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Improved biomass productivity in algal biofilms through synergistic interactions between photon flux density and carbon dioxide concentration

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HIGHLIGHTS

- At a PFD of 100 μmol/m^2/s inorganic carbon limitations occur below 2% CO_2.
- There is an interaction effect between CO_2 and PFD on algae biofilm growth.
- The model predicts optimal growth conditions to be 7.1% CO_2 and 440 μmol/m^2/s PFD.
- The model predicted maximal biomass productivities of 4.4 g/m^2/d, respectively.
- Increasing growth parameters beyond their optimal ranges adversely affected growth.

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ABSTRACT

Algal biofilms were grown to investigate the interaction effects of bulk medium CO_2 concentration and photon flux density (PFD) on biomass productivities. When increasing the CO_2 concentration from 0.04% to 2%, while maintaining a PFD of 100 μmol/m^2/s, biomass productivities increased from ~0.5 to 2.0 g/m^2/d; however, the productivities plateaued when CO_2 concentrations were incrementally increased above 2–12%. Statistical analysis demonstrates that there is a significant interaction between PFD and CO_2 concentrations on biomass productivities. By simultaneously increasing PFD and CO_2 concentrations, biomass productivities were significantly increased to 4.0 and 4.1 g/m^2/d in the experimental and modeled data, respectively. The second order model predicted increases in biomass productivities as both PFD and CO_2 simultaneously increased yielding an optimum at 440 μmol/m^2/s and 7.1%; however, when conditions were extended to the highest end of their respective ranges, the conditions were detrimental to growth and productivities decreased.

1. Introduction

Over the past several decades, researchers around the world have been studying algal growth systems to produce renewable bioresources such as bioenergy, bioplastics, fish and farm feed, nutraceuticals and pharmaceuticals. Algae are an attractive feedstock for these bioresources because they grow rapidly compared to most organisms, and the biomass has high concentrations of valuable biocompounds such as lipids/fatty acids and proteins; therefore, overall productivity of these biocompounds can be extraordinarily high under ideal growth conditions (Chisti, 2007). Currently, however, algal growth systems are not commercially viable for the production of most of these bioresources. There are several reasons for this, but perhaps the most significant is the high cost of harvesting and de-watering algal biomass grown planktonically. As such, several groups of researchers are focusing on growing algae as a biofilm, rather than a suspension culture. These algal biofilms offer potential harvesting and de-watering advantages because the algal biomass is: 1) immobilized, rendering it easily harvestable with novel reactor designs; 2) highly concentrated – up to 100 times more concentrated than planktonic growth systems (Christenson and Sims, 2012) – thus requiring less energy

Abbreviations: CO_2, carbon dioxide; CPCC, Canadian Phycological Culture Centre; RABR, rotating algal biofilm reactor; CCM, carbon concentrating mechanism.

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for de-watering the biomass before processing. Before algal biofilm growth systems can be scaled-up to industrial capacity, however, optimal conditions for growth must be established.

As with planktonic algal growth systems, carbon dioxide (CO_2) concentration is an important factor in controlling the productivity of algal biofilm growth systems. In planktonic systems, significant increases in growth rates with increased dissolved CO_2 concentrations have been observed, up until a threshold is reached where no further growth rate increase was observed (Chinnasamy et al., 2009; de Morais and Costa, 2007; Yun et al., 1996). In fact, these same studies demonstrated that very high concentrations of CO_2 can become detrimental to growth. In contrast, studies of the effect of CO_2 concentration on algal biofilm productivities have produced conflicting results. For instance, Blanken et al. (2014) reported very significant increases in algal biofilm growth rates when increasing the concentrations of inorganic carbon in the growth systems. However, Gross et al. (2013) and Kessano et al. (2015), reported no increases in algal biofilm growth rates when increasing the exposure concentrations of inorganic carbon. The reasons for the apparent contradictions across various biofilm systems are unknown but these differences perhaps betray an underlying and unappreciated complexity. Furthermore, algal biofilms do not necessarily respond to changes in environmental parameters in the same manner and magnitude that suspension cultures do (e.g. Schnurr et al., 2013 in the case of nitrogen stress). Thus, there is a need for a detailed characterization of algal biofilm growth responses to changing environmental parameters.

