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Oxygen Harvesting from Eukaryotic Green Algae Cultivation on Moon's Surface

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The presence of oxygen in the Earth atmosphere represents the key resource for the human life. Outside that thin layer of atmosphere, every place is naturally unsuitable for life. Nowadays, the vital resources on board the ISS, the only manned outpost in space, are constantly resupplied directly from Earth in an open-loop cycle. Different strategies must be adopted for deep-space manned explorations in order to ensure the mission independence from Earth. The main idea behind this work is to support the incoming manned mission towards the Moon by recycling part of the emitted carbon dioxide and the urine produced by the human crew to feed a green algae cultivation in a dedicated photobioreactor aimed to close-loop oxygen production. Indeed, oxygen availability opens to a variety of new scenarios for planetary colonization and exploration. A great amount of work on this side has been carried out in the context of MELiSSA Project, whose main objective is to set-up a regenerative life support system to reach the highest degree of autonomy to produce water, food, and oxygen by the mission wastes. Leveraging on the MELiSSA Project experiences and on an ISS photobioreactor demonstrator developed by DRL, we propose to use a *Chlorella Vulgaris* cultivation in a photobioreactor placed in a space system, properly designed for its survival on the Moon's surface. In this work we present the basic principle of photosynthesis linked to the hyperparameters that mostly affect the *Chlorella Vulgaris* cultivation, the set-up of the numerical simulations used for the design of the photobioreactor capable to work in Moon environmental conditions and the preliminary sizing of the system from a thermal and power supply point of view.
keywords: Photobioreactor, Moon, Green Algae, Oxygen, Photosynthesis, Multi-physics Simulations

1. Introduction

Oxygen availability represents a key resource for humans survival on other planets. The most widely diffused strategies for resources management in space [1, 2] aim to set-up a closed-loop system for the most basic resources needed to support the human life in space, with the objective to ensure the independence of manned missions from Earth. Leveraging on these strategies, the basic idea behind the work presented here is to support incoming manned missions to other celestial bodies (like the Moon and Mars) by recycling part of the emitted carbon dioxide and the urine nitrites produced by a human crew through a dedicated photobioreactor (PBR) for closed-loop oxygen production.

Mass-production of chlorophytes for biotechnological purposes is an already established activity on Earth. Green algae are widely cultivated for biofuel production [3], wastewater treatment [4], fertilizers [5] and also food integrators [6]. Also in the space sector the oxygen production via green algae cultivation is an accepted strategy. A great amount of work has been carried out in the context of the MELiSSA Project [7]. The main objective of the MELiSSA project is to set-up a regenerative life support systems aiming to the highest degree of autonomy and consequently to produce food, water and oxygen from mission wastes. The full-plant developed for this purpose is extremely complex and relies on several processes build one up to the other, thus it is difficult to be fully tested in space. A more compact technology demonstrator has been developed by DLR [8]. The demonstrator consists in a photobiorector filled with green algae whose main objective was to test the PBR capability of producing oxygen on the International Space Station (ISS), thus exposed to a controlled space environment.

For our purpose, leveraging on the works already done in the context of MELiSSA Project and by DLR, the *Chlorella Vulgaris*, a selected chlorophyte species,

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is here proposed as the alga suitable for the oxygen production. *C. Vulgaris* is a spherical single cell organism that can grow in a wide range of pH, temperature, and CO_2 concentration ranges [9,10]. Moreover, it shows a high resistance to cross contamination and to mechanical shear stress [9, 10], making it an ideal organism for a long-term Life Support System (LSS).

Several studies investigated the possibility of setting-up a cultivation of *C. Vulgaris* on Earth by using urea as nitrogen source [11, 12] and the possibility of cultivating green algae in properly designed PBR [13–15]. These aspects have been considered in the selection of this species in order to make possible recycling the urine of the astronauts.

Besides the biological aspects, the design of the cultivation system is also crucial. Up to now there have been only few attempts to cultivate algae in space and some points are still to be investigated in depth. Among them, the ones related to the space environment concerns the assessment of the radiation effects on algae, the operations of the PBR and cellular growth in microgravity, system automation, and long-term performances and stability [16].

Taking advantage from the next missions that aim to reach the surface of the Moon [17, 18], this paper proposes and verify the feasibility of a system with an integrated PBR that will allow to investigate the effects of the space environment on the oxygen production via *C. Vulgaris* cultivation with urea as nutrient. The system proposed includes other subsystems for nutrient supply, correct lighting, gas exchange, thermal control, growth medium control, harvesting and processing.

The next sections are organized as follows: the basic principles of photosynthesis and a review of the hyperparameters that most affects the green algae cultivation are reported in Sec. 2; the set-up of the numerical simulations needed to design a PBR capable of operating the Moon environment are discussed in Sec. 3. The results of the numerical simulations carried out are analysed in Sec. 4, while the subsystems preliminary sizing are reported in Sec. 5. The main outcomes of this work and possible future development are discussed in Sec. 6.

2. Photosynthesis and Green Algae Cultivation

Chlorophyll photosynthesis is a chemical process by which green plants and other organisms produce organic substances. During photosynthesis, under the mediation of chlorophyll, artificial or solar energy allows the conversion of six molecules of carbon dioxide (CO_2) and six molecules of water (H_2O) into a molecule of glucose ($C_6H_{12}O_6$), an essential sugar for the survival of the plant, and six oxygen (O_2) molecules (see Eq. 1), that are released into the environment.

$$6CO_2 + 6H_2O + energy \rightarrow C_6H_{12}O_6 + 6O_2 \quad [1]$$

To involve living organisms in a bioregenerative Environmental Control and Life Support System (ECLSS) as realistic future components, the following requirements have to be considered: high cultivation system utilization or efficiency (includes high light utilization yields and high volumetric biomass productivities), cultivability in microgravity, robustness/tolerance towards cosmic radiation, low susceptibility to bacterial contaminations, reliability of the biological system towards malfunction, controllability and reproducibility of growth processes, and low crew interaction effort or high level of automation. Microalgae meet the requirements best, as they can be cultivated in axenic or xenic conditions [19] in µg-capable closed PBRs with efficient conversion rates of extracellular substances into biomass. The most characterized algae worldwide is the green alga genus *Chlorella*: it has a variety of application possibilities, such as animal feed and aquaculture, human nutrition, biofuel components or wastewater treatment. This alga is easily available because it can be found in various habitats, such as marine water, fresh or brackish water and soil. *Chlorella vulgaris* is a type of this genus: it's unicellular with a mean cell diameter of 2–20 µm [20]. Because of their small cell size and spherical shape, these cells have the potential to be cultivated in all known PBRs types. It has a thick and rigid cell wall structure as common among several *Chlorella* species. It is also the most commonly used alga for sequestration of CO_2, due to its high biomass productivity and strong CO_2 fixation ability [15]. Fast growth, simple and flexible culture conditions, and resistance to interfering factors are other advantages that make this microalgae suitable for our scope. Therefore, plants or plant-like microorganisms provide a convincing potential to produce fresh oxygen in-situ in closed environmental systems at any place and at any time.

