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A New Modeling Approach for Predicting the Growth of Filamentous Algae in Outdoor Algae-Based Wastewater Treatment Systems

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ABSTRACT

Filamentous algae (FA) can form readily harvestable floating mats or attached turfs that facilitate their application in wastewater treatment systems. However, large-scale implementation is hindered by our inability to predict performance as a function of key operational parameters. A predictive mathematical model would be a valuable tool for designing efficient FA-based systems. Developing accurate models is challenging due to dynamic environmental conditions and the spatial complexities of FA cultures. In this work, a model was developed to mathematically describe the biomass productivity of static FA cultures (mats and turfs) in relation to the incident light intensity and temperature. The model was validated against published data to investigate the influence of time-dependent inhibition (inhibition from sustained light exposure) on productivity. When time-dependent inhibition was included in the model, predictions were within ~10% of experimental values, however, without including time-dependent inhibition there was a sixfold overestimation of biomass productivity. The model could also generate predictions of the effects of time-dependent inhibition during diurnal light fluctuations using experimentally determined rate constants. The model represents a powerful tool for optimizing the design and operational parameters in FA cultures that could be further expanded to incorporate the influence of nutrient and CO_2 availability.

1 | Introduction

The use of filamentous algae (FA) in algae-based wastewater treatment systems has many potential benefits over microalgae, including ease of harvesting, high resistance to predation, competitive dominance over competing organisms, and the ability to maintain monocultures beneficial for downstream biomass utilization (Lawton, de Nys, and Paul 2013; Lawton et al. 2021; Liu et al. 2020; Neveux et al. 2016; Roberts, de Nys, and Paul 2013). However, as more focus has been given to the use of microalgae, there are major research gaps in aspects such as species performance, nutrient and carbon uptake, algae-bacteria interactions, and performance prediction that hinder the use of FA-based wastewater treatment systems at large scale (Liu et al. 2020). The biomass productivity of FA is a key performance parameter to be optimized when scaling up FA-based wastewater treatment systems (Lawton et al. 2021; Neveux et al. 2016). Selecting design and operating parameters that optimize biomass productivity requires mathematical models that can relate FA growth to key variables. Biomass productivity of FA cultures is multidimensional and depends on many factors including nutrient concentrations, light intensity, temperature, pH, and pond depth (Béchet, Shilton, and Guieysse 2013).

In outdoor systems, light intensity and temperature have a controlling impact on algal productivity (Béchet, Shilton, and

Guieysse 2013). Studies on various FA species indicate the significant impact of temperature and light on photosynthesis and respiration, and consequently the biomass productivity of outdoor cultures (Graham, Arancibia-Avila, and Graham 1996; Graham et al. 1982; Graham et al. 1995; Necchi Jr 2004; Pitawala et al. 2024; Pitawala et al. 2023). Although photosynthesis is dependent on both light intensity and temperature, the respiration response consists of two separate components – light-enhanced respiration and dark respiration, of which the latter only depends on temperature (Duarte et al. 2013; Falkowski and Owens 1978; Pitawala et al. 2023). Light intensity mainly influences the reactions generating the energy needed for growth and maintenance of cellular functions, while temperature influences enzymatic activity within the algal cell (Manhaeghe et al. 2019). A mathematical model must be able to represent the effect of light and temperature on the growth of FA in relation to both photosynthesis and respiration.

Importantly, light and temperature are not practically controllable in outdoor cultures. Cultures are subject to both short-term diurnal fluctuations and long-term seasonal variations in light intensity and temperature. Ideally, a model can account for these variations when predicting productivity. In addition to contemporaneous effects of variations in light and temperature, long-term seasonal fluctuations present in temperate regions, lead to variations in the average growth conditions (average light exposure, daylight hours, and temperature). These long-term variations in the average growth conditions lead to biochemical compositional changes in the algal cells due to acclimation which directly influences the cellular mechanisms affecting the biomass productivity levels (Pitawala et al. 2024; Pitawala et al. 2023). Therefore, the influence of both short-term and long-term variations in the light intensity and temperature need to be accounted for when modeling the performance of outdoor FA-based wastewater treatment systems.

A mathematical model that could account for all these variations would be a valuable tool for understanding and predicting the influence of key parameters on biomass productivity. Numerous growth models have been developed for microalgae to aid in large-scale design and operation (Darvehei, Bahri, and Moheimani 2018; Shoener et al. 2019). These models can account for various aspects affecting microalgae growth, including light availability, temperature, nutrient and carbon concentration, and pH. Among the available models, the algal growth model developed by Huesemann et al. (2013) to predict microalgal productivity by considering the light attenuation by biomass due to self-shading, has proven to be useful (Gao et al. 2018; Huesemann et al. 2016; Van Wagenen et al. 2014). However, in comparison to microalgae (which are unicellular and therefore evenly distributed in a well-mixed growth pond), modeling the growth of FA is complicated by its uneven distribution in a pond. FA consists of cylindrical filaments formed by the longitudinal joining of cells that result in cultures containing spatially heterogenous macroscopic structures. These include tumble cultures, where clumps of filaments are tumbled around as a suspension within the culture (Cole, de Nys, and Paul 2014; Cole et al. 2016; Lawton et al. 2021), turf mats in which filaments grow attached to a surface in algal turf scrubbers (ATS) (Hariz, Lawton, and Craggs Lawton, and Craggs 2023a, 2023b) and filamentous algal nutrient scrubbers (FANS), or as stable floating mats located on the surface of the pond (Pikosz, Messyasz, and Gąbka 2017; Piotrowski, Graham, and Graham 2020; Pitawala et al. 2024). Mathematical models must be able to account for the effect of the differences in physical attributes of the various FA cultures.

Despite the extensive development of models describing microalgae growth (Darvehei, Bahri, and Moheimani 2018), to date no model has been specifically designed to predict the productivity of FA-based wastewater treatment systems and there are gaps in the available data required to develop one. Furthermore, the applicability of modified microalgal growth models for FA tumble cultures, where the FA cells experience rapid fluctuations in light due to mixing similar to cells in microalgal cultures, has not been investigated. Although there is no mathematical growth model specifically designed for static FA-based wastewater treatment systems (turf or floating mat cultures), there exist several versions of a model developed to predict the attached growth of FA species *Cladophora* in the Great Lakes of Canada (Auer and Canale 1982; Higgins, Hecky, and Guildford Hecky, and Guildford 2005, 2006; Kuczynski et al. 2020). In the literature, the model is known as the Great Lakes Cladophora Model (GLCM). The latest version of GLCM (GLCM v3) developed by Kuczynski et al. (2020) follows a similar discretized procedure to that used in the microalgal model developed by Huesemann et al. (2013). Both models consider the algal biomass to consist of separate layers instead of one homogenous unit, allowing for the model to account for the stratified self-shading effect caused by algae. In the microalgal model, the number of layers in a culture remains constant over time, as the microalgal cell suspension occupies the entire volume of the culture. In contrast, the number of layers in a mat-forming FA model can change with time, as the mat continues to grow in thickness (i.e. in the vertical direction). The GLCM v3 incorporates the increase in the depth of the attached growth mat by defining a constant biomass density to every layer, which once reached, triggers expansion into a new vertical layer (Kuczynski et al. 2020). The use of a constant biomass density is one potential limitation of the GLCM that may lead to unrealistic predictions of mat thickness, as in actuality the mat becomes more compacted as biomass accumulates (Kuczynski et al. 2022). As such, it would be preferable to have a model that could account for such variations in biomass density. This approach was implemented by Kuczynski et al. (2022) by replacing the fixed value for biomass density with a non-linear equation describing biomass density as a function of mat thickness that accounts for the mat compression observable in their experimental data.

In addition to the spatial inhomogeneity of FA cultures, the static nature of mats and turfs has important implications that should be considered in a mathematical model. In particular, as FA cells are fixed in relation to incident light, cells near the surface of the mat or turf will be continuously exposed to high-intensity light. It has been shown that time-dependent inhibition of photosynthetic activity which occurs due to sustained light exposure, significantly impacts static FA cultures where the cells are exposed to higher photooxidative and heat stresses for prolonged periods (Belay 1981; Pitawala et al. 2023). As such, in systems where the algal biomass remains static in stable floating mats or attached growth turfs, the influence of

sustained light exposure time should be considered for accurate model predictions.

The aim of this study was to develop mathematical growth models to describe two types of static FA systems: (i) filamentous algae nutrient scrubbers (FANS) and (ii) surface filamentous algal mats (FAM), where FA is attached to the bottom of a pond or float on the surface, respectively. The mathematical framework used in GLCM v3 to discretize the algal mat and determine the change of biomass in the system were the chosen basis for developing the model presented here. In this work, three significant modifications were made to the GLCM model, involving (i) incorporating mat compaction due to biomass accumulation (similar to what was recently done in Kuczynski et al. (2022), (ii) inclusion of time-dependent inhibition of photosynthetic activity, and (iii) for surface mats (FAM), inverting the orientation. The freshwater FA *Oedogonium* was selected based on the findings from previous studies, which have shown its potential for wastewater treatment and downstream biomass utilization (Cole et al. 2016; Lawton et al. 2017; Lawton et al. 2021; Neveux et al. 2016). The model utilizes species-specific parameters of *Oedogonium* grown under average winter conditions in Melbourne, Australia, based on the experimental data obtained in the study done by Pitawala et al. (2023). The model accounts for the influence of light intensity and temperature on the biomass productivity of FA *Oedogonium* grown in floating mats or turf beds. It can be applied to other species and seasonal conditions for which parameter values can be determined. The model can be used as a tool to help understand and predict the performance of outdoor FA-based systems where the biomass experiences short-term diurnal fluctuations in light intensity and temperature and can be applied to account for long-term seasonal variations.

2 | Materials and Methods

2.1 | Filamentous Algae Cultivation

Freshwater filamentous alga *Oedogonium*, sourced from stock cultures previously described (Pitawala et al. 2023), was initially acclimated to the average winter conditions in Melbourne, Australia (300 ± 25 $\mu mol.m^{-2}.s^{-1}$ with 13:11 dark: light cycle at 15°C) inside a light and temperature controlled growth chamber (BI-RIC-600, Biora, Australia). For this purpose, 600 mL transparent plastic containers filled with 450 mL of modified MLA medium (Liu et al. 2023) were inoculated with the algal biomass from the stock cultures (~300 mg, fresh weight) and transferred into the growth chamber for acclimation for 7 days. During the growth period, aeration was provided to the algal cultures using air stones placed at the bottom of the container with an airflow rate of around 100 mL. min^{-1} to promote the formation of the FA mat.

