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DTU Skylab Foodlab

# Valorising organic waste for the production of high-value molecules by purple non-sulphur bacteria

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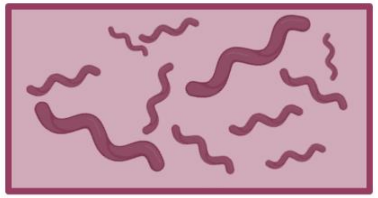
PROTMIC ...for a sustainable future

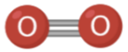
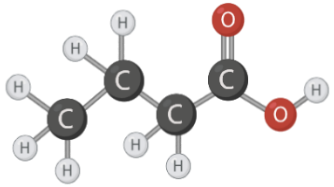
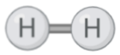
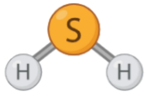
UMONS University of Mons

# Declaration of Interests

| Affiliation / Financial interests | Organisation |
| --- | --- |
| Employment | Department of Proteomics and Microbiology, University of Mons |
| Grants / Research support | European fund for regional development (FEDER) 2021-2027 program under the WalBioPower project (DECARBOWAL portfolio) and the Bioprofiling project cofinanced by the EU and Wallonia |
| Scientific Advisory Board / Consultant | none |
| Government | none |
| Other | none |



# Purple non-sulphur bacteria: Versatile metabolism

| | | |
|---|---|---|
| |   |   |
| C source: Organic compounds<br>e- source: Organic compounds<br> | **Photoheterotrophy** | Chemoheterotrophy |
| C source: <br>e- source: Inorganic compounds<br>  | Photoautotrophy | Chemoautotrophy |

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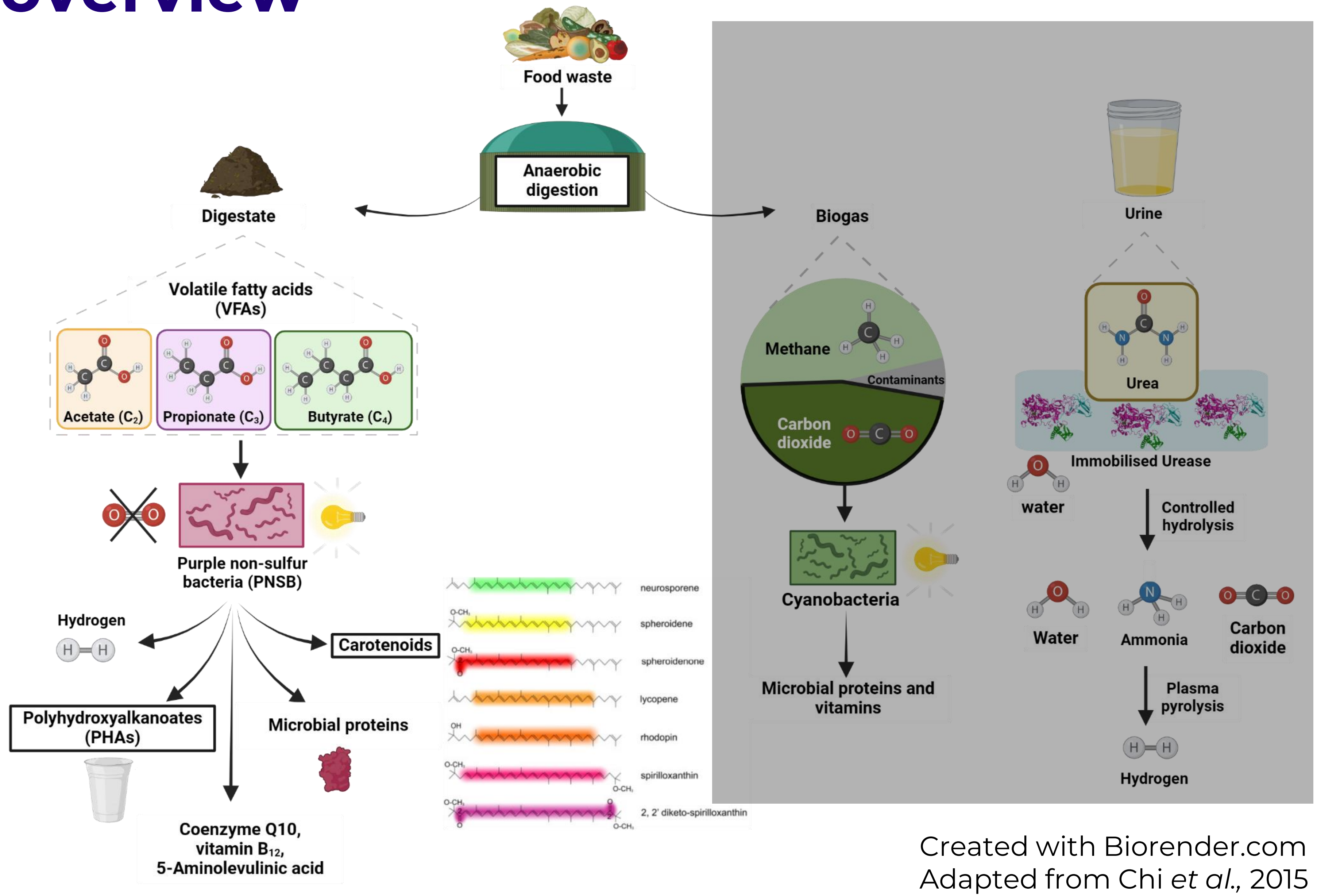
# Project overview




