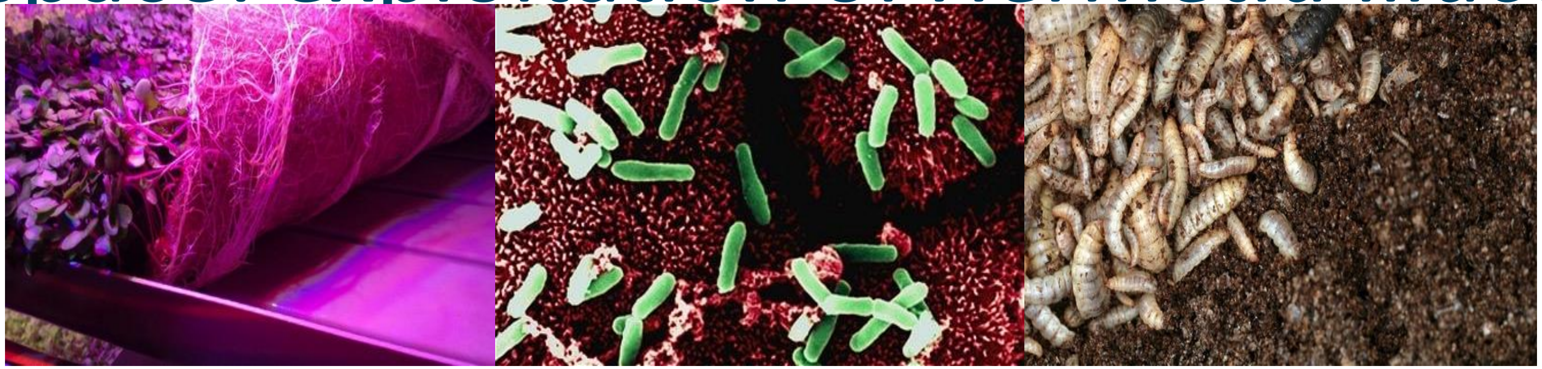
2022 MELISSA CONFERENCE
8-9-10 NOVEMBER 2022

CREATING A CIRCULAR FUTURE

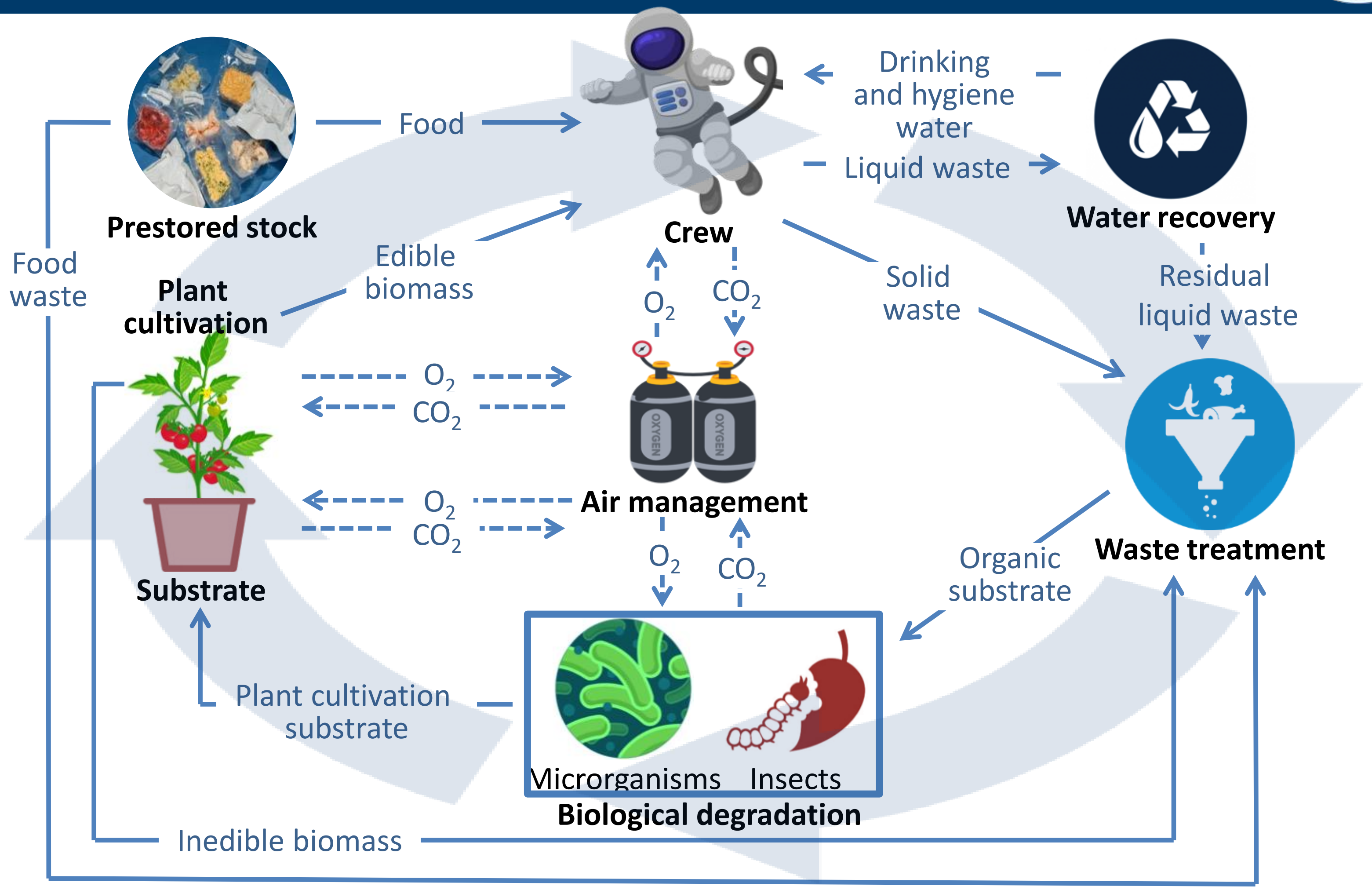
Insect based biorigenerative systems for space: exploitation of *Hermetia illucens*