Under any given environmental condition, photosynthesis requires balanced light absorption by photosystem II and photosystem I to provide a consistent supply of ATP and NADPH, in the appropriate ratio, to support optimal rates of algal CO_2 fixation and growth. Dynamic fluctuations in light and CO_2 concentration affect the photosynthesis rate and evoke acclimation responses from algal cells that readjust the balance between light harvesting complexes and the CO_2 fixation system over short and long term time scales (Huner et al., 1998; Falkowski and Raven, 2007). For example, excess excitation energy (light) may lead to photo-oxidative damage to the photosynthetic apparatus, short-term photo-inhibition of CO_2 fixation and slower growth (Huner et al., 1998; Takahashi and Murata, 2008); however, these effects are mitigated through dissipation of excess excitation energy by non-photochemical quenching mechanisms, the xanthophyll cycle in eukaryotic algae (Jahns and Holzwarth, 2012), photosystem II repair mechanisms (Takahashi and Murata, 2008; Nixon et al., 2010; Järvi et al., 2015) and/or the use of alternative electron acceptors beyond CO_2. Increasing CO_2 will also dissipate excess electrons by increasing rate of photosynthetic carbon reduction if intermediates of the cycle (i.e., ribulose, 1–5, bisphosphate) are available and sufficient to support increased activity. Enhanced CO_2 supply may arise from abiotic as well as biotic factors such as increased bacterial respiration or induction of an algal CO_2 concentrating mechanism, which permits superior utilization of existing CO_2 resources while at the same time using the excess light to energize the system (de Araujo et al., 2011; Wang et al., 2011). In contrast to planktonic algae, algal biofilms present a unique environment where the effect of CO_2-photon flux density (PFD) interaction is amplified over small distances due to the concentrated nature of the biomass that rapidly attenuates both light and CO_2, disturbing the photosynthetic balance. The interactive effects of light and CO_2 on algal biofilm growth have been modeled mathematically using a variety of approaches, which indicate a very complex relationship with respect to growth. Light-limitation frequently is the determining factor in algal biofilm growth while CO_2 limitation becomes more acute as the thickness of the biofilm increases (Liehr et al., 1990; Flora et al., 1995). Few studies, however, have experimentally assessed the direct response of algal biofilm growth rates in response to co-varying light and CO_2 with the purpose to identify interaction effects, which we address here.

In this study, two hypotheses were tested: 1) algal biofilm growth rates will increase as dissolved carbon dioxide concentrations are increased in the growth medium until a threshold is met; 2) there is an interaction effect of PFD and CO_2 concentration on algal biofilm growth kinetics. The objectives of this paper, therefore, were to study the effects of CO_2 concentrations on algal biofilm growth kinetics from a one-variable-at-a-time approach i.e. only CO_2 concentration is changed between experiments, and to study the interaction effects of CO_2 concentration and PFD on algal biofilm growth kinetics.

2. Materials and methods

2.1. Flat-plate parallel horizontal algae biofilm culturing systems

Two similarly designed semi-continuous flat plate parallel, horizontal photobioreactors (PBR) were used in this study. System #1 (Schnurr et al., 2013) was comprised of 18 glass growth coupons (Fig. 1), each housed in a separate compartment, with separate inlet and outlet flow ports for the circulation of growth media. System # 2 was comprised of a common housing containing 24 polycarbonate growth coupons (Fig. 2). Circulation of growth medium through the housing occurred via a single inlet and outlet port. System #1 was used in experiments where CO_2 concentration alone was manipulated while System # 2 was used in experiments where both CO_2 and PFD were varied simultaneously. The growth medium was CHU-10 amended to 75 mg/L K_2HPO_4, 175 mg/L KH_2PO_4, 120 mg/L NH_4Cl and 20.2 mg/L $Ca(NO_3)_2{\cdot}4H_2O$.

Both systems had approximately 95% (Q/Q) of the growth medium re-circulated through the system, and approximately 5% (Q/Q) as a fresh feed entering and waste feed exiting the system. Shear rates across the reactors/growth surfaces were $\sim 4\ s^{-1}$ as calculated in Schnurr et al. (2013). Cole Parmer Masterflex peristaltic pumps, model #7523-90 and 7520-50, controlled the liquid flow rates in both growth systems. Carbon dioxide (CO_2) concentration was controlled by vigorously sparging growth medium, contained in a mixing vessel that preceded the PBR, with a predefined CO_2/compressed air mixture. Pyrex gas dispersion tubes, model #39533 were used for this purpose. Rotameters, model #EW-32044-65 and RK-32457-44, controlled the CO_2 and compressed air flow rates, respectively. Red light emitting diodes (LED) were used to irradiate the biofilms from the water-side (above) on a diurnal cycle of 16 h/8 h light/dark. The center wavelength of the LEDs was 630 nm and had a wavelength range of 620–640 nm. Reactor temperature was maintained at 25 °C ±2 °C. The pH of the growth medium was maintained at 7 ± 0.2 by additions of 0.5 M NaOH as required.

2.2. Experimental approach

In both growth systems the reactors/growth surfaces were pre-conditioned with unsterile secondary wastewater effluent, which provided bacteria and extracellular polymeric substances. The wastewater was collected from Ashbridge's Bay Wastewater Treatment Plant in Toronto, ON. The 48 h pre-conditioning procedure was followed by the replacement of ~90% of the wastewater with synthetic, modified CHU-10 growth medium (Schnurr et al., 2013). Following pre-treatment, the reactors were inoculated with their respective algal cultures.

