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Evaluation of microalgae cultivation at air-CO_2 equilibrium pH for improving carbon utilization efficiency☆

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ABSTRACT

Microalgae are often cultivated at near-neutral pH to optimize growth. However, this results in significant CO_2 loss through outgassing in open-pond cultivation systems, leading to low CO_2 utilization efficiency and higher biomass production costs. One potential solution is algal cultivation at air-CO_2 equilibrium pH, which minimizes CO_2 outgassing but may inhibit growth due to stresses at higher pH levels. In this study, we evaluated the viability of this approach by growing *Picochlorum celeri* and *Tetraselmis striata* under outdoor relevant conditions at equilibrium pH. Compared to pH 7 cultures, biomass productivity declined by 35 % for *P. celeri* and 57 % for *T. striata*. Although CO_2 outgassing could be minimized, the significant loss in productivity led to a higher overall minimum biomass selling price (MBSP). Increased ammonia toxicity at the higher pH was found to be one of the growth-limiting factors and was mitigated by reducing ammonium fertilizer concentration. This adjustment resulted in a more moderate productivity decline of 13 % (instead of 35 %) for *T. striata*, and consequently, a lower MBSP was achieved. These findings suggest that equilibrium pH cultivation may be a viable method for reducing CO_2 loss without significantly compromising biomass productivity. Depending on the strain, strategies to mitigate stressors, such as ammonia toxicity, may be necessary.

1. Introduction

Microalgae constitute a diverse group of microorganisms that via photosynthesis harness sunlight and CO_2 to produce biomass. Their remarkable versatility and adaptability enable them to grow in diverse environments, including non-arable land and water bodies, making them good resources for generating sustainable biofuels and bioproducts [1,2]. Despite facing many challenges, microalgal biomass holds promise as a sustainable feedstock for biofuel production, offering a potential avenue for reducing reliance on fossil fuels and mitigating CO_2 emissions [1,3].

Biomass productivity is one of the key factors affecting the economic viability of microalgae-based biofuels [4,5]. Therefore, in the past, considerable research has been carried out to identify strains that can grow fast for feedstock production [6–9]. In these studies, the strains were provided with sufficient CO_2, allowing the culture pH to be maintained near 7. Although such conditions supported high growth rates, CO_2 loss due to outgassing has been overlooked, which could contribute significantly to the overall production cost [5,10].

For microalgae cultivation in open systems such as raceway ponds, CO_2 dissolved in the aqueous phase is constantly in exchange with CO_2 in the air. Maintaining the pH at near-neutral levels (pH 7) keeps the dissolved CO_2 concentration significantly higher than the equilibrium concentration. This creates a driving force across the gas–liquid interface, leading to CO_2 loss to the air. As a result, CO_2 utilization efficiency (CO_2UE), defined as the fraction of CO_2 assimilated in the biomass to the total amount of CO_2 provided to the culture, can be low. Although, depending on operational conditions, CO_2UE may reach up to 90 % [11], most studies have reported values only between 10 % and 40 % [12–15].

To mitigate such loss via CO_2 outgassing, one approach is to reduce the concentration of dissolved CO_2 to or below the equilibrium level by elevating the pH, in which case the carbon equilibrium shifts toward bicarbonate and carbonate. Fig. 1 shows the relationship between

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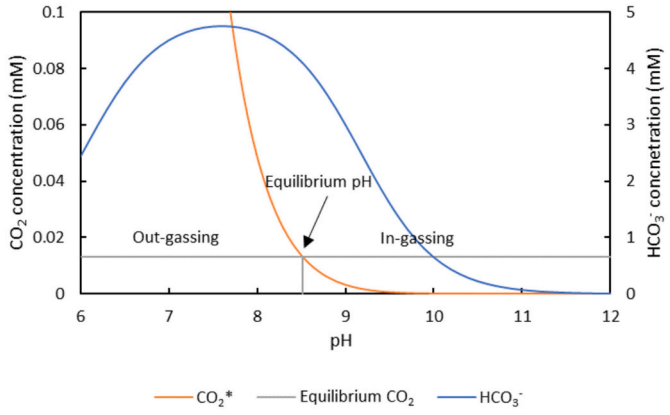

Fig. 1. CO_2 ingassing and outgassing as a function of pH, generated using equilibrium constants determined by Millero et al. [16]. The orange line represents the change in dissolved CO_2 concentration as a function of pH in seawater with additional 2.5 mM dissolved inorganic carbon (total alkalinity = ca. 5 meq L^{-1}) at 20 °C. The blue line represents the bicarbonate concentration as a function of pH. The horizontal line represents the equilibrium CO_2 concentration in the aqueous phase at 400 ppm atmospheric CO_2 concentration. It crosses the dissolved CO_2 line at pH around 8.6, the equilibrium pH. At a pH lower than the equilibrium pH, the CO_2 concentration in the aqueous phase is greater than the equilibrium CO_2 concentration, resulting in net CO_2 outgassing and carbon loss. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dissolved CO_2 concentration and pH. As the pH increases, the dissolved CO_2 concentration decreases. At pH 8.6, where the dissolved CO_2 concentration equals the air-CO_2 equilibrium concentration, referred to as the equilibrium pH in this study, the driving force across the gas–liquid interface is zero. If microalgae could be cultivated at the equilibrium pH, there will be minimal net CO_2 loss via outgassing, lowering the demand and cost of CO_2 supply.

An immediate concern with proposed cultivation method at the equilibrium pH is carbon limitation. At the higher pH, the dissolved CO_2 concentration is lower than at near-neutral pH by an order of magnitude or more. As an essential substrate for photosynthesis, CO_2 at a low concentration can limit biomass growth. In this case, microalgae must use bicarbonate as the primary carbon source. Although many microalgae species have demonstrated the ability to use bicarbonate for photosynthesis, there is no assurance that all the algal species can utilize bicarbonate as efficiently as CO_2 and maintain high biomass productivity [17–20]. While our goal is to enhance CO_2UE to reduce the cost of supplied CO_2, maintaining high biomass productivity is still critical for its significant impact on supporting cost-effective biomass. A high CO_2UE but a low biomass productivity could still lead to a high biomass production cost. Therefore, the strain's ability to grow fast at equilibrium pH is important for the economic feasibility of the proposed equilibrium pH cultivation approach.

