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RESEARCH PAPER

Experimental Study and Numerical Modeling of CO_2 Bio-Fixation in a Continues Photobioreactor

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Abstract

A dynamic numerical model was developed to predict the biomass concentration, pH, and carbon dioxide fixation rate in the continuous culture of cyanobacteria in a photobioreactor. The model is based on the growth rate equation of microalgae combined with mass transfer equations for gas and liquid phases in the photobioreactor as well as thermodynamic equilibrium of inorganic carbon ions in the culture media. The model was validated by comparing its predictions with experimental results obtained from turbidostat cultivation of Synechocystis in a flat-plate photobioreactor. Optical density, pH, and CO_2 concentration in outlet gas were measured continuously in this photobioreactor. The model was used to simulate this system at the same conditions that the experiments were performed at two light intensities of 75 mE/m2/s and 150 mE/m2/s. Although the growth rate and outlet gas CO2 concentration were quite different at these two light intensities, the model predicted the system behavior accurately. The average error in the prediction of biomass concentration, pH, and outlet gas CO_2 concentration was 0.40%, 0.61%, and 0.34%, respectively.

Keywords:
CO_2 fixation,
Dynamic Model,
pH,
Turbidostat Culture,
Synechocystis

Introduction

Excessive use of fossil fuels will release a huge amount of environmental pollutions which can cause health problems and climate change [1]. Therefore, finding alternative renewable energy sources is always in the focus of attention. Microalgae and cyanobacteria are one of the promising alternative sources for producing green fuels. These microorganisms have the ability to store significant amounts of energy-rich compounds such as lipids which can be used in biofuels production. They also have a high capacity for CO_2 capture and they can grow in nonagricultural lands [2].

Microalgae are generally cultivated photoautotrophically, which means that they use light as the energy source and inorganic carbon like carbon dioxide as the carbon source [3]. In this type of cultivation, the emission of carbon dioxide as a greenhouse gas reduces exceedingly [4]. The microalgae biomass production and carbon dioxide consumption are influenced by a large number of factors. Different microalgae species have a distinct ability of biomass production. Cultivation conditions like light, pH, and nutrients also affect the algal growth and CO_2 fixation [5-8].

Numerous kinetic models have been developed to understand microalgae growth. Most of these studies use individual Monod kinetic models, which are mostly developed based on regression analysis of measured data [9]. These kinetic models can be used to study the effect

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of multiple parameters on microalgae growth, such as light intensity, nutrient availability, dissolved CO_2 concentration, temperature, and dissolved oxygen concentration [10]. Some of these parameters, like pH and CO_2 concentration that are still worthy of being studied. It has been shown that pH is related to the concentration of dissolved CO_2 in the medium. Tamburic et al. [11] have demonstrated that algal growth is enhanced by increasing CO_2 concentration. However, at high CO_2 concentration growth rate is inhibited because of a reduction in the pH of the culture. Therefore, it is crucial to develop a dynamic model of microalgae to monitor and control the system and also understand different phenomena involved in the cultivation system. Having such a model will also assist in predicting and understanding carbon limitation in algal growth and bioproduct accumulation systems, as inorganic carbon availability plays a critical role in algal growth and product formation.

In this study, a dynamic numerical model was developed to predict biomass concentration, pH, and carbon dioxide fixation in flat-plate photobioreactors. The model was developed by combining growth rate equations, equilibrium relations of inorganic carbon ions in culture media, and mass transfer equations of liquid and gas phase in the photobioreactor. The accuracy of the model was examined by comparing its prediction results with experimental data obtained from turbidostat cultivation of *Synechocystis* in a flat-plate photobioreactor.

Experimentation

Algal strain and culture media

Synechocystis sp. PCC6803 was firstly inoculated in 100-mL flasks containing 25 mL autoclaved modified BG-11 medium [12] with supplemented with 5 mM $NaHCO_3$ and were incubated in a shaking incubator under continuous fluorescence illumination (50–70 µE/m^2/s) and at 120 rpm and 30 °C. Cells were transferred to the photobioreactor when they reached the logarithmic phase as determined by optical density (OD) at 730 nm using a spectrophotometer, model Novaspec II. The wavelength of 730 nm was chosen since OD in this wavelength has a good correlation with cell concentration [13].

