2022 MELISSA CONFERENCE
8-9-10 NOVEMBER 2022

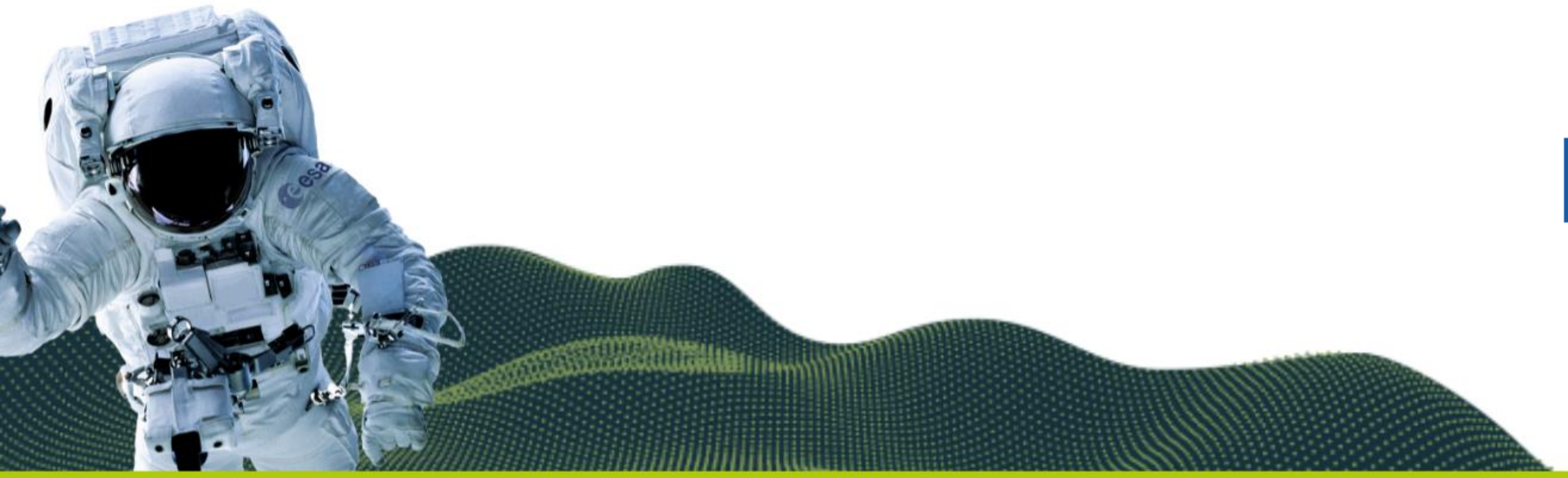
CREATING A CIRCULAR FUTURE

Space organic waste degradation: a new approach to microgreens cultivation

Luigi Chiarini, Lorenzo Nolfi, Angiola Desiderio, Marco Garegnani, Luca Nardi, Maria Elena Villani, Stefano Moscatello, Simona Proietti, Alberto Battistelli, Silvia Tabacchioni

ENEA

IRET

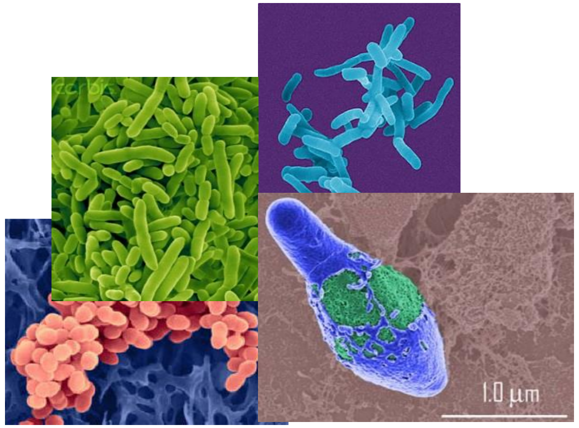


ReBUS project WP 2100: objective

Microbial conversion of organic waste in a suitable substrate for plant growth in space

