



A mathematical model of microalgae growth in a pilot scale thin-layer photobioreactor

Giuseppe Gargano^{a,b,*}, Alessandro Concas^{c,e}, Giacomo Cao^{c,e,f},
Francisco Gabriel Ación Fernández^{a,d}

^a Department of Chemical Engineering, Universidad de Almería, E04120, Almería, Spain

^b Biorizon Biotech S.L., Parque Científico-Tecnológico de Almería (PITA), 04131 Retamar-Almería, Spain

^c Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Piazza d'Armi, 09123, Cagliari, Italy

^d CIESOL Solar Energy Research Centre, Joint Centre University of Almería-CIEMAT, 04120, Almería, Spain

^e Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc. Piscina Manna, Building 1, 09050, Pula, CA, Italy

^f Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, 09124, Cagliari, Italy

ARTICLE INFO

Keywords:

Microalgae
Modelling
Photosynthesis rate
Photobioreactors
Thin-layer

ABSTRACT

Thin-layer photobioreactors represent a promising technology for high-productivity microalgae cultivation, yet their scale-up and optimization remain limited by the lack of validated dynamic models. In this work, a comprehensive first-principles dynamic model describing the growth of *Nannochloropsis gaditana* in a pilot-scale thin-layer photobioreactor (2.4 m³, 63 m² illuminated surface, 0.023 m liquid depth) is developed, calibrated, and validated for the first time. The model integrates biological kinetics with mass and energy balances, explicitly accounting for photosynthetically active radiation, ambient temperature, gas–liquid mass transfer, CO₂ injection, and oxygen stripping. Model predictions accurately reproduce the temporal evolution of biomass concentration, pH (7.5–8.5), temperature (15–27 °C), and dissolved oxygen (up to 200% air saturation) under outdoor operating conditions. Validation against independent experimental data confirms the predictive capability of the model under both batch and semi-continuous regimes. Model-based analysis indicated that dissolved oxygen accumulation acts as a measurable inhibitory factor affecting photosynthetic activity along the thin-layer channel. Although local oxygen inhibition may reduce photosynthetic activity by up to 50% during peak daylight hours, its overall impact on daily biomass productivity under the operating conditions investigated remained moderate. Simulations predicted that maintaining dissolved oxygen concentrations close to air saturation through enhanced oxygen stripping would increase biomass productivity by approximately 5%. In contrast, dilution rate optimization emerged as the most effective operational strategy, with theoretical productivity improvements of up to 70–95%, while more practical operating conditions were predicted to increase productivity by approximately 45–60% relative to the baseline operation. This study provides the first dynamic model validated at pilot scale for thin-layer photobioreactors, offering a robust tool for their design, optimization, and control toward large-scale microalgae production.

1. Introduction

Microalgae are an extremely diverse group of primary producers found in nearly all ecosystems on Earth, from marine or freshwater, desert sands, hot springs, snows and glaciers. They are unicellular organisms capable of using solar energy, carbon dioxide and ions dissolved in water for the synthesis of complex organic molecules through the process of photosynthesis and are responsible for more than half of the primary productivity underlying the food chain [1]. Currently,

microalgae are used in high-value applications from the pharmaceutical to nutraceutical, cosmetic and food industries [2,3]. Furthermore, these microorganisms have also been proposed as potentially useful for other applications such as biofuel production, wastewater treatment and CO₂ mitigation [4,5]. Moreover, the cultivation of microalgae can be combined with the bio-capture of the carbon dioxide emitted by industrial activities that make use of fossil fuels for energy production [6].

Regardless of the purpose, the most important part of the production process is the photobioreactor which must be designed based on several

* Corresponding author at: Department of Chemical Engineering, Universidad de Almería, E04120, Almería, Spain.

E-mail address: giuseppe@biorizon.es (G. Gargano).

<https://doi.org/10.1016/j.algal.2026.104871>

Received 10 February 2026; Received in revised form 5 July 2026; Accepted 29 July 2026

Available online 30 July 2026

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factors (the microorganism, biomass quality and production capacity). The most widely used photobioreactors at the industrial scale, now, are raceways ponds and tubular reactors. The first ones are currently the most used equipment to produce microalgae worldwide; over 95% of world production is achieved using these reactors, mainly due to their low cost, easy scalability and low energy requirements [7]. In this type of photobioreactor, the biomass concentration is around 1.0 g L^{-1} due to the relatively high depth of the culture medium. This aspect facilitates contamination of the culture by other microorganisms and increases the risk of failure which leads to a significant reduction in the overall productivity of these systems. To minimize such drawbacks, these reactors are used to produce only robust and fast-growing strains that are tolerant to extreme conditions. On the other hand, tubular photobioreactors are typically used to improve biomass productivity independently of the microalgae strain [8]. However, in these reactors, which are more difficult to scale up and more expensive [9], the biomass concentration range was $1.0\text{--}3.0 \text{ g L}^{-1}$ due to the shallower water depth determined by the diameter of the tube. Compared to raceway reactors, the tubular ones are closed and achieve higher biomass concentration thus reducing the issues related to contamination as well as the risk of failure.

The need to obtain higher productivity in a more economically sustainable way has led to the development of two other types of reactors which, however, are not currently used at an industrial level, as they are not optimized: thin-layers and flat-panels reactors. Their main advantage is to be able to minimize the water depth, even below 0.01 m , thus maximizing the availability of light to which cells are exposed, and therefore the productivity of the biomass. A key advantage of thin-layer reactors is efficient mixing, which induces a rapid light-dark (L / D) cycle of the cells. In these systems, cells are exposed to light every few milliseconds, even at liquid velocities of 0.5 m s^{-1} , and high biomass concentrations up to 20 g L^{-1} are achievable [10]. In addition, the higher biomass concentrations typically achieved in thin-layer photobioreactors can facilitate downstream harvesting and reduce biomass recovery costs compared with conventional raceway systems. The relevant availability of light in these reactors leads to a higher oxygen production due to the intense photosynthetic activity. In 80 m long thin-layer reactors, the concentration of dissolved oxygen along the channel increases so much due to photosynthesis, that it can reach values close to 250% of the saturation one at the end of the channel thus leading to a relevant worsening of the performance of microalgae cells [11]. Despite these drawbacks, the biomass productivity already reported in this type of reactor is one of the highest compared to other types and can still be doubled [11].

In general, while extremely promising, thin-layer reactors have not been yet optimized, therefore major problems are typically observed such as inadequate pH control, low-efficiency CO_2 transfer and excess temperature [11]. In this regard, mathematical models capable of suitably describing the phenomena taking place are very helpful for designing, optimising, and operating these systems correctly [12]. Although several dynamic models have been proposed for raceway and outdoor photobioreactors, spatially distributed models specifically developed for outdoor thin-layer reactors remain scarce in the literature. Unlike conventional raceway reactors, which are commonly represented as ideally mixed systems or a series of mixed compartments due to the strong recirculation induced by paddlewheel operation, thin-layer photobioreactors combine a shallow liquid film with a high surface-to-volume ratio and an elongated flow path. These characteristics promote the development of longitudinal gradients of dissolved gases, temperature, light availability, and photosynthetic activity along the reactor channel. Consequently, a spatially distributed modelling approach is required to adequately describe the coupled transport, thermal, and biological processes occurring in thin-layer systems under outdoor operating conditions. For this reason, a comprehensive dynamic model of microalgae growth in thin-layer reactors is proposed in this work for the first time. The model, based on the first principles of mass

and energy conservation, takes into account mass transfer, chemical reactions, light penetration, ion speciation and biological phenomena occurring within the reactor thus allowing to simulate the temporal evolution of biomass concentration, pH, dissolved oxygen and total inorganic carbon in the liquid phase, as well as oxygen and carbon dioxide variations in the gas phase. The model was calibrated and validated using experimental data from a 2.4 m^3 thin-layer reactor and was used to carry out performance analysis and identify possible optimization strategies. The originality of the proposed framework lies in the coupled spatial modelling of biological, thermal, and gas-liquid transport phenomena under outdoor conditions in pilot-scale thin-layer photobioreactors.

2. Materials and methods

2.1. Microorganism and culture medium

The algal strain used was *Nannochloropsis gaditana* (CCAP 849/5), which is an autotrophic organism growing at pH values ranging from 7.5 to 8 [13] and salinity varying from 3.6 to 44 g L^{-1} [14] although the best condition of this latter parameter was estimated to be 31 g L^{-1} [15]. Experiments were performed using Mann & Meyers saltwater medium, prepared using fertilizers instead of pure chemicals: CaNO_3 0.8 g L^{-1} , KH_2PO_4 0.1 g L^{-1} , MgSO_4 0.35 g L^{-1} , Karentol 0.03 g L^{-1} .

2.2. Photobioreactor and growth conditions

The thin-layer reactor used in this research (Fig. 1) was located in the IFAPA research centre (Almeria, Spain) as part of the SABANA project. The experiments were conducted in late spring. As far as the configuration is concerned, the reactor was composed of three main parts, i.e. a degassing column, a channel and a recirculation tank. The degassing column (230 L , 1.8 m high, with a diameter of 0.40 m) allows to reduce the concentration of dissolved oxygen, through the continuous air injection (100 L min^{-1}) and to keep the pH constant at 8, through CO_2 injections (10 L min^{-1}) managed by the control system. The latter one is an On/Off type and is built so that air and CO_2 cannot be injected at the same time. Downstream of the degassing column, the culture flows through a thin-layer channel 40 m long and 1.575 m wide, with an average liquid film thickness of 0.023 m , resulting in an operating volume of 1.45 m^3 and an illuminated surface area of 63 m^2 . The channel is constructed as a single continuous fiberglass section with a 1% slope and does not include intermediate cascade steps or segmented compartments. Under the operating conditions adopted in this study, the culture circulated at an average superficial velocity of 0.19 m s^{-1} , corresponding to a residence time of 210 s along the illuminated section. Based on the characteristic liquid depth and flow velocity, the Reynolds number was 1.75×10^4 , for the falling film. The Reynolds number was calculated based on the hydraulic diameter ($D_h = 4\delta$) as $Re = \frac{4\delta \cdot v}{\nu}$, assuming the fluid properties of water at 20°C ($\nu = 1.0 \cdot 10^{-6} \text{ m}^2\text{s}^{-1}$). This value confirms that the flow operates safely within a fully developed turbulent regime ($Re > 1500$). These hydrodynamic conditions promote continuous renewal of the illuminated liquid layer, enhance gas-liquid mass transfer, and facilitate the development of longitudinal gradients of dissolved oxygen, carbon dioxide, temperature, and photosynthetic activity along the flow path. The high surface-to-volume ratio characteristic of the thin-layer configuration further improves light availability and mass transfer compared with conventional raceway reactors. These spatial gradients constitute one of the distinctive features of the reactor and are explicitly represented in the modelling framework. At the end of this section, there are dissolved oxygen (Mettler Toledo In / Pro 6050/120), pH and temperature (Crison 5342 T) sensors that continuously record the value of these parameters. The last section is a recirculation tank, whose main purpose is to collect the culture and then send it back to the column through a fixed speed pump (Astralpool 38,776, 1.4 kW ,

