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Research Note

Chlorella vulgaris as a Model Organism for Microgravity Cultivation in a CubeSat

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A B S T R A C T

Chlorella vulgaris is one of the most common and characterized algae genus with several applications, including carbon sequestration, biofuel, food production and wastewater treatment. *Chlorella* sp. are considered suitable to be used as model organisms in space research due to their cultivation flexibility. Many studies have been carried out to ensure better conditions for supporting human life on long-term missions in deep space or on planetary surfaces, minimizing the need for resupplies. Regardless of the resilience of the genus *Chlorella* to space conditions has already been demonstrated, model organisms are useful in the improvement of new technologies. This research aimed to develop the culture conditions and a monitoring system for *C. vulgaris*, under microgravity, using an image capture device for CubeSats. The image acquisition system consisted of a digital microscope, with remote access, a Single Board Computer, a monitoring computer, and an image processing algorithm. Three microalgae colonies, under laboratory conditions, were evaluated in real time (every 30 minutes) using the size of the colonies as a parameter for evaluating growth rates. The highest microalgae biomass production for the three monitored colonies (C1-C3) was: increase of 28% for C1 after 90 h; 21% for C2 after 84 h; and 36% for C3 after 120 h. The results indicated that the system was able to monitor the growth of microalgae colonies. A specific support is being developed, which allows the installation of this image acquisition system for algae cultivation in a CubeSat, for future studies of algae growth in real microgravity conditions.

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INTRODUCTION

A CubeSat is a nanosatellite primarily developed as an educational demonstration device. However, CubeSat was demonstrated to be a technology tool for real low-cost missions with high value regarding science and commercial return. CubeSats decrease the complexity of development and launch once compared to traditional satellites [1].

CubeSat payloads is also a cost-effective tool for microbiology experiments in space. Since 2006, NASA has been launched several different CubeSat adapted for biological studies proposal [2]. These devices enable microorganism exposure to the space environment, allowing the observation of its behavior and survival under solar vacuum ultraviolet and ionizing radiation. Thus, CubeSat experiments could be monitored continuously for many months and in orbit higher than that of the International Space Station (ISS) [3].

In long-term space travel or habitation, it is critical to understand how persistent exposure to space radiation can affect living organisms. The successful of long terms space missions depends on significant technological and biomedical development to protect the crew from the effects of chronic radiation exposure. CubeSat missions can unveil space environments conditions with robust biological systems monitored by autonomous technology and can thus play an important role in enabling future human exploration of deep space [2].

The green alga *Chlorella vulgaris* is one of the most investigated and well characterized algae with diverse application possibilities such as component for biofuels [4], human nutrition, and wastewater treatment.

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Microalgae are effective in pollutants removal in wastewater, such as nitrogen/phosphate, organic matter, pharmaceutical compounds, textile dye compounds, and heavy metals. *Chlorella sp*. presents cultivation flexibility and resistance to different stress simulations and thus is suitable to be used as model organism for space application. Regarding utilization in Earth orbit or deep space, microalgae require cosmic radiation resistance. The survival of the genus *Chlorella* to extreme conditions has been already documented. In several radiation experiments, *Chlorella sp*. maintained its photosynthetic capacity after continuous exposure to ionizing irradiation [5]. However, as a model organism, *C. vulgaris* is still valuable in help to developed new methodologies, such the ones associated to space exploration. In addition to presenting high biomass production and excellent CO_2 fixation, *C. vulgaris* is also a nutritious food supplement, rich in proteins, unsaturated fatty acids, carotenoids, dietary fibers, vitamins, minerals and contains all essential amino acids [4] that can be useful for crew nutrition on long-term space travel. The development of a biological CubeSat will allow to test different species of extremophile microorganisms, including bacteria and yeasts. The exposure of extremophile organisms to extreme conditions should contribute to biotechnological applications for human space exploration. The aim of this study was to develop culture conditions to analyze the growth of *C. vulgaris* biomass in the laboratory using a remote sensing image capture system adapted to a CubeSat device.

MATERIAL AND METHOD

Algae culture and biomass production

The unicellular microalgae *Chlorella vulgaris* was cultivated and maintained in incubators with photoperiod and temperature controlled according to the ISO 8692 [6]. The microalgae species cultivated in Rio de Janeiro State University (UERJ) were purchased from the algae bank of the Linnaeus University, Sweden. Cultures were replicated at a monthly frequency and growth and development of the cultures were monitored biweekly by counting under a microscope.

