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Experiment and Kinetic Modeling of Soil Extract Effects on *Dunaliella primolecta* Growth

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Recent studies have shown that extracted soil has the potential to enhance microalgae growth. An experiment was conducted, and a kinetic model was developed to understand and predict the growth rate of *Dunaliella primolecta* with consideration of soil extract effects. *Dunaliella primolecta* was cultured and mixed with extracted soil from the Raja Musa Forest Reserve, Malaysia. At present, no model of microalgal growth associated with the soil extract effect has been developed to predict cell density and growth rate. A mathematical model was derived to describe the growth rate and cell density production of microalgae with soil extract in the cultured microplate. The prediction model of microalgae concentration agrees with the experimental data, with R^2 ranging from 0.94 to 0.98. Culturing microalgae with 1% of soil extract concentration yielded a significant increment of growth rate. However, the growth rate remained constant at a higher concentration, suggesting the percentage as an optimal value. Thus, the soil extract acts as a growth enhancer that doubles the growth rate of cultured microalgae. A parametric study was conducted to characterize the light intensity and temperature effect on the growth model concerning soil extract effect.

Microalgae have great potential and are widely used to produce various compounds of interest for several industrial sectors such as aquaculture and animal feed, human nutrition, cosmetics, nutraceutics, and pharmaceutics. In environmental sustainability, these microorganisms exhibit a great potential for CO_2 capture and biofuel production, such as biodiesel[1]. Biofuels produced by microalgae have become a promising renewable energy alternative source to fossil fuels and other biofuel production crops. In

addition, microalgae-based biofuel is more economical, as it does not compete with traditional crops for land. From the point of sustainability, their production involves low-carbon emissions processes[2].

According to [3], there are at least 30,000 microalgal species on earth, but only four species, *Chlorella sp.*, *Spirulina sp.*, *Dunaliella sp.*, and *Haematococcus sp.* have been commercially produced. Several other species can be commercialized, but their production has not been ventured beyond the laboratory level[4-6].

Despite the advantages, microalgae biofuel production is facing challenges related to optimizing growth conditions while maintaining a high growth rate. Several factors affect microalgal growth, and the most influential parameters are light intensity, photosynthetic rate, temperature, nutrient availability, and pH[7,8].

Recently, several types of extracted soil have been studied as a new parameter with the potential to enhance the microalgal growth rate and cell density. The addition of soil extracts to cultured media has significantly affected nutritional values and subsequently increased microalgae concentration. According to [9], one of the tested marine sediments added into the media has resulted in rapid methane production by the brown algae. Anaerobically treated sediments from the dairy waste area were also used as a nutrient source for bioenergy production by algae[10]. Studies by [11] and [12] have shown that soil extract from aquaculture sludge and forest soil has great potential as the enrichment media for microalgae cultivation. Therefore, soil extracts demonstrate a promising result in improving the growth rate of microalgae.

A mathematical model is relatively an inexpensive approach for the prediction of microalgal growth. For example, in an upscale microalgae production[13], the predicted model assisted the optimization of the microalgal processes by monitoring the interaction of influential parameters with the microalgae. Furthermore, the important interaction between influential parameters and cell growth can be quantitatively studied if the mathematical model is accurately modeled[14].The development of the microalgae growth model closely incorporates the investigation of influential factors that affect microalgal growth. A growth model by [15] was the first model used in establishing the relationship between the nutrient limitation and specific growth rate. Later, [16] modified the Monod formulation by considering internal cell quota measurements in its formulation. Since then, many models were developed based on their formulation, associating substrate concentration with microalgal growth rate in culture media[17-19].

These growth models can be grouped based on the number of their dependent parameters, i.e., single parameter or multiple parameters. The former models have been developed to predict the effect of nutrients with a process that depends on a single parameter, such as temperature[20,21], light intensity[22-24], pH[25,26], and CO_2[27,28]. Later, in the quest to predict microalgae growth by optimizing operation conditions, multiple parameters were integrated into a kinetic term of the model growth equations[29-33]. The growth models become more complex when the effect of fluid dynamics on microalgal growth is considered. The model required the Navier-Stoke equations and solved using the simulation software[34,35]. Despite its complex solution, the simulation model is favored for its capability to optimize the design of either raceway or other open pond systems[36-38]. These models consider fluid velocity, water depth, and atmospheric conditions, composed with kinetic growth parameters in its formulation.

