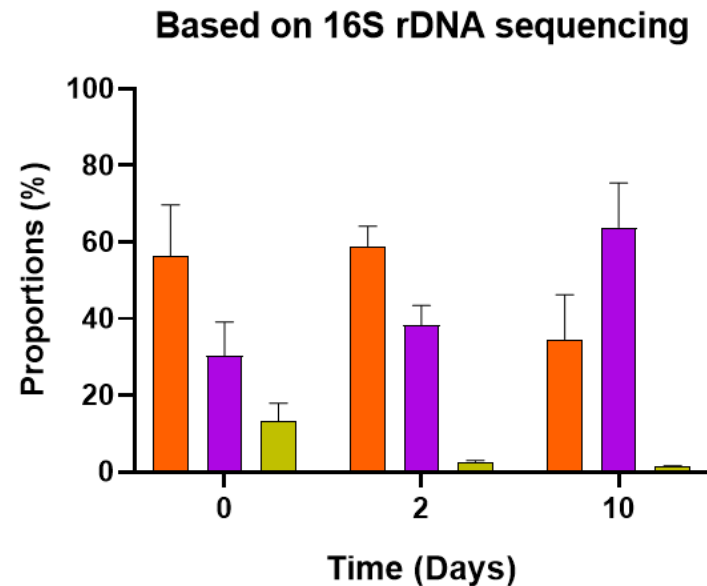
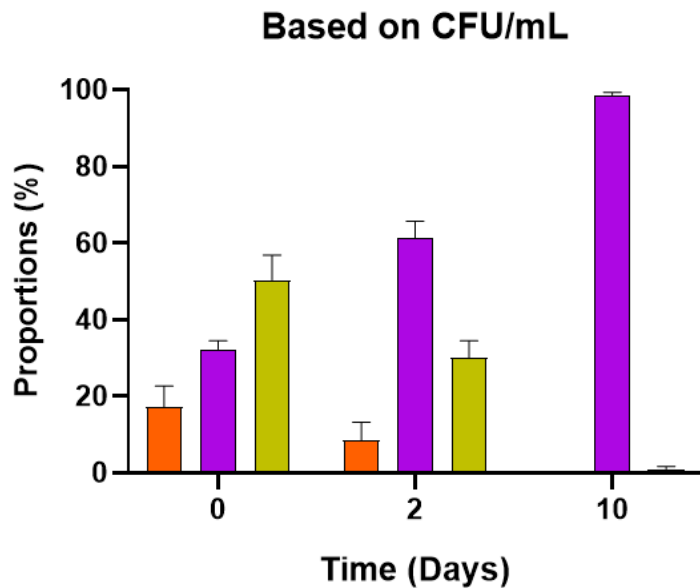
Oxford Nanopore GridION  
+ 16S rDNA primers from Oxford  
Nanopore