Created with Biorender.com
Adapted from Chi *et al.*, 2015

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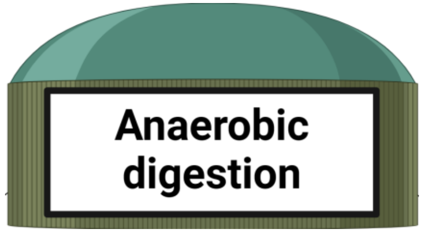
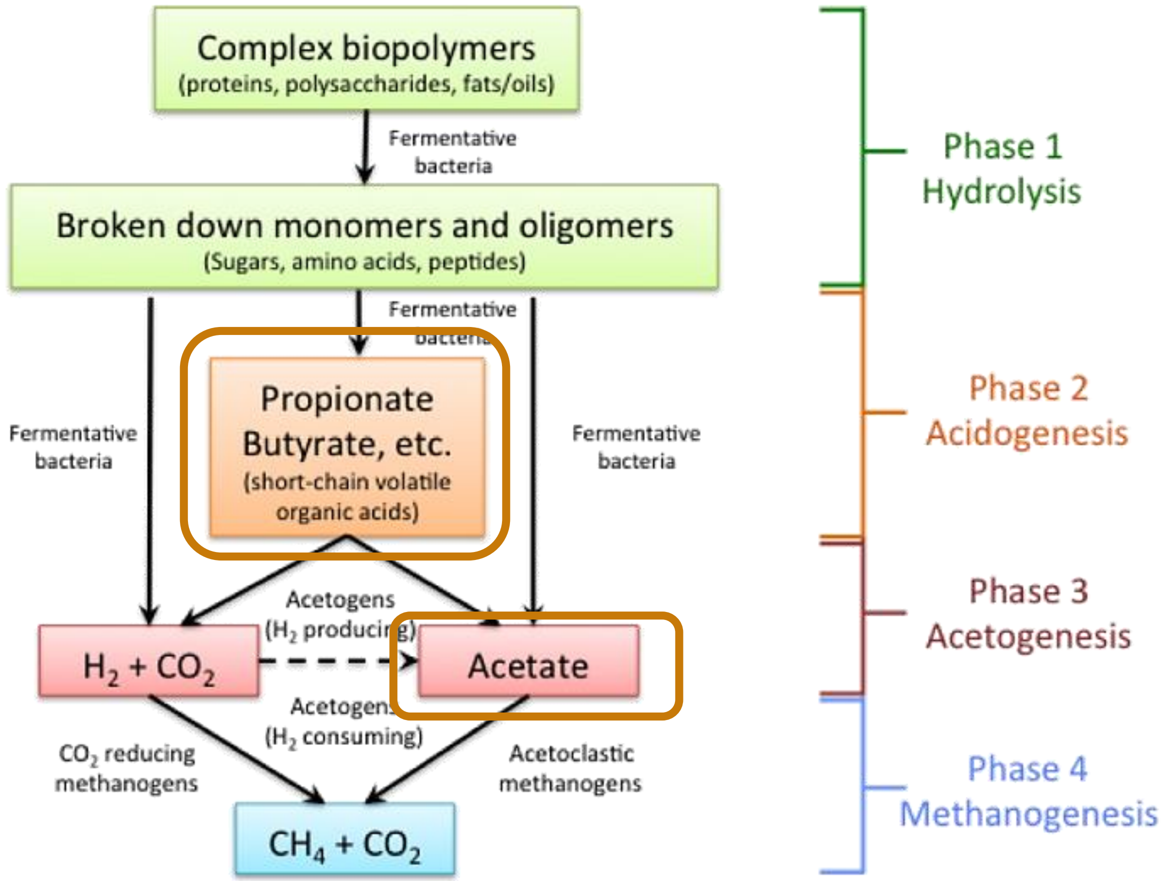
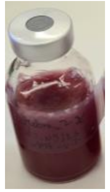
# Anaerobic digestion (AD)




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

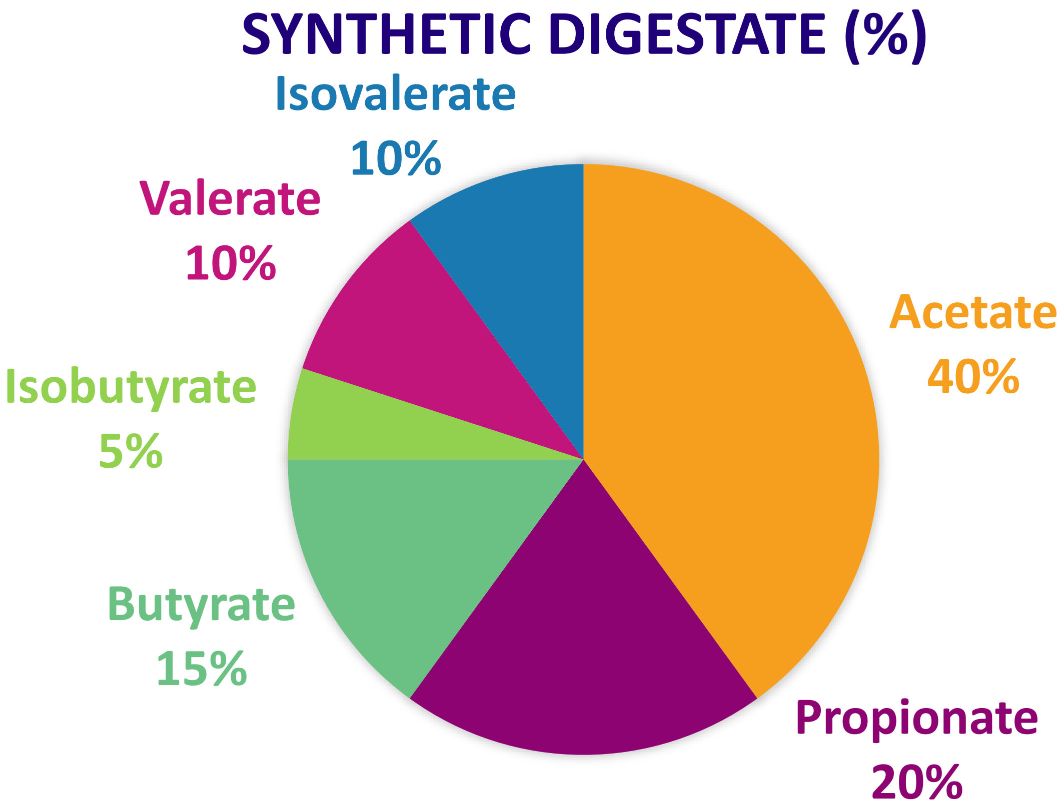
Short-chain fatty acids (SCFAs) = Volatile fatty acids (VFAs)
- Acetate
- Propionate
- (Iso)butyrate
- (Iso)valerate



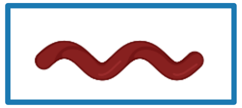
Purple non-sulphur bacteria (PNSB)

Credit: BEEMS Module B7 - Anaerobic Digestion

# Culturing PNSB in Synthetic Digestate




*Rhodospirillum rubrum*



Co-culture *crs:*

- *Rhodobacter capsulatus*
- *Rhodospirillum rubrum*
- *Cereibacter sphaeroides*

120 mM C equivalents

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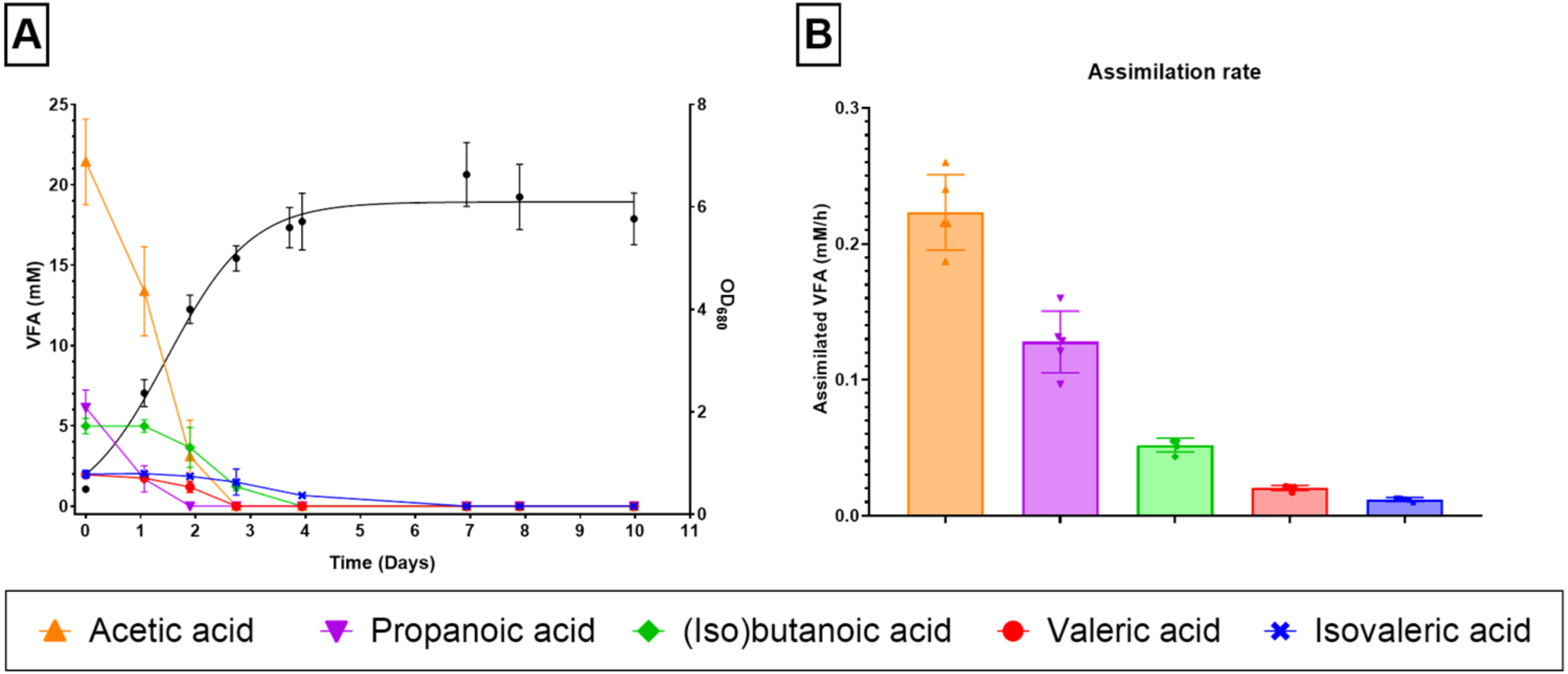
# Culturing PNSB in Synthetic Digestate

