applied sciences

MDPI

Article

A Novel Methodological Approach to Simulating the Growth of Photosynthetic Organisms Using Long-Term Meteorological Sequences: A Case Study of Microalgae (*Chlorella vulgaris*)

Ousmane Wane [1,2,*], Luis F. Zarzalejo [2,*], Francisco Ferrera-Cobos [2], Ana A. Navarro [2] and Rita X. Valenzuela [2]

[1] E.T.S.I. Agronómica, Alimentaria y Biosistemas, Universidad Politécnica de Madrid, Avda. Puerta Hierro 2-4, 28040 Madrid, Spain
[2] CIEMAT Energy Department, Renewable Energy Division, Avda. Complutense 40, 28040 Madrid, Spain; francisco.ferrera@ciemat.es (F.F.-C.); a.navarro@ciemat.es (A.A.N.); r.valenzuela@ciemat.es (R.X.V.)
* Correspondence: ousmane.wane@alumnos.upm.es or ousmane.wane@.ciemat.es (O.W.); lf.zarzalejo@ciemat.es (L.F.Z.)

Abstract: The growth of photosynthetic organisms requires specific ranges of temperature and photosynthetically active radiation. Monitoring and maintaining these conditions is technically difficult, especially in outdoor cultures. In such cases, a typical meteorological sequence can be a useful tool for estimating the growth of photosynthetic organisms. This study proposes a new methodology based on long-term meteorological sequences to simulate the growth of photosynthetic organisms. This case study addresses microalgae growth simulation (*Chlorella vulgaris*) in Riosequillo in the north of the Madrid region (Spain) for the four seasons of the year. Then, these estimates are compared with the observed results of an experimental culture of microalgae in domestic wastewater. The results also show strong agreement with the probability distribution function of the daily biomass concentration, giving the best results for typical summer and spring meteorological sequences. The methodology seems to confirm the representativeness of typical meteorological sequences, allows for the identification of the most likely production scenarios for project feasibility analyses, and may be applied to decision-making processes.

Keywords: long-term meteorological sequences; simulation; photosynthetic organisms; wastewater treatment; high-rate algae pond; microalgae; *Chlorella vulgaris*

Citation: Wane, O.; Zarzalejo, L.F.; Ferrera-Cobos, F.; Navarro, A.A.; Valenzuela, R.X. A Novel Methodological Approach to Simulating the Growth of Photosynthetic Organisms Using Long-Term Meteorological Sequences: A Case Study of Microalgae (*Chlorella vulgaris*). *Appl. Sci.* **2024**, *14*, 10580. https://doi.org/10.3390/app142210580

Academic Editors: Paweł Kiełbasa, Anna Miernik and Akinniyi Akinsunmade

Received: 30 August 2024
Revised: 7 November 2024
Accepted: 9 November 2024
Published: 16 November 2024

1. Introduction

The use of algae for the removal, biotransformation, or mineralization of various nutrients and heavy metals from wastewater is an environmentally friendly process, as no secondary pollution occurs if the biomass produced is used as feedstock and the treated wastewater is reused. According to [1,2], research in this field is not new and has demonstrated the ability of microalgae to efficiently use nitrogen, phosphorus, and other impurities in wastewater to promote their growth [3–5]. In addition to these nutrients that come with wastewater, microalgae also depend on other external parameters, such as light, which is absorbed and used in the photosynthesis process [6], and temperature, which has an effect on photosynthesis and cell division [7]. Furthermore, since microalgae are autotrophic microorganisms, they contribute to reducing the concentration of greenhouse gases by fixing CO_2 during their growth [8]. These efforts over the years allowed for an advanced level of mastery of this technology, which has led to its implementation in a rural community of 300 people [9].

A considerable number of research organizations [10,11] have studied the effects of temperature and solar irradiance on the growth of many different strains of microalgae grown in open or closed systems. A study of the impact of temperature on microalgae

showed a decrease in viable cells at high temperatures and an increase from 20 °C to 28 °C, the optimal temperature range [12]. This optimal temperature range varies depending on the strain of microalgae [11]. Regarding incident light, three different levels of solar irradiance (6213, 2741, and 3799 Wh m^{-2} d^{-1}) were investigated to understand the influence of solar irradiance on a microalgae–bacteria consortium grown in 80 L domestic wastewater in an outdoor high-rate algae pond (HRAP) [13]. Similarly, the effect of both parameters on microalgae growth was studied in [14] by modeling and validating the variation in the growth rate as a function of temperature at different light intensity levels. In addition to the importance of light intensity, its quality and photoperiodicity also play a key role in the metabolism of microalgae. For example, the work presented in [15] showed a faster growth rate and a higher lipid content in algae biomass when the medium received white light instead of red light.

Large-scale outdoor microalgae cultivations under natural daily or seasonal solar irradiance and temperature produced satisfactory results for understanding the impact of both culture parameters [16–18]. However, it is important to know the long-term temporal variability in these parameters when planning the installation of an HRAP system at a given site. To consider climatic variability in outdoor mass cultures, typical meteorological sequences (TMSs) have been developed as a preliminary step to evaluate the effects of temperature and solar irradiance on the growth of microalgae in HRAPs, as in [19]. This allows for the consideration of the extreme conditions of these variables when assessing the long-term viability of a proposed project.

However, the economic viability of an open pond cultivation system in a given location is strictly related to climatic variability. When microalgae are grown in an open pond, meteorological parameters, among others, are beyond our control. Daily and seasonal fluctuations in culture weather parameters significantly affect microalgae metabolism [18]. In some cases, this could have negative effects on the productivity of these microorganisms and therefore on the quality of the recycling of water, energy, and fertilizer nutrients. In an open-pond system for a microalgae culture, temperature and photosynthetically active radiation (PAR) are the most relevant meteorological parameters. In the case of solar radiation, assuming the representativeness of the period covered by the available meteorological database, the corresponding empirical probability distribution function [20] allows for the characterization of its long-term temporal variability. Previous works in the field of solar thermal power generation [21,22] have presented, from the point of view of economic viability, probabilistic analyses of different production scenarios.

This work presents the average daily productivities (in terms of microalgae concentration) corresponding to n-growth meteorological sequences (1204 in our case study) simulated from hourly PAR and temperature measurements recorded in situ using a simplified production model. This series of 'biomass productivities' for a given season was compared with the productivity corresponding to the representative TMS for the same period of the year [19]. This manuscript is structured as follows. Section 2 introduces the case study and describes the measured data set used, the growth model proposed in this work, and the simulation model utilized. Section 3 presents the main results and a discussion of them. Finally, Section 4 presents the conclusions and future work.

2. Materials and Methods

The growth of photosynthetic organisms (plants, protists, and bacteria) requires conditions that are difficult to achieve, especially when this bioprocess is carried out in a device exposed to weathering. In this case, crop yield would be significantly affected by environmental impacts that change throughout the year. However, optimal crop development depends on many factors, some of which directly influence growth characteristics. This is the case with temperature and light or photosynthetically active radiation (PAR) in massive outdoor cultivation. In this situation, a representation of the long-term variability in these two parameters at a given site may be advantageous when using growth simulation tools

on photosynthetic organisms or conducting laboratory-scale studies for application at that site.

A growth meteorological sequence (GMS) corresponds to a sequence of consecutive days at a given site and in a given season; the number of consecutive days of each GMS can be adapted by taking into account the growth period of the photosynthetic species under study. This adaptive tool has been designed to obtain input data for simulating different crop types that have a growth period of a few days to a few weeks. Therefore, the cultivation of microalgae or other plant species could be a case study.

On the other hand, it provides a general representation of long-term variability in solar irradiance and temperature over a period corresponding to the time needed for the crop species to reach harvest. The simulation of microalgae productivity from long-term meteorological data sets offers an opportunity to explore crop profitability in different scenarios before application on-site. In this framework, the cultivation of microalgae, tomatoes, or any other crops could be an example of a numerical simulation case study that includes PAR and temperature as input data.

2.1. Case of Study

A microalgae-based wastewater treatment model is the focus of our case study. Modeling such a system can be beneficial in anticipating potential obstacles and making decisions regarding its implementation.