To determine the physical mat properties relevant to the model, the acclimated biomass was allowed to form surface-covering floating mats in the 600 mL plastic containers (surface area ~0.0085 m^2) under the same growth conditions. The entire culture medium was replaced weekly to ensure that no nutrient depletion occurred. Once a surface-covering mat was formed, the air stone was removed, 24 h prior to taking the measurements to ensure that the mat spread throughout the entire surface of the container. Thereafter, the formed filamentous algal mats were used to parameterize variations in mat depth and light attenuation through the algal mat as a function of biomass density, as described in the following section.

2.2 | Determination of Filamentous Algae Mat Properties

The cultured FA mats were used to quantify the variation of mat depth and light attenuation through the mat with respect to the areal biomass density. Each culture was used to obtain one data point for both parameters. Initially, images were taken to determine the mat depth and the mat surface area using the image processing software, ImageJ. As the algal mats were not uniform throughout, four images of the side of the transparent plastic container were taken orthogonal to each other (Fig. S1). The thicknesses of the mat in the four images were determined using the image processing software and the average was taken as the mat depth. Additionally, the surface area of the mat was determined from aerial images using the same software. After taking the images, the transparent plastic container containing the FA mat was used to determine the light attenuation through the algal mat.

Light attenuation through a medium can be represented by Beer-Lambert's law, which defines light intensity as a function of depth (Eq. 1) (Béchet, Shilton, and Guieysse 2013; Kuczynski et al. 2020).

$$I_z = I_o \bullet e^{-k_a \bullet z} \tag{1}$$

where, I_o is the photosynthetically active radiation (PAR) at the surface ($\mu mol.m^{-2}s^{-1}$), I_z is the PAR at a position (z) within the medium ($\mu mol.m^{-2}s^{-1}$), k_a is the light extinction coefficient through the medium (m^{-1}) and z is the depth from the surface to the considered point in the medium (m).

To determine the light extinction coefficient of the FA mat, the transparent container was placed inside another container with the sides covered to ensure only vertically penetrating light was registered. Then, the light intensity reaching the bottom of the setup (I_1) was measured using an underwater full-spectrum quantum sensor (MQ-510, Apogee, USA) placed under the two containers during illumination from the top (Fig. S2). To eliminate the light absorbed by the culture medium and the container material, the light intensity at the bottom of the setup (I_2) was measured again after replacing the container with the algal mat with a container with a blank culture medium. Then, the light extinction coefficient of the algal mat was determined using Eq. 2, which was obtained by reordering Eq. 1.

$$k_{alg} = -\frac{1}{D_{mat}} \bullet \ln\left(\frac{I_1}{I_2}\right) \tag{2}$$

where, k_{alg} is the light extinction coefficient through the FA mat (m^{-1}), D_{mat} is the mat depth (m), I_1 and I_2 are the light intensities ($\mu mol.m^{-2}s^{-1}$) measured at the bottom of the setup with the algal mat and blank medium, respectively.

Finally, to determine the areal biomass density (X in g DW.m^{-2}), the biomass was harvested by passing the culture through a stainless-steel mesh strainer (pore size $< 700\,\mu m$). The retained biomass was then centrifuged using methods previously described (Liu et al. 2023) and the dewatered biomass was weighed to obtain the fresh weight (FW). A small portion of the dewatered biomass was weighed, and oven-dried for 48 h at 60°C and the dry weight (DW) was measured to determine the FW: DW ratio. The remaining portion of dewatered biomass was reinoculated into a 600 mL container with fresh modified MLA medium and transferred to the growth chamber to allow the floating mat to form, to obtain another data point for these investigations. The FW: DW ratio obtained was used to calculate the areal biomass density relevant to the determined mat depth and light extinction coefficient. The data points for mat depth and light extinction coefficient were plotted against the respective areal biomass density to obtain functions representative of the variations in these mat-related properties.

2.3 | Theory

As discussed, the proposed filamentous algae growth models build upon the basic concepts of the latest version of the Great Lakes Cladophora Model (GLCM v3) (Kuczynski et al. 2020), which models the growth of attached FA mats on the bottom of lakes. The models presented here were developed on the premise of the formation of a continuous FA mat, either floating on the surface (FAM) or attached to the bottom of a pond (FANS) that will continue to grow vertically as the areal biomass density increases with time. A graphical comparison of a microalgal model (Huesemann et al. 2013), GLCM v3, FANS, and FAM models is illustrated in Figure 1.

2.3.1 | Model Framework

The developed models predict the variation in the areal biomass density and FA mat thickness of freshwater FA *Oedogonium* with time by considering the light intensity and temperature variation across the prediction period. To simplify the modeling approach, the following assumptions were applied:

- Light intensity and temperature are the only limiting factors for the productivity and cellular responses of the FA (e.g. no nutrient or CO_2 limitation).
- Filamentous algae biomass forms a stable mat either floating on the water surface (FAM) or attached to the bottom (FANS) of a pond.
- No mixing occurs that disturbs the formation of the algal mat.
- The algal mat is filled layer by layer, with additional newly formed biomass being added to the bottom (FAM) or top layer (FANS) (Lawton, de Nys, and Paul 2013).
- Light is only incident on the water surface perpendicularly.
- Temperature is the same throughout the culture medium and FA mat.

The modeling procedure summarized by the model framework illustrated in Figure 2 is as follows. Initially, the algal mat is discretized into layers and the light intensity incident at the midpoint of each of the layers is determined, thus incorporating self-shading into the models. Then, the incremental change in the areal biomass density of each layer for a given timestep is calculated from the areal biomass density at the beginning of the timestep and the respective growth due to photosynthetic activity and loss due to respiration at the given temperature and incident light at the beginning of the timestep. Then, the total areal biomass density of the mat is calculated by summing the areal biomass densities of all the layers. Finally, before moving on to the next timestep, the algal mat is redivided into discrete layers of equal thickness, while adding any remaining biomass to the bottom layer (see Section 3.2.1). With time, as the biomass density and mat thickness increase, the number of layers will also increase, while the thickness of each layer remains the same. The above steps are repeated until the model has been run for the specified period.

The models were executed using MATLAB 2023b software, with input parameters that can be categorized as (i) user-defined and (ii) species-specific parameters (Figure 2). The species-specific parameters are determined experimentally and used to calibrate the model for the FA species being considered. It is important to note that the species-specific parameters will change with the algal species used and also with the average growth conditions due to seasonal acclimation. In this study, the model was

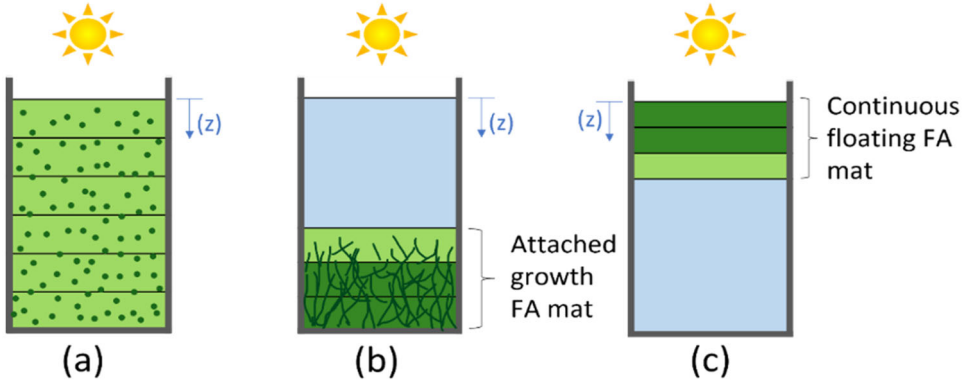

FIGURE 1 | Graphical representation of (a) microalgal growth model, (b) the latest version of the Great Lakes Cladophora Model (GLCM v3) and the FANS model, and (c) the FAM model.

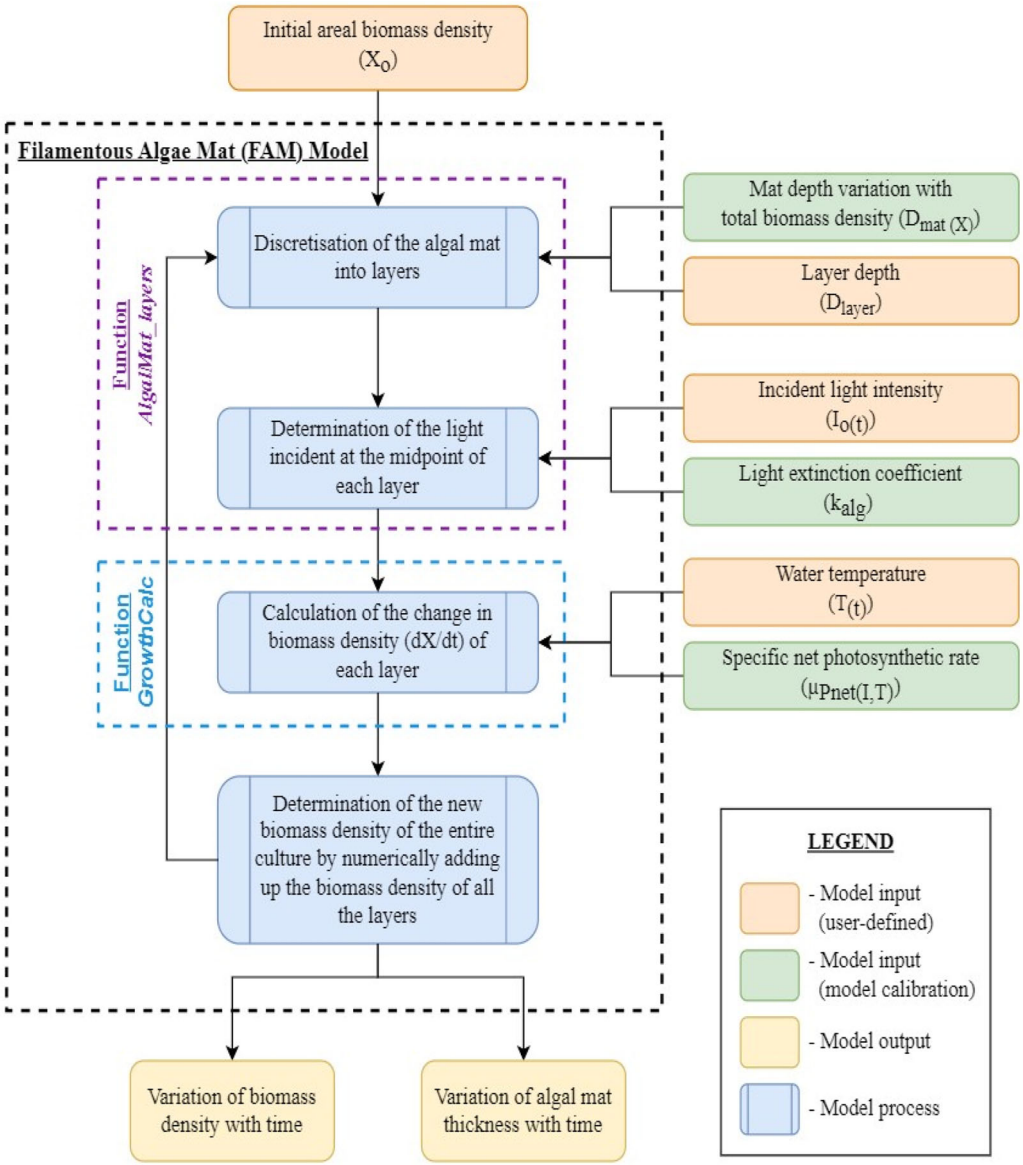

FIGURE 2 | The framework of the filamentous algae growth models.

calibrated using data relevant to FA *Oedogonium* grown under average winter conditions in Melbourne, Australia. For ease of modeling, the coding of the FAM model was embedded with two core functions: *AlgalMat_layers* and *GrowthCalc* (Figure 2). The main script was developed using MATLAB live scripts providing an interactive interface where users can enter the user-defined parameters easily. The MATLAB codes for all three sections of the models are included in the supporting information.

