Technical insight on the requirements for CO₂-saturated growth of microalgae in photobioreactors

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Abstract Microalgal cultures are usually sparged with CO₂-enriched air to preclude CO₂ limitation during photoautotrophic growth. However, the CO₂ vol% specifically required at operating conditions to meet the carbon requirement of algal cells in photobioreactor is never determined and 1–10% v/v CO₂-enriched air is arbitrarily used. A scheme is proposed and experimentally validated for *Chlorella vulgaris* that allows computing CO₂-saturated growth feasible at given CO₂ vol% and volumetric O₂ mass-transfer coefficient (k_{La})_O. CO₂ sufficiency in an experiment can be theoretically established to adjust conditions for CO₂-saturated growth. The methodology completely eliminates the requirement of CO₂ electrode for online estimation of dissolved CO₂ to determine critical CO₂ concentration (C_{crit}), specific CO₂ uptake rate (SCUR), and volumetric CO₂ mass-transfer coefficient (k_{La})_C required for the governing CO₂ mass-transfer equation. C_{crit} was estimated from specific O₂ production rate (SOPR) measurements at different dissolved CO₂ concentrations. SCUR was calculated from SOPR and photosynthetic quotient (PQ) determined from the balanced stoichiometric equation of growth. Effect of light attenuation and nutrient depletion on biomass estimate is also discussed. Furthermore, a simple design of photosynthetic

activity measurement system was used, which minimizes light attenuation by hanging a low depth (ca. 10 mm) culture over the light source.

Keywords Microalgae · CO₂-saturated growth · CO₂-enriched air · Photoautotrophic growth · Photobioreactor · *Chlorella vulgaris*

Introduction

With the developments in microalgal biotechnology towards the production of sustainable and clean bioenergy, it is becoming increasingly important to improve the kinetics and yield of microalgal cultures. Low cell density and productivity heavily constrain the utilization of algae to its full potential (Eriksen 2008; Ugwu et al. 2008). Understanding the microalgal culture is challenging, but vital for improving the process performance. Light, which drives the photochemical reactions to produce usable energy for carbon fixation in algal photosynthesis, has been researched utmost to maximize the rate of photosynthesis in photobioreactors, where the cells are often light-limited due to self shading (Olivieri et al. 2014). Mathematical models of photosynthesis–light relationship, description of light regime (periodic variations in light intensity experienced by individual cells as they traverse through light gradient inside culture), and prediction of photosynthetic response to light regime are the cornerstones of photobioreactor engineering (Brindley et al. 2011; Fernández et al. 1997; Molina-Grima et al. 1999; Merchuk et al. 2007; Pottier et al. 2005; Pruvost et al. 2008; Yun and Park 2003). Carbon dioxide—the carbon source—is another crucial factor affecting photoautotrophic growth. The effect of CO₂ concentration on the rate of photosynthesis has been

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known since the pioneering experiments of the British plant physiologist F. F. Blackman in 1905. Like the depletion of oxygen in aerobic cultures, CO₂ may get limiting with the increase in algal cell density under inappropriate operating conditions.

