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Sustaining a Mars Colony through Integration of Single-Cell Oil in Biological Life Support Systems

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***Abstract* – As humanity sets its sights on establishing a sustainable and prosperous colony on Mars, the main challenges to be overcome are ensuring a reliable and nutritious food supply for settlers, feedstock for 3D printing, fuel and pharmaceuticals. While various solutions for production of essential products on Mars have been proposed, there is growing interest in the use of microorganisms as the main production units. This scientific review article proposes a novel concept of using single cell oil (SCO) as a versatile feedstock for various applications in a bioregenerative life support system (BLSS) for space missions. The authors suggest using outputs from autotrophic systems, such as cyanobacteria biomass and oxygen, to cultivate SCO-producing microorganisms from the class Labyrinthulomycetes. The produced SCO can be used for food, fuel, 3D printing materials, and pharmaceuticals. This approach can potentially reduce the importance of carbohydrates in space foods, offering various benefits, including a reduction in food weight, simpler, lightweight, more compact bioreactors, launch cost reduction, potentially improved mental and cognitive performance, and reduced fatigue for the crew. The authors also suggest using SCO as the feedstock for the production of 3D printable filaments and resins and as a supplementary fuel source for space colonies. While the concept is hypothetical, the theoretical foundation is solid, and this approach could potentially become an important element required for the establishment of a successful Mars colony.**

***Keywords* – 3D printing materials; autotrophic systems; biological life support systems; food; fuel; Labyrinthulomycetes; Mars colony; single cell oil; space missions.**

1. INTRODUCTION

Exploring the cosmos can help us better understand the universe we live in, including its origin, evolution, and potential for supporting life beyond Earth. This knowledge can help us gain a better understanding of our place in the universe and answer fundamental questions about our existence. Colonizing other planets and moons could provide a backup plan in case of a planetary disaster or extinction event on Earth. Establishing human settlements on other celestial bodies could ensure the survival of the human species and protect our collective knowledge and cultural heritage. Space exploration and colonization have various practical applications in fields such as medicine, materials science, and environmental sustainability [1], [2]. For example, the technology developed for space travel has led to numerous advances in medical imaging [3]. Space exploration and colonization have the potential to spur innovation and inspire future generations to pursue scientific and technological breakthroughs. By pushing the limits of human knowledge and capability, space exploration can drive progress and create new opportunities for discovery and growth.

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Humans are considering going to Mars for various reasons, including scientific exploration, technological advancement, and the potential for human colonization of other planets [4]. Mars has long been of interest to scientists due to its similarity to Earth in terms of its geology and potential for supporting life [5], [6]. Exploring Mars and studying its history and geology could help us better understand the evolution of the Solar System and the potential for life beyond Earth. Furthermore, technological advances made in developing the infrastructure and systems required for a human mission to Mars could have numerous applications in fields such as biotechnology, medicine, materials science, food science, environmental sustainability etc. The potential for human colonization of Mars has been a topic of interest for many years [7], [8]–[10], with some seeing it as a way to establish a permanent human presence beyond Earth and a backup plan in case of a planetary disaster. While there are currently no concrete plans for colonizing other planets, there have been discussions and proposals for potential future missions to other planetary bodies in our solar system. For example, some scientists have proposed missions to the moons of Jupiter [11] and Saturn [12], such as Europa and Enceladus, which may have subsurface oceans that could potentially harbour life [13]. However, due to the technical challenges and high costs associated with such missions, any potential colonization plans for other planets or moons are likely to be far in the future and depend on significant advances in space technology and infrastructure. Mars is a good choice for exploration and potential colonization. Mars is the most Earth-like planet in our solar system and has a similar day-night cycle and seasonal changes to Earth [14]. Also, Mars has a relatively thin atmosphere and a weaker gravitational force compared to Earth, making it a more accessible target for human exploration and potentially colonizing in the long run [15]. Additionally, Mars is believed to have had liquid water on its surface in the past, such as water ice in the polar caps and subsurface, which could potentially be used to support human settlements and exploration, indicating that it may have had conditions that could have supported life [16].

Mars presents a tempting target for exploration and potential colonization due to its relative similarity to Earth and its potential scientific and economic benefits. And as humanity steadily overcomes the technological obstacles to deep space missions, space agencies and business organizations are seriously considering the prospect of exploring and settling interplanetary settlements [8], [17]. There are ongoing research projects related to Mars exploration and colonization. Organizations such as NASA, the European Space Agency (ESA), and private companies like SpaceX are actively engaged in Mars-related research projects [18]–[20]. Some examples of current and planned missions to Mars include the NASA Mars Perseverance rover mission [21], which launched in July 2020, and the upcoming ESA ExoMars mission [22], which was planned for launch in 2022. Private companies like SpaceX have also announced plans for missions to Mars. Scientists have been working on various research projects related to Mars, such as studies on the planet's geology [5], the atmosphere [15], [23] potential for supporting life [24], [25], and other projects. Researchers are exploring technologies and strategies for making human missions to Mars possible and sustainable with new projects and discoveries being announced regularly. Mars is technically colonisable, but it would be an extremely challenging and complex endeavour [17]. Yet regardless of who is the first to set foot on Mars, we will need to develop our independence from Earth if we intend to stay there for an extended period of time and collect valuable scientific data.

There are some significant challenges on Mars that we need to consider. Mars has an extremely harsh environment, with a thin atmosphere, extreme temperature fluctuations, high levels of radiation, and no magnetic field to protect against solar and cosmic radiation [26], [27]. These conditions would make it difficult for humans to survive without significant technological support. Mars has no breathable atmosphere, and the water on the planet is

mainly locked up in ice caps or underground. To establish a colony on Mars, humans would need to be able to generate breathable air and extract and purify water. Mars is an average of 140 million miles away from Earth, which means that communication and resupply missions would be delayed and difficult [8].

Despite these challenges, ongoing efforts exist to explore the possibility of colonizing Mars. Some proposed strategies include terraforming the planet to make it more habitable, establishing self-sustaining habitats, and using advanced technologies to support human survival [17], [28]. However, there are still many unknowns and obstacles to overcome before humans can successfully colonize Mars on a permanent basis. Technology is the major barrier to going to Mars now and in the foreseeable future. Future technological advancement may be offset by rising risks [29]. The main objectives of Mars exploration projects mostly are on producing and sustaining food, fuel, 3D printing materials, and pharmaceuticals. Some research groups have identified these as key research topics on colonizing Mars [30]–[32]. These are essential for the success of a Mars colony because it reduces reliance on Earth, promoting customization and flexibility, and promoting sustainability. Pharmaceutical production is critical to the success of a Mars colony by providing medical care for the colonists, promoting self-sufficiency, enabling research and development, and ensuring quality control over medications [1]. 3D printing materials are necessary for the rapid prototyping and repair of equipment and structures, which is essential in a remote location like Mars, where there may be limited access to replacement parts and repairs [33]. Producing fuel is important for cost savings, resource utilization, exploration capabilities, and interplanetary transportation. It would enable the colony to become more self-sufficient and provide further exploration and scientific research opportunities. Furthermore, producing fuel on Mars could also reduce the risk of relying solely on Earth for supplies and resources. This would increase the sustainability and resilience of the colony in the long term.