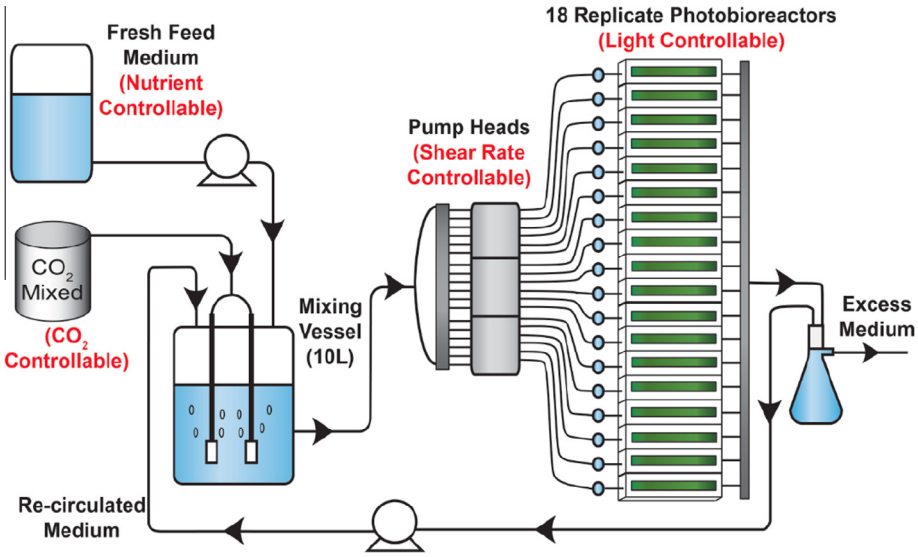

Fig. 1. Schematic of algal biofilm culturing used to determine the effect of bulk medium CO_2 concentration on algal biofilm biomass productivities (System #1). Highlighted in red are the growth parameters controllable by this growth system. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

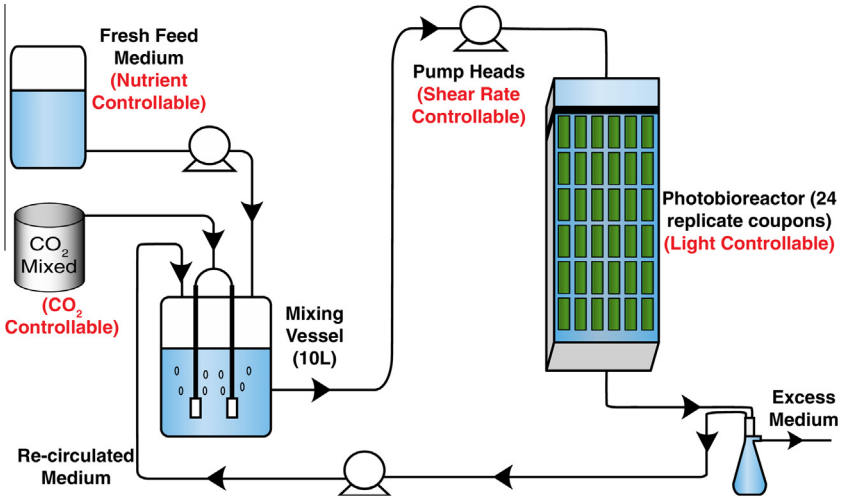

Fig. 2. Schematic of algal biofilm culturing system used to determine the interaction effects of bulk medium CO_2 concentration and photon flux density on algal biofilm biomass productivities (System #2). Highlighted in red are the growth parameters controllable by this growth system. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2.1. Growth System #1

System #1 was inoculated with the green alga *Scenedesmus obliquus* (CPCC 5), acquired from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, ON, Canada. The inoculum cultures were grown and maintained in an incubator at 25 °C ± 2 °C, in flasks on a shaker operating at 120 rpm, and at a PFD of 50 μmol/m^2/s supplied by red LEDs (620–640 nm). Each reactor was inoculated with 15 mL of algal suspension (~0.5 g/L dry weight) and left to stand with no circulation for the next 24-h. This period facilitated initial adhesion of cells to the pre-conditioned growth surface to seed the biofilm. The pumps were then activated and the experiment commenced.

Carbon dioxide one-variable-at-a-time (CO_2 manipulation only) experiments were conducted in Growth System #1. In these 26 day experiments, the effect of CO_2 concentration on algal biomass productivities was determined at 7 different CO_2 concentrations between 0.04 and 12.0% (v/v) in the growth medium. Experiments were independently replicated 3 times at 0.04, 2.0, 8.0 and 12% (v/v) CO_2, to provide an estimate of between-experiment variability, but only once at 1.0, 6.0 and 10.0% v/v CO_2. In the latter cases, we assumed that between-experiment variability would be similar to that found for the replicated concentrations. The PFD irradiating the biofilms was kept at a constant 100 μmol/m^2/s of red LED light (620–640 nm).

2.2.2. Growth System #2

System #2 was utilized to carry out the CO_2 concentration – photon flux density interaction studies. The inoculum (~0.5 g/L dry weight algae) for these experiments was an equal portions mixture (~333 mL each) of *Scenedesmus obliquus* (CPCC 5), *Chlamydomonas reinhardtii* (CPCC 243), and *Chlorella vulgaris* (CPCC 90), all acquired from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, ON, Canada. The inoculum cultures were grown and maintained in an incubator at

25 °C ± 2 °C, in flasks on a shaker operating at 120 rpm, and at a PFD of 50 μmol/m^2/s supplied by red LEDs (620–640 nm). The 1L inoculum was poured into the reactor on day zero while the pumps continued to run, thus the growth surfaces were colonized by recruitment through gravity and adhesion from the re-circulating suspension of algal cells in the system.

