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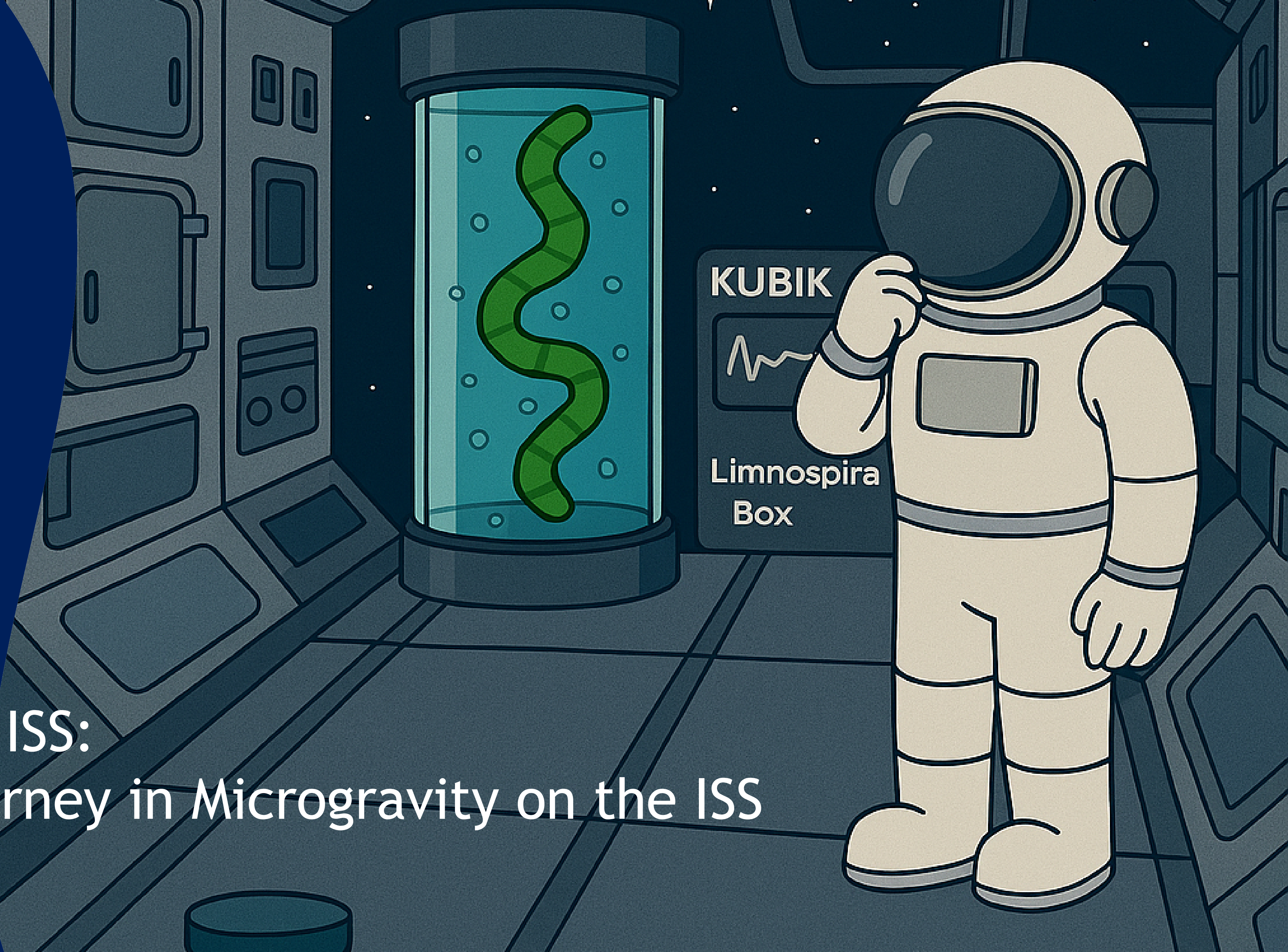
8th October 2025

