

Terraforming Mars with Diatoms: The Perspective of Life on a New Planet

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Abstract

During the last century, the space age was initiated, fueled by numerous efforts to extend our knowledge beyond the solar system and our existence beyond Earth. Since the seventies, many telescopes covering a wide range of electromagnetic radiation, space and planet probes, satellites, and crewed orbital missions have been launched, while humans seek to reach and colonize the Moon and Mars. Therefore, a crew's survival on a spaceship during long-term missions has become a critical issue requiring extensive research and innovations. Diatoms, the most abundant silica-walled microalgae, are considered the most successful primary producers on Earth, with extraordinary diversity and outstanding multifaceted applications, especially when considering their siliceous frustules. Moreover, some diatoms are potentially known as psychrophilic. Since Mars has a thin atmospheric layer consisting of pressure of 6–7 mbar, 95% CO₂, 2.8% N₂, 2.1% Ar with only 0.13% O₂ compared to 21% O₂ on Earth, 78% N₂, 0.04% CO₂ and ~1000 mBar of atmospheric pressure. Therefore, in this chapter we discuss the prospects of simulating life on Mars, starting with the most populating O₂-producing organism on Earth, *diatoms*, which we are proposing as SpaceM©.

Keywords: Diatoms, life on Mars, terraformation, SpaceM©

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12.1 Introduction

Since the beginning of time, when *Homo sapiens* started gazing at the sky, observing the Moon, twinkling stars, and other celestial objects, many questions have arisen which remain unanswered. During humankind's childhood, no one imagined how big the universe was, and most thought that our planet was the core of everything. This idea probably remained until the early sixteenth century, when Nicolaus Copernicus published his work, "*De revolutionibus orbium coelestium*" (*On the Revolutions of the Heavenly Spheres*), which introduced the suggestion that the Earth is not the solar system core, but the Sun. This initiated the Copernican revolution that inspired Kepler, Galileo, and Newton to set off the basics of modern Astronomy [12.11] [12.20], giving humanity the desire to delve into space, starting with the Moon [12.33].

In the 20th century, humankind's knowledge reached a remarkable turning point, allowing Yuri Gagarin (the first human astronaut) to step up into space for almost two hours, declaring the beginning of the Space Age [12.9]. Later, many space missions were launched, including Sputnik 1 and Apollo, seeking, on the one hand, glory, and on the other hand, answering our ancestors' questions [12.29]. Throughout this era, our understanding of the universe has been simultaneously changing as well as our imagination for living outside Earth. It is obvious that mankind is facing existence-threatening problems on Earth, including population increase and resources limitation, besides the dramatic climate change. Therefore, it is rational to expand our existence in the solar system and find new opportunities and alternatives. As an example, in the worst-case scenario, we leave Earth temporarily to save our species during catastrophic events on Earth [12.26]. This logic has eventually led to the idea of colonizing space, starting from the potential planets and moons in our solar system [12.46]. Such ideas have attained increasing momentum once we knew the size of the observable universe (estimated diameter of 93 billion light-years) [12.41] and since we discovered more exoplanets in the habitable zone [12.26].

The vastness of our universe also opens the door for further questions on the uniqueness of life on Earth. This has resulted in the emergence of astrobiology as a field of science trying to address the open questions regarding the existence of alien extra-terrestrial life in the expanding universe [12.32]. The probability of finding living organisms somewhere is increasing with the discovery of additional exoplanets in the habitable zone [12.26], various potential biosignatures, and organic molecules in

interstellar space [12.15] through space telescopes such as Hubble and James Webb [12.24]. Recently, probable signs of past life on Mars have been reported and are under investigation [12.54]. Such arguments about the presence of alien life are essential, especially when considering colonizing Mars or other planets in the future. On the one hand, this would give us faith in the idea of colonizing other planets (as life might work outside Earth), and on the other hand, gives us concerns about the security of new extra-terrestrial colonies.

To colonize other planets, such as Mars, by transporting humanity there, it is essential to first survive the lengthy space travel so as to get there and start life on Mars to be known as SpaceM®. This includes challenges such as microgravity, a lack of supplies, and the adaptation to life support systems. Suppose we can surmount the current limitations pertaining to spacecraft stability, the space environment (high cosmic radiation), life support systems, the time required to communicate with Earth, energy sources, and speed restrictions still remain a real challenge. In this circumstance, humans can likely exceed the current realistic limits.

To surmount some of the current limitations confronting crewed long-term missions, developing new concepts and materials for essential crew life applications is necessary. Bio-regenerative life support systems (BLSS) were an extensively proposed concept, where autotrophic organisms (such as plants or algae) have to be integrated for the production of oxygen and food for the artificial ecosystem [12.21]. Here, we focus on diatoms, an important class of microalgae flourishing in many aquatic habitats on Earth. However, Gordon *et al.* [12.13] discussed the idea of cultivating diatoms in space as a source of nanostructured biosilica for nanotechnological applications. Furthermore, Vinayak [12.49] discussed diatoms and other algae as a potential food and oxygen source for the space crew in space shuttles, due to the fact that they alone are responsible for fixing about 25% of CO₂ and are thus considered as primary producers on Earth [12.38]. Later, in Gordon *et al.* [12.14] and Rai *et al.* [12.38] have suggested diatoms as a primary producer for the BLSS for consuming CO₂, and producing O₂, biomass, and valuable metabolites. To turn this into a reality, it is necessary to resolve the survival of diatoms in space and how to incorporate and cultivate them into the BLSS. In this chapter, we intend to initiate discussions on the viability of diatom cultivation in space in order to encourage this endeavor. Furthermore, the chapter narrates the challenges for cultivation and a list of possible species that could survive and be productive in that Martian environment.

12.2 Instrumentation to Artificially Simulate SpaceM© Conditions

12.2.1 SpaceQ

The past few years have seen an increase in developing interest to not only mark missions to Mars but also to design instruments and ways for having a sustainable SpaceM©. Because Mars undoubtedly has an extreme environment and temperature, Kurt J. Lesker Company designed a stainless steel cubic-shaped chamber known as SpaceQ (Figure 12.1) for simulating life on Mars and space environment for utilization in many technical and scientific applications [12.47]. It can handle pressures up to 10^{-5} mbar and temperatures between 163 K and 423 K by chilling the plate with liquid nitrogen (LN_2) or heating the walls (using an external heating jacket), respectively. This chamber has an interior volume of 27,000 cm³ and is a cubical facility composed of stainless steel with an aluminum door (27 L). The working table plate has the following dimensions: 20 cm x 20 cm x 1.8 cm. The chamber includes a number of optical and electrical feedthroughs, including two quartz window viewports, two thermocouple feedthroughs, a vacuum-compatible UV lamp, a cold cathode/Pirani combination gauge to measure pressure, two gas inlets, ports for a vacuum pump, connections for a vacuum pump, USB and DB25 ports to read data in real-time, and connectors for the optical cable to spectrometer. Additionally, there is an additional connector that may be utilized to add more sensors if necessary.