The experimental design for these interaction studies was a fractional factorial design (5^2) called a rotatable octagonal design (Fig. 3). A range of CO_2 concentrations (0.04–12%) and red light PFDs (40–560 μmol/m^2/s of red LED light (620–640 nm)) were chosen from the results of experiments in which CO_2 at constant PFD (this work) or PFD at constant [CO_2] (Schnurr et al., 2016) was manipulated and biomass productivities determined. These growth condition ranges were chosen because they began at near minimal growth conditions, and extended well beyond what was determined to be optimal in the one-variable-at-a-time experiments previously conducted. The test values of the two independent variables (x_1 = [CO_2] and x_2 = PFD) were chosen within this operational range such that, when transformed into coded values, the combinations correspond to a rotatable octagonal experimental design (Fig. 3). The actual and coded values of [CO_2] (x_1) and PFD (x_2) are presented in Table 1. The rotatable octagonal experimental design was taken from Himmelblau (1970), and was chosen because this 5-level design enables unbiased determination of non-linear (second order) interaction terms. By running quadruplicate experiments around the center point (0,0), and assuming that variance is approximately the same at all conditions, statistical confidence was ensured without having to replicate all the other growth conditions.

2.3. Sampling and analysis

For experiments in which CO_2 was varied at a constant PFD, duplicate samples were harvested every 2–5 days during the 26–27 day time course of an experiment. For the CO_2 – PFD interaction studies (System #2) the experiments were run for 14–15 days while collecting triplicate samples every 2–5 days. Upon harvest of a coupon, each sample of algal biomass was scraped from the growth surface, lyophilized with a Labconco Lyph Lock 6 freeze dryer and weighed. The dry weight per unit area of growth surface was then determined. This procedure was followed across the entire experimental growth period to determine growth kinetics (dry weight per area per unit of time).

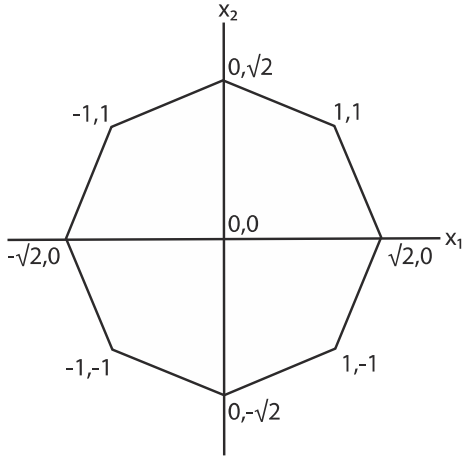

Fig. 3. Rotatable octagonal design for 2 variables, in this case CO_2 concentrations and red light PFD.

Table 1
Experimental design growth conditions for interaction experiments of coded and uncoded CO_2 concentrations and red light photon flux densities (PFD).

Experiment	% CO_2	x_1: (% CO_2) coded	PFD (μmol/m^2/s)	x_2: (PFD coded)
1	1.8	−1	116	−1
2	10.2	1	116	−1
3	1.8	−1	484	1
4	10.2	1	484	1
5	12	√2	300	0
6	0.04	−√2	300	0
7	6	0	560	√2
8	6	0	40	−√2
9	6	0	300	0
10	6	0	300	0
11	6	0	300	0
12	6	0	300	0

2.4. Statistical analysis

Biofilm biomass productivities at various CO_2 concentrations were determined by linear regression analysis (95% confidence intervals) of plots of biofilm yields across the time course of the experiment. StatPlus:mac software, supported through Microsoft Excel 2011, was used to determine productivities and confidence intervals.

A model was created using surface response methodology (RSM). The data used included experimentally determined algal biofilm growth kinetics data after 11 days of growth (Table 2). The response or independent variable (y) was biomass productivity, measured for each combination of photon flux density (x_1) and CO_2 (x_2). The model was generated using a statistics software program (R 1.15.1) and the graphics were created using rsm package in R 1.15.1. The purpose this model is to uncover interactions between independent variables and visualize their contributions, in graphical form as a response surface. A rotatable central composite design was chosen. The general equation for a model with two variables (x_1 and x_2) is $y = x_1 + x_2 + x_1x_2 + x_1^2 + x_2^2$. The model predicts algal biofilm biomass productivities (dependent variable) at specific CO_2 concentrations and PFDs (independent variables). All linear and quadratic combinations of PFD and CO_2 were tested. Variables with $p < 0.01$ were kept in the final model. Overall model p-value and a lack of fit test contributed in choosing the best model ($p < 0.001$). An analysis of residuals was used to justify linear and quadratic assumptions in the model (residuals analysis available in supplemental material as Fig. S3).

Table 2
Comparing the experimentally determined biofilm biomass productivities data to the productivities predicted by the model at each of the PFD and CO_2 concentrations tested. The biomass productivity model data was determined from the equation of the line, which was determined from the coefficients shown in Table 3.

