Dynamics of Carbon Dioxide Capture in Two Halotolerant Strains of *Chlorella vulgaris*

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Abstract

Microalgae-based systems provide a renewable approach for carbon capture to address the escalating challenge of rising global carbon dioxide (CO_2) levels. This study examined carbon availability and uptake in two halotolerant *Chlorella vulgaris* strains under varying nutrient and carbon supply regimes. CO_2 supply defined saturation levels in algae-free culture media; a 2-8 fold increase in saturation was noticed for 1-100% CO_2 in the inlet supply. In microalgae cultures, continuous carbon supply was seen to lead to carbon depletion, affecting growth and carbon uptake efficiency. Continuous air supply (0.04% CO_2), led to a decrease of dissolved inorganic carbon (DIC) from 1.4 mM at the start to near zero by day 8. Nutrient supplementation enhanced DIC uptake and biomass productivity; an 8 fold increase in nitrates and phosphates supplied led to a 1.7 fold increase in DIC for both strains and a 2-fold increase in biomass productivity for one of the strains. Passive carbon supply regimes exhibited higher carbon fixation efficiency compared to active regimes. Nitrate significantly increased carbon capture, with a quadratic relationship identified between DIC levels and biomass production. These findings reveal for the first time within species differences in the dynamics of carbon availability and uptake, offering a basis for microalgal CO_2 capture into scalable systems such as flue gas utilization or wastewater-fed photobioreactors. Whilst large-scale deployment requires further techno-economic validation, this study highlights a sustainable, biobased CO_2 capture process that avoids hazardous chemicals, offering a scalable alternative aligned with green chemical engineering principles for climate mitigation.

Keywords

Microalgae, Dissolved Inorganic Carbon, Carbon Sequestration, Carbon Fixation Efficiency, Nutrient Supplementation

1. Introduction

In recent decades, human activities such as burning fossil fuels and deforestation have led to a significant increase in atmospheric CO_2 levels. For the first time in human history, the daily average concentration of atmospheric CO_2 surpassed 400 parts per million (ppm) in May 2013 [1] and it has rapidly risen to a new record of 423 ppm currently [2]. The rise in atmospheric CO_2 levels is a cause for concern because it contributes to the warming of the planet and the resulting impacts such as sea level rise, more frequent and severe heat waves, droughts, and storms, as well as the loss of biodiversity [3]. The Intergovernmental Panel on Climate Change (IPCC) has warned that limiting global warming to 1.5°C above pre-industrial levels, the target agreed upon by the Paris Agreement, will involve rapid, deep and immediate greenhouse gas emissions reductions this decade [4]. The continued increase in CO_2 levels underscores the urgent need for immediate and sustained action to not only reduce greenhouse gas emissions, but also urgently and actively develop strategies to capture CO_2 from the atmosphere as well as from process flue gases and transition to a low-carbon economy [5,6].

In addition to using chemical solvents and sorbents [7], capturing CO_2 can also involve the use of biological approaches that rely on the natural process of photosynthesis. One such approach is the cultivation of microalgae, which are microscopic photosynthetic organisms that can capture CO_2 from the atmosphere and convert it into organic matter. Microalgae can be grown in a variety of habitats, making them a potentially sustainable and low-cost solution for carbon capture and utilization. Furthermore, the cultivation of microalgae can also provide additional benefits such as the production of biofuels, animal feed, and wastewater remediation. The use of biological approaches to carbon capture and storage, including microalgae, holds great promise for reducing atmospheric CO_2 levels and mitigating the impacts of climate change.

Whilst the use of microalgae for carbon capture and storage shows promise, several challenges remain to make it economically and environmentally feasible [8], prompting the adoption of various strategies - both biological and non-biological - to enhance the carbon uptake rate (CUR). Examples of biological strategies include appropriate strain selection [9], co-culturing with other microbes [10,11] and inoculation density used [12]. Non-biological strategies include carbon supply [13,14], nutrient supply [15–17], bioreactor design [18] and process modifications [19–21].

Despite numerous studies on microalgal growth under various CO_2 supply regimes, a systematic understanding of carbon availability and uptake by microalgae is lacking. Carbon availability and uptake by microalgae is complex and is defined by (a) CO_2 dissolution in the medium from the gas to the liquid phase, (b) chemical equilibrium between the different dissolved states of inorganic carbon (carbonic acid, bicarbonate and carbonate) and (c) the uptake of available carbon by microalgae and the resulting biological equilibrium between carbon supply and uptake. Whilst reports often concentrate on assessing carbon capture by microalgae through the accumulation of biomass, there's a noticeable gap in our understanding of how carbon and nutrient supply align with the biological uptake and overall availability within the culture.

It is worth noting that an increase in carbon supply does not guarantee a corresponding increase in availability, and even when carbon is available, not all of it will be fixed as biomass [22]. This complex interplay between carbon supply and uptake remains an underexplored aspect of microalgae research. Thus, this study will address these gaps by exploring the efficiency of passive versus active carbon supply regimes and their impact on dissolved inorganic carbon (DIC) availability, which is an underexplored aspect in the field of algal biotechnology.

The assessment of DIC is a widely used method for quantifying the concentration of inorganic carbon species in a medium, including CO_2 (gas), bicarbonate ions (HCO_3^-), and carbonate ions (CO_3^{2-}) aiding in the monitoring of carbon dynamics in a culture [23]. To enhance the reliability and replicability of carbon uptake measurements, this study introduces modifications to the DIC measurement method, including reducing sample volume and enabling sample storage without compromising accuracy.

This study will focus on *Chlorella vulgaris*, a halotolerant microalga known for its adaptability, fast growth, and robust performance under diverse environmental conditions. Previous studies have shown that two chosen halotolerant strains of *C. vulgaris* from the same habitat absorb different amounts of key nutrients such as nitrates and phosphates, depending on the supply of carbon, nitrogen, and phosphorus in the growth medium [24]. As a halotolerant microalga that can thrive in diverse salinity levels, *C. vulgaris* was selected as the target species in this study due to its well-documented adaptability, fast growth rate, and robust performance under various environmental conditions. Given the need to explore strain-specific differences in carbon uptake behaviour, *C. vulgaris* is an ideal model organism, with several well-characterized strains available from global culture repositories.

This study examines carbon availability and uptake by *C. vulgaris* strains through optimized DIC and CUR measurements. By first analysing carbon dissolution in algae-free medium and then studying the carbon uptake dynamics of the two strains, the study aims to assess existing carbon capture efficiencies and explore strategies to enhance them. We chose two *C. vulgaris* strains isolated from similar habitats, albeit from two geographically distinct culture repositories i.e. UK and India. The strain from the UK is referred to as CCAP (from The Culture Collection of Algae and Protozoa in Oban), while the strain from India is referred to as BDUG (from Bharathidasan University). Whilst molecular typing has indicated genetic similarity between the strains, their physiological responses, including CUR can vary significantly [24,25].

A key novelty of this work is the systematic assessment of microalgal carbon capture in relation to nutrient supply in two isolates of the same species. By measuring DIC concentrations over time, it is possible to optimize culture conditions for maximum growth and productivity. Understanding these dynamics is crucial for developing strategies to maximize the photosynthetic capacity of microalgae for carbon sequestration and transformation. Such insights are critical for improving the efficiency and sustainability of microalgae-based carbon capture and storage methods, thereby contributing to global efforts in combating climate change.

2. Experimental

2.1 Microalgae strains

Two brackish water strains of *Chlorella vulgaris* species (CCAP 211/21A and BDUG 91771) were investigated. CCAP 211/21A was obtained from The Culture Collection of Algae and Protozoa, based in the UK. BDUG 91771 was from an Indian repository (NRMC - Marine, Bharathidasan University, India). The taxonomic relationship between the two strains assessed by molecular typing has been elaborated before [24].

2.2 Microalgae cultivation and experimental set-up

Both the strains were maintained in *f*/2 medium [26], the composition of which was as follows (in 1 L): 33.6 g artificial seawater salts (Ultra Marine Synthetic Sea Salt, Waterlife), 75 mg $NaNO_3$, 5.65 mg $NaH_2PO_4.2H_2O$, 1 ml trace elements stock and 1 ml vitamin mix stock. The trace elemental solution (in 1 L) consisted of 4.16 g Na_2EDTA, 3.15 g $FeCl_3$. $6H_2O$, 0.18 g $MnCl_2$. $4H_2O$, 10 mg $CoCl_2$. $6H_2O$, 10 mg $CuSO_4$. $5H_2O$, 22 mg $ZnSO_4$. $7H_2O$ and 6 mg $Na_2MoO_4.2H_2O$. The vitamin mix solution (in 1 L) consisted of 100 mg vitamin B1, 0.5 mg vitamin B12 and 0.5 mg biotin, sterilized by filtration through a 0.2 µm polycarbonate filter in a flow cabinet. The *f*/2 medium without vitamins was sterilized by autoclave prior to the addition of the vitamin solution under sterile conditions.

All experiments were carried out in 1 L glass (Duran) photobioreactors with LED lighting. Three different media were examined, i.e. *f*/2, 2*f* and 4*f*. *f*/2 media were experimented by using four different types of carbon supply i.e. C_0, $C_{0.01M}$, C_{air} and $C_{1\%}$ which represents passive limited, passive excess, active limited and active excess carbon supply respectively.

C_0 is a microalgae cultivation regime, in which no additional carbon is added while $C_{0.01M}$ is another condition in which initial 0.01M of bicarbonate was added initially during inoculation. Meanwhile, C_{air} refers to the culture condition which is aerated continuously with air while $C_{1\%}$ refers to the culture condition which is aerated continuously with a 1% CO_2. 2*f* and 4*f* media were made by increasing the nitrate and phosphate contents to 4-fold and 8-fold, respectively, whilst keeping the other ingredients the same as in *f*/2. The nitrate and phosphate concentrations in 2*f* was 3.6 mM and 0.15 mM, respectively, whilst it was 7.2 mM and 0.29 mM, respectively, in 4*f*. The *f*/2 media without the presence of any microalgae will be called blank media, henceforth.

