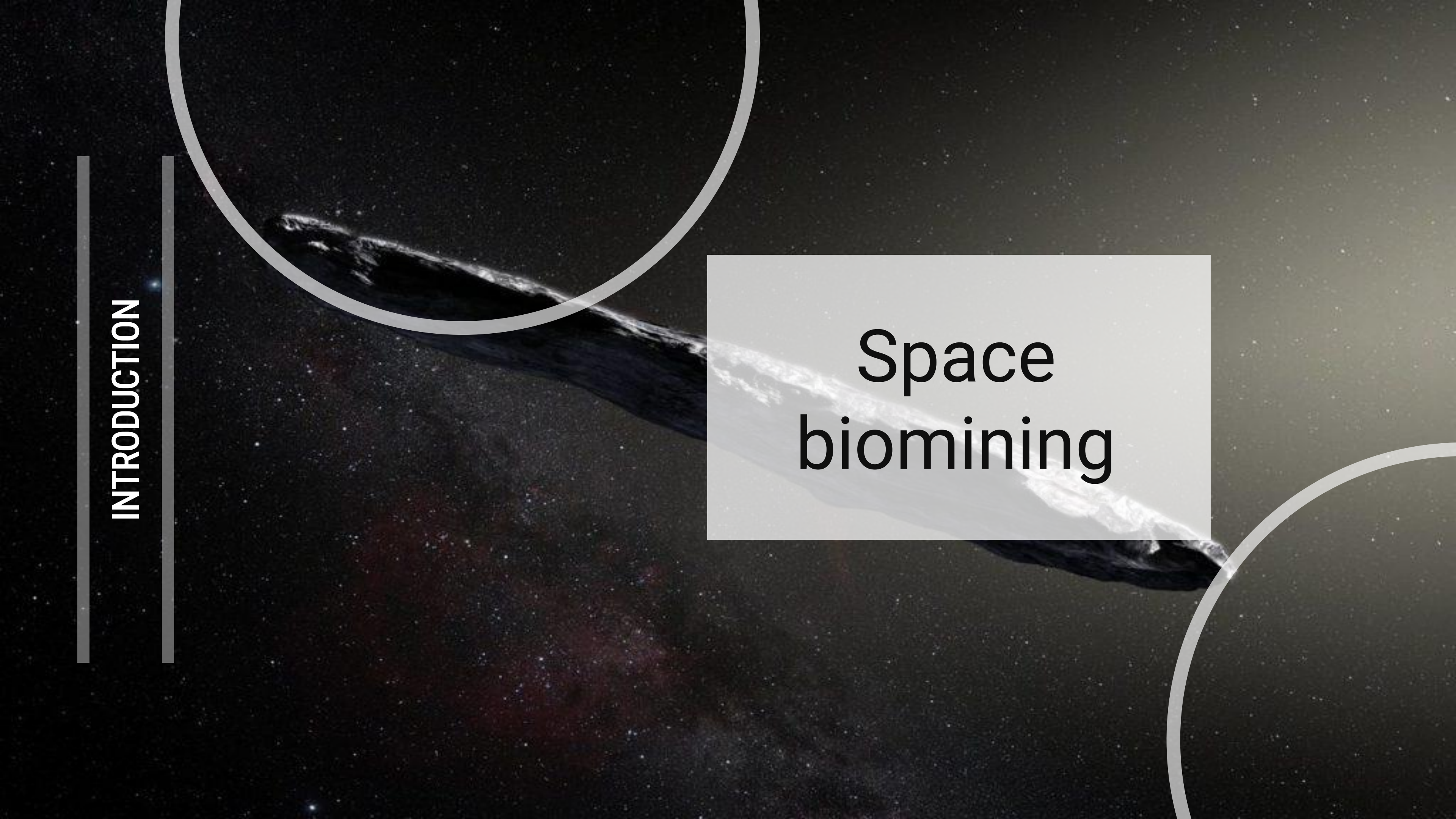
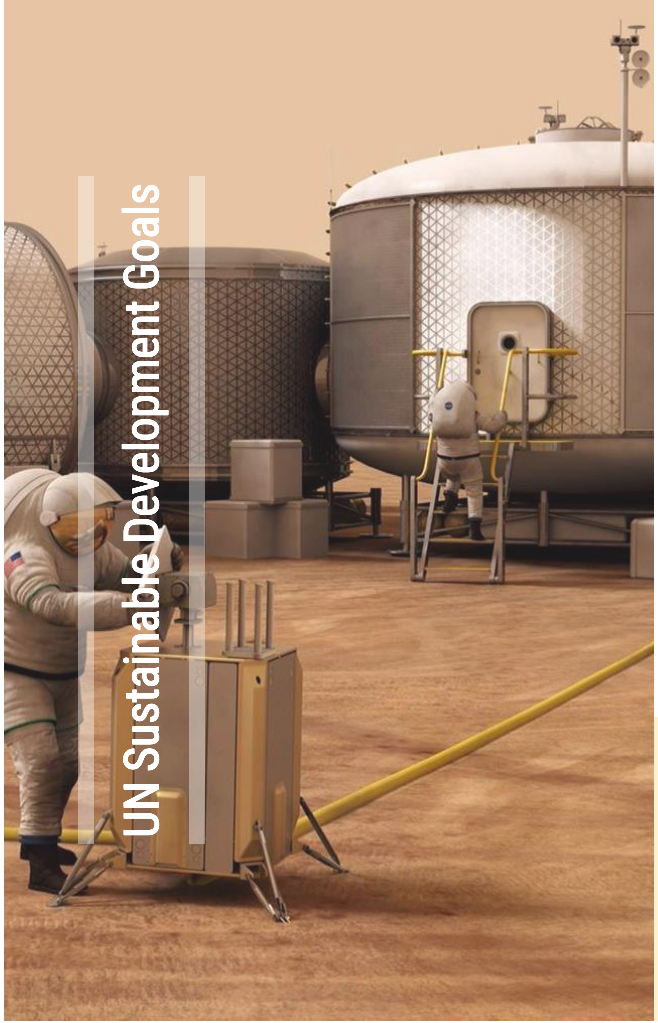
Quantification of metal extraction from a substrate by biomining processes in bacteria of the genus *Bacillus* under microgravity conditions

ICE CUBE

INTRODUCTION

Space biomining





UN Sustainable Development Goals

Industry, innovation and infrastructure:

As space exploration will require to cover long distances, alternatives to obtain the needed materials to avoid carrying heavy loads from the beginning are necessary.

Affordable and clean energy:

Using materials in abundance in the locations to explore, and generating close to no waste.

Sustainable cities and communities:

The use of *in situ* materials in a controlled manner with little waste produced will facilitate missions and the establishment of colonies.

