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Cultivation of Cyanobacteria and Microalgae using Simulated in-situ Available Resources for the Production of useful Bio-compounds on Mars: Modelling of Experiments

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To increase the likelihood of successful long-term manned missions to Mars, it is necessary to explore the potential for utilizing in-situ resources to cultivate microalgae for food and supplement production. This study examines the feasibility of growing *Spirulina platensis* in a medium consisting of high volume percentages of Martian Medium, which is produced using resources available on Mars, such as regolith, atmospheric CO_2, and astronauts' urine. An experimental activity is performed to simulate the microalgae growth process on Mars, demonstrating good productivity. A mathematical model is developed to describe biomass growth dynamics as a function of pH, light intensity, microgravity, and nutrient concentration. The model is validated and then utilized to identify optimal operating conditions for maximizing biomass productivity on Mars and meeting finding the nutritional and supplement needs of a six-member crew.

1. Introduction

Earth resources depletion and the climate crisis require the identification of new sustainable strategies to produce the resources humanity needs. A long-term solution to this problem could be the exploitation of extra-terrestrial resources. According to ISRU (In Situ Resource Utilization) paradigm, Mars is the best candidate to host humanity, due to the availability of resources such as atmospheric CO_2, water and regolith which might be suitably processed to produce useful crucial consumables such as oxygen, water and food (Fais et al., 2022a). Nevertheless, most ISRU technologies so far proposed rely on physic-chemical methods to produce oxygen and propellants but cannot contribute to food production (De Man et al., 2019). Therefore, further research activity is needed to investigate the potential use of ISRU technologies to obtain dry food and, then, sustain manned missions on Mars. In this regard, the use of microalgae to obtain photosynthetic oxygen and edible biomass on Mars is gaining increasing interest (Billi et al., 2021). In this view, a recent patent describes the possibility to cultivate microalgae in the framework of the process to be implemented on Mars that exploits local natural resources (Fais et al., 2022a). This technology is based on the coupling of a chemical-physical section with a biological one that working in synergy can produce water, oxygen, fuel for extra vehicular activity (EVA), building material, and food (Figure 1a). In-situ available resources and metabolic wastes are used along with small amounts of materials brought from Earth are used. Such features of the process determine low payloads that in turn result in a increased techno-economic feasibility of the mission. The main innovative aspect of this technology is the capability to produce food from microalgae in the biological section according to the simplified process schematized in Figure 1b. As it can be seen here photobioreactors are fed with the Martian Medium (MM) produced by mixing regolith leachate and astronauts' urine from the environmental control life system

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(ECLSS) and the fertilizers (ammonia nitrate) produced in chemical physical section. The cultivated microalgae are then used to meet a percentage of the crew food needs as well as oxygen to regenerate cabin air in the ECLSS.

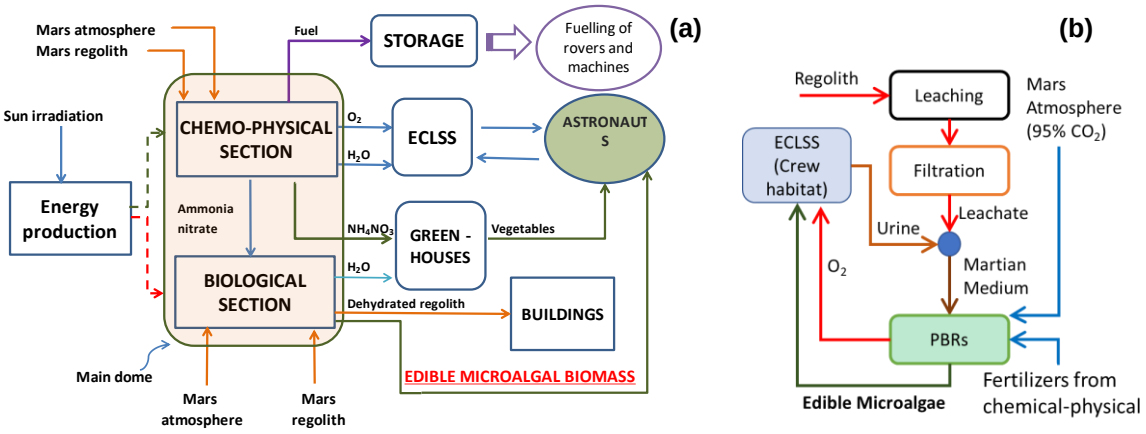

Figure 1. Scheme of the ISRU process to produce useful materials on Mars (a) and focus on the production of microalgae in the biological section (b). Part (a) of the Figure is adapted from Concas et al. (2012).