Cultivation System

A flat-plate photobioreactor with a working volume of 960 ml was used for the turbidostat cultivation of *Synechocystis*. The schematic diagram of this photobioreactor is shown in Fig.1. The temperature of the culture was kept constant at 30 ±0.2 °C. The photobioreactor was illuminated from one side by a LED panel illuminating red light with λ_{max} of 635 nm. The red light was chosen since based on previous studies it is the most efficient color in the visible spectrum for the cultivation of *Synechocystis* [14]. The temperature and pH of the culture were measured continuously using a combined pH/temperature probe. Nitrogen gas containing 0.5% CO_2 (v/v) was injected into the photobioreactor at a flow rate of 150 mL/min. Absorbed carbon dioxide was determined by measuring the CO_2 concentration at the outlet gas using the Senserion carbon dioxide sensor (SCD30, Switzerland). After inoculation, the culture was subsequently grown and the optical density (OD_{730}) of the culture was measured continuously using by an inbuilt densitometer. OD Measurements of all sensors were recorded at one-minute intervals. A peristaltic pump with a flow rate of 6.35 ml/min was used to inject the modified BG-11 medium to the photobioreactor. The pump was regulated by using an ON/OFF controller to maintain the OD_{730} within 0.4±0.004. The growth rate of the culture is calculated from the curve of the OD_{730} slope between the dilutions as $\mu = \Delta \ln(OD_{730}) / \Delta t$.

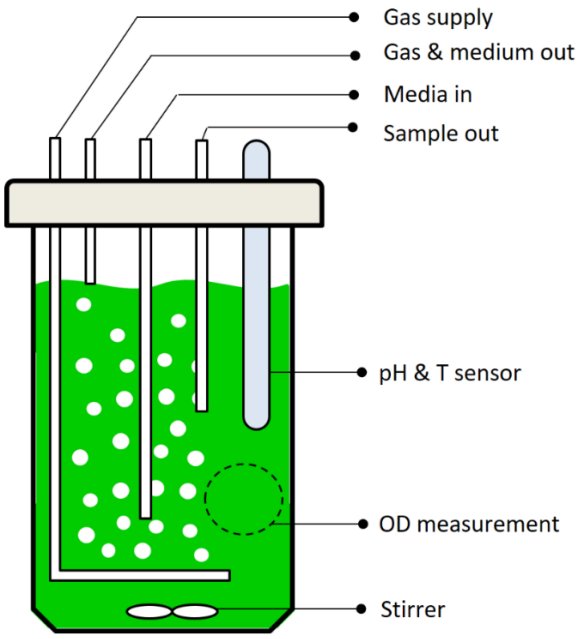

Fig. 1. Schematic diagram of the photobioreactor used for turbidostat experiments.

Dynamic Numerical Model

To accurately estimate the growth behavior of *Synechocystis* as well as dynamic variations of pH and absorbed CO_2 in the photobioreactor, a mathematical model is developed and presented in this section. The model was developed by combining the mass balance equations for gas and liquid phase in a photobioreactor, the growth rate kinetics, and algebraic equations describing the equilibrium of compounds in the culture media.

Mass Balance Model

Liquid Phase

The global volumetric growth rate of microalgae is expressed as follows [15]:

$$\frac{dX}{dt} = (\mu - D)X \tag{1}$$

where μ is the specific growth rate (in h^{-1}), X is the biomass concentration (in 10^9 cells/lit), and D is the dilution rate (in h^{-1}), the ratio of medium flow rate F to the culture volume V_l in the photobioreactor. The specific growth rate (μ) is described by the association of the Monod equation for the light effect and the Contois equation for the effect of total inorganic carbon (TIC) in culture media [16]:

$$\mu = \mu_{max}\left(\frac{E}{K_E + E}\right)\left(\frac{C_{TIC}}{K_{CL}X + C_{TIC}}\right) \tag{2}$$

