THE EFFECT OF LIGHT INTENSITY ON THE PRODUCTIVITY OF THE DIATOM *PHAEODACTYLUM TRICORNUTUM* CULTIVATION IN A COMPACT TUBULAR PHOTOBIOREACTOR

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Abstract. *Alternative and renewable sources are currently needed to replace fossil fuels mainly used in transportation. In recent years, Brazil has been investing heavily in the research and development of biodiesel as a substitute for petrodiesel. Since January 2010 the Brazilian legislation established that diesel sold in the country should contain at least 5% biofuel (B5). The raw materials used in Brazil for producing biodiesel in September 2009 according to the Brazilian National Oil Agency (ANP) consisted of: 77.13% soybean oil, 17.07% beef fat, 4.62% cottonseed oil and 1.18% from other sources, which reveals the low diversity and high dependence on soybeans. With the goal of contributing to the diversification of raw materials for biodiesel production, the Center for Research and Development of Sustainable Energy at UFPR has been working to establish the production of biodiesel from oil extracted from microalgae grown in compact photobioreactors. The microalgae are considered to have major advantages compared to larger photosynthetic organisms due to large growth rate, and high biomass production of lipids according to species. A key component to the growth of microalgae is the availability of an appropriate system to allow for efficient photosynthesis, which requires abundant light and adequate temperature. However, the occurrence of the phenomenon of photoinhibition should be taken into account, in which excess light causes photosynthesis inhibition and reduces microalgae growth rate. The photoinhibition is a phenomenon that is caused by ultraviolet light (UV), visible (V) and combinations of UV-V light. The absorption of photons is directly proportional to the photons flow density, which results in a linear response. However, the use of these photons by the photosynthetic process shows a hyperbolic response, demonstrating that there exists an optimal flow density which is determined by the saturation of photosynthesis. Therefore, the main scope of this study is to determine the effects of light on photosynthesis during the growth of Phaeodactylum tricornutum microalgae in the tubes of a compact photobioreactor with a volume of 11,000 m^3. For that, the microalgae growth is simulated computationally with a transient mathematical model to predict the microalgae growth in a batch cultivation system for a duration of ten days. The idea is to demonstrate how a maximum yield of biomass from a good photosynthetic efficiency can be achieved, and thus contribute to the production of biofuel in a sustainable manner. The concentration of microalgae in the medium was modeled with the species conservation principle inside volume elements (VE), distributed throughout the photobioreactor tubes, yielding a system of equations to be solved simultaneously to obtain the microalgae concentration spatial distribution in the reactor. The phenomenon of photoinhibition caused by excess light intensity was considered by the source term, μ (growth rate), calculated according to the light intensity inside the tubes. In order to investigate the effect of light intensity in isolation in the present study, the medium temperature was kept constant in the simulations, and set to an experimentally reported optimal value for the species. The utilized photobioreactor geometry followed the design proposed by Vargas (2007). The results show that the maximum productivity of microalgae can be achieved with respect to light intensity. The optima are sharp and should be taken into account by any method of cultivation of microalgae.*

Keywords*: Microalgal Growth Rate, Numerical Simulation, Phenomenon of Photoinhibition.*

1. INTRODUCTION

Microalgae are organisms found in various aquatic systems and require favorable conditions to carry out their vital activities. When the pH is appropriate and nutrients are not limiting the growth of biomass, the effect of light intensity and temperature become dominant factors. Richmond (1997) noted that the availability of light for each cell in a photoautotrophic culture is a function of intensity and duration of the incident light and the cell concentration, or density, which affects the growth process through mutual shading. According to Serenotti *et al.* (2004) under appropriate light conditions the cells can store energy and produce intermediate products (such as ATP) that are used for the fixation of carbon dioxide and biomass synthesis whether the cells are in light conditions or conditions of lightness or darkness.