Mixing is employed by a stirrer in each of the photobioreactors to ensure homogeneous distribution of cells and nutrients. The schematic representation and details of the set-up and the rationale used in the experimentation has been elaborated elsewhere [24].

Aeration was obtained from gas lines (air and CO_2) in the laboratory through Teflon tubing. The flowrate was maintained by connecting a rotameter between the gas supply and the reactor. 1% (v/v) CO_2 concentration was obtained either from the gas cylinder tank of 1% CO_2 or by pre-mixing 100% CO_2 with air at different flowrates. The CO_2/air mixture was adjusted to achieve the desired concentration of CO_2 in the air stream through three rotameters that measured the flow rates of CO_2, the air, and the mixture of gases, respectively. To monitor the CO_2 concentration, gas supply to the culture was split to create one extra flow to a BlueInOne gas analyser (BlueSens, GmbH, Germany) which logged the CO_2 concentration every hour using an infrared sensor. Calibration was done as described by the manufacturer (bluesens.com), using ambient air as 0.04 vol% CO_2.

All strains were maintained in air aerated medium. The cells were acclimated to the CO_2 concentration and nutrient condition of the experiment for at least two generations prior to experimentation. This procedure is to aid the microalgae adaptation to the new environment minimising any shock responses of the algal cell when introduced to the new cultivation conditions, thus maximising the chances of a more stable response to be examined. This strategy has been adopted earlier [13].

2.3 Analytical Methods

Biomass concentration was determined based on optical density (OD), measured using a UV/Visible Spectrophotometer (SPECTROstar Nano, BMG Labtech, Germany) at 595 nm [27,28]. This optical density was converted to dry weight (g/l) using the following calibration equation (Eq. 1).

$$\text{Dry weight (g/l)} = 0.1719 * (\text{Optical Density}_{595\text{nm}}) - 0.0229 \qquad \text{(Eq. 1)}$$

DIC Measurement: DIC concentration in culture was estimated as detailed elsewhere [23], with minor modifications as detailed below. The method used eliminates non-carbonate alkalinity (e.g. those contributed by OH^-, NH_3^+ and H_3PO_4). In brief, the samples were harvested, and several relevant parameters (i.e. salinity, pH and temperature) were measured. The pH of the sample is an important parameter for this measurement and was measured using a pH meter (S40 SevenMulti™ pH meter, Mettler Toledo). The calibration was carried out using a buffer containing a similar salt concentration to avoid errors due to liquid junction potential. Based on the salinity and temperature, the pH where bicarbonate species is dominant (pH_{HCO3}) was determined. The samples were later brought to this pH. The first titration was carried out to pH 3.5, whilst the second (back) titration was carried out back to the initial pH_{HCO3} after nitrogen purging. The amount and concentration of titrant was recorded, which was then translated to Total Alkalinity and Non-carbonate Alkalinity. Based on the correlation, Carbonate Alkalinity obtained was converted to total CO_2 (T_{CO2}) or DIC. Speciation of this value to CO_2, bicarbonate and carbonate was done by inputting the parameters to CO2SYS program available online. It should be noted that equilibrium-based models may underestimate pCO_2 under highly dynamic or alkaline conditions and that direct validation against measured pCO_2 would provide stronger confidence in the computed values. Nevertheless, for all practical purposes, the system we are investigating can be considered to be in pseudo-equilibrium when the measurement is made. This is a valid assumption, as the chemical changes in response to biological activity are reported over day intervals, which is a long enough time period for us to assume pseudo-equilibrium conditions to be prevailing (with respect to the chemical changes), at a given time point. The original method was modified and optimised for (a) a reduced sample volume (5 mL as opposed to 10 mL, originally proposed) and (b) sample storage (as opposed to immediate analysis). The results of this optimisation exercise are discussed in the Results and Discussion section.

Carbon Uptake Rate (CUR) Measurement: Carbon uptake by microalgae was calculated in one of two ways, depending on the carbon supply. For the cultivations without continuous gas supply (C_0 and $C_{0.01M}$) the difference in DIC concentration between two time-points was used to measure CUR, assuming that this difference directly relates to consumption by microalgae. A control experiment on carbonated blank media in reactors left for several days was run to monitor the changes in DIC concentration, if any. No noticeable difference in DIC concentrations in the blank media was observed, at the time points measured, and the concentration was stable for the duration of the experiment.

For cultures with continuous gas supply (C_{air} and $C_{1\%}$), a dynamic method was employed, where the gas supply was stopped at a given time point and switched to nitrogen supply for up to 60 minutes. Similar methods to measure gas uptake have been reported in the literature [29–31]. The DIC readings were recorded just before (0 minutes) and after 60 minutes of nitrogen passage, when the carbon supply was resumed. Carbon uptake by algae was measured by considering the difference in DIC change in both blank media (treated similarly) (starting pH 7.2) and also the algal culture as shown in the Eq. 2.

Algal DIC Uptake rate (mM/hr) =

$$\frac{DIC_0 - DIC_t}{t_t - t_0}_{culture} - \frac{DIC_0 - DIC_t}{t_t - t_0}_{media} \qquad \text{(Eq. 2)}$$

where DIC_0 and DIC_t are the DIC readings taken at time t_0 (when the carbon supply was switched to nitrogen) and at time t_t, after which the carbon supply was resumed, respectively.

The time duration for passing nitrogen was optimised by measuring DIC at different time intervals between 0 and 60 minutes, to arrive at a time that showed no further changes.

Carbon fixation and uptake efficiencies: The CO_2 fixation efficiency was estimated based on biomass concentration, assuming a simple elemental stoichiometry of $C_5H_7O_2NP_{0.5}$ for the algal biomass produced [32], and a simple reactor mass balance (Eq. 3). The CO_2 uptake efficiency was estimated similarly, but based on the CUR measured, instead of the CO_2 fixed in the biomass. Whilst the former measure indicates the efficiency of fixing the carbon as biomass, the later indicates the amount of carbon taken up by the system overall, only considering uptake and does not account for any loss of CO_2 post uptake.

Carbon in the reactor + Carbon supplied = Carbon in the medium + Carbon fixed in the algae + Carbon loss to the atmosphere (Eq. 3)

CO_2 Dissolution behaviour in blank media*:* Three levels of CO_2 gas (v/v in air) were tested, i.e. 100%, 10% and 1%. Before pumping of CO_2, pH was set to the desired value by using 0.1M sodium hydroxide, to have approximately similar speciation of carbon in the media at the beginning. Gas containing different levels of CO_2 was sparged into the blank medium (culture medium with no algae in it), and the DIC measured at intervals until it saturates. Saturation is defined when three recorded readings are within the 10% range.

Statistical Analysis: Each experiment was conducted in a minimum of three replicates. The data points reported in all graphs are mean of replicate measurements, whilst the error bars denote the standard errors about the mean. When comparing two datasets, only data with a significant difference of $p < 0.05$ calculated using a *t*-test are reported.

3. Results and Discussions

3.1 CO_2 dissolution dynamics and measurement of CUR

DIC was quantified using a back titration method as previously outlined [23]. An optimization process was aimed to reduce the required sample volume for estimation and facilitate sample storage before analysis. The results, depicted in Figure 1A, indicate that halving the sample volume for titration (treatment 1) and storing samples at -20 ºC for 3 days (treatment 2) or 7 days (treatment 3) did not yield a statistically significant change in measured DIC values. Consequently, the original method was modified to utilize a 5 mL sample for titration and DIC measurement within 7 days of storage at -20 ºC.

Another optimization process was aimed to get a suitable duration of N_2 sparging. For cultures with continuous gas supply, CUR was estimated by measuring the difference in DIC before and after switching the supply of gas from 1% CO_2 to N_2 for a fixed period of time (Figure 1B). Switching from gas containing CO_2 to N_2 will result in release of dissolved CO_2, which needs to be accounted for. This is done by measuring the baseline, using a blank media, and accounting for the changes in the CUR estimation (Figure 1C).

It is essential to maintain an active gas supply whilst recording DIC measurements for the CUR estimation. Switching the gas supply off caused unstable DIC readings as the carbon in the reactor's headspace began to equilibrate and dissolve back into the medium, thus an error. To prevent this error, continuous gas sparging is maintained by supplying inert nitrogen gas (N_2) at the same flow rate as 1% CO_2. Sparging with nitrogen for a fixed period establishes a stable condition without additional CO_2 supply, allowing us to measure CO_2 uptake by tracking changes in the medium's DIC when the cultures are not actively receiving CO_2.

Five time-points (5, 10, 20, 30 and 60 minutes) were investigated to determine the optimal duration for N_2 sparging. During N_2 sparging, some DIC (as dissolved CO_2) is lost due to the purging action of nitrogen, which was accounted for in the analysis. This loss caused greater deviations in DIC readings at the initial time points, which diminished over time (Figure 1D). The first data point (5 minutes) is lower than the second likely due to the initial instability in DIC readings caused by the equilibration process between the reactor's headspace and the liquid medium when the gas supply is switched. The 60-minute time point showed the smallest percentage change in DIC and the highest reproducibility, making it the optimal duration for N_2 sparging (Figure 1D). Although N_2 purging has been reported in the literature before [29–31], this has not been investigated to sufficient detail to understand changes in DIC.

3.2 CO_2 dissolution behaviour in algae-free media

Continuous supply of gas to the algae-free medium results in an increase of medium DIC followed by saturation. The dissolution behaviour of CO_2 in algae-free *f*/2 medium was studied by sparging the medium with air containing CO_2 at different levels (0%, 1%, 5%, 10% and 100% CO_2), and measuring DIC at different time intervals. To the best of our knowledge, this has not been sufficiently explored in the investigations reported in the literature thus far and is important to help understand and establish DIC uptake by algae.