The biomass production was carried out with a real-time monitoring system. Microalgae preculture was started 3-5 days before the tests or until microalgae presented exponential growth. On the test day, five petri plates (90 x 15 mm) containing 20 mL of L.C. Oligo medium according to the Standard ABNT NBR 12648 [7], containing 1.5% of agar were inoculated with 10 µl of the inoculum solution of *C. vulgaris* (Figure 1). The initial microalgae biomass applied for the test was 10^7 cells mL^{-1}. The cells counting was performed under an optical microscope with 400x magnification, using the Neubauer chamber.

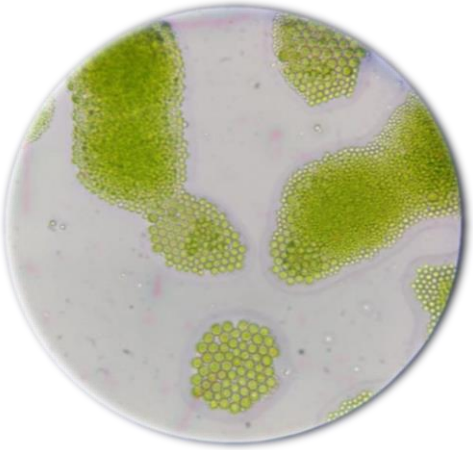

Figure 1. Microalgae *Chlorella vulgaris* culture in solid medium under an optical microscope (400x magnification)

Image acquisition system for CubeSat

An image acquisition system was developed to monitor *C. vulgaris* remotely through photo capture, as a cost-effective tool for microbiology experiments in space. A digital microscope with a microcomputer, commonly used in CubeSats studies, was chosen for this system. The biomass growth of the microalgae was evaluated through colonies observation in solid medium (L.C. Oligo medium + agar, 1.5%) using an image processing algorithm. The biological growth determination system was built based on a previous study [8] and consisted of a digital microscope with remote access and image processing algorithms, developed for this specific purpose. The optical system consisted of a microscope objective lens (40x magnification), digital camera (model OmniVision 5647, 5MP resolution), LED light source, stand with focus adjustment, in addition to a microcomputer (Raspberry PI 3). Microscope lens were calibrated with a calibration scale and achieved a total magnification of 750 times and a microscope field of view (FOV) of 1 square millimeter. A back light was adjusted to guarantee the quality of the photos. The algorithm on the microcontroller took photos of the colonies and stored the images in a flash memory. A high-speed broadband link using a Wireless Local Area Network, WLAN, were used to guarantee a rapid transmission of the images to the base station simulating a continuous monitoring system in a CubeSat experiment for long periods in orbit.

Many different factors must be considered to improve an image acquisition device for space cruises, such as the survival of organisms, the configuration of the imaging system and the functionality of CubeSat. Based on these concepts and for that purpose, this system was initially designed and built specifically for laboratory tests with microalgae, however, in the future the goal is to be able to be installed in meteorological Weather Balloons, as well as in space mission CubeSats.

Image processing

The microalgae cells were quantified by direct counting of image objects using digital image processing algorithms [6, 8]. One of the functions developed in the Matlab program made it possible to count the cells of the single-celled microalgae in each of the three monitored colonies, associated with each green circle. Based on the cell count, an attempt was made to establish a new cell growth index in each colony. The colony with the highest number of new cells in a given period of time was considered as the highest growth rate. The image processing system followed an algorithm performed in MATLAB, where this algorithm was able to identify and quantify the growth of a microalgae culture, through its area. The cell growth was estimated by the surface area, where the Petri dish was 90 mm in diameter and the microscope field of view (FOV) was limited to 1 square millimetre. The cell growth rate was calculated by determining the area occupied by a group of cells. Three cell colonies (C1-C3) were chosen for this purpose (Figure 2). The relative surface area was calculated by the area of the colony (C1, C2 or C3) monitored in real time (A), by the initial area of this colony at the initial time (A_0, at 0h), according to the following equation: A/A_0. However, it is extremely important to note that the growth analysis was done only on two-dimensional axes. The surface area of a microalgae colony can indicate the number of cells in a limited way, as the cells can stack, growing in height instead of horizontally. The calculation of the area of each cell colony and the processing of the images were done using the MATLAB image processing toolbox and specially developed scripts [8].

RESULTS AND DISCUSSION

CubeSat and image acquisition system overview

Regarding a consistent operation of microorganism monitoring during a CubeSat cruise an appropriate structure is crucial for a successful operation. CubeSat should be equipped with an attitude control for stabilization of CubeSat in the desirable orbit; a power supply for CubeSat remain powerful in orbit; and a board computer to control the entirely system. For microbiology analyses is also necessary a digital microscope to make the images capture; automated focus system; an apparatus to support the microalgae colonies; light source for images acquisition; microcomputer (Raspberry Pi) to control and integrate the whole system; and a radio transceiver for transmission of the acquired images (Figure 3).