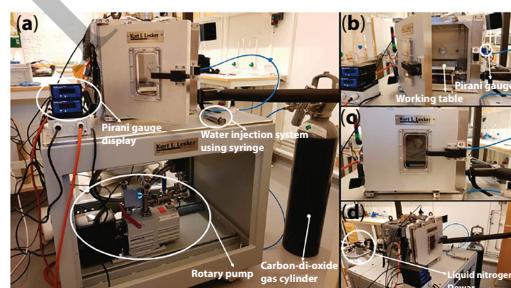


Figure 12.1 Overview of the SpaceQ chamber. (a) This figure shows the entire setup of the chamber, including the external Pirani gauge display, the rotary pump, the CO_2 gas cylinder (to insert a Mars-like atmosphere) and the syringe for water injection. (b) Detail of the experimental table where samples are cooled down to the desired temperature and the KJLC® Cold Cathode/Pirani combination gauge on the right side. (c) Viewport at the front of the chamber, used to take pictures during the experiment. (d) LN_2 Dewar used to cool down the working table. (Copyright [12.48]).

A Vaisala HMT334 sensor, which can be operated in vacuum and under Martian conditions, is installed at 10.2 cm above the plate and 5.2 cm from the sidewalls to measure the atmospheric temperature and relative humidity inside the chamber, using the legacy from the REMS RH sensor [12.45]. This sensor can measure temperatures in the range of 203.15 K to 453.15 K, with an accuracy of ± 2 K, and the relative humidity in the range of 0–100% RH, with an accuracy of $\pm 1\%$ RH. The sensor probe is exposed to the interior environment and is mounted in the chamber using an M22 x 1.5 thread. Vacuum-tight installations have been tested for the thread. The compartment is filled with water using a stainless-steel syringe. It is attached to a Swagelok $\frac{1}{4}$ " connector on the right side of the chamber, which is linked by a tube to a manually driven Swagelok $\frac{1}{4}$ " fitting ball valve, which has a 20 mL capacity. Since the water is connected to the chamber by a valve, it can be injected numerous times during the experiment. When the valve is opened, the water is injected into the chamber's depressurized atmosphere. A cold cathode Pirani gauge, which is intended for the vacuum measurement of gases in the range of 1×10^9 mbar to 1000 mbar, regulates the pressure inside the chamber. In order to lower the pressures to 10^5 mbar, two phases are needed. First, we use the Pfeiffer 1-phase DUO 10 M rotary pump to create a vacuum level of 10^3 mbar, and then we couple it with a turbomolecular pump to obtain higher orders of vacuum. In order to manage the flow of the LN₂ supply, a solenoid valve is installed on the working table's liquid nitrogen (LN₂) feedthrough, which is linked to a 50 L Dewar. The thermal control unit receives data from the temperature sensor installed on the working table and uses that information as feedback to cool the table down to the desired temperature. The top and left quartz silica viewports allow us to watch the experiment or start an operation utilizing a certain wavelength on the material. With a viewing surface of 65 mm, the window is constructed of Kodial glass [12.48].

The studies on SpaceQ involved simulating environmental conditions of Mars transitioning from night to day, as seen in Figure 12.2. The temperature on ground (T_g) and air (T_a) were directly measured along with pressure (P) and relative humidity of ice (RH_{gⁱ}) and liquid (RH_{g^l}) from Equations 12.1–12.4 [12.31] described below were derived by using the Formulae 12.1–12.4 [12.47]:

$$\text{RH}(T) = \frac{P}{ew_{(T)}} \times \frac{\text{vmr}}{1 + \text{vmr}} \times 100 \quad (12.1)$$

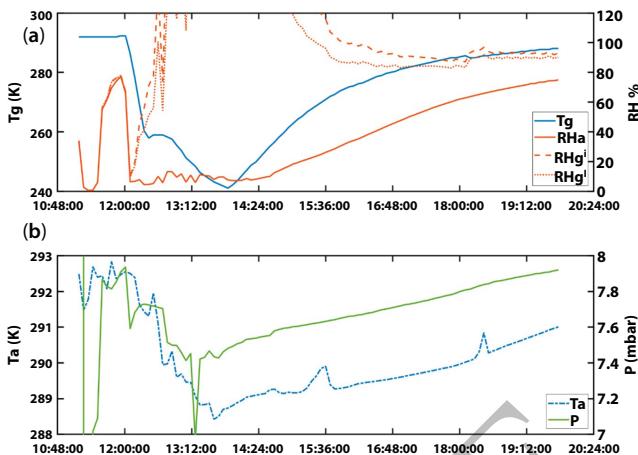


Figure 12.2 Time evolution of the simulated environmental parameters inside the SpaceQ chamber during the transition from a Martian night to a Martian day. Note: In this configuration, the relative humidity on the surface is saturated to allow for frost formation simulating the ground temperature under 260 K. (Copyright [12.47]).

$$ew = 6.112 \times e \left(17.62 \times \frac{T - 273.14159}{243.12 + (T - 273.14159)} \right) \quad (12.2)$$

$$ew_{ice(T)} = 6.112 \times e \left(22.5 \times 1 - \frac{273.14159}{T} \right) \quad (12.3)$$

$$vmr = \frac{W}{1000} \times \frac{M_d}{M_w} \quad (12.4)$$

where RH(Ta) refers to relative humidity of air, to retrieve volume mixing ratio (vmr) to calculate RHg (Tg). Here, RH_i represents the relative humidity with respect to ice, and RH_l represents the relative humidity of liquid.

RH: relative humidity in %

P: Pressure in mbar

vmr: volume mixing ratio in parts per million

T_g: table temperature in K

$P_{\text{ewliq}}(T_g)$: saturation partial pressure over liquid water at a given temperature

$P_{\text{ewice}}(T_g)$: saturation partial pressure over ice at a given temperature

M_w = 18.0160 (molecular weight of water)

M_d = 43.3400 (molecular weight of dry air on Mars)

W = water mass mixing ratio.

When RH_{gi} is above the saturation, atmosphere condenses, when RH_a is zero the water in Martian atmosphere condenses, while as the surface temperature increases slowly water is lost to the atmosphere which increases the chamber pressure P, and raises the RH_a . In such a case, pressure in the simulating chamber SpaceQ is maintained within 8 mbar with the help of a valve. Such simulations thus help in *in-situ* resource utilization (ISRU), which would be required for testing the corrosiveness of materials to be used for Martian architecture as well as to initiate life on Mars by growing our own food. Growing our own food in the form of plants has been researched to be best with the tiniest plants, i.e., microalgae which not only are responsible for consuming and fixing maximum CO₂ but also giving O₂ in return and acting as primary producers. Among microalgae, as previously discussed, diatoms stand out best among existing microalgae in fixing 25% of global CO₂ and as primary producers.

