

Arthrospira-B First photobioreactor for oxygen and edible biomass production in space

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# Bio-based life support for human space exploration

- From the start of human space travel, research has been done to develop 'bioregenerative <u>closed loop</u> life support systems'
- especially for air revitalisation : CO<sub>2</sub> removal, and O<sub>2</sub> production
  → algae & cyanobacteria, for the photosynthesis process
- Examples from all over the world :

https://en.wikipedia.org/wiki/MELiSSA

- **BIOS** (1960's, IBP, **Russia**)
  - algae Chlorella
- MELiSSA MicroEcological Life Support System Alternative (1987, ESA, France & Belgium)
   cyanobacterium (Nostoc) & Arthrospira
- CELSS Closed Ecological Life Support System (1989, NASA, US)
   cyanobacterium Arthrospira
- CEBAS Closed Equilibrated Biological Aquatic System AQUARACK unit (1992, DLR, Germany) – algae Chlamydomonas
- **CERAS** Closed Ecological Recirculating Aquaculture System (1997, Japan)
  - algae Euglena and Chlorella en cyanobacterium Arthrospira
- CAES Closed Aquatic Ecosystem (2004, China)
  algae Chlorella

...

Currently 'Research and Development' on Earth, no system "in use" in space

# SCK•CEN founding member of



Since 1989

Dr. Max Mergeay (SCK•CEN)

1 of the 4 'fathers' of MELiSSA

Director of MELiSSA Foundation www.melissafoundation.org

FOUNDATION



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# The concept, inspired of an ecosystem



# The challenges are big

Dissecting the earth ecosystem to its essential components & understand how they work

Then putting them back together, but smaller, with less complexity, less buffers or back-ups, ...

> Nevertheless predictable & reliable Engineered to targeting maximum yield

Using biotechnology which can be functional in space conditions (launch & radiation & microgravity)

A mineaturised sustainable synthetic earth ecosystem transplantable to space

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#### **MELiSSA** team at work

# 30 years of MELiSSA ...



#### MELiSSA team ... after work









# Oxygen production in space with cyanobacteria



# Arthrospira









6 µm x 400 µm

- Blue-green filamentous cyanobacteria
- Lives in warm alkaline salty lake water, water rich in carbonates, at pH 9 & 35°C
- Cylindrical cells, in helicoidale strings, called 'trichomes' or filaments
- Continuous illumination, no need for dark
- Very high photosynthetic efficiency
- High cell density Bioreactor cultivation
- Also known as Spirulina food supplement

In SPACE - life support system - MELiSSA

#### **PHOTOSYNTHESIS** for

- CO<sub>2</sub> removal from the air
- O<sub>2</sub> Production of from water,
- Nitrogen from waste water into proteins
- Edible biomass production

# Arthrospira – food supplement

#### • Edible

- Consumption by Aztecs, documented since 1500
- Still used by several tribes in Africa
- In Europe/Asia known as 'super food' Spirulina

### • Highly interesting Food value

- thin cell wall, full organism digestible, no need for processing or cooking, no waste (↔ algae, plant)
- Single cell protein source, Low DNA/proteincontent (↔ other bacteria, algae, plant)
- Does not contain phyco-toxines (↔ other cyano)
- Rich in essential fatty acids
- Contains vitamins, minerals, antioxidants, immunomodulators, prebiotic, ...









# Bio-engineering – Arthrospira in photo-bioreactor



### Arthrospira is an efficient oxygen producer e.g. BIORAT experiment



- The rat consumes O<sub>2</sub> and produces CO<sub>2</sub>; the bacterium produce O<sub>2</sub> and consume CO<sub>2</sub> with light energy input
- the photosynthetic yield  $(O_2/CO_2)$  of the algae matches the respiratory quotient of the rodent  $(CO_2/O_2)$ ; by playing on light energy supply & predictive model
- A ground demonstrator has validated the concept : 2-months experiment

DEMEY D. *et al.* 2000 "BIORAT : preliminary evaluation of biological life support in space environment developments". SAE paper 2384, 30th International Conference on Environmental Systems, Toulouse, France July 10-13th, 2000.

