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Carbon use efficiency diagnostics in *Nannochloropsis salina*

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ABSTRACT

Carbon dioxide (CO_2) can be a significant resource input affecting the cost of algae biomass production on an industrial scale. Improvements in biofuel productivity therefore require characterization of CO_2 use efficiency (CUE). RuBisCO saturation with CO_2 is an important factor influencing biomass productivity. During CO_2 fixation by RuBisCO, fractionation of carbon isotopes occurs, with preferential fixation of $^{12}CO_2$, resulting in assimilation of the lighter isotope in algae. This photosynthetic discrimination ($\Delta_{\text{DIC-algae}}$), approximated by the difference between the $\delta^{13}C$ of external medium and that of algae, is a function of the proportion of CO_2 fixed relative to supply. $\Delta_{\text{DIC-algae}}$ has been applied to the study of photosynthesis in algae over the past few decades and we have adopted the tool to characterize improvements in conditions optimized for biofuel productivity, such as controlled photobioreactors. We report the use of $\Delta_{\text{DIC-algae}}$ as a tool for characterizing CO_2 dynamics and RuBisCO saturation in *Nannochloropsis salina* CCMP 1776, a benchmark strain in algal biofuels research. We use $\Delta_{\text{DIC-algae}}$ to describe the conditions under which RuBisCO saturation can be achieved to maximize biomass productivity. Our results suggest that determination of $\Delta_{\text{DIC-algae}}$ can provide important feedback to support engineering and cultural improvements that can impact carbon use efficiency and biomass productivity.

1. Introduction

Nannochloropsis salina is a heterokont microalga of interest in biofuel production due to its high oil content. The efficiency of photosynthetic carbon fixation is one of the major factors affecting biomass productivity and biofuel production from algae. It is imperative to develop a diagnostic tool that will efficiently provide informative feedback of CO_2 assimilation dynamics to optimize and maximize sustainable productivity. The fractionation of carbon isotopes during photosynthesis is a useful tool in studying CO_2 dynamics [1]. Research dating back to the 1950s shows that tissues of photosynthetic organisms tend to be depleted in ^{13}C relative to atmospheric CO_2 due to discrimination against ^{13}C during photosynthesis [2–5]. Estimation of photosynthetic discrimination includes dissolution, equilibration with the rest of the inorganic carbon system (i.e. $^{13}C/^{12}C$ increases in the order atmospheric CO_2 < dissolved CO_2 < equilibrium HCO_3^-), diffusion ($^{13}CO_2$ diffuses more slowly than $^{12}CO_2$), and carboxylation (for example, the CO_2 fixing enzyme ribulose bisphosphate carboxylase oxygenase (RuBisCO) discriminates strongly in favor of $^{12}CO_2$ during carboxylation) [6–8].

Carbon isotope fractionation results in different isotopic signatures in photosynthetic organisms, and these differences can be exploited in the study of carbon assimilation. The natural abundance of the two carbon isotopes ^{12}C and ^{13}C is expressed as the $\delta^{13}C$ of the substance, a ratio of the carbon isotopes relative to the standard Vienna Pee Dee Belemnite (PDB), measured in parts per thousand (‰);

$$\delta^{13}C = 1000\left(\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{V-PDB}}} - 1\right)$$

High $\delta^{13}C$ values indicate a greater proportional amount of the heavier ^{13}C isotope. Carbon isotopic composition has been applied in many studies in biology. For example, the fact that C4 plants have less negative (heavier) $\delta^{13}C$ values than C3 plants has provided a way to distinguish between these two photosynthetic groups of plants [reviewed by 8] leading to the ability to classify paleo-herbivore diets [9]. In addition, $\delta^{13}C$ values have been used as indices for several physiological parameters, including water use efficiency [10–12] and growth [13]. Raven et al. [14] offered an interpretation of $\delta^{13}C$ values of marine organisms based on CO_2 or bicarbonate use, suggesting that organisms with $\delta^{13}C$ values more positive than −10‰ use only bicarbonate. Generally, in marine plants and macroalgae, $\delta^{13}C$ values are higher than those of terrestrial plants [2–4,8].