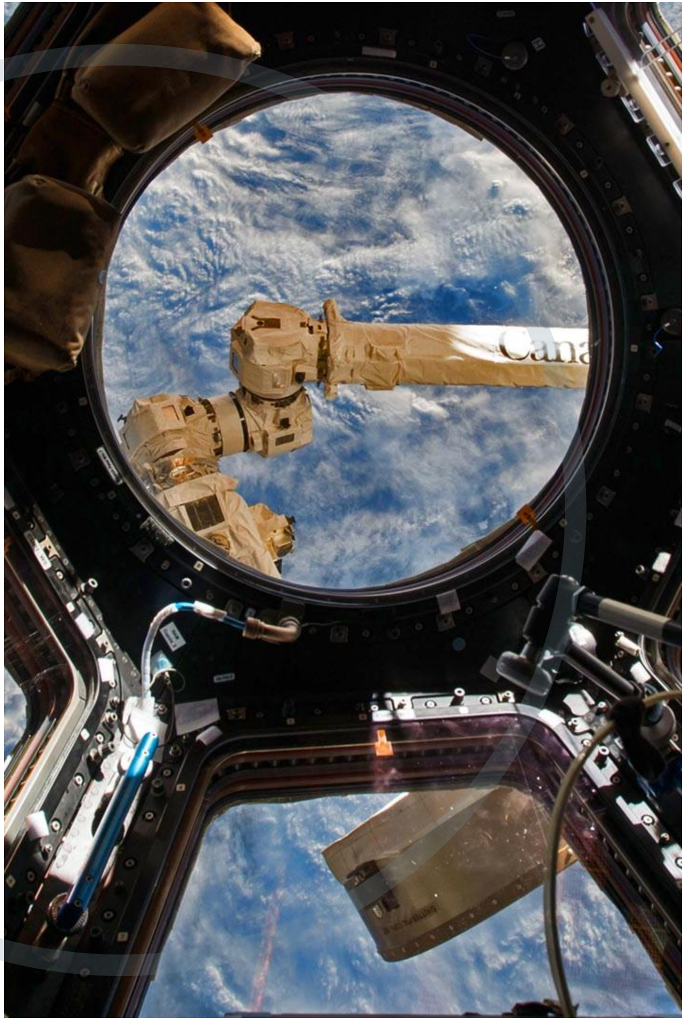
MISSION OBJECTIVES

DEVELOP
an innovative method for the study of biomining under microgravity conditions

QUANTIFY
the metal extraction from substrates for two *Bacillus* species.

ANALYZE
the data of the CO2 concentration measurements produced by the bacteria.

COMPARE
the biomining rate.



EXPERIMENTAL CONCEPT AND SETUP

CONCEPT

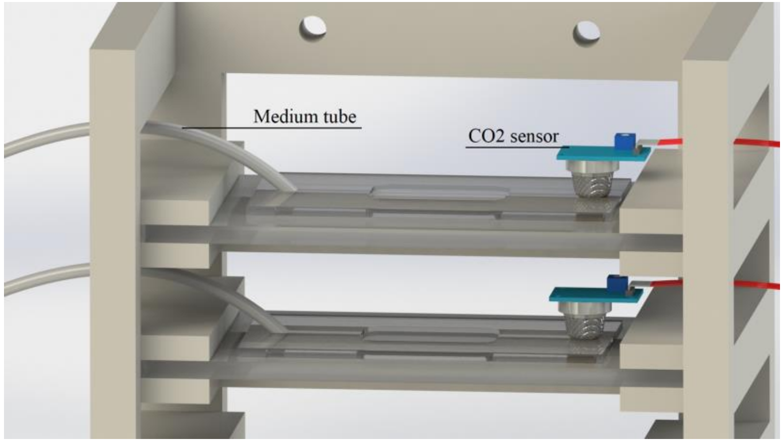
Bacillus species will be studied in different substrates to evaluate the biomining activity in microgravity conditions through the ICE Cube service.

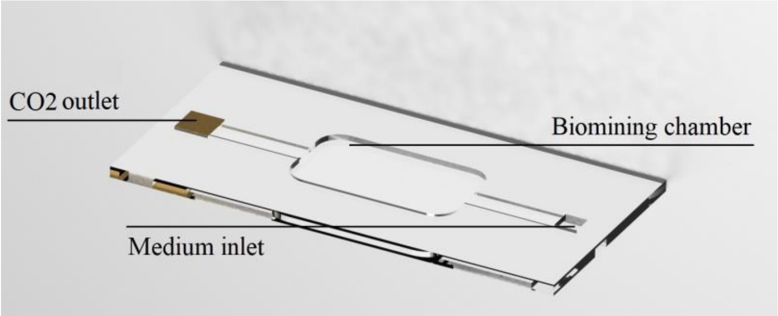
MEASUREMENT

Concentration of CO2 produced by the bacteria as a secondary product of its anaerobic metabolism during the biomining process.
Telemetry packets sent with a 0.01 Hz frequency.

MICROFLUIDIC CHIPS

Medium reaches the chamber through the microchannel.
The CO2 produced will be collected in the CO2 outlet.





KEY PERFORMANCE PARAMETERS

The pump passes the culture medium from the reservoir to the microplates.

Bacterial spores recover and produce biomining of the substrate.

The temperature sensors control the microenvironment of the plates.

CO_2 sensor is activated to measure indirectly the biomining process.

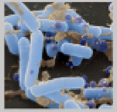
The information obtained is sent to Earth for their respective analysis.

01 SPACE SEGMENT DESCRIPTION

Experiment Subsystem

Selection of the bacterial species

Bacteria Safety Checklist

Description	*Bacillus subtilis* 	*Bacillus pumilus* 
Safe for space travel	✓	✓
Safe pH levels	✓	✓
Growth at safe temperature	✓	✓
Low pathogenicity	✓	✓
Biomining production on planet Earth	✓	✓
Safe culture medium composition	✓	✓

German TRBA 466

Risk group 1, that is the group of the lowest risk of pathogenicity.

Survive to adverse conditions of space

Capable of surviving in the form of endospores with high resistance, if they are protected against solar UV irradiation.

Biomining production on Earth

In anaerobic conditions, Fe (III) or Mn (IV) are used as a final alternative electron acceptor. The metals are reduced and CO2 is released.

Selection of the Culture Medium

pH
Between 7.0-7.4 at 25°C.

Previous studies
Was used in studies of space biomining.

Higher recovery rates
Of *Bacillus* spores after spaceflight.

Composition
Not hazardous and ideal for *Bacillus* species.

