

Potential for CO₂ Fixation and Novel Food Production in **Purple Non-Sulphur Bacteria**

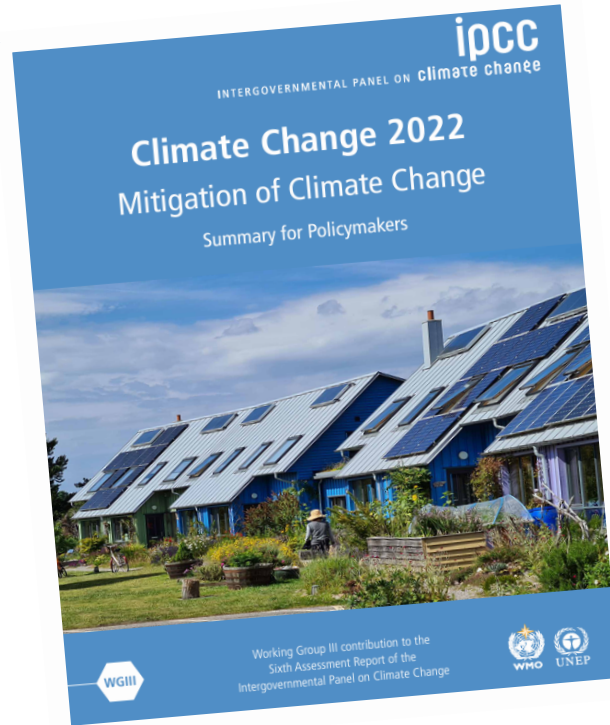
*Exploratory Study in Low-Cost Bag Photobioreactors
under Controlled and Resource-Scarce Analog Conditions*

Master thesis

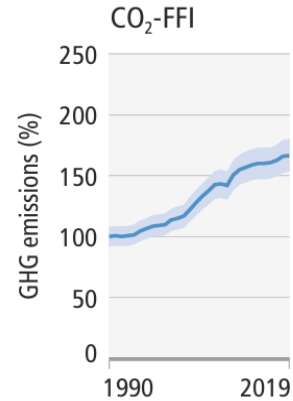
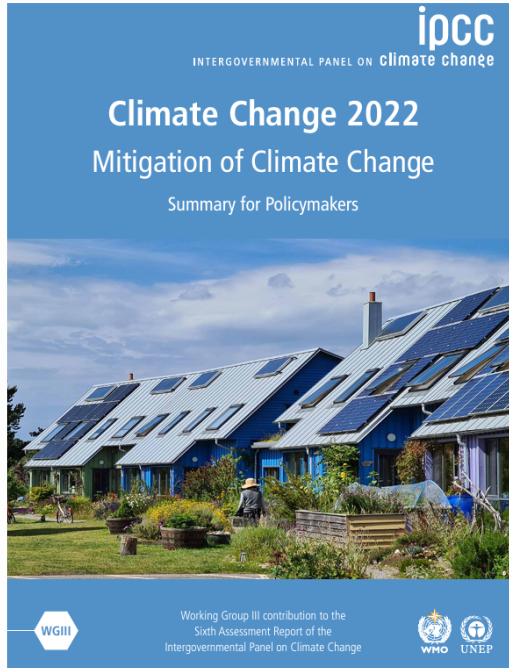
Guillaume GÉGO

Academic Year 2023-2024

CO_2 emissions - *Global Carbon Budget 2023*



CO₂ emissions - Global Carbon Budget 2023



	2019 emissions (GtCO ₂ -eq)	1990–2019 increase (GtCO ₂ -eq)	Emissions in 2019, relative to 1990 (%)
CO ₂ -FFI	38 ± 3	15	167

Fig 1-4. Friedlingstein et al. (2023). *Global Carbon Budget 2023*.
Earth System Science Data, 15(12), 5301-5369.

CO₂ emissions - Global Carbon Budget 2023

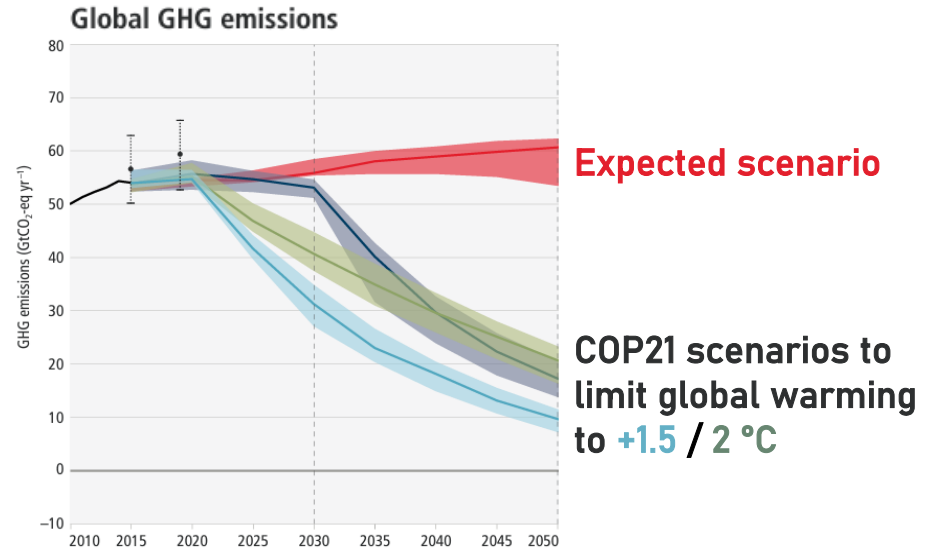
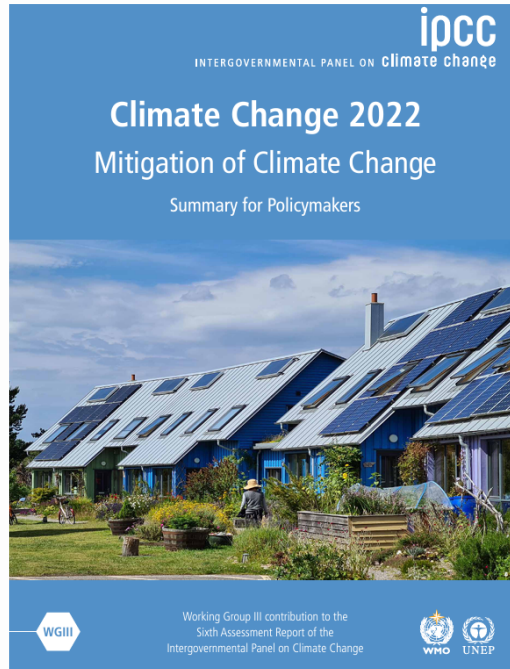
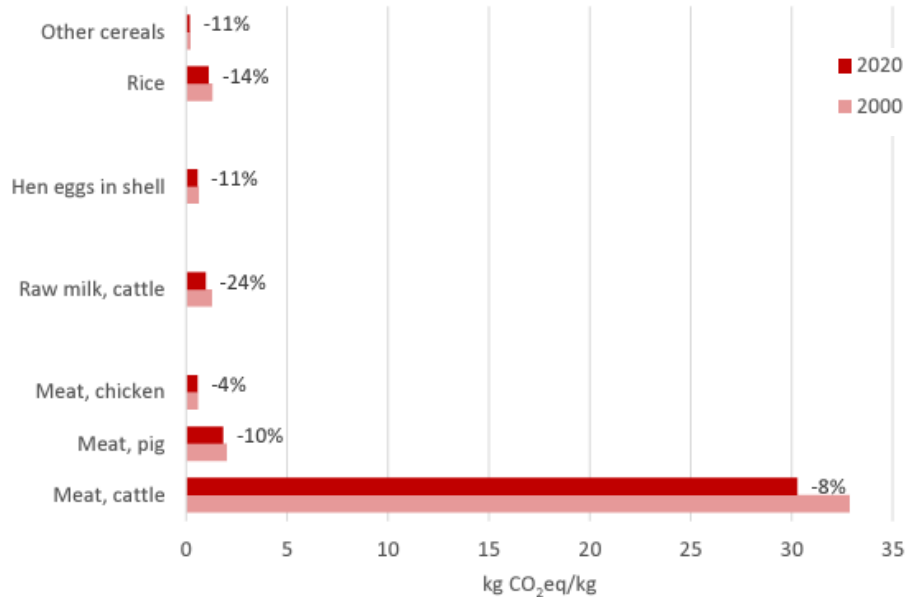


Fig 1-4. Friedlingstein et al. (2023). *Global Carbon Budget 2023*.
Earth System Science Data, 15(12), 5301-5369.

CO₂ emissions - Agrifood sector contribution

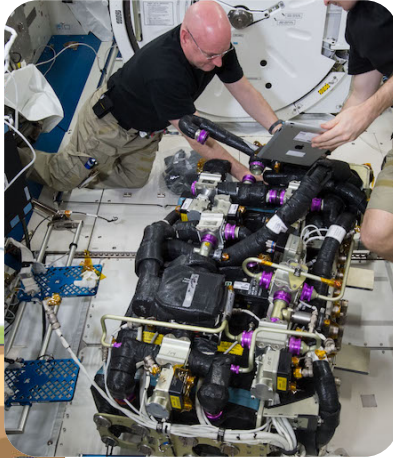
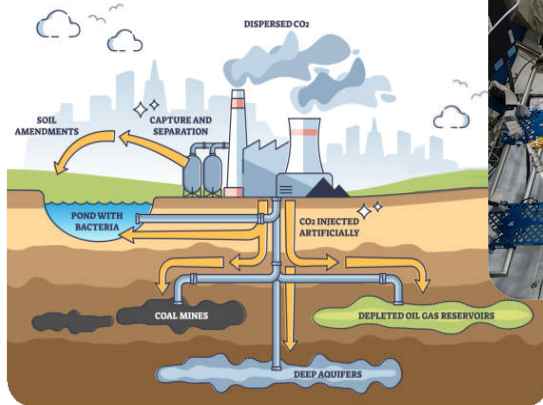


- **±1/3 of total GhG emissions in 2020** (FAO, 2022)
 - **+9% increase since 2000**
- **Mostly for protein-rich food**
 - **+50% demand by 2050** (Boland, 2013)
- **Need for low-CO₂ protein-rich food production systems!**

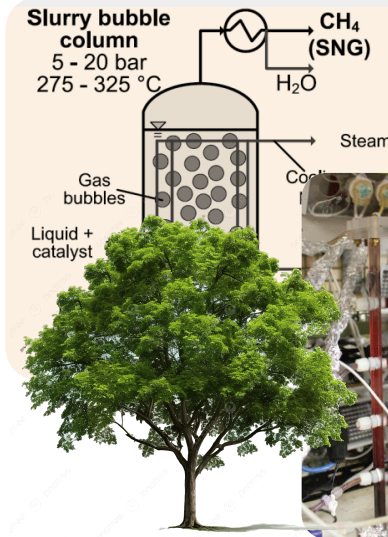
Fig 5. Greenhouse gas emissions from agrifood systems, evolution between 2000-2020. (FAO, 2022).