Growth conditions (CO_2%, PFD μmol/m^2/s)	Experimental data (g/m^2/d)	Model data (g/m^2/d)
1.8, 116	2.3 ± 0.34	1.2
10.2, 116	2.5 ± 0.47	2.2
1.8, 484	3.4 ± 0.49	2.8
10.2, 484	3.7 ± 0.70	3.9
12, 300	3.6 ± 1.1	2.8
0.04, 300	0.9 ± 0.12	1.4
6, 560	4.0 ± 0.99	4.1
6, 40	1.3 ± 0.31	1.7
6, 300	3.0 ± 0.47	4.0
6, 300	3.5 ± 0.44	4.0
6, 300	3.5 ± 0.54	4.0
6, 300	3.2 ± 1.3	4.0

3. Results and discussion

3.1. The effect of carbon dioxide concentration on algal biofilm growth kinetics

Algal biofilm biomass productivities significantly increased with increasing CO_2 concentration until a threshold was met and growth rates plateaued (Fig. 4). Linear regression analysis of algal biomass data collected over 26 day growth periods (Fig. S1) showed significant increases in overall productivities when CO_2 concentrations were increased from atmospheric (0.04%) to 2% (v/v). When CO_2 concentrations were increased beyond 2% up to 12%, no further increase in biomass productivities was observed.

The increase, and subsequent plateau, of algal biofilm biomass productivities with increasing CO_2 concentrations is not unexpected given the importance of CO_2 as a nutrient for algae, the inherent limitations of mass transport into biofilms, and because all organisms have inherent growth rate limitations. Cells within algal biofilms are immobilized and highly concentrated, thus making mass transport a potential challenge – increasing CO_2 concentrations subsequently increases the flux into the biofilm (Liehr et al., 1990), thereby increasing overall carbon availability and biomass productivity. Maximum growth rates are intrinsic to all biological systems in general (Monod Growth Kinetics); therefore, carbon fixation/biofilm biomass productivities are bound to eventually plateau. From the data above, CO_2 concentrations below 2% (v/v) caused inorganic carbon limitations within the biofilm at the PFDs, temperatures, etc., provided; however, CO_2 concentrations between 2% and 12% (v/v) yielded algal biofilm biomass productivities that were not significantly different ($p > 0.05$). Although the biofilm biomass productivity response to increasing CO_2 concentrations observed in these studies were similar to those reported in planktonic algal growth systems (Chinnasamy et al., 2009; De Morais and Costa, 2007; Ryu et al., 2009; Yun et al., 1996), the plateau in growth response occurred at lower CO_2 concentrations in our biofilm studies (2% CO_2) compared to the planktonic studies (~6% CO_2). It is likely, especially given the results in the following section on CO_2 and PFD interactions, that the plateau of growth rates at 2% CO_2 in the current study was a result of insufficient photons to fix CO_2 at higher concentrations i.e. the PFD was too low.

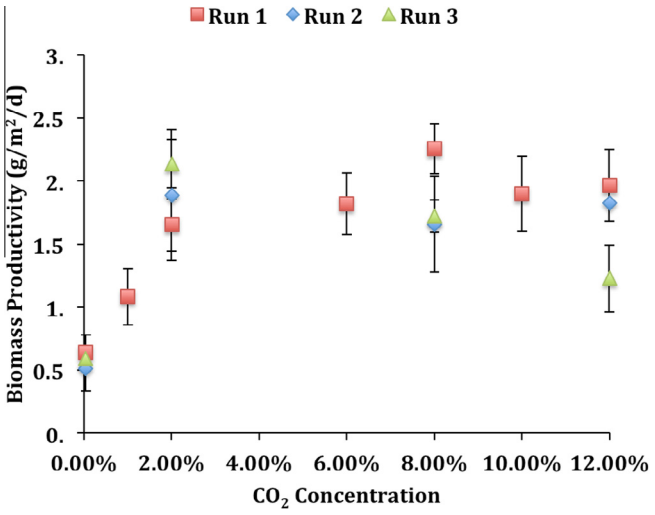

Fig. 4. Algal biofilm biomass productivities at various carbon dioxide concentrations. Throughout each experimental run shear rates were maintained at 4 s^{-1}, pH at 7 ± 0.2, temperature at 25 °C ± 2 °C, and photon flux density at 100 μmol/m^2/s. The productivities were determined by linear regression of 18 individual samples taken in duplicate and across 26 days of growth. The error bars represent the 95% confidence interval of the linear regression. Inoculum was a pure culture of *Scenedesmus obliquus*.