The solubility of CO_2 in algal growth media is studied by measuring its DIC content. Upon supply with increasing concentrations of CO_2 gas, DIC concentration in the algae-free *f*/2 media showed increasing saturation values. However, in the time duration monitored, none of the values reached the theoretical maximum of 33 mM at 20 °C and 0.1 MPa [33], which is equivalent to 1.5 g/L. For the record, saturation by 100% CO_2 at the mentioned condition with 0.2 vvm gave the experimental DIC saturation value of 16 mM, which appears to be the value at equilibrium with the atmosphere.

As shown in Figure 2A, media aerated with 100% CO_2 showed an 8-fold increase in DIC, at saturation, whilst 10% CO_2 gave a 2.5-fold increase and 1% CO_2 a 2-fold increase in DIC at saturation, compared to the starting values. Meanwhile, sparging the medium with air decreased the DIC by 30%.

Theoretically, a supply of 100% CO_2 of 0.2 vvm for an hour in 1-litre media is equivalent to a cumulative supply of 500 mM CO_2, over the period. Similarly, a supply of 1% CO_2 is equivalent to 5 mM carbon (0.218 g $L^{-1}CO_2$). However, this is not necessarily what is dissolved into the medium as there is always an equilibrium between dissolved and gas-phase CO_2, in addition to the escape of CO_2 out to the surroundings/outlet. This is the reason the supply of 1% CO_2 concentration at 0.2 vvm resulted in a maximum DIC of 2.6 mM (CO_2 of 0.114 g L^{-1}) experimentally. In this one-hour supply, the efficiency of around 50% of CO_2 dissolution into the medium due to the reason mentioned before. As the medium reaches carbon saturation, this efficiency decreases further since more and more carbon supplied to the media will escape to the surrounding.

Most of the saturation kinetics happened in the first hour of carbon supply. Supply of 100% CO_2 gave fluctuating readings that took longer to settle down, i.e. 40 minutes, compared to 10% CO_2, at the employed volumetric flow rate. This fluctuation may be due to the high proportion of CO_2 gas already present in the media, which will easily diffuse out from the medium to the surrounding. This is because for a medium at higher CO_2 concentrations, the liquid-gas equilibrium is more prone to disturbances caused by further addition of CO_2, leading to temporary imbalances in dissolved CO_2 levels.

It is also found that sparging with air (0.04% CO_2, 21% O_2, 78% N_2) does not contribute towards the increase in CO_2 dissolution value but instead results in loss of DIC from the medium (Figure 2A). After all, aeration is a known method of CO_2 removal [34].

Since pH of the medium defines the final CO_2 saturation conditions, CO_2 saturation curves were obtained under different initial pH of the algae-free medium. The results are plotted in Figure 2B for 100%, 10%, 1% CO_2 in air, as well as for air (0.04% CO_2). It is found that as the initial pH of the medium increases, the DIC value at saturation also increases, for all the levels of CO_2 supplied. It can also be observed that the DIC saturation value increases with the concentration of CO_2 supplied. Besides, it can be seen that 100% CO_2 gives the highest gradient, i.e. 2.7 mM/pH unit, followed by 10%, CO_2 (1 mM/pH), 1% CO_2 (0.9 mM/pH), and air (0.3 mM/pH), following the trend of the amount of CO_2 dissolved into the medium. As can be seen from Figure 2B, the relationship between initial pH of dissolution and the final

DIC at saturation is fairly predictable for all cases and can be estimated from the linear relationships indicated in Table 1.

3.3 Dynamics of DIC availability and uptake in microalgae cultures

DIC of a standard carbonate system in seawater is ~2 mM [35], which was also the initial DIC molarity in most of the conditions tested here, except for $C_{0.01M}$ culture, where a DIC of 10 mM of bicarbonate was added to the 2 mM already present, so ~12 mM was the starting point. As for the amount of carbon being supplied by air and 1% CO_2 (0.2 vvm for nine days), gravimetric analysis shows that carbon supplied to the culture is equivalent to 0.04 M and 1.07 M of T_{CO2}, respectively. This conversion is made assuming a pressure of 1 atm and a temperature of 23 °C (laboratory experimental conditions) (Eq. 4).

$$CO_2\ Concentration\ (mol/litre) = \frac{Amount\ of\ CO_2\ supplied\ in\ 9\ days\ at\ 0.2\ litre/minute\ (g)}{CO_2\ molecular\ weight\ (44\ g/mol) \times experimental\ volume\ (litre)} \quad \text{(Eq. 4)}$$

Thus, we may estimate the amount of carbon added in C_0 as negligible, 10 mM in the bicarbonate-supplied condition (sufficient at the start, but decreasing with time), < 40 mM in air (a low suboptimal supply), and < 1070 mM in 1% CO_2 (well exceeding required levels and saturating). This is because not all of the supplied carbon is fully available for consumption as some of the gas supplied escapes into the surrounding. This allows us to estimate the behaviour of carbon uptake by the algae with carbon availability ranging from negligible to saturating conditions, as well as static (or passive) (i.e. C_0 and $C_{0.01M}$) and dynamic continuous supply (or active) (i.e. C_{air} and $C_{1\%}$). In addition, we included two nutrient excess conditions, where the nitrogen and phosphorus supplied was 4-fold (2*f*) and 8-fold (4*f*) that of the other conditions (*f*/2), to assess the difference in the behaviour of the two chosen strains in a range of carbon supply regimes, including nutrient variations, as reported earlier [24]. For these latter high nutrient conditions, we employed the carbon excess condition ($C_{1\%}$) to assess the influence of nutrient availability on carbon uptake.

In C_0 and $C_{0.01M}$ cultures (passive supply), carbon was utilised rapidly at the beginning of the experiment, i.e. during the exponential phase, as can be seen in Figure 3. For the BDUG strain, C_0 cultures become carbon limited at day 6, whilst for $C_{0.01M}$, carbon-limitation happens at day 8. For the CCAP strain, DIC in C_0 culture was consumed fast in the first two days before the uptake rate slowed. The culture becomes carbon limited at day 8. Meanwhile, $C_{0.01M}$ culture becomes carbon limited at day 4, coinciding with cessation of growth. This observation indicates that both the strains require CO_2 supply in excess of the 10 mM bicarbonate initially added, or in other words, there is more capacity for carbon uptake beyond the duration of the culture than supplied at the start.

In the active carbon supply regimes (C_{air} and $C_{1\%}$), CO_2 was continuously supplied. In C_{air} culture, carbon starvation can be observed in both cultures (despite a continuous supply), as the DIC values were consistently below the corresponding saturation values in the algae-free medium. In the BDUG culture, DIC decreases at day 2 before plateauing at day 4, at around 0.5 mM DIC. In the CCAP culture, the DIC decreases slowly from 1.4 mM at the beginning to nearly zero at the end of the experiment (day 8). Clearly in both cases, the carbon at the end of the culture has reached limiting levels, indicating more capacity for the cultures to take up carbon than was made available. Such limiting conditions have been reported in the literature [36], where cultures aerated with ambient air was reported to have undetectable CO_2, high pH and low bicarbonate levels throughout the course of study.

In $C_{1\%}$ culture, DIC equilibrium was reached for both BDUG and CCAP culture after day 2. More importantly, the DIC saturation values were much above the corresponding saturation values in algae-free medium. Thus, the presence of algae has apparently increased the amount of DIC in the culture. We can interpret a DIC higher than the baseline as an indicator that carbon being consumed by the algae is less than the carbon being supplied. This also implies that the algae are able to enable sequestration of CO_2 in the culture. On the contrary, a reading lower than the baseline (as seen with C_0 and $C_{0.01M}$ cultures) shows that the amount of carbon being consumed is higher than the carbon being supplied, assuming no other contributions to the apparent change in DIC.

The four carbon supply regimes (C_0, $C_{0.01M}$, C_{air}, and $C_{1\%}$) were tested in *f*/2 culture medium, where the concentration of two of the key macronutrients, nitrogen and phosphorus were initially at 0.88 mM and 0.04 mM, respectively. It was noted that nitrogen and phosphorus in the medium were depleted in the dynamic carbon supply regimes (C_{air} and $C_{1\%}$), as discussed elsewhere [24], suggesting that this could have limited the algae's capacity to take up CO_2, especially in the $C_{1\%}$ condition, where excess CO_2 was available and apparently left unutilised. We therefore tested the 2*f* and 4*f* regimes in $C_{1\%}$, where excess N and P is available to allow the consumption of the excess CO_2. As can be seen from Figure 3, these conditions resulted in an increase in the DIC, as opposed to saturation, commensurate with the excess supply of nutrients, for both the strains. Nitrate and phosphate were both available throughout the cultivation period for these two conditions (2*f* and 4*f*), just approaching depletion at the end of cultivation, in the 2*f* condition, but still available in excess in the 4*f* condition, discussed elsewhere [24]. It is known that an increase in DIC in nitrate-fed algal cultivation is associated with consumption of nitrate and a concomitant increase in alkalinity of the medium [32,37].

Figure 3 also depicts the speciation of the DIC for the 6 conditions, in the BDUG and CCAP culture, respectively. The pH of the cultivation in C_0, $C_{0.01M}$, C_{air} conditions was alkaline, whilst for $C_{1\%}$ it equilibrated to 7.1 [24]. Most of the carbon in the culture medium existed as bicarbonate (HCO_3^-) at all times. This can be seen to be decreasing in C_0, $C_{0.01M}$, C_{air} conditions, whilst increasing in the $C_{1\%}$, 2*f* and 4*f* conditions, the latter conditions showing presence of CO_2, given the decreasing pH. In the passive supply regimes (C_0 and $C_{0.01M}$), the BDUG strain appears to be consuming the available carbon more rapidly than the CCAP strain. In C_{air}, HCO_3^- consumption plateaus for the BDUG strain, whilst there is a steady consumption to depletion for the CCAP strain, suggesting that when being supplied with continuous carbon supply, the CCAP is able to sequester more carbon than supplied, such that the carbon concentration in the continuous air supply is still below the uptake capacity of CCAP. Reports on *Desmodesmus* sp and *Acutodesmus* sp. cultivations [36] have shown that the bicarbonate concentration plateau in the C_{air} culture but decreases throughout the experiment in the culture with 20% CO_2. However, in the cultivation of *Kirchneriella cornuta*, bicarbonate concentration nearly stays the same throughout the experiment, both for C_{air} and culture with 20% CO_2 supply [36].