CO₂ emissions - *Main remediation strategies*

CO₂ CAPTURE AND STORAGE (CCS)



**No transformation
= Not sustainable**



CO₂ CAPTURE AND UTILISATION (CCU)



**CO₂ reduction
= Sustainable**

Could **PNSB** answer the protein demand while removing CO₂ ?

Fig 6-10. CO₂ remediation strategies (iStock; NASA; Sun, 2016; Hulsen, 2016)

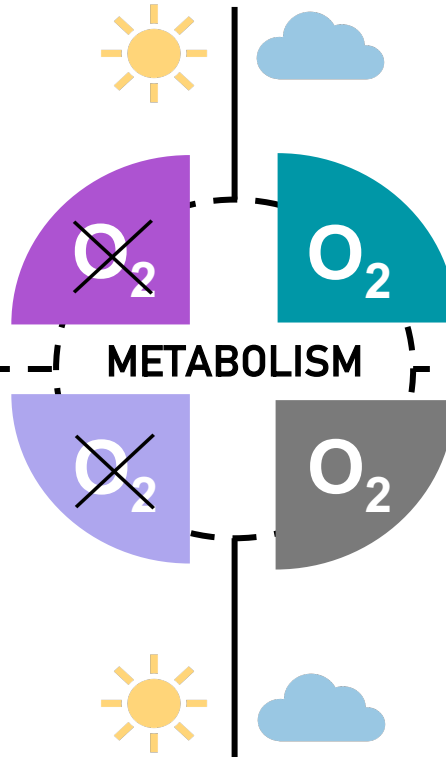
Purple non-sulphur bacteria - For CO₂ removal

Photoheterotrophy

C source: **organic** compounds
e⁻ source: **organic** compounds

Chemoheterotrophy

C source: **organic** compounds
e⁻ source: **organic** compounds



Photoautotrophy

C source: CO₂
e⁻ source: **inorganic** compounds

Chemoautotrophy

C source: CO₂
e⁻ source: **inorganic** compounds

Purple non-sulphur bacteria - For CO₂ removal

Photoheterotrophy

C source: } Butyrate
 e⁻ source: } (EthylMalonylCoA-Krebs)
 e⁻ sink: CO₂ (via CBB)

Photoautotrophy

C source: CO₂
 e⁻ source: inorganic compounds

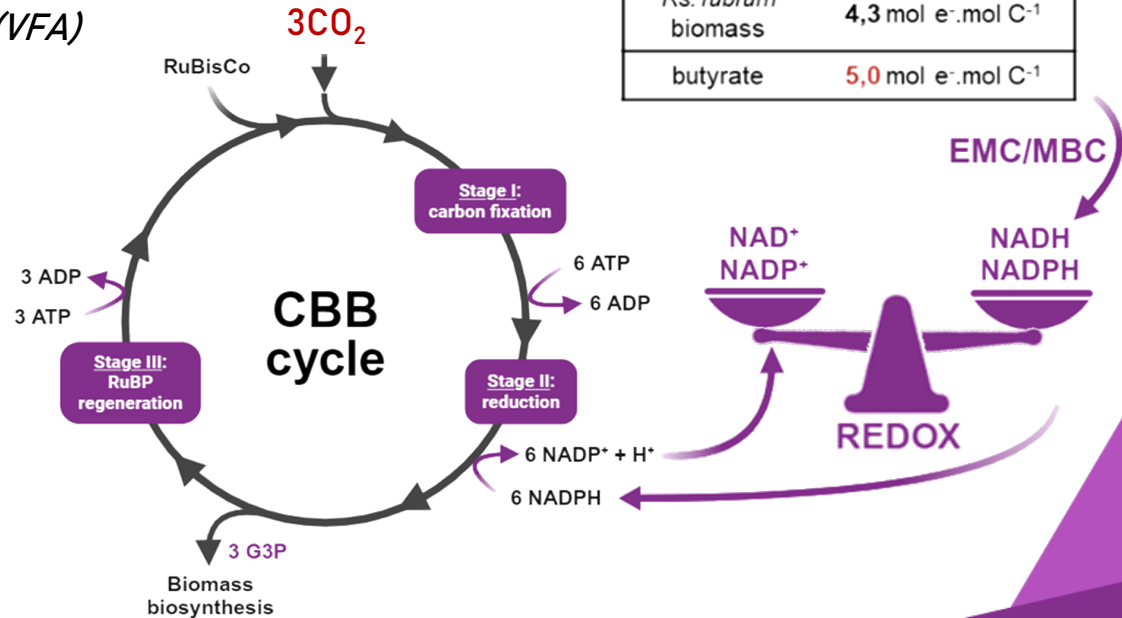


Fig 11. Redox homeostasis regulation by CO₂ fixation (Wang et al., 2011; Biorender, 2024).

Purple non-sulphur bacteria - For CO₂ removal

Photoheterotrophy

with highly reduced Volatile Fatty Acids (VFA)

C source: } Butyrate
 e⁻ source: } (EthylMalonylCoA-Krebs)
 e⁻ sink: CO₂ (via CBB)

Photoautotrophy

with H₂ gas (hydrogenotrophy)

C source: CO₂ (via CBB)

e⁻ source: inorganic compounds
 H₂ (via hydrogenases)

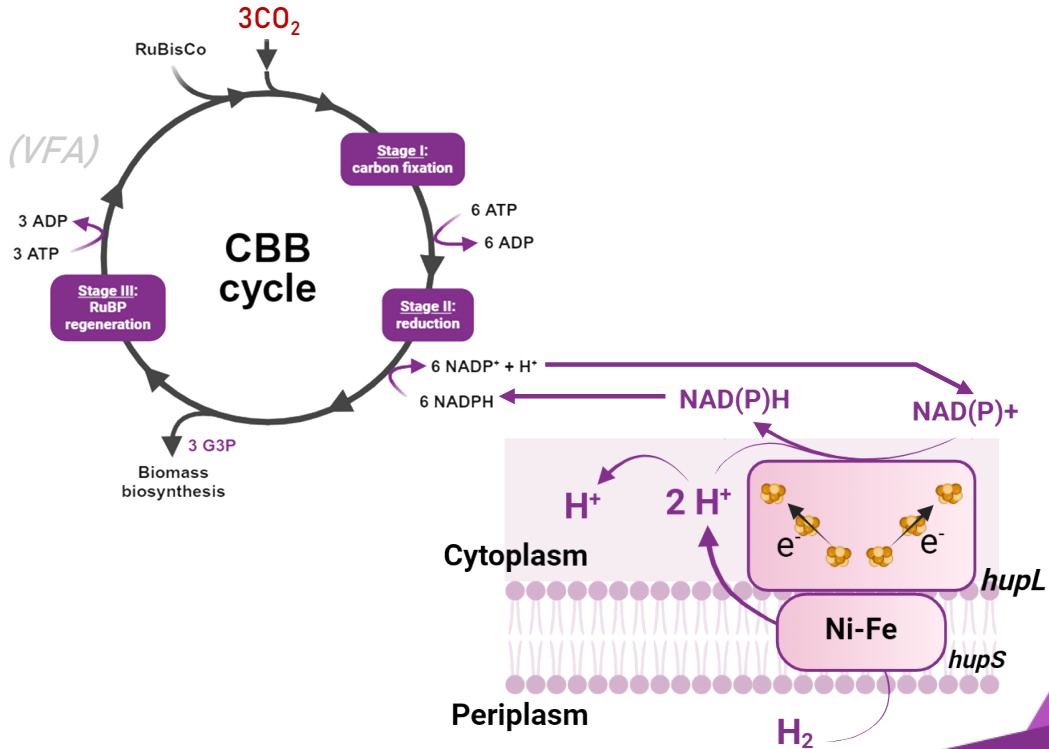


Fig 12. PNSB membrane-bound uptake hydrogenase (Zhu, 2010; Biorender, 2024).

Purple non-sulphur bacteria - For protein demand

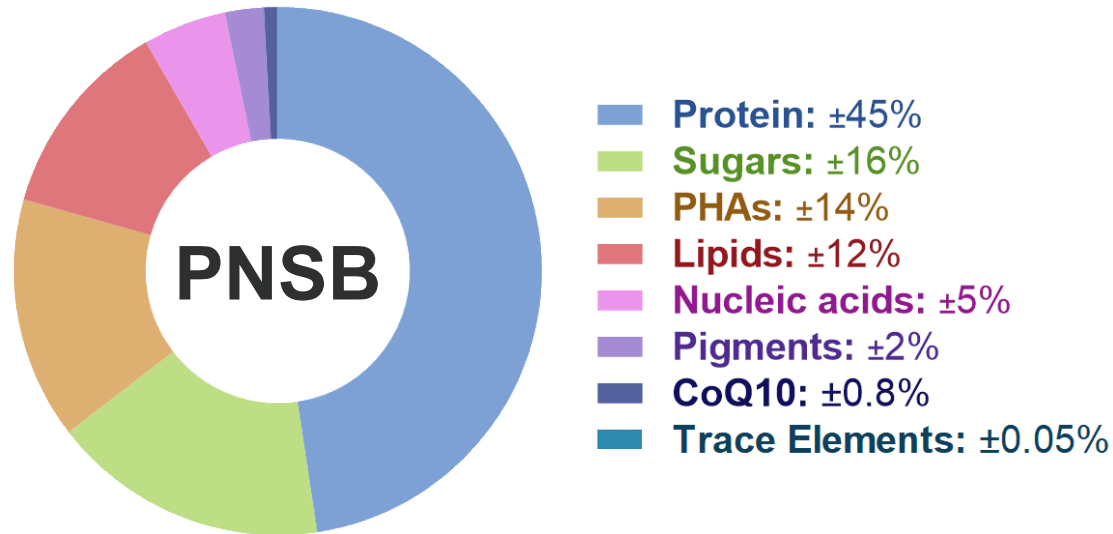


Fig 13. Average macromolecular composition of PNSB biomass.
(Multiple sources, see thesis; GraphPad Prism 8.0.1., 2024).