Microalgae have a wide range of $\delta^{13}C$ values compared to C3 plants,

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in part because carbon in the aquatic environment may be obtained from bicarbonate, which is enriched in ^{13}C (by approximately 8.4‰ at 30 °C, [15]) compared to atmospheric CO_2 [3,4,16,17]. Carbon isotopic fractionation studies delineating both the carbon source and the product, i.e. dissolved inorganic carbon (DIC) and algae, are particularly useful for assessing photosynthetic carbon supply and demand [18]. The isotopic difference between algae and their carbon source has been termed photosynthetic discrimination ($\Delta_{\text{DIC-algae}}$) [19].

The magnitude of $\Delta_{\text{DIC-algae}}$ is affected by DIC supply and demand and by physiological characteristics of algae, such as growth rate, respiration and cell geometry [20–24]. Kerby and Raven [24] showed that when CO_2 is limiting, fractionation can approach zero, whereas it can be > 20‰ when CO_2 concentrations are high. Interestingly, using online $^{13}CO_2$ gas exchange from bubbled cultures, Hanson et al. [25] reported an increase in $\Delta_{\text{DIC-algae}}$ with increase in CO_2 for *Chlamydomonas* while in *N. salina* $\Delta_{\text{DIC-algae}}$ was lower under high CO_2 than under low CO_2.

Generally, when CO_2 is unlimited, the fractionation associated with RuBisCO (29‰) will be fully expressed, and will determine the isotopic difference between the inorganic carbon source and the final bioproduct [26]. Correlations between $\Delta_{\text{DIC-algae}}$ and growth have been previously reported in literature (e.g. [13,27]). Carvalho et al. [27] suggested use of $\Delta_{\text{DIC-algae}}$ as an indexing system in kelp while Condon et al. [13] found a positive correlation between growth and $\Delta_{\text{DIC-algae}}$ in wheat, and suggested use of $\Delta_{\text{DIC-algae}}$ in selecting for wheat yield. The enzyme RuBisCO is characterized by competing carboxylation and oxygenation reactions, which depend on the concentrations of CO_2 and O_2 around the enzyme. Algae have evolved complex carbon concentrating mechanisms (CCMs) for enriching CO_2 around RuBisCO. These mechanisms are often responsive to low CO_2 concentrations and are controlled by regulated genetic elements. CCM responses will also have an impact on $\Delta_{\text{DIC-algae}}$. Understanding the complex CCMs is essential for designing the most efficient use of carbon inputs for algae biomass production on an industrial scale. Recently, Hanson et al. [25] reported that *N. salina* has a leaky CCM induced at low CO_2, similar to CCMs reported in other *Nannochloropsis* species [28,29]. As such, methods for measuring CO_2 efficiency are needed in order to optimize growth conditions and characterize improvements. This study attempts to harness $\Delta_{\text{DIC-algae}}$ as a tool in algal biofuel research.

1.1. The model

It is not our intention to produce a model that fully explains the isotopic variation of algae and DIC in our optimized photobioreactor systems. Rather, we wish to utilize a simple model that can provide feedback as to those conditions that increase the relative saturation status of RuBisCO with respect to CO_2. A closed system model is clearly inappropriate as new CO_2 is constantly added to the culture systems. Neither can the system be considered a priori to be open with unlimited substrate (DIC) available for algal growth. Modeling of a partially closed system also presents several difficulties. In a typical partially closed system, the degree of isotopic enrichment of the substrate (i.e. DIC; assuming product is isotopically lighter than substrate) is reduced due to continual new inputs of substrate. In our system, we not only have new inputs of carbon, but, under some conditions, the rate of carbon addition can increase as a function of time due to alkalinization associated with assimilation of nitrate (see Results for more detailed explanation). Moreover, the cumulative product pool is not isotopically fixed as new algae grows. Older algae continue to respire, photosynthesize, and turnover at some unknown rate. The photobioreactor system is very complex to model and requires some simplifying assumptions.

