Dynamical Simulation, Sensitivity, and Productivity Analysis of a Light-Photoacclimation Model for Microalgae-Based Carbohydrate Production in Continuous Photobioreactors

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Abstract: The world’s human population is increasing as is the demand for new sustainable sources of energy. Accordingly, microalgae-based carbohydrates for biofuel production are being considered as an alternative source of raw materials for producing biofuels. Microalgae grow in photobioreactors under constantly changing conditions. Models improve our understanding of microalgae growth. In this paper, a photoacclimated model for continuous microalgae cultures in photobioreactors was used to study the time-varying behavior and sensitivity of solutions under optimal productivity conditions. From the perspective of dynamic simulation in this work, light intensity was found to play an influential role in modifying metabolic pathways as a cell stressor. Enhancing carbohydrate productivity by combining nutritional deficiency and light intensity regulation modeling strategies could be helpful to optimize the process for the highest yield in large-scale cultivation systems. Under the proposed simulation conditions, a maximum carbohydrate productivity of 48.11 gCm⁻³d⁻¹ was achieved using an optimal dilution rate of 0.2625 d⁻¹ and 350 µmolm⁻²s⁻¹ of light intensity. However, it is important to note that, a particular set of manipulated inputs can generate multiple outputs at a steady state. A numerical solution of the sensitivity functions indicated that the model outputs were especially sensitive to changes in parameters corresponding to a minimum nitrogen quota, maximum nitrogen intake rate, dilution rate, and maximum nitrogen quota compared to other model parameters.

Keywords: dynamic simulation; photoacclimation model; photobioreactors; microalgae; carbohydrate pool; sensitivity; productivity

1. Introduction

Global energy consumption is continuously rising due to the rapid growth of the world’s population. As a consequence, the widespread use of fossil fuels worldwide has resulted in their depletion and near exhaustion due to their nonrenewable and unsustainable nature [1,2]. In order to satisfy this energy demand, researchers are investigating the possibility of producing biofuels from biomass. Biomass refers to any organic matter capable of serving as an energy source [3]. This could includes wood, crops, and even garbage [4]. Nevertheless, the selection of appropriate feedstock for biofuel production plays a major role in the success of the process [5]. As bulk commodities, the production of biofuels requires abundant and cost-effective feedstocks, which are essential for the process to be economically viable, as well as other equally important social, environmental, geographical, and industrial factors [6,7]. Microalgae are considered to be more ecofriendly and sustainable energy sources due to their low emissions of greenhouse gases and other
pollutants [8]. Thus, the use of microalgae as a renewable source can also contribute to the mitigation of climate change [9–11]. In addition, microalgae require a fraction of the space required for most land-based energy crops; for example, producing algal biomass to meet 50% of the U.S. transport fuel demand would require only 1 to 3% of the total U.S. cropping area [12]. Moreover, microalgae have the advantage of being able to produce a larger amount of biomass in a shorter time compared to other sources. In fact, most strains are capable of doubling their cell mass in less than 24 h [13,14]. Within this context, microalgae have been found to be the most efficient organisms for converting sunlight into valuable energy-producing organic compounds; among the most important organic compounds in algae biomass are carbohydrates, proteins, and lipids [15–18]. Carbohydrates are the primary products of photosynthesis. In photosynthesis, nicotinamide adenine dinucleotide phosphate (NADPH) is utilized to fix and convert the carbon dioxide (CO$_2$) into glucose through a metabolic pathway known as the Calvin cycle. Although carbohydrates are lower in energy than other microalgae compounds, such as lipids, they are considered the most suitable material to produce biofuels by combining biotechnology with thermochemical conversion [19,20]. In general, biomass can be converted to energy through a variety of biological as well as thermochemical methods [21]. Biological conversion includes the fermentation of degradable substances to produce renewable energy sources, such as bioethanol, biobutanol, biohydrogen [22]. Accordingly, microalgae-based carbohydrates for biofuel production, are being considered as an alternative source of raw materials for producing biofuels [23–25]. Even though carbohydrates are lower in energy than are other microalgae compounds, such as lipids, they are considered to be the most suitable material for conversion into biofuels through a thermochemical conversion process since they contain a low level of lignin [26]. In light of this, microalgae-based carbohydrates are considered a promising alternative source of raw materials for the production of biofuels. The photobioreactor (PBR) is designed to convert luminous energy into valuable products. They are typically used in large-scale outdoor operations for the cultivation of microalgae and to provide precise control over the growth environment. There are four phases in a typical PBR: (1) solid phase (microalgal cells), (2) liquid phase (growth medium), (3) gaseous phase CO$_2$ and oxygen (O$_2$), and (4) a superimposed light-radiation field [27,28]. Moreover, microalgal cultures in photobioreactors (PBRs) are highly responsive to light intensity and require consistent light intensity to optimize biomass productivity and metabolic activity. Several types of PBRs have been developed so far, including bubble column, airlift reactor, flat-plate, stirred-tank, and tubular, among others [29,30]. There is a close relationship between light distribution, hydrodynamics, mass transfer, and growth kinetics that complicates the design process [31–33]. In-depth research efforts, especially those that combine theory and practice, remain necessary for the success of large-scale cultivation systems. Dynamic models are critical tools for optimizing and controlling microalgae-based carbohydrate systems in the laboratory and on large scales. Dynamic models are of utmost importance in achieving optimal carbohydrate production and other valuable metabolites in microalgae. To bridge the gap between theoretical predictions and industrial photosynthetic productivity, it is vital to identify the limiting factors that impact the conversion of light energy into biomass. This understanding allows for the development of effective strategies to optimize microalgal production and align theoretical insights with practical implementation [34–36]. Over the past decade, a variety of mathematical models that describe the growth kinetics of microalgae have been developed to understand their application in large-scale microalgae production [37]. These models examine the influence of process parameters such as light, temperature, nutrients, oxygen concentration, salinity, pH, and organic or inorganic carbon on microalgal growth rates [38]. In most of the models, these conditions change over time, and the effects of these variations on growth are not considered; these variations are extremely important factors to be investigated in order to address large-scale problems [39] and constitute the frontier of knowledge and the contribution to research. Photobioreactors are characterized by multivariable conditions for microalgae cultivation. The physical, chemical, and biological processes occurring inside
a photobioreactor are difficult to study because most of them take place simultaneously and are strongly interdependent. Proper control of the environmental variables governing these processes is essential to the successful design and operation of a photobioreactor [40]. As mentioned above, few parameters are nearly constant; as a result, identifying the parameters that have the greatest impact on the model through sensitivity analysis can help determine which parameters are most critical during parameter calibration [41,42]. In this study, a dynamic photoacclimated model for continuous microalgae culture systems was used to study time-varying state variables behavior as well as the first-order effects of solutions to parameter variations under optimal productivity conditions. To this end, it was crucial to recognize that the productivity of a photobioreactor is largely determined by the dynamic control of its environmental factors. Additionally, having a thorough understanding of how model sensitivities vary during different phases of growth can help to significantly improve their efficiency and productivity.

1.1. Dynamic Photoacclimation Model

New techniques from biotechnology and the control field are necessary for large-scale microalgal cultivation to ensure robustness, durability, and optimization of the process. A critical challenge in converting solar light energy into chemical energy is providing sufficient light to sustain cell growth. As biomass concentration increases, so does light absorption. However, the maximal biomass attainable is limited by a critical biomass concentration, where all impinging photons are absorbed. The limitation to achieving a high biomass yield in microalgal cultivation is not a straightforward matter, as it is affected by the adaptive mechanism of cells to optimize light harvesting through pigments [43]. The concentration of pigments is dependent on light intensity, which in turn determines light attenuation. Moreover, an excess of photons can cause irreversible damage to a key protein involved in the photosystem II reaction centers in the chloroplasts, leading to photodamage and photoinhibition. These challenges require the development of new modeling and control strategies for microalgal-based processes. Specific dynamic models have been designed to understand and model this process, taking into account the close relationship between photoinhibition and photoacclimation [44]. Geider et al. [45] were the pioneers in proposing a simple model that includes chlorophyll (Chl) as a variable, along with microalgal carbon and nitrogen, to account for the response of photosynthesis to light and nitrogen status [46]. From this model, other models have been proposed [47]. These additional models aim to improve the understanding of microalgae behavior and optimize their biomass production. The primary characteristic of these models is the process of photoacclimation, which enables the microalgae to adjust the synthesis of pigments, particularly chlorophyll, in response to changes in light intensity [47–50]. One of the main challenges in the development of microalgal models is their calibration, validation, and application for control purposes. Despite these challenges, these models have a crucial role in enhancing our understanding of microalgal behavior and optimizing their growth and biomass production.

