



Research review paper

Cyanobacteria and microalgae in supporting human habitation on Mars

Lydia J. Mapstone ^{a,1}, Mara N. Leite ^{b,1}, Saul Purton ^a, Ian A. Crawford ^c, Lewis Dartnell ^{b,*}^a Algal Research Group, Department of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK^b Department of Life Sciences, University of Westminster, 115 New Cavendish St., London W1W 6UW, UK^c Department of Earth and Planetary Sciences, Birkbeck College London, Malet Street, London WC1E 7HX, UK

ARTICLE INFO

Keywords:
 Microalgae
 Cyanobacteria
 Mars
 Synthetic biology
 Space
 In-situ resources
 Food
 Biopolymers
 Pharmaceuticals
 Cultivation

ABSTRACT

Establishing the first human presence on Mars will be the most technically challenging undertaking yet in the exploration beyond our planet. The remoteness of Mars from Earth, the inhospitable surface conditions including low atmospheric pressure and cold temperatures, and the need for basic resources including water, pose a formidable challenge to this endeavour. The intersection of multiple disciplines will be required to provide solutions for temporary and eventually permanent Martian habitation. This review considers the role cyanobacteria and eukaryotic microalgae (collectively referred to here as 'microalgae') may have in supporting missions to the red planet. The current research using these microorganisms in biological life support systems is discussed, with a systematic analysis of their usage in each system conducted. The potential of microalgae to provide astronauts with oxygen, food, bio-polymers and pharmaceuticals is considered. An overview of microalgal experiments in space missions across the last 60 years is presented, and the research exploring the technical challenges of cultivation on Mars is discussed. From these findings, an argument for culturing microalgae in subterranean bioreactors is proposed. Finally, future synthetic biology approaches for enhancing the cyanobacterial/microalgal role in supporting human deep-space exploration are presented. We show that microalgae hold significant promise for providing solutions to many problems faced by the first Martian settlers, however these can only be realised with significant infrastructure and a reliable power source.

1. Introduction

1.1. Mars: The next frontier

Crewed missions to Mars are widely considered as the next step in human space exploration following a planned return to the moon in the mid-2020s (ISECG, 2018). There are several driving forces for the human exploration of Mars, including science, technology, human curiosity, and zest for discovery (e.g., Cockell, 2004; Horneck et al., 2006; Nangle et al., 2020; Rapp, 2008).

Previous robotic missions to Mars have helped us gain substantial knowledge regarding the planet's geology and atmosphere, and identified the many challenges for human exploration. A comparison of environmental parameters reveals stark differences between Earth and Mars (Fig. 1). Mars' thin atmosphere (6–7 mbar average at the datum surface altitude; Jakosky et al., 2018), consists of 95% CO₂, 2.8% N₂, 2.1% Ar, with only 0.13% O₂, compared to the 21% O₂ 78% N₂ and

0.04% CO₂ of the Earth's ~1000 mBar atmosphere (Franz et al., 2017). The thin atmosphere also results in large temperature variability between day and night, with an average surface temperature estimated to be –60 °C (Liu, 2003; Osczevski, 2014). Additionally, the occurrence of dust storms can significantly reduce sunlight on the surface and can last for months (Leovy, 2001). The combination of low temperature and low air pressure means that liquid water is generally not stable under current Martian surface conditions (Richardson and Mischna, 2005). Finally, the surface is bombarded by ionizing particle radiation (Dartnell et al., 2007; McKenna-Lawlor et al., 2012; Simonsen et al., 1990) and high doses of damaging wavelengths of solar ultraviolet light (Patel et al., 2002). Considering these significant constraints, a mission to Mars will be technically challenging and extremely expensive, mainly due to the volume of consumables such as oxygen, water, food and shelter required for astronauts' survival while *en route* to Mars, on the surface, and during the return to Earth.

Although current plans to establish human settlements on Mars are

Abbreviations: PBR, Photobioreactor; ISRU, In-Situ Resource Utilization; AM, Additive Manufacturing; BLSS, Biological Life Support Systems..

* Corresponding author.

E-mail address: lewis@lewisdartnell.com (L. Dartnell).

¹ Both these authors contributed equally.

<https://doi.org/10.1016/j.biotechadv.2022.107946>

Received 26 October 2021; Received in revised form 21 February 2022; Accepted 15 March 2022

Available online 19 March 2022

0734-9750/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

not yet sufficiently developed, national space agencies and private companies have shown interest in sending humans to Mars in the next few decades (e.g., NASA, 2020, 2009; Rapp, 2008; ISECG, 2018). Mission proposals vary significantly in the number of crew members (2–12) and length of stay. Before the first astronauts' arrival, habitational facilities offering shelter, oxygen and a water supply should already have been emplaced robotically. Farming will play a key role in the establishment of a permanent colony, but even with pressurised habitats this will be challenging due to the lack of fertile soil, essential minerals, and the presence of toxic compounds such as perchlorates on the Martian surface (Verseux et al., 2016).

1.2. The path to self-sufficiency

Missions to Mars will be resource-constrained since they depend significantly on the cargo's volume and mass. Larger payloads will require more storage and fuel, with fuel being expected to be about two-thirds of the mass of roundtrip missions (Human Spaceflight Plans Committee, 2010).

A significant degree of self-sufficiency will therefore be key to the success of Martian missions. Unlike the International Space Station (ISS), resupply from Earth is impractical, with a one-way trip taking somewhere between six to nine months (Helisch et al., 2020). Furthermore, travel opportunities are restricted by the "launch window," a limited period during which a spacecraft can be launched to reach the target orbit. If this window is missed, one must wait approximately two years for the next opportunity to launch to Mars. Critically, this means that in cases of emergency, Earth cannot send rescue missions and crew will not be able to return early. It is therefore imperative that Mars colonies become self-sufficient in their early stages.

One way to overcome Earth dependency is to gather and process the resources already available on Mars. This approach is called In-Situ Resource Utilization (ISRU). ISRU approaches include water harvesting from Martian ice, compressing and processing atmospheric gases, and mining minerals and constructing infrastructure from Martian regolith (Menezes et al., 2015; Nangle et al., 2020). The Martian atmosphere is rich in inorganic carbon (CO_2) that can be harvested through ISRU and transformed into organic products (Fig. 2). Although Mars' atmosphere is relatively deficient in nitrogen (~2.8%), it may be

possible to compress N_2 to sufficient quantities to be available for conversion to ammonium, either by the Haber-Bosch chemical process or via N_2 fixing microorganisms (Valgardson, 2020). The current methods and proposed approaches to conduct ISRU are discussed in detail in a recent review by Starr and Muscatello (2020), and here we will assume that considerations such as obtaining water and producing energy have been solved prior to the first crewed mission.

Another innovative solution that can help overcome issues of component availability and storage is Additive Manufacturing (AM), also known as three-dimensional (3D) printing. Through this technique, layers of material (e.g. plastic) are deposited successively to produce items on-demand, including replacement hardware, medical devices, and even large-scale structures such as habitats (Isachenkov et al., 2021; Menezes et al., 2015). Printing *in situ* with Martian raw materials and locally-synthesised biopolymers will reduce the required launch mass (Menezes et al., 2015). *In situ* production can also aid the return journey to Earth: synthesizing methane and oxygen for rocket propellant from atmospheric CO_2 and water ice. For example, the SpaceX mission plans include the construction of a local propellant plant for this purpose (Musk, 2018).

At the core of the practicability of such deep-space missions is the recycling of both organic and inorganic wastes. On Mars, regenerative life support systems that allow the efficient recovery of valuable products from waste will be implemented to help attain a closed-loop approach. As will be discussed in this review, microalgae will have a large role to play in achieving self-sufficiency through utilising the elements available on Mars, generating biopolymers for 3D-printing, and providing regenerative life support systems and recycling. Therefore, infrastructure for microalgal cultivation and harvesting should be considered as one of the core design requirements for long-term Martian habitation (Fig. 2).

1.3. Cyanobacteria and microalgae

Most of Earth's ecosystems are underpinned by photoautotrophs that harness energy from light to fix carbon dioxide into organic biomolecules, and in the process split water to release O_2 . Oxygenic photoautotrophs include terrestrial and aquatic plants, macroalgae (i.e. seaweeds), microalgae (i.e. microscopic species of eukaryotic algae),

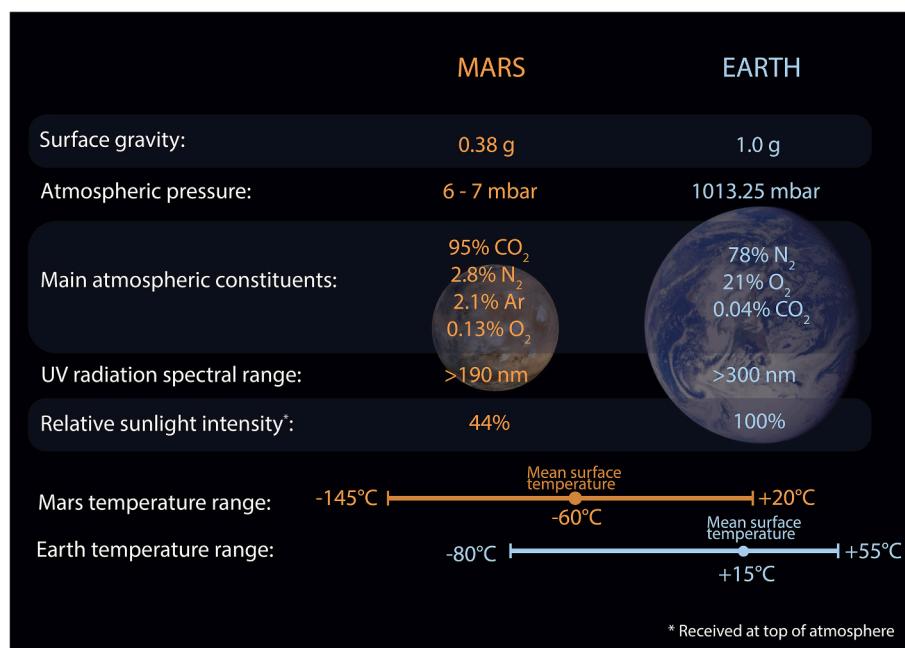


Fig. 1. Comparison between Earth and Mars of representative environmental parameters. Atmospheric pressure and temperature measurements are for Earth sea and Mars datum level recordings (although it is noted that Martian atmospheric pressure varies with season as well as altitude).

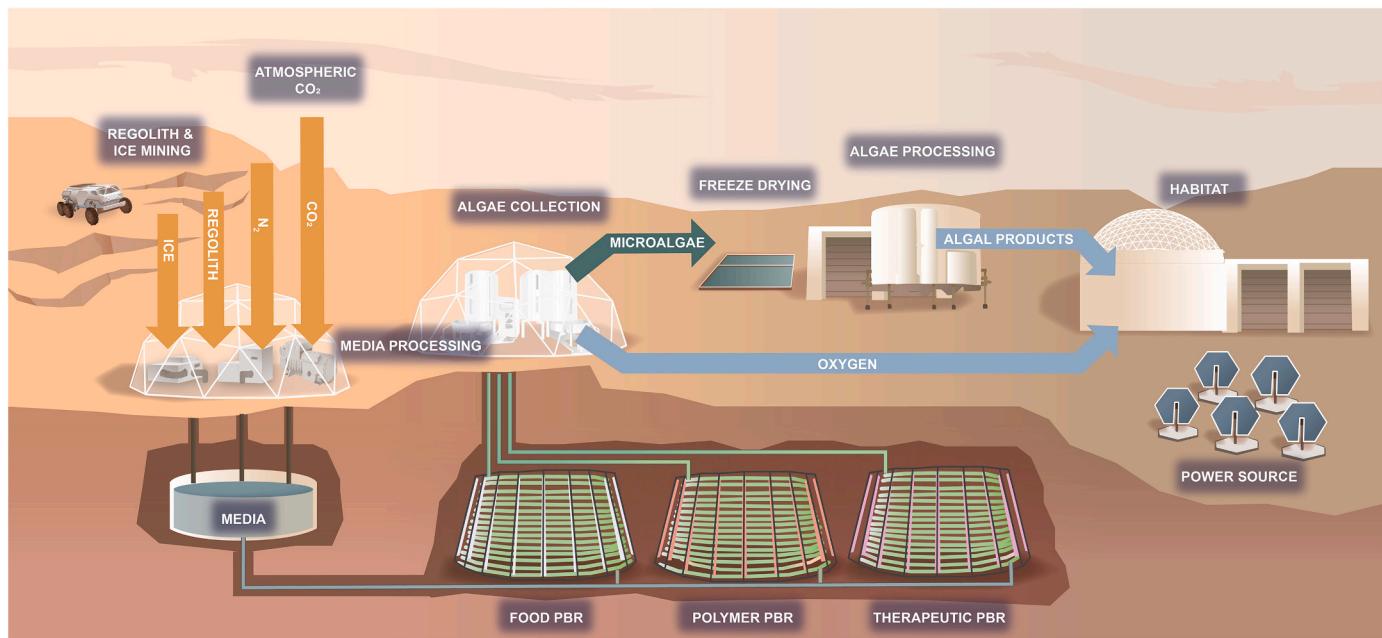


Fig. 2. Artist impression of a Martian base. Atmospheric CO₂, and ice and nutrients from regolith form the basis of growth media for microalgae, which are cultivated in various sub-surface photobioreactors (PBR). These PBRs are illuminated using artificial lighting banks powered from photovoltaic systems located on the planet's surface. The oxygen produced by the algae can be used directly for life support, and the microalgal biomass collected, dried and processed to provide food, polymers and therapeutics.

and photosynthetic bacteria termed cyanobacteria. For brevity, the term 'microalgae' is used in this review to refer to both cyanobacteria and microalgae (Fig. 3). Although plants are an important source of nutrition

and oxygen, there are many benefits that make cyanobacteria and microalgae attractive as a future commercial technology on Earth and for space exploration. This includes greater growth rates, minimal

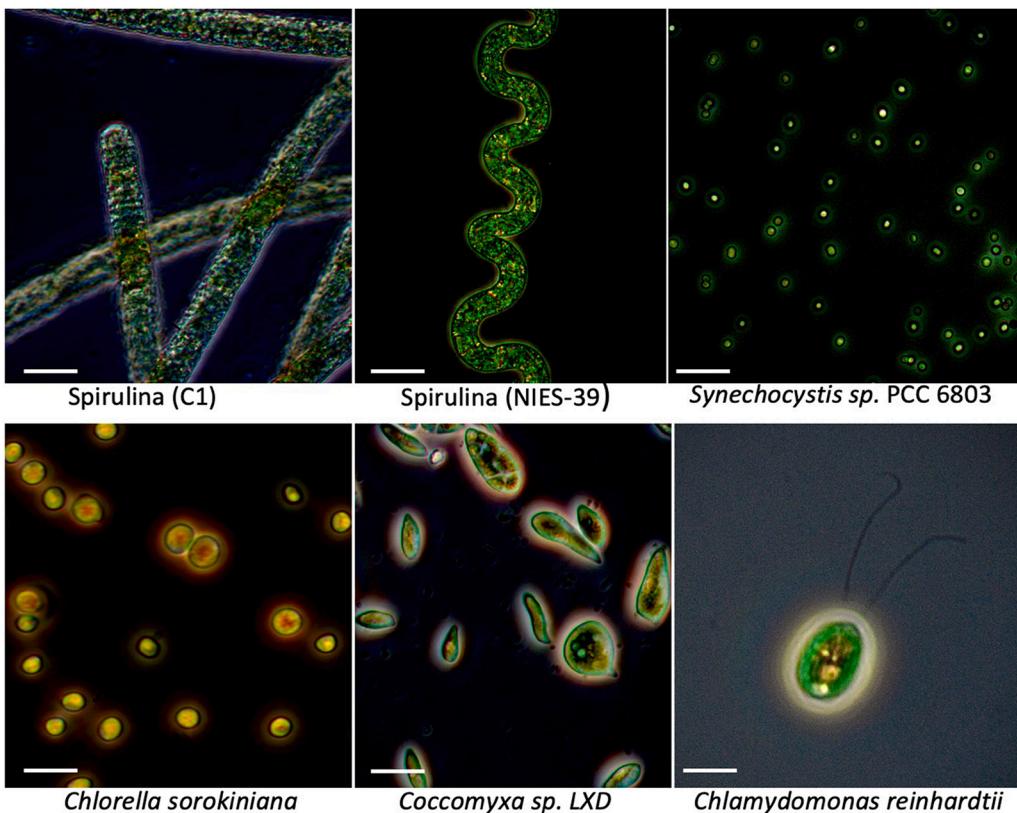


Fig. 3. Micrographs of different microalgae discussed here. Cyanobacteria on top row: two species of the filamentous *Limnoспора fusiformis* (commonly referred to as *Spirulina*) and the single-celled *Synechocystis* sp. PCC 6803. Single-celled eukaryotic microalgae on bottom row: *Chlorella sorokiniana*, *Coccomyxa* sp. LXD and *Chlamydomonas reinhardtii*. Scale bar size: 5 μm.

nutritional requirements, the ability to be grown on non-arable land and the production of several valuable by-products (Fabris et al., 2020; Janssen et al., 2014).

The use of various autolithotrophic microorganisms has been proposed (including those genetically engineered) for the support of human habitation of Mars, for example in biomining regolith for metals; providing chemical feedstocks for subsequent bioreactor cultures; as well as producing nutrients for human consumption (Averesch, 2021; Cockell, 2010; Nangle et al., 2020). Such lithotrophic or fermentation cultures can be sustained in simple stir-tank bioreactors and can achieve short harvest times, and so offer advantages for supporting human habitation, especially in the earliest stages of establishment (Nangle et al., 2020). Anoxygenic phototrophs, such as certain purple non-sulfur alphaproteobacteria, have also been discussed for their ability to fix nitrogen or provide products such as bioplastics or hydrogen propellant when cultured on a wide range of substrates (Averesch, 2021; Berliner et al., 2021; McKinlay and Harwood, 2010). Here, we focus on the applications of oxygenic photoautotrophs, specifically microalgae. The bioreactors needed for their growth are more complex than those for lithotrophic or heterotrophic cultures, requiring access to Martian sunlight or artificial lighting. However, cultivation on Mars will benefit from the simplicity of pressurising the CO₂-rich atmosphere to support photoautotrophic growth, and can provide multiple resources at once, such as oxygen and food.

Microalgae are emerging as alternative feedstocks in different industries, ranging from clothing to aquafeed to pharmaceuticals (Rizwan et al., 2018). Many of these applications will be useful on Mars. For example, recyclable bioplastics derived from algal biomass can serve as sustainable substrates for use in 3D printers. The *in situ* production of the raw materials required for fabrication will result in considerable reductions in launch mass (Menezes et al., 2015). Microalgae could also facilitate biomining on Mars, with metals extracted and recovered from Martian rocks through the action of cyanobacteria (Cockell, 2010). Table 1 lists potential microalgae for space applications and their most relevant features which will be discussed in the following sections. Although there has been substantial research into algal production of biodiesel or bioethanol for sustainability on Earth (Lam and Lee, 2012; Li et al., 2018; Peng et al., 2020; Qari et al., 2017), such biofuels are not practical as the major energy source for Martian surface habitation: fuel generation from microalgae is currently far less efficient than other power sources (Carneiro et al., 2017), and the oxygen required for their combustion is itself a limited resource needed for crew survival. Other energy sources (i.e. photovoltaics and nuclear power) will be needed to power a Mars habitat, although ISRU-produced oxygen and propellants (such as hydrogen or methane) have been discussed for ascent vehicles from the surface (NASA, 2009; Starr and Muscatello, 2020) and may be produced biologically (Menezes et al., 2015).

The genera *Chlorella*, *Limnospira* and *Chlamydomonas* are the most well characterized and studied microalgae in space exploration. *Limnospira* is a newly formed genus into which the commercial strains of Spirulina (previously called *Arthrospira platensis*) have been re-classified as *Limnospira fusiformis* (Nowicka-Krawczyk et al., 2019). For simplicity this species will be referred to as Spirulina throughout this review. The easy cultivation of *Chlorella*, *Limnospira* and *Chlamydomonas* makes them suitable candidates for Mars-specific biological life support systems (BLSS) (Helisch et al., 2020). All three genera have been tested in different life support systems, mainly for food and air revitalization, as shown in Table 2. *Anabaena* and *Nostoc* have biomining potential and can offer nitrogen-fixation. *Euglena gracilis* is also an established model organism in space research (Hauslage et al., 2018; Richter et al., 2014). It is an emerging dietary source with commercially relevant products (Gissibl et al., 2019), and can also withstand extremophilic conditions, such as low temperatures and desiccation (Hauslage et al., 2018). *Euglena* is frequently flown in space missions and has successfully provided enough oxygen for fish in a bioregenerative life support system, as summarised in Table 2 (Hauslage et al., 2018; Richter et al., 2014).

Table 1

List of promising microalgae for space exploration and their most attractive features.

Microalgae	Type of microalgae	Most relevant features	References
<i>Anabaena/Nostoc</i>	Filamentous cyanobacterium	Extremophile; genetic tools available; edible species; can use very high concentrations of carbon dioxide and Martian regolith simulant; biomining potential; capable of nitrogen fixation	(Arai et al., 2008; Bothe et al., 2010; Cockell et al., 2011; Murukesan et al., 2016; Olsson-Francis et al., 2012; Olsson-Francis and Cockell, 2010; Versey et al., 2021)
<i>Limnospira fusiformis</i> (Spirulina)	Filamentous cyanobacterium	Edible with excellent nutritional and nutraceutical properties	(Rumpold and Schlüter, 2013; Wells et al., 2017)
<i>Chlamydomonas reinhardtii</i>	Green microalgae	Great set of genetic tools available (e.g., for production of pharmaceuticals)	(Dyo and Purton, 2018; Taunt et al., 2018)
<i>Chlorella vulgaris</i>	Green microalgae	Edible; air revitalization; well-characterized; most studied under spaceflight conditions	(Niederwieser et al., 2018; Wang et al., 2008; Wells et al., 2017)
<i>Chroococcidiopsis</i>	Unicellular cyanobacterium	Extremophile (including desiccation and radiation tolerance)	(Billi et al., 2000; Cockell et al., 2011; Olsson-Francis and Cockell, 2010; Versey et al., 2016)
<i>Euglena gracilis</i>	Green microalgae	Edible with excellent nutritional properties; air revitalization; model organism in gravitational research; extremophile	(Gissibl et al., 2019; Hauslage et al., 2018; Martínez et al., 2017; Richter et al., 2014; Strauch et al., 2008; Suzuki, 2017)
<i>Synechocystis/Synechococcus</i>	Unicellular cyanobacterium	Well-studied metabolism; vast array of genetic tools (e.g., boost biopolymer content); survival in very high concentrations of carbon dioxide	(Carpine et al., 2017; Murukesan et al., 2016)

Additional applications of these microalgae are provided in Table 1.

1.4. Studies on microalgae in space

Many of the microalgae listed in Table 1 with potential applications for supporting human habitation on Mars have already been studied in space. Fahrion et al., 2021 and Niederwieser et al., 2018 provide recent reviews of microalgae studies conducted on space stations, space shuttle missions, and recoverable satellites, which we summarise in Fig. 4 as an overview of all space platforms that have flown microalgae culturing experiments. (For clarity we are not exhaustively listing all species flown, but focussing on the seven most prominent microalgae for supporting human habitation of Mars as argued in Table 1). However, as Fahrion et al. (2021) note, many of these space-based studies lack key reported information, including parameters such as pH and light intensity, which makes comparison of the results between different studies difficult. In future, such experiments would benefit from real-time

Table 2
Microalgae tested in Biological Life Support Systems.