where μ_{max} is the maximal specific growth rate, K_E is the half-saturation constant, E is light intensity available per cell (in $\mu E\ s^{-1}m^{-2}$), and K_{CL} is the half-saturation constant for TIC (in mol /(10^9 cells)). The parameter E is described by the light transfer modeling:

$$E = \frac{(I_{in} - I_{out})A_r}{V.X} \tag{3}$$

where I_{in}, I_{out} and A_r are the incident and outgoing light intensities, and the reactor illuminated area, respectively. The outgoing light intensity is calculated by an analytical expression as a function of the biomass concentration and the incident light intensity through the following empirical expression:

$$I_{out} = C_1 I_{in} X^{C_2} \tag{4}$$

where C_1 and C_2 are constants depending mainly on the reactor geometry. Total inorganic carbon balance in the media can be determined by:

$$\frac{dC_{TIC}}{dt} = -\langle r_{TIC} \rangle + N_{CO_2} + D(C_{TIC,in} - C_{TIC}) \tag{5}$$

where $C_{TIC,in}$ is TIC concentration in inlet media, $\langle r_{TIC} \rangle$ is TIC consumption rate given by Eq. 6:

$$\langle r_{TIC} \rangle = \frac{1}{M_x} \langle r_x \rangle \tag{6}$$

Where M_x is biomass to TIC conversion yield.

Gas Phase

Based on the ideal gas law and gas balance equations, the molar fractions of the output gases can be computed, assuming that the flow and concentration of all inlet gases are known and measurable. Thus, the time variation of mole fraction of carbon dioxide in the gas phase, $y_{out}^{CO_2}$ is expressed by:

$$\frac{dy_{out}^{CO_2}}{dt} = \frac{RT}{PV_g}\left(Gy_{in}^{CO_2} - Gy_{out}^{CO_2} - V_l N_{CO_2}\right) \tag{7}$$

where R is the universal gas constant, T is the temperature, and V_g is gas volumes. G is the volumetric flow rate of gas to the photobioreactor. N_{CO_2} is the gas-liquid mass transfer rate of carbon dioxide given by Eq. 8:

$$N_{CO_2} = K_{La,CO_2}\left(\frac{y_{avg}^{CO_2} P}{H_{CO_2}} - [CO_2]\right) \tag{8}$$

where P is the total pressure in the gas phase, H_{CO_2} is Henry's constant, $(K_{La})_{CO_2}$ is the overall volumetric mass-transfer coefficient for carbon dioxide and $y_{avg}^{CO_2}$ is the average mole fraction between inlet and outlet gasses.

Equilibrium Model of Components in Culture Media

Upon dissolution in culture media, carbon dioxide presented as four different compounds (CO_2, H_2CO_3, $HCO_2^{-1,}$ and CO_3^{2-}) and their equilibrium concentrations were pH-dependent. The carbonate equilibriums between them and the corresponding equilibrium constants (K_1, K_2, and K_w) were as follows [17]:

$$k_1 = \frac{[H^+][\mathrm{HCO_3^-}]}{[\mathrm{CO_2}]} = 10^{-6.381} \tag{9}$$

$$k_2 = \frac{[H^+][\mathrm{CO_3^{2-}}]}{[\mathrm{HCO_3^-}]} = 10^{-10.377} \tag{10}$$

$$k_w = [H^+][OH^+] = 10^{-14} \tag{11}$$

In our system of study, TIC is the summation of $[CO_2]$, $[H_2CO_3]$, $[HCO_3^-]$, which based on Eqs. 9 and 10 can be calculated as follows:

$$C_{TIC} = [\mathrm{CO_2}] + [\mathrm{H_2CO_3}] + [\mathrm{HCO_3^-}] = [\mathrm{CO_2}]\left[1 + \frac{k_1}{[H^+]} + \frac{k_1 k_2}{[H^+]^2}\right] \quad (12)$$