- LC-MS analysis of VFA assimilation



# Bacterial strain proportions

- $OD_{680}$ = 0.133 of each strain => Achieve Start $OD_{680}$ = 0.4

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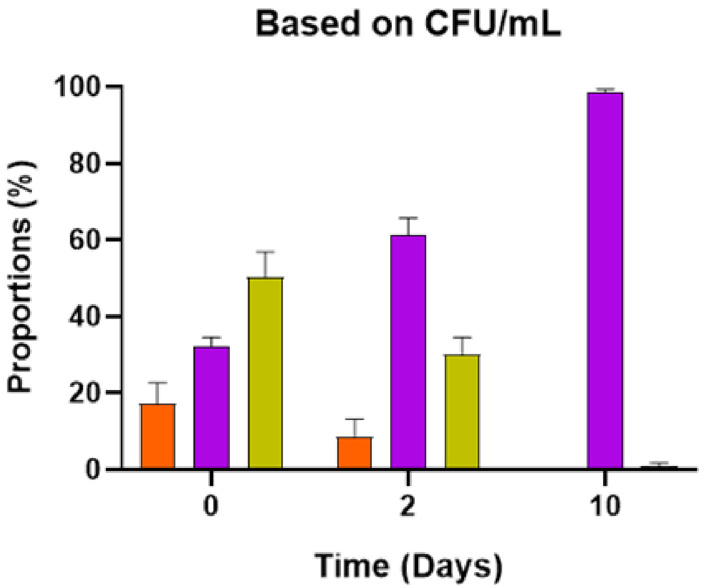
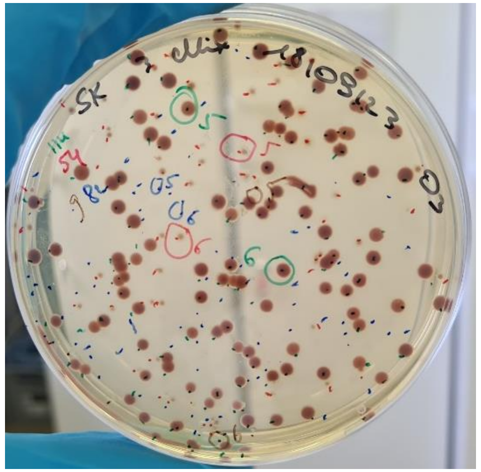
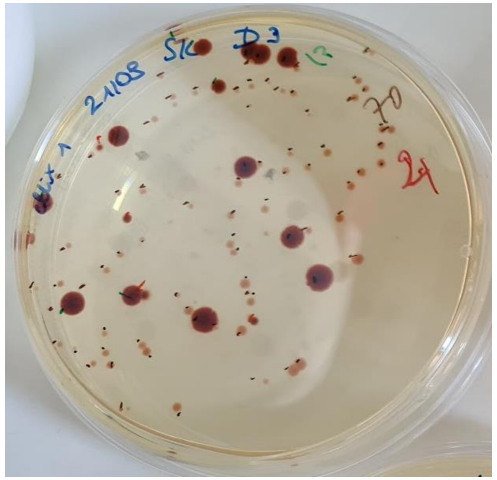
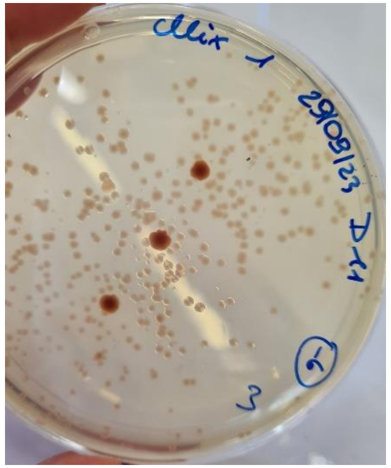


Day 0



Day 2



Day 10

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

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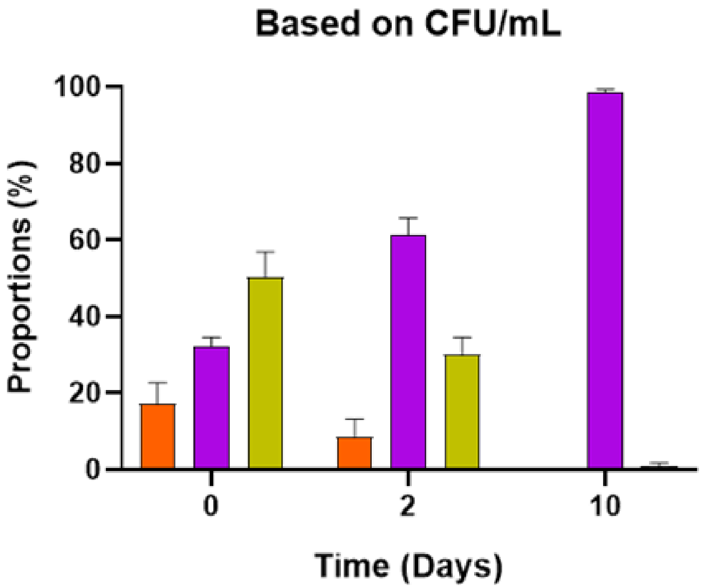
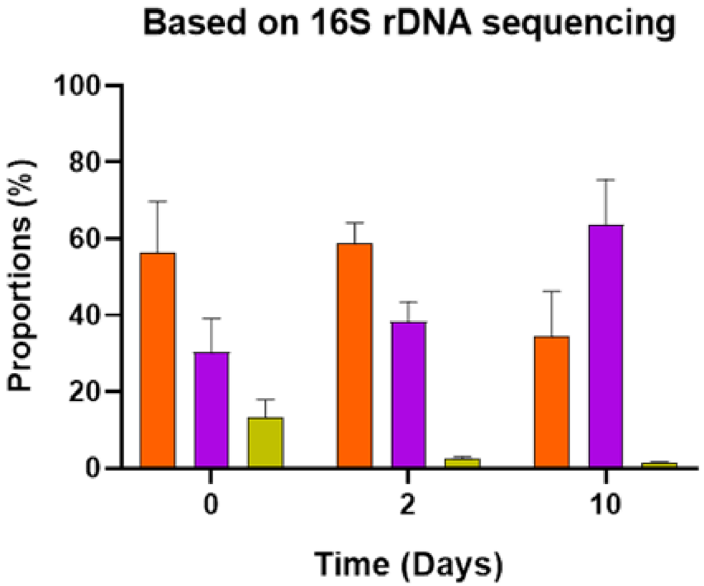
Similarity of colonies on agar plates