12.2.2 GraviSat Platform

The microgravity in space may cause varied effects on life in space. It was observed in various space experiments for sustainability of growing micro-organisms. It has been observed that *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium* showed varied cell densities in space flights which was higher than the control; however *E.coli* in GeneStat (nanosatellite) and yeast (*Saccharomyces cerevisiae*) in PharmaSat card (nanosatellite) grew slowly in space [12.40]. The nanosatellite with microalgae cells was linked to a low overall rate of atmospheric CO₂ input to the liquid medium, which led to carbon restriction. To make CO₂ available to the algal cells, it must first diffuse through the 50-mm-thick polystyrene barrier and then travel a variety of lengths inside the liquid media. Microalgae strains were cultivated on medium supplemented with sodium bicarbonate as a source of inorganic carbon to postpone the onset of carbon limitation. Some cyanobacteria were inhibited by bicarbonate at the highest tested concentrations (1.0 mM). In contrast to the non-bicarbonate supplemented controls,

all cyanobacteria in medium added with 0.1 mM sodium bicarbonate showed faster growth rates, greater photosynthetic efficiencies, and longer growth periods. Higher gas generation was made possible by adding more bicarbonate. The absence of comparable gas generation in control wells with media containing only 0.1 mM sodium bicarbonate suggests that the gas production was caused by photosynthesis rather than dissolved gas nucleating out of the medium. Although gas generation increased when bicarbonate was added to medium, bicarbonate concentrations had little to no impact on the growth or photosynthetic efficiency of green algal strains (*C. vulgaris* and *D. salina*). In terms of the effects of bicarbonate supplementation on growth ($F_{7,112} = 59$; $p < 0.001$) and photosynthetic efficiency ($F_{7,112} = 4.1$; $p < 0.001$), cyanobacteria (*Synechocystis*) and algae (*C. vulgaris*) differ from one another [12.7].

In yet another experiment, an 85 mL algal culture (the basic media) and two snails were placed in a 120 ml culture container to create the two-element closed aquatic environment (CAES). The guiding concepts for developing this straightforward CAES were that an algal culture (producer) supplies food and oxygen for the snails (consumer), and the algae culture may utilize the waste products and carbon dioxide created by the snails for photosynthesis and development. In addition, a control chamber was added to the 1 g onboard centrifuge. The culture containers were put into the spacecraft with the CAES chambers enclosed and installed. The culture pots gave the algae light. The transmitted light was proportional to the OD 665 nm of the algae, and it was measured by a light sensor on the other side of the tank. This made it possible to calculate the evolution of the algal population over time. All information was recorded before being downlinked. In January 2001, the Chinese spacecraft Shenzhou-II carried out the space experiment's culture chambers (microgravity group and space-exposed 1g centrifuge group). After entering orbit, the chambers were operated remotely, and the spacecraft used a 12-hour cycle to supply illumination [12.52].

The Chinese Academy of Sciences' Freshwater Algae Culture Collection donated the microcystin-producing *Microcystis aeruginosa*, which was employed in our investigation as the study's material. MC-LR was the primary MCs variation, and BG-11 was the growing medium. To inoculate, cells in exponential phase were employed, and the starting cell concentration was changed to $OD_{665} = 0.20$. In clinostats, samples were cultivated at 20 °C with a 12:12 h light/dark cycle. The clinostat's light intensities ranged from 5 $\mu\text{mE}(\text{m}^2 \cdot \text{s})^{-1}$ (areas with low light) to 26 $\mu\text{mE}(\text{m}^2 \cdot \text{s})^{-1}$ (areas facing bright light). Microgravity simulation was created by the use of rotating cell culture technique. The NASA Johnson Space Center developed the

improved suspension culture technique known as the rotating cell culture system (RCCS). The RCCS produced a continuous low-shear by maintaining the cells in a gently fluid orbit [12.52] [12.55].

12.3 Diatoms for Long-Term Space Missions

Diatoms are thus one of the most diverse and successful classes of unicellular microalgae on Earth [12.17] [12.42]. One of the most outstanding aspects of diatoms is their frustule, a hard shell made of amorphous hydrated silica that encloses the living cell. The frustule is made up of two halves that fit together like a Petri dish, with unique species-specific geometry and pore patterns [12.42] that give it exceptional mechanical strength [12.19], hydrokinetic characteristics [12.12], and assumed photobiological roles [12.10]. Despite their small size, diatoms have a significant impact on the planet [12.2]. Their vital roles in various aquatic ecosystems were unveiled and became evident through their significant primary production contribution to organic carbon and oxygen [12.23].

Given the outstanding adaptions of some diatom species to extreme environments and their widespread diversity within a relatively short evolutionary time, diatoms have been suggested as a primary producer for the BLSS in the long-term space missions for multipurpose utilization, as extensively explained in [12.13], [12.38], and [12.14]. Although many projects have suggested or tested green and cyanobacteria microalgal taxa for the BLSS, none of them have, to the best of our knowledge, ever considered diatoms [12.5] [12.38]. Therefore, testing diatom cultivation under space conditions should be considered in future work. Before testing diatoms for the BLSS, several key parameters need to be considered first to successfully grow them or any other microalgae in space, including:

- i. **Light:** Like plants, algae need light for photosynthesis. Adequate light sources, such as LEDs or other artificial lighting, are required since Mars is farther from the sun, and therefore receives comparatively less sunlight than Earth, as seen in Figure 12.2 [12.44].
- ii. **Nutrients:** Algae need essential macronutrients such as nitrogen, phosphorus, potassium, and trace elements for growth [12.35]. The growth medium should be supplemented with these nutrients and controlled to prevent nutrient loss due to stress or microgravity.

- iii. **Temperature:** Algae thrive within specific temperature ranges. Maintaining the appropriate temperature is crucial to ensure optimal growth and prevent stress on the algae.
- iv. **Carbon Dioxide (CO₂):** Algae, like plants, require CO₂ for photosynthesis. The CO₂ levels should be monitored and maintained at suitable concentrations to support growth.
- v. **pH Levels:** Algae tolerate specific pH ranges for optimal growth. The pH levels in the growth medium need to be carefully managed.
- vi. **Stirring/Mixing:** Algae require proper agitation or mixing of the growth medium to ensure even distribution of nutrients and prevent settling of algae cells in the cultivation vessel.
- vii. **Harvesting Methods:** Developing efficient and nondisruptive methods for harvesting algae biomass is essential for continuous growth and sustainable production.
- viii. **Microgravity Adaptation:** Diatoms behave differently in microgravity than on Earth. Researchers should study their behavior and adapt growth systems to accommodate their unique responses to weightlessness.

12.4 A Potential List for the BLSS: Taxa Tolerant to Extreme Conditions

In order to select the potential diatom species (out of the estimated species up to 10⁵) [12.16] for cultivation within the BLSS, specific criteria should be primarily considered. These criteria include their ability to (i) thrive with minimum cultivation requirements to minimize energy and materials required for the cultivation and maintenance of their cultures and, hence, the payload costs (i.e., they can grow efficiently under low light conditions, low temperature, and low nutrient concentrations) and to (ii) withstand extreme space conditions. Although the BLSS is expected to be well-maintained and protected in terms of temperature, pressure, cosmic radiation, etc., the ability of selected diatom species to overcome such extreme conditions is highly recommended for any accidental malfunctions in the BLSS systems. Thus, the cultivated species should be able survive under environmental stress conditions like temperature and light. Not all species of diatoms are expected to survive in these extreme conditions. Therefore, identifying the most potential species among the available taxa is crucial to prepare a short list for future work.

