

# Experimental and Modelling Investigation on the Use of In-Situ Available Resources to Produce Food and Supplements on Mars Using Microalgae

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To enhance the prospects of long-term human missions to Mars, it is crucial to investigate the use of locally available resources to cultivate microalgae as a sustainable source of food for astronauts. This research investigates the growth of *Chlorella vulgaris* in a culture medium potentially obtainable on Mars using only in-situ available resources, i.e. regolith, atmospheric CO<sub>2</sub> and metabolic wastes of crew. Experiments were performed in this view. The obtained results were then interpreted by a novel mathematical model. The nutritional characteristics of *C. vulgaris* biomass cultivated in such a “Martian” medium were also investigated.

## 1. Introduction

As humanity faces the depletion of Earth's resources and an escalating climate crisis, the need for alternative methods to produce essential supplies has become increasingly urgent. A long-term solution involves relocating human populations to other planets, where survival would depend on harnessing locally available resources. To achieve this, extended crewed missions beyond Earth must be conducted.

Mars has emerged as a promising candidate for sustaining human life, offering key resources such as CO<sub>2</sub>, water, and regolith. Through in-situ processing, these materials could be converted into vital consumables, including oxygen, water, and food (Casula et al., 2024). However, while current in-situ resource utilization (ISRU) technologies on Mars primarily focus on generating oxygen and propellants via physicochemical processes, they remain insufficient for supporting food production—an essential requirement for long-term human missions. Microalgae, particularly *Chlorella vulgaris*, have gained attention due to their ability to produce oxygen, edible biomass, and nutritional supplements for Mars crews. Recent advancements suggest that microalgae could be a viable solution for producing essential materials, including food, with minimal reliance on Earth-derived resources (Fais et al., 2022). This approach could significantly reduce the payload required for Mars missions, thereby enhancing their technical and economic feasibility.

To evaluate the practicality of this method, our study focuses on cultivating *C. vulgaris* in a simulated Martian environment using Martian regolith leachate, atmospheric CO<sub>2</sub>, and synthetic human urine. Experiments conducted under Mars-like conditions achieved favorable productivity levels, demonstrating the potential of this cultivation approach. Additionally, we developed a mathematical model to predict biomass growth dynamics under Martian conditions, considering factors such as pH, carbon availability, and limiting nutrient concentrations. This model could help determine optimal conditions for maximizing biomass productivity to meet the nutritional needs of a long-duration Mars mission crew.

Ultimately, this study aims to demonstrate that *C. vulgaris* can thrive and generate substantial amounts of algae-based food using only in-situ resources during a crewed Mars mission. This breakthrough would

significantly improve mission feasibility by drastically reducing payload requirements and increasing self-sufficiency for long-term human habitation on Mars.

## 2. Materials and Methods

### 2.1 Microalgal strain

Cells of *C. vulgaris* CCALA 269 were obtained from the Culture collection of autotrophic organisms (CCALA, Czech Republic). The strain was maintained in Bold Basal Medium (BBM) at 20 °C in a 5-L laboratory bottle under a light intensity, measured by a suitable luxmeter, of 40  $\mu\text{mol}_{\text{ph}} \text{m}^{-2} \text{s}^{-1}$ . A photoperiod of 12 h/12 h day/night was adopted. Cultures were agitated at 70 rpm by using a rotary shaker.

### 2.2 Simulant of culture medium obtainable on Mars

A growth medium potentially attainable on Mars (MM) using in-situ available resources was produced by mixing the leachate of a regolith simulant (RL) with diluted synthetic human urine (SU) as reported by Fais et al. (2022). The regolith leachate (RL) was obtained by contacting a suitable amount of JSC Mars-1 regolith simulant with a given volume of distilled water (pH 6.80) and then stirring the solid-liquid mixture for 24 h at 25 °C. SU was produced according to Sarigul et al. (2019) and diluted with ultrapure water at a ratio of 1:1e-0 v/v. Subsequently, RL and diluted SU were mixed (1:1 v/v) to obtain the MM. Different dilutions of MM with bicarbonate-enriched BBM were then evaluated during as growth media for *C. vulgaris* cultivation. Specifically, the dilutions are reported in the following table.