There are two main categories of factors affecting the growth of microalgae and the generation of oxygen by photosynthethic microalgae cultures: nutritional factors (chemical) and environmental factors (physical), such as temperature, nitrogen concentration, light intensity, salinity, etc.

Nitrogen is one of the most important limiting nutrients. Nitrogen control is essential for the inten-

sive cultivation of algae because it plays an important role in growth and regulation of metabolism. Different nitrogen sources for *Chlorella* were studied in past researches [14], including potassium nitrate, ammonium sulfate, urea (CH_4N_2O), ammonium nitrate, peptone and simultaneous effect of pH on the growth of the system under mixotrophic conditions. The best results were obtained for urea and potassium nitrate. Potassium had higher specific growth rate and biomass productivity, but, especially due to the lower price of urea, the latter was indicated as the best nitrogen source for cultivating algae. In addition, urea has no effect on the final chlorophyll of the culture. For future developments of the PBR, the urea extracted from the urine of astronauts crews could be used directly, given the high presence in this liquid, while the effects of direct addiction of urine are still to be assessed. The urea needed for the experiments is made available by a tank designed in order to ensure the correct supply for all the duration of the experiments.

Illumination is another fundamental aspect in algae growth: it consists of two subjects, intensity and wavelength of light. Experiments suggests that the light acts as a guide and helping factor to cell proliferation and it helps cellular respiration and photosynthesis [9]. Algae assimilate inorganic carbon for conversion into organic matter and light is the source of energy which drives this reaction. Considering light as the most important energy source for the photoautotrophic algae, many studies have focused on the effect of light intensity. The study in [13] analysed the effect of different light wavelengths on the growth of *Chlorella vulgaris*, using in particular the red (600-700 nm), blue (400-500 nm), clear (white) and green (500-600 nm) wavelengths to test their effect. It was discovered that green and red light don't show a great trend in the growth rates compared to clear and blue light wavelengths. Therefore, precise control of the light source is important for controlling the growth of algae.

Temperature is another important environmental factor affecting various aspects of growth for several microorganisms. Low temperature limits cell growth speed and therefore reduces the biomass production [21]. The optimal temperature for *Chlorella* is about 30°C, in which the maximum biomass productivity is achieved. An excessive rise in temperature to 38°C leads to an abrupt halt in microalgae growth and cells die. Temperature turns out to be crucial for the many aspects correlated to the thermal control of the space system. Indeed, the sizing described in the next chapters had the goal of keeping the temperature as constant as possible around a range value considered excellent (20-30°C). In this way it is possible to guarantee the adequate environmental conditions for *Chlorella* development.

Also pH results to be crucial for the growth of *Chlorella* [22]: an alkaline environment with an high pH value (pH = 9-10) ensures the best results for cellular growth. In our simulations we are not going to consider this parameter, but it could be an interesting aspect to be further analyze in future studies.

It is important to underline that the parameters that affect the development of algae are not independent one from each other: in fact, each factor influences other factors and changes their optimal range. Therefore, in a more accurate study, all these correlations should be considered in order to develop a simulation that is as realistic as possible. We will not evaluate all the mentioned parameters at the same time, but we will only evaluate some of them individually. All the other parameters will be ignored.

In this work, two simulations are performed: the first focuses on the study of fluid dynamics inside the PBR, with the aim of sizing the PBR and its components in order to ensure the correct mixing of the fluid and algae. The second simulation tests the consumption of urea by algae, in order to determine the inlet and outlet channels size for the fluid, the initial cellular density needed and the concentrations of nutrients to be supplied to the cells to guarantee the survival and growth. Finally, the total amount of hypothetical fluid required to produce oxygen to meet the average daily demand of a standard person will also be evaluated. COMSOL Multiphysics® Version 5.6, a software for multiphysics simulation, was used to perform the simulations and size the PBR and the input conditions. The main feature of this software is that it can carry out multiple physical communications at the same time. Both of the two simulations used 3D models and different physics, and each of them was implemented according to the simulation in question and carried out through a transient analysis.

3. Model of the PBR and Chemical Reactions

In the first simulation, the fluid dynamics inside the PBR was studied to verify that an appropriate recirculation of the fluid within the entire volume of the tank is established also in the Moon environment. To study this aspect a physics named *Laminar Flow (spf)* was used. It implements the Navier Stokes equation and the continuity equation (in the hypoth-

esis of Newtonian and incompressible fluid) as in Eqs. 2-3).

$$\rho\frac{\partial u}{\partial t} + \rho(u \cdot \nabla)u = \nabla \cdot [-\rho I + K] + F \qquad [2]$$

$$\rho\nabla \cdot u = 0 \qquad [3]$$

Navier Stokes describes the conservation of motion in a fixed volume. In the case of fixed volume and incompressible material, the continuity equation is valid, and the divergence of speed is zero. For this simulation we modeled a cylindrical tank with a small thickness. An impeller with two propellers (obtained directly from the software library) was inserted in the center of the PBR: the parameters of the propellers were modified and tuned for the model described here. The final geometry of the model was obtained through Boolean operations: this step is necessary to then define the moving mesh in order to determine the rotation of the blades (see Fig. 1). The input and output channels are the only geometries not present in this model. These structures are instead present in the real model in order to guarantee the insertion of urea through a fluid pump.

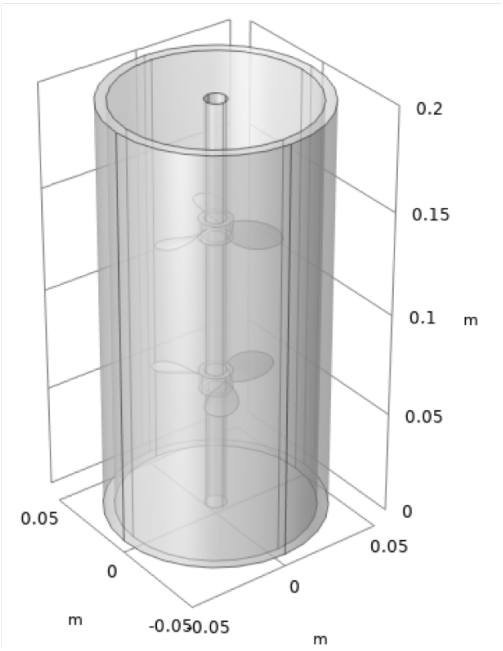

Fig. 1: Geometry model: first simulation.