Besides the rate of illumination, another important aspect when dealing with the heterotrophic cultivation of microalgae is the temperature of cultivation. Like any organism, the cultivation of microalgae at temperatures outside of the optimum temperature may cause a decrease in cell growth (at low temperatures) or inhibition and death (at high temperatures). However, microalgae can adapt to different growing conditions from which the increase or decrease in temperature occurs gradually. Sudden increases in temperature will invariably provide cell death. In addition to directly affecting cellular metabolism and causing a decrease in growth rate and reproduction of microalgae, temperature, as described in many studies in literature promotes a change in the composition of fatty acids present in lipids synthesized, in particular the lipid membrane (Chen *et al.*, 2008). This fact is explained by changes imposed by temperature on the fluidity of cell membranes. With increase in temperature, the replacement of lipids with unsaturated fatty acids with lipids with saturated fatty acids, which are more compact in structure, give this case a lower fluidity of the plasma membrane. In the case of a decrease in temperature the opposite occurs, i.e. a replacement of saturated fatty acids by unsaturated fatty acids in these membrane lipids, allowing the plasma membrane to provide fluidity at low temperatures. This same behavior is repeated in other organisms such as bacteria and even in mammals.

Having a mathematical and computational model showing agreement with the behavior of a culture of microalgae in a compact photobioreactor is of paramount importance. This model is especially important since microalgae are being considered as a potentially useful source for biodiesel production in the future. Microalgae are extremely biodiverse, able to produce more biodiesel oilseeds and occupy less space than the space used to grow oilseed plants (Pérez, 2007; Lawrence, 2006).

2. MATHEMATICAL MODEL OF THE SPEED OF GROWTH OF CELLULAR MICROALGAL GROWTH (μ)

Based on literature review, the kinetic model proposed by Molina Grima *et al.* (1994), Eq.1, was examined - where the increase of cell growth is proportional to the average light intensity inside the photo bioreactor. This mathematical model calculates the specific growth rate of microalgae and will serve as the basis for a new mathematical model that incorporates the effects of light and temperature in the fluid, together with the inhibition caused by excess or lack of them. According to the kinetic model of Eq.1 when more luminous intensity focuses on microalgae, the specific growth rate will tend towards the maximum growth rate of microalgae. The parameter μ_{MAX} represents the maximum specific growth rate that it can reach (h^{-1}), I_K represents the relationship of the alga with solar radiation ($\mu Em^{-2}s^{-1}$), n is a dimensionless parameter obtained experimentally, and $I_{médio}$ is the variable representing the average light intensity inside the tubes of the photo bioreactor ($\mu Em^{-2}s^{-1}$).

$$\mu = \frac{\mu_{MAX} I_{médio}^{n}}{I_{médio}^{n} + I_{K}^{n}} \tag{1}$$

The equation that calculates the average light intensity is defined as:

$$I_{médio} = \frac{I_0}{r\pi}\int_0^r\int_0^\pi e^{-CK_a\left((r-s)cos\varphi+\sqrt{r^2-(r-s)^2 sin^2\varphi}\right)} d\varphi ds \tag{2}$$

where K_a is the coefficient of light absorption ($m^2g^{-1}biomassa$), r the radius of the tube (m), s is the distance from the surface of the tube until an internal point (m) and φ is the angle of incidence of the path of light.

The light intensity on the surface of the photo bioreactor tubes is calculated with a sine function, and $I_{0,Max}$ is the maximum luminous intensity at the surface of the tubes and the photo bioreactor, α represents the angle of the sun in the photobioreactor in the range of $0 < \alpha < \pi$ during the 12 hours the culture is exposed to sunlight (Eq.3).

$$I_0 = I_{0,Max}\, sin(\alpha) \tag{3}$$

If the average light intensity is very high within the photobioreactor, cell growth should not tend towards maximum growth rate, but photoinhibition occurs. Photoinhibition occurs because most microalgal pigments serve as complex antennas, collecting light and transferring energy to the complex reaction centers, where the chemical reactions of oxidation and reduction lead to energy storage in the long term (Taiz & Zeiger, 2006). If a high intensity light is received, the electron transport in photosystem II (complex responsible for converting light energy into chemical) is affected. The D1 protein of the reaction center of photosystem II (PSII) undergoes photodamage mechanisms of photoinhibition. Photoinhibition may be a consequence of limiting the transport of electrons in the donor side of PSII or a limitation on the acceptor side of PSII.