Limnospira on ISS:
Spirulina’s Journey in Microgravity on the ISS



Limnospira on ISS

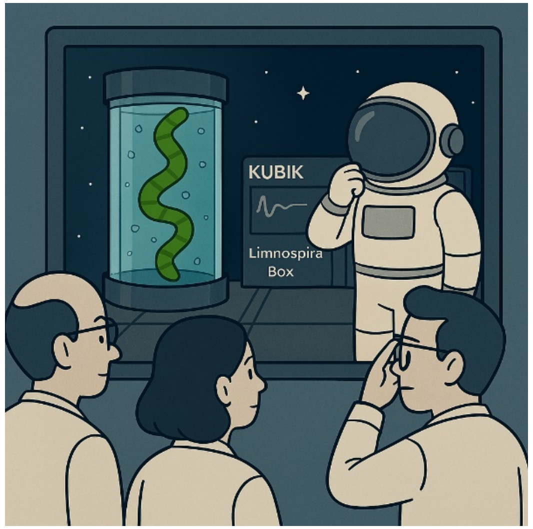
Experiment Goal

At the MELiSSA Conference, we are witnessing how a simple cyanobacterium like Spirulina (*Limnospira Indica*) is key to human survival beyond Earth

Limnospira indica not only produces oxygen and edible biomass, but serves as the core of regenerative life support systems for long-duration space missions. If it works under microgravity conditions, we could replicate self-sustaining ecosystems beyond Earth

And that is the objective of Limnospira on ISS experiment:

Studying Spirulina's behavior under microgravity conditions



Limnospira on ISS

Benefits of *Limnospira Indica* for Space Missions?

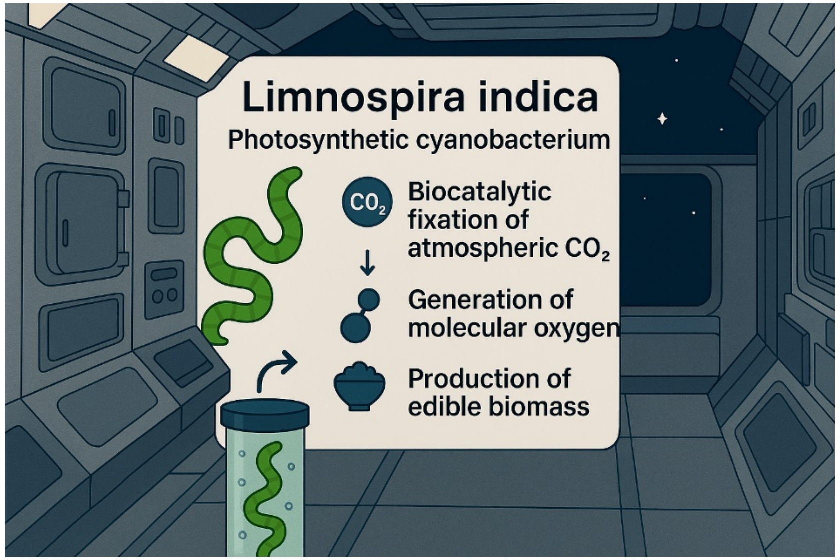
Limnospira indica is a photosynthetic cyanobacterium with three essential bioprocessing functions:

- ✓ Biocatalytic fixation of atmospheric CO_2
- ✓ Generation of molecular oxygen to sustain crew respiration
- ✓ Production of high-value, protein-rich edible biomass—validated as a nutritional supplement on Earth

These 3 main bio-functions and the growth performance of *Limnospira indica* has been successfully evaluated under terrestrial conditions at the MELiSSA Pilot Plant but…

… Will we be able to replicate it on-orbit?