Biomass processing technologies such as hydrothermal liquefaction (HTL) or biochemical fractionation approaches convert biomass into fuel intermediates and other co-products, and a large portion of the nitrogen in the biomass ends up in the aqueous waste stream as ammonium [21–23]. Recycling the waste ammonium into the culture for biomass growth has the potential to reduce net fertilizer costs and the overall production expenses. However, utilizing an ammonium-based medium presents a challenge at higher pH levels due to ammonia toxicity. In aqueous solution, the equilibrium between ammonium and ammonia is pH dependent (Eq. 1). Raising the culture pH from 7 to the equilibrium pH (e.g., pH 8.6) can result in an increase of ammonia concentration by more than 30 times [24]. The non-ionized form of ammonia is more toxic than the ionized form due to its rapid transport across cellular membranes [25]. Ammonia disrupts photosynthesis by damaging the manganese cluster of the oxygen evolution complex of photosystems II [26,27]. Additionally, the diffusion of ammonia uncouples ΔpH across the thylakoid proton gradient, negatively affecting ATP and ADP conversion [28,29]. Therefore, the successful implementation of the equilibrium pH cultivation approach for improving CO_2UE without severe biomass productivity loss relies on understanding the ammonia toxicity tolerance of the cultivated strain and operating the system within the non-toxic range.

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \quad (1)$$

The Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR) consortium project was initiated in 2016, and one of its main areas of focus has been on screening for the most productive strains in indoor and outdoor cultivation systems [30]. *Picochlorum celeri* TG2-WT-CSM/EMRE and *Tetraselmis striata* LANL1001 emerged as the top-performing strains from this research effort by demonstrating 50 % improvement in annual biomass productivity when cultivated year-round in outdoor ponds in Arizona, USA [31]. However, the high productivities of the two strains were achieved in ponds operated within near-neutral pH ranges, e.g., pH 7–7.5. The CO_2UE, although not reported in these studies, is generally near 30 % (data not shown). Currently, there is insufficient information on how these two fast-growing strains respond to cultivation at higher pH levels where CO_2 and bicarbonate become more limited. To assess the viability of the proposed cultivation approach, we initiated the present study by optimizing ammonium concentration so that ammonia toxicity is reduced and biomass productivity could be maximized at the equilibrium pH. After identifying the optimal ammonium concentration, each strain was cultivated at different pH levels to ensure that growth inhibition at high pH is within an acceptable range compared to the pH 7 baseline. Strains that passed the pH screening underwent testing in a bench-scale climate simulation photobioreactor, where the diurnally varying water temperature and light conditions in outdoor raceway ponds were replicated, to provide an estimation of pond-relevant biomass productivity. Following the determination of biomass productivity, the data served as input to a process model and an associated framework to evaluate the economic feasibility and the environmental impacts of implementing equilibrium pH cultivation.

2. Materials and methods

2.1. Strains

Two microalgal strains were grown in the present study: *Picochlorum celeri* TG2-WT-CSM/EMRE and *Tetraselmis striata* LANL1001. *P. celeri* was originally isolated from Texas coast of the Gulf of Mexico and was obtained from Dr. Matthew Posewitz of Colorado School of Mines. The strain showed outstanding growth rates at elevated temperatures with 30–40 °C being the optimal range; hence, it is primarily grown in the warm season. *T. striata* LANL1001 was originally isolated from a contaminated *Nannochloropsis salina* CCMP1776 pond culture and was obtained from Dr. Scott Twary of Los Alamos National Laboratory [32]. The strain tolerates temperatures as low as 5 °C and showed better growth than many other strains in the cool seasons [33–35]. The strains were revived in 150 mL Erlenmeyer flasks placed on a shaker table running at 120 rpm at room temperature (22–25 °C). Light was provided at ca. 450 μmol m^{-2} s^{-1} light intensity (12-h light: 12-h dark). Medium for reviving the cultures was prepared following the same composition used in previous work [33].

2.2. Identifying the optimal ammonium concentration

After revival, the two strains were grown in the DISCOVR medium with modification [33]. Instant Ocean® sea salt was added to the medium at 39.2 g L^{-1} to achieve a salinity of 35 practical salinity unit. The bicarbonate concentration was lowered to 1.5 mg L^{-1}. Combined with

the background alkalinity from the sea salts, the medium had a total alkalinity of approximately 5 meq L^{-1}. With 12 mg L^{-1} diammonium phosphate and 200 mg L^{-1} ammonium sulfate, the basal medium had a total ammonium concentration of 57.6 mg L^{-1}. The equilibrium pH was determined to be ca. 8.6 by sparging the medium with ambient air until a stable pH was observed. To determine their respective response to ammonium concentration, the two strains were grown over an ammonium concentration gradient from 3.24 to 220.7 mg L^{-1} by adding 0 (0×), 39.9 (0.1×), 99.8 (0.25×), 199.5 (0.5×), 399.1 (1×), 798.1 (2×), and 1596.3 (4×) mg L^{-1} ammonium sulfate while keeping the diammonium phosphate concentration unchanged. The media were denoted as 0×AS, 0.1×AS, 0.25×AS, 0.5×AS, 1×AS, 2×AS and 4×AS.

The two strains were grown in the Multi-Cultivator photobioreactor system (MC1000, Photon System Instrument, Czech Republic), which contains eight glass columns with a working volume of 80 mL and a diameter of 2.7 cm. The columns were submerged in a temperature-controlled water-bath and illuminated from the side with LED lighting (Fig. S1). For the warm season strain *P. celeri*, the temperature was maintained at 30 °C. For the cool season strain *T. striata*, the temperature was maintained at 15 °C. The cultures were grown on a 12 h:12 h light: dark cycle, with the light intensity set to 250 μmol m^{-2} s^{-1} during the light period. The cultures were mixed by constant sparging with ambient air at 80 mL min^{-1}. The medium pH was maintained at the equilibrium level (pH 8.6) via on-demand CO_2 addition using a pH-feedback control loop—if the medium pH increased above the setpoint, CO_2 was injected to lower the pH to the setpoint. All the cultures were started at the same optical density near 0.1, measured at 720 nm by the MC1000. The experiment was repeated once to confirm the results.

Volumetric biomass productivity was used to identify the optimal ammonium concentration. Samples were taken at the beginning and the end of each experiment to determine the initial and the final biomass concentrations. Biomass concentrations were measured and reported on an ash-free dry weight (AFDW, mg L^{-1}) basis. A 20 mL sample was filtered through a Whatman GF/F microfiber filter, dried at 105 °C overnight, and combusted at 540 °C for 2 h. The mass change before and after the combustion, divided by the sample volume (20 mL), was then reported as biomass concentration in AFDW. After obtaining the initial and final AFDW, the volumetric biomass productivity (g L^{-1} day^{-1}) was calculated by dividing the change in biomass concentration (between $t = 0$ and $t = 5$ days) by time (5 days, Eq. 2). The ammonium concentration that resulted in the highest volumetric biomass productivity was considered the optimum and was used to determine the biomass productivity at different pH levels and under simulated outdoor conditions, as described in Sections 2.3 and 2.4, respectively.

$$Biomass\ productivity = \frac{AFDW_{final} - AFDW_{initial}}{t} \quad (2)$$

where, $AFDW_{final}$ is the final biomass concentration in AFDW (mg L^{-1}), $AFDW_{initial}$ is the initial biomass concentration in AFDW (mg L^{-1}), and t is the growth period from time 0 to the final sampling (days).

