CO_2 Sequestration and Growth Characteristics of Euglena Gracilis in a Photo Bioreactor

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Abstract

The The impact of CO_2 in atmosphere can be alleviated by CO_2 sequestration using green algae which is receiving a lot of attention nowadays. CO_2 fixation or CO_2 sequestration through photosynthesis by microalgae is one of the most promising biological technologies. Algal samples from pure culture of Euglena gracilis were used for the CO_2 sequestration in bubble column reactor. The factors affecting Biological fixation of CO_2 bubble column were optimized. Light intensity of 5.0 Klux, flow rate of 1.5lpm with CO_2 concentration of 35.9%, were found to be optimum. Sparger was designed to optimize the size of bubbles for better gas hold up, to increase the resident time and mass transfer rate thereby CO_2 sequestration rate. In this study biomass production rate, effects of CO_2 on chlorophyll content and antioxidant activity of biomass produced were also carried out to understand the CO_2 sequestration by microalgae.

Keywords: *CO_2 sequestration, Euglena gracilis, Microalgae, Photobioreactor, Modeling.*

1. Introduction

The ever increasing consumption of fossil fuels by humankind has resulted in a rapid increase of carbon dioxide (CO_2) in the atmosphere [1]. Atmospheric increases of CO_2 are positively correlated with the amount of fossil fuels being burned [2].The amount of CO_2 has increased from 316 ppm in 1959 to approximately 370 ppm in 2000 and 390 ppm in 2010 as per the Keeling curve, Mauna Loa Observatory (Hawaii, US) [3]. Increased concentration of CO_2 in the atmosphere is considered as one of the main causes of global warming.

In order to reduce the impact of CO_2 on global warming sequestrations of CO_2 from the industries are today's demand [4].Sequestration of CO_2 by physical methods found to have disadvantages, hence in order to alleviate this problems biological method of CO_2 sequestration using algae was used. This has several advantages like mitigating CO_2, producing biofuels and other interesting secondary metabolites. [5].

To realize workable biological CO_2 fixation systems, selection of optimal microalgae species is vital. The selection of optimal microalgae species depends on specific strategies employed for CO_2 sequestration [6]. For this study, *Euglena gracilis* was used for CO_2 sequestration. *Euglena gracilis* is a common microalga, easy to grow and it have 45% CO_2 tolerance [7]. Although a tremendous number of studies exist in the literature, bubble columns are still not well understood due to the fact that most of these studies are often confined only with gas or liquid phases [8]. In the present work a bubble column reactor along with few important parameters were studied for growth kinetic modeling and production of some pigments and antioxidants

2. Materials and Methods

2.1. Experimental Growth chamber set up

A cuboidal glass chamber of dimensions 60.96 cm L x 45.72 cm W x 45.72 cm H with aluminium top illuminated with fluorescent tubes each of light intensity 450-500 lux fixed on the inner walls of the chamber was fabricated. The temperature was set at 22 ^oC and a 12 h light/dark cycle was maintained for all experiments. All the lab scale experiments were conducted inside this chamber at the optimized conditions.

2.2. Reactor studies

A glass cylinder of 97cm length 8.2 cm inner diameter and 9.9 cm outer diameter with glass inlet at the bottom and one outlet at the top as an exhaust was used. It was connected to two gas flow rotameters, one for air and other for CO_2.

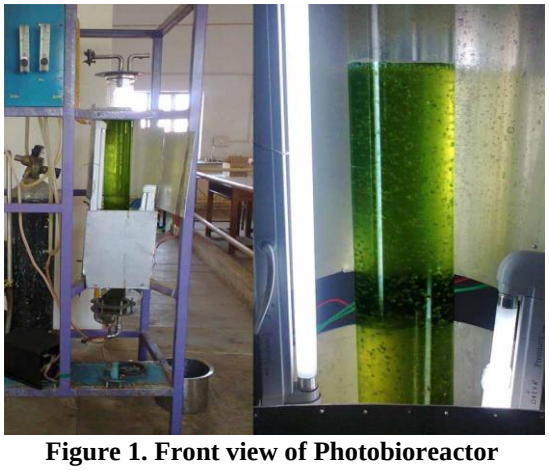

Figure 1. Front view of Photobioreactor

The control trails were conducted by sparging ambient air containing CO_2 concentration of 0.03% throughout the incubation period in the photo reactor. Successive trails were conducted to optimize the flow rate of CO_2, light intensities and bubble diameter with three different sparger assemblies (sparger 1 & sparger 2 and sparger 2 with 2 mesh plates) as shown in figure below.