Maurizio Calvitti

Italian National Agency for New Technologies, Energy and Sustainable Economic Development

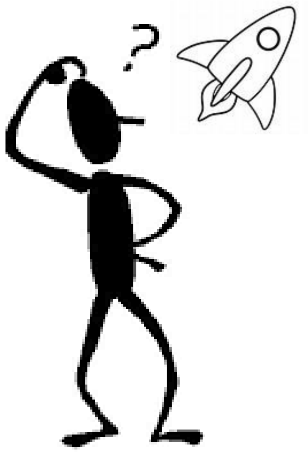
Bioregenerative Life Support Systems (BLSSs): Bioprocesses for primary resource recycling



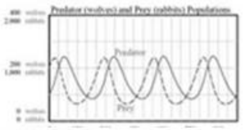
Resources recycling is an urgent need on Earth, but in space this requirement becomes a pillar of sustanaible missions. This complex process should involve organisms, capable of transforming waste into a substrate for plant growth, thus closing the bioregenerative cycle. With this perspective we proposed to study *H. illucens* in ReBUS Project

Biodegrading insects: may be a solution for space?

Insects are fundamental components of terrestrial ecosystems where they perform different bio-ecological functions, including **degradation and biocoversion of organic waste**.



- Predators and phytophagous



- Pollinators

- Biorigenerators

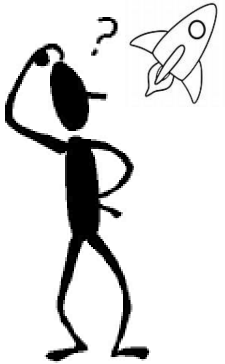
It is among these species that we must identify those with biological characteristics potentially exploitable in space context.

Preliminary attempts to exploit insects for space

Different insect species have been proposed in space experimental systems, such as

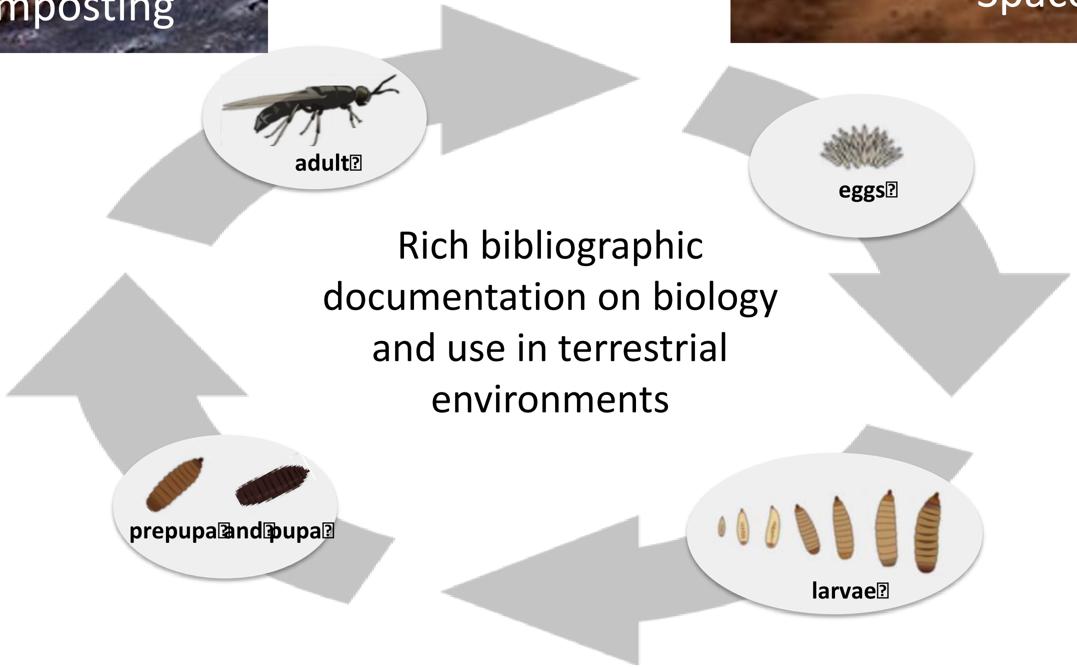
- *Tenebrio molitor* (Li et al., 2016)

- *Bombyx mori* (Liang et al., 2014)



Different insect species have been proposed in BLSS experimental systems, such as *Tenebrio molitor* and *Bombyx mori* the latter not properly a decomposer insect as it feeds on fresh plant material

***Hermetia illucens*, the black soldier fly (BSF) , is a common and widespread Dipterus of the family Stratiomydae**



Hermetia illucens is a master biodegrading insect. Its function has been extensively validated in the terrestrial environment

Why propose *H. illucens* for a space bio-regenerative system?