2.3.2 | Model Equations and Parameters

The equations incorporated into the models can be categorized into three groups:

1. Discretization of the algal mat
2. Light attenuation within the algal mat
3. Determination of the change in the areal biomass density with time

Parameter values were defined using experimental data collected in this study and from the study conducted by Pitawala et al. (2023) to calibrate the models, as discussed in the following sections. It is important to note that the model is thereby best suited to predicting the performance of *Oedogonium* in outdoor systems with similar growth conditions to that of winter in Melbourne, Australia.

2.3.3 | Discretization of the Algal Mat

The first set of equations is used at the start of each time step to discretize the algal mat into layers. As previously mentioned, the GLCM v3 assumes a constant biomass density in each layer, which can lead to unrealistic mat thicknesses as the biomass density increases. FA mats can be expected to be compacted with an increase in areal biomass density, which was observed in the experimental work conducted in this study (Figure 3a). This compaction is represented in the variation in mat depth with areal biomass density, which was described mathematically by Eq. 3.

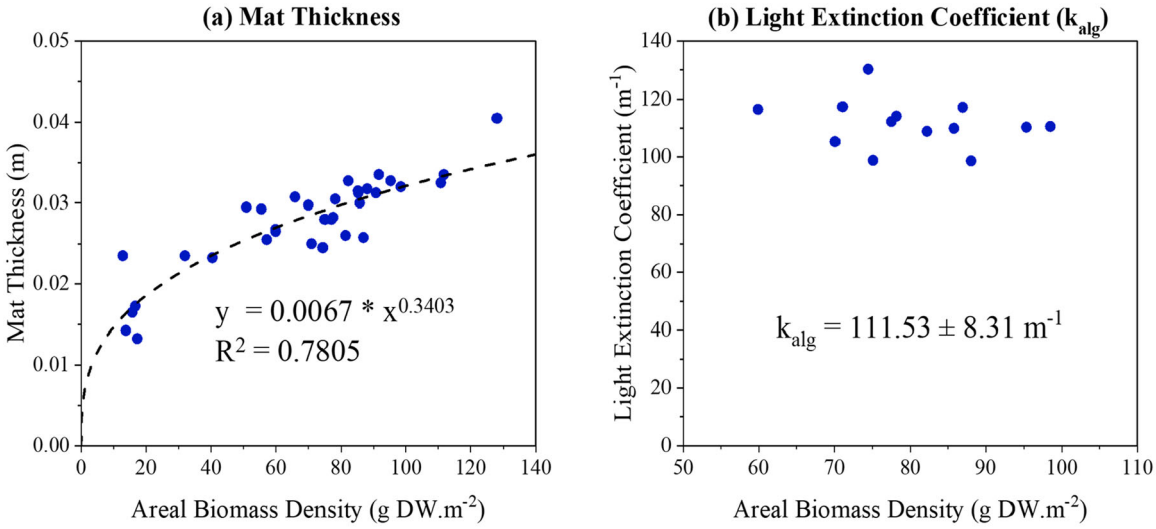

FIGURE 3 | Experimentally determined variation in (a) algal mat thickness and (b) light extinction coefficient (k_{alg}) of filamentous alga *Oedogonium* as a function of areal biomass density (X).

$$D_{mat\,(X)} = 0.0067 \bullet X^{0.3403} \tag{3}$$

where, D_{mat} is the FA mat depth (m) and X is the areal biomass density (g DW. m^{-2}). This equation has the same form to the equation presented in Kuczynski et al. (2022). The values are different, which could be explained by fitting the curves to data sets obtained on different FA (*Oedogonium* and *Cladophora*). At the start of each timestep, the model uses Eq. 3 to calculate the depth of the FA mat based on the areal biomass density at the start of that timestep. Then, using a user-defined layer thickness that governs the resolution of the model, the number of layers of the FA mat is calculated using Eq. 4.

$$n = \frac{D_{mat\ \ (X)}}{D_{layer}} \tag{4}$$

where, $D_{mat\ \ (X)}$ is the depth of the FA mat calculated using Eq. 3, D_{layer} is the user-defined layer depth (or thickness) (m), and n is the number of layers that the algal mat is discretized into (rounded up to a whole number with any additional biomass added to the bottom-most (FAM) or uppermost (FANS) layer the thickness of which will be less than D_{layer}).

2.3.4 | Light Attenuation Within the Algal Mat

Once the algal mat has been discretized, the next set of equations determines the attenuated light reaching the mid-point of each of the layers. This procedure has been used in both microalgal models and GLCM v3 to account for the self-shading created by the algal cells (Huesemann et al. 2013; Kuczynski et al. 2020).

For FAM, as the FA mat is on the surface of the pond in this model, attenuation of the light is predominantly due to absorption and scattering by the algal biomass. In microalgal growth models, light attenuation by self-shading has been parameterized using a light extinction coefficient (k_{alg}) that is proportional to a specific extinction coefficient and the biomass concentration of the culture (Béchet, Shilton, and Guieysse 2013; Higgins, Hecky, and Guildford 2006; Huesemann et al. 2013; Kuczynski et al. 2020). Interestingly, the light extinction coefficients of the *Oedogonium* mats were found to remain relatively consistent at $111.5 \pm 8.3\ m^{-1}$ (Figure 3b), with no significant variation within the range of areal biomass densities tested ($p > 0.05$). This is difficult to explain but is in correspondence with other observations of a constant light extinction coefficient through attached FA mats in previous studies (Flynn 2014; Malkin 2008).

It has been found that 10% of the incident light on a water surface is lost from reflection (Higgins, Hecky, and Guildford 2005), which is not included in the Beer-Lambert's law (Eq. 6.1). Therefore, Eq. 1 can be modified by incorporating a 10% reduction of incident light due to reflection at the pond surface as follows (Eq. 5).

$$I_z = 0.9 \ \bullet \ I_o \bullet e^{-k_{alg} \bullet z} \tag{5}$$

where, I_o is the PAR at the surface (in $\mu mol.m^{-2}s^{-1}$), I_z is the PAR at a position (z) within the algal mat (in $\mu mol.m^{-2}s^{-1}$), k_{alg} is the light extinction coefficient through the algal mat (in m^{-1}) and z is the depth from the surface to the considered point in the algal mat (m). This equation is used in the models to determine the light intensity at the midpoint of each of the layers of the discretized algal mat.

For the FANS model, it is also necessary to account for light attenuation due to the water column above the mat. As such, the equation used to determine the light incident at the midpoint of each layer (Eq. 5) is replaced by Eq. 6 to account for the shading caused by the water column above the biomass.

$$I_z = 0.9 \ \bullet \ I_o \bullet e^{-k_{alg} \bullet z} \ \bullet \ e^{-k_{water} \bullet h} \tag{6}$$

where, k_{water} is the light extinction coefficient through the water column (m^{-1}) and h is the height of the water column above the attached growth turf (m). It is important to note that in the proposed model the use of Beer Lambert's Law only accounts

for the variation in light distribution with depth of the culture. It does not take into account other complexities such as partial cloud cover that can make the light distribution uneven in large-scale outdoor systems.

2.3.5 | Determination of Areal Biomass Density

The final set of equations is used to calculate the change in areal biomass density with time. Similar to the GLCM v3 model, the rate of change of areal biomass density is determined at each timestep using an exponential growth model based on gross photosynthetic rate (P_{gross}) and losses due to respiration (R_{tot}) (Eq. 7).

$$\frac{dX}{dt} = (\mu_{Pgross} - \mu_{Rtot}) \bullet X \tag{7}$$

where X is the areal biomass density (g DW. m^{-2}), μ_{Pgross} is the specific gross photosynthetic rate (d^{-1}) and μ_{Rtot} is the specific total respiration rate (d^{-1}). The net photosynthetic rate (P_{net}) is represented by the difference between P_{gross} and R_{tot}. Therefore, Eq. 7 can be rearranged to Eq. 8.

$$\frac{dX}{dt} = \mu_{Pnet} \bullet X \tag{8}$$

Photosynthesis-irradiance (PI) response curves are used to study the cellular responses of both microalgae (MacIntyre et al. 2002; Schwaderer et al. 2011) and FA (Kuczynski et al. 2020; Necchi Jr 2004; Vieira and Necchi 2003) in response to variations in light intensities at a given temperature, providing insights into the biomass productivity under fluctuating light conditions (Duarte et al. 2013; Kuczynski et al. 2020). The equation developed by Platt, Gallegos, and Harrison (1981) and used by Kuczynski et al. (2020) to represent the three distinct regions of PI curves (light-limited, light-saturated, and photoinhibited region) as a continuous function, was modified here by including a dark respiration term so as to represent the variation in the specific net photosynthetic rate with the irradiance level (Eq. 9):

$$\mu_{P,net\,(I,T)} = \mu_{Pnet,s\ \ (T)}\left(1 - e^{-\frac{\alpha_{\mu,Pnet\,(T)} \bullet\ I}{\mu_{Pnet,s\ \ (T)}}}\right) \bullet e^{-\frac{\beta_{\mu,Pnet\ \ (T)}\ \bullet\ I}{\mu_{Pnet,s\ \ (T)}}} - \mu_{Rd\ \ (T)} \tag{9}$$

where $\mu_{P,net}$ is the specific net photosynthetic rate (day^{-1}), $\mu_{Pnet,s}$ is the maximum specific net photosynthetic rate when there is no photoinhibition (day^{-1}), $\alpha_{\mu,Pnet}$ is the initial slope in the linear range ($day^{-1}\mu mol^{-1}m^2s$), $\beta_{\mu,Pnet}$ is the component representing photoinhibition (when photoinhibition is not present this component is equal to zero) ($day^{-1}\mu mol^{-1}m^2s$), I is the light intensity ($\mu mol.m^{-2}.s^{-1}$) and μ_{Rd} is the light independent specific dark respiration rate (day^{-1}). The form of Eq. 9 is graphically illustrated in Figure 4.