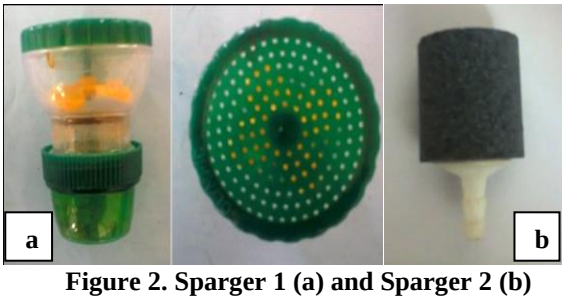

Figure 2. Sparger 1 (a) and Sparger 2 (b)

2.2.1. Mass Transfer Coefficient

Gas holdup is a dimensionless key parameter and plays an important role in design and analysis of bubble columns [9]. Volumetric mass transfer coefficient (K_La) is important design parameter for bubble columns. K_La was calculated for three trials at optimized flow rate of 35.3 % CO2 with three different bubble diameters formed by sparger1, 2 and sparger 2 with mesh.

To calculate K_L value an equation proposed by Hughmark for individual bubbles was used and this is the highly recommended equation when the bubbles have large diameters (say above 2.5 mm) [10].

$$K_L = \frac{D_{CO_2}}{d_B}\left[2 + 0.061(Re_B)^{0.779}\left(\frac{\mu_L}{\rho_L D_{CO_2}}\right)^{0.546}\left(\frac{d_B g^{0.33}}{D_{CO_2}^{0.66}}\right)^{0.116}\right] \quad (1)$$

$$Re_B = \frac{d_B V_t \rho_L}{\mu_L} \quad (2)$$

Where,

$$V_t = \sqrt{\frac{2\sigma}{d_B \rho_L} + \frac{g d_B}{2}} \quad (3)$$

The bubble mean diameter (d_B) can be calculated using image analysis software and gas holdup is a volume fraction so it can be easily calculated. From these values, it is possible to obtain the interfacial area per unit volume [11], viz.

$$a = \frac{6\varepsilon_g}{d_B} \quad (4)$$

2.2.2. Determination of Biomass and CO_2 Fixation Rate

The biomass concentration was estimated from the optical density of the culture, which was measured at 680 nm wavelength with a 1cm light path in a spectrophotometer. The relationship between the biomass concentration and the turbidity (as optical density) had previously found to be [12].

$$C_{bm} = 0.68 \times OD_{680} \quad (5)$$

$$P = \frac{C_{bm}}{\text{incubation period in days}} \quad (6)$$

$$P_{CO_2} = \frac{0.5 \times P \times 44}{12} \quad (7)$$

CO_2 fixation rates were calculated from the biomass productivity by using 50% as the carbon content of dried cells [13].

2.3. Pigment Extraction and Analysis

Chlorophylls exhibit two major light absorption bands, one on the blue side of the visible spectrum (< 460 nm) and one in the red (630–670 nm). The Chlorophylls and Carotenoids were extracted from the algal biomass with acetone and analyzed spectrophotometrically by the method of Alain Aminot [14]. Biomass was filtered by glass-fibre filter (Whatman GF/F type, 25 mm or 47 mm in diameter). The dried vacuum filtered samples were macerated with a mortar and pestle and steeped in cold 90% acetone to extract pigments from algal cells. The extracts were transferred to the graduated centrifuge tubes and made up to exactly 10 ml with 90% acetone

and the centrifuged for 10 minutes at 9000 rpm at 4 ^{0}C. The supernatants were carefully decanted into other set of graduated tubes and volumes of supernatant were noted. Since chlorophyll is sensitive to light and heat, graduated tubes were wrapped with aluminum foil and spectrophotometer readings were taken in dark room under subdued light and low temperature. Wavelength scan of the supernatants was done between 350 nm to 750 nm and optical densities were measured at 665, 661.5, 645, 630 and 470nm. The chlorophyll a, chlorophyll b and the total chlorophyll content were calculated by the following formulae called 'trichromatic equations' [15].