To account for the phenomenon of photoinhibition, terms were added to Eq (1) to incorporate the phenomenon of photoinhibition. Molina Grima *et al.* (1996a) analyzed how the excess of light influences the growth of microalgal culture in this form. The new equation for the microalgal growth rate including photoinhibition becomes:

$$\mu = \frac{\mu_{MAX} I_{médio}^{b+\frac{c}{I_0}}}{I_{médio}^{b+\frac{c}{I_0}} + \left(I_K \left(1 + \left(\frac{I_0}{K_i} \right)^a \right) \right)^{b+\frac{c}{I_0}}} \tag{3}$$

The new parameters shown in Eq (3) are: I_0 representing the light intensity on the surface of the photobioreactor tubes ($\mu Em^{-2}s^{-1}$), K_i which is a parameter of photoinhibition ($\mu Em^{-2}s^{-1}$), and a, b and c which are experimentally determined dimensionless parameters. Growth is also inhibited or stopped outside the temperature range to which each species supports. The kinetic reaction constants typically fit the Arrhenius equation (Roels, 1983). This is supported by the findings of Perez *et al.* (2008) and Sánchez *et al.* (2008). However, in their studies, the parameter μ_{MAX}, was represented by a constant. This work proposes to replace the constant specific growth rate (μ_{MAX}) with specific growth rate (μ) as a function of temperature (T) and average light intensity inside the tubes ($I_{médio}$), as shown in Equation 4.

$$\mu = \frac{\left(A_1 e^{\left(\frac{E_a}{RT} \frac{T-T_0}{T_0} \right)} - A_2 e^{\left(\frac{E_b}{RT} \frac{T-T_0}{T_0} \right)} \right) I_{médio}^{b+\frac{c}{I_0}}}{I_{médio}^{b+\frac{c}{I_0}} + \left(I_K \left(1 + \left(\frac{I_0}{K_i} \right)^a \right) \right)^{b+\frac{c}{I_0}}} \tag{4}$$

where A_1 and A_2 are frequency factors of pre-exponential value (h^{-1}), E_a e E_b represent the activation energy (*Kcal/mol*), R is the general constant of gases (*Kcal/mol*), T is the absolute temperature (K) and T_0 is the reference temperature (K). The mathematical model that shows the growth of the concentration of a culture of microalgal biomass as a function of time is given by the differential equation shown in Equation 5:

$$\frac{dC}{dt} = C(\mu - m) \tag{5}$$

where C represents the concentration of biomass (m^2/g), μ specifies the rate of growth (h^{-1}), and m the maintenance fee (h^{-1}).

2.1 NUMERICAL SIMULATION

The computer model uses data related to the microalgae Phaeodactylum tricornutum acquired from the literature (Perez et al, 2008; Grima *et al.*, 2001, Fernández *et al.* 1997; Sánchez *et al.*, 2008) and photobioreactor parameters proposed by Vargas (2007). These parameters are summarized in Table 1 below.

Table 1. Constants used in numerical simulation

$C_0 = 200\ g/m^3$	$\alpha = (0,\pi)$
$T_{final} = 120$ hours (5 days)	$m = 0.00385\ h^{-1}$
$I_{0,Max} = 2500\ \mu Em^{-2}s^{-1}$	$T_0 = 293\ K$
$I_K = 94.3\ \mu Em^{-2}s^{-1}$	$A_1 = 0.26\ h^{-1}$
$a = 3.04$	$A_2 = 0.18\ h^{-1}$
$b = 1.209$	$E_a = 117040\ Jmol^{-1}$
$c = 514.6$	$E_b = 163020\ Jmol^{-1}$

The numerical code was developed in FORTRAN 95, where the mathematical model consisting of Equations 1 to 5was implemented computationally. Equation 5 is an ordinary differential equation of first order demonstrates the growing concentration of the culture through time and was solved using the classical Runge-Kutta model with fourth

order accuracy. Equation 2, where the average light intensity is calculated with a double integral, was approximated by a double Riemann sum, which is an approximation of the actual volume by "*n*" volume columns.

Photosynthesis in nature is composed of two stages called: reaction course (or photochemical step) and dark reactions. The photochemical step is characterized by light absorption by chlorophyll, synthesis of adenosine triphosphate (ATP) and photolysis of water. During the dark phase, there is no need for direct light. However, the substances synthesized in the photochemical stage of photosynthesis are needed in the sequential reactions to occur the absorption of CO_2 molecules, which undergo a reduction, is obtained and glucose molecules. In analyzing this process, it is observed that there was not a net production of substances during the dark phase of photosynthesis. To support this observation the computational model simulated 12 hours of sunlight exposure and 12 hours of darkness. Several simulations were performed based on a compact photobioreactor proposed by Vargas (2007) through a batch culture by varying the temperature in the photobioreactor in the range of $274 \leq T \leq 300$ K in order to analyze the optimal temperature at which a high photosynthetic rate allows achieving better productivity.