Although the CO₂ is far more soluble in water than oxygen, the equilibrium concentration of CO₂ in water at 25 °C and 1 atm is ca. 0.6 ppm and that of O₂ is 15 times, since the volume percent of CO₂ in air (0.04%) is negligible compared to O₂ (21%). In addition, presence of salts in growth medium can reduce solubility of the gas. Microalgal cultures in PBRs, therefore, are usually sparged with CO₂-enriched air (1–10% v/v CO₂, generally 5%) to avoid carbon limitation. However, this practice has a serious shortcoming which has been overlooked. Sparging the culture with CO₂-enriched air cannot ensure that the cells will never be CO₂-limited throughout the course of cultivation. Analogous to critical O₂ concentration for aerobically respiring cells, microalgal cells will not be CO₂-limited as long as the dissolved CO₂ concentration is maintained above the critical CO₂ concentration (C_{crit}). Below C_{crit} , the specific CO₂ uptake rate (SCUR) should decrease. It implies that to maintain non-limiting concentration of CO₂ in microalgal cultures, C_{crit} should be determined and dissolved CO₂ should be maintained above C_{crit} . Apparently, there are at least three technical problems associated with the determination of C_{crit} and maintaining the dissolved CO₂ above it. First, measuring SCUR at different dissolved CO₂ concentrations to determine C_{crit} and monitoring dissolved CO₂ require online measurement of dissolved CO₂ with CO₂ electrode. Unlike the ease in measuring dissolved oxygen (DO) with common oxygen electrodes integrated in bioreactors, sterilizable CO₂ electrodes are uncommon and expensive. Second, even if the CO₂ electrode is available, the study could be perplexing, as the rate of photosynthesis, and hence the rate of carbon fixation, is not only dependent on dissolved CO₂ but also largely on light intensity. Third, since the growth of microalgae is very slow, it is likely that the SCUR measurements could be very long in duration. However, the experiments for the measurement of SCUR must be short for the same reason which requires short experiments for measurement of specific oxygen uptake rate for aerobically respiring cells. These inherent difficulties in the measurement of C_{crit} probably prevented algal researchers to determine C_{crit} and to assure CO₂ saturation by maintaining dissolved CO₂ greater than C_{crit} . Rather, sparging the microalgal culture with an optimized level of CO₂ vol% or an arbitrary high CO₂ level is a common practice. Microalgal cells will be CO₂-saturated throughout the course of cultivation if the concentration of dissolved CO₂ in equilibrium with the gas phase CO₂ vol% is greater than

C_{crit} and the prevailing CO₂ mass-transfer rate prevents the dissolved CO₂ to fall below C_{crit} . As the mass-transfer rate of CO₂ from gas to culture which replenishes depleted CO₂ is controlled by CO₂ vol% and volumetric CO₂ mass-transfer coefficient ($k_L a$)_C, the optimized CO₂ vol% should be specific for the prevailing mass-transfer. Hence, a sub-optimal CO₂ vol% may provide CO₂-replete condition in a relatively enhanced mass-transfer condition. On the other hand, since the cost of CO₂ in large-scale algal culture is substantial, using an arbitrary high CO₂ vol% will be uneconomical if a relatively lower fraction of CO₂ can maintain the same CO₂-replete condition (Chisti 2013; Tapie and Bernard 1988). Moreover, there is another common practice which needs reassessment. Since pH changes with the dissolved CO₂, it is considered as an indicator of dissolved CO₂ level in microalgal culture and is usually monitored to identify any significant depletion of CO₂ with growth. However, assimilation of nitrogen source also changes the pH of microalgal culture. Therefore, the assessment of CO₂ level in microalgal culture by pH-monitoring is not appropriate.

Realizing the need to ensure CO₂-replete condition in microalgal photoautotrophic cultures and the challenges in determination of C_{crit} , the objective of the present study is to develop a scheme to determine C_{crit} and SCUR using DO measurements instead of dissolved CO₂, and to estimate CO₂-saturated growth from species-specific parameters (C_{crit} , SCUR) and operating conditions (CO₂ vol%, CO₂ mass-transfer) to determine CO₂ sufficiency in a photobioreactor culture. CO₂ enrichment and mass-transfer required to keep microalgal cells CO₂-saturated throughout desired level of growth in photobioreactor can be, therefore, estimated. The green microalga *Chlorella vulgaris* has been used in the study to demonstrate the proposed methodology for estimation of C_{crit} and SCUR, and to validate the estimates of CO₂-saturated growth.

Materials and methods

Strain and cultivation conditions

Chlorella vulgaris (211/11B) was purchased from Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, United Kingdom. Cells were grown in Bold's Basal Medium (BBM) with twofold iron content to prevent iron limitation. Incubation temperature was 25 ± 1 °C and customized white LED light sources of 200 W with pulse width modulation (PWM) controlled brightness were used to provide the required photosynthetic photon flux density (PPFD, μmol/m²/s) to microalgal cultures.