2.3. Determining biomass productivity as a function of pH

The two strains were grown in the MC1000 photobioreactor system under the same temperature and light conditions as specified in Section 2.2. The ammonium concentration was adjusted to the optimal level as determined in Section 2.2 for each of the two strains. Four pH levels were tested in duplicate photobioreactor columns, including 7.0, 8.0, 8.6 (equilibrium pH) and 9.0. The pH was maintained by changing the pH setpoint in each column. The volumetric biomass productivity was also calculated using Eq. 2 by measuring the biomass accumulation during the 5-day cultivation. Because the two strains were grown at pH near 7 in previous indoor and outdoor DISCOVR research [33–35], the biomass productivity in the pH 7.0 culture was used as the control.

2.4. Measuring biomass productivity under simulated outdoor conditions

The Laboratory Environmental Algae Pond Simulator (LEAPS) photobioreactor was employed to measure biomass productivity under conditions simulating outdoor raceway ponds [36] (Fig. S2). The diurnally varying light and water temperature in the outdoor ponds were replicated in the LEAPS using scripts generated by the Pacific Northwest National Laboratory (PNNL) Biomass Assessment Tool [37], based on 30-year meteorological data collected at Mesa, Arizona, USA for a 20 cm deep raceway pond. The July 1 script (Fig. 2a) was used to grow *P. celeri*, and the May 1 script (Fig. 2b) was used to grow *T. striata*. The average temperature during the light period was 27 °C for the July 1 script and 21 °C for the May 1 script. The scripts were repeated daily to represent typical incident sunlight and outdoor pond water temperature conditions for the summer and late spring seasons at Mesa, Arizona, USA.

The LEAPS cultures were started via inoculum addition at a biomass concentration between 100 and 200 mg AFDW L^{-1}. Three conditions were tested for each strain in duplicate bioreactor columns, including pH 7 in the original medium (1×AS, control), pH 8.6 (equilibrium pH) in original medium (1×AS), and pH 8.6 in the medium with optimal ammonium concentration determined in Section 2.2. The pH was maintained at the respective setpoints by adding CO_2 via pH feedback control. Sparging with ambient air was provided at 100 mL min^{-1} during the daytime to generate vertical mixing through the 20 cm deep water column. Sparging and CO_2 were shut off for the night period because no photosynthesis occurs during the dark period. The stir bar at the bottom of each culture was running at 200 rpm to prevent biomass

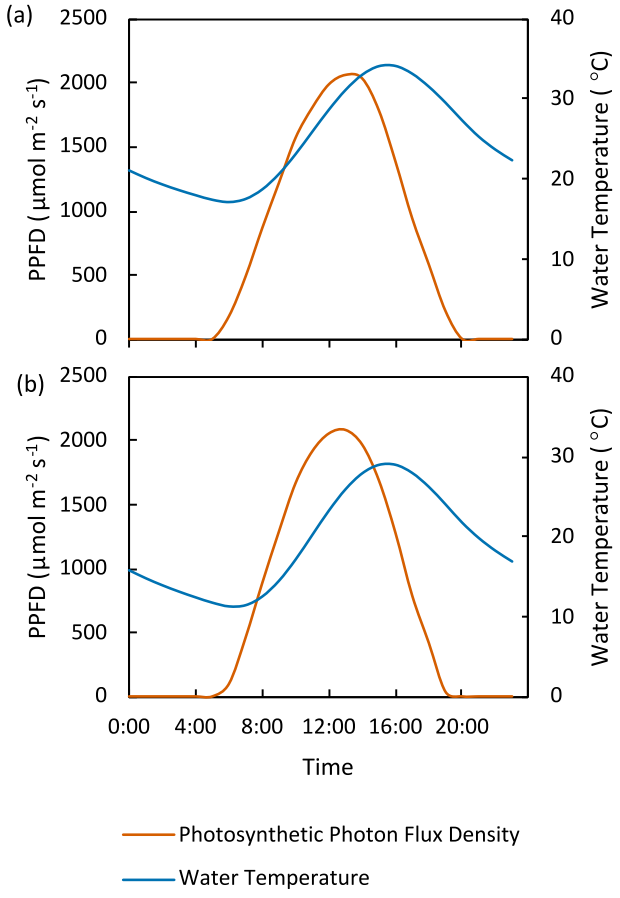

Fig. 2. The daily-repeated light intensity (photosynthetic photon flux density [PPFD]) and water temperature scripts used in this study. (a) The scripts for growing *P. celeri*, representing a typical summer day (July 1) in Mesa, Arizona. (b) The scripts for growing *T. striata*, representing a typical late spring day (May 1) in Mesa, Arizona.

from settling. Biomass concentration in AFDW (mg L^{-1}) was measured daily as described in Section 2.2. The experiment was continued until the controls reached a biomass concentration above 400 mg L^{-1} and repeated by diluting the cultures back to the initial biomass concentration (100–200 mg AFDW g^{-1}). The ammonium concentration was measured daily using the Nitrogen-Ammonia nitrogen test kit (Hach®, Loveland, Colorado, USA). Once the ammonium concentration decreased below 1.8 mg L^{-1} (0.1 mM), 66.7 mg L^{-1} (0.5 mM) ammonium sulfate was added to keep the cultures from becoming nitrogen depleted. The volumetric biomass productivity was calculated via Eq. 2. To compare results to previously reported pond productivities [31,35], the volumetric biomass productivity (g L^{-1} day^{-1}) was converted to areal biomass productivity (g m^{-2} day^{-1}) by multiplying the volumetric biomass productivity by culture depth (0.2 m).

2.5. Data analysis

Multivariate analysis of variance (MONOVA) was used to compare the growth curves and assess the effects of ammonium concentration and pH.