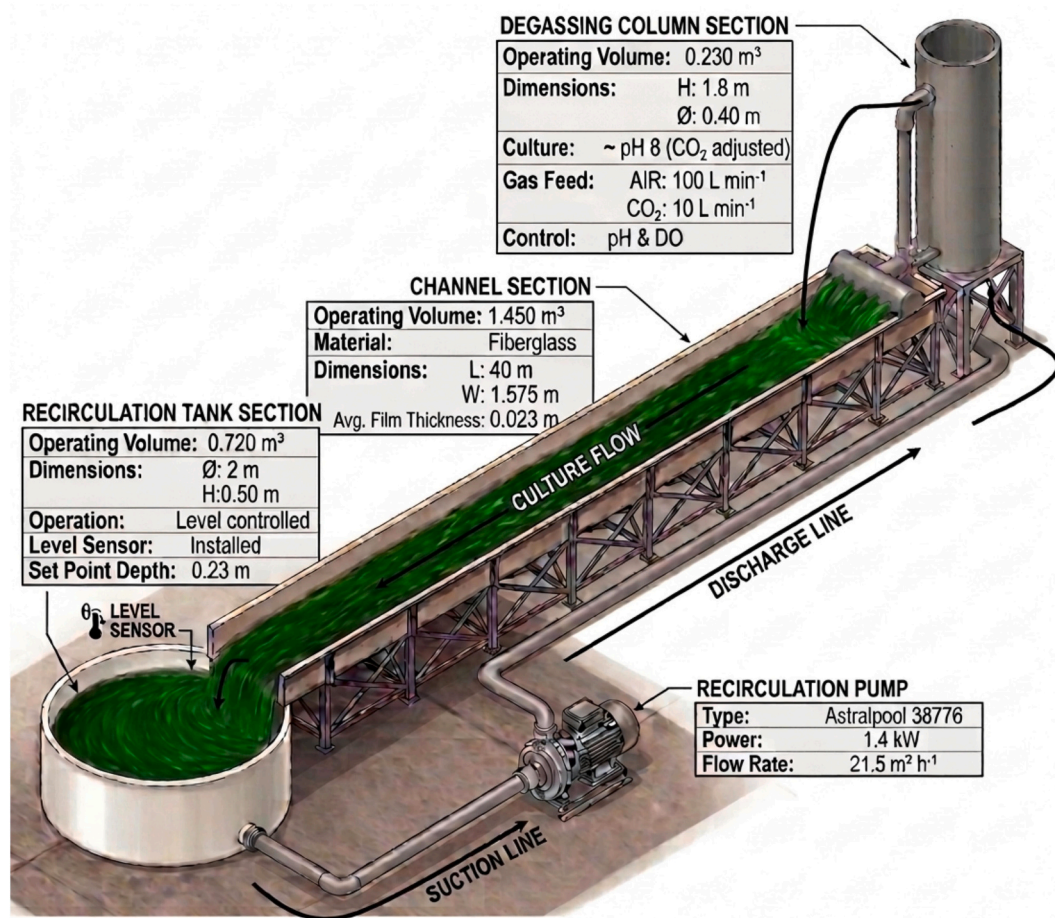


Fig. 1. Schematic representation of the reactor under analysis showing the main sections: degassing column, channel and recirculation tank.

21.5 m³ h⁻¹). The tank has a diameter of 2 m and a depth of 0.50 m. This section is equipped with a level sensor used to manage and compensate for evaporation, with a set-point fixed at 0.23 m, corresponding to an operating volume of 0.72 m³. The reactor under analysis was placed inside a greenhouse provided with continuous sensors for photosynthetic active radiation (PAR) and temperature. The photobioreactor was operated in batch mode for one week and subsequently switched to semi-continuous operation by daily withdrawing 0.72 m³ of culture from a total operating volume of 2.4 m³ (dilution rate: 0.3 d⁻¹) and replacing it with an equal volume of fresh medium.

3. Mathematical model

The model consists of two parts: a growth model and an engineering model. The first one describes the phenomenon of photosynthesis coupled with the growth of the alga. The second section takes into account the physical and chemical phenomena that occur inside the reactor. In addition to photosynthetic activity, phenomena of mass transfer between liquid and gaseous states and energy exchanges between the reaction volume and the external environment are quantitatively described.

3.1. Growth model

The growth model is based on the photosynthetic rate (P_{O_2}) of the concerned microorganism expressed in terms of oxygen developed in the unit of time per unit of algal mass-produced. For photosynthetic organisms, this rate is mainly related to the average density of photon flux in the culture I_{av} ($\mu E m^{-2} s^{-1}$), which can be calculated through Eq. 1 and depends on the incident external radiation (I_0 , $\mu E m^{-2} s^{-1}$), the water

depth (d , m) into the reactor and the concentration of biomass (C_b , gm⁻³) that absorb and scatters the incident light [16], where symbols' significance is reported in the notations section.

$$I_{av} = \frac{I_0}{KaC_b d} (1 - \exp(-KaC_b d)) \quad (1)$$

The average photon flux density within the culture (I_{av} , $\mu E m^{-2} s^{-1}$) represents the effective light available to the microalgal cells after attenuation by absorption and scattering phenomena. This variable is calculated from measurable reactor-scale parameters, namely the incident photosynthetically active radiation (PAR), the culture depth, and the biomass concentration. The extinction coefficient (Ka , m²g⁻¹) accounts for the combined effects of light absorption and scattering by the microalgal suspension, while the culture depth (d , m) and biomass concentration (C_b , gm⁻³) determine the optical path and, consequently, the degree of light attenuation within the reactor. Therefore, Eq. (1) provides a reactor-scale estimate of the average light environment experienced by the culture and establishes the link between external irradiance measurements and the biological growth model. Although individual cells are exposed to rapidly fluctuating light conditions due to mixing and self-shading effects, the use of average irradiance is a widely adopted approximation in dynamic photobioreactor models. While local light fluctuations and light-dark cycles are not explicitly resolved, this approach was considered appropriate for the reactor-scale simulation and optimization objectives of the present study, providing a reasonable compromise between biological realism and computational efficiency. The photosynthetic rate also considers other factors that affect microalgae growth such as dissolved oxygen, pH and temperature of the culture. In particular, under conditions of excess nutrients, the rate of oxygen production, P_{O_2} (gO₂g_{biomass}⁻¹s⁻¹), mainly depends on the average

density of photon flux and the physiological state of the culture, namely its dissolved oxygen level, temperature, and pH (Eq. 2, [17]):

$$P_{O_2} = P_{O_2}(I_0) \bullet \overline{P_{O_2}(O_2)} \bullet \overline{P_{O_2}(pH)} \bullet \overline{P_{O_2}(T)} - rP_{O_2,MAX} \quad (2)$$

The model also accounts for respiratory losses through the parameter r , expressed as the fraction of the maximum photosynthetic activity consumed by respiration (Eq. 2). Consequently, the term P_{O_2} represents the net oxygen production rate after subtraction of respiration effects. The photosynthetic oxygen production rate (P_{O_2}) is expressed as the net oxygen generation rate per unit biomass and constitutes the primary biological variable describing microalgal activity in the model. The formulation incorporates the effects of light availability, dissolved oxygen concentration, pH, and temperature through multiplicative correction factors. These variables correspond to quantities that are either continuously measured in the reactor (temperature, dissolved oxygen, and pH) or calculated from operational measurements (average photon flux density), thereby establishing a direct connection between the mathematical model and experimentally accessible process variables. The dependence on the average photon flux density, $I_{av}(\mu Em^{-2} s^{-1})$, was described according to the formulation proposed by Molina Grima [16]. In this formulation, the oxygen production rate is expressed as a function of the maximum oxygen production rate, $P_{O_2,MAX}(g_{O_2} g_{biomass}^{-1} s^{-1})$, the saturation constant, $K_I(\mu Em^{-2} s^{-1})$, and a dimensionless shape parameter, n , which controls the curvature of the light-response relationship.

The effect of dissolved oxygen concentration, $C_{O_2}(g m^{-3})$, was represented through an inhibition function relating the local oxygen concentration to the strain-specific inhibitory oxygen concentration, $K_{O_2}(g m^{-3})$. A dimensionless shape parameter, Z , was included to describe the sensitivity of the culture to oxygen accumulation (Eq. 4; [17]). The dissolved oxygen correction was applied only to the photosynthetic oxygen production term, in order to account for the negative effect of excessive oxygen accumulation on photosynthetic activity. This correction was not applied to the respiration term, since dissolved oxygen inhibition of respiration would not be physiologically justified under the conditions considered in this study. The effects of pH (Eq. 5, [17]) and culture temperature (Eq. 6, [18]), $T(K)$, were modelled by relating the instantaneous operating conditions to the minimum, optimum, and maximum values characteristic of the considered microalgal strain. These functions account for the progressive reduction in oxygen production when pH or temperature deviate from their optimal ranges.

$$P_{O_2}(I_0) = P_{O_2,MAX} \frac{I_{av}^n}{K_I^n + I_{av}^n} \quad (3)$$

$$\overline{P_{O_2}(O_2)} = \left(1 - \left(\frac{C_{O_2}}{K_{O_2}} \right)^Z \right) \quad (4)$$

$$\overline{P_{O_2}(pH)} = \left(\frac{(pH - pH_{MAX})(pH - pH_{MIN})^2}{(pH_{OPT} - pH_{MIN})[(pH_{OPT} - pH_{MIN})(pH - pH_{OPT}) - (pH_{OPT} - pH_{MAX})(pH_{OPT} + pH_{MIN} - 2pH)]} \right) \quad (5)$$

$$\overline{P_{O_2}(T)} = \left(\frac{(T - T_{MAX})(T - T_{MIN})^2}{(T_{OPT} - T_{MIN})[(T_{OPT} - T_{MIN})(T - T_{OPT}) - (T_{OPT} - T_{MAX})(T_{OPT} + T_{MIN} - 2T)]} \right) \quad (6)$$

The parameters of the cardinal model were obtained through calibration using experimental data previously reported for *Nannochloropsis gaditana* under outdoor cultivation conditions, mainly from Moraes et al. (2020) [19], who evaluated the effect of culture pH on the performance of *N. gaditana* in tubular photobioreactors, and Jiménez Veuthey et al. (2022) [20], who reported pilot-scale cultivation in thin-layer cascade photobioreactors using seawater-based media and pig slurry diluted with seawater. These studies provided the basis for calibrating the pH, productivity and operation-related parameters, including biomass productivity, culture pH, dilution rate, photosynthetic performance, reactor configuration, and environmental conditions.

3.2. Transport equations

In this section, the mass and energy balances describing the physical and chemical phenomena occurring within the photobioreactor are presented. The system was divided into three main compartments: the degassing column, the thin-layer channel, and the recirculation tank. The degassing unit was represented as a cylindrical column 1.8 m high and 0.4 m in diameter, corresponding to the 230 L column described in Section 2.2. For modelling purposes, both the degassing column and the recirculation tank were considered perfectly mixed compartments, whereas the thin-layer channel was represented as a plug-flow reactor. This assumption was supported by the hydrodynamic conditions prevailing in both units. In the degassing column, air was continuously injected through a bottom gas distributor at a flow rate of $100 L min^{-1}$, corresponding to a superficial gas velocity of $0.013 m s^{-1}$. This aeration rate promotes efficient oxygen stripping, vigorous gas-induced circulation, and rapid homogenization of dissolved gases within the liquid phase. Simultaneously, the culture was recirculated through the system at a flow rate of $21.5 m^3 h^{-1}$, resulting in a liquid residence time of 38 s within the column. Under these conditions, concentration and temperature gradients inside the column were considered negligible at the scale of the model. Additional mixing occurred in the recirculation tank, where the culture returning from the thin-layer channel entered through a cascade-type inlet configuration, generating turbulence and enhancing homogenization. The combination of the high recirculation flow rate and the cascade inflow minimized concentration gradients and supported the assumption of spatially uniform conditions within the tank. Consequently, both the degassing column and the recirculation tank were modelled as perfectly mixed volumes, while spatial gradients were only considered in the thin-layer channel, where they are expected to be significant.