2000-01-2379

Biological Life Support System Demostration Facility: The Melissa Pilot Plant

Joan Albiol, Francesc Gòdia, José Luis Montesinos, Julio Pérez, Anne Vernerey, Fernando Cabello, Nuria Creus, Anna Morist and Xavier Mengual Universitat Autónoma de Barcelona

Christophe Lasseur

closed gas loop between CVI a Arthrospira & 3 rats Contineous reactor, ca. 1 year



The ESA MELISSA pilot plant @ UAB, Barcelona, Spain

**CVI**a

= 40



# Cosmic radiation in space



# → Biological impact ?

Dose rates in space higher than on earth

- On Earth ~ 0.1 μSv/h x250
- On ISS (LEO) ~ 25 μSv/h
- On the way to mars ~ 75 µSv/h
- On surface of mars ~  $25 \mu$ Sv/h
- ⇒ Necessary to monitor radiation doses in space

# Type of Radiation in space different then on earth

- Electrons, protons, helium nuclei, heavier nuclei, neutrons, photons, muons, pions, ...
- Up to extremely high energies around 10<sup>12</sup> MeV
- Strong dependence on location, solar cycle and shielding
- ⇒ Very challenging to assess radiation doses in space

# Reduced/Altered gravity in space $\rightarrow$ Biological impact ?

Earth	Space	
Sedimentation, Convection, Diffusion	Diffusion only $\rightarrow$ Fluid quiescence, 'low shear'	
		d so fl 8
1g (Earth)	- Micro-g (space)	
e.g. boiling water in space	ce	

lifferent olid/liquid/gas luid dynamics

& separation

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# Testing impact of space conditions... on Earth



Simulation of space radiation and low gravity on earth ....



# Testing impact of space conditions... in LEO





Parameters	Earth	LEO (~4*10 <sup>2</sup> km dist.) ISS	Interplanetary Space (>7*10⁴ km dist.)	<b>Moon</b> (4*10 <sup>5</sup> km dist.)	Mars (4*10 <sup>8</sup> km dist.)
Ionizing radiations (µGy/day) Gravity (g)	2-4	160-500 <sup>a</sup>	220-1270	~ 400	200-300 (max.20,000)
	1	10 <sup>-6</sup> – 10 <sup>-3</sup>	< 10 <sup>-6</sup>	~ 0.2	~ 0.4

LEO: low Earth orbit, Dist.: distance from the Earth, a: inside space station



# Impact of space flight on microbial cells

SCK•CEN



# Impact of space flight on microbial cells



Bibliography @ http://www.melissafoundation.org/

### Anticipating the impact of spaceflight



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Arthrospira GeneExpression study and mathematicalModelling on cultures grown in theInternationalSpaceStationL. Hendrickx, 2004





# **ArtEMISS project**

"First bioreactor with cyanobacteria in space"

#### For controlled microbial oxygen and food production in space



- Investigating
  - Oxygen production
  - Biomass production
  - Biomass Biochemical composition (nutritive value)
- In a controlled photo-bioreactor,
  - axenic (1 strain),
  - batch & continuous,
  - defined continuous illumination (no day/night),
  - Fixed temperature and
  - synthetic waste water
- Under spaceflight conditions : gravity, radiation, magnetism ...

### Bio-engineering – Arthrospira in mini photo-bioreactor

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Capteurs de pression



Figure 5.3 : Photographie du réacteur.

Figure 5.2 : Schéma du réacteur.

