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A novel process to grow edible microalgae on Mars by exploiting in situ-available resources: Experimental investigation

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ABSTRACT

The achievement of manned missions on Mars is one of the main challenges the humanity is going to face in the next future. In this context, the possibility of growing Spirulina (*Arthrospira Platensis*) intended to produce food for crew members on Mars has been investigated in this work. The experiments have been carried out in a novel device capable to simulate microgravity and an inner atmosphere very similar to the Martian one in terms of chemical composition. This device simulates the conditions taking place within a Martian dome hosting the relevant photobioreactors according to a novel technology recently proposed in the patent literature. The growth medium has been obtained using a mixture, called Martian Medium, consisting of a mixture of Mars regolith leachate and astronauts' urine simulants to verify the possibility of exploiting in-situ available resources and reducing the payload associated to the mission. The obtained results have shown that *A. platensis* was capable to grow with a good productivity in a medium containing up to 40 %vol of Martian Medium. Moreover, when using this mixture in the developed device the obtained biomass productivity (~0.048 g L^{-1} day^{-1}) was higher than the one correspondingly gained using optimal growth medium and Earth conditions (~11 g L^{-1} day^{-1}). Ultimately, the use of Martian Medium in the developed device led to a growth rate much higher than the one achievable on Earth with classical media likely because the CO_2 rich atmosphere was capable to avoid carbon starvation phenomena while microgravity conditions reduced settling and aggregation of cells, thus leading to a better diffusion transport of dissolved nutrients to algae. Considered the obtained productivity and astronauts needs it was estimated that by taking advantage of this technology, a culture of about 15 m^3 available within pressurized domes would be sufficient to meet the protein needs of a crew of six members.

1. Introduction

Overpopulation, Earth resources depletion, pandemics and climate crisis call for the identification of new strategies to save the future of humanity. The current strategies to tackle these concerns involve the use of renewable energies and materials, recycling of wastes, water savings, economic degrowth etc. While these strategies can for sure help humanity on the short-medium term, it is now well established that, in the long term, humanity should be capable to travel and live on other planets to survive [1].

The accomplishment of this target necessitates the identification of new technologies for the sustainment of long duration manned missions in planets beyond the Lower Earth Orbit (LEO) [2]. So far, several studies have been devoted to developing Environmental Control and Life Support Systems (ECLSSs), also involving microalgae, cyanobacteria and microorganisms, which permit to produce water, oxygen, and food by totally recycling crew metabolic wastes including exhausted cabin air [3–6]. However, in view of their use on other planets, the current ECLSSs are not completely self-sustaining and thus require the integration of external inputs of supplies to meet the astronauts needs [3,7,8]. Since interplanetary trips are quite expensive [7], the integrative amounts of consumables cannot be continuously replenished from Earth but must be produced on the hosting planet by exploiting the approach represented by the acronym ISRU (In Situ Resource Utilization). Therefore, the ideal

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strategy for sustaining long duration manned missions beyond LEO involves the synergistic coupling of ECLSSs and ISRU technologies [3].

Among the planets where human life might be made possible by using this approach in the short-midterm, Mars represents for sure the better candidate due to its proximity to Earth, which would allow even some re-supply trip, the corresponding temperatures that are close to those of continental winters (~−14 °C on average in the equator), the day duration (~25 h), the average solar irradiance levels (~20 mol m^{-2} sol^{-1}) and the presence of resources such as atmospheric CO_2, water and regolith which might be transformed and in situ exploited to produce useful consumables [8]. For these reasons the main Space Agencies, gathered in the International Space Exploration Coordination Group, have listed manned missions to Mars as a common target [7].

Accordingly, one of the main challenges is today the development of technologies integrating ISRU and ECLSSs systems for sustaining crewed missions to Mars. In fact, while different regenerative ECLSSs have been developed and tested in the International Space Station [6], and several ISRU technologies are being investigated on Earth [9,10], only few papers or patents have been devoted to integrating of these systems for crewed missions to Mars [8]. In this regard, the paper by Abney et al. [11], discusses different possible technologies and architectures at the proof of concept stage to integrate ECLSS and ISRU, along with the challenges to face for their implementation on Mars. A detailed design of a self-sufficient smart colony on Mars relying on the coupling ISRU and ECLSSs systems was also presented by Miyajima et al. [12,13], in the framework of the SpaceX mission. However, these works consist of simulations that often rely on assumptions about the potential performances of the ELCSS-ISRU system that are not corroborated by experimental data obtained through suitable apparatuses operated under environmental conditions that really simulate the Martian ones.

Furthermore, while the use of bio-engineering techniques involving microalgae, macroalgae, bacteria and fungi is currently well established in the realization of bioregenerative ECLSS [2,3,6,7], very few works exist in the literature dealing with the possibility of using microorganisms to transform in-situ available resources such as regolith and atmosphere into useful supplies on Mars. In fact, most ISRU technologies so far proposed consists of ~~physic~~physico-chemical methods for oxygen and propellants production from Martian regolith and atmosphere, but cannot contribute to the production of food [9,10]. On the other hand, in-situ food production is the main bottleneck for manned mission since ECLSSs can contribute only to a limited extent to the needs of a crew, which have been estimated to be ~3000 cal sol^{-1} when the latter one is composed by 6 members [8]. Thus, ISRU technologies for food production on Mars are needed. Albeit several works envision the cultivation of crops, algae and fungi on Mars only few papers deal with the transformation of Martian resources into edible biomass under operating conditions that simulate the Martian ones.

In this regard, the few bio-ISRU technologies proposed to produce food on Mars can be divided into two main categories, i.e. the ones relying on methylotrophic bacteria and the ones using litho-autotrophic microorganisms. The first ones consist of using methanol, which can be produced in-situ via ~~physic~~physico-chemical ISRU processes [9], to obtain protein-rich edible biomass by means microorganisms such as *Pichia pastoris* and *Methylophilus methylotrophus*or engineered *Escherichia coli* and *Bacillus subtilis* [8,14]. The second category of bio-ISRU processes are based on rock weathering cyanobacteria which can photosynthetically convert N_2 and CO_2, along with S, P, Fe and several micronutrients, available in the Mars atmosphere and the regolith, respectively, into newly formed edible biomass by relying on the water and the light available in-situ [7,15]. In fact, several cyanobacteria or microalgae are capable to tolerate extreme environmental conditions such as low temperature and pressure, high CO_2 concentration and low pH values [16,17]. Moreover, the use of cyanobacteria and microalgae leads to the further positive effect of producing photosynthetic oxygen which is crucial for the crew and can integrate the amounts of oxygen produced via physico-chemical methods.