# Bacterial strain proportions

- $OD_{680}$ = 0.133 of each strain => Achieve Start $OD_{680}$ = 0.4



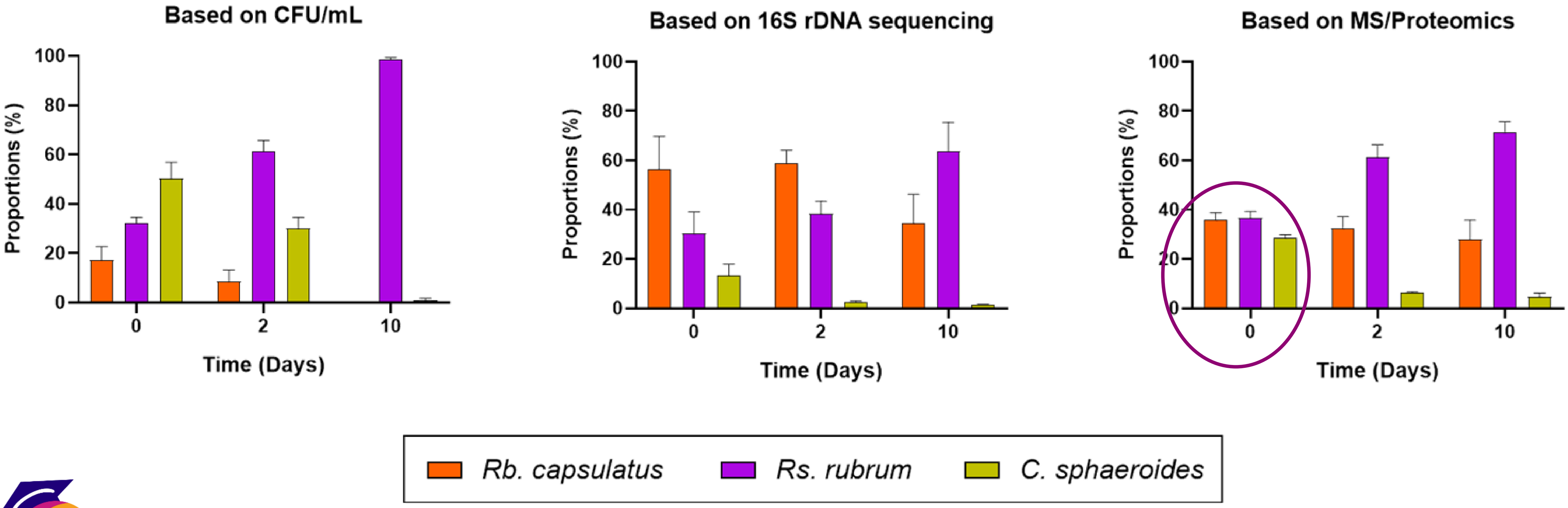

Oxford Nanopore GridION + 16S rDNA primers from Oxford Nanopore

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

Primers have a higher affinity for *Rb. capsulatus*

# Bacterial strain proportions

- $OD_{680}$ = 0.133 of each strain => Achieve Start $OD_{680}$ = 0.4




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

# Ammonium in Food-waste Digestates

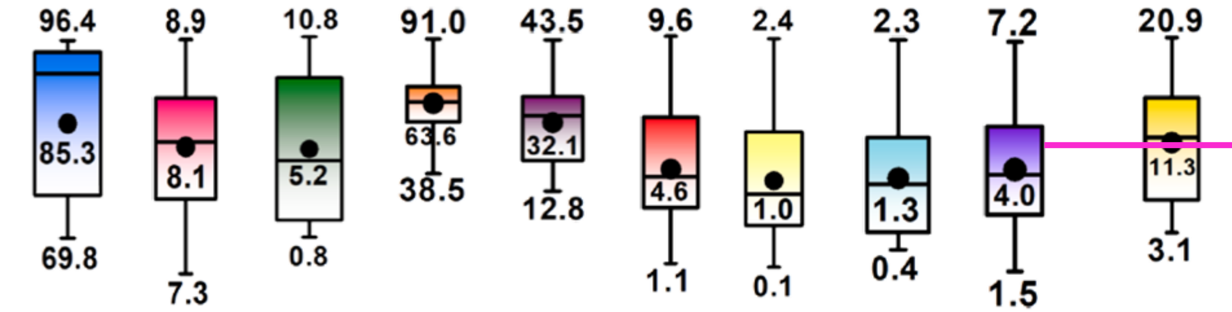
Standard MELiSSA Medium:
35 mM $NH_4Cl$
→ 0.5 g/L N
→ 0.635 g/L $NH_4^+$