INGREDIENTS R2A:

- Casein acid hydrolysate
- Dextrose
- Dipotassium phosphate
- Magnesium sulfate
- Proteose peptone
- Sodium pyruvate
- Starch, soluble
- Yeast extract

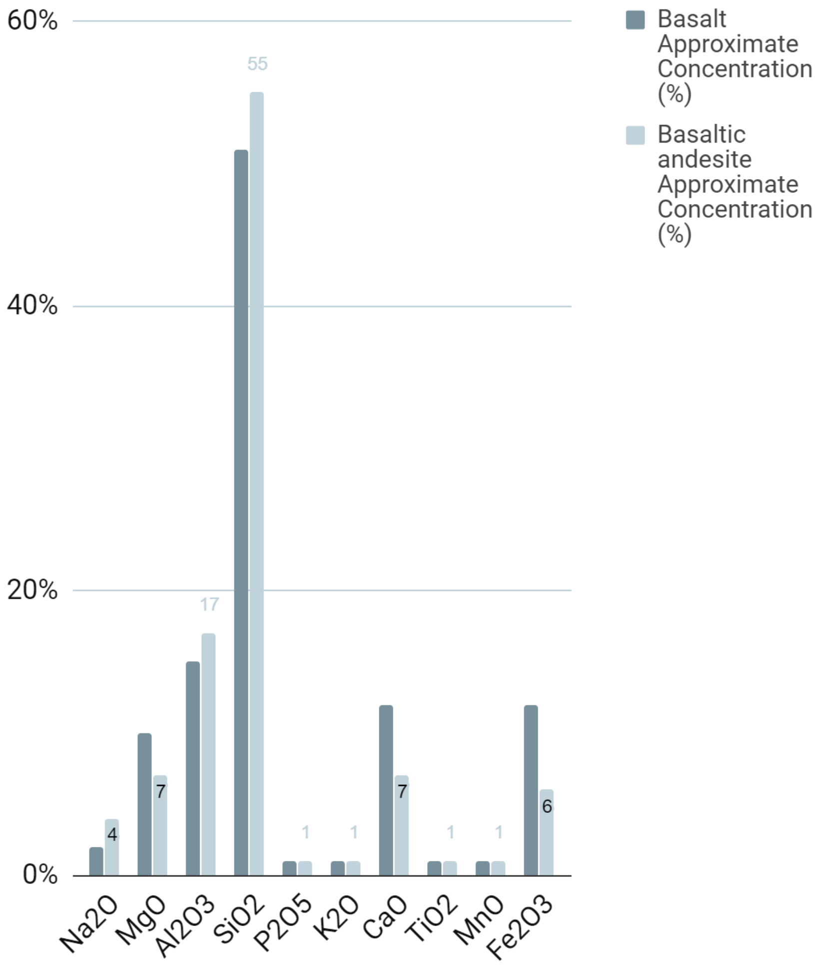
Selection of the substrate

51-55%
SiO_2

15-17%
Al_2O_3

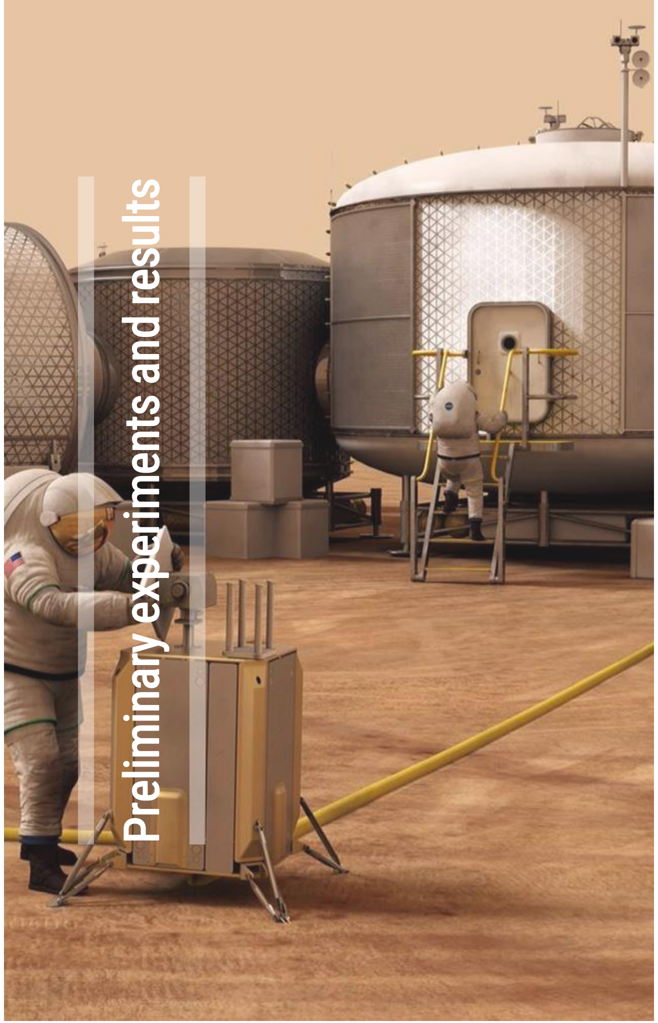
6-12%
Fe_2O_3

7-12%
CaO



Approximate chemical analysis of basaltic minerals by X-ray fluorescence

Preliminary experiments and results



Bacterial strain

Bacillus subtilis ATCC 6633, provided by the Faculty of Microbiology of the Universidad de Costa Rica.

Composition of the culture medium

- Casein
- Dextrose
- Dipotassium phosphate
- Magnesium sulfate
- Peptone
- Yeast extract