BLSS	Contributors	Microalgal species	Objective(s)	Reference(s)
BIOS (BIOlogical closed life support System) ^a	Russia	<i>Chlorella vulgaris</i>	Air revitalization/ Water recycling	(Gitelson et al., 1989; Salisbury et al., 1997)
MELiSSA (Micro-Ecological Life Support System Alternative) ^b	Belgium/ France/ Spain/ Canada	<i>Limnospira fusiformis</i> (<i>Spirulina</i>)	Air revitalization/ Food production/ Water recycling	(Hendrickx et al., 2006)
EU:CROPIS (Euglena and Combined Regenerative Organic food Production on Space) ^b	Germany/ Russia	<i>Euglena gracilis</i>	Air revitalization/ Water (urine recycling)	(Hauslage et al., 2018)
AQUACELLS ^b	Germany/ Russia	<i>E. gracilis</i>	Air revitalization	(Häder et al., 2006; Porst et al., 1997)
CAES (Closed Aquatic Ecosystem) ^b	China	<i>Chlorella pyrenoidosa</i>	Air revitalization/ Food production	(Wang et al., 2008)
OMEGA HAB (<i>Oreochromis Mossambicus-Euglena Gracilis</i> -Aquatic HABitat) ^b	Germany/ Russia	<i>E. gracilis</i>	Air revitalization	(Strauch et al., 2008)
CERAS (Closed Ecological Recirculating Aquaculture System) ^a	Japan	<i>C. vulgaris/ L. fusiformis/ Scenedesmus quadricauda</i>	Air revitalization/ Water recycling	(Endo et al., 1999; Omori et al., 2001; Takeuchi and Endo, 2004)
CyBLiSS (Cyanobacterium-Based Life Support Systems) ^a	Germany/ USA/ Italy	<i>Anabaena</i> PCC 7938	Substrate for other BLSSS organisms	(Billi et al., 2021; Verseux et al., 2021)
SIMBOX (Science in Microgravity Box) ^b	China/ Germany	<i>E. gracilis/ C. pyrenoidosa</i>	Air revitalization/ Food production/ Water recycling	(Li et al., 2017; Preu and Braun, 2014)
PBR@LSR (Photobioreactor at the Life Support Rack) ^b	Germany/ Russia	<i>C. vulgaris</i>	Air revitalization/ Food production	(Detrell et al., 2019)

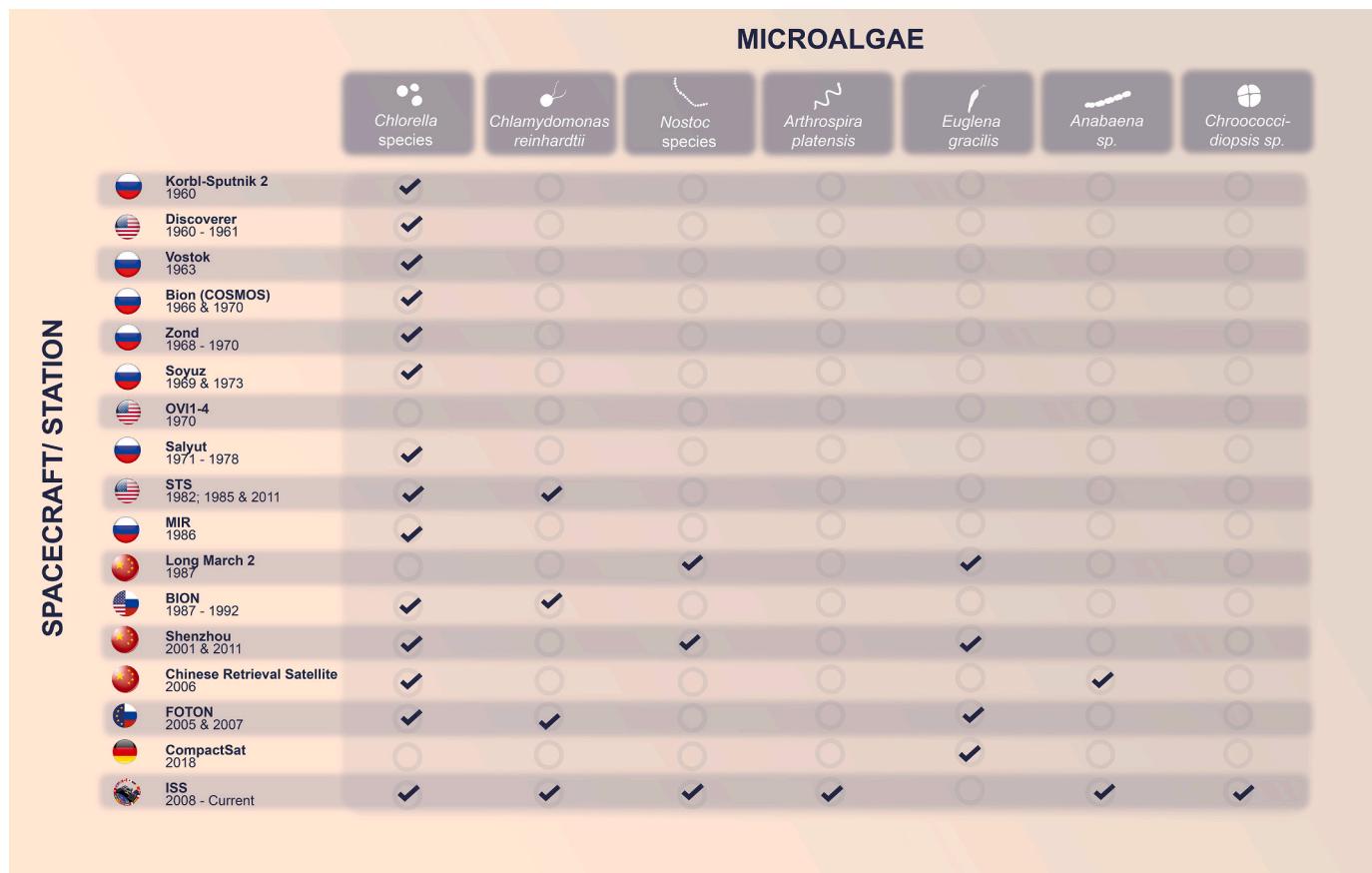
^a Earth-based simulations.^b Tested in space.

Fig. 4. List of all space platforms that have flown microalgae cultivation experiments. The most prominent microalgae used in these studies are indicated.

monitoring of all critical parameters including temperature, gas exchange rates, light intensity, nutrient concentrations, and biomass production.

The first microalga to be launched into low-Earth orbit (LEO) was *Chlorella pyrenoidosa* in 1960, and through that decade *Chlorella* cultures were flown on both short and long-term (22 day) missions by the Soviet

Union (Alexandrov, 2016; Niederwieser et al., 2018). After no significant differences were observed between test and ground cultures, *Chlorella* was sent to orbit the moon aboard the Zond spacecraft in 1968 and was later subjected to different experiments inside the Salyut space stations, which showed that microgravity does not interfere with algal growth (Alexandrov, 2016). (Growth under conditions of microgravity,

and its effects on the handling of liquids, are relevant to the use of microalgae-based systems during interplanetary transit (Huang et al., 2018), although not while on the Martian surface). On the basis of these early studies, and its robustness and flexibility of cultivation, *Chlorella* became one of the preferred candidates for incorporation into BLSSs (Wheeler, 2011), and the genus has subsequently been shown to be highly resilient to space conditions (Helisch et al., 2020).

New systems are being developed to facilitate the study of microalgae in the context of space exploration. For example, a simple protocol for liquid cultures of *Chlamydomonas reinhardtii* has been recently developed for adaption to the Veggie plant growth chamber used to grow vascular plants aboard the ISS (Zhang et al., 2020a, 2020b). With this system, batch cultures can be maintained for at least a month at room temperature, without agitation, inside gas-permeable, commercial tissue culture bags made of fluorinated ethylene propylene. Though this study was conducted on the ground, the system has since been operated on the SpaceX CRS-15 mission, although details are not yet published. The whole genome sequences of the adapted strains have been uploaded to NASA's GeneLab repository, making *C. reinhardtii* the first microalga to be included in this open-access database for genomics, proteomics, epigenomics, transcriptomics and metabolomics in support of biological spaceflight experiments (Settles, 2020).

Although microgravity experiments are relevant to the interplanetary transfer between Earth and Mars, whilst on the Martian surface both crew and components of biological life support systems will be subject to partial gravity of 0.38 g (see Fig. 1). The number of studies investigating microalgal growth and metabolic responses to fractional gravity have so far been limited (Santomartino et al., 2020). Mars gravity can be experimentally simulated in orbit using a centrifuge. For example, Japan's Multiple Artificial-gravity Research System (MARS) was used for the first microbial study under simulated Mars gravity, aboard the ISS. The authors reported no significant differences in the final cell

concentrations of three different bacteria after 21 days of growth (Santomartino et al., 2020). Focusing specifically on microalgae, researchers at the University of Stuttgart have designed TIME SCALE, a concept of an experimental platform for studying microalgae under fractional gravity conditions for future applications in regenerative life support systems (Detrell et al., 2018).

Fig. 5 presents a summary of the main scientific developments in algal space research in chronological order.

The organisational framework we use for this review is based on an adaptation of Maslow's Hierarchy of Needs (HoN) applied to survival on the Martian surface. Abraham Maslow first proposed his classification system in 1943 for the motivations for human behaviour (Maslow, 1943). Often represented as a pyramid, this hierarchy moves from requirements for survival at the base level to psychological needs for personal relationships, and self-fulfilment at the top. As illustrated in Fig. 6, we adapt the concept to propose a hierarchy of requirements for human survival during deep space exploration that can be met by microalgae, ordered based on those that would have the most immediate and grave life-critical effects in the event of failure. These applications of microalgae thus progress from maintaining a breathable atmosphere within pressurised crew areas, to supplying sufficient cultivated food and purified water, with production of biopolymers for clothing or 3D-printed articles and synthesis of pharmaceuticals providing for longer-term necessities. After treating these topics, the last sections of the review will consider the challenges for microalgae cultivation on Mars and what role synthetic biology may play in the future.

2. Biological life support systems and oxygen generation

Currently aboard the ISS, the Environmental Control and Life Support System (ECLSS) produces breathable oxygen by electrolysis of water as well as a Sabatier reactor employing the catalytic conversion of

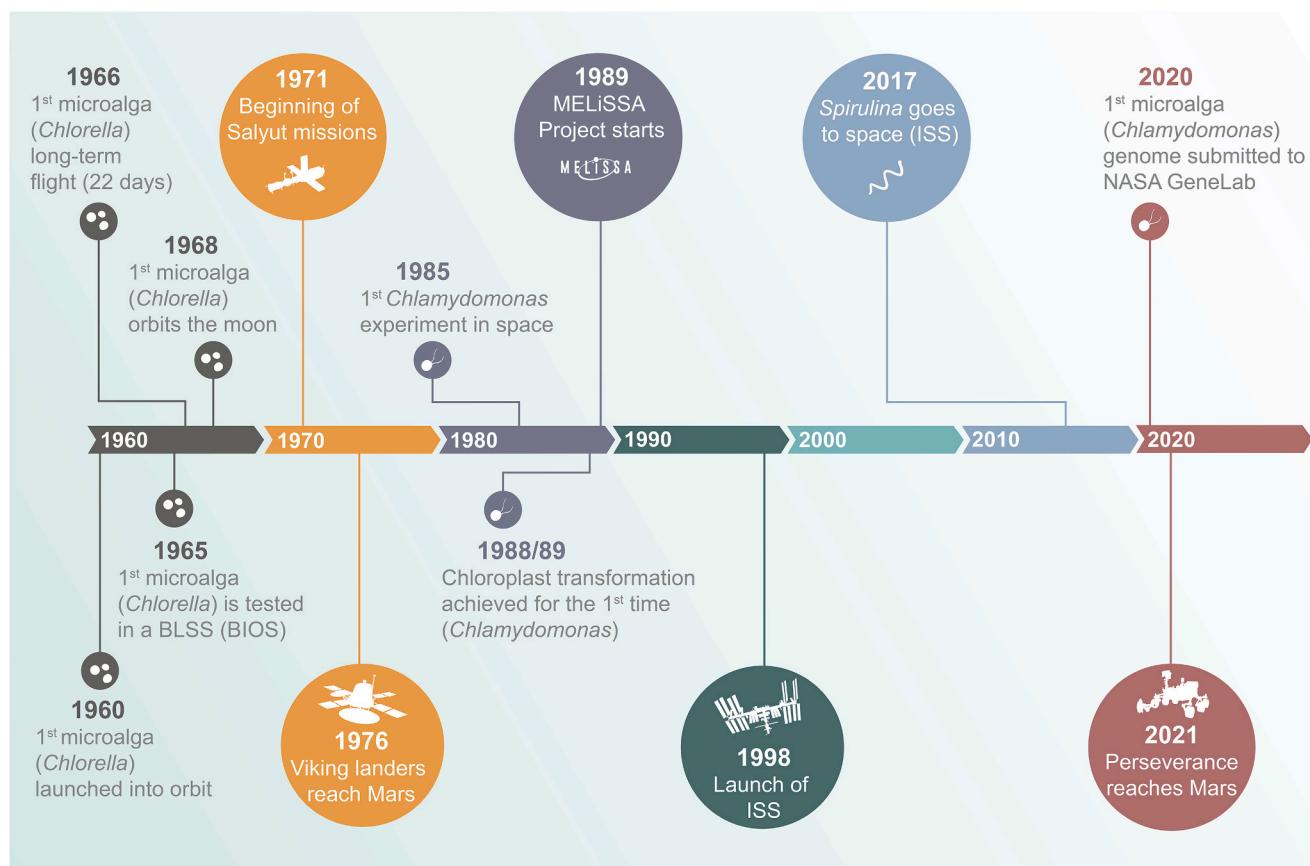


Fig. 5. Chronological timeline of major scientific achievements in algal space research and in space and Mars exploration.

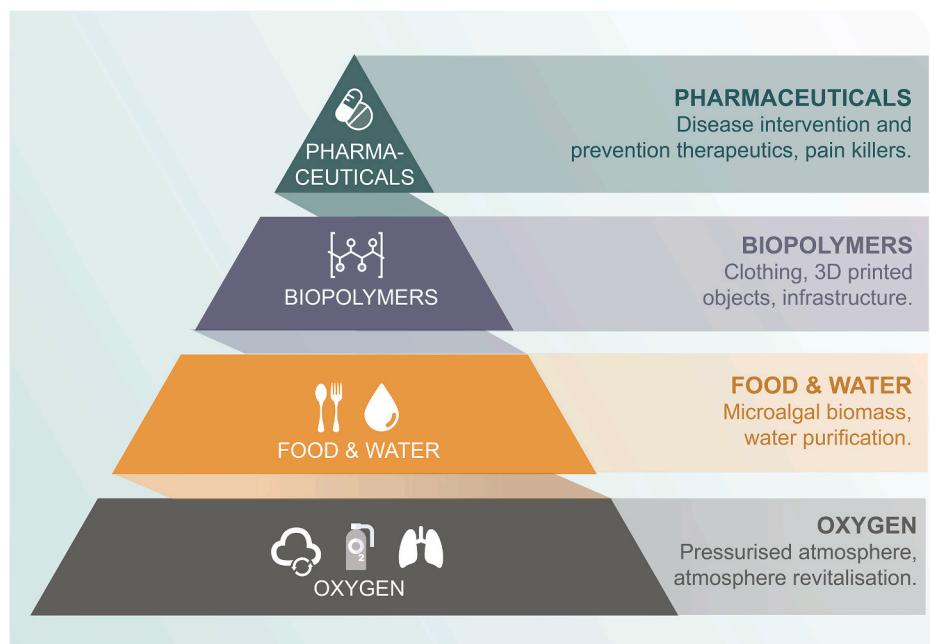


Fig. 6. Hierarchy of microalgae-derived products discussed in this paper. This conceptualisation highlights the ranking of concerns for supporting human survival in deep space exploration (i.e. where failure would have the most immediate and grave consequences for the crew) and serves as a visualisation of the order of topics covered in this review.

CO₂, and additional CO₂ scrubbing from the atmosphere. Most of the water from urine is recovered by vacuum distillation to recycle drinkable water, whereas solid human waste, and other waste, is loaded into resupply vehicles and destroyed upon their re-entry into Earth's atmosphere. Such life support systems are described as being open as they do not completely recycle matter and so entail the loss of resources as waste and necessitate resupply from Earth. While this is acceptable for short-term and low Earth orbit flights and space stations, crewed deep-space missions, including the habitation of Mars, will require loop closure of life support systems to more completely recycle the biological products of the astronauts and minimise the launch mass of necessary consumables (Alexandrov, 2016; Bagdigian et al., 2015; Barta and Henninger, 1994).

BLSS which utilise living organisms growing in bioreactors to recycle resources as an artificial ecosystem, offer a solution for closing the loop, as depicted schematically in Fig. 7.

While BLSSs are more complex than traditional life support systems, the incorporation of photoautotrophs expands life support system functionalities to include food production on top of the treatment of air, water, and waste. Microalgae have been incorporated into a number of BLSS, and a list of current or past investigations in BLSSs and their respective tasks are summarised in Table 2.

The most critical resource for the immediate survival of a crew on Mars, shown as the base layer of Fig. 6, is the generation of breathable oxygen. A crew of six astronauts consumes between 4–6 kg of oxygen a day, taking into consideration daily intense exercise routines of two hours (Horneck et al., 2006). Several physico-chemical methods are available to produce oxygen *in situ* on Mars. Oxygen can be generated by the electrolysis of water, as employed aboard the ISS. Oxide minerals in the Martian regolith can be physically or chemically separated (Sibille and Dominguez, 2012). Carbon dioxide captured from the atmosphere can also be split into oxygen and carbon monoxide, as has been successfully demonstrated on Mars by the MOXIE (Mars Oxygen ISRU Experiment) instrument aboard NASA's Mars 2020 'Perseverance' rover (Hecht et al., 2021). Alongside these options, microalgal photosynthesis may also provide oxygen for long-duration habitation of the Martian surface, as well as within the interplanetary transfer vehicle, and most BLSS systems tested so far (Table 2) have had this functionality at their

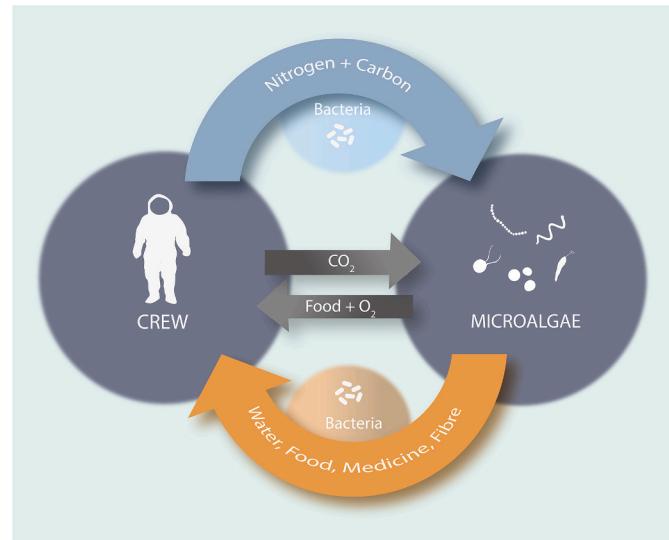


Fig. 7. Simplified schematic representation of a closed-loop biological life support system involving microalgae as a main component. The figure includes the products that humans can obtain directly from microalgae (i.e. food and oxygen) and indirectly through other microorganisms (i.e. used as feedstock to aid fermentation of bacterial products).

core.

The Soviet BIOS-3 (BIOlogical closed life support System) facility begun in 1965 was one of the earliest to employ microalgae (Salisbury et al., 1997). BIOS-3 was used to study gas exchange between a crew of 1–3 humans, and an 18 L algal bioreactor containing *Chlorella vulgaris*. By absorbing carbon dioxide and releasing oxygen during photosynthesis, the *Chlorella* was responsible for 20% of the overall system closure and later 80%–85% by helping recycle water in addition to air.

More recently, the MELiSSA (Micro-Ecological Life Support System Alternative) project, started in 1989, has been refining a more complex BLSS. Its pilot plant is based in Barcelona, Spain, and is coordinated by

the European Space Agency (ESA). MELiSSA mimics an aquatic ecosystem, with Spirulina as the key microalgal component (Hendrickx et al., 2006). MELiSSA's closed-loop has five compartments with different components, such as plants and microalgae, bacteria and humans, responsible for performing different tasks (Hendrickx et al., 2006). The aim is for solid waste, including faecal matter and inedible plant material, to be first processed with thermophilic anaerobic bacteria that can thrive at very high temperatures capable of destroying pathogens. In this stage the waste is converted into ammonium, minerals, and volatile fatty acids, which are then treated by other bacteria in subsequent compartments for the recycled carbon and nitrogen to support growth of higher plants and Spirulina (Clauwaert et al., 2017), which in turn provide food and oxygen for the astronauts (Hendrickx et al., 2006).

The PBR@LSR (Photobioreactor at the Life Support Rack), which was launched to the ISS in 2019, evaluated the performance of a hybrid system. The two-stage process first extracts and concentrates CO₂ produced by astronauts from the cabin's atmosphere using the ESA Life Support Rack, with the CO₂ then fed to a photobioreactor containing *Chlorella vulgaris*, to produce oxygen and biomass. Due to power failure and leakage, the experiment was terminated early and oxygen production could not be evaluated. Nonetheless, continuous growth and higher biomass than the control parallel experiment were observed at the beginning of the experiment (Detrell et al., 2019).

Spirulina's oxygen production has also been evaluated aboard the ISS inside the ArtEMISS (*Arthospira* sp. gene Expression and mathematical Modelling on cultures grown in the International Space Station) photobioreactor as part of the MELiSSA program (Poughon et al., 2020). Data from the ISS and ground bioreactors used as controls were comparable, and the authors concluded that microgravity has no major effect on the oxygen production rate. The Arthospira-B experiment (also using a Spirulina strain) was the first experiment in space to allow online remote monitoring of cellular metabolism, specifically oxygen production and growth rate. Direct measurements helped assess the effect of microgravity at a cellular level by giving insights into time variables instead of integral variables such as biomass concentration (Poughon et al., 2020).

For long-term missions, life support systems must not only regenerate breathable atmosphere and water, but also recapture valuable chemical elements from waste, such as nitrogen and phosphorus, which are in short supply on Mars. Nangle et al. (2020) argue that biotechnology will be key to closing this loop, with urine, being easier to process, to be the first waste stream to be solved. Microalgae can serve this function, with multiple studies having shown that *Chlorella* and *Spirulina* can utilise the urea in urine as a nitrogen source (Hodson and Thompson, 1969; Avila-Leon et al., 2012; Chang et al., 2013; Khalili et al., 2015; Jaatinen et al., 2016; Sukumaran et al., 2018), and more recently, Tuanet et al., 2019 demonstrated continuous growth of *Chlorella sorokiniana* in urine beyond 8 months.

3. Microalgae as food

3.1. The food challenge on Mars

A crew of six will need 13–15 t of food for a 500 day return mission to the Martian surface (Nangle et al., 2020). While the first missions will likely rely on food payloads, this core supply may be supplemented by food grown using *in situ* resources during the mission, and for longer-duration Martian habitation, self-sufficiency for nutritional intake will become imperative.

