ADVANCED MASTER IN SPACE STUDIES
LABORATORY OF APPLIED SPACE MICROBIOLOGY

Design of Cyanobacterium Photobioreactors for Mars *In-Situ* Resource Utilisation

Prototyping and Testing with Anabaena *sp. PCC 7938*

Master thesis

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Academic Year 2024-2025

SUPERVISORS:

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Mission To Mars - *Available* in-situ *resources*

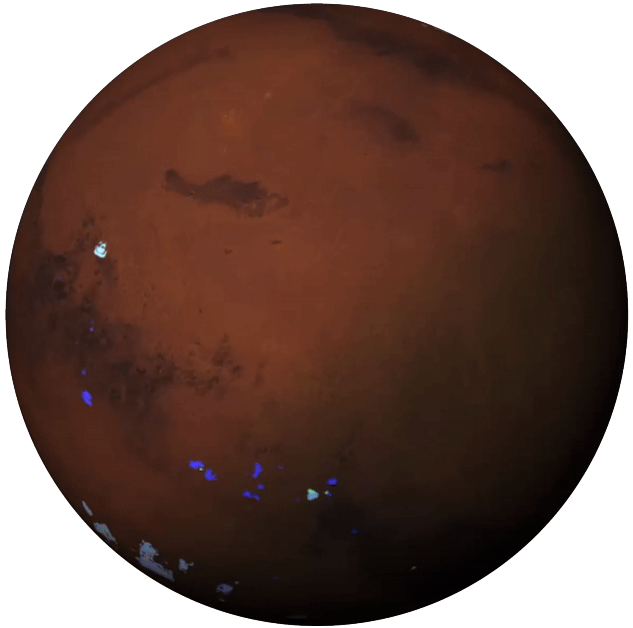
Atmosphere

(CO_2/N_2)

- ARGON — 1.9%
- NITROGEN — 1.9%
- CARBON DIOXIDE — 96%

TRACE GASES, INCLUDING:

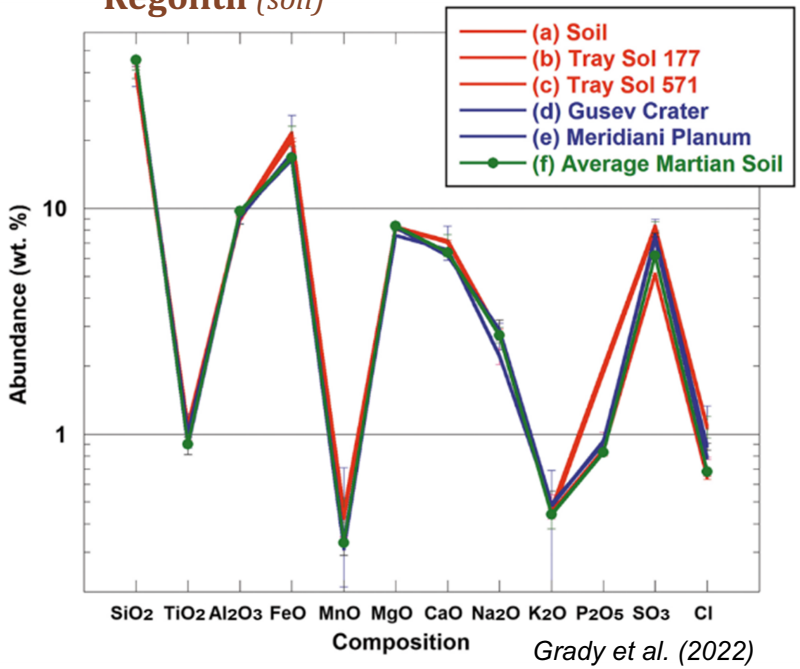
- ACETYLENE
- CARBON MONOXIDE
- KRYPTON
- METHANE
- NEON
- NITROGEN OXIDE
- OXYGEN
- OZONE
- WATER VAPOUR
- XENON



Water ice

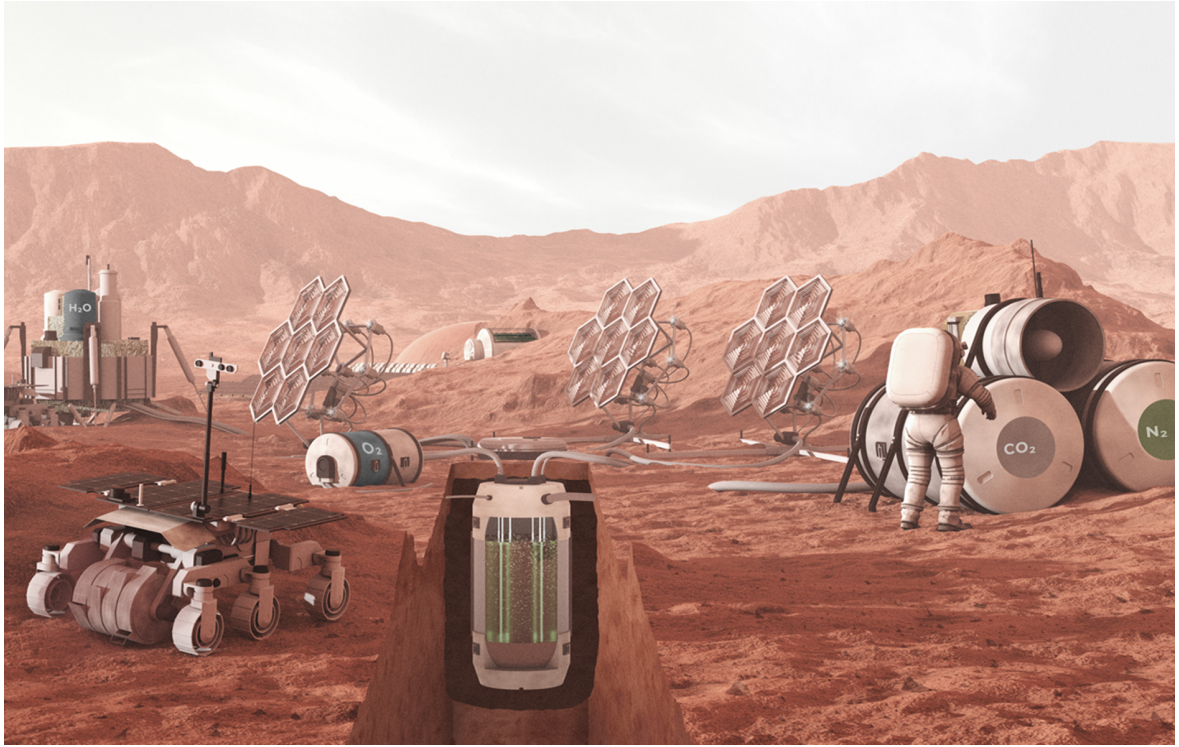
(caps, ground, H_20-minerals)

Regolith *(soil)*

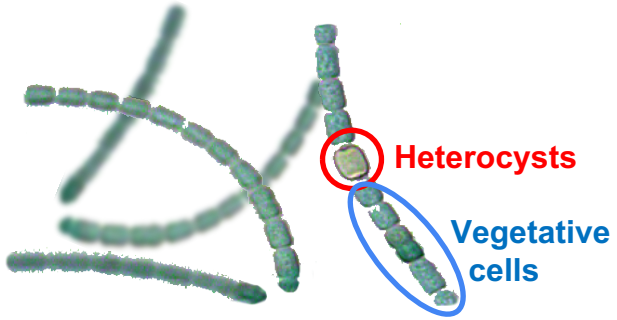


Grady et al. (2022)

Mission To Mars - *Biological* in-situ *resource utilisation*



***Anabaena* sp. PCC 7938**:



- **Regolith-leaching**
  → Extracts nutrients
- **Diazotrophic**
  → Fixes N_2
- **Photosynthetic**
  → Fixes CO_2 using light
  → Produces O_2

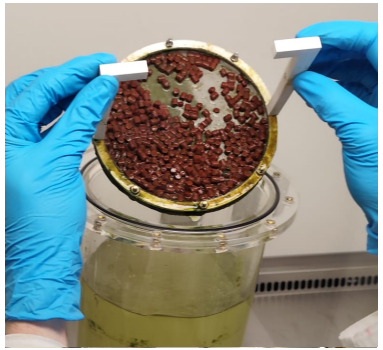
Biological ISRU - *Tri-phasic challenges for bioreactors*

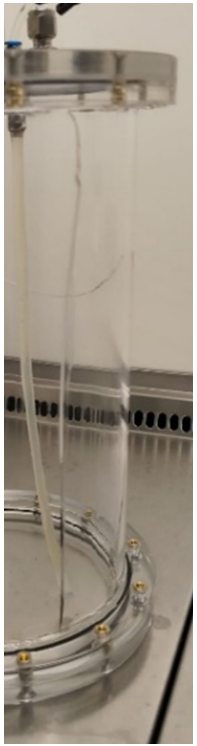
Three phases:

MGS-1

H_2O

CO_2/N_2



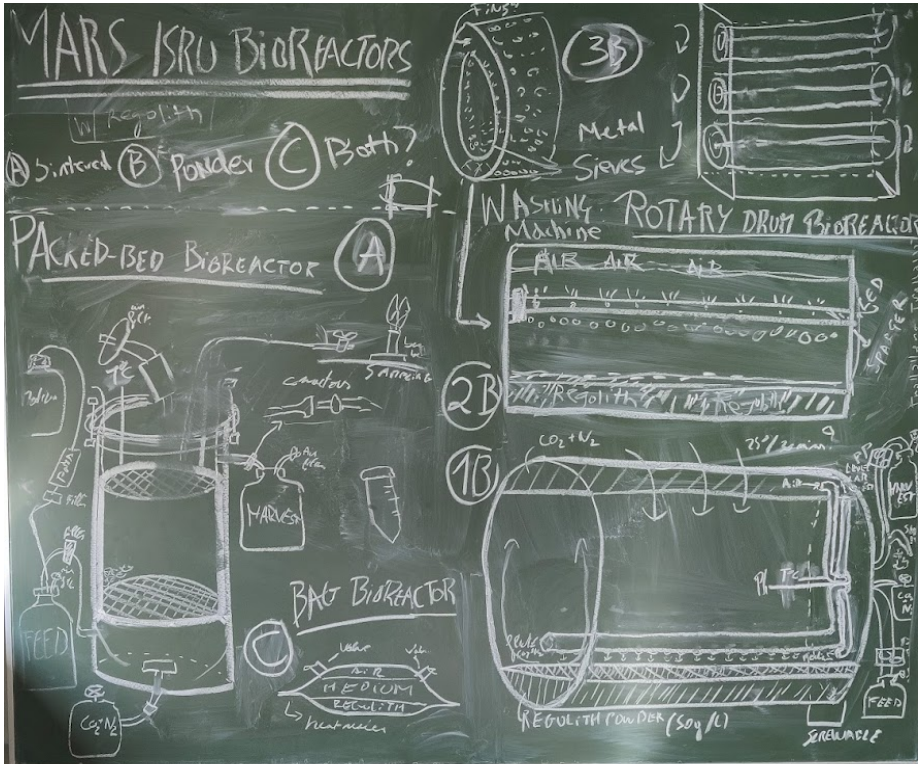


Design considerations:

- Regolith shadowing → photosynthesis?
- Agitation/bubbling → shadowing/homogenisation?
- Contamination → sterilisation?
- MGS-1/H_2O renewal → bioreactor type?
- Watertightness/leakproofness?

MGS-1 = Mars Global Simulant 1 (Space Resource Technologies)

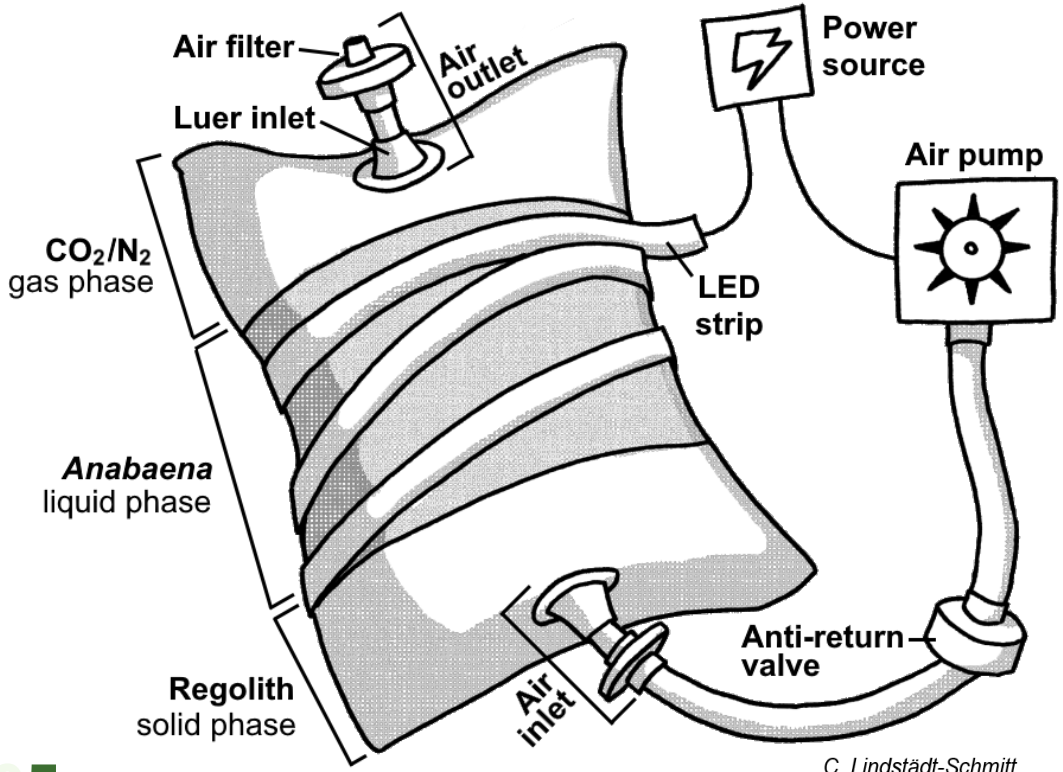
Objectives - *Cyanobacteria ISRU on Mars*



- **Cyanobacterium photobioreactors** designs for ISRU on Mars:
  - **Sterilisable:** vessel, connections, medium, regolith,…
  - **Operational support:** inoculation, medium filling, sampling,…
  - **Parameter control**: temperature, pH and light flux.
  - **Full ISRU capability:** CO_2/N_2 bubbling, regolith leaching.
- Study the **growth dynamics** of *Anabaena* sp. in the developed bioreactors.

