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Mathematical Modelling of the Effects of Circadian Rhythm on Microalgal Growth in Phototrophic and Mixotrophic Cultures

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This work presents a kinetic model that quantifies the effects of the algae's circadian rhythms and natural light-dark cycle on algal growth in photobioreactors. Such a model is necessitated by the fact that the classical Monod growth model applies to algal growth obtained under continuous exposure to light. However, any large scale production of algae under natural light in photobioreactors or raceway ponds subjects the algae to a day-night cycle, switching on the algae's natural circadian rhythm through upregulation of certain genes. This circadian rhythm results in a biphasic algal growth pattern during alternating light-dark cycle – a phenomenon that cannot be quantified by the classical Monod growth model. Our new growth model, capable of quantifying the algae's circadian rhythm in the presence of day-night photo-cycles, lends itself to both analytical and numerical solutions, both of which are obtained and compared in this study. Our growth kinetic model has been validated by comparing with experimental data on the effect of light-dark photoperiods on the growth of the microalgae *Chlorella sorokiniana* in phototrophic and mixotrophic culture media in air-sparged bubble-column photobioreactors. Our kinetic model shows that the growth during the light dependent phase can be studied as a biomass concentration dependent negative sigmoid function, whereas the dark phases shows a constant decrease in the biomass concentration which has been termed as 'night biomass loss'.

1. Introduction

Currently available literature studies on algal growth in photobioreactors focus on the implementation of available models to study the inhibitory effects of various abiotic growth factors on algal growth. Major stress is laid on the optimization of the nutrient and light inputs given to the system in order to maximize the available biomass yield. The classical Monod models enable us to describe the kinetics microalgal growth for short time spans. However, the deviation from the Monod kinetics becomes predominant in case of longer cultivation periods, where the effect of biotic regulation of growth becomes evident due to the saturation of nutrient effects. The current work presents a kinetic model for algal growth, which is applicable for longer growth period.

The mathematical modelling of algae has since long been a topic of interest to maximize the growth of algae under controlled conditions. A similar work on modelling was carried out (Concas et.al., 2013) using Monod Kinetics to simulate the growth of algal communities with *Chlorella vulgaris*. It also proposed a balance equation to take care of the oxygen and carbon dioxide material balances in the photobioreactors. This represents the amount of greenhouse gases being fixed by means of the growth process. A major problem however in large scale algal cultures is the presence of limiting factors, both abiotic and biotic. Photoinhibition and photosaturation are predominant causes which can be effectively removed by proper mixing of the culture medium. In dense algal cultures, the light intensity is said to attenuate with the depth of penetration which has been incorporated into an overall model for growth of the same (Sanchez Miron et al., 2000). It has been found that the effect of intermittent regions of non-exposure to light are effective in maintaining a high growth rate. In this case a bubble column photobioreactor can be used to fulfill this need by acting as a riser and down comer section separately, where the biomass is not continuously exposed to direct light giving rise to an increased growth rate and lower fatigue in the system (Shariff and Chakraborty, 2015). The experimental results have been compared with our model simulations to predict the growth of microalgae under different

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culture conditions. The extent of mixing induced in the system by the input air stream acts as an important controlling factor for growth. The system acts in the laminar zone for 2LPM airflow rate at which the experiment is performed. The mathematical model incorporates a light intensity factor, mixing time for the laminar regime and the specific growth rate as parameters to provide a generalized study for algal growth in photobioreactors. It is observed that the growth of microalgae is not a continuous process but occurs in regular periods referred to as circadian rhythms with alternating dark and illuminated periods. Continuous exposure to light for the same time provides less growth than the setup with alternating light and dark exposure, proving the presence of 'fatigue' in the biomass growth which cannot be accounted for.

2. Modelling of Microalgal Growth

The culture of *Cholorella sorokiniana* was cultivated in 1 lit (working volume 800 ml) bubble column photobioreactor (Kumar and Das, 2012) at 30 °C under continuous light intensity of 1800Lux provided by white fluorescent lamps under in mTAP[-acetate] medium for autotrophic growth, and in mTAP [+acetate] media for mixotrophic growth. The reactor was sparged with air at the rate of 2 LPM, and three different illumination regimes were investigated for the cultivation of algae in both autotrophic and mixotrophic growth. The illumination regimes include 24 h Light, 12 h Light: 12 h Dark, and 12 h Dark: 12 h Light.

