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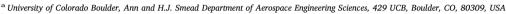
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# A review of algal research in space

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Spaceflight experiment Abbreviations: BLSS Bioregenerative Life Support System **ECLSS** Environmental Control and Life Support System FSW Fanhui Shi Weixing ISS International Space Station LEO Low Earth Orbit

NR Not Reported OD Optical Density PCR Polymerase Chain Reaction RNA

Space Transportation System

#### ABSTRACT

With the continued expansion of human presence into space, typical mission durations will routinely exceed six months and extend to distances beyond the Moon. As such, sending periodic resupply vehicles, as currently provided to the International Space Station, will likely no longer be feasible. Instead, self-sustaining life support systems that recycle human waste products will become increasingly necessary, especially for planetary bases. The idea of bioregenerative life support systems using algal photobioreactors has been discussed since the beginning of the space age. In order to evaluate how such a system could be implemented, a variety of space flight studies aimed at characterizing the potential for using algae in air revitalization, water recycling, food production, and radiation shielding applications have been conducted over the years. Also, given the recent, growing interest in algal research for regenerative fuel production, food supplements, and cosmetics, many algal strains are already well documented from related terrestrial experiments. This paper reviews past algal experiments flown in space from 1960 until today. Experimental methods and results from 51 investigations utilizing either green algae (Chlorophyta), cyanobacteria (Cyanophyta), or Euglenophyta are analyzed and categorized by a variety of parameters, including size, species and duration. The collected data are summarized in a matrix that allows easy comparison between the experiments and provides important information for future life support system requirement definition and design. Similarities between experiment results are emphasized. Common problems and shortcomings are summarized and analyzed in terms of potential solutions. Finally, key research gaps, which must be closed before developing a functional life support system, are identified.

#### 1. Introduction

Ribonucleic Acid

STS

To date, all human spaceflight missions have either been in close proximity to Earth, relatively short in duration, or received periodic resupply cargo delivery. The Environmental Control and Life Support Systems (ECLSS) employed in early short-duration missions relied purely on physicochemical systems using consumables [1]. For the continuous mission increments on the International Space Station (ISS), regenerable life support systems are employed that achieve oxygen recovery rates of up to 50% and water recovery rates of up to 70% [2,3]. The proximity to Earth, however, still allows resupply missions to provide the remaining consumables and spare parts. With further long

term missions to Mars and beyond on the horizon, however, this approach will likely no longer be feasible, so a greater degree of closure is needed [4]. Biological systems have been studied in this regard as they are the only feasible means of providing oxygen and water recovery of up to 100% while also allowing the production of food [5-7]. Within this biological domain, algal systems are promising candidates for human life support due to their fully edible biomass, ease of handling, and fast growth rates. Since the beginning of spaceflight, algae have been intensively studied for this purpose, even before the first human flew in space [8].

Numerous factors can influence the growth of algae in a spacecraft cabin environment, with radiation and microgravity being the most

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unique to spaceflight [9]. Outside of the cabin habitat, temperature extremes as well as the exposure to space vacuum must also be taken into account [10]. In order to design an ECLSS utilizing biological systems, it is important to understand the response and adaptation of algae to these effects. As microgravity and the specific radiation environment cannot be fully replicated on Earth, it is especially important to conduct spaceflight experiments addressing those two parameters.

Previous reviews found in the literature either do not focus specifically on algae, cover a limited time span, or are specific to certain countries and their space programs [11–15]. Therefore, to thoroughly document the quantity and quality of results already obtained, it is advantageous to list and analyse the prior spaceflight data comprehensively.

#### 2. Material and methods

The data presented in this review article were mainly obtained through published literature. Flight details were extracted from the journal article or technical report on the flown experiment, where provided. Investigations that did not publish, or where a cited publication could not be found, are included using secondary information from flight manifests, earlier review papers, or books. All orbital flights to date that reported carrying some strain of algae, including green algae, euglenophytes and/or cyanobacteria, are listed in chronological order in Table 1.

An attempt was made to identify all experiments flown in space since the first known flight in 1960. Due to the wide variation in time, language, and organizations, data acquisition was challenging. Even though this work was done as thoroughly as possible, it cannot be guaranteed that the information presented here is comprehensive.

#### 3. Results

The earliest verifiable record for algae flown in orbit was in 1960, together with a variety of other biological organisms, on board the Soviet Korabl-Sputnik 2 spacecraft – the 5th satellite ever launched into space. For this first mission, the algae cultures were grown in two groups for a total duration of 25 h: the first on agar in darkness and the second in liquid media under periodic illumination. Even though a higher number of dead cells, as well as a decreased photosynthetic rate compared to ground controls was seen in post-flight analysis, it was concluded that algae can perform the main physiological processes of photosynthesis, growth, development, and reproduction in space [16].