Arable cultivation supports human populations on Earth, and the farming of staple crops on regolith has been proposed to support the nutritional requirements for long-term Martian habitation (Eichler et al., 2021; Wamelink et al., 2014). Microalgae may play a supportive role in such crop cultivation by biofertilisation of regolith soil (Fackrell et al., 2021). The Martian regolith is mostly composed of eroded basaltic rock

and so represents only the pulverised mineral grains within a terrestrial soil and none of the dark organic material, or humus. Humus enhances soil stability and the availability of water and nutrients to roots and so supports plant growth. While experiments have demonstrated successful germination and plant growth using Martian regolith stimulant (Wamelink et al., 2014), it is not well suited to productive crop cultivation without nutrient enrichment (Eichler et al., 2021; Wamelink et al., 2019). Barren soils on Earth can be improved by the addition of cyanobacterial or algal biomass as a biofertiliser or soil conditioner (Chamizo et al., 2018; Chatterjee et al., 2017; Do Nascimento et al., 2019; Metting and Rayburn, 1983) and the addition of PBR-cultured microalgae to prepared regolith to create organic-rich artificial soil may enable more productive cultivation of food crops in greenhouses for future Martian colonists. Nitrogen-fixing (diazotrophic) cyanobacteria, such as *Anabaena* and *Nostoc*, would be particularly useful for recycling this crucial plant nutrient for biofertilisation. Martian regolith also contains chemical species toxic to plant growth, including excess salts, and in particular perchlorate, that would need to be purged by washing or bioremediation to prepare a soil suitable for crop cultivation (Davila et al., 2013; Fackrell et al., 2021; Oze et al., 2021).

Traditional terrestrial staple crops such as cereals and root vegetables, however, have multiple disadvantages for cultivation on Mars, especially in the early stages of establishment of a human presence. Firstly, these staples typically have poor nutritional profiles, being rich in carbohydrates but poor in minerals and protein. Secondly, a large proportion of the plant matter of most traditional crops is inedible – for example, only 29% of the wheat plant and 61% of the potato plant is consumed (Lehto et al., 2006) – and this makes them inefficient for cultivation when available space is limited. Thirdly, most plant crops require cooking to break down cellulose cell walls to release nutrients, making their conversion into food a more energy intensive process. Finally, plants are relatively slow growing, typically taking months from seed to harvest.

Culturing microbes for human nourishment solves many of these problems. Microbial fermentation, typically employing stains of lactobacillus or yeast, is widespread in cuisine around the world, producing, for example: bread, alcohol, yoghurt, miso, soy sauce, kombucha, and pickles such as sauerkraut and kimchi. While most of these ingredients typically form only a small fraction of the calorific intake in terrestrial nourishment, microbes will have a much larger role to play in feeding the crew of deep-space missions.

Lithoautotrophic or methylotrophic microbes (or model organisms engineered for methanol-based heterotrophic fermentation) have been proposed as suitable microbes for supplementing transported food in the earliest stages of human habitation on Mars. They can be grown on readily obtainable raw materials – redox couples within the regolith and atmospheric CO₂, or methanol derived from the catalytic reduction of CO₂, respectively – in simple stirred reactors and could be engineered to synthesise protein, polysaccharides and fatty acids, as well as vitamins and flavourings for human consumption (Nangle et al., 2020; Schrader et al., 2009). Microalgae, on the other hand, would require more complex photobioreactors with access to focussed Martian sunlight or artificial lighting (Averesch, 2021), but there are already several well-established microalgae suitable for human nutrition. Spirulina, *Chlorella vulgaris* and *Chlamydomonas reinhardtii* have all been certified as GRAS (Generally Recognized As Safe) status by the U.S. Food and Drug Administration (FDA, 2018). The trade-offs are between the operation of simpler fermentors vs. more complex photobioreactors on the Martian surface, and the microalgae already well established for safely producing food vs. extensively engineering lithoautotrophs or methylotrophs (and then certifying that their metabolism does not produce human toxins under any growth conditions). Here, we focus on the applications of microalgae.

3.2. *Spirulina* and *Chlorella* as space crops

The cyanobacterium *Spirulina* and microalga *Chlorella vulgaris* both have a long tradition as food and food ingredients (Wells et al., 2017) and are attractive candidates for food cultivation on space missions. The nutritional profiles of these microalgae are compared in Fig. 8 to those of the cereal crop wheat and the tuber sweet potato, both of which have been considered for off-world cultivation. Wheat has been tested in life support systems, with a variety of cultivars developed for space travel (Zhang et al., 2020a). All parts of the sweet potato plant are edible (Li et al., 2019) and it produces a greater quantity of nutritional biomass per unit area than any other food crop (Patil, 2020), making it space efficient for Martian cultivation. The farming of insects has also been proposed for providing animal protein, as they can be reared quickly in

small spaces on plant waste inedible to humans, or on cultured microalgae (Rumpold and Schlüter, 2013; Katayama and Yamashita, 2016). The nutritional profile of the house cricket, *Acheta domesticus*, is also shown as a comparator in Fig. 8.

Fig. 8 also shows the Acceptable Macronutrient Distribution Range (AMDR) for a healthy human diet (Trumbo et al., 2002). The AMDR is one of the parameters set by the USA's National Academy of Medicine for calculating the Dietary Reference Intake, and specifies the typical percentage of protein, carbohydrate and fat required by humans to have a healthy diet. It is immediately apparent from Fig. 8 that neither the staple crops nor insects provide a distribution of macronutrients that matches human nutritional requirements. As no one crop meets this exact profile, it would be necessary to cultivate a range of plants in space to fit human nutritional requirements.

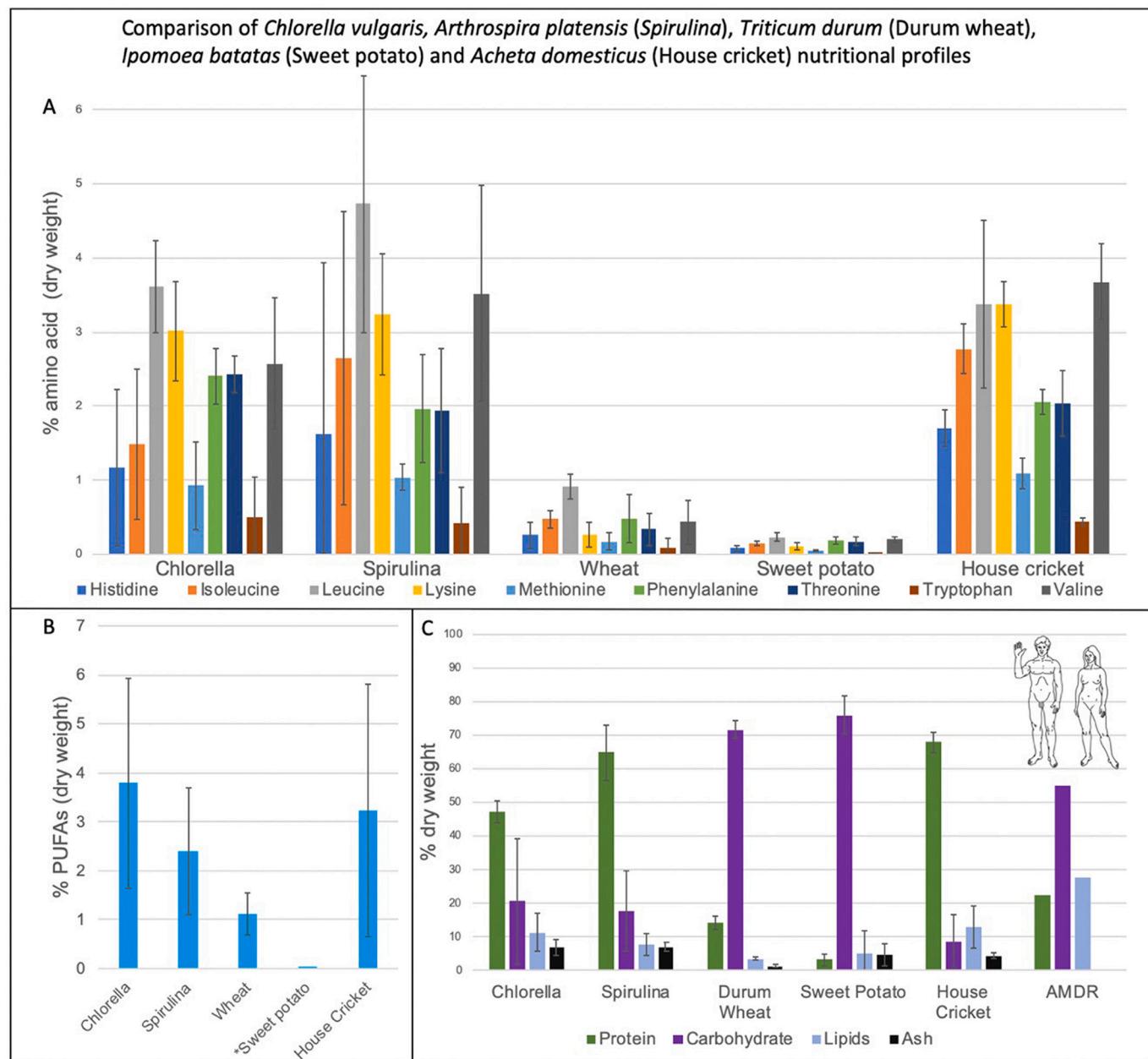


Fig. 8. Nutrient composition of five species. A: Essential amino acid distribution; B: Polyunsaturated fatty acids distribution (values for sweet potato PUFAs could not be determined from literature search); C: Macronutrient distribution. The AMDR (Acceptable Macronutrient Distribution Range) is the recommended proportion of a person's daily calories which should come from protein, carbohydrate and fats. The large error bars are due to the substantial variation in nutritional composition between different growth conditions and strains of each species. This figure was produced for this review by literature search, with the data and reference list available at (Mapstone, 2021).

The culturing of microalgae offers many advantages over plant crops. *Chlorella* and *Spirulina* contain far greater protein than typical cereal and root crops, and with high quantities of each essential amino acid (Fig. 8A). Importantly, these microalgae, in particular *Spirulina*, have protein content comparable to that of the house cricket, one of the most protein-dense edible insects (Rumpold and Schlüter, 2013), which represents a large energy saving to produce comparable dietary protein intake.

Spirulina and *Chlorella* also contain high levels of poly-unsaturated fatty acids (PUFAs) when compared to traditional crops (Fig. 8B). This includes the three essential fatty acids, which cannot be synthesised in humans: Alpha-linolenic acid [18:3n-3] (ALA), Eicosapentaenoic acid [20:5n-3] (EPA) and Docosahexaenoic acid [22:6n-3] (DHA). These fats have multiple roles in metabolism, including promoting brain health and reducing neuropsychiatric disorders (Derbyshire, 2018; Reimers and Ljung, 2019), aiding in immune system signalling (Gutiérrez et al., 2019) and maintaining the cardiovascular system (Pizzini et al., 2018; Watanabe and Tatsuno, 2017). The percentage of ALA in the total lipid of *Chlorella* and *Spirulina* ranges between 0.62 and 1.97% (Bertoldi et al., 2006; Tokuoglu and Ünal, 2003) and 0.62–1.3% respectively (Roohani et al., 2019; Tokuoglu and Ünal, 2003). The EPA percentage in total lipid ranges between 0.62 and 7.86% in *Chlorella* (Bertoldi et al., 2006; Tokuoglu and Ünal, 2003) and 1.79–7.77% in *Spirulina* (Diraman et al., 2009; Roohani et al., 2019; Tokuoglu and Ünal, 2003). Finally, DHA composition in the total lipid makes up between 0.58 and 20.94% for *Chlorella* (Bertoldi et al., 2006; Tokuoglu and Ünal, 2003) and 2.28–3.51% in *Spirulina* (Diraman et al., 2009; Tokuoglu and Ünal, 2003). For comparison, only ALA has been reported in Durum wheat at 1.72–2.48% total lipid (Narducci et al., 2019; Zengin et al., 2017) and Sweet potato at 0.022% total lipid (Shen et al., 2018). The house cricket also lacks DHA (Tzompa-Sosa et al., 2014; Udomsil et al., 2019), although does contain ALA at 0.105–1.59% total lipid and EPA at 0.46–0.55% total lipid (Tzompa-Sosa et al., 2014; Udomsil et al., 2019). On Earth, humans obtain the essential oils mainly from oily fish, flaxseed, beef and lamb liver, kidney, brain and seeds such as walnuts, brazil nuts or chia seeds. It is unrealistic to bring large animals and seed producing trees on space missions, and so microalgae present an attractive source of these essential oils.

The mineral content, or ash percentage, is also higher in *Chlorella* and *Spirulina* than the comparison staple crops (Fig. 8C), with greater than 50 mg / 100 g biomass of Na, K, Ca, Mg, Fe and P trace elements in both microalgae (Tokuoglu and Ünal, 2003). Iron in particular is better absorbed from microalgae: Puyfoulhoux et al. (2001) found that consuming *Spirulina* biomass resulted in a 6.5 fold higher iron assimilation than beef, which was the next best food source when tested using a human intestinal cell line Puyfoulhoux et al., 2001. Iron is also present in much greater quantities in *Chlorella* and *Spirulina* than in the other comparator crops. Both microalgae have high levels of B, C, E and A vitamins, as well as antioxidants such as carotenoids (Furmaniak et al., 2017; Safi et al., 2014; Venkataraman, 1997). As a consequence, both microalgae have also been examined as potential supplements and therapeutics (Karkos et al., 2011), which is discussed in more detail in Section 5.

Despite their rising popularity as a terrestrial food source, microalgae are considered a supplement and rarely reach more than 1% of the total daily diet. The highest reported intake of dietary *Spirulina* is in locations where it has been traditionally produced. In Chad, *Spirulina* is collected from alkaline lakes, sun dried and then used to produce Dihé cakes. Dihé can make up to 10–15% of the local diet when *Spirulina* is in season (six months / year) (Food and Agriculture Organization of the United Nations., 2007). Locals who do not eat Dihé cakes are significantly more likely to have vitamin A deficiency, highlighting the nutritional benefit of *Spirulina* consumption (Soudy et al., 2018). There have been numerous studies on supplementing animal feed with *Spirulina*, with reports of rabbits, rats, pigs and chickens being supplemented with *Spirulina* to form up to 20% of their total diet (Holman and

Malau-Aduli, 2013). *Spirulina*-supplemented animal feed studies generally report improved fertility, growth and nutritional quality of the animal products (Holman and Malau-Aduli, 2013). Though more experiments are required on microalgae providing higher fractions of a human diet, for long-duration space missions *Spirulina* or *Chlorella* could serve as a major protein source, with the additional benefit of supplying essential PUFAs and vitamins for astronauts.

3.3. Future microalgae space crops

Spirulina and *Chlorella vulgaris* are the best characterized microalgal species from a foodstuff perspective (Bito et al., 2020; Lafarga et al., 2020). However, there are other edible microalgae that could be relevant, following further studies. This includes *Euglena* species such as the model organism *Euglena gracilis* (Gissibl et al., 2019). This eukaryote contains 14–34% protein and 18–22% lipids (Wang et al., 2018), including all essential long chain fatty acids (ALA, EPA, DHA) at high concentrations (up to 50% of the lipid profile is PUFAs) (Korn, 1964; Wang et al., 2018). An advantage of *E. gracilis* its cell wall which is protein-rich rather than the rigid polysaccharide-based wall of *C. vulgaris*, making *E. gracilis* easier to digest (Vismara et al., 2000). It is consumed widely as a food supplement in Asian markets, in particular Japan (Gissibl et al., 2019; Suzuki, 2017). However, the first reported mass cultivation of *E. gracilis* was only in 2005 by Japanese company *Euglena* Co., Ltd., (Suzuki, 2017), whereas large scale production of *C. vulgaris* and *Spirulina* has been on-going since the 1960s and 1970s. Therefore, more research is needed on growing *E. gracilis* at scale to establish this microalga as a space crop contender.

Dunaliella species are another edible eukaryotic alga with potential as a space crop. Research on this genus has focused mostly on its beta-carotene production, which can make up 14% of dried biomass (Pourkarimi et al., 2020). Again, *Dunaliella* species such as *D. salina* hold an advantage over *C. vulgaris* by lacking a rigid cell wall, and have been shown to be highly digestible in rats (Herrero et al., 1993). Analysis of *D. salina* biomass shows it to have an equivalent protein content (~57%) to *C. vulgaris* and *Spirulina* (Pourkarimi et al., 2020). Despite being available as a food supplement, *Dunaliella* are not yet considered a ‘mainstream’ microalgal food, though a recent call for more nutritional research into this genus may change this (Sui and Vlaeminck, 2020).

Finally, species of filamentous cyanobacteria in the order *Nostocales*, namely, *Aphanizomenon flos-aquae* (AFA) and *Nostoc*, could hold promise as future space crops, with both currently available as food supplements. Although some strains from these species are toxic, edible *Nostoc* species have been reported as being highly nutritious (Gao, 1998; Han et al., 2013; Li and Guo, 2018), although a nutritional profile of AFA is not available in the scientific literature. AFA is harvested and sold as a supplement from lake Klamath in Oregon, US, but it has not yet been developed for mass cultivation in commercial ponds or PBRs (Carmichael et al., 2000). More research has been conducted on cultivating edible *Nostoc* species, specifically *N. commune*, *N. sphaeroides*, and *N. flagelliforme*, although none of these are currently grown at the mass scale required to make them profitable – largely due to their slow growth rate (Han et al., 2013). However, this slow growth rate is probably due to *Nostoc*’s ability to fix nitrogen, an energy intensive process, which would be a useful characteristic for regenerative life support on Mars.

3.4. Disadvantages of microalgae as food

One disadvantage of *C. vulgaris* consumption is its cellulose cell wall which requires cooking in order to release its full nutritional range, although this is also true for the majority of plant crops. *Spirulina*, and other potential microalgae food sources such as *E. gracilis* and *Dunaliella* species, do not however need cooking (Herrero et al., 1993; Van Eykenburg, 1977; Vismara et al., 2000).

Despite their nutritional advantages, microalgae have problems of palatability and texture (Douglas et al., 2020). For example, the most

consumed microalga, Spirulina, is still rarely eaten at >10 g a day. This is partly due to its poor palatability (Verseux et al., 2016), with a flavour that is reported to be earthy and mildly sulfuric. This could be countered by genetically modifying microalgae to contain more palatable compounds. For example, the model cyanobacterium *Synechocystis* sp. PCC 6803 has been engineered to produce the terpenoid responsible for lemon flavouring, limonene, which, if expressed in Spirulina, could enhance its flavour (Lin et al., 2017; Pattanaik and Lindberg, 2015). Strain engineering has been conducted by several algal companies to produce white or yellow mutants of *Chlorella* that lack chlorophyll, which reportedly improves flavour, smell and appearance (Schüler et al., 2020). However, as this requires growing the *Chlorella* heterotrophically, the energy saving advantage of such a crop would be lost.

Alternatively, microalgae could be processed prior to consumption. For example, Kose et al. (2017) hydrolysed raw Spirulina and *Chlorella* biomass with pancreatic enzymes to produce protein hydrolysates that can be added to food as a protein powder, and which may also remove some of the compounds responsible for unpalatable odours (Kose et al., 2017). If palatability and texture issues are not overcome, microalgae can also be used to feed the next trophic level – such as house crickets – for human consumption (Masojídek and Torzillo, 2008).

Finally, some essential vitamins are missing from cyanobacteria, most notably the cobalamin vitamin B₁₂ (Helliwell et al., 2016; Miyamoto et al., 2006; Tanioka et al., 2010; Watanabe et al., 1999). Furthermore, these cyanobacteria synthesise a non-bioavailable version of cobalamin, termed pseudocobalamin, that can reduce the uptake of B₁₂ contained in other components of the diet (Stupperich and Nexo, 1991). It would therefore be necessary to genetically engineer the cobalamin pathway in cyanobacteria to produce B₁₂ rather than pseudocobalamin.

4. Biopolymers

4.1. In-Space manufacturing and 3D printing

3D printing is a revolutionary technology that offers huge utility to Mars missions, both during interplanetary transit and on the surface. A diversity of products – including spare parts for repair and maintenance, components for habitat construction, as well as medical items and textiles (Liu et al., 2019) – can be manufactured on demand, using different feedstock materials, and so the technique has the potential to significantly reduce the necessary launch mass. The first object to be 3D printed in space was a plastic wrench aboard the ISS in 2014. The design was transmitted remotely from the ground to a printer developed by Made in Space (MIS), with no significant property differences found between the test objects printed in space and on Earth (Prater et al., 2019). MIS continued working with NASA to install the Additive Manufacturing Facility (AMF) aboard the ISS in 2016, and ESA has supported the development of the Manufacturing of Experimental Layer Technology (MELT) 3D printer.

Despite recent successes in this field, challenges remain to be overcome before 3D printing matures to a state suitable for supporting Martian habitation. Most prominently, printers will need to use recyclable or locally synthesised feedstocks. Advances are being made here, with the Refabricator printer, able to recycle plastic, installed in the ISS in 2019, and the Made In Space Recycler aiming to reprocess waste polyethylene into feedstock (Srinivasan et al., 2018). For Martian habitation, 3D printers would need to operate with bioplastics synthesised within a closed loop system.

In contrast to conventional plastics, microalgal biomass can be exploited as a carbon feedstock for culturing heterotrophic microorganisms that in turn produce high yields of intracellular biopolymers (Averesch, 2021). The cost of providing exogenous carbon sources (e.g. sugars) and other raw ingredients to grow heterotrophic bacteria is high (Afreen et al., 2021; Löwe et al., 2017; Rahman et al., 2014), and more economic biopolymer production can be attained by direct coupling of photoautotrophic and heterotrophic microorganisms (Afreen et al., 2021; Löwe et al., 2017). In two-step systems, microalgae are first grown under phototrophic conditions. Their biomass is then harvested and used by heterotrophic bacteria for PHB production. Improved PHA yields have been reported using *Scenedesmus obliquus* biomass as a substrate for genetically engineered *E. coli* (Rahman et al., 2014). Sucrose produced during photosynthesis and released into the medium can also feed heterotrophs (Rahman et al., 2014; Sathish et al., 2014). PHB production by recombinant *E. coli* grown on an algal-based medium has been demonstrated (Sathish et al., 2014). PHB production by a co-culture of *Synechococcus*

proposed for *in situ* space construction include regolith and renewable polymers (Schuldt et al., 2021). During NASA's Centennial Challenge for 3D-Printed Habitat, participants were requested to develop habitat models for Mars made out of indigenous resources and recyclable materials (Prater et al., 2018). A composite with the recyclable Polylactic Acid (PLA) reinforced with basalt fibre developed by AI SpaceFactory was used in one of the winning models (Prater et al., 2018). 3D printing of microalgae-derived materials has been demonstrated for a variety of household items and components (Balasubramanian et al., 2021; Chia et al., 2020; Pownall, 2018). PHAs also stand out in the medical field thanks to their low toxicity, antimicrobial activity, biodegradability, and relatively good cellular and tissue biocompatibility which can reduce immunogenic rejection by the patient's body (Liu et al., 2019). Microalgae-produced PHB can potentially be 3D-printed aboard spacecraft into various medical devices such as medical patches, prosthetic implants, and drug-delivery systems (Liu et al., 2019). Moreover, PHAs are also frequently used in the food sector as packaging (Keshavarz and Roy, 2010). On Mars, they can be utilized to prolong storage life of Martian harvests.

