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BIOCHEMICAL RESPONSES FROM BIOMASS OF ISOLATED *CHLORELLA SP.*, UNDER DIFFERENT CULTIVATION MODES: NON-LINEAR MODELLING OF GROWTH KINETICS

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Abstract - In this study, the effect of different modes of cultivation viz., photoautotrophic (AT), heterotrophic (HT) and photomixotrophic (MT), on the growth and biochemical responses of *Chlorella sp.* isolated from local ponds. The performance of microalgal growth was quantified using nonlinear growth models such as Gompertz, logistic, Baranyi-Roberts, Morgan under different cultivation conditions. The results revealed that microalgae could grow better in MT than in other cultivation modes with a major increase in biochemical responses for carbohydrates, which showed higher accumulation under HT. The maximum biomass concentration was 1.24 g L^{-1} (MT), 1.16 g L^{-1} (HT), 0.76 g L^{-1} (AT) with maximum specific growth rates of 0.0083 h^{-1} (HT), 0.0078 h^{-1} (MT) and 0.0055 h^{-1} (AT) respectively. The biomass concentration was higher in the order of MT>HT>AT for which MT yielded 8.8 fold higher biomass, compared with the initial biomass concentration, at the end of experiments (16 days). Concomitant increases in biochemical responses were observed in the three cultivation conditions. Protein and lipid accumulation in the MT mode was higher (1.2 fold) compared with the initial protein yield as well as the other cultivation modes. However, the carbohydrate yield was higher (1.12 fold) in the heterotrophic mode than in other cultivation conditions.

Keywords: Microalgal isolate, cultivation condition, biomass applications, bioenergy.

INTRODUCTION

Overexploitation of fossil fuels and lack of availability of alternate resources to meet the energy demand in the present scenario is increasing day by day (Vasudevan and Briggs, 2008). However, fluctuations in crude oil prices and the predicted lack of availability of resources are the best comfort zone to make the other alternate renewable energy sources viable. On the other hand, global warming due to fossil fuel utilization is another major problem concerning the world (Chisti, 2007). To overcome the problems associated with global warming and fossil fuel demand, the need for alternative renewable sources (biological based biofuels) has increased in attention as is evident from the literature (Scragg, 2002). Biofuel generation from various resources (rapeseed, pongamia, jatropha, and solid waste) has been regarded potential materials by the global fuel market (Kirrolia et al., 2013; Sandhya et al., 2013). Additionally, the use of microorganisms to produce lipid has shown remarkable positive advantages such as higher biochemical characteristics and short

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growth time (Brennan and Owende, 2010). Microalgae are among the important feedstocks for various applications such as biofuels, nutraceuticals, fertilizers etc., as they are produced in non-arable lands (Li et al., 2014). The growth of microalgae primarily depends upon light, media components (esp., carbon, nitrogen) and growth conditions (Ogbonna and Tanaka, 2000; Li et al., 2008; Liu et al., 2008) Usually, microalgae are cultivated in open ponds and photobioreactors under photoautotrophic condition. However, due to certain limitations like self-shading (light limitation) which hinders the growth of algae and biomass production, heterotrophic-based cultivation systems were introduced to avoid light and use a carbon source to support the growth of microalgae (Liang et al. 2009). However, certain limitations such as supply of oxygen and long-term commercialization are a few issues that limit the use of heterotrophy cultivation systems for microalgae cultivation (Spolaore et al., 2006). Hence, to address the issues and concern raised form autotrophic and heterotrophic systems, a mixotrophic mode of cultivation for microalgae systems was adopted to make algae cultivation simple, easier, and attractive (Li et al., 2008). Thus, several studies have been conducted for microalgae cultivation focused on biomass production and applications (Lee et al., 1996; Bhatnagar et al., 2011; Li et al., 2012). In mixotrophic mode, microalgae tend to use both organic and inorganic carbons sources for growth; thereby they tend increase more biomass and other biochemical features depending upon the substrate availability (Chojnacka and Noworyta, 2004; Liang et al., 2009). The main objective is to study the native isolate of microalgae subject to different modes of cultivation in a bubble column photobioreactor (PBR) focusing on biomass production and biochemical composition. In this paper, the microalgae were isolated from nearby ponds and subjected to cultivation under photoautotrophic (AT), photomixotrophic (MT) and heterotrophic (HT) conditions by the supply of atmospheric air as source of CO_2 and glucose (organic carbon source) in the growth medium in an enclosed bubble column photobioreactor (PBR). Glucose helps algae reach higher growth rates and possesses more energy content compared to other organic substrates (Perez-Garcia et al., 2011). The biochemical responses such as carbohydrates, proteins and lipids were recorded in all the cultivation modes of microalgae cultivation.