In this study, a dynamic model explaining photoinhibition and photoacclimation was used [51–53]. The model employs the classical Droop expression [54]. In a photobioreactor, the Droop equations describe the growth of microorganisms by combining three coupled, nonlinear ordinary differential equations. The Droop model uncouples growth from substrate uptake, resulting in defining an internal cell quota (i.e., the amount of internal nutrients present in each unit of biomass), which determines growth rate. The amount of nutrients taken up by cells is determined by the external concentration of nutrient y (nitrogen) [55]. The subsistence quota restricts algae growth below a certain amount. The photon flux density of photoacclimation is introduced as a state variable, $I^*$, to represent the light intensity of photoacclimation at a given physiological level [56]. The model proposed in [56,57] presents an analytical integration based on the average growth rate of the entire culture. This model, in its simplified form, has potential for process control, especially when the expression of photoinhibition is changed to a Haldane-inhibitory formulation.
A microalgae photobioreactor is a complex system, primarily due to its nonlinear and time-varying behavior, and most studies do not account for photoacclimation, so it would be valuable to examine its dynamic behavior.

By incorporating the mathematical description of photoacclimation and applying appropriate kinetic expressions for the nitrogen intake rate ($\rho$) and the specific growth rate ($\mu$), the Droop’s mass balance differential equation for biomass $x$, substrate $y$, and intracellular nitrogen quota $z$ are determined for an assumed perfectly mixed PBR [56,58,59]; the model nomenclature is described in detail in Table 1.

\[
\begin{align*}
\dot{x} &= \mu(x, z, l_0)x - Dx - Rx \\
\dot{y} &= Dy_{in} - \rho(y, z)x - Dy \\
\dot{z} &= \rho(y, z) - \mu(x, z, l_0)(z - z_c) + Rz \\
\end{align*}
\]  

(1)

In this equation, $D$ is the dilution rate (the ratio of the flow rate of the influent over the volume of the photobioreactor), and $Dy_{in}$ is the influent nutrient concentration of nitrate in the influent. The respiration rate is expressed by $R$. The uptake rate assumed in this model corresponds to that defined by the Michaelis–Menten law, and the specific growth rate is expressed in terms of the intracellular nitrogen quota ($z$) [54–56,58,60]. In order to calculate the remaining parameters of the model, the expressions in (2)–(11) are used.

<table>
<thead>
<tr>
<th>Table 1. List of Functions, State Variables, and Parameter Nomenclature.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functions Definition Unit</td>
</tr>
<tr>
<td>$\mu$ Specific growth rate gCm$^{-3}$</td>
</tr>
<tr>
<td>$\bar{\mu}$ Maximum specific growth rate gNm$^{-3}$</td>
</tr>
<tr>
<td>$k_{sl}$ Normalized growth half saturation constant gN(gC)$^{-1}$</td>
</tr>
<tr>
<td>$\theta$ Chlorophyll quota gChl(gC)$^{-1}$</td>
</tr>
<tr>
<td>$\xi$ Light-attenuation rate light-attenuation coefficient d$^{-1}$</td>
</tr>
<tr>
<td>$\lambda$ Optical depth m$^{-1}$</td>
</tr>
<tr>
<td>$Chl$ Chlorophyll concentration gCm$^{-3}$</td>
</tr>
<tr>
<td>State variables Definition Unit</td>
</tr>
<tr>
<td>$x$ Biomass concentration gCm$^{-3}$</td>
</tr>
<tr>
<td>$y$ External nutrient concentration gNm$^{-3}$</td>
</tr>
<tr>
<td>$z$ Intracellular nitrogen quota gN(gC)$^{-1}$</td>
</tr>
<tr>
<td>$l^*$ Photon flux density acclimation $\mu$molm$^{-2}$s$^{-1}$</td>
</tr>
<tr>
<td>$q_g$ Carbohydrate quota gC(gC)$^{-1}$</td>
</tr>
<tr>
<td>Parameters Definition Unit</td>
</tr>
<tr>
<td>$l_0$ Light intensity $\mu$molm$^{-2}$s$^{-1}$</td>
</tr>
<tr>
<td>$D$ Dilution rate d$^{-1}$</td>
</tr>
<tr>
<td>$y_{in}$ Nitrogen concentration in the reactor inlet gNm$^{-3}$</td>
</tr>
<tr>
<td>$I$ Average photon flux density throughout the culture $\mu$molm$^{-2}$s$^{-1}$</td>
</tr>
<tr>
<td>$\rho$ Nitrogen intake rate gN(gC)$^{-1}$d$^{-1}$</td>
</tr>
<tr>
<td>$\rho_m$ Maximum specific growth rate gNm$^{-3}$</td>
</tr>
<tr>
<td>$k_s$ Substrate-uptake half-saturation constant gNm$^{-3}$</td>
</tr>
<tr>
<td>$k_{sl}$ Growth half-saturation constant gChl(gN)$^{-1}$</td>
</tr>
<tr>
<td>$k_{sl}$ Photon flux density saturation constant over growth gChl(gN)$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$ Chlorophyll saturation function gChl(gN)$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_m$ Maximum chlorophyll saturation function gChl(gN)$^{-1}$</td>
</tr>
<tr>
<td>$k_{sl}$ Chlorophyll saturation function constant gChl(gN)$^{-1}$</td>
</tr>
<tr>
<td>$K_g$ Average photon flux density saturation function constant $\mu$molm$^{-2}$s$^{-1}$</td>
</tr>
<tr>
<td>$L$ Culture depth m</td>
</tr>
<tr>
<td>$a$ Attenuation coefficient due to chlorophyll m$^2$(gChl)$^{-1}$</td>
</tr>
<tr>
<td>$b$ Attenuation coefficient due to biomass m$(gC)$$^{-1}$</td>
</tr>
<tr>
<td>$c$ Attenuation coefficient due to background turbidity m$^{-1}$</td>
</tr>
<tr>
<td>$\alpha$ Protein synthesis coefficient gC(gN)$^{-1}$</td>
</tr>
<tr>
<td>$\beta$ Fatty acid synthesis coefficient gC(gN)$^{-1}$</td>
</tr>
<tr>
<td>$z_{cm}$ Maximum nitrogen quota gN(gC)$^{-1}$</td>
</tr>
<tr>
<td>$z_{c0}$ Minimum nitrogen quota gN(gC)$^{-1}$</td>
</tr>
<tr>
<td>$R$ Respiration rate d$^{-1}$</td>
</tr>
</tbody>
</table>
1.1.1. Specific Growth Rate

The specific growth rate is recalled from [56] in order to take into account the light intensity.

\[ \mu(x, z, I_0) = \beta(I) \left(1 - \frac{z_0}{z}\right) \tag{2} \]

\[ \beta(I) = \bar{\mu} \frac{I}{I + K_{sI} + \frac{I^*}{K_{il}}} \tag{3} \]

\[ k_{sI} = \frac{k_{sI}^*}{\theta} \tag{4} \]

\[ \theta = \frac{Chl}{x} \tag{5} \]

\[ \xi = aChl + bx + c \tag{6} \]

\[ \lambda = \xi L \tag{7} \]

\[ Chl = \gamma(I^*)xz \tag{8} \]

\[ \gamma(I^*) = \gamma_m \frac{k_{1I}^*}{k_{1I}^* + I^*} \tag{9} \]

1.1.2. Nutrient Uptake

According to the Michaelis–Menten law, the uptake rate can be defined as follows:

\[ \rho(y, z) = \rho_m \frac{y}{y + k_y \left(1 - \frac{z}{z_m}\right)} \tag{10} \]

For modeling the time evolution of this additional variable, Beer–Lambert expressions for the absorbance of light in the culture medium have been proposed [60]. The average light irradiation (i.e., the photon flux density that affects the culture mass on average) is calculated with \( \bar{I} \) and is used to drive the dynamics of the acclimation phenomenon through a factor proportional to \((\bar{I} - I^*)\) [58].

1.1.3. Average Photon Flux Density throughout the Culture

\[ \bar{I} \approx I_0 \frac{K_g \lambda}{K_g + K_\lambda} \tag{11} \]

1.2. Carbon Flux and Carbohydrates Dynamics

In order to explain how nitrogen limitations affect carbohydrate and neutral lipid accumulations, Mairet [57] has proposed a dynamical model based on a simplified carbon metabolism, as shown in Figure 1.