 *Rb. capsulatus*     *Rs. rubrum*     *C. sphaeroides*

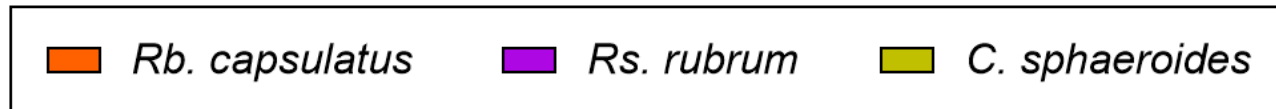


# Bacterial strain proportions

- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$



Oxford Nanopore GridION  
+ 16S rDNA primers from Oxford  
Nanopore

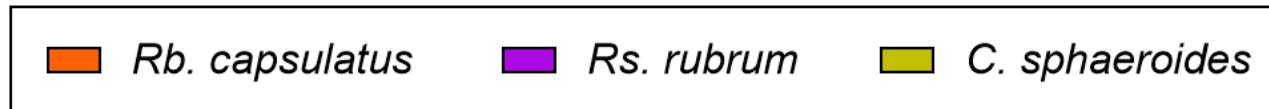
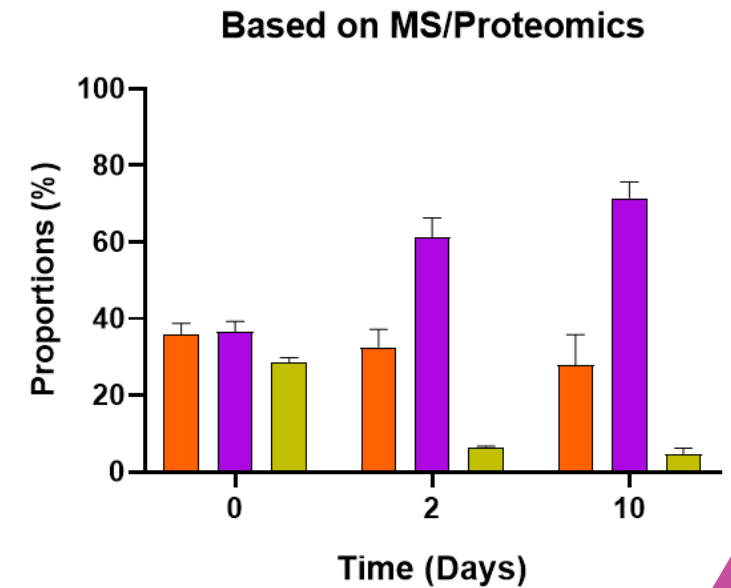
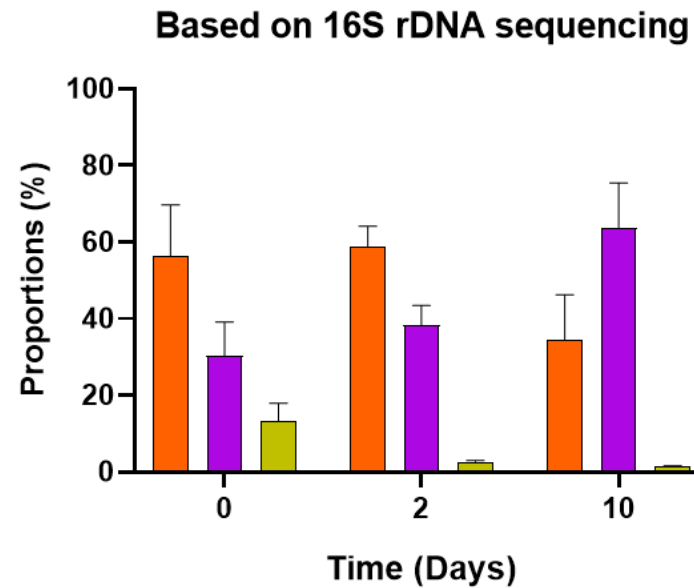
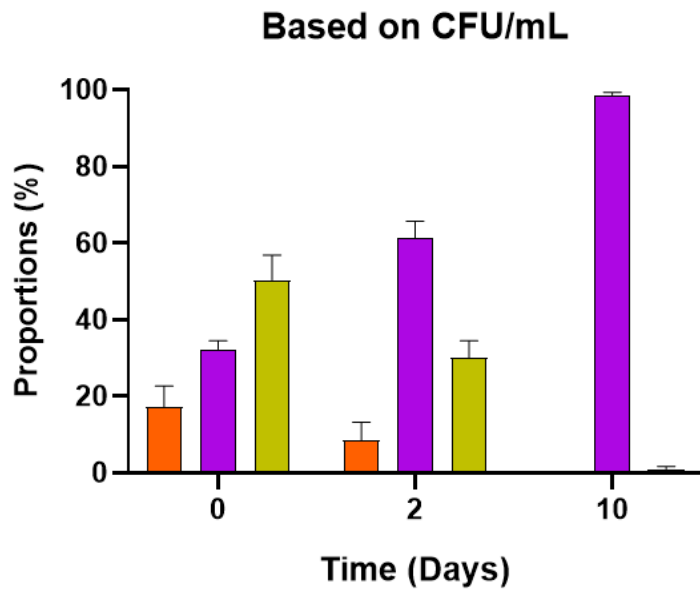