Microalgal culture identification, isolation and inoculum preparation

Water samples were collected in pre-sterilized polyethylene glass bottles from a freshwater source near VIT University, Vellore, India. These isolated samples, when observed under microscope with 40 X magnification, were identified as *Chlorella* sp. (Figure 1) and stored by plating on BG11 (*Chlorella*) under axenic condition (UTEX 2009). The cultures were developed from a small volume (10 mL) to a greater volume (50 mL). 50 mL each algae was then separately inoculated into growth medium and maintained at 25ºC, with 3000 lux measured using a lux meter (TES corp.,) under constant illumination.

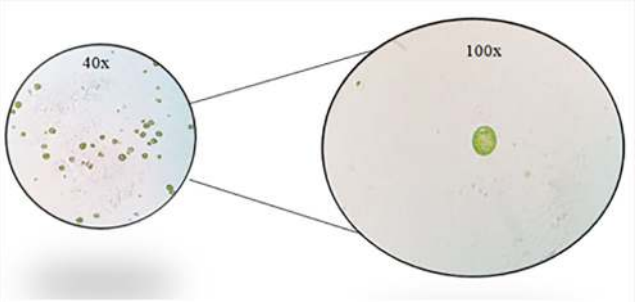


Figure 1. Microscopic image (40x, 100x) of the native isolates of microalgae *Chlorella sp*.

Experimental design

Batch scale experiments were carried out in bubble column photobioreactor (Schematic representation shown in Figure 2) with a working volume of 1.5 L and with organic substrate (glucose 1 g L^{-1} in growth medium). The reactor (made of acrylic material) under atmospheric air from a compressor at the flow rate of 0.03 vvm (~380 ppm CO_2) to mimic a mixotrophic condition supported with baffles at the top for mixing. Log phase cells of microalgal samples were used as the inoculum with a size of 10% (v v^{-1}) with an initial cell density of 20 × 10^5 cells mL^{-1} for experiments in the photobioreactor. Suspended microalgal cultures were separated from the growth media by centrifugation at 8000 rpm for 20 minutes. All the analyses were carried out in triplicates and the average was used for data analysis.

Analytical experiments

Microalgae samples collected from the photobioreactor after mixing were subjected to growth and biochemical analysis. For growth (dry weight) the microalgae were centrifuged at 8000 rpm for 15 minutes. The obtained pellet was washed with distilled water, again centrifuged and dried in an oven at 80ºC for 24 hours until constant weight was reached. The anthrone-sulfuric acid method was used to quantify the carbohydrates (Pons et al., 1980). The protein content of microalgae cells was analyzed by Lowry's method