Light intensity and temperature are two parameters generally expressed as a function of temperature in kinetic model expressions[39,40]. However, under the controlled condition in which nutrient composition is constant, the interaction of influential parameters like light intensity and temperature can be studied[41-43]. Alternatively,[44] had reviewed several microalgae simulation models for modeling algal productivity concerning light intensity and temperature effect. They categorized the light intensity simulation models into three types; average light, light gradient, and light gradients and short cycles. Furthermore, the categorized models were defined as a coupled model if the effect of temperature is considered in the model and known as an uncoupled model if contrary.

In the present study, the growth model was developed, focusing on the simulation of microalgal growth affected by the soil extract enhancer. The model was validated and compared with the experimental data. The experimental data is the first published data of the microalgae growth with soil extract effect, collected at Raja Musa Forest Reserve, Malaysia. Furthermore, a parametric study was performed for light intensity and temperature effect on the growth model. These parameters were considered in the modeling due to their importance in assessing outdoor algae cultivation optimization. An open-source tool package environment, Python, was used to model and simulate the growth model. The growth model results were obtained on an Intel Core i5, 8 GB RAM, laptop computer.

Materials and methods

Experimental data. Two kg of soil was collected from the Raja Musa Forest Reserve, Malaysia (3°26′45.2″N101°19′20.9″E) at three (triangle) 1-m distance points. Sampling was permitted by the Hulu Selangor Forest Department of Malaysia. The soil was oven-dried at 60°C, and subsequently grind, sieved, and homogenized. The extraction process known as aqueous extraction treatment was used on the dried soil samples[45]. Milli-Q (MQ) water was used as a solvent to prepare the soil extract (SE). The soil samples were autoclaved at 105°C for 1 hour. The temperature-treated samples were then centrifuged at 700 × g for 15 minutes, filtered through a 0.7 μm glass fiber filter and stored at 4°C.

Dunaliella primolecta used in this study was isolated from Kapas Island, Terengganu, Malaysia. Conway media was prepared from five basic solutions as described by[46], comprising the mineral solution – 100 g of $NaNo_3$, 45 g of disodium EDTA ($C_6H_{16}N_2O_8$), 33.6 g of H_3BO_3, 20 g of $NaH_2PO_4{\cdot}4H_2O$, 1.3 g of $FeCl_3{\cdot}6H_2O$, 0.36 g of $MnCl_2{\cdot}4H_2O$, and 1 mL trace metal solution in 1 L of MQ water; trace metal solution – 0.21 g of $ZnCl_2$, 0.2 g of $CoCl_3{\cdot}6H_2O$, 0.09 g of $(NH_4)_6MO_7O_2{\cdot}4H_2O$, and 0.2 g of $CuSO_4{\cdot}5H_2O$ in 100 mL MQ water; vitamin solution – 0.2 g of thiamine (B1), cyanocobalamin (B12) in 100 mL of MQ water; silicate solution – 2 g of Na_2SiO_3 in 100 mL of MQ water; and nitrate solution – 2 g of KNO_3 in 100 mL of MQ water. The media was prepared by adding 1 mL of main mineral, silicate, and nitrate solution to the Schott bottle to prepare 1 L volume media. After autoclaving, the prepared media, 1 mL of NH_4Cl and vitamin solution were added into the cooled medium to give a final medium concentration of 5.0×10^{-4} M. The cultures were grown at 25 ± 0.5°C under a light intensity of 33.75 μmol m^{-2} s^{-1} on a 12 h light:12 h dark cycle. The stock cultures were acclimatized to the experimental conditions prior to the experiment before the strains were tested on sludge extracts.

The microplate-incubation technique[11] was conducted for the microalgae at 5 different SE volumes and concentration; 1, 2, 3, 6, and 10 mL, with the percentage (%) of 0%, 0.5%, 1%, 1.5%, 3%, and 5%, respectively. Media without SE was used as the control media in another microplate. The microplates were incubated for nine days, and the cell concentration was determined by cell counting via optical density (OD) at 680 nm for every 24 h using the microplate reader Infinite M200 PRO, representing cell density (cells·mL^{-1}). The results of the microalgae density in different SE concentrations are tabulated in Table 1. Each sample was measured in triplicate, and the average value was acquired.