Gas-liquid mass transfer, heat exchange, and limited light penetration were explicitly modelled within the degassing column, and the calibrated volumetric mass-transfer coefficient (K_{La}) was used to characterize the oxygen stripping performance of this unit. In contrast, the thin-layer channel was modelled using mass and energy balances formulated as partial differential equations in both time and space,

allowing the prediction of the temporal and spatial evolution of the main process variables along the flow path. This modelling framework differs from conventional raceway reactor models, which generally assume ideally mixed behaviour as a consequence of paddlewheel-driven circulation and greater liquid depths. In thin-layer photobioreactors, the combination of a shallow liquid film (0.023 m), a high surface-to-volume ratio (43.4 m² m⁻³), and an elongated flow path (40 m) promotes the development of longitudinal gradients of dissolved oxygen, carbon dioxide, temperature, and photosynthetic activity. Furthermore, the culture flows through the channel at an average velocity of 0.19 m s⁻¹, corresponding to a residence time of 210 s and a Reynolds number of 1.75×10^4 indicative of a fully developed turbulent regime. Under these conditions, a spatially distributed representation is required to adequately capture the transport phenomena and biological responses occurring along the channel. Although the proposed framework captures the dominant biological, thermal, and mass-transfer processes occurring in thin-layer photobioreactors, local turbulence structures and free-surface hydrodynamic effects are not explicitly resolved and are incorporated through lumped transport parameters. Although the biological sub-model can be transferred to different photobioreactor configurations, the transport equations, hydrodynamic assumptions, and geometry-dependent parameters must be adapted to account for differences in mixing, light distribution, and gas-liquid mass transfer characteristics.

It should be noted that within the degassing column no photosynthetic activity could take place due to the scarce penetration of light that was, in turn, determined by the specific configuration of the column. The latter one is a cylinder (230 L, 1.8 m high, with a diameter of 0.40 m) made of opaque material where light can penetrate only from the upper base in the first 20–30 cm thus leaving most of the culture in the dark. The mass and energy balances in the column, along with their initial conditions, are therefore as follows:

$$\frac{dC_b^{Col}}{dt} = \frac{Q_{liq}^{Col}}{V_{liq}^{Col}} (C_b^T - C_b^{Col}) \quad C_b^{Col} = C_b^{Col,0} \quad @t = 0 \quad (7)$$

$$\frac{dC_{O_2}^{Col}}{dt} = \frac{Q_{liq}^{Col}}{V_{liq}^{Col}} (C_{O_2}^T - C_{O_2}^{Col}) + K_L a_{L,O_2}^{Col} (C_{O_2}^{*,Col} - C_{O_2}^{Col}) \quad C_{O_2}^{Col} = C_{O_2}^{Col,0} \quad @t = 0 \quad (8)$$

$$\frac{dC_{C_T}^{Col}}{dt} = \frac{Q_{liq}^{Col}}{V_{liq}^{Col}} (C_{C_T}^T - C_{C_T}^{Col}) + \frac{Y_{C_T}}{CO_2} K_L a_{L,CO_2}^{Col} (C_{CO_2}^{*,Col} - C_{CO_2}^{Col}) \quad C_{C_T}^{Col} = C_{C_T}^{Col,0} \quad @t = 0 \quad (9)$$

$$\frac{dT^{Col}}{dt} = \frac{Q_{liq}^{Col}}{V_{liq}^{Col}} (T^T - T^{Col}) + \frac{h_c^{Col} S_c^{Col}}{\rho c_p V_{liq}^{Col}} (T_{amb} - T^{Col}) \quad T^{Col} = T^{Col,0} \quad @t = 0 \quad (10)$$

The parameter $K_L a_L$ (s⁻¹) represents the volumetric gas-liquid mass-transfer coefficient of the degassing column and accounts for the combined effects of gas-liquid interfacial area and liquid-side mass-transfer resistance on oxygen exchange between the liquid and gas phases. Biological reactions were neglected in this unit because its liquid residence time (38 s) is negligible compared with the characteristic time-scales of photosynthesis and respiration. Consequently, the degassing column was modelled exclusively as a gas-exchange and heat-transfer compartment.

Since perfect mixing was assumed, the values of all state variables were considered spatially uniform within the column, and the outlet concentrations were therefore equal to those inside the compartment. Furthermore, because the reactor sections are directly connected, the inlet conditions of each section correspond to the outlet conditions of the preceding one. The liquid volume V_{liq} is evaluated through the following

Eq. 11 where ϵ_{Col} is the fraction of gas within the column and is evaluated through Eq. 12 as a function of the flow rate of incoming liquids and gases:

$$V_{liq}^{Col} = V_{Tot}^{Col} (1 - \epsilon_{Col}) \quad (11)$$

$$\epsilon_{Col} = \frac{Q_{gas}}{Q_{gas} + Q_{liq}} \quad (12)$$

Concentrations of dissolved oxygen and carbon dioxide at equilibrium are calculated as a function of the molar fraction in the gas phase using Henry's law as shown in Eqs. 13 and 14, respectively:

$$C_{O_2}^{*,Col} = M_{O_2} H_{O_2} P_{Tot} Y_{O_2} \quad (13)$$

$$C_{CO_2}^{*,Col} = M_{CO_2} H_{CO_2} P_{Tot} Y_{CO_2} \quad (14)$$

The CO₂ mass transfer coefficient has been then evaluated through Eq. 15 as a function of the oxygen's one:

$$K_L a_{L,CO_2}^{Col} = \frac{K_L a_{L,O_2}^{Col}}{0.91} \quad (15)$$

The coefficient 0.91 corresponds to the ratio between the liquid-side mass-transfer coefficients of CO₂ and O₂ and is derived from the ratio of their molecular diffusivities in water, according to classical gas-liquid mass-transfer theory under identical hydrodynamic conditions.

The gas flowrate, consisting of air or CO₂, is also fed to the inlet of the column in addition to the recirculated liquid. Accordingly, the gas phase balances of CO₂ and O₂ were coupled to the ones related to the liquid phase. It should be highlighted that gas-phase molar fractions have been normalized concerning the nitrogenous fraction according to a procedure proposed by Fernández et al. [21], since nitrogen is constant over time as it does not participate in the gas absorption process. The equilibria of the gas phase are shown in eqs. 16 and 17, where Y_{O_2} and Y_{CO_2} are the normalized molar fractions of oxygen and carbon dioxide, respectively:

$$\frac{dY_{O_2}}{dt} = \frac{Q_{gas}}{V_{gas}^{Col}} (Y_{O_2,IN} - Y_{O_2}) - \frac{V_{liq}^{Col}}{Y_{N_2} \epsilon_{Col}} K_L a_{L,O_2}^{Col} ([O_2^{*,Col}] - [O_2^{Col}]) \quad Y_{O_2} = Y_{O_2}^0 \quad @t = 0 \quad (16)$$

$$\frac{dY_{CO_2}}{dt} = \frac{Q_{gas}}{V_{gas}^{Col}} (Y_{CO_2,IN} - Y_{CO_2}) - \frac{V_{liq}^{Col}}{Y_{N_2} \epsilon_{Col}} K_L a_{L,CO_2}^{Col} ([CO_2^{*,Col}] - [CO_2^{Col}]) \quad Y_{CO_2} = Y_{CO_2}^0 \quad @t = 0 \quad (17)$$

$$Y_i = \frac{Y_i}{Y_i + 1} \quad (18)$$

where the symbols' significance is reported in the notations section. As far as the channel section is concerned, the phenomena that play a key role are mass transfer mass and energy exchange with the surrounding environment. In addition, since the following section is characterized by a large area exposed to light and a shallow water depth, the average light intensity in the culture is high enough to trigger and sustain photosynthesis. The latter is suitably described by a generative term composed of the oxygen production rate multiplied by the biomass concentration and a yield representing the mass of algae produced per unit weight of oxygen generated. For the present case a value of the yield equal to 0.7273 $g_{biomass} g_{O_2}^{-1}$ was adopted according to the literature [21]. About CO₂ consumption, it was considered as a 1:1 M ratio to oxygen production. The relevant mass balances, along with their boundary conditions, are reported as follows:

$$\frac{\partial C_b^{Chan}(x,t)}{\partial t} = -\frac{Q_{liq}^{Chan}}{A_{liq}^{Chan}} \frac{\partial C_b^{Chan}(x,t)}{\partial x} + Y_X P_{O_2} C_b^{Chan}(x,t) \quad (19)$$

$$\frac{C_b^{Chan}}{C_b^{Chan}} = C_b^{Chan,0} \quad @t = 0 \quad \forall x \in]0, L]$$

$$\frac{C_b^{Chan}}{C_b^{Chan}} = C_b^{Col} \quad @x = 0 \quad \forall t > 0$$

$$\frac{\partial C_{O_2}^{Chan}(x,t)}{\partial t} = -\frac{Q_{liq}^{Chan}}{A_{liq}^{Chan}} \frac{\partial C_{O_2}^{Chan}(x,t)}{\partial x} + P_{O_2} C_b^{Chan}(x,t) + K_L a_{L,O_2}^{Chan} (C_{O_2}^{*,Chan}(t) - C_{O_2}^{Chan}(x,t)) \quad (20)$$

$$\frac{C_{O_2}^{Chan}}{C_{O_2}^{Chan}} = C_{O_2}^{Chan,0} \quad @t = 0 \quad \forall x \in]0, L]$$

$$\frac{C_{O_2}^{Chan}}{C_{O_2}^{Chan}} = C_{O_2}^{Col} \quad @x = 0 \quad \forall t > 0$$

$$\frac{\partial C_{C_T}^{Chan}(x,t)}{\partial t} = -\frac{Q_{liq}^{Chan}}{A_{liq}^{Chan}} \frac{\partial C_{C_T}^{Chan}(x,t)}{\partial x} - \frac{Y_{C_T}}{O} P_{O_2} C_b^{Chan}(x,t) + \frac{Y_{C_T}}{CO_2} K_L a_{L,CO_2}^{Chan} (C_{CO_2}^{*,Chan}(x,t) - C_{CO_2}^{Chan}(x,t)) \quad (21)$$

$$\frac{C_{C_T}^{Chan}}{C_{C_T}^{Chan}} = C_{C_T}^{Chan,0} \quad @t = 0 \quad \forall x \in]0, L]$$

$$\frac{C_{C_T}^{Chan}}{C_{C_T}^{Chan}} = C_{C_T}^{Col} \quad @x = 0 \quad \forall t > 0$$