Water was assigned as material for the domain of the fluid, in order to simulate the fluid and algae inside the tank. This choice is due to the fact that algae are composed almost entirely of water [23], so modeling the whole system as water turns out to be a justified choice. Glass was assigned to the tank wall. The parameters of the physics involved in this simulation (*Laminar Flow (spf)*) have been defined. The flow in the mixer is considered laminar due to the relatively low speed of the impeller. As initial values, the fluid was set at a zero velocity condition in all the domain. A *Flow Continuity* condition has been imposed in the contact surface between the fluid and the wall so that there is continuity between the fluid dynamics of the internal mobile mesh and the non-mobile mesh of the wall. Finally, a *Moving Mesh* was defined to simulate the rotation of the fluid: the internal domain relating to the fluid was selected as the rotating domain, and the number of revolutions per minute of the propellers *rpm* was then set. This parameter simulates the fact that initially, before the motor reaches the full speed of $100rpm$, there is a transitional phase of 100 seconds where the angular velocity gradually increases. This value ensures an optimal condition for algal growth, in agreement with [11]. The last important parameter to consider is the gravity that on the Moon is about 1/6th of the one on the Earth. Gravity causes sedimentation within the reactor and this must be avoided by creating a continuous movement of the algae-suspension, but ensuring at the same time a good-mixing. In order to ensure this type of fluid dynamics, special blades have been selected for the propeller so that they have a sufficient angle to ensure the movement of the fluid also in the vertical direction.

In the second simulation, the consumption of urea by the cells, which is the selected source of nitrate, was studied. To do this, a simplified geometry compared to the previous one, was modeled as in Fig. 2. The reason behind the use of this simplified geometry was the will to put more attention on the simulation parameters with respect to the fluid dynamics inside the PBR. This leads to a reduction of the computation complexity and allows to evaluate in the most linear way possible the consumption of urea by the algae. A possible future implementation should include the development of a model in which both the simulations performed here are evaluated simultaneously. Therefore here, the simplified geometry only includes a cylinder (with no wall thickness) and two inlet and outlet channels (the central portion of the impeller and the propellers are also excluded). The purpose of this simulation is to determine the right initial density of cells inside the tank and the inlet speed with which the fluid is inserted in order to ensure a sufficient supply of urea for the survival of cells. A further simplification made is that the rotation of

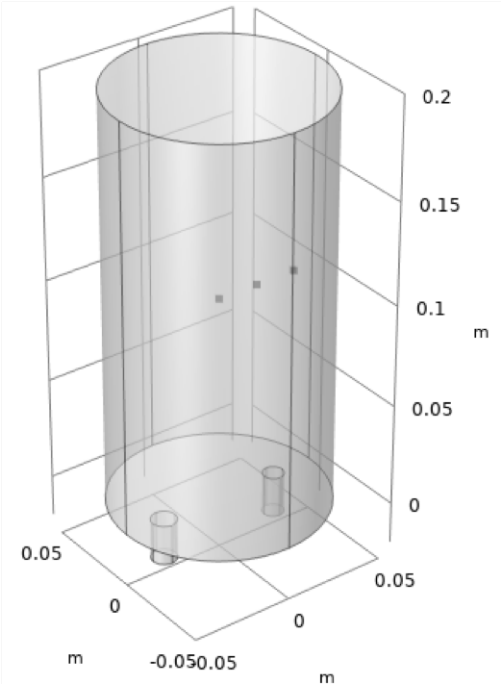

Fig. 2: Geometry model: second simulation.

the fluid, given by the propellers, is not taken into account. The whole volume has been modeled as water, since the walls do not contribute to the results.

Unlike before, this simulation involves the use of several physics that communicate with each other. The first physics implemented is *Laminar Flow (spf)* in which different conditions have been defined, compared to the previous case: in this case there is also an inlet boundary condition that simulates the fluid inlet and one outlet boundary condition for fluid leakage. The inlet has been applied to the larger channel in the bottom of the PBR, considered as the input channel, while the outlet has been assigned to the other cylinder: assigning an outlet boundary condition is fundamental at computational level since in this way the software manages to reach convergence. Also in this case the gravity of the Moon is taken into account.

The second physics used is named *Transport of Diluited Species (tds)*: it enables to simulate the transport of chemical species and its consumption through a specific reaction. The constitutive equations of this physics are Fick's laws (see Eqs. 4-5):

$$J_i = -D_i \nabla c_i \qquad [4]$$

$$\frac{\partial c_i}{\partial t} + \nabla \cdot J_i + u \cdot \nabla c_i = R_i \qquad [5]$$

Fick's laws are constitutive relations that describe the concentration variations in the materials in which molecular diffusion phenomena are taking place, in absence of thermal diffusion. The first law describes the anisotropic diffusion of an i-th species in spatial dimensions in homogeneous media. The Fick's second law describes the diffusion process in the time dimension and it is actually a simplified form of the matter balance equation. In our example, the species of interest is urea. Assuming that the reaction kinetics is equal to the oxygen consumption of the cell, its consumption is simulated. This simplification was carried out based on data of oxygen and urea uptake by nitrogen-starved *Chlorella* cells, according to [12], where it is reported that these two values are almost equal. So, at software level, this reaction involving urea was simulated by assuming a Michaelis Menten kinetics (see Eq. 6), which describes the rate trend of a reaction catalyzed by enzymes, as the concentration of the substrate and the enzyme varies:

$$R = \frac{-UCR \cdot \rho \cdot c}{c + k_m} \qquad [6]$$

where UCR is the consumption of moles of urea per second per cell, ρ is a function representing cell density, c is the urea concentration over time and k_m is the Michaelis Menten constant.

The density was assumed variable over time: according to [11] the trend of the growth of cell density over time as a function of the concentration of urea is linear-like in the first six days and then slows down until the eighth day. The values of minimum and maximum density reported in [11] have been set in this simulation. The function implemented for the cell density ρ as function of the time t is reported in Eq. 7:

$$\rho = \rho_{min} + (\rho_{max} - \rho_{min}) \frac{t}{t_{phase-1}} \qquad [7]$$

where t is the time variable, $t_{phase-1} = 520000$ seconds is the duration of the first six days of experiment, $\rho_{min} = 5e10 cell/m^3$ and $\rho_{max} = 1.57e12 cell/m^3$. The initial urea concentration was assumed to be zero. Instead, a condition of constant concentration of urea c_0 in the inlet channel was considered in order to simulate the constant supply of this nutrient, which is introduced through the inlet flow. This value was used according to [11] where it is shown that for this concentration the cells survive and proliferate in a good way.