![Figure 1. Metabolic route for carbohydrate dynamics.](image)

First, carbon dioxide is shown to be incorporated into a carbohydrate pool. These carbohydrates are mobilized by microalgae to produce proteins and nucleic acids, which
are dependent on nitrogen. Moreover, the carbohydrate compartment \((g)\) is used in a parallel pathway to synthesize free fatty acids (FFAs). The rate of fatty acid synthesis is determined by the rate of photosynthesis, as well as the nitrogen quota. Further, the FFAs may either be stored as neutral lipids \((l)\) or mobilized for the purpose of creating functional carbon \((f)\) (membranes) \([56,57]\).

**Carbohydrate Dynamics Deduction**

On the basis of the previously described simplified metabolic network shown in Figure 1, the dynamics of carbohydrate production could be derived as follows:

\[
\dot{g} = (1 - \beta z)\mu(x, z, I_0)x - \alpha \rho(y, z)x - Dg - Rx
\]  
(12)

As shown in Figure 1, biomass \((x)\) is composed of functional carbon \((f)\), carbohydrates \((g)\), and lipids \((l)\). Based on this information, Equation (13) represents the sum of these three sources of carbon:

\[
x = f + g + l
\]  
(13)

Based on the above, the proportion that corresponds to the carbohydrate share of total biomass can be calculated as follows:

\[
q_g = \frac{g}{x}
\]  
(14)

By dividing both sides of Equation (12), Equation (15) becomes

\[
\frac{\dot{g}}{x} = \frac{(1 - \beta z)\mu(x, z, I_0)x - \alpha \rho(y, z)x - Dg - Rx}{x}
\]  
(15)

By solving \(D\) of (1) in steady state, we obtain Equation (16) as follows:

\[
D = \mu(x, z, I_0) - R
\]  
(16)

By substituting the value of \(D\) into Equation (15), Equation (17) is obtained as follows:

\[
\frac{\dot{g}}{x} = \frac{(1 - \beta z)\mu(x, z, I_0) - \alpha \rho(y, z) - (\mu(x, z, I_0) - R)\frac{g}{x} - R}{x}
\]  
(17)

Substituting \(\frac{\dot{g}}{x}\) for \(q_g\) and \(\frac{\dot{g}}{x}\) for \(q_g\) in Equation (17) results in (18) in the following:

\[
\dot{q}_g = (1 - \beta z - q_g)\mu(x, z, I_0) - \alpha \rho(y, z) - (\mu(x, z, I_0) - R)q_g - R
\]  
(18)

This Equation (18) can be simplified to provide the following Equation (19), which describes the dynamics of the carbohydrate quota:

\[
\dot{q}_g = (1 - \beta z - q_g)\mu(x, z, I_0) - \alpha \rho(y, z) + R(q_g - 1)
\]  
(19)

2. Materials and Methods

2.1. The Fermentation Process Simulation

Photobioreactors have been designed to produce high algal biomass density and rapid multiplication at a low cost \([27]\). The representation shown in Figure 2 illustrates the main inputs and outputs of the microalgae photobioreactor considered in this report.

The fermentation process simulation described in this study aims to model and analyze the dynamic behavior of microalgae cultivation. The simulation utilizes a photobioreactor (PBR) in which, instead of a sole reliance on the absolute volume of the reactor, the control parameter chosen for this simulation is the dilution rate. By employing the dilution rate as a control parameter, the simulation addresses concerns related to reactor scale. The
dilution rate provides a more meaningful measure of the rate of dilution and medium renewal, allowing for better control and optimization of the fermentation process. It enables precise adjustments of nutrient concentration and other critical parameters throughout the simulation, ensuring accurate representation of the actual conditions.

![Figure 2. Conceptual illustration of the cultivation of microalgae in a continuous photobioreactor system. The variables $y$, $x$, $z$, $q_g$, and $I^*$ represent external nutrients, biomass, intracellular nitrogen quota, carbohydrate quota, and photoacclimation respectively. The dilution rate and initial light intensity are given by $D$ and $I_0$, respectively.](image)

Meanwhile, the influent nutrient concentration ($y_{in}$) in the simulation of the fermentation process refers to the concentration of nutrients in the culture medium or input medium before cells initiate growth, as depicted in Figure 2. This concentration can either remain constant or vary over time depending on the experimental design or system conditions. In this study, the variable ($y_{in}$) was set to be equal to the initial concentration of the external nutrient ($y_0$), which represents the nutrient concentration within the microalgal cells at the beginning of the cultivation.

2.1.1. Methodology for Dynamic Simulation

Summarizing, the PBR model (1)–(19) describes the evolution of biomass and the ones of carbohydrates and photoacclimation factor, and prompts the close coupling between these states. This model enables the analysis of the effect of light in biomass and carbohydrate production, which is carried out in the following. The effect of nutrients has been examined in several other studies with only Droop-type models. The set of ordinary differential equations (ODEs) of the model was solved through a solver called LSODE in GNU Octave version 7.1.0. The methods included for numerical solution can be found in [61]. The model parameters are given in Table 2, which were recalled from [56,58] to simulate the cultivation of *Isochrysis galbana* strain. The initial conditions for the Droop model were estimated using data obtained from culture runs reported in [58]. These runs involved simulations with the Droop model, which was also utilized in this study. The collected experimental data were utilized for parameter identification and cross-validation tests. The measured state variables in [58], including biomass concentration, intracellular nitrogen quota, external nutrient concentration, and chlorophyll concentration, were analyzed along with their corresponding 95% confidence intervals. These experimental results were compared to the model predictions based on the identified parameters, allowing for a visual comparison. By incorporating the identified parameters and comparing the experimental and model results, more realistic initial conditions for the state variables could be established for the dynamic simulation analysis in this study. Additionally, the initial condition for the carbohydrate quota was estimated using simulations from a previous study [62].
Table 2. Model parameters for simulating the cultivation of *Isochrysis galbana* [56,58].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\bar{\mu})</td>
<td>1.7</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>(k_y)</td>
<td>0.0012</td>
<td>gNm(^{-3})</td>
</tr>
<tr>
<td>(z_{c0})</td>
<td>0.05</td>
<td>gN(gC)(^{-1})</td>
</tr>
<tr>
<td>(z_{cm})</td>
<td>0.25</td>
<td>gN(gC)(^{-1})</td>
</tr>
<tr>
<td>(p_m)</td>
<td>0.073</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>(k_{sI})</td>
<td>1.4</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>(k_{I})</td>
<td>295</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>R</td>
<td>0.0081</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>(\gamma_m)</td>
<td>0.57</td>
<td>gChl(gN)(^{-1})</td>
</tr>
<tr>
<td>(k_{I}^{*})</td>
<td>63</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>a</td>
<td>16.2</td>
<td>m(^2)(gChl)(^{-1})</td>
</tr>
<tr>
<td>b</td>
<td>0.087</td>
<td>m(^2)(gChl)(^{-1})</td>
</tr>
<tr>
<td>c</td>
<td>0</td>
<td>m(^{-1})</td>
</tr>
<tr>
<td>(K_g)</td>
<td>10.6</td>
<td>–</td>
</tr>
<tr>
<td>(a)</td>
<td>2.6</td>
<td>mgC(mgN)(^{-1})</td>
</tr>
<tr>
<td>(\beta)</td>
<td>4.8</td>
<td>mgC(mgN)(^{-1})</td>
</tr>
</tbody>
</table>

\(^1\) Parameter taken from [58].

In order to analyze the effect of light intensity, the analysis of equilibrium and productivity, as well as a parameter sensitivity analysis, were carried out considering four scenarios of light intensity, as shown in Table 3. Considering the light-intensity constant was first methodological study step.

Table 3. Scenarios of initial light intensity for continuous photobioreactor simulations of *Isochrysis galbana* strain growth.

<table>
<thead>
<tr>
<th>Simulation Scenario</th>
<th>Initial Light Intensity ((I_0)) (^1)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>B</td>
<td>150</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>C</td>
<td>250</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>D</td>
<td>500</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
<tr>
<td>E</td>
<td>750</td>
<td>(\mu)molm(^{-2})s(^{-1})</td>
</tr>
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</table>

\(^1\) Simulated under constant initial light intensity.

