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An empirical process model to predict microalgal carbon fixation rates in photobioreactors

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ABSTRACT

An empirical process model was developed to infer the instantaneous net photosynthesis and carbon fixation rates from continuous pH and dissolved oxygen measurements during microalgal cultivation in photobioreactors. The model is based on the physical and chemical processes that govern the relationship between inorganic carbon supplied to a microalgal culture and the organic carbon fixed into microalgal biomass, with a particular focus on carbonate chemistry and mass transfer. Bayesian statistics were used to estimate the uncertainty in state variables, such as pH, net photosynthesis rate, and bicarbonate ion concentration, based on the constraints imposed by prior knowledge about these variables.

The model was verified by batch-culturing the chlorophyte microalga *Chlorella vulgaris* in a photobioreactor under both bicarbonate-replete and bicarbonate-limiting conditions in order to test its predictive ability under different operational settings. The replicate photobioreactors were set up to simulate a scaled-down vertical cross-section of a typical raceway pond. This model could be used to test the activity and efficiency of carbon concentrating mechanisms in different microalgal species. It also provides a detailed understanding of how the rate of photosynthesis depends on dissolved inorganic carbon concentration, which could lead to better management of carbon supply in large-scale microalgal cultivation facilities.

1. Introduction

The key performance indicator for any large-scale microalgal culturing system is the aerial/volumetric biomass productivity of that system [1]. The higher the biomass productivity, the greater quantity of the desired bioproduct is synthesized per unit time. The most common limiting factors to photoautotrophic microalgal biomass productivity are light and carbon availability [2]. Light availability is a complex function of incident irradiance, the geometry and mixing regime of the cultivation system, and the cell density and absorption properties of the chosen microalga (reviewed recently in [3]). This article will focus on identifying the variables that affect carbon availability and it will develop a process model to describe the relationship between inorganic carbon supplied to a microalgal culture and organic carbon fixed into microalgal biomass.

Carbon bioavailability is dictated by the carbonate chemistry of the microalgal culture medium [4]. Carbon is usually supplied as gaseous CO_2, which dissolves in water to form carbonic acid (H_2CO_3) and then dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions according to the following equilibrium equations:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \quad (1a)$$

$$H_2CO_3 \overset{K_1}{\leftrightarrow} H^+ + HCO_3^- \quad (1b)$$

$$HCO_3^- \overset{K_2}{\leftrightarrow} H^+ + CO_3^{2-} \quad (1c)$$

where K_1 and K_2 are the respective chemical equilibrium constants [5].

The sum of dissolved CO_2, HCO_3^- and CO_3^{2-} is referred to as dissolved inorganic carbon (DIC). The dissolution of CO_2 results in the release of H^+ ions, which reduces the pH of the solution. A common strategy to manage carbon availability is to continuously measure pH and inject gaseous CO_2 when the pH increases above a set threshold, thereby maintaining high DIC [6].

The carbonate chemistry of a microalgal culture grown in freshwater/seawater supplemented with nutrients is primarily affected by the total alkalinity (TA) and the total phosphorus (TP) of the solution. In addition to light and carbon, microalgal cultures also require inorganic sources of nitrogen and phosphorus for growth; these are normally supplied as nitrate/phosphate salts. Nitrate consumption leaves behind anionic counterions (OH^-) that increase TA [7,8] whereas

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phosphate consumption reduces TP (the change in TA is negligible). Provided that DIC, TA, and TP are known, the carbonate chemistry system can be solved for a given pH to calculate the concentration of all of its constituents [9].

Microalgae consume DIC to produce biomass and dissolved oxygen (DO) by photosynthesis. The complication is that microalgae have a preference for specific DIC species. Ultimately, photosynthetic carbon fixation relies on delivering CO_2 molecules to the active site of the RuBisCO enzyme in the stroma tissue within microalgal cells [10]. Since dissolved CO_2 constitutes only a tiny fraction of DIC [11], most microalgae have evolved carbon concentrating mechanisms (CCMs) to actively pump HCO_3^- into their cells and convert it to CO_2 [12]. Most aquatic photosynthesis, therefore, involves CCMs and proceeds according to the following photosynthetic quotient:

$$H_2O + HCO_3^- \leftrightarrow CH_2O_\alpha + \frac{3-\alpha}{2}O_2 + OH^- \quad (2)$$

where $\alpha = 1$ corresponds to the synthesis of glucose (*i.e.,* carbohydrates), whereas $\alpha \sim 0$ corresponds to the synthesis of lipids, and $\alpha \sim 0.3$ to protein synthesis [13].

The net photosynthetic oxygen production rate (P) depends most strongly on the number of photons absorbed by a microalgal culture, which is determined by the incident irradiance. At a given incident irradiance, photosynthesis also depends on HCO_3^-, if the HCO_3^- concentration is sufficiently low. The photosynthesis saturation at high HCO_3^- concentration and proportional dependence on HCO_3^- at low concentration can be understood by the Monod equation:

$$P = P_{max}\left[HCO_3^-\right] \Big/ \left(\left[HCO_3^-\right] + K_{HCO3}\right) \quad (3)$$

where P_{max} is the maximum carbon-replete photosynthesis rate at a given irradiance and K_{HCO3} is the HCO_3^- half-saturation constant [14].