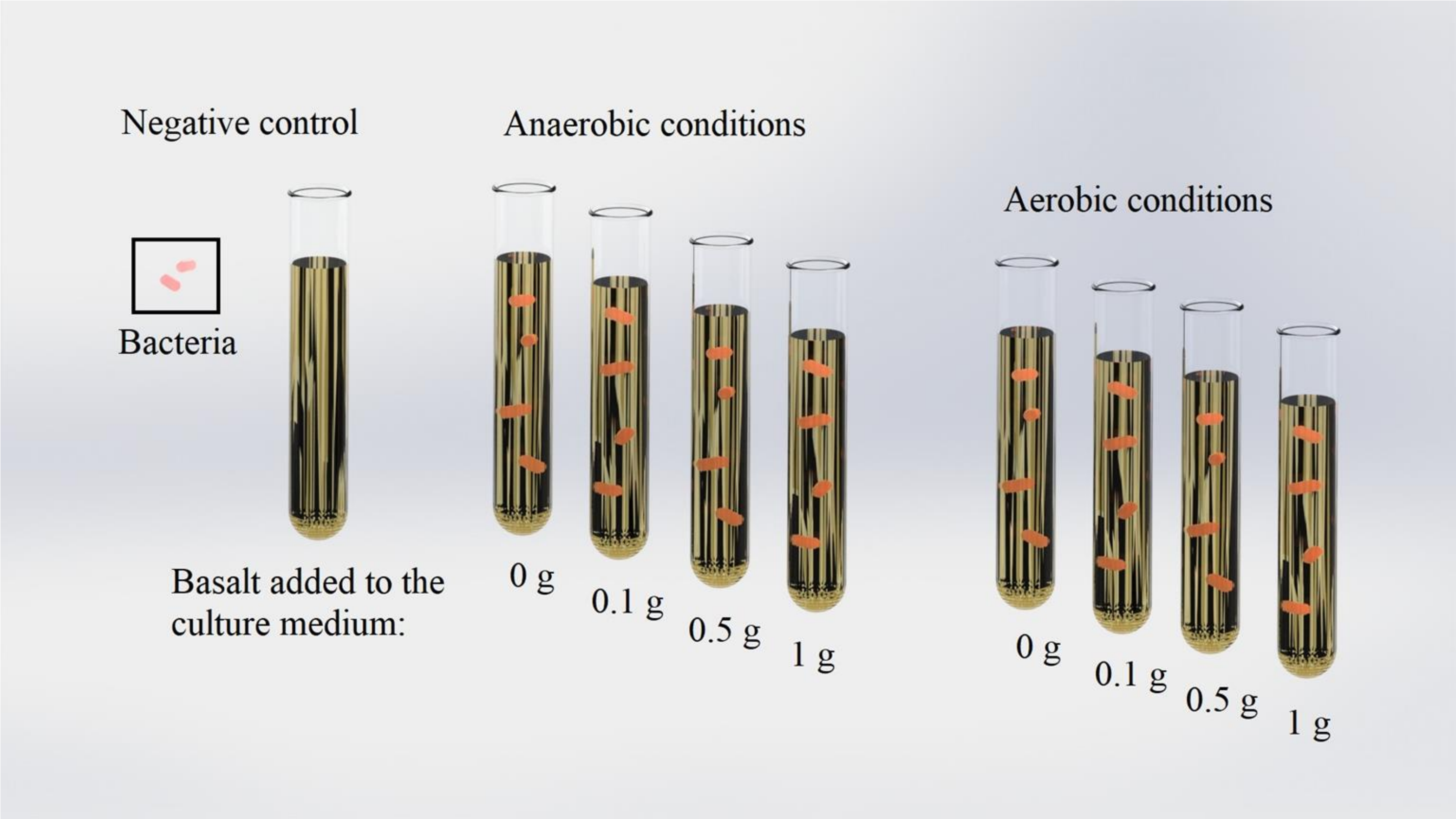
Inoculum of bacteria

Was prepared in saline solution 0.85%, using the McFarland 0.5 turbidity standard equivalent to 1.5 x 10^8 bacteria/mL

Volumes

20 mL of culture medium was dispensed into 9 tubes.

100 µL of bacteria was inoculated into the 8 tubes.



The experiment was incubated at 35°C with an atmosphere of 5% CO2 for 15 days.

Preliminary experiments and results

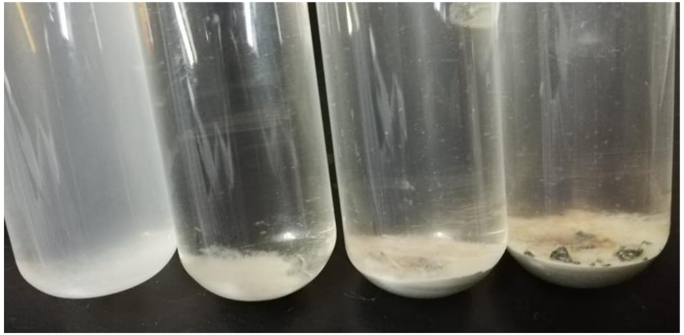
Formation of lumps in the lower part of the culture tubes on the basalt.

Bacteria may be forming a biofilm: external Fe in the microenvironment influences biofilm formation in *Bacillus subtilis*, needed to acquired Fe from the medium and growth normally.

Directly under the microscope mobile bacilli and formation of spores.

A sample of the biofilm of each tube was inoculated in Blood Agar. All the samples growth well.

Bacteria remain viable after 15 days of incubation.



Preliminary experiments and results

Sample	Analysis	Result
All samples	Measurement of soluble Mn*	No detection
0g basalt - anaerobic	Measurement of soluble Fe*	No detection
0g basalt - aerobic		No detection
0,1g basalt - anaerobic		0,27±0,03
0,1g basalt - aerobic		0,23±0,03
0,5g basalt - anaerobic		0,22±0,03
0,5g basalt - aerobic		0,19±0,03
1g basalt - anaerobic		0,29±0,03
1g basalt - aerobic		0,25±0,03

*by atomic absorption spectroscopy with a SprectrAA 220 FS Varian equipment.

Preliminary experiments and results

Culture Medium

35 ml of the culture medium R2A in each tube.

Addition of Agar

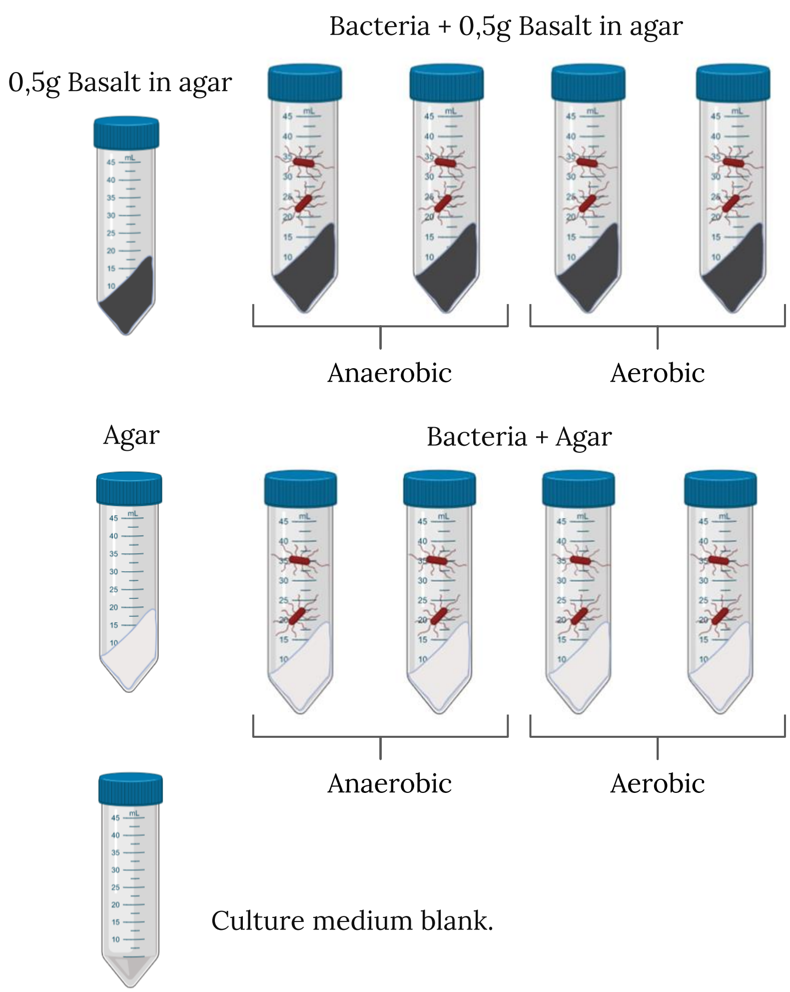
To immobilize the basalt in the tube, simulating the conditions in the chips.

Growth conditions

35°C with an atmosphere of 5% CO_2 for 15 days.