- **Appreciably shorter development times** (45-50 days in optimal conditions) compared to *T. molitor* (130-220 gg), which is important for optimizing process efficiency and reduce the proliferation of microorganisms in the degrading substrate (Lalander et al., 2015).

- **Capacity of degrading virtually all organic waste** (food, vegetable, paper, human and animal waste), while *T.molitor and B.mori* are specialized on cereals/starch and fresh leaves (mulberry) respectively.

- **Production of digestive enzymes** that transform the organic biomass while reducing the bacterial load which causes unpleasant or toxic gases (Kim et al., 2011; Meneguz et al., 2018).

- **Degradation product enriched in ammonium ions**, which enhance its fertilizing properties and can be used as compost for plant cultivation or as soil improver (Green & Popa, 2012).

Phases of research

Insect adaptation to artificial rearing conditions

Efficiency of organic wastes biodegradation compatible with mission conditions

Quality of the degradation product as a plant growing substrate

Modular controlled unit for the study of bioprocesses

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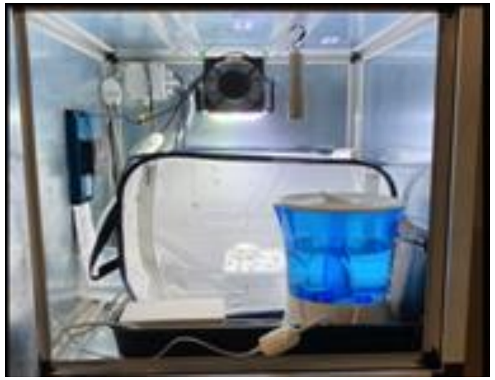
Insect adaptation to artificial rearing conditions

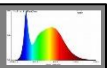
Standardize rearing conditions

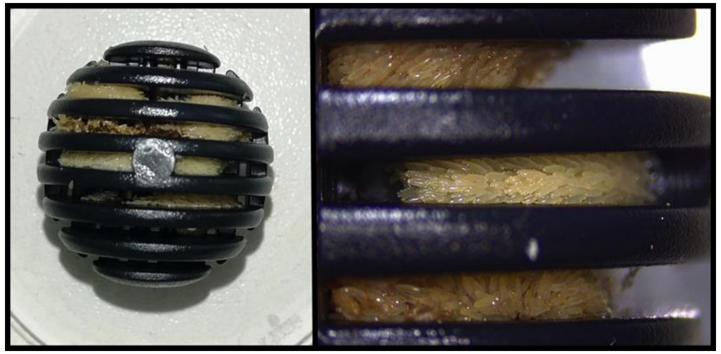
Solutions for enhancing ovideposition



GRM Module (Metelli et al., 2022)

Fitness enhancement through specific spectrum LED lighting (fixing UV wavelenght)