The work on algal biofilm biomass productivities exposed to various concentrations of inorganic carbon has been contradictory, and consequently inconclusive. As discussed in Schnurr and Allen (2015), these contradictions could be the result of the following phenomena: 1) interaction effects between CO_2 and PFD i.e. a lack of photons to assimilate the increased inorganic carbon concentrations; 2) photobioreactor design and the effective rates of mass transport of CO_2 they facilitate. It is likely that both factors contributed to the contradictory results in the effect of inorganic carbon concentrations on algal biofilm growth seen in the literature. Gross et al. (2013) and Kessano et al. (2015) may not have observed increases in growth rates with increased CO_2 concentrations because, at the PFDs provided, there was already maximum carbon assimilation at the lowest inorganic carbon concentrations provided. Moreover, their rotating algal biofilm reactor (RABR) may have provided sufficient mass transfer from the gaseous CO_2 exposures to saturate the Calvin cycle even at the lowest inorganic carbon exposures at their respective PFDs supplied. The rotating biological contactor used by Blanken et al. (2014) also exposed algal biofilms directly to air for extended periods of time, however, the PFDs used in this study were more than twice that of the two studies mentioned above. These higher PFDs may have facilitated the increased carbon fixation and biofilm biomass productivities when exposed to increased inorganic carbon concentrations. The results reported in the present study indicated significant inorganic carbon limitations below 2% CO_2 sparging concentrations. This suggests insufficient carbon saturation in the photosynthetically active regions of the biofilms, which may have been a result of mass transfer limitations across the boundary layers and through the biofilm. Although unclear, it possible that the different reactor designs that were used above facilitate different capabilities for inorganic carbon mass transfer. This may explain the inconsistencies in the reported effects of inorganic carbon concentrations on algal biofilm biomass productivities.

Although not evident in the data, biofilms treated with CO_2 concentrations between 8 and 12% (v/v) were physically much less stable and 'intact' than other biofilms grown at lower CO_2 concentrations i.e. the biofilm came apart when disturbed. These less 'intact' biofilms may have been a result of increased additions of monovalent ions (Na^+) as NaOH that was introduced for pH balancing. In studies of floc chemistry it has been demonstrated that additions of Na^+ resulted in more fragile and weak flocs (Kara et al., 2008). This is because monovalent cations displace divalent and trivalent cations by ion exchange, and the multivalent cations form stronger flocs through bridge structures of negatively charged biopolymers. These results have implications for reactor design if sloughing events are unfavourable for growth systems.

3.2. Interaction effects of PFD and carbon dioxide concentration on algal biofilm growth kinetics

We conducted 12 independent measurements of algal biofilm biomass productivity over a range of CO_2 concentrations and PFD values defined by our 5^2 fractional factorial experimental design (Tables 1 and 2). Growth kinetics over the 15 day experiments were linear in all cases (eg. Fig. S2). Experimentally determined biomass productivities increased 4.4-fold above the baseline value to a maximum measured value of 4.0 g/m^2/d. This productivity level was about 2-fold higher than the maximum observed in Fig. 4. Biomass productivities for four replicate experiments of the 6% [CO_2] × 300 PFD condition were in good statistical agreement with one another with an average value of 3.3 g/m^2/d (±0.25), indicating that biofilm growth within System #2 can be readily replicated. Comparison of productivity values for the 1.8%

$[CO_2]$ × 116 PFD condition and the 10.2% $[CO_2]$ × 116 PFD condition (2.3 & 2.5 g/m^2/d, Table 2) with those from near – comparable conditions from Fig. 4 (2% $[CO_2]$ × 100 PFD & 10.0% $[CO_2]$ × 100 PFD; 1.9 & 1.9 g/m^2/d) indicate that the biofilm biomass productivities are about 20–25% higher in the former case. The higher productivities can likely be accounted for by the 16% higher PFD level used in the System #2 experiments (Table 2). Given the approximate agreement, these data suggest that there are no large scale, systemic differences between the experimental outcomes achieved using System #2 and System #1, and that the data acquired are directly comparable.

Results from the studies investigating PFD and CO_2 concentration interactions demonstrated an interaction effect on algal biofilm biomass productivities. PFD, CO_2, PFD^2 and CO_2^2 all significantly contributed to predicting approximately 70% of the variation in biomass productivity (Table 3, $p < 0.01$ for individual variables and <0.001 overall model). The remaining 30% variation may be due to different photosynthetically active regions of the biofilm growing at different rates (Schnurr and Allen, 2015), interactions of bacterial and algal populations in the biofilm i.e. biomass consumption through cellular respiration, and/or to random variation inherent in biological systems.

Individually increasing PFD and CO_2, and simultaneously increasing both, resulted in higher biofilm biomass productivity in both the experimental data and the model (Figs. 5 and 6, and Tables 2 and 3). CO_2^2 and PFD^2 variables captured the optimal CO_2 and PFD range in these experiments where intermediate levels of each outperformed lower or higher levels of each condition (Figs. 5 and 6, Table 2). Statistically, this is demonstrated by the correlation coefficients of −0.55 and −0.96 for PFD^2 and $[CO_2]^2$, respectively. As demonstrated by the magnitude of the coefficient for each independent variable, PFD contributed more to overall algal biofilm productivities than CO_2 (Table 2). The modeled data in Figs. 5 and 6 show significant biofilm biomass productivity increases as CO_2 concentrations and PFDs were increased to their optimal values. The optimal values (stationary points) of 7.1% $[CO_2]$ and 440 µmol/m^2/s PFD were determined from the model, which predicted an optimal biofilm biomass productivity of 4.4 g/m^2/d. The model also predicts that biofilm biomass productivity decreases to 3.0 g/m^2/d at the highest end of the ranges used in this study i.e. 12% CO_2 and 560 µmol/m^2/s of red LED light (620–640 nm). Figs. 5 and 6, and Table 2 also demonstrated that algal biofilm biomass productivities were significantly lower under high PFD-low CO_2 and high CO_2-low PFD conditions. The contour lines in Fig. 6 can be used to predict biofilm biomass productivities at various PFD and CO_2 concentration growth conditions.