Since this section is open, the factors that affect the temperature are mainly related to the external environment. Therefore, outside convection, irradiation, radiation and evaporation are the phenomena considered in the following energy balance [22]:

$$\frac{\partial T^{Chan}(x,t)}{\partial t} = -\frac{Q_{liq}^{Chan}}{A_{liq}^C} \frac{\partial T^{Chan}(x,t)}{\partial x} + \frac{h_c^{Chan} S_c^{Chan}}{cpV_{Tot}^{Chan}} (T_{amb}(t) - T^{Chan}(x,t)) + \frac{I_0 S_c^{Chan} a^{Chan}}{\rho cp V_{Tot}^{Chan}} + \frac{\sigma S_c^{Chan} e}{\rho cp V_{Tot}^{Chan}} (T_{sky}^4 - T^{Chan}(x,t)^4) \quad (22)$$

$$\frac{S_c h(p^{Chan} - p_{amb})}{\rho cp V_{Tot}} \quad \frac{T^{Chan} = T^{Chan,0} \quad @t = 0 \quad \forall x \in]0, L]}{T^{Chan} = T^{Col} \quad @x = 0 \quad \forall t > 0}$$

It should be noted that, unlike models developed for conventional raceway ponds, Eq. 22 does not include a soil conduction term. This is because the thin-layer channel consists of a fiberglass structure that is not in direct contact with the ground, making conductive heat losses negligible compared with the dominant heat-transfer mechanisms, namely solar radiation, convective exchange with the atmosphere, and evaporative cooling. In addition, axial diffusion terms were neglected in all transport equations (Eqs. 19–22) because transport within the channel was strongly advection dominated under the operating conditions investigated. The high liquid velocity (0.19 m s⁻¹) and the elongated channel geometry result in high Péclet numbers, indicating that convective transport largely exceeds axial diffusion and supporting the plug-flow approximation adopted in the model.

In the recirculation tank, the relevant phenomena affecting the time evolution of model output variables are the same as those of the channel. In addition, in this section the daily harvest of the culture and the introduction of the medium takes place. The balances of matter and energy, with the relative limit conditions, are then as follows:

$$\frac{dC_b^{Tank}}{dt} = \frac{Q_{liq}^{Tank}}{V^T} (C_b^{Chan}(L,t) - C_b^{Tank}) + Y_X P_{O_2} C_b^{Tank} - \frac{Q_m}{V} C_b^{Tank} \quad C_b^{Tank} = C_b^{Tank,0} \quad @t = 0 \quad (23)$$

$$\frac{dC_{O_2}^{Tank}}{dt} = \frac{Q_{liq}^{Tank}}{V^T} (C_{O_2}^{Chan}(L,t) - C_{O_2}^{Tank}) + P_{O_2} C_b^{Tank} + K_L a_{L,O_2}^{Tank} (C_{O_2}^{*,Tank} - C_{O_2}^{Tank}) \quad (24)$$

$$+ \frac{Q_m}{V^T} (C_{O_2m} - C_{O_2}^{Tank}) \quad C_{O_2}^{Tank} = C_{O_2}^{Tank,0} \quad @t = 0$$

$$\frac{dC_{C_T}^{Tank}}{dt} = \frac{Q_{liq}^{Tank}}{V^T} (C_{C_T}^{Chan}(L,t) - C_{C_T}^{Tank}) - Y_{C_T} P_{O_2} C_b^{Tank} + Y_{C_T} \frac{K_L a_{L,CO_2}^{Tank}}{CO_2} (C_{CO_2}^{*,Tank} - C_{CO_2}^{Tank}) + Y_{C_T} \frac{Q_m}{CO_2 V^T} (C_{CO_2m} - C_{CO_2}^{Tank}) \quad C_{C_T}^{Tank} = C_{C_T}^{Tank,0} \quad @t = 0 \quad (25)$$

$$\frac{\partial T^{Tank}}{\partial t} = \frac{Q_{liq}^{Tank}}{V^T} (T^{Chan}(L,t) - T^{Tank}) + \frac{h_c^{Tank} S_c^{Tank}}{cpV^T} (T_{amb}(t) - T^{Tank}) + \frac{I_0 S_c^{Tank} a^{Tank}}{\rho cp V^T} + \frac{\sigma S_c^{Tank} e}{\rho cp V^{Tank}} (T_{sky}^4 - T^{Tank,4}) - \frac{S_c h(p^{Tank} - p_{amb})}{\rho cp V_{Tot}} + \frac{Q_m}{\rho cp V_{Tot}} (T_m - T^{Tank}) \quad T^{Tank} = T^{Tank,0} \quad @t = 0 \quad (26)$$

Finally, pH was not modelled as an independent state variable but was calculated algebraically at each spatial node and time step. Following the approach proposed by Rubio et al. [23], the local pH was determined from the electroneutrality condition using the dynamically calculated concentrations of total inorganic carbon (CCT) and dissolved CO₂. The influence of nutrient uptake on alkalinity was neglected because short-term pH dynamics were dominated by inorganic carbon speciation, photosynthetic CO₂ consumption, gas–liquid mass transfer, and controlled CO₂ injection. Although the assimilation of nutrients such as nitrate and phosphorus can affect alkalinity and, consequently, pH, these effects occur over longer timescales and were considered negligible relative to the rapid pH fluctuations driven by carbon dynamics under the operating conditions investigated. Therefore, assuming a constant background alkalinity reduced model complexity without compromising the accuracy of short-term pH predictions.

3.3. Model resolution, calibration and validation

The equations constituting the model described in the previous sections were implemented in MATLAB R2021a (MathWorks, Massachusetts, USA). The partial differential equations (PDEs) describing transport phenomena along the thin-layer channel were solved using the method of lines, whereas the ordinary differential equations (ODEs) describing the dynamics of the perfectly mixed compartments were solved using a first-order forward finite-difference scheme. Model parameters were classified into three categories according to their source. Operational variables, including incident photosynthetically active radiation (PAR), ambient temperature, pH, dissolved oxygen concentration, and CO₂ injection rate, were obtained directly from experimental measurements and used as model inputs. Biological and physicochemical parameters were obtained from literature sources or previous experimental characterization of the cultivation system. Only two parameters were estimated through model calibration: the culture absorptivity coefficient and the volumetric gas-transfer coefficient (K_La_L) of the degassing column.

Parameter estimation was performed using a genetic algorithm [24]. The culture absorptivity coefficient was constrained within the physically meaningful range of 0–1, while the search interval for the gas-transfer coefficient was established from preliminary order-of-magnitude estimates derived from thin-layer photobioreactor operation, providing a sufficiently broad parameter space for optimization. The calibration procedure employed two independent experimental datasets. The culture temperature profile was used to estimate the culture absorptivity coefficient, owing to its direct influence on light absorption and heat accumulation within the culture. The dissolved oxygen (%DO) measurements recorded at the outlet of the thin-layer channel were used to estimate the volumetric gas-transfer coefficient of the degassing column. The optimization was driven by a multi-objective fitness function that minimized the normalized sum of

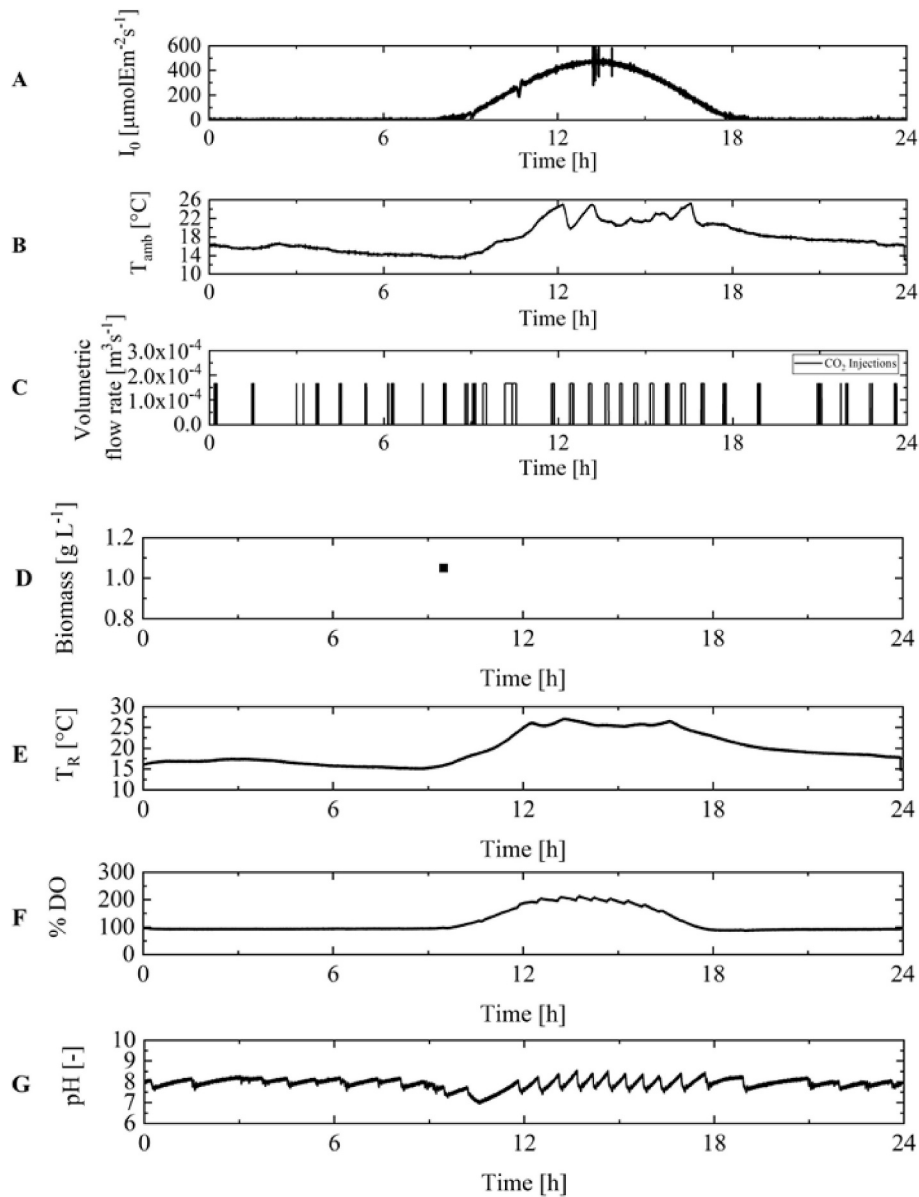


Fig. 2. Daily temporal profiles of the environmental and culture conditions during reactor operation. (A) Incident photosynthetically active radiation (PAR). (B) Ambient temperature inside the greenhouse. (C) CO₂ injection pulses regulated by the control system to maintain the culture pH within the desired operating range. (D) Measured biomass concentration in the reactor. (E) Measured culture temperature profile. (F) Measured dissolved oxygen concentration (%DO) profile. (G) Measured culture pH profile.

squared errors between simulated and experimental values of culture temperature and dissolved oxygen concentration. Following calibration, the model was validated using three independent experimental datasets obtained under different operating conditions, including semi-continuous cultivation, to assess its predictive capability and robustness.