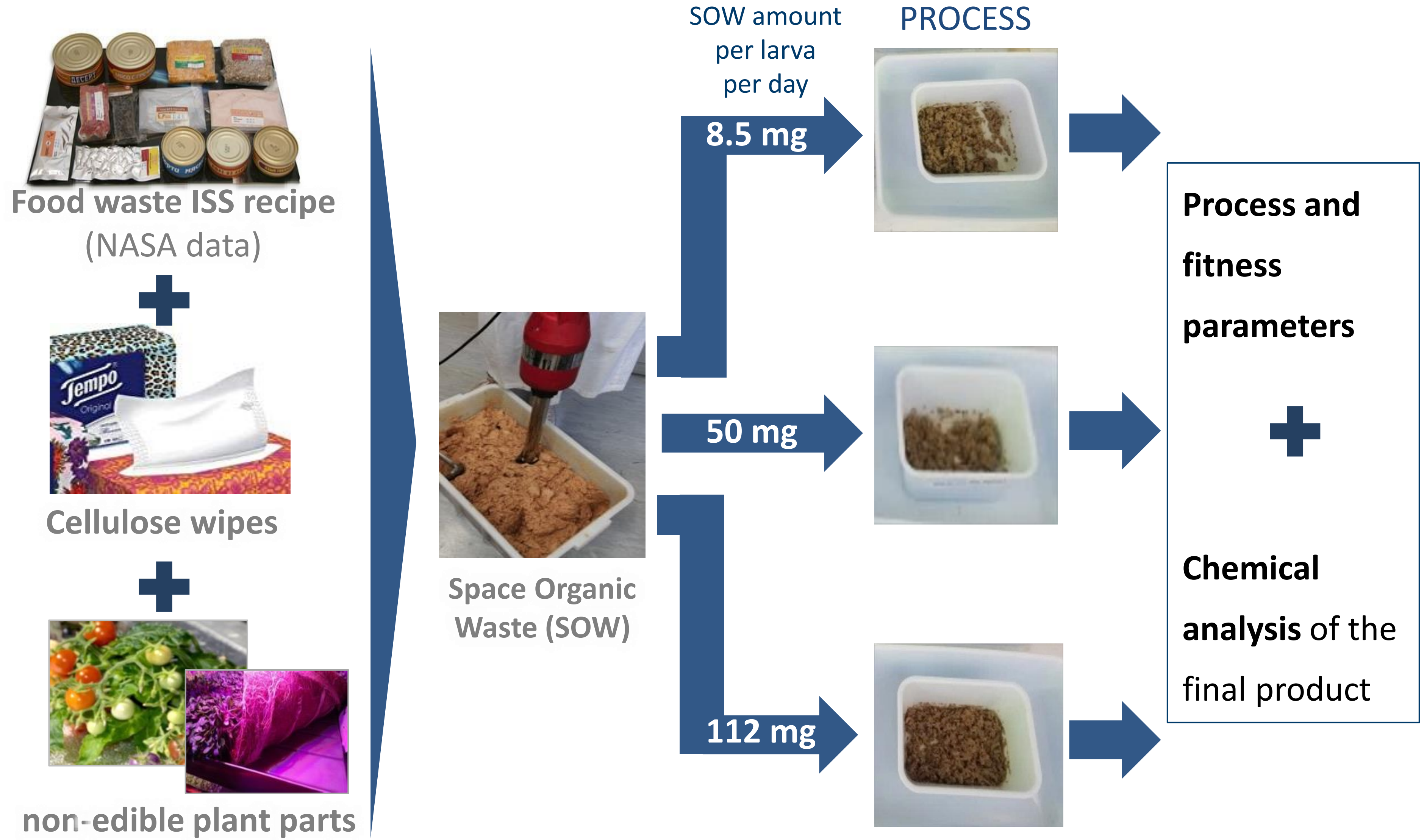




Outcomes of adaptation studies

- ✓ Ease of rearing in a confined environment
- ✓ Spontaneous removal of the prepupae from the degraded substrate
- ✓ Limited tendency to fly
- ✓ Mating posing on surfaces
- ✓ Egg laying in confined spaces for easy retrieval
- ✓ Pupae can be quiescent for long time

Preparation of organic waste that mimics those produced during space mission for studying the efficiency of biodegradation



Bioconversion efficiency evaluation

in relation to the amount of food substrate supplied

PROCESS

***Metabolism** = S-(B+PS)*

Complex of biochemical reactions of synthesis and degradation

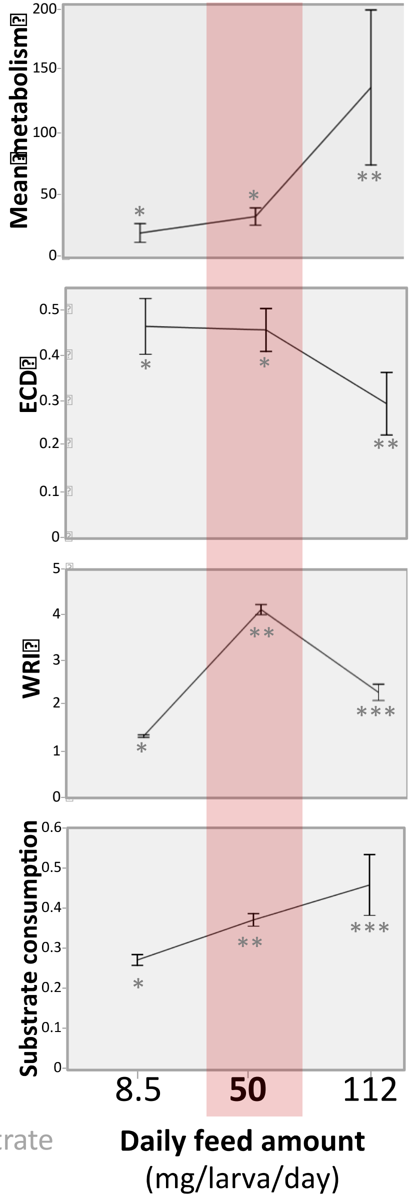
***Efficiency of Conversion of Digested feed** (ECD) = B/(S-PS)*