2.1.2. Methodology for Equilibrium and Productivity Analysis

Biomass productivity has long been recognized as a key indicator of continuous bioreactor performance. In this section, we leverage the model’s capability to accurately describe carbohydrate accumulation to identify the optimal operational parameters, namely light intensity and dilution rate. By doing so, we can simulate and attain a maximum carbohydrate yield under the specific conditions outlined in this study. The resulting productivity values are then employed as nominal parameters in our comprehensive sensitivity analysis. Equations (20) and (21) illustrate the calculation of diagrams showing the equilibria and productivity of biomass \((x)\) and carbohydrates \((g)\) in relation to simulations that vary the parameters of light intensity and dilution rate. The simulation was conducted for a period of 100 days. The calculation of biomass and carbohydrate productivity was carried out by computing the following equations:

\[
P_C = \bar{x}\bar{D}
\]

\[
P_g = \bar{x}\bar{\mu}_k\bar{D}
\]

\(P_C\) is the biomass productivity curve, and \(P_g\) is the carbohydrate productivity curve. \(\bar{x}\) represents the vector of solutions for biomass at steady state. The expression \(\bar{D}\) represents the range of dilution rates from 0.02 to 0.5 with increments of 0.02 units. There was a range
of variation between 50 and 1000 molm$^{-2}$s$^{-1}$ with increments of 25 units. A vector of carbohydrate quota solutions at a steady state is denoted as $\vec{q}^g$, which is obtained from simulations of a continuous culture system using the Isochrysis galbana strain. The data used for these simulations are presented in Table 2.

2.1.3. Methodology for Sensitivity Analysis

To provide first-order estimates of the effect of parameter variations on the state variables solutions, the sensitivity function (22) was solved by the following steps [63]: First, the nominal state equation was solved for nominal parameters. Then, the Jacobian matrices (23) and (24) of the considered model were calculated and evaluated. Finally, the sensitivity equations were numerically solved using Equation (25).

The function $S(t)$ is called a sensitivity function, and (22) is called a sensitivity equation. The nomenclature list is shown in Table 4.

\[
\dot{S}(t) = A(t, \lambda_0)S(t) + B(t, \lambda_0)
\]  
(22)

where:

\[
A(t, \lambda) = \left. \frac{\partial f(t,x,\lambda)}{\partial x} \right|_{x=x(t,\lambda)}
\]  
(23)

\[
B(t, \lambda) = \left. \frac{\partial f(t,x,\lambda)}{\partial \lambda} \right|_{x=x(t,\lambda)}
\]  
(24)

\[
\begin{align*}
\dot{x}_1 &= f(t, x, \lambda) & x(t_0) &= x_0 \\
\dot{x}_\lambda &= \left[ \frac{\partial f(t,x,\lambda)}{\partial x} \right] x_\lambda + \left[ \frac{\partial f(t,x,\lambda)}{\partial \lambda} \right] x_\lambda(t_0) &= 0
\end{align*}
\]  
(25)

Table 4. Nomenclature list of symbols used in the sensitivity function.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S(t)$</td>
<td>Sensitivity function</td>
</tr>
<tr>
<td>$x$</td>
<td>State variables</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>System evaluated parameters</td>
</tr>
<tr>
<td>$\lambda_0$</td>
<td>Nominal value of $\lambda$</td>
</tr>
<tr>
<td>$A$</td>
<td>First-order partial derivatives with respect to $x$</td>
</tr>
<tr>
<td>$B$</td>
<td>First-order partial derivatives with respect to $\lambda$</td>
</tr>
</tbody>
</table>

The aforementioned sensitivity function enables us to estimate the first-order effect of parameter variations on solutions. Additionally, it can be used to approximate the solution when the parameter $\lambda$ is sufficiently close to its nominal value $\lambda_0$. A Taylor series expansion around the nominal solution $x(t, \lambda_0)$ can be used to obtain a good approximation for small values of $\|\lambda-\lambda_0\|$, as shown in Equation (26) [63].

\[
x(x, \lambda) = x(t, \lambda_0) + S(t)(\lambda - \lambda_0) + \text{higher order terms}
\]  
(26)

By neglecting the higher-order terms [63], the solution $x(t, \lambda)$ can be approximated as follows:

\[
x(x, \lambda) \approx x(t, \lambda_0) + S(t)(\lambda - \lambda_0)
\]  
(27)

Equation (27) is the first-order approximation of the differential equation and serves as the foundation for the procedure. A more detailed explanation and justification for this approximation can be found in [63]. The approximation is valid if the differential equations are differentiable and continuous, satisfying the existence and uniqueness theorem [42,63]. In this context, sufficient conditions for the existence and uniqueness of the Droop model have been established in previous studies, such as in [64].
To calculate the sensitivity, it is convenient to rename the parameters and state variables in Equation (1). One of the simplest approaches is to define the aforementioned parameters and variables as follows:

The arrangement (28) is a representation of the parameters represented by the symbol \( \lambda \), and the array (29) is a representation of the nominal parameters \( \lambda_0 \).

\[
\lambda = \begin{bmatrix}
\lambda_1 & \lambda_6 & \lambda_{11} \\
\lambda_2 & \lambda_7 & \lambda_{12} \\
\lambda_3 & \lambda_8 & \lambda_{13} \\
\lambda_4 & \lambda_9 & \lambda_{14} \\
\lambda_5 & \lambda_{10} & \lambda_{15}
\end{bmatrix} = \begin{bmatrix}
D & K_{iI} & a \\
0 & \rho_m & \beta \\
\gamma_m & K_S & a \\
K_i^* & zc_m & b \\
K_I^* & zc_0 & k_S
\end{bmatrix}
\tag{28}
\]

\[
\lambda_0 = \begin{bmatrix}
\lambda_{01} & \lambda_{06} & \lambda_{011} \\
\lambda_{02} & \lambda_{07} & \lambda_{012} \\
\lambda_{03} & \lambda_{08} & \lambda_{013} \\
\lambda_{04} & \lambda_{09} & \lambda_{014} \\
\lambda_{05} & \lambda_{10} & \lambda_{015}
\end{bmatrix} = \begin{bmatrix}
D_0 & K_{iI0} & a_0 \\
0 & \rho_{m0} & \beta_0 \\
\gamma_{m0} & K_{S0} & a_0 \\
K_{i0}^* & zc_{m0} & b_0 \\
K_{I0}^* & zc_{00} & k_{S0}
\end{bmatrix}_{\text{nominal}}
\tag{29}
\]

The arrangement (30) presents the nomenclature of the variables involved in the sensitivity analysis.

\[
x = \begin{bmatrix}
\dot{x}_1 \\
\dot{x}_2 \\
\dot{x}_3 \\
\dot{x}_4 \\
\dot{x}_5
\end{bmatrix} = \begin{bmatrix}
\dot{x} \\
\dot{y} \\
\dot{z} \\
\dot{I}^* \\
\dot{q}
\end{bmatrix}
\tag{30}
\]

By using the lsode function in GNU Octave v. 7.1.0, sensitivity equations were computed and simulated using the nominal parameters shown in Table 2.

The initial conditions of the above renamed state variables \( x_{10}, x_{20}, x_{30}, x_{40}, \) and \( x_{50} \) for the simulation of the Droop model’s sensitivity functions in a continuous culture system of Isochrysis galbana strain are shown in Table 5. It is worth noting that the dynamic simulations in Section 3.1 were performed using the initial conditions corresponding to \( (C_1) \) as shown in Table 5.

<table>
<thead>
<tr>
<th>Initial Conditions</th>
<th>( x_{10} )</th>
<th>( x_{20} )</th>
<th>( x_{30} )</th>
<th>( x_{40} )</th>
<th>( x_{50} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_1 )</td>
<td>10.1</td>
<td>33.2</td>
<td>0.055</td>
<td>50</td>
<td>0.3</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>30.7</td>
<td>41.3</td>
<td>0.055</td>
<td>50</td>
<td>0.3</td>
</tr>
<tr>
<td>( C_3 )</td>
<td>225</td>
<td>49.9</td>
<td>0.055</td>
<td>50</td>
<td>0.3</td>
</tr>
</tbody>
</table>

3. Results and Discussion

3.1. Dynamics Simulation

According to the FBR model presented in the Equations (1)–(19), this section presents the simulation results and analysis of the time-varying behavior of the light-photoacclimation model.

Figure 3 illustrates the time-varying behavior of biomass concentration and external nutrient concentration under different scenarios of light intensity shown in Table 3.