# **QINETIQ** ArtEMISS Space photobioreactor



Portable miniaturized photo-bioreactor (50ml liquid volume) Size 147 x 125 x174 mm Weight 4.2 kg (full) Energy 2,2 W (standby) - 3,4 W (incubation)

### **Complete hardware for one ArtEMISS space bigreactor**

2x Sample reservoirs

Medium ↓ supply

Rad sensors gas chamber & pressure sensor Reactor programming & commanding

Data logging & communication

Power converter from Biolab line 5V to 12V

OMU cuvette : cell density (absorbance) photosynthetic activity (fluorescence)

QINETIQ

liquid pumping

Liquid culture chamber gas membrane mixing (magnetic stirrer)

temperature sensor Hermetically sealed box

Illumination (white LED array)

External source of energy and heat required.

# Space bioreactor – intensive training and testing

Breadboards - Science models – Ground models – Flight models (2009 – 2017)



# **Challenges for BioProduction in Space**



#### **Bio-compatibility/Bio-toxicity**

- Biotoxicity of individual materials, and full assembly, with sterilization !
- Biotoxicity prevention, & removal

#### **Sterility**

- Complete sterilisation of manufactured space bioreactor materials
- Fully sterile assembly of the liquid loop & reactor components
- Maintain sterility through upload & crew handling

#### **Reversible 'stasis' of cells & proces**

- Inactivation & storage of cells
- Successful reactivation of biological cultures & bioprocess in space

# **Challenges for BioProduction in Space**





#### **Gas/liquid separation**

- high oxygen production capability cyanobacteria
- engineering appropriate gas control/removal mechanisms
- bubble free liquid loop for measurements

#### **Culture conditions**

#### Optimization of cell culture parameters for

- hermetically sealed bioreactor,
- gas overpressure (oxygen inhibition),
- LED illumination (light spectrum & intensity),
- mixing by magnetic stirring (cell integrity),
- liquid pumping (cell integrity),
  - ••

allowing good oxygen release over membrane, & cell dispersion without flocculation or biofilms, for optimal growth and production in space

# **Challenges for BioProduction in Space**





#### **Remote reactor control**

- Automatic activation & stop
- Automated data collection via temp & pressure sensors, absorbance and fluorescence measurements in liquid loop
- Data logging & data communication
- Bioreactor commanding from ground : light intensity, liquid circulation.

#### Sampling & Storage & Analysis

- Automated sampling
- Cell fixation and effluent preservation
- Sample storage in ISS and return to earth
- Methods for small sample volumes.

# ArtEMISS Space photobioreactor

# - in flight measurements & sampling for post flight analysis -

#### Measurement of



- CO<sub>2</sub> consumption by pH
- O<sub>2</sub> production by Pressure
- Cell production by OD
- Cell photosynthetic activity by fluorescence

#### **Sampling for**

post flight

- Microscopy in flight
- BioChemical analysis (frozen)
- DNA, RNA, protein, pigment analysis (fixed)
- Post-flight regrowth (live)



#### LAUNCH SpaceX CRS-13

#### 30<sup>th</sup> of November 2017 4<sup>th</sup> of December 2017

# Time to go space












Thursday 7<sup>th</sup> of December : Finally : filling the hardware ! Mers. 'filling' (the biologist !) & 4 pers. checking

OR DRINI

0 ---- 0 Row



#### LAUNCH

30<sup>th</sup>-of November 2017 4<sup>th</sup>-of December 2017 8<sup>th</sup>-of December 2017 9<sup>th</sup>-of December 2017

12<sup>th</sup> of December 2017

"the cold storage team" a special team (4 pers.) from NASA Houston, to pack all the experiments in special 'aerogel' insulator boxes with cold packs 'bricks' containing 'heavy water', which can keep passively the temperature in the box for several days at 4°C (not needing any power), according to the launch and safety regulations, for SpaceX launches.

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- Wednesday 20 the of December 2017, at ca. 10h Belgian time, the crew started their activities on our experiment on orbit in space.
- All 4 bioreactors were integrated inside the glove box next to the Biolab, and thereafter placed in the Biolab incubator.
- Thereafter from ground, the contact with our bioreactors in Biolab was restored and at ca. 12:45h the timeline of the 4 reactor was activated.