At ground station, the system would have a monitoring computer, a radio transceiver, and an antenna. It is also worth mentioning the importance of some controllers for the system, such as: temperature and humidity controllers.

CubeSat devices cannot be recovered when launched into space orbit, thus all equipments can be use in just one experiment. The proposed approach aimed to develop a methodology for determination of microalgae growth in a solid medium of low cost and low technological complexity.

Microalgae growth monitoring

The monitoring of microalgae was performed and its analysis was satisfactory according to the theoretical analyses, with some caveats, previously predicted, mainly in biomass production. Three colonies were chosen for biomass production determination, correlating colonies growth with respect to time (168 hours) (Figure 4). According to Figure 4, the three colonies showed a similar growth pattern. However, these presented different slope coefficients. This fact may be related to some factors, such as: light intensity, algae self-shading, availability of nutrients, two-axis counting method, among other factors. Also according

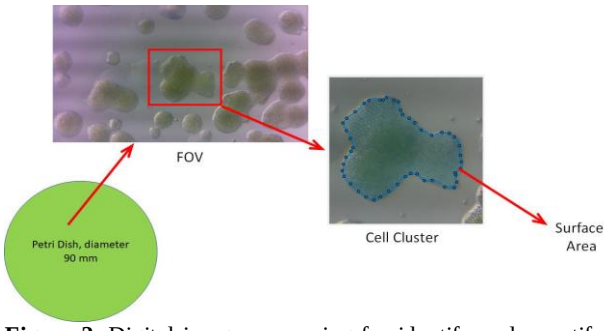

Figure 2. Digital image processing for identify and quantify the *C. vulgaris* colonies growth

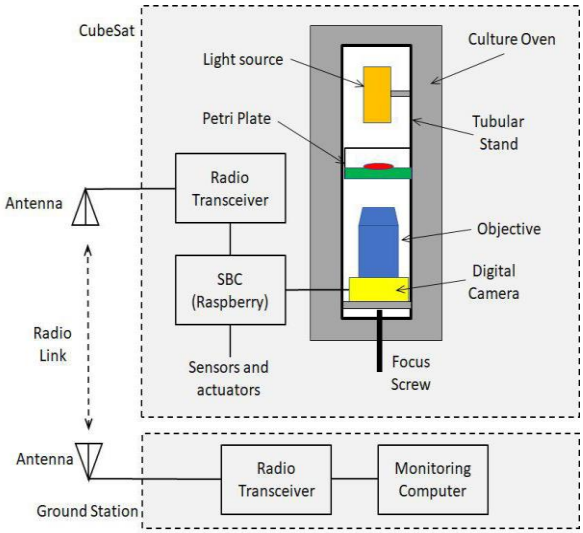

Figure 3. Block diagram of image acquisition system, for CubeSat tests with microalgae

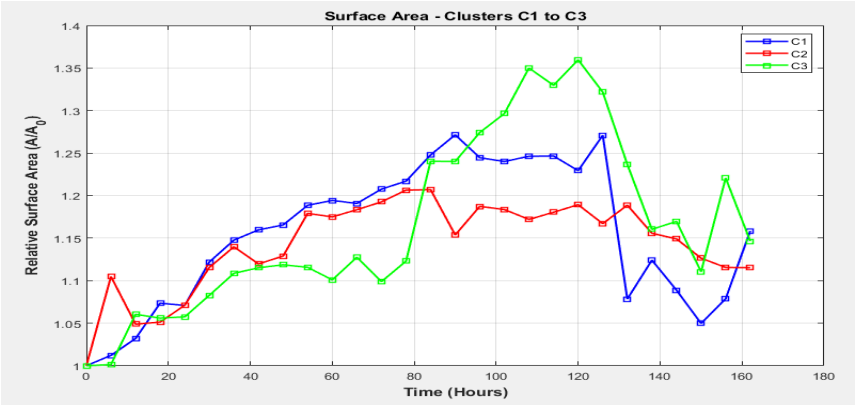

Figure 4. Surface area *vs* time of a microalgae colonies C1, C2 and C3. The relative surface area represents the area of the colony (C1, C2 or C3) monitored in real time (A), by the initial area of this colony at the initial time (A0, at 0h).