Figure 3b illustrates that scenario A achieved a lower steady-state biomass concentration (448.78 gCm\(^{-3}\)) than did scenarios B, C, D, and E, corresponding to 539.65, 558.12, 558.86, and 544.54 gCm\(^{-3}\), respectively. In scenario B, the biomass concentration was higher than the concentration obtained in scenario A. As compared to scenario B, scenario C had a higher biomass concentration. Considering the highest specific growth rate reached during the simulation of scenarios B and C, there was a difference of 47.19%.
Figure 3. Simulation of (a) external nutrient concentration and (b) biomass concentration in a continuous culture system of microalgae of the Isochrysis galbana strain. The simulation input parameters are presented in Table 2 and the initial conditions in Table 5. The light intensity settings for A, B, C, D, and E correspond to 50 µmolm$^{-2}$s$^{-1}$, 150 µmolm$^{-2}$s$^{-1}$, 250 µmolm$^{-2}$s$^{-1}$, 500 µmolm$^{-2}$s$^{-1}$, and 750 µmolm$^{-2}$s$^{-1}$, respectively.

Additionally, based on simulations conducted in scenarios D and E, an increase in light intensity led to a slight reduction in biomass concentration (2.56%) and growth rates (11.81%). Despite the fact that the concentration reached in the steady state in scenario D did not differ significantly from that found in scenario C, a slight reduction in growth rate (6.86%) was observed in scenario D compared to scenario C. Each simulation case maintained a constant initial concentration of biomass and nutrients. Similar findings were reported in a previous study [65], where it was observed that while growth saturation was rapidly achieved at irradiance levels exceeding 200 µmolm$^{-2}$s$^{-1}$, no indications of photoinhibition were observed, even at higher irradiance levels of up to 400 µmolm$^{-2}$s$^{-1}$. The findings of our study are consistent with the research conducted by Sukenik and Wahnon (1991) [66] and by Tzovenis et al. (2003) [67]. These studies also reported that growth saturation occurred at irradiance levels above 300 and 200 µmolm$^{-2}$s$^{-1}$, respectively, and no photoinhibition was observed until a light intensity of µmolm$^{-2}$s$^{-1}$ [67]. These findings highlight the robustness and reproducibility of the observed growth saturation phenomenon and the absence of photoinhibition under the specified irradiance conditions. These results suggest that our simulation outcomes are in line with the existing
literature and contribute to our understanding of the growth and photoinhibition dynamics of microalgae under varying light conditions.

Figure 3a illustrates that the concentration of external nutrients was removed more rapidly in scenario B (0.056 gN(gC)^{-1}d^{-1}) than in scenarios A, C, D, and E.

As a result, the biomass was obtained at a faster growth rate than in any other scenario, as shown in Figure 3b. However, as indicated in Figure 3a, in the exponential growth phase, scenario C resulted in a 14.3% slower consumption of external nutrients compared to scenario B despite a higher metabolization of the nutrient as biomass. These results suggest that although microalgae depend on light for photosynthesis and energy production, excessive light intensity can negatively impact their nutrient intake and metabolism. Therefore, it is essential to optimize the light conditions in microalgae cultures to promote efficient nutrient utilization and enhance overall growth and productivity. Furthermore, the rate at which the nutrient concentration was depleted was significantly affected by the intensity of the light, as shown in scenarios D and E in Figure 3a. Essentially, the mathematical simulation indicated that light affects the efficiency of photosynthetic energy capture to assimilate and metabolize nitrogen from the external culture medium. Furthermore, a decrease in nitrate concentration leads to a significant reduction in biomass content, indicating that nitrogen starvation negatively affects the metabolic activity and cell division in *I. galbana*. These findings are consistent with previous studies [68,69]. In microalgae culture systems, nutrient and carbon utilization are closely linked. In order to generate energy from stored carbon, microalgae use the tricarboxylic acid cycle. As a result of this energy, inorganic nitrogen (ammonium) is absorbed to produce glutamine and glutamate amino acids, which require energy in the form of ATP and NADPH, as well as carbon skeletons in the form of 2-oxoglutarate and oxaloacetate [70,71].

These results are consistent with the previous literature highlighting the importance of nitrogen in various biological macromolecules, such as proteins, chlorophyll, photosystems, enzymes, and genetic materials [72,73]. Nitrogen deprivation has been identified as a significant limiting factor that impacts both growth and biomass production [74,75]. Studies have observed that a lack of nitrogen leads to a decrease in the synthesis of photosynthetic pigments, which subsequently hampers the processes of photosynthesis and assimilation [76,77]. Therefore, our findings support the notion that light plays a critical role in the efficiency of photosynthetic energy capture for nitrogen assimilation from the external culture medium.

The mathematical simulation illustrated in the Figure 4 showed that the dynamic behavior of the assimilation of the intracellular nitrogen quota (z) varied with the illumination conditions used in each scenario. It was observed that the internal quota of nutrients increased in scenarios of higher light intensity (D and E), achieving a maximum saturation value as the external nutrient concentration decreased. In addition, the simulation indicated that once external nutrients were removed from the culture medium, the cells began to metabolize the nitrogen that the cells had stored.
**Figure 4.** Simulation of internal nutrient quota in a continuous culture system of microalgae of the *Isochrysis galbana* strain. The simulation input parameters are presented in Table 2 and the initial conditions in Table 5. The light intensity settings for A, B, C, D, and E correspond to 50 \(\mu\text{mol}\text{m}^{-2}\text{s}^{-1}\), 150 \(\mu\text{mol}\text{m}^{-2}\text{s}^{-1}\), 250 \(\mu\text{mol}\text{m}^{-2}\text{s}^{-1}\), 500 \(\mu\text{mol}\text{m}^{-2}\text{s}^{-1}\), and 750 \(\mu\text{mol}\text{m}^{-2}\text{s}^{-1}\), respectively.

Meanwhile, Figure 5 illustrates the results of a dynamic simulation of photoadaptation and chlorophyll concentration under different light-intensity conditions.

The photoacclimation dynamics described by \(I^*\), varied according to the simulated light-intensity scenarios. As can be observed in Figure 5a, the greater the intensity of incident light in the photobioreactor was \(I_0\), the greater the degree of photoacclimation. Conversely, as shown in Figure 5b, the higher the intensity of incident light in the photobioreactor was, the lower the chlorophyll concentration. In Figure 5b, it can be seen that in scenario A, the concentration of chlorophyll was higher over time than in any other scenario. In scenario E, there was a lower concentration of chlorophyll than in any other scenario. In accordance with the literature, the dynamic behavior observed in this study provides a consistent description of the photoacclimation mechanism [56]. The photoacclimation is the process by which chlorophyll synthesis is adapted to the intensity of light. There is a strong dynamic correlation between chlorophyll concentration and nutrient removal [78]. In order to maximize their ability to absorb light energy from sources of light, microalgae tend to produce a higher concentration of chlorophyll during low lighting conditions as shown in scenario A.

According to the results of the mathematical simulation of the carbohydrate quota in Figure 6a, scenario C had a higher carbohydrate quota concentration than did scenarios A, B, D, and E.
Figure 5. Simulation of (a) photoacclimation and (b) chlorophyll concentration in a continuous culture system of microalgae of the *Isochrysis galbana* strain. The simulation input parameters are presented in Table 2 and the initial conditions in Table 5. The light-intensity settings for A, B, C, D, and E correspond to 50 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), 150 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), 250 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), and 750 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), respectively.

In a mathematical simulation of carbohydrate quota dynamics, light intensity was shown to influence carbohydrate production. This may be a result of the fact that light intensity alters the way in which microalgae assimilate inorganic carbon. Consequently, low light intensities lead to a reduced capacity for carbon assimilation, which increases the ability of photosynthetic organisms to fix carbon dioxide by stimulating the synthesis of chlorophyll. In such poor lighting conditions, the assimilated carbon structures are mainly used to synthesize chlorophyll rather than other organic compounds, as shown in Figure 6b. On the other hand, nitrogen starvation in the culture medium induces a decrease in the production of pigments and an increase in the production of carbohydrates. In relation to this, the authors in [79] reported an unexpected increase in carbohydrate content in the microalgal culture under nitrogen deprivation, which aligns with the findings of [80] who observed an increase in relative carbohydrate content in *Picochlorum* under nitrogen deprivation.

In relation to the decrease in pigment production, a study conducted by [65] revealed that reducing the nitrate concentration in the cultivation medium from 8 mM to 0.5 mM led to a significant reduction in total chlorophyll and carotenoid content. Specifically, the total
chlorophyll content decreased by approximately 89.4% to 83.8% (DW), while the carotenoid content decreased by 78.1% to 67.7% (DW) under different light regimes.