Net photosynthesis during the day results in a gradual increase in pH coupled to a rapid increase in DO, whereas net respiration during the night works in the opposite direction. Both pH and DO can be measured instantaneously, continuously and non-invasively using sensors (see [15]). This is in sharp contrast to the traditional techniques used to quantify microalgal biomass productivity, such as dry weight and cell density measurements [16]. In order to measure dry weight for example, a microalgal sample must first be filtered, dried overnight and desiccated, resulting in a time lag of at least one day before the dry weight is known. Empirical modelling based on pH and DO measurements provides an opportunity to infer instantaneous carbon fixation and biomass productivity rates at unprecedented temporal resolution. This approach is complementary to pulse amplitude modulated (PAM) fluorometry, which is widely used to estimate biomass productivity from instantaneous chlorophyll fluorescence measurements [17].

A complication arises due to the mass transfer of O_2 and CO_2 between the gas and liquid phases during microalgal culturing. Most cultivation facilities drive mass transfer by aerating the microalgal culture to supply CO_2 and remove toxic DO from solution. DO, therefore, becomes a function of both photosynthesis and mass transfer, as described by:

$$d(\mathrm{DO})\Big/dt = \frac{3-\alpha}{2}P + k_L a\,(\mathrm{DO} - \mathrm{DO_H}) \quad (4)$$

where α is the photosynthetic quotient (see Eq. (2)), k_La is the volumetric mass transfer coefficient for O_2 and DO_H is the Henry's Law equilibrium DO [18]. Provided that k_La has been measured and it is relatively constant, it becomes possible to relate changes in DO directly to changes in net photosynthesis rate.

The aim of this article is to develop and describe an empirical process model to calculate microalgal carbon fixation rates in photobioreactors (PBRs) using continuous pH and DO measurements. This model is verified using experimental data collected by culturing the microalga *Chlorella vulgaris* at different levels of carbon bioavailability in PBRs, including a bicarbonate-replete treatment [C+] and a bicarbonate-limiting treatment [C−].. The PBRs were set up to simulate a scaled-down vertical cross-section of a typical raceway pond (see Materials and Methods).

2. Materials and methods

2.1. Chlorella strain and stock culture

Chlorella vulgaris strain CS-42 (Australian National Algae Culture Collection) was cultured in MLA medium [19]. *C. vulgaris* is a green microalga in the class Trebouxiophyceae of the division Chlorophyta. Stock cultures were maintained in 200 mL Erlenmeyer flasks in an incubator (Labec Pty. Ltd.) under a 12 h/12 h light/dark cycle at an incident irradiance of 40 μmol photon $m^{-2}\,s^{-1}$ and at a temperature of 20 °C. They were diluted weekly at 10% v/v to maintain active cell growth.

2.2. Photobioreactor setup and pH measurement

Experimental cultures were grown in cylindrical PBRs (Phenometrics Inc.) with top-side cool-white LED illumination and a light path of 25 cm [20,21]. A 2-pi quantum sensor (LI-COR Inc.) was used to calibrate surface irradiance. PBRs were inoculated with 10% v/v of stock *C. vulgaris* culture in MLA medium to a working volume of 450 mL. PBRs were maintained at a temperature of 20 °C and a surface irradiance of 1200 μmol photon $m^{-2}\,s^{-1}$ (following a 2-day acclimation period at 600 μmol photon $m^{-2}\,s^{-1}$) under a 12 h/12 h light/dark cycle ("day" period 08:00–20:00 h). 1 mL of sample was extracted daily from each PBR for optical density and nutrient concentration measurements. The PBR vessel was mixed using a magnetic stirrer at a rate of 110 rpm, and bubbled with 0.2 μm filtered and humidified air that was delivered through a 0.4 mm hypodermic needle pressurized to 12 kPa.

An electrochemical probe (Van London Co.) was used to measure pH every 5 min; the pH electrode was calibrated using three pH standards (Sigma-Aldrich). Batch culture experiments were performed over a period of 11 days and samples were extracted daily at 10:00 h, *i.e.,* 2 h into the light cycle ("day").

2.3. Dissolved oxygen and net photosynthesis

DO was measured every 1 min (FireSting logger; PyroScience GmbH) using robust optical minisensor probes with optical isolation (OXROB10; PyroScience GmbH). A two-point calibration against air-saturated water and sodium sulfate-saturated water (zero DO) was performed. A solenoid valve (SMC Pneumatics Pty. Ltd.) was activated at regular 2 h intervals using a microcontroller (Arduino) to stop air bubbling for a period of 10 min. The linear increase in DO in the light during this period was used to calculate net photosynthetic oxygen production rate (P); negative P values represent net respiration [4].

2.4. Carbon dioxide treatments

Experiments were performed in triplicate either with [C+] or without [C−] carbon dioxide enrichment. All treatments were continuously aerated with ambient air, which had a CO_2 concentration of 0.04%. In the [C+] treatment, a pulse (1 min) of 5% CO_2 in air was periodically bubbled through the *C. vulgaris* culture from a gas cylinder (BOC Australia) through a second 0.4 mm hypodermic needle to maintain a pH-stat at pH 8.5 ± 0.5.

2.5. Optical density, dry weight and elemental microanalysis

C. vulgaris biomass samples were extracted daily from each PBR and their optical density was measured at 750 nm (OD_{750}) using a spectrophotometer (Spectronic 200; Thermo Scientific). Dry weight (DW)