3.2.1. Physiological basis for CO_2 and PFD interaction

From what is known about the physiology of algae cells, the interaction between PFD and CO_2 concentrations on algal biofilm biomass productivities is not surprising. During photosynthesis energy from ATP and electrons from NADPH are used to reduce

Table 3
A summary of statistically significant coefficients that describe surface response model used to determine the impact of $[CO_2]$ × [PFD] on biomass productivity.

Variable	Coefficients	P-value
Intercept	4.02	4.05E−13
PFD	0.84	5.79E−06
CO_2	0.52	1.63E−03
PFD^2	−0.55	8.99E−03
CO_2^2	−0.96	4.27E−05

n = 30, Adjusted.
R^2 = 0.695.
p Value = 5.722e−07.
Lack of fit P = 0.117.

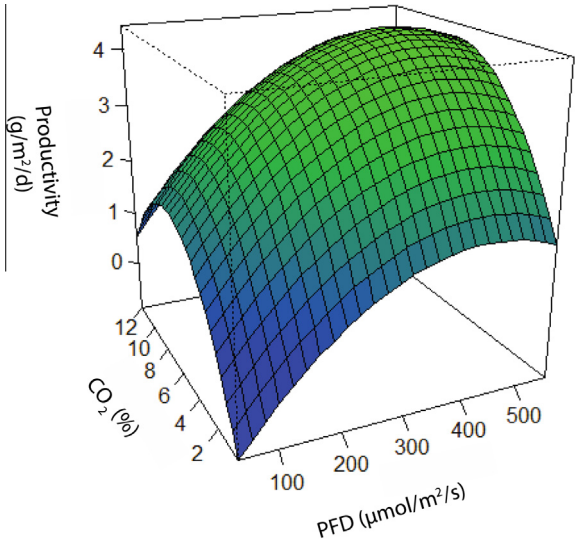

Fig. 5. Response surface of modeled biofilm biomass productivity data from coded CO_2 and PFD values after 11 days of growth. Biofilm biomass productivities increased with increasing CO_2 concentrations and PFDs until a threshold was surpassed at the high end of each range, adversely affecting biofilm growth.

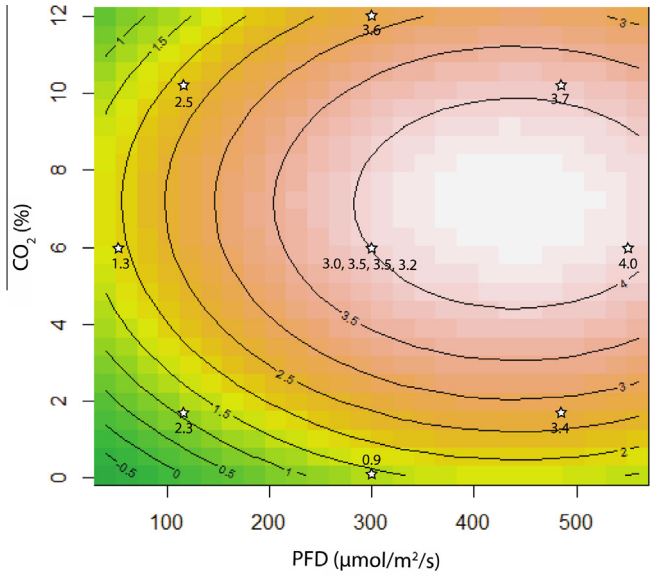

Fig. 6. Contour plot of modeled biofilm biomass productivity data from coded CO_2 and PFD values after 11 days of growth. The values on the contour lines represent the models predicted biofilm biomass productivity at the specific PFD and CO_2 concentration growth conditions. The stars and their respective numbers represent the experimental data productivities determined at the specific PFD and CO_2 concentrations. Optimal growth conditions predicted by the model are at the center of the oval contour line.

CO_2 in the Calvin cycle to produce biomass. The ATP and NADPH are produced in the electron transport chain from the collection of light energy (photons) by light harvesting antennae chlorophyll molecules and reaction centres in photosystem I and Photosystem II (Martinko and Clark, 2009). Therefore, the rates of photosynthesis, and hence growth, reproduction, and biomass accumulation, are dependent on the availability of photon energy and CO_2. On a cellular level, due to timescales of reactions, it is typically not the primary photochemical reactions or electron transfer that is the major limitation to photosynthesis, but rather, the availability of inorganic carbon through transport mechanisms and enzymatic

reactions in the Calvin cycle (Henley, 1993; Huner et al., 1998). However, algae cells have developed carbon-concentrating mechanisms (CCM) that accumulate and store inorganic carbon to be used in the Calvin cycle. These CCMs are increasingly engaged as PFDs are increased (de Araujo et al., 2011), which causes more carbon assimilation into biomass. Additionally, increasing CO_2 concentrations down regulates the energy consuming CCMs, which enhances growth rates as more energy is available to the cell (Sarker et al., 2013). An increased bulk medium CO_2 concentration likely facilitates enhanced growth by enhancing CO_2 transport into algal cells. At a cellular level, basic algae physiology explains the existence of interactive effects between PFD and CO_2 concentrations.