SE Concentration (mL)	Cell Density (×10^6 cell/mL)								
	Day								
	1	2	3	4	5	6	7	8	9
0*(0%)	0.46	0.63	0.89	1.00	1.40	1.49	2.57	3.74	3.49
1(0.5%)	0.26	0.85	1.35	1.30	1.75	2.83	5.03	7.29	6.54
2(1%)	0.24	0.92	1.38	1.33	1.73	2.56	4.55	7.41	6.78
3(1.5%)	0.35	0.88	1.27	1.07	1.49	2.14	4.15	6.59	6.10
6(3%)	0.33	0,86	1.25	1.05	1.47	2.12	4.13	6.57	6.08
10(5%)	0.76	0.55	1.31	1.56	2.18	2.80	4.26	6.76	5.76

Table 1. Cell density growth of *D. primolecta* with various soil extract (SE) concentrations.
**ControlMedia*

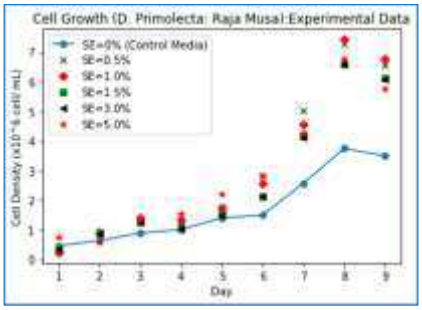


Figure 1. Experimental data of cell density with different SE concentrations (in %)

Figure 1 shows the plotted data of *D. primolecta* density for nine days cell counting observation in different SE concentrations (in percentage). The control media, represented by the curved line, is also plotted in the graph to show the differences between with and without soil extract effects regarding cell density. The soil extract interaction significantly affected *D. primolecta* growth with the highest density

($7.41 \cdot 10^6$ cells·mL^{-1}) on day 8 from the 1% SE concentration, i.e., twice the growth of the control media density result. The highest cell density for control media is $3.74 \cdot 10^6$ cells·mL^{-1}. It is noticed that the microalgae density is decreased after day 8 for all soil concentration variations, including from the control media.

Microalgae kinetic model. Having a simulation model for algae growth is advantageous, as it is more economical to study, compare, and upscale the production based on the effect of algal growth parameters. The present work is concerned with the kinetic model of microalgae growth under SE influences, where light intensity and temperature are constant. The growth of *D. primolecta* is described by the growth model (simple rate laws) as in Equation (1) and revised using the Monod equation to include other parameters considered in the present work. This is done by considering the specific growth rate constant, μ, in Equation (1) as a function of a new SE parameter, with the inclusion of light intensity (LI) and temperature (T) effects, shown in Equation (2).

$$\frac{\partial X}{\partial t} = \mu X \quad (1)$$

$$\frac{\partial X}{\partial t} = \mu(SE, LI, T)X \quad (2)$$

where $\frac{\partial X}{\partial t}$ is the microalgal growth rate, X (mL^{-1}) is cell concentration, t (days) is time, and $\int$ (day^{-1}) is a specific growth rate constant. The specific growth rate constant was calculated from the observed data in Table 1, using Equation (3) as follows, and the result is given in Table 2.

$$\mu = \frac{ln(X_2 - X_1)}{t_1 - t_2} \quad (3)$$

where X_1 and X_2 are the cell density on days t_1 and t_2, respectively. The t_1 is taken at day 1, while t_2 is at day 8, i.e., the interval time of microalgae growth phase.

SE Concentration (mL)	Maximum density $\times 10^6$ (cell/mL)	Specific Growth Rate (day^{-1})
0*(0%)	3.74	0.299
1(0.5%)	7.30	0.478
2(1%)	7.40	0.487
3(1.5%)	6.60	0.417
6(3%)	6.57	0.425
10(5%)	6.75	0.3119

Table 2.0. Specific growth and maximum cell density of *D. primolecta* with various soil extract (SE) concentrations. **ControlMedia*

In general, the Monod equation is used to describe the effect of nutrient concentration solely[15,17] because other parameters, such as light intensity and temperature, are kept constant during an experiment. However, light intensity and temperature are also the other two major parameters affecting microalgal growth, regarding the amount of absorbed energy for their growth and affecting biochemical enzymes in the reproduction process[47,48].

The Monod equation[15] was amended to include light intensity and temperature terms to improve the comparability of the current model. In Equation (4), the SE effect and function of light intensity and temperature parameters are considered in Monod's kinetics model. Consequently, the specific growth rate becomes a function of SE, light intensity, and temperature.

$$\mu(SE, LI, T) = \mu_{maxPRM} \cdot f(SE) \cdot f(LI) \cdot f(T) \qquad (4)$$

Here, μ_{maxPRM} is the maximum specific growth rate under optimal condition, $f(SE)$ is the effect of suboptimal for soil extract, $f(LI)$ is the effect of suboptimal for light intensity, and $f(T)$ is the effect of suboptimal for temperature.