According to [23], more than 80% of total nitrogen and 70% of total phosphorus in wastewater are removed in 5 days, with a biomass productivity equal to 0.64 $gL^{-1}d^{-1}$ for batch culture. In addition, Zou et al. obtained a significant result of 1.72 $gL^{-1}d^{-1}$ of microalgal biomass produced during batch treatment and proposed that a hydraulic retention time of 7 to 9 days could be efficient for nutrient removal and microalgal biomass production during continuous treatment [24]. In our case study, long-term climate variability of air temperature and PAR was taken into account by generating a set of n-GMS consisting of seven consecutive days generated according to the methodology proposed in [19]. The methodology offers a significant number of seven-day sequences for every season of the year, with 1204 for spring, 1204 for summer, 1190 for autumn, and 1184 for winter, taking the following approach.

$$EA = \{d_1,\ d_2,\ d_3,\ \ldots d_n\}$$
$$S_1 = \{d_1,\ d_2,\ \ldots d_7\},\ \ S_2 = \{d_2,\ d_3,\ \ldots d_8\}\ \ \ldots\ \ S_m = \{d_{n-6},\ d_{n-5},\ \ldots d_n\}$$

where EA corresponds to all the days available (n) for a given season and S_m is a set of a sequence of 7 consecutive days (with $m = n - 6$).

The Filkenstein–Schafer statistic (FS) and a persistence criterion were applied to determine the most typical sequence in the series:

$$FS = \frac{1}{N}\sum_{i=1}^{N}\delta_i \tag{1}$$

where N is the number of days in a week (N = 7). $\delta_i = |CDF_e(x_i) - CDF_{e,s}(x_i)|$, with CDF_e and $CDF_{e,s}$ as the long-term and short-term CDFs for each weather parameter x. For each sequence, Equation (2) was calculated for all seasons and all weather parameters studied. According to the FS statistics, the CDF of each parameter x with n observations and then arranged in ascending order $x_1, x_2, \ldots, x_{k-1}, x_k$ is given by a function $S_k(x)$ defined by:

$$S_n(x) = \begin{cases} 0 & for\ x < x_1 \\ (k - 1/2)/n & for\ x_k \le x \le x_{k+1} \\ 1 & for\ x > x_n \end{cases} \tag{2}$$

The weighted sum (WS) of the FS statistics corresponding to each parameter FS_i was calculated by applying a weight factor wf_i.

$$WS = \sum_{i=1}^{m} wf_i \cdot FS_i \quad (3)$$

The persistence is evaluated by determining the number of occurrences of a given sequence. In this case study, each of these sequences was used to obtain algal biomass production and evaluate the variability of productivity. This approach is described in more detail in Sections 2.3 and 2.4.

In addition, a seven-day PAR and temperature variation data set was obtained from 15-year remote sensing data, with a spatial resolution of $0.125^\circ \times 0.125^\circ$. PAR data were obtained from Kato bands, provided by the spectral resolved irradiance (SRI) of the Satellite Application Facility on Climate Monitoring (CMSAF), which belongs to the European Organisation for the Exploitation of Meteorological Satellites (EUMETSAT). The temperature values were sourced from the Copernicus Climate Change Service (2023): ERA5 hourly data.

2.2. Microalgae Growth Model

In this study, a high-rate algae pond (reactor) model was just simplified to focus on microalgae growth as a function of air temperature (T), photosynthetically active radiation (PAR), and a reduced number of impurities present in the cultivation medium. Phosphorus (P) and nitrogen (N) are two of the impurities in wastewater (cultivation medium) that microalgae can remove for their growth. Wastewater contains a number of nitrogen compounds, such as ammonium, organic nitrogen, nitrate, and nitrite. In this study, total nitrogen is considered equal to the sum of total Kjeldahl nitrogen, nitrite, and nitrate. Similarly, wastewater is relatively rich in phosphorus compounds, including phosphate ions, inorganic forms (ortho and polyphosphates), and organic forms (organically bound phosphates). Therefore, total phosphorus is given here as a combination of these different phosphorus compounds. These parameters were taken as limiting substrates for the growth of these photosynthetic microorganisms.

In addition, the initial concentrations of nitrogen and phosphorus in the culture medium are favorable to the growth of microorganisms. These substrates are consumed exclusively by microalgae. Only PAR and temperature are beyond our control and can sometimes act as inhibitors. This simplification has made it possible to reduce the complexity of the model and study biomass productivity including climatic variability.

The remaining micropollutants, heavy metals, other nutrients, and organic pollutants are considered non-limiting nutrients. The model does not include the population of bacteria or the maintenance of microalgae. The energy balance [25] and the gas exchange [8,26] between the system and its environment were also neglected. Finally, the depth of the pond was the only geometric parameter of the system included in the model and was equal to 10 cm. This was used to calculate the mean PAR value in the culture medium.

The cell mass concentration of the microalgae in the culture medium was determined using the material balance approach. For a batch culture model, the influent and effluent of the materials in the medium are null, that is, it does not add nutrients to the culture medium, and the volume is considered constant. Furthermore, assuming that all cells have the same mass, the growth kinetics of microalgae is proportional to its specific growth rate (μ) which depends on the availability of a limiting substrate. A proportion of microalgae that die during the cultivation period was added to the model formulation and represented by the specific mortality rate (μ_D).

$$\frac{dX}{dt} = \mu(P, N, PAR, T) \cdot X - \mu_D \cdot X \quad (4)$$

$$\mu(PAR, T, P, N) = \mu_{max} \cdot \mu(P) \cdot \mu(N) \cdot \mu(PAR) \cdot \mu(T)$$

where $X(\mathrm{mg/L})$ is microalgae concentration, μ_{max}, in day^{-1}, is the maximum specific growth rate of microalgae. To calculate the specific growth rate, these factors $\mu(P)$ and $\mu(N)$ were estimated from the Monod model [27], $\mu(PAR)$ from modified Monod model [28,29], and $\mu(T)$ from the so-called cardinal temperature model [14,30]. The empirical Monod model, which is easy to calibrate and links growth with a culture parameter, is widely used to simulate wastewater treatment using microorganisms [31]. For PAR, Monod's modified model involved adding a term to the denominator to account for inhibition when the substrate (PAR) is highly above the optimal limit. The same consideration was included in the cardinal temperature model.

Equation (5) gives the expression of the factors $\mu(P)$ and $\mu(N)$, where S represents the concentration of phosphorus (P) or nitrogen (N). K_S is a constant, expressed in mg/L, which represents the half-saturation concentration of the limiting nutrients (P and N).

$$\mu(S) = \frac{S}{K_S + S} \tag{5}$$

For Equations (6) and (7), an intermediate calculation was performed to find the mean values for the available PAR and the medium temperature of cultivation, respectively. Regarding the photosynthesis rate (Equation (6)), the Lambert–Beer law was applied to obtain the average value of PAR $\left(\mu\mathrm{E}\cdot\mathrm{m}^{-2}\cdot\mathrm{s}^{-1}\right)$. This value represents the intensity of light to which cells are exposed in culture medium and was estimated as proposed in [32,33].

$$\mu(PAR) = \frac{PAR}{K_I + PAR + \frac{PAR^2}{K_i}} \tag{6}$$

K_i and K_I (in $\mu\mathrm{E}\cdot\mathrm{m}^{-2}\cdot\mathrm{s}^{-1}$) are inhibition constant and saturation PAR intensity at which the specific growth rate is half the maximum, respectively.

Figure 1 shows the experience of growing *Chlorella vulgaris* in the laboratory from an open container representative of a pond. The type of wastewater selected for this study came from a secondary wastewater treatment plant in a municipality in the Madrid region and was therefore domestic. Air was injected into the bottom of the container to ensure that the water remained in movement, preventing the possible eutrophication of the latter.

Figure 1. Experimental start-up of microalgae cultivation in domestic wastewater.

The experimental conditions are established to ensure that the light received by the culture is primarily obtained from an adjustable lamp. This calibrated lamp was connected

to an electronic device programmed to manage the photoperiod and PAR values corresponding to the TMS of each season. The temperature of the culture medium was not controlled. However, the experiments were carried out at periods of the year that offered ranges that were not too far from the values obtained with the TMYs.

For data acquisition, full-spectrum PAR sensors from Apogee Instruments and appropriate temperature sensors were used, respectively, to measure the light intensity and temperature inside and outside the medium. From these measurements, the average of the extinction coefficient found in the Lambert–Beer equation was estimated by relating the absorbance to the transmittance of light. This extinction coefficient was experimentally estimated for each season. A calibrated lamp was programmed with PAR values corresponding to the TMS for each season.

The productivity of microalgae is also affected by outdoor temperature. The remaining factor (Equation (7)) models the growth kinetics of the microalgae as a function of the culture temperature (T). Having an open system, this temperature is influenced by the recorded surrounding air temperature. Furthermore, the water that makes up the culture system is constantly in motion. The idea is to obtain the temperature of the culture medium from that of the air using the function established in [34] that associates these two parameters. Equation (7) also includes minimum (T_{min}), maximum (T_{max}), and optimum (T_{opt}) temperature values that are specific to the selected microalgae (*Chlorella vulgaris*).

$$\mu(T) = \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]} \quad (7)$$

2.3. Simulation Process

The computational implementation of the modeling described above was prepared using MATLAB and Simulink. This allowed us to combine textual and graphical programming in the same environment to perform a multitude of parallel simulations (Figure 2) [35]. Therefore, for a given meteorological season (spring, summer, autumn, or winter) in the Madrid region, batch production was simulated for all GMS generated during this period. In our case, as indicated previously, a GMS corresponds to a sequence of seven consecutive days formed by the *PAR* and *T* data. According to previous calculations [19], there were 1204 GMS for the spring season, 1204 GMS for the summer, 1190 GMS for the autumn and 1184 GMS for the winter. Therefore, the average daily concentration of microalgae was determined in each of them. The average daily *PAR* and *T* data of these GMSs were taken as input parameters for the microalgae production simulation. These are the external parameters involved in the growth of microalgae that characterize environmental conditions. The simulations were run in parallel with this 7-day package workflow, considering the other input data as initialization parameters of the process.