The translation of carbon uptake to growth and biomass accumulation can be seen in Figure 4. No statistically significant differences in the specific growth rate were noticed between the conditions for CCAP, the median specific growth rate hovering around 0.3-0.4 d^{-1} (Figure 4A). However, an active supply of sufficient carbon ($C_{1\%}$) resulted in a dramatic increase in the maximum specific growth rate of the BDUG strain that was not seen with the CCAP strain. The median specific growth rate for this strain increases nearly 3-fold in $C_{1\%}$, compared to C_0, $C_{0.01M}$, and C_{air}. Interestingly, excess nutrient supply (2*f* and 4*f*) did not result in increase in growth rates for the BDUG strain. Here, a high growth rate was achieved under relative nutrient limited conditions (*f*/2), indicating differences in the capacity of the two strains to respond to the different conditions tested. The growth performance of these setups and the corresponding nutrient concentrations, along with their relationship to nutrient consumption, has been previously discussed and compared with existing literature [24].

The average biomass productivity was the lowest for the C_0 condition for both the strains (Figure 4B), as would be expected from the low and depleting levels of CO_2 in this condition, and reported elsewhere [38]. This scenario is improved in $C_{0.01M}$, with the availability of excess carbon at the start, in both the strains. A continuous supply of CO_2, in C_{air}, results in a similar average biomass productivity for CCAP, as $C_{0.01M}$, but one that was lower for BDUG. Interestingly, BDUG responds more drastically to the supply of excess carbon ($C_{1\%}$), indicating that this strain is more sensitive to the supply of carbon than the CCAP strain, as there was no statistically significant difference in average biomass productivity, between $C_{0.01M}$, C_{air}, and $C_{1\%}$, for the CCAP strain. Different species show differences in the influence of carbon supply towards biomass productivity, as can be observed from reports in the literature. *Chlorella variabilis* showed improved biomass productivity in continuous air supply that was supplemented by intermittent bicarbonate addition, compared to supplementation with intermittent supply of 1% CO_2, or with continuous supply of 1% CO_2 [39]. *Botryococcus baunii* showed better growth with continuous 10% CO_2 supply, compared to continuous air-sparged or 20% CO_2 supplied cultures, whilst *Scenedesmus* sp. showed better growth with incremental carbon supply, 20% CO_2 supplied cultures showing best results, in this regard [40]. *Chlorella kessleri* cultivated at different CO_2 concentrations (0, 2, 6 and 10%), showed higher biomass productivity with higher carbon supply (2-10% CO_2) compared to air (0%) [41]. This has also been observed with other *Chlorella* sp. [42]. This strengthens the observation that for a given species, supplementing the culture with more CO_2 does not necessarily mean it will translate to a higher biomass productivity, as has been noted earlier. In fact, CO_2 concentration tolerance of microalgae differs from species to species [40,43].

Increasing the nutrient supply (2*f* and 4*f*) made no significant difference to the biomass productivity in BDUG, but this resulted in a significant increase in CCAP in the 2*f* condition that wasn't improved in 4*f*. Thus, whilst BDUG was more sensitive to carbon supply, CCAP appears to respond more to changes in N and P. These observations are also reflected in the levels of the final biomass produced at the end of the cultivation period (Figure 4C), where increasing supply of C, N and P results in incremental biomass accumulation for both the strains, with a statistically significant difference in response between the strains in C_{air}, and $C_{1\%}$ ($p < 0.05$). This can be compared to literature data, where species level differences have been reported along the lines observed here for the two strains. *Scenedesmus obliquus* showed a final biomass concentration that was 1.5-fold higher when the carbon supply was changed from air to 2.5% CO_2 [44], and an increase in N supply of the culture by 4 fold resulted in an increase in final biomass by more than 4 fold, in *Chlorella vulgaris* [45].

CUR can be approximated in C_0 and $C_{0.01M}$ cultures to the difference in DIC between two time points, given that there is no "active" supply of CO_2, other than at the start, which is consumed by the organism with passage of time. However, in C_{air}, and $C_{1\%}$ cultures, CUR estimation has to account for the dynamic scenario of continuous active supply of CO_2. We adopted the strategy of switching the CO_2 supply to N_2 and monitoring the decrease in DIC for a fixed period of 1 h to enable us to estimate CUR in the $C_{1\%}$ cultures. We did not estimate CUR in C_{air}, as we assessed this method to be less reliable for monitoring changes in the lower CO_2 concentrations seen in air (0.04%).

In both BDUG and CCAP, maximum CUR in C_0 and $C_{0.01M}$ were recorded as 1 mM d^{-1} and 4 mM d^{-1}, respectively, for both BDUG and CCAP. This value is higher than reports in literature for static cultures (equivalent to C_0), for example, for freshwater *Chlorella* sp., in which HCO_3^- concentration changed at a rate of 0.63 mM d^{-1} [46], and, in *N. salina* culture initially supplied with 5 mM bicarbonate (equivalent to $C_{0.01M}$ condition in this investigation), where a rate of around 0.7 mM d^{-1} was reported [23]. A carbon sequestration capacity of 15 mg L^{-1} d^{-1} was reported for *Chlorella* sp., when supplied with ammonium bicarbonate [47]. The maximum CUR for both the strains in this study increases dramatically with more available carbon and a more active supply of CO_2 ($C_{1\%}$), as can be seen from Figure 5A. This is seen more with the BDUG than the CCAP strain, in *f*/2 and 2*f* conditions, indicating the sensitivity of this strain to CO_2 supply, as was noted earlier. Increase in CUR with increase in carbon supply has been reported for freshwater *C. vulgaris* [48,49].

With both the strains, the maximum CUR did not increase with the additional nutrient supply (2*f* and 4*f*), which is also observed elsewhere for this species [50]. In our study, the maximum specific CO_2 uptake rates for the $C_{1\%}$ carbon supply regime clearly exceeded that in C_0 and $C_{0.01M}$, indicating that the cells have the capacity to absorb more CO_2 than supplied in these static regimes that is improved substantially on supply of excess nutrients (2*f*, 4*f*) (Figure 5B). This latter point was noted better with the CCAP strain, which showed a significantly higher value in the 2*f* regime, compared to the BDUG strain. An increase in CUR with increased CO_2 supply has been reported in *C. vulgaris* and *Scenedesmus* sp. [51].

The median CO_2 sequestration capacity of the cultures, over the cultivation period, can be seen to be higher for the $C_{1\%}$ carbon supply regime, with a significant increase in capacity compared to C_{air}, for both the strains (Figure 6A). Interestingly, the nutrient excess conditions (2*f* and 4*f*) do not appear to help in improving the sequestration capacity for these two strains of *C. vulgaris*. As noted earlier [24], phosphate gets depleted whilst nitrate is in excess in 2*f*, but both the nutrients are in excess in 4*f*. It is possible that other limitations, such as shading may have contributed to the response. It is worth noting that the cultures were acclimatised in the respective conditions they were grown in before the experiment.

Increased carbon supply results in an increased assimilation of CO_2, as has been demonstrated in many instances [52,53]. Nevertheless the efficiency of CO_2 assimilation in relation to supply appears to be poorly characterised. In this study, the CO_2 fixation efficiency for each of the regimes was calculated based on the total amount of carbon supplied over the cultivation period and the amount of carbon fixed into biomass. This is plotted for each of the regimes in Figure 6B, for the two strains. As can be seen the passive supply regimes are the most efficient in terms of sequestering available carbon into biomass. Under these conditions, very little carbon is available to the biomass, but nearly all of the available carbon is fixed in the biomass.

However, the active carbon supply regimes fare poorly. The efficiency drops to ~20% in C_{air} and is <5% in $C_{1\%}$. This suggests that the higher CO_2 supply regimes, as in the case of using flue gases, which typically contain CO_2 in excess of 10%, will need improvement in CO_2 fixation efficiencies by at least an order of magnitude, with considerable scope for research and development. There are reports on CO_2 fixation efficiency with other species of algae such as *Scenedesmus* sp., at 9.8%, for a CO_2 supply 5% (v/v) [54], peaking between 5-7% for a CO_2 supply of 10-20% with *Scenedesmus* sp. [40], and peaking at 20%, for a CO_2 supply of 20% with *B. braunii* [40].

Interesting differences in the evolution of the fixation efficiencies over time can be noted between the strains investigated in our study (Figure 6C). Whilst in C_0 the BDUG strain displays a lower fixation efficiency throughout compared to the CCAP strain, the trend reverses in $C_{0.01M}$, where the efficiency drops for CCAP, but increases for BDUG, suggesting the different capacities to uptake bicarbonate, between these two strains. Such differences are noted between species [55–57] but seldom reported between strains of the same species. BDUG is however found to be less efficient in C_{air}, where the efficiency drops to <10% with time.

3.4 Increased carbon capture is afforded in nitrate-containing media

The increasing algal biomass produced in culture correlated well with an increase in the DIC measured in the medium for 2*f* and 4*f* conditions, where higher nitrate consumption was possible (Figure 7A). This suggests that the growth of algae biomass results in a greater availability of DIC in the medium. This has been reported in the past with cyanobacteria [58] and green algae [32] and attributed to the presence of nitrate in the culture medium. The linear relationship projected for cyanobacteria [58] was used to predict the DIC, for a given biomass of the two strains of green algae used in the current study. This is plotted as DIC_{pred}. However, the DIC calculated from the experiments ($DIC_{expt.}$) appeared to show a second order polynomial fit to the biomass produced, for the two green algae strains used in this study, suggesting that the relationship cannot be generalised. A correlation plot of nitrate consumed against the increase in DIC (Figure 7B) shows that this is not a universal simple relationship, as can be seen by the different linear relationships for each condition, even for these closely associated strains (as seen in 4*f*). A relationship that is linear (R^2>0.9) can only be seen at higher nitrate concentrations (4*f*), for the two strains of *C. vulgaris*, investigated in this study.