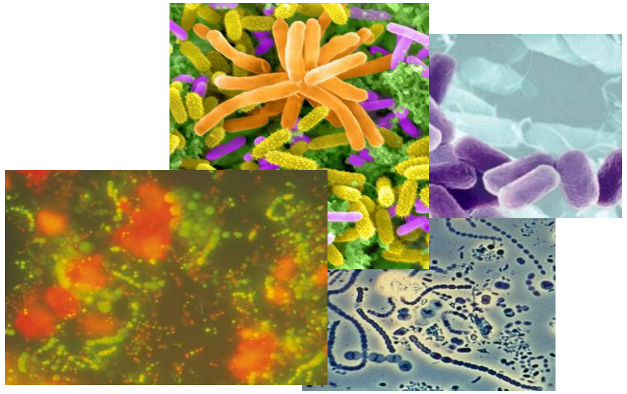
Use of microbial consortia for space organic waste processing

Pure culture vs mixed cultures



Single strain

vs



Microbial consortia

More effective than single strains for degradation of complex organic matter

- Synergistic activity among microrganisms with similar or different functions
- Higher potential in terms of stability and resilience following environmental perturbations

Composition of organic waste

Prepackaged food

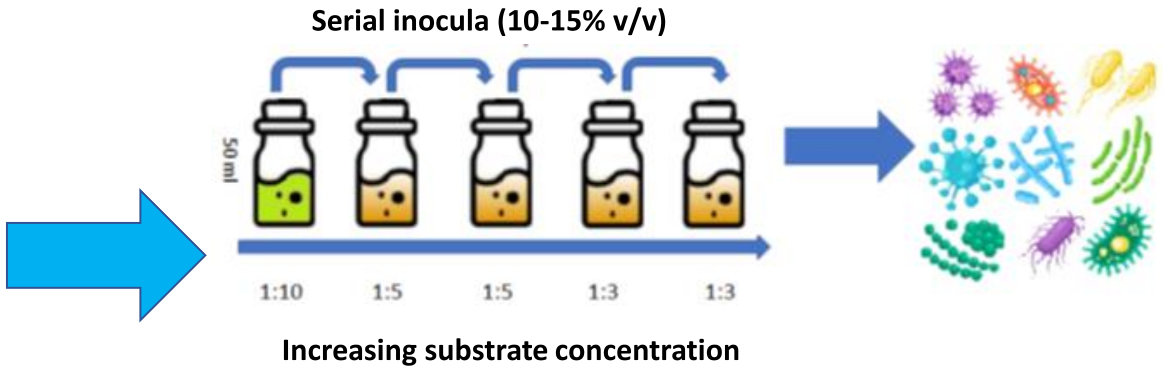
Cellulose tissues

Vegetable wastes

Space organic waste (SOW)