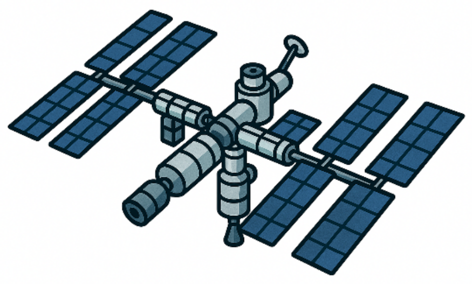
Limnospira on the ISS: the experiment that holds the answer



Limnospira on ISS

Who is involved? – Experiment details

- Principal investigator (PI): Francesc Gòdia & MELiSSA Team
- Main Payload Developers: SENER Aeroespacial & CNM
- Experiment Sponsor: Agencia Espacial Española (AEE)
- Estimated Flight to ISS: Private Astronaut Mission (PAM-5)
- Estimated Flight Date: September 2026

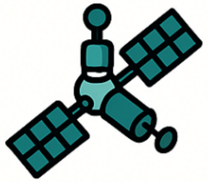


Limnospira on ISS

Expected results

Assess *Limnospira Indica* growth and metabolism in microgravity

Generate images and growth data



Validate the payload for future studies under similar approach

Support MELiSSA pilot plant and experiments increasing the knowledge on the current activities

Limnospira on ISS

Heritatge - *Limnospira Indica* research

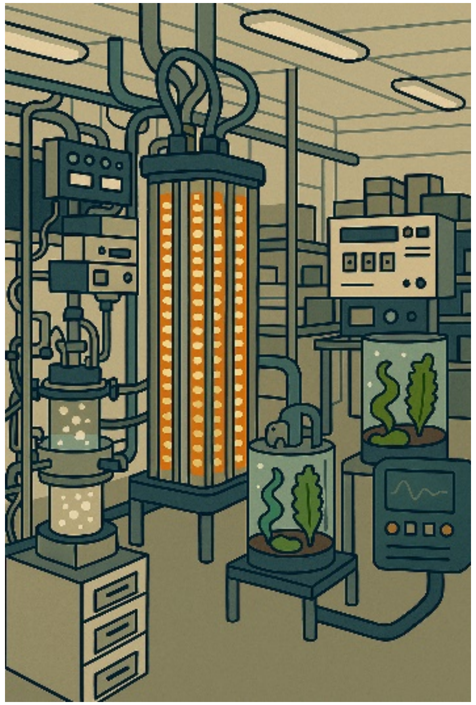
MELiSSA Pilot Plant

Location: Universitat Autònoma de Barcelona (UAB)

Objective: Integration and validation of regenerative life support technologies under controlled conditions

Applications:

- Development of self-sustaining habitats
- Water and waste recycling systems
- Nutrient recovery technologies with direct relevance to Earth sustainability



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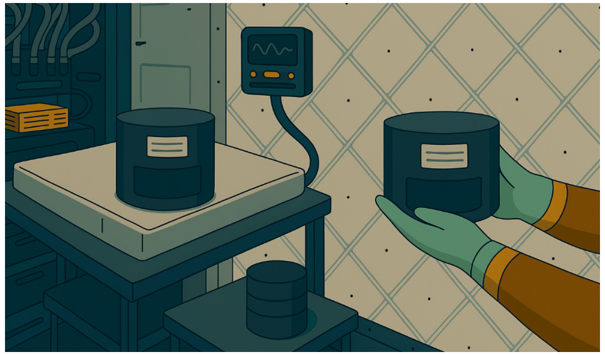
Heritatge - Hardware previous use



NASA Seedling Growth-2

Joint NASA-ESA study aboard the ISS using the European Modular Cultivation System (EMCS)

Objective: Investigate how light and gravity influence plant growth in *Arabidopsis thaliana*

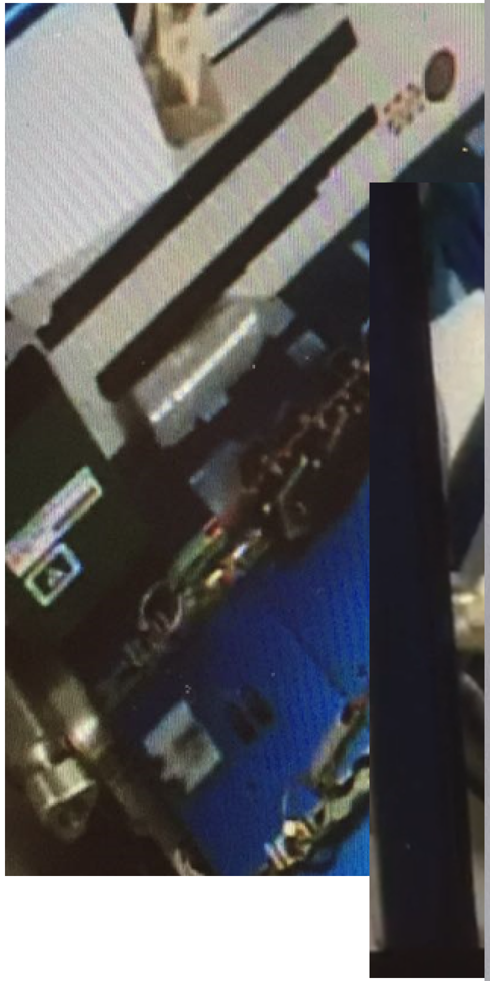
FixBox: SENER developed hardware used in SG-2 for on-orbit plant growth chemical fixation