2.6. Techno-economic analysis and life cycle assessment

Techno-economic analysis (TEA) and life cycle assessment (LCA) were carried out based on the National Renewable Energy Laboratory (NREL) algae farm model, described in NREL's 2016 algae design report [5]. For this exercise, the accompanying algae farm tool was set to simulate open-pond biomass cultivation in 10-acre individual ponds. The employed framework considered a 5000-acre algae farm with a harvested density setpoint of 0.5 g L^{-1} and a three-step dewatering approach to recover and concentrate algae biomass to 20 wt% solids. All scenarios were assessed as having a year-round flat productivity for two main reasons: (1) to avoid the addition of an uncertainty factor to the analysis in the form of productivity variability among seasons, and (2) to provide results that can be more directly applied by researchers because cultivation trials carried out in small scale usually yield individual productivity numbers representative of a single seasonality condition. A similar framework has been successfully applied in previous studies which aimed at providing a comparative analysis between microalgae cultivation strategies [38,39]. The main parameters varied in the simulations are tied to experimentally derived biomass productivity data and CO_2UE, the latter of which is assessed as a sensitivity: 20–40 % for pH 7 cultivations and 70–90 % for pH 8 and 8.6 cultivations (encompassing typical ranges observed for neutral pH cultivation while reflecting higher CO_2UE achievable under elevated pH conditions). The production of algae biomass using the previously described conditions was then assessed from both economic and environmental standpoints. A discounted cash flow rate of return analysis was employed to consider both operational costs and capital expenses incurred by the algae farm to yield the minimum biomass selling price (MBSP) as the main metric of interest, given in 2020 U.S. dollars per kg of AFDW biomass. In this effort, no additional costs associated with increasing the pH to the desired levels (i.e., either 8 or 8.6 for high pH cultivations in the data presented herein) were taken into account as pH control in large-scale facilities would make use of CO_2 and nutrient supply strategies that are already in place to deliver macronutrients to cultivations without incurring significant additional expenses [40]. LCA was also carried out using the outcomes from the algae farm models to estimate the greenhouse gas (GHG) emissions associated with the production of algae biomass as a function of varying biomass productivity and CO_2UE levels. The carbon intensities for chemical and energy inputs reflected in the 2022 Greenhouse gases, Regulated Emissions, and Energy use in Technologies (GREET) model were leveraged to estimate the carbon footprint of the microalgae biomass, reported in g of CO_2-equivalent (gCO_2e) per kg of AFDW biomass [41]. The reader is referred to Tables S1 and S2 in the Supplementary Material for additional details associated with the TEA and LCA procedures.

3. Results and discussions

3.1. Optimal ammonium concentration at equilibrium pH

Adding the correct amount of ammonium to grow the algae requires careful balancing: On one hand, excessive ammonium inhibits growth due to the presence of ammonia toxicity at elevated pH values. On the other hand, nitrogen is an essential nutrient for microalgal growth, and insufficient supply of ammonium also leads to productivity loss. To sustain a fast-growing high-productivity culture, ammonium should be added at the concentration that provides enough nitrogen without causing significant growth inhibition. The sensitivity to ammonia toxicity varies considerably among different microalgae species. For instance, the specific toxicity of ammonia (EC_{50}, 24-h exposure) with respect to a green alga, *Nephroselmis pyriformis*, was measured as low as 39.8 μg L^{-1} [42]. Ammonia concentrations of 2.3 and 3.3 mg L^{-1} significantly inhibited the growth of *Neochloris oleoabundans* and *Dunaliella tertiolecta* [29]. *Nannochloropsis oculata* and *Dunaliella tertiolecta* showed 50 % growth inhibition at near 8 mg L^{-1} free ammonia, with complete mortality observed at 16 mg L^{-1} free ammonia [29]. Källqvist and Svenson [42] reported that 25.5 mg L^{-1} ammonia could halve the carbon assimilation rate of *Scenedesmus obliquus*. In contrast, research on microalgae for nitrogen removal from wastewater identified strains that tolerate higher ammonia concentrations. For example, in manure-free piggery wastewater, 19.9 mg L^{-1} free ammonia did not affect the growth of *Chlorella pyrenoidosa* [43], and *C. vulgaris* exhibited the best growth at 110 mg L^{-1} ammonium [44]. A specifically isolated *Chlorella* dominated microalgae consortium demonstrated stable growth at 800–1600 mg ammonium L^{-1} at pH 8 [45]. The basal medium used in this study has 57.6 mg L^{-1} total ammonium, and depending on other factors such as temperature [24], the ammonia concentration ranges between 4.3 and 10.7 mg L^{-1} at the equilibrium pH 8.6. If *P. celeri* and *T. striata* are sensitive to ammonia as some of the above-mentioned species, their growth could be substantially inhibited.

Fig. 3 shows the volumetric biomass productivity of the two species in response to varying ammonium concentrations at pH 8.6. The biomass productivity of *P. celeri* increased from 20.6 to 68.1 mg L^{-1} day^{-1} as ammonium concentration increased from 3.2 to 57.6 mg L^{-1}, suggesting nitrogen insufficiency is the primary growth-limiting factor within this range. As ammonium concentration increased further, biomass productivity started to decrease. At 220.7 mg L^{-1}, ammonia toxicity was so severe that *P. celeri* failed to grow after the first light

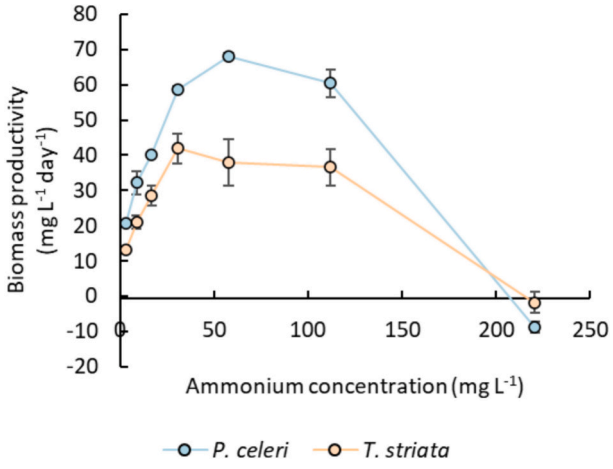

Fig. 3. Volumetric biomass productivity of *P. celeri* and *T. striata* as a function of the initial ammonium concentration at air-CO_2 equilibrium pH (8.6). Error bars represent one standard error ($n = 2$).

cycle. The dying culture ended up with a negative overall biomass productivity (−8.8 mg L^{-1} day^{-1}). Because the best productivity was observed at 57.6 mg L^{-1} (1×AS medium), subsequent experiments on *P. celeri* were carried out at this ammonium concentration.