One of the most difficult aspects of establishing a human presence on Mars is guaranteeing a sustainable and stable food source. The main food challenges on Mars stem from the difficulty of supplying food to sustain a long-term human presence on the planet (up to 18 months) [34]. Given the distance between Earth and Mars, resupplying food from Earth would be difficult, expensive, and impractical for a long-term mission or colony [34]. This means that any human presence on Mars would need to find a way to produce its own food sustainably using local resources. One approach that has been proposed is the use of bioregenerative life-support systems (BLSS) [35], which would involve recycling food, water, and gases using plants and other biological systems. However, it is not clear whether it will be possible to sustainably grow crops on Mars using local soil, which is known to be high in salts and other minerals that can be toxic to plants [36]. This means that any farming or food production on Mars would need to be done in a controlled environment using carefully designed soil and fertilizers. Another challenge is providing a varied and nutritious diet for astronauts on Mars. Unlike on Earth, where a wide range of fruits, vegetables, and other food sources are available, any human presence on Mars would likely have to rely on a limited set of crops and processed foods that can be produced and stored locally. This could potentially lead to nutritional deficiencies and health problems if not managed carefully [34], [37]. Overall, while there are promising approaches for sustainably producing food on Mars, such as using BLSS and hydroponics [30], [38], significant research and development will be needed to overcome the unique challenges posed by the Martian environment and ensure a reliable food supply for any long-term human presence on the planet.

Biotechnology offers a range of tools and techniques that can be used to develop sustainable and efficient production systems for essential resources on a Mars colony. By harnessing the power of biotechnology, we can create a more self-sufficient and resilient

human presence on Mars [30], [31]. Research on life support systems for space exploration and Mars colonization has led to many innovations and technological breakthroughs [30], [39]–[42]:

1. Closed Ecological Life Support Systems (CELSS): CELSS technology involves using plants and other biological systems to recycle air, water, and nutrients, creating a closed-loop system that can sustain human life in space. CELSS technology has been used on the International Space Station (ISS) and could be a key component of future Mars colonies.
2. Hydroponics: Hydroponics involves growing plants in nutrient-rich water instead of soil. This technique has been used to successfully grow crops in space, where traditional soil-based farming is impractical. Hydroponic systems could be used on Mars to produce food for human settlements sustainably.
3. Water recycling systems: Water is a critical resource for human survival, and recycling systems that can purify and reuse wastewater have been developed for space exploration. These systems could be adapted for use on Mars, where water is scarce and any human presence would need to conserve and recycle water resources.
4. Regenerative life support systems: Regenerative life support systems use advanced technologies to recycle air, water, and other resources with nearly 100 % efficiency. These systems have been proposed for future Mars colonies, where they could help ensure a sustainable and self-sufficient human presence.
5. In-situ resource utilization (ISRU): ISRU involves using local resources on Mars, such as water, ice, and minerals, to support human settlements and exploration. Technologies for extracting and processing these resources have been developed and tested on Earth and in space and could be critical for sustainable long-term human presence on Mars.

Many of innovative products and technologies have practical applications beyond space exploration. These advances demonstrate the power of scientific and technological research to drive progress and create new opportunities for discovery and growth. Humans will be able to walk on Mars thanks to breakthroughs in applied physics, and we can settle there thanks to advances in applied biology [43].

2. CURRENTLY CONSIDERED SOLUTIONS

Before we look at the technological solutions currently being considered to promote the production of food, fuel, polymers, pharmaceuticals, and other products in Martian colonies, we need to establish what resources and energy sources are available on Mars.

2.1. Resources on Mars

Solar power would most likely be the primary source of energy for technological food production systems on Mars [26], [44]. Solar panels could be used to collect solar energy and convert it into electricity, which could then be used to power food production machinery and other systems. Because Mars receives only about 40 % of the solar energy that Earth receives due to its greater distance from the Sun, efficient use of this resource would be essential for a long-term human presence on Mars [44]. In the future, other potential energy sources, such as nuclear energy [45] or geothermal energy [46], could be used in the Martian colony.

The atmosphere is an important resource on Mars, containing carbon dioxide, nitrogen, argon, oxygen, carbon monoxide, water vapor, and nitrogen oxide. Atmospheric pressure on Mars fluctuates depending on location and season, but a general average value is

0.007 atm [15]. Most plans for *In situ* resource use on Mars relate to atmospheric CO_2 [47]–[50]. The atmosphere of Mars is rich in CO_2 (95.3 %). There is enough water in the form of ice and hydrated minerals in the Martian regolith, as well as other elements necessary for the growth of autotrophs, such as P, K, Mg, Mn, Fe, S, Cl, Fe, Ca, Zn, Ni, Mo, etc. However, the Martian regolith must be processed physically, chemically, and/or biologically to extract these elements in a usable form. The extracted water must also be pretreated to reduce its salt content. A major challenge for successful food production on Mars is the nitrogen deficit, as it is present in the atmosphere in very low concentrations (only 2.7 %) [48], [50]–[53].

2.2. Food production

The production and delivery of food in a Martian colony is now the subject of various space research projects [54], [55]. Traditional agricultural practices and cutting-edge technical innovations are some of the options. A sustainable and self-sufficient Mars colony will only be possible with the effective use of proposed technology ideas. Bringing fresh food to Mars is challenging since it takes six months for spacecraft to go there from Earth [34]. Therefore the cost of food delivery can be very high [31]. Additionally, storing and delivering food on Mars may be challenging due to hostile conditions, including high radiation levels and temperatures [26]. Therefore, novel approaches are required to guarantee a steady food supply for a future Mars settlement.

In scientific studies, the main biological methods considered for food and oxygen production for colonies on Mars include growing photoautotrophs such as cyanobacteria, microalgae, and plants. The main advantage of these photosynthetic organisms is their ability to be cultivated *In situ* from available resources [56]. At the early stages of colonization, the option of delivering nitrogen from Earth is considered. Subsequently, nitrogen fixation from the atmosphere using a chemical processes or nitrogen-fixing cyanobacteria, as well as extraction from processed waste from astronaut life support systems and biological waste from farms of plants and fermenters of microalgae and cyanobacteria, are possible [48], [57].

Autotrophs, like as photosynthetic bacteria and plants, may be utilized to produce food on Mars [58], although the arid soils there may call for the addition of cyanobacterial or algal biomass as a biofertilizer or soil conditioner to allow for more proper development of food crops in greenhouses [28]. Plants and microorganisms can both fixate CO_2 on Mars and can be used to produce food, oxygen, and other resources. Plants can also create a more Earth-like environment, which may benefit residents mentally [37], [59]. The ability of autotrophic organisms to fix atmospheric carbon dioxide and turn it into organic matter through photosynthesis makes them a promising option for biomass production on Mars. However, Mars has low atmospheric pressure, low temperatures, and intense radiation, making plant cultivation difficult. Because of that, plants need more resources and infrastructure than microbes.

The soil on Mars is unsuitable for producing crops because it lacks the necessary nutrients and contains high levels of hazardous compounds [60]. Hydroponic systems, however, do not require soil and may feed plants with the essential nutrients directly through water. With their highly efficient resource utilization and ability to function in a controlled environment, hydroponic systems have several benefits for agriculture in a Mars colony [61]. However, there are possible drawbacks to consider, such as high initial costs, energy requirements, technical complexity, system malfunctions, and a limited variety of crops [62]. To guarantee its success, these must be thoughtfully studied and addressed, such as growing plants in low atmospheric pressure and microgravity conditions [63], [64].