In this regard, the possibility of using several cyanobacteria, exposed to simulated Martian conditions (−27 °C, 0.8 kPa, pure CO_2) has been investigated by Olsson-Francis and Cockell [15]. The experimental results indicated that the strains *Anabaena cylindrica PCC 6309Chroococcidiopsis 029*, *Gloeocapsa* sp. *strain OU_20*, *Phormidium* sp. *strain OU_10 Leptolyngbya* sp. *strain OU_13* were able to survive several days under Mars simulated conditions and using a regolith simulant as growth substrate [15]. Therefore, albeit neglecting the effects of microgravity, this study demonstrated that cyanobacteria can be used for ISRU purposes.

Recently Verseux et al. [7] investigated the diazotrophic growth of *Anabaena* sp. PC7983 under an artificial low-pressure (~101 hPa) atmosphere composed by N_2 (96%) and CO_2 (4%) which the authors envisioned to be produced by using the Martian atmosphere. The results showed that this strain was capable to vigorously grow by taking C and N from such atmosphere and other micronutrients from a Martian regolith simulant immersed in the growth medium BG-11 [7]. Thus, while the possibility to obtain such kind of atmosphere within closed domes or greenhouses on Mars still needs to be evaluated, and the effects of microgravity were neglected, even this relevant work demonstrated that cyanobacteria cultivation by exploiting in-situ available resource on Mars might be feasible.

In the work by Billi et al. [18] the capability of the autotrophic strain *Chroococcidiopsis* sp. CCMEE 029 to tolerate perchlorate salts, that are typically found in Mars regolith, was investigated. The obtained experimental results showed that exposition to Mars-relevant concentrations of Mg or Ca perchlorate did not affect the growth of this strain under simulated Martian conditions thus demonstrating that *Chroococcidiopsis* is a good candidate for bio-ISRU contexts on Mars.

To the best of our knowledge, aside the works cited above only few and less comprehensive papers dealing with the use of Mars environmental resources to sustain the growth of microalgae and cyanobacteria can be found in the literature. Moreover, all these papers had the common lack that the effect of microgravity (~1/3 g on Mars) was neglected during the experiments.

Thus, further research activity is needed to verify the possibility to use microalgae and cyanobacteria as potential food source in the framework of manned missions on Mars that rely on ISRU technologies. Accordingly, in this work, by means of a novel experimental apparatus, we investigate the possibility of using Mars regolith and atmosphere to grow a cyanobacterium called *Arthrospira Platensis (A. platensis)* under microgravity conditions, within pressurized and heated domes, in the framework of a process which also exploits the urine simulant produced by crew members as a potential component of the growth medium for this alga. The envisioned ISRU-ECLSS process which the experimental results refer to is the one recently proposed in the patent by Cao et al. [19] and shown in Fig. 1.

Therefore, the experimental results achieved in this work also serve to evaluate the in-situ feasibility of this process. The strain *Arthrospira Platensis* was chosen among the other because characterized by a higher nutritional power and the high content of proteins which make it as suitable food for astronauts. In fact, several studies showed that *A. platensis* is considered as a superfood and it is also known as the “food of the future” for its high nutritional value [20]. Nowadays it is already used to produce several types of food [21]. Due to its impressive protein content and its fast growing in mineral environments, also space agencies and industries have expressed interest including such strain in their research program by exploiting the ISRU-ECLSS process [22]. *A. platensis* contains up to 70% complete protein with high biological value and digestibility, about 10% of lipids and 20% of carbohydrates on a dry weight. *A. platensis* showed an antioxidant activity due to its content in Vitamin E and carotenoids, it has also anti-inflammatory, antitumor and immunostimulant activity [21].

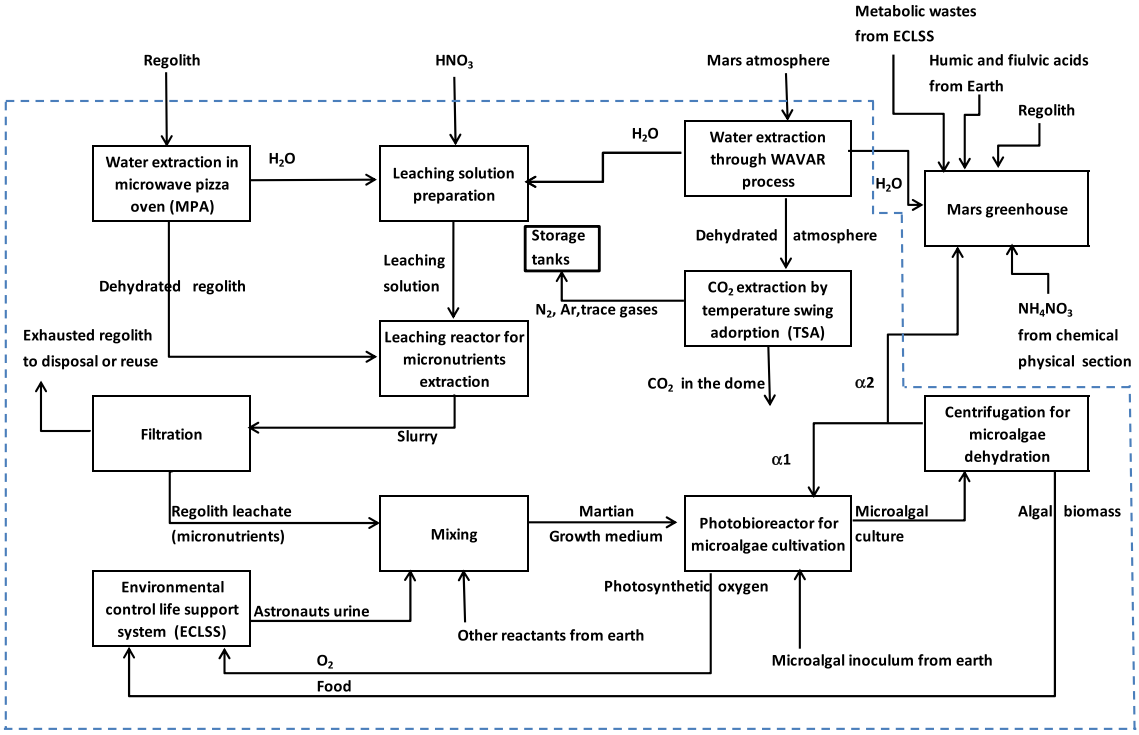


Fig. 1. Patented process integrating ECLSS and bio-ISRU to produce food and oxygen on Mars through microalgae. The process is envisioned to take place within a pressurized dome wherein a temperature >10 °C is ensured.

2. Materials and methods

2.1. Microorganism maintenance conditions

Unialgal culture of cyanobacterium A. *platensis* was obtained from the operating plant of TOLO Green located in Arborea, Sardinia, Italia. The strain was maintained at the laboratory of the Interdepartmental Centre of Environmental Sciences and Engineering (CINSA), University of Cagliari, Sardinia, Italy, under axenic conditions. The cultures were kept in 250 mL Erlenmeyer flask, containing 150 ml Zarrouk's medium (ZM) sealed with an aluminium foil that covered cotton wool. The chemical composition of ZM is reported in Table 1.