T. striata is a cool season strain and was grown at a lower temperature (15 °C). Its productivity was generally lower than that of *P. celeri*, but its growth response to ammonium concentration followed a similar pattern. The maximum biomass productivity of *T. striata* was observed at 30.42 mg L^{-1} ammonium, approximately half of the optimal ammonium concentration for *P. celeri*. There was little reduction in productivity within the ammonium concentration range of 30.4 and 112.0 mg L^{-1} (p-value <0.05). However, *T. striata* also failed to survive at 220.7 mg L^{-1} ammonium due to toxic effects, resulting in a negative productivity of −1.8 mg L^{-1} day^{-1}. Given that 30.4 mg L^{-1} ammonium supported the best growth, *T. striata* was grown at this concentration (0.5×AS) in the subsequent experiments.

3.2. Biomass productivity as a function of pH

Even though results in Section 3.1 showed that the two strains could grow at the equilibrium pH, it is important to ensure that biomass growth is not severely inhibited compared to the pH 7 baseline. Previous studies have shown that increasing the pH could lead to a substantial reduction in biomass growth [46,47]. In such cases, the productivity decreases relative to the control at pH 7 may outweigh the benefits of improved CO_2UE at equilibrium pH, thereby resulting in increased biomass production costs.

Fig. 4 shows the biomass productivities of the two strains as a function of pH. According to the results in Section 3.1, the ammonium concentration remained at 57.6 mg L^{-1} (1×AS) for *P. celeri* but was reduced to 30.4 mg L^{-1} (0.5×AS) for *T. striata*. Compared to the pH 7 control, only a 4 % reduction in biomass productivity was observed in *P. celeri*, and no effect was observed in *T. striata* cultures at pH 8.6. At pH 8.6, bicarbonate becomes the dominant carbon species, accounting for approximately 80 % of the dissolved inorganic carbon (DIC), and the concentration of dissolved CO_2 becomes negligible (ca. 1 % DIC). The minimal growth reduction of the two strains at pH 8.6 indicated these two strains can utilize bicarbonate efficiently as the primary carbon source, which was observed in many other microalgae species such as *Nannochloropsis*, *Chlorella*, and *Scenedesmus* [48–50]. However, as pH further increased to 9, significant growth inhibition occurred in both

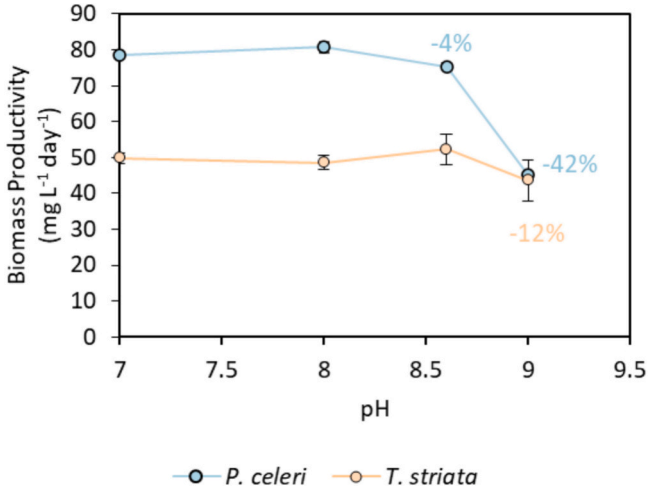

Fig. 4. Biomass productivities of *P. celeri* and *T. striata* as a function of pH at the respective optimal ammonium concentrations. Error bars represent one standard error (n = 4). The 4 % and 42 % decline represent the growth penalty at pH 8.6 and pH 9 for *P. celeri* with respect to the pH 7 control. The 12 % decline represents the growth penalty at pH 9 for *T. striata* with respect to the pH 7 control.

strains. Compared to the pH 7 control, the biomass productivity at pH 9 was reduced by 42 % and 12 % for *P. celeri* and *T. striata*, respectively. From pH 8.6 to 9, the ammonia concentration almost doubled, while bicarbonate concentration decreased by 20 %. Both ammonia toxicity and carbon limitation could have contributed to the decline in growth rates. Given the small growth penalty observed at pH 8.6, the proposed cultivation at equilibrium pH has the potential to be a feasible approach to improve CO_2UE without incurring a significant loss in biomass productivity.

3.3. Measuring biomass productivity under simulated outdoor conditions

The results above were achieved in small bioreactor columns (ca. 80 mL) under constant temperature and light conditions. For instance, the temperature for *P. celeri* was continuously maintained at 30 °C, which is within the optimal temperature range of this strain (30–40 °C) [33]. The light intensity was kept at 250 μmol m^{-2} s^{-1} without variation throughout the light period. Also, the cultures in the bioreactor columns were thoroughly mixed by air sparging at a 1:1 v:v min^{-1} rate, which effectively removed excess photosynthetically generated dissolved oxygen (DO) and maintained the DO concentration at the air-saturation level of ca. 8 mg L^{-1}. While these conditions are well suited for quickly evaluating strains, outdoor cultures face more challenges such as large daily temperature and light intensity fluctuations, supersaturated DO concentrations due to limited mixing and O_2 outgassing, and other nonideal conditions [51,52]. Therefore, to provide a more reliable prediction of strain performance in outdoor ponds, the two strains were evaluated in the LEAPS photobioreactor where the outdoor diel water temperature and light intensity variations were replicated.

As mentioned in Section 2.2, the three originally planned conditions for the LEAPS growth simulation were (a) pH 7 in the 1×AS medium (control), (b) pH 8.6 in the 1×AS medium, and (c) pH 8.6 at the optimal ammonium concentration. However, based on the results shown in Section 3.1, the optimal ammonium concentration for *P. celeri* is 57.6 mg L^{-1} (1×AS). As a result, treatment b and c would have the same condition. To avoid duplication, condition for treatment b was changed to pH 8 in the 1×AS medium for *P. celeri*.

There were two repeated batch culture runs carried out for each strain in the LEAPS photobioreactors (Fig. 5a and b). The pH was maintained at near the setpoints for all cultures during the day (Fig. S3). For *P. celeri*, each batch culture run lasted 4 days. The baseline biomass productivity in the pH 7 control was 18.5 g m^{-2} day^{-1}. Increasing the pH to 8 and 8.6 lowered biomass productivity to 16.4 g m^{-2} day^{-1} (a 13 % reduction relative to the baseline) and 7.9 g m^{-2} day^{-1} (a 57 % reduction relative to the baseline), respectively (Fig. 5c). The significant growth penalty at pH 8.6 was in disagreement with the minimal impact observed in the pH effect evaluation experiment (Fig. 4). The discrepancy was likely due to the drastic difference in growth conditions between the two systems. In the LEAPS, the culture experienced a temperature variation from 15 °C to 34 °C and light intensity variation from 0 to 2058 μmol m^{-2} s^{-1} (Fig. 1). Additionally, during the light period, the DO concentration in the LEAPS cultures peaked at near 18 mg L^{-1} in the afternoon, which is more than 200 % greater than the air-saturation level as observed in the well-sparged small columns. As reported in previous studies, supersaturated DO can cause severe oxidation stress and inhibits photosynthetic growth [52–54]. These nonideal and fluctuating conditions in temperature, light, and DO concentration in the LEAPS photobioreactors could have weakened the culture, making it more sensitive to high pH and ammonia toxicity; hence, growth was substantially inhibited at pH 8.6.