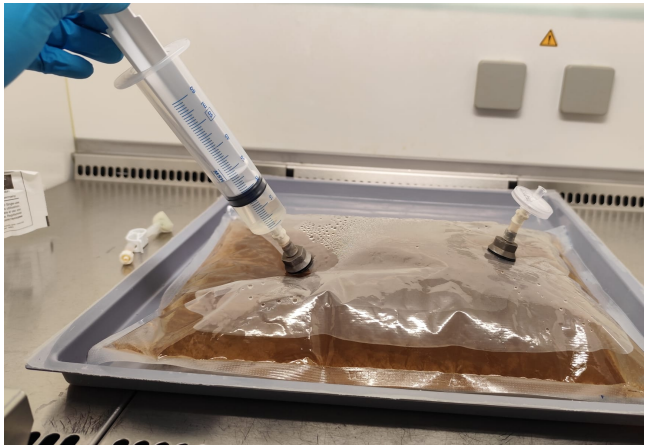
ISRU = In-Situ *Resource Utilisation*

Materials & Methods - Bag bioreactors



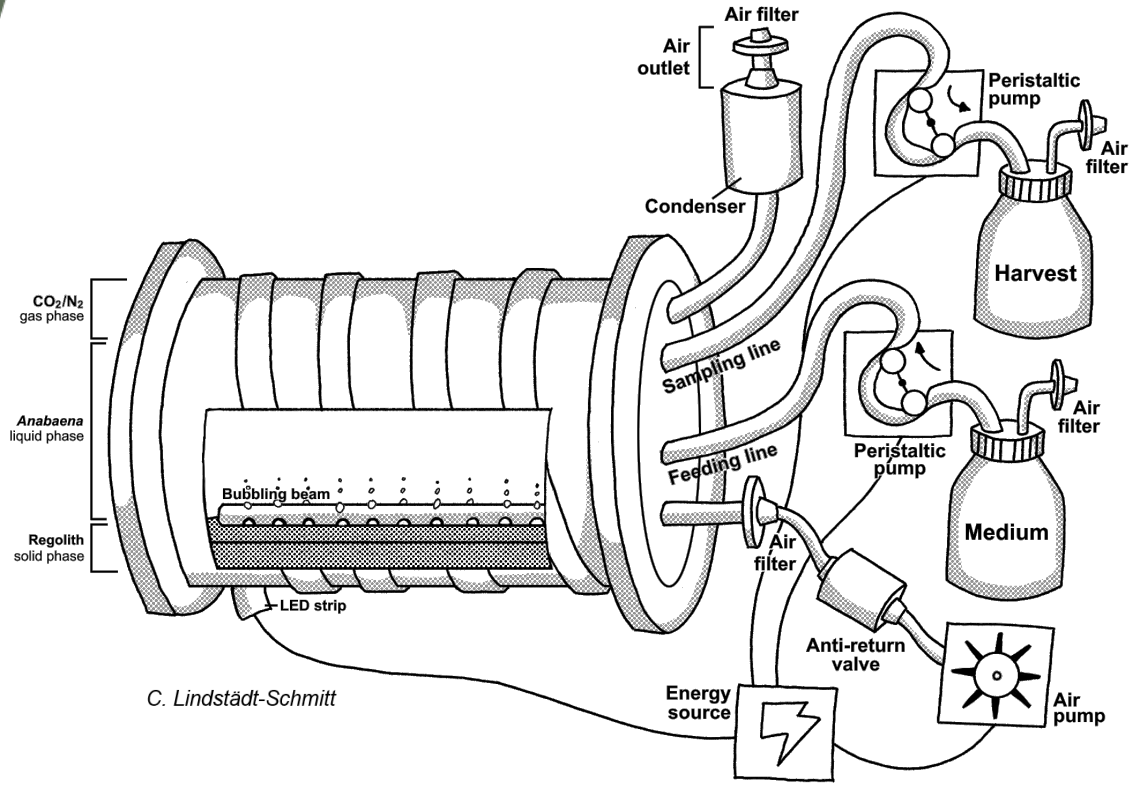
C. Lindstädt-Schmitt

- Heat-sealed plastic bags = ↓ESM
- Cheap & easy for prototyping
- Scalable and reusable

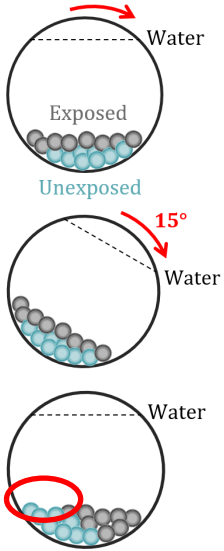


ESM = Equivalent System Mass

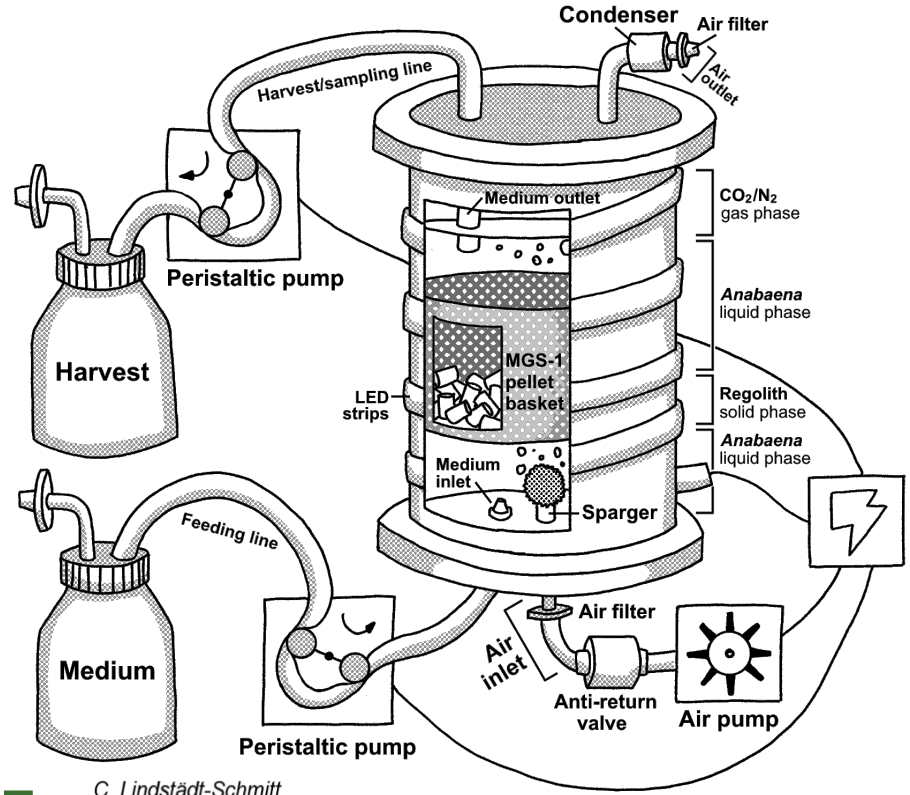
Materials & Methods - Rotary drum bioreactors



- Robust acrylic vessel = ↑ESM
- Bubbling = ↑homogeneity
- Swiveling = regolith renewal



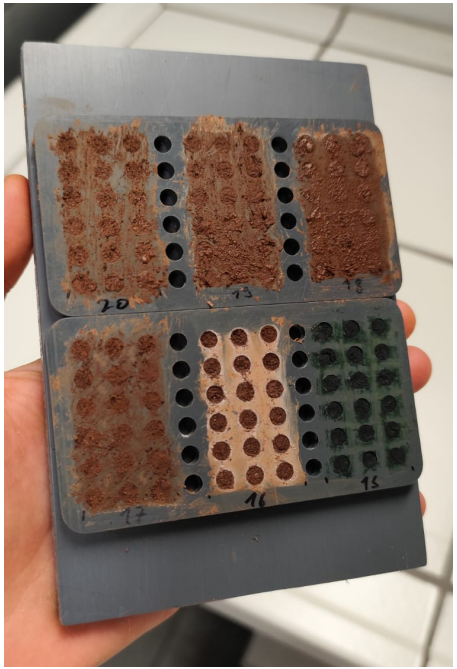
Materials & Methods - *Packed-bed bioreactors*

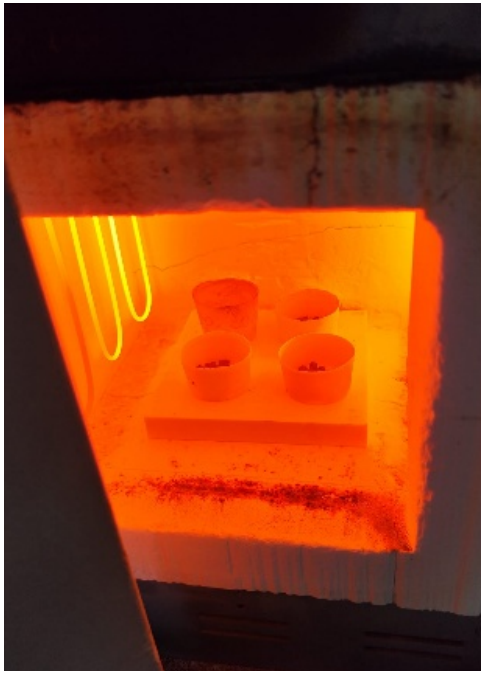


C. Lindstädt-Schmitt

- Robust acrylic vessel = ↑ESM
- **Sintered regolith =** ↓shadowing = ↑**ESM**
- Sintered pellet basket = regolith renewal
- Bubbling + recirculation loop = ↑ homogeneity

Materials & Methods - Sintered regolith pellets



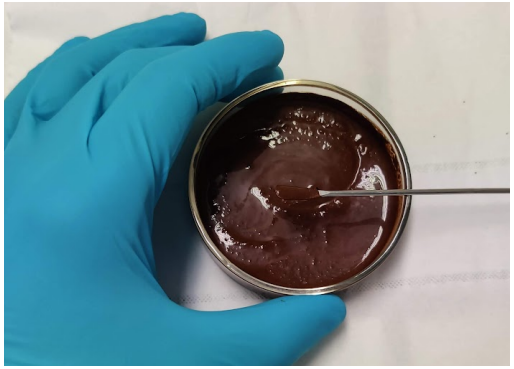


→ **Sintering**: Heat-based regolith agglomeration (MGS-1 + H_2O + binder)

Materials & Methods - Sintered regolith pellets

27 pellet batches tested: MGS-1 + H_2O + (binders)

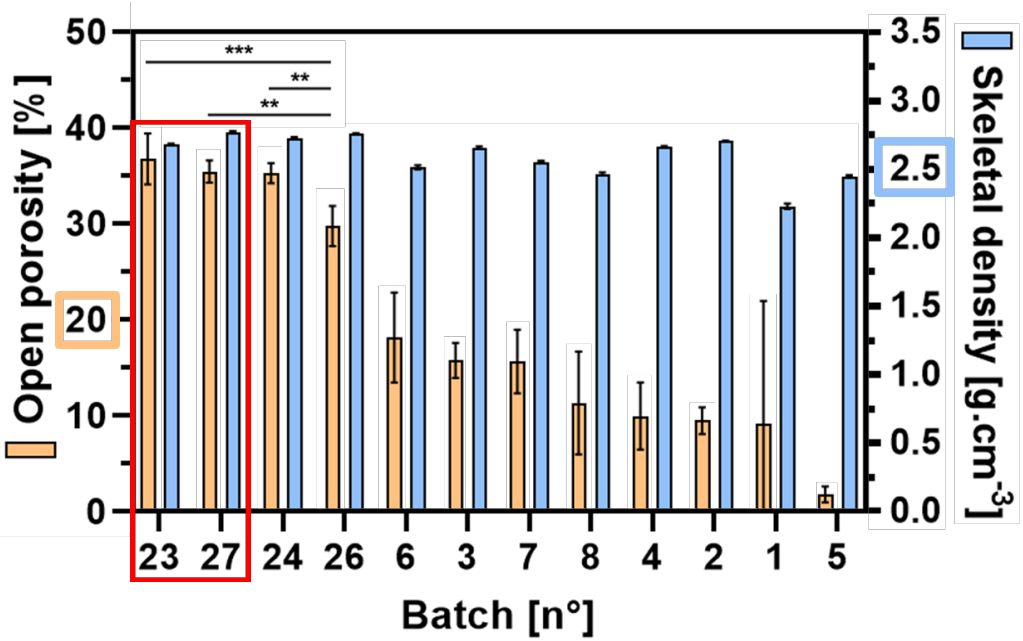
- Binders: Spirulina powder, ammonium carbonate, sodium carbonate.
- Iterations: recipe, pellet size, moulding method, sintering time and temperature, etc.



Protocol development:

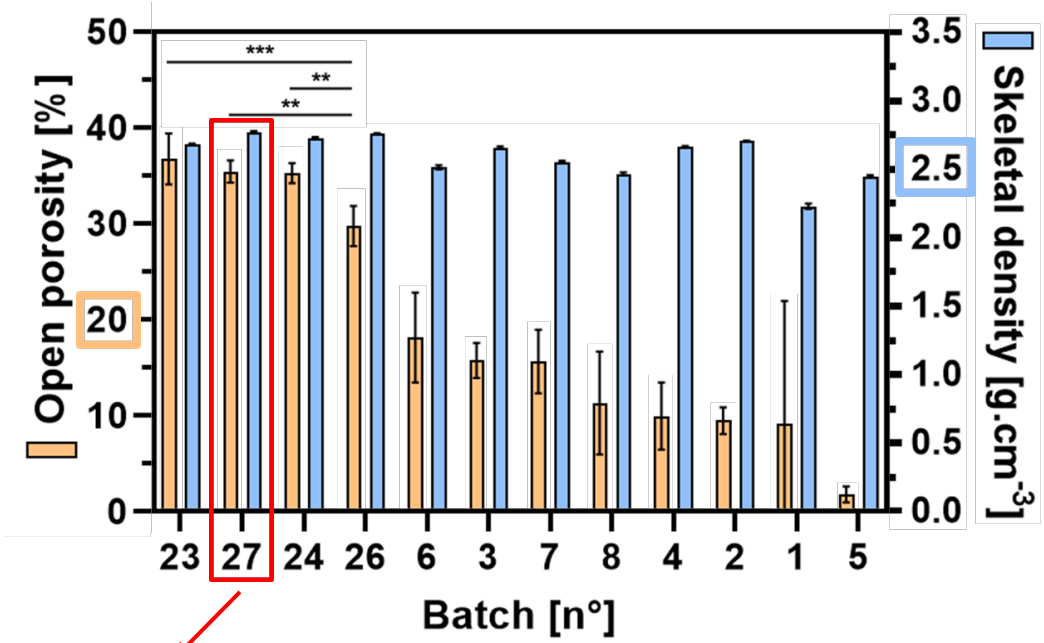
- Mix MGS, water and the binder in **?:?:? ratio**
- Feed slurry into **mould?** Syringe **extrusion?**
- Dry for **? hours** at **?°C**.
- Sinter at **?°C** for **? hours**.

Results - Water intrusion & Helium pycnometry



→ **Best batch: n°27** (23 = brittle)

Results - Water intrusion & Helium pycnometry



Skeletal density: 2.71 ± 0.06 g.cm^{-3}
Average porosity: 35.48 ± 1.17%

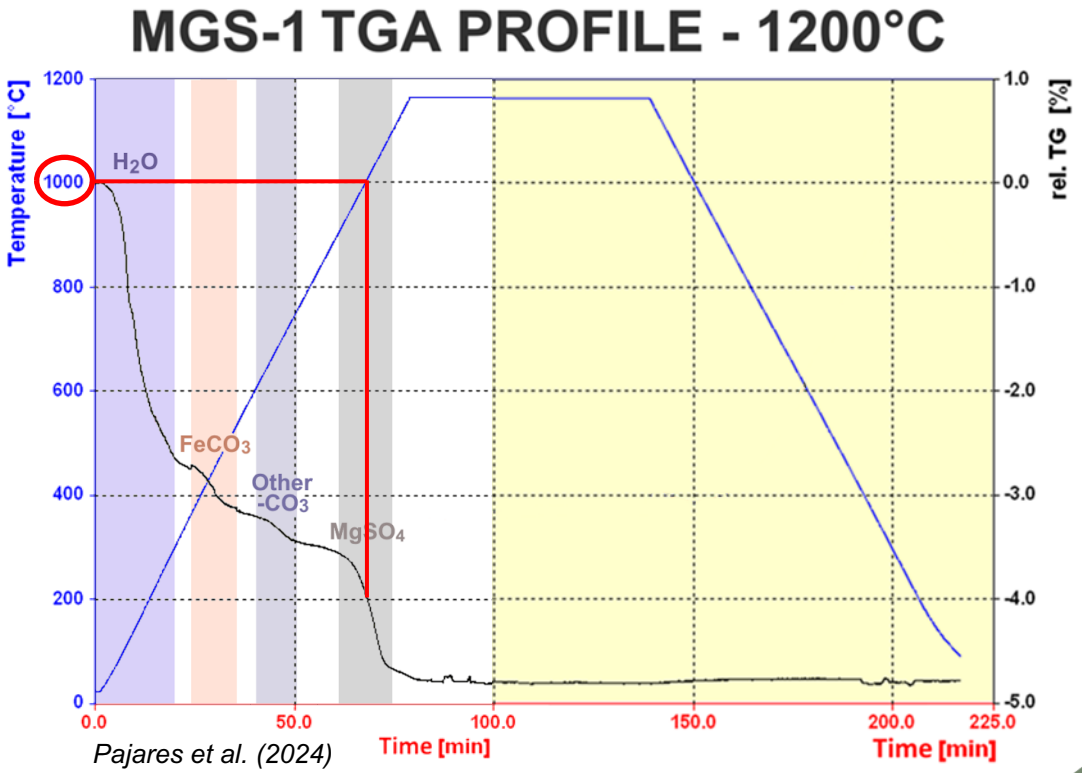
Recipe n°27: 4:1 MGS-1:H_2O, ~~binder~~, moulded, dried overnight then 70°C for 2h before sintering.