Flasks containing the culture media were autoclaved at 121 °C for 15 min prior to inoculation. The cultures were kept under photoautotrophic conditions and incubated in a thermostatically controlled chamber at 20 ± 1 °C. The photoperiod was fixed at 12:12 h light and dark periods with white light illumination of 25 μmol m^{-2} s^{-1} (Light meter Delta OHM HD 2302.0). Stirring was set at 100 rpm.

2.2. Preparation and composition of the martian medium (MM)

The JSC Martian regolith simulant (JSC MARS-1) utilized in this work was provided by Orbital Technologies Corporation (Madison, WI, USA). MM was prepared by mixing a leachate of Martian regolith simulant and synthetic human urine (MP-AU) to simulate astronauts' one.

2.2.1. Preparation of the regolith leachate (RL)

Main constituents of JSC MARS-1 used in the present study are reported in Table 2 in terms of oxides wt%.

According to the literature [23], the mineral phases of JSC MARS-1 identified by XRD analysis consisted primarily of tectosilicate plagioclase feldspar, pyroxene, iron oxides (magnetite and hematite), ilmenite and olivine.

The regolith leachate (RL) was prepared by contacting 15 g of

Table 1
Composition of the Zarrouk's medium (ZM).

Components	(g/L)
NaCl	1.00
EDTA	0.08
$FeSO_4$ x $7H_2O$	0.01
$MgSO_4$ x $7H_2O$	0.20
$NaHCO_3$	16.80
$CaCl_2$ x $2H_2O$	0.04
K_2HPO_4	0.50
$NaNO_3$	2.50
K_2SO_4	1.00
Micronutrients	(mg/L)
H_3BO_3	2.86
$Na_2Mo_7O_4$	0.0177
$CuSO_4$ x $5H_2O$	0.079
$ZnSO_4$ x $4H_2O$	0.222
$MnCl_2$ x $4H_2O$	1.81

Table 2
Composition of Martian Regolith simulant JSC Mars-1A in terms of oxides.

Major element composition[a]	wt %
Silicon dioxide (SiO_2)	34.5–44
Titanium dioxide (TiO_2)	3–4
Aluminum oxide (Al_2O_3)	18.5–23.5
Ferric oxide (Fe_2O_3)	9–12
Iron oxide (FeO)	2.5–3.5
Magnesium oxide (MgO)	2.5–3.5
Calcium oxide (CaO)	5–6
Sodium oxide (Na_2O)	2–2.5
Potassium oxide (K_2O)	0.5–0.6
Manganese oxide (MnO)	0.2–0.3
Diphosphorus pentoxide (P_2O_5)	0.7–0.9

[a] The normal convention for data presentation uses oxide formulae from an assumed oxidation state for each element (with the exception of Fe) and oxygen is calculated by stoichiometry.

regolith simulant (<1 mm diameter size) with 150 ml of ultrapure water having a pH 6.80 within a 250 ml Erlenmeyer flask with cap. The solid liquid mixture was stirred at 200 rpm through an orbital shaker (Stuart SSM1, Bio sigma) for 24 h at 25 °C. The resulting solution was filtered by gravity by means of bibulous paper.

Analysis of the supernatant was carried out using an inductively coupled plasma (ICP) optical emission spectrometry (Varian 710-ES ICP OES) for the determination of Al, Ca, Fe, K, Mg, Mn, Na, P, Si, and Ti. ICP operating conditions were as follows: radiofrequency generator power 1.2 kW, frequency 40 MHz; Ar (99.996% purity) was used both for plasma (15 L/min), nebulizer (200 KPa), and optic supply (1.5 L/min). The spray chamber was a double-pass, glass cyclonic. The power and pressure applied were 600 W and 100 PSI for 13 min. Calibration curves were calculated on five points and were considered acceptable for $R^2 \geq 0.999$. The results of RL analysis through ICP are shown in Table 3.

2.2.2. Preparation of synthetic urine

Synthetic human urine (MP-AU) was produced according to the literature [24] and then diluted with ultrapure water at a ratio of 1:10 to both simulate the diluting effect of flushing water in ECLSS systems and meet the nitrogen requirement of microalgae. The chemical composition of diluted human urine simulant is shown in Table 4.

2.2.3. Preparation of martian medium (MM)

Finally, one-part of the leachate of Martian regolith and one-part of diluted urine were mixed (1:1 *v:v*) to produce the so-called Martian Medium (MM). Conductivity was 850 μS/cm and pH 7.4 at 25 °C. Dilutions of MM (20, 40, 60, and 100 %v/v) were made with ZM which was also the experimental control medium. MM and its dilutions have been sterilized at 121 °C for 15 min prior to use. Table 5 shows the composition of the resulting MM in terms of macronutrients and metals. Some of the metals, such as Zn, Fe, Mg, Si, Mn and K, can serve as micronutrients for algae [25,26].

2.3. Growth experiments for the identification of optimal content of MM in the culture medium and the identification of the effects of simulated martian conditions

Preliminary tests were performed, using growth media consisting of mixtures of MM and ZM wherein the volume percentages of MM were equal of 0, 20, 40, 60and 100% v/v, respectively. From these experiments (Fig. S2 in the Supplementary Materials) the best growth medium involving the use of MM was identified to be the one containing 40 %v/v of MM and 60%v/v of ZM (MM40), respectively. Subsequently, different experiments were performed to assess the effects of operating conditions that simulate the ones of the process to be implemented on Mars (cf. Fig. 1). Both the isolated and the synergic effect of all the operating conditions in the process to be realized on Mars were assessed. Table 6 summarizes all the experiments performed in this case. These experiments permitted us to identify the feasibility of the invented process.

The features common to all these experiments are reported in what follows. The batch culture experiments were carried out within transparent vented cap flasks filled up to 40 ml. The experiments were set in triplicates with an illumination of 100 μmol m^{-2} s^{-1} on the irradiated surface of the culture flask. The optical density at the beginning of the experiments was about 0.2 at a wavelength of 650 nm.

Cells morphology was examined using magnification of 40 and 100 X (Leica DM750) optical microscope interfaced with Leica EC3 digital camera (Leica Microsystems, Wetzlar, Germany), and using the Leica Application Suite (version 3.4.0, Leica Microsystems). All operations were carried out avoiding environmental contamination and under a suitable microbiological safety cabinet. Atmosphere consisted of air and gravity was equal to 1 g. During experiments, the growth of cyanobacteria was monitored through absorbance microplate reader ELISA (TECAN, Sunrise™, Tecan Trading AG, Switzerland) of the chlorophyll-a optical density (OD) of the culture at 650 nm wavelength. The biomass

Table 3
Heavy Metals and micronutrients in the Martian Regolith leachate (RL).