Mark Thomas Vande Hei





# Every Week – start new batch







#### ESA OPS & MUSC & BIOTESC & AIRBUS & DLR, Cologne, DE





#### It can work as on ground & as predicted



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# BIOLAB COLD SPOT SPONGE To much humidity in Biolab





09Jan18 12:44:08 INF0 : Opening display C:\Users\Admin\.centre-connector\displays\ops\CUL312190004\APM\USOC\MUSC\BIOLAS\_CONTROL\SYNOPTICS\USS\FD\LSS.uss

Raw Data  $\rightarrow$  further quantitative and qualitative analysis ongoing





Parallel Ground Experiment 4 GM bioreactors, operated by PI & PD only @ SCK•CEN, Mol, Belgium





Controlled sample storage & return SpaceX CRS-13 & SpaceX CRS-14



High-throughput Technologies, on small sample volumes, low biomass conc

Analysing samples returned on 5th of May 2018 (SpaceX CRS-14)

### LessonsLearned/Actions

### TimeLine

	15 Nov. 2017	Shipping all hardware from Belgium to KSC, Florida US		SpaceX CRS-13 Launch	
	23 Nov 2017 - 08 Dec. 2017	4 FM & 4 GM Experiment preparation @ KSC, Florid	a US	<del>30<sup>th</sup> Nov. 2017</del> 4 <sup>th</sup> <del>Dec. 2017</del> <sup>8th</sup> <del>Dec. 2017</del> 9 <sup>th</sup> <del>Dec. 2017</del> <del>12<sup>th</sup> Dec. 2017</del> <del>13<sup>th</sup> Dec. 2017</del> 15 <sup>th</sup> Dec. 2017	
	7- 15 Dec. 2017	4 FM Storage for SpaceX CRS-13 @ KSC, Florida U 4 GM transport to Belgium (dark, 4°C)	S		
	15 Dec. 2017	4 FM Launch SpaceX CRS-13 @ KSC, Florida US (dark, 4°C)		In Space !	
	17 Dec. 2017	4 FM Arrival ISS & Storage (dark, 4°C)			
START	20 Dec. 2017	4 FM Installation in Biolab ISS by Mark Vande Hei (NASA) & Reactivation of bioreactors after 13 days via remote ground control, via ESA-OPS/MUSC/BIOTESC, Cologne 4 GM manual activation, SCKCEN, Belgium (medium, light 35µE, temp 35°C)		Reactors are ON !	
				Impact storage & delay !	
				Data connection ON	
	21 Dec. 2017	After 24h first set of daily data down link, via MUSC/BIOTESC, Cologne		Data connection ON	
Wait					



# **Successful bioreactor in space**



Mark Vande Hei working on the bioreactor in Biolab glove box in ISS (Source : ESA)

First time mini photobioreactor with gas-liquid separation built, certified, uploaded and running in space, with live data feedback from space and ground commanding

- 4 bioreactors launched to ISS with SpaceX CRS-13 (15.12.2017)
- 13 days of inactivation (cooled) during upload & storage (7– 20.12.2017)
- Installation in the Biolab incubator in the Columbus module of ISS by crew (Mark Vande Hei, NASA & Joseph Acaba, NASA) (20.12.2017)
- Automated bioreactor reactivation by commanding from ground
- Total bioreactor operation duration in space of 35 days (20.12.2017 24.01.2018)
- 4x sampling of reactor effluent and biomass during the 5 weeks run
- 4x new feed supply to the reactor & sample storage in ISS by crew
- Good Gas Liquid separation, at least for some phases
- Live bioreactor monitoring from ground & daily data download link
  - Ground commands to collect samples and adjust settings (temp, light intensity, fluid circulation)
  - Sample return with SpaceX CRS-13 (13.01.2017) & SpaceX CRS-14 (5.5.2018)