4.2. Microalgae as producers of biopolymers

Microalgae-based bioplastics can be obtained by different approaches, as outlined in detail in recent reviews (Chia et al., 2020; Cinar et al., 2020). PHB and starch are produced by some microalgae for carbon storage, and can be extracted and purified from the culture (Chia et al., 2020; Cinar et al., 2020). The cyanobacterium *Synechocystis* sp. is a well-studied PHB-producer, and its yield can be increased through genetic engineering of the biosynthesis pathway (Koch et al., 2020; Osanai et al., 2013) to ~12% (w/w) (Carpine et al., 2017). Non-natural PHB producers can also be engineered to synthesise the bioplastic. Chaogang et al. (2010) demonstrated PHB production by transgenic *Chlamydomonas reinhardtii* expressing the PhbB and PhbC enzymes from *Ralstonia eutropha* (also known as *Cupriavidus necator*) (Chaogang et al., 2010).

Current research is focussed on optimising the intracellular production of biopolymers through the screening of algae strains with the highest yields, and evaluating the effect of different carbon sources and limiting conditions (principally nitrogen limitation) (Cassuriaga et al., 2020). *Chlorella*, *Chlamydomonas*, *Nostoc*, *Spirulina*, *Synechocystis* and *Synechococcus* have all been investigated for PHB synthesis. *Synechocystis* PCC6803 has become the strain most commonly used in PHB production (Afreen et al., 2021; Carpine et al., 2017; Troschl et al., 2017); it has helped researchers better understand PHB metabolism (Afreen et al., 2021; Troschl et al., 2017). The *Synechococcus* genus encompasses the cyanobacteria with the highest PHB yield known to date under autotrophic conditions (Afreen et al., 2021); *Synechococcus* sp. MA19 accumulated up to 55% of dry cell weight (dcw) and produced 2.4 g/L of PHB in phosphate-limited media (Nishioka et al., 2001).

Alternatively, microalgal biomass can be exploited as a carbon feedstock for culturing heterotrophic microorganisms that in turn produce high yields of intracellular biopolymers (Averesch, 2021). The cost of providing exogenous carbon sources (e.g. sugars) and other raw ingredients to grow heterotrophic bacteria is high (Afreen et al., 2021; Löwe et al., 2017; Rahman et al., 2014), and more economic biopolymer production can be attained by direct coupling of photoautotrophic and heterotrophic microorganisms (Afreen et al., 2021; Löwe et al., 2017). In two-step systems, microalgae are first grown under phototrophic conditions. Their biomass is then harvested and used by heterotrophic bacteria for PHB production. Improved PHA yields have been reported using *Scenedesmus obliquus* biomass as a substrate for genetically engineered *E. coli* (Rahman et al., 2014). Sucrose produced during photosynthesis and released into the medium can also feed heterotrophs (Rahman et al., 2014; Sathish et al., 2014). PHB production by recombinant *E. coli* grown on an algal-based medium has been demonstrated (Sathish et al., 2014). PHB production by a co-culture of *Synechococcus*

3D printing can facilitate habitat construction. Some of the materials

elongatus and *Pseudomonas putida* was also successful (Löwe et al., 2017). Furthermore, a collaborative effort between NASA and German Aerospace Center (DLR) proposes the culturing of the nitrogen-fixing cyanobacterium *Anabaena* using Martian in situ resources such as sunlight, and further use the cell lysate prepared from this, rich in organic compounds such as sugars, to serve as feedstock for growing genetically engineered *Bacillus subtilis* (Rothschild, 2016).

The biomass of some microalgal species can also be mixed with conventional plastics, cellulose, or starch to achieve polymer products with enhanced properties, including prolonged lifespan, improved chemical properties, and mechanical performance (Otsuki et al., 2004; Mathiot et al., 2019; Chia et al., 2020; Cinar et al., 2020). For example, Zeller et al. (2013) demonstrated that bioplastic blends of *Chlorella* or Spirulina biomass with polyethylene exhibit superior tensile strength (Zeller et al., 2013).

Polylactic Acid (PLA), whose properties are comparable to those of synthetic plastics, is expected to become one of the leading biopolymers (Bussa et al., 2019). A wide range of terrestrial feedstock (corn, potatoes, etc.) has been explored in PLA manufacturing (Bussa et al., 2019). Due to space and resource limitations, microalgae-based PLA is more suitable for Mars. Algal biomass can be used in composites of PLA as both fillers (Ahmed and Sultana, 2020; Bussa et al., 2019; Johnson and Shivkumar, 2004) or as reinforcing fibres to increase tensile strength (Bulota and Budtova, 2015). In addition to the whole algal biomass, proteins and other biomass components can also help manufacture bioplastics. For example, cyanobacterial lipopeptides have been proposed as PLA feedstock (Bussa et al., 2019).

5. Health and pharmaceutical uses of microalgae

5.1. Health challenges on space missions

Protecting the health of the crew in the face of numerous hazards during a Mars mission is of critical importance. The microgravity environment during interplanetary transit will cause bone and muscle loss and immune dysregulation (Crucian et al., 2016; Kast et al., 2017; Pavletić et al., 2022). Susceptibility to infectious disease can be further aggravated by increased microbial pathogenicity under microgravity and a higher risk of microbial transmission as a result of confinement (Kast et al., 2017). Exposure to the ionizing radiation of cosmic rays during transit as well as while on the Martian surface is also of great concern as it can result in carcinogenesis, tissue degeneration, and potentially acute radiation syndrome (Chancellor et al., 2014; Pavletić et al., 2022). The year-long study of an astronaut compared to his earth-bound twin showed persistent changes in health, including increased DNA damage, shortened telomeres, and reduced cognition (Garrett-Bakelman et al., 2019).

Aboard the ISS, the medical conditions most frequently reported are allergies and hypersensitivities, followed by infectious diseases and latent viral reactivation (Crucian et al., 2016). Sleeping and motion sickness pills, antihistamines, and pain relievers are among the pharmaceuticals most commonly taken by astronauts (Kast et al., 2017). Some medications have been reported to be less effective in space due to altered stability, which could compromise astronaut health on long-duration missions (Du et al., 2011; Kast et al., 2017). During a Mars mission the crew will face further pharmaceutical challenges of limited medication selection and storage, and the difficulty of resupply.

5.2. Nutraceutical and pharmaceutical properties of cyanobacteria and microalgae

5.2.1. Functional foods

Any food which can provide medical or health benefits can be labelled as a 'functional food' or 'nutraceutical'. Other than exercise, the optimal method to offset disease risk is for astronauts to have a diet rich in these foods. Research has shown that microalgae have a range of

nutraceutical effects, as recently reviewed by Jha et al. (2017). Further in-depth literature reviews discussing the nutraceutical properties of specific microalgae have been conducted on Spirulina (de la Jara et al., 2018), *Chlorella* (Panahi et al., 2015), and *Euglena* (Kottuparambil et al., 2019), and found all to demonstrate antimicrobial, antiviral and antioxidant properties when consumed.

Spirulina in particular has a large body of research supporting claims of its nutraceutical properties, having been tested in 129 animal studies and more than 25 human clinical trials (de la Jara et al., 2018). These studies have assessed its benefit in reducing risk for a range of metabolic diseases such as diabetes, hypertension and the blood lipid disorder dyslipidaemia. Spirulina can aid immune responses such as reducing inflammation, precancerous lesions and allergies, and has also been shown to have antiviral properties. Alongside its value as a protein source, its high antioxidant content should be considered an equally important reason to consume Spirulina (de la Jara et al., 2018).

Developing cancer is a potential hazard for astronauts on long-duration during space missions due to radiation exposure. Having a diet rich in microalgae shows promise in offsetting this risk, as many species have exhibited anti-cancer properties (Abd El-Hack et al., 2019; Martínez Andrade et al., 2018). For example C-phycocyanin from Spirulina triggers apoptosis in breast and cervical (MCF7 and HeLa) cancer cells (Li et al., 2006), and crude extracts of *Chlorella* and *Aphanizomenon flos-aquae* have been shown to trigger selective apoptosis of liver cancer (HepG2) and leukaemia (AML) cells (Bechelli et al., 2011; Yusof et al., 2010).

5.2.2. Pharmaceuticals

Pharmaceutical challenges such as drug degradation and limited storage can be overcome by producing pharmaceuticals *in situ* and on demand. This section lists some examples of cyanobacteria and microalgae which contain active molecules that could be a useful source for developing therapeutics during missions.

Some species of cyanobacteria are highly toxic if consumed. However, a number of these toxins have shown to have potent anti-cancer properties (Vijayakumar and Menakha, 2015). These include cytotoxins Cryptophycin I from *Nostoc* sp. GSV224 (Golakoti et al., 1995; Singh et al., 2011) and Borophycin from *Nostoc spongiaeforme* var. *Tenuie* (Banker and Carmeli, 1998). *Anabaena* species have been shown to have a similar effect, one study showing crude cell extracts could selectively cause apoptosis in leukaemia AML cells while not affecting nearby non-malignant cells (Oftedal et al., 2010).

Astronauts can suffer from latent viral reactivation infections (Crucian et al., 2016). Some cyanobacteria and microalgae contain specific compounds which have been shown to have potent antiviral properties. This includes red algae lectins such as griffithsin from marine *Griffithsia* sp. (Lee, 2019) and cyanobacterial lectins such as Cyanovirin-N from freshwater *Nostoc ellipsosporum* (Lotfi et al., 2018). In total ten lectins have been described from cyanobacteria and microalgae, as reviewed by Romero et al. (2021). Other active compounds in microalgae such as astaxanthin from green microalga *Haematococcus pluvialis* and sulfated polysaccharides from red microalga *Porphyridium* sp. additionally possess antiviral properties (Alam et al., 2021).

In addition to possessing anti-cancer and anti-viral potential, extracts from a number of species from *Charophyta*, *Chlorophyta*, and *Cyanobacteria* have been shown to inhibit biofilm production in the pathogenic yeast *Candida albicans* and bacterium *Enterobacter cloacae* (Cepas et al., 2019). There are many more cyanobacterial and microbial compounds which have been shown to aid in anti-inflammatory, anti-epilepsy and anti-osteoporosis activities, all of which have been extensively reviewed previously (Saide et al., 2021).

5.2.3. Pharmaceutical bio factories

In addition to natively synthesizing antiviral, antibacterial, and anticancer agents as secondary metabolites, engineered microalgae can serve as factories to produce other compounds with medical

significance.

Microalgae have a number of advantages for serving as pharmaceutical biofactories, including autotrophic growth, high protein yield and ability to synthesise complex proteins, and a range of genetic manipulation tools have already been developed (Rasala and Mayfield, 2011). For example, the model algal species *Chlamydomonas reinhardtii* has been used as a platform for the production of vaccines, hormones, immunotoxins, and antimicrobial proteins such as endolysins (Dyo and Purton, 2018), and the organism has been granted GRAS status by the U.S. Food and Drug Administration (Fields et al., 2020). The whole biomass of *Chlamydomonas reinhardtii* could be used for oral delivery of these pharmaceuticals without needing processing. (Rosales-Mendoza et al., 2020). Spirulina is also being investigated as a potential edible vaccine, with one strain genetically modified to produce an antibody against campylobacter, a common cause of gastrointestinal infections in developing countries. The antibody within this engineered Spirulina strain has been shown to be active against this pathogen in a mouse study and is safe for human consumption (Jester et al., 2022). Microalgal synthetic biology applications are discussed further in Section 7.

6. Technical challenges in culturing microbial photoautotrophs on Mars

6.1. Martian resources

Mars provides many of the resources required for the cultivation of microalgae, accessible by ISRU techniques. Large quantities of water exist on Mars as ice (Clifford, 1987; Feldman et al., 2004; Kieffer et al., 1976), within hydrated minerals (Ehlmann and Edwards, 2014), and as atmospheric water vapour (Martínez et al., 2017; Trokhimovskiy et al., 2015). Subsurface ice deposits, if present at the habitat location, may be accessed by drilling, and adsorbed or mineral water extracted by chemical means (Abbud-Madrid et al., 2016; Starr and Muscatello, 2020) and purified for drinking or making culture media for bioreactors.

Mars' atmosphere contains 95% CO₂ and 2.8% N₂ (Franz et al., 2017). Pressurisation of the atmosphere will support the growth of phototrophs, and their carbon fixation provide organics for other microbes. These gases can be compressed from the atmosphere and purified (Starr and Muscatello, 2020). Carbon fixation of CO₂ by phototrophs as discussed in this review will provide the main carbon source on the planet. As aforementioned, N₂ fixation could be performed by chemical fixation or by microorganisms, such as edible *Nostoc flagelliforme* (Bothe et al., 2010), or *Anabaena* sp. (Verseux et al., 2021). Small quantities of nitrates are also present in Martian sedimentary deposits (Stern et al., 2015). However, due to the scarcity of nitrogen on Mars, it may be difficult to provide sufficient quantities from *in situ* methods alone, and even with careful recycling may require supplementary payloads from Earth.

Regolith analysis has shown the presence of P, S, Mg and Na, with other key trace elements (K, Mn, Cl, Fe, Ca) also available in rocks on the surface (Achilles et al., 2017; Meslin et al., 2013).

Crude regolith, water, and gases from the atmosphere mixed with water may be sufficient to grow some microalgae (See Section 6), however for the others, technologies will need to be developed to extract and purify elements. For example, the microorganism *Shewanella oneidensis* has been shown to be capable of biomining iron from lunar and Martian simulant (Volger et al., 2020). Microorganisms *Sphingomonas desiccabilis*, *Bacillus subtilis* and *Cupriavidus metallidurans* have also shown biomining abilities from basaltic rock in simulated Martian gravity and microgravity environments for rare element extraction (Cockell et al., 2020).

There is one major processing obstacle which will need to be addressed before mined minerals and water can be utilized from Mars, which is removing highly soluble perchlorate (ClO₄⁻) ions. In humans, trace quantities of ClO₄⁻ competitively inhibit iodine ions from being taken up in the thyroid gland, resulting in disruption of hormone output

which eventually can lead to severe disruption of organ systems (Leung et al., 2010). Perchlorate has been detected at 3–4 orders of magnitudes greater on Mars than is typical in rocks on Earth (Hecht et al., 2009). Plant crops have shown to take up ClO₄⁻ and accumulate it to high levels, which will be absorbed by the astronauts if eaten (Van Aken and Schnoor, 2002; Yu et al., 2004). Methods to reduce ClO₄⁻ levels are under investigation. For example, one biochemical approach might be to mix the redox enzymes perchlorate reductase (Pcr) and chlorite dismutase (Cld) with a regolith slurry to convert ClO₄⁻ into O₂ and Cl₂. It has been estimated that with 6 kg of Martian regolith, this system could provide an hour of O₂ for an astronaut (Davila et al., 2013). The purified slurry would then be ready for refinement into fertilisers and water. As various microalgal species are already being explored as candidates for bioremediation of soil and water on Earth (Leong and Chang, 2020), these species should also be tested for their ClO₄⁻ accumulation and remediation capabilities. These strains could also be genetically engineered to produce and secrete the redox enzymes, to further enhance Martian water and regolith ClO₄⁻ purification technologies.

As a number of microalgal species only require a minimal growth medium and can grow without oxygen, they stand one of the best chances for successful cultivation from the crude resources available on the planet. Therefore, with research into new *in situ* technologies for mineral extraction, a system for ClO₄⁻ removal and a robust nitrogen recycling system, Mars has the potential to support life for extended periods.

6.2. Microalgae grown under simulated Martian conditions

There have been a number of microalgal growth experiments that have simulated some of the conditions that microalgae may be exposed to on Mars. The simplest study is to mimic the composition of the Martian atmosphere but at ambient Earth pressure (1000 mBar) rather than the Martian pressure of 6 mBar. However, a 1000 mBar atmosphere comprising 95% CO₂ as found on Mars significantly reduces microalgal growth. This is because CO₂ dissolves into the media, creating highly acidic conditions (Thomas et al., 2005). More promise has been shown in microalgal growth experiments investigating the Martian atmospheric composition under lower pressures. Murugesan et al. (2016) tested growth of *Synechocystis* sp. PCC 6803, Spirulina and *Anabaena cylindrica* cultures in 100% CO₂ down to 50 mBar (Murugesan et al., 2016). The authors showed an inverse relationship between CO₂ and pressure; 100% CO₂ atmospheres produced highest growth in the 100 mBar lower pressure environments. These results show promise as it will be an engineering challenge to operate pressurised bioreactors within the low-pressure Martian atmosphere. Construction costs, material usage and the risk of ruptures would all be minimised if the microalgae cultures could be reliably grown at pressurisation levels much less than 1000 mBar. Other low pressure cultivation experiments at 100 mBar, one with an Earth atmosphere supplemented with 1–5% CO₂ (Kanervo et al., 2005) another using 96% N₂, 4% CO₂ (Verseux et al., 2021) and a third at 80 mBar with 100% CO₂ (Cycil et al., 2021), have supported findings that microalgae can tolerate low pressure conditions. Of these, Verseux et al., 2021 demonstrated that they could obtain equivalent biomass of the nitrogen fixing cyanobacterium, *Anabaena* sp. PCC 7938 in low pressures compared to ambient, and successfully grew this species in media produced with Martian regolith simulant (MGS-1).

Other experiments have shown *Anabaena cylindrica* and a number of other extremophile cyanobacteria are capable of growth on a variety of Mars-like rocks, and can also survive exposure to simulated Martian environments for 28 days (Olsson-Francis et al., 2012; Olsson-Francis and Cockell, 2010). New species of cyanobacteria isolated from iron-depositing hot-springs have also been shown to have significant bio-weathering and nutrient-extraction abilities on Martian rock analogues (Brown et al., 2008; Brown, 2008). Another nitrogen fixing cyanobacterium, *Nostoc* sp. has also been grown in Martian regolith simulant MRS (Arai et al., 2008).

Microalgal and cyanobacterial biomass can also be used to produce media for other valuable heterotrophic bacteria, such as *E. coli*, a key cell platform used for generating a multitude of valuable recombinant products (Rosano et al., 2019). Verseux et al., 2021 used the dried biomass produced from *Anabaena* cultures grown under a Martian simulated atmosphere and in a medium produced from simulated Martian regolith (which grew from an optical density of approximately 0.15 to 0.9 after 10 days) to produce a growth medium for *E. coli*. The authors showed their *Anabaena*-sourced medium produced equivalent *E. coli* cell concentrations as its standard medium (Verseux et al., 2021).

Taken together, these early results show immense promise for the viability of growing microalgae on Mars; their photobioreactors will not require as much reinforcement as living quarters and significantly less O₂ supplementation. The nitrogen fixing cyanobacteria grown on Martian regolith can also provide nutrients to other heterotrophic organisms.

6.3. Photobioreactors on Mars

One of the difficulties in growing microalgae at scale is the necessity for light penetration through the entire culture. The denser the culture becomes, the more light is blocked and the growth rate reduced. Therefore, microalgae are grown in different vessels than those commonly used for bacterial growth. There are two main designs, referred to as open and closed PBRs, which have been compared in detail by Gupta et al. (2015). The critical design to make productive PBRs is to maximise surface area to a light source, however, a consequence of a large surface area is rapid heat loss. The functionally useful microalgae discussed in this review have limited temperature ranges in which they can grow, typically between 15 and 30 °C. Although some transparent (to visible light, but shielding ultraviolet) insulation materials, such as 2–3 cm thick silicon aerogels (Wordsworth et al., 2019), have been proposed to potentially cover a surface PBR, this material would need to cover a substantial area and would not be able to contain a significantly elevated pressure. Directing sunlight into subterranean caverns using mirrors for light and heating has been discussed in a Luna context (Woolf and Angel, 2021) however could not be relied on as the major light source for PBRs on Mars as long duration dust storms block significant quantities of sunlight (Forget and Montabone, 2017; NASA's Goddard Space Flight Center, 2016).

The optimal Martian PBR design therefore is likely to be tubular PBRs lit by panels of Light Emitting Diodes (LEDs) (see Bugbee et al., 2020 for review of potential lighting systems on Mars) maintained in subterranean caverns (Fig. 2) or lava tubes (Paris et al., 2020; Perkins, 2020). LED lighting has been optimised to be more energy efficient for cyanobacterial and microalgal photosynthetic growth in two ways. Firstly, the light can be altered to match different species' preferred spectra, with some preferring red or blends of blue and red light (Glemser et al., 2016). Secondly, LEDs can be flashed or strobed at high intensity to allow greater light penetration into dense cultures whilst being synchronised to the light and dark reactions of photosynthesis (Schulze et al., 2017). The bright flash enables the electron carrier chain to contain the optimal number of electrons to drive the light reaction while the dark period prevents photoinhibition by giving the electron carriers time to shift any excess electrons from becoming reactive oxygen species (Schulze et al., 2017). Flashing light experiments have been shown to increase cell concentration in final cultures of *Chlorella kessleri* (Park and Lee, 2000) and *Dunaliella salina* (Abu-Ghosh et al., 2015), and hold immense promise for optimising large scale cultivation. The duty cycle of strobing also has an additional benefit of saving energy (i.e. during the off periods).

7. Engineering cyanobacteria and microalgae using synthetic biology

Synthetic biology aims to apply design principles such

standardisation, modularity and abstraction hierarchy to the genetic engineering of organisms (Church et al., 2014). Here, bespoke strains of microorganisms with desired phenotypes are designed *in silico* and rapidly created by standardised assembly of validated DNA parts to create novel genes. The combining of these synthetic genes within the cell into functional genetic networks results in strains with new outputs. These could be novel products such as proteins, bioactive compounds, chemicals, biopolymers, etc., or improved functionalities such as greater abiotic tolerances or an ability to fix nitrogen.

Whilst the development of basic genetic engineering technologies for key species of microalgae (e.g. those listed in Table 1) has traditionally lagged behind more established microbial systems such as *E. coli* and yeast (Ruffing, 2011; Spicer and Purton, 2016) the last few years has seen rapid developments in microalgal synthetic biology. DNA toolkits for modular assembly of gene clusters are increasingly becoming standardised and shared in the community (Fabris et al., 2020; Santos-Merino et al., 2019). For comprehensive reviews on studies applying synthetic biology approaches to developing new microalgal strains, see Fajardo et al. (2020), Vavitsas et al. (2021) and Ng et al. (2020). Many of these toolkits use Golden Gate methods that utilise type IIS restriction enzymes for rapid 'one-pot' assembly of DNA parts at each complexity level, allowing the creation of elaborate gene clusters in *E. coli* within a few days. Transformation of the microalgae with these clusters then allows evaluation of different designs through a iterative cycle of 'design-build-test-learn' (Jackson et al., 2021). Examples of Golden Gate methods developed for microalgae include MoClo (Crozat et al., 2018), Start-Stop (Taylor et al., 2019) and Cyanogate (Vasudevan et al., 2019), and their libraries of validated DNA parts such as promoters and terminators are expanding rapidly (e.g. Geisler et al., 2021; Kelly et al., 2019; Taylor et al., 2021). Combined with these are new CRISPR-cas based technologies for precision genome editing and gene regulation in microalgae (Lu et al., 2021; Patharaprachayakul et al., 2020) and the discovery of faster growing and more robust species that offer the next generation of cell platforms (e.g. Włodarczyk et al., 2020).