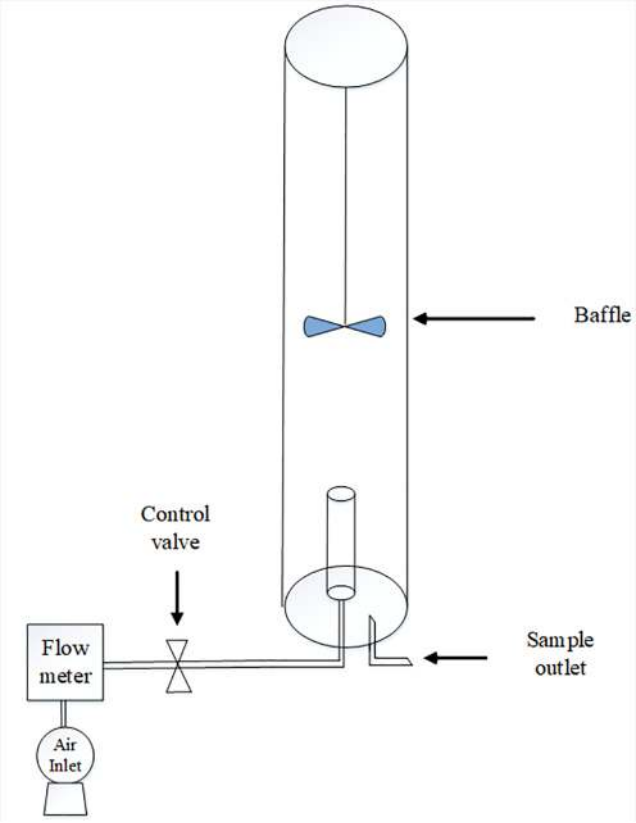


Figure 2. Schematic representation of the photobioreactor (PBR) for the study

(Lowry, 1951). Following the Bligh and Dyer method (Bligh and Dyer, 1959) total lipids were extracted.

Non-Linear model

Four microbial nonlinear models, namely logistic, Gompertz, Baryani-Roberts, and Morgan were used in the study (Table 1).

The logistic model defines the microbial populations as a function of the initial population (biomass), growth rate and final population. The Gompertz model defines a large number of the growth functions and was later modified (Zwittering et al., 1990) to include biological relevant parameters .The Morgan model is another expression for the microbial biomass growth estimation. The Baranyi-Roberts model is geometrically different because it exhibits a quasi-segment during the log phase of cells. A nonlinear regression technique was used to fit the growth models using SPSS 21.0 and the correlation coefficient (R^2), root mean square value (RMSE) and standard error of prediction (%SEP) were calculated for determining the goodness of fit using the formula given in the literature (Mohamed et al., 2014).

RESULTS AND DISCUSSION

Growth behavior in cultivation modes

The microalgae *Chlorella sp.,* showed good growth in all the cultivation modes depicted in Figure 3. The biomass concentration in MT cultivation exhibited an 8.8-fold increase (1.2 g L^{-1}) at the 12th day compared to the initial biomass content. Likewise, the AT mode exhibited good growth but less compared to the HT & MT modes. At the 14th day of cultivation, the biomass was ~ 5-fold increased compared to the initial day. However, the growth attained ~ 8-fold increase in HT at the 11th day of cultivation. Nevertheless, the specific growth rate was slightly higher in HT compared to MT, despite higher biomass accumulation in MT compared

Table 1. Non-Linear model for the algal growth curve.

Model	Equation	Reference
Gompertz	$Y(t) = A\exp\left(-\exp\left(\frac{\mu \exp(1)}{A}(\lambda - t) + 1\right)\right)$	Zwittering et al. (1990)
Logistic	$Y(t) = \frac{A}{1+\exp\left(\frac{4\mu}{A}(\lambda - t) + 2\right)}$	
Baranyi-Roberts	$Y(t) = \mu X(t) - In\left(1 + \frac{\exp(\mu X(t)) - 1)}{\exp(A)}\right)$ where, $X(t) = t + \frac{1}{\mu} In(\exp(-\mu t) + \exp(-\mu\lambda) - \exp(-\mu(t+\lambda)))$	Baranyi and Roberts (1995)
Morgan	$Y(t) = \frac{A * t^{\vartheta}}{K^{\vartheta} + t^{\vartheta}}$	Morgan et al. (1975)

*exp(1)=2.718; Y(t) is the predicted biomass yield with respect to time (t); A is the maximum biomass produced at various cultivation conditions, μ is the specific growth rate (d $^{-1}$); λ is the lag phase observed; X(t) is the adjustment function for cell growth as described in literature (Mohamed et al., 2014). K is the half maximum growth with respect to time (t) and ϑ is the curvature parameter.