Simulation of soil extract influences. Equation that reflects the SE effect on the microalgal growth is based on the Monod equation and given below.

$$f(SE) = \frac{SE_c}{K_{PRM}+SE_c} \quad (5)$$

where SE_c (mL) is the soil extract concentration and K_{PRM} (mL) is the soil extract half-saturation constant. The K_{PRM} is determined from the specific growth rate versus the SE concentration graph, where the data are taken from Table 2 and plotted in Figure 2.

Referring to Figure 2, the maximum specific growth rate is 0.487 day^{-1} at 2 mL (1%) SE concentration. The half-saturation dashed line at $0.5\mu_{maxPRM}$ is where the SE concentration equals the half-saturation constant growth by 50%. The $0.5\mu_{maxPRM}$ is equal to 0.393 day^{-1} and yields K_{PRM} equal to 0.5 mL. Figure 2 shows that any value higher than 1% soil concentration will result in the decline of growth rate, suggesting that the 2 mL (1%) SE concentration as the optimal value. In evaluating the optimal soil extract concentration value, the fixed value of 25°C temperature is under a fixed light intensity of 33.75 µmol·m^{-2}s^{-1}.

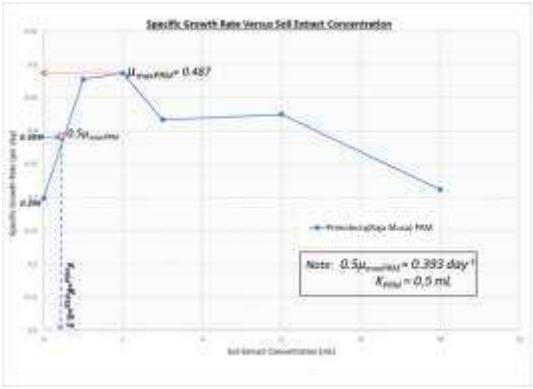


Figure 2. Specific growth rate constant of *D. primolecta* with respect to various soil extract concentration from Raja Musa Forest Reserved (PRM)

Simulation of light intensity influences. Simulation of light intensity and temperature influences is derived according to the Cardinal Temperature Model with Inflexion (CTMI) and has been applied in the present work. The CTMI model can represent the temperature and different light intensities on microalgal growth for a broad range of temperature and various phytoplankton species[41]. The model was proposed and applied to bacterial growth by[49] and applied to microalgal growth by[41]. Thus, the model has a great

potential to be coupled with a physical model in predicting the temperature in a raceway or a photobioreactor to upscale.

Light is regarded as the primary factor that significantly affects microalgal growth. The light intensity effect of the average light intensity is expressed and shown in Equation (6). Even though the light intensity is dependent on the depth, it is tolerable to assume that the depth effect is negligible because a microplate was used in the experiment.

$$f(LI) = \frac{I}{I + \frac{\mu_{maxLIT}}{\alpha}\left(\frac{I}{I_{opt}} - 1\right)^2} \quad (6)$$

The equation is used to simulate the effect of light intensity on the growth of *D. primolecta* assumed in a well-mixed system, where I (μmol·m^{-2}s^{-1}) is light intensity, α (day^{-1}) is the initial slope of the light response curve, I_{opt}(μmol·m^{-2}s^{-1}) is light intensity for which growth is maximal (with respect to light), μ_{maxLIT} (day^{-1}) is the maximum growth rate for the optimal light intensity and temperature T_{opt}(°C).

Simulation of temperature influences. In a well-mixed system, the temperature is uniformly distributed in the culture media. Thus, the temperature effect on microalgal growth rate is defined by the optimal temperature for growth and the minimum and maximum water temperature, as described in Equation (7).

$$f(T) = \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[f(t) - g(t)]} \quad (7)$$

with

$$f(t) = (T_{opt} - T_{min})(T - T_{opt}) \quad (8)$$

$$g(t) = (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T) \quad (9)$$

where T(°C) is water temperature, T_{min}(°C) is the temperature below which the growth is assumed to be zero, T_{max}(°C) is the temperature above which there is no growth, T_{opt}(°C) is the optimal temperature at the maximal growth rate. The equation could simulate at any temperature range, i.e., above and below the optimum temperature (Mayo 1997).