Development of microbial consortia

Organic waste:
Prepackaged foods, microgreens, micro Tom cellulose tissues



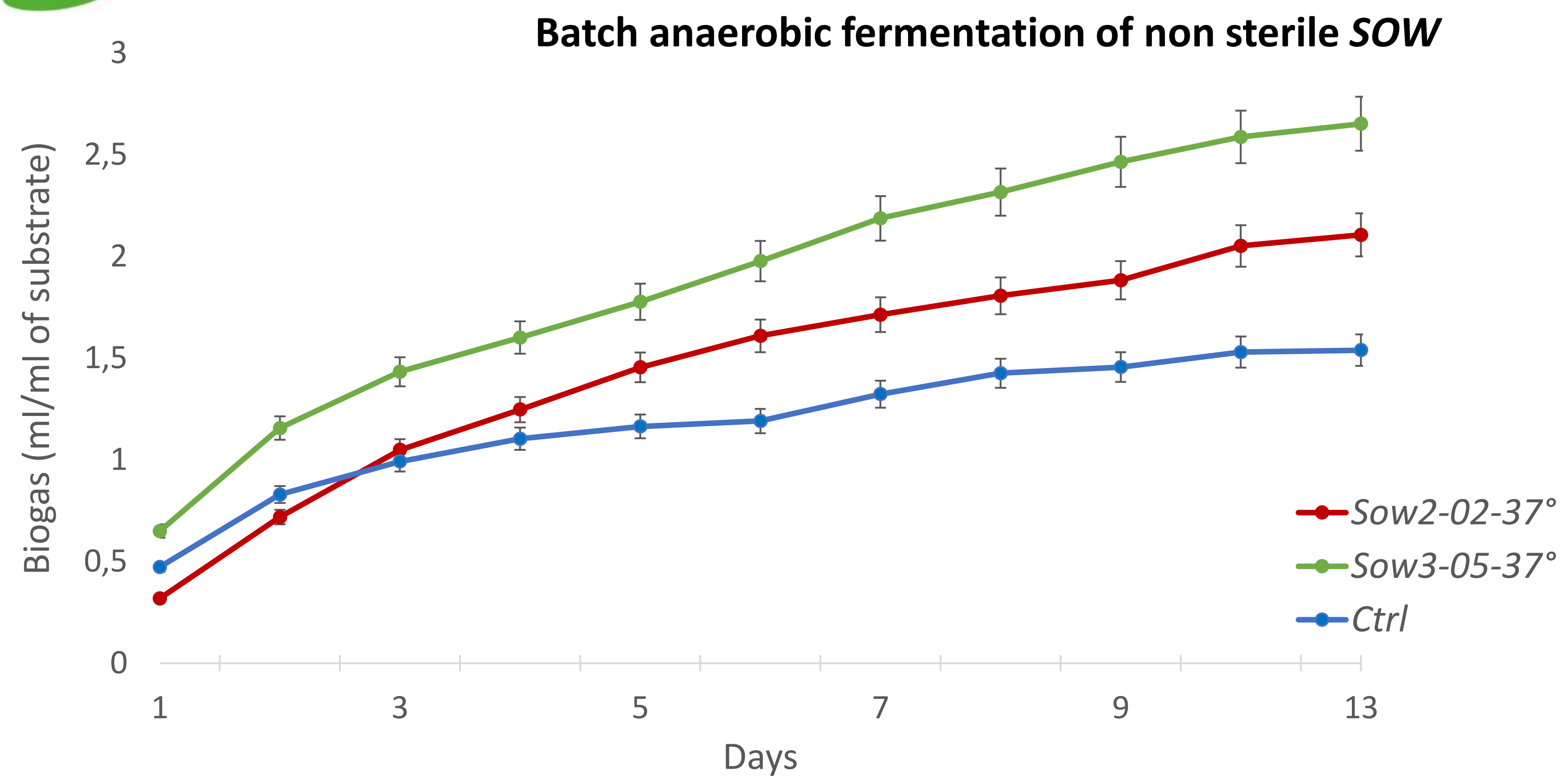
Enrichment generations : 5
Microbial consortia stored at -80°C: 15 for each T

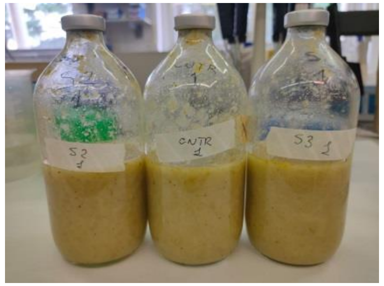
- *Anaerobic enrichment at 28 and 37°C*
- *No starter as inoculum*

Selected microbial consortia (S2-02-37° - S3-05-37°)

- Molecular characterization
- Degradative efficiency (500 ml flasks)

SOW degradation: biogas production





Biogas composition: mainly H_2 and CO_2

No CH_4 was detected

30-40% reduction of total solids by microbial consortia

Structure and diversity of microbial consortia

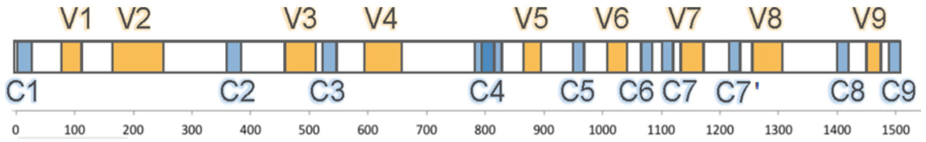
DNA estraction from not fermented *SOW* and microbial consortia *Sow2-02-37°* and *Sow3-05-37°*

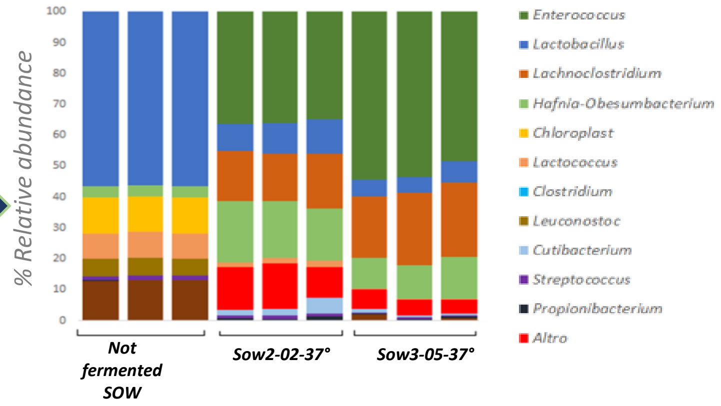
Sequencing (MiSeq – Illumina)