In order to compare the performance of the model in calibration and validation phase, the IAE (Integral Absolute Error), ISE (Integral Square Error), ITAE (Integral of Time multiplied Absolute Error), ITSE (Integral of Time multiplied Square Error) were calculated, following the expressions shown in Eq. (27). Subsequently, to filter out distortions due to different scales and provide dimensionless performance indices, each metric was normalized by dividing its cumulative value by the respective mean experimental value of the monitored variable, yielding the normalized metrics (nIAE, nISE, nITAE, and nITSE).

$$IAE = \int_0^T |e(t)| dt; ITAE = \int_0^T |e(t)| \cdot t dt; ISE = \int_0^T e(t)^2 dt; ITSE = \int_0^T e(t)^2 \cdot t dt. \quad (27)$$

4. Result and discussion

4.1. Daily variation of culture conditions

Concerning the reactor temperature (Fig. 2E), minimum and maximum values of 15° and 27 °C were detected respectively. Similar values are reported for the same reactor during the spring season in [25] and for raceway-type reactors in summer [26]. It is interesting to observe that the curve is quite similar to the one of external temperature even if the maximum values are slightly lower (Fig. 2B). This is because,

in addition to a strong correlation with the external temperature, the reactor temperature also depends on solar radiation (Fig. 2A). During daylight hours, the culture temperature is positively influenced by external conditions due to the reactor being almost entirely open, except for the degassing column. Heat is transferred into the system through convection, driven by the ambient temperature (Fig. 2B), and solar radiation (Fig. 2A), through irradiance. In contrast, energy loss of the culture were attributed to evaporative losses and outgoing radiation. At night, with no incoming energy, the system tends to lose heat, resulting in a gradual drop in temperature until thermal equilibrium with the surrounding environment is reached.

As for the dissolved oxygen (DO) levels in the culture (Fig. 2F), saturation values reached up to 200% of air saturation. Values of 300% saturation in spring have been reported for the same reactor [25]. The oxygen produced by microalgae during photosynthesis can negatively affect system performance, which is why removing oxygen from the culture is important. Indeed, oxygen concentrations increase more rapidly when using thin-layer cascade reactors [26]. The reason for high

oxygen saturation in such reactors is their greater productivity, which nearly doubles oxygen production compared to raceways. Significantly lower values, however, have been reported for raceway reactors, where reduced productivity leads to lower oxygen levels [25]. Daily DO profiles revealed a consistent behaviour: during daylight hours, high photosynthetic activity led to oxygen accumulation in the culture. This suggests that aeration provided by the degassing column was insufficient to remove all the oxygen produced. At night, in the absence of photosynthesis, the excess oxygen was gradually removed via the degassing column until equilibrium was achieved. While there is some gas exchange with the environment in both the channel and the recirculation tank, these contributions are minor compared to the exchange in the degassing column.

With regard to biomass concentration (Fig. 2D), a value of $1.2 \text{ g} \cdot \text{L}^{-1}$ was achieved, which is lower than those reported in the literature [25]. It should be noted that this concentration corresponds to a pseudo-steady-state condition maintained during semi-continuous operation at a dilution rate of 0.3 d^{-1} , rather than to a stationary phase reached

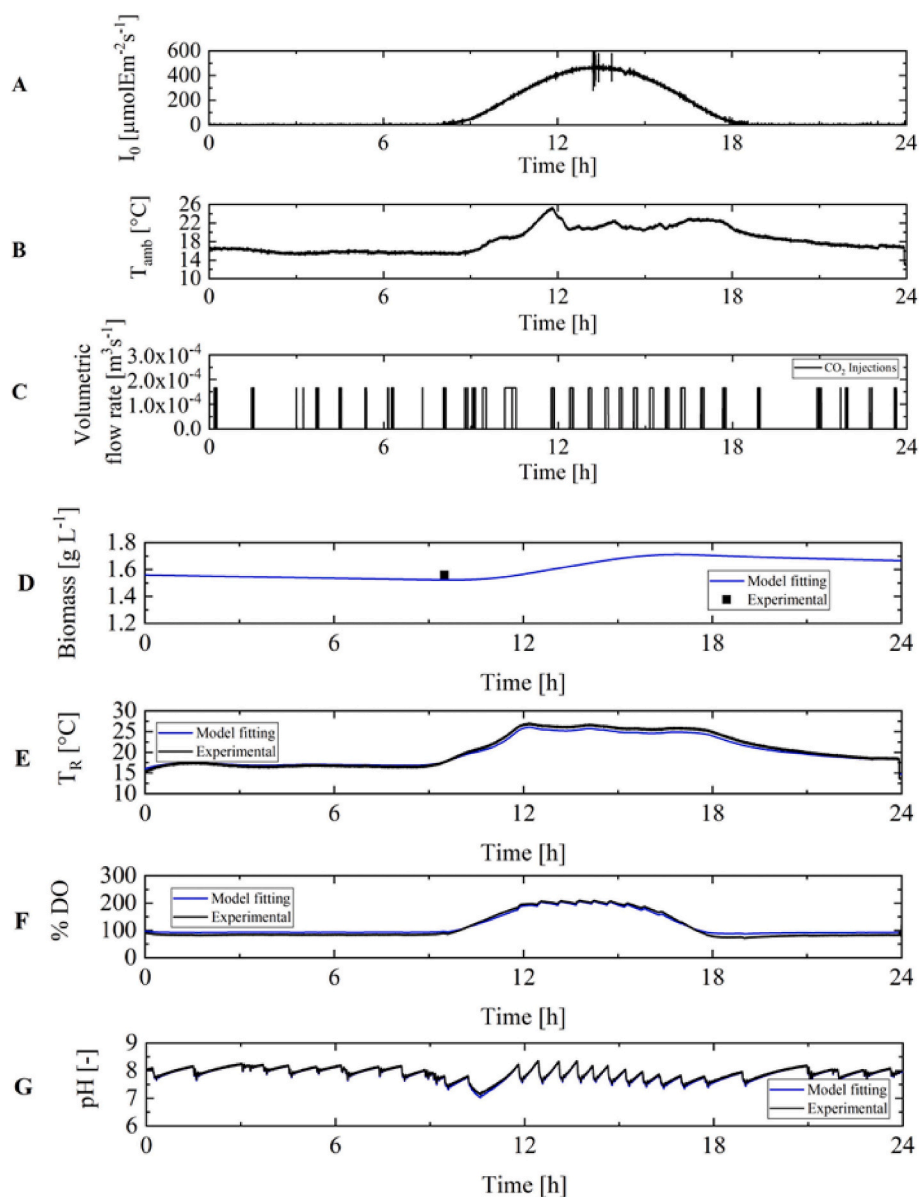


Fig. 3. Experimental and simulated data of biomass concentration (D), temperature inside the reactor (E), dissolved oxygen concentration (F) and pH (G) at the end of the channel. The simulation was done as a function of the PAR irradiance (A), the external temperature (B) and the CO₂ injections (C) of the second day of the experimental campaign, used for calibration.

during batch cultivation. Therefore, the culture was maintained under active growth conditions throughout the experimental period. With regard to pH (Fig. 2G), its fluctuations are influenced by photosynthetic activity during daylight hours and by respiration during the night. pH control is achieved through CO₂ injections into the degassing column using an On/Off system (Fig. 2C). The setpoint was established at pH 8, which is optimal for the strain under investigation. As can be observed, the system operates with high precision during the night, as the effect of respiration is minimal and thus CO₂ injections are infrequent. The slight increase in pH observed during the night represents an interesting dynamic that warrants further investigation. A plausible explanation is related to the operation of the pH control system, in which air and CO₂ supply are mutually exclusive. When CO₂ injection is inactive, continuous aeration in the degassing column (100 L min⁻¹) may strip dissolved CO₂ from the culture faster than it is generated by respiratory activity, resulting in a temporary increase in pH. Further studies will be required to confirm the mechanisms responsible for this behaviour. During daylight hours, however, the system becomes less efficient, with pH values fluctuating between 7.5 and 8.5. This is because the sensor is positioned in the next section of the reactor, 40 m from the injection point; consequently, due to the mixing times, there is a slight delay and more CO₂ than necessary is injected.

4.2. Calibration and validation

Only two parameters were suitably adjusted using the genetic algorithm in order to fit the model outputs to the experimental data for the four variables shown in Fig. 3. The gas exchange parameter in the degassing column was calibrated, as it depends on the instantaneous gas fraction within the column. Significantly lower values have been reported for raceway reactors (0.0063 s⁻¹; [21]). The calibrated K_La_L value obtained for the degassing unit was significantly higher than those typically reported for raceway ponds. This result is expected because the degassing column operates hydrodynamically as a gas-sparged bubble column rather than as a surface-aerated system. Continuous air injection through a bottom gas distributor at a flow rate of 100 L min⁻¹ generates intense gas-induced mixing and a large gas-liquid interfacial area, substantially enhancing oxygen transfer rates. Consequently, the mass-transfer performance of the degassing unit is more appropriately

compared with bubble-column and airlift photobioreactors than with conventional raceway reactors [27–29].

The culture absorptivity coefficient was also estimated during model calibration. This parameter represents the effective fraction of incident solar radiation absorbed by the cultivation system and therefore directly influences the predicted culture temperature through the energy balance. Its value integrates the combined effects of reactor material properties, optical characteristics of the microalgal suspension, surface reflection losses, and environmental operating conditions. Since these contributions cannot be independently quantified under outdoor conditions, a lumped absorptivity parameter was adopted and calibrated by minimizing the deviation between simulated and measured culture temperature profiles. The calibrated value was consistent with those reported for outdoor microalgae cultivation systems, being lower than values reported for tubular photobioreactors (0.9725; [30]) and comparable to those reported for raceway reactors (0.7; [22]).

Regarding the growth model of the strain (Eqs. 1 to 6), most characteristic values were measured experimentally in the laboratory through respirometry tests. Exceptions include the pH-related parameters, namely the minimum, optimum, and maximum values specific to this strain; as well as the oxygen-related parameters, such as the oxygen inhibition constant and its shape parameter, and the extinction coefficient. These were instead obtained from existing literature [31,32]. As for gas exchange processes, it was assumed that the recirculation channel and tank could be treated analogously to the channel and pond of a raceway reactor. Consequently, characteristic values reported in the literature [21] for this reactor type were adopted in Eqs. 20 and 24. Finally, for the energy balance (Eqs. 10, 22 and 26), it was assumed that the culture medium behaves similarly to water. Thus, the thermophysical properties of water were used. Table 1 lists all the model parameters along with their respective sources. It should be noted that the biological parameters reported in Table 1 were independently determined through laboratory characterization experiments and were used as fixed model inputs. Consequently, only the culture absorptivity coefficient and the volumetric mass-transfer coefficient of the degassing column were estimated through model calibration.

Model parameters were classified into three categories: (i) measured operational inputs, photosynthetically active radiation (PAR) (Fig. 3A), the temperature of the environment surrounding the reactor (Fig. 3B), and the CO₂ injection rates (Fig. 3C), expressed as volumetric flow rates, which are used to regulate the pH of the culture.; (ii) parameters obtained from laboratory characterization or literature sources; and (iii) calibrated parameters estimated through the genetic algorithm. Based on these variables and the defined parameters, the model was able to estimate the instantaneous concentration of microalgal biomass and dissolved oxygen in the culture, as well as the culture temperature and pH.