Figure 2 shows the block diagram model of microalgae growth (single microalgae cells, initial process) in a wastewater container without the addition of another product: where X, N and P represent the concentrations of microalgae, nitrogen and phosphorus, respectively. μ and μ_D are the specific growth and mortality rates, respectively. The nitrogen and phosphorus removal coefficients are given by Y_N and Y_P. T and PAR are the temperature and photosynthetically active radiation.

The initialization of the simulation process was based on the concentration of microalgae inoculum and nutrients initially present in the wastewater sample used for the cultivation experiment (Figure 1). To achieve this, it is necessary to know the characteristics of the aqueous medium, a wastewater sample, and microalgae species. The type of wastewater selected for this study came from a wastewater treatment plant in a municipality in the Madrid region and was therefore domestic. Its overall nitrogen and phosphorus compositions are given in Table 1. *Chlorella vulgaris* is the microalgae strain inoculated in the culture medium, and its concentration is also shown in Table 1. Other specific parameters are also given in Table 1. In addition, $Y_{S,a}$, which are constants for all seasons, were obtained after adjusting the proposed model. The results of this model were compared with those

obtained in the study presented by Eze et al. [27]. The minimum temperature corresponds to the apparent threshold for any microalgae growth, which is approximately 8 °C [36]. The optimum and maximum temperatures were taken from [12]. The mortality rate was arbitrarily chosen to be low compared to the growth rate. The same value proposed by [27] was adopted, although it worked with a different type of microalgae. Finally, inhibition and half-saturation coefficients were estimated to be constant throughout the simulation.

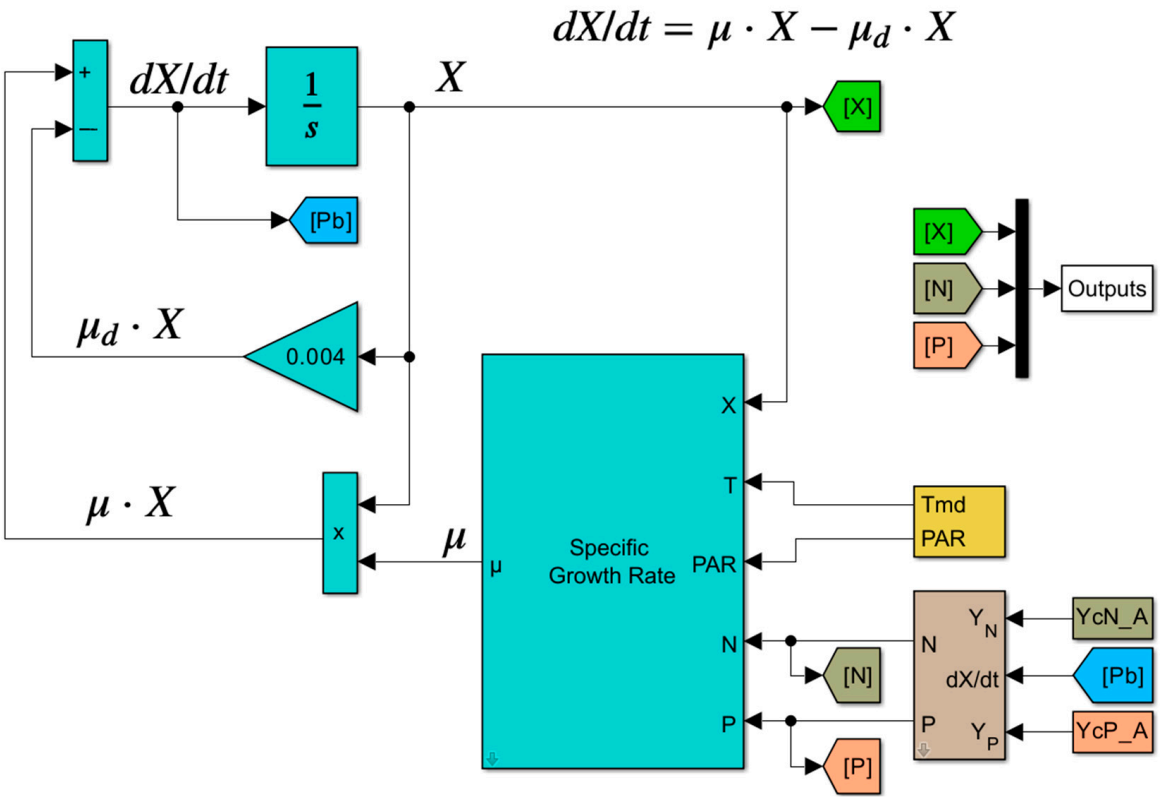

Figure 2. Block diagram model of microalgae growth in batch culture.

Table 1. Parameter values to perform the simulation.

Parameter	Value	References
Initial total nitrogen (N)	224 mg/L	This study
Initial total phosphorus (P)	16.1 mg/L	This study
Microalgae inoculum	100 mg/L	This study
Minimum temperature	8 °C	
Optimal temperature	23.3 °C	[12,36]
Maximum temperature	35 °C	
Maximum-specific growth rate (μ_{max})	0.948 day^{-1}	[5]
Nitrogen removal coefficient $Y_{N,a}$	0.71	This study
Phosphorus removal coefficient $Y_{P,a}$	0.035	This study
Mortality rate (μ_D)	0.004 day^{-1}	[27]
Inhibition constant (K_i)	2400 µE m^{-2}s^{-1}	[11]
Half-saturation constant for light (K_I)	124.115 µE m^{-2}s^{-1}	[37]
Half-saturation constant for Nitrogen (K_N)	31.5 mg/L	[38]
Half-saturation constant for Phosphorus (K_P)	10.5 mg/L	

2.4. Dataset for Statistical Analysis

Having the biomass concentration for each day of the GMS that makes up a season, the next step was to concatenate the daily production per day. Building the data set per day allows statistical analysis by calculating the probability distribution function (PDF)

of the biomass concentration of each data group per day (D2, D3, D4, D5, D6, or D7). For example, the biomass concentration is put together for all days 2 (D2) of those GMSs, and the same for days 3 (D3), etc. Day 1 (D1) was not taken as it corresponds to initialization. The idea was to calculate the PDF of the microalgae concentration for each of these datasets, grouped by day. The final step of the methodology consists of observing whether the biomass concentration on a given day (D2, D3, D4, D5, D6, or D7) of the TMS selected in [19] is, for the same day, in the bin with the largest PDF.

For a given season, the determination of the average daily biomass concentration was carried out for all GMS generated. The model outputs for identical days (D2, D3, D4, D5, D6 or D7) were used to calculate their daily PDFs. The PDFs of biomass concentrations obtained from all D2 of the GMS for the representative summer TMS used as input data are plotted and discussed in Section 3.2. The same was applied to the other TMS days. Beyond showing the range of probable results for a given day in terms of their frequency of occurrence, this graphical representation includes the graphic concordance of the biomass concentration for the corresponding day of the same day in the TMS. Therefore, it was possible to compare whether this biomass concentration position belonged to the concentration bin with the highest PDF value. For greater precision, the length of the biomass concentration bin is reduced to 2 mg/L.

3. Results and Discussion

3.1. Seasonal Biomass Production

Microalgae growth is not only affected by solar irradiance, temperature, phosphorus, and nitrogen but also depends on other parameters such as pH, dissolved carbon dioxide, bacteria, etc. These parameters not mentioned in this work are each maintained at their optimum value, which corresponds to a specific growth rate equal to unity. In summary, the focus was exclusively on the influence of these physical parameters on the variation in microalgal growth. Furthermore, this disregards the interactions between microorganisms and the various components of the culture system, which are essential for model fidelity.

Figure 3 illustrates the typical meteorological sequences (TMSs) for each season, detailing the PAR and temperature estimates for each day of the sequence.