Following Georicke et al. [30], we made the simplifying assumption of approximate steady state at each of the given time points of sampling. Goericke et al. [30] allowed for a growth term in their derivation, though that term is effectively eliminated by assuming that fractionation associated with respiration is 0‰. We further assume rapid turnover of carbon within the photobioreactors. Further justification of these assumptions based on experimental results is provided in the 'Discussion' along with considerations of the effects of these assumptions on our interpretations and the utility of $\Delta_{\text{DIC-algae}}$ as a feedback tool in algal cultivation.

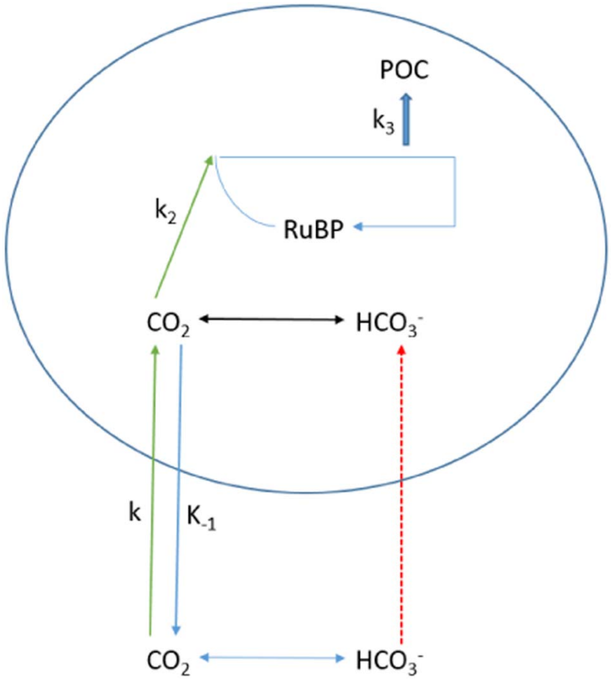

Fig. 1. Conceptual model for carbon uptake in algae showing possible fractionation steps (modified from Sharkey and Berry, 1985), where k = uptake of inorganic carbon from the external pool of dissolved inorganic carbon, k_{-1} = back diffusion of inorganic carbon, k_2 = incorporation of intracellular carbon into algae (carbon fixation by RuBisCO), k_3 = dark respiration, POC (particulate organic carbon) represents the carbon in the organisms. *N. salina* has been suggested to activate a bicarbonate pump under low CO_2 [25].

In the two-step model proposed by Park and Epstein [5], the main isotope-discriminating steps during carbon fixation are uptake and biosynthesis of cellular components.

The initial diffusion is reversible (k, k_{-1}, Fig. 1), since CO_2 can leak back out, while the carboxylation step is irreversible. Additionally, the proportion of CO_2 that leaks out of the cell also affects the overall isotope signature of algae [19,30] (Fig. 1).

Based on the model by Goericke et al. [30], and assuming that isotopic fractionation associated with CO_2 forward- and back-diffusion are equal, and that fractionation associated with respiration is 0‰, the isotopic signature of the particulate organic matter (POC) can be calculated as

$$\delta_{\text{POC}} = \delta_{\text{DICexternal}} - f_{\text{effective}}(\varepsilon_{\text{carboxylation}}) \tag{1}$$

where $f_{\text{effective}}$ is the ratio of CO_2 leakage out of the cell to gross inorganic carbon uptake. This equation can be rearranged, substituting $\Delta_{\text{DIC-algae}}$ to give:

$$\Delta_{\text{DIC}-\text{algae}} = f_{\text{eff}}(\varepsilon_{\text{carboxylation}}) \tag{2}$$

We have slightly modified the model of Goericke et al. [30] by expressing f as effective f (f_{eff}) rather than as physiological f (the inherent 'leakiness' of the algae) to acknowledge that the actual ratio of back diffusion of CO_2 to forward diffusion may be less than that determined by algae physiology under conditions of photobioreactors optimized for enhanced photosynthesis and biofuel production. The maximum fractionation predicted by Eq. (2) is 37.4‰, assuming a fractionation of 8.4‰ for CO_2 in equilibrium with bicarbonate at 30 °C and a value of 29‰ for $\varepsilon_{\text{carboxylation}}$, the fractionation associated with