The two physics have been coupled through the *Multiphysics* module in order to simulate both of

them simultaneously. The time-dependent solution of urea concentration over time was calculated, assuming a total time of six days ($t_{phase-1} = 520000$ seconds).

After this simulation, another similar to this one was implemented: in this case the values were evaluated from the sixth to the eighth day. For this reason, the last value of the simulation relative to the first six days was set as the initial concentration value at time zero. A different law of cell density growth has been set (see Eq. 8), always according to [11]:

$$\rho' = \rho_{max} + \rho_{max} \cdot 1.1 \cdot \frac{t}{t_{phase-2}} \quad [8]$$

where $t_{phase-2} = 172800$ seconds, t is the time variable and $\rho_{max} = 1.57e12 cell/m^3$. The last aspect evaluated, but of relevant importance, is the production of oxygen in the PBR. The cells are kept inside the tank in an aqueous medium with the right initial concentrations of basic nutrients, then oxygen is produced thanks to the chlorophyll photosynthesis that occurs in the algae. To make this possible, it is necessary to provide energy to the reaction and this is guaranteed by the sunlight rays that pass through the tank wall. In this case, oxygen production was fixed at $200 fmol/(h \cdot cell)$, according to [24].

To evaluate the total amount of moles produced during the experiment, the calculation was divided into two parts: the first takes into account the first six days, using an average density between the minimum and the maximum used in the previous simulation. The second part instead considers the maximum density since, as stated before, the growth after the sixth day is almost null.

4. Results of simulations

In the first simulation, the fluid dynamics of the system was investigated. The values obtained from the tuning process are reported in Tab. 1. The results of the simulation are showed in Fig. 3, where the streamlines of the velocity field are plotted. It can be noticed that the flow lines are distributed throughout the entire domain of interest, guaranteeing the mixing of algae and nutrients, an essential condition for an optimal algae growth. In this way, sedimentation is avoided and algae can grow in an optimal environment. It is worth to remark that in this model there are no input and output channels for the introduction of urea, but since they are perpendicular to the lower plate, they will ensure further mixing of the fluid. The simulation also confirms that by using two propellers with properly modeled blades placed in a prescribed position, it is possible to obtain a correct fluid dynamics inside the PBR.

Parameter	Value
Tank height	0.2 m
Tank diameter	0.1 m
Tank wall thickness	0.005 m
Central axis height	0.2 m
Central axis diameter	0.01 m
Number of propellers	2
Gravity (z component)	-1.62 m/s^2

Table 1: Parameters for first simulation.

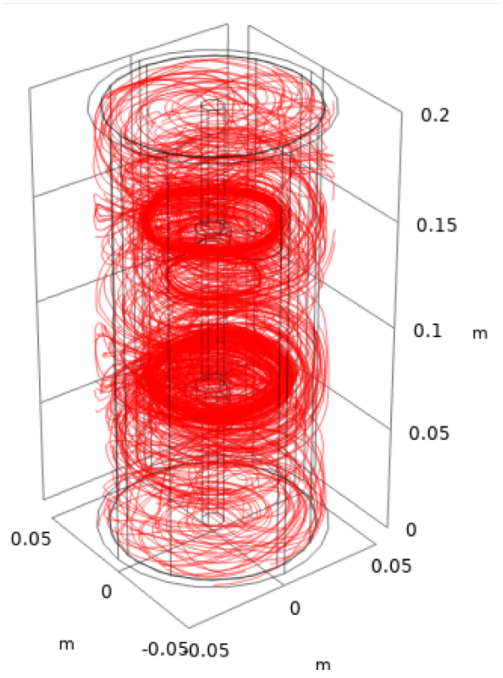

Fig. 3: Velocity streamlines.

The uptake of urea by the algae is investigated in the second simulation. The parameters used in this simulations are reported in Tab. 2. The parameter c_0 is obtained from [11], where a concentration of $800 mg/l$ of urea is proven to be optimal for the cellular growth. In Fig. 4 the linear growth over time set for the cell density is reported, in agreement with Eq. 7 previously defined.

The values in Fig. 4 are already related to the optimal minimum and maximum density values chosen for the experiment. The reference ρ values from [11] have been scaled by a factor 100: in this way, as proven by the results here obtained, it is possible to

Parameter	Value
Tank height	0.2 m
Tank diameter	0.1 m
Inlet channel height	0.02 m
Inlet channel diameter	0.012 m
Outlet channel height	0.02 m
Outlet channel diameter	0.01 m
Inlet fluid velocity	0.005 m/s
UCR	1e-17 mol/(cell s)
k_m	0.201 mol/m^3
ρ_{min}	5e10 cell/m^3
ρ_{max}	1.57e12 cell/m^3
Simulation time	6 days (520000 s)
c_0	13 mol/m^3
Gravity (z component)	-1.62 m/s^2

Table 2: Parameters for second simulation.

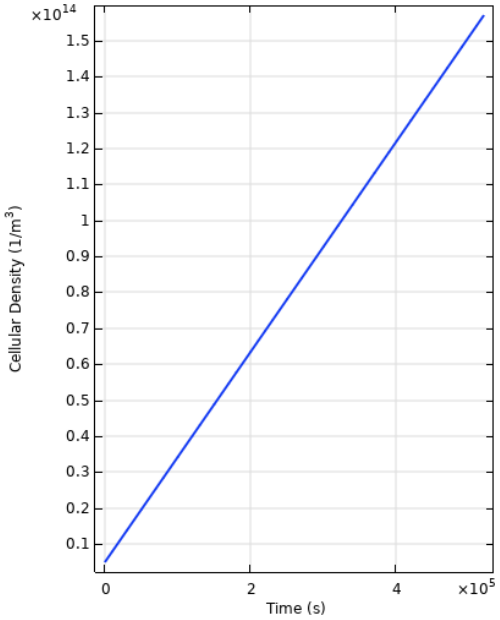

Fig. 4: Cellular density growth.

guarantee a sufficient concentration of urea throughout all the experiment duration. Using the corrected density values, it is possible to visualize the streamlines of urea concentration over time (Fig. 5). These values refer to the final instant of the simulation. From Fig. 5 it is possible to notice that even at the final instant there is still the right urea concentration, close to the initial value c_0. This demonstrates that a good supply of urea for the cells is maintained for the entire duration of the experiment. In Fig. 6 the variation in urea concentration over time is shown, in