Taxonomy

Alpha and Beta diversity

Structure and diversity of microbial consortia



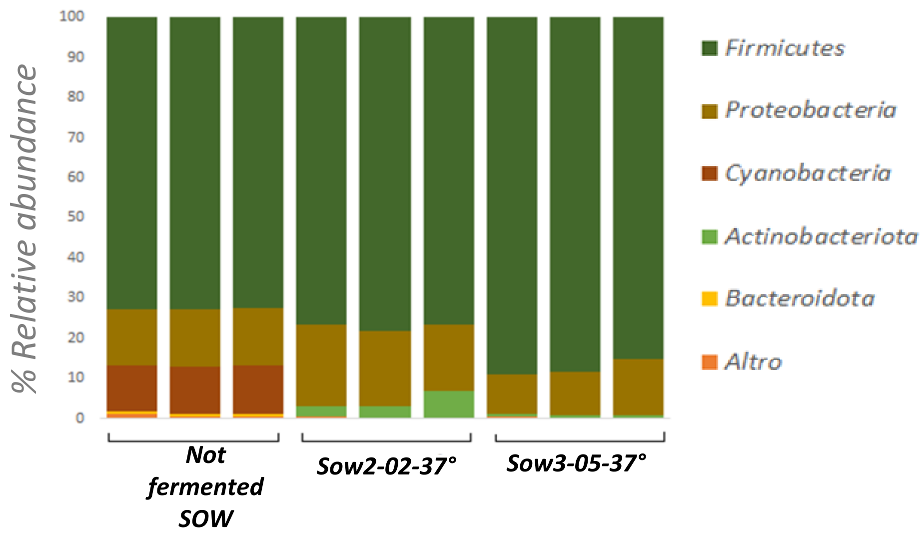


Primer 515F - 806R

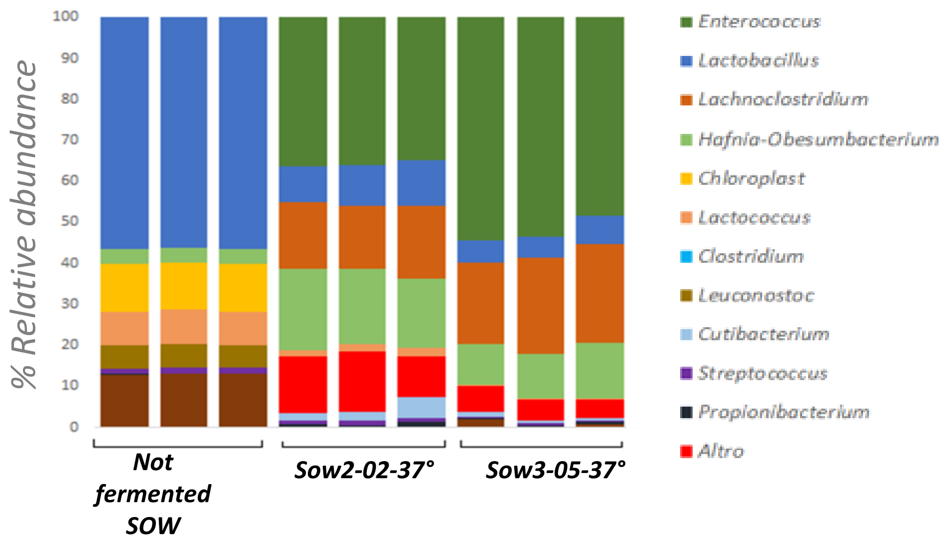
Caporaso *et al.*, 2011-PNAS 108:4516-4522
https://doi.org/10.1073/pnas.1000080107