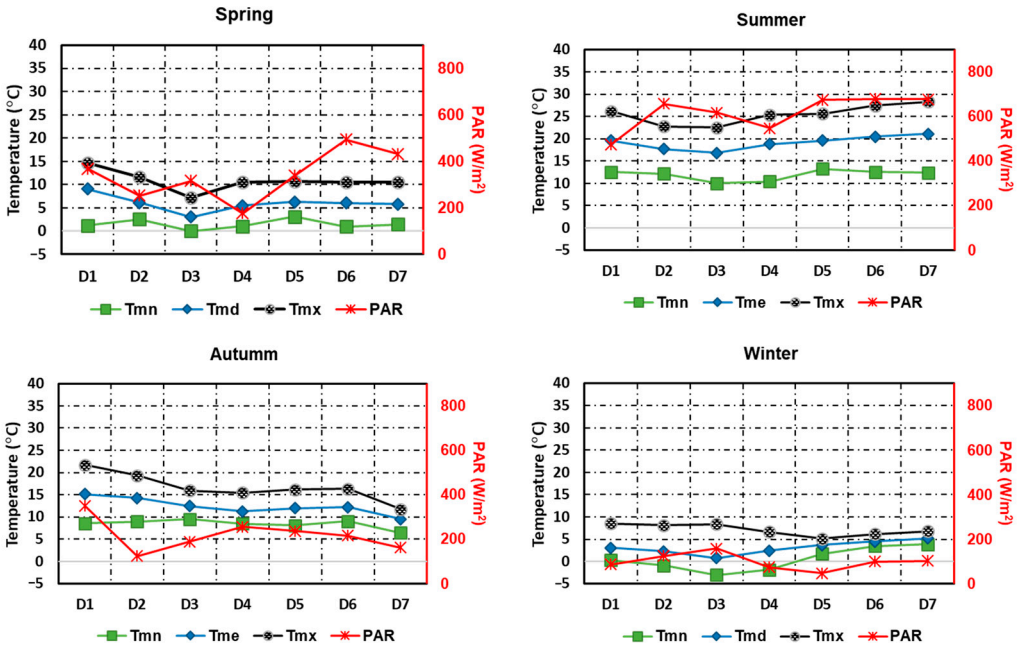

Figure 3. Typical meteorological sequences (TMSs) data from a northern locality in the Madrid region, Riosequillo, to produce the biomass concentration curves shown in Figure 4. T_{mn} and T_{mx} represent the average daily minimum and maximum temperature.

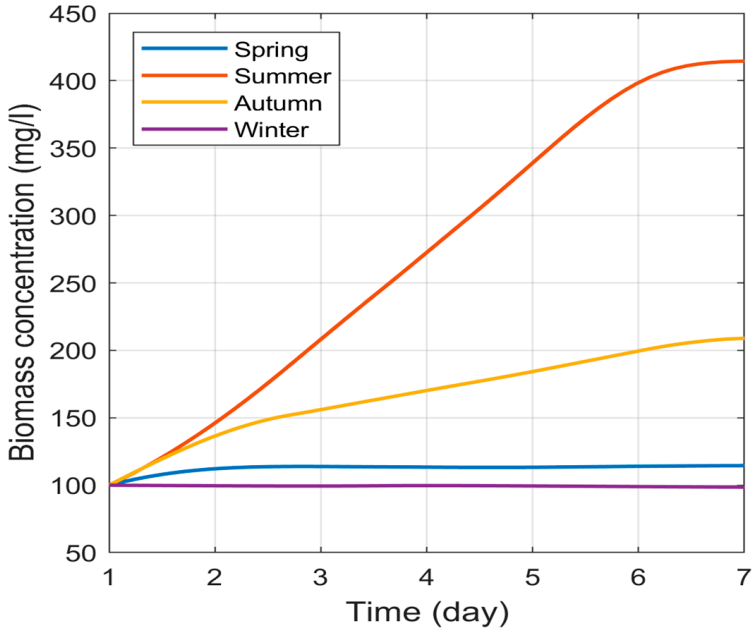

Figure 4. Seasonal variation in the concentration of algal biomass as a function of meteorological parameters (typical meteorological sequence, TMS) from the northern region of Madrid (Riosequillo). These curves were derived by applying, as input data to the model, the average daily temperature (T_{me}) and *PAR* values of selected TMS, Figure 3 [2].

Figure 4 shows the growth curves for the four meteorological seasons of the year using selected TMS from a site in the north of the Madrid region (Riosequillo). The result in Figure 4 reveals the difference in the production of algal biomass between the four seasons of the year, with a higher concentration in summer. This may be explained by the fact that the growth of these microorganisms is intrinsically dependent on the intensity of these two physical parameters. Figure 4 also shows that biomass production is higher in the autumn than in the spring. This difference in production between these two periods of the year appears to be mainly due to the temperature, which is higher in the autumn (Figure 3). A comparison of the PAR over these two periods shows that it is higher in spring. In spring and winter, the microalgae concentration varies very little, even decreasing to 98.5 mg/L on the seventh day in the lowest temperature period (winter). However, in Figure 4, it appears stationary for these two seasons due to the relatively large variation obtained in summer. Furthermore, a general analysis of the curves in all seasonal figures (Figures 5 and A1–A3) also shows the biomass concentrations that are the most likely to be obtained throughout the day of cultivation.

The approach of this case study produced statistically acceptable results. By comparing the results with those obtained by [27], although the latter worked with a different species of algae, the order of magnitude of the final concentration of microalgae produced for the summer season was 400 mg/L. Furthermore, the study carried out by [39] on *Chlorella vulgaris* growth under four culture conditions with different physicochemical properties gave approximate results in the same range. However, these results were obtained over a period ranging from 8 to 14 days.

3.2. Comparative Analysis of Algae Production

In this section, the PDF of the microalgal productivity for each day that forms each GMS for the summer season is represented for the study location. The spring, autumn, and winter figures are in Appendix A. In Figure 5 (and the figures included in Appendix A), the asterisk symbol represents the biomass concentration obtained for each day of TMS and was placed in the middle of the corresponding biomass concentration bin.

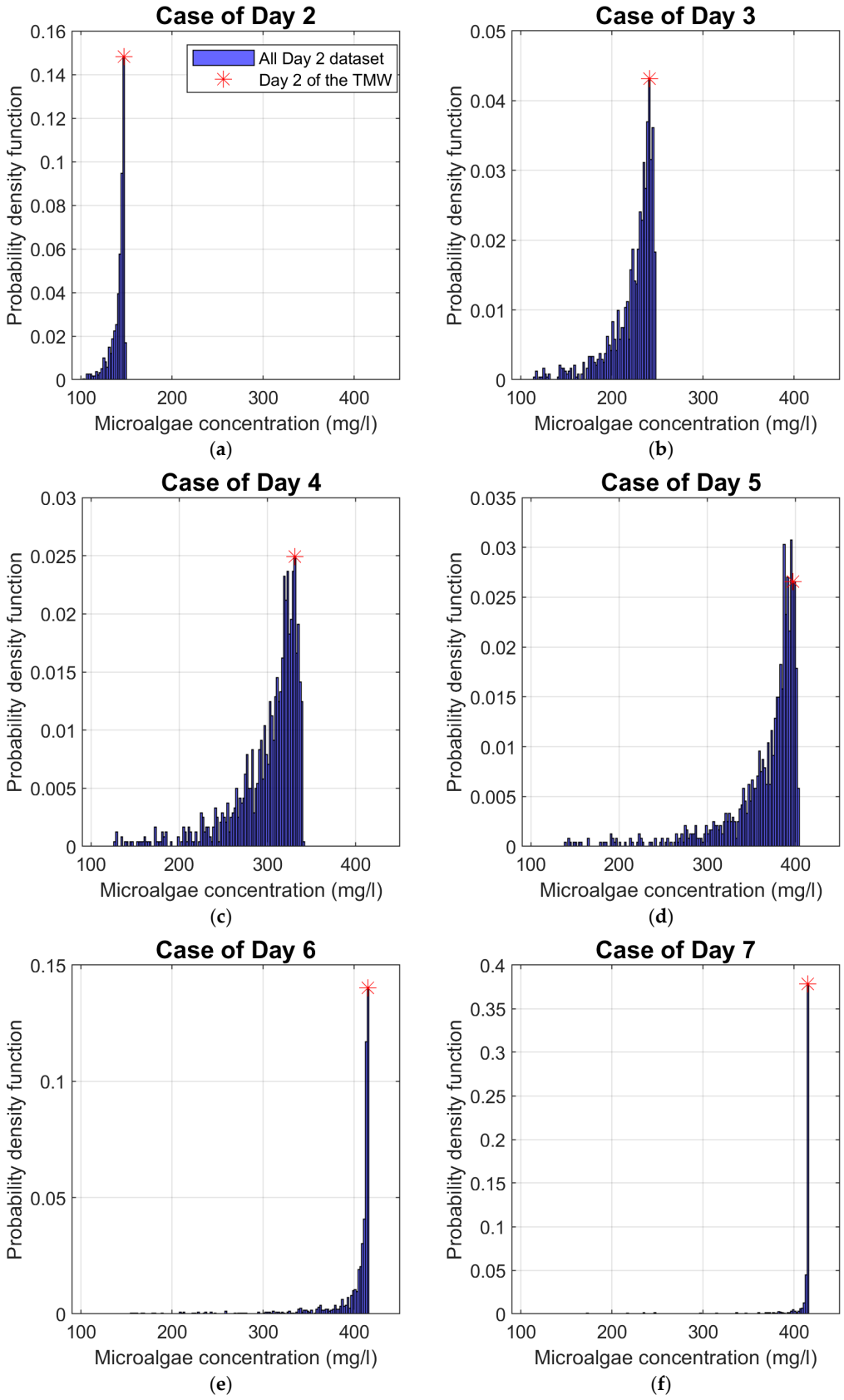

Figure 5. Probability distribution function (PDF) of the sequence of day-by-day biomass concentrations using PAR and temperature data from each GMS input for the model of the summer season at Riosequillo (Madrid). (**a**–**f**) Represent the PDF of the biomass concentration of each dataset from days D2, D3, D4, D5, D6, and D7, respectively.