Referring to Equation (4) and substituting it with all terms in Equation (5), (6), and (7), a complete kinetic model is yielded, representing the SE, light intensity, and temperature influences on microalgal growth, Equation (10).

$$\mu(SE,LI,T) = \mu_{maxPRM} \cdot \underbrace{\frac{SE}{K_{PRM}+SE}}_{Soil\ Extract} \cdot \left(\underbrace{\frac{I}{I+\frac{\mu_{maxLIT}}{\alpha}\left(\frac{I}{I_{opt}}-1\right)^2}}_{Light\ Intensity} \right) \cdot \left(\underbrace{\frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[f(t)-g(t)]}}_{Temperature} \right) \quad (10)$$

The governing equation is established by substituting Equation (10) into Equation (2), as shown in Equation (11).

$$\frac{\partial X}{\partial t} = \mu_{maxPRM} \cdot \frac{SE}{K_{PRM}+SE} \cdot \left(\frac{I}{I+\frac{\mu_{maxLIT}}{\alpha}\left(\frac{I}{I_{opt}}-1\right)^2} \right) \cdot \left(\frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[f(t)-g(t)]} \right) \cdot X \quad (11)$$

Equation (11) was solved by the Runge-Kutta method, an approximate numerical method, in the Python open-source environment. The solution simulates the influence of soil extract, light intensity, and temperature, using parameter values in Table 3.

Parameter	Notation	Value	Unit
Maximum specific growth constant	μ_{max}	0.487	day-^{1}
Soil extract concentration	SE_c	2	mL
Soil extract half-saturation constant	K_{PRM}	0.5	day-^{1}
Temperature	T	25	ºC
Optimal temperature	T_{opt}	26.7	ºC
Minimum temperature	T_{min}	0.2	ºC
Maximum temperature	T_{max}	33.5	ºC
Light intensity	I	33.75	µmol·m^{-2}s^{-1}
Maximum growth rate of light intensity	μ_{maxLI}	1.85	µmol·m^{-2}s^{-1}
Initial slope of light response	α	0.12	µmol·m^{-2}s^{-1}
Optimal light intensity	I_{opt}	1.7×10^{3}	µmol·m^{-2}s^{-1}

Table 3. Parameters Values of the growth model

Results and discussion

Figure 3 shows light intensity limitation, part of the terms in Equation (11). The SE and temperature are fixed at 2 mL and 25°C, respectively. The light intensity is bound between 5 to 1700 µmol·m^{-2}s^{-1}. A high increase in the growth rate can be observed between 5 and 75 µmol·m^{-2}s^{-1}. At these lower intensities of light, where the growth rate is usually proportional to light intensity, photosynthesis activity is limited by the rate of photons captured. Light intensity reaches a saturation condition, and the growth rate is maximal when algae become lightly saturated[44]. This condition is reflected in the graph, where the light intensity

term gives constant (between 0.691 to 0.702 day-^{1}) growth rate at 450 μmol·m^{-2}s^{-1} and higher. This indicates an optimum light intensity point regarding the maximum growth rate, suggesting that the optimum light intensity value is not greater than 450 μmol·m^{-2}s^{-1}. Nevertheless, the light intensity term in Equation (10) could not simulate the decrease rate of microalgae due to the deactivation of protein in photosynthesis[50].

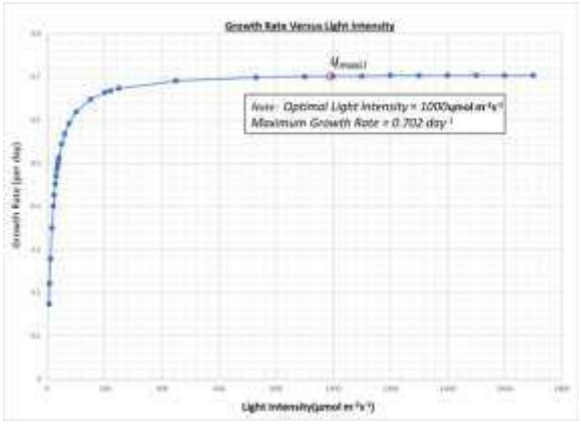


Figure 3. *D. primolecta* growth limitation in various light intensity (LI) value with fixed soil extract (SE) and temperature (T)

Temperature limitation in Equation (10) is plotted in Figure 4. The figure shows the gradual inclination of growth rate until it reached a peak and gradual decline towards the maximum temperature, T_{max}. The peak point is identified as the optimal temperature point, T_{opt}., a temperature where the growth rate is maximum. The temperature is bounded between T_{min} = 5°C to T_{max} = 33.5°C.