Purple non-sulphur bacteria - *For protein demand*

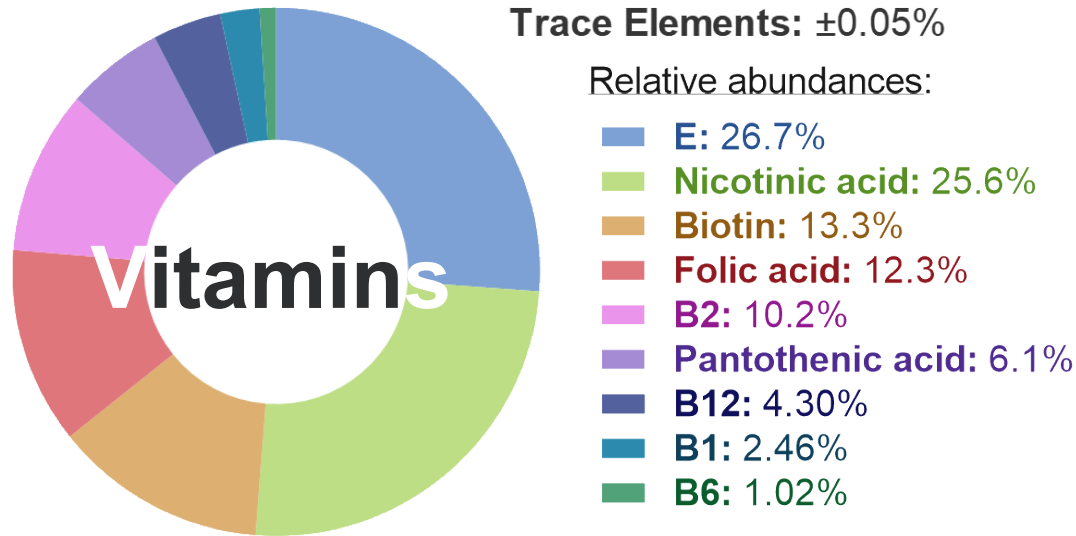
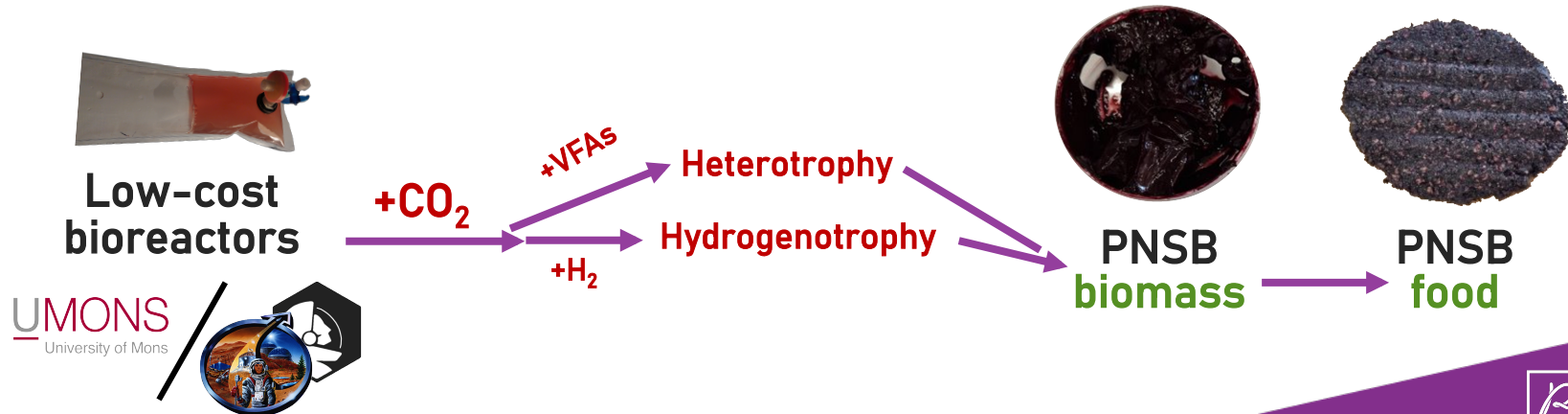


Fig 14. Average vitamin composition of PNSB biomass.
(Sasaki et al., 1998; GraphPad Prism 8.0.1., 2024).

Objectives - *Purple bacteria + CO₂ + byproduct → Food*

1. Design, manufacture and test **low-cost bioreactors**.
2. Assess **reliability** in controlled (UMONS) and scarce (AATC/MDRS) conditions.
3. Compare **heterotrophy/hydrogenotrophy for CCU**.
4. Characterise **PNSB biomass for food applications**.



Low-cost system - *Development timeline*

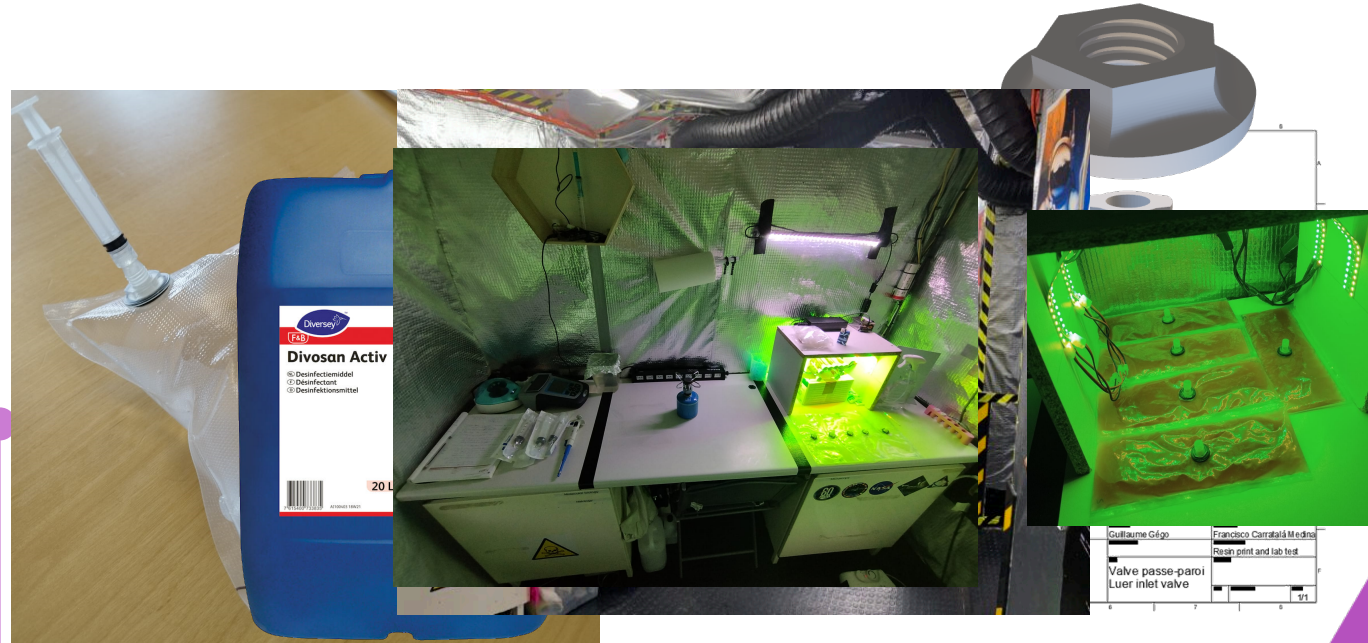


Fig 15. Materials & Methods, part 2 (own production).

Low-cost system - *Development timeline*

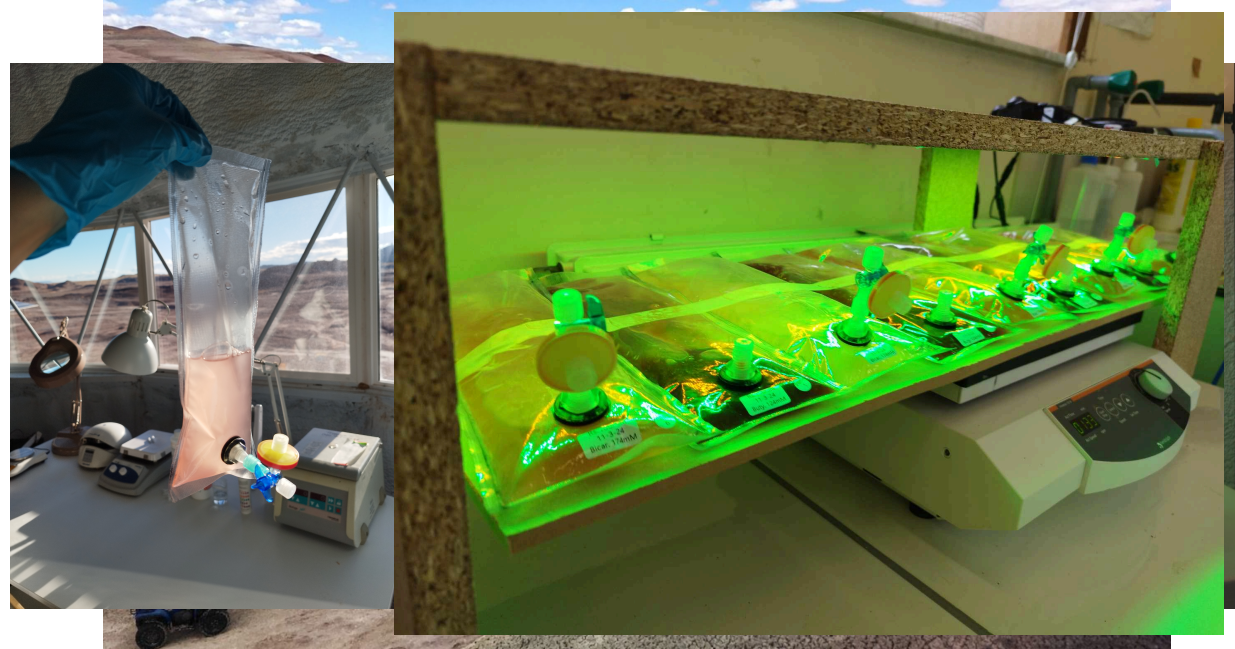


Fig 16. Materials & Methods, part 3 (own production).