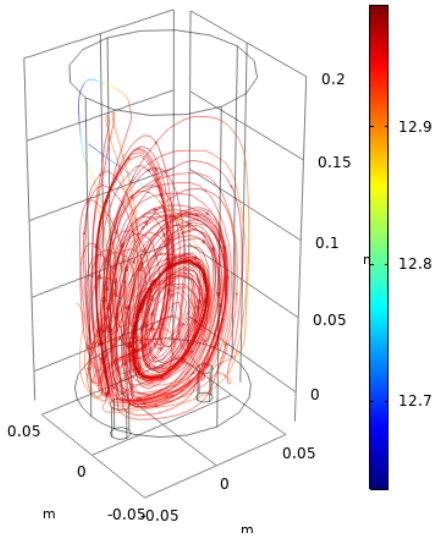

Fig. 5: Urea streamlines.

the three reference points highlighted in Fig. 2 (the small dots). The lines with greater slope are rela-

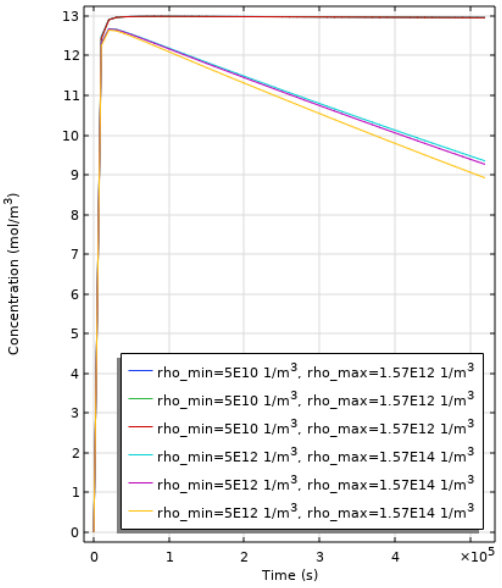

Fig. 6: Urea concentration over time.

tive to the minimum and maximum densities set as in [11], while the lines with an almost constant trend are relative to the optimal value obtained here. A density higher than the optimal value identified does not lead to an acceptable solution, as the urea concentration decreases too much over time, leading to a

condition of deficit of nutrients for the cells. On the other hand, the scaled values guarantee an adequate concentration for the entire duration of the experiment, since as can be seen, even at the end of the experiment there is a concentration value close to c_0. In that way, also the value set for the inlet velocity results correct for the simulation.

In Fig. 7 the results relative to the simulation of urea uptake from the sixth to the eighth day of experiment are reported. Also in this case the concentration remains at a good level during time. There is a first phase of settling of the concentration values, due to different scales of the plots, then, as the simulation proceeds, the curve goes from a value of about $13 mol/m^3$ to a slightly lower value at the end of the eighth day. The results obtained gave the parameters

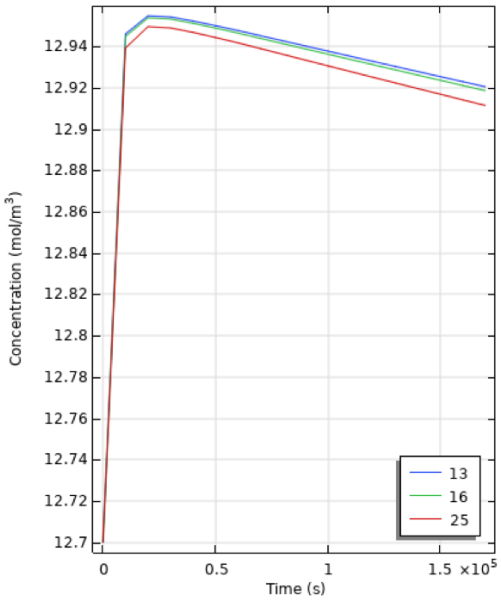

Fig. 7: Urea concentration from day six to eight.

for the correct sizing of the fluid inlet speed, the right initial cellular density, and the concentration of urea to be supplied.

The last results reported are related to the oxygen producted by the PBR of 1.57 l of capacity. The moles of O_2 produced during the first six days are computed as in Eq. 9:

$$\begin{aligned} M_{phase-1} &= K_{O_2} \cdot t_{phase-1} \cdot \rho_m \cdot V_{PBR} \\ &= 0.03436 \ mol_{O_2} \end{aligned} \quad [9]$$

where $K_{O_2} = 200 * 10^{-15} \ \frac{fmol}{h*cell}$ is the oxygen produced by photosynthesis, $t_{phase-1} = 144h$ is the time duration of this phase of the experiment, $\rho_m = 7.6 * 10^{11} \ \frac{cell}{m^3}$ is the average of cell density between ρ_{max} and ρ_{min}, $V_{PBR} = 0.00157 \ m^3$ is the volume of the PBR. For the second part we have the same equation, but with different values (see Eq.10):

$$\begin{aligned} M_{phase-2} &= K_{O_2} \cdot t_{phase-2} \cdot \rho_{max} \cdot V_{PBR} \\ &= 0.02366 \ mol_{O_2} \end{aligned} \quad [10]$$

where $t_{phase-2} = 44h$ is the time duration of this second part of the experiment and $\rho_{max} = 1.57 * 10^{12} \ \frac{cell}{m^3}$ is the maximum cellular density. In total, in eight days, there is a production of $0.0580 mol_{O_2}$. This value is equal to the moles of CO_2 that react, since as seen, in photosynthesis six molecules of CO_2 react and six molecules of O_2 are obtained. The quantity of moles produced has been used for the sizing of the tank which must contain the oxygen produced.

Therefore, the liters of fluid that are necessary for the production of sufficient oxygen to guarantee the wellness of an adult man per day on Earth ($0.85 Kg/day$, according to [25]) were evaluated: to do this the maximum density of algae was considered. Therefore, every day with our system it is possible to produce (see Eq.11):

$$\begin{aligned} M_{day} &= K_{O_2} \cdot t_{day} \cdot \rho_{max} \cdot V_{PBR} \\ &= 0.01183 \ mol_{O_2} \end{aligned} \quad [11]$$

where $t_{day} = 24h$, $\rho_{max} = 1.57 * 10^{12} \ \frac{cell}{m^3}$ is the maximum cellular density. The $0.01183 mol_{O_2}$ produced per day corresponds to $0.18927 g_{O_2}$ per day. With this rate of production, by proportionality and by assuming a medium consumption of $0.85 Kg/day$ of oxygen for a man on Earth [25], it is possible to compute that it would be needed a PBR of about $7050 liters$. This value demonstrates that obviously the experimental PBR designed is not sufficient to provide enough oxygen per day for a human, and it was not aimed to to this, but it will serve as experimental tests before setting up a properly sized oxygen production plant. It must be noticed that the volume of the production plant could be reduced by increasing the density of the algae present in the PBR. However, this would involve other considerations on the survival of the cells themselves, and the PBR here proposed could serve for this purpose.