To satisfy the electroneutrality constraint, the concentration of anions weighed by their charges must equal the concentration of cations weighed likewise [18]:

$$2[CO_3^{2-}] + [HCO_3^-] + [OH^-] + [An^-] = [H^+] + [Cat^+] \quad (13)$$

$[An^-]$ and $[Cat^+]$ are the concentration of anions and cations in the solution, respectively. Combination of Eq. 9 with Eqs. 11 and 13 gives:

$$\frac{2k_1 k_2[\mathrm{CO_2}]}{[H^+]^2} + \frac{k_1[\mathrm{CO_2}]}{[H^+]} + \frac{k_w}{[H^+]} + [An^-] = [H^+] + [Cat^+] \quad (14)$$

Rearranging Eq. 14 results to Eq. 15:

$$[\mathrm{CO_2}] = \frac{\frac{-k_w}{[H^+]} + [H^+] + [Cat^+] - [An^-]}{\frac{2k_1 k_2}{[H^+]^2} + \frac{k_1}{[H^+]}} \quad (15)$$

Combining Eqs. 12 and 15 gives:

$$C_{TIC} = \frac{\frac{-k_w}{[H^+]} + [H^+] + [Cat^+] - [An^-]}{\frac{2k_1 k_2}{[H^+]^2} + \frac{k_1}{[H^+]}}\left[1 + \frac{k_1}{[H^+]} + \frac{k_1 k_2}{[H^+]^2}\right] \quad (16)$$

Having C_{TIC} from the Dynamic Numerical Model section, dynamic variation in pH can be calculated by solving Eq. 16.

The parameters' values of the numerical model are summarized in Table 1. Except μ_{max} and $(K_{La})_{CO_2}$, the other parameters are either geometrical parameters or they are obtained from the literature. μ_{max} was measured by off-line washout experiments and $(K_{La})_{CO_2}$ was fitted based on the experimental data.

Table 1. Model Parameters

Parameter	value	unit	Definition
R	8314	Pa.lit/mol/K	Ideal gas constant
K_{La,CO_2}	187.563	h^{-1}	Overall volumetric mass-transfer coefficient of CO_2
H_{CO_2}	2.904×10^6	Pa.lit/mol	Henry's constant of CO_2
T	303	K	Temperature
P	101300	Pa	Pressure
V_l	0.960	lit	Culture media volume in photobioreactor
V_g	0.120	lit	Gas volume in photobioreactor
M_x	27.8	g.C/mol	Biomass to TIC conversion yield
μ_{max}	0.158	1/h	Maximal specific growth rate
K_E	0.08	µE $s^{-1}m^{-2}$	Half saturation constant of light
K_{CL}	3.8×10^{-3}	mol/(10^9 cell)	Half saturation constant of TIC
A_r	0.016	m^2	Radiating area
C_1	0.49	-	Radiation model constant
C_2	-0.92	-	Radiation model constant

Results and Discussions

Turbidostat experiments were done at two different light intensities of 75 and 150 mE/m^2/s. The numerical model described in the preceding was used to simulated the dynamic behavior of the system for those experiments.

Estimation of Biomass Concentration

Biomass concentrations for turbidostat experiments using two different light intensities of 75 mE/m^2/s and 150 mE/m^2/s are shown in Fig. 2. OD_{730} was continuously measured during these experiments and once the OD_{730} of the culture reached higher than 0.404, the peristaltic pump was turned on to dilute the culture. The pump was turned off once the OD_{730} reduced to 0.396. These ON/OFF cycles were repeated for about 20 hours and the mean growth rate was calculated by averaging the growth rate at each cycle. Mean growth rate for 75 mE/m^2/s and 150 mE/m^2/s illumination were 0.0317±0.0024 1/h and 0.0598±0.0039 1/h, respectively. These results are in agreement with the results of Zavrel et al. [19] which reported a growth rate of 0.085 1/h for *Synechocystis* under 220 mE/m^2/s red light illumination. The biomass concentration during these ON/OFF cycles was predicted using the numerical model described in section 2 and the results are shown in Fig. 2. As can be seen, the perfect prediction was obtained by the model for both light intensities. The average absolute deviation in these simulations was 0.4 %. Mean growth rate predicted from simulations for 75 mE/m^2/s and 150 mE/m^2/s were 0.0335±0.0043 1/h and 0.0626±0.0054 1/h, respectively. These values are quite similar to the ones obtained from experimental measurements.