Results - CO_2 capture capacity & CO_2 capture rate

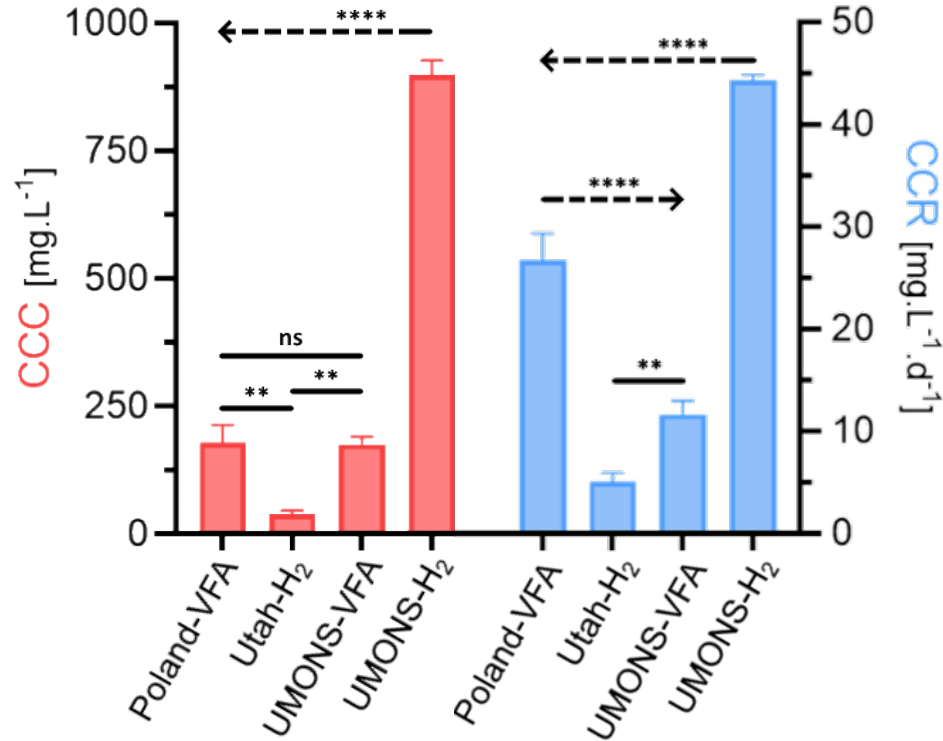


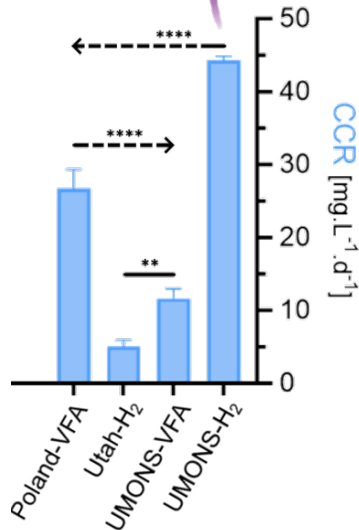
Fig 17. CCC and CCR across tests, ANOVA at p-value = 0.5 (own data, GraphPad 8.0.1.).

Applications - For CO₂ removal

Rs. rubrum in photoautotrophy + H₂

CCR: **±0.0443** g CO₂/L.d

Productivity: **0.11** g/L.d



Limnospira indica in photoautotrophy

CCR: **0.32-1.13** g CO₂/L.d (MELiSSA, 2024)

Productivity: **>1.5** g/L.d



Fig 18. Carbon Dioxide Removal Assembly (CDRA) on the ISS (NASA, Wikimedia Commons)

CCS system: CDRA on ISS

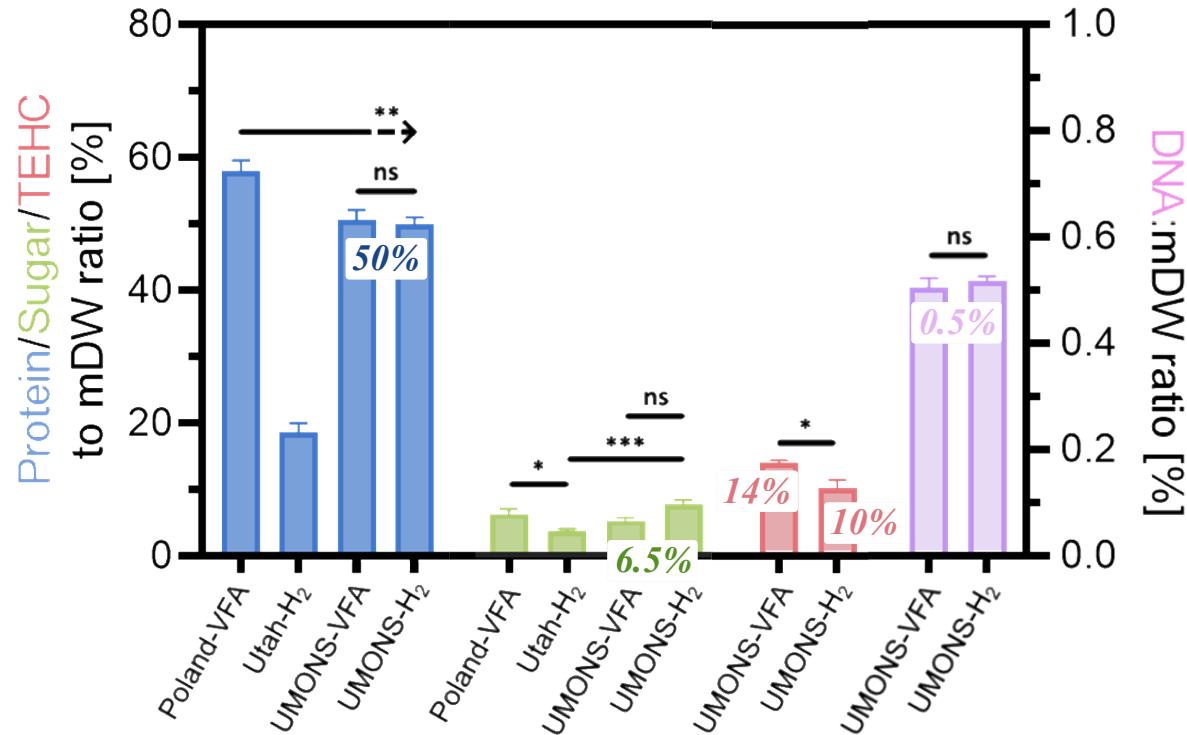
CCR: **±23** g CO₂/L.day (El Sherif & Knox, 2005)

Volume: 205 "L"

Electrical consumption: 1 kWh

→ **CO₂ is not recycled**

Results - Macromolecular analyses



Literature:

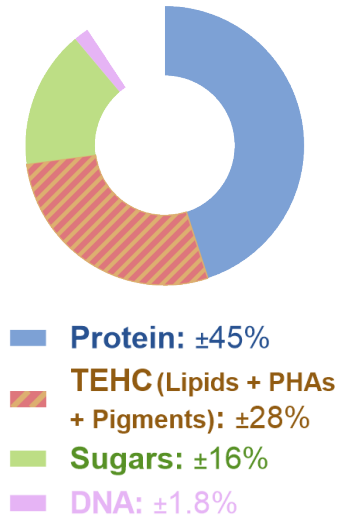


Fig 19. Macromolecular analyses across tests, ANOVA at p-value = 0.5 (own data, GraphPad 8.0.1.).

Applications - *For protein demand*

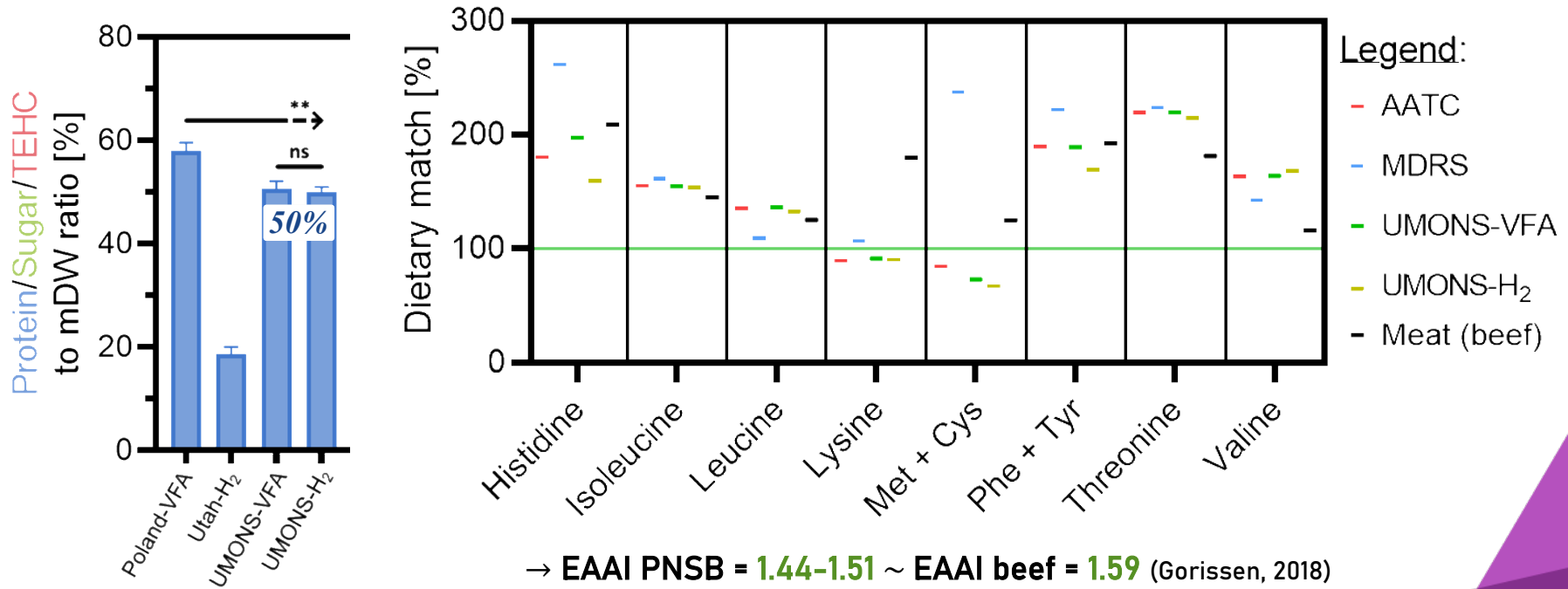


Fig 20. Dietary match of PNSB EAAI compared to beef (own data/Gorissen, 2018, GraphPad 8.0.1.).