Estimation of CO₂-saturated growth

Equation (1) can be used to estimate CO₂-saturated growth ($x_{c \text{ sat}}$), if C_{crit} and SCUR for the microalga (k_{La})_C and the equilibrium CO₂ concentration (C^*) in the growth medium at the operating CO₂ vol% are known:

$$x_{c \text{ sat}} = \frac{(k_{\text{La}})_C \cdot (C^* - C_{\text{crit}})}{\text{SCUR}}. \quad (1)$$

Henry's law (Eq. 2) can be used to approximate C^* , since the growth medium is like water:

$$C^* = \frac{m_{\text{CO}_2, \text{gas}} \cdot P_T}{H_{\text{CO}_2}}, \quad (2)$$

where $m_{\text{CO}_2, \text{gas}}$ is the mole fraction of CO₂ in the gas phase, P_T is total gas pressure, and H_{CO_2} is Henry's constant for CO₂ which is a function of temperature (H_{CO_2} in pure water at 25 °C is 29.41 L atm/mol). Determining other parameters of Eq. (1) without online measurement of dissolved CO₂ is challenging. Relationship (Eq. 3) proposed by Fair (1967) can be used to estimate (k_{La})_C from experimentally measurable (k_{La})_O (Babcock et al. 2002; Boogerd et al. 1990):

$$\begin{aligned} (k_{\text{La}})_C &= (k_{\text{La}})_O \left[\frac{D^{\text{CO}_2}}{D^{\text{O}_2}} \right]^{0.5} \\ &= (k_{\text{La}})_O \left[\frac{2.0 \times 10^{-9} \text{ m}^2/\text{s}}{2.4 \times 10^{-9} \text{ m}^2/\text{s}} \right]^{0.5}_{25^\circ\text{C}} = 0.9 (k_{\text{La}})_O, \end{aligned} \quad (3)$$

where D^{CO_2} and D^{O_2} are the molecular diffusivities of CO₂ and O₂ in water, respectively. Diffusivities are temperature dependent and their values at 25 °C are substituted in Eq. (3). SCUR can be estimated theoretically from Eq. (4) if specific O₂ production rate (SOPR) and photosynthetic quotient (PQ) are known:

$$\begin{aligned} \frac{\text{SOPR}}{\text{SCUR}} &= \frac{\text{O}_2 \text{ produced/biomass/time}}{\text{CO}_2 \text{ assimilated/biomass/time}} \\ &= \frac{(\text{Moles of O}_2 \text{ produced}) 32 \text{ g/mol}}{(\text{Moles of CO}_2 \text{ assimilated}) 44 \text{ g/mol}} = \frac{8}{11} \text{ PQ}, \end{aligned} \quad (4)$$

SOPR can be calculated from online measurement of DO (described in “Analytical methods”), whereas PQ (moles of O₂ produced per unit mole of CO₂ assimilated) can be estimated from the stoichiometric equation of growth (Eq. 5):

where z , the stoichiometric coefficient of O₂, needs to be evaluated as it is the measure of PQ. On solving H, O, and N balance equations, z (or PQ) can be completely written in terms of p , q , and r (Eq. 6) which can be easily determined from C, H, O, and N mass fractions of the biomass:

$$\text{PQ} = z = \frac{1}{2} \left[\frac{p}{2} + \frac{5r}{2} - q \right]. \quad (6)$$