In the present work, the results of an experimental and modelling activity aimed to evaluate the real feasibility of the process proposed by Fais et al., 2022., are reported. In particular, the experimental activity consisted in growing Spirulina platensis under operating conditions that simulate the ones theoretically occurring in the process implemented on Mars, i.e. pure CO_2 fed to the photobioreactors (PBR), microgravity and MM as growth medium. A mathematical model was then developed to simulate the obtained results and infer useful information about the application of such process on Mars.

2. Materials and methods

2.1 Strain maintenance and preparation of the Martian Medium

The cyanobacterium *Spirulina platensis* was chosen as test organism due to its high nutritional properties that make it a promising candidate to feed the astronauts during long term missions (Fais et al., 2022b). Unialgal culture of cyanobacterium *S. platensis* was obtained from TOLO Green Srl (Arborea, Italy). The strain was maintained under axenic conditions at the laboratory of the Center for Engineering and Environmental Sciences (CINSA) in Cagliari, Italy. The cultures were kept in 250 mL flask, containing 150 mL Zarrouk-medium (ZM). Synthetic Martian Medium (MM) was prepared by mixing a leachate of Martian regolith simulant (JSC MARS-1) and synthetic human urine (MP-AU) to simulate astronauts' urine. Briefly, the regolith leachate was prepared within a 250 mL flask with a cap by contacting 15 g of regolith simulant with 150 mL of ultrapure water. The solid liquid mixture was stirred at 200 rpm with an orbital shaker (Stuart SSM1, Bio sigma) for 24 hours at 25°C, then the solution was filtered by gravity by means of filter paper. MP-AU was produced according to available protocols and diluted with ultrapure water at a ratio of 1:10. Finally, the leachate and diluted urine were mixed (1:1 v/v) to produce MM. A more detailed description of the procedure to produce MMand the final growth media is reported elsewhere (Fais et al., 2022a)

2.2 Growth experiments in microgravity and pure CO_2 atmosphere conditions

Two main growth experiments were carried out. EXP-I: MM40 (MM 40%v/v, ZM 60%v/v), 100% CO_2 atmosphere and microgravity (μg); EXP-II: ZM, 100% CO_2 and μg. Both experiments were meant to simulate the operating conditions taking place on Mars within the dome hosting the process patented by Cao et al (2021). However EXP-I Involved 40% of medium produced in-situ while EXP-II involved only Zarrouk's medium whose components would be brought from Earth. The experiments were carried out in a clinostat (3D Random Positioning Machine, Fokker Space, Netherlands) to simulate μg conditions; to simulate Martian atmosphere a jar (2.5 L) containing pure CO_2 was mounted, allowing to carry 8 culture flasks. The batch culture experiments were carried out into transparent vented cap flasks filled up to 80 mL. The experiment was set in triplicates with an illumination of I_0 =150 μmol m^{-2} s^{-1} and the photoperiod fixed at 12:12 hours light and dark periods. The growth was monitored through absorbance spectrophotometric measurements (Genesys 20 spectrophotometer, Thermo Scientific, Walthmanm, USA) of the chlorophyll-a optic<.al density (OD) of the

culture at 650 nm wavelength. The biomass concentration X (g L^{-1}), calculated through OD measurements and a calibration curve, obtained by gravimetry. Also in this case, a detailed description of the experimental procedure is reported elsewhere (Concas et al., 2023).