Applications - ECLSS in Space

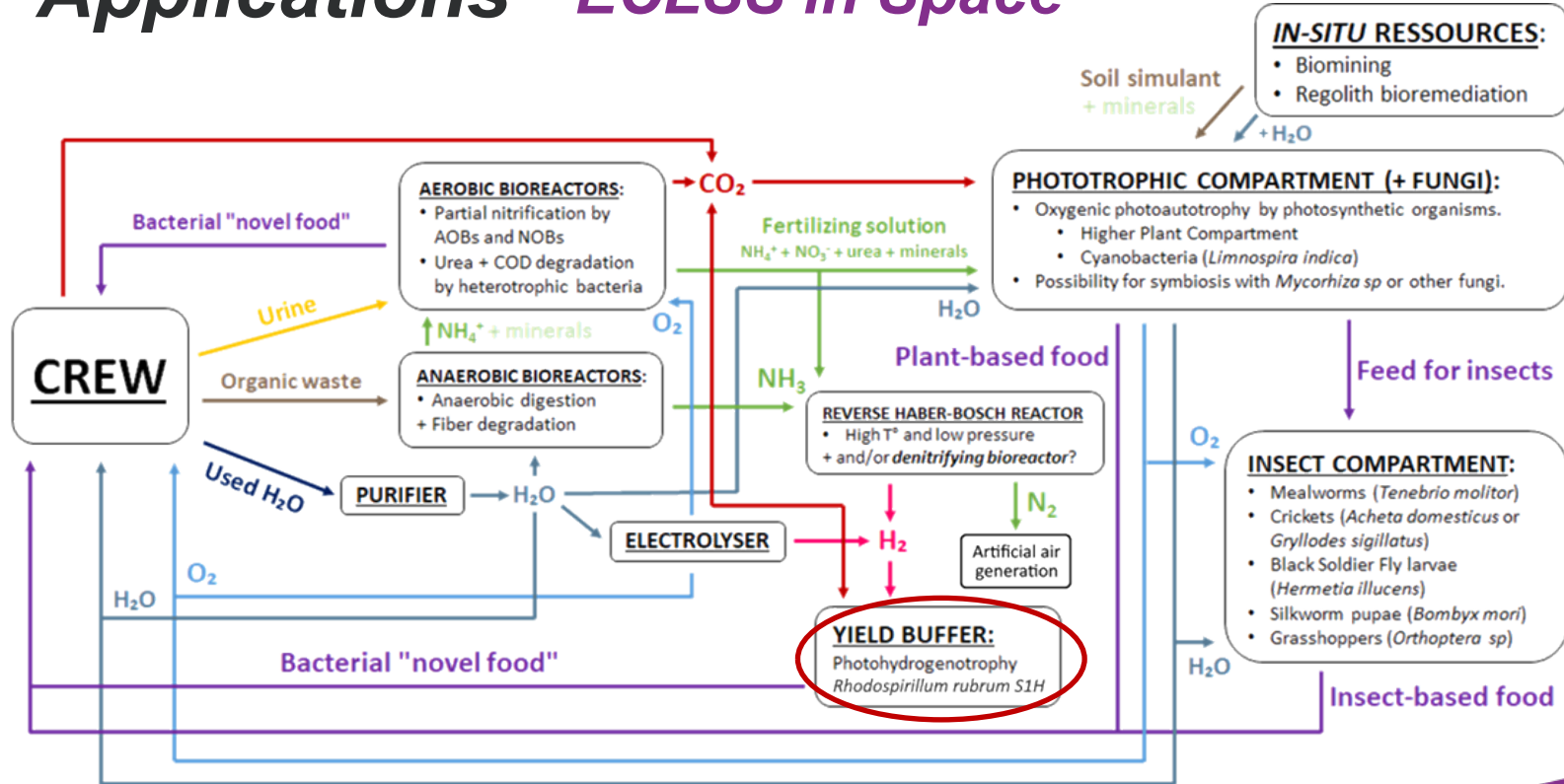


Fig 21. Photohydrogenotrophic PNSB system as a yield buffer for space exploration ECLSS (own production).

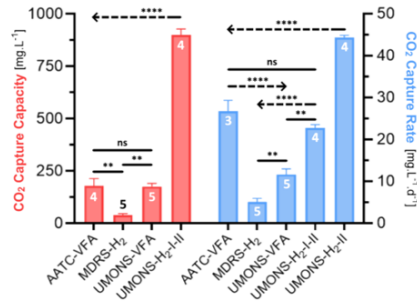
Applications - ECLSS in Space

Litres of culture needed to meet the daily physiological needs of one human					
Exper.	Protein	Sugar	Lipids	DNA	CO ₂
UMONS-VFA	260.06 ± 24.46 L	1326.23 ± 377.20 L	1345.31 ± 83.33 L	835.77 ± 93.31 L	91859.47 ± 22748.40 L
UMONS-H ₂ -II	949.99 ± 52.98 L	3304.71 ± 806.98 L	6683.00 ± 1719.84 L	2979.25 ± 133.70 L	24198.65 ± 671.88 L

Table 1. Relative maximal instantaneous productivity of the four main macromolecules between photohydrogenotrophy (UMONS-H₂-II) and photoheterotrophy (UMONS-VFA). (own data).

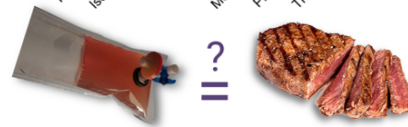
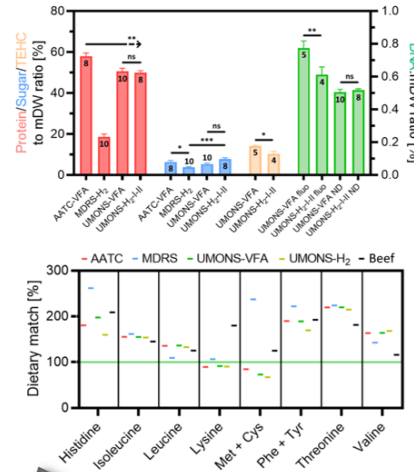
Conclusion - Achievements and prospects

AXIS 1. Assessment of CO₂ fixation efficiency by Rhodospirillum rubrum in phototrophy



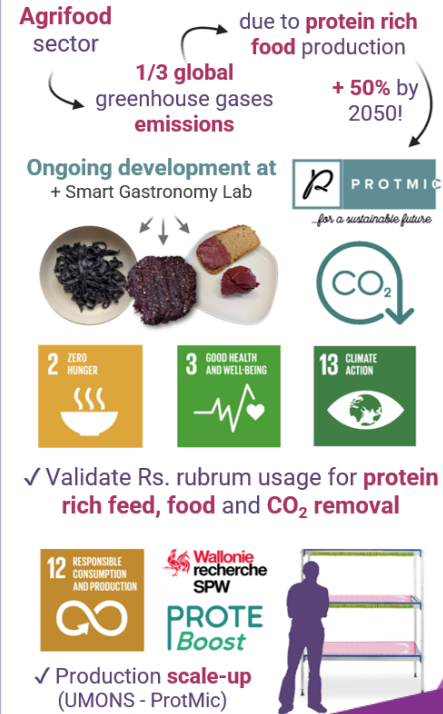
- ✓ Test in photoheterotrophy CO₂ + Volatile Fatty Acids (VFAs)
- ✓ Test in photohydrogenotrophy CO₂ + Hydrogen gas (H₂)
- ✓ Test in **controlled & scarce** conditions (UMONS & analog missions)

AXIS 2. Assessment of the nutritional value of Rhodospirillum rubrum biomass



- ✓ Assessment of **protein, sugar, lipid** and **nucleic acid** content
- ✓ Assessment of **protein quality** by essential amino acid **dietary match**

AXIS 3. Validation and commercialisation of Rhodospirillum rubrum in the agrifood sector





Guillaume Gégé

HRE-HS Trainee Radiation Microbiology at
ESA-ESTEC | Co-Founder and Project Manag...



MELISSA


Thank you!

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Hülse, T., Barry, E. M., Lu, Y., Puyol, D., Keller, J., & Batstone, D. J. (2016). Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. *Water Research*, 100, 486–495. <https://doi.org/10.1016/j.watres.2016.04.061>

Zhu, R. & Li, J. Hydrogen metabolic pathways of *Rhodospirillum rubrum* under artificial illumination. *Chinese Science Bulletin* 55, 32–37 (2010).