Understanding the relationship between microalgal biomass and DIC can aid in developing strategies to mitigate nitrate pollution (runoff from agriculture fertilizer usage) and eutrophication. Recognizing the role of nitrate and phosphate in promoting algal growth and understanding the relationship between microalgae biomass and available DIC in relation to nutrient availability, might help develop strategies to allow for more efficient consumption of nutrients by microalgae. This will be useful in developing sustainable wastewater treatment opportunities.

Table 2 provides a comparison of carbon sequestration capacity observed in this investigation with that of chemical alternatives, on a gram per gram basis. Among the chemical alternatives, the highest recorded sequestration capacity is shown by NaOH (1.1 g CO_2 / g). This value is 0.7 g less than the stoichiometric capacity frequently reported for microalgae, i.e. ~1.8 gram CO_2 being uptake per gram algae produced.

This mass difference may highlight the potential of microalgae as an agent for CO_2 utilization. In addition, microalgae have the advantage of producing value-added chemicals, under atmospheric temperature and pressure, with the option of retaining active ingredients inside the cell in dry powder form, enabling storage that can minimise associated process challenges. These advantages highlight the usefulness and potential of microalgae cultivations for CO_2 sequestration. This table, however, does not necessarily suggest that the gram per gram basis is the only criteria for comparison since there are other aspects to consider such as overall cost, process complexity and life cycle assessments.

Understanding the dynamics of CO_2 dissolution, the availability of DIC for uptake by microalgae, and the influence of nutrients on carbon capture in microalgae cultures is vital for optimizing microalgae-based carbon sequestration strategies. This study has investigated how CO_2 is absorbed and dissolved in an algae-free medium to provide baseline data to better understand carbon uptake by microalgae. Observations on the availability and uptake of DIC by two strains of *C. vulgaris* in media with different levels of nutrients indicated that the presence of nitrate in the culture medium enhances the uptake of DIC by the microalgae, promoting photosynthetic activity and biomass production. It is believed that the presence of nitrate facilitates the consumption of carbon by the microalgae. This investigation provides the basis for developing effective cultivation techniques, nutrient management strategies, and culture conditions that maximize carbon capture efficiency enhancing the overall sustainability of using microalgae-based systems and strategies.

4. Conclusions

This study investigated the dynamics of CO_2 dissolution, availability and uptake in two halotolerant strains of *Chlorella vulgaris*, influenced by nutrient and carbon supply. Dissolution of CO_2 in the algae-free medium reached different saturation values that depended on the concentration of CO_2 supplied. However, equilibrium conditions do not allow for a theoretical maximum to be reached, even with a supply of 100% CO_2. This has implications with respect to CO_2 that can be made available for consumption that is controlled by supply. Subsequent examination of CO_2 uptake in the two halotolerant strains showed that continuous carbon supply regimes result in varying levels of carbon starvation, which can affect growth and carbon uptake efficiency. Carbon availability has been shown to change when excess nutrients (nitrogen and phosphorus) are available, offering an attractive avenue for further exploration and potential utilization. In addition, nitrogen and phosphorus supply was found to enhance the specific CUR by both the strains, leading to increased photosynthetic activity and total biomass produced. Differences in behaviour was observed between the two strains that were isolated from a similar habitat, suggesting the significance of examining specific strains carefully. Passive carbon supply regimes result in lower carbon availability but allow for maximal carbon fixation, resulting in relatively higher efficiency of carbon fixed with respect to supply. Although the active carbon supply regimes resulted in greater availability of carbon, these were less efficiently fixed into biomass, suggesting considerable scope in development of strategies to maximise uptake. A key finding of the study is the establishment of a nonlinear correlation between increase in medium DIC with biomass produced. The study contributes to understanding carbon availability and uptake in microalgae, offering insights for developing strategies in carbon sequestration and sustainable wastewater treatment.

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Faqih Shuhaili: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Mathumathy Srinivasan**: Investigation, Methodology, Writing - original draft. **Rashmi Vijayaraghavan**: Methodology, Writing - review & editing. **Mariona Segura-Noguera**: Data curation, Validation, Writing- review & editing. **Uma Laksmanan**: Data curation, Funding Acquisition, Resources, Writing- review & editing. **Dharmar Prabaharan**: Data curation, Funding Acquisition, Resources, Writing-review & editing. **Seetharaman Vaidyanathan**: Conceptualization, Data curation, Funding acquisition, Project Administration, Resources, Supervision, Validation, Visualization, Writing - original draft, review & editing.

Data Availability

Data will be made available on request.

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Table 1. Analysis of relationship between concentration of inorganic carbon at saturation (y) and its initial pH (x) post aeration with respective CO_2 levels supplied, shown in Figure 2B.

CO_2 Levels Supplied	**Equation**	**R^2**
100%	$y = 2.7x - 1.7$	0.8
10%	$y = 1.0x - 2.5$	1.0
1%	$y = 0.9x - 4.4$	0.8
0.04% (Air)	$y = 0.3x - 0.1$	0.8

Table 2. Comparison of carbon uptake capacity for microalgae compared to several chemicals

Sequestration Agents	**Amount Reported in the Literature**	**Equivalent g CO2 per g Algae Produced or Present**	**Note**
Chlorella vulgaris (This experiment)	4 – 25	4 – 25	Calculation based on the DIC in the culture media
Microalgae (in general)	1.8 ton/ton CO2	1.8	Commonly Cited [59]
NaOH[a]	0.9 ton/ton CO2	1.1	Theoretical Value [60]
MEA[b]	1.39 ton/ton CO2	0.7	Theoretical Value [60]
MEA	581.3 g CO2/kg MEA	0.6	Experimental Value [61]
IPA[c] / DA[d]	0.499 g CO2/g amine	0.5	Experimental Value [62]
PZ[e] / AMP[f]	1.0 mol CO2/mol	0.5	Experimental Value [63]
AEEA[g] / MDEA[h] / DET[i]	1.0 mol CO2/mol	0.4	Experimental Value [63]
Graphene	7.95 mmol CO2/g	0.4	Adsorption capacity [63]
Ionic liquids [TETAH][BF4] containing 40% water	2.04 mol CO2/mol	0.4	Experimental Value [64]

TETA[j]	170 g CO2/kg	0.2	Experimental Value [65]
DEA[k]	0.5 mol CO2/mol	0.2	Experimental Value [66]
Deep Eutectic Solvent [2DBN[l]:Tz[m]]	0.2 g CO2 / g	0.2	Experimental Value [67]
Arginine	0.8 – 1.7 mol CO2/mol	0.2 - 0.4	Experimental Value [66]

[a] Sodium hydroxide [b] Monoethanolamine [c] Isopropylamine [d] Diethylamine [e] Piperazine [f] aminomethyl propanol [g] Aminoethylethanolamine [h] Methyl diethanolamine [i] Diethyltryptamine [j] Triethylenetetramine [k] diethanolamine [l] Diazabicyclo non-5-ene [m] Triazole

Fig. 1 Measurement of DIC and carbon uptake rate (CUR). **(A)** Effect of three method modifications (reduced sample volume and measurement after cold storage for 3 and 7 days) on the DIC measurement, compared with control; C – control (10 mL sample volume, measured immediately), 1 – 5 mL sample volume, measured immediately, 2 – 5 mL sample volume, measured after storage at – 20 ºC for 3 days, 3 – 5 mL sample volume, measured after storage at – 20 ºC for 7 days; **(B)** CUR measurement methodology. At t_1, CO_2 (1%) supply is stopped and replaced with nitrogen until t_2, and DIC measured at different time points. CUR is measured as the DIC difference between t_1 and t_2 subtracted from the baseline change in DIC after nitrogen purge in algae-free *f*/2 medium (indicated as ΔC_{ib}); **(C)** DIC species distribution before (t_1) and after (t_2) nitrogen purge in algae-free *f*/2 medium; **(D)** DIC % change rate at different t_2 with respect to t_1.

Fig. 2 Dissolution dynamics of CO_2 in the algae-free culture medium (*f*/2). **(A)** Fold change in DIC (relative to the start) over time at different gas concentrations of CO_2 in air (v/v); **(B)** relationship between DIC saturation ($[C_i]_{sat}$) values and pH of culture medium at the start of the dissolution, for gases with 100% CO_2, 10% CO_2, 1% CO_2 in air (v/v), and air (0.04% CO_2). The respective relationship equations are provided in Table 1.

Fig. 3 Dissolved inorganic carbon (DIC) profiles in the microalgal culture medium for each of the *Chlorella* strains, in the different carbon and nutrient supply regimes (top panels). The green dotted line indicates the pH corrected baseline saturation value in algae free medium for air, and the black dotted line for 1% CO_2. The secondary axis on the right is the DIC (mmoles/kg) for $C_{0.01M}$. The individual carbon species profiles for each carbon and nutrient supply regimes are shown in the bottom panels for the two strains. Left hand panels indicate the response for the BDUG strain and the right hand panels for the CCAP strain.

Fig. 4 Growth and biomass productivity under the tested conditions. **(A)** Specific growth rate ; **(B)** Average biomass productivity and **(C)** Total biomass produced over the cultivation period.

Fig. 5 Carbon uptake rates under the tested conditions, measured using N_2 purging. **(A)** Maximum carbon dioxide uptake rate (CUR); **(B)** Maximum specific CUR.

Fig. 6: (A) CO_2 sequestration capacity of the two cultures under the tested conditions. The range displayed is the minimum and maximum in each condition for each strain, with the bar representing the median value over the cultivation period. The carbon sequestered is a sum of the carbon fixed into biomass (based on biomass productivity) and that retained in the culture medium as DIC. **(B)** Maximum efficiency of CO_2 fixation into biomass (based on stoichiometric levels of CO_2 fixed in the biomass formed) at the different culture conditions. **(C)** The time profiles of the fixation efficiencies are also shown for the two strains under each of the carbon and nutrient supply regimes tested.