Currently, most algal genetic engineering studies have focussed on a handful of model systems such as *Chlamydomonas reinhardtii*, the diatom *Phaeodactylum tricornutum*, and the unicellular cyanobacteria *Synechocystis* sp. PCC6083 and *Synechococcus elongatus* PCC7942 since efficient methods for DNA delivery and transformant selection are well established (Sproles et al., 2021; Sun et al., 2018). Of the eukaryotic microalgae, *C. reinhardtii* is the most versatile host since routine methods have been established for genetic manipulation of both the nuclear and chloroplast genomes (Dyo and Purton, 2018; Zhang et al., 2021; Purton et al., 2013). The chloroplast is a particularly attractive target for metabolic engineering since this biosynthetic organelle is the site of synthesis of key precursors such as carbohydrates, fatty acids, terpenoids, amino acids and tetrapyrroles, and can also serve as a sub-cellular compartment for hyper-accumulation of the recombinant product (Jackson et al., 2021). On-going efforts to develop chloroplast engineering methodology for other GRAS species such as *Chlorella vulgaris*, *Dunaliella salina* and *Haematococcus pluvialis* will pave the way for exploiting these microalgae as functionalised foods, and as oral delivery systems for synthesised drugs (Taunt et al., 2018). Similarly, we are witnessing the transfer of cyanobacterial transformation technology from model species to more industrially relevant species such as the robust, fast-growing *Synechococcus* sp. PCC 11901 (Włodarczyk et al., 2020), and to filamentous cyanobacteria such as *Spirulina* and *Phormidium lacuna* that grow under extreme conditions of high pH or high temperature and are easy to harvest (Dehgani et al., 2018; Nies et al., 2020; Jester et al., 2022).

The future advances in microalgal synthetic biology will bring numerous opportunities for creating a whole spectrum of designer strains that are able to support the habitation of Mars whilst requiring just local resources of sunlight, water and basic inorganic nutrients. For example, species might be engineered with new abiotic traits for improved survival in the Martian environment. These traits might

include cold tolerance, desiccation resistance, and an enhanced ability to cope with toxic compounds in the regolith, as discussed in detail by (Verseux et al., 2016). We might modify edible microalgal species to be more digestible, palatable and nutritious, thereby improving their suitability as food crops as discussed in Section 3. Meanwhile, other microalgae could be set to work producing biopolymers for 3D printing and other fabrication requirements (Section 4), or synthesizing pharmaceuticals and nutraceuticals to meet the health needs of the inhabitants (Section 5).

Having just one biosynthetic function per microalgal strain will be inefficient in the long term, so synthetic biologists could design multi-functional strains whereby different functionality are tightly controlled by genetic switches that are activated or repressed using simple inputs such as metals or small molecules (Santos-Merino et al., 2019). This is an active area of study for cyanobacterial platforms where inducible promoters, riboswitches and CRISPR-cas based regulation systems are all being developed for controlled synthesis of bio-products (Wang et al., 2020). Alternatively, co-culture systems might be exploited whereby the a microalga produces secreted sugars via photosynthesis, but the actual synthesis of the desired product is outsourced to a heterotrophic partner within the co-culture. This partner could be an engineered bacterium or yeast that is able to utilise the sugar as a carbon and energy source. Several studies have demonstrated the feasibility of such co-culture systems. For example, *Synechococcus elongatus* was engineered such that it exported 85% of its fixed carbon as sucrose into its surrounding media. Co-cultivation of this strain with *Halomonas boliviensis* resulted in active uptake of the sucrose allowing the *Halomonas* to produce significant quantities of the bioplastic precursor PHB (Weiss et al., 2017). In a second study, the *Synechococcus* strain was co-cultured with an engineered *Pseudomonas* bacterium capable of bio-converting the toxic compound 2,4-dinitrotoluene to non-toxic products (Fedeson et al., 2020). Such modular approaches, whereby the synthesis of one of a range of different products is decided by the Martian inhabitants by selecting a particular input into a microalgal culture or by choosing an appropriate heterotrophic partner, would meet the short lead times and flexibility needed for bio-manufacturing on the red planet.

8. Concluding remarks

This review has examined the role microalgae may have in setting up a Martian base and systematically presented research conducted on the different microalgal species in space programmes to-date. There is immense potential to use microalgae for a variety of roles, including providing oxygen, food, construction biopolymers (including feedstocks for 3D printers) and drugs. In order to reduce reliance on *in situ* resources, it will be important to maximise recycling of these materials in a circular economy. In any case, it appears that microalgae are well-suited to play a significant role in supporting the first human outposts on Mars (and elsewhere). Despite the large barriers which will need to be crossed before the first large scale cultivation of microalgae is possible (including the building of infrastructure, establishing a power source, and production of growth media), cyanobacteria and microalgae appear to be the best option for the first Martian crops due to the planet already holding many of the resources required to cultivate them (Section 6) and their fast growth rates. We can expect the future of the red planet to contain little pockets of green.

Data generated for Section 3.2, Fig. 8

<https://data.mendeley.com/datasets/3mh8m429pv/2>

Declaration of Competing Interest

None of the authors have any actual or potential conflicts of interest to disclose that could inappropriately influence, or be perceived to

influence, this submitted work.

Acknowledgements

LJM was supported by a PhD studentship funded through grant BB/J014567/1 from the UK's Biotechnology and Biological Sciences Research Council to the London Interdisciplinary Biosciences Consortium. Thanks to Eleanor K L Powell for illustrations.

References

- Abbud-Madrid, A., Beatty, David, Boucher, Dale, Bussey, Ben, Davis, Richard, Gertsch, Leslie, Hays, Lindsay, Kleinhenz, Julie, Meyer, Michael, Moats, Michael, Mueller, Robert, Paz, Aaron, Suzuki, Nantel, van Susante, Paul, Whetsel, Charles, Zbinden, Elizabeth, Beatty, D.W., Boucher, D., Bussey, B., Davis, R., Gertsch, L., Hays, Le, Kleinhenz, J., Meyer, Ma, Moats, M., Mueller, R.P., Paz, A., Suzuki, N., van Susante, P., Whetsel, C., Zbinden, Ea, 2016. Mars Water In-Situ Resource Utilization (ISRU) Planning (M-WIP) Study.
- Abd El-Hack, M.E., Abdellour, S., Alagawany, M., Abdo, M., Sakr, M.A., Khafaga, A.F., Mahgoub, S.A., Elnesr, S.S., Gebriel, M.G., 2019. Microalgae in modern cancer therapy: current knowledge. *Biomed. Pharmacother.* <https://doi.org/10.1016/j.bioph.2018.12.069>.
- Abu-Ghosh, S., Fixler, D., Dubinsky, Z., Solovchenko, A., Zigman, M., Yehoshua, Y., Iluz, D., 2015. Flashing light enhancement of photosynthesis and growth occurs when photochemistry and photoprotection are balanced in Dunaliella salina. *Eur. J. Phycol.* 50, 469–480. <https://doi.org/10.1080/09670262.2015.1069404>.
- Achilles, C.N., Downs, R.T., Ming, D.W., Rampe, E.B., Morris, R.V., Treiman, A.H., Morrison, S.M., Blake, D.F., Vaniman, D.T., Ewing, R.C., Chipera, S.J., Yen, A.S., Bristow, T.F., Ehlmann, B.L., Gellert, R., Hazen, R.M., Fendrich, K.V., Craig, P.I., Grotzinger, J.P., Des Marais, D.J., Farmer, J.D., Sarrazin, P.C., Morookian, J.M., 2017. Mineralogy of an active eolian sediment from the Namib dune, Gale crater. *Mars. J. Geophys. Res. Planets* 122, 2344–2361. <https://doi.org/10.1002/2017JE005262>.
- Afreen, R., Tyagi, S., Singh, G.P., Singh, M., 2021. Challenges and perspectives of polyhydroxyalkanoate production from microalgae/cyanobacteria and bacteria as microbial factories: an assessment of hybrid biological system. *Front. Bioeng. Biotechnol.* 9, 1–14. <https://doi.org/10.3389/fbioe.2021.624885>.
- Ahmed, B., Sultana, S., 2020. A Critical Review on PLA-Algae Composite: Chemistry, Mechanical, and Thermal Properties, 10. <https://doi.org/10.37421/jtese.2020.10.425>.
- Alam, M.A., Parra-Saldivar, R., Bilal, M., Afroze, C.A., Ahmed, M.N., Iqbal, H.M.N., Xu, J., 2021. Algae-derived bioactive molecules for the potential treatment of SARS-CoV-2. *Molecules* 26, 2134. <https://doi.org/10.3390/MOLECULES26082134>.
- Alexandrov, S., 2016. Algal research in space: history, current status, and future prospects. *Innovare J. Life Sci.* 4, 1–4.
- Arai, M., Tomita-Yokotani, K., Sato, S., Hashimoto, H., Ohmori, M., Yamashita, M., 2008. Growth of terrestrial cyanobacterium, *Nostoc* sp., on Martian Regolith Simulant and its vacuum tolerance. *Biol. Sci. Sp.* 22, 8–17. <https://doi.org/10.2187/bss.22.8>.
- Averesch, N.J.H., 2021. Choice of microbial system for in-situ resource utilization on Mars. *Front. Astron. Sp. Sci.* 8 <https://doi.org/10.3389/fspas.2021.700370>.
- Avila-Leon, I., Chuei Matsudo, M., Sato, S., de Carvalho, J.C.M., 2012. *Arthrosphaera platensis* biomass with high protein content cultivated in continuous process using urea as nitrogen source. *J. Appl. Microbiol.* 112, 1086–1094. <https://doi.org/10.1111/j.1365-2672.2012.05303.x>.
- Bagdigian, R.M., Dake, J., Gentry, G., Gault, M., 2015. International space station environmental control and life support system mass and crewtime utilization in comparison to a long duration human space exploration mission. In: 45th Int. Conf. Environ. Syst. pp. 1–16.
- Balasubramanian, S., Yu, K., Meyer, A.S., Karana, E., 2021. Bioprinting of regenerative photosynthetic living materials. *Adv. Funct. Mater.* 2011162 <https://doi.org/10.1002/adfm.202011162>.
- Banker, R., Carmeli, S., 1998. Tenuecyclamides A-D, cyclic hexapeptides from the cyanobacterium *Nostoc spongiaeformis* var. *tenue*. *J. Nat. Prod.* 61, 1248–1251. <https://doi.org/10.1021/np980138j>.
- Barta, D.J., Henninger, D.L., 1994. Regenerative life support systems—Why do we need them? *Adv. Sp. Res.* 14, 403–410. [https://doi.org/10.1016/0273-1177\(94\)90329-8](https://doi.org/10.1016/0273-1177(94)90329-8).
- Bechelli, J., Coppage, M., Rosell, K., Liesveld, J., 2011. Cytotoxicity of algae extracts on normal and malignant cells. *Leuk. Res. Treatment* 2011, 1–7. <https://doi.org/10.4061/2011/373519>.
- Berliner, A.J., Hilzinger, J.M., Abel, A.J., McNulty, M.J., Makrygiorgos, G., Averesch, N. J.H., Sen Gupta, S., Benvenuti, A., Caddell, D.F., Cestellos-Blanco, S., Doloman, A., Friedline, S., Ho, D., Gu, W., Hill, A., Kusuma, P., Lipsky, I., Mirkovic, M., Luis Meraz, J., Pane, V., Sander, K.B., Shi, F., Skerker, J.M., Styer, A., Valgardson, K., Wetmore, K., Woo, S.G., Xiong, Y., Yates, K., Zhang, C., Zhen, S., Bugbee, B., Clark, D.S., Coleman-Derr, D., Mesbah, A., Nandi, S., Waymouth, R.M., Yang, P., Criddle, C.S., McDonald, K.A., Seefeldt, L.C., Menezes, A.A., Arkin, A.P., 2021. Towards a biomanufactory on Mars. *Front. Astron. Sp. Sci.* 8, 1–14. <https://doi.org/10.3389/fspas.2021.711550>.
- Bertoldi, F.C., Sant'Anna, E., Da Costa Braga, M.V., Oliveira, J.L.B., 2006. Lipids, fatty acids composition and carotenoids of *Chlorella vulgaris* cultivated a in hydroponic wastewater. *Grasas Aceites* 57, 270–274. <https://doi.org/10.3989/gya.2006.v57.i3.48>.

- Billi, D., Friedmann, E.I., Hofer, K.G., Caiola, M.G., Ocampo-Friedmann, R., 2000. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Appl. Environ. Microbiol.* 66, 1489–1492. <https://doi.org/10.1128/AEM.66.4.1489-1492.2000>.
- Billi, D., Gallego Fernandez, B., Fagiarone, C., Chiavarini, S., Rothschild, L.J., 2021. Exploiting a perchlorate-tolerant desert cyanobacterium to support bacterial growth for in situ resource utilization on Mars. *Int. J. Astrobiol.* 20, 29–35. <https://doi.org/10.1017/S1473550420000300>.
- Bito, T., Okumura, E., Fujishima, M., Watanabe, F., 2020. Potential of chlorella as a dietary supplement to promote human health. *Nutrients* 12, 1–21. <https://doi.org/10.3390/nu12092524>.
- Bothe, H., Schmitz, O., Yates, M.G., Newton, W.E., 2010. Nitrogen fixation and hydrogen metabolism in cyanobacteria. *Microbiol. Mol. Biol. Rev.* 74, 529–551. <https://doi.org/10.1128/mmbr.00033-10>.
- Brown, I.I., 2008. Cyanobacteria to link closed ecological systems and in-situ resources utilization processes. In: 37th COSPAR Sci. Assem, 383.
- Brown, I., Sarkisova, S., Garrison, D., Thomas-Keppta, K., Allen, C., Jones, J., Galindo, C., McKay, D., 2008. Bio-weathering of lunar and martian rocks by cyanobacteria: a resource for moon and mars exploration. *Lunar Planet. Sci. XXXIX*, 1–2.
- Bugbee, B., Hardy, M., Wheeler, R., Ewert, M., Kusuma, P., 2020. Providing photons for food in regenerative life support: A comparative analysis of solar fiber optic and electric light systems. In: Proceedings in the 50th International Conference on Environmental Systems, Lisbon, Portugal, July 12–16, 2020.
- Bulota, M., Budtova, T., 2015. PLA/algae composites: morphology and mechanical properties. *Compos. Part A Appl. Sci. Manuf.* 73, 109–115. <https://doi.org/10.1016/j.compositesa.2015.03.001>.
- Bussa, M., Eisen, A., Zollfrank, C., Röder, H., 2019. Life cycle assessment of microalgae products: State of the art and their potential for the production of polyalactid acid. *J. Clean. Prod.* 213, 1299–1312. <https://doi.org/10.1016/j.jclepro.2018.12.048>.
- Carmichael, W.W., Drapeau, C., Anderson, D.M., 2000. Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. *J. Appl. Phycol.* 12, 585–595. <https://doi.org/10.1023/A:1026506713560>.
- Carneiro, M.L.N.M., Pradelle, F., Braga, S.L., Gomes, M.S.P., Martins, A.R.F.A., Turkovics, F., Pradelle, R.N.C., 2017. Potential of biofuels from algae: Comparison with fossil fuels, ethanol and biodiesel in Europe and Brazil through life cycle assessment (LCA). *Renew. Sust. Energ. Rev.* <https://doi.org/10.1016/j.rser.2017.01.152>.
- Carpine, R., Du, W., Olivieri, G., Pollio, A., Hellingwerf, K.J., Marzocchella, A., Branco, F., 2017. Genetic engineering of *Synechocystis* sp. PCC6803 for poly-β-hydroxybutyrate overproduction. *Algal Res.* 25, 117–127. <https://doi.org/10.1016/j.algal.2017.05.013>.
- Cassuriaga, A.P.A., Moraes, L., Morais, M.G., Costa, J.A.V., 2020. Polyhydroxybutyrate production and increased macromolecule content in *Chlamydomonas reinhardtii* cultivated with xylose and reduced nitrogen levels. *Int. J. Biol. Macromol.* 158, 875–883. <https://doi.org/10.1016/j.ijbiomac.2020.04.273>.
- Cepas, V., López, Y., Gabasa, Y., Martins, C.B., Ferreira, J.D., Correia, M.J., Santos, L.M.A., Oliveira, F., Ramos, V., Reis, M., Castelo-Branco, R., Morais, J., Vasconcelos, V., Probert, I., Guillou, E., Mehri, M., Soto, S.M., 2019. Inhibition of bacterial and fungal biofilm formation by 675 extracts from microalgae and cyanobacteria. *Antibiotics* 8, 77. <https://doi.org/10.3390/ANTIBIOTICS8020077>.
- Chamizo, S., Mugnai, G., Rossi, F., Certini, G., De Philippis, R., 2018. Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. *Front. Environ. Sci.* 11, 49. <https://doi.org/10.3389/FENVS.2018.00049>.
- Chancellor, J.C., Scott, G.B.I., Sutton, J.P., 2014. Space radiation: the number one risk to astronaut health beyond low Earth orbit. *Life (Basel, Switzerland)* 4, 491–510. <https://doi.org/10.3390/life4030491>.
- Chang, Yuanyuan, Wu, Zucheng, Bian, Lei, Feng, Daolun, Leung Y.C., Dennis, 2013. Cultivation of *Spirulina platensis* for biomass production and nutrient removal from synthetic human urine. *Appl. Energy* 102, 427–431. <https://doi.org/10.1016/j.apenergy.2012.07.024>.
- Chaogang, Wang, Zhangli, Hu, Anping, Lei, Baohui, Jin, 2010. Biosynthesis of Poly-3-hydroxybutyrate (PHB) in the transgenic green alga *Chlamydomonas reinhardtii*. *J. Phycol.* 46 (2), 396–402. <https://doi.org/10.1111/j.1529-8817.2009.00789.x>.
- Chatterjee, A., Singh, S., Agrawal, C., Yadav, S., Rai, R., Rai, L.C., 2017. Role of algae as a biofertilizer. In: Algal Green Chemistry: Recent Progress in Biotechnology, 1st ed. Elsevier. <https://doi.org/10.1016/B978-0-444-63784-0.00010-2>.
- Chia, W.Y., Ying Tang, D.Y., Khoo, K.S., Kay Lup, A.N., Chew, K.W., 2020. Nature's fight against plastic pollution: Algae for plastic biodegradation and bioplastics production. *Environ. Sci. Ecotechnol.* 4, 100065 <https://doi.org/10.1016/j.ese.2020.100065>.
- Church, G.M., Elowitz, M.B., Smolke, C.D., Voigt, C.A., Weiss, R., 2014. Realizing the potential of synthetic biology. *Nat. Rev. Mol. Cell Biol.* <https://doi.org/10.1038/nrm3767>.
- Cinar, S.O., Chong, Z.K., Kucuker, M.A., Wieczorek, N., Cengiz, U., Kuchta, K., 2020. Bioplastic production from microalgae: a review. *Int. J. Environ. Res. Public Health* 17, 3842.
- Clauwaert, P., Muys, M., Alloul, A., De Paepe, J., Luther, A., Sun, X., Ilgrande, C., Christiaens, M.E.R., Hu, X., Zhang, D., Lindeboom, R.E.F., Sas, B., Rabaeij, K., Boon, N., Ronsse, F., Geelen, D., Vlaeminck, S.E., 2017. Nitrogen cycling in Bioregenerative Life Support Systems: Challenges for waste refinery and food production processes. *Prog. Aerosp. Sci.* 91, 87–98. <https://doi.org/10.1016/j.paerosci.2017.04.002>.
- Clifford, S.M., 1987. Polar basal melting on Mars. *J. Geophys. Res.* 92, 9135. <https://doi.org/10.1029/jb092ib09p09135>.
- Cockell, C.S., 2004. Martian Expedition Planning, Science and Technology Series, Vol 107. American Astronautical Society.
- Cockell, C.S., 2010. Geomicrobiology beyond Earth: Microbe-mineral interactions in space exploration and settlement. *Trends Microbiol.* 18, 308–314. <https://doi.org/10.1016/j.tim.2010.03.005>.
- Cockell, C., Rettberg, P., Rabbow, E., Olsson-francis, K., 2011. Exposure of phototrophs to 548 days in low Earth orbit: microbial selection pressures in outer space and on early Earth. *ISME J.* 5, 1671–1682. <https://doi.org/10.1038/ismej.2011.46>.
- Cockell, C.S., Santomartino, R., Finster, K., Waajan, A.C., Eades, L.J., Moeller, R., Rettberg, P., Fuchs, F.M., Van Houdt, R., Leyds, N., Coninx, I., Hatton, J., Spaniman, L., Krause, J., Koehler, A., Caplin, N., Zuijderduijn, L., Mariani, A., Pellari, S.S., Carubia, F., Luciani, G., Balsamo, M., Zolesi, V., Nicholson, N., Loudon, C.M., Doswald-Winkler, J., Herová, M., Rattenbacher, B., Wadsworth, J., Craig Everroad, R., Demets, R., 2020. Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity. *Nat. Commun.* 11, 1–11. <https://doi.org/10.1038/s41467-020-19276-w>.
- Crozet, P., Navarro, F.J., Willmud, F., Mehrshahi, P., Bakowski, K., Lauersen, K.J., Pérez-Pérez, M.E., Auroy, P., Gorches Rovira, A., Sauret-Gueto, S., Niemeyer, J., Spaniol, B., Theis, J., Trösch, R., Westrich, L.D., Vavitsas, K., Baier, T., Hübner, W., De Carpenter, F., Cassarini, M., Danon, A., Henri, J., Marchand, C.H., De Mia, M., Sarkissian, K., Baulcombe, D.C., Peltier, G., Crespo, J.L., Kruse, O., Jensen, P.E., Schröder, M., Smith, A.G., Lemaire, S.D., 2018. Birth of a photosynthetic chassis: a MoClo toolkit enabling synthetic biology in the microalga *Chlamydomonas reinhardtii*. *ACS Synth. Biol.* 7, 2074–2086. <https://doi.org/10.1021/acssynbio.8b00251>.
- Crucian, B., Babiak-vazquez, A., Johnston, S., Pierson, D.L., Ott, C.M., Sams, C., 2016. Incidence of clinical symptoms during long-duration orbital spaceflight. *Int. J. Gen. Med.* 9, 383–391.
- Cycli, L.M., Hausrath, E.M., Ming, D.W., Adcock, C.T., Raymond, J., Remias, D., Ruehmmele, W.P., 2021. Investigating the growth of algae under low atmospheric pressures for potential food and oxygen production on Mars. *Front. Microbiol.* 12, 3171. <https://doi.org/10.3389/fmicb.2021.733244>.
- Dartnell, L.R., Desorger, L., Ward, J.M., Coates, A.J., 2007. Modelling the surface and subsurface Martian radiation environment: implications for astrobiology. *Geophys. Res. Lett.* 34 <https://doi.org/10.1029/2006GL027494>.
- Davila, A.F., Willson, D., Coates, J.D., McKay, C.P., 2013. Perchlorate on Mars: a chemical hazard and a resource for humans. *Int. J. Astrobiol.* 12, 321–325. <https://doi.org/10.1017/S1473550413000189>.
- de la Jara, A., Ruano-Rodriguez, C., Polifrone, M., Assunçao, P., Brito-Casillas, Y., Wägner, A.M., Serra-Majem, L., 2018. Impact of dietary *Arthrospira* (*Spirulina*) biomass consumption on human health: main health targets and systematic review. *J. Appl. Phycol.* 30, 2403–2423. <https://doi.org/10.1007/s10811-018-1468-4>.
- Dehghani, J., Adibkia, K., Movafeghi, A., Barzegari, A., Pourseif, M.M., Maleki Kakelar, H., Golchin, A., Omidi, Y., 2018. Stable transformation of *Spirulina* (*Arthrospira*) *platensis*: a promising microalga for production of edible vaccines. *Appl. Microbiol. Biotechnol.* 9267–9278 <https://doi.org/10.1007/s00253-018-9296-7>.
- Derbyshire, E., 2018. Brain health across the lifespan: A systematic review on the role of Omega-3 Fatty acid supplements. *Nutrients* 10, 1094. <https://doi.org/10.3390/nut10081094>.
- Detrell, G., Belz, S., Bretschneider, J., Jost, A.K., Gent, U., Chamber, A.C., Container, E., Station, I.S., 2018. Design of a test platform for algae cultivation research at different gravitation levels. In: 48th Int. Conf. Environ. Syst. ICES-2018-145.
- Detrell, G., Helisch, H., Keppler, J., Martin, J., 2019. PBR @ LSR: the algae-based photobioreactor experiment at the ISS – operations and results. In: International Conference on Environmental Systems.
- Diraman, H., Koru, E., Dibeklioglu, H., 2009. Fatty acid profile of *Spirulina platensis* used as a food supplement. *Isr. J. Aquacult. Bamidgeh* 61, 134–142.
- Do Nascimento, M., Battaglia, M.E., Sanchez Rizza, L., Ambrosio, R., Arruebarrena Di Palma, A., Curatti, L., 2019. Prospects of using biomass of N2-fixing cyanobacteria as an organic fertilizer and soil conditioner. *Algal Res.* 43 <https://doi.org/10.1016/J.ALGR.2019.101652>.
- Douglas, G.L., Zwart, S.R., Smith, S.M., 2020. The journal of nutrition issues and opinions space food for thought: challenges and considerations for food and nutrition on exploration missions. *J. Nutr.* 150, 2242–2244. <https://doi.org/10.1093/jn/nxaa188>.
- Du, B., Daniels, V.R., Vaksman, Z., Boyd, J.L., Crady, C., Putcha, L., 2011. Evaluation of physical and chemical changes in pharmaceuticals flown on space missions. *AAPS J.* 13, 299–308. <https://doi.org/10.1208/s12248-011-9270-0>.
- Dyo, Yulia, Purton, S., 2018. The algal chloroplast as a synthetic biology platform for production of therapeutic proteins. *Microbiology* 164, 113–121. <https://doi.org/10.1099/MIC.0.000599>.
- Ehlmann, B.L., Edwards, C.S., 2014. Mineralogy of the Martian surface. *Annu. Rev. Earth Planet. Sci.* <https://doi.org/10.1146/annurev-earth-060313-055024>.
- Eichler, A., Hadland, N., Pickett, D., Masaitis, D., Handy, D., Perez, A., Batchelder, D., Wheeler, B., Palmer, A., 2021. Challenging the agricultural viability of martian regolith simulants. *Icarus* 354, 114022. <https://doi.org/10.1016/J.ICARUS.2020.114022>.
- Endo, M., Takeuchi, T., Yoshizaki, G., Toyobe, M., Kanki, R., Ohmori, K., Suzuki Oguchi, M., Kibe, S., 1999. Studies on the development of closed ecological recirculating aquaculture system (CERAS) IV. A long term feeding experiment with oreochromis niloticus in the fish rearing closed tank. *CELSS J. 生態工学会* https://doi.org/10.11450/SEITAOKOGAKU1989.11.2_17.
- Fabris, M., Abbriano, R.M., Pernice, M., Sutherland, D.L., Commault, A.S., Hall, C.C., Labeeuw, L., McCauley, J.I., Kuzhiuparambil, U., Ray, P., Kahlik, T., Ralph, P.J., 2020. Emerging technologies in algal biotechnology: toward the establishment of a