In previous experiments by Pitawala et al. (2023), the P_{net} and dark respiration (R_d) of FA *Oedogonium* acclimated to average winter conditions in Melbourne, Australia was measured under short-term exposure to eight different light and five different temperature levels (between 10 and 1100 μmol. m^{-2}. s^{-1} and 15°–35°C, respectively). To incorporate the influence of both light intensity and temperature, this cellular response data was fitted to Eq. 9 to obtain the PI curve coefficient values at each temperature (Table 1). Subsequently, the temperature-specific PI curve coefficient values were used to obtain a function to describe the temperature dependence of each coefficient (Figure 5). All the data fitting activities were performed using the curve fitter app in MATLAB 2023b.

Finally, the variation in the specific net photosynthetic rate under varying light (I) and temperature (T) levels can be represented by Eq. 10–13, with the outputs from the equations visualized in Figure 6.

$$\mu_{Rd\ \ (T)} = 0.0565 \bullet 1.05^{(T-20)} \tag{10}$$

$$\alpha_{\mu,Pnet\ \ (T)} = 0.06 \ \bullet\ e^{\left(-\left(\frac{\frac{T-25}{7.906} - (-1.51)}{2.3666}\right)^2\right)} \tag{11}$$

$$\mu_{Pnet,s\ \ (T)} = 0.2255 \ \bullet\ e^{(0.0625\ \bullet\ T)} \tag{12}$$

$$\beta_{\mu,Pnet\ \ (T)} = 7.18 \ \bullet\ 10^{-10} \ \bullet\ e^{(0.4387\ \bullet T)} \tag{13}$$

The models incorporate these equations to represent the cellular responses to irradiance and temperature. The increase in the areal biomass density over a time interval of Δt is then computed in the models using Eq. 14:

$$X(t + \Delta t) = X(t) \ \bullet\ e^{(\mu_{Pnet\ \ (I,T)}\ \bullet\ \Delta t)} \tag{14}$$

2.3.6 | Incorporation of Time-Dependent Inhibition of Photosynthetic Activity

As previously mentioned, exposure to high light intensity levels for a prolonged period can increase photoinhibition damage to algal cells in static FA cultures (mats or turfs). In a recent experimental study, we found that time-dependent inhibition in *Oedogonium* exposed to constant light intensity was significant even at light-limiting conditions, and could be well represented by first-order kinetics (Pitawala et al. 2024). As the available data did not cover the complete range of temperature and irradiance levels of interest (18, 79, 337, and 1093 μmol. m^{-2}. s^{-1} and 15°C, 25°C and 35°C), it was extrapolated to provide the parameters needed for the model (Table S1). Using these values, the time-dependent inhibition of photosynthetic activity could be incorporated by modifying Eq. 8 which determines the rate of change of biomass density (Eq. 15).

$$\frac{dX}{dt} = \mu_{Pnet\ \ (I,T)} \bullet e^{-k_{inhibition\ \ (I,T)} \bullet\ t} \bullet X \tag{15}$$

where, $k_{inhibition}$ is the rate constant of the exponential decay due to time-dependent inhibition relevant to the exposed irradiance (I) and temperature (T) level (min^{-1}) and t is the exposure period to the irradiance level associated with the time-dependent inhibition (min). Based on the extrapolated data (Fig. S3– S4), the

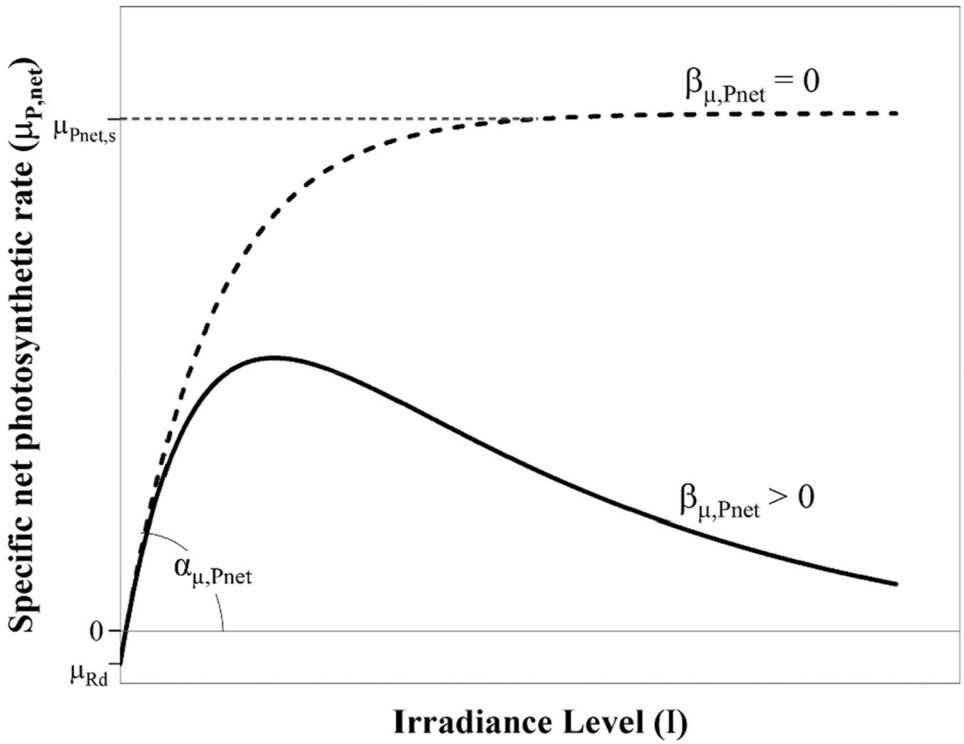

FIGURE 4 | Graphical representation of the variation in the specific net photosynthetic rate ($\mu_{p,\ net}$) with irradiance level (I) described by Eq. 9 under conditions without photoinhibition ($\beta_{\mu,\ Pnet} = 0$) and with photoinhibition ($\beta_{\mu,\ Pnet} > 0$).

TABLE 1 | The PI curve coefficient values were obtained for the winter acclimated dataset for each temperature at eight different light levels (18, 79, 178, 337, 491, 722, 871, and 1093 μmol. m^{-2}. s^{-1}). The individual PI curve fits at each temperature are presented in the supporting information (Figures S6– S10).

	PI curve coefficient values				
Temperature (°C)	$\mu_{Pnet,s}$ (day^{-1})	$\alpha_{\mu,\ Pnet}$ ($day^{-1}\mu mol^{-1}m^2s$)	$\beta_{\mu,\ Pnet}$ ($day^{-1}\mu mol^{-1}m^2s$)	μ_{Rd} (day^{-1})	R^2
15	0.6389	0.0592	0	0.0433	0.9593
20	0.7970	0.0531	0	0.0565	0.9677
25	1.1423	0.0389	0	0.0738	0.9488
30	1.2301	0.0274	0.0001	0.0964	0.8918
35	2.1160	0.0150	0.0034	0.1260	0.8624

inhibition rate constant ($k_{inhibition}$) could be described as a function of light and temperature exposure levels by Eq. 16.

$$k_{inhibition\ \ (I,T)} = ((1 \times 10^{-4}) \bullet \ln(T) - 0.0002) \bullet I \qquad (16)$$

3 | Results and Discussion

3.1 | Model Validation

The framework of the FANS and FAM models is built upon a mechanistic understanding of FA growth, with the models parameterized using data from controlled laboratory experiments. While this approach should yield a robust model, it is important to evaluate the predictiveness of the models against actual experimental data. Most previous studies done using freshwater FA *Oedogonium* for wastewater treatment (Cole et al. 2016; Lawton et al. 2021; Neveux et al. 2016) have used tumble cultures, where the algal biomass is mixed throughout the culture volume. As such, data from these studies cannot be used for validating these models in which the biomass is maintained as static mats. Fortunately, more applicable data is available from a recent study by Hariz, Lawton, and Craggs (2023a) investigating the influence of operational parameters on filamentous algae nutrient scrubbers (FANS). In the study, a monoculture of FA *Oedogonium* was grown attached to a textured liner fixed to the bottom of a nutrient scrubber under controlled environmental conditions (Table 2). Importantly, the flow rate within the system was maintained low enough to avoid detachment of the algal filaments from the liner. This means that the *Oedogonium* biomass in this study was maintained as a stable attached growth turf, as represented by the FANS model.

As a constant light intensity was maintained during the light period in this study (Hariz, Lawton, and Craggs 2023a), the

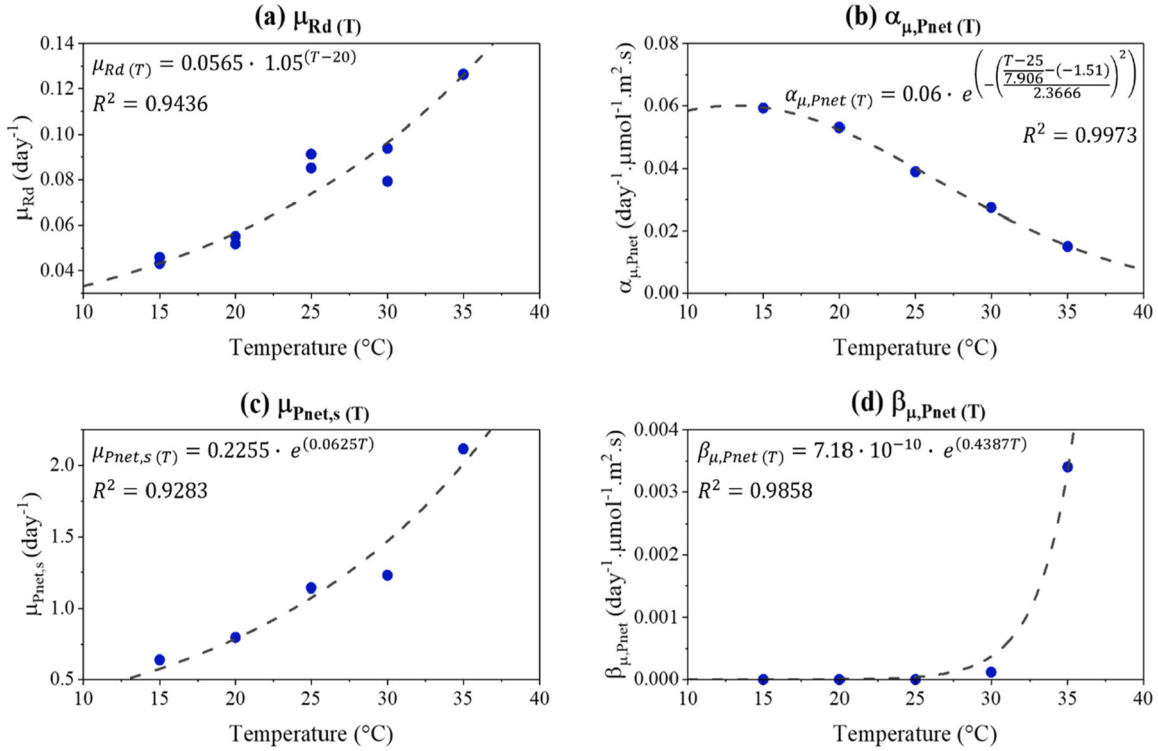

FIGURE 5 | PI curve coefficients as a function of temperature, with the resulting equations presented in the figures. The individual PI curve fits at each temperature level are presented in the supporting information (Figures S6– S10).