10 x

~350 mM $NH_4Cl$
→ 5 g/L N
→ 6.35 g/L $NH_4^+$

**Table 1**

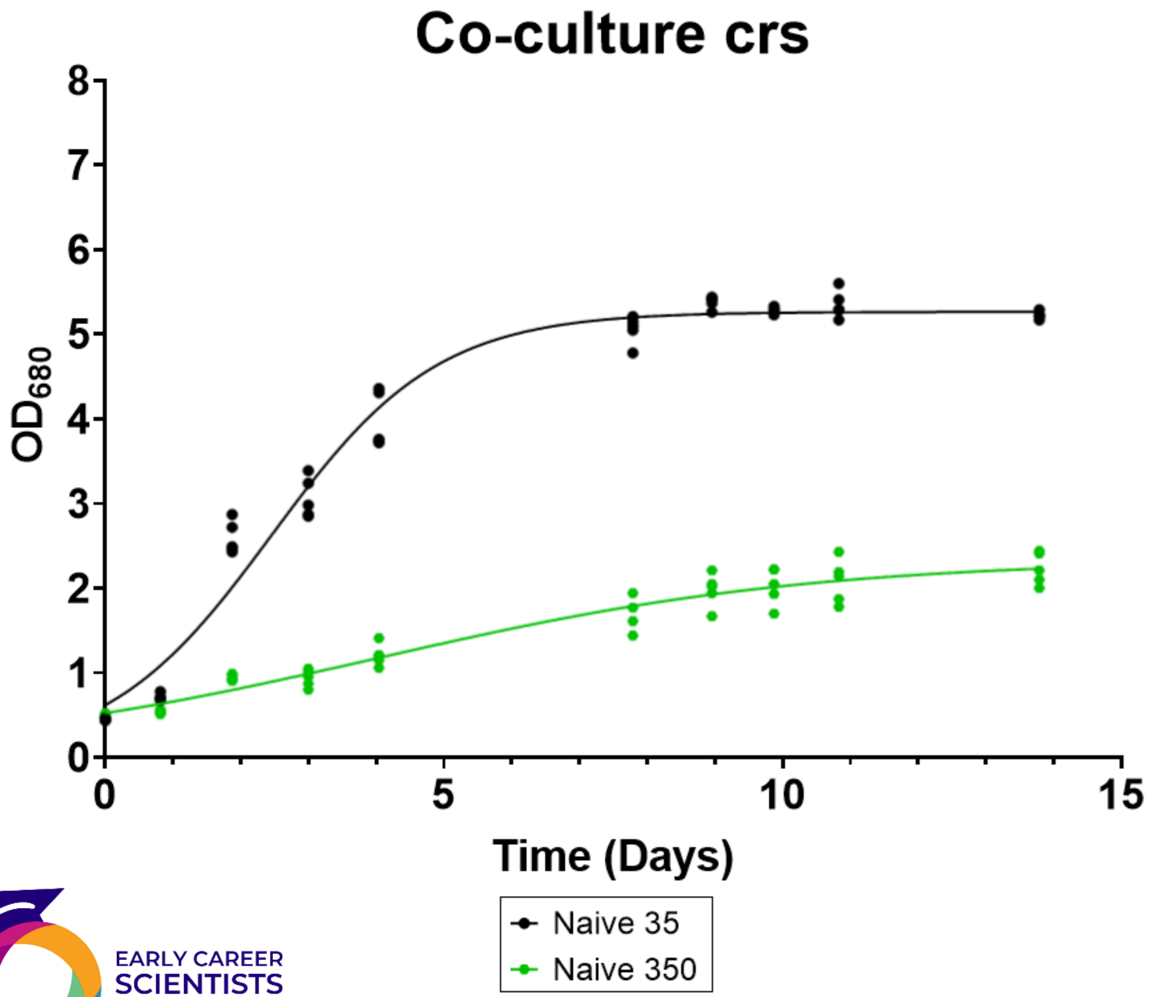
Food waste digestate characteristics (MC: moisture content; EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorous; TK: total potassium).




| Waste type | Country | MC (%) | pH | EC (dS/m) | OM (%) | TOC (%) | TN (%) | TP (%) | TK (%) | $NH_4^+$-N (g/kg) | C/N ratio | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 Drosg (2013) |
| Bio waste | Germany | – | 7.6–8.1 | – | – | – | 3–6.8 | – | – | 1.5–5.6 | – | Fuchs and Drosg (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) |

Manu *et al.*, 2021. *Bioresource Technology*, *334*, p.125032.

# Effects of High Ammonium Levels




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?*

⇒ *How could they adapt to these stringent conditions?*

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

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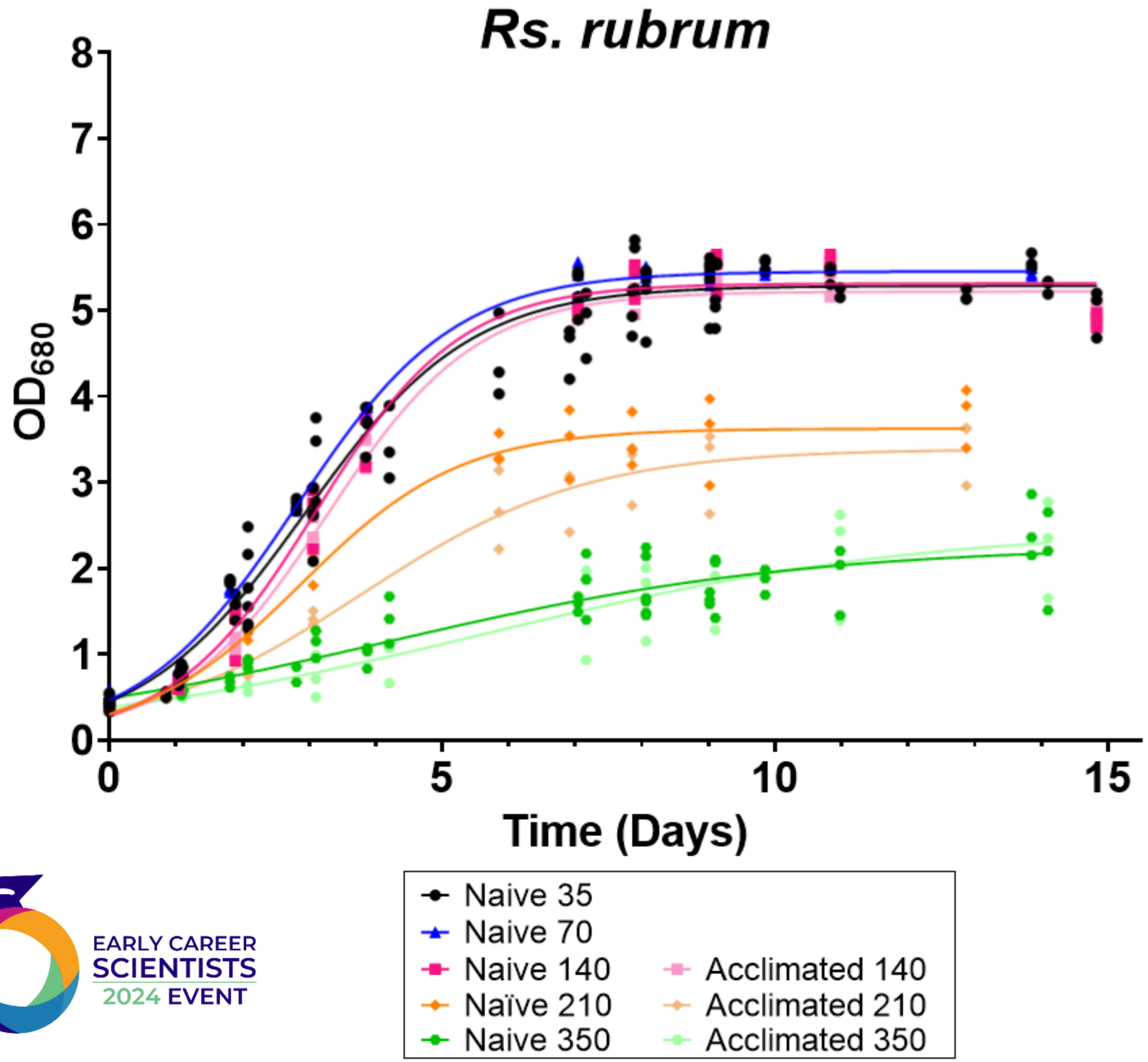
# Effects of High Ammonium Levels




⇒ Growth is impaired at 210 mM and 350 mM $NH_4Cl$ and the cultures did not reach the same $OD_{680}$ as in lower $NH_4Cl$ medium