- sustainable, algae-based bioeconomy. *Front. Plant Sci.* <https://doi.org/10.3389/fpls.2020.00279>.
- Fackrell, L.E., Schroeder, P.A., Thompson, A., Stockstill-Cahill, K., Hibbitts, C.A., 2021. Development of Martian regolith and bedrock simulants: Potential and limitations of Martian regolith as an in-situ resource. *Icarus* 354, 114055. <https://doi.org/10.1016/j.icarus.2020.114055>.
- Fahrion, J., Mastroleo, F., Dussap, C.-G., Leys, N., 2021. use of photobioreactors in regenerative life support systems for human space exploration. *Front. Microbiol.* 12 <https://doi.org/10.3389/fmich.2021.699525>.
- Fajardo, C., De Donato, M., Carrasco, R., Martínez-Rodríguez, G., Mancera, J.M., Fernández-Acero, F.J., 2020. Advances and challenges in genetic engineering of microalgae. *Rev. Aquac.* 12, 365–381. <https://doi.org/10.1111/RAQ.12322>.
- FDA, U., 2018. GRAS notice inventory [WWW Document]. GRAS Not. Invent. URL: <https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory>.
- Fedeson, D.T., Saake, P., Calero, P., Nikel, P.I., Ducat, D.C., 2020. Biotransformation of 2,4-dinitrotoluene in a phototrophic co-culture of engineered *Synechococcus elongatus* and *Pseudomonas putida*. *Microb. Biotechnol.* 13, 997–1011. <https://doi.org/10.1111/1751-7915.13544>.
- Feldman, W.C., Prettyman, T.H., Maurice, S., Plaut, J.J., Bish, D.L., Vaniman, D.T., Mellon, M.T., Metzger, A.E., Squyres, S.W., Karunatillake, S., Boynton, W.V., Elphic, R.C., Funsten, H.O., Lawrence, D.J., Tokar, R.L., 2004. Global distribution of near-surface hydrogen on Mars. *J. Geophys. Res. E Planets* 109, 9006. <https://doi.org/10.1029/2003JE002160>.
- Fields, F.J., Lejzerowicz, F., Schroeder, D., Ngoi, S.M., Tran, M., McDonald, D., Jiang, L., Chang, J.T., Knight, R., Mayfield, S., 2020. Effects of the microalgae Chlamydomonas on gastrointestinal health. *J. Funct. Foods* 65, 103738. <https://doi.org/10.1016/J.JFF.2019.103738>.
- Food and Agriculture Organization of the United Nations, 2007. Projet Pilote de développement de la filière Dihé au Tchad. <https://doi.org/10.3726/978-3-653-01927-8/2>.
- Forget, F., Montabone, L., 2017. Atmospheric dust on Mars: a review. In: *47th International Conference on Environmental Systems*. Charleston, South Carolina, pp. 1–13.
- Franz, H.B., Trainer, M.G., Malespin, C.A., Mahaffy, P.R., Atreya, S.K., Becker, R.H., Benna, M., Conrad, P.G., Eigenbrode, J.L., Freissinet, C., Manning, H.L.K., Prats, B. D., Raaen, E., Wong, M.H., 2017. Initial SAM calibration gas experiments on Mars: Quadrupole mass spectrometer results and implications. *Planet. Space Sci.* 138, 44–54. <https://doi.org/10.1016/j.pss.2017.01.014>.
- Furmaniak, M.A., Misztak, A.E., Franczuk, M.D., Wilmette, A., Waleron, M., Waleron, K. F., 2017. Edible cyanobacterial genus *Arthrospira*: actual state of the art in cultivation methods, genetics, and application in medicine. *Front. Microbiol.* <https://doi.org/10.3389/fmich.2017.02541>.
- Gao, K., 1998. Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. *J. Appl. Phycol.* 10, 37–39.
- Garrett-Bakelman, F.E., Darshi, M., Green, S.J., Gur, R.C., Lin, L., Macias, B.R., McKenna, M.J., Meydan, C., Mishra, T., Nasrini, J., Piening, B.D., Rizzardi, L.F., Sharma, K., Siampwala, J.H., Taylor, L., Vitaterna, M.H., Afkarian, M., Afshinnekoo, E., Ahadi, S., Ambati, A., Arya, M., Bezdan, D., Callahan, C.M., Chen, S., Choi, A.M.K., Chilpala, G.E., Contrepois, K., Covington, M., Crucian, B.E., De Vivo, I., Dinges, D.F., Ebert, D.J., Feinberg, J.I., Gandara, J.A., George, K.A., Goutsias, J., Grills, G.S., Hargens, A.R., Heer, M., Hillary, R.P., Hoofnagle, A.N., Hook, V.Y.H., Jenkinson, G., Jiang, P., Keshavarzian, A., Laurie, S.S., Lee-McMullen, B., Lumpkins, S.B., MacKay, M., Maienschein-Cline, M.G., Melnick, A.M., Moore, T.M., Nakahira, K., Patel, H.H., Pietrzyk, R., Rao, V., Saito, R., Salins, D.N., Schilling, J.M., Sears, D.D., Sheridan, C.K., Stenger, M.B., Tryggvadottir, R., Urban, A.E., Vaisar, T., Van Espen, B., Zhang, J., Ziegler, M.G., Zwart, S.R., Charles, J.B., Kundrot, C.E., Scott, G.B.I., Bailey, S.M., Basner, M., Feinberg, A.P., Lee, S.M.C., Mason, C.E., Mignot, E., Rana, B.K., Smith, S.M., Snyder, M.P., Turek, F. W., 2019. The NASA twins study: a multidimensional analysis of a year-long human spaceflight. *Science* (80–) 364. <https://doi.org/10.1126/science.aau8650>.
- Geisler, K., Scaife, M.A., Mordaka, P.M., Holzer, A., Tomsett, E.V., Mehrshahi, P., Mendoza Ochoa, G.I., Smith, A.G., 2021. Exploring the impact of terminators on transgene expression in chlamydomonas reinhardtii with a synthetic biology approach. *Life* 11, 1–19. <https://doi.org/10.3390/life11090964>.
- Gissibl, A., Sun, A., Care, A., Nevalainen, H., Sunna, A., 2019. Bioproducts from euglena gracilis: synthesis and applications. *Front. Bioeng. Biotechnol.* 7, 108. <https://doi.org/10.3389/fbioe.2019.00108>.
- Gitelson, I.I., Terskov, I.A., Kovrov, B.G., Lisovskii, G.M., Okladnikov, Y.N., Sid'ko, F.Y., Trubachev, I.N., Shilenko, M.P., Alekseev, S.S., Pan'kova, I.M., Tirranen, L.S., 1989. Long-term experiments on man's stay in biological life-support system. *Adv. Sp. Res.* 9, 65–71. [https://doi.org/10.1016/0273-1177\(89\)90030-6](https://doi.org/10.1016/0273-1177(89)90030-6).
- Glemser, M., Heinig, M., Schmidt, J., Becker, A., Garbe, D., Buchholz, R., Brück, T., 2016. Application of light-emitting diodes (LEDs) in cultivation of phototrophic microalgae: current state and perspectives. *Appl. Microbiol. Biotechnol.* <https://doi.org/10.1007/s00253-015-7144-6>.
- Golakoti, T., Ogino, J., Heltzel, C.E., Le Husebo, T., Jensen, C.M., Larsen, L.K., Patterson, G.M.L., Moore, R.E., Mooberry, S.L., Corbett, T.H., Valeriote, F.A., 1995. Structure determination, conformational analysis, chemical stability studies, and antitumor evaluation of the cryptophycins. isolation of 18 new analogs from nostoc sp. strain GSV 224. *J. Am. Chem. Soc.* 117, 12030–12049. <https://doi.org/10.1021/ja00154a002>.
- Gupta, P.L., Lee, S.M., Choi, H.J., 2015. A mini review: photobioreactors for large scale algal cultivation. *World J. Microbiol. Biotechnol.* 31, 1409–1417. <https://doi.org/10.1007/s11274-015-1892-4>.
- Gutiérrez, S., Svahn, S.L., Johansson, M.E., 2019. Effects of Omega-3 fatty acids on immune cells. *Int. J. Mol. Sci.* 20, 5028. <https://doi.org/10.3390/ijms20205028>.
- Häder, D.P., Richter, P.R., Strauch, S.M., Schuster, M., 2006. Aquacells - Flagellates under long-term microgravity and potential usage for life support systems. *Microgr. Sci. Technol.* 18, 210–214. <https://doi.org/10.1007/BF02870411>.
- Han, D., Deng, Z., Lu, F., Hu, Z., 2013. Biology and biotechnology of edible nostoc. In: Richmond, A., Hu, Q. (Eds.), *Handbook of Microalgal Culture*. John Wiley & Sons, Ltd, Oxford, UK, pp. 433–444. <https://doi.org/10.1002/9781118567166.ch23>.
- Hauslage, J., Strauch, S.M., Eßmann, O., Haag, F.W.M., Richter, P., Kr., J., Stoltze, J., Becker, I., Nasir, A., Bornemann, G., Hartmut, M., Delovski, T., Berger, T., Rutczynska, A., Marsalek, K., Lebert, M., 2018. Eu: CROPIS – “Euglena gracilis: combined regenerative organic-food production in space” - A space experiment testing biological life. *Microgr. Sci. Technol.* 30, 933–942.
- Hecht, M.H., Kounaves, S.P., Quinn, R.C., West, S.J., Young, S.M.M., Ming, D.W., Catling, D.C., Clark, B.C., Boynton, W.V., Hoffman, J., DeFlores, L.P., Gospodinova, K., Kapit, J., Smith, P.H., 2009. Detection of perchlorate and the soluble chemistry of martian soil at the phoenix lander site. *Science* (80–) 325, 64–67. <https://doi.org/10.1126/science.1172466>.
- Hecht, M., Hoffman, J., Rapp, D., McClean, J., Soohoo, J., Schaefer, R., Aboobaker, A., Mellstrom, J., Hartvigsen, J., Meyen, F., Hinterman, E., Voecks, G., Liu, A., Nasr, M., Lewis, J., Johnson, J., Guernsey, C., Swoboda, J., Eckert, C., Alcalde, C., Poirier, M., Khopkar, P., Elangovan, S., Madsen, M., Smith, P., Graves, C., Sanders, G., Araghi, K., de la Torre Juarez, M., Larsen, D., Agui, J., Burns, A., Lackner, K., Nielsen, R., Pike, T., Tata, B., Wilson, K., Brown, T., Disarro, T., Morris, R., Schaefer, R., Steinke, R., Surampudi, R., Werne, T., Ponce, A., 2021. Mars oxygen ISRU experiment (MOXIE). *Space Sci. Rev.* <https://doi.org/10.1007/s11214-020-00782-8>.
- Helisch, H., Keppler, J., Detrell, G., Belz, S., Ewald, R., Fasoulas, S., Heyer, A.G., 2020. High density long-term cultivation of *Chlorella vulgaris* SAG 211-12 in a novel microgravity-capable membrane raceway photobioreactor for future bioregenerative life support in SPACE. *Life Sci. Sp. Res.* 24, 91–107. <https://doi.org/10.1016/j.lssr.2019.08.001>.
- Helliwell, K.E., Lawrence, A.D., Holzer, A., Kudahl, U.J., Sasso, S., Kräutler, B., Scanlan, D.J., Warren, M.J., Smith, A.G., 2016. Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B12. *Curr. Biol.* 26, 999–1008. <https://doi.org/10.1016/j.cub.2016.02.041>.
- Hendrickx, L., De Wever, H., Hermans, V., Mastroleo, F., Morin, N., Wilmette, A., De Wever, H., Hermans, V., Mastroleo, F., Morin, N., Wilmette, A., Janssen, P., 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. *Res. Microbiol.* 157, 77–86. <https://doi.org/10.1016/j.resmic.2005.06.014>.
- Herrero, C., Abalde, J., Fabregas, J., 1993. Nutritional properties of four marine microalgae for albino rats. *J. Appl. Phycol.* 5, 573–580. <https://doi.org/10.1007/BF02184636>.
- Hodson, R.C., Thompson, J.F., 1969. Metabolism of urea by *Chlorella vulgaris*. *Plant Physiol.* 44, 691–696. <https://doi.org/10.1104/pp.44.5.691>.
- Holman, B.W.B., Malau-Aduli, A.E.O., 2013. *Spirulina* as a livestock supplement and animal feed. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 97, 615–623. <https://doi.org/10.1111/j.1439-0396.2012.01328.x>.
- Horneck, G., Facius, R., Reichert, M., Rettberg, P., Seboldt, W., Manzey, D., 2006. HUMEX, a study on the survivability and adaptation of humans to long-duration exploratory missions, part II: Missions to Mars. *Adv. Sp. Res.* 38, 752–759. <https://doi.org/10.1016/j.asr.2005.06.072>.
- Huang, B., Li, D.G., Huang, Y., Liu, C.T., 2018. Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. *Mil. Med. Res.* 5 <https://doi.org/10.1186/s40779-018-0162-9>.
- Human Spaceflight Plans Committee, 2010. *Seeking a Human Spaceflight Program Worthy of a Great Nation. Future of U.S. Human Spaceflight, Background and Issues*.
- Isachenkov, M., Chugunov, S., Akhatov, I., Shishkovsky, I., 2021. Regolith-based additive manufacturing for sustainable development of lunar infrastructure – An overview. *Acta Astronaut.* 180, 650–678. <https://doi.org/10.1016/j.actaastro.2021.01.005>.
- ISEC2, 2018. *The Global Exploration Roadmap*, pp. 1–36.
- Jaatinen, Sanna, Lakanemi, M., Aino, Rintala, Jukka, 2016. Use of diluted urine for cultivation of *Chlorella vulgaris*. *Environ. Technol.* 37 (9), 1159–1170. <https://doi.org/10.1080/09593330.2015.1105300>.
- Jackson, H.O., Taunt, H.N., Mordaka, P.M., Smith, A.G., Purton, S., 2021. The algal chloroplast as a testbed for synthetic biology designs aimed at radically rewiring plant metabolism. *Front. Plant Sci.* 12, 1–15. <https://doi.org/10.3389/fpls.2021.708370>.
- Jakosky, B.M., Brain, D., Chaffin, M., Curry, S., Deighan, J., Grebowksy, J., Halekas, J., Leblanc, F., Lillis, R., Luhmann, J.G., Andersson, L., Andre, N., Andrews, D., Baird, D., Baker, D., Bell, J., Benna, M., Bhattacharyya, D., Bouger, S., Bowers, C., Chamberlin, P., Chaufray, J.Y., Clarke, J., Collinson, G., Combi, M., Connerney, J., Connour, K., Corriera, J., Crabb, K., Crary, F., Cravens, T., Crismani, M., Delory, G., Dewey, R., DiBraccio, G., Dong, C., Dong, Y., Dunn, P., Egan, H., Elrod, M., England, S., Eparvier, F., Ergun, R., Eriksson, A., Eisman, T., Espley, J., Evans, S., Fallows, K., Fang, X., Fillingim, M., Flynn, C., Fogle, A., Fowler, C., Fox, J., Fujimoto, M., Garnier, P., Girazian, Z., Groeller, H., Gruesbeck, J., Hamil, O., Hanley, K.G., Hara, T., Harada, Y., Hermann, J., Holmberg, M., Holsclaw, G., Houston, S., Inui, S., Jain, S., Jolitz, R., Kotova, A., Kuroda, T., Larson, D., Lee, Y., Lee, C., Lefevre, F., Lentz, C., Lo, D., Lugo, R., Ma, Y.J., Mahaffy, P., Marquette, M.L., Matsumoto, Y., Mayyasi, M., Mazelle, C., McClinton, W., McFadden, J., Medvedev, A., Mendillo, M., Meziane, K., Milby, Z., Mitchell, D., Modolo, R., Montmessin, F., Nagy, A., Nakagawa, H., Narvaez, C., Olsen, K., Pawlowski, D., Peterson, W., Rahmati, A., Roeten, K., Romanelli, N., Ruhunusiri, S., Russell, C., Sakai, S., Schneider, N., Seki, K., Sharrar, R., Shaver, S., Siskind, D.E., Slipski, M.,