3. Mathematical model

The developed model aims to describe microalgae growth by considering the CO_2 mass transfer from gas to liquid phase and chemical composition of the medium. According to the literature (Concas et al., 2021b), the mass balance for biomass X, Eq. (1), is written by considering the death term and the growth term that depends on the limiting factors: average light intensity I_{av}, medium pH and nutrient's concentration, $[C_j]$ with j representing total inorganic carbon (TIC), nitrogen (TIN), phosphorous (TIP).

$$\frac{dX}{dt} = \mu_{max}\left[\prod_{j=1}^{n}\frac{[C_j]}{[C_j]+K_j}\right]\left[\frac{I_{av}}{I_{av}+I_{k,h}}\right]\left[\frac{\frac{k_{0,i}}{k_{1,i}}+\frac{[H^+]}{K_1}}{1+\frac{[H^+]}{K_1}+\frac{[H^+]^2}{K_1K_2}}\right]X - \mu_d X - \theta D X \quad \text{with} \quad X(0)=X^0 \tag{1}$$

The mass balance for TIN and TIP is expressed by Eq (2), where $\theta(I_{av})$ accounts for the growth process dependency on the light: $\theta(I_{av}) = 1$ if $I_{av} > 0$, otherwise $\theta(I_{av}) = 0$.

$$\frac{dC_j}{dt} = \theta\left[-Y_j\frac{dX}{dt} + D\left(C_j{}^0 - C_j\right)\right] \quad \text{with} \quad C_j(0)=C_j{}^0 \quad \text{and} \quad j = TIC,\ TIN,\ TIP \tag{2}$$

with Y_j representing the yield of biomass with respect to the limiting nutrient j. As reported in Eq (3), the mass balance of TIC has to take into account the carbon dioxide mass transfer from gas to the liquid phase:

$$\frac{dC_{TIC}}{dt} = V_r\ k_{l,a}\left(P_{CO_2}H_{CO_2} - [CO_2]\right) + \theta\left[-Y_{TIC}\frac{dX}{dt} + D\left(C_{TIC}{}^0 - C_{TIC}\right)\right] \quad \text{with} \quad C_{TIC}(0)=C_{TIC}{}^0 \tag{3}$$

where V_r is the reactor volume and $k_{l,a}$ the volumetric transfer coefficient. Moreover, the CO_2 dissolution gives rise to the production of CO_3^{2-} and HCO_3^-, as shown in Table 1.

Table 1. Equilibria involving carbon species in the liquid phase and values of equilibrium constants.

ID	Chemical Equilibrium	pK	Units	Ref.
R.1	$CO_2 + H_2O \overset{K_{e1}}{\longleftrightarrow} HCO_3^- + H^+$	4.15×10^{-7}	mol L^{-1}	Perrin et al., 1969
R. 2	$HCO_3^- \overset{Ke2}{\longleftrightarrow} H^+ + CO_3^{2-}$	2.74×10^{-10}	mol L^{-1}	Perrin et al., 1969
R. 3	$H_2O \overset{K_w}{\longleftrightarrow} H^+ + OH^-$	6.84×10^{-15}	mol^2 L^{-2}	Perrin et al., 1969

Accordingly, the total carbon and the electroneutrality equations (Eq.4-5) are considered.

$$C_{TIC} = CO_3^{2-} + HCO_3^- + CO_2 \tag{4}$$

$$0 = Alk + H^+ - OH^- - 2CO_3^{2-} - HCO_3^- \tag{5}$$

where Alk represents the non-carbonatic alkalinity of the medium (Concas et al., 2021a). By deriving Eq (4-5) with respect to the time and using the equilibrium relations to express the species concentration as a function of CO_2 and H^+, Eq (6) and (7) are obtained:

$$\frac{dC_{TIC}}{dt} = \frac{d[CO_2]}{dt}\left(1+\frac{K_{e1}}{[H^+]}+\frac{K_{e1}K_{e2}}{[H^+]^2}\right) + \frac{d[H^+]}{dt}\frac{[CO_2]}{[H^+]^2}\left(K_{e1}+\frac{2K_{e1}K_{e2}}{[H^+]}\right) \tag{6}$$

$$0 = \frac{-d[CO_2]}{dt}\frac{K_{e1}}{[H^+]}\left(1+\frac{2K_{e2}}{[H^+]}\right) + \frac{d[H^+]}{dt}\left(1+\frac{K_w}{[H^+]^2}+\frac{K_{e1}CO_2}{[H^+]^2}+\frac{4K_{e1}K_{e2}[CO_2]}{[H^+]^3}\right) + \frac{d\ Alk}{dt} \tag{7}$$