![Carbohydrates quota](image1.png)

![Chlorophyll quota](image2.png)

**Figure 6.** Simulation of (a) carbohydrate quota and (b) chlorophyll quota in a continuous culture system of microalgae of the *Isochrysis galbana* strain. The simulation input parameters are presented in Table 2 and the initial conditions in Table 5. The light intensity settings for A, B, C, D, and E correspond to 50 µmolm⁻²s⁻¹, 150 µmolm⁻²s⁻¹, 250 µmolm⁻²s⁻¹, 500 µmolm⁻²s⁻¹, and 750 µmolm⁻²s⁻¹, respectively.

As illustrated in Figure 6a, light intensity may affect nitrogen uptake rates and delay the onset of nutrient deficiency. Studies in the literature have reported that high light intensity stress and nitrate deprivation affect the cellular metabolic activity and redirect protein synthesis toward carbohydrate accumulation [81].

It has been observed that subjecting organisms to nitrogen-deficient conditions for a short duration results in a significant elevation in carbohydrate content. This is primarily attributed to the limitation of nitrogen availability, which restricts the rate of cell division and redirects anabolic pathways away from protein synthesis toward the production of reserve substances such as carbohydrates [82–85].

Since nitrogen is an essential component of protein synthesis, its deficiency caused by high light intensities reduces protein synthesis rates. Protein synthesis is a crucial component of both the photosystem reaction center and the electron transport system in photosynthesis. Thus, since chlorophyll is a nitrogenous compound, nitrate concentration plays a significant role in the synthesis of chlorophyll. For example, in [79], it was reported that decreasing the nitrogen concentration from 144 to 0 mg/L resulted in a decrease in the concentration of Chla from 0.41 to 0.11 mg/L, and Chlb decreased from 0.56 to 0.18 mg/L.

The limitation of nitrogen adversely affects photosynthesis indirectly as well. Furthermore, chlorophyll synthesis is also controlled by the photoacclimation process [56]. The dynamic simulation revealed that high light intensity leads to a decrease in chlorophyll content. This response has been reported in the literature and is believed to be a protec-
tive mechanism to prevent the absorption of excess light energy by the photosynthetic machinery [86,87].

Based on the carbohydrate quota simulations, there was a gradual increase in carbohydrate quota as chlorophyll quotas decreased over time. This observation aligns with a study conducted by [79] who investigated the growth rate and biochemical composition of Isochrysis galbana. The study reported a decrease in cell growth, pigments, and protein content of I. galbana biomass as the nitrogen concentration decreased. However, under conditions of total nitrogen deprivation, the carbohydrate content reached its highest value at 47%. In another study, it was observed that the carbohydrate quota of I. galbana was 0.48 mgN/mgC during the initial nitrogen-sufficient growth period. However, during nitrogen starvation, the carbohydrate quota increased significantly by 87.7% [88].

Based on the aforementioned information, it can be inferred that carbohydrate production is specifically triggered when external nutrient sources have been fully depleted, as depicted in Figure 6a. This dynamic behavior is consistent with numerous studies that demonstrated that under N-limited conditions, carbohydrate contents were improved in many algal species such as Neochloris oleoabundan [89], Thermosynechococcus sp., and Chlorella vulgaris [90], Scenedesmus obliquus CNW-N [91], Nannochloropsis sp., and Tetraselmis suecica [92].

3.2. Analysis of Productivity

Based on the methodology provided for the analysis of productivity, the following outcomes are presented.

Figure 7a illustrates the biomass productivity map based on variations in the light-intensity parameter ($l_0$) and the dilution rate ($D$).

The productivity map displays the yield of biomass concentration per unit time for the strain Isochrysis galbana. The equilibrium points were obtained by varying the light-intensity parameter ($l_0$) between 50 and 1000 µmolm$^{-2}$s$^{-1}$. The circles represent the steady state achieved multiplied by the dilution rate ($D = 0.1$). The symbol (●) represents the optimal point of light intensity and maximum biomass production. In terms of biomass productivity, the maximum value was 56.214 gCm$^{-3}$d$^{-1}$. The optimal light intensity ($l_0$) for maximum biomass yield was 350 µmolm$^{-2}$s$^{-1}$ under the simulation conditions proposed in this study. Biomass concentration productivity decreased when the parameter ($l_0$) exceeded 350 µmolm$^{-2}$s$^{-1}$. Our findings are in line with previous studies that have reported the ability of Isochrysis galbana to grow across a wide range of light conditions without experiencing significant stress that would require extensive cell adaptation. For instance, the literature has shown that a maximum growth rate was observed in cells grown at an irradiance level of 325 µmolm$^{-2}$s$^{-1}$, indicating the species’ capability to thrive under diverse light intensities [65]. Additionally, other studies have demonstrated that the production of cell biomass in Isochrysis galbana cultures decreases when the light intensity exceeds 400 µmolm$^{-2}$s$^{-1}$. Conversely, increasing the light intensity from 200 to 400 µmolm$^{-2}$s$^{-1}$ leads to an increase in cell biomass. However, at intensities higher than 400 µmolm$^{-2}$s$^{-1}$, photoinhibition occurs, resulting in a decrease in cell growth [93].

In productivity studies of Isochrysis galbana, Tzovenis et al. (2003) [67] reported productivities ranging from 28 to 62 gCm$^{-3}$d$^{-1}$ under continuous light. These values fall within the range found in our study.

Meanwhile, Figure 7b illustrates the productivity map of the biomass concentration of the strain Isochrysis galbana as a function of the dilution parameter ($D$).

This productivity map illustrates the simulation of biomass concentration per unit time of the strain Isochrysis galbana. The equilibrium points were determined by varying the dilution parameter ($D$) between 0.0125 and 0.4 d$^{-1}$. Circles represent the steady state reached in each simulation multiplied by the dilution rate ($D$) for each run between 0.0125 and 0.4 d$^{-1}$. Symbolically represented as (●), the point N(D, x) indicates the optimal point of dilution and maximum biomass production. In this study, the maximum biomass productivity was found to be 126.91 gCm$^{-3}$d$^{-1}$. The optimal value of dilution parameter
(D) for achieving maximum biomass yield was 0.325 d\(^{-1}\) under the simulation conditions described in this paper. For values of the parameter (D) greater than 0.325, the productivity of the biomass concentration decreased.

![Graph](image)

**Figure 7.** Steady-state productivity maps of biomass concentration with respect to (a) incident light intensity \(I_0\) and (b) dilution rate (D).

Figure 8a illustrates the productivity map for the carbohydrate concentration of the strain *Isochrysis galbana* with respect to changes in the light-intensity parameter \(I_0\).

The productivity map shows the simulations of the concentration of carbohydrates per unit of time for the strain *Isochrysis galbana*. The equilibrium points were determined by varying the light intensity parameter \(I_0\) between 50 and 1000 \(\mu\text{mol m}^{-2}\text{s}^{-1}\). Each circle represents the steady state achieved during each simulation multiplied by the dilution rate \((D = 0.1)\). In the figure, \(M(I_0, x)\) represents the optimal point of light intensity and biomass productivity, represented by the symbol (*). As shown in Figure 8, there was a maximum carbohydrate productivity of 30.352 g\(\text{C m}^{-3}\text{d}^{-1}\). The optimal value of the light intensity parameter \(I_0\) for maximum carbohydrate yield was 350 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) under the simulation conditions that were used in this study. For values of \(I_0\) greater than 350 \(\mu\text{mol m}^{-2}\text{s}^{-1}\), the productivity of biomass concentration decreased.

Figure 8b illustrates the productivity map of the carbohydrate concentration of strain *Isochrysis galbana* in response to changes in the dilution parameter (D).
Figure 8. Steady-state productivity maps of carbohydrates concentration with respect to (a) incident light intensity $I_0$ and (b) dilution rate $D$.

The equilibrium points were determined by varying the dilution parameter ($D$) between 0.0125 and 0.4 d$^{-1}$. Each circle represents the steady state reached in each simulation multiplied by the dilution rate ($D$) corresponding to each run between 0.0125 and 0.4 d$^{-1}$. As indicated by the point $O(D, x)$ and symbol (*), 0.2625 d$^{-1}$ was the optimal value of dilution rate to achieve a maximum productivity of 48.11 gCm$^{-3}$d$^{-1}$ under the proposed simulation conditions. Regarding this result, one study reported high sugar productivities of *Isochrysis galbana* at a dilution rate of 0.3 d$^{-1}$ and a nitrogen concentration of 6 gN(m$^3$) [62]. These optimal findings further highlight the significant role of nutrient deficiency in enhancing the carbohydrate content of *I. galbana*. The productivity of carbohydrates decreased for the parameter $D$ greater than 0.2625 d$^{-1}$. The optimal dilution rate value was lower than that obtained from the biomass, which may be the result of the maximum storage capacity of carbohydrates under optimal operating conditions.