Microalgae and lithoautotrophic or methylotrophic bacteria are also suggested as potential solutions for enhancing food delivery during the early phases of human residence on

Mars [51]. Methylotrophic or methylautotrophic microbes—or model organisms created for methanol-based heterotrophic fermentation – have been suggested as ideal bacteria to supplement delivered food [57]. Microalgae cultivation has numerous advantages over plant crops [65]. They can be cultivated in straightforward stirred reactors using readily available raw materials like atmospheric CO_2 and redox couples found in the regolith or methanol produced by the catalytic reduction of CO_2. Microorganisms can be engineered to synthesize human food ingredients like protein [66], polysaccharides [67], and fatty acids [68], as well as vitamins and flavourings [69]. For example, *Chlorella* sp. and Spirulina contain significantly more protein than traditional grain and root crops and high levels of each essential amino acid [51]. These microbes can be cultivated in bioreactors with reclaimed wastewater, nutrients from human waste, and natural or artificial light sources [70]. It offers a sustainable food source for the Mars colony as the technologies use ISRU (*In Situ* Resource Utilization) [71]. The primary uses for ISRU on Mars are for ascending propulsion from the Martian surface and water and oxygen for life support [50].

Autotrophic microorganisms could be exploited to make biofuels, biomaterials, and other vital substances that could support human life on Mars and serve as a source of food and oxygen. Furthermore, they can be genetically tweaked to operate better under Martian conditions and are quite simple to cultivate and maintain [37], [72]. These microorganisms can synthesize organic compounds from inorganic materials, CO_2 and energy sources such as sunlight [73], [74]. Some autotrophic microbes, such as cyanobacteria, can fix nitrogen from the atmosphere, providing an extra supply of nutrition [74]. Altogether, microorganisms can use resources more efficiently, take up less space, and thrive in a broader range of environmental circumstances.

2.3. Fuel production

As for fuel production on Mars, it would almost certainly entail gathering resources from the planet's surface and refining them using various processes [75]. One possible option for this is to use Mars' carbon dioxide atmosphere to make methane via the Sabatier reaction [76], in which hydrogen is coupled with CO_2 to produce methane and water. Methane can be utilized as a rocket propellant, while water can be used for a variety of uses, including plant growth. Another possibility is to recover water ice from the Martian soil, which can then be electrolyzed to separate hydrogen and oxygen [77]. The hydrogen and oxygen can then be mixed to make water for human consumption and plant development, or they can be used as rocket propellant. These solutions, however, would necessitate enormous infrastructure and energy resources to build and sustain. Some highly specialized microbes, such as *Chlamydomonas reinhardii*, may even release elemental hydrogen from water under specific cultivation conditions, which could be utilized to run fuel cells and generate electric energy on Mars [78].

2.4. 3D printing materials

The ability to manufacture 3D printing materials on Mars could be critical to the long-term success of a Mars colony. These polymers have a wide range of applications, including the manufacturing of tools, building materials, and even medical implants. Yet, due to a scarcity of readily available materials and resources, creating 3D printing materials on Mars can be difficult. One possibility is to use locally obtained materials such as regolith or even recycled plastics to create polymers for 3D printing [79]. Another option is to genetically engineer microbes to make biodegradable polymers from Martian materials [51], which can then be extracted and processed for a variety of purposes. Unlike conventional plastics, microalgae-

produced bioplastics made from polymers, including starch, cellulose, and polyhydroxyalkanoates (PHAs), are biodegradable [80].

2.5. Limitations of currently considered solutions

The cultivation of traditional agricultural crops, such as cereals and root vegetables, appears to be an obvious option. However, creating an adequate environment on Mars will be an incredibly challenging task in the early stages of colonization [72]. Firstly, to cultivate crops in soil, it is necessary to extract regolith, which requires additional time and infrastructure [48]. To use regolith as a substrate, it is essential to ensure the bioavailability of elements necessary for plant growth and remove inhibiting components such as excess salts and oxides. In addition, regolith has a low water-holding potential and lacks nitrogen. Therefore, not only processing of the regolith but also enrichment with nutrients will be required [72]. An alternative can be a soilless cultivation system, such as hydroponics, aquaponics or aeroponics. This system will allow for the cultivation of plants from the first days on Mars. However, additional payload will be required from Earth when delivering trays, pumps, and tanks [38]. Secondly, the majority of the vegetative biomass of most agricultural crops is inedible, which makes their cultivation inefficient in conditions of limited available space [51]. Thirdly, plants grow relatively slowly, usually taking weeks or months from planting to harvest [72]. Microorganisms are simpler and can grow in various habitats with fewer resources. Microorganisms could potentially be used to help maintain a sustainable food supply by producing organic matter from CO_2 in the Martian atmosphere. Not only food but also biofuels [47], 3D printing polymers [51], and pharmaceuticals [81] can be engineered from them.

2.6. Advantages of photosynthetic microorganisms

Compared to plants, photosynthetic cyanobacteria such as *Arthrospira* sp., *Limnospira* sp., *Chlamydomonas* sp., *Nostoc* sp., *Anabaena* sp., and others have several advantages, which make them better candidates for launching primary biological life support systems on Mars [49].

2.6.1. Capturing solar energy

Cyanobacteria more efficiently captures solar energy, producing more edible biomass and O_2. This is crucial when available resources and growing space are limited [72]. Studies show that certain species are capable of using the ‘flashing light effect’ to accumulate light energy near the surface of the reactor for subsequent biochemical processes in the dark away from the reactor surface under certain conditions [82]–[84]. Furthermore, *Cyanothece* sp. ATCC 51142 is capable of using the accumulated daytime light energy to continue growing at night. This strain carries out photosynthesis, accumulating glycogen during the day, and then breaking down its glycogen reserves during the night to provide energy for nitrogen fixation. Thus, available solar energy is utilized throughout the day [43]. Such characteristics can improve the efficiency of cultivating cyanobacteria, given the frequent Martian dust storms, as well as the fact that photosynthetically active radiation on Mars is 57 % lower than on Earth [48].

2.6.2. Cultivation conditions and resilience

Cyanobacteria are not demanding in terms of cultivation conditions and can grow on simple inorganic media, with many of them not requiring fixed nitrogen in their environment due to

their ability to fix N_2. For these purposes, nitrogen-fixing species such as *Anabaena variabilis*, *Anabaena azotica*, *Synechococcus* sp., *Trichodesmium* sp. [74], *Nostoc flagelliforme* [51], and *Scytonema javanicum* [85], are considered, which are capable of compensating for the nitrogen deficiency in the culture medium, thus making the process more energy-efficient. However, prior to this, it is necessary to concentrate the available nitrogen in the atmosphere since the partial pressure of nitrogen on Mars is 10 times lower than required for microbiological fixation [48].

Cyanobacteria are highly stress-tolerant and easily adaptable organisms capable of surviving in extreme conditions [52], [86]. Several studies have shown that the desert cyanobacteria of the genus *Chroococcidiopsis* can maintain viability for a long time when exposed to atmospheric composition, temperature, pressure, and UV radiation similar to those on Mars [86]–[88]. The microalga *Chlorella* sp. is also capable of surviving prolonged exposure to ionizing radiation while maintaining over 90 % of its initial photosynthetic capacity [89]. Some strains of cyanobacteria are able to maintain their ability to reproduce in an atmosphere with high levels of CO_2. As reported in reference [90], *Synechococcus* sp. and *Anabaena* sp. were able to grow at concentrations up to 55 % CO_2, after which reproduction ceased. Moreover, *Plectonema boryanum* demonstrated the ability to grow with a gradual increase in CO_2 concentration up to 100 % in the atmosphere, albeit at a slower rate than under normal air conditions, while still producing biomass [90].