Results - Thermogravimetry

Best batch: n°27

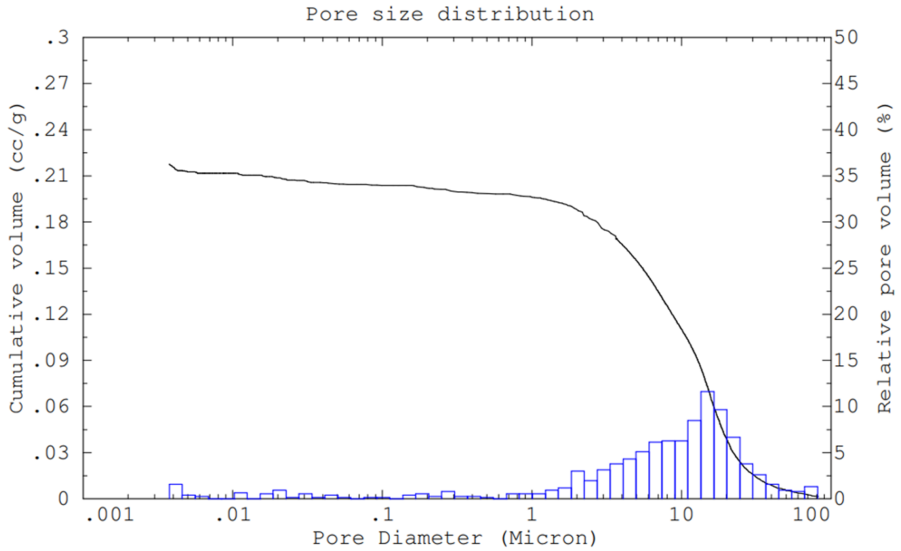
Sintering parameters:
1h ramp up (15°C.min^{-1})
1h hold at 1000°C ?
3h cooldown

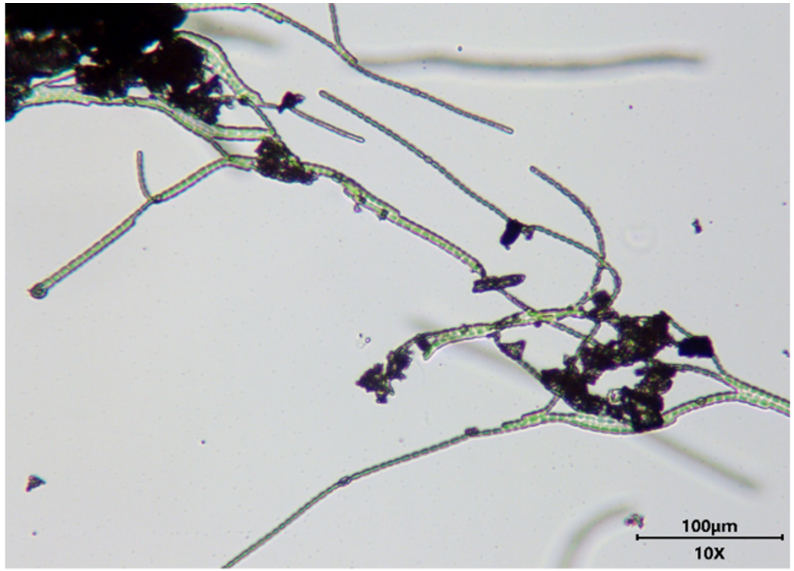
↑1000°C = glassy samples
↓1000°C = brittle samples



Pajares et al. (2024)

Results - *Mercury intrusion*





Best batch: n°27

→ **Average pellet pore size**: 15 µm (2-20 µm)

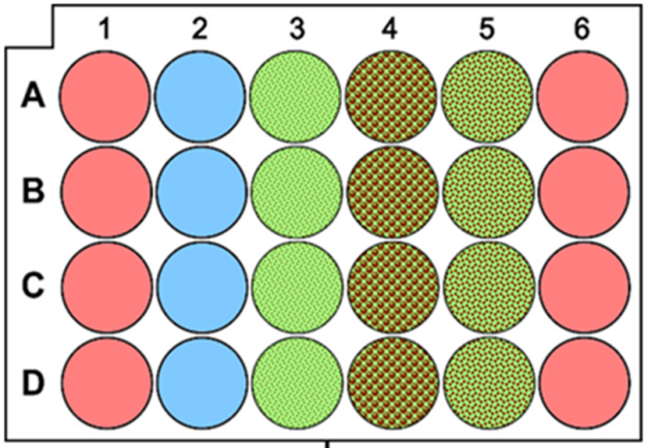
→ ***Anabaena* filament size**: 6x1000 µm

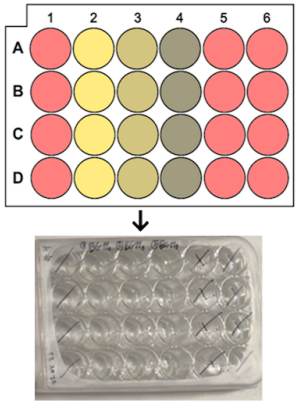
} **Penetration** of Anabaena in pores? → SEM?

→ Effect of sintering on **bioleaching**? → Multiwell plates

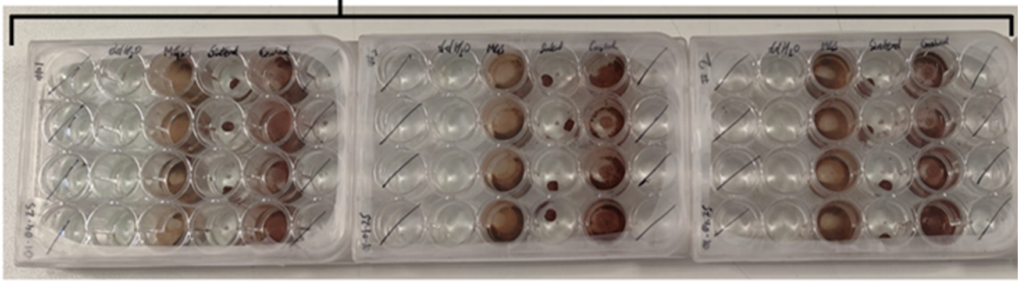
SEM = Scanning Electron Microscopy

Results - *Growth of* Anabaena *on sintered regolith*

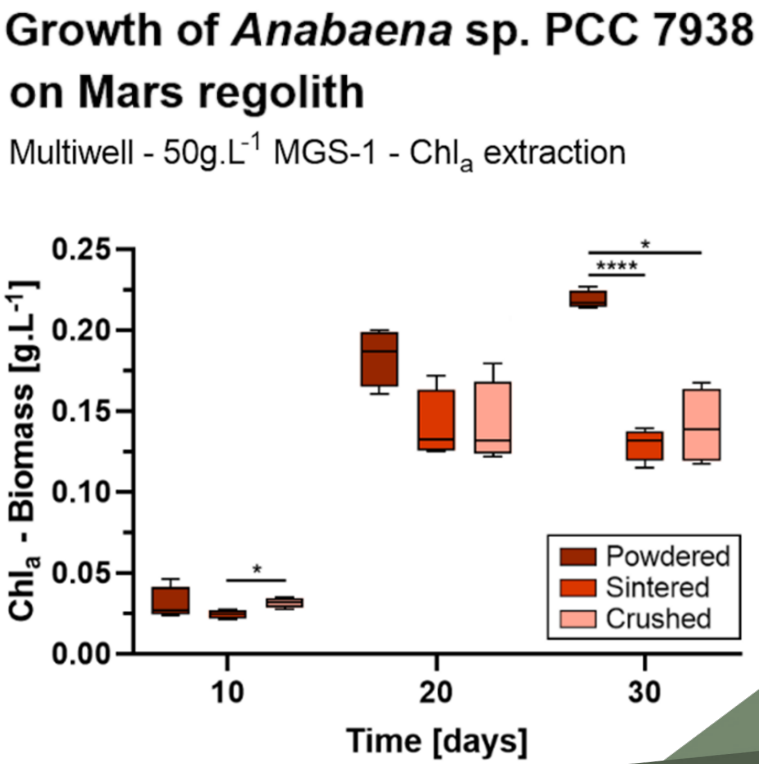




BG110 controls

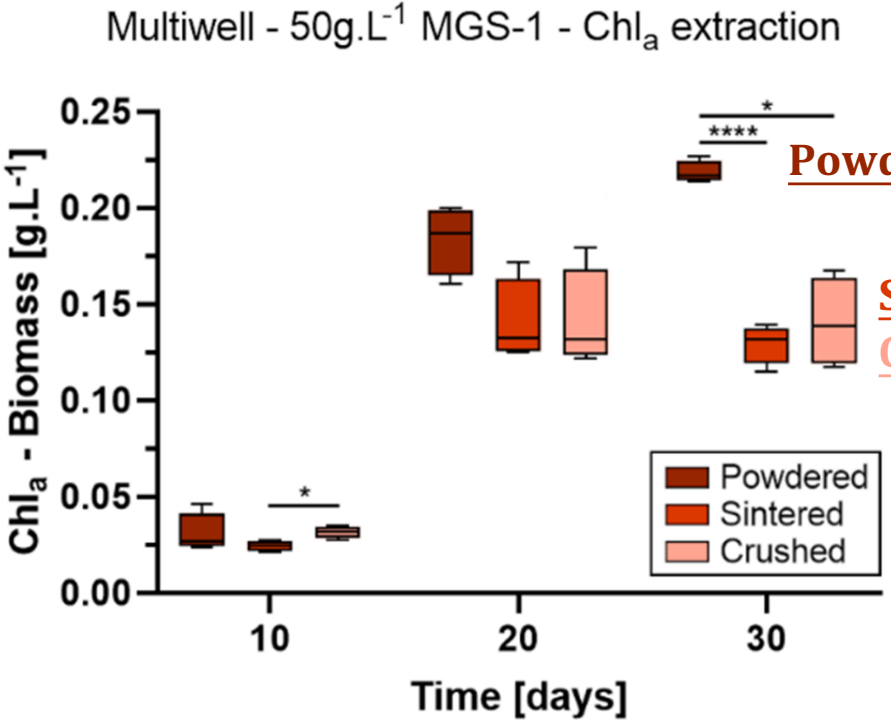


t = 10 days replicates t = 20 days replicates t = 30 days replicates



Chla = Chlorophyll alpha

Results - *Growth of* Anabaena *on sintered regolith*



Powdered: 218.5±5.0 mg.L^{-1}

Sintered: 129.5±8.8 mg.L^{-1}

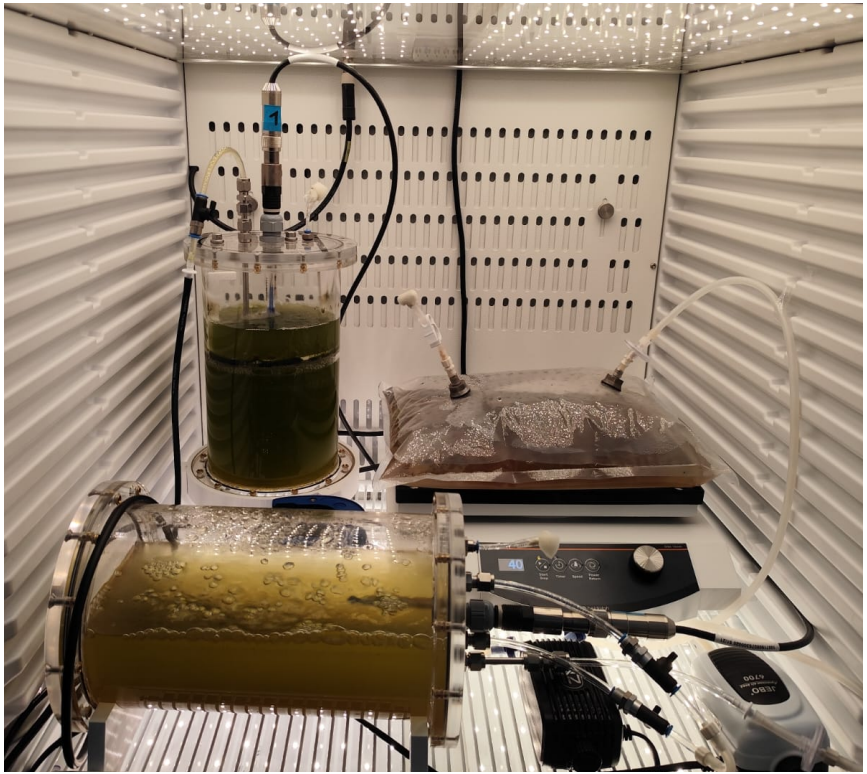
Crushed: 140.6±20.5 mg.L^{-1}

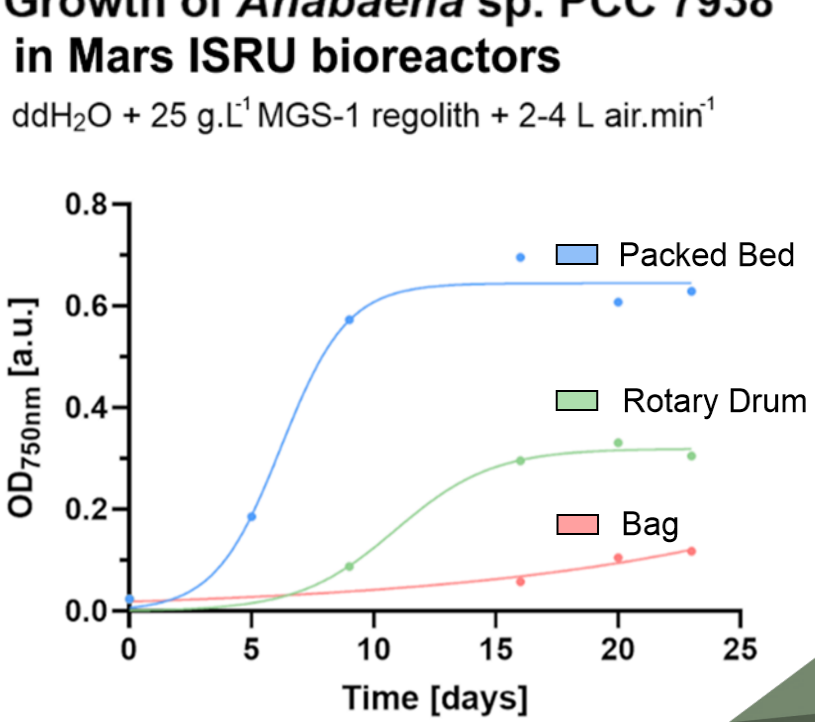
→ **Sintering impairs bioleaching**:

- Lowers specific surface area
- Alters chemical bioavailability

Chla = Chlorophyll alpha

Results - *Mars ISRU photobioreactors performance*



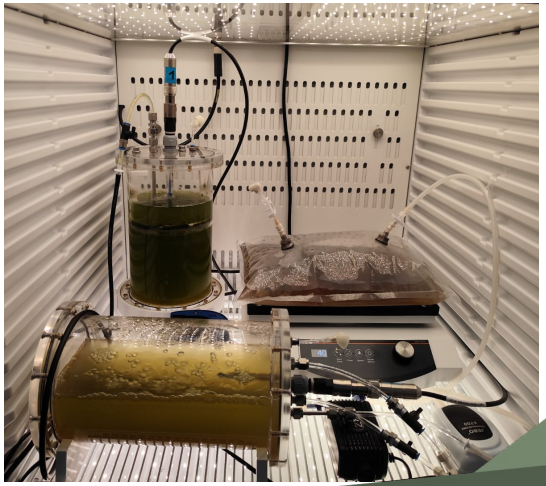


ddH₂O = double distilled water

Conclusion - *Mars ISRU bioreactors are feasible!*

1. **Mars ISRU photobioreactors:**
   - Bags: **-** complex operations (shadowing, leakage) / **+** low ESM / **-** low growth
   - Rotary drum: ~ better operations (shadowing) / ~ medium ESM / ~ medium growth
   - Packed bed: **+** best operations / **-** high ESM (sintering) / **+** high growth

→ **"Best" = ESM trade-off**… but **packed bed is promising.**



1. **Regolith agglomeration:**
   - Moulded cylindrical pellets, 4:1 MGS-1:H_2O, ~~binder~~.
   - 1h - 1000°C sintering (15°C.min^{-1}).
   - Properties: ±2.7 g.cm^{-3} density, 35.5% porosity.

→ **Sintering impairs bioleaching**… but **packed bed performed well**→ Publication in 2026.

Acknowledgements - esa academy

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Tuition fee

Experiments

REXUS/BEXUS

EuRoC

Fly your Satellite!

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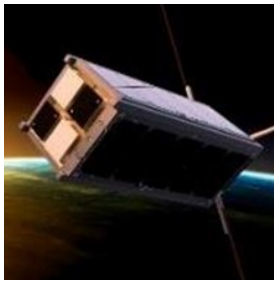
Experiments

REXUS/BEXUS

EuRoC



Fly your Satellite!

NEWSLETTER

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ESA-ESTEC | Co-Founder and Project Manag…

Thank you!