Taxonomic composition of microbial consortia

Phylum



Genus



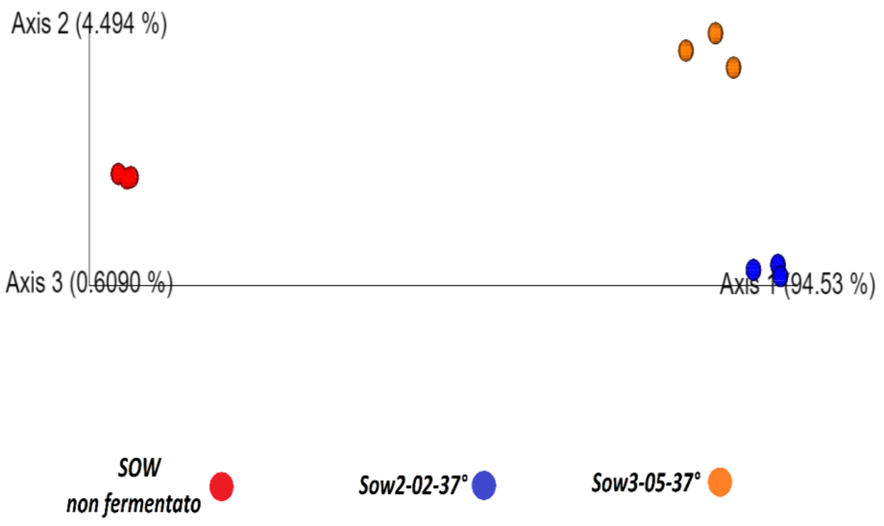
- No Archaea were detected among the ASVs from analysed samples
- The *Firmicutes* phylum is dominant in all samples
- Increase of the relative abundance of facultative/obligate genera during enrichment

Alpha and Beta diversity of microbial consortia

Alpha diversity

Samples	Diversity indexes		
	Chao1 (S)	Simpson (D)	Shannon (H')
SOW (not fermented)	88 99 92	0.651 0.657 0.654	2.531 2.554 2.551
Sow2-02-37°	24 30 26	0.781 0.774 0.792	2.678 2.675 2.747
Sow3-05-37°	15 22 26	0.592 0.619 0.665	1.795 1.846 2.018

Beta diversity



Principal Coordinates analysis (PCoA)

The enrichment process led to the selection of microbial communities genetically different with similar metabolic functions

SOW degradation: chemical analyses

Dry Material

Phase I°: extraction and determination procedure for soluble and non-structural components.

Phase II°: extraction procedure and chemical determination of structural components, using NREL protocols.

Parameters

Structural components of the converted material: (ash, total extractives, lignin, hemicellulose, cellulose)