2.6.3. High productivity

Cyanobacteria have a high growth rate, with reproduction occurring every 2–3 hours, which is significantly faster than that of microalgae, which can take up to 7 hours. Cyanobacteria produce edible biomass that is rich in all essential amino acids, polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), as well as carotenoids and B-group vitamins [48], [49], [74], [91]. A competitive option for space farming is considered to be three types of cyanobacteria – *Arthrospira platensis*, *Arthrospira maxima*, and *Limnospira indica*, which together form the supplement Spirulina [56], [92]. Spirulina demonstrates high productivity, with cultivation in open ponds in non-optimized conditions typically yielding 20 times more protein per hectare than soy [72]. It should be noted that cyanobacterial biomass is more easily digested and does not require additional processing, whereas the biomass of *Chlorella* sp. has poorly digestible cellulose-containing cell walls, therefore, cooking is necessary. That is also true for most plant crops [72]. Despite the numerous advantages, the use of cyanobacteria and microalgae has some drawbacks. For example, they contain less fibre than edible plant biomass and too few carbohydrates for a fully balanced diet [49]. Moreover, the main limiting factor is the high content of protein in their biomass. Thus, *Chlorella* sp. can be an additive to a human daily diet in the amount of 20–35 % [92] and Spirulina up to 10 g per day [93]. Additionally, Spirulina biomass has an unpleasant taste and odour, described as ‘earthy’ and ‘slightly sulphurous’ [51]. Therefore, it can only partially replace food intake, and other products are required for enriching the diet with nutrients and fibre, as well as for flavour and texture diversity [72].

2.6.4. Producing bioplastics and biofuels

Arthrospira platensis, *Synechocystis* sp., *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Nostoc* sp. are capable of producing bioplastics such as polyhydroxyalkanoates (PHA) and starch for carbon storage, which can be extracted and purified from the culture [51]. It has been found that the mechanical and thermal properties of bioplastics produced using microalgae and cyanobacteria are comparable to or better than those of conventional

petroleum-based bioplastics [94]. However, this method of bioplastic production is energy-intensive, so it is necessary to develop economically and energetically efficient approaches. Researchers also speculate that the physical and chemical properties of bioplastics may change over time, and their durability in harsh Martian conditions is not well understood [30], [80].

Some species of cyanobacteria, such as *Anabaena azotica*, *Anabaena variabilis*, *Anabaena cylindrica*, *Synechococcus* sp., *Synechocystis* sp., *Arthrospira maxima*, and *Nostoc punctiforme*, are capable of producing hydrogen under conditions of low atmospheric nitrogen content, which is advantageous in the case of the Martian atmosphere, which has low levels of N_2 [52], [74]. Nitrogen-fixing species such as *Nostoc* sp. and *Anabaena* sp. are particularly effective in this regard [51]. Their hydrogen production activity is derived from their nitrogenase enzymes, which catalyze the reduction of N_2 to $NH4^+$ and, in the absence of nitrogen, reduce H^+ to H_2 [72]. Theoretically, H_2 could be used directly as a propellant [51]. However, the efficiency of hydrogen production by cyanobacteria is currently not high enough for practical use on Mars [95]. Nevertheless, research in this area continues with the aim of improving the productivity and efficiency of the process. In addition to H_2, cyanobacteria can produce various biofuel precursors and components, including alkanes, ethylene, hydrogen peroxide, and lipids [72]. They can also provide organic substrates for the production of biofuel by heterotrophic microorganisms. This approach seems more realistic, as improved strains for chemical applications such as *Escherichia coli* and *Saccharomyces cerevisiae* are more efficient in fuel production [48].

2.6.5. Nutrient extraction and bioavailability

Cyanobacteria are able to extract nutrients directly from rock substrates, making them useful as bioweathering agents for releasing nutrients from Martian regolith [52], [72]. The biomass of cyanobacteria can also enrich the nutrient composition of regolith and increase its water-holding capacity for further use as a substrate for plants [96]. In a study by Nascimento *et al.* [97], the biomass of *Nostoc* sp. was able to completely replace the inorganic nitrogen source – urea during the cultivation of different crops. The addition of *Nostoc* sp. biomass to the substrate allowed for maximum growth of wheat *Triticum aestivum*, resulting in a compact root system and more branches [97].

Microalgae and cyanobacteria are capable of extracting valuable chemical elements such as nitrogen, phosphorus, and sulphur from waste such as wastewater, solid organic wastes, and urine. This is particularly important for life support systems on Mars [72], [98]. Numerous studies have shown that *Chlorella* sp. and Spirulina can use urea in urine as a nitrogen source, returning it to a bioavailable form as ammonium ions and bicarbonate [99]–[101].

2.6.6. Nutrient sources and substrates for microbial productions

The biomass of microalgae and cyanobacteria can also be used for the production of nutrient media for other heterotrophic microorganisms that produce products of high value for food, polymer, fuel, and pharmaceutical production. Nitrogen-fixing cyanobacteria are especially advantageous as they accumulate amino acids, nucleic acids, and ammonium in their cells. Heterotrophic microorganisms, in turn, are capable of utilizing these nitrogen sources in accessible form from lysed cells of cyanobacterial biomass [51], [73], [98]. The study by Verseux *et al.* [73] demonstrated that a medium based on dried biomass of *Anabaena* sp., grown in simulated Martian regolith, gave cell concentration of *Escherichia coli* equivalent to those in standard microbiological media [73].

Additionally, cyanobacterial biomass can be used as a carbon source for culturing heterotrophic microorganisms that produce high yields of intracellular biopolymers. It has been reported that using the biomass of *Scenedesmus obliquus* as a substrate for genetically engineered *Escherichia coli* improved the yield of PHA [51]. It is known that the biomass of cyanobacteria, such as Spirulina, is rich in proteins (60-70 % of dry weight) [80] and cannot boast a high content of lipids or carbohydrates. However, this statement is true under optimal cultivation conditions. Nevertheless, under certain cultivation conditions (light intensity, nitrogen and phosphorus limitations, atmospheric pressure, CO_2 concentration), metabolic pathways can be switched to the synthesis of carbohydrates and lipids [102], [103]. In the study by Markou [104], phosphorus starvation increased the accumulation of carbohydrates and lipids in the biomass of *Arthrospira platensis* from 9 % up to 65 % and from 4.9 % up to 7.5 %, respectively [104]. In another study, *Arthrospira platensis* showed a 2.8-fold increase in carbohydrate accumulation under nitrogen limitation compared to the control sample, while phosphorus limitation in the medium led to a 1.6-fold increase in starch accumulation. [105]. Also, it has been reported that *Arthrospira cylindrica* grown at low pressure and high CO_2 concentrations showed increased carbohydrate content, approaching the protein-to-carbohydrate ratio required by humans [72].

3. Novel Concept of Single-cell Oil Role in BLSS

3.1. Pros and cons of cultivating heterotrophic and autotrophic microorganisms

Heterotrophic microorganisms are widely used in various industries on Earth. They are used in food production, agriculture to provide symbiosis with plants, soil improvement, pharmaceuticals, synthesis of bioactive compounds, production of various industrially important chemicals, waste management, bioremediation, and more. Therefore, there are already available appropriate, tested, safe, and reliable solutions based on heterotrophic microorganisms for many processes that are also important for the formation of sustainable and self-sufficient colonies. Cultivating heterotrophic microorganisms is straightforward, and the used bioreactors are simple, compact, and easy to maintain [51]. Thanks to very high cell densities achievable in heterotrophic cultures, the volume of the respective bioreactors needs to be significantly smaller compared to photobioreactors [72]. For example, the culture of heterotrophic *Fusarium venenatum* A3/5 can be used to obtain protein-rich biomass [56], [106]. Due to the rapid heterotrophic growth of *Fusarium venenatum*, it is possible to produce approximately 24 g L^{-1} d^{-1} of dry weight biomass and approximately 12.48 g L^{-1} d^{-1} of protein [106]–[108], while one of the most effective autotrophic cultures, Spirulina, can produce approximately 1 g L^{-1} d^{-1} of dry weight biomass and 0.65 g L^{-1} d^{-1} of protein [51], [109]. Therefore, bioreactors designed for heterotrophic cultures can also significantly reduce volume and weight, which is a crucial factor for launch weight and costs. Of course, in general, cultivating *Fusarium venenatum* and other heterotrophic cultures is less energy efficient than Spirulina [56], [110]. However, launch costs are a crucial consideration, and there may be justification for finding a balance between overall process efficiency, launch weight, complexity of bioreactor systems, required maintenance, and the potential to increase bioreactor volume while being on an extraterrestrial planet.