Primers have a higher affinity  
for *Rb. capsulatus*



# Bacterial strain proportions

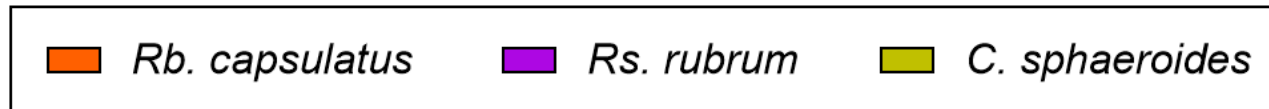
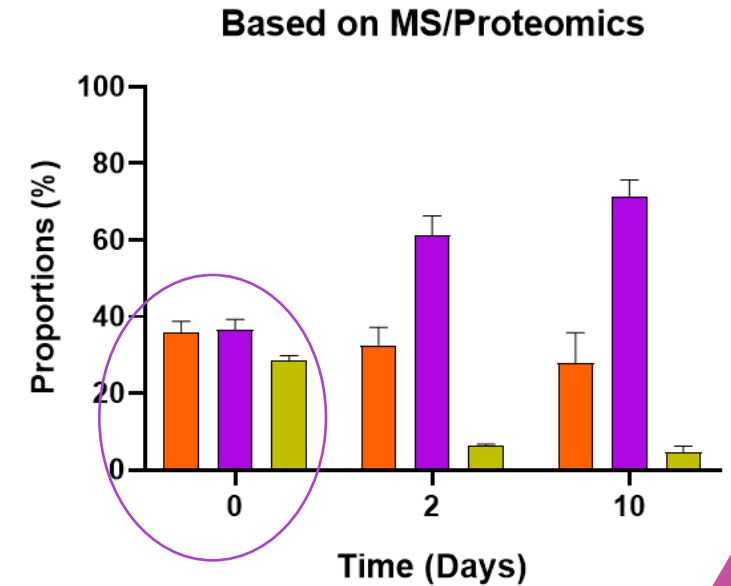
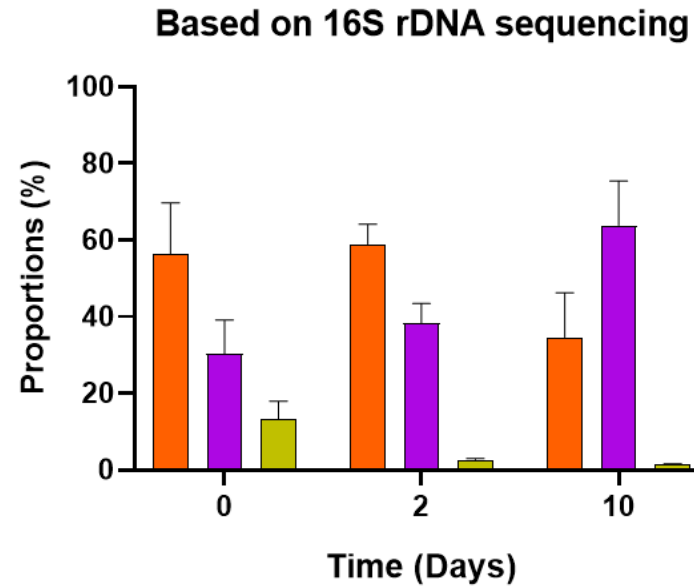
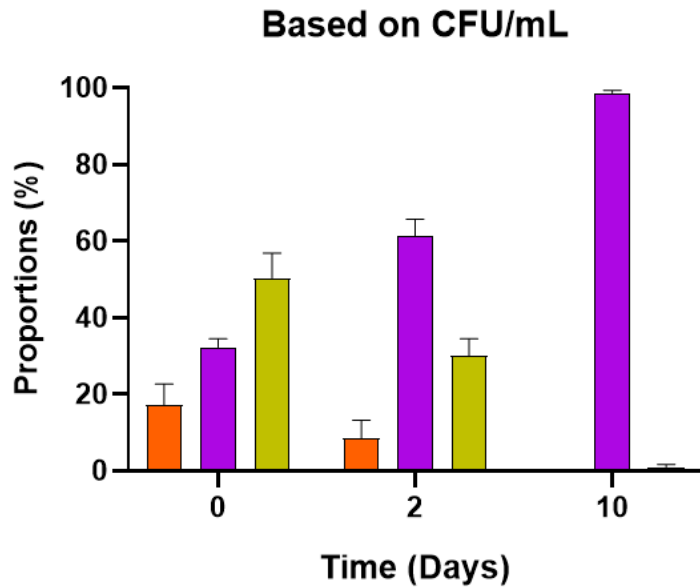
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# Bacterial strain proportions