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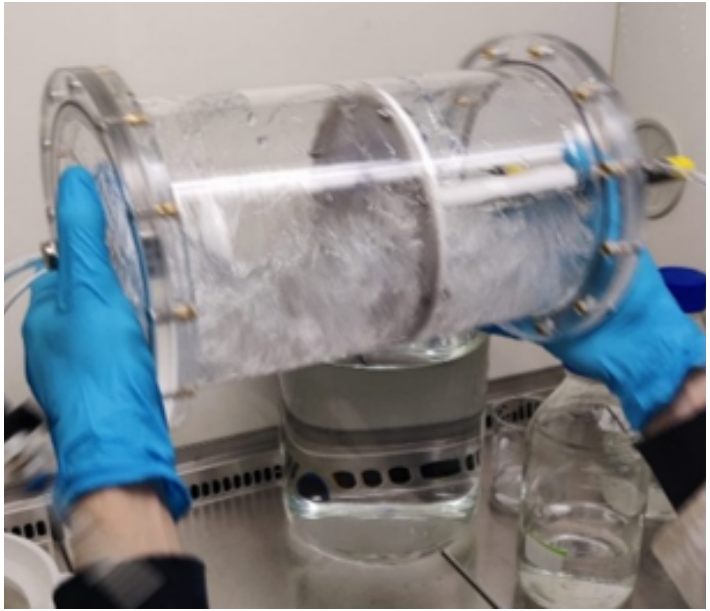
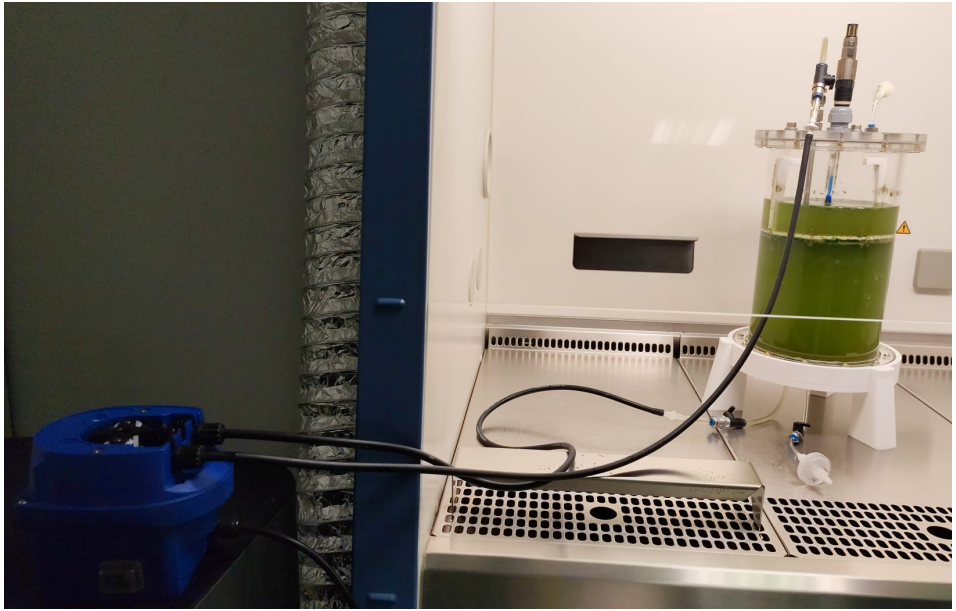
Dr. Cyprien VERSEUX (Laboratory of Applied Space Microbiology (LASM), Germany)

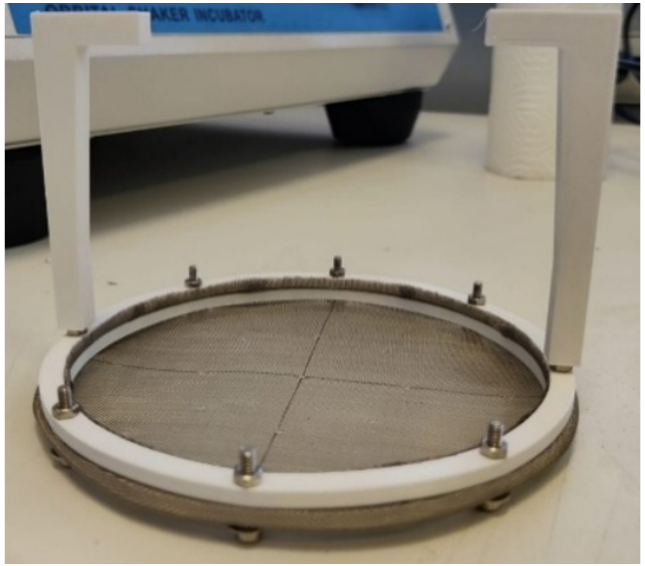
Materials & Methods - *Vessel operations*

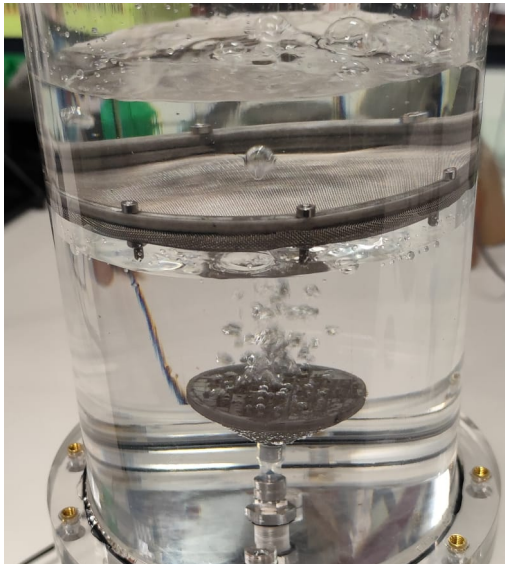




→ **Sterilisation, Inoculation & Sampling**: Rotary drum ≈ Packed-bed.
PBR: samples taken via recirculation loop (biosafety cabinet).

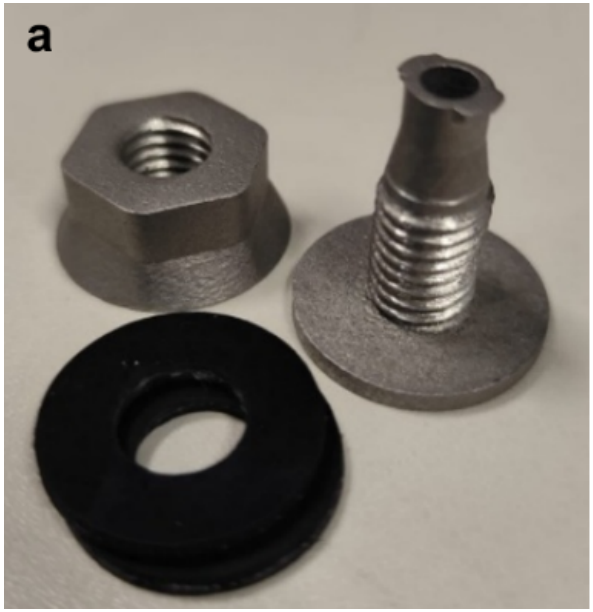
Materials & Methods - *Packed-bed bioreactor*





→ **Wire mesh sieve** for sintered pellets support.
→ **PETG** sparger & sieve (>< bacterial degradation).

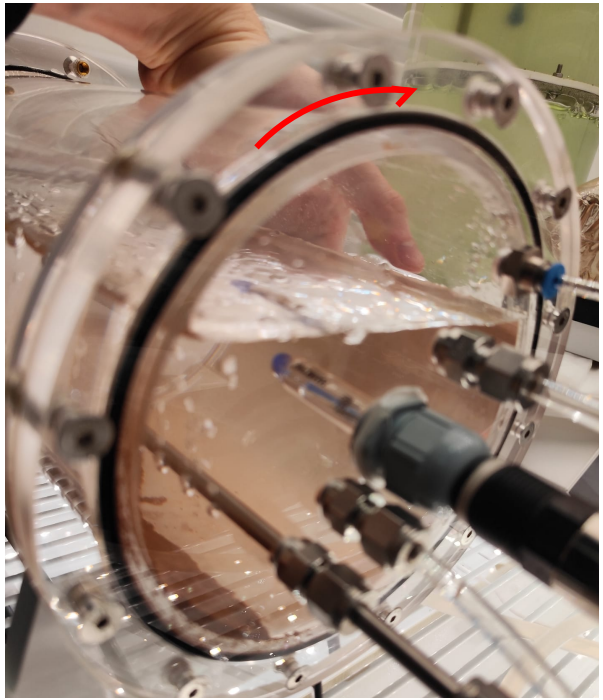
Materials & Methods - Bag bioreactor sterilisation

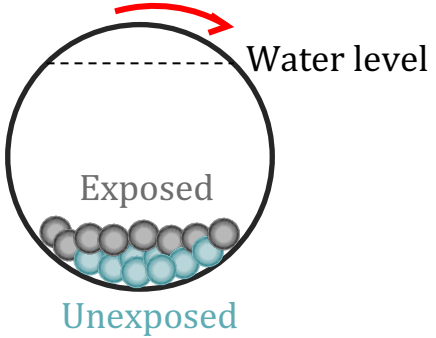


→ **Heat sterilisation**: aluminum *Luer Lock* inlets + PP bags = autoclavable.
→ Regolith autoclaved in the bag directly.

Materials & Methods - Rotary drum bioreactor

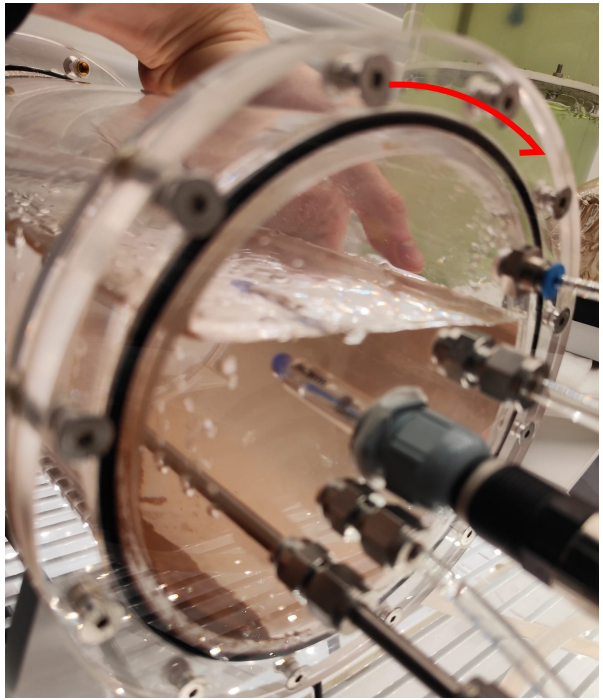
→ **Swiveling**: in-process "renewal" of regolith surface.

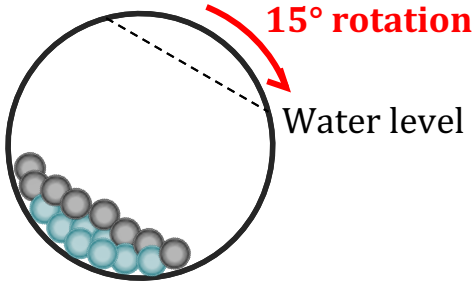




Materials & Methods - Rotary drum bioreactor

→ **Swiveling**: in-process "renewal" of regolith surface.





Materials & Methods - Rotary drum bioreactor

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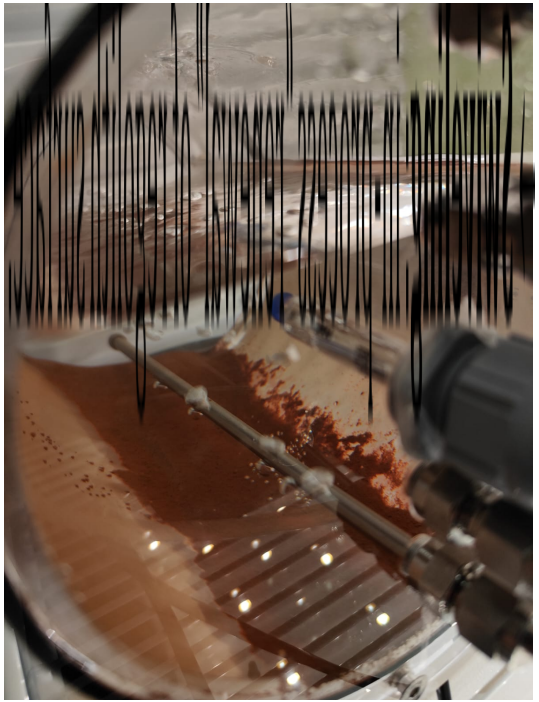
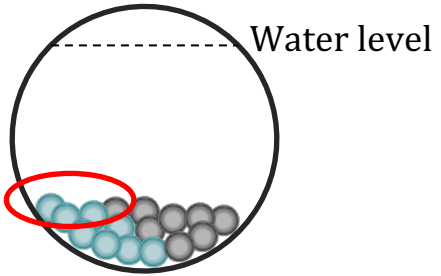
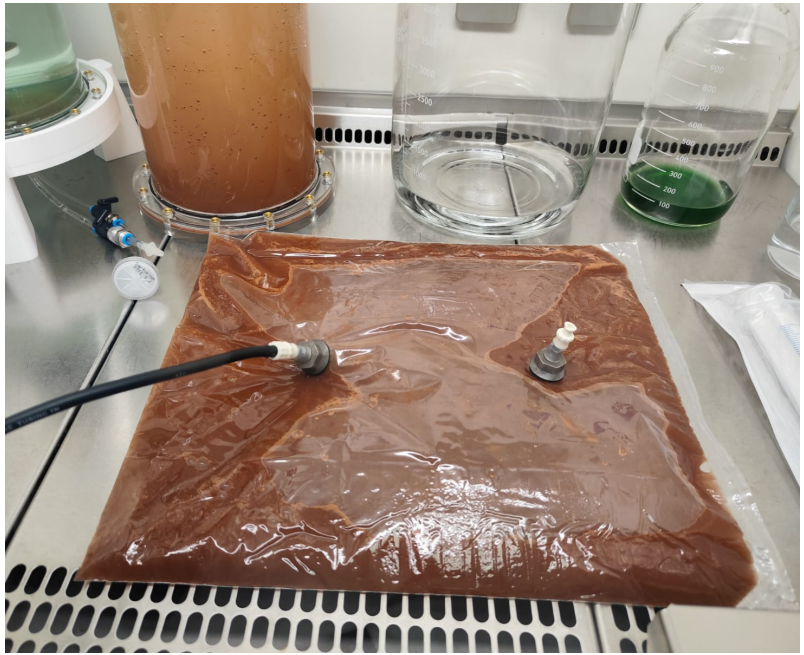
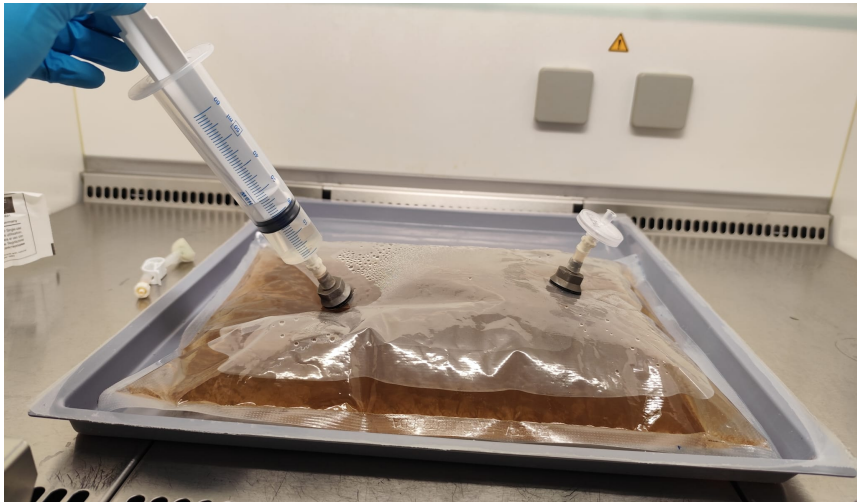




Materials & Methods - Bag bioreactor operations

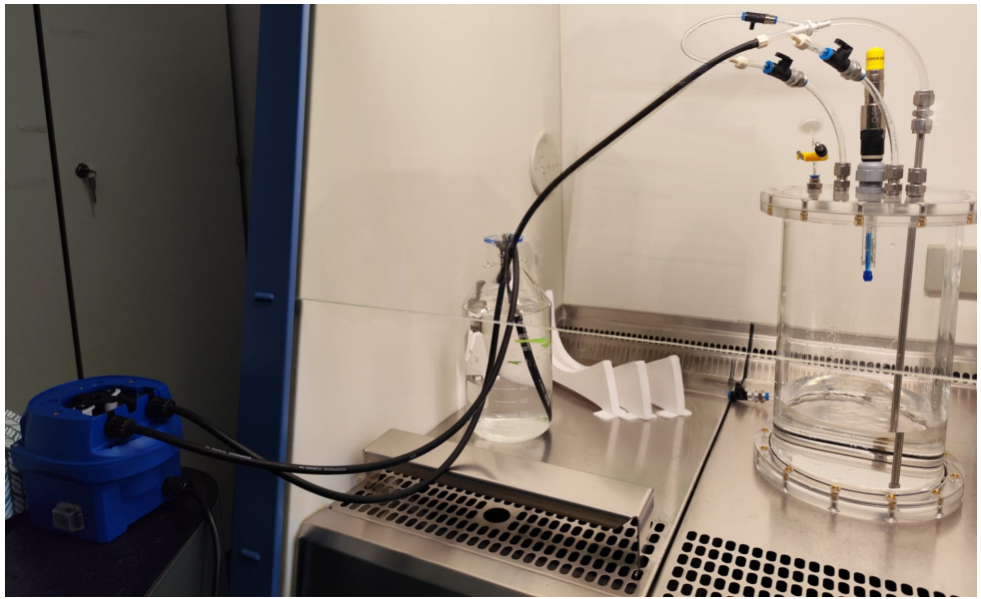


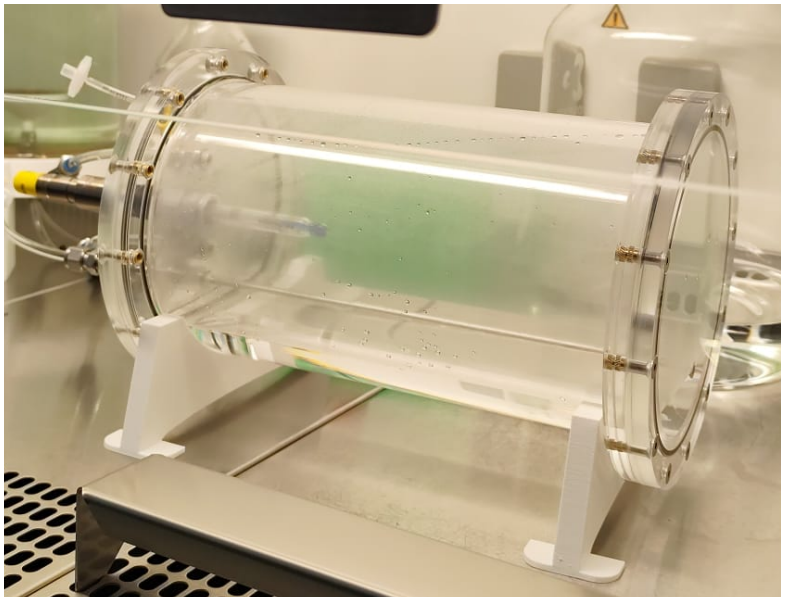


→ **Sampling**: In the biosafety cabinet, via the luer lock aluminium inlet.