As has been already reported and studied extensively in various sources the ability of microalgae to metabolize and multiply is heavily dependent on the availability of light which is evident from the experimental data collected as well. In order to understand the nature of mixing in the culture medium, a rough calculation of the flow parameters were made in the PBR which gives a fair idea on the nature of mixing prevalent in the reactor and the advantages of having the same. In the current work, the effect of both autotrophic and heterotrophic growth is being studied which adds to the overall productivity of the system. Heterotrophic growth is achieved by adding an acetate source which can be used to calibrate the effect of organic carbon sources in aiding the growth of algae.

Microalgal growth has been under the scanner for development of dynamic models based on ordinary differential equations. In the current realm of engineering microbial growth for biofuel production and CO_2 mitigation, the most commonly used model of algal growth is the Monod model(Eq(1)).

$$\mu = \frac{\mu_{max} S}{K_s + s} \tag{1}$$

Microalgae are known for their ability to uncouple the uptake of nutrients which form an essential part of understanding microalgal growth kinetics. It has been found in many cases that microalgal growth continuous for some time even after the nutrients are exhausted which is in violation of the Monod kinetics for modelling algal growth in living systems. An attempt to devise a more accurate model is done in this work by assuming the growth rate to be a function of the internal concentration of an individual nutrients. The behaviour and variation in the growth rates of algal cultures are being studied to understand the variation of the same with regard to the deviation from expected classical behaviour. Classical mathematical modelling of algal cultures usually take into account the fundamentals of a semi-batch stirred tank reactor. The bubble column photobioreactor which is designed in this case is defined as two phase system, where the liquid phase is stationary and the gas phase is operated in bubbled continuously through the liquid regime. To study the dynamics of algal growth the major parameters include biomass concentration, oxygen and carbon dioxide concentration which form important aspects in the model. The idea of studying algal circadian rhythms is to scale up and maximize the growth of microalgae for CCS and biofuel production. The availability of light is generally limited to about 12 hours per cycle which can be taken as the duration of the circadian rhythm in each of the growth conditions. The scope of the present work limits to the calibration of algal growth rates with respect to culture conditions with excess of nutrients. The following section mentions the modelling of algal growth with alternate light and dark periods, as well as with 24 hour long periods of illumination. Reynolds number is found to be in the laminar region for the above mentioned parameters, which give an ideal mixing time in the photobioreactor. The mixing time in a laminar system is given by: $t_{mix} = a^2/D_m$, where a is the diameter of the riser section and D_m is the liquid diffusion coefficient (taken to be $10^{-6}\ cm^2/s$ and is found to be of the order of 2-3 minutes.

2.1 Reasons for deviating from the classical Monod Model

It has been observed that the algal data cannot be modelled directly by using the Monod kinetics which can be used to normally define algal cultures. Although many explanations can be suggested for the same, it can be easily ascertained which factors are not affecting the variation in the growth rates. Nutrient Content is provided in abundance to make the dependence relatively less on the lack of nutrients in the growth medium as time progresses. Light Intensity factor is assumed to be at a constant value of 0.9 which can be assumed to be constant because of ample lighting provided at 2,000 lux. The death rate or metabolic loss of cell mass is

assumed to be constant with the growth of the biomass colony. It is found that using the Monod kinetics in the dynamic model brings about an over/under prediction in the rate production.

3. Results and Discussion

The following assumptions are taken to set up the modelling equations for the available experimental data. The photobioreactor is completely mixed and specific growth rate of algae is taken to be independent of nutrient concentration and light intensity factor. A light limited continuous flow culture was assumed for writing the energy balance, where all incident photosynthetically available radiance is absorbed. Although the size of gas bubbles are not constant inside the bioreactor and the gas-liquid mass transfer rate changes continuously, it is assumed that the bubbles size remains almost constant due to the short length of the reactor Temperature and pH are constant in the culture medium and the Yield coefficients are constants and the same as their theoretical values in the preliminary study.

It can be observed from the experimental data that the algal growth phase shows a slight difference in the nature of growth and does not follow a constant growth rate as the amount of biomass concentration(X) changes in the culture medium. From the available data, the instantaneous specific growth rate shows the highest rate of growth in the initial stages followed by a gradual decrease in the same. It can be safely assumed that in case of autotrophic cultures, the specific growth rate is function of the biomass concentration in the system which can be appropriately modelled along the line of an analytical solution. To understand the nature of growth in each of the cases, the data is fitted to a curve which provides the function of specific growth rate with biomass concentration.