Low-cost system - Cultivation & analyses

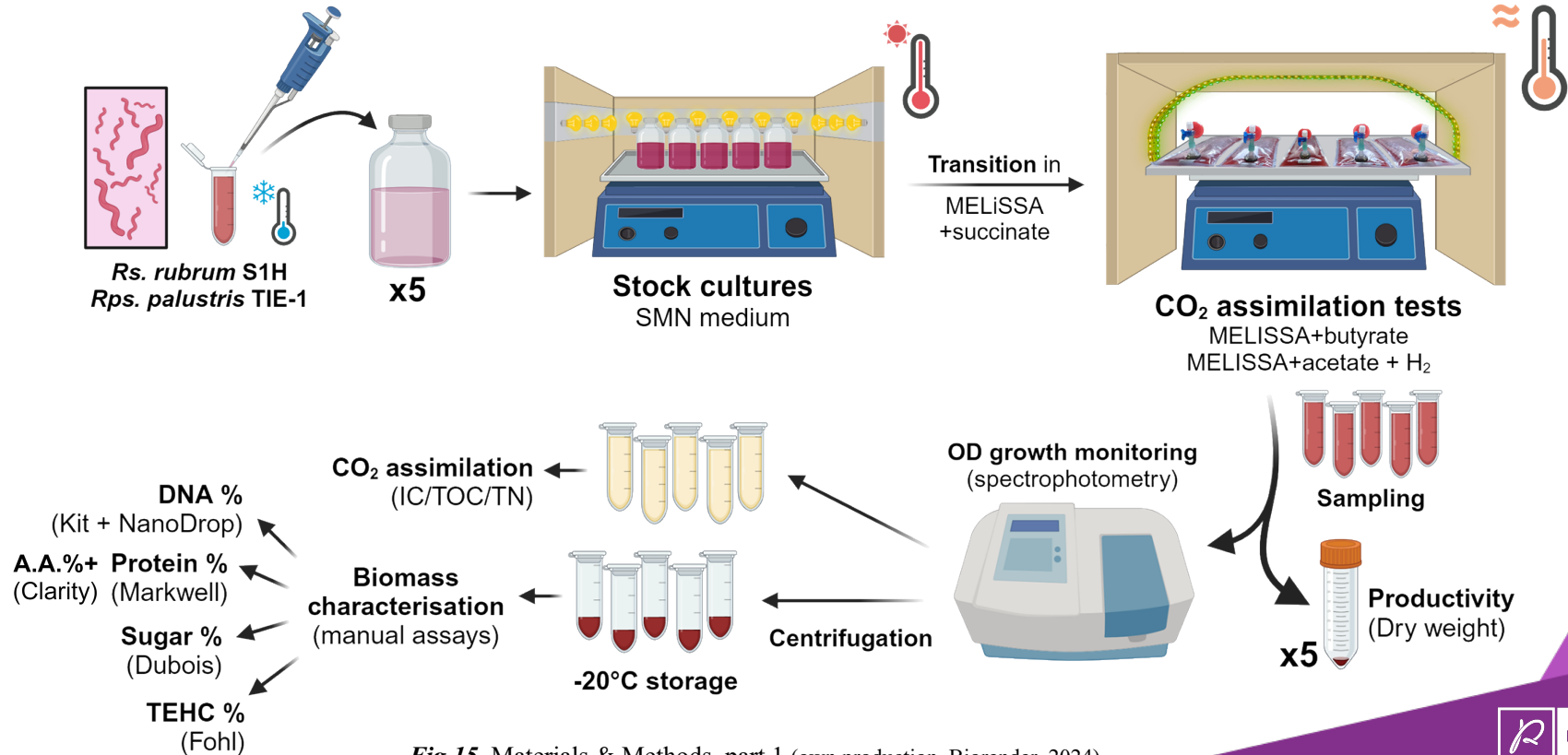
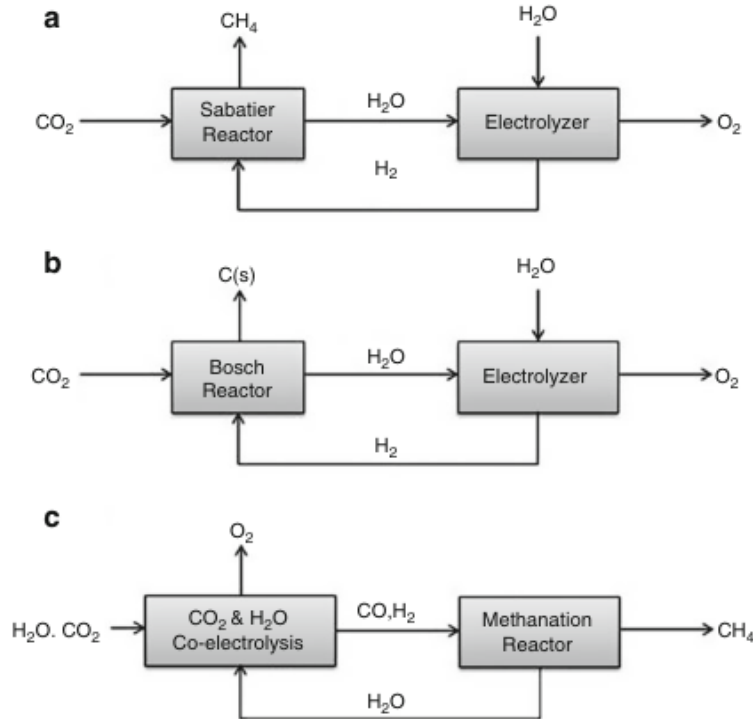
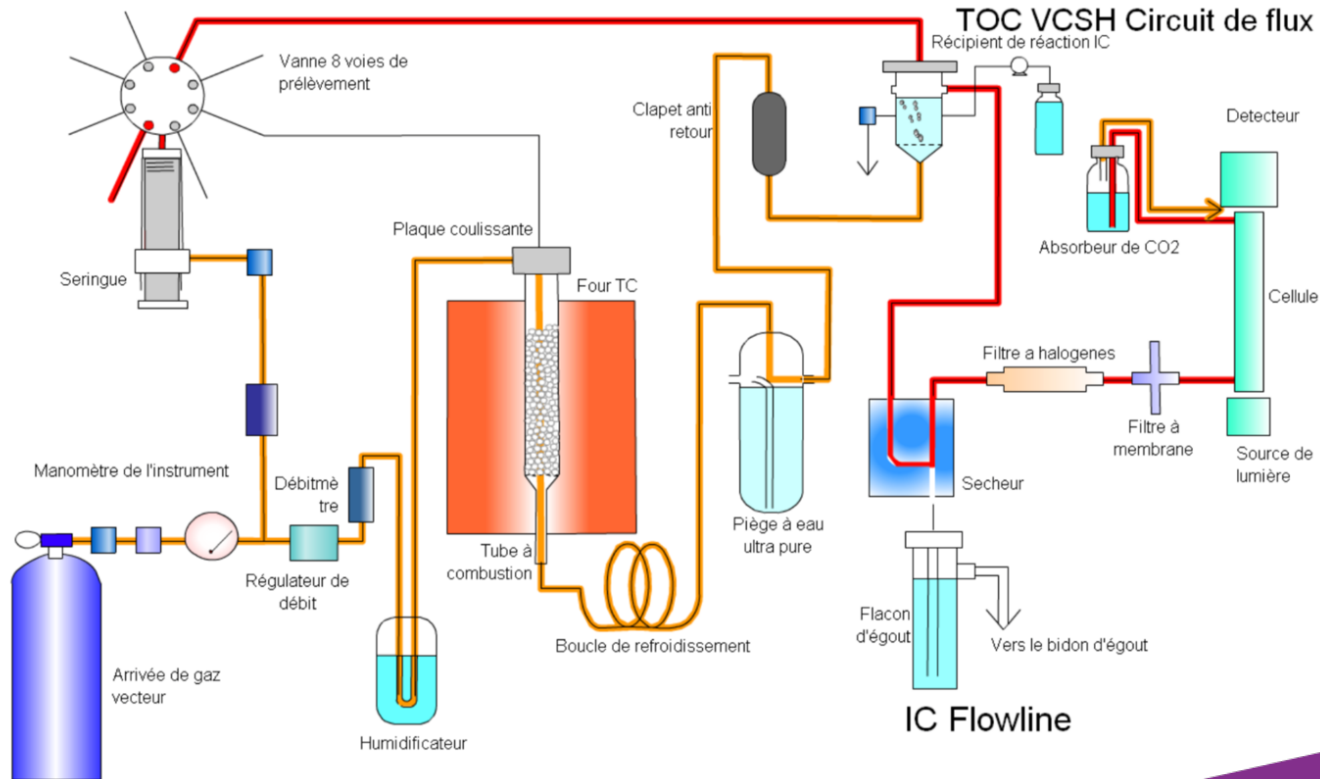


Fig 15. Materials & Methods, part 1 (own production, Biorender, 2024).

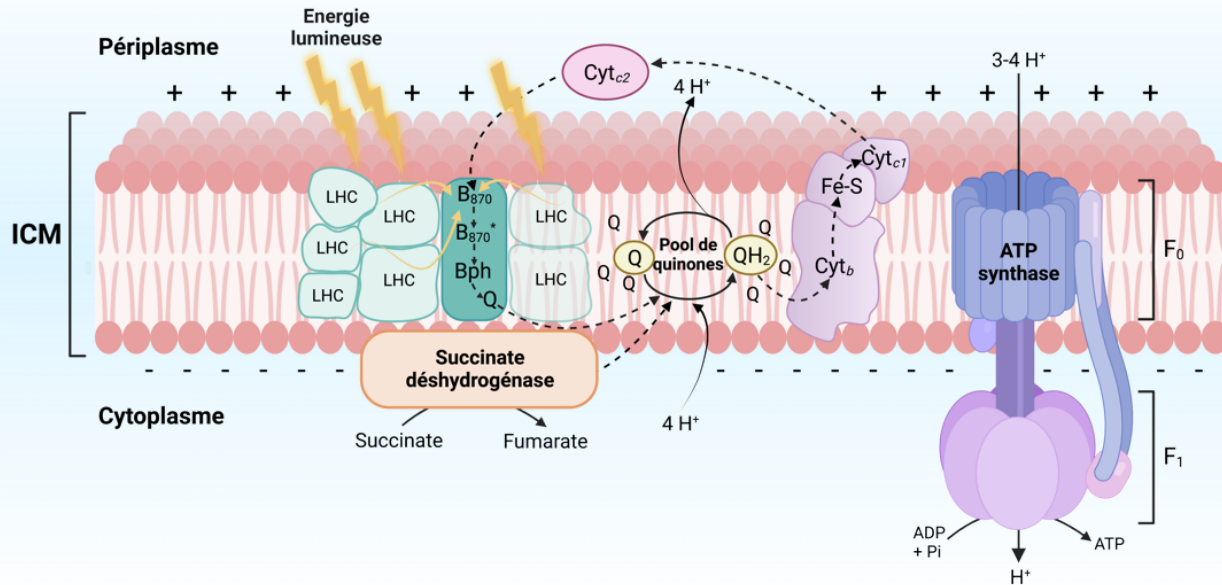
Annex A - Chemical systems for CO₂ reduction