Species of diatoms existing in polar and subarctic ecosystems might be the most suitable ones, as they would be able to withstand the freeze-thawing cycles from -80 °C to +12 °C [1.3]. Diatoms are particularly abundant in polar and subarctic regions, where they thrive in some of the harshest and most extreme environments on the planet. Among diatoms, certain species have developed exceptional adaptations that enable them to endure the challenging freeze-thawing cycles experienced in these frigid climates, ranging from -80 °C to +12 °C, for example, in *Phaeodactylum tricornutum* [12.3]. Polar and subarctic ecosystems present a unique set of challenges for life to flourish. Extreme temperatures, limited light availability during the winter months, and an ever-changing environment characterized by ice formation and melting are some of the adversities that organisms must confront. Therefore, diatoms inhabiting such habitats have evolved remarkable strategies to survive and thrive in these harsh conditions. One of the key features that make diatoms so well-suited to polar and subarctic ecosystems is their silica-based cell walls, known as frustules. These frustules provide structural integrity to the diatoms besides protecting them against freezing temperatures. When the water temperature drops below freezing, ice crystals may form in the surrounding water. The silica frustules of diatoms act as a natural antifreeze, preventing the cell damage that would be caused by ice crystal formation and expansion. This unique property allows them to endure the extreme cold and survive in a suspended state until conditions become favorable again. Furthermore, diatoms are known for their ability to adapt to fluctuations in light availability, which is critical in polar and subarctic regions where periods of complete darkness occur during the winter months. They possess light-harvesting pigments that can efficiently capture and utilize available light energy, enabling them to continue photosynthesizing even under dim light conditions. As the polar day lengthens during the summer, diatoms take full advantage of the increased light availability, rapidly growing and reproducing. In addition to their resilience in freezing temperatures and low light conditions, diatoms have developed robust life strategies to navigate the dynamic freeze-thawing cycles characteristic of polar and subarctic environments. During the warmer months, when temperatures rise above freezing, diatoms flourish and reproduce, building up their populations. When the colder months return and the temperatures drop, some diatoms form specialized spores or resting stages known as auxospores, which protect their genetic material and allow them to withstand adverse conditions. These auxospores remain dormant in the ice or sediment until favorable conditions return, at which point they germinate and restart the reproductive cycle. The ability of diatoms to endure extreme conditions and adapt to the harsh polar

and subarctic environments makes them essential players in the ecological dynamics of these regions. Their capacity to fix carbon through photosynthesis contributes significantly to primary production, supporting entire food webs. Moreover, as diatoms are sensitive indicators of environmental changes, studying their populations and distributions can provide valuable insights into the impacts of climate change on these delicate ecosystems. By persevering through the challenging environmental conditions, these diatoms contribute to the delicate balance and resilience of polar and subarctic ecosystems. Understanding their unique adaptations can help us appreciate the wonders of biodiversity and may also offer insights into mitigating the effects of climate change on vulnerable ecosystems on Mars and other planets away from Earth.

Diatoms adapt to their environment by changing their biovolume, silicification, pore morphology or arrangements. Change in silicification is often observed as a response to external stimuli, such as temperature, which may play an important role in climate change [12.27]. This may involve various morphological and physiological changes in their cell.

- i. **Biovolume:** Diatoms can adjust their cell size or overall biovolume in response to changes in environmental conditions. For example, when resources are abundant, diatoms may increase their biovolume to take advantage of the available nutrients for growth and reproduction. Conversely, under resource-limited conditions, they may decrease their biovolume to conserve energy and nutrients [12.50].
- ii. **Silicification:** This refers to the process of depositing silica in their cell walls, forming intricate and sturdy structures called frustules. This silica deposition is essential for the diatom's survival and protection [12.1]. Diatoms can regulate the thickness and composition of their frustules, enabling them to withstand different environmental stresses. Changes in silicification may occur as a response to factors like temperature, nutrient availability, or pH levels [12.25].
- iii. **Pore Morphology:** The frustules of diatoms have pores that allow for nutrient uptake and waste excretion. The morphology and arrangement of these pores can be altered in response to environmental cues. By modifying their pore structures, diatoms can regulate the exchange of

- substances with their surroundings, thus influencing their physiological processes.
- iv. **Cell Arrangements and Assemblages:** Diatoms can change the pattern of their cell arrangements, forming colonies' filaments in various shapes. This adaptation allows them to optimize light exposure, nutrient availability, and protection from predators. These changes in silicification could be observed as their response to external stimuli such as temperature. This means that diatom frustules can alter the composition and structure of their silica cell walls depending on changes in temperature.
 - v. **Temperature:** Temperature fluctuations can directly influence diatom metabolism and growth rates, and as a result, they may modify their frustules to better cope with the changing conditions.

Overall, diatoms exhibit a high level of adaptability through these various mechanisms, allowing them to thrive and persist in diverse aquatic environments. Their ability to adjust their biovolume, silicification, pore morphology, and arrangements is essential for their survival and success in fluctuating environmental conditions.

The diatom *Phaeodactylum tricornutum* Bohlin, a widely used model species when cultivated in turbidostat cultures, has shown an increase in the photosynthetic rate per cell. It is a pleiomorphic diatom due to the low concentration of silica on its cell wall as compared to other diatoms [12.39]. *P. tricornutum* Bohlin is a species of diatom, which are a type of unicellular algae characterized by their silica cell walls. Diatoms play a crucial role in aquatic ecosystems as they are primary producers, performing photosynthesis to convert sunlight into energy while releasing oxygen. In this case, *P. tricornutum* is widely used as a model organism for studying diatoms due to its well-understood biology, ease of cultivation, and its ability to represent the broader characteristics of diatoms. A turbidostat is a type of continuous culture system used in microbiology, where the culture density is maintained at a constant level by regulating the inflow and outflow of culture medium. This characteristic makes it an interesting subject for studying the factors affecting photosynthesis in diatoms. *P. tricornutum* is a pleiomorphic diatom as there is variability in the shapes and forms of individual cells within the same species. Unlike some other diatoms that may have a more consistent or uniform cell shape, *P. tricornutum* exhibits a greater range of cell morphologies [12.6]. This variability in cell shape is partly attributed to the low concentration of silica in its cell wall compared

to other diatoms. The cell wall of diatoms is made of silica, which provides them with a protective and rigid structure. In *P. tricornutum*, the lower concentration of silica in its cell wall contributes to the pleiomorphism, allowing for more flexibility in cell shape and morphology [12.8]. Overall, the unique characteristics of *P. tricornutum*, such as being a widely used model species and its ability to show increased photosynthetic rates per cell in certain culture conditions, make it an important organism for scientific research in the study of diatoms and their ecological significance.

Growth rate and the generated biochemical and molecular data would allow *P. tricornutum* to be considered as a model diatom species for diatom cultivation in space. It is the most promising taxa of diatom which is exploited for producing many biochemicals such as vitamins, nutraceuticals, amino acids, proteins, etc. A total of 12233 coding genes have been deciphered from the genome of this diatom. Analysis of metagenomic data reveals the existence of a temperature correlated transporter gene J43171, which regulates the thermal adaptation and climate resilience of *P. tricornutum* [12.30].