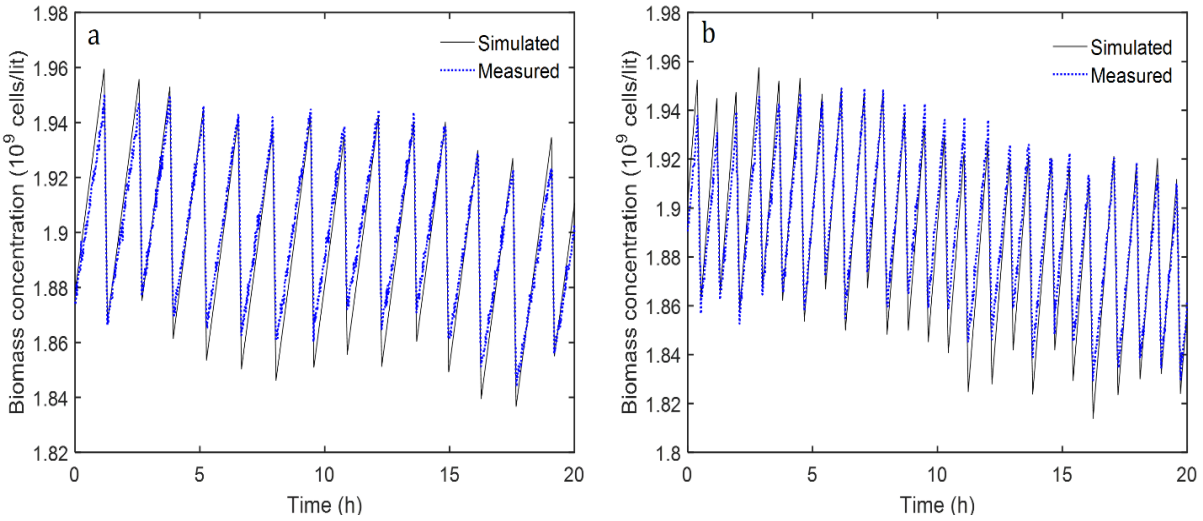

Fig. 2. Simulated biomass concentration compared to experimental data for turbidostat culture exposed to a) 75 mE/m^2/s red light and b) 150 mE/m^2/s red light.

Estimation of CO_2 Fixation Rate

The outlet gas CO_2 concentration was measured for the last four cycles of the turbidostat experiments and the results are shown in Fig. 3. As can be seen, a good prediction was obtained by the proposed model. The average absolute deviation of the model predictions was 0.34%. The outlet gas CO_2 concentration for the light intensity of 150 mE/m^2/s was lower than that of the 75 mE/m^2/s, which is due to the higher growth rate in the former case. In average, CO_2 concentration in outlet gas was 0.479 % and 0.448% for light intensities of 75 mE/m^2/s and 150 mE/m^2/s, respectively. By taking the photobioreactor footprint area of 0.0064 m^2, the CO_2 bio-fixation rates for these two light intensities were 23.4 g/m^2/day and 57.9 g/m^2/day, respectively. According to these results, while the growth rate at 150 mE/m^2/s light intensity was 89% higher

than that of 75 mE/m^2/s, the CO_2 bio-fixation rate at 150 mE/m^2/s light intensity was 147% higher than that of 75 mE/m^2/s. These results indicated that increasing the light intensity not only enhanced the growth rate but also enhanced carbon fixed per biomass. Zhang et al. [20] reported the CO_2 bio-fixation rate of 51 g/m^2/day for *Synechocystis*, which is in accordance with the present results.