3.2.2. Detrimental interactions

Our data shows that the algal species used in these experiments had a robust response to high CO_2 concentrations and PFDs with minimal detrimental effects on overall biofilm productivity. Shown in both the contour (Fig. 6) and response surface plots (Fig. 5), going beyond the maximal PFD and CO_2 concentrations could cause detrimental growth effects. In particular, for excessive PFDs the rate limiting carbon transport, enzymatic processes, and Calvin cycle can cause an accumulation of damaging reactive oxygen species (Huner et al., 1998). These oxygen species are well known to cause photoinhibition and photooxidation, and result in negative growth/productivities. This is likely occurring in the photosynthetically active region of the biofilm that is adjacent to the light source (Schnurr et al., 2014). Excessive CO_2 concentrations cause acidification of the growth medium as carbonic acid is produced. The reduced pH adversely affects algal biofilm growth as most green algae species thrive in near neutral conditions.

3.2.3. Mass and light transport limitations

Photon and CO_2 transport affect the interaction effects and overall algal biofilm productivities. It is well known that getting light/photon energy (Carvalho et al., 2011) and nutrients to algal cells in a PBR is an inherent challenge, but is particularly true of biofilms because of their immobilized and concentrated nature (Liehr et al., 1990). Increasing the PFDs irradiating the biofilms has the potential to increase photon penetration and hence the photosynthetically active region of the biofilms (Schnurr and Allen, 2015). Increasing bulk medium dissolved inorganic carbon concentrations (CO_2) increases mass transport into the biofilms, but increasing PFDs further increases total inorganic carbon (TIC) flux into algal biofilms (Liehr et al., 1990). By simultaneously increasing both PFDs and CO_2 concentrations in algal biofilm growth systems, more of the algae cells within the biofilms become engaged in the cellular physiological mechanisms described above. This significantly increases the overall productivity of the algal biofilms, and did so in this study.

3.2.4. Interaction effects in other aquatic photosynthetic organisms

Although there have been a few studies of PFD and CO_2 interactions on planktonic algae and other photoautotrophic aquatic organisms, there have been no such experiments conducted on algal biofilm systems. While studying the aquatic plant *Riccia fluitans*, Andersen and Pedersen (2002) found significant interaction effects of dissolved CO_2 concentrations and PFD on the relative growth rate. Specifically, as they increased dissolved CO_2 concentrations to 16, 150 and 950 μM, and PFDs to 23, 89 and 250 μmol/m^2/s, the authors observed significant increases in growth rates at all levels. The maximal growth rate achieved in this study was 12-fold greater than the lowest, and they concluded that light had a greater effect on growth than CO_2. As mentioned above, the results of the present study also indicated that PFD had a greater affect on algae biofilm growth than CO_2 concentration. Significant interaction effects similar to those reported by Andersen and Pedersen (2002) were reported by Madsen and Sand-Jensen (1994) when they studied the aquatic macrophytes *Elodea canadensis* and *Callitriche cophocarpa*. On the other hand, while studying relative growth rates of the marine red algae *Chondrus crispus* grown planktonically, Sarker et al. (2013) determined no significant interaction between irradiance and CO_2 concentration on growth rates. This may have been a result of several things: 1) they used relatively low CO_2 concentrations (280 and 700 ppm) and PFDs (10 and 70 μmol/m^2/s); 2) they only tested two levels of each of the independent variables, and those two levels may not have interacted with one another at the respective ranges tested; 3) they may have used ranges too narrow to demonstrate interactions. The results of the present study show significant interaction effects between PFD and CO_2 concentration, but because the chosen PFD range was not high enough, maximal productivities may not have been achieved. Future work should investigate a larger range of PFDs, and perhaps incorporate different species and temperatures, as temperature has been shown to be a significant interacting parameter with PFD using other algal species grown planktonically (Sandness et al., 2005; Zucchi and Necchi, 2001).

4. Conclusions

The results from this paper demonstrate that bulk medium CO_2 concentrations and the interacting effects of PFDs and CO_2 concentrations have significant affects on algae biofilm biomass productivities. At a fixed PFD of 100 μmol/m^2/s, algae biofilm biomass productivities were significantly increased from ~0.5 to 2 g/m^2/d as CO_2 concentrations were increased from atmospheric to 2%. Incrementally increasing CO_2 concentrations from 2% to 12%, however, caused no statistical difference in algae biofilm growth rates. An interaction effect was observed as biofilm biomass productivities significantly increased to 4.0–4.1 g/m^2/d when CO_2 concentrations and PFDs were simultaneously increased to their optimal values.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.06.129.

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