⇒ Acclimatation did not take place

⇒ *Organic acid contents and proteomic analyses will follow*

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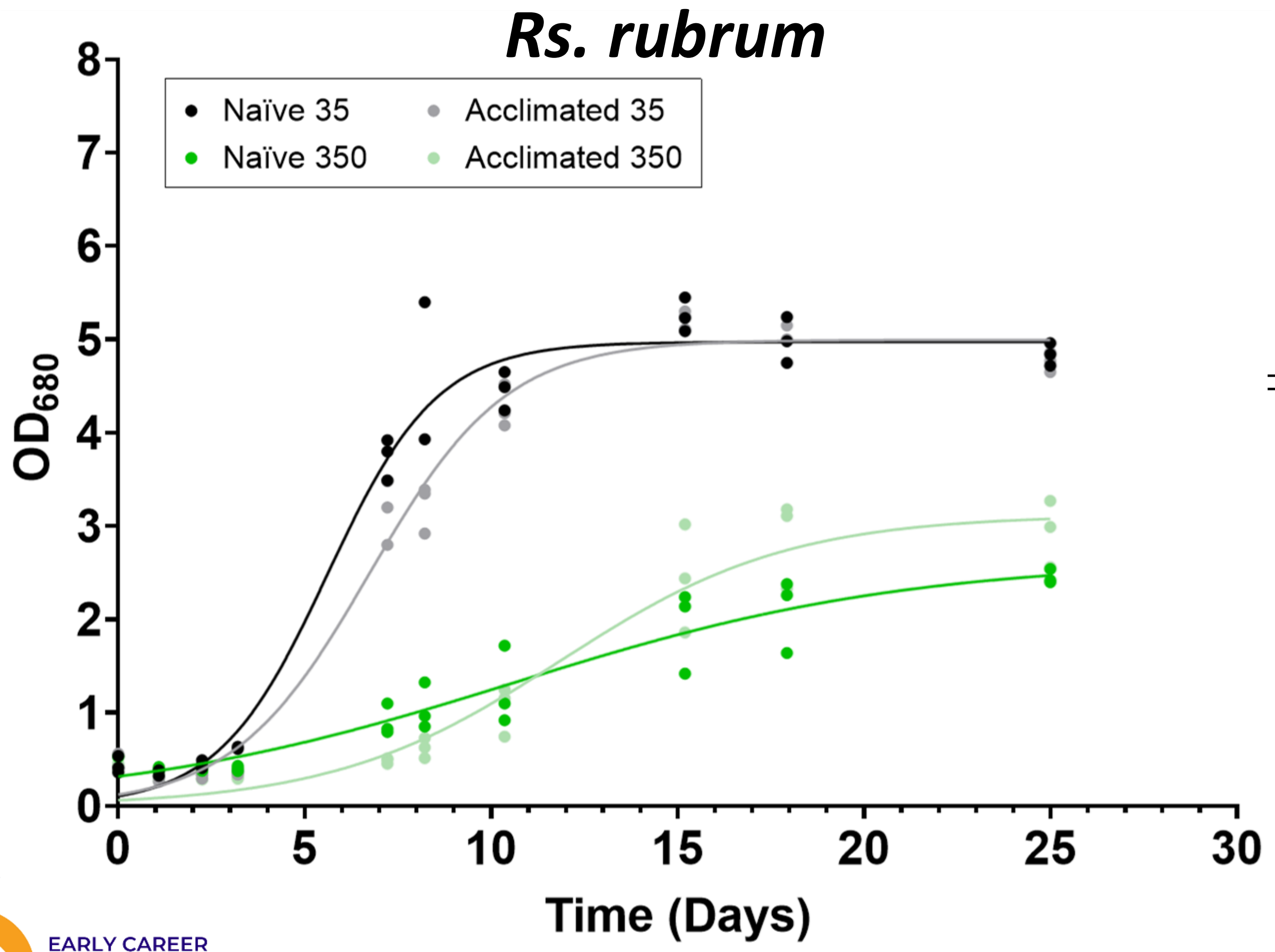
# Acclimatation to High Ammonium Levels




⇒ Is the acclimatation taking place at the moment?

⇒ *Organic acid contents and proteomic analyses will follow*

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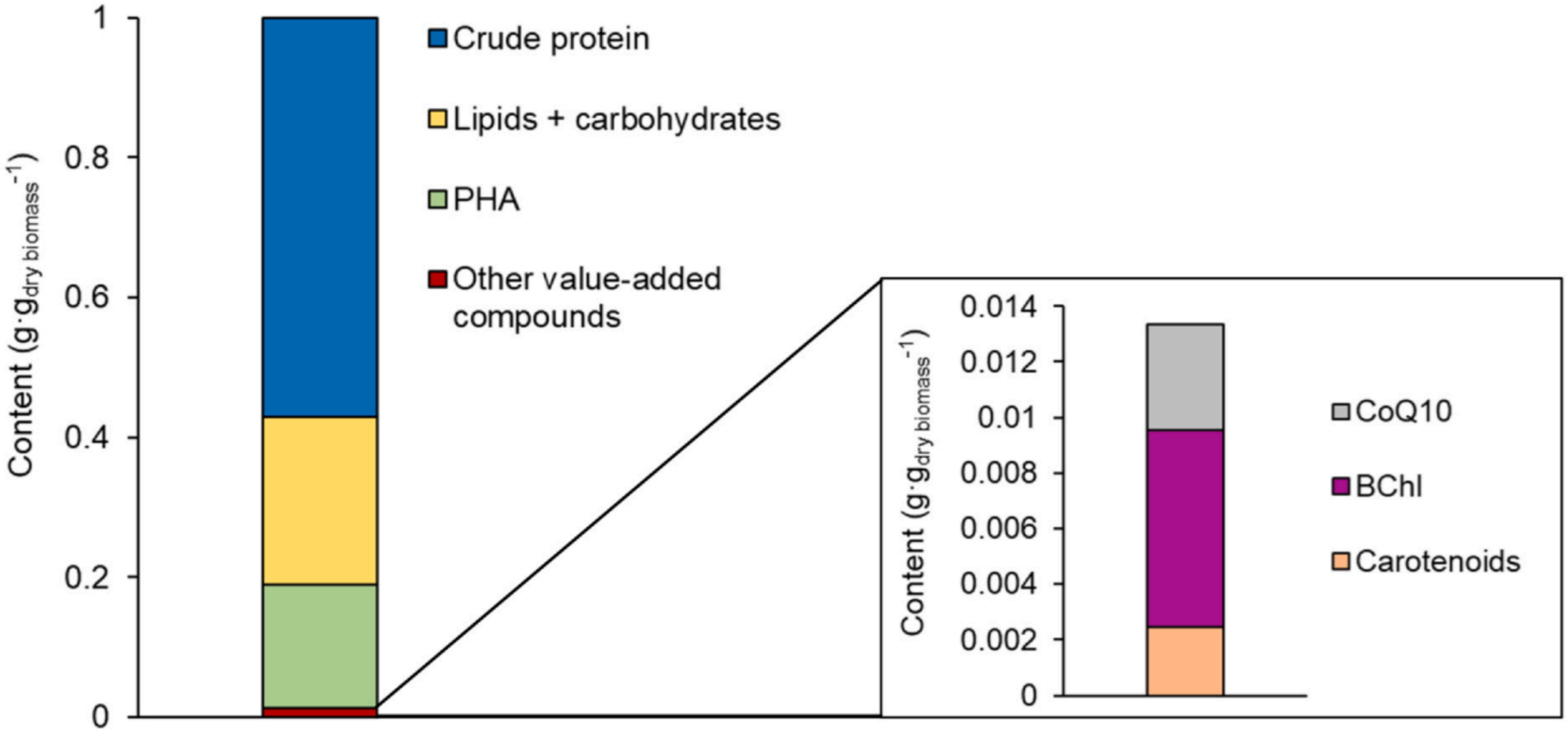
# Contents of PNSB