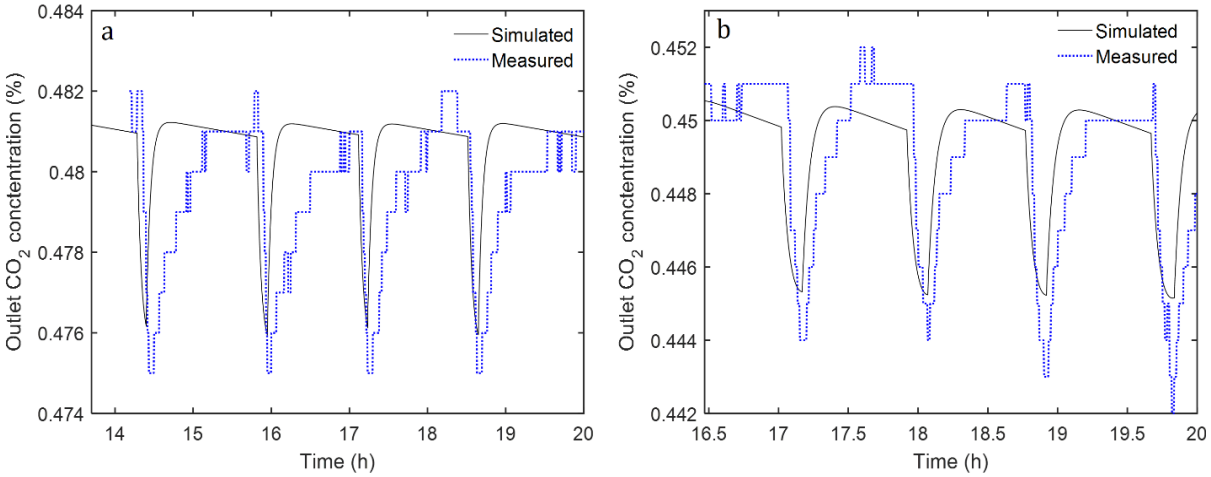

Fig. 3. Simulated outlet CO_2 concentration compared to experimental data for turbidostat culture exposed to a) 75 mE/m^2/s red light and b) 150 mE/m^2/s red light.

Estimation of pH

Simulated and measured pH of the culture for the light intensities of 75 mE/m^2/s and 150 mE/m^2/s was shown in Fig. 4. As can be seen, there is a good agreement between simulation results and experimental data. While the growth rate and CO_2 fixation rate were enhanced by increasing the light intensity, pH did not change significantly. This can be due to the presence of buffer in culture media.

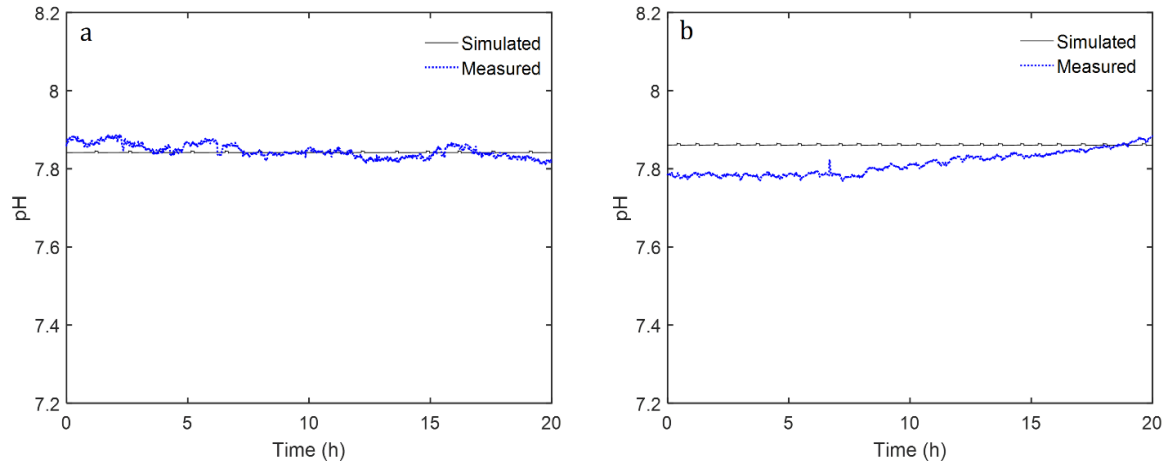

Fig. 4. Simulated pH compared to experimental data for turbidostat culture exposed to a) 75 mE/m^2/s red light and b) 150 mE/m^2/s red light.