→ **Inoculation**: Water + *Anabaena*, via the inlet, using a peristaltic pump.

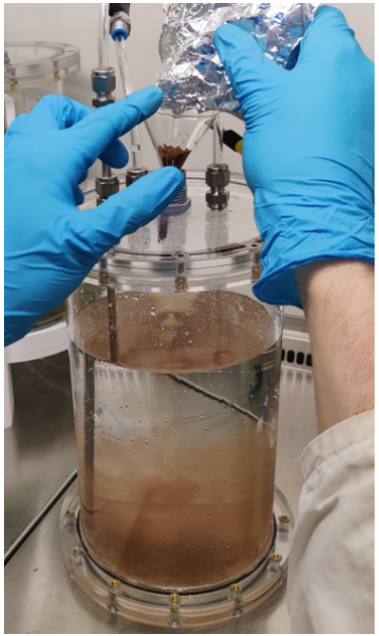
Materials & Methods - Rotary drum sterilisation

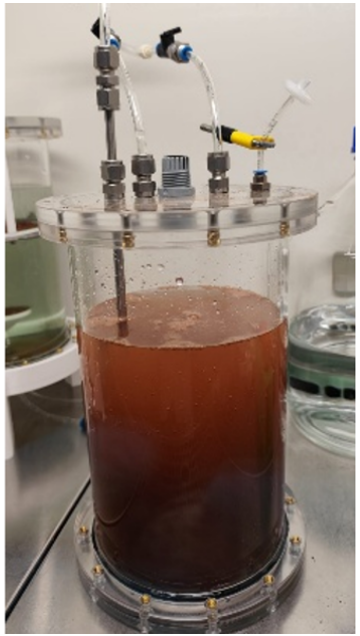


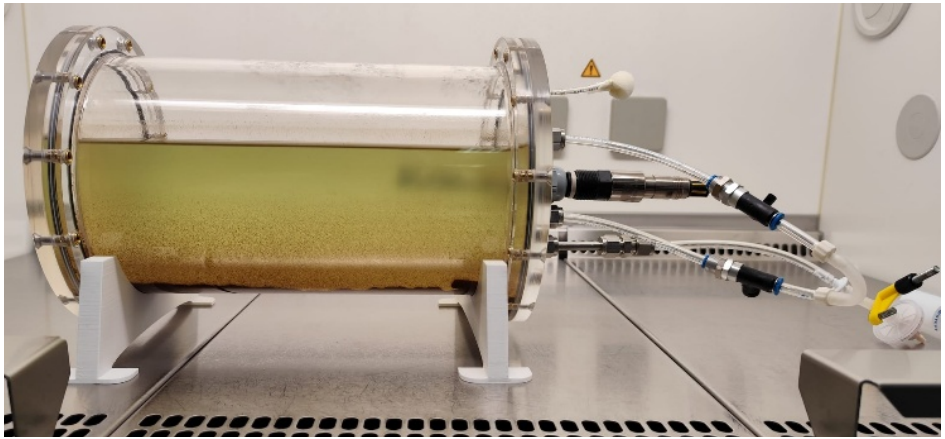


→ **Chemical sterilisation**: 10% H_2O_2 + 5x ddH_2O rinsing with peristaltic pump.

Materials & Methods - Rotary drum operations



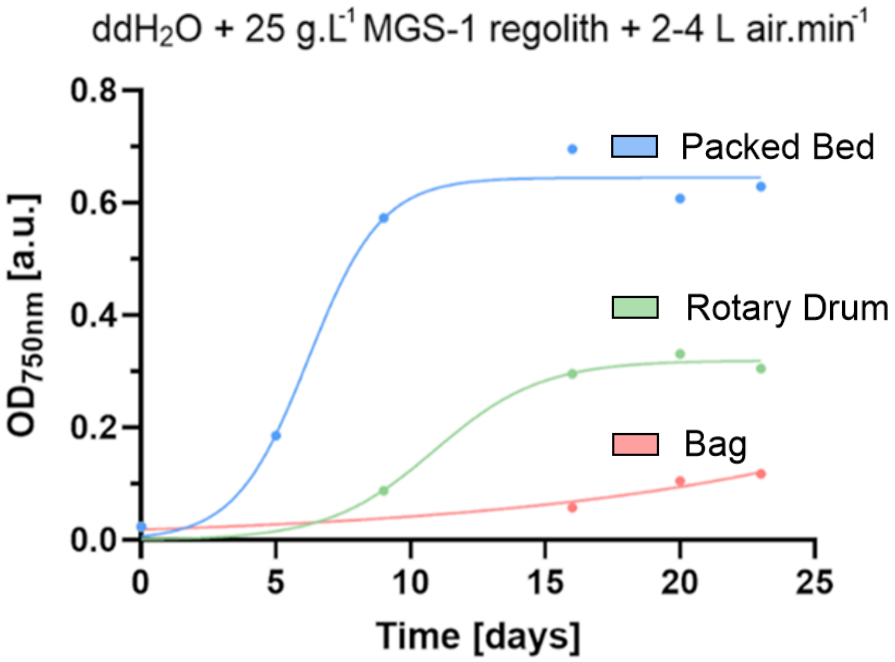




→ **Sampling**: In the biosafety cabinet, via the liquid outlet with a stopcock valve.

→ **Inoculation**: Regolith powder and inocula via the pH probe fitting, Water via the liquid inlet, using a peristaltic pump.

Results - *Mars ISRU photobioreactors performance*



Packed bed:
- Productivity: 4.55±0.06 mg.L^{-1}.d^{-1}
- ↓ shadowing, easy regolith removal.
- High ESM (sintering, acrylic).

Rotary Drum:
- Productivity: 1.34±0.03 mg.L^{-1}.d^{-1}
- ~ shadowing, hard regolith removal.
- Medium ESM (acrylic).

Bag bioreactor:
- Productivity: 1.27±0.04 mg.L^{-1}.d^{-1}
- ~ shadowing, hard regolith removal.
- Low ESM, but leakages.

Materials & Methods - ATMOS and Anabaena

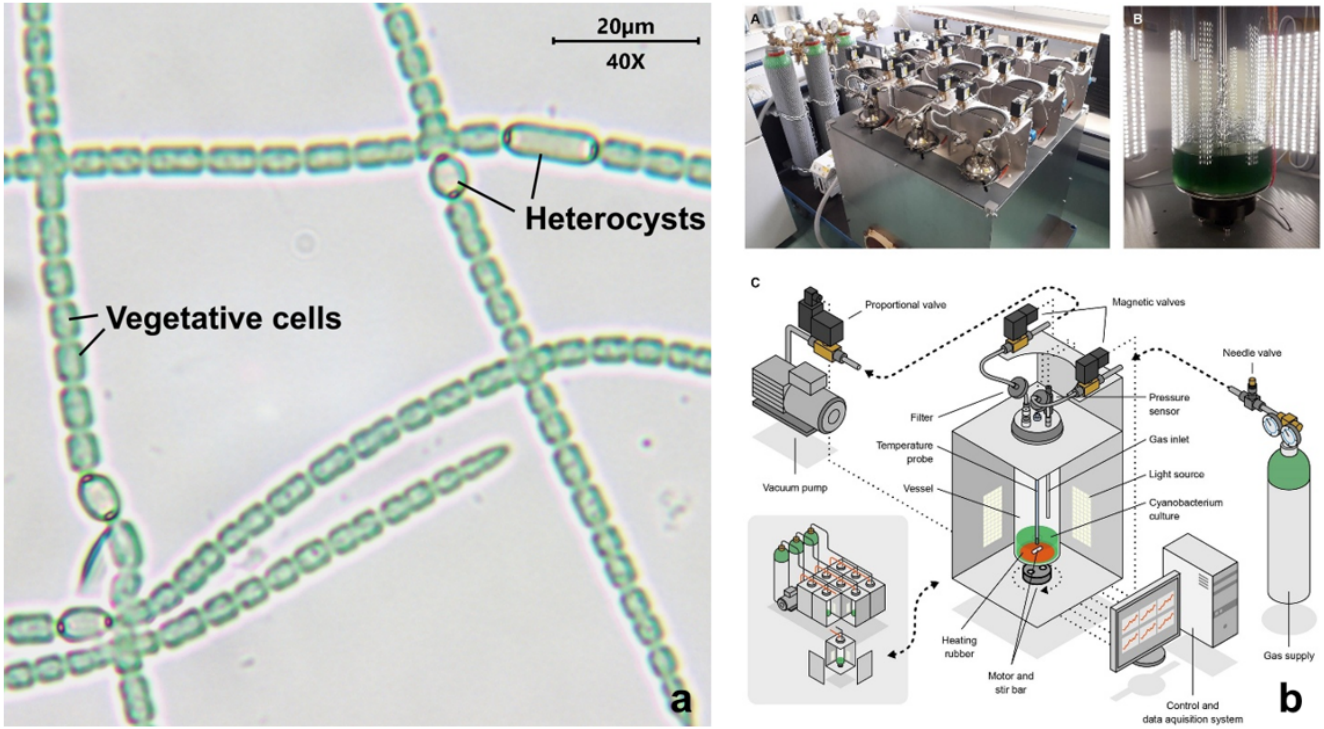
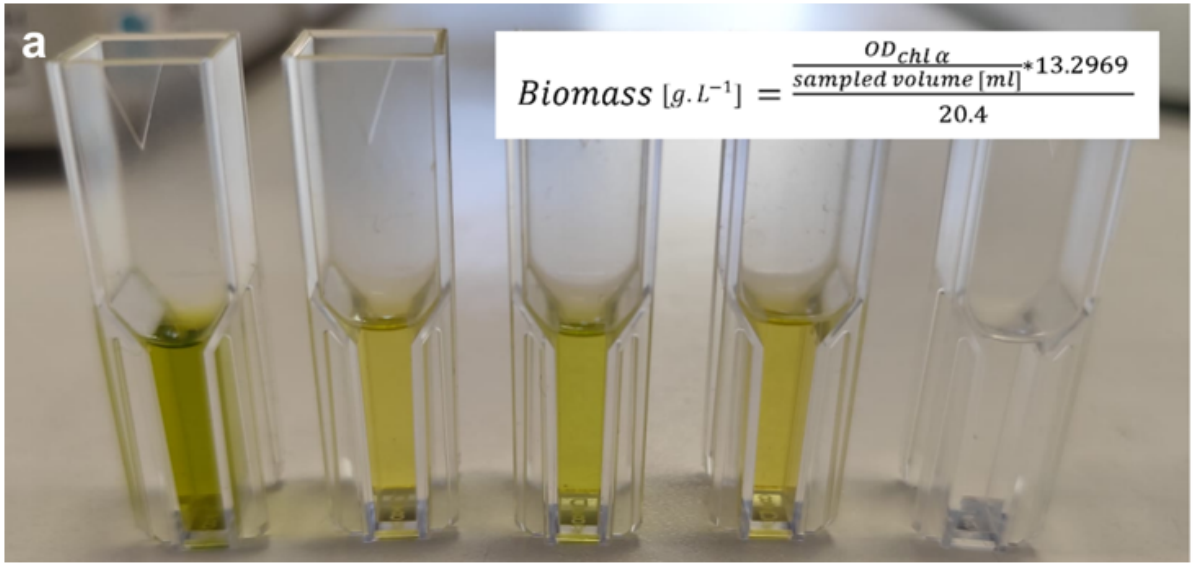


***Figure 6**: **a)** Vegetative cells and heterocyst of* Anabaena *sp. PCC 7938 under an optical microscope (40x). **b)** Low-pressure photobioreactor (ATMOS) developed for Mars ISRU research using* Anabaena *sp. PCC 7938. Taken from Verseux et al. (2021)[81].*

A.

Materials & Methods - Growth assessment



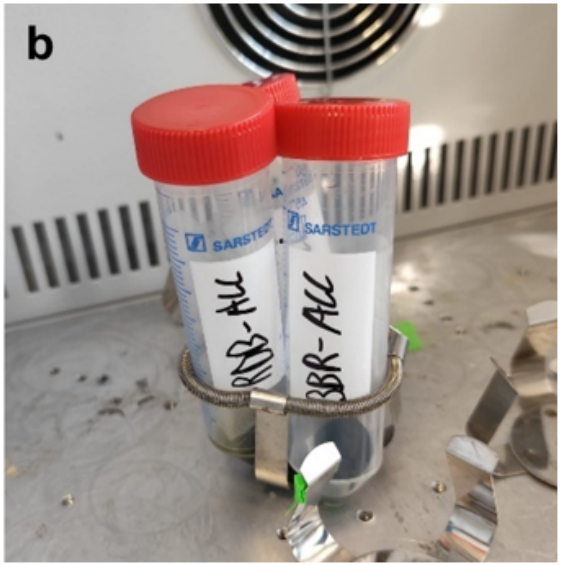


***Figure 25**: **a)** Ethanol-extracted chlorophyll α in spectrophotometry cuvettes with Chlorophyll α to biomass conversion equation. **b)** Tared tubes drying at 70°C for dry weight average productivity assessment.*

B.

Materials & Methods - Luer Lock *inlet and nut*

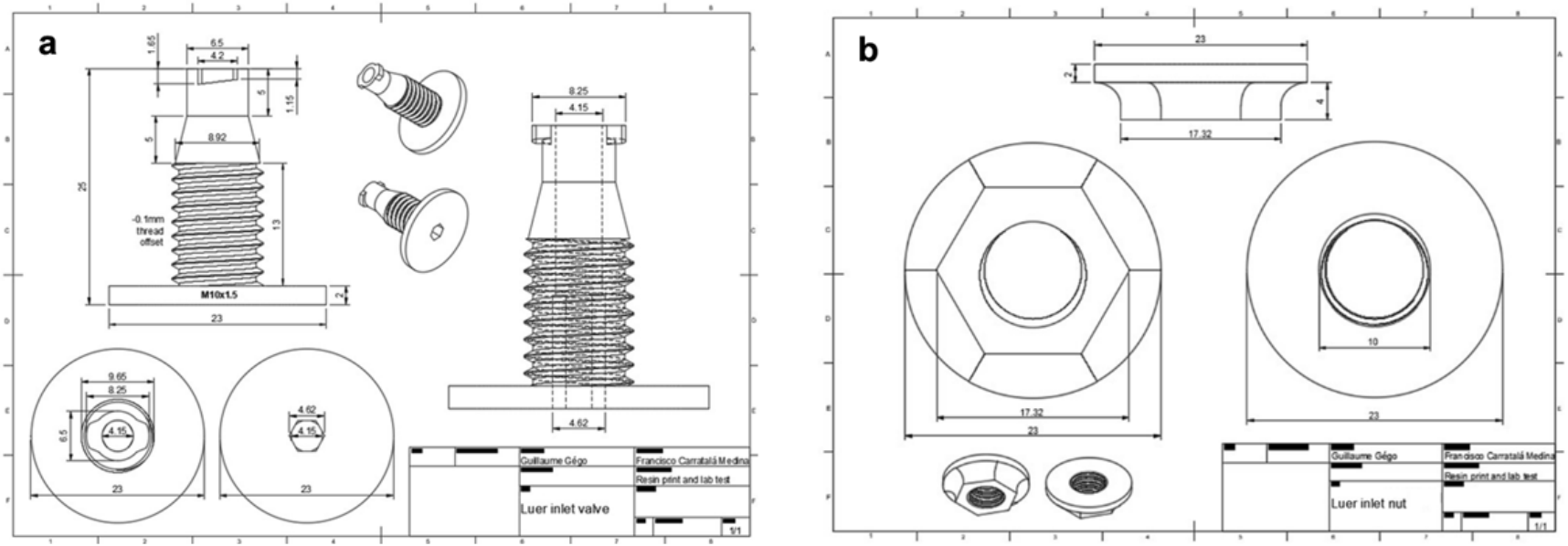


Figure 12: *Schematic diagrams of* ***(a)*** *the Luer Lock inlet and* ***(b)*** *the first iteration of the inlet nut. Made with Fusion360*[89].