There is limited available data on the carbohydrate productivity of *Isochrysis galbana* in standard laboratory cultures [67]. However, carbohydrates play a crucial role as intermediate reserves in certain algae, especially when nitrogen availability becomes limited, as they are required for lipid synthesis. Studies addressing this aspect have been conducted, such as the one by [78]. They reported a carbohydrate storage of 25% in *Isochrysis galbana* strains. Moreover, during the stationary growth phase, a decrease in protein content and a slight increase in carbohydrates were observed. These findings suggest that changes in the cellular biochemical composition of I. galbana are influenced by the nutrient concentration in the medium.
3.3. Sensitivity Analysis

The system of equations below (31) is a representation of the Droop model for continuous culture systems based on the nominal parameters.

\[
\begin{align*}
\dot{x} &= \mu(l_0, I^*, x, z, \lambda_0)x - D_0 x - R_0 x \\
\dot{y} &= D y_{in} - \rho(y, z, \lambda_0)y - D_0 y \\
\dot{z} &= \rho(y, z, \lambda_0) - \beta (l_0, I^*, x, z, \lambda_0) (z - z_{Col}) + R_0 z \\
\dot{q}_g &= (1 - \beta_0 z - g_0)\mu(l_0, I^*, x, z, \lambda_0) - \kappa_0 \rho(y, z, \lambda_0) + R(q_g - 1) \\
I^* &= \mu(l_0, I^*, x, z, \lambda_0) [I(l_0) - I^*]
\end{align*}
\]

This section describes the computation of the sensitivity equation using the arrangements (32) and (33), which summarize the Jacobian matrix elements \(J_{A_{ij}}\) and \(J_{B_{ij}}\). As \(n = 15\) and \(m = 5\), the matrix elements are denoted as \([A(1,n) \ldots A(m,n)]\), respectively.

\[
J_{A_{ij}} = \frac{\partial f_i(x, t, \lambda)}{\partial \lambda_j} = \begin{bmatrix}
\frac{\partial f_1(x, t, \lambda)}{\partial x_1} & \cdots & \frac{\partial f_1(x, t, \lambda)}{\partial x_n} \\
\vdots & \ddots & \vdots \\
\frac{\partial f_m(x, t, \lambda)}{\partial x_1} & \cdots & \frac{\partial f_m(x, t, \lambda)}{\partial x_n}
\end{bmatrix}
\begin{bmatrix}
A(1, 1) & \ldots & A(1, n) \\
\vdots & \ddots & \vdots \\
A(m, 1) & \ldots & A(m, n)
\end{bmatrix} \tag{32}
\]

\[
J_{B_{ij}} = \frac{\partial f_i(x, t, \lambda)}{\partial \lambda_j} = \begin{bmatrix}
\frac{\partial f_1(x, t, \lambda)}{\partial x_1} & \cdots & \frac{\partial f_1(x, t, \lambda)}{\partial x_n} \\
\vdots & \ddots & \vdots \\
\frac{\partial f_m(x, t, \lambda)}{\partial x_1} & \cdots & \frac{\partial f_m(x, t, \lambda)}{\partial x_n}
\end{bmatrix}
\begin{bmatrix}
B(1, 1) & \ldots & B(1, n) \\
\vdots & \ddots & \vdots \\
B(m, 1) & \ldots & B(m, n)
\end{bmatrix} \tag{33}
\]

In the arrangements (34) and (35), Jacobian matrices are represented by the values of the nominal parameters. The symbols \(\theta \ y \ \omega\) represent the elements A and B that were evaluated as nominal parameters.

\[
J_{\theta_{ij}}|_{\text{nominal}} = \begin{bmatrix}
\theta(1, 1) & \ldots & \theta(1, n) \\
\vdots & \ddots & \vdots \\
\theta(m, 1) & \ldots & \theta(m, n)
\end{bmatrix} \tag{34}
\]

and

\[
J_{\omega_{ij}}|_{\text{nominal}} = \begin{bmatrix}
\omega(1, 1) & \ldots & \omega(1, n) \\
\vdots & \ddots & \vdots \\
\omega(m, 1) & \ldots & \omega(m, n)
\end{bmatrix} \tag{35}
\]

Equation (36) was used to solve the dynamic sensitivity equations numerically.

\[
\dot{x}_1 = f(t, x, \lambda) \quad x(t_0) = x_0 \tag{36}
\]

\[
\dot{x}_\lambda = \left\{ \frac{\partial f(t, x, \lambda)}{\partial x} \right\}_x x_\lambda + \left\{ \frac{\partial f(t, x, \lambda)}{\partial \lambda} \right\}_x x(0) = 0
\]

The sensitivity equations obtained for the elements \(J_{\theta_{ij}}\) and \(J_{\omega_{ij}}\) are shown in the supplementary materials, specifically in the arrangements (S1) and (S2). The Equations (S3)–(S102) pertaining to these elements have been included in the provided link.

A representation of the solutions to the sensitivity functions is shown in the arrangement (37). Based on the order shown in the arrangement (38) (H), each element in the arrangement (S) represents the variation in state variables as a function of a given parameter. Based on the three initial conditions shown in the Table 5, the numerical solution of the sensitivity functions was evaluated. The simulation parameters are outlined in Table 2. The simulations were carried out using the optimal values found in the productivity analysis, corresponding to a dilution rate of 0.2625 \(d^{-1}\) and a light intensity of 350 \(\mu\text{mol m}^{-2}\text{s}^{-1}\). In the simulation, all other parameters were kept at the same values.
The solution of Equation (36) was computed for the three different initial states presented in Table 5. Figure 9 shows $x_6, x_{11}, x_{16}, x_{21}, x_{26}, x_{31}, x_{36}, x_{41}, x_{46}, x_{51}, x_{56}, x_{61}, x_{66}, x_{71}, x_{76}$, which are the sensitivities of biomass concentration $x_1$ with respect to the parameters shown in arrangement (38). Figures 9–13 showed the corresponding sensitivities for external nutrient concentration $x_2$, internal quota $x_3$, photolacclimation $x_4$, and the carbonhydrate quota $x_5$.

Inspection of these figures shows, in order of importance, that the solution of the state variables are more sensitive to variations in the parameters $\lambda_0$, $\lambda_L$, $\lambda_T$, and $\lambda_0$ than to all other parameters evaluated; these more sensitive parameters correspond to $z_{C_0}$, $\rho_m$, $D_0$, and $z_{m_0}$, respectively. These findings align with a previous sensitivity analysis conducted for nutrient-limited batch cultivation, which demonstrated a greater sensitivity of the model outputs to changes in the parameter associated with minimal cell quota [41].

The sensitivity analysis conducted in this study enables the identification of significant parameters and their interrelationships. Specifically, $z_{C_0}$, $\rho_m$, and $z_{m_0}$ are found to have a direct correlation with the nitrogen uptake rate $\rho(y,z)$. The nitrogen uptake rate represents the microalgaee's ability and affinity to utilize nitrogen from its surroundings for growth and metabolism, taking into account factors such as light stress and other environmental conditions. Consequently, these parameters have a direct influence on biomass productivity. Additionally, it can be observed that the model solutions are more sensitive under the scenario of low biomass concentration and initial nutrient levels ($C_1$) compared to the scenarios ($C_2$ and $C_3$). Another important point is that this type of sensitivity analysis allows us to dynamically observe the effect of parameter variations on the model solutions. It provides insights into the specific growth stages of the culture where parameter variations have a more pronounced impact.