Conversion efficiency rate of the ingested substrate

***Waste Reduction Index** (WRI) = %Rid/t*

***% Consumption** = PS/S*

Reduction rate of the substrate provided



S= substrate administered , B insect biomass, PS=processed substrate

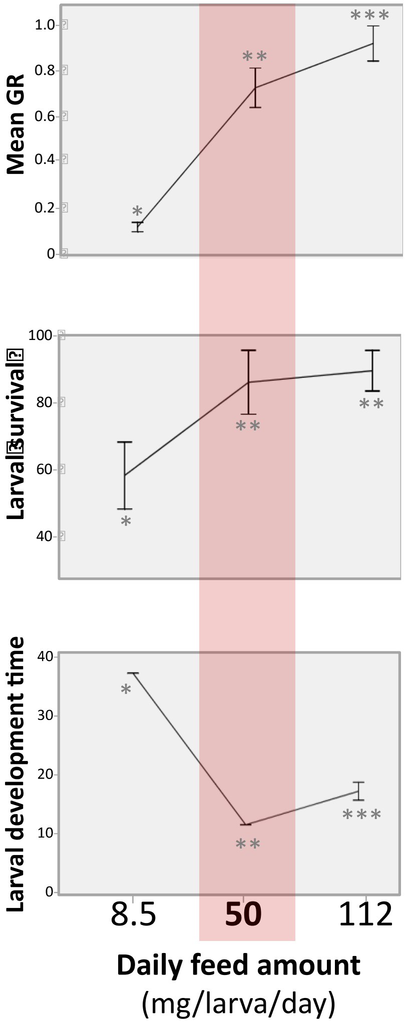
FITNESS

***Growing rate** (GR) = $(Wl_f - Wl_0)/(t)$*

Larval growth efficiency

***Larval survival** = l_f / l_i*

***Larval development time** = t*



Indices for efficiency evaluation

Conclusions

The intermediate quantity of substrate supplied = **50 mg/larva/day** (rather than 8.5 or 120 mg) was found to be the optimal compromise between the efficiency of the process and the biological response of the insect

- Highest conversion efficiency of digested feed
- Highest waste reduction index
- Shorter developmental time of larvae
- Sustanaible growing rate and larval survival

Quality of biodegradation: chemical characterization of SOW processed by *Hermetia illucens* larvae at the 3 different feeding rates (in collaboration with CNR)

Analytical method:

- SOW dry material.
- Developed an extraction procedure and chemical determination of structural components, using NREL protocols (National Renewable Energy Laboratory) for:

  1) soluble and non-structural components

  2) structural components

- HPAEC-PAD Ion Chromatography (HPAEC-PAD) for measurements of carbohydrates, inorganic ions, organic acids; spectrophotometric analysis; elemental analyzer.

Analyzed parameters:

- Structural components of the converted material: **ash**, **extractives**, **lignin**, **hemicellulose**, **cellulose**
- **Carbon** and **nitrogen** content
- **Soluble carbohydrate** content

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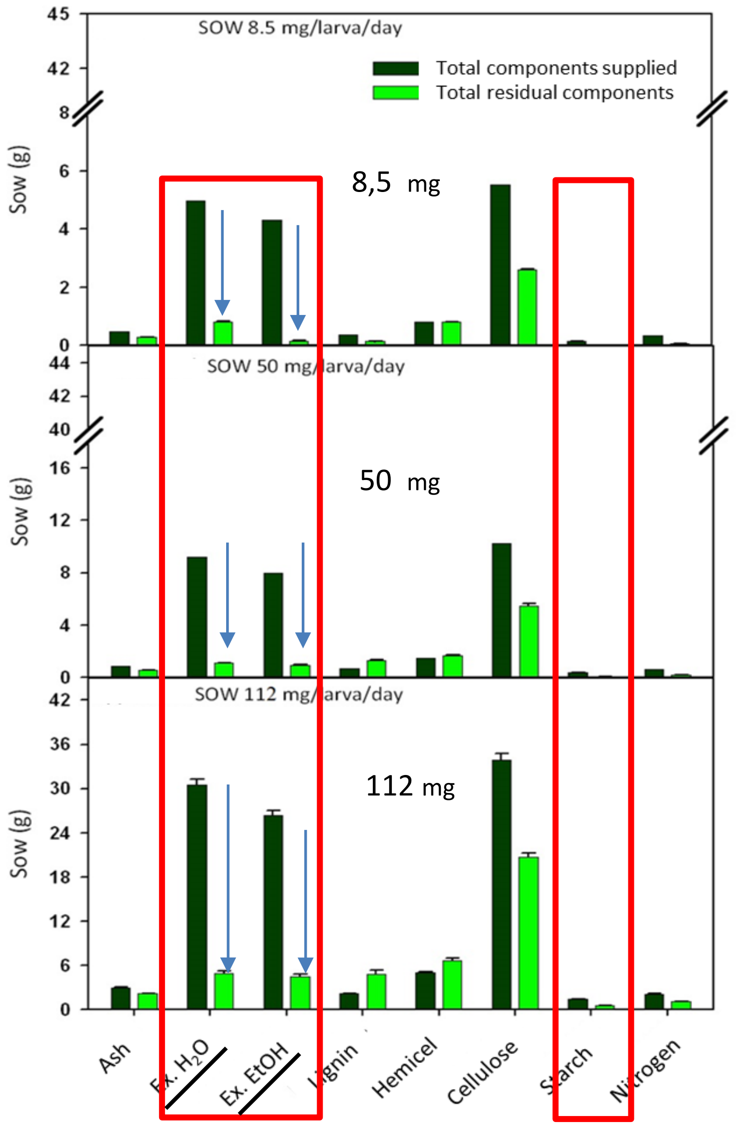
Differential use of feeding component by *H. illucens larvae (BSFL)*: Initial composition *vs* feeding residual



EXTRACTIVES

Water and ethanolic extractives include **non-structural carbohydrates**, **organic acids**, **soluble proteins**, and **fats**, representing the main components (52,9%) of SOW dry biomass and the easiest to metabolise.

Hermetia performed a significant reduction of extractives for all diets proposed.

