

ALGAL RESEARCH IN SPACE: HISTORY, CURRENT STATUS, AND FUTURE PROSPECTS

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ABSTRACT

The aim of this article is to give a brief review of the history and current status of research experiments with cyanobacteria, algae, and lichens concerning their adaptation in space or their potential use for the needs of astronauts. The future of algological space research is also being discussed with regard to creating self-sustainable stations and terraformation of Mars.

Keywords: Cyanobacteria, algae, space

INTRODUCTION

The idea to grow algae for human consumption is currently more than 70 years old after it was first suggested, in 1942, by Harder and von Witsch [1,2] that organisms such as diatoms could be source of lipids which could be used for food, with a detailed analysis published subsequently by Spoehr in 1951 [3]. The concept of growing algae in space for the needs of cosmonauts precedes even the launch of Earth's first cosmonaut. Lockhart [4] stated 2 years before the flight of Yuri Gagarin that there is little doubt that unicellular algae will be part of the diet of cosmonauts because they consume a minimum of space due to their ability to grow in closed systems. This author also advanced the idea stating the fact that you can eat the whole algal plant in contrast to higher plants where you have to discard a large amount of inedible roots and stems. In the early space age, it has also been proposed that algae could be used to regenerate oxygen from exhaled carbon dioxide in manned spacecraft [5]. The prominent visionary Carl Sagan even suggested the bold idea that algae could be used to terraform the other planets of the solar system and make them suitable for Earth life [6].

However, although decades have passed since then, nowadays the algae are still being considered "a novel example" with "tremendous potential for applications in space" [7], without actually being used as food or as a source of oxygen aboard the International Space Station. Alas, the prospect of terraforming the planets remains as distant as it was during the early space age. What is the current state of the algal research in space? What has been done and what still has to be done? We seek answers to these questions in this manuscript.

A BRIEF HISTORY OF STUDYING CYANOBACTERIAL AND ALGAL SURVIVABILITY IN SPACE

Algae flew in space as soon as the space race began and before the flight of Gagarin. On 19 August 1960, a Vostok rocket launched the spacecraft "Korabl-Sputnik 2." The most famous passengers aboard the spacecraft were the two dogs Belka and Strelka that later successfully returned to Earth. However, the dogs were accompanied by 40 white mice, two rats, numerous flies, seeds, fungi, and cultures of the alga *Chlorella pyrenoidosa* [8]. The mission duration was very short, and the spacecraft returned home after just a day, so it offered only a short-term assessment of how organisms react in space conditions. The results with *Chlorella* from this particular flight are well documented. It was estimated that launch, landing, and short-duration stay (25 hrs) in spaceflight conditions do not impact the main physiological processes such as growth, development, and reproduction. The only deviations were a higher number of dead cells in spaceflight cultures compared to control cultures and the photosynthetic activity in the spaceflight culture was lower than expected [9].

By the mid-60s, it became clear that short duration spaceflight is survivable by Earth organisms (including algae as cited above). However, what about long duration missions? On 22 February 1966, a Voskhod rocket launched the Soviet spacecraft Cosmos 110 with the dogs Veterok and Ugolyok and numerous other organisms, including *Chlorella* cultures (LARG-1, LARG-3) strains. The spacecraft stayed in orbit for 22 days before it safely landed, duration far longer than any of the previous missions. The occurrence frequency of visible mutations has been surveyed, and no significant differences have been found between test cultures and the control samples on Earth. Statistically significant reduced survival of *Chlorella* cells has been noted only for the LARG-3 strain [10], but otherwise, it was estimated that algae possess the ability to survive even after 22 days in space.

Not only algae flew in space before Gagarin, algal cells also went to the moon before first humans traveled there. On 15th September 1968, 10th November 1968 and 7th August 1969 Zond 5, Zond 6, and Zond 7 spacecraft were launched, and they flew the whole distance from the Earth to the Moon and back to the Earth. Each of those spacecraft had onboard *Chlorella* cultures. Results have been contradictory; cell survivability was lower on Zond 5 and Zond 6, whereas space had no effect on the cell viability in the experiment onboard Zond 7 [11].

Chlorella cells have been used in experiments aboard the Soviet space stations. Between 1977 and 1982, the Soviet Union operated the station Salyut 6. Aboard this station it has been proven that weightlessness does not affect a growing algal population, nor it affects the relationship between the alga and its environment. Results received from Soviet space stations show that weightlessness is not an obstacle to the normal growth and development of plants in general [12].