Table 1. Volumetric composition of the investigated media

Medium	MM0	MM20	MM40	MM60	MM100
BBM (vol %)	100	80	60	40	0
MM (vol %)	0	20	40	60	100

### 2.3 Growth experiments

An inoculum (5 mL) of *C. vulgaris* CCALA 269 in exponential phase was fed to 15 vented-cap, transparent flasks filled with 25 mL each of one among the five experimental growth media described above, in triplicate. The strain was cultivated at  $25 \pm 1$  °C under continuous stirring at 70 rpm using a white light intensity of 80  $\mu\text{mol}_{\text{ph}} \text{m}^{-2} \text{s}^{-1}$  provided from above. Microalgal growth was monitored through daily OD measurements. Biomass concentrations were obtained from OD via a calibration line providing OD as function of biomass. pH was also assessed daily. To reproduce the use of Mars atmosphere an apparatus was set up consisting of a transparent insulated jar wherein pure CO<sub>2</sub> (100% vol) was continuously blown in. The transparent flasks were disposed within the mentioned jar to simulate a Martian dome filled with pressurized Martian atmosphere according to the process proposed by Fais et al. (2022).

### 2.4 Harvesting and biomass characterization

After 17 days of cultivation time, microalgal cultures were harvested and centrifuged. Supernatant was then removed, and pellets were resuspended in distilled water. The procedure was repeated three times. The obtained pellets were subsequently frozen at -80 °C, then lyophilized and finally grinded to powder by mortar and pestle. Different amounts of microalgal powder were then subjected to further different processing depending on the biochemical parameter to be determined. Briefly, carbohydrates were analysed through a slightly modified version of the method by Dubois et al. (1956). The total content of soluble protein was evaluated as reported by Lowry et al. (1951). After cell disruption the total lipid content were extracted and determined according to the procedure by Folch et al., (1957). Finally, total carotenoids content was evaluated through the method by Zavrel et al. (2015). The details on the specific analyses being performed can be found elsewhere (Casula et al., 2024 b).

## 3. Mathematical Model

The evolution of main macro- and micro-nutrients' concentration during cultivation was analysed to identify the growth limiting nutrient (data not shown). At the end of the growth phase, when the stationary one took place, the intracellular concentration of iron, evaluated through proper analyses and mass balances (data not shown) was lower than the minimum iron cell quota for *C. vulgaris* growth according to the literature (Concas et al., 2021). Therefore, the following mathematical model was developed considering iron and inorganic carbon concentration along with pH as factors controlling *C. vulgaris* growth.

### 3.1 Model Equations

The model equations are the mass balances for: (1) total microalgal biomass concentration ( $b$ ); (2) the limiting substrate (i.e.  $S = [Fe]$ ) in the liquid bulk; (3)  $Fe$  in the intracellular compartment; (4)  $CO_2$  in the gas phase; (5) the non-carbonic alkalinity ( $Alk$ ); (6)  $[H^+]$  in the liquid bulk and finally (7)  $[CO_2]$  in the liquid bulk. Eqs (1) to (3) derive from the application of the well-known Droop model to the concerned system (Concas et al., 2021) while Eq. (7) is obtained from the application of the electroneutrality principle to the investigated system. Finally Eqs. (4) to (5) and (7) to (8) are obtained considering mass transfer of  $CO_2$  from gas phase to liquid and its subsequent speciation in a liquid with a given pH. Eq (5) also considers that inorganic carbon is consumed by microalgae in the liquid phase. Eqs (6) and (7) are obtained through the novel approach proposed by Atzori et al. (2023) that allows to avoid solving a differential algebraic system of equations.

$$\frac{dX}{dt} = \mu_{max} \left( 1 - \frac{q_{min}}{q} \right) \frac{[C_{TIC}]}{[C_{TIC}] + K_{TIC}} X \quad (1)$$

$$\frac{dC_{Fe}}{dt} = -\rho_{max} \frac{C_{Fe}}{K_{Fe} + C_{Fe}} X \quad (2)$$

$$\frac{dq}{dt} = \rho_{max} \frac{C_{Fe}}{K_{Fe} + C_{Fe}} - \mu_{max} \left( 1 - \frac{q_{min}}{q} \right) q \quad (3)$$

$$\frac{dP_{CO_2}}{dt} = -K_I a \frac{V_F}{V_F - V_L} (P_{CO_2} H_{CO_2} - [CO_{2,L}]) RT \quad (4)$$

$$\frac{d[C_{TIC}]}{dt} = \frac{V_F}{V_L} K_I a (P_{CO_2} H_{CO_2} - [CO_{2,L}]) - Y_{TIC} \frac{dX}{dt} \quad (5)$$