Extractives were the major component used. Moreover, soluble sugars were undetectable after BSFL action.

mg/larvae/day	8,55	50	112
% of total extractives reduction	89,8%	88,3%	83,5%

STARCH

Starch was also reduced by BSFL of 87,2% on average.

mg/larva/day	8,55	50	112
% of total starch reduction	87,4%	78,1%	61,5%

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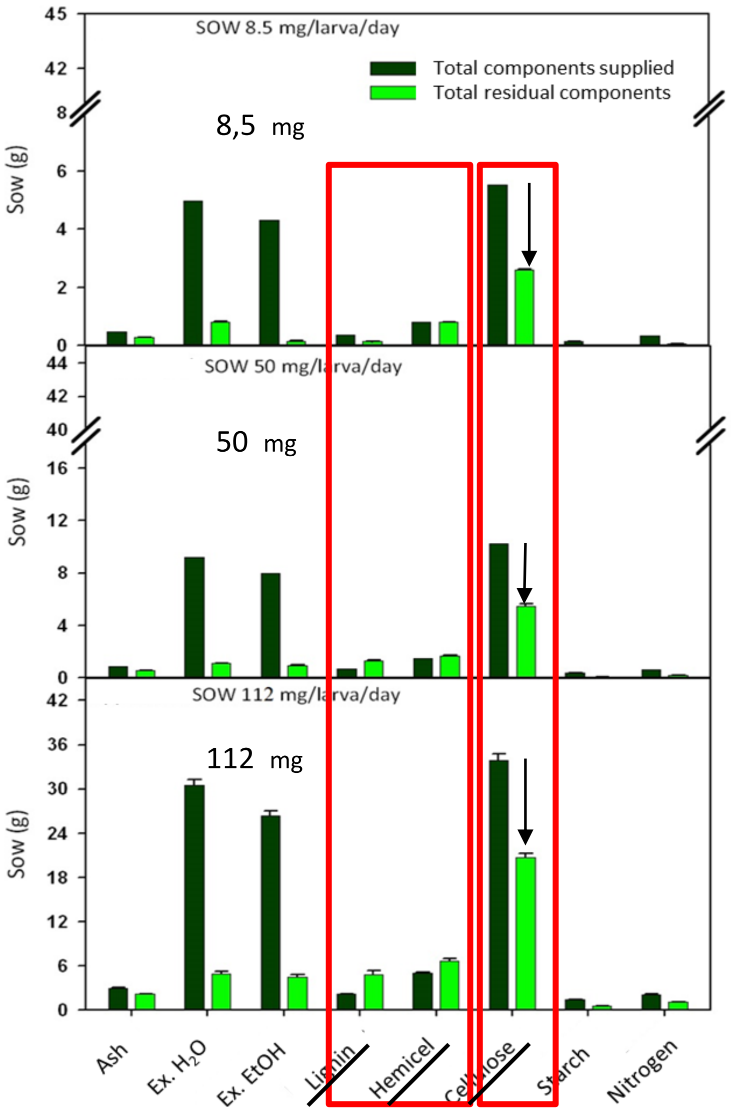
Differential use of feeding component by *H. illucens:* Initial composition *vs* feeding residual



CELLULOSE

The main recalcitrant components of the vegetable biomass.

Hermetia **performed a significant reduction of cellulose for all diet rates.**

mg/larvae/day	8,55	50	112
% of cellulose reduction	52,9%	46,7%	38,7%

HEMICELLULOSE and LIGNIN

Components in low percentage of the feeding biomass.

The increase after feeding clearly indicates that these components were not used by the insect, causing their relative accumulation in feeding residues after the digestion of the other SOW components.

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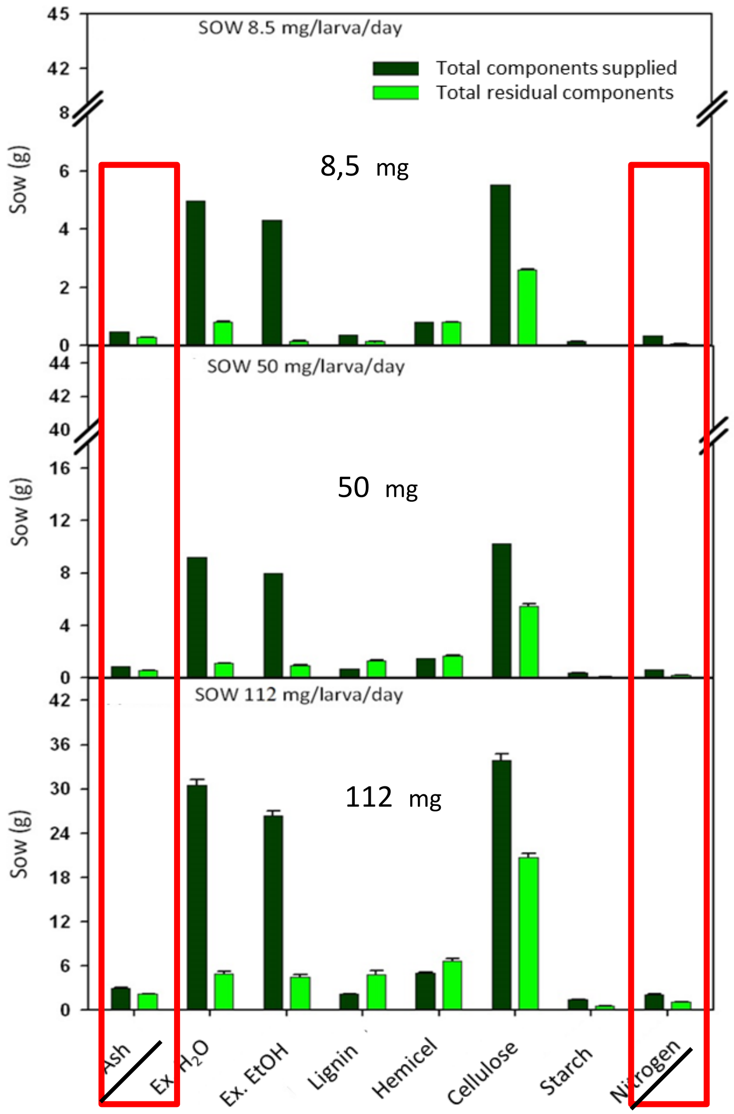
Differential use of feeding component by *H. illucens:* Initial composition *vs* feeding residual