In summer, the biomass concentration estimates by TMS coincide with the most probable values obtained with the proposed methodology, as Figure 5 shows. The same was observed with data from the spring season, except for the case of days D2 and D7, Figure A1. The position of the biomass concentration, for D5 in the summer (Figure 5) and for D2 and D7 in the spring TMS (Figure A1), is in the concentration bin with a relatively high probability and is directly preceded or followed by the bin with the highest PDF value. The methodology seems to confirm the representativeness of the TMS for the summer and spring seasons in the location studied. In fact, in these two seasons, almost all of the days of TMS already selected are in one of the ranges of biomass concentration that have the highest PDFs.

However, the PDF is more distributed for the autumn season (see Figure A2). In this case, the biomass concentration position of each of the TMS days is not within the bin with the highest PDFs or within the bins near the latter. In Figure A3, in addition to day D2, the biomass concentration for each of the other days of TMS representing winter is far from being among those with the highest probability of distribution, despite the low dispersion of the results. Furthermore, the concentration hardly increases during this period. Consequently, most of the concentrations with the highest probability of distribution are below the initial biomass concentration. The non-growth of the biomass concentration can be explained by the fact that the PAR and temperature values are relatively low during these periods of the year and are unfavorable to the proliferation of these microorganisms. For example, according to Figure 3, average daily temperatures are below the minimum required for the growth of *Chlorella vulgaris*.

The best results, in terms of productivity, were obtained for the summer and spring. The explanation may lie in the fact that certain values of the model parameter were taken from references that worked in conditions with the presence of light and an adequate temperature almost similar to those of these two periods of the year. As Figures 2 and 3 show, the other two seasons, autumn and winter, offer unsuitable conditions for microalgae cultivation.

Figure 6 shows four box plots for the different seasons, grouping the daily average biomass concentrations obtained from the simulation by day. This allows one to evaluate the ranges within which daily biomass production is recorded. It can be observed that the results are almost similar in spring and autumn with more spread boxes between the 25th and 75th percentiles for the latter. However, daily production is less dispersed during summer and winter, clearly showing optimal performance during summer and negligible production levels during winter.

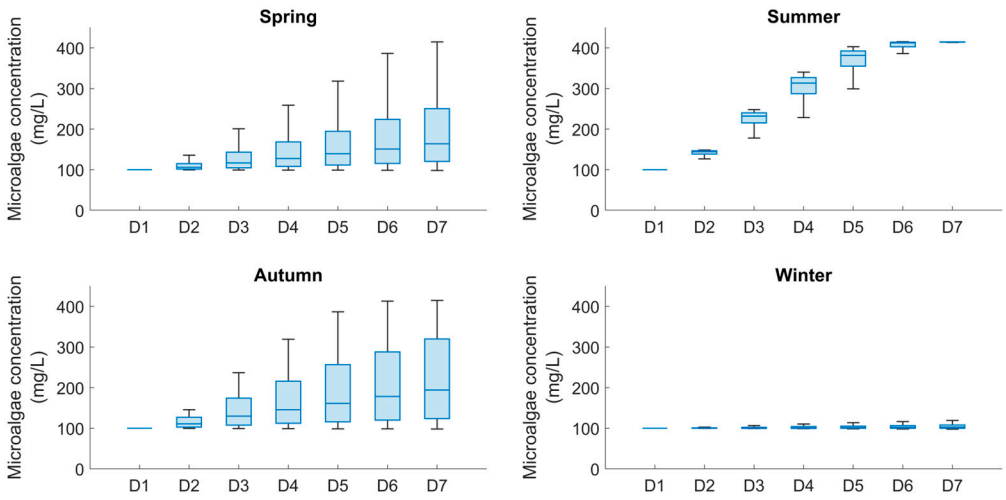

Figure 6. Box plots graphs to display statistics of the series of average daily productions of biomass obtained for each season.

4. Conclusions

In this article, we present a simulation and assessment methodology for plant growth and productivity that can be adapted to all types of crops. The application of this methodology using long-term meteorological data sets makes it possible to identify the most probable production scenarios, which is of great help in decision-making processes (project feasibility analysis, site selection, planning, and management, etc.). The results obtained for our case study confirm the representativeness of typical meteorological sequences, very close to the 50th percentile, for use with more complex simulation programs.

Author Contributions: Conceptualization, L.F.Z. and R.X.V.; methodology, L.F.Z., O.W. and A.A.N.; software, O.W.; validation, O.W. and L.F.Z.; formal analysis, L.F.Z., O.W. and F.F-C; investigation, O.W., L.F.Z., R.X.V., F.F.-C. and A.A.N.; resources, R.X.V. and L.F.Z.; data curation, O.W., R.X.V., A.A.N., L.F.Z. and F.F.-C.; writing—original draft preparation, O.W., R.X.V., A.A.N., L.F.Z. and F.F.-C.; writing—review and editing, O.W., R.X.V., A.A.N., L.F.Z. and F.F.-C.; visualization, O.W. and A.A.N.; supervision, R.X.V. and L.F.Z.; project administration, R.X.V. and L.F.Z.; funding acquisition, R.X.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) and the European Union "Next Generation EU"/PRTR, TEDDY (TED2021-130366B-I00).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data provided by ECMWF used in this study are openly available at https://www.ecmwf.int/en/forecasts/access-forecasts/access-archive-datasets, reference number [8] (accessed on 17 April 2021). CMSAF data used in this study are openly available at https://wui.cmsaf.eu/safira/action/viewProduktList?dId=2&d-1342877-p=6, reference numbers [9] (accessed on 14 April 2021). The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the Programa Iberoamericano de Ciencia y Tecnología (CYTED) (through RED RENUWAL P320RT0005). O.W. thanks Gdańsk University of Technology in the form of grant (grant DEC-6/1/2022/IDUB/II.1a/Au) under the Aurum Supporting International Research Team Building Programme.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Appendix A