$$C_a = \frac{[11.85 \times OD_{664}) - 1.54 \times (OD_{647}) - 0.08 \times (OD_{630})] \times V_1}{V_2 \times L} \quad (8)$$

$$C_b = \frac{[20.13 \times (OD_{645}) - 4.19 \times (OD_{661.5})] \times V_1}{V_2 \times L} \quad (9)$$

$$C_t = \frac{[7.05 \times (OD_{661.5}) + 18.09 \times (OD_{645})] \times V_1}{V_2 \times L} \quad (10)$$

2.4. Total anti-oxidant activity

Total antioxidant activity of the extracts was measured by ferric reducing antioxidant power (FRAP) assay. The stock solutions to conduct FRAP assay include 300 mM acetate buffer- pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl3{\cdot}6H_2O$ were prepared. The fresh working solution was prepared by mixing the above mentioned solutions in the ratio 10:1:1 (v/v/v) respectively. The dry algal biomass is mixed with methanol in the ratio 1:10 (w/v) and thoroughly macerated. The mixture was centrifuged at 8000 rpm for 10 minutes and the supernatant was collected. The volume of supernatant is noted. 100 µL of this methanol extract was mixed 3 ml of the FRAP solution and incubated for 6 min. Absorbance of the colored product (ferrous tripyridyltriazine complex) was read at 593 nm using UV. The calibration curve was obtained using Ascorbic acid standard. Results were expressed in µM Ascorbic acid/g dry mass of biomass.

2.5. Kinetic Modeling of *Euglena gracilis*

Various unstructured models were proved efficient for characterizing the fermentation kinetics. In an unstructured model, the cellular representations are single component representations [16]. The exponential growth phase may be characterized by the following first order equation which states that the rate of increase of biomass is proportional to the quantity of viable cell mass at any instant time,

$$\frac{dX}{dt} = \mu X \quad (11)$$

Where $\frac{dX}{dt}$ is the growth rate (g/L day); X is the concentration of biomass (g/L); µ is the specific cell growth rate (1/day).

The growth of cell is governed by a sigmoidal relationship and there is a limit to the maximum attainable cell mass concentration. Such growth kinetics is described by logistic equation,

$$\frac{dX}{dt} = \mu_{max}\left(1.0 - \frac{X}{X_{max}}\right)X \quad (12)$$

Where μ_{max} the maximum specific growth is rate (1/day) and X_{max} is the maximum biomass concentration (g/L). Eq. on integration using $X = X_0 (t = 0)$ gives a sigmoidal variation $X(t)$ that may empirically represent both an exponential and a stationary phase.

$$X(t) = \frac{X_0 e^{\mu_{max} t}}{1 - \left(\frac{X_0}{X_{max}}\right)\left(1 - e^{\mu_{max} t}\right)} \quad (13)$$

The kinetic parameter, μ_{max} in this equation was determined by rearranging equation as,

$$\mu_{max} t = \ln\left[\frac{X_{max}}{X_0} - 1.0\right] + \ln\left[\frac{X(t)}{X_{max} - X(t)}\right] \quad (14)$$

With the logistic equation, a plot of $\ln\left[\frac{X(t)}{X_{max} - X(t)}\right]$ vs time (t) gives straight line of slope μ_{max} and intercept $-\ln\left[\frac{X_{max}}{X_0} - 1\right]$.

3. Results and Discussions

Microscopic examination of *Euglena gracilis* after preliminary experimental trial in a growth chamber setup was carried out to check the purity of the culture using digital microscope (Gippo, Japan).

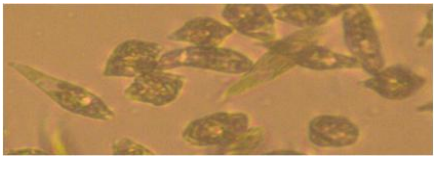

Figure 3. ***Euglena gracilis***

3.1. CO_2 sequestration

CO_2 sequestration rate was optimized by selecting suitable flow rate, decrement in bubble size, increasing resident time and optimization of intensity of light. Initial experimental trials were carried out to optimize the concentration of CO_2 for the production of biomass data as shown in Fig.4.