Bacteria

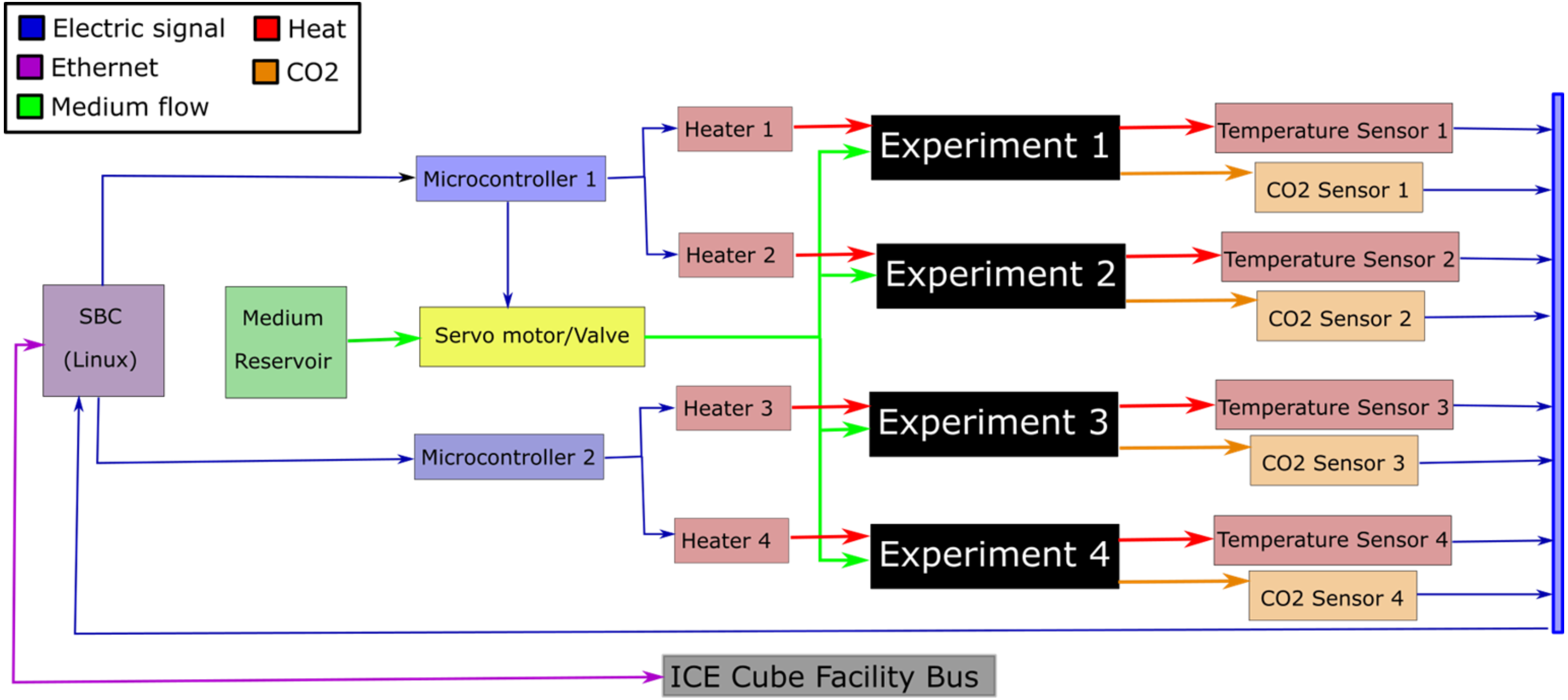
100 µL of an inoculum of *Bacillus subtilis* ATCC 6633, in saline solution 0.85%, using the McFarland of 0.5.



02 SPACE SEGMENT DESCRIPTION

Electrical Subsystem

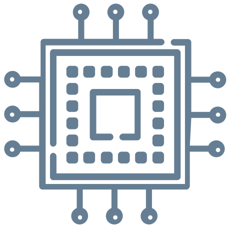
Framework


Electric signal
Heat
Ethernet
CO2
Medium flow
Microcontroller 1
Heater 1
Heater 2
Experiment 1
Experiment 2
Temperature Sensor 1
CO2 Sensor 1
Temperature Sensor 2
CO2 Sensor 2
SBC
(Linux)
Medium
Reservoir
Servo motor/Valve
Microcontroller 2
Heater 3
Heater 4
Experiment 3
Experiment 4
Temperature Sensor 3
CO2 Sensor 3
Temperature Sensor 4
CO2 Sensor 4
ICE Cube Facility Bus

Microcontrollers and SBC

Single Board Computer

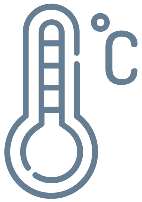
- ❖ Single board Raspberry Pi computer, with Linux Raspbian OS.
- ❖ Data recollection and communication with ground base.



Microcontrollers

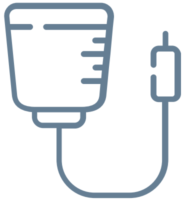
- ❖ Two ATmega328P microcontrollers.
- ❖ Control algorithms for the actuators.

Sensors and Actuators



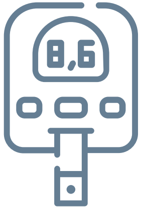
Temperature Control

- Integrated temperature sensors and heaters on each microfluidic chip.
- Imprinted by means of photolithography.



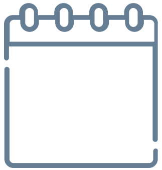
Medium Transport

- Medium reservoir with plug microvalve, that supplies the microfluidic chips.
- Operated by a servo motor.



CO2 Measurement

- One dedicated CO2 sensor for each microfluidic chip.
- Coupled to the experiment chamber.



Other Measurements

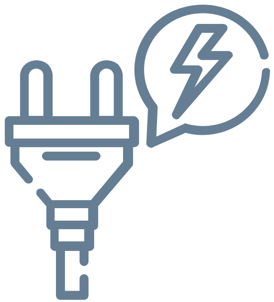
- Magnetic, gyroscopic, humidity and pressure sensors.
- Integrated on the framework.