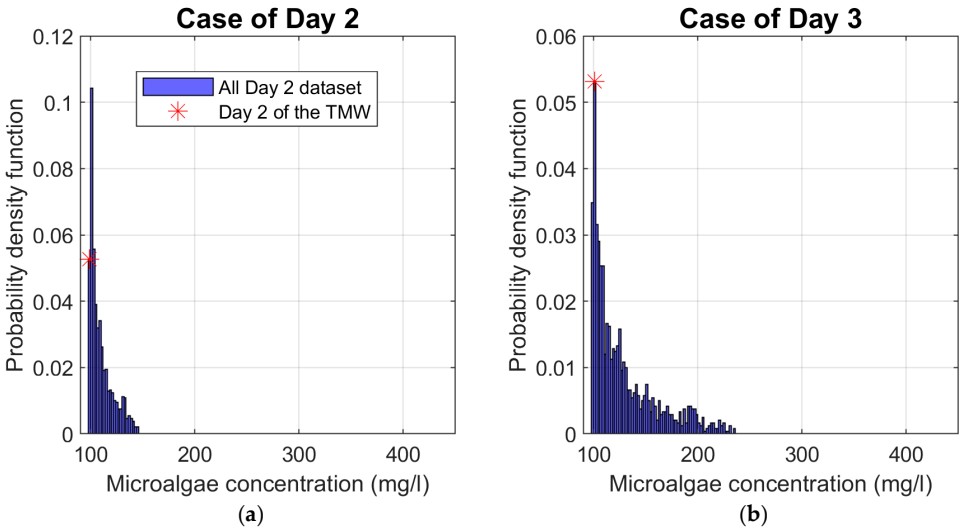

Figure A1. *Cont.*

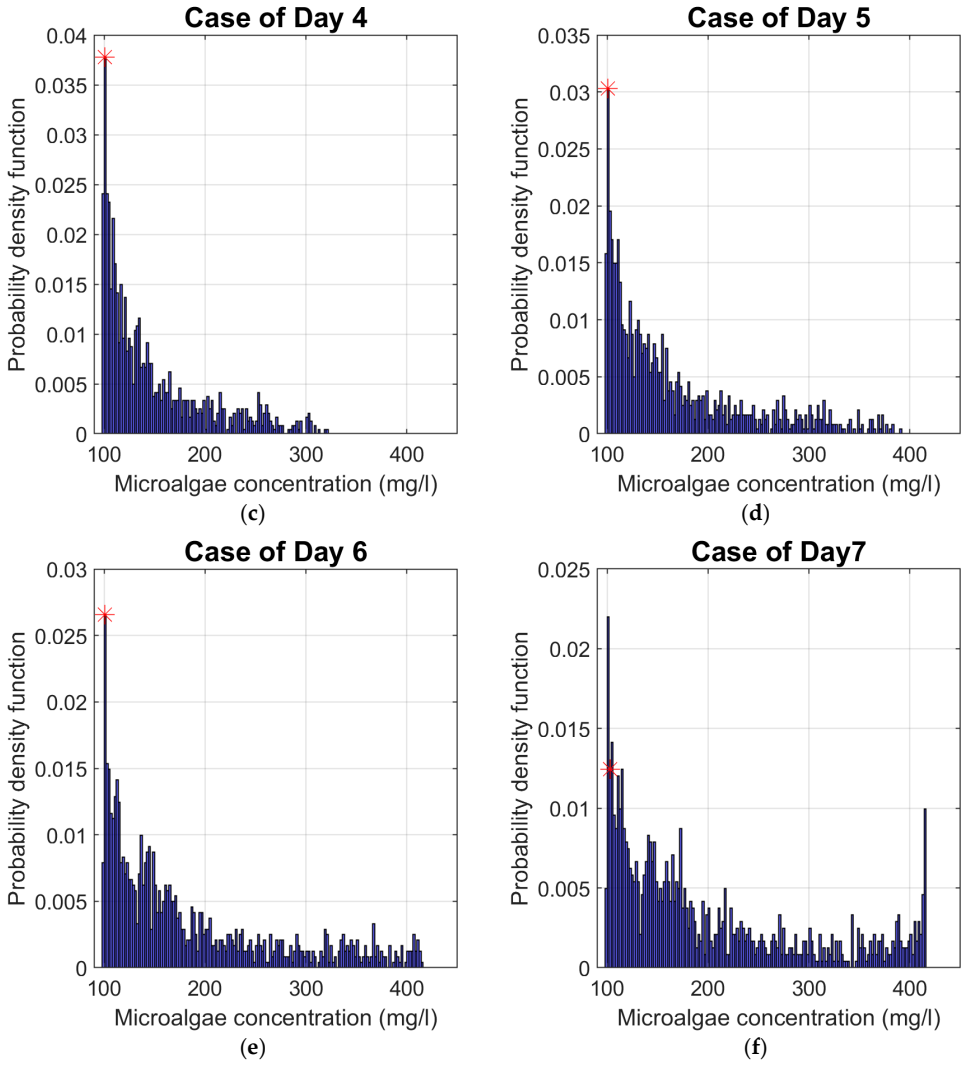

Figure A1. Probability distribution function (PDF) of the sequence of day-by-day biomass concentrations using as model input PAR and temperature data from each GMS of the spring season at Riosequillo (Madrid). (**a**–**f**) represent the PDF of the biomass concentration of each dataset from days D2, D3, D4, D5, D6, and D7, respectively.

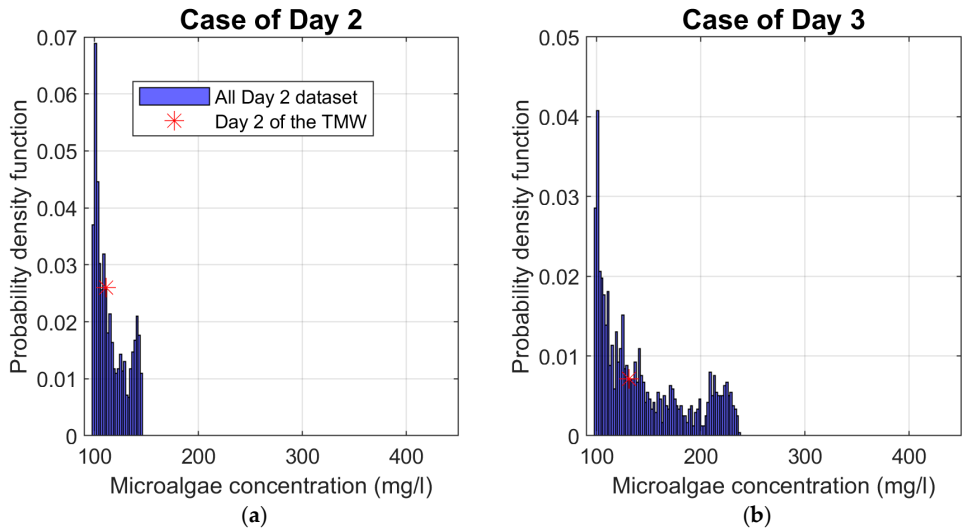

Figure A2. *Cont.*

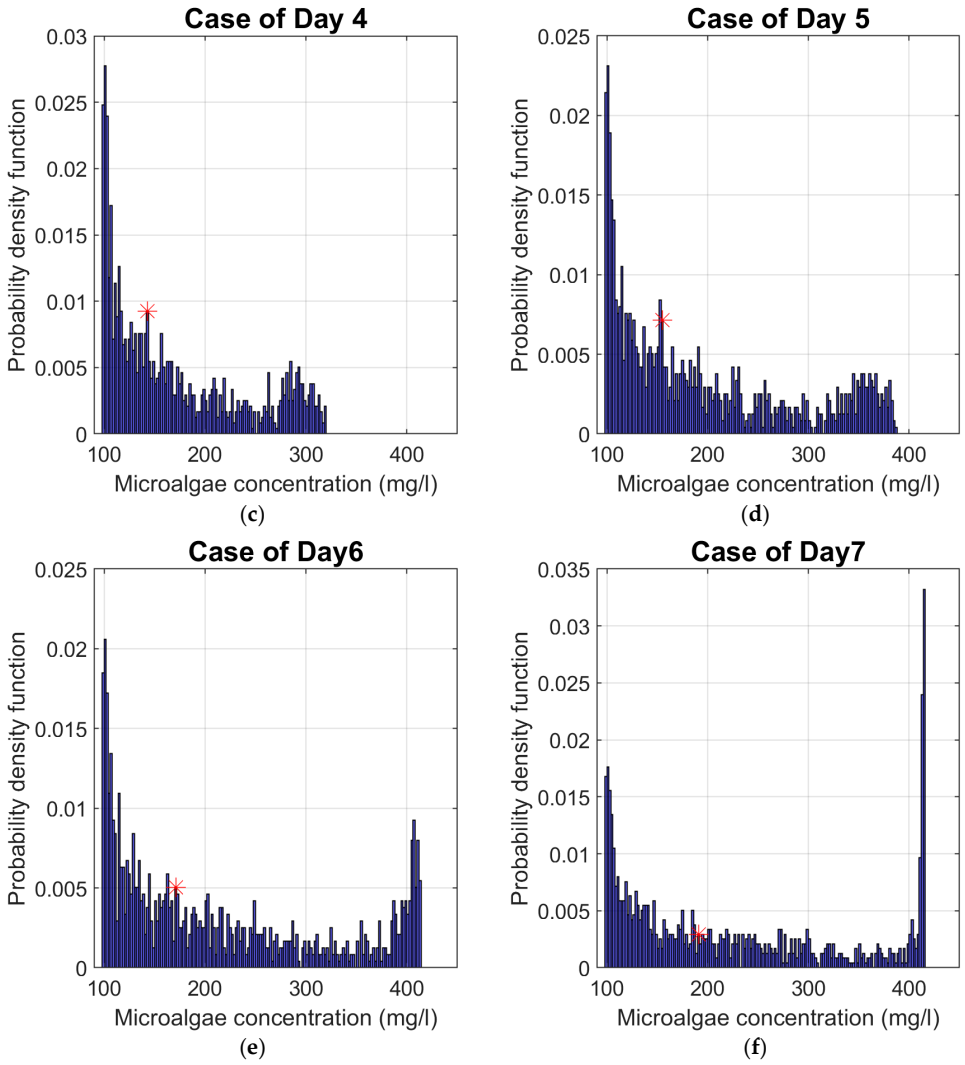

Figure A2. Probability distribution function (PDF) of the sequence of day-by-day biomass concentrations using as model input PAR and temperature data from each GMS of the autumn season at Riosequillo (Madrid). (**a**–**f**) represent the PDF of the biomass concentration of each dataset from days D2, D3, D4, D5, D6, and D7, respectively.