The total moles of urea needed in eight days have been also evaluated. By starting from a condition of zero concentration at time zero and by considering the constant inlet concentration of $c_0 = 13 \ \frac{mol}{m^3}$, the fluid inlet speed of $v_{in} = 0.005 \ \frac{m}{s}$ and the diameter $D = 0.012m$ of the input channel, Eq.12 can be used

to evaluates the moles of urea per second needed:

$$U_{urea} = c_0 \cdot v_{in} \cdot \pi \cdot (D/2)^2 = 7.35 * 10^{-6} \ \frac{mol}{s} \quad [12]$$

Thus for the eight days of the experiments, $5.08mol$ of urea would be needed. Converted to a mass, it corresponds about to $305g$ of urea.

From the system point of view, notice that the oxygen is not present at the beginning of the experiment, but a system to collect and store it was included in our design. All these values are useful for evaluating the correct parameters to build a PBR able to support human life on the moon.

5. Subsystems Sizing

To verify the feasibility of the experimental setup described above, the subsystems sizing is needed. The following subsections report the main outcomes.

5.1 *Configuration*

The configuration for the system proposed is reported in Fig. 8 with all the main components localized in the figure. This configuration is mainly driven by the needs for large solar arrays (in order to have enough power generation especially at high latitude) and large surfaces for radiators. The 12U CubeSat structure has been selected since it allows to accomodate all the components, included the photobioreactor.

From the model of the chemical reaction expected for the entire duration of the experiment, the total amount of oxygen produced and both CO_2 and urea needed are computed. From these values (marginated), the tanks have been designed. In particular the two O_2 tanks needed have an envelope of 1U and 0.25U each, the CO_2 tank occupies about 0.5U, and the urea tank has an envelope of about 0.5U. The other components in the lowest part of Fig. 8 have a total envelope of about 1.25U.

5.2 *Thermal Control System*

A preliminary sizing of the thermal control subsystem was performed since one of the main objectives is to verify the feasibility of setting up a microalgae culture on the soil of the Moon and since the temperature is one of the hyperparameters that mostly affects the growth of the microalgae. As previously said, several studies conducted in a controlled environment on Earth [9, 10] demonstrated that an average temperature of 25 °C is optimal for the growth of microalgae in a cylindrical photobioreactor as the one considered here. Anyway, the same studies demonstrated that comparable grown-rates and molecular oxygen production can be obtained also in a range between 20 °C and 30 °C with a constant illumination of 5000 lux [11].

The temperature on the surface of the Moon is strongly dependent on the Sun illumination and, due to the almost total absence of an atmospheric layer, the temperature gradient during day and during night are strong, with a maximum temperature in sunlight of 397 K at the equator and a minimum in shadow of 94.3 K [26]. The system proposed is in direct contact with the soil of the Moon thus a correct estimation of the actual temperature depending on the position of where the system is deployed is needed. From the analyses of the data collected by the Diviner Lunar Radiometer, the following equations describing the mean daytime temperature (see Eq. 13) and the mean albedo coefficient (see Eq. 14) as function of the latitude on the surface θ of the Moon has been derived in [26]:

$$T(\theta) = [S(1 - A(\theta)) \cos(\theta)/\epsilon\sigma]^{1/4} \quad [13]$$

$$A(\theta) = A_0 + a(\theta/45)^3 + b(\theta/90)^8 \quad [14]$$

Where in Eq. 13 $S = 1370W/m^2$ is the solar constant at 1AU, $\epsilon = 0.95$ is the emissivity, and σ is the Stefan-Boltzmann constant. In Eq. 14 $a = 0.045$, $b = 0.14$, and $A_0 = 0.08$. The temperature of the surface of the Moon affects the thermal fluxes acting on the spacecraft both by conduction (conductive heat fluxes are established between the soil of the Moon and all the surfaces that are in direct contact with it, e.g. the back side of the solar array panels and the bottom part of the main body of the system) and by infrared (IR) radiative heat transfer (radiation heat fluxes are established between the soil of the Moon and all the surfaces that are "in view" of the soil).

The heat fluxes coming directly from the Sun and from its reflection on the surface of the moon have been included in the balances performed to obtain the temperature of the most significant surfaces. The heat coming from the direct solar radiation has been computed as [27]:

$$Q(\theta) = A_{ref} \alpha S \cos\theta \quad [15]$$

Where A_{ref} is the reference area of the illuminated object and α its absorptivity coefficient. The albedo on the Moon is computed by using the heat flux computed for the direct Sun illumination as in 15 and by applying a scaling factor A computed with Eq. 14 as function of the latitude. By using an analogous formulation (with $A = 0.35$ [28])]), it is possible to

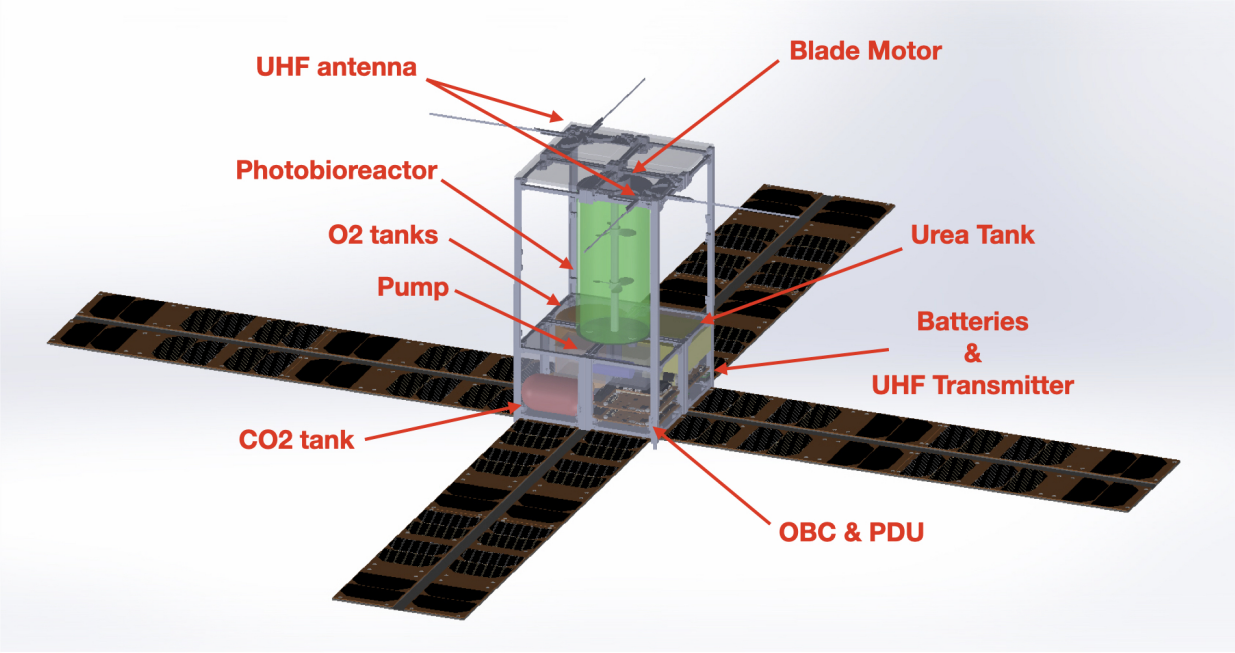

Fig. 8: System configuration and components.