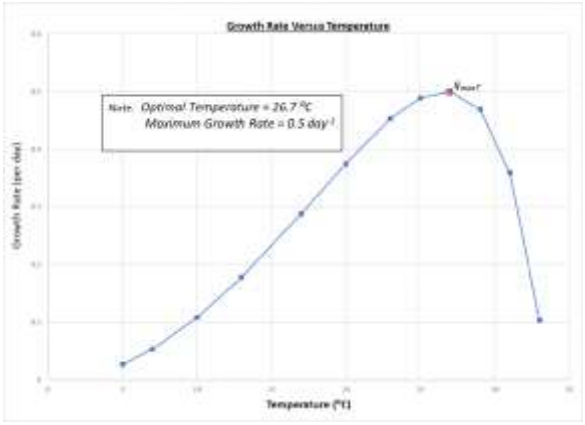
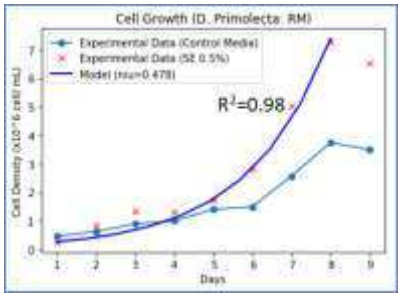


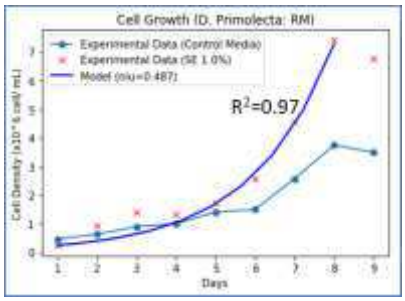
Figure 4. *D. primolecta* growth limitation in various temperature (T) value with fixed soil extract (SE) and light intensity (LI)

Microalgal growth model with SE effect. The growth model output is produced from the expression of Equation (1). The specific growth rate is computed by Equation (3) using the experimental data from Table 1. The growth model is plotted with the experimental result from microalgal growth in control media and media with SE concentration. Figure 5 shows the growth of D. *primolecta* at different soil extract concentrations, 0%, 0.5%, 1%, 1.5%, 3%, and 5%.

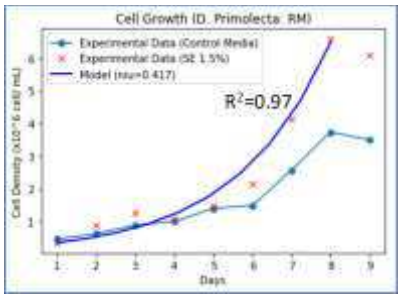
The relationship between *D. primolecta* growth and SE concentration can be described by the present growth model. The model output agrees well with the experimental data over a wide range of soil concentration percentages, where the lowest R^2 equals 0.94, and the highest R^2 equals 0.98. Furthermore, from Figure 5, the model accurately predicts the cell density value at the initial stage and the maximum density value. Meanwhile, Table 4 shows that the deviation between the growth model and experimental result at maximum density is too small. However, a slight discrepancy is noticeable at the internal stage of growth.



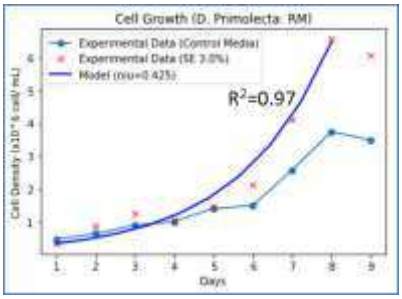
a) Soil Extract 0.5%



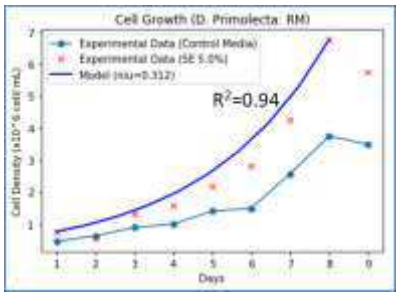
b) Soil Extract 1.0%



c) Soil Extract 1.5%



d) Soil Extract 3.0%



e) Soil Extract 5.0%

Figure 5. Comparison of growth model and experimental results of *D. primolecta* growth with different SE concentrations.

In all soil extract concentrations, the growth curve exhibits the well-known exponential curve growth. As expected, the organic matter in SE contributes to the higher growth rate of the microalgae. It is observed that the SE has a significant effect on the cell density of *D. primolecta*. From the growth curve in Figure 5(a)–(d), a lag phase is observed on days 1–4, and the algae started their log phase after day 4 until day 8. During the lag phase, the cell densities in the SE and control media exhibit are almost similar, revealing that the nutrient concentration from both cultured media could promote the growth of *D. primolecta* at almost similar rates. However, when the log phase started after day 4, the higher nutrient content in the

SE media supported the higher rate of cell multiplication demand. This pattern can be observed from the graphs by comparing the control media curve with the SE growth curve, with a significantly higher rate in terms of cell density observed from day 4 until day 8, during the log phase. After day 8, *D. primolecta* growth entered the stationary phase and achieved its maximum nutrient intake.