$$\frac{d[Alk]}{dt} = -Y_{alk} \frac{dX}{dt} \quad (6)$$

$$\frac{d[H^+]}{dt} = \frac{[H^+]^2}{g([H^+])} \left\{ \begin{aligned} & \left[ [H^+]^2 + K_{1C}[H^+] + K_{1C}K_{2C} \right] \frac{d[Alk]}{dt} \\ & + \left[ K_{1C}[H^+] + 2K_{1C}K_{2C} \right] \frac{d[C_{TIC}]}{dt} \end{aligned} \right\} \quad (7)$$

$$\frac{d[CO_{2,L}]}{dt} = \frac{[H^+]}{g([H^+])} \left\{ \begin{aligned} & \left[ [H^+]^3 \frac{d[C_{TIC}]}{dt} + [H^+] k_w \frac{d[C_{TIC}]}{dt} \right. \\ & + K_{1C}[CO_{2,L}][H^+] \frac{d[C_{TIC}]}{dt} + K_{1C}[CO_{2,L}][H^+] \frac{d[Alk]}{dt} \\ & \left. + 4[CO_{2,L}]K_{1C}K_{2C} \frac{d[C_{TIC}]}{dt} + 2[CO_{2,L}]K_{1C}K_{2C} \frac{d[Alk]}{dt} \right] \end{aligned} \right\} \quad (8)$$

$$g([H^+]) = [H^+]^2 + K_C [H^+]^2 + K_{1C} K_C [H^+] + K_{1C} K_{2C} K_C \quad (9)$$

The detailed symbol significance is reported in the nomenclature. The system of ordinary differential equations (ODEs) is solved along with the initial conditions of each variable via numerical routines available in MATLAB.

## 4. Results and discussion

Figure 1 shows the comparison between experimental model results obtained when cultivating *C. vulgaris* in the investigated media (Table 1) under a pure  $CO_2$  atmosphere. In all cases, the biomass concentration starts growing without showing a significant lag phase until the growth stops when the internal cell quota of Fe achieved the minimum value capable to sustain growth. From the practical point of view, the relevant aspect deducible from Figure 1 is that the *C. vulgaris* cultivated in MM100, i.e. a medium obtainable by exploiting only in-situ available resources on Mars, grew till the end of the experiment at a rate very close to the one observed when using BBM medium. Indeed, the biomass productivities (Figure 1) evaluated at the end of the experiments were all very close (around  $0.1 \text{ g L}^{-1} \text{ day}^{-1}$ ) without a statistically significant difference. The capability of *C. vulgaris* to grow in medium obtainable using only in-situ available resources only is very important in view of its application on Mars also because it would reduce dramatically the need of compounds to bring from Earth and, correspondingly, the payload of the mission.