evaluate the effects of both the Earth IR emission and the albedo coming from the reflection of the Sun on the Earth. These two additional terms have been neglected here since the corresponding heat fluxes have been computed to be well below $0.5W/m^2$, thus their contribution to the balances are safely negligible in first approximation.

The additional terms coming from the radiative heat exchange between surfaces that are "in view" of each other have been considered in the balances. To correctly evaluate these additional heat fluxes, the evaluation of the view factors is needed. It has been assumed that a single planar surface perpendicular to the surface of the Moon has a view factor equal to 0.5 both with respect to the surface of the Moon and the Deep Space (assumed to be at a temperature $T_{space} = 0$ K), in agreement with [29]. Due to the simple geometries involved here, all the view factors have been computed by using the assumption made above, the algebra of the view factors reported in [27] and the equations for computing the view factor for several geometries both in 2D and 3D given in [30].

To perform a preliminary feasibility study, 3 nodes have been considered in the equivalent Oppenheim's network that refers to the most critical components of the system. The nodes refers to the solar arrays panels, the radiators needed, and the external surface of the photobioreactor respectively. The service module is assumed to be in thermal equilibrium with the photobioreactor. The maximum and minimum operational temperature for the nodes are reported in Tab. 3. To properly model the radiation heat exchanges of the radiators, the upper surface and the side surfaces of the service module (that are covered by radiators) have been considered as separate surfaces with different view factors. The "pseudo" Oppenheim's network developed is reported in Fig. 9. In the pseudo-thermal network are reported the connections and equivalent resistors for the radiation heat exchange as in a classical Oppenheim's network, but also the conduction heat exchange and the external heat input have been inserted. From Fig. 9 it can be noticed that Node 2, the radiator, is composed by three different surfaces that exchanges heat in three different ways depending on the view factors. In the

Node	T range [K]	Critical Component
1	223 – 383	Solar Array Panel
2	273 – 313	Radiator [27, 31]
3	293 – 303	PBR inner temperature

Table 3: Temperature Ranges for each node.

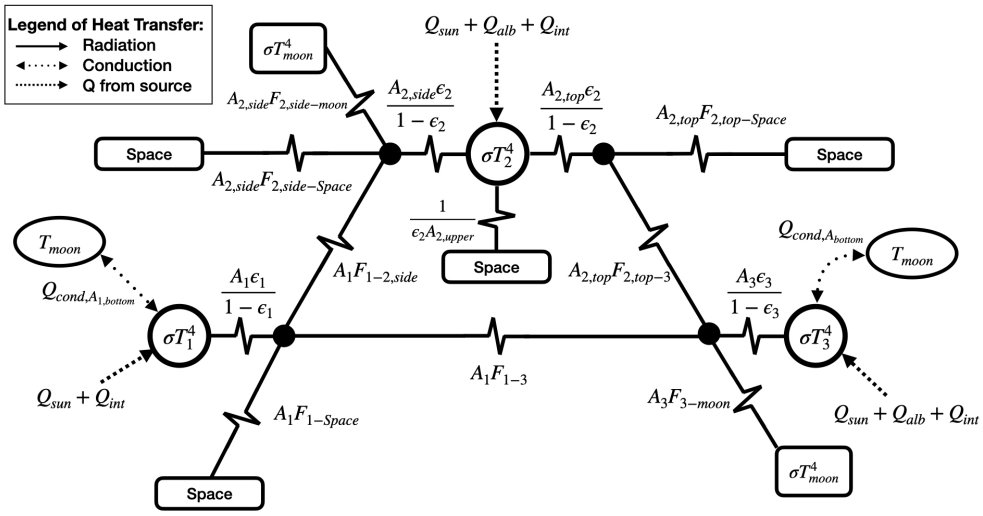

Fig. 9: Pseudo-Oppenheim's Network.

worst hot case it is considered that the internal heat generated by electrical components is directly discharged on the radiator by using a thermal strap, while in the cold case the connection is interrupted (this is possible by using a thermal switch that allow the conductive heat flux only above a certain temperature) and the internal heat contribute to warm-up the system.

The worst "hot-case" for the system has been identified as the case in which it is located on the Moon surface at low altitude ($\theta_{min} = 65\,\text{deg}$, thus high soil temperature) with all the subsystems and components switched on (the total Q_{int} marginated of +20% is equal to 20.8W) and in sunlight. The worst "cold-case" is identified by $\theta_{max} = 75\,\text{deg}$ in sunlight. In the cold case the only components switched on are the OBC, the blade-engine and the pump for the feeding (the total Q_{int} marginated of +20% is equal to 11W). Both the hot and cold case are in sunlight to allow the microalgae to grow. The limiting values for θ_{min} and θ_{max} reported above have been given by the capacity of the thermal control system in the hot case and by the electric power availability in the cold case. The surfaces in contact with the soil of the Moon have been insulated by using a layer of SiO2 Aerogel ($k = 0.01\frac{W}{m\ K}$ [32]). The ϵ and α parameters of the selected materials are reported in Tab. 4. The surfaces of Node 2 are louvered (with louvers opened in the hot case and closed in the cold case) to allow a strong heat dissipation in the hot case and a lower one during the cold case by the rotation of their blades [33]. By solving the network reported in Fig. 9 with the material properties in Tab. 4 for the steady state case, the limits in the worst hot case are fully respected, while in worst cold case the heaters are needed to maintain the strict limits imposed in Node 2, even with louvered radiators. The heat flux coming from the heaters has been computed to be equal to $Q_{heaters} = 17W$ in the worst case. To have a more refined control on the temperature of the solution inside the PBR, the heaters should be placed in order to affect the solution exiting the pump and entering the PBR. The temperature obtained for each node marginated by +15K in the hot case and -15K in the worst case are reported in Tab. 5. From Tab.