Several days of experimental trials were conducted. For the model calibration, a day on which no biomass harvesting was performed was selected. The absence of this operation simplified the modelling process, as it allowed for the exclusion of one term from the equations, thereby streamlining the calibration procedure. Following completion of the calibration, the model was validated over three consecutive days. Biomass harvesting occurred on the first and third of these days. This validation approach was designed to assess the model's predictive capabilities both under batch conditions and when accounting for the harvesting process.

Table 2

Performance indexes from the model calibration. Errors are normalized by dividing each metric by the respective mean experimental value to provide a dimensionless comparison across variables.

	nIAE	nISE	nITAE	nITSE
pH	0.1143	0.0057	1.5255	0.0756
% DO	1.3338	12.6357	16.5782	160.3523
Temperature	0.6014	0.4170	8.1352	6.0418

Table 1
Model parameters.

Parameter	Value	Units of measurement	Source
K _a	0.1	m ² g ⁻¹	[17]
P _{O₂} , _{MAX}	0.139	gO ₂ g ⁻¹ Biomass m ³ h ⁻¹	Tuned based on [19,20]
K _i	151	μEm ⁻² s ⁻¹	Tuned based on [19,20]
n	2	–	Tuned based on [19,20]
K _{O₂}	21.2494	g m ⁻³	Set based on [31,32]
z	2	–	Set based on [31,32]
T _{opt}	32	°C	Tuned based on [19,20]
T _{MAX}	41	°C	Tuned based on [19,20]
T _{MIN}	4.6	°C	Tuned based on [19,20]
pH _{opt}	8	–	[13]
pH _{MAX}	9.1	–	Set based on [31,32]
pH _{MIN}	4	–	Set based on [31,32]
rr	0.0396	–	Set based on previous studies [21]
K _L a _L , _{O₂} , _{Col}	31.0009	s ⁻¹	Tuned
K _L a _L , _{O₂} , _{Chan}	2.5092e ⁻⁴	s ⁻¹	Set based on previous studies [21]
K _L a _L , _{O₂} , _{Tank}	3.4125e ⁻⁴	s ⁻¹	Set based on previous studies [21]
h _c , _{Col}	10	Wm ⁻² °C ⁻¹	Set based on the value for water
h _c , _{Chan}	12.6733	Wm ⁻² °C ⁻¹	Set based on the value for water
h _c , _{Tank}	62.0716	Wm ⁻² °C ⁻¹	Set based on the value for water
α _{Chan}	0.7	–	Tuned

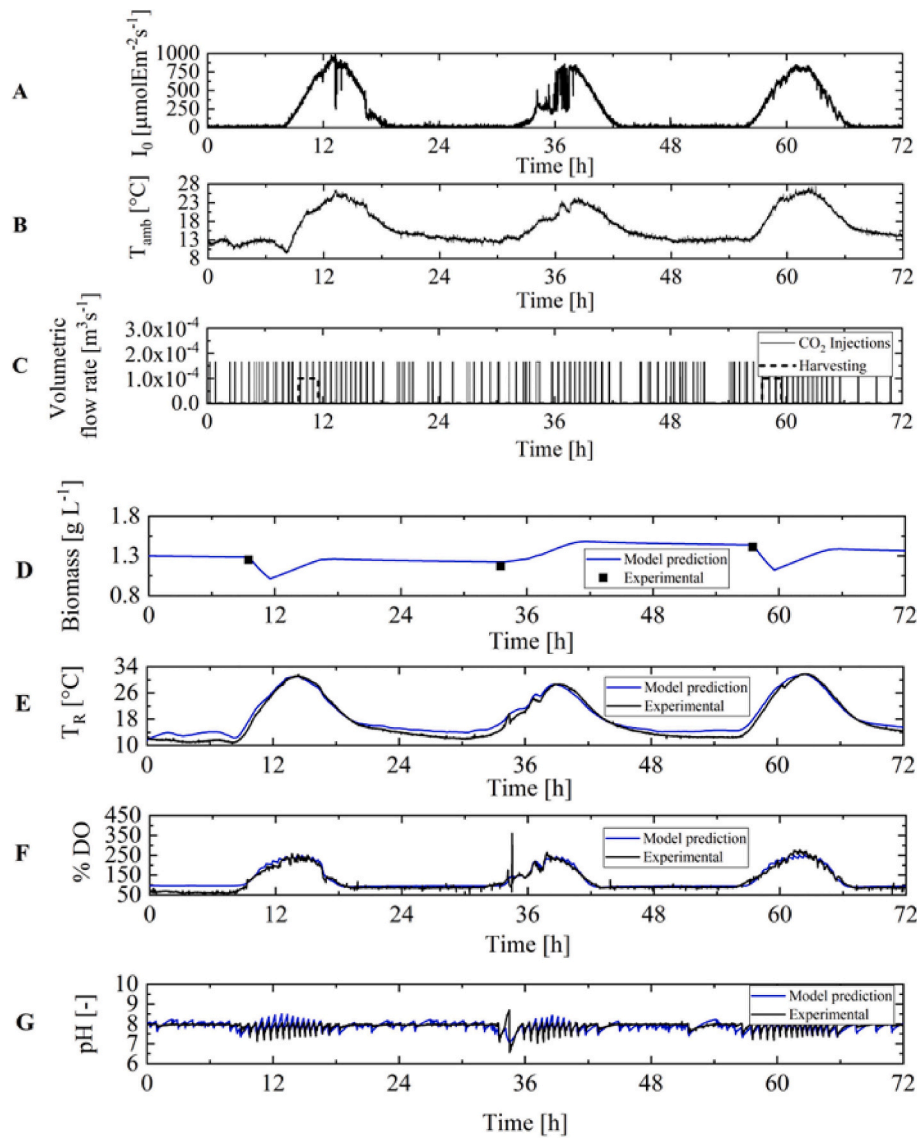


Fig. 4. Experimental and simulated data of biomass concentration (D), temperature inside the reactor (E), dissolved oxygen concentration (F) and pH (G) at the end of the channel. The simulation was done as a function of the PAR irradiance (A), the external temperature (B) and the CO₂ injections (C) of the three days of the experimental campaign, used for the validation.

Table 3

Performance indexes from the model validation. Errors are normalized by dividing each metric by the respective mean experimental value to provide a dimensionless comparison across variables.

	nIAE	nISE	nITAE	nITSE
pH	1.2543	0.3137	42.3843	10.5355
% DO	5.9753	125.1257	162.3455	2558.3464
Temperature	4.1296	6.5778	137.4961	200.4159

The calibration results are shown in Fig. 3. As it can be observed, the model is capable of capturing the experimental data accurately. To evaluate the model's performance on a rigorous and comparable basis, the integral error metrics (IAE, ISE, ITAE, and ITSE) were normalized by dividing each value by the corresponding mean experimental variable, as reported in Table 2. This mathematical normalization filters out distortions caused by the different physical units and numerical scales of temperature ($^{\circ}\text{C}$), pH, and dissolved oxygen (% air saturation). Considering that the real data originate from continuously operating sensors, which are inherently affected by measurement noise, the

interpretation of these normalized performance indices must be evaluated accordingly.

As shown in Table 2, the normalized Integral Absolute Error (nIAE) values demonstrate the remarkable predictive accuracy of the calibrated model. The relative error achieved for dissolved oxygen, with an nIAE of 1.34%, confirming that both the biological photosynthetic kinetics and the gas-liquid mass transfer mechanism were successfully captured. The dynamic trend of pH was also successfully simulated with a fully acceptable relative error (nIAE of 0.1143%), which is considered highly satisfactory given the complexity of the non-linear equilibria governing the carbonate system in microalgae cultures. Finally, the thermal balance exhibited high robustness, yielding an nIAE of 0.6414% for the culture temperature, proving that the low nIAE and nITAE values reflect a highly accurate tracking with minimal deviation over time.

Regarding the squared and time-weighted metrics, the low values of nISE and nITAE consistently confirm that the discrepancy between simulated profiles and experimental data remains minimal throughout the entire calibration period. Conversely, the nITSE values expectedly reflect higher numbers due to the quadratic penalization of errors over cumulative time. In fact, error indices that square the error (ISE, ITSE)

are known to be significantly more sensitive to high-frequency measurement noise and transient sensor disturbances, even when the underlying predictive performance is robust and well-tuned [34]. It is thus necessary to interpret these squared-error results with caution, as they tend to mathematically overestimate the actual tracking error due to continuous sensor noise rather than genuine model deficiencies [34].

As shown in Fig. 4, the model accurately predicts the system dynamics even under conditions that differ from those used during calibration. Given that the data were acquired from continuous sensors over a period of three consecutive days (72 h), the influence of sensor noise and environmental fluctuations must be carefully considered when evaluating these normalized performance indices. Continuous measurements of pH and dissolved oxygen in outdoor cultivation systems typically include high-frequency noise that can inflate integral error metrics unless proper filtering or state estimation is applied [35].

As shown in Table 3, the normalized Integral Absolute Error (nIAE) values consistently demonstrate the remarkable robustness of the model during the validation phase. The lowest relative error over the 72-h period was achieved for pH, with an nIAE of just 1.25%, closely followed by the culture temperature and dissolved oxygen, which yielded a highly accurate nIAE of 4.12% and 5.97%. These results confirm that the thermal balance and the core biological and gas-transfer kinetics remain stable and reliable under different operating modes. While this values are slightly higher than in the model calibration, it is expected due to the longer integration time, the semi-continuous operational mode, and the accumulating effect of small non-linear oscillations in the signal.

Regarding the squared and time-weighted metrics, the cumulative effect of a longer 72-h integration period naturally leads to an inflation of the indices, particularly for the time-weighted ones (nITAE and nITSE). This trend is consistent with the known sensitivity of squared-error-based indices to high-frequency measurement noise in continuous monitoring conditions—tiny, continuous oscillations in the sensor signals can lead to disproportionately large squared errors over long integration periods [36]. This phenomenon is especially visible in the nITSE values across all variables. Nonetheless, given the nature of continuous outdoor measurements and the known limitations of

physical sensors in outdoor bioprocesses, it can be concluded that a significant portion of these squared errors arises from high-frequency measurement noise rather than an inherent model deficiency [35], confirming that the underlying predictive model remains stable and well-validated.

Although the model was validated on a single pilot-scale system, its structure is general and can be extended to other thin-layer configurations. Reactor-specific parameters are mainly related to geometry and gas–liquid transfer coefficients, while the biological sub-model and transport framework remain unchanged. Although the biological sub-model may be transferable to different photobioreactor configurations, the transport equations and hydrodynamic assumptions adopted in this work are specifically tailored to thin-layer systems. In conventional raceway reactors, the larger liquid depth and stronger paddlewheel-driven mixing reduce longitudinal gradients and may justify ideally mixed or compartmentalized modelling approaches instead of the plug-flow representation adopted here. Moreover, different reactor geometries would require recalibration of transport and gas exchange parameters as well as modified descriptions of light distribution and hydrodynamics.