Fig. 7 (A) Correlation between DIC and biomass. Experimental data is plotted as closed circles (blue), which fits a quadratic model and the predicted result based on equation is shown in open circles (red), which fits a linear model; **(B)** Increase in DIC correlated with the decrease in nitrate in the medium for each strain, under each of the three nitrate supply regimes, in $C_{1\%}$.

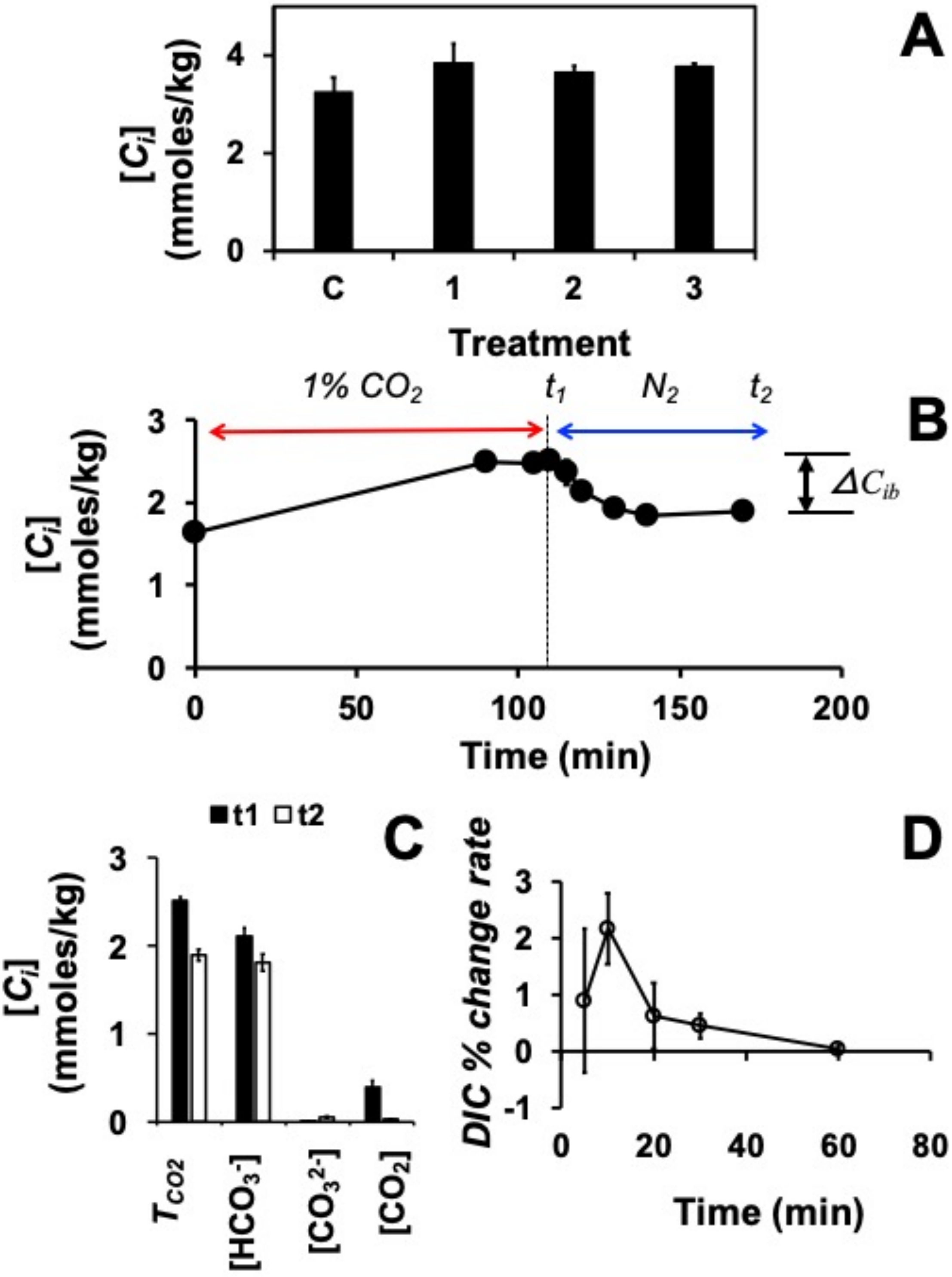

A
[C_i] (mmoles/kg)
0
2
4
C
1
2
3
Treatment
B
1% CO_2
t_1
N_2
t_2
ΔC_{ib}
[C_i] (mmoles/kg)
0
1
2
3
0
50
100
150
200
Time (min)
C
■t1 □t2
[C_i] (mmoles/kg)
0
1
2
3
T_{CO2}
[HCO_3^-]
[CO_3^{2-}]
[CO_2]
D
DIC % change rate
-1
0
1
2
3
0
20
40
60
80
Time (min)

Figure 1

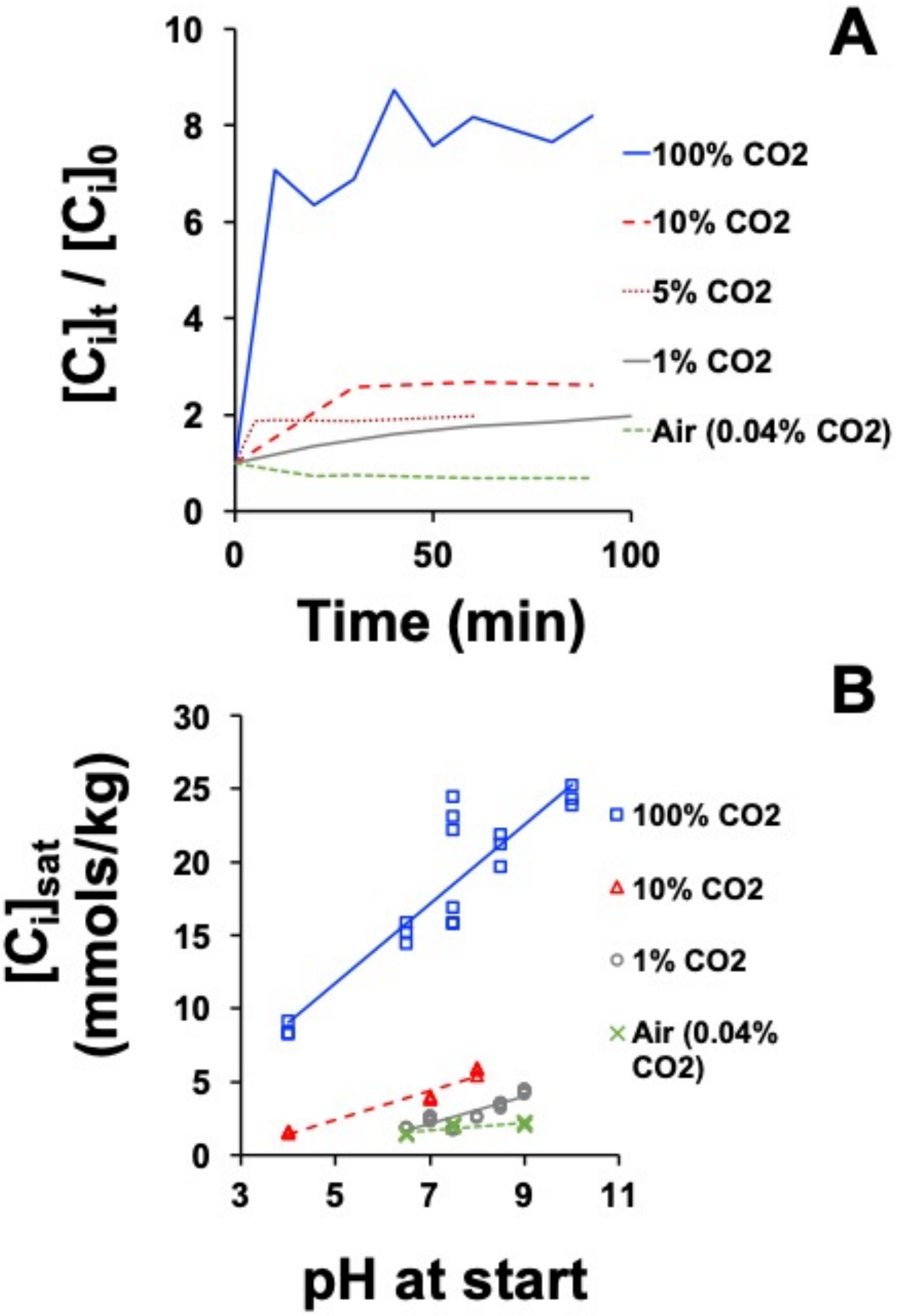
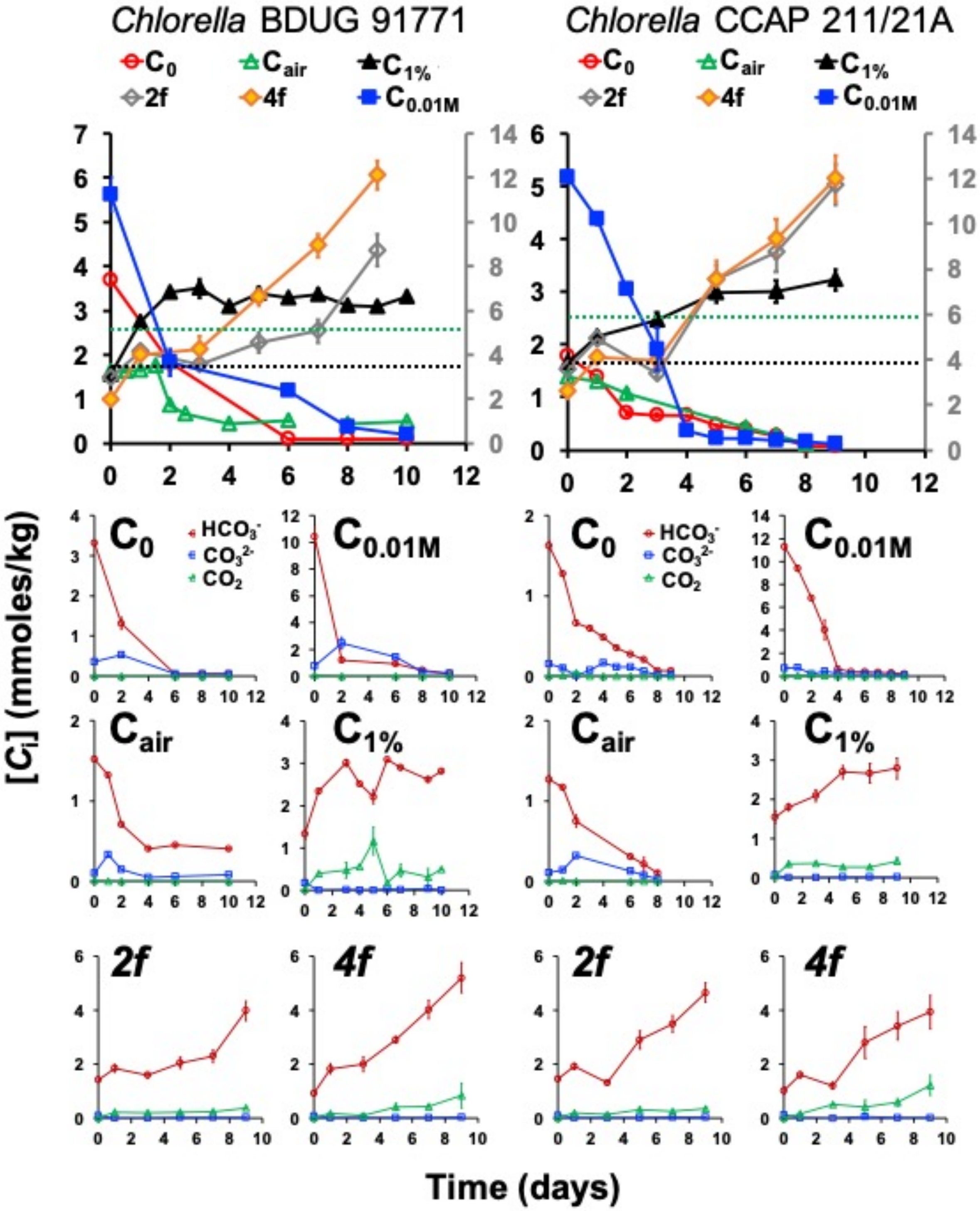