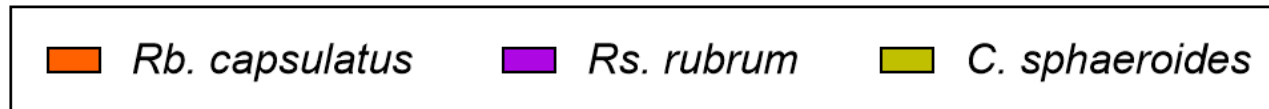
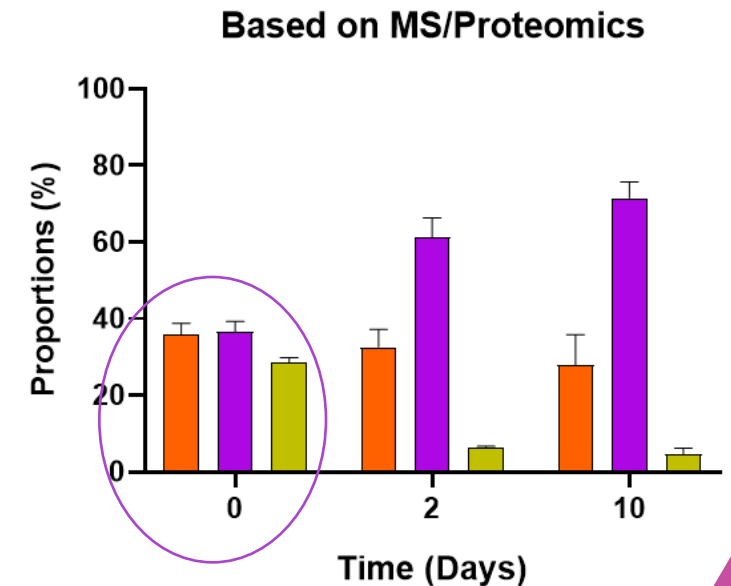
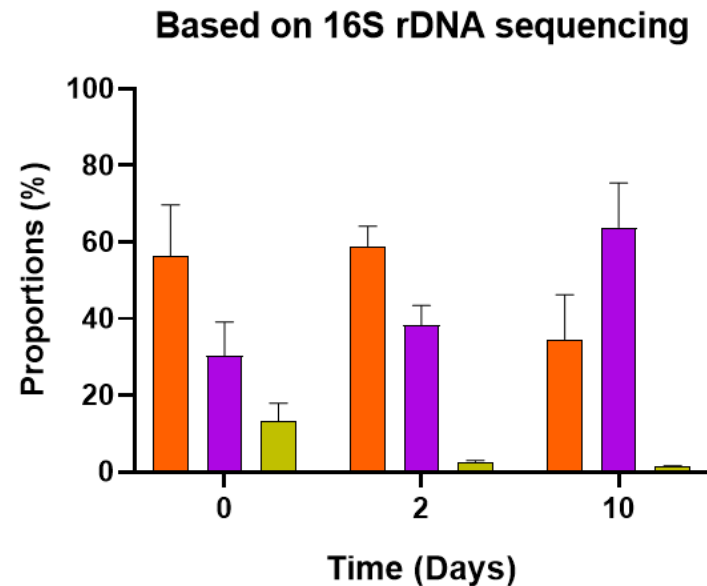
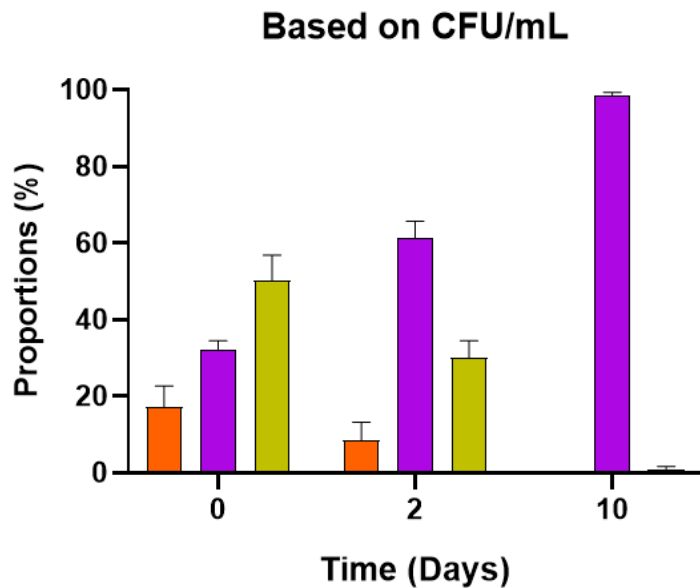
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# Bacterial strain proportions