Results - Scaling-up with bag photobioreactors

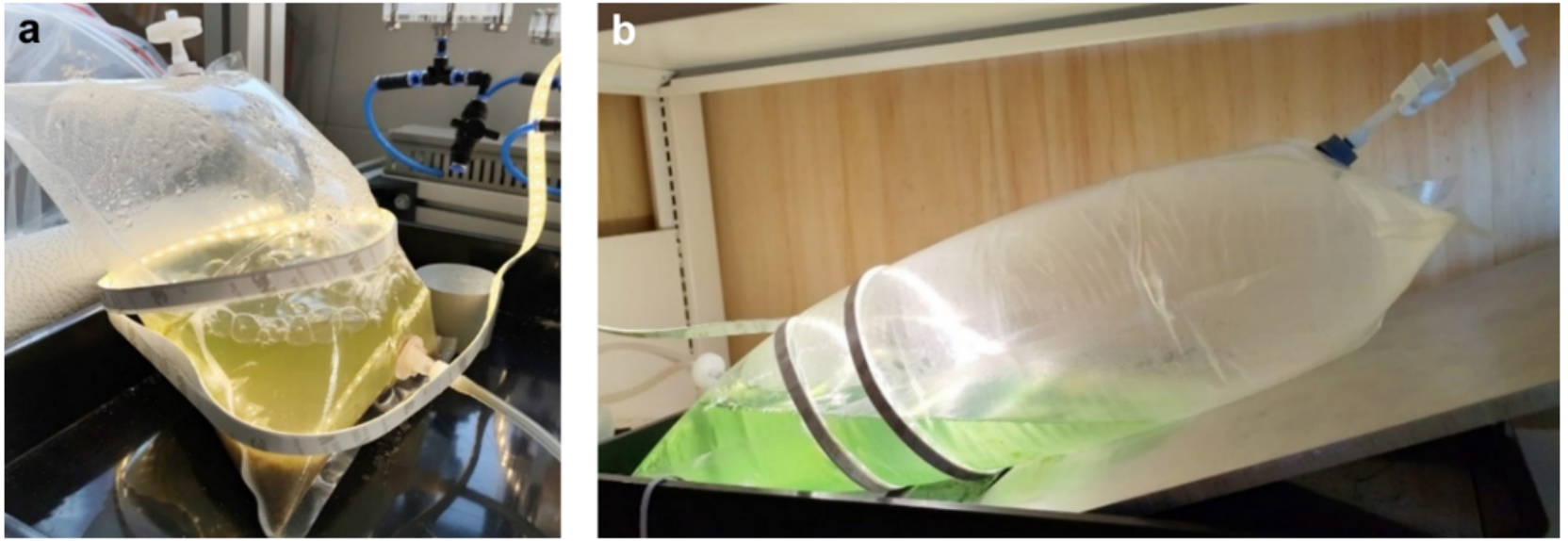


*Figure 13: Tests using clear resin **(a)** and grey resin **(b)** inlets for regolith processing (0.5 L) and biomass production (5 L).*

Materials & Methods - *Inoculation process*

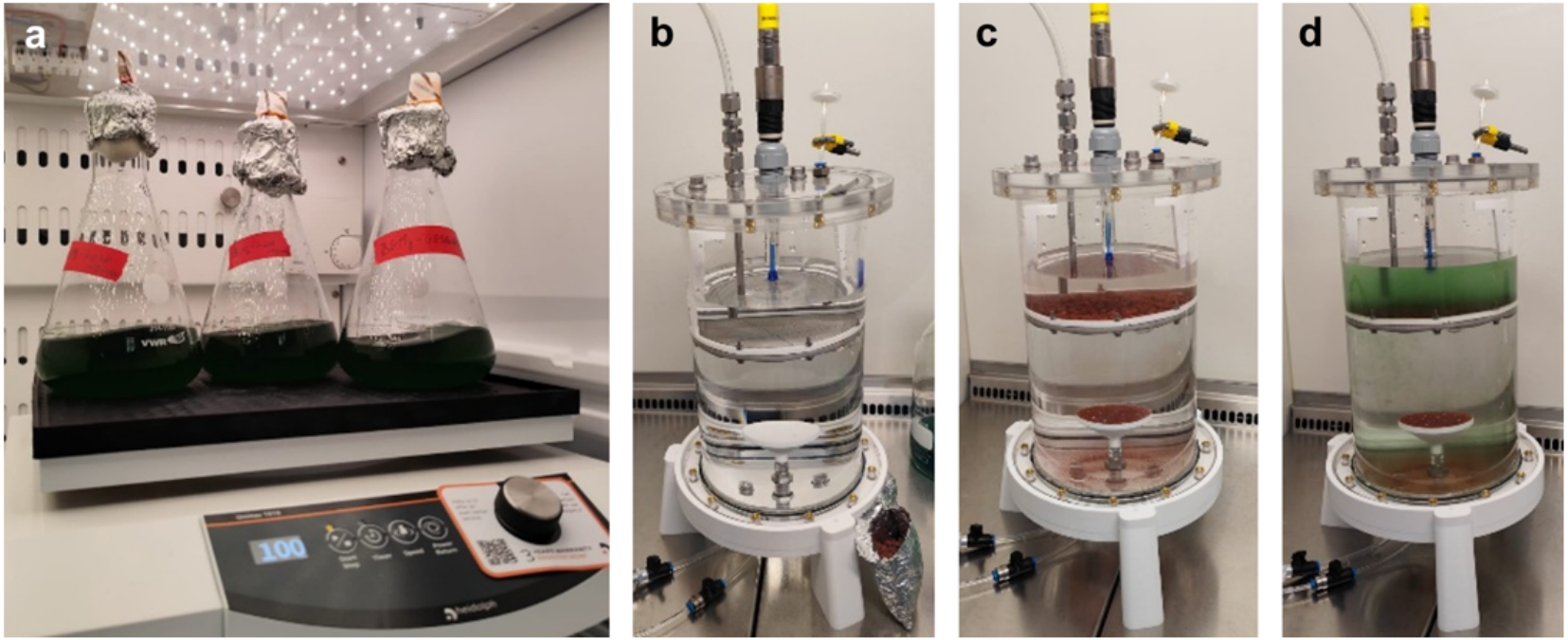


***Figure 21**: **a)** Three 250 mL inocula of* Anabaena *sp. PCC 7938. **b)** Packed bed bioreactor filled with 2.9 L of ddH_2O, then **(c)** with 75 g of sintered regolith pellets and **(d)** with 100 mL of inoculum at an OD of 0.71 for a final OD of 0.0237.*

Materials & Methods - Bioreactor sampling

***Figure 41**: Sampling operations for the **(a)** bag, **(b)** rotary drum, and **(c)** packed bed bioreactors in the biosafety cabinet.*

Materials & Methods - Regolith handling

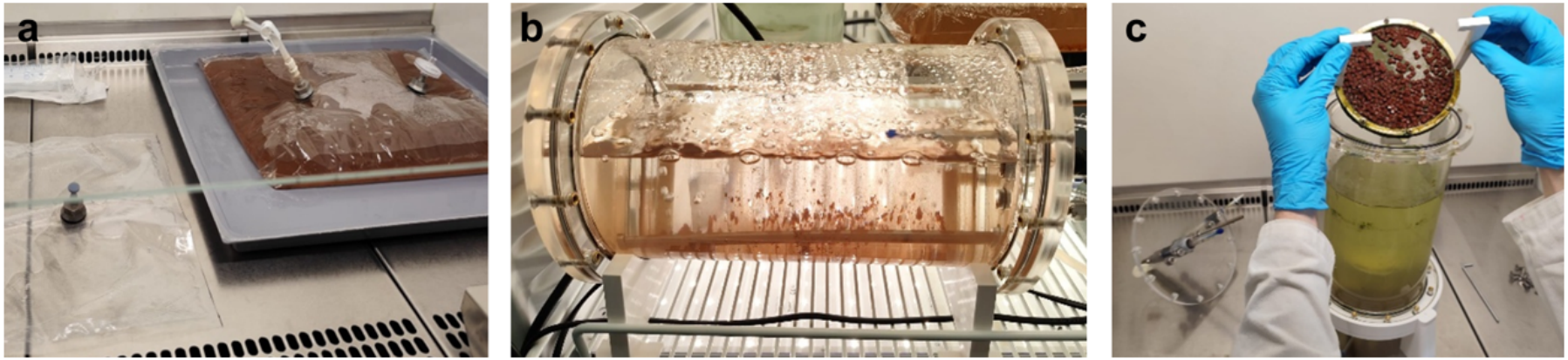


Figure 44: *Regolith handling in the bioreactors.* ***a)*** *Bag transfer difficulties.* ***b)*** *Clearness of the rotary drum due to the biomass having been dragged down by the regolith during inoculation.* ***c)*** *Pellets handling with the sieve of the packed bed.*

Materials & Methods - Regolith subsystem

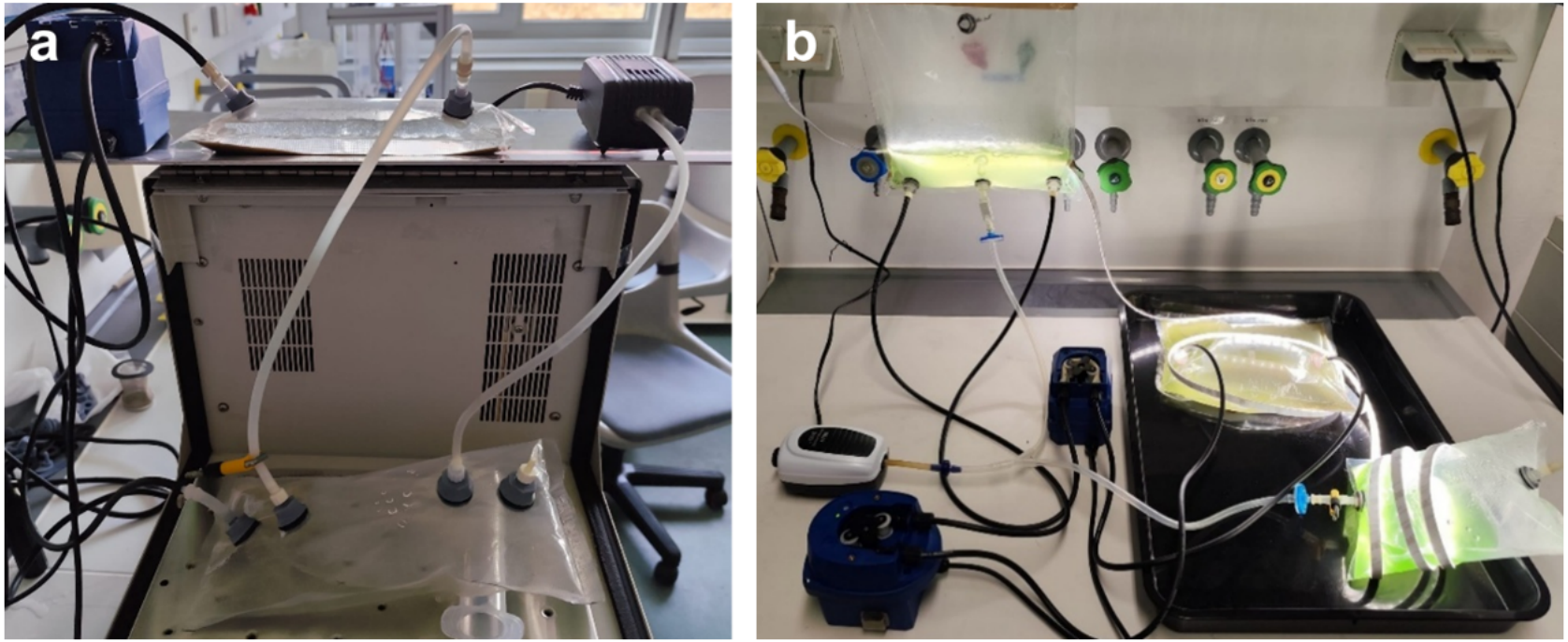


***Figure 42**: a) External regolith sub-system bag bioreactor prototype. b) Feasibility test with Anabaena and powdered MGS-1*

Materials & Methods - MGS-1 composition

a

Oxide	RN bulk[1]	Calc. MGS-1[2]	RN amorph.[3]	Calc. MGS-1 amorph.[2]	MGS-1 prototype
SiO_2	43.0	48.3	34.2	47.0	50.8
TiO_2	1.2	0.2	2.1	0.6	0.3
Al_2O_3	9.4	9.5	5.4	7.0	8.9
Cr_2O_3	0.5	0.1	1.4	0.2	0.1
FeO_T	19.2	16.9	23.0	21.0	13.3
MnO	0.4	0.1	1.2	0.2	0.1
MgO	8.7	12.1	4.0	9.9	16.7
CaO	7.3	6.7	4.4	4.5	3.7
Na_2O	2.7	2.6	3.3	1.0	3.4
K_2O	0.5	0.1	1.4	0.3	0.3
P_2O_5	1.0	0.2	2.7	0.6	0.4
SO_3	5.5	3.2	13.9	7.7	2.1
Cl	0.7	0.0	2.0	0.0	–
SUM	100.1	100.0	99.9	100.0	100.0

b

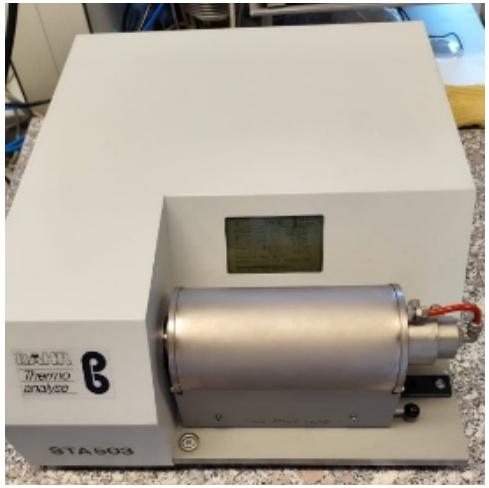
Component	MGS-1 (wt.%)	Rocknest[1'] crystalline + amorphous
Crystalline phases	65.0%	65.0%
Plagioclase	27.1	26.3
Pyroxene	20.3	19.7
Olivine	13.7	13.3
Magnetite	1.9	1.8
Hematite	1.1	1.0
Anhydrite	0.9	0.9
Quartz	0.0	0.8
Ilmenite	0.0	0.9
Amorphous phases	35.0%	35.0%
Basaltic Glass	22.9	–
Hydrated Silica	5.0	–
Mg-sulfate	4.0	–
Ferrihydrite	1.7	–
Fe-carbonate	1.4	–
Sum	100.0	100.0%

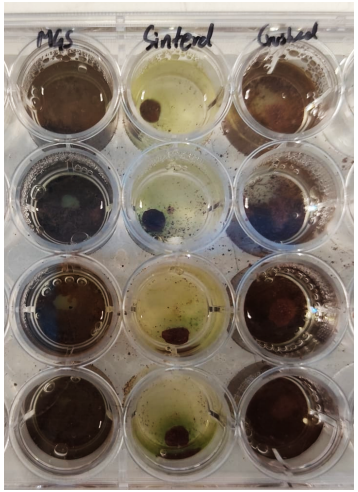
***Table 1**: MGS-1 composition. **a)** Bulk major element oxide compositions (in wt%) for Rocknest (RN) soil and the MGS-1 Martian regolith simulant, including both bulk and amorphous phases, as well as the MGS-1 prototype composition. Values for the simulant are based on calculated mineral recipes and idealised mineral formulas derived from silicate crystal chemistries reported in the literature: (1) from Table 6, Achilles et al. (2017)[28]. (2) calculated in Cannon et al. (2019)[26]. (3) from Table 8, Achilles et al. (2017)[28]. **b)** Mineral recipe for the Rocknest soil and the MGS-1 standard. (1') from Table 1, Achilles et al. (2017)[28]. Table 1a and 1b are adapted from Cannon et al. (2019)[26].*

Materials & Methods - Pellet moulding techniques

Figure 26: *MGS-1 regolith pellets moulding techniques.* ***a)*** *Syringe pressed,* ***(b)*** *cylinder pressed,* ***(c)*** *mould pressed.*

Materials & Methods - MGS-1 pellets analyses





- **Helium pycnometry & Water intrusion**: skeletal density & porosity
- **Thermogravimetry**: gas release during sintering (**?**°C & ?h)
- **Mercury intrusion**: pore size distribution
- **Multiwell:** effect of sintering on bioleaching efficiency

Materials & Methods - Mercury intrusion formulas

In capillaries, the Laplace equation (*Eq. 3*) shows that the pressure on the concave side of a curved surface (p_i) is always greater than the pressure on the convex side (p_a), proportionally to the radius of the capillary (r), and the surface tension of the liquid (γ).

$$dw = \gamma d\sigma \quad [Eq.\ 2] \qquad p_i = p_a + 2.\frac{\gamma}{r} \quad [Eq.\ 3]$$

The rising of wet liquids in capillaries due to surface tension is called the capillary force. As a non-wetting liquid, mercury atoms are attracted more strongly to one another than to the inner side of capillary. As a result, the liquid surface bends upward, meaning that the concave side and its associated overpressure) faces towards the pore opening (*Fig. 29*).