Almost at the same time in the United States, a variety of studies with *Chlorella* cells were performed on the Discoverer spacecraft series for up to three days. These experiments launched photosynthetically inactive cells in darkness and performed microscopic and growth dynamic analyses during post-flight cultivation. Due to temperature variations, both in ground and flight cultures, it was difficult to draw any firm conclusions, but it was confirmed that algae were capable of surviving exposure to spaceflight conditions [17,18].

Encouraged by the initial success, Russian scientists launched a variety of *Chlorella* strains in the early 1960s on board the manned Vostok 5 and 6 missions, as well as the unmanned Cosmos 109 and 110 spacecraft. The Cosmos experiments employed photosynthetic inactive cells of up to five different *Chlorella* strains on agar, whereas the methods used in the Vostok experiments were not described in sufficient detail. All four flights conducted post-flight analysis using macroand microcolony methods and reported no differences in survival or mutation frequency between flight and controls [19–21]. One notable experiment among those is the one performed on Cosmos 110. As all previous experiments had been relatively short (less than five days) and the effects of space environment are thought to be increased with longer mission durations, an experiment was flown on board the Cosmos 110 satellite for 22 days. This was almost three times longer than any prior experiment. Post-flight culturing showed a trend in

delayed growth and reduced survival of the flight cultures but were statistically significant only for one strain of *Chlorella* (LARG-3) [21].

The first experiment employing in-flight analysis on an active photosynthetic algal culture was planned by Ward et al. (1970) using *Chlorella sorokiniana* in liquid medium for 30 days. Unfortunately, the reactor developed a leak and exposed the algae to vacuum, which prevented any data collection [22].

Different from the previously described experiments conducted in Low Earth Orbit (LEO), the Zond missions flew on a trajectory around the Moon. This had a unique influence on the environment that the algae were exposed to, since it went outside the radiation-protecting Van Allen belts. The Zond missions around the Moon were hence exposed to both deep space radiation as well as to the radiation trapped in the Van Allen belts during the transit, making it difficult to distinguish between radiation and microgravity as the cause for potentially altered behaviour. On the Zond 5, 6, 7, and 8 missions, Chlorella vulgaris cells were flown on agar in darkness for six or seven days. In post-flight culturing, no statistically significant differences were seen between flight and ground cultures using macro- and microcolony methods. Trends in survival and mutability were contradictory between the different experiments, which could have been due to unstable temperatures during the transport of the samples to the launch site. A very similar experimental setup and approach was consequently used by the same research group on board Cosmos 368, Soyuz 5, and Salyut 1 for a duration of up to 72 days with similar inconclusive results and failures [23-27].

In 1970, Soyuz 9 carried the first successful attempt to grow a photosynthetically active culture in space for different times of 1, 6, and 14 days. Living samples were investigated back in the laboratory and analysed by post-flight culturing for four days. It was reported that the duration of exposure to microgravity did not influence the cells sensitivity to flight factors, as no morphological or structural changes were seen in microscopic examinations. Post-flight cultivation dynamics followed the same patterns between the different flight groups as well as with the ground controls. The only difference observed using the microcolony methods was that productivity and sporulation were slightly decreased in the flight experiments, attributable to the increased death of cells exposed to the space environment [28].

A total of five different algal experiments were conducted on the Salyut 6 space station, including different strains of *Chlorella* and *Scenedesmus obliquus*. The experiments were carried out both in liquid and on agar for durations between 4 and 18 days. All experiments employed post-flight analysis on the living algal cells and reported no changes between flight cultures and ground controls in microscopy, microcolony methods, and electron microscopy [29–32].

Algal experiments were also conducted on board the Space Shuttle from the very beginning of the program. Axenic Chlorella vulgaris cultures were grown and fixed in-flight on STS-4 and an algae-kefir ecosystem with in-flight monitoring was flown on STS-51-G, but no resulting publications from either experiment were found in our literature search [33]. In 1985, on board the German Spacelab mission (D-1), an experiment was carried out looking at the circadian rhythm of Chlamydomonas reinhardtii. This experiment was unique, as it successfully employed an in-flight measurement during active growth of the algae. Besides proving that the algae expressed a 24-h period that was not significantly different from the ground controls during the 6.5 days of the experiment, it was also noted that the absorption amplitude of the space culture was larger. The amplitudes were in fact so large that they exceeded the range of the data acquisition system and therefore could not be recorded. It was hypothesized that the microgravity environment allowed the cells to maintain a more uniform suspension due to the lack of sedimentation. This potentially caused a more even illumination that distributed the light energy better to each cell. Additionally, some of the spaceflight cultures showed an increase in cell numbers and higher survival rate than the control cultures on the ground [34].

The longest experiment using photosynthetically active algal cells in

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