- $OD_{680} = 0.133$  of each strain  $\Rightarrow$  Achieve Start  $OD_{680} = 0.5$



**MS/Proteomics could be a great alternative to the classical methods!**



# Ammonium in Food-waste Digestates

Standard MELiSSA Medium:  
 35 mM NH<sub>4</sub>Cl  
 → 0.5 g/L N  
 → 0.635 g/L NH<sub>4</sub><sup>+</sup>

10x

Table 1

Food waste digestate characteristics (MC: moisture content; EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorous; TK: total potassium).

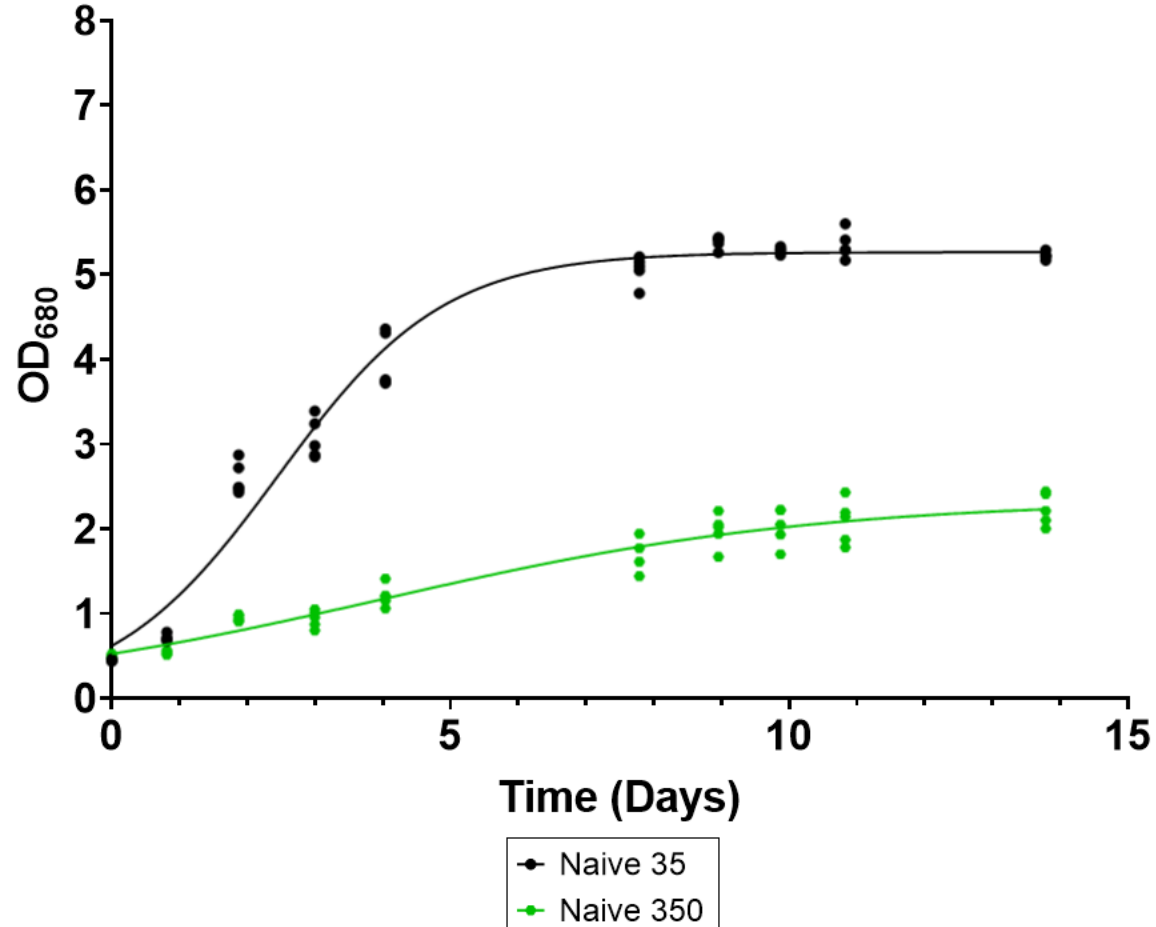
Waste type	Country	MC (%)	pH	EC (dS/m)	OM (%)	TOC (%)	TN (%)	TP (%)	TK (%)	NH <sub>4</sub> <sup>+</sup> -N (g/kg)	C/N ratio	References
		96.4	8.9	10.8	91.0	43.5	9.6	2.4	2.3	7.2	20.9	
		85.3	8.1	5.2	63.6	32.1	4.6	1.0	1.3	4.0	11.3	
		69.8	7.3	0.8	38.5	12.8	1.1	0.1	0.4	1.5	3.1	
OFMSW	Spain	71.8	8.5	6.8	45.3	-	3.2	0.75	-	-	-	García-Albacete et al. (2014)
Source selected biowaste	Spain	75.6	8.31	-	63	34.1	4.3	-	-	-	11.9	Cerda et al. (2019)
OFMSW	Spain	76	8.3	-	63	34	4.3	-	-	-	11.8	Rodríguez et al. (2019)
Food waste	Ireland	80	-	-	64.7	-	-	-	-	-	-	Chen et al. (2019)
OFMSW	Italy	92.1	7.69	-	65	35.7	-	-	-	-	-	Beggio et al. (2019)
Food waste	UK	92.5	8.05-8.24	-	-	-	3.3-4.4	0.56-0.76	1.05-1.55	2.9-3.6	-	Sánchez-Rodríguez et al. (2018)
Food waste	China	-	-	-	66.1	43.5	1.92	-	-	-	-	Liu et al. (2020)
Food waste	UK	93.9-95.6	8.1-8.8	-	-	-	5.4-8	-	-	3.9-6.3	-	Nicholson et al. (2017)
Food waste	China	96.4	8.6	-	91	-	4.8	0.53	-	2.54	-	Wang et al. (2020)