Typically, SOPR increases with the dissolved CO₂ concentration and levels off at C_{crit} . Moreover, C_{crit} and its corresponding SOPR increases with the intensity of light available for photosynthesis, and are maximal at saturating intensity. Substituting maximum values of C_{crit} and SOPR in Eqs. (1) and (4) makes $x_{c \text{ sat}}$ minimal to represent minimum biomass which can be produced without CO₂ limitation and maximum biomass which can be produced in CO₂- and light-saturated culture. Moreover, since the biomass taken for measurement of SOPR in our experiments was grown in nutrient-replete condition, PQ is also minimal. Therefore, precisely, $x_{c \text{ sat}}$ estimates the maximum biomass possible in CO₂-, light-, and nutrient-replete culture. In general, as the cell density increases inside photobioreactor, light gets attenuated and the nutrient levels, particularly nitrogen began to fall gradually resulting in accumulation of lipid or carbohydrates, thereby changing biomass composition. These changes decrease the values of C_{crit} and SOPR and increase the value of PQ (Eriksen et al. 2007). Consequently, the actual biomass produced under CO₂ saturation will be greater than $x_{c \text{ sat}}$ when light intensity and nutrient concentration drop below their saturation level.

Experimental setups

For the measurement of SOPR, a photosynthetic activity measurement system has been designed which minimizes light attenuation by hanging a low depth culture over the light source (Fig. 1a). It consists of two borosilicate transparent glass units, viz., lid and hanging chamber, which are easy to assemble and clean. The lid has three glass joints—a centre joint for glass stirrer and two side joints for oxygen and pH electrodes. The gaps between the glass joints and electrodes or stirrer were sealed with the customized PTFE fittings to provide containment during experiment. A glass paddle stirrer was attached to a speed controlled 12 V DC geared motor overhead for mixing the culture. The hanging chamber is essentially a Petri dish of depth less than 10 mm and fits upright inside the lid cavity. The gap between the lid and hanging chamber was sealed with a silicone gasket. The whole apparatus was placed over the LED light source. Fans were installed near the light source to dissipate heat.

Experiments to validate the accuracy of calculated $x_{c \text{ sat}}$ were conducted in tube reactors. These reactors were essentially 60 mL glass culture tubes (25 mm outer diameter and 200 mm length) with screw caps bored to pass through silicone air tubes for culture aeration and exhaust gas removal. The gaps between bores and tubes were

Fig. 1 Photosynthetic activity measurement system. **a** Diagram of the experimental setup. **b** Photograph of a *C. vulgaris* culture sparged with 2% CO₂-enriched air before inoculation

sealed with silicone sealant. Rotameter was installed to measure and control gas flow to the reactor. Air filters were installed in the inlet and exhaust gas lines. LED light was mounted behind the tube reactors to illuminate cultures. Cooling fans were installed near the reactors to prevent heat accumulation.

Experimental design

Two sets of experiments were performed in the photosynthetic activity measurement system to determine the maximum values of C_{crit} and SOPR. In the first set, saturating CO₂ level was determined at a supposed saturating/supersaturating intensity by measuring SOPR at different dissolved CO₂ concentrations obtained by sparging medium with air or CO₂-enriched air (0.14, 0.48, 1.09, 2.12, 3.07, 3.95, and 10.08% v/v) before inoculation. The experiment will provide maximum SOPR and C_{crit} at the saturating intensity. The second set of experiments was performed to confirm the saturating intensity supposed in the first set. At the dissolved CO₂ level greater than the maximum C_{crit} , SOPR should increase with the light intensity and must level off at saturating intensity. SOPR was measured at different light intensities (100–600 $\mu\text{mol}/\text{m}^2/\text{s}$), at the dissolved CO₂ level much higher than the determined C_{crit} . Inoculums were prepared in 250 mL Borosil glass bottle sparged with filtered sterilized air/CO₂-enriched air, depending on the requirements. Light intensity and CO₂ vol% were the same as supposed to be used in the experiment. Cells were harvested for inoculation at low cell density (OD_{750} between 0.2 and 0.3) to prevent any undesirable change in biomass composition due to nutrient