The instantaneous specific growth rate (μ) is caculated as $\frac{1}{X}\left(\frac{dX}{dt}\right)$ where the derivative is calculated as the backward difference form. The autotrophic (light dependent) and the dark region of the two sets of data: light/dark cycle and dark/light cycle are seen to have a difference in the nature of the curves which are represented as a three parameter model for the data. A comparison between the three parameter and two parameter models show a greater accuracy in representing the data.

An important aspect of the current work is exploring the dynamics of algal growth in mixotrophic cultures. Looking closely into the metabolism of microalgae such as *C. sorokiniana* , it has been established that the growth and metabolism in the presence of organic carbon sources leads to a higher lipid content which is beneficial for biofuel production. The behaviour of microalgal colonies has been studied under the influence of organic acetate sources. The modelling of the growth dynamics of microalgae under phototrophic conditions and in the presence of organic carbon is being studied in three major parts, namely: 24 hour illumination ,12 hour Dark: 12 hour Light cycle and 12 hour Light: 12 hour Dark Cycle

3.1 Phototrophic Growth Model

The calibration of phototrophic biomass growth rates provide the following empirical model for describing the growth of biomass in PBRs under phototrophic conditions. The algal colony is unable to perform any photosynthesis during the absence of light which can be said to have a negative effect on the biomass in terms of a metabolic loss which has been represented in Eqns (3). The need for numerical verification of the analytical model for the autotrophic microalgal growth is imperative, to decide the set of parameters for which the growth curve provides the closest fit with the experimental data. In most cases it has been found that the algal growth takes an initial lag time to get acclimated to the culture conditions where the growth rate is found to be very low. This region is described as the lag time and can be used to modify the final numerical model(Eq(2)).

$$\frac{dX}{dt} = f_I[\mu_0\left(1 - V_m\frac{X^n}{k^n+X^n}\right)X - k_D X] \quad \text{for } t > t_{lag} \quad \text{.....Phototrophic growth during light phase} \quad (2)$$

$$\frac{dX}{dt} = -k_M X \quad \text{.....Night biomass loss} \quad (3)$$

3.2 Analytical and Numerical Solution of Phototrophic Growth Rate

The main driving force behind studying growth dynamics and the deviation from ideal Monod Kinetics is the need for developing an analytically robust model that can determine the nature and extent of growth in algal cultures, under bubble column Photobioreactor setups. As discussed earlier as well, the nature of the curves are heavily dependent on the illumination available in the setup, and has been studied separately for each of the cases of alternating dark/light periods and continuous exposure to light.

The current model discusses autotrophic growth dynamics which are connected to the nature of the curves available. This shows the absence of growth or rather 'Night Biomass Loss' which is caused to the internal metabolism and respiration of algae in the absence of light. The death rate of algae is assumed to be a

constant under both illuminated and dark conditions which can be used as a constant decreasing factor to account for the necessary changes.

$$\int \frac{dX}{\left[\mu_0\left(1-V_m\left(\frac{X^n}{k^n+X^n}\right)\right)X-k_D X\right]} = \int f_I dt \tag{4}$$

$$\frac{1}{Nn} ln\left(\frac{M+NX^n}{M+NX_0^n}\right) - \frac{P}{Mn} ln\left(\frac{M+NX^n}{M+NX_0^n}\right) + \frac{P}{M} ln\left(\frac{X}{X_0}\right) = f_I \mu_0 t \quad \text{Analytical expression for phototrophic growth} \tag{5}$$

$$M = 1 - V_m - k', N = P(1-k'), k' = \frac{k_D}{\mu_0}, P = k^n, f_I: \ Light\ intensity\ factor\ (0.92)$$

The analytical solution is an implicit function of the biomass concentration(Eq(5)) which can be used to predict the growth of a microalgal culture under the influence of autotrophic conditions. The model can be extended to other algal cultures from *Chlorella sorokiniana* by varying the parameters. The final form of the analytical function fitted to the experimental data is shown in the Figure (1a, 2a, 3a) which can be used to test the goodness of fit for the analytical solution of the system. The numerical solution of the differential equation describing microalgal growth is calculated in MATLAB using the ode15s solver for stiff ODEs. The setup however has an initial lag time which has to be incorporated in the system and is evident from the experimental data as well. The R^2 value is found to be of the order of 0.98 which is sufficiently good in this case.