Eq (6) and (7) represent an algebraic equation system where the time derivatives of $[CO_2]$ and $[H^+]$ are the unknowns whose solution is expressed with Eq (8) and (9).

$$\frac{d[CO_2]}{dt} = \frac{[H^+]}{g([H^+])}\left\{\frac{dC_{TIC}}{dt}\left[[H^+]^3 + K_w[H^+] + k_{e1}[CO_2][H^+] + 4k_{e1}k_{e2}[CO_2]\right] + \frac{d\ Alk}{dt}k_{e1}[CO_2]\left[[H^+] + 2k_{e2}\right]\right\} \quad (8)$$

$$\frac{d[H^+]}{dt} = [H^+]^2\left[\frac{[H^+]^2 + k_{e1}[H^+] + k_{e1}k_{e2}}{g([H^+])}\right]\frac{dAlk}{dt} + \left[\frac{k_{e1}[H^+] + 2k_{e1}k_{e2}}{g([H^+])}\right]\frac{dC_{TIC}}{dt} \quad (9)$$

$$g([H^+]) = \left(K_w + [H^+]^2\right)\left([H^+]^2 + k_{e1}[H^+] + k_{e1}k_{e2}\right) + k_{e1}k_{e2}[CO_2]\left(k_{e1} + 4[H^+]^2\right) \quad (10)$$

Moreover, according to Concas et al. (2021b). the alkalinity variation is described through Eq (10).

$$\frac{d\ Alk}{dt} = -Y_{alk}\frac{dX}{dt} + \theta D\left(Alk^0 - Alk\right) \quad \text{with} \quad Alk(0) = Alk^0 \quad (11)$$

From the mathematical point of view the model consists of an ODEs system which can be easily solved by mens of specific routines available in MATLAB. It should be noted that the value of the dilution rate D was set equal to 0 when using the model to interpret the experimental data since the latter ones were obtained by operating the lab-scale photobioreactors in batch mode. On the contrary, when extrapolating the possibility to use the reactors on Mars in fed-batch mode different values of D were tested.

4. Results and discussions

Figure 2a shows the comparison between the experimental data obtained when cultivating the algae in MM40 or ZM under a CO_2 atmosphere and microgravity conditions. In both cases, the biomass concentration starts growing without showing a significant lag phase until the growth stops when the biomass concentration achieves a kind of steady state witnessing the occurred equivalence of growth and death rate in Eq. 1.