Table 12.1 Different diatoms that thrive under different extreme temperature conditions.

S. no.	Diatom sp.	Optimal temp. range (°C)	Extreme cold tolerance (°C)	Extreme heat tolerance (°C)
1	<i>Thalassiosira antarctica</i>	0–5	-2	20–25
2	<i>Pseudo-nitzschia multiseries</i>	10–15	5	30–35
3	<i>Chaetoceros muelleri</i>	15–20	10	35–40
4	<i>Fragilariopsis cylindrus</i>	-1–4	-5	18–22
5	<i>Nitzschia frigida</i>	0–6	-2	25–30
6	<i>Thalassiosira weissflogii</i>	20–25	15	35–40

Antarctic diatoms show a higher content of Rubisco as compared to temperate diatoms as an adaptation for photosynthesis at low temperature. Thus, the psychrophilic species compensates for the reduced enzyme activity at low temperature by increasing the concentration of Rubisco in them [12.56]. Another polar diatom, *Fragilariaopsis cylindrus*, is well studied for its adaptation to extremely low temperatures and is known to produce an ice-binding protein (IBP) and extracellular polymeric substance, capable of altering the structure of ice [12.34]. On the other hand, antarctician species *Thalassiosira gravida* has been reported to contain UV light-absorbing compounds, such as mycosporine-like amino acids (MAAS), which act as photoprotectants in their frustules. Table 12.1 shows different diatoms that survive and thrive under different extreme climate and temperature conditions.

Diatoms are incredibly diverse and adaptable organisms, so their temperature preferences can vary based on their geographical location and the conditions they experience in their natural habitats.

12.5 Testing Diatom Growth Under Microgravity Conditions

12.5.1 Microgravity and Living Organisms

On Earth, different life forms have evolved and developed under specific gravitational conditions ($g \approx 9.8 \text{ m/s}^2$), where gravity plays crucial roles in the living organisms. Therefore, the change in the gravitational conditions is expected to alter different biological activities. The satellites or International Space Station (ISS) which travels around the Earth's orbit, experiences freefall conditions, which is conventionally called microgravity. Away from the Earth, e.g., in interplanetary space, the spaceship travels in between celestial objects and does not experience a specific gravity from them if it does not enter their gravitational field. The experience on cultivation of plants and microalgae in space conditions showed microalgae cultivation has been briefly carried out. No doubt, microalgae face problems in culturing in microgravity.

Microgravity is the condition experienced by objects in space, where the force of gravity is significantly reduced, often resulting in a near-weightless environment. This condition is commonly experienced aboard the ISS or during spaceflight missions. Testing microalgae in a microgravity environment can be of scientific interest for several reasons:

- i. **Space Exploration:** Understanding how microorganisms like microalgae respond to microgravity is essential for long-duration space missions, such as interplanetary travel or establishing colonies on other celestial bodies. Microalgae could potentially serve as a source of food, oxygen, and life support systems in these environments (see Table 12.2).
- ii. **Bioregenerative Life Support Systems:** Microalgae can be used in BLSS, where they can help regenerate essential resources like oxygen and remove carbon dioxide from the air. Evaluating their behavior in microgravity can help optimize such systems for space habitats.
- iii. **Biofuel Production:** Microalgae have the potential to be a renewable source of biofuels, and studying their growth and lipid production in microgravity may offer insights into their potential as a sustainable energy source for space missions.
- iv. **Environmental Applications:** Microalgae play a vital role in carbon capture and nutrient cycling in Earth's ecosystems. Understanding how they behave in microgravity can provide insights into how they might contribute to environmental remediation in space or future space habitats.

Overall, studying microalgae in microgravity can offer valuable data and insights that have implications for both space exploration and various other practical applications. These experiments help scientists better understand the behavior of microorganisms under extreme conditions, paving the way for advancements in space research and various terrestrial fields.

Employing the EXPOSE-E launched on 22 October 2008, the European Space Agency allowed long exposures to space conditions and solar UV radiation on ISS. In this facility outside of the ISS, various rock samples with microbial populations on them were kept for 18 months outside the European Columbus laboratory module, one of the nine payloads of the European Technology Exposure Facility (EuTEF). While being subjected to space vacuum and severe temperatures, samples were maintained in darkness. A cyanobacterium (*Gloeocapsa* sp.) and two green algae (*Chlorella* and *Rosenvingiella radicans*) from the microbial community were reported to have endured the space conditions [12.4]. In yet another study, *Chlamydomonas reinhardtii* cells were carried on the Space Shuttle Endeavour's last trip (STS-134) in 2011 [12.36], both inside the cabin and

Table 12.2 List of some microalgae experimented in space missions and their purposes/findings.

S. no	Microalgae species	Mission	Experiment purpose/findings
1	<i>Chlorella vulgaris</i>	Svetlana-2 (Bion-M1)	Studied microgravity's effect on growth rate
2	<i>Scenedesmus</i>	Foton-M2	Investigated photosynthetic changes
3	<i>Chlamydomonas</i>	Space Shuttle STS-77	Examined cell wall structure and motility
4	<i>Nannochloris</i>	Space Shuttle STS-95	Studied photosynthesis and gene expression
5	<i>Haematococcus</i>	ISS-Expedition 4	Explored changes in pigment production
6	<i>Phaeodactylum</i>	Foton-M3	Investigated growth and lipid production
7	<i>Euglena</i>	Kibo (ISS)	Studied the impact of microgravity on motion
8	<i>Chlorella vulgaris</i>	ISS, other space missions	Space-based life support system development
9	<i>Dunaliella salina</i>	ISS	Nutritional content analysis, stress response
10	<i>Nannochloropsis</i> sp.	ISS	Lipid production for biofuel research
11	<i>Euglena gracilis</i>	ISS	Phototaxis and movement behavior
12	<i>Scenedesmus dimorphus</i>	Space Shuttle missions, ISS	Cellular changes in microgravity

(Continued)

Table 12.2 List of some microalgae experimented in space missions and their purposes/findings. (*Continued*)

S. no	Microalgae species	Mission	Experiment purpose/findings
13	<i>Chlamydomonas reinhardtii</i>	Space Algae 1 2014	Studied effects of microgravity on photosynthesis and growth rates
14	<i>Haematococcus pluvialis</i>	Space Algae 2 2016	Investigated astaxanthin production and stress responses in microgravity
15	<i>Scenedesmus</i> sp.	Algae Bioreactor 2017	Tested microalgae cultivation in space for potential use in life support systems
16	<i>Chlorella vulgaris</i>	Space Algae 3 2018	Examined lipid production and potential for biofuel applications in microgravity
17	<i>Dunaliella salina</i>	Algae SAT 2019	Studied the effects of space radiation on the growth and physiology of microalgae

in a hermetically sealed container in the cargo compartment. Through a transparent cover, the latter exposed a variety of viable algal mutants that were enclosed in a pressurized chamber to space radiation. When compared to the original strain, the mutants showed stronger photosynthetic activity and a faster rate of regrowth, indicating a higher capacity for stress recovery, according to post-flight investigation utilizing PCR, oxygen evolution measurements, and fluorescence measurements [12.51].