The main disadvantage of heterotrophic microorganisms is that they depend on products from autotrophic and lithotrophic systems, such as sources of carbon, nitrogen, oxygen, leached macro and micronutrients, etc. Therefore, although heterotrophic systems have a number of advantages, they are fundamentally not self-sustaining and will always need to be linked to autotrophic systems.

This is also the greatest advantage of autotrophic systems, as they are self-sustaining systems capable of utilizing limited resources available on Mars. Autotrophic systems such as cyanobacteria are capable of using solar energy, inorganic nitrogen, and CO_2 to produce a wide range of valuable products. Other authors [51], [56], [72] have described in detail how cyanobacteria outperform other producers in various directions, including for their use in production of food, fuel, 3D printing materials, pharmaceutical active compounds, and other solutions.

Considering both the indispensability of autotrophic systems and the advantages of heterotrophic systems, an interesting approach is to simplify the outputs of autotrophic systems (such as biomass and oxygen) and diversify the final products through heterotrophic systems. Heterotrophic systems can be sustained using two main products of autotrophic systems – biomass and oxygen. This concept has been widely discussed in several other studies [51], [56], [72] and research articles [73], [111]–[114], where primarily cyanobacterial and microalgae biomass lysates or their extracellular products are used as the main nutrients for culturing heterotrophic microorganisms. Meanwhile, engineered and wild-type bacteria such as *Saccharomyces cerevisiae* [111], [114], *Escherichia coli* [73], [115], Pseudomonas [116], [117], and others, are used as the main heterotrophic cultures, which are adapted for the synthesis of various products, such as ethanol [111], [114], recombinant proteins [51], [118], polyhydroxyalkanoates [116], [119], etc. As seen from these examples, the solutions currently offered, which combine outputs of autotrophic systems to produce specific products through heterotrophic microorganisms, could be improved, in our opinion, by utilizing heterotrophic systems that synthesize precursors for a variety of different products, which are essential for fulfilling colony's needs and simplifying the overall system.

In any case, some products will require the use of dedicated cultures solely for the production of a single specific product, but the essential products, such as food, fuel, feedstock for 3D printing, and pharmaceuticals, theoretically can be produced from a single raw material – single-cell oil, which in turn can be obtained only from a single heterotrophic system – single-cell oil (SCO)-rich microorganisms, mainly from class Labyrinthulomycetes.

Therefore, our proposed idea is to use the outputs of autotrophic systems, biomass (most likely cyanobacteria), and oxygen, to cultivate SCO-producing microorganisms, whose produced single-cell oil could be further utilized for the production of food, fuel, 3D printing feedstocks, and pharmaceuticals (see Fig. 1). In the following sections, we will discuss in further detail the theoretical rationale for the application of SCO in the production of each of these product categories.

3.2. Application of SCO in Food and Pharmaceuticals Production

Acceptable macronutrient distribution ranges (AMDRs) recommended for human nutrition consist of 55–70 % carbohydrates, 7–20 % protein, and 15–25 % fats [129]. Carbohydrates, which are broken down to or converted into glucose, are the main source of energy in this diet, utilized through glycolysis and the citric acid cycle to produce adenosine triphosphate (ATP) [130]. Almost all carbohydrates and a majority of total calories in modern diets come from plants. Plants contribute to approximately 70 % of daily caloric intake [131], and the most widely consumed crops are rice, wheat, maize, potatoes, and cassava [132]. However, in the context of space exploration, the use of plants for meeting daily caloric needs is problematic in space stations, spacecraft, and colonies. Despite being autotrophic systems, plants are considerably less efficient than cyanobacteria and microscopic algae in terms of CO_2 fixation, energy consumption, space requirements, growth rate, environmental demands, labour requirements, stress tolerance, proportion of edible biomass, and other factors [2], [51], [72], [133]–[138].

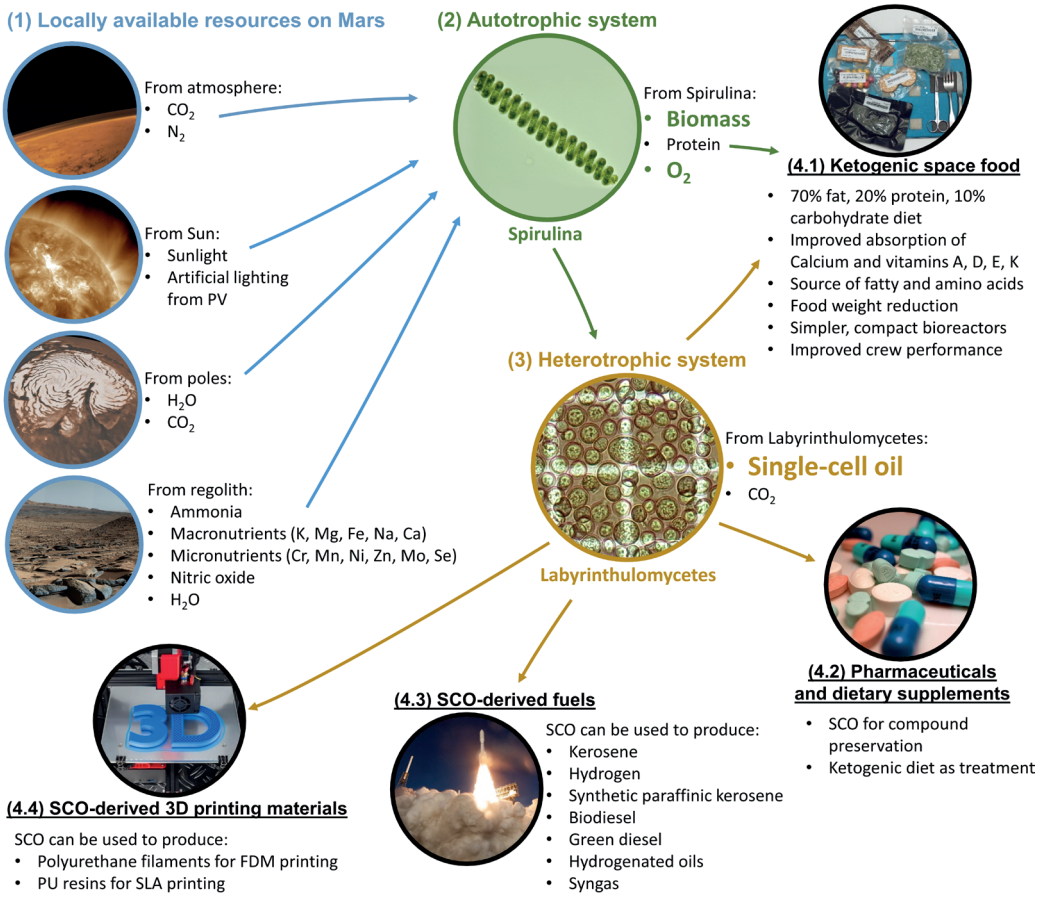


Fig. 1. Scheme for the proposed concept of autotrophic-heterotrophic hybrid system in BLSS for single cell oil production and utilization as multipurpose feedstock for food, pharmaceutical, fuel and 3d printing material production. Sources of the images: [120]–[128].