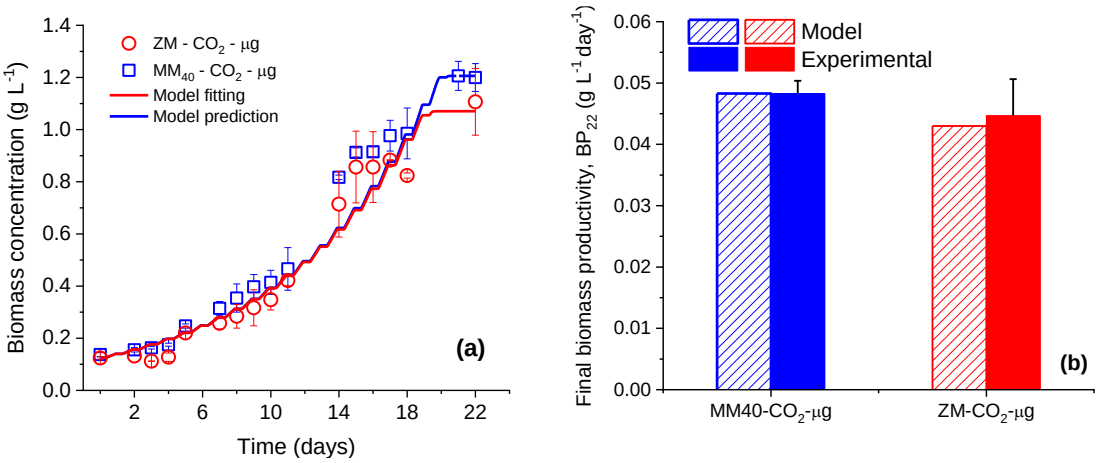

Figure 1: Comparison between experimental and model results in terms of biomass concentration evolution (a) and biomass batch productivity after 22 days (b).

The relevant aspect inferable from Figure 2 is that the alga cultivated in MM40 achieves a plateau at higher concentration in comparison to the ones cultivated in ZM. This means that, as shown in Figure 2b, by using in-situ available resources biomass productivity (0.048 g L^{-1} day^{-1}) higher than the ones obtained with the ZM (0.044 g L^{-1} day^{-1}), which relies only on compounds brought from Earth, could be achieved, with a relevant positive effect on the payload of the mission. It should be noted that the batch biomass productivity (BP_{22}) is calculated at 22 days since this was the time needed by the cultures to achieve the steady state. This higher biomass concentration at the steady state could be due to the higher concentrations of crucial elements (likely iron) in the MM that are probably consumed during the growth in pure ZM. The experimental data related to the use of ZM were fitted by the proposed model by using literature values for specific model parameters and tuning remaining ones. In particular, the kinetic, thermodynamical and biological parameters of the model equations inferred from literature are those reported in Table 2 along with a specific reference while the yield coefficients

Y_{TIP} and Y_{Alk} were identified by fitting the experimental data (in terms of biomass and pH) through the function *fmincon* in MATLAB.

Table 2. Model parameters

Symbol	Value	Units	Refs.
H_{CO_2}	3.89×10^{-4}	$\mathrm{mol\ Pa^{-1}L^{-1}}$	Sander et al., 2015
$I_{k,h}$	6.00×10^{1}	$\mu\mathrm{mol\ m^{-2}s^{-1}}$	Grima et al., 1994
$K_{l,a}$	2.86×10^{1}	$\mathrm{hr^{-1}}$	Klöckner et al., 2012
K_1	5.00×10^{-8}	$\mathrm{mol}\ L^{-1}$	Concas et al. 2021b
K_2	1.00×10^{-8}	$\mathrm{mol}\ L^{-1}$	Concas et al. 2021b
$k_{0,i}$	1.50×10^{-1}	$\mathrm{hr^{-1}}$	Concas et al. 2021b
$k_{1,i}$	1.30×10^{-1}	$\mathrm{hr^{-1}}$	Concas et al. 2021b
K_{TIC}	1.00×10^{-6}	$\mathrm{mol}\ L^{-1}$	Marsullo et al., 2015
K_{TIN}	3.78×10^{-5}	$\mathrm{mol}\ L^{-1}$	Baldia et al., 2007
K_{TIP}	9.04×10^{-7}	$\mathrm{mol}\ L^{-1}$	Baldia et al., 2007
X_0	1.25×10^{-1}	$\mathrm{g\ L^{-1}}$	Measured
μ_{max}	1.00×10^{-2}	$\mathrm{hr^{-1}}$	Jeffryes et al., 2013
μ_d	1.00×10^{-5}	$\mathrm{hr^{-1}}$	Concas et al., 2013
Y_{TIC}	3.70×10^{-2}	$\mathrm{mol\ g^{-1}}$	Cornet et al., 1992
Y_{Alk}	-1.23	$\mathrm{mol\ g^{-1}}$	Tuned
Y_{TIP}	9.27×10^{-5}	$\mathrm{mol\ g^{-1}}$	Tuned

A good matching between experimental and model results was obtained as confirmed by the statistical values of R^2_{adj} = 0.954 and MSE =5.1×10^{-3}. Then the experimental data obtained when using MM40 were correctly predicted (R^2_{adj} = 0.9853 and MSE =7.7×10^{-3}) by the proposed model by keeping fixed the parameter values obtained as previously discussed. Such a good predictivity capability of the model is confirmed by the good between the experimental and model results even in terms of biomass productivity shown in Figure 2b.