When we talk about studying plants in space, we must clarify which spaceflight conditions are being tested in experiments. In the cases cited above, we are talking about algae which have flown on the inside of spacecraft, i.e., pressurized containers, with strictly controlled conditions. Thus, only two factors could be studied - weightlessness and increased radiation. Of these usually, the focus is on weightlessness. Studying radiation is more challenging because radiation conditions on the inside of spacecraft depend on orbital parameters and structure of the spacecraft. Experiments about survivability of algae on the inside of spacecraft are useful, but they say nothing about how algae react in harsh conditions of space with all other factors - vacuum, direct solar illumination, and extreme temperatures. This is being reviewed in the following paragraphs.

A Russian FOTON-M spacecraft launched successfully from the Baykonur cosmodrome on May 31, 2005, via a Soyuz rocket. Aboard

the spacecraft flew lichens *Rhizocarpon geographicum* and *Xanthoria elegans*, symbiotic organisms composed of algal and fungal cells. Lichens stayed space for 16 days before returning safely to Earth after full exposure to space conditions including vacuum, UV illumination, extreme temperatures, and weightlessness. It was confirmed that lichens were able to recover within 24 hrs to their full metabolic activity [13]. Much longer experiments with lichens have also been conducted aboard the International Space Station for a total of 1.5 years. It was confirmed that lichens *X. elegans* and *Aspicilia fruticulosa* can maintain their photosynthetic and physiological activities even after a year and a half exposure, but *R. geographicum* was described as "slightly damaged" [14]. Aboard the external EXPOSE facility of the International Space Station was also conducted experiments with different algae as separate organisms, not part of lichen symbiosis, and it was estimated that cells of *Chlorella* and *Rosenvingiella* and cyanobacterium *Gloeocapsa* sp. survived after a year and a half exposure to vacuum in dark conditions. In experiments aboard, the same EXPOSE facility which included exposure to both space vacuum and extraterrestrial UV spectrum, *Chroococcidiopsis* cells survived, but cells of *Nostoc commune* did not [15].

GROWING ALGAE IN SPACE AS PART OF REGENERATIVE LIFE SUPPORT SYSTEMS

To keep the astronauts alive in space, life support systems must be utilized. So far, all space stations (those in the past plus the currently present International Space Station) have been constructed in low Earth orbit, and they have relied on continuous resupply of food, water, and oxygen delivered by spacecraft. Open loop life support systems have been successful on short duration missions. However, when space missions get longer, the supply load gets heavier and resupply becomes prohibitive [16]. This will be the case when we need to construct bases on the Moon and send people to Mars. We will need closed loop technologies which will provide regenerative functions. So far, there are some regenerative devices already developed, like the Water Recovery System aboard the International Space Station, which is able to convert urine back into drinking water. However, this is still not enough. In order for self-sufficient stations to become reality, reliable regenerative life support systems must be created first.

Regenerative life support systems can be divided to physico-chemical and biological. So far, all life support systems used for the needs of the present or past space stations have been physico-chemical. According to Belz *et al.* [17], biological regenerative life support will not be available in the next 50 years, as there is much fundamental research to be done. This is quite a long time, but the authors state that there is a logical intermediate step between physico-chemical and bioregenerative life support system, and this is the hybrid life support system which uses integration of physico-chemical and simple biological system components. Such biological components, according to the authors, could be algae which are grown in photobioreactors.

Despite the hardships, biological life support systems have been studied by several space agencies during the last decades including NASA, JAXA, and ESA. In Europe, the effort is mainly performed within the MELiSSA, an international project led by ESA. The aim is to create a closed loop via the use of five main compartments (a compartment for waste liquefaction, compartment for carbon transformation, compartment for nitrification, compartment for food, water, and oxygen production, and crew compartment). An algal photobioreactor is being used as part of the compartment for food, water, and oxygen production, inhabited by a culture of cyanobacteria *Arthrospira platensis* [18]. All of the mentioned compartments include living organisms and the project MELiSSA as a whole is based on the principle of an "aquatic" lake ecosystem where waste products are processed using the metabolism of plants and algae which in return provide food, air revitalization, and water purification [19]. Christophe Lasseur, project manager of MELiSSA, has stated publicly: "We are looking into the future. Let us give MELiSSA another 25 years" [20].