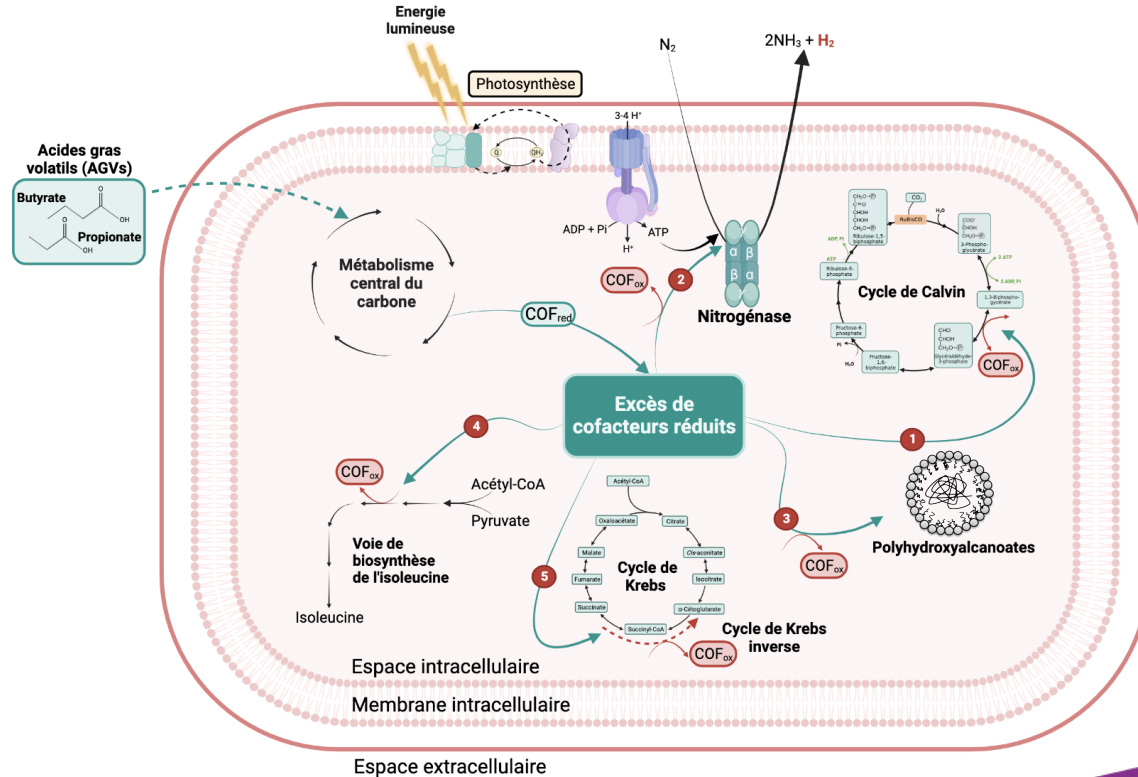
Annex B - TIC assay principle



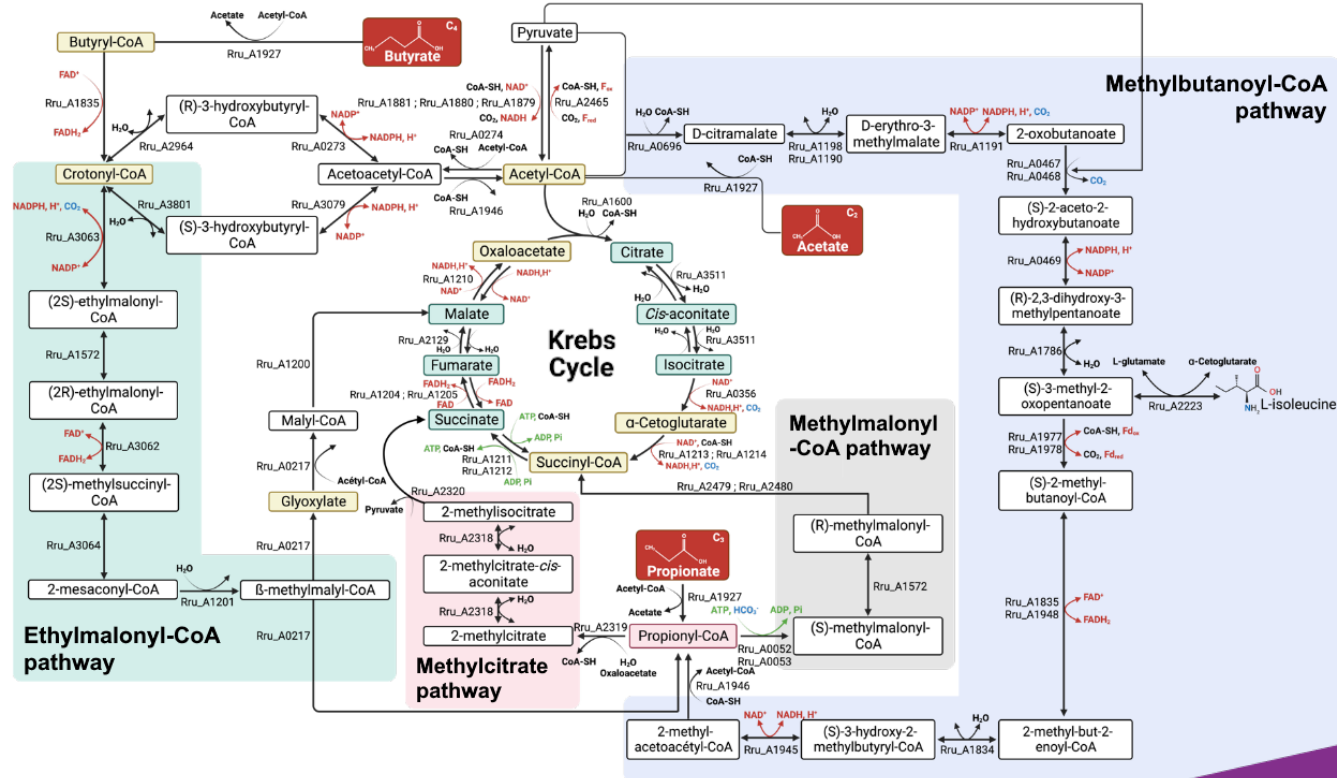
Annex C - Anoxygenic photosynthesis



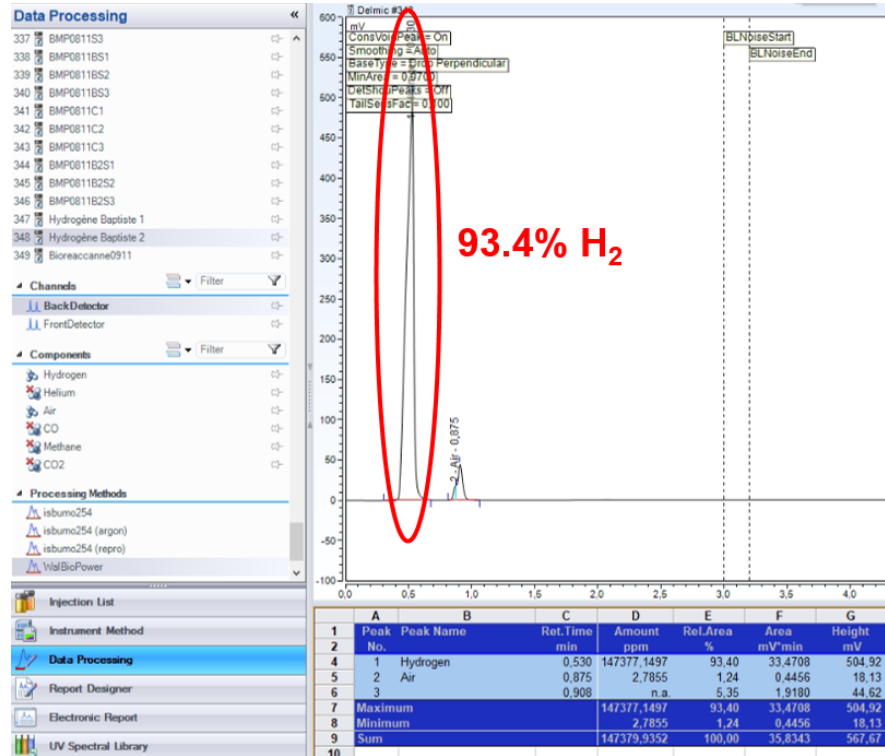
Annex D - Other electron sinks



Annex E - VFA assimilation in *Rs. rubrum*



Annex F - Hydrogen production via electrolysis



Annex G - Productivities

Exper.	Max. inst. theo. prod. [mg.L ⁻¹ .d ⁻¹]	Protein prod. [mg.L ⁻¹ .d ⁻¹]	Sugar prod. [mg.L ⁻¹ .d ⁻¹]	TEHC prod. [mg.L ⁻¹ .d ⁻¹]	DNA (ND) prod. [mg.L ⁻¹ .d ⁻¹]	CCR [mg.L ⁻¹ .d ⁻¹]
UMONS- VFA	397.0	200.7 ± 18.88	21.30 ± 6.06	56.02 ± 3.47	2.03 ± 0.23	11.67 ± 2.89
UMONS- H ₂ -II	110.0	54.95 ± 3.06	8.55 ± 2.09	11.28 ± 2.90	0.57 ± 0.03	44.30 ± 1.23

Annex H - EAAI & Dietary match

The dietary match between the EAA profile of PNSB biomass and the EAA requirement of human adults was calculated using *Equation {3}* (from *Spanoghe et al, 2021*)⁷⁸.

$$\text{Dietary match [\%]} = \frac{EAA_{\text{biomass}} \left[\frac{\text{g EAA}}{100 \text{ g protein}} \right] \times \text{digestibility [\%]}}{EAA_{\text{requirements}} \left[\frac{\text{g EAA}}{100 \text{ g protein}} \right]} \quad \{3\}$$

Eventually, the protein quality was assessed following the **EAA index** (hrta. EAAI), where it can be defined as “superior” (>1), “high” (0.95-1), “good” (0.86-0.95), “useful” (0.75-0.86) or “inadequate” (<0.75)⁹⁴. *Equation {4}* was used (from *Spanoghe et al, 2021*)⁷⁸.

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \dots \times \frac{aa_n}{AA_n}} \quad \{4\}$$

Where: n is the number of EAA; aa_n is the EAA content in the PNSB protein (g EAA.g⁻¹ protein); AA_n is the EAA content requirement for humans (g EAA.g⁻¹ protein).

Annex I - Large scale bag bioreactors

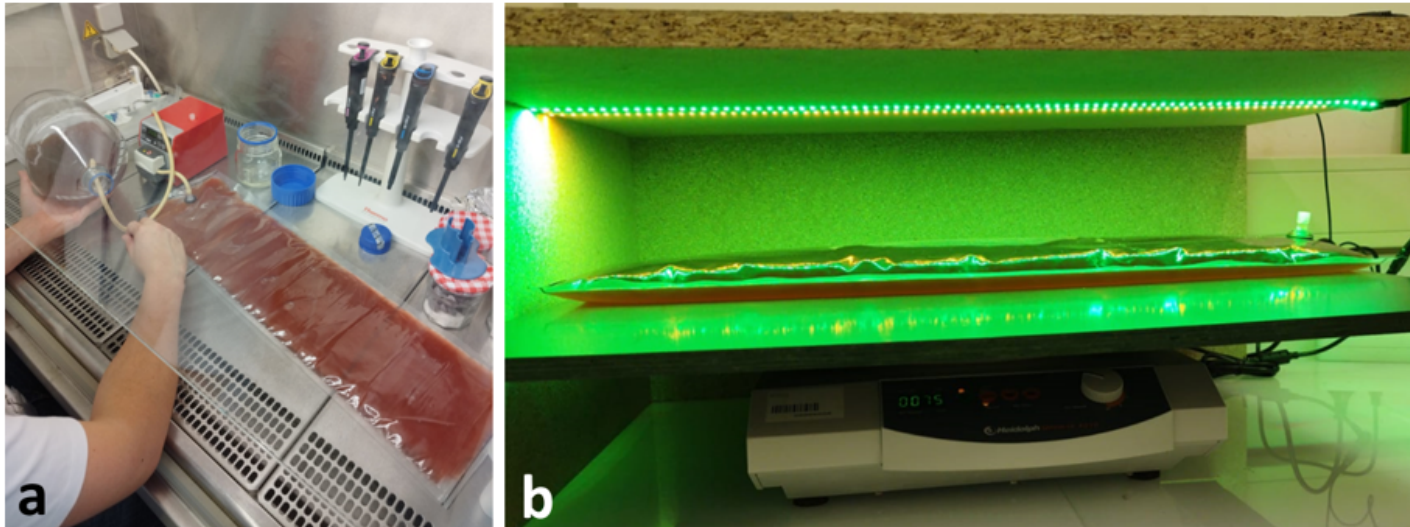
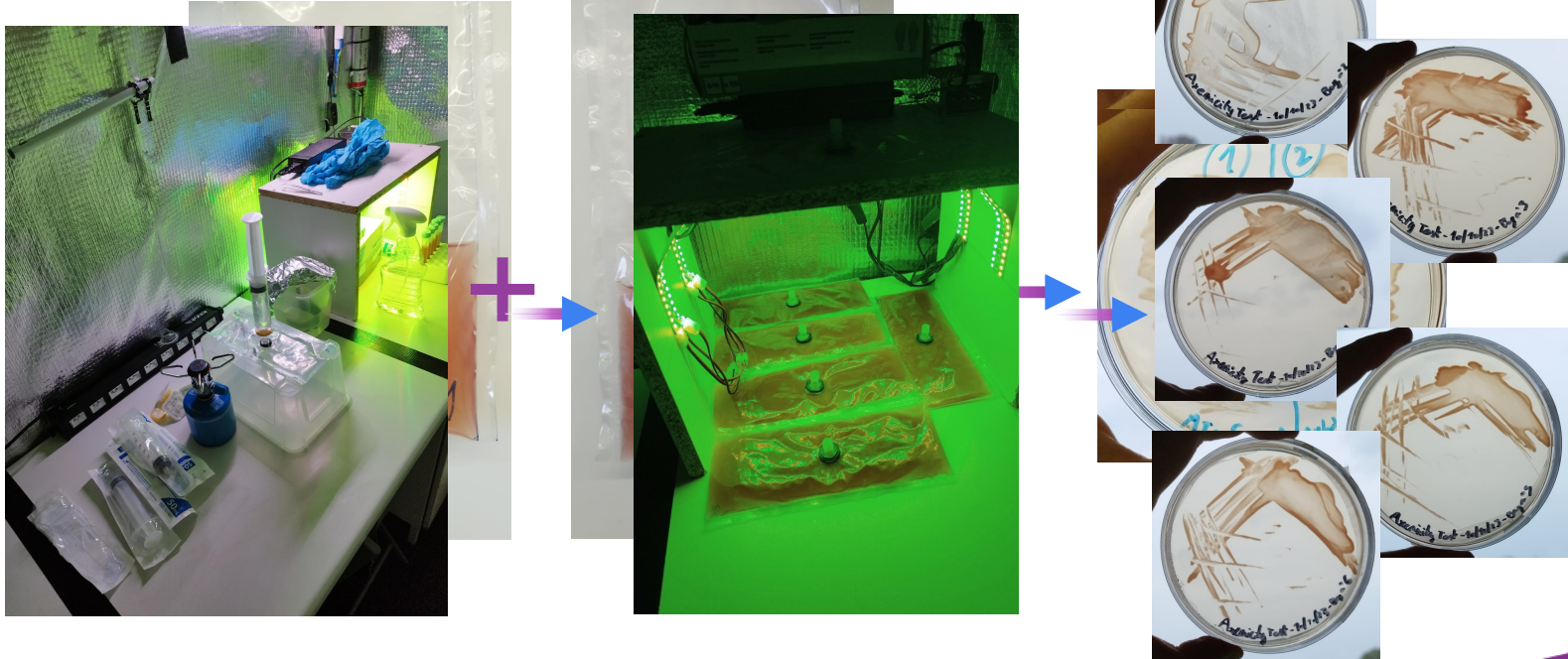