- Soobiah, Y., Steckiewicz, M., Stevens, M.H., Stewart, I., Stiepen, A., Stone, S., Tenishev, V., Terada, N., Terada, K., Thiemann, E., Tolson, R., Toth, G., Trovato, J., Vogt, M., Weber, T., Withers, P., Xu, S., Yelle, R., Yiğit, E., Zurek, R., 2018. Loss of the Martian atmosphere to space: present-day loss rates determined from MAVEN observations and integrated loss through time. *Icarus* 315, 146–157. <https://doi.org/10.1016/j.icarus.2018.05.030>.
- Janssen, P.J.D., Lambreva, M.D., PlumerÃ©, N., Bartolucci, C., Antonacci, A., Buonasera, K., Frese, R.N., Scognamiglio, V., Rea, G., 2014. Photosynthesis at the forefront of a sustainable life. *Front. Chem.* 2, 36. <https://doi.org/10.3389/fchem.2014.00036>.
- Jester M, Benjamin, Zhao, Hui, Gewe, Mesfin, Adame, Thomas, Perruzza, Lisa, Bolick, David, Agosti, Jan, Khuong, Nhi, Kuestner, Rolf, Gamble, Caitlin, Cruickshank, Kendra, Ferrara, Jeremy, Lim, Rachelle, Paddock, Troy, Brady, Colin, Ertel, Stacey, Zhang, Mia, Tasch, Michael, Saveria, Tracy, Doughty, David, Marshall, Jacob, Carrieri, Damian, Lee, Jamie, Goetsch, Lauren, Dang, Jason, Sanjaya, Nathaniel, Fletecher, David, Martinez, Anissa, Bryce, Kadis, Kristjan, Signar, Afreen, Esha, Nguyen, Tammy, Randolph, Amanda, Taber, Alexandria, Krzeszowski, Ashley, Robinett, Brittney, Grassi, Fabio, Guerrant, Richard, Spigarelli, Michael, Takeuchi, Ryo, Finrow, Brian, Behnke, Craig, Roberts, James, 2022. Development of spirulina for the manufacture and oral delivery of protein therapeutics. *Nature Biotechnol.* <https://doi.org/10.1038/s41587-022-01249-7>.
- Jha, D., Jain, V., Sharma, B., Kant, A., Garlapati, V.K., 2017. Microalgae-based pharmaceuticals and nutraceuticals: an emerging field with immense market potential. *ChemBioEng Rev.* 4, 257–272. <https://doi.org/10.1002/cben.201600023>.
- Johnson, M., Shivkumar, S., 2004. Filamentous green algae additions to isocyanate based foams. *J. Appl. Polym. Sci.* 93, 2469–2477. <https://doi.org/10.1002/app.20794>.
- Kanervo, E., Lehto, K., Stähle, K., Lehto, H., Mäenpää, P., 2005. Characterization of growth and photosynthesis of *Synechocystis* sp. PCC 6803 cultures under reduced atmospheric pressures and enhanced CO₂ levels. *Int. J. Astrobiol.* 4, 97–100. <https://doi.org/10.1017/S1473550405002466>.
- Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji, N., Assimakopoulos, D.A., 2011. Spirulina in clinical practice: evidence-based human applications. In: *Evidence-Based Complement. Altern. Med.* <https://doi.org/10.1093/ecam/nen058>.
- Kast, J., Yu, Y., Seubert, C.N., Wotring, V.E., Derendorf, H., 2017. Drugs in space: Pharmacokinetics and pharmacodynamics in astronauts. *Eur. J. Pharm. Sci.* 109, S2–S8. <https://doi.org/10.1016/j.ejps.2017.05.025>.
- Katayama, Naomi, Yamashita, Masamichi, 2016. Usefulness of the insect food in the long-term space stay. 41st COSPAR Scientific Assembly.
- Kelly, C.L., Taylor, G.M., Satkuté, A., Dekker, L., Heap, J.T., 2019. Transcriptional terminators allow leak-free chromosomal integration of genetic constructs in cyanobacteria. *Microorganisms* 7. <https://doi.org/10.3390/microorganisms7080263>.
- Keshavarz, T., Roy, I., 2010. Polyhydroxyalkanoates: bioplastics with a green agenda. *Curr. Opin. Microbiol.* 13, 321–326. <https://doi.org/10.1016/j.mib.2010.02.006>.
- Khalili, A., Najafpour, G.D., Amini, G., Samkhaniyani, F., 2015. Influence of nutrients and LED light intensities on biomass production of microalgae Chlorella vulgaris. *Biotechnol. Bioprocess Eng.* 20, 284–290. <https://doi.org/10.1007/s12257-013-0845-8>.
- Kieffer, H.H., Chase, S.C., Martin, T.Z., Miner, E.D., Palluconi, F.D., 1976. Martian north pole summer temperatures: Dirty water ice. *Science* (80–). 194, 1341–1343. <https://doi.org/10.1126/science.194.4271.1341>.
- Koch, M., Bruckmoser, J., Scholl, J., Hauf, W., Rieger, B., Forchhammer, K., 2020. Maximizing PHB content in *Synechocystis* sp. PCC 6803: a new metabolic engineering strategy based on the regulator PirC. *Microb. Cell Factories* 19. <https://doi.org/10.1186/s12934-020-01491-1>.
- Korn, E.D., 1964. The fatty acids of *Euglena gracilis*. *J. Lipid Res.* 5, 352–362. [https://doi.org/10.1016/s0022-2275\(20\)40204-4](https://doi.org/10.1016/s0022-2275(20)40204-4).
- Kose, Ayse, Ozen O, Mehmet, Elibol, Murat, Oncel, S, Suphi, 2017. Investigation of in vitro digestibility of dietary microalga Chlorella vulgaris and cyanobacterium Spirulina platensis as a nutritional supplement. *3 Biotech* 7 (3), 170–177. <https://doi.org/10.1007/s13205-017-0832-4>.
- Kottuparambil, S., Thankamony, R.L., Agusti, S., 2019. Euglena as a potential natural source of value-added metabolites. A review. *Algal Res.* <https://doi.org/10.1016/j.algal.2018.11.024>.
- Lafarga, T., Fernández-Sevilla, J.M., González-López, C., Acién-Fernández, F.G., 2020. Spirulina for the food and functional food industries. *Food Res. Int.* 137, 109356. <https://doi.org/10.1016/j.foodres.2020.109356>.
- Lam, M.K., Lee, K.T., 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnol. Adv.* <https://doi.org/10.1016/j.biotechadv.2011.11.008>.
- Lee, C., 2019. Griffithsin, a highly potent broad-spectrum antiviral lectin from red algae: from discovery to clinical application. *Mar. Drugs* 17, 576. <https://doi.org/10.3390/MD17100567>.
- Lehto, K.M., Lehto, H.J., Kanervo, E.A., 2006. Suitability of different photosynthetic organisms for an extraterrestrial biological life support system. *Res. Microbiol.* 157, 69–76. <https://doi.org/10.1016/j.resmic.2005.07.011>.
- Leong, Y.K., Chang, J.S., 2020. Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. *Bioresour. Technol.* 303, 122886. <https://doi.org/10.1016/j.biotech.2020.122886>.
- Leovy, C., 2001. Weather and climate on Mars. *Nature*. <https://doi.org/10.1038/35084192>.
- Leung, A.M., Pearce, E.N., Braverman, L.E., 2010. Perchlorate, iodine and the thyroid. *Best Pract. Res. Clin. Endocrinol. Metab.* 24, 133–141. <https://doi.org/10.1016/j.beem.2009.08.009>.
- Li, Z., Guo, M., 2018. Healthy efficacy of *Nostoc commune* Vaucher. *Oncotarget*. <https://doi.org/10.18632/oncotarget.23620>.
- Li, B., Gao, M.-H., Zhang, X.-C., Chu, X.-M., 2006. Molecular immune mechanism of C-phycocyanin from *Spirulina platensis* induces apoptosis in HeLa cells in vitro. *Biotechnol. Appl. Biochem.* 43, 155. <https://doi.org/10.1042/BA20050142>.
- Li, X., Richter, P.R., Hao, Z., An, Y., Wang, G., Li, D., Liu, Y., Strauch, S.M., Schuster, M., Haag, F.W., Lebert, M., 2017. Operation of an enclosed aquatic ecosystem in the Shenzhou-8 mission. *Acta Astronaut.* 134, 17–22. <https://doi.org/10.1016/j.actaastro.2017.01.014>.
- Li, S., Sun, T., Xu, C., Chen, L., Zhang, W., 2018. Development and optimization of genetic toolboxes for a fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973. *Metab. Eng.* 48, 163–174. <https://doi.org/10.1016/j.ymben.2018.06.002>.
- Li, J., Zhang, N., Luo, J., Yu, Q., Ai, W., Zhang, L., Tang, Y., 2019. Growth and biomass yield of 25 crops in the 4-subject 180-day integrated experiment. *Acta Astronaut.* 162, 336–343. <https://doi.org/10.1016/j.actaastro.2019.06.028>.
- Lin, P.C., Saha, R., Zhang, F., Pakrasi, H.B., 2017. Metabolic engineering of the pentose phosphate pathway for enhanced limonene production in the cyanobacterium *Synechocystis* sp. PCC. *Sci. Rep.* 7. <https://doi.org/10.1038/s41598-017-17831-y>.
- Liu, J., 2003. An assessment of the global, seasonal, and interannual spacecraft record of Martian climate in the thermal infrared. *J. Geophys. Res.* 108, 5089. <https://doi.org/10.1029/2002JE001921>.
- Liu, J., Sun, L., Xu, W., Wang, Q., Yu, S., Sun, J., 2019. Current advances and future perspectives of 3D printing natural-derived biopolymers. *Carbohydr. Polym.* 207, 297–316. <https://doi.org/10.1016/j.carbpol.2018.11.077>.
- Lotfi, H., Sheervailouli, R., Zarghami, N., 2018. An update of the recombinant protein expression systems of Cyanovirin-N and challenges of preclinical development. *Bioimpacts* 8, 151. <https://doi.org/10.15171/BL2018.16>.
- Löwe, H., Hobmeier, K., Moos, M., Kremling, A., Pfütter-Grau, K., 2017. Photoautotrophic production of polyhydroxyalkanoates in a synthetic mixed culture of *Synechococcus elongatus* cscB and *Pseudomonas putida* cscAB. *Biotechnol. Biofuels* 10, 1–11. <https://doi.org/10.1186/s13068-017-0875-0>.
- Lu, Y., Zhang, X., Gu, X., Lin, H., Melis, A., 2021. Engineering microalgae: transition from empirical design to programmable cells. *Crit. Rev. Biotechnol.* 41, 1233–1256. <https://doi.org/10.1080/07388551.2021.1917507>.
- Mapstone, L., 2021. Nutritiona profiles Spirulina Chlorella Wheat SweetPotat HouseCricket.
- Martínez Andrade, K.A., Lauritano, C., Romano, G., Ianora, A., 2018. Marine microalgae with anti-cancer properties. *Mar. Drugs*. <https://doi.org/10.3390/mdl6050165>.
- Martínez, G.M., Newman, C.N., De Vicente-Retortillo, A., Fischer, E., Renno, N.O., Richardson, M.I., Fairén, A.G., Genzer, M., Guzewich, S.D., Haberle, R.M., Harrí, A.M., Kemppinen, O., Lemmon, M.T., Smith, M.D., de la Torre-Juárez, M., Vasavada, A.R., 2017. The modern near-surface martian climate: a review of in-situ meteorological data from viking to curiosity. *Space Sci. Rev.* <https://doi.org/10.1007/s11214-017-0360-x>.
- Maslow H, Abraham, 1943. A theory of human motivation. *Psychol. Rev.* 50 (4), 370–396. <https://doi.org/10.1037/H0054346>.
- Masojídek, J., Torzillo, G., 2008. Mass cultivation of freshwater microalgae. In: *Encyclopedia of Ecology, Five-Volume Set*. Elsevier Inc, pp. 2226–2235. <https://doi.org/10.1016/B978-008045405-4.00830-2>.
- Mathiot, C., Ponge, P., Gallard, B., Sassi, J.F., Delrue, F., Le Moigne, N., 2019. Microalgae starch-based bioplastics: screening of ten strains and plasticization of unfractionated microalgae by extrusion. *Carbohydr. Polym.* 208, 142–151. <https://doi.org/10.1016/j.carbpol.2018.12.057>.
- McKenna-Lawlor, S., Gonçalves, P., Keating, A., Morgado, B., Heynderickx, D., Nieminen, P., Santini, G., Truscott, P., Lei, F., Foing, B., Balaz, J., 2012. Characterization of the particle radiation environment at three potential landing sites on Mars using ESA's MEREM models. *Icarus* 218, 723–734. <https://doi.org/10.1016/j.icarus.2011.04.004>.
- McKinlay, J.B., Harwood, C.S., 2010. Photobiological production of hydrogen gas as a biofuel. *Curr. Opin. Biotechnol.* 21, 244–251. <https://doi.org/10.1016/j.cobi.2010.02.012>.
- Menezes, A., Cumbers, J., Hogan, J., Arkin, A., 2015. Towards synthetic biological approaches to resource utilization on space missions. *J. R. Soc. Interface* 12. <https://doi.org/10.1098/rsif.2014.0715>.
- Meslin, P.-Y., Gasnault, O., Forni, O., Schröder, S., Cousin, A., Berger, G., Clegg, S.M., Lasue, J., Maurice, S., Sautter, V., Le Mouëlic, S., Wiens, R.C., Fabre, C., Goetz, W., Bish, D., Mangold, N., Ehmann, B., Lanza, N., Harrí, A.-M., Anderson, R., Rampe, E., 2013. *Soil diversity and hydration at gale crater, Mars*. *Science* (80–). 341, 1–9.
- Metting, B., Rayburn, W.R., 1983. The influence of a microalgal conditioner on selected washington soils: an empirical study. *Soil Sci. Soc. Am. J.* 47, 682–685. <https://doi.org/10.2136/SSAJ1983.03615995004700040015X>.
- Miyamoto, E., Tanioka, Y., Nakao, T., Barla, F., Inui, H., Fujita, T., Watanabe, F., Nakano, Y., 2006. Purification and characterization of a corrinoid-compound in an edible cyanobacterium *Aphanizomenon flos-aquae* as a nutritional supplementary food. *J. Agric. Food Chem.* 54, 9604–9607. <https://doi.org/10.1021/jf062300r>.
- Murugesan, G., Leino, H., Mäenpää, P., Stähle, K., Rakšajit, W., Lehto, H.J., Allahverdiyeva-Rinne, Y., Lehto, K., 2016. Pressurized martian-like pure CO₂ atmosphere supports strong growth of cyanobacteria, and causes significant changes in their metabolism. *Orig. Life Evol. Biosph.* 46, 119–131. <https://doi.org/10.1007/s11084-015-9458-x>.
- Musk, E., 2018. Making life multi-planetary. *New Sp.* 6, 2–11. <https://doi.org/10.1089/space.2018.29013.emu>.
- Nangle, S.N., Wolfson, M.Y., Hartsough, L., Ma, N.J., Mason, C.E., Merighi, M., Nathan, V., Silver, P.A., Simon, M., Swett, J., Thompson, D.B., Ziesack, M., 2020. The case for biotech on Mars. *Nat. Biotechnol.* 38, 401–407. <https://doi.org/10.1038/s41587-020-0485-4>.

- Narducci, V., Finotti, E., Galli, V., Carcea, M., 2019. Lipids and fatty acids in Italian durum wheat (*Triticum Durum Desf.*) cultivars. *Foods* 8. <https://doi.org/10.3390/foods8060223>.
- NASA, 2009. Human Exploration of Mars Design Reference Architecture 5.0 Addendum. NASA Johnson Space Center, Houston, Texas.
- NASA, 2020. FY 2021 Budget Estimates [WWW Document]. URL. https://www.nasa.gov/sites/default/files/atoms/files/fy_2021_budget_book_508.pdf.
- NASA's Goddard Space Flight Center, 2016. Aerosol Optical Thickness, MODIS, 2000–2016 [WWW Document]. URL. <https://svs.gsfc.nasa.gov/12302> (accessed 4.19.21).
- Ng, I-Son, Keskin B, Batuhan, Tan, Shih-I, 2020. A Critical Review of Genome Editing and Synthetic Biology Applications in Metabolic Engineering of Microalgae and Cyanobacteria. *Biotechnol. J.* 15 (8) <https://doi.org/10.1002/biot.201900228>.
- Niederwieser, T., Kociolek, P., Klaus, D., 2018. A review of algal research in space. *Acta Astronaut.* 146, 359–367. <https://doi.org/10.1016/j.actaastro.2018.03.026>.
- Nies, F., Mielke, M., Pochert, J., Lamparter, T., 2020. Natural transformation of the filamentous cyanobacterium Phormidium lacuna. *PLoS One* 15, 1–18. <https://doi.org/10.1371/journal.pone.0234440>.
- Nishioka, M., Nakai, K., Miyake, M., Asada, Y., Taya, M., 2001. Production of poly-β-hydroxybutyrate by thermophilic cyanobacterium, *Synechococcus* sp. MA19, under phosphate-limited conditions. *Biotechnol. Lett.* 23, 1095–1099. <https://doi.org/10.1023/A:1010551614648>.
- Nowicka-Krawczyk, P., Mühlsteinová, R., Hauer, T., 2019. Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). *Sci. Rep.* 9, 1–12. <https://doi.org/10.1038/s41598-018-36831-0>.
- Oftedal, L., Selheim, F., Wahlsten, M., Sivonen, K., Døskeland, S.O., Herfindal, L., 2010. Marine benthic cyanobacteria contain apoptosis-inducing activity synergizing with daunorubicin to kill leukemia cells, but not cardiomyocytes. *Mar. Drugs* 8, 2659–2672. <https://doi.org/10.3390/md8102659>.
- Olsson-Francis, K., Cockell, C.S., 2010. Use of cyanobacteria for in-situ resource use in space applications. *Planet. Space Sci.* 58, 1279–1285. <https://doi.org/10.1016/j.pss.2010.05.005>.
- Olsson-Francis, K., Simpson, A.E., Wolff-Boenisch, D., Cockell, C.S., 2012. The effect of rock composition on cyanobacterial weathering of crystalline basalt and rhyolite. *Geobiology* 10, 434–444. <https://doi.org/10.1111/j.1472-4669.2012.00333.x>.
- Omori, K., Watanabe, S., Endo, M., Takeuchi, T., Oguchi, M., 2001. Development of an airtight recirculating zooplankton culture device for closed ecological recirculating aquaculture system (CERAS). *J. Sp. Technol. Sci.* 17, 11–17. https://doi.org/10.11230/jsts.17.1_11.
- Osanai, T.A., Numata, K.E., Oikawa, A.K., Kuwahara, A.Y., Iijima, H.I., Doi, Y.O., Tanaka, K.A.N., Saito, K.A., Hirai, M.A.Y.O., 2013. Increased bioplastic production with an RNA polymerase sigma factor SigE during nitrogen starvation in *synechocystis* sp. PCC 6803. *DNA Res.* 20, 525–535. <https://doi.org/10.1093/dnaregs/dst028>.
- Osczevski, R., 2014. Martian windchill in terrestrial terms. *Bull. Am. Meteorol. Soc.* 95, 533–541. <https://doi.org/10.1175/BAMS-D-12-00158.1>.
- Otsuki, Toshi, Zhang, Farao, Kabeya, Hiroshi, Hirotsu, Takahiro, 2004. Synthesis and tensile properties of a novel composite of Chlorella and polyethylene. *Journal of Applied Polymer Science* 92 (1), 812–816. <https://doi.org/10.1002/app.13650>.
- Oze, C., Beisel, J., Dabsys, E., Dall, J., North, G., Scott, A., Lopez, A.M., Holmes, R., Fendorf, S., 2021. Perchlorate and agriculture on mars. *Soil Syst.* 5, 1–10. <https://doi.org/10.3390/soilsystems5030037>.
- Panahi, Y., Darvishi, B., Jowzi, N., Beiraghdar, F., Sahebkar, A., 2015. Chlorella vulgaris: a multifunctional dietary supplement with diverse medicinal properties. *Curr. Pharm. Des.* 22, 164–173. <https://doi.org/10.2174/138161282266151112145226>.
- Paris, A.J., Davies, E.T., Tognetti, L., Zahniser, C., 2020. Prospective lava tubes at hellas planitia. *arXiv*, pp. 1–16.
- Park, K.H., Lee, C.G., 2000. Optimization of algal photobioreactors using flashing lights. *Biotechnol. Bioprocess Eng.* 5, 186–190. <https://doi.org/10.1007/BF02936592>.
- Patel, M.R., Zarnecki, J.C., Catling, D.C., 2002. Ultraviolet radiation on the surface of Mars and the Beagle 2 UV sensor. *Planet. Space Sci.* 50, 915–927. [https://doi.org/10.1016/S0032-0633\(02\)00067-3](https://doi.org/10.1016/S0032-0633(02)00067-3).
- Patil, B.L., 2020. Plant viral diseases: economic implications. In: Reference Module in Life Sciences. Elsevier. <https://doi.org/10.1016/b978-0-12-809633-8.21307-1>.
- Pattanaik, B., Lindberg, P., 2015. Terpenoids and their biosynthesis in cyanobacteria. *Life* 5, 269–293. <https://doi.org/10.3390/life5010269>.
- Pattharapraphayakul, N., Lee, M., Incharoensakdi, A., Woo, H.M., 2020. Current understanding of the cyanobacterial CRISPR-Cas systems and development of the synthetic CRISPR-Cas systems for cyanobacteria. *Enzym. Microb. Technol.* 140, 109619 <https://doi.org/10.1016/j.enzmictec.2020.109619>.
- Pavletić, B., Runzheimer, K., Siems, K., Koch, S., Cortesão, M., Ramos-Nascimento, A., Moeller, R., 2022. Spaceflight virology: what do we know about viral threats in the spaceflight environment? *Astrobiology* 22, 210–224. <https://doi.org/10.1089/ast.2021.0009>.
- Peng, L., Fu, D., Chu, H., Wang, Z., Qi, H., 2020. Biofuel production from microalgae: a review. *Environ. Chem. Lett.* <https://doi.org/10.1007/s10311-019-00939-0>.
- Perkins, S., 2020. Lava tubes may be havens for ancient alien life and future human explorers. *Proc. Natl. Acad. Sci. U. S. A.* 117, 17461–17464. <https://doi.org/10.1073/pnas.2012176117>.
- Pizzini, A., Lunger, L., Sonnweber, T., Weiss, G., Tancevski, I., 2018. The role of Omega-3 fatty acids in the setting of coronary artery disease and COPD: a review. *Nutrients* 10, 1864. <https://doi.org/10.3390/nu10121864>.
- Porst, M., Lebert, M., Hader, D., 1997. Long-term cultivation of the flagellate Euglena gracilis - PubMed. *Microgr. Sci. Technol.* 10, 166–169.
- Poughon, L., Laroche, C., Creuly, C., Dussap, C., Paille, C., Lasseur, C., Monsieurs, P., Heylen, W., Coninx, I., Mastroleo, F., Leyls, N., 2020. Limnospira indica PCC8005 growth in photobioreactor: model and simulation of the ISS and ground experiments. *Life Sci. Sp. Res.* 25, 53–65. <https://doi.org/10.1016/j.lssr.2020.03.002>.
- Pourkarimi, S., Hallajisian, A., Nourashahi, A., Alizadehdakheel, A., Golzary, A., 2020. Factors affecting production of beta-carotene from Dunaliella salina microalgae. *Biocatal. Agric. Biotechnol.* <https://doi.org/10.1016/j.biab.2020.101771>.
- Pownall, A., 2018. No Title [WWW Document]. 10 bioplastic Proj. made from algae, corn starch other Nat. Mater. URL. <https://www.dezeen.com/2018/10/09/bioplastic-projects-algae-corn-starch-beetle-shells/> (accessed 2.10.22).
- Prater, T.J., Kim, T., Roman, M., Mueller, R., 2018. NASA's Centennial Challenge for 3D-Printed Habitat: Phase II Outcomes and Phase III Competition Overview. In: *Proceedings of the 2018 AIAA SPACE Forum*. Orlando, Florida. 17–110 September 2018.
- Prater, T., Werkheiser, N., Ledbetter, F., Timucin, D., Wheeler, K., Snyder, M., 2019. 3D Printing in Zero G Technology Demonstration Mission: complete experimental results and summary of related material modeling efforts. *Int. J. Adv. Manuf. Technol.* 101, 391–417. <https://doi.org/10.1007/s00170-018-2827-7>.
- Preu, P., Braun, M., 2014. German SIMBOX on Chinese mission Shenzhou-8: Europe's first bilateral cooperation utilizing China's Shenzhou programme. *Acta Astronaut.* 94, 584–591. <https://doi.org/10.1016/j.actaastro.2013.08.022>.
- Putron, S., Szabó, J.B., Wannathong, T., Young, R., Economou, C.K., 2013. Genetic engineering of algal chloroplasts: progress and prospects. *Russ. J. Plant Physiol.* 60, 491–499. <https://doi.org/10.1134/S1021443713040146>.
- Puyfoulhoux, Grégoire, Rouanet, Jean Max, Besançon, Pierre, Baroux, Bruno, Baccou, Jean Claude, Caporiccio, Bertrand, 2001. Iron availability from iron-fortified spirulina by an in vitro digestion/Caco-2 cell culture model. *Journal of Agricultural and Food Chemistry* 49 (3), 1625–1629. <https://doi.org/10.1021/jf001193c>.
- Qari, H., Rehan, M., Nizami, A.S., 2017. Key issues in microalgae biofuels: a short review. In: *Energy Procedia*. Elsevier Ltd, pp. 898–903. <https://doi.org/10.1016/j.egypro.2017.12.144>.
- Rahman, A., Anthony, R.J., Sathish, A., Sims, R.C., Miller, C.D., 2014. Effects of wastewater microalgae harvesting methods on polyhydroxybutyrate production. *Bioresour. Technol.* 156, 364–367. <https://doi.org/10.1016/j.biortech.2014.01.034>.
- Rapp, D., 2008. Human missions to Mars: enabling technologies for exploring the Red Planet. Springer, Chichester.
- Rasala, B.A., Mayfield, S.P., 2011. The microalgae *Chlamydomonas reinhardtii* as a platform for the production of human protein therapeutics. *Bioeng. Bugs* 2, 50–54. <https://doi.org/10.4161/bbug.2.1.13423>.
- Reimers, A., Ljung, H., 2019. The emerging role of omega-3 fatty acids as a therapeutic option in neuropsychiatric disorders. *Ther. Adv. Psychopharmacol.* 9 <https://doi.org/10.1177/2045125319858901>, 2045125319858901.
- Richardson, M.I., Mischna, M.A., 2005. Long-term evolution of transient liquid water on Mars. *J. Geophys. Res. Planets* 110, n/a-n/a. <https://doi.org/10.1029/2004JE002367>.
- Richter, P.R., Strauch, S.M., Ntefidou, M., Schuster, M., Daiker, V., Nasir, A., Haag, F.W., Lebert, M., 2014. Influence of different light-dark cycles on motility and photosynthesis of euglena gracilis in closed bioreactors. *Astrobiology* 14, 848–858. <https://doi.org/10.1089/ast.2014.1176>.
- Rizwan, M., Mujtaba, G., Memon, S.A., Lee, K., Rashid, N., 2018. Exploring the potential of microalgae for new biotechnology applications and beyond: a review. *Renew. Sust. Energ. Rev.* <https://doi.org/10.1016/j.rser.2018.04.034>.
- Romero, J.A.F., Paglini, M.G., Priano, C., Koroch, A., Rodríguez, Y., Sailer, J., Teleshova, N., 2021. Algal and cyanobacterial lectins and their antimicrobial properties. *Mar. Drugs* 19, 678. <https://doi.org/10.3390/MD19120687>.
- Roohani, A.M., Abedian Kenari, A., Fallahi Kapoorchali, M., Borani, M.S., Zorie Zahra, S. J., Smiley, A.H., Esmaili, M., Rombenso, A.N., 2019. Effect of spirulina *Spirulina platensis* as a complementary ingredient to reduce dietary fish meal on the growth performance, whole-body composition, fatty acid and amino acid profiles, and pigmentation of Caspian brown trout <1>Salmo trutta caspi. *Aquac. Nutr.* 25, 633–645. <https://doi.org/10.1111/anu.12885>.
- Rosales-Mendoza, S., Solís-Andrade, K.I., Márquez-Escobar, V.A., González-Ortega, O., Bañuelos-Hernández, B., 2020. Current advances in the algae-made biopharmaceuticals field. *Expert. Opin. Biol. Ther.* <https://doi.org/10.1080/14712598.2020.1739643>.
- Rosano, G.L., Morales, E.S., Ceccarelli, E.A., 2019. New tools for recombinant protein production in Escherichia coli: A 5-year update. *Protein Sci.* 28, 1412–1422. <https://doi.org/10.1002/pro.3668>.
- Rothschild, L.J., 2016. Synthetic biology meets bioprinting: enabling technologies for humans on Mars (and Earth). *Biochem. Soc. Trans.* 44, 1158–1164. <https://doi.org/10.1016/BST20160067>.
- Ruffing, A.M., 2011. Engineered cyanobacteria: Teaching an old bug new tricks. *Bioeng. Bugs* 2, 136–149. <https://doi.org/10.4161/bbug.2.3.15285>.
- Rumpold, B.A., Schlüter, O.K., 2013. Nutritional composition and safety aspects of edible insects. *Mol. Nutr. Food Res.* <https://doi.org/10.1002/mnfr.201200735>.
- Safí, C., Zebib, B., Merah, O., Pontalier, P.Y., Vaca-García, C., 2014. Morphology, composition, production, processing and applications of Chlorella vulgaris: a review. *Renew. Sust. Energ. Rev.* <https://doi.org/10.1016/j.rser.2014.04.007>.
- Saide, A., Martínez, K.A., Ianora, A., Lauritano, C., 2021. Unlocking the health potential of microalgae as sustainable sources of bioactive compounds. *Int. J. Mol. Sci.* 22, 4383. <https://doi.org/10.3390/IJMS22094383>.
- Salisbury, F.B., Gitelson, J.I., Lisovsky, G.M., 1997. Bios-3: Siberian Experiments in Bioregenerative Life Support: Attempts to purify air and grow food for space exploration in a sealed environment began in 1972. *Bioscience* 47, 575–585.
- Santomartino, R., Waajanen, A.C., de Wit, W., Nicholson, N., Parmitano, L., Loudon, C.M., Moeller, R., Rettberg, P., Fuchs, F.M., Van Houdt, R., Finster, K., Coninx, I., Krause, J., Koehler, A., Caplin, N., Zuijderduijn, L., Zolesi, V., Balsamo, M.,