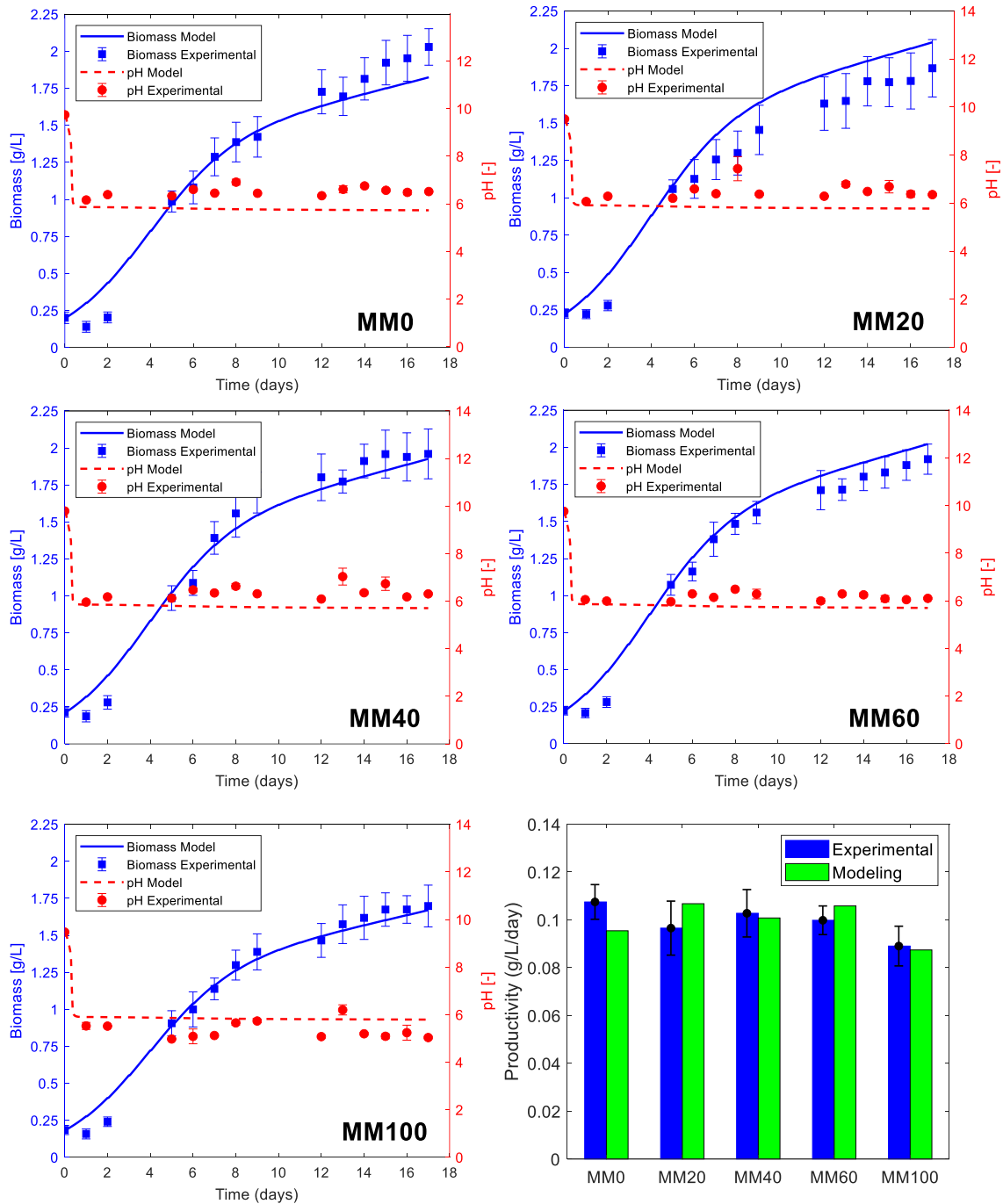


Figure 1. Microalgae biomass concentration and pH time evolution in the cultures with different percentage of Martian Medium. Last figure summarizes the biomass productivities achieved with the different media.

The experimental data in Figure 1 were well simulated by the proposed model when using the model parameters in Table 1. It should be noted that most parameters values were taken from the literature since only two of them,  $\mu_{max}$  and  $\rho_{max}$ , were suitably tuned by using the function `fmincon` in MATLAB. It should be noted that the values obtained of the two adjusted parameters are consistent with those reported in the literature for similar systems. It should be noted that fitting was performed using only two series of data, i.e. pH Vs time and X Vs time for MM0, while in the remaining simulations the obtained values of the above parameters were kept constant so that simulations could be considered predictions. The mean square errors (MSE) were in the range 0.02 - 0.12 for the biomass variable and 0.32-0.38 for pH thus demonstrating the good predictive capability of the model.

Table 2. Model parameters

Parameter	Value	Units	Reference
$H_{CO_2}$	$3.4 \times 10^{-4}$	$M Pa^{-1}$	Atzori et al., 2023
$K_{Ia}$	$2.93 \times 10^{-4}$	$hr^{-1}$	Klöckner et al. 2013
$Y_{TIC}$	$4.1 \times 10^{-2}$	$mol g^{-1}$	Concas et al., 2021
$Y_{Alk}$	$1.00 \times 10^{-5}$	$mol g^{-1}$	Concas et al., 2021
$k_w$	$1.01 \times 10^{-14}$	$M^2$	Bates and pinching 1949
$K_C$	$10^{-2.77}$	$M$	Atzori et al., 2023
$K_{1C}$	$10^{-6.1}$	$M$	Atzori et al., 2023
$K_{2C}$	$10^{-9.2}$	$M$	Atzori et al., 2023
$b_0$	$1.4 - 2.2 \times 10^{-1}$	$g L^{-1}$	Experimental value
$s_0$	$1.3 - 1.9 \times 10^{-3}$	$g L^{-1}$	Experimental value
$q_0$	$5.5 \times 10^{-3}$	-	Concas et al., 2021
$K_S$	$9.5 \times 10^{-6}$	$g L^{-1}$	Concas et al., 2021
$q_{min}$	$4 \times 10^{-4}$	-	Concas et al., 2021
$\mu_{max}$	$2.73 \times 10^{-2}$	$hr^{-1}$	This work
$\rho_{max}$	$6.49 \times 10^{-7}$	$hr^{-1}$	This work

In Figure 2 the content of total carbohydrates, proteins, lipids and carotenoids in the biomass cultivated in the different media is shown. Except for slight and not statistically significant variations, the macro-biochemical composition of the obtained biomass was not affected by the percentage of MM.