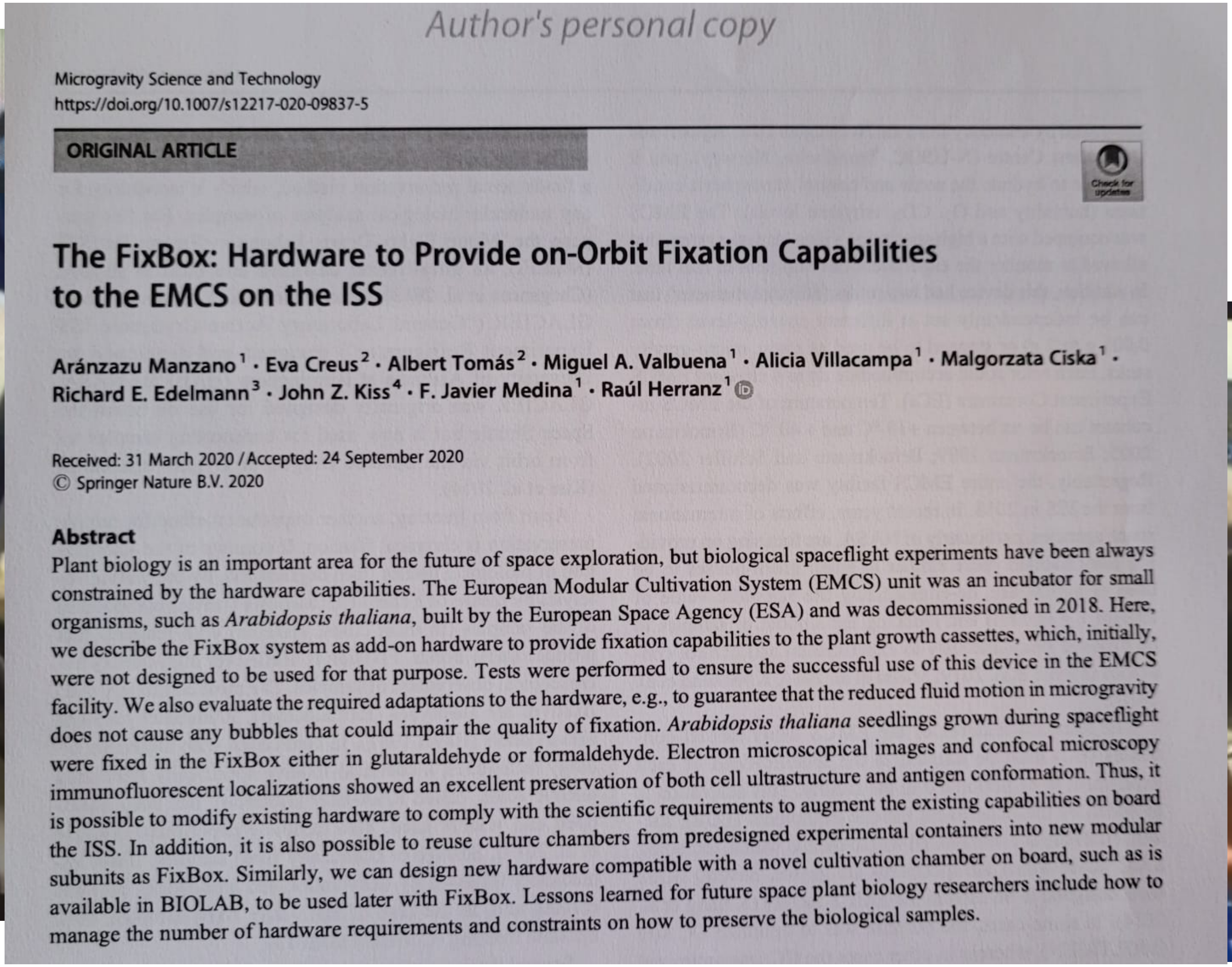
Functions:

- Automated injection of fixatives (e.g., glutaraldehyde, formaldehyde) in microgravity
- Triple containment for crew safety and leak prevention (even at $-130\,^{\circ}\mathrm{C}$)
- Housing for 5 seed cartridges, compatible with ISS freezers
- 12 units used on-orbit in 2016

Limnospira on ISS

Heritatge SG-2 – Experiment





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ORIGINAL ARTICLE

The FixBox: Hardware to Provide on-Orbit Fixation Capabilities to the EMCS on the ISS

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Abstract
Plant biology is an important area for the future of space exploration, but biological spaceflight experiments have been always constrained by the hardware capabilities. The European Modular Cultivation System (EMCS) unit was an incubator for small organisms, such as *Arabidopsis thaliana*, built by the European Space Agency (ESA) and was decommissioned in 2018. Here, we describe the FixBox system as add-on hardware to provide fixation capabilities to the plant growth cassettes, which, initially, were not designed to be used for that purpose. Tests were performed to ensure the successful use of this device in the EMCS facility. We also evaluate the required adaptations to the hardware, e.g., to guarantee that the reduced fluid motion in microgravity does not cause any bubbles that could impair the quality of fixation. *Arabidopsis thaliana* seedlings grown during spaceflight were fixed in the FixBox either in glutaraldehyde or formaldehyde. Electron microscopical images and confocal microscopy immunofluorescent localizations showed an excellent preservation of both cell ultrastructure and antigen conformation. Thus, it is possible to modify existing hardware to comply with the scientific requirements to augment the existing capabilities on board the ISS. In addition, it is also possible to reuse culture chambers from predesigned experimental containers into new modular subunits as FixBox. Similarly, we can design new hardware compatible with a novel cultivation chamber on board, such as is available in BIOLAB, to be used later with FixBox. Lessons learned for future space plant biology researchers include how to manage the number of hardware requirements and constraints on how to preserve the biological samples.

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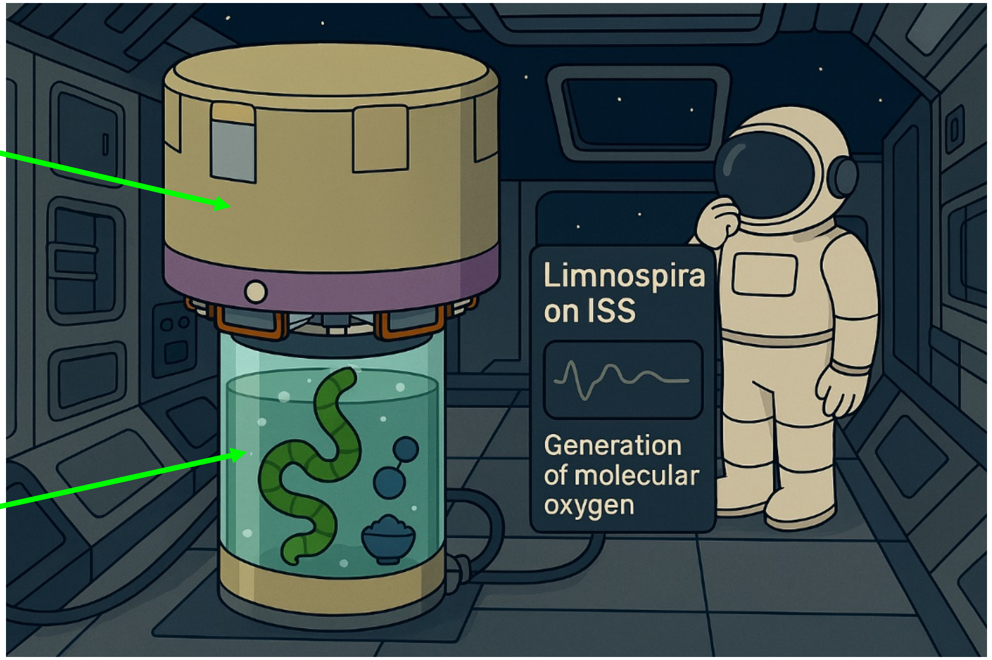
Experiment key elements