The *Chlamydomonas reinhardtii* strain was most notable for being transported on board the Russian Foton-M2 and Foton-M3 missions in 2005 and 2007, the wild type together with four different mutants were tested [12.37]. The D1 protein, crucial for photosynthesis, has different

amino acid changes in the mutations and plays a crucial role in stabilizing and enhancing photosystem II under extreme conditions. Photosynthetic performance was measured in flight using fluorescence measurements on cultures that were grown in artificial light. Two mutants showed greater photosynthetic efficiency in space and were able to regrow after returning to Earth, but the wild type and two mutants showed lower photosynthetic activity [12.37]. Researcher then conducted an experiment on the Chinese retrieved satellite on the spacecraft Shenzhou-II in an effort to improve the control system, cultural circumstances, and data gathering system's functionality [12.53]. This spacecraft had freshwater snail (*Bulinus asutralianus*) and *Chlorella pyrenoidosa* on board. Real-time data on the closed aquatic ecosystem (CAES) performance in microgravity was gathered using a variety of sensors to monitor the CAES. To investigate gravity-related issues, an on-board 1 g centrifuge was also included. It was discovered that the main element impacting the CAES performance in space is microgravity. Each day in microgravity, the main producer's biomass changed more than that of the control groups. The mean biomass concentration per day in the microgravity group declined, whereas it grew for a few days before leveling out in the control groups. Microgravity effects that increase the consumer's metabolic rate while decreasing photosynthesis may cause space impacts on a primary producer's biomass [12.52].

In an *in-situ* resource utilization (ISRU) and regenerative life support system experiment for long-duration human space voyage, the majority of microalgal strains were biocompatible with nanosatellite materials; however, due to CO₂ deprivation, microalgal development was severely restricted in sealed microwells (~1 week) without dissolved bicarbonate. Further microalgae also produced oxygen, which led to the creation of bubbles inside the wells, which tampered the sensor readings. By reducing light intensities (2–10 mol photons m⁻² s⁻¹) and temperatures (4–12 °C), researchers were able to extend the development periods of two microalgal strains, *Chlorella vulgaris* and *Dunaliella bardawil*, without producing excessive amounts of oxygen. Though the studies detailed above were carried out to create the GraviSat platform, the outcomes of these studies could be helpful for the introduction of microalgae into other satellite payloads using low-volume microfluidic systems [12.7].

The expected effects of microgravity on diatom growth are in fact the need of the hour due to the many solid reasons discussed above. Gravity can be important for diatoms, which due to their heavy silica wall, generally sink to the bottom. The microgravity may allow diatom to float on the surface. Since diatoms contribute to 25% global O₂ production and Q2

CO₂-fixation. Testing diatom growth during a space mission is limited by the availability and the costs incurred. Alternatively, starting with microgravity simulator experiments on Earth may be the first promising step [12.22].

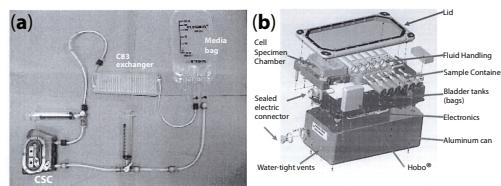
Since the introduction of the classical clinostat in 1879 by Julius von Sachs, a number of GBFs have been designed to simulate the condition of “weightlessness” or “free fall” in laboratories on Earth [12.28].

12.6 Life Support Systems for Space Missions

One of the most promising life support systems was prepared by NASA, known as the controlled life support system (CELSS) for Mars, which would use controlled environmental agriculture (CEA) to grow plants in a thick-walled pressure hull buried inside the Martian surface. However, simulating the Martian atmosphere on Earth has given different results for different plants; for instance, tomato plant growth was inhibited [12.43]. In another study conducted decades back it was observed that winter rye could germinate at pressure as low as 101 mb but failed to grow at pressure below 31 mb. The advances in bioreactors ever since to simulate Martian atmosphere has been an important field of study for various scientists and researchers.

The NASA single loop cell culture reactor was used to grow three diatoms whose genomes have been sequenced: *Phaeodactylum tricornutum*, *Fragilariopsis cylindrus*, and *Thalassiosira pseudonana*. Among these, *F. cylindrus* is abundant in the Artic and Antarctic, hence a suitable diatom to be tested for Mars atmosphere. In 2007, Gordan *et al.* [12.13] first reported the fabrication of a single loop cell culture reactor in which the type of diatom cells at a particular stage and live cell types were selected via fluorescence-activated cell sorting (FACS) or cell cytometry. In this study, the first Compustat was also built, which examined all diatoms in the chamber but destroyed cells which were farthest away by UV or laser light. The Cell Specimen Environment Assembly, which holds up to eighteen Cell Specimen Chambers (CSCs, each with 3 to 30 ml capacity), permits up to 60 samples to be drawn (or injected) and stored under preprogrammed ground control, and provides growth medium perfusion, additive delivery, and heat and gas exchange for each chamber individually;

- 1) Electronics Assembly, which contains all computer and signal conditioning.



Q3 **Figure 12.3** Single Loop for Cell Culture (SLCC): The single loop consists of a CSC (Cell Specimen Chamber), media bag, gas exchanger, and sample and injection syringes (pump, Q4 stir bar motor and controller not shown). (A ; B; Ref)

- 2) Video Microscopy Subsystem, which provides 40x and 200x optical magnification views of the specimen cultures to the crew or video downlink to scientists on the ground.
- 3) Structural Containment Assembly, which supports all the other assemblies and provides interface mounting with the shuttle and ISS host systems.

The temperature was maintained at 4 °C for the psychrophilic diatoms to be cultivated, however before the project could be completed, the cell culture unit (CCU) was modified. The SLCC, which was delivered to NASA in 2006, never flew. The free-swimming Euglena was cultured in a closed SLCC having a porous membrane. The light source was 3500–4000 lux which was economical as the system just needed an initial launch cost except filter tapes, nutrient salts from Provasoli-Guillard National Center for Culture of Marine Phytoplankton, and diatom inoculum in fresh chemostat chambers.

The payload characteristics are: size: 3.4" x 5" x 10" (2.8 liters); power: each SLCC unit requires 2 Watt (165 mA at 12 VDC) steady-state power, with up to 3.3 Watts of peak power when additional pumps and valves are operating; weight: 5.5 lbs (2.5 kg); automation: it is automated, except at the end of the test, a crew member is needed to help for last sample drying, takes less than half an hour. An exploded view of the SLCC is also shown.

Researchers have been constantly working on designing ways to produce oxygen on the Red Planet. A recent study by Gupta *et al.* showed that production of oxygen on Mars can be done *in situ* by an advanced bioreactor system [12.18]. This system has potential to support life on Mars by converting high-grade iron ores by some biological mechanism and the most advanced seems to be that of a photobioreactor.