Power Consumption

Device		Mass (g)	Power (mW)
Raspberry Pi 3		50	2500
ATmega328P	(2)	8.38	52
CO_2 sensor	(4)	10	14
Temperature sensor	(4)	8	12.6
Humidity sensor	(2)	5.4	10
IMU	(2)	4.2	9.23
Servo motor		9	-
		94.98	**2589.9**

Power

2590 mW

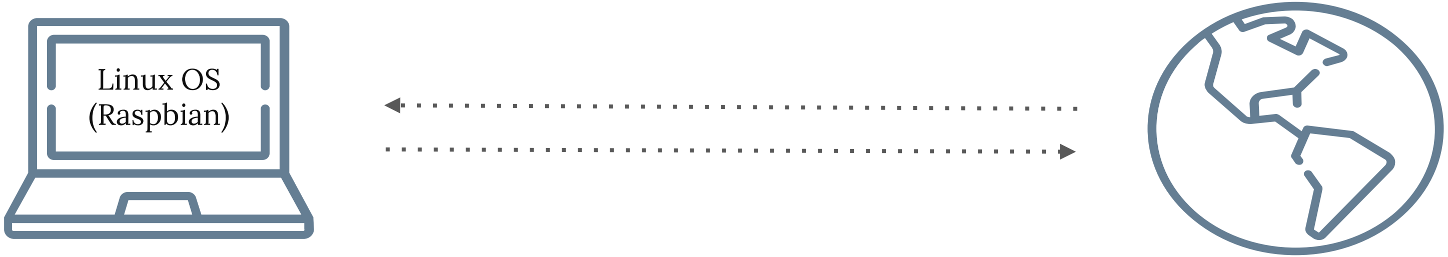


Peak Power

4158.24 mW
security factor: 1.6

Communications and Data Handling

Raspberry Pi 3

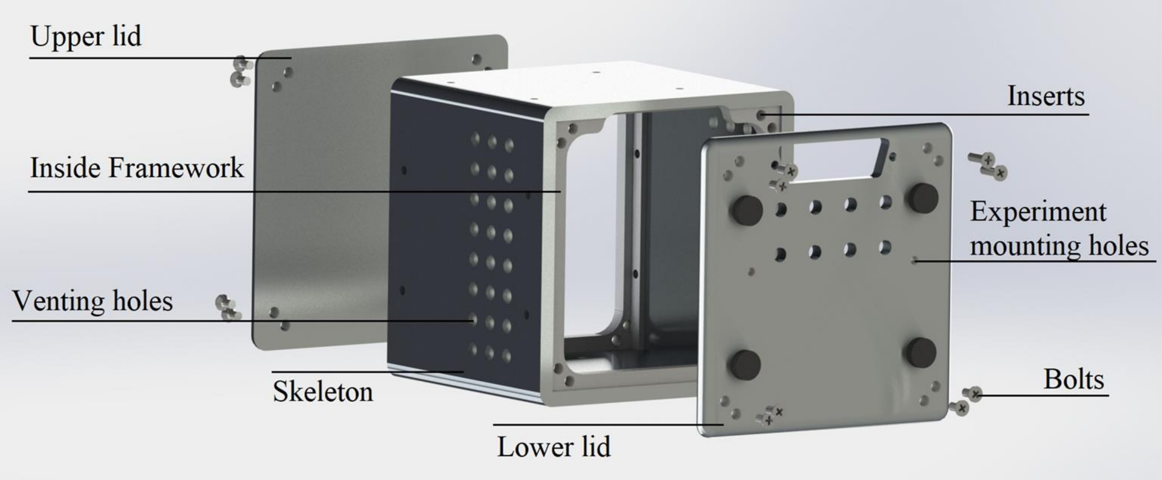


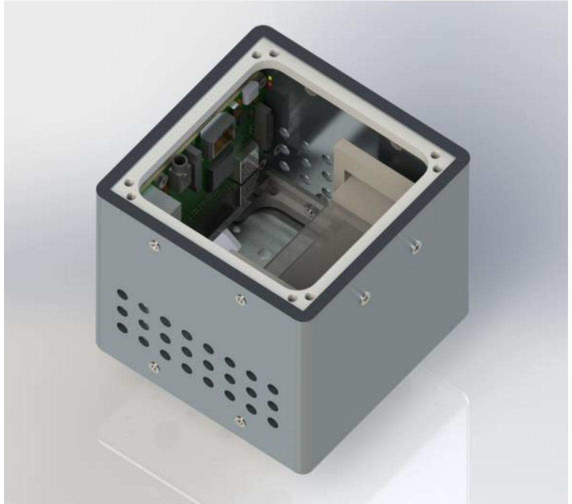
- ❖ TCP protocol
- ❖ IP address assigned by ICE Cubes Mission Control Centre
- ❖ Obtained data periodically sent via SFTP.