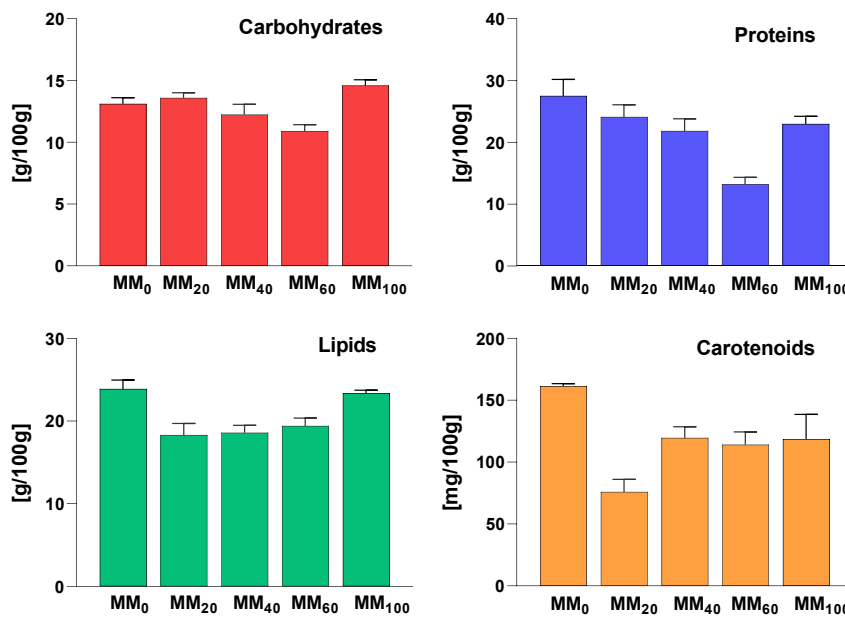


Figure 2. Effect of MM percentage in the growth media on the biochemical composition of the obtained biomass.

Since the composition of *C. vulgaris* biomass obtained using only MM as the growth medium (MM100) was more or less similar to the one obtained using typical growth medium (MM0 that is BBM) and the latter is generally recognized as safe (GRAS) by the US Food and Drugs Administration (Gouda et al., 2022), it can be deduced an edible biomass capable to feed the astronauts can be potentially produced by relying only on in-situ available resources on Mars.

## 5. Conclusions

*C. vulgaris* was capable to grow in a medium potentially obtainable using only in-situ available resources during a crewed mission (MM100) on Mars without relevant differences respect to the growth in a classical growth medium. Good productivities and nutritional characteristics were obtained at the end of cultivation period thus demonstrating the feasibility of using *C. vulgaris* as a source of food for the astronauts involved in the mission. A suitable mathematical model was capable to well capture the experimental results thus representing a potentially useful tool to design the implementation of this technology on Mars.

## Nomenclature

a – interfacial area, $\text{m}^2 \text{m}^{-3}$	q – iron cell quota, $\text{g g}^{-1}$
Alk – non carbonic Alkalinity, $\text{g L}^{-1}$	S – iron bulk concentration, $\text{g L}^{-1}$
$C_{\text{Fe}}$ – iron bulk concentration, $\text{g L}^{-1}$	T – temperature, K
$C_{\text{TIC}}$ – total inorganic carbon, $\text{g L}^{-1}$	$V_{\text{F}}$ – volume of flasks, L
$H_{\text{CO}_2}$ – henry constant $\text{CO}_2$ , $\text{M atm}^{-1}$	$V_{\text{L}}$ – volume of liquid, L
$K_{1\text{C}}$ – dissociation constant of $\text{H}_2\text{CO}_3$ , M	X – biomass concentration, $\text{g L}^{-1}$
$K_{2\text{C}}$ – dissociation constant of $\text{HCO}_3^-$ , M	$Y_{\text{alk}}$ – Alkalinity yield, $\text{mol g}^{-1}$
$K_{\text{I}}$ – mass transfer coefficient, $\text{m s}^{-1}$	$Y_{\text{alk}}$ – Alkalinity yield, $\text{mol g}^{-1}$
$K_{\text{TIC}}$ – half saturation of C, $\text{mol L}^{-1}$	$\mu_{\text{max}}$ – Max specific growth rate, $\text{day}^{-1}$
$K_{\text{Fe}}$ – half saturation of Fe, $\text{g L}^{-1}$	$\rho_{\text{max}}$ – Max specific absorption rate of Fe, $\text{day}^{-1}$
$P_{\text{CO}_2}$ – pressure of $\text{CO}_2$ , atm	

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