Metal	(mg/L)
Al	4.80
Ca	8.12
Fe	6.41
K	8.32
Mg	1.48
Mn	0.19
Na	4.66
P	0.25
Si	10.28
Ti	1.27

Table 4
The composition of synthetic urine (MP-AU) [a].

Components	(g/L)
Na_2SO_4	1.700
$C_5H_4N_4O_3$	2.500
$Na_3C_6H_5O_7$ x $2H_2O$	0.720
$C_4H_7N_3O$	0.881
CH_4N_2O	15.000
KCl	2.308
NaCl	1.756
$CaCl_2$	0.185
NH_4Cl	1.266
$K_2C_2O_4$ x H_2O	0.035
$MgSO_4$ x $7H_2O$	1.082
NaH_2PO_4 x $2H_2O$	2.912
Na_2HPO_4 x $2H_2O$	0.831

[a] $C_5H_4N_4O_3$ = Uric acid; $Na_3C_6H_5O_7$ x $2H_2O$ = Sodium citrate dihydrate; $C_4H_7N_3O$ = Creatinine; CH_4N_2O = Urea.

Table 5
Resulting composition of the Martian Medium (MM) in terms of macro-nutrients and metals acting as micronutrients.

Macronutrients		Micronutrients	
Component	(g/L)	Component	(mg/L)
Na_2SO_4	0.085	Al	2.4
$C_5H_4N_4O_3$	0.012	Ca	4.06
$Na_3C_6H_5O_7$ x $2H_2O$	0.036	Fe	3.205
$C_4H_7N_3O$	0.044	K	4.16
CH_4N_2O	0.750	Mg	0.74
KCl	0.115	Mn	0.095
NaCl	0.087	Na	2.33
$CaCl_2$	0.009	P	0.125
NH_4Cl	0.063	Si	5.14
$K_2C_2O_4$ x H_2O	0.002	Ti	0.635
$MgSO_4$ x $7H_2O$	0.054		
NaH_2PO_4 x $2H_2O$	0.146		
Na_2HPO_4 x $2H_2O$	0.041		

Table 6
Experiments performed to assess the feasibility of the process.

Experiment ID	Medium[a]	Atmosphere	Gravity
ZM_Air_1 g	ZM	Air	1 g
MM40_Air_1 g	ZM (60 %v) + MM (40 %v)	Air	1 g
ZM_Air_μg	ZM	Air	μg
MM40_Air_ μg	ZM (60 %v) + MM (40 %v)	Air	μg
ZM_CO_2_1 g	ZM	CO_2	1 g
MM40_CO_2_1 g	ZM (60 %v) + MM (40 %v)	CO_2	1 g
ZM_CO_2_ μg	ZM	CO_2	μg
MM40_CO_2_ μg	ZM (60 %v) + MM (40 %v)	CO_2	μg

[a] ZM = Zarrouk's medium, MM = Martian medium.

concentration C_x (g L^{-1}) was calculated from OD measurements using the calibration curve C_x vs OD shown in Fig. S1 of Supplementary Material. The calibration line was obtained as follows:

A spirulina culture that was growing in a well-mixed 2 L photobioreactor was used. A specific volume V (mL) of the liquid was withdrawn from the reactor and its OD650 was measured using a spectrophotometer (Genesys 20, Thermo Scientific, Walthmanm Waltham, USA). The liquid sample was then centrifuged at 4000 rpm for 15 min and dried at 105 °C for 24 h. Then the dry weight W (g) of biomass originally contained in the liquid volume V (ml) was gravimetrically measured. This way the dry biomass concentration (g/L) corresponding to the OD measured in liquid culture in the reactor could be evaluated. By repeating the procedure for different values of OD achieved in the reactor a series of data of biomass concentration Vs OD was obtained. By linearly fitting these data the calibration line in Fig. S1 was obtained. The pH was measured daily by pH-meter (XS instrument, Carpi, MO, Italy).

2.3.1. Simulating the atmosphere in the martian dome (whitley jar gassing system)

To investigate the possibility to use CO_2 from Mars atmosphere, further experiments were performed by growing microalgae in an atmosphere consisting of pure CO_2. To this aim a workstation (Don Whitley Scientific Ltd, UK), capable to provide excellent conditions for the processing, incubation, and examination of samples without exposure to atmospheric oxygen, was used. The workstation allowed to manipulate samples in a sustainable environment where parameters can be altered to create the required conditions for growing the cultures in presence of ~100% CO_2 into the jars in just 2 min. A full colour touch screen control panel allowed the operator to monitor, in real time, that the criteria necessary for the culture's growth have been met. The workstation was connected to both a CO_2 cylinder and a polycarbonate jar. The capacity of the jar was 2.5 L, (Height 24 cm, Diameter 17 cm) being capable to accommodate 8 flasks with a base section of 75 cm^2. The jar had an in-built fault detector to create an alert if leaks occur.

2.3.2. Simulating microgravity conditions

To verify whether microalgae growth and metabolism could be affected by microgravity conditions achieved in space and Martian conditions further experiments were performed at the laboratory of the Departmental of Biomedical Sciences, University of Sassari, Sardinia, Italy. In order to simulate microgravity (μg) a 3D random positioning machine (RPM) built by Dutch Space (former Fokker Space, Leiden, The Netherlands) was used. The 3D Random Positioning Machine (RPM) is a micro-weight ('microgravity') simulator that is based on the principle of 'gravity-vector-averaging', constructed from two perpendicular frames that rotate independently. The direction of the gravity vector is constantly changed so that the average of the gravity vector simulates a microgravity environment, i.e. less than 10^{-3} g. The dimensions of the 3D RPM are limited to 1000 × 800 × 1000 mm (length × width × height). A mechanical stage can accommodate a maximum of 12 flasks with a base section of 75 cm^2 simultaneously, all flasks resided within 10 cm from the centre of rotation.

2.3.3. Simulating the simultaneous effect of all the operating conditions of the process to be implemented on mars

To simulate the effect of all operating conditions of the patented process on Mars, the following procedure was adopted. The flasks were carefully filled (approximately 80 ml) with medium a containing 40 %v/v of MM (MM40_) without air bubbles to avoid shearing of the fluid. Subsequently, the flasks were fixed inside the jar, filled with CO_2, that in turn was mounted on the 3D RPM. The latter one was operated for at least 23 days in a dedicated room at 25 °C. The same cultures were grown in parallel at 1 g, comprised the control cultures and placed in the static bar to undergo the same vibration of the sample under μg conditions. The 3D RPM is connected to a computer and through a specific software the mode and speed of rotation were selected. Random Walk mode with a 60°/sec (rpm) was chosen. The cultures were enlightened with a white illumination of 150 μmol m^{-2} s^{-1} for 12 h and CO_2 has been provided to microalgae during the light hours. A simplified scheme of the experiment's steps is shown in Fig. 2 while Fig. 3 shows a graph of the vectorial components of the acceleration achieved by the RPM and a table where the vectorial sum (G-res) of such components (whose values are always close to zero) is reported.