limitation which can affect CO₂-uptake and O₂-production rates. Prior to inoculation inside laminar hood, the auto-claved medium (without iron) was sparged with sterilized air/CO₂-enriched air for at least 10 min to achieve CO₂ equilibrium. Under aseptic conditions, a 200 mL of the sparged medium was taken out for titration to quantify dissolved CO₂ and a 100 mL, supplemented with auto-claved iron stock, was inoculated with cells harvested from the inoculum to obtain OD_{750} of ca. 0.05 to prevent light attenuation. A 50 mL of the culture was poured into the sterilized hanging chamber of the photosynthetic activity measurement system and the rest 50 mL was used to determine the dry cell weight (DCW), as described in “[Analytical methods](#)”, to calculate biomass titer of the culture. Electrodes and stirrer were inserted into their respective openings on the sterilized lid, and the hanging chamber with the culture was carefully fitted inside the lid cavity with the silicone gasket. A thin needle connected to gas tube was inserted inside from the silicon gasket to flush the air trapped inside the system with the same CO₂-enriched air which was used to sparge the growth medium before inoculation. The whole assembly was placed over the LED light source in a dark room (Fig. 1b) and the changes in DO were recorded to determine SOPR as described in the “[Analytical methods](#)”. All experiments were performed in triplicate.

In validation experiments, 20 mL cultures were grown in illuminated tube reactors with CO₂-enriched air sparging. Two air flow rates (20 and 60 mL/min) of the CO₂-enriched air were used separately to vary the $(k_L a)_O$. Specific growth rate of the culture was determined after 12 h incubation using Eq. (7).

Analytical methods

PPFD was measured by Quantum sensor (LI-COR LI-190SB, USA). Dissolved CO₂ concentration of the medium was determined by the titrimetric method for free carbon dioxide (APHA 1998) immediately after sparging medium (without iron) with CO₂-enriched air. A 200 mL of the sparged medium with added phenolphthalein was titrated with N/44 NaOH till the end point, indicated by pink colour. Iron stock was sterilized separately to prevent its interference in titration and was added to the remaining portion of the sparged medium for culturing cells. In addition, medium pH was maintained at 7 before sterilization to prevent the interference of constituent acids during titration with NaOH. Elemental (CHON) composition of biomass was determined by element analyzer Vario EL III (Elementar Analysensysteme, Hanau, Germany). DO and pH electrodes (Easy sense O₂ 21, Easy sense pH 31, Mettler Toledo, India) were installed to a transmitter (M200 Mettler Toledo India). DO electrode was calibrated in air-saturated distilled water at 25 °C (8.69 mg O₂/L at equilibrium) and pH electrode was calibrated with pH buffers of pH 4, 7, and 10. Exhaust gas analyzer (EGAS-L, Sartorius BBI Systems GmbH) was used to determine the CO₂ vol% of the CO₂-enriched air.

DCW was determined by centrifuging (10 min at 10,000 rpm) culture in a pre-weighed dry centrifuge tube followed by drying the washed pellet at 80 °C for 24 h. SOPR was calculated by dividing the Oxygen Production Rate (OPR, mg O₂/L/h) with the biomass titer (mg DCW/L). OPR (mg O₂/L/h) was obtained from the slope of the linear portion of the DO curve (generally observed after an initial short acceleration phase) determined by linear regression. The dynamic gassing-out method was used to measure ($k_L a$)_O of the tube reactor under the experimental conditions. Specific growth rate (μ) was determined in the validation experiments according to the following equation:

$$\mu(1/\text{day}) = \frac{\ln x_f - \ln x_0}{t/24}, \quad (7)$$

where x_0 and x_f are the biomass titers (mg/L) at the initial and after t h, respectively.