4.3. Performance and characteristic analysis

Once the model was validated, it was used to analyse reactor performance and variable distribution along the reactor length. As a first step, spatial profiles of key state variables were extracted at 12:30 of the first experimental day (Fig. 5). The reactor was conceptually divided into three sections: degassing column (0–0.5 m), channel (0.5–40.5 m), and recirculation tank (40.5–42.5 m) to assess the contribution of each segment to the overall reactor behaviour. Photosynthetic activity (Fig. 5A), as expected, occurs primarily in the channel region, which combines large illuminated surface and shallow liquid depth, leading to significantly higher average irradiances compared to the recirculation tank. This agrees with recent findings on light distribution and productivity in thin-layer photobioreactors and shallow systems, where light penetration and surface irradiance largely determine biomass

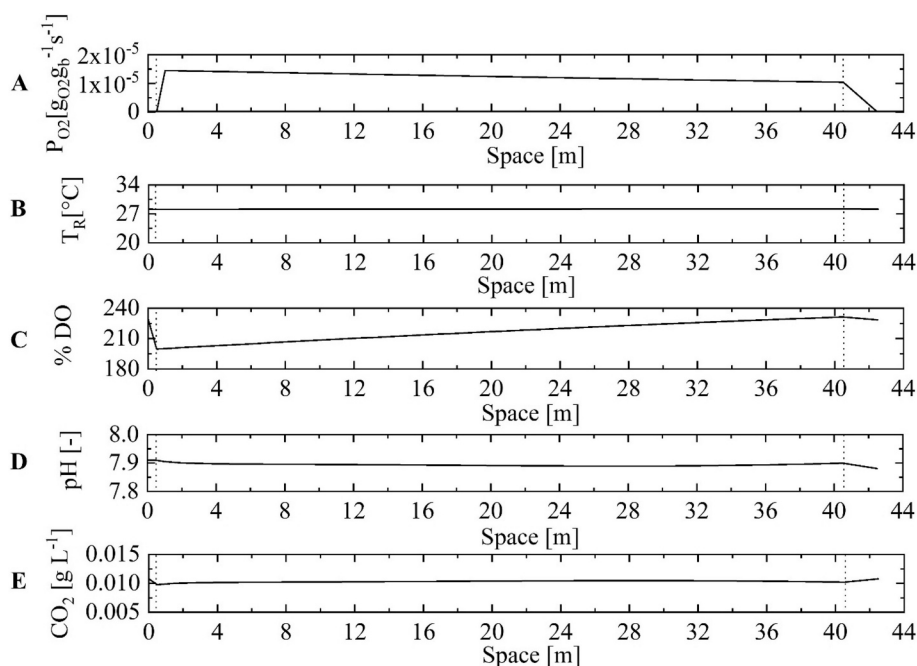


Fig. 5. Profiles along the entire reactor of: A photosynthetic activity, B temperature inside the reactor, C dissolved oxygen, D pH, E CO_2 . Data obtained from the implemented model with the respective characteristic parameters. The values shown for the degassing column and recirculation tank correspond to perfectly mixed compartments and therefore represent spatially uniform conditions. The line segments displayed in these sections are included solely for visualization purposes and do not represent spatial gradients. In contrast, only the thin-layer channel is described by spatially distributed profiles.

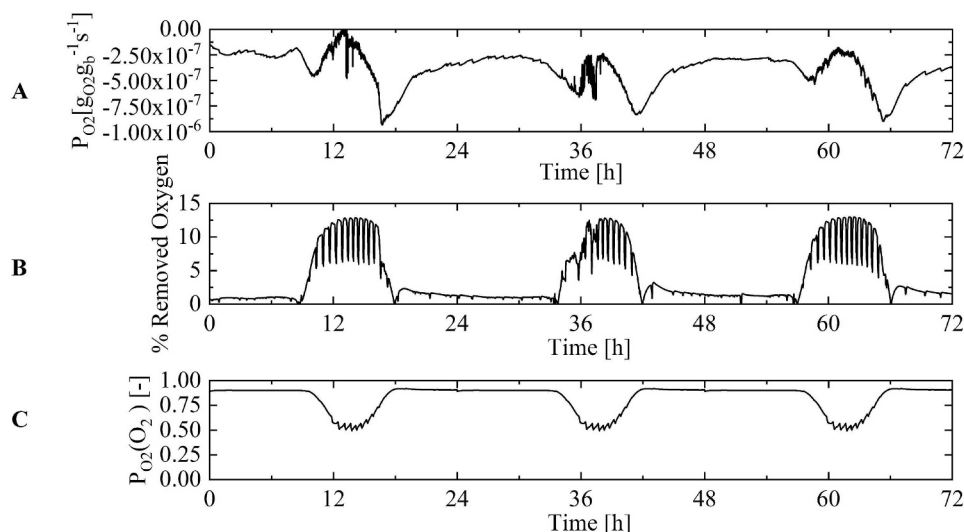


Fig. 6. Model-derived analysis of oxygen-related limitations in the photobioreactor. (A) Temporal profile of photosynthetic activity in the recirculation tank. (B) Oxygen stripping efficiency of the degassing column during the experimental campaign. (C) Effect of dissolved oxygen concentration on the photosynthetic activity, where 0 indicates complete inhibition and 1 indicates the absence of oxygen inhibition. Results were obtained from model simulations using the calibrated parameter set.

growth patterns [37–39]. Temperature profiles (Fig. 5B) were relatively uniform across the reactor, although the channel exhibited the greatest sensitivity to ambient conditions due to its larger exposed surface and shallower depth. Similar observations have been reported in comparative studies of outdoor photobioreactors, where temperature stratification is minimized in well-mixed thin-layer systems but surface exposure remains a key driver of thermal variability [38].

Dissolved oxygen (DO) exhibited an initial decrease in the column followed by an increase along the channel and a slight decline in the recirculation tank (Fig. 5C). This pattern reflects the coupling between oxygen production and gas–liquid mass transfer, consistent with recent discussions on oxygen accumulation and degassing limitations in outdoor algal cultivation systems [40]. The pH profile remained relatively constant throughout the reactor (Fig. 5D), with a slight increase in the recirculation tank accompanied by a corresponding decrease in CO_2 concentration (Fig. 5E), consistent with CO_2 uptake during photosynthesis. Similar trends have been documented in shallow raceway and thin-layer photobioreactors, where pH and carbon dynamics are closely linked to biomass activity and mixing conditions [41].

From Fig. 5, it can be observed that in the recirculation tank the dissolved oxygen concentration decreases, while the CO_2 concentration increases. Moreover, photosynthetic activity was absent at the specific time at which the spatial profiles were plotted. Fig. 6A shows the temporal evolution of the photosynthetic rate in the recirculation tank. It can be observed that throughout the entire experimental campaign the photosynthetic oxygen production rate (P_{O_2}) was always negative, indicating that only respiratory activity occurred, leading to net oxygen consumption and CO_2 production. This behaviour can be attributed to the relatively high liquid depth (0.3 m) combined with the short residence time in the recirculation tank, which limits light availability and prevents effective photosynthetic activity, as commonly reported for deep or poorly illuminated sections of photobioreactors.

As shown in Fig. 5C, the degassing column represents the reactor section primarily responsible for the removal of dissolved oxygen from the culture. This operation is essential to prevent the accumulation of excessive oxygen concentrations, which are known to inhibit microalgal growth and photosynthetic efficiency in outdoor cultivation systems [42]. Fig. 6B reports the percentage of oxygen removed in the degassing column relative to the incoming concentration. During nighttime operation, oxygen removal remained nearly constant at approximately 1%, while during daylight hours values up to about 13% were reached.

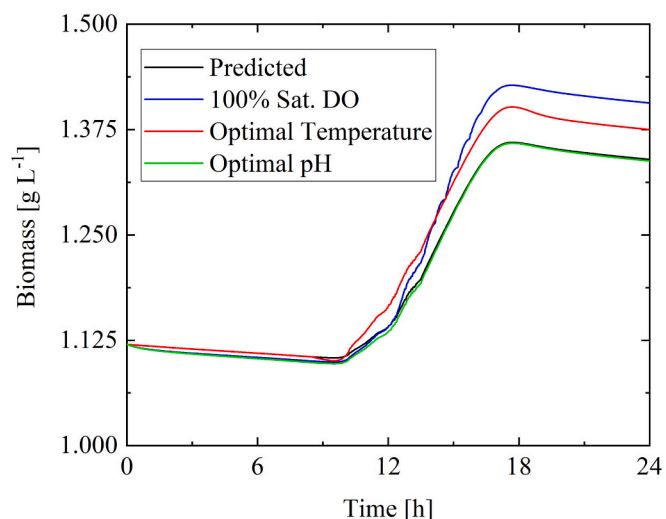


Fig. 7. Growth simulation with optimal growth parameters: dissolved oxygen, pH and temperature. Data obtained from the implemented model with the respective characteristic parameters.

During the light period, oscillations in oxygen removal efficiency were observed, with values ranging from approximately 13% to 6%. These fluctuations are a direct consequence of the configuration of the pH control system, which does not allow the simultaneous injection of CO_2 and air. Consequently, during periods of CO_2 injection the airflow in the column was significantly reduced, limiting oxygen stripping and leading to temporary oxygen accumulation until air supply was restored. Similar interactions between CO_2 injection strategies, aeration, and oxygen removal efficiency have been described in recent studies on gas management and control strategies in outdoor photobioreactors [40]. The limited removal efficiency of the degassing column leads to a significant accumulation of dissolved oxygen within the photobioreactor. Fig. 6C illustrates the temporal profile of the dissolved oxygen inhibitory effect on the photosynthetic activity, represented by the dimensionless term $P_{O_2}(O_2)$. This parameter ranges between 0 and 1, where 0 represents complete photosynthetic inhibition and 1 indicates no oxygen-induced limitation on the photosynthesis rate. The values displayed in the

graph are estimated at the end of the channel. As can be observed, the high dissolved oxygen concentrations accumulated during daylight hours result in a 50% reduction in photosynthetic activity during peak operating hours.

4.4. Possible improvements

As shown in the previous sections, the performance of the reactor can still be improved. To this aim, the novel model presented in this work can be considered a valuable tool for system optimization, allowing the identification of new operational strategies for microalgae cultivation. In this context, the model was exploited to predict the system response when the main operating variables (temperature, dissolved oxygen and pH) are maintained at their optimal values, an approach commonly adopted in recent modelling and optimization studies on photobioreactors [43]. Specifically, simulations were carried out by fixing each operating variable at its optimal value and analysing the variation in biomass concentration after one day of cultivation. All optimization results presented correspond to daily average values calculated after the system reached periodic steady-state operation. Fig. 7 shows the results obtained for dissolved oxygen, whose concentration was maintained at 100% saturation, corresponding to the assumption that excess oxygen is continuously removed from the culture. Such a condition could be achieved in practice by increasing the airflow supplied to the degassing column or by modifying the geometry of the degassing section or the channel [40]. Although dissolved oxygen accumulation was identified as the main inhibitory factor affecting photosynthetic activity along the channel, the magnitude of this effect under the evaluated operating conditions was moderate. Model simulations indicate that maintaining dissolved oxygen concentrations close to air saturation would increase biomass productivity by approximately 5%, suggesting that oxygen management can improve reactor performance but is not the dominant factor controlling productivity under the conditions investigated (Fig. 7). This result is consistent with recent findings showing that excessive dissolved oxygen accumulation may inhibit microalgal growth and that improved oxygen removal can enhance productivity [44]. Microalgae growth is also known to be inhibited at excessively high or low pH values. Fig. 7 also reports the results of a simulation in which pH was maintained at its optimal value. It can be observed that operating the system at a constant optimal pH does not lead to a significant improvement in biomass growth. This is because the imposed pH value (8) is close, but not equal, to the true optimum. Temperature optimization could theoretically lead to an enhanced algal growth rate through

the installation of a heat exchange system capable of maintaining the culture at its optimal temperature. To selectively promote photosynthetic activity without increasing dark respiration rates, a temperature control strategy operating solely during daytime hours was implemented in the simulation. Based on this approach, a 2.6% increase in daily biomass productivity was estimated compared to the current operating conditions.