Ash and **nitrogen** contents decreased after *Hermetia* degradation in all diet rates, **since a fraction was used by the larvae.**

ASH

mg/larvae/day	8,55	50	112
% of ash reduction	41,5%	37,4%	25,9%

NITROGEN

mg/larvae/day	8,55	50	112
% of nitrogen reduction	80,9%	71,9%	46,7%

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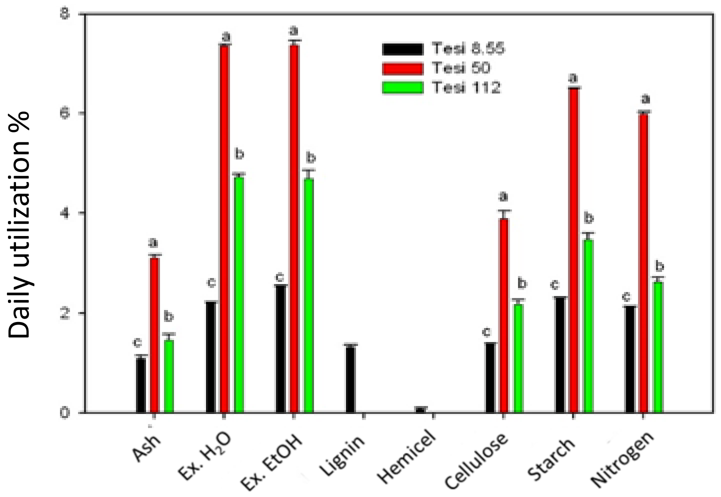
Rate of SOW components degradation on a daily basis depending on by black soldier larvae feeding rate



SOW degradation by *H. illucens* larvae showed a great potential in waste recycling, quickly reducing complex components into molecules more easily usable by plants.

Recycling efficiency varied among SOW components and was significantly affected by the feeding rate.

8.55 mg SOW/larva/day = higher % of components degradation, but a slow development time from larvae to pre-pupa (38 days) decreases efficiency.

50 mg SOW/larva/day = Faster development time from larvae to pre-pupa (12 days) results in a higher % daily use efficiency of different chemical components

112 mg SOW/larva/day = Lower % of component degradation combined with an intermediate development time from larvae to pre-pupa (18 days)

Modular controlled unit for studying bioprocesses: towards solutions for BLSS in Space.

Designing a BLSS for future missions requires careful studies before the introduction of new candidate organisms, to predict:

I. *their adaptation to not natural conditions*

I. *exclude the possibility that they could alter the environment in an unhealthy way for the crew (e.g., by altering atmosphere composition or producing toxic substances).*