For *T. striata*, if ammonium was kept at the original level (1×AS, 57.6 mg L^{-1}), increasing the pH from 7 to 8.6 caused biomass productivity to decrease from 12.7 g m^{-2} day^{-1} to 8.3 g m^{-2} day^{-1}, a 35 % reduction due to ammonia toxicity under high pH conditions (Fig. 5d). Using the medium with the optimized ammonium concentration (0.5×AS, 30.4 mg L^{-1}), *T. striatia* achieved a biomass productivity of

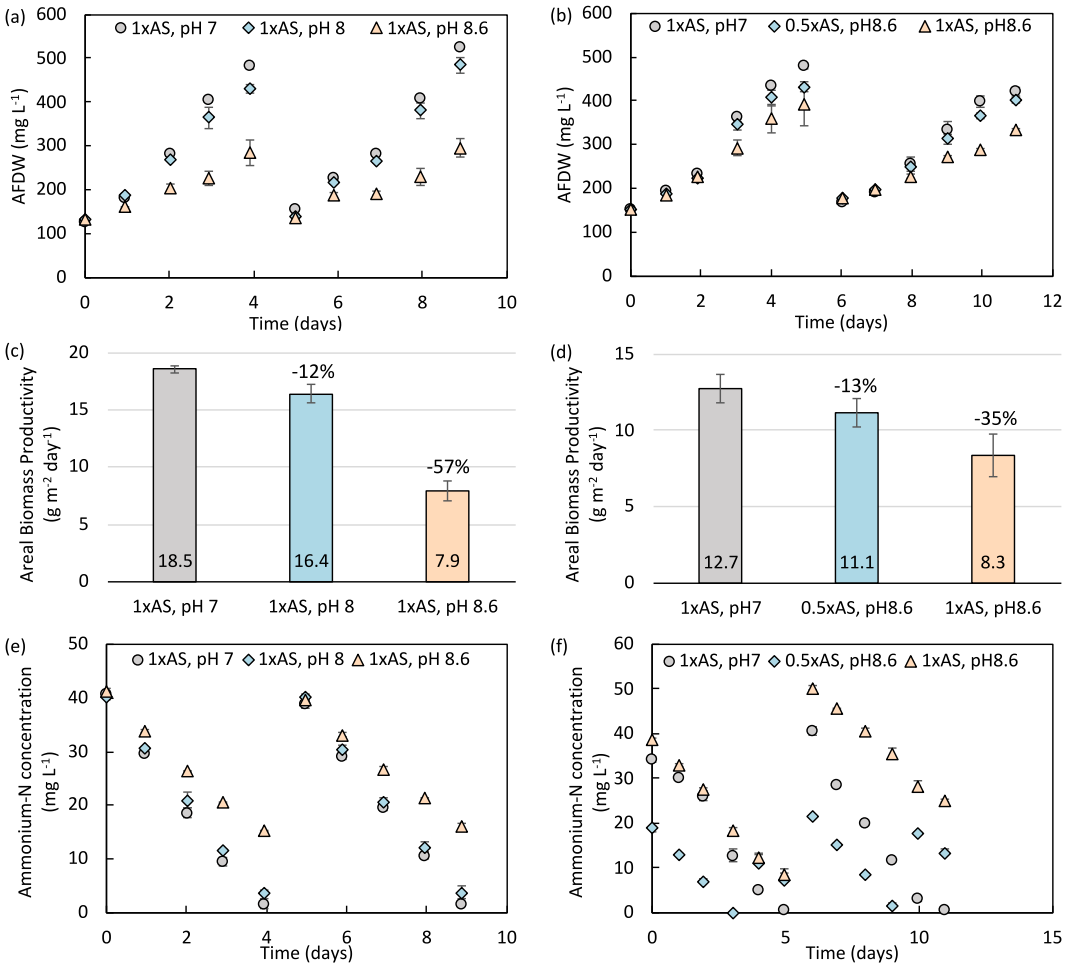

Fig. 5. Biomass growth curves, biomass productivity, and ammonium concentration as a function time for *P. celeri* (left panel, a, c, e) and *T. striata* (right panel, b, d, f) in the LEAPS photobioreactor experiments simulating outdoor pond conditions. Error bars represent one standard error ($n = 2$ for growth curves and ammonium concentration, and $n = 4$ for biomass productivities). The percentages in (c) and (d) show the productivity loss of each condition with respect to the control (1×AS, pH 7).

11.1 g m^{-2} day^{-1} at pH 8.6. Despite being 13 % lower than the control productivity at pH 7, halving the addition of ammonium sulfate noticeably mitigated ammonia toxicity and prevented severe losses in biomass productivity. There is a potential to further moderate ammonia toxicity by changing the fertilization strategy to more frequent addition of small doses of ammonium, so that ammonium concentration is reduced while ensuring a steady nitrogen supply. The exploration of this fed-batch ammonium addition method was not pursued in this study because determining the optimal dosing frequency and concentration requires more extensive research. Nevertheless, further investigation into this approach would be worthwhile if the equilibrium pH cultivation shows potential to be economically feasible.

Microalgae are known for their efficient nitrogen removal capabilities. During the 4-day climate simulation experiment, the ammonium concentration decreased from approximately 53 mg L^{-1} to near 2 and 5 mg L^{-1} for *P. celeri* cultures at pH 7 and 8, respectively (Fig. 5e). Due to the inhibited growth at pH 8.6, about 20 mg L^{-1} ammonium was left in the culture on the last day. The ammonium consumption rate at pH 8.6 was 8.1 mg L^{-1} day^{-1}, which is 35 % slower than the 12.6 mg L^{-1} day^{-1} in the pH 7 control culture (Table 1). In the *T. striata* 0.5×AS pH 8.6 culture, ammonium was fully consumed within three days. Additional ammonium sulfate was added to prevent nitrogen depletion on Day 3 and Day 9 (Fig. 5f). The ammonium consumption rate ranged from 7.2 mg L^{-1} day^{-1} in the 1×AS, pH 8.6 culture to 9.6 mg L^{-1} day^{-1} in the 1×AS pH 7 control culture (Table 1).