3.3 Modelling of Mixotrophic Growth

The expected growth model is taken as Eq(6) , where μ_M is the specific growth rate for 24 hour illumination in mixotrophic regime. $[Ac]$ is the Acetate concentration at any time in the reaction broth and K_{Ac_M} is the associated Michaelis-Menten constant for the 24 hour Mixotrophic model. In order to obtain the model parameters from the experimental data, the data is plotted in the form of

$$\mu = \mu_M \frac{[Ac]}{k_{Ac_M}+[Ac]} \quad \text{....Effect of acetate on specific growth rate} \tag{6}$$

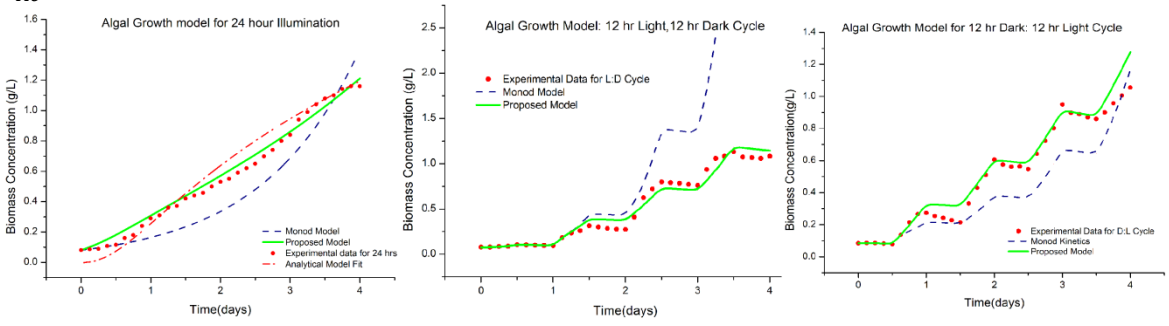
The value of K_{Ac_M} is used to calculate the value of the Acetate term in each case and divide the effective growth rate by the same in order to obtain the light dependent growth term. On obtaining the effective growth rates due to autotrophic growth in the 24 hour illuminated system, the data is fitted to the relation derived in the previous section for autotrophic growth. The final consolidated model for the growth of algae in a mixotrophic culture under the continuous illumination and dark phase is given in Eq(7) and (8).

$$\frac{dX}{dt} = f_{I_M} \mu_M \left(1 - V_m \frac{X^n}{k^n+X^n}\right)\left\{\frac{[Ac]}{k_{Ac_M}+[Ac]}\right\}X \quad \text{.Mixotrophic algal growth for continuous illumination} \tag{7}$$

$$\frac{dX}{dt} = \mu_H \frac{[Ac]}{K_{Ac_M}+[Ac]} \quad \text{.....Heterotrophic growth in the absence of light} \tag{8}$$

Likewise under intermittent illumination cycles of 12 hours each, the nature of the growth rate is similar with the exception of the absence of the light dependent growth factor in case of the dark cycles. This can be treated as a decreasing factor for the growth in dark regions which utilises the presence of only organic carbon in the nutrient medium. The acetate attenuation factor which takes into account the variation of acetate concentration(Eq(9)) in the growth expression can be modelled as a linear function of the biomass concentration. This in turn increase the efficiency of the model as a single variable model for the system. (Figure 2)

$$\frac{[Ac]}{k_{Ac}+[Ac]} = A + BX \quad \text{..... Acetate attenuation factor} \tag{9}$$



(a) (b) (c)

Figure 1: Temporal Dynamics of Algal Cultivation in Phototrophic Growth: Model-Experiment Comparison

3.4 Numerical Verification of the Mixotrophic Model

The robustness of the proposed model can be numerically verified by fitting with the necessary parameters to match the available experimental data. As evident from the plots in fig 3(a,b,c), the model is successful in predicting the algal growth with great precision over a the growth period taken into account. The gradual variation in the growth rate from the initial lag phase to the final saturation phase can be efficiently taken into consideration with this model for both phototrophic and heterotrophic medium. The numerical model converts the variation of both the acetate concentration and the growth rate as a function of the biomass accumulated in the system. The complete parameter set for each of the numerical growth models for algal growth are given in (Table 1). An analytical model for this system cannot be derived given the implicit nature of the growth function which provides an improper integral for the same (Eq(10)).