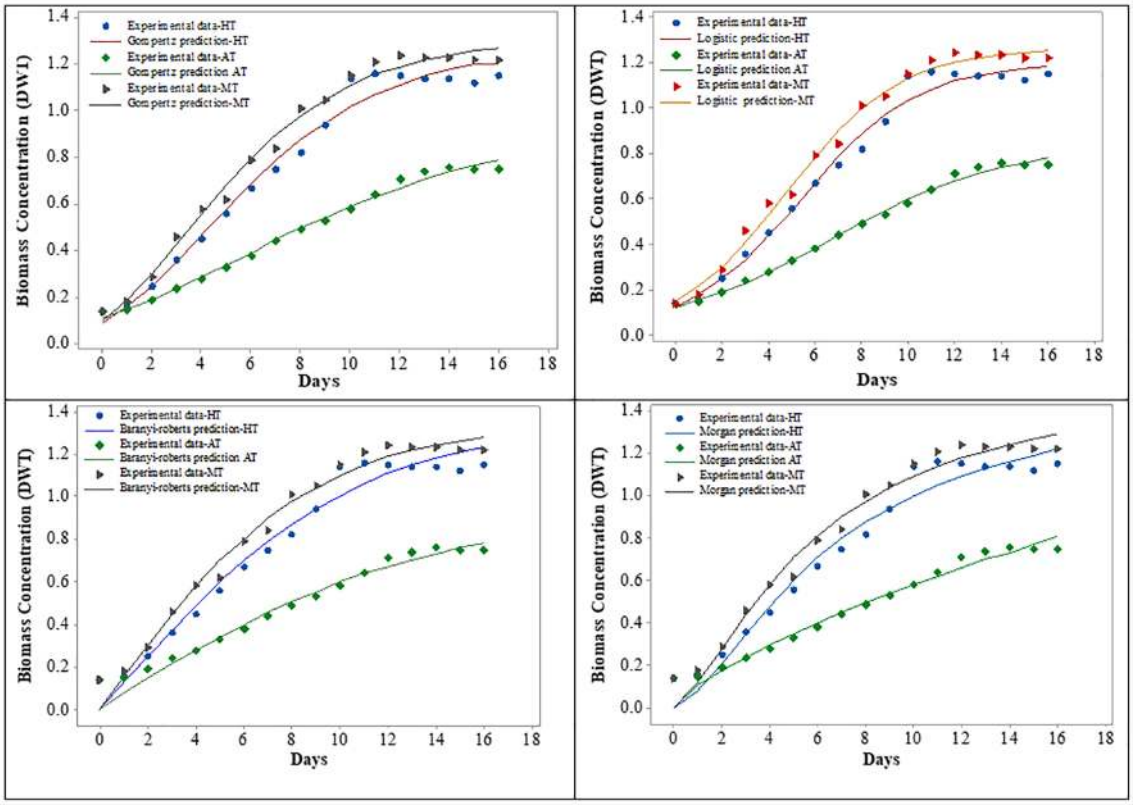


Figure 3. Non-Linear model for the growth of *Chlorella sp.*, under various cultivation modes.

to the other condition, indicating the affirmative role of organic and inorganic carbon sources, which help enhance the biomass rate (Nigam et al., 2011). The four nonlinear models fit the experimental data (Figure 3) using adjusted parameters is shown in Table 3. All the models were in good agreement with cultivation modes expect for MT, which exhibited an R^2 value of 0.85 for the logistic model. In addition, Table 2 portrays the performance indices for the nonlinear microbial models. The correlation was in the order of Gompertz > logistic > Morgan = Baranyi for HT and AT whereas Gompertz> Baranyi> Morgan> logistic for MT, supporting the above statement. RMSE is a good fit to extent the correctness and the lower value specifies the finest model agreement (Nigam et al., 2011). In this study, the RMSE value was lower to all the models, which is perfectly aligned with the %SEP values, respectively. Overall, the logistic and the Gompertz models described the growth kinetics effectively in this study. Similar results with lower SEP values shown during cultivation of *Tetraselmis sp*. under in media (Mohamed et al., 2014).

Variation of biochemical composition in different cultivation modes of microalgae