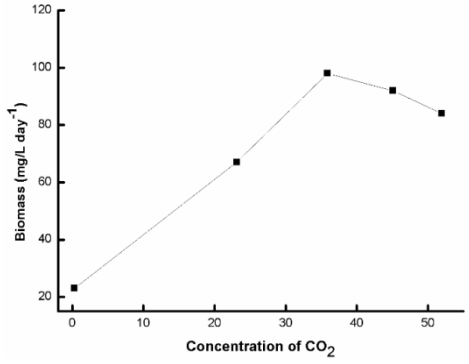

Figure 4. Effect of CO_2 on Biomass Production

3.1.1. Flow rate

In this study to select a suitable flow rate five different flow rates ranging from 1.0 to 2.0 lpm of the air – CO_2 mixture under same proportion were tried. As shown in Fig. 5, biomass production rate (P) and CO_2 fixation rate (P_{CO2}) were calculated for the above mentioned flow rates. At 1.5 lpm both P and P_{CO2} were found to be higher compared to 1.0 and 1.25 lpm, this may due to the increase in mass transfer rate [17]. However for higher flow rates i.e, 1.75 and 2.0 lpm a decreasing trend in the P and PCO_2 were observed which may be due to lower resident time and increased shear forces that might affected the growth of *Euglena gracilis*. Thus an optimum flow rate of 1.50 lpm was used for further studies.

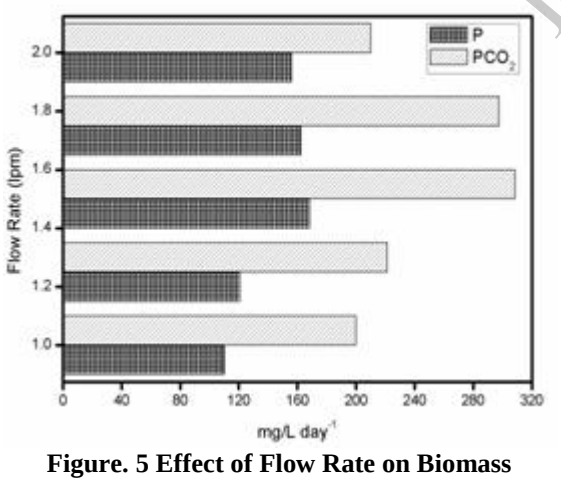

Figure. 5 Effect of Flow Rate on Biomass Production and CO_2 Fixation

3.1.2. Effect of Bubble Diameter on Biomass Production and CO_2 fixation rate

The gas to liquid mass transfer depends on the interfacial area between the two phases which can be increased by the reducing the bubble diameter. Thus different geometry and orientation of the spargers where tried in the bubble column to optimize the bubble size and its resident time. The bubble size distribution was analyzed using visual aids and Image analysis software. Experiments were performed at optimized CO_2 concentration (35.9%), light intensity (5.0 Klux) and gas flowrate (1.5 lpm). Approximately 7.53 and 4.21 mm diameter of bubbles were obtained using sparger 1 and sparger 2. Whereas bubbles of approximately 4.02 mm diameter were achieved throughout the column with the use of two mesh plates placed at equal intervals to break the coalesced bubbles and increase their resident time. Thus the estimated mass transfer rate found to increase for the reduced bubble size and increased resident time. Hence an enhancement in biomass production and CO_2 fixation rate can be observed from Fig. 6.

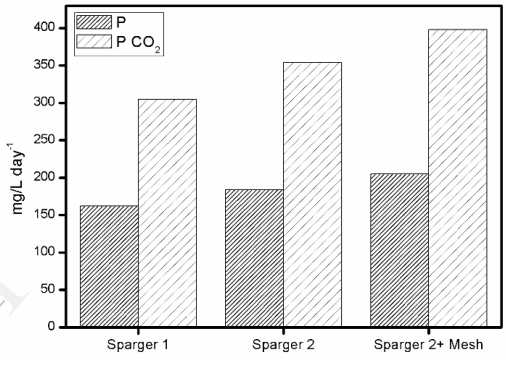

Figure 6. Effect of Bubble Diameter on Biomass Production and CO_2 Fixation rate

3.1.3. Light Intensity

Light intensity plays an important role in photosynthesis, in plants and microalgae. Five trials were performed at optimized CO_2 concentration of 35.9% and at three different light intensities (1.72, 3.28, 5.0, 6.72 and 8.44 Klux) as shown in Fig.6. It was observed that at 5.0 Klux the biomass production rate and CO_2 sequestration rate was high than at the other trials. This may be due to the uniform distribution of light intensity throughout the chamber. Though the biomass production rate and CO_2 fixation rate at 6.9 Klux light intensity was the same as that of 5.0 Klux, it had a negative effect on the pigment production