Node	Material	ϵ - α
1	Solar Array	0.82 - 0.8
2	Optical Solar Reflector [27]	0.71 - 0.07
2	Louvers Blade Material [27]	0.115 - 0.18
3	Opal Glass Coating [27]	0.87 - 0.24

Table 4: Material ϵ and α values for each node.

Node	T Hot Case [K]	T Cold Case [K]
1	311	249
2	299	283
3	303	293

Table 5: Temperature ranges for each node.

5 it can be noticed that for the PBR the temperature computed are exactly equal to the ones given as limiting temperature. This is not foreseen to be an issue since the temperature reported are already marginated and since there is still the possibility to add a thermal strap between Node 2 and Node 3 to reduce the temperature of the PBR (notice that for the hot case $T_{node-3} > T_{node-2}$) or to improve the heater capacity of few watts in the cold case.

We acknowledge that the design proposed here is only a preliminary design but, due to the margins applied, it confirms the feasibility of the experiment on the Moon soil in a given range of latitudes. Anyway, since the PBR processes are strongly affected by the temperature, a more refined model will be developed in the next phases. Moreover, once that the experiment site will be selected with an higher accuracy, it will be possible to refine the models by taking into account also the temperature variations during the daytime on the Moon.

5.3 *Electrical Power System*

In order to assess the feasibility of the mission in such Moon environment, also a preliminary sizing of the electrical power subsystem has been performed. The design of the EPS subsystem derives from some criteria such as the robustness of the subsystem, the risk and failure minimization and the ability to provide the correct amount of power to the other subsystems and components in all the possible environmental scenarios considered. In particular, the design strictly depends on the Sun condition that can be faced by the unit. As mentioned in Section 5.2, the Moon latitude range selected for the mission sizing is between 65° and 75°. During the design process the solar panels have been selected as the primary power source; the motivation behind this choice is double: the first is related to the fact that solar panels allow longer life and re-usability of the system once a single experiment is concluded, the second takes into consideration some configuration problems; indeed, using batteries as primary power source would have needed a lot of space to place them inside the unit. Therefore the major trade-off to be done for the EPS subsystem consisted in the solar panels size and configuration. In Table 6 a preliminary power budget is presented considering the power requested by the onboard computer, the UHF system, the blades engine, the pump and all the sensors needed to maintain the algae reactions and nutrition.

The choice to adopt different margins for each of the components is driven by the common application of the component in a Moon/space environment. If the reference for the component is COTS then the margins is lower. In order to size the dimension of the solar panels, an average value of the solar cell power at beginning of life has been considered, in particular $P_{cell} = 1.05$ W. Considering θ as the Moon latitude and N_{cell} the total number of solar cells, the total power generated can be retrieved with Eq. 16.

$$P_{panels} = N_{cell} P_{cell} cos(\theta) \quad [16]$$

The configuration designed, as presented in Section 5.1, shows 16 unfolded panels. Each panels has a surface of 3Ux1U with 7 solar cells on it, that are typical values for cubesats missions. Therefore, at 65° the power available is 49.7W while at 75° is 30.44W for a total of 112 solar cells. Combining the outcome of this sizing together with the resulting power balance came out from the thermal analysis, we can assure the correct amount of power in each of the possible conditions selected for the unit to live in.

Even if the current solar panels configuration is capable to generate the correct power level, a stack of batteries has been placed to help if necessary the EPS in the most expensive moment of the mission. To fulfill the mission there is no need to recharge the selected secondary battery, that can be considered expendable. Nevertheless, especially for the lower latitude case, in which the total amount of power generated is quite above the power effectively requested, part of that can be used to recharge the batteries.

6. Conclusion

In the previous sections of the paper it has been proposed a preliminary sizing of a system with an integrated photobioreactor capable of working in Moon's environmental conditions and the preliminary sizing of the most important subsystems. From the multiphysics simulations carried out a preliminary sizing of a photobioreactor capable of ensuring a correct mixing and fluid dynamics conditions for the cultivation of *C. Vulgaris* within it has been proposed. The proper fluid dynamics conditions can be established in the Moon environment (thus with a reduced gravity acceleration with respect to the Earth) by using two propellers properly designed and placed at the prescribed location along the central rotating axis of the photobioreactor. The transient analysis revealed that a rotation of 100 rpm of the central axis can ensure the desired mixing and recirculation of the fluids in the entire volume of the photobioreactor. As a second achievement, a numerical model to

Components	Peak Power [W]	Margin [%]	Margined Power [W]
Main OBC	2	10	2.2
UHF board	5	10	5.5
UHF antenna	0.05	10	0.1
Blades Engine	4	20	4.8
Fluid Pump	3.2	20	3.84
Mass Flow Rate Sensor	0.5	100	1
Lab-on-chip (pH, EC, T)	1e-5	100	2e-5
Lab-on-chip (O2)	1e-5	100	2e-5
Total Power			17.44 W
Margin			20 %
Total Power Budget			20.93 W

Table 6: Preliminary Power Budget.

Scenario	Power Requested [W]	Heaters [W]	Power Available [W]
Hot Case at 65°	20.93	-	49.7
Cold Case at 75°	10.84	17	30.44
Nominal at 75°	20.93	5	30.44

Table 7: Power Availability in different Environment Conditions.

describe the chemical reactions and the oxygen production via *C. Vulgaris* has been set-up. The numerical simulations carried out make possible to evaluate the consumption of both urea and CO_2 and the production of O_2 of a growing concentration of green algae inside the photobioreactor. Moreover, by using multiphysics numerical simulations, the sizing of the urea feeding system has been performed and the correct amount of urea to be supplied in order to reach a steady state condition in the photobioreactor without lack or excess of nutrients has been evaluated. From the subsystem point of view, the thermal control subsystem and the electric power subsystem have been sized in order to guarantee the proper working condition of the entire system on the surface of the Moon in a prescribed range of latitudes (from 65° to 75°). The preliminary sizings performed and the results obtained prove the feasibility of the system proposed that would make possible to study the effects of the Moon environment on an oxygen production system based on green algae cultivation.

The system proposed would allow to perform several experiments with the integrated photobioreactor, but it is acknowledged that some points should be still explored. In particular as future works, a more refined sizing of all the subsystems will be performed and, moreover, an actual test on Earth of the photobioreactor will be performed in a controlled environment in order to characterize and prove its real performances and acquire data to be compared with those that could be obtained from the experiments on the Moon. Finally, another future goal of this work will be to develop a similar system that is able to do the same kind of experiments on the Martian surface, taking advantage of a stronger gravity acceleration and of the CO_2-rich atmosphere of Mars. Another interesting application to be explored could be the coupling of the *C. Vulgaris* cultivation with a plant and/or an animal organism capable of producing proteins in a close-loop system.

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