3. RESULTS

The computer model effectively models the phenomenon of inhibition as shown in Figure 1. This phenomenon occurs when the internal temperature of the photobioreactor tubes undergo an abrupt change in relation to the optimum temperature, causing poor cell growth (temperature drop) or even killing of microorganisms (with temperatures well above the optimum). The growth part of the curve represents a stimulation of temperature dependent photosynthesis. The decline of the curve is associated with deleterious effects, some of which may be reversible. The highest photosynthetic rates observed in response to temperature changes are called the response to optimal temperature. When such temperatures are exceeded, photosynthetic rates begin to decrease again (Taiz & Zeiger, 2006).

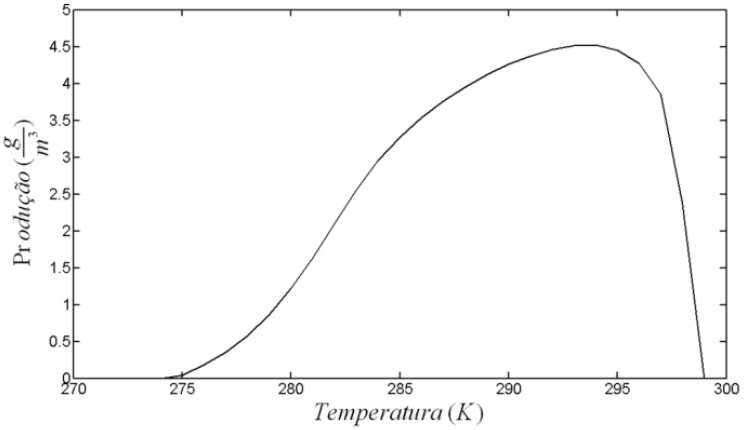

Fig. 1 Productivity of microalgal biomass for each temperature used in the simulation.

Figures 2a and 2b show a 3D graph and with contour lines of the concentration of microalgae over time for a temperature range of 274 K to 300 K. The temperature that generated the highest yield of microalgae was 294 K with a yield of 4.52g/ (m^3h) as seen in Figure 1. The times in which the highest yields of biomass occur can be seen in Figure 2b. These times coincide with the period of greatest illumination of photobioreactor. The decrease in the concentration of microalgae during periods without light can also be seen in Figure 2b. In a traditional cultivation of microalgae, the period of illumination is the period in which the microalgae consume their energy reserves accumulated during the light period. In the mathematical model this phenomenon is represented with a constant called the maintenance fee $m = 0.00385\ h^{-1}$.

Equation 3 is displayed graphically in Figure 3. It can be seen clearly in these figures that during the 12 hours of darkness no microorganism growth occurs, therefore its specific growth rate is zero. At the temperatures 298 K, 299 K and 300 K the specific growth rate is negative and Figure 3 shows that the cells are dying instead of multiplying. Cell death occurs because for this particular microalga, *Phaeodactylum tricornutum*, these temperatures (298K, 299K, and 300K) are above the threshold for cell growth. The influence of temperature on the growth of microalgae was evaluated by Goldman and Ryther (1976). In this work the authors concluded that if different species of microalgae are subjected to mixed culture simultaneously, only the microalgae adapted to growing conditions (especially temperature) showed significant growth performance.

The phenomenon of photoinhibition occurs when the intensity of light used in the growth of microalgae is approaching the maximum intensity of the system (usually at 12 hours). Photoinhibition may also occur in the middle of the period of 12 hours of light in experiments that simulate a natural light cycle. In the computational model that simulates these conditions it is possible to observe this effect by the decrease in growth during the most luminous intensities. This phenomenon can be seen very clearly in Figure 3.