Limnospira on ISS Box

- Repurposed ESA flight hardward (FixBox, Seedling Growth-2 heritage), re-engineered for cyanobacterial cultivation in microgravity
- Modified to monitor the *Limnospira Indica* growth and to adapt to new functional requirements

Limnospira indica

- Cyanobacterium strategically selected for its essential role in oxygenic photoautotrophic bioprocessing within closed-loop regenerative life support systems
- Proven MELiSSA program heritage as a core component for autonomous human exploration missions



Limnospira on ISS

Experiment key elements

Cassettes

- 5 independent cultivation chambers Located inside the Limnospira on ISS Box, will act as housing for the *Limnospira Indica* growth.
  Note: No interface with the crew
- Autonomous data acquisition for real-time monitoring of cell proliferation and pigment content under controlled illumination
- Enables precise quantification of photosynthetic performance and stress biomarkers, supporting future integration into bioregenerative life support subsystems

Limnospira on ISS Launch Clips:

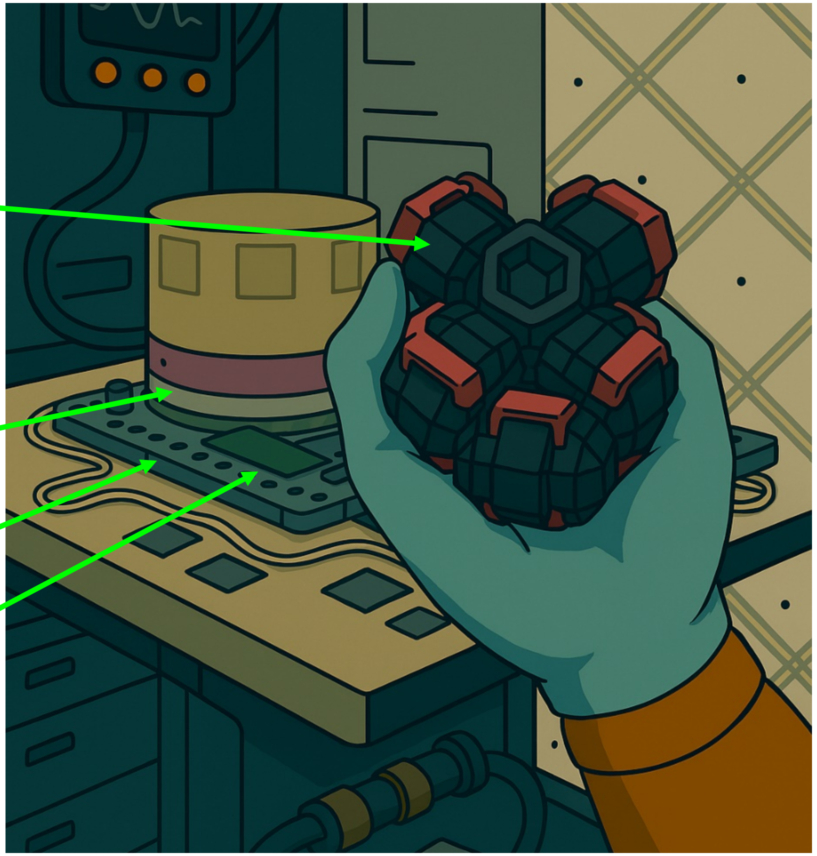
Prevention for the premature mechanical engagement

Limnospira on ISS KUBIK interface plate (KIP):

Structural support and electrical interconnection platform designed for docking with ISS incubator payload (KUBIK)