#### Successful bioprocess in space - Yes it also works in space !

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Oxygen and biomass production in bioreactor FM4-B4 in space, compared to predictive model

First long-term culture of an axenic photosynthetic bacterium in ISS

First microbial production of oxygen and edible biomass in space

- Culture & Bioprocess successfully reactivated in space after 13 days of stasis
- Active photosynthetic culture of *Arthrospira* for more than 1 month in ISS
- Culture axcenicity maintained in space for at least 3 weeks (final timepoints to be analysed)
- 'Good' oxygen and biomass productivity in the bioreactor in space, compared to model – quantitated analysis ongoing
- Oxygen produced was provided to the cabin, for consumption by the crew
- Post-flight biochemical and biomolecular analysis (genomic, transcriptomic, proteomic) analysis of reactor effluent and biomass harvested from the bioreactors is ongoing

### 'spirulina' as part of the low-weight, low-waste, high-nutrient diet of astronauts during space flights

# Space Food

NASA and the European Space Agency are studying spirulina as optimum food for astronauts due to its remarkable nutritional profile.

http://www.esa.int/Our\_Activities/Human\_Spaceflight/Education/Feeding\_our\_future\_nutrition\_on\_Earth\_and\_in\_space



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### Arthrospira as food supplement for space

#### **Development of recipes & healthy menu**



Microbiome & health studies on mice, with irradiation





Universiteit Antwerpen

#### Acceptance test during Bedrest studies



Microbiome studies on human volunteers in isolation



Belgian PE Antactica station, Dec. 2017

Mars Desert Research Station (MDRS), Crew 190 (UCL to Mars), March 2018



Bibliography @ http://www.melissafoundation.org/

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# MELiSSA- Step-wise Space flight strategy

- FEMME concept paper study on small bioreactor for O2 production by Arthro + O2 consumption by BACTERIA (design) – ESA, NL
- MASK bioreactor with Arthro & control by light (mathematical model, molecular) (ground exp.) UBP, France
- ARTEMISS bioreactor with Arthro O<sub>2</sub> production (mathematical model, molecular) (ISS flight exp.)
  SCK•CEN, B (+QinetiQ, UCA, UMons)
  - » Arthrospira-B : fed-batch reactor (flight Dec. 2017 Jan. 2018)
  - » Arthrospira-C : continuous reactor (flight TBD)
- BIORAT-1 Coupling Photobioreactor compartment with a RAT/Mouse consumer compartment bioreactor, controlled by light – RUAG, CH
- URINIS Urine treatment compartment Ugent, B (+ SCK•CEN, UMons)
- BIORAT-2 Biorat-1 + Oxygen control with two Nitrogen sources Umons, B
- BIOMAN bioreactor for O2 production by Arthro + O2 consumption by MAN



### **Molecular mechanisms of ionising radiation resistance**

- how to protect against & repair from radiation damage -



# Spirulina as food supplement in DR Congo

- SCK•CEN contribution to the organisation of 'Enterpreneurs for Enterpreneurs'
- project to help fight the malnutrition of Congolese c development of local spirulina culture.
- 'Pilot' culture & harvest & cooking at SCKCEN run b
- construction of an Arthrospira sp. pilot farm in the re

Presentation Felice Mastroleo 'Mission to Mars Inspires food project in Congo'



Dr. Felice Mastroleo



#### sponsors at Belspo/ESA





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# Only possible with the help of many

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THANK YOU ALL

for supporting the preparation and operation of this bioreactor in space

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academy.sckcen.be Upcomming events Deadline June 4th, abstract 18th may



Space Summer School June 25 - July 6, 2018 SCK•CEN, Mol, BELGIUM

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https://www.sckcen.be/en/Technology\_future/Space

http://www.melissafoundation.org/

http://www.esa.int/Our\_Activities/Space\_Engineering\_Technology/Melissa

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