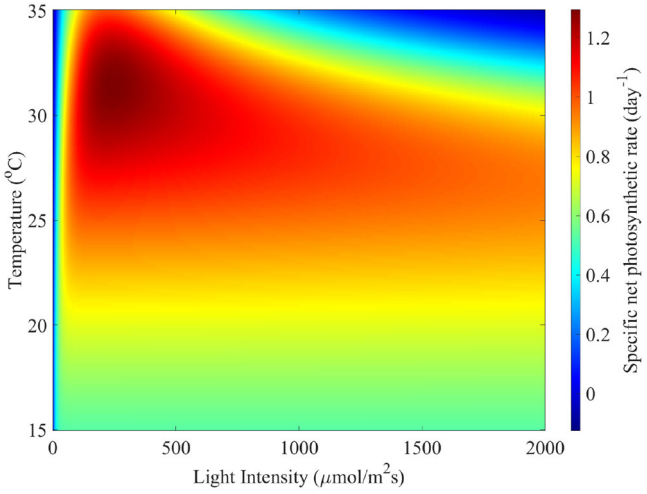

FIGURE 6 | Specific net photosynthetic rate of winter acclimated FA *Oedogonium* as a function of light intensity and temperature obtained using Eqs. 10–14.

functions developed to represent time-dependent inhibition present in static cultures using extrapolated data can be incorporated into the FANS model. This provides the opportunity to understand and validate the influence of time-dependent inhibition on system performance in static FA cultures. Based on the available laboratory data for *Oedogonium* (Pitawala et al. 2024), no inhibition was observed at the lowest light level (18 μmol.m^{-2}.s^{-1}) while at the other three tested light levels (79, 337, and 1093 μmol.m^{-2}.s^{-1}), the inhibition rate constant was found to linearly increase with the exposed light level. This suggests the presence of a threshold light exposure level, only above which time-dependent inhibition occurs. To gain preliminary insights into the influence of this phenomenon on the performance of outdoor systems with static biomass, a threshold light exposure level of 50 μmol.m^{-2}.s^{-1} was defined. This value was chosen as it represents an approximate midpoint between the two light levels where time-dependent inhibition was not present (18 μmol.m^{-2}.s^{-1}) and where it was present (79 μmol.m^{-2}.s^{-1}). Future work expanding this dataset would allow for this phenomenon to be incorporated into the FAM model to more fully account for the influence of diurnal fluctuations in outdoor culture systems, thereby increasing the accuracy and utility of the model.

Based on this, two versions of both the FAM and FANS models were developed for validation: one with and the other without incorporating time-dependent inhibition. Table 2 lists the input conditions for the FANS model compared to those maintained in the study by Hariz, Lawton, and Craggs (2023a). For validation, the biomass productivity values predicted by the models were compared with the value of 9.3 ± 1.7 g DW. m^{-2}. day^{-1} reported by Hariz, Lawton, and Craggs (2023a).

The results from the model simulations are reported in Figure 7. The versions of FAM and FANS models without the time-dependent inhibition predicted biomass productivity values which were over 6-fold higher than the reported experimental values. In comparison, the version that included time-dependent inhibition predicted biomass productivities of 10.59 and 11.05 g DW. m^{-2}. d^{-1}, respectively, aligning closely with the experimental value of 9.3 ± 1.7 g DW. m^{-2}. d^{-1} (Hariz, Lawton, and Craggs 2023a). These findings emphasize the importance of considering time-dependent inhibition in static cultures, as neglecting this phenomenon in predictive modeling

TABLE 2 | Operational parameters reported in Hariz, Lawton, and Craggs (2023a) and the corresponding values used for the model simulations.

Parameter	Values reported in Hariz, Lawton and Craggs (2023a)	Values used for the model simulations
Light intensity ($\mu mol.m^{-2}.s^{-1}$)	600–650	625
Light: dark cycle	14:10	14:10
Temperature (°C)	22	22
Initial biomass density (g DW. m^{-2})	70–80	75
Harvesting frequency (days)	4	4
Depth of the nutrient scrubber (cm)	5	5

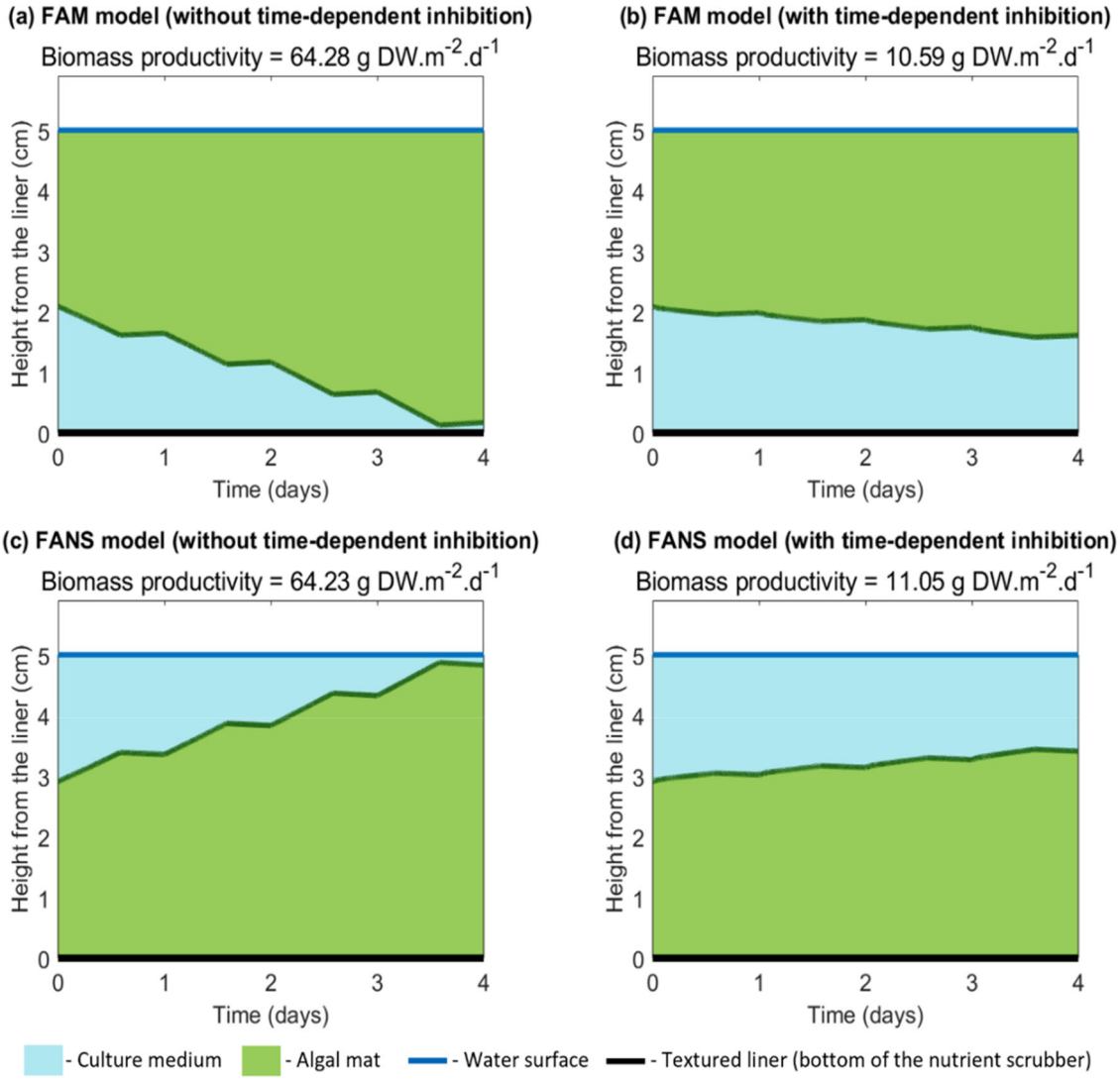

FIGURE 7 | Biomass productivity and mat thickness predictions for a 4-day culture of *Oedogonium* using the (a) FAM model version without time-dependent inhibition, (b) FAM model version with time-dependent inhibition, (c) FANS model version without time-dependent inhibition and (d) FANS model version with time-dependent inhibition. The culture conditions used for the model simulations are defined in Table 2. The user-defined initial areal biomass density was set at 75 g $DW.m^{-2}$, which is within the optimum initial standing crop reported for unialgal nutrient scrubbers with FA *Oedogonium* (Hariz, Lawton, and Craggs 2023a).

can lead to dramatic overestimation of system performance. The relatively small differences in the values predicted by the FANS and FAM models suggest that both stable floating mats and attached growth turfs can be expected to perform similarly under given temperature and light conditions.

The effect of time-dependent inhibition on different layers within the algal mat was further investigated using the FAM and FANS model versions with time-dependent inhibition incorporated (Figure 8). The rate of exponential decay in biomass productivity due to photoinhibition was highest in layer 1

and layer 28 of the FAM and FANS model, respectively, which were the layers closest to the water surface and therefore receiving the highest light levels. Further, the rate of exponential decay was found to be progressively lesser in layers deeper into the mat/turf that receives less light, with the bottom-most layers showing no time-dependent inhibition (Figure 8). The reduction in the rate of change in areal biomass density observed at bottom-most layer in the FANS model is due to the reduction in the incident light with time due to the addition of a new layer at the top as the overall areal biomass density of the system increases, rather than time-dependent inhibition.

3.2 | Model Simulations

Following validation, the developed FANS model with and without time-dependent inhibition was used to run predictions of outdoor cultures of *Oedogonium* by incorporating diurnal variations in light and temperature levels over periods where the average growth conditions were similar to the biomass acclimation conditions (winter in Melbourne, Australia). These types of simulations can offer insights into how fluctuations in light intensity and temperature affect overall system performance.