As a final analysis, the influence of daily harvesting was investigated (Fig. 8), representing the daily averages obtained once the system reached a periodic steady state under cyclic day/night environmental inputs. In this operational configuration, the harvesting process implies that as the microalgae culture is continuously or semi-continuously removed from the system, an equal flow rate of fresh nutrient medium is simultaneously supplied to maintain a strictly constant working volume inside the reactor loop. This simultaneous feeding and harvesting mechanism explains how the system can physically operate at daily dilution rates greater than 1.0 d^{-1} , meaning that a volume greater than the total reactor capacity is renewed within a 24-h period [45]. The objective of this analysis was to identify an optimal compromise between biomass growth within the reactor and harvested biomass, namely the optimal dilution rate. Excessive harvesting (high dilution rate) may result in culture wash-out, while insufficient harvesting (low dilution rate) leads to poor biomass productivity. This trade-off between growth and harvesting rate is well documented in recent fed-batch and semi-continuous microalgae cultivation studies [46].

As can be observed in Fig. 8, biomass productivity exhibits a characteristic parabolic trend with respect to the dilution rate, revealing a classic trade-off between nutrient availability, light integration, and cell density [47]. At low dilution rates, such as the current operating baseline of approximately 0.2 d^{-1} , productivity is constrained by self-shading phenomena caused by high biomass concentrations. This high cell density limits light penetration within the thin layer, reducing the net photosynthetic efficiency of the culture [25]. Conversely, at excessively high dilution rates (above 1.6 d^{-1}), the progressive over-dilution of the culture drastically reduces the overall light absorption efficiency of the system, steering the reactor toward the kinetic washout zone. The model successfully identifies the absolute optimal operating point at a daily dilution rate of approximately 1.2 d^{-1} . At this optimal point, the biomass concentration settles around 0.65 g L^{-1} , which maximizes light penetration while maintaining a sufficient number of cells to optimize photon capture. Operating at this optimal dilution rate yields a simulated maximum daily productivity of approximately $0.68 \text{ g L}^{-1} \text{ Days}^{-1}$, representing a significant improvement compared to the current operating baseline. However, from an industrial and downstream processing perspective, achieving the absolute maximum productivity at the peak of the curve might not represent the most economically viable strategy. As predicted by the model, operating at the optimal dilution rate of 1.2 d^{-1} leads to a 50% reduction in the steady-state biomass concentration, which drops from the baseline of 1.2 g L^{-1} down to approximately 0.65 g L^{-1} . Such a dilute culture significantly increases the volume of water to be processed during the harvesting phase, thereby escalating the energy requirements and operational costs of downstream operations like centrifugation or filtration [45]. To mitigate this drawback, the developed model can be used to identify a sub-optimal but operationally superior sweet spot. By selecting a more conservative intermediate dilution rate—for instance, between 0.6 and 0.8 d^{-1} —the system allows the culture to remain significantly more concentrated, maintaining a biomass concentration of 1.0 g L^{-1} . Interestingly, even at this more concentrated state, the model predicts that biomass productivity would still increase by approximately 45% to 60% compared to the current baseline operation (0.3 d^{-1}). This compromise configuration offers an excellent balance for pilot and industrial-scale operations, as it captures a substantial portion of the potential productivity gains while preventing the economic penalties associated with processing highly diluted cultures.

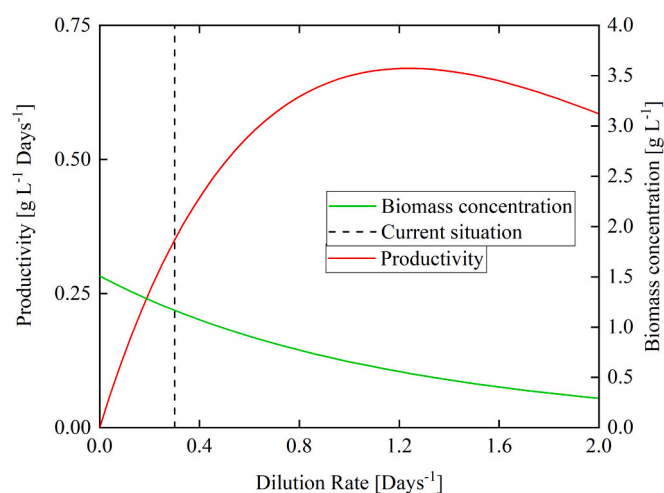


Fig. 8. Simulated daily biomass productivity as a function of the daily dilution rate evaluated across different harvesting operational windows (1 to 4 h). The results represent the daily averages computed after the model achieved a periodic steady state under cyclic day/night environmental inputs.

5. Conclusions

A comprehensive dynamic model for a pilot-scale thin-layer photobioreactor was developed, calibrated, and validated using outdoor experimental data. The model integrates biological kinetics, gas–liquid mass transfer, heat transfer, and spatial transport phenomena, enabling the prediction of biomass growth, dissolved oxygen concentration, pH, and culture temperature under both batch and semi-continuous operating conditions. Unlike conventional raceway reactor models, the proposed framework incorporates a spatially distributed description of the thin-layer channel, allowing the analysis of longitudinal gradients of dissolved gases, temperature, and photosynthetic activity. Validation against independent datasets demonstrated good agreement between experimental observations and model predictions, confirming the capability of the model to describe the main biological and physico-chemical processes occurring in thin-layer photobioreactors.

Model simulations revealed that dissolved oxygen accumulation can locally inhibit photosynthetic activity under high irradiance conditions, although its overall impact on biomass productivity remained moderate under the operating conditions investigated, with improved oxygen stripping increasing productivity by approximately 5%. In contrast, operational optimization, particularly through adjustment of the dilution rate, was identified as a key strategy for enhancing reactor performance, with predicted productivity improvements of up to 70–95% relative to the baseline operation. Overall, the proposed model provides a robust tool for the design, optimization, and control of thin-layer photobioreactors and establishes a quantitative framework for the analysis and scale-up of microalgae cultivation systems under outdoor conditions.

Notations

Letters

A	Cross section	$[m^2]$
a	Absorptivity	$[-]$
C	Concentration	$g\ m^{-3}$
$[CO_2]$	Molar concentration of CO_2	$[mol\ m^{-3}]$
c_p	Heat capacity	$[J\ g^{-1}\ ^\circ C^{-1}]$
e	Emissivity	$[-]$
h_c	Thermal transmittance of the culture	$[W\ m^{-2}\ ^\circ C^{-1}]$
H	Henry's constant	$[mol\ m^{-3}\ atm^{-1}]$
$[H^+]$	Molar concentration of hydrogen ions	$[mol\ m^{-3}]$
I_{av}	Average photon flux density in culture	$\mu E\ m^{-2}\ s^{-1}$
I_0	Incident radiation	$\mu E\ m^{-2}\ s^{-1}$
K_a	Extinction coefficient	$m^2\ g^{-1}$
K_I	Saturation constant	$\mu E\ m^{-2}\ s^{-1}$
$K_L a_L$	Volumetric gas-liquid mass transfer coefficient	$[s^{-1}]$
K_{O_2}	Oxygen inhibition constant	$g\ m^{-3}$
M	Molar mass	$[g\ mol^{-1}]$
n	Shape parameter	$[-]$
$[O_2]$	Molar concentration of dissolved oxygen in the culture	$[mol\ m^{-3}]$
P	Pressure	$[atm]$

(continued on next column)

(continued)

p	Vapor pressure	$[atm]$
pH	pH inside the culture	$[-]$
P_{O_2}	Photosynthetic rate	$[g_{O_2}\ g_{biomass}^{-1}\ s^{-1}]$
$P_{O_2}(I_0)$	Influence of incident radiation on photosynthetic velocity	$[g_{O_2}\ g_{biomass}^{-1}\ s^{-1}]$
$\overline{P_{O_2}(O_2)}$	Influence of dissolved oxygen concentration in the culture on the photosynthetic rate	$[-]$
$\overline{P_{O_2}(pH)}$	Influence of the pH present in the culture on the photosynthetic rate	$[-]$
$\overline{P_{O_2}(T)}$	Influence of the temperature present in the culture on the photosynthetic rate	$[-]$
Q	Volumetric flow rate	$[m^3\ s^{-1}]$
r	Relationship between respiratory activity and maximum photosynthetic rate	$[-]$
S_c	Surface of the equipment in contact with the outside	$[m^2]$
T	Temperature present in the crop	$[^\circ C]$
t	Time	$[s]$
V	Section volume	$[m^3]$
x	Space	$[m]$
Y	Normalized molar fraction with respect to the nitrogenous fraction	$[-]$
$\frac{Y_{C_T}}{O}$	Ratio of molar mass of carbon to oxygen	$[g_C\ g_{O_2}^{-1}]$
$\frac{Y_{C_T}}{CO_2}$	Ratio of molar mass of carbon to CO_2	$[g_C\ g_{CO_2}^{-1}]$
$\frac{Y_X}{O}$	Relationship between biomass production and O_2 production	$[g_{biomass}\ g_{O_2}^{-1}]$
y	Molar fraction in gas phase	$[-]$
Z	Shape parameter	$[-]$

Greek letters

ε	Gas fraction	$[-]$
ρ	Density	$g\ m^{-3}$
σ	Boltzman constant	$[W\ m^{-2}\ K^{-4}]$

Superscripts

0	Initial
$*$	Equilibrium conditions
$Chan$	Referring to the channel
Col	Referring to the degassing column
$Tank$	Referring to the recirculation tank

Subscripts

Amb	Referring to the external environment
b	Referring to biomass
CO_2	Referring to CO_2
C_T	Referring to total inorganic carbon
gas	Gas
Liq	Liquid
m	Referring to the culture medium
MAX	Maximum
MIN	Minimum
N_2	Referring to nitrogen
O_2	Referring to oxygen
OPT	Optimal
Tot	Total

CRediT authorship contribution statement

Giuseppe Gargano: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Data curation. **Alessandro Concas:** Writing – review & editing, Supervision, Data curation. **Giacomo Cao:** Writing – review & editing, Supervision. **Francisco Gabriel Acien Fernández:** Writing – review & editing, Validation, Supervision, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giuseppe Gargano reports financial support was provided by Spanish Ministry of Science and Innovation. Francisco Gabriel Acien Fernandez reports financial support was provided by REALM project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

G. Gargano acknowledges financial support from the Spanish Ministry of Science and Innovation through grant DIN2021-011774. This work was carried out as part of the REALM project, which G. Gargano and F. G. Acien Fernández gratefully acknowledge for its support.

Data availability

Data will be made available on request.

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