3. Results and discussion

3.1. Isolated effect of the use of Martian Medium (MM40)

This experiment was aimed to verify whether the replacement of a specific volume of ZM with the same volume of MM could affect the growth of *A. platensis*. To this aim preliminary tests were performed, using growth media consisting of mixtures wherein the volume percentages of MM were equal of 0, 20, 40, 60, and 100 %v/v, respectively (Fig. S2 in supplementary materials). These experiments were performed using atmospheric air and Earth gravity. From these experiments the best growth medium involving the use of MM was identified to be the one containing 40 %v/v of MM and 60%v/v of ZM (MM40), respectively. Higher percentages resulted in a reduction of the growth rate of the culture. In Fig. 4a the comparison between the biomass concentration evolution obtained when using only ZM and MM40 is shown.

No relevant effect resulted from the re-placement of 40%v/v of ZM with a corresponding volume of MM up to 14 days when both cultures achieved a concentration close to 0.45 g L^{-1}. Rather a slight improvement of the growth could be observed up to 13 days of cultivation. After the 15th day of cultivation both cultures start to decrease (data not shown) probably due to carbon starvation or the inhibition determined by the high pH achieved (close to 11). Therefore, to be productive when operated under batch mode, these cultures should be stopped after 15 days of cultivation.

It should be noted that biomass productivity is a crucial variable to verify the possibility to eventually feed a crew. For this reason, a further comparison of the two experiments is shown in Fig. 4b in terms of productivities achieved after 14 days (Pb_{14}) of cultivation. This figure further confirms that no relevant difference can be detected between the performances of these cultures since their corresponding productivities were both close to 0.022 g L^{-1} day^{-1}. It is then apparent that the replacement of 40%v/v of ZM with a corresponding volume of MM is feasible without affecting the production of biomass. On the contrary a relevant payload associated to the nutrients to bring from Earth, could be saved during a potential manned mission on Mars. In this regard, it should be noted that MM40 includes minerals and water. Indeed, part of the water would be obtained from urine while the remaining part would be obtained by extraction of the hydration and adsorbed water of Mars regolith as well as from Mars atmosphere according to the process depicted in Fig. 1 and patented by Cao et al. [19].

3.2. Isolated effect of the use of simulated martian atmosphere

The goal of this experiment was to isolate the effect of using an atmosphere that simulates the one that will be obtained in the pressurized domes hosting the process to be implemented on Mars and depicted in Fig. 1. According to the process of the invention by Cao et al. [19], the latter one will be obtained from the Mars atmosphere and will consist of almost pure CO_2 pressurized at a pressure equal to at least 0.8 bar. For this reason, these experiments were performed by inserting the laboratory scale photobioreactors (flasks) containing the microalgal cultures within a jar wherein pure CO_2 was then fluxed until its partial pressure was equal to 1 bar. The obtained results are shown in Fig. 5a where the medium was invariably the ZM.

No relevant effect resulted from the re-placement of air with CO_2 simulating Martian atmosphere in the dome simulant could be observed

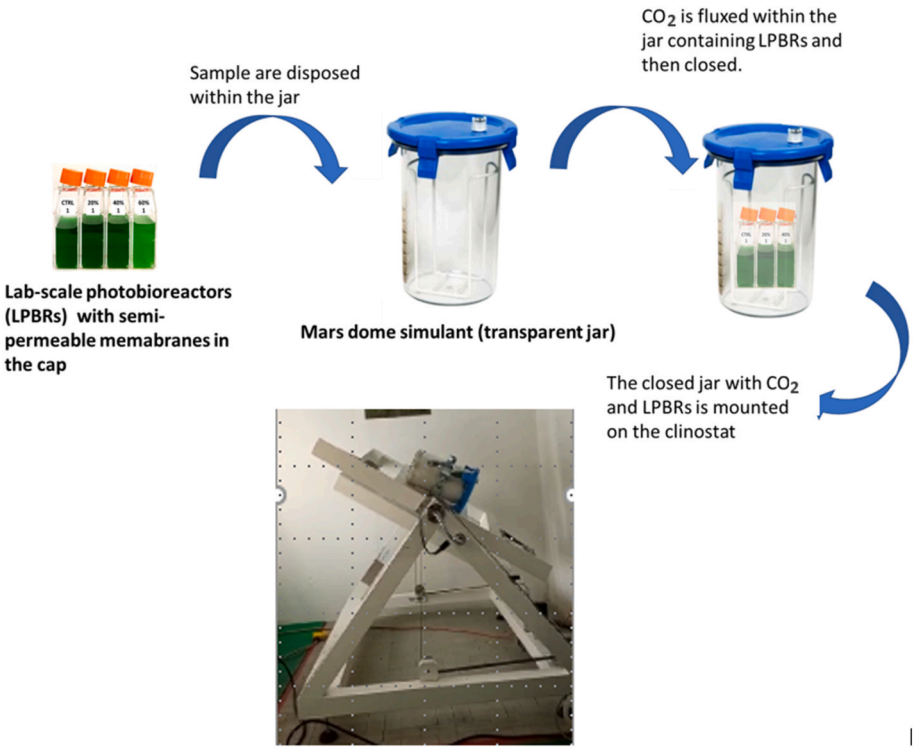
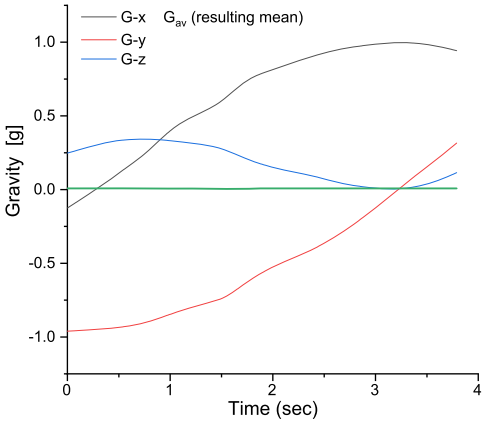


Fig. 2. Schematic representation of the experimental activity performed to investigate the growth of microalgae when using simulated Martian resources under microgravity conditions.