While the results so far obtained are promising, it should be stressed that the possible application of the technology described by Fais et al. (2022a) on Mars needs to be further corroborated by additional research activity aimed to verify the effect of the strong variation of operating conditions, such as temperature, light intensity and medium composition, that might take place on Mars. In this regard, the availability of a mathematical model might be strategic since it can provide an "a – priori" information about the response of the system to changing operating conditions. An example of how the model can be viably exploited to infer useful information is to assume that the algae will be grown in an open pond (Figure 3a) operated in fed-batch mode up until a specific time (t_f) on Mars. Indeed, this working mode allows to produce biomass continuously removing the dead-times associated to the operations of growing the culture, reactor emptying and recharging typical of the batch processes. This way higher biomass productivity could be also achieved. For this reason, further simulations were carried out by considering the biomass productivities achievable after different operating for different times (t_f) and using different dilution rates (D) in the equations already reported in the mathematical model section. The corresponding productivity was calculated according to the following equation.

$$BP_{t_f} = \frac{\int_{t_i}^{t_f} D\,X(t)\,dt + X(t_f)}{t_f - t_i} \tag{12}$$

The results obtained by running the model for several values of tf and D are summarized in contour plot of Figure 3b. It can be observed that the best results (5 g m^{-2} hr^{-1}) are obtained by adopting a dilution rate ranging from 3 to 6 hr^{-1} and final times greater than 40 days. The corresponding biomass could be used to feed the crew.

5. Conclusions

The objective of this paper is to simulate and evaluate the growth of microalgae on Mars accordingly to a process recently patented to achieve this, an experimental study is conducted where the Martian atmosphere is replicated, and a growth medium is synthesized using the planet's resources according to the ISRU paradigm. A mathematical model, capable to well predict the experimental results, is developed to accurately simulate the growth process under different operating conditions. The model is then utilized to predict the potential biomass productivity of an open pond located in a Martian dome and operated in fed-batch mode. Future studies can integrate temperature-dependent functions and assess specific kinetic parameters.

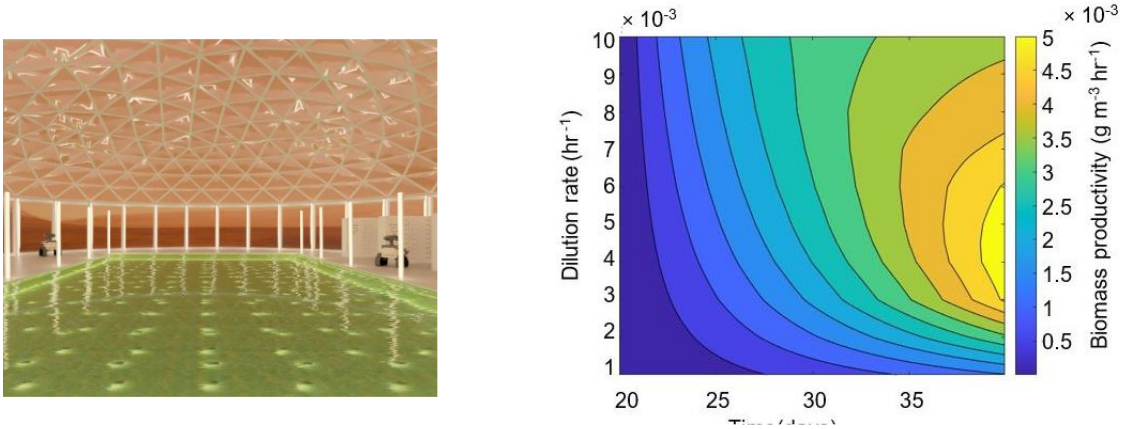

Figure 3. Render of a possible open pond on a Martian dome (a) and simulated effect of the cultivation of S. platensis on Mars in the pond fed-batch mode.

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