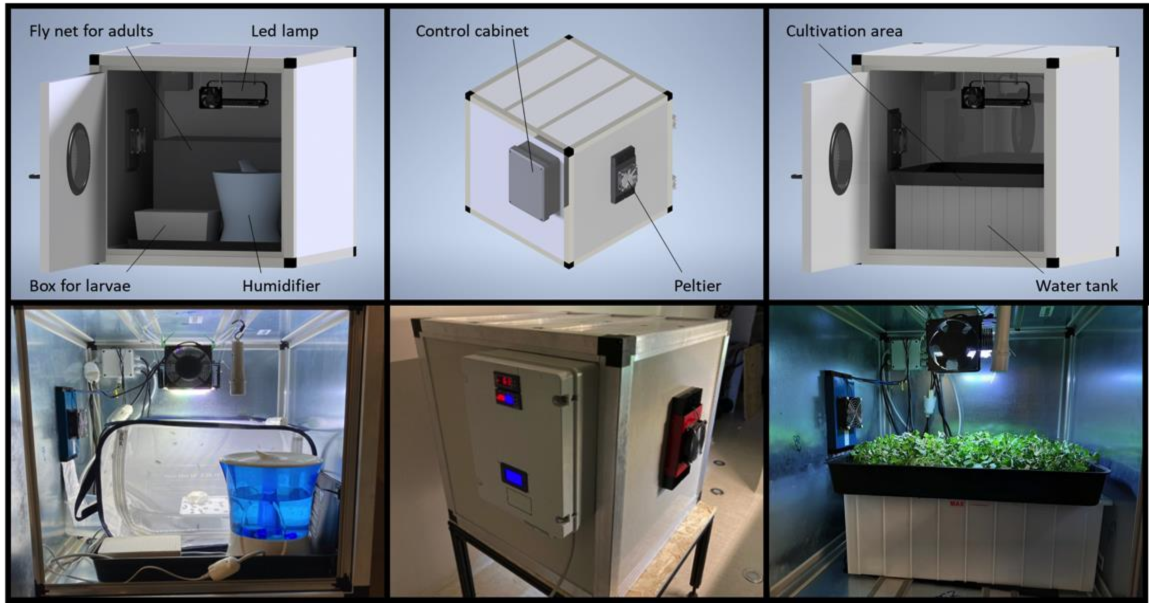
ELSEVIER

Life Sciences in Space Research
Available online 28 October 2022
In Press, Journal Pre-proof

Design of a modular controlled unit for the study of bioprocesses: Towards solutions for Bioregenerative Life Support Systems in Space.

Giulio Metelli [1, 2]*, Elena Lampazzi [1]*, Riccardo Pagliarello [1, 2], Marco Garegnani [1, 3], Luca Nardi [1], Maurizio Calvitti [1], Luca Gugliermetti [4], Riccardo Restivo Alessi [5], Eugenio Benvenuto [1], Angiola Desiderio [1]

Modular controlled unit for studying bioprocesses: towards solutions for BLSS in Space.



Growing/Rearing Module (GRM):

- completely isolated and equipped with micro-environmental monitoring and control systems
- specifically intended to study single bioprocesses, which can be composed to design functional BLSSs
- equipped and implementable with specific devices for different biological system (validated in experiments of microgreen cultivation and waste bioconversion by *H. illucens*)
- compatible with the International Standard Payload Racks present on the ISS and easily scalable.

Modular controlled unit for studying *H. illucens* bioconversion

System validated for:

- Rearing and study the black soldier fly, which completes its life cycle under these conditions
- Degradation trials of organic waste mimicking those produced during missions (previosly showed).

(for furhter information please refer to Metelli *et al.*, 2022)

ONGOING RESEARCH

Use of compost from *H. illucens* in plant cultivation: experiments in progress


Mix of mission organic waste (SOW)


Degradation product

Lunar regolith



Martian regolith

Use as soil improver to give Lunar and Martian simulants chemical-physical characteristics suitable for the cultivation of microgreens.

Experiments in collaboration with **University "Federico II"** Naples, Italy

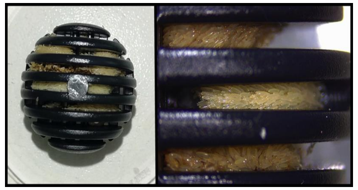
Develop protocols for cryoconservation of BFS fertilised eggs

-Embryo pemeabilization

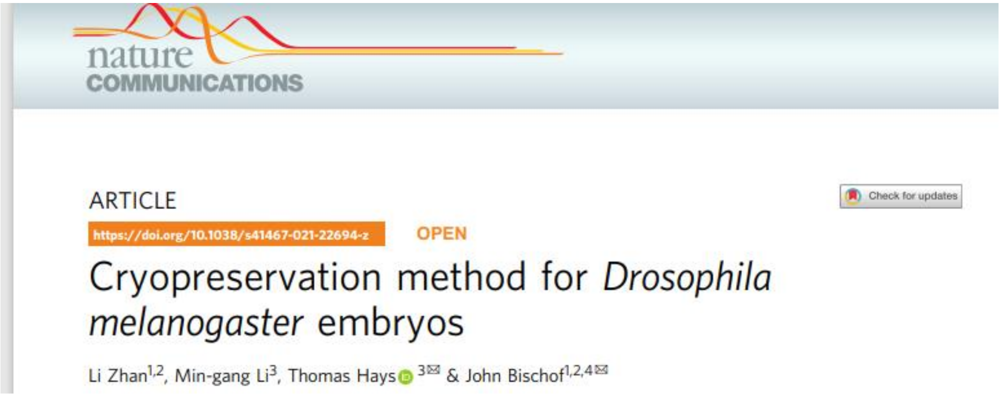
-Cryoprotectant agent loading

-Vitrification

-Rewarming



Main reference literature



nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-22694-z OPEN

Check for updates

Cryopreservation method for *Drosophila melanogaster* embryos

Li Zhan[1,2], Min-gang Li[3], Thomas Hays[3] & John Bischof[1,2,4]

Concurrently analytical studies to evaluate H.illucens larvae as food source are going on because:

- Reported use of *H.illucens* larvae in human and animal nutrition in some countries.
- Fresh food source of animal protein, with essential amino acids.
- Larvae rich in fatty acids.
- Possibility of conditioning the nutritional composition through larval diet.
- Possibility of obtaining flour that can be easily integrated as an ingredient in meatballs or hamburgers.

[Shumo et al., Sci Reports (2019) 9:10110; Spranghers et al. J Sci Food Agric (2017) 97:2594]

ReBUS - "In-situ REsource Bio-Utilization for Life Support in Space"

Project funded by the Italian Space Agency (ASI)

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- Kayser Italia Srl
- Telespazio TPZ SpA

Thank you for your attention !