Time (s)	G-X	G-Y	G-Z	G-res
0	-0.1255	-0.9607	0.2475	0.0075
0.39	0.0516	-0.9442	0.3252	0.0075
0.55	0.1394	-0.9313	0.3364	0.0075
0.77	0.2425	-0.9062	0.3465	0.0075
1.05	0.4469	-0.8309	0.3289	0.0073
1.38	0.5426	-0.7642	0.3011	0.0076
1.54	0.6171	-0.7386	0.2714	0.0015
1.76	0.7616	-0.6165	0.1999	0.0075
2.03	0.8191	-0.5086	0.1411	0.0075
2.36	0.9016	-0.421	0.0995	0.0075
2.58	0.9419	-0.3312	0.0563	0.0075
2.75	0.9653	-0.2591	0.0292	0.0075
3.13	0.9986	-0.0535	-0.0025	0.0075
3.35	0.9971	0.0757	0.0111	0.0075
3.57	0.9801	0.1896	0.0474	0.0075
3.79	0.9421	0.3153	0.1143	0.0075

Fig. 3. Plot of the gravity vector in X, Y and Z direction and Resulting gravity vector.

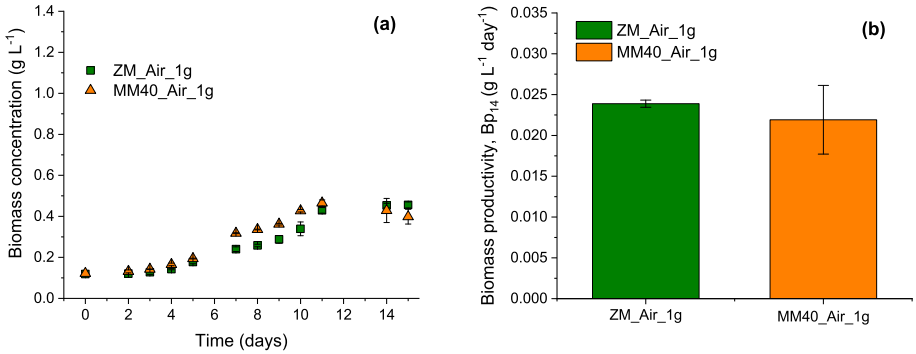


Fig. 4. Effect of the use replacement of 40%v/v of ZM with MM on the time evolution of *A. platensis* concentration (a) and on the biomass productivity after 14 days of cultivation (b).

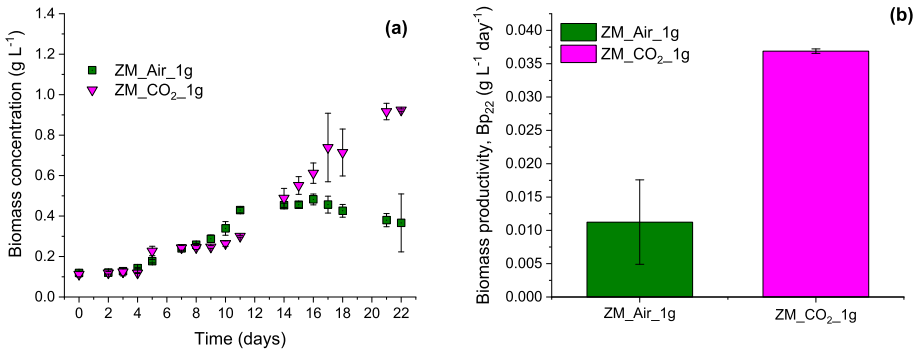


Fig. 5. Effect of the use of pure CO_2, simulating Martian atmosphere, instead of air on the time evolution of *A. platensis* concentration (a) and on the biomass productivity after 22 days of cultivation (b).

up to the 14th day of cultivation. However, from that moment on, the culture using the simulated Martian atmosphere, i.e. CO_2, kept on growing while the biomass concentration of the culture grown in air started to decrease. This result demonstrates that from the 14th day, carbon likely becomes the main limiting factor for *A. platensis* growth and the use pure CO_2 can overcome carbon starvation phenomena. Therefore, the use of an atmosphere simulating the one foreseen in the proposed process is not only feasible but even advantageous with a consequent reduction of the payload needed from the Earth. This result is further confirmed by Fig. 5b where the comparison between the cultures is shown in terms of productivities achieved after 22 days (Bp_{22}) of cultivation. It is apparent that the use of simulated Mars atmosphere (CO_2) led to relevant improvement of the culture performance since it allows achieving a Bp_{22} of about 0.036 g L^{-1} day^{-1} vs the 0.010 g L^{-1} day^{-1} obtained under the control conditions. Therefore, the use of local atmosphere as source of carbon for growing algae not only would lead to significant saving of the mission payload but would also permit to produce larger amounts of food for the crew. In this regard it is worth noting that, while the pressure of Mars atmosphere is about 6.5 mbar, in the experiment ZM_CO_2_1 g a CO_2 pressure of about 1 bar was adopted in order to simulate the operating conditions occurring in the pressurized domes that would host the process depicted in Fig. 1 according to the patent by Cao et al. [19].

3.3. Isolated effect of simulated microgravity

This experiment was aimed to evaluate how simulated microgravity could affect the growth of *A. platensis*. Hence, it was performed by mounting the flasks containing culture of *A. platensis* in ZM on the Random Positioning Machine and monitoring growth periodically. The corresponding results are shown in Fig. 6a.

After 18 days of cultivation biomass concentration of both ZM_Air_μg and ZM_Air_1 g cultures decreased and thus the former one was interrupted. In Fig. 6a, a slight improvement of the growth rate was observed when cultivating the sample under microgravity conditions. This might be due to a reduced effect of settling and aggregation determined by the gravity. The latter phenomena might in fact hinder the diffusion transport of nutrients dissolved to the algae.

Moreover, microgravity likely resulted in the improvement of CO_2 transfer from the gas phase to the liquid one since according to the literature [27] the mass transfer coefficient k_La in shaken flasks is typically a function of several factors among which the gravity acceleration. (cf. Eq. S-1 in Supplementary Materials). In particular, in the relationship proposed by Klöckner et al. [27] k_La is proportional to a negative power g_a and thus mass transfer coefficient increases as the gravitational acceleration decreases (cf. Eq. S-2 in Supplementary Materials). Accordingly, when the gravity acceleration g_a is reduced to $0.0075 \times g$ (with g being 9.8 m s^{-2}) like in our experiments, the k_La would be correspondingly increased 12 times with respect to case where a gravity equal to $1 \times g$ is adopted, thus resulting in a potentially great enhancement of CO_2 transfer to the liquid phase. While the biological and physico-chemical mechanisms underlying the effects of microgravity on the growth of *A. platensis* should be better investigated, the experimental evidence reported in Fig. 6b further confirms that microgravity conditions resulted in the improvement of biomass productivity with respect to case where growth was carried out under Earth gravity. Ultimately, although the gravity on Mars is slightly higher than the one adopted in this experiment, the latter one demonstrates that reduced gravity conditions that would take place on Mars not only don't affect the growth of *A. platensis* but can even improve its growth.