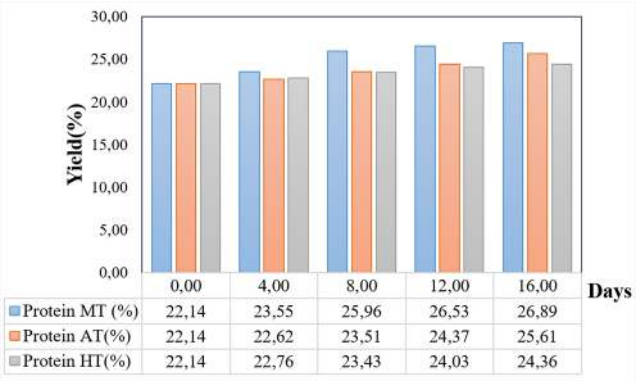
The protein profiles for microalgae under different modes of cultivation are shown in Figure 4. Initially, *Chlorella sp.,* possessed 22.14 % of protein, and during the 16th day of cultivation in the HT condition, it can be noted that accumulation of protein content was 24.36% with relative increase of 1.1-fold, with respect to the initial day concentration. Likewise, the AT cultivation system exhibited a 25.61% yield of protein, which is a 1.15-fold relative increase. However, protein contents were higher in the MT condition than in the other modes, which indicated the role of both organic and inorganic substrate favorable for the accumulation of protein content. This may be due to the fact that the presence of organic carbon (glucose) promoted the cell metabolic pathway of algae thereby increasing protein content (Endo et al., 1974). Carbohydrate is an important energy source in microalgae as compared to lipids, which play a crucial role for bioethanol production. The carbohydrate profiles of microalgae are shown in Figure 5, respectively. At the 16th day of cultivation MT, AT and HT exhibited 21.71%, 20.82% and 22.05% with relative increases in 1.1 fold, 1.06 fold and 1.12 fold with respect to initial day of microalgae culture. Hence, the increase in accumulation of carbohydrate was higher in HT than in the MT mode of cultivation, indicating the role of the organic source in accumulation of carbohydrates such as starch in green microalgae and glycogen in cyanobacteria (Shekharam et al., 1987). Lipids are the major prime

Table 2. Performance indices of the nonlinear model for Chlorella sp. under various cultivation modes.

Cultivation mode	Error Model	Gompertz	Logistic	Baranyi-Roberts	Morgan
HT	**R^2**	0.98	0.98	0.97	0.96
	RMSE	0.05	0.04	0.07	0.07
	%SEP	6.7	5.8	8.9	14.87
	B_f	1.23	1.23	1.23	1.23
AT	**R^2**	0.99	0.99	0.96	0.96
	RMSE	0.02	0.02	0.04	0.05
	%SEP	4.2	3.6	9.6	14.86
	B_f	1.23	1.23	1.22	1.22
MT	**R^2**	0.99	0.99	0.98	0.97
	RMSE	0.04	0.03	0.05	0.06
	%SEP	4.3	3.9	6.2	14.86
	B_f	1.23	1.23	1.23	1.23

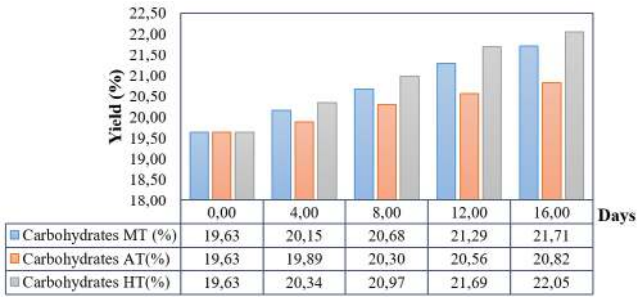
Table 3. Parameter values in nonlinear model.

Model	Equation
Gompertz	$$HT(t) = 1.16\exp\left(-\exp\left(\frac{0.198 * \exp(1)}{1.16}(1-t)+1\right)\right)$$
	$$AT(t) = 0.76\exp\left(-\exp\left(\frac{0.12 * \exp(1)}{0.76}(1-t)+1\right)\right)$$
	$$MT(t) = 1.24\exp\left(-\exp\left(\frac{1.187 * \exp(1)}{1.24}(1-t)+1\right)\right)$$
Logistic	$$HT(t) = \frac{1.16}{1+\exp\left(\frac{4(0.198)}{1.16}\right)(1-t)+2}$$
	$$AT(t) = \frac{0.76}{1+\exp\left(\frac{4(0.12)}{0.76}\right)(1-t)+2}$$
	$$MT(t) = \frac{1.24}{1+\exp\left(\frac{4(0.187)}{1.24}\right)(\lambda-t)+2}$$
Baranyi-Roberts	$$HT(t) = 0.198X(t) - In\left(1+\frac{\exp(0.198X(t)-1)}{\exp(1.16)}\right)$$
	$$X(t) = t + \frac{1}{0.198}In(\exp(0.198t)+\exp(-0.198*1)-\exp(-0.198(t+1)))$$
	$$AT(t) = 0.12X(t) - In\left(1+\frac{\exp(0.12X(t)-1)}{\exp(0.76)}\right)$$
	$$X(t) = t + \frac{1}{0.12}In(\exp(0.12t)+\exp(-0.12*1)-\exp(-0.12(t+1)))$$
	$$MT(t) = 0.187X(t) - In\left(1+\frac{\exp(0.187X(t)-1)}{\exp(1.24)}\right)$$
	$$X(t) = t + \frac{1}{0.187}In(\exp(0.187t)+\exp(-0.187*1)-\exp(-0.187(t+1)))$$
Morgan	$$HT(t) = \frac{1.16t^{9}}{5^{9}+t^{9}}$$
	$$AT(t) = \frac{0.76t^{12}}{6^{12}+t^{12}}$$
	$$MT(t) = \frac{1.24t^{10}}{5^{10}+t^{10}}$$