Furthermore, there is not yet a complete understanding of how plant growth will be affected in reduced gravity conditions [139], [140], or whether plant cultivation will be possible using the available Mars regolith. For example, it is known that Martian regolith contains high levels of chemical species such as perchlorate, which is toxic to plants [51], [141]–[143]. Therefore, relying extensively on plant utilization for food production would not be desirable, especially in the early stages of colony development, and full-scale plant utilization would require much more extensive and complicated systems.

As the options of using plants, at least in the early stages of the colony, are limited, it is theoretically possible to meet caloric needs by using cyanobacteria and microscopic algae such as *Chlorella* and *Euglena* [51], [102], [144], [145]. However, these microorganisms are characterized by high protein concentrations, with Spirulina ranging from 55–70 %, Chlorella from 44–50 %, and Euglena from 14–34 % [51], [72], which is a very good property. However, proteins cannot serve as the main source of energy in the diet, as it can lead to health problems such as gout, kidney stones, protein poisoning, coronary artery disease, cancer, and osteoporosis [146]–[151]. In addition, only a few microorganism cultures are able

to produce significant amounts of suitable carbohydrates, such as *Arthrospira platensis* (Spirulina), which is able to produce up to 60 % carbohydrates of the total biomass in phosphorus limiting conditions [103], [104]. However, obtaining such elevated carbohydrate concentrations under phosphorus limiting conditions is also very inefficient, as it significantly reduces biomass production [103].

In general, microorganisms are not as efficient carbohydrate producers, and carbohydrates are the main nutrients for most microorganisms. Therefore, offsetting high protein concentrations by using other carbohydrate-synthesizing microorganisms to balance the diet can be very difficult. The wider use of microorganism-derived carbohydrates for food production is an interesting research direction that should be further explored, but it is beyond the scope of this review paper and will not be further discussed.

One possible solution is to replace carbohydrates as the primary source of energy with fats. By changing the diet to consist of 70 % fat, 20 % protein, and only 10 % carbohydrates [152], it is possible to 'switch' the human metabolism to a state of ketogenesis [153]. In a ketogenic state the body starts breaking down fats and synthesizing ketone bodies, which then replace glucose in ATP production [154]–[156]. Ketone bodies have been called the most energy-efficient fuel, yielding more ATP per mole of substrate than pyruvate (glucose metabolism intermediate product) and increasing the amount of energy released during ATP hydrolysis [157], [158]. However, this has also been disputed [158], [159], indicating that there might be no scientific consensus on the topic and that further studies are needed. Nevertheless, the use of a ketogenic state has been extensively studied in the treatment of epilepsy, digestive tract, microbiome, psychological, digestive, allergic, autoimmune, arthritis, hormonal, neurodegenerative diseases, and other disorders [154]–[158], [160]–[174]. In addition, significant improvement in mental and physical performance and reduction of fatigue has been reported with a ketogenic diet [157], [166], [167]. Furthermore, the volume of food consumed in a ketogenic metabolism is significantly smaller, as fats are 2.25 times more energy-dense (9 kcal/g) than carbohydrates and proteins (4 kcal/g) [166]. Assuming that a ketogenic diet consists of 70 % fat, 20 % protein, and 10 % carbohydrates, and an AMDR diet consists of 20 % fat, 15 % protein, and 65 % carbohydrates, 100 g of a ketogenic meal has 750 kcal, while a standard AMDR meal has 500 kcal. This factor could also be exploited more widely in preparing food for missions where the whole food is sent along with the crew, thus reducing the launch cost of food by about 33 % while maintaining the same energy content.

A diet rich in fats also promotes the absorption of several mineral elements [167] and vitamins [168] into the bloodstream. For example, fats enhance the absorption of calcium [167] which is extremely important due to the loss of bone density in microgravity conditions [169]–[171]. Fats also dissolve and improve the absorption of vitamins A [172], D [173], E [174] and K [175]. These properties could be utilized to produce various pharmaceuticals and nutritional supplements.

Of course, the high-fat ketogenic diet is also widely criticized. It has been reported that the ketogenic diet reduces whole-body efficiency, cognitive performance [175], [176], time to exhaustion [177], and impairs cardiac high-energy phosphate metabolism [176]. In NASA's book 'Human adaptation to space flight: The role of nutrition' Smith *et al.* writes that 'A ketotic state would likely impair performance of crewmembers, as seen in studies conducted by the military ...' [178]. However, this assumption is based on a publication by Phillips [179], which analyses a soldier's performance in starvation conditions. Although the human body is in a ketogenic metabolic state in both starvation conditions and while on a high-fat diet, creating a correlation in this way between starvation and a high-fat diet is not a proper comparison.

Despite the criticism, when gathering information from the available literature, which has evaluated the effect of low carbohydrate/high fat and low carbohydrate ketogenic diets on the human athletic performance, it was concluded that these diets do not cause significant improvement or impairment on endurance and strength performance [180]. However, trends suggest that these diets improve body composition by increasing lean body mass, which can be beneficial not only for athletes where strength-to-weight ratio is important [180], but it could also be beneficial for astronauts and colonists. Additionally, whole native populations of Inuit people have shown no impairment while being on a carbohydrate-free diet [181]. The same assumption has also been made for the majority of hunter-gatherer communities, for which carbohydrate consumption was opportunistic and humanity in general, before the rise of agriculture, mainly depended on fat and protein as primary sources of nutrition [181], which makes the ketogenic state the default metabolic state for most of humanity throughout the history. Phinney argues that optimizing potassium and sodium intake and constraining protein intake makes it possible to have unimpaired performance while on a ketogenic diet [181].

Overall, this conflicting information indicates that additional research is needed to fully understand the benefits and drawbacks of the ketogenic diet. It also indicates that there are likely to be conditions and situations in which the ketogenic diet is beneficial and conditions and situations in which it is not. Still, it does not deny that the ketogenic diet has great potential for use in space food, which should be further evaluated for its implementation possibilities in astronaut food. Although available information on relevant study is limited, the ketogenic diet is already being tested as part of NASA's Extreme Environment Mission Operations (NEEMO) project [182], [183].

In general, the ketogenic diet provides an opportunity to replace carbohydrates with fats, which can be obtained in large quantities from lipid-producing microorganisms, such as Labyrinthulomycetes. The use of lipids extracted from Labyrinthulomycetes microorganisms has been extensively researched for its application in food and has several nutritional advantages [184]–[195]. Lipids derived from Labyrinthulomycetes are rich in high-quality polyunsaturated omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [196]–[198]. These fatty acids can be used not only as an energy source in ketogenic metabolism, but EPA and DHA also play a crucial role in brain function, nervous system, immune system, cardiovascular, and other organ system functions [199]–[205]. Labyrinthulomycetes SCO are used as an alternative to fish oils in baby formula, as DHA is essential for the development of infant brain and nervous system [184], [186]–[188], [199], [200]. The lipid content of Labyrinthulomycetes microorganisms is typically 60-80 % of the total cell biomass [196]–[198], thus mixing such and similar oils with spirulina biomass can provide appropriate macromolecular ratios for adequate nutrition in a ketogenic diet.