Even though as cited above, researches give decades before the biological life support systems to mature, work in this field continues. In fact, some interesting experiments with algae have been conducted in the past few years, and they have been described below.

An assessment study has been conducted about the integration of an algal photobioreactor into an environmental control and life support system of a space station. The study is focused on the performance of the photobioreactor as a food production system in addition to air revitalization. Researchers conclude that it is unfavorable to convert all the metabolically produced carbon dioxide within the photobioreactor, but it does contribute to the closure of the carbon cycle. They claim that resupply mass savings of 16% compared to physico-chemical life support systems are achievable. The photobioreactor would add to the oxygen and food supply for the astronauts. The authors also conclude that the cultivation of higher plants is advantageous when we talk about further closure of the carbon cycle, reducing resupply requirements, and providing more variety to the nourishment [21].

One of the most challenging hurdles to the development of bioregenerative life support systems is that it is very hard to maintain stable concentrations of O_2 and CO_2 . Indeed, sometimes, the balance between O_2 and CO_2 could be greatly disrupted, could algae be used to regulate the balance during scheduled maintenance, or be implemented as a compensatory system during emergency conditions? According to Li *et al.* [22], the alga *Chlorella vulgaris* is a promising organism in such cases. Sometimes, cultivation of higher plants could fail and subsequently lead to an imbalance of O_2 and CO_2 . Stabilizing the habitat just by recultivating higher plants would be slow, and the use of algae could be advantageous. The authors conclude that using *C. vulgaris*, the original productivity could recover within 5 days, and this would ensure stability until the higher plant is regrown.

PROSPECTS OF USING CYANOBACTERIA AND ALGAE FOR TERRAFORMING MARS

In contrast to the first chapter, in which the focus was more on survivability of algae during spaceflight and less on growing them, in this chapter, we talk about terraforming of other planets and moons, or changing their conditions so they could be suitable for life. This implies something different - algae should not just survive but should be photosynthetically active, able to grow, reproduce, and accumulate biomass. Although the word "terraforming" might imply changing other planets (or moons) to be like Earth in a strict sense, making them suitable for human life, it is not necessary to be so. It could simply make just a fraction of the foreign world inhabitable, not the whole world, or by "terraforming" we could mean changing the planetary (moon) surface to the point we are able to produce food, although it may still not be ideal for humans to live there [23].

It has been widely accepted among the society that the most probable world for colonization and settlement in our foreseeable future is Mars. Unfortunately, Mars has a strong oxidative atmosphere, low temperatures, extremely dry conditions and high UV radiation, which makes its surface a harsh place for life [24]. Of these, the UV radiation is the most deleterious factor because UV rays are able to directly damage the DNA structure - a result which has been confirmed after tests with viruses, bacterial, or fungal spores [25]. UV radiation conditions are a bit different on the surface on Mars, compared to open space, because CO_2 , which comprises 95% of the atmosphere, is able to absorb short-wavelength UV radiation and no radiation at wavelength below 200 nm reaches the surface of Mars. The main problem with the Martian atmosphere, however, is the extremely low concentration of O_3 and most of the time UV radiation above 200 nm penetrates the atmosphere and reaches the surface [26]. This may have rather unfortunate consequences in the efforts to colonize Mars and its terraforming with the use of algae. Yes, it is true that cyanobacteria possess various UV-absorbing or screening compounds [27], and while they allow for them to thrive in extreme conditions on Earth, UV conditions could be a

problem for algae to live on unprotected planetary surface. Mars is more extreme than the most extreme places on Earth such as deserts, arctic, and antarctic conditions which are typically inhabited by algae. That is why, it has been suggested that since you cannot just inoculate cultures from Earth and expect them to thrive on Mars, initial engineering (ecopoiesis) would be needed to change the planet just enough to allow the organisms to live there [28].

It is possible to create simulation chambers on Earth where you can mimic Martian conditions to study how organisms adapt. This could affirm which algal organisms are suitable for terraforming the planet. Mars in the process of ecopoiesis would probably resemble the conditions of early Earth, so most probably primitive cyanobacteria which are considered similar to the earliest forms of cyanobacteria that appeared on the surface on our planet will be prime candidates for terraformation. An example of such organisms are the rock-inhabiting cyanobacteria from the genus *Chroococcidiopsis*, which grow in the most extreme dry habitats on our planet, such as McMurdo Dry Valleys in Antarctica and the Atacama Desert in Chile. *Chroococcidiopsis* cyanobacteria have been suggested for growing on Mars due to their ability to grow in the deserts, inside porous rocks and on the lower surface of translucent stones, as well as due to its resistance to desiccation and high salinity [29]. Experiments have been conducted under simulated Mars conditions which confirm that cells retain their membrane integrity, photosynthetic pigments, autofluorescence, and dehydrogenase activity, but it has also been reported that organisms are unable to form colonies [30]. Still, this is a promising result because an alga will not be able to grow on Mars if it is not photosynthetically active.