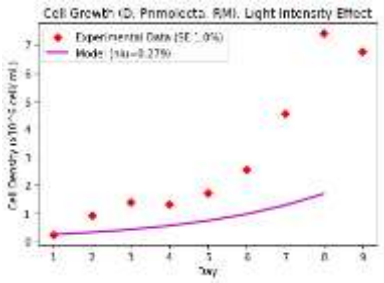
SE Concentration (µL)	Predicted Maximum Density $\times 10^6$ (cell/µL)	Experimental Maximum Density $\times 10^6$ (cell/µL)	Deviation
0*(0%)	3.73	3.74	0.26%
1(0.5%)	7.381	7.29	1.2%
2(1%)	7.256	7.41	2.1%
3(1.5%)	6.483	6.59	1.62%
6(3%)	6.465	6.57	1.6%
10(5%)	6.745	6.76	0.22%

Table 4. Growth model output and experimental values for maximum growth. *Control Media

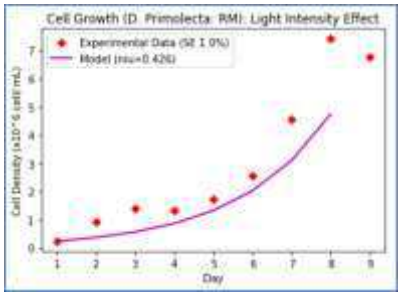
Parametric study of light intensity and temperature effects. The kinetic growth of *D. primolecta* was modeled according to Equation (11) concerning different light intensity and temperature values and the constant soil extract concentration (SE: 1% = 2mL). The 1% concentration in soil was selected based on the maximum growth rate occurring at the concentration value. The parametric study on light intensity and the temperature were conducted in different parameter values: 10, 23, 33.7, 50, and 450 $\mu mol \cdot m^{-2}s^{-1}$ and 10, 17, 25, 31, and 33°C, respectively. The parametric study was performed using estimated values in Table 3, synthesized from[42,44,51]. The validity of the growth model was confirmed by comparing it with experimental data in a similar environment, i.e., 33.7$\mu mol \cdot m^{-2}s^{-1}$ and 25°C for 1% SE concentration.

Effect of light intensity on a microalgae growth model. The parametric study shows that the growth model in Figure 6(c) obtained comparable results with the experimental data at 33.7 $\mu mol \cdot m^{-2}s^{-1}$. For light intensity that is lower than 33.7 $\mu mol \cdot m^{-2}s^{-1}$, the model shows a lower growth rate with cell density that did not exceed the maximum cell density ($7.41 \cdot 10^6$ cell/mL), as shown in Figures 6(a) and (b). Figures

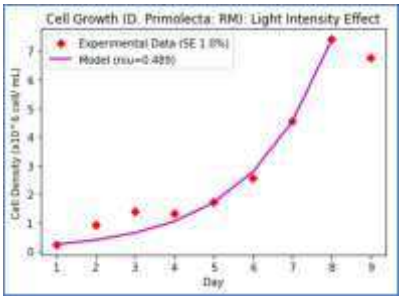
6(d) and (e) show that a higher growth rate and cell density are obtained for light intensity beyond 33.7 $\mu mol \cdot m^{-2}s^{-1}$.



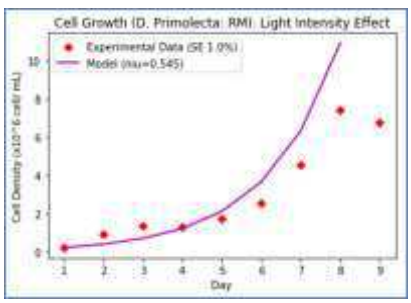
a) Light Intensity: 10 $\mu mol\ m^{-2}s^{-1}$



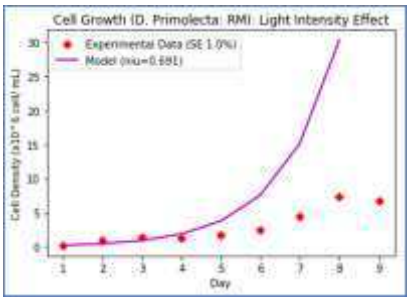
b) Light Intensity: 23 $\mu mol\ m^{-2}s^{-1}$



c) Light Intensity: 33.7 $\mu mol\ m^{-2}s^{-1}$



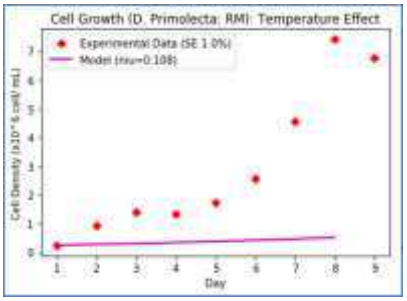
d) Light Intensity: 50 $\mu mol\ m^{-2}s^{-1}$