A
10
8
6
4
2
0
$[C_i]_t / [C_i]_0$
0
50
100
Time (min)
100% CO2
10% CO2
5% CO2
1% CO2
Air (0.04% CO2)
B
30
25
20
15
10
5
0
$[C_i]_{sat}$ (mmols/kg)
3
5
7
9
11
pH at start
100% CO2
10% CO2
1% CO2
Air (0.04% CO2)

Figure 2



Chlorella BDUG 91771
Chlorella CCAP 211/21A
C_0
C_{air}
$C_{1\%}$
2f
4f
$C_{0.01M}$
HCO_3^-
CO_3^{2-}
CO_2
$C_{0.01M}$
C_{air}
$C_{1\%}$
2f
4f
[C_i] (mmoles/kg)
Time (days)

Figure 3

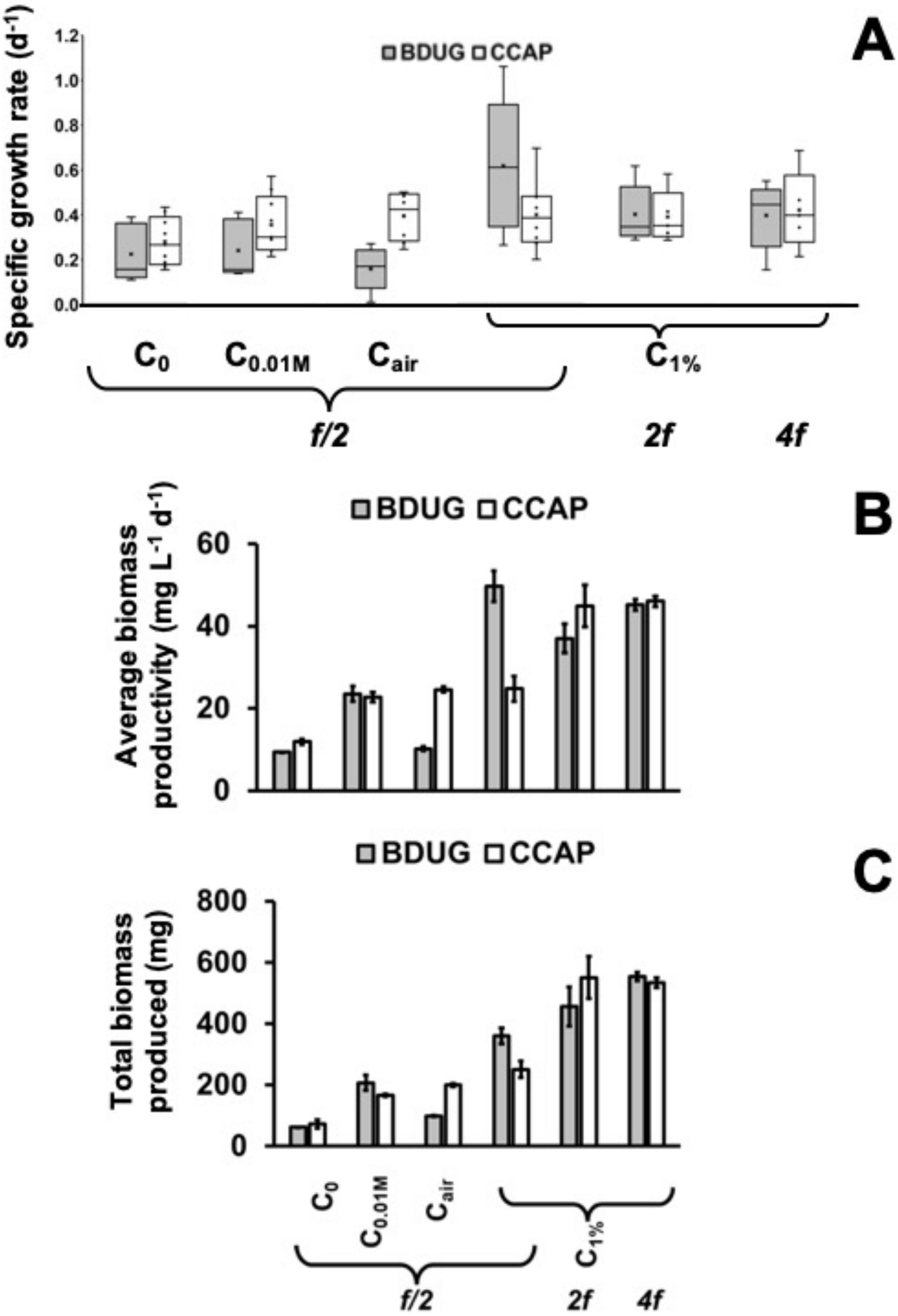

A
BDUG
CCAP
Specific growth rate (d^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C_0
$C_{0.01M}$
C_{air}
$C_{1\%}$
f/2
2f
4f
B
BDUG
CCAP
Average biomass productivity (mg L^{-1} d^{-1})
60
40
20
0
C
BDUG
CCAP
Total biomass produced (mg)
800
600
400
200
0
C_0
$C_{0.01M}$
C_{air}
$C_{1\%}$
f/2
2f
4f