Hülsen *et al.*, 2022 https://doi.org/10.1016/j.copbio.2022.102726

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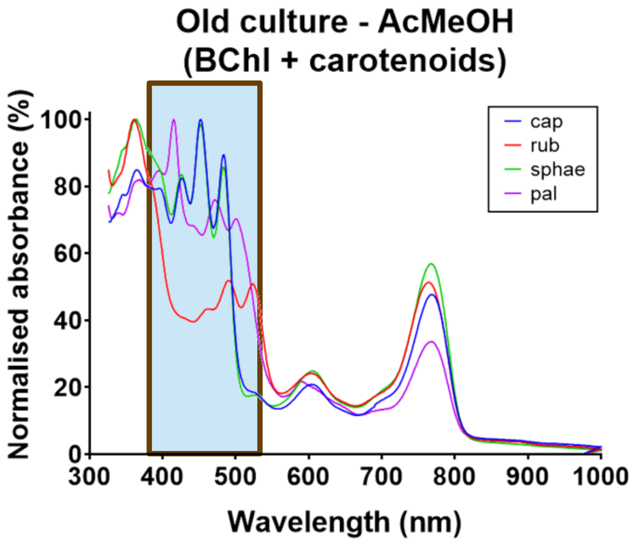
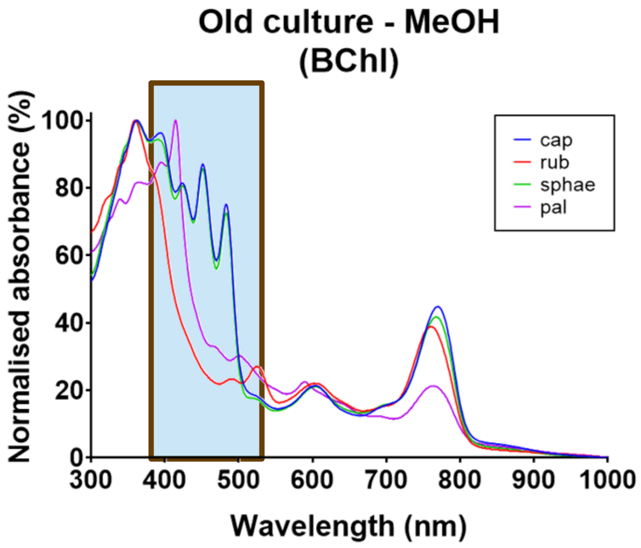
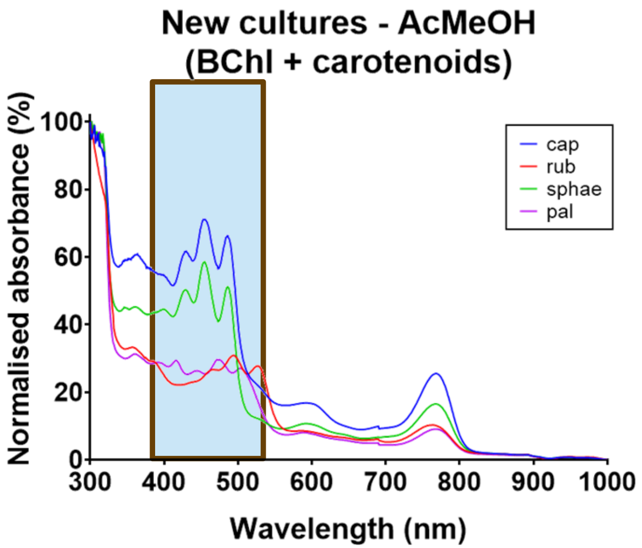
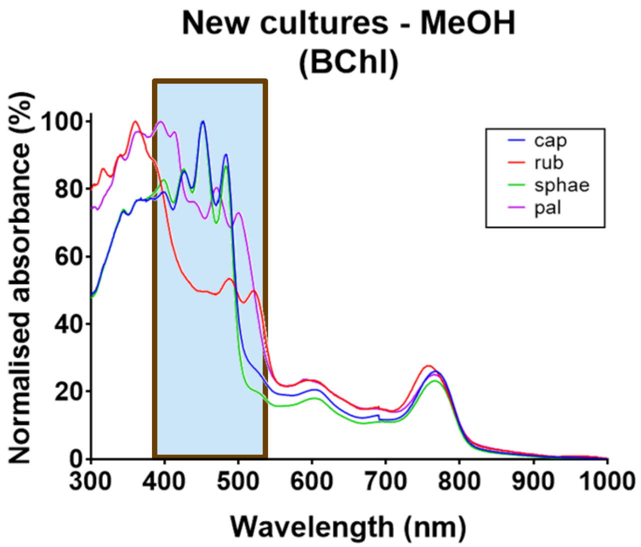
# Carotenoid extractions

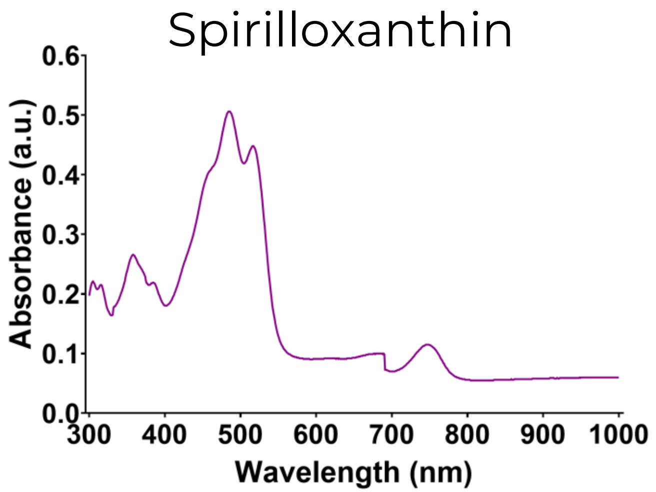



*Currently testing the antioxidant effects of carotenoids on walnut oil (UCLouvain)*