3.4. Synergic effects of the combination of two out of three of process operating conditions to be implemented on mars

The goal of these experiments was to explore the effect of the simultaneous application of two out of three operating conditions simulating the ones taking place in the process to be realized on Mars, for example Martian atmosphere together with microgravity, MM

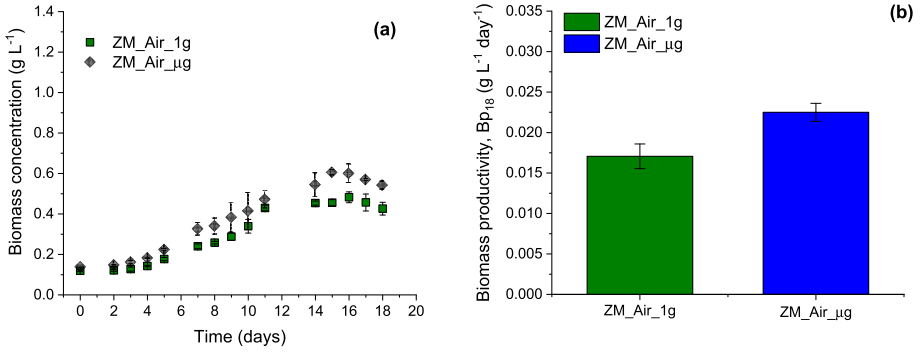


Fig. 6. Effect of microgravity (0.0075 g) on the time evolution of *A. platensis* concentration (a) and on the biomass productivity after 18 days of cultivation (b).

together with microgravity or MM together with Mars atmosphere. Fig. 7a shows some of the possible combinations of operating conditions resulted in synergic effect providing a better growth of microalgae when compared to the base case experiment (ZM_air_1 g).

Up to the 15th day of growth the culture using MM under microgravity (MM40_Air_μg) was the better one but from the 16th day on it started to decrease probably due to the lack of carbon. On the contrary the two cultures using CO_2, i.e. the simulated Martian atmosphere used in the process, kept on growing for all the experiment duration thus demonstrating the capability of this strain to benefit of the high carbon concentration available in the liquid phase. The best performance was then obtained when both CO_2 and microgravity were used (ZM_CO_2_μg). In fact, in the latter experiment a biomass concentration of about 1.2 g L^{-1} was achieved after 22 days of cultivation. It is then apparent that the use of pure CO_2 was the discriminant factor in view of the obtainment of high biomass productivities. The latter ones, calculated at the 22nd day of cultivation are shown in Fig. 7b which clearly highlights that the better productions were obtained when using the simulated Martian atmosphere, pressurized according to the process by Cao et al. [19], rather than air as a mean to provide carbon to microalgae. In particular, using ZM and CO_2 under microgravity a final biomass productivity of about 0.045 g day^{-1} L^{-1}.

3.5. Simulating all the operating conditions taking place on mars according to the process of the invention

In this experiment all the operating conditions of the process by Cao et al. [19], to be implemented on Mars, i.e. microgravity, pressurized CO_2 atmosphere and MM40, are simultaneously applied to verify its feasibility. The obtained results are shown in Fig. 8a.

It can be observed that the simultaneous use of all the operating conditions of the process not only did not affect microalgae cultivation but also resulted in an important improvement thus showing a synergic effect. This is probably because when the biomass concentration becomes high (at the end of the experiment) the culture requires more CO_2 to perform photosynthesis while microgravity conditions avoid aggregation and settling. Moreover, the strain *A. platensis* well tolerate high CO_2 concentrations because its photosynthesis is capable to significantly increase culture pH and counteract the acidification effects of CO_2 which potentially inhibit microalgae growth. While the reasons underlying such an improvement should be better investigated in further studies, the experimental evidence is univocal and further confirmed by the comparison of the productivities achieved by the two curves after 22 days (cf. Fig. 8b).

Ultimately, based on these results, it can be reasonable stated that, although the gravity on Mars is slightly higher than the one adopted in this experiment, the process proposed by Cao et al. [19] is advantageously feasible allowing the production of food and oxygen on Mars. This statement is further corroborated by the comparison reported in Fig. 9 wherein the results obtained under simulated Martian conditions with MM40 and pure ZM are reported.

It can be observed that, under the simulated process conditions that should take place on Mars *A. platensis* grew slightly better when using the MM40 medium rather than ZM. Therefore, the use of local resources to cultivate algae would result not only in a significant reduction of the mission's payload but even into a higher biomass productivity that would permit to better satisfy the astronauts needs in terms of food.

In Table 7 the volumes of *A. platensis* culture to be cultivated in order to meet the needs of crew consisting of 6 astronauts are evaluated based on the biomass productivities obtained in this work, the nutritional needs in the ISS reported by Bychkov et al. [28] and the average composition of *A. platensis* in terms of total proteins, carbohydrates and lipids reported by Tokuşoglu and Ünal [29].

Even considering a diet consisting only of *A. platensis*, relatively small volumes of cultures should be cultivated. To meet the protein needs of astronauts about 15 m^3 of culture would be enough while to obtain the carbohydrates needs larger volumes would be necessary. If we then consider that a diet consisting only of *A. platensis* is not feasible, the volumes of culture to be cultivated further decrease. For instance, if we assume to satisfy 40% of the crew protein needs with *A. platensis*, only 6 m^3 of culture would be necessary. The geometry and set-up of photobioreactors wherein performing cultivation could be different but in principle growth might be carried out also in suitable open pond systems realized indoor within the pressurized domes foreseen in the process by Cao et al. [17], as shown in Fig. 10. This way the in-situ realization of the culture would consist of digging the regolith up to a deep of about 30–40 cm and then lining the soil with an impermeable material.

To ensure a culture volume of 6 m^3 an area of about 20 m^2 would be enough. Such a design would result in a reasonable payload consisting of the liner and the pump. It should be noted that the present study assumes that the dome is capable to ensure a suitable shielding of dangerous radiation and an inner temperature suited to sustain the growth of microalgae. Several designs and materials have been already proposed in the literature to realize inflatable domes on Mars capable to permit the growth of algae and higher plants [30–34].