- Mariani, A., Pellari, S.S., Carubia, F., Luciani, G., Leys, N., Doswald-Winkler, J., Herová, M., Wadsworth, J., Everroad, R.C., Rattenbacher, B., Demets, R., Cockell, C. S., 2020. No effect of microgravity and simulated mars gravity on final bacterial cell concentrations on the international space station: applications to space bioproduction. *Front. Microbiol.* 11 <https://doi.org/10.3389/fmcb.2020.579156>.
- Santos-Merino, M., Singh, A.K., Ducat, D.C., 2019. New applications of synthetic biology tools for cyanobacterial metabolic engineering. *Front. Bioeng. Biotechnol.* 7, 33. <https://doi.org/10.3389/fbioe.2019.00033>.
- Sathish, A., Glaittli, K., Sims, R.C., Miller, C.D., 2014. Algae biomass based media for poly(3-hydroxybutyrate) (PHB) production by escherichia coli. *J. Polym. Environ.* 22, 272–277. <https://doi.org/10.1007/s10924-014-0647-x>.
- Schrader, J., Schilling, M., Holtmann, D., Sell, D., Filho, M.V., Marx, A., Vorholt, J.A., 2009. Methanol-based industrial biotechnology: current status and future perspectives of methylotrophic bacteria. *Trends Biotechnol.* 27, 107–115. <https://doi.org/10.1016/j.tibtech.2008.10.009>.
- Schuldt, S.J., Jagoda, A., Hoisington, A.J., Delorit, J.D., Administration, V.H., Mountain, R., Illness, M., Mountain, R., Veterans, R., Medical, A., 2021. Automation in construction A systematic review and analysis of the viability of 3D-printed construction in remote environments. *Autom. Constr.* 125, 103642 <https://doi.org/10.1016/j.autcon.2021.103642>.
- Schüler, L., Gueque de Moraes, E., Trovão, M., Machado, A., Carvalho, B., Carneiro, M., Maia, I., Soares, M., Duarte, P., Barros, A., Pereira, H., Silva, J., Varela, J., 2020. Isolation and characterization of novel chlorella vulgaris mutants with low chlorophyll and improved protein contents for food applications. *Front. Bioeng. Biotechnol.* 8, 469. <https://doi.org/10.3389/FBIOE.2020.00469>.
- Schulze, P.S.C., Guerra, R., Pereira, H., Schüler, L.M., Varela, J.C.S., 2017. Flashing LEDs for microalgal production. *Trends Biotechnol.* <https://doi.org/10.1016/j.tibtech.2017.07.011>.
- Settles, M.A., 2020. GLDS-265: Selecting for Chlamydomonas Reinhardtii Fitness in the KSC Veggie Unit [WWW Document]. GeneLab. URL <https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-265/> (accessed 5.26.21).
- Shen, Y., Sun, H., Zeng, H., Prinyawiwatkul, W., Xu, W., Xu, Z., 2018. Increases in phenolic, fatty acid, and phytosterol contents and anticancer activities of sweet potato after fermentation by lactobacillus acidophilus. *J. Agric. Food Chem.* 66, 2735–2741. <https://doi.org/10.1021/acs.jafc.7b05414>.
- Sibile, L., Dominguez, J., 2012. Joule-heated molten regolith electrolysis reactor concepts for oxygen and metals production on the Moon and Mars. In: 50th AIAA Aerospace Sciences Meeting Including the New Horizons Forum and Aerospace Exposition.
- Simonsen, L., Nealy, J., Townsend, L., Wilson, J., 1990. Radiation Exposure for Manned Mars Surface Missions.
- Singh, R.K., Tiwari, S.P., Rai, A.K., Mohapatra, T.M., 2011. Cyanobacteria: an emerging source for drug discovery cyanobacteria: an emerging source for drug discovery. *J. Antimicrob. (Tokyo)*. 64, 401–412. <https://doi.org/10.1038/ja.2011.21>.
- Soudy, I.D., Minet-Quinard, R., Mahamat, A.D., Ngoua, H., Izzedine, A.A., Tidjani, A., Bum, E.N., Lambert, C., Pereira, B., Desjeux, J.F., Sapin, V., 2018. Vitamin A status in healthy women eating traditionally prepared spirulina (Dihé) in the Chad Lake area. *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0191887>.
- Spicer, A., Purton, S., 2016. Genetic Engineering of Microalgae: Current Status and Future Prospects.
- Sproles, A.E., Fields, F.J., Smalley, T.N., Le, C.H., Badary, A., Mayfield, S.P., 2021. Recent advancements in the genetic engineering of microalgae. *Algal Res.* 53, 102158. <https://doi.org/10.1016/j.algal.2020.102158>.
- Srinivasan, V.G., Krishna, V., Rupesh, M., Yalamanchili, V.K.M.R., Awwad, M., 2018. Revolutionizing additive manufacturing in space logistics - a path towards self-reliant nature. In: Proceedings of the International Annual Conference of the American Society for Engineering Management, pp. 1–10.
- Starr, S.O., Muscatello, A.C., 2020. Mars in situ resource utilization: a review. *Planet. Space Sci.* <https://doi.org/10.1016/j.pss.2019.104824>.
- Stern, J.C., Sutter, B., Freissinet, C., Navarro-González, R., McKay, C.P., Archer, P.D., Buch, A., Brunner, A.E., Coll, P., Eigenbrode, J.L., Fairen, A.G., Franz, H.B., Glavin, D.P., Kashyap, S., McAdam, A.C., Ming, D.W., Steele, A., Szopa, C., Wray, J. J., Martin-Torres, F.J., Zorzano, M.P., Conrad, P.G., Mahaffy, P.R., Thiemens, M.H., 2015. Evidence for indigenous nitrogen in sedimentary and aeolian deposits from the Curiosity rover investigations at Gale crater. *Mars. Proc. Natl. Acad. Sci. U. S. A.* 112, 4245–4250. <https://doi.org/10.1073/pnas.1420932112>.
- Strach, S.M., Schuster, M., Lebert, M., Richter, P., Schmittnägel, M., Häder, D.P., 2008. A closed ecological system in a space experiment. *J. Gravitational Physiol. A. J. Int. Soc. Gravitational Physiol.* 15.
- Stupperich, E., Nexo, E., 1991. Effect of the cobalt-N coordination on the cobamide recognition by the human vitamin B12 binding proteins intrinsic factor, transcobalamin and haptocorrin. *Eur. J. Biochem.* 199, 299–303. <https://doi.org/10.1111/j.1432-1033.1991.tb16124.x>.
- Sui, Y., Vlaeminck, S.E., 2020. Dunaliella microalgae for nutritional protein: an undervalued asset. *Trends Biotechnol.* <https://doi.org/10.1016/j.tibtech.2019.07.011>.
- Sukumaran, P., Nulit, R., Halimoon, N., Simoh, S., Omar, H., Ismail, A., 2018. Formulation of cost-effective medium using urea as a nitrogen source for arthrosira platensis cultivation under real environment. *Annu. Res. Rev. Biol.* 22, 1–12. <https://doi.org/10.9734/arrb/2018/38182>.
- Sun, T., Li, S., Song, X., Diao, J., Chen, L., Zhang, W., 2018. Toolboxes for cyanobacteria: recent advances and future direction. *Biotechnol. Adv.* 36, 1293–1307. <https://doi.org/10.1016/j.biotechadv.2018.04.007>.
- Suzuki, K., 2017. Large-scale cultivation of Euglena. In: Advances in Experimental Medicine and Biology. Springer New York LLC, pp. 285–293. https://doi.org/10.1007/978-3-319-54910-1_14.
- Takeuchi, T., Endo, M., 2004. Recent advances in closed recirculating aquaculture systems. *Eco-Engineering* 16, 15–20. <https://doi.org/10.11450/seitaihogaku.16.15>.
- Tanioka, Y., Miyamoto, E., Yabuta, Y., Ohnishi, K., Fujita, T., Yamaji, R., Misono, H., Shigeoka, S., Nakano, Y., Inui, H., Watanabe, F., 2010. Methyladenylcobamide functions as the cofactor of methionine synthase in a Cyanobacterium, Spirulina platensis NIES-39. *FEBS Lett.* 584, 3223–3226. <https://doi.org/10.1016/j.febslet.2010.06.013>.
- Taunt, H.N., Stoffels, L., Purton, S., 2018. Green biologics: the algal chloroplast as a platform for making biopharmaceuticals. *Bioengineered* 9, 48–54. <https://doi.org/10.1080/21655979.2017.1377867>.
- Taylor, G.M., Mordka, P.M., Heap, J.T., 2019. Start-Stop Assembly: a functionally scarless DNA assembly system optimized for metabolic engineering. *Nucleic Acids Res.* 47, 17. <https://doi.org/10.1093/nar/gky1182>.
- Taylor, G.M., Hitchcock, A., Heap, J.T., 2021. Combinatorial assembly platform enabling engineering of genetically stable metabolic pathways in cyanobacteria. *Nucleic Acids Res.* 49 <https://doi.org/10.1093/nar/gkab791>.
- Thomas, D.J., Sullivan, S.L., Price, A.L., Zimmerman, S.M., 2005. Common freshwater cyanobacteria grow in 100% CO₂. *Astrobiology* 5, 66–74. <https://doi.org/10.1089/ast.2005.5.66>.
- Toksozoglu, Ö., Ünal, M.K., 2003. Biomass nutrient profiles of three microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrysis galbana. *J. Food Sci.* 68, 1144–1148. <https://doi.org/10.1111/j.1365-2621.2003.tb09615.x>.
- Trokhimovskiy, A., Fedorova, A., Koralev, O., Montmessin, F., Bertaux, J.L., Rodin, A., Smith, M.D., 2015. Mars' water vapor mapping by the SPICAM IR spectrometer: five martian years of observations. *Icarus* 251, 50–64. <https://doi.org/10.1016/j.icarus.2014.10.007>.
- Troschl, C., Meixner, K., Drosig, B., 2017. Cyanobacterial PHA production—review of recent advances and a summary of three years' working experience running a pilot plant. *Bioengineering* 4, 26. <https://doi.org/10.3390/bioengineering4020026>.
- Trumbo, P., Schlicker, S., Yates, A.A., Poos, M., 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J. Am. Diet. Assoc.* 102, 1621–1630. [https://doi.org/10.1016/S0002-8223\(02\)90346-9](https://doi.org/10.1016/S0002-8223(02)90346-9).
- Tuanet, K., Temmink, H., Zeeman, G., Wijffels, R.H., Buisman, C.J.N., Janssen, M., 2019. Optimization of algae production on urine. *Algal Res.* 44, 1–11. <https://doi.org/10.1016/j.algal.2019.101667>.
- Tzompa-Sosa, D.A., Yi, L., van Valenberg, H.J.F., van Boekel, M.A.J.S., Lakemond, C.M. M., 2014. Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Res. Int.* 62, 1087–1094. <https://doi.org/10.1016/j.foodres.2014.05.052>.
- Udomsil, N., Imsoonthornruksa, S., Gosalawit, C., Ketudat-Cairns, M., 2019. Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). *Food Sci. Technol. Res.* 25, 597–605. <https://doi.org/10.3136/fstr.25.597>.
- Valgardson, K., 2020. DigitalCommons @ USU Utilizing Earth's Microbiology to Develop the Framework for a Manufactured Martian Nitrogen Cycle.
- Van Aken, B., Schnoor, J.L., 2002. Evidence of perchlorate (ClO₄⁻) reduction in plant tissues (poplar tree) using radio-labeled 36ClO₄. *Environ. Sci. Technol.* 36, 2783–2788. <https://doi.org/10.1021/es020560t>.
- Van Eykelburg, C., 1977. On the morphology and ultrastructure of the cell wall of *Spirulina platensis*. *Antonie Van Leeuwenhoek* 43, 89–99. <https://doi.org/10.1007/BF00395664>.
- Vasudevan, R., Gale, G.A.R., Schiavon, A.A., Puzorjov, A., Malin, J., Gillespie, M.D., Vavitsas, K., Zulkower, V., Wang, B., Howe, C.J., Lea-Smith, D.J., McCormick, A.J., 2019. Cyanogene: a modular cloning suite for engineering cyanobacteria based on the plant moco syntax. *Plant Physiol.* 180, 39–55. <https://doi.org/10.1104/pp.18.01401>.
- Vavitsas, K., Kugler, A., Satta, A., Hatzinikolaou, D.G., Lindblad, P., Fewer, D.P., Lindberg, P., Toivari, M., Stensjö, K., 2021. Doing synthetic biology with photosynthetic microorganisms. *Physiol. Plant.* 173, 624–638. <https://doi.org/10.1111/PPL.13455>.
- Venkataraman, L.V., 1997. *Spirulina platensis (Arthrospira): physiology, cell biology and biotechnology*, edited by Avigad Vonshak. *J. Appl. Phycol.* 9, 295–296. <https://doi.org/10.1023/A:1007911009912>.
- Verseux, C., Baqué, M., Lehto, K., De Vera, J.P.P., Rothschild, L.J., Billi, D., 2016. Sustainable life support on Mars - The potential roles of cyanobacteria. *Int. J. Astrobiol.* 15, 65–92. <https://doi.org/10.1017/S14735504150021X>.
- Verseux, C., Heinicke, C., Ramalho, T.P., Determann, J., Duckhorn, M., Smagin, M., Avila, M., 2021. A low-pressure, N₂ / CO₂ atmosphere is suitable for systems on Mars. *Front. Microbiol.* 12, 611798. <https://doi.org/10.3389/fmcb.2021.611798>.
- Vijayakumar, S., Menakha, M., 2015. Pharmaceutical applications of cyanobacteria d A review. *J. Acute Med.* 5, 15–23. <https://doi.org/10.1016/j.jacme.2015.02.004>.
- Vismara, R., Barsanti, L., Lupetti, P., Passarelli, V., Mercati, D., Dallai, R., Gualtieri, P., 2000. Ultrastructure of the pellicle of *Euglena gracilis*. *Tissue Cell* 32, 451–456. [https://doi.org/10.1016/S0040-8166\(00\)80001-4](https://doi.org/10.1016/S0040-8166(00)80001-4).
- Volger, R., Pettersson, G.M., Brouns, S.J.J., Rothschild, L.J., Cowley, A., Lehner, B.A.E., 2020. Mining moon & mars with microbes: Biological approaches to extract iron from Lunar and Martian regolith. *Planet. Space Sci.* 184, 104850. <https://doi.org/10.1016/j.pss.2020.104850>.
- Wamelink, G.W.W., Frissen, J.Y., Krijnen, W.H.J., Verwoert, M.R., Goedhart, P.W., 2014. Can plants grow on mars and the moon: a growth experiment on mars and moon soil simulants. *PLoS One* 9, e103138. <https://doi.org/10.1371/JOURNAL.PONE.0103138>.
- Wamelink, G.W.W., Frissen, J.Y., Krijnen, W.H.J., Verwoert, M.R., 2019. Crop growth and viability of seeds on Mars and Moon soil simulants. *Open Agric.* 4, 509–516. <https://doi.org/10.1515/opag-2019-0051>.

- Wang, G., Liu, Y., Li, G., Hu, C., Zhang, D., Li, X., 2008. A simple closed aquatic ecosystem (CAES) for space. *Adv. Sp. Res.* 41, 684–690. <https://doi.org/10.1016/j.asr.2007.09.020>.
- Wang, Y., Seppänen-Laakso, T., Rischer, H., Wiebe, M.G., 2018. Euglena gracilis growth and cell composition under different temperature, light and trophic conditions. *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0195329>.
- Wang, F., Gao, Y., Yang, G., 2020. Recent advances in synthetic biology of cyanobacteria for improved chemicals production. *Bioengineered* 11, 1208–1220. <https://doi.org/10.1080/21655979.2020.1837458>.
- Watanabe, Y., Tatsuno, I., 2017. Omega-3 polyunsaturated fatty acids for cardiovascular diseases: present, past and future. *Expert. Rev. Clin. Pharmacol.* 10, 865–873. <https://doi.org/10.1080/17512433.2017.1333902>.
- Watanabe, F., Katsura, H., Takenaka, S., Fujita, T., Abe, K., Tamura, Y., Nakatsuka, T., Nakano, Y., 1999. Pseudovitamin B12 is the predominant cobamide of an algal health food, spirulina tablets. *J. Agric. Food Chem.* 47, 4736–4741. <https://doi.org/10.1021/jf90541b>.
- Weiss, T.L., Young, E.J., Ducat, D.C., 2017. A synthetic, light-driven consortium of cyanobacteria and heterotrophic bacteria enables stable polyhydroxybutyrate production. *Metab. Eng.* 44, 236–245. <https://doi.org/10.1016/j.ymben.2017.10.009>.
- Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Hellawell, K.E., Smith, A.G., Camire, M.E., Brawley, S.H., 2017. Algae as nutritional and functional food sources: revisiting our understanding. *J. Appl. Phycol.* 29, 949–982. <https://doi.org/10.1007/s10811-016-0974-5>.
- Wheeler, R.M., 2011. Plants for human life support in space: from Myers to Mars. *Gravitation. Sp. Biol.* 23.
- Włodarczyk, A., Selão, T.T., Norling, B., Nixon, P.J., 2020. Newly discovered *Synechococcus* sp. PCC 11901 is a robust cyanobacterial strain for high biomass production. *Commun. Biol.* 3 <https://doi.org/10.1038/s42003-020-0910-8>.
- Woolf, N., Angel, R., 2021. Pantheon habitat made from regolith, with a focusing solar reflector. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 379, 20200142. <https://doi.org/10.1098/rsta.2020.0142>.
- Wordsworth, R., Kerber, L., Cockell, C., 2019. Enabling Martian habitability with silica aerogel via the solid-state greenhouse effect. *Nat. Astron.* 3, 898–903. <https://doi.org/10.1038/s41550-019-0813-0>.
- Yu, L., Cañas, J.E., Cobb, G.P., Jackson, W.A., Anderson, T.A., 2004. Uptake of perchlorate in terrestrial plants. *Ecotoxicol. Environ. Saf.* 58, 44–49. [https://doi.org/10.1016/S0147-6513\(03\)00108-8](https://doi.org/10.1016/S0147-6513(03)00108-8).
- Yusof, Y.A.M., Saad, S.M., Makpol, S., Shamaan, N.A., Ngah, W.Z.W., 2010. Hot water extract of *Chlorella vulgaris* induced DNA damage and apoptosis. *Clinics* 65, 1371–1377. <https://doi.org/10.1590/S1807-59322010001200023>.
- Zeller, A., Mark, HUNT, Ryan, Jones, Alexander, Sharma, Suraj, 2013. Bioplastics and their Thermoplastic Blends from Spirulina and Chlorella Microalgae. *J. Appl. Polymer Sci.* 130 (5), 3263–3275. <https://doi.org/10.1002/app.39559>.
- Zengin, G., Nithiyananthan, S., Sarikurkcü, C., Uysal, S., Ceylan, R., Ramya, K.S., Maskovic, P., Aktumsek, A., 2017. Identification of phenolic profiles, fatty acid compositions, antioxidant activities, and enzyme inhibition effects of seven wheat cultivars grown in Turkey: A phytochemical approach for their nutritional value. *Int. J. Food Prop.* 20, 2373–2382. <https://doi.org/10.1080/10942912.2016.1238391>.
- Zhang, J., Müller, B.S.F., Tyre, K.N., Hersh, H.L., Bai, F., Hu Jr., Y., M.F.R.R., Rathinasabapathi, B., Settles, A.M., Graham, T., 2020a. Competitive Growth Assay of Mutagenized *Chlamydomonas reinhardtii* Compatible With the International Space Station Veggie Plant Growth Chamber. *Front. Plant Sci.* 11, 631. <https://doi.org/10.3389/fpls.2020.00631>.
- Zhang, N., Li, J., Luo, J., Yu, Q., Ai, W., Zhang, L., Tang, Y., 2020b. Wheat cultivation and nutrient control for the 180-day CELSS integrated experiment. *Life Sci. Sp. Res.* 26, 46–54. <https://doi.org/10.1016/j.lssr.2020.04.001>.
- Zhang, M.P., Wang, M., Wang, C., 2021. Nuclear transformation of *Chlamydomonas reinhardtii*: a review. *Biochimie* 181, 1–11. <https://doi.org/10.1016/j.biochi.2020.11.016>.