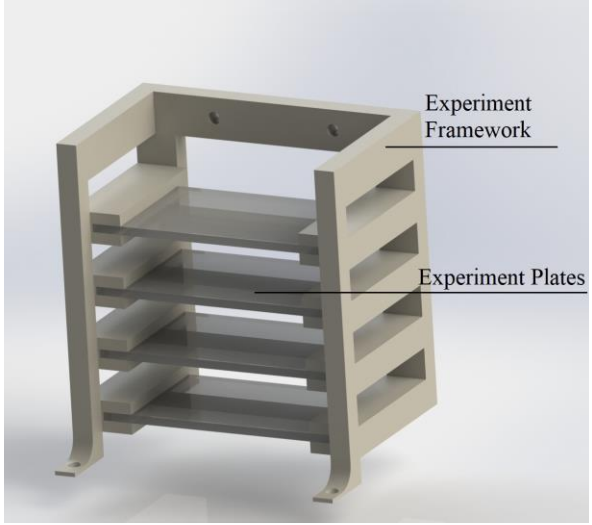
03 SPACE SEGMENT DESCRIPTION

Mechanical Subsystem

CASING DESIGN







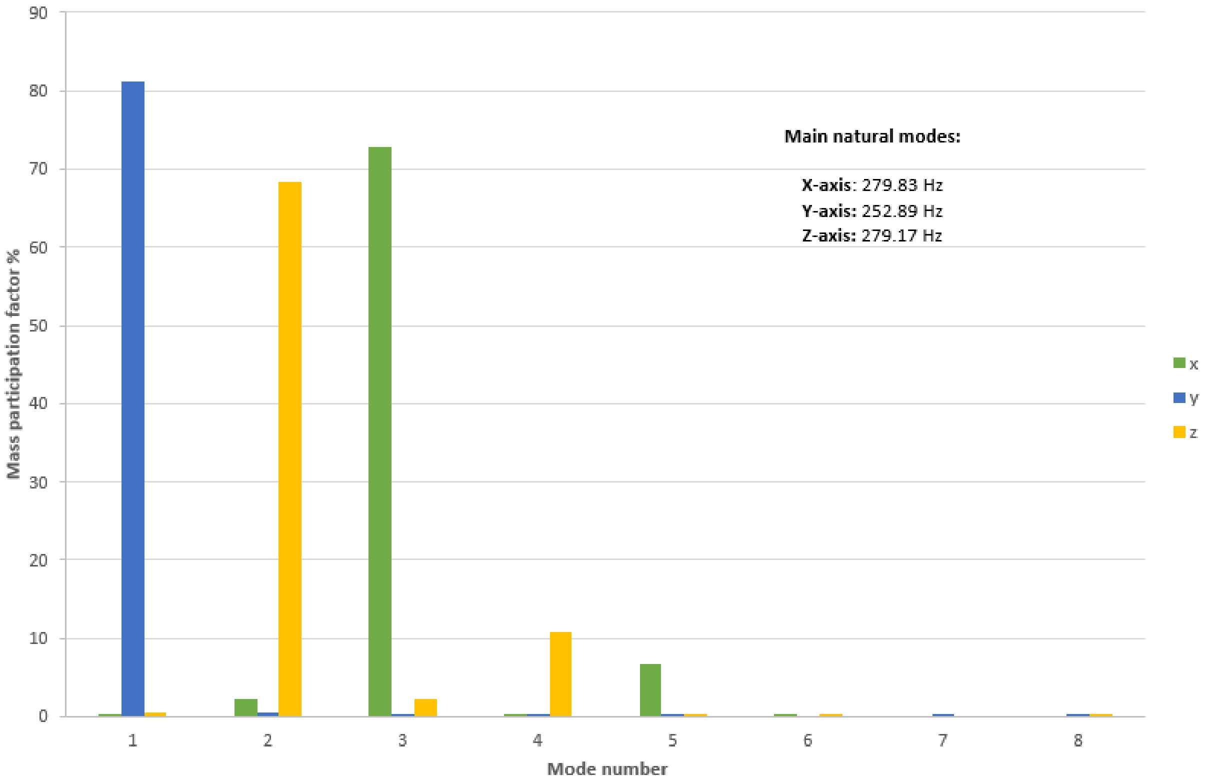
STRESS ANALYSIS

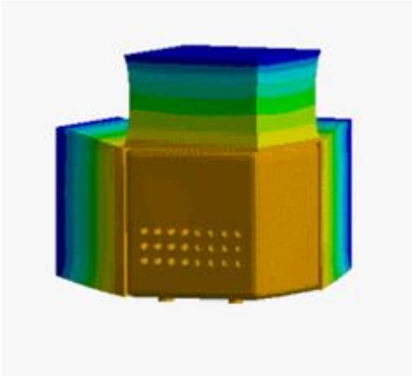
Component	Mass (g)	Material
Skeleton and lids	435	Aluminum 7075
Bolts	<25	Stainless Steel
Inside framework	25	Alumide
Experiment framework	60	Alumide
Experiment plates	35	Glass
Inserts	<5	Stainless Steel
Total	**585**	

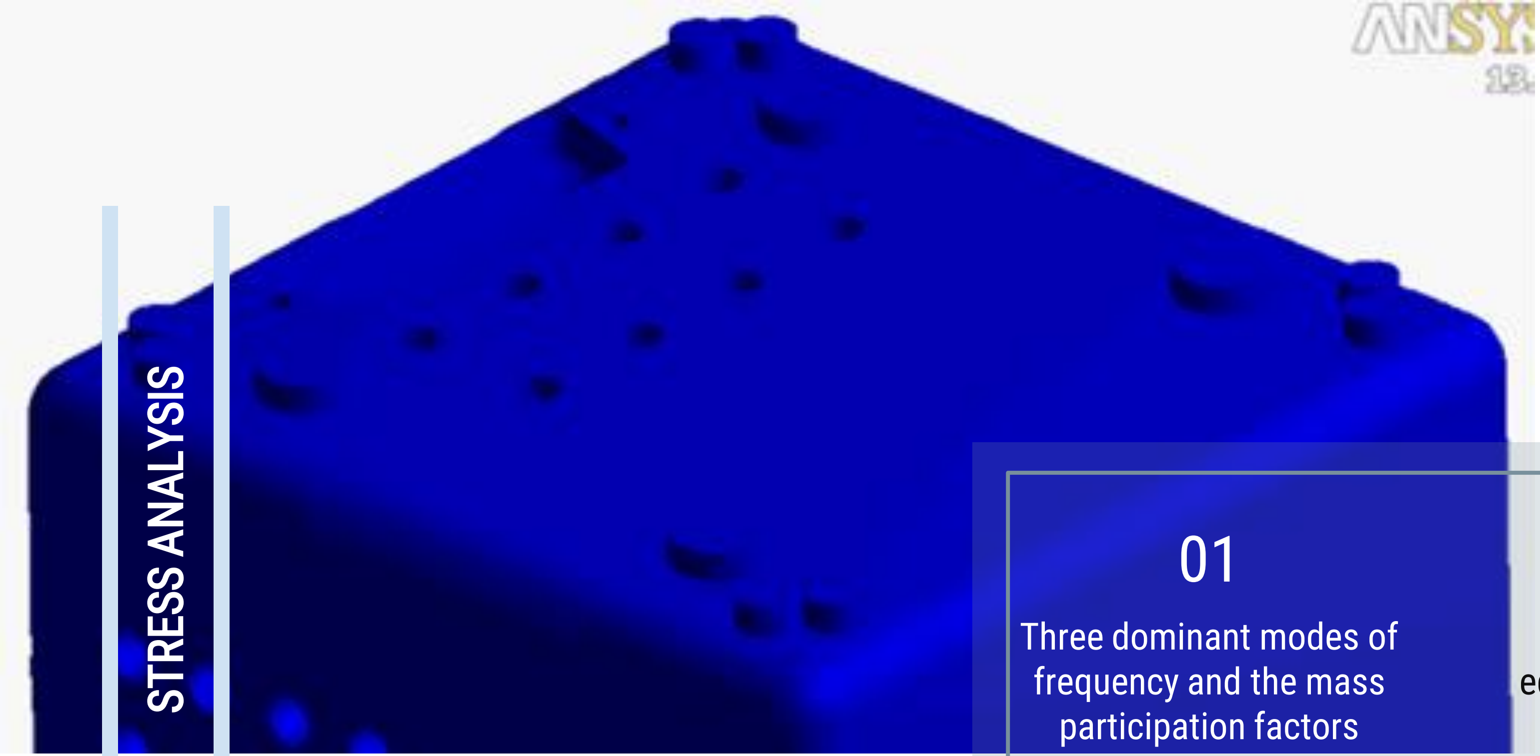
Total structure mass

585 g

STRESS ANALYSIS







STRESS ANALYSIS

01

Three dominant modes of frequency and the mass participation factors

02

Random vibration equivalent static inertia loads (RVL)