Limnospira on ISS SD Card:

Primary experiment data acquisition



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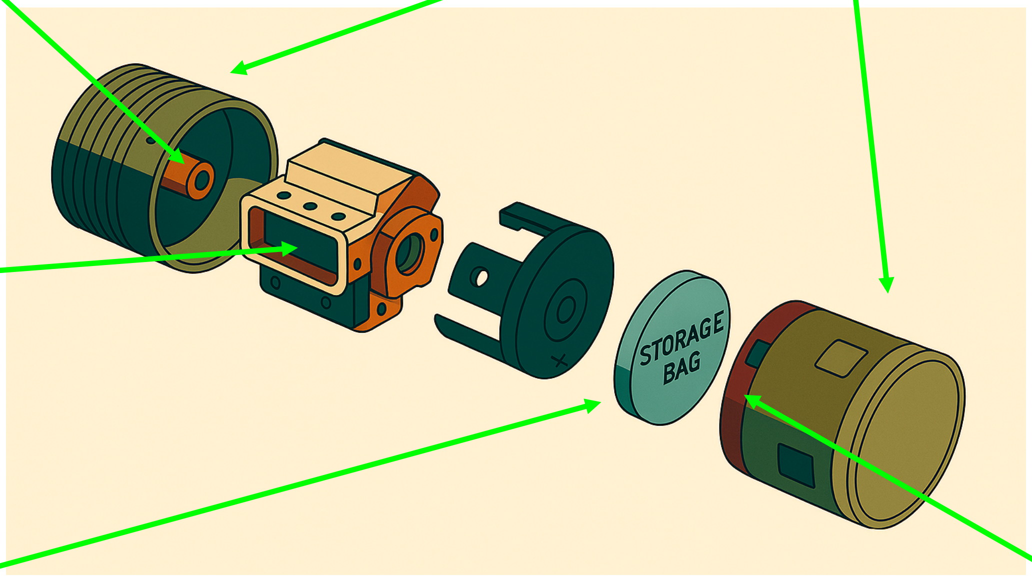
Limnospira on ISS Box – Key elements

2 cylindrical threaded halves: **Base and Cover**

Cannula
Used to puncture the Aluminium Bag to start liquid transfer

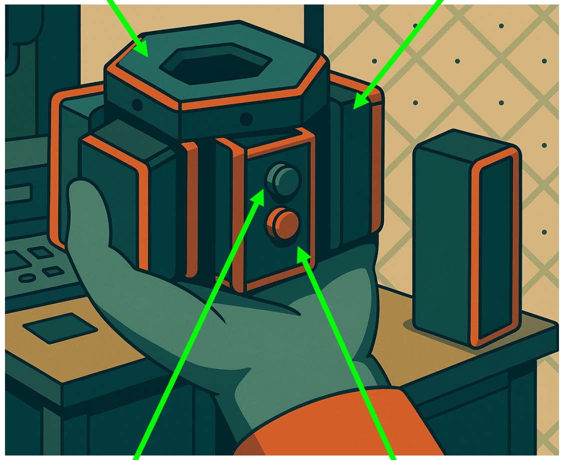
Carrousel
It is the 5 cassettes holder during the growing process