Results and discussion

The first set of experiments in the photosynthetic activity measurement system, to determine C_{crit} , were performed at 400 $\mu\text{mol}/\text{m}^2/\text{s}$, since photosynthesis in *C. vulgaris* has been found to peak at intensities below or about 400 $\mu\text{mol}/\text{m}^2/\text{s}$ (Lloyd et al. 1977). As expected, pH of the medium decreased with the increase in CO₂ level (Fig. 2). Since it

Fig. 2 SOPR and pH as a function of dissolved CO₂ concentration in optically thin hanging cultures of *C. vulgaris* at 400 $\mu\text{mol}/\text{m}^2/\text{s}$. Data are mean $\pm$ SD of triplicate determinations

was observed in a separate set of shake flask experiments that optimum pH for growth of the microalga is 6 and 7 (Fig. S1), pH variations between 6 and 7 arising due to varying degrees of CO₂ enrichment will not affect SOPR. All the experiments were initiated with an initial culture OD₇₅₀ of ca. 0.05 (corresponds to a biomass titer of ca. 30 mg DCW/L). Low biomass titer and low culture depth in the direction of propagation of light assure non-shading condition and the light intensity is virtually the same throughout the culture volume—optically thin culture. We ran each experiment for 20 min. All DO curves were linear following an initial short phase of 2–3 min (Fig. S2). In each case, slope of the DO curve (measure of OPR) between 4 and 14 min has been used to calculate SOPR. During this period, change in pH was negligible and oxygen accumulation (maximum 9.6 mg/L) was insignificant for the inhibition of photosynthesis. Maximum SOPR is 0.142 mg O₂/mg DCW/h and the value of C_{crit} is taken as 7 mg/L, since it is apparent from Fig. 2 that C_{crit} lies between 2 and 7 mg/L dissolved CO₂. Similar levels of C_{crit} were earlier reported for *Chlorella* (Markl et al. 1985; Warburg 1919). Dissolved CO₂ concentration determined by titration was lower than the value obtained from Henry's Law in each case; however, the difference is not significant in any case (maximum difference is 6.4 mg/L for the titration value of 144.4 mg/L). Therefore, Henry's law can be used to estimate dissolved CO₂ instead of performing titration.

To verify the assumption that 400 $\mu\text{mol}/\text{m}^2/\text{s}$ is the saturating/supersaturating intensity, the second set experiments were performed at a high saturating CO₂ level of 30.5 mg/L (Fig. 3) (obtained by sparging ca. 2% v/v CO₂-enriched air). The same trend in the DO curves was observed as in the first set of experiments—an initial short acceleration phase followed by a linear region (Fig. S3). SOPR increased with the light intensity and levelled off at

Fig. 3 SOPR as a function of light intensity in optically thin hanging cultures of *C. vulgaris* at 30.5 mg/L dissolved CO₂ (obtained by sparging 2% CO₂-enriched air before inoculation). Data are mean $\pm$ SD of triplicate determinations

300 $\mu\text{mol/m}^2/\text{s}$ reaching the same maximum value of 0.142 mg O₂/mg DCW/h (Fig. 3). This verifies that 400 $\mu\text{mol/m}^2/\text{s}$ is the supersaturating intensity. Elemental formula of the strain (CH_{1.649}O_{0.373}N_{0.127}) is determined from the composition of the biomass (54% C, 7.42% H, 26.05% O, and 8% N) grown in inoculums with low cell density and dissolved CO₂ levels above C_{crit} to prevent changes in biomass composition due to CO₂ limitation. The value of PQ calculated from Eq. (6) is 0.3845 and the value of SCUR obtained by substituting SOPR (0.142 mg O₂/mg DCW/h) and PQ in Eq. (4) is 0.5078 mg CO₂/mg DCW/h. From the values of C_{crit} and SCUR, $x_{c \text{ sat}}$ can be determined from Eq. (1) for the given CO₂ fraction of the sparged air and $(k_L a)_C$ of the photobioreactor determined from Eq. (3).