12.7 Management of the Culture Vessel and Elements

As space exploration advances, innovative and sustainable biowaste management solutions will be essential to support future crewed missions to distant destinations like Mars or beyond. Managing the culture vessel and elements is an important aspect of diatom cultivation. It is also important to maintain proper temperature and pH levels within the culture vessel, as diatoms have specific requirements for these parameters. The culture medium, which is the liquid in which the diatoms are grown, must also be regularly monitored and replenished as needed. Nutrients, such as nitrate, phosphate, and silicate, must also be added to the culture medium in appropriate concentrations to support diatom growth. Additionally, the light level and intensity must be controlled to maintain the optimal light intensity for the diatoms to survive and grow. By properly managing the culture vessel and elements, a healthy and thriving diatom culture can be maintained.

Managing biowaste in a space vessel is crucial to ensure the health and safety of astronauts and the proper functioning of the spacecraft. Biowaste refers to biological waste, such as human waste (urine and feces), food scraps, and other organic materials generated during space missions. Efficient biowaste management is essential for minimizing contamination, controlling odors, and recycling valuable resources. Continuous monitoring of waste management systems is essential to ensure their efficiency and safety. Research on advanced biowaste management technologies is ongoing to improve waste handling and resource recycling in space. Effective biowaste management is critical not only for the health and comfort of astronauts but also for the sustainability and success of long-duration space missions.

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References

- [12.1] Ahirwar, A., Meignen, G., Khan, M.J., Khan, N., Rai, A., Schoefs, B., Marchand, J., Varjani, S. and Vinayak, V. (2021) Nanotechnological approaches to disrupt the rigid cell walled microalgae grown in wastewater for value-added biocompounds: commercial applications, challenges, and breakthrough. *Biomass Conversion and Biorefinery*, 1-26.
- [12.2] Armbrust, E.V. (2009) The life of diatoms in the world's oceans. *Nature* 459(7244), 185-192.
- [12.3] Bernal-Bayard, P., Puerto-Galán, L., Yruela, I., García-Rubio, I., Castell, C., Ortega, J.M., Alonso, P.J., Roncel, M., Martínez, J.I. and Hervás, M. (2017) The photosynthetic cytochrome c 550 from the diatom *Phaeodactylum tricornutum*. *Photosynthesis Research* 133, 273-287.
- [12.4] Cockell, C.S., Rettberg, P., Rabbow, E. and Olsson-Francis, K. (2011) Exposure of phototrophs to 548 days in low Earth orbit: microbial selection pressures in outer space and on early earth. *The ISME journal* 5(10), 1671-1682.
- [12.5] Cycil, L.M., Hausrath, E.M., Ming, D.W., Adcock, C.T., Raymond, J., Remias, D. and Ruemmele, W.P. (2021) Investigating the growth of algae under low atmospheric pressures for potential food and oxygen production on Mars. *Frontiers in Microbiology*, 3171.
- [12.6] De Martino, A., Bartual, A., Willis, A., Meichenin, A., Villazán, B., Maheswari, U. and Bowler, C. (2011) Physiological and molecular evidence that environmental changes elicit morphological interconversion in the model diatom *Phaeodactylum tricornutum*. *Protist* 162(3), 462-481.
- [12.7] Fleming, E.D., Bebout, B.M., Tan, M.X., Selch, F. and Ricco, A.J. (2014) Biological system development for GraviSat: A new platform for studying photosynthesis and microalgae in space. *Life Sciences in Space Research* 3, 63-75.
- [12.8] Fu, W., Shu, Y., Yi, Z., Su, Y., Pan, Y., Zhang, F. and Brynjolfsson, S. (2022) Diatom morphology and adaptation: Current progress and potentials for sustainable development. *Sustainable Horizons* 2, 100015.
- [12.9] Gagarin, Y. (2001) *Soviet Man in Space*. The Minerva Group, Inc.
- [12.10] Ghobara, M.M., Ghobara, M.M., Mazumder, N., Vinayak, V., Reissig, L., Gebeshuber, I.C., Tiffany, M.A., Gordon, R. and Gordon, R. (2019) On light and diatoms: A photonics and photobiology review. *Diatoms: Fundamentals and Applications*, 129-189.
- [12.11] Gingerich, O. (1993) *The Eye of Heaven: Ptolemy, Copernicus, Kepler*. Springer.
- [12.12] Goessling, J.W., González, A.A.S., Raj, V.S.P., Ashworth, M.P., Manning, S.R. and Lopez-Garcia, M. (2020) Biosilica slab photonic crystals as an alternative to cleanroom nanofabrication? *Faraday Discussions* 223, 261-277.

Q5 [12.13] missing

- [12.14] Gordon, R., Merz, C.R., Gurke, S. and Schoefs, B. (2019) Bubble farming: scalable microcosms for diatom biofuel and the next green revolution. *Diatoms Fundam. Appl.*, 583-654.
- [12.15] Guélin, M. and Cernicharo, J. (2022) Organic molecules in interstellar space: Latest advances. *Frontiers in Astronomy and Space Sciences* 9, 787567.
- [12.16] Guiry, M.D. (2012) How many species of algae are there? *Journal of phycology* 48(5), 1057-1063.
- [12.17] Guiry, M.D., Guiry, G.M., Morrison, L., Rindi, F., Miranda, S.V., Mathieson, A.C., Parker, B.C., Langangen, A., John, D.M. and Bárbara, I. (2014) AlgaeBase: an on-line resource for algae. *Cryptogamie, Algologie* 35(2), 105-115.
- [12.18] Gupta, E., Kanu, N.J., Agrawal, M.S., Kamble, A.A., Shaikh, A.N., Vates, U.K., Singh, G.K. and Chavan, S.S. (2021) An insight into numerical investigation of bioreactor for possible oxygen emission on Mars. *Materials Today: Proceedings* 47, 4149-4154.
- [12.19] Hamm, C.E. (2005) The evolution of advanced mechanical defenses and potential technological applications of diatom shells. *Journal of Nanoscience and Nanotechnology* 5(1), 108-119.
- [12.20] Hanslmeier, A. and Hanslmeier, A. (2014) Zustandsgrößen der Sterne. *Einführung in Astronomie und Astrophysik*, 279-307.
- [12.21] Hendrickx, L., De Wever, H., Hermans, V., Mastroleo, F., Morin, N., Wilmette, A., Janssen, P. and Mergeay, M. (2006) Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long-haul space exploration missions. *Research in microbiology* 157(1), 77-86.
- [12.22] Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C., de Geest, M., Hauslage, J., Hilbig, R., Hill, R.J. and Lebert, M. (2013) Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. *Astrobiology* 13(1), 1-17.
- [12.23] Ishak, S., Cheah, W., Waiho, K., Salleh, S., Fazhan, H., Manan, H., Kasan, N. and Lam, S. Aquatic Role of Diatoms: From Primary Producers and Aquafeeds. In: *Diatoms*. CRC Press: 87-104.
- [12.24] Kalirai, J. (2018) Scientific discovery with the James Webb space telescope. *Contemporary Physics* 59(3), 251-290.
- [12.25] Khan, M.J., Singh, R., Shewani, K., Shukla, P., Bhaskar, P., Joshi, K.B. and Vinayak, V. (2020) Exopolysaccharides directed embellishment of diatoms triggered on plastics and other marine litter. *Scientific reports* 10(1), 18448.
- [12.26] Kopparapu, R.K. (2018) The habitable zone: the climatic limits of habitability. *Handbook of Exoplanets*, 58.