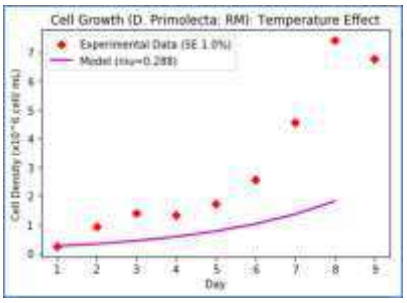
e) Light Intensity: 450 µmol $m^{-2}s^{-1}$

Figure 6. Comparison of growth model and experimental results of *D. primolecta* growth with different light intensity effects

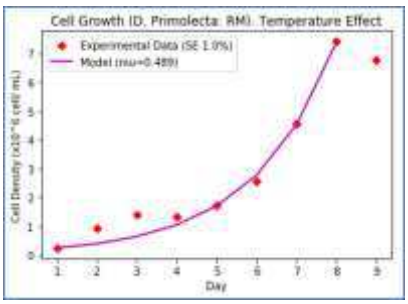
Effect of temperature on microalgae growth model. Most microalgae can carry out the photosynthesis process; hence, the temperature might influence microalgal growth efficiency. The effect of different temperatures was studied on microalgae growth using Equation (11) to analyze its effect with constant soil extract concentration (1%) and light intensity (33.7µmol·$m^{-2}s^{-1}$) values. The growth pattern of *D. primolecta* is observed at 5 different temperatures: 10°C, 17°C, 25°C, 31°C, and 33°C, as shown in Figure 7 (a–e). The equation can describe the temperature effect either below or above the optimal temperature. Figure 7 shows that at 25°C, i.e., a similar environment temperature, a good correlation between the growth model and experimental data is noted, acquired by Equation (11). Meanwhile, lower growth rates and cell densities are observed in low temperatures, as illustrated in Figures 7(a) and (b). Subsequently, at temperatures exceeding optimal value, the microalgal growth rate decreased, as depicted in Figures 7(d) and (e). This is generally due to the effect of enzyme or modified proteins involved in the photosynthetic process affected by heat stress[52].



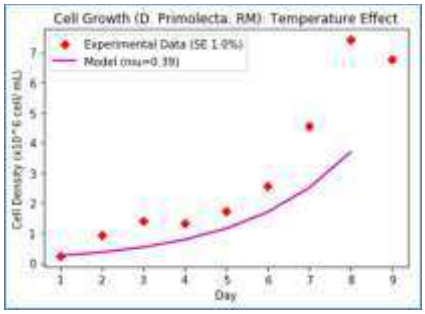
a) Temperature: 10 ºC



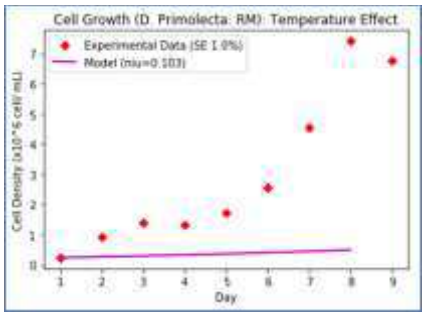
b) Temperature: 17 ºC



c) Temperature: 25 ºC



d) Temperature: 31 ºC



e) Temperature: 33 ºC

Figure 7. Comparison of mathematical model and experimental results of *D. primolecta* growth with various temperature effects.

Conclusion

In this study, a growth model of *D. primolecta* with soil extract (SE) effects was developed. The Monod model was integrated to consider the soil extract, light intensity, and temperature effects in the growth model. The developed model has permitted the obtention of the cell density and growth rate at any soil extract concentration. The model growth rate compared well with the experimental data, with R^2 ranging from 0.94 to 0.98. The maximum growth rate for microalgae with soil extract is 0.487 day^{-1}, i.e., double growth compared to the growth rate without SE (0.299 day^{-1}). The optimum light intensity and temperature of 33.75 $\mu mol \cdot m^{-2}s^{-1}$ and 25°C have been verified in the parametric study.

(a) (b)

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Author contributions

D.A.: Conceptualization, Writing original draft, kinetics modelling and numerical solution. I.FA.G and N.S.Y.: Writing, reviewing and editing. M.F.A.: Methodology, investigation and synthesis of materials. M.H.M and M.I.B.: Data verification and numerical solution.

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