Aluminum Bag
Original storage of the ‘sleeping’ cyanobacteria



Ratchet System
Used to avoid unscrewing process once the unit is closed

Carrousel **Cassette**

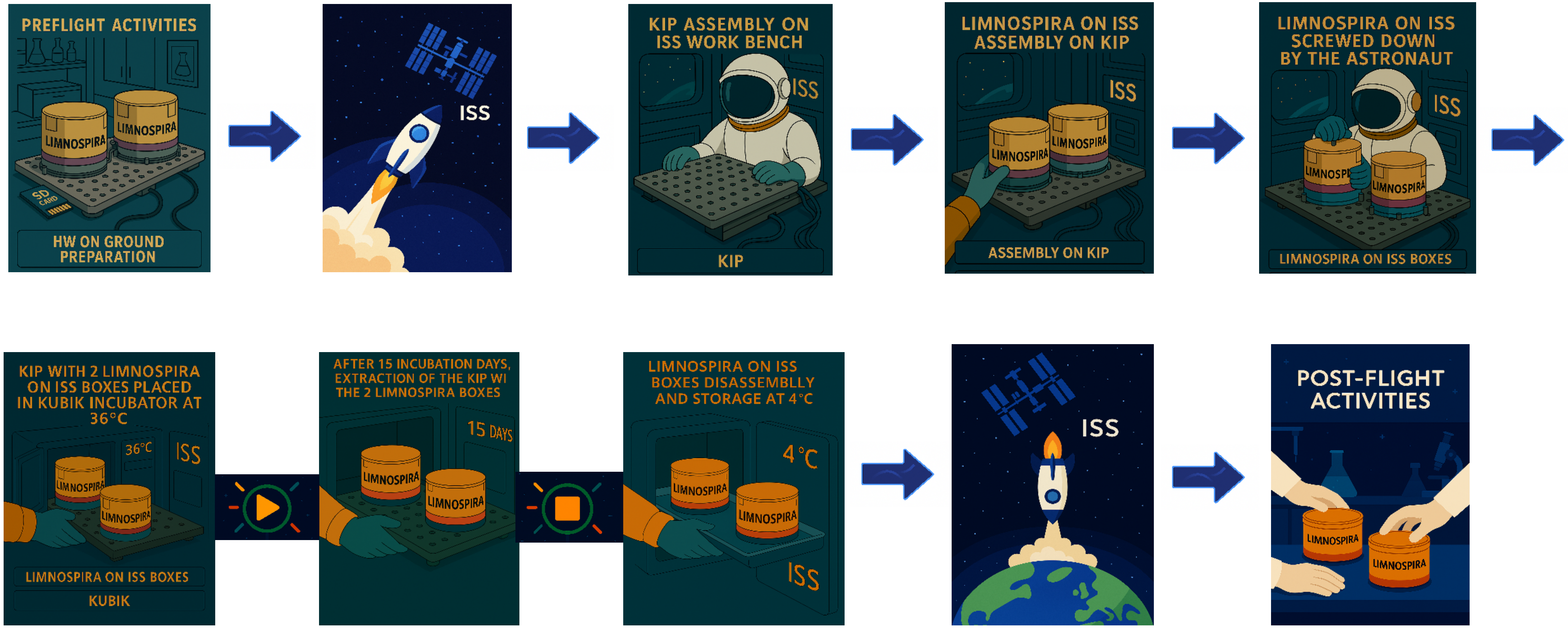


Injection port Positioning port

Note: Seals & Septa are used to avoid liquid release during the experiment and to provide crew safety against toxic materials release

Limnospira on ISS

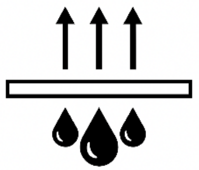
Experiment sequence summary on ISS

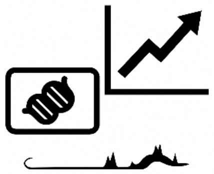
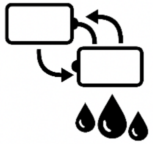


Limnospira on ISS

Main technical challenges

The most technically demanding challenges of the new FixBox application—now renamed as Limnospira on ISS Box—are the following:

- ✓ Design of hydrophobic membranes for air venting while maintaining liquid containment 
- ✓ Screwing torque reduction 

- ✓ Cassette design 
- ✓ *Limnospira Indica* growth monitoring 
- ✓ Fluidics control inside Limnospira on ISS Boxes 
- ✓ Crew safety first! 

Limnospira on ISS

What's next? - FluxSen

The growing demand for microgravity scientific research highlights the need for effective liquid transfer systems.

Yet, limited understanding of fluid behavior in space hinders robust industrial solutions and slows scientific progress but…

The solution is within reach—and it has a name:

sener

Limnospira on ISS

What’s next? - FluxSen

FluxSen will offer a modular solution for liquid transfer in microgravity, enabling controlled liquid and air injection and extraction, mixing, and bubble management. Designed for compatibility with sensitive biological experiments, it will support advanced research across diverse scientific fields

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