- [12.27] Kuefner, W., Ossysek, S., Geist, J. and Raeder, U. (2020) The silicification value: a novel diatom-based indicator to assess climate change in freshwater habitats. *Diatom research* 35(1), 1-16.
- [12.28] Kutschera, U. and Niklas, K.J. (2018) Julius Sachs (1868): The father of plant physiology. *American Journal of Botany* 105(4), 656-666.
- [12.29] Launius, R.D. (2000) The historical dimension of space exploration: reflections and possibilities. *Space Policy* 16(1), 23-38.
- [12.30] Liu, S., Storti, M., Finazzi, G., Bowler, C. and Dorrell, R.G. (2022) A metabolic, phylogenomic and environmental atlas of diatom plastid transporters from the model species *Phaeodactylum*. *Frontiers in Plant Science* 13, 950467.
- [12.31] Martín-Torres, F.J., Zorzano, M.-P., Valentín-Serrano, P., Harri, A.-M., Genzer, M., Kemppinen, O., Rivera-Valentin, E.G., Jun, I., Wray, J. and Bo Madsen, M. (2015) Transient liquid water and water activity at Gale crater on Mars. *Nature Geoscience* 8(5), 357-361.
- [12.32] McMahon, S. (2021) Astrobiology (overview). *Oxford Research Encyclopedia of Planetary Science*.
- [12.33] Mendell, W.W. (1985) *Lunar bases and space activities of the 21st century*. Lunar and Planetary Institute.
- [12.34] Mock, T., Otillar, R.P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A. and Ward, B.J. (2017) Evolutionary genomics of the cold-adapted diatom *Fragilariaopsis cylindrus*. *Nature* 541(7638), 536-540.
- [12.35] Mourya, M., Khan, M.J., Ahirwar, A., Schoefs, B., Marchand, J., Rai, A., Varjani, S., Rajendran, K., Banu, J.R. and Vinayak, V. (2022) Latest trends and developments in microalgae as potential source for biofuels: The case of diatoms. *Fuel* 314, 122738.
- [12.36] missing
- [12.37] Niederwieser, T., Kocielek, P. and Klaus, D. (2018) A review of algal research in space. *Acta Astronautica* 146, 359-367.
- [12.38] Rai, I., Ahirwar, A., Rai, A., Varjani, S. and Vinayak, V. (2022) Biowaste recycling strategies for regenerative life support system: An overview. *Sustainable Energy Technologies and Assessments* 53, 102525.
- [12.39] Remmers, I.M., D'Adamo, S., Martens, D.E., de Vos, R.C., Mumm, R., America, A.H., Cordewener, J.H., Bakker, L.V., Peters, S.A. and Wijffels, R.H. (2018) Orchestration of transcriptome, proteome and metabolome in the diatom *Phaeodactylum tricornutum* during nitrogen limitation. *Algal research* 35, 33-49.
- [12.40] missing
- [12.41] Roukema, B. (1996) On determining the topology of the observable Universe via three-dimensional quasar positions. *Monthly Notices of the Royal Astronomical Society* 283(4), 1147-1152.
- [12.42] Round, F.E., Crawford, R.M. and Mann, D.G. (1990) *Diatoms: biology and morphology of the genera*. Cambridge university press.

- [12.43] Rule, D. and Staby, G. (1981) Growth of Tomato Seedlings at Sub-atmospheric Pressures1. *HortScience* 16(3), 331-332.
- [12.44] Sagan, C. and Pollack, J.B. (1974) Differential transmission of sunlight on Mars: biological implications. *Icarus* 21(4), 490-495.
- [12.45] Shirke, Y.M., Abou-Elanwar, A.M., Choi, W.-K., Lee, H., Hong, S.U., Lee, H.K. and Jeon, J.-D. (2019) Influence of nitrogen/phosphorus-doped carbon dots on polyamide thin film membranes for water vapor/N₂ mixture gas separation. *RSC advances* 9(55), 32121-32129.
- [12.46] Smith, C.M. and Davies, E.T.S. (2012) *Emigrating beyond Earth: Human adaptation and space colonization*. Springer.
- [12.47] Vakkada Ramachandran, A., Nazarious, M.I., Mathanlal, T., Zorzano, M.-P. and Martín-Torres, J. (2020) Space environmental chamber for planetary studies. *Sensors* 20(14), 3996.
- [12.48] Vakkada Ramachandran, A., Zorzano, M.-P. and Martín-Torres, J. (2021) Experimental investigation of the atmosphere-regolith water cycle on present-day Mars. *Sensors* 21(21), 7421.
- [12.49] Vinayak, V. (2022) Algae as sustainable food in space missions. In: *Biomass, Biofuels, Biochemicals*. Elsevier: 517-540.
- [12.50] Vinayak, V., Bhaskar, P., Pandey, L.K. and Khan, M.J. (2022) Diatoms: the Living Jewels and their Biodiversity, Phycosphere and Associated Phenotypic Plasticity: A Lesson to Learn from the Current Pandemic of Coronavirus. In: *Biodiversity in India: Status, Issues and Challenges*. Springer: 385-429.
- [12.51] Vukich, M., Ganga, P.L., Cavalieri, D., Rizzetto, L., Rivero, D., Pollastri, S., Mugnai, S., Mancuso, S., Pastorelli, S. and Lambreva, M. (2012) BIOKIS: a model payload for multidisciplinary experiments in microgravity. *Microgravity Science and Technology* 24(6), 397-409.
- [12.52] Wang, G., Liu, Y., Li, G., Hu, C., Zhang, D. and Li, X. (2008) A simple closed aquatic ecosystem (CAES) for space. *Advances in Space Research* 41(5), 684-690.
- [12.53] Wang, J., Shanahan, J., Qi, G. and Wang, R.K. (2022) *The Making of The Wandering Earth: A Film Production Handbook*. Routledge India.
- [12.54] Webster, C.R., Mahaffy, P.R., Atreya, S.K., Moores, J.E., Flesch, G.J., Malespin, C., McKay, C.P., Martinez, G., Smith, C.L. and Martin-Torres, J. (2018) Background levels of methane in Mars' atmosphere show strong seasonal variations. *Science* 360(6393), 1093-1096.
- [12.55] Xiao, Y., Liu, Y., Wang, G., Hao, Z. and An, Y. (2010) Simulated microgravity alters growth and microcystin production in *Microcystis aeruginosa* (cyanophyta). *Toxicon* 56(1), 1-7.
- [12.56] Young, J.N., Goldman, J.A., Kranz, S.A., Tortell, P.D. and Morel, F.M. (2015) Slow carboxylation of R ubisco constrains the rate of carbon fixation during Antarctic phytoplankton blooms. *New Phytologist* 205(1), 172-181.

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