Figure 4

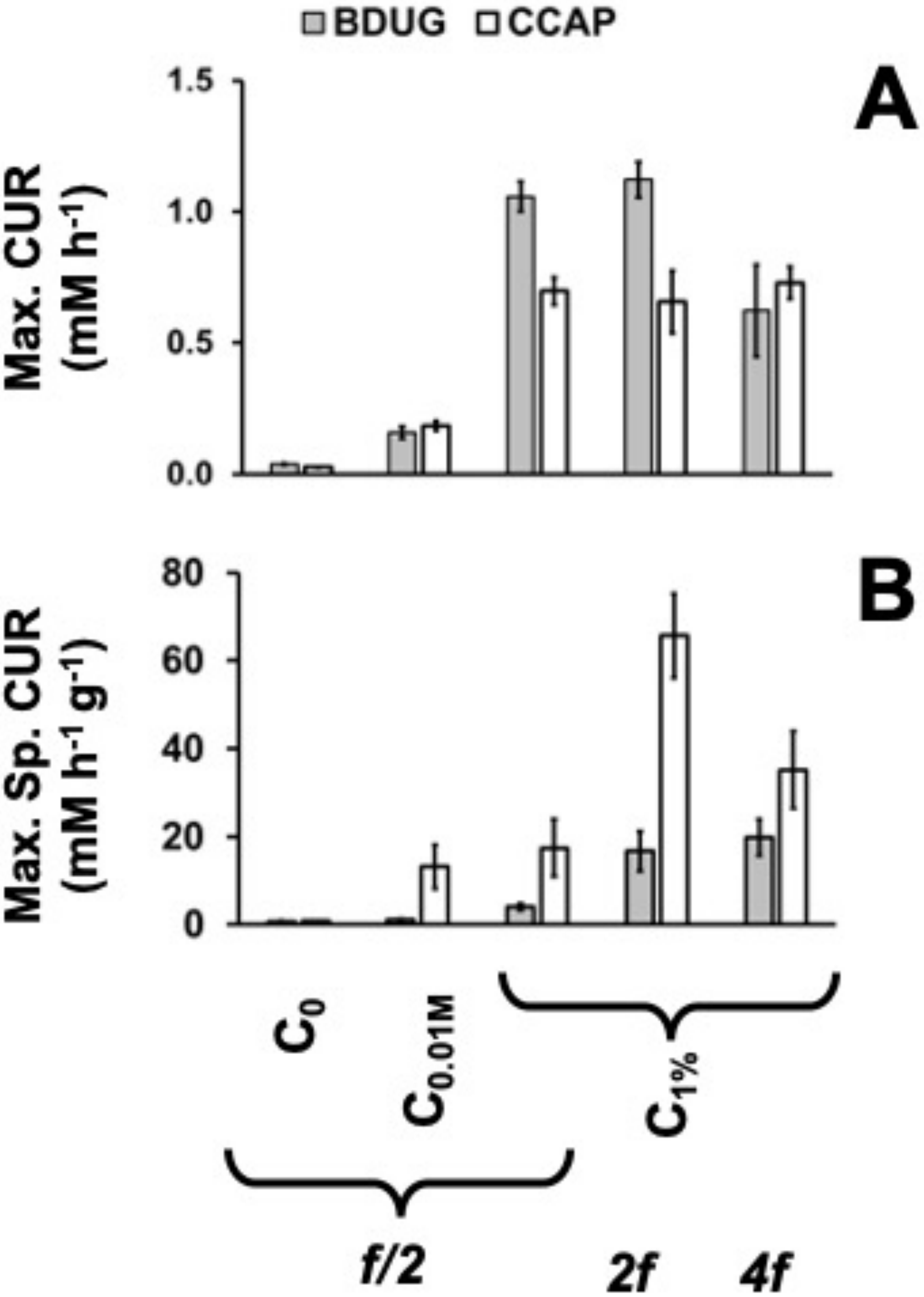

BDUG
CCAP
A
Max. CUR (mM h^{-1})
1.5
1.0
0.5
0.0
B
Max. Sp. CUR (mM h^{-1} g^{-1})
80
60
40
20
0
C_0
$C_{0.01M}$
$C_{1\%}$
f/2
2f
4f

Figure 5

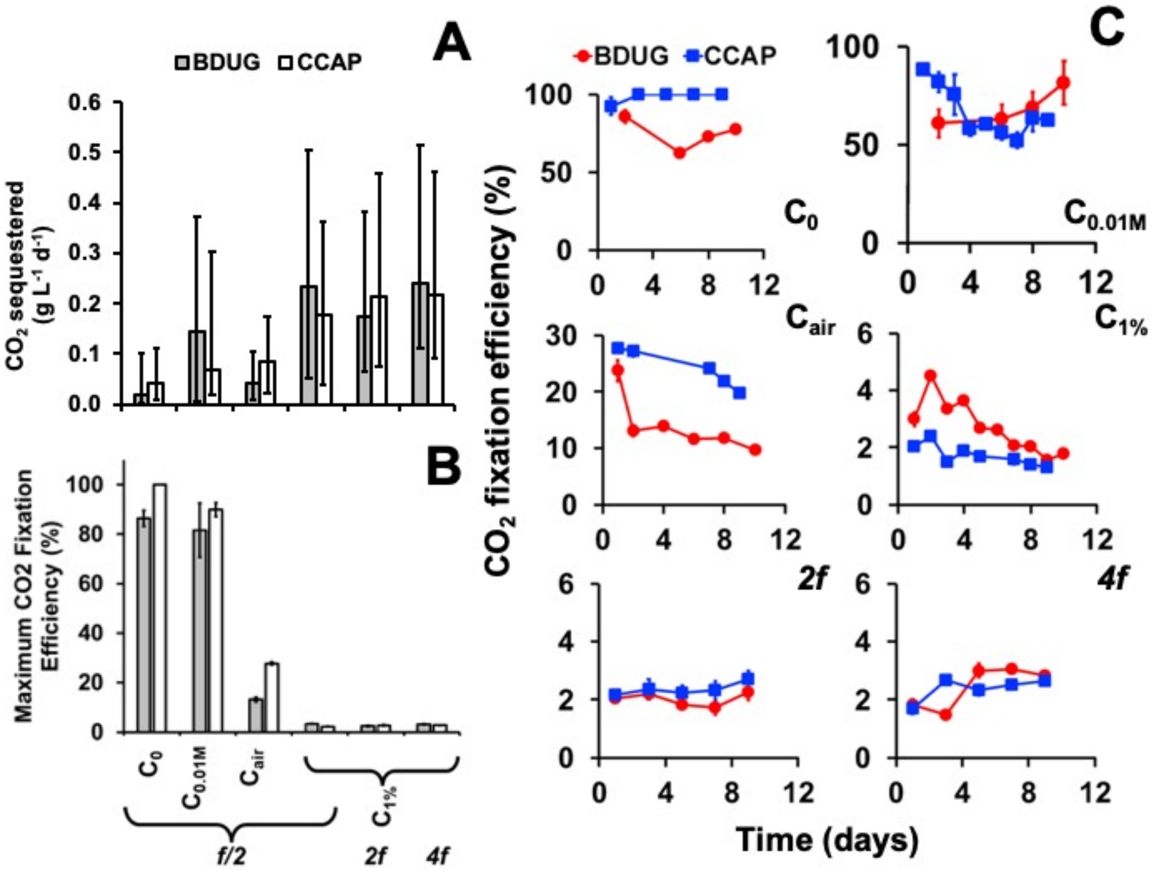
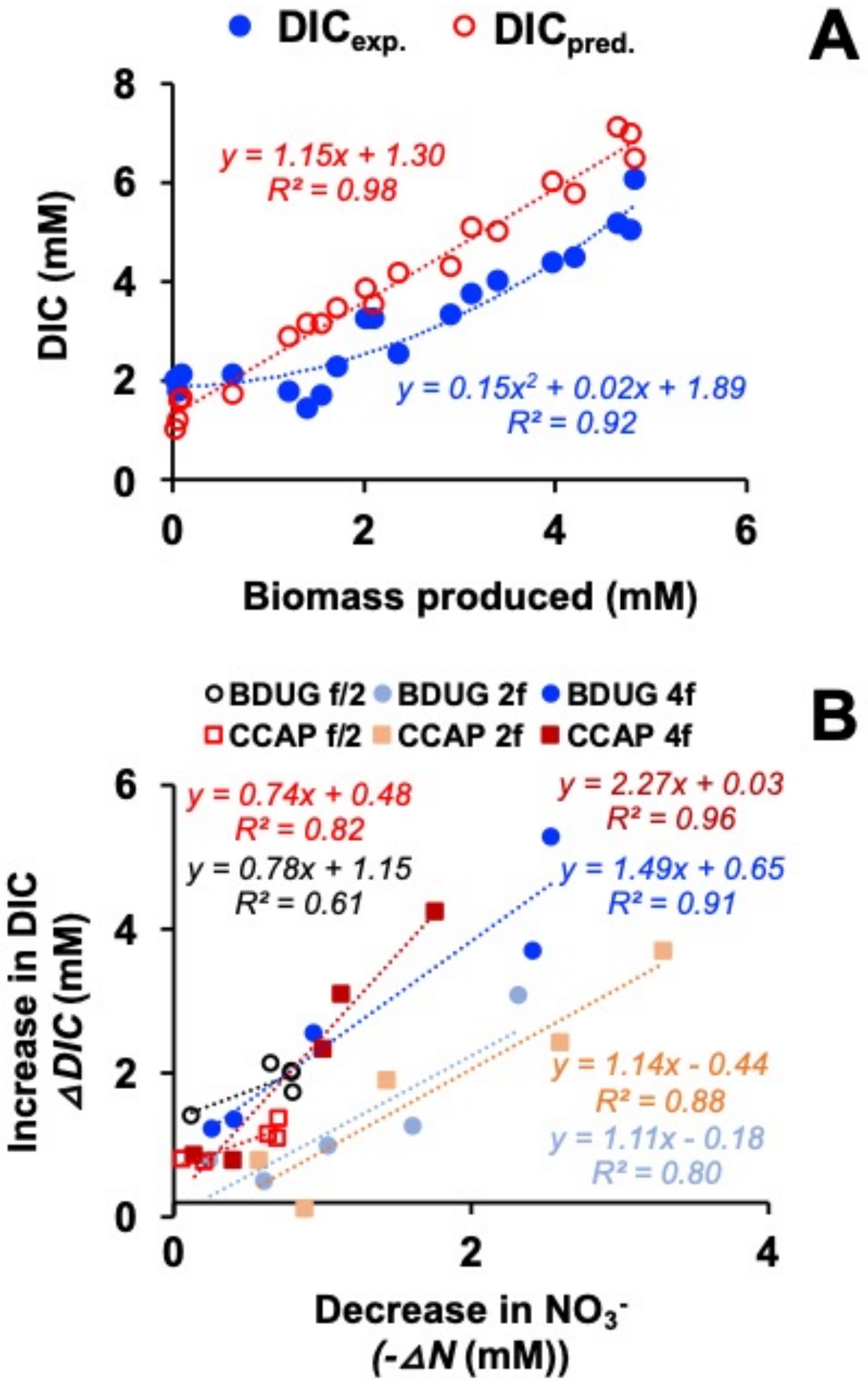

A
▨BDUG □CCAP
CO2 sequestered (g L-1 d-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
B
Maximum CO2 Fixation Efficiency (%)
0
20
40
60
80
100
C0
C0.01M
Cair
C1%
f/2
2f
4f
C
●BDUG ■CCAP
CO2 fixation efficiency (%)
C0
C0.01M
Cair
C1%
2f
4f
0
4
8
12
Time (days)

Figure 6



A
● DIC$_{exp.}$ ○ DIC$_{pred.}$
y = 1.15x + 1.30
R² = 0.98
y = 0.15x² + 0.02x + 1.89
R² = 0.92
DIC (mM)
8
6
4
2
0
0
2
4
6
Biomass produced (mM)
B
○ BDUG f/2 ● BDUG 2f ● BDUG 4f
□ CCAP f/2 ■ CCAP 2f ■ CCAP 4f
y = 0.74x + 0.48
R² = 0.82
y = 0.78x + 1.15
R² = 0.61
y = 2.27x + 0.03
R² = 0.96
y = 1.49x + 0.65
R² = 0.91
y = 1.14x - 0.44
R² = 0.88
y = 1.11x - 0.18
R² = 0.80
Increase in DIC
ΔDIC (mM)
6
4
2
0
0
2
4
Decrease in NO₃⁻
(-ΔN (mM))

Figure 7