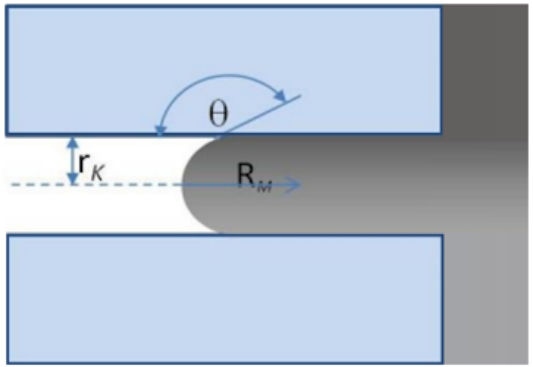


Figure 29: *Mercury intrusion in a capillary, with: R_M the radius of curvature, r_K the capillary radius and θ the contact angle.*

Materials & Methods - Water intrusion process

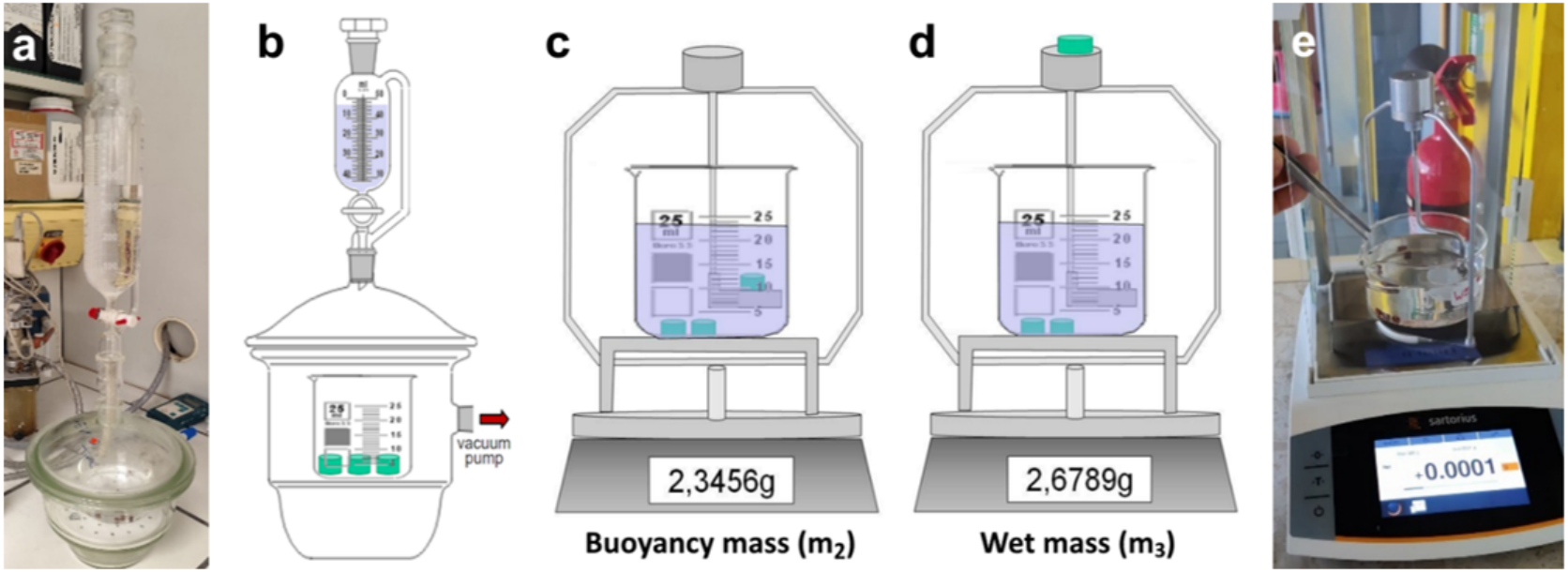


Figure 31: ***a)*** *Water intrusion test setup and* ***b)*** *schematic view.* ***c)*** *Buoyancy (m_2) and* ***(d)*** *wet mass (m_3) determination method. The pellet (in green) is placed on a suspended platform inside the water for m_2, and on top of the metal contraption for m_3. Samples are slightly wiped before m_3 weighing. The dry mass (m_1) is measured beforehand on the same precision balance. Analysis performed by master student Sahil Bhatia.*

Materials & Methods - Water intrusion formulas

Water intrusion works on the basis that a porous structure gains weight when its open pores are filled with liquid. To calculate this open porosity, three masses must be weighed: the dry mass (m_1), the buoyancy mass (m_2, *Fig. 31c*) and the wet mass (m_3, *Fig. 31d)*. Then, multiple properties can be determined from these masses: the open porosity (*Eq. 4*), which takes into account all the pores water can seep into; the apparent volume (*Eq. 5*), which is the total volume taken up by the pellet without the volume of the pores; the bulk density (*Eq. 6*), which is the ratio of mass to the total volume of the pellet, including the pores; and other less relevant parameters.

$$P_{op} = \frac{(m_3 - m_1)}{(m_3 - m_2)} \quad [Eq.\ 4] \qquad V_{sch} = \frac{(m_1 - m_2)}{\rho_w} \quad [Eq.\ 5] \qquad \rho_{roh} = \rho_w \frac{m_1}{(m_3 - m_2)} \quad [Eq.\ 6]$$

Materials & Methods - MGS-1 pellets degradation

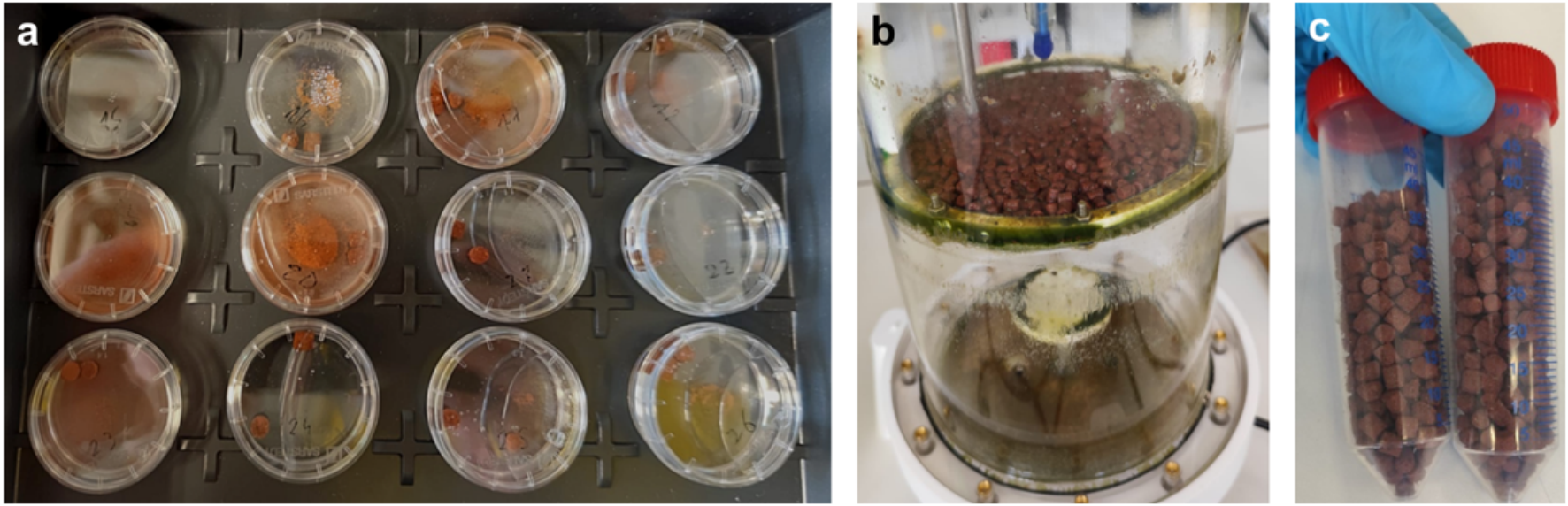


***Figure 38**: Effect of water on sintered pellets. **a)** Water dissolution test on duplicate samples from batches 15 to 26 (left to right, top to bottom) after a week of agitation. **b)** Aspect of pellets (batch 27) after 23 days of operation in the packed bed. **c)** State of the pellets used in the packed bed for 23 days (batch 27), after thorough rinsing and drying at 70°C.*

Materials & Methods - *Multiwell experiment*

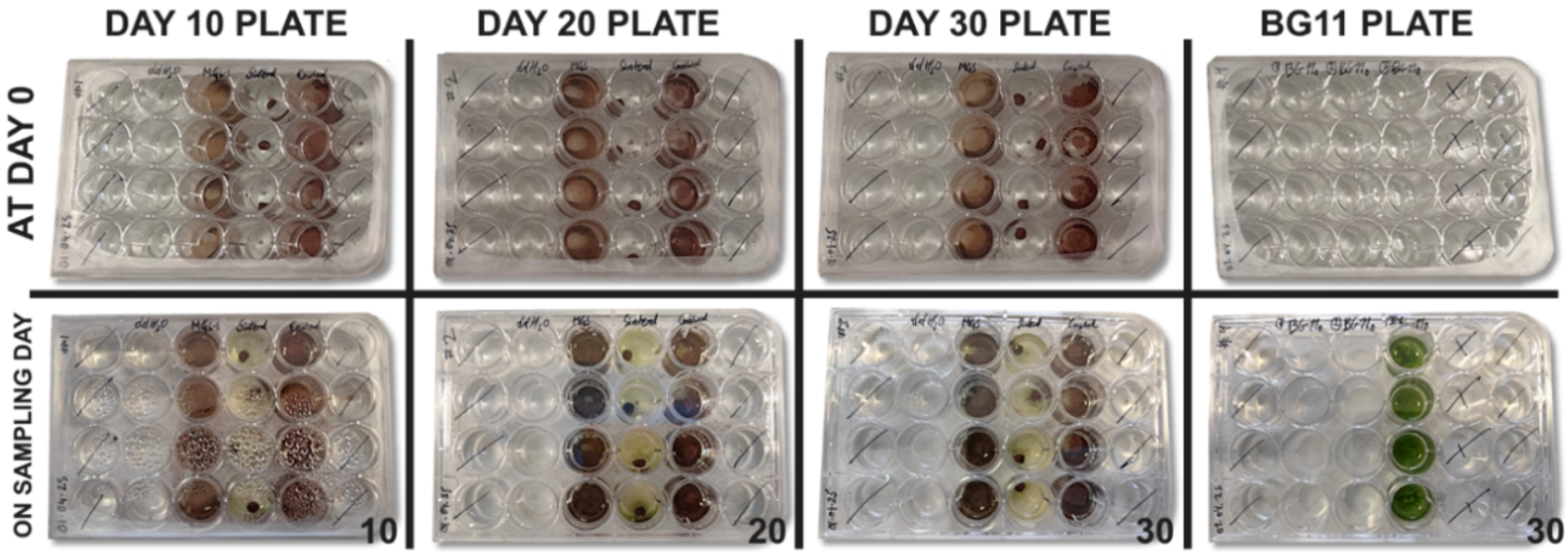


Figure 39: *Composite image of the multiwell experiment. Top row: day 10, 20, 30 and BG11 positive control plates at day 0. Bottom row: same day 10, 20, 30 and BG11 plates, but on sampling day (BG11 at day 30, two previous columns sampled on day 10 and 20). Performed in four biological replicates (four rows per plate).*

Results - MGS-1 thermogravimetric profile analysis

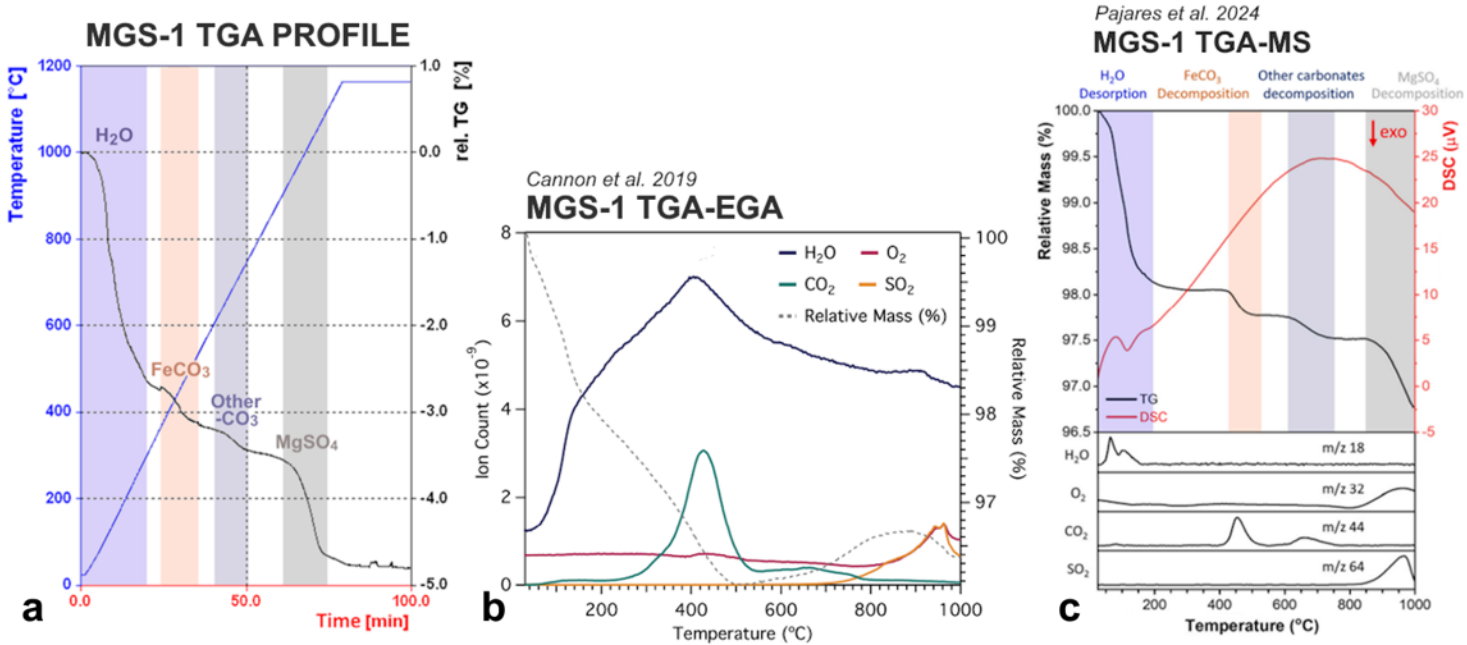


***Figure 35**: **a)** MGS-1 TGA, 1200°C, 60 min hold, 15°C/min (STA 503AHR, BÄHR-Thermoanalyse), with indications of the nature of the compounds affected by temperature during the four TG dips. **b)** MGS-1 TGA-Evolved Gas Analysis (EGA) performed by Cannon et al. (2019)[26]. **c)** MGS-1 TGA-Mass Spectrometry (MS) analysis performed by Pajares et al. (2024)[96] showing the same TG dips. The nature of the gases released was determined by subsequent MS analysis.*