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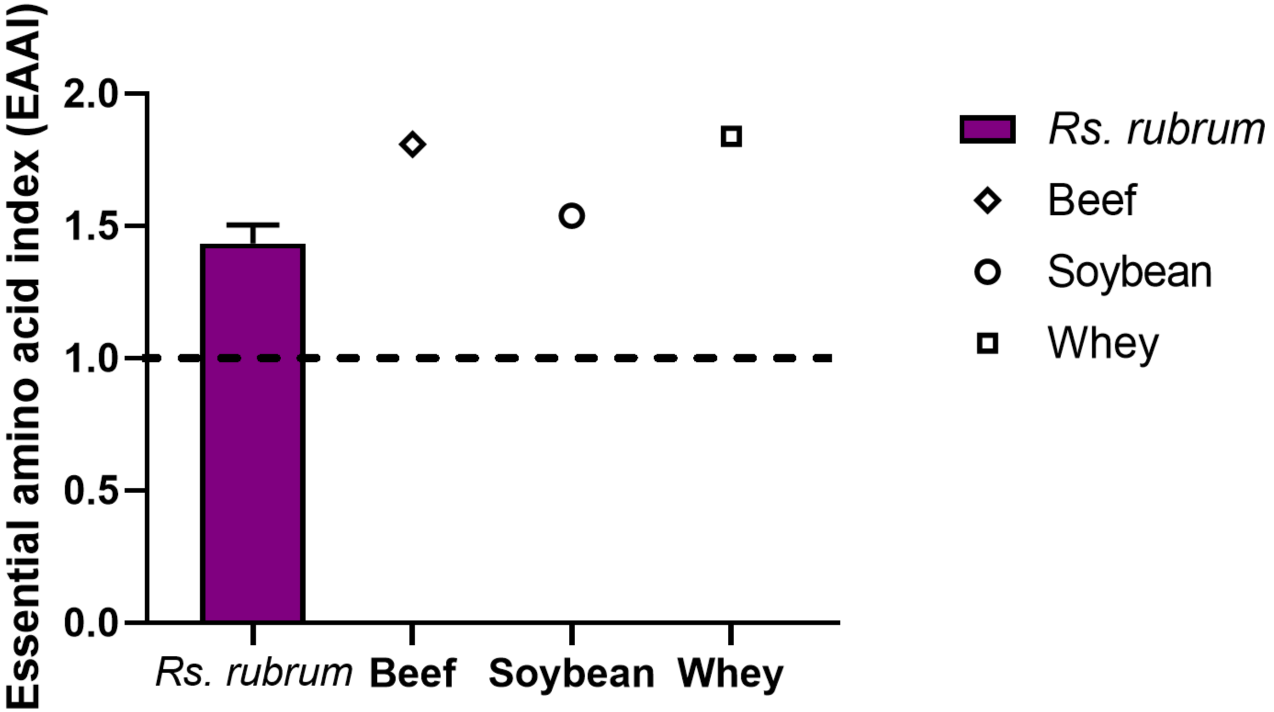
# Protein-rich part of *Rhodospirillum rubrum*

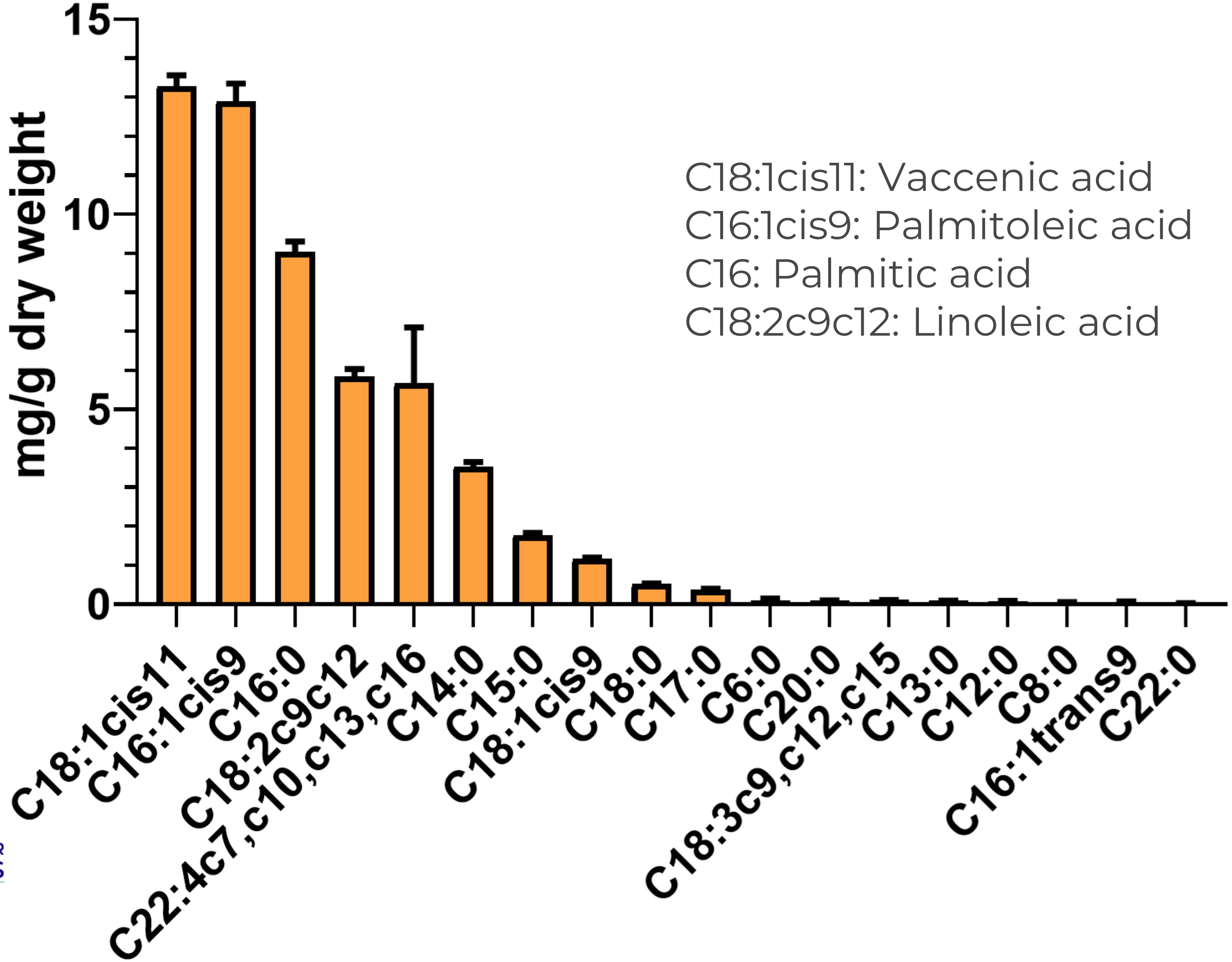



Conversion of molasses into protein-rich *Rhodospirillum rubrum* biomass

Essential amino acid contents are similar to other protein sources

Results from Dr Guillaume Bayon-Vicente

# Fatty acids in *Rhodospirillum rubrum*




C18:1cis11: Vaccenic acid
C16:1cis9: Palmitoleic acid
C16: Palmitic acid
C18:2c9c12: Linoleic acid

Many *cis* fatty acids

# Take-home messages

- **Co-cultures** of *Rhodospirillum rubrum, Rhodobacter capsulatus* and *Cereibacter sphaeroides* assimilate **acetic and propionic acid** first, followed by **(iso)butyric acid and valeric acid** and lastly, **isovaleric acid**

- **Mass spectrometry** is a great alternative method to determine **bacterial proportions** in mixed cultures

- ***Rhodospirillum rubrum*** is able to survive to **high ammonium** levels of 140 mM $NH_4Cl$ (= **2.54 g/L $NH_4^+$**) without reduction in growth

- ***Rhodospirillum rubrum*** is able to produce **microbial proteins (containing essential amino acids) and *cis* fatty acids**, which **could have health benefits**

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# Acknowledgements

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Cofinancé par l'Union européenne

Wallonie

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PURPLEGAIN

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# Thank you!

**Contact**

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