Therefore, a sensitivity analysis can help optimize the parameter adjustment of the model, enhancing its predictive capability and understanding of the underlying biological processes. The highly sensitive parameters identified in this study hold promising applications in extremum seeking control (ESC) [94], an adaptive control strategy that actively and continuously seeks optimal values of a variable in a system by adjusting system parameters based on real-time feedback to optimize performance. A dedicated investigation by [95] explored the feasibility of dynamically adjusting light intensity to consistently track optimal biomass productivity. This study aimed to achieve this objective in a nearly model-free setup, following the principles of the extremum seeking control approach. The experimental setup focused specifically on cultures of Isochrysis galbana, employing the model proposed by Bernard [56] and Mairet [57]. In this evaluation, the parameters associated with the influence of light ($K_{il}, K_{il'}, K_m, K_l$) were systematically tested and demonstrated significant effects on the system performance [95].
Figure 9. Sensitivity functions of (a) \( x_1 \) with respect to (b) \( x_{6r} \), (c) \( x_{11} \), (d) \( x_{16r} \), (e) \( x_{21r} \), (f) \( x_{26r} \), (g) \( x_{31r} \), (h) \( x_{36r} \), (i) \( x_{41} \), (j) \( x_{46r} \), (k) \( x_{51} \), (l) \( x_{56r} \), (m) \( x_{61r} \), (n) \( x_{66r} \), (o) \( x_{71} \), and (p) \( x_{76} \). The solid line corresponds to the simulation with \( C_1 \) initial conditions, the dashed line corresponds to the simulation with \( C_2 \) initial conditions, and the dotted line corresponds to the simulation with \( C_3 \) initial conditions.
Figure 10. Sensitivity functions of (a) $x_2$ with respect to (b) $x_{77}$, (c) $x_{127}$, (d) $x_{177}$, (e) $x_{227}$, (f) $x_{277}$, (g) $x_{327}$, (h) $x_{377}$, (i) $x_{427}$, (j) $x_{477}$, (k) $x_{527}$, (l) $x_{577}$, (m) $x_{627}$, (n) $x_{727}$, and (p) $x_{777}$. The solid line corresponds to the simulation with $C_1$ initial conditions, the dashed line corresponds to the simulation with $C_2$ initial conditions, and the dotted line corresponds to the simulation with $C_3$ initial conditions.
Figure 11. Sensitivity functions of (a) $x_3$ with respect to (b) $x_8$, (c) $x_{13}$, (d) $x_{18}$, (e) $x_{23}$, (f) $x_{28}$, (g) $x_{33}$, (h) $x_{38}$, (i) $x_{43}$, (j) $x_{48}$, (k) $x_{53}$, (l) $x_{58}$, (m) $x_{63}$, (n) $x_{68}$, (o) $x_{73}$, and (p) $x_{78}$. The solid line corresponds to the simulation with $C_1$ initial conditions, the dashed line corresponds to the simulation with $C_2$ initial conditions, and the dotted line corresponds to the simulation with $C_3$ initial conditions.
Figure 12. Sensitivity functions of (a) $x_4$ with respect to (b) $x_9$, (c) $x_{14}$, (d) $x_{19}$, (e) $x_{24}$, (f) $x_{29}$, (g) $x_{34}$, (h) $x_{39}$, (i) $x_{44}$, (j) $x_{49}$, (k) $x_{54}$, (l) $x_{59}$, (m) $x_{64}$, (n) $x_{69}$, (o) $x_{74}$, and (p) $x_{79}$. The solid line corresponds to the simulation with $C_1$ initial conditions, the dashed line corresponds to the simulation with $C_2$ initial conditions, and the dotted line corresponds to the simulation with $C_3$ initial conditions.
4. Conclusions

In this report, a dynamic photoacclimated model for continuous microalgae culture systems was used for the study of time-varying state variables under various light scenarios as well as the first-order effects of solutions to parameter variations under optimal productivity conditions. The results of this study demonstrated that the dynamic photoacclimated
model is an effective tool for studying the time-varying behavior of microalgae culture systems and can be used to identify optimal parameters to maximize productivity. Under the proposed simulation conditions, a maximum carbohydrate productivity of 48.11 gCm$^{-3}$d$^{-1}$ was achieved using an optimal dilution rate of 0.2625 d$^{-1}$ and 350 µmolm$^{-2}$s$^{-1}$ of light intensity. However, a particular set of manipulated inputs can generate multiple outputs at a steady state. Based on the optimal operating conditions identified as nominal parameters, sensitivity analysis was conducted. Sensitivity functions were solved to provide first-order estimates of the effect of parameter variations over time in solutions. The resulting model solutions were more sensitive to variations in the parameter of minimum nitrogen quota, maximum nitrogen intake rate, dilution rate, and maximum nitrogen uptake rate. The sensitivity pattern was consistent when solved for other initial states. Furthermore, sensitivity increased during the physiological stage of exponential growth, and the model solutions were more sensitive under conditions of low cell and nutrient concentrations. This analytical tool could be considered highly useful, as it facilitated model development, validation, and the reduction of uncertainty, ultimately enhancing the reliability of the results. Through the use of a photoacclimated model, a dynamic simulation of a continuous culture system of the strain *I. galbana* was conducted. The photoacclimation process interacts dynamically with chlorophyll synthesis, nutrient incorporation, and carbohydrate accumulation. Two independent but closely related processes for carbohydrate synthesis were observed to be affected by light: nitrogen assimilation and protein formation. Based on the underlying model, chlorophyll is proportional to cellular proteins (i.e., linearly correlated to particulate nitrogen). Furthermore, dynamic simulations have shown that microalgae cells stopped dividing as nutrient deprivation increased their carbohydrate content. Observations indicated a tradeoff between high light intensities, nitrogen assimilation, and carbohydrate synthesis. Given the interdependence between these processes and the fact that microalgae cells undergo metabolic switching from protein to carbohydrate synthesis under nitrate deprivation, excess light can delay nitrogen depletion and subsequent carbohydrate synthesis. On the other hand, low light intensities decrease chlorophyll production, resulting in insufficient energy absorption for photosynthesis. This approach could be useful for developing models suitable for optimizing industrial processes for microalgae-based carbohydrate production. Light plays an influential role in modifying metabolic pathways as a stress factor. Combining cell stress strategies such as nutritional deficiency and light intensity regulation in order to improve carbohydrate productivity in continuous photobioreactors for microalgae cultivation. In order to quantify the impact of other factors that can cause stress to the cell, it is recommended to mathematically integrate the effects of these factors as well. This approach could be useful for developing models suitable for optimizing industrial processes for microalgae-based carbohydrate production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11071866/s1, Arrangements (S1) and (S2): Sensitivity equations obtained from the photoacclimated model utilized in this study; Equations (S3)–(S102): The elements $f_{ij}$ and $f_{ij}$ of the sensitivity equations.


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Abbreviations

The following abbreviations are used in this manuscript:

- NADPH Nicotinamide Adenine Dinucleotide Phosphate
- PBR photobioreactor
- FFA free fatty acid
- ODE ordinary differential equation
- LSODE Livermore solver for ordinary differential equations
- ATP adenosine triphosphate
- ATP adenosine triphosphate

References


35. Formighieri, C.; Franck, F.; Bassi R. Regulation of the pigment optical density of an algal cell: Filling the gap between photosynthetic productivity in the laboratory and in mass culture. *J. Biotechnol.* 2012, 162, 115–123. [CrossRef]

36. Briones-Baez, M.F.; Aguiler-Vazquez, L.; Rangel-Valdez, N.; Martinez-Salazar, A.L.; Zufiga, C. Multi-Objective Optimization of Microalgal Metabolism: An Evolutive Algorithm Based on FBA. *Metaboletes* 2022, 12, 603. [CrossRef]


49. Zonneveld, C. A cell-based model for the chlorophyll a to carbon ratio in phytoplankton. Ecol. Model. 1998, 113, 55–70. [CrossRef]
57. Mairet, F.; Bernard, O.; Masci, P.; Lacour, T.; Sciandra, A. Modelling neutral lipid production by the microalga Isochrysis aff. galbana under nitrogen limitation. Bioresour. Technol. 2011, 102, 142–149. [CrossRef]
64. Martinez, C.; Mairet, F.; Bernard, O.; Dynamics of the periodically forced light-limited Droop model. J. Differ. Equ. 2020, 269, 3890–3913. [CrossRef]


77. Serpa, R.; Calderón, A. Efecto de diferentes fuentes de nitrógeno en el contenido de carotenoides y clorofila de cuatro cepas peruanas de *Dunaliella salina* TEOD. *J. Appl. Ecol.* 2006, 5, 93–99. [CrossRef]


87. Ak, I. Effects of light intensity, salinity and temperature on growth in CAMALT1 strain of *Dunaliella viridis* Teodoresco from Turkey. *J. Biol. Sci.* 2008, 8, 1356–1359. [CrossRef]


89. Sun, X.; Cao, Y.; Xu, H.; Liu, Y.; Sun, J.; Qiao, D.; Cao, Y. Effect of nitrogen-starvation, light intensity and iron on triacylglycerol and fatty acid profile of *Neochloris* oleoabundans HK-129 by a two-stage process. *Bioresour. Technol.* 2014, 155, 204–212. [CrossRef]


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