4. Conclusions

The growth of *Arthrospira Platensis* within a novel device simulating microgravity along with pure CO_2 atmosphere and using a culture medium containing suitable amounts of a mixture of regolith leachate and urine simulants has been investigated with the goal of verifying the possibility of producing food on Mars by using in situ available resources. The obtained results showed that the considered strain was capable to growth better under Martian simulated conditions using the in-situ produced growth medium than under Earth ones using optimal growth medium. In fact, in the former case a biomass productivity of

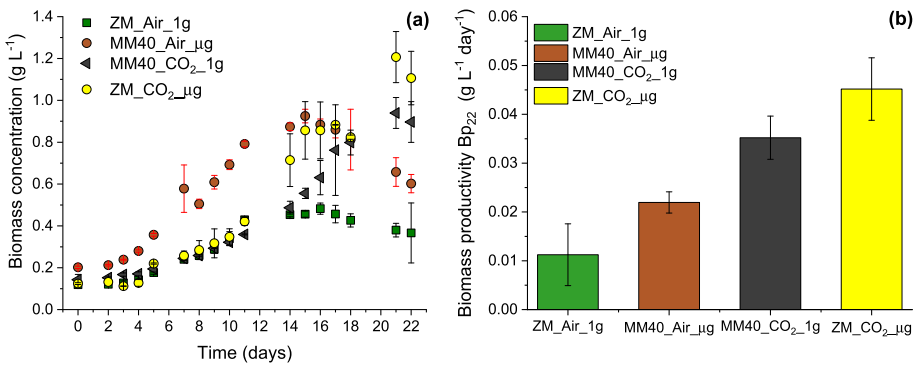


Fig. 7. Synergic effects of the combination of two out of three of the process operating conditions on Mars on the time evolution of *A. platensis* concentration (a) and on the biomass productivity after 22 days of cultivation (b).

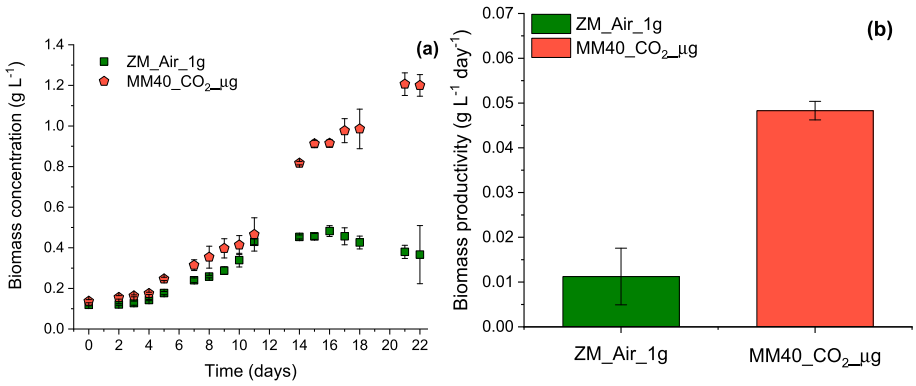


Fig. 8. Synergic effects of all the operating conditions that would take place on Mars according to the process by Cao et al. [17] on the time evolution of *A. platensis* concentration (a) and on the biomass productivity after 22 days of cultivation (b).

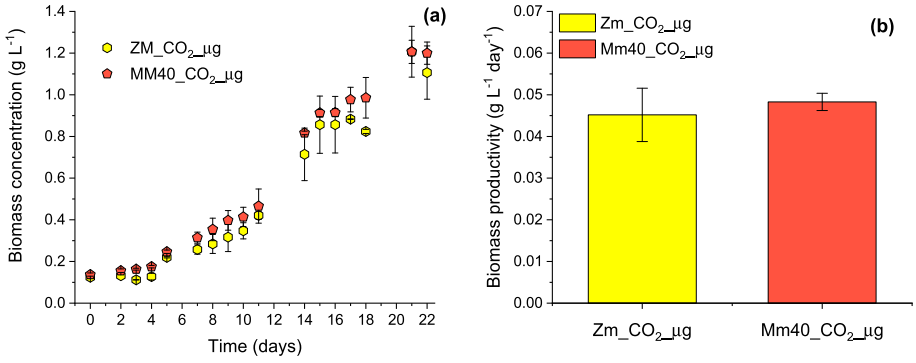


Fig. 9. Comparison of the evolution of *A. platensis* concentration (a) and the biomass productivities after 22 days of cultivation (b) obtained when using MM_40 and ZM under simulated process conditions on Mars.

Table 7
Evaluation of the volumes of culture needed to meet the nutritional needs of a crew of 6 astronauts.

	Nutrient's need per man* (g man^{-1} day^{-1})	Crew members (men)	Total daily amount of nutrient needed (g day^{-1})	Average Nutrient's content in *A. platensis* (g g^{-1})	*A. platensis* to produce to meet astronauts needs (g day^{-1})	Volume of culture to meet crew member needs (m^3)
Proteins	78	6	468	0.643	728	15.0
Carbohydrates	58.4	6	350	0.151	2321	47.8
Lipids	64.4	6	386	0.714	541	11.2

Values obtained from Bychkov et al. [28] referring to the astronauts needs in the ISS.
**Value taken from Tokuşoglu and Ünal [29].

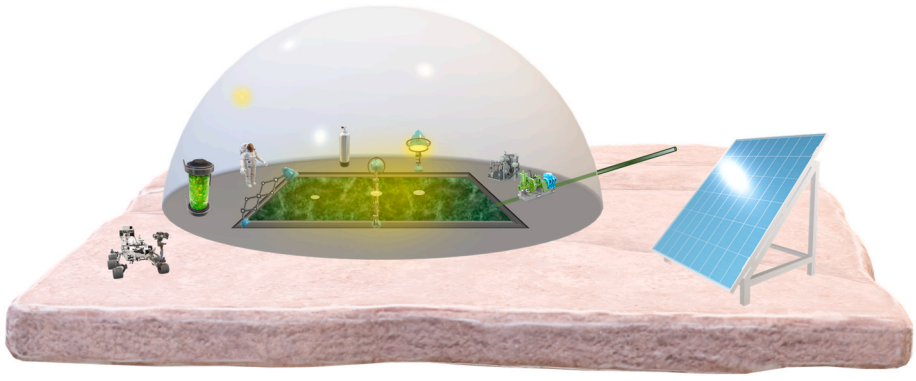


Fig. 10. Scheme of the possible open pond to be available within the Martian dome according to the process by Cao et al. [17].

about 0.048 g L^{-1} day^{-1} was achieved while in the latter one the productivity was equal to about 11 g L^{-1} day^{-1}. Such a productivity could allow to meet the proteins needs of a crew of six members with about 15 m^3 of culture. While different phenomena could have contributed to this result, in our opinion the most relevant are that the CO_2 rich atmosphere hinders carbon starvation while microgravity reduces the effects of settling and aggregation produced by the gravity thus allowing a better diffusion of nutrients towards single cells. Further investigations should be performed to better understand these aspects as well as to assess the effects of cosmic radiation and low temperatures available on Mars which have been neglected by the present study. In this regard, it should be noted that algae cultivation could be theoretically carried out within a heated and pressurized inflatable domes in an open pond whose availability would require digging and subsequent lining of the soil. Such a design would result in a low payload associated to the realization of the pond.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actaastro.2022.09.058.

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