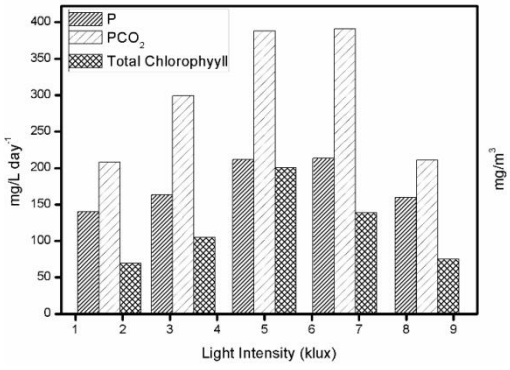

Figure 7. Effect of Light Intensity on Biomass Production and CO2 Fixation rate

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3.1.4. Gas hold up and Volumetric Mass Transfer Coefficient

The superficial gas velocity in the photobioreactor was studied to analyze its effect on the gas holdup and mass transfer coefficient. The experimental gas holdups for three different superficial gas velocities were shown in Fig.8.

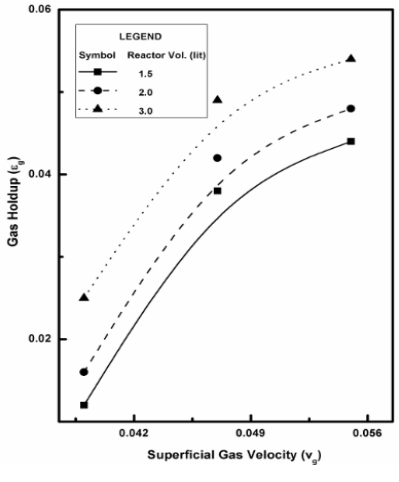

Figure 8. Gas holdup at different gas velocities

The volumetric mass transfer coefficient is a key parameter in the characterization and design of both industrial stirred and non-stirred gas–liquid reactors [18]. To check the consistency of the experimental values, empirical correlations were used from the literature. The three different bubble diameters obtained using different spargers were used. K_La was calculated using correlation by Hughmark which is suitable for bigger bubbles and it was compared with experimental mass transfer coefficient values.

Table 1. Values for K_La at different bubble diameters

Bubble size (mm)	Theo. K_La (s^{-1})	Expt. K_La (s^{-1})	a (m^{-1})	ε	ρ_L (kg/m^3)
7.53	0.019	0.021	39.58	0.049	975
4.21	0.034	0.029	72.68	0.051	990
4.02	0.036	0.031	76.84	0.052	992

Volumetric mass transfer coefficient also depends on gas liquid interfacial area (a) and it was increased as bubble size decreases. Bubble diameter also affects gas holdup and bubble rise velocity. The estimates obtained for the various (related) coefficients are summarized in Table 1. Experimental and theoretical values for K_La were plotted as shown in Fig.9. It was observed that the mass transfer rate decreased as bubble size increased, because bigger bubble gives less gas-liquid interfacial. The overall mass transfer coefficients calculated for CO_2 transfer into the culture of microalgae; mass transfer is enhanced by the uptake of CO_2 by the algal cells. In fact, several authors [19] have claimed that the transfer of CO_2 in micro algal cultures may be as much as 80% higher than the one observed for a purely physical absorption process.

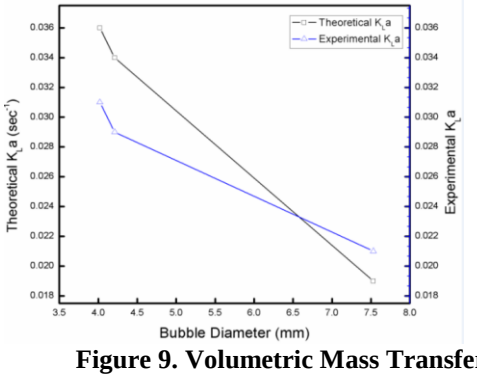

Figure 9. Volumetric Mass Transfer Coefficient

3.2. Effect of CO_2 on pigments and antioxidant activity

Five experimental trials were performed to check the effect of different concentrations of CO_2 (0.3%, 23.12%, 35.9%, 45.08% and 51.95%) on pigments and antioxidant activity at the optimized light intensity of 5.18 Klux in the sparger 2 with mesh at the optimized flow rate of 1.5 lpm. It clearly indicates that *Euglena gracilis* utilize CO_2 from the gas mixture for photosynthesis. Utilization of CO_2 was enhanced by decreasing the bubble size using sparger 2 with mesh which thereby increases the mass transfer rate in the bubble column photobioreactor.