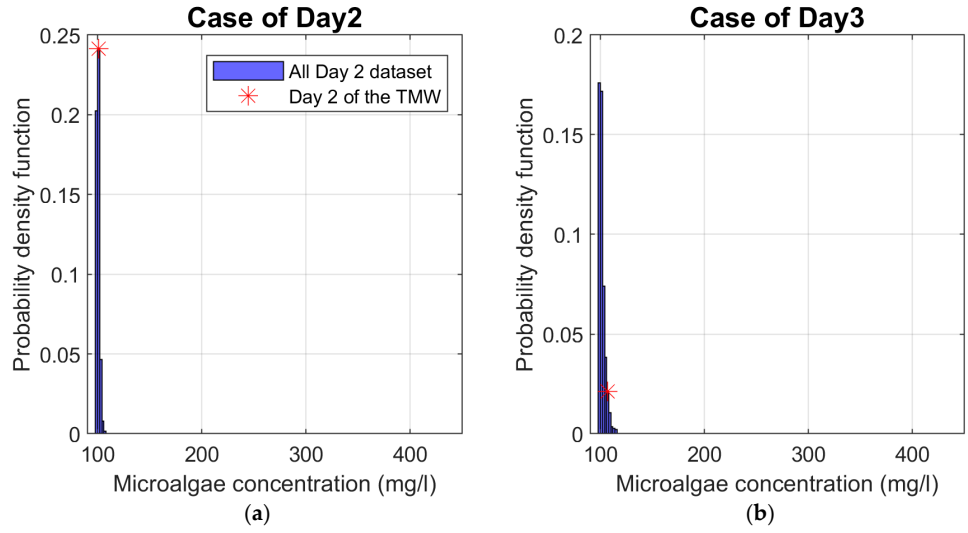

Figure A3. *Cont.*

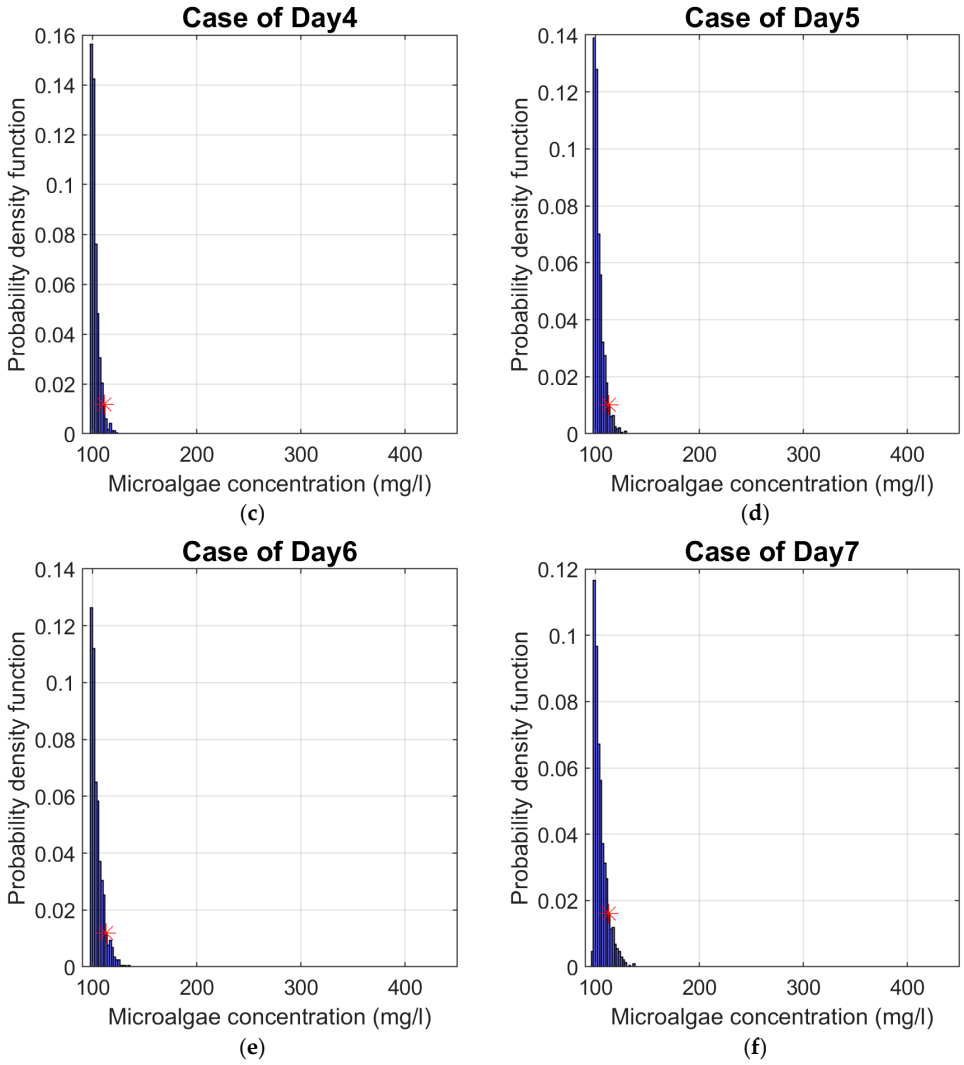

Figure A3. Probability distribution function (PDF) of the sequence of day-by-day biomass concentrations using as model input PAR and temperature data from each GMS of the winter season at Riosequillo (Madrid). (**a**–**f**) Represent the PDF of the biomass concentration of each dataset from days D2, D3, D4, D5, D6, and D7, respectively.

References

1. Oswald, W.J.; Gotaas, H.B. Photosynthesis in Sewage Treatment. *Trans. Am. Soc. Civ. Eng.* **1957**, *122*, 73–97. [CrossRef]
2. García, J.; Green, B.F.; Lundquist, T.; Mujeriego, R.; Hernández-Mariné, M.; Oswald, W.J. Long Term Diurnal Variations in Contaminant Removal in High Rate Ponds Treating Urban Wastewater. *Bioresour. Technol.* **2006**, *97*, 1709–1715. [CrossRef] [PubMed]
3. AlMomani, F.A.; Örmeci, B. Performance Of *Chlorella Vulgaris*, Neochloris Oleoabundans, and Mixed Indigenous Microalgae for Treatment of Primary Effluent, Secondary Effluent and Centrate. *Ecol. Eng.* **2016**, *95*, 280–289. [CrossRef]
4. Sacristán de Alva, M.; Luna-Pabello, V.M.; Cadena, E.; Ortíz, E. Green Microalga Scenedesmus Acutus Grown on Municipal Wastewater to Couple Nutrient Removal with Lipid Accumulation for Biodiesel Production. *Bioresour. Technol.* **2013**, *146*, 744–748. [CrossRef]
5. Wang, L.; Min, M.; Li, Y.; Chen, P.; Chen, Y.; Liu, Y.; Wang, Y.; Ruan, R. Cultivation of Green Algae *Chlorella* sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1174–1186. [CrossRef]
6. Janssen, M. Microalgal Photosynthesis and Growth in Mass Culture. In *Advances in Chemical Engineering*; Academic Press Inc.: Cambridge, MA, USA, 2016; Volume 48, pp. 185–256. ISBN 9780128036617.
7. Ras, M.; Steyer, J.P.; Bernard, O. Temperature Effect on Microalgae: A Crucial Factor for Outdoor Production. *Rev. Environ. Sci. Biotechnol.* **2013**, *12*, 153–164. [CrossRef]
8. Gerotto, C.; Norici, A.; Giordano, M. Toward Enhanced Fixation of CO_2 in Aquatic Biomass: Focus on Microalgae. *Front. Energy Res.* **2020**, *8*, 213. [CrossRef]