Figure 14: Large-scale prototype developed for the upcycling of molasses. (a) Filling operation of the 4 L bag under the biosafety cabinet. (b) New enclosure with a 4 L bag under LED light.

Annex J - Axenicity



Annex K - Results AATC-VFA

Markwell analysis of protein content evolution (AATC)

Rs. rubrum - MELiSSA medium (124 mM C butyrate, 50 mM NaHCO₃)

Amino acid group abundances [%]						
Bag ID	OD	mDW in sample [µg]	Sugar TR1 [µg]	Sugar TR2 [µg]	Mean sugar:mDW ratio [%]	Overall mean sugar:mDW ratio [%]
3	5.61	1389.19	155.72	70.59	8.15 ± 4.33	6.38 ± 2.11
4	5.49	1566.56	117.38	84.47	6.44 ± 1.49	
5	5.96	1700.81	94.44	93.23	5.52 ± 0.05	
6	6.09	1650.76	98.06	81.15	5.43 ± 0.72	

Table 9: Sugar:DW ratio calculation based on the Dubois analysis of the sugar content of *Rs. rubrum* cultures in MELiSSA butyrate medium (124 mM C butyrate, 50 mM NaHCO₃) at AATC, Kraków, Poland. Data based on four biological replicates, each assessed in two technical replicates.

D: aspartic acid, N: asparagine, E: glutamic acid, Q: glutamine, H: histidine, K: lysine, R: arginine.

~ ∞ duplicates (one min and one max) for each nested box. Protein:tDW ratio calculated using the tDW standard conversion curve (see section 3.5.3). Figure made using GraphPad Prism 8.0.1.

Annex L - Results UMONS-VFA

Markwell analysis of protein content evolution (UMONS-VFA)

Rs. rubrum - MELiSSA medium (124 mM C butyrate, 50 mM NaHCO₃)

n in

Bag ID	OD	mDW in sample [μg]	QIAGEN® DNA TR avg. [μg]	QIAGEN® mean DNA:mDW ratio [%]	QIAGEN® ovr. mean DNA:mDW ratio [%]	Fluoresc. DNA TR1 [μg]	Fluoresc. mean DNA:mDW ratio [%]	Fluoresc. ovr. mean DNA:mDW ratio [%]
1	6.45	3353.5	15.03 ± 0.55	0.45 ± 0.02	0.51 ± 0.06	25.30	0.75	0.77 ± 0.10
2	6.58	3475.1	16.72 ± 0.84	0.48 ± 0.02		32.10	0.92	
3	6.29	3600.5	20.03 ± 1.48	0.56 ± 0.04		26.00	0.72	
4	6.46	3315.5	19.42 ± 0.14	0.59 ± 0.004		26.60	0.80	
5	6.19	3444.7	16.63 ± 0.60	0.48 ± 0.02		23.10	0.67	

Table 16: DNA:DW ratio calculation based on the QIAGEN-NanoDrop/fluorescence analysis of the TEHC content of *Rs. rubrum* cultures in MELiSSA butyrate medium (124 mM C butyrate, 50 mM NaHCO₃) at UMONS, Belgium. Data based on five biological replicates.

Belgium. Data obtained using a S 433 Amino Acid Analyzer (Sykam). Amino acid code in Table 8.

Figures 1

obtained via Markwell assays on five samples across five biological replicates, each in technical duplicates (one min and one max) for each nested box. Protein:tDW ratio calculated using the tDW standard conversion curve (see section 3.5.3). Figure made using GraphPad Prism 8.0.1.

Annex M - Results MDRS- H_2

Markwell analysis of protein content evolution (MDRS)

Rps. palustris - Freshwater medium (10 mM C acetate, 83.33 mM NaHCO_3)

80

palustris

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Bag ID	OD	mDW in sample [μg]	Sugar TR1 [μg]	Sugar TR2 [μg]	Mean sugar:mDW ratio [%]	Overall mean sugar:mDW ratio [%]
6	1.200	1150.27	34.36	26.21	2.63 ± 0.47	3.77 ± 1.10
7	1.290	808.03	25.30	28.93	3.36 ± 0.35	
8	1.265	521.40	20.78	17.76	3.70 ± 0.85	
9	1.440	781.38	25.00	47.34	4.63 ± 0.48	
10	1.315	800.29	41.30	31.04	4.52 ± 0.28	

Table 21: Sugar:DW ratio calculation based on the Dubois analysis of the sugar content of *Rps.*

palustris cultures in freshwater medium (10mM C acetate, 83.3mM NaHCO_3 , 3:2 H_2 -medium ratio)

at MDRS, Utah, US. Data based on five biological replicates, assessed in two technical replicates.

palustris cultures in freshwater medium (10mM C acetate, 83.3mM NaHCO_3 , 3:2 H_2 -medium ratio) at MDRS, Utah, US. Data obtained using a S 433 Amino Acid Analyzer (Sykam). Amino acid code in Table 8.

& ASI
 standard conversion curve (see section 3.5.3). Figure made using GraphPad Prism 8.0.1.

Annex N - Results UMONS-H₂

Markwell analysis of protein content evolution (UMONS-H₂)

Rs. rubrum - MELiSSA medium (169mM NaHCO₃, 10mM C acetate)

60

Bag ID	OD	mDW in sample [µg]	QIAGEN® DNA TR avg. [µg]	QIAGEN® mean DNA:mDW ratio [%]	QIAGEN® ovr. mean DNA:mDW ratio [%]	Fluoresc. DNA TR1 [µg]	Fluoresc. mean DNA:mDW ratio [%]	Fluoresc. ovr. mean DNA:mDW ratio [%]
7	3.40	1814.5	9.75 ± 0.38	0.54 ± 0.02	0.52 ± 0.02	13.20	0.73	0.61 ± 0.10
8	3.23	1810.7	9.45 ± 0.08	0.52 ± 0.004		11.40	0.63	
9	3.49	1867.7	9.80 ± 0.24	0.52 ± 0.01		11.10	0.59	
10	3.33	1778.4	8.63 ± 0.15	0.49 ± 0.01		8.80	0.49	

Table 29: DNA:DW ratio calculation based on the QIAGEN-NanoDrop/fluorescence analysis of the TEHC content of *Rs. rubrum* cultures in MELiSSA acetate medium (10 mM C acetate, 169 mM NaHCO₃, 3:2 H₂-medium ratio) at UMONS, Belgium. Data based on four biological replicates.

obtained in technical duplicates (one min and one max) for 'I-V, Shim each nested box. Protein:tDW ratio calculated using the tDW standard conversion curve (see section 3.5.3). Figure made using GraphPad Prism 8.0.1.

Annex O - CCU/macromolecule ratio results

80		1.0				
Exper.	Max. inst. theo. prod. [mg.L ⁻¹ .d ⁻¹]	Protein prod. [mg.L ⁻¹ .d ⁻¹]	Sugar prod. [mg.L ⁻¹ .d ⁻¹]	TEHC prod. [mg.L ⁻¹ .d ⁻¹]	DNA (ND) prod. [mg.L ⁻¹ .d ⁻¹]	CCR [mg.L ⁻¹ .d ⁻¹]
UMONS-VFA	397.0	200.7 ± 18.88	21.30 ± 6.06	56.02 ± 3.47	2.03 ± 0.23	11.67 ± 2.89
UMONS-H ₂ -II	110.0	54.95 ± 3.06	8.55 ± 2.09	11.28 ± 2.90	0.57 ± 0.03	44.30 ± 1.23
Litres of culture needed to meet the daily physiological needs of one human (70 kg, 2000 kcal diet) = 46.2 g protein; 25 g sugar; 66.7 g lipids; 1.5 g TNA; -1072 g CO ₂ ¹²⁴ .						
Exper.	Protein	Sugar	Lipids	DNA	CO ₂	
UMONS-VFA	260.06 ± 24.46 L	1326.23 ± 377.20 L	1345.31 ± 83.33 L	835.77 ± 93.31 L	91859.47 ± 22748.40 L	
UMONS-H ₂ -II	949.99 ± 52.98 L	3304.71 ± 806.98 L	6683.00 ± 1719.84 L	2979.25 ± 133.70 L	24198.65 ± 671.88 L	

Table 30: Relative maximal instantaneous productivity of the four main macromolecules between photohydrogenotrophy (UMONS-H₂-II) and photoheterotrophy (UMONS-VFA). Data based on the same replicate number as Figure 30. Maximal instantaneous theoretical productivity (productivity at μ_{max}) calculated following the method described in section 3.4.3. Only NanoDrop (ND) data was considered for DNA productivity assessment (less error). The daily physiological needs of one human were defined as described in section 1.4.