Results - *Growth of* Anabaena *on sintered regolith*

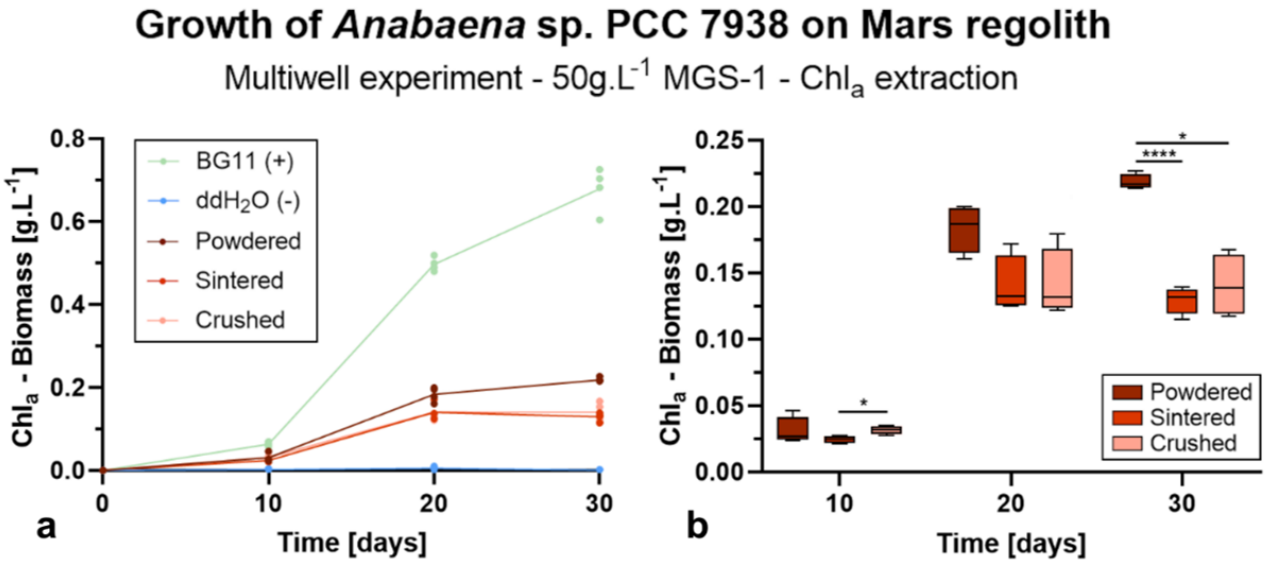


Figure 40: *Growth curves of Anabaena sp. PCC 7938 on powdered, sintered and crushed MGS-1 regolith simulant.* ***a)*** *Biomass evolution of the BG11₀ (positive control), ddH₂O (negative control), powdered, sintered and crushed wells assessed from chlorophyll α extraction over 10, 20 and 30 days.* ***b)*** *Boxplot of the biomass of the three states of regolith, with analysis of significance determined using a two-way ANOVA and a Tukey post-hoc test (see Annex D.1). The α threshold was set at 5% (p-value > 0.05); * = significant at p-value < 0.05; **** = significant at p-value < 0.0001. All others = not significant. Data available in four biological replicates, see Annex F. Figure made using GraphPad Prism 8.0.1.*

Results - Mars ISRU photobioreactors performance

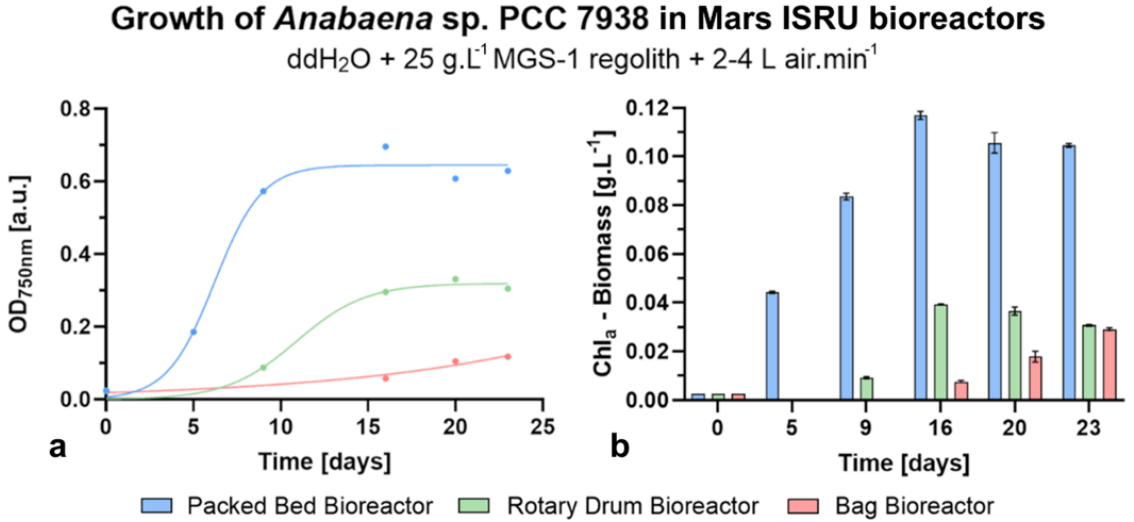


***Figure 47**: Growth of* Anabaena *sp. PCC 7938 in ddH_2O with 25 $g.L^{-1}$ of MGS and 2 $L.min^{-1}$ (until day 10) to 4 $L.min^{-1}$ (until day 23) of air. **a)** Direct OD measurement (section 3.2.5). The OD data was fitted to the GraphPad built-in logistic growth model using a least squares nonlinear regression. Packed bed bioreactor: YM = 0.6447; Y0 = 0.006552; k = 0.7376; Xint = 1.356; R^2 = 0.9881. Rotary drum bioreactor: YM = 0.3195; Y0 = 0.001316; k = 0.5036; Xint = 1.986; R^2 = 0.9887. Bag bioreactor: YM = unstable*; Y0 = 0.01848; k = 0.08164; Xint = 12.25; R^2 = 0.9541. *unstable: since the YM refers to the highest OD reached in a logistic equation curve, and the curve did not reach a plateau due to the premature end of the experiment, the YM is not computed by GraphPad. Data obtained from one biological replicate due to size constraints. **b)** Chlorophyll α assay results for biomass content after $g.L^{-1}$ conversion from OD_{665nm} measurements (section 3.2.5). Data obtained from four technical replicates, available in Annex G. Figure made using GraphPad Prism 8.0.1.*

Results - Comparison of growth across experiments

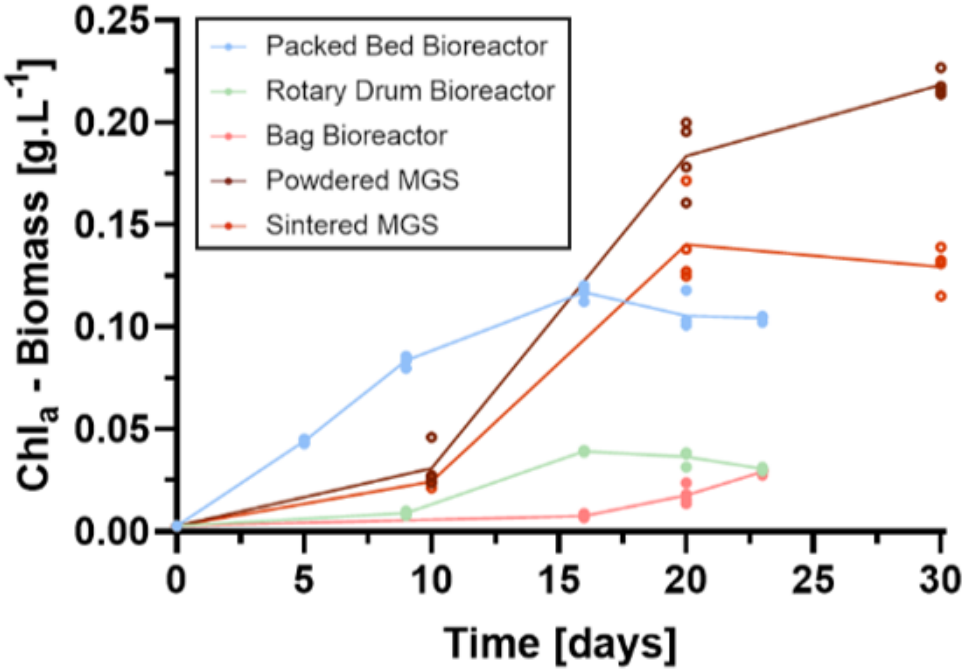


***Figure 49**: Comparison of the growth of Anabaena in bioreactors and multiwell plates. See details in* Fig. 40b *and* 47b.

Results - Statistical analyses - Multiwell

ANNEX D.1: Two-way ANOVA and Tukey post-hoc tests for the multiwell experiment

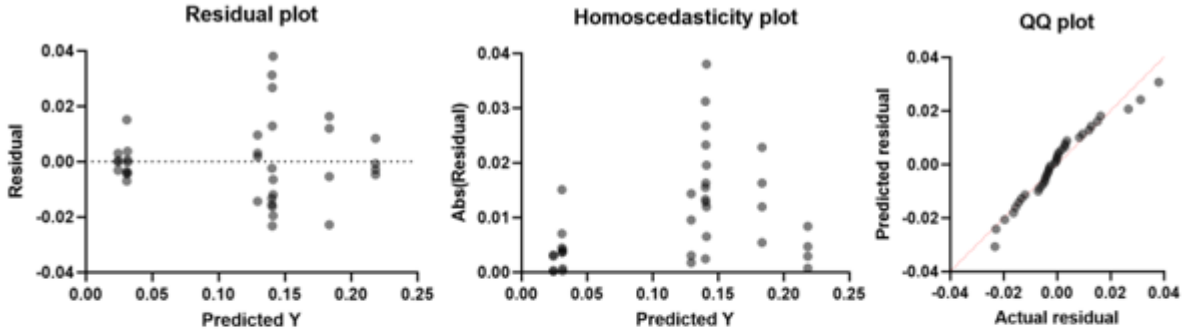


***Figure 50**: Residuals, homoscedasticity and QQ plot of the multiwell experiment data analysed with a two-way ANOVA and a Tukey post-hoc test. Since GraphPad does not support normality and homoscedasticity tests with two-way ANOVA, they were assessed visually on the plots above and computed separately for days 10, 20 and 30. These separate computations confirmed a parametric test was adequate to use. **Homoscedasticity verified for day 10** (Brown-Forsythe test: 0.6881, ns at p-value = 0.5271. Bartlett's test: 5.906, ns at p-value = 0.0522), **Homoscedasticity verified for day 20** (Brown-Forsythe test: 0.01152, ns at p-value = 0.9886. Bartlett's test: 0.3553, ns at p-value = 0.8372) **Homoscedasticity slightly compromised for day 30** (Brown-Forsythe test: 7.312, * at p-value = 0.0130. Bartlett's test: 4.878, ns at p-value = 0.0873). **Normality slightly compromised for day 10** (Anderson-Darling test: 0.6639, ns at p-value = 0.0619. D'Agostino-Pearson omnibus test: 11.93, ** at p-value = 0.0026. Shapiro-Wilk test: 0.8495, * at p-value = 0.0361. Kolmogorov-Smirnov test: 0.2046; ns at p-value 0.1000), **Normality verified for day 20** (Anderson-Darling test: 0.5309, ns at p-value = 0.1376. D'Agostino-Pearson omnibus test: 1.993, ns at p-value = 0.3692. Shapiro-Wilk test: 0.8960, ns at p-value = 0.1407. Kolmogorov-Smirnov test: 0.2154; ns at p-value 0.1000), **Normality verified for day 30** (Anderson-Darling test: 0.1692, ns at p-value = 0.9121. D'Agostino-Pearson omnibus test: 0.1349, ns at p-value = 0.9348. Shapiro-Wilk test: 0.9815, ns at p-value = 0.9889. Kolmogorov-Smirnov test: 0.1175; ns at p-value 0.1000).*

Results - Statistical analyses - Multiwell

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DAY 10					
Sintered vs. Powdered	-0.006520	-0.02694 to 0.01390	No	ns	0.5021
Crushed vs. Powdered	0.0005450	-0.01950 to 0.02059	No	ns	0.9943
Crushed vs. Sintered	0.007065	0.0006978 to 0.01343	Yes	*	0.0336
DAY 20					
Sintered vs. Powdered	-0.04318	-0.08662 to 0.0002500	No	ns	0.0511
Crushed vs. Powdered	-0.04237	-0.09246 to 0.007732	No	ns	0.0882
Crushed vs. Sintered	0.0008175	-0.05141 to 0.05304	No	ns	0.9987
DAY 30					
Sintered vs. Powdered	-0.08897	-0.1083 to -0.06961	Yes	****	<0.0001
Crushed vs. Powdered	-0.07789	-0.1255 to -0.03032	Yes	*	0.0115
Crushed vs. Sintered	0.01109	-0.03451 to 0.05668	No	ns	0.6901

***Table 2**: Results of the Tukey post-hoc test for analysis of significance for the multiwell plate experiment.*

Results - Statistical analyses - Open porosity

ANNEX D.2: Two-way ANOVA and Tukey post-hoc tests for the open porosity

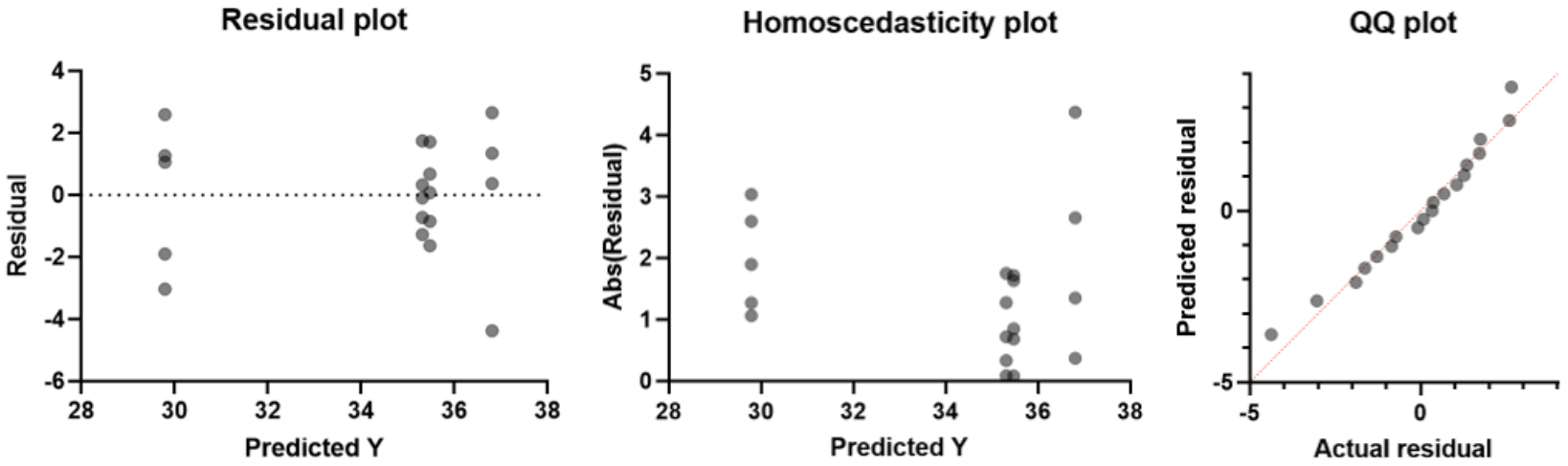


Figure 52: *Residuals, homoscedasticity and QQ plot of the open porosity dataset analysed with a one-way ANOVA and a Tukey post-hoc test.* ***Homoscedasticity verified*** *(Brown-Forsythe test: 0.7389, ns at p-value = 0.5451. Bartlett's test: 4.097, ns at p-value = 0.2512).* ***Normality verified*** *(Anderson-Darling test: 0.2559, ns at p-value = 0.6859. D'Agostino-Pearson omnibus test: 2.104, ns at p-value = 0.3492. Shapiro-Wilk test: 0.9590, ns at p-value = 0.5520. Kolmogorov-Smirnov test: 0.1130; ns at p-value 0.1000),*

Results - *Statistical analyses - Open porosity*

Tukey's multiple comparisons test	Mean diff.	Significant?	Summary	Adjusted P Value	
Batch 27 vs. 26	5.686	Yes	**	0.0026	A-B
Batch 27 vs. 24	0.1613	No	ns	0.9993	A-C
Batch 27 vs. 23	-1.334	No	ns	0.7654	A-D
Batch 26 vs. 24	-5.525	Yes	**	0.0033	B-C
Batch 26 vs. 23	-7.021	Yes	***	0.0006	B-D
Batch 24 vs. 23	-1.495	No	ns	0.6990	C-D

Table 3: *Results of the Tukey post-hoc test for analysis of significance for the open porosity dataset.*