Table 1
Ammonium consumption rate and nitrogen-biomass yield for the two strains under the different pH and medium conditions.

Strain	Medium condition	Ammonium-N consumption rate (mg L^{-1} day^{-1})	Ammonium biomass yield (mg AFDW mg^{-1} ammonium-N)
P. celeri	1×AS, pH 7	12.6	7.4
P. celeri	1×AS, pH 8	12.0	6.8
P. celeri	1×AS, pH 8.6	8.1	4.8
T. striata	1×AS, pH 7	9.6	6.7
T. striata	0.5×AS, pH 8.6	7.3	7.6
T. striata	1×AS, pH 8.6	7.2	5.8

The observed difference between the two strains is consistent with the varying rates of ammonium uptake across different species and cultivation conditions. Chen et al. [55] demonstrated that *T. chui* exhibited a three-fold faster ammonium consumption rate compared to three other marine microalgae under the same condition. As temperature increased from 15 °C to 34 °C, the ammonium nitrogen removal rate of *Scenedemus* increased from 4.3 to 17 mg L^{-1} day^{-1} [56]. Ji et al. reported that *Desmodesmus* sp. consumed ammonium nitrogen at a rate of 3.7 mg L^{-1} day^{-1} in batch cultivation and 8.9 mg L^{-1} day^{-1} in fed-batch cultivation [57]. Moreover, the different growth conditions could also

result in different ammonium-biomass yield. The third column in Table 1 presents the amount of biomass generated based on the consumption of ammonium nitrogen for the two strains under different cultivation conditions. For both strains, the cultures grown in the 1×AS medium at pH 8.6 had the lowest biomass yield per unit of ammonium nitrogen consumed. Compared to the other conditions, the ammonia concentration in the 1×AS medium at pH 8.6 is 2–60 times higher. The non-ionized ammonia can easily permeate into the cells and accumulate at a higher level intracellularly, leading to a stronger growth inhibition and higher nitrogen content per unit biomass, and hence, a low ammonium-biomass yield [25,58]. It wass also possible that the decrease in medium ammonium concentration was not only attributed to biological uptake but also ammonia outgassing. With more nitrogen in the form of volatile ammonia and continuous sparging in the photobioreactors, nitrogen loss to the air in the 1×AS pH 8.6 cultures was likely the highest among all cultures. Because the primary focus of this study was biomass productivity, nitrogen utilization was not fully investigated. However, future research should be carried out to explore efficient nitrogen utilization during cultivation at equilibrium pH, in conjunction with the frequent addition of small doses of fertilizers.

The LEAPS photobioreactor was designed to replicate the outdoor light and water temperature conditions, enabling predictions of biomass growth in outdoor ponds under defined pH setpoints and medium compositions [36]. However, aside from photosynthetic carbon assimilation, CO_2UE is determined by CO_2 provided to the culture, stored in the medium, and outgassing and ingassing across the gas-liquid interface. The gas exchange dynamics are heavily influenced by system geometry and operational conditions, which differ substantially between the bench-scale photobioreactor and outdoor raceway ponds. For example, cultures in raceway ponds are driven by a paddlewheel, whereas mixing in the LEAPS relies on sparging with air and a spinning stir bar at the bottom. With the bubbles constantly rising in the cylindrical water column and bursting at the surface, CO_2 ingassing and outgassing in the LEAPS are drastically different than in the raceway ponds where active sparging is absent. As a result, CO_2UE in the LEAPS does not represent CO_2UE in the ponds. Therefore, CO_2UE was not quantified in this study. Nonetheless, even though the LEAPS cannot replicate the abiotic gas mass transfer characteristics of a pond culture,