Initial simulations were conducted using the FANS model based on the 3-month winter season (June to August) in Melbourne, Australia which has average temperature and light intensity profiles that align with the acclimation conditions applied in the acquisition of data used for the model calibration. The average diurnal light intensity profile was obtained from a 20-year dataset of 1-min solar data collected at the Melbourne Airport by the Bureau of Meteorology, Australia. A constant temperature of 15°C was selected to represent an expected average water temperature over these months. For these simulations, the initial areal biomass density and the culture period was set to 75 g DW. m^{-2} and 4 days, respectively which is similar to the parameters used in previous studies of outdoor FANS systems using FA *Oedogonium* (Hariz, Lawton, and Craggs Lawton, and Craggs 2023a, 2023b).

Incorporating time-dependent inhibition into the FANS model required consideration of how to mathematically represent two aspects: (1) accumulation of photoinhibitory effects under inconstant light intensity, and (2) recovery from photoinhibition upon reduced light exposure. Parameterization of time-dependent inhibition requires experiments to be performed under constant light so that decay constants can be determined as a function of a given light intensity. As above, these values were then used to establish an equation describing $k_{inhibition}$ as a function of light intensity and temperature (Eqn 16). The incorporation of this equation into the FANS model enabled the cumulative effect of time-dependent inhibition during inconstant light to be directly computed as an integral of the light intensity-time profiles from the meteorological data. This methodology represents a bridge between experimentally obtainable parameters describing time-dependent inhibition and the practically relevant manifestation of this phenomenon – diurnal inhibition. Such an approach is not limited to static filamentous algae cultures but could be incorporated into models describing microalgal or plant growth.

With respect to recovery from photodamage sustained during inhibitive light, it was assumed that complete recovery occurred during the night, rather than assuming instantaneous recovery following reduction to non-inhibitory light levels. The chosen assumption was based on observations from our experiments with FA *Oedogonium*, in which recovery was not instantaneous when irradiance levels were reduced from above to below an inhibitory level (Fig. S5). The assumption also corresponds with observations made for phytoplankton assemblages, which showed full recovery from exposure to inhibitory irradiance levels within 4 to 20 h when transitioned to lower light conditions (Belay 1981).

The FANS model without time-dependent inhibition predicted a biomass productivity of 16.53 g DW. m^{-2}. d^{-1} which was 2.8 times greater than the prediction from the model that incorporates time-dependent inhibition (Figure 9). Based on the findings from the model validation, the predicted productivity derived from the FANS model without time-dependent inhibition is likely to be significantly overestimated. The origins of the overestimation in biomass productivity resulting from neglecting time-dependent inhibition are illustrated in Figure 9. In the FANS model without time-dependent inhibition, the layers closest to the water surface, which experience prolonged exposure to high light levels, show no reduction in the rate of change in areal biomass density. In contrast, the model incorporating time-dependent inhibition demonstrates a progressive decay in biomass productivity in the upper layers, consistent with experimental observations (Pitawala et al. 2024). The slight increase in biomass productivity predicted later in the day can be attributed to the termination of photoinhibition once the light levels drop. However, the productivities are still less than those predicted without time-dependent inhibition, showing the effects of the cumulative photodamage that is not repaired until the next day. These simulations emphasize the significant impact of time-dependent inhibition on productivity predictions in static FA cultures, highlighting the necessity of incorporating this factor when developing predictive models of static FA cultures.

Further, the FANS model can provide detailed insights into variations in light, temperature, and time-dependent inhibition affecting biomass productivity at different locations within the algal mat. To explore this, the model was employed to generate spatial-temporal variations in light intensity and the rate of change in areal biomass density (dX/dt) over a 24-h period (Fig. 10). The resulting graphs illustrate a reduction in light intensity within the depth of the algal mat during the light period, due to self-shading. This shading effect reduces the severity of time-dependent inhibition and the associated decline in biomass productivity, resulting in higher productivity levels in the lower layers of the algal mat compared to those at the top (Figure 10). These simulations emphasize the significant impact of self-shading on biomass productivity, suggesting that deeper layers may benefit from reduced light stress, thereby enhancing overall productivity. Understanding these dynamics will be essential for optimizing the performance and operation of FA cultures, for example in relation to determining an appropriate harvesting frequency and mat depth. The developed model can also serve as a predictive tool for optimization and for exploring the functional mechanisms of such systems.

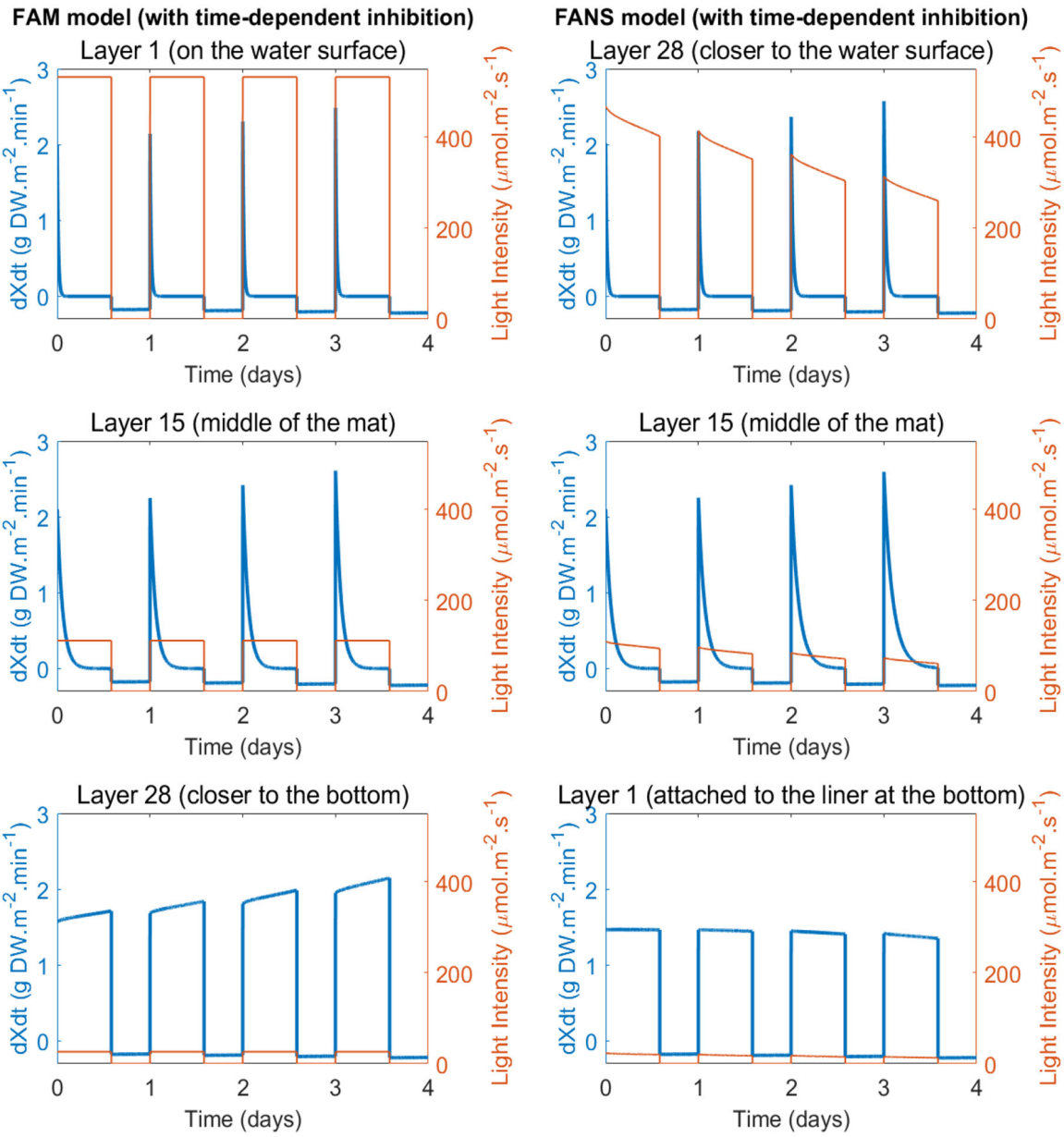

FIGURE 8 | Rate of change in areal biomass density (dX/dt) and the incident light intensity at the midpoint of different layers within the FA mat as predicted by the FAM model (left panels) and FANS model (right panels).

3.2.1 | Further Opportunities for Model Development and Improvement

Overall, the developed models provide a new foundation for the predictive modeling of filamentous algal cultures and can be applied to either floating mats on the surface of ponds or attached growth turfs, in both engineered and natural systems. However, the models still have some predictive limitations due to the limited availability of data needed to assign parameters spanning the full range of environmental conditions that could be encountered outdoors. These limitations can be addressed through future studies to fully exploit the potential of this tool.

Most algal growth models have only been applied using varied model input parameters specific to the algal species being considered. However, as previously discussed, the key findings from previous studies highlight the importance of considering variations in growth parameters due to seasonal changes when predicting the year-round performance of outdoor algal cultures (Pitawala et al. 2024; Pitawala et al. 2023). Accounting for long-term seasonal fluctuations that lead to changes in both the biochemical and cellular responses of algae is important for future applications of growth models.

The current model was calibrated using species-specific data obtained for a single strain of *Oedogonium* that was acclimated under the average winter conditions in Melbourne, Australia. As a result, the model cannot be expected to accurately predict the year-round performance of an outdoor culture system in Melbourne, Australia, or other temperate regions subjected to seasonal variations that affect the biochemical composition and cellular responses. Broadening the applicability of this model to different climates requires data on the cellular responses and species-specific mat properties to be obtained on biomass