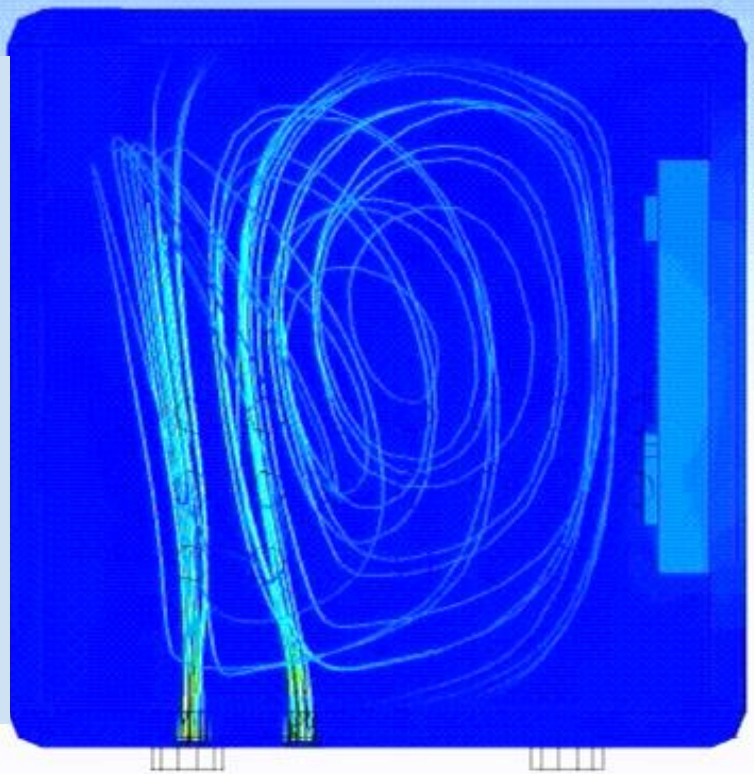
03

Von Mises stresses

04

Yield and ultimate margins of safety

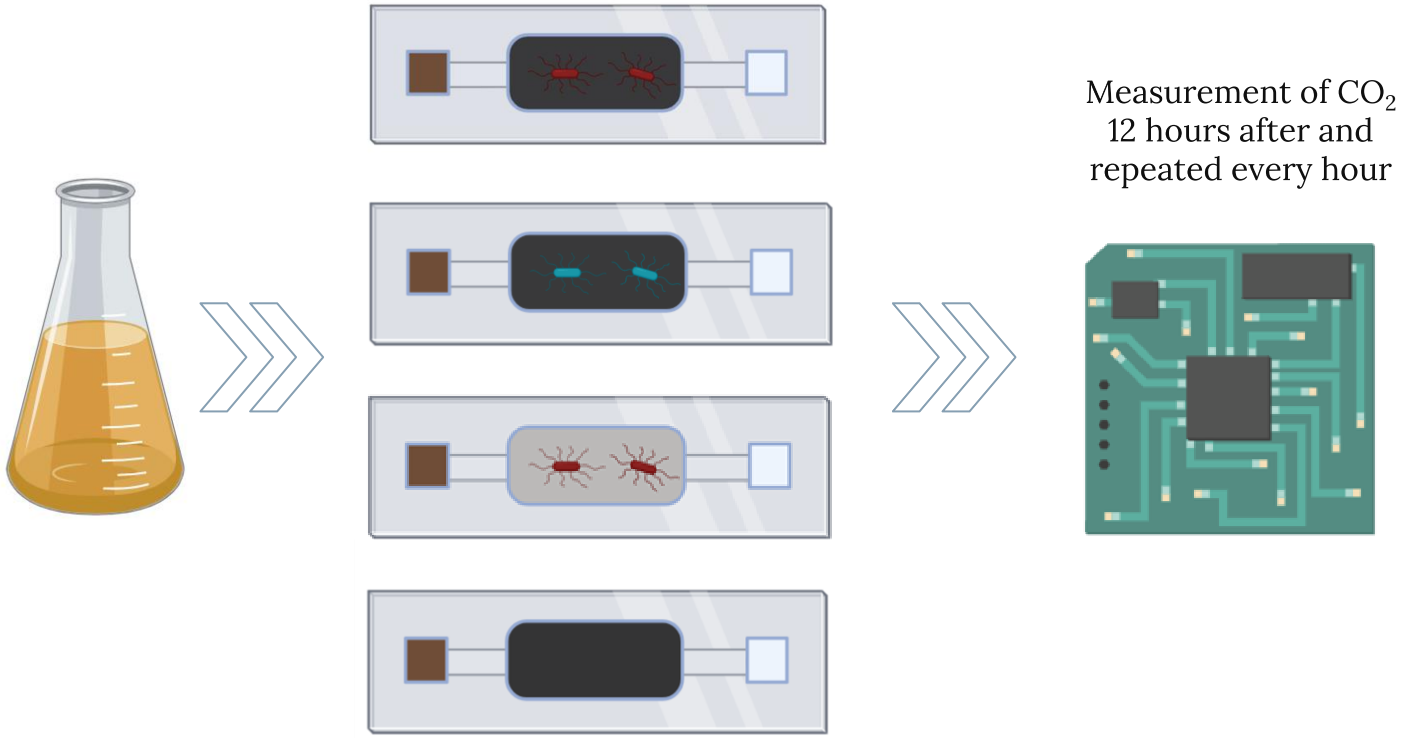
THERMAL ANALYSIS



- Finite element simulation of the effect of the forced convection provided by the hosting rack.

- Equilibrium temperature of around 13 degrees higher than the surrounding temperature.

CONCEPT OF OPERATIONS



Communication of the ICE Cube with ground: TCP protocol with a private IPv4 address, via SFTP service.
Work station with software running under Linux Ubuntu.

IMPLEMENTATION PLAN

- ICE Cube: Universidad de Costa Rica (UCR) Mechanical Engineering Workshop.
- Microfluidic chips: UCR Laboratory of Solid State Physics (CICIMA).
- Cleanroom in the Center of Electrochemistry and Chemical Energy (CELEQ) and Semi clean room in the Center of Investigation in Atomic, Nuclear and Molecular Sciences (CICANUM).
- Faculty of Microbiology, UCR.

Total cost of the mission: $60000

- $55000 for a 4 month life cycle on the ISS
- $2000 for the electrical and mechanical parts of the ICE Cube
- $3000 for experimental testing on bacteria, culture medium and substrate

Funded partly by the UCR, private sponsors and governmental entities.

IMPLEMENTATION PLAN

Task	2020				2021				2022	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Funding	■	■	■	■						
Mission Feasibility Evaluation	■									
Development and Construction		■	■	■						
ICE Cube Integrated Tests					■	■				
Launch							■			
Start of Mission							■			
Data Analysis							■	■		
End of Mission (Return/Disposal)								■		
ICE Cube Examination									■	
Publication of results										■

IMPLEMENTATION PLAN

Risk	Probability	Impact	Risk Score
Failure in retrieval of data measured.	2	2	4
Problems in manufacturing and integrating stages	1	5	5
Insufficient funding for the project	3	2	6
Contamination of culture samples	2	5	10
Behavioral change not considered normal in bacterias	3	4	12

ACKNOWLEDGMENTS

ESCUELA CENTROAMERICANA DE GEOLOGÍA

EIM Escuela de Ingeniería Mecánica

grupo de ingeniería aeroespacial

Vicerrectoría de Vida Estudiantil

EIE Escuela de Ingeniería Eléctrica

Mi Ba

CELEQ Centro de Electroquímica y Energía Química Universidad de Costa Rica

UNIVERSIDAD DE COSTA RICA

CICIMA

CICANUM

FMic Facultad de Microbiología

LASA Laboratorio de Servicios Analíticos

ESCUELA DE QUÍMICA

Quantification of metal extraction from a substrate by biomining processes in bacteria of the genus *Bacillus* under microgravity conditions

ICE CUBE