In validation experiments, cultures (OD₇₅₀ ca. 0.05) were grown in tube reactors (optically thin cultures) at supersaturating intensity (500 $\mu\text{mol/m}^2/\text{s}$) for 12 h to prevent light and nutrient limitation. Results of the four experiments performed are given in Table 1. It can be noted that all the cultures with final biomass titer $x_f < x_{c \text{ sat}}$ grew at an specific growth rate of ca. 2.2/day, whereas the culture with $x_f > x_{c \text{ sat}}$ grew at comparatively lower rate (2.02/day). The growth is limited by CO₂ when $x_f > x_{c \text{ sat}}$ under light and nutrient-replete condition. Decrease in the specific growth rate when $x_f > x_{c \text{ sat}}$ is a manifestation of CO₂-limited growth. Although the difference of this scale is generally not considered, but it is significant in this case, since the photoautotrophic algal growth is too slow to manifest prominent change in specific growth rate due to changes in CO₂ levels. Moreover, very low standard deviations in all the four cases (Table 1) indicate the precision in measurement and support the significance of the observed difference in specific growth rates. The results validate the estimated $x_{c \text{ sat}}$ under different operational conditions.

Table 1 Results of validation experiments on *C. vulgaris*. $x_{c \text{ sat}}$, x_0 , and x_f are in mg DCW/L, and μ is in 1/day

$(k_L a)_O$ (1/h)	Dissolved CO ₂	
	8.01 mg/L (0.55% CO ₂)	57.24 mg/L (4% CO ₂)
11.63		
$x_{c \text{ sat}}$	20.82	1035.57
x_0	31.67 $\pm$ 0.08	32.26 $\pm$ 0.06
x_f	86.90 $\pm$ 0.14	96.24 $\pm$ 0.13
μ	2.02 $\pm$ 0.002	2.19 $\pm$ 0.001
55.02		
$x_{c \text{ sat}}$	98.49	4899.14
x_0	31.08 $\pm$ 0.09	30.45 $\pm$ 0.08
x_f	93.17 $\pm$ 0.11	91.93 $\pm$ 0.07
μ	2.20 $\pm$ 0.003	2.21 $\pm$ 0.004

Data are mean $\pm$ SD of triplicate cultures

Fig. 4 Contour plot of $x_{c \text{ sat}}$ for *C. vulgaris* within typical range of $(k_L a)_O$ and CO₂ vol %

Based on the measured C_{crit} and SCUR, a contour map of $x_{c \text{ sat}}$ for typical range of $(k_L a)_O$ and CO₂ volume fractions was prepared (Fig. 4). C* was calculated from Henry's law (Eq. 2) at atmospheric pressure and 25 °C. Combinations of CO₂ vol% and $(k_L a)_O$ required to sustain CO₂-replete growth of *C. vulgaris* up to high biomass titers can be figured out directly from the contour map. It is evident from the contour plot that even at low CO₂ vol%, relatively high CO₂-saturated growth is feasible in stirred tank reactors ($(k_L a)_O$ 100–300/h) than in bubble columns ($(k_L a)_O < 100$ /h). In addition, in stirred tank reactors with $(k_L a)_O > 100$ /h, 5–10% CO₂ can support biomass titer too high to achieve practically. However, irrespective of the type of photobioreactor used for cultivation, relatively low quantity of algal biomass is generally produced in photoautotrophic batch cultivation as the growth is limited by several factors. In addition to inadequate CO₂-enrichment and mass-transfer, the microalgal growth is generally

limited by light attenuation, nitrogen depletion, and oxygen accumulation at inhibitory levels. It is, therefore, essential to determine the CO₂ vol% just required to provide CO₂ sufficiency throughout the cultivation. Under the prevailing mass-transfer condition inside the photobioreactor, a minimum CO₂ vol% required to support a target CO₂-saturated growth should be first calculated. If the biomass produced at calculated CO₂ vol% is less than the target, then the growth is limited by factors other than CO₂ and consequently a relatively lower CO₂ vol% can be selected, such that the cells are limited by other factors before CO₂ could limit the growth. Lowering CO₂ vol% is economically favourable, especially at large scale. However, high CO₂ concentrations may be useful when oxygen accumulates to inhibitory levels.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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