Residual household waste	France	69.8	8.2	-	38.5	-	-	-	-	4.18	-	Zeng et al. (2016)
Organic household waste	Germany	-	-	-	85.1	34.3	1.9	-	-	-	-	Cao et al. (2019)
Food waste	USA	-	-	2.5	-	-	6.96	1.2	1.9	3.4	-	Barzee et al. (2019)
Food waste	Italy	-	8.4-8.9	-	50.1-58.8	30.2-31.3	1.6-3.5	0.5-0.9	0.4-0.5	2.3-2.4	8.9-18.9	Grigatti et al. (2020)
Food waste	Poland	-	8.1	-	63	-	6.6	1.6	1.63	-	-	Czekala et al. (2020)
Segregated biodegradable waste	Italy	-	7.7	-	64	31.4	4.9	-	-	3.48	-	Peng et al. (2018)
Food waste	Singapore	-	7.81	-	-	-	-	0.5	-	4.7	-	Cheong et al. (2020)
Food waste	Austria	-	7.8	-	-	39.7	1.4	-	-	5.1	-	Franke-Whittle et al. (2014)
OFMSW	Canada	-	8.5	10.8	48	24.5	1.6	1.5	1.5	6.19	-	Arab and McCartney (2017)
Food waste	Finland	-	7.6-8.3	-	50.2-63.7	25.9-26.9	7.8-8.7	0.1-0.3	1.9-3.2	3.2-4.5	3.1-3.3	Tampio et al. (2016)
OFMSW	Canada	-	8.5	0.8	-	-	-	1	-	5	-	McLachlan et al. (2004)
Food waste	Australia	-	8.02	-	-	42.1	5.8	1.97	0.62	1.5	-	Opatokun et al. (2016)
Food waste	Italy	-	7.97	-	80.4	-	3.97	0.9	2.3	-	-	Peng and Pivato (2019)
OFMSW	Italy	-	8.8	-	68-71	12.8-22.7	1.09	1.49	0.78	-	12.1-20.9	Peng and Pivato (2019)
Food waste	Germany	-	7.3-8.3	-	-	-	4.2-6.7	-	-	5.1-7.2	-	Fuchs and Drogg (2013)
Bio waste	Germany	-	7.6-8.1	-	-	-	3-6.8	-	-	1.5-5.6	-	Fuchs and Drogg (2013)
Fruit waste	Australia	94.8	-	-	73.1	43.5	9.6	0.8	1	-	-	Serrano et al. (2020)
Household organic waste	Switzerland	-	-	-	64.7	33.1	4.7	2.45	0.63	-	-	Loes et al. (2018)