$$\int \frac{dX}{\mu_0\left(1-V_m\frac{X^n}{k^n+X^n}\right)\{A+BX\}X} = \int f_{I_M} dt \qquad \text{..... Mixotrophic growth- numerical verification} \qquad (10)$$

f_{I_M} : Light intensity factor in mixotrophic growth (0.92)

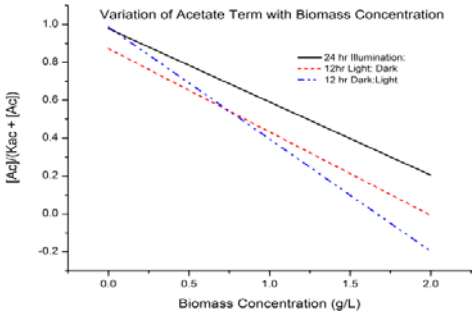
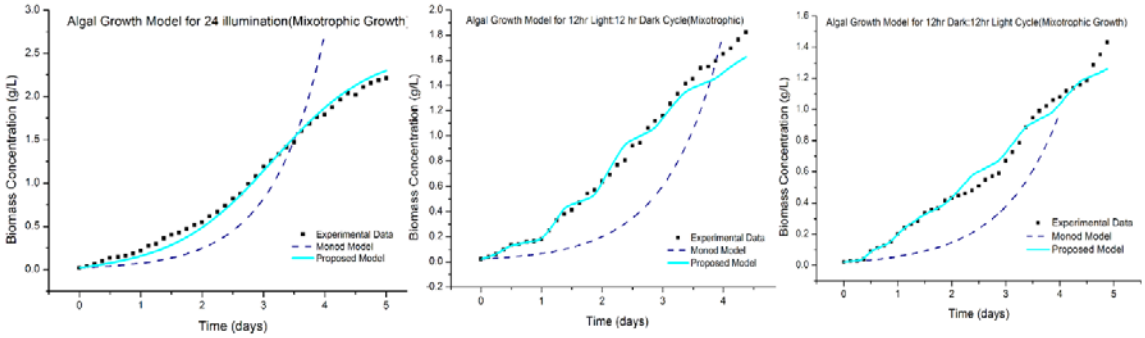


Figure 2: Variation of Acetate attenuation factor with Biomass Concentration (X)



(a) (b) (c)

Figure 3: Temporal Dynamics of Algal Cultivation in Mixotrophic Growth: Model-Experiment Comparison

Table 1: List of fitting parameters for the proposed algal growth models

Parameters	Phototrophic Growth			Mixotrophic Growth		
	24 h L	12 h L: D	12 h D:L	24 h L	12 h L: D	12 h D:L
V_m	0.86	0.84	0.89	0.72	0.8	0.88
n	2.2	1.5	1.75	2.2	2	4.218
k	0.218	0.162	0.1634	0.037	0.707	0.1844
$\mu_0(\mu_M)$	2.0366	4.74	5.261	4.5144	3.6	3.7
A	-	-	-	0.97724	0.8723	0.9866
B	-	-	-	-0.3866	-0.44	-0.592
μ_H	-	-	-	-	-	-
μ_{Monod}	0.714	2.35	1.203	1.2	1.1	0.95
f_I(Illumination factor)	0.92	0.92	0.92	0.92	0.92	0.92

4. Conclusions

In theory, algae can be made to perform photosynthesis and add to the biomass continuously but in practice, natural light can only be provided during the day for large scale cultures of algae. To mimic this natural environmental phenomenon, experiments have been performed to quantify the circadian rhythm of microalgae. The algal growth curves are oscillatory in nature, and are biphasic during a single light-dark cycle, in sync with the algae's circadian rhythm. We quantify the effects of photo-periods on algal growth under autotrophic and mixotrophic conditions, and derive new growth models that account for effect of the algae's circadian rhythm on its growth. Our analytical model quantifies the temporal dynamics of biomass growth, for a set of given operating and growth parameters in the photobioreactor, and lends itself amenable to both analytical and numerical solutions, both of which have been obtained and compared. Our model shows that the growth during the light dependent phase can be studied as a biomass concentration dependent negative sigmoid function, whereas the dark phases shows a constant decrease in the biomass concentration which has been termed as 'night biomass loss'. This work provides a theoretical framework to quantify the kinetics of algal growth in large scale production in photobioreactors and raceway ponds, under natural light and during alternating light-dark photo-cycles.

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