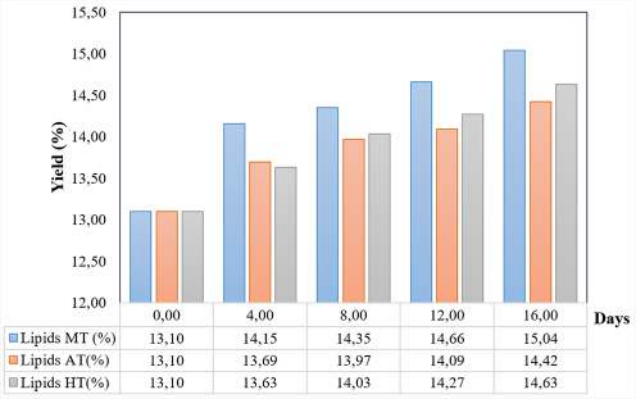
	0,00	4,00	8,00	12,00	16,00
Protein MT (%)	22,14	23,55	25,96	26,53	26,89
Protein AT(%)	22,14	22,62	23,51	24,37	25,61
Protein HT(%)	22,14	22,76	23,43	24,03	24,36

Figure 4. Protein profile of *Chlorella sp.* under various cultivation modes.



	0,00	4,00	8,00	12,00	16,00
Carbohydrates MT (%)	19,63	20,15	20,68	21,29	21,71
Carbohydrates AT(%)	19,63	19,89	20,30	20,56	20,82
Carbohydrates HT(%)	19,63	20,34	20,97	21,69	22,05

Figure 5. Carbohydrates profile of *Chlorella sp.* under various cultivation modes.



	0,00	4,00	8,00	12,00	16,00
Lipids MT (%)	13,10	14,15	14,35	14,66	15,04
Lipids AT(%)	13,10	13,69	13,97	14,09	14,42
Lipids HT(%)	13,10	13,63	14,03	14,27	14,63

Figure 6. Lipid profile of *Chlorella sp.* under various cultivation modes.

factors for microalgal biodiesel production (Chen et al., 2011). The lipid profiles of three microalgae isolates are presented in Figure 6. Initially, the lipid content was 13.10%. Upon subjecting to the MT mode there was a higher lipid content of 15.04% than for the other modes HT or AT modes that exhibited 14.63% and 14.42%, respectively. This might be due to the lower availability of substrate (nitrogen source), which may subject microalgae to a stress condition and thereby increase the lipid content (Mairet et al., 2011; Jian et al., 2012). Thus, the higher lipid in the mixotrophic condition (MT) might be an influence of nitrogen limitation as the C: N ratio of the microalgae cell was 106:16.

CONCLUSIONS

In this study, the mixotrophic cultivation mode of microalgae *Chlorella sp.* showed a better yield in growth parameters and biochemical responses than the autotrophic mode. Growth parameters were significant with nonlinear model kinetics, revealing the MT is the ideal mode of cultivation. Likewise, biochemical responses such as protein and lipids showed >1 fold increase in MT than HT and AT at the 16th day of cultivation. However, carbohydrate was higher in heterotrophic cultivation, which is due to the participation of glucose in the metabolic reaction of algae than other modes. Based on the results, the isolated microalgae species is an ideal candidate for various value added product applications under mixotrophic condition.

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NOMENCLATURE

μ	Specific growth rate
A	Maximum biomass concentration
t	time
λ	lag phase
ϑ	shape parameter

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