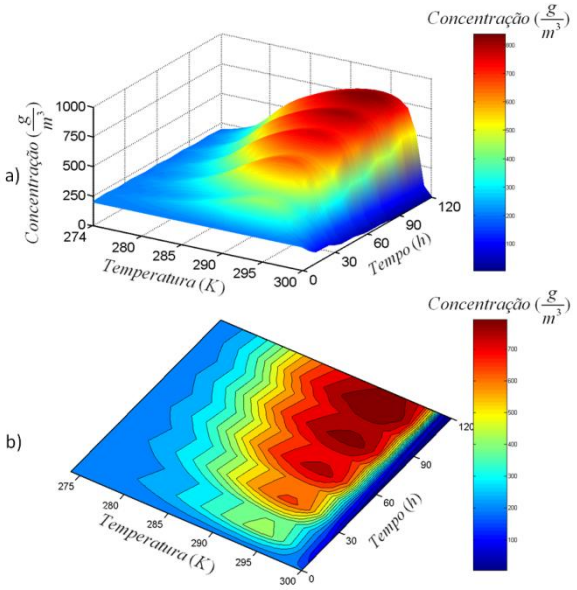

Fig. 2 a) 3D graphics, showing the concentration of microalgae over time for different temperatures
b) contour lines of the 3D chart.

High temperatures cause a reduction in the strength of hydrogen bonds and electrostatic interactions between the polar groups of proteins in the aqueous phase of the membrane, reducing membrane stability. High temperatures also alter the composition and structure of the membrane and can cause a loss of ions. The rupture of membranes also causes the inhibition of processes such as photosynthesis and respiration, which depend on the activity of electron transporters and enzymes associated with membranes. It can be seen in Figure 3a that the growth rate below zero due to the high temperature is the inhibition of photosynthesis, as a consequence of decreased productivity. Photosynthesis and respiration are inhibited at high temperatures, but with the temperature increase, photosynthetic rates fall before respiration rates. The temperature at which the amount of CO_2 fixed by photosynthesis equals the amount of CO_2 released by respiration at a given time interval, is called the *compensation temperature point*. At temperatures above the compensation temperature, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, the reserves of carbohydrates decrease. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effect of high temperatures (Taiz & Zeiger, 2006).

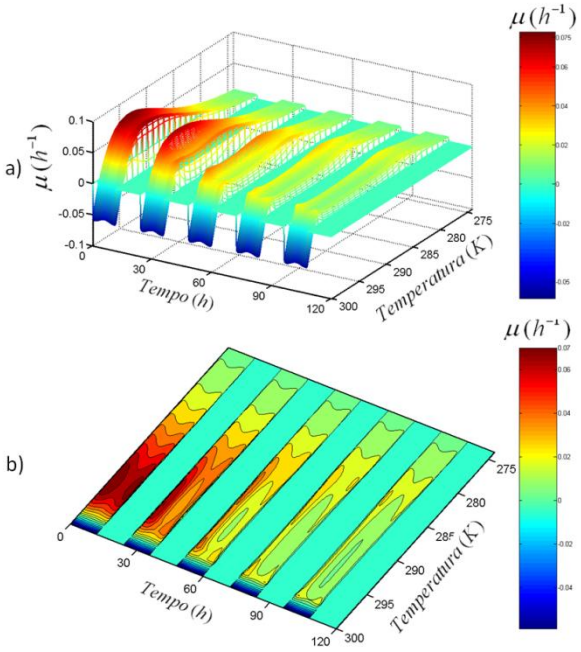

Fig. 3 a) 3D graph showing the specific growth rate over time for various temperatures.
b) contour lines of the 3D chart

4. CONCLUSION

This paper reported on the importance of assessing the productivity of microalgae subjected to varying temperatures inside the tubes of photobioreactors by numerical simulation. It is important to know the temperature range tolerable by microalgae when cultured in the photobioreactor in order to avoid massive death of a culture of microalgae. The compensation temperature point is essential in order to obtain maximum productivity and a higher production of fatty acids. When the temperature reaches its threshold, photosynthetic rates become higher and have the highest rate of productivity, in which the rate of CO_2 fixed by photosynthesis is equivalent to the amount of CO_2 released by respiration. Yet when the compensation temperature point is exceeded, there is cellular inequilibrium and cellular reserves decrease and consequently the oils produced also tend to decrease. Numerical simulation can be used to investigate the tolerable temperature range for optimal metabolic activity of microalgae. Data from numerical simulations can help ensure that the cultivation of microalgae occurs under favorable conditions of temperature (respecting a range of values) so that production of biomass can yield greater productivity and oil content to be used as feedstock for the production of biodiesel.

5. ACKNOWLEDGEMENTS

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