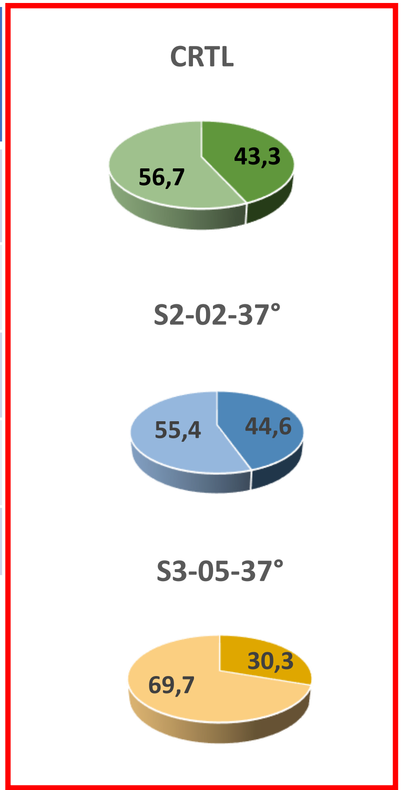
carbon (C) and nitrogen (N) content

soluble carbohydrate content

Total extractives and cellulose consumption by microbial consortia

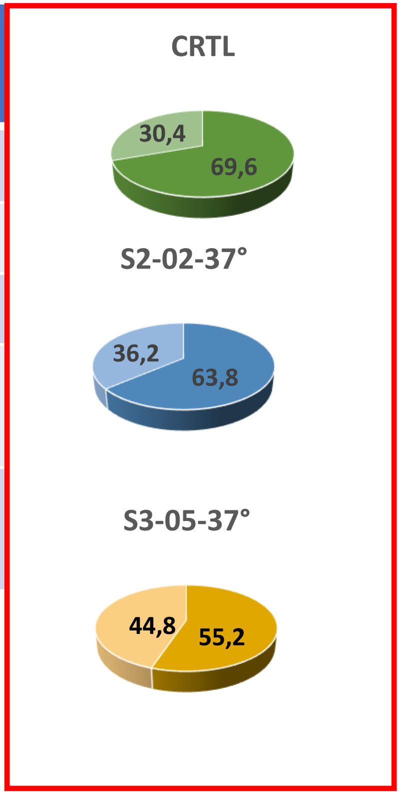
Total extractives
% utilization

Samples	Total extractives (% dry weight)	
	Mean	se
SOW	52.90	1.21
CRTL	22.88	5.11
S2-02-37°	23.59	6.04
S3-05-37°	16.03	0.29



Cellulose
% utilization

Samples	Cellulose (% dry weight)	
	Mean	se
SOW	31.57	0.88
CRTL	21.96	1.00
S2-02-37°	20.14	0.48
S3-05-37°	17.42	2.32



- Total Extractives were the main components used (61% on average) during the fermentation process
- On average 37% of cellulose was degraded
- The microbial consortium S3-05-37° showed the best degradation efficiency for both components

Hemicellulose, lignin, ash and inorganic ions after microbial degradation

Sample	Hemicellulose (% dry weight)		Lignin (% dry weight)		Ash (% dry weight)		Cloride mg g dw^{-1}		Nitrate mg g dw^{-1}	
	Mean	se	Mean	se	mean	se	mean	s.e	mean	s.e
SOW	4.65	0.21	2.48	0.28	2.75	0.04	7.43	0.18	1.04	0.19
CRTL	4.94	0.47	2.28	0.75	12.62	2.75	2.41	0.39	2.02	0.33
S2-02-37°	4.56	0.32	1.31	0.10	11.43	1.77	2.64	0.19	1.79	0.27
S3-05-37°C	3.66	1.03	2.18	0.23	8.56	2.34	0.56	0.06	0.56	0.06

The content of hemicellulose and lignin were substantially unchanged

Chloride and nitrate are present in all samples (initial, and fermented SOW)

Conclusions and future perspectives

Two different microbial consortia have been selected to degrade space organic wastes

- Significant reduction ($p < 0.05$) of total solids
- Significant decrease ($p < 0.05$) of some components

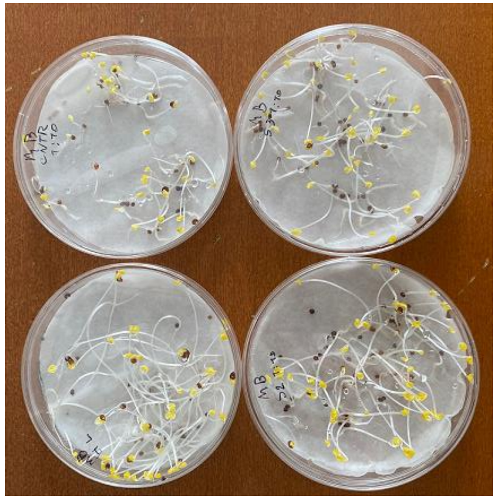
Are the fermentation products obtained from the two microbial consortia suitable to support the growth of microgreens in the space environment?

Work in progress

Potential phytotoxicity of liquid digestate to the germination of microgreens seeds/chemical analysis

Microgreens:

- Radish green daikon (*Raphanus sativus)*
- Mizuna boy *(Brassica Rapa Nipposinica)*
- Mustard Red carpet *(Brassica Juncea)*
- Mustard green frills *(Brassica Juncea)*
- Salad rocket victoria *(Eruca vesicaria)*



Liquid fraction of digestate 1:10
EC = 1.7-2.3 (mS cm^{-1})

The % of germination of Mizuna boy, Salad rocket victoria and radish green daikon seeds did not decrease using liquid digestate from microbial consortia compared to Hoagland solution

Seeds treated with the liquid digestate from the consortium S2-02-37° showed the highest % of germination

Preliminary results of chemical analysis indicate that liquid digestate may be a good substrate for microgreens growth

MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

2022 MELISSA CONFERENCE
8-9-10 NOVEMBER 2022

www.melissafoundation.org

Follow us

THANK YOU.

Silvia Tabacchioni
ENEA-Department for Sustainability
silvia.tabacchioni@enea.it