9. Buchanan, N.A.; Young, P.; Cromar, N.J.; Fallowfield, H.J. Performance of a High Rate Algal Pond Treating Septic Tank Effluent from a Community Wastewater Management Scheme in Rural South Australia. *Algal Res.* **2018**, *35*, 325–332. [CrossRef]
10. Darvehei, P.; Bahri, P.A.; Moheimani, N.R. Model Development for the Growth of Microalgae: A Review. *Renew. Sustain. Energy Rev.* **2018**, *97*, 233–258. [CrossRef]
11. Singh, S.P.; Singh, P. Effect of Temperature and Light on the Growth of Algae Species: A Review. *Renew. Sustain. Energy Rev.* **2015**, *50*, 431–444. [CrossRef]
12. Serra-Maia, R.; Bernard, O.; Gonçalves, A.; Bensalem, S.; Lopes, F. Influence of Temperature on *Chlorella Vulgaris* Growth and Mortality Rates in a Photobioreactor. *Algal Res.* **2016**, *18*, 352–359. [CrossRef]
13. Arcila, J.S.; Buitrón, G. Influence of Solar Irradiance Levels on the Formation of Microalgae-Bacteria Aggregates for Municipal Wastewater Treatment. *Algal Res.* **2017**, *27*, 190–197. [CrossRef]
14. Bernard, O.; Rémond, B. Validation of a Simple Model Accounting for Light and Temperature Effect on Microalgal Growth. *Bioresour. Technol.* **2012**, *123*, 520–527. [CrossRef]
15. Metsoviti, M.N.; Papapolymerou, G.; Karapanagiotidis, I.T.; Katsoulas, N. Effect of Light Intensity and Quality on Growth Rate and Composition of *Chlorella Vulgaris*. *Plants* **2020**, *9*, 31. [CrossRef]
16. Hoyo, Á.; Rodríguez-Miranda, E.; Guzmán, J.L.; Acién, F.G.; Berenguel, M.; Moreno, J.C. A Computer-Based Tool to Simulate Raceway Photobioreactors for Design, Operation and Control Purposes. *Comput. Chem. Eng.* **2022**, *156*, 107572. [CrossRef]
17. Huesemann, M.; Crowe, B.; Waller, P.; Chavis, A.; Hobbs, S.; Edmundson, S.; Wigmosta, M. A Validated Model to Predict Microalgae Growth in Outdoor Pond Cultures Subjected to Fluctuating Light Intensities and Water Temperatures. *Algal Res.* **2016**, *13*, 195–206. [CrossRef]
18. Díez-Montero, R.; Belohlav, V.; Ortiz, A.; Uggetti, E.; García-Galán, M.J.; García, J. Evaluation of Daily and Seasonal Variations in a Semi-Closed Photobioreactor for Microalgae-Based Bioremediation of Agricultural Runoff at Full-Scale. *Algal Res.* **2020**, *47*, 101859. [CrossRef]
19. Wane, O.; Zarzalejo, L.F.; Ferrera-Cobos, F.; Navarro, A.A.; Rodríguez-López, A.; Valenzuela, R.X. Generation of Typical Meteorological Sequences to Simulate Growth and Production of Biological Systems. *Appl. Sci.* **2023**, *13*, 4826. [CrossRef]
20. Dobos, A.; Gilman, P.; Kasberg, M. *P50/P90 Analysis for Solar Energy Systems Using the System Advisor Model Preprint*; National Renewable Energy Lab.(NREL): Denver, Colorado, 2012.
21. Fernández Peruchena, C.M.; Ramírez, L.; Silva-Pérez, M.A.; Lara, V.; Bermejo, D.; Gastón, M.; Moreno-Tejera, S.; Pulgar, J.; Liria, J.; Macías, S.; et al. A Statistical Characterization of the Long-Term Solar Resource: Towards Risk Assessment for Solar Power Projects. *Sol. Energy* **2016**, *123*, 29–39. [CrossRef]
22. Fernández-Peruchena, C.M.; Vignola, F.; Gastón, M.; Lara-Fanego, V.; Ramírez, L.; Zarzalejo, L.; Silva, M.; Pavón, M.; Moreno, S.; Bermejo, D.; et al. Probabilistic Assessment of Concentrated Solar Power Plants Yield: The EVA Methodology. *Renew. Sustain. Energy Rev.* **2018**, *91*, 802–811. [CrossRef]
23. Su, H.; Zhang, Y.; Zhang, C.; Zhou, X.; Li, J. Cultivation of Chlorella Pyrenoidosa in Soybean Processing Wastewater. *Bioresour. Technol.* **2011**, *102*, 9884–9890. [CrossRef]
24. Zou, G.; Liu, Y.; Zhang, Q.; Zhou, T.; Xiang, S.; Gu, Z.; Huang, Q.; Yan, H.; Zheng, H.; Wu, X.; et al. Cultivation of *Chlorella Vulgaris* in a Light-Receiving-Plate (LRP)-Enhanced Raceway Pond for Ammonium and Phosphorus Removal from Pretreated Pig Urine. *Energies* **2020**, *13*, 1644. [CrossRef]
25. Behera, B.; Aly, N.; Balasubramanian, P. Biophysical Modeling of Microalgal Cultivation in Open Ponds. *Ecol. Modell.* **2018**, *388*, 61–71. [CrossRef]
26. Ali, H.; Park, C.W. Numerical Multiphase Modeling of CO_2 Absorption and Desorption in Microalgal Raceway Ponds to Improve Their Carbonation Efficiency. *Energy* **2017**, *127*, 358–371. [CrossRef]
27. Eze, V.C.; Velasquez-Orta, S.B.; Hernández-García, A.; Monje-Ramírez, I.; Orta-Ledesma, M.T. Kinetic Modelling of Microalgae Cultivation for Wastewater Treatment and Carbon Dioxide Sequestration. *Algal Res.* **2018**, *32*, 131–141. [CrossRef]
28. Aiba, S. Growth Kinetics of Photosynthetic Microorganisms. In *Microbial Reactions*; Springer: Berlin/Heidelberg, Germany, 1982; pp. 85–156.
29. Schirmer, M.; Posten, C. Modeling of Microalgae Bioprocesses. In *Advances in Chemical Engineering*; Academic Press Inc.: Cambridge, MA, USA, 2016; Volume 48, pp. 151–184. ISBN 9780128036617.
30. Rosso, L.; Lobry, J.R.; Flandrois, J.P. An Unexpected Correlation between Cardinal Temperatures of Microbial Growth Highlighted by a New Model. *J. Theor. Biol.* **1993**, *162*, 447–463. [CrossRef] [PubMed]
31. Shoener, B.D.; Schramm, S.M.; Béline, F.; Bernard, O.; Martínez, C.; Plósz, B.G.; Snowling, S.; Steyer, J.P.; Valverde-Pérez, B.; Wágner, D.; et al. Microalgae and Cyanobacteria Modeling in Water Resource Recovery Facilities: A Critical Review. *Water Res. X* **2019**, *2*, 100024. [CrossRef] [PubMed]
32. García-Malea, M.C.; Acién, F.G.; Fernández, J.M.; Cerón, M.C.; Molina, E. Continuous Production of Green Cells of Haematococcus Pluvialis: Modeling of the Irradiance Effect. *Enzym. Microb. Technol.* **2006**, *38*, 981–989. [CrossRef]
33. Pawłowski, A.; Guzmán, J.L.; Berenguel, M.; Acíen, F.G.; Dormido, S. Application of Predictive Feedforward Compensator to Microalgae Production in a Raceway Reactor: A Simulation Study. *Energies* **2018**, *11*, 123. [CrossRef]
34. Kamarianakis, Y.; Ayuso, S.V.; Rodríguez, E.C.; Velasco, M.T. Water Temperature Forecasting for Spanish Rivers by Means of Nonlinear Mixed Models. *J. Hydrol. Reg. Stud.* **2016**, *5*, 226–243. [CrossRef]

35. MathWorks Centro de Ayuda—Ejecutar Varias Simulaciones. Available online: https://es.mathworks.com/help/simulink/run-multiple-parallel-simulations.html (accessed on 5 November 2024).
36. Aleya, L.; Dauta, A.; Reynolds, C.S. Endogenous Regulation of the Growth-Rate Responses of a Spring-Dwelling Strain of the Freshwater Alga, Chlorella Minutissima, to Light and Temperature. *Eur. J. Protistol.* **2011**, *47*, 239–244. [CrossRef] [PubMed]
37. Pires, J.C.M.; Gonçalves, A.L.; Martins, F.G.; Alvim-Ferraz, M.C.M.; Simões, M. Effect of Light Supply on CO2 Capture from Atmosphere by *Chlorella Vulgaris* and Pseudokirchneriella Subcapitata. *Mitig. Adapt. Strateg. Glob. Chang.* **2014**, *19*, 1109–1117. [CrossRef]
38. Shaw, A.; Takács, I.; Pagilla, K.R.; Murthy, S. A New Approach to Assess the Dependency of Extant Half-Saturation Coefficients on Maximum Process Rates and Estimate Intrinsic Coefficients. *Water Res.* **2013**, *47*, 5986–5994. [CrossRef]
39. Ajala, S.O.; Alexander, M.L. Assessment of *Chlorella Vulgaris*, Scenedesmus Obliquus, and Oocystis Minuta for Removal of Sulfate, Nitrate, and Phosphate in Wastewater. *Int. J. Energy Environ. Eng.* **2020**, *11*, 311–326. [CrossRef]