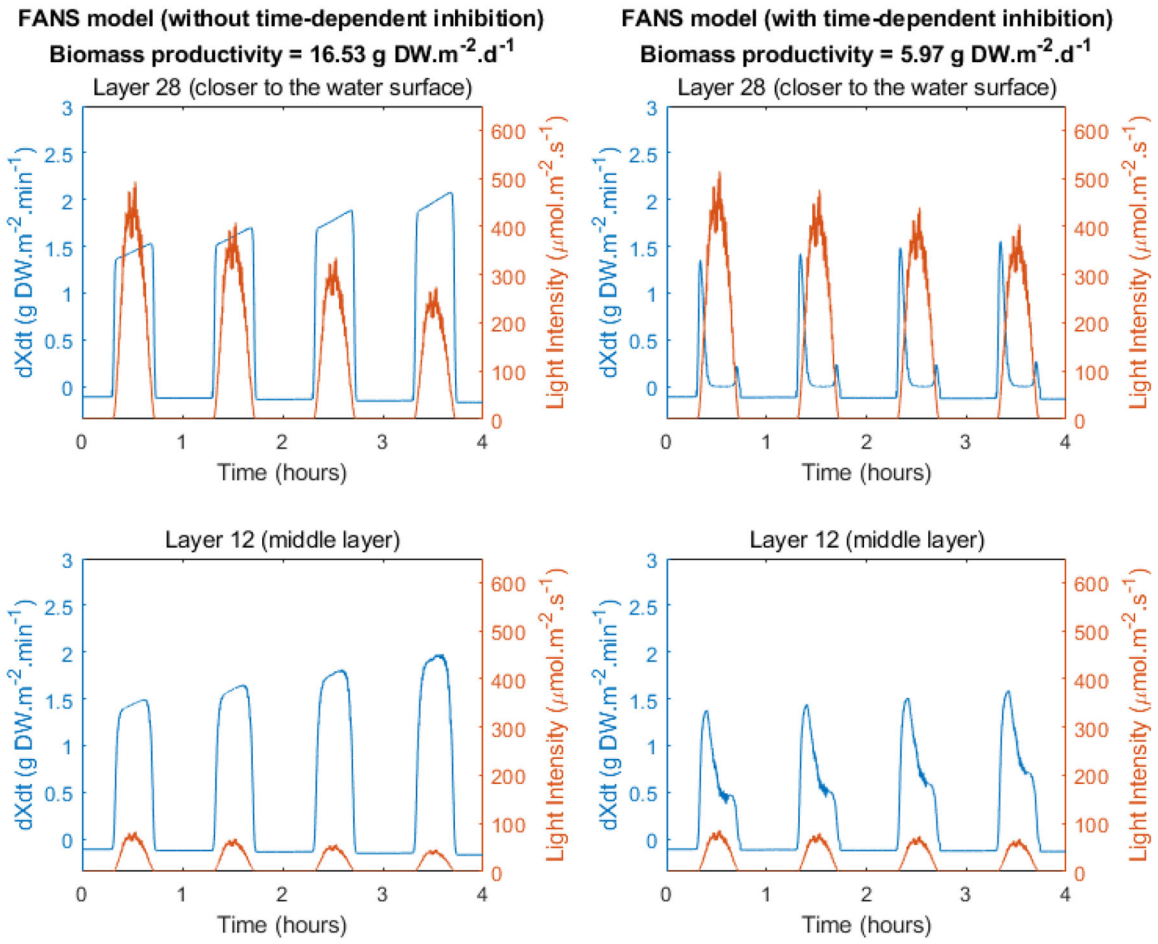

FIGURE 9 | Rate of change in areal biomass density (dX/dt) and the incident light intensity at the midpoint of different layers within the FA mat as predicted by the FANS model with (right panels) and without (left panels) time-dependent inhibition. The average diurnal light intensity profile in winter in Melbourne, Australia, and a temperature of 15°C was used to run the simulations. The user-defined initial areal biomass density was set at 75 g DW. m^{-2}.

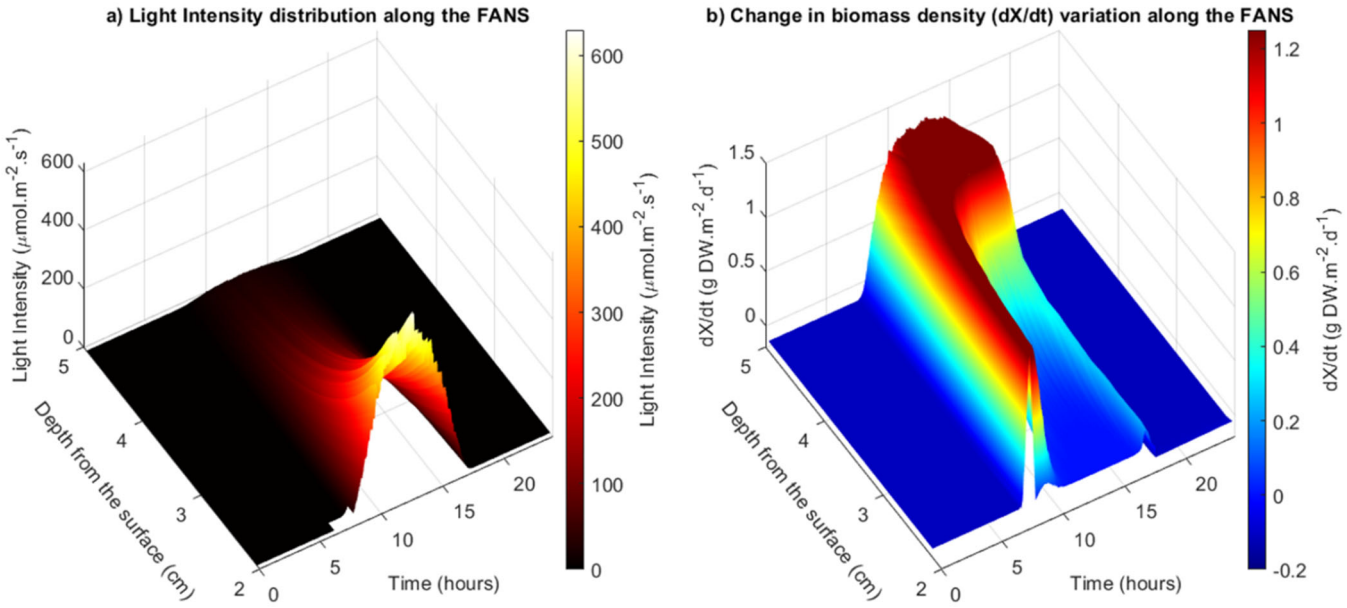

FIGURE 10 | (a) Light intensity distribution and (b) the rate of change of areal biomass density (dX/dt) at different locations within the algal mat in the filamentous algal nutrient scrubber (FANS) over 24 h when exposed to the average diurnal light intensity profile in winter in Melbourne, Australia at 15°C. The user-defined initial areal biomass density was set at 75 g DW. m^{-2}.

acclimated to different seasonal conditions. Incorporated parameters based on this data into the model would improve the ability to predict the year-round performance of an outdoor culture system and optimize operational parameters like the harvesting frequency for different periods of the year. Further, future studies investigating the effect of multiple recurring exposures to high photooxidative stresses due to diurnal fluctuations and how algae respond to such variations over time through cellular adaptations would enable further enhancements to the model.

Importantly, this model can provide new insights into algae-based systems where the biomass remains static (e.g. floating mats or attached growth biofilms/turfs). These results illustrate the importance of incorporating time-dependent inhibition into future models of algal growth, particularly static FA cultures in which the biomass is exposed to high light levels for prolonged periods. Currently, the only available data on time-dependent inhibition for any type of FA are those from the recent experimental work conducted in our laboratory (Pitawala et al. 2024). This dataset needs to be expanded to other FA species to obtain species-specific parameters.

Based on observations of time-dependent inhibition in static cultures of FA, it can be inferred that algae in fixed or attached systems may experience higher stress, potentially resulting in lower productivity levels when compared to mixed tumble cultures. However, the shading from the upper layers of the mat could offer sufficient protection against photooxidative stress, potentially enhancing productivity in the lower regions as shown in the model simulations. This shading effect might lead to higher overall productivity compared to tumble cultures. Therefore, fully developed fixed model predictions can be used to compare against a well-mixed model representing tumble FA cultures to gain valuable insights into selecting the optimal FA cultivation method for achieving the highest productivity levels.

In addition to light intensity and temperature, the model can be further built upon by incorporating nutrient and carbon limitations to expand the predictive capabilities. It is important to note that the model currently assumes that the biomass productivity is not limited by nutrient or CO_2 availability. However, in outdoor algae-based wastewater treatment systems, these can be limiting and can significantly impact the actual productivity of the system. Furthermore, the presence of ammonia can create toxic conditions for algae, negatively impacting the cellular responses and affecting biomass productivity (Dai, Qiu, and Forchhammer 2014; Liu et al. 2023; Markou, Vandamme, and Muylaert 2014; Wang et al. 2019). The possible impact of grazers on biomass decomposition (Salovius and Bonsdorff 2004) could also be considered, particularly in the context of natural systems.

4 | Conclusions

The models developed in this study provide a foundation for the predictive modeling of both mat-forming and attached FA growth systems and can be applied to engineered or natural systems. Furthermore, by incorporating time-dependent inhibition for the first time into an algal growth model, the validation studies highlighted the important influence of this phenomenon on static cultures of FA. This approach provides a bridge between experimentally obtainable rate constants describing time-dependent inhibition and the diurnal fluctuations experienced outdoors, which can be applied to any model of algal or plant growth.

Future studies to characterize year-round seasonal acclimation effects and time-dependent inhibition under diurnal fluctuations will provide data needed to expand the applicability and increase the accuracy of the models. Additionally, the model can be extended by incorporating the influence of nutrients and carbon dioxide present in outdoor culture systems, on biomass productivity. A fully developed model has the potential to be a powerful tool for understanding the influence of key environmental parameters on the growth of FA. This can be used to help optimize operational parameters to maximize FA biomass productivity in outdoor FA-based cultures in engineered systems and to better understand and predict the behavior of FA in natural systems.

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Sulochana Pitawala performed the experiments, wrote and developed the model code, and drafted the manuscript. Peter Scales and Gregory Martin provided supervision and technical guidance. All authors were involved in the conceptual development of the work, manuscript revision, and final approval.

Author Statement

The authors declare no commercial or proprietary interest in any product or concept discussed in this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available upon request. The source code for the model has been provided in the Supplementary Information.

References

Auer, M. T., and R. P. Canale. 1982. "Ecological Studies and Mathematical Modeling of Cladophora in Lake Huron: 3. The Dependence of Growth Rates on Internal Phosphorus Pool Size." *Journal of Great Lakes Research* 8, no. 1: 93–99.

Béchet, Q., A. Shilton, and B. Guieysse. 2013. "Modeling the Effects of Light and Temperature on Algae Growth: State of the Art and Critical Assessment for Productivity Prediction during Outdoor Cultivation." *Biotechnology Advances* 31, no. 8: 1648–1663.

Belay, A. 1981. "An Experimental Investigation of Inhibition of Phytoplankton Photosynthesis at Lake Surfaces." *New Phytologist* 89, no. 1: 61–74.

Cole, A. J., N. Neveux, A. Whelan, et al. 2016. "Adding Value to the Treatment of Municipal Wastewater Through the Intensive Production of Freshwater Macroalgae." *Algal Research* 20: 100–109.

Cole, A. J., R. de Nys, and N. A. Paul. 2014. "Removing Constraints on the Biomass Production of Freshwater Macroalgae by Manipulating Water Exchange to Manage Nutrient Flux." *PLoS One* 9, no. 7: e101284.

Dai, G.-Z., B.-S. Qiu, and K. Forchhammer. 2014. "Ammonium Tolerance in the Cyanobacterium S Ynechocystis Sp. Strain PCC 6803 and the Role of the psbA Multigene Family." *Plant, Cell & Environment* 37, no. 4: 840–851.