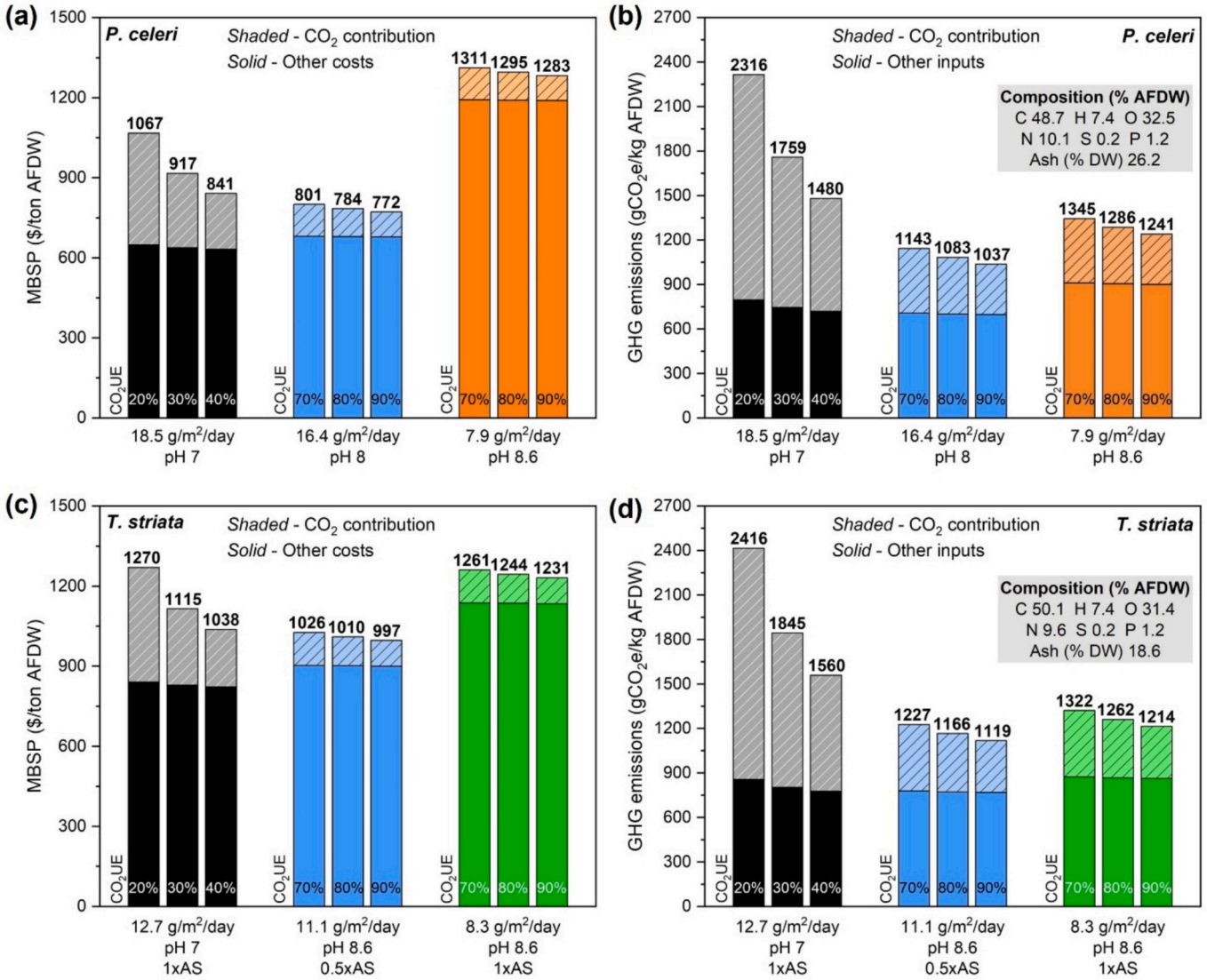

Fig. 6. MBSP and GHG emissions for the cultivation of *P. celeri* (a, b) and *T. striata* (c, d) at different average biomass productivities observed under low and high pH conditions, reflected over assumed ranges of CO_2UE from 20 to 40 % for neutral pH cultivation increasing to 70–90 % for high pH cultivation. For *P. celeri*, ammonium was kept at 1×AS for pH 7, 8, and 8.6 to generate the experimental productivity data as discussed above. For *T. striata*, experimental cases were run for pH 7, pH 8.6 in 1×AS medium, and pH 8.6 at reduced ammonium concentration (0.5×AS). The compositional profiles of *P. celeri* and *T. striata* are indicated in panels b and d, respectively.

as long as the critical environmental factors were simulated accurately, e.g., pH, nutrient concentrations, light intensity, and temperature, LEAPS can reliably predict biomass growth in outdoor ponds. As an initial assessment of the proposed equilibrium pH cultivation method, we focused on one of the most impactful variables in determining the economic viability of microalgal biofuels—biomass productivity [5]. If biomass productivity observed in this study led to reduced MBSP and carbon intensity in the following TEA and LCA analyses, further validation experiments and CO_2UE measurements should be conducted in outdoor raceway ponds.

3.4. TEA results

Fig. 6a and c illustrate the effects of elevated pH conditions on the MBSP for *P. celeri* and *T. striata*. Fig. 6b and d depict the corresponding impacts on GHG emissions for the same strains. In previous studies, these microalgal strains showed different biomass compositions [35] and exhibited distinct areal biomass productivities when grown under varying pH conditions (Fig. 5). The MBSP and GHG emission metrics, by incorporating the strain-specific biomass composition and productivity, were determined to quantify the contributions of CO_2 uptake and other influencing factors on the overall economic and environmental performance [5].

The panels in Fig. 6 show the economic and environmental benefits of increasing the pH of the cultivation medium for generating algae biomass. In the case of *P. celeri*, there is an expected net decrease both in MBSP and in GHG emissions when the pH in cultivations is modified from 7 to 8 (Fig. 6a, b), even when comparing the worst case for pH 9 (CO_2UE = 70 %, MBSP = 801 $/ton) for pH 8 to the best case for pH 7 (CO_2UE = 40 %, MBSP = 841 $/ton) for pH 7. For cultivations carried out under pH 8.6, however, the resulting net MBSP is anticipated to be higher than that of the baseline. Even though CO_2UE is in the 70–90 % range, the decrease in biomass productivity is large enough to offset any gains from a more efficient utilization of CO_2. In terms of GHG emissions, the biomass cultivated at pH 8.6 would have a slightly higher carbon intensity than that obtained at pH 8, with both being lower than the baseline cultivations at pH 7, implying a stronger dependency on CO_2UE relative to productivity for the GHG emissions metric than for MBSP. For *T. striata*, the trend is similar for both economic (Fig. 6c) and environmental results (Fig. 6d), suggesting that moving from low pH to higher pH is a practical strategy to improve both MBSP and GHG emissions associated with algal biomass production. However, a caveat to this conclusion is that a net reduction in cost and carbon intensity is contingent on maintaining a certain cultivation productivity level within reason. In the *T. striata* case, passing from a biomass productivity of 11.1 g m^{-2} day^{-1} at the equilibrium pH with reduced ammonium concentration (0.5×AS) to 8.3 g m^{-2} day^{-1} at the same pH but with the original ammonium concentration (1×AS) employed experimentally virtually negates the economic benefits from achieving a higher CO_2UE (Fig. 6c). Such results indicate that optimizing the addition of nutrients is necessary to mitigate stress for algal strains that are sensitive to ammonia toxicity. Analogously, the decrease in productivity moving from 0.5×AS to 1×AS incurs a slight increase in GHG emissions, although the latter is still estimated to be better than the carbon intensity of biomass cultivated under low pH conditions (Fig. 6d). Finally, it is noted that the bench-scale photobioreactor LEAPS cannot represent gas exchange in the ponds; hence, the analysis shown herein was carried out under the assumption that CO_2UE increases from 20 to 40 % at pH 7, a commonly reported range [12–15], to 70–90 % at the equilibrium pH where there is little driving force for CO_2 outgassing. Such an effect, as already observed experimentally, is a direct product of the water chemistry employed in cultivations, which influences the equilibrium of inorganic carbon species available for microalgae uptake [11,59,60].

4. Conclusion

This study evaluated air-CO_2 equilibrium pH cultivation as a way to improve CO_2 utilization efficiency and reduce carbon delivery costs for microalgae biomass production. Under the air-CO_2 equilibrium pH conditions, a decrease in biomass productivity was observed compared to baseline cultivation at pH 7. Without mitigation strategies, the substantial productivity loss could offset the benefit of improved CO_2UE and lead to an increased MBSP. However, if stress could be mitigated—for example, by optimizing fertilization strategies to lower ammonia toxicity as demonstrated by *T. striata*—the equilibrium pH cultivation method could achieve an optimal balance of minimally lower productivity versus higher CO_2UE, translating to more favorable overall production costs and GHG emissions for cultivation.

CRediT authorship contribution statement

Song Gao: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicholas Kalamaris:** Visualization, Investigation, Data curation. **Bruno Klein:** Writing – original draft, Visualization, Methodology, Formal analysis. **Scott Edmundson:** Writing – review & editing, Methodology, Conceptualization. **Geetanjali Yadav:** Writing – review & editing, Formal analysis. **Ryan Davis:** Writing – review & editing. **Michael Huesemann:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2025.104392.

Data availability

Data will be made available on request.

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