Concentration of Inorganic Carbon Ions in Culture Media

The concentration of inorganic carbon ions in the culture media including CO_2, CO_3^{2-} and HCO_3^- are calculated based on the equilibrium model described in section 3.3 and the results are shown in Fig. 5. According to these results, there is no significant difference in ions concentration for light intensities of 75 mE/m^2/s and 150 mE/m^2/s. This is due to the fact that inorganic carbon

concentration is highly dependent on pH, and pH was almost the same for the two light intensities. According to Fig. 5, the main inorganic carbon ion in culture media was HCO_3^- and CO_2 and CO_3^{2-} are not dominant in culture media. This is in agreement with previous studies [17], which stated that in the pH range of 6 to 11, all the inorganic carbon ions are converted to HCO_3^-.

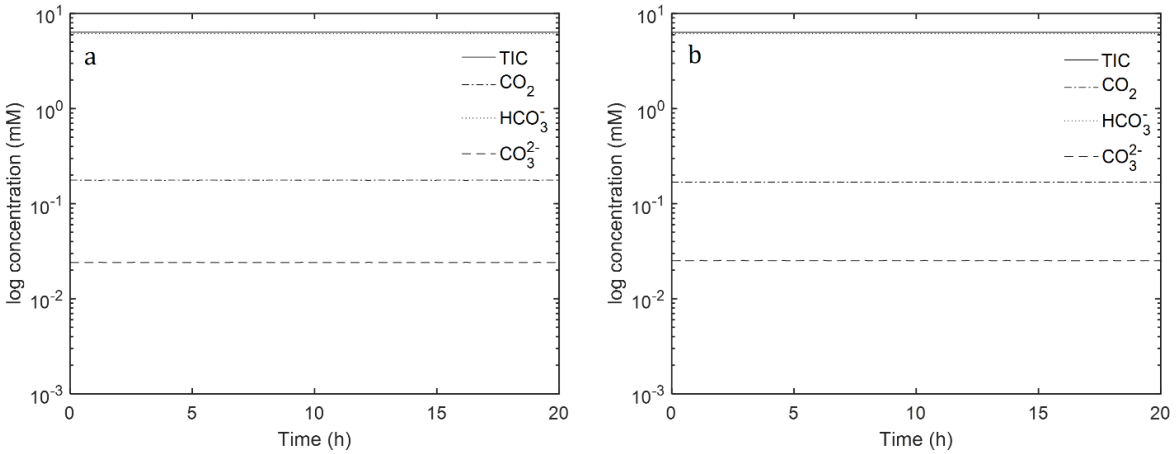

Fig. 5. Simulated concentration of inorganic carbon ions in turbidostat culture exposed to a) 75 mE/m^2/s red light and b) 150 mE/m^2/s red light.

Conclusions

Recently, there has been increasing interest in using biological methods for sequestering greenhouse gasses. Cultivation of microalgae and cyanobacteria is a promising approach that not only can capture CO_2 from the atmosphere, but also can be used for the production of various biological products. In this study, dynamic variation in biomass concentration, pH, and CO_2 fixation of cyanobacteria were investigated numerically and experimentally. A flat-plate photobioreactor is used for turbidostat cultivation of *Synechocystis* radiated by red LEDs at two different light intestines of 75 mE/m^2/s and 150 mE/m^2/s. Optical density, pH, and CO_2 concentration in outlet gas were measured continuously in the photobioreactor. According to the experimental results, the growth rate of *Synechocystis* under 75 mE/m^2/s red light was 89% higher than the growth rate under 150 mE/m^2/s illumination. On the other hand, the CO_2 fixation rate was increased by 147% once the light intensity increased from 75 mE/m^2/s to 150 mE/m^2/s. A numerical model is developed by taking mass transfer equations for gas and liquid phases in the photobioreactor, growth rate, absorbed radiation, and equilibrium of inorganic carbon ions in the culture media. The model was used to predict the experimental results obtained from turbidostat cultivation. The results indicate that model predictions have a perfect match with experimental data for biomass concentration, pH, and outlet gas CO_2 concentration.

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