Darvehei, P., P. A. Bahri, and N. R. Moheimani. 2018. "Model Development for the Growth of Microalgae: A Review." *Renewable and Sustainable Energy Reviews* 97: 233–258. https://doi.org/10.1016/j.rser.2018.08.027.

Duarte, P., M. Ramos, G. Calado, and B. Jesus. 2013. "Laminaria Hyperborea Photosynthesis Irradiance Relationship Measured by Oxygen Production and Pulse-Amplitude-Modulated Chlorophyll Fluorometry." *Aquatic Biology* 19, no. 1: 29–44.

Falkowski, P. G., and T. G. Owens. 1978. "Effects of Light Intensity on Photosynthesis and Dark Respiration in Six Species of Marine Phytoplankton." *Marine Biology* 45, no. 4: 289–295.

Flynn, K. F. 2014. *Methods and Mathematical Approaches for Modeling Cladophora Glomerata and River Periphyton*. Tufts University.

Gao, S., P. Waller, G. Khawam, S. Attalah, M. Huesemann, and K. Ogden. 2018. "Incorporation of Salinity, Nitrogen, and Shading Stress Factors into the Huesemann Algae Biomass Growth Model." *Algal Research* 35: 462–470.

Graham, J. M., P. Arancibia-Avila, and L. E. Graham. 1996. "Physiological Ecology of a Species of the Filamentous Green Alga Mougeotia Under Acidic Conditions: Light and Temperature Effects on Photosynthesis and Respiration." *Limnology and Oceanography* 41, no. 2: 253–262.

Graham, J. M., M. T. Auer, R. P. Canale, and J. P. Hoffmann. 1982. "Ecological Studies and Mathematical Modeling of Cladophora in Lake Huron: 4. Photosynthesis and Respiration as Functions of Light and Temperature." *Journal of Great Lakes Research* 8, no. 1: 100–111.

Graham, J. M., C. A. Lembi, H. L. Adrian, and D. F. Spencer. 1995. "Physiological Responses to Temperature and Irradiance Inspirogyra (Zygnematales, Charophyceae)." *Journal of Phycology* 31, no. 4: 531–540.

Hariz, H. B., R. J. Lawton, and R. J. Craggs. 2023a. "Effects of Operational Parameters on the Performance of Unialgal Oedogonium Sp. Filamentous Algae Nutrient Scrubbers Under Controlled Environmental Conditions." *Journal of Environmental Management* 326: 116705.

Hariz, H. B., R. J. Lawton, and R. J. Craggs. 2023b. "Nutrient Uptake and Biomass Productivity Performance Comparison Among Freshwater Filamentous Algae Species on Mesocosm-Scale FANS Under Ambient Summer and Winter Conditions." *Ecological Engineering* 189: 106910.

Higgins, S. N., R. E. Hecky, and S. J. Guildford. 2005. "Modeling the Growth, Biomass, and Tissue Phosphorus Concentration of Cladophora Glomerata in Eastern Lake Erie: Model Description and Field Testing." *Journal of Great Lakes Research* 31, no. 4: 439–455.

Higgins, S. N., R. E. Hecky, and S. J. Guildford. 2006. "Environmental Controls of Cladophora Growth Dynamics in Eastern Lake Erie: Application of the Cladophora Growth Model (CGM)." *Journal of Great Lakes Research* 32, no. 3: 629–644.

Huesemann, M., B. Crowe, P. Waller, et al. 2016. "A Validated Model to Predict Microalgae Growth in Outdoor Pond Cultures Subjected to Fluctuating Light Intensities and Water Temperatures." *Algal Research* 13: 195–206.

Huesemann, M. H., J. Van Wagenen, T. Miller, A. Chavis, S. Hobbs, and B. Crowe. 2013. "A Screening Model to Predict Microalgae Biomass Growth in Photobioreactors and Raceway Ponds." *Biotechnology and Bioengineering* 110, no. 6: 1583–1594.

Kuczynski, A., M. T. Auer, W. D. Taylor, S. C. Chapra, and M. Dijkstra. 2022. "Keeping up With the Math: Advancing the Ecological Foundation of the Great Lakes Cladophora Model." *Ecological Modelling* 473: 110118.

Kuczynski, A., A. Bakshi, M. T. Auer, and S. C. Chapra. 2020. "The Canopy Effect in Filamentous Algae: Improved Modeling of Cladophora Growth Via a Mechanistic Representation of Self-Shading." *Ecological Modelling* 418: 108906.

Lawton, R. J., A. J. Cole, D. A. Roberts, N. A. Paul, and R. de Nys. 2017. "The Industrial Ecology of Freshwater Macroalgae for Biomass Applications." *Algal Research* 24: 486–491.

Lawton, R. J., C. R. K. Glasson, P. M. Novis, J. E. Sutherland, and M. E. Magnusson. 2021. "Productivity and Municipal Wastewater Nutrient Bioremediation Performance of New Filamentous Green Macroalgal Cultivars." *Journal of Applied Phycology* 33: 4137–4148.

Lawton, R. J., R. de Nys, and N. A. Paul. 2013. "Selecting Reliable and Robust Freshwater Macroalgae for Biomass Applications." *PLoS One* 8, no. 5: e64168.

Liu, J., B. Pemberton, J. Lewis, P. J. Scales, and G. J. O. Martin. 2020. "Wastewater Treatment Using Filamentous Algae–A Review." *Bioresource Technology* 298: 122556.

Liu, J., B. Pemberton, P. J. Scales, and G. J. O. Martin. 2023. "Ammonia Tolerance of Filamentous Algae Oedogonium, Spirogyra, Tribonema and Cladophora, and Its Implications on Wastewater Treatment Processes." *Algal Research* 72: 103126.

MacIntyre, H. L., T. M. Kana, T. Anning, and R. J. Geider. 2002. "Photoacclimation of Photosynthesis Irradiance Response Curves and Photosynthetic Pigments in Microalgae and Cyanobacteria 1." *Journal of Phycology* 38, no. 1: 17–38.

Malkin, S. 2008. "The Ecology of the Nuisance Macroalga, Cladophora Glomerata, and Its Resurgence in Lake Ontario." Thesis, University of Waterloo.

Manhaeghe, D., S. Michels, D. P. L. Rousseau, and S. W. H. Van Hulle. 2019. "A Semi-Mechanistic Model Describing the Influence of Light and Temperature on the Respiration and Photosynthetic Growth of Chlorella Vulgaris." *Bioresource Technology* 274: 361–370.

Markou, G., D. Vandamme, and K. Muylaert. 2014. "Ammonia Inhibition on Arthrospira Platensis in Relation to the Initial Biomass Density and pH." *Bioresource Technology* 166: 259–265.

Necchi Jr., O.,. 2004. "Light-Related Photosynthetic Characteristics of Lotic Macroalgae." *Hydrobiologia* 525, no. 1: 139–155.

Neveux, N., M. Magnusson, L. Mata, A. Whelan, R. De Nys, and N. A. Paul. 2016. "The Treatment of Municipal Wastewater by the Macroalga Oedogonium Sp. and Its Potential for the Production of Biocrude." *Algal Research* 13: 284–292.

Pikosz, M., B. Messyasz, and M. Gąbka. 2017. "Functional Structure of Algal Mat (Cladophora Glomerata) in a Freshwater in Western Poland." *Ecological Indicators* 74: 1–9.

Piotrowski, M. J., L. E. Graham, and J. M. Graham. 2020. "Temperate-Zone Cultivation of Oedogonium in Municipal Wastewater Effluent to Produce Cellulose and Oxygen." *Journal of Industrial Microbiology and Biotechnology* 47, no. 2: 251–262.

Pitawala, S., T. J. Kim, P. J. Scales, and G. J. O. Martin. 2024. "Time-Dependent Inhibition of Photosynthetic Activity in Filamentous Alga Oedogonium and Its Implications for Outdoor Cultures." *ACS Sustainable Chemistry & Engineering* 12, no. 40: 14902–14909. https://doi.org/10.1021/acssuschemeng.4c06340.

Pitawala, S., Z. Trifunovic, N. D. Crosbie, P. J. Scales, and G. J. O. Martin. 2024. "Biochemical Changes Due to Photothermal

Acclimation of Oedogonium and Associated Implications for Photosynthetic Growth and Biomass Utilisation." *Algal Research* 82: 103666. https://doi.org/10.1016/j.algal.2024.103666.

Pitawala, S., Z. Trifunovic, J. R. Steele, et al. 2023. "Variation of the Photosynthesis and Respiration Response of Filamentous Algae (Oedogonium) Acclimated to Averaged Seasonal Temperatures and Light Exposure Levels." *Algal Research* 74: 103213. https://doi.org/10.1016/j.algal.2023.103213.

Platt, T., C. Gallegos, and W. G. Harrison (1981). Photoinhibition of Photosynthesis in Natural Assemblages of Marine Phytoplankton.

Roberts, D. A., R. de Nys, and N. A. Paul. 2013. "The Effect of CO_2 on Algal Growth in Industrial Waste Water for Bioenergy and Bioremediation Applications." *PLoS One* 8, no. 11: e81631.

Salovius, S., and E. Bonsdorff. 2004. "Effects of Depth, Sediment and Grazers on the Degradation of Drifting Filamentous Algae (Cladophora Glomerata and Pilayella Littoralis)." *Journal of Experimental Marine Biology and Ecology* 298, no. 1: 93–109. https://doi.org/10.1016/j.jembe.2003.08.006.

Schwaderer, A. S., K. Yoshiyama, P. de Tezanos Pinto, N. G. Swenson, C. A. Klausmeier, and E. Litchman. 2011. "Eco-Evolutionary Differences in Light Utilization Traits and Distributions of Freshwater Phytoplankton." *Limnology and Oceanography* 56, no. 2: 589–598.

Shoener, B. D., S. M. Schramm, F. Béline, et al. 2019. "Microalgae and Cyanobacteria Modeling in Water Resource Recovery Facilities: A Critical Review." *Water Research X* 2: 100024.

Vieira Jr., J., and O. Necchi, Jr.. 2003. "Photosynthetic Characteristics of Charophytes From Tropical Lotic Ecosystems." *Phycological Research* 51, no. 1: 51–60.

Van Wagenen, J., S. L. Holdt, D. De Francisci, B. Valverde-Pérez, B. G. Plósz, and I. Angelidaki. 2014. "Microplate-Based Method for High-Throughput Screening of Microalgae Growth Potential." *Bioresource Technology* 169: 566–572.

Wang, J., W. Zhou, H. Chen, J. Zhan, C. He, and Q. Wang. 2019. "Ammonium Nitrogen Tolerant Chlorella Strain Screening and Its Damaging Effects on Photosynthesis." *Frontiers in Microbiology* 9: 3250.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.