It is known that both Spirulina and *Chlorella vulgaris* are capable of synthesizing DHA, EPA, and other polyunsaturated fatty acids [51]. However, the concentration of EPA in Spirulina and *Chlorella vulgaris* is approximately 4 % of the total fat content [51], [206]–[208], and the concentration of DHA in Spirulina is approximately 3 % and in *Chlorella vulgaris* approximately 10 % of the total fat content [51], [207]–[209]. This is significantly lower compared to microorganisms of the class Labyrinthulomycetes, whose cells contain approximately 45–65 % of DHA and 5–15 % of EPA of the total fats [210], [211]. Additionally, the total fat composition in biomass of Spirulina and *Chlorella vulgaris* is 2–11 % and 6–18 %, respectively [51], [212], whereas it is 50–84 % in Labyrinthulomycetes microorganisms [197], [211], [213]–[216]. Therefore, although it is theoretically possible to obtain essential high-quality Omega-3 fatty acids from Spirulina and *Chlorella*, their

concentration and total fat content are too low to compensate for the high proportion of protein in Spirulina and *Chlorella* biomass.

Other research groups have repeatedly emphasized the high potential of Spirulina for use in the diet of astronauts and colonists [51], [56], [72]. However, Spirulina's very high protein concentration, its undesirable texture, smell, and taste limit its wider use. Currently, it is not recommended to consume more than 10 g per day [51], [72], although reports are available of cases where Spirulina is used in the diet up to 10–15 % of the total food intake [51], [217]. Mixing Spirulina with SCO oil or with the biomass of the entire SCO-producing microorganism would allow for balancing and reducing the overall proportion of protein and significantly increasing the proportion of fat. For example, Spirulina biomass with a macromolecular composition of 63 % protein, 22 % carbohydrates, and 2 % fat [212] could be mixed with *Schizochytrium* sp. biomass with a macromolecular composition of 12 % protein, 10% carbohydrates, and 65 % fat [218], in a proportion of 1 part Spirulina biomass to 9 parts *Schizochytrium* sp. biomass, thus providing a final macromolecular proportion of 20 % protein, 13% carbohydrates, and 67 % fat, which is close to the ideal proportion for ketogenic diet. Of course, such a mixture would need to be evaluated for odor, texture, taste, and other properties, as well as the proportions of other additives, concentrations of microelements, vitamins, and other essential nutrients in the diet. It is important to note that the feasibility and safety of such an approach would require careful consideration and further research. However, this example clearly demonstrates that a complete diet can be provided by significantly reducing the proportion of carbohydrates and by using only microorganisms as the main food producers.

In addition, the taste and aroma of the biomass and oils synthesized by microorganisms of the class Labyrinthulomycetes resemble fresh saltwater fish or fish roe (Spalvins *et al.*, unpublished organoleptic assessment), which allows to create variations in the overall flavour characteristics of the food. By extracting the oil and then refining it by using degumming, neutralization, bleaching, filtration, deodorization, winterization, and drying techniques [219], it is possible to customize the taste, aroma, consistency, preparation, and other properties of the oil according to the desired application.

On the other hand, it has been argued that Spirulina biomass is not suitable as a stable food due to limited recommended consumable amount of only 10 g per day, unappealing taste, inappropriate carbohydrate-to-protein ratio, high vitamin A levels and lack of essential fatty acids and vitamin C [72].

In this regard, *Chlorella* has even more limitations for its use in food, as its cellulose cell wall significantly hinders its digestibility [72] and additional pre-processing is required to fully obtain nutrients from *Chlorella* biomass [51]. *Chlorella* also has undesirable taste and texture properties [72], which further limits its broader use in food supply. On the other hand, the biomass of microorganisms belonging to the class Labyrinthulomycetes is highly digestible [220], and their cells are considerably larger, which facilitates cell lysis and oil extraction, especially when it is not desirable to consume the entire biomass of the microorganism, but only the oil itself.

Despite widely available information on the use of single-cell oil and especially Labyrinthulomycetes oils in both human and animal nutrition, it should be emphasized that in general the use of single-cell oil in the diet of astronauts and colonists is poorly studied. There is only one publication available on this topic by [221]. This work studies the possibility of using plant residues as the main raw material for cultivating oil-producing microorganisms. As we discussed before, plant biomass, at least in the early stages of colony development, is likely to be a very limited resource, and the main producer in colony's BLSS would be cyanobacteria. Furthermore, this study explored the potential of *Cryptococcus*

curvatus as an oil-producing microorganism known to contain high levels of saturated fatty acids such as oleic acid, palmitic acid, and stearic acid [222]. The oil produced by *Cryptococcus curvatus* is unsuitable for 3D printing material production due to a lack of unsaturation sites in its triglycerides. Additionally, the oil lacks essential polyunsaturated fatty acids and contains high concentrations of saturated fatty acids, making it unsuitable for human consumption.

Since there is a large amount of CO_2 available in the atmosphere of Mars, it would be technologically simple to perform supercritical fluid extraction, which allows for efficient extraction of nonpolar compounds such as fats from various types of biomass [219], [223], [224]. Moreover, supercritical CO_2 extraction also enables fractionation of extracts [219]. By applying specific pressures in separator vessels, it is possible to separate the extract into fractions, thus obtaining highly purified oils composed of specific fatty acids with a very high degree of purity [219]. Several studies have reported successful fractionation and acquisition of high-purity DHA oil using supercritical CO_2 extraction [219], [225]–[228]. Fractionation of oils not only allows for concentration of specific fatty acids, but also provides broader opportunities for combining extraction processes with fractionation to obtain feedstocks for more specific purposes. If a specific proportion of fatty acid mixture is needed for nutrition, fuel, 3D printing feedstock, or medical purposes, fractionation of supercritical CO_2 extraction allows us to obtain high-purity oils or specific fatty acid mixtures for such purposes.

In general, wider utilization of SCO in food could help mitigate the shortcomings currently identified in popular autotrophic cultures. Therefore, conducting further research where SCO-producing microorganisms are integrated into existing bioregenerative life support systems (BLSS) such as Micro Ecological Life Support System Alternative (MELiSSA) is desirable.

3.3. Application of SCO in Fuel Production

Fuel production from locally available resources, especially rocket fuel production, is a vital resource not only for colonies but for any space exploration mission. ISRU fuel production allows for a significant reduction in initial launch costs and promotes colony sustainability. Other studies [51], [56], [72], [229]–[231] have extensively described how methane-oxygen and nitrous oxide-hydrocarbon could be obtained using methanogenic and cyanobacterial cultures, which could then be used as rocket fuels. However, competitive rocket fuels can also be produced by using SCO as the main feedstock. SCO can be used as a feedstock to produce synthetic paraffinic kerosene through hydrotreating [232], [233] and synthetic Fischer-Tropsch fuel, hydrogen, kerosene, and other fuels by using gasification followed by Fischer-Tropsch synthesis and consequent upgrading steps [232].

SCO can be used as a feedstock to obtain other types of fuels as well, such as biodiesel through the transesterification process [234], green diesel through the hydrocracking process [235], [236], hydrogenated oils through the hydrogenation process [237], syngas through gasification [238], and more. The concept of using SCO to meet a variety of fuel needs is a crucial aspect, and fundamentally, SCO-derived fuels do not exclude the simultaneous production of other fuels using different feedstocks and technologies, including those that require methanogenic and cyanobacterial cultures. The ability to use SCO as a feedstock for fuel production strengthens the overall viability and resilience of the colony, as it allows for the production of different types of fuels for various purposes, ensuring a robustness in fuel production, as fuels can be obtained from multiple sources. Furthermore, since SCO-producing microorganisms can contain up to 60–80 % lipids, such high purity of the feedstock not only simplifies the overall extraction process, but also reduces the need for other feedstocks. In addition, fractionation through supercritical solvent extraction provides even

more opportunities to tailor the SCO extraction process for specific fuel production processes and end-use purposes.