to Figure 4, five distinct phases in the growth of microalgae colonies can be observed [9, 10]: Latency phase (lag phase), relatively short phase, characterized by no growth or slow growth (C1 and C3 T= 0 to 6 h) or even a decline in culture (C2 T= 6 to 18 h). Exponential phase (Exp. phase), there is a constant increase in the number of organisms in the culture (C1 T = 6 to 90 h; C2 T = 18 to 78 h; C3 T = 6 to 108 h); Deceleration phase, a decline in growth rate occurs, usually due to some limiting factor such as nutrients, carbon dioxide or light (algae self-shading) (C1 T = 90 to 102 h; C2 T = 78 to 96 h; C3 T = 108 to 114 h). Stationary phase, characterized by lack of growth and in a short time the cells begin to undergo biochemical changes (C1 T = 102 to 120 h; C2 T = 96 to 132 h; C3 T = 108 to 120 h). Decline or death phase, occurs when cell metabolism can no longer be maintained due to depletion of nutrients or warning conditions (C1 T > 126 h; C2 T > 138 h; C3 T > 126 h). The peak production or the maximum biomass production rate of the three microalgae colonies monitored was: 28% for C1 after 90 h of monitoring; 21% for C2 after 84 h; and the greatest increase was observed in C3 with 36% after 120 h. It should be noted that the monitoring of the colonies occurred after five days of storage of Petri dishes at temperatures of 4 ° C and in the absence of light, and a few hours were necessary for the microalgae to break dormancy and again show satisfactory growth.

CONCLUSIONS

This research aimed to evaluate, analyse, and simulate system of a CubeSat with an embedded biological experiment being analysed by a digital microscope and a transmission, reception, and analysis system. Therefore, the obtained results were satisfactory, from the structure established in the laboratory with all available equipment to biomass growth analysis. However, some challenges have yet to be overcome. As long as the improvement of the project, it is sought to perform: a remote temperature controller, where it will be responsible for maintaining the ideal temperature for the system; a remote focus controller, responsible for adjusting the focus of the image to be obtained by the camera. For testing, it is thought to carry out launches of meteorological Weather Balloons (balloonSats) with embedded microalgae. Being this a perfect occasion to test, in real conditions, the present system of remote acquisition of images of the growth of microalgae. The objective is to allow the monitoring of microorganisms on small satellites using low-cost equipment, enabling small research groups focused on the development of rockets and small satellites to incorporate instruments for biological experiments into their devices.

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Persian Abstract

چکیده

کلرلا یکی از انواع جلبکی است که با کاربردهای مختلفی از جمله ترسیب کربن، سوخت‌های زیستی، تولید مواد غذایی و تصفیه فاضلاب مورد بررسی و توصیف ترین جلبک‌ها قرار گرفته است. گونه‌های *کلرلا* به دلیل انعطاف‌پذیری در کشت، برای استفاده به عنوان ارگانیسم‌های نمونه در تحقیقات فضایی مناسب شناخته می‌شوند. مطالعات زیادی برای اطمینان از شرایط بهتر برای حمایت از زندگی انسان در مأموریت‌های طولانی مدت در فضای عمیق و یا در سطح سیارات انجام شده است، و نیاز به منابع مجدد را به حداقل می‌رساند. صرف نظر از انعطاف‌پذیری جنس *کلرلا* در برابر شرایط فضایی قبلاً نشان داده شده است، ارگانیسم‌های مدل در بهبود فن‌آوری‌های جدید مفید هستند. این تحقیق با هدف توسعه شرایط محیط کشت و سیستم نظارت بر *کلرلا ولگاریس*، تحت ریز جاذبه، با استفاده از یک دستگاه ضبط تصویر CubeSats انجام شده است. سیستم اکتساب تصویر شامل یک میکروسکوپ دیجیتال، با دسترسی از راه دور، یک کامپیوتر تک برد، یک کامپیوتر مانیتور و یک الگوریتم پردازش تصویر بود. سه کلنی ریز جلبک، تحت شرایط آزمایشگاهی، در زمان واقعی (هر ۳۰ دقیقه) با استفاده از اندازه کلنی ها به عنوان یک پارامتر برای ارزیابی نرخ رشد، مورد ارزیابی قرار گرفتند. بالاترین تولید زیست‌توده ریز جلبک برای سه کلنی تحت نظارت (C1-C3) بود: افزایش ۲۸٪ برای C1 پس از ۹۰ ساعت. ۲۱٪ برای C2 بعد از ۸۴ ساعت؛ و ۳۶٪ برای C3 بعد از ۱۲۰ ساعت. نتایج نشان داد که این سیستم قادر به رشد کلنی‌های ریز جلبک بود. یک پشتیبانی خاص در حال توسعه است، که امکان نصب این سیستم کسب تصویر برای کشت جلبک‌ها در CubeSats برای مطالعات آینده رشد جلبک‌ها در شرایط ریز جاذبه واقعی را فراهم می‌کند.