Another experiment has been conducted with the extremophile lichen *Pleopsidium chlorophanum*, and it was estimated that the organism adapts differently if it is on an unprotected surface, exposed to harsh conditions, or in protected niches such as cracks and fissures in rocks. Under unprotected surface, researchers were not able to confirm successfully the photosynthesis of the algal symbiont, but the organism remained photosynthetically active in protected site conditions. De Vera *et al.* concluded that terrestrial life most likely can adapt physiologically to live on Mars [31]. Here, it must be pointed out that De Vera *et al.* conclusions were not used to justify terraforming the planet, but rather they stated the exact opposite - stringent measures should be taken to prevent human activities from infecting Mars with terrestrial organisms.

It should be stressed that there are two major public opinions concerning colonization of Mars with regards to the possible existence of life there. The opinions are often expressed in a stark and polarized way in the public space. The first one is that if life on Mars does exist, we should do everything to protect it from Earth organisms which could displace it. This would mean strict measures and sterilization of anything that goes to Mars, even refraining from sending humans to the surface of the planet and keep exploring it only robotically. The second opinion is that spreading humanity to other planets is a must for the long-term survival of the human species, even if this could bring some dangers to the possible local life.

Terraforming Mars is the exact opposite idea to preservation - the idea is plant life on Mars, so the planet would become Earth-like. This is the position I support as an author of this review.

In order for algae and lichens to grow on Mars, specific chemical compounds must be present. Of course, the most important one for photosynthesis is the carbon dioxide, which is abundant in the Martian atmosphere. However, it is not enough. Various macro- and micro-elements must also be ensured. Recent research, conducted on the Martian surface, confirms that many important chemicals are already present there. It is already estimated that the soil of Mars contains Fe-, Ca-, and Mg-sulfates [32]. Of these, the presence of Mg-sulfates is especially important because Mg has many vital roles in the plant

organism, including the one of being a critical part of the chlorophyll molecule. Mars Rover Curiosity, which is working on the Martian surface since 2012, recently confirmed the presence of "fixed" nitrogen in the form of nitrates, as opposed to atmospheric nitrogen which is inaccessible for most living organisms [33]. These findings could mean what if we are to grow algae on the surface on Mars; we will not need to bring certain chemicals with Earth spacecraft. However, the soil of Mars also contains chemicals that are not very common on Earth - perchlorates. Two different landers (Phoenix and Curiosity) on two different locations (Vastitas borealis and Gale crater, respectively) have found evidence for their presence [34]. The possible culprit for the formation of perchlorates may be UV photooxidation of chlorides [35]. Unfortunately, it is hard to access how the presence of perchlorates in the Martian soils could affect the prospects of growing algae for food on Mars or for terraforming the planet. Uptake of perchlorate and perchlorate reduction in plant tissues have been studied in land plants [36,37], however, detailed studies how perchlorates affect growth and development of algae have yet to be conducted. This is a task a biologist could be able to conduct in the future.

CONCLUSIONS

It is a common sentiment among people that the pace of the scientific development is very slow. This is especially true about space exploration. Although it has been suggested early in the space age that algae could be grown for the needs of astronauts, we can say that years, even decades will yet have to pass before this turns into reality. Still, important research has been conducted. We know that microgravity does not significantly affect the growth of algae. It has been proven that some strains are able to survive even after a long exposure to harsh conditions including vacuum and extreme temperatures. Work is being conducted to develop bioregenerative support systems, even though basic research still needs to be done. Terraforming Mars with the use of algae is still an idea worth to be considered.

Robotic space probes have made important discoveries about the conditions of the planets in our solar system. We have already determined important facts about the composition of the surface of Mars. This gives us some directions of fundamental research that could be conducted during the next years. How, for example, would commonly meet chemicals in the soil of Mars affect growth of algae? This is an important question that awaits its answer.

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