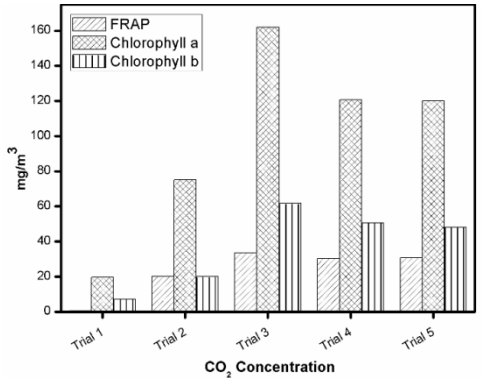

Figure 10. Effect of CO_2 on pigments and antioxidant activity

It was observed that the production of chlorophylls was not influenced by CO_2 as shown in Fig.9. Pigment production was successfully increased with increasing growth of *Euglena gracilis.* Production of chlorophyll a and chlorophyll b increased as the CO_2 concentration increased upto the 3rd trial then it decreased. It clearly indicates the response of *Euglena gracilis* against the approach of CO_2 towards the tolerance limit (45% CO_2 in air).

The antioxidant activity increased 8 times in the 2nd trial compared to the 1st. In every trial antioxidant activity was increased because with increasing growth of *Euglena gracilis* along with CO2 sequestration level of 35.9% beyond which it decreased.

3.3. Kinetic Modeling of *Euglena gracilis*

Kinetic study of the algal biomass is an important parameter for determining its suitability for commercial or large scale production for the extraction of oil, pigments and other value added products.

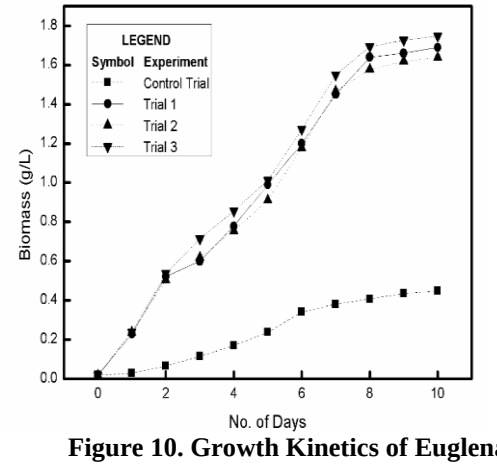

Figure 10. Growth Kinetics of Euglena gracilis in a photobioreactor

Fig. 10 indicates that for the curves, control trial (air + 0.3% CO_2) and test trial (air + 35.9% CO_2) respectively, the variation in algal density as a function of time exhibits sigmoidal growth kinetics. The Euglena's growth rate increases with CO_2 concentration and increasing the mass transfer rate. This was evident in the log phase of the test trial (sparger-2 with 2 mesh plates).

The parameter μ_{max} in Logistic equation was determined from the plot ln $(X/X_{max}-X)$ vs time (t). The slope of straight line which represents μ_{max} was found to be 0.87 (day^{-1}). X_0 and X_{max} was found to be 0.038 (g/L), 1.665 (g/L) respectively from intercept. Using the values of μ_{max} and X_0 biomass concentration was predicted according to equation. Fig.12 shows the comparison of experimental and the logistic model predicted biomass concentration. The correlation coefficient (R^2) value of 0.992 was obtained with logistic model for biomass.

Nomenclature

a	gas-liquid interfacial area per unit volume of liquid (L^{-1})
C_a	chlorophyll a (M/L^3)
C_b	chlorophyll b (M/L^3)
C_{bm}	biomass concentration (M/L^3)
C_t	total chlorophyll (M/L^3)
d_B	bubble diameter (L)
D_{CO2}	diffusivity of CO_2 (L^2/T)
g	acceleration of gravity ($L\ T^2$)
K_L	volumetric liquid side mass transfer coefficient (L/T)
L	path length of cuvette used in spectrophotometer (L)
P	biomass productivity (M/L^3T)
Re	Reynolds number (dimensionless)
V_1	volume of supernatant after centrifugation (ml)
V_2	volume of culture filtered (lit.)
V_t	bubble rising velocity (L/T)
μ_L	viscosity of liquid (M/LT)
ρ_L	density of liquid (M/L^3)
σ	surface tension of liquid (M/T^2)
ε	gas holdup (dimensionless)

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