3.4. Application of SCO in 3D Printing Material Production

3D printing technologies have immense potential for use in space missions, in space stations, spacecraft, and surface colonies. 3D printing allows for the production of a wide range of products, including spare parts for repairs, parts for equipment maintenance, production of consumable materials, packaging, production of hard-to-transport materials, habitat components, medical supplies, textiles, and many other everyday necessities [51], [239]. If the raw materials for 3D printing can be produced on site, for example, using resources available in Mars colonies, then 3D printing also offers the opportunity to significantly reduce launch mass and cost, as a significant portion of products can be manufactured on site. In addition, the introduction of reliable 3D printing technologies that utilize 3D printing materials obtained from local resources (regolith, CO_2, N_2, H_2O, O_2, H_2, etc.) increases colony's self-sufficiency and resilience.

Both recyclable and locally synthesized feedstocks are considered as raw materials for 3D printing. An interesting approach is the production of microalgae-based bioplastics, as this allows for obtaining bioplastics from various pathways [51], [80], [240]. For example, polyhydroxybutyrate (PHB) has been extensively studied for its application in 3D printing [51], [80], [240], [241], it can be synthesized by microalgae [51], [80] and its use has been demonstrated in 3D printing for space exploration needs [51], [239]. The use of PHB and other bioplastics as feedstock for 3D printing is a very promising industry, and further development of this field will promote colony self-sufficiency. However, SCO also has a potential role in the production of bioplastics from resources available on Mars.

Epoxidized vegetable oils are entering the market as an alternative to conventional fossil-based polymers, used for production of bio-based epoxides and bio-based polymers such as polyurethanes [242]–[244]. However, some of these materials have been reported to have unsatisfactory material properties [244]–[247]. La Scala and Wool [247] proposed that triglycerides with a higher number of unsaturation sites could be used to improve the mechanical and thermal properties of bio-based polymers. Building upon this model, Negrell et al, [248] suggested that algal triglycerides could be a promising alternative to vegetable oil-based polymers. But it's not just algal oils that contain high concentrations of long polyunsaturated fatty acids. As discussed before, Labyrinthulomycetes class microorganisms have very high concentration of such polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and eicosatetraenoic acid (ETA) [197], [210], [249]. Therefore, single-cell oils are a promising alternative for the production of bio-based epoxy resins and polymers [244], which in turn can be used as a raw material in the production of 3D printing materials.

Several research groups have explored the use of single cell oils as an alternative raw material for bio-based polymer production [244]. For instance, Negrell *et al.* [248] utilized DHA-rich oil from *Schizochytrium* sp. to synthesize polymer materials and foams with various properties [248]. Roesle *et al.* [250] obtained a polymer with high melting and crystallization temperature by using EPA-rich oil from the microalga *Phaeodactylum tricornutum* [250]. Petrovic *et al.* [251] obtained polyol and polyurethane foams from microalgae oil [251]. Pawar *et al.* [252] used *Chlorella* sp. microalga oil to produce rigid polyurethane foam [252]. Later, Pawar *et al.* also reported on the synthesis of an epoxidized material using cottonseed and *Chlorella* sp. oil, which exhibited competitive thermal stability properties [252]. Arbenz *et al.* used *Schizochytrium* sp. oil to obtain epoxide and polyol foams [253], and Hidalgo *et al.* [254] used microalga *Nannochloropsis gaditana* oil to obtain

epoxidized microalgae lipids, although toxic fossil-resource-derived bisphenol A was used to improve the mechanical properties of the material [244], [254].

In the context of space exploration, both recycled plastics and PHB are mainly discussed in the context of its application in fused filament fabrication (FDM) 3D printing technology, which is suitable for printing large and relatively simple parts. On the other hand, various resins can be used for stereolithography (SLA) 3D printing technology, which primarily utilizes ultraviolet lasers to cure photosensitive resin. SLA is better suited for printing complex parts, molds, and tools that require high precision [255]. Biobased epoxy resins and polyurethane can be produced from SCO [244]. There are already a range of polyurethane 3D printing resins available in the market for SLA printing [256], and polyurethanes can also be used for FDM printing [257], which makes SCO-derived polymers great candidates for use as feedstock in various types of 3D printing technologies.

Wide variety of biomaterials can be synthesized from vegetable oils, that can be used for 3D printing [258], [259]. Use of polyurethane synthesized from epoxidized soybean oil has been mixed with acrylates and used in stereolithographic 3D printing [260]. However, in the scientific literature, the potential of utilizing SCO-derived polymers for 3D printing has not been explored, neither in the context of conventional applications on Earth nor in space exploration. Therefore, this approach can open up new directions for further research in the future, both in terms of technology development here on Earth and technologies that will serve space exploration.

4. CONCLUSION

In general, single cell oil has great potential for applications in BLSS. We propose to use the outputs from autotrophic systems, such as biomass of cyanobacteria and oxygen, to cultivate SCO-producing microorganisms, mainly from the class Labyrinthulomycetes, whose produced single cell oil could be further used for food, fuel, 3D printing materials, and production of various pharmaceuticals. To the best of the authors' knowledge, this is the first time such a concept has been proposed, that focuses on a cyanobacteria-SCO-producing microorganism hybrid system.

It also appears that this is the first proposal in the scientific literature, to use SCO-based nutrition centred on the utilization of ketogenic diet. Of course, factors such as the nutritional composition, stability, and safety of SCO as a food ingredient, as well as its potential effects on astronaut health and performance, would need to be thoroughly investigated before it could be implemented in actual space missions. However, this approach can serve as a basis for a paradigm shift, reducing the importance of carbohydrates in space foods. This, in turn, could unlock full range of potential benefits that a high-fat diet, ketogenic state and SCO in general may offer, such as: (1) significant reduction in food weight, (2) simpler, more compact and lightweight bioreactors, (3) launch cost reduction, (4) ketone bodies as potentially more efficient metabolic fuel, (5) improved mental and cognitive performance, (6) reduced fatigue, (7) serve as treatment for various physiological and psychological disorders, (8) source of essential fatty acids, (9) fats as medium for improved nutrient absorption.

To the best of the authors' knowledge, this is also the first publication where the possibility of using single-cell oils as the feedstock for the production of 3D printable filaments and resins has been reviewed. With the emergence of various vegetable oil-derived polymers on the market and the growing commercial utilization of polyurethane filaments and resins for 3D printing, it becomes evident that materials derived from single-cell oil and other bio-based lipids are poised for development and application in 3D printing, both for consumer markets on Earth and for use in space exploration.

In this review article, we also briefly discussed the possibility of using SCO for production of rocket fuel and other fuels that could find application in operation of equipment, systems, and vehicles essential for sustaining a colony. The use of SCO for fuel production certainly requires further investigation to assess its efficiency. It is likely that SCO-derived fuels may not be the most efficient, considering the inefficiencies arising from cyanobacterium to SCO conversion and the production process of SCO to fuel itself. However, the possibility of utilizing SCO for this purpose provides an opportunity to potentially provide a supplementary fuel source for the colony when needed, enhancing system resilience and simplifying the production chain.

The overall concept is highly hypothetical and relies on a number of assumptions that would need to be tested. However, the theoretical foundation is solid, and incorporating the diversification of products (such as food, fuel, 3D printing materials, and pharmaceuticals) within a production chain that utilizes a single feedstock, namely single cell oil, can be a crucial component for establishing a resilient, independent, self-sufficient, and robust biological life support system. In the future, this approach could potentially become one of the essential elements required for the establishment of a successful Mars colony.

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