~350 mM NH<sub>4</sub>Cl  
 → 5 g/L N  
 → 6.35 g/L NH<sub>4</sub><sup>+</sup>



# Effects of High Ammonium Levels

## Co-culture crs



Reduced growth of co-cultures of *Rb. capsulatus*, *Rs. rubrum*, *C. sphaeroides* in high-ammonium medium

⇒ Why do the bacteria suffer in high-ammonium medium?

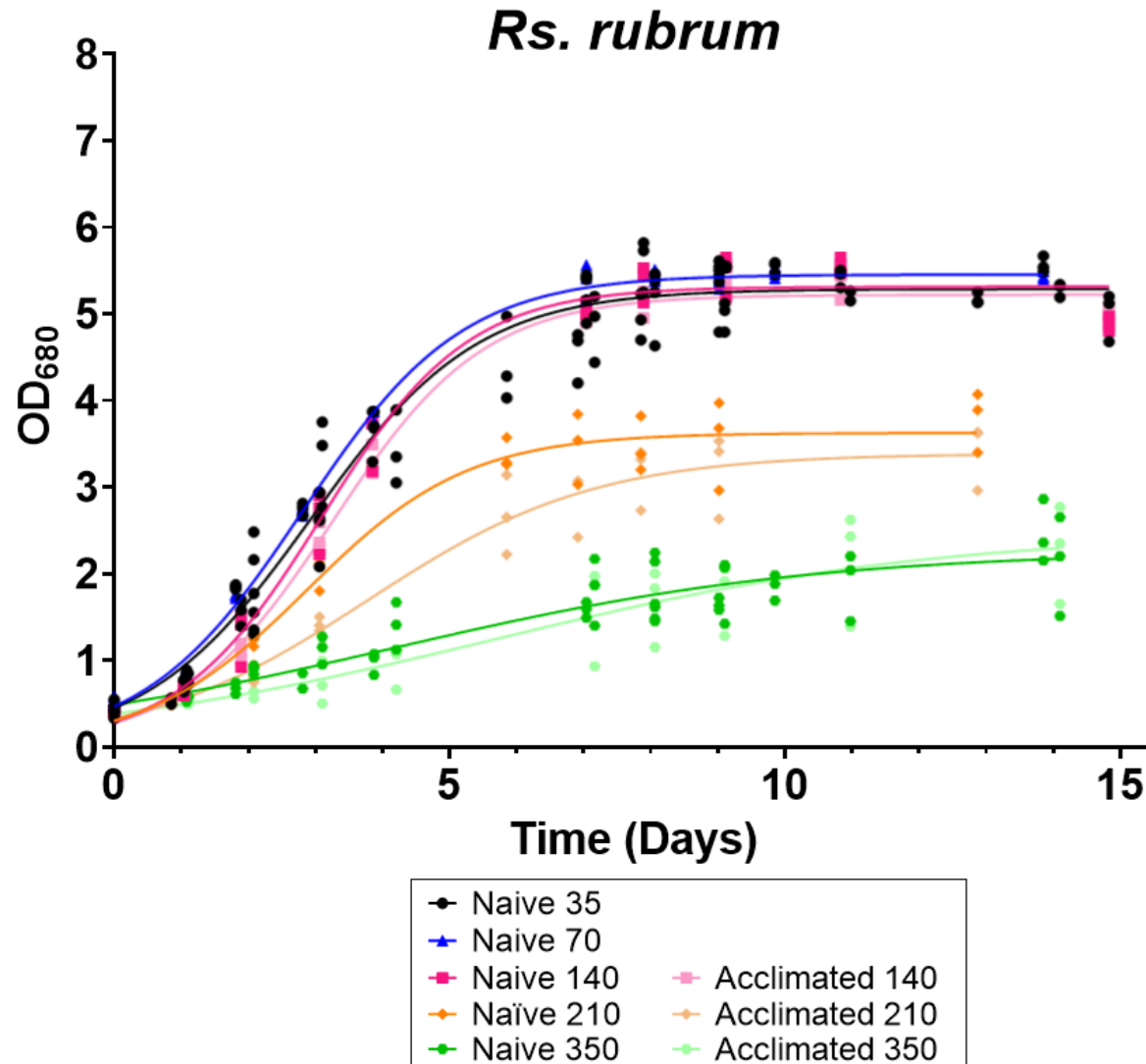
⇒ What is the minimal inhibitory concentration?

⇒ How can they adapt to these stringent conditions?

⇒ Organic acid contents, proteomic analysis and bacterial strain proportions will follow



# Effects of High Ammonium Levels



⇒ Growth is impaired at 210 mM and 350 mM NH<sub>4</sub>Cl and the cultures did not reach the same OD<sub>680</sub> as in lower NH<sub>4</sub>Cl medium

⇒ Acclimatation did not take place

⇒ *Organic acid contents and proteomic analyses will follow*

# Acknowledgements



**Thank you for  
your attention!**



This research is supported by the European fund for regional development (FEDER) through the WalBiopower project (DECARBOWAL portfolio) and Bioprofiling project cofinanced by EU and Wallonia.